MINI-PAM-II

Photosynthesis Yield Analyzer

Manual

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1 Safety Instructions

1.1 General Safety Instructions

- Read safety instructions and the operating instructions prior to operation of the device and its accessories.
- Pay attention to all safety warnings.
- Keep device and its accessories away from water or high moisture areas.
- Keep the device and its accessories away from dust, sand, and dirt.
- Do not put the device and its accessories near sources of heat.
- Ensure that neither liquids nor foreign bodies get inside the device or its accessories.
- Ensure sufficient ventilation.
- Connect the device only to the power source indicated in the operating instructions or on the device. If the device is not in use, remove the mains plug from the socket.
- The device and its accessories should only be repaired by qualified personnel.

1.2 Special Safety Instructions

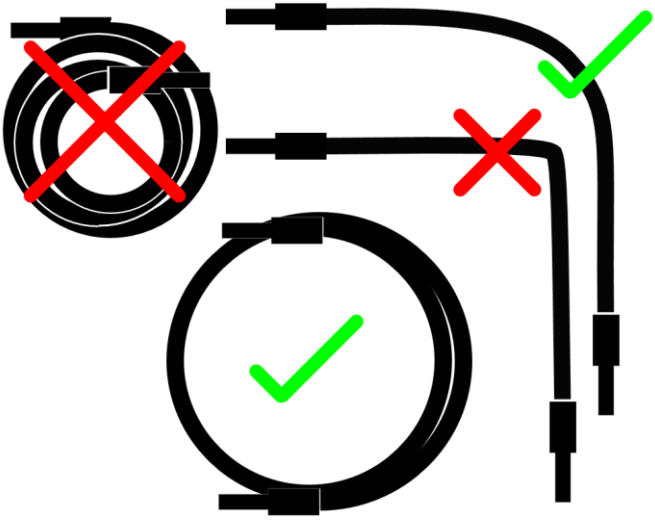
- The MINI-PAM-II is a highly sensitive instrument which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual in

order to avoid potential harm to the user and damage to the instrument.

- The MINI-PAM-II can emit very strong light! To avoid harm to your eyes, never look directly into the light port of the MINI-PAM-II or its fiberoptics.
- Switch off MINI-PAM-II before connecting or disconnection 2054-L External LED Source.

1.3 Handling of Fiberoptics

The MINI-PAM/F Fiberoptics of the MINI-PAM-II is made of delicate glass fibers. Each glass fiber extents over the entire length of the fiber optics. Do not sharply bend, stretch, or crush the fiber optics as this can break the glass fibers. Each broken fiber reduces light transmission by the fiber optics. If many fibers are broken, the fiber optics must be replaced. Place protection caps on fiber ends when the fiber optics is not in use.



2 Introduction

- The “Photosynthesis Yield Analyzer MINI-PAM-II” has been designed for highly sensitive saturation pulse analysis of photosystem II (PS II) in the field as well as in the laboratory. The automatically calculated parameters are F_V/F_M (maximum photochemical yield), Y(II) (effective photochemical yield) and its complementary yields Y(NPQ) and Y(NO), as well as parameters of photochemical (q_L, q_P) and non-photochemical quenching (q_N, NPQ) (see Table 26, page 166).
- The instrument continues the tradition of the preceding MINI-PAM chlorophyll fluorometer. The major technical advancements of the MINI-PAM-II are the consistent use of energy-efficient LEDs, an internal PAR sensor, and stand-alone operation by a touchscreen which is well readable under natural light conditions. Also, a far-red LED has been added for selective excitation of photosystem I.
- A further technical progress is the newly designed leaf clip sensor (2035-B) which measures photosynthetically active radiation (PAR) at leaf level with high accuracy and, thus, provides reliable light intensity data for calculations of electron transport rates (ETR).
- A variety of add-ons make the MINI-PAM-II a highly versatile measuring system which can be configured to meet the needs of your research goal. The range of accessories includes a multi-colored lighting unit (Section 3.4.3, page 29), an optical oxygen sensor (Section 3.4.12, page 36) and a miniature spectrometer (Section 3.4.1, page 21).
- For long-term field campaigns, the memory capacity has been upgraded to keep data of more than 27,000 saturation pulse

analyses. The fluorometer is powered by of-the-shelf AA (Mignon) batteries which are easily replaceable even under field conditions. One set of batteries lasts for up to 1000 saturation pulse analyses.

- The MINI-PAM-II can be operated in the stand-alone mode or by the well-proven WinControl-3 software. WinControl-3 has been introduced with the JUNIOR-PAM fluorometer and now operates many other fluorometers like the DIVING-PAM, MONITORING-PAM, and WATER-PAM. In addition to the features available in the stand-alone mode, the software allows evaluations of light-response curves by a non-linear fitting routine and automatic execution of custom-designed experiments using the built-in batch file feature.

2.1 Overview

The MINI-PAM-II fluorometer provides a vast range of settings and protocols for measuring fluorescence. To make full use of these opportunities, it is necessary to become acquainted with terminology and principles of saturation pulse analysis. Therefore, the present manual provides a chapter dealing with the basics of saturation pulse analysis (Chapter 1, page 157).

Chapter 1 also provides a short list of review papers on PAM chlorophyll fluorescence and saturation pulse analysis (page 165). Further, a small section is included providing some hints for beginners (Chapter 7, page 153).

In the field, the MINI-PAM-II is mostly operated in the stand-alone mode by its touchscreen. Chapter 4 (page 41) provides detailed instructions on how to use the touchscreen interface including advice on fluorescence induction and light curve programs.

Also, this manual includes a section on safe handling of the MINI-PAM-II (Chapter 1, page 7), and on the extent of delivery of the basic fluorescence system and its accessories (Chapter 3, page 13). Further, technical information (Chapter 1, page 169) and guarantee conditions (Chapter 1, page 181) are provided.

In the laboratory, the WinControl-3 software offers a convenient user surface for working with the MINI-PAM-II. Chapters 1 and 6 (page 99 and 105, respectively) introduce the many facets provided by the WinControl-3 software.

3 Components and Setup

3.1 Extent of Delivery (Basic System)

Optoelectronic Unit	MINI-PAM-II/B or -/R
Fiber optics	MINI-PAM/F
Power Supply	MINI-PAM-II/N
Battery charger	000190101101
Battery (12 x)	000160101990
USB cable type A to Mini B	000130606252
Distance Clip 60°	2010-A
Sloped Plexiglas rack	000240313614
Stylus	000160201311
Carrying strap	000150401922
Software WinControl-3	
MINI-PAM-II Manual	
WinControl-3 Software	

3.2 Optoelectronic Unit

Sockets, fiber optics port and on/off switch of the back side of the MINI-PAM-II are outlined and numbered in Fig. 1, their properties and functions are explained in Table 1, page 15. Fastening of the carrying belt is illustrated in Fig. 2, page 16.

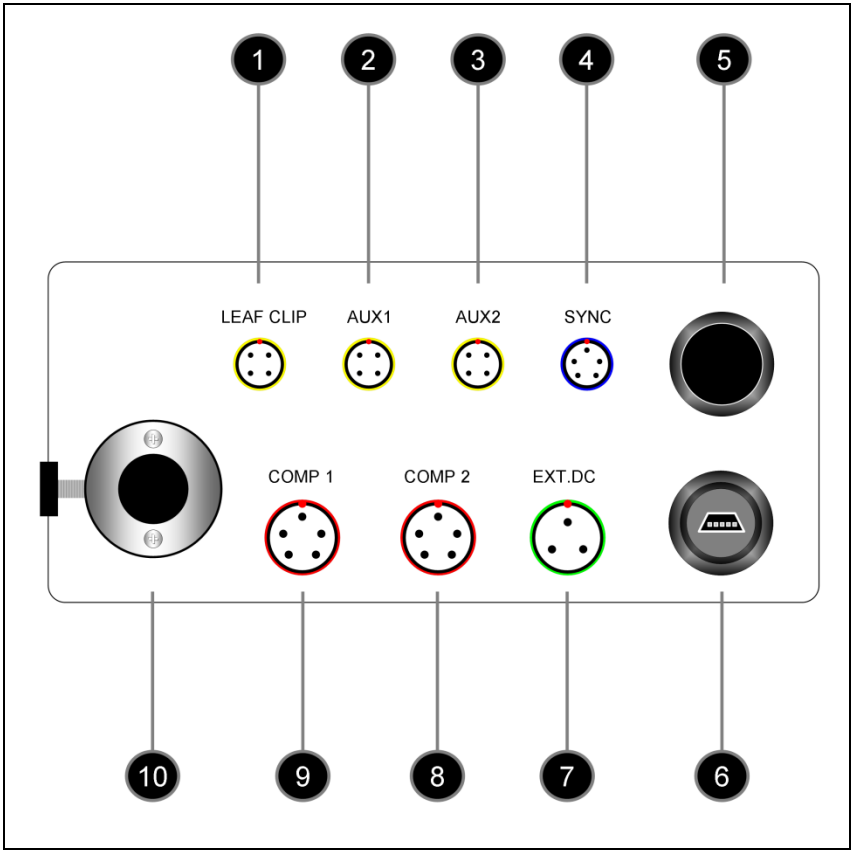


Fig. 1: Back Panel of MINI-PAM-II Power-and-Control-Unit

Table 1: Back Panel of MINI-PAM-II.

Numbering refers to Fig. 1, page 14	Function
1	LEAF CLIP Socket for 2035-B Leaf-Clip Holder
2	AUX 1 Electronically configured as LEAF CLIP socket
3	AUX 2 Electronically configured as LEAF CLIP socket
4	SYNC Socket for external light source which emits synchronized with MINI-PAM-II measuring light
5	ON/OFF MINI-PAM-II switch
6	USB SOCKET Receptacle for MINI-B USB plug.
7	EXT. DC Socket for Power Supply MINI-PAM-II/N
8	COMP 2 Prepared for peripherals mastering RS232 communication (e.g. bar code scanner)
9	COMP 1 Electronically configured as COMP 2
10	LIGHT PORT Port for Fiberoptics MINI-PAM/F

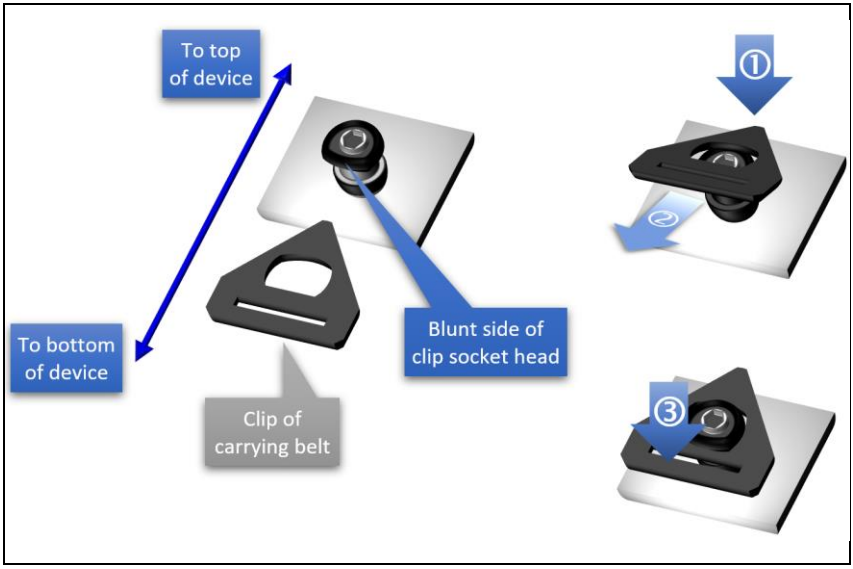


Fig. 2: **Carrying Belt Fastening**

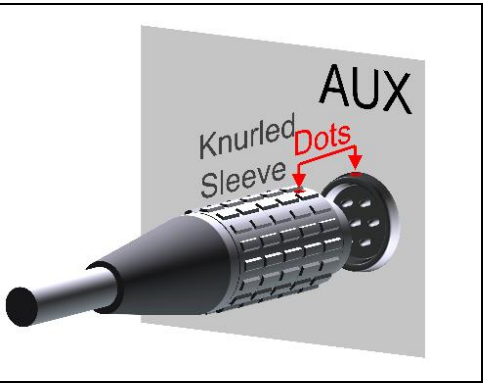
Note Noses for the clips of the carrying belt are mounted on each side of the MINI-PAM-II device.
From top to bottom, the nose consists of a bluntly shaped head, a circular groove, and a cylindrical base.

Instruction ① Position apex of belt clip above the groove on the opposite of the blunt site.
② While keeping the position of the apex, pull base of the belt clip to further move the clip apex into the groove.
③ While maintaining the pull, press down the base of the clip.

Note Great caution should be exercised to prevent dirt or foreign matter from entering the ports or sockets of the MINI-PAM-II. Do not force a plug into the wrong socket. Orientate each plug so that the red dot on the plug coincides with the red dot of the socket. Do not try to disconnect a plug by pulling at the cable. Disconnect plug by pulling at the rippled bushing of the plug.

Fig. 3: Self-locking connector

To lock connection, align red dots of plug and socket and push in. To disconnect, hold the knurled sleeve and pull out. Applies for connection numbers 1 - 4 and 7 - 9 Fig. 1.



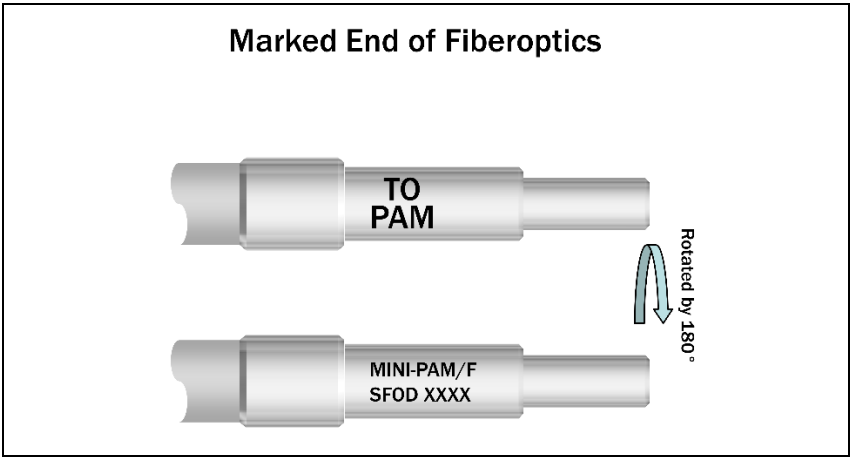


Fig. 4: Fiberoptics

Insert the end marked “TO= PAM” into fiberoptics port.

Two versions of the MINI-PAM-II fluorometer are available. The MINI-PAM-II/B fluorometer is equipped with a blue LED which in the MINI-PAM-II/R fluorometer is replaced by a red LED. This LED is electronically driven to act as measuring and as actinic light source. In addition, both versions of the MINI-PAM-II offer a far-red LED. Normalized spectra of blue, red, and far-red LEDs are shown in Fig. 5.

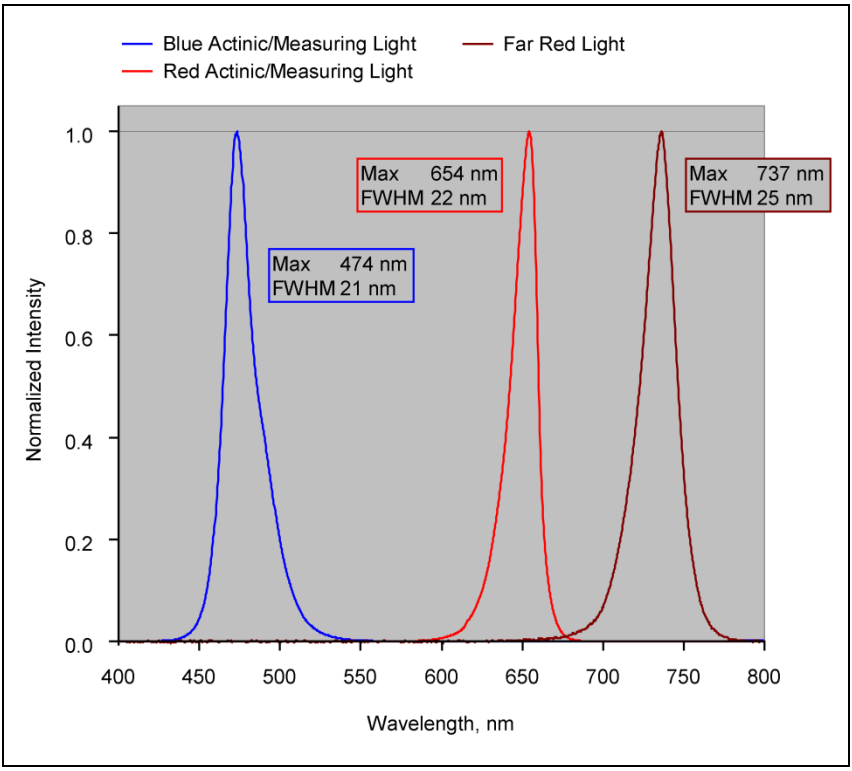


Fig. 5: Emission Spectra of MINI-PAM II LEDs.

Normalized emission spectra of blue LED (MINI-PAM-II/B), red LED (MINI-PAM-II/R), and far-red LED (MINI-PAM-II/B and R).

3.2.1 Batteries

When operated independently, the system is powered by six AA (Mignon) rechargeable batteries (1.2 V/2 Ah). The system can also be powered by non-rechargeable batteries. The battery compartment of the opto-electronic unit does not have a charging function. Therefore, the device can be connected to line power even in the presence of non-rechargeable batteries.

The battery compartment is closed by an aluminum plate. Its locking mechanism functions properly if the label “INNER FACE” on the aluminum plate faces the batteries.

3.3 2010-A Distance Clip 60°

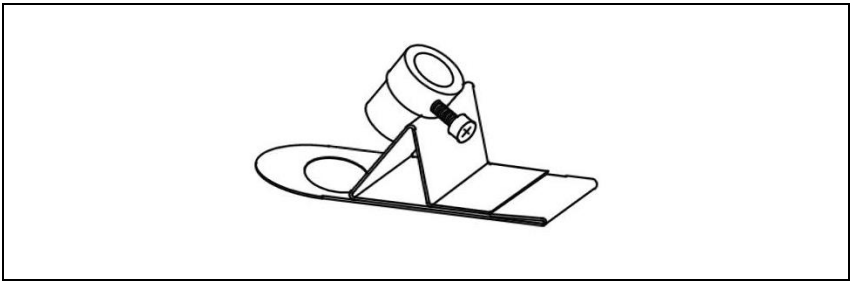


Fig. 6: Distance Clip 60° 2010-A

The 2010-A clip positions the fiberoptics end-piece relative to the sample. The axis of the end-piece is positioned at a 60° angle relative to the sample plane. Two different spacer rings may be used to increase the distance between fiberoptics and sample.

In case of relative thick leaves, or when lichens and mosses are examined, the sample may be placed below the hole of the 2010-A clip. Normal leaves are usually examined above this hole. In the

latter case, the leaf can be held between the folded parts of the clip.

The distance between fiberoptics exit plane and sample has considerable influence on signal amplitude and effective light intensities (Fig. 7, page 20). With a 60° angle between sample plane and fiberoptics, the distance between leaf surface and fiber optics varies. Hence, the leaf surface is exposed to slightly heterogeneous light intensities when actinic light is applied *via* the fiberoptics. A much more pronounced intensity gradient exists inside the leaf due to shading by the top chloroplast layers. In essence, the measured signal will be dominated by that part of the leaf which receives maximal intensity, as this also is most strongly excited by the measuring light and emits most of the fluorescence which is received by the fiberoptics.

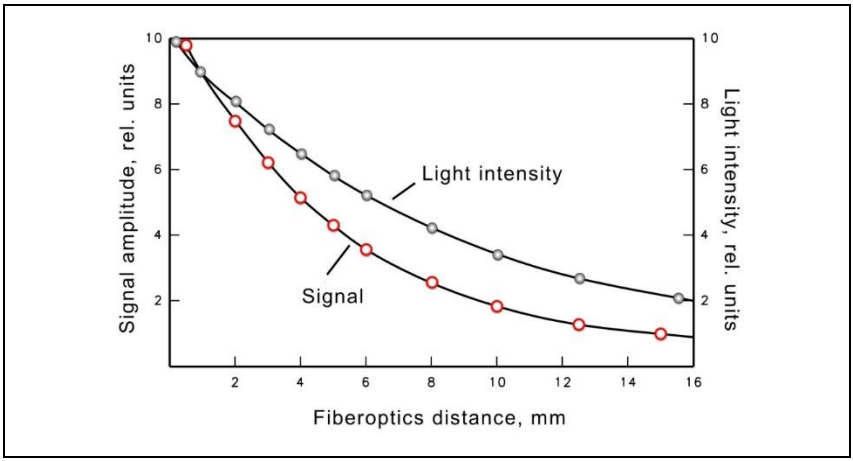


Fig. 7: Measuring Light and Signal

Signal amplitude and light intensity as a function of the distance between fiberoptics and sample level

3.4 Accessories

3.4.1 MINI-SPEC/MP Miniature Spectrometer

Originally, the spectrometer MINI-SPEC/MP (Fig. 11, page 25) has been introduced as accessory for the DIVING-PAM-II. For this reason, the MINI-SPEC/MP possesses an underwater-type connector. Proper connection to the MINI-PAM-II requires that the cable plug is completely inserted before the screw is tightened (see Fig. 8).

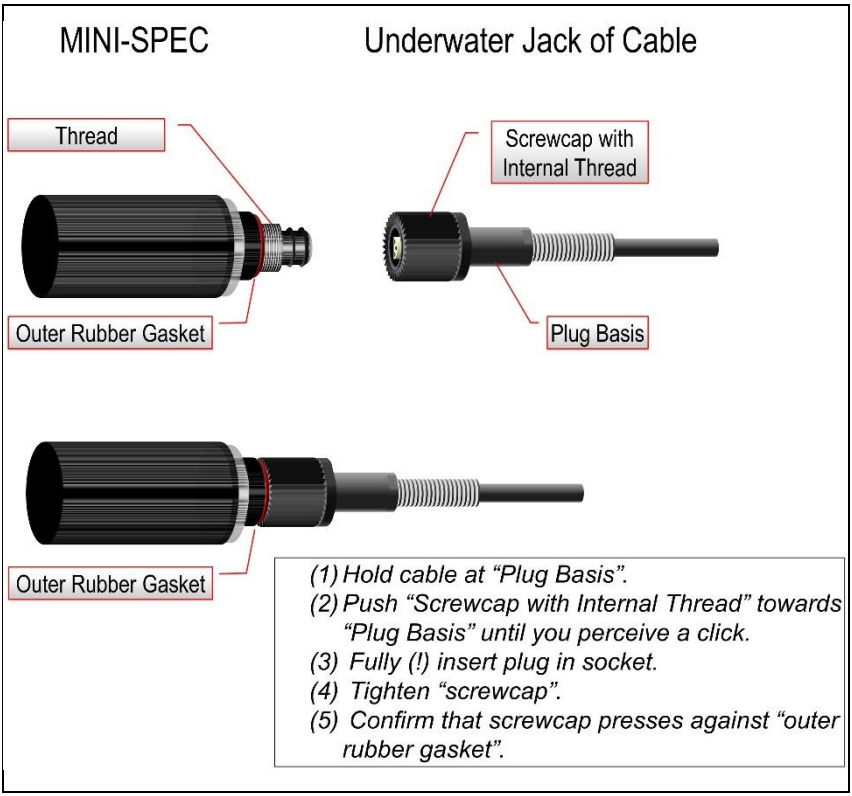


Fig. 8: Connection of MINI-SPEC/MP

The spectrometer is calibrated to measure spectra of quantum fluxes. Integration of these spectra over the visible range (400 – 700 nm) yields PAR data equivalent to those recorded by Walz quantum sensors.

Like the PAR sensor of the 2035-B or 2065-M devices (Section 3.4.1, page 21 and Section 3.4.11, page 36), the spectrometer can be employed to calibrate the internal PAR sensor of the MINI-PAM-II (see Section 4.3.4.1, page 78). To this aim, the MINI-PAM-II light guide and the entrance optics of the spectrometer are inserted in the PAR calibration block (Fig. 9, page 22). The light guide can be inserted either in the 60° or the 90° port according to the two possible orientations of the light guide in the Leaf Clip Holder 2035-B. With both pieces fully inserted, the distance between fiber optics end and diffusing disk of the spectrometer matches the corresponding standard distances between fiber optics end and sample level in the Leaf Clip Holder 2035-B.

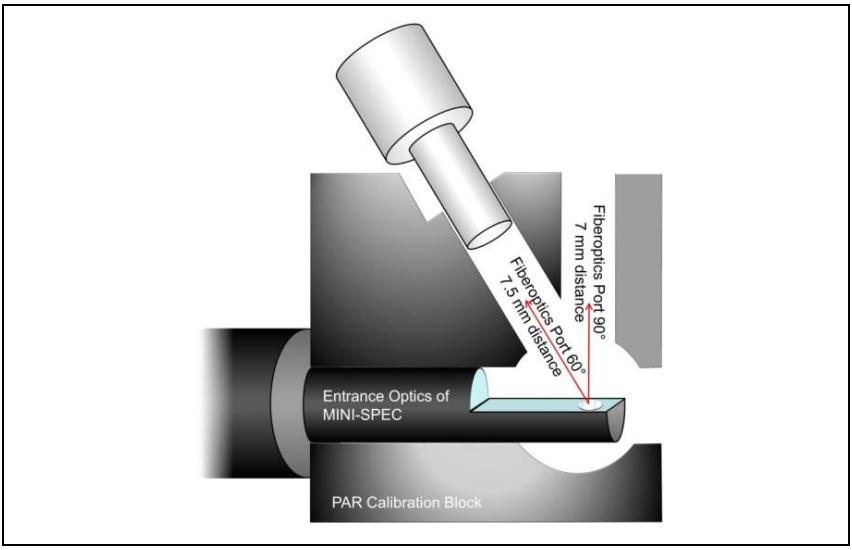


Fig. 9: PAR Calibration Block

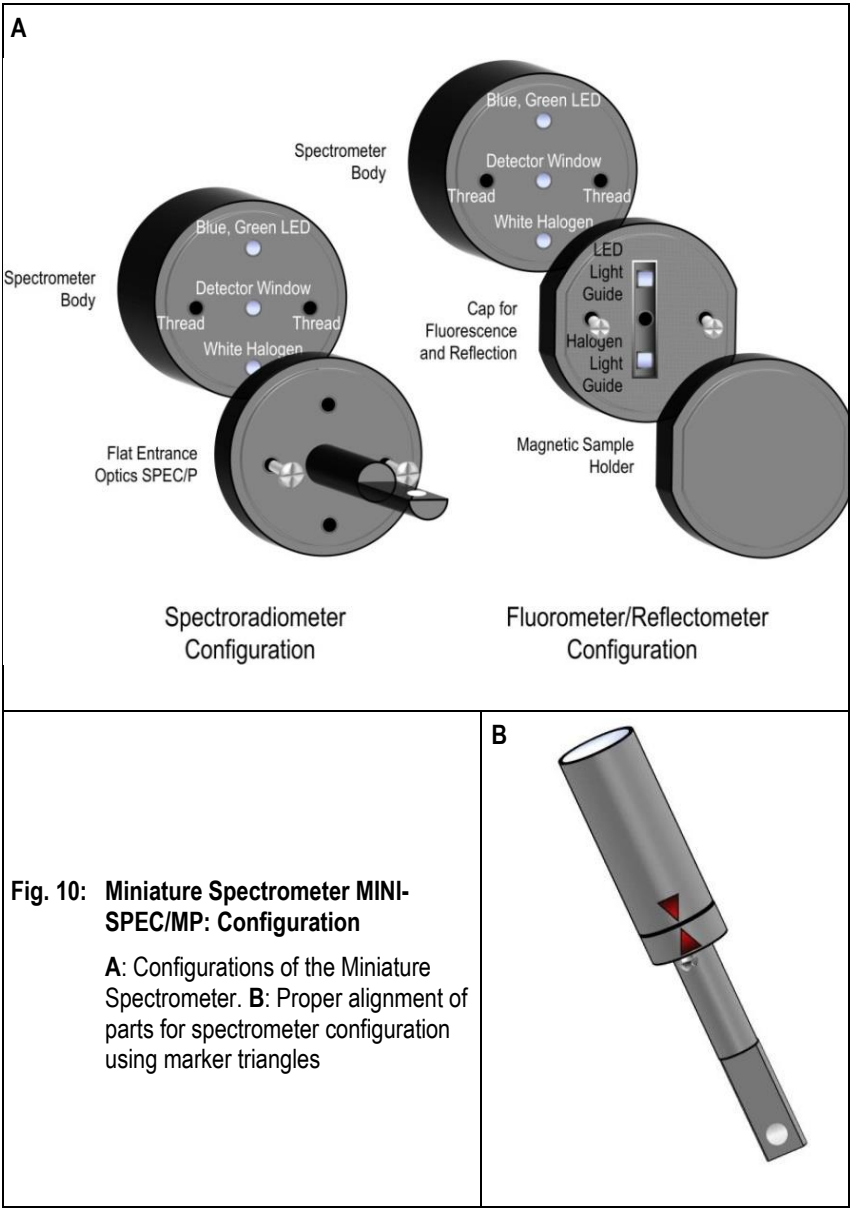


Fig. 10: Miniature Spectrometer MINI-SPEC/MP: Configuration

A: Configurations of the Miniature Spectrometer. **B**: Proper alignment of parts for spectrometer configuration using marker triangles

Replacing the entrance optics used for evaluation of light by the cap for fluorescence and reflection (Fig. 10, page 23) considerably extents the range of spectral information attainable by the miniature spectrometer.

In the fluorescence mode, a light guide in the cap leads light from a blue or a green LED located inside the spectrometer body to the sample. The excited fluorescence reaches the spectrometer body through a central hole in the cap. Green light penetrates deeper into photosynthetic tissue than blue or red light (Terashima et al (2009) Plant Cell Physiol 50:684-697). Therefore, the two excitation colors permit probing the effect of varying depths of penetration of excitation light on the shape of fluorescence spectra. The spectrometer automatically compensates for the spectral sensitivity of the detector.

For reflection measurements, a halogen lamp is employed whose emission is transferred through another light guide to the sample (Fig. 10, page 23).

From reflection measurements, reflectance (R) is derived by dividing a sample spectrum (r_{sample}) by the spectrum of a white light-diffusing reflector consisting of a fluoropolymer with very high diffuse reflectance in the entire visible range ($r_{reference}$):

$$R(\lambda) = \frac{r_{sample}(\lambda)}{r_{reference}(\lambda)}$$

The reference material is part of delivery. To maintain its optical properties, do not touch the white surface, keep away dirt, dust, and humidity, and store the reflection standard in a closed container when not used.

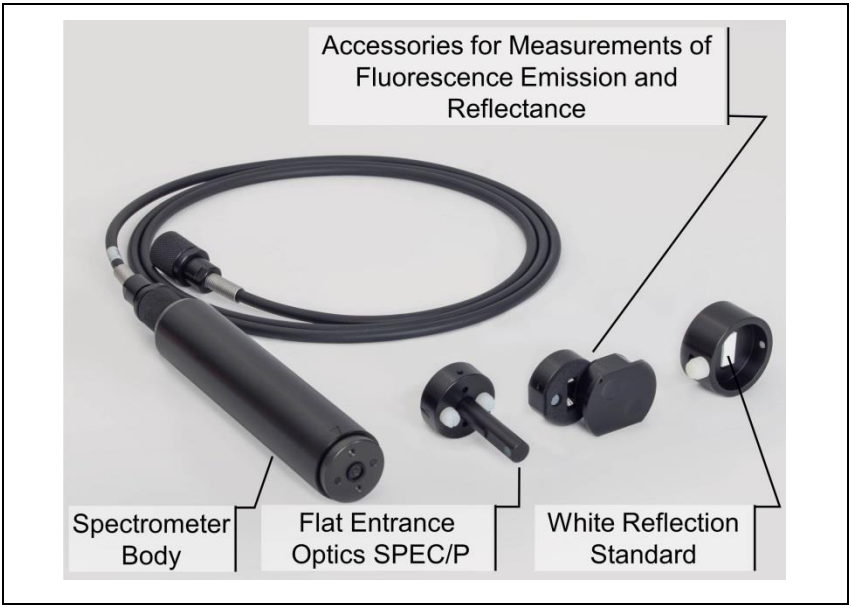


Fig. 11: MINI-SPEC/MP

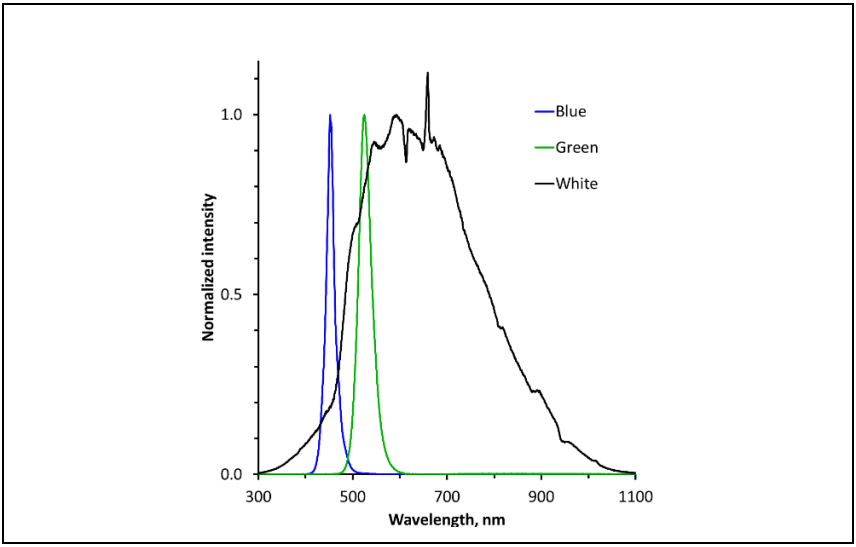


Fig. 12: Emission Spectra of Miniature Spectrometer Light Sources.

3.4.2 2035-B Leaf-Clip Holder

The Leaf-Clip Holder 2035-B must be connected to the LEAF CLIP socket (Fig. 1, page 14) to record PAR, leaf temperature and ambient humidity in parallel with chlorophyll fluorescence. In the stand-alone mode, readings of environmental data are taken with every saturation pulse analysis, but these data can be continuously recorded when the MINI-PAM-II is operated by the WinControl-3 software.

The Leaf-Clip Holder 2035-B is almost indispensable for field investigations, when ambient conditions may vary considerably. It substitutes for the standard "Distance Clip" (2010-A) as a device for defined positioning of the fiberoptics relative to the leaf plane.

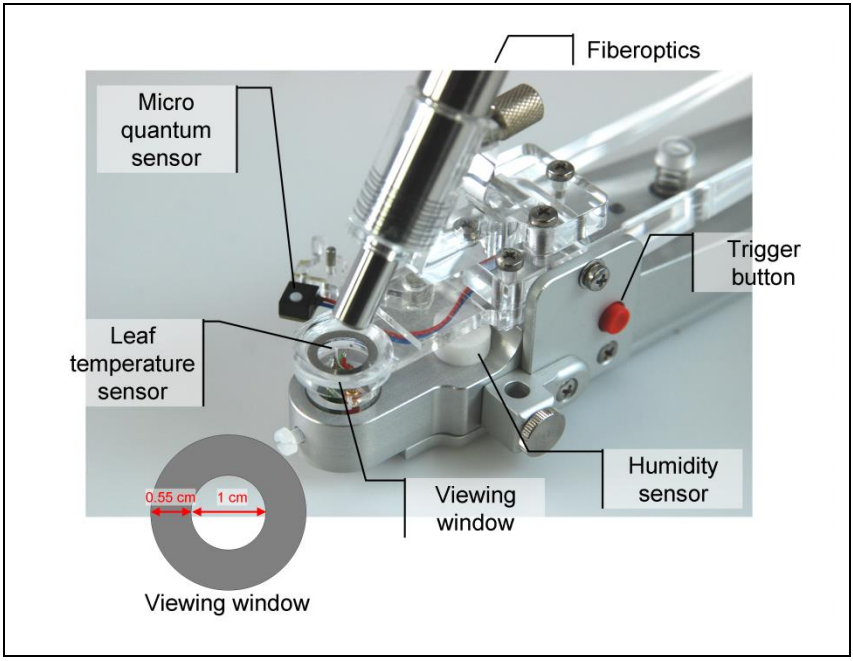


Fig. 13: 2035-B Leaf-Clip Holder

Further, using the PAR sensor of the leaf clip, the internal PAR sensor of the MINI-PAM-II can be readily calibrated.

In the 2035-B holder, the leaf is resting on a Perspex tube with widened crest. The tube can be vertically adjusted to account for different leaf thicknesses. The fiberoptics axis forms a 60° angle with the leaf plane. Optionally, a 90° fiberoptics adapter (2030-B90) is available for applications requiring homogenous illumination by actinic light applied *via* the fiberoptics. The distance between fiberoptics and leaf can be varied. For most applications the minimal distance is recommended (maximal signal). Larger distances can be defined by spacer rings. The illuminated leaf area is limited by a steel ring with 10 mm ∅ opening.

At the bottom of the Leaf-Clip Holder 2035-B, a tripod mounting thread is provided. Mounting the device on a tripod (e. g. Compact Tripod ST-2101A) facilitates long term measurements with the same plant.

The handle of the Leaf-Clip Holder 2035-B features a red push-button for remote control of the MINI-PAM-II. Pressing the button triggers a saturation pulse and associated measurements of fluorescence levels for “fluorescence quenching analysis”.

Micro-Quantum-Sensor

A micro quantum sensor is integrated into the Leaf-Clip Holder 2035-B to monitor the photosynthetic active radiation (PAR, between 400 and 700 nm) to which the sample is exposed. The micro-quantum-sensor measures light intensity in µmol quanta m^{-2} s^{-1}. The µmol quanta m^{-2} s^{-1} is the unit of photon flux density. Hence, the micro-quantum-sensor measures photosynthetic photon flux density (PPFD).

Essential optoelectronic elements of this micro-quantum-sensor are:

- A 3 mm ∅ diffusing disk.
- High stability silicon photovoltaic detector with filter set for PAR correction, magnetically attached to 2035-B Leaf Clip Holder.
- Cosine response characteristics (Angular dependence: Error < 3 % for angle between -30 ° and +30 ° from normal axis).

The sensor is factory calibrated and calibration factors are stored in the internal memory of the 2035-B leaf clip. The stability of calibration depends on keeping the diffuser clean. It is advisable to check calibration regularly by comparison with a standard quantum sensor. Any deviation can be corrected by entering a new calibration factor in WinControl-3 (Section 6.11.4, page 151) or on the touch screen (Section 4.3.4.2, page 80). A substantial increase of the calibration factor from its original value indicates dirt-deposition on the diffuser, which may be reversed by gentle cleaning using a cotton tip applicator, moistened with some diluted ethanol.

Thermocouple

A NiCr-Ni thermocouple is mounted in the Perspex tube on which the leaf area is resting. The thermocouple is forming a loop that gently presses against the lower surface of the leaf. This arrangement results in effective temperature equilibration between leaf and thermocouple and protects the thermocouple from direct sun radiation.

The reference couple is located on the circuit board, near the thermovoltage amplifier, enclosed in the bottom part of the holder. The relationship between thermovoltage and temperature is almost linear. With decreasing temperatures there is a small decline of ΔV/ C. Calibration was performed at 25 °C. At 0 °C or –15 °C the deviation amounts to 0.5 or 0.8 °C, respectively.

Humidity Sensor

A calibrated, capacitive-type humidity sensor measures humidity conditions close to the sample surface.

Data Display

All sensor data are displayed on the touchscreen window “Primary Data” (Fig. 30, page 47).

Signal LED

See Table 2.

Table 2: Signal Code of LED on 2035-B Leaf Clip Holder.

LED action	Status
Flashing green	Normal operation.
Continuous green	Communication from MINI-PAM-II to 2035-B clip interrupted. This happens temporarily during firmware update of MINI-PAM-2.
Flashing red	(a) Broken thermocouple: inspect and ask for repair kit. (b) Internal error on 2035-B EPROM: contact Walz.
Continuous red	As “flashing red” plus communication interrupted.

3.4.3 2054-L External LED Source

For experiments requiring different actinic light colors, we offer an external light source which can be attached to the 2035-B leaf clip (Fig. 14, page 30). The light source consists of four four-chip LED RGBW sources each capable of emitting red, green, blue, and white light. Total intensity and color composition can be regulated by the software of the MINI-PAM-II or by WinControl-3. The maximum PAR of each light quality is 1500 µmol m^{-2} s^{-1}.

Note Switch off MINI-PAM-II before connecting or disconnecting 2054-L External LED Source.

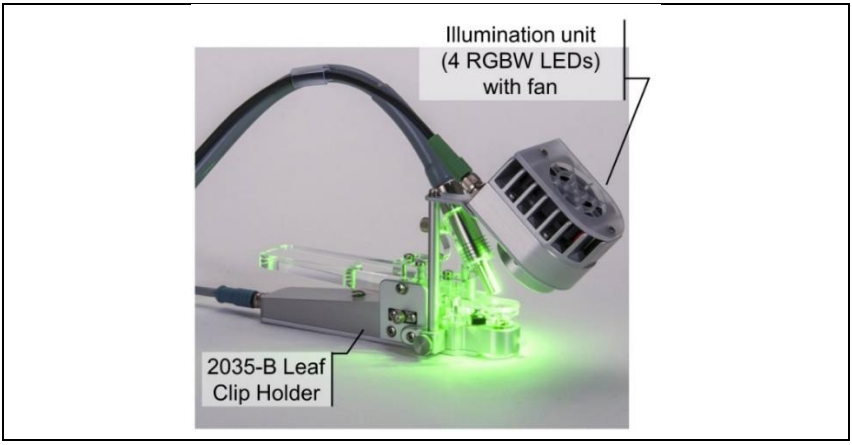


Fig. 14: 2054-L External LED Source

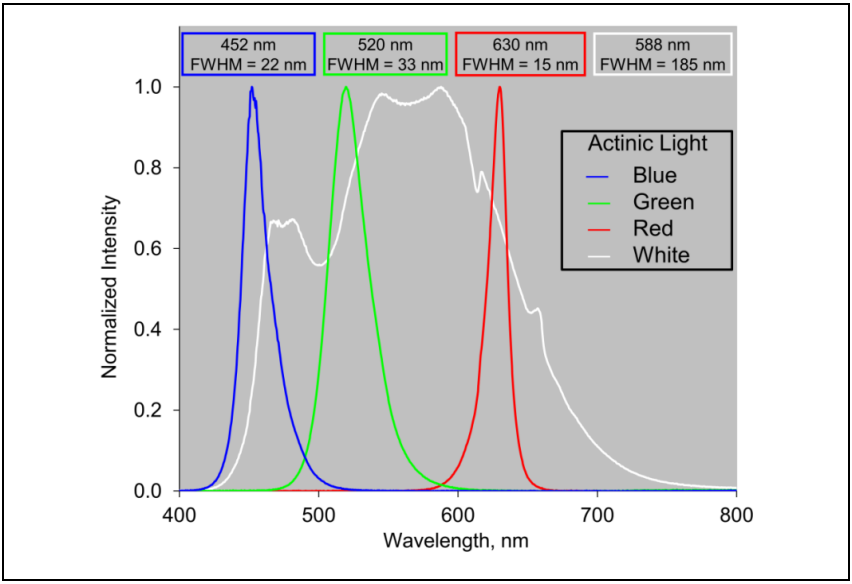


Fig. 15: Emission spectra of the 2054-L External LED Source

3.4.4 2035-B/RLC Conversion Kit for 2030-B/2035-B Leaf Clips

The 2035-B/RLC kit shields the sample area of 2030-B/2035-B Leaf Clips from external light. The accessory has been designed for field experiments with the fluorometer's internal light source. The 2035-B/RLC kit is especially suitable for rapid light curve experiments (RLC) in outdoor studies.

Fig. 16: 2035-B/RLC Conversion Kit

3.4.5 2060-B Arabidopsis Leaf Clip

This leaf clip is made to position small samples in the beam of the fiberoptics of the MINI-PAM-II. Usually, the 2060-B clip is combined with the 2065-M Mini Quantum/Temp.-Sensor to measure PAR at sample level and lower leaf temperature (see Fig. 17, page 32).

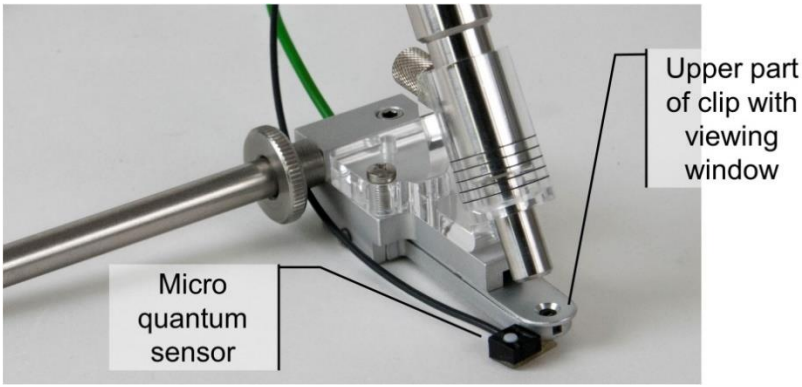


Fig. 17: 2060-B & 2065-M Arabidopsis Clip with Quantum/Temp.-Sensor

3.4.6 Fiberoptics Adapter 90º 2030-B90

The fiberoptics adapter 90º 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.

3.4.7 Leaf Positioning Setup DUAL-BA

The DUAL-BA has been designed to position the fiber optics of a PAM fluorometer easily and quickly on attached leaves. The accessory is compatible with the DUAL-PAM/F, MINI-PAM-II and PAM-2500 fluorometers. It includes a duct with laterally mounted neodymium magnet and a screw to lock the fiber optics tip. An angle bracket made of a spring steel strip positions the leaf in front of the fiber optics, where the steel strip is held by the magnet of the duct. The DUAL-BA includes a fiber optics guide with metal rod to mount the guide on a stand.

Fig. 18: Leaf Positioning Setup DUAL-BA

3.4.8 2060-A Fiberoptics Holder for Surfaces

The holder positions the fiberoptics of the MINI-PAM-II on bulky samples. When combined with the 2065-M Mini Quantum/Temp.-Sensor, temperature and PAR of the surface can be measured (Fig. 19).

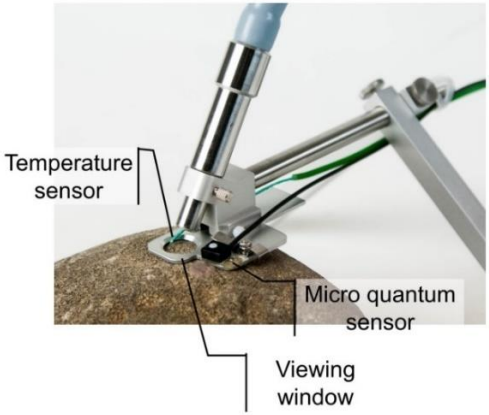


Fig. 19: 2060-A & 2065-M, Surface Holder with Quantum/Temp.-Sensor

3.4.9 2065-M Mini Quantum/Temp.-Sensor

The light and temperature sensors of the 2065-M device can be mounted on both the 2060-B Arabidopsis Leaf Clip and the 2060-A Fiberoptics Holder for Surfaces. Sensors and electronics of the 2065-M device are identical to the 2035-B clip (Section 3.4.1, page 21).

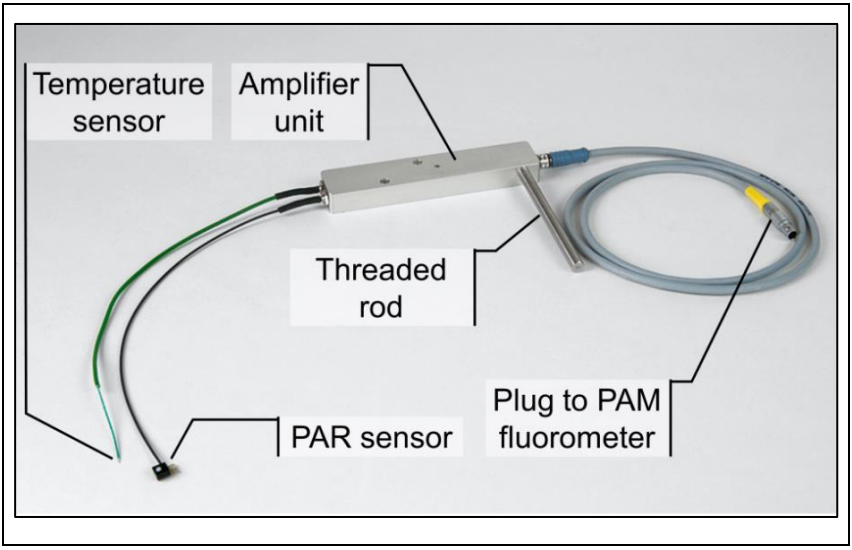


Fig. 20: Mini Quantum/Temp.-Sensor 2065-M

3.4.10 DLC-8 Dark Leaf Clip

The DLC-8 leaf clip permits dark-acclimation of small leaf areas in the field which is essential for proper determination of the maximal quantum yield F_V/F_M and for recording of dark-light induction kinetics. The Dark Leaf Clip DLC-8 weighs approx. 4 g and, hence, can be attached to most types of leaves without any detrimental effects.

Fig. 21: DLC-8 Dark Leaf Clip

The tip of the fiberoptics of the MINI-PAM-II fits snugly into 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.

Using the Dark Leaf Clip DLC-8, the fiberoptics is positioned at right angle with respect to the leaf surface at the relatively short distance of 7 mm. Consequently, signal amplitude is distinctly higher (factor of 2.4) compared to the Leaf-Clip Holder 2035-B with 60° fiberoptics angle. To avoid signal saturation, the settings of measuring light intensity and gain have to be lowered with respect to the standard settings (Fig. 41, page 64).

When the shutter is still closed and the measuring light is on, an artifactual Ft signal is observed. This signal is due to a small fraction of measuring light which is reflected from the closed shutter to the photodetector. However, this background signal is of no concern as the reflection is much smaller when the shutter is opened, and the measuring light is strongly absorbing by the leaf sample instead of being reflected by the metal surface of the shutter.

3.4.11 KS-2500 Suspension Cuvette

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

Fig. 22: KS-2500 Suspension Cuvette and MKS-2500 Magnetic Stirrer

3.4.12 Oxygen Package

The oxygen package consists of an optode sensor, an oximeter (FSO2-1) and an interface (FSO2-AK). The sensor is inserted through the needle port of the KS-2500 cuvette (Fig. 22, Fig. 23). The oximeter controls the sensor and transmits the oxygen signal via the interface to the MINI-PAM-II. A separate manual is provided for this package.

Fig. 23: O_2 measurement. System Overview.

3.4.13 BCS-9590 Barcode Scanner

The barcode scanner is the ideal add-on when many differed samples are probed. Simply convert your sample IDs into barcodes, print labels, and mark your samples. Then, the BCS-9590 Barcode Scanner writes your sample ID into the memory of the MINI-PAM-II or the report data in WinControl-3.

In Report table, barcodes are printed in the Aux1 column in the data line of the subsequently executed saturation pulse analysis. Writing barcode and measuring results in the same line facilitates data sorting when data of many samples are collected.

Fig. 24: BCS-9590 Barcode Scanner

3.4.14 MINI-PAM/F1 Miniature Fiberoptics

The MINI-PAM/F1 fiberoptics is useful for investigation of small areas. It consists of a single coated plastic fiber with an active diameter of 2 mm.

Fig. 25: Miniature Fiberoptics MINI-PAM/F1

3.4.15 MQS-B/A Adapter Set for Thin Fiberoptics

The MQS-B/A Adapter Set is designed to measure the light intensity of the MINI-PAM/F1 fiberoptics using an MQS-B light sensor together with a data logger having a high-impedance BNC input, for example, the Walz ULM-500 Universal Light Meter & Data Logger.

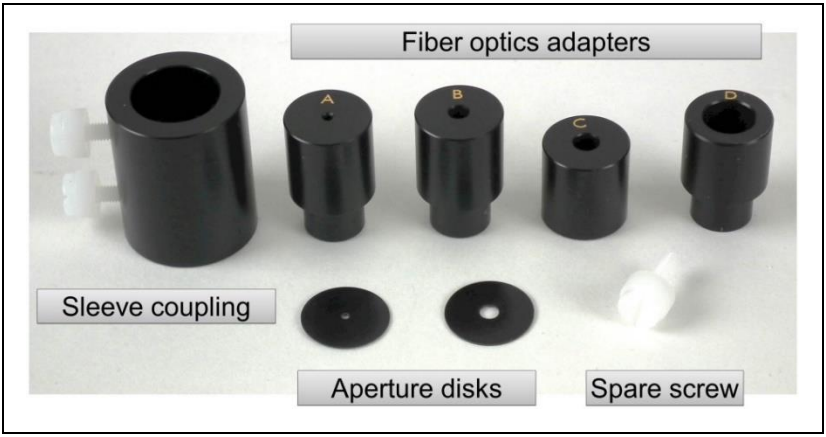
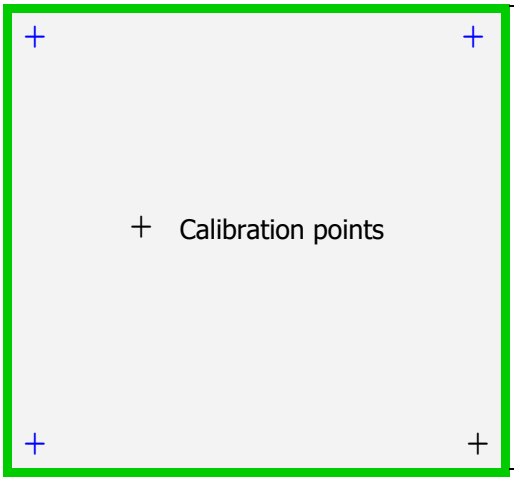


Fig. 26: MQS-B/A adapter set

4 Touchscreen Operation

Touchscreen operation of the MINI-PAM-II allows saturation pulse analysis of photosynthesis independent from a computer. Using the touchscreen, single F_V/F_M or Y(II) measurements as well as more complex experiments like induction and light curves are feasible. Continuous recording of steady state fluorescence, however, requires operation of the MINI-PAM-II by WinControl-3 running on an external computer.



To open the calibration window, start MINI-PAM-II with finger pressed on touchscreen. To calibrate, use pointed but not edged tool like a wooden toothpick. Touch target point (+) at bottom right corner. Consecutively, three more target points have to be touched. Finally, a message “Press anywhere to continue” indicates end of calibration.

Fig. 27: Touch Screen Calibration

4.1 Calibration

The MINI-PAM-II can be operated autonomously using its resistive touchscreen. The touchscreen is factory-calibrated to match its two-dimensional response with the LED screen underneath. If

touch operation is imprecise, calibration can be performed as described in Fig. 27.

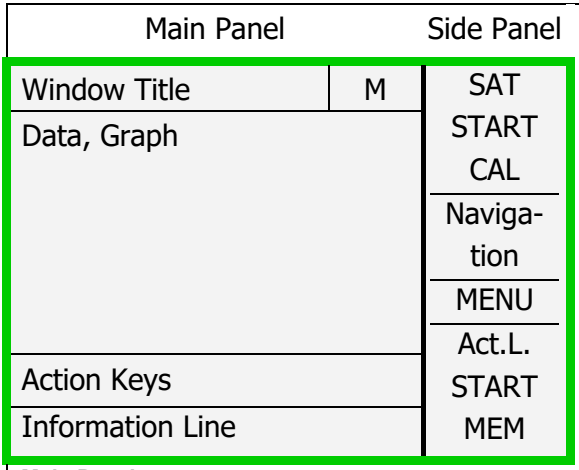
4.2 Top-level Windows

The MINI-PAM-II provides various windows for control and data display. Frequently used commands, fluorescence data and the actinic light list (PAR list) are presented in 10 top-level windows (Table 3, page 42).

Table 3: Overview of Top-level Windows

	Window	Content
1	**Basic Data**	Minimum data set and basic action keys for filed operation.
2	**Primary Data**	Data of last saturation pulse analysis and current levels of fluorescence and additional measured parameters.
3	**Quenching Analysis**	Fluorescence levels of last saturation pulse analysis and corresponding fluorescence ratios (Y(II), F_V/F_M, …).
4	**Ft-Chart**	Trace of fluorescence, total x axis interval: 25 or 125 s (see Fig. 63, page 89).
5	**Spectrometer**	Measurement and display of light, fluorescence and reflectance spectra.
6	**Actinic + Yield**	Short illumination program with saturation pulse analysis.
7	**Induction Curve**	Graphics of saturation pulse analysis of current curve.
8	**Light Curve**	Graphics of saturation pulse analysis of current light response curve.
9	**Recovery Curve**	Recovery graphics after induction curve and light response curve.
10	**Actinic Light List**	Set values of photosynthetically active radiation PAR in µmol m^{-2} s^{-1}.

Accessible from all top-level windows is the Main Menu and its submenus which allow adjusting settings of the MINI-PAM-II and its peripherals (Fig. 39, page 62; Fig. 40, page 63).



Side Panel

The panel provides a button for saturation pulses (SAT) which is in some windows replaced by a start/stop button for automated experiments or by a CAL button to start PAR calibration. Navigation keys, a "MENU" key and action keys are located on the side panel.

Main Panel

From top to bottom: Window title and currently selected character to mark measurements, numerical and/or graphical data, action keys and information line. Action keys are absent or differently arranged in some windows. The information line displays date + time, or PAM activity, or informs on critical fluorometer states. Alphanumeric data are displayed in the "Cursor Mode" graphics windows

Fig. 28: Principal Screen Layout of Top-level Windows

Generally, all top-level windows consist of the "main panel" and the "side panel" (Fig. 28, page 43). The top line of the main panel displays window title and a capital letter which is added as a mark to each saturation pulse analysis.

The bottom of the main panel provides various action keys. Depending on window, these action keys trigger saturation pulse analysis, control light conditions or affect graphic display. The "Information Line" at the bottom edge of the main panel shows cur-

rent date and time. The Information Line can also include alphanumeric data when graphics windows are in the “Cursor Mode” which is started by touching the graph area.

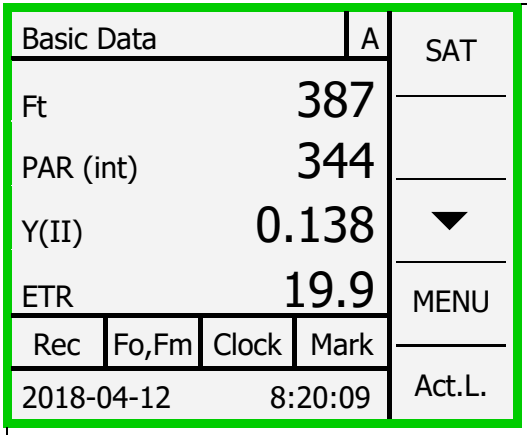
The side panel provides arrow keys to change windows, control keys for fluorometer functions, and a (MENU) key to access the main menu. Control keys are different between windows. For instance, the uppermost button in the sidebar triggers saturation pulses in case of the first three top-level windows (cf. Table 3, page 42), but the same button starts automated experiments in windows Actinic + Yield, Induction Curve, Light Curve and Recovery Curve. Sections 4.2.1 to 4.2.10 will introduce all 10 top-level windows in detail.

4.2.1 Basic Data

The Basic Data window (Fig. 29, page 45) displays a reduced set of four data for fast sampling under field conditions. From these data, the Ft and PAR represent current measurements but the Y(II), or F_V/F_M, and the ETR are derived from the last saturation pulse analysis.

The bottom of the window provides keys for frequently used commands: Rec starts a new data set (Record), Fo,Fm determines maximum PS II photochemical yield, Clock starts repetitive triggering of saturation pulses or an automated experimental routine, and Mark opens the window “New Marker” in which the letter saved with each saturation pulse analysis (“mark”) can be defined.

To change the marker letter, use up and down keys in the window “Change Marker”. The currently selected letter is shown on the top edge of the window. Touching the SET key confirms the current selection and returns to the Basic Data window.



Side Panel

SAT, trigger saturating pulse to determine Y(II).

▼ , go to next window (Primary Data).

MENU, go to Main Menu.

Act.L., actinic light switch.

Main Panel

Ft, current fluorescence value (mV).

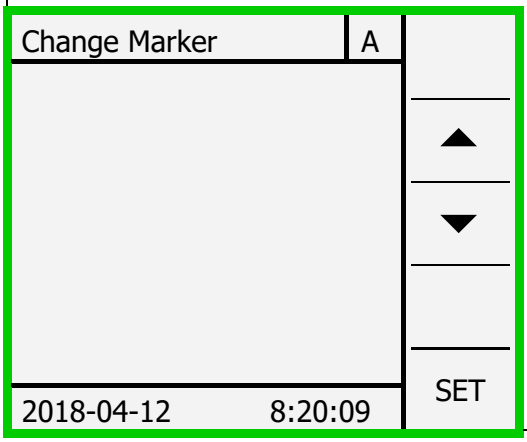
PAR (int), PAR value (µmol·m^{-2}·s^{-1}) from internal PAR sensor; **PAR (ext)** is displayed if an external sensor is active.

Y(II), effective photochemical quantum yield of PS II; **F_V/F_M** is displayed after touching Fo,Fm.

ETR, (relative) electron transport rate (µmol e$^-$·m^{-2}·s^{-1}).

Rec, Fo,Fm, Clock, Mark: Keys to start a new data section (Record), to trigger a saturation pulse for F_0 and F_M determination, to start repetitive triggering of an event, and change the marker letter of data.

Touch Mark open the window below



Side Panel

▲ , go to previous character in alphabet.

▼ , go to next character in alphabet.

SET, use current letter and return to Basic Data.

Fig. 29: Basic Data, Change Marker

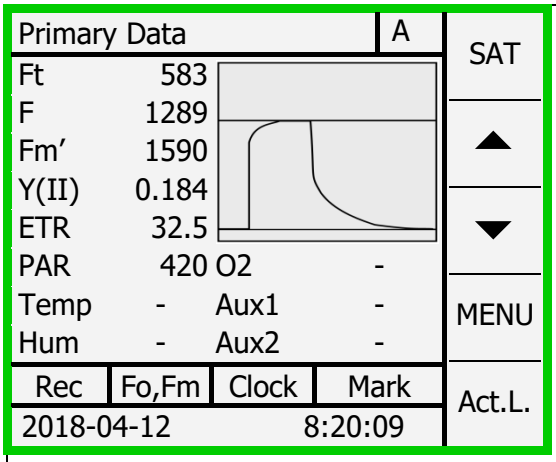
The side panel of the Basic Data window offers four commands: the SAT key determines effective PS II photochemical yield, and the Act.L. represents a switch for actinic light. The MENU key leads to the Main Menu window as in all other top-level windows. The downward arrow key switches to the next window. Two arrow keys (up and down) are present in all other top-level windows except the last (Actinic Light List; Table 3, page 42) which requires only the upward key.

Comment on Rec (New Record)

Starting a new Record disconnects current saturation pulse analyses from that of the previous Record. Hence, a SAT command in a Record without F_0 and F_M determinations will only result in data of Y(II), q_P, and q_L because calculations of these parameters do not require F_0 and F_M (see Table 26, page 166). Calculations of q_P and q_L parameters require F_0': the two parameters will not be calculated if the Fo' mode is inactive and F_0 and F_M is not available because the latter two fluorescence levels are needed for Fo' calculations according to Oxborough and Baker (Chapter 1, page 157).

In the presence of F_0 and F_M data, the fluorescence ratio parameters requiring F_0 and F_M data will be calculated. If a Record holds more than one F_0, F_M determination, the latest F_0, F_M will be used to calculate fluorescence coefficients. Such calculations are only valid for Fo,Fm and SAT measurements with the same sample. Sometimes, Fo,Fm and SAT measurements with different samples even result in fluorescence ratio parameters that exceed their valid range (compare Table 26, page 166). These invalid data will be displayed in grey on the touch screen.

4.2.2 Primary Data



Side Panel

Same as Basic Data window (Fig. 29, page 45) plus upward arrow key.

Main Panel

Ft, current fluorescence level

Displayed after Fo,Fm: **Fo**, **Fm**, and **Fv/Fm**; minimum and maximum fluorescence levels of dark-acclimated sample, maximum photochemical PS II yield. Displayed after SAT: **F**, **Fm'**, and **Y(II)**; fluorescence level just before saturation pulse and maximum fluorescence of light-exposed sample, and effective photochemical quantum yield of PS II

Insert, fluorescence trace during last saturation pulse.

PAR, Temp, and **Hum**, photosynthetic active radiation in µmol·m^{-2}·s^{-1} and temperature in °C, respectively.

O2, **Aux1**, **Aux2**, data measured by optional oxygen, humidity and other sensors.

Rec, Fo,Fm, Clock, Mark: see Fig. 29, page 45.

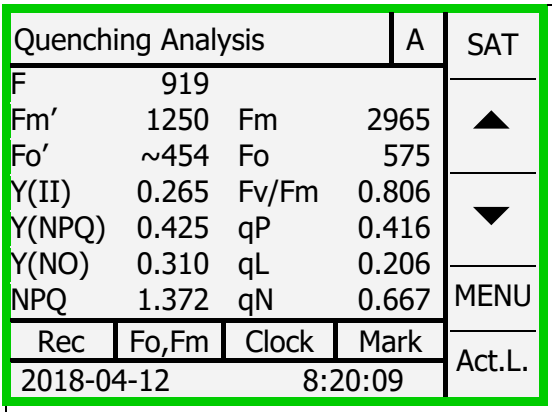
Fig. 30: Primary Data

The insert in the Primary Data window display the fluorescence trace caused by the last saturation pulse. In addition, the window repeats the data of the previous one (Ft, F_V/F_M or Y(II), ETR, PAR). New information of the Primary Data window is the F_0 and F_M signal levels (after Fo,Fm was pressed) or the F and F_M' signal levels (after SAT was pressed). Additionally, temperature, °C

(Temp) and relative humidity, % (Hum) as measured by the MINI-PAM-II leaf clip (2035-B) are displayed. Data from an optional optode oxygen sensor (O2) and two more auxiliary sensors (Aux1 and Aux2) can be displayed.

4.2.3 Quenching Analysis

The window provides a complete overview on fluorescence levels and the fluorescence ratio quotients calculated by the MINI-PAM-II or WinControl-3. Data line 2 to data line 4, compare data of the light exposed sample (left) with data of the dark-acclimated sample (right).



Side Panel

Same as Basic Data window (Fig. 29, page 45) plus upward arrow key.

Main Panel

Numerical data: see Fig. 89, page 159 and Table 26, page 166.

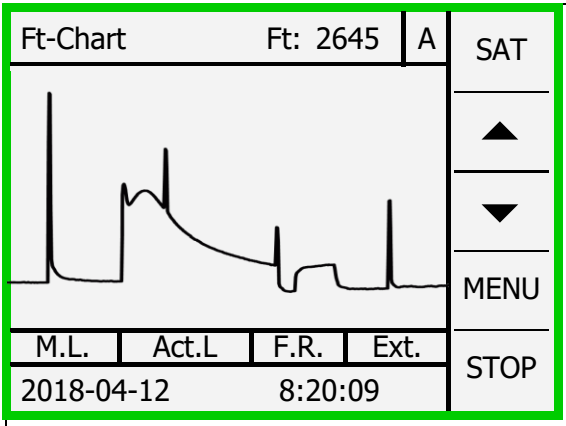
Rec, Fo,Fm, Clock, Mark: see Fig. 29, page 45.

Fig. 31: Quenching Analysis

4.2.4 Ft-Chart

The Ft-Chart displays a 25 or 125 s interval of Ft where the right-most level of the graph corresponds to the current Ft value. The X axis interval can be adjusted in the menu “MINI-PAM-II Settings (Fig. 63, page 89).

Continuous Ft values are not saved when the MINI-PAM-II is operated in the stand-alone mode. Recording of Ft requires operation of the MINI-PAM-II by WinControl-3.



Side Panel

Same as above but Act.L. replaced by STOP/RUN.

Main Panel

Graph. Trace of current fluorescence measurement. Total X axis: 25 or 125 s: see Fig. 63, page 89.

M.L, **Act.L** , **F.R.** , **Ext.**. On/off switches for measuring light, actinic light, far-red light and external (synchronized) light source, respectively. The **Ext.** appears only if “Ext. Panel Mode” is active (see Fig. 44, page 70)

Fig. 32: Ft Chart

4.2.5 Spectrometer

The SPEC command of the Spectrometer window triggers a spectrometric measurement. In the standard mode, the spectrometer is equipped with a tube containing the optical entrance for external radiation (Fig. 10, page 23).

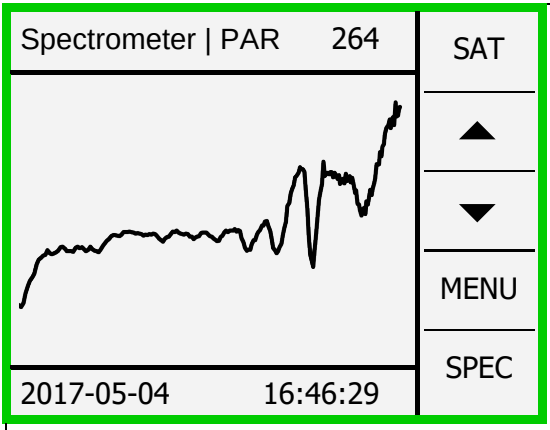
Spectra of photon flux densities are measured in units of nmol photons m^{-2} s^{-1} nm^{-1}. PAR corresponds to the integral of the spectrum from 400 to 700 nm. PAR is derived from the spectrometer when “Use Ext. PAR” in Menu Sensor Settings is checked (available by selecting “Sensors” in the Main Manu), and the spectrometer is selected as external PAR sensor (Main Menu → Sensor → Leaf Clip/Ext PAR → External PAR sensor → Spectrometer; Fig. 53, page 81).

Note that the spectrometer measures the PAR of the MINI-PAM-II internal light correctly only in the CAL mode (Fig. 38, page 59) because only in the CAL mode the LEDs are operated continuously. In the measuring mode, actinic light is pulse-width modulated and fluctuating PAR is displayed.

To record fluorescence or reflectance spectra, change spectrometer configuration as described in Fig. 10 (page 23). Then select operation mode: Main Menu → Sensor → Spectrometer → Operation Mode.

In menu Operation Mode, the items “Fluores. (blue)”, “Fluores. (green)” and “Reflectance” set the spectrometer mode to fluorescence spectra with blue excitation, fluorescence spectra with green excitation and reflectance spectra, respectively.

For reflectance measurements, first measure dark current in complete darkness, then the 100% reflection signal with the white reference material (Fig. 11, page 25), and finally the sample.



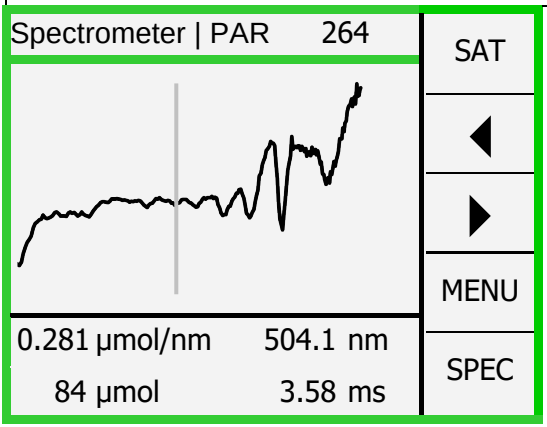
Side Panel

Same as window Quenching Analysis but Act.L. replaced by SPEC.

Main Panel

Spectrum. Type of spectrum identified by title line: PAR, light spectrum; Fluor Mode, fluorescence emission spectrum; Refl. Mode, reflection spectrum.

Touch chart to open/close the window below.



Side Panel

As above except arrow keys ◀, ▶. Go to previous, next position in spectrum (current position is marked by vertical line). The first line below the graph indicates the PAR per nm at the wavelength indicated in the same line. The second line shows the PAR (400-700 nm) of the spectrum depicted and the current integration time

Main Panel

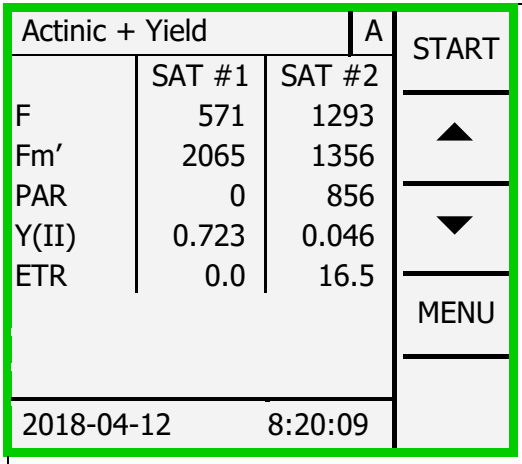
Spectrum and its numerical values.

Fig. 33: Spectrometer

In the Spectrometer window, touching the graph display area show a vertical cursor line as well as the x-y data of the intersection between cursor line and spectrum. Use the arrow keys to navigate through the spectra. Touching the graph display again returns to the original function of arrow keys.

4.2.6 Actinic + Yield

The “Actinic + Yield” window is the first of four windows for automated measuring routines. The parameters of the Actinic + Yield routine can be set in the menu “Program/Clock” (Fig. 39, page 62).



Side Panel

START. Start Actinic + Yield routine.

▲ Previous window (Ft-Chart).

▼ Next window (Induction Curve).

MENU. Go to Main Menu.

Main Panel

SAT #1, SAT #2. Column for data of first and second saturation pulse analysis, respectively.

F, Fm’. Fluorescence level before and at saturation pulse. For dark-acclimated samples: $F=F_0$ and $F_M'=F_M$.

Y(II). Effective photochemical quantum yield of PS II (F_V/F_M of dark-acclimated samples).

PAR, ETR. Photosynthetically active radiation (µmol·photons $m^{-2}\cdot s^{-1}$), electron transport rate (µmol·electrons $m^{-2}\cdot s^{-1}$).

Fig. 34: Actinic + Yield

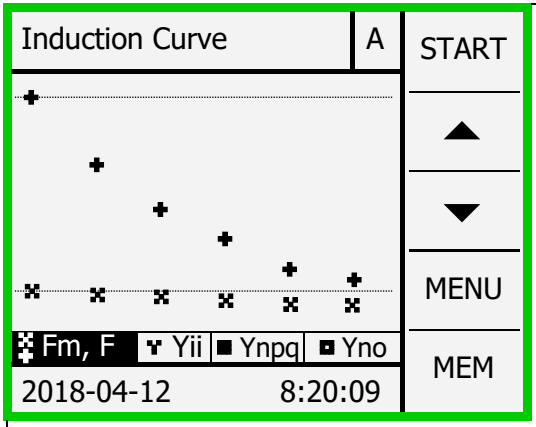
The routine illuminates a sample with actinic light of a defined period. Depending on settings, saturation pulse analysis is carried out prior and after actinic light exposure, or only after actinic light exposure. Because the Actinic + Yield experiment is rather short, it is frequently employed when response to light exposure needs to be evaluated for many samples.

4.2.7 Induction Curve

This window controls fluorescence induction curve experiments (Fig. 35, page 54). The graphics panel provides a qualitative picture of induction curve properties, Because of the screen resolution, it is difficult to quantitatively evaluate graphical data.

The Induction Curve window provides numerical data of fluorescence ratio parameters and fluorescence levels. These numerical data appear in response to touching the graphics panel. The navigation keys move the cursor (grey vertical line) from one saturation pulse analysis to another. The cursor indicates the position within the induction curve of the currently displayed set of numerical data.

The parameters of an induction curve experiment, number of saturation pulse analyses and interval between them, can be adjusted in the menu “Induction Curve Settings” (Fig. 47, page 74) which is available over the Program/Clock line of the Main Menu (Fig. 39, page 62). In the “Induction Curve Settings” menu, one can choose to continue fluorescence monitoring after the induction curve in the dark (recovery curve). The MEM of the Induction Curve window opens the “Induction Curve Memory” window. In this window, the navigation keys scroll through stored induction curves.



Side Panel

START/STOP. Switch to start or stop fluorescence induction curve.

▲, ▼ .Go to previous, next window.

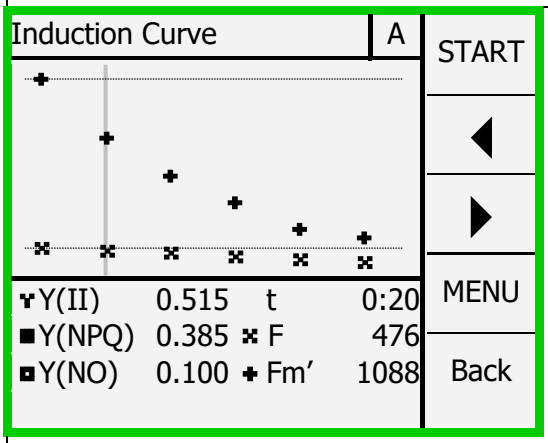
MENU. Go to Main Menu.

MEM. Open "Induction Curve Memory", view saved induction curves.

Main Panel

Graphics panel and buttons Fm, F, Yii, Ynpq, Yno to control display of data (F_M,F_M' plus F, Y(II), Y(NPQ) and Y(NO)). The Y(NPQ) and Y(NO) can be exchanged by parameters NPQ and qL in the menu Program/Clock Settings (4.3.3, page 71).

Touch chart to toggle between windows. BACK closes lower window.



Side Panel

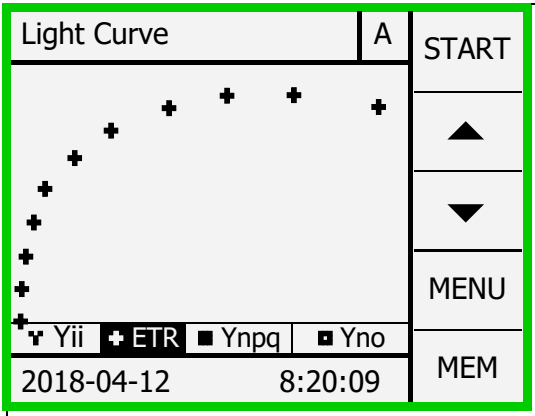
As above except arrow keys

◀, ▶. Go to previous, next saturation pulse analysis (current position is marked by vertical line).

Main Panel

Numerical values of fluorescence ratio parameters and fluorescence levels.

Fig. 35: Induction Curve



Side Panel

START/STOP. Switch to start or stop light curve.

▲, ▼ .Go to previous, next window.

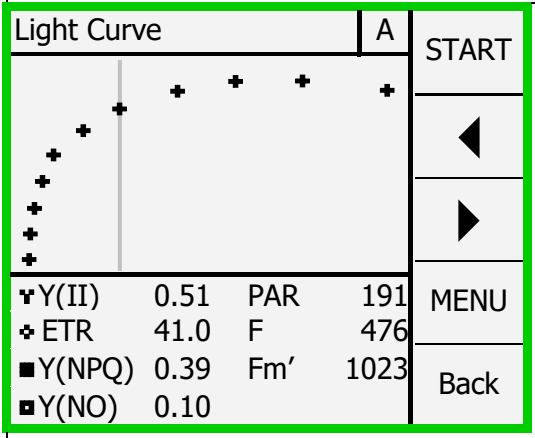
MENU. Go to Main Menu.

MEM. Open "Light Curve Memory", view saved light curves.

Main Panel

Graphics panel and buttons Yii, ETR, Ynpq, Yno to control display of data (Y(II), ETR, Y(NPQ) and Y(NO)). The Y(NPQ) and Y(NO) can be exchanged by parameters NPQ and qL in the menu Program/Clock Settings (4.3.3.7, page 76).

Touch chart to toggle between windows. BACK closes lower window.



Side Panel

As above except arrow keys

◀, ▶. Go to previous, next saturation pulse analysis (current position is marked by vertical line).

Main Panel

Numerical values of fluorescence ratio parameters and fluorescence levels.

Fig. 36: Light Curve

4.2.8 Light Curve

In analogy to the previous window (Induction Curve), the “Light Curve” window provides buttons to start and stop light curves and to survey them (Fig. 36, page 55). Also, the Light Curve window provides numerical data of saturation pulse analysis which can be accessed by touching the graphics panel. Navigation between different saturation pulses analyses and selection of displayed data works as described for the previous window (Induction Curve).

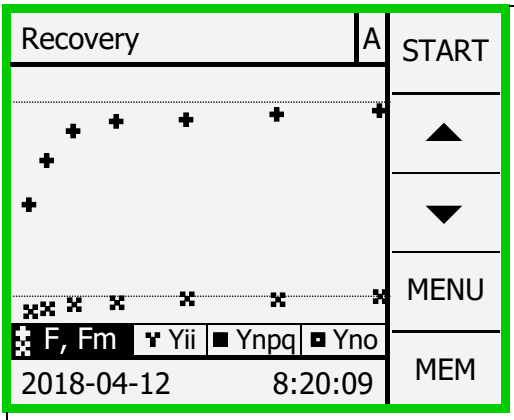
Light curve parameters (number and duration of light steps, initial PAR, and recovery curve) can be adjusted in the menu “Light Curve Settings” (Fig. 48, page 75). Touching the MEM button and scrolling using the arrow keys on the side bar permits viewing stored light curves.

4.2.9 Recovery

Often, fluorescence recovery experiments are automatically appended to an induction or a light curve (cf. 4.2.7 and 4.2.8). In these cases, the recovery curves are started automatically. Otherwise, recovery curves can be initiated (and cancelled) manually using the START/STOP button on the side panel of the Recovery window (see Fig. 37, page 57).

As in previous Induction and Light Curve windows, recovery data are represented graphically and numerically. The time course of recovery curves is fixed: each curve last 39 min during which 7 saturation analysis are carried out. In case of a preceding induction or light curve, the last saturation pulse analysis of the induction or light curve corresponds to the first one of recovery. The

interval between neighboring saturation pulse analysis roughly doubles with time (Table 4, page 15).



Side Panel

START/STOP. Switch to start or stop recovery curve.

▲, ▼ .Go to previous, next window.

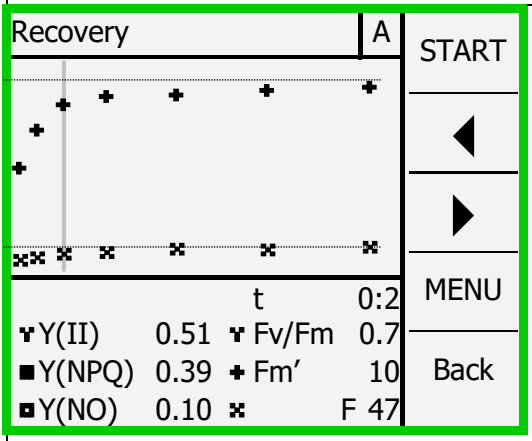
MENU. Go to Main Menu.

MEM. Open "Recovery Memory", view saved light curves.

Main Panel

Graphics panel and control buttons as described in Fig. 35, page 54.

Touch chart to toggle between windows. BACK closes lower window.



Side Panel

As above except arrow keys

◀, ▶. Go to previous, next saturation pulse analysis (current position is marked by vertical line).

Main Panel

Numerical values of fluorescence ratio parameters and fluorescence levels.

Fig. 37: Recovery

In the Recovery window, the MEM button opens the window “Recovery Memory” in which all recovery kinetics can be viewed independent if they are connected to an induction or light curve or if they represent separate experiments.

Table 4: Sequence of Saturation Pulse Analyses in a Recovery Curve

SAT number	Time in darkness, min
1	0.00
2	0:30
3	1:30
4	4:00
5	9:00
6	19:00
7	39:00

4.2.10 Actinic Light List

The window “Actinic Light List” contains PAR values (in µmol m^{-2} s^{-1}) which increase roughly exponentially with intensity settings 1 to 12. The entire PAR list can be multiplied by the internal actinic factor (“Int. Act. Factor, Fig. 43, page 69).

In the factory, the internal actinic light is adjusted so that the PAR values of the Actinic Light List apply to the sample level of the 2035-B leaf clip with the MINI-PAM-II fiber optics fully inserted (i.e. distance between fiber optics tip and sample level of 7 mm; angle between end piece of fiber optics and sample level of: 60°). Because of similar geometrical arrangement, these factory settings are also valid for the 2060-B Arabidopsis Leaf Clip when distance rings 2 mm plus 4 mm are used, and the 2010-A Distance Clip 60°.

External versus Internal Actinic Light

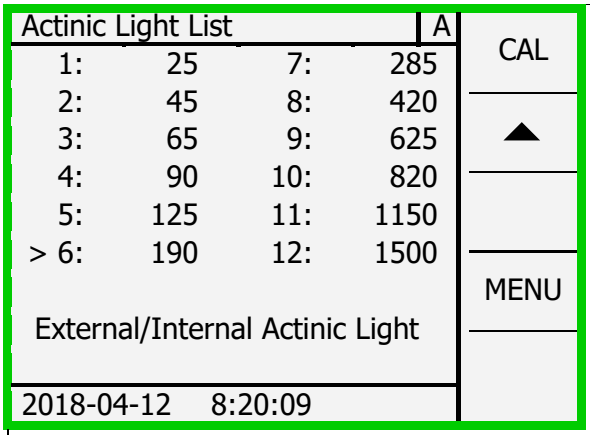
The main panel of the window Actinic Light List (Fig. 38, page 59) indicates the light source selected for actinic illumination.

External Actinic Illumination is displayed when:

- An external lamp (2054-L External LED Source) is connected to the SYNC port of the MINI-PAM and “PAM Mode (Act)” is selected as operation mode (see 4.3.2.6, page 69).

Internal Actinic Illumination is displayed when:

- An external lamp (2054-L External LED Source) is connected to the SYNC port of the MINI-PAM and “Ext. Panel Mode” is selected as operation mode.
- An external lamp is not connected.



Side Panel

CAL. Calibrate Actinic Light List.

▲ Go to previous window.

MENU, go to Main Menu.

Main Panel

PAR target values (µmol m^{-2} s^{-1}) of the 12 settings for actinic light intensity. The PAR target values are valid for an actinic light factor of 1.00 (see menu “Light Sources” Fig. 43, page 69). The “>” indicates the currently selected PAR level.

Fig. 38: Actinic Light List

The CAL command adjust internal actinic or external actinic light so that its PAR at sample level corresponds to the PAR values displayed in window “Actinic Light List”.

Internal Actinic Illumination

To calibrate internal actinic light, the **internal PAR sensor** is employed. The internal sensor is exposed to a small and constant fraction of internal actinic light. Following its calibration, the internal PAR sensor can be utilized to measure continuously internal light intensity. In the factory, the internal sensor has been adjusted to indicate the PAR values at sample level of the 2035-B leaf clip when the fiber optics is fully inserted.

When the sample level differs from the standard situation (2035-B leaf clip), the internal PAR sensor must be recalibrated. For this purpose, a calibrated PAR sensor is positioned at the currently selected sample level. Note that the PAR sensor of the 2035-B leaf clip is magnetically attached and can easily be disconnected to adjust the internal PAR sensor for various measuring configurations.

If the calibrated PAR sensor feeds its data in the MINI-PAM-II (as is the case with the PAR sensor of 2035-B leaf clip), the internal PAR sensor can be calibrated automatically. To do so, select “Calibrate” in window “Int. PAR Sensor Settings” and start automatic adjustment of the calibration factor (see Section 4.3.4.1, page 78).

If the calibrated PAR sensor is not compatible with the MINI-PAM-II, switch light on, and vary in the window “Int. PAR Sensor Settings” (Fig. 51, page 79) the “Calibration Factor” until internal PAR matches external PAR.

If "**External Actinic Illumination**" is displayed by the window "Actinic Light List", the CAL command adjusts the PAR emitted by the 2054-L External LED Source as measured by an external sensor (typically that of the 2035-B leaf clip) to the PAR values of the Actinic Light List.

The CAL command switches the PAR sensor of the 2035-B leaf clip automatically to the "Mixed Mode" (see Section 4.3.4.2, page 80), and adjusts each of the four light types of the 2054-L lamp. After calibration, the measuring mode of the PAR sensor is returned to its previous mode. Therefore, make sure that the Mixed Mode is selected when checking the light intensities after calibration.

4.3 Main Menu

The “Main Menu” (Fig. 39, page 62) forms the central hub to access settings, calibration data, hardware information and the memory of the MINI-PAM-II. The Main Menu consists of seven items. Items are selected using the arrow keys of the side panel followed by the SET command, or by directly touching a menu line. Most of these items lead to submenus that itself link to lower-level menus. The complex architecture of the Main Menu and its submenus is outlined in Fig. 40, page 63.

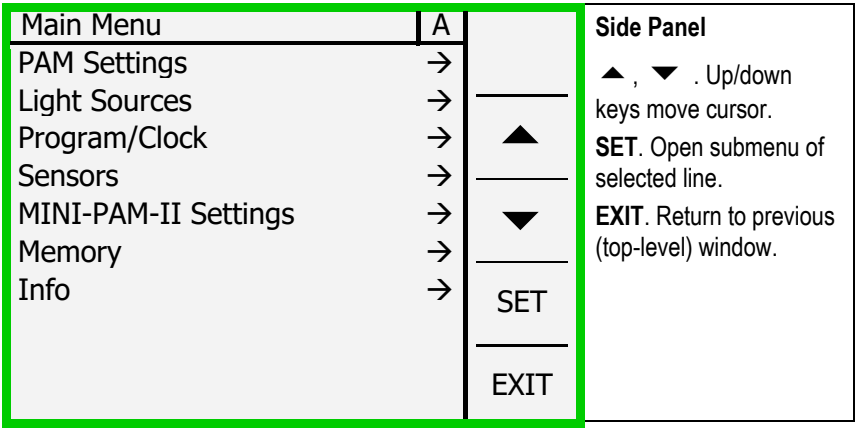


Fig. 39: Main Menu

4.3.1 PAM Settings

“PAM Settings” include adjustments of the way how the MINI-PAM-II acquires PAM fluorescence. The menu contains seven selectable items (Fig. 41, page 64) but also displays the current Ft value and the currently active offset which is automatically subtracted from the raw signal to obtain Ft.

4.3.1.1 Meas. Light

On/Off switch for measuring light (weak excitation light consisting of µs pulses). Measuring light can be switched by selecting “Meas. Light” and touching SET. Independent of the selected line in the menu, the OFF/ON key turns measuring light off or on.

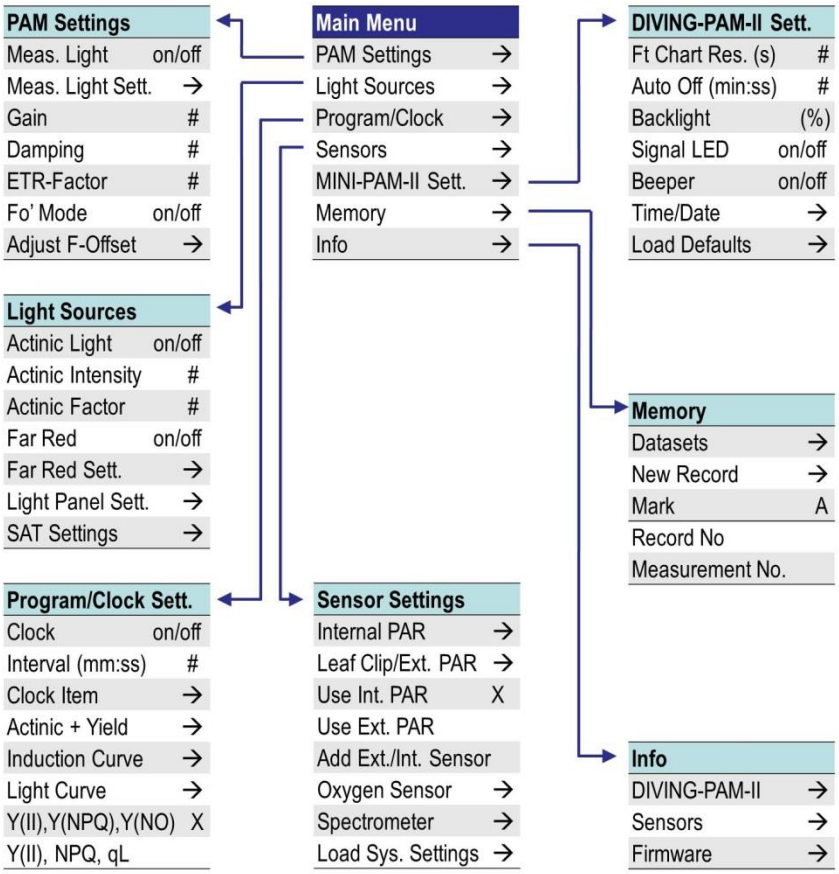


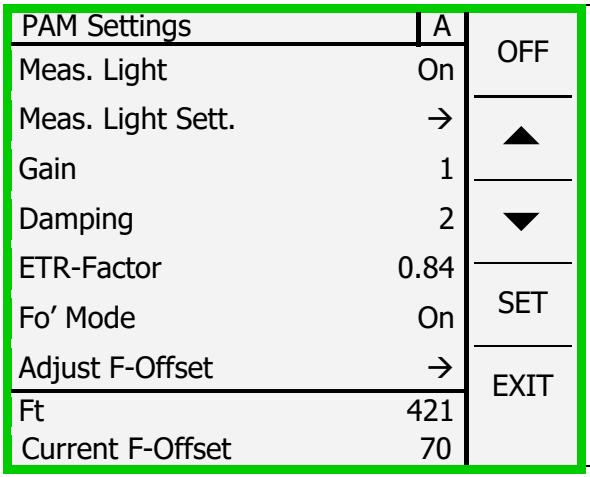
Fig. 40: Main Menu with all seven submenus

4.3.1.2 Meas. Light Sett.

This command opens the menu “Measuring Light Settings” in which measuring light intensity (Meas. Light Int.) can be marked by the Up/Down arrows, selected by the SET key and then adjusted using the arrow keys again. At constant frequency, measuring light intensity can be considered as proportional to intensity settings 1 to 12.

Table 5: Measuring Light Frequencies

Setting	Frequency, Hz
1	5
2	10
3	15
4	20
5	25
high	100



Side Panel

ON/OFF. Switch for measuring light.

▲, ▼ . Up/down keys move cursor.

SET. Open submenu (→) or select parameter, change parameter value by up/down keys.

EXIT. Return to Main Menu.

Fig. 41: PAM Settings

The two remaining items of the menu “Measuring Light Settings” concern measuring light frequency. Selecting the line “Meas. Light. Freq.” by SET allows choosing between 5 frequency levels using arrow keys. Table 5 (page 64) lists the measuring light frequencies of settings 1 to 5.

The highest measuring light frequency of 100 Hz can be activated by selecting by the SET key the third line in the menu (ML-F High). The ML-F High command overrules settings made under “Meas. Light. Freq.” High measuring light frequency improves signal quality but bears the risk that its higher intensity drives photosynthesis, that is, the measuring light becomes actinic. In this case, the F_0 may be overestimated. Measuring light frequency is automatically switched to “high” for saturation pulse analysis. Measuring light frequency does not affect the frequency of acquisition of Ft by the WinControl-3 software.

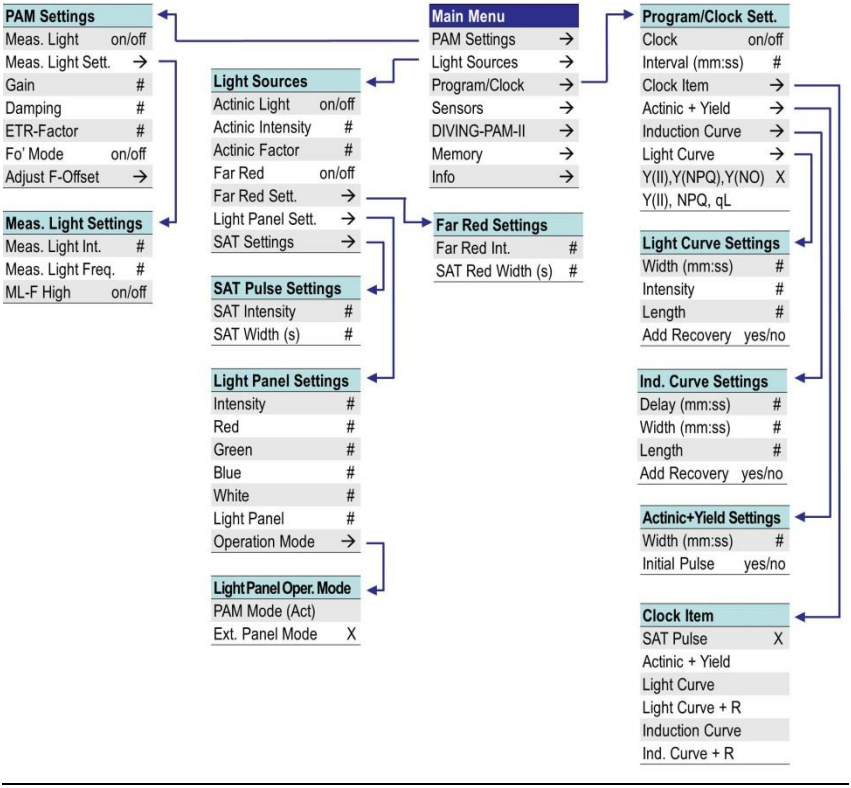


Fig. 42: Main Menu and First Three Submenus

The average PAR of measuring light at highest frequency and highest intensity setting was measured to be 1.5 µmol m^{-2} s^{-1} by the PAR sensor of the 2035-B leaf clip and MINI-PAM-II fiber in the fully inserted position. For the same geometrical arrangement, average measuring light intensities can be estimated using the subsequent equation:

$$I_{ML}(\frac{\mu mol}{m^2 \cdot s}) = 1.5\,(\frac{\mu mol}{m^2 \cdot s}) \cdot \frac{f\,(Hz)}{100\,(Hz)} \cdot \frac{Int.\,Sett.}{12}$$

where I_{ML}, *f* and *Int. Sett.* is the current measuring light intensity in µmol m^{-2} s^{-1}, the current measuring light frequency, and the current intensity setting for measuring light, respectively.

4.3.1.3 Gain

Selecting “Gain” by the SET gives access to four electronic amplification factors (1 to 4) which can be adjusted by the arrow keys.

4.3.1.4 Damping

Damping is a software-based filter that specifically suppresses high frequency noise and, thus, can improve signal quality. Changing damping settings uses the same principle as described for “Gain”. Default setting for damping is 2 (two). Changing damping to higher values can make the MINI-PAM-II response slow.

4.3.1.5 ETR-Factor

This factor is used for ETR calculations and corresponds to the fraction of incident PAR absorbed by a leaf; its default value is 0.84 (cf. Section 8.3, page 164).

4.3.1.6 Fo' Mode

The "Fo' Mode" replaces after saturation pulses actinic light by far-red light to quickly open PS II reaction centers (cf. Chapter 1, page 157). The measured F_0' is the minimum Ft during far-red illumination. Interval and intensity of far-red illumination can be adjusted in menu "Light Sources" (Fig. 43, page 69).

4.3.1.7 Adjust F-Offset

The "Adjust F-Offset" command determines the background signal for subtraction from the total signal. Background signals must possess the modulation characteristics of measuring light to be recognized by a PAM fluorometer. These signals can arise from:

- Fluorescence from suspension media or detector filter excited by measuring light.
- Traces of modulated excitation light transmitted by the detector filter.
- Non-optical modulated "electronic noise".

Usually, the background signal increases with measuring light intensity and signal amplification (gain). Therefore, the Adjust F-Offset command determines the background signal for all measuring light intensities and all gain settings. The currently active offset is

displayed in the bottom line of the PAM Settings window (Fig. 41, page 64).

Procedure

- Choose dim environment.
- Switch off any flickering light sources like fluorescent lamps or computer screens.
- Point fiber tip away from any objects, keep fiber tip clear.
- Run “Adjust F-Offset”

4.3.2 Light Sources

4.3.2.1 Actinic Light

On/off switch for actinic illumination.

4.3.2.2 Actinic Intensity

Intensity regulation for actinic light. Select menu item by SET and choose setting using arrow keys. Settings 1 to 12 are available. PAR information of settings is shown in window “Actinic Light List” (Fig. 38, line 59).

4.3.2.3 Actinic Factor

Factor multiplying target PAR values in window “Actinic Light List”. Factor range is 0.5 to 2.0. Maximum intensity might be constraint by LED limits.

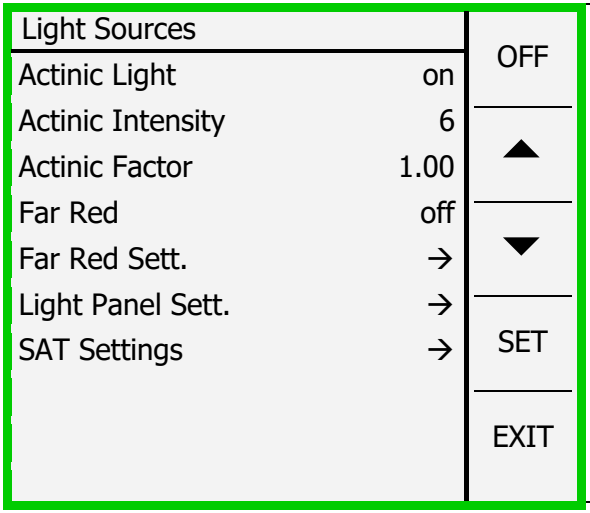


Fig. 43: Light Sources

4.3.2.4 Far-red

On/off switch for far-red light.

4.3.2.5 Far-red Sett.

Opens menu to adjust intensity and duration of far-red illumination. These settings are active in F_0' determinations.

4.3.2.6 Light Panel Sett.

Use SET to enter submenu “Light Panel Settings” (Fig. 44, page 70). The functionality of this window depends on the “operation mode”. The operation mode is selected via the last line in the window.

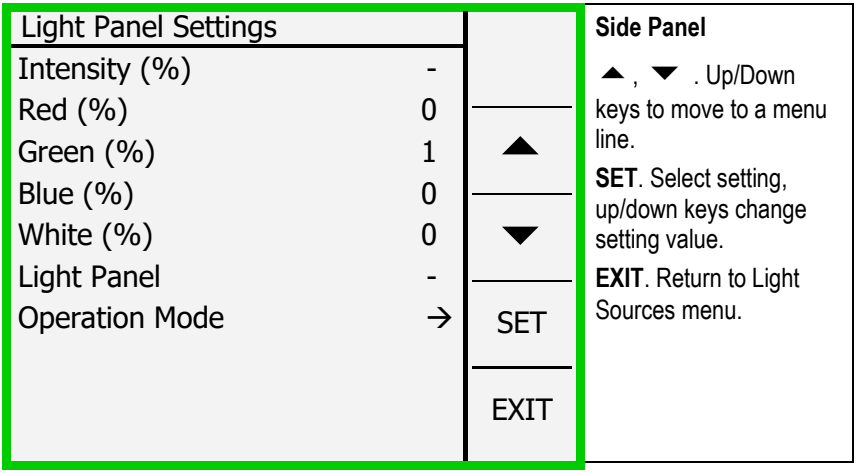


Fig. 44: Light Panel Settings

Two operation modes are available: “PAM Mode (Act.)” and “Ext. Panel Mode”. “PAM Mode (Act.)” means that actinic light is provided by an external source like the 2054-L External LED Source (Fig. 14, page 30). “Ext. Panel Mode” means that the MINI-PAM-II internal light is used as actinic light to which an external light source may be added (see Table 20, page 139).

Only when “Ext. Panel Mode” is chosen, the item “Intensity” and the Light Panel On/Off switch are available in the previous window’s (Light Panel Settings). In this case, “Intensity” determines the percentage of maximum intensity for all LED groups (red, green, blue, and white). The numbers entered for an individual LED group correspond to percentages of the scale from zero to “Intensity”. Typically, the maximum intensity of all four LED groups is greater than 5000 µmol m^{-2} s^{-1}. In “Ext. Panel Mode”, the external actinic light can be operated in parallel.

Note: Operation of the external light source (2054-L External LED Source) requires line power.

Note: In absence of an external light source, “PAM Mode Act.” is not available.

With “PAM Mode (Act.)”, the total intensity of all four LED groups is determined by the setting for actinic light intensity (see above). In the window “Light Panel Settings”, the numbers for the four LED groups now represent ratio numbers determining the fraction with which each LED group contributes to total illumination.

4.3.2.7 SAT Settings

Moving to “SAT Settings” and SET opens the submenu “SAT Pulse Settings” (see below) in which relative intensity (1 to 12) and duration (width, 0.2 to 2.0 s) of saturation pulses can be set.

At sample level, at intensity setting 12 the SAT intensity corresponds to 6000 µmol m^{-2} s^{-1} under the geometrical conditions of a 2035-B leaf clip (distance fiber optics tip to sample level: 7 mm; angle between end piece of fiber optics and sample level: 60°). The saturation pulse intensity can be adjusted at increments of 500 µmol m^{-2} s^{-1}.

4.3.3 Program/Clock Settings

The menu “Program/Clock Settings” provides all options to configure automated experimental routines including Actinic + Yield, Induction Curve and Light Curve experiments as well as repetitive triggering of single measurements and experimental protocols.

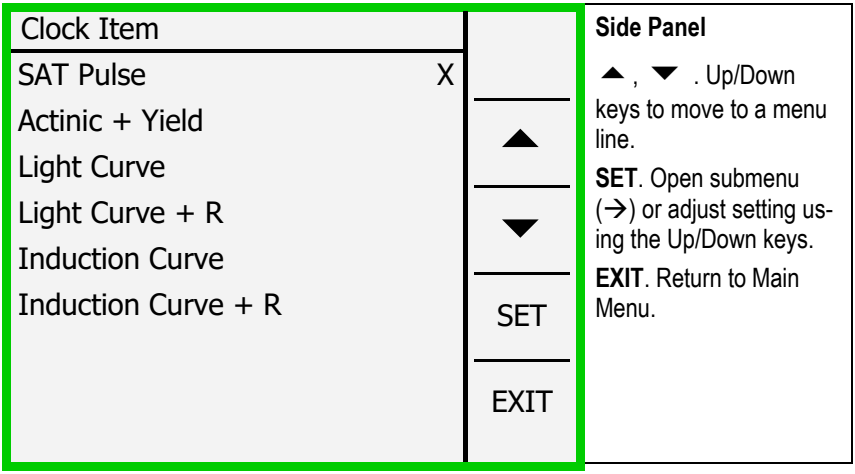


Fig. 45: Clock Item

4.3.3.1 Clock

On/off switch of clock. The “Clock” triggers repetitively an event at a defined interval. The interval is specified in “Clock Interval” and the event in “Clock Item”.

4.3.3.2 Clock Interval

Adjust clock interval between 10 s and 60 min by selecting “Interval” (up/down keys and SET, respectively) and adjusting time interval (up/down keys).

4.3.3.3 Clock Item

Saturation pulse analyses and the programs Actinic + Yield, Induction Curve and Light Curve can be repetitively performed under clock control. Also, recovery experiments can be performed after induction and light curves (item Light Curve + R and Induction Curve + R, respectively). To select one of the six items in menu “Clock Item” (Fig. 45), move cursor to the item of interest and touch SET. The selected item is then marked by an X (“SAT pulse is selected in Fig. 45).

4.3.3.4 Actinic + Yield

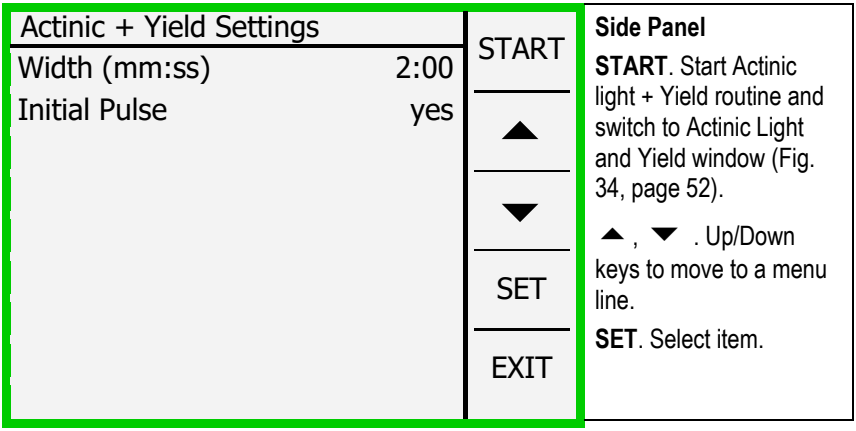


Fig. 46: Actinic + Yield Settings

The behavior of the Actinic + Yield program is defined by two factors (Fig. 46, page 73): the duration (width) of actinic illumination (possible settings from 5 s to 5 min) and the option to start actinic illumination without preceding saturation pulse analysis (Initial pulse). Width of actinic illumination is adjusted as described above

for clock interval and initial pulse is selected by the SET command. Further, actinic light intensity is adjusted in the window "Light Sources" (Fig. 43, page 69).

An Actinic + Yield routine can be started using the START button on the side panel of Fig. 46. In this case, the screen display will automatically switch to the Actinic + Yield experimental window (Fig. 34, page 52).

4.3.3.5 Induction Curve

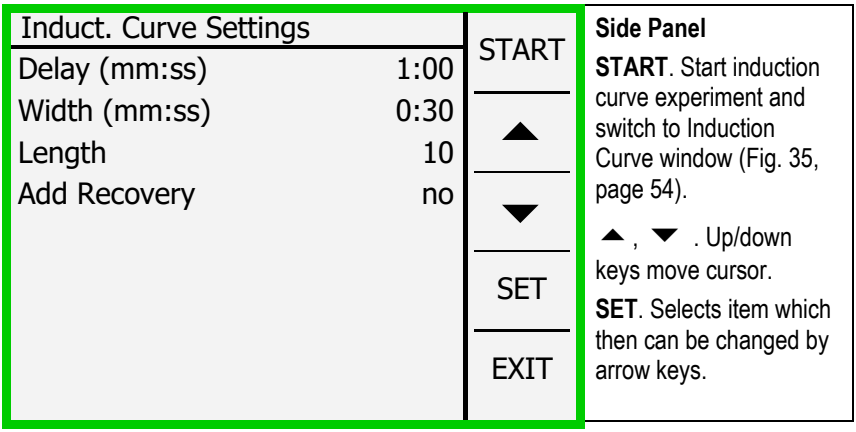


Fig. 47: Induction Curve Settings

Induction curve experiments are configured in the window "Induction Curve Settings" (Fig. 47, page 74).

Delay (range 5 s to 10 min) defines the dark interval between saturation pulse analysis with the dark-acclimated sample (F_0, F_M determinations, Chapter 1, page 157) and beginning of actinic illumination.

Width (range 5 s to 10 min) is the time interval between two successive saturation pulse analyses during illumination.

Length is the number of saturation pulse analyses carried out during actinic illumination. Thus, the duration of actinic illumination is "Length – 1" times Width.

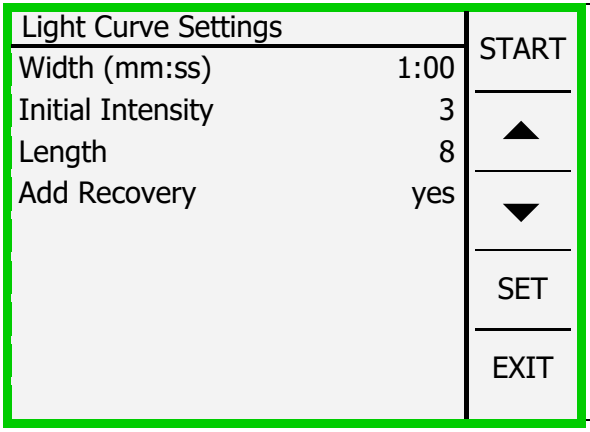
Add recovery appends a recovery curve to an induction curve (see Section 4.2.9, page 56 for information on recovery times).

4.3.3.6 Light Curve

Light Curves are defined in "Light Curve Settings" (Fig. 48, page 75). Properties of Width and Add Recovery are identical for light and induction curves.

Intensity specifies the actinic intensity setting for the first light step (range 1 to 5, for PAR values see Fig. 38, page 59).

Length is the number of light steps which can range from 2 to 12. If length = 5 and intensity = 2, 5 light steps with intensity settings 2, 3, 4, 5, and 6 will be performed. The time required for a light curve results from Length times Width.



Side Panel

START. Start light curve experiment and switch to Light Curve window (Fig. 36, page 55).

▲, ▼ . Up/down keys move cursor.

SET. Selects item which then can be changed by arrow keys.

Fig. 48: Light Curve Settings

4.3.3.7 Select (Y(II), Y(NPQ), Y(NO)) or (Y(II), NPQ, qL)

The last two lines of the menu “Program/Clock Settings” affect graphical and numerical presentation of data in windows Induction Curve, Light Curve, and Recovery Curve. Specifically, selecting “Y(II), Y(NPQ) Y(NO)” displays three yield parameters that are used in analyzing energy partitioning. Choosing Y(II), NPQ, and qL displays the classical NPQ parameter and a parameter for indicating the reduction state of PS II (q_L). Selection between lines works as described for “Clock Item” (Fig. 45, page 72). See Table 26, page 166 for definitions of fluorescence parameters.

4.3.4 Sensors

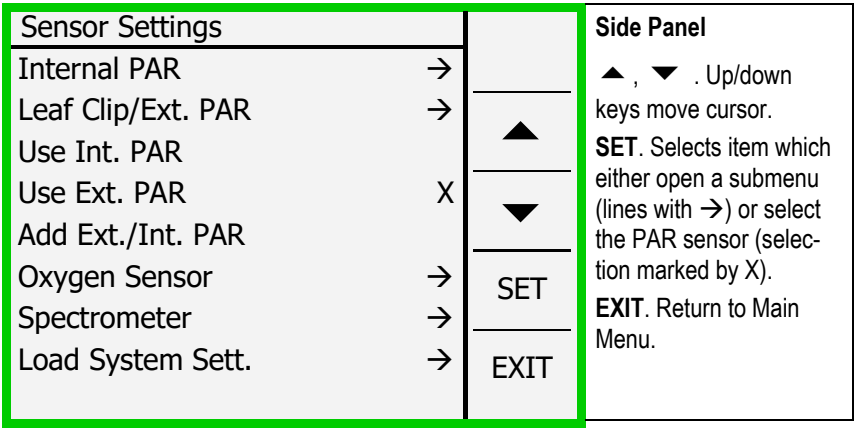


Fig. 49: Sensor Settings

Selection of “Sensors” in the Main Menu opens the window “Sensor Settings” (Fig. 49). This window consists of five links leading to submenus (distinguished by →) and the option to select for light

measurements the MINI-PAM-II internal PAR sensor or the external sensor of the 2035-B leaf clip (Use Ext. PAR/Use Int. PAR). An overview on window Sensor Settings and its complex structure of submenus gives Fig. 50 (page 77).

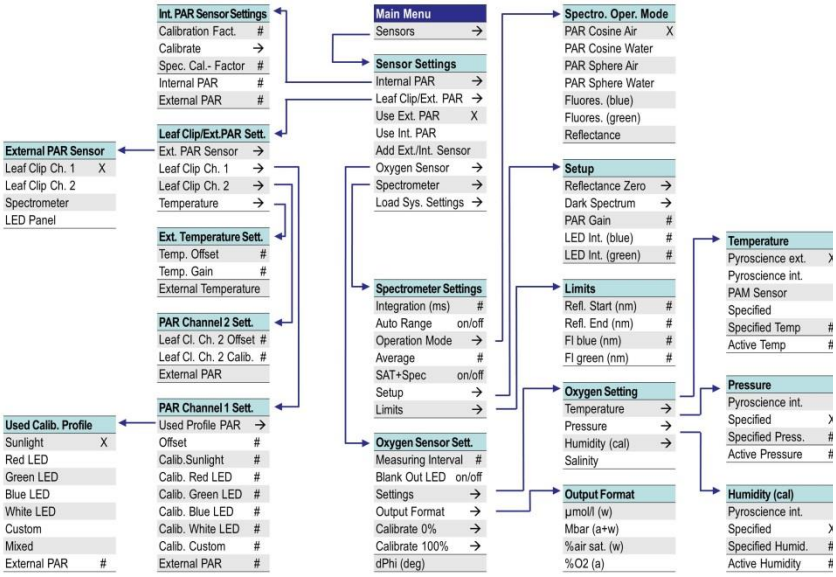


Fig. 50: Sensor Settings Menu and its Submenus

Selecting “Internal PAR” opens the window “Internal PAR Sensor Settings” (Fig. 51, page 79). This window is dedicated to calibration of MINI-PAM-II internal PAR sensor. The window displays the current “Calibration Factor” of the internal PAR sensor. If the miniature Spectrometer should be used for calibration of the internal PAR sensor, the spectrometer calibration factor (Spec. Cal. Factor) is important. This factor corrects for the effect of inhomogeneous illumination by actinic light of the diffusing disk of the miniature spectrometer. Typically, the Spec. Cal. Factor is adjusted by comparing the Calibration Factor determined by the PAR sensor of the

2035-B clip with the Calibration Factor determined by miniature spectrometer in the PAR block arrangement (Fig. 9, page 22). The Spec. Cal. Factor is correct if both setups result in similar Calibration Factors. Ask Walz for the Spec. Cal. Factor when a 2035-B clip is not at hand.

4.3.4.1 Internal PAR

“Internal PAR” opens the menu “Int. PAR Sensor Settings” (Fig. 51, page 79). In this menu, the last two lines display the PAR measured by the internal and by the external PAR sensor (µmol m^{-2} s^{-1}), when internal actinic light is switched on.

Remember that calibration of the internal PAR sensor is done by comparison with a calibrated external sensor. The external sensor is positioned at a defined distance and angle relative to the end of the fiber optics. Therefore, the readout of the internal PAR sensor applies only for the position of the external PAR sensor. With the external PAR sensor in calibration position, proper calibration of the internal sensor is apparent from similar readouts of internal and external PAR.

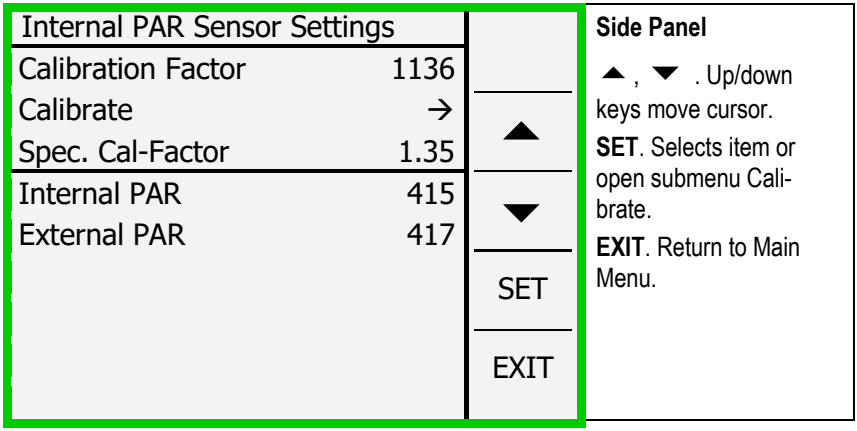


Fig. 51: Internal PAR Sensor Settings

If calibration of the internal PAR sensor has previously been carried out but the two PAR values differ clearly, check if:

(1) External PAR sensor is in the center of the actinic light beam.

(2) Setup of previous calibration was different from the current one. For instance, if in previous calibration the external PAR sensor was 3 mm away from fiber tip and now the 2035-B leaf clip geometry is used (7 mm instead of 3 mm distance) the internal PAR readout would be much higher than the external one.

(3) External PAR sensor not connected or incorrect PAR channel selected in window “External PAR Sensor” (Fig. 53, page 81).

PAR sensor compatible with MINI-PAM-II: To calibrate the internal PAR sensor using the PAR sensor of the 2035-B clip connected to the MINI-PAM-II, go to “Calibrate” and establish calibration factor automatically.

Other PAR sensor: To calibrate the internal PAR sensor using an external PAR sensor which cannot be read by the MINI-PAM-II,

adjust calibration factor manually by selecting “Calibration Factor”, pressing SET and changing the factor by arrow keys until internal PAR matches that of the external sensor.

4.3.4.2 Leaf Clip/Ext. PAR

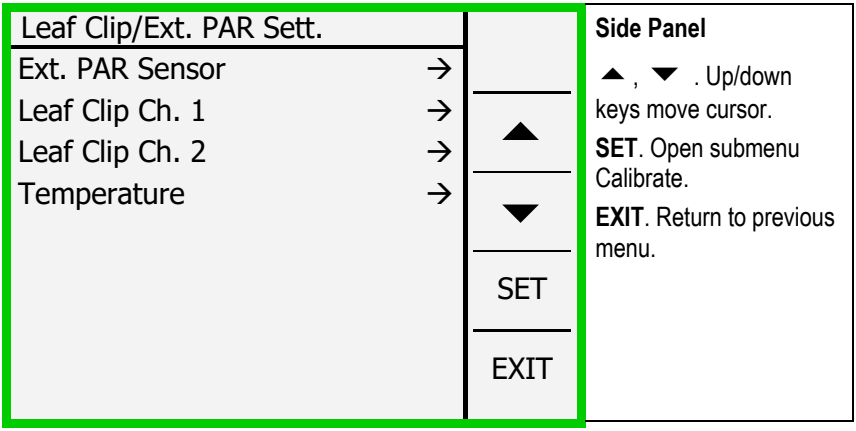


Fig. 52: Leaf Clip/Ext. PAR Sett.

Ext PAR Sensor of the menu “Leaf Clip/Ext. PAR” opens a submenu that selects the active external PAR channel (Fig. 53, page 81). Four possible PAR sensors are considered: PAR sensors 1 and 2 of 2035-B leaf clip, the miniature spectrometer, and a PAR sensor connected to the LED-Panel RGBW-L084. In the normal configuration of the MINI-PAM-II, Leaf Clip Ch 1 is selected.

Leaf Clip Ch. 1 opens “Leaf Clip Channel 1 Settings” which contains various calibration factors for PAR channel 1, that is, the mini quantum sensor of Leaf Clip Holder 2035-B. The different calibration factors (“Calib. Sunlight to Calib. White”, see Fig. 54) were factory-established and are characteristic for each PAR sensor.

By considering the spectral variations in sensitivity of the PAR sensor, these calibration values are optimized to measure various light qualities. Specifically, Calib. Sunlight is optimized to measure sunlight under clear skies.

Calib. Red, Calib. Green, Blue and Calib. White LED applies to measurements of light from Red, Green, Blue, and White LEDs. Calib. Custom can be chosen by the user. The “Offset” in the window “Leaf Clip Channel 1 Settings” applies to all PAR measurements.

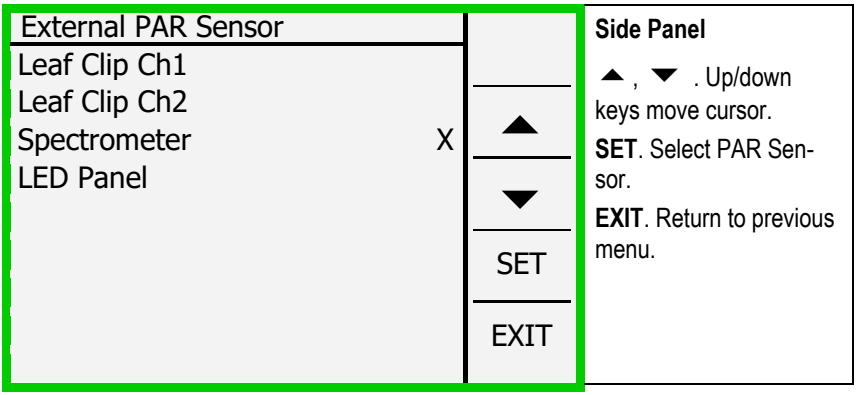


Fig. 53: External PAR Sensor

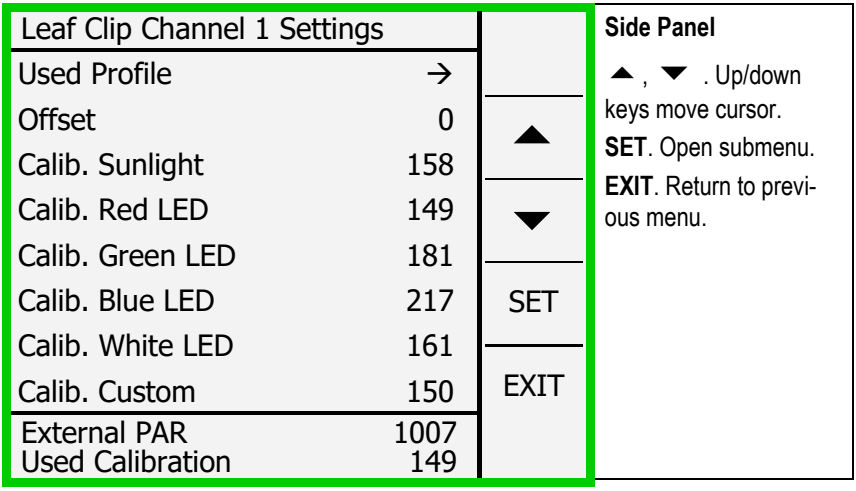


Fig. 54: Leaf Clip Channel 1 settings

The item Used Profile in window “Leaf Clip Channel 1 Settings” opens another menu (Used Cal. Profile, Fig. 55, page 83) in which a calibration factor or a calibration profile can be selected. In “Used Cal. Profile” the items “Sunlight” to “Custom” correspond to comparable items in the previous window., For example, selecting profile “Sunlight” activates the factor “Calib Sunlight” for PAR measurements.

The profile “Mixed” is adjust the calibration factor dynamically depending on the contributions of the red, green blue and white LED of the External LED Light Source 2054-L.

Leaf Clip Ch. 2 in window “Leaf Clip/Ext. PAR Sett” (Fig. 56, page 83) opens the calibration menu for a second PAR sensor connect to the SMA socket on the side of the 2035-B leaf clip. This menu simply consists of slope (Calib.) and offset of the calibration line are given. In the lowest line, the PAR readout of the active PAR sensor is shown.

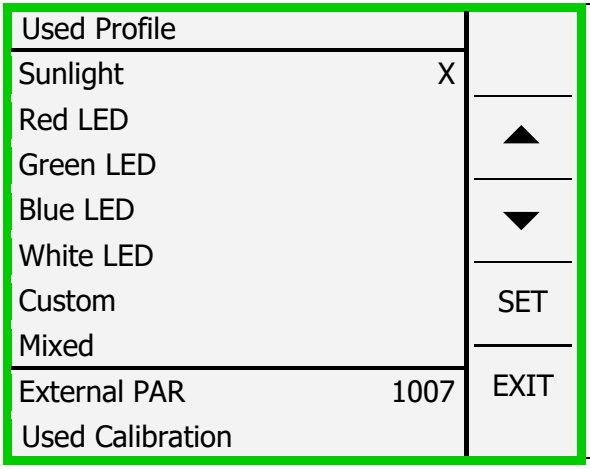


Fig. 55: Used Cal. Profile

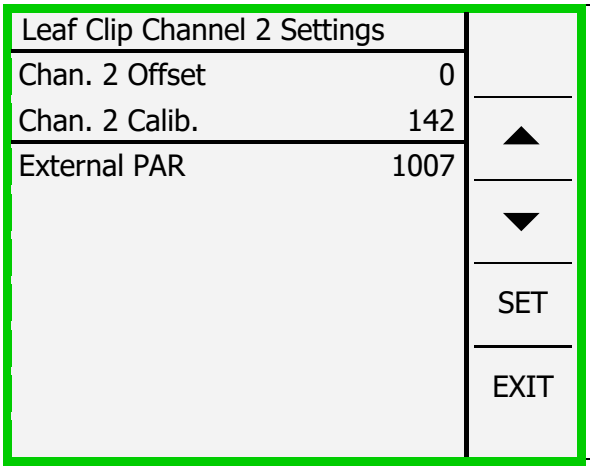


Fig. 56: Leaf Clip Channel 2 settings

Temperature leads to the calibration data for the leaf temperature sensor of the 2035-B leaf clip (External Temp. Sensor Sett., Fig. 57, page 84). This window lists slope (Gain) and offset of the calibration line.

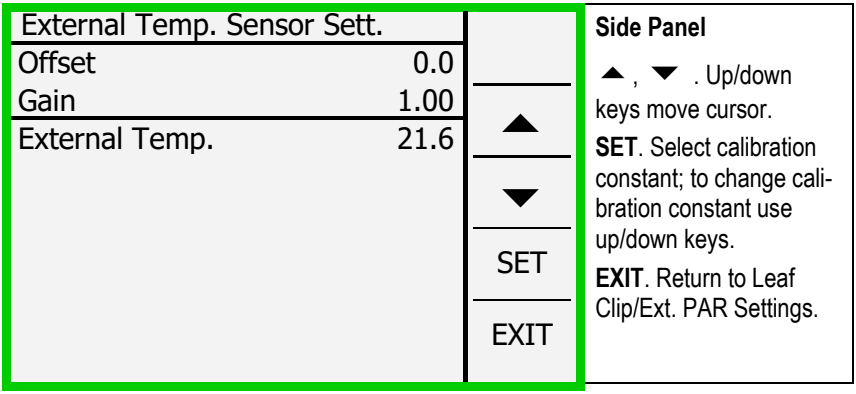


Fig. 57: External Temperature Sensor Settings

4.3.4.3 Oxygen Sensor

The window “Oxygen Sensor Settings” is prepared for connection of an optode sensor (oxygen package for MINI-PAM-II) where “Meas. Interval” defines the frequency of oxygen readings and “Blank Out LED” switches of M-PAM-II internal light in the case that it interferes with oxygen measurements.

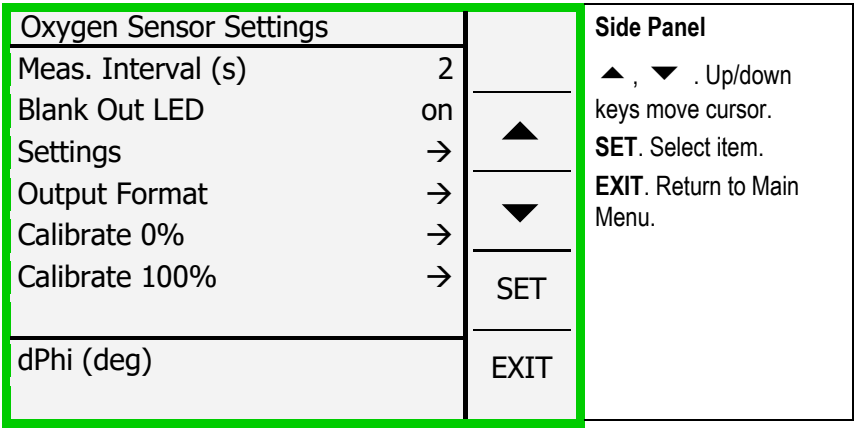


Fig. 58: Oxygen Sensor Settings

"Settings" allows entering temperature, pressure, and humidity data for correct sensor calibration. For each of these three parameters, an extra submenu is provided. "Output Format" opens a menu for selection of the parameter used for oxygen representation in the chart.

For details see the instructions for the oxygen measurements with the MINI-PAM-II:

https://www.walz.com/products/chl_p700/mini-pam-II/downloads.html

and the manual for the FireStingO2 oxymeter:

(https://www.pyroscience.com/en/).

4.3.4.4 Spectrometer

The item "Spectrometer" in menu "Sensor Settings" leads to the window "Spectrometer Settings (Fig. 59, page 86) which is dedicated to configuration on the miniature spectrometer.

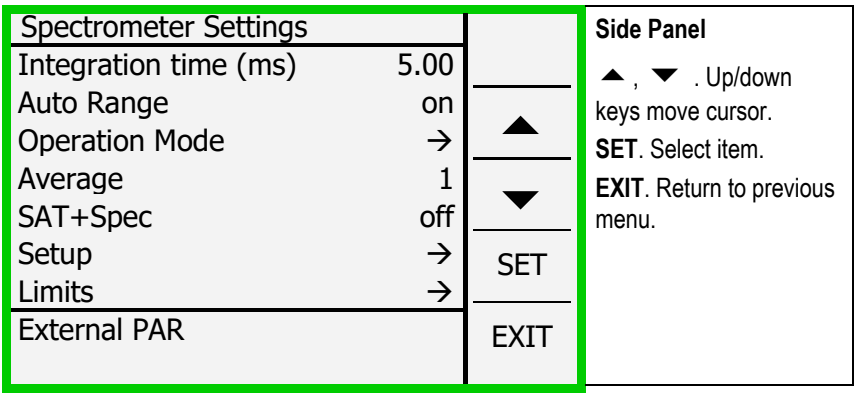


Fig. 59: Spectrometer Settings

Integration time (ms) determines the integrated measuring time in ms for a single spectrum.

Auto Range optimizes the integration time for a spectrum depending on incoming light. Auto Range ON is the default setting. Auto Range OFF is for special applications. When spectra look unrealistic, switch on Auto Range.

Operation Mode opens another menu (Fig. 60, page 87) in which the type of spectrum (light, fluorescence or reflectance) and, for light spectra, the entrance optics (flat=cosine versus spherical) and the environment in which measurements are performed (air or water) can be specified.

The cosine configuration employs a diffusing disk as light entrance (Fig. 10). This configuration shows an approximate cosine response toward incoming radiation. The sphere configuration measures light from all directions with similar weight. At the time of writing of this manuscript, the spherical sensor is under development.

Average determines the number of measurements used to calculate the final spectrum.

SAT+Spec active: each saturation pulse analysis is followed by recording of a spectrum.

Setup opens a menu for recording of the dark spectrum of the miniature spectrometer (Dark Spectrum →). The dark current of the spectrometer at room temperature is measured in the factory and stored on the flash memory of the device. To newly establish the dark current, fully darken the entrance optics (cv. Fig. 10, page 23).

The reference spectrum for reflectance measurements with the white standard is recorded by selecting “Refl. 100%”. The “amplification factor “Gain” is automatically adjusted depending on operation mode of the spectrometer. For example, switching the operation mode from air to water increases the Gain from 1.00 to 1.03 to consider the lower light flow to the detector under water. The last two items in the Setup menu allows adjusting the intensity of the blue and green LED used as excitation source for fluorescence emission spectra.

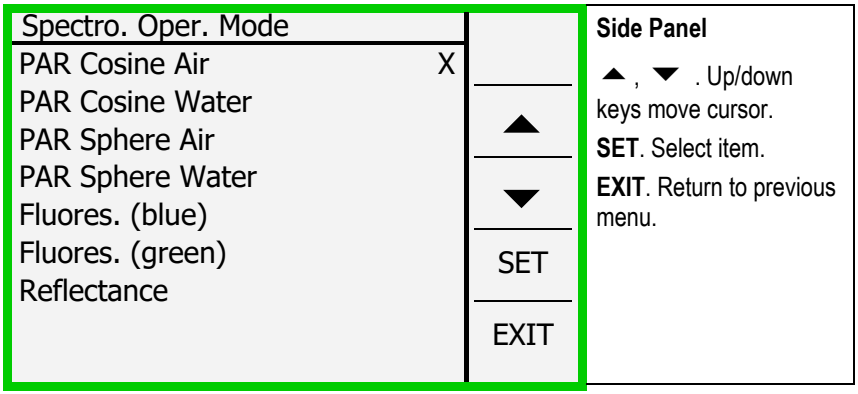


Fig. 60: Spectro. Oper. Mode

Limits: In the menu “Limits” short and long wavelength borders of reflectance spectra can be defined, and also the short wavelength limits of fluorescence emission spectra.

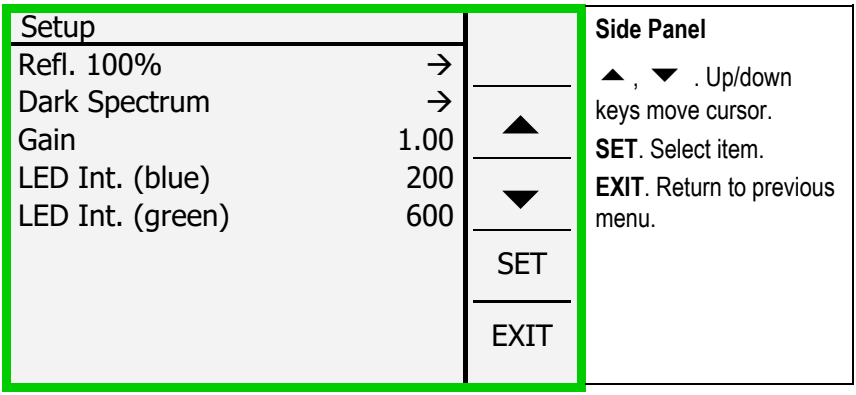


Fig. 61: Setup

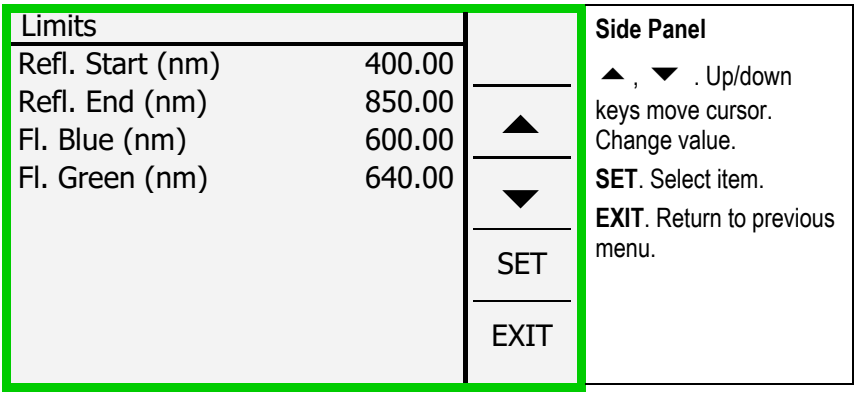


Fig. 62: Limits

4.3.4.5 Load System Settings

“Load System Settings” restores settings like calibration factor of internal PAR sensor (calibrated for geometry of 2035-B leaf clip), measuring light current and calibration factors of external devices

like those stored on the 2035-B leaf clip. “Load System Settings” must not be confused with “”Load Defaults” (Fig. 63, page 89).

4.3.5 MINI-PAM-II Settings

The menu “MINI-PAM-II Settings” permits adjusting of fluorometer settings, choosing between two time intervals for the Ft chart settings, and retrieving the default configuration for measurements.

Ft Chart Resolution (s)

Ft chart resolution can be either 0.2 or 1.0 s/dot corresponding to 25 or 125 s/total time axis.

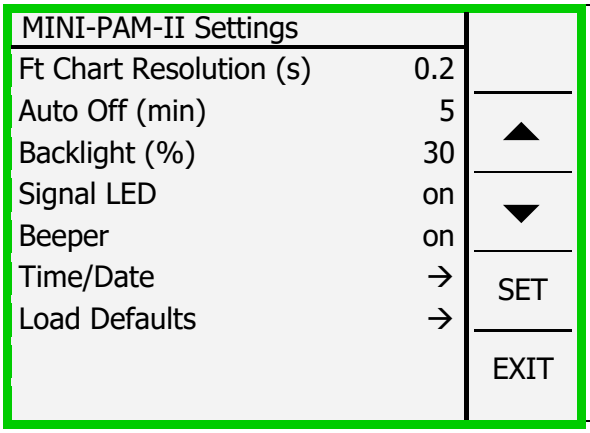


Fig. 63: MINI-PAM Settings

Auto Off (min)

Time interval without saturation pulse analysis after which the MINI-PAM-II powers off.

Backlight (%)

Percentage of maximum intensity of the display's backlight LED array.

Signal LED

On/off switch for LED on top of the MINI-PAM-II (green flash every 2 s, normal operation; green double flash every 2 s, clock-controlled operation; continuous light, saturation pulse analysis; green flash every 10 s, sleep mode).

Beeper

On/off switch for beeper which acoustically confirms keystrokes and saturation pulse analysis.

Time/Date

Simple menu for setting time and date.

Load Defaults

The MINI-PAM-II keeps on its internal memory the default settings for the variables listed in Table 6, page 91, left and center column. Current settings of some variables are saved when the MINI-PAM-II is shut off. These variables are marked by "Yes" in the rightmost column of Table 6. Settings of these variables are restored next use when the MINI-PAM-II is used.

Table 6: Default Settings

	Default Setting	Current Setting (saved and restored)
Measuring Light		
Status	On	No
Intensity	6, relative unit	Yes
Frequency	3, see Table 5	Yes
Frequency high status	Off	No
Actinic Light		
Status	Off	No
Intensity	6, relative unit	Yes
Factor	1.00	Yes
PAM Signal		
Gain	1, relative unit	Yes
Damping	2, relative unit	Yes
Far-red Light		
Width 5	5, s	Yes
Intensity	8, relative unit	Yes
Saturation Pulse		
Intensity	10, relative unit (≙ 5000 µmol m^{-2} s^{-1})	Yes
Width	0.6, s	Yes
Program Actinic Light and Yield		
Actinic light width	30, s	Yes
Initial Pulse	Yes	Yes
Program Induction Curve		
Delay	40, s	Yes
Width	20, s	Yes
Length	12	Yes
Program Light Curve		
Width	20, s	Yes
Intensity	3, relative unit	Yes
Length	8, light steps	Yes
Clock		
Item	Saturation pulse	Yes
Interval	60, s	Yes

Table 6: Default Settings

	Default Setting	**Current Setting** (saved and restored)
Hardware		
Signal LED status	On	Yes
Beeper status	On	Yes
Automatic power down	15, min	Yes
Background light	60%	Yes
Graphics		
Ft chart time resolution	0.2, s/dot	Yes
External Light		
Status	Off	No
Total intensity	1%	Yes
Red LED	10%	Yes
Green LED	10%	Yes
Blue LED	10%	Yes
White LED	10%	Yes
Stirrer		
Status	Off	No
Speed	10%	Yes
Pre-SAT off	10, s	Yes
Reverse	0, s	Yes
Interval mode	Off	Yes
Interval	2, min	Yes
Stirring interval	5, s	Yes
Stir in program	Off	Yes
F_0' Mode		
Status	Off	Yes
PAR Sensor		
Status	Internal	No
Mark		
Character	A	Yes

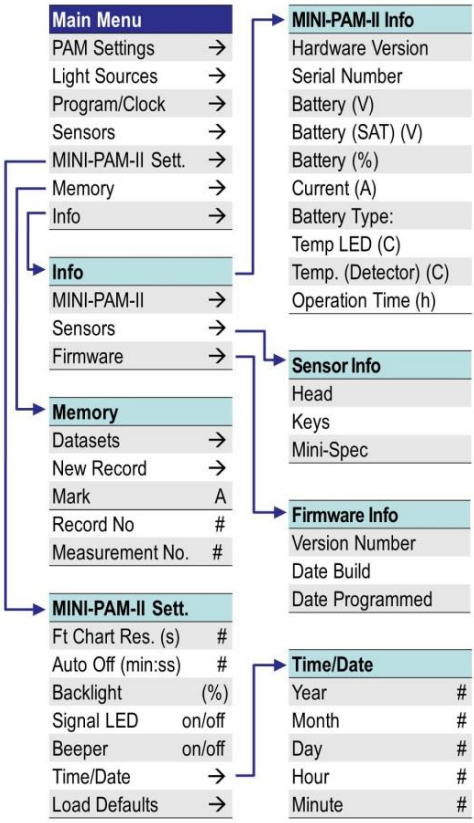


Fig. 64: MINI-PAM-II, Memory and Info Menus

4.3.6 Memory

The “Memory” window provides an overview on stored saturation pulse analyses (Datasets). Importantly, here fluorescence kinetics induced by saturation pulses can be viewed. To see stored induction and light curves, use the MEM key of window “Induction Curve” (Fig. 35, page 54) and window “Light Curve” (Fig. 36, page

55), respectively. Also in this window, new records can be started and the mark of saturation pulse data can be changed.

Deletion of data from the MINI-PAM-II internal memory is not allowed in the stand-alone mode. However, the memory can be cleared using the software WinControl-3.

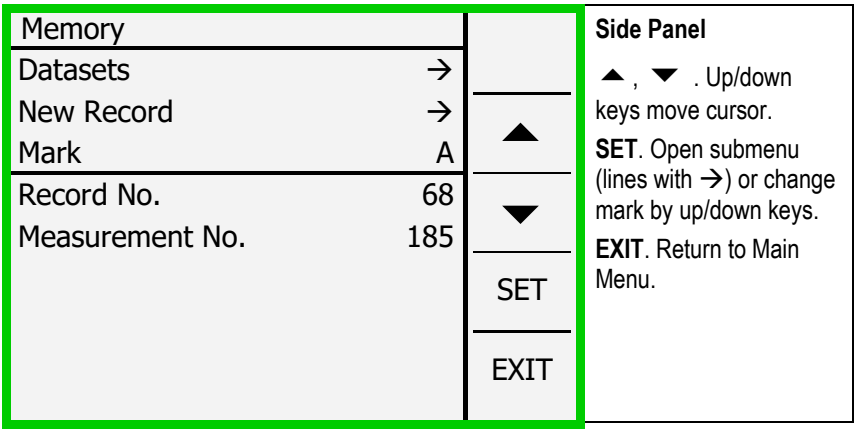


Fig. 65: Memory

4.3.7 Info

"Info" (Fig. 66, page 95) consists of 3 links to submenus of which three list hardware and software information:

- MINI-PAM-II provides hardware information of the fluorometer.
- Sensors lists serial numbers of sensors connected to the MINI-PAM-II.
- Firmware shows serial number and date of the firmware of the MINI-PAM-II.

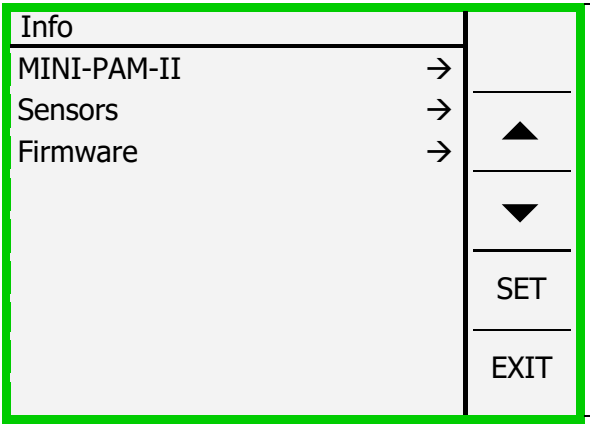


Fig. 66: Info

4.3.7.1 MINI-PAM-II Info

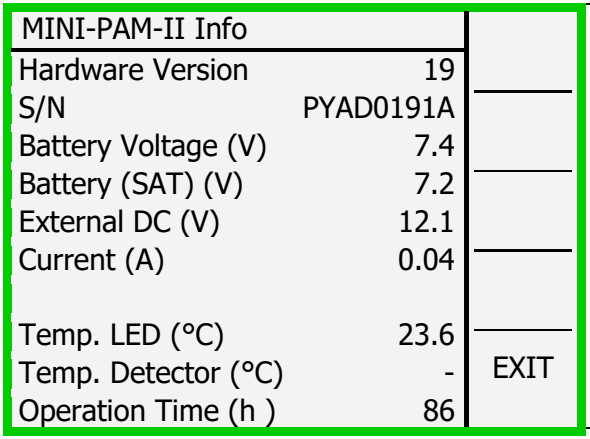


Fig. 67: MINI-PAM-II Info

Information available on this window is (1) hardware version of fluorometer, (2) serial number of fluorometer, (3) battery voltage at normal operation, (4) battery voltage during a saturation pulse, (5)

charge status in percent, (6) present current consumption (7) temperature (in °C) of actinic LED, (9) temperature (in °C) of detector, and (9) operation time of device.

4.3.7.2 Sensor Info

Serial number and hardware information of MINI-PAM-II measuring head and accessories.

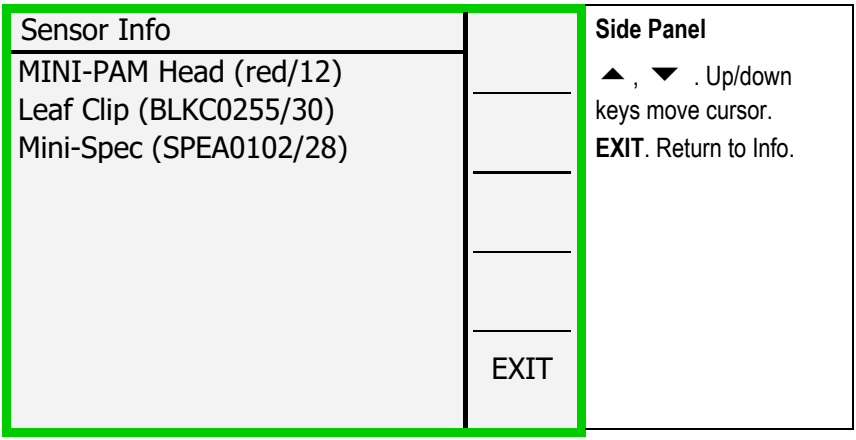


Fig. 68: Sensor Info

4.3.7.3 Firmware Info

Version and times of completion of firmware.

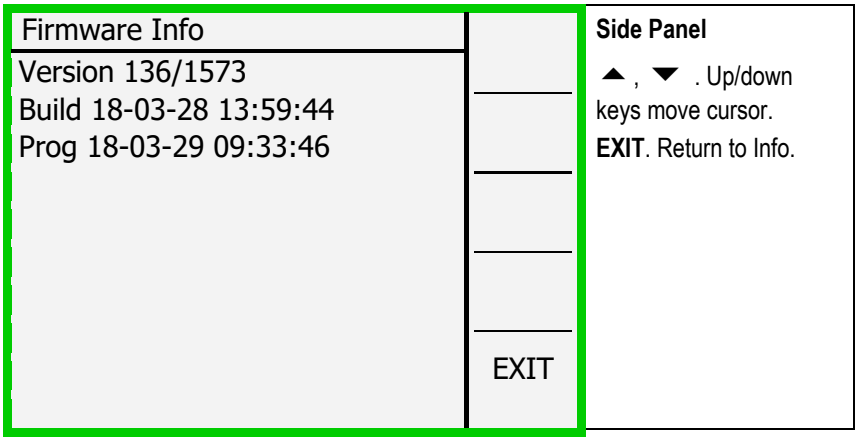


Fig. 69: Firmware Info

5 WinControl-3 Installation

The WinControl-3 software is provided on a Walz USB flash drive. The WinControl-3 software are regularly optimized. The latest software version is available on the Walz website:

https://www.walz.com/products/chl_p700/mini-pam-II/downloads.html

WinControl-3 can be installed from the Walz USB flash drive or using the setup software downloaded from the Walz website.

5.1 Installation process

Installation of WinControl-3 is mostly automatic. Dialog boxes appearing during setup provide advice or allow configuration of WinControl-3. To install WinControl-3, proceed as follows:

- Close other programs as advised by the setup wizard.
- Execute setup file: double-click on file or right-click on file and choose “run” from context menu.
- A pop-up windows must appear which identifies the Heinz Walz GmbH as verified publisher.
- Accept default folder for program installation or choose different folder after clicking Browse… .
- Select “Standard” Installation. (The “JUNIOR-PAM Teaching Edition” runs only with JUNIOR-PAM fluorometers.)
- Install USB driver and select optional WinControl-3 links (icon or shortcut).

- Connect MINI-PAM-II to computer and run PAM Firmware Update. If the current firmware* is outdated, PAM Firmware Update will automatically replace it by the recent version. Running PAM Firmware Update after installation of WinControl-3 is important because new software properties may function only in the presence of the latest firmware.

  *The term firmware denotes a piece of software residing on a flash memory of the MINI-PAM-II. The firmware is integrated in the WinControl-3 software.

5.2 WinControl-3 Program Group

Setup of WinControl-3 creates the WinControl-3 program group (Table 7, p. 100) in the Windows Start menu. The WinControl-3 program group is comprised of 5 items. The items are introduced in sections 5.2.1 through 5.2.5.

Table 7: WinControl-3 in Windows Start Menu

 WinControl-3
 PAM Firmware Update
 Uninstall WinControl-3
 WinControl-3
 WinControl-3 – Network Mode
 WinControl-3 – Offline

5.2.1 PAM Firmware Update

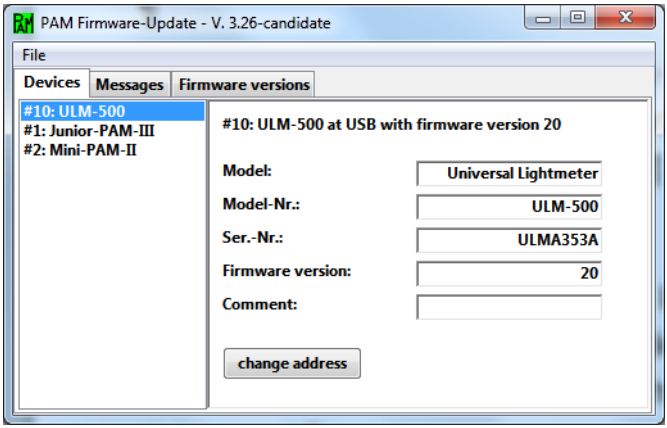


Fig. 70: PAM Firmware Update
Several devices connected to the same computer must have different addresses (channel numbers).

Initialization of PAM Firmware Update triggers a search for PAM devices connected to the computer. The result is displayed in the right panel of window “Devices” (Fig. 70, page 101). Each device name is preceded by its address number (between hash and colon).

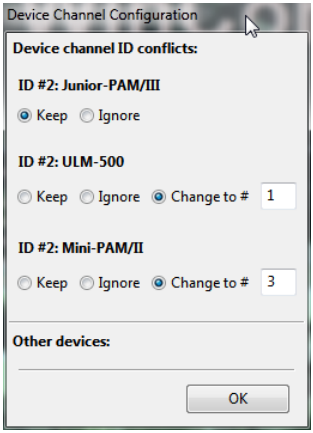
“PAM Firmware Update” compares the firmware in the device with the firmware included in the WinControl-3 software. If WinControl-3 includes newer firmware, the device is automatically updated.

“PAM Firmware Update” cannot update firmware of first-generation devices (DIVING-PAM, MICROFIBER-PAM, MICROSCOPY-PAM, MINI-PAM, WATER-PAM). In these devices, firmware resides on an EPROM chip and firmware update requires exchange of this chip.

A device can be selected by mouse click. The currently selected device is highlighted (white letters on blue background). The main panel of the window shows information on the device selected. The first four information lines define the hardware and software state of the device. The last line displays a comment associated with the device and typed in using the WinControl-3 software.

Devices with identical addresses cannot be operated simultaneously. If WinControl-3 detects identical addresses, the window “Device Channel Configuration” (Fig. 71, page 102) pops up offering a working address configuration and the option to change addresses manually. Note that address number is synonymous to channel number in the software WinControl-3.

Fig. 71: Device Channel Configuration



Address numbers can be changed manually via the button change address. Then, determine new address by picking a number from the drop-down list “New address:”. If several devices are connected, the drop-down list offers only unused address numbers. The window “Messages” displays the protocol of activities including firmware update of devices. The window “Firmware

Versions" compiles all software version provided by PAM Firmware Update.

5.2.2 Uninstall WinControl-3

This program removes WinControl-3 and all its links. It does not remove the USB driver software.

5.2.3 WinControl-3

This command starts WinControl-3 in the default mode. When devices which are compatible with the WinControl-3-type software are detected, WinControl-3 enters the measure mode. Clicking Offline-Mode interrupts the search process and WinControl-3 is started in the offline mode. When the search process fails to find compatible devices, a pop-up window provides three options: Yes triggers another search for devices, No launches the offline mode of WinControl-3, and Cancel ends the whole process. Measuring mode and offline mode, and several instances of WinControl-3 in the offline mode, can run in parallel.

After detection of the MINI-PAM-II, measuring of fluorescence is automatically started. With a green leaf placed in the 2035-B leaf clip, fluorescence values of 400 to 600 are observed (Ft value, bottom of window). Check "Rec. Online" or click Start Online to display continuously the Ft on the Chart window. If the Ft is much lower than 400, make sure that the optical fiber is properly inserted. Click Autoscale if data are not visible. Trigger saturation pulse analyses by pressing Fo, Fm or SAT A healthy leaf, which was kept dark before, should show a value for F_V/F_M of 0.8 or higher.

5.2.4 WinControl-3 Network Mode

The Network mode requires MONITORING-PAM measuring heads and connection via a special interface. It is not available for the MINI-PAM-II.

5.2.5 WinControl-3 Offline Mode

This command launches Wincontrol-3 without the initial search for available PAM devices.

6 WinControl-3 Operation

WinControl-3 offers the same functions as touchscreen operation of the MINI-PAM-II, except that continuous data acquisition and light curve analysis is confined to WinControl-3.

WinControl-3 functions are arranged in 11 Windows (Table 8). The Chart window appears at software start. When more than one device is connected, the additional window “Moni-Bus” appears.

Table 8: Windows of WinControl-3

	Window	Availability	Main panel	Content
1	Chart	Online and Offline	Graphics	Data versus time of all experiments
2	Induct. Curve	Online and Offline	Graphics	Data versus time of fluorescence induction curves
3	Light Curve	Online and Offline	Graphics	Data versus time of light response curves
4	SAT-Chart	Online and Offline	Graphics	Saturation pulse kinetics
5	Spectrum	Online and Offline	Graphics	Spectra
6	Report	Online and Offline	Alphanumerics	Saturation pulse data and data collected at the same time
7	Memory	Online only	Alphanumerics	Information on files stored on the MINI-PAM-II flash memory
8	Batch	Online and Offline	Commands	Site for automatic execution of experiments
9	Control	Online only	Settings	Configuration of external light panel PAR sensor and stirrer control
10	Sensors	Online only	Settings	Control of PAR sensor, spectrometer, and oxygen sensor
11	Settings	Online only	Settings	Device settings

6.1 Chart Window

Fig. 72 divides the Chart window into six areas. Most of the side bars are also present when other windows are viewed.

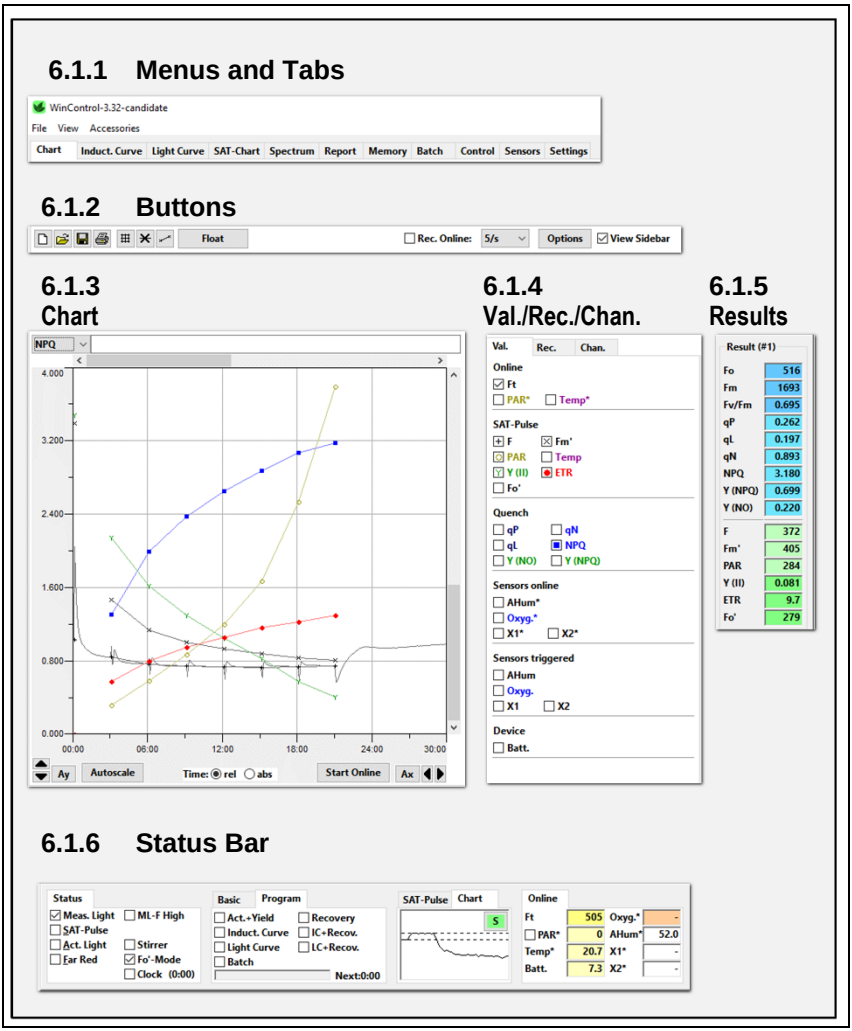


Fig. 72: Chart Window

6.1.1 Menus and Tabs

The top bar includes three menus (File, View, and Accessories) and all window tabs (compare Table 8, page 105). The three menus are explained in Table 9.

Table 9: Menu Overview

Menu	Item	Comment
File	Load Data	Opens WinControl-3 files. Save current data first and switch off online recording of data.
	Save Data	Saves present data in WinControl-3 format.
	Save Settings	Saves all current instrument settings in a batch file. The settings can be restored by executing this batch file.
	Export Report	Exports the data displayed on Report window. For information on configuration of export data see Table 10 (page 108).
	Export Chart Record	Exports the data displayed on Chart.
	Quit	Exit WinControl-3.
View	Results Panel	Switches Results panel (Section 6.1.3) on or off.
	Status Panel	Switches Status panel (Section 6.1.6) on or off.
	Warnings	Prompts the display of the 'program starting time' and non-critical errors.
	Batch Window	Switches Batch File window on or off.
Accessories	Temperature Units	Toggles between °Celsius and °Fahrenheit. The temperature unit affects only the numerical display on the Status bar (6.1.6, p.116).
	Plugins	Does not apply for the MINI-PAM-II.
	Record File	Saves data continuously to reduce data loss in case of program failure. The command prompts for a folder in which the file should be saved. The file name is created automatically using data and time of record start: WinControl-Record-YYYY-MM-DD-hh_mm_ss.pam.

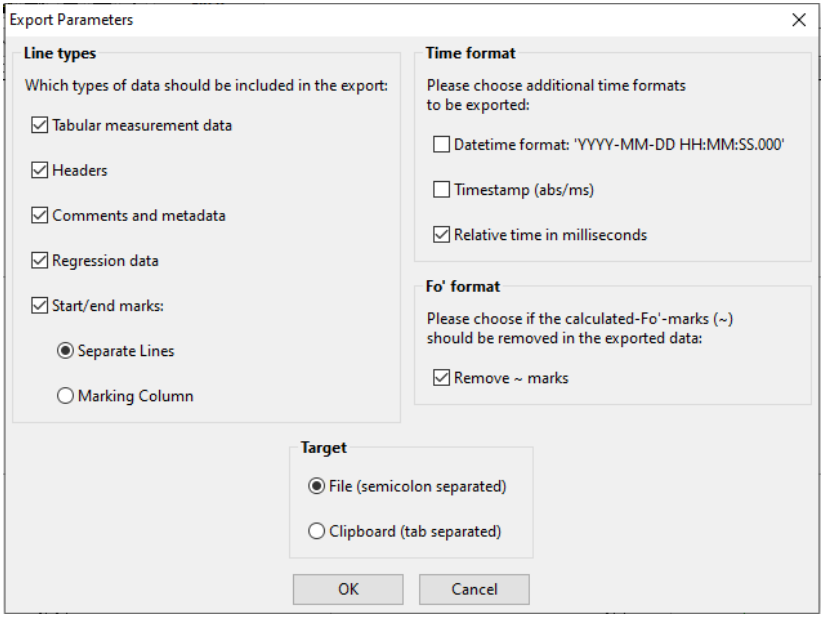


Fig. 73: Export Format for Reports

Table 10: Export Format for Reports

Parameter	Action when checked
☑ Tabular measurement data	Exports data of saturation pulse analysis and all other data recorded at the same time.
☑ Headers	Exports column headers.
☑ Comments and metadata	Exports user and software comments (=”metadata”, e.g., device information).
☑ Regression data	Exports the cardinal numbers of light curves.
☑ Start/end marks	
◉ Separate Lines	Writes start and end of an experiment in separate lines.
○ Marking Column	Writes start and end of an experiment in a separate column.

Table 10: Export Format for Reports	
Parameter	**Action when checked**
☑ Datetime format	Exports date and time. Format: day/month/year hours:minutes:seconds.ms. You may need to custom-format the datetime cell by entering dd/mm/yyyy hh:mm:ss.000 in the Type line.
☑ Timestamp	Export UTC time in ms (Timestamp 0 corresponds to 1970/01/01 00:00:00).
☑ Relative time in ms	Export time of experiment in ms.
☑ Remove "~" marks	Remove tilde (swung dash) signs. WinControl-3 marks calculated F'_0 values by a tilde.
⦿ File (semicolon separated)	Creates a file in which individual data are separated by semicolons.
○ Clipboard (tab separated)	Copies the data to the clipboard. Data are separated by tab characters. Use the paste command to copy the data into a spread sheet program.

6.1.2 Buttons

The elements of the buttons bar are introduced in Table 11 and Table 12.

Table 11: Buttons		
Icon	**Meaning**	**Comment**
	Delete	Deletes all current data.
	Load	Same function as "Load Data" in the File menu (Section 6.1.1.).
	Save	Same function as "Save" in the File menu (Section 6.1.1.).
	Print	Prints current chart view.
	Grid on/off	Controls display of chart grid.
	Lines	Connects data points with lines.

Table 11: Buttons

Icon	Meaning	Comment
	Symbols	Controls the display of symbols.
Float	Add Chart	Creates an additional chart window with separate view settings.
☑ Rec. Online	Continuous recording	Controls continuous recording of fluorescence, PAR, and temperature.
5/s	Sampling frequency	Sets sampling frequency for online data. Open drop-down menu by the downward arrow. Available sampling frequencies are 5/s, 1/s, and 1/10s. 5/s is available only for fluorescence.
Options **Or right-click on chart.**		See Table 12.
☑ View Sidebar		Controls display of side bar (see 6.1.4, Val./Rec./Chan.)

Table 12: Options

Menu Item	Present	Comment
Export Record	Always	Exports the data displayed.
Select current record	Always	Highlights data of a Record on the chart, and also on windows SAT-chart and Report.
Split Selection	After selection of interval.*	Put selection in separate record.
Zoom to Selection	After selection of interval.*	Displays the interval enlarged.
Export Selection	After selection of interval.*	Exports the data selected.

***** How to select an interval on the chart: keep the left-hand mouse button pressed, move cursor across x-interval and release.

6.1.3 Chart

Fig. 74 outlines the tools to adjust the chart. Section 6.1.4 explains how to select data for display.

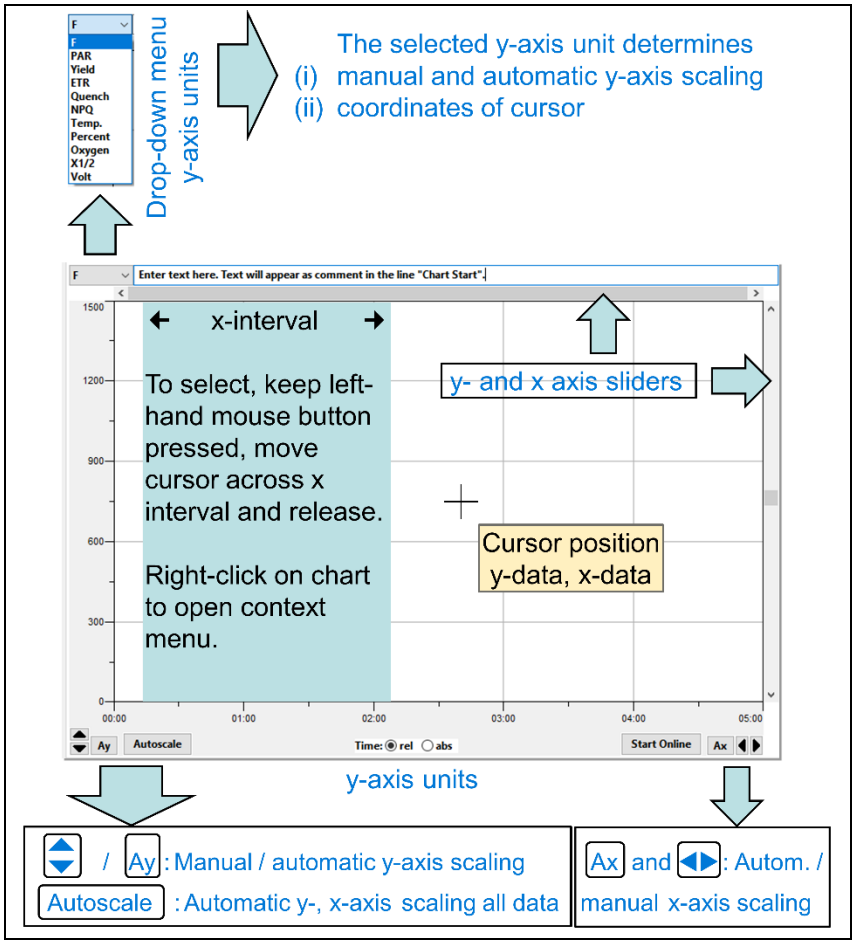


Fig. 74: Chart Tools

The scale of y- and x-axis can be manually changed: a vertical pair of arrows affects the y axis and a horizontal pair of arrows adjusts the x-axis (see lower border of Fig. 74). Right next the two pairs of arrows are buttons for automatic axis scaling.

Manual and automatic y-axis scaling affects only the currently active y-axis unit. In contrast, the command Autoscale adjusts y- and x-axis so that all data fit in the chart area.

The y-axis unit can be picked from a drop-down menu located in the upper left corner of the chart. The active y-axis unit also determines the y-data of the cursor position on the chart.

A way to zoom in to a particular x-axis interval is to mark the x-interval of interest (see Fig. 74), open a context menu by right-click on the chart area, and select the command “Zoom to Selection”.

The selection of data on the chart results in highlighting the corresponding saturation pulse kinetics in the window SAT-Chart and the corresponding data lines in the window Report.

6.1.4 Val./Rec./Chan.

The field “Val. Rec. Chan.” includes three different sidebars (Fig. 75). Data which should be displayed on the chart are selected on the Val. (= values) sidebar. The Val. sidebar distinguishes continuously recorded data (data groups: Online and Sensors Online) from saturation pulse data and data taken at the time of a saturation pulse (data groups SAT Pulse, Quench, and Sensors Triggered).

The Rec. (= Record) sidebar lists the number of individual charts of the present data file (= Report). Additional Records can be started by the button New Record and existing Records can be

deleted by Delete Record. The latest Record, or the Record selected by mouse click, will be displayed on chart. The windows “Induction Curve” and “Light Curve” also possess the Rec. sidebar but in these cases the Induction Curve and Light Curve experiments, respectively, are listed.

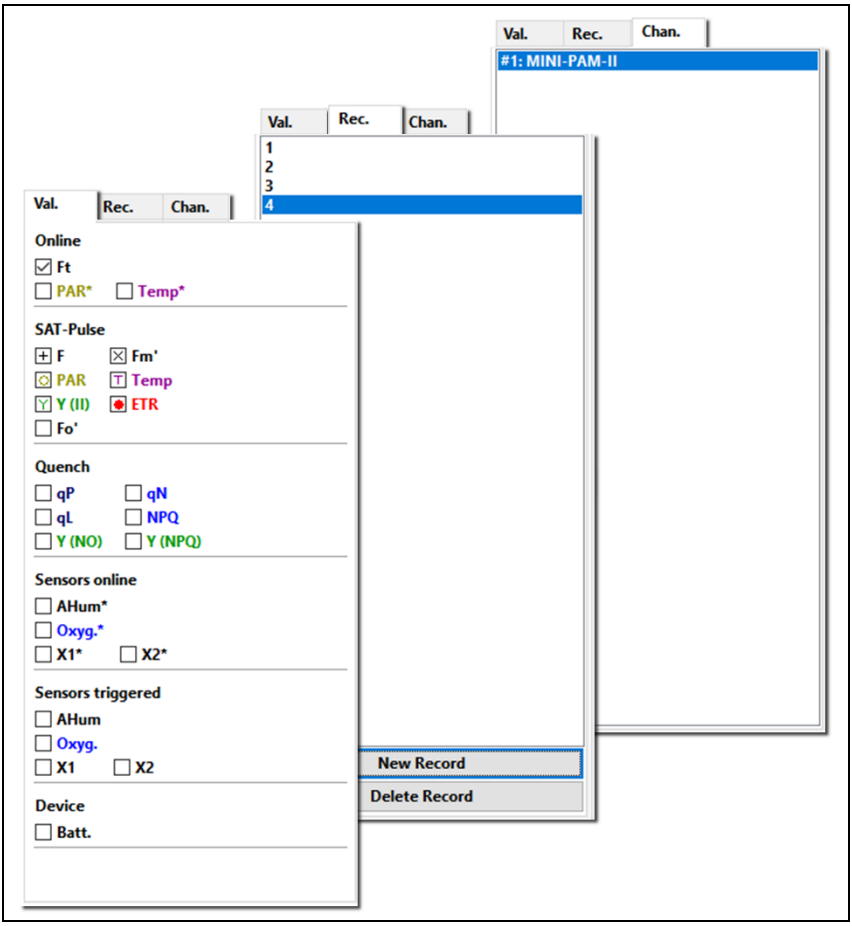


Fig. 75: Val. Sidebar

The Chan. sidebar displays all devices detected by the WinControl-3 software. Only a MINI-PAM-II was connected in Fig. 75.

On the Val. sidebar, the Ft represents a continuously recorded PAM fluorescence signal (online signal). The fluorescence level “F” is the corresponding saturation pulse signal (the F is the Ft measured right before the saturation pulse).

PAR* (photosynthetic active radiation, µmol photons $m^{-2} \cdot s^{-1}$) and Temp* (Temperature, °C) are two further online signal. They are distinguished by a superscript asterisk from their pendants which are measured together with saturation pulses. In the same way, data of additional sensors are marked (Fig. 75). The additional sensors available measure humidity (AHum) or oxygen concentration (Oxyg.). The sensor channels X1 and X2 are prepared for future use.

Besides F level fluorescence, the SAT-Pulse fluorescence levels are maximum fluorescence F_M' (including F_M) and minimum fluorescence F_0' (F_0). Derived from these fluorescence levels is the photochemical quantum yield of photosystem II, Y(II) (including F_V/F_M). The electron transport rate (ETR, µmol electrons/($m^2 \cdot s$) is calculated with Y(II) and PAR. The section “Quench” of the Val. sidebar includes six more saturation pulse parameters. All fluorescence parameters are explained in Chapter 1 (page 157).

6.1.5 Results

The Results sidebar (Table 13) shows numerical values of the current experiment. The upper part of the sidebar displays data of the Fo, Fm determination. These data are maintained throughout the current experiment. All other data are updated with each saturation pulse analysis. All fluorescence levels and ratios are explained in Chapter 1 (page 157).

Table 13: Sidebar

Parameter	Sample Data	Comment
Fo	441	Fluorescence properties of the dark-acclimated sample.
Fm	1818	
Fv/Fm	0.757	
qP	0.237	Fluorescence quotients describing the state of the light acclimated sample.
qL	0.147	
qN	0.816	
NPQ	2.212	
Y(NPQ)	0.616	
Y(NO)	0.278	
F	506	Fluorescence properties of the light-acclimated sample.
Fm'	566	
PAR	285	Actinic light intensity, µmol m^{-2} s^{-1}.
Y(II)	0.106	Fluorescence property of the light-acclimated sample.
ETR	12.7	Relative electron transport rate, µmol m^{-2} s^{-1}.
Fo'	313	Fluorescence property of the light-acclimated sample.

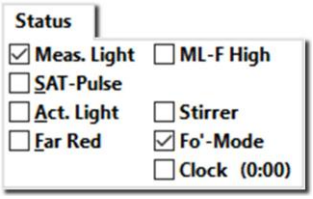
6.1.6 Status Bar

The “Status” field indicates the state of light sources and special functions (Table 14). The checkboxes function both as indicator and as on/off switches.

The “Basic” and the “Program” fields are stacked and can be selected by tabs. Actinic light and the clock frequency is adjusted on the Basic field, all automatic routines of the WinControl-3 software can be started in the Program field (Table 14).

The stacked “SAT-Pulse” and “Chart” field provide buttons to trigger F_V/F_M or Y(II) analyses, and graphic areas displaying fluorescence kinetics induced by saturation pulses. The “Online” field display live data numerically.

Table 14: Status Bar



Meas. Light: Low frequency PAM measuring light.

ML-F high: High frequency measuring light. Measuring light changes automatically to high frequency when actinic light is switched on.

SAT-Pulse: Saturation pulse analysis to determine Y(II); equivalent to SAT button (see below).

Act. Light: Actinic light to drive photosynthesis.

Far-red: Light at wavelengths > 700 nm.

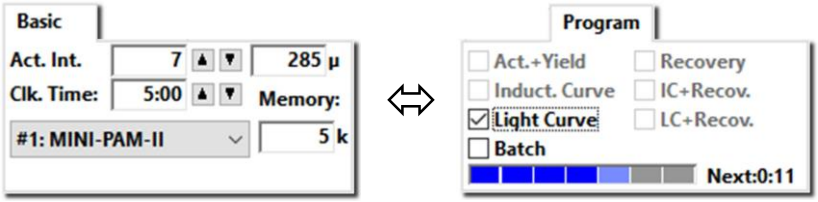
Stirrer: Does not apply for the MINI-PAM-II.

F_0'-Mode: Automatically takes as F_0' fluorescence the minimum fluorescence in a period of far-red-light illumination following a saturation pulse.

Clock: Repetitive trigger of the event specified on

Settings Window (0, page 142). See below on how to adjust the interval between trigger events.

Table 14: Status Bar



Act. Int.: Setting and intensity of MINI-PAM-II light source in µmol m^{-2} s^{-1}.

Clk. Time: Time interval between automatically triggered events in minutes : seconds.

#1: MINI-PAM-II: Device connected (channel).

Memory: Current file size in kilobytes (k).

Experimental Routines

Experimental routines are defined on the

Settings Window (0, page 142). Most routines can be triggered by the clock.

Act.+Yield: Period of actinic illumination terminated by saturation pulse analysis.

Recovery: Dark phase with saturation pulse analyses performed at increasing intervals.

Induct. Curve: F_0, F_M determination followed by illumination by actinic light with repeated saturation pulse analysis.

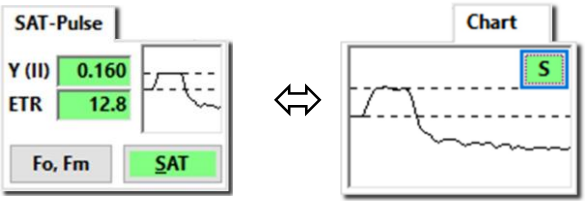
IC+Recov.: Induction curve plus dark phase with saturation pulse analyses performed at increasing intervals.

Light Curve: F_0, F_M determination followed by illumination with stepwise increasing light intensities where each step is terminated by a saturatin pulse analysis.

LC+Recov.: Light Curve followed by a dark phase with saturation pulse analyses.

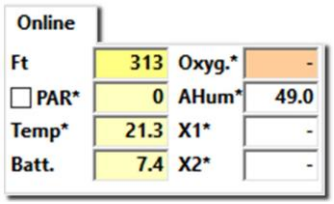
Batch: Execution of batch file program.

Table 14: Status Bar



Fo, Fm: Saturation pulse analysis to determine F_0, F_M, and F_V/F_M.

SAT = S: Saturation pulse analysis to determine F, F'_0, F'_M, and Y(II).



Online are continuously recorded with the frequency defined (see Table 11, page 109).

Ft, PAR* and Temp*: see 6.1.4, page 112. When PAR* is unchecked, the intensity is taken from the internal PAR list (Section 0, page 142); when checked, the actually measured value is displayed. Batt., battery voltage.

6.2 Induct. Curve/Light Curve Windows

The icons and bars of the Chart window introduced above are also present in the Induction Curve and Light Curve windows. In contrast to the Chart window, which displays all data of a Record, the Induction Curve window displays individual induction curves, and the Light Curve window displays individual Light Curves. The same Record can include several Induction and Light Curves. Use up and down arrow keys to scroll through the list of curves.

Table 15 summarizes further differences between the three windows. The windows have different start buttons and only the Light Curve windows possesses PAR as x-axis unit. The button Start IC starts an experiment in which a sample is exposed to a single defined light intensity, the button Start IC initiates a routine in which a sample is exposed to incrementally increasing

light intensities. The x-axis radio button “Time” of the Light Curve window corresponds to the radio button “rel” (relative time) in the two other windows.

The Light Curve window allows fitting model functions to experimental data. The experimental data are the electron transport rates (ETR) plotted against the photon flux density, PAR. WinControl-3 provides two model functions called REG1 and REG2 which are introduced in Fig. 76 and Fig. 77, respectively. The function REG1 can decrease at high PAR values. Therefore, REG1 can consider photoinhibition of photosynthesis, where β is a photoinhibition parameter. In contrast, REG2 is a rectangular hyperbola which cannot describe photoinhibition.

Both models calculate the three cardinal parameters of a light curve:

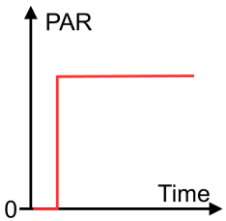
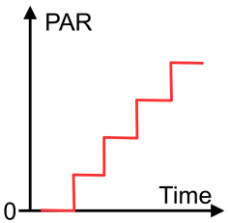
(i) α, electrons/photons: Initial slope of RLC which is related to the quantum efficiency of photosynthesis.

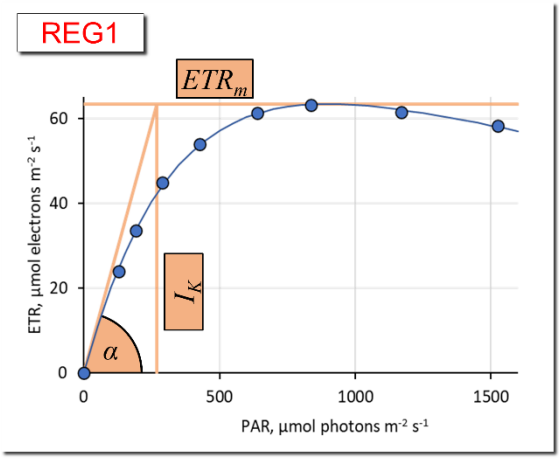
(ii) ETR_m, µmol electrons $m^{-2} \cdot s^{-1}$: Maximum electron transport rate.

(iii) I_K, µmol photons $m^{-2} \cdot s^{-1}$: Idealized PAR value at which light-limited photosynthesis becomes light-limited.

The cardinal parameters are written into the Report. To export cardinal parameters separately, right click on the chart of the Light Curve window and select from the menu “Export Regression Data” (see Table 15). The item “Select current light curve” in the same menu highlights the data of the currently displayed light curve in the Chart and Report windows. A similar function is available for the window Induction Curve.

Table 15: Three Graphics Windows

Chart Window	Induct. Curve Window	Light Curve Window
Rec. Sidebar Val. Rec. Chan. 1 2 3 … Start Online	Rec. Sidebar Val. Rec. Chan. IC 1 IC 2 IC 3 … Start IC	Rec. Sidebar Val. Rec. Chan. LC 1 LC 2 LC 3 … Start LC
Lighting 	Lighting 	Lighting 
Right click on chart Export Record Select current record	Right click on chart Export Record Select current induction curve	Right click on chart Export Record Select current light curve Export Regression Data
Val. Sidebar Quench qP qN qL NPQ Y (NO) Y (NPQ) Sensors online	Val. Sidebar Quench qP qN qL NPQ Y (NO) Y (NPQ) Sensors online	Val. Sidebar Quench qP qN qL NPQ Y (NO) Y (NPQ) Regression REG1 REG2 Sensors online
x-axis units Time: rel abs	x-axis units Time: rel abs	x-axis units Time PAR



$$ETR = ETR_{mPot} \cdot (1 - e^{-\frac{\alpha \cdot PAR}{ETR_{mPot}}}) \cdot e^{-\frac{\beta \cdot PAR}{ETR_{mPot}}}$$

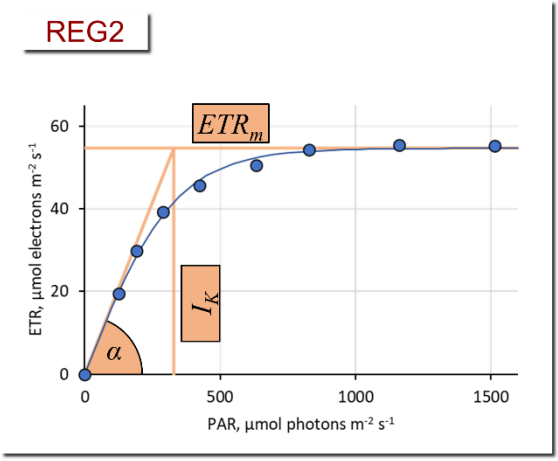
$$ETR_m = ETR_{mPot} \cdot (\frac{\alpha}{\alpha + \beta}) \cdot (\frac{\beta}{\alpha + \beta})^{\frac{\beta}{\alpha}}$$

$$I_K = \frac{ETR_m}{\alpha}$$

$$I_b = ETR_{mPot} / \beta$$

Fig. 76: Model Function REG1

The three cardinal points of the light curve are indicated (α, ETR_m, and I_K). The decrease at PAR>1000 µmol m^{-2} s^{-1} is frequently attributed to photoinhibition of photosystem II by strong light. I_b is the theoretical PAR at which the light curve reaches 1/e of ETR_{mPot}. ETR_{mPot} is the ETR_m in the absence of photoinhibition. According to: Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38: 687-701



$$ETR = ETR_m \cdot tanh(\frac{\alpha \cdot PAR}{ETR_m})$$

$$I_K = \frac{ETR_m}{\alpha}$$

Fig. 77: Model Function REG2

The three cardinal points of the light curve are indicated (α, ETR_m, and I_K). According to Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21: 540-547.

6.3 SAT-Chart Window

The SAT-Chart window displays saturation pulse kinetics of fluorescence (Fig. 78). The automatically determined values of F and F_M' are shown as dashed horizontal lines. Fluorescence traces are automatically scaled to fit into the coordinate system. The x-axis covers about 2 s, the saturation pulse starts at 200 ms, and the distance between data points is 50 ms. The mouse wheel scrolls through graphs.

Each graph is accompanied by a protocol panel. The protocol panel lists the pulse number (Nr) and the line number of the Report (Rep. Nr). Select a fluorescence trace by double-click in the protocol window. The selected graph automatically moves to the top of the window.

The Options menu (upper right corner of the SAT-Chart window) links the actual view of saturation pulse kinetics to the saturation pulse events selected in one of the three previous windows. The option “Follow Selection” automatically displays on top of the window the saturation pulse kinetics of marked events. When Follow Selection” is inactive, the option “Jump to Selection” brings the selected kinetics into view.

The Val. sidebar is similarly configured as described in Section 6.1.4 (page 112), except “Online Data” are omitted and the electron transport factor (ETR-F.) is added. The ETR-F. is the fraction of absorbed photons by the sample relative the PAR to which the sample is exposed. The ETR-F. is a factor of the equation for electron transport (Chapter 1, page 157). Each item selected on the Val. sidebar is numerically displayed below the corresponding saturation pulse kinetics.

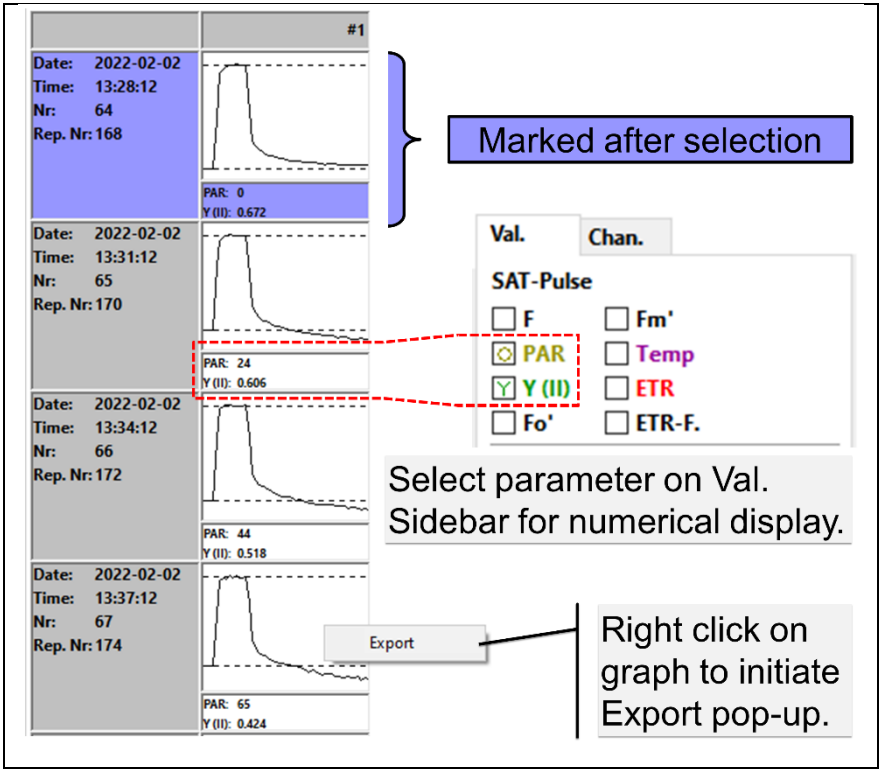


Fig. 78: SAT-Chart Window

Table 16: SAT Kinetics in Raw File

To extract a series of saturation pulse kinetics, open PAM file with spread sheet program, sort by SG and copy data right of SG.

	A	B	C	D	…	AD	AE	AF	AG	AH
1	Date	Datetime	Type	No.						
2	08/03/2022	08/03/2022 09:17:42.997	F	2	…	**SG**	50	419	419	…
3	08/03/2022	08/03/2022 09:17:44.389	F	3	…	**SG**	50	383	383	…
						SAT Graph Label	Time interval in ms	Data of Saturation pulse kinetics		

Single saturation pulse kinetics can be exported by right-click in its chart field. To export a series of saturation pulse kinetics, open the PAM file with a spread sheet program. In the text file, saturation pulse kinetics are preceded by an SG (Table 16). Sort the lines of the file by SG. Create a time scale by considering the neighboring points are spaced apart by 50 ms.

6.4 Spectrum Window

The window is active in the presence of spectral data. With the cursor placed inside the drawing area, or below the x-axis, the mouse wheel has a zoom function for the x-axis. When the cursor is placed left of the y-axis, the mouse wheel has a zoom function for the y-axis.

Right click on the drawing area to open a menu containing zoom and export commands (Fig. 79). The “Zoom to selection” is available after selection of an x-axis interval described before (Fig. 74).

An application menu is available in the top right corner of the window (Fig. 79). To change between the three principal applications (PAR, fluorescence, reflectance), the spectrometer MINI-SPEC/MP has to be reconfigured (see Fig. 10, page 23).

The dark current of the spectrometer at room temperature is measured in the factory and stored on the flash memory of the device. To newly establish the dark current, fully darken the entrance optics (cv. Fig. 10, page 23) and press Calibr. Dark.

The standard PAR configuration is “PAR cosine”. This configuration employs a diffusing disk as light entrance (Fig. 10). The configuration shows an approximate cosine response toward incoming radiation. The spectrometer calibration differs between measurements in air and in water. Therefore, choose PAR cosine air or

PAR cosine water for proper measurements under the two conditions.

“PAR sphere” employs a spherical light entrance. The sphere configuration measures light from all directions with similar weight. At the time of writing of this manuscript, the spherical sensor is under development.

The setting “PAR Open sensor” is used by service engineers.

“Fluorescence (blue)” and “Fluorescence (red)” measures fluorescence emission spectra excited by blue or red light, respectively. Measuring “Reflectance” requires that the 100% reflection signal has been established with the highly reflective reference material provided with the spectrometer (Fig. 11, page 25).

The sidebar also display the Integration time (ms) which is the integrated measuring time for a single spectrum. ☑ Auto Range optimizes the integration time for a spectrum depending on incoming light. Auto Range ON is the default setting. Auto Range OFF is for special applications. When spectra look unrealistic, switch on Auto Range. Average determines the number of spectra averaged to yield the final spectrum. ☑ SAT+Spec measures a spectrum for saturation pulse analysis.

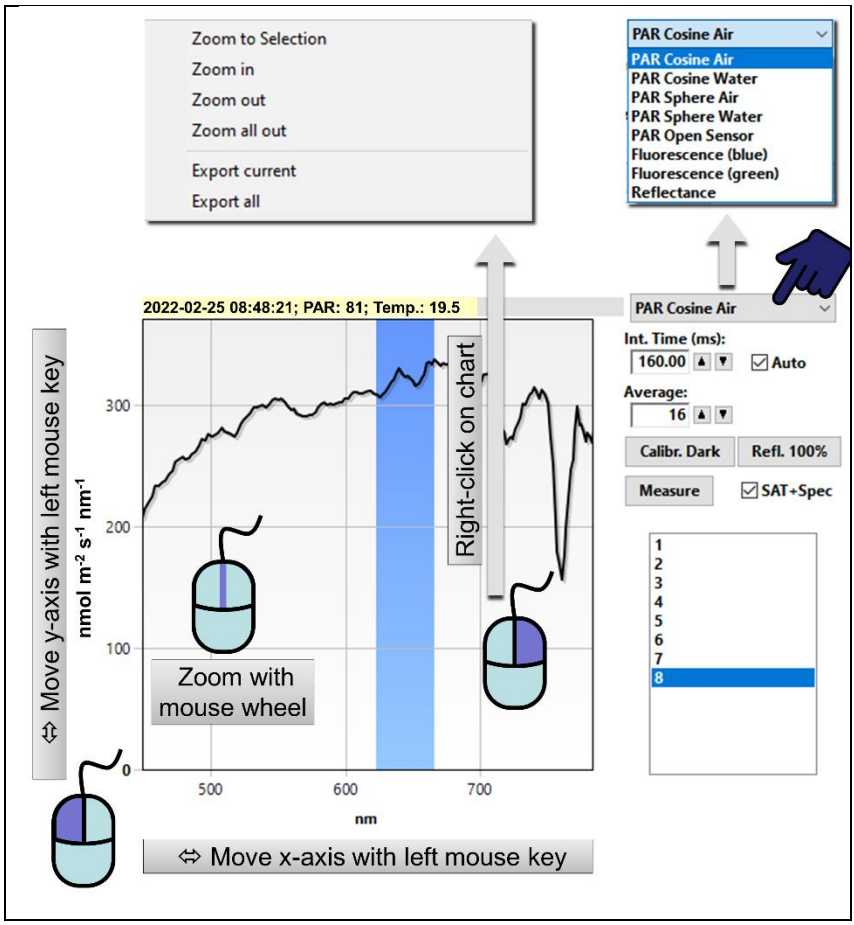


Fig. 79: Spectrum Window

6.5 Report Window

The Report window lists all data associated with saturation pulse analyses. Continuously recorded signals, saturation pulse kinetics and spectra are not listed. The fact that a spectrum was recorded is indicated by “SPEC” in the column “Type” (Table 17). The Report window also displays start and end of induction and light curves, as well as the cardinal points of light curves (Table 17).

The types of data displayed is controlled by the Val. side bar, which is configured as described for the SAT-CHART window (Section 6.3, page 123). Only data displayed on the Report table are exported.

Table 17: Report Table

Type: D, device. FO, F_0 and F_M determination. F, determination of F, F_0', and F_M'. SPEC, spectrum. SICWS and SICSE, induction curve start and end, respectively. SLCS and SLCE, light curve start and end, respectively. REG1 and REGS, cardinal parameters of regression analysis 1 and 2, respectively (see 6.2). **Mark**: Letters assigned to data (see 4.2.1, page 44). Hide Mark column via option menu. Here, the MINI-PAM-II has the channel number #2. “#2” is preceding the cardinal values of light curve analysis, REG1 and REG2.

	Date	Time	Type	No.	2:Mark	2:F	2:Fm'	2:PAR	2:Y (II)	2:ETR
1			D			Device Nr: #2, MINI-PAM-II (PYAD0191A)				
2	2022-03-08	09:07:11	SCHS							
3	2022-03-08	09:16:20	FO	1	A	461	1932	6	0.761	1.9
4	2022-03-08	09:16:50	F	2	A	680	774	6	0.121	0.3
5	2022-03-08	09:17:24	SPEC		PAR: 12; Temp.: 21.2					
6	2022-03-08	09:17:42	SICS		Induction Curve start					
7	2022-03-08	09:17:48	SICE		Induction Curve end					
8	2022-03-08	09:17:59	SLCS		Light Curve start					
9	2022-03-08	09:18:07	REG1		#2: alpha: 0.362, ETRm: 13.99, Ik: 38.634 (beta: 0.001, ETRmPot: 14.179)					
10	2022-03-08	09:18:07	REG2		#2: alpha: 0.272, ETRm: 13.665, Ik: 50.280					
11	2022-03-08	09:18:05	SLCE		Light Curve end					

The options menu of the Report window can be opened by clicking the Options button or by right-click in the Report field. The options menu contains various tools for handling the Report table. All menu items and their function are summarized in Table 18.

Command “Insert Settings” of the options menu writes the current device settings in the report. The abbreviations used for the various settings are compiled in Table 19.

Table 18: Options Menu **Options Menu Item**	**Comment**
	General Report Management
Follow Selection	Automatically displays the data associated with events marked in other windows
Show Mark	Display marker letters (see “Mark” in Table 17)
Insert Settings	Writes settings of MINI-PAM-II in Report table (see Table 19)
	Manipulation of All Data
Export All	For details see Section 6.1.1, page 107.
Delete All Measure Data	Self-explaining
Page Setup for Printing	Basic configuration of print layout. Checks if output fits on page
Preview Printing	Self-explaining
Print Report	Selects and configures printer, prints current Report
	Manipulation of Selected Data
	Requires that saturation pulse events on a chart or lines of the Report have been selected. To select, move the cursor with left mouse key pressed over events
Export Selected Lines	Confines export to lines of interest

Table 18: Options Menu Options Menu Item	Comment
Jump to Selection	Brings data into view, when “Follow Selection” is off
Delete Selected Data	As above. Affects only selected lines.
Preview Print Selection	As above. Affects only selected lines.
Print Selected Data	As above. Affects only selected lines.
Mark as Light Curve	Combines a series of saturation pulse analysis into a light curve and performs regression analysis REG1 and REG2. The series must not contain start or end marks of previous light or induction curves.

Table 19: Abbreviations for Settings

MEA	Measuring Light Intensity
MI	Measuring Light Frequency
DI	Damping
GA	Gain
EF	ETR-Factor
FZ	F Offset
CW	Clock Interval
SI	Saturation Pulse Intensity
SW	Saturation Pulse Width
AI	Actinic Light Intensity
AF	Actinic light factor
FRI	Far-red Intensity
FRW	Far-red Width
AW	Length of exposure of “Act. + Yield” routine
ICD	Delay of Induction Curve
ICW	Interval Between Saturation Pulses of Induction Curve
ICL	Length of Induction Curve
LCW	Step Length of Light Curve
LCI	Initial Intensity Setting of Light Curve
LCL	Length of Light Curve
LO	Offset of External PAR Sensor
LG	Calibration Factor of External PAR Sensor
LO2	Offset of External PAR Sensor 2

Table 19: Abbreviations for Settings

LG2	Calibration Factor of External PAR Sensor 2
TO	Offset of External Temperature Sensor
TG	Gain of External Temperature Sensor
ILO	Offset of Internal PAR Sensor
ILG	Gain of Internal PAR Sensor
PARGAIN_RED	Calibration Factor for Red LED of 2054-L
PARGAIN_GREEN	Calibration Factor for Green LED of 2054-L
PARGAIN_BLUE	Calibration Factor for Blue LED of 2054-L
PARGAIN_WHITE	Calibration Factor for White LED of 2054-L
PARGAIN_CUSTOM	Calibration Factor for Other light Sources
ITO	Offset of Internal Temperature Sensor
ITG	Gain of Internal Temperature Sensor
MLC	Fm Correction Factor 1
MLC2	Fm Correction Factor 2
TRM	Trim Value for Measuring Light
TRA	Trim Value for Actinic Light
TRSA	Tim Value for Saturation Pulse
TRFR	Trim Values for Far-red Light

6.6 Memory Window

The Memory window accesses the data of the internal memory of the MINI-PAM-II. The MINI-PAM-II memory is a circular buffer which can store 512 files or 30 000 data entries maximally. The memory is full when one of the two limits is reached. For instance, if each file contains only two data entries, the full memory contains 512 files but only 1024 data entries, or, if each file contains 1000 data entries, then only 30 files fit into the memory.

When the memory is full, the oldest data are overwritten with the latest ones. Usually, data acquired in the offline mode are downloaded (6.6.2) and the memory is cleared (0) which avoids that all memory is used.

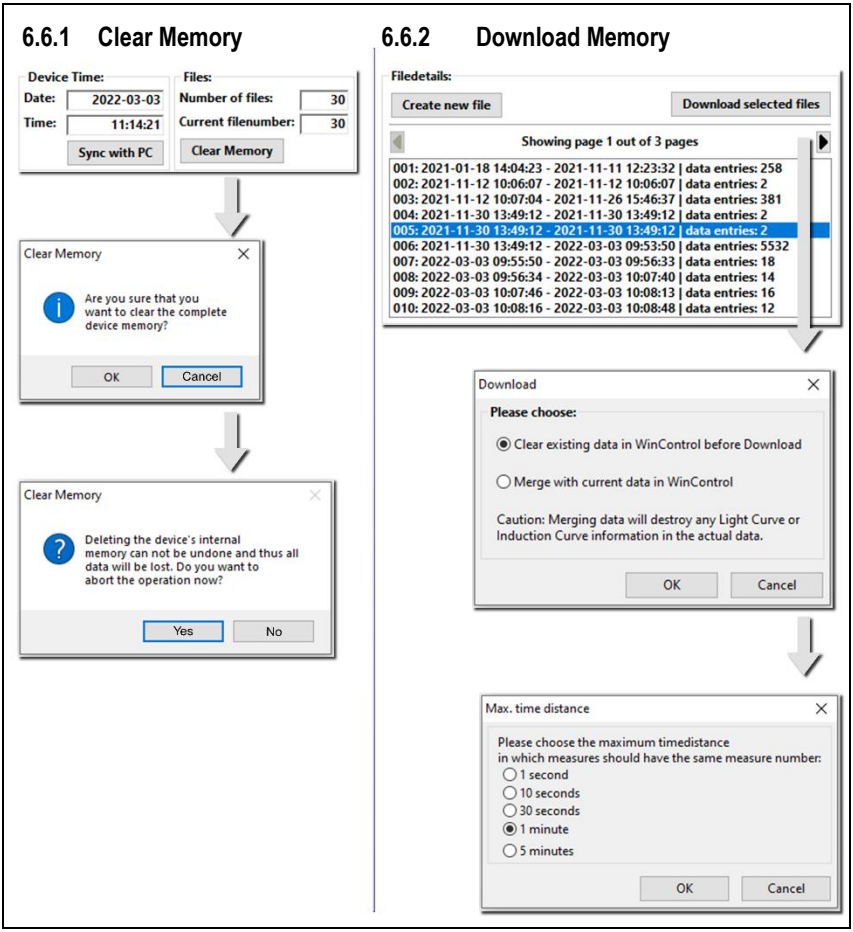


Fig. 80: Memory Window

6.6.1 Clear Memory

Clearing the memory is irreversible. To avoid inadvertent deletion of data, two warnings must be passed before the command Clear Memory is executed.

Above the Clear Memory button, memory information is given: “Number of Files” is the file count, “Current file number” is the serial number of the file to which data are currently written. The Current file number can be greater than the number of files when the memory is full and old files are overwritten by new ones.

The Clear Memory field display date and time of the internal computer of the MINI-PAM-II. Click Sync with PC to apply the computer time settings to the MINI-PAM-II.

6.6.2 Download Memory

A list of files is displayed in the Download field of the Memory window (Fig. 80). You can download individual or groups of files. To pick several files, hold down Ctrl key and click on files of interest. To pick consecutive files, hold down Shift key and click on first and last files of the row. Then click Download selected files.

Usually, data are written in an empty Report window. Selecting “Clear existing data …” in the download dialogue box, empties the Report window.

When parallel measurements of two different fluorometers should be combined, choose “Merge with current data …”. The newly imported data will be sorted so that time points of import data matches time points of existing data. Two time points are consid-

ered as matching when their time difference is smaller as the interval selected from the box “Max. time distance” (cf. Fig. 80). The merge operation deletes the report entries for begin and of an induction or a light curve.

6.7 Batch Window

Batch files automatically execute experimental procedures. To activate the Batch window, click icon new batch or open existing batch file (Fig. 81, “Batch Start Buttons”). The click Edit and choose between “Add command” and “Record Macro”. The command “Update indentation” is an automatic editing tool to improve readability.

6.7.1 Add Command

Add command leads to five groups of commands (Fig. 81). A command can be inserted into a batch file by left click.

Measure commands

Saturation pulse	Saturation pulse analysis of light-exposed sample
F_0, F_M determination	Saturation pulse analysis of dark acclimated sample

Light Sources commands

Controls for actinic and measuring light. The light ON command asks for the light intensity: simple enter the light intensity setting in the grey-shaded field. New intensity settings can be chosen using the commend “Change intensity”.

Settings commands

“Reset settings to default” installs the settings described in Table 6 (page 91).

Repetition commands

The group contains two commands. “Repeat specific number of times” and “Repeat indefinitely”. Both commands write two lines on the batch file sheet. In case of the first command, these lines are:

Line 1:

```
for $loopvar = 1 to count // Start repetition block with specified number of repetitions
```

Line 2:

```
next                      // End of repetition block
```

Write commands to be repeated between Line 1 and 2. Define how often the commands should be repeated by the number entered in field count.

The second command (Repeat indefinitely) repeats the commands placed between the two lines until the batch program is stopped manually.

Line 1:

```
while 1   // Start block repeating indefinitely
```

Line 2:

```
wend      // End of repetition block
```

Timing

The command “Delay” inserts a time interval after the previous command has been terminated. The next command is executed when the time interval ends.

The command “Continue Delay” takes the end of the previous delay phase as starting point. Actions within this time interval are

performed without affecting the interval defined for Continue Delay. A continuous time scale can be built by a series of Continue Delay commands.

“Wait until time of day” delays start of the batch program until the time specified.

6.7.2 Record Macro

The function “Record Macro” converts your manually entered commands into batch file lines. Simply click “Record Macro”, perform experiment, and click “Record Macro” again.

6.7.3 Options

All items of the options menu of the Batch window are related to the MONI-DA data acquisition system.

Check MONI-DA Compatibility

Some batch file commands cannot be executed by the MONI-DA. This command searches for such incompatibilities.

Upload to Device

Transfers a batch file to the MONI-DA memory.

Export

Export the current batch file as “WinControl-3 Compiled Batch File” (*.wccb). This file format is for future use. Upcoming MONI-DA versions will be enabled to directly download wccb files, that is, without being connected to WinControl-3. The final goal is to install batch files by remote control.

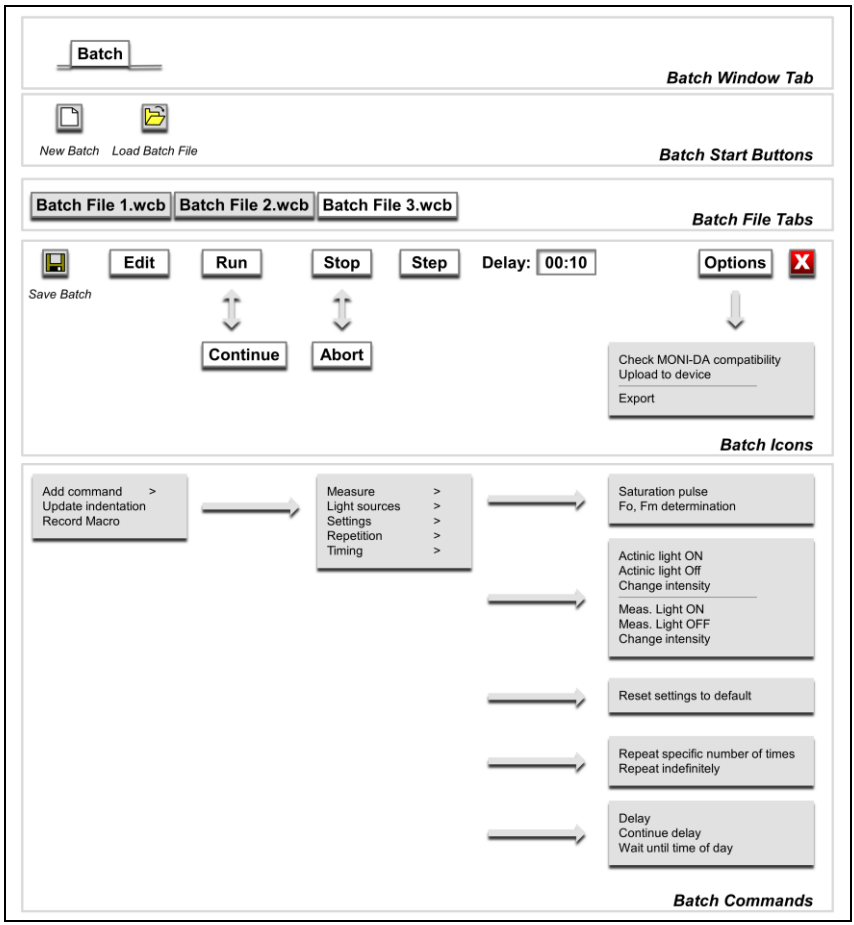


Fig. 81: Batch Window Overview

6.8 Control Window

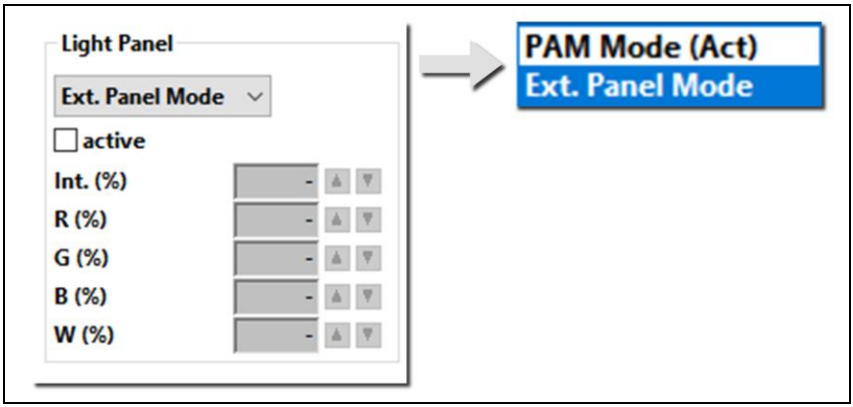


Fig. 82: Control Window

Fig. 82 shows the section of the Control Window which is important for the MINI-PAM-II fluorometer. The drop-down menu on top of the Light Panel area consists of two points: “PAM Mode (Act.)” and “Ext. Panel Mode”. Ext. Panel Mode is the standard mode in which the fluorometer’s internal light source is used as actinic light to which an external light source may be added (see Table 20). Activating PAM Mode (Act.) selects the external light source as the sole actinic light. The meaning of numerical inputs (Int. (%) to W (%) varies depending on the mode chosen (Table 20).

Typically, the maximum intensity of all four LED groups is 5000 µmol m^{-2} s^{-1} or higher. Operation of the 2054-L External LED Source requires line power.

Table 20: External Light Source

	Ext. Panel Mode	PAM Mode (Act.)
Internal light source of MINI-PAM-II		
Function	Main actinic source	Not available
Intensity setting	Actinic light intensity (Settings window)	Not available
External LED Light Source 2054-L		
Requirement	Not mandatory	Required
Function	Background illumination	Main actinic source
Intensity setting	Int %	Actinic light intensity (Settings window)
Control		
Int. (%)	Relative intensity (0-100%). The number multiplies the intensity of all light sources of the External LED Light Source 2054-L	Not active
R (%), G (%), B(%), W (%)	Absolute intensity in percent (is multiplied by Int. (%))	Factor defining the intensity with which a light source contributes to total intensity.

6.9 Sensors Window

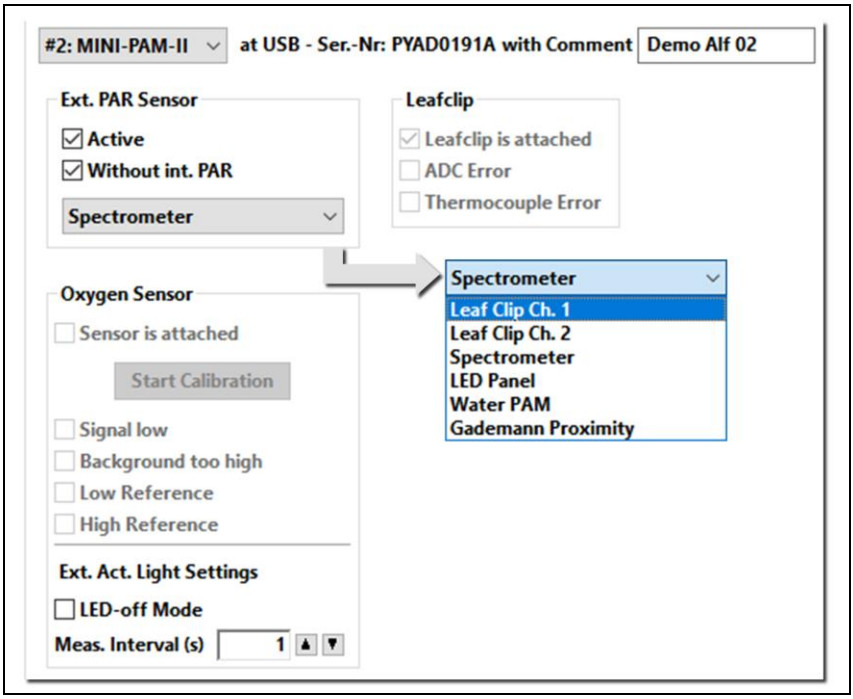


Fig. 83: Sensors Window

External PAR Sensor

The internal PAR sensor measures the light intensity of internal light at sample level. Internal actinic light is used for short-term illumination (Act. + Yield), or when induction and light curve experiments are carried out. External PAR sensors are designed to measure the intensity of external light at sample level. External PAR sensors are employed to estimate electron transport rates driven by external artificial or natural light.

The checkbox “Active” selects sensing of external PAR. Checking “Without int. PAR” ignores measurements by the internal PAR sensor. The drop-down menu below these checkboxes lists external PAR sensors.

Drop-Down Menu

“Leaf Clip Channel 1” corresponds to the micro quantum sensor of the 2035-B clip (Fig. 13, page 26). “Leaf Clip Channel 2” is the signal of a PAR sensor connected to the side port of the 2035-B clip. “Spectrometer” uses PAR spectra integrated over the visible range as PAR value. “LED panel” is the PAR sensor connected to the LED-Panel RGBW-L084. (Does not apply for External LED Light Source 2054-L). “WATER-PAM” is available when working with this PAM fluorometer. “Gademann Proximity” is a sensor under development.

Leaf Clip

The checkbox of “Leafclip is attached” automatically indicates that a 2035-B clip is connected to the MINI-PAM-II. Erroneous conversion of the analogue measuring signal to digital information is signaled by “ADC Error”. A defective thermocouple activates the checkbox of “Thermocouple error”.

Oxygen sensor

This section, including “External Actinic Light Settings” is dedicated to the accessory Fiber-Optic Oxygen Meter FireStingO2 (see separate manual).

6.10 Settings Window

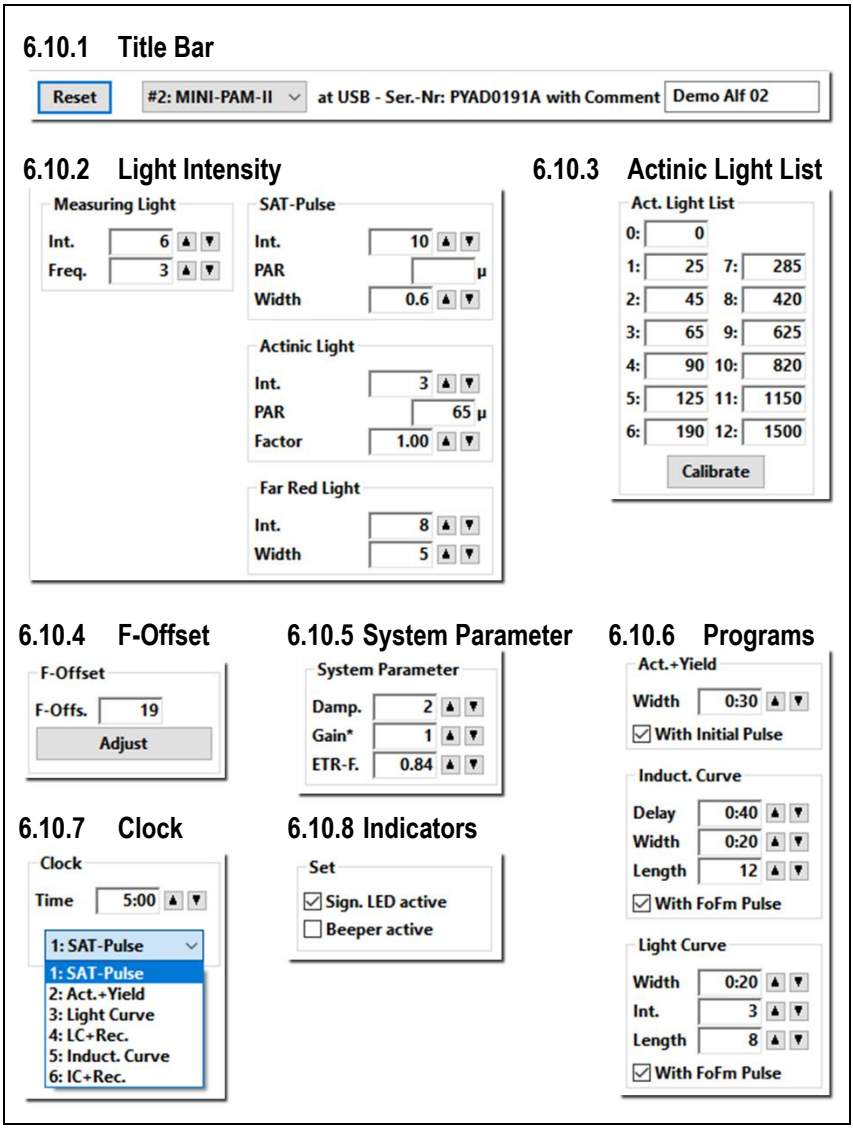


Fig. 84: Settings Window

6.10.1 Title Bar

The Reset button restores factory values for all settings of the current window. Located right of the Reset button is a drop-down menu listing all devices connected. The settings displayed on the current window belong to the device selected, and also the serial number which is displayed right of the drop-down menu. In the text box right of the serial number, up to 20 characters can be entered. Serial number and text box content are written in the first line of each record file.

6.10.2 Light Intensity

When actinic light is switched off, µs measuring pulses are delivered at 5 to 25 Hz depending on frequency setting “Freq.“ (Table 5, page 64). Switching on actinic light automatically increases the measuring light frequency to 100 Hz. The measuring light frequency can also be manually set to 100 Hz by checking “MF-F High” on the Status Bar (Table 14, page 116).

The intensity setting “Int.” adjusts measuring light intensity. Twelve intensity levels are available. The numerical value of the setting is proportional to the measuring light intensity. How to estimate the integrated measuring light intensity for the frequency and intensity settings used is described in Section 4.3.1.2 (page 64).

Note that an increase in flash intensity increases the F_0 fluorescence level (the fluorescence intensity, in general is, proportional to the flash intensity). An increase in the number of flashes/pulse increases the F_0 level only if some PS II reaction centers become closed by the increased integrated measuring light intensity (the measuring light becomes actinic). If this is the case, the integrated

measuring light intensity must be reduced either by reducing intensity or by reducing frequency.

Like measuring light, the intensity of saturation pulses, actinic light and far-red light can be adjusted in 12 levels. The PAR of saturation pulses is indicated in newer versions of WinControl-3. The PAR of actinic light is taken from the light list (see below). Most of far-red emission is not absorbed by chlorophylls. Therefore, intensity data for far-red radiation (PAR or photon flux density) is not given as it would strongly overestimate its actual actinic effect.

6.10.3 Actinic Light List

Twelve actinic light levels are listed. These values are target PAR values. As delivered from the factory, the light emission of the LED is adjusted so that these target values are present at the sample level of a 2035-B Leaf Clip Holder. The target values can be varied by changing the “Factor” in the field “Actinic Light” (Section 6.10.2).

6.10.4 F-Offset

See Section 4.3.1.7 (page 67).

6.10.5 System Parameter

Damping (Damp.) Function for data smoothing. The smoothing effect increases with increasing numerical value, at the same time, but instrument response decreases. See 4.3.1.4 (page 66).

Gain Factor (Gain). See 4.3.1.3 (page 66). The Gain factor amplifies signal and noise. At low signal levels, increasing the gain improves digital resolution.

ETR-Factor (ETR-F.). The ETR-Factor is the percentage of light absorbed by the sample and is a parameter for calculating the electron transport rate (Section 8.3, page 164).

6.10.6 Programs

Act. + Yield: Light exposure followed by saturation pulse analysis

"Width" is the duration of light exposure. The actinic light intensity adjusted in the field "Actinic Light" applies (Section 6.10.2, page 143). A saturation pulse analysis is always carried out at end of illumination. Checking "With Initial Pulse" places an additional saturation pulse analyses before illumination. The fluorescence levels of the initial saturation pulse analysis are defined as F_0 and F_M. All fluorescence ratio parameters requiring these two fluorescence levels (Table 26, page 166) are only available with initial saturation pulse.

Induct. Curve: Fluorescence curve following the onset of light

Three parameters determine the sequence of events. "Delay" indicates the dark time interval between F_0 and F_M determination and onset of light. The delay time can be adjusted between 5 seconds and 10 minutes. Default value is 40 seconds. "Width" is the time interval between neighboring saturation pulses in the light period. Length is the number of saturation pulse analyses carried out. Time interval of actinic illumination is approximately "Width" times "Length". A dark period can be appended to an induction experiment (Table 14, page 116 and Table 4, page 58).

Light Curve: Saturation pulse analyses after exposure to different light intensities

The “Light Curve” program exposes a sample to consecutively increasing actinic light intensities. Three input values define the procedure. “Width” is the duration of exposure to a particular light intensity. “Int.” is the first light level of the light curve. “Length” is the number of light steps. The duration of a light curve is determined by “Width” times “Length”. A dark period can be appended to illumination (see above).

☑ **With FoFm pulse**: Both Induction and Light Curves can be performed without initial saturation pulse (F_0 and F_M measurement).

6.10.7 Clock

The clock utility repetitively triggers one out of 6 different measuring routines. functions: Saturation pulse analysis, Act.+Yield, Induct. Curve, IC+Recov., Light Curve and LC+Recov (sse Above). The interval time between two events (Clk. Time) can be adjusted between 10 s and 50 min. The time interval must be longer than the time required by the triggered event.

6.10.8 Indicators

The signal LED located above the touchscreen indicates the operational state of the MINI-PAM-II as listed in Table 21. The beeper acoustically marks begin and end of a saturation pulse analysis.

Table 21: LED Signal Code

LED Color Measuring	Frequency mode	Process
Green	1 Hz	Normal operation
Green double flash	1 Hz	Normal operation and clock running
Green	continuous	Normal operation and saturation flash
Additional codes during firmware update		
Green/red alternating	high	Waiting for software
Red	continuous	Update running

System information is displayed on the bottom of the Settings and the System Settings windows (see Table 22 below)

Table 22: System Information

System information displayed on bottom of Systems and Systems Settings window.

Headline	Model	Model Number	Serial Number
Display	MINI-PAM-II (blue) or MINI-PAM-II (red)	MINI-PAM-II/B or MINI-PAM-II/R	PYAC####(A) or PYAD####(A)
Information	Color version	Order code	S/N convention

6.11 System Settings Window

Different from all other windows of WinControl-3, the window System Settings is not represented in the row of tabs. To access system settings, open Settings window and click “System Settings” (Fig. 87). The functional elements of System Settings are shown in Fig. 86 together with their section numbers.

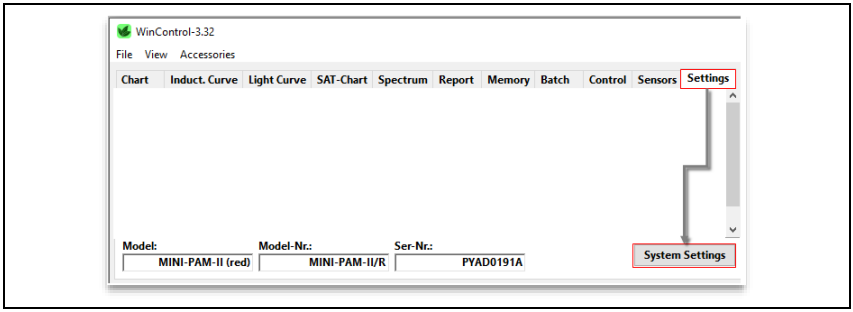


Fig. 85: System Settings Button

Click to open System Settings window.

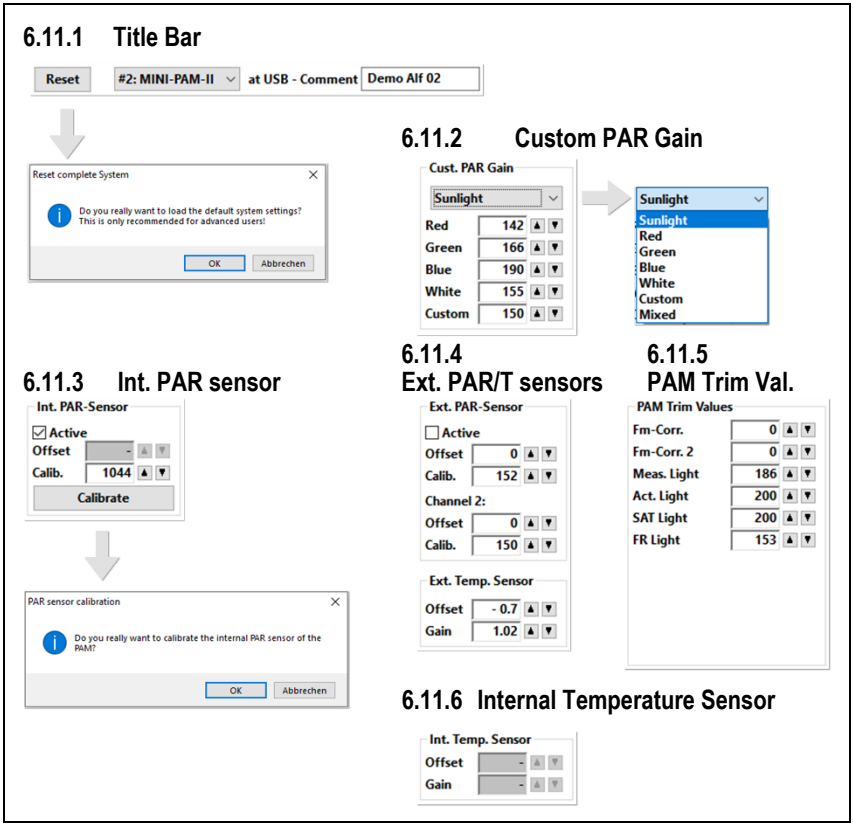


Fig. 86: System Settings

6.11.1 Title Bar

The Reset button of the System Settings page recalls all calibration data established at the factory. This information is taken from the flash memories of the MINI-PAM-II and of the external sensor (e.g., the 2035-B Leaf-Clip Holder). The original calibration data cannot be changed by the user. The command Reset on the System Settings window is equivalent to the command “Load System Settings” of the MINI-PAM-II menu (Section 4.3.4.5, page 88). The command Reset on the Settings windows (Section 6.10.1, page 143) is equivalent to the command “Load default” of the MINI-PAM-II menu (Section 4.3.5, page 89).

The other elements of the Title Bar are described in Section 6.10.1, page 143.

Table 23: Custom PAR Gain

The table compiles the types of gain factors with their application, which are available for the PAR sensor of the 2035-B leaf clip or the PAR sensor of the 2065-M device.

Gain factor	Application
Sunlight	Outdoor natural light. Factor displayed in “Ext. PAR Sensor/Calib.”.
Red, green, blue, white	Red, green, blue, white LED light from MINI-PAM-II internal light source or from 2054-L external light source.
Mixed	2054-L external light source. The active factor is dynamically adjusted depending on the color mixture selected.
Custom	User-defined factor.

6.11.2 Custom PAR Gain

Different calibration factors for different light sources are provided. The calibration factor varies because of slight spectral variations in sensitivity of the PAR sensor. Table 23 summarizes the factors available.

6.11.3 Int. PAR sensor

The internal PAR sensor received a small fraction of the actinic light emitted by the internal LED of the MINI-PAM-II. The calibration factor of the internal PAR sensor is displayed in the “Calib.” numerical field (Fig. 86).

The calibration of the internal PAR sensor is done by comparison with a calibrated external PAR sensor. The reading of the external PAR sensor depends on its position relative to the end of the optical fiber of the MINI-PAM-II. Factory calibration was established with a 2035-B leaf clip in which the fiber was fully inserted. For other configurations, the internal sensor must be newly calibrated. (See also Section 4.3.4.1, page 78.)

PAR sensor compatible with MINI-PAM-II: To calibrate the internal PAR sensor using the PAR sensor of the 2035-B clip, connect clip to MINI-PAM-II, position light sensor relative to fiber identical to the experimental situation (with the light sensor in the sample plane) and click Calibrate.

Other PAR sensor: To calibrate the internal PAR sensor using an external PAR sensor which cannot be read by the MINI-PAM-II, proceed as described above but adjust calibration factor manually until the readout of the internal sensor matches that of the calibrated external sensor.

6.11.4 Ext. PAR/T sensors

The boxes “Ext. PAR Sensor” and “Ext. Temp. Sensor” (Fig. 86) display the factory-established calibration factors of the 2030-B clip or the 2065-M Mini Quantum/Temp.-Sensor, depending on which device is connected. In box “Ext. PAR Sensor”, the “Channel 2” calibration applies to a second PAR sensor connected to the 2035-B leaf clip. Calibration factors must be manually entered when a PAR sensor does not support automatic installation of its calibration data.

6.11.5 PAM Trim Val.

Trim values have been set at the factory so that the MINI-PAM-II meets its specifications.

Table 24: PAM trim values

The table compiles the types of gain factors with their application, which are available for the PAR sensor of the 2035-B leaf clip or the PAR sensor of the 2065-M device.

Type	Application
Fm-Corr.	Factor to compensate a signal decrease during a saturation pulse. The factor is determined with a Walz fluorescence standard foil. Signal compensation is obsolete (value=0) for the latest generation of LEDs.
Fm-Corr.2	Second factor to compensate a signal decrease during a saturation pulse. Two factors are required when a biphasic signal decrease occurs. Signal compensation is obsolete (value=0) for the latest generation of LEDs.
Meas. Light Act. Light SAT Light FR Light	Factors adjusting the intensities of measuring light/actinic light/saturation pulse light/far-red light to meet the respective specification.

6.11.6 Internal Temperature Sensor

Does not apply to the MINI-PAM-II device.

7 Hints & Troubleshooting

7.1 Instrument Settings

Instrument settings were adjusted at the factory for optimum performance of the MINI-PAM-II. For example, LED currents have been adjusted to meet target PAR values for the measuring setup of the 60° distance clip or the 2035-B leaf clip (Fig. 6 and Fig. 13). For a different geometry, the internal PAR sensor requires recalibration to correctly measure internal actinic light (see Fig. 51, page 79). Estimation of PAR according to Fig. 7 (page 20) is rather inaccurate.

Also, the fluorescence offset (F-Offset) of your system has been measured and was saved on the MINI-PAM-II memory. That means that the MINI-PAM-II should show a fluorescence value close to zero with the fiber fully inserted and in the absence of a sample. If this signal deviates clearly from zero, newly adjust F-Offset (see Fig. 41, page 64).

7.2 Default settings

For fluorescence measurements with most healthy green leaves, default settings for measuring light and saturation pulses are well suited. Some samples, however, require special settings. The following sections will provide some hints to adjust settings properly.

7.3 F_0 Fluorescence

Usually, measuring light intensity is adjusted to reach F_0 fluorescence levels around 500 mV (for a definition of F_0 see Chapter 1, page 157). Theoretically, the F_0 should stay below 640 mV. The latter upper value is derived from the assumption that the maximum F_V/F_M of any sample is 0.84 und from the fact that signal saturation occurs at 4000 mV (see equation below, Table 25). If required, measuring light can be adjusted (Fig. 41, page 64).

Table 25: Maximum F_0 of a Dark-acclimated Leaf

$\left(\frac{F_V}{F_M}\right)_{Max} = \frac{(F_M)_{Max} - (F_0)_{Max}}{(F_M)_{Max}}$ $with\ (F_V/F_M)_{Max} = 0.84$ $(F_0)_{Max} = 640$	$(F_M)_{Max}$, maximum possible F_M value = 4000. $(F_0)_{MAX}$, unknown maximum F_0 value (the F_M associated with this F_0, or with smaller F_0, is not saturating). $(F_V/F_M)_{Max}$, assumed maximum possible PS II photochemical yield.

At low signal levels, signal height can be increased by increasing measuring light intensity. At too high intensities, the measuring light might drive photosynthesis to some degree. Therefore, test if switching on measuring light results in a stable signal or if any signal increase occurs. In the latter case, the effective measuring light intensity must be decreased either by reducing the amplitude of measuring light (measuring light intensity) or by reducing measuring light frequency or both (Section 4.3.1, page 62, and Section 6.10.2, page 143).

7.4 F_M Fluorescence

The F_M and F_M' levels are determined as the maximum of the fluorescence signal induced by a saturation pulse. Factory settings of saturation pulse width and intensity are adjusted to arrive at a plateau with normal green leaves (Fig. 87A). Some samples (for instance, high light grown leaves) do not reach a plateau with standards settings (Fig. 87B). In this case, saturation pulse intensity or/and length should be increased. Also, fluorescence kinetics can reach its maximum clearly before end of the saturation pulse (Fig. 87C). The latter does not result in erroneous F_M or F_M' values because these values correspond to the maximum of fluorescence kinetics. In case of Fig. 87C, saturation pulse intensity or/and length might be decreased.

Some samples, particularly low light grown or senescing plants, exhibit with standard settings somewhat decreased F_V/F_M values but show normal fluorescence kinetics. These samples increase the F_V/F_M with decreasing saturation pulse intensity. Therefore, testing the F_V/F_M at saturation pulse intensities also below and above standard settings is important to optimize your saturation pulse settings.

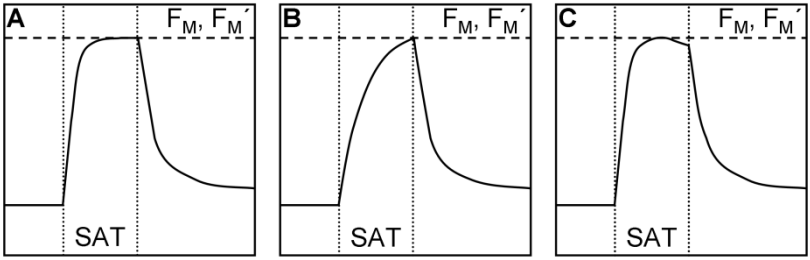


Fig. 87: Fluorescence Kinetics Induced by Saturation Pulse.

7.5 Signal Noise

Check if fluctuating light sources (fluorescent tubes, computer screens) affect the signal.

Exclude that automatic scaling of Y axis at low Y(II) has amplified signal noise.

7.6 System Hangs

Disconnect line power and remove batteries. Power on again.

7.7 External PAR Sensor is not Responding

Check the following:

External sensor activated (Fig. 49, page 76. Section 6.11.4, page 151)?

Correct external PAR sensor selected (Fig. 53, page 81. Section 0, page 140)?

Sunlight profile selected when measuring external light (Fig. 55, page 83. Section 6.9, page 140)?

When the miniature spectrometer is used, make sure that the spectrometer in the light measuring mode (not fluorescence or reflectance).

7.8 Intensity of external lamp cannot be adjusted

Repeat lamp calibration.

8 Saturation Pulse Analysis

8.1 Pulse-amplitude Modulated (PAM) Fluorescence

The PAM principle is illustrated by Fig. 88. The top part shows the total fluorescence of a sample. µs-measuring flashes are given throughout the experiment starting with “Pulse on”. These flashes cause the spikes in the fluorescence trace. From left to right, an external effect induces a “False Signal” of continuous fluorescence in the darkened sample. Then the sample is exposed to a period of actinic illumination (“Actinic on” and “Actinic off”), and, finally, the sample is kept in the dark again.

During actinic illumination, an effect of stray light on the fluorescence signal is additionally assumed. The fluorescence level at onset of stray light plus actinic light is denoted “Actinic F_0“. The further increase of continuous fluorescence during illumination is denoted “Actinic F_V”, where the V stands for variable fluorescence. The “Actinic F_V“ reflects changes of the fluorescence yield in the sample because stray light and actinic light are constant during the illumination period.

In Fig. 88, not only continuous fluorescence varies but also the amplitude of fluorescence spikes. PAM fluorometers ignore the changes of continuous fluorescence and measure only the amplitude of fluorescence spikes. This is achieved by subtracting the fluorescence level just before the µs-measuring flash from the fluorescence level at the µs-measuring flash. In Fig. 88, the PAM fluorescence amplitude during the initial dark phase is denoted “Pulsed F_0”, and the maximum variable fluorescence at the end of actinic illumination is denoted “Pulsed F_V”.

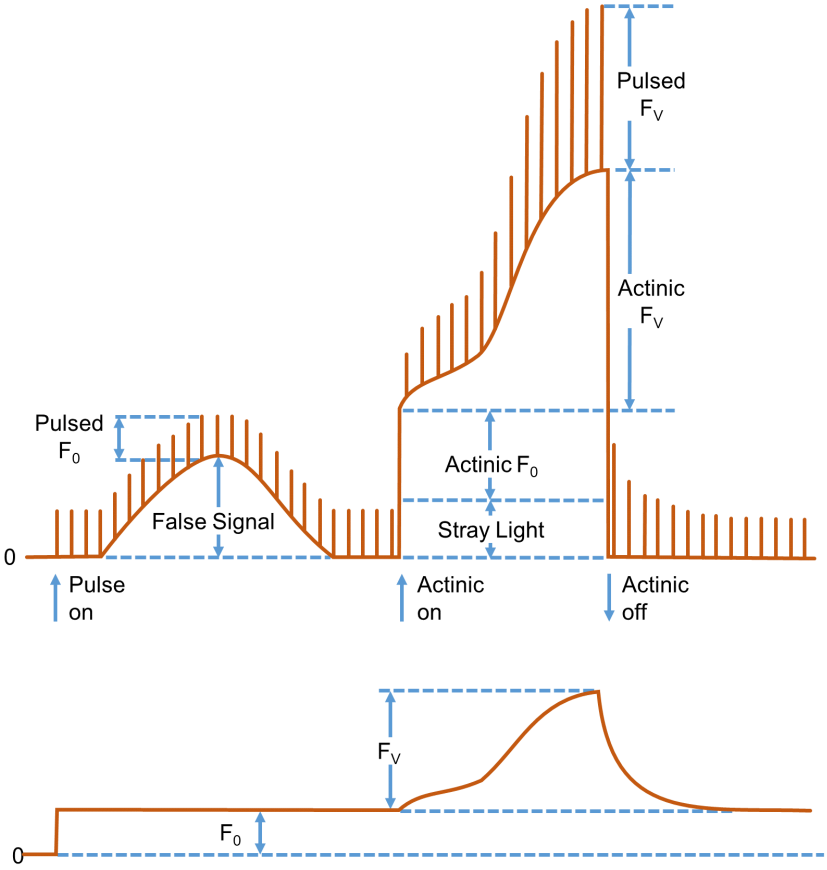


Fig. 88: Illustration of the PAM measurement principle

Figure redrawn from Dr. Ulrich Schreiber, Manual for PAM-101.

Because the µs-measuring flashes have constant amplitude, the varying amplitudes of fluorescence spikes is a measure of how efficient excitation light is converted into fluorescence. In other words, PAM fluorescence is proportional to the fluorescence yield.

The lower trace in Fig. 88 outlines the PAM fluorescence trace. Obviously, PAM fluorescence irons out the “False Signal” of total

fluorescence at the beginning of the experiment, and also the fluorescence jumps when actinic light is switched on and off. The course of continuous fluorescence within the range "Actinic F_V" resembles the corresponding trace of PAM fluorescence, because both measuring light and actinic illumination are constant.

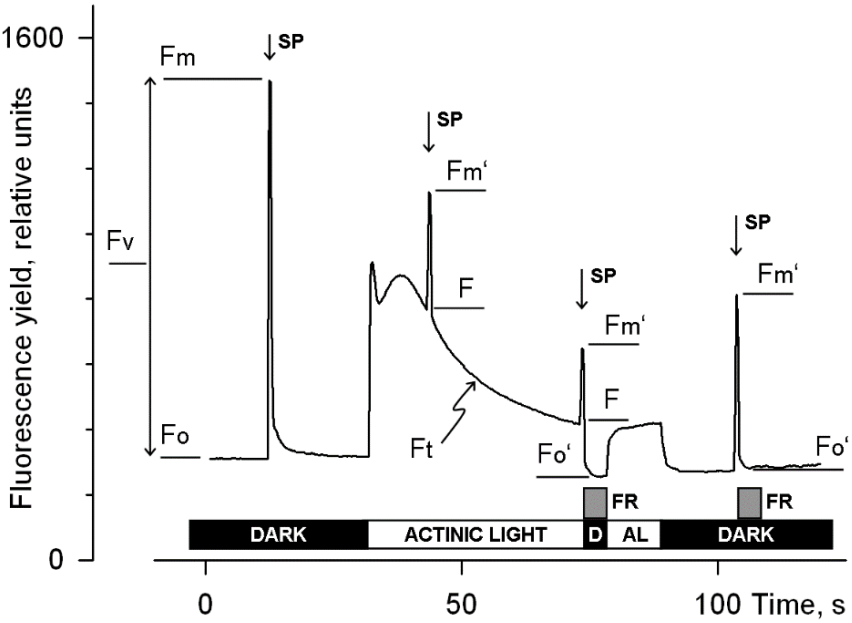


Fig. 89: Fluorescence Levels of Saturation Pulse Analysis

Y-axis (Fluorescence yield) corresponds to PAM fluorescence, see 8.1. AL, Actinic Light; D, dark; SP, Saturation Pulse; Ft, continuously recorded PAM fluorescence; FR, far-red illumination.

8.2 Saturation Pulse Analysis

The five principal levels of PAM fluorescence which are used for saturation pulse analysis are shown in Fig. 89. Two of these levels (F_0 and F_M) must be measured with the dark-acclimated sample. The three other levels (F_0', F, and F_M') are measured with the actinic light-exposed sample or in a dark period following this light treatment. Some parameters of saturation pulse analysis require fluorescence measurement of the same sample in both the dark-acclimated and light-exposed state (Table 26, page 166).

Because PAM fluorescence is excited by µs pulses of constant amplitude, variations between fluorescence levels are usually interpreted as variation in chlorophyll fluorescence yield. This applies for variations between different types of fluorescence levels (e.g. between F_0 and F_M) and for variations of the same type of fluorescence level (e.g. the change of F_M' during a fluorescence induction curve).

Measurements with Dark-Acclimated Samples

F_0 Minimum fluorescence level excited by very low intensity of measuring light to keep photosystem II reaction centers open.

F_M Maximum fluorescence level elicited by a pulse of saturating light (Saturation Pulse) which closes all photosystem II reaction centers.

Measurements with Illuminated Samples

F_0' Minimum fluorescence level of illuminated sample. The F_0' is lowered relative to F_0 by non-photochemical quenching. The measuring routine for F_0' (see 4.3.1.6, page 67) determines the F_0' level during a dark interval following a Saturation Pulse. In this dark interval, far-red

light is applied which selectively drives photosystem I. As a consequence, electrons are removed from the intersystem electron transport chain and opening of photosystem II reaction centers is efficiently accelerated

If the F_0' Mode is switched off, the F_0' will be calculated according to Oxborough and Baker:

$$F_0' = \frac{1}{\frac{1}{F_0} - \frac{1}{F_M} + \frac{1}{F_M'}}$$

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. https://doi.org/10.1023/A:1005936823310

In the Record table, the calculated value F_0' is preceded by a tilde sign (~).

F_M' Maximum fluorescence level of the illuminated sample. The F_M' is induced by a Saturation Pulse which temporarily closes all photosystem II reactions centers. F_M' is decreased relative to F_M by non-photochemical quenching.

F The F corresponds to the momentary fluorescence level (Ft) of an illuminated sample shortly before application of a Saturation Pulse.

To quantify photochemical use and non-photochemical losses of absorbed light energy, fluorescence ratio expressions have been derived which use two or more of the five relative fluorescence yields introduced above. Table 26 (page 166) compiles the fluorescence ratio parameters available in the DualPAM software. Below, these parameters will be explained briefly.

F_V/F_M and Y(II) Maximum and effective photochemical quantum yields of photosystem II

The F_V/F_M and Y(II) estimate the fraction of absorbed quanta used for photosystem II photochemistry. F_V/F_M corresponds to the maximum photochemical yield of photosystem II, Y(II) is the effective photochemical yield of photosystem II. Measurements of F_V/F_M require that samples are acclimated to darkness or dim light so that all reactions centers are in the open state and non-photochemical dissipation of excitation energy is minimal.

In algae and cyanobacteria, however, the dark-acclimated state often is not showing maximal photosystem II quantum yield, as the photosystem II acceptor pool may be reduced in the dark by stromal reductants and, consequently, the so-called state 2 is formed exhibiting low photosystem II quantum yield. In this case, preillumination with moderate far-red light should precede determinations of F_0 and F_M.

The Y(II) value estimates the photochemical use of excitation energy in the light. It is lowered with respect to F_V/F_M by partial closure of photosystem II centers and various types of non-photochemical energy losses induced by illumination.

q_P and q_L Coefficients of photochemical fluorescence quenching

Both parameters estimate the fraction of open photosystem II reaction centers. The q_P is based on the concept of separated photosystem II antenna units (puddle model), whereas the q_L assumes interconnected photosystem II antenna units (lake model) which was assumed to be present in leaves (*cf.* Kramer *et al.*, 2004). Determinations of q_P an q_L do not require fluorescence measurements with the dark-acclimated sample, except the F_0'

mode is switched of and F_0' is calculated according to Oxborough and Baker (1997).

q_N and NPQ Parameters of non-photochemical quenching

Both parameters are associated with non-photochemical quenching of excitation energy, mainly involving a low thylakoid lumen pH- and a zeaxanthin-dependent quenching mechanism. The qN and the NPQ parameters require fluorescence measurements with the sample in the dark-acclimated and in the light-exposed states (cf. Table 26, page 166).

Calculation of NPQ (or SV_N; Gilmore and Yamamoto, 1991) corresponds to the Stern-Volmer equation for fluorescence quenching which predicts proportionality between fluorescence quenching (NPQ) and the concentration of fluorescence-quenching centers in the photosynthetic antennae (e.g. zeaxanthin).

Y(NO), Y(NPQ) and Y(II) Complementary photosystem II yields

Genty *et al.* (1996) and Kramer *et al*. 2004 have presented expressions describing the partitioning of absorbed excitation energy in photosystem II between three fundamental pathways the sum of which adds up to one:

Y(NO) non-regulated losses of excitation energy including heat dissipation and fluorescence emission,

Y(NPQ) regulated energy losses of excitation energy by heat dissipation involving ΔpH- and zeaxanthin-dependent mechanisms, and

Y(II) use of excitation energy for charge separation.

This concept of "complementary photosystem II quantum yields" is useful to analyze the partitioning of absorbed light energy in photosynthetic organisms. For instance, in the presence of strong

light, a much higher Y(NPQ) than Y(NO) indicates that excess excitation energy is safely dissipated at the antenna level and that photosynthetic energy fluxes are well-regulated.

In variance, high values of Y(NO) would signify that excess excitation energy is reaching the reaction centers, resulting in strong reduction of photosystem II acceptors and photodamage, e.g. via formation of reactive oxygen species.

8.3 Relative Electron Transfer Rate (ETR)

Relative electron transfer rates for photosystem II are calculated according to:

$ETR(II) = PAR \cdot ETR\text{-}Factor \cdot P_{PS2}/P_{PS1+2} \cdot Y(II)$.

The basic idea of the ETR equation is to multiply Y(II), the effective photochemical quantum yield of photosystem II, by an estimate for the photon flux density absorbed by all photosystem II in the sample. The latter estimate is derived from three numbers:

(1) PAR Quantum flux density of photosynthetically active radiation (PAR) impinging on the sample.

(2) ETR-Factor Sample absorptance (= 1 – transmittance)

The ETR-Factor describes the fraction of incident photons absorbed by the sample. The most frequently used default value for green leaves is 0.84 meaning that 84% of incoming light is absorbed. The ETR-Factor can be lower in bleached leaves or leaves containing considerable amounts of non-photosynthetic pigments like anthocyanins.

(3) P_{PS2}/P_{PS1+2} Relative distribution of absorbed PAR to photosystem II

The default P_{PS2}/P_{PS1+2} is 0.5 which assumes the photosystem II contributes 50% to total sample absorptance. The P_{PS2}/P_{PS1+2} may deviate from the idealized factor of 0.5 depending on wavelength of light and acclimation status of the sample.

8.3.1 Reviews on Saturation Pulse Analysis of Photosystem II

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Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42: 313-349. https://doi.org/10.1146/annurev.pp.42.060191.001525

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Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 64: 3983-3998. https://doi.org/10.1093/jxb/ert208

Schreiber U (2004) Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. In: Papageorgiou GC, Govindjee (eds) Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Springer, Dordrecht, pp 279-319. https://doi.org/10.1007/978-1-4020-3218-9_11

Table 26: Fluorescence Ratio Parameters.

Source	Equation	Sample State	Range [Theory] [Experiment]
Maximum photochemical quantum yield of PS II (Kitajima and Butler, 1975)	$\frac{F_V}{F_M} = \frac{F_M - F_0}{F_M}$	Dark	[0, 1] [0, ~0.84]
Effective photochemical quantum yield of PS II (Genty *et al*., 1989)	$Y(II) = \frac{F'_M - F}{F'_M}$	Light	[0, 1] [0,~ 0.84]
Quantum yield of light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Genty *et al*. 1996, Kramer *et al*. 2004)*	$Y(NPQ) = \frac{F}{F'_M} - \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Quantum yield of non-regulated heat dissipation and fluorescence emission: this type of energy loss does not involve the action of a transthylakoid ΔpH and zeaxanthin (Genty *et al*. 1996, Kramer *et al*. 2004)*	$Y(NO) = \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Stern-Volmer type non-photochemical fluorescence quenching (Bilger and Björkman, 1990; Gilmore and Yamamoto, 1991))	$NPQ = \frac{F_M}{F'_M} - 1$	Dark and Light	[0, ∞] [0, ~4]
Coefficient of photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_P = \frac{F'_M - F}{F'_M - F'_0}$	Light. If F_0' calculated, Dark and Light	[0, 1] [0, 1]
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (Kramer *et al.* 2004)	$q_L = q_P \cdot \frac{F'_0}{F}$	As q_P.	[0, 1] [0, 1]
Coefficient of non-photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_N = 1 - \frac{F'_M - F'_0}{F_M - F_0}$	Dark and Light	[0, 1] [0, ~0.95]

* Kramer *et al.* (2004) have derived more complex equations for Y(NO) and Y(NPQ). Klughammer and Schreiber (2008) have transformed the equations by Kramer *et al.* (2004) into the simple equations of Genty *et al*. (1996).

Table 27: References Cited in Table 26

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res 25:173-185. https://doi.org/10.1007/BF00033159

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Gilmore AM, Yamamoto HY (1991) Zeaxanthin Formation and Energy-Dependent Fluorescence Quenching in Pea Chloroplasts under Artificially Mediated Linear and Cyclic Electron Transport. Plant Physiol 96:635-643. https://doi.org/10.1104/pp.96.2.635

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9 Specifications MINI-PAM-II

9.1 Basic System

9.1.1 Optoelectronic Unit

MINI-PAM-II/B (Blue Version)

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 µmol m^{-2} s^{-1}. Fluorescence at wavelengths greater than 630 nm is measured

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

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

MINI-PAM-II/B and 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 160x104 dots (78 x 61 mm) transflective 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

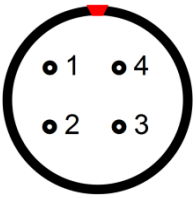
Power supply: 6 AA (Mignon) rechargeable batteries (Eneloop 1.2 V/2 Ah), providing power for up to 1000 yield measurements; 6 spare batteries, automatic power/off, battery charger (100 to 240 V AC, 50-60 Hz, 0.35 A) for 1 to 8 AA/AAA NI-MH/NI-CD batteries, 12 V 5,5 A power supply MINI PAM-II/N

Operating temperature: -5 to +45 °C, non-condensing

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

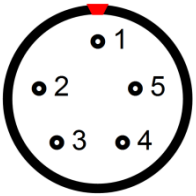
Weight: 1.5 kg (incl. battery)

Table 28: Pin Assignments. View from Outside.



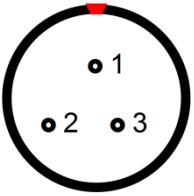
LEAF CLIP/AUX 1/AUX 2

1	GND
2	Serial Clock
3	Serial Data
4	+ 5 V Output



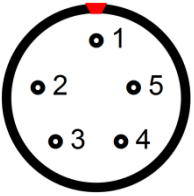
1	SYNC
2	GND
3	SYNC
4	RS 485 A
5	RS 485 B
6	Voltage output of current power supply (line or battery)

Table 28: Pin Assignments. View from Outside.



EXT. DC

1	Not used
2	GND
3	+ 12 V Input



COMP 2/ COMP 1

1	+ 5 V Output (= 4 of LEAF CLIP)
2	Voltage output of current power supply (= 5 of SYNC)
3	RS 232 TxD
4	RS 232 RxD
5	GND

9.1.2 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

9.1.3 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: -5 to +45 °C, (non-condensing)

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

Weight: 350 g including cables

9.1.4 Battery Charger 000190101101

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

Output: 12 V DC, 1.0 A

Operating temperature: -5 to +45 °C, (non-condensing)

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

Weight: 300 g including cable

9.1.5 Distance Clip 60° 2010-A

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

9.1.6 Complementary Items

Sloped Plexiglas rack for convenient desktop operation. Stylus for touchscreen. Carrying strap for optoelectronic unit

9.1.7 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, F_0, F_M, F, F_0' (also calculated), F_M'. PAR, leaf temperature and relative humidity using 2035-B Leaf-Clip Holder. Calculated: F_0' (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.

9.1.8 Transport Case MINI-PAM/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

9.2 Accessories

9.2.1 2035-B Leaf-Clip Holder

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

Micro quantum sensor: LS-C sensor for selective PAR measurement, range 0 to 7000 µmol m^{-2} s-1, cosine corrected for light incident at an angle between -30 ° to +30 from surface normal, internal preamplifier

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)

9.2.2 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

9.2.3 External LED Source 2054-L

Accessory to Leaf Clip Holder 2035-B to illuminate the sample plane at an angle of 45° with red, green, blue or white light, or

mixtures of these four light qualities. The intensity of each light quality is adjusted individually and the maximum PAR per light quality is 1500 µmol m^{-2} s^{-1}.

Design: LED holder for 4 RGBW LEDs made of anodized aluminum with cooling ventilator on the back side. The device can be fastened by two 7 cm long aluminum rods whose ends fit in drilled holes of the 2035-B clip

Dimensions: 9 cm (max.) x 4.5 cm x 12 cm (max.) (L x W x H)

Weight: 210 g without cable

9.2.4 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

9.2.5 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 3.0 cm (max.) x 5.2 cm (max.) (L x W x H)

Weight: 55 g

9.2.6 Conversion Kit for 2030-B/2035-B Leaf Clips 2035-B/RLC

Design: Duct and mounting bar, made of Plexiglas, replacing the standard 60° fiberoptics duct of the 2030-B clip (PAM-2500) or the 2035-B clip (MINI-PAM-II). The duct positions the fiberoptics 2010-F (2030-B clip) or fiberoptics MINI-PAM/F (2035-B clip) perpendicularly to the sample surface. With lateral set screw. Including two circular light-shielding elements made from cellular rubber and a 3 cm x 2 cm (L x W) black plastic base plate with cellular rubber rim

Dimensions, max: 7 cm x 2.6 cm x 2.7 cm (L x W x H)

Weight: 12 g

9.2.7 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) steal 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

9.2.8 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 µmol m^{-2} 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)

9.2.9 Miniature Spectrometer MINI-SPEC/MP

Design: POM tube, at one side, port for light detection, port for fluorescence excitation by blue (452 nm max) or green (525 nm max) LEDs, and port for white light from a tungsten lamp for reflection measurements; at the opposite side, 4-pole underwater socket.

Spectrometer: Hamamatsu micro-spectrometer, spectral range: 400 to 800 nm, spectral resolution: between 8 and 10 nm**.**

Maximum PAR: 4000 µmol m^{-2} s^{-1} for illumination having spectral characteristics similar to sunlight

Dimension: 3.25 cm diameter, 175 cm length max

Weight: 135 g

9.2.9.1 Flat Entrance Optics SPEC/P

Design: Hard-anodized aluminum rod of 10 mm diameter and 50 mm length, at one end with lateral light entrance through a 5 mm diameter diffusor and the opposite end inserted in a mounting plate (diameter 33 mm, height 5 mm). Aluminum rod with internal light guide

9.2.9.2 Fluorescence and Reflection Optics SPEC/R

Design: Spectrometer cap consisting of POM: maximum diameter 35 mm, height 13 mm, weight 16 g. With central 5 mm x 16 mm groove which accommodates at one end a Perspex light guide for fluorescence excitation by blue or green light, and at the other end a Perspex light guide for white light for reflection measurements. With 3 mm diameter central drilled hole as light channel to the detector window of the spectrometer. The sample is fixed between the cap part and another disk (maximum diameter 40 mm, height 10 mm, weight 8 g). The sample side of cap and disk is padded with foam rubber, both parts have magnets build in to attract each other and, thus, hold the sample. Including a 10 mm thick Zenith Polymer reflectance standard

9.2.9.3 PAR Calibration Block 000160101439

Design: POM block with drill hole for entrance optics of the Miniature Spectrometer. Oriented at an angle of 60° and 90° relative to the spectrometer port are drill holes for the Fiber Optics DIVING-F

Dimensions: 4.15 cm x 2 cm x 5 cm (L x W x H)

Weight: 40 g

9.2.10 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 POM body with injection port for microliter syringes and hose nozzles for connecting an external flow-through water bath (not included). Including three 6.0 x 1.5 mm magnetic stir bars

Dimensions: 11.5 cm x 9 cm x 8.5 cm (L x W x H, including ports)

Weight: 252 g

9.2.11 Magnetic Stirrer with Fiberoptics Holder MKS-2500

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

9.2.12 Compact Tripod ST-2101A

Stand for 2035-B Leaf-Clip Holder.

Height: Adjustable between 24 cm and 87 cm

Weight: 400 g

9.2.13 Barcode Scanner BCS-9590

Design: Single-line handheld laser scanner with trigger button and 1 m, partially coiled cord; to be connected to the Photosynthesis Yield Analyzer MINI-PAM-II. Bar codes are stored together with fluorescence data

Dimensions: 9 cm (max.) x 6 cm (max.) x 16 cm (max.) (L x W x H)

Weight: 335 g

9.2.14 MINI-PAM/F1 Miniature Fiberoptics

Active diameter 2 mm, length 1.5 m. Including adapter for attachment to Leaf-Clip Holder 2035-B.

Subject to change without prior notice

10 Guarantee

10.1 Manufacturer's Guarantee

Under this Manufacturer's Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product's features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product's respective brochure, data sheet, or respective tab on the Manufacturer's website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer's option, and this Guarantee does not include any other rights or remedies.

10.2 Conditions

This Guarantee shall not apply to:

- Any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.
- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.

- Damage caused from improper packaging during shipment or any acts of God.
- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps, thermocouples, and underwater cables.
- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.
- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

10.3 Instructions

- To obtain guarantee service, please follow the instructions below:
- The Walz Service Information Form available at http://www.walz.com/support/repair_service.html must be completed and returned to Heinz Walz GmbH, Germany.
- The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.

- All products being returned for guarantee service must be carefully packed and sent freight prepaid.
- Heinz Walz GmbH, Germany is not responsible or liable for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

10.4 Applicable law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.

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