

MONI-HEAD MONI-DA

Manual

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1 Safety Instructions

1.1 General Safety Instructions

- Read and follow safety and operating instructions prior to operation of the device. Pay attention to all safety warnings.
- The data acquisition systems MONI-DA and MONI DA/S, as well as the fluorometers (measuring heads) MONI-HEAD/485 and MONI-HEAD/S are designed for outdoor use. However, avoid exposure of devices to dust, sand, and dirt as much as possible.
- Always, care must be taken to keep sockets clean. During field operation, the sockets must be closed either by screw caps or by cable plugs. Plugging and unplugging of lines, as well as handling the MicroSD card require dry and clean conditions.
- Keep all ports of the MONI-DA and MONI DA/S, as well as of the MONI-HEAD/485 and MONI-HEAD/S clean and dry.
- Run all cables so that stepping on or stumbling over them is excluded.
- Ensure that neither liquids nor foreign bodies get inside the device.
- Do not put these devices near sources of heat.
- Connect the device only to the power source indicated in operating instructions or on the device. If the device is not in use, remove the mains plug from the socket.

- Do not open housing of MONI-DA and MONI DA/S, as well as of the MONI-HEAD/485 and MONI-HEAD/S. There are no serviceable parts inside. The device may only be repaired by the manufacturer.

1.2 Special Safety Instructions

- A MONI-PAM system is a highly sensitive measuring system which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual to avoid potential harm to the user and damage to the instrument.
- The measuring heads MONI-HEAD/485 and MONI-HEAD/S can emit very strong light! To avoid harm to your eyes, never look directly into the light port or fiber optics of the measuring heads.

2 Introduction

The MONI-PAM systems are designed for long-term monitoring of photosynthesis either above ground or underwater. The above-ground system is referred to as TERRESTRIAL version, the underwater system is referred to as AQUATIC version (see Chapter 3, page 5).

Both versions are available as systems with a battery-powered data acquisition unit (STAND-ALONE configuration), or without data acquisition unit (ONLINE configuration). The STAND-ALONE configurations can be upgraded with a WiFi or a satellite modem. These accessories send data to the user's computer via a Walz server.

All MONI systems are multi-site fluorometers. This means that they consist of several measuring heads (cf. Table 1). The simultaneous long-term monitoring of several sites is now a well-proven concept to obtain statistically significant information about photosynthesis under natural conditions.

Version	Configuration	Maximum number of measuring heads	Comment
TERRESTRIAL	ONLINE	4	Several systems can be combined
TERRESTRIAL	STANDALONE	16	Measuring head numbers from 8 to 16 require MICRO-HUB interface(s).
AQUATIC	ONLINE	4	Several systems can be combined
AQUATIC	STANDALONE	7	

Table 1: Maximum Number of Measuring Heads

The individual measuring head of a MONI-PAM system is a sealed tube which contains all optical and electrical components. One end of the tube consists of an optical lens which focusses the fluorometer's internal light on the sample.

Source for internal light is a high-performance LED which emits actinic light, modulated measuring light, and saturation pulses. MONI measuring heads are available with either a blue or a white LED (see Section 5.2, page 18). Measuring light and saturation pulses are employed to determine the photochemical quantum yield of photosystem II ($Y(II)$), see Table 12, page 87).

A special feature of MONI measuring heads is a diffusing pad mounted parallel to the sample surface. This pad reflects light into the housing, where the quantum flux density (PAR) is measured. The sensor is calibrated to output the light intensity at sample plane. From PAR and $Y(II)$, the photosynthetic electron transport (ETR) can be derived.

3 Extent of Delivery

All systems	
Power supply 19 V DC, 3.7 A	
USB cable (1.8 m)	
Software	
Transport box	
Manual	
Systems: Surface MONI-PAM	Systems: Underwater MONI-PAM
MONI-SET3 or MONI-SET4	MONI-SET3/S or MONI-SET4/S
3 or 4 MONI-HEAD/485	3 or 4 MONI-HEAD/S
PC Interface Box MONI IB4/LAN	PC Interface Box MONI IB4/LANS
MONI-SET3-DA or MONI-SET4-DA	MONI-SET3-DA/S or MONI-SET4-DA/S
3 or 4 MONI-HEAD/485 (see below)	3 or 4 MONI-HEAD/S (see below)
Data Acquisition System MONI-DA	Underwater Data Acquisition System MONI-DA/S
PC Interface Box MONI-IB1	PC Interface Box MONI-IB1/S
Outdoor RS-485 cable (10 m) (plus 3 or 4 cables included in MONI-HEAD/485)	Underwater RS-485 cable (10 m) (plus 3 or 4 cables included in MONI-HEAD/S)
Power supply 19 V DC, 3.7 A	Power supply 19 V DC, 3.7 A
2 Solar Panels MONI-SP	
Measuring Heads	
MONI-HEAD/485 MONITORING-PAM Measuring Head	MONI-HEAD/S Underwater MONITOR- ING-PAM Measuring Head
Aluminum housing and sample clip	POM Housing and POM/Titanium sample clip
Outdoor RS-485 cable (10 m)	Underwater RS-485 cable (10 m)
Ring holder with rod	Flexible stand with baseplate (MONI-ST)
Fluorescence standard	Fluorescence standard
Spare O-rings	Spare O-rings

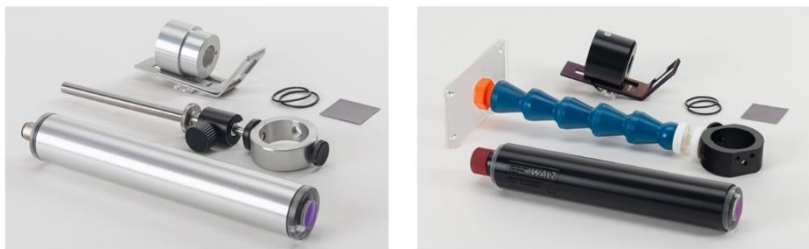


Fig. 1: Measuring Heads – Extent of Delivery

Left: MONI-HEAD-485. Right, MONI-HEAD/S. 10 m RS-485 cable not shown.

4 Systems

MONITORING-PAM systems can be differentiated according to their areas of application. The TERRESTRIAL version is designed for research of photosynthetic organisms growing on land, from layers of cyanobacteria or eukaryotic algae to mosses, ferns, and seed plants. In comparison, the AQUATIC Version is made to study photosynthesis underwater. The organisms investigated are macroalgae, seagrasses, and corals.

The parts of the TERRESTRIAL and AQUATIC version cannot be interchanged because of different connectors.

Both versions can be configured to work in the absence of power supply. These “STAND-ALONE Configurations” include a battery-powered data acquisition system. When a power supply is present, the ONLINE Configuration is suitable. In this case, a computer replaces the data acquisition system.

4.1 TERRESTRIAL Version

4.1.1 ONLINE Configuration

The ONLINE configuration of the TERRESTRIAL version (Fig. 2, page 8) requires permanent power supply to the PC Interface Box MONI-IB4/LAN (Fig. 16, page 32). The MONI-IB4/LAN provides input sockets for four RS-485 lines connecting to MONI-HEAD/485 emitter-detector heads. The LAN function of the MONI-IB4/LAN interface permits integration of the MONITORING-PAM in a local area network. In addition, RS-232 and USB communications are available for direct connection of the MONITORING-PAM to a Windows PC.

The length of communication and power cables determines the distances between components. For example, samples can be about 10 m away from the computer in case of USB or RS-232 communication, but this distance increases to more than 100 m when Ethernet communication is used.

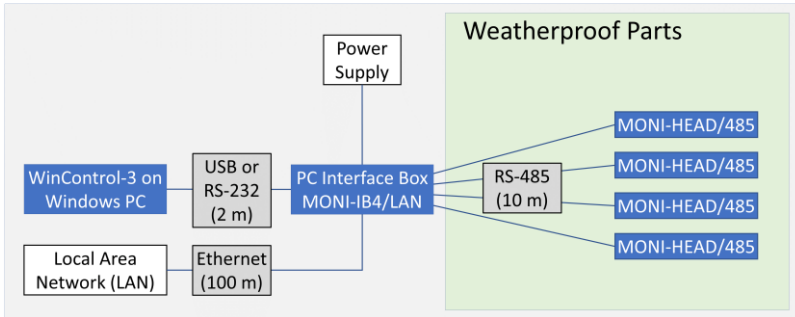


Fig. 2: TERRESTRIAL Version, ONLINE Configuration

The figure shows the system package MONI-SET4. PC, Ethernet cable and RS-232 cable must be ordered separately. Weatherproof parts are drawn on light-green background.

4.1.2 STAND-ALONE Configuration

The STAND-ALONE configuration of the TERRESTRIAL version of the MONITORING-PAM is suited for independent long-term monitoring of photosynthesis in the absence of power supply. The system consists of the battery-powered data acquisition system MONI-DA (Fig. 13, page 25, and Fig. 15, page 30), two solar panels MONI-SP, and up to seven emitter-detector heads MONI-HEAD/485 (Fig. 8, page 17). The number of MONI-HEAD/485 heads per MONI-DA can be increased using a MICRO-HUB interface (Fig. 3, page 9).

The MONI-DA logs data on a microSD flash card and, additionally, on an 8 MByte internal ring buffer. Online connection between MONI-DA and a Windows computer is established using the interface MONI-IB1 (Fig. 17, page 32) and USB communication. A WiFi or satellite modem transmits data to distant computers with little time delay.

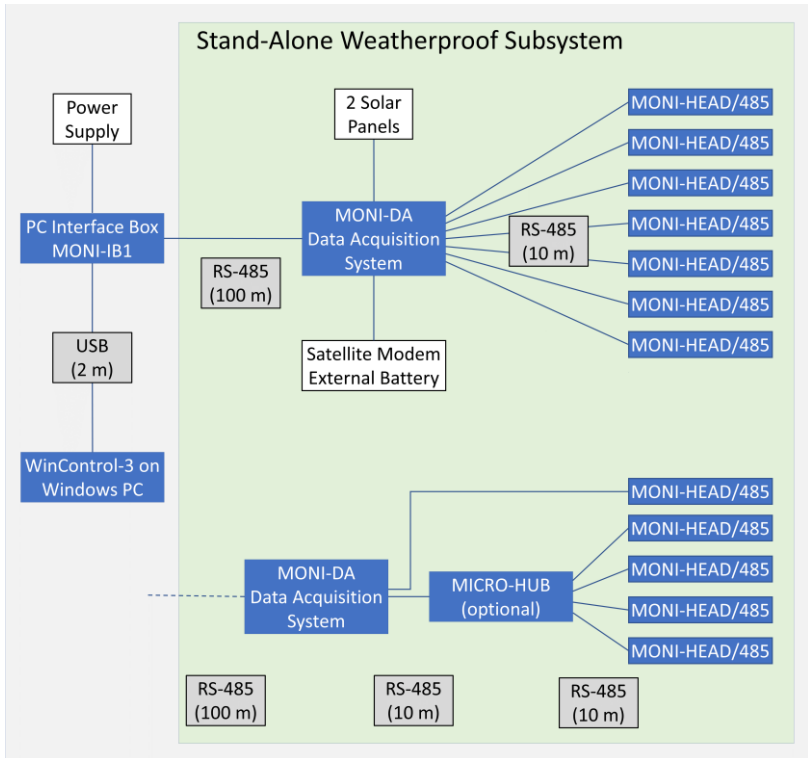


Fig. 3: TERRESTRIAL Version, STAND-ALONE Configuration

Upper part, setup with seven measuring heads connected to the MONI-DA. Basic system packages include three or four measuring heads (MONI-SET3-DA and MONI-SET4-DA, respectively). Lower part: up to 16 measuring heads can be connected to the MONI-DA when the optional MICRO-HUB is used. Weatherproof parts are drawn on light-green background.

4.2 AQUATIC Version

The AQUATIC versions of the MONITORING-PAM are suited for long-term monitoring of chlorophyll fluorescence at water depths down to 75 m.

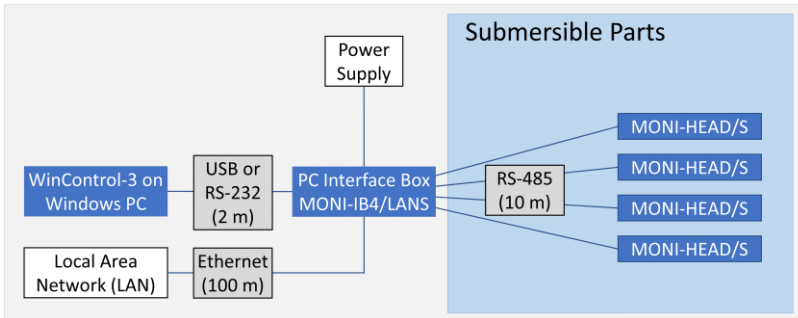


Fig. 4: AQUATIC Version, ONLINE Configuration

The scheme shows the standard package MONI-SET4/S. PC, Ethernet cable or RS-232 cable must be ordered separately. Waterproof parts are drawn on light-blue background.

4.2.1 ONLINE Configuration

The ONLINE configuration of the AQUATIC version of the MONITORING-PAM requires external power supply and is operated by a Windows computer. Up to four waterproof MONI-HEAD/S measuring heads can be linked to the interface MONI-IB4/LANS. The interface MONI-IB4/LANS connects to a computer via Ethernet, RS-232 or USB communication. The water depth of measuring sites is determined by the length of the RS-485/S underwater cables, which is 10 m by default.

4.2.2 STAND-ALONE Configuration

The STAND-ALONE configuration of the AQUATIC version of the MONITORING-PAM consists of the waterproof battery-powered data acquisition system MONI-DA/S, the PC interface MONI-IB1/S, and up to seven waterproof emitter-detector heads MONI-HEAD/S (Fig. 5, page 11).

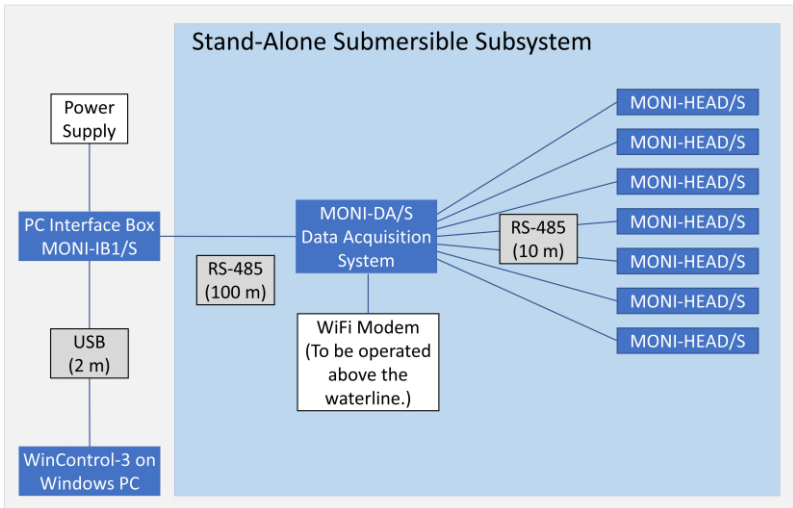


Fig. 5: AQUATIC Version, STAND-ALONE Configuration

Setup with seven measuring heads connected to the MONI-DA/S. Standard packages include three or four measuring heads (MONI-SET3-DA/S and MONI-SET4-DA/S, respectively). Waterproof parts are drawn on light-blue background.

The MONI-DA/S runs for more than 4 weeks on battery with seven MONI-HEAD/S connected and a saturation pulse interval of 15 minutes. Solar panels with underwater connectors to charge the battery are available.

Data are logged on an internal ring buffer and on a non-removable microSD card. Data are transferred from the MONI-DA/S to a computer via the interface MONI-IB1/S. Wireless data transfer is feasible using a watertight WiFi modem. The WiFi

modem must be operated above the waterline because water absorbs the WiFi signal.

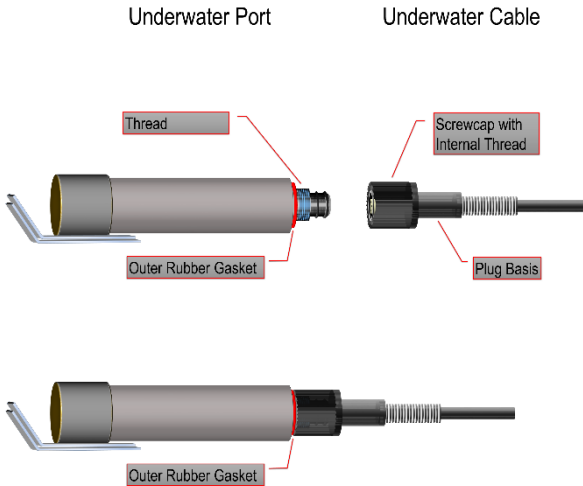


Fig. 6: Watertight Connection of RS 485 Underwater Cable

- (1) Hold cable end at "Plug Basis".
- (2) Pull back "Screwcap" until it clicks.
- (2) Fully (!) insert plug in socket.
- (3) Tighten "Screwcap".
- (4) Confirm that screwcap presses against "Outer Rubber Gasket".

These instructions apply to the RS 485 connectors of the MONI-HEAD/S Underwater MONITORING-PAM Measuring Head, the Underwater Data Acquisition System MONI-DA/S, the PC Interface Box MONI IB4/LANS, and the PC Interface Box MONI IB1/S.

4.2.3 Underwater Cables

Unlike RS 485 cables of TERRESTRIAL versions, RS 485 underwater cables cannot be connected to one another. The reason is that both ends have identical plugs. Cables longer than the standard lengths must be ordered separately. Check data transfer and charging of the data acquisition unit, for cable lengths longer than 10 m.

Underwater connections are specially designed to be watertight. How to proceed to properly connect cables is described in Fig. 6 (page12).

5 MONI-PAM Measuring Heads

5.1 MONI-HEAD/485 & MONI- HEAD/S

The measuring heads of MONI systems have a tubular housing which is made either of aluminum (MONI-HEAD/485) or of POM plastic (MONI-HEAD/S, Fig. 7A, page 16). POM (Polyoxymethylene) was selected for underwater measuring heads MONI-HEAD/S because it does not corrode. By comparison, aluminum would corrode in warm saltwater.

At one end of the tube, the socket for the RS 485 data and charge cable is located. The other end is an optically transparent window. The measuring and actinic light passes through this window to the sample. Through the same window, ambient light, reflected from the diffuser pad of the leaf clip, reaches the PAR sensor, and sample fluorescence reaches the fluorescence sensor. Appropriate filters inside the MONI-HEAD prevent that ambient light reaches the fluorescence detector, and that fluorescence light reaches the PAR detector.

The sample clips for the two types of sensors are also made from different materials (Fig. 7A, page 16). The aluminum of the clip for the MONI-HEAD/485 is replaced by POM and titanium in the clip for the MONI-HEAD/S.

To mount the sample clip, insert the measuring head into the ring of the sample clip. Turn the ring until the plastic screw of the housing inserts into the hole of the ring (Fig. 8, page 17). Fasten sample clip with plastic setscrew(s).

Attached to the inner side of the leaf clip frame is an 8 x 12 mm piece which exhibits very high diffuse reflection of radiation in

the visible range. The intensity of the reflected light is measured by a PAR sensor inside the measuring head. This internal PAR sensor was adjusted by comparison with a calibrated light sensor which was mounted next to the white patch and parallel to the sample plane. Thus, the measuring head reads the PAR which is effective at the sample level.

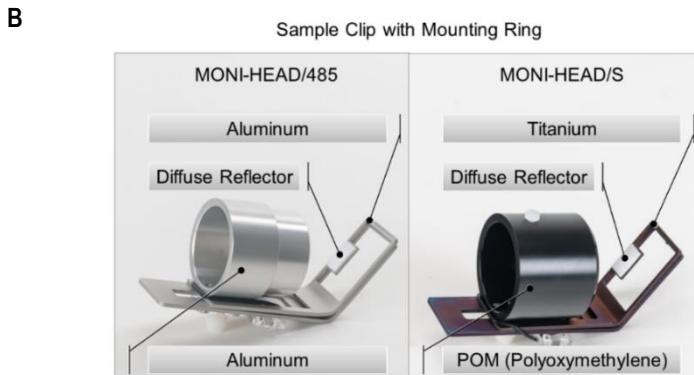


Fig. 7: MONI-HEAD/485 & MONI HEAD/S

- A:** Full view of MONI-HEAD/485 and underwater MONI-HEAD/S measuring heads. The MONI-HEAD/S is made from corrosion resistant POM.
- B:** Leaf clip of (left) MONI-HEAD/485 and (right) MONI-HEAD/S. Corrosion resistant material has been used for the MONI-HEAD/S leaf clip.

Sample Clip with Mounting Ring

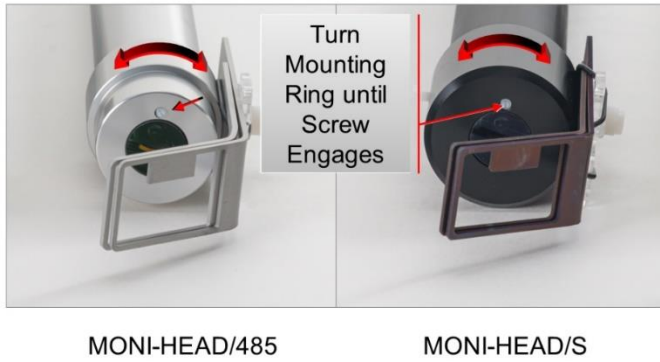


Fig. 8: Mounting the Leaf Clip

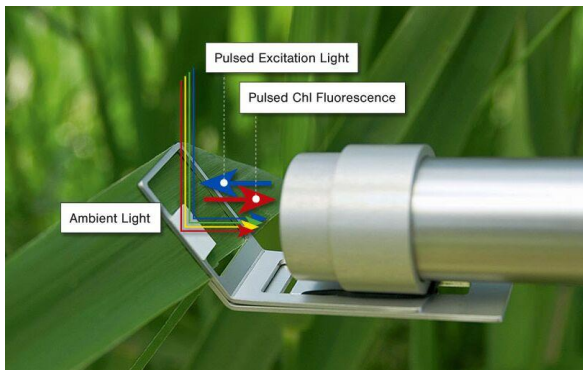


Fig. 9: Schematic Drawing of Light Paths

Red to blue colored narrow arrows symbolize external light in the visible region (PAR). The bigger blue arrow stands for blue pulsed excitation light, and the parallel red arrow symbolized the pulsed “amplitude modulated” fluorescence.

Each measuring head contains a temperature sensor. Because the sensor is not in direct contact with the environment, the temperature readings follow changes in ambient temperature with

some delay. When operated by a data acquisition system (MONI-DA or MONI-DA/S), measuring heads are sent to the hibernation mode between saturation pulse analyses. This mode minimizes a possible temperature increase by LED heat emission. When a measuring head is directly exposed to sun radiation, the temperature readout might be higher than the actual air temperature.

5.2 BLUE and WHITE Version

MONI measuring heads can be equipped with a blue LED (BLUE Version) or a white LED (WHITE version). The blue LED emits between 400 and 500 nm with a maximum around 445 nm. The white LED shows a narrow-band blue emission peaking around 445 nm plus a broad band emission ranging from 475 to 650 nm (Fig. 10).

The spectral window for fluorescence detection is different for the two version. The BLUE Version detects fluorescence at wavelengths > 630 nm and the WHITE Version detects fluorescence at wavelengths > 650 nm (Fig. 10).

The BLUE version is the standard type of MONI heads, and it is suitable for fluorescence measurements of most eukaryotic algae and higher plants. For samples absorbing poorly in the blue, such as species of the phylum cyanobacteria, the WHITE version gives better fluorescence signals than the BLUE version.

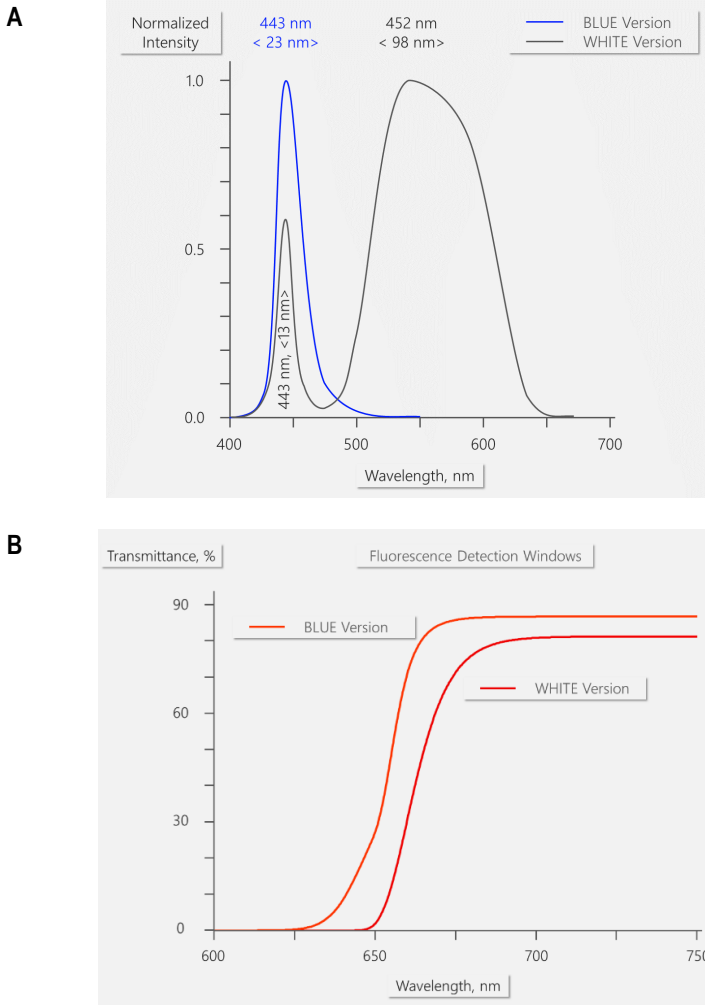


Fig. 10: BLUE & WHITE Version

- A:** Typical LED emission spectra normalized to their maxima. The blue curve represents the spectrum of the blue LED (BLUE Version), the black curve represents the spectrum of the white LED (WHITE Version). Peak wavelengths and full width at half maximum (bracketed) in nm are displayed.
- B:** Transmittance spectra of detection filters in measuring heads containing a blue LED (BLUE Version, red line) and measuring head containing a white LED (WHITE Version, dark red line).

5.3 Advice/Maintenance

Diffuser plate

Take care that the diffuser plate for PAR measurements is exposed to the same radiation as the sample, e.g., prevent coverage of the plate by a neighboring leaf.

Keep the diffuser plate as clean as possible. Avoid any contact with oily substances (do not touch with fingers). Clean dust and dirt by rinsing with distilled water. Very careful sanding (using emery cloth) may remove stubborn dirt. If the diffuser plate cannot be completely cleaned, ask Walz for replacement.

Saturation pulse analysis

Do not carry out saturation pulse analysis at high frequency as this might stress the sample, particularly during the night. Intervals between 30 and 60 min are sufficient for most studies. On request, we provide a batch file which reduces the SAT pulse frequency automatically during dark periods.

Measuring head lost

When data of individual heads or of the entire system are repeatedly missing, check if water has got in any of the connections. When needed, blow dry with pressured air.

6 Accessories

6.1 Four-Way Distributor MICRO-HUB

Input port expander when more than 7 MONI-PAM measuring heads are to be connected to a MONI-DA data acquisition system. The maximum number of measuring heads per MONI-DA is 16 corresponding to 12 heads connected via MICRO-HUB and 4 directly connected heads. For system TERRESTRIAL version, STAND-ALONE configuration.



Fig. 11: Four-Way Distributor MICRO-HUB

6.2 MONI-DA/WIFI and MONI-DA/SWIFI (WiFi Modems)

In the presence of WiFi network coverage, the WiFi modem transfers MONI-DA data via internet and a special Walz server to your computer. When WiFi is not available but cellular network coverage is present, the WiFi modem can be connected to the Walz data server using a mobile hotspot. The MONI-

DA/WIFI is compatible with the system TERRESTRIAL version, STAND-ALONE configuration. The MONI-DA/SWIFI is equipped with a connector for underwater cables and is therefore suited for the system, AQUATIC version, STAND-ALONE configuration.

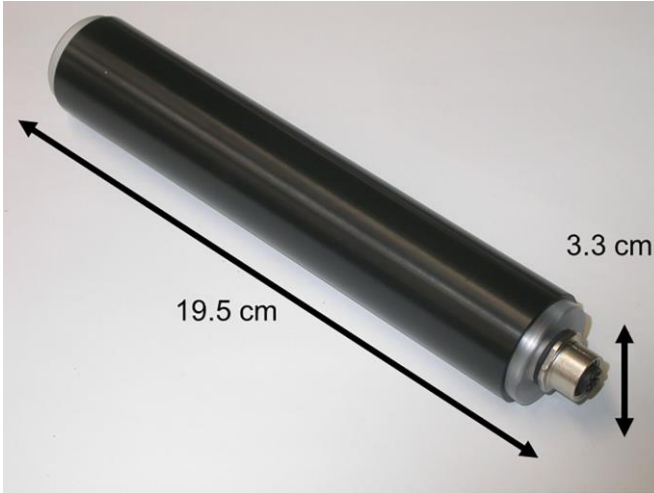


Fig. 12: MONI-DA/WIFI

The underwater WiFi modem MONI-DA/SWIFI is identical to the MONI-DA/WIFI except its terminal is configured for underwater cables.

6.3 Satellite Modem

When terrestrial wireless communication is unavailable, a satellite modem can be used to transfer data via communications satellite and Walz server to your office. Inquire specifications with Walz.

7 MONI-DA Data Acquisition System

Both STAND-ALONE configurations (see Sections 4.1.2, page 8, and Section 4.2.2, page 11) include a data acquisition system. A data acquisition system controls the measuring heads and stores data. It is battery powered and can be charged by solar panels, which are also available for the underwater version MONI-DA/S. For long nights at polar sites, an external high-capacity battery can support the internal battery of the MONI-DA.

The MONI-DA(/S) has seven so-called MONI-BUS ports to which MONI-PAM measuring heads can be connected. In case of the MONI-DA, the MONI-HUB permits connecting four measuring heads to a single MONI-BUS port. Thus, the number of measuring heads can exceed the number of seven. Because of memory-limitations, the maximum number of measuring heads is 16 (Fig. 3, page 9).

7.1 General Description

The data acquisition systems have been developed for unattended, long-term and multi-site monitoring of photosynthesis. The data acquisition systems MONI-DA can control both measuring heads MONI-HEAD/485 and MICRO-PAM. Only MONI-DA/S measuring heads are compatible with the MONI-DA/S. Common properties of the two data acquisition systems are:

- Waterproof and robust housing.
- Low power consumption.
- Dual data storage on ring buffer chip and memory card.

Major differences between MONI-DA and MONI-DA/S are:

- To avoid water leaks, the MONI-DA/S lacks the memory card port of the MONI-DA and, hence, the card cannot be removed. For the same reason, signal LEDs are omitted on the rear face of the MONI-DA/S. For data download see Section 7.4.2.
- All sockets of the MONI-DA are M12 5-pole but in the MONI-DA/S, waterproof 6-pole sockets are employed.

7.1.1 Battery and Power Consumption

Following the rapid development in battery technique, we have equipped data acquisition systems with different batteries (Table 2).

Table 2: MONI-DA Batteries

Serial number	Date	Battery type	Voltage, Charge
CFMH#### (MONI-DA) CFMK#### (MONI-DA/S)	Before June 2011	Sealed lead-acid (contact Walz to have the lead-acid battery exchanged for a lithium iron-phosphate battery)	12.0 V, 7.0 Ah (Low battery warning at 11.5 V, auto shut down at 7.5 V)
CFMP#### (MONI-DA) CFMS#### (MONI-DA/S)	After June 2011	Lithium manganese oxide	7.2 V, 4.8 Ah (Low battery warning at 6.8 V, auto shut down at 6.5 V)
	After November 2012	Lithium iron-phosphate	12.0 V, 7.5 Ah (Low battery warning at 11.5 V, auto shut down at 11.0 V)

Charging of MONI-DA batteries requires that the PC interface box of the system (MONI-IB1, MONI-IB4/LAN, MONI-IB1/S or MONI-IB4/LANS) is connected to the mains and to the data acquisition system.

A full charge cycle of the lithium iron-phosphate battery requires approximately 6 hours. The readout of the MONI-DA indicates the battery charging state (see Table 5, line e).

Power consumption of a MONI-DA system is 5 mW in the standby mode. In the measuring mode, this value is increased depending on the number of the emitter-detector heads connected and on measuring conditions. To give an example, with 7 emitter-detector heads delivering saturation flashes every 15 minutes the system runs on lithium iron-phosphate batteries for more than 4 weeks.

In most cases, power supply by the two solar panels delivered together with MONI-DA systems (MONI-SET-3-DA or MONI-SET-4-DA) is sufficient for long-term operation of the system without line power.

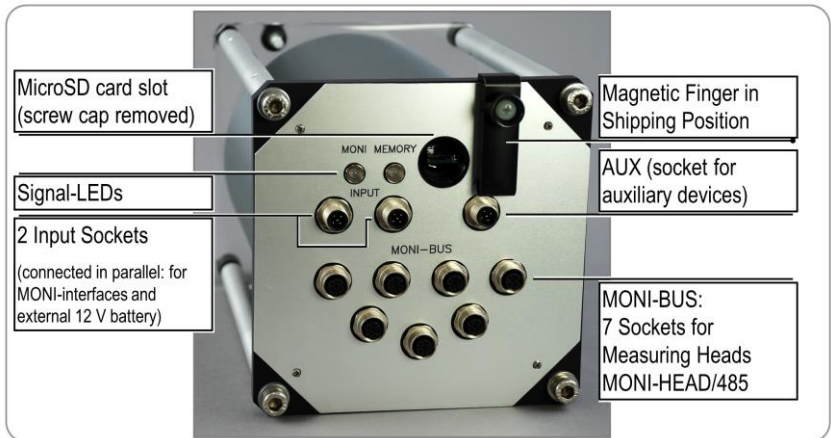


Fig. 13: MONI-DA Rear Face

7.1.2 MONI-DA, MONI-DA/S: Rear Face

Located on the back side of the data acquisition system are the signal LEDs, a memory card slot (not available for MONI-DA/S), a fixing screw for the magnetic finger and the connections for measuring heads, accessories, and interface.

LEDs

The status of the MONI-DA is indicated by the MONI (Monitoring-LED) and the MEM LEDs (Memory-LED). The pair of LEDs is present on both the front and rear face in case of the MONI-DA. The MONI-DA/S possesses the LEDs only on the front face. See Table 3 for the LED codes.

MicroSD Card

By default, both, the MONI-DA and the MONI-DA/S are equipped with an industrial grade 0.5 GByte MicroSD flash card. The MONI-DA also handles 1 and 2 GByte MicroSD cards. For data transfer to a PC, plug in the MicroSD flash card of the MONI-DA in the card reader of your computer.

In the MONI-DA/S unit, accessing the MicroSD card requires opening of the housing: therefore, the card serves for backup of data. Download of data is described in Section 7.4.2 (page 41).

MicroSD Card Handling

Only high-quality Industrial Grade MicroSD cards should be used for data storage. They need to be FAT16 formatted. The FAT32 format as well as SDHC and SDXC are not supported by the MONI-DA.

Do not remove or insert a memory card while writing or reading data that is shortly before, during, and shortly after saturation pulses. Also, do not remove the memory card during data restoration or formatting.

Table 3: Signal LEDs Modem

Status	Event	MONI (Monitoring-LED)	MEM (Memory-LED)
Start	Searching devices	Fast green flashes	Fast green flashes
	Detecting devices	Lights up red	Fast green flashes
Measure mode	Batch or clock running, and device detected	Double green flashes every 2 seconds	
	Batch or clock not running, and device detected	Single green flash every 2 seconds	
	No device detected = Searching devices	Fast green flashes	Fast green flashes
	Memory flash card ok		Double green flashes every 2 seconds
	Memory flash card error		Double red flashes every 2 seconds
	Writing to memory flash card		Fast green flashes
	Saturation pulse execution	Light constantly green	
Hibernation	Batch or clock running, and device detected	Double green flashes every 10 seconds	
	Batch or clock not running, and device detected	Single green flash every 10 seconds	
	No device detected	Double red flashes every 10 seconds	
	Memory flash card damaged		Double red flashes every 10 seconds
	Memory flash card ok		Double green flashes every 10 seconds

To remove a MicroSD card from the MicroSD card connector, gently push and release the SD card. Do not attempt to pull out the MicroSD card without prior unlocking it by a “push-to-release” action. Also, installing the MicroSD card requires push and release.

Memory cards have limited lifetime and should be replaced regularly. The lifetime depends on the number of the storage processes.

Do not touch the contact area of the MicroSD card. When fingerprints or stains are found on the contact area, wipe the area with a soft dry cloth.

Magnetic Finger

The MONI-DA (also MONI-DA/S) is delivered with a “magnetic finger”. The magnetic finger is used to operate the MONI-DA via the 7 magnetic proximity switches at the MONI-DA front face (Fig. 15, page 30). For transport, the MONI-DA system can be completely switched off by placing the magnetic finger in the transport position at the rear face of the MONI-DA. In the off-state, signal LEDs do not flash.

Input and AUX Socket

Two parallel-connected input sockets and one AUX (auxiliary) socket are provided. All three are male M12 5-pole sockets in case of the MONI-DA but the MONI-DA/S uses special waterproof 6-pole sockets. Table 4 (page 29) summarizes the options for connecting various devices to the MONI-DA.

After a computer connection has been established using an interface (see Section 7.2), the standby mode of the MONI-DA is disabled, and the device is operated by the WinControl-3 software which also acquires all currently measuring data. In parallel, writing on the MicroSD card and in internal flash memory continues.

Note that the option to select different measuring light intensities for different measuring heads is available only when the MONI-DA(/S) is operated in the stand-alone mode. Operation by WinControl-3 will set measuring light intensities of all measuring

heads to the intensity level of the measuring head detected first by the data acquisition system.

The MONI-DA battery is charged via a RS 485 communication cable. For charging, the cable is plugged into one of the input sockets of the MONI-DA.

For powering the MONI-DA by an external 12 V battery, the relevant pins are marked in Fig. 14.

Table 4: Assignment of Input and AUX Sockets of MONI-DA

Device	Socket	Input 1 and 2	AUX
PC Interface Box MONI-IB1		yes	no
PC Interface Box MONI-IB4/LAN		yes	no
WiFi Modem/Satellite Modem		no	yes
Solar Panel SP		yes	yes
12 V External Battery		yes	no

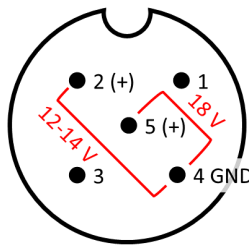


Fig. 14: Pin Assignment for Connection of External Power Sources to MONI-DA

View from the outside. To charge battery, use positions 4 and 5 of Input 1 or 2, or AUX. To connect an external battery, use positions 4 and 2 of Input 1 or 2. Compare Table 4.

MONI-Bus

The MONI-Bus consists of 7 female M12 5-pole sockets for communication with measuring heads MICRO-HEAD/3B (MONI-DA/S: 7 special waterproof 6-pole sockets for connection of MONI-HEAD/S).

7.1.3 MONI-DA, MONI-DA/S: Front Face

The front face of the data acquisition system contains the user interface consisting of a b/w display and a number of magnetic proximity switches. On the front face positioned is a fixing screw for the magnetic finger when the machine is in the acquisition mode.

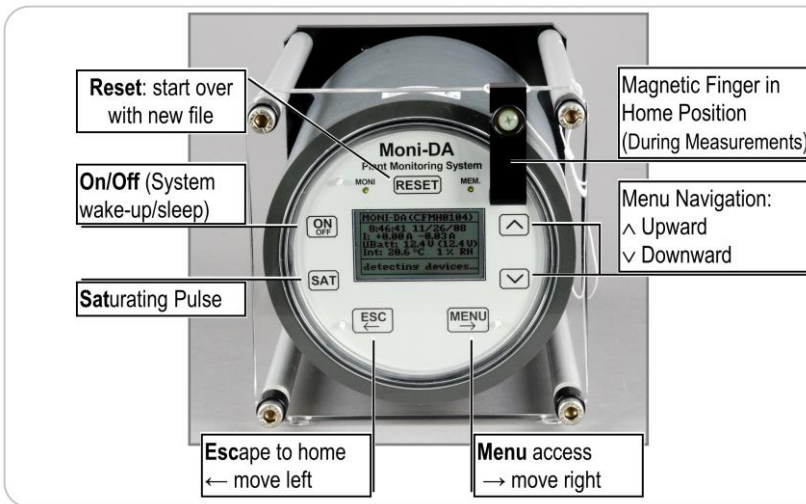


Fig. 15: MONI-DA Front Face

Magnetic Finger

During measurements, the magnetic finger is kept in the home position (see Fig. 15).

Control of the MONI-DA, MONI-DA/S

The MONI-DA can be configured using WinControl-3 software via an interface. A large number of commands and data, however, are directly accessible by the 7 magnetic proximity switches of the front plate. The option to adjust certain settings for different measuring heads individually is only available in the stand-alone mode of the MONI-DA, that is, in the absence of computer control.

To operate the proximity switches, hold the magnetic finger horizontally and move the rounded end of the finger vertically over the switch area. For instructions on operation see Section 7.3, page 35.

7.2 Interface Devices

7.2.1 PC Interface Boxes MONI-IB4/LAN and MONI-IB4/LANS

The MONI-IB4/LAN and MONI-IB4/LAN interface boxes are part of the ONLINE configurations (Section 4.1.1, page 7, and Section 4.2.1, page 10). The boxes have four connectors for RS 485 cables on one side (Fig. 16). These connectors are different for the surface and the underwater systems.

On the opposite site, the charge cable can be plugged in. In addition, three ports are allowing connection to a Windows computer via RS 232, USB, or Ethernet cable.



Fig. 16: MONI-IB4/LAN, MONI-IB4/LANS

The PC Interface Boxes MONI-IB4/LAN and MONI-IB4/LANS possess the same charge, RS 232, Ethernet, and USB connectors, but different RS 485 connectors.

7.2.2 PC Interface Boxes MONI-IB1 and MONI-IB1/S



Fig. 17: PC Interface Box MONI-IB1

The PC Interface Boxes MONI-IB1 and MONI-IB1/S have identical charge and USB ports, but different RS 485 connectors.

For online operation, the PC Interface Box MONI-IB1 connects the MONI-DA data acquisition system to a Windows computer

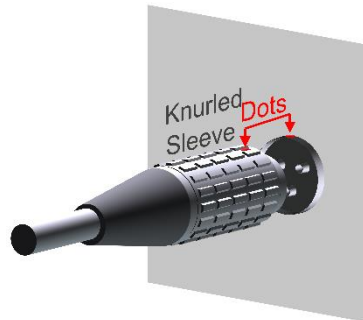
(Fig. 3, page 9. MONI-IB1/S requires MONI-IB1/S, Fig. 5, page 11). The box is equipped with an RS 485 and a USB port (Fig. 17). When connected to a computer, the STAND-ALONE configuration is operated in the online mode, that is, the software WinControl-3 controls the MONI setup and acquires data.

Data stored on the MicroSD card cannot be downloaded via the MONI-IB1 box. Instead, the MicroSD card in the MONI-DA must be removed and read by a computer.

The MONI-IB1 (MONI-IB1/S) interface box is used to charge the MONI-DA battery. Note that the charge cable is plugged into the interface box with a self-locking connector. To connect, align red dots of plug and socket. To disconnect, hold the knurled part of the plug and pull. Never try to disconnect by pulling on the cable.

Fig. 18: Self-locking connector

Applies for power connectors of interface boxes. To lock connection, align red dots of plug and socket and push in. To disconnect, hold the knurled sleeve and pull out.



7.2.3 Four-Way Distributor MICRO-HUB

The MICRO-HUB (Fig. 11, page 21) allows to connect more than 7 measuring heads MONI-HEAD/485 to a data acquisition system MONI-DA. The maximum number of measuring heads per MONI-DA is 16 corresponding to 12 heads connected via MICRO-HUB and 4 directly connected heads. A similar option is not available for the underwater data acquisition system MONI-DA/S.

7.2.4 WiFi Modem MONI-DA/WIFI

The WiFi modem (Fig. 12, page 22) transfers data from the MONI-DA data acquisition system via a Walz server. The data can be downloaded to the user's computer (see Section 7.5, page 47). The WiFi modem requires access to a network, or connection to a hotspot.

7.3 MONI-DA Operation

Table 5: MONI-DA Operation

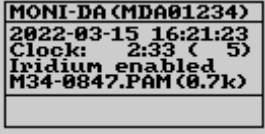
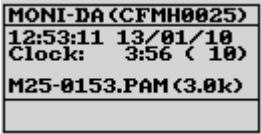

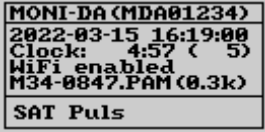
	KEY COMMAND	SCREEN	COMMENT
a	RESET Detects devices connected (here satellite modem) and starts a new data file on MicroSD card	Home Screen 	
b	On/Off ON switches the home screen on. OFF puts the MONI-DA into the standby mode	Home Screen 	
c	 Starting from the home screen, the keys scroll through four pages (d to f , and h)		Pages accessible: Home Screen (see d) MONI-DA values (see e) Measuring Head values (see f) Fast Kinetics Screens (see h)
d		Home Screen 	1st line. Serial number of MONI-DA (in brackets) 2nd line. Time & date 3rd line. Time until next event (mm:ss); In <u>clock mode</u> : time till next flash & clock interval. In <u>batch mode</u> : time till next flash 4th line. Display of device connected (here WiFi modem) 5th line. File name, file size 6th line. Message row

Table 5: MONI-DA Operation

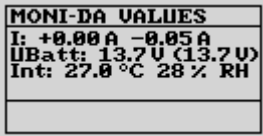
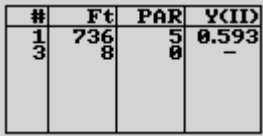

	KEY COMMAND	SCREEN	COMMENT
e		<p style="text-align: center;">MONI-DA Values</p> 	<p>1st line. Screen title</p> <p>2nd line. Charging & consumption current</p> <p>3rd line. Battery voltage during measurement and saturation pulse (bracketed)</p> <p>4th line. Temperature & relative humidity inside MONI-DA</p>
f		<p style="text-align: center;">MONI-HEAD Values</p> 	<p>Column Content</p> <p># Measuring Head ID</p> <p>Ft Fluorescence level</p> <p>PAR Photosynthetically active radiation, $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$</p> <p>Y(II) Effective photochemical quantum yield of photosystem II</p>
g	<p>➤ and “MONI-HEAD Values” screen displayed (see f)</p>	<p style="text-align: center;">PAM Settings</p> 	<p>1st line. Screen title</p> <p>2nd line. Key ➤ followed by ⬆ and ⬇ keys adjust saturation pulse length. Key ⬅ escapes.</p> <p>3rd line. Keys ⬇ and ➤, selection of “Yes” by ⬇ followed by ➤ restores factory settings in all measuring heads</p>

Table 5: MONI-DA Operation



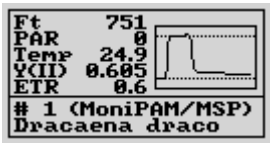
	KEY COMMAND	SCREEN	COMMENT
h	<p>Use  and  key to select MONI-HEAD</p> <p>SAT and “Fast Kinetics” displayed triggers a saturation pulse only in the measuring head selected</p> <p>SAT triggers a saturation pulse in all measuring heads when “Fast Kinetics” is NOT displayed</p>	<p style="text-align: center;">Fast Kinetics</p> 	<p>Ft, PAR, and Y(II): see f</p> <p>Temp: Temperature</p> <p>ETR: Electron transport rate</p> <p>Last two lines: Device number and microprocessor typ. Comment written in WinControl-3</p> <p>Graph: Fluorescence trace during saturation pulse</p>

Table 5: MONI-DA Operation


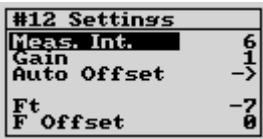












KEY COMMAND	SCREEN	COMMENT
<p>i  and “Fast Kinetics” screen (h) displayed</p>	<p style="text-align: center;">MONI-HEAD Settings</p> 	<p>1st line. Measuring Head ID</p> <p>2nd line. Intensity setting of PAM excitation light. To adjust, use key  followed by  and  keys. Key  escapes</p> <p>3rd line. Amplification factor. To change use keys  and  and subsequently the  and  keys</p> <p>4th line. Automatic determination of background signal. To determine background signal, direct optical window away from objects and light sources, select Auto Offset by  and , and choose Yes ( and )</p> <p>Ft and Offset are the currently measured fluorescence level and the currently used background signal, respectively</p>

Table 5: MONI-DA Operation


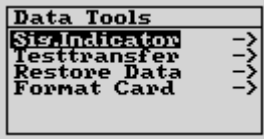
	KEY COMMAND	SCREEN	COMMENT
j	<p>➤ (Menu) and home screen (a) displayed, or ➤ (Menu) and MONI-DA values (e) displayed</p> <p>To navigate, use up/down (▲▼) and left/right (◀▶) buttons</p>	<p style="text-align: center;">Main Menu</p>  <p><i>* In the batch mode, the MONI-DA is controlled by the currently loaded batch file (see . Section 8.2.5, page 69)</i></p> <p><i>** UTC=Coordinated Universal Time. "Time Offset" determines the time stored on flash memory, and the time displayed. UTC is used for internal data processing.</i></p>	<p>1st line. Screen title</p> <p>2nd line. Access to MONI-DA firmware and flash memory information: Revision number and date, flash memory size in data sets, and operating hours</p> <p>3rd line. Adjustment of intervals between saturation pulses</p> <p>4th line. Setting of saturation pulse control: either clock or batch mode*</p> <p>5th line. Opens menu Data Tools (see k)</p> <p>6th line. Setting date and time</p> <p>7th line. Offset relative to UTC**</p>
k	<p>To navigate, use up/down (▲▼) and left/right (◀▶) buttons</p>	<p style="text-align: center;">Data Tools</p> 	<p>1st line. Screen title</p> <p>2nd line. Displays signal strength detected by modem</p> <p>3rd line. Tests data transfer by modem</p> <p>4th line. Writes data from internal memory to MicroSD card. Capacity: 10 days with 4 measuring heads and clock interval of 5 minutes</p> <p>5th line. To FAT16 format a MicroSD card</p>

Table 5: MONI-DA Operation

	KEY COMMAND	SCREEN	COMMENT
I	➤ and “Sig. Indica- tor” selected	<pre>Datatransfer Iridium enabled signal: 0/5</pre> <hr/> <pre>Datatransfer WiFi enabled signal: -76 dBm</pre>	<p>Satellite Modem Relative signal strength. 0, n signal. 5, high sig- nal strength.</p> <p>WiFi Modem Signal strength. -30 dBm, maximum. -60 to -67 dBm, fair. -80 dBm, check net- work.</p>
m	➤ and “Test Trans- fer” selected	<pre>Datatransfer Iridium enabled connection ok</pre> <hr/> <pre>Datatransfer WiFi enabled connection ok cloud ok sending ok</pre>	<p>Satellite Modem Checks satellite con- nection.</p> <p>WiFi Modem Checks the following. <u>Connection</u>, connection to network. <u>Cloud</u>, con- nection to server. <u>Send- ing</u>, acceptance of data by server.</p>

7.4 Data Storage and Transfer

7.4.1 MicroSD Card

By default, both, the MONI-DA and the MONI-DA/S are equipped with an industrial grade 0.5 GByte MicroSD flash card. The MONI-DA also handles 1 and 2 GByte MicroSD cards. For data transfer to a PC, plug in the MicroSD flash card of the MONI-DA in the card reader of your computer.

When the MicroSD card is removed and plugged back, the MONI-DA will resume writing on the last data file. Saturation flash data in the absence of a card can be retrieved from the MONI-DA internal flash memory (see Section IV). New data files are created with each clock start or by a system reset.

Data transfer from memory card to computer requires removal of the MicroSD memory card and readout using the card reader of a computer. Alternatively, data can be read from an internal flash memory chip (see next, Section 7.4.2).

7.4.2 Internal Flash Memory Chip

Properties

Parallel to saving data on MicroSD, data are stored on an internal 2, 4, or 8 Mbyte flash memory chip, depending on the version of MONI-DA (MONI-DA/S). The flash memory acts as ring buffer which means that, once filled, the latest data overwrite the oldest ones.

Flash Memory Size

The “Info” page of the MONI-DA menu indicates the size of the flash memory chip as maximum number of “data sets” that can be stored. Per saturation flash analysis, one data set is created by the MONI-DA and also one data set per each of the measuring heads connected to the MONI-DA. The relationship between flash memory sizes in Mbyte and data sets is given in Table 6:

Table 6: Internal Flash Memory

Size of flash memory chip, Mbyte	Maximum data sets
2	12000
4	28000
8	60000

The maximum available experimental time can be assessed according to:

$$\text{Time}_{\max}[\text{days}] = \frac{\text{Memory Size}[\text{data sets}] \cdot \text{Clock Interval}[\text{min}]}{1440 \cdot (\text{Number of Measuring Heads} + 1)}$$

The same equation can be used to assess the capacity of MicroSD cards which usually can hold data of many years but will stop storing data when full.

Preparations for data transfer

Connection between an input socket of data acquisition unit and an RS 485 socket of a PC interface (MONI-DA: MONI-IB1, MONI-IB4/LAN. MONI-DA/S: MONI-IB1/S or MONI-IB4/LANS).

USB connection between the PC interface box and computer using an AB-type USB cable

Previous installation of WinControl-3 to have the necessary drivers installed.

The executable program **bustool.exe**.

Data Transfer

Open DOS input window by clicking on the Windows start button and then on the run icon (or enter RUN in the search line right of the Windows start button).

Type cmd. This opens the command prompt window.

Start program `bustool.exe` by entering location of the program and program name. For example, `C:\Directory1\Directory2\bustool.exe` commands

To list all data files in flash memory, type: **`bustool -a 0 -l`** (`bustool`, space, minus letter `a`, space, number zero, space, minus letter `l`) and hit the enter key. The **`-a`** followed by **`0`** specifies the device address which is always zero in the case of the MONI-DA, and the **`-l`** is the list command.

The file list appearing in the command prompt window contains the information specified in Table 7:

Table 7: File Information

id	measurement start	measurement start	file size	filename	comment
MONI-DA file number	date	Time	number of data sets	file name	incomplete file note
integer	yyyy-mm-dd	hh:mm:ss	integer	Mss-iiii.PAM where ss = MONI-DA device number and iiii = consecutive file counter	(partial)

Note: the number of data sets per saturation pulse analysis is 1 + the number of measuring heads connected to the MONI-DA. The comment "(partial)" indicates that the file is incompletely present because it has been partially overwritten by newer data.

To download the file with the MONI-DA id number **X**, type **`bustool -a 0 -d X`** and press the enter key. The command downloads file **X** into the directory of the program `bustool.exe`. Communication has been optimized to work properly even for long data lines: therefore, a Baud rate of 19200 was selected which corresponds to about 5 data sets per seconds.

Delete

Delete: files on the flash memory cannot be deleted. Oldest data will be automatically overwritten by the newest ones.

Help

Type **bustool -h** and enter key

7.4.3 Network connection via Ethernet

Connections

Connect MONI-IB4/LAN to a network, thereafter connect line power.

Find IP address/DHCP host name

The DHCP hostname and Hardware Address are printed on the MONI-IB4/LAN. The DHCP hostname is identical to the serial number (S/N) and has the format CFMIXXXX (the X stands for Arabic numerals. MONI-IB4/LANS: CFMMXXXX).

Start network mode of WinControl-3

Click on Windows Start button.

Select Programs and WinControl-3.

Select WinControl-3 Network Mode.

Now the Network connect window appears. Enter the DHCP host name and click OK. The connection to the PAM device will be established.

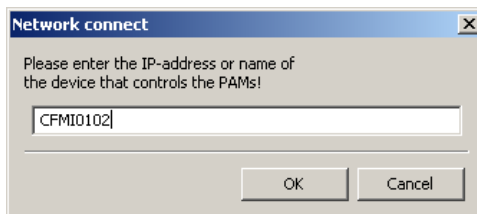


Fig. 19: Network Connect Window

The IP address also can be entered in the Network connect window but only the DHCP host name provides a permanent address when different IP addresses are assigned to the MONI-IB4/LAN by the DHCP server.

Find MONI-IB4/LAN addresses

In case the procedure described above was not successful, you can check the address of the MONI-IB4/LAN as described next:

Download from DeviceInstaller program from Lantronix webpage: <http://www.lantronix.com/device-networking/utilities-tools/device-installer.html> (Note that the DeviceInstaller requires the Microsoft .NET framework which is present on up-to-date computers but is also available via the Lantronix webpage.

Install and launch DeviceInstaller. The MONI-IB4/LAN should now appear as XPort with IP and hardware address (Fig. 20).

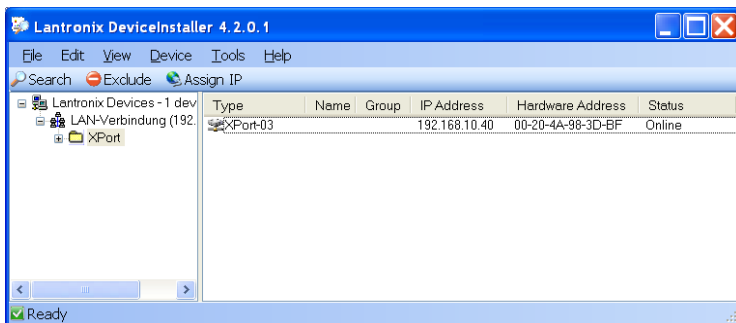


Fig. 20: DeviceInstaller 1

Click on XPort until the right window displays the “Device Details”, “Web Configuration” and “Telnet Configuration” tabs (see Fig. 21).

Select <Web Configuration> and navigate to XPort by clicking the white arrow (→) on green background.

Hit <Enter> key to ignore the User/Password window.

Click <Network> on the XPort window (upper left corner).

The next window shows the DHCP host name of MONI-IB4/LAN.

If network connection with new address information still cannot be established, contact your network administrator to assign an IP address manually using the <Assign IP> function of the DeviceInstaller software.

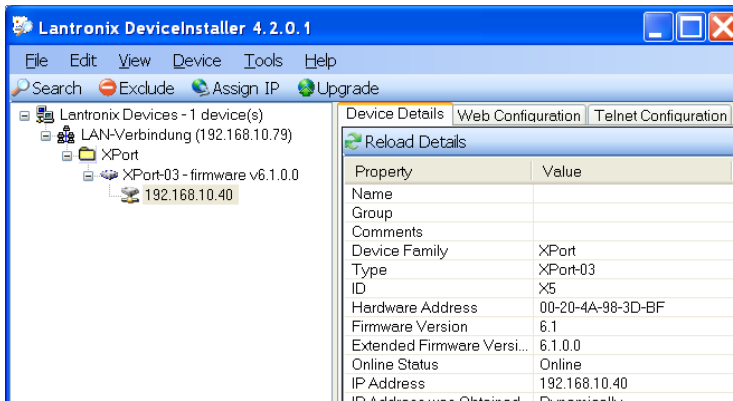


Fig. 21: DeviceInstaller 2

7.4.4 WinControl-3

The MONI-DA can be operated under WinControl-3 using a PC interface. When controlled by WinControl-3, the magnetic proximity switches of the MONI-DA are unresponsive except the RESET button.

In WinControl-3, information on MONI-DA and connected devices are displayed in the MONI-Bus window of WinControl-3. Data on the MONI-DA can be viewed under settings when MONI-DA is selected in the device drop-down list (upper left corner of settings window). Similarly, physical information on

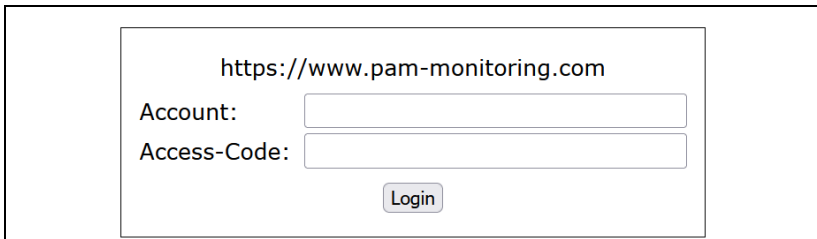
devices connected to the MONI-DA can be retrieved by selecting the device in the device drop down list.

7.5 Modem

7.5.1 Download MONI-DA Data

Wireless transfer of MONI-DA data can be carried out via WiFi or Iridium satellite modem. Data transfer is also performed by the earlier GPRS modems which are no longer produced.

In all cases, a Walz server receives the MONI-DA data. Walz provides account name and access code to download your data. The server's URL is <https://www.pam-monitoring.com/>. Fig. 22 displays the welcome window of the Walz server.



https://www.pam-monitoring.com

Account:

Access-Code:

Login

Fig. 22: Login to Walz Server

After login, the server displays your account(s) and the time elapsed since the last transfer of MONI-DA data (Fig. 23). Transfer activity is also listed for images when a camera is installed at the experimental site (Column “Last image update”, Fig. 23). The frequency of data transfer is additionally indicated by a color code, where green indicates normal traffic.

To download the data of the last 30 days, click on the file name in column “Last Upload (CET/CEST)” (Fig. 23). To download data of the last year or of the last 24 hours, click on [1 year](#) or [24 h](#), respectively, and then the download button on top of the

graph. To download data of other intervals, copy link for 30 days download (right-click on filename in column “Last Upload (CET/CEST)”). The example link below is for account “walz1” and the last 30 days of data:

<https://www.pam-monitoring.com/cgi-bin/download?user=walz1×pan=30>

Paste link in any text editing software and replace the “30” by the number of previous days that you want to download. Then copy the modified link and paste it in the address line of your web browser. Hit enter.

Account	PAM Data Transfer Date, Time - Time Elapsed		Video Image Transfer Date, Time - Time Elapsed		Download Graphics	
Account	Last upload (CET/CEST)		Last image upload		Download Chart	
walz1	2022-03-18 21:01:11	2 min	2022-03-18 21:01:07	2 min	1 year	24 h
walz2	2021-04-13 08:47:00	339 d	-	-	1 year	24 h
walz3	2018-10-22 18:00:31	1243 d	-	-	1 year	24 h
walz4	2018-11-27 06:40:02	1207 d	-	-	1 year	24 h
	< 1h	< 6h	< 48h	> 48h	No data	

Current time: 2022-03-18 21:03:19

Fig. 23: Data Information on Walz Server

7.5.2 WiFi Modem Upload

Connect WiFi modem to the AUX port of the MONI-DA, and MONI-DA to computer. The modem is automatically detected by the MONI-DA. The WiFi/GPRS area on the Settings window of WinControl-3 has four input fields (Fig. 24 and Table 8). The Walz server information (Account and Access Code) is preprogrammed and should automatically appear. If this information is absent, enter the access information provided by Walz. SSID

and key are properties of the WiFi network or the hotspot. Check data transfer using the MONI-DA screen (Table 5, l and m).

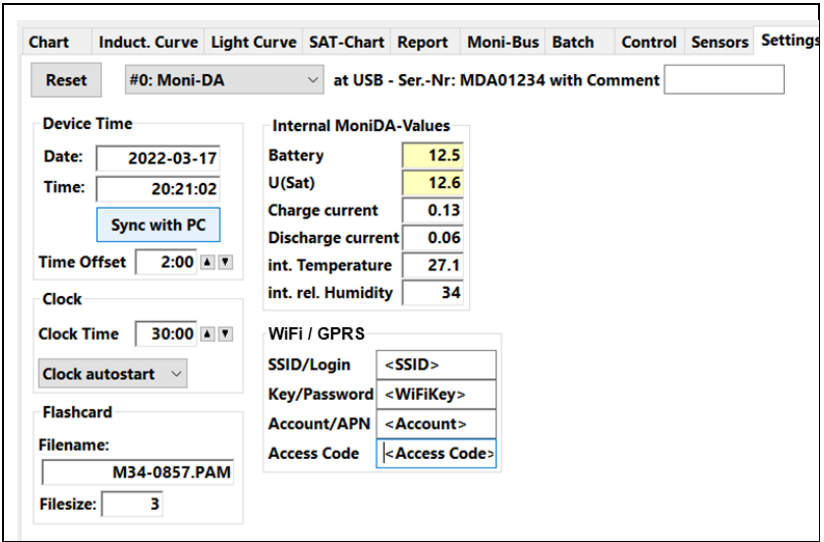


Fig. 24: Modem Settings

Table 8: Login Information

	WiFi	GPRS
SSID/Login	SSID: Access data of WiFi network.	Login: As instructed by your SIM card provider.
Key/Password	Key: Access data of WiFi network.	Password: As instructed by your SIM card provider.
Account/APN	Account: Access data of Walz server	APN (access point name): As instructed by your SIM card provider.
Access Code	Access Code: Access data of Walz server	

7.5.3 GPRS Modem Upload

The production of GPRS modems was ceased. The instructions given is for users having acquired the GPRS modem earlier.

7.5.3.1 Cost Considerations

At each saturation flash analysis, the GPRS modem sends data of the previous saturation flash analysis. The data files sent are rather small. Typically, one set of saturation pulse data of a MONI system comprising six measuring heads corresponds to about 1 Kbyte; the amount of data varies roughly proportionally with the number of measuring heads of the MONI system.

GPRS providers offer flat rates, or they charge according to the amount of data transferred. In the latter case, the total amount of data is calculated as the sum of packages of definite size, that is, always the complete package size will be added even if the actual size of data transferred is smaller than the package size.

Let us consider that a GPRS provider uses a rather small package size of 10 Kbyte. This package size is clearly greater than the saturation pulse data of even complex MONI systems. If saturation pulse analyses are carried out every 10 minutes, the data size per month is

$$10 \text{ Kbyte} \cdot (6 \cdot 24 \text{ packages/day}) \cdot 30 \text{ days} = 43 \text{ Mbyte}$$

Hence, a contract for 50 Mbyte per month would be sufficient to cover the data transfer of 43 Mbyte calculated above. Often, the minimum total transfer volume of GPRS providers is 100 Mbyte. Still, these 100 Mbyte might be cheaper than GPRS flat rates.

7.5.3.2 Operation

The Walz GPRS modem uses serial data transfer to communicate with the MONI-DA. For insertion or replacement of the SIM card, the weatherproof case of the modem has to be disassembled as described overleaf. Standard SIM cards (“Mini-SIM”) can be used in the modem. Also, Micro- and Nano-SIM-cards can be employed using appropriate adapters.

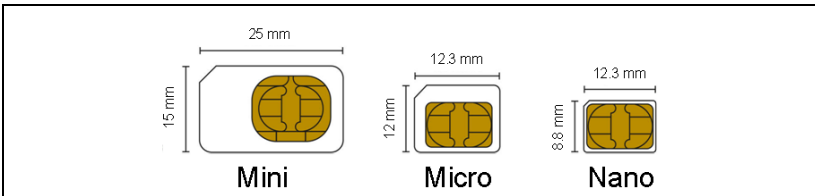


Fig. 25: SIM Cards

Any PIN code protection of the SIM card needs to be inactivated before the SIM card is installed in the GPRS modem. To inactivate the PIN code, insert SIM card in a suitable mobile phone and proceed as described in the phone’s manual.

To mount the SIM card, unscrew the four hex head screws at the front panel which holds the connectors and the status LED (cf. drawing). Carefully draw out electronics. Pay attention to keep the two gaskets of the front plate in place (Fig. 26).

The main circuit board holds a smaller board which contains the modem as well as the SIM card slot (drawn in red, Fig. 26). To install a SIM card, simply push the card into the slot and release. To remove SIM card, gently push and release card. Do not attempt to pull out the SIM card without prior unlocking by a “push-to-release” action.

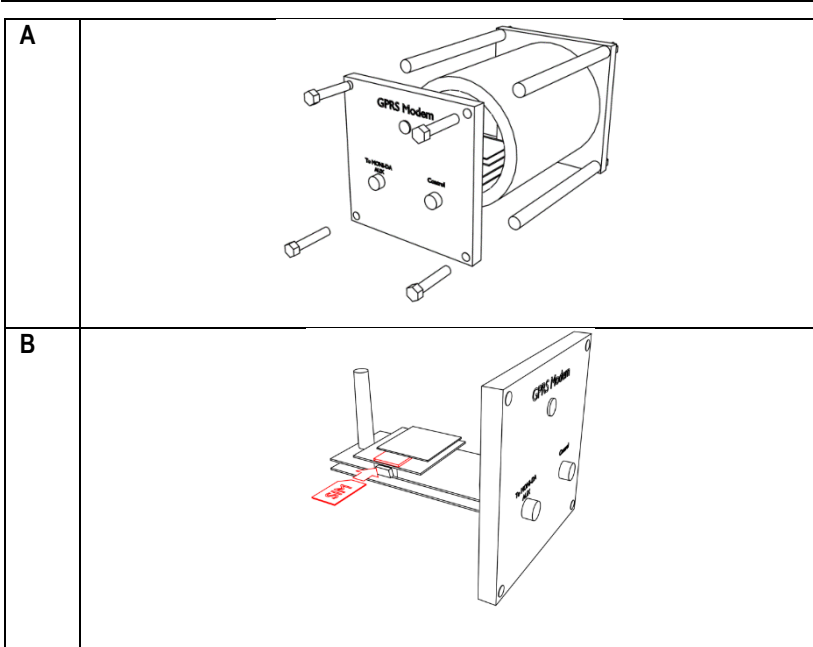


Fig. 26: SIM Card Mounting

A, opening of GPRS modem housing. B, insertion of SIM card

To establish the GPRS connection, connect GPRS modem to AUX port of the MONI-DA, and connect MONI-DA to computer. To connect, only three input fields of the WiFi/GPRS area have to be filled in: Login, Password, and APN (Fig. 24 and Table 8). These data are delivered with the GRPS service of your provider.

7.5.4 Satellite Modem

Connect satellite modem to the AUX port. The satellite modem is fully preprogrammed (Including “International Mobile Station Equipment Identity”, IMEI) and the WiFi/GPRS area has no entries. Test data transfer as instructed in Table 5, sections l and m.

8 WinControl-3 Software

8.1 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/monitoring-pam/downloads.html

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

8.1.1 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 .
- 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 MONI-DA 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.
- Connect MICRO-HEAD/3B to computer and run PAM Firmware Update. Measuring heads must be connected via interface (MONI-IB4/LAN or MONI-IB4/LAN) to the computer. Firmware update does not work when the MICRO-HEAD/3B are connected to the MONI-DA.

*The term firmware denotes a piece of software residing on a flash memory of the MINI-PAM-II. Newest firmware is delivered as part of the WinControl-3 software.

Table 9: WinControl-3 in Windows Start Menu



WinControl-3



PAM Firmware Update



Uninstall WinControl-3



WinControl-3



WinControl-3 – Network Mode

WinControl-3 – Offline

8.1.2 WinControl-3 Program Group

Setup of WinControl-3 creates the WinControl-3 program group (Table 9, p. 54) in the Windows Start menu. The 5 items of the WinControl-3 program group are.

(1) PAM Firmware Update

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. 27, page 56). 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. 28, page 57) 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.

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.

(2) Uninstall WinControl-3

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

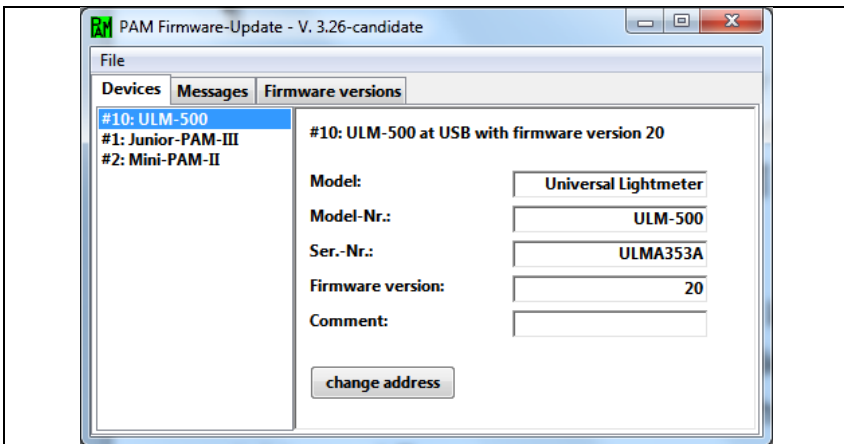
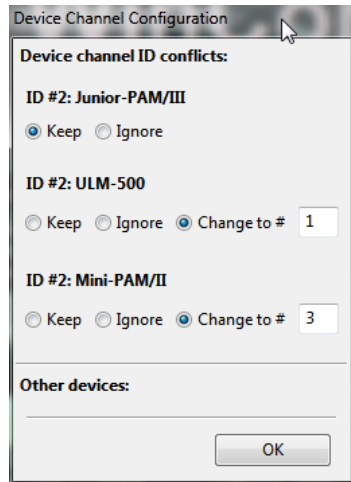


Fig. 27: PAM Firmware Update

Several devices connected to the same computer must have different addresses (channel numbers).

Fig. 28: Device Channel Configuration



(3) WinControl-3

Subfolder “WinControl-3” 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 devices, measuring of fluorescence is automatically started. To continuously display the fluorescence signal (Ft), check “Rec. Online” or click **Start Online**. If the Ft is much lower than 250, check leaf position and increase measuring light intensity and/or gain. 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.

(4) WinControl-3 Network Mode

See 7.4.3, page 44.

(5) WinControl-3 Offline Mode

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

8.2 WinControl-3 Operation

Launch software via desktop icon or the WinControl-3 folder in the Start menu of Windows. The software will automatically detect all devices connected and being compatible with the WinControl-3 software. The following section will provide an overview on the operation of a MICRO-PAM system by WinControl-3. More details are available in the latest edition of the Manual for the MINI-PAM-II.

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

8.2.1 Chart Window

The Chart window (Fig. 29) continuously records PAM fluorescence (F_t) and the results of saturation pulse analysis. The amount of data produced by a MICRO-PAM system can be overwhelming. To get an overview, reduce the number of data displayed by selecting parameters on the “Val.” panel, or by selecting channels on the “Rec.” panel or by both.

In the same way, data display can be controlled in other windows (Induct. Curve, Light Curve, and Report). In the latter case, the data selection also corresponds to the data exported as CSV file.

On the chart, an X axis interval can be marked by the cursor with the left mouse button pressed. A right click in the marked area opens a context menu. All marked data are also highlighted in the SAT Chart window and the Report.

Table 10 summarizes and explains the commands of the Chart Window.

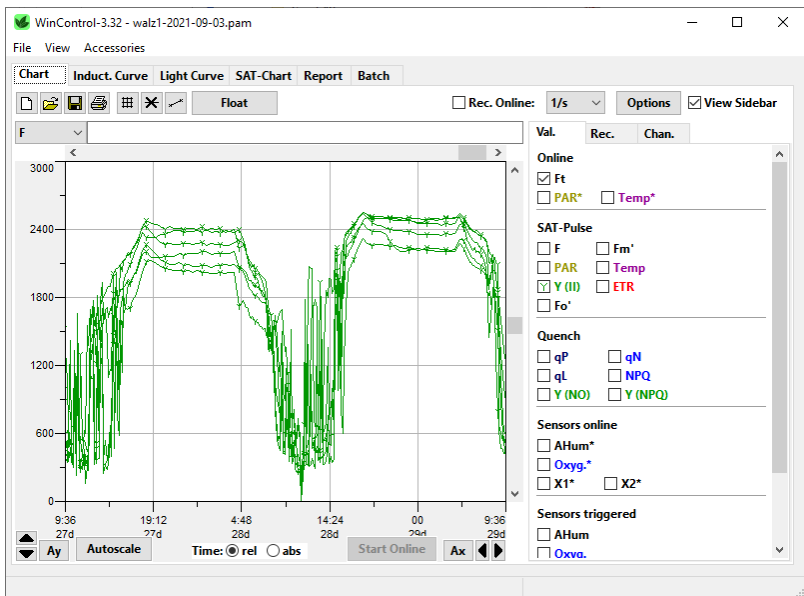










Fig. 29: Chart Window

Table 10: Chart Commands		
Icon	Command	Comment
	Delete	Delete current data (all records and report data)
	Load	Delete current data and load previously saved data. Same function as Load Data in File menu.
	Save	Save all records of the current experiment. Same function as Save Data in File menu
	Print Chart	Print current chart
	Switch Grid on/off	Graph design command
	View Symbols on/off	Graph design command
	View Lines	Graph design command
Float	Float	Create a floating chart window. Graph settings and data selection of the new chart are independent of the WinControl-3 main window.
<input checked="" type="checkbox"/> Rec. Online	Record Online	Record continuously Ft, PAR* and Temp*. For long-term measurements, Rec. Online may be turned off when memory size is small.
5/s	Sampling Frequency	Dropdown menu with three different sampling frequencies for online record: <5/s>, <1/s>, and <1/10s>. The frequency setting affects only online data (Ft, PAR*, and Temp*, see below). The highest sampling frequency of <5/s> applies only to Ft: sampling of PAR* and Temp* occurs with <1/s> maximally. The actual intervals between measurements vary depending on communication between fluorometer and computer.

Icon	Command	Comment
	Options	Menu with 4 items of which two (Zoom to Selection and Export Selection) are available only after chart data have been selected. To select data, place mouse cursor in the Chart area, move mouse cursor with left mouse button pressed parallel to the x-axis across the data of interest: the selection will be highlighted. The options menu is also available by placing the cursor within the selected area and clicking the right mouse button. A single left-button click in the chart area removes an existing selection. Note that a selection made in the chart highlights the associated data in the SAT-Chart and the Report windows.
Export All	Export All	Export all data currently graphed (online and saturating pulse analysis data) as CSV file.

8.2.2 Moni-Bus Window

The Moni-Bus Window provides an overview to all devices connected to the MICRO-PAM (Fig. 30).

(p. 62) depicts the situation of 6 measuring heads connected to a MONI-DA. The numbers preceded by a hash in column 1 are channel numbers. Graphical and numerical data in WinControl-3 are always associated to the channel number.

The second column (Comment) can be edited. These comments are stored in the MICRO-PAM flash memory.

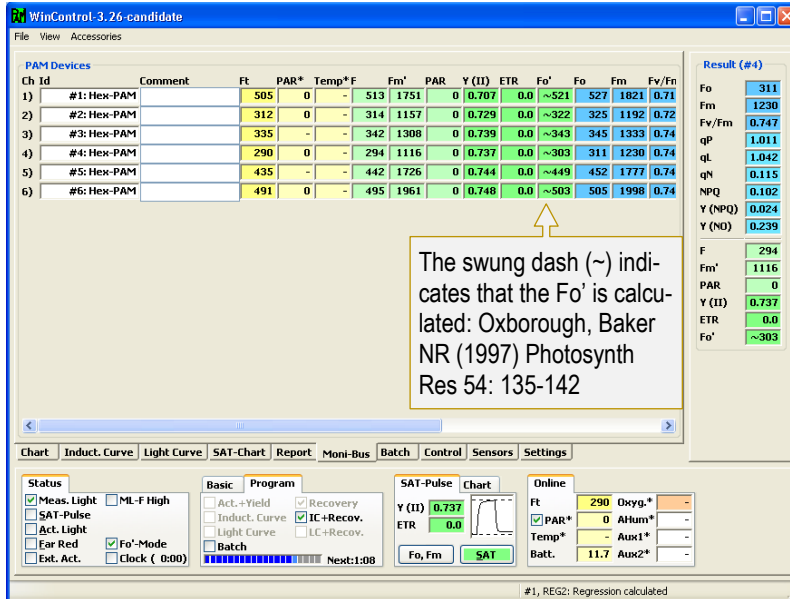


Fig. 30: Moni-Bus Window

Screenshot taken with predecessor model HEX-PAM.

8.2.3 SAT Chart Window

The SAT chart window displays fluorescence kinetics of the current experiments. In saturation pulse analysis, the F_M and F_M' levels are determined as the maximum of these curves. Factory settings of saturation pulse width and intensity are adjusted reach a plateau with normal green leaves (Fig. 32A). Some samples do not reach a plateau with standards settings (Fig. 32B). In this case, the saturation pulse intensity or/and length should be increased. Also, fluorescence kinetics can reach a maximum clearly before the end of the saturation pulse (Fig. 32C). This does not result in erroneous F_M or F_M' values because these values correspond to the maximum of fluorescence

kinetics. In this case, saturation pulse intensity or/and length might be decreased.



Fig. 31: SAT Chart Window

Some samples, particularly low light grown or senescing plants, exhibit somewhat decreased F_V/F_M values with standard settings 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 below (and above) standard settings is worthwhile to optimize your saturation pulse settings.

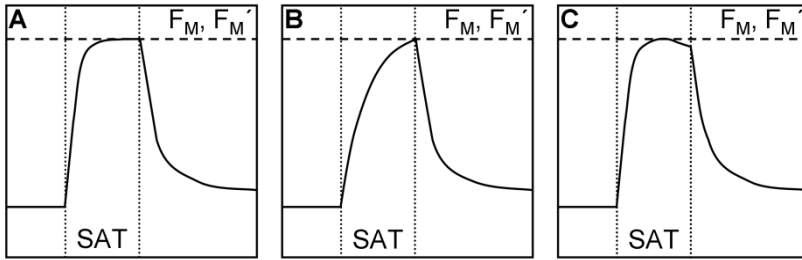


Fig. 32: SAT Pulse Kinetics

SAT pulse length is fine (A), too short (B), too long (C).

8.2.4 Light Curve Window

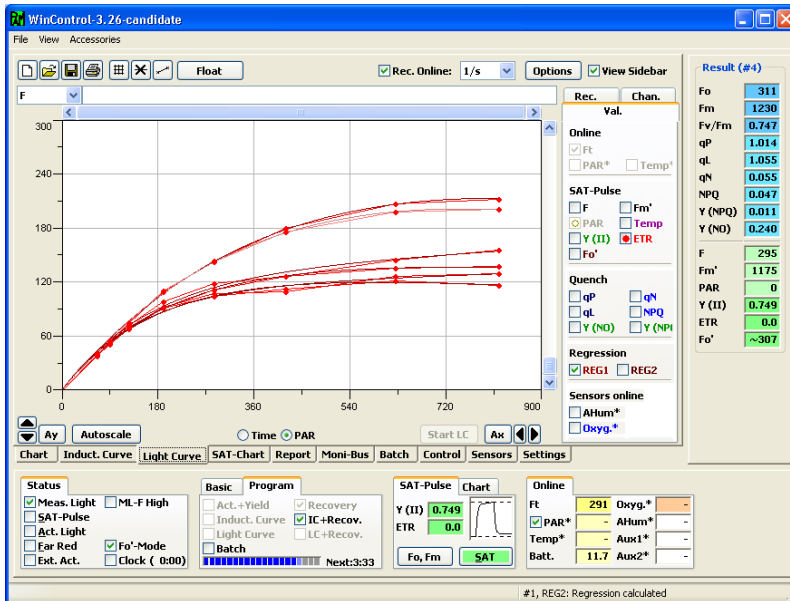


Fig. 33: Light Curve Window

Screenshot taken with predecessor model HEX-PAM.

The Light Curve window provides the button to start a light curve and to view the results of saturation pulse analysis plotted

against PAR (Fig. 33, p. 64). The experimental course of a light curve is defined on the settings page.

A light curve program exposes a sample to stepwise increasing intensities of actinic illumination. In “Rapid Light Curves” (RLC), the time interval of each light step is short (down to 10 s) and full equilibration of photosynthetic reactions is not reached within an illumination interval. Typically, the RLC starts at a PAR value somewhat below that of the natural environment. RLC measurements are carried out with samples in their momentary acclimation status, that is, without dark-acclimation period to determine F_0 and F_M . This way, RLC data can provide information on the present acclimation state of photosynthesis. Obviously, without F_0 and F_M determination, those fluorescence ratio parameters requiring F_0 and F_M (like NPQ) are not available.

If illumination steps are long enough to reach steady state of photosynthesis, fluorescence-based light curves may be compared with classical light response curves (P-I curves).

8.2.4.1 Light Curve Theory

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. 34 and Fig. 35, 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.

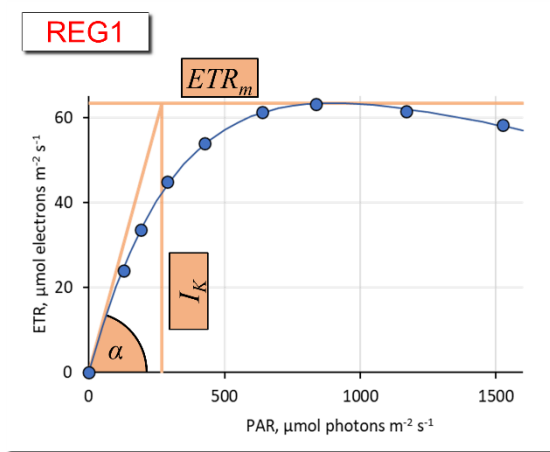
Both models calculate the three cardinal parameters of a light curve:

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

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

(iii) I_K , $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$: 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".



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

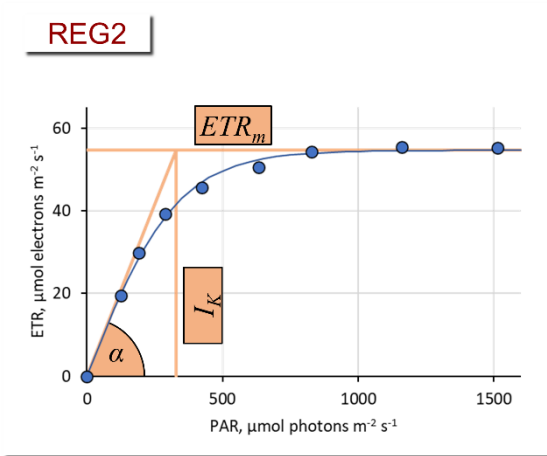
$$ETR_m = ETR_{mPot} \cdot \left(\frac{\alpha}{\alpha + \beta}\right) \cdot \left(\frac{\beta}{\alpha + \beta}\right)^{\frac{\beta}{\alpha}}$$

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

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

Fig. 34: Model Function REG1

The three cardinal points of the light curve are indicated (α , ETR_m , and I_K). The decrease at $PAR > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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



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

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

Fig. 35: 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.

8.2.5 Batch File Window

Batch files automatically execute experimental procedures. To activate the Batch window, click icon new batch or open existing batch file (Fig. 36, “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.

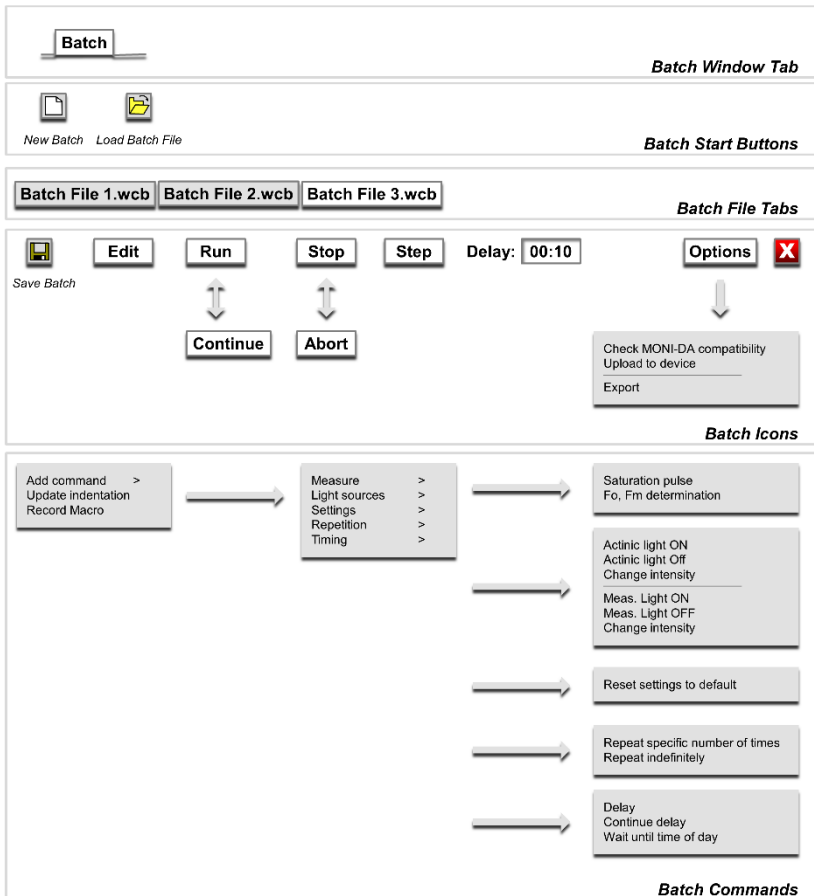


Fig. 36: Batch Window Overview

8.2.5.1 Add Command

Add command leads to five groups of commands (Fig. 36). A command can be inserted into a batch file by left-click.

Measure commands

Saturation pulse	Saturation pulse analysis of light-exposed sample
Fo, Fm 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: simply enter the light intensity setting in the grey-shaded field. New intensity settings can be chosen using the command “Change intensity”.

Settings commands

“Reset settings to default” installs default setting.

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.

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.

8.2.5.2 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.

8.2.5.3 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.

8.2.6 Settings Window

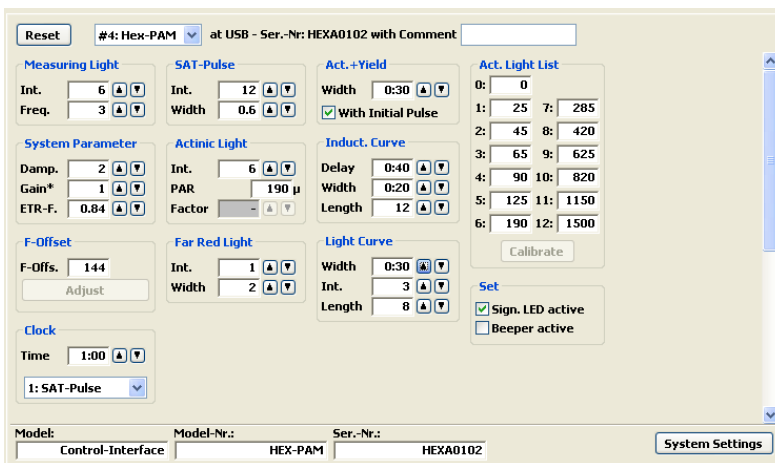


Fig. 37: Settings Window

Screenshot taken with predecessor model HEX-PAM.

The Settings window provides all options to configure instrument settings and the automated experimental routines Actinic + Yield, Induction Curve and Light Curve. These routines can be started manually by corresponding check boxes in the field “Program” on the bottom of all WinControl-3 windows. Also, they can be automatically triggered by the clock function.

Measuring light

Int. (intensity) and Freq. (frequency, see Table 11) adjustment. Frequency high (100 Hz) is active during saturation pulse analysis.

Table 11: Measuring Light Frequencies

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

Damping

Damping is a software-based filter that specifically suppresses high frequency noise and, thus, can improve signal quality. Default setting for damping is 2 (two). Changing damping to higher values can make the MICRO-PAM response slow.

Gain

Gain corresponds to an electronic amplification factor. Settings 1 to 4 are available.

Adjust F-Offset

The 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 command determines the background signal for all measuring light intensities and all gain settings. The currently active offset is displayed in the Moni-Bus Window with disconnected measuring heads.

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"

Clock Interval and Item

Adjust clock interval between 10 s and 60 min by adjusting the time interval (up/down keys) or enter numbers after double click. Choose clock item from drop down menu.

Clock items are saturation pulse analysis, Actinic + Yield, Induction Curve and Light Curve. A recovery experiment can be performed after induction and light curves (LC+Rec and LC+Rec).

SAT-Pulse

Adjust intensity (settings 1 to 12) and interval (width, 0.2 to 2.0 s) of saturation pulses.

Actinic Light

Adjust intensity of actinic light (settings 1 to 12). Values of the actinic light list are used.

Far Red Light

Does not apply for standard measuring heads of MICRO-PAM systems.

Actinic + Yield

The behavior of the Actinic + Yield program is defined by two factors: 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). The current actinic light intensity is used.

Induction Curve

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) and beginning of actinic illumination.

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

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

Light Curve

Width defines the interval between two successive saturation pulse analyses in the light phase. Intensity specifies the actinic intensity setting for the first light step (see “Act. Light List”). 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.

Calibrate

Calibration routine for actinic light. Requires measuring head with PAR sensor.

9 Saturation Pulse Analysis

9.1 Pulse-amplitude Modulated (PAM) Fluorescence

The PAM principle is illustrated by Fig. 38. 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. 38, 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. 38, 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 ”.

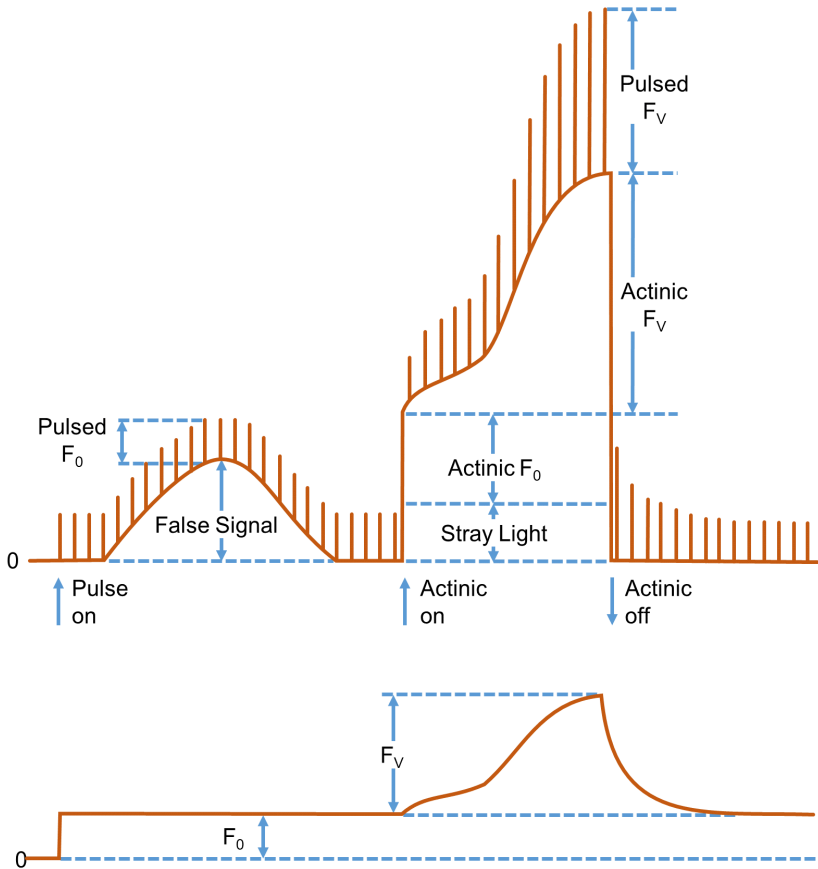


Fig. 38: Illustration of the PAM measurement principle

Figure redrawn for 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. 38 outlines the PAM fluorescence trace. Obviously, PAM fluorescence irones 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.

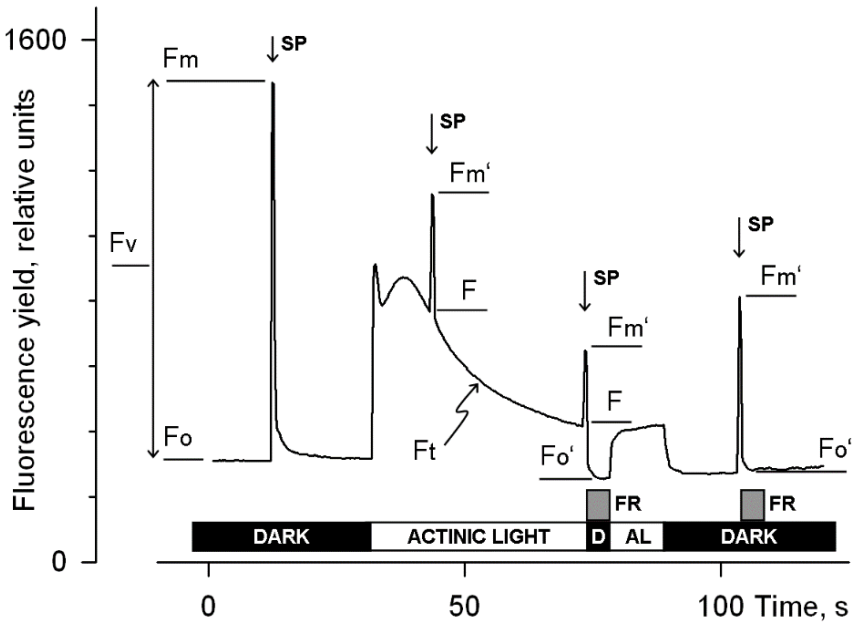


Fig. 39: Fluorescence Levels of Saturation Pulse Analysis

Y-axis (Fluorescence yield) corresponds to PAM fluorescence, see 9.1. AL, Actinic Light; D, dark; SP, Saturation Pulse; F_t , continuously recorded PAM fluorescence; FR, far red illumination.

9.2 Saturation Pulse Analysis

The five principal levels of PAM fluorescence which are used for saturation pulse analysis are shown in Fig. 39. 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 12, page 87).

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.

Under laboratory conditions, the F_0' level is determined during far red illumination in a dark interval following a saturation pulse. In this dark interval, far red light selectively drives photosystem I. Consequently, electrons are removed from the intersystem electron transport chain and opening of photosystem II reaction centers is efficiently accelerated. As a result, photochemical quenching quickly becomes maximal as is the case for F_0 fluorescence. The non-photochemical quenching, which was built up in the light, dissipates slower and is noticeably as reduction of the initial F_0 to the F_0' . See Table 12 (page 87) for fluorescence quotients calculated with F_0' .

Under field conditions, natural light intensities drive photosystem II to a degree which renders far red action ineffective. Therefore, the MICRO-PAM and MONITORING-PAM measuring heads are not equipped with a far red light source. The F_0' is 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 (F_t) 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 12 (page 87) compiles the fluorescence ratio parameters calculated by the 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 reaction 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 and q_L do not require fluorescence measurements with the dark-acclimated sample, except the F₀' mode is switched off and F₀' 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 q_N and the NPQ parameters require fluorescence measurements with the sample in the dark-acclimated and in the light-exposed states (*cf.* Table 12, page 87).

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.

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 causes strong reduction of photosystem II acceptors and photodamage, e.g., via formation of reactive oxygen species.

9.3 Relative Electron Transfer Rate (ETR)

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

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

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 absorbance (= 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 absorbance. 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.

9.4 Reviews on Saturation Pulse Analysis of Photosystem II

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https://doi.org/10.1007/978-1-4020-3218-9_11

Table 12: 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 <i>et al.</i> , 1989)	$Y(II) = \frac{F'_M - F}{F'_M}$	Light	[0, 1] [0, ~0.84]
Quantum yield of light-induced (Δ pH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Genty <i>et al.</i> 1996, Kramer <i>et al.</i> 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 trans-thylakoid Δ pH and zeaxanthin (Genty <i>et al.</i> 1996, Kramer <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2004)	$q_L = q_P \cdot \frac{F'_0}{F}$	As q_P .	[0, 1] [0, 1]
Coefficient of non-photochemical fluorescence quenching (Schreiber <i>et al.</i> 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).

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10 Specifications

Specifications are subject to change without notice.

10.1 TERRESTRIAL Version, ONLINE Configuration

10.1.1 Measuring Head MONI-HEAD/485

General Design

Housing: Water-tight aluminum cylinder with one end featuring a ball lens for focusing modulated measuring light, actinic light and saturating light pulses on the sample, and collecting fluorescence. Opposite end of tube: M12 5-pole socket for RS485/power line

Sample Clip: Consisting of 2 aluminum frames (3.5 x 2.5 cm), held together by a special O-ring, and mounted at a distance of 2.5 cm from the MONI-HEAD/485 optical window. Angle between optical axis of the MONI-HEAD/485 and sample clip plane: 120°

Cables: Data/power cable, 10 m standard length (between MONI-HEAD/485 and MONI-IB4/LAN, MONI-HEAD/485 and MONI-DA, or MONI-DA and MONI-IB1)

Dimensions: Cylinder with diameter of 3 cm and length of 18 cm

Power consumption: Peak loads during saturating pulses 7 W. During measuring mode 0.1 W

Operating temperature: -15 to +40 °C

Weight: 165 g

Light Emission

Modulated fluorescence excitation: Blue power LED (typical peak wavelength 470 nm, full width at half maximum 22 nm). Photosynthetically active radiation (PAR) of measuring light at level of the sample clip range from 0.15 to 1.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at low modulation frequencies (5 to 25 Hz), and from 1.5 to 22.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at high modulation frequency (100 Hz)

Actinic light: Same power LED as for modulated light. At level of sample clip, maximum photosynthetically active radiation of actinic light and saturating flashes of 1500 and more than 8500 $\mu\text{mol m}^{-2} \text{s}^{-1}$

Sensors

Fluorescence: PIN-photodiode protected by long pass filter (50% transmittance at 645 nm). Selective window amplifier to measure pulse amplitude modulated (PAM) fluorescence.

Photosynthetically active radiation (PAR): Integrated quantum sensor (photodiode protected by near infrared filters) measuring the radiation reflected by a 1.3 x 0.7 cm area of an optically diffuse Teflon sheet, 1 mm thick, mounted at the edge of the leaf clip

Temperature: Integrated-circuit temperature sensor on circuit board

10.1.2 PC Interface Box MONI-IB4/LAN

Housing: Aluminum case with RS-232, USB-B, Ethernet, power supply sockets, and four M12 5-pole sockets for RS-485 communication

Interfacing: The interface box connects a computer with up to four MICRO-HEAD/3B (or one MONI-DA). RS-485 serial data

communication is used between interface box and MICRO-HEAD/3B or MONI-DA. RS232, USB or Ethernet communication is used between interface box and computer

Recommended maximum cable lengths: To computer via USB and RS-232: 2 m. To computer via Ethernet, 100 m. To MICRO-HEAD/3B via RS-485: 10 m. To MONI-DA via RS-485, 100 m

Dimensions: 12 x 9.3 x 3 cm (L x W x H)

Weight: 400 g

Operating temperature: 0 to +40 °C

Power supply: Input: 100 to 240 V AC, 50 to 60 Hz. Output: 19 V DC, 3.7 A. Dimensions: 13.2 x 5.8 x 3 cm (L x W x H). Weight: 310 g

10.1.3 Fluorometer Software

WinControl-3 System Control and Data Acquisition Program (Microsoft Windows 10 and 11) for operation of measuring system via PC, data acquisition and analysis. Not compatible with Windows 10 on ARM. Measured and calculated parameters: F_0 , F_M , F_M' , F , F_0' (calculated), F_v/F_M (max. Yield), $\Delta F/F_M'$ (Yield), q_P , q_N , q_L , NPQ, Y(NPQ), Y(NO), ETR (i.e. PAR x $\Delta F/F_M'$), PAR and °C. Two different fitting routines for ETR versus light intensity curves

10.1.4 Computer Requirements

Processor, 1 GHz RAM, 1 GB hard disc space, screen resolution 1280 x 1024 pixels. Interface, USB, RS-232, or Ethernet. Operating system: Microsoft Windows 10 and 11

10.1.5 Transport Box

Design: Aluminum box with custom foam packing for MONITORING-PAM

Dimensions: 60 cm x 40 cm x 25 cm (L x W x H); 42 liters

Weight: 4.7 kg

10.2 TERRESTRIAL Version, STAND-ALONE Configuration

10.2.1 Measuring Head MONI-HEAD/485

See Section 10.1.1

10.2.2 Data Acquisition System MONI-DA

Housing: Robust water-proof cylinder consisting of a polyvinyl chloride (PVC) tube and poly-oxymethylene (POM) endplates. One endplate with 2 male M12 5-pole sockets connected in parallel (MONI-IB4/LAN communication, charging voltage), one male M12 5-pole socket for auxiliaries, and 7 female M12 5-pole sockets (MICRO-PAM, MONI-HEAD/485 communication)

Dimensions: Cylinder with diameter of 16 cm and length of 24 cm

Data management: Dual data storage on internal 8 MByte circular flash buffer and an industrial grade 512 MByte removable microSD flash card. Wireless data transfer via cellular phone or satellite modem. Online data transfer using RS-485 serial data communication.

Power consumption: 5 mW in standby mode. Operating mode, depends on the number of measuring heads connected

Battery: 12 V / 7.5 Ah (96 Wh) LiFePO₄ battery.

Operating temperature: -30 to +60 °C

Weight: 5.4 kg

10.2.3 Transport Box

Design: Aluminum box with custom foam packing for MONITORING-PAM including MONI-DA

Dimensions: 80 cm x 40 cm x 34 cm (L x W x H); 60 liters

Weight: 4.9 kg

10.2.4 PC Interface Box MONI-IB1

Housing: Aluminum case with USB-B socket, power supply socket, and M12 5-pole socket for RS-485 communication

Properties: The interface box connects a computer with a MONI-DA using RS-485 serial data communication. The same line is used to charge the MONI-DA battery. USB communication is used between interface box and computer

Recommended maximum cable lengths: To computer via USB: 2 m. To MONI-DA via RS-485, 100 m

Dimensions: 9.7 x 6.3 x 3.5 cm (L x W x H)

Weight: 270 g

Operating temperature: 0 to +40 °C

10.2.5 Solar Panel MONI-SP

Design: Polycrystalline silicon panel. Highly resistant to water, abrasion, hail impact and other severe weather conditions. Equipped with a 4 m cable and plug for the AUX or INPUT socket of the Data Acquisition System MONI-DA. Several panels can be connected in parallel to provide sufficient power under conditions of low insolation

Electrical characteristics: V_{max} , 15.0 V, I_{max} , 0.63 A (at 1000 W/m² sunlight). V_{OC} , 19.6 V. I_{SC} , 0.80 A

Dimensions: 50 x 35 cm (L x W)

Weight: 1.2 kg (incl. cable and plug)

10.2.6 Fluorometer Software and Computer Minimum Requirements

As described in Sections 10.1.3 and 10.1.3, except RS 232 and Ethernet communication

10.2.7 Accessories

10.2.7.1 Four-Way Distributor MICRO-HUB

Housing: Aluminum case with one M12 5-pole socket to connect the MONI-DA data acquisition system and four M12 5-pole sockets to connect MONI-HEAD/485 Measuring Heads

Links: The interface box connects up to four MICRO-HEAD/3B Measuring Heads to one single MONI-BUS port of a MONI-DA data acquisition system

Recommended maximum cable lengths: 10 m between MICRO-HEAD/3B and MICRO-HUB, and between MICRO-HUB and MONI-DA

Dimensions: 10 x 6 x 3.5 cm (L x W x H)

Weight: 228 g

Operating temperature: -30 to +40 °C

10.2.7.2 WiFi Modem MONI-DA/WIFI

Design: Weatherproof cylinder made of POM (Polyoxymethylene) containing a standard WiFi module. One endplate made of POM, the other endplate made of Plexiglas with 5-pole M12 plug connector. Includes a 10 m cable to connect the modem to the AUX port of the MONI-DA. Data transfer requires connection to a WLAN network or to a hotspot

Dimensions: 19.5 cm (L) 3.27 cm (Ø)

Weight: 140 g (modem), 320 g (cable)

10.2.7.3 Satellite Modem

Specifications depend on available electronic components at the time of order

10.3 AQUATIC Version, ONLINE Configuration

10.3.1 Measuring Head MONI-HEAD/S

General Design

Housing: As described for MONI-HEAD/485 (Section 10.1.1) but polyoxymethylene (POM) replaces aluminum as housing material and a special underwater 6-pole socket replaces the standard 5-pole RS485 socket. Waterproof down to a depth of 75 m

Sample Clip: As described for MONI-HEAD/485 (Section 10.1.1) but titanium replaces aluminum

Cables: Special underwater cables for communication and power. Standard length: 10 m (MONI-HEAD/S to MONI-IB4/LANS, MONI-HEAD/S to MONI-DA/S, or MONI-DA/S to MONI-IB1/S)

Dimension, power consumption, and operating temperature: As described for MONI-HEAD/485 (Section 10.1.1)

Weight: 180 g

Light Emission and Sensors

As described for MONI-HEAD/485 (Section 10.1.1)

10.3.2 PC Interface Box MONI-IB4/LANS

As described for PC Interface Box MONI-IB4/LAN (Section 10.1.2) but four special waterproof 6-pole sockets replace the M12 5-pole sockets. Special underwater cable for communication and power included. Standard length: 10 m

10.3.3 Fluorometer Software and Computer Minimum Requirements

As described in Sections 10.1.3 and 10.1.3

10.3.4 Transport Box

As described in Sections 10.1.5

10.4 AQUATIC Version, STAND-ALONE Configuration

10.4.1 Measuring Head MONI-HEAD/S

As described in Sections 10.3.1

10.4.2 Data Acquisition System MONI-DA/S

As described for Data Acquisition MONI-DA (Section 10.2.2) but waterproof 6-pole sockets replace M12 5-pole sockets and microSD card non-removable. Waterproof down to a depth of 75 m

10.4.3 Transport Box

As described in Section 10.2.3

10.4.4 PC Interface Box MONI-IB1/S

As described in Section 10.2.4 but a waterproof 6-pole socket replaces the M12 5-pole socket for RS-485 communication

10.4.5 Fluorometer Software and Computer Minimum Requirements

As described in Section 10.2.6

11 Guarantee

All products supplied by the Heinz Walz GmbH, Germany, are warranted by Heinz Walz GmbH, Germany to be free from defects in material and workmanship for two (2) years from the shipping date (date on invoice).

11.1 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.

11.2 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 (halogen, LED), 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.

11.3 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.

11.4 Applicable law

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

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