DIVING-PAM-II

Underwater Chlorophyll Fluorometer

Manual

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Contents

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.
- 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 put the device near sources of heat.
- Expose the device to dust, sand, and dirt as little as possible.
- Ensure that neither liquids nor foreign bodies get inside the device.
- The device may only be repaired by the manufacturer.

1.2 Special Safety Instructions

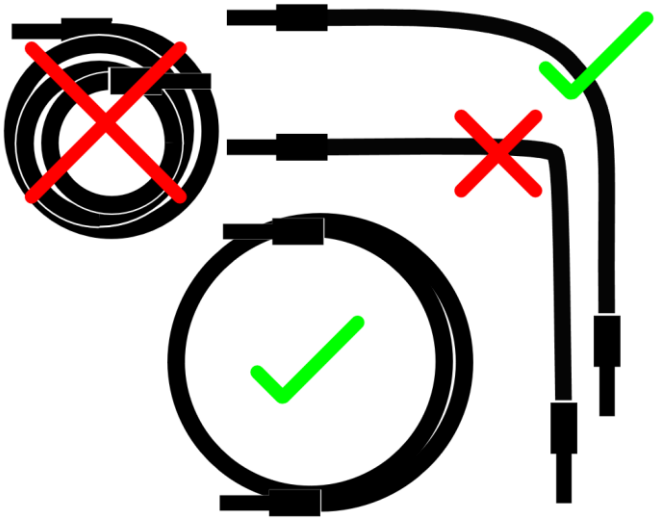
- The DIVING-PAM-II is a highly sensitive instrument which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual to avoid potential harm to the user and damage to the instrument.
- The DIVING-PAM-II can emit very strong light! To avoid harm to your eyes, never look directly into the light port of the DIVING-PAM-II or its fiberoptics.
- Keep all ports and plugs clean (see Section 9.1, page 157). Do not expose open cable ends to water or high moisture.

- Do not force a plug into the wrong socket (cf. Fig. 3, page 12). Make sure that the connection is watertight (Fig. 4, page 14).
- Do not attempt to open pressure and temperature sensor (Fig. 3, page 12).
- Run all cables so that stepping on or stumbling over them is excluded.
- Do not open housing of the DIVING-PAM-II for repair. There are no serviceable parts inside the DIVING-PAM-II. Exception: battery change (Section 9.3, page 158).
- Before immersing the DIVING-PAM, make sure that all cable plugs are properly connected (the rippled cylinder of the cable end must be completely screwed in so that it presses against the rubber ring of the cable port: Fig. 4, page 14). Make sure that each unused cable port is properly sealed by a red riffled cap (screw in completely).
- Do not exceed the maximum diving depth of 50 m.
- The PC Interface Box DIVING-PAM-II/I is not watertight; keep it away from water or high moisture areas.
- At the interface box, orientate charge plug so that the red dot on the plug coincides with the red dot of the socket. Do not try to disconnect the plug by pulling at the cable. Disconnect plug by pulling at the rippled bushing of the plug.

1.3 Correct Handling of Fiber Optics DIVING-F

This fiber optics DIVING-F is made of delicate glass fibers. Each glass fiber extends over the entire length of the fiber optics. Do not sharply bend, stretch, or crush the fiber optics as this can break the glass fibers. Each broken fiber reduces light transmission by the fiber optics. If many fibers are broken, the fiber optics must be

replaced. Place protection caps on fiber ends when the fiber optics is not in use.



1.4 Instructions for Battery Handling

Only for DIVING-PAM-II with Lithium-Ion Battery

- The DIVING-PAM-II Info window indicates if a lithium battery is built in (Fig. 69, page 92).
- For long-term storage of the DIVING-PAM, discharge battery to about 30% of its capacity. The charge status is indicated in the top right corner of the Basic Data window (Fig. 24, page 39).

Only for DIVING-PAM-II with Lithium-Ion Battery

For shipping of the DIVING-PAM-II:

- Discharge battery to 30% (see above).
- Use original DIVING-PAM-II/T transport case.
- Download from Walz website the testing protocol for the DIVING-PAM-II battery (Test_Report_UN38-3_Transport.pdf).

https://www.walz.com/downloads/?filter=diving-pam-ii

- Print entire protocol (6 pages) and enclose this protocol in the box.
- Visibly label package with lithium battery label (Lithium Battery Label-UN3481.pdf, downloadable at the link above).

Fig. 1: Lithium Battery Label

2 Introduction

2.1 Features DIVING-PAM-II

- The “Underwater Chlorophyll Fluorometer DIVING-PAM-II” has been designed for saturation pulse analysis of photosystem II in aquatic organisms. Typically, these organisms include sea grass species, macroalgae, zooxanthellae in corals, and cyanobacterial layers.
- Compared to its predecessor (“first-generation” DIVING-PAM), the chief technical advancements are the consistent use of energy-efficient LEDs, a far-red light source for selective excitation of photosystem I, an internal PAR sensor, a touchscreen for outdoor application, and WiFi communication.
- The fluorometer measures the efficiency of photosystem II under dark conditions (F_V/F_M) and in the light (Y(II)). Further parameters measured characterize photochemical fluorescence quenching (q_L, q_P), non-photochemical fluorescence quenching (q_N, NPQ, Y(NPQ), Y(NO)).
- The DIVING-PAM-II is equipped with a spectroradiometer which is calibrated to measure spectra of photon flux density. The software automatically integrates photon flux density in the range from 400 to 700 nm to obtain PAR, which, together with Y(II), is used to calculate relative rates of photosynthetic electron transport (ETR).
- The spectrometer can also record reflectance and fluorescence spectra of samples.
- An underwater oxygen sensor using optical detection of O_2 completes the range of accessories.

- The capacity of the internal memory corresponds to data of more than 27,000 data sets of saturation pulse analyses.
- A high-capacity lead acid battery lasts for up to 1300 saturation pulse analyses.
- In the lab, the DIVING-PAM-II can be combined with the same oxygen measuring system developed for the MINI-PAM-II (https://www.walz.com/products/mini-pam-ii/ & scroll down).

2.2 Structure of Manual

The DIVING-PAM-II fluorometer provides a vast range of settings and protocols. To make full use of these opportunities, become acquainted with terminology and principles of saturation pulse analysis (see Chapter 10, page 165). Section 10.4 (page 173) provides a list of review papers on PAM chlorophyll fluorescence and saturation pulse analysis.

Prior to working with the Diving-PAM-II, note the Safety Instruction (Chapter 1, page 1). Chapter 3 (page 9) deals with the setup of the basic system and the accessories available.

In the field, the DIVING-PAM-II is normally operated in the stand-alone mode. Chapter 4 (page 35) provides detailed instructions on how to use the screen interface including advice on fluorescence induction and light curve programs.

The DIVING-PAM-II can also be operated by the software WinControl-3 running on a computer with Windows operating system. Installation of WinControl-3 is introduced in Chapter 4.1, page 36, and the features of WinControl-3 are dealt with in Chapter 6, page 103.

Chapter 7 (page 151) provides hints for beginners, Chapter 8 (page 155) contains instructions for trouble shooting, and Chapter 9 (page 157) gives guidelines for maintenance of the device.

Further, technical information (Chapter 10, page 165) and guaranty conditions (Chapter 12, page 187) are provided.

This manual ends with lists of keywords (Chapter 13, page 191) figures (Chapter 14, page 197) and tables (Chapter 15, page 201).

3 Components and Setup

3.1 Basic System

3.1.1 Extent of Delivery (Basic System)

Item	Order Code
Optoelectronic Unit	DIVING-PAM-II/B or -/R
Fiberoptics	DIVING-F
PC Interface Box	DIVING-PAM-II/I
Power Supply	MINI-PAM-II/N
Underwater Cable	DIVING-PAM-II/K5
Miniature Spectrometer	MINI-SPEC
PAR Calibration Block	160101439
Distance Clip 60°	2010-A
Dark Leaf Clip (3 pcs)	DIVING-LC
Surface Holder	DIVING-SH
Software	WinControl-3
Transport Case	DIVING-PAM-II/T
Standard USB-A to USB-B cable	
Silicone grease	
Fuses	
Manual	

3.1.2 System Overview

An overview to the principal components of the DIVING-PAM-II system and connections between them is given in Fig. 2.

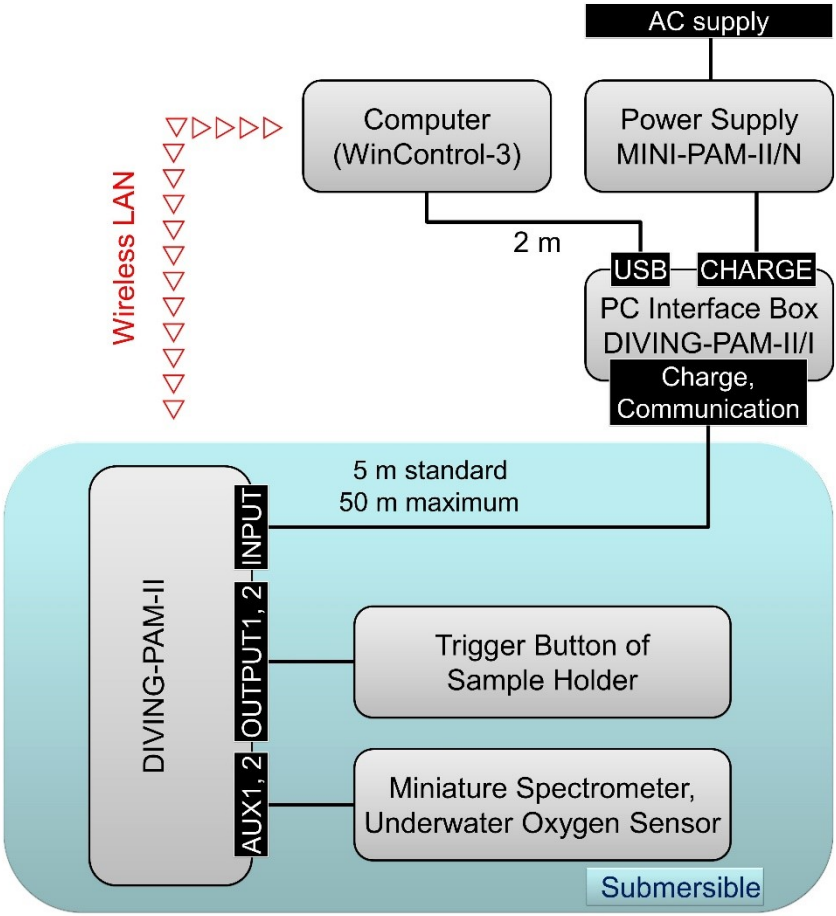


Fig. 2: DIVING-PAM-II: System Components

In Fig. 2, all submersible components of the system are drawn on aquamarine background.

Submersible components, which are part of the basic system, are the central unit of the DIVING-PAM-II, the Fiberoptics DIVING-F, which is inserted into the central optical port of the right end plate (not shown in Fig. 2), and the Miniature Spectrometer MINI-SPEC. Submersible accessories, which are not part of the basic system, are the Underwater Oxygen and pH Sensor DIVING-PAM-II/O2PH, and the Universal Sample Holder DIVING-II-USH.

To measure light spectra and total photon flux in the visible range (PAR), the MINI-SPEC is plugged into the AUX 1 or AUX 2 port of the fluorometer's right end plate. The DIVING-PAM-II/O2PH uses the same ports.

The Universal Sample Holder DIVING-II-USH includes a trigger cable which can be plugged into port OUT 1 or OUT 2 on the right end plate. When plugged in, the trigger button of the DIVING-II-USH has the same function of the START button on the right end plate of the fluorometer (Fig. 3).

The function of both the trigger button of the DIVING-II-USH and the START button depends on the window selected on the DIVING-PAM-II screen, e.g., when the window "Basic Data" is visible, a saturation pulse analysis will be triggered, or when the window "Induction Curve" is visible an induction curve will be performed.

Fig. 2 also outlines that the DIVING-PAM-II can be operated in the stand-alone mode or by the software WinControl-3 running on a computer with Microsoft Windows operating system. Operation by WinControl-3 requires connection between computer and fluorometer via the system's interface box (DIVING-PAM-II/I). Computer and interface box are linked by a standard USB cable; interface box and fluorometer are connected by a special underwater cable.

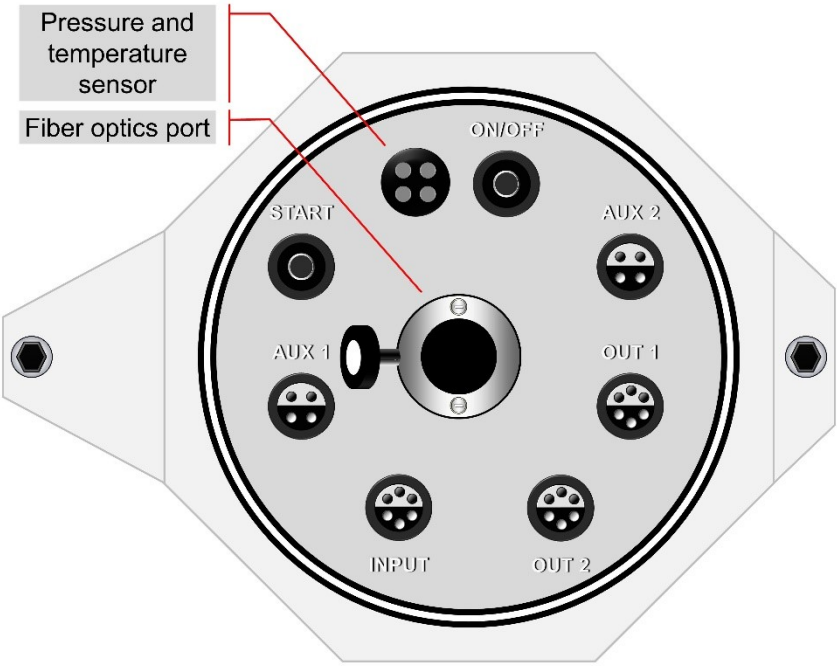


Fig. 3: Right End Plate of DIVING-PAM-II

Instructions on how to properly connect underwater cables are given by Fig. 4 (page 14). Note that the interface box is not submersible.

For communication between computer and fluorometer, the interface box does not need line power. When the interface box is connected to the mains, the DIVING-PAM-II battery is charged via the underwater cable. Advantages of WinControl-3 operation are the option to remotely control the DIVING-PAM-II, and to continuously record steady state chlorophyll fluorescence. (Note that continuous fluorescence is not recorded in the stand-alone mode.)

Table 1: Side panel of DIVING-PAM-II: Summary of Elements

Key or port (see Fig. 3)	**Function**	**Comment**
ON/OFF	On/off switch and Keyboard lock switch	Press briefly to switch on, press 2 seconds to switch off DIVING-PAM-II. When the DIVING-PAM-II is switched on, briefly pressing locks or unlocks keyboard (the red LEY LED indicates a lock state of the keyboard).
START	Trigger button	The button triggers the command displayed in the top right corner of the current display.
AUX 1/AUX 2	Connectors for auxiliary devices	Connect here MINI-SPEC, Underwater Oxygen Sensor DIVING-PAM-II/O2PH, or Fiber-Optic Oxygen Meter FireStingO2 (special adapter required).
OUT 1/OUT 2	Connectors for external light	Sockets for synchronized external light source (light is blanked out when PAM fluorescence is measured). The sockets work also as input for the trigger signal from Sample Holder DIVING-II-USH.
INPUT	Interface connector	To charge battery and to operate DIVING-PAM-II by WinControl-3.
Pressure and temperature sensor	Housing for pressure and temperature sensor. The digital sensor chip is second order temperature-compensated. Do not attempt to open.	
Port for fiber-optics with locking screw	Optical window for internal light sources and fluorescence	

As new feature, the DIVING-PAM-II has a Wireless LAN Interface built-in, which permits convenient data download at the experimental site. See Table 2, page 15.

Note that all peripherals may only be connected in dry and clean environment.

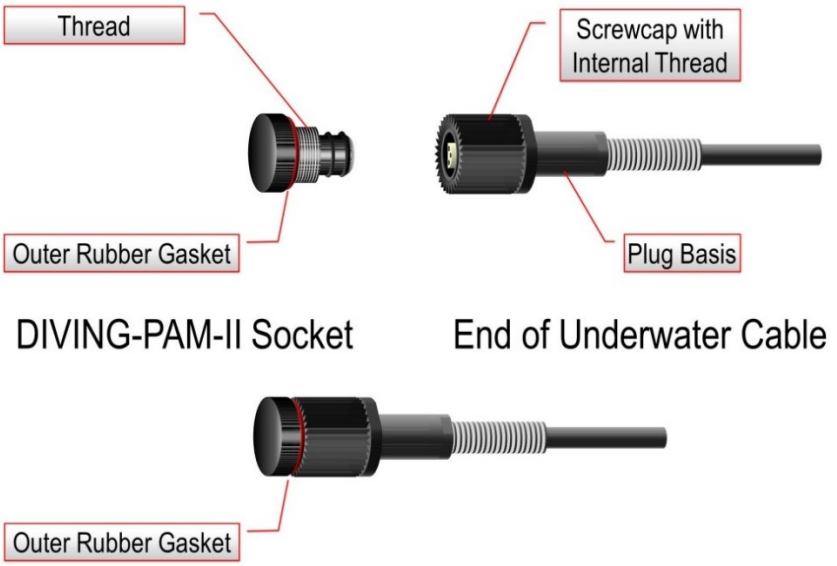


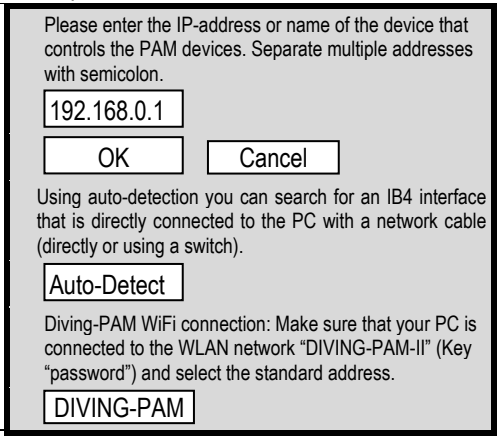
Fig. 4: **Watertight Connection of Cable**
(1) Hold cable end at “plug basis”.
(2) Fully (!) insert plug in socket.
(3) Tighten “screwcap”.
(4) Confirm that screwcap presses against “outer rubber gasket”.

The waterproof fluorometer housing consists of a Plexiglas tube with Plexiglas end plates. The right end plate accommodates the fiberoptics port, various plug connectors, two push buttons (START and ON/OFF), and a combined pressure and temperature sensor (Fig. 3, page 12). All ports and buttons are explained in Table 1 (page 13).

On the front of the DIVING-PAM-II, a compact, low-energy black/white screen displays measured data and provides field elements to control the fluorometer. The waterproof design of the fluorometer requires that these field elements are selected by optical switches which are arranged in two rows of 5 keys on the right

side of the screen (Fig. 5A, page 16). Just touching a key area activates the command of the associated element on the screen. The LED located right of the screen shines green when an optical switch is activated.

Table 2: WLAN Connection

Establish WLAN Connection Computer to DIVING-PAM-II	
DIVING-PAM-II	Switch on WLAN When WLAN is set to “Auto” in window “Wireless”, the WLAN is active 5 minutes after powering on the fluorometer; WLAN is always active when “On” is selected “Wireless” (Fig. 66, page 89). The “W” in the first line of Windows “Basic Data” indicates that WLAN is active.
Computer	Select DIVING-PAM-II as access point. - Enter security key “**password**”. - Ignore error message “The connection is limited”.
Connect WinControl-3 to DIVING-PAM-II	
Computer	Start Network Mode of WinControl-3. - Windows 7: Click “Start”→Select “All Programs”, “WinControl-3”. - Windows 10 and newer: Click tile “WinControl-3-Network Mode”. - General alternative option: Search “WinControl-3 – Network Mode”. The network mode opens the window below.
Click “DIVING-PAM” Click OK Wait for DIVING-PAM-II detection by WinControl-3	

3.1.3 Fluorometer

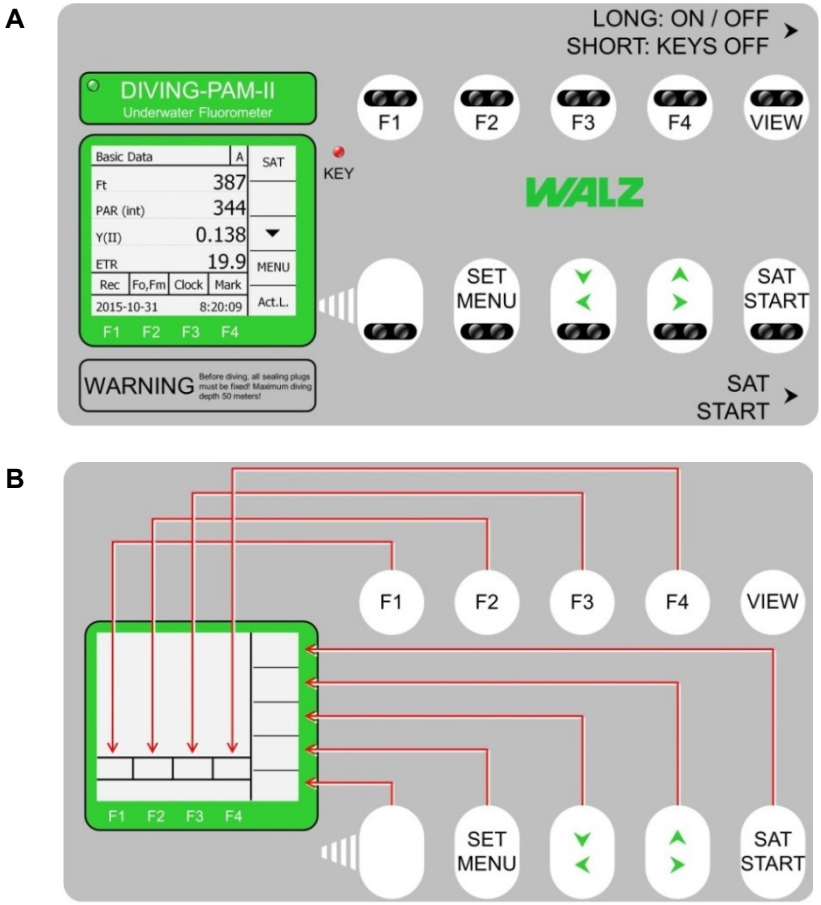


Fig. 5: Front View of DIVING-PAM-II

A, keys and screen. **B**, relation of keys and fields elements.

The relation of optical switches and field elements are outlined in Fig. 5B (page 16). The function keys of the upper row of keys (F1 through F4) are associated with the 4 control elements located on the lower edge of the DIVING-PAM-II screen. The rightmost key in the upper row, the VIEW button, is not connected to with a field

element but changes appearance and properties in graphical windows (e.g., induction curve or spectral data) so that more numerical values are displayed and selection of individual points in a graph becomes possible.

The keys of the lower row from left to right correspond to the field elements on the right side of the screen from bottom to top. The function of the rightmost optical key of the lower row is identical to the START button on the right fluorometer end plate (Fig. 3, page 12) or the trigger button of the DIVING-II-USH (Fig. 17, page 28).

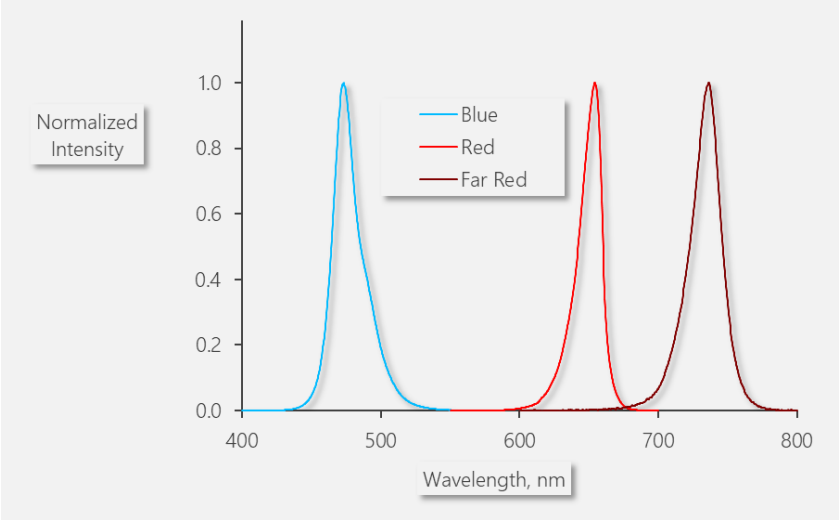


Fig. 6: Normalized Emission Spectra of DIVING-PAM-II LEDs

Typical LED emission spectra normalized to their maxima. The blue curve corresponds to the spectrum of the blue LED of the DIVING-PAM-II/B, the red curve represents the red LED in the DIVING-PAM-II/R. Both DIVING-PAM-II versions possess a far-red LED which emits maximally above 700 nm (rightmost curve). Peak wavelength and full width at half maximum (in brackets) are indicated.

In the DIVING-PAM-II/R, a red LED acts as measuring light and as actinic light source. In the blue version (DIVING-PAM-II/R), a blue LED is used. Both versions of the DIVING-PAM-II are equipped with a far-red LED. Normalized emission spectra of blue, red, and far-red LEDs are shown in Fig. 6.

Another difference between the two versions is the spectral window for fluorescence detection. The BLUE version detects fluorescence at wavelengths > 630 nm but the RED version detects fluorescence at wavelengths > 700 nm (Fig. 7).

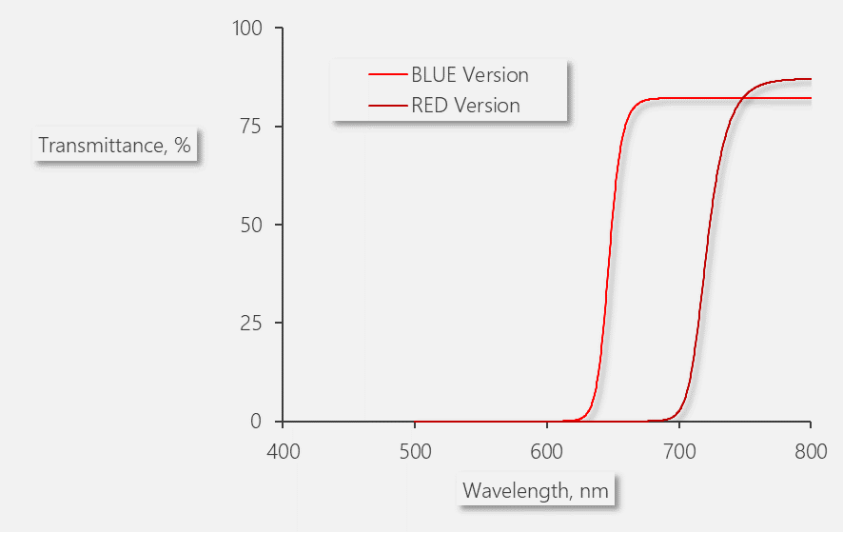


Fig. 7: Transmittance Spectra of DIVING-PAM-II Versions

Transmittance spectra of detection filters in the DIVING-PAM-II/B (BLUE Version, red line) and DIVING-PAM-II/R (RED Version, dark red line) are shown.

3.1.4 Miniature Spectrometer MINI-SPEC

The Miniature Spectrometer MINI-SPEC belongs to the basic system of the DIVING-PAM-II. The MINI-SPEC is calibrated to measure spectra of quantum fluxes. Integration of these spectra over the visible range results in PAR data like those recorded with Walz quantum sensors. Compared to standard quantum sensors, the spectrometer provides additional information on the spectral composition of photosynthetically active radiation which changes with water depth and can be influenced by photosynthetic organisms close by. During transport, the spectrometer is mounted on the fluorometer as show in Fig. 8, page 20.

The spectrometer is used to calibrate the internal PAR sensor of the DIVING-PAM-II (see Section 3.1.4, page 19). To this aim, the DIVING-PAM-II light guide and the entrance optics of the spectrometer are inserted in the PAR calibration block (Fig. 10, page 22). The light guide can be inserted either in the 60° or the 90° port according to the two possible orientations of the light guide in the Universal Sample Holder DIVING-II-USH. With both pieces fully inserted, the distance between fiber optics end and diffusing disk of the spectrometer matches the corresponding standard distances between fiber optics end and sample level in the DIVING-II-USH (7 mm, compare Fig. 16, page 26).

Replacing the entrance optics used for evaluation of light by the cap for fluorescence and reflection (Fig. 9, page 21) extents considerably the range of spectral information attainable by the miniature spectrometer.

In the fluorescence mode, a light guide in the cap leads light from a blue or a green LED located inside the spectrometer body to the sample. The excited fluorescence reaches the spectrometer body through a central hole in the cap. Green light penetrates deeper into photosynthetic tissue than blue or red (Terashima et al (2009)

Plant Cell Physiol 50:684-697). Therefore, the two excitation colors permit probing the effect of varying depths of penetration of excitation light on the shape of fluorescence spectra. The spectrometer automatically compensates for the spectral sensitivity of the detector.

For reflection measurements, a halogen lamp is employed whose emission is transferred through another light guide to the sample (Fig. 9, page 21). From reflection measurements, reflectance (R) is derived by dividing a sample spectrum (r_{sample}) by the spectrum of a white light-diffusing reflector consisting of a fluoropolymer with very high diffuse reflectance in the entire visible range ($r_{reference}$):

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

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

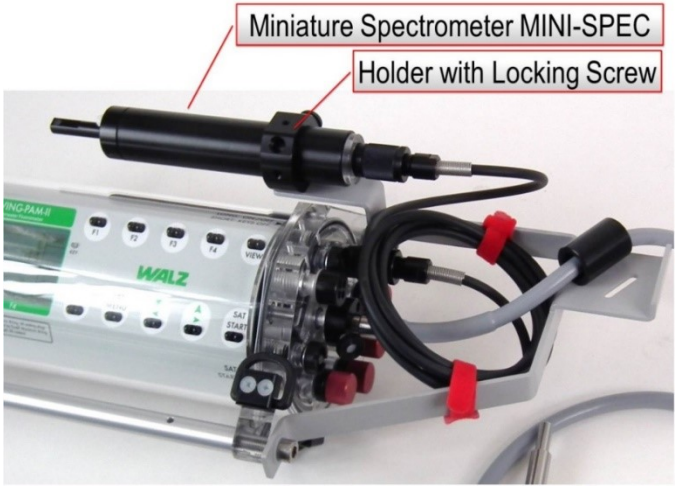


Fig. 8: DIVING-PAM-II with MINI-SPEC in Transport Position

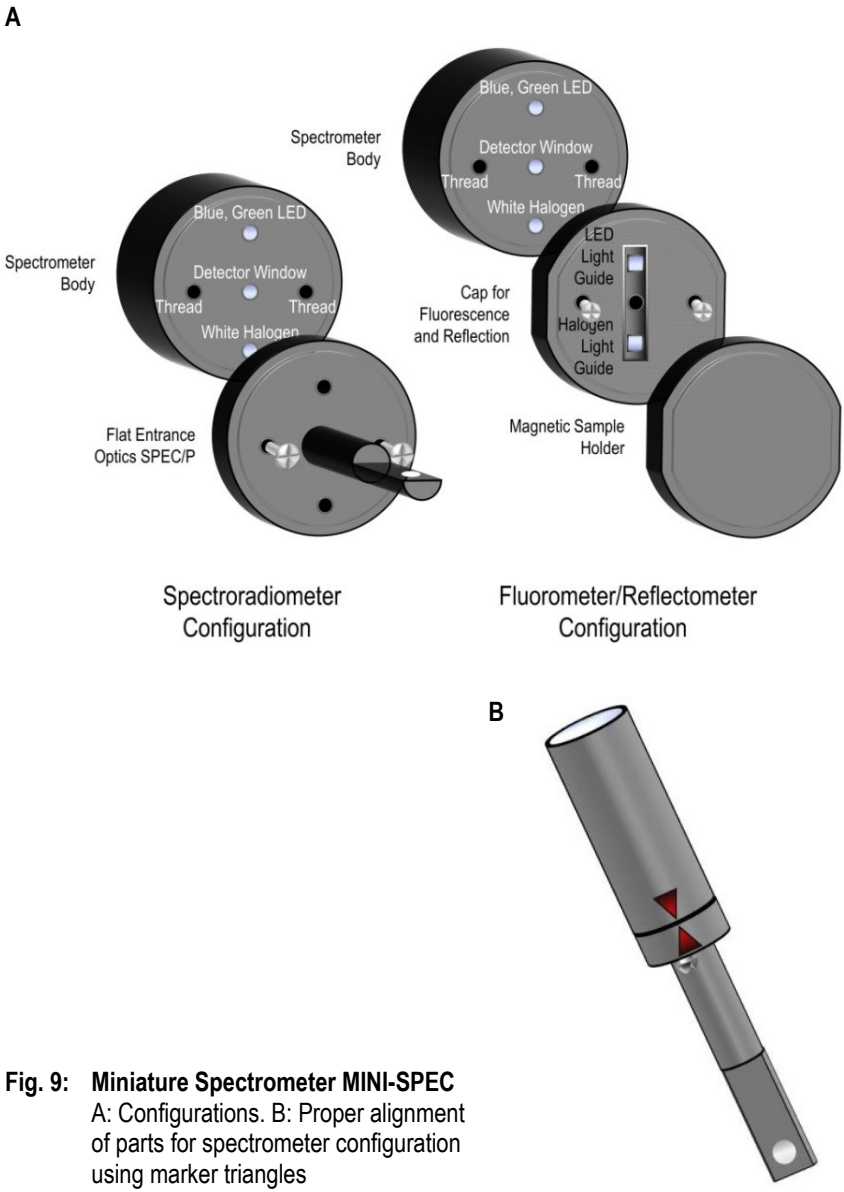


Fig. 9: Miniature Spectrometer MINI-SPEC
A: Configurations. B: Proper alignment of parts for spectrometer configuration using marker triangles

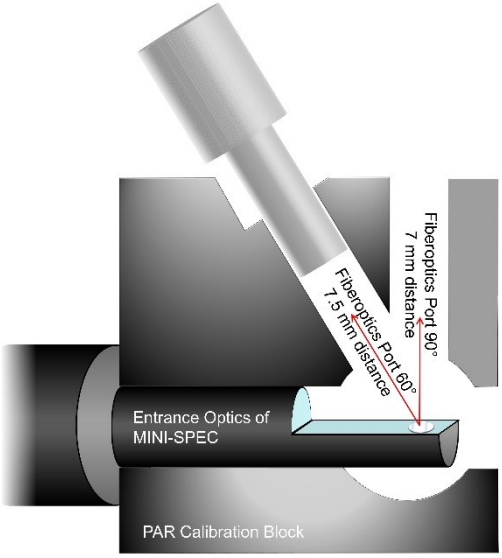


Fig. 10: PAR Calibration Block

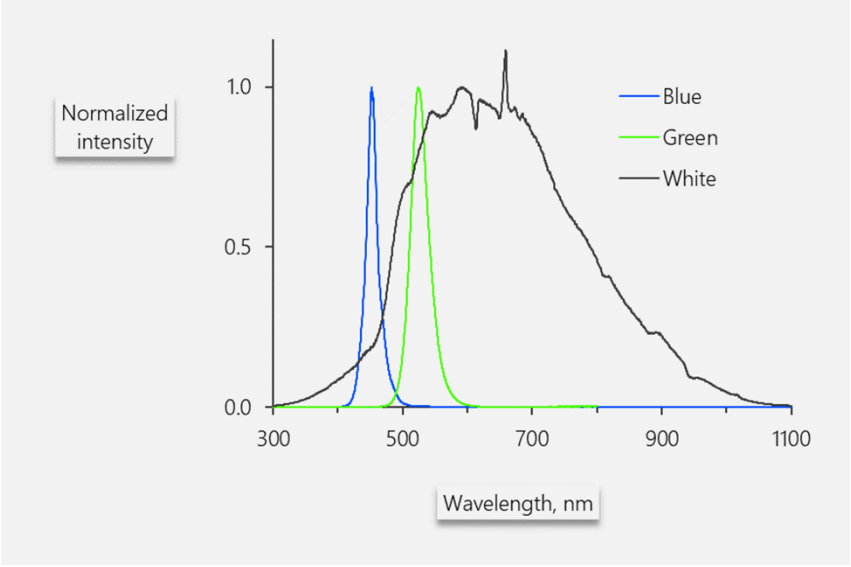


Fig. 11: Normalized Emission Spectra of MINI SPEC Light Sources

3.1.5 Distance Clip 60° 2010-A

The 2010-A clip positions the fiberoptics end-piece relative to the sample. The axis of the end-piece is positioned at a 60° angle relative to the sample plane. Two different spacer rings may be used to increase the distance between fiberoptics and sample. The distance between fiberoptics exit plane and sample has considerable influence on signal amplitude and effective light intensities (Fig. 14, page 25).

Normally, a specimen is examined above the viewing hole of the clip and it is held between the jaws of the clip. In case of thick or bulky specimens, the sample is placed below the hole of the 2010-A clip.

From the 60° angle between sample and fiberoptics results slightly heterogeneous light intensities at leaf surface because the distance between fiber optics tip and leaf surface varies. The measured signal will be dominated by that part of the leaf, which receives maximal intensity, as this is most strongly excited and emits most of the fluorescence.

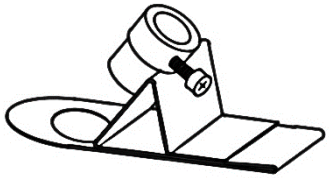


Fig. 12: Distance Clip 60° 2010-A

3.1.6 Dark Leaf Clip DIVING-LC

The Dark Leaf Clip DIVING-LC weighs 6.5 g and can be attached to most flat samples without detrimental effects. It is equipped with a miniature sliding shutter which prevents light access to the leaf during dark acclimation. The shutter is opened for the measurement after the fiberoptics has been inserted to prevent exposure to external light. Proper dark adaptation is essential for determination of the maximal quantum yield F_V/F_M.

The dark leaf clip plus adapter (Fig. 13, page 24) positions the fiberoptics tip at the relatively short distance of 3 mm above leaf surface. As a consequence, measuring light intensity and, thus, signal amplitude are higher than with the standard distance for vertical illumination of 7 mm (see Fig. 16, page 26). If signal saturation occurs at 3 mm distance, reduce measuring light intensity and gain. The background signal (F-Offset) will be automatically adjusted (cf. Section 4.2.1.7, page 63, and Section 6.10.4, page 142).

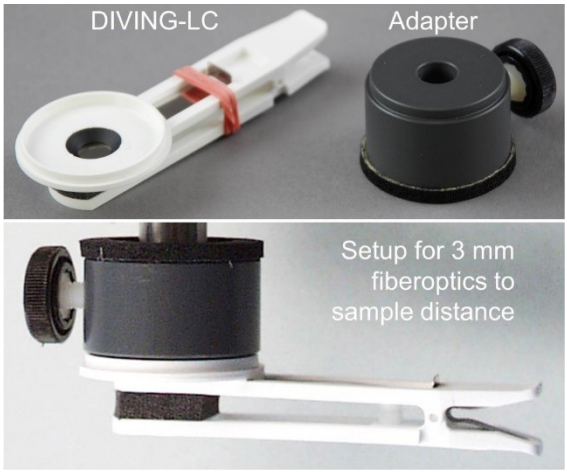


Fig. 13: DIVING-LC and Adapter

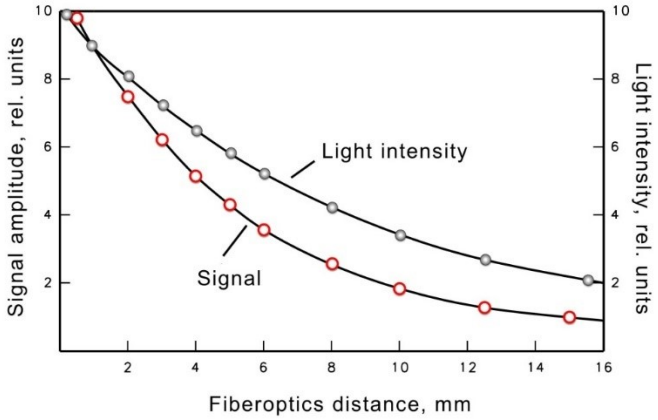


Fig. 14: Signal/Distance Relationship
Relationship between signal amplitude/light intensity and distance between fiberoptics tip and sample

3.1.7 Surface Holder DIVING-SH

To study corals or epilithic plants, the surface holder can be attached by three hooks and rubber bands to uneven, creviced surfaces. The three screws holding the rubber bands can be moved up and down to adjust the surface holder relative to the sample the desired distance.

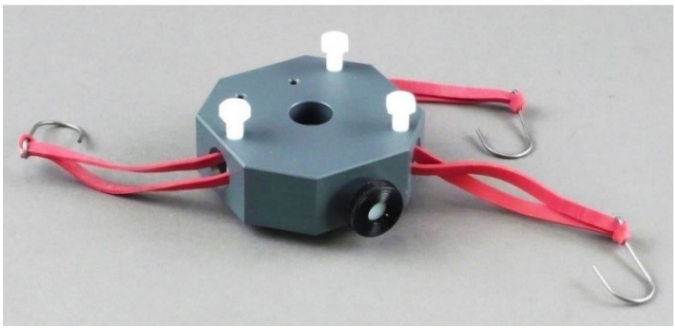


Fig. 15: .Surface Holder DIVING-SH

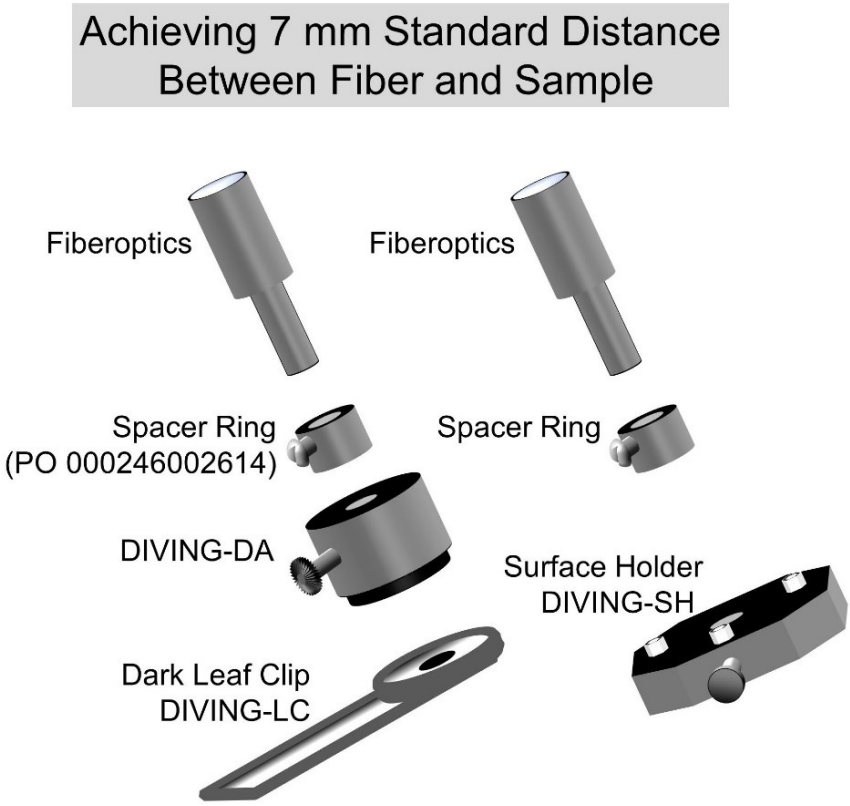


Fig. 16: **The Standard Distance of 7 mm**
Combinations of adapters and leaf clip to achieve the standard distance of 7 mm between fiberoptics end and sample level.

3.2 Accessories

3.2.1 Universal Sample Holder DIVING-II-USH

The Universal Sample Holder DIVING-II-USH was designed for underwater investigations of on samples like sea grass, macroalgae, corals, algal mats and periphyton.

The trigger button of the sample holder permits one-hand operation. The trigger signal is transmitted by a cable to ports OUT1 or OUT2 of the DIVING-PAM-II (see Table 1, page 13). The fiberoptics can be positioned perpendicularly to the sample or in the 60° arrangement. In both orientations, the distance between fiber end and sample surface corresponds to the standard conditions as shown in Fig. 10 (page 22) that is 7.5 mm for 60° and 7 mm for 90° angle between fiberoptics and sample surface.

Trigger cable, spectrometer cable and the fiber optics are loosely held together by a nylon-mesh-cover featuring a zipper. When not in use, the whole device can be hooked to the diver's jacket.

The general setup of the DIVING-II-USH is depicted Fig. 17, (page 28), connection to the DIVING-PAM-II is illustrated by Fig. 18 (page 29). The various modes of application are addressed in the following section.

DIVING-USH Applications

Leaves and macroalgae

With flat samples, the sample holder with clip attached can be used. The clip is connected to the holder with two screws (Fig. 17, page 28). The clip can be opened by a handle with a single finger of the hand holding the device.

Fig. 17: Universal Sample Holder DIVING-II-USH

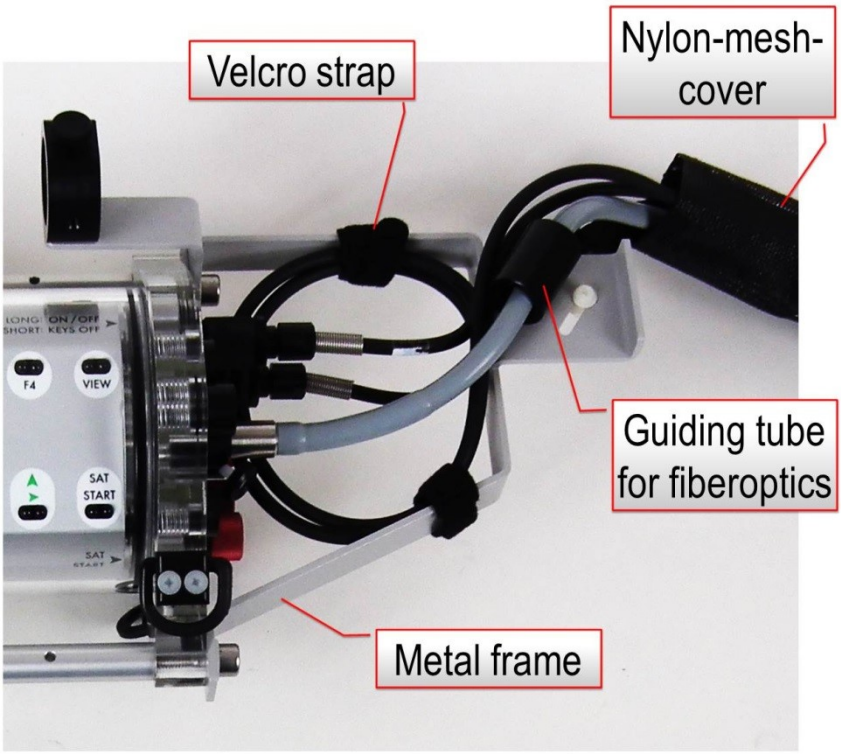


Fig. 18: Arrangement of Fiberoptics and Cables

Two rubber O-rings constitute the elastic part of the clip, holding the upper and lower parts together. The O-rings are held by nylon screws positioned sideward and at the lower side of the clip. O-rings can be hooked into either of the two lower screws resulting in different stretching of the O-ring. Depending on stretching, the pressure of the clip put on a sample varies. Different pressures can be also obtained with O-rings of various thicknesses.

Furthermore, it is possible to adjust the distance between upper and lower clip part by two vertical nylon screws (Fig. 17, page 28). In this way, the clip can be adapted to the sample's pressure sensitivity and thickness. The stripe-like contact areas on the edges

of the clip edge are covered with neoprene rubber foam (bottom jaw) and a plastic “pin-cushion” (top jaw), which assures a good grip even with slippery objects like kelp and other seaweeds.

The fiberoptics can be mounted at angles of either 60° or 90° with respect to the sample plane. Changing the angle just takes a couple of seconds. At an angle of 60° ambient light can freely reach the site at which fluorescence is measured. The quantum flux density of this light can be measured by the Miniature Spectrometer MINI-SPEC. Knowledge of quantum flux density is important for estimation of relative electron transport rate (ETR) and for evaluation of measured effective quantum yields.

The 90° arrangement is employed for illumination by the actinic light of the DIVING-PAM-II fluorometer. Well-defined light response and induction curves can be measured when the external light is fully excluded. For this purpose, an adapter is provided which slips over the fiberoptics metal endpiece (see Fig. 16, page 26, “Adapter”). One side of this adapter is covered with neoprene rubber, making provision for light-tight, gentle contact with the sample. To prevent access of light from the bottom side, a “darkening plate” can be fixed in the lower part of the clip (part of delivery, not shown).

Bulky samples

For measurements of bulky samples (e.g., corals, sea anemones, periphyton and microphytobenthos), the bottom part of the clip can be removed. For this purpose, two rubber O-rings are pulled off. Then, the lower part is pulled out of its bearings. It is also possible to remove the entire clip and to use the adapter for dark acclimation only.

Sample holders for dark acclimation

Dark acclimation of a sample for at least several minutes is required for assessment of the maximal quantum yield (F_V/F_M). For

this purpose, two different types of dark sample holders are available, the Magnet Sample Holder DIVING-MLC and the Dark Leaf Clip DIVING-LC. The former is suited for the study of relatively large and robust samples, whereas the latter is better suited for more fragile leaf-like samples.

3.2.2 Underwater Oxygen and pH Sensor

The DIVING-PAM-II/O2PH sensor measures oxygen and pH optically. Sensor spots carrying fluorescent dye change their fluorescence properties dependent on oxygen or pH concentration. For Details see Instruction Manual for Oxygen Measurements:

https://www.walz.com/files/downloads/diving_pam_ii_o2ph_01.pdf

The sensor measures oxygen concentration in fresh and seawater. The pH sensor was developed for seawater, and it is not recommended for freshwater. The sensor is an accessory for the DIVING-PAM-II but can also be operated by the MINI-PAM-II.

Fig. 19: Underwater Oxygen and pH Sensor DIVING-PAM-II/O2PH

3.2.3 Magnet Sample Holder DIVING-MLC

The Magnet Sample Holder DIVING-MLC consists of two circular halves each containing magnets (Fig. 20, page 32). A flat sample sandwiched between the two halves is hold by magnetic pull. Typical samples are seagrasses, kelp and other large macroalgae.

The upper halve of the DIVING-MLC has a central hole which can be closed by a sliding shutter. The upper halve also provides a seat for the adapter DIVING-DA. With the shutter closed, the sample part below the central hole is shielded from light. Using an adapter DIVING-DA, the DIVING-PAM-II fiberoptics can be connected to the DIVING-MLC. Measurements with the still dark-acclimated sample can start after opening of the shutter.

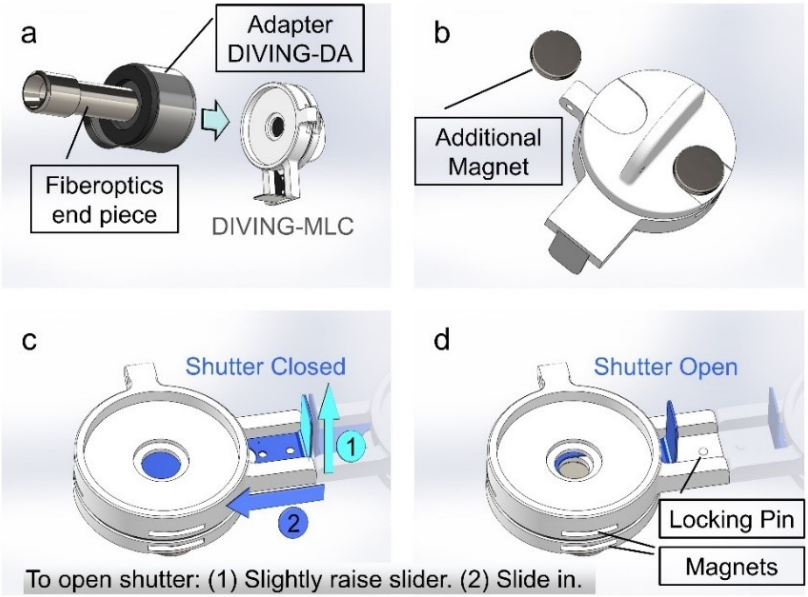


Fig. 20: DIVING-MLC

a, putting the fiberoptics with DIVING-DA adapter on a DIVING-MLC. **b**, positioning an additional magnet. **c**, DIVING-MLC with closed shutter. **d**, DIVING-MLC & open shutter.

The lower halve provides seats for additional magnets. The magnetic pull can be adapted to the sample by varying the number of additional magnets.

3.2.4 Miniature Fiberoptics DIVING-F1

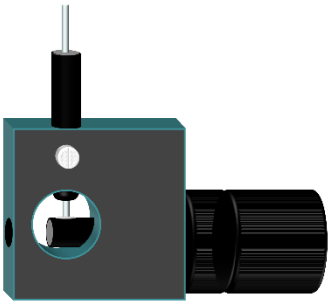
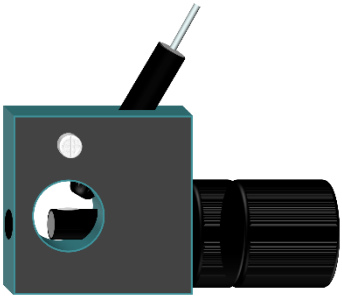
The Miniature Fiberoptics DIVING-F1 has been designed to investigate small surfaces. The light guide consists of a single coated plastic fiber, which has an active diameter of 2 mm and a length of 1.50 m.

Fig. 21: Miniature Fiberoptics DIVING-F1

Fiber optics with adapters and Polishing Set (order number 000160103450, supplied with DIVING-F1).

3.2.4.1 Calibration of the internal light sensor and establishing the internal PAR list

Table 3: Miniature Fiberoptics DIVING-F1

What is measured?	**PAR at fiberoptics tip**	**PAR at sample level** (Standard geometry, distance to sample level 7.5 mm, angle 60°. See Fig. 10, page 22.)
Setup	 Fiber touches center of the diffusing disk of the MINI-SPEC.	 Fiber flush with adapter which is fully inserted in 60° port of the calibration block.
Calibration of internal light sensor	Spec. Cal. Factor **= 3.01**	Spec. Cal. Factor **= 1.22**
	See Section 4.2.4.5, page 83. The Spec. Cal. Factor can only be entered on the DIVING-PAM screen.	
Calibration of actinic light list	Go to “Actinic Light List” on MINI-PAM-II display (Section 4.1.10, page 52) or Settings Window of WinControl-3 (Section 6.10.3, page 142), execute “Calibrate”.	

3.2.5 Underwater Cables DIVING-II/K25 and DIVING-II/K50

Underwater cables of two different lengths are provided for remote control and long-term measurements.

4 Stand-alone Operation

Stand-alone operation of the DIVING-PAM-II allows saturation pulse analysis of photosynthesis independent of a computer. Using water-tight reflection switches, single F_V/F_M or Y(II) measurements can be triggered, but more complex experiments like induction and light curves are also feasible. Continuous recording of steady state fluorescence, however, requires operation of the DIVING-PAM-II by WinControl-3 running on an external computer.

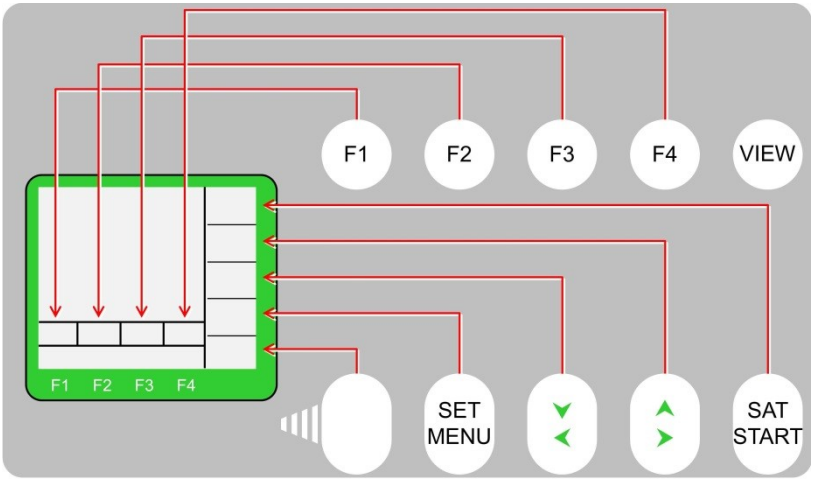


Fig. 22: Optical Switches and Display Fields
Note that "VIEW" is not linked to a display field but changes the appearance and opens navigation options of windows displaying graphical information (induction curve, light curve, spectra).

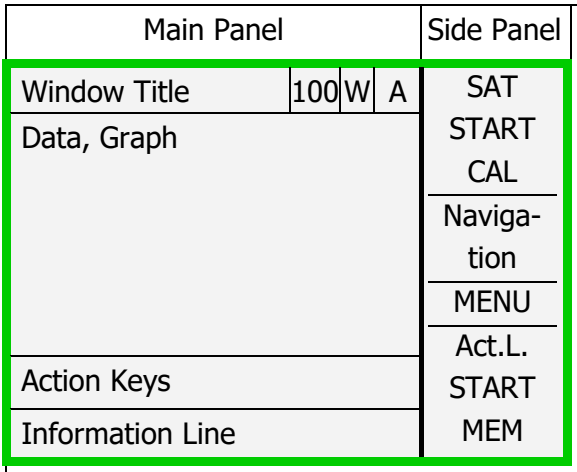
4.1 Top-level Windows

The DIVING-PAM-II provides various windows for control and data display. Frequently used commands, fluorescence data and the intensity list of actinic light (PAR list) are presented in 10 “top-level” windows (Table 4, below)

Accessible from all top-level windows is the Main Menu and its submenus which allow adjusting settings of the DIVING-PAM-II and its peripherals (Fig. 36, page 57; Fig. 37, page 58).

Table 4: Overview of Top-level Windows

	Window	Content
1	**Basic Data**	Minimum data set and basic action keys for filed operation.
2	**Primary Data**	Data of last saturation pulse analysis and current levels of fluorescence and additional measured parameters.
3	**Quenching Analysis**	Fluorescence levels of last saturation pulse analysis and corresponding fluorescence ratios (Y(II), F_V/F_M, …).
4	**Ft-Chart**	Fluorescence signal over 25 or 125 s (see Fig. 66, page 89).
5	**Spectrometer**	Control and display of light, fluorescence and reflectance spectra (only when a MINI-SPEC/MP is connected).
6	**Actinic + Yield**	Short illumination program with saturation pulse analysis.
7	**Induction Curve**	Graphic of saturation pulse analyses of the last induction curve experiment.
8	**Light Curve**	Graphic of saturation pulse analyses of the last light response curve.
9	**Recovery Curve**	Graphic of saturation pulse analyses of the last dark phase following either an induction or a light response.
10	**Actinic Light List**	Set values of photosynthetically active radiation PAR in µmol m^{-2} s^{-1}.



Side Panel

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

Main Panel

Upper field: Window title, battery charge (%), WLAN status (W), and currently selected character to mark measurements. Central field: Numerical and/or graphical data. Lower fields: action keys and information line. Action keys are absent or differently arranged in some windows. The information line displays date + time or PAM activity or informs on critical fluorometer states.

Fig. 23: Principal Screen Layout of Top-level Windows

All top-level windows consist of the "main panel" and the "side panel" (Fig. 23, page 37). The top line of the main panel displays window title, the battery charge percentage, existing WiFi connection, and the capital letter which is currently added as a mark to each saturation pulse analysis.

The bottom of the main panel provides various action keys. Depending on the window, these action keys trigger saturation pulse analysis, control light conditions or affect graphic display. The "Information Line" at the bottom edge of the main panel shows current date and time. The Information Line can also include alphanumeric data when graphics windows are in the "Cursor Mode" which is started by touching the graph area.

The side panel provides arrow keys to change windows, control

keys for fluorometer functions, and a [MENU] key to access the main menu. Control keys are different between windows.

An example is the top button on the sidebar. It triggers saturation pulses on the first three top-level windows (cf. Table 4, page 36), but the same button starts an experimental routine in the windows Actinic + Yield, Induction Curve, Light Curve, and Recovery Curve. Sections 4.1.1 to 4.1.10 will introduce all top-level windows in detail.

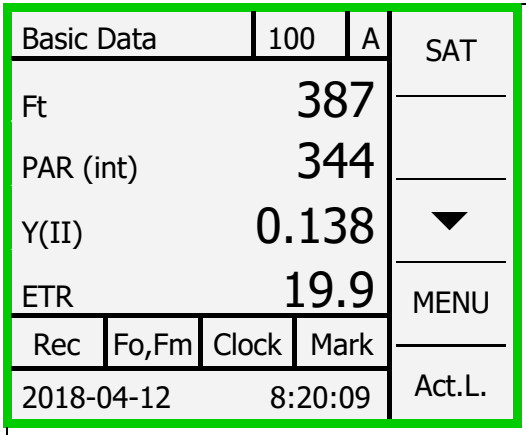
4.1.1 Basic Data

The Basic Data window (Fig. 24, page 39) displays a reduced set of four data for fast sampling under field conditions. From these data, the Ft and PAR represent current measurements but the Y(II), or F_V/F_M, and the ETR are derived from the last saturation pulse analysis.

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

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

The side panel of the Basic Data window offers four commands: the [SAT] key determines effective PS II photochemical yield, and the [Act.L.] represents a switch for actinic light.



Side Panel

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

▼ , go to next window (Primary Data).

MENU, go to Main Menu.

Act.L., actinic light switch.

Main Panel

Ft, current fluorescence value (mV).

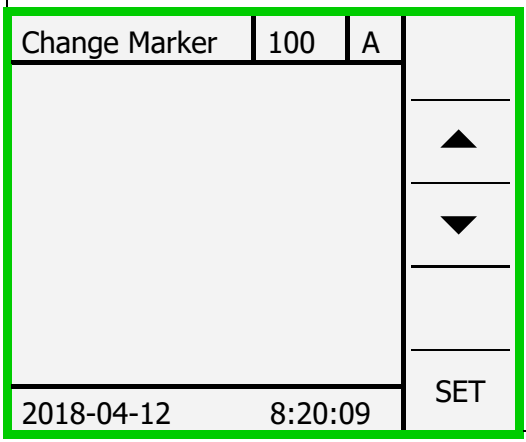
PAR (int), PAR value (µmol·m^{-2}·s^{-1}) from the internal PAR sensor;
PAR (ext) is displayed if an external sensor is active.

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

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

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

Touch [Mark] to open the window below



Side Panel

▲ , go to previous character in alphabet.

▼ , go to next character in alphabet.

SET, use current letter and return to Basic Data.

Fig. 24: Basic Data, Change Marker

The [MENU] key leads to the Main Menu window as in all other top-level windows. The downward arrow key switches to the next window. Two arrow keys (up and down) are present in all other top-level windows except the last (Actinic Light List; Table 4, page 36) which requires only the upward key.

Comment on [Rec] (New Record)

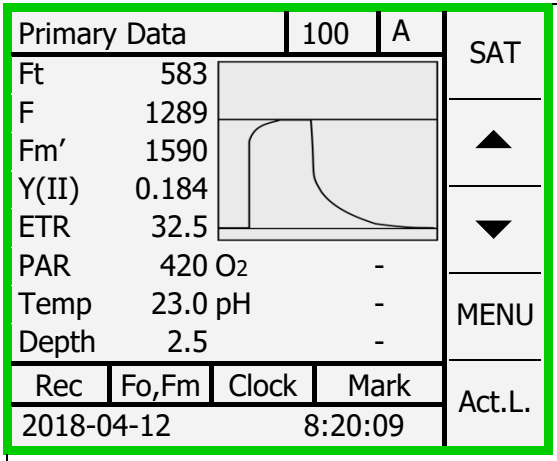
Starting a new Record disconnects current saturation pulse analyses from that of the previous Record. Hence, without F_0 and F_M determinations after [Rec], a [SAT] command will only result in data of Y(II), q_P, and q_L, because only these parameters can be calculated without F_0 and F_M (see Table 28, page 174).

However, the calculation of q_P and q_L requires a F_0' determination. F_0' can be measured by the routine "F_0' mode". If the "F_0' mode" is inactive, F_0' can be calculated according to Oxborough and Baker (see Chapter 10, page 165). These calculations, however, require F_0 and F_M data. Note that all calculated F_0' values are preceded by a swung dash (tilde).

If a Record holds more than one F_0, F_M determination, the latest F_0, F_M will be used to calculate fluorescence coefficients. All calculations using both [Fo,Fm] and [SAT] data are only valid if the two measurements were made with the same sample and under the same measuring conditions. Violating these conditions sometimes results in fluorescence ratio parameters exceeding their valid range (compare Table 28, page 174). Invalid data will be displayed in grey on the touch screen.

4.1.2 Primary Data

The insert in the Primary Data window displays the fluorescence trace of the last saturation pulse. In addition, the window repeats the data of the previous one (Ft, F_V/F_M or Y(II), ETR, PAR). New information of the Primary Data window is the F_0 and F_M signal levels (after [Fo,Fm] was pressed) or the F and F_M' signal levels (after [SAT] was pressed). Additionally, temperature, °C (Temp) and diving depth, as derived from the pressure sensor, are displayed.



Side Panel

Same as Basic Data window (Fig. 24, page 39) plus upward arrow key.

Main Panel

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

Insert, fluorescence trace of last saturation pulse.

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

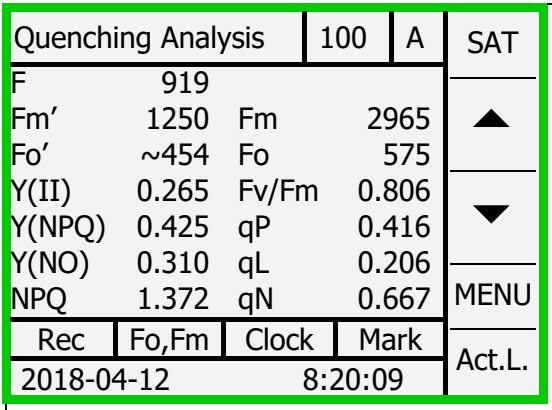
O2, **pH**, display present when an oxygen sensor and a pH sensor are connected.

[Rec], [Fo,Fm], [Clock], [Mark]: see Fig. 24, page 39.

Fig. 25: Primary Data

4.1.3 Quenching Analysis

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



Side Panel

Same as Basic Data window (Fig. 24, page 39) plus upward arrow key.

Main Panel

Numerical data: see Fig. 98, page 167 and Table 28, page 174.

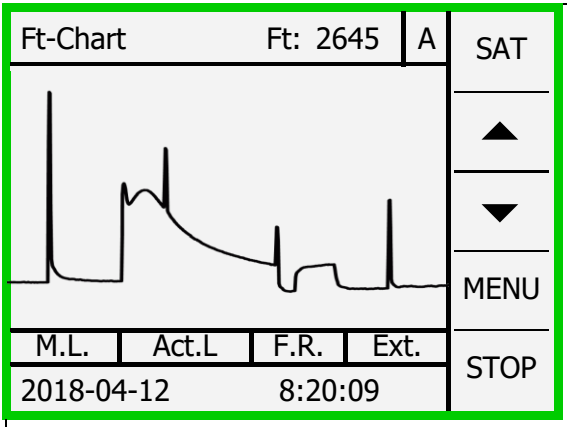
Rec, Fo,Fm, Clock, Mark: see Fig. 24, page 39. The swung dash (tilde) before the F_0' indicates that the value was calculated.

Fig. 26: Quenching Analysis

4.1.4 Ft-Chart

The Ft-Chart displays a 25 or 125 s interval of Ft where the rightmost level of the graph corresponds to the current Ft value. The X axis interval can be adjusted in the menu “DIVING-PAM-II Settings (Fig. 66, page 89).

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



Side Panel

Same as above (Fig. 26) but Act.L. replaced by STOP/RUN.

Main Panel

Graph. Trace of current fluorescence measurement. Total X axis: 25 or 125 s: see Fig. 66, page 89.

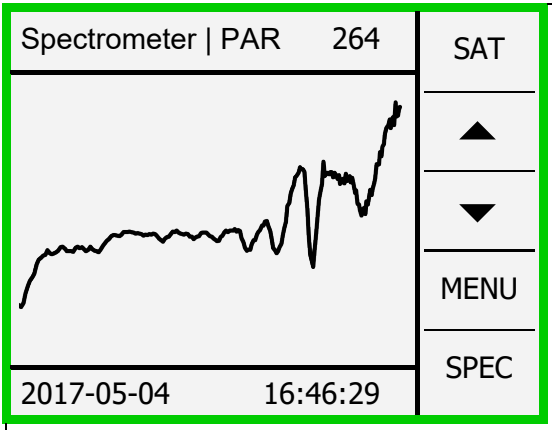
M.L, **Act.L** , **F.R.** , **Ext.**. On/off switches for measuring light, actinic light, far-red light and external (synchronized) light source, respectively. The **Ext.** appears only if "Ext. Panel Mode" is active (see Fig. 42, page 65)

Fig. 27: Ft Chart

4.1.5 Spectrometer

The SPEC command of the Spectrometer window triggers a spectrometric measurement. In the standard mode, the spectrometer is equipped with a tube containing the optical entrance for external radiation (Fig. 9, page 21).

Spectra of photon flux densities are measured in units of nmol photons m^{-2} s^{-1} nm^{-1}. PAR corresponds to the integral of the spectrum from 400 to 700 nm. PAR is derived from the spectrometer when "Ext. PAR Sensor" is set to "On" in menu "Sensor Settings" (available by selecting "Sensors" in the Main Manu), and the spectrometer is selected as external PAR sensor (Main Menu → Sensors → External PAR sensor → Spectrometer; Fig. 50, page 74).



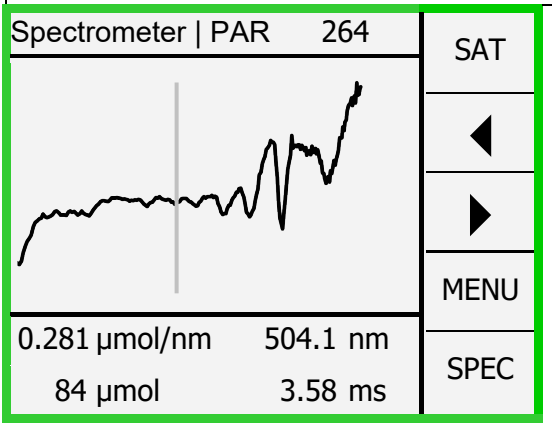
Side Panel

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

Main Panel

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

Touch chart to open/close the window below.



Side Panel

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

Main Panel

Spectrum and its numerical values.

Fig. 28: Spectrometer

Note that the spectrometer measures the PAR of the DIVING-PAM-II internal light correctly only in the CAL mode (Fig. 33, page 52) because only in the CAL mode the LED emits continuous light.

In the measuring mode, actinic light is pulse-width modulated, and the PAR value fluctuates strongly.

To record fluorescence or reflectance spectra, change spectrometer configuration as described in Fig. 9 (page 21). Then select operation mode: Main Menu → Sensor → Spectrometer → Operation Mode.

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

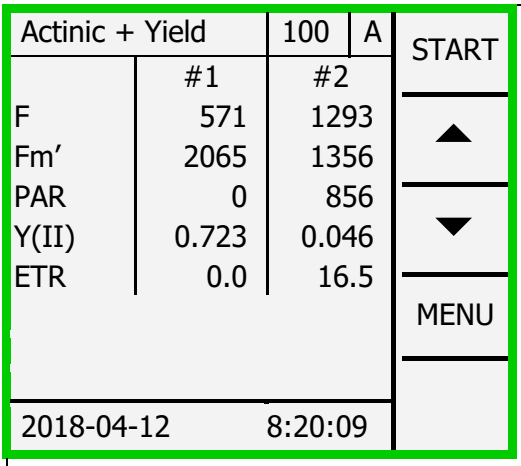
For reflectance measurements, first measure dark current in complete darkness, then the 100% reflectance signal with the white reference material (Fig. 8, page 20), and finally the sample.

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

4.1.6 Actinic + Yield

The “Actinic + Yield” window is the first of four windows for automated measuring routines. The parameters of the Actinic + Yield routine can be set in the menu “Actinic + Yield Settings” (Fig. 45, page 69) which is accessible via “Program/Clock” of the Main Menu.

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



Side Panel

START. Start Actinic + Yield routine.

▲ Previous window (Ft-Chart).

▼ Next window (Induction Curve).

MENU. Go to Main Menu.

Main Panel

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

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

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

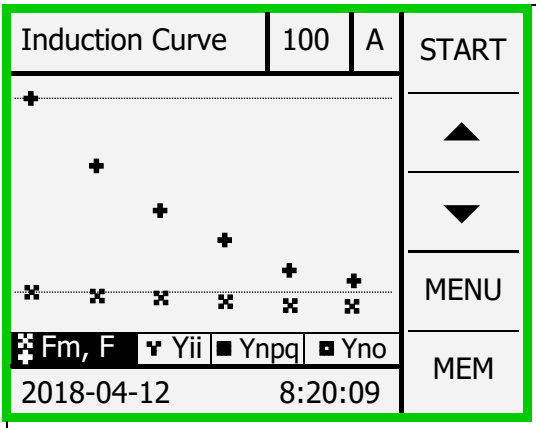
PAR, ETR. Photosynthetically active radiation (µmol·photons $m^{-2}\cdot s^{-1}$), electron transport rate (µmol·electrons $m^{-2}\cdot s^{-1}$).

Fig. 29: Actinic + Yield

4.1.7 Induction Curve

The "Induction Curve" window controls fluorescence induction curve experiments (Fig. 30, page 47). The graphics panel provides a qualitative picture of induction curve data.

The window also provides numerical data of fluorescence ratio parameters and fluorescence levels. These numerical data appear by pressing the VIEW optical switch of the DIVING-PAM-II. The navigation keys move the cursor (grey vertical line) from one saturation pulse analysis to another. The cursor indicates the position within the induction curve of the currently displayed set of numerical data.



Side Panel

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

▲ , ▼ .Go to previous, next window.

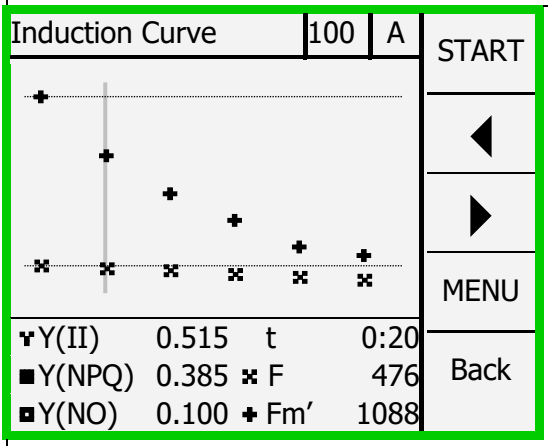
MENU. Go to Main Menu.

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

Main Panel

Graphics panel and buttons Fm, F, Yii, Ynpq, Yno to control display of data (F_M,F_M' plus F, Y(II), Y(NPQ) and Y(NO)). The Y(NPQ) and Y(NO) can be exchanged by parameters NPQ and qL in the menu Program/Clock Settings (4.2.3, page 67).

Press the VIEW optical switch on the DIVING-PAM-II to toggle windows.



Side Panel

As above except arrow keys

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

Main Panel

Numerical values of fluorescence ratio parameters and fluorescence levels.

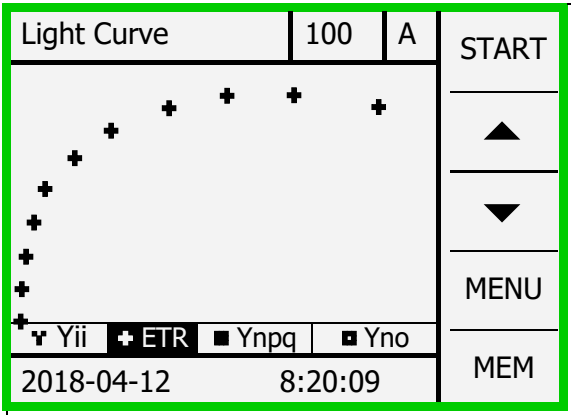
Fig. 30: Induction Curve

The parameters of an induction curve experiment, number of saturation pulse analyses and interval between them, can be adjusted in the menu “Induction Curve Settings” (Fig. 46, page 70) which is available over the Program/Clock line of the Main Menu (Fig. 36, page 57). In the “Induction Curve Settings” menu, you can choose to continue fluorescence monitoring after the induction curve in the dark (recovery curve). The MEM of the Induction Curve window opens the “Induction Curve Memory” window. In this window, the navigation keys scroll through stored induction curves.

4.1.8 Light Curve

In analogy to the previous window (Induction Curve), the “Light Curve” window provides buttons to start and stop light curves and to survey them (Fig. 31, page 49). Also, the Light Curve window provides numerical data of saturation pulse analysis which can be accessed by VIEW optical switch on the DIVING-PAM-II. Navigation between different saturation pulses analyses and selection of displayed data works as described for the previous window (Induction Curve).

Light curve parameters (number and duration of light steps, initial PAR, and recovery curve) can be adjusted in the menu “Light Curve Settings” (Fig. 47, page 71). Touching the MEM button and scrolling using the arrow keys on the side bar permits viewing of stored light curves.



Side Panel

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

▲, ▼ .Go to previous, next window.

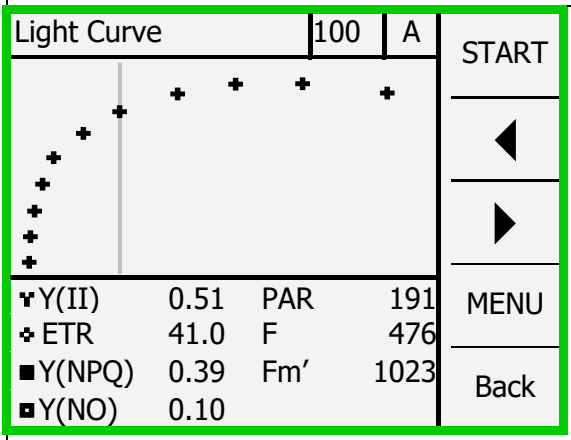
MENU. Go to Main Menu.

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

Main Panel

Graphics panel and buttons Yii, ETR, Ynpq, Yno to control display of data (Y(II), ETR, Y(NPQ) and Y(NO)). The Y(NPQ) and Y(NO) can be exchanged by parameters NPQ and qL in the menu Program/Clock Settings (4.2.3.7, page 72).

Press the VIEW optical switch on the DIVING-PAM-II to toggle windows.



Side Panel

As above except arrow keys

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

Main Panel

Numerical values of fluorescence ratio parameters and fluorescence levels.

Fig. 31: Light Curve

4.1.9 Recovery

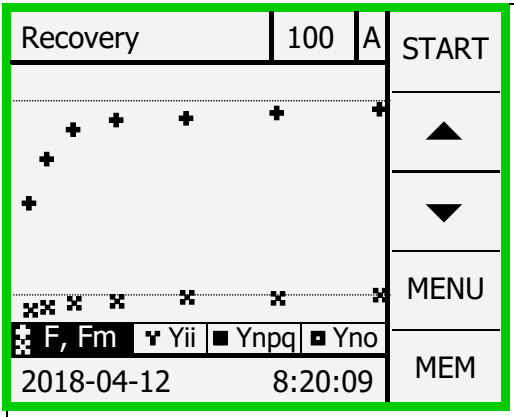
Fluorescence recovery experiments can be automatically appended to an induction or a light curve (see 4.2.3.5, page 70 and Section 4.2.3.6, page 71. Recovery curves can also be initiated (and cancelled) manually using the START/STOP button on the side panel of the Recovery window (see Fig. 32, page 51).

As in previous Induction and Light Curve windows, recovery data are represented graphically and numerically. The time course of recovery curves is fixed: each curve last 39 min during which 7 saturation analysis are carried out. In case of a preceding induction or light curve, the last saturation pulse analysis of the induction or light curve corresponds to the first one of recovery. The interval between neighboring saturation pulse analysis roughly doubles with time (Table 5, page 13).

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

Table 5: Sequence of Saturation Pulse Analyses in a Recovery Curve

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



Side Panel

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

▲, ▼ .Go to previous, next window.

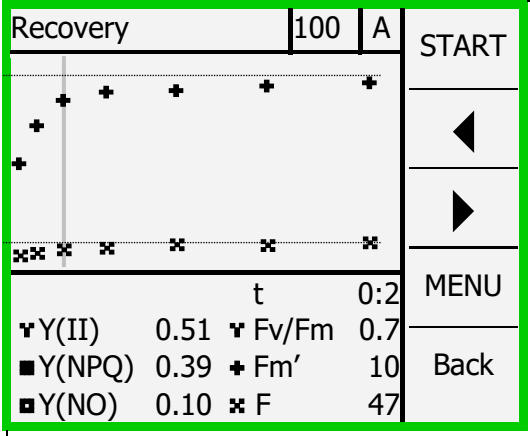
MENU. Go to Main Menu.

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

Main Panel

Graphics panel and control buttons as described in Fig. 30, page 47.

Press the VIEW optical switch on the DIVING-PAM-II to toggle windows.



Side Panel

As above except arrow keys

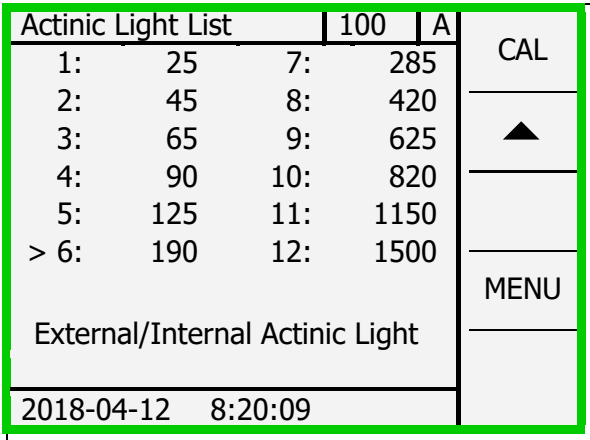
◀, ▶. Go to previous, next saturation pulse analysis (current position is marked by vertical line).

Main Panel

Numerical values of fluorescence ratio parameters and fluorescence levels.

Fig. 32: Recovery

4.1.10 Actinic Light List



Side Panel

CAL. Calibrate Actinic Light List.

▲ Go to previous window.

MENU, go to Main Menu.

Main Panel

PAR target values (µmol m² s¹) of the 12 settings for actinic light intensity. The PAR target values are valid for an actinic light factor of 1.00 (see menu "Light Sources" Fig. 41, page 65). The ">" indicates the currently selected PAR level.

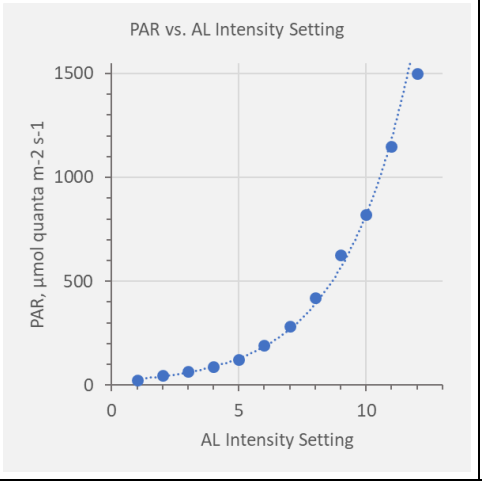


Fig. 33: Actinic Light List

The window “Actinic Light List” displays PAR values (in µmol m^{-2} s^{-1}) which increase roughly exponentially with intensity settings from 1 to 12. The entire PAR list can be multiplied by the Actinic Factor (Fig. 41, page 65).

In the factory, blue or red LED emissions are adjusted so that these PAR values apply for the sample level of the Universal Sample Holder DIVING-II-USH with the DIVING-PAM-II fiber optics fully inserted (i.e. distance between fiber optics tip and sample level is around 7.5 mm; angle between end piece of fiber optics and sample level: 60°). (Because of similar geometrical arrangement, these factory settings also apply to the Leaf Clip Holder 2035-B, 2010-A Distance Clip 60°, and the 2060-B Arabidopsis Leaf Clip when distance rings 2 mm plus 4 mm are used.)

External versus Internal Actinic Light

The main panel of the window Actinic Light List (Fig. 33, page 52) indicates the light source used for actinic illumination.

External Actinic Light is displayed when:

- An external lamp (2054-L External LED Source) is connected to the SYNC port of the MINI-PAM and “PAM Mode (Act)” is selected as operation mode (see 4.2.2.6, page 66).

Internal Actinic Light is displayed when:

- An external lamp (2054-L External LED Source) is connected to the SYNC port of the MINI-PAM and “Ext. Panel Mode” is selected as operation mode (see 4.2.2.6, page 66)..
- An external lamp is not connected.

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

Clicking CAL opens the window “Calibrate Light List” in which an external or internal light sensor can be selected (Fig. 34). The option “Use External Light Sensor” requires that an external light

sensor is connected. If “Use External Light Sensor” is attempted in the absence of an external sensor, an error message pops up (Fig. 35, page 56). The same error message appears when the external sensor is not exposed to the actinic light, or when the external sensor to be used was not set as active sensor (see Fig. 50, page 74). The option “Use Internal Light Sensor” cannot be used to calibrate an external light source. Both the external and internal light sensor options are only available for the internal actinic light source.

Table 6: The CAL Command

Light source being calibrated.	**Ext. PAR Sensor** (e.g., 2035-B Leaf-Clip Holder).	**External Light Source** (e.g., LED Light Source 2054-L).	**Action.**
Internal actinic light.	Connected	Absent OR Present and “Ext. Panel Mode” active.	If “Using Ext. Sensor” selected: Calibration of internal light sensor. Then, calibration of the Actinic Light List with the internal light sensor.
Internal actinic light.	Absent or connected	As above.	If “Using Intern. Sensor” selected: Calibration of the Actinic Light List with the internal light sensor.
External actinic light.	Connected	Present and “Ext. Panel Mode” active.	Calibration of the Actinic Light List with the external light sensor.*

Using the MINI-SPEC Miniature Spectrometer as external PAR sensor: See Section 3.1.4, page 19). Calibration of the internal light sensor: See Section 4.2.4.5, page 83.

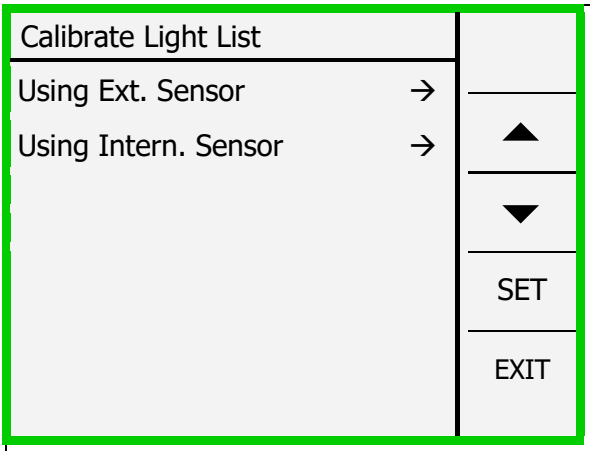


Fig. 34: Calibrate Light List

Calibration of an external light source. If "External Actinic Illumination" is displayed by the window "Actinic Light List", the CAL command adjusts the PAR emitted by the 2054-L External LED Source to the PAR values of the Actinic Light List. Typically, the external sensor is the PAR sensor of 2035-B leaf clip.

To separately adjust each of the four light types of the 2054-L lamp, CAL command switches the PAR sensor of the 2035-B leaf clip automatically to the "Mixed Mode" (see Section 4.2.4.4, page 80). After calibration, the measuring mode of the PAR sensor is returned to its previous mode. When checking the light intensities of the 2054-L lamp, make sure that the Mixed Mode is selected.

Internal Light Sensor. The internal sensor is exposed to a small and constant fraction of internal actinic light. The properly calibrated internal PAR sensor measures continuously internal light intensity. In the factory, the internal sensor has been adjusted to

indicate the PAR values at sample level of the 2035-B leaf clip when the fiberoptics is fully inserted.

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

If a calibrated PAR sensor feeds its data in the DIVING-PAM-II (as is the case with the MINI-SPEC or the PAR sensor of 2035-B leaf clip), the internal PAR sensor can be calibrated automatically. To do so, select “Calibrate” in window “Int. PAR Sensor Settings” and start automatic adjustment of the calibration factor (see Section 4.2.4.5, page 83).

If the calibrated PAR sensor is not compatible with the MINI-PAM-II, switch light on, and vary in the window “Int. PAR Sensor Settings” (Fig. 63, page 84) the “Calibration Factor” until internal PAR matches external PAR.

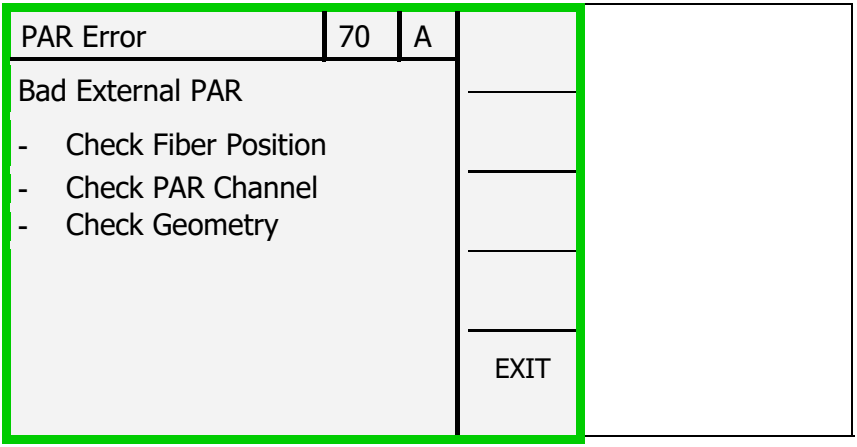


Fig. 35: PAR Error

4.2 Main Menu

The “Main Menu” (Fig. 36, page 57) is the central hub to access settings, calibration data, hardware information and the memory of the DIVING-PAM-II. The Main Menu consists of eight items. An item is selected by the arrow keys of the side panel followed by the SET command, or by directly touching a menu line. Most of these items lead to submenus that themselves link to lower-level menus. The complex architecture of the Main Menu and its sub-menus is outlined in Fig. 37, page 58.

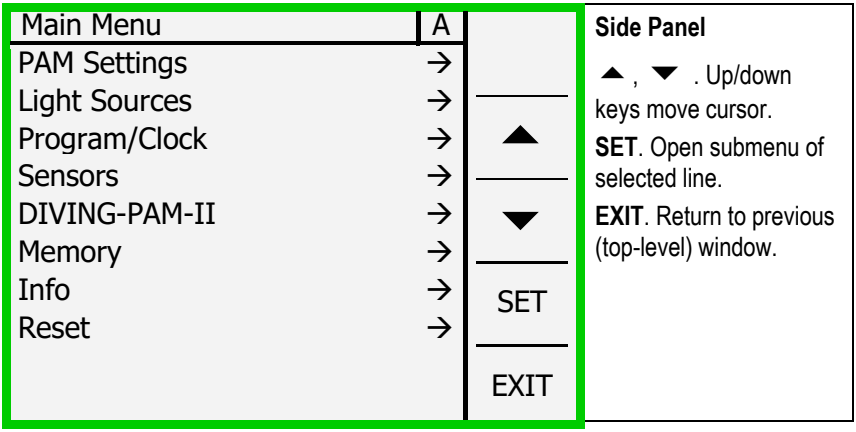


Fig. 36: Main Menu

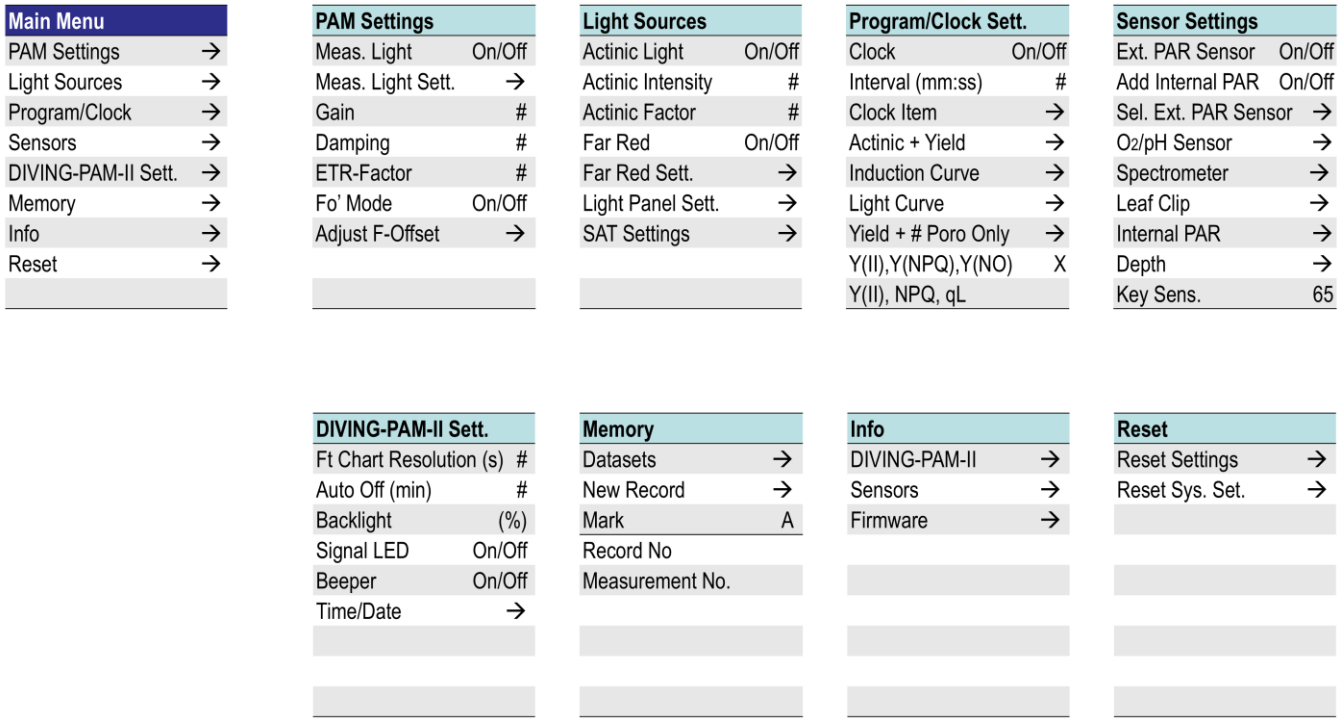


Fig. 37: Main Menu with all eight submenus

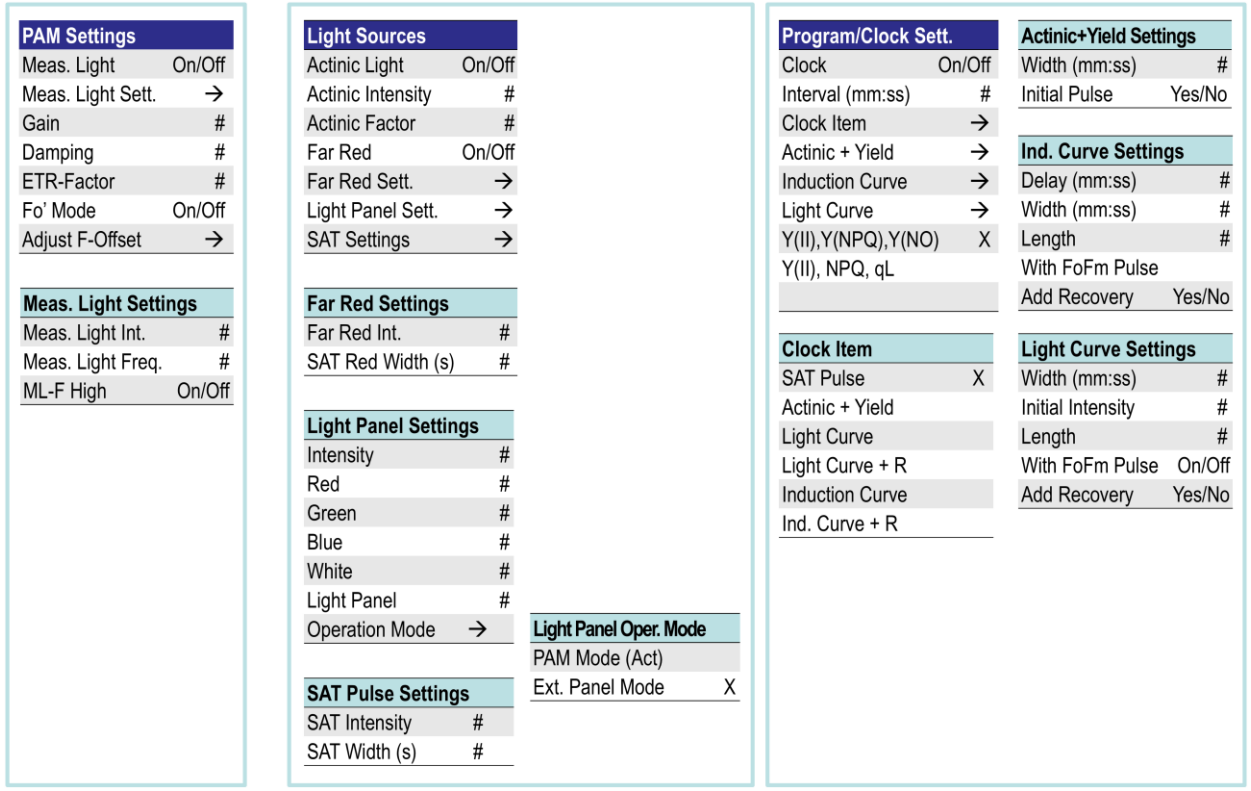


Fig. 38: First Three Submenus in Detail

4.2.1 PAM Settings

The menu item “PAM Settings” includes adjustments of the way how the DIVING-PAM-II acquires PAM fluorescence. The PAM Settings menu contains seven items (Fig. 39, page 60) but also displays the current Ft value and the currently active offset, which is automatically subtracted from the raw signal to obtain Ft.

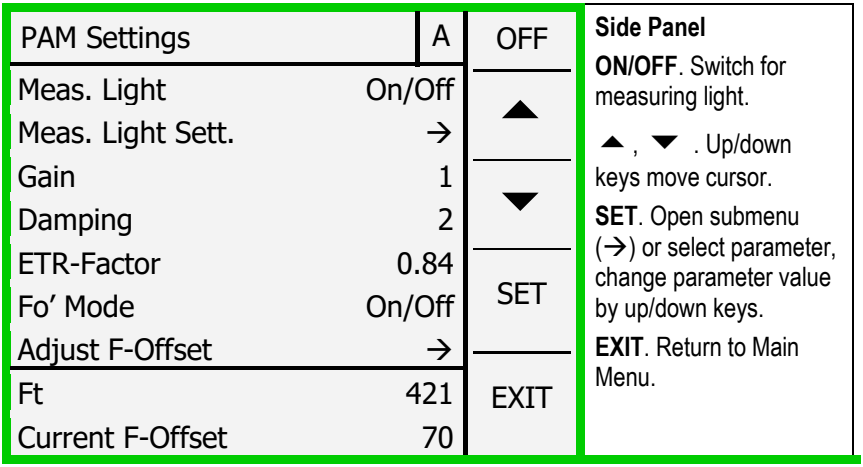


Fig. 39: PAM Settings

4.2.1.1 Meas. Light

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

4.2.1.2 Meas. Light Sett.

The item “Measuring Light Settings” opens a menu for configuring the measuring light. The variables are measuring light intensity (Meas. Light Int.), measuring light frequency (Meas. Light. Freq.), and the option to set measuring light frequency to high (MF-F

High). To adjust, select variable by the Up/Down arrows and press SET . If several options are available, select desired setting by Up/Down arrows followed by SET .

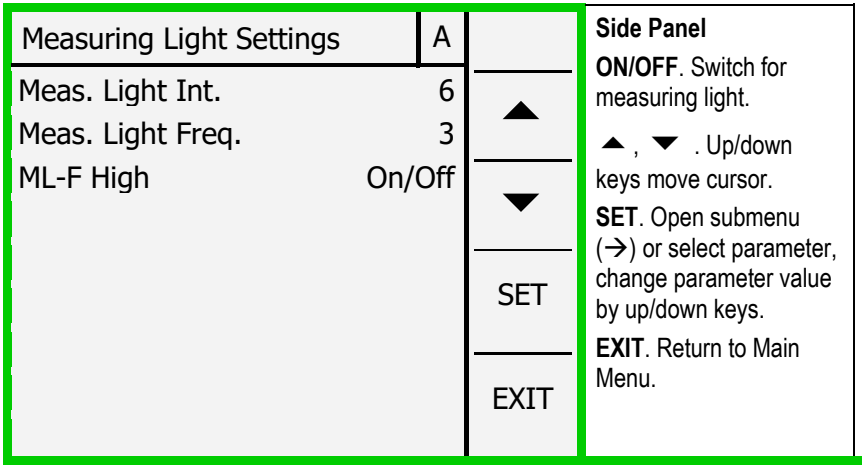


Fig. 40: Measuring Light Settings

Measuring light intensity: At constant frequency, measuring light intensity can be considered as proportional to settings 1 to 12. The fluorescence signal increases with measuring light intensity. High measuring light intensities can interfere with the correct F_0 fluorescence measurements.

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

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

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

Measuring light frequency: Five frequency levels are available (see Table 7, page 62). Increasing the measuring light frequency improves the signal quality but can also be actinic and therefore interfere with F_0 fluorescence measurements. Measuring light frequency does not affect the frequency of acquisition of Ft by the WinControl-3 software.

High measuring light frequency: The highest measuring light frequency is 100 Hz. The “ML-F High” command overrules settings made under “Meas. Light. Freq.” High measuring light frequency improves signal quality but bears the risk that its higher intensity drives photosynthesis, that is, the measuring light becomes actinic. In this case, the F_0 may be overestimated. Measuring light frequency is automatically switched to “high” for saturation pulse analysis.

4.2.1.3 Gain

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

Table 7: Measuring Light Frequencies

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

4.2.1.4 Damping

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

4.2.1.5 ETR-Factor

This factor is used for ETR calculations and corresponds to the fraction of incident PAR absorbed by a leaf; its default value is 0.84 (cf. Section 10.3, page 172).

4.2.1.6 Fo’ Mode

After saturation pulses, the “Fo’ Mode” replaces actinic light by far-red light to quickly open PS II reaction centers (cf. Chapter 10, page 165). The measured F_0’ is the minimum Ft during this far-red period. Interval and intensity of far-red illumination can be adjusted in menu “Light Sources” (Fig. 41, page 65).

4.2.1.7 Adjust F-Offset

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

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

Usually, the background signal increases with measuring light intensity and signal amplification (gain). Therefore, the Adjust F-Offset command determines the background signal for all measuring light intensities and all gain settings. The currently active offset is displayed in the bottom line of the PAM Settings window (Fig. 39, page 60).

Procedure

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

4.2.2 Light Sources

4.2.2.1 Actinic Light

On/off switch for actinic illumination. Instead via the menu item, actinic light can be switched on and off by the OFF/ON key on the side panel of the window (Fig. 41, page 65).

4.2.2.2 Actinic Intensity

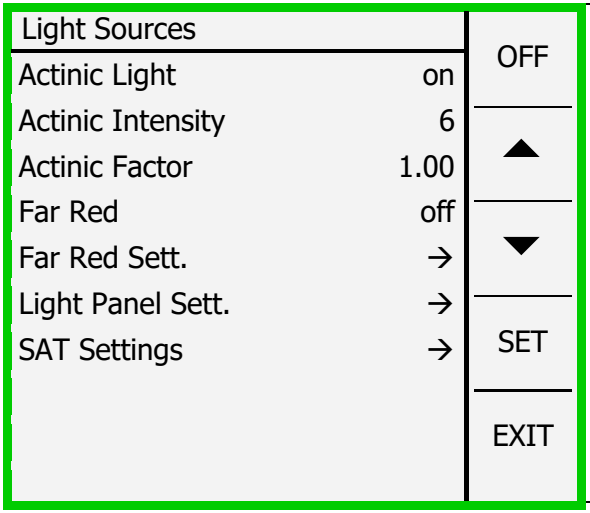
Intensity regulation for actinic light. Select menu item by SET and choose setting using arrow keys. Settings 1 to 12 are available. PAR information of settings is shown in window “Actinic Light List” (Fig. 33, line 52).

4.2.2.3 Actinic Factor

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

4.2.2.4 Far-red

On/off switch for far-red light.



Side Panel

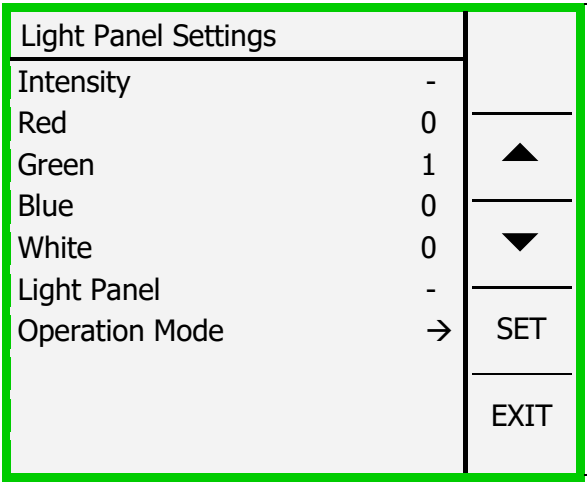
ON/OFF, actinic light switch.

▲, ▼ . Up/down keys move cursor.

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

EXIT. Return to Main Menu.

Fig. 41: Light Sources



Side Panel

▲, ▼ . Up/Down keys to move to a menu line.

SET. Select setting, up/down keys change setting value.

EXIT. Return to Light Sources menu.

Fig. 42: Light Panel Settings

4.2.2.5 Far-red Sett.

Opens the menu to adjust intensity and duration of far-red illumination. These settings are active for F_0' determinations (Section 10.2, page 168).

4.2.2.6 Light Panel Sett.

Use SET to enter submenu "Light Panel Settings" (Fig. 42, page 65). The function of this window depends on the "operation mode". The operation mode is selected in the last line in this window.

Two operation modes are available: "PAM Mode (Act.)" and "Ext. Panel Mode". In the first mode (PAM Mode (Act.)), actinic light is provided exclusively by an external source (e.g., the MINI-PAM-II accessory "2054-L External LED Source") In the second mode (Ext. Panel Mode), the DIVING-PAM-II internal light is used as actinic light to which an external light source may be added (see Table 22, page 137).

Note: Operation of the external light source 2054-L requires line power.

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

For **PAM Mode (Act.)**, proper calibration of the external light source is mandatory. In this mode, the item "Intensity" and the Light Panel On/Off switch in Fig. 42 are not available.

In the "PAM Mode (Act.)" mode, the total intensity of all four LED colors is adjusted as described above for internal actinic light, that is, 12 intensity settings are available with the intensities defined in the "Actinic Light List" (Fig. 33, page 52), and the intensities can be modified by the Actinic Factor (Section 4.2.2.3, page 64).

In the Light Panel Settings window (Fig. 42, page 65), the numbers entered for the four LED colors (red to white) determine the fraction with which each LED color contributes to the actinic light. For example, if red=1, green=1, blue=3 and white=0, the actinic light contains 20% each of red and green, 60% of blue and no white.

In the "**Ext. Panel Mode**", in the window Light Panel Settings (Fig. 42), the item "Intensity" and the Light Panel On/Off switch are available. The intensity scales range from 0% to 100% (maximum intensity). The typical maximum value (100%) for the individual LEDs is about 1500 µmol m^{-2} s^{-1}. When all four LED intensities are set to 100%, the maximum total intensity is > 5500 µmol m^{-2} s^{-1}. Exact PAR values of the external panel are not available in the Ext. Panel Mode.

4.2.2.7 SAT Settings

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

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

4.2.3 Program/Clock

The menu "Program/Clock Settings" provides all options to configure automated experimental routines (Actinic + Yield, Induction Curve, and Light Curve) well as repetitive triggering of single measurements and experimental routines.

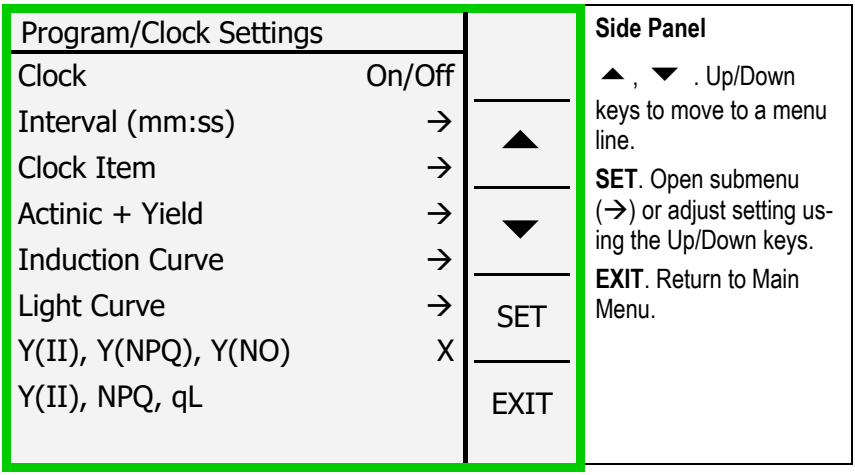


Fig. 43: Program/Clock Settings

4.2.3.1 Clock

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

4.2.3.2 Clock Interval

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

4.2.3.3 Clock Item

Saturation pulse analyses, and the programs Actinic + Yield, Induction Curve and Light Curve can be repetitively performed under clock control. Recovery experiments can be appended to induction and light curves (item Light Curve + R and Induction Curve + R, respectively). To select one of the six items in menu “Clock Item” (Fig. 44), move cursor to the item of interest and touch SET. The selected item is then marked by an X.

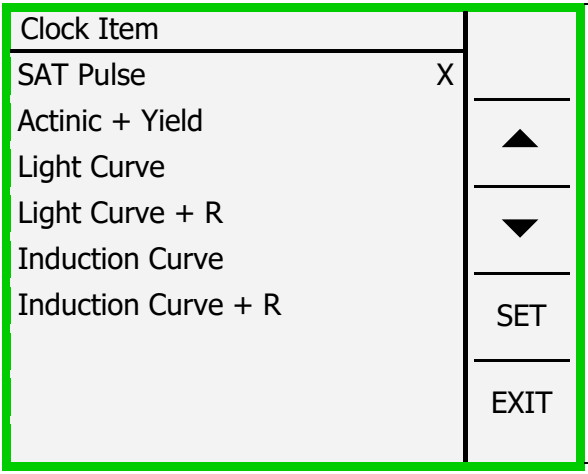


Fig. 44: Clock Item

4.2.3.4 Actinic + Yield

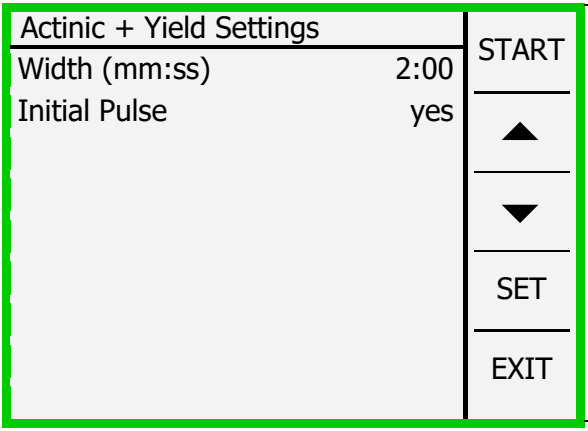


Fig. 45: Actinic + Yield Settings

The behavior of the Actinic + Yield program is defined by two factors (Fig. 45, page 69): 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 width of actinic illumination is adjusted as described above for clock interval and initial pulse is selected by the SET command. Actinic light intensity is adjusted in the window “Light Sources” (Fig. 41, page 65).

An Actinic + Yield routine can be started using the START button on the side panel of Fig. 45. In this case, the screen display will automatically switch to the Actinic + Yield experimental window (Fig. 29, page 46).

4.2.3.5 Induction Curve

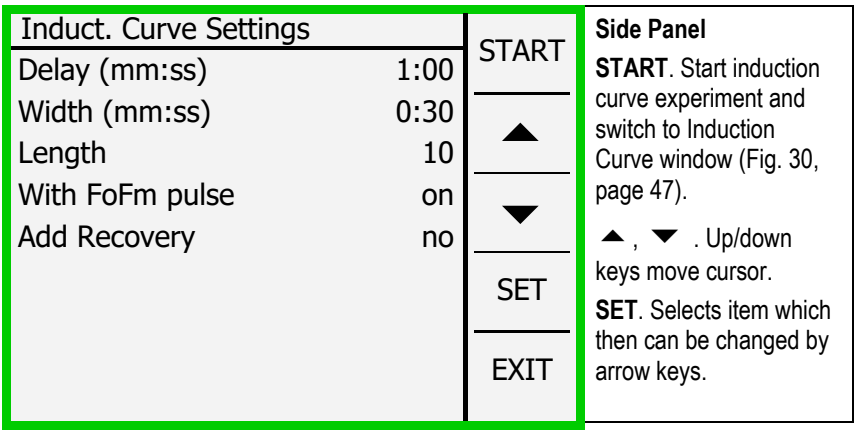


Fig. 46: Induction Curve Settings

Induction curve experiments are configured in the window “Induction Curve Settings” (Fig. 46, page 70).

Delay (range 5 s to 10 min) defines the dark interval between saturation pulse analysis with the dark-acclimated sample (F_0, F_M determinations, Chapter 10, page 165) and beginning of actinic illumination.

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

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

With FoFm Pulse performs a saturation pulse analysis before actinic light is switched on.

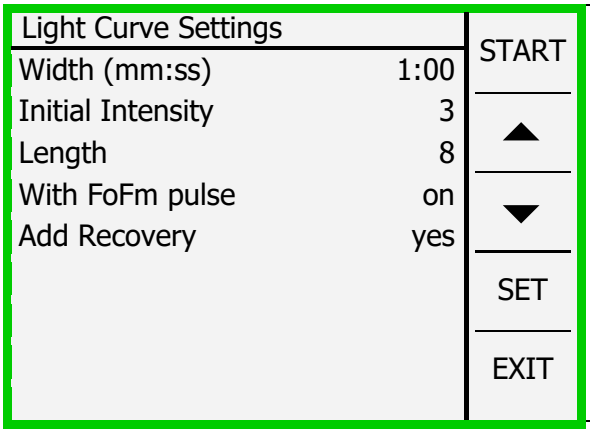
Add recovery appends a recovery curve to an induction curve (see Section 0, page 49 for information on recovery times).

4.2.3.6 Light Curve

Light Curves are defined in "Light Curve Settings" (Fig. 47, page 71). Properties of Width and Add Recovery are identical for light and induction curves.

Intensity specifies the actinic intensity setting for the first light step (range 1 to 5, for PAR values see Fig. 33, page 52).

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 is Length times Width.



Side Panel

START. Start light curve experiment and switch to Light Curve window (Fig. 31, page 49).

▲, ▼ . Up/down keys move cursor.

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

Fig. 47: Light Curve Settings

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

The last two lines of the menu “Program/Clock Settings” affect graphical and numerical presentation of data in windows Induction Curve, Light Curve, and Recovery Curve. Selecting “Y(II), Y(NPQ) Y(NO)” displays three yield parameters that are used in analyzing energy partitioning. Choosing Y(II), NPQ, and qL displays the classical NPQ parameter and a parameter for indicating the reduction state of PS II (q_L). Selection between lines works as described for “Clock Item” (Fig. 44, page 69). See Table 28, page 174 for definitions of fluorescence parameters.

4.2.4 Sensors

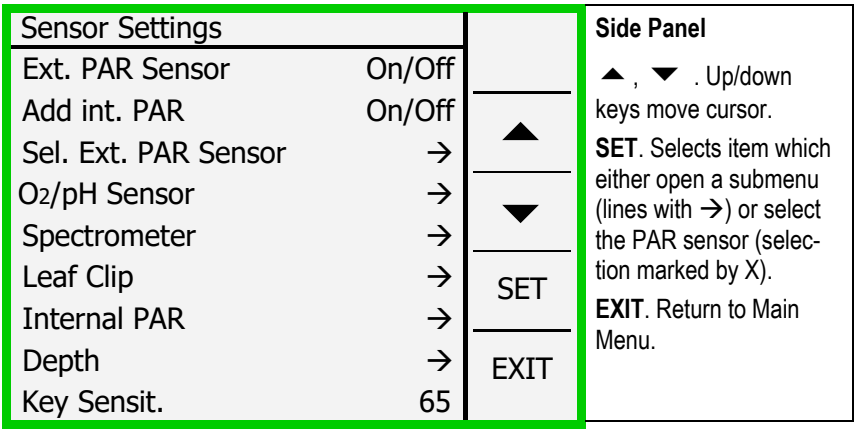


Fig. 48: Sensor Settings

The Main Menu item “Sensors” leads to the window “Sensor Settings” (Fig. 48). The external PAR sensor (if connected) can be set as the light sensor via the first window item (Ext. PAR Sensor). The second window item allows adding the intensity of the internal light source to the external measurement. In addition, “Sensor Settings” includes eight links leading to submenus (distinguished by

→). An overview of Sensor Settings and its complex structure of submenus gives Fig. 49.

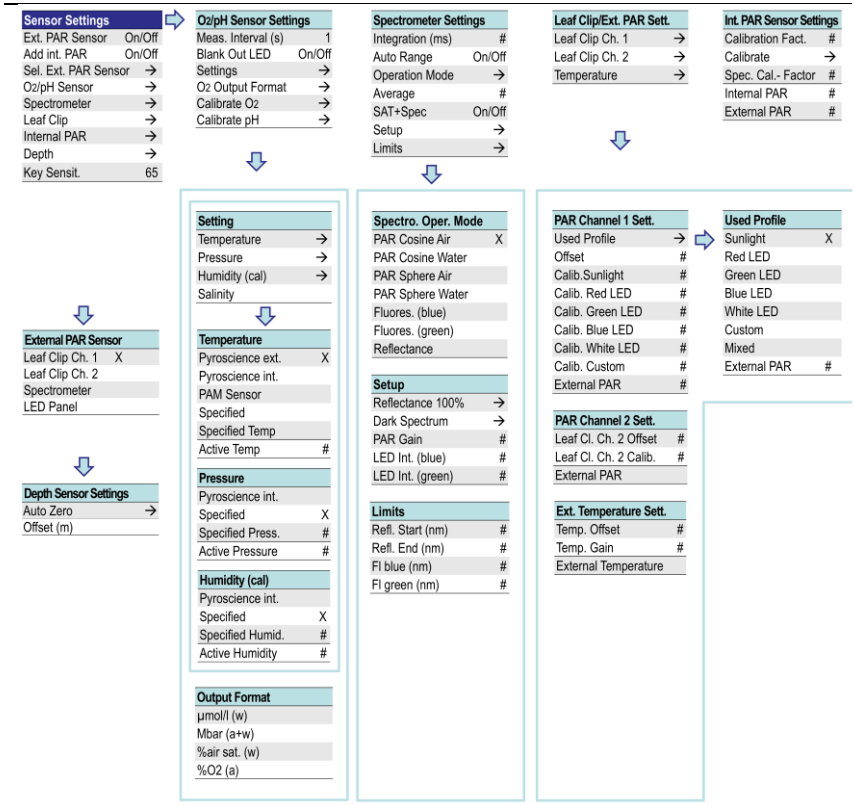


Fig. 49: Sensor Settings Menu and its Submenus

4.2.4.1 External PAR Sensor

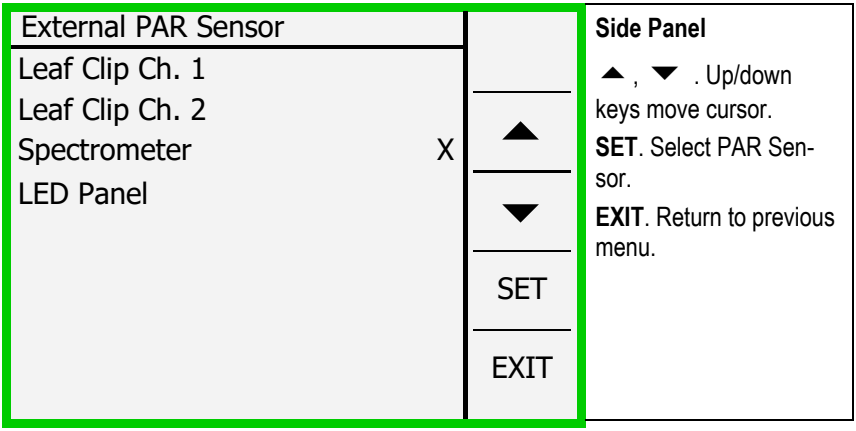


Fig. 50: External PAR Sensor

Ext PAR Sensor opens a list of PAR sensors (Fig. 50, page 74): PAR sensors 1 and (optional) 2 of 2035-B leaf clip, the miniature spectrometer, and a PAR sensor connected to the LED-Panel RGBW-L084. Select PAR sensor via Up/down keys and SET. In the default configuration, Leaf Clip Ch 1 is selected.

4.2.4.2 O2/pH Sensor Settings

The window “O2/pH Sensor Settings” is dedicated to optode sensors measuring either oxygen concentration of pH. (At the time of printing this manual, the market launch of the pH sensor for the DIVING-PAM-II device is in preparation.)

Here, the subject “O2/pH Sensor Settings” is shortly introduced. Details on O_2 optode measurements are provided by a separate manual:
https://www.walz.com/products/chl_p700/mini-pam-II/downloads.html
and by the manual for the FireStingO2 oxymeter:
(https://www.pyroscience.com/en/).

In the window “O2/pH Sensor Settings”, “Meas. Interval” defines the frequency of oxygen readings and “Blank Out LED” switches of DIVING-PAM-II internal or external light in the case that it interferes with oxygen measurements.

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

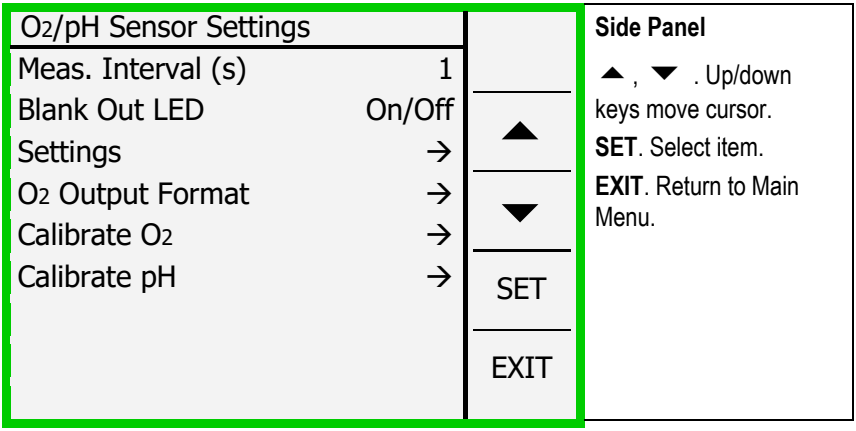


Fig. 51: Oxygen Sensor Settings

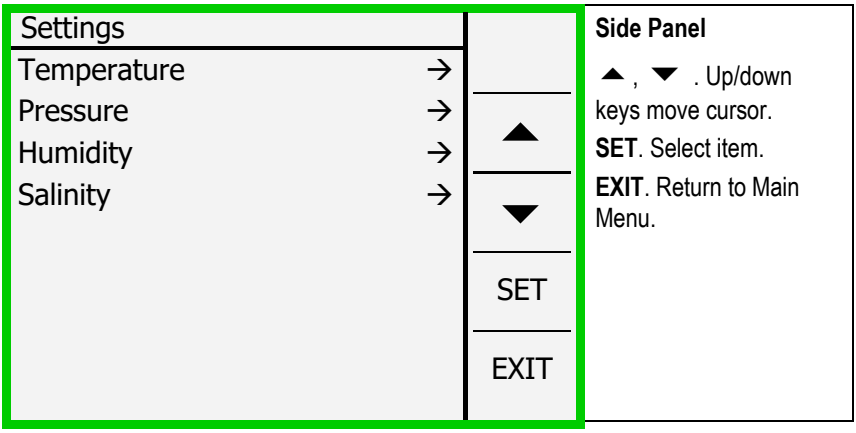


Fig. 52: Settings

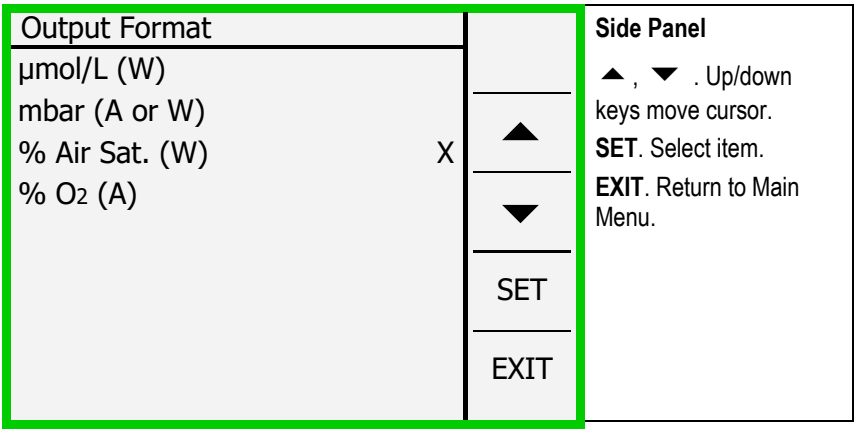


Fig. 53: Output Format

The last two menu items (Calibrate O2 and Calibrate pH) give access to the windows for calibration of the oxygen and the pH sensor, respectively.

4.2.4.3 Spectrometer

The item “Spectrometer” in menu “Sensor Settings” leads to the window “Spectrometer Settings (Fig. 54, page 77) which is dedicated to configuration on the miniature spectrometer.

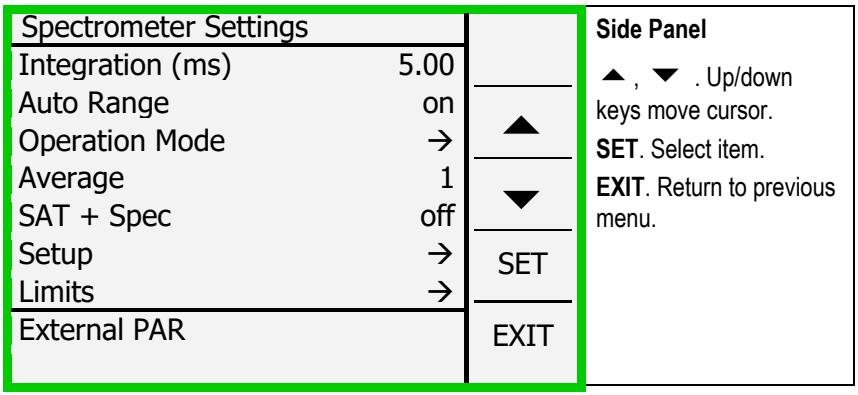


Fig. 54: Spectrometer Settings

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

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

Operation Mode opens another menu (Fig. 55, page 78) in which the type of spectrum (light, fluorescence or reflectance) and, for light spectra, the entrance optics (flat=cosine versus spherical) and the environment in which measurements are performed (air or water) can be specified.

The cosine configuration employs a diffusing disk as light entrance (Fig. 9). This configuration shows an approximate cosine response toward incoming radiation. The sphere configuration

measures light from all directions with similar weight. At the time of writing of this manuscript, the spherical sensor is under development.

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

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

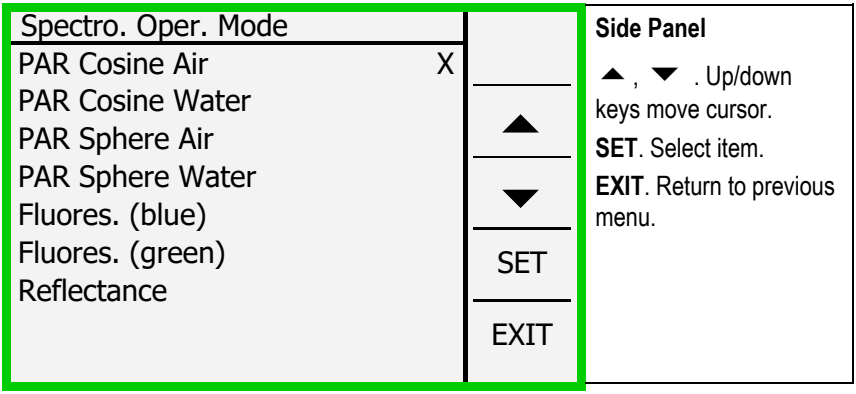


Fig. 55: Spectro. Oper. Mode

Setup opens a menu for recording of the dark spectrum of the miniature spectrometer (Dark Spectrum →). The dark current of the spectrometer at room temperature is measured in the factory and stored on the flash memory of the device. To newly establish the dark current, fully darken the entrance optics (cv. Fig. 9, page 21).

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

the blue and green LED used as excitation source for fluorescence emission spectra.

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

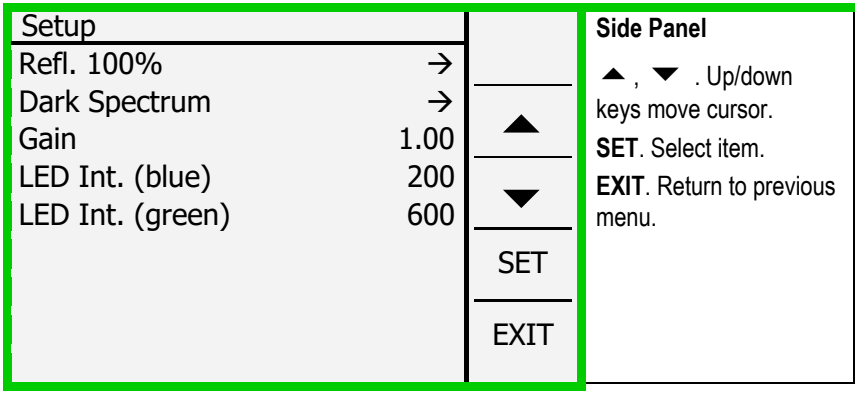


Fig. 56: Setup

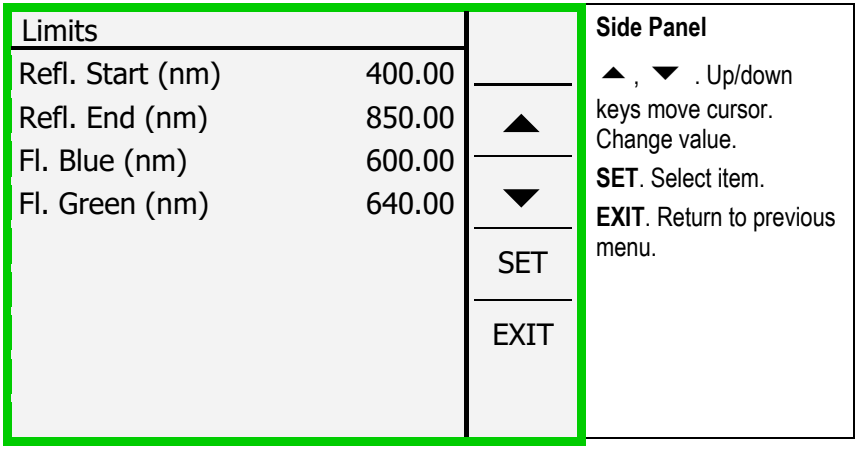


Fig. 57: Limits

4.2.4.4 Leaf Clip

In the Sensors menu, the item “Leaf Clip” opens the Window “Leaf Clip / Ext. PAR Sett.” which deals with the calibration of the Leaf Clip Holder 2035-B (Fig. 58). The factory calibration data are stored on the flash memory of the Leaf Clip Holder 2035-B.

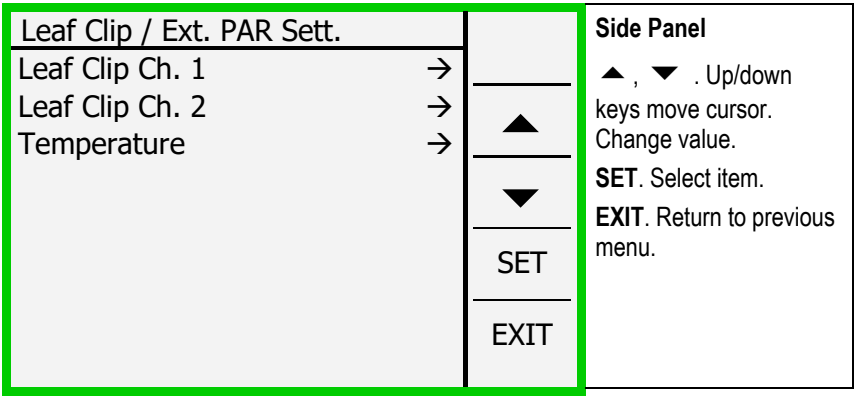


Fig. 58: Leaf Clip / Ext. PAR Sett.

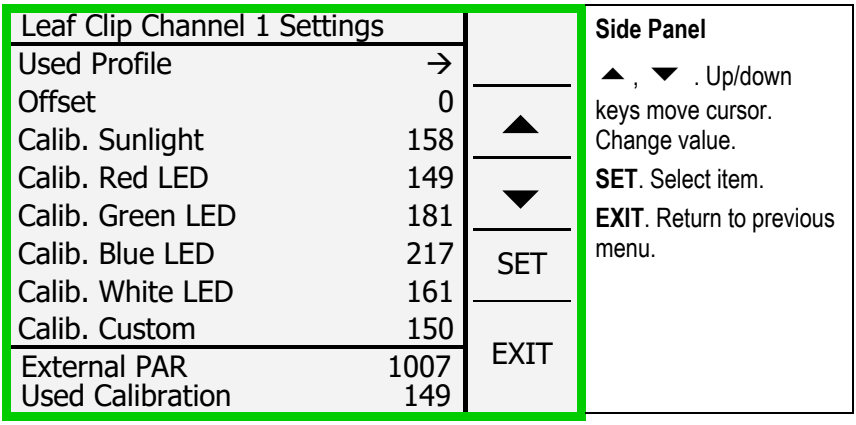


Fig. 59: Leaf Clip Channel 1 Settings

Leaf Clip Ch. 1 opens “Leaf Clip Channel 1 Settings” which contains various calibration factors for PAR channel 1, that is, the mini

quantum sensor of Leaf Clip Holder 2035-B. The different calibration factors (“Calib. Sunlight to Calib. White”, see Fig. 59) were factory-established and are characteristic for each PAR sensor.

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

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

The item Used Profile in the “Leaf Clip Channel 1 Settings window” opens another menu (Used Cal. Profile, Fig. 60, page 82) in which a calibration factor or a calibration profile can be selected. In “Used Cal. Profile”, the items “Sunlight” to “Custom” correspond to comparable items in the previous window., For example, selecting profile “Sunlight” activates the factor “Calib Sunlight” for PAR measurements.

The ‘Mixed’ profile is designed for the 2054-L external LED light source. It adjust the calibration factor dynamically depending on the contributions of the red, green blue and white LEDs.

Leaf Clip Ch. 2 in window “Leaf Clip/Ext. PAR Sett” (Fig. 61, page 82) opens the calibration menu for a second PAR sensor, which can be connected to the SMA socket on the side of the 2035-B leaf clip. This menu consists of slope (Calib.) and offset of the calibration line. Also, the PAR readout of the active PAR sensor is displayed.

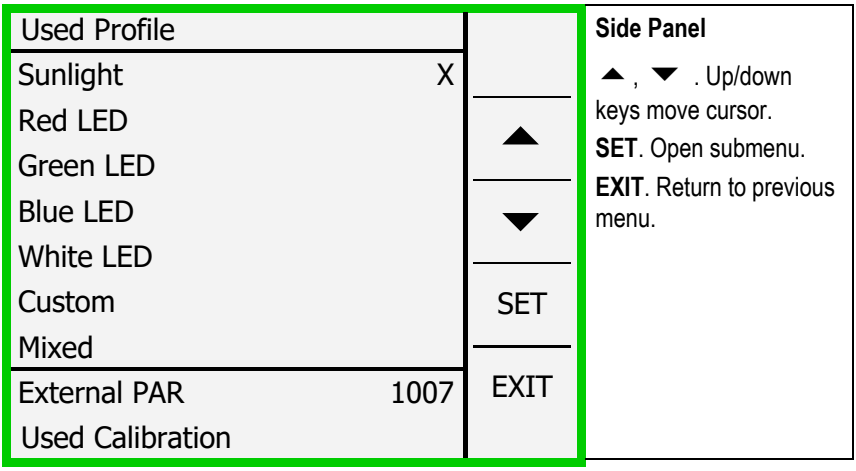


Fig. 60: Used Cal. Profile

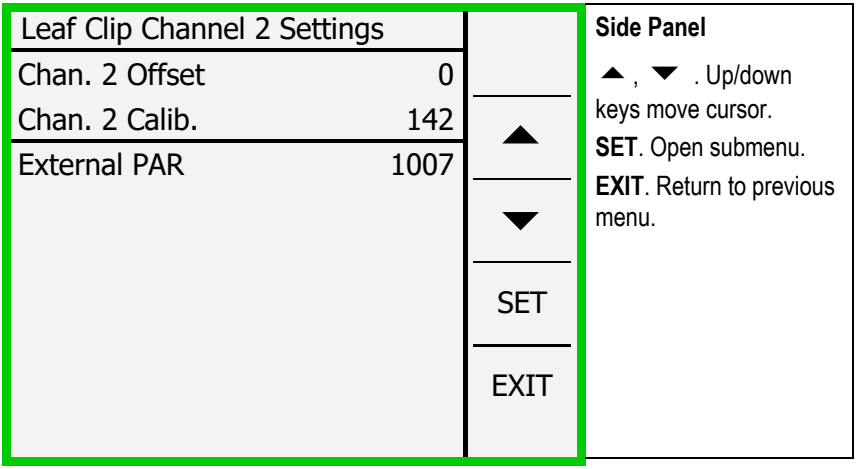


Fig. 61: Leaf Clip Channel 2 Settings

Temperature leads to the calibration data for the leaf temperature sensor of the 2035-B leaf clip (Fig. 62, page 83). This window lists slope (Gain) and offset of the calibration line.

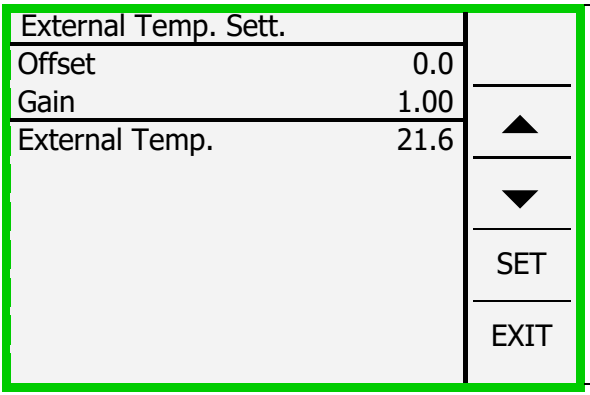


Fig. 62: External Temperature Sensor Settings

4.2.4.5 Internal PAR

"Internal PAR" of the "Sensors" menu opens the "Internal PAR Sensor Settings" window (Fig. 63, page 84). This window is used to calibrate the DIVING-PAM-II internal PAR sensor which receives continuously a small fraction of internal actinic light. The window displays the current "Calibration Factor", the specific calibration factor (Spec. Cal-Factor), as well as the readouts (µmol m^{-2} s^{-1}) of internal and external PAR.

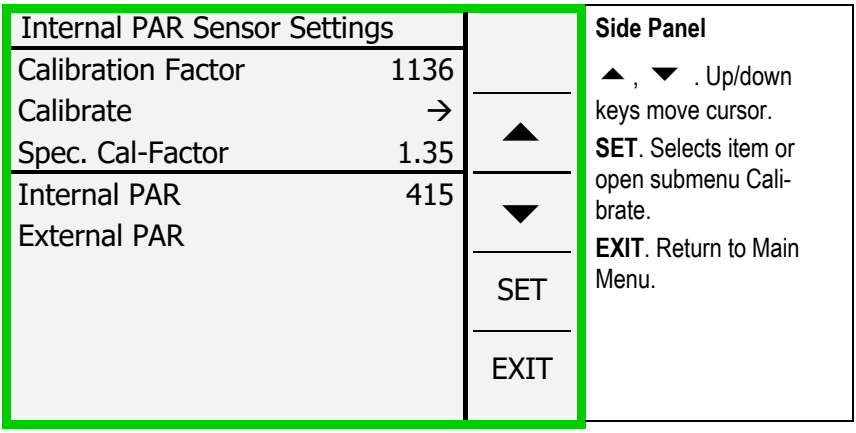


Fig. 63: Internal PAR Sensor Settings

The Spec. Cal. Factor corrects the effect of inhomogeneous illumination by actinic light of the diffusing disk of a PAR sensor. The factor is greater than 1 in the case of the Miniature Spectrometer MINI-SPEC. The factor equals one in the case of the Leaf Clip Holder 2035-B.

The Spec. Cal. Factor of the MINI-SPEC has been determined by comparing the PAR measurement of the 2035-B leaf clip with that of the miniature spectrometer in the PAR block (Fig. 10, page 22). The Spec. Cal. Factor is stored on the DIVING-PAM-II internal flash memory, and it is automatically considered when the internal sensor is calibrated against the miniature spectrometer, MINI-SPEC.

CALIBRATION

Calibration of the internal PAR sensor is done by comparison with a calibrated external sensor. The external sensor is positioned at a defined distance and angle relative to the fiberoptics. The

readout of the internal PAR sensor applies only for the position which the external PAR sensor had during calibration.

PAR sensor compatible with DIVING-PAM-II: To calibrate the internal PAR sensor using the MINI-SPEC connected to the DIVING-PAM-II, click [Calibrate] and establish calibration factor automatically (see Section 3.1.4, page 19 for further instructions). To calibrate the internal PAR sensor when the MINI-PAM/F1 Miniature Fiberoptics is used, see Section 3.2.4.1 (page 34).

When the 2035-B leaf clip is used for calibration, click Select External PAR Sensor (Sel. Ext. PAR Sensor) in window Sensor Settings (Fig. 48, page 72) and pick “Leaf Clip Ch. 1” in window External PAR Sensor (Fig. 50, page 74). When the blue light of a DIVING-PAM/B fluorometer is used for calibration of the internal sensor, the calibration factor for blue light (stored in the flash memory of the 2035-B leaf clip) will automatically be used. Similarly, when calibration is performed with a DIVING-PAM/R fluorometer, the “red” calibration factor will be used.

Other PAR sensor: To calibrate the internal PAR sensor using an external PAR sensor which cannot be read by the DIVING-PAM-II, adjust calibration factor manually by selecting “Calibration Factor”, pressing [SET] and changing the factor by arrow keys until internal PAR matches that of the external sensor.

Proper calibration of the internal light sensor can be checked by comparing the actinic light intensity measured by this sensor with that measured by the 2035-B leaf clip. (The MINI-SPEC/MP cannot be used as the external sensor here, because its reading fluctuates in the presence of the discontinuous internal actinic light source. Internal light is continuous during the calibration process.)

If internal and external PAR differ clearly, check whether:

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

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

(3) External PAR sensor is connected, or incorrect PAR channel is selected in window “External PAR Sensor” (Fig. 50, page 74).

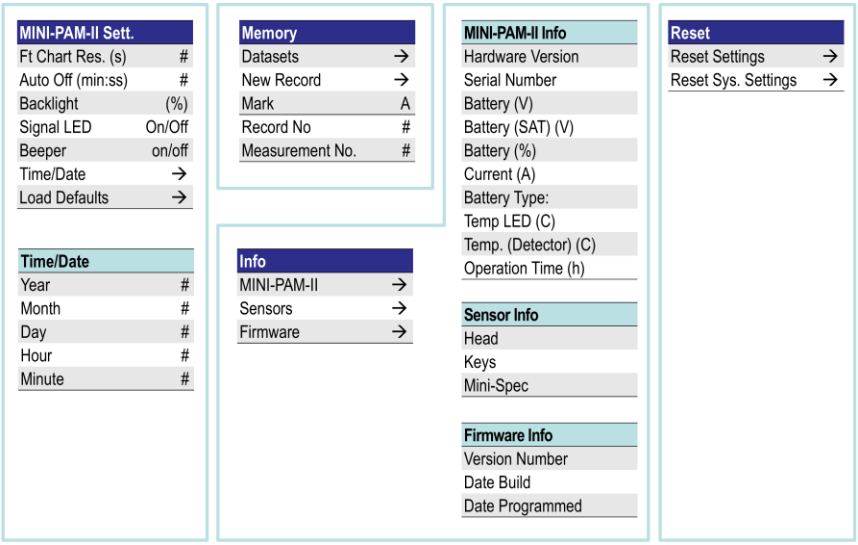


Fig. 64: Overview: DIVING-PAM-II, Memory, Info, and Reset Menus

4.2.4.6 Depth

In the menu Sensor Settings, the item “Depth” opens a submenu in which the “Offset” for depth measurements is adjusted (Fig. 65). “Offset“ is the actually measured air pressure given as barometric

pressure in meter (hydrostatic pressure). “Auto Zero” defines the current pressure condition as 0 m diving depth.

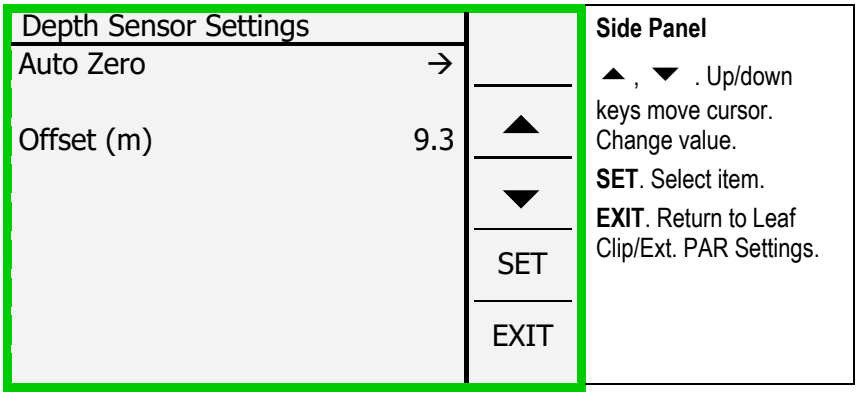


Fig. 65: Depth Sensor Settings

4.2.4.7 Key Sensitivity

Increasing the numerical value increases sensitivity of optical switches of the DIVING-PAM-II. Usually, a higher sensitivity is employed underwater compared to the use in air.

4.2.5 DIVING-PAM-II

The menu “DIVING-PAM-II Settings” allows choosing between two time intervals for the Ft chart (Ft Chart Resolution), setting device parameters (Auto Off through Beeper), and setting of date and time.

Ft Chart Resolution (s)

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

Auto Off (min)

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

Backlight (%)

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

Signal LED

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

Beeper

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

Time/Date

Simple menu for setting time and date.

Wireless

Select to open submenu "Wireless". Where:

On WLAN always on, which is battery-consuming on the long run

Off WLAN always off

Auto WLAN is activated with powering up the DIVING-PAM and deactivated after 5 minutes.

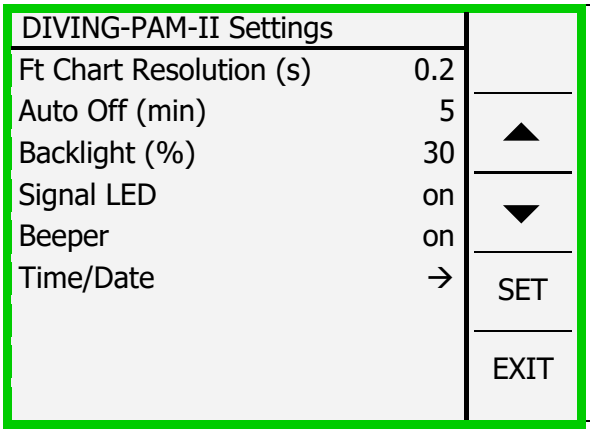


Fig. 66: MINI-PAM Settings

4.2.6 Memory

The item “Datasets” of the “Memory” window gives access to stored saturation pulse analyses including fluorescence transients induced by saturation pulses. Scroll through data sets using the up and down key.

Also, in the “Memory” window, new records can be started and the mark of saturation pulse data can be changed. To see stored induction and light curves, use the MEM key of window “Induction Curve” (Fig. 30, page 47) and window “Light Curve” (Fig. 31, page 49), respectively.

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

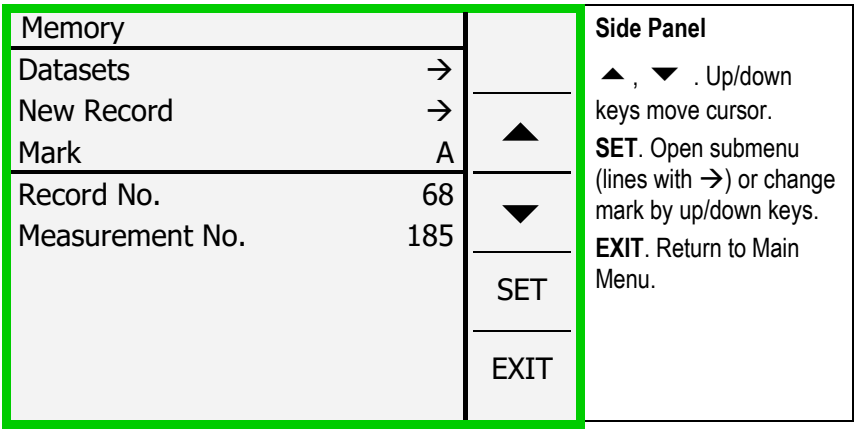


Fig. 67: Memory

4.2.7 Info

"Info" (Fig. 68, page 91) consists of 3 links to submenus of which three list hardware and software information:

DIVING-PAM-II provides hardware information for the fluorometer.

Sensors lists serial numbers of sensors connected to the DIVING-PAM-II.

Firmware shows serial number and date of the firmware of the DIVING-PAM-II.

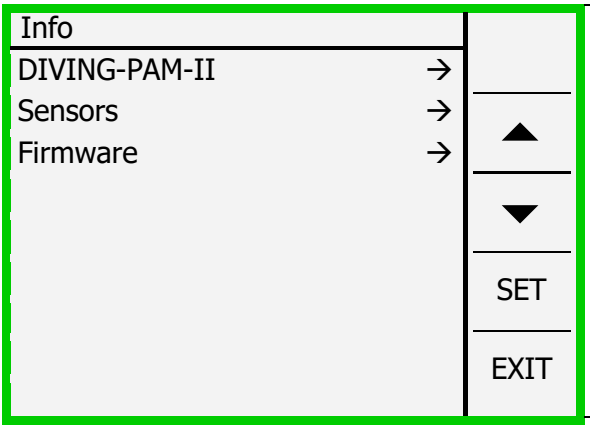


Fig. 68: Info

4.2.7.1 DIVING-PAM-II Info

Information available on this window is (1) hardware version of fluorometer, (2) serial number of fluorometer, (3) battery voltage at normal operation, (4) battery voltage during a saturation pulse, (5) charge status in percent, (6) present current consumption (7) type of built-in battery(Li-ion or lead acid), (8) temperature (in °C) of actinic LED, (9) temperature (in °C) of detector, and (10) operation time of device.

Note that the DIVING-PAM-II should be charged when the current battery voltage drops below 7.6 V.

When connected to line power, the external DC voltage display replaces all battery information except the battery type.

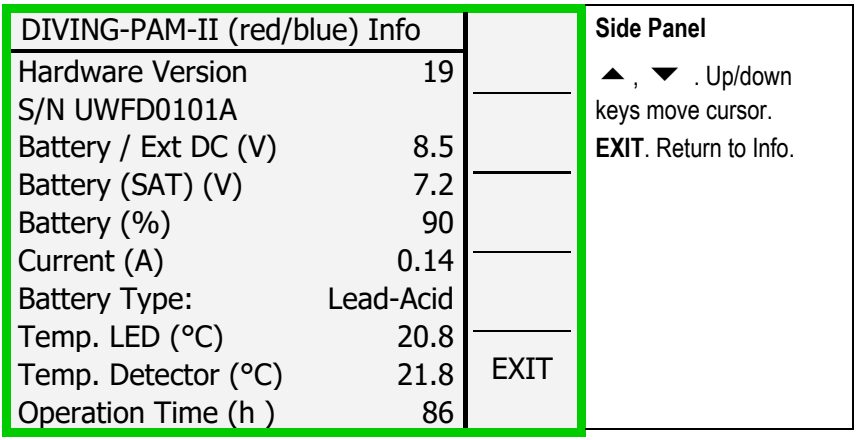


Fig. 69: DIVING-PAM-II Info

4.2.7.2 Sensor Info

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

4.2.7.3 Firmware Info

Version and times of completion of firmware.

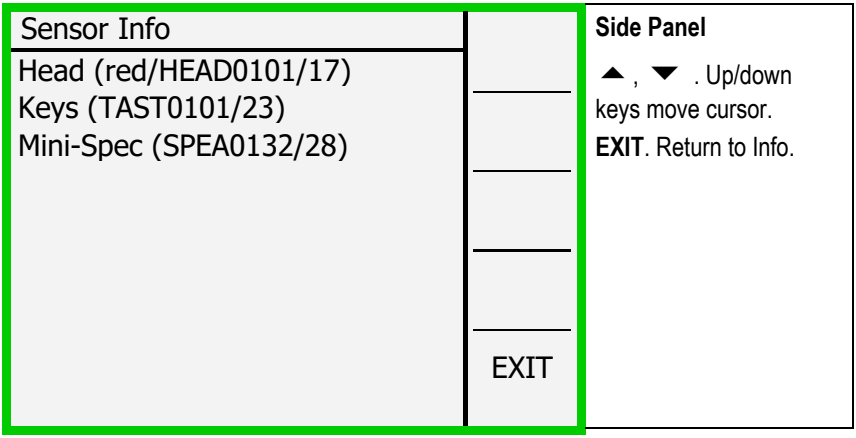


Fig. 70: Sensor Info

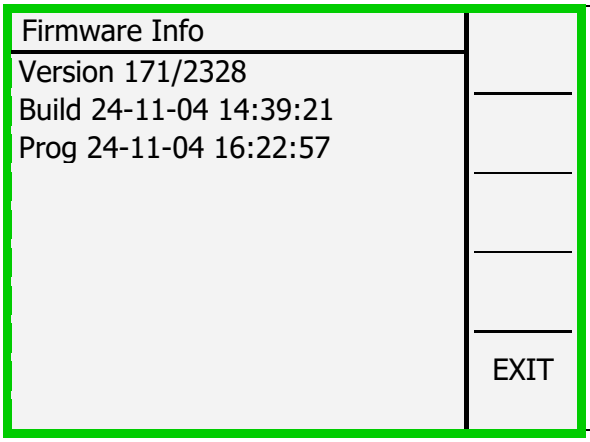


Fig. 71: Firmware Info

4.2.8 Reset

The „Reset“ menu consists of two items and is used to install default settings:

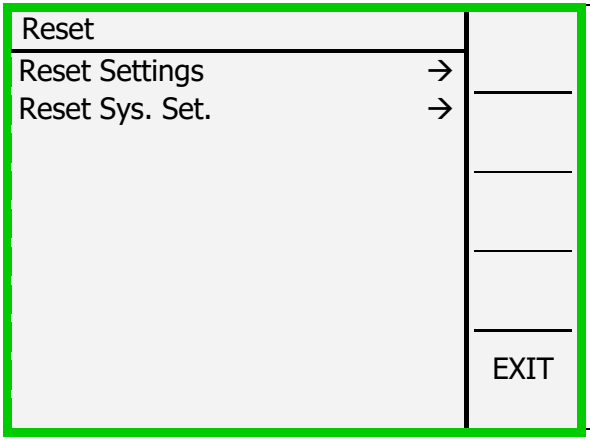


Fig. 72: Reset

4.2.8.1 Reset Settings

Installs the default DIVING-PAM-II setting (Table 8, page 94) left and center column. Current settings of some variables are saved when the DIVING-PAM-II is shut off. These variables are marked by “Yes” in the rightmost column of Table 8.

Table 8: Default Settings

	Default Setting	**Current Setting** (saved and restored)
Measuring Light		
Status	On	No
Intensity	6, relative unit	Yes
Frequency	3, see Table 7	Yes
Frequency high status	Off	No
Actinic Light		
Status	Off	No
Intensity	6, relative unit	Yes
Factor	1.00	Yes
PAM Signal		
Gain	1, relative unit	Yes
Damping	2, relative unit	Yes
Far-red Light		
Width 5	5, s	Yes
Intensity	8, relative unit	Yes
Saturation Pulse		
Intensity	10, relative unit (≙ 5000 µmol m^{-2} s^{-1})	Yes
Width	0.6, s	Yes
Program Actinic Light and Yield		
Actinic light width	30, s	Yes
Initial Pulse	Yes	Yes
Program Induction Curve		
Delay	40, s	Yes
Width	20, s	Yes
Length	12	Yes
Program Light Curve		
Width	20, s	Yes
Intensity	3, relative unit	Yes
Length	8, light steps	Yes

Table 8: Default Settings

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

4.2.8.2 Reset Sys. Settings

Restores device settings like calibration factor of internal PAR sensor (calibrated for geometry of 2035-B leaf clip), measuring light current and calibration factors of external devices like those stored on the 2035-B leaf clip.

5 WinControl-3 Installation

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

https://www.walz.com/downloads/?filter=diving-pam-ii

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

5.1 Installation process

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

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

Update will automatically replace it by the recent version. Running PAM Firmware Update after installation of WinControl-3 is important because new software properties may function only in the presence of the latest firmware.

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

5.2 WinControl-3 Program Group

Setup of WinControl-3 creates the WinControl-3 program group (Table 9, p. 98) in the Windows Start menu. The WinControl-3 program group is comprised of 5 items. The items are introduced in sections 5.2.1 through 5.2.5.

Table 9: WinControl-3 in Windows Start Menu

WinControl-3

PAM Firmware Update

Uninstall WinControl-3

WinControl-3

WinControl-3 – Network Mode

WinControl-3 – Offline

5.2.1 PAM Firmware Update

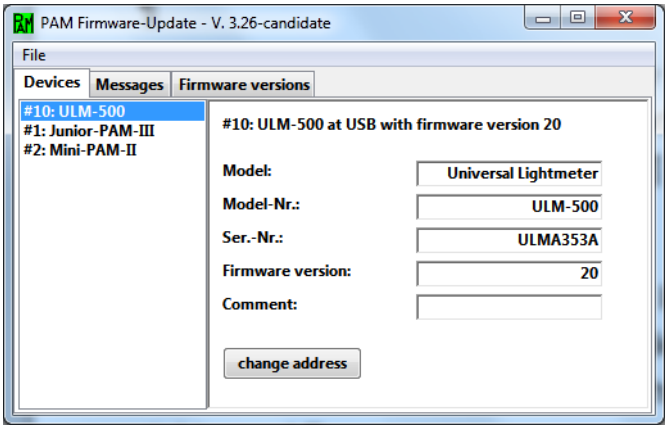


Fig. 73: PAM Firmware Update
Several devices connected to the same computer must have different addresses (channel numbers).

Initialization of PAM Firmware Update triggers a search for PAM devices connected to the computer. The result is displayed in the right panel of window “Devices” (Fig. 73, page 99). Each device name is preceded by its address number (between hash and colon).

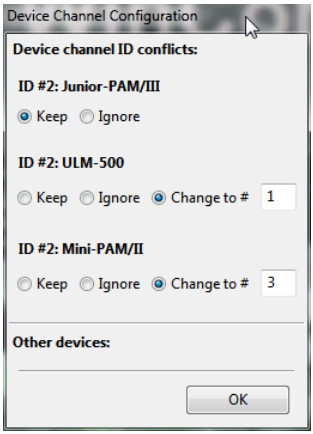
“PAM Firmware Update” compares the firmware in the device with the firmware included in the WinControl-3 software. If WinControl-3 includes newer firmware, the device is automatically updated.

“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. 74, page 100) pops up offering a working address configuration and the option to change addresses manually. Note that address number is synonymous to channel number in the software WinControl-3.

Fig. 74: Device Channel Configuration



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

5.2.2 Uninstall WinControl-3

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

5.2.3 WinControl-3

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

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

5.2.4 WinControl-3 Network Mode

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

5.2.5 WinControl-3 Offline Mode

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

6 WinControl-3 Operation

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

WinControl-3 functions are arranged in 11 Windows (Table 10). The Chart window appears at software start. When more than one device is connected, the additional window “Moni-Bus” appears.

Table 10: Windows of WinControl-3

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

*When data loaded.

6.1 Chart Window

Fig. 75 divides the Chart window into six areas. Most of the side bars are also present when other windows are viewed.

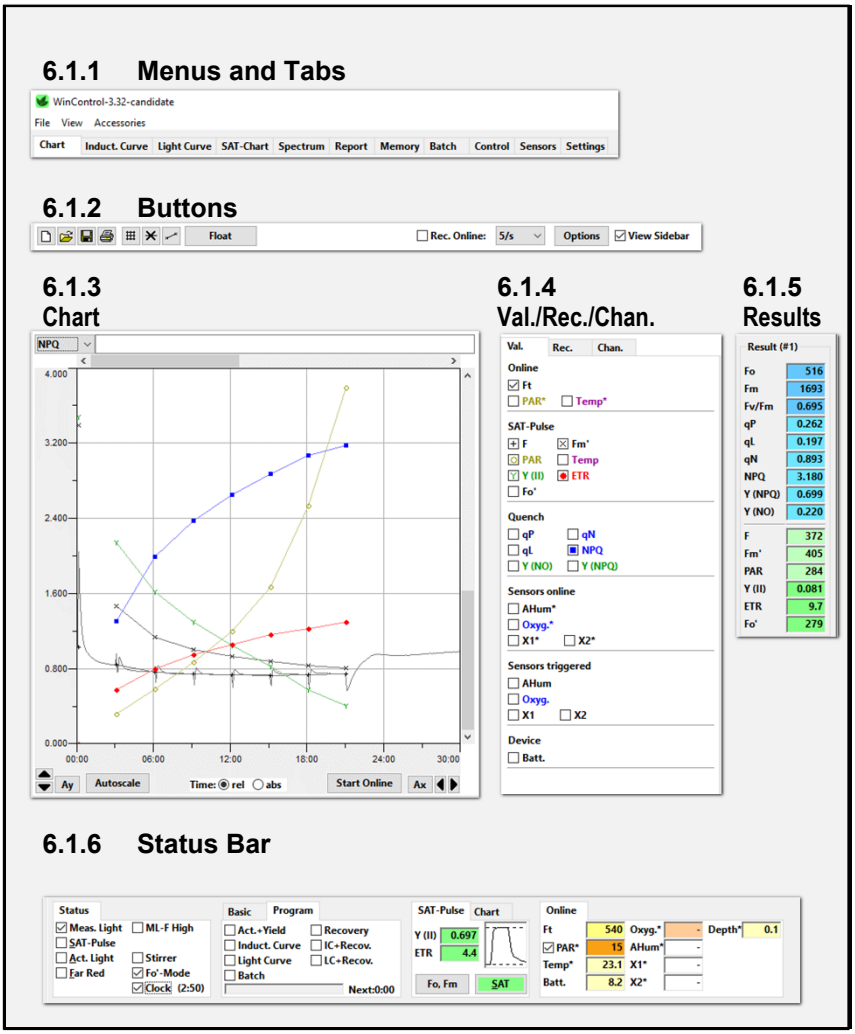


Fig. 75: Chart Window

6.1.1 Menus and Tabs

The top bar includes three menus (File, View, and Accessories) and all window tabs (compare Table 10, page 103). The three menus are explained in Table 11.

Table 11: Menu Overview

Menu	Item	Comment
File	Load Data	Opens WinControl-3 files. Save current data first and switch off online recording of data.
	Save Data	Saves present data in WinControl-3 format.
	Save Settings	Saves all current instrument settings in a batch file. The settings can be restored by executing this batch file.
	Export Report	Exports the data displayed on Report window. For information on configuration of export data see Table 12 (page 106).
	Export Chart Record	Exports the data displayed on Chart.
	Quit	Exit WinControl-3.
View	Results Panel	Switches Results panel (Section 6.1.3) on or off.
	Status Panel	Switches Status panel (Section 6.1.6) on or off.
	Warnings	Prompts the display of the 'program starting time' and non-critical errors.
	Batch Window	Switches Batch File window on or off.
Accessories	Temperature Units	Toggles between °Celsius and °Fahrenheit. The temperature unit affects only the numerical display on the Status bar (6.1.6, p.114).
	Plugins	Does not apply for the DIVING-PAM-II.
	Record File	Saves data continuously to reduce data loss in case of program failure. The command prompts for a folder in which the file should be saved. The file name is created automatically using data and time of record start: WinControl-Record-YYYY-MM-DD-hh_mm_ss.pam.

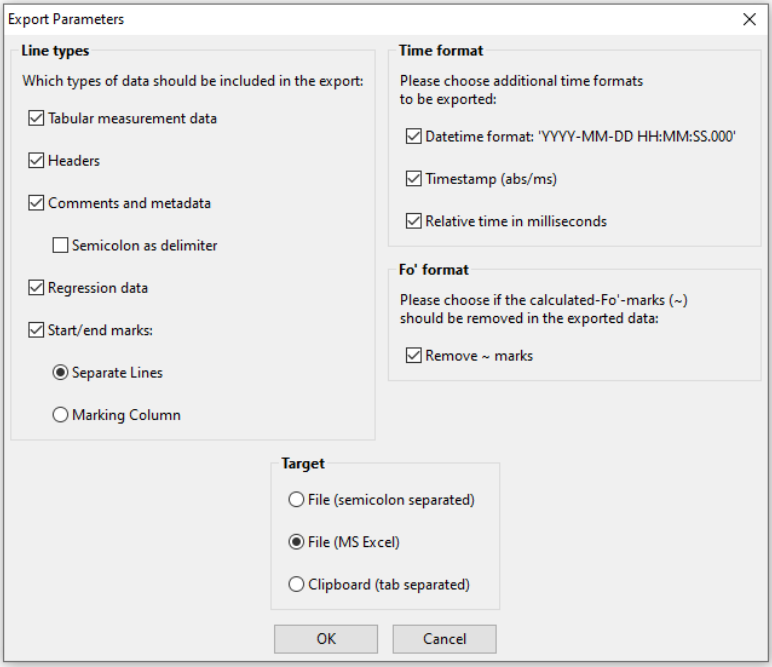


Fig. 76: Export Format for Reports

Table 12: Export Format for Reports

Parameter	Action when checked
☑ Tabular measurement data	Exports data of saturation pulse analysis and all other data recorded at the same time.
☑ Headers	Exports column headers.
☑ Comments and metadata	Exports user and software comments (=”metadata”, e.g., device information).
☑ Semicolon as delimiter	Applies to comments and metadata. Information after a semicolon is written in a new cell.
☑ Regression data	Exports the cardinal numbers of light curves.

Table 12: Export Format for Reports

Parameter	Action when checked
☑ Start/end marks	
⦿ Separate Lines	Writes start and end of an experiment in separate lines.
○ Marking Column	Writes start and end of an experiment in a separate column.
☑ Datetime format	Exports date and time. Format: day/month/year hours:minutes:seconds.ms. In Excel, custom-formatting may be needed: dd/mm/yyyy hh:mm:ss.000.
☑ Timestamp	Exports UTC (Coordinated Universal Time) time in ms. To convert into date/time use: $=\frac{SourceCell + HoursTimeShift * 3.6 \cdot 10^6}{86.4 \cdot 10^6} + 25569$ Format target cell, e.g.: dd/mm/yyyy hh:mm:ss.000
☑ Relative time in ms	Export time of experiment in ms.
☑ Remove "~" marks	Removes tilde (swung dash) signs. WinControl-3 marks calculated F'_0 values by a tilde.
⦿ File (semicolon separated)	Creates a file in which individual data are separated by semicolons.
⦿ File (MS Excel)	Creates an Excel file. Note that columns "Date" and "Time" are differently formatted but contain the same information: dd/mm/yyyy hh:mm:ss.000
○ Clipboard (tab separated)	Copies the data to the clipboard. Data are separated by tab characters. Use the paste command to copy the data into a spread sheet program.

6.1.2 Buttons

The elements of the buttons bar are introduced in Table 13 and Table 14.

Table 13: Buttons

Icon	Meaning	Comment
	Delete	Deletes all current data.
	Load	Same function as “Load Data” in the File menu (Section 6.1.1.).
	Save	Same function as “Save” in the File menu (Section 6.1.1.).
	Print	Prints current chart view.
	Grid on/off	Controls display of chart grid.
	Lines	Connects data points with lines.
	Symbols	Controls the display of symbols.
Float	Add Chart	Creates an additional chart window with separate view settings.
☑ Rec. Online	Continuous recording	Controls continuous recording of fluorescence, PAR, and temperature.
5/s ▼	Sampling frequency	Sets sampling frequency for online data. Open drop-down menu by the downward arrow. Available sampling frequencies are 5/s, 1/s, and 1/10s. 5/s is available only for fluorescence.
Options		See Table 14.
Or right-click on chart.		
☑ View Sidebar		Controls display of side bar (see 6.1.4, Val./Rec./Chan.)

Table 14: Options

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

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

6.1.3 Chart

Fig. 77 outlines the tools to adjust the chart. Section 6.1.4 explains how to select data for display.

The scale of y- and x-axis can be manually changed: a vertical pair of arrows affects the y axis, and a horizontal pair of arrows adjusts the x-axis (see lower border of Fig. 77). Right next the two pairs of arrows are buttons for automatic axis scaling.

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

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

A way to zoom in to a particular x-axis interval is to mark the x-interval of interest (see Fig. 77), open a context menu by right-click on the chart area, and select the command “Zoom to Selection”.

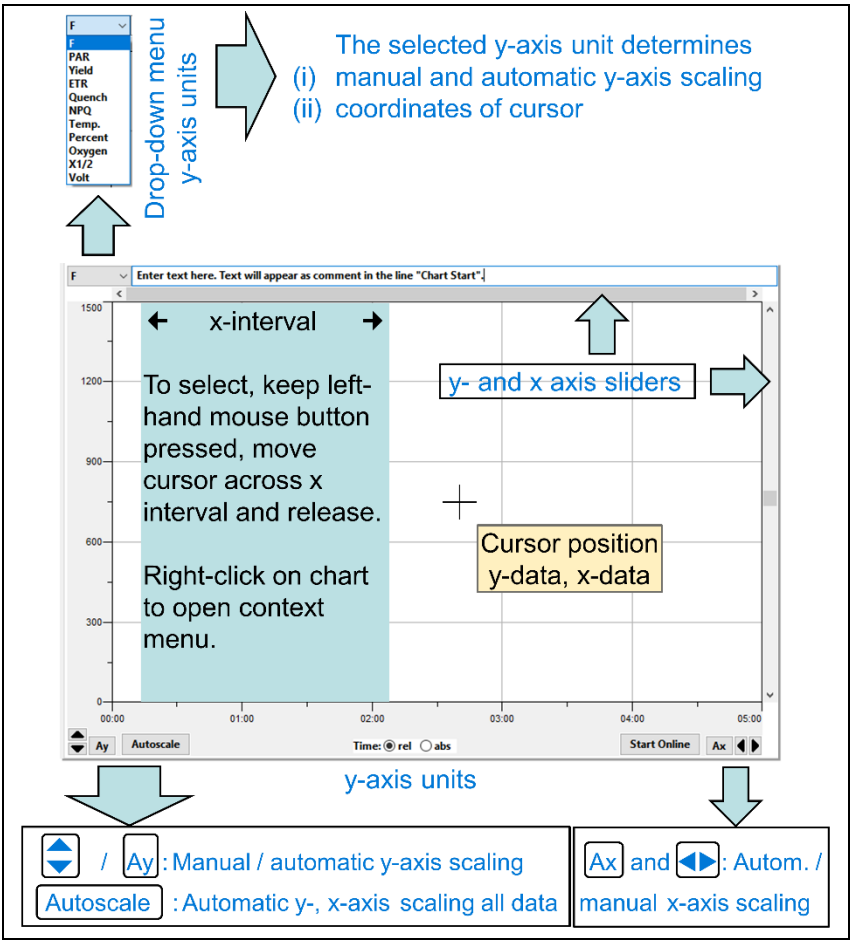


Fig. 77: Chart Tools

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

6.1.4 Val./Rec./Chan.

The field “Val. Rec. Chan.” includes three different sidebars (Fig. 78). Data which should be displayed on the chart are selected on the Val. (= values) sidebar. The Val. sidebar distinguishes continuously recorded data (data groups: Online and Sensors Online) from saturation pulse data and data taken at the time of a saturation pulse (data groups SAT Pulse, Quench, and Sensors Triggered).

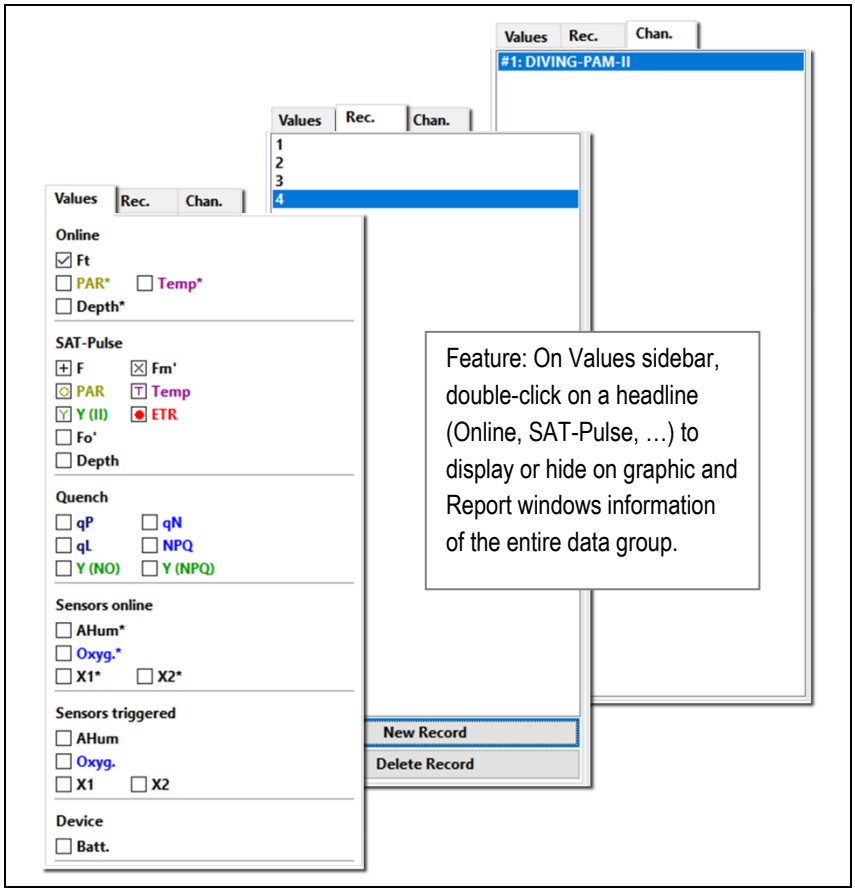


Fig. 78: Val. Sidebar

The Rec. (= Record) sidebar lists the number of individual charts of the present data file (= Report). Additional Records can be started by the button New Record and existing Records can be deleted by Delete Record. The latest Record, or the Record selected by mouse click, will be displayed on chart. The windows “Induction Curve” and “Light Curve” also possess the Rec. sidebar but in these cases the Induction Curve and Light Curve experiments, respectively, are listed.

The Chan. sidebar displays all devices detected by the WinControl-3 software. Only a DIVING-PAM-II was connected in Fig. 78.

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

PAR* (photosynthetic active radiation, µmol photons $m^{-2} \cdot s^{-1}$) and Temp* (Temperature, °C) are two further online signal. They are distinguished by a superscript asterisk from their pendants which are measured together with saturation pulses. In the same way, data of additional sensors are marked (Fig. 78). The additional sensors available measure humidity (AHum) or oxygen concentration (Oxyg.). The sensor channels X1 and X2 are prepared for future use.

Besides F level fluorescence, the SAT-Pulse fluorescence levels are maximum fluorescence F_M' (including F_M) and minimum fluorescence F_0' (F_0). Derived from these fluorescence levels is the photochemical quantum yield of photosystem II, Y(II) (including F_V/F_M). The electron transport rate (ETR, µmol electrons/($m^2 \cdot s$) is calculated with Y(II) and PAR. The section “Quench” of the Val. sidebar includes six more saturation pulse parameters. All fluorescence parameters are explained in Chapter 10 (page 165).

6.1.5 Results

The Results sidebar (Table 15) shows numerical values of the current experiment. The upper part of the sidebar displays data of the Fo, Fm determination. These data are maintained throughout the current experiment. All other data are updated with each saturation pulse analysis. All fluorescence levels and ratios are explained in Chapter 10 (page 165).

Table 15: Sidebar

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

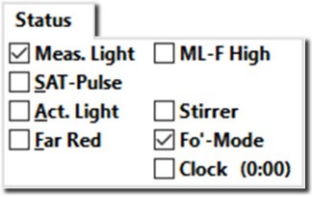
6.1.6 Status Bar

The “Status” field indicates the state of light sources and special functions (Table 16). The checkboxes work both as indicator and as on/off switches.

The “Basic” and the “Program” fields are stacked and can be selected by tabs. Actinic light and the clock frequency is adjusted on the Basic field, all automatic routines of the WinControl-3 software can be started in the Program field (Table 16).

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

Table 16: Status Bar



Meas. Light: Low frequency PAM measuring light.

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

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

Act. Light: Actinic light to drive photosynthesis.

Far-red: Light at wavelengths > 700 nm.

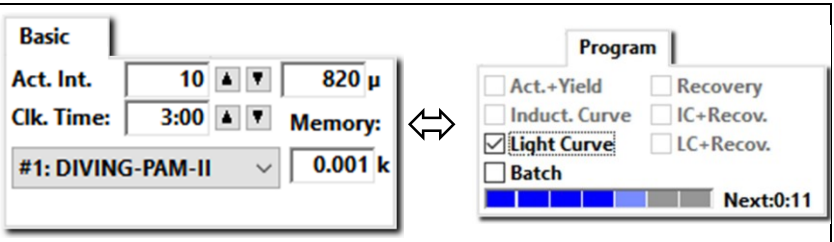
Stirrer: Does not apply for the DIVING-PAM-II.

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

Clock: Repetitive trigger of the event specified on

Settings Window (Section 6.10, page 140). See below on how to adjust the interval between trigger events.

Table 16: Status Bar



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

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

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

Memory: Current file size in kilobytes (k).

Experimental Routines

Experimental routines are defined on the

Settings Window (Section 6.10, page 140). Most routines can be triggered by the clock.

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

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

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

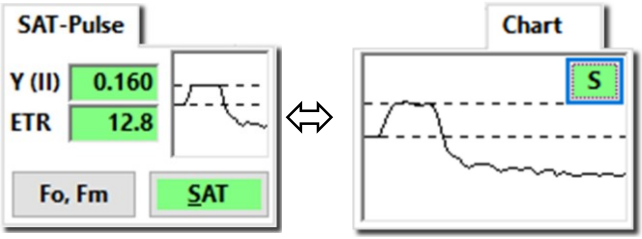
IC+Recov.: Induction curve plus dark phase with saturation pulse analyses performed at increasing intervals.

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

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

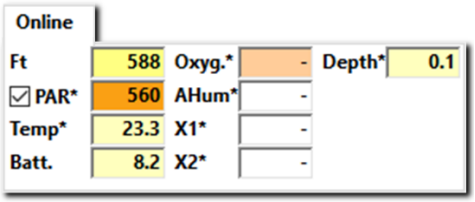
Batch: Execution of batch file program.

Table 16: Status Bar



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

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



Online are continuously recorded with the frequency defined (see Table 13, page 108).

Ft, PAR* and Temp*: see 6.1.4, page 111. When PAR* is unchecked, the intensity is taken from the internal PAR list (Section 6.10, page 140); when checked, the actually measured value is displayed. Batt., battery voltage.

6.2 Induct. Curve/Light Curve Windows

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

Table 17 summarizes further differences between the three windows. The windows have different start buttons and only the Light

Curve window possesses PAR as x-axis unit. The button Start IC starts an experiment in which a sample is exposed to a single defined light intensity, the button Start LC initiates a routine in which a sample is exposed to incrementally increasing light intensities. The x-axis radio button “Time” of the Light Curve window corresponds to the radio button “rel” (relative time) in the two other windows.

The Light Curve window allows fitting model functions to experimental data. The experimental data are the electron transport rates (ETR) plotted against the photon flux density, PAR. WinControl-3 provides two model functions called REG1 and REG2 which are introduced in Fig. 79 and Fig. 80, respectively. The function REG1 can decrease at high PAR values. Therefore, REG1 can consider photoinhibition of photosynthesis, where β is a photoinhibition parameter. In contrast, REG2 is a rectangular hyperbola which cannot describe photoinhibition.

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

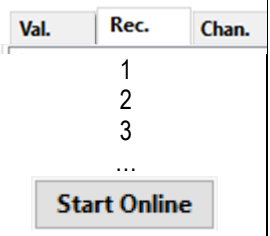
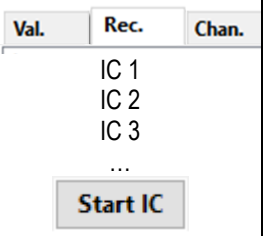
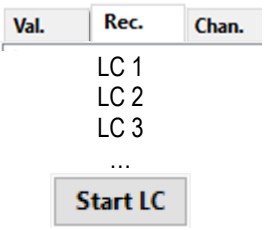
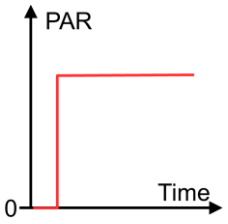
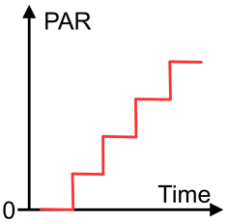
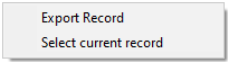
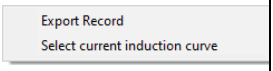
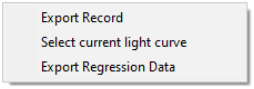
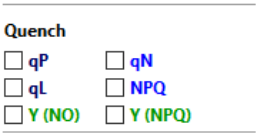
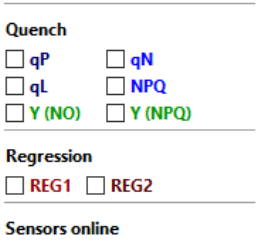
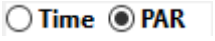
(i) α, electrons/photons: Initial slope of RLC which is related to the quantum efficiency of photosynthesis.

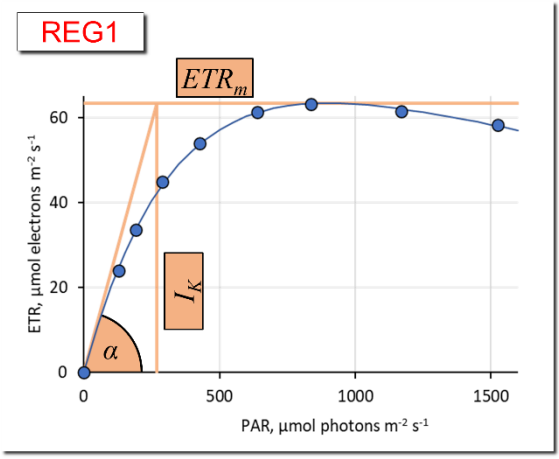
(ii) ETR_m, µmol electrons $m^{-2} \cdot s^{-1}$: Maximum electron transport rate.

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

The cardinal parameters are written into the Report. To export cardinal parameters separately, right click on the chart of the Light Curve window and select from the menu “Export Regression Data” (see Table 17). The item “Select current light curve” in the same menu highlights the data of the currently displayed light curve in the Chart and Report windows. A similar function is available for the window Induction Curve.

Table 17: Three Graphics Windows

Chart Window	Induct. Curve Window	Light Curve Window
Rec. Sidebar 	Rec. Sidebar 	Rec. Sidebar 
Lighting 	Lighting 	Lighting 
Right click on chart 	Right click on chart 	Right click on chart 
Val. Sidebar 	Val. Sidebar 	Val. Sidebar 
x-axis units 	x-axis units 	x-axis units 



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

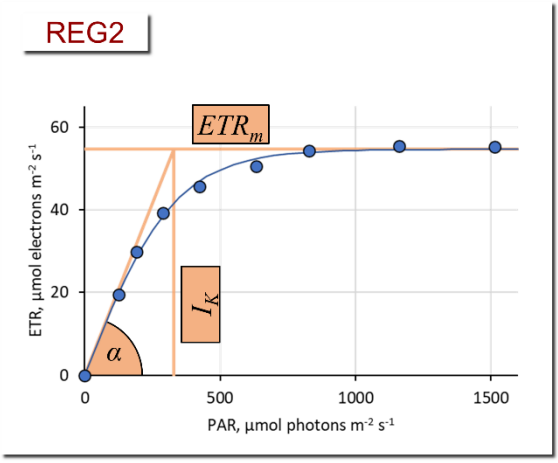
$$ETR_m = ETR_{mPot} \cdot (\frac{\alpha}{\alpha + \beta}) \cdot (\frac{\beta}{\alpha + \beta})^{\frac{\beta}{\alpha}}$$

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

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

Fig. 79: Model Function REG1

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



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

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

Fig. 80: Model Function REG2

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

6.3 SAT-Chart Window

The SAT-Chart window displays saturation pulse kinetics of fluorescence (Fig. 81). The automatically determined values of F and F_M' are shown as dashed horizontal lines. Fluorescence traces are automatically scaled to fit into the coordinate system. The x-axis covers about 2 s, the saturation pulse starts at 200 ms, and the distance between data points is 50 ms. The mouse wheel scrolls through graphs.

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

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

The Val. sidebar is similarly configured as described in Section 6.1.4 (page 111), except “Online Data” are omitted and the electron transport factor (ETR-F.) is added. The ETR-F. is the fraction of absorbed photons by the sample relative the PAR to which the sample is exposed. The ETR-F. is a factor of the equation for electron transport (Chapter 10, page 165). Each item selected on the Val. sidebar is numerically displayed below the corresponding saturation pulse kinetics.

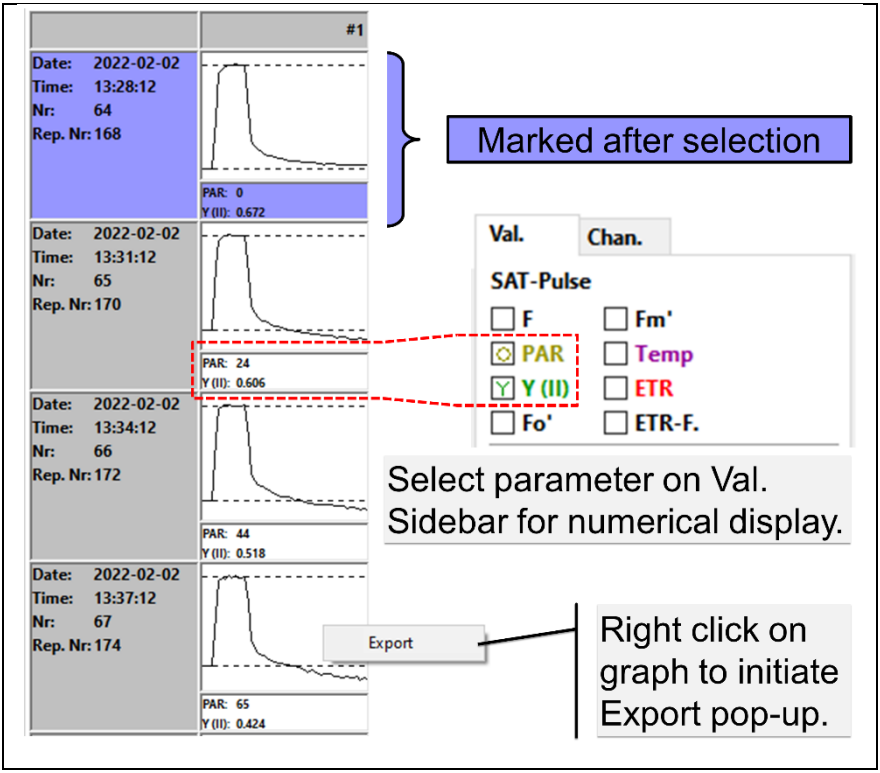


Fig. 81: SAT-Chart Window

Table 18: SAT Kinetics in Raw File

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

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

Single saturation pulse kinetics can be exported by right-click in its chart field. To export a series of saturation pulse kinetics, open the PAM file with a spread sheet program. In the text file, saturation pulse kinetics are preceded by an SG (Table 18). Sort the lines of the file by SG. Create a time scale by considering the neighboring points are spaced apart by 50 ms.

6.4 Spectrum Window

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

Right click on the drawing area to open a menu containing zoom and export commands (Fig. 82). The “Zoom to selection” is available after selection of an x-axis interval described before (Fig. 77).

An application menu is available in the top right corner of the window (Fig. 82). To change between the three principal applications (PAR, fluorescence, reflectance), the spectrometer MINI-SPEC has to be reconfigured (see Fig. 9, page 21).

The dark current of the spectrometer at room temperature is measured in the factory and stored on the flash memory of the device. To newly establish the dark current, fully darken the entrance optics (cv. Fig. 9, page 21) and press Calibr. Dark.

The standard PAR configuration is “PAR cosine”. This configuration employs a diffusing disk as light entrance (Fig. 9). The configuration shows an approximate cosine response toward incoming radiation. The spectrometer calibration differs between measurements in air and in water. Therefore, choose PAR cosine air or

PAR cosine water for proper measurements under the two conditions.

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

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

“Fluorescence (blue)” and “Fluorescence (red)” measures fluorescence emission spectra excited by blue or red light, respectively. Measuring “Reflectance” requires that the 100% reflection signal has been established with the highly reflective reference material provided with the spectrometer (Fig. 8, page 20).

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

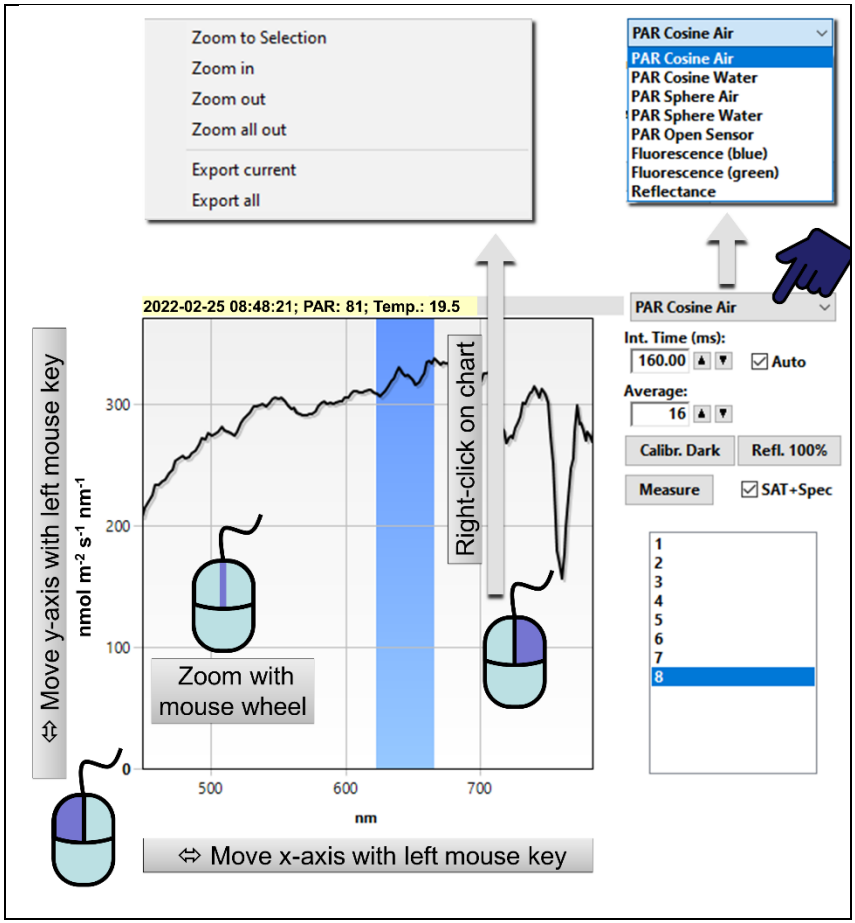


Fig. 82: Spectrum Window

6.5 Report Window

The Report window lists all data associated with saturation pulse analyses. Continuously recorded signals, saturation pulse kinetics and spectra are not listed. The fact that a spectrum was recorded is indicated by “SPEC” in the column “Type” (Table 19). The Report window also displays start and end of induction and light curves, as well as the cardinal points of light curves (Table 19).

The types of data displayed is controlled by the Val. side bar, which is configured as described for the SAT-CHART window (Section 6.3, page 121). Only data displayed on the Report table are exported.

Table 19: Report Table

Type: D, device. FO, F_0 and F_M determination. F, determination of F, F_0', and F_M'. SPEC, spectrum. SICWS and SICSE, induction curve start and end, respectively. SLCS and SLCE, light curve start and end, respectively. REG1 and REGS, cardinal parameters of regression analysis 1 and 2, respectively (see 6.2). **Mark**: Letters assigned to data (see 4.1.1, page 38). Hide Mark column via option menu. Here, the DIVING-PAM-II has the channel number #2. “#2” is preceding the cardinal values of light curve analysis, REG1 and REG2.

	Date	Time	Type	No.	2:Mark	2:F	2:Fm'	2:PAR	2:Y (II)	2:ETR
1			D			Device Nr: #2, DIVING-PAM-II (UWFD0131A)				
2	2022-03-08	09:07:11	SCHS							
3	2022-03-08	09:16:20	FO	1	A	461	1932	6	0.761	1.9
4	2022-03-08	09:16:50	F	2	A	680	774	6	0.121	0.3
5	2022-03-08	09:17:24	SPEC		PAR: 12; Temp.: 21.2					
6	2022-03-08	09:17:42	SICS		Induction Curve start					
7	2022-03-08	09:17:48	SICE		Induction Curve end					
8	2022-03-08	09:17:59	SLCS		Light Curve start					
9	2022-03-08	09:18:07	REG1		#2: alpha: 0.362, ETRm: 13.99, Ik: 38.634 (beta: 0.001, ETRmPot: 14.179)					
10	2022-03-08	09:18:07	REG2		#2: alpha: 0.272, ETRm: 13.665, Ik: 50.280					
11	2022-03-08	09:18:05	SLCE		Light Curve end					

The options menu of the Report window can be opened by clicking the Options button or by right-click in the Report field. The options menu contains various tools for handling the Report table. All menu items and their function are summarized in Table 20.

Command “Insert Settings” of the options menu writes the current device settings in the report. The abbreviations used for the various settings are compiled in Table 21.

Table 20: Options Menu **Options Menu Item**	**Comment**
	General Report Management
Follow Selection	Automatically displays the data associated with events marked in other windows
Show Mark	Display marker letters (see “Mark” in Table 19)
Insert Settings	Writes settings of DIVING-PAM-II in Report table (see Table 21)
	Manipulation of All Data
Export All	For details see Section 6.1.1, page 105.
Delete All Measure Data	Self-explaining
Page Setup for Printing	Basic configuration of print layout. Checks if output fits on page
Preview Printing	Self-explaining
Print Report	Selects and configures printer, prints current Report
	Manipulation of Selected Data
	Requires that saturation pulse events on a chart or lines of the Report have been selected. To select, move the cursor with left mouse key pressed over events
Export Selected Lines	Confines export to lines of interest
Jump to Selection	Brings data into view, when “Follow Selection” is off

Table 20: Options Menu **Options Menu Item**	**Comment**
Delete Selected Data	As above. Affects only selected lines.
Preview Print Selection	As above. Affects only selected lines.
Print Selected Data	As above. Affects only selected lines.
Mark as Light Curve	Combines a series of saturation pulse analysis into a light curve and performs regression analysis REG1 and REG2. The series must not contain start or end marks of previous light or induction curves.

Table 21: Abbreviations for Settings

MEA	Measuring Light Intensity
MI	Measuring Light Frequency
DI	Damping
GA	Gain
EF	ETR-Factor
FZ	F Offset
CW	Clock Interval
SI	Saturation Pulse Intensity
SW	Saturation Pulse Width
AI	Actinic Light Intensity
AF	Actinic factor
FRI	Far-red Intensity
FRW	Far-red Width
AW	Length of exposure of “Act. + Yield” routine
ICD	Delay of Induction Curve
ICW	Interval Between Saturation Pulses of Induction Curve
ICL	Length of Induction Curve
LCW	Step Length of Light Curve
LCI	Initial Intensity Setting of Light Curve
LCL	Length of Light Curve
LO	Offset of External PAR Sensor
LG	Calibration Factor of External PAR Sensor
LO2	Offset of External PAR Sensor 2
LG2	Calibration Factor of External PAR Sensor 2
TO	Offset of External Temperature Sensor
TG	Gain of External Temperature Sensor
ILO	Offset of Internal PAR Sensor

Table 21: Abbreviations for Settings

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

6.6 Memory Window

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

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

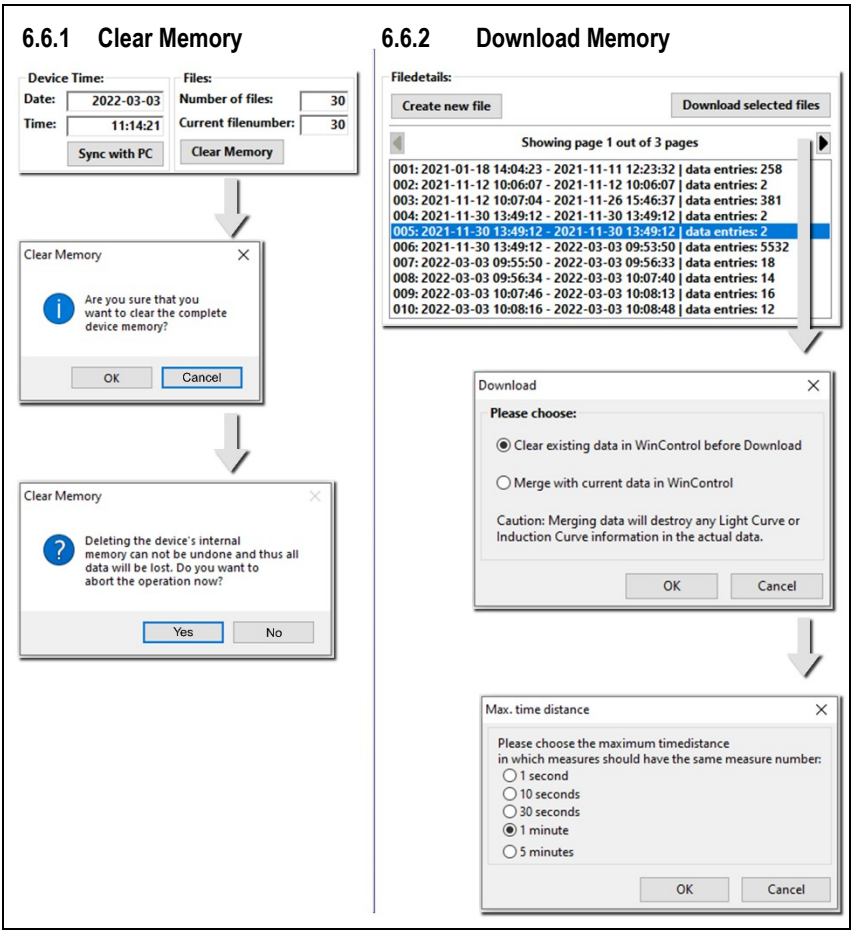


Fig. 83: Memory Window

6.6.1 Clear Memory

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

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

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

6.6.2 Download Memory

A list of files is displayed in the Download field of the Memory window (Fig. 83). You can download individual or groups of files. To pick several files, hold down Ctrl key and click on files of interest. To pick consecutive files, hold down Shift key and click on first and last files of the row. Then click Download selected files.

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

When parallel measurements of two different fluorometers should be combined, choose “Merge with current data …”. The newly imported data will be sorted so that time points of import data matches time points of existing data. Two time points are considered as matching when their time difference is smaller as the interval selected from the box “Max. time distance” (cf. Fig. 83). The

merge operation deletes the report entries for begin and of an induction or a light curve.

6.7 Batch Window

Batch files automatically execute experimental procedures. To activate the Batch window, click icon new batch or open existing batch file (Fig. 84, “Batch Start Buttons”). The click Edit and choose between “Add command” and “Record Macro”. The command “Update indentation” is an automatic editing tool to improve readability.

6.7.1 Add Command

Add command leads to five groups of commands (Fig. 84). A command can be inserted into a batch file by left click.

Measure commands

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

Light Sources commands

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

Settings commands

“Reset settings to default” installs the settings described in Table 8 (page 94).

Repetition commands

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

Line 1:

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

Line 2:

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

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

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

Line 1:

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

Line 2:

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

Timing

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

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

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

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

6.7.2 Record Macro

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

6.7.3 Options

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

Check MONI-DA Compatibility

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

Upload to Device

Transfers a batch file to the MONI-DA memory.

Export

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

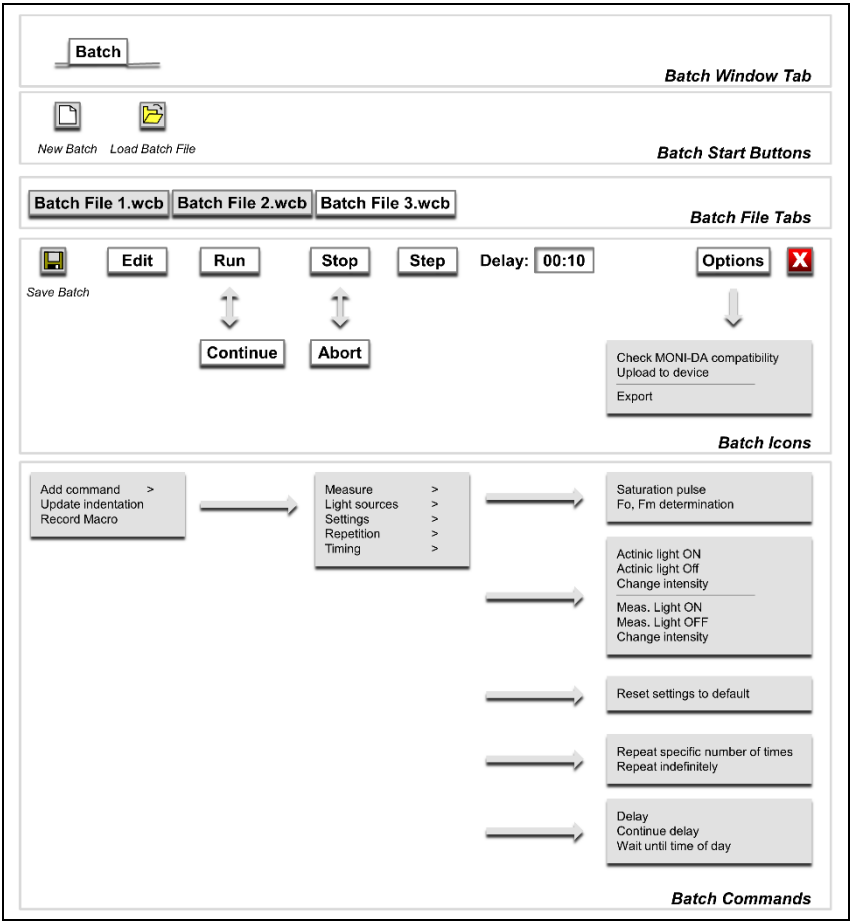


Fig. 84: Batch Window Overview

6.8 Control Window

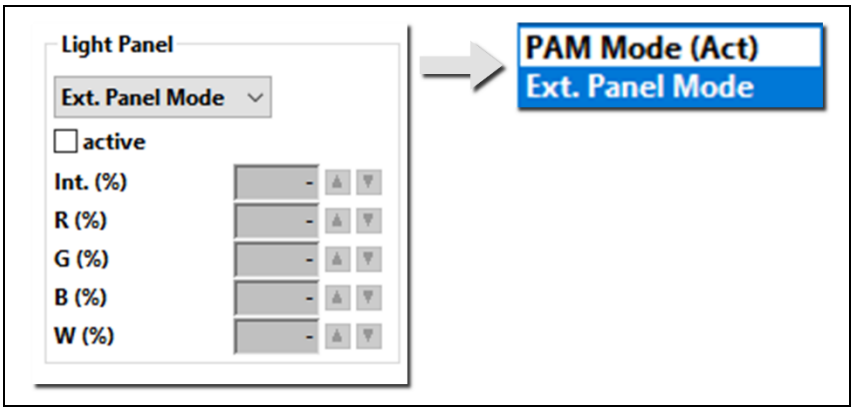


Fig. 85: Control Window

Fig. 85 shows the section of the Control Window which applies to the DIVING-PAM-II fluorometer. The drop-down menu on top of the Light Panel area consists of two points: “PAM Mode (Act.)” and “Ext. Panel Mode”. Ext. Panel Mode is the standard mode in which the fluorometer’s internal light source is used as actinic light to which an external light source may be added (see Table 22). Activating PAM Mode (Act.) selects the external light source as the sole actinic light.

With the External LED Light Source 2054-L is connected, the meaning of numerical inputs (Int. (%) to W (%) varies depending on the mode chosen (see Table 22). Typically, the maximum intensity of all four LED groups is 5000 µmol m^{-2} s^{-1} or higher. Operation of the 2054-L External LED Source requires line power.

Table 22: External Light Source			
		Ext. Panel Mode	**PAM Mode (Act.)**
Internal light source of DIVING-PAM-II			
	Function	Main actinic source	Not available
	Intensity setting	Actinic light intensity (Settings window)	Not available
External LED Light Source 2054-L			
	Requirement	Not mandatory	Required
	Function	Background illumination	Main actinic source
	Intensity setting	Int %	Actinic light intensity (Settings window)
Control			
	Int. (%)	Relative intensity (0-100%). The number multiplies the intensity of all light sources of the External LED Light Source 2054-L	Not active
	R (%), G (%), B(%), W (%)	Absolute intensity in percent (is multiplied by Int. (%))	Factor defining the intensity with which a light source contributes to total intensity.

6.9 Sensors Window

External PAR Sensor

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

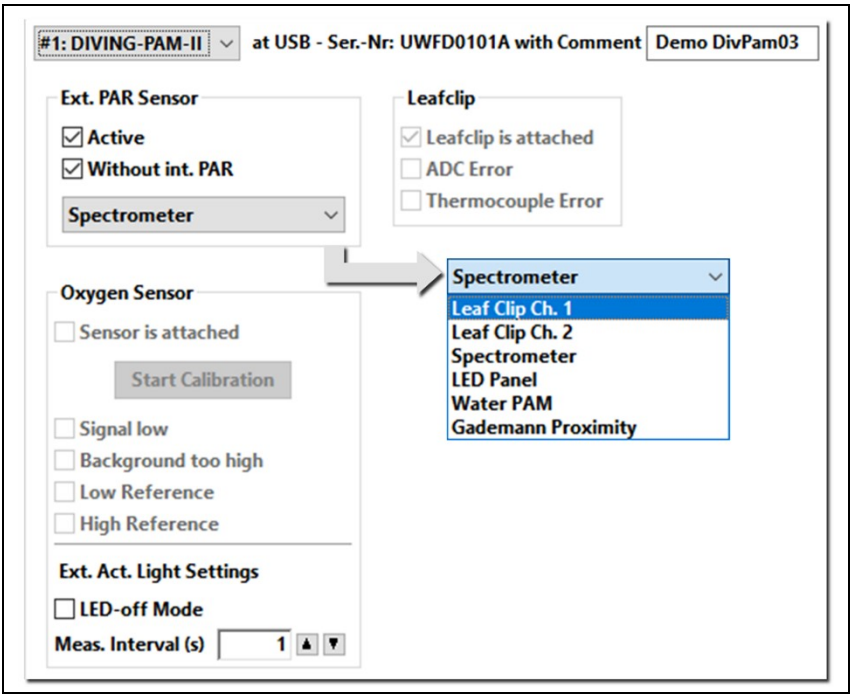


Fig. 86: Sensors Window

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

Drop-Down Menu

“Leaf Clip Channel 1” corresponds to the micro quantum sensor of the 2035-B leaf clip (MINI-PAM-II accessory). Selectable when connected via special adapter cable (operation only under dry lab conditions). “Leaf Clip Channel 2” is the signal of a PAR sensor connected to the side port of the 2035-B clip. “Spectrometer” uses PAR spectra integrated over the visible range as PAR value. “LED panel” is the PAR sensor connected to the LED-Panel RGBW-L084. (Does not apply for External LED Light Source 2054-L).

“WATER-PAM” is available when working with this PAM fluorometer. “Gademann Proximity” is a sensor under development.

Leaf Clip

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

Oxygen sensor

This section, including “External Actinic Light Settings” is dedicated to the accessory “Underwater Oxygen and pH Sensor DIVING-PAM-II/O2PH”. For details, see separate manual:

https://www.walz.com/files/downloads/diving_pam_ii_o2ph_01.pdf

6.10 Settings Window

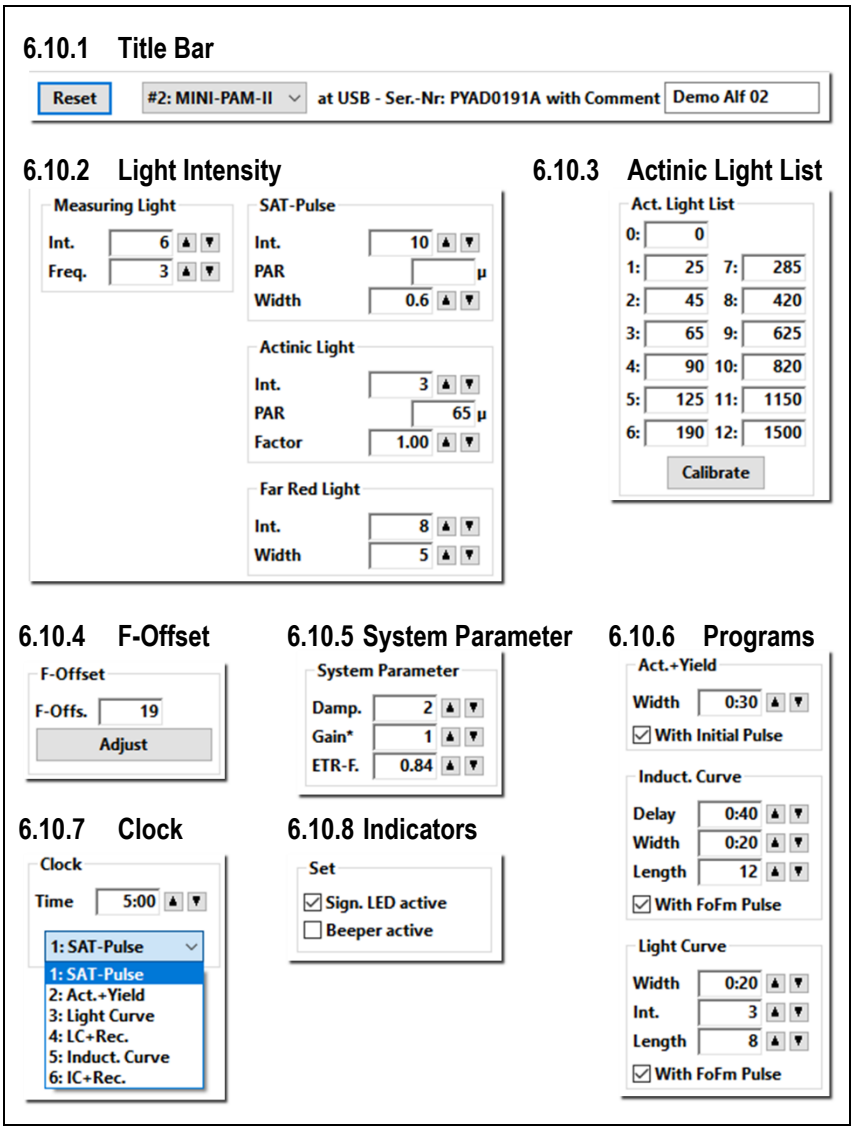


Fig. 87: Settings Window

6.10.1 Title Bar

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

6.10.2 Light Intensity

When actinic light is switched off, µs measuring pulses are delivered at 5 to 25 Hz depending on frequency setting “Freq.“ (Table 7, page 62). Switching on actinic light automatically increases the measuring light frequency to 100 Hz. The measuring light frequency can also be manually set to 100 Hz by checking “MF-F High” on the Status Bar (Table 16, page 114).

The intensity setting “Int.” adjusts measuring light intensity. Twelve intensity levels are available. The numerical value of the setting is proportional to the measuring light intensity. How to estimate the integrated measuring light intensity for the frequency and intensity settings used is described in Section 4.2.1.2, page 60.

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

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

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

6.10.3 Actinic Light List

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

Calibrate newly adjusts the emission of the LED. Calibration must be performed when the sample level is closer or further away than the sample level (7 – 7.5 mm, see Section 3.1.4, page 19). Calibration is also necessary when the DIVING-F1 Miniature Fiberoptics is used. (See Section 3.2.4.1, page 34.)

6.10.4 F-Offset

Correction for background fluorescence. See Section 4.2.1.6 (page 63).

6.10.5 System Parameter

Damping (Damp.) Function for data smoothing. The smoothing effect increases with increasing numerical value, at the same time, instrument response decreases. See Section 4.2.1.4 (page 63).

Gain Factor (Gain). The Gain factor amplifies signal and noise. At low signal levels, increasing the gain improves digital resolution. See Section 4.2.1.4 (page 63).

ETR-Factor (ETR-F.). The ETR-Factor is the percentage of light absorbed by the sample and is a parameter for calculating the electron transport rate (Section 10.3, page 172).

6.10.6 Programs

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

“Width” is the duration of light exposure. The actinic light intensity adjusted in the field “Actinic Light” applies (Section 6.10.2, page 141). A saturation pulse analysis is always carried out at end of illumination. Checking “With Initial Pulse” places an additional saturation pulse analyses before illumination. The fluorescence levels of the initial saturation pulse analysis are defined as F_0 and F_M. All fluorescence ratio parameters requiring these two fluorescence levels (Table 28, page 174) are only available with initial saturation pulse.

Induct. Curve: Fluorescence curve following the onset of light

Three parameters determine the sequence of events. “Delay” indicates the dark time interval between F_0 and F_M determination and onset of light. The delay time can be adjusted between 5 seconds and 10 minutes. Default value is 40 seconds. “Width” is the

time interval between neighboring saturation pulses in the light period. Length is the number of saturation pulse analyses carried out. Time interval of actinic illumination is approximately “Width” times “Length”. A dark period can be appended to an induction experiment (Table 16, page 114 and Table 5, page 50).

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

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

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

6.10.7 Clock

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

6.10.8 Indicators

The signal LED located above the touchscreen indicates the operational state of the DIVING-PAM-II as listed in Table 23. The

beeper acoustically marks begin and end of a saturation pulse analysis.

Table 23: LED Signal Code

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

System information is displayed on the bottom of the Settings and the System Settings windows (see Table 24 below)

Table 24: System Information

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

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

6.11 System Settings Window

Different from all other windows of WinControl-3, the window “System Settings” is not represented in the row of tabs. To access system settings, open Settings window and click “System Settings” (Fig. 89). The functional elements of System Settings are shown in Fig. 88 together with their section numbers.

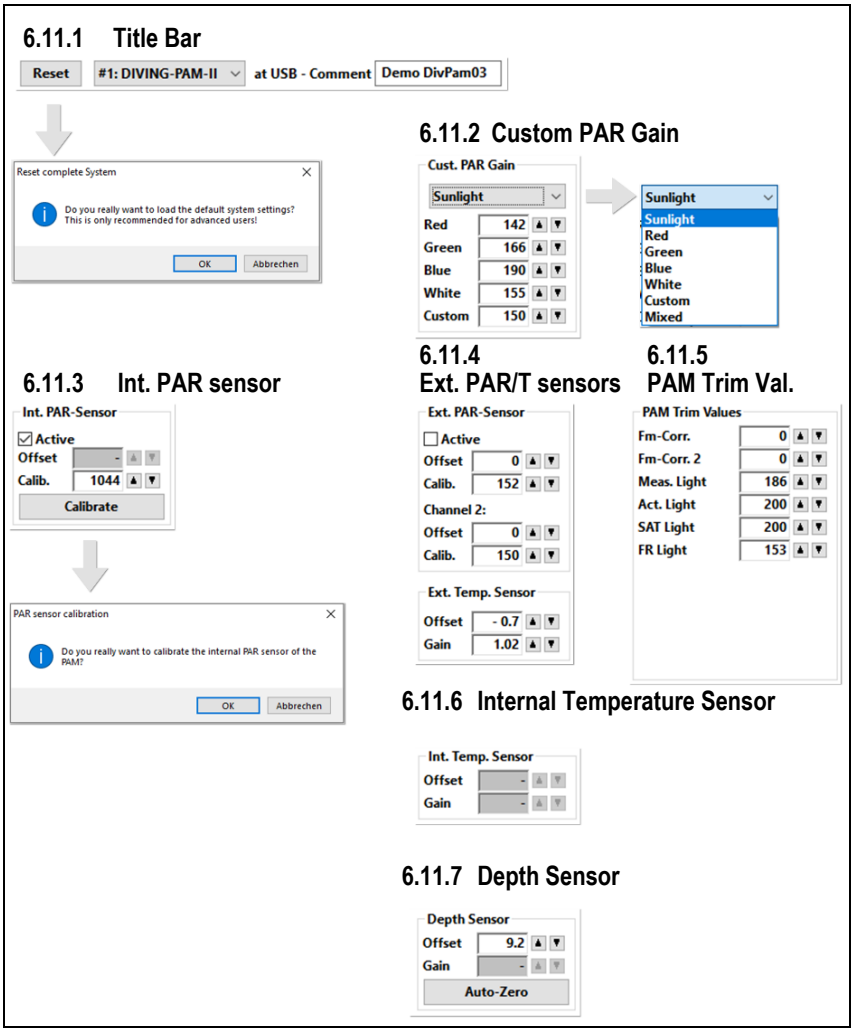


Fig. 88: System Settings

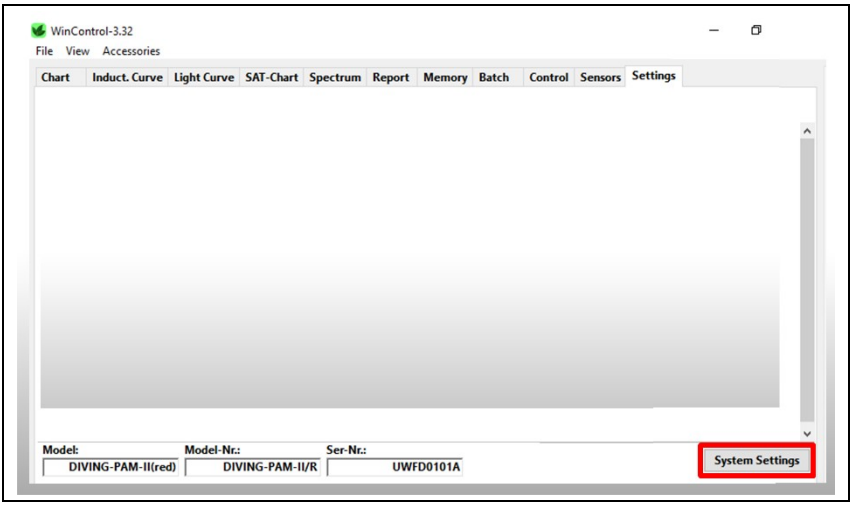


Fig. 89: System Settings Button

Click to open System Settings window.

6.11.1 Title Bar

The Reset button of the System Settings page recalls all calibration data established at the factory. This information is taken from the flash memories of the DIVING-PAM-II and of the external sensor (e.g., the 2035-B Leaf-Clip Holder).

The original calibration data cannot be changed by the user. The command Reset on the System Settings window is equivalent to the command “Reset System Settings” of the DIVING-PAM-II menu (Section 4.2.8.2, page 96). The command Reset on the Settings windows (Section 6.10.1, page 141) is equivalent to the command “Reset Settings” of the DIVING-PAM-II menu (Section 4.2.8.1, page 94).

The other elements of the Title Bar are described in Section 6.10.1, page 141.

Table 25: Custom PAR Gain

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

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

6.11.2 Custom PAR Gain

Different calibration factors for different light sources are provided. The calibration factor varies because of slight spectral variations in sensitivity of the PAR sensor. Table 25 summarizes the factors available.

6.11.3 Int. PAR sensor

The internal PAR sensor received a small fraction of the actinic light emitted by the internal LED of the DIVING-PAM-II. The calibration factor of the internal PAR sensor is displayed in the “Calib.” numerical field (Fig. 88).

The calibration of the internal PAR sensor is done by comparison with a calibrated external PAR sensor. The reading of the external PAR sensor depends on its position relative to the end of the optical fiber of the DIVING-PAM-II. Factory calibration was established with a 2035-B leaf clip in which the fiber was fully inserted.

For other configurations, the internal sensor must be newly calibrated. (See also Section 6.11.3, page 148.)

PAR sensor compatible with DIVING-PAM-II:

To calibrate the internal PAR sensor using the Miniature Spectrometer MINI-SPEC, proceed as described in Section 3.1.4, page 19, and click Calibrate.

To calibrate the internal PAR sensor using the PAR sensor of the 2035-B clip (DIVING-PAM-II accessory, requires adapter cable, operation only under dry conditions), insert fiber into clip, position light sensor relative to fiber identically to the experimental situation (with the light sensor in the sample plane) and click Calibrate.

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

6.11.4 Ext. PAR/T sensors

The boxes “Ext. PAR Sensor” and “Ext. Temp. Sensor” (Fig. 88) display the factory-established calibration factors of the 2030-B clip or the 2065-M Mini Quantum/Temp.-Sensor, depending on which device is connected. In box “Ext. PAR Sensor”, the “Channel 2” calibration applies to a second PAR sensor connected to the 2035-B leaf clip. Calibration factors must be manually entered when a PAR sensor does not support automatic installation of its calibration data.

6.11.5 PAM Trim Val.

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

Table 26: PAM Trim Values

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

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

6.11.6 Internal Temperature Sensor

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

6.11.7 Depth Sensor

Offset is adjusted in the factory under the prevailing conditions. Adjust offset using Auto-Zero if the readout above the waterline deviates from zero. Gain does not apply for the digital and temperature-compensated sensor of the DIVING-PAM-II.

7 Hints

7.1 Instrument Settings

Instrument settings are adjusted at the factory for optimum performance of the DIVING-PAM-II. For example, LED currents have been adjusted to meet target PAR values for the 60° measuring setup in the Universal Sample Holder DIVING-II-USH. For a different geometry, the internal PAR sensor requires recalibration to correctly measure internal actinic light (see Fig. 63, page 84). Estimation of PAR according to Fig. 14 (page 25) is rather inaccurate.

Also, the fluorescence offset (F-Offset) of your system has been measured and was saved on the DIVING-PAM-II memory. That means that the DIVING-PAM-II should show a fluorescence value close to zero in the absence of a sample. If this signal deviates clearly from zero, newly adjust F-Offset (see Fig. 39, page 60 ff).

7.2 Default settings

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

7.3 F_0 Fluorescence

Usually, measuring light intensity is adjusted to reach F_0 fluorescence levels around 500 mV (for a definition of F_0 see Section 10.2, page 168). Theoretically, the F_0 should stay below 640 mV.

The latter upper value is derived from the assumption that the maximum F_V/F_M of any sample is 0.84 und from the fact that signal saturation occurs at 4000 mV (see equation below, Table 27). If required, measuring light can be adjusted (Fig. 39, page 60 or Section 6.10.2, page 141).

Table 27: Maximum F_0 of a Dark-acclimated Leaf

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

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

7.4 F_M Fluorescence

The F_M and F_M' levels are determined as the maximum of the fluorescence signal induced by a saturation pulse. Factory settings of saturation pulse width and intensity are adjusted to arrive at a plateau with many macro algae and green leaves (Fig. 90A). Some high light grown samples do not reach a plateau with standards settings (Fig. 90B). In this case, saturation pulse intensity or/and length should be increased. Also, fluorescence kinetics can

reach its maximum clearly before end of the saturation pulse (Fig. 90C). The latter does not result in erroneous F_M or F_M' values because these values correspond to the maximum of fluorescence kinetics. In case of Fig. 90C, saturation pulse intensity or/and length might be decreased.

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

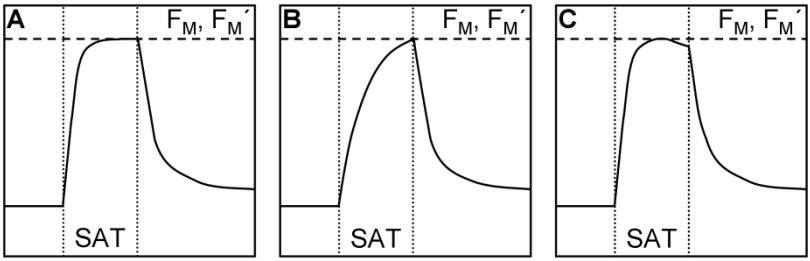


Fig. 90: Fluorescence Kinetics Induced by a Saturation Pulse

8 Trouble Shooting

8.1 Device does not switch off

Check if clock is running.

Check if device is connected to computer via USB or WLAN.

8.2 Device is not charged

Make sure that interface cable is connected to INPUT (there are several 6 pin connectors matching the interface cable, Fig. 3, page 12).

Make sure that the interface in connected to line power (Fig. 2, page 10).

8.3 “Overflow” message in window primary data

Reduce measuring light intensity and signal amplification (see “PAM Settings”, Fig. 39, page 60)

8.4 Fluorescence Signal Noise

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

Exclude that automatic scaling of Y axis after very low Y(II) has extremely amplified the fluorescence signal.

9 Maintenance

9.1 Cleaning

Depending on experimental site, the DIVING-PAM-II is exposed to various degrees of dirt. In marine environments, salt crystals may deposit on the fluorometer. To retain normal function, regular clearing is recommended. Suggested measures are:

- Shower off dirt and saltwater. Let air dry. Avoid scratches and do not wipe the surface unless all particles have been removed.
- Remove fiberoptics. Rinse fiberoptics and fiberoptics port with freshwater. Let air dry. Do not mechanically clean the fiberoptics port as this might scratch the fluorometer's optical window.
- Unscrew pushbuttons ON/OFF and START. Rinse port, plastic parts and spring. Let air dry. An aluminum tool to unscrew pushbuttons is part of delivery.
- Thoroughly rinse pressure sensor with freshwater. Let air dry. Only if particles remain after rinsing, remove protective cap. Use fingers to unscrew. When screwed very tightly, use small water pipe tongs (prevent scratches by wrapping the protective cap with a cloth). Rinse with freshwater. Never touch the sensor surface! Replace protective cap. Let air dry.

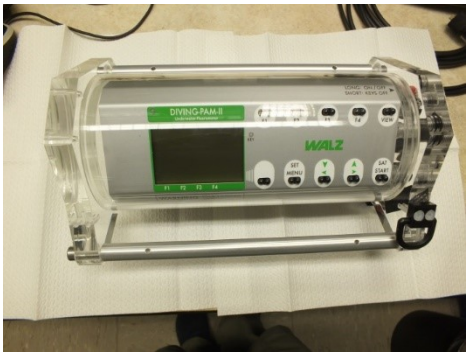
9.2 Battery Maintenance

Even when the fluorometer is not used, the status of the battery charge decays due to self-drain. Although self-drain currents are very low, the battery can become deeply discharged over long time periods. A deep discharge stresses the battery and can significantly reduce the battery capacity. Therefore, when the fluorometer is not used, charge battery every 6 months.

9.3 Battery Replacement

a Preparation

Disconnect all cables. Place DIVING-PAM-II on solid surface with display in front. Use paper towels or other padding to avoid scratches.



b Removing left end plate (01)

Use 6 mm Allen key (included in delivery) to remove the Allen screw connected to front handlebar.

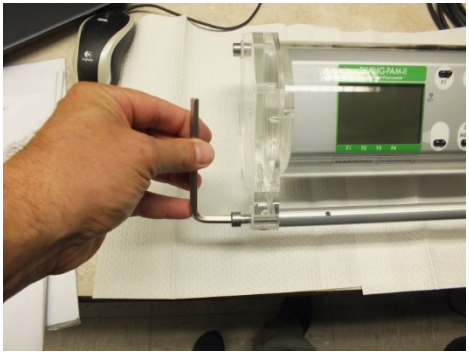
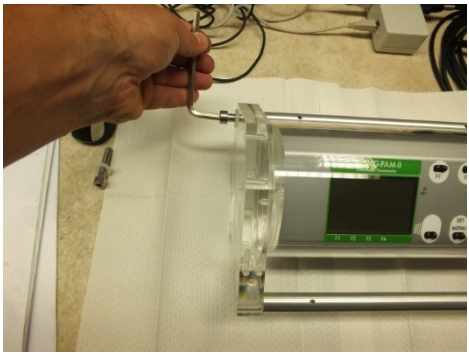


Fig. 91: Battery Replacement 01

c Removing left end plate (02)

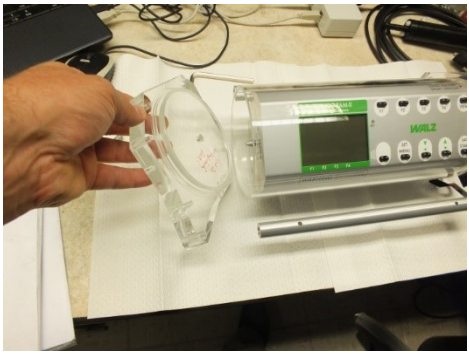
Remove the Allen screw connected to rear handle-bar.

NOTE FOR REASSEMBLY
When reassembling the end plate, Tighten the two screws alternately and in small increments.



d Removing left end plate (03)

Take off end plate.



e Removing Plexiglas tube (01)

Pull out tube.

NOTE FOR REASSEMBLY
Lightly grease sealing rings (lubricant included in delivery). Check correct position of sealing rings. Check if sealing rings are all flat and not folded or twisted.

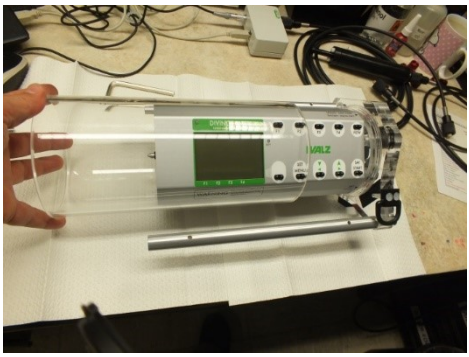
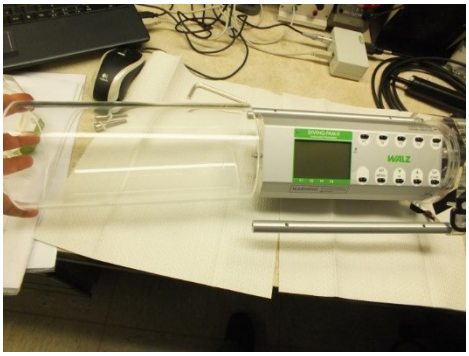


Fig. 92: Battery Replacement 02

f Removing Plexiglas tube (02)

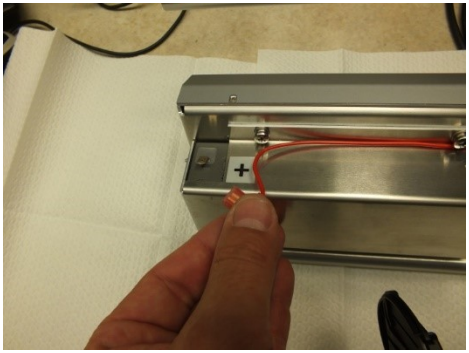
Put tube aside.



g Disconnecting battery (01)

Turn fluorometer upside down.

Pull off battery plug connected to **RED** cable (+ sign, positive pole).



h Disconnecting battery (02)

Pull off battery plug connected to **BLACK** cable (- sign, negative pole).

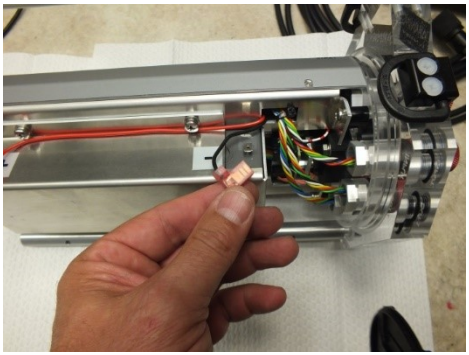
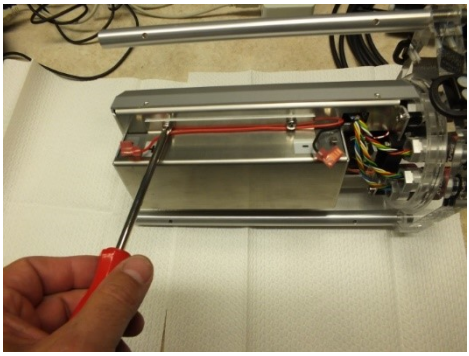


Fig. 93: Battery Replacement 03

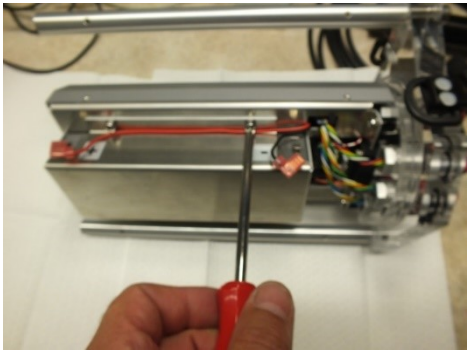
i Removing battery holder (01)

Use 6 mm Phillips screw-driver to undo the screw close to plus sign.



j Removing battery holder (02)

Undo screw close to minus sign.



k Removing battery holder (03)

Rotate fluorometer until the two remaining screws of the battery holder are easily accessible. Undo any of the two screws.

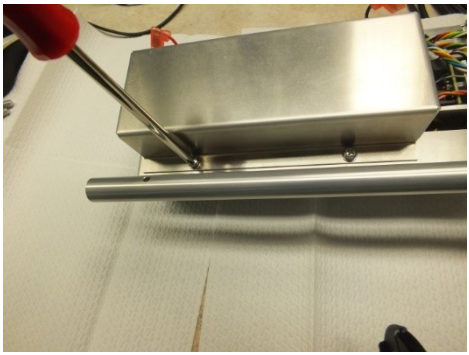
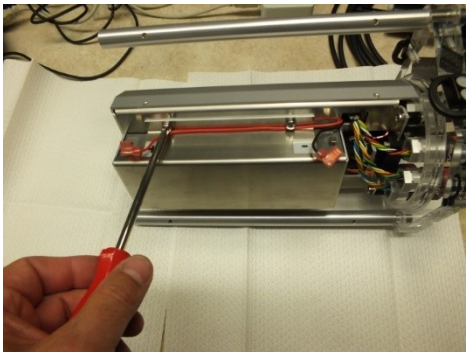


Fig. 94: Battery Replacement 04

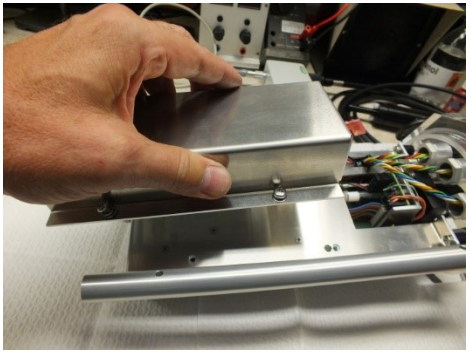
l Removing battery holder (04)

Undo last screw of battery holder.



m Removing battery holder (05)

Lift off battery holder.



n Removing battery (01)

Put aside the four screws.
Flip over battery holder.

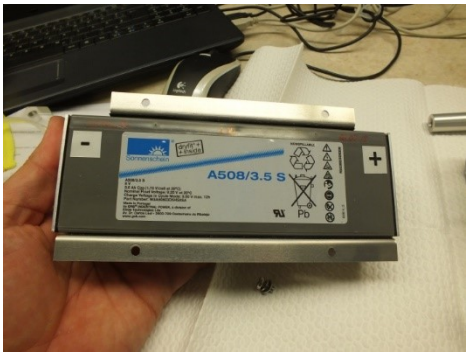


Fig. 95: Battery Replacement 05

- Removing battery (02)

  Release battery by gently striking the holder onto the hand.

  NOTE FOR REASSEMBLY
  Match plus and minus signs of new battery and battery holder. Proceed in reverse order (n→a).

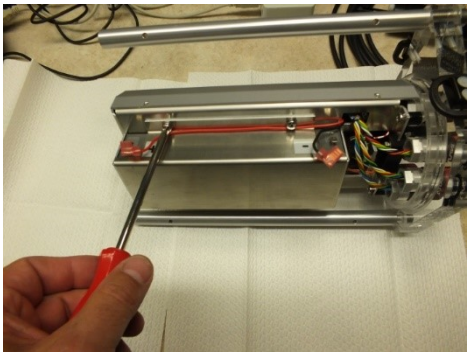


Fig. 96: Battery Replacement 06

10 Saturation Pulse Analysis

10.1 Pulse-amplitude Modulated (PAM) Fluorescence

The PAM principle is illustrated by Fig. 97. 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. 97, 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. 97, 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”.

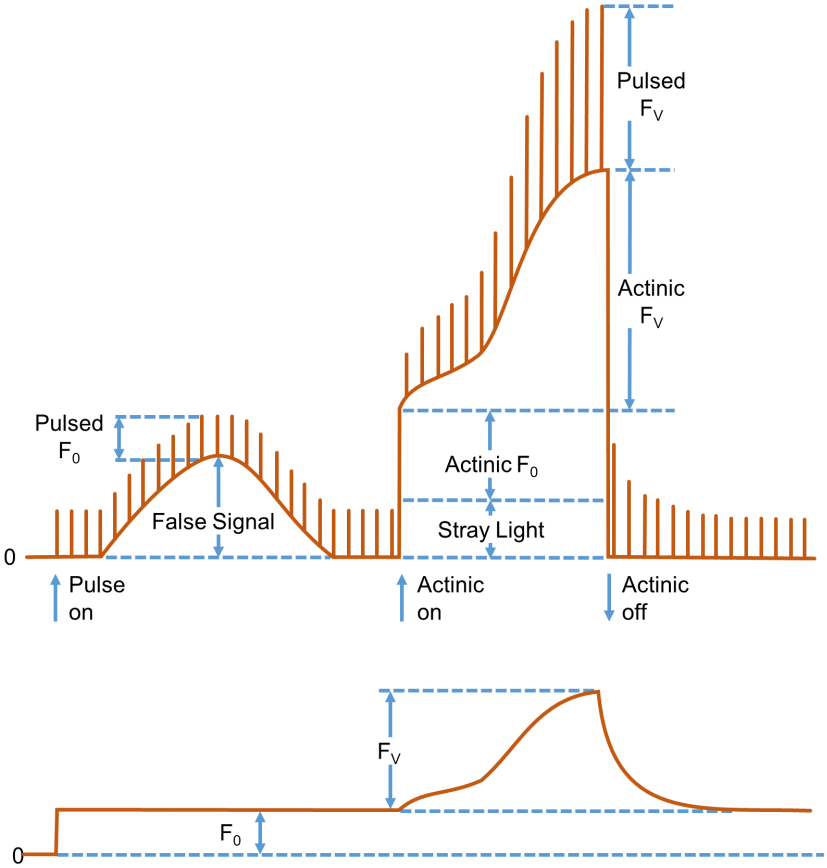


Fig. 97: **Illustration of the PAM measurement principle**
Figure redrawn from Dr. Ulrich Schreiber, Manual for PAM-101.

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

The lower trace in Fig. 97 outlines the PAM fluorescence trace. Obviously, PAM fluorescence eliminates 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.

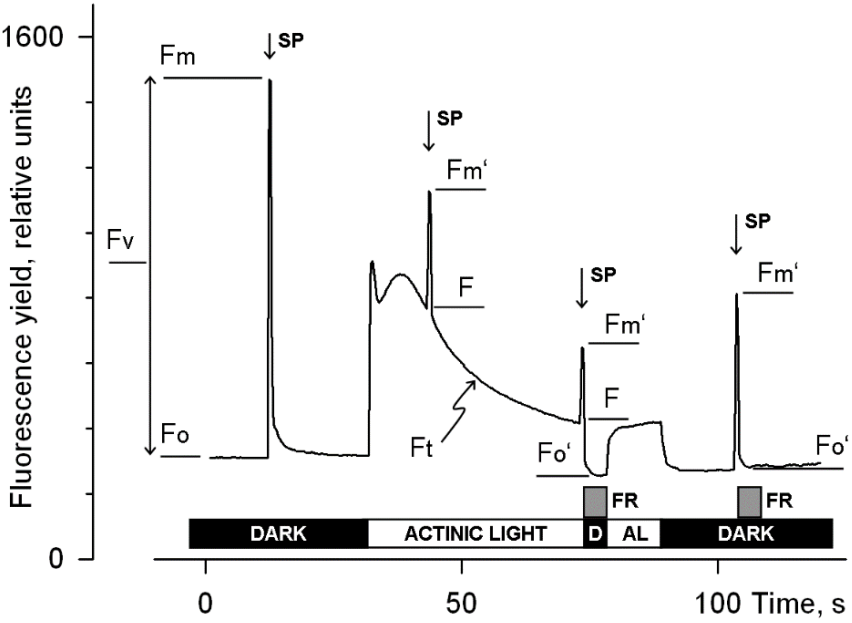


Fig. 98: Fluorescence Levels of Saturation Pulse Analysis

Y-axis (Fluorescence yield) corresponds to PAM fluorescence, see 10.1. AL, Actinic Light; D, dark; SP, Saturation Pulse; Ft, continuously recorded PAM fluorescence; FR, far-red illumination.

10.2 Saturation Pulse Analysis

The five principal levels of PAM fluorescence which are used for saturation pulse analysis are shown in Fig. 98. 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 28, page 174).

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

Measurements with Dark-Acclimated Samples

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

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

Measurements with Illuminated Samples

F_0' Minimum fluorescence level of illuminated sample. The F_0' is lowered relative to F_0 by non-photochemical quenching. The measuring routine for F_0' (see Fig. 98, page 167) determines the F_0' level during a dark interval following a Saturation Pulse. In this dark interval, far-red light is applied which selectively drives photosystem I. As

a consequence, electrons are removed from the intersystem electron transport chain and opening of photosystem II reaction centers is efficiently accelerated

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

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

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54 135-142. https://doi.org/10.1023/A:1005936823310

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

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

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

To quantify photochemical use and non-photochemical losses of absorbed light energy, fluorescence ratio expressions have been derived which use two or more of the five relative fluorescence yields introduced above. Table 28 (page 174) compiles the fluorescence ratio parameters available in the DualPAM software. Below, these parameters will be explained briefly.

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

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

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

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

q_P and q_L Coefficients of photochemical fluorescence quenching

Both parameters estimate the fraction of open photosystem II reaction centers. The q_P is based on the concept of separated photosystem II antenna units (puddle model), whereas the q_L assumes interconnected photosystem II antenna units (lake model) which was assumed to be present in leaves (*cf.* Kramer *et al.*, 2004). Determinations of q_P an q_L do not require fluorescence measurements with the dark-acclimated sample, except the F_0' mode is switched of and F_0' is calculated according to Oxborough and Baker (1997).

q_N and NPQ Parameters of non-photochemical quenching

Both parameters are associated with non-photochemical quenching of excitation energy, mainly involving a low thylakoid lumen pH- and a zeaxanthin-dependent quenching mechanism. The qN and the NPQ parameters require fluorescence measurements with the sample in the dark-acclimated and in the light-exposed states (cf. Table 28, page 174).

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

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

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

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

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

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

This concept of "complementary photosystem II quantum yields" is useful to analyze the partitioning of absorbed light energy in photosynthetic organisms. For instance, in the presence of strong light, a much higher Y(NPQ) than Y(NO) indicates that excess excitation energy is safely dissipated at the antenna level and that photosynthetic energy fluxes are well-regulated.

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

10.3 Relative Electron Transfer Rate (ETR)

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

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

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

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

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

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

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

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

10.4 Reviews on Saturation Pulse Analysis of Photosystem II

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

Table 28: Fluorescence Ratio Parameters.

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

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

Table 29: References Cited in Table 28

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11 Specifications

11.1 Basic System

11.1.1 Optoelectronic Unit

Design: Plexiglas tube with Plexiglas end plates, one with waterproof fiberoptics port

Mountings: Anodized aluminum rod (diameter 15 mm) mounted parallel to the fluorometer body below the screen at a distance of 30 mm, and another one mounted on the opposite side at a distance of 5 mm. Anodized aluminum holder for fiber optics and cables at one end of the fluorometer (maximum dimensions 20.5 cm x 18.5 cm x 5 cm; L x W x H). Two plastic loops to fasten a carrying belt, and a poly-oxymethylene (POM) ring with outer diameter of 46 mm to store the miniature spectrometer MINI-SPEC

Display: Backlit 160 x 104 dots (78 mm x 61 mm) transflective B/W screen

Control elements: 10 infrared reflection switches to operate control fields on the screen, pushbutton for saturation pulses, pushbutton to switch device on/off and to lock/unlock reflection switches

Pressure and temperature sensor: Module including a high linear pressure sensor and a temperature sensor. With gel protection and antimagnetic stainless steel cap

Ports equipped with watertight caps:

AUX1 and AUX2, 4-pole, for miniature spectrometer MINI-SPEC and, via special adapter, for MINI-PAM-II accessories Fiber-Optic Oxygen Meter FireStingO2 or Leaf Clip Holder 2035-B (connected via adapter cable)

OUT1 and OUT2, 6-pole, for operation of an external light source synchronized with PAM measuring light. Works also as input for or trigger signal from Sample Holder DIVING-II-USH

INPUT, 6-pole, for RS-485 communication and charging of internal battery. Works also as input for or trigger signal from Sample Holder DIVING-II-USH

Battery: Lead acid battery 8.0 V / 3.5 Ah (28 Wh) providing power for more than 1300 yield measurements

Maximum diving depth: 50 m

Operating temperature: -5 to +45 °C

Dimensions: Diameter 19 cm, length 39 cm

Weight: 3.9 kg

11.1.2 Light sources

DIVING-PAM-II/B (Blue Version)

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

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

DIVING-PAM-II/R (Red Version)

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

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

DIVING-PAM-II/B and DIVING-PAM-II/R

Far-red light: Peak emission at 735 nm

11.1.3 Data acquisition

Fluorescence: PIN photodiode protected by long-pass and a short-pass filters, 12 bit signal resolution

Other parameters: Piezo-resistive pressure sensor and temperature sensor. Pressure is converted in meters of diving depth, range 0 to -50 m, displayed at 0.1 m intervals. Temperature, range -10 °C to +60 °C, displayed at 0.1 °C intervals

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

11.1.4 WLAN

Wireless LAN Interface, IEEE 802.11 b/g/n (2.4 GHz), Access Point Mode

11.1.5 Fiberoptics DIVING-F

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

Dimensions: Active diameter 5.5 mm, outer diameter 8 mm, length 150 cm

Weight: 340 g

11.1.6 PC Interface Box DIVING-PAM-II/I

Housing: Aluminum case with USB-B port, socket for power supply MINI-PAM-II/N, and waterproof 6-pole socket for RS-485 communication

Function: The interface box connects computer and DIVING-PAM-II. RS-485 serial data communication is used between box and DIVING-PAM-II, USB communication is employed between interface box and computer. Recommended maximum cable lengths are: 100 m RS-485 cable between DIVING-PAM-II and interface box, 2 m USB cable between interface and box computer. Standard USB-A to USB-B cable included

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

Weight: 270 g

Operating temperature: -5 °C to + 40 °C

11.1.7 Power Supply MINI-PAM-II/N

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

Output: 12 V DC, 5.5 A

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

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

Weight: 350 g including cables

11.1.8 Underwater Cable DIVING-PAM-II/K5

Underwater cable for RS-485 communication and charging of the DIVING-PAM internal battery

Length: 5 m

Weight: 500 g

11.1.9 Miniature Spectrometer MINI-SPEC

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

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

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

Dimension: 3.25 cm diameter, 17.5 cm length max

Weight: 135 g

11.1.9.1 Flat Entrance Optics SPEC/P

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

11.1.9.2 Fluorescence and Reflection Optics SPEC/R

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

other and, thus, hold the sample. Including a 10 mm thick Zenith Polymer reflectance standard

11.1.9.3 PAR Calibration Block 000160101439

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

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

Weight: 40 g

11.1.10 Distance Clip 60° 2010-A

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

Fiber holder: 1.2 cm length, mounted 0.7 cm above base, with lateral screw to fix fiber optics. Angle between fiber optics axis and sample plane: 60°. Two spacer rings to vary the distance between fiber end and leaf surface

11.1.11 Dark Leaf Clip DIVING-LC

Design: Three clips made of white plastic with gasket contact areas and sliding shutter for light-tight closure.

Dimensions: Diameter 3.2 cm, length 8 cm

Weight: 6.5 g

11.1.12 Surface Holder DIVING-SH

Design: Holder made of grey PVC, equipped with 3 rubber bands and hooks to be attached to creviced surface (e.g. of coral); nylon screws for distance adjustment

Dimensions: 6 cm x 6 cm x 2.5 cm (L x W x H)

Weight: 95 g

11.1.13 Software WinControl-3

Program: WinControl-3 System Control and Data Acquisition Program (Windows 7, 8, 10) for operation of DIVING-PAM-II via PC, data acquisition and data analysis

Saturation Pulse Analysis: Measured: Ft, F_0, F_M, F, F_0' (also calculated), F_M'. PAR, water temperature and depth (derived from pressure). Calculated: F_0' (also measured), F_V/F_M and Y(II) (maximum and effective photochemical yield of PS II, respectively), q_L, q_P, q_N, NPQ, Y(NPQ), Y(NO) and ETR (electron transport rate)

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

Programmed Features: Automatic determination of signal offset for all light intensities and all gain levels. Automatic calibration of internal PAR sensor against PAR from MINI-SPEC sensor

Communication Protocol: USB and IEEE 802.11 b/g/n

Computer Requirements: Processor, 1 GHz. RAM, 512 MB. Screen resolution, 1024 x 600 pixels. Interface, USB 2.0/3.0.

11.1.14 Transport Case DIVING-PAM-II/T

Design: Rugged, hard plastic outdoor case with wheels, pull-out handle and custom foam packing

Dimensions: 57 cm x 47 cm x 27 cm (L x W x H)

Weight: 7.7 kg

11.2 Accessories

11.2.1 Universal Sample Holder DIVING-II-USH

Design: Plexiglas bar (15 cm x 4.5 cm) with upward curved end possessing a port for positioning at 60° or 90° relative to the sample level the DIVING-PAM-II fiber optics. Mounted to the curved end is a 5.5 cm x 7.5 cm (W x H) sample clip consisting of a Plexiglas plate (lower part) and an aluminum frame open to the top (upper part). Featuring a 10 cm long plastic grip with button for triggering measurements via a 1.5 m trigger cable. Including a 1 m long tubular net with zipper to keep together trigger cable and fiber optics. With holder to for spectrometer MINI-SPEC

Dimensions: 25 cm x 4.5 cm x 21 cm (L x W x H)

Weight: 380 g

11.2.2 Underwater Oxygen and pH Sensor DIVING-PAM-II/O2PH

Design: POM tube, one end equipped with an O2 and a pH optical sensor spot fixed by a Perspex disk having funnel-shaped holes for water access to the sensor spots, and a PT100 resistance thermometer, at the opposite end with 4-pole underwater socket. Temperature-compensated oxygen and pH measurements by a high precision optical pH and oxygen meter, respectively, both connected to the respective sensor spot by fiberoptics. Including a 2 m underwater cable 000130204945, two spare oxygen sensor spots OXSP5, and two spare pH sensor spots PHSP5-PK8T, a holder (weight 75 g, maximum dimensions 6.5 cm x 6 cm x 12 cm, L x W x H) to attach both the DIVING-PAM-II/O2PH and the spectrometer MINI-SPEC to the DIVING-PAM-II optoelectronic unit, consisting of the mounting brackets 000246001714 and 000246003914 and 1 ring holder 000244905514.

Dimension: 3.25 cm diameter, 29.5 cm length max

Weight: 205 g

11.2.3 Magnet Sample Holder DIVING-MLC

Design: Two plastic halves with magnets, one with sliding shutter made of spring steel and seat for the adapter DIVING-DA. The other halve with seats for additional magnets. Inner sides of both halves covered with black fabric. Including 4 additional magnets.

Dimensions: Diameter 30 mm, height 28 mm

Weight: 10 g

11.2.4 Surface Holder DIVING-SH

Design: Holder made of grey PVC, equipped with 3 rubber bands and hooks to be attached to creviced surface (e.g. of coral); nylon screws for distance adjustment

Dimensions: 6 cm x 6 cm x 2.5 cm (L x W x H)

Weight: 95 g

11.2.5 Miniature Fiberoptics DIVING-F1

Design: Both ends with adapter which fits to the fiber port of the DIVING-PAM-II and the various accessories for fiber positioning of the DIVING-PAM-II system.

Dimensions: Active diameter 2 mm, length 1.5 m

11.2.6 Underwater Cable DIVING-II/K25

Dimensions: 25 m length, 6 mm diameter

Weight: 1.25 kg

11.2.7 Underwater Cable DIVING-II/K50

Dimensions: 50 m length, 6 mm diameter

Weight: 2.5 kg

Including charger **DIVING-II/L15**:

Input: 100 V to 240 V AC, 47 to 63 Hz

Output: 15 V DC, 4.65 A

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

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

Weight: 350 g including cables

Subject to change without prior notice

12 Guarantee

12.1 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.

12.2 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.

12.3 Instructions

- To obtain guarantee service, please follow the instructions below:
- The Walz Service Information Form available at https://www.walz.com/files/downloads/walz_serviceinformationform.pdf 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.

12.4 Applicable law

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

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