DIVING-PAM-II

Underwater Chlorophyll Fluorometer

Manual

For Standalone Use

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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.
- Keep the device away from dust, sand and dirt.
- 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 in order to avoid potential harm to the user and damage to the instrument.
- The DIVING-PAM-II can emit very strong light! In order to avoid harm to your eyes, never look directly into the light port of the DIVING-PAM-II or its fiberoptics.
- The PC Interface Box DIVING-PAM-II/I is not water tight; keep it away from water or high moisture areas.

- Keep all ports and plugs clean. Do not expose open cable ends to water or high moisture.
- Do not force a plug into the wrong socket. Orientate each plug so that the red dot on the plug coincides with the red dot of the socket. Do not try to disconnect a plug by pulling at the cable. Disconnect plug by pulling at the rippled bushing of the plug.
- Run all cables so that stepping on or stumbling over them is excluded.
- Do not open housing of the DIVING-PAM-II. There are no serviceable parts inside the DIVING-PAM-II. Do not attempt to open pressure and temperature sensor (Fig. 3, page.9).
- Before immersing the DIVING-PAM, make sure that all cable plugs are properly connected (the rippled cylinder must be completely screwed in so that it pressed against the rubber ring of the cable port). Also, make sure that each unused cable port is properly sealed by a red riffled cap (screw in completely). See **Fig. 4**, page 11.
- Do not exceed the maximum diving depth of 50 m.

1.3 Instructions for Battery Handling

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

- DIVING-PAM-II fluorometers up to serial number UWFD0110 contain a Lithium manganese oxide battery.
- 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. 20, page 33).

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.
    http://www.walz.com/products/chl_p700/diving-pam-II/downloads.html
    (Testing according to UN 3.83: Recommendations on the Transport of Dangerous Goods. UN Manual of Tests and Criteria. Part III, Subsection 38.3: Lithium metal and lithium ion batteries, Fifth revised version, 2009, United Nations, New York and Geneva):
  - Print entire protocol (6 pages) and enclose this protocol in the box.
  - Visibly label package with lithium battery label (downloadable at http://www.walz.com/products/chl_p700/diving-pam-II/downloads.html).

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 underwater plants, including sea grasses, macroalgae, and zooxanthellae in corals.
- The instrument continues the tradition of its predecessor DIVING-PAM. Technical advancements of the DIVING-PAM-II include the consistent use of energy-efficient LEDs, an internal PAR sensor, and a backlit transflective B/W screen which is readable even under sunlight.
- Also, the DIVING-PAM-II features a far red LED for selective photosystem I excitation.
- 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.
- 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 us with the MINI-PAM-II (http://www.walz.com/products/chl_p700/mini-pam-II/accessories_O2fluo_configuration.html).
- The DIVING-PAM-II can be operated by the well-proven WinControl-3 software which has been first introduced for the JUNIOR-PAM fluorometer and now operates many other fluorometers like the MONITORING-PAM, and WATER-PAM.

2.2 Structure of Manual

The DIVING-PAM-II fluorometer provides a vast range of settings and protocols for measuring fluorescence. To make full use of these opportunities, it is necessary to become acquainted with terminology and principles of saturation pulse analysis. Therefore, the present manual provides a chapter dealing with the basics of saturation pulse analysis (Chapter 5, page 81).

Chapter 5 also provides a list of review papers on PAM chlorophyll fluorescence and saturation pulse analysis (page 93). Further, a short section is providing some hints for beginners (Chapter 6, page 95) and another one gives some instructions for trouble shooting (Chapter 7, page 98).

In the field, the DIVING-PAM-II is mostly operated in the stand-alone mode. Chapter 4 (page 29) provides detailed instructions on how to use the screen interface including advices on fluorescence induction and light curve programs.

Also, this manual includes a section on safe handling of the DIVING-PAM-II (Chapter 1, page 1), and on the extent of deliv-

ery of the basic fluorescence system and its accessories (Chapter 3, page 7). Further, technical information (Chapter 8, page 99) and warranty conditions (Chapter 9, page 109) are provided.

3 Components and Setup

3.1 Basic System

3.1.1 Extent of Delivery (Basic System)

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	000160101439
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 (page 8). The figure outlines that, in addition to operation in the stand-alone mode, the DIVING-PAM-II can be operated by the software WinControl-3 running on a computer with Microsoft Windows op-

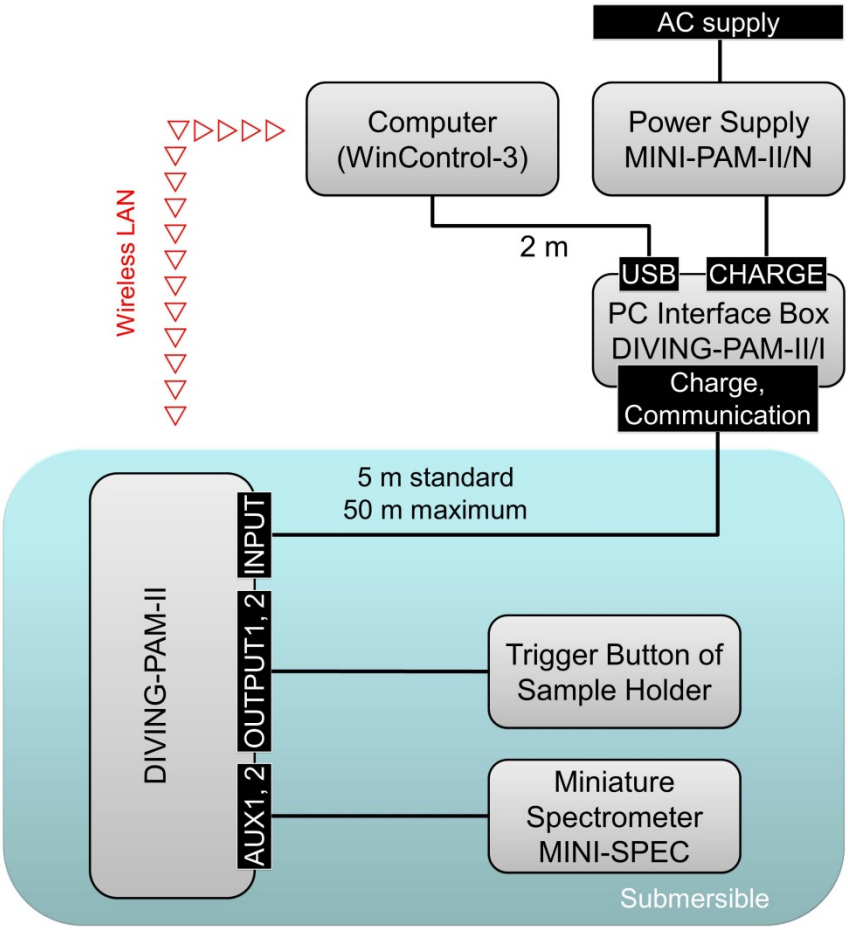


Fig. 2: DIVING-PAM-II: System Components

erating system. This online mode requires connection between computer and fluorometer via the system's interface box. Computer and interface box are linked by a standard USB cable; interface box and fluorometer are connected by a special underwater cable. Instructions on how to properly connect underwater cables are given by Fig. 4 (page11). 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.)

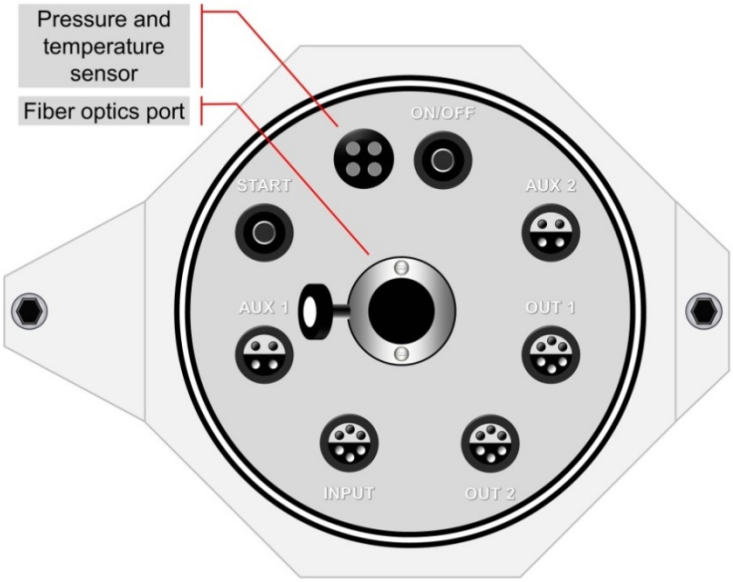


Fig. 3: Right end plate of fluorometer DIVING-PAM-II

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 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. 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 12.

Further submersible components are the Miniature Spectrometer MINI-SPEC and the optional Universal Sample Holder DIVING-II-USH (not part of basic system). 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.

When the cable of the DIVING-II-USH is plugged into port OUT 1 or OUT 2 on the right end plate, pressing the trigger button of the DIVING-II-USH executes the command currently selected in the DIVING-PAM-II screen. The START button on the right end plate of the fluorometer (Fig. 3, page 9) has the same function as the trigger button of the DIVING-II-USH.

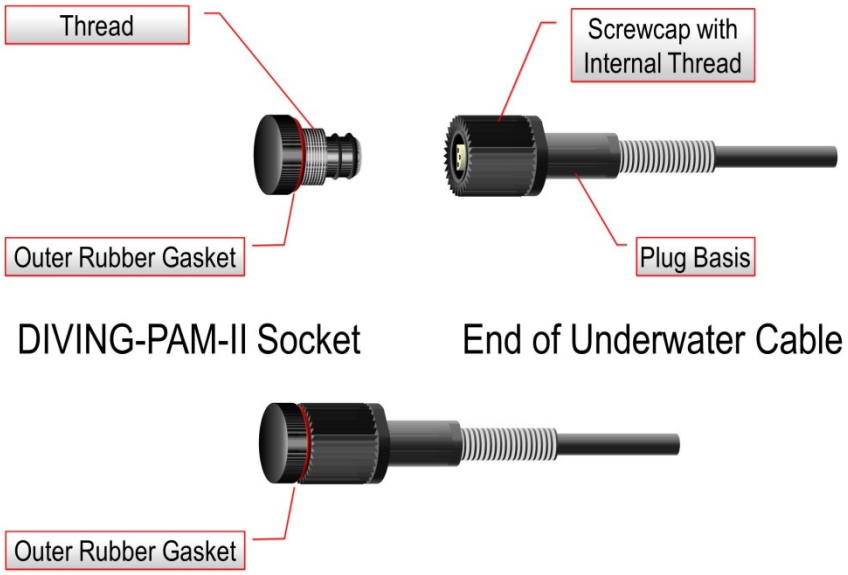
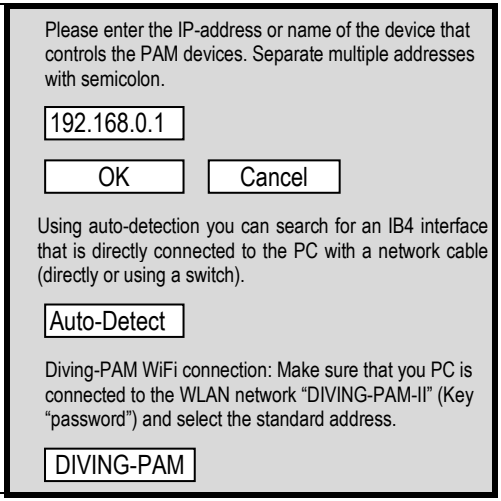


Fig. 4: **Watertight connection of cables.**
(1) Hold cable end at "plug basis".
(2) Fully (!) insert plug in socket.
(3) Tighten "screwcap".
(4) Confirm that screwcap presses against "outer rubber gasket".

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. 57, page 74). 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”, “Win-Control-3”. - Windows 8 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	

Another submersible component is the Fiberoptics DIVING-F which is inserted into the central optical port of the right end plate (not shown in Fig. 2).

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

3.1.3 Fluorometer

The water proof 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 9). All ports and buttons are explained in Table 1 (page 10).

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 14). 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.

The associations between optical switches and field elements are outlined in Fig. 5B (page 14). Basically, the function keys of the upper row of keys (F1 through F4) are associated with the 4 control elements displayed close to the bottom of the DIVING-PAM-II screen. The rightmost key in the upper row, the VIEW button, is not associated with a field element but changes appearance and properties if graphical windows (e.g. induction curve or spectral data) so that more numerical values are dis-

played and selection of individual points in a graph becomes possible.

The optical keys of the lower row from left to right correspond to the field elements of the right side 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 or the trigger button of the DIVING-II-USH.

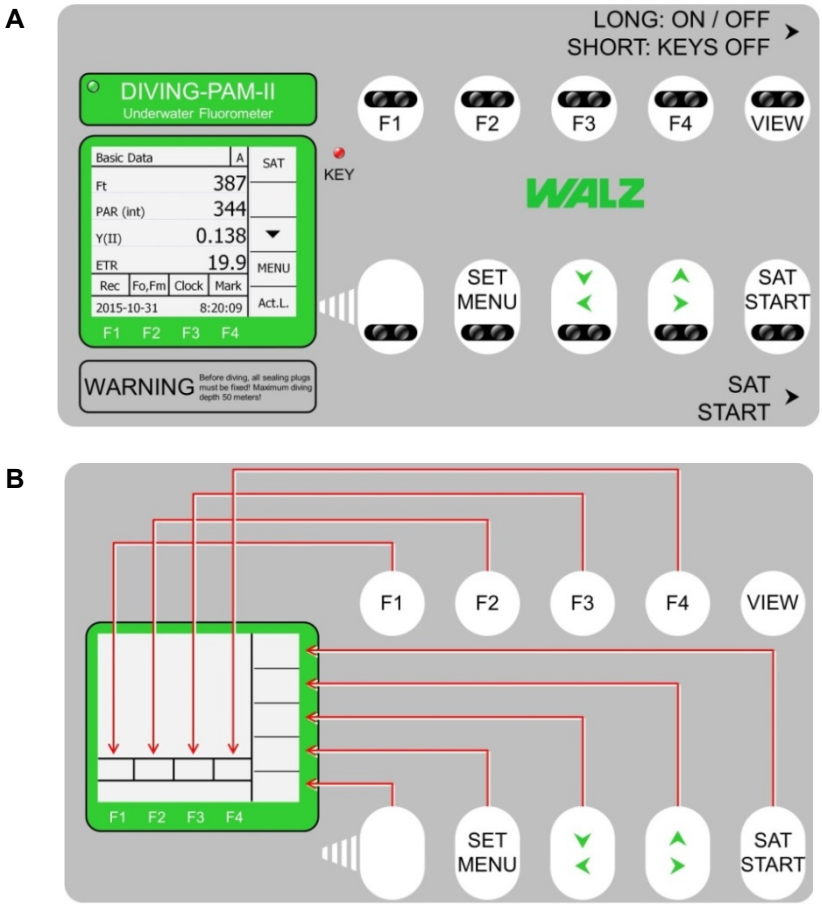


Fig. 5: Front view of DIVING-PAM-II and association of keys with fields elements.

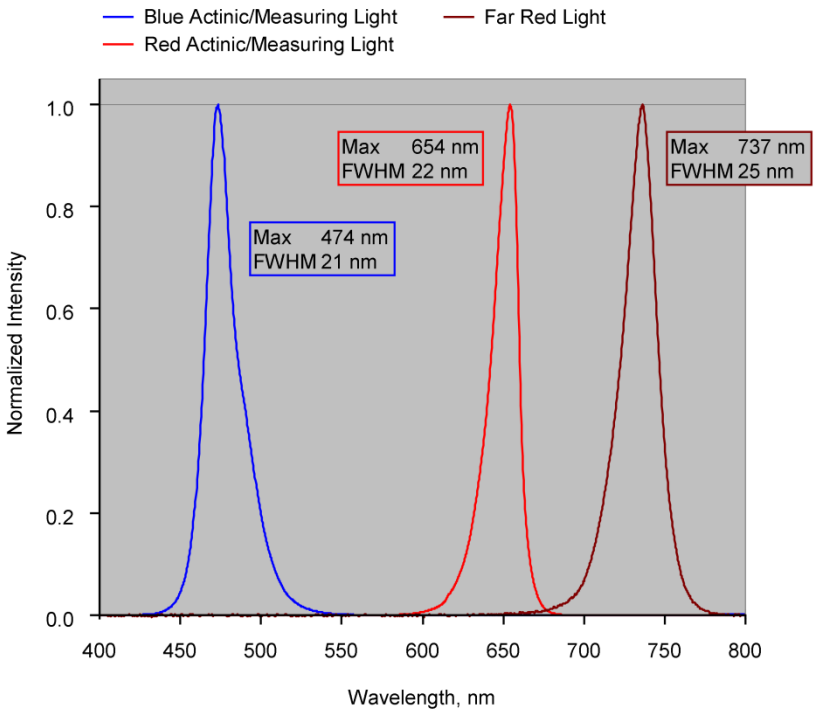


Fig. 6: Normalized emission spectra of DIVING-PAM II LEDs. Normalized emission spectra of blue LED (DIVING-PAM-II/B), red LED (DIVING-PAM-II/R) and far red LED (DIVING-PAM-II/B and R).

In the DIVING-PAM-II/R, a red LED is electronically driven to act as measuring light and, at the same time, as actinic light source. In the blue version (DIVING-PAM-II/R), a blue LED replaces the red one. In addition, both versions of the DIVING-PAM-II offer a far red LED. Normalized emission spectra of blue, red and far red LEDs are shown in Fig. 6.

3.1.4 Miniature Spectrometer MINI-SPEC

The DIVING-PAM-II is the first Walz chlorophyll fluorometer equipped with a spectrometer. This device is calibrated to measure spectra of quantum fluxes. Integration of these spectra over the visible range results in PAR data similar to those recorded with Walz quantum sensors. Beyond measuring PAR, the spectrometer provides 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. 7, page 16.

By its ability to measure PAR, the spectrometer can calibrate the internal PAR sensor of the DIVING-PAM-II (see Section 4.2.4.1, page 62). To this aim, the DIVING-PAM-II light guide and the en

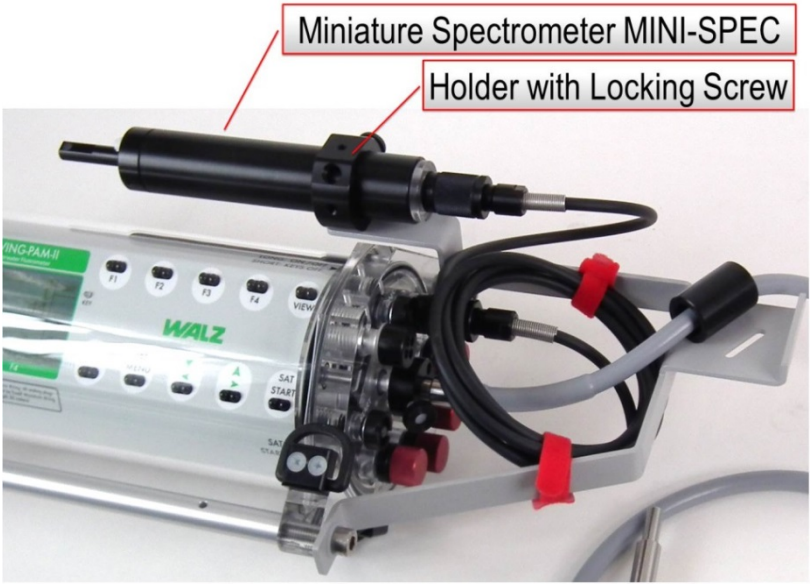


Fig. 7: DIVING-PAM-II with miniature spectrometer in transport position.

trance optics of the spectrometer are inserted in the PAR calibration block (Fig. 9, page 18). 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-

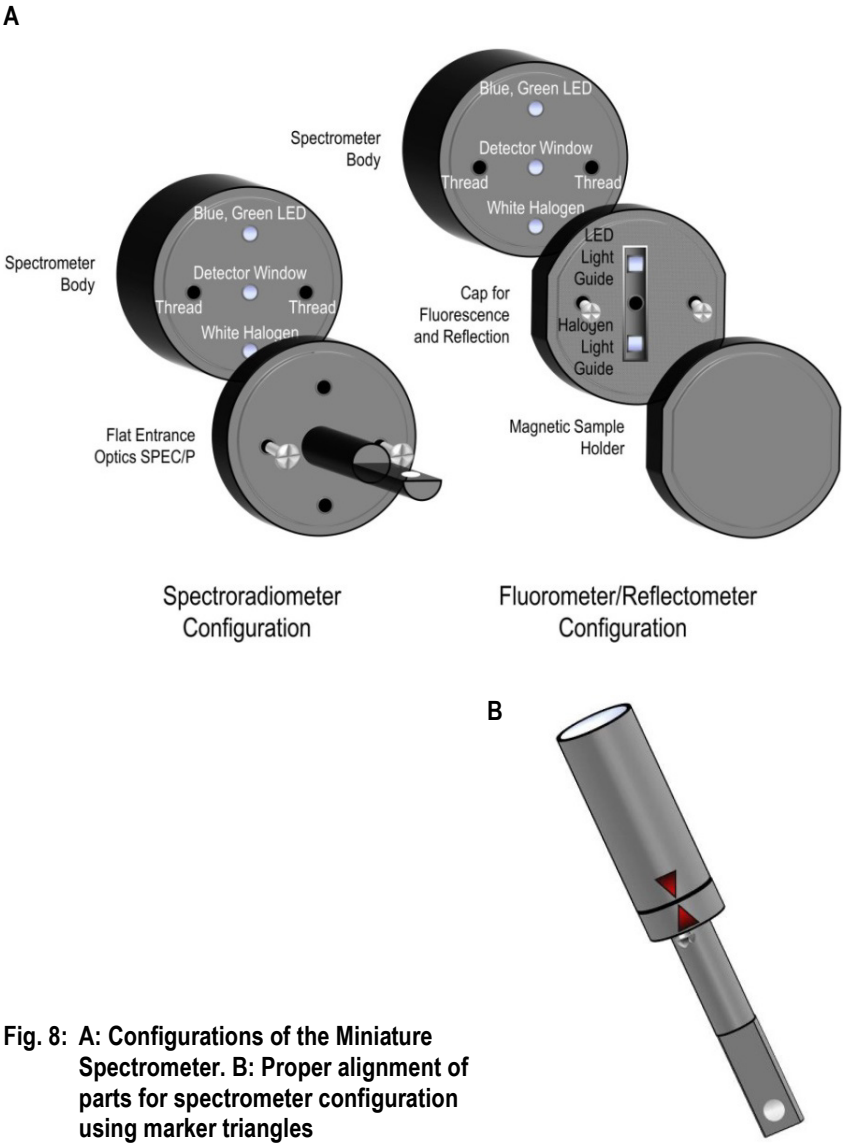


Fig. 8: A: Configurations of the Miniature Spectrometer. B: Proper alignment of parts for spectrometer configuration using marker triangles

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

Replacing the entrance optics used for evaluation of light by the cap for fluorescence and reflection (Fig. 8, page 17) 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.

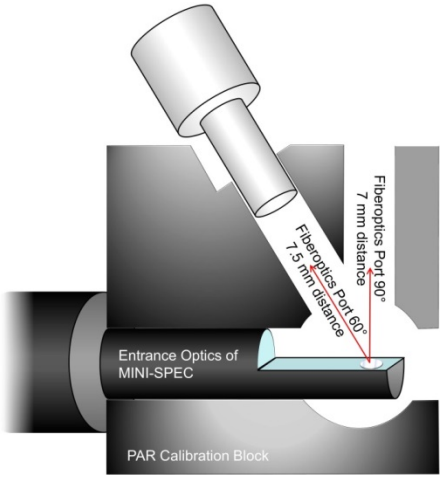


Fig. 9: PAR Calibration Block.

For reflection measurements, a halogen lamp is employed whose emission is transferred through another light guide to the sample (Fig. 8, page 17). 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

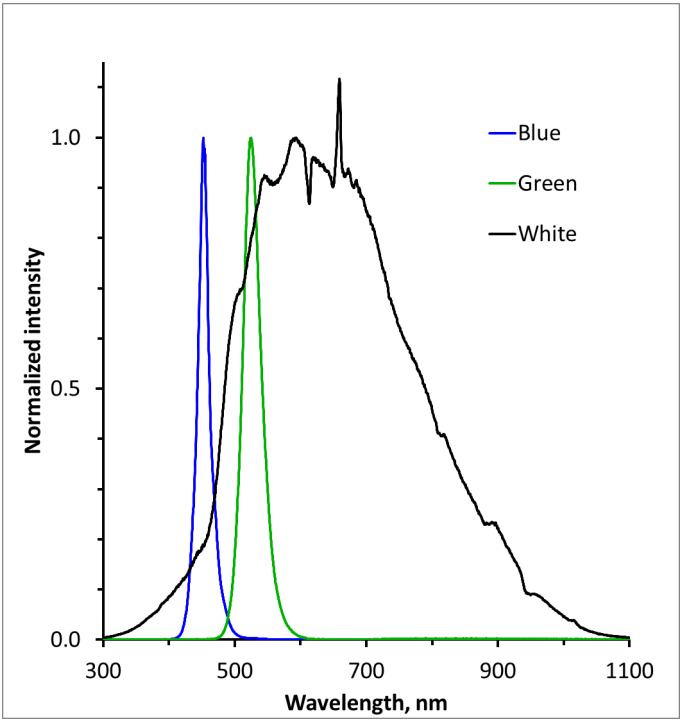


Fig. 10: Normalized Emission Spectra of Miniature Spectrometer Light Sources.

and humidity, and store the reflection standard in a closed container when not used.

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. 13, page 22).

Normally, a specimen is examined above the viewing hole of the clip and it is hold between the folded parts 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.

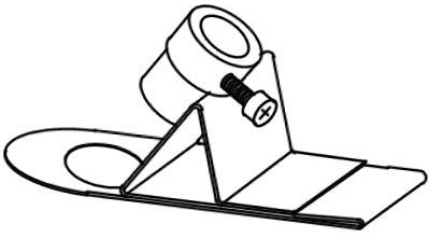


Fig. 11: Distance Clip 60° 2010-A.

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. 12, page21) positions the fiberoptics tip at the relatively short distance of 3 mm above leaf surface. As a consequence, measuring light intensity and, thus,

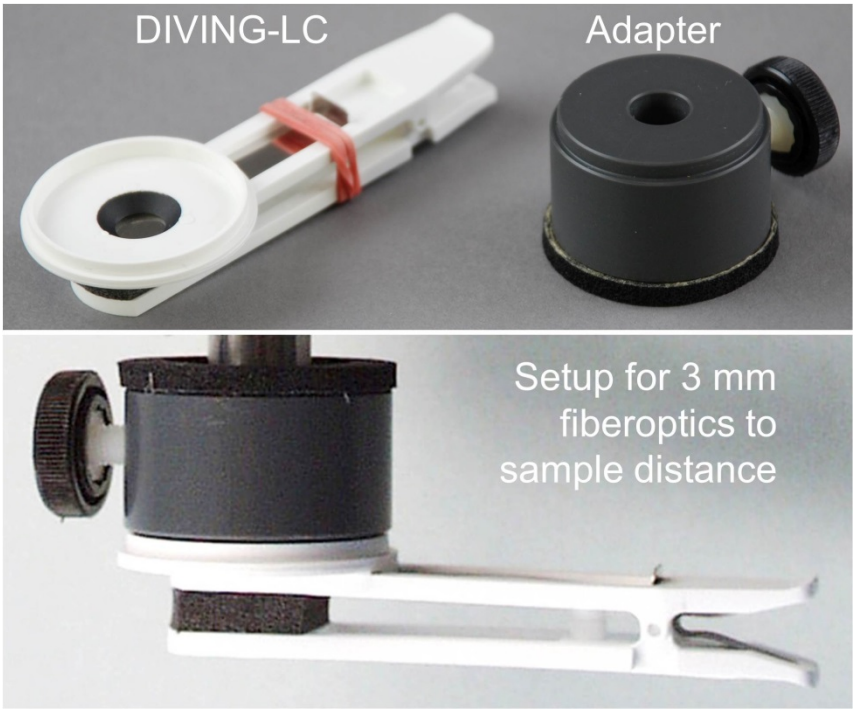


Fig. 12: DIVING-LC and Adapter.

signal amplitude are higher than with the standard distance for vertical illumination of 7 mm (see Fig. 15, page 23). If signal saturation occurs at 3 mm distance, reduce measuring light intensity and gain.

The closed shutter increases the signal level to some degree because a small fraction of measuring light is reflected by the shutter and transmitted to the photodetector. Therefore, carry out compensation of the background signal (F-Offset, Section 4.2.1, page 50) with the fiberoptics end directed into the air.

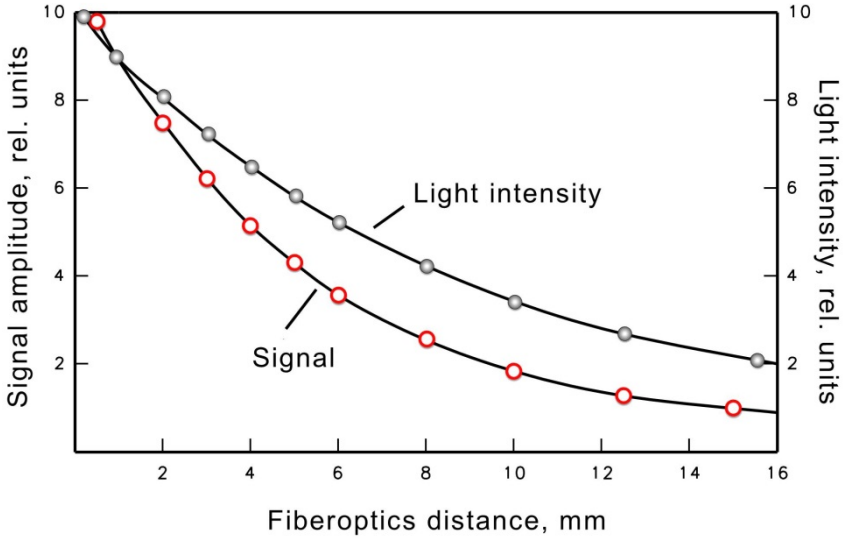


Fig. 13: Relationship between signal amplitude/light intensity and distance between fiberoptics tip and sample

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.

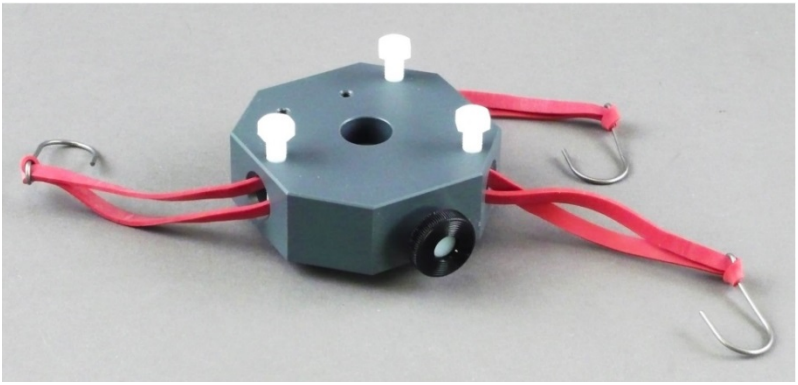


Fig. 14: Surface Holder DIVING-SH.

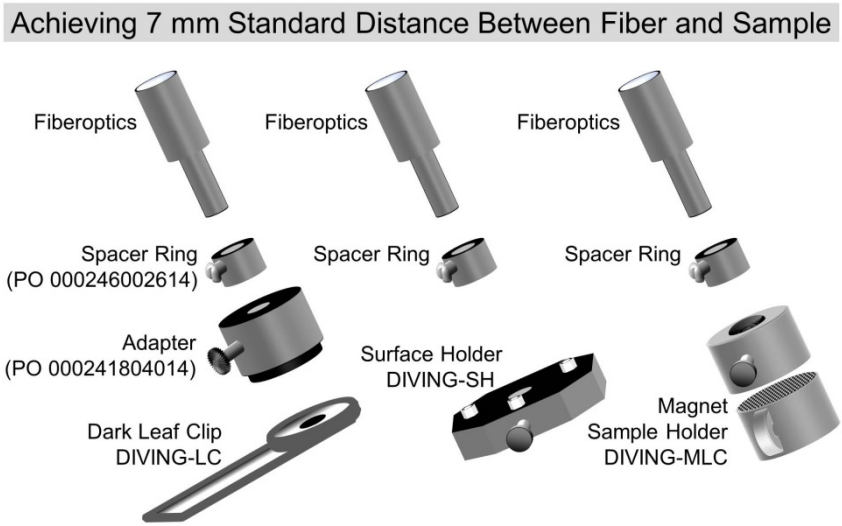


Fig. 15: Combinations of adapters and leaf clip to achieve the standard distance of 7 mm between fiberoptics end and sample level.

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 10). 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. 9 (page18) 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. 16, (page 25), connection to the DIVING-PAM-II is illustrated by Fig. 17 (page 26). The various modes of application are addressed in the following section.

3.2.2 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. 16, page 25). The clip can be opened by a handle with a single finger of the hand holding the device.

Fig. 16: Universal Sample Holder DIVING-II-USH.

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 or at the lower side of the clip. O-rings can be hooked into to 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. 16, page 25). In this way, the clip can be adapted for samples of various strength and thickness. The stripe-like contact area on the lateral

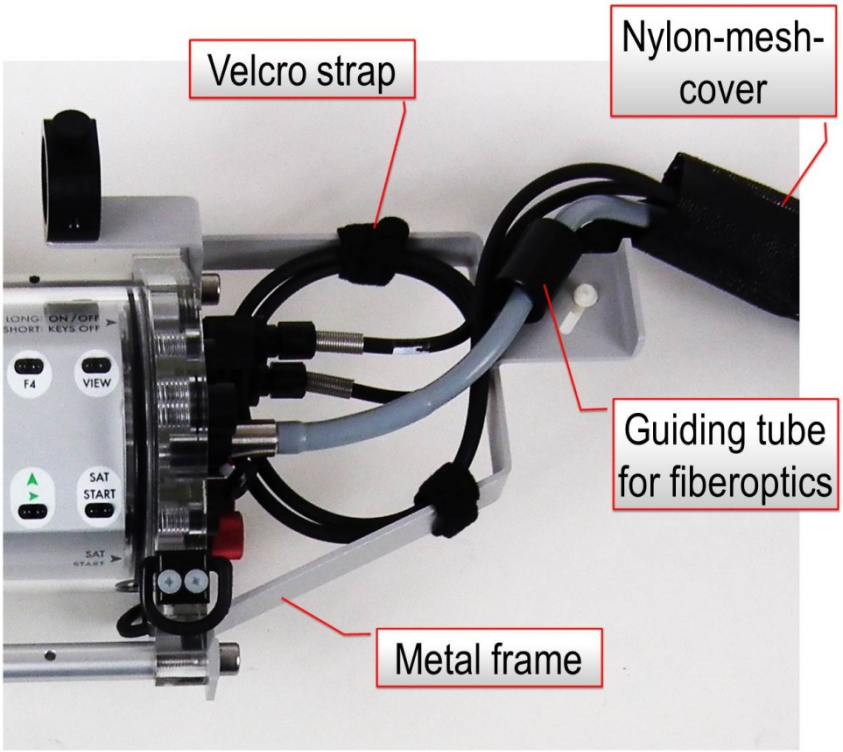


Fig. 17: DIVING-II-USH, arrangement of fiberoptics and cables.

clip edge is covered with neoprene rubber foam (bottom) and a plastic “pin-cushion” (top), 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 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. 15, page 23, “Adapter”). One side of this adapter is covered with neoprene rubber, making provision for a light-tight, gentle contact with the sample. In order 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.

3.2.3 Magnet Sample Holder DIVING-MLC

The Magnet Sample Holder is suited for the study of relatively large and robust leaf-like samples, as sea-grasses, kelp and other large macroalgae. It consists of two halves with ring magnets, between which a flat sample can be held. One half provides the adapter for the fiberoptics, whereas the other half serves the purpose of creating buoyancy, such that the holder will float in the water. The opening for the fiberoptics is covered by a split black rubber hood, such that the investigated sample area may dark adapt while the fiberoptics are not yet connected.

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

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

4 Stand-alone Operation

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

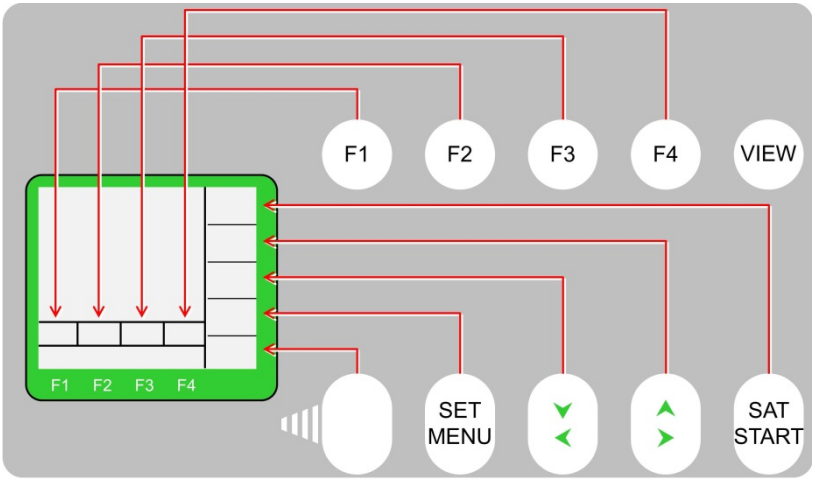


Fig. 18: **Connection between optical switches and display fields.** Note that VIEW is not linked to a display field but changes the appearance and opens navigation options of windows displaying graphical information (induction curve, light curve, spectra).

4.1 Top Level Windows

The DIVING-PAM-II provides various windows for control and data display. Frequently used commands, fluorescence data and the actinic light list (PAR list) are presented in ten top level windows (Table 3, page 30).

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. 30, page 48).

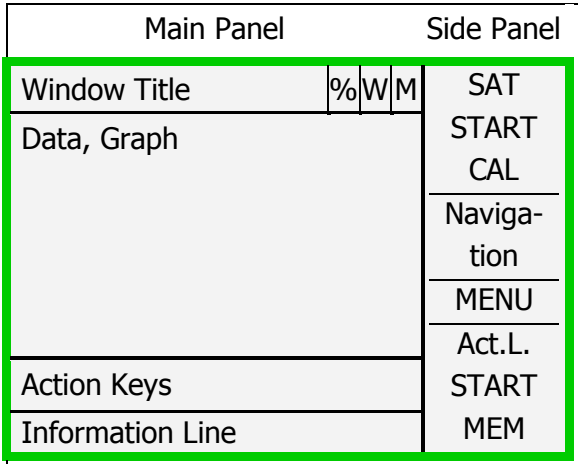
Table 3: Overview of Top Level Windows

	Window	Content
1.	Basic Data	Minimum data set and basic action keys for filed operation.
2.	Primary Data	Data of last saturation pulse analysis and current levels of fluorescence and additional measured parameters.
3.	Quenching Analysis	Fluorescence levels of last saturation pulse analysis and corresponding fluorescence ratios (Y(II), F_V/F_M, …).
4.	Ft-Chart	Trace of fluorescence, total x axis interval: 25 or 125 s (see Fig. 57, page 74).
5.	Spectrometer	Measurement and display of light, fluorescence and reflectance spectra.
6.	Actinic + Yield	Short illumination program with saturation pulse analysis.
7.	Induction Curve	Graphics of saturation pulse analysis of current curve.
8.	Light Curve	Graphics of saturation pulse analysis of current light response curve.
9.	Recovery Curve	Recovery graphics after induction curve and light response curve.
10	Actinic Light List	Set values of photosynthetically active radiation PAR in µmol m^{-2} s^{-1}.

Generally, all top level windows consist of a “main panel” and a “side panel” (Fig. 19, page 31). The top line of the main panel displays window title and three small fields which from left to right display:

- Charge status of internal battery in %. When connected to the mains, “ext” is displayed.
- The WLAN status, where “W” indicates that the WLAN interface of the DIVING-PAM is active. WLAN can be activated via the item “Wireless” in menu “DIVING-PAM-II Settings” (Fig. 57, page 74).
- Mark. A capital letter which is added as a mark to each saturation pulse analysis in the Report file.

The bottom of the main panel provides various action keys. Depending on window, these action keys trigger saturation pulse



Side Panel

The panel provides a button for saturation pulses (SAT) which is in some windows replaced by a start/stop button for automated experiments, or by a CAL button to start PAR calibration. Also, navigation keys, a “MENU” key and action keys can be 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. 19: Principal Screen Layout of Top Level Windows

analysis, control light conditions or affect graphic display. The “Information Line” at the bottom edge of the main panel shows current date and time.

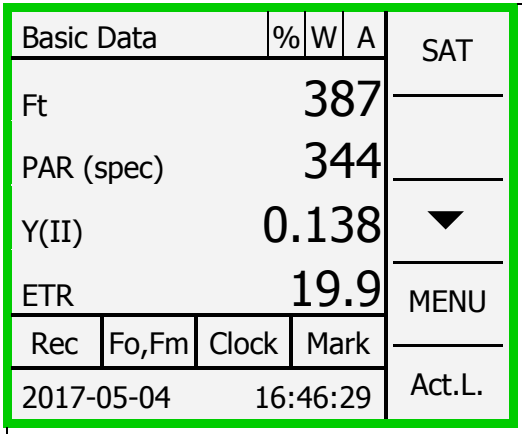
The side panel provides arrow keys to switch windows, control keys for fluorometer functions, and a (MENU) key to access the main menu. Control keys are different between windows. For instance, the uppermost button in the sidebar triggers saturation pulse analyses in case of the first five top level windows (cf. Table 3, page 30) but the same button starts automated experiments in windows Actinic + Yield, Induction Curve, Light Curve and Recovery Curve.

Sections 4.1.1 to 4.1.10 will introduce all ten top level windows in detail.

4.1.1 Basic Data

The Basic Data window (Fig. 20, page 33) displays a reduced set of data which allows fast sampling under field conditions. Of 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 Basic Data 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 the marker letter, use up and down keys in the window “New Marker”. The currently selected letter is shown on the top edge of the window. Se



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).

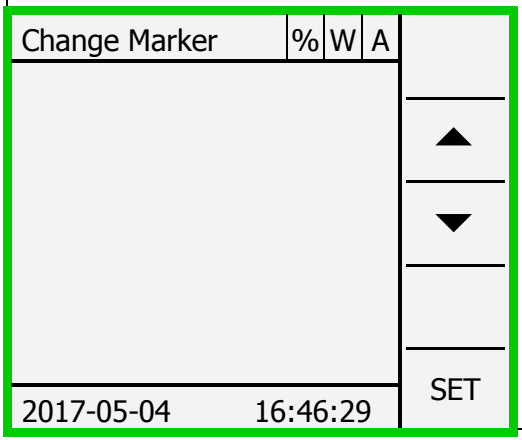
PAR (spec), PAR value (µmol·m^{-2}·s^{-1}) from miniature spectrometer. **PAR(ext1)**, **PAR(ext2)**, or **PAR(int)** is displayed if external sensor 1 or 2 is active, or when the internal PAR sensor is selected in Main Menu→Sensors.

Y(II), effective photochemical quantum yield of PS II; **F_V/F_M** is displayed after ing 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 determinations, to start repetitive triggering of an event, and marker of data.

Select Mark open the window below



Side Panel

▲ , go to previous character in alphabet.

▼ , go to next character in alphabet.

SET, accept current letter and return to Basic Data.

Fig. 20: Basic Data and Change Marker

lecting SET key confirms 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. The MENU key leads to the Main Menu window as in all other top level windows. Finally, a downward arrow key switches to the next window. Two arrow keys (up and down) are present in all other top level windows except the last (Actinic Light List; Table 3, page 30) 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. Without initial Fo,Fm, a SAT command in the new Record will only yield data of Y(II). In addition, q_P, and q_L will be calculated provided that the Fo' Mode is active. All parameters requiring F_0 and F_M (see Table 7, page 84) cannot be calculated.

If the new Record includes an Fo,Fm command, the F_0' can be also be derived from F_0, F_M and F_M' (Section 5.1.2, page 82). Hence, all parameters of Table 7 can be calculated with the Fo' Mode being active.

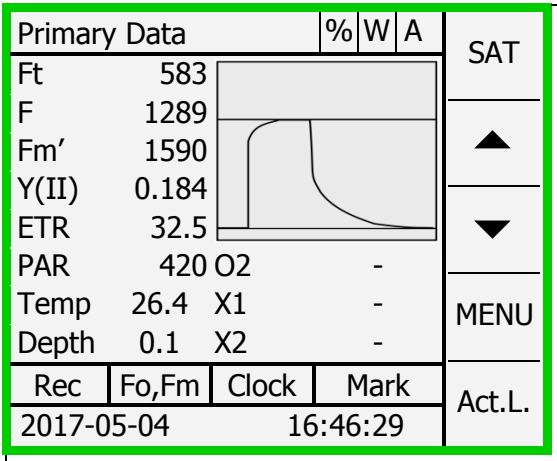
If a Record holds more than one F_0, F_M determination, the last one will be used to calculate fluorescence coefficients. Such calculations, however, are only valid when measurements of Fo,Fm and SAT are carried out with the same sample.

Sometimes, Fo,Fm and SAT measurements with different samples even result in fluorescence ratio parameters that exceed their valid range (compare Table 7, page 84). These invalid data will be displayed in grey on the screen.

4.1.2 Primary Data

The central feature of the Primary Data window is a monitoring graph displaying the fluorescence trace during and right after 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 selected) or the F and F_M' signal levels (after SAT was selected). Ad-



Side Panel

Same as Basic Data window (Fig. 20, page 33) plus upward arrow key.

Main Panel

Ft, current fluorescence level

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

Insert, fluorescence trace during last saturation pulse.

PAR, Temp, and **Depth**, photosynthetic active radiation in $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature in °C, and diving depth in m, respectively.

O2, **X1**, **X2**, data measured by optional oxygen and auxiliary sensors.

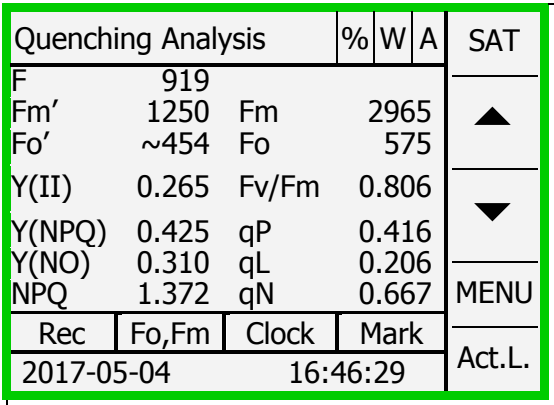
Rec, Fo,Fm, Clock, Mark: see Fig. 20, page 33.

Fig. 21: Primary Data

ditionally, temperature, °C (Temp) and relative humidity, % (Hum) as measured by the MINI-PAM-II leaf clip (2035-B) are displayed (connectable via adapter to the DIVING-PAM-II). Also, data from an optional optode oxygen sensor (O2) and two more auxiliary sensors (X1 and X2) can be displayed.

4.1.3 Quenching Analysis

The window provides an overview on fluorescence levels and 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. 20, page 33) plus upward arrow key.

Main Panel

Numerical data: see Fig. 61, page 81 and Table 7, page 84.

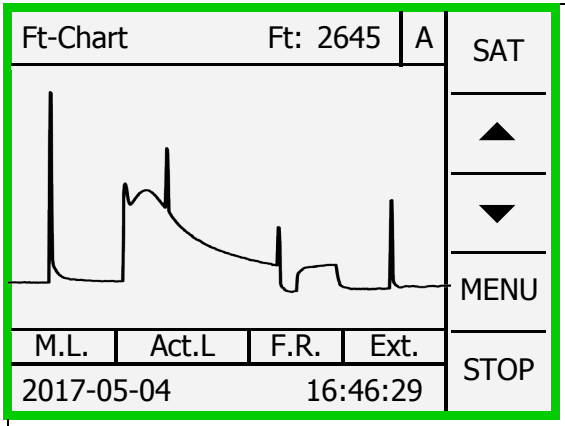
Rec, Fo,Fm, Clock, Mark: see Fig. 20, page 33.

Fig. 22: 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. 57, page 74).

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 but Act.L. replaced by STOP/RUN.

Main Panel

Graph. Trace of current fluorescence measurement. Total X axis: 25 or 125 s: see Fig. 57, page 74.

M.L, **Act.L** , **F.R.** , **Ext.**. On/off switches for measuring light, actinic light, far red light and external (synchronized) light source, respectively.

Fig. 23: Ft Chart

4.1.5 Spectrometer

The SPEC command of the Spectrometer window triggers reading data from the spectrometer. In the standard mode, the

spectrometer is equipped with an optical entrance for external radiation (Fig. 8, page 17). Spectra of photon flux densities are measured in units of µmol photons m^{-2} s^{-1} nm^{-1}. PAR corresponds to the integral of the spectrum from 400 to 700 nm. Spectrometer-derived PAR will be used for ETR calculations if "Use Ext. PAR" in Menu Sensor Settings is checked (available by selecting "Sensors" in the Main Manu). Also, make sure that the spectrometer is selected as external PAR source (Main Menu → Sensor Settings → Leaf Clip/Ext PAR Sett → External PAR sensor).

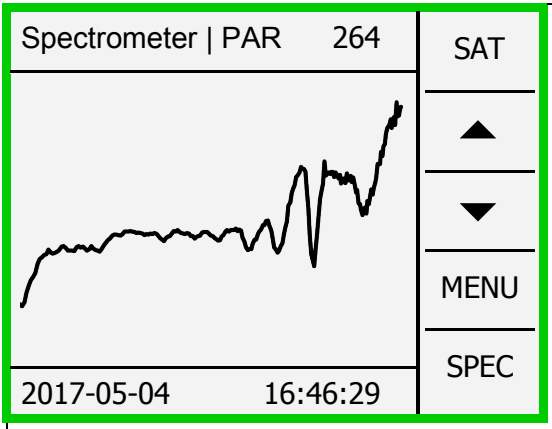
Note that the spectrometer measures the PAR of the DIVING-PAM-II internal light correctly only in the CAL mode (Fig. 29, page 48). Only in the CAL mode the LEDs are operated continuously. In the measuring mode, actinic light is pulse-width modulated and fluctuating PAR is displayed.

To record fluorescence or reflectance spectra, change spectrometer configuration as described in (Fig. 8, page 17). Then select operation mode: Main Menu → Sensor → Spectrometer → Operation Mode.

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

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

In the Spectrometer window, touching the VIEW key displays 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. Another VIEW command returns to the original function of arrow keys.



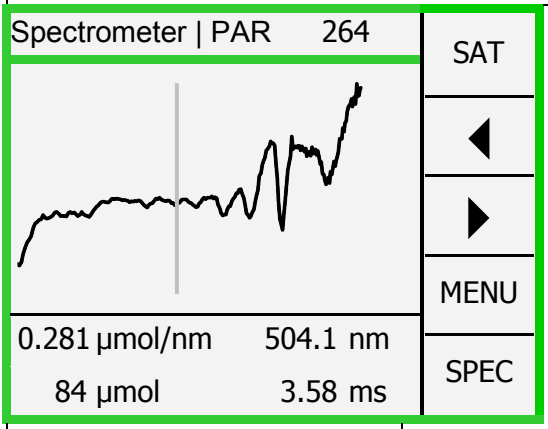
Side Panel

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

Main Panel

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

Press VIEW KEY to open/close the window below.



Side Panel

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

Main Panel

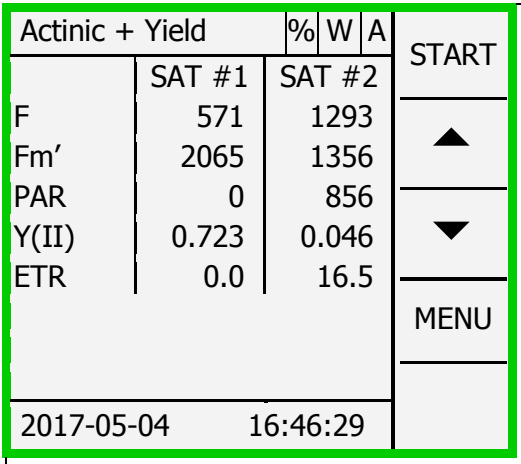
Spectrum and its numerical values.

Fig. 24: Spectrometer

4.1.6 Actinic + Yield

The “Actinic + Yield” window is the first of four windows designed for starting and monitoring automated measuring routines. The parameters of the Actinic + Yield routine can be set in the menu “Program/Clock” (Fig. 36, page 57). 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 light response of photosynthesis needs to be compared 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

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

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

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

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

Fig. 25: Actinic + Yield

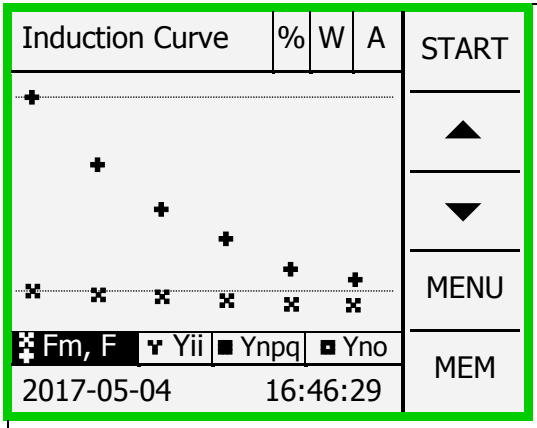
4.1.7 Induction Curve

This window permits starting and aborting fluorescence induction curves (Fig. 26, page 42). The graphics panel provides a qualitative picture of induction curve properties. Because of the given screen resolution, it is difficult to quantitatively evaluate graphical data. Therefore, the Induction Curve window also provides numerical data of fluorescence ratio parameters and fluorescence levels.

These numerical data appear in response to selecting the graphics panel. Navigation keys permit passing from one saturation pulse analysis to another. The position in the induction curve of the current set of numerical data is indicated by a grey vertical line.

The parameters of an induction curve experiment, like number of saturation pulse analyses and interval between them, can be adjusted in the menu “Induction Curve Settings” (Fig. 39, page 60) which is available over the Program/Clock line of the Main Menu (Fig. 30, page 48). In the “Induction Curve Settings” menu, a fluorescence recovery experiment (in the absence of actinic illumination) can be placed right after an induction curve.

The MEM of the Induction Curve window opens the “Induction Curve Memory” window. In this window, the navigation keys permit scrolling through stored induction curves.



Side Panel

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

▲, ▼ .Go to previous, next window.

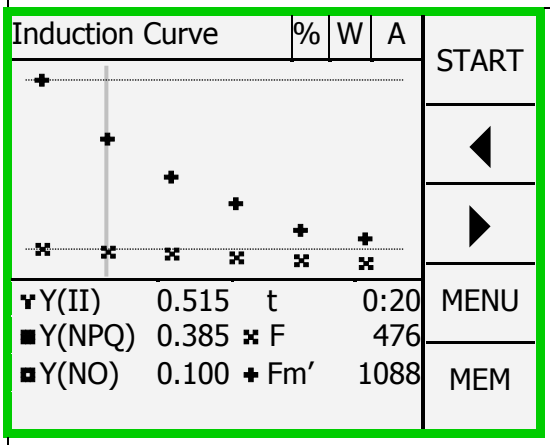
MENU. Go to Main Menu.

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

Main Panel

Graphics panel and buttons Fm, F, Yii, Ynpq, Yno to control display of data (F_M,F_M' plus F, Y(II), Y(NPQ) and Y(NO)). The Y(NPQ) and Y(NO) can be exchanged by parameters NPQ and qL in the menu Program/Clock Settings (Fig. 36, page 57).

Press VIEW KEY to open/close the window below.



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. 26: Induction Curve

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. 27, page 44). Also, the Light Curve window provides numerical data of saturation pulse analysis which can be accessed by pressing the VIEW key. Navigation between different saturation pulses analyses and selection of displayed data works as described for the previous window (Induction Curve).

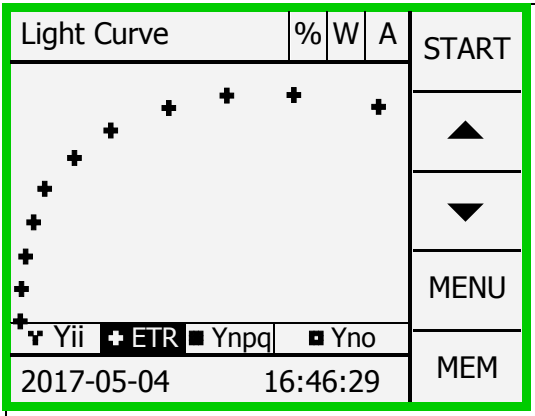
Light curve parameters (number and duration of light steps, initial PAR, and recovery curve) can be adjusted in the menu “Light Curve Settings” (**Fig. 40**, page 60). Selecting the MEM button and scrolling using the arrow keys on the side bar permits viewing stored light curves.

4.1.9 Recovery

Fluorescence recovery experiments can be automatically appended to an induction or a light curve (see item Program/Clock of the Main Menu; Fig. 30, page 48). When selected, the recovery curve is started automatically. Otherwise, recovery curves can be initiated (and cancelled) manually using the START/STOP button on the side panel of the Recovery window (see Fig. 28, page 45).

As in previous Induction and Light Curve windows, recovery data are represented graphically and numerically. The time course of recovery curves is determined: 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 recov-

ery. The interval between neighboring saturation pulse analysis roughly doubles with time (Table 4).



Side Panel

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

▲, ▼. Go to previous, next window.

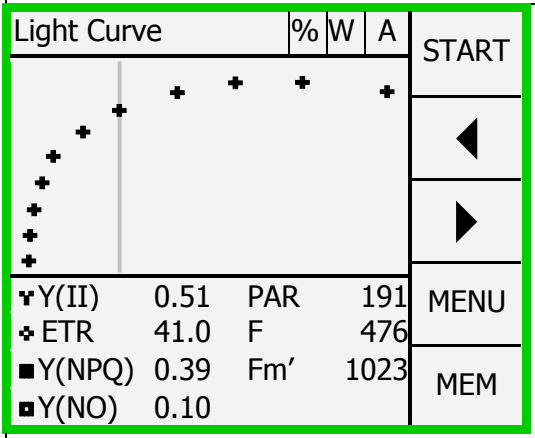
MENU. Go to Main Menu.

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 (Fig. 36, page 57).

Press VIEW KEY to open/close the window below.



Side Panel

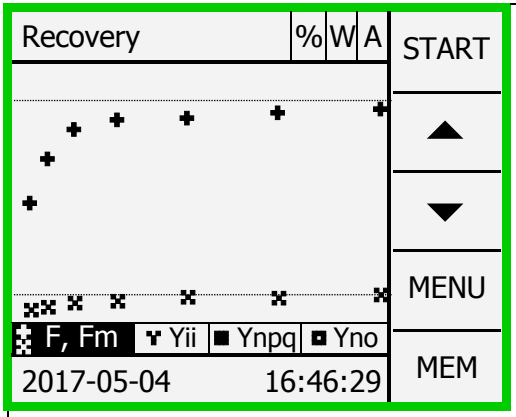
As above except arrow keys.

◀, ▶. 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. 27: Light Curve



Side Panel

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

▲, ▼ .Go to previous, next window.

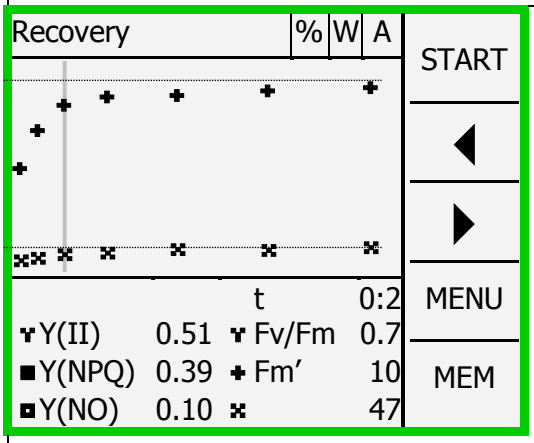
MENU. Go to Main Menu.

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

Main Panel

Graphics panel and control buttons as described in Fig. 26, page 42.

Press VIEW KEY to open/close the window below.



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. 28: Recovery

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

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

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

4.1.10 Actinic Light List

The window "Actinic Light List" contains PAR values (µmol m^{-2} s^{-1}) which increase roughly exponentially with intensity settings. In the factory, blue or red LED emission is 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.)

The CAL command

The DIVING-PAM-II has an internal PAR sensor which is exposed to a small and constant fraction of LED emission. In the factory, this internal sensor is adjusted so that it displays the

same PAR values as a calibrated cosine-response PAR sensor at sample level of a Universal Sample Holder DIVING-II-USH.

The CAL command varies LED emission until for each actinic intensity stetting (1 to 12) the readout of the internal PAR sensor matches the corresponding value of the light list. Therefore, after carrying out CAL, the PAR values of the light list apply to the sample level of a Universal Sample Holder DIVING-II-USH 2035-B leaf clip, and devices with similar geometry. This way the CAL command can compensate reduced light transmission of older fiber optics or intensity losses of LEDs used for long periods. Note that the entire PAR list can be multiplied by the internal actinic factor ("Int. Act. Factor, Fig. 34, page 54). In this case, the CAL command adjusts light intensities to the modified PAR values.

Various measuring configurations

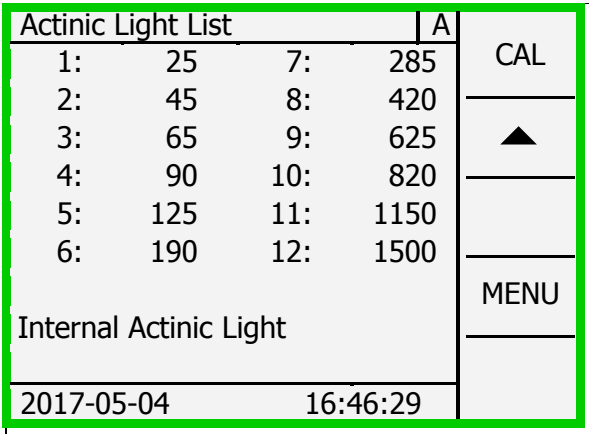
The use of the CAL command for other than factory configuration requires new adjustment of the internal sensor. For this purpose, a calibrated PAR sensor is positioned at sample level of the selected measuring setup and the internal PAR sensor is adjusted as described in Section 4.2.4.1, page 62 (see also Fig. 9, page 18). In short:

Using a calibrated PAR sensor which is not connectable to the DIVING-PAM-II requires manual adjustment. This is done by switching actinic light on and varying in the window "Int. PAR Sensor Settings" (Fig. 43, page 63) the "Calibration Factor" until internal PAR readout matches that of the external sensor.

If the calibrated PAR sensor feeds its data in the DIVING-PAM-II (e.g. the MINI-SPEC spectrometer of 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 Set-

tings" (Main Manu → Sensors → Internal PAR) and start automatic adjustment of calibration factor.

To calibrate the internal sensor with the MINI-SPEC spectrometer, use the PAR calibration block (Fig. 9, page 18).



Side Panel

CAL. Calibrate Actinic Light List.

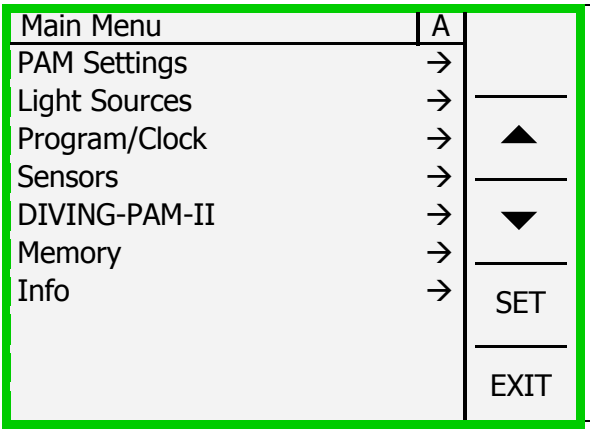
▲ Go to previous window.

MENU, go to Main Menu.

Main Panel

PAR target values (µmol m^{-2} s^{-1}) of the 12 settings for actinic light intensity.
The PAR target values are valid for an actinic light factor of 1.00 (see menu "Light Sources" Fig. 34, page 54).

Fig. 29: Actinic Light List



Side Panel

▲ , ▼ . Up/down keys move curser.

SET. Open submenu of selected line.

EXIT. Return to previous (top level) window.

Fig. 30: Main Menu

4.2 Main Menu

The “Main Menu” (Fig. 30, page 48) forms the central hub to access settings, calibration data, hardware information and the memory of the DIVING-PAM-II. The Main Menu consists of seven items (Fig. 30, page 48). Selection of lines in the Main Menu is achieved by the arrow keys of the side panel. Most of these items lead to submenus that itself link to lower-level menus.

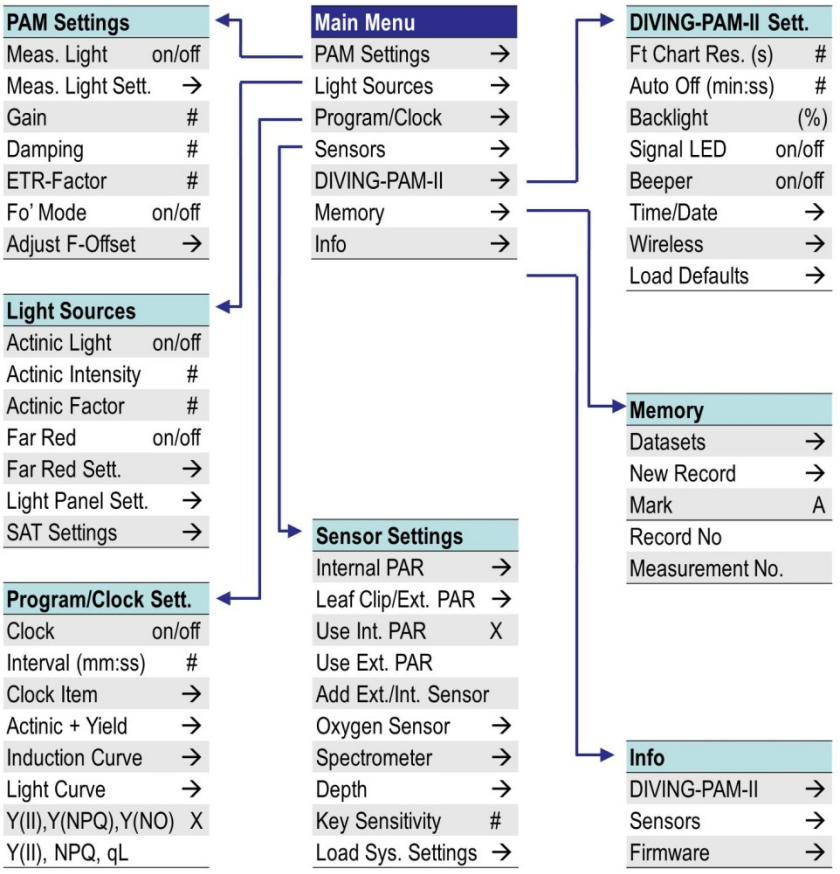
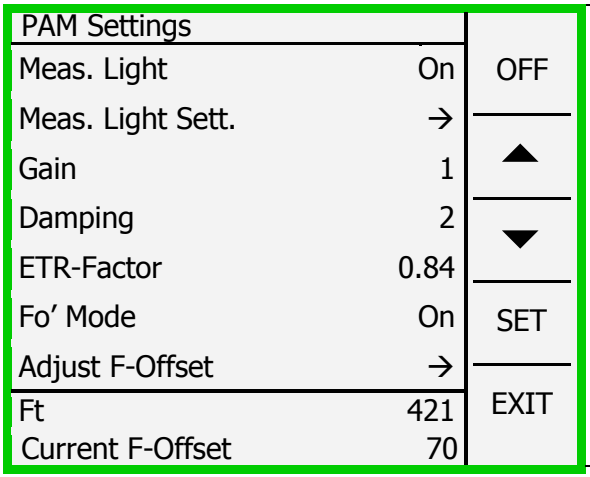


Fig. 31: Main Menu with all seven submenus

4.2.1 PAM Settings



Side Panel

ON/OFF. Switch for measuring light.

▲, ▼ . Up/down keys move curser.

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

EXIT. Return to Main Menu.

Fig. 32: PAM Settings

"PAM Settings" include adjustments of the way how the DIVING-PAM-II acquires PAM fluorescence. The menu contains seven selectable items (Fig. 32, page 50), the window also displays the current Ft value and the currently active offset which is automatically subtracted from the raw fluorescence.

Meas. Light

On/Off switch for measuring light (weak excitation light consisting of µs pulses). Measuring light can be switched on by selecting "Meas. Light" and selecting SET.

Meas. Light Sett.

This command opens the menu "Measuring Light Settings" in which measuring light intensity (Meas. Light Int.) can be selected by the SET key and then adjusted using the arrow keys. At

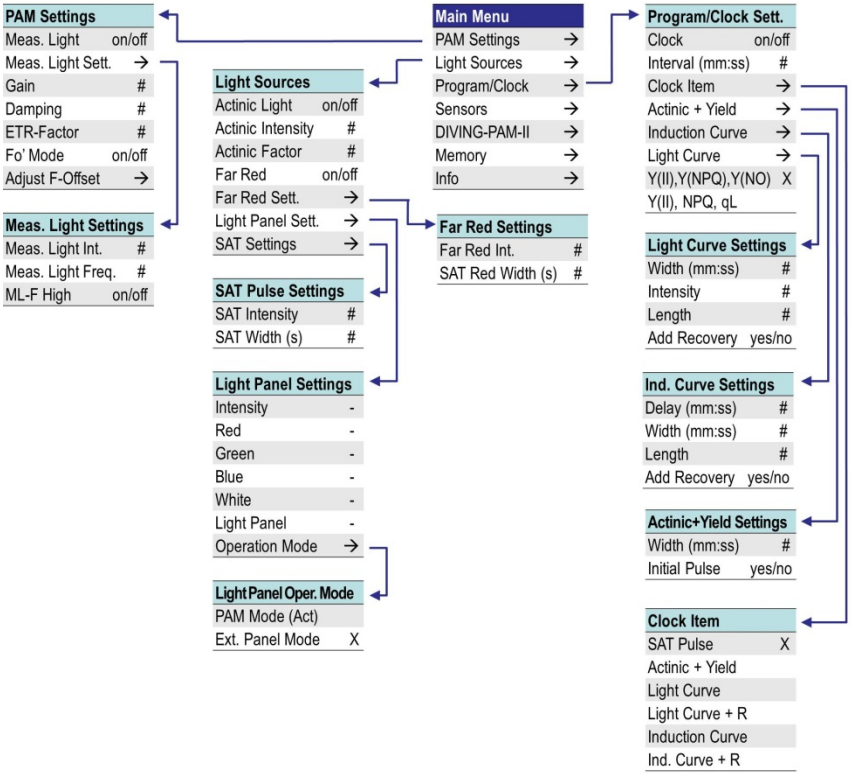


Fig. 33: Submenus PAM Settings, Light Sources, and Program/Clock

constant frequency, measuring light intensity can be considered as proportional to intensity settings 1 to 12.

The two remaining items of the menu “Measuring Light Settings” concern measuring light frequency. Selecting the line “Meas. Light. Freq.” by SET allows choosing between 5 frequency levels using arrow keys (see Table 5 below).

The highest measuring light frequency of 100 Hz can be activated by selecting by the SET key the third line in the menu (ML-F High). The ML-F High command overrules settings made under “Meas. Light. Freq.” High measuring light frequency improves

signal quality but bears the risk that its higher intensity drives photosynthesis, that is, the measuring light becomes actinic. In this case, F_0 measurements may be wrong.

Generally, measuring light frequency is automatically switched to high during a saturation pulse, that is, for F_M and F_M' determinations. Measuring light frequency does not affect the frequency of Ft data acquisition by the WinControl-3 software.

Table 5: Measuring Light Frequencies

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

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:

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

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

Gain

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

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.

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 5.3, page 88).

Fo’ Mode

The “Fo’ Mode” replaces after saturation pulses actinic by far red light to quickly open PS II reaction centers (cf. Section 5.1.2, page 82). The measured F_0’ is the minimum Ft during far red illumination. Interval and intensity of far red illumination can be adjusted in menu “Light Sources” (Fig. 34, page 54).

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. 32, page 50).

Procedure

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

4.2.2 Light Sources

(Fig. 34, page 54)

Actinic Light

On/off switch for actinic light.

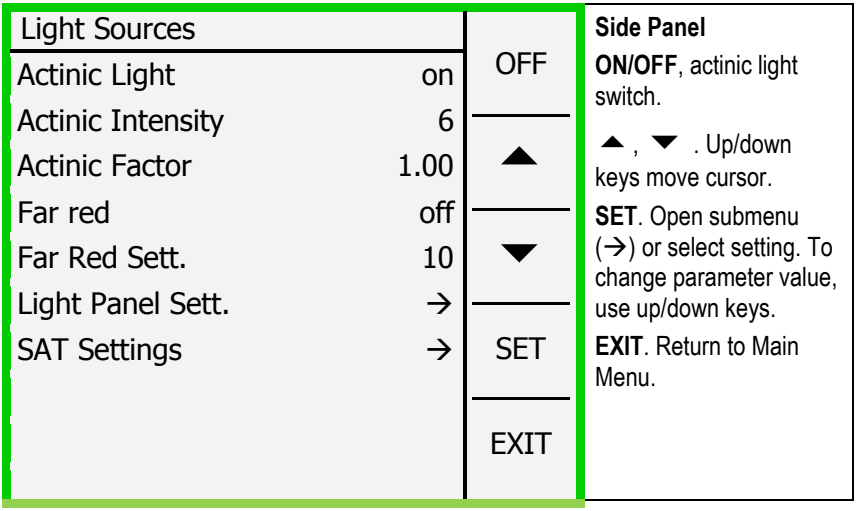


Fig. 34: Light Sources

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. 29, line 48).

Actinic Factor

Factor multiplying target PAR values in window "Actinic Light List". Factor range is 0.5 to 2.0.

Far Red

On/off switch for far red light.

Far Red Sett.

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

Light Panel Sett.

Use SET to enter submenu "Light Panel Settings" (see below). Each LED type of the light panel is independently adjustable between 0 and 100%. The effective relative intensity depends on setting Intensity (Ext. Actinic Int.).

"Operation Mode" enters a submenu consisting of two items: "PAM Mode (Act.)" and "Ext. Panel Mode". The terms are counter-intuitive: The "PAM Mode (Act.)" means that actinic light is provided by an external source like the 2054-L External LED Source (MINI-PAM-II accessory) which under dry lab conditions can be operated by the DIVING-PAM-II using a special adapter. "Ext. Panel Mode" means that the DIVING-PAM internal light is used as actinic light to which an external light source may be added. The Ext. Panel Mode is the default setting for the DIVING-PAM-II.

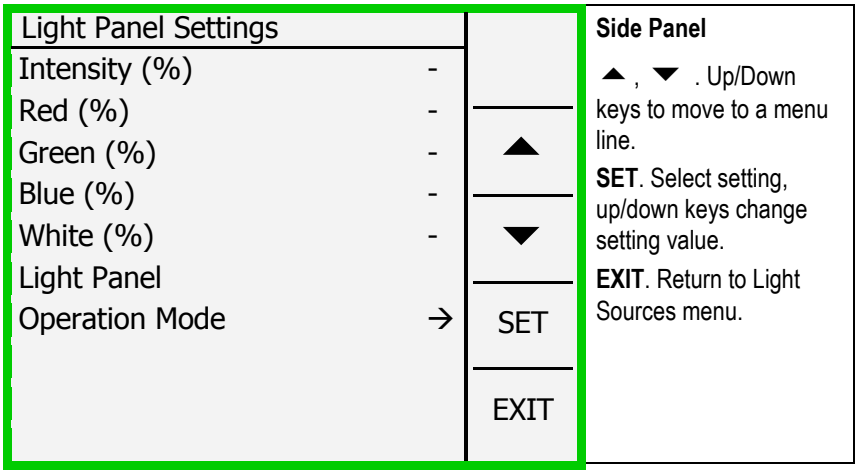


Fig. 35: Light Panel Settings

SAT Settings

"SAT Settings" and SET opens the submenu "SAT Pulse Settings" 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, intensity setting 12 corresponds to 6000 µmol m^{-2} s^{-1} under standard geometrical conditions (distance fiber optics tip to sample level: 7.5 mm; angle between end piece of fiber optics and sample level: 60°). Saturation pulse intensity can be adjusted at increments of 500 µmol m^{-2} s^{-1}.

4.2.3 Program/Clock Settings

The menu "Program/Clock Settings (Fig. 36, page 57) provides all options to configure automated experimental routines including Actinic + Yield, Induction Curve and Light Curve experiments as well as repetitive triggering of single measurements and experimental protocols.

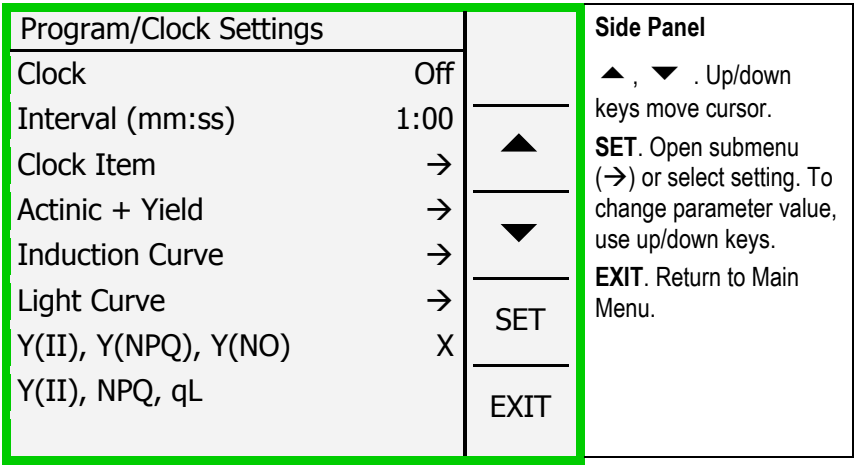


Fig. 36: Program/Clock Settings

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”.

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).

Clock Item

Saturation pulse analyses and the programs Actinic + Yield, Induction Curve and Light Curve can be repetitively performed under clock control. Also, recovery experiments can be performed after induction and light curves (item Light Curve + R and Induction Curve + R, respectively). To select one of the six items in menu “Clock Item” (Fig. 37), move cursor to the item of interest

and select SET. The selected item is then marked by an X ("SAT pulse is selected in Fig. 37).

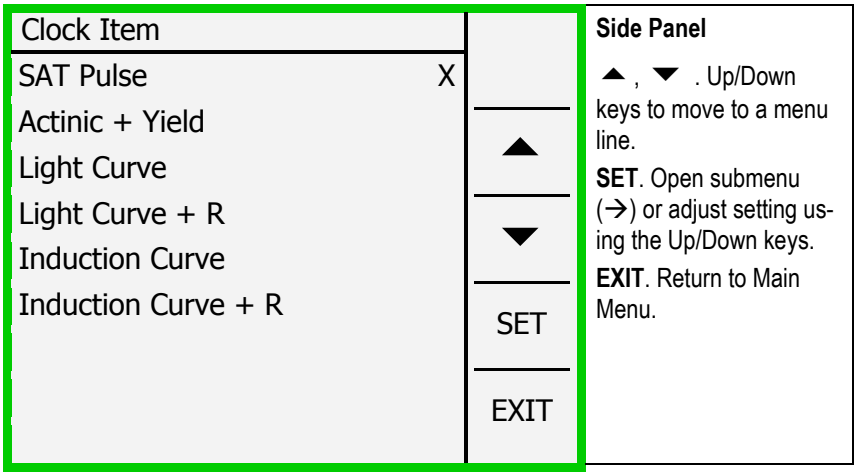


Fig. 37: Clock Item

Actinic + Yield

The behavior of the Actinic + Yield program is defined by two factors (Fig. 38, page 59): the duration (width) of actinic illumination (possible settings from 5 s to 5 min) and the option to start actinic illumination without preceding saturation pulse analysis (Initial pulse). Width of actinic illumination is adjusted as described above for clock interval and initial pulse is selected by the SET command. Further, actinic light intensity is adjusted in the window "Light Sources" (Fig. 34, page 54).

An Actinic + Yield routine can be started using the START button on the side panel of Fig. 38. In this case, the screen display will automatically switch to the Actinic + Yield experimental window (Fig. 25, page 40).

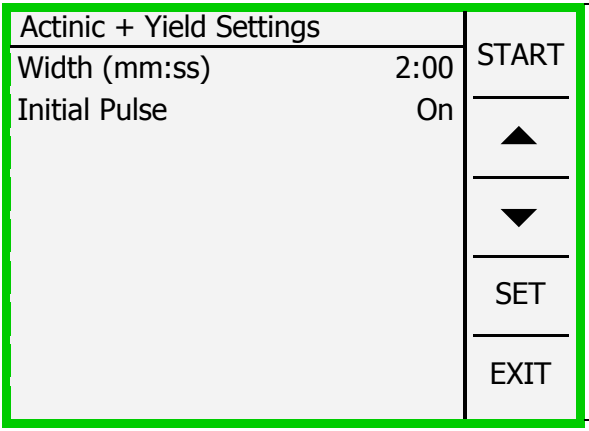


Fig. 38: Actinic + Yield Settings

Induction Curve

Induction curve experiments are configured in the window "Induction Curve Settings" (Fig. 39, page 60).

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, Section 5.1.1, page 82) and beginning of actinic illumination.

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

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

Add recovery appends a recovery curve to an induction curve (see Section 4.1.9, page 43 for information on recovery times).

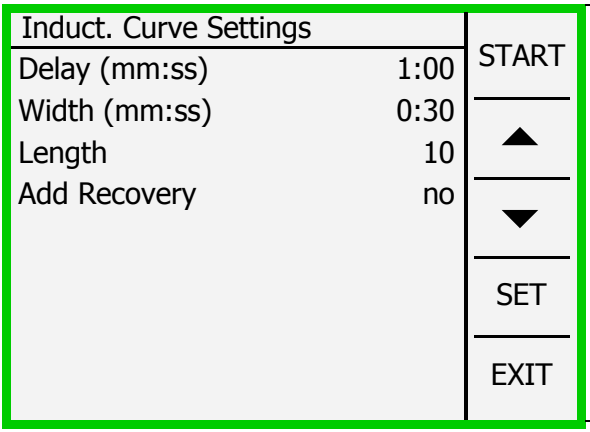


Fig. 39: Induction Curve Settings

Light Curve

Light Curves are defined in “Light Curve Settings” (Fig. 40, page 60). 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. 29, page 48).

Length is the number of light steps which can range from 2 to 12.

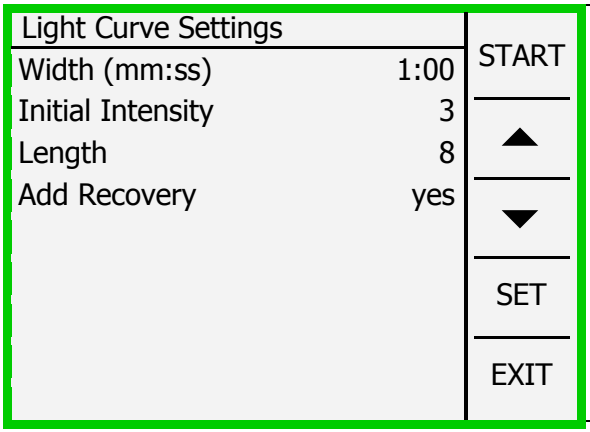


Fig. 40: Light Curve Settings

If length = 5 and intensity = 2, 5 light steps with intensity settings 2, 3, 4, 5, and 6 will be performed. The time required for a light curve results from Length times Width.

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

The last two lines of the menu "Program/Clock Settings" affect graphical and numerical presentation of data in windows Induction Curve, Light Curve, and Recovery Curve. Specifically, selecting "Y(II), Y(NPQ) Y(NO)" displays three yield parameters that are used in analyzing energy partitioning. Choosing Y(II), NPQ, and qL displays the classical NPQ parameter and a parameter for indicating the reduction state of PS II (q_L). Selection between lines works as described for "Clock Item" (Fig. 37, page 58). See Table 7, page 84 for definitions of fluorescence parameters.

4.2.4 Sensors

Selection of "Sensors" in the Main Menu opens the window "Sensor Settings" (Fig. 41, page 61). This window includes six links leading to submenus (distinguished by →) and it allows to select for light measurements the DIVING-PAM-II internal PAR

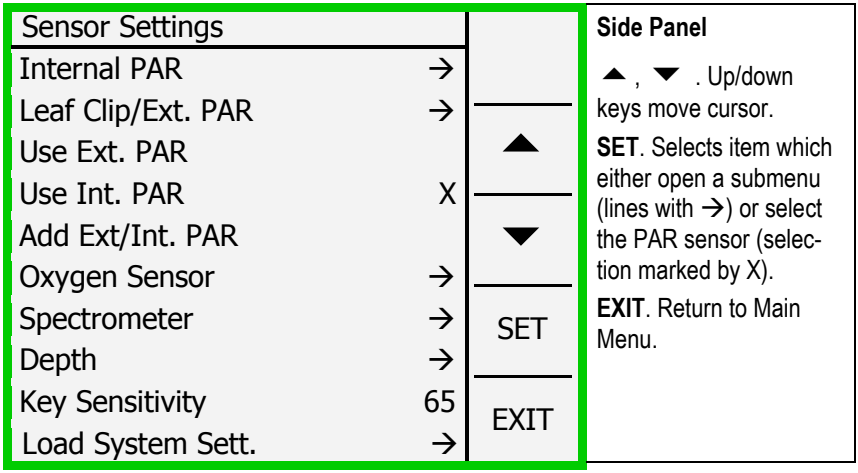


Fig. 41: Sensor Settings

sensor or the external sensor of the 2035-B leaf clip (Use Ext. PAR/Use Int. PAR). The currently active mode is marked by an X, to change selection, move cursor to the other sensor and select SET.

4.2.4.1 Internal PAR

Selecting “Internal PAR” opens the window “Internal PAR Sensor Settings” (Fig. 43, page 63). This window is dedicated for calibration of the DIVING-PAM-II internal PAR sensor which receives continuously a small fraction of internal actinic light. The window displays the current “Calibration Factor”, as well as the readouts (µmol m^{-2} s^{-1}) of internal and external PAR.

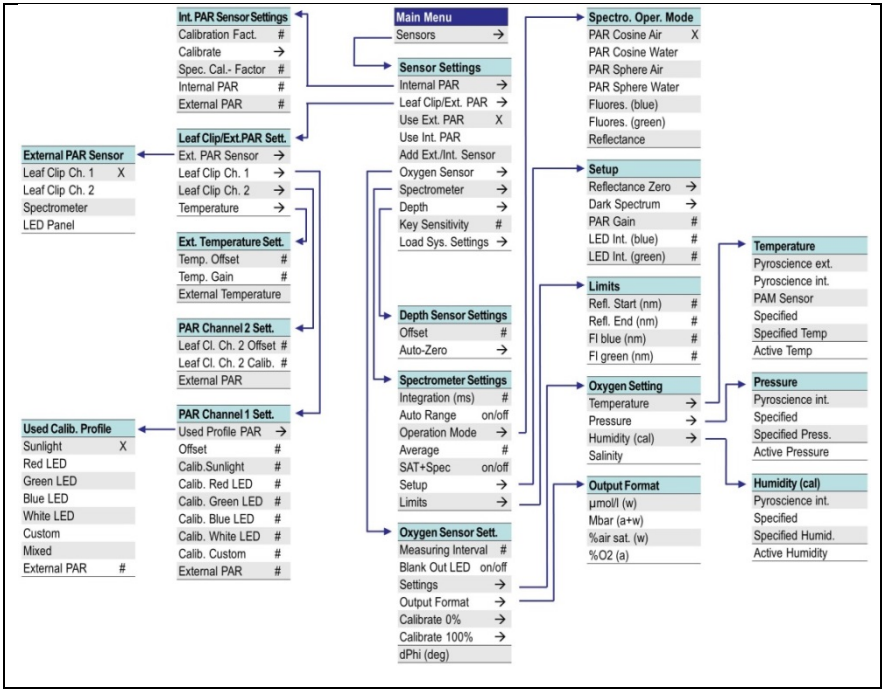


Fig. 42: Sensor Settings Menu and its Submenus

The specific calibration factor (Spec. Cal-Factor) corrects for the effect of inhomogeneous illumination by actinic light of the diffus-

ing disk of the miniature spectrometer in the PAR block arrangement (Fig. 9, page 18). The specific calibration factor is established at factory and automatically considered when the internal sensor is calibrated against the miniature spectrometer.
The specific calibration factor is ignored when a 2035-B leaf clip is used for calibration and the leaf clip is registered in Window (Fig. 45, page 64). The external sensor calibration for blue and red light will automatically be used for DIVING-PAM/B and DIVING-PAM-II/R fluorometers, respectively.

Proper calibration of internal sensor is apparent from same readouts of internal and external PAR. If calibration of the internal PAR sensor has previously been carried out but the two PAR values differ clearly, check if:

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

(2) Setup of previous calibration was different from the current one, for instance, in the previous calibration the external PAR sensor was not at standard distance from fiber tip.

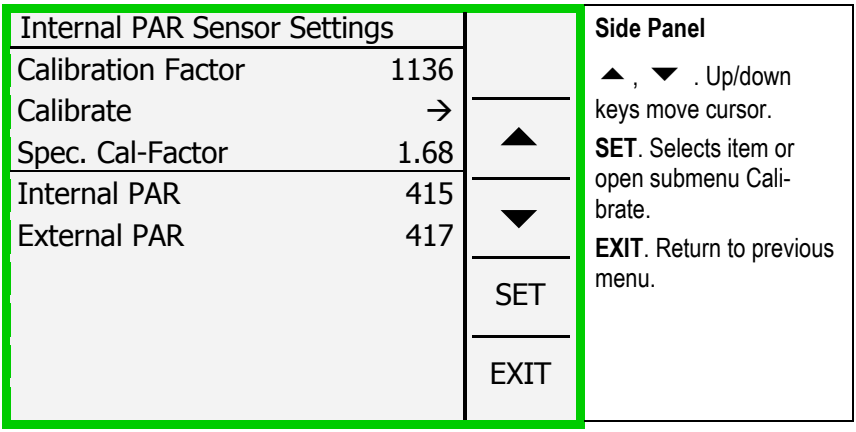


Fig. 43: Internal PAR Sensor Settings

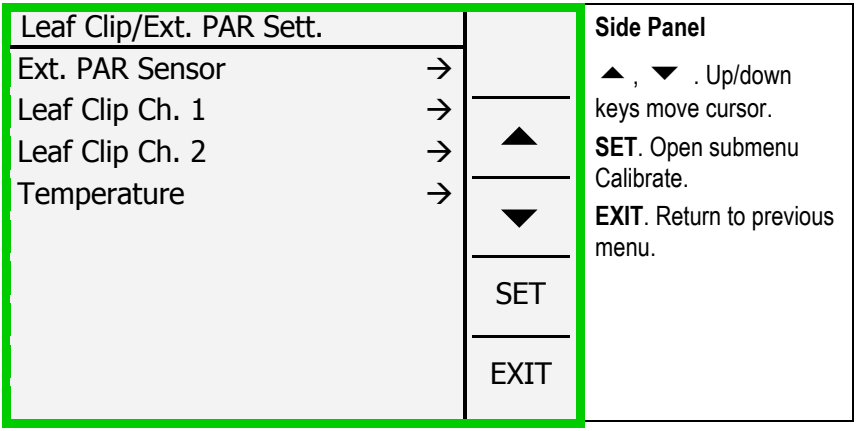


Fig. 44: External PAR Sensor

(3) External PAR sensor is connected or incorrect PAR channel is selected in window “External PAR Sensor” (Fig. 45, page 64).

PAR Sensor compatible with DIVING-PAM-II: To calibrate the internal PAR sensor using an external PAR sensor connected to the DIVING-PAM-II (e.g. miniature spectrometer in PAR block): go to “Calibrate” and establish calibration factor automatically.

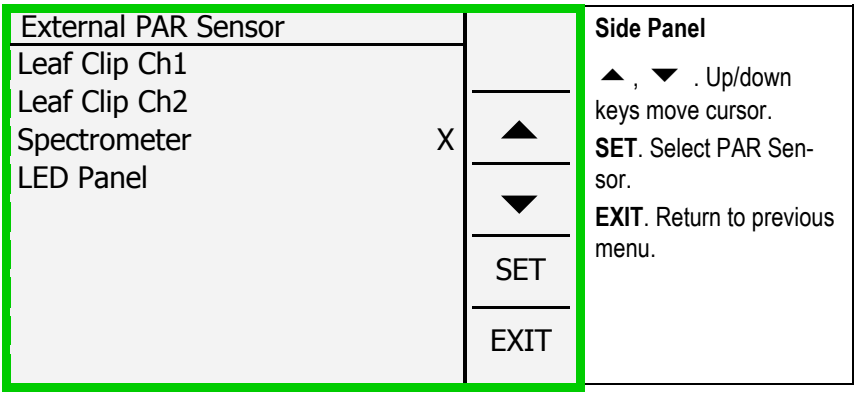


Fig. 45: External PAR Sensor

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.

4.2.4.2 Leaf Clip/Ext. PAR

Selecting “Leaf Clip/Ext. PAR” opens the window “Leaf Clip/Ext. PAR Sett” (Fig. 44, page 64). The menu considers that various PAR sensors can be connected to the DIVING.PAM.II including the 2035-B leaf clip via a special adapter. The window provides four items:

Ext PAR Sensor opens a menu that selects the active external PAR channel (Fig. 45, page 64). Four possible PAR sensors are considered: PAR sensors 1 and 2 of 2035-B leaf clip, the miniature spectrometer, and a PAR sensor connected to the LED-Panel RGBW-L084. In the normal configuration of the DIVING-PAM-II, Spectrometer is selected.

Leaf Clip Ch. 1 opens “Leaf Clip Channel 1 Settings” which con-

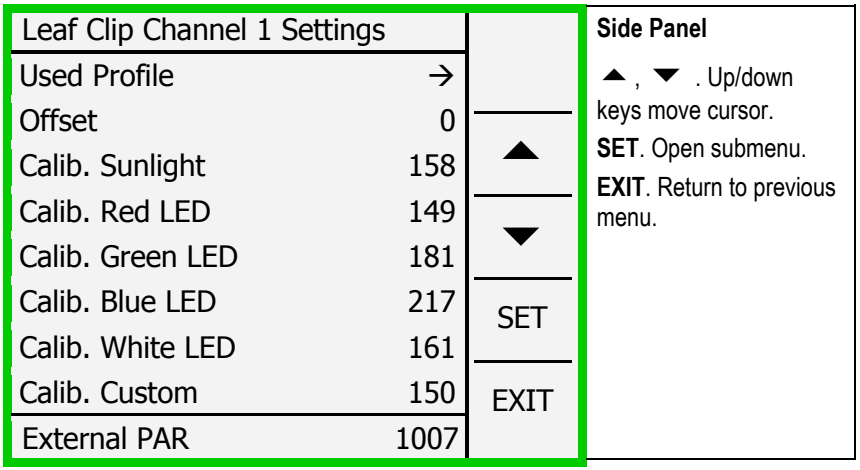


Fig. 46: Leaf Clip Channel 1 settings

tains 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. 46) 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 window "Leaf Clip/Ext. PAR" opens another menu (Used Cal. Profile, Fig. 47, page 66) 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.

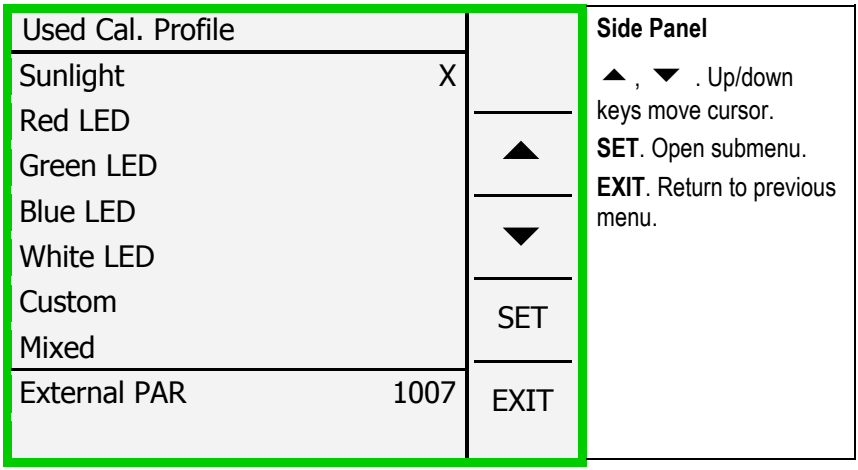


Fig. 47: Used Cal. Profile

The profile “Mixed” is particular as it adjust the calibration factor dynamically depending on the contributions of the red, green blue and white LED of the External LED Light Source 2054-L (compatible with DIVING-PAM-II with special adapter for dry lab use).

Leaf Clip Ch. 2 in window “Leaf Clip/Ext. PAR Sett” (Fig. 44, page 64) opens the calibration menu for a PAR sensor connect to the SMA socket on the side of the 2035-B leaf clip. This menu simply consists of slope (Calib,) and offset of the calibration line are given. In the lowest line, the PAR readout of the active PAR sensor is shown.

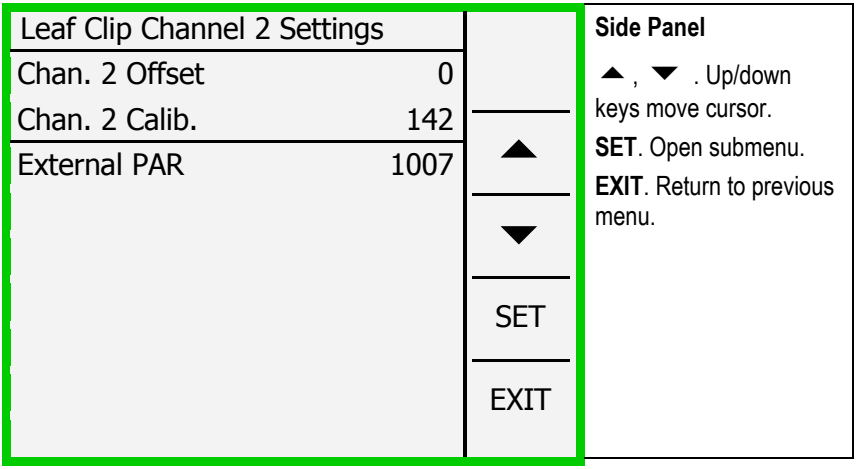


Fig. 48: Leaf Clip Channel 2 settings

Temperature in window “Leaf Clip/Ext. PAR Sett” (Fig. 44, page 64) leads to the calibration data for the leaf temperature sensor of the 2035-B leaf clip (External Temp. Sensor Sett., Fig. 49, page 68). This window lists slope (Gain) and offset of the calibration line. Factory settings are Gain = 1.00, Offset = 0.

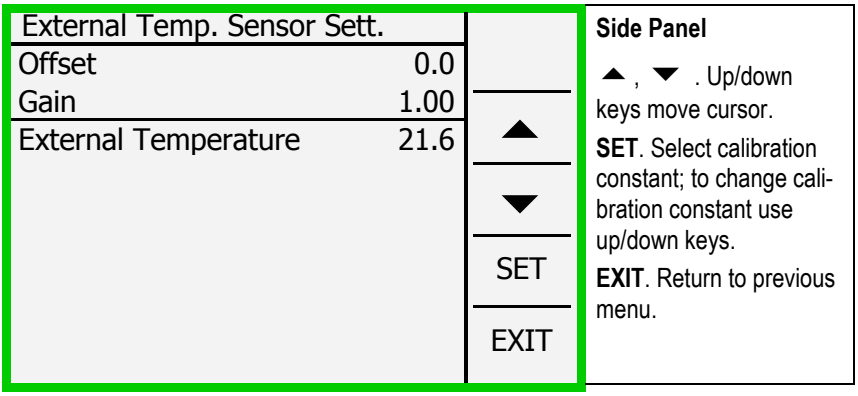


Fig. 49: External Temperature Sensor Settings

4.2.4.3 Oxygen Sensor

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

Selecting “Settings” allows entering temperature, pressure, and humidity data for correct sensor calibration. For each of these

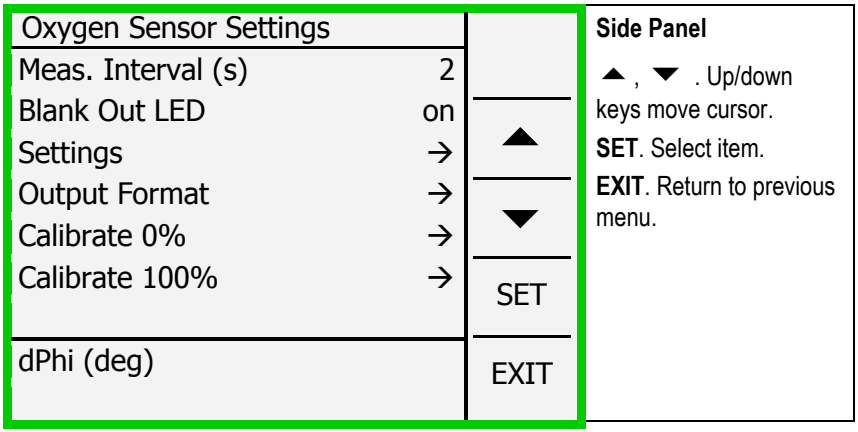


Fig. 50: Oxygen Sensor Settings

three parameters, an extra submenu is provided. For details see the instructions for the oxygen measurements with the MINI-PAM-II (http://www.walz.com/downloads/manuals/mini-pam-II/Oxygen_MINI-PAM-II_02.pdf) and the manual for the FireStingO2 oxymeter (http://www.pyro-science.com/). "Output Format" opens a menu for selection of the parameter used for oxygen representation in the chart.

4.2.4.4 Spectrometer

The item "Spectrometer" in menu "Sensor Settings" leads to the window "Spectrometer Settings (Fig. 51, page 69) which is dedicated to configuration on the miniature spectrometer.

Integration time (ms) determines the measuring time in ms for a single spectrum used to integrate light on the spectrometer in milliseconds.

Auto Range in the active state ignores the integration time set above and determines the optimal integration time automatically.

Operation Mode opens another menu (Fig. 52, page 70) in which the type of spectrum (light, fluorescence or reflectance) and, for light spectra, the entrance optics (flat versus spherical) and the

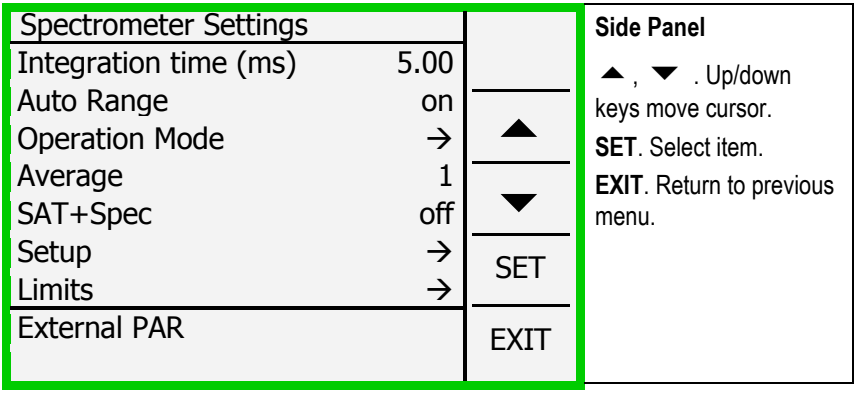


Fig. 51: Spectrometer Settings

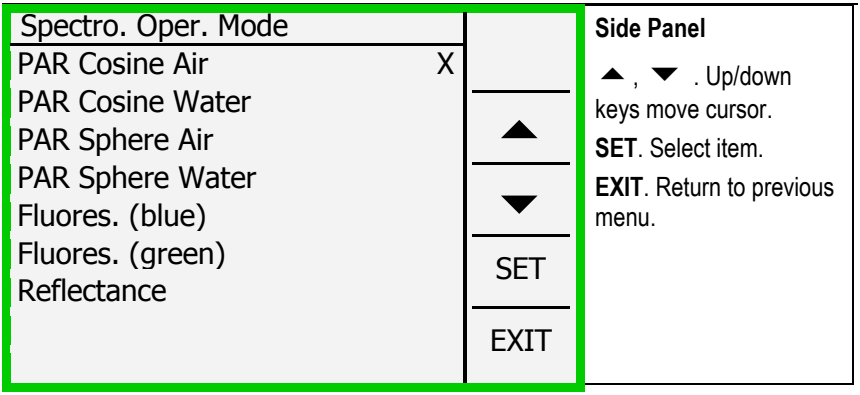


Fig. 52: Spectro. Oper. Mode

environment in which measurements are performed (air or water) can be specified.

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

SAT+Spec active links recording of a spectrum to a saturation pulse analysis.

Setup opens a menu for recording of the dark spectrum of the miniature spectrometer (Dark Spectrum →). The reference spectrum for reflectance measurements with the white standard is

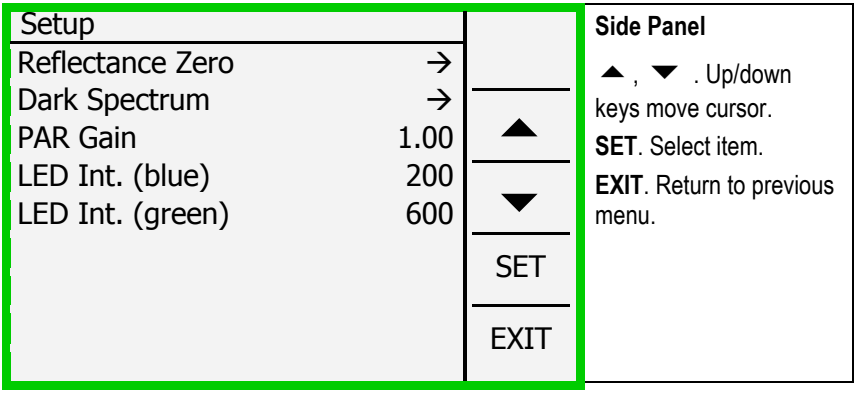


Fig. 53: Setup

recorded by selecting “Reflectance Zero”. This menu display an amplification factor (PAR Gain) that is automatically adjusted depending on Operation mode of the spectrometer. For example, switching the operation mode from air to water increases the PAR 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.

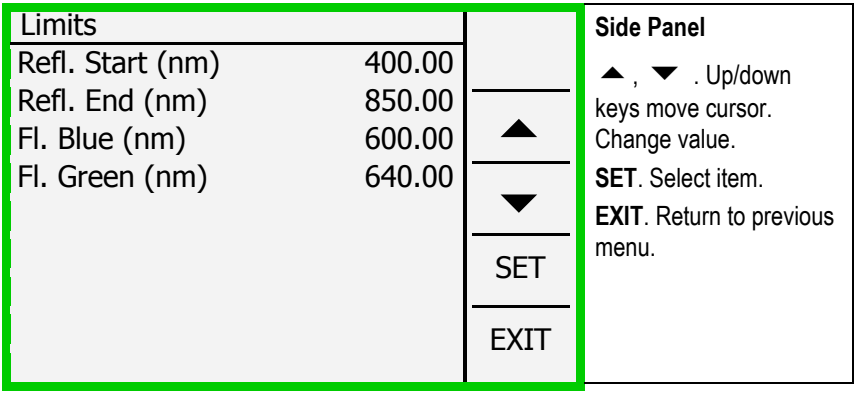


Fig. 54: Limits

4.2.4.5 Depth

Selecting Depth opens a submenu in which the offset for depth measurements is adjusted.

4.2.4.6 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.

4.2.4.7 Load System Sett.

"Load System Settings" restores settings like calibration factor of internal PAR sensor (calibrated for geometry of 2035-B leaf clip), measuring light current and calibration factors of external devices like those stored on the 2035-B leaf clip. "Load System Settings" must not be confused with ""Load Defaults" (Fig. 57, page 74).

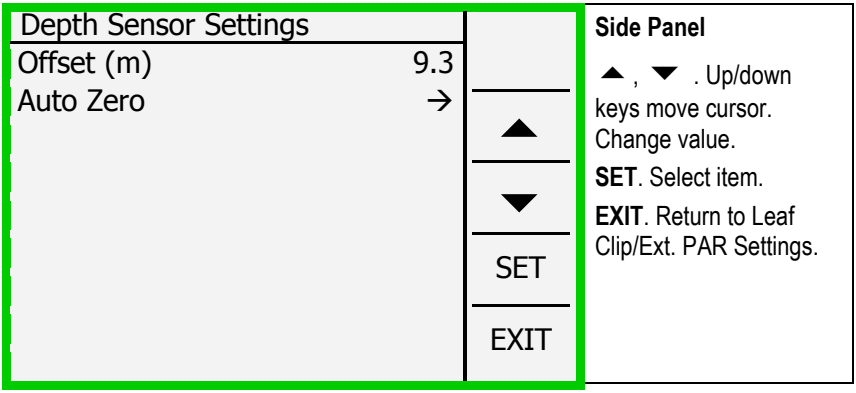


Fig. 55: Depth Sensor Settings

4.2.5 DIVING-PAM-II

The menu "DIVING-PAM-II Settings" permits adjusting of various fluorometer settings.

Ft Chart Resolution (s)

Time resolution of Ft Chart (Fig. 23, page 37) 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. The DIVING-PAM stays on when the

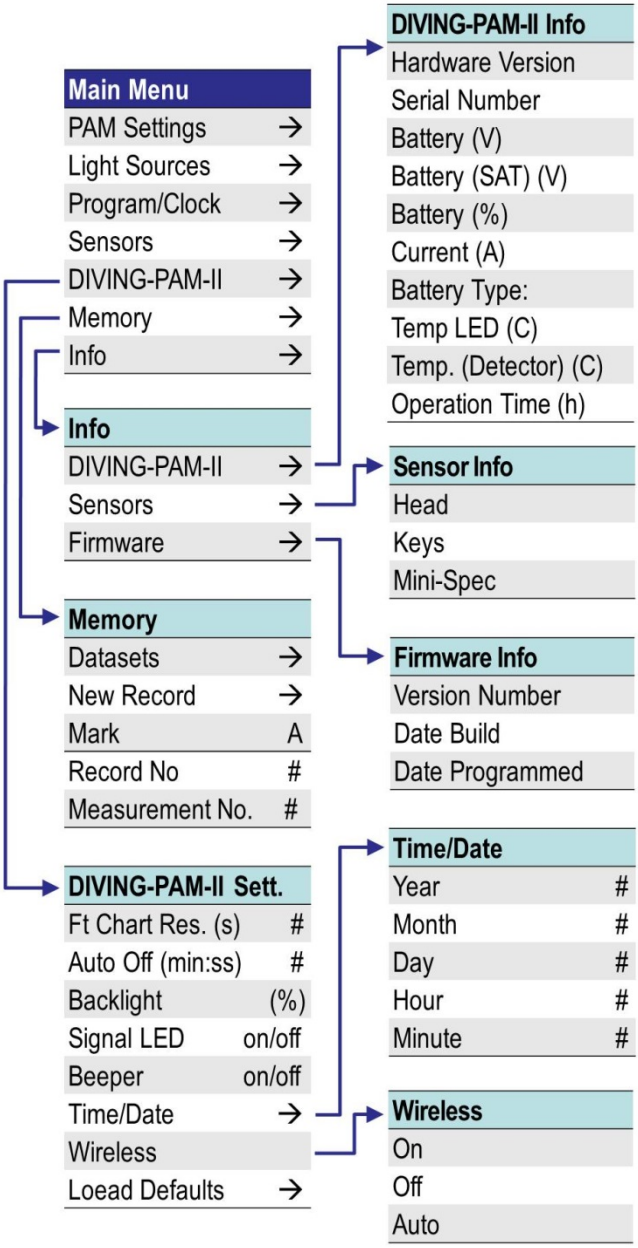


Fig. 56: DIVING-PAM-II, Memory and Info menus

clock is running or when communicating with an external computer either via cable or using WLAN.

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

Menu for setting time and date.

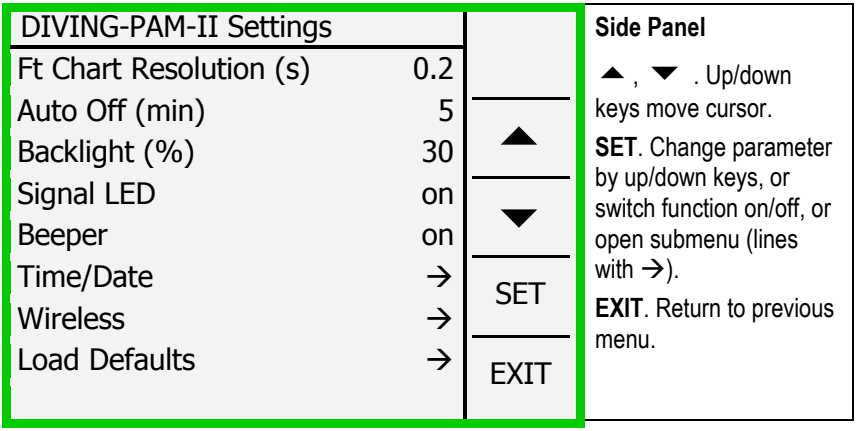


Fig. 57: DIVING-PAM Settings

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.

Load Defaults

The DIVING-PAM-II keeps on internal memory the default settings for the variables listed in Table 6, page 75. Virtually all parameters for which default settings are defined are also saved when the DIVING-PAM-II is shut off (Table 6). This way, the settings used last are activate when powering on the DIVING-PAM-II. Defaults setting can be resored by , the variable line is marked by “Yes” in the column on the right (Table 6). “Yes” implies that these settings are restored on following the use of the DIVING-PAM-II.

Table 6: Default Settings

	Default Setting	**Custom Setting** (saved and restored)
Measuring Light		
Status	On	No
Intensity	6, relative unit	Yes
Frequency	3, see Table 5	Yes
Frequency high status	Off	No
Actinic Light		
Status	Off	No
Intensity	6, relative unit	Yes
Factor	1.00	Yes
PAM Signal		
Gain	1, relative unit	Yes
Damping	2, relative unit	Yes

Table 6: Default Settings

	Default Setting	Custom Setting (saved and restored)
Far Red Light		
Width 5	5, s	Yes
Intensity	8, relative unit	Yes
Saturation Pulse		
Intensity	10, relative unit (≙ 5000 µmol m^{-2} s^{-1})	Yes
Width	0.6, s	Yes
Program Actinic Light and Yield		
Actinic light width	30, s	Yes
Initial Pulse	Yes	Yes
Program Induction Curve		
Delay	40, s	Yes
Width	20, s	Yes
Length	12	Yes
Program Light Curve		
Width	20, s	Yes
Intensity	3, relative unit	Yes
Length	8, light steps	Yes
Clock		
Item	Saturation pulse	Yes
Interval	60, s	Yes
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

Table 6: Default Settings

	Default Setting	**Custom Setting** (saved and restored)
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

4.2.6 Memory

The “Memory” window provides an overview on stored saturation

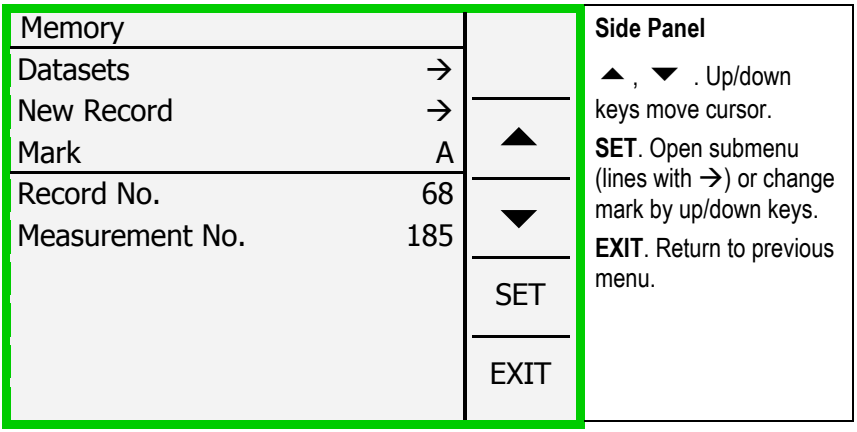


Fig. 58: Memory

pulse analyses (Datasets). Importantly, here fluorescence kinetics induced by saturation pulses can be viewed. To see stored induction and light curves, use the MEM key of window “Induction Curve” (Fig. 26, page 42) and window “Light Curve” (Fig. 27, page 44), respectively. Also in this window, new records can be started and the mark of saturation pulse data can be changed.

Deletion of data from the DIVING-PAM-II internal memory is possible using software WinControl-3.

4.2.7 Info

“Info” (Fig. 59, page 78) consists of three links to submenus of which three list hardware and software information:

- DIVING-PAM-II provides hardware information of the fluorometer including type of internal battery and operation hours.
- Sensors lists serial numbers of sensors connected to the DIVING-PAM-II.

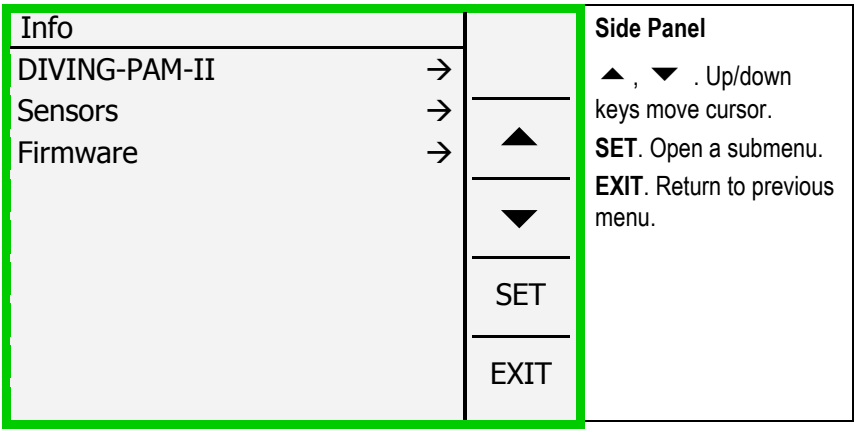


Fig. 59: Info

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

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.

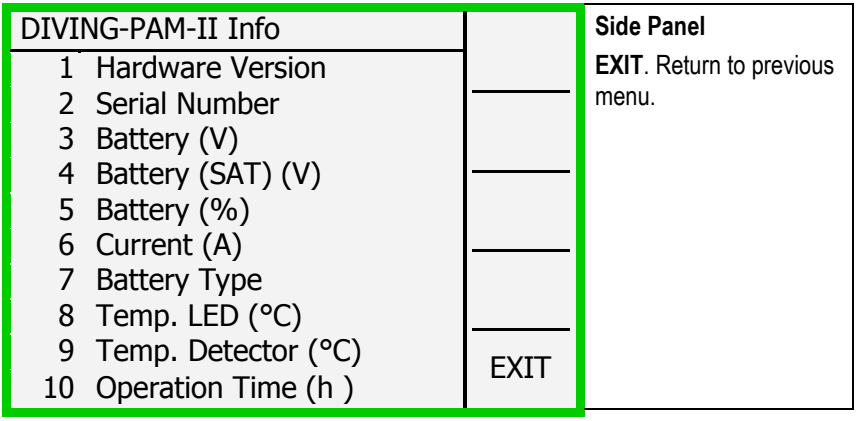


Fig. 60: DIVING-PAM-II Info

5 Saturation Pulse Analysis

5.1 Five Fluorescence Levels

Usually, five different levels of modulated fluorescence are acquired by PAM fluorometers. 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 (see Fig. 61, page 81). Some parameters of saturation pulse analysis require fluorescence measurement of the same sample in both the dark-acclimated and light-exposed state (Table 7, page 84).

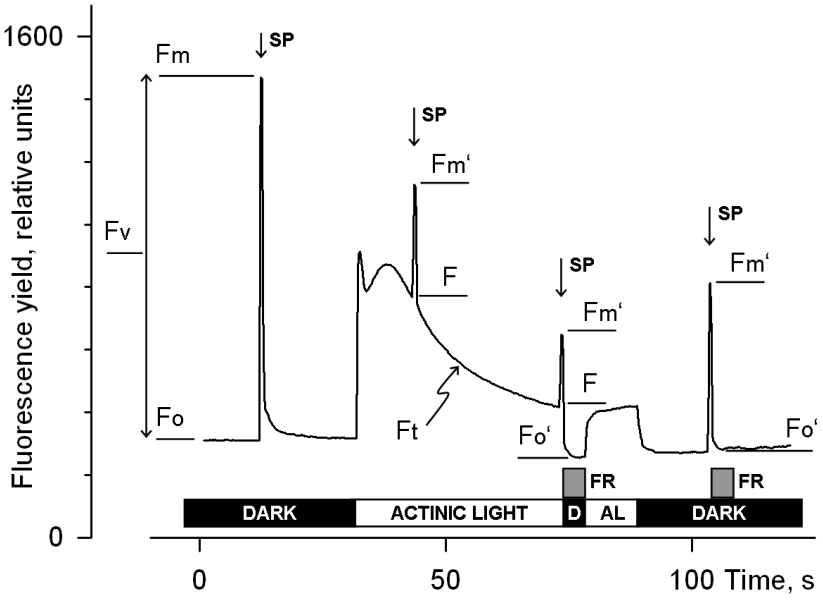


Fig. 61: **Measurements for Saturation Pulse Analysis. AL, Actinic Light; D, dark; SP, Saturation Pulse; FR, Far red illumination.**

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).

5.1.1 Measurements with Dark-Acclimated Samples

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

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

5.1.2 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' (F_0' Mode, Fig. 32, page 50) 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 PS I. As a consequence, electrons are removed from the intersystem electron transport chain and opening of PS II reaction centers is efficiently accelerated (see Fig. 61, page 81, time point 75 s). Ideally, far red opens PS II in less than 5 s and it is often assumed that during this short in-

terval light-driven energization of the photosynthetic membrane decays very little.

If the F_0' Mode is switched off, the F_0' will be calculated according to Oxborough and Baker (1997, reference in Table 8, page 85):

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

On the screen (Fig. 22, page 36) and in WinControl-3, the numerical value of the calculated F_0' is preceded by a tilde sign (e.g. ~456).

F_M' Maximum fluorescence level of the illuminated sample. The F_M' is induced by a Saturation Pulse which temporarily closes all PS 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 measured shortly before application of a Saturation Pulse.

Table 7: 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)*	$Y(NPQ) = \frac{F}{F'_M} - \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Quantum yield of non-regulated heat dissipation and fluorescence emission: this type of energy loss does not involve the action of a trans-thylakoid ΔpH and zeaxanthin (Genty *et al*. 1996)*	$Y(NO) = \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Stern-Volmer type non-photochemical fluorescence quenching (Bilger +and Björkman, 1990)	$NPQ = \frac{F_M}{F'_M} - 1$	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 demonstrated that the equations by Kramer *et al.* (2004) can be transformed into the simple equations of (Genty *et al*. 1996) which are used by the DIVING-PAM-II and WinControl-3.

Table 8: References Cited in Table 7, page 84.

- Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosynth Res 25:173-185
- Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87–92
- Genty B, Harbinson J, Cailly AL and Rizza F (1996) Fate of excitation at PS II in leaves: the non-photochemical side. Presented at: The Third BBSRC Robert Hill Symposium on Photosynthesis, March 31 to April 3, 1996, University of Sheffield, Department of Molecular Biology and Biotechnology, Western Bank, Sheffield, UK, Abstract P28
- Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376:105-115
- Klughammer C and Schreiber U (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. PAM Application Notes 1: 27-35 (http://www.walz.com/e_journal/pdfs/PAN078007.pdf)
- Kramer DM, Johnson G., Kiirats O, Edwards GE (2004) New flux parameters for the determination of Q_A redox state and excitation fluxes. Photosynth Res 79: 209-218
- Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of pho-tosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54 135-142
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62
- van Kooten O, Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147–150

5.2 Fluorescence Ratio Parameters

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 7 (page 84) compiles the fluorescence ratio parameters available in DIVING-PAM-II and WinControl-3. Below, these parameters will be explained briefly.

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

The F_V/F_M and Y(II) estimate the fraction of absorbed quanta used for PS II photochemistry. F_V/F_M corresponds to the maximum photochemical yield of PS II, Y(II) is the effective photochemical yield of PS 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 PS II quantum yield, as the PS II acceptor pool may be reduced in the dark by stromal reductants and, consequently, the so-called state 2 is formed exhibiting low PS 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 PS 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 PS II reaction centers. The q_P is based on the concept of separated PS II antenna units (puddle model), whereas the q_L assumes interconnected PS II antenna units (lake model) which appears to be the more realistic situation 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 that the 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 7, page 84).

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 PS II yields

Genty *et al.* (1996) presented expressions describing the partitioning of absorbed excitation energy in PS 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 PS 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 PS II acceptors and photodamage, e.g. via formation of reactive oxygen species.

5.3 Relative Electron Transfer Rate (ETR)

Relative electron transfer rates are calculated according to:

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

The basic idea of the ETR equation is to multiply Y(II), the effective photochemical quantum yield of PS II, by an estimate for the photon flux density absorbed by all PS 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 PS 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.

5.4 Light Curves

The light curve program exposes a sample to stepwise increasing intensities of actinic illumination. In “Rapid Light Curves”

(RLC), the time interval of each light step is short (down to 10 s) and full equilibration of photosynthetic reactions is not reached within an illumination interval. Typically, the RLC starts at a PAR value somewhat below that of the natural environment.

RLC measurements are carried out with samples in their momentary acclimation status, that is, without dark-acclimation period to determine F_0 and F_M. This way, RLC data can provide information on the present acclimation state of photosynthesis. Obviously, without F_0 and F_M determination, those fluorescence ratio parameters requiring F_0 and F_M (like NPQ and qP) are not available.

If illumination steps are long enough to reach steady state of photosynthesis, fluorescence-based light curves may be compared with classical light response curves (P-I curves). Naturally, any limitation by insufficient CO_2 supply must be avoided during such long-term light curves.

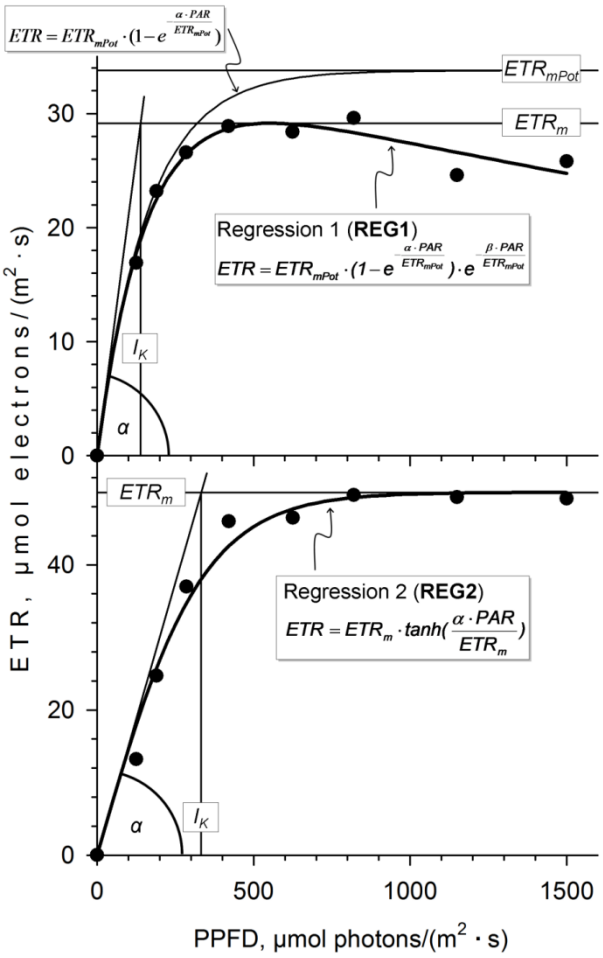


Fig. 62: Model Functions of Rapid Light Curves.

In both cases, RLC and long-term light curves, plotting ETR versus PAR yields light response curves which are often described by the following three cardinal points.

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

- ETR_{max}, µmol electrons/(m^2 · s): Maximum electron transport rate.
- I_K, µmol photons/(m^2 · s): Minimum saturating irradiance.

Evaluation of cardinal points requires WinControl-3. The software uses two empirical functions to estimate these cardinal data: the functions **REG1** and **REG2** have been introduced by Platt *et al.* (1980) and Jassby and Platt (1976), respectively, to describe classical light response curves of photosynthesis.

REG1

In case of REG1, the cardinal point α results directly from fitting the ETR below to experimental data.

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

The latter equation considers photoinhibition of photosynthesis by high light intensities. Therefore, the fitting procedure also yields an estimate for β, the "photoinhibition parameter"(Platt *et al.*, 1980), and for ETR_{mPot}, the maximum potential light-saturated electron transport rate which would be observed if photoinhibition was absent.

Platt *et al.* (1980) have introduced the "Photoinhibition Index" (I_b) to quantify photoinhibition. The authors define I_b as the PAR value required to photoinhibit ETR_{mPot} by the factor of 1/e according to:

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

With the results from curve fitting, WinControl-3 computes the ETR_{max} and I_K according to:

$$ETR_{\max} = ETR_{mPot} \cdot (\frac{\alpha}{\alpha + \beta}) \cdot (\frac{\beta}{\alpha + \beta})^{\frac{\beta}{\alpha}} \quad \text{and} \quad I_K = \frac{ETR_m}{\alpha} \quad .$$

REG2

The function REG2 is monotonically nondecreasing and, hence, does not allow for photoinhibition:

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

Here, the cardinal points, α and ETR_{max}, are estimated by the fitting procedure. With the latter two parameters, the I_K is calculated as described for REG1.

5.5 Some Light Curve References

Long-term Light Curves

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21: 540-547

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38: 687-701

Rapid Light Curves

Fouqueray M, Mouget J-L, Morant-Manceau A, Tremblin AG (2007) Dynamics of short-term acclimation to UV radiation in marine diatoms. J Photochem Photobiol B: Biology 89: 1–8

Perkins RG, Mouget J-L, Lefebvre S, Lavaud J (2006) Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms. Marine Biol 149: 703-712

Ralph PJ, Gademann R (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. Aquat Bot 82: 222-237

Rascher U, Liebig M, Lüttge U (2000) Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant Cell Environ 23: 1397-1405

Schreiber U, Gademann R, Ralph PJ, Larkum AWD (1997) Assessment of photosynthetic performance of Prochloron in Lissoclinum patella in hospite by chlorophyll fluorescence measurements. Plant Cell Physiol 38: 945-951

Serôdio J, Vieira S, Cruz S, Coelho H (2006) Rapid light-response curves of chlorophyll fluorescence in microalgae: relationship to steady-state light curves and non-photochemical quenching in benthic diatom-dominated assemblages. Photosynth Res 90: 29-43

White AJ, Critchley C (1999) Rapid light curves: A new fluorescence method to assess the state of the photosynthetic. Photosynth Res 59: 63-72

5.6 Some Reviews on Saturation Pulse Analysis

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59: 89–113

Dau H (1994) Molecular mechanisms and quantitative models of variable photosystem II fluorescence. Photochem Photobiol 60:1-23

Demmig-Adams B and Adams WW, III (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 43:599-626

Govindjee (1995) Sixty-three years since Kautsky: Chlorophyll a fluorescence. Aust J Plant Physiol 22:131-160

Krause GH and Weis E (1991) Chlorophyll fluorescence and photosynthesis: The basics. Annu Rev Plant Physiol Plant Mol Biol 42:313-349

Logan BA, Adams III WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. Funct Plant Biol 34, 853–859

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. J Exp Bot 51, 659–668.

Mohammed GH, Binder WD, Gillies SL (1995) Chlorophyll fluorescence: A review of its practical forestry applications and instrumentations. Scand J Forest Res 10:383-410

6 Hints

6.1 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. 43, page 63). Estimation of PAR according to Fig. 13 (page 22) 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. 32, page 50 ff).

6.2 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.

6.3 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

5.1.1, page 82). 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 9). If required, measuring light can be adjusted (Fig. 32, page 50ff).

Table 9: 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.

6.4 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. 63A). Some high light grown samples do not reach a plateau with standards settings (Fig. 63B). In this case, saturation pulse intensity or/and length should be increased. Also, fluorescence ki-

netics can reach its maximum clearly before end of the saturation pulse (Fig. 63C). 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. 63C, 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.

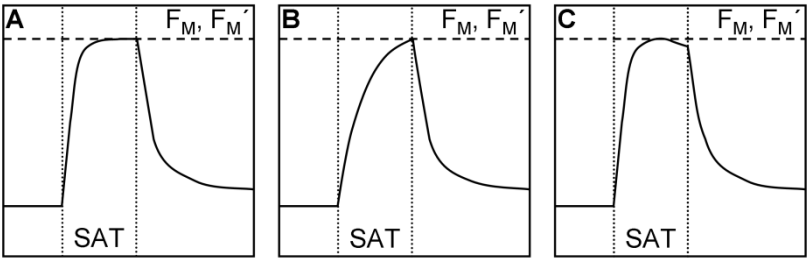


Fig. 63: Fluorescence Kinetics induced by a Saturation Pulse.

7 Trouble Shooting

7.1 Device does not switch off

Check if clock is running.

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

7.2 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 9).

Make sure that the interface in connected to line power (Fig. 2, page 8).

7.3 “Overflow” message in window primary data

Reduce measuring light intensity and signal amplification (see “PAM Settings”, Fig. 32, page 50)

7.4 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.

8 Specifications DIVING-PAM-II

8.1 Basic System

8.1.1 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

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: 3.4 kg

8.1.2 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

8.1.3 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

8.1.4 WLAN

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

8.1.5 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

8.1.6 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

8.1.7 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

8.1.8 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

8.1.9 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, 175 cm length max

Weight: 135 g

8.1.9.1 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

8.1.9.2 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

8.1.9.3 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

8.1.10 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

8.1.11 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

8.1.12 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

8.1.13 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.

8.1.14 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

8.2 Accessories

8.2.1 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

8.2.2 Magnet Sample Holder DIVING-MLC

Design: Two halves with ring magnets, one with fiberoptics adapter and split rubber hood (dark adaptation), the other serving as buoyancy body

Dimensions: Diameter 37 mm, height 48 mm

Weight: 60 g; floating underwater

8.2.3 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

8.2.4 Underwater Cable DIVING-II/K25

Dimensions: 25 m length, 6 mm diameter

Weight: 1.25 kg

8.2.5 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

9 Warranty

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

9.1 Conditions

This warranty applies if the defects are called to the attention of Heinz Walz GmbH, Germany, in writing within two (2) years of the shipping date of the product.

This warranty 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.
- to damage caused from improper packaging during shipment or any natural acts of God.
- to batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps, thermocouples, and underwater cables.

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. Warranty shall not apply for diving depths exceeding the maximum operating depth. Further, warranty shall not apply for

damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

9.2 Instructions

To obtain warranty service, please follow the instructions below:

- The Warranty Registration form 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 warranty service are at customer expense. Duty and taxes are covered by Walz. Accompany shipment by the Walz Service and Repair form available at:

  http://www.walz.com/support/repair_service.html
- All products being returned for warranty 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.

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