UNDERWATER FLUOROMETER
DIVING-PAM

Submersible Photosynthesis Yield Analyzer

Handbook of Operation

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DIVING-PAM Upgrade

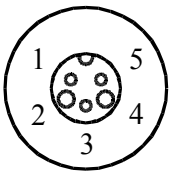
Valid for instruments with serial numbers UWFAxxxxA and UWFBxxxxA

The following important modifications and extensions were recently introduced in response to helpful suggestions of DIVING-PAM users:

1. Waterproof connectors

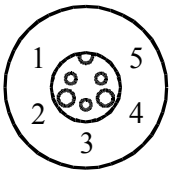
The DIVING-PAM now features the two waterproof connectors **INTERFACE BOX** and **AUX** which substitute for the previous connectors CHARGE and RS 232 (items 5 and 11 in Fig.4 of Handbook of Operation; see also new illustration below). These connectors are waterproof only if the corresponding special underwater cables are connected or the respective blind plugs are installed. The water-sealing O-rings on the cable connectors and plugs should be kept free of sand/dirt and occasionally greased. The connectors are not supposed to be opened underwater. In case a user should forget to seal the connectors with the plugs, this would not lead to the ruin of all electronics (as before), but would just cause corrosion of the connectors which then would have to be replaced.

"INTERFACE BOX"



1: External input +12 V (max. 13.8V)
ATTENTION: Internal battery cannot be charged via this input.
2: RxD
3: GND } RS 232
4: TxD
5: Charge input +18V

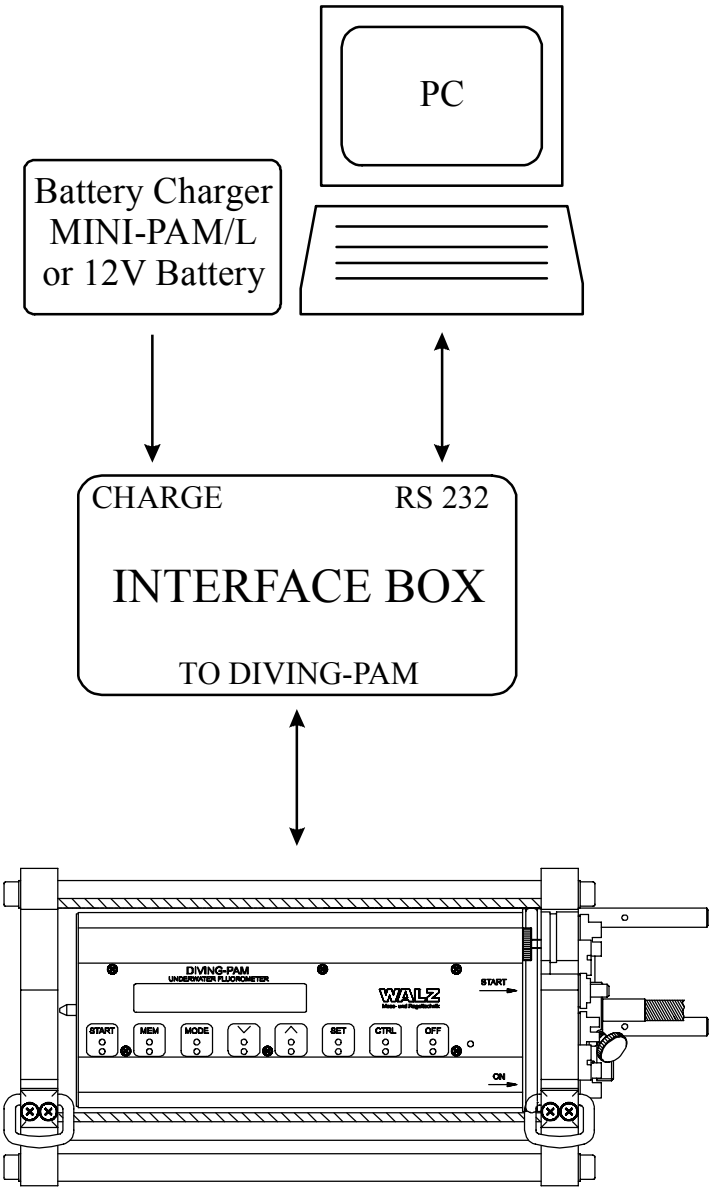
"AUX"



1: +12V Batt output
2: Remote 1 OUT
3: GND
4: Ext. Start Switch IN
5: +5V output

2. INTERFACE BOX and underwater cable for remote control and CHARGE

An INTERFACE BOX and a 5 m long underwater cable with waterproof connectors are provided for remote control and the CHARGE function. The 5 m long cable connects INTERFACE BOX and DIVING-PAM (please make sure to use the INTERFACE BOX - and not the AUX connector - of the DIVING-PAM). The INTERFACE BOX features two input sockets for the **CHARGE**- and **RS 232-cables**, which previously were directly connected to the DIVING-PAM (see illustration below). Optionally, cables up to 50 m length are available. In this way, the DIVING-PAM can be operated for longer periods of time underwater by remote control using a PC in conjunction with the **WinControl-software**. If battery power becomes exhausted, an **external 12 V battery** can be connected via the CHARGE-input socket, for which purpose a special cable (MINI-PAM/AK) is available. If line voltage is available, the **Battery Charger MINI-PAM/L** can be used for recharging. Please note that the internal battery cannot be recharged by an external 12 V battery. The INTERFACE BOX houses an internal 2A fuse (slow blow type).

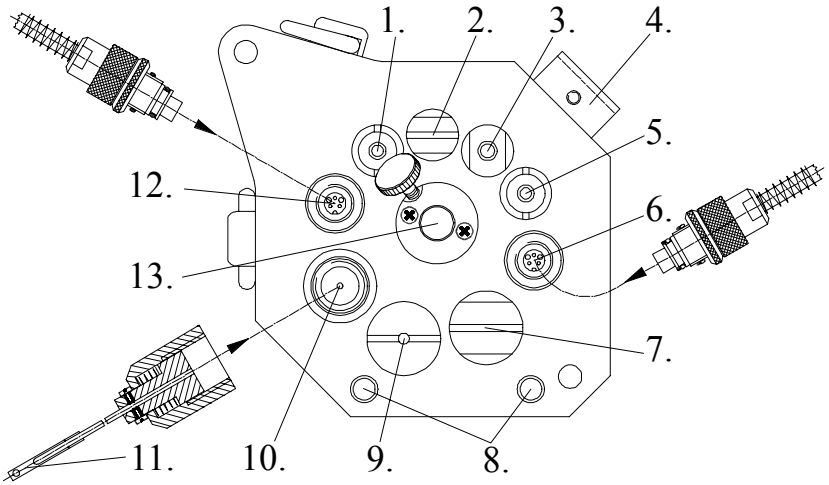

PC
Battery Charger
MINI-PAM/L
or 12V Battery
CHARGE
RS 232
INTERFACE BOX
TO DIVING-PAM
DIVING-PAM
UNDERWATER FLUOROMETER
WALZ
START
START
MEM
MODE
SET
CTRL
OFF
ON

3. AUX-connector

The waterproof AUX-connector is reserved for future applications. In the near future a special **Underwater Leaf/Thallus-Clip** will become available which features a **START-button** and can be connected via a waterproof cable/connector to the AUX-input of the DIVING-PAM. Using the START-button a saturation pulse or any other function featuring under **CLOCK ITEM** (Mode-menu point 29) can be started.

4. START-button

In Fig. 4 of the Handbook of Operation the item 2 so far featured two blind plugs (not used). In the upgraded DIVING-PAM version, item 2 represents „Access to service functions“ (adjustment of keyboard sensitivity and instrument reset). The new item 5 (see illustration below) features a separate START-button which is equivalent to the START-key on the keyboard and the START-button of the Leaf/Thallus-Clip (soon available). In practice, it is more easy to use this new START-button than the START-key on the keyboard.



1. ON-switch
2. Access to service functions (keyboard sensitivity and RESET)
3. Water temperature sensor
4. Holder for light guide
5. START-button
6. Connector for the INTERFACE BOX
7. Not used
8. Distance rods
9. Depth sensor
10. Connector for Fiber Quantum Sensor
11. Fiber Quantum Sensor
12. AUX (reserved for future applications)
13. Connector for Fiberoptics DIVING-F

5. KEYBOARD-off option

For routine measurements just involving the START of a saturation pulse (or of any other function selected as CLOCK ITEM) it may be advantageous to **disable the keyboard function** and to **use the new START-button** on the DIVING-PAM (see point 4) or on the Leaf/Thallus-Clip (soon available). This KEYBOARD-off option is now linked with the DISPLAY ILLUMINATION-off option

(Mode-menu point 9). When DISPLAY ILLUMINATION is off, also the keyboard is disabled, except for the **CTRL-key**. By pressing this key for ca. 4 s, KEYBOARD can be enabled again together with **DISPLAY ILLUMINATION**. Please note that KEYBOARD must be also enabled in order to switch the DIVING-PAM off via the OFF-key.

1 Safety instructions

1.1 General safety instructions

To avoid the risk of electric shock, do not remove the casing or open the back. There are no user serviceable parts inside. Leave servicing to the experts! To prevent fire or the risk of electric shock, keep this device out of the rain and away from moisture whenever it is connected to the mains. The lightning symbol with the arrow head inside an equilateral triangle means that there are live, uninsulated parts inside this device that may give you a dangerous electric shock if touched.

1. Instructions: Read all the safety instructions and all the operating instructions thoroughly before using the device for the first time. Keep these safety instructions and operating instructions somewhere safe in case you need to refer to them again in the future.

2. Safety warnings: In your own interest pay heed to all the safety warnings on the device and in the operating instructions. Follow the instructions on operation and use of the device in every respect.

3. Water and moisture: Never use the device near water, for example near a bath, a washing basin, a sink, a washing machine, in a damp cellar or near a swimming pool, if connected to the mains.

4. Ventilation: Wherever you put the device, always ensure there is sufficient ventilation. Never put the device on a bed, for example, or a sofa, a carpet or similar surface that might block the vents. Do not build it into furniture either, such as a bookcase or a cupboard.

5. Effect of heat: Do not put the device anywhere near sources of heat, such as radiators, hot-air shafts, ovens, etc.

6. Power source: Connect the device only to the power source indicated in the operating instructions or on the device.

7. Protecting the flex: Run the flex so that no one can step on it and nothing can rest on or against it. The flex is particularly at risk in the area of the plug, the socket and where it comes out of the device.

8. Cleaning: Follow the manufacturer's recommendations for cleaning the device.

9. Device not in use: If you are not going to use the device for some time, remove the plug from the socket.

10. Foreign bodies: Take great care to ensure that no liquids or other foreign bodies can find their way inside the device through the openings in the casing.

11. Repair in the event of damage: The device should only be repaired by qualified personnel. Never try to do more in the way of maintenance to your device than the operating instructions allow. Beyond that, always consult an expert for repair work.

12. Fixing to wall or ceiling: The device may only be fixed to a wall or ceiling in the manner specified by the manufacturer.

1.2 Special safety instructions

Before putting the DIVING-PAM into the water, please make sure that all sealing plugs are thoroughly fixed and that the RS 232 and charge cables are disconnected. The maximum diving depth is 50 meters.

In order to prevent excessive heating of the internal halogen lamp, it is recommended to avoid frequent successive illumination at high lamp currents.

2 General Information

The Underwater Fluorometer DIVING-PAM has been developed with special attention to the quick and reliable assessment of the effective quantum yield of photochemical energy conversion in photosynthesis. The most relevant information is obtained by a single key operation within a second and up to 4000 data sets can be stored for later analysis. Due to a novel opto-electronic design and modern microprocessor technology, the DIVING-PAM is compact and at the same time highly sensitive and selective. It is ideally suited for rapid screening of photosynthetic activity in the field, and it was particularly designed for underwater measurements down to 50 m depth.

The DIVING-PAM, like all PAM Fluorometers, applies pulse-modulated measuring light for selective detection of chlorophyll fluorescence yield. The actual measurement of the photosynthetic yield is carried out by application of just one saturating light pulse which briefly suppresses photochemical yield to zero and induces maximal fluorescence yield. The given photochemical yield then immediately is calculated, displayed and stored. Numerous studies with the previously introduced PAM Fluorometers have proven a close correlation between the thus determined YIELD-parameter ($\Delta F/Fm'$) and the effective quantum yield of photosynthesis in leaves, algae and isolated chloroplasts. With the help of the included miniature light sensor also the photosynthetic active radiation (PAR) can be determined close to the site of fluorescence measurement, such that an apparent electron transport rate (ETR) is calculated. In addition to this central information, the DIVING-PAM also provides the possibility of measuring fluorescence quenching coefficients (qP, qN, NPQ). Continuous actinic light can be applied for measurement of induction curves (Kautsky-effect) and for automatic recordings of light-saturation curves with quenching analysis. For these purposes,

an extensive MODE-menu is provided. The stored data can be transferred to a PC with the help of a special PamTrans Data Transfer Software. When operated above water the DIVING-PAM alternatively can be controlled on-line by a PC via the RS 232 interface using the optional WinControl software.

3 Basic Operation of the DIVING-PAM

The DIVING-PAM is very easy to be operated. It has a two-line LC-display and an optical sensor-keyboard with eight function keys (CTRL, OFF, MODE, MEM, ∧, ∨, START, SET). In order to get started, only the fiberoptics have to be connected and the ON-key at the right side of the case is pressed. Now the system is ready for recording fluorescence yield of any sample which is close (5-20 mm) to the free end of the fiberoptics. The actual measurement of the most relevant YIELD-parameter (quantum yield of photochemical energy conversion) just involves pressing the START-key. Then on the display, for example, the following information is shown:

```
  1:     445F 1739M-12.3m
F: 448   745Y   6.2E    20L
```

The meaning of the various displayed parameters is as follows:

`1:` Number denoting the standard MODE-menu position 1 which is automatically installed whenever the DIVING-PAM is switched on or a YIELD-determination is carried out via START.

`445F` Fluorescence yield (F) measured briefly before the last saturating light pulse triggered by START.

`1739M` Maximal fluorescence yield (M = Fm or Fm') measured during the last saturating light pulse triggered by START.

`-12.3m` Water depth measured with the built-in pressure sensor.

`F:448` Momentary fluorescence yield, which shows small fluctuations caused by electronic noise.

`745Y` The most relevant YIELD-parameter determined by the last saturating light pulse triggered by START, which is calculated as follows:

$$YIELD = Y/1000 = (M-F)/M = \Delta F/M = \Delta F/Fm'$$
(Genty-parameter)

With a dark-adapted sample $\Delta F/Fm$ = Fv/Fm, corresponding to the maximal yield of photochemical energy conversion.

`3.2E` Relative rate of electron transport (ETR). It is calculated by the formula:

$$ETR = E = YIELD \times PAR \times 0.5 \times ETR\text{-}factor$$

`20L` Light intensity in units of PAR (quantum flux density of photosynthetically active radiation, [µmol quanta m^{-2} s^{-1}]).

After every operation of START the obtained data set with the corresponding time and date is entered into a RAM-memory, with a storage capacity of 4000 data sets. The stored data can be called on the display via the MEM-key. Previously recorded data can be recalled by using the arrow-keys (∧ or ∨). Stored data can be transferred via an RS 232 interface on a PC for further analysis.

The DIVING-PAM has been pre-programmed at the factory with standard settings (see list in 7.1) for all relevant measuring parameters (for example Measuring Light Intensity, Gain, Damping, Saturation Pulse Intensity, Saturation Pulse Width etc.). These standard settings are optimized for measurements with standard samples at approx. 10 mm distance between fiberoptics and sample surface. For special applications, there is great flexibility for appropriate adjustment of all measuring parameters with the help of the extensive MODE-menu, using the arrow-keys (∧ and ∨) in combination with the SET-key. Details are given in the MODE-menu list below (see 7.2).

4 Description of the Keyboard Functions

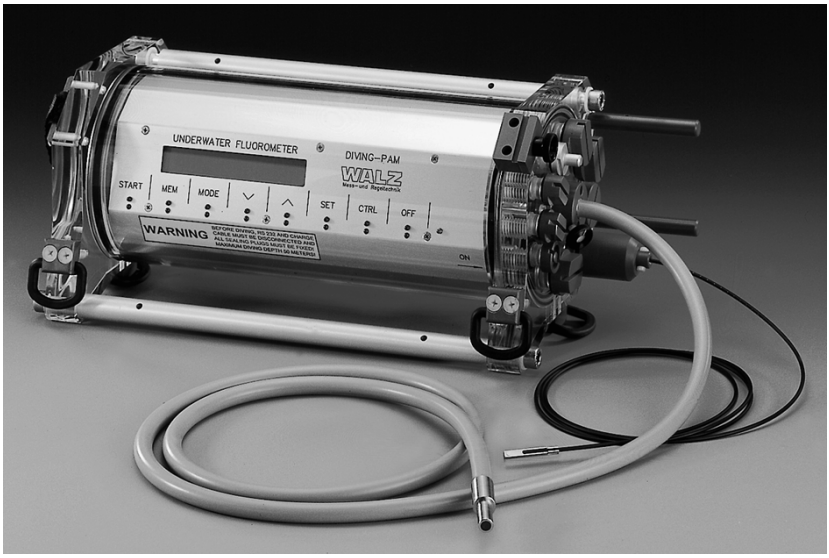


Fig. 1: Underwater Fluorometer DIVING-PAM

4.1 Single key operations

The DIVING-PAM is controlled by eight optical switches arranged in one line at the top side of the housing. Just touching the marked fields is sufficient to carry out a command. A green LED located at the right edge of the key panel serves as function control. It will light up only when a key was touched for more than 0.5 s. In this way, the instrument function is not affected by unintentional touching. The DIVING-PAM power is switched on by a separate mechanical push button switch, which is located on the right side cover of the housing (see ON → at the right hand side of the key panel). In principle, the DIVING-PAM may also be switched-on and controlled in all functions via the RS-232 interface using a PC and

the optional WinControl software. In this case, manual key control normally is out of function. It will be enabled again after pushing the ON-switch.

`START` To trigger a saturating light pulse for assessment of YIELD and related fluorescence parameters.

`MEM` To enter the MEMORY-level of stored data with the last stored data set being displayed.

`MODE` To return to MODE-menu after using the MEM- or SET-keys.

∧ Counting up

∨ Counting down

`SET` To start and stop selected function.

`CTRL` For special commands by double key operation (see following chapter).

To activate the backlighting of the display, requiring 3 s continuous pressing of the key. The light switches automatically off when no key operation for 50 s in order to save battery power.

`OFF` To switch DIVING-PAM manually off. It will occur automatically, if no key operation for 4 min (power saving for field use), unless disabled via menu point 10.

4.2 Double key operations

Besides the single key operations, there is a number of double key operations which can serve as short-cuts for selecting or carrying out certain items/commands in the MODE-menu. For this purpose,

the first key must be kept firmly pressed before briefly pressing the second key.

MODE+START	To return to standard display (menu position 1).
MODE+SET	To move from one functional block in the MODE-menu to the next (see list in 7.1).
MODE+∧	To move to MODE-menu point 17: LIGHT CURVE (carried out via SET).
MODE+∨	To move to MODE-menu point 21: IND.CURVE.
MODE+CTRL	To switch measuring light on/off.
MODE+MEM	To move to MODE-menu point 28: REP-CLOCK.
MEM+SET	To print out the headerline with assignments of data set parameters via a serial matrix printer.
CTRL+SET	To switch actinic light on/off.
CTRL+START	To start/stop actinic illumination with yield-measurement (see menu point 13).
CTRL+MEM	To start/stop the clock for repetitive triggering of selected function (e.g. saturation pulses when 29: CLOCK-ITEM in position SAT).
CTRL+∧	To start/stop a LIGHT CURVE (equivalent to menu point 17).
CTRL+∨	To start/stop an INDUCTION CURVE (equivalent to menu point 21).
SET+OFF	To reset program, if DIVING-PAM for some reason does not respond to key-operations.

If the DIVING-PAM is switched on by RS 232-access the key-controller may not respond. In this case push the ON-switch once.

5 Important Points for Correct YIELD-Measurements

The main purpose of the DIVING-PAM is the reliable determination of the YIELD-parameter ΔF/Fm (Genty-parameter). This task is carried out by the DIVING-PAM with exceptional sensitivity and reproducibility. Because of the central importance of this particular type of measurement, a special section is devoted to it in this handbook (see section 12.3). Here just the most important practical aspects are outlined which are essential for correct YIELD-measurements:

1) The distance between sample and fiberoptics should be approx. 10 mm, such that a normal sample at **standard settings gives a signal of 300-500 units**.

2) The AUTO-ZERO function (MODE-menu point 2) should be applied (while sample is removed), in order to suppress any unavoidable background signal which otherwise would cause some lowering of the YIELD-reading (see 12.3.3).

3) In practice, YIELD-measurements make sense only, if the light conditions of the sample are well controlled. For example, a sample may be severely damaged in Calvin cycle activity and still show a high YIELD-value when dark-adapted or in weak light. The overall photosynthetic performance should be assessed during steady state illumination at a photon flux density which is somewhat below saturation in a control sample. For highest accurracy it is essential that the PAR is measured close to the spot of the sample where also fluorescence is detected. On the basis of the measured YIELD- and PAR-data an apparent electron transport rate (ETR) is calculated and displayed (...E). The plot of ETR vs. PAR corresponds to a light-response curve of photosynthesis (see 12.3.9).

4) When YIELD is measured under field conditions, it is essential that the sample position and effective PAR are not inadvertently changed. During the actual measurement, the fiberoptics must be stably fixed with respect to the sample surface for ca. 2 s, e.g. with the help of the Surface Holder DIVING-SH.

5) Dark YIELD-measurements require special conditions (see also 12.3.1). As already pointed out in 3), such measurements cannot give information on the overall photosynthetic performance. They are useful to specifically assess the state of PS II, for example following light stress treatment. In this case, it is essential, that the measuring light does not induce any significant increase of fluorescence yield. For this purpose, the MODE-menu point 5 provides the possibility of applying the measuring light in short bursts of pulses, thus cutting its integrated intensity to 1/5 (see 12.3.2).

6 Description of the Memory-Function

All data recorded via START are automatically stored in RAM-memory with a capacity of 4000 data sets. They can be recalled on display via the MEM-key. Then, for example, the following information is shown:

```
MEM 382: 12:27 27/MAY/95
A:       322Y 21.1E  157L
```

In the top line it can be seen that the data set Nr. 382 of the current MEMORY was recorded at 12:27 o'clock on May 27th 1995. The bottom line shows that a sample of type A was used (see MODE-menu point 51), which displayed a YIELD-value (Y) of 0.322 and an apparent ETR-value (E) of 21.1 at an incident light intensity (L) of 157 µmol quanta m^{-2} s^{-1} of the photosynthetically active radiation (PAR).

More information relating to this particular data set can be displayed in the top line by SET-operation:

```
MEM 382:390F  576M-19.9m
A:       322Y 21.1E  157L
```

After the first SET, the top line shows that the fluorescence yield (F) measured briefly before the saturating light pulse was 390, that the maximal fluorescence (M) amounted to 576 and that water depth was 19.9 m.

```
MEM 382:645P 759N 1.557Q
A:       322Y 21.1E  157L
```

After the second SET, the top line shows the quenching coefficients qP=0.654, qN=0.759 and NPQ=1.557, which will be meaningful only if for this particular sample a Fo-Fm determination

(MODE-menu point 25) had been carried out beforehand (see 12.3.4).

Further operation of SET (2x) leads back to the original display with time and date.

Using the arrow keys ∧ and ∨ one can move within the memory and display any previously recorded data sets.

All data stored in MEMORY can be cleared by the CLEAR MEMORY function (MODE-menu point 39). For safety's sake, this command does not only require execution by SET, but in addition confirmation by the ∧-key. The memory is organized in form of a ring storage and its clearance normally is not required, as old data will be automatically overwritten.

The MEMORY-front normally corresponds to the MEM-No. under which the last set of data was stored. It can be moved to any number between 1 and 4000 with the help of MODE-menu point 38.

After any change in instrumental settings, the complete set of settings will be stored upon the next YIELD-measurement. This is indicated by "Settings" in the MEMORY-display.

7 The Mode-Menu

The MODE-menu contains 51 items corresponding to a variety of measured values, instrumental settings or special commands. The positions of the various menu points were arranged for optimal practicability, with the most frequently used functions being closest to the standard position 1.

Increasing or decreasing position numbers are selected by the ∧- or ∨-arrow key, respectively. Changes in settings are terminated via SET or MODE. Starting from position 1, at increasing numbers there are mostly MODE-points involving commands (for example, 2: AUTO-ZERO), while at decreasing numbers the MODE-points for instrumental settings prevail (for example, 50: MEASURING LIGHT INTENSITY). Some of the MODE-menu positions can be directly reached via double key operations (see list in section 4.2 above and subsection 7.1). In particular, the MODE+SET command allows to move in larger steps from one functional block to the next (see 7.1).

Irrespective of the selected menu position, a YIELD-measurement can be initiated at any time by pressing the START-key. Normally, the system then automatically returns to the menu position 1 where the measured data set is displayed. The only exceptions are menu-positions 11, 25-27 and 34, where the displayed measured values are of primary interest.

The operations related to the various points of the MODE-menu are either directly carried out via SET (e.g. 2: AUTO-ZERO) or initiated/terminated (e.g. 50: MEAS-INT: 8) by pressing SET. Settings are changed by arrow key operations (∧, ∨) and become immediately effective. The numbers following the double points show the momentary settings.

7.1 List of Menu points

The Menu points are organized in functional blocks. The starting point of each block can be reached by repetitive simultaneous pressing of MODE and SET. The frequently used positions MARK, MEAS-INT and GAIN can be readily selected by going backwards from position 1 using the ∨-key.

The below list shows the default settings, which can be reset at any time by the command 36: RES. SETTINGS. The first points of the functional blocks which can be quickly reached by the MODE+SET command, are emphasized by boldface printing.

Menu points:	Quick access via:
1. **Standard display**	MODE+START
2. AUTO-ZERO: 0(SET)	
3. MEAS.LIGHT: ON (SET)	MODE+CTRL
4. M.FREQ: LOW (SET)	
5. ML-BURST: OFF(SET)	
6. LIGHT AV15s:OFF(SET)	
7. EXT.LIGHT-S:ON (SET)	
8. LIGHT CALIB: (SET)	
9. DISP.ILLUM.:OFF(SET)	
10. AUTO-OFF: ON (SET)	
11. AV. YIELD and ETR	
12. **ACT-LIGHT: OFF(SET)**	
13. ACT+YIELD: OFF(SET)	
14. ACT-WIDTH 0:30 (SET)	
15. ACT-INT: 5 (SET)	
16. AL-FACT: 1:00 (SET)	
17. **LIGHT CURVE:OFF(SET)**	MODE+∧
18. L.CURVE+REC:OFF(SET)	
19. LC-WIDTH 0:10 (SET)	

20. LC-INT: 3 (SET)
21. **IND.CURVE: OFF(SET)** MODE+∨
22. IND.C+REC: OFF(SET)
23. IND-DELAY 0:40 (SET)
24. IND-WIDTH 0:20 (SET)
25. Fo and Fm (SET)
26. qP and qN (SET)
27. NPQ (SET)
28. **REP-CLOCK: OFF(SET)** MODE+MEM
29. CLOCK-ITEM:SAT(SET)
30. CLK-TIME:00:30(SET)
31. **TIME 17:32:56 (SET)**
32. DATE 17-OCT (SET)
33. YEAR 1997 (SET)
34. BATT: 12.4V (11.8)
35. WAT.TEMP: 23C
36. **RES.SETTINGS: (SET)**
37. PROGR.D2.07(280698)
38. MEMORY: 12 (SET)
39. CLEAR MEMORY (SET)
40. **LIGHT-OFFS: 0(SET)**
41. LIGHT-GAIN:1.00(SET)
42. DEP.OFFS: 0.0(SET)
43. DEP.GAIN: 1.00(SET)
44. ZERO-OFFS: 20(SET)
45. ETR-FAC: 0.84 (SET)
46. **SAT-WIDTH:0.8s(SET)**
47. SAT-INT: 8 (SET)
48. DAMP: 2 (SET)
49. GAIN: 2 (SET)

50. MEAS-INT: 8 (SET)
51. MARK: A (SET)

7.2 Description of the Mode-menu points

The following list briefly describes the items contained in the MODE-menu, some of which are outlined in more detail in section 12.3 (Assessment of photosynthesis yield with the DIVING-PAM). Standard settings are shown.

```
  1:    445F 1739M-12.3m
F: 448  745Y  6.2E   20L
```

Standard menu-position for display of the data measured by last saturating light pulse triggered by START. The 4 central parameters F, M, Y and E, the present fluorescence signal F: (with blinking ∗), water depth and ambient PAR are displayed.

```
  2: AUTO-ZERO:  20(SET)
F: 448  745Y  6.2E   20L
```

Command for determination of signal in absence of sample (background signal), the value of which is displayed and automatically subtracted, such that signal becomes zero without sample. This offset value remains effective for all following measurements until being deliberately changed. It has to be newly determined whenever 50: MEASURING LIGHT INTENSITY or 49: GAIN are modified. If this is not done there is a warning ?NEW OFFSET? when YIELD is determined by START. The warning will stop when a new offset is determined via menu point 2 or the given offset is confirmed in menu position 1 via SET.

```
  3:MEAS.LIGHT: ON (SET)
F: 448  745Y  6.2E   20L
```

On/off switch of measuring light. Under standard conditions the measuring light is on. When switched off, a negative signal indicates the AUTO-ZERO value (see menu point 2). The switch can also be operated via MODE + CTRL without entering the MODE-menu.

```
  4: M.FREQ:  LOW  (SET)
F: 448  745Y  6.2E   20L
```

Switch between the standard measuring pulse frequency of 0.6 kHz (LOW) and 20 kHz (HIGH). At 20 kHz the signal/noise is increased by a factor of 5-6. On the other hand, at this high frequency the measuring light intensity can induce substantial fluorescence changes. Hence, 20 kHz normally should be used only when its actinic effect can be neglected relative to a stronger ambient light (e.g. above 100 µmol quanta m^{-2} s^{-1}).

```
  5:  ML-BURST: OFF(SET)
F: 448  745Y  6.2E   20L
```

Switch between normal signal detection (continuously pulsed measuring light) and signal detection by short bursts of measuring light. In the latter case, pulse trains are 0.2 s with dark-intervals of 0.8 s, resulting in a reduction of integrated measuring light intensity by a factor of 5. This can be advantageous for assessment of the maximal photochemical yield after dark-adaptation (ΔF/Fm = Fv/Fm). In the ML-BURST mode the basic frequencies of 0.6 or 20 kHz are maintained.

```
  6:LIGHT AV15s:OFF(SET)
F: 448  745Y  6.2E   20L
```

When this function is enabled, the readings of the external light sensor are averaged over a period of 15 s, in order to account for the substantial fluctuations of light intensity underwater. It is important that the sensor remains fixed in a given position for 15 s before the actual measurement of quantum yield.

```
  7:EXT.LIGHT-S:ON (SET)
F: 448  745Y  6.2E   20L
```

Switch to enable display of readings of external LIGHT-SENSOR (in ON-position). When in OFF-position, the PAR-values stored in an internal list are effective. This list is created via the LIGHT-CAL function (see next menu point).

```
  8:LIGHT-CALIB:   (SET)
F: 448  745Y  6.2E   20L
```

Automized routine for determination of PAR-values of the 12 ACTINIC LIGHT settings in a given measuring geometry. These values are stored in a

list, which is effective whenever the EXT.LIGHT-SENSOR is OFF (menu point 7). For this determination the LIGHT-SENSOR must be fixed instead of the sample in front of the fiberoptics. When the routine is carried out, the LIGHT AVERAGING function (menu point 6) is disabled. If it is afterwards required, it must be manually enabled. After the LIGHT-CALIBRATION the EXT.LIGHT-SENSOR (menu point 7) is in the OFF-position.

```
  9: DISP.ILLUM:OFF(SET)
F: 448  745Y  6.2E   20L
```

When in ON-position, the DISPLAY is continuously illuminated. It should be noted, that this may cause considerable costs of battery power. When in OFF-position, DISPLAY ILLUMINATION can be transiently turned on for 40 s by pressing CTRL for 3 s.

```
 10: AUTO-OFF:  ON (SET)
F: 448  745Y  6.2E   20L
```

On/off switch to enable/disable the power saving automatics which turn off the DIVING-PAM after 4 min without key operation. It is advisable to disable the AUTO-OFF when the DIVING-PAM is connected to an external power supply (via CHARGE-socket). Whenever the instrument is switched off manually, the AUTO-OFF function is enabled again (automatic reset to ON-position). The AUTO-OFF function is also automatically enabled when battery voltage drops below 11.2 V.

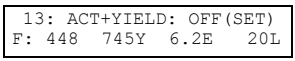
```
 11:AV. 564Y  5.9E   8No
F: 448  745Y  6.2E   20L
```

Function to average a number of consecutive YIELD- and ETR-determinations. The SET-key is used to reset the counter to 0 and to erase the averaged values of the preceding measurements. For safety's sake the reset must be confirmed by pressing the ∧-key. The averaged YIELD and ETR are shown in the top line, whereas in the bottom line the values of the last measurement are displayed.

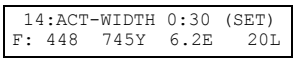
```
 12: ACT-LIGHT: OFF(SET)
F: 448  745Y  6.2E   20L
```

On/off switch of the internal actinic light source (halogen lamp). This can also be directly operated via CTRL + SET. The internal actinic lamp is not

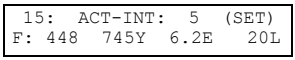
meant to be turned on for extended periods of time, as this may lead to excessive internal heating. Therefore, the illumination periods are restricted (see menu point 14: ACT-WIDTH). There is a blinking sign (ACT) in the upper left corner while actinic illumination is on.



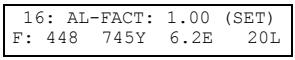
On/off switch of the internal actinic light source, with additional application of a saturation light pulse for YIELD-assessment at the end of the illumination time which is set by menu point 14: ACT-WIDTH. There is a blinking sign (A+Y) in the upper left corner of the display while actinic illumination with terminal YIELD-determination is running. This function can be also directly started from standard position 1 by double key operation CTRL + START.



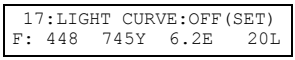
Setting of actinic illumination time. The setting can be modified via SET and the arrow-keys in 10 s steps. Maximal setting is limited to 5 min (5:00) in order to avoid excessive internal heating.



Setting of intensity of internal actinic light source (halogen lamp). The setting can be modified via SET and the arrow-keys between 0 and 12. The range covered by intensities 1-12 can be shifted up and down with the help of AL-FACT (menu point 16).

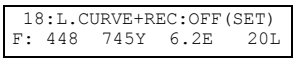


Actinic light factor by which the range of actinic intensities (ACT-INT, menu point 15) can be shifted up and down. The standard factor of 1.00 can be modified between 0.5 and 1.5 via SET and the arrow keys. The relationship between AL-FACT and PAR is non-linear.

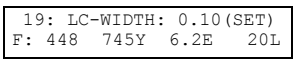


When switched on via SET, first the maximal YIELD in the absence of actinic light (Fv/Fm) is measured and then a series of 8 consecutive YIELD-measurements at increasing light intensities is started. This function

can be also directly started by double key operation CTRL + ∧. The time periods at the different intensities are set by menu point 19: LC-WIDTH. There is a blinking sign (LC) in the upper left corner of the display while a LIGHT CURVE is recorded. The series involves YIELD-determinations at 8 settings of actinic light. It starts with the intensity-setting, which is selected by 20: LC-INT, where one can choose between values from ACT-INT 1 to 5, with the standard setting being ACT-INT 3. The range of absolute PAR-values corresponding to these settings can be moved up and down with the help of menu point 16: AL-FACT or by changing the distance between fiberoptics and sample. The effective PAR-values at the sample surface may be calibrated by the LIGHT-CALIBRATION routine (menu point 8). A LIGHT CURVE can provide profound information on the overall photosynthetic performance of a plant, even if the illumination periods are too short to achieve true steady states. **Note:** Due to the unavoidable internal heating during recording of a LIGHT CURVE, assessment of absolute fluorescence signal amplitudes is problematic, but this does not affect correct determination of the ratio ΔF/Fm'.

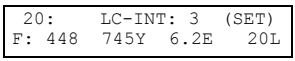


When switched on via SET, a LIGHT CURVE is measured as described for menu point 17 and in the following dark period the recovery of YIELD is assessed by 6 consecutive measurements at 10 s, 30 s, 60 s, 2 min, 5 min and 10 min following illumination. **Note:** Due to the unavoidable internal heating during recording of a LIGHT CURVE assessment of absolute fluorescence signal amplitudes is problematic, but this does not affect correct determination of the ratio ΔF/Fm'.

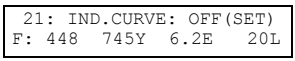


LC-WIDTH determines the illumination time at each intensity setting. 10 s are

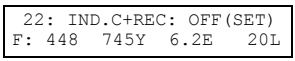
sufficient for so-called „rapid light curves“. It is limited to 3 min in order to avoid excessive internal heating.



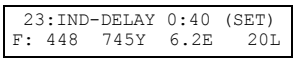
The LC-INT determines the starting intensity which can be chosen between settings 1 to 5. LIGHT CURVES always involve 8 intensities. Hence, more emphasis may be put either on the linear rise or on the plateau region of the curve.



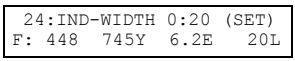
This function starts registration of a dark-to-light INDUCTION CURVE with Saturation Pulse Quenching Analysis. Normally dark-adapted samples are used. First a saturation pulse is given for determination of Fo, Fm and Fv/Fm. After a certain dark time, set by IND. DELAY (menu point 23), ACTINIC LIGHT at a given intensity (ACT-INT, menu point 15) is turned on and 8 saturation pulses are applied at intervals determined by IND.WIDTH (menu point 24).



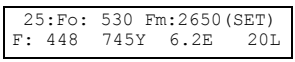
In addition to the recording of dark-to-light INDUCTION CURVE (as described for menu point 21), after turning off the ACT.-LIGHT 6 saturation pulses are applied at 10 s, 30 s, 60 s, 2 min, 5 min and 10 min to assess the dark recovery of fluorescence parameters.



Delay time between first saturation pulse and turning-on of ACT-LIGHT. The default setting is 40 s. Possible settings range from 5 s to 10 min.

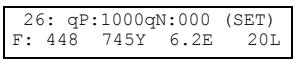


Time interval between two consecutive saturation pulses during recording of IND.CURVE. The default setting is 20 s. Possible settings range from 5 s to 3 min.



Function to sample the minimal fluorescence, Fo, and maximal fluorescence, Fm, of a dark-adapted sample by use of the SET-key.

The thus sampled values are stored until new values are sampled via SET. With START a normal YIELD-determination is carried out and the given Fo- and Fm-values are maintained. The stored Fo- and Fm-values are used for determination of the quenching coefficients qP, qN and NPQ (see menu points 26 and 27). In some applications, in order to obtain minimal Fo it is advantageous to make use of the ML-BURST function (see menu point 5).

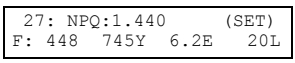


Coefficients of photochemical quenching, qP, and non-photochemical quenching, qN, as defined by the following equations:

$$qP=(M-F)/(M-Fo) \text{ and } qN=(Fm-M)/(Fm-Fo)$$

In order to obtain the usual values between 0 and 1, the displayed values have to be multiplied by 0.001. qP is set to 000 if M<F and qN is set to 000 if M>Fm. qN is set to 1.000 if M<Fo.

Note: M here represents the maximal fluorescence measured by a saturation pulse in any given light state (normally denoted Fm'), whereas Fm and Fo are the particular values sampled via menu point 25 after dark-adaptation. The thus determined values of qP and qN should be considered approximations only, as a possible non-photochemical quenching of Fo is not taken into consideration.



Parameter describing non-photochemical quenching defined by the equation:

$$NPQ = (Fm-M)/M$$

Note: M here represents the maximal fluorescence measured by a saturation pulse in any given light state (normally denoted Fm'), whereas Fm is the particular value sampled via menu point 25 after dark-adaptation. NPQ has been shown to be closely related to the excess light energy which is actively dissipated by plants into heat in order to avoid photodamage. Contrary to qN, NPQ-determination

does not require knowledge of Fo and is not affected by non-photochemical quenching of Fo. NPQ is set to 0.000 if M>Fm.

```
 28: REP-CLOCK: OFF(SET)
F: 448  745Y  6.2E   20L
```

On/off switch of repetition clock which serves to trigger a number of functions which are specified in menu point 29: CLOCK ITEM. This function can be also directly started by double key operation CTRL + MEM.

```
 29:CLOCK ITEM:SAT (SET)
F: 448  745Y  6.2E   20L
```

This menu point allows to choose between the following functions to be triggered by the REPETITION CLOCK:

SAT-PULSE, ACT-LIGHT, ACT + YIELD, LIGHT CURVE, L-CURVE + REC., IND. CURVE , IND.C + REC.

```
 30: CLK-TIME:00:30(SET)
F: 448  745Y  6.2E   20L
```

Setting of clock interval, which is the time between two consecutive saturation pulses (or other functions) triggered by the REP-CLOCK (menu point 28). The setting can be modified via SET and the arrow-keys in 10 s steps. Possible settings range from 0:10 to 42:30. When moving beyond the maximal time, the lowest values are reached and vice versa.

```
 31: TIME 14:43:51 (SET)
F: 448  745Y  6.2E   20L
```

Display of present time which can be modified via SET and the arrow-keys. With SET one can move from the hours to minutes and vice versa. The change is terminated via MODE.

```
 32:  DATE 17-OCT  (SET)
F: 448  745Y  6.2E   20L
```

Display of present date which can be modified via SET and the arrow-keys. With SET one can move from the days to months and vice versa. The change is terminated via MODE.

```
 33:  YEAR 1997    (SET)
F: 448  745Y  6.2E   20L
```

Display of present year which can be modified via SET and the arrow-keys. The change is terminated via MODE.

```
 34:  BATT: 12.8V (12.3)
F: 448  745Y  6.2E   20L
```

Display of battery voltage. The value in brackets shows the voltage observed during the last saturation pulse (transiently decreased value due to high current of halogen lamp). YIELD-measurements may become erroneous, if the voltage during a pulse drops below 8.0 V (Error message 6: CHECK BATTERY). The battery voltage is a non-linear function of the remaining battery capacity. When dropped below 11.2 V (without saturation pulse) the remaining capacity is approx. 20 % and recharging soon will become necessary. In this case there is a warning (BAT-sign blinking in the left corner of the upper display line).

```
 35:  WAT.TEMP: 24C
F: 448  745Y  6.2E   20L
```

Display of water temperature. Full equilibration of the temperature sensor with the water may require several minutes.

```
 36: RES.SETTINGS: (SET)
F: 448  745Y  6.2E   20L
```

Command to reset all instrument settings (which can be varied via the MODE-menu) to the standard settings preset at the factory (see in section 7.1).

```
 37: PROGR.D2.07(280698)
F: 448  745Y  6.2E   20L
```

Number and date of origin of current program version of the DIVING-PAM which is resident on EPROM.

```
 38:  MEMORY: 125  (SET)
F: 448  745Y  6.2E   20L
```

Function to move the present MEMORY-front to any number between 1 and 4000. This function may be important when the MEMORY is full and the user wants to avoid overwriting of certain older data.

Note: The MEMORY-front is identical to the MEM-number under which the last data set was stored. It advances by 1 with each following YIELD-determination.

```
 39: CLEAR MEMORY  (SET)
F: 448  745Y  6.2E   20L
```

Command to erase all data accumulated in MEMORY. For safety's sake this command

is not yet carried out by SET but requires confirmation by pressing the ∧-key. Then the MEMORY-front is reset to 0 and the data set recorded with the next saturation pulse will be in MEM position 1.

```
 40: LIGHT-OFFS: 20(SET)
F: 448  745Y  6.2E   20L
```

Function for adjustment of PAR-reading by comparison with calibrated device. Particular care must be taken that both sensors are exposed to the same photon flux density. After SET, the PAR-reading (L) can be adjusted by the arrow-keys in steps of 1 µmol quanta m^{-2} s^{-1}. For proper calibration over a wide range of PAR also adjustment of LIGHT-GAIN (menu point 41) may be required. This can be checked by comparison with calibrated device at a different PAR-value.

```
 41:LIGHT-GAIN:1.00(SET)
F: 448  745Y  6.2E   20L
```

Function for adjustment of PAR-reading. The adjustment via LIGHT-GAIN should be carried out after a preceding adjustment by LIGHT-OFFS (menu point 40) at a different light intensity, such that the slope of the response curve can be evaluated. For highest accuracy, the LIGHT-OFFS then may have to be adjusted once more (menu point 40).

```
 42: DEP.OFFS:  0.0(SET)
F: 448  745Y  6.2E   20L
```

Offset for compensation of changing values of atmospheric pressure.

```
 43: DEP.GAIN: 1.00(SET)
F: 448  745Y  6.2E   20L
```

Function for adjustment of water depth-reading by calibration against alternative device.

```
 44: ZERO-OFFS:  20(SET)
F: 448  745Y  6.2E   20L
```

Display of present zero offset value which normally is identical to the value obtained automatically via AUTO-ZERO (menu point 2). Following SET, this value can be manually modified using the arrow-keys.

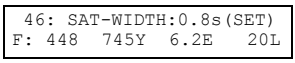
```
 45: ETR-FAC: 0.84 (SET)
F: 448  745Y  6.2E   20L
```

Display of current factor applied for calculation of relative electron transport rate (ETR) which for a standard leaf is defined as follows:

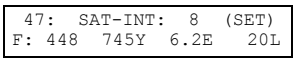
$$ETR = Yield \times PAR \times 0.5 \times 0.84$$

The standard factor 0.84 corresponds to the fraction of incident light absorbed by a leaf. The preset value, which corresponds to an average observed with a variety of leaf species, can be modified via SET and the arrow-keys.

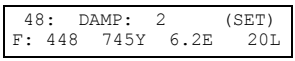
Note: For aquatic plants this factor may differ substantially from 0.84 and the user should enter a measured value or a reasonable estimate.



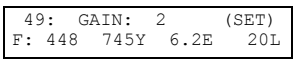
Setting of the width of saturating light pulses for YIELD-determination. The setting can be changed between 0.4 and 3.0 s in 0.2 s steps.



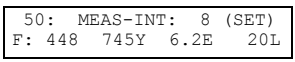
Setting of saturation pulse intensity for YIELD-determination. Settings can be changed between 0 and 12.



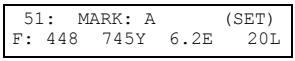
Setting of electronic signal damping. The three settings correspond to the following time constants (defined for 63.2 % of a signal change): 1: 0.05 s, 2: 0.2 s, 3: 1 s.



Setting of electronic signal gain (amplification factor) which can be varied between 1 and 12. By increasing GAIN not only the signal but also the noise increases in proportion. Any change in GAIN requires a new determination of the unavoidable background signal via AUTO-ZERO (menu point 2).



Setting of intensity of measuring light which can be varied between 0 and 12. Any change in MEAS-INT requires a new determination of the unavoidable background signal via AUTO-ZERO (menu point 2).



Letter from A to Z for identification of a particular type of sample. This MARK is entered into the MEMORY with every new data set measured in connection with a saturation pulse. It can be helpful when a number of different plants are assessed in the field.

8 Components of the DIVING-PAM

The basic functional system for measurements of fluorescence yield and of the effective yield of photosynthetic energy conversion consists of the DIVING-PAM Main Control Unit and the fiberoptics.

Additional peripheral components can be connected to sockets at the right side of the Main Control Unit. Fig. 2 shows a functional block diagram of the DIVING-PAM and its most essential accessories.

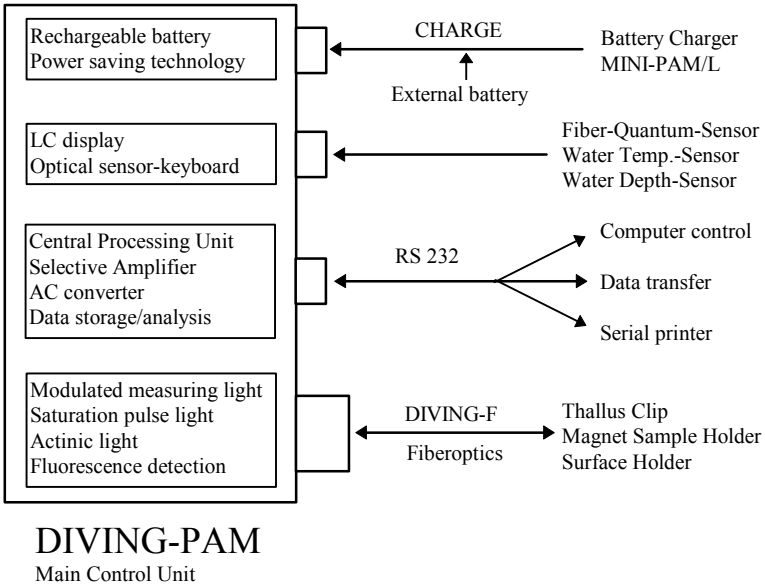


Fig. 2

8.1 Main Control Unit

Except for the fiberoptics and the light sensor cable the Main Control Unit contains all essential components of the DIVING-PAM Fluorometer. These include the optics for fluorescence excitation and

detection, the selective amplifier, the data acquisition and storage system, an actinic light source for saturation pulses and continuous illumination, a large rechargeable battery and the user interface, with the LC-display and keyboard. Details on some of these components are given in the following sections.

8.1.1 Fluorescence excitation and detection

Fluorescence is excited by pulse modulated red light from a light-emitting-diode (LED, 655 nm). The pulse-width is 3 μs and pulse frequency is 0.6 or 20 kHz. In the so-called "burst-mode" pulse trains of 0.2 s are alternating with 0.8 s dark-intervals. The LED-light is passed through a cut-off filter (Balzers DT Cyan, special) resulting in an excitation band peaking at 650 nm, with a very small "tail" at wavelengths beyond 700 nm. Fluorescence is detected with a PIN-photodiode (type BPY 12, Siemens) at wavelengths beyond 700 nm, as defined by a long-pass filter (type RG 9, Schott).

The effective intensity of the measuring light at the level of the sample is an important parameter for correct determination of the minimal fluorescence yield, Fo, of a dark-adapted sample. Its absolute value depends on

- intensity setting (menu point 50, preset value 8),
- measuring frequency (menu point 4, preset at 0.6 kHz),
- burst mode status (menu point 5, preset to be off),
- distance between fiberoptics and sample (standard 10 mm).

At the standard distance of 10 mm between fiberoptics and sample, and at measuring light intensity 8, the quantum flux density of photosynthetic active radiation typically amounts to 0.15 μmol quanta m^{-2} s^{-1} at 0.6 kHz and 5 μmol quanta m^{-2} s^{-1} at 20 kHz. These

values are lowered to 1/5 when the burst mode is active. At such low intensities an "actinic effect" of the measuring light normally can be excluded.

8.1.2 Internal halogen lamp as actinic light source

A miniature 8 V/20 W halogen lamp (type Bellaphot, Osram) serves as light source for saturation pulses and for continuous actinic illumination. The light is filtered two-fold by a heat-reflecting filter (Balzers, Calflex-X, special) and by a short-pass filter (Balzers, DT Cyan, special), such that white light with negligible content of wavelengths beyond 700 nm is obtained.

It is not recommended to operate the internal halogen lamp for extended periods of actinic illuminations as this would lead to excessive internal heating. This aspect must be taken serious particularly when light curves are automatically recorded (menu points 17 and 18) and when the range of actinic intensities is increased by AL-FACT (menu point 16). A temperature-sensor, which is mounted in the vicinity of the lamp, causes turn-off of the lamp power supply when 70 °C is reached. It is turned on again when temperature has dropped to approx. 55 °C. The internal temperature affects the output of the measuring light LED. A 1 °C temperature rise leads to approx. 1 % lowering of the measuring light intensity. While not affecting the actual YIELD-measurement (i.e. $\Delta F/Fm'$), this will lead to a corresponding drop in the fluorescence signal.

8.1.3 Rechargeable battery

A relatively large rechargeable lead acid battery (12 V/2.1 Ah) is mounted within the DIVING-PAM housing. For recharging, the

Battery Charger MINI-PAM/L is provided which is connected to the CHARGE-socket at the right side of the DIVING-PAM. The charger, which operates at input voltages between 90 and 260 V AC, features an overload protection. Full charging of an empty battery takes approx. 5 hours. Battery voltage is displayed under menu point 34. The warning 'BAT' is given in the upper left corner of the display when voltage drops below 11.2 V in the resting state. If in this situation the AUTO-OFF function (menu point 10) is disabled, it will be automatically enabled again. In addition, there is the warning Err. 3: 'LOW BATTERY' which, however, only occurs upon measurements involving START. After this error message approximately 20 further measurements can be made, but then the battery should be recharged. In brackets also the voltage is given which was measured during the last saturation pulse. It is normal that voltage drops by ca. 0.5 V during a saturation pulse. However, if it drops below 8 V, YIELD-measurements may become erroneous, as Fm' is likely to be underestimated. In this case, there is the warning Err. 6: 'CHECK BATTERY'.

With a fully charged battery the displayed voltage is 12.5 - 12.9 V. In first approximation, battery voltage can be taken as a measure of remaining battery power. The functional relationship between capacity (Ah) and voltage of a new battery is depicted in Fig. 3. It is apparent that battery voltage first drops steeply to about 12.3 V and then slowly decreases to about 11.8 V, from whereon there is a steep further drop to values below 11 V.

The DIVING-PAM can be also powered by an external 12 V battery for which purpose a special cable (MINI-PAM/AK, optional) is available which can be connected to the CHARGE-socket at the right side of the DIVING-PAM. It should be noted, that a recharging of the internal battery with a 12 V external battery is not possible.

Attention: Please make sure that after battery charging the sealing plug is fastened and, if necessary, the O-rings are greased, such that no water will penetrate into the housing under diving conditions.

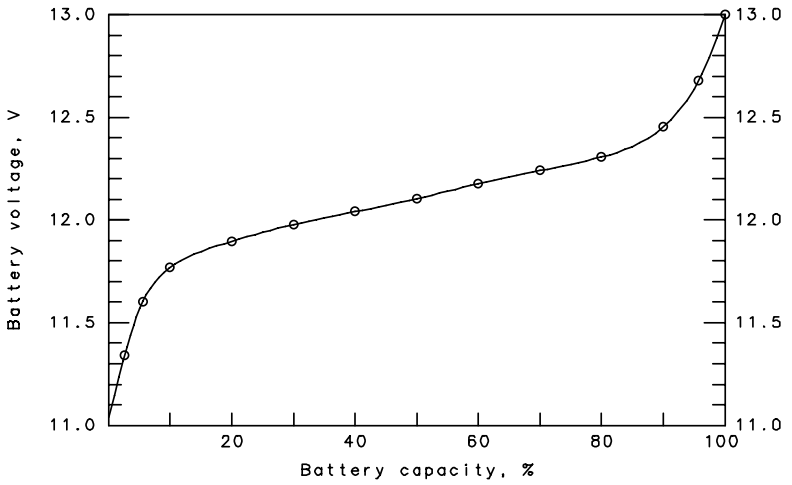


Fig. 3

8.1.4 LC-display

The data are displayed by a 24 x 2 character LC-display with backlight. The backlight, which switches on together with the instrument, automatically turns off again after 50 s, for the sake of saving battery power. It can be turned on again by pressing the CTRL-key for at least 3 s. Permanent display illumination can be switched on via MODE-menu point 9: DISP.ILLUM. However, it should be noted that it increases basic power consumption from 0.7 W to 2 W.

The information shown on the LC-display is intentionally restricted to the most relevant parameters. Additional information can

be called on display in connection with the 51 menu points and by entering the MEMORY.

8.1.5 Electronic components

The compact design of the DIVING-PAM is a consequence of recent progress in miniaturization of solid state integrated circuits. The central processing unit features a powerful CMOS microcontroller. The program software is stored in a CMOS EPROM. This EPROM can be exchanged by the user, if program updates become available (see 11.3). A CMOS RAM with 128 kB serves as data memory, providing storage capacity for 4000 data sets.

8.1.6 Description of the connectors

As indicated by corresponding arrows, the major electrical connectors at the right side of the DIVING-PAM are:

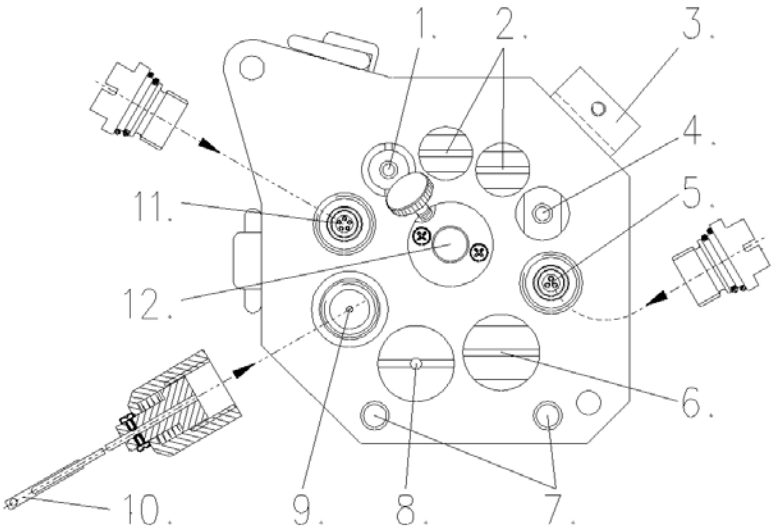
a) RS 232 (item 11 in Fig. 4)

An RS 232 interface cable is provided to connect the DIVING-PAM to IBM or IBM-compatible PCs for data transfer (see 9) or for remote control of the DIVING-PAM functions via PC keyboard operation (see 10).

b) CHARGE (item 5 in Fig. 4)

Together with the DIVING-PAM the Battery Charger MINI-PAM/L is delivered which connects to the CHARGE-input at the right side of the instrument. The charger can be used with line voltages of 90 to 260 V at 50-60 Hz. A special cable (MINI-PAM/AK) is available for connecting an external 12 V battery to the CHARGE-input. While the DIVING-PAM can be powered by this

external battery, it should be noted that the internal battery cannot be recharged in this way.



1. ON-switch
2. Not used
3. Holder for the light guide
4. Water temperature sensor
5. Connector for the battery charger
6. Not used
7. Distance rods
8. Depth sensor
9. Connector for the Fiber Quantum Sensor
10. Fiber Quantum Sensor
11. Connector for the RS 232 cable
12. Connector for the Fiberoptics DIVING-F

Fig. 4

8.2 Fiberoptics DIVING-F and Miniature Fiberoptics DIVING-F1 (optional)

The fiberoptics are inserted into the corresponding adapter at the right side of the DIVING-PAM (item 12 in Fig. 4). The active cross section of the standard version DIVING-F is 5.5 mm. A special version (DIVING-F1) with ∅ 2 mm is also available, consisting of a single plastic fiber. This is particularly recommended for use with small objects. In the standard version, numerous 70 µm fibers are thoroughly randomized over a 150 cm mixing pathway, such that a homogenous field of illumination is created.

The fiberoptics should be handled with care. Excessive bending, in particular close to the connector plug, should be avoided, as it would lead to fiber breakage with resulting loss in signal amplitude. The fibers are protected by a steel-spiral and plastic mantle which provides a natural resistance to strong bending.

Using the Miniature Fiberoptics DIVING-F1 (optional) the signal amplitude is particularly sensitive to the distance between fibertip and sample. A standard distance of 4 mm provides for a homogenous field of illumination and a very satisfactory signal amplitude, approximately equal to that obtained with the 5.5 mm ∅ fiberoptics at the standard distance of 10 mm. Signal amplitude can be further increased at least 4 fold, when the fibertip is advanced to the sample surface. It should be noted, however, that in this case the measuring light may show an actinic effect. This can be counteracted by use of the ML-BURST function (MODE-menu position 5, see 12.3.2).

Because of the strong influence of sample distance on signal amplitude, particularly with the 2 mm ∅ fiberoptics, it is recommended to clamp the fiberoptics tip at fixed distance to the sample surface. Otherwise there may be substantial errors, even in

the ratio measurement of ΔF/Fm, if there is a change of distance between the consecutive measurements of F and Fm, which are separated by approximately 1 s (see 12.3.6).

8.3 Fiber Quantum Sensor

The special Fiber Quantum Sensor is provided for assessment of the quantum flux density of photosynthetically active radiation. It is screwed on the corresponding connector (item 9 in Fig. 4) at the right side of the housing.

Essential opto-electronical elements of this micro-quantum-sensor are a 2.5 mm cross-section diffusing disk; a 1 mm diameter fiber guiding the scattered light via the connector to the detector within the DIVING-PAM housing; a filter combination selecting the photosynthetic active wavelength range between 380 and 710 nm; and a blue-enhanced silicon photodiode. Despite its small dimensions, the diffuser displays properties of 'cosine correction', i.e. also light impinging at rather small incidence angles is reliably monitored. The micro-quantum-sensor measures incident photosynthetic radiation in μmol quanta $m^{-2}s^{-1}$, i.e. in units of flux density. Hence, the measured parameter is identical to PPFD (photosynthetic photon flux density). It is displayed at the end of the second line of the LCD (...L) when the sensor holder is connected and MODE-menu point 7: EXT.LIGHT-S. is enabled (ON-position). The sensor was calibrated against a LI-COR Quantum Sensor (Type LI-190). The stability of calibration depends strongly on keeping the diffuser clean. It is advisable to check calibration regularly by comparison with a standard quantum sensor, like the LI-190. Any deviation can be corrected by entering a recalibration factor via menu point 41: LIGHT GAIN. A substantial increase of the calibration factor from its original value of 1.00 indicates dirt-deposition on the diffuser, which may be reversed by gentle cleaning using a cotton-

tip, moistened with some alcohol. In addition, it is possible to enter an offset value via menu point 40: LIGHT-OFFS.

The sensor head with the diffusing disk normally is mounted at the indicated site of the DIVING-PAM housing (item 3) or on top of the special Surface Holder provided with the DIVING-PAM. In both cases PAR is not measured at the same spot where fluorescence parameters are assessed and where the internally generated actinic light is effective. Hence, the PAR-readings relate to ambient actinic light intensity. However, a routine is provided (MODE-menu point 8: LIGHT CALIB.) by which the internally generated actinic light can be calibrated with the help of the Fiber Quantum Sensor.

Excessive bending of the fiber light guide of the sensor should be avoided. At a bending radius below 5 cm the signal will be attenuated. In most applications, it has proven practical to tape the Fiberoptics DIVING-F and the fiber light guide of the sensor together.

Rough underwater conditions may cause damage to the fiber light guide of the quantum sensor, particularly at the site where it connects to the DIVING-PAM housing. Such damage often can be repaired by the user simply by shortening the fiber at that end. For that purpose the two white screws have to be loosened, the fiber end pulled out and cut with a sharp knife. Then the fiber is fixed in the holder again, with its end slightly protruding from the hole at the inner side of the holder. Special polishing paper is provided in the green Utility Box. It should be applied with decreasing grade: grey (800 µm), yellow (12 µm), pink (3 µm) and white (0.3 µm). Eventually, the polished fiber end should be level with the inner surface of the holder. Then the sensor requires recalibration against a standard device, like the LI-COR Quantum Sensor.

It should be noted that for optical reasons the Quantum Sensor is less sensitive when used underwater as compared to air by a factor of

1.33. Therefore, if calibration was carried out in air, the LIGHT-GAIN (MODE-menu point 41) should be set to 1.33.

8.4 Depth Sensor

The water depth is monitored with the help of a pressure sensor mounted at the right side of the DIVING-PAM (site 8 in Fig. 4). It is part of the data set stored with every YIELD-measurement. As the 1.5 m long Fiberoptics separate DIVING-PAM and the site where fluorescence is assessed, the measured depth may differ up to 1.5 m from the true value.

8.5 Water temperature sensor

Water temperature is sensed via a metal "finger" protruding from a connector at the right side of the DIVING-PAM housing (site 4 in Fig. 4). It should be noted that several minutes may be required for a correct reading, due to the heat capacity of the housing.

8.6 Sample-Fiberoptics Holders

For correct YIELD-determinations it is required that during the measurement (i.e. for approx. 2 s) the distance between fiberoptics exit plane and sample is kept constant. Different types of sample holders are provided for various applications (e.g. dark adaptation) and various objects (e.g. corals, macroalgae).

8.6.1 Dark Leaf Clip DIVING-LC

The Dark Leaf Clip DIVING-LC weighs approx. 6.5 g and, hence, can be attached to most types of leaves without any

detrimental effects. It is equipped with a miniature sliding shutter which prevents light access to the leaf during a dark-adaptation period and which is opened for the actual measurement only, when exposure to external light is prevented by the fiberoptics. Proper dark-adaptation is essential for determination of the maximal quantum yield Fv/Fm (see 12.3.1).

With the Dark Leaf Clip the fiberoptics are positioned at right angle with respect to the leaf surface at the relatively short distance of 3 mm. As a consequence, measuring light intensity and signal amplitude are higher than with the standard distance of 10 mm. In order to avoid signal saturation, it may be necessary to lower the settings of MEAS-INT (menu point 50) and GAIN (menu point 49) with respect to the standard settings. For optimal results the burst mode of measuring light (menu position 5: ML-BURST) is recommended (see 12.3.2).

When the shutter is still closed and measuring light is on, an artifactual signal is observed, which is due to a small fraction of the measuring light which after reflection from the closed shutter penetrates to the photodetector. However, the reflection is much smaller when the shutter is opened and the measuring light hits the strongly absorbing leaf instead of the shiny metal. Therefore, it is recommended to carry out compensation of the unavoidable background signal by AUTO-ZERO (menu point 2) with the fiberoptics end directed into the air.

8.6.2 Surface Holder DIVING-SH (corals)

For the study of corals and epilithic plants a special "Surface Holder" is provided which can be attached with the help of three hooks and rubber bands to uneven, creviced surfaces, assuring a fixed distance between fiberoptics and sample. The three screws,

which hold the rubberbands, can be moved up and down to adjust the desired distance.

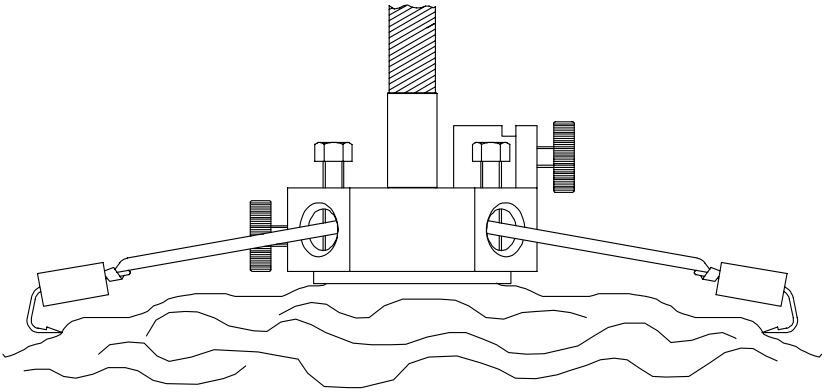


Fig. 5: Surface Holder DIVING-SH

The Fiber Quantum Sensor (see 8.3) can be mounted on the Surface Holder. For this purpose, the same PVC-holder can be used, which is mounted with 2 screws on top of the right side part of the DIVING-PAM housing upon instrument delivery.

8.6.3 Magnet Sample Holder DIVING-MLC (optional)

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.

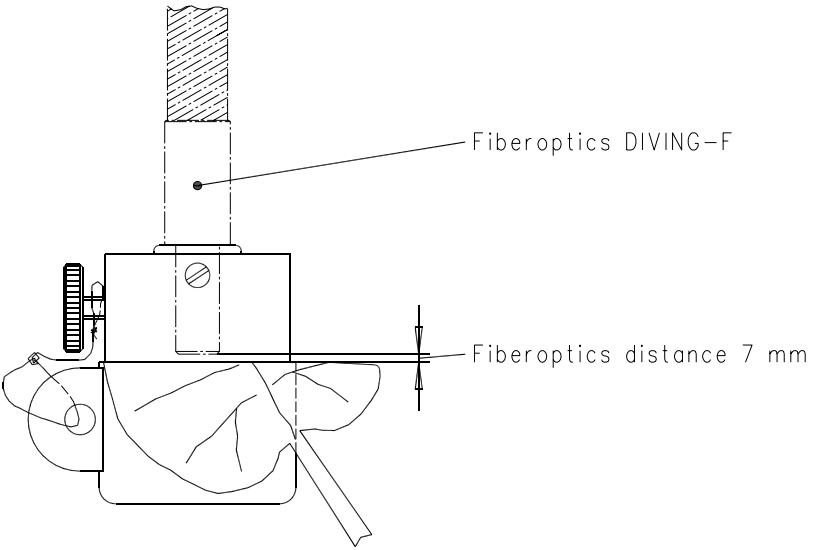


Fig. 6: Magnet Sample Holder DIVING-MLC

9 Data Transfer

Special programs are provided for the transfer of data from the DIVING-PAM via RS 232 interface cable to a PC. MS-DOS and Windows versions of the PamTrans Data Transfer Software are available on the disk which is delivered together with the DIVING-PAM. They are installed as follows:

MS-DOS version: Enter 'A:' and enter 'INSTALL'. After connection of the RS 232 cable and definition of the communication port (COM 1, 2, ...) the system is ready for data transfer.

Windows version: Enter 'A:\SETUP' at the Program Manager level of Windows (select first 'File' and then 'Run'). Then SETUP will be initialized and PAMTRANS.EXE installed. Before data transfer can be carried out, the RS 232 cable has to be connected. The communication port (COM 1, 2, ...) is selectet automatically.

With the Windows-version transfer of 1000 data sets takes ca. 90 s, as compared to 9 min with the MS-DOS version. The steps required for carrying out the transfer of defined data sets, which are almost identical for the two versions, shall be briefly described. Fig. 7 and Fig. 8 illustrate the screen layouts used with MS-DOS- and Windows-versions, respectively. After start of the program the last measured data set is entered by default into the 'Last Data' field. The user can enter MEMORY-numbers defining the limits of the transfer into the 'First Data' and 'Last Data' fields. Before starting the transfer, a Destination File must be entered into the corresponding parameter field. The data are processed as text file and the extension .TXT will be automatically added to the Destination File name.

Upon start of data transfer (F6 in DOS and START button in Windows) the data sets will be transferred starting with 'First Data'. Transfer can be stopped (Esc in DOS and Exit button in Windows).

If the address of 'Last Data' has a lower number than that of 'First Data', after MEM 4000 the transfer continues from MEM 1 upwards.

With the function VIEW you can have a look to the transferred data file. Shown is the file selected in the fileselect box and it can be changed.

```
MINI-PAM Data Transfer for MS-DOS V1.1

First Data:1    :  Data:   1,  A ,08:06:13, 21/01/98,  735, 1647,:
Last Data :18   :  Data:  18,  A ,15:07:49, 27/01/98,  760, 1842,:

Dest. File:C:\

+  -> Spin one data set up
-  -> Spin one data set down
#  -> Show selected data set
F6 -> Start transfer from PAM to destination file
F10-> Leave MINI-PAM Data Transfer
Esc-> Abort running transfer

Path and name of destination file. If no extension given, .TXT
will be added. Return jumps to first parameter.
```

Fig. 7: MS-DOS version of software PamTrans

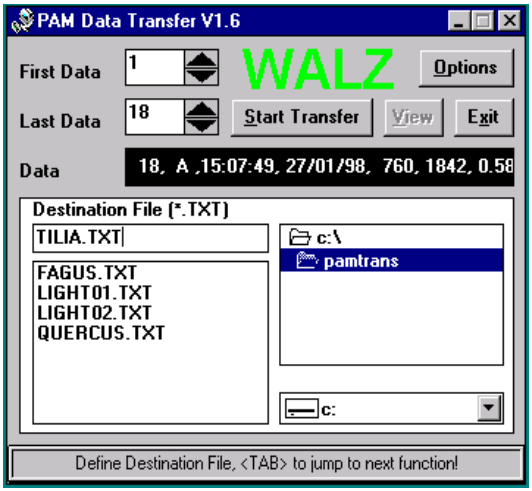


Fig. 8: Windows version of software PamTrans

Update versions of the PamTrans Data Transfer Software may be distributed in the future (e.g. with increased speed of transfer). In this

case, relevant information concerning the update will be contained in a Read-me file on the new disk.

10 Operation of the DIVING-PAM via a PC-Terminal and the RS 232 Interface

The DIVING-PAM is basically conceived as a stand-alone instrument, i.e. the most essential measurements can be carried out without the need for any peripheral instruments. There is, however, the possibility to connect the DIVING-PAM via the RS 232 interface to a PC and to control all functions by PC-keyboard operations. For this purpose, first a suitable terminal program must be installed which allows communication between the PC and the DIVING-PAM. In Windows the TERMINAL program is available (under Accessories in the Program Manager). In order to enable this program for communication with the DIVING-PAM the following steps are required:

- Define communication parameters (SETTINGS-menu)

Baud rate	9600
Data bits	8
Stopbits	1
Parity	none
Protocol	Xon/Xoff
Connector	Com 1, 2 ...

- Define terminal preferences (SETTINGS menu)

Colums	132
Terminal fond	Fixed sys

- Create DIVING-PAM.trm (SAVE AS ... in FILE-menu)

For the communication between PC and DIVING-PAM a special set of commands is provided which is listed in the Appendix (section 13.4). Each command consists of one or several low-case letters which may be followed by further specifications. Any command is executed via 'Return'.

The following examples may serve to illustrate the principle of DIVING-PAM TERMINAL operation (a Return is required after each command, which from here onward will not be mentioned anymore):

- 'a1' will switch on actinic light, which will be switched off again by 'a0'.
- 's' starts a saturation pulse.
- with 'f' and 'fmp' the values of the fluorescence parameters F and Fm', as measured with the last saturation pulse, can be called on display.
- 'o45' transfers dataset No. 45, 'o' transfers the next dataset.
- 'o+90' transfers datasets up to No. 90, 'o+' transfers all datasets up to the last one stored (memory front).
- 'of' shows the format of a dataline.
- 'pas' shows the momentary settings.
- 'pr1' activates the auto-print function, a new dataset will be transferred after each Yield-measurement (Sat.-Pulse).

In this way, it is possible to carry out all DIVING-PAM functions by remote control from a PC terminal and to transfer information from the DIVING-PAM to a PC. In principle, using the TERMINAL-program also a network of DIVING-PAM Fluorometers can be operated. For the transfer of a larger number of data sets from the DIVING-PAM to a PC, the PamTrans-software is recommended (see section 9). Alternatively, the optional WinControl software is available, by which all operations of data acquisition, transfer, recalculation and display can be handled in a most comfortable way.

11 Maintenance

11.1 Internal battery and its replacement

The internal battery is essentially 'maintenance free'. However, even when the instrument is switched off, there is some discharge, which is stimulated by elevated temperatures. If it is forseeable that the instrument will not be used for some months, the battery should be charged beforehand. Excessive discharge of the battery should be avoided, as this may cause irreversible damage. Such damage involves lowering of the capacity and increase of internal resistance, with the consequence that recharging becomes necessary after relatively short times of operation and that there is an excessive lowering of voltage during a saturation pulse. In this case, battery replacement is recommended.

The DIVING-PAM features a number of functions and warnings which make it highly unlikely that excessive discharge of the battery occurs inadvertently:

- AUTO-OFF (when there was no key operation for 4 min)
- Backlight-off (50 s after switching the instrument on)
- Menu point 34: BATT (display of battery voltage in the resting state as well as with application of a saturation pulse)
- Warning 'BAT' on the display, when battery voltage drops below 11.2 V in the resting state
- Error message 3: 'LOW BATTERY' when battery voltage drops below 11.2 V (coupled to measurements involving START).
- Error message 6: 'CHECK BATTERY' when battery voltage drops below 8.5 V during a saturation pulse.

- When battery voltage drops below 8.5 V the CLOCK is automatically turned off. This is important as the CLOCK disables the AUTO-OFF function.
- When battery voltage drops below 8.0 V, the DIVING-PAM immediately turns-off itself.

If replacement of the battery becomes necessary, the DIVING-PAM housing must be opened.

11.1.1 Opening the housing

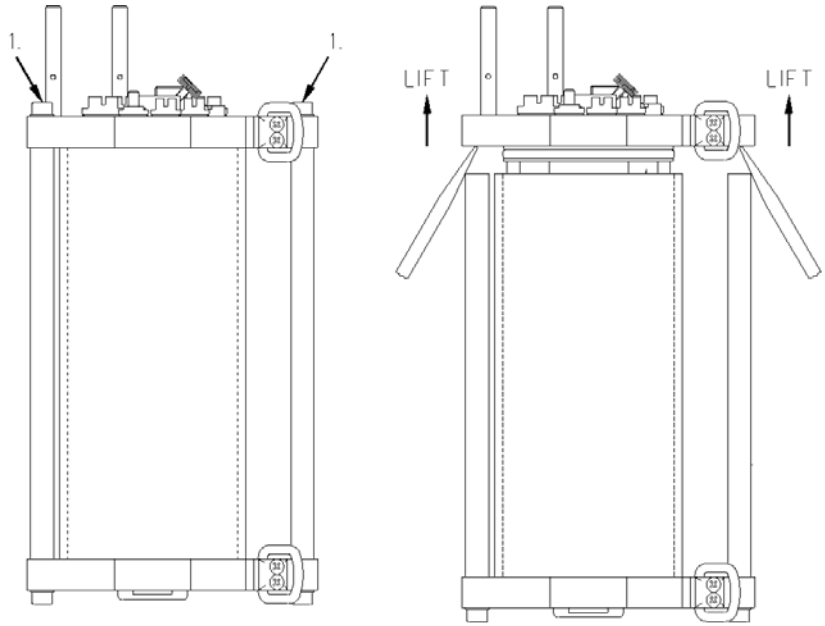


Fig. 9: Opening the DIVING-PAM housing

In order to open the DIVING-PAM housing, the instrument should be put with its right side (containing the various connectors) up. The perspex lid, which with the help of an O-ring tightly seals against the perspex tube of the housing, can be lifted up after

removing the two hex nut screws with which it is connected to the two aluminum bars. A special hex key fitting these screws is provided in the Utility Box. Loosening the lid requires some power, which can be exerted best, when the other side of the housing is firmly pressed down. Please do not use sharp-edged levers, like screw-drivers, as these may cause damage. Once the lid becomes loose, the whole interior of the instrument can be pulled out.

11.1.2 Exchanging the rechargeable battery

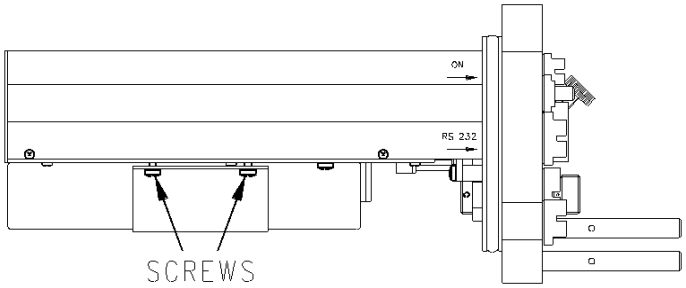


Fig. 10: Side view of the interior of the DIVING-PAM

After having pulled out the interior of the instrument from the perspex tube housing, the battery can be exchanged. It is fixed to the bottom of the instrument by a metal holder with four screws. When connecting the new battery, please note the proper polarities (red/positive and black/negative).

11.1.3 Closing the housing

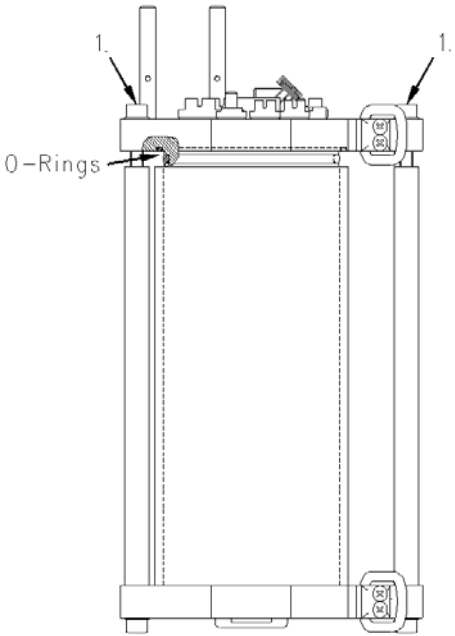


Fig. 11: Closing the DIVING-PAM housing

The housing must be carefully closed in order to avoid any leakage at high pressures under water. The sealing O-rings should be checked and, if necessary, some additional silicon grease applied. A syringe with silicon grease is provided in the Utility Box. Please make sure before closing the lid, that the little bag with silica gel is put back into the housing. The best place for this bag is next to the battery. If the silica gel is exhausted (pink color), it should be renewed or dried at 75 °C until it is blue again. The two screws marked with 1. should be tightened alternately with half turns.

11.2 Halogen lamp and its replacement

Due to a very efficient optical system, very high light intensities can be obtained with the internal 8 V/20 W halogen lamp, without applying the maximal allowable voltage. This results in a long life time of the lamp which is primarily meant to generate saturation pulses. Continuous operation is limited to 5 min periods in order to avoid excessive internal heating of the DIVING-PAM.

For replacement of the internal halogen lamp the DIVING-PAM must be opened and the interiour of the instrument pulled out (see subsection 11.1.1). The halogen lamp is located underneath the aluminum cover. In order to exchange the lamp, please proceed as follows:

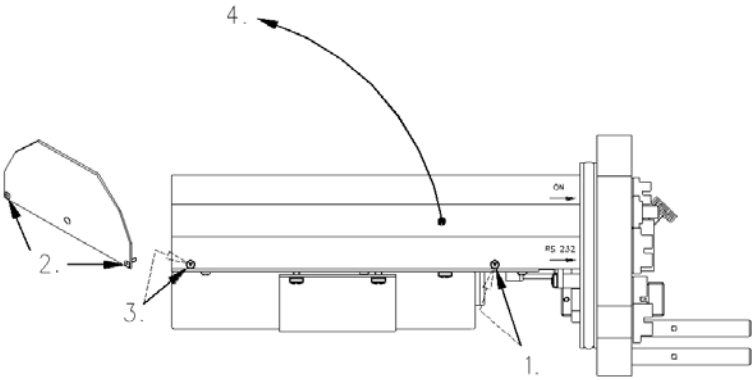


Fig. 12: Side view

a) Remove the two screws which are marked with 1. (one screw at each side)

b) Remove the two screws which are marked with 2. at the rear covering plate and detach this plate

c) Loosen (do not remove) the two screws marked with 3. (one screw at each side)

d) Lift the aluminum cover (direction indicated by 4.)

The position of the halogen lamp (and also of the EPROM, see 11.3) are shown in the following figure. It is held in a pre-focused position by an aluminum mounting-frame, which is fastened to the optical compartment by two screws. These screws can be removed with a small hex nut driver provided in the Utility Box. Spare Halogen Lamps (SL-8/20) with mounting-frame are available. When the housing is closed again, please proceed as outlined above (11.1.3).

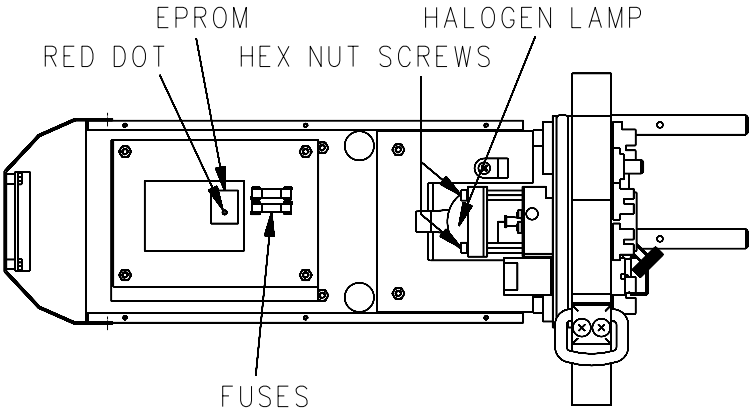


Fig. 13: Top view

11.3 EPROM and its replacement

The location of the EPROM on the microcontroller board is indicated in Fig. 13, which shows a top view on the interior of the DIVING-PAM after removing the lid (see 11.1.1) and lifting the aluminum cover (see 11.2). The EPROM contains the software of the current program version (see menu point 37). It can be readily exchanged against a new EPROM when program updates become available. Please note the position of the little red dot on the EPROM. For lifting the EPROM, a paper-clip can be useful. Put a finger on it, so that it does not jump up. When installing the replacement, make sure that the red dot is on the proper side (there is also an arrow on the EPROM socket). Push in the EPROM firmly, until there is a click and it sits level at all sides. After EPROM replacement it is recommended to reset the instrument settings and to clear the memory (menu point 36 and 39).

11.4 Fuse replacement

The DIVING-PAM contains two fuses which are mounted on the main board, at the right hand side of the EPROM (see Fig. 13):

315 mA: general electronics (top)

4 A: halogen lamp circuit (bottom)

In order to replace a fuse, the DIVING-PAM housing must be opened (see 11.1.1) and the aluminum cover must be lifted (see 11.2).

11.5 Cleaning

It is recommended to clean the DIVING-PAM carefully after every dive. A feasible way is to just take it under the shower. Please

make particularly sure that the plugs covering the CHARGE and RS 232 sockets are carefully cleaned. It is important that no sand or dirt sticks to the O-rings.

12 Chlorophyll Fluorescence Measurements with the DIVING-PAM

Chlorophyll fluorescence is a large signal and in principle its measurement is rather simple. Hans Kautsky already observed chlorophyll fluorescence changes by his bare eyes in 1931 and suggested that these are related to photosynthesis. In the following 50 years, with the progress of modern electronics and photooptics, highly sensitive and fast fluorometers were developed which contributed substantially to the elucidation of the basic mechanisms involved in the complex process of photosynthesis. Chlorophyll fluorescence always has been a pioneering tool. Many aspects which eventually were analyzed in great detail by more specific methods, were first discovered by chlorophyll fluorescence measurements. Such discoveries are still taking place, presently mostly at the level of regulation of the complex photosynthesis process under the control of changing environmental factors. This still is a widely open field of plant science, as only recently the instrumentation and methodology for *in situ* fluorescence measurements and analysis have become generally available. Progress in this field of research has been greatly stimulated by the invention of the patented Pulse-Amplitude-Modulation (PAM) measuring principle (see section 12.2 below). The first PAM-101 Chlorophyll Fluorometer, with its accessory modules PAM-102 and PAM-103, as well as the PAM-2000 Portable Fluorometer and the MINI-PAM Photosynthesis Yield Analyzer have been successfully used all over the world, as can be judged from the large number of publications based on investigations carried out with these instruments.

With the DIVING-PAM, for the first time it has become possible to extend the study of *in situ* photosynthesis into the underwater world. This has opened a completely new field of ecophysiological research, including such fascinating topics as coral photosynthesis

and the interaction between animal hosts and endosymbiotic phycobionts.

Just like the MINI-PAM, from which it was derived, the DIVING-PAM is optimized to perform one particular type of measurement with the greatest ease, accuracy and reliability, namely the determination of the effective quantum yield of photosynthetic energy conversion, ΔF/Fm', the so-called Genty-parameter. In the following sections some background information on this and other fluorescence parameters is given, and special aspects on fluorescence measurements with the DIVING-PAM are outlined, in order to make optimal use of this instrument.

12.1 Chlorophyl fluorescence as an indicator of photosynthesis

Photosynthesis involves reactions at five different functional levels:

- processes at the pigment level
- primary light reactions
- thylakoid electron transport reactions
- dark-enzymic stroma reactions
- slow regulatory feedback processes

In principle, chlorophyll fluorescence can function as an indicator at all of these levels of the photosynthesis process. Chlorophyll is the major antenna pigment, funneling the absorbed light energy into the reactions centers, where photochemical conversion of the excitation energy takes place.

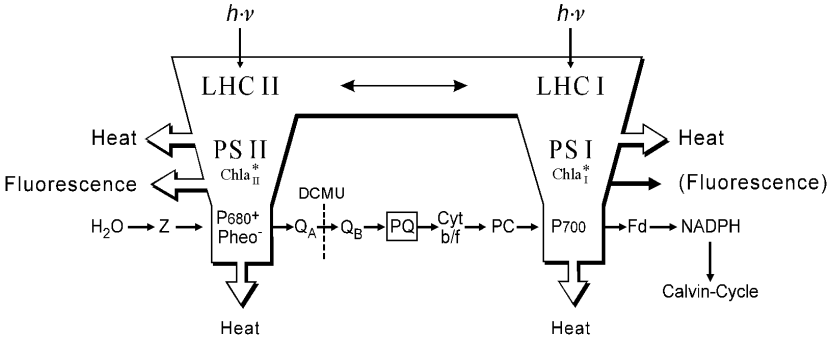


Fig. 14: Schematic view of primary energy conversion and primary electron transport in photosynthesis. LHC, light harvesting pigment-protein complex; P680 and P700, energy converting special chlorophyll molecules in the reaction centers of photosystem II (PSII) and photosystem I (PSI), respectively; Pheo, pheophytin; DCMU, PSII inhibitor (diuron); PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin

The indicator function of chlorophyll fluorescence arises from the fact that fluorescence emission is complementary to the alternative pathways of de-excitation, which are photochemistry and heat dissipation. Generally speaking, fluorescence yield is highest when the yields of photochemistry and heat dissipation are lowest. Hence, changes in fluorescence yield reflect changes in photochemical efficiency and heat dissipation. In practice, the variable part of chlorophyll fluorescence originates mainly from photosystem II and excitation transfer to photosystem I may be considered an additional competitive pathway of de-excitation.

Measuring chlorophyll fluorescence is rather simple: The emission extends from 660 nm to 760 nm, and if shorter wavelength excitation light is used, separation of fluorescence from the measuring light is readily achieved with the help of optical filters. The challenge arises with the wish to measure fluorescence in

ambient daylight and to use very strong light for the so-called 'quenching analysis'. For this purpose the PAM measuring principle has been developed which allows monitoring fluorescence against 10^6 times larger background signals (see 12.2).

From the viewpoint of fluorescence emission there are two fundamentally different types of competing de-excitation processes:

- photochemical energy conversion at the PS II centers
- non-photochemical loss of excitation energy at the antenna and reaction center levels

By both mechanisms, the maximal potential fluorescence yield is 'quenched' and, hence, 'photochemical' and 'non-photochemical fluorescence quenching' can be distinguished. For interpretation of fluorescence changes, it is essential to know the relative contributions of these two different quenching mechanisms to the overall effect. If, for example, fluorescence yield declines, this may be caused by

- an increase of the photochemical rate at the cost of fluorescence and heat-dissipation
- or an increase of heat-dissipation at the cost of fluorescence and photochemistry

These two possibilities can be distinguished by the so-called 'saturation pulse method':

With a very strong pulse of white light the electron transport chain between the two photosystems can be quickly fully reduced, such that the acceptors of PSII become exhausted. Hence, during the saturation pulse photochemical fluorescence quenching becomes zero and any remaining quenching must be nonphotochemical. It is assumed that changes in non-photochemical quenching are too slow

to become effective within the approx. 1 second duration of a saturation pulse.

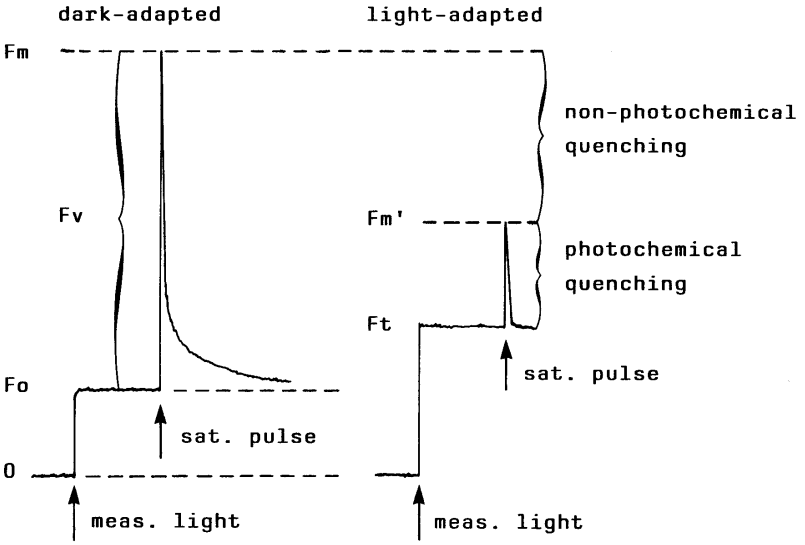


Fig. 15: Principle of fluorescence quenching analysis by the saturation pulse method

On the basis of these considerations so-called 'quenching coefficients' qP and qN were defined, which can be determined by simple fluorescence measurements (see 12.3.5). For qP- and qN-determination it is necessary to define the extremes of maximal and minimal fluorescence yield, which are given in the dark-adapted state (see 12.3.4). However, quenching analysis is not restricted to qP- and qN-determination and very relevant information can be also obtained without previous dark-adaptation of the samples. This is an important point for field investigations, for which the DIVING-PAM was optimized.

In recent years, evidence from a number of research groups has shown that the overall quantum yield of photochemical energy conversion can be assessed by the simple expression:

$$YIELD = (Fm'-F)/Fm' = \Delta F/Fm'$$

This expression, which was introduced by Genty et al. (1989) is identical to the YIELD-parameter measured by the DIVING-PAM (see 12.3.6). With this fluorometer, YIELD-determination has become exceedingly simple: The fiberoptics are held at short distance (ca. 10 mm) to a sample, and the START-key is pressed. Everything else is proceeding automatically within seconds:

- the present fluorescence yield F is sampled
- a saturation pulse is applied
- Fm' is sampled (displayed as ... M)
- YIELD = (Fm'-F)/Fm' is calculated and shown on the LC-display
- the obtained data are stored in the MEMORY.

The simplicity of this measurement is contrasted by the profound information it provides. In steady-state illumination, as prevailing under field-conditions, the YIELD-parameter reflects the efficiency of the overall process. Any change at the various functional levels (outlined at the start of this section) will be reflected in this parameter. The accuracy of this measurement is very high, and as recordings are quick, very detailed information on the photosynthetic performance of plants under varying environmental and physiological conditions can be obtained.

For full evaluation of the fluorescence information, some knowledge of the environmental parameters is required, in particular of light intensity and temperature. For example, if the measured YIELD of a sample A is lower than that of sample B, this does not

necessarily mean that sample A is photosynthetically less competent than sample B. The difference could as well arise from sample A being exposed to stronger light or to a lower temperature than sample B. The DIVING-PAM offers the possibility to measure photosynthetically active radiation (PAR) and temperature close to the site where also fluorescence is measured, such that together with every YIELD-value also the corresponding values of PAR and temperature are entered into the file of automatically stored data. When PAR is known, the apparent relative rate of electron transport (ETR) is calculated (displayed as ...E).

For assessment of overall photosynthetic performance, measurements in the steady-state are most informative. On the other hand, additional information on the various partial reactions can be obtained from analysis of so-called 'induction kinetics'. Upon a dark-light transition, fluorescence yield displays a series of characteristic transients, the so-called 'Kautsky effect', which reflect the complexity of the overall process. The rapid transients contain information on primary electron transport reactions, while the slow transients reflect reactions at the level of enzyme regulation. Analysis of the slow transients is greatly facilitated by use of the saturation pulse method, which allows to distinguish between the contributions of photochemical and nonphotochemical quenching.

Since the introduction of the PAM Fluorometer in 1985, there has been a boom in chlorophyll fluorescence research, at the basic as well as at the applied level. This is reflected in a large number of publications, due to which there has been considerable progress in understanding of the indicator function of chlorophyll, of photosynthesis as such, and of the regulation of photosynthesis under stress conditions. The review articles and some selected papers, which are listed in the Appendix (section 13.5) cover a representative part of the work which so far was carried out. This literature may be

useful to become informed in more detail about chlorophyll fluorescence and possible applications of the PAM Fluorometer.

12.2 The PAM measuring principle

With conventional chlorophyll fluorometers, the same light is used for driving photosynthesis and for exciting fluorescence. Separation of fluorescence from stray excitation light then is achieved by appropriate combinations of optical filters (e.g. excitation by blue light and protection of the detector by a red filter, which only passes the red fluorescence). Such conventional fluorometers are of rather limited use for ecophysiological research, as their function is severely disturbed by ambient daylight. In order to distinguish between fluorescence and other types of light reaching the photodetector, fluorescence excitation can be 'modulated': When a special 'measuring beam' is rapidly switched on/off, the fluorescence signal follows this on/off pattern and with the help of suitable electronic devices the resulting modulated signal can be separated. Standard devices for this purpose are lock-in amplifiers which tolerate background signals several hundred times larger than the fluorescence signal. For the extreme requirements of chlorophyll fluorescence quenching analysis by the so-called saturation pulse method (see 12.1), a new modulation principle was developed which tolerates a ratio of $1:10^5$ or even higher between fluorescence and background signal. This measuring principle is patented (DE 35 18 527) and licensed exclusively to the Heinz Walz GmbH.

The pulse-amplitude-modulation (PAM) principle displays the following essential features (see also Fig. 16):

Fluorescence is excited by very brief but strong light pulses from light-emitting diodes. With the DIVING-PAM, these pulses are 3 μs long and repeated at a frequency of 600 or 20000 Hz. The LED light

passes a short-pass filter (λ<670 nm) and the photodetector is protected by a long-pass filter (λ>700 nm) and a heat reflecting filter. A highly selective pulse amplification system ignores all signals except the fluorescence excited during the 3 µs measuring pulses. The photodetector is a PIN-photodiode which displays linear response with light intensity changing by factors of more than 10^9. Hence, this measuring system tolerates extreme changes in light intensity (up to several times the intensity of full sun light) even at weak measuring light intensities. This property is essential for correct determinations of photochemical quantum yield via the fluorescence parameters Fv/Fm or ΔF/Fm' and of minimal and maximal fluorescence yields, Fo and Fm (see 12.1 and 12.3.4).

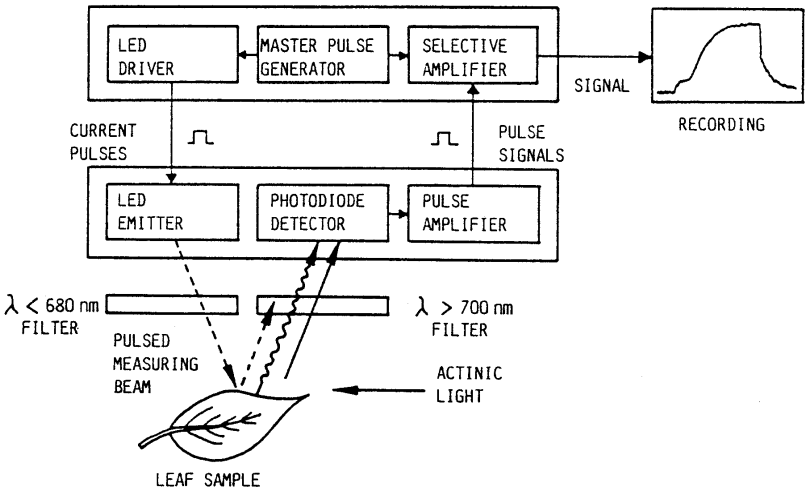


Fig. 16: Schematic view of the PAM measuring principle

Due to the PAM measuring principle, saturation pulse induced fluorescence changes can be very selectively and reliably analyzed in terms of photosynthetic activity. With the DIVING-PAM, just like with all PAM fluorometers, even small values of ΔF induced by a saturation pulse can be relied on. This can be simply tested by

applying a saturation pulse (via START) to a fluorescing sample, like the FLUORESCENCE STANDARD (Blue plastic filter delivered with the DIVING-PAM), which is not capable of photochemical energy conversion. With such a sample invariably YIELD = 0.000 is displayed. Such reliable performance, which is not possible with conventional amplifier systems, is of particular importance when photosynthesis yield is low due to stress conditions. In such cases it is essential to be sure that total inhibition really is indicated by YIELD = 0.000. These aspects are illustrated in Fig. 17.

Note: A small lowering of fluorescence yield observed upon application of a saturation pulse to the FLUORESCENCE STANDARD is a genuine effect which results from a transient temperature increase within the sample (see 12.3.4).

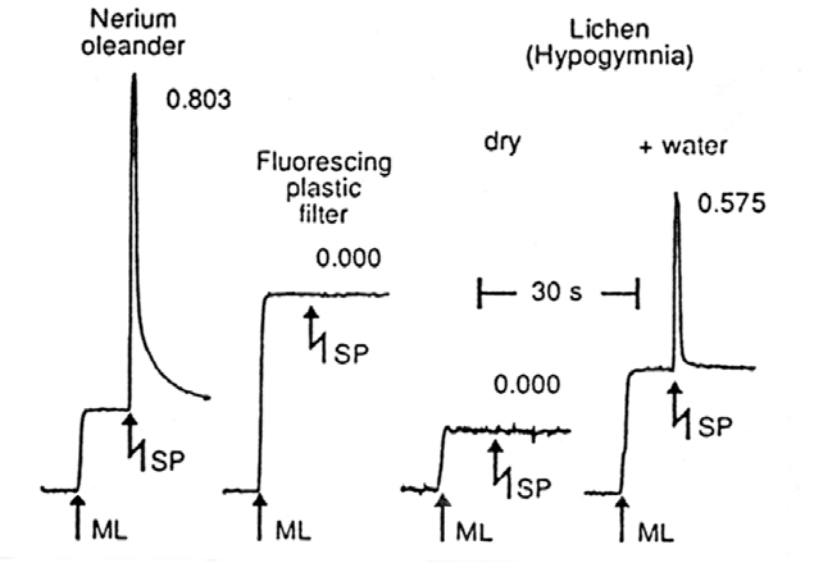


Fig. 17: Comparison of fluorescence responses of photosynthetically active (Nerium; Lichen, +water) and inactive (Lichen, dry) plant samples with those of a fluorescing plastic filter. ML, measuring light; SP, saturation pulse. The calculated values

of effective quantum yield of energy conversion in PSII (YIELD) are depicted.

12.3 Assessment of photosynthesis with the DIVING-PAM: Outline of the most important functions in practical applications.

As soon as the DIVING-PAM is switched on, it continuously monitors the fluorescence yield of a sample which is close to the fiberoptics exit. In section 12.1 it was outlined, in which way fluorescence yield relates to the effective quantum yield of photochemical energy conversion. Assessment of this very fundamental information is made automatically by two consecutive measurements of fluorescence yield (initiated by START), one briefly before and one during a short pulse of saturating light. The effective quantum yield of photochemical energy conversion (Y, YIELD) is then simply calculated from the equation $Y = \Delta F/Fm$. Although this sounds easy and straightforward, in practice certain aspects must be taken into consideration to obtain optimal and meaningful results (for a brief outline, see section 5). While it is almost trivial that the actual measurement must be correct, it is also important that the conditions are properly chosen to give meaningful information. Both of these two aspects are dealt with in the following sections, which outline the most important functions of the DIVING-PAM, corresponding to some selected points of the MODE-menu. A short description of all 51 points of the MODE-menu is given in section 7.

12.3.1 Maximal photochemical yield Fv/Fm

In green plants the maximal quantum yield of photosystem II is observed after dark adaptation when all reaction centers are open (all

primary acceptors oxidized) and heat dissipation is minimal. Then a saturation pulse induces maximal fluorescence yield, Fm, and maximal variable fluorescence, Fv, such that also ΔF/Fm = Fv/Fm is maximal. Fv/Fm, if properly assessed, is a reliable measure of the potential quantum yield of PS II. It is lowered by all effects which cause inhibition of PS II reaction centers and increase of heat dissipation. In this respect, photoinhibition is particularly relevant. Phenomenologically, both an increase of Fo or a decrease of Fm may contribute to a decrease of Fv/Fm = (Fm-Fo)/Fm. While an increase of Fo points to photodamage, a decrease of Fm reflects enhanced nonradiative energy loss (heat dissipation), which does not necessarily reflect irreversible damage, but often may be also viewed as an expression of photoprotection.

12.3.2 ML-BURST (menu point 5)

Plants can differ widely with respect to their requirements for dark-adaptation. With some extreme shade plants less than 0.1 μmol quanta m^{-2} s^{-1} may already be enough to cause substantial closure of PS II centers accompanied by a fluorescence increase, whereas most sun plants display close to minimal fluorescence yield and maximal Fv/Fm in the steady-state at 10-40 μmol quanta m^{-2} s^{-1}. Even if a sample before measurements is kept in absolute darkness, the actual fluorescence measurement necessarily involves illumination by the excitation light, which however, is very weak. With the DIVING-PAM, under standard conditions this amounts to ca 0.15 μmol quanta $m^{-2}s^{-1}$. It can be decreased by lowering the measuring light intensity (menu point 50: MEAS-INT) or by applying the 'burst mode' (menu point 5: ML-BURST).

The burst mode is particularly useful for a quick check whether the measuring light intensity is too high or not. By intermittent dark

periods the integrated intensity is cut down to 1/5, which will result in a lowering of fluorescence yield and an increase in Fv/Fm in very light sensitive plants. In this case, the MEAS-INT could be further lowered via menu point 50. Application of the burst mode has two advantages: First, there is no loss in signal/noise ratio. Second, there is no need to repeat AUTO-ZERO via menu point 2.

12.3.3 AUTO-ZERO (menu point 2)

The DIVING-PAM, like any other chlorophyll fluorometer, is not absolutely selective for chlorophyll fluorescence but also shows a small signal when no plant sample is in contact with the fiberoptics. This false signal originates from internal electronic "pick-up" and from traces of scattered measuring light which reach the photodetector despite the blocking filters. Most importantly, this signal is not dependent on properties of the investigated sample and, therefore, is constant as long as the measuring light intensity (menu point 50) and the gain (menu point 49) are not changed.

The false signal can be automatically subtracted from all measured fluorescence signals by the AUTO-ZERO function (menu point 2). For this purpose, the sample is removed and in menu position 2 AUTO-ZERO is carried out via SET. Thereafter the signal (F) without a sample fluctuates around 0 and sample specific fluorescence can be assessed. Any changes in GAIN (menu point 49) or MEAS-INT (menu point 50) lead to corresponding changes in the offset voltage caused by the false signal. Therefore, in this case AUTO-ZERO has to be repeated. If this is not done and a new YIELD-determination is made via START, there is the warning ?NEW OFFSET?, which reminds the user to first carry out AUTO-ZERO (without sample) before YIELD can be correctly determined. If the user prefers to keep the old offset value, the warning can be overruled simply by pressing SET (while in menu position 1).

Any false signal which is not compensated by AUTO-ZERO (menu point 2) or manually by ZERO-OFFS (menu point 44) will lead to underestimation of Y (ΔF/Fm' or Fv/Fm). Normally, i.e. with a leaf at 10-15 mm distance from the fiberoptics, the error is small (approximately 2 %). The error can increase considerably, when samples with low chlorophyll content and unfavorable geometries are assessed. In such cases, the signals can be increased by applying maximal measuring light intensity and maximal gain. However, in this way also the background signal is increased and AUTO-ZERO becomes very essential. For example, in an experiment with a 1 mm² piece of a leaf at maximal gain and measuring light intensity quite reproducibly an Fv/Fm = 0.610 to 0.630 was measured, when no offset was applied. However, when AUTO-ZERO was properly applied, Fv/Fm = 0.795 to 0.815.

12.3.4 Fo, Fm (menu point 25)

Fo and Fm are defined as the minimal and maximal fluorescence yields of a dark adapted sample, respectively. Knowledge of Fo and Fm is essential for determination of the quenching coefficients qP, qN and NPQ (see section 12.3.5). Fo and Fm determination is carried out in menu position 25 via SET. Then in menu position 26 there is automatic reset of qP to 1.000 and of qN to 000 and in menu position 27 NPQ is reset to 0.000. With all consequent applications of saturation pulses (via START), calculation of the quenching coefficients will be based on these Fo, Fm values, until they are re-determined via SET in menu position 25. As outlined in section 12.3.2, the threshold of light intensity below which a sample is dark-adapted can vary considerably. In most plants Fm/Fo = 5 to 6, which is equivalent to Y = Fv/Fm = 0.800 to 0.835. Such high values can be measured only when true dark-adaptation is reached and the

measuring procedure is optimized as outlined in the preceding sections 12.3.2 and 12.3.3.

For Fv/Fm, just as for YIELD-measurements in general, the absolute signal amplitudes are of no concern, as long as Fo and Fm are measured under the same conditions. However, measurements of absolute signal amplitudes are important for full assessment of photoinhibition (see 12.3.1) and also for calculation of the quenching coefficients qP, qN and NPQ (see 12.3.5). It must be emphasized, however, that it is not a simple matter to compare absolute fluorescence values of a sample measured at different times and under different conditions. While it is almost trivial that the sample must be in exactly the same position with respect to the fiberoptics (e.g. in a suitable sample-holder) and that the same settings of MEAS-INT (menu point 50) and GAIN (menu point 49) must be used, it is less obvious that the sensitivity of the fluorometer is affected by temperature. A 1 °C increase results in an approximately 1 % decrease in signal amplitude. This is due to the fact that the efficiency of the light-emitting-diode, which provides the pulse-modulated measuring light, slightly drops with increasing temperature. Hence, any internal heating of the fluorometer will lead to a corresponding decrease of the signal amplitude. In practice, this has to be accounted for whenever signal amplitudes are compared. It is of no concern for YIELD-measurements (signal ratios ΔF/Fm' or Fv/Fm), except for a small local temperature increase within the LED when pulse frequency is switched from 0.6 to 20 kHz during a saturating light pulse which affects selectively Fm.

Therefore, actinic illumination time (menu point 14: ACT-WIDTH) is limited to 5 min and not more than 2 min are recommended for recordings of LIGHT CURVES (with maximal time being limited to 3 min), which involve 8 consecutive illumination periods (see section 12.3.9). Nevertheless, internal

temperature increases of 10-20 °C are not uncommon, which will cause a decrease of measuring light intensity in the order of 10-20 %.

Small temperature related decreases of measuring light intensity also occur when the frequency is switched from 0.6 to 20 kHz. This is normally the case during actinic illumination with the internal halogen lamp. The extent of decrease depends on measuring light setting (menu point 50: MEAS-INT) and the length of the period at 20 kHz. It can be ignored at all measuring light settings for times below 2 s and amounts to approx. 1 % when a 3 s pulse is given at ML-setting 12. Hence, the effect on YIELD-determinations can be considered marginal. During prolonged 20 kHz operation at maximal ML-setting, the effective intensity drops by 2-3 %. This effect is reversible within a few minutes after returning to 0.6 kHz.

Changes in measuring light intensity induced by temperature changes, just like any effect on the sensitivity and selectivity of fluorescence measurements with the DIVING-PAM, can be also evaluated by monitoring the fluorescence signal of the FLUORESCENCE STANDARD delivered together with the instrument. This blue plastic filter (Roscolene Surprise Blue) emits red fluorescence with an intensity similar to that of a leaf. As there is no photochemical energy conversion, fluorescence yield of this sample is constant during illumination, provided temperature is not changing. A temperature increase of 10° corresponds to a fluorescence decrease of approximately 4 %. There is a similar effect on chlorophyll fluorescence when a plant sample is heated up by strong actinic illumination and during saturation pulses. Therefore the intensity and duration of saturation pulses should not be excessive. Otherwise there would be some underestimation of YIELD which is, however, rather small. For example, assumed the sample surface heats up by 5 °C, instead of Fv/Fm = 0.833 the measured value would be 0.830.

12.3.5 qP, qN and NPQ (menu points 26 and 27)

When a photosynthetically active sample is illuminated, its fluorescence yield can vary between two extreme values, Fo and Fm, which can be assessed after dark adaptation (see section 12.3.4). Any fluorescence lowering with respect to Fm may be caused either by enhanced photochemical energy conversion or by increased heat-dissipation (as compared to the dark state). As was outlined in section 12.1, saturation pulse quenching analysis allows to distinguish between these two fundamentally different types of fluorescence quenching. In brief, photochemical quenching can be suppressed by a pulse of saturating light (as photochemistry is saturated), whereas nonphotochemical quenching does not change during a saturation pulse (as changes in heat-dissipation involve relatively slow processes). The quenching coefficients are defined as follows (with Fm' being displayed as ...M):

$$qP = \frac{Fm' - F}{Fm' - Fo} \qquad qN = \frac{Fm - Fm'}{Fm - Fo} \qquad NPQ = \frac{Fm - Fm'}{Fm'}$$

qP and qN can vary between 0 and 1, whereas NPQ can assume values between 0 and approximately 10. The displayed quenching coefficients are meaningful only, if the values of Fo and Fm were previously measured with the same sample at the same sensitivity, i.e. with unchanged optical parameters, measuring light intensity (see 12.3.4) and gain.

The definitions of qP and qN imply that fluorescence quenching affects only the so-called variable fluorescence, Fm-Fo, and not Fo. In reality, at higher levels of qN (exceeding approx. 0.4) there can be also significant quenching of Fo, resulting in the lowered yield Fo'. This can be estimated upon light-off, when the acceptor side of PS II is quickly reoxidised (within 1-2 s), whereas relaxation of non-

photochemical quenching requires at least 5-10 s. Far-red light, which mainly excites PS I, can enhance Q_A-reoxidation and facilitate assessment of Fo'. However, the DIVING-PAM does not feature an intrinsic far-red light source (as e.g. the PAM-2000). Therefore, it should be realized that the measured values of qP and qN are valid in first approximation only, in particular when strong energy-dependent nonphotochemical quenching is given.

Fo-quenching is of no concern for NPQ-determination. The definition of NPQ implies a matrix model of the antenna system (Stern-Volmer quenching). With NPQ that part of non-photochemical quenching is emphasized which reflects heat-dissipation of excitation energy in the antenna system. NPQ has been shown to be a good indicator for 'excess light energy'. On the other hand, NPQ is relatively insensitive to that part of non-photochemical quenching which is associated with qN-values between 0 and 0.4, reflecting mainly thylakoid membrane energization. The different responses of qN and NPQ are illustrated in Fig. 18 in which a plot of qN vs. NPQ is shown. In this presentation, it is assumed that no Fo-quenching takes place. When Fo-quenching affects qN-calculation, the relationship extends to NPQ-values exceeding 4.

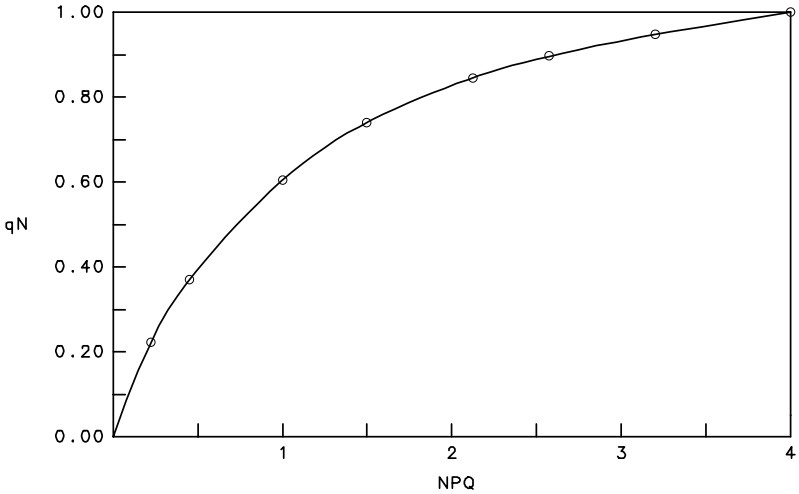


Fig. 18: Relationship between the coefficients of nonphotochemical quenching, qN and NPQ

12.3.6 YIELD-measurements of illuminated samples

With every application of START a YIELD-measurement is carried out and then on the LC-display of the DIVING-PAM in the standard menu position 1 the following signals measured in connection with each saturation pulse are shown:

- F, fluorescence yield measured briefly before triggering of the saturation pulse;
- M, fluorescence yield reached during the saturation pulse;
- Y, effective yield of photochemical energy conversion calculated as YIELD = (M-F)/M;
- E, apparent electron transport rate (ETR) calculated as ETR = YIELD x PAR x 0.5 x ETR-factor

This and additional information is stored in the MEMORY (see section 6) and can be transferred via the RS 232 interface in form of an ASCII-file to a PC (see sections 9 and 13.4).

Formally, there is no difference between YIELD-measurements with dark-adapted or illuminated samples (see Fig. 15). In the former case, F and M correspond to Fo and Fm (see 12.3.4) and Y corresponds to Fv/Fm, the maximal photochemical quantum yield (see 12.3.1). In practice, YIELD-determinations of illuminated samples are more easy, as the effect of measuring light intensity can be neglected. On the other hand, the interpretation of YIELD-data from illuminated samples requires somewhat more background knowledge. Whereas the dark-adapted state is well defined, there is an infinite number of light states, mainly determined by quantum flux density (PAR), illumination time, temperature and the physiological state of the sample. Therefore, YIELD-measurements should be carried out at defined light intensities and after defined periods of exposure to these intensities.

In one of its most common applications, the DIVING-PAM assesses the YIELD of plants in their natural light environment under steady-state conditions. In this case, use of the Fiber Quantum Sensor with automatic measurement of PAR is very convenient (see 8.3).

It should be noted that due to focusing effects of moving waves, the underwater measured PAR displays large variations. Furthermore, normally the diffusing disc of the light sensor cannot be placed at exactly the same spot where fluorescence is measured. Therefore, it is recommended to make use of the LIGHT AVERAGING function (MODE menu point 6). It may be assumed that the light adaptation state of a sample relates to the integral of the fluctuating light exposure.

Alternatively, there is the possibility to avoid illumination by ambient light and to use the internally generated light (halogen

lamp), the intensity of which is under instrument control. For this purpose, MODE-menu function 8: LIGHT CALIB is very useful, which allows to measure the effective PAR-values at the various intensity settings in a given geometry. These values are stored and applied for ETR-calculation when the EXT.LIGHT-SENSOR (menu point 7) is OFF.

Note: The intensity of the internal halogen lamp gradually declines with decreasing battery power. The decline amounts to ca. 0.3 % per LIGHT CURVE. When larger series of LIGHT CURVES are measured, it is recommended to repeat LIGHT CALIB. after approx. every 20 LIGHT CURVES. With the optional WinControl software it is possible to correct previously recorded data (see WinControl Manual).

12.3.7 YIELD- and ETR-averaging (menu point 11)

With normal samples under standard conditions the signal/noise ratio obtained with the DIVING-PAM is rather high, such that a single measurement results in the reliable determination of YIELD and ETR (see 12.3.6). In practice, the averaging function is most useful in order to obtain representative information on the photosynthetic performance of a heterogeneous sample. For example, repeated measurements at one particular sample site may give YIELD- and ETR-values fluctuating by no more than 0.001, whereas the values at another site may differ by more than 0.1 units. This is particularly true for outdoor measurements where the effective incident light intensity depends strongly on sample position, possible shading etc. In order to assess the effective quantum yield and the apparent electron transport rate of a sample in a given situation under natural conditions, the sample holder ideally must be attached such that there is no sample shading. As this ideal can be only more or less approached, unavoidably there is some

variability in the results, and averaging can be useful. The averaged data are not stored in the memory. On the other hand, as every individual data set is stored in MEMORY, which can be later transferred to a PC (see 9), users may prefer to evaluate statistical aspects of the data at a later stage, applying standard programs. The optional WinControl software also allows averaging of stored data in a most comfortable way.

Another case where averaging is advantageous relates to measurements under extreme environmental conditions which cause almost complete loss of variable fluorescence, i.e. when YIELD approaches zero, while ETR may still be substantial due to high PAR-values. Under such conditions, even the DIVING-PAM becomes limited by the signal/noise ratio in YIELD-determination, which can be improved by averaging.

Before use of the averaging function, the SET-key must be pressed in menu position 11 and AV. YIELD RESET must be confirmed by pressing the ∧-key. Then with every application of a saturation pulse the measured values of YIELD (Y) and ETR (E) will be averaged until another reset is carried out.

12.3.8 ACT-LIGHT and ACT+YIELD (menu points 12 and 13)

For shorter periods of actinic illumination also the internal halogen lamp can be used (see 8.1.2). As this leads to internal heating, illumination times are limited to 5 min. However, even much shorter times often are sufficient for reaching close to steady state, when the sample before has been kept in ambient light for some time. The actinic light can be turned on/off either by the double key operation CTRL + SET or in menu position 12 via SET. In the latter case the remaining illumination time is displayed. The DIVING-PAM also features the possibility of combining actinic

illumination and YIELD-determination. In menu position 13: ACT + YIELD, there is first actinic illumination and at the end of the chosen period a saturation pulse is applied for YIELD-determination. This function can be also started via the double key operation CTRL + START.

The intensity of actinic illumination can be varied via menu point 15: ACT-INT which features 12 settings, with consecutive settings differing by a factor of ca. 1.5. The effective quantum flux density at the sample depends on the distance from the fiberoptics. Using the Fiber Quantum Sensor at a standard distance of 10 mm the PAR amounts to approximately 40 µmol quanta m^{-2} s^{-1} at setting 1 and 2800 µmol quanta m^{-2} s^{-1} at setting 12 (see list of relative light intensities below). It should be noted that the absolute intensity is also determined by battery voltage, and the quality of the individual fiberoptics which may deteriorate due to rough conditions underwater. Hence, at a given setting of ACT-INT the effective intensity may vary and for quantitative work parallel measurement of PAR is recommended. For this purpose the Fiber Quantum Sensor in conjunction with the LIGHT CALIB routine (MODE-menu point 8) is useful.

ACT-INT Setting	1	2	3	4	5	6	7	8	9	10	11	12
Relative Intensity	1.0	1.5	2.5	3.5	5.5	8.0	11.5	17	26	38	57	87

The range of PAR-values covered by ACT-INT settings 1-12 can be shifted up or down via menu point 16: AL-FACT. In this way it is possible to account for special measuring conditions (absolute actinic intensities at sample site being exceptionally low or high) or for differences in light saturation properties of plants. The latter aspect is particularly relevant in conjunction with the automatic recording of light response curves (see 12.3.9). It is important to note that the

relationship between AL-FACT and PAR is non-linear. It depends on the setting of ACT-INT (menu point 15) and also on battery voltage. For example, at setting 10 and with a freshly charged battery the PAR is increased by a factor of ca. 1.7 when AL-FACT is increased from 1.0 to 1.5, and PAR is decreased by a factor of ca. 0.4 when AL-FACT is decreased from 1.0 to 0.5. In practice, it is recommended to measure the effective PAR with the Fiber Quantum Sensor, which should be placed in the same position as the sample.

The duration of the actinic illumination periods is set via menu point 14: ACT-WIDTH, with an upper limit of 5:00. When several actinic illumination periods are consecutively triggered, as with CLOCK-operation or LIGHT CURVE recordings (see 12.3.9), the ACT-WIDTH should be small, in order to avoid excessive internal heating of the DIVING-PAM. In these applications it is limited to 3 min.

The ACT + YIELD function provides very essential information on the state of the photosynthetic apparatus of a sample. At a given photon flux density of photosynthetically active radiation (PAR), which can be monitored by the Fiber Quantum Sensor, the measured values of YIELD and ETR of different samples can be directly compared and interpreted in terms of relative electron transport rates. The efficiency of photosynthetic electron transport can be limited by numerous steps in the long sequence of reactions between the primary process of photochemical energy conversion at the reaction centers and the export of the assimilates out of the chloroplasts. In the steady state, the overall yield of assimilation is equivalent to the yield of energy conversion at PS II. For a limitation to become apparent, the system must be 'put under light pressure'. For example, if some stress factor has caused a decrease in Calvin cycle activity, this will be only expressed in YIELD or ETR, if a sufficiently high PAR is applied to make dark enzymic steps of the Calvin cycle

limiting. The maximal YIELD of a dark-adapted sample, as measured by Fv/Fm (see 12.3.1) and by YIELD-values at low PAR, will be affected only, if the stress treatment has caused a limitation at the level of the primary reactions of energy conversion (excitation energy capture efficiency and charge separation efficiency at the reaction centers). This is, for example, the case after photoinhibitory treatment. Photoinhibition occurs, if a sample is exposed for longer time periods to excessive light intensities. To what extent a given light intensity is excessive depends on the physiological state of the sample and can be judged by YIELD-measurements (see following section 12.3.9 on LIGHT CURVES). A suppression of YIELD upon exposure of a sample to excessive light does not necessarily reflect permanent damage, but can also reflect a high potential for photoprotection by non-radiative energy dissipation. The latter is associated with high values of qN and NPQ (see 12.3.5).

12.3.9 LIGHT CURVE (menu point 17) and LIGHT-CURVE+REC (menu point 18)

The recording of a LIGHT CURVE involves 9 consecutive YIELD-measurements. The illumination series may start at ACTINIC INTENSITY 1, 2, 3, 4 or 5 (set via the LC-INT function, MODE-menu point 20). This feature allows to adjust the range of applied intensities to the light adaptation properties of the sample (sun or shade plant). When choosing the start-intensity, it should be also considered that a lower intensity range reduces the danger of instrument overheating with longer illumination times. An alternative possibility to vary the ACTININIC INTENSITY range is given by the ACT-FACT function (menu point 16). This shifts all intensities up or down.

Before starting a LIGHT CURVE recording, a sample should be well adapted to a moderate light intensity, which is close to the light

intensity experienced by the plant in its natural environment. In this way the requirement of long illumination periods for reaching steady-state can be avoided. The length of the actinic-light-periods is determined by LC-WIDTH (menu position 19). This is limited to 3:00 min in LIGHT CURVE recordings. With the 8 consecutive illumination periods applied during a LIGHT CURVE it is advisable not to exceed 2:00 min in order to avoid excessive internal heating of the DIVING-PAM.

A LIGHT CURVE is started either in menu position 17 via SET or in any other menu position by double key operation CTRL + ∧. The same commands apply for termination of a LIGHT CURVE. After starting a LIGHT CURVE there is first a YIELD-determination in the absence of actinic illumination for assessment of the maximal quantum yield. The sample should be sufficiently shaded, such that the external light does not contribute substantially to the PAR, which is approximately 100 μmol quanta m^{-2} s^{-1} at setting 3 of ACT-INT, when the distance is approximately 10 mm.

ACT-INT is automatically increased during the course of a LIGHT CURVE and YIELD is automatically determined at the end of each illumination period, the length of which is determined by the ACT-WIDTH. This results in a total of 9 YIELD- and ETR-values, which are stored in MEMORY (see 6) or transferred to a PC for further processing (see 9).

Additional information on the dark-recovery of YIELD-lowering during actinic illumination can be obtained by the function L-CURVE+REC. This function can be started either via SET in menu position 18 or by CTRL+∨. It can be terminated by the same commands. The actual illumination program with L - CURVE + REC is identical to that of a LIGHT CURVE. In addition, after termination of the last illumination period, in the absence of actinic light the recovery of YIELD in the dark is assessed by 6 consecutive

saturation pulses applied at 10 s, 30 s, 60 s, 2 min, 5 min, 10 min after light-off. In this way, different types of non-photochemical quenching can be distinguished which contribute to the lowering of the PS II quantum yield. It is generally assumed that the rapid recovery within the first 30-60 s reflects the disappearance of energy dependent nonphotochemical quenching, in parallel with the relaxation of the transthylakoidal ΔpH. The slower recovery within the first 10-30 min is considered to reflect a change of energy distribution in favor of PS II (so-called State Shift). The apparently irreversible YIELD-lowering (with respect to the original dark state) is expression of "photoinhibition".

LIGHT CURVES as measured with the DIVING-PAM contain somewhat different information than the conventional light response curves. Correct measurement of the latter requires the attainment of steady state at each PAR-value, which takes at least 10 min. LIGHT CURVES recorded with short illumination times (down to 5 s; so-called Rapid Light Curves, RLC) allow insight into the physiological flexibility with which a plant sample can adapt its photosynthetic apparatus to rapid changes of light intensity. Hence, RLC contain information on induction as well as saturation characteristics of photosynthesis. LIGHT CURVES measured during the course of a day (e.g. triggered by the Repetition Clock, see 12.3.11) may show largely different characteristics due to the fact that the physiological state of the photosynthetic apparatus is regulated by environmental factors in a highly dynamic manner. Whereas for proper recording of a conventional light response curve it is essential that all conditions (like temperature, CO_2-concentration, humidity) are kept constant over extended periods of time, LIGHT CURVES are sufficiently fast that they can characterize a momentary state of a plant in a naturally changing environment.

12.3.10 INDUCTION CURVE (menu point 21) and INDUCTION CURVE+RECOVERY (menu point 22)

Dark/light induction curves (Kautsky effect) contain complex information on the photosynthetic performance of a plant at different functional levels (see 12.1). By repetitive application of saturating light pulses and quenching analysis additional information is obtained which is essential for reliable interpretation of the Kautsky effect. After a longer period of darkness, Calvin-cycle enzymes are partially inactivated. They are light-activated during the first minutes of illumination. During this induction period oxygen instead of CO_2 serves as terminal electron acceptor. O_2 dependent electron flow (Mehler-Ascorbate-Peroxidase Cycle) as well as cyclic electron flow at photosystem I create a large proton gradient, which will be used for ATP-synthesis only after Calvin cycle has been light activated. This leads to strong "energy-dependent" nonphotochemical fluorescence quenching during the first minutes of illumination (characterized by low Fm'-values), which partially declines again when CO_2-fixation takes over and ATP is consumed.

In order to record an INDUCTION CURVE with the DIVING-PAM, a fixed geometry between sample and fiberoptics must be assured for the duration of the recording. The recording is started by MODE-menu function 21: IND.CURVE. It is also possible to record the light/dark recovery in addition to the dark/light induction (22:IND.CURVE+REC). In this case information on post-illumination reactions are obtained, in particular on the recovery of various components of nonphotochemical quenching (see 12.3.9), the extent of photoinhibition and also on dark electron flow between stroma (or cytoplasma) and the electron carrier in the thylakoid membrane.

Before recording of the actual induction curve, a single saturation pulse is applied for assessment of Fo, Fm and Fv/Fm after dark

adaptation. This is a prerequisite for correct quenching analysis (see 12.3.1, 12.3.2, 12.3.3). The delay between this saturation pulse and onset of illumination can be varied (23: IND.DELAY); its default value is 40 s. Another variable is the time interval between two consecutive saturation pulses during actinic illumination (24: IND.-WIDTH), with a default setting of 20 s.

Due to the outstanding role of molecular O_2 during the induction period, O_2 partial pressures within the sample has a strong influence on all features of the the induction curves. This aspect is particularly relevant for endosymbiotic phycobionts, as O_2 is consumed by their own and the host's dark-respiration and O_2-diffusion is restricted (see recent report by Schreiber, Gademann, Ralph and Larkum: Assessment of photosynthetic performance of Prochloron in Lissoclinum patella *in hospite* by chlorophyll fluorescence measurements. Plant Cell Physiol. 38(8), 945-951, 1997).

12.3.11 Repetition Clock (menu point 28: REP-CLOCK and double key function CTRL+MEM)

The Repetition Clock is primarily meant to trigger saturation pulses for YIELD-determination at defined time intervals which are set in menu position 30: CLK-TIME. The standard interval of 20 s is appropriate for the recording of fluorescence induction curves with repetitive YIELD-determination. The CLOCK can be started/stopped in menu position 28 via SET. Then on the display the remaining time to the next start of a function is shown. Start/Stop of the CLOCK is also possible in other menu positions via the double key operation CTRL+MEM.

Besides YIELD-measurements also other functions can be repetitively triggered by the CLOCK. For this purpose the MODE-

menu point 29 (CLOCK-ITEM) is provided which allows to choose between:

1: SATURATION PULSE (SAT)

2: ACT+YIELD (A+Y)

3: LIGHT CURVE (LC)

4: L-CURVE+REC. (LC+)

5: INDUCTION-CURVE (IC)

6: INDUCTION-C+REC. (IC+)

The CLOCK can be very useful for long term characterization of the photosynthetic performance of a plant in its natural environment, e.g. over the course of a day. As after every YIELD-determination the corresponding data set is stored in MEMORY, in principle the researcher just needs to start the CLOCK in the morning and collect the data at night. In this context it is important to note that the full capacity of a freshly charged battery allows approximately 12 hours continuous operation of the DIVING-PAM with standard YIELD-determination every minute (total of ca. 720 saturation pulses). When the CLOCK is running, the usual power saving function of AUTO-OFF is disabled, with the consequence that there could be excessive discharge of the battery. Therefore, in order to avoid battery damage (see 11.1) the CLOCK is automatically switched off when battery voltage drops below 8.5 V.

13 Appendix

13.1 Technical specifications

13.1.1 Basic System

Underwater Fluorometer DIVING-PAM

Measuring light source:	Red LED, emission maximum (in conjunction with short-pass filter) at 650 nm; 12 intensity settings, standard intensity 0.15 µmol $m^{-2}s^{-1}$ PAR; modulation frequency 0.6 or 20 kHz; Auto 20 kHz function; burst-mode, 1/5 integrated intensity
Halogen lamp:	8 V/20 W blue enriched, filtered to give λ<710 nm; 12 intensity settings, max. 6000 µmol $m^{-2}s^{-1}$ PAR with continuous actinic illumination, max. 18000 µmol $m^{-2}s^{-1}$ PAR during saturation pulses
Signal detection:	PIN-photodiode protected by long-pass filter (λ>710 nm); selective window amplifier (patented)
Microcontroller:	CMOS 80C52
Data memory:	CMOS RAM 128 kB, providing memory for 4000 data sets
Measured parameters:	Fo, Fm, Fm', F, Fv/Fm (max. Yield), ΔF/Fm' (Yield), qP, qN, NPQ, PAR (using Fiber Quantum Sensor), ETR (i.e. PAR x ΔF/Fm'), water temperature, water depth

Display:	2 x 24 character alphanumerical LC-display with backlight; character size 4.5 mm
User interface:	1 x 8 touch sensitive keypad
Water temperature measurement:	-10 °C to +60 °C, in steps of 1 °C
Water depth measurement:	0 to -70 m, in steps of 0.1 m
PC-terminal operation:	Via RS 232 interface using special command set; for remote control of all functions; alternatively, control by optional WinControl software
Data output:	Data transfer on PC via RS 232 as ASCII-file using DOS or Windows-version of PamTrans Data Transfer Sofware or by optional WinControl software
Power supply:	Internal rechargeable battery 12 V/2.1 Ah, providing power for up to 1000 yield measurements; Battery Charger MINI-PAM/L
Operating temperature:	-5 to +45 °C
Dimensions:	Diameter 19 cm, length 39 cm
Weight:	3.7 kg

Fiberoptics DIVING-F

Design:	Randomized 70 µm glass fibers forming single plastic shielded bundle with waterproof stainless steel adapter ends
Dimensions:	Active diameter 5.5 mm; outer diameter 8 mm; length 1.5 m
Weight:	340 g

Fiber Quantum Sensor

Design:	Single plastic fiber with miniature diffuser
PAR measurement:	0 to 20000 µmol $m^{-2}s^{-1}$ PAR in conjunction with DIVING-PAM
Dimensions:	Active diameter 1mm; length 1.5 m

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:	60 mm x 60 mm x 25 mm (L x W x H)
Weight:	95 g

Battery Charger MINI-PAM/L

Mains input:	90 to 260 V AC, 47 to 63 Hz
Output:	18 V DC, max. 30 W
Dimensions:	11.2 cm x 6 cm x 3.6 cm (L x W x H)
Weight:	235 g

Transport Case DIVING-T

Design:	Aluminium case with custom foam packing for DIVING-PAM and accessories
Dimensions:	58 cm x 38 cm x 25 cm (L x W x H)
Weight:	5 kg

13.1.2 Accessories (optional)

Dark Leaf Clip DIVING-LC

Design:	Clip made of white plastic with gasket contact areas and sliding shutter (light-tight closure)
Dimensions:	Diameter 32 mm, length 80 mm
Weight:	6.5 g

Magnet Sample Holder DIVING-MLC

Design:	Two halves with ring magnets, one with fiberoptics adapter and split back rubber hood (dark adaptation), the other serving as buoyancy body
Dimensions:	Diameter 37 mm, height 48 mm
Weight:	60 g, floating underwater

Miniature Fiberoptics DIVING-F1

Design:	Single plastic fiber with adapter for the DIVING-PAM
Dimensions:	Active diameter 2 mm, length 1.5 m

13.2 List of warnings and errors

Errors in DIVING-PAM performance and warnings concerning sub-optimal use of the instrument are signalled by messages in the upper left corner of the display line. The following list briefly describes the various error messages:

Err. OVERFLOW: >3500

Maximal signal level was exceeded. The distance to the sample should be increased. Alternatively, the GAIN (menu point 49) or the MEAS-INT (menu point 50) may be decreased. In the latter cases, the zero offset must be newly determined (2: AUTO-ZERO).

Err. SIGNAL LOW: <130

Signal/noise ratio can be improved by increasing the signal: For this purpose, decrease distance between fiberoptics and sample; or increase GAIN (menu point 49) or MEAS-INT (menu point 50). In the latter cases, the zero offset must be newly determined (2: AUTO-ZERO).

Err. LOW BATTERY

Battery voltage has dropped below 11.2 V which means that only 20-30 further measurements are possible: Recharge battery or connect external battery by special cable (MINI-PAM/AK, optional).

Err. ? NEW OFFSET ?

Last measurement may be erroneous as GAIN (menu point 49) or MEAS-INT (menu point 50) was changed without being followed by new zero offset determination (2: AUTO-ZERO). The warning can be overruled by pressing SET while in menu position 1.

Err. ! CHECK BATTERY !

Battery voltage drops during application of a saturation pulse below 8.5 V, which means that it is almost empty or too old: Recharge or possibly replace battery.

Err. MEMORY: 001

Maximal MEMORY-number of 4000 is reached. With further measurements, the new data sets will replace the old data sets starting from No. 1.

Additional warnings and information are given by messages in the left corner of the upper display line:

BAT Battery voltage has dropped below 11.2 V: Be prepared that the error message 3 (LOW BATTERY) will appear when START is applied.

ACT Actinic illumination is running.

A+Y Actinic illumination with terminal YIELD-determination (menu point 12) is running.

CLK REPETITION-CLOCK (menu point 28) is running.

LC Automatic recording of a LIGHT CURVE (menu point 17) is running.

LC+ Automatic recording of a LIGHT CURVE + RECOVERY (menu point 18) is running.

IC Automatic recording of an INDUCTION CURVE (menu point 21) is running.

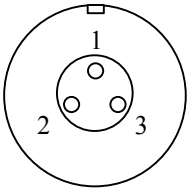
IC+ Automatic recording of an INDUCTION CURVE + RECOVERY (menu point 22) is running.

REC Recovery part of LIGHT INDUCTION CURVE is running.

SAT A saturating light pulse is applied for YIELD-determination.

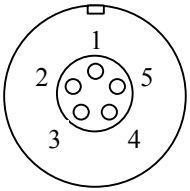
13.3 PIN-assignments

"CHARGE"



1: Charge input +18 V
2: GND
3: External input +12 V (max. 13.8V).
ATTENTION: Internal battery cannot be charged via this input.

"RS 232"



1: Not used
2: Not used
3: TxD
4: RxD
5: GND

13.4 List of commands for operation of DIVING-PAM via PC-terminal

As described in section 9, the DIVING-PAM can be operated by remote control from a PC terminal. For this purpose a suitable TERMINAL-program must be installed and the RS 232 interface cable connected to the corresponding communication port. The following commands are executed via 'Return'. Please note that only low-case letters are effective.

In custom applications it should be made sure that at least 50 ms elapse between two consecutively sent letters. The communication has lower priority than the measuring routines and at higher rates letters may get lost. For some commands the measuring program is transiently stopped. Hence, data transfer should not occur during measurements.

It should be mentioned that optionally the WinControl software is available, which has been optimized for the communication between PC and DIVING-PAM and features a number of most comfortable functions for data acquisition and analysis.

Command	**Corresponding point in MODE-menu**	**Description**
?	37:	Date of Software version (current EPROM)
a1/a0	12:	Act. light on/off
aix	15:	Act. Intensity with setting x (x = 0 ... 12)
awx	14:	Act. Width with setting x (x = 10 s ... 5 min)
a01/a00	10:	AUTO-OFF on/off
af or afx	16:	ACT.-FACTOR (x = 0.5 ... 1.5)
ay1/ay0	13:	Act. Light + YIELD on/off
b		BREAK, to stop all running functions
be1/be0		Enable/Disable beep-function
bp or bpx		Beep with length x (1 = 10 ms)
c1/c0	28:	CLOCK on/off
ct or ctx	30:	CLOCK time (interval x = 10 ... 990 s)
ci or cix	29	CLOCK item (1..6)
d or dx	48:	Damping setting (x = 1 ... 3)
dat or dat(ddmmyy)	32:	Date (day month year)
dl1/dl0	9:	Display light on/off
dsx		Display MODE-menu point x
e	1:	ETR (electron transport rate)
ea	11:	Averaged ETR (Leaf Clip)
ec1/ec0		Echo on/off
ef or efx	45:	ETR-factor (defined as x)
f	1:	Fluorescence yield before last

Command	Corresponding point in MODE-menu	Description
		sat.pulse, F
f*	1:	Momentary fluorescence yield, F*
fmp	1:	Max. fluor. yield during last sat.pulse, Fm'
fm	25:	Max. dark adapted fluor., Fm
fo	25:	Min. dark adapted fluor., Fo
fos	25:	Fo-Fm determination
fz or fzx	44:	ZERO-OFFSET (defined as x)
fzs	2:	AUTO-ZERO determination
g or gx	49:	Gain setting (x = 1 ... 12)
ic1/ic0	21:	Induction Curve on/off
ic+1/ic+0	22:	IC+Recovery on/off
idx	23:	Induction delay
iwx	24:	Induction width
l	1:	Light int. (PAR meas. with ext.light sensor)
la1/la0	6:	Light average on/off
lg or lgx	41:	Light gain (Leaf Clip)
lo or lox	40:	Light offset (Leaf Clip)
lc1/lc0	17:	Light curve on/off
lc+1/lc+0	18:	Light curve + Recovery on/off
le1/le0	7:	Ext. light sensor on/off
lec1	8:	Light cal
lr		Read light list
lw		Write light list
li or lix	20:	Light curve start-intensity
lw or lwx	19:	Light curve step width
m1/m0	3:	Measuring light on/off
ma or max	51:	Mark of sample (x = A ... Z)
mb1/0	5:	ML-BURST function on/off
mf1/0	4:	ML-frequency (20/0.6 kHz)
mi or mix	50:	ML-intensity (x = 1 ... 12)
me or mex	38:	MEMORY-number (x = 1 ... 999)
mez	39:	CLEAR-MEMORY (attention! data will be erased)

Command	Corresponding point in MODE-menu	Description
npq	27:	NPQ-parameter
o or ox		Display of data set Mem.x
o+ or o+x		Display of data sets from 1 to x
of		Display of data set format
pao		DIVING-PAM switched off
pas		Display of present DIVING-PAM settings
paz	36:	DIVING-PAM settings reset to standard
qn	26:	Display of present qN
qp	26:	Display of present qp
s		Start saturation pulse
si or six	47:	Sat. pulse intensity (x = 1 ... 12)
sw or swx	46:	Sat. pulse width (x = 0.4 ... 3.0)
t	1:	Water depth
ti	35:	Water temperature
tox	42:	Water depth offset
tgx	43:	Water depth gain
tim(hhmm)	31:	Time (hour minute)
ub	34:	Battery voltage
us	34:	Battery voltage during last sat. pulse
ver		No. of program version
vx		Voltage at channel x of A/D converter (x = 0 ... 7)
y	1:	YIELD measured with last sat. pulse
ya	16:	Averaged YIELD
yn	16:	No. of averaged YIELD-values
yz	16:	Reset YIELD-averaging function

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14 Rechargeable battery

The Underwater Fluorometer DIVING-PAM is equipped with a rechargeable sealed-lead acid battery.

The life time is 1-3 years and it depends on the specific application. A 10 °C rise of the temperature will decrease battery life by approx. 25%. Near the end-of-life the standby capacity of the battery will be reduced. When this reduction becomes persistently, please replace the battery.

The battery **cannot be overcharged**, when the battery charger supplied with the instrument is used! Do **not** use any other battery charger!

Never store the instrument with a discharged or partially discharged battery! It is recommended to charge the battery every three months during the storage period.

- **For optimum performance always recharge the battery immediately after discharging!**
- **Never leave the battery in a discharged stage!**
- **Never short-circuit the battery terminals!**

15 Warranty conditions

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 one (1) year from the shipping date (date on invoice).

The warranty is subject to the following conditions:

1. This warranty applies if the defects are called to the attention of Heinz Walz GmbH, Germany, in writing within one year (1) of the shipping date of the product.
2. 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.
3. This warranty shall not apply to any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.
4. This warranty does not apply to damage caused from improper packaging during shipment or any natural acts of God.
5. This warranty does not apply to underwater cables, batteries, fiberoptic cables, lamps, gas filters, thermocouples, fuses or calibrations.

To obtain warranty service, please follow the instructions below:

1. The Warranty Registration form must be completed and returned to Heinz Walz GmbH, Germany.
2. 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, custom duties,

and/or shipping costs incurred in returning equipment for warranty service are at customer expense.

3. All products being returned for warranty service must be carefully packed and sent freight prepaid.

4. 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.