PAM-CONTROL
Universal Control Unit

For Ultrasensitive
Chlorophyll Fluorescence Measurements

Handbook of Operation

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1 Safety instructions

1.1 General safety instructions

1. Read the safety instructions and the operating instructions first.
2. Pay attention to all the safety warnings.
3. Keep the device away from water or high moisture areas.
4. Keep the device away from dust, sand and dirt.
5. Always ensure there is sufficient ventilation.
6. Do not put the device anywhere near sources of heat.
7. Connect the device only to the power source indicated in the operating instructions or on the device.
8. Clean the device only according to the manufacturer's recommendations.
9. If the device is not in use, remove the mains plug from the socket.
10. Ensure that no liquids or other foreign bodies can find their way inside the device.
11. The device should only be repaired by qualified personnel.

1.2 Special safety instruction

The PAM-CONTROL Universal Control Unit is a highly sensitive research instrument which should be used only for research purposes, as specified in this manual. Please follow the instructions of this manual in order to avoid potential harm to the user and damage to the instrument.

2 Introduction

Chlorophyll fluorescence is an indicator of photosynthetic energy conversion in higher plants, algae and bacteria. Fluorescence originates from the same excited states, created by light absorption, which alternatively can be photochemically converted or dissipated into heat. Hence, the yield of chlorophyll fluorescence is complementary to the yields of photochemical energy conversion and heat dissipation:

fluorescence + photochemistry + heat = 1

PAM Chlorophyll Fluorometers are specialized to measure chlorophyll fluorescence yield with high sensitivity and selectivity. In conjunction with the so-called "saturation pulse quenching analysis", PAM-measurements allow to distinguish between fluorescence quenching by photochemistry (photochemical quenching) and heat dissipation (nonphotochemical quenching). All PAM (**P**ulse **A**mplitude **M**odulation) Fluorometers apply µsec-pulses of measuring light for fluorescence excitation. The actual measurement of the photosynthetic yield is carried out by application of a saturating light pulse which briefly suppresses photochemical yield to zero and induces maximal fluorescence yield.

The PAM-CONTROL Universal Control Unit has been developed for extremely sensitive chlorophyll fluorescence measurements in a variety of specialized optical geometries using a photomultiplier as fluorescence detector. Due to the use of a photomultiplier, the sensitivity is increased approximately 1000-fold with respect to standard PAM Fluorometers (like PAM-2500 or MINI-PAM) featuring photodiode detectors. At the same time, however, due to the extreme sensitivity, PAM-CONTROL based measuring systems do not tolerate nonmodulated background light, like ambient day light. For the same reason, standard light sources

commonly used for actinic illumination and saturation pulses with other PAM Fluorometers, are not suitable for PAM-CONTROL based systems. A special pulse technique has been developed (patent pending) for the application of strong actinic illumination which does not disturb measurements of fluorescence yield by the photomultiplier (see 8).

As suggested by its name, the PAM-CONTROL Universal Control Unit as such does not yet constitute a functional fluorometer. It has to be complemented by the following components:

1. Pulsed measuring light source, normally a light emitting diode (LED),
2. Special photomultiplier with pulse preamplifier,
3. Optical system, including special filters for selection of chlorophyll fluorescence.

In principle, these components can be arranged in a large variety of optical geometries and the same PAM-CONTROL Unit can be used for largely different purposes. So far, three different PAM-CONTROL based measuring systems have been realized:

- MICROSCOPY-PAM, with a blue LED mounted as pulsed light source in an Epifluorescence Microscope and the photomultiplier being mounted on top of the microscope ocular, for measurements at the level of single cells and chloroplasts.
- MICROFIBER-PAM, with a blue, green, yellow or red LED as pulsed light source and the photomultiplier connected to a microfiber coupler, for small spot measurements down to single cells in different tissue layers.
- WATER-PAM, with an array of measuring and actinic LEDs illuminating a water sample (e.g. from lakes, rivers, oceans) and

special optics collecting the fluorescence on the photomultiplier, for assessment of low levels of natural phytoplankton.

In the case of MICROSCOPY-PAM and MICROFIBER-PAM only very small sample areas are involved, such that a single LED provides enough light not only for measuring light, but for actinic and saturation pulse light as well. On the other hand, in the case of WATER-PAM a larger volume has to be illuminated, requiring a larger number of LEDs. The PAM-CONTROL Unit provides sufficient power to drive up to 3 measuring LEDs and 3 actinic LEDs. If more actinic LEDs are required, as with WATER-PAM, the required drivers are incorporated in a separate unit, connected to PAM-CONTROL.

The PAM-CONTROL is a compact, battery-powered unit, such that the derived fluorometers in principle are portable and suitable for stand-alone operation. This feature is particularly valuable in the case of the WATER-PAM. Up to 4000 data sets can be stored on RAM within the PAM-CONTROL. An extensive MODE-menu is provided for stand-alone operation. On the other hand, PAM-CONTROL based fluorometers may be also operated in conjunction with a PC. For this purpose, the dedicated WinControl-software was developed. When the PAM-CONTROL is connected to a PC, the information on instrument settings and data registration is continuously exchanged, such that both ways of operation are equivalent.

The WinControl-software provides so-called "tooltips" with short explanations of the numerous functions of the measuring system. Hence, the use of WinControl is recommended particularly to the beginner for becoming acquainted with the principles of operation and of chlorophyll fluorescence information. It should be emphasized that despite of the use of an ultrasensitive photomultiplier, there is no risk of serious mistakes causing damage.

The photomultiplier sensitivity is automatically turned down when being exposed to excessive light. Therefore, beginners may feel free to "play" with the system, trying out all functions. For this purpose, the Chart-window is particularly useful, as it records all fluorescence changes like a chart recorder.

This manual deals with the PAM-CONTROL unit. Short brochures are available describing the particular components and features of the special Chlorophyll Fluorometers on the basis of the PAM-CONTROL, namely MICROSCOPY-, MICROFIBER- and WATER-PAM. In addition a separate manual is provided for the WinControl-software.

3 Components of the PAM-CONTROL

As already outlined in the Introduction, the PAM-CONTROL as such does not yet constitute a functional fluorometer. As a universal control unit for different types of specialized fluorometers, it does not contain any optical components. In principle, obtaining a functional fluorometer based on PAM-CONTROL is rather simple:

- Connect measuring light (ML) LED
- Connect photomultiplier (PM)
- Arrange ML-LED and PM in an appropriate geometry with respect to the investigated sample.

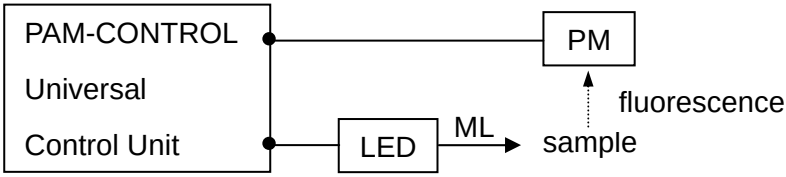


Fig. 1: Principle of PAM-CONTROL based fluorometers

The PAM-CONTROL is universal in the sense that it contains all the components which are required to derive a variety of specialized fluorometers with widely different geometries and applications. So far, three types of specialized chlorophyll fluorometers have been derived, which cover the most obvious applications: MICROSCOPY-, MICROFIBER- and WATER-PAM. In addition, researchers with experience in instrument development may construct their own specialized measuring system on the basis of the PAM-CONTROL.

3.1 Universal Control Unit

The major components of the PAM-CONTROL and the connections to various peripheral components are summarized in Fig. 2.

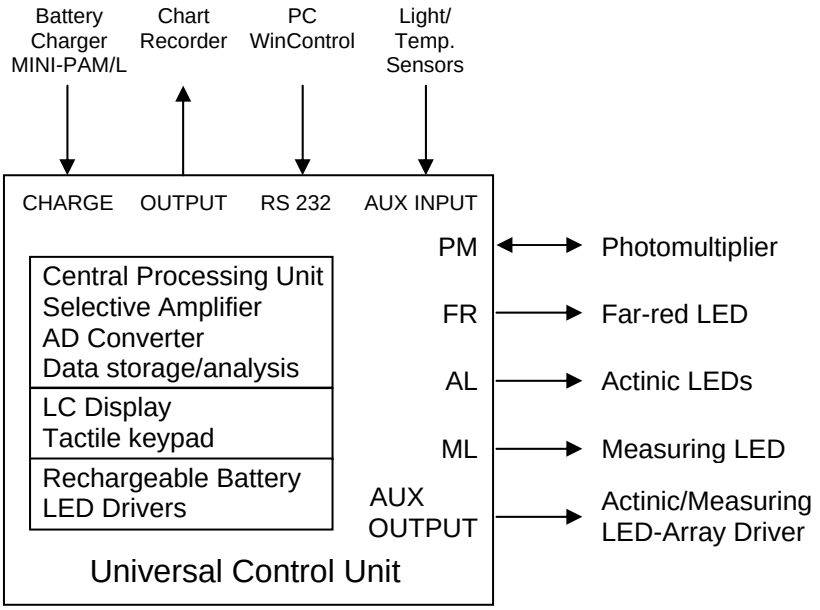


Fig. 2: Major components of PAM-CONTROL and connections to peripheral components

The PAM-CONTROL is a central processing unit with a selective amplifier, data acquisition and storage system, a variety of connectors, drivers for measuring and actinic LEDs, a large rechargeable battery and the user interface, with the LC-display and keypad. Details on some of the components are given in the following sections.

3.1.1 Electronic components

The central processing unit features a CMOS microcontroller. The program software is stored in a CMOS EPROM. This EPROM is readily accessible after removing the bottom of the PAM-CONTROL housing (see 10.3) and can be exchanged by the user, if program up-dates become available. A CMOS RAM with 128 kB serves as data memory, providing storage capacity for 4000 data sets.

3.1.2 Description of the connectors

3.1.2.1 Rear side of the PAM-CONTROL housing

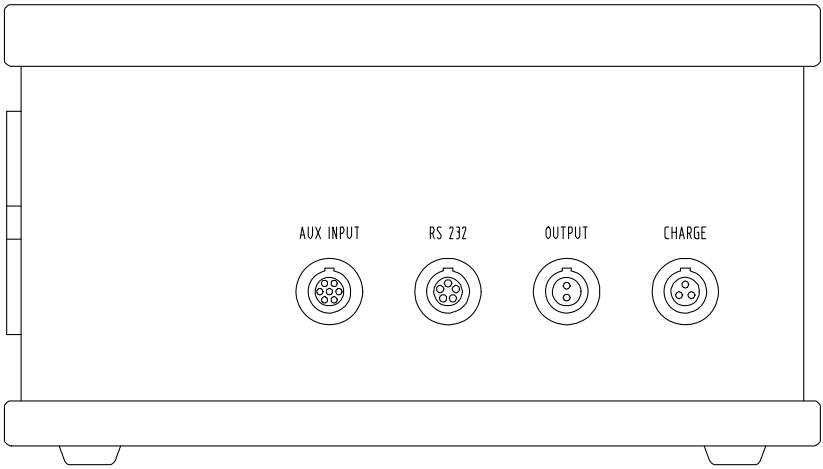


Fig. 3: Rear side of the PAM-CONTROL housing

At the rear side of the PAM-CONTROL housing the following electrical connectors are located:

a) AUX IN

The AUX IN socket can be used for connecting optional devices featuring a micro-quantum-sensor and/or temperature-sensor. For the MICROSCOPY-PAM the special MICRO Quantum Sensor MC-MQS was developed which measures quantum flux density of photosynthetically active radiation (PAR) in the focal plane of the microscope objective. For the WATER-PAM the Spherical Quantum Sensor US-SQS is available. For special applications, the combined Micro-Quantum/Temp.-Sensor 2060-M was developed. Please note that MODE-menu point 7: EXT.LIGHT-S must be activated for the externally measured PAR to be displayed.

Connection of a temperature sensor is required for display of C (leaf temperature in °C). Even if no light sensor is connected, PAR and the derived apparent relative electron transport rate, ETR, are displayed. In this case, the PAR-values are derived from an internal PAR-list based on previous calibration of PAR as function of actinic intensity setting (ACT-INT) (see 8.2).

b) RS 232

A RS 232 interface cable is provided to connect the PAM-CONTROL to a PC. Then the PAM-CONTROL can be operated via the PC-keyboard using the dedicated WinControl-software (see separate manual).

c) OUTPUT

A special cable is provided to connect the PAM-CONTROL analog output to a chart recorder. The output signal can vary between 0 and 4 volt.

d) CHARGE

Together with the PAM-CONTROL the Battery Charger MINI-PAM/L is delivered which connects to the CHARGE-input. The

charger can be used with line voltages of 100 to 240 V at 50-60 Hz. When used in the laboratory the charger can remain permanently connected. A special cable (MINI-PAM/AK) is available for connecting an external 12 V battery to the CHARGE-input. While the PAM-CONTROL can be powered by this external battery, it should be noted that the internal battery cannot be recharged in this way.

3.1.2.2 Right hand side of PAM-CONTROL housing

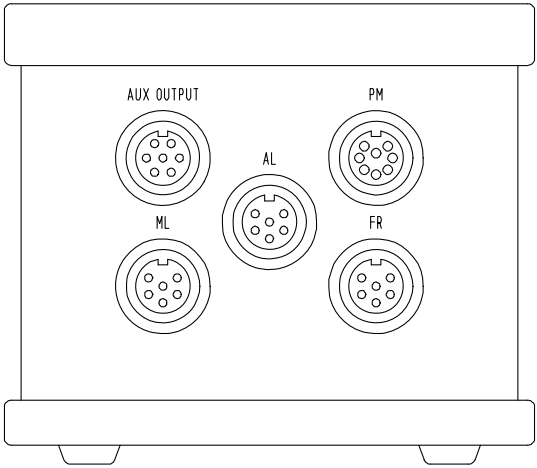


Fig. 4: Right hand side of the PAM-CONTROL housing

At the right hand side of the PAM-CONTROL housing five sockets are located, to which the peripheral optical components are connected.

a) PM

All PAM-CONTROL based fluorometers are equipped with a Photomultiplier Detector, which provides the extreme sensitivity required for ultrasensitive measurements. Via the PM-cable the

photomultiplier is supplied with a control voltage for adjustment of PM-GAIN. The same cable also carries back the preamplified pulse signal, which is further amplified by the Selective Amplifier within the PAM-CONTROL. The same Photomultiplier Detector, based on the Photosensor Module H-6779-01 (Hamamatsu) is used in conjunction with different mechanical adapters in MICROSCOPY- and MICROFIBER-PAM. The WATER-PAM does not feature a separate Photomultiplier Detector Unit, as all optical components are assembled around the sample cuvette within one compact housing.

b) FR

A far-red LED may be connected to the FR-socket. Far-red light can be useful in the assessment of chlorophyll fluorescence, as it causes preferential PSI excitation, thus oxidizing the PSII acceptor side and inducing a special state of the photosynthetic apparatus (state 1). The FR-LEDs are controlled by a special pulse program which assures that they are off during measuring periods. In this way, the increased PM-noise does not affect the signal/noise ratio. Nevertheless, if FR-light is turned on during fluorescence measurements, steps must be taken that it does not penetrate unhindered to the photomultiplier as this would cause artefacts by amplifier saturation (see 8.1). In principle, the FR-output is identical to the AL-output (actinic illumination). Up to 3 LEDs may be connected to it in series.

c) AL

Up to 3 actinic LEDs may be connected in series to the AL-socket. The AL-LEDs are controlled by a special pulse program which assures that they are off during measuring light periods. In this way, the increased PM-noise does not affect the signal/noise ratio. AL-LEDs are not required in the case of MICROSCOPY- and MICROFIBER-PAM when MODE-menu point 6: MEAS+ACT is activated. Then the measuring light (ML) LED provides both

measuring light and actinic light, as well as saturation pulses. In the case of WATER- PAM, more than 3 AL-LEDs are used, such that an additional LED-driver outside the PAM-CONTROL is required, which connects to the AUX OUTPUT (see below).

d) ML

Up to 3 measuring LEDs may be connected in series to the ML-socket. When MODE-menu point 6: MEAS+ACT is activated, ML-LEDs not only provide measuring light but actinic and saturation pulse light as well. In the case of MICROSCOPY- and MICROFIBER-PAM these three tasks are fulfilled by just one LED. Due to a special microprocessor-controlled pulse program and synchronized signal detection, the signal is not disturbed by the actinic/saturation pulses .

e) AUX OUTPUT

The AUX OUTPUT connects to separate external units with additional LED-Drivers. In case of the WATER-PAM, actinic and measuring light LEDs are connected by the same cable using the AUX OUTPUT instead of the ML-socket .

4 Operation- Basic Operations of the PAM-CONTROL

4.1 The basics

The PAM-CONTROL is very easy to operate. It has a two-line LC-display and a small tactile keypad with eight function keys (ON, OFF, MODE, MEM, ∧, ∨, START, SET). In this respect, it is identical to the well-known MINI-PAM Chlorophyll Fluorometer. However, while the MINI-PAM already contains all essential optical components, including measuring/actinic light sources and photodetector, these components first have to be connected to the PAM-CONTROL as described in the brochures of MICROSCOPY-, MICROFIBER- and WATER-PAM, in order to obtain a functional fluorometer.

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  ..C
F: 448  745Y   ..E  ..L
```

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 PAM-CONTROL 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.

`..C` Temperature in degree Celsius, display of which requires optional temperature sensor.

`F:448` Momentary fluorescence yield displaying small fluctuations.

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

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

(Genty-parameter)

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

`..E` Relative rate of electron transport (ETR), display of which requires optional micro quantum sensor. It is calculated by the formula:

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

`..L` Light intensity in terms of PAR (quantum flux density of photosynthetically active radiation), display of which requires optional micro quantum sensor.

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 ($\wedge$ or $\vee$). Stored data can be printed out via an RS 232 interface or transferred on a PC for further analysis.

The PAM-CONTROL has been pre-programmed at the factory with standard settings (see list in 7.1) for all relevant measuring

parameters (for example Measuring Light Amplitude, PM-Gain, Saturation Pulse Intensity, Saturation Pulse Width etc.). For various applications, there is great flexibility for appropriate adjustment of all measuring parameters with the help of the extensive MODE-menu, using the arrow-keys ($\wedge$ and $\vee$) in combination with the SET-key. Details are given in the MODE-menu list below (see 7.2).

4.2 Operation via PC

The PAM-CONTROL can also be operated via PC in a most comfortable way by the WinControl software. When a PC is connected via a RS 232 interface cable with the PAM-CONTROL (see 3.1.2.1), all settings and functions are accessible by mouse click. The user is free to give the commands either via the PC- or the PAM-CONTROL-keypad. Any change of parameter settings via PC results in a corresponding change in the PAM-CONTROL Mode menu (see 7.2) and *vice versa*. Data are automatically written into the "Report file" of WinControl and in the MEMORY of PAM-CONTROL. And data collected during stand-alone operation of the PAM-CONTROL can be readily transferred to the PC by WinControl.

The WinControl software offers a number of additional functions, which cannot be carried out by the PAM-CONTROL in stand-alone operation, like Chart-recording, display of light response curves and curve averaging. These functions not only enhance the analytical capacity of the PAM-CONTROL based devices, but also can help the beginner to become familiar with the properties of chlorophyll fluorescence changes. A separate manual for the WinControl software is available.

5 Operation - Description of Keypad Functions

Fig. 5: PAM-CONTROL Universal Control Unit

5.1 Single key operations

ON To switch PAM-CONTROL on (short pressing of the key).

To activate the backlighting of the display (switches automatically off when no key operation for 50 s; power saving for field use); requires 3 s pressing of the key.

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

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

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

∧, ∨ To select one of 55 points of the MODE-menu or one of 4000 data sets when MEMORY is activated.

For advancement by several steps these keys can be kept pressed.

To change a particular parameter setting in the MODE-menu after operating the SET-key.

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

SET To start and stop selected function.

To initialize and finish change of setting via arrow keys.

5.2 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 19: LIGHT CURVE (carried out via SET).

MODE+∨ To move to MODE-menu point 23: IND.CURVE (carried out via SET).

MODE+ON To switch measuring light on/off.

MODE+MEM To move to MODE-menu point 27: Fo and Fm (carried out via SET).

ON+SET To switch actinic light on/off.

ON+START To start/stop actinic illumination with yield-measurement (see menu point 13).

ON+MEM To start/stop the clock for repetitive triggering of selected function (e.g. saturation pulses when 31: CLOCK-ITEM in position SAT).

ON+∧ To start/stop a LIGHT CURVE (equivalent to menu point 19).

ON+∨ To start/stop an INDUCTION CURVE (equivalent to menu point 23).

SET+OFF To reset program, if PAM-CONTROL for some reason does not respond to key-operations.

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

Note: Whenever a command is given which involves the switching on and off of actinic light, a short beep-sound confirms that the command is carried out. In addition, there is a more extended beep for the duration of a saturating light pulse. The confirmation beeps can be switched off during the operation of PAM-CONTROL via WinControl software (Settings Window / Box: Set / Beeper active).

6 Operation-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/APR/99
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 April 27th 1999. The bottom line shows that a sample of type A was used (see MODE-menu point 55), 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 19.9C
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 an external temperature sensor measures a value of 19.9 °C.

```
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 27) had been carried out beforehand (see 11.3.4).

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 41). 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 40.

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

7 Operation - The Mode-Menu

The MODE-menu contains 55 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. It should be noted that alternatively the same items may be also accessed via the WinControl-software using a PC. While PC operation may be more practicable under laboratory conditions, stand-alone operation using the MODE-menu may be advantageous in the field.

Increasing or decreasing position numbers are selected by the ∧- or ∨-arrow keys, respectively. Changes 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, 54: PM-GAIN). Some of the MODE-menu positions can be directly reached via double key operations (see list in section 5.2 above). In particular, the MODE+SET double key operation allows quick access to various functional blocks of MODE-menu points (see below in section 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, 27-29 and 36, where the displayed parameters 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: 30) or a change of settings is initiated/terminated by pressing SET (e.g. 54: PM-GAIN: 15). Settings are changed by arrow key operations (∧, ∨)

and become immediately effective. The numbers following the double points show the present 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 and PM-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 38: RES. SETTINGS. The first points of the functional blocks which can be quickly reached by the MODE+SET command, are emphasized by boldface printing. Also the double key operations for quick access are listed.

Menu points:	Quick access via:
1. **Standard display**	MODE+START
2. AUTO-ZERO: 0(SET)	
3. MEAS.LIGHT: ON (SET)	MODE+ON
4. M.FREQ: LOW (SET)	
5. PHOTO-M: ON(SET)	
6. MEAS+ACT: ON(SET)	
7. EXT.LIGHT-S:OFF(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)**	ON+SET
13. ACT+YIELD: OFF(SET)	ON+START
14. ACT-WIDTH 0:10 (SET)	
15. ACT-INT: 6 (SET)	

16. **FAR-RED: OFF (SET)**
17. FR-WIDTH: 0:10 (SET)
18. FR-INT: 6 (SET)
19. **LIGHT CURVE:OFF(SET)** MODE+∧
20. L.CURVE+REC:OFF(SET)
21. LC-WIDTH 0:30 (SET)
22. LC-INT: 3 (SET)
23. **IND.CURVE: OFF(SET)** MODE+∨
24. IND.C+REC: OFF(SET)
25. IND-DELAY 0:40 (SET)
26. IND-WIDTH 0:20 (SET)
27. Fo and Fm (SET) MODE+MEM
28. qP and qN (SET)
29. NPQ (SET)
30. **REP-CLOCK: OFF(SET)**
31. CLOCK-ITEM:SAT(SET)
32. CLK-TIME:00:20(SET)
33. **TIME 17:32:56 (SET)**
34. DATE 17-OCT (SET)
35. YEAR 1999 (SET)
36. BATT: 12.4V (11.8)
37. INT.TEMP: 23C
38. **RES.SETTINGS: (SET)**
39. PROGR.D2.25(270998)
40. MEMORY: 12 (SET)
41. CLEAR MEMORY (SET)
42. **LIGHT-OFFS: 0(SET)**
43. LIGHT-GAIN:1.00(SET)
44. TEMP.OFFS: 0.0(SET)
45. TEMP.GAIN: 1.00(SET)
46. ZERO-OFFS: 0(SET)

47. ETR-FAC: 0.84 (SET)
48. ACT-AMPL: 12 (SET)
49. MEAS-AMPL: 8 (SET)
50. **SAT-WIDTH:0.8s(SET)**
51. SAT-INT: 10 (SET)
52. M.FREQ: 3 (SET)
53. OUT-GAIN: 2 (SET)
54. PM-GAIN: 15 (SET)
55. 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 11.3 (Assessment of photosynthesis yield using the PAM-CONTROL). Standard settings are shown. It should be noted that alternatively the same items may be also accessed via the WinControl-software using a PC.

```
1:    445F 1739M   ..C
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 ∗), ambient temperature (if temperature sensor connected) and PAR are displayed.

```
  2: AUTO-ZERO:   0(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 49: MEASURING LIGHT AMPLITUDE, 53: OUTPUT-GAIN or 54: PM-GAIN are modified. If this is forgotten 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 + ON without entering the MODE-menu.

```
  4: MEAS-FRQ: LOW (SET)
F: 448  745Y  6.2E   20L
```

Switch between the measuring pulse frequency set under Mode-point 52 (LOW) and maximal measuring pulse frequency (HIGH, corresponding to 688 Hz). In the High-position the signal/noise ratio is increased. On the other hand, at high frequency the measuring light intensity can induce substantial fluorescence changes. Therefore the High-position normally should be used only when the actinic effect of the measuring light can be neglected relative to a stronger actinic light. MEAS-FRQ is automatically increased from LOW to HIGH during a saturation pulse and during actinic illumination at ACT-INT settings 4-12.

```
  5: PHOTO-M:   ON (SET)
F: 448  745Y  6.2E   20L
```

Switch to turn photomultiplier (PM) voltage ON/OFF. It normally may be in the ON-position without risk of damage, as PM-voltage is automatically turned down when the PM-anode current becomes excessive.

```
  6: MEAS+ACT:  ON (SET)
F: 448  745Y  6.2E   20L
```

Switch between two fundamentally different programs determining pulse pattern of measuring light LED. In ON-position, which is to be used for MICROSCOPY- and MICROFIBER-PAM, the measuring light LED also provides actinic and saturation pulse illumination. In OFF-position, which should be used for the WATER-PAM, the measuring light LED just switches to HIGH-frequency during actinic and saturation pulse periods.

```
  7:EXT.LIGHT-S:OFF(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
```

Automatized 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 in the same position where normally the sample is placed. When using the MICRO Quantum Sensor MC-MQS in conjunction with the MICROSCOPY-PAM, this is placed instead of the sample underneath the same objective, with the 0.2 mm fluorescing diffuser being in focus. 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 costs battery power. This, however, is of no concern under laboratory conditions, when the battery charger can be connected. When in OFF-position, DISPLAY ILLUMINATION switches off 1 min after turning on the instrument. It can be transiently turned on again for 40 s by pressing ON 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 PAM-CONTROL after 4 min without key operation. It is recommended to disable the AUTO-OFF for laboratory work when the PAM-CONTROL is connected to the battery charger (via CHARGE-socket). The AUTO-OFF function is 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 actinic illumination. This can also be directly done via ON + SET. There is a blinking sign (ACT) in the upper left corner of the display while actinic illumination is on.

```
 13: ACT+YIELD: OFF(SET)
F: 448  745Y  6.2E   20L
```

On/off switch of actinic illumination, 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 ON + START.

```
 14:ACT-WIDTH 0:10 (SET)
F: 448  745Y  6.2E   20L
```

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 10 min.

```
 15:  ACT-INT:  6  (SET)
F: 448  745Y  6.2E   20L
```

Setting of intensity of actinic illumination. The setting can be modified via SET and the arrow-keys between 1 and 12. The range covered by intensities 1-12 is shifted up and down at different settings of ACT-AMPL (MENU-point 48), when as in the case of the WATER-PAM separate actinic LEDs are used, and of MEAS-AMPL, when as in the case of MICROSCOPY- or MICROFIBER-PAM, the measuring light LED is also used for actinic illumination (see 3.1.2.2).

```
16:   FAR-RED: OFF(SET)
F: 448  745Y  6.2E   20L
```

On/off switch of optional far-red light source. Please note, that the use of far-red light may cause artifacts, if too much of this light can reach the photomultiplier (see 8.1).

```
 17: FR-WIDTH 0:10 (SET)
F: 448  745Y  6.2E   20L
```

Setting of far-red illumination time. The setting can be modified via SET and the arrow keys.

```
 18:  FR-INT:    6 (SET)
F: 448  745Y  6.2E   20L
```

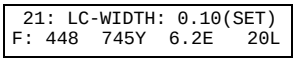
Setting of intensity of far-red illumination. The setting can be modified via SET and the arrow keys between 1 and 12.

```
 19:LIGHT CURVE:OFF(SET)
F: 448  745Y  6.2E   20L
```

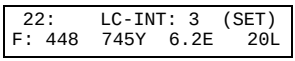
When switched on via SET, first the maximal YIELD in the absence of actinic light (Fv/Fm) is measured and then a series of 8 consecutive YIELD-measurements at increasing light intensities is started. This function can be also directly started by double key operation ON + ∧. The time periods at the different intensities are set by menu point 21: 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 22: LC-INT, where one can choose between values from ACT-INT 1 to 5, with the standard setting being ACT-INT 3. The effective PAR-values at the sample site are calibrated by the LIGHT-CALIBRATION routine (menu point 8). A LIGHT CURVE can provide profound information on the overall photosynthetic performance of a sample, even if the illumination periods are too short to achieve true steady states.

```
 20:L.CURVE+REC:OFF(SET)
F: 448  745Y  6.2E   20L
```

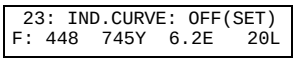
When switched on via SET, a LIGHT CURVE is measured as described for menu point 19 and in the following dark period the recovery of YIELD is assessed by 6 consecutive measurements at 10 s, 30 s, 60 s, 2 min, 5 min and 10 min following illumination.



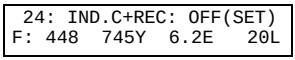
LC-WIDTH determines the illumination time at each intensity setting. 10 s are sufficient for so-called “rapid light curves”. It is limited to 10 min.



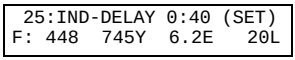
The LC-INT determines the starting intensity which can be chosen between settings 1 to 5. LIGHT CURVES always involve 8 intensities. Hence, more emphasis may be put either on the linear rise or on the plateau region of the curve.



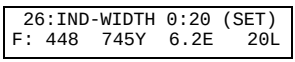
This function starts registration of a dark-to-light INDUCTION CURVE with Saturation Pulse Quenching Analysis. Normally dark-adapted samples are used. First a saturation pulse is given for determination of Fo, Fm and Fv/Fm. After a certain dark time, set by IND. DELAY (menu point 25), ACTINIC LIGHT at 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 26).



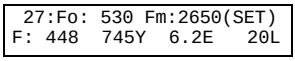
In addition to the recording of dark-to-light INDUCTION CURVE (as described for menu point 23), after turning off the ACT.-LIGHT 6 saturation pulses are applied at 10 s, 30 s, 60 s, 2 min, 5 min and 10 min to assess the dark recovery of fluorescence parameters.



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.

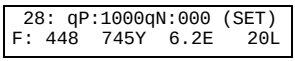


Time interval between two consecutive saturation pulses during recording of IND.CURVE. The default setting is 20 s. Possible settings range from 5 s to 3 min.



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 28 and 29). This function can be also directly accessed via the double key operation MODE+MEM.



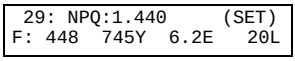
Coefficients of photochemical quenching, qP, and non-photochemical quenching, qN, as defined by the following equations:

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

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.

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 27 after dark-adaptation.

Note: Wincontrol calculates the coefficients qP and qN according to Schreiber et al. (1986) as formulated by van Kooten and Snel (1990): $qP = (Fm'-F')/(Fm'-F_0')$ and $qN = 1-((Fm'-F_0')/(Fm-F_0))$.

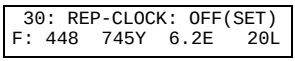


Parameter describing non-photochemical quenching defined by the equation:

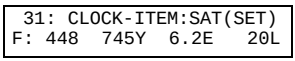
$$NPQ = (Fm-M)/M$$

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 27 after dark-adaptation. NPQ has been shown to be closely related to the excess light energy which is actively dissipated by the photosynthetic

apparatus 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.

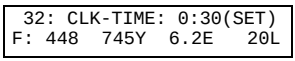


On/off switch of the repetition clock which serves to trigger a number of functions which are specified in menu point 31: CLOCK ITEM. This function can be also directly started by double key operation ON + MEM.

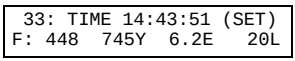


This menu point allows to choose between the following functions to be triggered by the REPETITION CLOCK:

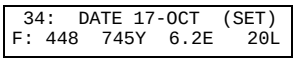
SAT-PULSE, ACT-LIGHT, ACT + YIELD, LIGHT CURVE, L-CURVE + REC., IND. CURVE , IND.C + REC.



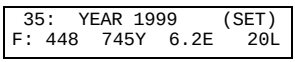
Setting of clock interval, which is the time between two consecutive saturation pulses (or other functions) triggered by the REP-CLOCK (menu point 30). The setting can be modified via SET and the arrow-keys in 10 s steps. Possible settings range from 0:10 to 42:30. When moving beyond the maximal time,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



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



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.



Display of present year which can be

modified via SET and the arrow-keys. The change is terminated via MODE.

```
 36:  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 (may be transiently decreased due to increased LED-current). 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).

```
 37:  INT.TEMP: 24C
F: 448  745Y  6.2E   20L
```

Display of temperature within the instrument.

```
 38: 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 section 7.1).

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

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

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

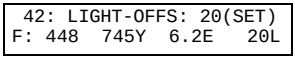
Function to move the present MEMORY-front to any number between 1 and 4000. This function may be important when the MEMORY is full and the user wants to avoid overwriting older data.

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.

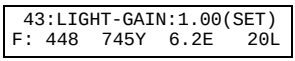
```
 41: CLEAR MEMORY  (SET)
F: 448  745Y  6.2E   20L
```

Command to erase all data accumulated in

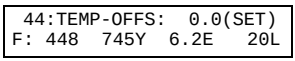
MEMORY. For safety's sake this command is not yet carried out by SET but requires confirmation by pressing the ∧-key. Then the MEMORY-front is reset to 0 and the data set recorded with the next saturation pulse will be in MEM position 1.



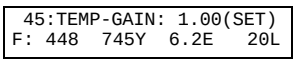
Function for adjustment of PAR-reading by comparison with calibrated device. Particular care must be taken that both sensors are exposed to the same photon flux density. After SET, the PAR-reading (L) can be adjusted by the arrow-keys in steps of 1 µmol quanta m^{-2} s^{-1}. For proper calibration over a wide range of PAR also adjustment of LIGHT-GAIN (menu point 43) may be required. This can be checked by comparison with calibrated device at a different PAR-value.



Function for adjustment of PAR-reading. The adjustment via LIGHT-GAIN should be carried out after a preceding adjustment by LIGHT-OFFS (menu point 42) 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 42).

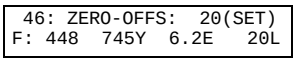


Function for adjustment of temperature-reading with optional temperature sensor in comparison with calibrated device. After SET, the temperature reading can be adjusted by the arrow-keys in 0.1 °C steps. For proper calibration over a wide temperature range also adjustment of TEMP-GAIN (menu point 45) may be required. This can be checked by comparison with a calibrated device at different temperatures.

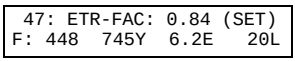


Function for adjustment of temperature-reading with optional temperature sensor. The adjustment via TEMP-GAIN should be carried out after a preceding adjustment by TEMP-OFFS (menu point 44) at a different temperature, such that the slope in the temperature response curve

can be evaluated. For highest accuracy, TEMP-OFFS then may have to be adjusted once more (menu point 44).



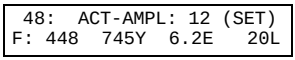
Display of present zero offset value which normally is identical to the value obtained automatically via AUTO-ZERO (menu point 2). Following SET, this value can be manually modified using the arrow-keys.



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

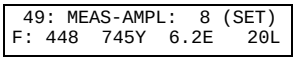
$$ETR = Yield \times PAR \times 0.5 \times 0.84$$

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:** In many practical cases (e.g. dealing with single cells or natural phytoplankton) this value is far from realistic and, hence, the calculated ETR can be only considered a relative measure of electron transport activity.



Setting of pulse amplitude of separate actinic LEDs, as e.g. used in WATER-PAM, which can be changed in 12 steps.

Note: This setting normally remains fixed (standard setting 12) and the intensity of actinic illumination is changed via ACT-INT (menu point 15). See chapter 8.2.



Setting of pulse amplitude of measuring light LEDs, which can be changed in 12 steps.

Note: This setting normally remains fixed (standard setting 8) and the intensity of the measuring light is changed via MEAS-FRQ

(menu point 4), with the advantage that signal amplitude is constant (see chapter 8).

```
 50: SAT-WIDTH:0.8s(SET)
F: 448  745Y  6.2E   20L
```

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.

```
 51:  SAT-INT:  8  (SET)
F: 448  745Y  6.2E   20L
```

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

```
 52:  MEAS-FRQ:  3 (SET)
F: 448  745Y  6.2E   20L
```

Setting of the frequency of measuring light pulses which can be changed in 12 steps. With increasing frequency the integrated measuring light intensity is raised and time resolution as well as signal/noise ratio are increased.

```
 53: OUT-GAIN:  2  (SET)
F: 448  745Y  6.2E   20L
```

Setting of output gain (amplification factor) which can be varied between 1 and 12. By increasing OUT-GAIN not only the signal but also the noise increases in proportion. Any change in OUT-GAIN requires a new determination of the unavoidable background signal via AUTO-ZERO (menu point 2). In most practical applications, it is advantageous to increase signal amplitude by PM-GAIN (menu point 54) instead of OUT-GAIN.

```
 54: PM-GAIN:   15 (SET)
F: 448  745Y  6.2E   20L
```

Setting of photomultiplier gain which can be varied in 30 steps. Increasing PM-GAIN will not only increase the signal but also the noise. Any change in PM-GAIN requires a new determination of the unavoidable background signal via AUTO-ZERO (menu point 2).

```
 55:  MARK: A      (SET)
F: 448  745Y  6.2E   20L
```

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 in experiments involving different types of samples.

8 Special features of illumination program

PAM-CONTROL based fluorometers differ from previous PAM-Fluorometers (like PAM-101/102/103, PAM-2000 or MINI-PAM) by a special pulse technique which allows to make optimal use of a photomultiplier (PM) as an ultrasensitive fluorescence detector. A PM cannot be exposed to ambient light, like a photodiode, without being damaged. In order to avoid such damage, in all PAM-CONTROL based devices the PM is protected by special circuitry which turns down supply voltage whenever the anode current becomes excessive. Furthermore, the output noise of a photomultiplier increases with light intensity, contrary to the situation with a photodiode. Fortunately, this noise disappears, as soon as the light disappears. These specific photomultiplier properties have led to the special pulse illumination technique with following features:

Actinic illumination is either achieved by the measuring light (ML) pulses applied at different frequencies or by special actinic light (AL) pulses, which are longer than the ML-pulses and positioned in the dark times between individual ML-pulses. For optimal signal/noise ratio, the ML-pulses should have a high amplitude and be applied at high frequency. However, then the measuring light will have an actinic effect. Therefore, in situations when no actinic effect can be tolerated, as assessment of fluorescence yield after dark adaptation, Fo, ML-pulses are applied at low frequency, and ML-pulse frequency is switched to high frequency during actinic illumination.

As PAM-CONTROL based devices do not tolerate ambient light and all actinic light is fully controlled, the effective intensity of photosynthetically active radiation, PAR, in principle can be

calculated, if the system is calibrated for a particular instrument and a specific optical situation. This aspect will be further dealt with below (see 8.2).

8.1 Different types of pulse light

As outlined above, an important feature of all PAM-CONTROL based instruments is the very special pulse modulation technique. While it is not necessary that the user is aware of all the details, some knowledge on the generation and pulse pattern of the various types of light will be useful:

a) Measuring light (ML) can be generated by 1-3 LEDs with a broad choice of wavelengths. For example, the MICROSCOPY-PAM in its standard version employs a single blue (470 nm) LED while with the MICROFIBER-PAM the user may choose between a single blue (470 nm), green (520 nm), yellow (590 nm) and red (650 nm) LED. On the other hand, in the WATER-PAM 3 ML-LEDs are employed, as much larger areas are illuminated. The ML-LED pulses are 5 µs long and repeated with frequencies ranging between 8 and 688 Hz (Meas.-Frequency settings 1-12) (MEAS-FRQ, Menu point 52). At low frequency (standard setting 3 corresponding to 18 Hz) the integrated light intensity at the sample level is very low, even when the beam is highly focused, as with the MICROSCOPY-PAM. This is important to assess the minimal fluorescence yield after dark-adaptation (see 11.1). As Meas.-frequency increases, the integrated light intensity increases proportionally, whereas the amplitude of the individual ML-LED pulses remains constant. Therefore, also the fluorescence signal remains constant, unless the integrated intensity is sufficiently high to induce an increase in fluorescence yield (see 11.1). The amplitude of the ML-LED pulses can also be varied (Meas.-Amplitude settings 1-12) (MEAS-AMPL, Mode-menu point 49). For the sake of a high signal amplitude, a high setting is

recommended (standard setting is 8). Normally, MEAS-AMPL is kept constant and the effective intensity of the measuring light is varied via its frequency (MEAS-FRQ).

b) Actinic light (AL) can be generated in two different ways. Either the same ML-LED, which generates measuring light, also serves for generating actinic light (as in the case of MICROSCOPY- and MICROFIBER-PAM) or a separate array of AL-LEDs is used (as with the WATER-PAM). In both modes of operation the actinic light is applied in the form of 22 µs long pulses which are positioned in the dark times between measuring pulses.

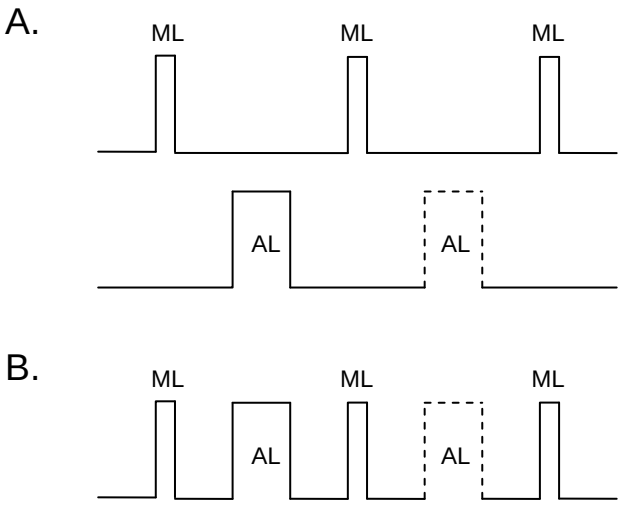


Fig. 6: Two different ways of actinic light (AL) generation.

A. AL-pulses are generated by a separate array of actinic LEDs and positioned in the dark periods between measuring light (ML)-LED pulses (e.g. WATER-PAM)

B. The same LED which generates ML-pulses also serves for generation of AL-pulses.

In both modes the effective actinic light intensity is determined by the frequency of AL-pulses. In addition, in mode A the amplitude of AL-LED pulses and in mode B the amplitude of ML-LED pulses (being identical for ML and AL) can be varied in order to shift the range of effective actinic intensities up and down.

Note: For proper operation of the PAM-CONTROL in conjunction with MICROSCOPY- and MICROFIBER-PAM it is essential that a special mode is activated (Menu-point 6: MEAS+ACT, see 7.2). This mode will be automatically activated when the WinControl-software is used and the MICROSCOPY- or MICROFIBER-PAM is selected as device (see WinControl manual).

While the effective light intensity during actinic illumination is determined by a number of parameters, these are normally kept constant, except for ACT-INT (Menu point 15, see 7.2) featuring 12 settings which cover a range of relative light intensities between 1 and 85. The effective intensity is not changed via the amplitude but via the frequency of the 22 µs LED pulses.

Note: The ACT-INT settings are not only valid for devices with separate actinic LEDs (like WATER-PAM), but for devices with a single LED as well (like MICROSCOPY-PAM). Besides the ACT-INT settings, in the latter case, the amplitude of ML-LED pulses (MEAS-AMPL, Mode-menu point 49, see 7.2) determines the actinic light intensity, whereas in the former case ACT-AMPL (Mode-menu point 48) applies. Normally, MEAS-AMPL and ACT-AMPL are kept constant and the actinic light intensity is varied via ACT-INT.

c) Saturation pulses (SP) are generated by the same LEDs which also generate actinic light, i.e. depending on the selected mode either by a separate AL-LED array or by a single LED (as in the case of MICROSCOPY- or MICROFIBER-PAM). During saturation pulses the 5 µs ML-pulses are applied at a frequency of 20 kHz and depending on the setting of SAT-INT (Menu-point 51) the frequency

of the 22 µs AL-pulses is varied to achieve different saturation pulse intensities.

Note: Just as with actinic light, the generation of saturation pulses by devices featuring a single LED (like MICROSCOPY- and MICROFIBER-PAM) requires activation of Menu-point 6: MEAS+ACT. Otherwise, there is just a transient increase of ML-pulse frequency to 20 kHz, which normally is not sufficient for saturation. Furthermore, it should be noted that the effective intensity of saturation pulse is not only determined by SAT-INT (Menu point 51), but by ACT-AMPL (Menu point 48, with devices featuring an actinic LED array) and MEAS-AMPL (Menu point 49, with devices featuring a single LED). But, normally MEAS-AMPL and ACT-AMPL are kept constant and saturation pulse intensity is varied via SAT-INT.

d) Generation of far red light (FR) requires an optional FR-LED source (see 3.1.2.2) which is controlled by the same pulse program (22 µs pulses) as AL-light.

Note: Although the FR-pulses, just like the AL-pulses are applied between ML-pulses, they may disturb the fluorescence measurement, if a substantial part of the FR-light can penetrate to the photomultiplier detector (amplifier saturation). This can be avoided by choosing an appropriate angle between the FR-LED beam and the optical axis between sample and detector. In principle, it is also possible to place a short pass filter ($\lambda<710$ nm) in front of the photomultiplier. This, however, not only absorbs the FR-light (peak at 735 nm) but the long wavelength fluorescence as well and, hence, lowers the signal.

8.2 Effective light intensity and PAR-calibration

It is a characteristic of all PAM-CONTROL based fluorometers that the investigated sample must be carefully protected from external light and that all light is under software control. As a consequence, all parameters determining the relative light intensity are known and the effective intensity of photosynthetically active radiation, PAR, can be calculated after proper calibration. Depending on the specific device and the particular optical situation (e.g. different objectives with the MICROSCOPY-PAM, different types of LED-lamps with the MICROFIBER-PAM) at the same instrument settings (e.g. ACT-INT, MEAS-AMPL) different absolute intensities at the sample level will occur. At the same time, the relative intensities in dependence of the various parameters are the same for all devices and optical situations.

In most practical applications, knowledge of absolute PAR is not required. Actually, even if absolute incident PAR were known, there would remain uncertainty about absorbed PAR. On the other hand, knowledge of relative PAR is very useful for an evaluation of fluorescence data, for example when comparing the effective quantum yields ($\Delta F/Fm'$, see 11.1 and 11.3.6) in different light states, as e.g. by recording of a light response curve (see 12.3.8).

With the help of special micro quantum sensors also the absolute incident PAR at the sample level can be determined. For the MICROSCOPY-PAM the special Micro Quantum Sensor MC-MQS was developed, which has the dimensions of an object carrier, featuring a 0.2 mm pin-hole with a fluorescing diffuser and a blue-enhanced photodiode. For the WATER-PAM the Spherical Micro Quantum Sensor US-SQS is available. This consists of a 3 mm Ø white diffusing plastic sphere connected via a flexible 1 mm Ø plastic fiber to a detector unit featuring a blue-enhanced photodiode and a special filter-set for selection of photosynthetically active

radiation (380-710 nm). It should be noted, that in the case of the Micro Quantum Sensor MC-MQS, the spectral sensitivity of the detector is not defined by filters. Therefore, calibration of this device was carried out with the blue LED Measuring Light Source, which is used in the MICROSCOPY-PAM and PAR-readings are valid only in conjunction with this light source.

The PAM-CONTROL offers a routine for light calibration (Menu point 8, LIGHT CALIB). Light calibration is valid only for one particular optical situation with the diffuser of the micro quantum sensor being in exactly the same position as normally the investigated sample. The routine involves illumination at the 12 settings of ACT-INT and storage of the PAR-readings in an internal PAR-list. Via Menu point 7 (EXT.LIGHT-SENSOR ON/OFF) the user may choose between PAR-values provided by the external sensor (ON) and PAR-values form the internal list (OFF). Normally it is not feasible to measure PAR during fluorescence measurements and, hence, following PAR-calibration the micro quantum sensor is replaced by the sample and EXT.LIGHT-SENSOR switched OFF.

9 Important Points for Correct YIELD-Measurements

Chlorophyll fluorescence provides very useful information for assessment of photosynthesis, particularly with the numerous functions offered by the extensive PAM-CONTROL Mode-menu and the WinControl-software. Probably the most important function is the determination of the YIELD-parameter ΔF/Fm (Genty-parameter). This task is carried out by the PAM-CONTROL with exceptional sensitivity and reproducibility. Important practical aspects for correct YIELD-measurements are:

1) The **signal should be in the order of 200-500 units**. If this is not the case at standard settings, the PM- or Output-Gain should be increased.

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 11.3.3).

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. In order to compare the YIELD-values of two samples, they should be exposed to the same PAR and this should be measured at the same site where also fluorescence is detected. For this purpose in conjunction with the MICROSCOPY-PAM the optional Micro Quantum Sensor MC-MQS is available, while in conjunction with the WATER-PAM the Spherical Quantum Sensor US-SQS may be

used. 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 11.3.7).

4) Dark YIELD-measurements require special conditions (see also 11.3.1). 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 which normally is assured at low measuring light frequency (Mode-menu point 52, MEAS-FRQ, standard setting 3).

5) An important requirement for correct YIELD-determination is that the saturation pulse is sufficiently intense to close all PSII reaction centers and to induce maximal fluorescence yield. In the case of the MICROSCOPY-PAM and the MICROFIBER-PAM, this task has to be fulfilled with the same LED which also provides the measuring light, whereas the WATER-PAM employs separate sets of LEDs for these different tasks (see 8.1). Hence, in the case of MICROSCOPY- and MICROFIBER-PAM the MODE-menu point 6 (MEAS+ACT) must be set to ON, whereas it must be set to OFF in the case of the WATER-PAM.

10 Maintenance

10.1 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 foreseeable 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 PAM-CONTROL 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)
- Display illumination-off (1 min after switching the instrument on unless disabled by menu point 9)
- Menu point 36: 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 8.0 V during a saturation pulse.

- When battery voltage drops below 8.0 V the CLOCK is automatically turned off. This is important as the CLOCK disables the AUTO-OFF function.

If replacement of the battery becomes necessary, this is readily accessible after removing the 4 screws at the bottom of the PAM-CONTROL housing. The battery is attached to the bottom part by double-sided adhesive tape. After disconnecting the cables, the battery can be detached by means of a screw-driver used as a lever. The replacement battery comes with adhesive tape. When connecting the cables, please note the proper contact polarities (red/positive and black/negative).

10.2 Fuse replacement

Two fuses are provided:
Si1: 2 AT for external components
Si2: 315 mAT for general electronics

For replacement, put the PAM-CONTROL upside down and remove the bottom part (4 screws). The fuses are located on the main board.

10.3 EPROM and its replacement

The location of the EPROM on the microcontroller board is indicated in Fig. 7, which shows a view on the interior of the PAM-CONTROL in its upside-down position after removing the bottom part (4 screws). The EPROM contains the software of the current program version (see menu point 39). It can be readily exchanged against a new EPROM when program updates become available. Please note the little red dot at the side of the EPROM which is directed to the back side of the PAM-CONTROL housing where e.g.

the RS 232 socket is located. A special tool for removing the EPROM is delivered with the PAM-CONTROL. When installing the new EPROM, 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 points 38 and 41).

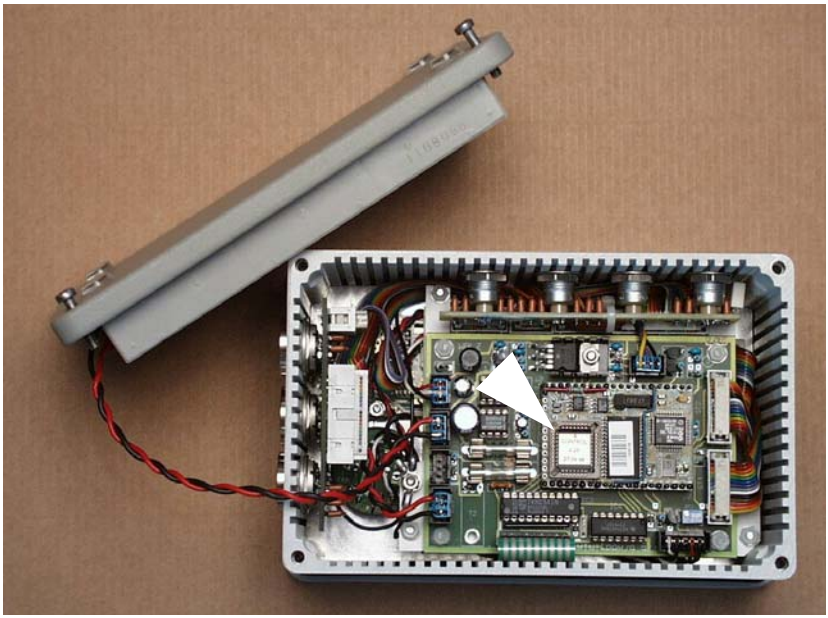


Fig. 7: Location of the EPROM

11 Chlorophyll Fluorescence Measurements with PAM-CONTROL Based Instruments

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. Progress in this field of research has been greatly stimulated by the invention of the Pulse-Amplitude-Modulation (PAM) measuring principle (see section 11.2 below). The first PAM-101 Chlorophyll Fluorometer, with its accessory modules 102 and 103, as well as 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 PAM-CONTROL a new generation of ultrasensitive Chlorophyll Fluorometers has been created which has opened the way for assessment of photosynthesis at the level of single cells and chloroplasts. Hence, chlorophyll fluorescence again can be applied as a pioneering tool, as e.g. in the study of single guard-cells. While from a technical point of view the new PAM-CONTROL based fluorometers differ considerably from previous PAM Fluorometers, the principles of measuring the yield of chlorophyll fluorescence and

deriving information on photosynthetic electron transport are the same. Just like with previous PAM Fluorometers, e.g. the MINI-PAM, the new PAM-CONTROL based fluorometers were 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 often referred to as Genty-parameter. In the following sections some background information on this and other fluorescence parameters is given, and special aspects on fluorescence measurements with PAM-CONTROL based fluorometers are outlined, in order to make optimal use of these instruments.

11.1 Chlorophyll 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.

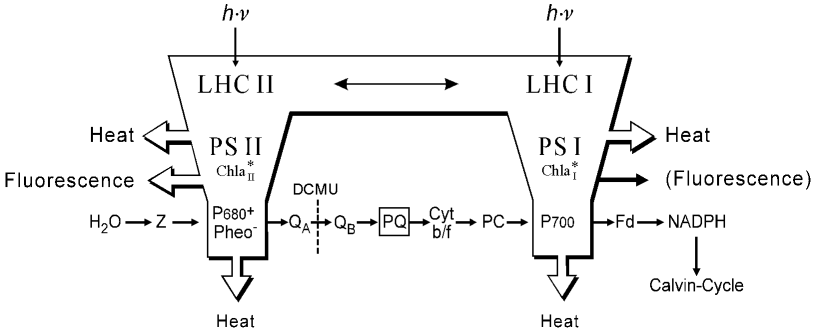


Fig. 8: Schematic view of primary energy conversion and primary electron transport in photosynthesis. LHC, light harvesting pigment-protein complex; P680 and P700, energy converting special chlorophyll molecules in the reaction centers of photosystem II (PSII) and photosystem I (PSI), respectively; Pheo, 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 in 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 yield (not just intensity) at largely changing actinic light intensities and to use

very strong light for the so-called 'quenching analysis'. For this purpose, first the original PAM measuring principle was developed which allows monitoring fluorescence against 10^6 times larger background signals (see 11.2). This had to be modified and extended for PAM-CONTROL based fluorometers employing a photomultiplier detector (see 8).

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 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.

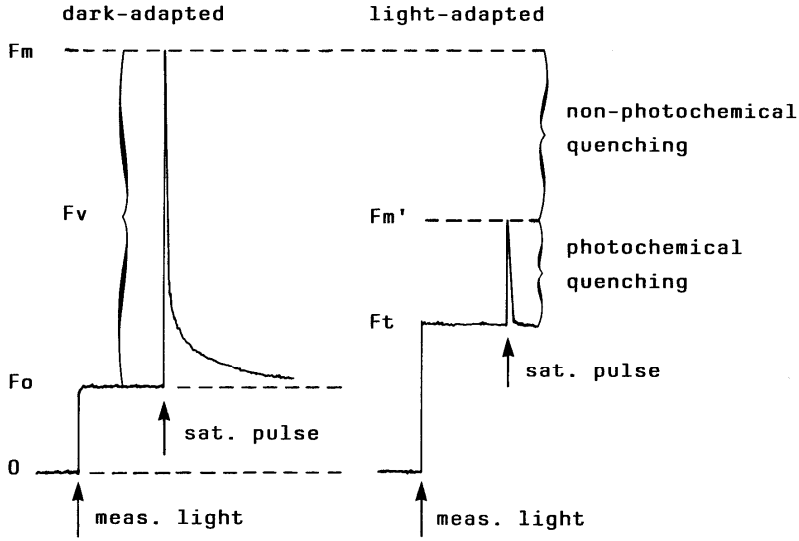


Fig. 9: Principle of saturation pulse quenching analysis

On the basis of these considerations so-called 'quenching coefficients' qP and qN were defined, which can be determined by simple fluorescence measurements (see 11.3.5). 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 11.3.4). 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.

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 PAM-Fluorometers (see 11.3.6). With the new types of microprocessor-controlled fluorometers, YIELD-determination has become exceedingly simple: One just has to press the START-key. 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, 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 samples under varying environmental and physiological conditions can be obtained.

For full assessment of fluorescence information, knowledge of environmental parameters is required, in particular of light intensity. 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 than sample B. Therefore, particular efforts were undertaken to develop also for PAM-CONTROL based fluorometers special quantum sensors which allow assessment of photosynthetically active radiation (PAR) at the very site where fluorescence is

measured (see 8.2). As with PAM-CONTROL based fluorometers the actinic illumination is under instrument control, after appropriate calibration the relevant PAR-values for different settings of actinic intensity are known and entered with each YIELD-determination into the file of automatically stored data. As PAR is known, the apparent rate of electron transport (ETR) can be 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 whole complexity of the process. Such transients can be either monitored with an analog chart recorder or by making use of the "Chart"-function of the WinControl-software with a PC. 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 non-photochemical 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 (see review articles, and the original papers cited in chapter 12.4).

11.2 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 measure the intensity of chlorophyll fluorescence. However, for so-called quenching analysis (see 11.1) it is necessary to measure chlorophyll fluorescence yield at largely different light intensities, which are applied with the purpose of affecting the yield rather than measuring it. 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 11.1), a new modulation principle was developed which tolerates a ratio of $1:10^5$ or even higher between fluorescence and background signal. Fluorescence is excited by very brief but strong light pulses from light-emitting diodes. With PAM-CONTROL based fluorometers, these pulses are 5 µs long and repeated at frequencies ranging between 8 and 20000 Hz. The LED light passes a short-pass filter and the photodetector is protected by a long-pass filter. A highly selective pulse amplification system ignores all signals except the fluorescence excited during the 5 µs measuring pulses.

However, while this measuring principle works very satisfactory with fluorometers equipped with photodiodes as fluorescence detectors, there is a distinct problem with photomultipliers, as their noise increases with background light and, unless properly protected, will be even damaged when the background light becomes excessive. Therefore, as all PAM-CONTROL based fluorometers employ a photomultiplier for the sake of sensitivity, the original PAM measuring principle had to be modified for this new generation of ultrasensitive instrument. External nonmodulated actinic light cannot be used anymore and instead actinic illumination as well as saturation pulses are applied in form of light pulses positioned in the dark periods between measuring light pulses (see 8.1).

11.3 Assessment of photosynthesis with PAM-CONTROL based fluorometers: Outline of the most important functions in practical applications.

In section 11.1 it was outlined, in which way fluorescence yield relates to the effective quantum yield of photochemical energy conversion. This very fundamental information is obtained 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) then is 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 9). While it is almost trivial that the actual measurement must be correct from a technical point of view, 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 PAM-

CONTROL, corresponding to some selected points of the MODE-menu. A short description of all 55 points of the MODE-menu was already given in section 7.

11.3.1 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 can be viewed as an expression of photoprotection. In practice, determination of Fv/Fm just involves pressing of the START-key to trigger a saturation pulse. However, in order to assess maximal Fv/Fm, it is essential that the measuring light intensity is sufficiently low and the sample is dark-adapted.

11.3.2 MEAS-FRQ (menu point 52)

Plants can differ widely with respect to their requirements for dark-adaptation. With shade plants, less than 1 µmol quanta $m^{-2}s^{-1}$ may already cause closure of PS II centers accompanied by a fluorescence increase, whereas sun adapted plants display close to minimal fluorescence yield and maximal Fv/Fm in the steady-state

even at 10-40 µmol quanta $m^{-2}s^{-1}$. For assessment of "dark" fluorescence yield the use of measuring light is unavoidable and particularly when dealing with very low amounts of chlorophyll, as in microscopically small samples or surface water samples, the measuring light pulses should not be too weak, in order to obtain a satisfactory signal/noise ratio. Even at a relatively high intensity of the individual measuring light pulses, the overall actinic effect of the measuring light can be kept low, if the frequency of the measuring light pulses is low (menu point 52: MEAS-FRQ). At the standard setting 3, corresponding to 18 Hz, there is sufficient time between individual pulses for reoxidation of reduced PSII acceptors. It should be noted, however, that at such low measuring frequency the response time of the fluorometer is rather slow (time constant in the s range). Normally, this is of no concern, as no rapid changes of fluorescence yield occur under dark conditions. During actinic illumination (at ACT-INT settings 4-12) and saturation pulses, MEAS-FRQ automatically is switched to the maximal setting 12 (corresponding to 700 Hz), such that rapid changes of yield can be followed. Furthermore, briefly after a saturation pulse and after turning off actinic light, for 0.2 s maximal MEAS-FRQ setting 12 is maintained, in order to follow the return of fluorescence yield to the dark level.

11.3.3 AUTO-ZERO (menu point 2)

PAM-CONTROL based fluorometers like other chlorophyll fluorometers, are not absolutely selective for chlorophyll fluorescence but also show without samples small signals. Such false signals originate from traces of scattered measuring light which reach the photodetector despite the blocking filters. Such signals do not depend on properties of the investigated sample and, therefore, are

constant as long as the measuring light amplitude (menu point 49) and the gain (menu points 53 and 54) are not changed.

A 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 OUT-GAIN (menu point 53), PM-GAIN (menu point 54) or MEAS-AMPL (menu point 49) 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) in order to determine YIELD correctly. 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 46) will lead to underestimation of Y ($\Delta F/Fm'$ or Fv/Fm). Normally the error is small (approximately 2 %). The error can increase considerably, when samples with exceptionally low chlorophyll content and unfavorable geometries are assessed. In such cases, in order to reach a practicable signal amplitude high measuring light intensity and maximal gain are applied. However, in this way also the background signal is increased and then AUTO-ZERO becomes very essential.

11.3.4 Fo, Fm (menu point 27)

Fo and Fm are defined as the minimal and maximal fluorescence yields of a dark adapted sample, respectively. Knowledge of Fo and

Fm is required for determination of the quenching coefficients qP, qN and NPQ (see section 11.3.5). Fo and Fm determination is carried out in menu position 27 via SET. This position can be quickly accessed via the double key operation MODE+MEM. After Fo-Fm determination, in menu position 14 there is automatic reset of qP to 1.000 and of qN to 000 and in menu position 15 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 27. As outlined in section 11.3.2, the threshold of light intensity below which a sample is dark-adapted can vary considerably. In most higher plants Fm/Fo = 5 to 6, which is equivalent to Y = Fv/Fm = 0.800 to 0.835. In algae Fv/Fm-values are somewhat lower. Optimal values can be measured only when true dark-adaptation is reached and the measuring procedure is optimized as outlined in the preceding sections 11.3.2 and 11.3.3.

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 11.3.1) and also for calculation of the quenching coefficients qP, qN and NPQ (see 11.3.5). 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 and that the same settings of MEAS-AMPL (menu point 49), PM-GAIN (menu point 54) and OUT-GAIN (menu point 53) 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.

11.3.5 qP, qN and NPQ (menu points 28 and 29)

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 11.3.4). Any fluorescence lowering with respect to Fm may be caused either by enhanced photochemical energy conversion or by increased heat-dissipation (as compared to dark state). As was outlined in section 11.1, 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 equivalent to the ...M on the PAM-CONTROL display):

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

Note: Wincontrol calculates the coefficients qP and qN according to Schreiber et al. (1986) as formulated by van Kooten and Snel (1990): qP = (Fm'-F')/(Fm'-F_0') and qN = 1-((Fm'-F_0')/(Fm-F_0)).

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 11.3.4) 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'. The PAM-CONTROL features an output for far-red illumination (FR-socket) to which an optional FR-LED may be connected. However, the use of far-red light in conjunction with PAM-CONTROL based fluorometers may be problematic, if a substantial part of this light can penetrate to the photomultiplier. In principle, this can be prevented when using short-wavelength LEDs (like blue LED in MICROSCOPY-PAM) by protecting the photomultiplier by a short-pass filter ($\lambda<700$ nm). This, however, cuts down the fluorescence signal to ca. 1/3, which may be considered too high a price for correct Fo-determination.

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 nonphotochemical 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 nonphotochemical 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. 10 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.

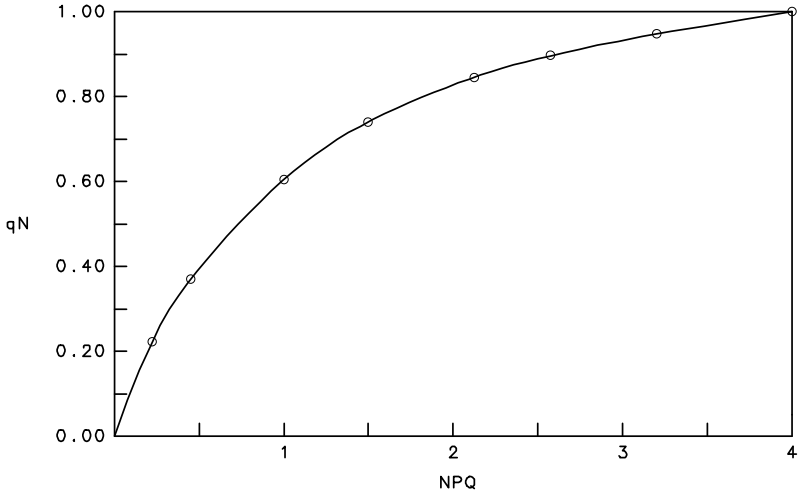


Fig. 10: Relationship between the fluorescence parameters of nonphotochemical quenching qN and NPQ

11.3.6 YIELD-measurements of illuminated samples

With every application of START a YIELD-measurement is carried out and on the LC-display of the PAM-CONTROL in the standard menu position 1 the following signals measured in connection with a particular 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 Y/1000 = YIELD = (M-F)/M = (Fm'-F)/Fm';

- E, apparent electron transport rate (ETR) calculated as ETR = YIELD * PAR * 0.5 * ETR-factor

This and additional information is stored in the MEMORY (see section 6).

Formally, there is no difference between YIELD-measurements with dark-adapted or illuminated samples. In the former case, F and M correspond to Fo and Fm (see 11.3.4) and Y corresponds to Fv/Fm, the maximal photochemical quantum yield (see 11.3.1). 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.

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

YIELD-measurements of illuminated samples do not necessarily involve prolonged illumination times with the PAM-CONTROL. Relatively short times down to less than a minute 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 ON + SET or in menu position 12 via SET. In the latter case the remaining illumination time is displayed. The PAM-CONTROL 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 ON + 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 type of instrument and the particular optical situation, like the choice of objective in the case of the MICROSCOPY-PAM. Hence, at a given setting of ACT-INT the effective intensity may vary and for quantitative work calibration of PAR is required. However, in most practical work, knowledge of relative light intensities and of relative electron transport rates is sufficient.

With PAM-CONTROL based fluorometers actinic illumination is exclusively applied by the instrument. Therefore the intensity of the actinic irradiation, as a function of ACT-INT, is known as relative PAR value" (using the internal PAR-List) or as absolute incident PAR values (after calibration with the MicroQuantum SensorsMC-MQS or US/SQS respectively).

With PAM-CONTROL based fluorometers featuring separate actinic LEDs (like the WATER-PAM) the menu point 48: ACT-AMPL offers the possibility to shift the range of PAR-values covered by ACT-INT settings 1-12 up and down. With instruments featuring one LED, which at the same time provides measuring and actinic light (like the MICROSCOPY-PAM), the range of PAR-values can be shifted via menu point 49: MEAS-AMPL. It should be noted that with any change in ACT-AMPL or MEAS-AMPL the PAR-list becomes obsolete and recalibration at the new setting will be required. Therefore, in practice the settings of ACT-AMPL or MEAS-AMPL should be kept fixed, unless there is a good reason for

the contrary, e.g. when dealing with particularly light-sensitive or insensitive samples (see also 8.1).

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), 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 11.3.1) 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 11.3.8 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 11.3.5).

11.3.8 LIGHT CURVE (menu point 19) and LIGHT CURVE+REC (menu point 20)

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 22). This feature allows to adjust the range of applied intensities to the light adaptation properties of the sample (sun or shade plant). In principle, an alternative possibility to vary the range of actinic intensities is by changing the settings of ACT-AMPL (menu point 48) and MEAS-AMPL (menu point 49). This shifts all intensities up or down. However, as outlined above (see 11.3.7), changing these settings also affects the internally stored PAR-list, such that recalibration is required (see 8.2).

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 sample 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 21). This is limited to 10:00 min in LIGHT CURVE recordings.

A LIGHT CURVE is started either in menu position 19 via SET or in any other menu position by double key operation ON + $\wedge$. 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. Then ACT-INT is automatically increased in 8 steps 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). When a PC is connected, the WinControl-software allows graphical display of previously stored LIGHT CURVES.

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 20 or by the double key command ON+∨. 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 nonphotochemical 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 PAM-CONTROL 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 11.3.10) 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 changing environment. The advantage of RLC with respect to conventional light response curves is obvious in the case of measurements with the MICROSCOPY- or MICROFIBER-PAM, where the physiological state of the samples would deteriorate severely with extended measuring times.

11.3.9 INDUCTION CURVE (menu point 23) and INDUCTION CURVE+RECOVERY (menu point 24)

Dark/light induction curves (Kautsky effect) contain complex information on the photosynthetic performance of a plant at different functional levels (see 11.1). 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 is 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.

An INDUCTION CURVE recording is started by MODE-menu function 23: IND.CURVE. It is also possible to record the light/dark recovery in addition to the dark/light induction (24: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 11.3.8), the extent of photoinhibition and also on dark electron flow between stroma (or cytoplasm) and the electron carriers 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 11.3.1, 11.3.2, 11.3.3). The delay between this saturation pulse and onset of illumination can be varied (25: IND.DELAY); its default value is 40 s. Another variable is the time interval between two consecutive saturation pulses during actinic illumination (26: 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 induction curves. This aspect is particularly relevant if the rate of respiratory O_2-uptake is high and rapid diffusion of O_2 is prevented. Such situation e.g. is encountered with respiring samples (like algae or guard cells) covered by a microscope slip.

11.3.10 Repetition Clock (menu point 30: REP-CLOCK and double key command ON+MEM)

The Repetition Clock is primarily meant to trigger saturation pulses for YIELD-determination at defined time intervals which are set in menu position 32: 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 30 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 command ON+MEM.

Besides YIELD-measurements also other functions can be repetitively triggered by the CLOCK. For this purpose the MODE-menu point 31 (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+)

12 Appendix

12.1 Technical specifications

PAM-CONTROL Universal Control Unit

Measuring light output:	For driving up to 3 Measuring Light LEDs which may also serve as actinic light sources; 12 intensity settings; 12 pulse frequency settings; Auto High Frequency function
Actinic light output:	For driving up to 3 Actinic Light LEDs
Far-red output:	For driving up to 3 Far-Red LEDs
Aux-output:	Control signals for external LED drivers
Microcontroller:	CMOS 80C52
Data memory:	CMOS RAM 128 kB, providing memory for 4000 data sets
Mode menu:	55 items for off-line control of instrument settings and measuring functions
Measured parameters:	Fo, Fm, Fm', F, Fv/Fm (max. Yield), ΔF/Fm' (Yield), qP, qN, NPQ, PAR (using special Micro Quantum Sensor), ETR (i.e. PAR x ΔF/Fm')
Display:	2 x 24 character alphanumerical LC-display with backlight; character size 4.5 mm
User interface:	2 x 4 touch sensitive keypad
PC-terminal operation:	Via RS 232 interface using special command set; for remote control of all functions using WinControl Software

Data output:	Data transfer via RS 232 using WinControl Software
Power supply:	Internal rechargeable battery 12 V/2 Ah, providing power for at least 10.000 Yield measurements; automatic power/off; Battery Charger MINI-PAM/L (100 to 240 V AC)
Operating temperature:	-5 to +45 °C
Dimensions:	17.6 cm x 11.5 cm x 9.5 cm (L x W x H)
Weight:	2 kg (incl. battery)

Battery Charger MINI-PAM/L

Power Supply:	100 to 240 V AC, 50/60 Hz
Output:	18 V/45 W
Dimensions:	13.5 cm x 6 cm x 3.6 cm (L x W x H)
Weight:	0.26 kg

Transport Box CONTROL-T

Design:	Aluminium box with custom foam packing for PAM-CONTROL and accessories
Dimensions:	60 cm x 40 cm x 25 cm (L x W x H)
Weight:	5 kg

12.2 List of warnings and errors

Errors in PAM-CONTROL performance and warnings concerning sub-optimal use of the instrument are signaled 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 PM-GAIN (menu point 54), OUT-GAIN (menu point 53) or the MEAS-AMPL (menu point 49) may be decreased. In all 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 the PM-GAIN (menu point 54), OUT-GAIN (menu point 53) or MEAS-AMPL (menu point 49) should be increased. In all 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 the battery soon should be recharged.

Err. ? NEW OFFSET ?

Last measurement may be erroneous as PM-GAIN (menu point 54), OUT-GAIN (menu point 53) or MEAS-AMPL (menu point 49) 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 13) is running.

CLK REPETITION-CLOCK (menu point 30) is running.

LC Automatic recording of a LIGHT CURVE (menu point 19) is running.

LC+ Automatic recording of a LIGHT CURVE + RECOVERY (menu point 20) is running.

IC Automatic recording of an INDUCTION CURVE (menu point 23) is running.

IC+ Automatic recording of an INDUCTION CURVE + RECOVERY (menu point 24) is running.

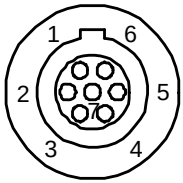
REC Recovery part of LIGHT INDUCTION CURVE is running.

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

12.3 PIN-assignments

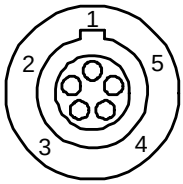
12.3.1 Connectors at the rear side

"AUX INPUT"



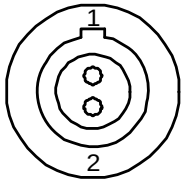
1: +5 V
2: GND
3: Signal input for quantum sensor
4: Signal input for quantum sensor
5: Signal input for temperature sensor
6: Remote control button
7: -5 V

"RS 232"



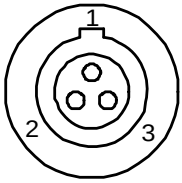
1: Not used
2: Not used
3: TxD
4: RxD
5: GND

"OUTPUT"



1: Signal output
2: GND

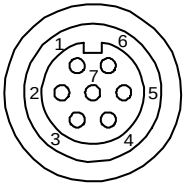
"CHARGE"



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

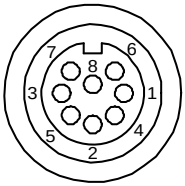
12.3.2 Connectors at the right hand side

"AUX OUTPUT"



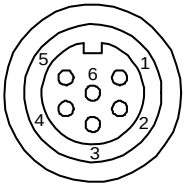
1: +5 V
2: ML (pulse signal)
3: GND
4: AL (pulse signal)
5: not used
6: +12 V
7: FR (pulse signal)

"PM"



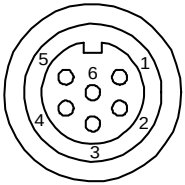
1: PM input current
2: GND
3: +12 V
4: PM signal (input)
5: not used
6: -5 V
7: +5 V
8: PM control voltage

"AL"



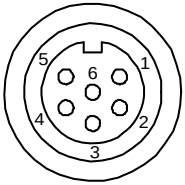
1: LED anode (+12 V)
2: LED anode (+12 V)
3: not used
4: LED cathode
5: LED cathode
6: not used

"ML"



1: LED anode (+12 V)
2: LED anode (+12 V)
3: not used
4: LED cathode
5: LED cathode
6: not used

"FR"



1: LED anode (+12 V)
2: LED anode (+12 V)
3: not used
4: LED cathode
5: LED cathode
6: not used

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13 Rechargeable battery

The Universal Control Unit PAM-CONTROL 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!**

14 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. Note: Water damage to non-submersible or water-proof instruments constitutes misuse.
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, connectors, batteries, fiberoptic cables, lamps, gas filters, thermocouples, fuses or calibrations.
6. All submersible instruments are tested and certified water-tight at Walz facility prior to shipment. Thus Warranty shall not apply to submersible/water-tight instruments that are damaged by leakage, interior flooding of the instrument housing or uncapped/improperly sealed port connections.

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.