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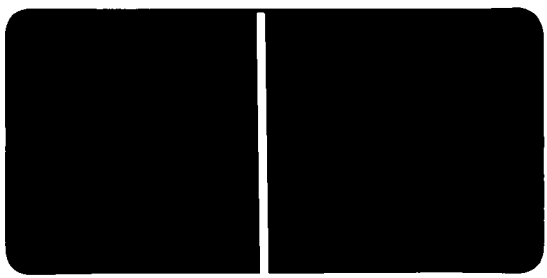
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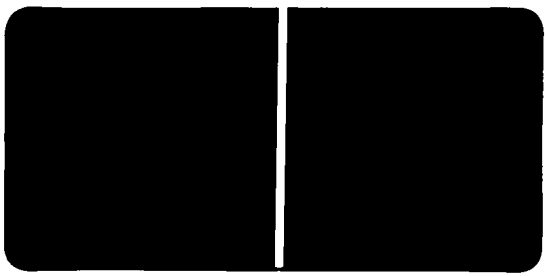
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PDA-100
PAM-Data Acquisition System

for
PAM-100 and XE-PAM
Fluorometer

Instructions for Users

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Rechargeable Batteries

What battery type is used in the PAM-2000/MINI-PAM/PAM-CONTROL/DIVING-PAM/PHYTO-PAM/PDA-100?

Rechargeable sealed-lead acid batteries.

Life time of the sealed-lead acid battery

The life time is 1-3 years. It depends on 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.

Overcharge

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

Storage

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!**

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1 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.

2 Introduction

The **PDA-100 PAM-Data Acquisition System** was specially developed for the widely **distributed PAM-100 Chlorophyll Fluorometer** and the **XE-PAM Fluorometer**, both of which originally were designed as manually operated stand-alone devices. For the PAM-100, which comprises the PAM-101 Main Control Unit as well as the Accessory Control Units PAM-102 and PAM-103, previously the DA-100 Data Acquisition System was developed featuring MS DOS-software, which now has become outdated with the general advance of Windows-Operating Systems. Instead during recent years the dedicated **Windows-software WinControl** has been developed which has proven quite successful for control of PAM Fluorometers and analysis of the obtained data. This software was first introduced in conjunction with the Universal Control Unit PAM-CONTROL and specialized chlorophyll fluorometers like the MICROSCOPY-PAM, MICROFIBER-PAM and WATER-PAM. Later its use was extended to the MINI-PAM and DIVING-PAM Chlorophyll Fluorometers. All of these fluorometers, which so-far could be run under WinControl, are microprocessor-based and, hence, are predestined for software-control. As this is not the case with the PAM-100 and the XE-PAM, until recently WinControl could not be applied with these fluorometers. **The PDA-100 PAM-Data Acquisition System** has filled this gap by providing a **microprocessor-based interface** between these fluorometers and the **WinControl-software** installed on a PC.

These Instructions for Users deal mainly with the PDA-100 as such and special aspects of its use in conjunction with the PAM-100 and the XE-PAM. A **separate Users Manual** is provided for **WinControl** in which the extensive features of this software are described in detail.

3 Features and components of the PDA-100

The PDA-100 is the mediator between the actual fluorometer (PAM-100 and XE-PAM) and the **WinControl-software** installed on a PC. It has **three main functions:**

It serves as an **analog/digital converter** of the PAM-signals, such that these signals can be processed and analyzed under WinControl with the help of a PC. In addition, it also serves as an interface for recording photosynthetically active radiation (PAR) and temperature using suitable sensors. The corresponding input-sockets are located on the front side of the PDA-100 housing.

The PDA-100 provides **trigger-signals** for **switching on/off** various sources of **Actinic Light** and **Saturation Pulses** via PC under WinControl.

It also provides **LED-drivers** for low power (up to 3 LEDs) **Actinic** and **Far-Red Light Sources**. Furthermore, control signals for various light qualities (Actinic Light, Far-red Light and Saturation Pulses) are provided, which may be applied to control on/off as well as intensity of special light sources developed for this purpose.

3.1 Components at the front side

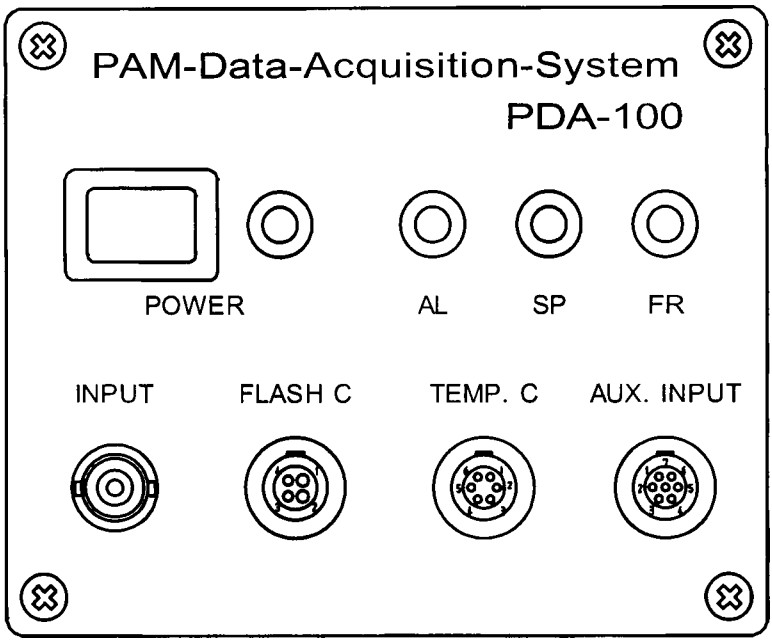


Fig. 1 Components at the front side of the PDA-100 housing

POWER Push button for switching on (or **reset**) the PDA-100. Power is automatically switched on when the PDA-100 is connected via an RS-232 interface cable to a PC and the WinControl program is started. When the PDA-100 is switched on, the **green status LED** at the right hand side of the push button is blinking with a frequency of 1 Hz. The PDA-100 turns off automatically after 4 min when not in use. Therefore, it does not feature an off-switch.

AL Yellow status LED which lights up for the time period **Actinic Light** is switched on.

SP Blue status LED which lights up for the duration of a **Saturation Pulse**

FR Red status LED which lights up for the time period **Far-Red Light** is switched on.

INPUT BNC-socket for input of **fluorescence signal**, which has to be connected with the OUT-socket at the front of PAM-100 or XE-PAM using a BNC-cable.

FLASH C Flash Compensation for correction of intensity fluctuations of individual measuring light flashes in the case of the XE-PAM (not yet implemented)

TEMP. C Socket for connecting temperature control unit (in preparation)

AUX. INPUT Socket for connecting suitable light and/or temperature sensors (e.g. 2030-B, 2060-M, US-SQS/W). The Spherical Micro Quantum Sensor US-SQS/W is particularly suited for measurements with suspensions in the Optical Unit ED-101US.

3.2 Components at the rear side

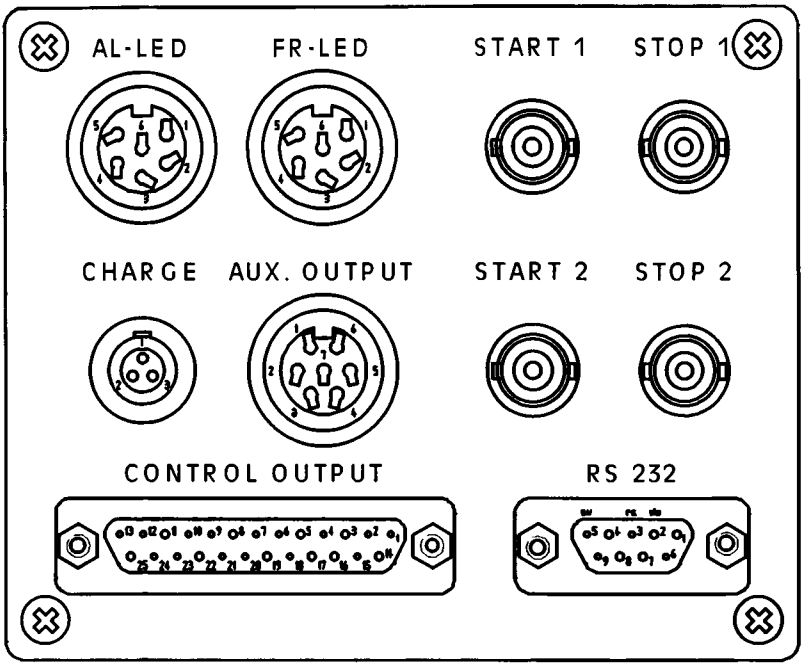


Fig. 2 Components at the rear side of the PDA-100 housing

AL-LED Socket for connecting an **Actinic LED Light Source** featuring up to 3 red LEDs or 2 blue LEDs (max. voltage 11 V, max. current 40 mA). **The LED Light Source 102-L** of the PAM-100 can be connected directly to AL-LED. As intensity and illumination time can be controlled via WinControl, all functions of the PAM-102 Actinic Light Control Unit are covered by the PDA-100/WinControl combination, such that the **PAM-102 is not required anymore.**

FR-LED Socket for connecting a **Far-Red LED Light Source** with up to 3 far-red LEDs (max. voltage 11 V, max. current 40 mA). The **Far-Red LED Light Source**

102-FR can be connected directly to FR-LED. As intensity and illumination time can be controlled via WinControl, all functions of the PAM-102 Actinic Light Control Unit of the PAM-100 are covered by the PDA-100/WinControl combination, such that the **PAM-102 is not required anymore**.

Note: In the case of the PAM-100, connecting the 102-L and 102-FR directly to the AL-LED and FR-LED sockets of the PDA-100 also has the advantage, that both light sources can be used at the same time. As to the AUTO 100 kHz (with PAM-100) and AUTO 64 Hz (with XE-PAM), these functions are supported by WinControl only, if the fluorometer is equipped with a REMOTE CONTROL connector at the rear side of the housing. This is the case with all new instruments. Older instruments can be updated at the factory. If the connector REMOTE CONTROL is missing and the Auto 100 kHz function is required, the 102-L or 102-FR should be connected to the PAM-102 module. Then the PAM-102 can be triggered using the START 1 trigger signal of the PDA-100.

START 1
STOP 1

Output trigger signals for Start/Stop of a Saturation Pulse Light Source or a **Flash Discharge Lamp** (equivalent to START OUT/STOP OUT of the PAM-103 and SAT.PULSES TRIG.OUT ON/OFF of XE-PAM). The FL-103/E, XMT-103 or XST-103 can be directly connected to START 1/STOP 1. The Auto 100 kHz and gating functions are supported only, if the fluorometer is equipped with a REMOTE CONTROL-connector at the rear side of the housing. This is the case with all new

instruments. Older instruments can be updated at the factory.

Note: In principle, the FL-103/E, XMT-103 or XST-103 can also be connected to the PAM-103 and triggered via START 1. This, however, is recommended only when the instrument is not yet equipped with a REMOTE CONTROL-connector and the Auto 100 kHz and gating functions are required. In this case START 1 at PDA-100 and START IN at the rear of PAM-103 (or TRIG.IN at front of XE-PAM) have to be connected. Then the Saturation Pulse Width is determined by the time interval selected at PAM-103 or XE-PAM. The Saturation Pulse Width set under WinControl should be identical and the CLOCK selection-switch at PAM-103 or XE-PAM should be in the STOP-position.

START 2
STOP 2

Output trigger signal for **Start/Stop** of an **Actinic Light Source** (equivalent to START OUT/STOP OUT of the PAM-102 and ACT.LIGHT TRIG.OUT ON/OFF of XE-PAM). In the case of the PAM-100, the Fiber Illuminator FL-103/E can be directly connected to START 2/STOP 2 and used for actinic halogen illumination. The Auto 100 kHz function is supported only, if the instrument (PAM-100 or XE-PAM) is equipped with a REMOTE CONTROL-connector at the rear side of the housing. This is the case with all new instruments. Older instruments can be updated at the factory.

Note: In principle, the FL-103/E (or any other triggerable lamp) can be also connected to the PAM-102 and triggered via START 2. This, however, is

recommended only when the instrument is not yet equipped with a REMOTE CONTROL-connector and the Auto 100 kHz function is required. In this case START 2 at PDA-100 and START IN at the rear of PAM-102 have to be connected. Then the Actinic Illumination Time is determined by the time interval selected at PAM-102. The Actinic Width set under WinControl should be identical.

CHARGE Socket for connecting the Battery Charger MINI-PAM/L.

AUX. OUTPUT Output socket for control signals of special external LED-lamps for Actinic Light, Saturation Pulses and Far-Red Light (a variety of such lamps is in preparation). The control signals define on/off as well as intensity of the different types of illumination, as set under WinControl.

CONTROL OUTPUT Output socket for special remote control cable which connects to the REMOTE CONTROL input-socket at the rear side of PAM-101 or XE-PAM. REMOTE CONTROL is implemented in all new instruments. Older instruments can be updated at the factory.

RS 232 RS 232 socket for connecting the PDA-100 with a PC.

3.3 The rechargeable battery

A relatively large rechargeable lead acid battery (12 V/2 Ah) is mounted in the bottom part of the PDA-100 housing. For recharging, the Battery Charger MINI-PAM/L is provided which is connected to the CHARGE-socket at the rear side of the PDA-100. It may remain permanently connected. The charger, which operates at input voltages

between 100 and 240 V AC, features an overload protection. Full charging of an empty battery takes approx. 5 hours. Battery voltage is displayed under WinControl (Online Parameters). A Low Battery warning is given when the voltage drops below 11.2 V.

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

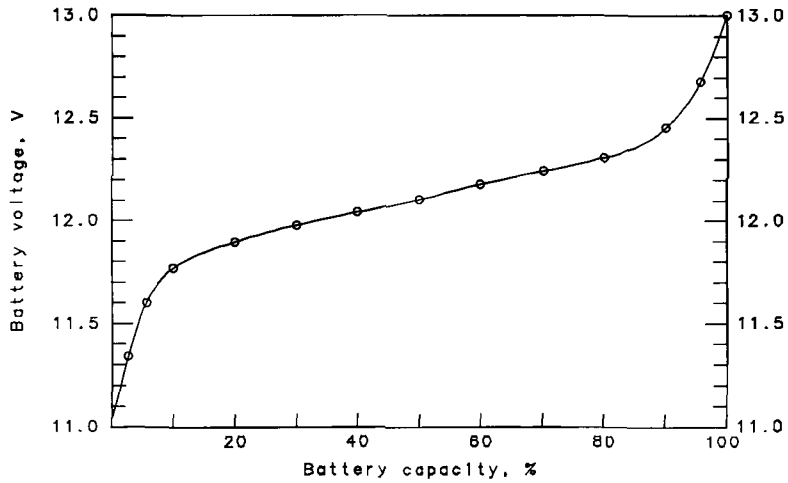


Fig. 3 Battery capacity vs. battery voltage

3.4 Update of instrument software on EPROM

All functions of the PDA-100 are under the control of the instrument software which is stored on the EPROM located on the main printed circuit board within the instrument. The number and date of origin of the current version of the EPROM-software is

displayed under WinControl (head line under Settings). It may be expected that this software will be further improved and extended in the future, also following suggestions by the users. In this case, updated software versions will be distributed in form of bin-files (current version pda-073-.bin) by e-mail attachment. The update can be carried out by the user with the help of the dedicated **update-program flash.exe** and a **special cable** (PDA-100/K1) provided with the PDA-100. For this purpose, the four top screws of the instrument have to be removed and the lid removed. Using the provided cable, the EPROM can be connected to the parallel printer interface of the PC. The EPROM-connector fits into the 6-pin socket of the printed circuit board (see Fig. 4).

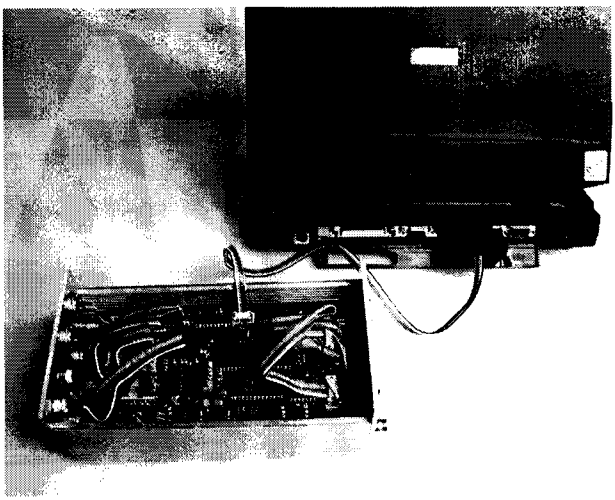


Fig. 4 Cable PDA-100/K1 connected to parallel printer interface of the PC

In order to start the update, the icon of the bin-file can be simply drawn with the mouse cursor on top of the icon of the flash.exe file in the WINCONT-directory. Then the previous program version will be overwritten by the new version.

4 Setting up the PDA-100

In order to make use of the PAM-Data Acquisition System PDA-100, the WinControl software first has to be installed on a PC and cable connections have to be made between PC, PDA-100 and fluorometer including various light sources. Please consult the **WinControl Users Manual** for instructions on software installation. As to the cable connections, these depend to on the type of instrument (PAM-100 or XE-PAM) and on whether the particular fluorometer is prepared for **REMOTE CONTROL**. This is the case with all new instruments. Older instruments can be updated at the factory. While such update is strongly recommended for taking full advantage of all features offered by the PDA-100, it is not necessary for some standard applications. It should be noted that there always remains the option of operating the PAM Fluorometer under manual control. Different cable connections also apply for the control of various types of light sources via PDA-100/WinControl. In the following sections proper connections are outlined for PAM-100 with and without REMOTE CONTROL as well as with and without PAM-102/103 modules, and for the XE-PAM with and without REMOTE CONTROL. The following light sources are covered:

- Actinic LED Light Source 102-L
- Far-Red LED Light Source 102-FR
- Fiber Illuminator FL-103/E
- Single and Multiple Turnover Flash Units XST-103 and XMT-103
- Single Turnover Flash Unit XE-ST
- High Power LED-Lamp HPL-C

4.1 Cable connections with PAM-100 featuring REMOTE CONTROL

When the PAM-100 is featuring REMOTE CONTROL, all functions of the PAM-102 and 103 modules are covered by the PDA-100/WinControl combination. Hence, in principle there is no need for these two modules and all lamps and cables can be connected to the PDA-100. The various cable connections are as follows:

1) Connection of the **Battery Charger MINI-PAM/L** to CHARGE-socket of the PDA-100 (see 7.3, Fig. 11)

2) **RS 232 connection** between PDA-100 and PC (see 7.3, Fig. 12)

3) Connection between INPUT-socket at the PDA-100 and OUT-socket at the front of the PAM-101 for **transfer of fluorescence signal** using BNC-cable (see 7.3, Fig. 13)

4) Connection between CONTROL OUTPUT at the PDA-100 and **REMOTE CONTROL** at the PAM-100 using Connecting Cable PDA-100/K2 (see 7.3, Fig. 14)

5) Connection of the **Actinic LED Light Source 102-L** to AL-LED-socket of the PDA-100 (see 7.3, Fig. 15)

6) Connection of the **Far-Red LED Light Source 102-FR** to FR-LED-socket of the PDA-100 (see 7.3, Fig. 16)

7) If to be used as **Saturation Pulse source**, the **Fiber Illuminator FL-103/E** has to be connected to START 1/STOP 1 at the PDA-100 using the special trigger cable provided with the FL-103/E (see 7.3, Fig. 17).

8) If to be used as an **Actinic Light source**, the **Fiber Illuminator FL-103/E** has to be connected to START 2/STOP 2 at the

PDA-100 using the special trigger cable provided with the FL-103/E (see 7.3, Fig. 18).

9) The TRIG-IN socket of the **Flash Lamps XST-103, XMT-103** and **XE-ST** has to be connected to START 1 at the PDA-100 using a BNC-cable (see 7.3, Fig. 19).

10) The AUX. OUTPUT socket of the PDA-100 is connected with the REMOTE CONTROL input-socket of the **High Power LED-Lamp HPL-C** using the cable provided with the HPL-C (see 7.3, Fig. 20). If the HPL-C is applied for Actinic Light as well as for Saturation Pulses, under System Parameters of **WinControl** the **check-box Act+Sat** has to be activated. If this is not the case, the HPL-C is applied for Actinic Light only.

If PAM-102 and 103 modules are installed in an instrument which is also equipped for REMOTE CONTROL, they may be used under manual control for operation of additional lamps, independent of WinControl.

4.2 Cable connections with PAM-100 not featuring REMOTE CONTROL

Also when the PAM-100 is not featuring REMOTE CONTROL, it can still be used in various applications in conjunction with the PDA-100 and the WinControl-software. Important restrictions are:

- Measuring Frequency cannot be changed under WinControl
- If no PAM-102 or PAM-103 module is installed, the AUTO 100 kHz function is not available
- If no PAM-103 module is installed, the detector-gating upon flash-triggering is not available

No REMOTE CONTROL is required for full control of low power Actinic and Far-Red LED light sources (up to 3 LEDs) as well as for triggering external Actinic Light sources and flash discharge lamps. Proper connection of these components in some cases depends on whether PAM-102 or PAM-103 modules are installed. If this modules are installed, use of the AUTO 100 kHz and the detector-gating functions can be made. In this case, however, the user must make sure that the time settings chosen at PAM-102 and PAM-103 agree with the corresponding settings under WinControl. Also the CLOCK of PAM-102 and PAM-103 should be in the STOP-position. Furthermore, if the High Power LED Lamp HPL-C is available, this features its own REMOTE CONTROL socket, such that for full control of Actinic and Saturation Pulse Light it is not necessary that REMOTE CONTROL is installed in the PAM-100 as such.

When the PAM-100 does not feature REMOTE CONTROL, the following connections apply:

1. Connection of the **Battery Charger** to CHARGE at the PDA-100 (see 7.3, Fig. 11)
2. **RS 232 connection** between PDA-100 and PC (see 7.3, Fig. 12)
3. Connection between INPUT-socket at the PDA-100 and OUT-socket at the front of the PAM-101 for **transfer of fluorescence signal** using BNC-cable (see 7.3, Fig. 13)
4. Connection of the **Actinic LED Light Source 102-L** to AL-LED-socket of the PDA-100 (see 7.3, Fig. 15). In this case, the AUTO 100 kHz function is not effective.

   or, if the PAM-102 module is installed,

   connection of the 102-L to the corresponding output-socket at the rear of the PAM-102 and connection between START 2 at

PDA-100 and the Trig.-IN socket at the rear of the PAM-102 using a BNC-cable.

5. Connection of the **Far-Red LED Light Source 102-FR** to FR-LED-socket of the PDA-100 (see 7.3, Fig. 16). While in this case the AUTO 100 kHz function is not effective, normally this is not desired during far-red illumination.

   or, if the PAM-102 module is installed,

   connection of the 102-FR to the 102-L output-socket at the rear of the PAM-102 and connection between START 2 at PDA-100 and the TRIG-IN socket at the rear of the PAM-102 using a BNC-cable.

6. If to be used as **Saturation Pulse source**, the **Fiber Illuminator FL-103/E** can be connected to START 1/STOP 1 at the PDA-100 using the special trigger cable provided with the FL-103/E (see 7.3, Fig. 17). In this case the AUTO 100 kHz function is not effective.

   or, if the PAM-103 module is installed,

   connection of the FL-103/E to the START and STOP TRIG-OUT sockets at the rear side of the PAM-103 using the special trigger cable provided with the FL-103/E and connection between START 1 at PDA-100 and the TRIG-IN socket at the rear of the PAM-103 using a BNC-cable.

7. If to be used as an **Actinic Light source**, the **Fiber Illuminator FL-103/E** has to be connected to START 2/STOP 2 at the PDA-100 using the special trigger cable provided with the FL-103/E (see 7.3, Fig. 18). In this case the AUTO 100 kHz function is not effective.

   or, if the PAM-102 module is installed,

connection of the FL-103/E to the START and STOP TRIG-OUT sockets at the rear side of the PAM-102 using the special trigger cable provided with the FL-103/E and connection between START 2 at PDA-100 and the TRIG-IN socket at the rear of the PAM-102 using a BNC-cable.

8. The TRIG-IN socket of the **Flash Lamps XST-103, XMT-103 and XE-ST** has to be connected to START 1 at the PDA-100 using a BNC-cable (see 7.3, Fig. 19). In this case the AUTO 100 kHz and detector-gating functions are not effective.

   or, if the PAM-103 module is installed,

   connection of the Flash Lamp TRIG-IN socket with the TRIG-OUT START socket at the rear side of the PAM-103 using BNC-cable and connection between START 1 at PDA-100 and the TRIG-IN socket at the rear of the PAM-103 using BNC-cable.

9. The AUX. OUTPUT socket of the PDA-100 is connected with the REMOTE CONTROL input-socket of the **High Power LED-Lamp HPL-C** using the cable provided with the HPL-C (see 7.3, Fig. 20). If the HPL-C is applied for Actinic Light as well as for Saturation Pulses, under WinControl System Parameters the check-box Act+Sat has to be activated. If this is not the case, the HPL-C is applied for Actinic Light only.

4.3 Cable connections with XE-PAM featuring REMOTE CONTROL

When the XE-PAM is equipped for REMOTE CONTROL, in principle all functions of FREE RUN PULSE Measuring Light, ACT.LIGHT, SAT.PULSE and CLOCK can be covered by the PDA-100/WinControl combination. For this purpose the PDA-100 and XE-PAM have to be connected via the special **Remote Control**

Cable provided with the XE-PAM. When this cable is installed and under WinControl the **check-box XE Remote** is activated, all manual controls of the XE-PAM are blocked except for GAIN, DAMPING, AUTO COMP. and ZERO OFFSET. It is up to the user to make use of these controls in addition to the corresponding controls offered by WinControl. The XE-PAM can generate signals up to 8 V, whereas PDA-100/WinControl accepts signals below 4 V only. Therefore under WinControl the GAIN is preset to Standard Setting 2, such that the same signal amplitudes are displayed by XE-PAM and under WinControl. When the XE-PAM signal exceeds 4 V, the GAIN under WinControl can be set to 1.

The following cable connections apply:

1. Connection of the **Battery Charger** to CHARGE-socket of the PDA-100 (see 7.3, Fig. 11)
2. **RS 232 connection** between PDA-100 and PC (see 7.3, Fig. 12)
3. Connection between INPUT-socket at the PDA-100 and OUT-socket at the front of the XE-PAM for **transfer of fluorescence signal** using BNC-cable (see 7.3, Fig. 21)
4. Connection between CONTROL OUTPUT at the PDA-100 and **REMOTE CONTROL** at the XE-PAM using provided special cable (see 7.3, Fig. 22). When this cable is installed, the XE Remote check-box is activated under WinControl and the **Actinic-/Saturation Light Unit XE-AL** is connected to the XE-PAM, the corresponding sources for Actinic Light and Saturation Pulses are fully controlled via WinControl. Under System Parameters the choice between **Halogen Lamp** (Act.=Halogen box checked) and **LED Lamp** (Act.=Halogen box not checked) can be made.

5. The AUX. OUTPUT socket of the PDA-100 is connected with the REMOTE CONTROL input-socket of the **High Power LED-Lamp HPL-C** using the cable provided with the HPL-C (see 7.3, Fig. 20). If the HPL-C is applied for Actinic Light as well as for Saturation Pulses, under WinControl System Parameters the check-box Act+Sat has to be activated. If this is not the case, the HPL-C is applied for Actinic Light only.

6. In order to trigger the **Single Turnover Flash Unit XE-ST**, the START 1 at the PDA-100 is connected to the TRIG-IN socket of the XE-ST using a BNC-cable. Please note that PDA-100/WinControl presently does not yet support application of the XE-PAM in the **Pump&Probe mode**. A corresponding update of the software is in preparation. The Pump&Probe mode can be used when under System Parameters XE Remote is inactivated. Then the **Chart-function** of WinControl can serve for recording of the Pump&Probe data.

7. Connection of the **Actinic LED Light Source 102-L** to AL-LED-socket of the PDA-100 (see 7.3, Fig. 15). While this light source originally was developed for the PAM-100, it can be applied in conjunction with XE-PAM/PDA-100/WinControl as well, if the Fiberoptics 101-F or 101-F5 are available. In this case the 102-L is synchronized with any other LED-source controlled by the PDA-100 via REMOTE CONTROL connection.

8. Connection of the **Far-Red LED Light Source 102-FR** to FR-LED-socket of the PDA-100 (see 7.3, Fig. 16). While this light source originally was developed for the PAM-100, it can be applied in conjunction with XE-PAM/PDA-100/WinControl as well, if the Fiberoptics 101-F or 101-F5 are available.

4.4 Cable connections with XE-PAM which does not feature REMOTE CONTROL

Also when the XE-PAM is not equipped for REMOTE CONTROL, it still can be used in various applications in conjunction with the PDA-100 and the WinControl-software. Important restrictions are:

- Measuring Light cannot be switched on/off under WinControl
- Measuring Frequency cannot be changed under WinControl
- Intensities of the Actinic-/Saturation Light Unit XE-AL cannot be changed under WinControl (i.e. automatic recording of Light Curves is not possible)
- Illumination times of the Actinic-/Saturation Light Unit XE-AL cannot be changed under WinControl

No REMOTE CONTROL is required for full control of low power Actinic and Far-Red LED light sources (up to 3 LEDs). Also the Actinic Light and Saturation Pulse Lamps of the Actinic-/Saturation Light Unit XE-AL can be triggered. In this case, however, the user must make sure that the time settings chosen at the ACT.LIGHT and SAT.PULSE units do agree with the corresponding settings under WinControl. Also the CLOCK should be in the STOP-position. Furthermore, if the High Power LED Lamp HPL-C is available, this features its own REMOTE CONTROL socket, such that for full control of Actinic and Saturation Pulse Light it is not necessary that REMOTE CONTROL is installed in the XE-PAM as such.

When the XE-PAM does not feature REMOTE CONTROL, the following connections apply:

1. Connection of the **Battery Charger** to CHARGE-socket of the PDA-100 (see 7.3, Fig. 11)
2. **RS 232 connection** between PDA-100 and PC (see 7.3, Fig. 12)
3. Connection between INPUT-socket at the PDA-100 and OUT-socket at the front of the XE-PAM for **transfer of fluorescence signal** using BNC-cable (see 7.3, Fig. 21)
4. The AUX. OUTPUT socket of the PDA-100 is connected with the REMOTE CONTROL input-socket of the **High Power LED-Lamp HPL-C** using the cable provided with the HPL-C (see 7.3, Fig. 20). If the HPL-C is applied for Actinic Light as well as for Saturation Pulses, under WinControl System Parameters the check-box Act+Sat has to be activated. If this is not the case, the HPL-C is applied for Actinic Light only.
5. In order to trigger the **Single Turnover Flash Unit XE-ST**, the START 1 at the PDA-100 is connected to the TRIG-IN socket of the XE-ST using a BNC-cable. Please note that PDA-100/WinControl presently does not yet support application of the XE-PAM in the **Pump&Probe mode**. A corresponding update of the software is in preparation. The Pump&Probe mode can be used when under System Parameters XE Remote is inactivated. Then the **Chart-function** of WinControl can serve for recording of the Pump&Probe data.
6. Connection of the **Actinic LED Light Source 102-L** to AL-LED-socket of the PDA-100 (see 7.3, Fig. 15). While this light source originally was developed for the PAM-100, it can be applied in conjunction with XE-PAM/PDA-100/WinControl as well, if the Fiberoptics 101-F or 101-F5 are available. In this case the 102-L is synchronized with any other LED-source controlled by the PDA-100 via REMOTE CONTROL connection.

7. Connection of the **Far-Red LED Light Source 102-FR** to FR-LED-socket of the PDA-100 (see 7.3, Fig. 16). While this light source originally was developed for the PAM-100, it can be applied in conjunction with XE-PAM/PDA-100/WinControl as well, if the Fiberoptics 101-F or 101-F5 are available.

5 WinControl in conjunction with PAM-100

The numerous functions of the WinControl-software are described in detail in the separate **WinControl manual** where preferential reference is made to applications in conjunction with the MICROSCOPY-PAM and MINI-PAM Chlorophyll Fluorometers, for which WinControl originally was developed. Here some special aspects in applications with the **PAM-100** are outlined. Such aspects are the **Remote Control** of PAM-100 functions via WinControl, the refinement of Saturation Pulse Quenching Analysis by the **Fo'-mode**, as well as the **Chart**- and **Kinetics-modes** of data acquisition.

The PAM-100 with its accessory modules PAM-102 and 103 originally was conceived as manually controlled instrument in contrast to e.g. the MICROSCOPY-PAM with its microprocessor-based PAM-CONTROL unit. Even when the PAM-100 is connected via the PDA-100 to a PC and the WinControl program is started, in principal all manual controls of the PAM-100 remain functional. In addition, however, some important functions of the PAM-100 can be also remotely controlled by WinControl. These functions are:

- Switching between 1.6 and 100 kHz Measuring Light Frequency
- Start of Actinic Illumination
- Start of Saturation Pulse
- Triggering of discharge flash with gating of detector system
- Triggering of PAM-102 and 103 by Repetition Clock

While Measuring Light Intensity remains under full manual control (PAM-101), Actinic, Far-Red and Saturation Pulse Light Intensities can be controlled under WinControl if suitable light sources are connected to the PDA-100 (e.g. 102-L, 102-FR and HPL-C, see sections 4.1 and 4.2).

5.1 WinControl user surface and Main Settings

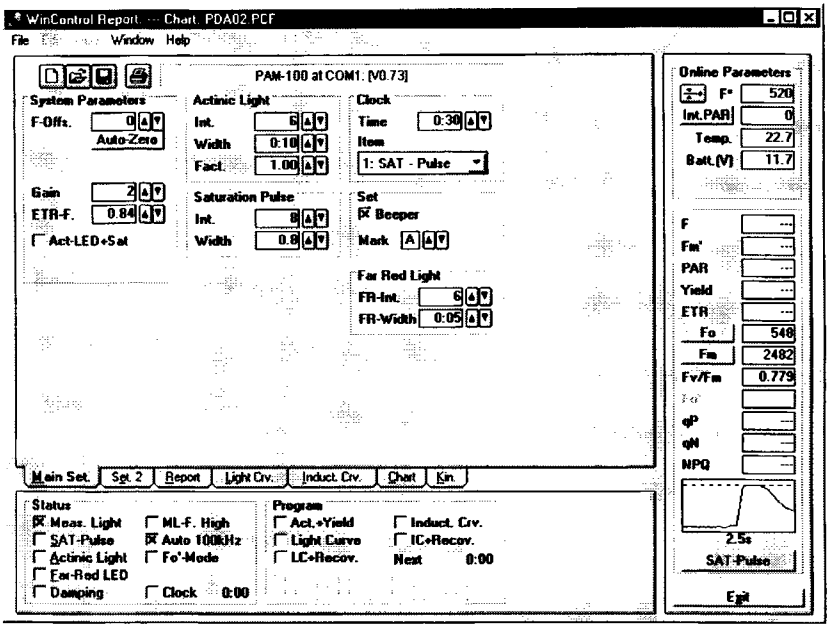


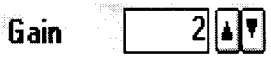
Fig. 5 WinControl user surface with Main Settings window

Fig. 5 shows the WinControl user surface with the Main Settings window (default settings) when a PAM-100 is connected to the PDA-100. The instrument automatically is recognized by the program. The following information applies specifically to applications with the PAM-100:

System Parameters



The **Auto-Zero** functions independent (i.e. on top of) the ZERO OFFSET carried out manually at the PAM-101.

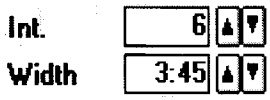


At standard **Gain 2** the same fluorescence values are displayed under WinControl (F* under Online Parameters) and at the PAM-101



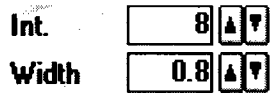
When **Act-LED+Sat** is checked, Actinic Light as well as Saturation Pulses are given by the same special LED-source, e.g. the HPL-C

Actinic Light



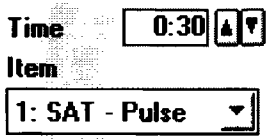
The settings of **Intensity** and **Width** of Actinic Light apply to a LED-light source connected to the PDA-100 (e.g. 102-L or HPL-C)

Saturation Pulse



The settings of **Intensity** and **Width** of Saturation Pulses apply to the HPL-C being connected to the PDA-100

Clock



If the **Clock Time** applies to a function **(Clock Item)** carried out by a lamp (as e.g. Sat-Pulse), this lamp must be controlled by trigger signals from the PDA-100: either Start 1 (e.g. FL-103/E) or REMOTE CONTROL (e.g. HPL-C).

Far Red Light



The settings of **FR-Int.** and **FR-Width** apply to to a FR-LED source (like 102-FR) connected to the PDA-100. FR-Width also determines the duration of FR-illumination in the Fo'-mode.

Status



The checkboxes for **SAT-Pulse, Actinic Light** and **Far-Red LED** apply for corresponding light sources being connected to the PDA-100 or being triggered by the PDA-100 via PAM-102 and PAM-103.



This software-controlled **Damping** is automatically deactivated during a Saturation Pulse.



In the **Fo'-mode** after Saturation-Pulse the Actinic Illumination is transiently interrupted and Far-Red light is applied in order to determine the minimal fluorescence yield.

Program



The checkbox for **Act+Yield** applies for an Actinic Light source connected to the PDA-100 or triggered by the PDA-100 via PAM-102. At the end of Actinic Illumination a Saturation Pulse is applied for assessment of Yield.



The recording of **Light Curve** and **LC+Recovery** is supported by WinControl only if a suitable Actinic LED-source (e.g. 102-L or HPL-C) is connected to the PDA-100.

☐ Induct. Crv. ☐ IC+Recov. In order to record **Induction Curve** and **IC+Recovery** suitable Actinic and Saturation Pulse Light sources have to be connected to the PDA-100 or triggered by the PDA-100 via PAM-102 and 103, respectively.

Online Parameters

F* 510 The **F*-value** corresponds to the momentary fluorescence yield which is also displayed at the PAM-101 (identical values at standard Gain 2 only).

Int.PAR 250 The **Internal PAR** is shown when no External PAR-sensor is connected (corresponding box under Settings 2 not checked). In this case the previously stored Internal PAR-list applies.

Ext.PAR 252 The **External PAR** applies when a suitable External PAR-sensor (e.g. 2030-B, 2060-M or US-SQS/W) is connected to the AUX. INPUT of the PDA-100 and the corresponding box under Settings 2 is checked.

Temp. 27.0 For display of **Temperature** a suitable Temperature Sensor (e.g. provided in 2030-B and 2060-M) must be connected to the PDA-100 AUX. INPUT.

Fo' 727
qP 0.552
qN 0.581

When the **Fo'-mode** is active, after every Saturation Pulse the minimal fluorescence yield **Fo'** is determined while Actinic Illumination is transiently interrupted and **Far-Red** is applied for the time set under FR-Width. Calculation of **qP** and **qN** is based on Fo' and not on original Fo.

5.2 Fo'-mode, Far-Red illumination and Chart Recording

Outstanding advantages of PDA-100/WinControl in conjunction with the PAM-100 are the possibilities of automated Saturation Pulse Quenching Analysis and Chart Recording of time dependent fluorescence changes. While the principle of Quenching Analysis is quite simple (see chapter 6), in practice there is the phenomenon of so-called Fo-quenching which may complicate the calculation of the quenching coefficients qP and qN. For correct determination of qP and qN the value of Fo' must be known, which corresponds to the minimal fluorescence yield with all PS II reaction centers being open (acceptor side fully oxidized) in a given state of nonphotochemical quenching. Fo' can be measured by taking advantage of the fact that the relaxation of nonphotochemical quenching upon darkening is relatively slow (fastest phase in the order of 15 sec), while the reoxidation of PS II acceptors can be speeded up by Far-RED LIGHT to be completed within a few seconds. WinControl supports a routine of Fo'-determination involving the following steps:

- simultaneously with a Saturation Pulse the Actinic Light is switched off
- simultaneously Far-Red light is switched on with its intensity and duration being defined under Main Settings
- during the last 1.3 sec of Far-Red illumination the fluorescence yield is averaged and the averaged value is entered as Fo'-value into the corresponding Parameter Field and into the Report-file
- the quenching coefficients qP and qN are calculated on the basis of Fo' instead of Fo
- upon cessation of Far-Red illumination the Actinic Light is switched on again.

It should be pointed out that for correct determination of Fo' by this routine the PS I acceptor side should be activated, i.e. there must be a suitable sink for the electrons pumped away from the PS II acceptor side by Far-red. Therefore, the Fo'-mode should not be applied during the first minute of a dark-light induction, when the PS I acceptor side is not yet fully light activated.

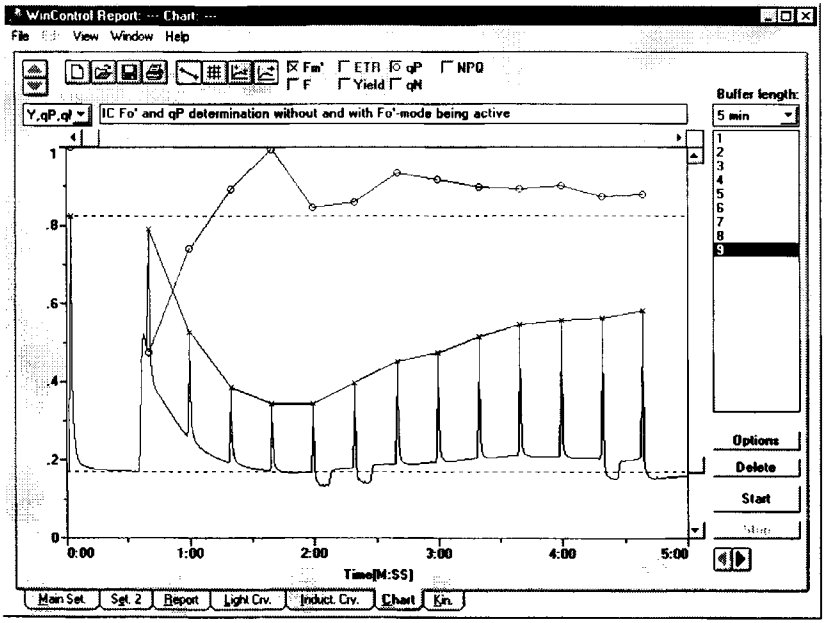


Fig. 6 Chart recording illustrating Fo'-determination under WinControl

In Fig. 6 an example of Fo'-determination is given using the Chart recording function of WinControl. In this example, the Fo'-mode is started ca. 1.5 min after onset of actinic illumination. A standard dark/light Induction Curve of a green leaf is recorded. First a Saturation Pulse is applied for Fo-Fm determination (doted lines). Then Actinic Light is switched on (at ca. 40 s) and repetitive Saturation Pulses are given every 20 sec for quenching analysis. First

the Fo'-mode is not active. Strong fluorescence quenching builds up during illumination, such that fluorescence yield even drops below the Fo-line, which indicates that there is Fo-quenching and that the calculated qP-value of 1 cannot be correct. Then, ca. 1.5 min after light-on, the Fo'-mode is activated in order to measure Fo' and to determine the correct qP-values for the next two Saturation Pulses. In the absence of Actinic Light and presence of Far-Red Light the fluorescence yield rapidly drops ca. 20% below the original Fo-line. The calculated qP-value is 0.85, which is significantly lower than the 1.00 determined when the Fo'-mode is not active. With continuing actinic illumination nonphotochemical quenching relaxes, as reflected by the rise of the maximal fluorescence yield measured during a Saturation Pulse, Fm'. This reflects activation of Calvin cycle enzymes and increasing consumption of ATP in the process of CO_2-fixation. When at the end of the recording the Fo'-mode again is activated, it is found that Fo' now is only ca. 7% below the Fo-line and qP is 0.87 as compared with a value of 0.90 determined when the Fo'-mode is not active. This example illustrates that the Fo'-mode is particular important under conditions of strong nonphotochemical quenching.

5.3 Recording of rapid induction kinetics

The PAM-100 features two Measuring Light Frequencies, 1.6 kHz for assessment of fluorescence yield under quasi-dark conditions and 100 kHz for assessment of fluorescence yield during actinic illumination. The AUTO 100 kHz function assures that Measuring Light Frequency automatically is switched from 1.6 to 100 kHz upon onset of actinic illumination, such that rapid induction kinetics can be resolved. WinControl supports rapid kinetics recording with a special **Kinetics-window**.

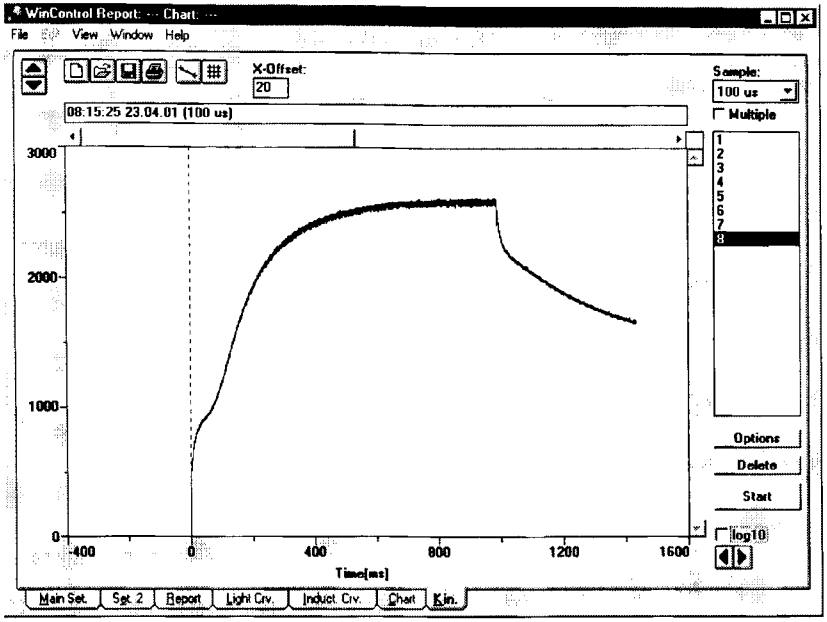


Fig. 7 Kinetics-window with display of rapid dark-light-dark induction kinetics as measured with a green leaf using the PAM-100

Fig. 7 shows the Kinetics-window displaying rapid induction kinetics which were measured with a PAM-100 in conjunction with a High-Power-LED Lamp (HPL-C plus HPL-655). The illumination time (Act-Width) was set to 1 sec, so that with a total recording of 1.4 sec (14000 data points at a sampling rate of 100 µs/point) not only the light-on but also the light-off kinetics were recorded.

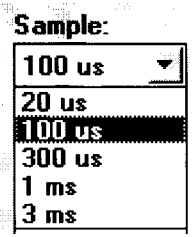
The following points refer to some specific features of WinControl in conjunction with recordings on the Kinetics-window.



Icon for enabling/disabling **point averaging** of recorded data. When enabled the rapid noise is reduced. At the same time also rapid transients may become damped.

X-Offset: 20

Offset of recorded data points with respect to the x-axis (time axis). The entered number corresponds to the **number of data points** by which all **x-values are shifted** to the right (longer times). This function allows to correct for small delays between the light-on trigger signal and the precise moment of light-on (or flash firing). The X-Offset value can also be changed after recording. It is saved together with the recording.



Time period corresponding to the sampling of one out of 14000 data points. **5 different sampling times** are for choice. The product of sampling time and total number of points (14000) corresponds to the total recording time.

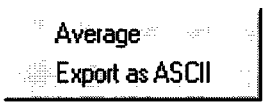
Multiple

When the Multiple-box is checked, **several recordings can be displayed** on top of each other by marking the selected numbers by left mouse click.

Delete

By Delete a **marked recording can be erased.** After deletion of a recording all recordings with higher numbers are renumbered.

Options



Marked recordings can be averaged. The averaged curve is added at the end to the list of recordings.

Marked recordings can be exported in form of ASCII-files. An ASCII-file then can be imported into other programs, like Excel.



When the log10 box is checked, marked recordings are displayed on a log10 time scale. In this way rapid transients are stretched and slow transients are compressed. A **logarithmic time scale** is particularly useful for analysis of the polyphasic fluorescence rise upon onset of saturating light.

6 Chlorophyll fluorescence as an indicator of photosynthesis and Saturation Pulse quenching analysis

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 photo optics, 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. Progress in this field of research has been greatly stimulated by the invention of the Pulse-Amplitude-Modulation (PAM) measuring principle.

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 photosynthesis. Chlorophyll is the major

antenna pigment, funneling the absorbed light energy into the reactions centers, where photochemical conversion of the excitation energy takes place.

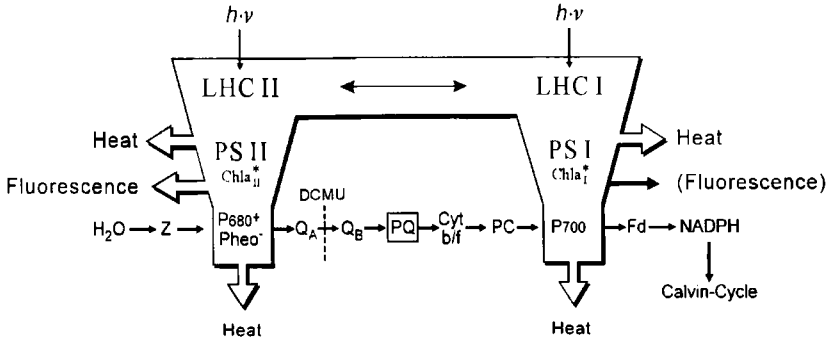


Fig. 8 Schematic view of primary energy conversion and primary electron transport in photosynthesis. LHC, light harvesting pigment-protein complex; P680 and P700, energy converting special chlorophyll molecules in the reaction centers of photosystem II (PS II) and photosystem I (PS I), respectively; Pheo, pheophytin; DCMU, PS II 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 enhanced when the yields of PS II photochemistry and heat dissipation are decreased and *vice versa*. Hence, changes in fluorescence yield reflect changes in PS II photochemical efficiency and heat dissipation. The variable part of chlorophyll fluorescence originates mainly in photosystem II. Excitation transfer to photosystem I may be considered an additional competitive pathway of de-excitation, i.e. any increase will cause fluorescence quenching.

Measuring chlorophyll fluorescence is rather simple: The emission extends from 660 nm to 760 nm, and if shorter wavelength

excitation light is used, separation of fluorescence from the measuring light is readily achieved with the help of optical filters. The challenge arises with the wish to measure fluorescence in the presence of strong background light and to use very strong light for the so-called 'Saturation Pulse quenching analysis'. For this purpose the PAM measuring principle has been developed which allows monitoring fluorescence against 10^6 times larger background signals.

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 PS II 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 Saturation Pulse quenching analysis:

With a very strong pulse of white light the electron transport chain between the two photosystems can be quickly fully reduced, such that the acceptors of PS II 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.

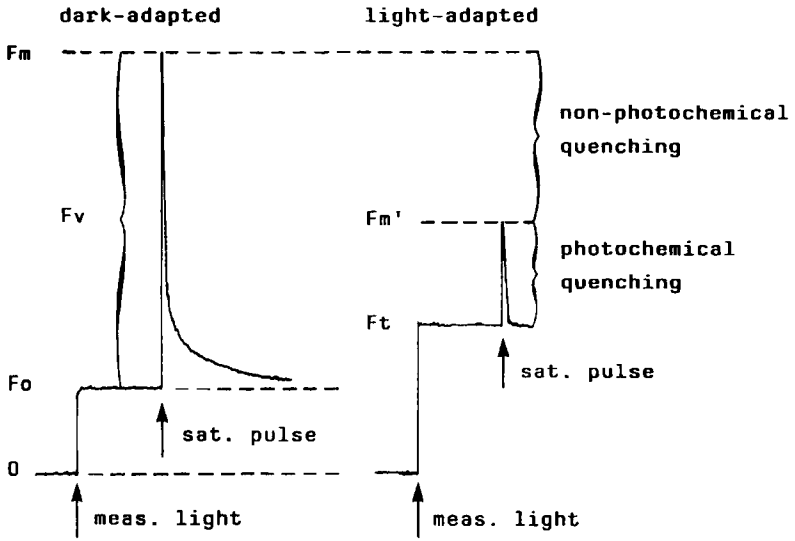


Fig. 9 Principle of Saturation Pulse Quenching Analysis

On the basis of these considerations so-called 'quenching coefficients' qP and qN were defined, which can be determined by simple fluorescence measurements (see 6.3). 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 6.2). 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:

$$\mathbf{Yield = (Fm'-F)/Fm' = \Delta F/Fm'}$$

This expression, which was introduced by Genty et al. (1989) is identical to the Yield-parameter determined with the help of a Saturation Pulse under WinControl. With PDA-100/WinControl Yield-determination has become exceedingly simple: After the SAT-Pulse key is pressed, everything else proceeds automatically within seconds:

- the momentary fluorescence yield F is sampled
- a Saturation Pulse is applied
- Fm' is sampled
- Yield = (Fm'-F)/Fm' is calculated and displayed
- the obtained data are stored in the Report-file of WinControl

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 plants 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 and temperature. For example, if the measured Yield of sample A is lower than that of sample B, this does not necessarily mean that sample A is photosynthetically less competent than sample B. The

difference could as well arise from sample A being exposed to stronger light or to a lower temperature than sample B. The PDA-100 features an AUX. INPUT where suitable PAR- and temperature-sensors can be connected, such that together with every Yield-value also the corresponding values of PAR and temperature are entered into the Report-file. When PAR is known, the apparent rate of electron transport (ETR) is calculated and displayed.

For assessment of overall photosynthetic performance, measurements during steady-state illumination 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. The rapid transients contain information on primary electron transport reactions, while the slow transients reflect reactions at the levels of enzyme regulation and adjustment of energy distribution between the two photosystems (so-called pigment state shifts). Analysis of the slow transients is greatly facilitated by use of the method, which allows to distinguish between the contributions of photochemical and non-photochemical quenching.

6.1 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 Yield = $\Delta F/Fm$ = 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 an 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.

6.2 Fo, Fm

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 6.3). Fo and Fm determination is carried out under WinControl via the Fo and Fm-keys. Upon pressing the Fm-key both Fo and Fm as well as Fv/Fm are determined. At the same time there is an automatic reset of qP to 1.000 as well as of qN and NPQ to 0.000. With all consequent applications of Saturation Pulses (via SAT-Pulse key), calculation of the quenching coefficients will be based on these Fo, Fm values, until they are redetermined via a new Fo-Fm measurement. 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. Such high values can be measured only when true dark-adaptation is reached and the measuring procedure is optimized.

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 and also for calculation of the quenching coefficients qP, qN and NPQ (see 6.3).

6.3 qP, qN and NPQ

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 6.2). When the momentary fluorescence yield, F, is lower than Fm, this may be caused either by photochemical energy conversion or by enhanced heat-dissipation (as compared to dark state). 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 the rate of photochemical energy conversion is saturated), whereas non-photochemical quenching does not change during a Saturation Pulse (as changes in heat-dissipation involve relatively slow processes). The quenching coefficients are defined as follows:

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

qP and qN can vary between 0 and 1, whereas NPQ can assume values between 0 and approximately 10. The displayed quenching coefficients are meaningful only, if the values of Fo and Fm were previously measured with the same sample at the same sensitivity, i.e. with unchanged optical parameters, Measuring Light intensity and Gain-settings.

The above 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 reoxidized (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 thus facilitates the assessment of Fo'. The PDA-100/WinControl combination supports determination of Fo'. A special Fo'-mode is provided (see 5.2).

Fo-quenching is of no concern for NPQ-determination. The definition of NPQ implies a matrix model of the antenna system (Stern-Volmer quenching). With NPQ that part of non-photochemical quenching is emphasized which reflects heat-dissipation of excitation energy in the antenna system. NPQ has been shown to be a good indicator for 'excess light energy'. On the other hand, NPQ is relatively insensitive to that part of non-photochemical quenching which is associated with qN-values between 0 and 0.4, reflecting mainly thylakoid membrane energization.

6.4 YIELD-measurements of illuminated samples

A Yield-measurement is triggered via the SAT-Pulse key. After application of a Saturation Pulse under the following measured parameters are shown:

- F, fluorescence yield measured briefly before triggering of the Saturation Pulse
- Fm', maximal fluorescence yield reached during the Saturation Pulse
- Yield, effective yield of photochemical energy conversion calculated as

  Yield = (Fm'-F)/Fm'

- ETR, apparent electron transport rate calculated as

ETR = Yield * PAR * 0.5 * ETR-factor

This and additional information is stored in the Report-file under WinControl.

There is no principal difference between Yield-measurements with dark-adapted or illuminated samples. The same Saturation Pulses are applied. Fv/Fm just constitutes the maximal Yield after dark-adaptation. 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. While 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.

6.5 Light Curve and LC+Recovery

The recording of a Light Curve (LC) involves 9 consecutive Yield-measurements. The illumination series may start at Actinic Intensity 1, 2, 3, 4 or 5 (set via LC-Int under Light Curve Parameters, Settings 2). This feature allows to adjust the range of applied intensities to the light adaptation characteristics of the sample (sun or shade plant). When an Actinic Halogen Source is used, the Actinic Intensity range alternatively can be also modified by the Actinic Light Factor, which shifts all intensities up or down (Actinic Light Fact. under Main settings).

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

experienced by the plant in its natural environment. In this way the requirement of long illumination periods for reaching steady-state during each intensity step of the LC can be avoided. The length of the actinic-light-periods is determined by LC-Width (set under Settings 2).

After start of a LC recording there is first a Yield-determination in the absence of actinic illumination for assessment of the maximal quantum yield. Actinic Intensity is automatically increased during the course of a LC and Yield is automatically determined at the end of each illumination period, the length of which is determined by the LC-Width. This results in a total of 9 Yield- and ETR-values, which under WinControl are stored in the Report-file and plotted in the Light Curve window.

Additional information on the dark-recovery of Yield-lowering during actinic illumination can be obtained by the function LC+Recovery. The illumination program with LC+Rec. is identical to that of LC. In addition, after termination of the last illumination period, in the absence of actinic light the recovery of Yield in the dark is assessed by 6 consecutive Saturation Pulses applied at 10 s, 30 s, 60 s, 2 min, 5 min, 10 min after light-off. In this way, different types of non-photochemical quenching can be distinguished which contribute to the lowering of the PS II quantum yield. It is generally assumed that the rapid recovery within the first 30-60 s reflects the disappearance of energy dependent nonphotochemical quenching, in parallel with the relaxation of the transthylakoidal ΔpH. The slower recovery within the first 10-30 min is considered to reflect a change of energy distribution in favor of PS II (so-called State Shift). The apparently irreversible Yield-lowering (with respect to the original dark state) is expression of 'photoinhibition'.

LC as measured under WinControl may contain somewhat different information than 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. LC recorded with short illumination times (down to 5 s; so-called Rapid Light Curves, RLC) allow insights into the physiological flexibility with which a plant sample can adapt its photosynthetic apparatus to changes of light intensity. Hence, RLC contain information on induction as well as saturation characteristics of photosynthesis. LC measured on the same sample during the course of a day 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, LC are sufficiently fast that they can characterize a momentary adaptation state of a plant in a naturally changing environment.

6.6 Induction Curve and IC+Recovery

Dark/light induction curves (Kautsky effect) contain complex information on the photosynthetic performance of a plant at different functional levels. By repetitive application of saturating light pulses and quenching analysis additional information is obtained which is essential for reliable interpretation of the Kautsky effect. After a longer period of darkness, Calvin-cycle enzymes are partially inactivated. They are light-activated during the first minutes of illumination. During this induction period oxygen instead of CO_2 serves as terminal electron acceptor. O_2 dependent electron flow (Mehler-Ascorbate-Peroxidase Cycle) as well as cyclic electron flow

at photosystem I create a large proton gradient, which will be used for ATP-synthesis only after Calvin cycle has been light activated. This leads to strong 'energy-dependent' nonphotochemical fluorescence quenching during the first minutes of illumination (characterized by low Fm'-values), which partially declines again when CO_2-fixation takes over and ATP is consumed.

After an Induction Curve is started (via the corresponding check box under WinControl), 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. The delay between this Saturation Pulse and onset of illumination can be varied (IC-Delay under Settings 2); its default value is 40 s. Another variable is the time interval between two consecutive Saturation Pulses during actinic illumination (IC-Width under Settings 2), with a default setting of 20 s (for an example of a standard Induction Curve see Fig. 5).

It is also possible to record the light/dark recovery in addition to the dark/light induction (IC+Recovery). In this case information on post-illumination reactions are obtained, in particular on the recovery of various components of nonphotochemical quenching (see 6.3), the extent of photoinhibition and also on dark electron flow between stroma (or cytoplasma) and the electron carrier in the thylakoid membrane. An example of an IC+Recovery recording with the PAM-100 is given in Fig. 10.

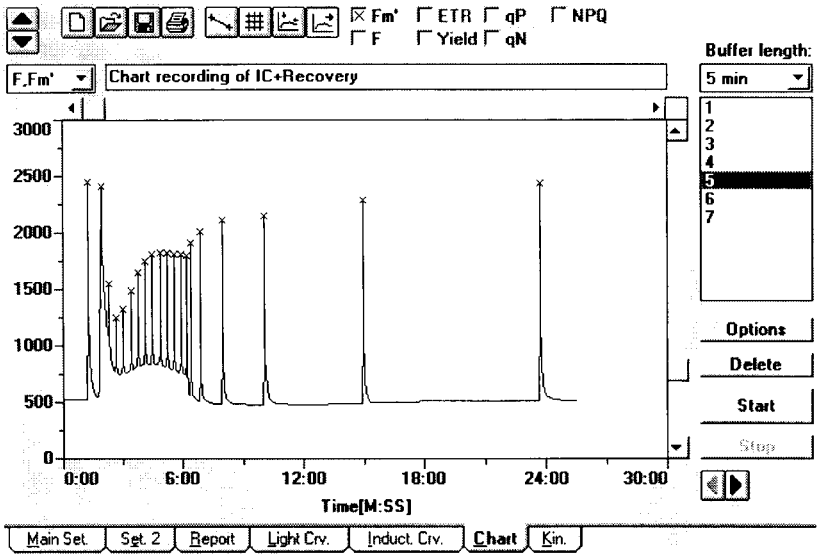


Fig. 10 Example of Chart registration of IC+Recovery with PAM-100 Chlorophyll Fluorometer

7 Appendix

7.1 Technical specifications

PAM-Data Acquisition System PDA-100

Design:	Control box to be connected with signal outputs and Remote Control inputs of XE-PAM or PAM-100 as well as with PC; housing CMOS micro controller 80C52, 12-bit A/D converter with 20 µs acquisition time, various drivers for external LED-sources and trigger signals; featuring internal battery
Fluorescence signal input:	0 – 8 V (XE-PAM) or 0 – 4 V (PAM-100)
Aux. input:	For Leaf-Clip Holder 2030-B, Micro Quantum/Temp.-Sensor 2060-M or Spherical Micro Quantum Sensor US-SQS/W
Actinic light output:	For driving up to 3 red LEDs, e.g. LED Light Source 102-L or up to 2 blue LEDs; output voltage max. 11 V; output current max. 40 mA
Far-red light output:	For driving up to 3 far-red LEDs, e.g. Far-Red Led Light Source 102-FR; output voltage max. 11 V; output current max. 40 mA
Trigger outputs I:	Start- and Stop-pulses (12 V DC) synchronized with start and stop of illumination period, e.g. for Fiber Illuminator FL-103/E; featuring BNC sockets
Trigger outputs II:	Start- and Stop-pulses (12 V DC) synchronized with start and stop of illumination period; featuring BNC sockets
Aux. output:	Control signals (Actinic, Far-red and Saturation Pulses) for external lamp drivers, e.g. Power-and Control-Unit HPL-C (High Power LED Lamp)
User interface:	IBM-compatible Pentium PC with WinControl-software; connection via RS 232, 19200 Baud; keyboard operation; monitor screen display

Power supply:	Internal rechargeable battery 12 V/ 2 Ah; automatic power/off; Battery Charger MINI-PAM/L (100 – 240 V AC)
Power consumption:	Basic operation 40 mA, max. 120 mA
Dimensions:	22 cm x 10.5 cm x 8.5 cm (L x W x H)
Weight:	approx. 1.85 kg (incl. battery)

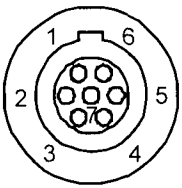
Battery Charger MINI-PAM/L

Input 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

Technical specifications are subject to change without prior notice

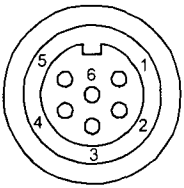
7.2 PIN-assignments

"AUX INPUT"



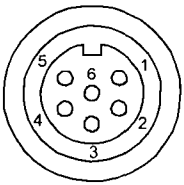
1: +5 V
2: GND
3: } Analog inputs for 2030-B, 2060-M or US-SQS/W
4: } Analog inputs for 2030-B, 2060-M or US-SQS/W
5: } Analog inputs for 2030-B, 2060-M or US-SQS/W
6: Remote control button
7: -5 V

"AL-LED"



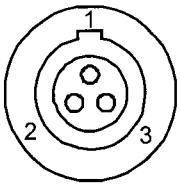
1: LED anode
2: LED anode
3: not used
4: LED cathode
5: LED cathode
6: not used

"FR-LED"



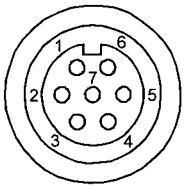
1: LED anode
2: LED anode
3: not used
4: LED cathode
5: LED cathode
6: not used

"CHARGE"



1: Charge input +18 V
2: GND
3: not used

"AUX OUTPUT"



1: +5 V
2: SP (pulse signal)
3: GND
4: AL LED (pulse signal)
5: AL HAL (pulse signal)
6: +12 V
7: FR (pulse signal)

7.3 Figures with cable connections

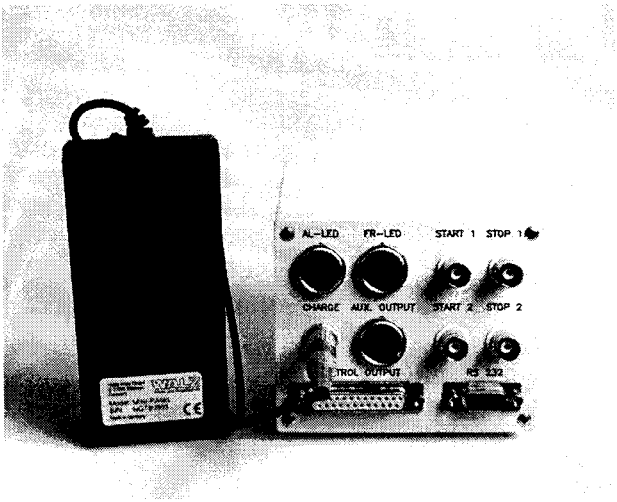


Fig. 11 Battery Charger MINI-PAM/L connected to PDA-100 CHARGE

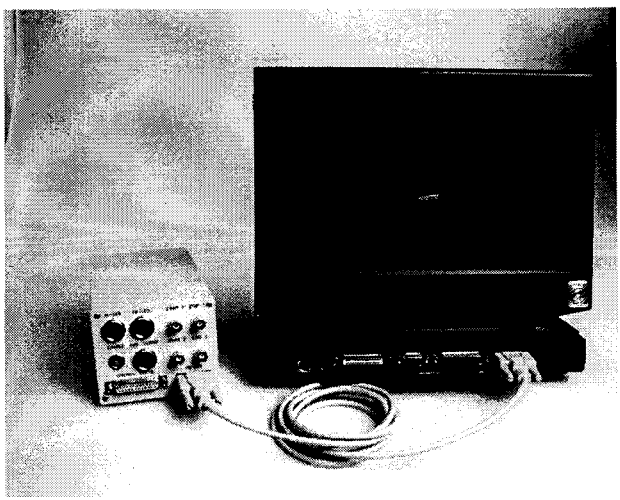


Fig. 12 PC connected to PDA-100 via RS 232

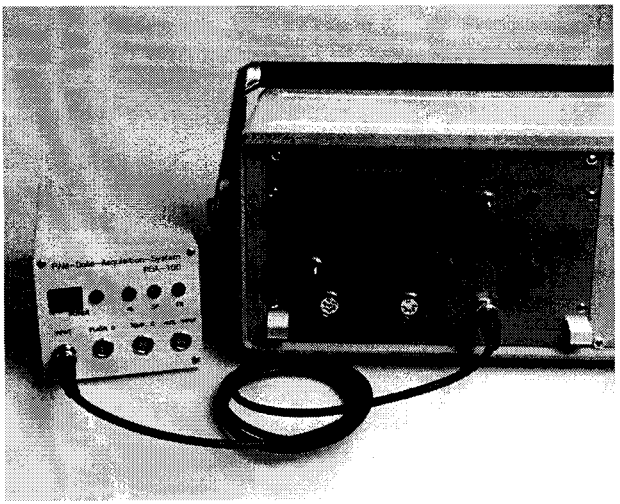


Fig. 13 PAM-101 OUTPUT connected to PDA-100 INPUT

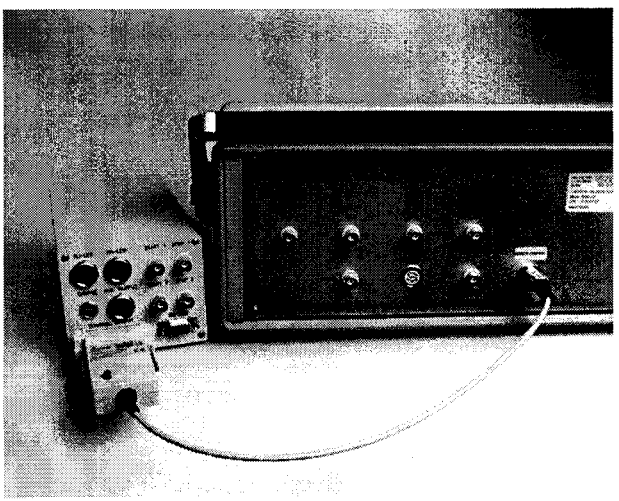


Fig. 14 PAM-101 connected to PDA-100 via REMOTE CONTROL

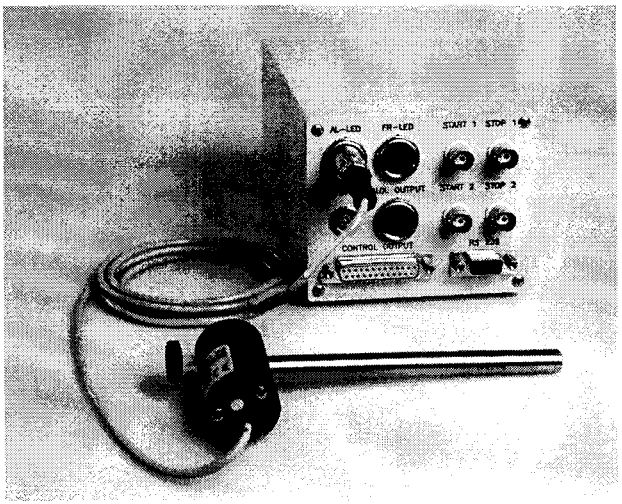


Fig. 15 Actinic Light Source 102-L connected to PDA-100 AL-LED

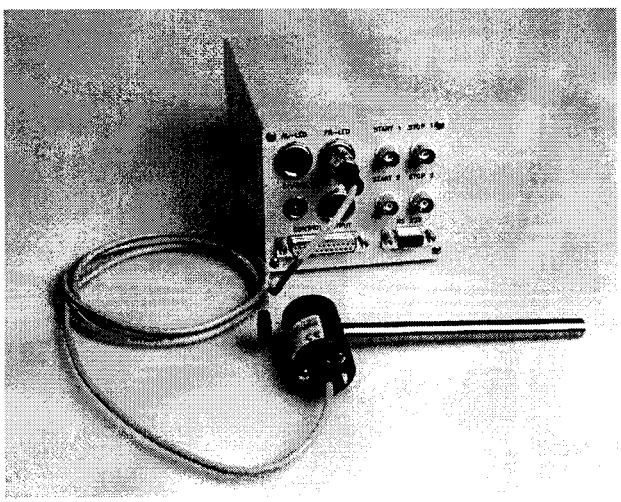


Fig. 16 Far-Red Light Source 102-FR connected to PDA-100 FR-LED



Fig. 17 Fiber Illuminator FL-103/E connected to PDA-100 START 1/STOP 1



Fig. 18 Fiber Illuminator FL-103/E connected to PDA-100 START 2/STOP 2

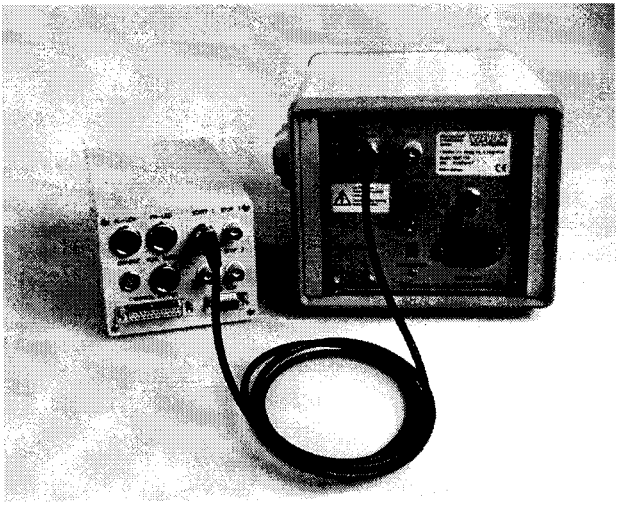


Fig. 19 Flash Lamp XMT-103 connected to PDA-100 START 1

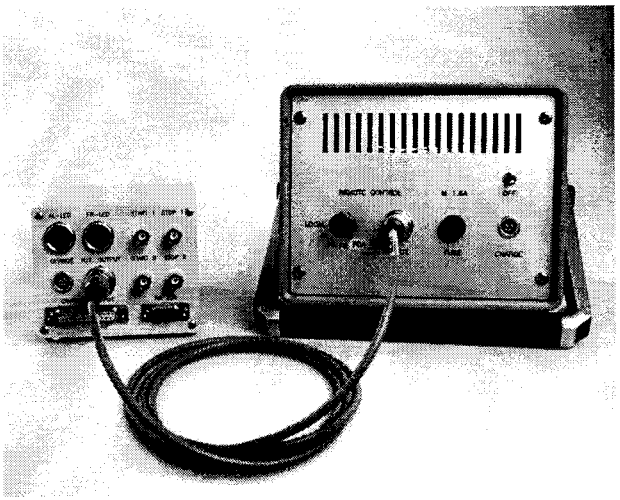


Fig. 20 High Power LED Lamp HPL-C connected to PDA-100 AUX. OUTPUT

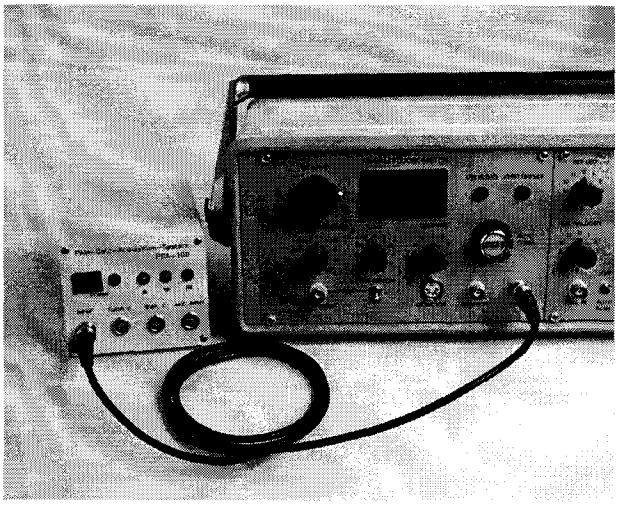


Fig. 21 XE-PAM OUTPUT connected to PDA-100 INPUT

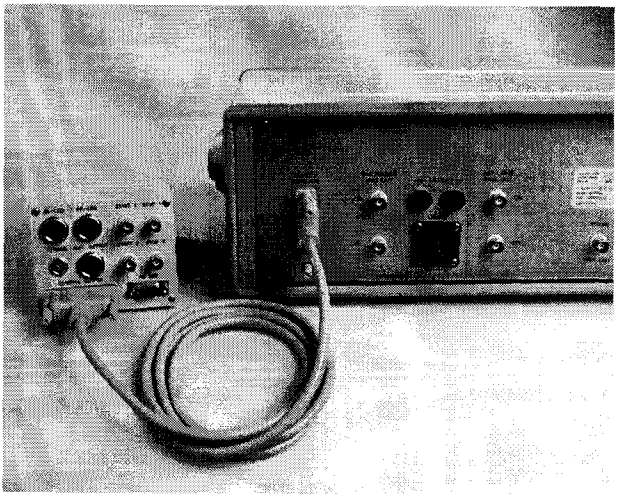


Fig. 22 XE-PAM connected to PDA-100 via REMOTE CONTROL

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:

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2. This warranty shall not apply to any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.
3. This warranty shall not apply to any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.
4. This warranty does not apply to damage caused from improper packaging during shipment or any natural acts of God.
5. This warranty does not apply to underwater cables, batteries, fiberoptic cables, lamps, gas filters, thermocouples, fuses or calibrations.

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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.

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