Portable
Chlorophyll Fluorometer
PAM-2100

Handbook of Operation

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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. Ensure that no liquids or other foreign bodies can find their way inside the device.
10. The device should only be repaired by qualified personnel.

2 Introduction

The **PAM-2100 Portable Chlorophyll Fluorometer** is the follow-up model of the well-known **PAM-2000** that was introduced in 1992 as the first portable PAM fluorometer and since then has been sucessfully applied by numerous scientists all over the world. In the development of the PAM-2100 particular care was taken to maintain all properties appreciated by PAM-2000 users over many years and at the same time to take account of the recent technical progress. Essentially the proven hardware and optical system are unchanged. Also all accessories previously introduced for the PAM-2000 can be used in conjunction with the PAM-2100 (except for the 2050-H, which is replaced by the 2150-H). While instrument operation is still based on the same Data Acquisition software (DA-2100 equivalent to DA-2000), the program was **extended for stand-alone operation** (without external PC using a panel-PC) as well as for **PC-operation under Windows** using the new **PamWin** software.

Major **points of progress** of the PAM-2100 with respect to the PAM-2000 are:

- The PAM-2100 features a **built-in PC** and, hence, has become even more compact and suited for stand-alone operation under **field conditions**.
- An **LCD-display** and a **20-key board** are integrated into the top cover of the instrument, thus simplifying operation under **field conditions**.
- Alternatively, particularly for work under **laboratory conditions**, an **external keyboard** and an **external monitor** can be connected. This facilitates data analysis and system programming considerably.

- A **Li-ion battery** provides increased power for extended time of field work.
- The new **Trans2100** software is provided for transfer of data from the PAM-2100 to an external PC running under Windows.
- The new **PamWin** software can serve for **display of the transferred data** under Windows (including Windows XP) as well as for carrying out **measurements under Windows.**
- The instrument software DA-2100 is stored on a **programmable "flash card"** and can be readily **updated via the RS 232 interface** without opening the instrument.

Chlorophyll fluorescence can be measured in a number of different ways and depending on the given application the results may be evaluated by numerous analytical routines. The Portable Fluorometer PAM-2100 displays a **high degree of flexibility** in measuring and analysing fluorescence. However, this does not necessarily mean that all features of this multifunctional instrument must be understood before measurements can be started. Actually, due to the "intelligent" central control of all functions by the special DA-2100 software, serious operational mistakes harming the instrument are highly unlikely. Also, at first there is no need to care about the numerous settings of instrument parameters, because these are pre-set for standard measurements and, if changed, can be reset at any time. Hence, even the unexperienced user can start measuring with a minimum of background knowledge, and will be gradually guided to deeper understanding and more profound applications. This handbook tries to cover all of the numerous features and applications of the PAM-2100 Fluorometer, some of which probably are not of immediate interest to many users, but probably will become relevant, as new questions arise on the basis of the obtained results. If time is no problem, the best way to become acquainted

with all features of the PAM-2100 Fluorometer is to read this handbook section by section, trying out all described functions and reproducing the given examples. On the other hand, in order to get a quick start it will suffice to read **Chapter 4** on **How to get started.** Reading this chapter, the user can learn within a few minutes how to connect the components of the measuring system and how to carry out simple measurements. Hence, this section may serve as a first, condensed outline of system properties and operation.

3 Components of the Portable Chlorophyll Fluorometer PAM-2100

The PAM-2100 Fluorometer consists of two basic parts forming the minimal functional unit of this measuring system:

- **Main Control Unit**
- **Special Fiberoptics 2010-F**

The Main Control Unit contains the actual fluorometer with various light sources, detectors and electronic hardware. The fiberoptics form the optical link to the plant sample. The **DA-2100 software** provides the framework for operation of the fluorometer via the **integrated PC** and for on-line analysis of the fluorescence data. Essential accessories are the **Battery Charger 2020-L** and the **Leaf-Clip Holder 2030-B**. Further accessories for special applications are the **Micro Quantum/Temp.-Sensor 2060-M**, the **External Halogen Lamp 2150-H** and the **Dark Leaf Clip DLC-8**. The various components are described in the following sections.

3.1 PAM-2100 Control Unit

Fig. 1 PAM-2100 Control Unit

The PAM-2100 Control Unit measures 23 x 10.5 x 10.5 cm (LxWxH) and weighs 2.7 kg (including internal battery). Fig. 1 shows front (right side) and rear (left side) views of the instrument. The following elements are located at the right side:

- **POWER ON**

This switch primarily serves for turning power on and starting the internal PC. Switching power off normally should be carried out under software control, as it is coupled with quitting the DA-2100 program and shutdown of the PC operating system. After switching POWER ON the **green STATUS LED** starts blinking and the built-in monitor screen briefly shows the PAM-2100 logo. Please note that it takes about **40 sec for booting the internal PC**, i.e. before the DA-2100 user surface is displayed. When the POWER-ON switch is pressed for more than about 2 sec, power is switched off without closing the DA-2100 program. In this case the instrument settings are not saved in an INI-file and, hence, will not necessarily be identical when the instrument is switched on again. This "hardware way" of switching power off can be used, if for some reason the "software way" (via Com-menu or Alt-X command using an external keyboard) does not work.

- **STATUS (green LED)**

The pulsing green light of this LED signals that the microcontroller is operating alright. When the LED stays off or lights continuously, the microprocessor functioning is disturbed. In this case renewed switching of POWER ON should restore normal functioning. The green LED will also start blinking when the instrument is connected via an RS 232 interface cable with an external PC. In this case power is switched on for the PAM fluorometer only, but not for the internal PC. Power is automatically switched off again, if there is no data transfer via the RS 232 interface for more than 5 min. It also can be

turned off by pressing the POWER-ON switch for longer than about 2 sec.

- **CHARGE (red/green LED)**

  This dual-color LED provides information on the charging status of the internal battery. While the internal battery is being charged by the **Battery Charger 2120-N**, the red LED chip lights up. When the battery is fully charged, the green LED chip lights up.

- **LEAF CLIP**

  To connect the Leaf-Clip Holder 2030-B or the Micro Quantum/Temp.-Sensor 2060-M.

- **RS 232**

  To connect the PAM-2100 with an external Windows-PC via an RS 232 interface cable for data transfer using the Trans2100 program or to run the instrument under the PamWin program.

- **EXT. DC**

  To connect the **Battery Charger 2120-N** for charging the internal Li-ion battery or an external 12 V battery via the **Battery Cable 2125-A**. Please note that an external 12 V battery cannot recharge the internal Li-ion battery; but it can provide power for running the instrument when the internal Li-ion battery is empty. **Avoid charging the internal Li-ion battery, when the PAM-2100 is switched on**.

- **EXT. HALOGEN**

  Power output for the **External Halogen Lamp 2150-H**, which can be mounted on the **Leaf-Clip Holder 2030-B**. Use of the External Halogen Lamp is recommended for longer illumination at high intensities, as e.g. for photoinhibition of a sample or extended Light Response Curves.

- **Fiberoptics connector**

To connect the **Special Fiberoptics 2010-F** (see 3.2).

Note: The four cable connector sockets (LEAF CLIP, RS 232, EXT.DC and EXT.HALOGEN) should not be mixed up. Do not force a plug into the wrong socket. The proper positioning is indicated by the red dots. Do not try to disconnect a plug by pulling at the cable; it can be readily disconnected by pulling at the rippled metal part of the plug.

At the left side of the PAM-2100 two sockets for connecting optional external hardware are located:

- **MONITOR**

To connect an external monitor screen that can be recommended for laboratory work or data analysis.

- **KEYBOARD**

To connect an external keyboard that is useful for laboratory work or data analysis. The **Ultra-Compact Keyboard 2170-K** is provided, which does not occupy much space and is also suited for work under field conditions. Many commands, particularly for data analysis, are more easily given via an external keyboard than with the 20 keys integrated into the top of the PAM-2100 Control Unit.

3.2 Special Fiberoptics 2010-F

The Special Fiberoptics 2010-F are connected to the front side of the PAM-2100 Main Control Unit with the help of a special plug that resembles an electrical connector. There are three "fiber pins" with different optical cross-sections, which fit into the corresponding holes at the front side of the PAM-2100 housing, where they interface the three essential optical devices (from left to right):

Optical device **Active fiber cross-section**

LED-cone	4 mm
Photodiode detector	3.5 mm
Halogen lamp	3 mm

Within the "interface plug" the three fiber branches are joint to a common fiberbundle and randomized via a 100 cm mixing pathway. The total active cross-section amounts to 6 mm. A so-called **Distance Clip** is provided with the fiberoptics for convenient positioning of the fiberoptics end-piece relative to the sample.

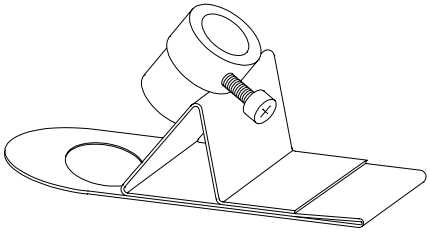


Fig. 2 Distance Clip for positioning leaf sample with respect to fiberoptics

Two spacer rings may be used to define fixed distances. The fiberoptics exit plane is positioned at a 60° angle relative to the sample plane. In this way shading of the sample is minimized, if the fiberoptics are pointing towards the sample from the side opposite to incident light. The sample may be placed either below the hole or, preferentially with normal leaves, above the hole. In the latter case, the leaf can be held between the folded part of the clip. The former possibility applies e. g. to thick leaves, lichens and mosses. The distance between fiberoptics exit plane and sample has considerable influence on signal amplitude and effective light intensities. Unavoidably, with a 60° angle between sample plane and fiberoptics there is a range of distances between fiberoptics and leaf, which will result in an effective light intensity gradient. However, this point should not be of too much concern, as in any case there is a much larger vertical light gradient within the leaf due to chloroplast

shading by the top chloroplast layer. Also, the measured signal will be dominated by that part of the leaf which receives maximal intensity, as this also is most strongly excited by the measuring light and emits most of the fluorescence which is received by the fiberoptics. The following figure depicts the signal amplitude and light intensity in dependence of the distance between fiberoptics and sample.

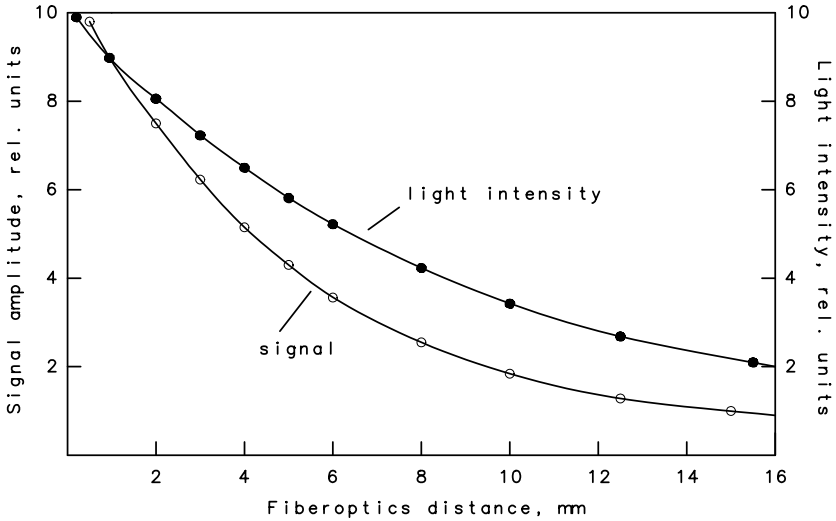


Fig. 3 Relationship between signal amplitude/light intensity and distance between fiberoptics exit plane and sample

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

3.3 Leaf-Clip Holder 2030-B

Fig. 4 Leaf-Clip Holder 2030-B with Fiberoptics 2010-F and External Halogen Lamp 2150-H mounted on tripod.

The Leaf-Clip Holder 2030-B may be considered the most important accessory of the PAM-2100, which is almost indispensable for efficient field investigations, when ambient light and temperature conditions may vary considerably. It substitutes for the standard "Distance Clip" as a device for defined positioning of the fiberoptics relative to the leaf plane. It features special **microquantum** and

temperature sensors, the readings of which are transferred to the PAM-2100 with every measurement.

In this holder, the leaf is resting on a perspex tube with widened crest, which can be vertically adjusted, to account for different leaf thicknesses. The fiberoptics axis forms a 60° angle with the leaf plane. Optionally, a 90° fiberoptics adapter (2030-B90) is available. The distance between fiberoptics and leaf can be varied. Standard distances are defined by spacer rings. The illuminated leaf area is limited by a steel ring with 10 mm ∅ opening.

At the bottom of the Leaf-Clip Holder 2030-B a **tripod mounting thread** is provided. Mounting the device on a tripod (e. g. **Compact Tripod ST-2101**) facilitates long term measurements with the same plant. Such recordings can be automated by using the Clock-function (6.1.11) and the pre-programmed Run-files (6.1.13).

Two holes are provided in the front bottom part of the holder for mounting the optional **External Halogen Lamp 2150-H** (see 3.4). This lamp allows long periods of illumination with strong light, as e. g. required for photoinhibitory treatment or extended Light Response Curves. It is not recommended to use the internal halogen lamp for this purpose, as this would lead to excessive internal heating and rapid depletion of battery power.

3.3.1 Micro-Quantum-Sensor

A tiny micro quantum sensor is integrated into the Leaf-Clip Holder 2030-B, which is unique in monitoring the **photosynthetic active radiation (PAR)** at the very spot where also fluorescence is measured and at which photosynthetic performance is assessed. This function already is fulfilled, when only 4 mm^2 of the total 80 mm^2 measuring area are occupied by the sensor. The resulting loss in signal amplitude is small. If wished, the sensor can also be moved

out of the measuring field that is limited by the10 mm ∅ opening of the steel ring. With its tip resting on this ring, even without penetrating into the measuring field the sensor will accurately monitor **incident light intensity** under natural day light conditions, when the leaf-clip holder is positioned such that light incidence is mainly from the front.

Essential opto-electronical elements of this micro-quantum-sensor are:

- a 1.5 mm cross-section **diffusing disk**;
- a **0.5 mm diameter fiber** guiding the scattered light to the detector;
- a **filter combination** selecting the photosynthetic active wavelength range between 380 and 710 nm;
- a **blue-enhanced silicon photodiode**.

Despite of its small dimensions, the diffuser assures that also light impinging at rather small incidence angles (e. g. with rising or setting sun) is reliably monitored. Due to the equalization of leaf and sensor planes, automatically achieved by fixing the leaf in the clip, the measured PAR very closely corresponds to the PAR at that spot of the leaf where fluorescence is measured. The micro-quantum-sensor measures incident photosynthetic radiation in µmol quanta $m^{-2}s^{-1}$, i.e. in units of flux density. Hence, the measured parameter **PAR** is **identical to PPFD** (photosynthetic photon flux density). The PAR is displayed in the **PAR- parameter field** of the monitor screen when the Leaf-Clip Holder 2030-B is connected. The sensor was **calibrated against a LI-COR Quantum Sensor**. The stability of calibration depends on keeping the diffuser clean. It is advisable to check calibration regularly by comparison with a standard quantum sensor. Any deviation can be corrected by entering a recalibration factor via the DA-2100 program (Ctrl C command or Shift+Com Menu). A substantial increase of the calibration factor from its

original value of 1.000 indicates dirt-deposition on the diffuser, which may be reversed by gentle cleaning using a cotton-tip, moistened with some ethanol.

It may be pointed out that in most applications the interpretation of measured fluorescence parameters requires knowledge of the PAR at the very site were fluorescence is measured. Therefore the micro quantum sensor of the Leaf-Clip Holder 2030-B may be considered the most important accessory of the PAM-2100 fluorometer. Measured PAR-values are automatically written into the **Report-file** (6.1.17).

3.3.2 Thermocouple Monitoring Leaf Temperature

A **NiCr-Ni thermocouple** is mounted in the perspex tube on which the investigated leaf area is resting. Its tip is forming a loop that gently presses against the lower surface of the leaf. In this way there is effective temperature equilibration and the thermocouple is protected from direct sun radiation. The reference couple is located on the circuit board, in close proximity to the thermovoltage amplifier, enclosed in the bottom part of the holder. The relationship between thermovoltage and temperature is almost linear. With decreasing temperatures there is a small decline of ΔV/°C. Calibration was performed at 25 °C. At 0 °C or –15 °C the deviation amounts to 0.5 or 0.8 °C, respectively. An offset value can be entered via the DA-2100 program (Ctrl O-command or Shift+Com Menu) with a resolution of 0.3 °C. The measured temperature is displayed in the Tmp-parameter field of the monitor screen when the Leaf-Clip Holder 2030-B is connected. Temperature resolution is 0.3 °C. The temperature as well as the PAR data are automatically stored in the **Report-file** after every saturation pulse, together with the on-line calculated quenching parameters.

3.3.3 Remote Control

The handle of the Leaf-Clip Holder 2030-B features a **red push-button** for remote control of the PAM-2100. Pressing this button is equivalent to a **Return (↵) on the keyboard**. In practice, this offers the advantage, that one hand can be used for positioning the leaf within the holder, while the Leaf-Clip is held with the other hand, with which at the same time a recording can be triggered by remote control. In this way, field measurements are considerably facilitated, which is particularly helpful when many recordings are averaged to increase the accuracy of determinations.

The specific command carried out by Return or remote control depends on the cursor position on the Parameter Screen (see 6.1). The remote control function is particularly useful in conjunction with Run-file 1 (see 6.1.13) to determine overall quantum yield and apparent electron transport rate at given PAR and temperature. Approx. 1 second elapses between pushing the remote control button and triggering of the saturation pulse. The actual start of the measurement is announced by a beep-sound. From that moment onward the leaf clip should be held steady for approx. one second.

3.4 External Halogen Lamp 2150-H

The External Halogen Lamp 2150-H can be **mounted on the Leaf-Clip Holder 2030-B**, as shown in Fig. 4, and is connected to the **EXT. HALOGEN** output socket at the front of the PAM-2100. It is suited for extended illumination times at high intensity settings, in contrast to the internal halogen lamp, longer application of which is limited by the unavoidable heat development within the PAM-2100 housing. The External Halogen Lamp, just like the Internal Halogen Lamp and the LED-lamp, is **controlled via the DA-2100 program**. For this purpose, in the **H-parameter field** on the Parameter Screen

"ext. Hal." has to be selected. The 20 W halogen lamp is equipped with a heat-reflecting window. In addition, for standard applications a short-pass filter (λ < 700 nm) is provided, which is mounted directly on the lamp. This filter passes almost all visible light, whereas eliminates the long wavelength radiation, against which the fluorescence detector is not protected. For special applications, other filters (e. g. daylight or blue) are available which, however, decrease the maximal possible intensities, which are in the order of 5500 µmol quanta $m^{-2}s^{-1}$.

In its standard application, the External Halogen Lamp is mounted on the Leaf-Clip Holder 2030-B, with the light (8° beam divergence angle) shining at approx. 60° incident angle with respect to the leaf plane, selectively illuminating the site where fluorescence and PAR are measured. The optimum angle, giving maximal PAR and minimal shading by the fiberoptics can be manually adjusted.

A major application of the External Halogen Lamp is the adjustment of defined light intensities for measurements of Light Response Curves under field conditions. For this purpose, the light obtained from this lamp may substitute or complement the natural daylight. At maximal settings, the obtained intensity exceeds that of full sun light. Hence, this light source can be applied for photoinhibitory treatment of leaves and of other photosynthesising organisms in the field. It should be noted that application of such high light intensities will cause a substantial rise of leaf-temperature, which is monitored by the thermosensor integrated in the Leaf-Clip Holder 2030-B and can be read off the PC parameter screen.

Please note that operation of the External Halogen Lamp is not supported under the Windows-software PamWin.

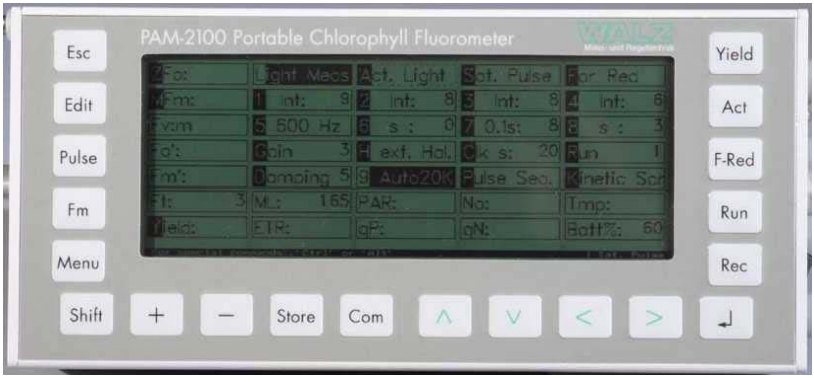


Fig. 5 Keyboard of PAM-2100

While the area on the top of the PAM-2100 is too small to give room for all the keys available with an external keyboard, all instrument functions can be controlled via the 20 keys on top of the instrument. Besides **single key operations** also a number of **dual key operations** are possible (see list below). Furthermore, also some **selection menus** featuring **special commands** can be called up. Before explaining the function of each key systematically, for a start the most essential key operations from a practical point of view shall be briefly described:

Depending on the ambient temperature, the display may be more or less dark. Higher temperatures cause darkening. The darkness of the display can be readily adjusted by the user:

Shift + When Shift is pressed and then the + key is clicked, the display darkens

Shift - When Shift is pressed and then the - key is clicked, the display lightens up

On purpose the **PAM-2100 does not feature on OFF switch**. Because of the integrated PC, the program has to be quit and the

operating system shut down properly. This step is software-controlled:

Com When the Com key is pressed, a window with the **Command selection menu** is opened:

∨ and ∧ A particular point of this menu can be selected with the help of the **∨ and ∧**-keys. For shutdown of the system **Quit Program** has to be selected. Please note that on the normal display the vertical as well as the horizontal arrow-keys serve for moving the cursor in order to select a particular command-field.

↵ The **Enter** (or **Return**) key serves for **executing a command.** This is not only true for a command in a menu, but also for a command on the normal display, which can be selected via the cursor with the help of the arrow keys.

During a dialog the Com-key serves for opening a selection menu.

∧	To move the **cursor up** and to move up in the **alphabet** or **number list** during a dialog (e.g. writing filename). To move one line up in the Report file.
∨	To move the **cursor down** and to move down in the **alphabet** or **number list** during a dialog (e.g. writing filename). To move one line down in the Report file.
<	To move the **cursor to the left**. On the Kinetic Screen to move curve limit to the left.

>	To move the **cursor to the right**. On the Kinetic Screen to move curve limit to the right.
↵	Equivalent to **Enter** or **Return**. To carry out a function selected by the cursor and to switch between alternative functions (e.g. 600 Hz and 20 Khz, different types of Actinic Lamps)
Rec	To start a **Kinetic Recording** when the Kinetic Screen is active. To move over to the **Kinetic Screen** when the Parameter Screen is active,
Run	To start a **Run**.
F-Red	To switch on **Far Red Light** at the given settings of intensity and duration of illumination.
Act	To switch on the selected **Actinic Light** source (LED, Int. Halogen or Ext. Halogen) at the given settings of intensity and duration of illumination.
Yield	To apply a **Saturation Pulse** for assessment of the effective PS II quantum yield of a sample in the illuminated state, **ΔF/Fm'**.

Please note that when an **external keyboard** is connected and **Scroll Lock** is active, the function of part of the 20-keys of the PAM-2100 is changed in order to allow use of the **Function-keys** F1-F10 in the **Triggered** and **Continuous modes**.

3.5 Micro Quantum/Temp.-Sensor 2060-M

The Micro Quantum/Temp.-Sensor 2060-M essentially displays the same features as outlined above for the Leaf-Clip Holder 2030-B (see 3.3), except that the micro-sensors of PAR and temperature are

not mounted in a leaf-clip. This device is rather designed for experiments with objects which are not leaf-shaped, like crustose lichens and cushions of moss. The two miniature sensors can be attached to the site where fluorescence is monitored without interfering with the actual measurement. A defined position with respect to the object and the fiberoptics exit plane can be achieved with the help of a special holder, in analogy to the "Distance Clip" (see Fig. 2).

It should be pointed out that the sensitivity of the micro quantum sensor is affected by bending the relatively long, flexible light guide that bridges the distance between the small diffusing disk at the object and the detector in the metal housing. Therefore, this device cannot substitute for a reliable quantum sensor like the LI-COR Quantum Sensor, against which it was originally calibrated. Recalibration (see 6.1.19) is recommended after bringing the sensor and the metal housing into a fixed position with respect to the object.

3.6 Dark Leaf Clip DLC-8

The Dark Leaf Clip DLC-8 weighs approx. 4 g and, hence, can be attached to most types of leaves without any detrimental effects. It is equipped with a miniature sliding shutter which prevents light access to the leaf during a dark-adaptation period. This shutter is opened for the actual measurement only, when exposure to external light is prevented by the fiberoptics. Proper dark-adaptation is essential for determination of the maximal quantum yield Fv/Fm and for recording of dark-light induction kinetics

Using the Dark Leaf Clip DLC-8, the fiberoptics are positioned at right angle with respect to the leaf surface at the relatively short distance of 7 mm. As a consequence, signal amplitude is distinctly higher than when the Leaf-Clip Holder 2030-B with 60° fiberoptics angle is used. In order to avoid signal saturation, the settings of

Measuring Light Intensity and Gain (6.1.8 and 6.1.9) have to be correspondingly lowered with respect to the standard settings.

When the shutter is still closed and the measuring light is on, an artifactual Ft signal is observed. This signal is due to a small fraction of the Measuring Light which is reflected from the closed shutter to the photodetector. However, this background signal is of no concern as the reflection is much smaller when the shutter is opened and the Measuring Light hits the strongly absorbing leaf instead of the metal surface of the shutter that acts like a mirror.

4 How to Get Started

4.1 Connection of the Basic Components

The PAM-2100 Fluorometer consists of two basic components forming the minimal functional unit of this measuring system:

- **Main Control Unit**
- **Special Fiberoptics 2010-F**

The Main Control Unit contains the actual fluorometer with various light sources, detectors and electronic hardware. The fiberoptics form the optical link to the plant sample. The **DA-2100 software** provides the framework for stand-alone operation of the fluorometer via the **integrated PC** and for on-line analysis of the fluorescence data. For a start it is not necessary to connect the instrument to an external PC. Essential accessories are the **Leaf-Clip Holder 2030-B** and the **Battery Charger 2020-L**.

Fig. 6 Front panel of PAM-2100

For getting started, the fiberoptics have to be connected to the Control Unit using the special **three-pin optical connector** at the front of the unit. The other end of the fiberoptics is connected to the

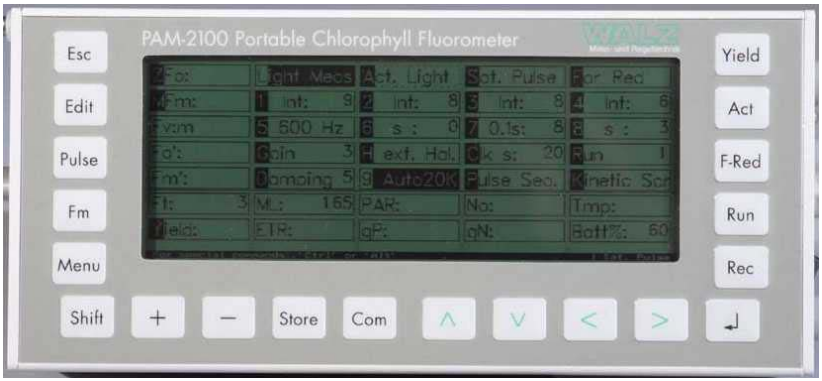
Leaf-Clip Holder 2030-B, provided this is available. If this is the case, please connect it to the corresponding socket at the front side of the instrument, for recording of PAR and temperature in parallel with chlorophyll fluorescence. For the time being, while the internal battery is still full, no battery charger needs to be connected to the EXT.DC socket. Also the EXT.HALOGEN-socket for an External Halogen Lamp and the RS 232 socket for the serial interface cable can remain empty.

4.2 Start of the DA-2100 Program

The DA-2100 program starts automatically after switching on the internal PC via the **POWER ON** switch (at front side of Control Unit). Please note that the internal PC, like any other PC, requires some **time for booting**. Immediately after switching POWER ON, **the green STATUS LED** (front panel of Control Unit) starts blinking, thus signalling that the microprocessor is working properly. Then on the display at the top side of the Control Unit the PAM-2100 logo is briefly shown. But it takes a total of about 40 sec until the user surface of the DA-2100 program is displayed.

4.3 PAM-2100 Keyboard

The keyboard that is integrated in the top cover of the instrument, features a total of 20 keys. The use of these keys is particularly important for measurements in the field, under conditions when an external keyboard would be a burden. Under lab conditions, an external keyboard as well as an external monitor may facilitate data analysis considerably. They can be connected to the corresponding sockets at the left side of the PAM-2100.



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↵ The **Enter** (or **Return**) key serves for **executing a command.** This is not only true for a command in a menu, but also for a command on the normal display, which can be selected via the cursor with the help of the arrow keys.

Probably at this point the user does not yet want to quit the program, but instead wants to learn a little bit more about the operation of the instrument.

Esc The Esc-key allows to escape from the selection menu and to return to the normal display.

Before starting first measurements, the user may want to make sure that the instrument settings are appropriate for standard applications. For this purpose the Command selection menu is called up again via the Com-key and **Standard Settings** is selected and confirmed by ↵.

After selection of **Standard Settings**, all light sources of the instrument are switched off and the cursor is on the **Light Meas box**, which functions as on **on/off switch** of the **Measuring Light** in conjunction with the ↵ key. It may be noted that the Measuring Light is also automatically switched on, whenever a measurement is carried out which requires that the Measuring Light is on. Therefore, in view of the limited space on top of the PAM-2100, no separate key for switching on/off Measuring Light is provided. On the other hand, there are separate keys for control of the other light qualities:

Yield Application of a Saturation Pulse for determination of the effective PS II quantum yield, ΔF/Fm'

Act ON/OFF switch of Actinic Light that under Standard Settings is derived from an LED array featuring the same type of red LEDs (650 nm) as the Measuring Light. The user may select between three different types of Actinic Light sources (see below).

F-Red ON/OFF switch of Far-Red Light that serves for selective excitation of photosystem I.

A major application of the PAM-2100 is **Saturation Pulse Quenching Analysis**. For this method it is essential that the quasi-dark fluorescence parameters Fo and Fm are determined. For this purpose the Fm-key is provided.

Fm Application of a Saturation Pulse for determination of the quasi-dark fluorescence parameters Fo and Fm, as well as the derived parameter of PS II quantum yield, Fv/Fm.

In many applications it is useful to apply Saturation Pulses repetitively with a defined interval between consecutive pulses. Pulse sequences can be started/stopped using the Pulse-key.

Pulse Start/Stop of a Pulse Sequence for repetitive determination of the effective PS II quantum yield, ΔF/Fm'.

4.4 PAM-2100 Display

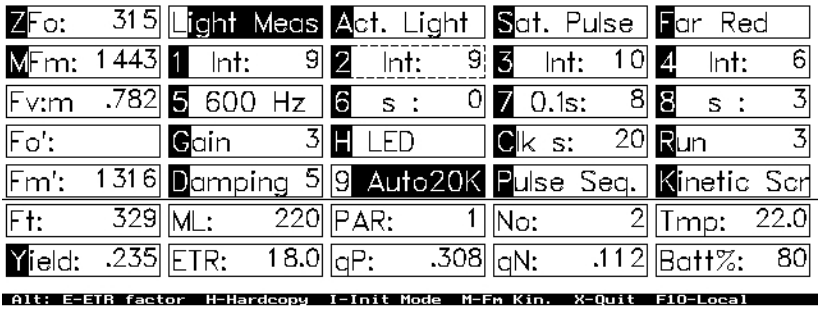


Fig. 8 Display of PAM-2100 showing the "Parameter Screen" with Standard Settings

Fig. 8 shows the PAM-2100 display featuring the **Standard Settings**. Five columns of **"parameter fields"** are displayed in seven lines. Different types of parameters are involved: The top fields of columns 2-5 refer to the status of four different light sources. The first letters (**L, A, S** and **F**) are inverted when the light sources are off. The initial character keys may be visualized as **ON/OFF switches**, operation of which is particularly simple when an **external keyboard** is used. In this case, simply pressing L, A, S, or F will activitate the corresponding lamp and will cause inversion of the letters in the given parameter field. Using the keys on top of the PAM-2100, it takes some more effort to achieve the same goal by moving the cursor to the corresponding parameter fields with the help of the arrow keys (∨ **and** ∧) and carrying out the command via Return (↵). Therefore, the direct keys **Yield, Act and F-Red** are available, which can be used instead.

In the second line of columns 2-5 parameter fields with the function of **dial switches** for changing the **intensities of the various light sources** are located. The pre-set values are suited for standard experiments. To **change settings**, first the corresponding parameter field is selected and then the **+ or - keys** are used to increase or decrease the settings, respectively. Selection of a parameter field is indicated by the cursor. Field selection can be achieved by cursor-movement using the arrow-keys. When an **external keyboard** is connected, simply the corresponding numbers of the parameter fields have to be typed (e.g. 2 for selecting Act. Light Intensity). An external keyboard is particularly helpful for users who have been used to the original PAM-2000 Chlorophyll Fluorometer and, hence, are accustomed to the keyboard commands. Use of the 20 keys and of an external keyboard is fully equivalent. Hence, under laboratory conditions the keyboard may be permanently connected and the user may choose one or the other way, whichever is more convenient. In the following description, the commands for both the integrated 20-key board and the external keyboard are specified.

4.5 First Measurements

While so-far only a small part of the numerous functions of the PAM-2100 and its complex user software were briefly outlined, this will be sufficient explanation for starting chlorophyll fluorescence measurements. To do so, the Measuring Light should be switched on. If this is not yet the case, please move the cursor to the L-field (using arrow keys) and switch on via Return (or L-command with external keayboard). When you now look at the fiberoptics exit, you will see the weak Measuring Light that serves for exciting chlorophyll fluorescence. As long as there is no chlorophyll containing object, the **Ft parameter field** shows values close to 0. When you approach a leaf with the fiberoptics, fluorescence is excited and guided via the

fiberoptics to the detector system. Depending on the distance, more or less Ft will be measured. For reproducible measurements the distance between fiberoptics exit and leaf should be defined. For this purpose a small adjustable **"Distance Clip"**, delivered with the PAM-2100, can be mounted on the fiberoptics. Alternatively **the Leaf-Clip Holder 2030-B** can be used. The latter can be particularly recommended, as it features sensors for measuring light-intensity and temperature.

Relevant information is obtained when the yields of fluorescence in different states of illumination are compared. For this purpose the PAM-2100 contains "Actinic Light" sources, which can be switched ON/OFF by key operations. When you press **Act (**equivalent to pressing the A-key on an external keyboard) you will see that the leaf is illuminated by a relatively strong red light. At the same time the value of Ft quickly rises and then slowly decays again. Here you witness the so-called **"Kautsky-effect"**. Pressing **Act** again, the actinic red light is turned off and you can see Ft decreasing.

When you now apply **F-Red** (or F-key with external keyboard), a far-red light source is switched on for a pre-set time of 3 s and the decrease of Ft is speeded up, with Ft approaching the original value before application of actinic light. The opposite effects of red and far-red light on fluorescence yield can be readily explained in the framework of the so-called Z-scheme of photosynthesis and by the theory of fluorescence quenching. The minimal fluorescence yield, called **Fo**, is observed when all PS II reaction centers are open, which is the case after dark-adaptation. The maximal fluorescence yield, called **Fm**, is observed when all PS II centers are closed. Full closure of reaction centers and consequent Fm-determination is achieved by a **Saturation Pulse** that is applied by pressing the **Fm-key** (or M-key using external keyboard). Actually, with this command both Fo and Fm are determined quickly one after the other.

At the same time also the value of **Fv/Fm** is calculated and entered into the **Fv:m field**. This parameter corresponds to the ratio (Fm-Fo):Fm, which gives information on the photochemical quantum yield of open PS II reaction centers. With a healthy and dark-adapted leaf, Fm is about five times higher than Fo, and Fv:m amounts to approx. 0.8.

Instead of pressing **Fm**, you can also trigger a Saturation Pulse via the **Yield-key** (or S-key using external keyboard). Then with each measurement a new value is entered into the **Yield parameter field** (first column). As long as Actinic Light is off, these values will be very close to the Fv:m value. However, as soon as actinic illumination is started (Act-key), you will see consecutively measured Yield values first decrease and then rise again, stabilizing in the steady state at a constant value that is characteristic for the photosynthetic performance of the given leaf sample. If you get tired of pressing the Yield-key you can press the **Pulse-key** (or P-key using external keyboard) to trigger a sequence of Saturation Pulses that will be applied at defined 20 s intervals (**Clk-parameter** in column 4) until the Pulse-key is pressed again to stop it. While the Pulse Sequence is still active, you notice that after each pulse not only the displayed Yield parameter is up-dated, but also the values in a number of other parameter fields are changing, namely **No, qP, qN, ETR** and **Fm'**. **No** simply denotes the current number of a pulse in the sequence. **qP** and **qN** are the coefficients of photochemical and nonphotochemical fluorescence quenching. **ETR** is a relative measure of apparent photosynthetic electron transport rate. And **Fm'** corresponds to the maximal fluorescence yield measured during a Saturation Pulse, which normally is lowered with respect to Fm measured after dark-adaptation.

The fluorescence data which you have measured using the Fm-, Yield- and Pulse-keys were automatically stored in a so-called

Report-file that can be opened with the help of the **Edit-key** (Ctrl E with external keyboard). In order to return from the Report-file to the Parameter screen, the **Esc-key** has to be pressed.

Sometimes it is most informative to see the recorded data plotted versus time (Kinetic Recording). For this purpose, the normal **Parameter Screen** is exchanged against the **Kinetic Screen** by pressing the **Rec-key** (or K-key using external keyboard). Once the Kinetic Screen is installed, with the same **Rec-key** a Kinetic Recording can be started (C-key using external keyboard). A recording can be stopped at any time via the **Esc-key**. There are a large number of functions that apply to Kinetic Recordings, which are particularly useful for the experienced researcher and will be explained in some detail below.

In the meantime, you not only got started with the PAM-2100 Fluorometer, but also learnt how to perform the basic and most important types of measurements. In the section 6.1 (Using the Parameter Screen) the various operations are described systematically in more detail and also those parameter fields are explained which so far were not yet mentioned. If you are interested in learning more about Kinetic Recordings, reading sections 6.2 and 6.3 is recommended.

5 Different Modes of Data Acquisition

The PAM-2100 offers **two different modes of data acquisition**. In the **stand-alone mode** the system makes use of the built-in PC with the DA-2100 program running under MS-DOS, just like in the case of the original DA-2000. As in this mode no external PC and other external hardware components like display and keyboard are required, it is particularly well suited for **field applications**.

Alternatively, the PAM-2100 can also be operated with **an external Windows-PC** using the **PamWin program.** While the **MS-DOS operating system** has the advantage of supporting **real-time measurements**, in contrast to **Windows**, the latter offers a **more comfortable user surface** for system operation and data analysis. In practice, a combination of both modes of operation is optimal: For the actual measurements, unless carried out in the laboratory, stand-alone operation provides most flexibility. While it may take some time to memorize all possible commands that can be given with the help of the 20-key board (see 4.3), under field conditions this is more practicable than using an external PC. The most important commands, like giving a Saturation Pulse, starting a Kinetic Recording or storing the data just require pressing single keys. After returning to the laboratory, the user can transfer the stored data from the internal PC to an external Windows-PC using the **Trans2100** program (see 5.3) and view the data with the help of the **PamWin program** or export the data to **spread-sheet programs**, like Excel, for further analysis.

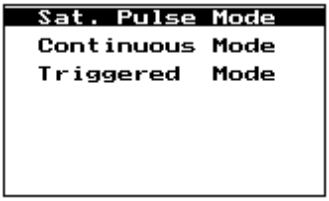
5.1 Data Acquisition in the Stand-alone Mode

The **POWER ON** switch at the front panel of the PAM-2100 applies for stand-alone operation only, i.e. when the instrument is not

connected to an external PC. When the POWER ON switch is briefly pressed, the internal PC is booted (which takes about 40 sec) and then the **DA-2100 program** is started. After start of the program automatically the so-called **Saturation Pulse Mode** is installed. This mode of operation is by far the most common with the PAM-2100 Fluorometer, particularly in the case of field measurements, for which this instrument primarily was designed. In addition, under MS-DOS there are two other modes of operation, namely the **Continuous Mode** and the **Triggered Mode**, which are most useful for kinetic recordings. There is a short **Mode Selection Menu** which can be called up as follows:

1) Open the **Command Menu** using the **Com-key**
2) Select the second line **Mode Selection** using the **∨-key**
3) Call up the **Mode Selection Menu** via Return (↵ key)

Now the user can select one of the three **Modes of Data Acquisition** using the arrow- and Return-keys:



The choice of a particular Mode of Data Acquisition depends on the envisaged application:

- **Saturation Pulse Mode**

For all applications that involve quenching analysis by the **Saturation Pulse Method**, i. e. on-line calculation of the quenching coefficients qP and qN (or NPQ), of Fv/Fm, ΔF/Fm' (Yield) and apparent electron transport rate (ETR). In principle, Saturation Pulses can also be applied in the two other modes, but then no quenching analysis is performed.

- **Continuous Mode**

This mode of operation is analogous to standard registration with a chart recorder or a digital storage oscilloscope. It is best suited for measurements of **Slow Induction Kinetics**. When Saturation Pulses are applied, all Ft data points during Fm- (or Fm') determination are recorded, contrary to the Saturation Pulse Mode, where for technical reasons a certain "fade out time" is required.

- **Triggered Mode**

For recording of **Rapid Induction Kinetics**, with a maximal time resolution of 150 µs/point, whereas maximal resolution in the two other modes is 10 ms/point. After start of a recording, the onset of actinic illumination (LED source) is automatically triggered. Data acquisition in the Triggered Mode fundamentally differs from that in the two other modes in that it is not on-line. Rather the **data are transiently stored in RAM-memory** before they are transferred to the panel-PC after the recording. In this way higher time resolution and sampling rates are possible.

For stand-alone operation of the PAM-2100 without external keyboard some knowledge of the numerous key functions is required. Operation is most simple in the Saturation Pulse mode that is most frequently used for field measurements. While in principle also all operations in the Continuous and Triggered modes can be carried out with the 20 keys on top of the instrument, for extended kinetic measurements in the stand-alone mode an **external keyboard** (like the Ultra-Compact Keyboard 2170-K) is recommended. Since November 2003 (date of issue of this manual) this keyboard is delivered as a standard component together with the PAM-2100.

In the following sub-sections lists of the **20 single-key functions** and of the **double-key functions** are presented.

5.1.1 List of Single-key Operations Using 20-key Board

Esc	To **quit** a menu or the Report File; and to **stop** a kinetic recording.
Edit	To open the **Report File.**
Pulse	To start and stop a sequence of **Saturation Pulses** controlled by the **Clock**.
Fm	To apply Saturation Pulse after dark-adaptation for assessment of **Fo and Fm** as well as **Fv/Fm** (Fv:m)
Menu	To open the **Main Menu** on the Kinetic Screen.
Shift	This key is effective only in combination with a number of other keys in **double-key operations**.
+	To **increase** the setting of a parameter which is selected by the cursor.
-	To **decrease** the setting of a parameter which is selected by the cursor.
Store	To call up the **Write-function** in the Main Menu (under Data) in order to store a Kinetic Recording.
Com	To open the **Command-menu:**

At the Report-file level the Com-key serves for opening a character selection menu.

During a dialog the Com-key serves for opening a selection menu.

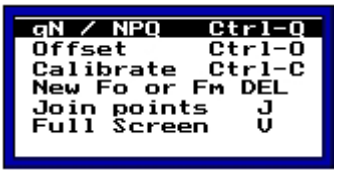
^	To move the **cursor up** and to move up in the **alphabet** or **number list** during a dialog (e.g. writing filename). To move one line up in the Report file.
v	To move the **cursor down** and to move down in the **alphabet** or **number list** during a dialog (e.g. writing filename). To move one line down in the Report file.
<	To move the **cursor to the left**. On the Kinetic Screen to move curve limit to the left.
>	To move the **cursor to the right**. On the Kinetic Screen to move curve limit to the right.
↵	Equivalent to **Enter** or **Return**. To carry out a function selected by the cursor and to switch between alternative functions (e.g. 600 Hz and 20 Khz, different types of Actinic Lamps)
Rec	To start a **Kinetic Recording** when the Kinetic Screen is active. To move over to the **Kinetic Screen** when the Parameter Screen is active,
Run	To start a **Run**.

F-Red	To switch on **Far Red Light** at the given settings of intensity and duration of illumination.
Act	To switch on the selected **Actinic Light** source (LED, Int. Halogen or Ext. Halogen) at the given settings of intensity and duration of illumination.
Yield	To apply a **Saturation Pulse** for assessment of the effective PS II quantum yield of a sample in the illuminated state, **ΔF/Fm'**.

5.1.2 List of Double-key Commands Using 20-key Board

Using the 20-key board 12 double-keys can be used, which **all involve the Shift-key**.

Shift + Fm	To activate and deactivate a routine for Fo' determination with every Saturation Pulse (equivalent to the **Ctrl S** command using an external keyboard).
Shift + +	To **darken** the LCD-display
Shift + –	To **lighten** the LCD-display
Shift + Store	For a single measurement of Fo' in conjunction with the next Saturation Pulse (equivalent to the **Ctrl Z** command using an external keyboard).
Shift + Com	To open the **Shift-Command menu** with the following functions (external keyboard commands):



Shift + ^	In the **Report file** to move one **page up**. On the **Kinetic Screen** to increase the step size for cursor movement and definition of curve limits.
Shift + v	In the **Report file** to move one **page down**. On the **Kinetic Screen** to decrease the step size for cursor movement and definition of curve limits.
Shift + <	To move the cursor to the **Light Meas** parameter field (Parameter Screen) or to the **Z (Fo)** field (Kinetic Screen).
Shift + >	To move the cursor to the **Kinetic Scr** parameter field (Parameter Screen) or to the **Screen (N)** field (Kinetic Screen).
Shift + Run	To **stop a Run** (equivalent to B-key with external keyboard). When the Cursor is on Run-field: to open the **Write/Read** selection menu for **User Runs**.
Shift + ↵	To open the **Shift-Return menu** with the following functions (and corresponding commands using external keyboard):

During a dialog to **erase letters** going backwards in a text line.

Shift + Yield	To activate or deactivate **averaging** of Yield, PAR and Tmp values (equivalent to the **Ctrl Y** command using an external keyboard).

Please note that when an **external keyboard** is connected and **Scroll Lock** is active, the function of part of the double-key commands of the PAM-2100 is changed in order to allow use of the **Function-keys** F1-F10 in the **Triggered** and **Continuous modes**.

5.2 Operation under PamWin

The **PamWin** software is provided for running the PAM-2100 in conjunction with a **Windows-PC**. Before starting the PamWin program, the **PAM-2100** has to be **switched off**, in order to **shut down the internal PC**. When Pamwin.exe is started, the instrument automatically is turned on (green status LED blinking).

A **separate manual** is provided for measurements with the PAM-2100 using the PamWin program. This program can be also used in the **View-mode**, i.e. for analysing previously recorded data without the instrument being connected to the PC. Data recorded in the stand-alone mode can be transferred from the internal PC with the help of the **Trans2100 program** (see below) and then can be viewed and analysed under PamWin.

As operation via PamWin requires a Windows-PC, this mode of operation is particularly suited for laboratory work, when weight of the measuring system does not play a major role. Operation under PamWin is more simple and, hence, can be recommended to beginners, who want to become acquainted with the basics of the chlorophyll fluorescence approach and the possibilities of the instrument.

5.3 Data Transfer Using the Trans2100 Program

The Trans2100 program serves for **transfer** of data from the internal PC of the PAM-2100 to an **external Windows-PC**. Upon installation of the PamWin-software the Trans2100.exe file is copied into the main **PamWin directory**. Transferred data can be viewed using the **PamWin** user surface in the **View-mode**. Furthermore, under PamWin the data can be **printed out** or **exported** to spreadsheet programs, like Excel.

Before data can be transferred with the help of Trans2100, the **Fileserver** within the PAM-2100 has to be activated. For this purpose, the **Main Menu on the Kinetic Screen** has to be called up (Menu-key or F10 on external keyboard) and under **Data** the function **Transfer Files** has to be selected. This function is started via Return (↵). When the message **"Fileserver active."** is shown on the PAM-2100 display, **Trans2100.exe** in the PamWin-directory of the Windows-PC can be started.

First a window is opened for selection of the **Com-port** to which the RS 232 cable is connected. After selection of the active Com-port, another window is opened showing the **data files** presently stored within the PAM-2100. A file has to be doubleclicked in order to be transferred.

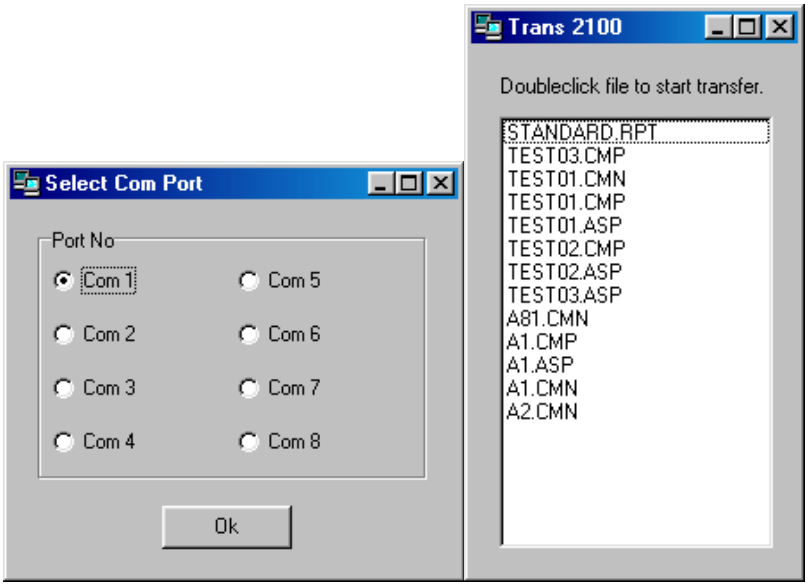


Fig. 9 User surface for data transfer from PAM-2100 to external PC

The program asks to which directory a selected file shall be transferred. The Data-directory of the PamWin folder is proposed, which is o.k., if the file is going to be viewed under PamWin. Please note that after transfer of a file to an external PC, the file is not stored in the internal PC anymore.

After closing the Trans2100 program the communication with the PAM-2100 is interrupted and on the PAM-2100 display the Mode Selection Menu is shown.

5.4 Update of DA-2100 program

The DA-2100 program is directly derived from the original DA-2000 program that was developed for the PAM-2000. It was adapted to the special requirements of the new PAM-2100, in particular with respect to its operation via the 20-key board in the stand-alone mode. The DA-2100 is resident on the "hard disk" of the internal panel-PC

(flash card). It is likely that in the future new program versions will be issued. The program can be updated via the **RS232** interface cable without opening the instrument using the special Windows software **Update.exe**

The Update-software with a new program version can be loaded down from the Walz homepage (www.walz.com/Support/ Downloads), preferentially into the PamWin folder of the Windows-PC. The Update-software contains the Update.exe and a new **DA-2000.ovr**, with which the old DA-2000.ovr is overwritten. For carrying out the update, please proceed as follows:

- Switch on the PAM-2100
- Connect the PAM-2100 to a Windows-PC with the RS-232 cable
- Open the Data-submenu in the Main Menu
- Select the function Update DA-2100
- Start Update.exe (normally in PamWin main directory)

The user is guided through the Update procedure by dialogs on the PAM-2100 display and the Windows-PC monitor. The Update is finalized by pressing the Return-key of the PAM-2100. For the new program version to become effective, the PAM-2100 has to be switched off (via the point Quit program in the Com-menu or the Alt-X-command using an external keyboard) and then started again.

Please note that in some instances it may be necessary to carry out the Update procedure twice with the same Update-software. This applies, when several files have to be updated, one of which requires the presence of another updated file. If this is the case, a corresponding note will be attached to the Update-software available from the Walz-homepage.

6 Measurements in the Saturation Pulse Mode

The Saturation Pulse Mode is the most common Mode of Data Acquisition with the PAM-2100 Fluorometer. It is automatically installed upon start of the program. The recorded data can be displayed in numerical form using the so-called **Parameter Screen** or in graphical form using the **Kinetic Screen**. In addition, all Saturation Pulse data are stored in a so-called **Report-file** that is accessible via the **Edit-key**. For becoming acquainted with the numerous functions of the PAM-2100 it is recommended to make use of an external keyboard (e.g. Ultra-Compact keyboard 2170-K). While all commands in principle can be also given using the 20 keys integrated into the top cover of the PAM-2100, in many cases the use of an external keyboard is more comfortable. This is particularly true for work under laboratory conditions.

6.1 Using the Parameter Screen

When the instrument is switched on, the internal PC is booted (after about 40 sec) and the DA-2100 program is started. Initially the LCD-display (or external monitor) shows by default the **Parameter Screen** in the Saturation Pulse Mode of data acquisition (see Fig. 7). Five columns of **"parameter fields"** are displayed, with each featuring seven lines. Different types of parameters are involved: The top fields of columns 2-5 refer to the status of four different light sources. The first letters (L, A, S and F) are white on black when the light sources are off. The initial character keys may be visualized as on/off switches. When an **external keyboard** is available, pressing **L, A, S** or **F** will activitate the corresponding lamp. At the same time the letters in the given parameter field are inverted, thus indicating the status of the light source. When the same key is pressed again, in the case of L and A the given lamp is turned off again. In the case of

S and F the lighting is only transient, 0.8 s with the Saturation Pulse lamp and 3 s with the Far-Red lamp (at default settings).

Use of the **20-key board** on top of the instrument is equivalent to the use of the external keyboard, i.e. clicking e.g. **Act** has the same effect as pressing **A**. But, due to the limited number of keys, only the most important **parameter field commands** can be directly given by **single key operation**. The other fields have to be selected by **cursor movement** with the help of the **arrow-keys**. After a parameter field has been selected, the command is carried out via Return (**↵ key**).

On the Parameter Screen the **second line** of columns 2-5 relates to the **intensities** of the 4 different types of light. These parameter fields function like **dial switches**. The pre-set values are suited for standard experiments. To change settings, first the corresponding parameter field is selected by typing the characteristic number (1-4) and then the **+ or - keys** are used to **increase or decrease** the settings, respectively. Selection of a parameter field is indicated by a "broken box" (cursor). Using the 20-key board on top of the instrument, field selection is achieved by cursor-movement using the arrow-keys. Further parameters with dial switch function for instrument settings will be detailed in the corresponding sub-sections below.

The other major type of displayed parameters relates to the **measured fluorescence data** and the **on-line calculated values** of photochemical yield and apparent electron transport rate. All **measured parameters** are organized in **column 1** and in the **two bottom lines** of the parameter screen. A special role is played by **Z (Fo)**, **M (Fm)** and **Y (Yield)**. Using these keys (external keyboard or 20-key board, in brackets) the most relevant determinations of

fluorescence and quenching parameters are performed (see corresponding sub-sections).

When an external keyboard is connected, additional commands and special functions can be activated by **Ctrl** and **Alt key combinations**. They are listed in the bottom information line when Ctrl or Alt is pressed. The **Ctrl-E** command is particularly important as it opens the **Report-file** in which the relevant data are stored and which can be edited by the user (see 6.1.17). Using the 20-key board, the **Edit-key** has the same function as the Ctrl E command. With the help of the double-key operation **Shift + Com** a little menu can be called on display, which allows to select further **Ctrl-commands** as well as a number of other commands, which were defined for use of an external keyboard with the original PAM-2000 and now are also valid with the PAM-2100:

qN / NPQ	Ctrl-Q
Offset	Ctrl-O
Calibrate	Ctrl-C
New Fo or Fm	Del
Join points	J
Full Screen	U

The meaning of these commands will be explained below, where the various functions are described to which they relate.

The Shift+Com menu, like all menus, can be quit with the help of the **Esc-key**.

In the following sub-sections the various functions linked to the different parameter fields are outlined in detail. Each of these sub-sections may be read separately in order to become introduced to the special features and suggested applications. Numerous cross-references are made to point out the functional links to the other

parameters. For a quick overview of the meaning and function of all parameter fields the reader is referred to the list in the Appendix.

6.1.1 Fo, Fm and Fv:m

Fo and Fm are defined as minimal and maximal fluorescence yields of a **dark-adapted sample**, with all PS II reaction centers fully open or closed, respectively. The **Fv:m-parameter** corresponds to the well-known parameter **Fv/Fm** that is a measure of the **maximal quantum yield of Photosystem II** (i.e. under optimal conditions after dark adaptation). It is calculated from the given Fo- and Fm-values using the equation:

Fv:Fm = (Fm-Fo):Fm

With fully active, dark-adapted samples Fv:m may reach values around 0.86 corresponding to a Fm/Fo ratio of about 7. "Dark adaptation" does not necessarily involve prolonged strict darkness. As far as Fo is concerned, the ambient background light should be sufficiently low not to cause accumulation of reduced PS II acceptors, accompanied by a fluorescence increase. This can be readily checked after covering the sample with a dark cloth. At 600 Hz modulation frequency, even at the highest setting the measuring light will induce only a minor fluorescence increase. As far as Fm is concerned, definition of dark-adaptation is less straight-forward. There are several mechanisms of light-induced Fm-quenching, the dark relaxation of which displays several phases with vastly different rates. Actually, part of this relaxation is enhanced by moderate light (e. g. room light at about 20-40 µmol quanta $m^{-2}s^{-1}$). In field experiments, Fo and Fm can be measured most reliably in the early morning, before direct sun light hits the leaves.

Fo can be determined separately via the point **Fo-determination** in the **Shift+Return menu** or via the **Z-command** using an external

keyboard. If the measuring light (L) is not yet turned on, this will be done automatically before Fo-sampling. Normally Fo is determined **in conjunction with Fm** using the **Fm-key** (20-key board) or via the **M-command** (using an external keyboard). In this way, these basic fluorescence parameters are sampled under identical conditions, and the on-line calculated **Fv:m** is intrinsically normalized. It will not be influenced by sensitivity factors, as the distance between sample and fiberoptics, chlorophyll content, sample size etc.

It is recommended to **adjust Fo** routinely to a value between 200 and 400 by appropriate choice of **Measuring Light intensity, Gain** or **fiberoptics-to-sample distance**. In this way, optimal resolution is provided without any risk of amplifier saturation. With Saturation Pulse quenching analysis, there are warnings when the signal level is too low (***Attention, low accuracy due to low signal level***) or/and when the Saturation Pulse induced fluorescence change is very small (***Attention, low accuracy due to small Fv***). The first type of warning comes for Fm- or Fm'-values lower than 33 x Gain-setting, whereas the second type of warning is given whenever the Saturation Pulse induced Fv (in mV) is smaller than the Gain-setting. These warnings take account of the fact that any electronic noise will be increased by Gain to the same extent as the signal. At standard conditions (G3, D5) the noise amounts to approx. 1 mV at 20 kHz modulation frequency. It should be mentioned that such low noise levels are obtained by on-line averaging of data points. The fluctuations of the Ft-values in the corresponding parameter field are considerably higher, as these values involve less averaging.

The intensity and duration of the Saturation Pulse triggered via M (or Fm) is pre-set at settings 10 and 0.8 s, respectively. These standard settings have proven suitable for most applications. However, to avoid artifacts and to optimize the measurements, it is recommended to check the detailed kinetics of the Saturation Pulse

induced fluorescence change, which is accessible via the **Com-menu (Pulse Kinetics)** or via the **Alt M command**, if an external keyboard is connected (see 6.1.18).

The values of Fo, Fm and Fv/Fm are written automatically into the **Report-file** (see 6.1.17) which can be opened via **Edit** or by the **Ctrl E-command** (external keyboard). When only Fo is determined via Z (external keyboard), the measured value is entered in the Ft-column.

6.1.2 Fo', Shift+Store (Ctrl Z) and Shift+Fm (Ctrl S)

The parameter **Fo'** corresponds to the minimal fluorescence yield of a pre-illuminated sample, with all PS II centers fully open. Special routines are available for Fo'-determination in conjunction with saturation pulse quenching analysis:

- **Shift + Store (Ctrl Z)**

With the Shift+Store command (or Ctrl Z using external keyboard) a **single Fo'-determination** is carried out. The determined value of Fo' applies for quenching analysis with the **next Saturation Pulse** only. Upon Shift+Store (or CtrlZ) the following operations are carried out:

- Actinic Light is turned off;
- simultaneously with Actinic Light-off, Far-red light is turned on for 5.5 sec;
- 0.5 sec after onset of Far-red illumination, the data points in five consecutive 1 sec periods of Far-red illumination are averaged; the lowest of the 5 values is entered as Fo' in the corresponding parameter field and into the Report-file;
- simultaneously with termination of Far-red illumination Actinic Light is turned on again;

- when the next Saturation Pulse is given, quenching analysis will be based on Fo' and not on the original Fo.

- **Shift + Fm (Ctrl S)**

The Shift+Fm key (or CtrlS-command using external keyboard) operates like an on/off switch for a routine to determine Fo' with every application of a Saturation Pulse. When this **Fo'-mode** is active, an **asterix** appears in the **Far Red parameter field** and the following sequence of operations is started with every triggering of a Saturation Pulse:

- first the Saturation Pulse is given;
- 3 sec following termination of the Saturation Pulse the Actinic Light is turned off and Far-red light is turned on;
- 0.5 s after turning on the Far-red light, the first of 5 consecutive 1 sec-periods for data point averaging starts; the lowest of the 5 values is entered as Fo' in the corresponding parameter field and into the Report file.
- 5.5 sec after the Far-red light was turned on, it is turned off again and simultaneously the actinic light is turned on again.

When the Fo'-mode is active, the shortest interval between Saturation Pulses (Clock-parameter), is 20 s. In the Fo'-mode the quenching coefficients qP and qN are calculated on the basis of Fo' and not on the basis of the original Fo. Due to Fo-quenching during actinic illumination, Fo'-values often are considerably lower than the original Fo-value and consequently qP and qN values are decreased. On the other hand, determinations of Yield and ETR are not affected by Fo'-measurements. Using the Fm-key (or M-command) there is normal Fo- and Fm-determination, also when the Fo'-mode is active.

Fo'-determination is recommended for quenching analysis of samples that have reached a steady-state in the light. Steady state fluorescence yield, as indicated by Ft often is close to the original Fo or even below that. And when the Actinic Light is switched off,

fluorescence yield drops even lower to the Fo'-level. This so-called Fo-quenching may have different mechanistic causes. An important mechanism of Fo-quenching is related to the presence of the xanthophyll zeaxanthin, which may serve as a quencher of excess excitation energy in the antenna system. Hence, Fo'-determinations are of practical importance to assess the regulation of energy dissipation in photosynthesis. Such measurements are of considerable ecophysiological relevance.

The existence of Fo-quenching has consequences on qP- and qN-determination. In the original expressions for calculation of these quenching coefficients the Fo-level is assumed to be constant. In practice, this assumption may be considered almost correct as long as qN does not exceed a value of approx. 0.4. At higher values it becomes essential to use Fo' instead of the original Fo, i. e. to make use of the Shift+Store (Ctrl Z) or Shift+Fm (Ctrl S)-functions.

Under field conditions, normally actinic illumination is provided by the ambient day light that cannot be simply turned off as envisaged with the Shift+Store (Ctrl Z) or Shift+Fm(Ctrl S)-functions. Then, shortly before the 5 s time period during which Far-red is applied and Fo' is determined, the sample should be transiently covered with a dark cloth, without obstructing the light path between leaf and fiberoptics. Darkening does not need to be perfect, as with light-activated samples the far-red light will be efficient to counteract the accumulation of reduced acceptors.

In many applications, the user may prefer not to bother about Fo-quenching and Fo'-determination. With Yield and NPQ two parameters are provided the determination of which does not require knowledge of Fo'. On the other hand, for proper assessment of the proportion of open PS II centers via qP-determination, determination of Fo' may be indispensable.

6.1.3 Fm'

The parameter Fm' is defined as the **maximal fluorescence yield** reached in a pulse of saturating light when the **sample is preilluminated**. In green plants, Fm' generally is lower than Fm that is determined after dark adaptation. Per definition, with Fm' as well as with Fm the yield of photochemical energy conversion at PS II centers is zero. The quenching of Fm' with respect to Fm is defined as non-photochemical quenching, for which the quenching coefficient qN was introduced (see 6.1.7). The extent of non-photochemical quenching can also be expressed by the NPQ-parameter (see 6.1.7). After every Saturation Pulse the current value of Fm' is written into the Report-file along with the on-line calculated parameters.

6.1.4 Ft

The parameter Ft represents the measured **fluorescence yield at any given time**. It is determined by the redox and energy status of the sample. For so-called quenching analysis, given Ft-values can be defined as Fo and Fm or Fo' and Fm' by special commands (e.g. Z, M, S, Y, Ctrl Z, Ctrl S using external keyboard). With every Saturation Pulse the current Ft, measured briefly before the pulse, is written into the Report-file along with the on-line calculated parameters. Ft can vary **between 0 and 2557**, with the maximal value corresponding to the saturated amplifier output in millivolts. When Ft exceeds the value of 2450, there is a warning "overload". Ft-resolution to some extent is limited by digital noise, the amplitude of which is independent of Gain-setting and signal amplitude. Hence, signal disturbance by this type of noise will decrease with increasing fluorescence signal. On the other hand, reaching 2500 mV should be avoided. In practice, it is recommended to adjust Fo to a value between 200 and 400 mV. In recordings involving the Kinetics

Screen, with the pre-set Y-axis limit at 2 V, the Y-axis is divided into 10xFo to 5xFo and normal induction curves are unlikely to exceed the Y-limit.

6.1.5 Yield and Ctrl Y (Averaging)

The Yield-parameter may be considered the most important piece of information obtained with the PAM-2100 Fluorometer. It represents the **essence of fluorescence quenching analysis** by the **Saturation Pulse method**. Its information value is particularly high when combined with that of effective light intensity (PAR) and leaf temperature. Yield-determinations are most commonly made under steady-state illumination, as encountered under field conditions. Then the **effective quantum yield of photosystem II** is close to the overall quantum yield of photosynthesis. The Yield-parameter is calculated according to the equation:

Y = (Fm'-Ft): Fm' = ΔF : Fm'

For Yield-determination knowledge of **Fo is not required**, which is a great practical advantage. In practice, Yield-determination is rather simple. After switching on the PAM-2100 Fluorometer just the **Yield-key** has to be pressed. The system then automatically turns on the Measuring Light, measures Ft and immediately afterwards applies a Saturation Pulse to assess Fm'. If the **Leaf-Clip Holder 2030-B** is available, Yield-determination can also be started by **Remote Control** (red push button) after activating **Run-file 1** (see 6.1.13) or after positioning the **cursor** on the **S parameter field**. Furthermore, with the integrated micro-quantum-sensor the effective light-intensity (PAR) is measured and used for the on-line calculation of apparent electron transport rate (ETR) (see 6.1.6).

With every Saturation Pulse, the measured and the on-line calculated data are written into the **Report-file** that is accessible via

the **Edit-key** (or Ctrl E-command). In this file head-lines with date, time etc. introduce every new set of data and are installed with the first application of a Saturation Pulse after initialization of the **Saturation Pulse Mode** and with every Fm-command.

The Yield-values are displayed with an **accuracy of 0.001 units**. The actual data fluctuation depends on a number of factors:

- it increases with the distance between leaf and fiberoptics;
- it increases with the extent of sample heterogeneity.
- it decreases with the size of ΔF = Fm'-Ft;
- it decreases with the signal amplitude.

It is possible to make full use of the possible accuracy of 0.001 units when a number of Y-values are averaged. **Averaging** is initiated by **Shift+Yield (**or **Ctrl Y-command** using external keyboard). This command functions like an **on/off switch**. After initialization of the Saturation Pulse Mode the averaging function is off; via Shift+Yield (Ctrl Y) it is switched on and stays on until Shift+Yield (Ctrl Y) is pressed again. On the Parameter-Screen, installation of the averaging mode is indicated by the appearance of the ^ **-symbol** in the parameter fields of Yield and ETR.

Besides **Yield** and **ETR** also **PAR** and **Tmp** are **averaged**. However for these two parameters the averaged values are only displayed in the **Report-file**, so that with each measurement the user can assess the present temperature and light intensity on the parameter screen. The current number of averages is shown in the **No-field**. After averaging is started via Shift+Yield (Ctrl Y), the No is reset to 1 upon application of the following Saturation Pulse. When averaging is stopped via Shift+Yield (Ctrl Y), in the Report-file a line with the averaged values is written.

Application of Pulse Sequence in Averaging Mode; spinach leaf in steady state										
averaging			Tmp.	PAR		ETR	Yield			
11:28:27	1	172	23.0	443	0.535	110.1	0.529	0.877	0.507	1.310
11:28:57	2	171	23.0	443	0.530	111.0	0.597	0.883	0.505	1.314
11:29:27	3	171	23.0	443	0.529	111.4	0.599	0.885	0.503	1.318
11:29:57	4	171	22.9	442	0.529	111.4	0.600	0.885	0.500	1.323
11:30:27	5	170	22.9	442	0.529	111.7	0.602	0.886	0.498	1.328
averaged			22.9	442		111.0	0.598			

When Yield-averaging is applied using dark-adapted samples, the obtained results are equivalent to Fv/Fm-averages. With dark-adapted samples Fo=Ft and Fm=Fm' and, hence, the expressions for Yield and Fv:m become identical.

Measurements of Yield and Fv:m profit from the fact that both parameters represent ratios of fluorescence yields, with the consequence that the data are independent of measuring sensitivity. This is of great advantage in field measurements on objects with variable chlorophyll content and morphology, as e. g. lichens, algae and mosses. Reliable results can be obtained with largely varying sample size and at variable distance, as long as there are no changes in the period between the Ft (or Fo) and Fm' (or Fm) determination. Therefore, the fiberoptics should be held steady facing the given object for about 2 s during the actual measurement. This is facilitated by using the **Distance-Clip**, which is mounted to the fiberoptics end-piece (see Fig. 2), or by using the **Leaf-Clip Holder 2030-B**, which is equipped with a **Remote Control** button (see 3.3.3).

6.1.6 ETR, PAR and Alt E

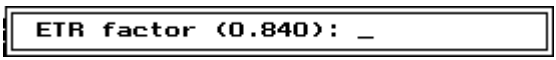
Measurements of the ETR- and PAR-parameters require the **Leaf-Clip Holder 2030-B** (see 3.3) or the Micro Quantum/Temp.-Sensor 2060-M (see 3.5) to be connected to the PAM-2100 Fluorometer. ETR represents the relative apparent photosynthetic electron transport rate in µmol electrons $m^{-2}s^{-1}$, which is calculated on the basis of the measured values of Yield and of PAR using the equation:

ETR = Yield x PAR x 0.5 x 0.84

The following assumptions are made:

- Yield represents the overall photochemical quantum yield
- PAR corresponds to the flux density of incident photosynthetically active radiation, measured in µmol quanta $m^{-2}s^{-1}$
- transport of one electron requires absorption of two quanta, as two photosystems are involved (factor 0.5)
- 84 % of the incident quanta are absorbed by the leaf (factor 0.84).

In practice, the last assumption is not always valid. Although an absorption coefficient close to 0.84 was reported for leaves of numerous species, this value obviously depends on a number of variables, such as leaf reflectance, chlorophyll content and spectral composition of the incident light. These aspects should be considered when ETR-data are evaluated. If the true absorption coefficient of the given leaf material is known, this may be entered via the point **ETR factor** in the **Shift+Return menu** (or the Alt E-command using an external keyboard). When this command is given, a dialog field appears in the center of the monitor screen:



The current coefficient is shown (in brackets), which is 0.840 upon instrument delivery. A new value can be entered, on which consequent ETR measurements will be based. A new value is most readily entered using an external keyboard. If this is not available, the value can be entered with the help of the four arrow keys. Numbers ranging between 0 and 9 as well as a point can be selected using the ∧ and ∨ keys. With the < and > keys the position is shifted to the left or the right. The value is confirmed by Return. The dialog can be quit via Esc. Any entered coefficient is stored in the

Configuration-file (see 6.1.22) when the program is quit via Alt X (or via Quit program in the Com-menu).

The ETR may be compared to the rate of CO_2-assimilation or of O_2-evolution. For such comparison the following aspects are relevant:

- 4 e^- must be transported for every CO_2 assimilated or O_2 evolved
- the value of ETR/4 is not necessarily identical to CO_2-fixation rate or O_2-evolution rate; discrepancies e. g. may arise from photorespiratory electron flow, nitrite reduction or electron cycling at PS II
- fluorescence information primarily originates from the topmost chloroplast layers, while gas exchange integrates over all layers; on the other hand, the topmost layers absorb most of the light and, hence, are also responsible for most of the gas exchange, unless photoinhibited.

The PAR can be measured at the same spot of the leaf where fluorescence is measured, when the micro-quantum-sensor is moved into the beam. This is possible without substantial loss in signal amplitude. The properties of the micro-quantum-sensor are such that its response to spectral composition and incidence angle of the impinging light approximates that of the leaf. The PAR-reading of the micro-quantum-sensor has been calibrated against a LI-COR Quantum Sensor. See section 6.1.19 for details on recalibration and section 6.1.20 for the possibility of applying a constant offset to the PAR-reading.

Measurement of ETR, just as that of Yield, occurs with every Saturation Pulse applied in the Saturation Pulse Mode. After activation of the Yield-averaging mode (Shift+Yield or Ctrl Y with external keyboard) (see 6.1.5), ETR values are averaged. The averaged values are based on averages of Yield as well as of PAR. At the same time, also Tmp-values are averaged. PAR- and Tmp-

averages are not displayed on the parameter screen, but are recorded in the Report-file which is accessible via the Edit-key (or Ctrl E using external keyboard).

The combined information of ETR, PAR and Tmp provides profound insight into the photosynthetic performance of a plant. Plots of ETR versus PAR at different temperatures respond in a very sensitive manner to changes at all levels of the photosynthetic process. The measurement of such **"light saturation curves"** is facilitated by using the pre-programmed **Run-files 8 and 9** (see 6.1.13).

6.1.7 qP, qN, NPQ and Ctrl Q

qP and qN are defined as the **coefficients of photochemical and non-photochemical fluorescence quenching**, respectively:

qP = (Fm'-Ft) : (Fm'-Fo) qN = (Fm-Fm') : (Fm-Fo)

These coefficients may vary between 0 and 1. Their on-line calculation **requires previous Fo, Fm-determination** via the **Fm-command**. qP and qN are then calculated with every Saturation Pulse. The calculated values are written into the Report-file which can be opened using the Edit-key (or Ctrl E using external keyboard).

The original definition of qP and qN implies that fluorescence quenching affects only the so-called *variable fluorescence*, Fm-Fo, and not to the minimal fluorescence yield, Fo. However, it has turned out that with qN exceeding values of approx. 0.4 there is also significant **quenching of Fo**. This has to be considered for correct calculation of qP and qN. For this purpose, the PAM-2100 offers special procedures for **Fo'-determination**, which involve transient darkening and Far-red illumination, activated via Shift+Store or Shift+Fm (Ctrl Z or Ctrl S using external keyboard) (see 6.1.12 and

6.1.2). In the **Fo'-mode**, the quenching coefficients are calculated on the basis of Fo' instead of Fo:

qP = (Fm'-Ft) : (Fm'-Fo') qN = (Fm-Fm') : (Fm-Fo')

Besides this expression for qN, also the following definition has been used:

qN = 1 - (Fm'-Fo') : (Fm-Fo) = 1 - Fv' : Fv

Although somewhat different in their theoretical derivations, numerically the two expressions provide almost identical values.

It should be pointed out that for correct qP and qN-determinations in the Fo'-mode it is essential that the far-red light is effective in reoxidizing the PS II acceptor side. This requires activation of the PS I acceptor side, which is not given with dark-adapted samples. Hence, these functions should be used only after induction of photosynthesis, i. e. normally after approx. 2 min illumination.

The NPQ-parameter represents another expression of non-photochemical quenching. It is calculated according to the equation:

NPQ = (Fm-Fm') : Fm'

Mathematically speaking, NPQ can vary between 0 and ∞. However, in practice NPQ is unlikely to exceed a value of 10. The user may switch between NPQ and qN via the point **qN/NPQ** in the **Shift+Com** menu (or the **Ctrl Q-**command using an external keyboard). The choice between NPQ and qN depends on applications. With NPQ that part of non-photochemical quenching is emphasized which reflects heat-dissipation of excitation energy in the antenna system. Hence, NPQ is a convenient indicator for "excess light energy". Notably, **NPQ-determination does not require knowledge of Fo'**. The same is true for Yield and ETR-determinations. On the other hand, NPQ is relatively insensitive to

that part of non-photochemical quenching which is associated with qN-values between 0 and 0.5. This part of qN is closely correlated with thylakoid membrane energization, an important aspect of photosynthesis regulation. The different responses of qN and NPQ are illustrated in the following figure in which qN is plotted vs. NPQ. In this presentation, it is assumed that no Fo-quenching takes place. In reality, when Fo-quenching occurs, NPQ may well exceed the value of 4.

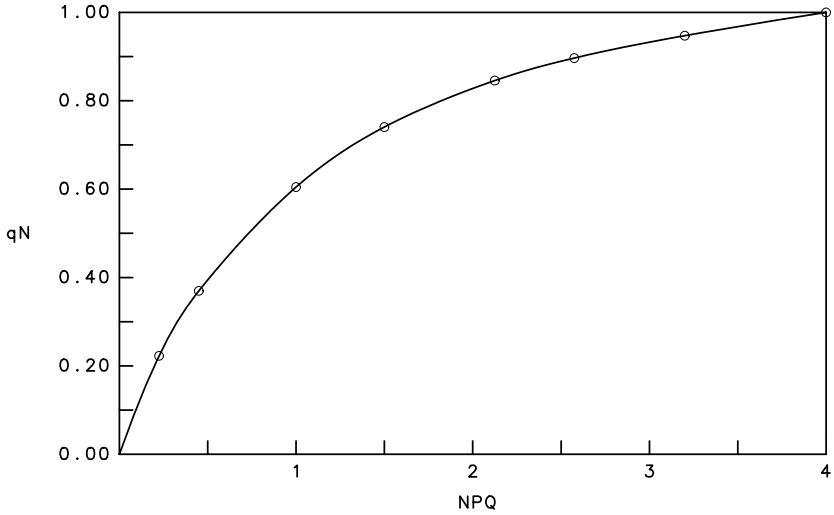
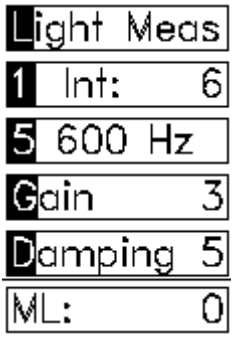


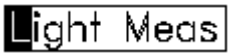
Fig. 10 Relationship between the coefficient of non-photochemical quenching qN and the NPQ-parameter.

6.1.8 Measuring light parameters

The PAM-2100 Fluorometer operates with a **pulse modulated measuring beam** and a **selective amplifier system** that amplifies only signals which originate from fluorescence excited by the measuring light pulses. After program start and initialization of the Saturation Pulse Mode the parameter fields related to measuring light properties are displayed as follows:

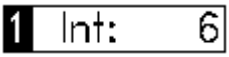


The first letter or number in these parameter fields (except for ML) is inverted and by pressing the corresponding key the pre-set status of the given parameter can be changed (provided an external keyboard is connected; otherwise the particular parameter field first has to be selected via cursor movement using the arrow keys and then the status is changed via Return or the settings changed via the +/- keys):

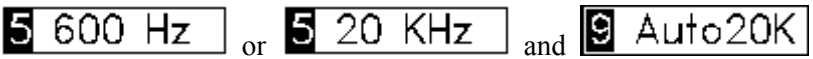


Via **L** the **Measuring Light** is switched on and off. As long as it is off, the measured fluorescence signal Ft is close to 0. If this is not the case, the displayed value corresponds to an **offset** entered via the **Ctrl O-command** (or point **Offset** in the **Shift+Com menu)** (see 6.1.20). When Measuring Light is switched on, an Ft-signal appears, the size of which (at the pre-set intensity) depends strongly on the distance between sample and fiberoptics. Measuring light is also automatically activated with a number of commands, the execution of which require Measuring Light to be on. Such are: **Z** (using external keyboard)**, Fm** (or **M** using external keyboard), **Yield (Y** using external keyboard), **Act (A** using external keyboard), **S** (using external keyboard) and **Pulse (P** using external keyboard). When Measuring Light is **switched on automatically**, a period of 0.6 s is given between onset of Measuring Light and execution of the given

command. After Standard-initialization (point Standard Settings in Com-menu or 0-command using external keyboard) the Measuring Light is off.



The **Int-field** is selected by pressing 1 (with external keyboard only). Alternatively this field may be selected by **cursor (arrow key) operation**. The pre-set value can be increased or decreased by using the **+ or - keys**, respectively. The maximal setting obtained in this way is 10, which can be used for some time without substantial loss in LED output. The **"super-setting" 11** can be installed via the point **Setting 11** in the **Com-menu** or by pressing the **! - key** using an external keyboard. There is nothing wrong with using this setting in applications requiring special sensitivity, in particular at 600 Hz modulation frequency and when measuring light periods are kept short. However, longer periods of operation at setting 11 and 20 kHz modulation frequency would cause irreversible loss in LED output. Actually, the **ML-parameter** provides a convenient monitor for such changes in LED output. The relative intensities at the different settings increase linearly from 1 to 11 (see illustration of Run-file 10 below). The absolute measuring light intensity at a given setting depends on the distance between sample and fiberoptics.



Switching from **600 Hz to 20 kHz modulation frequency** (and vice versa) is carried out via the **5-key** (using external keyboard). Alternatively the 5-field can be selected using the arrow-keys and the switch operated via the return key. At 600 Hz even the strongest measuring light will not induce much fluorescence increase. On the other hand, at 20 kHz the measuring light displays an appreciable actinic effect. Hence, 600 Hz should be selected for Fo-measurements. By switching from 600 Hz to 20 kHz the

signal/noise ratio is increased by a factor of 5.7, advantage of which can be taken whenever the sample is illuminated by light substantially stronger than the Measuring Light. This is normally the case with Yield-measurements. Whenever a **Saturation Pulse** is applied, during the pulse the measuring frequency is switched **automatically to 20 kHz**. This occurs irrespective of whether the Auto 20 K-function is activated or not.

The **Auto 20 K-function**, which can be switched off and on by the **9-key** (using external keyboard), couples measuring light frequency with the **status of the Act. Light**. When Auto 20 K is activated (pre-set status) frequency will automatically jump from 600 Hz to 20 kHz when Act. Light is switched on and return to 600 Hz when it is switched off again. This feature is particularly important for recordings of induction kinetics (see 0).

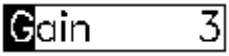
The value in the **ML-parameter field** corresponds to the light output of the Measuring Light LED source. This intensity is measured within the PAM-2100 housing. The original ML-values at the standard measuring light intensity 9 should be noted with a new instrument, such that long term loss in **Measuring Light LED output** can be assessed. It differs between individual instruments and normally amounts to values around 200. It is normal that with prolonged operation the LED output drops. This ageing process is enhanced by use of higher currents, i. e. by operation at high Int-settings (in particular setting 11) and 20 kHz. However, even if the ML-values eventually should drop to 1/2 of the original values, the signal/noise ratio still would be very satisfactory.

The ML-values display **reversible temperature dependent changes**. A temperature increase of 10 °C within the PAM-2100 housing may produce a 10-20 % decrease in Measuring Light

intensity. Actually, this aspect is quite relevant for field measurements, where temperature may vary by as much as 30 °C during the course of a day. With every Fo, Fm-determination, and generally with every Saturation Pulse associated with quenching analysis, the ML-parameter is entered automatically into the **Report-file** together with the fluorescence data. In this way, there is a continuous check of measuring LED output and, if necessary, data can be later corrected for possible variations. These considerations do not play any role for all measurements of fluorescence signal ratios (i. e. Yield, Fv:m, qP, qN, NPQ and ETR). They are, however, most **relevant for absolute Fo- and Fo'-measurements**.

6.1.9 Gain and Damping

Signal quality depends to some extent on proper settings of 'Gain' and 'Damping'. The pre-set values are optimized for standard applications in different recording modes.



The **Gain-setting** should be adjusted to the given signal amplitude, which depends on measuring light intensity, distance between sample and fiberoptics, sample size, chlorophyll content and relative extent of fluorescence quenching. Signal resolution should not be limited by digital noise which is negligibly small, if the signal amplitude is in the order of 0.5 to 2 V.

Generally speaking, the Gain-setting should be such that the Fo-level amounts to approx. 200-400 mV. Then, even with a 6-fold increase of fluorescence yield in a Saturation Pulse, there is no risk of amplifier saturation, which will occur at 2.5 V. The Gain can be adjusted in 10 steps with linear increments. For an illustration, see Run-file 10. By increasing the Gain, not only the signal but **also the noise becomes amplified**. Therefore, before increasing the Gain, the

user should consider whether an equivalent signal increase cannot be reached as well by decreasing the sample-to-fiberoptics distance without noise-amplification. Whether this is preferable or not depends on possible shading of the sample by the fiberoptics, when measurements are in ambient light. Also an increase of Measuring Light intensity may be considered, which is particularly feasible with applications in which the Measuring Light is on for short periods only.

After selection of the **Damping-parameter field** via D (using external keyboard) (or arrow keys and Return) one of 8 different time constants can be chosen with the **+ and - keys**. The logarithm of the time constants increases linearly with increasing settings. The Damping limits the maximal rate of signal changes and suppresses any signal disturbance (noise) that is faster than the set time constant.

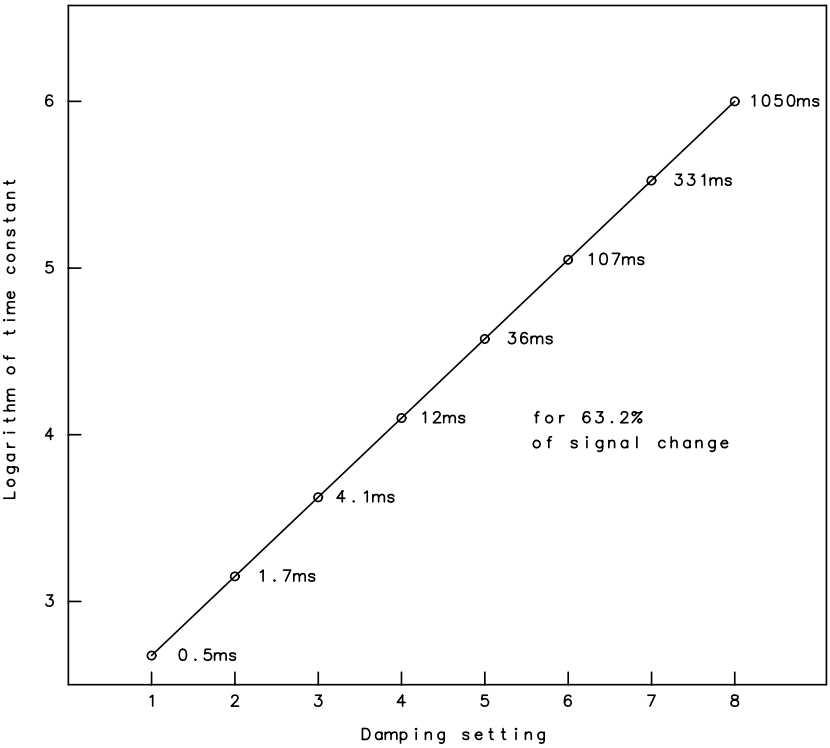


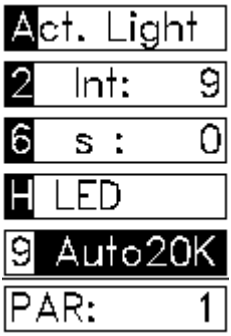
Fig. 11 Relationship between time constant of signal response and setting of Damping

In general, Damping should be selected such that its time constant is somewhat smaller than the most rapid expected fluorescence changes. In the Saturation Pulse Mode the fastest fluorescence changes are induced during the Saturation Pulse. When 0.8 s pulses are used, **Damping 5** is appropriate, which corresponds to the Standard-setting in **the Saturation Pulse Mode** and **Continuous Mode** of data acquisition. The lower settings are required for the registration of rapid induction kinetics in the **Triggered Mode** where the Standard-setting is **Damping 2**. In addition to signal damping by the electronic hardware, it is also

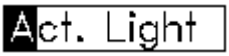
possible to smoothen stored curve traces with the help of the DA-2100 software (see below).

6.1.10 Actinic light parameters

After program start and initialization of the Saturation Pulse Mode the parameter fields related to Actinic Light properties are displayed as follows:

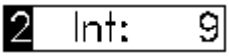


The first letter or number in these parameter fields is inverted and by pressing the corresponding key the pre-set status of the given parameter can be changed (provided the external keyboard is connected; otherwise the particular parameter field first has to be selected via cursor movement using the arrow keys and then the status is changed via Return or the settings via the +/- keys):



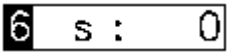
Via **A** the Actinic Light is switched on and off. Without external keyboard the **Act-key** is used for this purpose. After Standard-initialization ('0'-command using external keyboard or via point Standard Settings in the Com-menu), the **LED light source** is installed as active lamp. This lamp produces red light peaking around 655 nm. The on/off characteristics of the LEDs are very rapid (in the µs-time range) and, hence, well suited for **registration of rapid**

induction kinetics. The alternative **halogen light sources** that can be selected via the **H-command**, displays **slow on/off characteristics** in the time range of 100 ms. It is not intended to be used for triggered kinetic recordings. Whenever Act. Light is switched on also the Measuring Light will be activated. When the Auto 20 K-function is active (Standard status), the frequency of the pulse modulated measuring light automatically is **increased from 600 Hz to 20 kHz**.

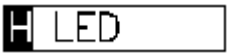


The **Int-parameter field** for Actinic Light is selected by pressing 2 (or cursor movement). The given setting can be increased or decreased by + or - operation. The maximal setting obtained in this way is 10. The **"super-setting" 11** can be installed via the point **Setting 11** in the **Com-menu** or by pressing the **! - key** using an external keyboard. It is not recommended to operate the LED lamp for longer time periods at this setting, as it will heat up too much. This would cause irreversible loss in LED lamp output. As far as lamp life time is concerned, with the halogen lamp the setting 11 is still moderate; much higher currents are applied for Saturation Pulse generation with the same lamp. However, it should be considered that this lamp consumes an excessive amount of battery power (about 10 times more than LED lamp) and that its operation is accompanied by considerable heat-development. This consideration is of no concern when the External Halogen Lamp is used (see 3.4).The relative intensities at settings 1-11 increase exponentially, with a **factor of approx. 1.5 between consecutive settings**. In this way, relative intensities cover a range from 1 to 58. The PAR produced at a given setting with the halogen source is approx. 10 times higher than with the LED source.

The effective light intensity at the sample surface depends on the distance between fiberoptics and sample. If the **Leaf-Clip Holder 2030-B** is connected, **Actinic Light intensity** at the relevant measuring site is indicated in the **PAR-parameter field**. At a standard distance of 7 mm and with the fiberoptics fixed at 60° angle, light intensities range from approx. 20 to 500 µmol quanta $m^{-2}s^{-1}$ with the LED lamp and from approx. 60 to 4500 µmol quanta $m^{-2}s^{-1}$ with the Internal Halogen lamp. If desired, these ranges can be considerably shifted up and down by appropriate movement of the fiberoptics, changing its distance to the sample. It should be noted that there is some reversible drop of actinic intensity associated with lamp heating which is particularly pronounced with the halogen lamp. At setting 10 within the first 5 min of lamp operation the output may drop by approx. 4 % with the LED lamp and 15 % with the halogen lamp. For most applications, Actinic Light intensity changes in this order of magnitude are of no concern.



This parameter field, which is selected by the 6-key (or cursor movement), refers to the **duration of actinic illumination**. When s-values above 0 are set, Actinic Light turned on by the **Act-key** (or A-key using external keyboard) is automatically turned off after the set number of seconds. With the pre-set status (denoted with 0), termination of actinic illumination requires **manual operation** via the Act-key. The s-settings can be changed by 1 s steps using the + and - keys. Also in the **Triggered Mode** (see 0) the s-settings are effective. Then, however, at setting 0, the duration is automatically adjusted to approx. 2/3 of the total recording time that depends on the sampling rate.



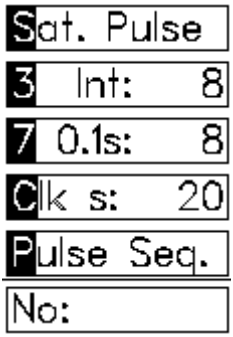
The **H-parameter field** serves for selection of one out of three different Actinic Light sources: **LED lamp**, **Internal Halogen Lamp** and optional **External Halogen Lamp** (see 3.4). Selection is possible only when Actinic Light is off. In many applications it is advantageous to use the LED lamp for the following reasons:

- much less consumption of battery power
- smaller drop of output during operation
- no internal heating of the PAM-2100
- spectral composition independent of intensity settings
- much steeper on/off characteristics
- possibility of triggered operation.

Particularly in view of the much higher power consumption and the substantial heat development, the internal halogen lamp should preferably be used for short illumination periods only (i. e. a few minutes). Actually, with prolonged operation at high settings the power supply will be automatically turned off, when the internal temperature of the PAM-2100 in the vicinity of the halogen lamp exceeds 70 °C. If long term illumination with strong white light is essential, e. g. for photoinhibitory treatment, we recommend use of the External Halogen Lamp 2150-H.

6.1.11 Saturation Pulse parameters

After program start and initialization of the Saturation Pulse Mode the parameter fields related to the Saturation Pulse properties are displayed as follows:

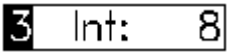


The first letter or number in these parameter fields is inverted and by pressing the corresponding key the pre-set status or setting of the given parameter can be changed (provided the external keyboard is connected; otherwise the particular parameter field first has to be selected via cursor movement using the arrow keys and then the status is changed via Return or the settings via the +/- keys):

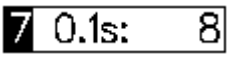
Single Saturation Pulses are triggered by operation of the **S-command** (using external keyboard). Alternatively, in the Saturation Pulse Mode single pulses can also be given via the **Yield-key** (**Y-command** using external keyboard) or the **Fm-key** (**M**-command using external keyboard). In all cases, first the Measuring Light is turned on, then pulse modulation frequency is increased **to 20 kHz**, the **Saturation Pulse** is given, **Fm'** or **Fm** are determined and eventually **Yield** or **Fv:m** are calculated. Saturation Pulses can also be triggered in the two other modes of data acquisition (see 0). However, then no on-line quenching analysis occurs. A **sequence of Saturation Pulses** can be started by the **Pulse-key** (or P-command using external keyboard).

Note: On the Parameter Screen, operation of S is fully equivalent to operation of Y, i. e. on-line quenching analysis takes place. This is not the case with kinetic recordings (see 6.2)

where quenching analysis requires Y- or P-operation (Yield- or Pulse-keys).



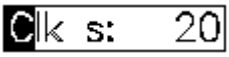
The Int-field for Saturation Pulses is selected by the 3-key (or cursor movement). The pre-set value can be changed by the + or - keys. The maximal setting is 10. The optimal intensity setting depends primarily on the distance between leaf and fiber optics endpiece and the light adaptation state of the sample. Often the Saturation Pulse intensity required for Fm- and Fv:m-determination (after dark-adaptation) is considerably less than that required for Fm'- and Yield-determination (in the steady-state). Generally speaking, the intensity and duration of the pulse should be such that fluorescence reaches a peak plateau briefly before pulse termination. It is possible to display the kinetics of the Saturation Pulse induced fluorescence increase by using the **Alt M-command** (using external keyboard) (or via the point **Pulse Kinetics** in the **Com-menu**) (see 6.1.18). By assessment of these kinetics the proper setting of pulse intensity and length can be chosen. In section 6.1.18 some typical examples are given for illustration.



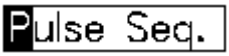
This parameter field, which is selected by the 7-key (or cursor movement), refers to the length of the Saturation Pulse. The pre-set value can be changed in 0.2 s steps using the +- and - keys. Maximal value is 1.4 s and minimal value is 0.4 s. Too long Saturation Pulses should be avoided for a number of reasons:

- to save battery power
- for the sake of longer lamp life
- to minimize strong light effects on the sample.

On the other hand the pulse has to be sufficiently long to induce maximal Fm or Fm'. This may be checked via the fluorescence kinetics which can be displayed via the Alt M-command (or via Pulse Kinetics in the Com-menu) (see 6.1.18).



The **Clock-parameter field**, which is selected by the **C-key** (or cursor movement), displays the **time interval between two consecutive Saturation Pulses** triggered by the **Pulse Sequence** function. The pre-set value can be changed using the + and - keys first in 10 s steps (between 10 and 60 s), then in 1 min steps (up to 10 min) and finally in 10 min steps (up to 120 min). The Clk-settings cannot be changed while Pulse Seq. is activated. Using **the Alt F10-command** (see 6.1.21) in conjunction with Pulse sequence (see below), it is also possible to define other functions (Act. Light, Far-red light, Runs) to be triggered at the given Clock-intervals.



Using the **P-command** (or **Pulse-key**) normally a sequence of Saturation Pulses is started or stopped. The **interval** between pulses is determined by the **Clk-parameter**. Pulse sequences are most commonly applied for recordings of so-called **Saturation Pulse Induction Curves** (see 6.2.3) and for **automatic averaging of Yield- and ETR-values** via Shift+Yield (or the Ctrl Y-command using an external keyboard) (see 6.1.5). With the **Alt F10-command** (using external keyboard) (or via the point Local Menu in the Shift+Return menu) (see 6.1.21) instead of Saturation Pulses also other functions (**Act. Light**, **Far-red light**, **Runs**) can be defined to be triggered by Pulse sequence.

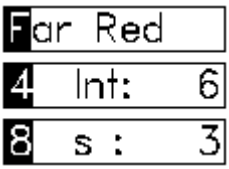
Note: A number of commands (like C for changing the clock interval and K for moving over to the Kinetic Screen)

cannot be used while a Pulse Sequence is running. The attempt to start a Run-file during a Pulse Sequence may lead to malfunctioning of the instrument. In this case the Run should be stopped by Shift+Run (or the B-command using an external keyboard) and the Pulse Sequence stopped by Pulse (or the P-command using an external keyboard).

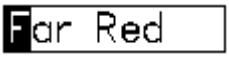
The displayed number corresponds to the number of applied Saturation Pulses following initialization of the Saturation Pulse Mode. **No** is **reset to 1 after every Fo, Fm-determination**. After initialization of **averaging** of Yield-and ETR-values via Shift+Yield (or the Ctrl Y-command using an external keyboard) No is set to 1 upon the first Saturation Pulse and thereafter corresponds to the **number of averages**.

6.1.12 Far-red light parameters

After program start the parameter fields related to the far-red light source are displayed as follows:

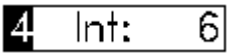


The first letter or number in these parameter fields is inverted and the pre-set status of the given parameter can be changed by pressing the corresponding key (provided the external keyboard is connected; otherwise the particular parameter field first has to be selected via cursor movement using the arrow keys and then the status is changed via Return or the settings via the +/- keys):

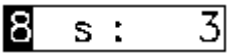


Far red illumination is turned on/off by operation of the **F-Red key** or the **F-command** (using an external keyboard). A LED with an emission peak around **735 nm** is used as Far-red source. At this wavelength there is almost selective excitation of photosystem I with the consequence of an enhanced reoxidation rate of photosystem II acceptors. The effect of this PS I light is most significant when PS II light intensity is weak (up to approx. 50 µmol quanta $m^{-2}s^{-1}$) and immediately after turning-off strong PS II light. Normally a brief period of Far-red illumination at moderate intensity is sufficient for effective acceptor pool reoxidation under condition of dark relaxation. This aspect is taken into account with the pre-set standard value of 3 s Far-red illumination at intensity setting 6.

Far-red background illumination is essential for ensuring quick acceptor oxidation with Fo' determinations in the steady state. In the **Fo'-mode** Far-red illumination is applied (see 6.1.2). When this mode is active, an asterix appears in the right hand corner of the Far Red parameter field.



The Int-field for Far-red light is selected by the 4-key using an external keyboard or by cursor movement and the pre-set value can be increased or decreased using the + or - keys. Relatively small intensities already are quite effective. The optimal setting depends on the leaf-to-fiberoptics distance and on the amount of actinic background light. The optimum is reached when the observed fluorescence yield (shown in Ft-field) becomes minimal.



Following selection of this parameter by the 8-key using an external keyboard or by cursor movement, the pre-set value of 3 s

can be changed in 1 s-steps with using the + and - keys. A defined **Far-red pre-illumination** period can be useful to pre-oxidize the PS II acceptor pool before assessment of Fo' and preceding the recording of induction kinetics. When the s-value is set to 0, the **F-Red key** operates like an on/off switch for manual definition of Far-red illumination times.

6.1.13 Run

or


The **Run-parameter field** is selected by the **Ctrl R-**command using an external keyboard or by cursor movement (Shift+> followed by ∧). A particular Run is started by pressing the **Run-**key. One out of 10 settings, which correspond to the 10 **Standard Run-files**, can be selected using the + and - keys. A Run-file represents a **pre-programmed sequence of commands** that are separated by defined time periods. In addition, for each Standard **Run-file specific pre-settings** of instrument parameters (like Gain, Damping, etc.) are defined to provide optimal conditions for the given experiment. These settings can be initialized before the start of a particular Run via the point **Run-specific Settings** in the **Com**-menu or by the **I-**command using an external keyboard. The start of a Run is triggered via the Run-key (via R-command or Return using external keyboard) or by remote control, using the Remote button on the Leaf-Clip Holder 2030-B (see 3.3.3).

The **10 Standard Runs** represent the most frequent types of measurements carried out with the PAM-2100 Fluorometer. Details on these **"standard experiments"** are given below. Running these experiments can be helpful to become acquainted with the various measuring functions and modes of data acquisition. By the purpose-tailored setting of instrument parameters even the unexperienced user

can make full use of the numerous features of the PAM-2100 measuring system.

In addition to the Standard Run-files, also **User-Runs** can be programmed by the researcher himself (see below). With the cursor position on the Run-field upon **Shift+Run** (or **Alt F10** using an external keyboard) a selection menu for **Write/Read Run-files to/from disk** is accessible. When a certain Standard Run is replaced by a User-Run, this is indicated by a **(u)** in the Run-field.

6.1.14 Kinetic Screen

The **Kinetic Scr-field** serves as a switch to quit the normal Parameter Screen and to install the Kinetic Screen. Using an external keyboard, this switch can be simply operated via the **K-command** and to return to the Parameter Screen the **N-command** is used. Using the 20-key board of the PAM-2100, the cursor is moved to the Kinetic Scr field by **Shift** + >. The Kinetics Screen can be directly installed via the **Rec**-key. Once the Kinetic Screen is installed, with the same Rec-key a Kinetics recording is started. On the Kinetic Screen the fluorescence data are displayed graphically and a special menu for Kinetics data analysis is offered (see 6.2.4). For measurements in the Saturation Pulse Mode the Kinetics Screen in comparison with the Parameter Screen plays a lesser role. With the other two modes of data acquisition the Kinetics Screen is of major importance. Details on using the Kinetics Screen are presented in sections 6.2 and 7.

6.1.15 Temperature, Tmp

The **Tmp-field** indicates the temperature at the lower surface of the leaf at the site where fluorescence is monitored. Tmp-measurement requires connection of **the Leaf-Clip Holder 2030-B**

or of the **Micro Quantum/Temp.-Sensor 2060-H** to the PAM-2100 (see 3.3 and 3.5). The Tmp as well as the PAR-data are entered with each Saturation Pulse measurement together with the on-line calculated fluorescence parameters into the **Report-file** for later data analysis. When Yield and ETR are **averaged** (after Shift+Yield or Ctrl Y using an external keyboard) also the corresponding Tmp- and PAR-values are averaged. It may be noted that photosynthetic capacity and, hence, Yield and ETR depend strongly on temperature. Air temperature and leaf temperature may differ considerably, and leaf temperature may increase by several degrees with illumination. A **constant offset** can be applied to the measured Tmp-value via the point **Offset** in the **Shift+Com menu** or using the **Ctrl O-command** using an external keyboard (see 6.1.20),.

6.1.16 Batt% and Volt

The Batt%-field indicates the momentary loading state of the **internal Li-ion battery**. The %-value refers to the remaining battery capacity. When this value approaches 0 %, there is a warning beep and the message **"Low battery!"**. In this situation the PAM-2100 Fluorometer still functions normal. However, with every application of the halogen lamp there is a substantial further drop in remaining battery capacity. Eventually the high currents required for Saturation Pulses cannot be delivered any more and all operations involving Saturation Pulses become malfunctioning.

When the **Battery Charger 2120-N** is connected, the Batt% value moves to 100 within a few minutes, irrespective of the fact that fully charging of the battery takes longer. The charging status is indicated by the **red/green Charge LED** at the front side of the PAM-2100. Charging is not yet completed while the red LED chip is lighted. Only when the green LED is lighted the battery is full and a

Batt% value of 100 will continue to be displayed after the Battery Charger is disconnected.

Batt%: 80	(int. Halogen)	or	**Volt: 12.3**	(ext. Halogen)

When an **external 12 V battery** is connected via the **Battery Cable 2125-A** to the **EXT.DC** input socket of the PAM-2100, the Batt% field is substituted by the **Volt-field**. While the external 12 V Battery is connected the internal Li-ion battery is disconnected and the displayed voltage corresponds to that of the external battery. In first approximation, battery voltage can be taken as a measure of remaining power of the external battery. The functional relationship between capacity (Ah) and voltage of a new battery is depicted in the following figure. It is apparent that battery voltage first drops steeply down to about 12.4 V and then slowly decreases down to about 11.8 V, from whereon there is a steep drop to values below 11 V. When voltage drops below 11 V there is a "Low battery" warning.

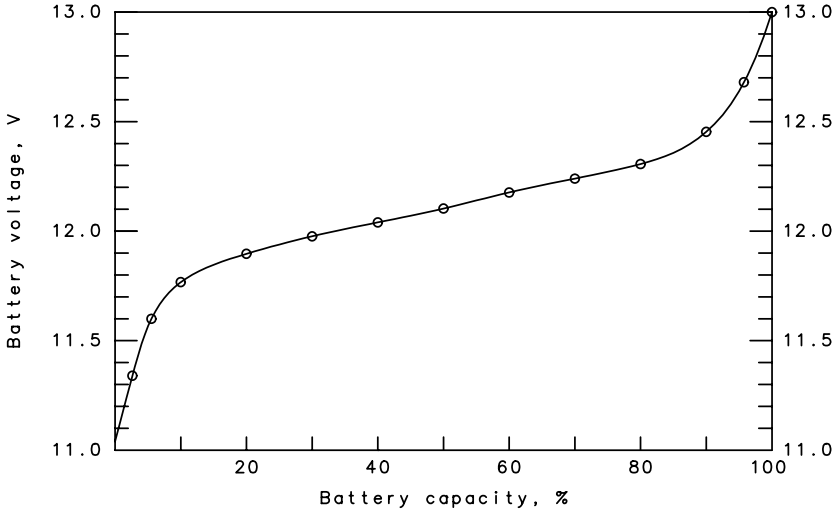


Fig. 12 Relationship between voltage of external lead-acid (12 V) battery and remaining battery capacity

6.1.17 Edit (Ctrl E) and the Report-file

With the help of the **Edit-key** (or via **Ctrl E** using an external keyboard) the user can leave the Parameter or Kinetic Screen and enter the **Report-file** where all data measured by the Saturation Pulse method are listed. This file is automatically installed upon initialization of the **Saturation Pulse Mode** of data acquisition. The Report-file can be edited by the user, e.g. comments on a particular set of experimental data can be entered and data can be erased. For editing similar commands as with Wordstar are effective (see 0)). To characterize a particular set of experimental data it is advisable to enter a line of explanatory text before running the experiment. For making optimal use of the Report-file and the Edit function an external keyboard is recommended.

```
Demonstration of Report-file information in experiment with spinach leaf
07-June-93        ML     Tmp.   PAR    Fo              Fv/Fm                    Fm
11:20:28          176    22.8   0      0.426           0.808                    2.220

Time          No  ML     Tmp.   PAR    Ft          ETR   Yield    qP       qN       Fm'      Fo'
11:21:25       2  176    22.8   462    1.455       57.1  0.294    0.371    0.088    2.061
11:21:54       3  175    23.1   453    0.630       54.4  0.286    0.553    0.746    0.883
11:22:24       4  174    23.1   450    0.524       75.9  0.401    0.783    0.750    0.875
11:22:54       5  173    23.1   448    0.543       87.1  0.463    0.801    0.675    1.010
11:23:24       6  174    23.1   448    0.535       93.1  0.498    0.830    0.643    1.066
11:26:08       7  172    23.0   445    0.534      107.6  0.576    0.838    0.526    1.259    0.394
11:26:37       8  171    23.1   445    0.534      108.1  0.578    0.838    0.522    1.266    0.393
11:27:07       9  171    23.0   445    0.525      110.1  0.589    0.851    0.516    1.278    0.394
11:27:27      10  172    23.0   445    0.529      110.7  0.592    0.882    0.515    1.296
11:27:57      11  172    23.0   444    0.530      110.4  0.592    0.881    0.513    1.299
averaging                Tmp.   PAR               ETR           Yield
11:28:27       1  172    23.0   443    0.535      110.1  0.529    0.877    0.507    1.310
11:28:57       2  171    23.0   443    0.530      111.0  0.597    0.883    0.505    1.314
11:29:27       3  171    23.0   443    0.529      111.4  0.599    0.885    0.503    1.318
11:29:57       4  171    22.9   442    0.529      111.4  0.600    0.885    0.500    1.323
11:30:27       5  170    22.9   442    0.529      111.7  0.602    0.886    0.498    1.328
averaged                 22.9   442               111.0  0.598

11:31:11          173    22.2   0      0.415
```

Fig. 13 Typical Report-file

A typical Report-file shows in the first two lines the information which is stored in conjunction with the Fm-command (Fo-Fm determination of dark-adapted sample). The first line shows the date and the notations of the measured parameters ML, Tmp, PAR, Fo, Fv/Fm and Fm. In the second line the time and the corresponding parameter values are written. In the given example, the actinic light was off (PAR=0) when the Fm-command was given. Then the actinic light was turned on and first a sequence of 5 Saturation Pulses was given, before the Fo'-mode was activated via Shift+Fm (or Ctrl S using an external keyboard), initiating Fo'-determination with every Saturation Pulse (see 6.1.2). Eventually, after having reached a quasi-stationary state, the averaging function was activated via Shift+Y (or Ctrl Y using an external keyboard) (see 6.1.5), which is indicated by a line saying "averaging". Averaging is terminated by another Shift+Y (or Ctrl Y) command. After that the averaged values are displayed.

The measured parameters are arranged in columns. Values of Tmp, PAR and ETR are entered only when the Leaf-Clip Holder 2030-B or the Micro Quantum/Temp.-Sensor 2060-M are connected. The No. refers to the current number of Saturation Pulses applied

after Fo, Fm determination. The No. is reset to 1 with the start of averaging .

When Fo is determined without associated Fm-determination (point Fo-determination in Shift+Return menu or Z-command using an external keyboard), its value is entered into the column in which normally Ft is listed.

The Report-file is quit via the **Esc-key**. It is stored in the internal PC under the name **STANDARD.RPT**. This can be renamed and transferred to an external PC using the **Trans2100 program** (see 5.3) and then viewed under Windows with the help of the **PamWin** program. From there it also can be exported to a spreadsheet program, like Excel, where it can be further analysed. After transfer of the Report-file, with the next initialization of the Saturation Pulse Mode a new, empty Report-file automatically is installed.

6.1.18 Display of Pulse Kinetics (Alt M)

The fluorescence kinetics induced by the last Saturation Pulse can be displayed in a pop-up window at the right hand corner of the Parameter-Screen via the point **Pulse Kinetics** in the **Com-Menu** or the **Alt M-command** using an external keyboard. While the Pulse Kinetics window is shown, the instrument does not react to key-commands. It can be closed again via Return (↵ **key**). The displayed curves are normalized by **Autoscaling**, such that the maximal fluorescence levels are identical. Hence, with decreasing Fm'-values, the noise increases. The Fm- and Fm'-values are determined by averaging 16 data points during the last 160 ms before termination of a Saturation Pulse. During the Saturation Pulse the modulation frequency is increased to 20 kHz. These features assure a high accuracy of Fm- and Fm'-determination. In the case of Fo-Fm measurements, the assessed levels of Fo and Fm are indicated by

dotted horizontal lines. Fm-determination can be considered correct, when the Fm-line coincides with a distinct plateau. In the case of Fm'-determination, the dotted horizontal line represents the assessed value of Ft. The numerical values of Fm or Fm' are displayed at the top of the curves. The total time scale of the inset amounts to 1500 ms. The first vertical line marks the termination of the Saturation Pulse, whereas the second vertical line indicates the end of the so-called "fade-out" time. During this time, starting with the onset of the Saturation Pulse, the graphical recording of Ft on the Kinetics Screen is suppressed.

Assessment of the Pulse kinetics is important to ascertain that intensity and length of the Saturation Pulses are appropriate for a given sample under the given conditions. The pre-set standard values of Int:10 and 0.8 s are well suited for most applications with leaf samples. Generally speaking, a plateau should be reached before termination of the Saturation Pulse (at first vertical line). Whether this is the case or not also depends on the light adaptional state of the plant sample. If the signal transiently increases after pulse termination, this is indicative of excessive pulse intensity. Relevant examples are given in the following figures.

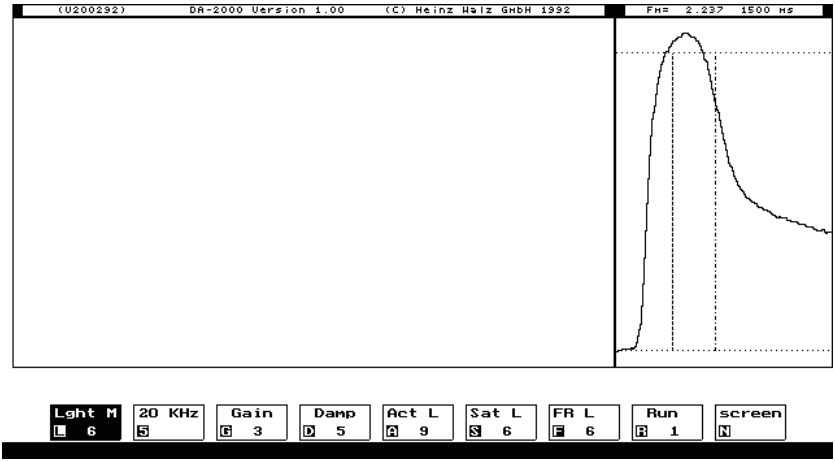


Fig. 14 Saturation Pulse settings: Int: 6 and 0.1 s: 4

In Fig. 14 a pulse length of 0.4 s at intensity setting 6 is too short to reach a plateau. It may be noted that, due to the slow on/off characteristics of the halogen lamp, the onsets of fluorescence rise and decay are delayed by approx. 150 ms with respect to the switching times. Because of the 160 ms averaging period, the thus determined Fm-value is lower than the peak value.

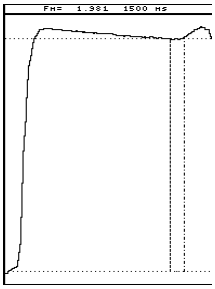


Fig. 15 Saturation Pulse settings: Int: 10 and 0.1 s: 12

In the example of Fig. 15 the intensity setting 10 is too high and the pulse length 1.2 s is too long. During the Saturation Pulse the Fm-level already declines, thus resulting in underestimation of Fm.

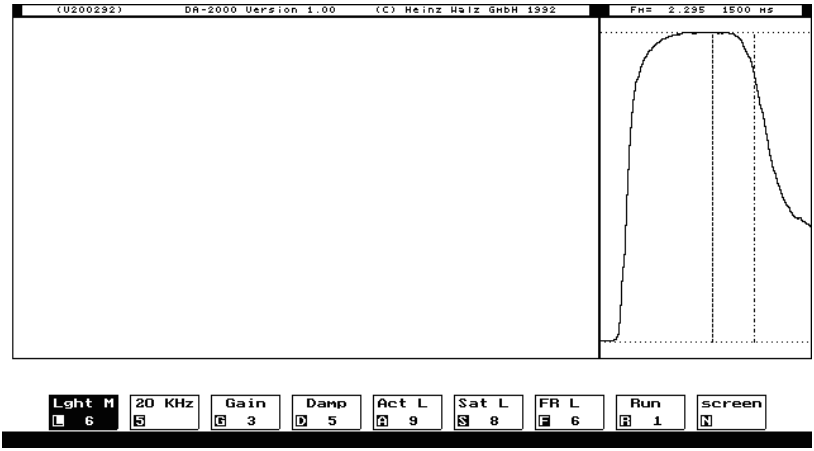


Fig. 16 Saturation Pulse settings: Int: 8 and 0.1 s: 8

Fig. 16 shows a correct Fm-determination at 0.8 s pulse length and intensity 8. A clear-cut plateau is reached, which defines the correct Fm-value.

Please note that Saturation Pulse parameters that are correct for Fm-determination after dark-adaptation may well be unsuitable for Fm'-determination after light adaptation. This is particularly true when in conjunction with illumination there is an increase in leaf temperature. Both light adaptation and elevated temperature lead to substantial stimulation of the rate with which electrons can be transported away from the PS II acceptor side via PS I into the electron sink of CO_2-fixation.

6.1.19 PAR re-calibration (Ctrl C)

The **micro-quantum-sensor** for PAR-measurement can be recalibrated via the point **Calibrate** in the **Shift+Com** menu or via the **Ctrl C-command** using an external keyboard. This only applies when the **Leaf-Clip Holder 2030-B** or the **Micro Quantum/Temp.-**

Sensor 2060-M are connected (see 3.5). When this function is called up, a dialog-field appears in the center of the monitor screen:

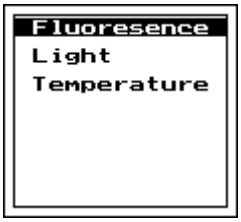
```
Calibration factor for PAR (1.000): _
```

The current calibration factor is shown (in brackets), which is 1.000 upon instrument delivery. A new value is most readily entered using an external keyboard. If this is not available, the value can be entered with the help of the four arrow keys. Numbers ranging between 0 and 9 as well as a point can be selected using the ∧ and ∨ keys. With the < and > keys the position is shifted to the left or the right. The value is confirmed by Return. The dialog can be quit via Esc. Any entered coefficient is stored in the Configuration-file (see 6.1.22) when the program is quit via Alt X (or via Quit program in the Com-menu).

Re-calibration may become necessary after ageing of the micro-quantum-sensor and dirt-deposition on the diffuser-disk. Original calibration was against a LI-COR Quantum Sensor, using day-light illumination. Care should be taken that the two types of sensors receive the same light at the same incidence angle. With the Micro Quantum/Temp.-Sensor 2060-M frequent recalibration is recommended, as the sensitivity of the PAR-sensor is affected significantly by fiber bending.

6.1.20 Definition of Offset-values (Ctrl O)

Via the point **Offset** in the **Shift+Com menu** or via the **Ctrl O-command** (external keyboard) a small menu can be opened to select **Fluorescence**, **PAR** or **Temperature** for definition of a certain offset-value.



- **Fluorescence**

Offset for measured Ft-value

After selection by Return or F (external keyboard), a dialog-field appears in the center of the monitor screen:

```
Offset for Fluorescence in mV [-300..+300] (    0): _
```

The given offset is shown (in brackets) which normally is 0. Values between -300 and +300 can be entered. A new value is most readily entered using an external keyboard. If this is not available, the value can be entered with the help of the four arrow keys. Numbers ranging between 0 and 9 can be selected using the ∧ and ∨ keys. With the < and > keys the position is shifted to the left or the right. The value is confirmed by Return. The dialog can be quit via Esc. Any entered coefficient is stored in the Configuration-file (see 6.1.22) when the program is quit via Alt X (or via Quit program in the Com-menu).

The offset value is indicated in the Ft-field when the measuring light is off. It shifts all measured fluorescence values by the same amount. A negative offset can be useful to correct for a constant background signal, e. g. observed with an empty cuvette.

- **Light (PAR)**

Offset for measured PAR

After selection and Return (or via P using an external keyboard) the corresponding dialog-field appears:

```
Offset for measured light intensity in uE [-100..+100] (    0): _
```

If necessary an Offset value between -100 and +100 can be entered (as described above for Fluorescence).

- **Temperature**

Offset for measured Tmp

After selection and Return (or T using an external keyboard) an offset value can be entered into the corresponding dialog-field (as described above for Fluorescence) for recalibration of the temperature sensor. The device originally was calibrated at 25°C. As the ΔV/°C of the thermocouple shows some variation with temperature, for operation at much higher or lower temperatures recalibration by offset-application will be useful.

6.1.21 Local menus (Alt F10)

"Local menus" are selection menus specifying the details of an instrument function. So far, such selection menus are installed for the **Pulse Sequence** and the **Run**-functions. They can be opened by first moving the cursor to the Pulse Seq.- or Run-parameter field and then selecting the point **Local menu** in the **Shift+Return** menu (or via the **Alt F10-command** using an external keyboard).

- **Local menu of Pulse Sequence (Alt F10)**

Select unit to be repetitively triggered

One of four possible units can be selected by entering the initial letter (or using up/down arrow keys) and confirmation by Return. The Pulse sequence-function (see 6.1.11) then applies to the selected item. This can be particularly useful for semi-automated field

measurements, when certain parameters are assessed repetitively during a longer time period. In this way, an almost unlimited flexibility can be achieved, when the complex experimental protocols, which are programmed in the Run-files, are repeated by the Pulse Sequence-function.

- **Local menu of Run (Alt F10)**
  The Local menu of the Run-function is essential for the definition and application of so-called **User-Runs**:

Writes current Runs to disk.
Reads new Runs from disk.

Upon start of the DA-2100 program automatically a set of **10 Standard Runs** is installed. In addition any number of User Runs can be defined, which may be derived from the Standard Runs or programmed independently (see 0).

A user-defined Run will become active after reading the corresponding file (Name. Run) via the Run Local menu. A User-Run is active when for a given Run-number a (u) is displayed in the Run-field.

6.1.22 Initialization of instrument settings and Configuration-file

Three different commands apply to different types of initialization of instrument settings. In the stand-alone mode without external keyboard these commands are accessible via the points **Standard Settings, Run-specific Settings** and **Update Settings** in the **Com-**

menu. When an external keyboard is available, the following simple key-commands apply:

0 (zero)	-	Standard settings
I	-	Initialization of Run-file specific settings
U	-	Update of displayed settings

The **0-command** for initialization of **Standard settings** is automatically given with every reset (cold or warm start) of the computer. These settings have proven most useful for common applications. It is good practice to apply 0 before start of an experiment and to modifiy just a few settings for the specific requirements of a particular type of measurement. The Standard settings are identical for the three modes of data acquisition except for the Damping setting, which is 2 for the Triggered Mode and 5 for Continuous and Sat. Pulse modes.

With the **I-command** appropriate instrument settings for a particular run-file are installed. Hence, first the desired Run-file is selected and then the I-command is applied. If wished, before the actual start of a Run certain settings still may be changed manually.

With application of the **U-command** (Update), the user can make sure that the settings displayed in the various parameter fields are indeed effective. Under normal conditions this is not necessary. It may be required after the PAM-2100 was automatically turned off due to low battery power..

Normally, when the DA-2100 program is quit via the point **Quit program** in the **Com-menu** (or **Alt X** using an external keyboard), the current instrument settings are stored in a **Configuration-file** (DA-2100.CFG). The same settings are installed, when the program is started again. In this way it is assured that a set of instrument

settings, which has proven useful for a certain type of experiment, is maintained irrespective of turning the measuring system on/off.

Note: If due to instrument malfunctioning the program is quit in an uncontrolled way, the values entered into the Configuration-file are erroneous. In this case, first the Standard settings should be installed (via the point Standard Settings in the Com-menu or the 0-command using an external keyboard), which then can be modified by the user.

6.2 Using the Kinetic Screen

The **Kinetic Screen** is installed via the **Rec-key** or the **K-command** when using an external keyboard. It substitutes for the Parameter Screen. All instrument settings and parameter values are maintained when switching between these two screens. Immediately after changing over to the Kinetic Screen the cursor is on the Normal Screen-field. Then a Return (↵) is sufficient for returning to the **Parameter Screen.** Later, when the cursor has moved, it can be put back on the Normal Screen field via **Shift+>** and and the Parameter Screen re-installed via ↵. Using an external keyboard the Parameter Screen can be re-installed via the **N-command**. The Kinetics Screen is primarily used in conjunction with the **Continuous Mode** and **Triggered Mode** of data acquisition. In the Saturation Pulse Mode it allows registration of so-called **Saturation Pulse Induction Curves** (see 6.2.3).

6.2.1 Screen Layout

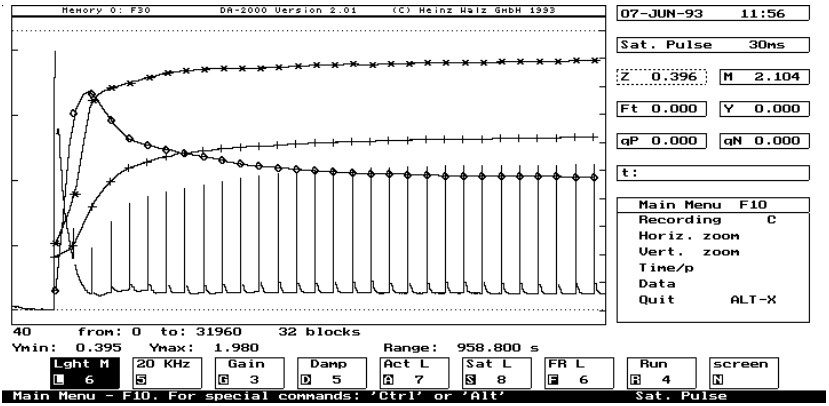


Fig. 17 Kinetic Screen with typical recording of Saturation Pulse Induction Curve

The Kinetic Screen is divided into four main areas:

- graphics area
- measured parameter area
- menu area
- instrument parameter area

In the **graphics area**, the measured curve of Ft vs time and the on-line calculated quenching parameters are displayed. Above the graphics area the current **Memory number** and **name** (left hand corner) and the DA-2100 program version are indicated. Below the graphics area relevant information concerning the recorded graphical data is presented in two lines:

The first line relates to the **data point addresses**. In the given example, the number "40" means that each image point represents 40 stored data points, which are automatically averaged. "from: 0 to 31 960" expresses that data points corresponding to addresses 0 to 31 960 are displayed. "32 blocks" means that the screen memory

contains **32 blocks of 1000 points each**. Data are generally stored in block fomat.

The second line relates to the **scaling of Y-and X-axes**. Ymin and Ymax are the minimal and maximal signal amplitudes, which may differ slightly from Fo and Fm, the deviations arising from the electronic noise. Fo-and Fm-determinations involve extensive data point averaging, which eliminates extreme values caused by noise. The **"Range"** indicates the time between first and last data points.

The values in the two information lines are changed when new **curve limits** are defined via the point **Limit** in the **Shift+Return menu** or via the **O-command** using an external keyboard.

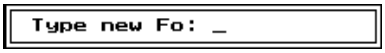
The graphics area can be expanded over the **full screen** via the point **Full Screen** in the **Shift+Com menu** or the **V-command** using an external keyboard (Full screen display). Via Return (↵) the normal display is re-installed. Using an external keyboard, the kinetic data can be erased by the /-command.

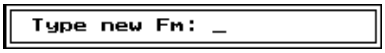
The **measured parameters** are shown in the upper right hand area:

- date and time
- mode and sampling rate (time per data point)
- Z (Fo) and M (Fm)
- Ft (fluorescence yield at time t) and Y (effective quantum yield)
- qP and qN (quenching coefficients)
- t (time associated with currently selected fluorescence data point)

Before a kinetic recording is started, normally Fo and Fm are sampled by pressing the **Fm-key** (or the **M-command** using an external keyboard). In this way Fo as well as Fm are determined, and the fluorescence rise kinetics induced by the Saturation Pulse are automatically displayed. The latter corresponds to the information

that normally can be called up via the point **Pulse Kinetics** in the **Com-menu** (or via the Alt M-command when using an external keyboard) (see 6.1.18). It is also possible to enter values for **Fo and Fm** manually via the point **New Fo or Fm** in the **Shift+Com menu** (or the **Del-command** using an external keyboard). Before this function can be carried out, first either the Fo or Fm field has to be selected with the cursor. The cursor jumps to the **Fo-field** when **Shift+<** is pressed. It automatically is on the **Fm-field** after an Fo-Fm determination. The new values can be written into the corresponding dialog-fields:





Note: The values of Fo and Fm are expressed in Volt, which is in contrast to the Offset-values (see 6.1.20) which are expressed in mV.

A new value is most readily entered using an external keyboard. If this is not available, the value can be entered with the help of the four arrow keys. Numbers ranging between 0 and 9 can be selected using the $\wedge$ and $\vee$ keys. With the < and > keys the position is shifted to the left or the right. A value is confirmed by Return. The dialog can be quit via Esc.

In the Saturation Pulse Mode the **Main Menu** for data acquisition and analysis comprises 5 sub-menus, which are detailed in section 6.2.4.

The parameter fields representing the major instrument settings and operational functions are arranged in the **Parameter Line** at the bottom of the screen. The commands for parameter setting and operation are outlined in section 6.2.2.

A **Cursor** is provided, the position of which is indicated by a dotted box and which can be moved by arrow-key operations

between the fields of measured parameters, of the Main Menu and of the Parameter Line. The corresponding functions then can be activated via ↵ (Return). The Cursor automatically jumps to the parameter field related to the last carried out function. In this way modifications in the settings of these functions can be most readily carried out, e.g. with the help of the +/- keys.

At the very bottom of the screen after certain key operations an **Information Line** is displayed, which informs the user about steps to be taken and also presents information on special commands involving the Alt- and Ctrl-keys (using an external keyboard). In the Information Line also a brief description of each point in the Main Menu is provided (see 6.2.4).

6.2.2 Commands for Parameter Setting

Due to the limited space and for the sake of clarity only 9 out of 20 instrument parameters are represented in the **Parameter Line** of the Kinetic Screen.

The character in the lower left corner of each parameter field is identical that of the corresponding functions on the Parameter Screen. When an external keyboard is available, either the functions are directly carried out using the corresponding keys (as in the case of L, 5, A, S, F and N) or a function is selected in order to change instrument settings (as in the case of G, D and R). Without external keyboard a parameter field is selected via cursor movement (arrow-keys). Please note that a parameter field is also automatically selected upon carrying out the related function (e.g. Act or F-Red). Settings are increased by + and decreased by -. The "super-setting" 11, possible only with L, A and F, is reached via the !-command

using an external keyboard or via the point Setting 11 in the Com-menu using the 20-key board.

Although not represented by a parameter-field on the Kinetic Screen, the status of the actinic light source can be switched between LED, Internal and External Halogen lamps by the H-command, provided an external keyboard is connected.

For more details on the various instrument settings and functions, please consult the corresponding paragraphs in section 6.1.

6.2.3 Saturation pulse induction curves

In conjunction with the **Saturation Pulse Mode** of data acquisition, the **Kinetic Screen** is most frequently used for recordings of so-called **Saturation Pulse Induction Curves**. These represent the induction kinetics upon a dark-light transition with repetitive application of Saturation Pulses and **on-line quenching analysis** (a typical example was already given in Fig. 17). Such induction curves contain complex information on the dynamic interplay between light- and dark-reactions of photosynthesis. For the experienced researcher such curves bear insights into regulatory mechanisms which cannot be obtained by steady-state studies.

Saturation Pulse Induction Curves recorded at 10 ms/point and at 30 ms/p are included in the so-called Run-files of Standard Experiments (Run 3 and 4) described in detail below. **Sampling rate** is changed via the **Main Menu** (see 6.2.4). For quenching analysis, previous determination of Fo and Fm via the **Fm-command** (M-command using an external keyboard) is prerequisite. On the Kinetic Screen upon Fm-determination automatically the fluorescence rise kinetics induced by the Saturation Pulse (**Pulse Kinetics)** are displayed. In this way, the correct determination of Fm can be assessed (see 6.1.18). The measured Fm-value has to be confirmed

by ↵ **(Return).** After that a recording can be started using the **Rec-key** (or **C-command** using an external keyboard**)**. The screen shows dotted lines representing Fo and Fm. In the absence of actinic illumination a trace of Ft is drawn close to the Fo-line. The Fm-line is autoscaled close to the upper limit of the screen. When actinic light is switched on (Act-key or A-command using an external keyboard), Ft first rapidly increases and then slowly decays again (Kautsky effect). For quenching analysis, Saturation Pulses are given. Single Saturation Pulses are applied with the **Yield-key** (or **Y-command** using an external keyboard). For repetitive Saturation Pulses the **Pulse Seq. Clock-function is** used (via **Pulse-key** or **P-command** using external keyboard (see 6.1.11). The first Saturation Pulse should be given in the peak of Ft. In this way, the state of minimal photochemical and non-photochemical quenching is assessed. No data points are registered during the Saturation Pulse and the so-called fade-out time (see 6.1.18), except for a single point marking Fm'. Depending on the **Join-status** the Saturation Pulse induced spikes (peaking in Fm') can be made visible or not (see 0).

The on-line calculated values of Y, qP and qN are first displayed by stepped-lines. When the recording is stopped via **Esc**, the various data points corresponding to these parameters are characterized by different symbols and connected with curved segments using a special spline interpolation. The left and right curve limits can be defined **by Horizontal zoom** (see 0) or, more quickly via the **O-command** (using external keyboard). In particular, when a recording was stopped before reaching the end of the screen, the command sequence O Return Return (using external keyboard) can be used for stretching the curve segment horizontally, such that it extends over the whole screen.

It is possible to remove any of the four parameter kinetics. For that purpose, the corresponding parameter field is selected by the

cursor and ↵ (Return) is applied. Then, the screen can be redrawn without the undesired parameter via the command sequence O Return Return (using external keyboard). The same procedure is used to restore the display of a particular parameter after the corresponding field is marked by the cursor..

The on-line calculated parameters are automatically written into the Report-file (see 6.1.17). The Ft-kinetics, on the other hand, have to be manually saved via the **Store-key** or via the W-command using an external keyboard. In the Saturation Pulse Mode a full kinetic recording involves 32,000 data points. This information is transiently stored in RAM memory (so-called Memory 0) until it is overwritten by the next kinetics recording. For further information on the saving of kinetic recordings, see 0.

6.2.4 Main Menu

In the Saturation Pulse Mode of data acquisition the Main Menu consists of 5 sub-menus. After selection of the Main Menu via the **Menu-key** (or the **F10-command** using an external keyboard), the sub-menus can be selected by cursor movement, using the arrow keys and Return, or more quickly by pressing the initial character key (if external keyboard available). Some menu-points also can be activated directly without entering the menu (if external keyboard available). These **short-cut commands** are:

C - Recording (start)
O - Limits (definition of left and right limits)
J - Join (switching Join function on/off)
V - Full screen (for screen filling graph)
Q - Read (to display stored data)
W - Write (to store displayed data)

Using the **20-key board** corresponding direct commands are:

Rec - Recording (start), C
Store - Write (to store displayed data), W

In the Saturation Pulse Mode, use of the Kinetic Screen and of the Main Menu is rather the exception. The Parameter Screen (see 6.1) in conjunction with the Report-file (see 6.1.17) provides a most convenient framework for data acquisition and analysis in this mode. This is in contrast to the Continuous Mode and the Triggered Mode where the Kinetic Screen is mainly used. Hence, a more detailed description of the Kinetic Screen and of Menu-guided data acquisition and analysis is presented in the following section 6.3 which deals with these modes.

7 Measurements in Continuous or Triggered Mode

When the PAM-2100 is switched on, automatically the Saturation Pulse Mode is installed, as this is the most frequently used mode of data acquisition. The **Continuous Mode** or **Triggered Mode** can be selected via the point **Mode Selection** in the **Com-menu** or via the **Alt I-command** using an external keyboard (see 5.1). As the Continuous and Triggered modes are used for the recording of kinetics, upon initialization of these modes the **Kinetics Screen** is installed. Details on the features of the Kinetics Screen were already presented in sections 6.2.1 and 6.2.2.

The **Parameter Screen** can be installed via the **Screen-field** in the Parameter Line of the Kinetics Screen. The cursor can be moved to this field via **Shift+>**. Upon Return the screen is changed. Alternatively, if an external keyboard is available, changing over to the Parameter Screen is achieved via the N-command and returning to the Kinetics Screen by the K-command.

The Parameter Screen is most convenient for changing the pre-set values of instrument settings. All settings are maintained upon returning to the Kinetics Screen. **Standard settings** can be initialized via the point **Standard Settings** in the **Com-menu** or via the **0-command** using an external keyboard. They are identical in the three different modes of data acquisition, except for the value of Damping, which is 2 in the Triggered Mode and 5 in the two other modes. The instrument settings for Standard Experiments, pre-programmed in the so-called **Run-files**, are initialized via the point **Run-specific Settings** in the **Com-menu** (or the I-command using an external keyboard.

There are some differences between data acquisition in the Cont. Mode and Trig. Mode, which are briefly outlined below:

- **Data recording**
  Cont. Mode: data are recorded on-line like with a **chart recorder**, i. e. the kinetics can be viewed on the screen during the recordings.
  Trig. Mode: data sampled at high rates first stored in **RAM-memory** within the PAM-2100. Only after the actual recording the data are transferred to the PC and then displayed on the Kinetics Screen.

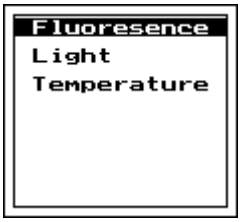
- **Sampling rates**
  Cont. Mode: sampling rates **10 ms/point** and **30 ms/point**, which are suited for relatively **slow kinetic recordings**.
  Trig. Mode: sampling rates extending from **150 µs/point** to **3000 µs/point**. These rates are suited for recordings of **rapid induction kinetics**.

- **Total number of recorded data points**
  Cont. Mode: 32 000 points

- **Start of induction curve**
  Cont. Mode: via the **Act-key** or the **A-command** (using external keyboard) after the recording was already started via the **Rec-key** or the **C-command** (external keyboard).
  Trig. Mode: start of Act. Light is automatically coupled with the start of the recording.

- **Use of Halogen lamp**
  Cont. Mode: possible during kinetic reordings
  Trig. Mode: not possible with kinetic recordings

- **Pulse Kinetics display**



- **Fluorescence**
  Cont. Mode: Following Fm-determination the Pulse Kinetics are automatically displayed.
  Trig. Mode: Pulse Kinetics cannot be displayed

7.1 Menu-guided data acquisition and analysis

When the PAM-2100 is operated in the stand-alone mode, irrespectively of whether an external keyboard is connected or not, almost all commands can be carried out with the integrated 20-key board. Hence, in principal commands given via integrated or external keyboards are equivalent. The most common commands, like Yield (Y), Act (A), Run (R), Rec (C), Menu (F10), Fm (M) and Pulse (P), are as quickly carried out via the 20-key board as via an external key board. This is, however, not true for **Menu-guided data acquisition** and **Menu-guided data analysis**, which involve numerous commands that are much more readily carried out with the help of an external keyboard than with the 20-key board. Therefore, it is recommended that an **external keyboard** (like the **Ultra-Compact Keyboard 2170-K**) is used for Menu-guided data acquisition and analysis. Consequently, in the following description emphasis is put on the commands given with an external keyboard. Where feasible, the equivalent command using the 20-key board is given in parentheses.

When an **external keyboard** is connected in the **Triggered** or **Continuous Mode** of data acquisition, the use of the **Function-keys**

F1-F9 requires **Scroll Lock** to be active. This normally applies to data analysis under laboratory conditions. Please note that when an **external keyboard** is connected and **Scroll Lock** is active, the function of part of the 20-keys of the PAM-2100 is changed. For example, the the Com-key corresponds to F9 and Shift+Com corresponds to F8.

When an external keyboard is connected, on one hand, there are **immediate key-commands**, like C, O or W (see 6.2.4) and on the other hand, there is the **Main Menu** with its various **sub-menus**, in which the user may move by cursor or **initial character operation**. The former, which has the advantage of being quick, is advantageous to the experienced user, who knows all commands by heart. The latter can be recommended to beginners, who will profit from the explanatory text accompanying every menu-point (Information Line at the bottom of the screen).

Fig. 18

In the Continuous Mode and Triggered Mode the Main Menu consists of 7 sub-menus. After selection of the Main Menu via **F10** (or **Menu**-key), the sub-menus can be either selected by cursor-movement using the arrow keys or by typing the initial character. In the former case, they are activated by **Return**, whereas in the latter case they are directly carried out. The **Esc-command** is used to return from a sub-menu point (e.g. Limits) to the corresponding Main

Menu point (e.g. Horiz. zoom) and also to leave the Main Menu. The direct commands (like C, O and W) do not work while in the Main Menu. In the following sub-sections the various sub-menus are outlined. Below the various menu and sub-menu points a corresponding explanatory text is written. The same text is also displayed in the Information Line at the bottom of the screen.

7.1.1 Recording C (Rec-key)

Triggered kinetic recording of max. 4000 data points at given sampling rate (Trig. Mode)

Kinetic recording of max. 32 000 data points at given sampling rate (Cont. Mode)

This menu-point may serve to start a kinetic recording. Actually the same purpose can be more easily achieved without entering the menu by using the **C-command (Rec-key)**. The start of a recording should be preceded by Fo-determination for Y-axis scaling (see 7.1.3) and, at least in the Sat. Pulse Mode, also by Fm-determination. Both Fo and Fm are determined with the **M-command (Fm-key)**. In the Triggered Mode, the measured kinetics are displayed some time after the actual recording. In this mode the data are transiently stored before they are transferred for display on the Kinetics Screen.

7.1.2 Horiz. zoom

For horizontal expansion by selecting curve segment and Full Screen display

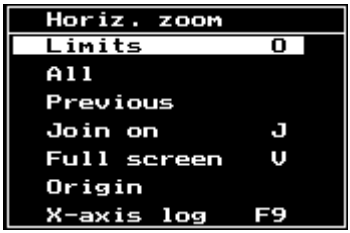


Fig. 19

- **Limits O** (point **Limits** in **Shift+Return menu**)
  Define first/last point of curve segment which is to be displayed by zooming
  Select left limit by typing address or using arrow keysSelect right limit by typing address or using arrow keys

By appropriate choice of the **left and right curve limits** the horizontal stretching of a curve can be freely varied. Curve limit selection can be made by cursor movement using the arrow keys or by typing the desired address, which can be written into the information line. A selected address is entered by Return. Numerical limit selection may be advantageous for the following reasons:

--- to reproduce defined curve segments
--- to display curves starting at the trigger point that is at address 512
--- to define a curve segment in block format (multiples of 1000 data points) for storage on disk or RAM (see 9).

In the Triggered Mode curve limits are maintained for consecutive recordings until they are re-defined. To return to the complete recording, the **All-command** is used (see below).

Limits selection is also possible without entering the Main Menu via the **O-command** or via the point **Limits** in the **Shift+Return menu**.

When in the Continuous Mode a recording was terminated by Esc before the maximal amout of 32000 data points was collected, the

recording can be horizontally stretched to the full screen width by the sequential key operation **O Return Return**.

- **All**
  To return to the original recording with all data points
  The same command can be given without entering the menu by the sequential key operation **O A**.

- **Previous**
  To return to the curve segment defined by previous Limits determination
  This function is useful if, on search of a suitable segment, an optimal extent of horizontal stretching was exceeded. The addresses of up to 16 segments are stored.
  The same command is possible without entering the menu by the sequential key operation **O P**.

- **Join off J**
  To draw single data points
  Join on J
  To connect data points

  When the Join-function is active, the single data points are connected by lines. This can be particularly useful for hardcopies of curves displaying steep slopes.

  The status of Join can be also changed by the **J-command** without entering the Main Menu. The Join-status cannot be changed with curves that were obtained by mathematical transformation using the special Function-commands (see 7.1.6). When such a transformed curve is displayed, via the J-command the original curve or curve segment is redrawn with altered Join-status. Hence, the J-command provides a convenient way to return to the original curve or curve segment.

The Join-function is also useful to display the saturation pulse induced fluorescence spikes when quenching analysis is applied in the saturation pulse mode, by drawing vertical connecting lines to the Fm'-points.

- **Full screen**
  Display of screen filling curve

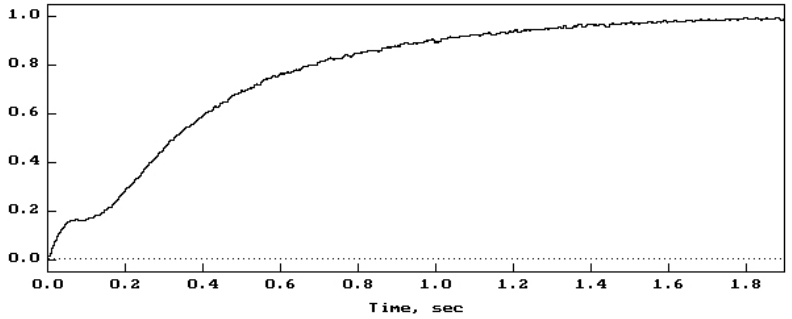


Fig. 20

The above figure shows a triggered recording displayed in **Full screen graphics**. In the given example, before application of the V-command the curve origin was defined at the trigger point. In this way of presentation the Y-axis is divided into 10 units from 0.0 to 1.0, where 0.0 corresponds to the Fo-line and 1.0 corresponds to the chosen Y-limit. The X-axis is divided in 10 time units the length of which depends on the sampling rate. Pressing Return or any other key will restore the normal Kinetics Screen.

- **Origin**
  To define point of new "origin", at which t=0 and Ft=0
  Select new origin by typing address or using arrow keys

With the Origin-function the first data point of a selected curve segment is transposed into the left lower corner (origin of coordinates). At the same time, the Ft and t values associated with all data addresses are changed, such that the **new curve origin** is represented by **t=0 and Ft=0**. Hence, if the new origin is put on the original Fo-line, the newly defined Ft-values correspond to variable fluorescence yield. Contrary to Ft, the Y-values associated with the original data points are not changed. In many applications it is useful to define the curve origin at the **trigger point** which corresponds to **address 512**. An example was already given above in conjunction with the full screen display of an induction curve. The new origin will be maintained in consequent recordings until the normal display is re-installed via the All-command (or the sequential key operations O A).

The Origin-function can also be applied without entering the Main-Menu by the sequential key operations **O O**. In this case, the left limit should have been previously defined.

- **x-axis log F9**
  Logarithmic time scale to stretch early kinetics and compress late kinetics
  Particularly in the Continuous Mode, with 32 000 data points, a recording contains kinetic information at vastly different time scales. In order to evaluate rapid as well as slow transients in the same figure, the time scale can be stretched at the beginning of a recording and then increasingly compressed towards the end by using a **logarithmic time scale.** For this purpose, the time axis is divided into 800 intervals which increase from n • x to n • x^{799}. Each time interval then corresponds to one image point. Furthermore, all data points belonging to one interval are averaged.

The x-axis log function can be also applied without entering the Main-Menu by use of the **F9-command**.

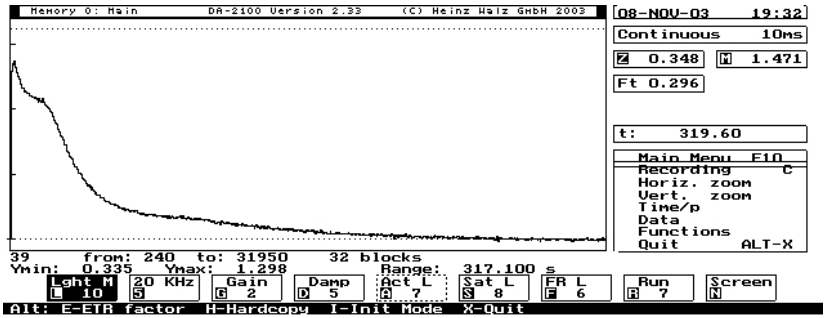


Fig. 21

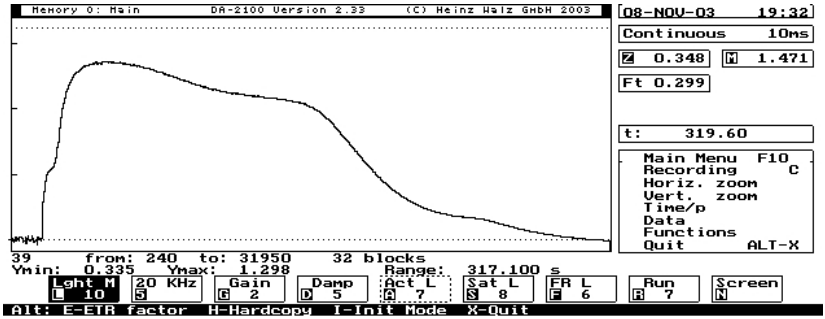


Fig. 22

In order to obtain effective stretching of the rapid light-on transients, it is advisable to select an address shortly before light-on or at the trigger point as left curve limit (see Origin sub-menu point above). A modification of the right curve limit is not advisable, as the routine profits from a large set of data points. At the end of a plot with logarithmic time base some points are missing, corresponding to the

pre-trigger points that were omitted when the left curve limit was defined.
The original curve with **linear time base** is reinstalled via **O Return Return** or more simply via **J** (see 7.1.2).

7.1.3 Vertical zoom

For vertical stretching of given curve or change of y-axis scaling

Fig. 23

- **Fo offset off**
  To display complete fluorescence signal, starting with zero
  Fo offset on
  To display variable fluocescence only, starting with Fo
  This command operates like an on/off switch. When Fo offset is active, fluorescence values between zero and Fo are suppressed and only variable fluorescence is displayed. This is the normal situation, which is pre-set upon program initiation. Turning off the Fo offset is advantageous when there is strong non-photochemical quenching and the Fo'-mode is activated (see 6.1.2).

- **Join off J**
  To draw single data points
  Join on J
  To connect data points

When the Join-function is active the single data points are connected by lines. The status of Join can be also changed by the **J-command** without entering the Main Menu. The Join-status cannot be changed with curves that were obtained by mathematical transformation using the special Function-commands (see 7.1.6). When such a transformed curve is displayed, upon application of J, the original curve or curve segment is redrawn with altered Join-status. Hence, the J-command provides a convenient way to return to the original curve or curve segment.

- **Stretching F7**
  Factor for vertical stretching of a given record

Fig. 24

The pre-set value is 1.0. It can be changed by typing the desired factor and Return. The Fo-line stays in its original position. The stretching factor always refers to the original curve that can be recalled at any time by returning to the factor 1.0.
The stretching factor is automatically re-set to 1.0 when a new recording is started. This is in contrast to the vertical scaling which can be modified via Y scale F8 (see below).
Changing the vertical stretching factor is also possible via the **F7-command** without entering the Main Menu.

- **Y scale F8**
  To change scaling of Y-axis for this and following recordings

Fig. 25

The scaling of the Y-axis is based on the **Y-limit (maximal amplitude).** The pre-set value is 2.0 volt, which is divided into Fo-units (up to 12Fo). With Fm/Fo ≈ 5 for most healthy leaves, and with Fo being adjusted to a value slightly below 400 mV, the Y-axis will be divided into 5xFo and an induction curve is unlikely to exceed the Y-limit. In principle, it is also possible to work with a lower Fo and a correspondingly lower Y-limit. However, in this case the signal/noise ratio may become limited by digital noise. When **"0"** is entered as Y-limit, there is **autoscaling**, i. e. for any chosen curve segment the Ymax value is automatically taken as Y-limit. Autoscaling is figuring as a separate point in the Vertical zoom sub-menu.

A given scaling is maintained as long as it is not changed or the program is newly initialized. In this respect, there is a basic difference to Stretching F7.

Changing Y-axis scaling is also possible the **F8-command** without entering the Main Menu.

- **Autoscaling**

  For maximal vertical expansion of this and following recordings

  Choosing this function, it is possible to perform autoscaling without entering the brief dialog involved in F8 (see above).

- **Origin**

  To define point of new "origin", at which t=0 and Ft=0

  Select new origin by typing address or using arrow keys

  With the Origin-function the first data point of a selected curve segment is transposed into the left lower corner (origin of coordinates). At the same time, the Ft and t values associated with the data addresses are changed, such that the **new curve origin** is represented by **t=0 and Ft=0**. Hence, if the new origin is put on the original Fo-line, the newly defined Ft-values correspond to variable fluorescence yield. Contrary to Ft, the Y-values

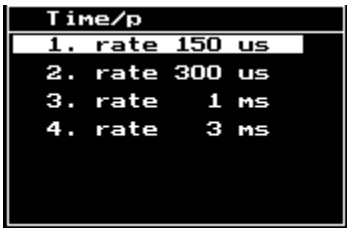
associated with the original data points are not changed. In many applications it is useful to define the curve origin at the trigger point which corresponds to address 512. An example was already given in conjunction with the full screen display of an induction curve.

The new origin will be maintained in consequent recordings until the normal display is re-installed via the All-command (or sequential key-operations OA).

7.1.4 Rate

Rate of data acquisiton in time/data point

Cont. Mode



Trig. Mode

Fig. 26

The choice of possible recording rates is different in the Triggered Mode (150, 300, 1000 and 3000 µs/point) and the Continuous Mode/Saturation Pulse Mode (10 and 30 ms/point). In the Triggered Mode, a total of **4000 data points** are sampled, whereas in the two other modes a full recording consists of **32 000 data points**. Hence the following **total recording times** are given:

Triggered Mode		**Continuous Mode**	
Sampling time (µs/p)	Recording time (s)	Sampling time (µs/p)	Recording time
150	0.6	10	5 min 20 s
300	1.2		
1000	4.0	30	16 min
3000	12.0		

7.1.5 Data

The PAM-2100 features two types of "memory" of kinetic Ft-data. The data are first stored in a transient **Main Memory (Mem. 0)** that is overwritten with every new recording and is lost when the instrument is switched off. In order to save the kinetic data permanently, they have to be manually stored on the hard-disk of the integrated panel-PC via the **Write-function** (see below) or using the **Store-key** (20-key board). In **the Triggered Mode** it is possible to transfer the data from Mem.0 to one out of **Mem.1-4**, in order to be able to compare kinetic recordings. In contrast to Mem.0, Mem.1-4 are not automatically overwritten with every new recording. However, also Mem.1-4 are lost upon switching off the PAM-2100, unless saved via the Write-function.

Fig. 27

- **Read Q**
  Load data from disk to be displayed on screen

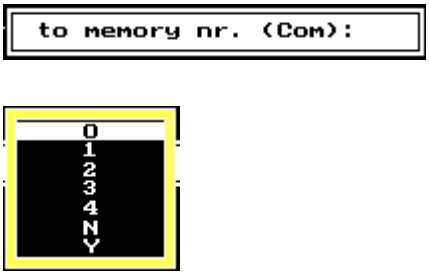


Fig. 28

After selection of **Read**, in the **Trig. Mode** first the Memory to which the file shall be read has to be entered, which is Mem. 0 or one out of Mem. 1-4. (In Cont. and Sat Pulse Modes Mem. 0 only.) The number can be either typed or selected by up/down arrow keys from a menu that is opened by the **Com-key**. The latter way is preferable in the stand-alone mode. The Mem. number is entered via Return. Now the name of the file to be read can be selected by cursor movement from a list of names shown at the bottom of the screen. After Return the name of the selected file is shown, which is confirmed by another Return. The Read-function can also be directly called up via the **Q-command** without entering the menu. The name of the loaded data file is displayed in the upper left corner of the screen.

- **Write W**
  Save data by writing into disk file

Fig. 29

After selection of **Write**, first in the **Trig. Mode** first the Memory number has to be entered, which is 0 or 1-4. (In Cont. and Sat Pulse

Modes Mem. 0). As described above for the Read-function, the number can be selected from a list after pressing the Com-key. After entering the number, the free storage space on the active disk is indicated in kBytes and the names of already existing data files are displayed. Either the current Memory 0 (up to 32 000 points in the Cont. Mode and up to 4000 points in the Trig. Mode) or (in the Trig. Mode only) one out of Memories 1-4 (up to 4000 points each) can be saved. For Mem. 1-4 the corresponding name is proposed as filename, which may be deleted by **Esc** and replaced by a new name, before entering by Return. With Mem. 0, the current recording number, e.g. Rec001, is proposed. Generally, selected curves or curve segments are stored in block units, with one block consisting of 1000 points. If a curve segment consists of less than 1000 points or of less than a multiple thereof, the right curve limit is automatically shifted to a higher address, such that the next higher block is completed.

A new name is most readily written with an external keyboard. It also can be written with the integrated 20-key board using the up/down and left/right arrow keys. With the **up-arrow-key** the letters from **A to Z**, the **characters "_", "-" and "."** as well as the **numbers 0-9** can be selected. After 9 the alphabet starts again with A. With the **down-arrow-key** the same characters can be selected the other way around. With the **right-arrow-key** the position is shifted to the right, whereas with the **left-arrow-key** the position is not only shifted to the left, but also the previous letter on this position is erased.

In the Trig. Mode, the **X-command** initiates a special routine for writing all **Mem. 1-4** to disk. After applying X, first the name of Mem. 1 is proposed as filename. When this is entered, the same happens for Mem. 2 and so on for all Memories.
The Write function can also be directly called up by the **W-command** or via the **Store-key**, without entering the menu.

- **Transfer Files**
  The special Windows software **Trans2100** is provided for transfer of data files from the panel-PC of the PAM-2100 to an **external PC** (see 5.3). When the data are transferred to the PamWin-directory, they can be viewed using the **PamWin** user surface in the **View-mode** (see separate PamWin manual).

- **Update DA-2100**
  The DA-2100 software resident on the flash-card within the PAM-2100 can be updated using the Update.exe program provided with the instrument (see 5.4).

- **Memory**
  Select, store and overlay memories

Fig. 30

Kinetic data are first stored in the transient **Memory 0 (Main Memory)** that is overwritten with each new recording. In the **Trig. Mode** of data acquisition curves or curve segments can be transferred from Mem. 0 into the **Mem. 1-4**, where they are saved until leaving the Trig. Mode or the program. For permanent storage of kinetic data on disk, the **Write-command (W)** as well as the **Store-key** are provided (see above). In addition, with the **X-command** a routine can be started for saving all of Mem. 1-4 on disk.

The number and name of the current Memory (Mem. 0 or Mem. 1-4), the data points of which are displayed on the Kin. Screen, are displayed in the upper left corner of the screen.

- **Next memory F5**
  Select next memory
  With this command, kinetic curves stored in Mem. 0-4 one after the other are called on the screen in a circular way, i.e. Mem. 0 is installed after Mem. 4 again. The Memory number and name are displayed in the upper left corner of the screen. Use of the Function key 'F5' normally will be preferable to entering the menu.

- **Store to memory** (direct execution via Ctrl M)
  To store measured curve in memory

Fig. 31

In the Trig. Mode of data acquisition, curves or curve segments defined by Horizontal Zoom (see 7.1.2) can be saved in Mem. 1-4 via the **Ctrl M-command**. After selecting the Memory number (e.g. via the selection menu opened with the **Com-key**) and Return, this Memory number is proposed as Memory name, which can be entered by Return. Alternatively, the proposed Memory name can be first erased by **Esc** and then a new name can be typed, which is entered by Return. These names will be proposed when applying one of the Write-commands (W or X or Store-key, see above) for saving kinetic data on disk.
Data storage in Mem. 1-4 is always in block format, with one block consisting of 1000 data points, and each Memory containing up to 4 blocks (as does the original recording first stored in Mem. 0). If a curve segment consists of less than 1000

points or of less than a multiple of 1000 points, automatically the right curve limit is shifted such that the next higher block is completed. The left curve limit remains unchanged. The numbers of the occupied Memories, as well as the amount of blocks that are involved, are indicated in a line below the Main Menu.

- **Erase memory**
  To clear a memory

Fig. 32

After selecting this function in the Memory-submenu, the nr. of the Memory, which shall be erased, can be typed in. Then the command is immediately executed. Memories can be also erased by overwriting.

- **Overlay memory**
  To overlay a memory to displayed curve

Fig. 33

With the Overlay-command any curve from **Mem. 1-4** can be superimposed over the curve (or curves) currently displayed on the screen. The originally displayed Memory, the number and name of which is shown in the upper left corner of the screen, will determine **Y-axis scaling**. Also, when **Autoscaling** is active (see 7.1.3) this Memory is decisive. Mem. 0 cannot be superimposed on other Memories. The superimposed curves or curve segments should consist of the same amount of data blocks.

- **All memories**
  To show all memories

Using this command, **all Mem. 1-4** can be displayed on top of each other. The originally displayed Memory, the number and name of which is shown in the upper left corner of the screen, determines the **Y-axis scaling**. Also, when **Autoscaling** is active (see 7.1.3) this Memory is decisive. In order to return to the display of single Memories, the **F5-command** is used.

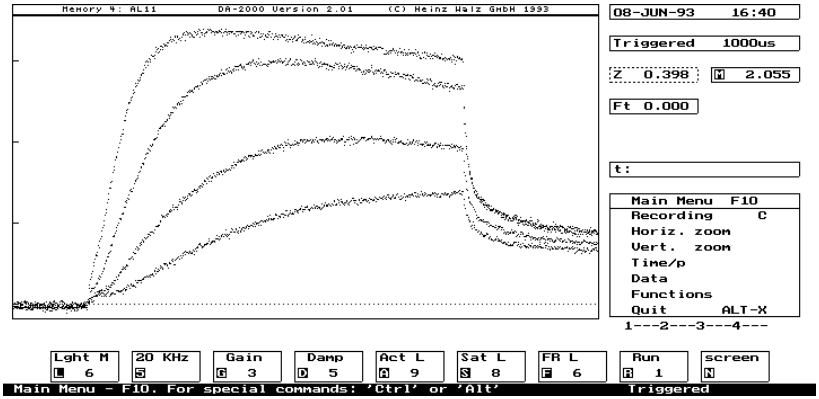


Fig. 34

The above figure shows an example of four superimposed rapid induction kinetics recorded at different actinic light intensities, which were stored in Mem. 1-4 and then displayed on top of each other via the **All memories-function**. In this example, Mem. 4 (A11) was called on the screen before the All memories-command was given. Hence, with Autoscaling being active, this curve (with the highest fluorescence values) is decisive.

- **Write all mem. X**
  To write Mem 1-4, one after the other, to disk

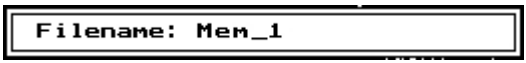


Fig. 35

By pressing the X-key, a routine is started, which allows quick storage of **Mem. 1-4** on disk. The Memory-names are proposed as Filenames that can be entered by Return, one after the other. The proposed names can be also deleted by Esc and new names can be entered.

7.1.6 Functions

Special mathematical transformations of displayed curves or curve segments

All 10 Function-keys are used with the Data Acquisition Program DA-2100. However, only 7 of these are listed in the Functions sub-menu. F10 installs the Main Menu. F5 is reserved for the Next Memory command in the Trig. Mode (see 7.1.5) and F9 is listed in the Horiz. zoom sub-menu (X-axis log function) (see 7.1.2).

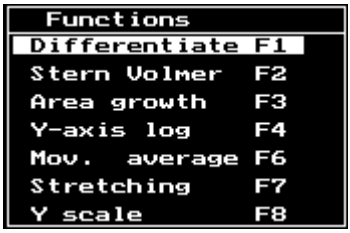


Fig. 36

When an **external keyboard** is connected, all Functions can be also directly selected by the corresponding **Function keys** without entering the menu. Use of the Functions is not possible in the Sat. Pulse Mode of data acquisition. Please note that the use of the **Function-keys** F1-F9 requires **Scroll Lock** to be active. This normally applies to data analysis under laboratory conditions. When an **external keyboard** is connected and **Scroll Lock** is active, the function of part of the 20-keys of the PAM-2100 is changed. For

example, the the Com-key corresponds to F9 and Shift+Com corresponds to F8.

All Functions can be also directly selected by the corresponding Function keys without entering the menu. Use of the Functions is not possible in the Sat. Pulse Mode of data acquisition.

- **Differentiate F1**
  To evaluate slopes as well as maxima and minima of displayed curves
  With this function, curves or curve segments are differentiated. The properties of this function are adapted to the special requirements of the analysis of fluorescence induction curves:

- As the underlying data points display some electronic noise, before differentiation automatically data point averaging is applied and after differentiation curve smoothing via F6 is carried out (see below).

- The scale of the differentiated curve is given in Fo/sec, representing a generally valid measure for the slope of a fluorescence change, which does not change when curves are vertically or horizontally stretched.

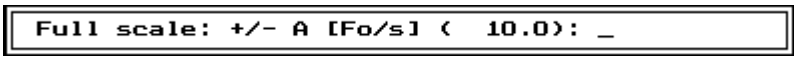


Fig. 37

After initiating Differentiate, a scaling proposal is given (see above figure), which either can be accepted by Return or changed by typing-in the desired value. The entered value will be valid also in subsequent experiments unless it is changed or the program is newly initialized.

After the scaling is entered the differentiated curve is displayed with a dotted zero-line dividing the screen into two equal halves (+/-

A). In the information line, the chosen scaling, A, in [Fo/sec] units and the time length of the displayed curve segment ("Range") are indicated.

In most cases, it is necessary to further smoothen the differentiated curves by applying F6. This may lead to suppression of peak values, and if these values are of interest, it is advisable to determine them separately after appropriately narrowing down the curve segments by horizontal zoom (see 7.1.2). The sampling rate (see 7.1.4) of the original recording should match the rate of the transients that are analysed.

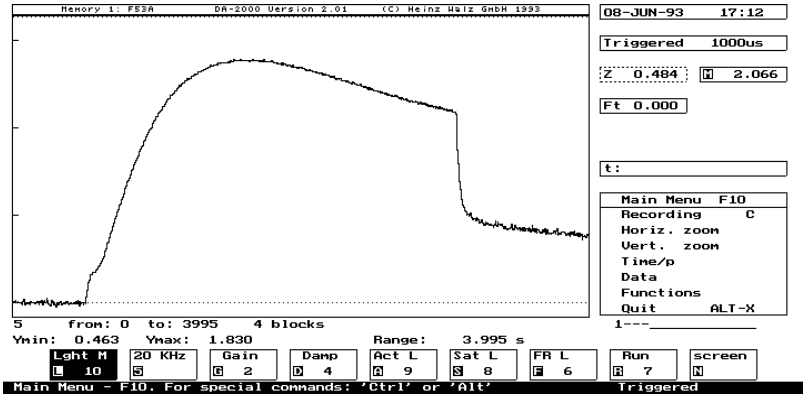


Fig. 38

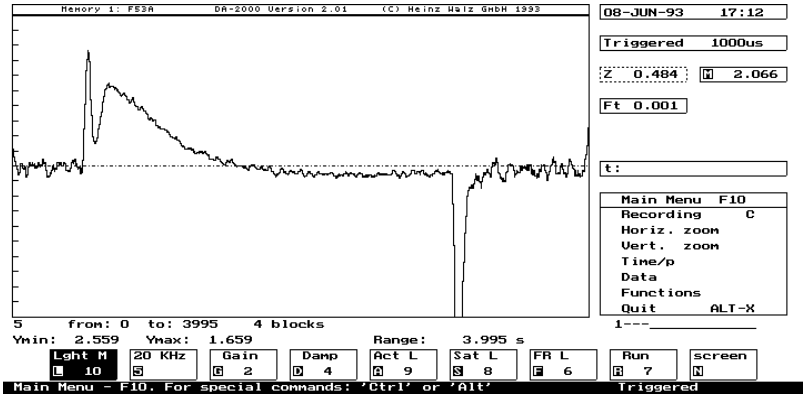


Fig. 39

Figure 53 A, B shows an example of application. Relevant points of information are the peak-amplitudes (max. slopes) and the peak-times. In the given case, the max. slope of the I-P rise is reliably displayed, whereas with the selected large curve segment quantitative slope determinations of the O-I rise or of the "light-off" response are not possible, as the corresponding peak values are lowered by the smoothing operations.

- **Stern-Volmer F2**

  Inverse plot of Fm/Ft vs. time to account for statist. pigment bed properties

  This function serves to transform curves according to the Stern-Volmer equation: The function Fm/Ft vs. time is displayed inversely, with the Y-axis divided into 6 units, starting with 6 (below) and ending with 1 (up). This division was chosen, as Fm/Ft is unlikely to exceed the value of 6. It is maximal for Ft=Fo and becomes 1 for Ft=Fm. Hence, in this presentation a fluorescence rise in the original Ft curve corresponds to a rise of the inversely displayed Fm/Ft curve.

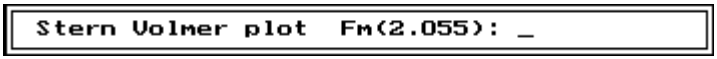


Fig. 40

When this function is selected, there is a request to enter Fm. If Fm was previously determined via the M-command, this value is proposed in brackets and accepted by Return. Alternatively, a fictive Fm-value of 6xFo may be assumed, in which case the displayed curve starts at the origin of the Y-axis (Fm/Fo=6).

Transformation of fluorescence data according to the **Stern-Volmer equation** (Fm/Ft = 1 + k [Q], with [Q] representing the concentration of quenching reaction centers) causes linearization of fluorescence yield with respect to the concentration of closed PS II reaction centers. This equation is based on the "statistical pigment bed model", assuming that excitation energy can move from closed reaction centers to open centers. Due to this interunit energy transfer, fluorescence yield at first rises to a much lesser extent than Q is reduced. And, hence, in fluorescence induction curves a certain change in Q- concentration will be expressed to a much stronger extent in the range of high fluorescence yield than in the range of low yield. This is not the case when Fm/Ft is plotted.

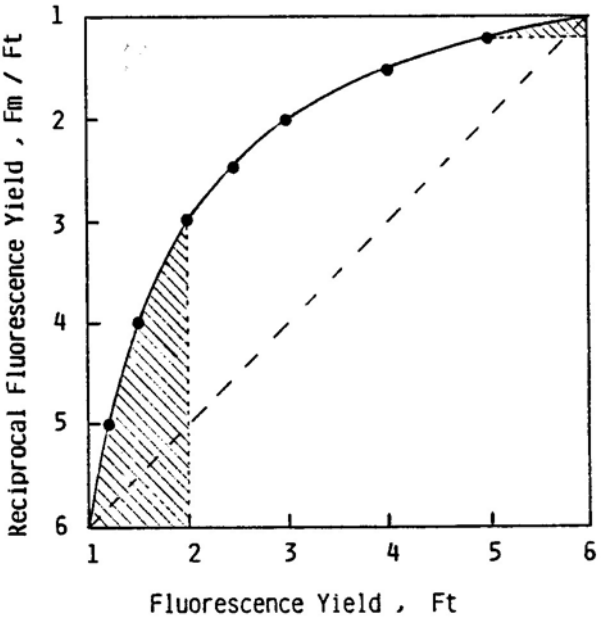


Fig. 41

The above figure shows the relationship between Fm/Ft and Ft, assuming a ratio Fm/Fo=6. With a change of Ft at low variable fluorescence yield (e. g. from 1 to 2) the corresponding changes of Fm/Ft are much larger than with the same change of Ft at high fluorescence yield (e. g. from 5 to 6).

In the following figures an Ft induction curve is compared with the corresponding Fm/Ft curve, which was obtained by application of F2.

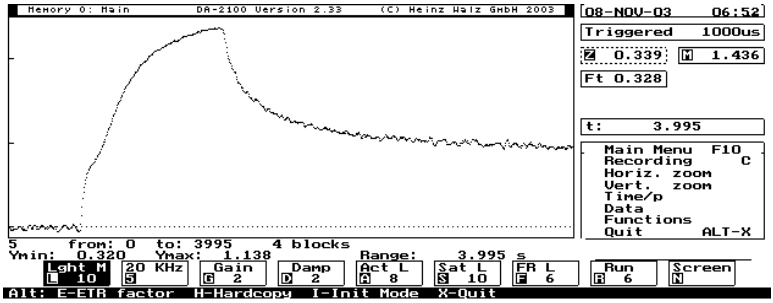


Fig. 42

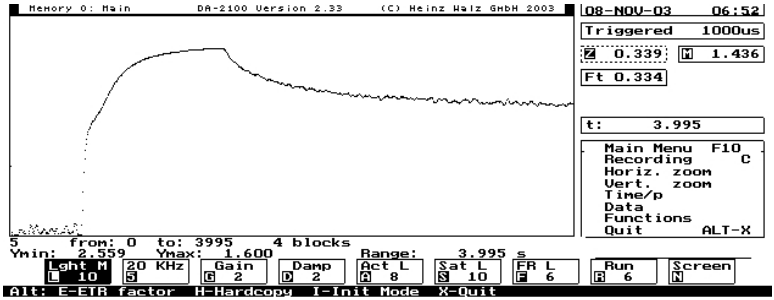


Fig. 43

- **Area growth F3**

  Integr. area betw. curve and Fm-line plotted vs. time to assess reduc. kinet.

  The fluorescence yield at a given light intensity corresponds to a defined turnover rate of PS II reaction centers. At Fo the rate is maximal, while it becomes zero at Fm. When a certain fluorescence yield is observed over a given time period, this corresponds to a defined number of transported electrons. A measure for the amount of transported electrons is the area bounded by the Ft curve and the Fm line. With an induction curve, in which fluorescence yield rises from Fo to Fm (e. g. in presence of inhibitors or at high light intensity), the area between the Ft curve and the Fm line is a measure for the acceptor pool size.

The "area growth" function is based on integration of the area between the Ft curve and the Fm line. When F3 is activated, a Fm line is automatically drawn through the last data point at the right curve limit. The proposed Fm value may be manually corrected ("Select Fm by arrow keys") and eventually stored by Return. Then the program calculates the area integral, the value of which is displayed in units of [mV x ms]. This corresponds to an area Amax, representing the total number of transported electrons.

Fig. 44

For the sake of a kinetic analysis of the so-called **"area growth curve"**, it is possible to correct this Amax ("delta [+/- in %]). Normally, a small positive correction (about 1 %) is appropriate, such that At approaches Amax at the right curve limit, without reaching it. When the calculated or corrected total area Amax is entered by Return, the "area growth curve" is displayed.

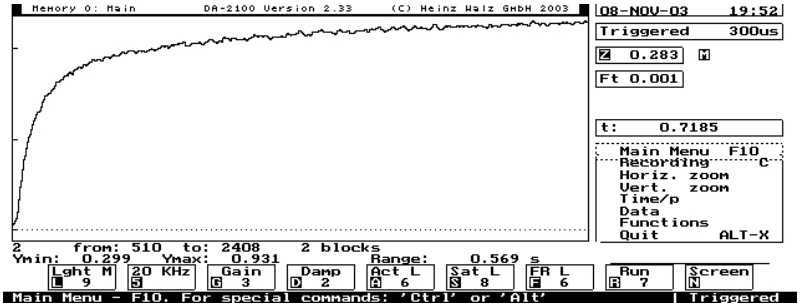


Fig. 45

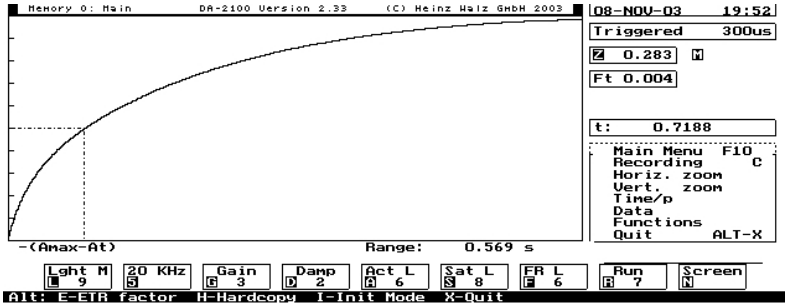


Fig. 46

At any time, t, a certain partial area, At, is given, which is zero at the left curve limit (normally trigger point) and eventually approaches Amax at the right curve limit. The resulting "area growth curve" corresponds to the rise in At, which can be directly compared to the rise in Ft.

The area growth kinetics reflect the Q-reduction kinetics, provided that reoxidation of Q is negligible (e. g. due to presence of DCMU). These kinetics may be further analysed by a logarithmic plot, making use of the F4-function (y-axis log). With F4 any selected curve or curve segment is transformed according to the function ln(Yt-Ymin) (see below). In the given application, (Yt-Ymin) corresponds to the area function (Amax-At), as Yt=Amax-At and Ymin=0. The ordinate scale is divided into 4 units, from 0 (top) to (-4). The slope of the displayed log-function is a measure for the rate constant of photochemical charge separation (provided there is no Q-reoxidation).

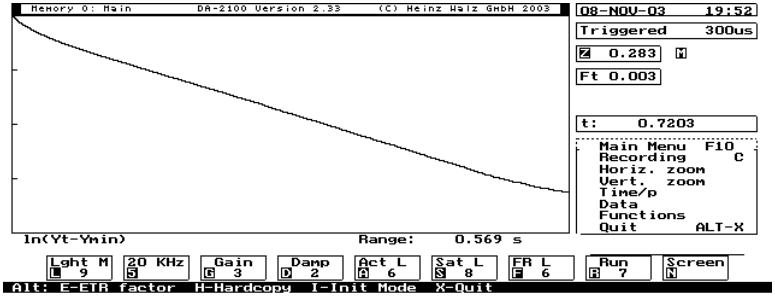


Fig. 47

With PS II heterogeneity (e. g. different unit sizes of so-called α- and β-units), several phases may be apparent in the logarithmic plot. Often the slower component displays a linear phase (particularly following appropriate manipulation of Fm and of Amax) which can be extrapolated to time zero. In this way, it is possible to estimate how much of the total area (i. e. the total acceptor pool) is corresponding to the slower ß-units. For example, when extrapolation yields ln(Amax-At)=-1, this means that (Amax)β=e-1=0.368, i. e. 37 % of all centers are β-centers.

- **Y-axis log F4**
  Semilog. plot to linearize exponential kinetic components
  With F4 a given curve or curve segment is plotted according to the function ln [(Yt-Ymin)/(Ymax-Ymin)].
  This function becomes **ln(Yt-Ymin)** when the normalization parameter (Ymax-Ymin)=1. The ordinate scale is divided into 4 units, from 0 (top) to (-4) below. This logarithmic plot is particularly useful for the analysis of decay kinetics. Exponential kinetic components become linearized and the corresponding rate constants can be determined. For this purpose, first the trigger address is defined as left curve limit (see 7.1.2) with the corresponding function value being Ymax. The right curve limit should be selected such that Yt asymptotically approaches Ymin.

An example for application of F4 was already given above in conjunction with the area growth analysis. In another example below the fluorescence relaxation kinetics following a 1 s pulse of strong actinic light (setting 11 with LED source) are shown. The logarithmic plot ln(Yt-Ymin) displays two line segments with different slopes corresponding to exponential decay components with different rate constants. With the (Yt-Ymin) value approaching zero, inevitably the electronic noise of the measuring system becomes more emphasized.

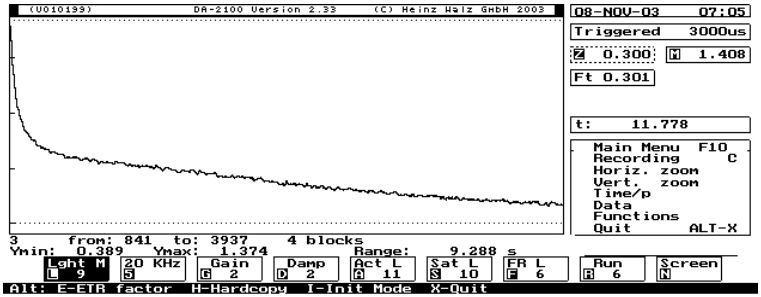


Fig. 48

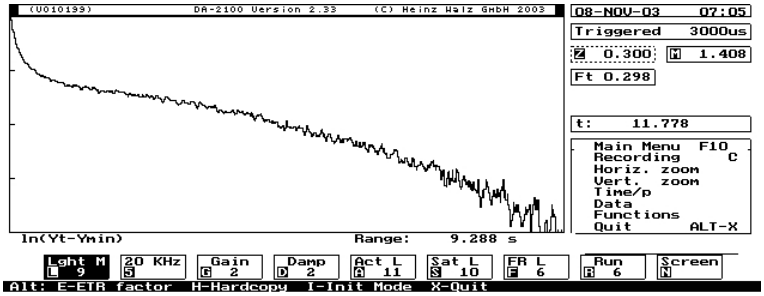


Fig. 49

- **Next memory F5**
  Select next memory

This function is not shown in the Functions sub-menu, but rather in the Memory sub-menu (see 7.1.5).

- **Mov. average F6**
  Curve smoothing by averaging (4, 8, 16 or 32) neighboring points
  In addition to curve smoothing by data point averaging that is automatically performed after every recording, the F6 function serves for additional smoothing by **"moving average"**. The F6 function may be applied several times on the same curve, with the special feature that the number of averaged points is increased with each application. Naturally, there is a limit when the smoothing affects the kinetics that are of interest.
  It is the principle of the "moving average" routine that for each image point its average with the neighboring points is calculated. With the first application of F6 four neighboring points are taken, with the second 8, than 16 and eventually 32 neighboring points.

- **Stretching F7**
  Factor for vertical stretching of a given record
  This function is also a point in the **Vertical zoom sub-menu** (see 7.1.3).
  The pre-set value is 1.0. It can be changed by typing the desired value and Return. The Fo-line stays in its original position, in analogy to the "autocenter" function commonly used with digital oscilloscopes. The stretching factor always refers to the original curve that can be recalled at any time by returning to the factor 1.0.
  The stretching factor is automatically re-set to 1.0 when a new recording is started. This is in contrast to the vertical scaling that can be modified via the Y scale-function (F8).
  Changing the vertical stretching factor is also possible via F7 without entering the Main Menu.

- **Y-scale F8**

To change scaling of Y-axis for this and following recordings

The scaling of the Y-axis is based on the **Y-limit (maximal amplitude).** The pre-set value is 2.0 volt, which is divided into Fo-units (up to 12Fo). With Fm/Fo ≈ 5 for most healthy leaves, and with Fo being adjusted to a value slightly below 400 mV, the Y-axis will be divided into 5xFo and an induction curve is unlikely to exceed the Y-limit.

In principle, it is also possible to work with a lower Fo and a correspondingly lower Y-limit. However, in this case the signal/noise ratio may become limited by digital noise. When **"0"** is entered as **Y-limit**, there is **autoscaling**, i. e. for any chosen curve segment the **Ymax** value is automatically taken as Y-limit. Autoscaling is also figuring as a separate point in the Vertical zoom sub-menu.

A given scaling is maintained as long as it is not changed or the program is newly initialized. In this respect, there is a basic difference to Stretching F7.

Changing Y-axis scaling is also possible via F8 without entering the Main Menu.

- **x-axis log F9**

  Logarithmic time scale to stretch early kinetics and compress late kinetics

  This function is not shown in the Functions sub-menu, but rather in the **Horizontal zoom sub-menu** (see 7.1.2).

7.1.7 Quit Alt X

To leave program and to switch off PAM-2100

By entering this command, the user leaves the DA-2100 program and switches off the PAM-2100. Doing so, all instrumental settings are stored in a **Configuration file** (see 6.1.22) and are re-installed when the instrument is switched on again.

8 Standard experiments (Run-files)

A Run-file represents a pre-programmed sequence of commands that are separated by defined time periods. In addition, for each Run-file specific instrument settings are defined, which provide optimal conditions for a particular experiment. With the help of the Run-files standard experiments can be carried out, which are outlined in the following sub-sections. Such standard experiments are useful for a number of reasons:

- the unexperienced user is introduced to the various types of measurements possible with the PAM-2100 Fluorometer.
- experimental protocols and instrument settings are strictly reproducible
- running standard experiments, in particular Run-file 10, provides a convenient means of testing proper functioning of the instrument.

The given Run-files may be modified by the user and completely new **User-Runs** can be defined (see 8). Before selection of a Run-file (see 6.1.13), the appropriate mode of data acquisition must be installed via the point **Mode Selection** in the Com-menu or **the Alt I-command** using an external keyboard (see 5.1). For Runs 1-5 and Runs 8-9, this is the Saturation Pulse Mode, for Runs 3 and 4 optionally also the Continuous Mode, for Runs 6-7 the Triggered Mode and for Run 10 the Continuous Mode. Before starting a Run-file for the first time after mode selection, the Run-file specific instrument settings are initialized via the point **Run-specific Settings** in the Com-menu or the **I-command** using an external keyboard.

The Run-parameter field can be selected by the cursor or the **Ctrl R-command** (with external keyboard), then the Run-number is chosen by '+' and '-' operation and the run-specific instrument settings initialized as described above. With the standard experiments a

sample-to-fiberoptics distance of 5-10 mm is assumed. The most appropriate distance depends on the particular Run-type. For example, using Run 1 in field experiments a larger distance is recommended, in order to avoid sample shading by the fiberoptics. On the other hand, with those Runs, which only involve artificial illumination, a closer distance is appropriate. In any case, it must be avoided that Fm or Fm' exceed the saturation value of 2500.

A selected Run can be started by Return, via the **Run-key** or via the **R-key** using an external keyboard. Furthermore it can be also started by remote control, when the cursor is on the R-parameter field, using the **remote button** of the **Leaf-Clip Holder 2030-B** (see 3.3.3). The latter is particularly useful with Runs 1-2. Runs operating in the Trig. or Cont. Mode can be started while still on the Par. Screen. The Kin. Screen and the appropriate sampling time are automatically installed.

During the course of a Run, instrument settings cannot be changed manually. A Run can be interrupted via the Shift+Run key or by the B-command (break) using an external keyboard. In this case manual control is re-installed and a kinetic recording will continue with the given instrument settings until stopped via Esc. It should be avoided to start a Run while a Pulse sequence is activated, because this will result in mulfunctioning (see 6.1.11).

8.1 Run 1: Determination of 'Yield' (ΔF/Fm')

Mode:	Sat. Pulse Mode		
Settings:	1 Int:	9	(Meas. light intensity)
	20 kHz is permanently installed		
	Auto 20 K is not active		
	G	4	(Gain)
	3 Int:	9	(Sat. pulse intensity)
	Other settings standard		

After Run selection please initialize the Run-specific settings.

Run 1 is particularly useful for rapid screening of photosynthetic yield in conjunction with the Leaf-Clip Holder 2030-B (see 3.3.3). Then yield-measurements can be triggered by remote control. The on-line calculated fluorescence parameters Yield (ΔF/Fm') and ETR (see 6.1.6) are automatically written into the Report-file (see 6.1.17). For highest accuracy the data from a number of measurements may be averaged (see 6.1.5).

Attention: The pre-set Gain 4 may be too high, if the leaf-to-fiberoptics distance is small and/or non-photochemical fluorescence quenching is low. In any case, it must be assured that Fm' does not exceed the saturation value of 2500 (see 6.1.4).

8.2 Run 2: Determination of Fv/Fm

Mode: Sat. Pulse Mode

Settings: Standard, except that the measuring light is on
After Run selection please initialize the Run-specific settings.

Run 2 is particularly useful in conjunction with the Leaf-Clip Holder 2030-B (see 3.3.3). The Fo, Fm-measurements and Fv:m-determination can be triggered by remote control. The measured parameters are automatically written into the Report-file (see 6.1.17).

Run 2 should be used with samples after defined periods of dark-adaptation that does not necessarily involve strict darkness (see 6.1.1). For the sake of evaluating the quantum yield of open PS II reaction centers, it is sufficient to shade the sample from direct sun or sky light. PAR-levels of 20-40 µmol quanta $m^{-2}s^{-1}$ normally are tolerable. For strict dark-control special leaf-clips are available (see 3.6), which can be attached beforehand to the leaves.

A relevant application of Run 2 is for assessment of photoinhibition at the level of PS II, which is characterized by a type of non-photochemical fluorescence quenching that recovers only slowly (if at all) in the dark (see also 8.5).

8.3 Run 3: Induction curve with quenching analysis at 10 ms/p sampling rate

Mode: Sat. Pulse Mode or Cont. Mode

Settings: Standard

After Run selection please initialize the Run-specific settings.

Depending on the light conditions during growth the standard actinic intensity (2 Int: 9) may require modification for optimal induction behaviour.

Run 3 normally involves the measurement of a fluorescence induction curve with on-line quenching analysis. (In principle, this experiment can be also run without quenching analysis, in which case Cont. Mode must be activated). After start of Run 3, the Kinetics Screen and a sampling rate of 10 ms/p are automatically installed (if not already selected), the measuring light is switched on, and a saturation pulse is applied for Fo, Fm-determination. Then the kinetic recording is started. At the given sampling rate of 10 ms/p the complete run takes 5 min 20 s.

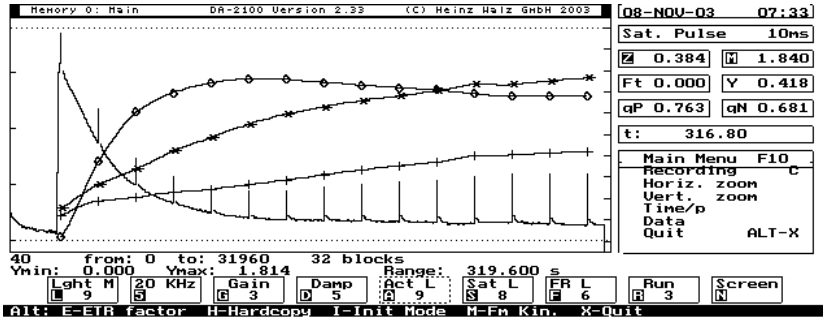


Fig. 50

As shown in the figure, fluorescence yield relaxes after the Fm-determination and approaches the Fo-line before actinic illumination is started and the sequence of Sat. pulses is initialized. After every Sat. pulse, the values of Y, qP and qN are calculated and displayed numerically in the corresponding fields and in form of stepped lines in the graphics area. After termination of the recording, the Y, qP and qN lines are substituted by different symbols (+ for Y, * for qP and ◊ for qN). The relevant information is also automatically stored in the Report-file (see 6.1.17).

When Run 3 is started in the Cont. Mode, the experimental protocol is identical, but no quenching analysis is performed.

8.4 Run 4: Induction curve with quenching analysis at 30 ms/p sampling rate

Mode: Sat. Pulse Mode

Settings: 7 0.1s : 12 (Sat. pulse length)
Other settings standard
After Run selection please initialize the Run-specific settings.

Depending on light conditions during growth, the standard actinic intensity (2 Int: 9) may require modification for optimal induction behaviour.

Run 4 involves the measurement of a fluorescence induction curve with on-line quenching analysis. After start of Run 4 the Kinetics Screen and a sampling rate of 30 ms/p are installed (if not already selected), the measuring light is switched on, and a Sat. pulse is applied for Fo, Fm-determination. Then the kinetic recording is started. At the given sampling rate of 30 ms/p the complete run takes 16 min.

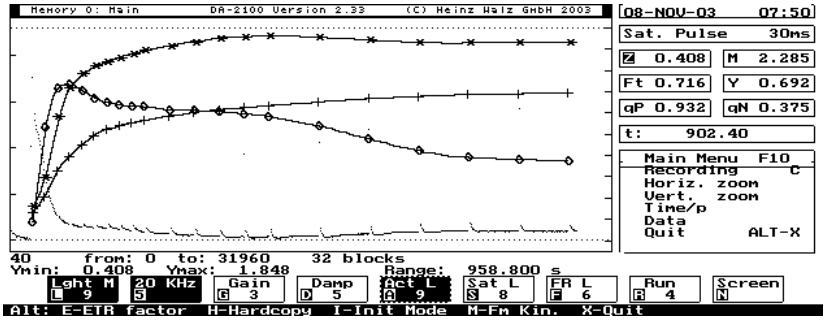


Fig. 51

The figure shows some fluorescence relaxation towards Fo before the actinic light is switched-on and a sequence of Sat. pulses is started. After 10 pulses at 20 s intervals, 5 pulses at 40 s intervals and finally 6 pulses at 80 s intervals are applied. After every Sat. pulse, the values of Y, qP and qN are calculated and displayed numerically in the corresponding fields and in form of stepped lines in the graphics area. After termination of the recording, the Y, qP and qN lines are substituted by different symbols (+ for Y, * for qP and ◊ for qN). The relevant information is also automatically stored in the Report-file (see 6.1.17).

Run 4 differs from Run 3 in three respects:

- the sampling rate is 30 ms/p instead of 10 ms/p
- the interval between consecutive saturation pulses is not constant at 20 s (as with Run 3), but increases first to 40 s and then to 80 s
- the saturation pulse length is 1.2 s instead of 0.8 s.

8.5 Run 5: Relaxation kinetics of qN

Mode: Sat. Pulse Mode

Settings: Measuring light at setting 6 is on
Actinic light at setting 9 initially is on
7 0.1s : 12 (Sat. pulse length)
Other settings standard
After Run selection please initialize the Run-specific settings.

It may be advantageous, to modify the intensity of the actinic light, in order to induce a differen extent of non-photochemical quenching, the relaxation of which is studied. Also the duration of actinic illumination may be varied. The user may prefer to apply Run 5 shortly after Run 3 or Run 4, during the course of which a sample has already reached a steady light-state and has developed non-photochemical fluorescence quenching. In this case, the actinic light should be turned-on again, after Run 3 or Run 4 is terminated. The kinetic information obtained during Run 3 or 4 may be first stored (see 6.1.5) before Run 5 is started. In any case, the on-line calculated quenching parameters are stored in the Report-file. Fo and Fm-values obtained during Run 3 or 4 remain valid for quenching analysis in Run 5. If not determined in preceding Runs 3 or 4, Fo and Fm must have been determined (Fm-key or M-command using external keyboard) with a dark-adapted sample before actinic illumination. Fo and Fm values may also be entered manually using the Del-command (see 6.2.1).

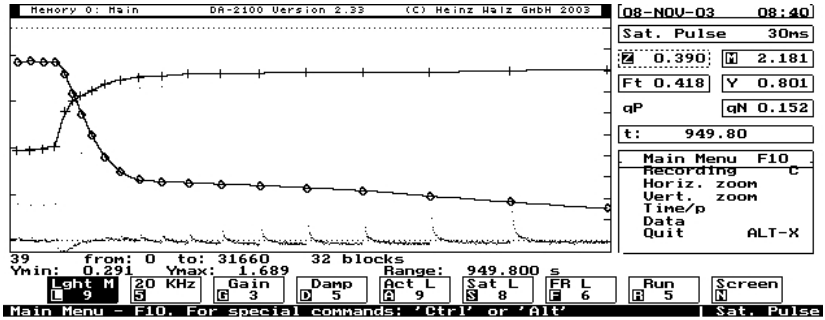


Fig. 52

Following start of Run 5 the Kinetics Screen and a sampling rate of 30 ms/p are selected (if not already installed) and a recording is started. First actinic illumination is continued and two Sat. pulses are applied to assess quenching parameters in the light-state. Then actinic light is switched off and in the following dark period a number of Sat. pulses is applied with exponentially increasing time intervals between consecutive pulses: 10 s, 12 s, 14 s etc. according to the function:

$$y = 10 \times 1.2n\text{-}1$$

Run 5 is terminated after 16 min. The data are automatically stored in the Report-file (see 6.1.17). On the graphics screen, for each Sat. pulse the on-line calculated values of Y, qP and qN are displayed. Actually, in the given context qP is of no interest and the corresponding data points may be removed by selecting the qP-parameter field via cursor movement and Return (see 6.2.3). In the given example, in order to produce strong qN, the halogen lamp was used.

The relaxation kinetics of qN bear information on different types of non-photochemical quenching developed during illumination. Non-photochemical quenching reflects an increased yield of non-

radiative dissipation of excitation energy. Such dissipation lowers the quantum yield of open PS II centers, which after turning off the actinic light is indicated by the Yield-parameter (ΔF/Fm'). The second phase of Yield-recovery parallels qN-relaxation (see also 6.1.7).

8.6 Run 6: Rapid induction kinetics at 1000 µs/p

Mode: Trig. Mode

Settings:

1 Int:	10	(Meas. light intensity)
2 Int:	7	(Act. light intensity)
6 s :	2	(Act. illumination time)
8 s :	0	(Far-red illumination time)
G	1	(Gain)

Other settings standard

For maximal sensitivity, a short distance between sample and fiberoptics is recommended. The induction transients strongly depend on the dark-adaptational state.

After Run selection please initialize the Run-specific settings.

With Run 6 the rapid induction kinetics at a sampling rate of 1000 µs/p are measured. When the Run is started, the Kinetics Screen and 1000 µs/p are selected (if not yet installed). Then the measuring light is switched on and Fo is determined shortly before the actinic light is switched on for 2 s. During actinic illumination the modulation frequency is increased to 20 kHz. As the total recording lasts 4 s, not only the fluorescence rise but also the relaxation kinetics are recorded.

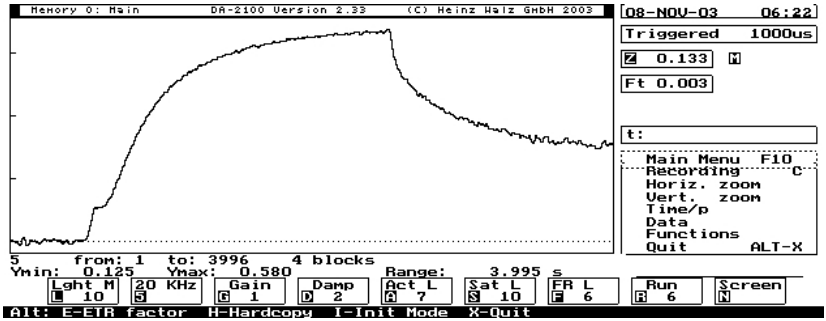


Fig. 53

The rapid induction kinetics display two major rise components as well as two well separated relaxation components, which give information on the light-driven reduction and dark-reoxidation of different pools of PS II acceptors. When this experiment is carried out in the presence of far-red background light, reoxidation is accelerated due to electron pumping by PS I.

For further analysis of the rapid induction kinetics special Functions are provided (see 7.1.6).

8.7 Run 7: Rapid induction kinetics at 300 µs with log time scale

Mode:	Trig. Mode		
Settings:	1 Int:	10	(Meas. light intensity)
	2 Int:	10	(Act. light intensity)
	G	1	(Gain)
	6 s:	2	(Act. illumination time)
	8 s:	0	(Far-red illumination time)

Other settings standard

To obtain maximal sensitivity and actinic intensity, it is recommended to choose a short distance between sample and fiberoptics. After Run selection please initialize the Run-specific settings.

With Run 7 the rapid induction kinetics are measured at a sampling rate of 300 µs/p, resulting in a total recording time of 1.2 s. As the actinic illumination period is set to 2 s, no dark relaxation kinetics are recorded. This Run may serve to demonstrate a number of special features of kinetic analysis with the PAM-2100: When Run 7 is started, the Kinetics Screen and 300 µs/p are selected (if not yet installed). Then the measuring light is switched on and Fo is determined shortly before the actinic light is switched on for 2 s. During actinic illumination the modulation frequency is increased to 20 kHz. After the recording, automatically there is autoscaling (see 7.1.3) and definition of the curve origin at the trigger point (see 7.1.2). Furthermore, the curve is redrawn with a logarithmic time scale (see 7.1.2 and 7.1.6).

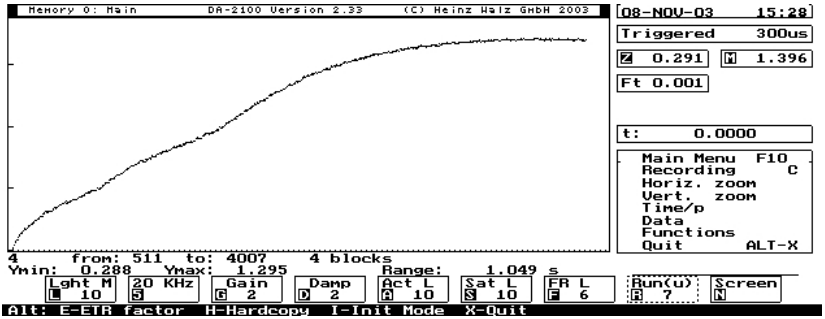


Fig. 54

With the logarithmic time scale the resolution of the early fluorescence rise phases is enhanced. In this presentation at least three well separated transients with two intermediate plateaus can be distinguished. The various phases contain information on donor- and acceptor-side properties of photosystem II. For returning to the linear time scale, the user may redraw the original curve via O Return Return or via the J-command, in which case the Join-status is changed (see 7.1.2). Getting back to the log time scale requires the

commands F 10, H and X, which can be given in quick sequence (external keyboard required).

8.8 Run 8: Light response curve (running 76 min)

Mode: Sat. Pulse Mode

Settings: 2 Int: 8 (Initial actinic light intensity)
Auto 20 K-function off
3 Int: 9 (Sat. pulse intensity)
8 s: 0 (Far-red illumination time)
Other settings standard
After Run selection please initialize the Run-specific settings.

Run 8 provides a convenient means for assessment of the light intensity dependence of a sample. Taking into consideration the photosynthetic capacity of a sample, the absolute range of actinic intensities may be changed by modifying the distance between fiberoptics and sample surface. It is not recommended to use the halogen lamp as actinic light source for this Run, as with the long illumination times involved this would produce excessive heating of the instrument. Other reasons in favor of the LED actinic source are the much lower power consumption, less sample heating and the constancy of spectral output that only in the case of the LED lamp is independent of intensity settings. On the other hand, in many cases (particularly with outdoor plants) the maximal intensity obtained with the LED lamp may be too low to saturate photosynthetic electron transport.

```
Run-file 8: Standard experiment with dark-adapted dandelion leaf;
        Fo-Fm determination before start of Run 8; Fo'-mode inactive

08-NOV-03      ML   Tmp.   PAR    Fo          Fv/Fm              Fm
09:55:34      189   22.1     1  0.416         0.809            2.175
```

Time	No.	ML	Tmp.	PAR	Ft	ETR	Yield	qP	qN	Fm'	Fo'
10:12:05	2	190	22.0	23	0.439	7.2	0.747	0.983	0.249	1.736	
10:18:35	3	191	21.9	33	0.464	10.2	0.738	0.965	0.229	1.771	
10:25:05	4	191	21.7	48	0.471	14.7	0.731	0.959	0.240	1.753	
10:31:35	5	191	21.7	68	0.476	20.7	0.726	0.954	0.249	1.738	
10:38:05	6	191	21.9	104	0.494	31.1	0.712	0.940	0.262	1.714	
10:44:35	7	191	21.9	148	0.508	43.5	0.700	0.928	0.274	1.693	
10:51:05	8	190	21.9	222	0.535	62.7	0.673	0.902	0.307	1.635	
10:57:35	9	189	21.9	320	0.553	84.4	0.628	0.873	0.392	1.486	
11:04:05	10	189	21.7	465	0.506	109.8	0.562	0.878	0.579	1.156	
11:10:35	11	188	22.1	665	0.493	141.2	0.506	0.868	0.670	0.996	
11:17:05	12	188	22.1	863	0.454	168.1	0.464	0.913	0.755	0.846	

Fig. 55

During the course of Run 8 the changing actinic intensities and the on-line calculated data can be followed on the Parameter Screen. At the end of Run 8 the Report-file is automatically loaded and the measured data are displayed. In the given example, before start of the Run Fo and Fm were determined. The correctness of these values depends on the dark-adaptation state of the sample. Actually, in many cases it is preferable to use light-adapted samples and concentrate on Yield- and ETR-measurements, for which previous Fo, Fm-determination is not required. It is also possible to enter Fo and Fm values manually using the Del-command (see 6.2.1). After start of the Run there is first a 10 min illumination period at an intermediate light intensity (2 Int: 8), which serves for light adaptation of the sample and activation of Calvin cycle enzymes. Then actinic intensity is switched to setting 1 and the setting number is increased every 6.5 min until setting 11 is reached. During the last minute at each setting the quenching parameters are assessed with a Sat. pulse.

For automatic recordings of PAR, Tmp and ETR the Leaf-Clip Holder 2030-B is required. If this is not available, it is sufficient to determine actinic intensity at one setting. All other intensities can be calculated on the basis of the table presented in section 6.1.10. For correct determination of qP and qN it is necessary to install the Fo'-mode before start of Run 8 in order to determine Fo' at each light intensity (Shift+Fm-key or Ctrl S-command using an external keyboard) (see 6.1.2).

A principal problem with light intensity dependencies of steady-state parameters is caused by the fact that the times to reach a "true" steady-state may be rather long. Hence, the 10 min adaptation time at an intermediate intensity and the 6.5 min periods at each intensity should be considered a compromise for the sake of practical feasibility. Still shorter illumination times are used in Run 9 (see 8.9). There is the possibility to modify the Standard Runs by defining e. g. different adaptation times in so-called User-Runs (see 9).

8.9 Run 9: Light response curve (running 33 min)

Mode: Sat. Pulse Mode

Settings: Meas. and Act. Light initially on
2 Int: 1 (Initial actinic intensity)
Auto 20 K function off
3 Int: 9 (Sat. pulse intensity)
8 s: 0 (Far-red illumination time)
Other settings standard
After Run selection please initialize the Run-specific settings.

Run 9 provides a rapid means of assessing the light saturation properties of a sample. It is assumed that the sample already has been light activated by pre-illumination, preferentially in its natural light environment. Therefore, the Run as such does not include Fo, Fm

determination. If besides Yield also qP and qN are of interest, Fo and Fm should be determined beforehand. It is also possible to enter values for Fo and Fm via the Del-command (see 6.2.1). For correct qP and qN determinations Fo-quenching must be considered. In this case, before starting Run 9, the Fo'-mode should be installed (Shift+Fm-key or Ctrl S-command using an external keyboard). Also, the user should make sure that actinic light at setting 1 is already on before starting the Run. This is the case after initialization using the I-command.

The absolute range of actinic intensities may be changed by modifying the distance between fiberoptics and sample surface. For automatic recordings of PAR, Tmp and ETR the Leaf-Clip Holder 2030-B is required. If this is not available, it is sufficient to determine actinic intensity at one setting. All other intensities can be calculated on the basis of the table presented in section 6.1.10. It is not recommended to use the halogen lamp as actinic light source in this Run, although contrary to Run 8 the heating of the instrument is less excessive, because of the shorter illumination times. With a single Run 9 more than half of the internal battery power would be used up. Further arguments in favor of the LED actinic source are less sample heating and the constancy of spectral output that only in the case of the LED lamp is independent of intensity settings. On the other hand, in many cases (particularly with outdoor plants) the maximal intensities obtained with the LED lamp may be too low to saturate photosynthetic electron transport.

```
Run-file 9: Standard experiment with preilluminated dandelion leaf
        Fo-Fm determination before preillumination; Fo'-mode

08-NOV-03      ML   Tmp.   PAR    Fo           Fv/Fm                Fm
11:32:37      189   22.2     1  0.421          0.817              2.300

  Time     No.  ML   Tmp.   PAR    Ft     ETR   Yield   qP    qN     Fm'  Fo'
```

11:48:06	2	191	21.7	23	0.411	7.5	0.778	0.979	0.234	1.850	0.380
11:51:11	3	191	21.9	33	0.416	10.7	0.775	0.976	0.236	1.846	0.381
11:54:16	4	191	22.0	48	0.433	15.4	0.764	0.965	0.244	1.831	0.381
11:57:21	5	191	22.0	68	0.440	21.7	0.759	0.959	0.247	1.825	0.381
12:00:26	6	191	22.2	103	0.475	31.7	0.733	0.931	0.271	1.779	0.379
12:03:31	7	190	22.2	148	0.479	43.4	0.698	0.909	0.369	1.588	0.368
12:06:36	8	189	22.4	221	0.473	58.8	0.634	0.864	0.516	1.290	0.344
12:09:41	9	189	22.5	319	0.458	77.4	0.578	0.832	0.618	1.084	0.331
12:12:46	10	189	22.8	464	0.444	102.3	0.525	0.789	0.687	0.934	0.313
12:15:51	11	189	22.9	663	0.448	132.3	0.475	0.741	0.726	0.853	0.306
12:18:56	12	188	23.3	862	0.453	158.5	0.438	0.696	0.747	0.805	0.299

Fig. 56

During the course of Run 9 the changing actinic intensities and on-line calculated data can be followed on the Parameter Screen. After Run-termination the Report-file is automatically loaded and the measured data are displayed. Starting at intensity 1, the sample is illuminated at each intensity setting for 3 min, at the end of which a saturation pulse is applied to assess fluorescence parameters.

During the relatively short illumination periods at the various light intensities a "true" steady-state cannot be reached. This should be considered with evaluation of the results. Still, the obtained information is characteristic for the quantum efficiency and photosynthetic capacity of a given plant sample. Longer illumination times are provided by Run 8 (see 8.8). Furthermore, the pre-programmed times may be modified by definition of so-called User-Runs (see 9).

8.10 Run 10: Instrument self-test

Mode: Cont. Mode or preferentially recorded via analog output on chart paper

Object: Blue-plastic fluorescence standard

Initial settings:

1 Int:	1	(Meas. light intensity)
G	10	(Gain)
D	7	(Damping)
3 Int:	10	(Sat. pulse intensity)
4 Int:	10	(Far-red light intensity)
7 0.1s:	12	(Sat. pulse duration)
8 s:	0	(Far-red illumination time)

Other settings standard

After Run selection please initialize the Run-specific settings.

Before Run 10 is started, the following preparations have to be made: The Cont. Mode is selected. After selection of Run 10 the run-specific settings should have been initialized. The distance between the blue-plastic fluorescence standard and the fiberoptics is adjusted such that at the given measuring light intensity (1 Int: 1) and gain (G 10) the signal amplitude is 500-800 mV. When this is the case, 1/10 of this value is entered manually as Fo-value using the Del-command (see 6.2.1) and the y-axis limit is set to 1 V using the F8-command (see 7.1.3). Before the Run is started the measuring light should be off.

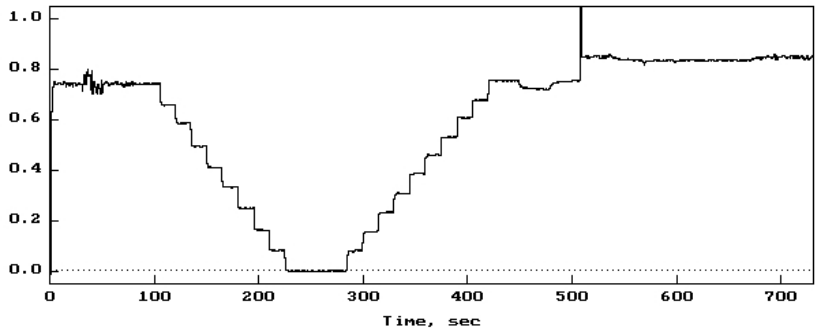


Fig. 57

Run 10 provides a self-test of some essential instrument functions, including system sensitivity, signal/noise ratio, gain and measuring light settings, selectivity to modulated signal and dynamic performance. The various aspects are denoted by numbers in the presented example and shall be briefly outlined:

1 Signal amplitude at L1 G10. At a given distance this amplitude is a measure of system sensitivity, which will decrease with ageing of the measuring light LED (see 6.1.8) and of the fiberoptics (see 3.2). The former can be more conveniently assessed by the ML-Parameter (see 6.1.8).

2 Signal/noise ratio at L1 G10 D3 600 Hz. Under the given unfavorable conditions the noise band is rather broad.

3 Improvement of signal/noise by increasing modulation frequency to 20 kHz. The system performs normal, if the noise band is decreased by a factor of approx. sqrt (20 : 0.6) = 5.7

4 Gain at 10 different settings. In good approximation the 9 steps should be equal.

5 Measuring light intensity at 11 different settings. In first approximation the 10 steps should be equal. For technical reasons, absolute linearity is not possible.

6 Signal decline at maximal measuring light intensity upon switching to 20 kHz modulation frequency. A 1-2 % reversible decline is normal; it reflects the transient heating of the measuring light LED and is also indicated by the ML-parameter (see 6.1.8).

7 Lack of appreciable signal decline upon switching to 20 kHz at standard measuring light intensity. Under standard measuring conditions the signal amplitude should not be affected by switching to high modulation frequency.

8 Signal decline caused by high intensity saturation pulses. It is normal that at a high Sat. pulse intensity setting (S10) the signal amplitude is decreased by 1-2 %. This effect reflects a small non-linearity of the detecting system at very high signal levels.

9 Lack of appreciable signal decline by moderate intensity Sat. pulses. The decrease caused by S6 should be less than 1 %. In this order of magnitude the effect on Fm determination may be considered negligible.

10 Lack of a noticeable increase of noise when high intensity far-red light is applied. As no filters protect the detector against far-red light, this is a good test for the system selectivity.

Running this self-test (total recording 15 min) will help to assess the normal performance of the instrument and can also be of use for trouble-shooting.

9 User-Run files

Whereas the standard Run-files (see 8) are incorporated in a fixed form in the DA-2100 program, so-called User-Runs can be programmed by the user. User Runs then can substitute for the Standard Runs. A special routine is provided to **Write/Read User Run-files to/from disk**. When the cursor is on the Run parameter field (e. g. via Ctrl R using an external keyboard), the selection menu for Write/Read Runs is opened via **Shift+Run** or the **AltF10-command** using an external keyboard (see 6.1.21).

The total number of active Runs is always 10, with each number representing either a Standard or a User Run. The latter are recognised by the program and indicated by a **(u)** in the Run parameter field.

9.1 Modification of Standard Runs

It is recommended to define User Runs by modification of the existing Standard Runs at a given mode of data acquisition. Hence, e.g. Runs involving the Triggered Mode of data acquisition should carry the numbers 6 or 7. This has the advantage that the initial instrument settings, which are installed via the point **Run-specific Settings** in the **Com-menu** or by the **I-command** using an external keyboard, will be close to the desired settings.

For modification of a Standard Run at a given mode of data acquisition, the following steps have to be carried out (use of an **external keyboard** is assumed):

1. Move the Cursor on the **Run-parameter field** via the **Ctrl R-command**.

2. Enter **Write/Read selection menu** via **Alt F10** (see 6.1.21), while in the relevant mode of data acquisition.

3. **Activate Write** and enter the name **Standard** (type Standard and confirm by Return). Now the Standard Run files that apply to the given mode of data acquisition are written to the "harddisk" of the PAM-2100 (flash-card) under the name **Standard. Run**.

4. Open the **Editor** via the **Edit-key** or the **Ctrl E-command** (see 6.1.17).

5. Type the Wordstar-command **Ctrl KR** (see 12.4). Now at the top of the screen the **File to read** can be typed, which is **Standard.Run**. After this name was entered via Return, the definitions of those Standard-Runs are displayed, which can be used with the given mode of data acquisition. For the Sat. Pulse Mode, this is Runs 1-5 and 8-10. For the Cont. Mode, this is Runs 3, 4 and 10. And for the Trig. Mode, this is Runs 6 and 7.

6. For modification of the given Run-files normal **Wordstar editor commands** apply (see 12.4) and a **simple syntax** is used (see 9.2).

7. After modification, the text block corresponding to the edited Run is marked with the help of the **Ctrl KW-command**: Definition of block start (entered via Return) and definition of the block end (entered via return).

8. For **File to write** (dialog line at the top of the screen) type the new name, e. g. R3U1.Run (i. e. the first modification of the Standard Run 3) and enter via Return. In this way, an almost unlimited number of such user defined Runs can be derived and stored on disk (flash card within the PAM-2100).

9. Leave the Editor via **Esc** and return to the Parameter Screen or Kinetic Screen levels. Before a User Run can be applied, it has to be defined to replace the Standard Run.

10. Move the Cursor on the **Run-parameter field** via the **Ctrl R-command** and enter the **Write/Read selection menu** via **Alt F10** (see 6.1.21), while in the relevant mode of data acquisition.

11. Activate **Read** and enter the name of the User Run (e.g. type R3U1 and confirm by Return). Now a **(u)** appears in the Run parameter field showing that the corresponding User Run is successfully installed.

9.2 Syntax for User-Runs

User-Runs are defined as ASCII-files (Name.Run). The DA-2100 provides a routine for editing already existing Standard-Runs (see 9.1) or defining new User-Runs. At the editor-level (opened via the Edit-key or the Ctrl E-command) the following **syntax** is used for **programming a User Run**:

#1 ... 10	;	Run number (first program line)
1 ... 6200,	;	time point in sec (preceding the commands)
A ... Z	;	single key commands
CR	;	Return
ESC	;	Escape
HOME	;	Cursor home (Pos. 1)
END	;	Cursor end
LEFT	;	Cursor left
RIGHT	;	Cursor right
UP	;	Cursor up
DOWN	;	Cursor down
F1 ... F10	;	Function keys
^	;	Ctrl key
"...."	;	String delimiter (to enter text)

Each command is written into a new line. The time at which the command is given, is written before the command (in sec after the start of the Run), separated by a ' , ' (comma). In addition, after each command an explanatory text can be written, separated by a ' ; ' (semicolon). The maximal number of command-lines is 256.

A Run consists of a sequence of commands, which are programmed to be carried out one after the other at defined times following Run-start with an accurracy of +/- 0.5 sec. In practice, it has to be considered that the time required to carry out a certain command is variable and also depending on the type of computer used.

Writing a User-Run program with the routine provided by the DA-2100 is quite simple, if the user is already experienced with the key operations controlling instrument settings and data analysis (see 6 and 7). In order to get acquainted with the syntax and command structure, it is recommended to study the definition of the Standard Runs (see 8 and 9.1).

In the following list, the **commands** involved in the **10 Standard Runs** are briefly explained:

#1	;	**Run 1, Sat. Pulse Mode**
1, N	;	change over to Par. Screen
1, L	;	turn measuring light on, immediately after finishing previous command
1, Y	;	apply Sat. pulse for quenching analysis, immediately after carrying out previous command
1, L	;	turn measuring light off, immediately after carrying out previous command
1, ^R	;	move cursor back on Run-field, immediately after carrying out previous command.

Note: The five commands involved in this Run are programmed to be carried out as fast as possible. However, as a command will be only initiated when the previous one is completed, the whole sequence takes substantially more than 1 sec, depending also on the type of computer used.

#2	;	**Run 2, Sat. Pulse Mode**
1, M	;	determine Fo and Fm
1, ^R	;	move cursor back on Run-field
#3	;	**Run 3, Sat. Pulse Mode**
1, K	;	change over to Kin. Screen
1, L	;	turn on measuring light
1, F10	;	enter Main Menu
1, T	;	select Time-submenu
1, 1	;	select 10 ms/point
7, M	;	determine Fo and Fm at 7 sec following Run-start
14, CR	;	accept Fm-determination by Return
15, C	;	start kinetic recording at 15 sec following Run-start
40, A	;	turn on actinic light at 40 sec following Run-start
42, P	;	start saturation pulse sequence for repetitive quenching analysis
335, A	;	turn off actinic light
335, L	;	turn off measuring light
335, ESC	;	stop recording and redraw kinetic traces
#4	;	**Run 4, Sat. Pulse Mode**
1, K	;	change over to Kin. Screen
1, L	;	turn on measuring light
1, F10	;	enter Main Menu
1, T	;	select Time-submenu
1, 2	;	select 30 ms/point
7, M	;	determine Fo and Fm
14, CR	;	accept Fm-determination by Return

```
45, C          ;   start kinetic recording
75, A          ;   turn on actinic light
77,Y...937,Y   ;   apply Sat. pulses at variable intervals for
                   repetitive quenching analysis
950, A         ;   turn off actinic light
950, L         ;   turn off measuring light
950, ESC       ;   stop recording and redraw kinetic traces

#5             ;   Run 5, Sat. Pulse Mode
1, K           ;   change over to Kin. Screen
1, F10         ;   enter Main Menu
1, T           ;   select Time-submenu
1, 2           ;   select 30 ms/point
10, C          ;   start kinetic recording
60, Y          ;   apply Sat. pulse for quenching analysis
80, Y          ;   apply Sat. pulse for quenching analysis
80, A          ;   turn off actinic light
90,Y...954,Y   ;   apply Sat. pulses at increasing intervals for
                   quenching analysis during dark relaxation
959, ESC       ;   stop recording and redraw kinetic traces

#6             ;   Run 6, Triggered Mode
1, K           ;   change over to Kin. Screen
1, F10         ;   enter Main Menu
1, T           ;   select Time-submenu
1, 3           ;   select 1000 µs/point
3, Z           ;   determine Fo
4, C           ;   start kinetic recording
5, L           ;   turn off measuring light
5, ^R          ;   move cursor on Run-field

#7             ;   Run 7, Triggered Mode
1, K           ;   change over to Kin. Screen
1, F10         ;   enter Main Menu
1, T           ;   select Time-submenu
1, 2           ;   select 300 µs/point
3, Z           ;   determine Fo
4, C           ;   start kinetic recording
```

```
5, L            ;   turn-off measuring light
5, F10          ;   enter Main Menu
5, H            ;   select Horizontal Zoom-submenu
5, O            ;   enter Origin-Routine
5, 5            ;   type      the trigger address 512
5, 1                (5,"512" would be equivalent)
5, 2
5, CR           ;   accept the typed address by Return
5, F8           ;   apply F8 to define Y-axis scale
5, 0            ;   type ' 0 ' for Autoscaling
5, CR           ;   carry out Autoscaling via Return
5, F9           ;   apply F9 for X-axis log scale
5, ^R           ;   move cursor back on Run-field

#8              ;   Run 8, Sat. Pulse Mode
1, N            ;   change over to Parameter Screen
1, A            ;   turn on actinic light
600, -          ;   lower actinic intensity setting by 7 steps
                    (from 8 to 1)
1, -                after 10 min illumination at setting 8
1, -
1, -
1, -
1, -
1, -
970, Y          ;   apply Sat. pulse for quenching analysis
1000, +         ;   increase actinic intensity by one step
                    (from 1 to 2)
1360,Y...4480,Y;    apply Sat. pulses at 390 s intervals with
                    actinic intensity settings being stepwise
                    increased
4510, !         ;   increase actinic intensity to setting 11
4870, Y         ;   apply last Sat. pulse
4890, A         ;   turn off actinic light
1, L            ;   turn off measuring light
1, ^E           ;   change over into Report-file

#9              ;   Run 9, Sat. Pulse Mode
```

```
1, N              ;   change over to Parameter Screen
1, 2              ;   move cursor on Act. Int. parameter-field
180, Y            ;   apply Sat. pulse for quenching analysis
185, +            ;   increase act. intensity setting by one step
365,Y...1845,Y    ;   apply Sat. pulses at 185 s intervals with act.
                      intensity settings being stepwise increased
1850, !           ;   increase act. intensity to setting 11
2030, Y           ;   apply last Sat. pulse
2050, A           ;   turn off actinic light
1, L              ;   turn off measuring light
1, ^E             ;   change over into Report-file

#10               ;   Run 10, Cont. Mode
1, K              ;   change over to Kin. Screen
1, F10            ;   enter Main Menu
1, T              ;   select Time-submenu
1, 2              ;   select 30 ms/point
5, C              ;   start kinetic recording
5, L              ;   turn on measuring light
35, D             ;   move cursor on Damping-field
1, -              ;   immediately decrease Damping-setting
                      by four
1, -                  steps (from D7 to D3)
1, -
1, -
55, 5             ;   switch to 20 kHz modulation frequency
85, D             ;   move cursor on Damping-field
1, +              ;   immediately increase Damping-setting by
                      four steps
1, +
1, +
1, +
110, G            ;   move cursor on Gain-field
1, -              ;   immediately decrease Gain-setting by
                      one step
125, - ... 230, - ;   stepwise decrease Gain-setting (from G 9 to
                      G 1) with 15 s between consecutive steps
260, 5            ;   switch to 600 Hz modulation frequency
```

```
290, LEFT                move cursor two positions to the left to place
                         it on Meas. Light field
1, LEFT
1, +             ;       immediately increase meas. light intensity by
                         one step
305, + ... 410, + ;      stepwise increase of meas. light intensity
                         (from 2 to 10) with 15 s between consecutive
                         steps
425, !           ;       increase meas. light intensity to setting 11
455, 5           ;       switch to 20 kHz modulation frequency
485, 5           ;       switch back to 600 Hz
515, -           ;       decrease measuring light intensity from
                         setting 11 to 6
1, -
1, -
1, -
1, -
1, G             ;       immediately increase Gain-setting by one
                         step
1, +
1, D
1, -             ;       immediately decrease Damping-setting by
                         two steps
1, -
545, 5           ;       switch to 20 kHz
575, S           ;       apply two Sat. pulses
605, S
1, -             ;       decrease Sat. pulse intensity by 4 steps (from
                         10 to 6)
1, -
1, -
1, -
635, S           ;       apply two Sat. pulses
665, S
680, 5           ;       switch to 600 Hz
710, F           ;       turn on far-red light
740, ESC         ;       stop recording and redraw kinetic traces
1, F             ;       immediately turn off far-red light and
                         measuring light
```

```
1, L
1, 0          ;     apply horizontal zoom by definition of curve
                    limits at first and last point of recording
1, CR
1, CR
1, V          ;     redraw recorded curve at "full screen
                    display"
```

After studying these Standard Runs, the user should be prepared to program his own User Runs, either by modification of the Standard Runs (see 9.1) or by completely new definitions. As only few storage place is involved in the Run-files, an almost unlimited amount of such User-Runs can be stored on disk.

10 Data storage and transfer

10.1 Saturation Pulse Mode

All data that are measured in the Sat. Pulse Mode are automatically saved in a so-called **Report-file** (see 6.1.17), which is created upon program installation with the name **STANDARD.RPT** and stored on the hard disk memory of the PAM-2100 (flash card).

It is recommended to empty the current Report-file after accumulation of a certain amount of data by transfer to an external Windows PC using the **Trans2100 program** (see 5.3). There are numerous ways for **editing data stored in the Report-file**. For this purpose normal **Wordstar-commands** are effective (see 12.4).

In the Sat. Pulse mode data are automatically stored in the Report-file irrespective of whether the Parameter Screen or the Kinetics Screen is active. With **kinetic recordings**, however, there is the additional possibility of storing the kinetic information via the **Store-key** or the **Write-command** using an external keyboard (see 7.1.5). In this case, actually two files are created when a file-name is entered, i.e.

- **NAME.CMP** , the actual kinetic data file,
- **NAME.ASP** , the corresponding ASCII-file of the on-line calculated quenching parameters

A CMP-file occupies a considerable amount of memory space (approx. 65000 kByte whereas an ASP-file is relatively small (approx. 700 kByte). CMP- as well as ASP-files can be transferred to an external Windows PC using the **Trans2100 program** (see 5.3). When transferred to the **PamWin-directory**, the kinetics can be

viewed and analysed under PamWin in the **View-mode.** The selection menu of CMP-files can be opened via the Import-speedbutton (see separate PamWin manual).

10.2 Triggered Mode and Continuous Mode

Kinetic data recorded in the Triggered or Continuous Mode are saved via the **Store-key** or using the **W-** or **X-commands**, if an external keyboard is available (see 7.1.5). In this way the data files **NAME.TMN** (Trig. Mode) and **NAME.CMN** (Cont. Mode) are created and stored on the hard-disk of the PAM-2100 (flash card). Data are stored in block-units, with one block consisting of 1000 points. As a block takes somewhat more than 2 kByte memory, full kinetic recordings occupy approx. 8200 kByte in the Trig. Mode and 65000 kByte in the Cont. Mode.

TMN- and CMN- files (just like CMP- and ASP-files in the Sat-Pulse Mode) can be transferred to an external Windows PC using the **Trans2100 program** (see 5.3). When transferred to the **PamWin-directory**, the kinetics can be viewed and analysed under PamWin in the **View-mode** (see separate PamWin manual).

11 Maintenance

Although the PAM-2100 Fluorometer is designed for a wide range of work environments some precautions are required to prevent malfunctioning and to extend the lifetime of its components:

- Avoid getting the PAM-2100 Main Control Unit and the Leaf-Clip Holder 2030-B wet or sandy. Even in extreme environments system operation is still possible when the sensitive parts are sealed in plastic bags.
- Avoid excessive bending of the fiberoptics, in particular close to the connector-plug.
- Make it a good habit to turn the lamps off when they are not used, thus extending their lifetime.
- Turn the PAM-2100 off (via the point Quit program or the AltX-command using an external keyboard, when no immediate measurements are planned, thus extending battery life time.
- Avoid charging the internal Li-ion battery, when the PAM-2100 is switched on.

11.1 Internal battery and its replacement

The internal battery essentially is "maintenance free". However, even when the instrument is switched off, there is some unloading that is substantially stimulated by elevated temperatures. Therefore, it is good practice to recharge the internal battery every 3 months, using the Battery Charger 2120-N. If it is foreseeable that the instrument will not be used for a period of months, the battery should be charged beforehand. Battery lifetime depends primarily on the "capacity flux", i.e. the number of full charging cycles. Under otherwise normal conditions about 200 de- and re-charging cycles may be expected.

Excessive discharge of the battery should be avoided, as it may cause irreversible damage. Such damage involves a pronounced lowering of capacity, which means that recharging is required after relatively short operation times. In this case, battery replacement is recommended.

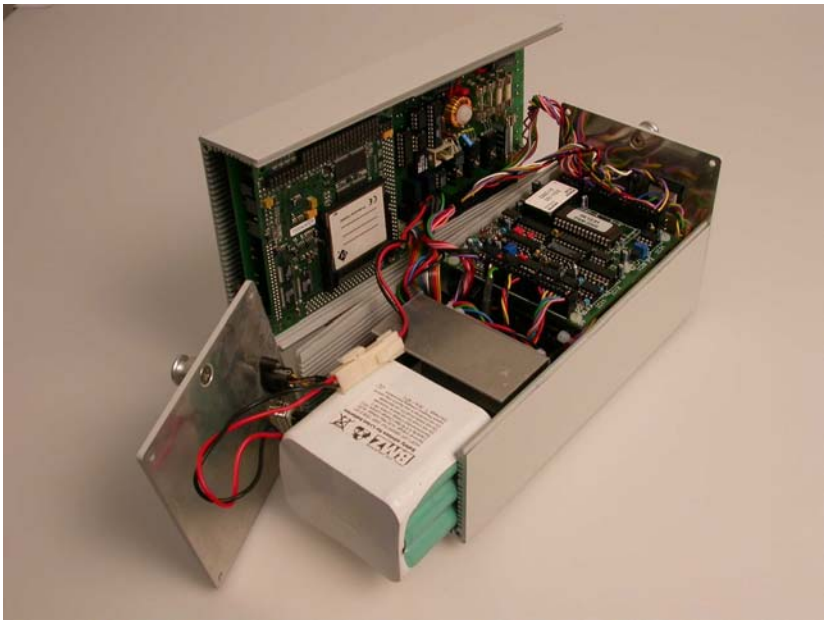


Fig. 58

The above photograph allows a view into the opened PAM-2100 housing for demonstration of battery replacement. Make sure that the PAM-2100 power switch is off. The 4 top screws and the 2 bottom screws at the left (rear) side of the PAM-2000 housing are removed. The top of the housing is folded to the back. A book or something similar should be used, on which the top of the housing can rest on. When the rear-plate is folded to the side, the internal battery can be pulled out and can be replaced.

11.2 Halogen lamp and its replacement

The halogen lamp lifetime depends strongly on the maximal current drawn during its operation. This lamp is primarily meant to generate saturation pulses. Prolonged continuous operation should be avoided.

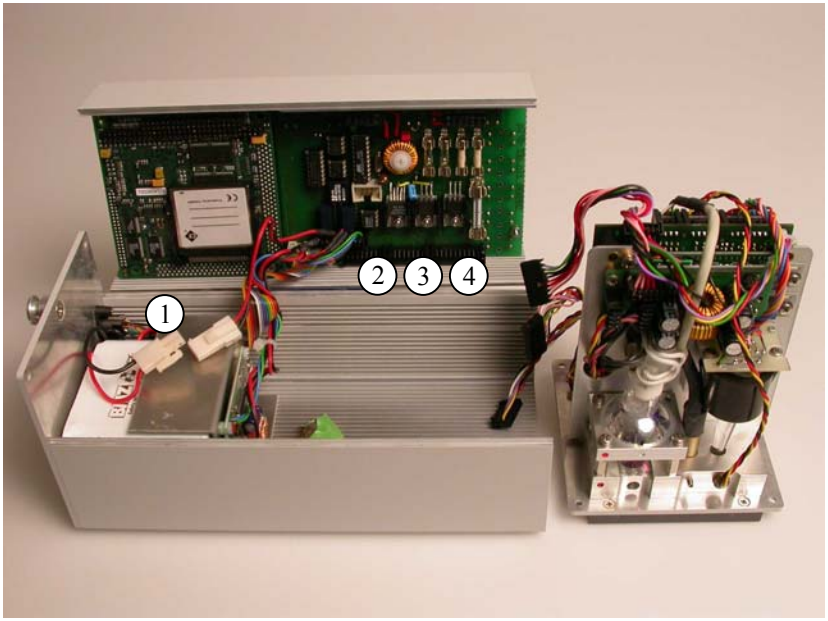


Fig. 59

The above photograph allows a view into the opened PAM-2100 housing for demonstration of halogen lamp replacement. Make sure that the PAM-2100 power switch is off. The 4 top screws and the 2 bottom screws at the right (front) side of the PAM-2000 housing are removed. The top of the housing is folded to the back. A book or something similar should be used, on which the top of the housing can rest on. First the battery should be disconnected (1), then the three connectors (2), (3) and (4) should be unplugged. When the front plate is pulled out, the lamp compartment becomes accessible. The halogen lamp is held in pre-focused position by an aluminum

mounting-frame. Spare halogen lamps also come with this mounting-frame. The mounting frame is fixed by 3 or 4 screws, which have to be removed for lamp replacement. Make sure that the red dots come together. It is sufficient to fix the halogen lamp by 3 screws, because the 4th screw is hardly accessible.

11.3 Fuse replacement

Four fuses are provided:

- 4 A (2 times)
- 0.5 A
- 1 A

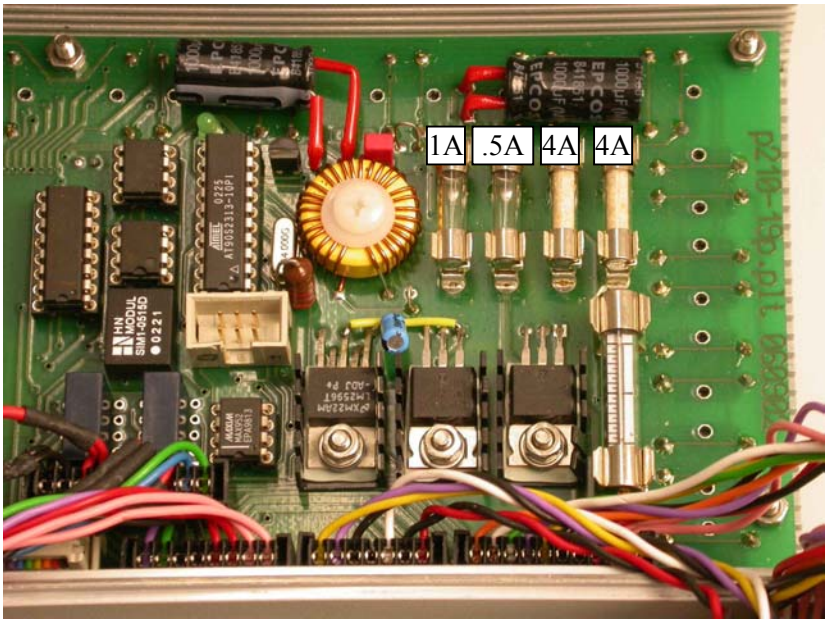


Fig. 60

For fuse replacement make sure that the PAM-2100 power switch is off. The 4 top screws of the PAM-2000 housing are removed. The top of the housing is folded to the back. A book or something similar

should be used, on which the top of the housing can rest on. The fuses are located on the board in the top of the housing.

11.4 EPROM and its replacement

Besides the various hardware components, the PAM-2100 Main Control Unit also contains software residing in an EPROM on the microcontroller board. It can be expected that this software will be up-dated in conjunction with new DA-2100 program versions. As soon as such up-dates are available, they will be sent free of charge to the customers. The current EPROM version is indicated in the left hand corner of the 'Kinetics Screen' headline, when this screen is first installed after program start or initialization of one of the three modes of data acquisition.

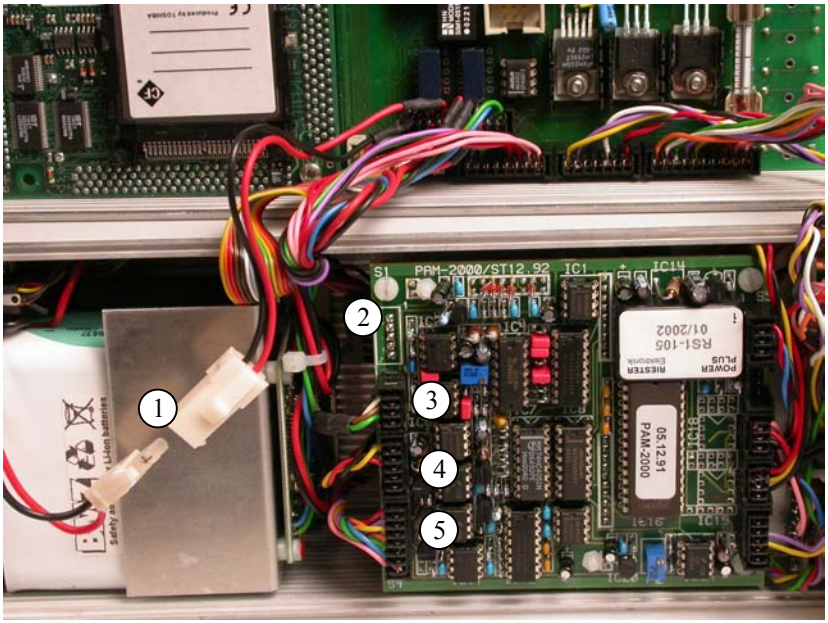


Fig. 61

Before its replacement, first power should be switched off. Then the lid of the housing is folded to the back after removing the 4 top

screws (see Fig. 61). First the battery (1), then the four plugs (2), (3), (4) and (5) should be disconnected. There are little 'noses' at the right hand bottom of the plugs, which together with a small screw-driver will be useful to lift the plugs. Then the four plastic screws holding the circuit board must be removed before it can be folded to the right side and the microcontroller board becomes accessible.

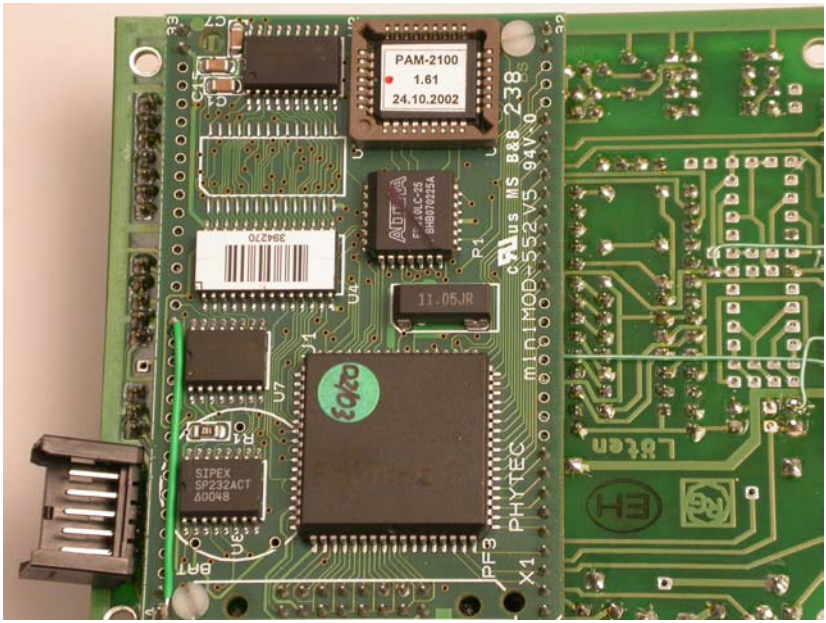


Fig. 62

With the circuit board being up-side down, the EPROM is located in the upper right hand corner of the microcontroller board (see Fig. 62). Please note the little red dot at the upper side of the EPROM. For lifting the EPROM, a paper-clip can be useful. Put a finger on the EPROM, so that it does not jump up. When putting in the new EPROM, make sure that the red dot of the EPROM is at the same side as the arrow that is shown at the EPROM socket. Push-in the EPROM firmly, until there is a click and the EPROM sits level at

all sides. Then all steps are followed backwards to re-assemble the board and cable connectors. Before closing the housing make sure that the microcontroller is functioning alright by switching-on power. EPROM replacement was successful if the green "STATUS" LED pulses.

12 Appendix

12.1 General environmental conditions

The general environmental conditions are valid for all instruments outlined in section 12.2. The values refering to the mains voltage apply only if the instrument features a mains connector.

Permissible environmental temperature

During operation:	-5 °C to +45 °C
In resting state:	-30 °C to +60 °C

Environmental humidity: up to 31 °C ≤ 80%, linearly decreasing to 50 % at 40 °C

Maximal altitude

During operation:	4000 m
In resting state:	15000 m

Mains voltage fluctuations: max. ±10 %

Overvoltage category: II

Contamination level: 1

12.2 Technical Specifications

12.2.1 Basic System

Portable Fluorometer PAM-2100

Design:	Aluminum housing featuring graphical LC-display, keypad, large size built-in Li-ion battery, shoulder strap, connector for Special Fiberoptics 2010-F, as well as sockets for cable connections with PC, external PC-keyboard, external PC-monitor, Battery Charger 2120-N or external battery, Leaf Clip Holder 2030-B or Micro Quantum/Temp.-Sensor 2060-M and External Halogen Lamp 2150-H
Measuring light source:	Red LED, 650 nm, standard intensity 0.1 µmol m^{-2} s^{-1} PAR; modulation frequency 0.6 or 20 kHz; Auto 20 kHz function
Actinic light sources:	Red LED-array, 665 nm, max. 600 µmol m^{-2} s^{-1} PAR and far-red LED, 730 nm, max. 15 W m^{-2}
Halogen lamp:	8 V/20 W, blue-enriched, $\lambda<710$ nm, max. 8500 µmol m^{-2} s^{-1} PAR with continuous actinic illumination, max. 15000 µmol m^{-2} s^{-1} PAR during saturation pulses
Signal detection:	PIN-photodiode protected by long-pass filter ($\lambda>710$ nm); selective window amplifier (patented)
Measured and calculated parameters:	Fo, Fm, Fm', F, Fo', Fv/Fm (max. Yield), ΔF/Fm' (Yield), qP, qN, NPQ, PAR and °C (using Leaf-Clip Holder 2030-B or Micro Quantum/Temp.-Sensor 2060-M), ETR (i.e. PAR x ΔF/Fm')
Built-in PC:	486/33

Data memory:	32 MB
Display:	Graphic LC-display (640 x 240 dots), effective display area 15.5 cm x 6 cm
Keypad:	20 keys, sealed from dust and moisture with membrane overlay
Power supply:	Internal rechargeable Li-ion battery 14.4 V/6 Ah; external 12 V battery
Power consumption:	Basic operation 300 mA, with all internal light sources (LED and halogen) turned on max. 2.5 A
Recharging time:	Approx. 4 hours (PAM-2100 turned off) via Battery Charger 2120-N
PC-terminal operation:	Via RS 232 interface using dedicated Windows Software PamWin
Operating temp.:	-5 to +40 °C
Dimensions:	24 cm x 10.5 cm x 11 cm (L x W x H)
Weight:	2.7 kg (incl. battery)

Special Fiberoptics 2010-F

Design:	Flexible, plastic shielded bundle with three-pin "optical connector"
Joint end (meas. site):	Active diameter 6 mm, outer diameter 8 mm
Length:	100 cm
Weight:	300 g

Battery Charger 2120-N

Input:	100 to 264 V AC, 50/60 Hz
Output:	19 V DC, 3.7 A
Dimensions:	15 cm x 6 cm x 3 cm (L x W x H)

Weight: 300 g

Transport Box 2040-T

Design:	Aluminum box with custom foam packing for PAM-2100 and accessories
Dimensions:	60 cm x 40 cm x 25 cm (L x W x H)
Weight:	5 kg

Ultra-Compact Keyboard 2170-K

Layout:	US-english with 86 keys
Cable Connector:	1.7 m long with PS/2 plug
Dimensions:	28 cm x 13 cm x 2.4 cm (L x W x H)
Weight:	500 g

12.2.2 Accessories (optional)

Leaf-Clip Holder 2030-B

Micro quantum sensor:	Selective PAR measurement, 0 to 20000 µmol $m^{-2}s^{-1}$ PAR
Thermocouple:	Ni-CrNi, dia. 0.1 mm, -20 to +60 °C
Power supply:	Via PAM-2100 (5 V/4 mA)
Output:	PAR, 0 to 1000 and 0 to 20000 µmol $m^{-2}s^{-1}$ PAR (0 to 2.5 V each); Leaf temperature, -20 to +60 °C (0 to 0.8 V); Remote trigger button
Cable length:	100 cm

Dimensions:	17 cm x 5.7 cm (max.) x 8 cm (max.) (L x W x H)
Weight:	310 g

Arabidopsis Leaf Clip 2060-B

Dimensions:	7.6 cm x 4.9 cm (max.) x 5.2 cm (max.) (L x W x H)
Weight:	65 g

Dark Leaf Clip DLC-8

Design:	Clip made of aluminum with felt contact areas and sliding shutter (closure)
Dimensions:	6.5 cm x 2 cm (max.) x 1.5 cm (max.) (L x W x H)
Weight:	3.6 g

Micro Quantum/Temp.-Sensor 2060-M

Micro quantum sensor:	Selective PAR measurement, 0 to 20000 µmol $m^{-2}s^{-1}$ PAR
Thermocouple:	Ni-CrNi, dia. 0.1 mm, -20 to +60 °C
Sensor cable length:	30 cm
Power supply:	Via PAM-2100 (5 V/4 mA)
Output:	PAR, 0 to 1000 and 0 to 20000 µmol $m^{-2}s^{-1}$ PAR (0 to 2.5 V each); Leaf temperature, -20 to +60 °C (0 to 0.8 V)
Cable length:	100 cm
Dimensions:	16 cm x 3 cm x 1.7 cm (L x W x H)
Weight:	220 g

External Halogen Lamp 2150-H

Halogen lamp:	12 V/20 W, blue-enriched, λ<710 nm, 0 to 3000 µmol m^{-2} s^{-1} PAR
Power supply and light intensity control:	Via PAM-2100
Cable length:	120 cm
Weight:	200 g

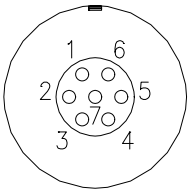
Compact Tripod ST-2101A

Adjustable height:	In steps between 24 cm and 87 cm
Weight:	400 g

Technical specifications are subject to change without prior notice

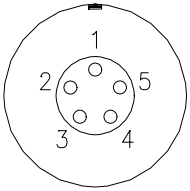
12.3 Pin assignments of PAM-2100 connectors

"LEAF CLIP"



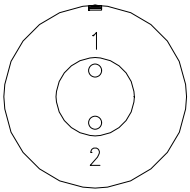
1: +5 V output
2: GND
3: } Analog inputs Leaf-Clip Holder
4: } 2030-B or Micro Quantum/Temp.-
5: } Sensor 2060-M
6: Remote control button
7: -5 V output

"RS 232"



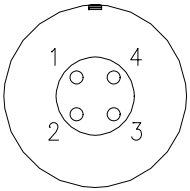
1: Not used
2: Not used
3: TxD
4: RxD
5: GND

"EXT.HALOGEN"



1: Analog control output External Halogen Lamp 2150-H
2: GND

"EXT.DC"



1: +19 V input } Battery Charger 2120-N
2: GND }
3: +12 V input } External 12 V battery
4: GND }

12.4 List of warnings and error messages

'Low battery'
Internal Li-ion battery is close to being exhausted.
Voltage of external 12V battery has dropped below 11 V.

'Low battery during saturation pulse'
Risk of erronously low Fm- or Fm'-values, because of voltage drop during Sat. pulse.

'Overload'

Faulty fluorescence readings as the limit of 2450 mV was exceeded.

'Attention, low accuracy due to low signal level'
Fm- or Fm'-values measured by a Sat. pulse are smaller than 33 times Gain setting; therefore, quenching analysis is inaccurate; if possible, increase Fm/Gain by decreasing sample distance and increasing measuring light.

'Attention, low accuracy due to small Fv'
Saturation pulse induced Fv (in mV) is smaller than Gain-setting; therefore, quenching analysisis inaccurate; if possible, increase Fv/Gain by decreasing sample distance and increasing measuring light.

'Function F1-F9 not operative while in Sat. Pulse Mode'
To remind the user that the Special Functions can be applied only in the Trig. and the Cont. Mode of data acquisition.

'File doesn't exist'
A faulty file-name was entered.

12.5 List of editor commands

In connection with the Report-file (see 6.1.17) within the DA-2100 program a simple editor is provided for which essentially the same commands as in Wordstar apply. The following list summarizes some of the more frequently used commands:

Block-commands

Ctrl KR : to read a file from disk
Ctrl KW : to write a file to disk, print out when file-name prn is entered
Ctrl KY : to erase a block

Ctrl KC : to copy a block
Ctrl KV : to move a block into new position

Cursor-commands

Ctrl QR : cursor to file-start
Ctrl QC : cursor to file-end
Ctrl QS : cursor to left margin
Ctrl QD : cursor to right margin
Ctrl X : cursor one line down
Ctrl E : cursor one line up

Roll-commands

Ctrl W : to roll screen one line down
Ctrl Z : to roll screen one line up
Ctrl C : advance one page
Ctrl R : go back one page

Edit-commands

Ctrl Y : to erase line
Ctrl T : to erase word
Ctrl QY : to erase to end of line
Ctrl V : switch between insert/overtype

12.6 List of key commands using external keyboard

The following list of commands provides an overview of all commands that are executed by the DA-2100 program and which can be applied with the help of an external keyboard. Lists of single- and double-key operations using the integrated 20-key board were already provided in sections 5.1.1 an 5.1.2, respectively.

The commands are listed in numerical and alphabetical order, with a brief explanatory text outlining the essential information on their function. For a more detailed description see chapters 6 and 7. If the use of a particular command is restricted to a certain part of the Data Acquisition Program DA-2100, this is indicated in parentheses (Par. Scr. for Parameter Screen, Kin. Scr. for Kinetics Screen).

Single key commands:

0	:	To initialize standard instrumental settings
1	:	Selection of measuring light intensity
2	:	Selection of actinic light intensity (dial switch)
3	:	Selection of Sat. pulse intensity (dial switch)
4	:	Selection of far-red light intensity (dial switch)
5 (Par. Scr.)	:	To switch modulation frequency from 600 Hz to 20 kHz or vice versa
6 (Par. Scr.)	:	Selection of act. illumination time (dial switch)
7 (Par. Scr.)	:	Selection of Sat. pulse length (dial switch)
8 (Par. Scr.)	:	Selection of far-red illumination time (dial switch)
9 (Par. Scr.)	:	To switch Auto 20 K function on/off
A	:	Actinic light on/off
B	:	'Break', to stop Run-file
C (Par. Scr.)	:	Selection of dial switch to set time interval between consecutive sat. pulses
C (Kin. Scr.)	:	To start a kinetic recording
D	:	Damping, to select time constants (dial switch)
F	:	Far-red light on/off
G	:	Gain, to select amplification (dial switch)

H	:	Halogen lamp, to switch from LED lamp to halogen lamp or vice versa
I	:	Initialize, to install the current Run-file specific instrument settings
J (Kin. Scr.)	:	Join, to connect data points by line segments or to return to single point display
K (Par. Scr.)	:	Kinetics Screen, to change over from Parameter Screen to Kinetic Screen
L	:	Light, measuring light on/off
M	:	Maximal fluorescence yield, to determine Fm and Fo
N (Kin. Scr.)	:	Normal Screen, to change over from Kinetics Screen to Parameter Screen
O (Kin. Scr.)	:	To define the time axis limits of a curve to be horizontally zoomed
P	:	Pulse Sequence, to start/stop a sequence of Sat. pulses
Q (Kin. Scr.)	:	To read a kinetic data file saved on disk
R	:	To start a Run
S	:	Saturation Pulse, to apply a single Sat. pulse (on the Kin. Screen without quenching analysis)
T (Par. Scr.)	:	Terminal, for changing instrumental settings by machine code (for service only)
U (Par. Scr.)	:	To up-date the displayed instrumental settings
V (Kin. Scr.)	:	Full screen display of kinetic recording
W (Kin. Scr.)	:	Write, to save kinetic data in a disk file
X (Kin. Scr.)	:	To save Mem. 1-4 in disk files
Y	:	Yield, to apply a single Sat. pulse with quenching analysis (yield, qP, qN-determination)

Z	:	Zero, to determine Fo
/	:	To clear Kin. Screen
+	:	To increase a parameter setting, marked by cursor position, by one step
-	:	To decrease a parameter setting, marked by cursor position, by one step
!	:	To increase light intensity setting (marked by cursor position) from 10 to 11 (not for Sat. Pulse)
Esc(Kin. Scr.)	:	To stop recording To leave menu or sub-menu To delete proposed file-name
Esc (Edit. Scr.):		To quit Report-file
Del (Kin. Scr.):		To enter dialog for changing Fo- or Fm-values

Sequential key commands:

O A (Kin. Scr.):	To display original curve with all data points
O P (Kin. Scr.):	To display curve segment with previously defined limits
O O (Kin. Scr.):	To define left limit as origin for Fv=0 and t=0
O Return Return (Kin. Scr.):	To redraw original curve or curve segment

Special key commands:

Alt E	:	To enter dialog for ETR-factor definition
Alt F10	:	To enter local menus in conjunction with User Runs and Pulse Seq. definition

Alt I	:	To enter menu for selection and initialization of one of three different modes of data acquisition
Alt M	:	To display the fluorescence kinetics induced by the last Sat. pulse in the right corner of the parameter screen
Alt X	:	To quit the DA-2100 program and switch off the PAM-2100
Ctrl C	:	To enter dialog for up-dating calibration factor of PAR-determination
Ctrl E	:	To access the Report-File for assessment and editing of data measured in the Saturation Pulse mode
Ctrl O	:	To enter dialog for definition of offset values
Ctrl Q	:	Switch to select between qN and NPQ-determination for assessment of non-photochemical quenching
Ctrl R	:	To position cursor on Run-field
Ctrl S	:	On/off switch for special Saturation Pulse mode (Fo'-mode) involving far-red illumination to determine Fo'
Ctrl Y	:	On/off switch for averaging mode of Yield-determination; with Leaf-Clip Holder 2030-B also averaging of ETR, PAR and Tmp
Ctrl Z	:	To determine Fo' in conjunction with next Sat. pulse, involving far-red illumination

12.7 List of parameter fields and associated key commands

The following list of parameter fields and associated key commands is organized according to the position of the various parameter fields on the so-called Parameter Screen in five columns. It is meant for quick reference. In the description of key-commands the

use of an external keyboard is assumed. For a more detailed description see section 6.1.

Column 1

Z Fo : Minimal fluorescence yield sampled after dark-adaptation via Z-command

M Fm : Maximal fluorescence yield after dark-adaptation which is sampled together with Fo via M-command

Fv:m : (Fm-Fo):Fm calculated after M-command, representing photochemical quantum yield of open PS II centers in dark-adapted sample

Fo' : Minimal fluorescence yield of illuminated sample, measured via Ctrl Z-command or after activation of Ctrl S with every saturation pulse

Fm' : Maximal fluorescence yield of illuminated sample, measured with every saturation pulse after previous sampling of Fm via M-command

Ft : Fluorescence yield at a given time, t

Y ield : (Fm'-Ft):Fm' calculated after Y or S command (on Par. Screen only), representing overall photochemical quantum yield of PS II

Column 2

L ight Meas : On/off switch for measuring light operated by the -command

[1] Int : Change of measuring light intensity between 11 settings; selection via 1-command and use of +/- keys; setting 11 only via !-key

[5] 600 Hz : Switch for selection of modulation frequency between 600 Hz and 20 kHz via 5-command

[G] ain : Change of amplifier gain setting after selection via G-command and use of +/- keys

[D] amping : Change of signal damping setting after selection via D-command and use of +/- keys

ML : Relative intensity of measuring light before entering the fiberoptics

ETR : Apparent rate of electron transport calculated from Yield x PAR x 0.5 x 0.84

Column 3

[A] ct. Light : On/off switch for actinic light operated by the A-command

[2] Int : Change of actinic light intensity between 11 settings; selection via 2-command and use of +/- keys; setting 11 only via !-key

[6] s : Setting of actinic illumination time in seconds; selection via 6-command; setting 0 for manual termination; change of settings with +/- keys

[H] LED : Switch for selection of actinic light source choosing between LED and halogen lamp via the H-command

[9] Auto 20 K : On/off switch for Auto 20 K-function operated by 9-key

PAR : Photosynthetically active radiation measured by micro-quantum-sensor at leaf surface with Leaf-Clip Holder 2030-B

qP : Coefficient of photochemical fluorescence quenching: (Fm'-Ft) : (Fm'-Fo') or (Fm'-Ft) : (Fm'-Fo) depending on Fo' being determined or not, respectively.

Column 4

[S] at. Pulse : To trigger a single Sat. pulse via the S-command

[3] Int : Change of Sat. pulse intensity between 10 settings; selection via 3-command and use of +/- keys

[7] 0.1 : Length of Sat. pulse in 0.1 seconds; variable between settings 4 and 14 by 0.2 s steps; selection via 7-command and use of +/- keys

[C] lk s : Time between consecutive Sat. pulses in a pulse

[C] lk m : sequence initiated by the P-command; selection of multiples of 10 sec, 1 min and 10 min via C-command and use of +/- key

No : Number of Sat. pulses applied after initial Fo and Fm determination via the M-command

qN : Coefficient of non-photochemical fluorescence quenching: (Fm-Fm'):(Fm-Fo') or (Fm-Fm'):(Fm-Fo) depending on Fo' being determined or not, respectively.

NPQ : Expression for non-photochemical quenching defined as: NPQ = (Fm-Fm') : Fm'. NPQ substitutes for qN after operation of Ctrl Q and vice versa (not on Kin. Screen)

Column 5

[F]ar Red : On/off switch for far-red light operated by F-command

[4]Int : Change of far-red light intensity between 11 settings; selection via 4-command and use of +/- keys

[8]s : Length of far-red illumination period in seconds; with setting 0 manual termination; selection by 8-command and +/- key operation

[R]un : Change of Run-number after selection via Ctrl R-command and use of +/- keys; start Run via R-command or Return

[K]in. Scr. : Switch to select between normal Parameter Screen and Kinetics Screen

Tmp : Leaf temperature in °C, as measured with thermocouple at lower leaf surface, using Leaf-Clip Holder 2030-B

Volt : Voltage of external 12 V battery; warning 'Low battery!' below 11 V

12.8 PAM-2100 command language

This section of the Appendix will be useful for users with practical experience in the digital control of measuring devices. The

commands listed below provide a direct means of controlling the instrument functions of the PAM-2100, without involvement of the DA-2100 program.

The commands use lower-case first letters. Numerical values are transmitted in hexadecimal form (upper-case letters). The format is fixed, whereby the various entries must appear at the given positions of a command line. Each command line must be terminated by a 'RETURN' (0Dh) If not specially mentioned, there is no echo and no reply.

12.8.1 Command overview

Single-byte commands:

<CR>	Current fluorescence (10 bits)
10h	Average of fluorescence (12 bits)
11h	External temperature (12 bits)
12h	Light I (0 ... 20000 µE, 12 bits)
13h	Light II (0 ... 1000 µE, 12 bits)
14h	Intensity measuring light
18h	Battery voltage at the end of last sat. pulse (10 bits)

Terminate commands with RETURN (0Dh):

?	Version number (e. g. 220392)
axx	Actinic light on/off. "xx" = duration in seconds
b	Break: disables all functions
dx	Damping (0 ... 7)
fxx	Far red light on/off. "xx" = duration in seconds

gxx	Gain
hxx	Halogen light on/off
iAxx	Intensity of the actinic light
iFxx	Intensity of the far red light
iHxx	Intensity of the halogen light
iLxx	Intensity of the measuring light
iSxx	Intensity of the saturation pulses
jx	Input: fast storage
kx	Measuring frequency 20 kHz: 1=on; 0=off
lx	Measuring light: 1=on; 0=off
paaxx	Program starting at address (aa) pattern (xx)
sxx	Saturation pulse. xx = number of pulses
taa	Transfer starting at address (aa)
vx	Reading AD converter
wRxx	Interval between saturation pulses (rate)
wSxx	Width of the saturation pulse (in 0.2 sec)
xx	Auto 20 kHz: 1 = on; 0 = off

12.8.2 Command description

The following single-byte commands need not be terminated with RETURN. The reply is immediate.

0Dh <RETURN> Current fluorescence (10 bits with flags)

If only a RETURN (0Dh) is entered, the current fluorescence value is transmitted. It consists of two bytes, whereby the lower 10 bits contain the measured value in binary form and the higher 5 bits reflect the status of the PAM-2100:

Bit 15	Measuring light: H= on; L= aus
Bit 14	Measuring light: H= 20 kHz; L= 0.6 kHz
Bit 13	Actinic light: H= on; L= off
Bit 12	Saturating light: H= on; L= off
Bit 11	Far red light: H= on; L= off
Bit 10	Input (fast storage) active, and status of the button on the leaf-clip holder

The button switches to ground. The flag is inverted.

First the lower byte is transmitted and then the higher byte. The A/D converter works with 10 bits and a minimum resolution of 2.5 mV.

10h	Average of fluorescence (12 bits without flags, 16 points with 10 ms/point)
11h	External temperature (12 bits)
12h	Light I (0 ... 20000 µE, 12 bits)
13h	Light II (0 ... 1000 µE, 12 bits)
14h	Intensity measuring light

The following commands must be terminated with RETURN (0Dh).

? Version number:

This command informs the user of the last date of revision.

axx Actinic light:

'xx' indicates the duration of the actinic light in seconds. The maximum value is '32h' (50 sec). '00' switches the light off and 'FF' (256) leaves the light on until it is switched off with 'a00' or 'b' (Break). If no value xx is specified, the light is switched on or off.

b Break:

'b' is used to stop all currently active functions. These are: actinic light, far red light, saturating pulses and programs. The measuring light is switched back to 0.6 kHz.

dx Damping:

Damping can be adjusted in 8 stages by entering decimal numbers (0...7) for Damping-settings 1...8.

fxx Far red light:

'xx' indicates the duration of the far red light in seconds. The maximum value is '32' (50 sec). '00' switches the light off and 'FF' (256) leaves it on until it is switched off with 'f00' or 'b' (Break).
If no value xx is specified, the light is switched on or off.

gxx Gain:

'gg' specifies the gain.
Values: 00 = 1; 7F = 2; AA = 3; BF = 4; CC = 5; D5 = 6; DB = 7; DF = 8; E3 = 9; E6 = 10

hxx Halogen light:

'xx' specifies the duration of the halogen light in seconds. The maximum value is '32E' (50 sec). 'h00' switches the light off.
If no value xx is specified, the light is switched on or off.

iAxx **Intensity of the actinic LED light:**

This command is used to change the intensity of the actinic LED light. The maximum value at setting "FF" corresponds to 45 mA.

iFxx **Intensity of the far red light:** (as actinic light)

iHxx **Intensity of the halogen light:** (0 ... 4.5 V) (as actinic light)

iLxx **Intensity of the measuring light:** (as actinic light)

iSxx **Intensity of the saturating light:** (0 ... 8 V) (as actinic light)

jx **Data sampling:**

This command starts the rapid sampling of measured values. A pattern must be entered beforehand with the command "p". "x" specifies one of the following times between measuring points: 1 = 150 □sec; 2 = 300 □sec; 3 = 1000 □sec; 4 = 3 msec; 5 = 10 msec.
If x is not specified, the measurement is carried out with the old value.

kx **Measuring frequency:**

"1" switches to 20 kHz, "0" switches to 0.6 kHz.

lx **Measuring light:**

"1" switches the measuring light on, "0" switches it off

paaxx **Program:**

Starting with the data address specified with aa x32, the bit pattern specified with xx is written to memory. The bit pattern corresponds to the 2nd byte of the 16-bit data format (see RETURN). Switching operations currently include only the actinic light, the measuring light and the measuring frequency.

sxx **Saturating light pulses:**

"xx" indicates the number of pulses. "FF" produces an unlimited number. "00" stops the timer. If no value "xx" is entered, this corresponds to the command "s01"

taa **Transfer stored data:**

256 measured values are transferred starting with the data address aa x32.

vx **Volt:**

This channel reads one of the 8 channels of the A/D converter. The channel assignment is given below. The format corresponds to that of the RETURN command (10 bits + flags).

0: Supply voltage
3: Fluorescence value
4: Ext. 1 (external temperature)
5: Ext. 2 (light 0 ... 20000 µE)
6: Ext. 3 (light 0 ... 1000 E)
7: Interior temperature

13 Warranty conditions

All products supplied by the Heinz Walz GmbH, Germany, are warranted by Heinz Walz GmbH, Germany to be free from defects in material and workmanship for one (1) year from the shipping date (date on invoice).

The warranty is subject to the following conditions:

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

To obtain warranty service, please follow the instructions below:

1. The Warranty Registration form must be completed and returned to Heinz Walz GmbH, Germany.
2. The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, custom duties,

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

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

4. Heinz Walz GmbH, Germany is not responsible or liable, for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.