PORTABLE FLUOROMETER
PAM-2000
and
DATA ACQUISITION SOFTWARE
DA-2000

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Software Version 2.00

Handbook of Operation,
with
Examples of Practical Applications

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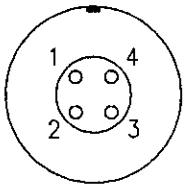
Subject: **Modifications of the PAM-2000 Main Control Unit from S/N 93 08 088 upwards**

From S/N 93 08 088 (year month number) upwards the PAM-2000 Main Control Unit shows the following modifications compared to the model described in this handbook:

The internal charging circuitry and the LED 'RECHARGE' (red) are discontinued (see 2.1.). In consequence of that the internal battery is not recharged when an external battery is connected. In this case the battery with the higher voltage provides the power for running the instrument. Please notice that the input voltage should not be higher than +14 V. The new pin assignment of the connector "EXT.DC" (compare 5.2.) is now:

"EXT.DC"



1: +12 V } internal
2: GND } battery
3: Input +12 V } external battery
4: Input GND } e. g. NP-3/12

These modifications were necessary in order to comply with the newest EMC regulations (Europe's Electromagnetic Compatibility). To keep to these regulations, please obey the following rules:

- If the HP 200LX is used in conjunction with the PAM-2000, it must always be on top of the PAM-2000 Main Control Unit.
- It is not allowed to extend the connecting cables delivered with the PAM-2000.
- Alternatively to the HP 200LX we recommend to use the laptop of the company COMPAQ. If another computer is used, we cannot guarantee that the PAM-2000 is still following the EMC regulations.

Palmtop Computer HP 200LX

Production of the POQET PC and POQET PC PLUS palmtops has been discontinued. They are being replaced by the Hewlett Packard HP 200LX. The manual for the PAM-2000 applies to the Poqet PC. The chapters 2.9., 3.7.2. and 3.7.3. are therefore no longer of any relevance. The following pages give a brief information on how to use the HP 200LX in combination with the PAM-2000. The HP 200LX offers a lot of applications. If you want to use those or whenever a problem occurs, then please refer to the original user's guide (HP 200LX User's Guide), which contains detailled information on function, operation and maintenance of the HP 200LX.

The HP 200LX delivered together with the PAM-2000 differs from the commercially available HP 200LX and includes additional equipment. The package consists of the following parts:

- HP 200LX with a system frequency of 16 MHz (the usual HP 200LX has a system frequency of 8 MHz) including HP 200LX User's Guide and HP 200LX Quick Start Guide,
- RS 232 interface cable for the PAM-2000,
- Connectivity Pack including a user's guide and a RS 232 interface cable for data transfer between HP 200LX and PC,
- 512 kB Flash EPROM Card with the program DA-2000.

1. Initial installation of the HP 200LX

The following measures are required for the initial installation of the software DA-2000:

- Insert the Flash EPROM Card which contains the program DA-2000, a special AUTOEXEC.BAT, a special CONFIG.SYS and drivers for the 16 MHz version of the HP 200LX. Because those files are necessary for booting the HP 200LX, it is important that the Flash EPROM card is inserted before the batteries are inserted!
- Insert batteries, first the two main AA batteries and then the backup battery (button cell). As soon as the two main AA batteries are inserted the HP 200LX is booted. After finishing booting the HP

200LX is on DOS level.

- It is advantageous to enter correct time via the command "time" and correct date via the command "date".

2. Important details on the HP 200LX

The Flash EPROM Card must be inserted whenever the HP 200LX is booted or whenever the program DA-2000 is started!

The HP 200LX has a 2 MB RAM, 1.4 MB are available for disk space (RAM disk C) and 0.65 MB are reserved for main memory. The HP 200LX offers a lot of applications such as appointment book, phone book, memo editor, database, Lotus 1-2-3 etc., which are described in detail in the user's guide.

When the HP 200LX is installed according to chapter 1, the System manager with the above mentioned applications is not loaded. If you want to have access to those applications you have to start the System manager via the command "200". The System manager occupies part of the main memory. However, to run the program DA-2000 the whole main memory must be available. Therefore, if you have loaded the System manager via the command "200" and you want to start the program DA-2000, first all applications of the HP 200LX must be terminated and the System manager must be closed. Under no circumstances the program DA-2000 should be started from the DOS level of the System manager or from the Filer of the System manager.

The applications are terminated and the System manager is closed as follows:

- By pressing the key "&.." the Application manager [More Applications] is activated.
- Via the key "ALT" or "MENU" the menu is activated and then the menu point "Application/Terminate All..." must be executed.
- Now the HP 200LX is on DOS level. With the command "MEM" the memory size can be checked. If largest executable program size is greater than 620 kB, the program DA-2000 can be started without problems.
- Whenever you want to use applications of the HP 200LX, the

System manager can be loaded via the command "200".

Software reset and Hardware reset of the HP 200LX:

If the HP 200/LX crashes two possibilities exist to reset the HP 200/LX, a software reset and a hardware reset. Whenever a reset is carried out, the Flash EPROM Card must be inserted in the PCMCIA slot. First you should try to solve the problem carrying out a software reset via the key combination "CTRL+ALT+DEL". The software reset has no affect on the data on drive C (RAM disk). If the software reset cannot be carried out or does not solve the troubles, then a hardware reset should be executed via the key combination "CTRL+Shift+ON". This hardware reset has the advantage that data on RAM disk C can be preserved. To preserve data the question "Initialize RAM disk?" has to be answered with "N". If the RAM disk is initialized data are erased. A hardware reset can also be carried out by removing both the two AA batteries and the backup battery (button cell). But in this case the RAM disk C will be automatically initialized, that means that data on drive C are erased. After a hardware reset with an initialization of the RAM disk C it is advantageous to enter correct time and date (see chapter 1).

3. Starting the program DA-2000

If the System manager has been activated with the command "200", all applications must be terminated before starting the program DA-2000 (see previous chapter 2).

The program DA-2000 is in the directory DA-2000 on the Flash EPROM Card, which must be inserted in the PCMCIA slot (drive A). When the program DA-2000 is started, it accesses two files ("DA-2000.CFG" and "STANDARD.RPT"), which are updated when the program is left. The file "DA-2000.CFG" contains the instrumental settings and the file "STANDARD.RPT" contains the data sets measured in the "Sat. pulse mode". When running the program DA-2000 on the HP 200LX the RAM disk C is the only drive, on which data can be written. Therefore the "DA-2000.CFG", the "STANDARD.RPT" and all kinetic recordings are stored to drive C. The program DA-2000 can be

started via the command "DA-2000". A batch file "DA-2000" exists to start the program DA-2000 from any drive or directory. But please notice that the program DA-2000 searches for the "DA-2000.CFG" and "STANDARD.RPT" in the directory of drive C, which was opened when the program DA-2000 is started. Also all kinetics will be written into this directory. This allows the user to work with different configuration files and to sort his data simply by opening a certain directory before starting the program.

4. Quick Start Guide for transfer of data between HP 200LX and the host PC (detailled description in the HP 200LX User's Guide or HP 200LX Quick Start Guide)

4.1. Quick Start Guide for installation of the software 'Connectivity Pack' on the host PC (detailled description in the guide 'HP 200LX/HP 100LX Connectivity Pack')

The following installation has to be carried out on your PC only once.

- Insert diskette 1
- Enter "a:"
- Enter "install"
- Installation according to the screen instructions (Largely automatic)
- After installation is finished, remove diskette

4.2. Start the transfer software on the HP 200LX

- Activate the System manager of the HP 200LX via the command "200"
- Press the key "&..." to start the Application manager
- Enter "F" to start the Filer
- If the screen is not splitted into two columns enter "F7"
- Enter "MENU", "C" and "R" (Communication/Remote Settings...), then the menu 'Remote Settings' appears
- Check and, where necessary, change the following parameters: 'Baud' 19200; 'Interface' Com1; 'Server-Mode' Enabled

- End with "F10"

4.3. Start the transfer software on the host PC:

- Enter "c:"
- Enter "cd\cpack200"
- Enter "app200", then the Application manager ('More Applications') appears
- Enter "F", then the Filer appears
- If the screen is not splitted into two columns enter "F7"
- Enter "ALT", "C" and "R" (Communication/Remote Settings...), then the menu 'Remote Settings' appears
- Check and, where applicable, change the following parameters: 'Baud' 19200; 'Interface' (free serial port, e.g. Com1); 'Server-Mode' Enabled
- End with "F10"
- Connect the cable supplied in the 'Connectivity Pack' to the HP 200LX and the host PC (to the free serial port)
- Enter "F6" on the host PC
- The following message appears briefly 'Please wait... Establishing remote connection'
- The following display appears on the HP 200LX 'In server mode: Processing...'
  If no connection is established, check serial line, check the remote settings ("Alt", "C", "R") and consult the User's Guide
- Transfer files from the HP 200LX to the host PC: On the host PC change to the column 'Remote', change to the drive and directory with the files which should be transferred. With the cursor keys the files can be selected and with the space bar the files can be marked. Transfer is carried out with "F2" (copy) or "F3" (move).
- End with "F10"
- Quit Filer on host PC with "ALT", "F" and "X"
- Quit Application manager on host PC with "ALT", "A" and "X"

1. INTRODUCTION

1.1. How to use this handbook

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-2000 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 one can start measuring. Actually, due to the 'intelligent' central control of all functions by the special DA-2000 software, you cannot make serious operational mistakes which would harm the instrument. Also, at first there is no need to care about the numerous settings of instrumental parameters, because these are pre-set for standard measurements. 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-2000 Fluorometer, most 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-2000 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 part of the sections in the following suggested order:

see 3.1. How to get started

Reading this section, you can learn within a few minutes how to connect the components of the measuring system and how to carry out simple measurements. Actually, this section may serve as a first, condensed outline of system operation.

see 3.3.1. Using the Parameter Screen

Here the essential points of instrument operation are systematically explained: How to switch on/off the various light sources, how to change instrumental settings and how to measure the basic fluorescence parameters. It is not necessary to study all sub-sections one after another.

For a quick overview of all parameter fields and functions there is a corresponding list at the end of this manual:

see 5.6. List of parameter fields and associated key commands

The sub-sections of greatest practical relevance for most users are:

see 3.3.1.1. Fo, Fm and Fv:m
see 3.3.1.5. Yield and Ctrl Y (Averaging)
see 3.3.1.6. ETR, PAR and Alt E

In these sub-sections the exceedingly simple measurements of those fluorescence parameters are outlined which provide the most essential information on yield and rate of photosynthetic energy conversion.

Of considerable practical relevance are also the sections dealing with data storage and transfer:

see 3.3.1.17. Ctrl E and the 'Report-file'

Here the automatic storage of data in a so-called 'Report-file' is outlined.

see 3.7. Data storage, transfer and output

This section describes how to save data and to transfer them to a Desktop computer for further analysis or for making print-outs.

A substantial part of this handbook deals with graphical registration and kinetic analysis of time dependent fluorescence changes. Knowledge of this part is not prerequisite for basic applications of the PAM-2000 Fluorometer. However, it may be expected that sooner or later the advanced researcher will wish to analyse the additional information contained in the kinetic properties of light-induced fluorescence changes. For this purpose the following sections should be studied:

see	3.2.	Different modes of data acquisition
see	3.3.2.	Using the 'Kinetics Screen'
see	3.4.	Measurements in the 'Continuous Mode' or 'Triggered Mode'

A convenient way to become introduced to the multiple functions of the PAM-2000 Fluorometer is by running pre-programmed 'standard experiments' which are stored in so-called 'Run-files':

see	3.3.1.13.	Run
see	3.5.	Standard experiments (Run-files)

Running these standard experiments is simple and instructive. At the same time it provides a test for proper functioning of the instrument.

Parts of this handbook are stored in Documentation-files on the DA-2000 program disk. In this way, the most essential information on instrumental operation is always available even if the handbook is not at hand:

see	2.3.	Data Acquisition Software DA-2000

1.2. Chlorophyll fluorescence as an indicator of photosynthesis

Photosynthesis involves reactions at five different functional levels:

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

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

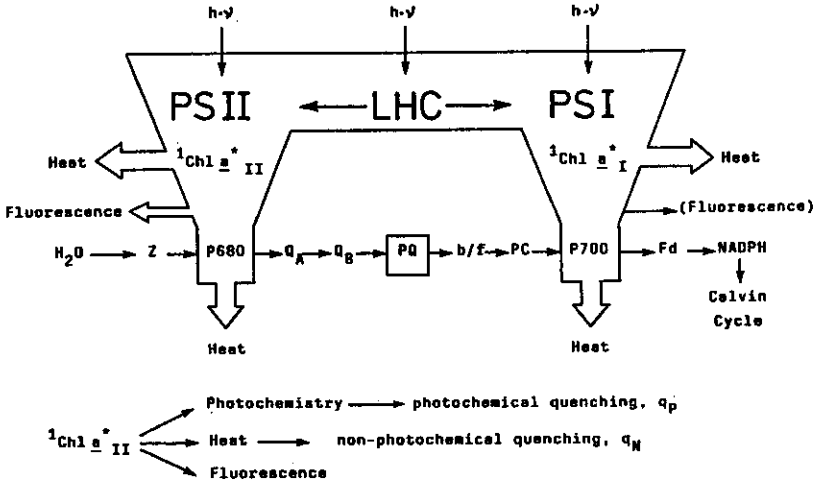


Fig. 1

The indicator function of chlorophyll fluorescence arises from the fact that fluorescence emission is complementary to the alternative

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

Measuring chlorophyll fluorescence is rather simple: The emission extends from 660 nm to 760 nm, and if shorter wavelength excitation light is used, separation of fluorescence from the measuring light is readily achieved with the help of optical filters. The challenge arises with the wish to measure fluorescence in ambient daylight and to use very strong light for the so-called 'quenching analysis'. For this purpose the PAM measuring principle has been developed which allows monitoring fluorescence against 10^6 times larger background signals (see 1.3. and 1.4.).

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

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

By both mechanisms, the maximal potential fluorescence yield is 'quenched' and, hence, 'photochemical' and 'non-photochemical fluorescence quenching' are distinguished. For interpretation of fluorescence changes, it is essential to know the relative contribution of these two different quenching mechanisms to the overall effects. If, for example fluorescence yield declines, there are the opposing possibilities of:

- increasing photochemical rate
- increasing dissipation rate, paralled by decreasing photochemical rate

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

With a very strong pulse of white light the electron transport chain between the two photosystems can be quickly fully reduced, such that the acceptors in PSII photochemical charge separation are exhausted. Hence, for the duration of the saturation pulse, photochemical fluorescence quenching becomes zero and all remaining quenching must be non-photochemical. It is assumed that changes in non-photochemical quenching are too slow to become effective within the approx. 1 second duration of a saturation pulse.

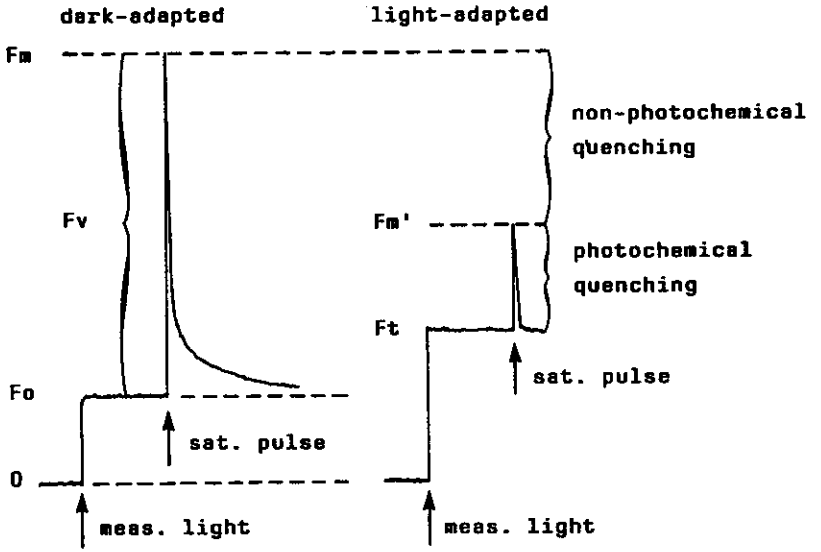


Fig. 2

On the basis of these considerations so-called 'quenching coefficients' qP and qN were defined, which can be determined by simple fluorescence measurements (see 3.3.1.7.). For qP- and qN-determination it is necessary to define the extremes of maximal and minimal fluorescence yield, which are given in the dark-adapted state

(see 3.3.1.1.). However, quenching analysis is not restricted to qP- and qN-determination and very relevant information can be obtained without previous dark-adaptation of the samples. This is an important point for field investigations.

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

$$(Fm'-Ft) : Fm' = \Delta F : Fm'$$

This expression, which was introduced by Genty et al. (1989) (see list of literature in Appendix 5.8.) is identical to the 'Yield'-parameter, for the measurement of which the PAM-2000 Fluorometer has been optimized (see 3.3.1.5.). With this fluorometer, 'Yield'-determination has become exceedingly simple: The fiberoptics are held at short distance to a sample, and the 'Y'-key is pressed on the PC keyboard. Everything else is proceeding automatically within seconds:

- measuring light is switched on
- Ft is sampled
- a saturation pulse is applied
- Fm' is sampled
- Yield = (Fm'-Ft):Fm' is calculated and displayed on the screen
- the obtained data are stored in computer memory.

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

For full assessment of fluorescence information, knowledge of

environmental parameters is required, in particular of light intensity and temperature. For example, if the measured 'Yield' of leaf A is lower than that of leaf B, this does not necessarily mean that leaf A is photosynthetically less competent than leaf B. The difference could as well arise from leaf A being exposed to stronger light or to a lower temperature than leaf B. The PAM-2000 Fluorometer offers the possibility to measure photosynthetic active radiation (PAR) and temperature at the same spot of a leaf where also fluorescence is measured (see 2.5.), such that together with every 'Yield'-value also the corresponding values of PAR and temperature are entered into the file of automatically stored data (see 3.3.1.17.). When PAR is known, it is only a small step to estimate the apparent rate of electron transport (see 3.3.1.6.).

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

Since the introduction of the PAM Fluorometer in 1984, there has been a boom in chlorophyll fluorescence research, at the basic as well as at the applied level. This is reflected in a large number of publications, due to which there has been considerable progress in understanding of the indicator function of chlorophyll, of photosynthesis as such, and of the regulation of photosynthesis under stress conditions. The list of relevant literature given in the Appendix covers a representative part of the work which so far was carried out with the PAM Fluorometer. This list may be useful to become informed

in more detail about chlorophyll fluorescence and possible applications of the PAM Fluorometer. Many more references are found in the cited literature. The critical reader will notice that despite the great progress reflected in this literature, there are still many open questions, extending from more applied to basic aspects. In this sense, the PAM-2000 Fluorometer is not only a means to obtain quick information based on well established models, but also an instrument for continuing basic research on the relationship between fluorescence and photosynthesis.

1.3. The PAM measuring principle

With conventional chlorophyll fluorometers, the same light is used for driving photosynthesis and for exciting fluorescence. Separation of fluorescence from stray excitation light is achieved by appropriate combinations of optical filters (e. g. excitation by blue light and protection of the detector by a red filter which only passes the red fluorescence). Such conventional fluorometer is of rather limited use for ecophysiological research, as its function is severely disturbed by ambient daylight. In order to distinguish between fluorescence and other types of light reaching the photodetector, fluorescence excitation can be 'modulated': When a special 'measuring beam' is rapidly switched on/off, the fluorescence signal follows this on/off pattern and with the help of suitable electronic devices the resulting modulated signal can be separated. Standard devices for this purpose are lock-in amplifiers which tolerate background signals several hundred times larger than the fluorescence signal. For the extreme requirements of chlorophyll fluorescence quenching analysis by the so-called saturation pulse method (see 1.2.), a new modulation principle was developed which tolerates a ratio of $1:10^5$ or even higher between fluorescence and background signal (Schreiber 1986, Schreiber et al. 1986, see list of literature in the Appendix, section 5.8.).

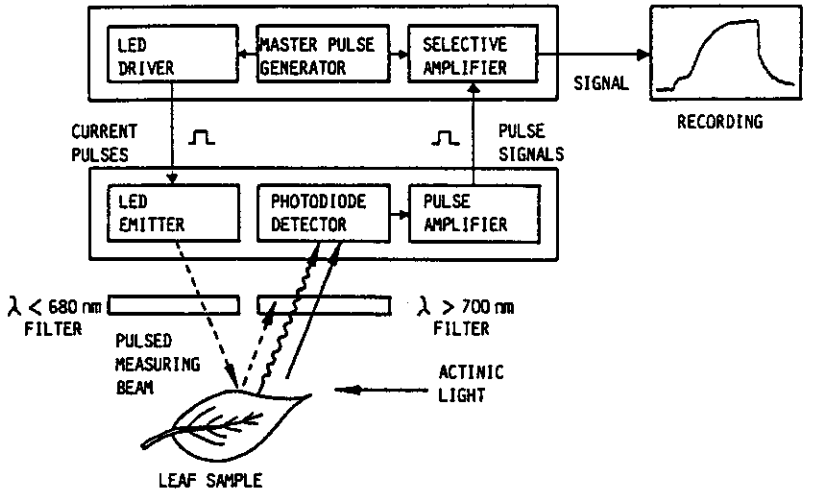


Fig. 3

The pulse-amplitude-modulation (PAM) principle displays the following essential features:

Fluorescence is excited by very brief but strong light pulses from light-emitting diodes. With the PAM-2000, these pulses are 3 µsec long and repeated at a frequency of 600 or 20000 Hz. The LED light passes a short-pass filter (λ<670 nm) and the photodetector is protected by a long-pass filter (λ>700 nm) and a heat absorbing filter. A highly selective pulse amplification system ignores all signals except the fluorescence excited during the 3 µsec measuring pulses. The photodetector is a PIN-photodiode which displays linear response with light intensity changing by factors of more than 10^9. Hence, this measuring system tolerates extreme changes in light intensity (up to several times the intensity of full sun light) even at weak measuring light intensities. This property is essential for correct determination of minimal and maximal fluorescence yield, Fo and Fm (see 1.2. and 3.3.1.1.).

1.4. Special features of the Portable Fluorometer PAM-2000

The incentive for development of the PAM-2000 Fluorometer arose with the realization that

- quantitative information on photosynthetic quantum yield and electron transport rate can be derived from simple fluorescence measurements
- present knowledge on fluorescence analysis suggests numerous practical applications in ecophysiological field studies
- for harvesting the available information it is not sufficient to measure fluorescence, but essential to apply the full range of diagnostic tools
- in order to be useful for in situ field studies, a fluorometer must be self-contained and readily portable, without major sacrifice in its diagnostic potential.

The PAM-2000 Fluorometer, as it eventually was developed, is far more than just a fluorometer. In combination with a Notebook PC, it rather may be considered 'a portable laboratory' for fluorescence analysis. It consists of a multitude of components each of which could well be as large or even larger than the whole PAM-2000, such as:

- modulated measuring light source
- red actinic light source
- far-red actinic light source
- white actinic light source
- saturation pulse source and pulse generator
- regulated power supplies for various light sources
- on/off controls for various light sources
- photodetector and pre-amplifier
- selective amplifier for modulated signal
- microcomputer
- transient recorder and data logger
- battery with charging circuitry

The PAM-2000 is unique in being exclusively operated via a computer key terminal. Fortunately, very small Notebook size PCs are available, which do not add much to the total weight, and provide extraordinary flexibility in system operation, data analysis and storage. The PAM-2000 Fluorometer takes full advantage of the enormous progress made in micro-computer technology. A considerable part of the former electronics and general hardware now resides in microcomputer and PC software. With the combination of computer, microcontroller and solid-state optoelectronic devices a new generation of scientific instruments has evolved, which offers exceptional possibilities of system control. All instrumental settings and measuring parameters are computer controlled, by virtue of which measuring reliability is high, even with unexperienced users following complex experimental protocols. This is achieved by pre-programmed sequences of commands which can be started by operation of single keys (see 3.3.1.13. and 3.5.).

A feature of considerable importance, particularly for field investigations, is the large data storage capacity offered by RAM cards in the portable PC. All data obtained by on-line quenching analysis (see 1.2.) are automatically stored. In this way, the researcher can concentrate on the actual measurements, collecting large amounts of data in a short time and postponing detailed analysis to a later time. This will be particularly appreciated with measurements involving samples in a rapidly changing environment or at locations with low accessibility. In these cases, it will be also advantageous that the PAM-2000 offers the possibility of on-line recording of PAR and temperature at the leaf level (see 2.5.).

Probably the most outstanding feature of the PAM-2000 is its extreme miniaturization with respect to the original PAM Chlorophyll Fluorometer which was achieved without any loss in performance and signal quality. Its smallness and simple operation should act 'catalytically' on its potential use by lowering the 'activation energy' for taking the instrument out into the field, where photosynthesis takes place under natural conditions.

2. COMPONENTS OF THE PORTABLE FLUOROMETER PAM-2000

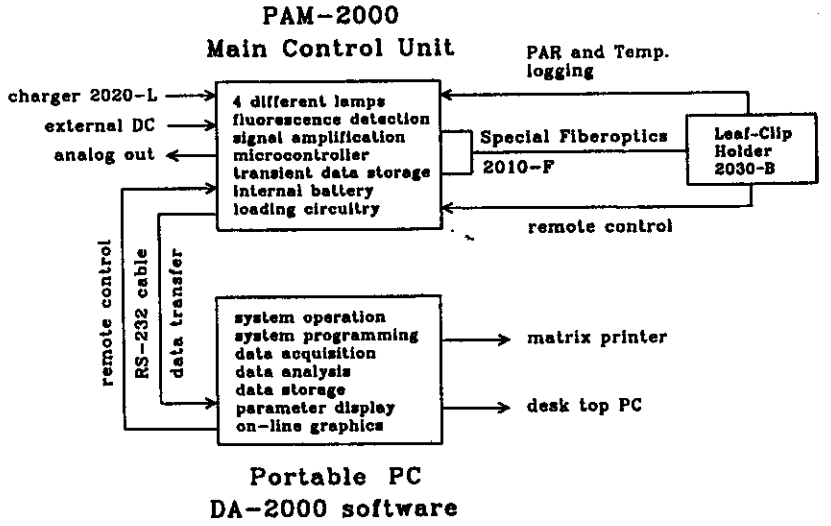


Fig. 4

The PAM-2000 Fluorometer consists of three basic parts forming the minimal functional unit of this measuring system

- Main Control Unit
- Special Fiberoptics 2010-F
- Data Acquisition Software DA-2000, to be used in conjunction with a Poqet PC, IBM or IBM-compatible PC.

The Main Control Unit contains the actual fluorometer with various light sources, detectors and electronics hardware. The fiberoptics form the optical link to the plant sample. The DA-2000 software provides the framework for operation of the fluorometer via a PC keyboard 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 2050-H and the Dark Leaf Clip DLC-8. The various components are described in the following sections.

2.1. PAM-2000 Main Control Unit

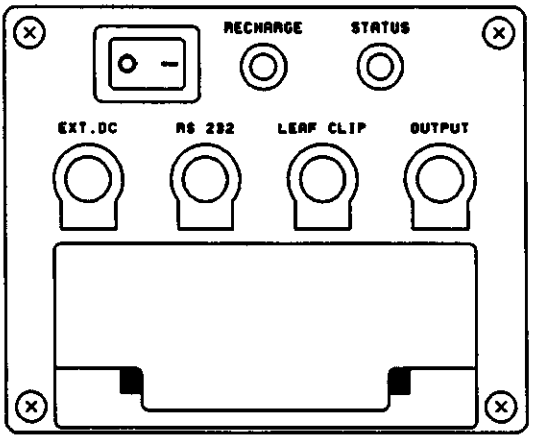


Fig. 5

The PAM-2000 Main Control Unit measures 20 x 10.5 x 8.5 cm (LxWxH) and weighs 2.0 kg (including internal battery). All parts communicating with the outside are located at the front side of the aluminum housing:

- **Switch 0/1**
  Power on/off switch. With power-on there is a basic current of 90 mA with all lamps-off. This current is increased to max. 130 mA when the Poqet PC is connected. When power is switched off while the DA-2000 program is running, the computer reads zero Volt and 'Low battery!' is announced (see 3.3.1.16.).

- **LED 'RECHARGE' (red)**
  This LED provides information whenever the internal battery is connected to an external battery for recharging using the internal charging circuitry. There are three situations:

Continuous light	:	charging is going on
Pulsing light	:	charging stops because external voltage is below 10.5 V
Light off	:	charging is completed, as internal battery has reached its maximal capacity

  When the Battery Charger 2020-L is connected, charging does not involve the internal charging circuitry and, therefore, the 'RECHARGE' LED is not activated.

- **LED 'STATUS' (green)**
  Normally 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 switching power off/on at the PAM-2000 should restore normal functioning.

- **Connector 'EXT. DC'**
  To connect an external 12 V DC source, as e. g. the Battery NP-3/12 or the Battery Charger 2020-L, for recharging of the internal battery (see also 2.4.). The pin-connections at the plugs of the battery cable and of the Battery Charger cable are different, such that with the Battery Charger the internal charging circuitry is by-passed and the 'RECHARGE' LED does not light up.

- **Connector 'RS 232'**
  For connection with the Poqet PC, IBM or IBM-compatible PC via the RS 232 interface cable. When the DA-2000 program is started without this cable being connected, there is a warning 'No PAM-2000 connected! Press 'Return' to continue. After connection Alt-I'. The connection is not required for analysis of data stored in disk or

RAM memory (see 3.4.1.5. and 3.7.1.).

- **Connector 'LEAF CLIP'**
  To connect the Leaf-Clip Holder 2030-B (see 2.5.) or the Micro Quantum/Temp.-Sensor 2060-M (see 2.6.).

- **Connector 'OUTPUT'**
  Analog signal output to chart recorder, 0-2.5 volt

- **Fiberoptics connector**
  To connect the Special Fiberoptics 2010-F (see 2.2.).

Note: The four cable connector plugs should not be mixed up. Do not force a plug into the wrong port. The proper positioning is indicated by the red dots. Do not try to disconnect a plug by pulling at the cable; it will be readily disconnected by pulling at the rippled metal part of the plug.

The interior of the PAM-2000 Main Control Unit is divided into three compartments with largely differing functions:

In the rear, approx. one third of the total space is occupied by a 12 V/1.2 Ah rechargeable battery. The other 2/3 of the total space split into two levels, with the lower level occupying the various light sources, fuses, detectors, pre-amplifiers and optical component, and the upper level housing printed circuit boards with the microcontroller, A/D and D/A converters, lamp drivers, amplifiers and other electronic components. To open the PAM-2000, e. g. for fuse, lamp or EPROM replacement (see section 4), first the four top screws and the lid are removed, so that the printed circuit boards and cables become visible. To access the optical part, also the two bottom screws at the front side are removed. Then the front 2/3 of the interior can be pulled out and, turning the instrument upside-down the components at the 'optical level' can be inspected.

In the following some details on the various opto-electronical components are given:

- **LED cone**
  This is a lucite cone which funnels the light of 7 LEDs into one arm of the tri-furcated fiberoptics (see 2.2.). The center LED (H-3000 Stanley) emits red pulsed measuring light (peak wavelength 650 nm) which passes through a Balzers DT Cyan short-pass filter (λ<670 nm). Another five H-3000 LEDs serve for red actinic illumination (unfiltered, peak at 655 nm). The seventh LED (KL 571, Shinko) provides far-red light (peak wavelength 735 nm); wavelengths below 700 nm are cut off by a RG 9 filter (Schott). Close to the end of the lucite cone, where it interfaces the fiberoptics, a special light-sensor is installed at 90° angle, which serves to monitor the measuring light intensity (see 3.3.1.8.). Selective measurement of the pulsed measuring light is accomplished by the same approach as with the actual fluorescence measurement (see 1.3.). There are slow, temperature-dependent changes in measuring light intensity, knowledge of which can be important for assessment of genuine changes in the minimal fluorescence yield, Fo (see 3.3.1.1. and 3.3.1.2.).

- **Photodiode detector**
  The photodiode (BPY 12, Siemens) together with the pre-amplifier (SMD-technology) is enclosed in a 10 mm metal tube, the front of which is covered by a RG 9 (Schott) long-pass filter and a heat filter.

- **Halogen lamp**
  A miniature 8 V/20 W halogen lamp (Bellaphot, Osram) is focused on one of the three fiberoptics endpieces. Wavelengths above 710 nm are removed by a short-pass filter (Balzers, Calflex-X special). The halogen lamp serves for generation of saturation pulses (max. voltage 8 V) and for continuous actinic illumination (max. voltage

4.5 V). Prolonged continuous operation of the halogen lamp is not recommended for two reasons: Even at low settings its power consumption is about 10 times higher than that of the whole rest of the instrument. Furthermore, its heat generation raises the internal temperature, which affects the measuring light intensity; a temperature-switch mounted directly underneath the lamp will turn off the lamp power supply when 70 °C is reached. It will be turned on again when temperature has dropped to approx. 55 °C. Behind the short-pass filter a light-sensor is mounted which monitors the halogen lamp output. At present the resulting signal is not yet further processed.

The PAM-2000 Main Control Unit is normally enclosed in a padded carrying bag with the Poqet PC mounted on top of it. The front panel with power switch, status LED and various connectors is accessible via an opening at the right hand side. In this way, carrying the bag at his front, the user can guide the fiberoptics to the sample with his right hand and operate the keyboard with his left hand. At the bottom of the housing of the PAM-2000 Main Control Unit a thread-adaptor for tripod mounting is provided which may be useful for stationary measurements in the field.

A Transport Case 2040-T is provided which offers sufficient room for transport of the PAM-2000 with most of its accessories.

2.2. Special Fiberoptics 2010-F

The Special Fiberoptics 2010-F are connected to the front side of the PAM-2000 Main Control Unit with the help of a special plug, which resembles an interface connector. There are three 'fiber pins' with different optical cross-sections, which fit into the corresponding holes at the front side of the PAM-2000 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 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.

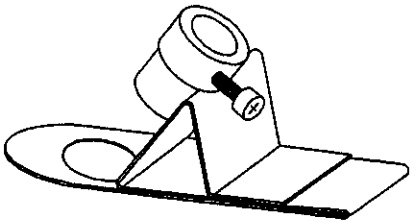


Fig. 6

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. The relative magnitude of this gradient is reduced with increasing fiber distance. However, this point should not be of too

much concern, as there is anyways 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.

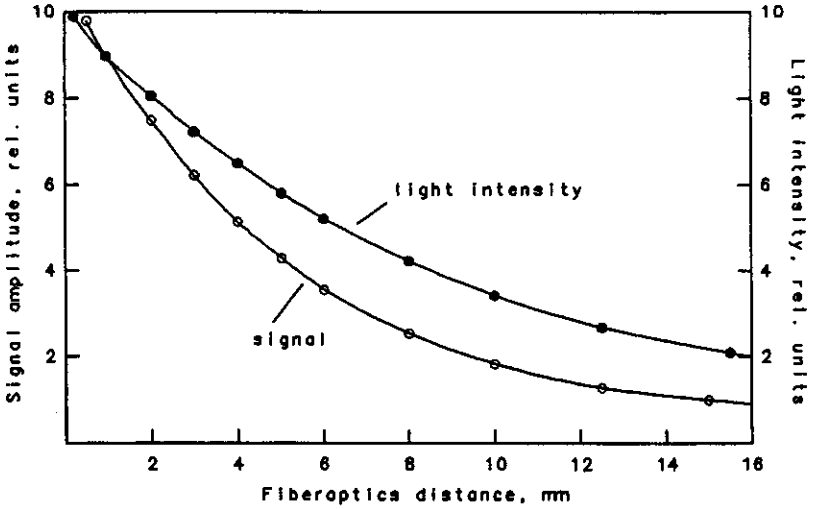


Fig. 7

For measurements with leaves the special Leaf-Clip Holder 2030-B was developed, featuring an integrated micro-quantum-sensor and a thermocouple (see 2.5.).

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

2.3. Data Acquisition Software DA-2000

The Data Acquisition Software DA-2000 is delivered in two versions:

- for IBM or IBM-compatible computers in form of 5 1/4" and 3 1/2" program disks (desktop version)
- for the Poqet PC in form of a program EPROM, which is already installed in this PC upon delivery (see separate manual for Poqet PC).

Due to some peculiarities of the Poqet PC, the Poqet version of the DA-2000 differs in various respects from the normal version:

- graphics are monochrome instead of colored
- hard copy print-outs of kinetic recordings require the Kodak Diconix 180 si printer with serial interface
- on the Kinetics Screen the instrumental settings are displayed in abbreviated form
- the DA-2000 supports an on/off switch for the Poqet power management
- no 'Drive' sub-menu point is given for the Poqet PC, as the DA-2000 program is always on drive B (EPROM card) and data storage is on drive A (RAM card).

To operate the DA-2000 with IBM or IBM-compatible PCs, the following requirements must be fulfilled:

- processor type 286 or higher
- hard disk
- EGA, VGA or Hercules graphics adapter
- 640 kB
- at least 540 kB free memory should be available under MS-DOS

Installation of the DA-2000 proceeds by the following steps:

- insertion of the program disk
- definition of the drive into which program disk was inserted as 'current drive' (e. g. A:)
- entering of 'Install' to call up the INSTALL-program which transfers the DA-2000 program on hard disk (drive C:). A directory C: PAM-2000 is created into which all files essential for DA-2000 operation are copied. Then the program is automatically started. Following a 'cold start', first the PAM-2000 directory must be entered (cd PAM-2000) and then DA-2000 has to be called up.

The following files are present on the program disk:

INSTALL	.BAT	-	Installs the DA-2000 on hard disk (in C:\PAM-2000) (only desktop version)
DA-2000	.EXE	-	The actual data acquisition program
DA-2000	.OVR	-	Overlays of data acquisition program
LORES	.FON	-	First print set for Hercules card
HIRES	.FON	-	Second print set for Hercules card
HERC	.BGI	-	Graphics driver for Hercules card (only desktop version)
EGAVGA	.BGI	-	Graphics driver for EGA/VGA graphics cards (only desktop version)
SANS	.CHR	-	1. Graphics character set
TRIP	.CHR	-	2. Graphics character set
DOASCII	.EXE	-	Program to transform CMN-file into ASCII-file
DATA	.DOC	-	Documentation text file on data acquisition software
COMMAND	.DOC	-	Documentation text file on DA-2000 commands
PARFIELD	.DOC	-	Documentation text file on parameter fields and associated key commands
RPT2WKS	.EXE	-	Program to transform RPT-files

Two additional files are automatically created by the program:

DA-2000	.CFG	-	Configuration file containing sets of instrumental settings (see 3.3.1.22.)
STANDARD	.RPT	-	Report-file containing data and on-line calculated parameters measured in the 'Saturation Pulse Mode' (see 3.3.1.17.).

Three more files are opened by the user in conjunction with the 'Write'-command:

NAME	.CMN	-	Data file originating from kinetic recordings in the 'Continuous Mode' and 'Triggered Mode' (see 3.4.1.5.)
NAME	.CMP	-	Data file originating from kinetic recordings in the 'Saturation Pulse Mode' (see 3.3.2.4.)
NAME	.ASC	-	ASCII-file of the data-file with identical name, containing the on-line calculated quenching parameters (see 3.7.).

When the DA-2000 program is started by entering 'DA-2000', the normal color version is installed. Alternatively, there is the possibility of entering 'DA-2000 MONO', in which case the monochrome version is installed. This version gives higher contrast with LCD-displays and, hence, is advantageous with most Laptop PCs. Another advantage is given with hardcopies, print-out of which is speeded up, because there is no need of redrawing the screen in monochrome (see 3.4.1.5.). Furthermore, only with the MONO-version it is possible to print out superimposed curves from Mem. 1-4 (see 3.4.1.5.).

2.4. Battery Charger 2020-L

The Battery Charger 2020-L is designed for simultaneous charging of the internal battery in the PAM-2000 Main Control Unit and of an external 12 V battery (e. g. NP-3/12) with a capacity not exceeding 10 Ah. Approx. 4 h are required to recharge an empty internal battery.

For charging of the internal battery the 'EXT.DC' cable is connected to the 'EXT.DC' connector at the front side of the PAM-2000 Main Control Unit. The other cable is used for charging of an external 12 V battery, with the red contact connected to '+' and the black contact connected to '–'.

Charging of the batteries is fully automatic due to the voltage limited charging process. Overcharging is impossible and, hence, the charger may remain continuously connected when the PAM-2000 Fluorometer is used in the laboratory. Charging starts as soon as the charger is connected to the mains supply. When a DC output is produced, the indicator LED for the Battery Charger 2020-L lights up. The intensity of this LED diminishes with decreasing charge current. It extinguishes at an approx. current of 10-20 mA.

It should be noted that the red 'RECHARGE' LED at the front panel of the PAM-2000 Main Control Unit does not light up upon charging with the Battery Charger 2020-L. It only lights up when the internal charging circuitry is used, e. g. with recharging from a external 12 V battery. Using the Battery Charger 2020-L the internal charging circuitry is by-passed.

2.5. Leaf-Clip Holder 2030-B

The Leaf-Clip Holder 2030-B may substitute for the standard 'Distance Clip' as a device for defined positioning of the fiberoptics relative to the leaf plane. 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. The distance between fiberoptics and leaf can be varied. Standard distances are defined by spacer rings. In addition, the Leaf-Clip Holder 2030-B displays the following features:

- **Micro-quantum-sensor monitoring PAR**
  This tiny sensor 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 79 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 which is limited by a metal ring of 10 mm inner diameter. 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; and a blue-enhanced silicon photodiode. Despite its small dimensions, the diffuser displays properties of 'cosine correction', i. e. also light impinging at rather small incidence angles (e. g. with rising or setting sun) is reliably monitored. Due to the equalization of leaf and sensor planes, automatically achieved by fastening the leaf in the clip, the measured effective 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 corresponding parameter field of the monitor screen (see

3.3.1.6.) when the leaf-clip holder is connected. The sensor was calibrated against a LI-COR Quantum Sensor (Type LI-190). The stability of calibration depends strongly on keeping the diffuser clean. Also, it must be pointed out that there is some decrease in sensitivity (approx. 10%) when the sensor is moved from the center of the measuring field to its peripherie. It is advisable to check calibration regularly by comparison with a standard quantum sensor, like the LI-190. Any deviation can be corrected by entering a recalibration factor via the 'Alt C'-command of the DA-2000 software (see 3.3.1.19.). 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 alcohol. In addition, it is possible to enter an offset value via the 'Ctrl O'-command (see 3.3.1.20.).

- **Thermocouple monitoring leaf temperature**
  A NiCr-Ni thermocouple is mounted in the perspex tube on which the studied leaf area is resting. Its tip is forming a loop which 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 (AD), 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 $\Delta V/°C$. 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 'Ctrl O'-command with a resolution of 0.3 °C. The measured temperature is displayed in the 'Tmp'-parameter field of the monitor screen (see 3.3.1.15.) 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 (see 3.3.1.17.).

- **Remote control push button**
  Pressing the 'remote' control push button on the handle of the Leaf-Clip Holder 2030-B is equivalent to a 'Return' with the PC keyboard. In practice, this offers the advantage, that the experimenter can use both hands for positioning the leaf within the holder and at the same time trigger a recording by remote control. In this way, sampling is considerably facilitated, which is particularly helpful when many recordings are averaged to increase the accuracy of determinations (see 3.3.1.5.).
  The specific command carried out by 'Return' or remote control depends on the cursor position on the 'Parameter Screen' (see 3.3.1.). The remote control function is particularly useful in conjunction with Run-file 1 (see 3.3.1.13. and 3.5.1.) 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.

- **Tripod mounting thread**
  Mounting the Leaf-Clip Holder 2030-B on a tripod (e. g. Compact Tripod ST-2101) facilitates long term recordings with the same plant. Such recordings can be automated by using the Clock-function (see 3.3.1.11.) and the pre-programmed Run-files (see 3.5.).

- **Holes for mounting External Halogen Lamp 2050-H**
  Two holes are provided in the front bottom part of the holder for mounting the optional External Halogen Lamp 2050-H (see 2.7.). This lamp allows long periods of illumination with strong light, as e. g. required for photoinhibitory treatment. It is <u>not</u> recommended to use the internal halogen lamp for this purpose (see 3.3.1.10.), as this would lead to excessive internal heating and rapid depletion of battery power.

2.6. 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 2.5.), 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' of Fig. 6.

It should be pointed out that the sensitivity of the micro quantum sensor is affected by bending the relatively long, flexible light guide which 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 (Type LI-190), against which it was originally calibrated. Recalibration (see 3.3.1.19.) is recommended when bringing the sensor and the metal housing into a fixed position with respect to the object.

2.7. External Halogen Lamp 2050-H

The External Halogen Lamp 2050-H provides a strong light source for prolonged illumination periods, for which purpose the internal halogen lamp is not suited because of the heat developing within the PAM-2000 housing. A 20 W Philips Masterline lamp is powered by an external battery (e. g. NP-3/12). Its intensity can be varied steplessly via a 15-turn potentiometer. Power consumption is minimised by special electronic circuitry. The Masterline lamp is equipped with a heat-reflecting, sealed 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 and only 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 with which, however, the maximal possible intensities are lower.

The External Halogen Lamp is meant to be used in conjunction with PAR-measurements, as performed with the Leaf-Clip Holder 2030-B. In its normal application, it is mounted on the Leaf-Clip Holder, with the light (8° beam divergence angle) shining at an approx. 60° incident angle with respect to the leaf plane on the site where fluorescence and PAR are measured. The optimum angle, giving maximal PAR and minimal shading by the fiberoptics can be manually adjusted, preferentially using a white piece of paper instead of a leaf. With the 15-turn potentiometer defined PAR-values can be chosen, which are read off the PC parameter screen (see 3.3.1.6.). A switch is provided to turn the lamp on/off.

A major application of the External Halogen Lamp is the adjustment of defined light intensities for measurements of light saturation curves under field conditions. For this purpose, the light obtained from this lamp may substitute or complement the natural daylight. Intensities corresponding to PAR-values of more than 3000 µmol quanta $m^{-2}s^{-1}$ can be achieved, exceeding the intensity of direct sun light. Hence, this light source can be also useful 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.

2.8. 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 and which is opened for the actual measurement only, when exposure to external light is prevented by the fiberoptics. Proper dark-adaptation is essential for determination of the maximal quantum yield Fv/Fm (see 3.3.1.1) and for recording dark-light induction kinetics (see 3.3.2.).

Different from the other leaf clips, 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 amplitudes are approx. 2-3 times higher than when the Leaf-Clip Holder 2030-B is used. In order to avoid signal saturation, the settings of measuring light intensity and gain (see 3.3.1.) have to be correspondingly lowered with respect to the standard settings.

When the shutter is still closed and measuring light is on, an artifactual Ft signal is observed. This signal is due to a small fraction of the measuring light which after reflection from the closed shutter penetrates to the photodetector. However, this 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 shiny metal.

2.9. Poqet PC

The Poqet PC presently is the smallest available IBM-compatible computer to be used with the DA-2000 Data Acquisition System. Due to its extremely low weight and low power consumption it is ideally suited for the portable PAM-2000 Fluorometer. In its normal operation, the Poqet PC is located on top of the PAM-2000 Main Control Unit,

contained in the carrying bag.

A separate User's Guide is provided for the Poqet PC, which gives detailed information on the properties, operation and maintenance of this computer. Here only some points will be outlined which refer to the use of the Poqet PC in conjunction with the PAM-2000 Fluorometer.

Memory cards

You have received two types of Memory Cards together with your Poqet PC, an EPROM-card containing the DA-2000 Data Acquisition software and a formatted 1 MByte RAM-card for data storage. These two cards were already installed in the Poqet PC at the company. The EPROM-card (labelled 'DA-2000') has been inserted into the right (as viewed from top) card drawer (drive B:) and the RAM-card (labelled '1 MB RAM') into the left drawer (drive A:).

RS 232 interface cable

A special cable is provided with the PAM-2000 which not only serves for serial data transfer but also connects the Poqet PC with the internal battery of the PAM-2000. In this way, the major power for operation of the Poqet PC is provided by the large, rechargeable battery of the PAM-2000. The two small AA batteries of the Poqet PC still are required for powering the monitor screen. The interface cable can remain permanently connected to the Poqet PC, thus protecting the delicate 80-pin connector. The other cables for Poqet Link and print-out are made to match the connector which normally links to the PAM-2000. When the connection between Poqet PC and the PAM-2000 or the printer is made, both instruments should be switched off. After the connection between the Poqet PC and the PAM-2000 is made, drive B: should be selected. Then the program is started by entering 'DA-2000'.

Data transfer with Link Cable for Poqet PC

With this cable, which directly connects to the RS 232 interface cable, data can be transferred from the Poqet PC to a Desktop

computer (see 3.7.2.).

Printer cable PQ-DK

This optional cable directly connects to the RS 232 cable of the Poqet PC. It allows serial data transfer to IBM or IBM compatible printers (see 3.7.3.).

Power saving management

The Poqet PC consumes very low power due to a special power saving management. When the DA-2000 program is installed, the normal Poqet PC key combination for power management is not active and a special command in the Data sub-menu (see 3.4.1.5.) is provided to enable and disable power management.

Unfortunately, in the present combination of the Poqet PC power management and the DA-2000 program, there is a tendency of the Poqet PC to "awake" from its "sleep" mode, just as if the I/O key were pressed. Therefore, it is recommended to leave the DA-2000 program via 'Alt X' before turning off the Poqet PC via I/O. Alternatively, if the present fluorescence information shall be retained, the user may disable power management or simply enter the editor level via Ctrl E, before turning off the PC. At the editor level, power management is not active.

Replacement of AA batteries

When the batteries are running low, a bar is displayed above the battery symbol on the status indicator line of the Poqet PC and the computer will beep once (see Poqet User's Guide). It is essential to replace the two AA batteries despite the fact that most of the power consumed by the Poqet PC is provided by the PAM-2000 via the RS 232 interface cable. The two batteries are required for operation of the monitor screen, which draws a current of approx. 5 mA. Even when the Poqet PC is turned off, it draws a weak current of approx. 0.5 mA. Therefore, it is normal that batteries need replacement every 2-3 months. Continued use without replacing the batteries may cause loss of stored data.

3. OPERATION OF THE PORTABLE FLUOROMETER PAM-2000

3.1. How to get started

With your Portable Fluorometer PAM-2000 you have obtained a number of components and accessories which are described in section 2. To get started, you have to proceed as follows:

- Install the Data Acquisition Software DA-2000 on your PC (see 2.3.). If you have purchased the Poqet PC together with the PAM-2000, the software is resident in an EPROM-card which should be installed in the right hand card drawer (drive B:) of the Poqet PC.
- Connect PC and PAM-2000 via the RS 232 cable, while both intruments are turned off.
- Connect the Special Fiberoptics 2010-F to the PAM-2000.
- Switch on power at PAM-2000 and PC.

A flashing green 'STATUS' LED indicates normal functioning of the PAM-2000 processor module. After proper installation of the DA-2000 program, the PC monitor screen will display a list of 'parameters' which are arranged in 5 columns with 7 lines each. The first column and the two bottom lines display measured parameters, whereas the remaining parameters in columns 2-5 represent instrumental settings, primarily relating to the four different light sources for measuring light (L), actinic light (A), saturation pulse light (S) and far-red light (F). For quick reference on the meaning of the various parameters you may consult the list in section 5.6. In section 3.3.1. a detailed outline is presented.

If you first started the DA-2000 program before making the connection between computer and PAM-2000, there was a message "No PAM-2000 connected! Press 'Return' to continue. After connection 'Alt-I", as the computer does not get any signal from the PAM-2000.

The DA-2000 program provides this kind of explanatory text in numerous situations in order to assist the user in proper system operation. In this case, after pressing 'Return' and 'Alt I', a mode selection menu (see 3.2.) is entered and after another 'Return' the 'Saturation Pulse Mode' with the 'Parameter Screen' is installed.

You are now ready for measuring chlorophyll fluorescence. To do so, you switch on the measuring light by pressing 'L' on the PC keyboard. (Details on the measuring principle of the PAM fluorometer are presented in section 1.3.). When you look at the fiberoptics exit, you will see the weak measuring light which excites chlorophyll fluorescence in a green leaf. As long as there is no leaf, 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-2000, can be mounted on the fiberoptics (see 2.2.). Alternatively the Leaf-Clip Holder 2030-B can be used which also provides sensors for light-intensity and temperature measurements (see section 2.5.).

Relevant information is obtained when the yields of fluorescence in different states of illumination are compared. For this purpose the PAM-2000 contains a number of so-called 'actinic' light sources (see section 2.1.), which are switched on/off by key operations. When you press 'A' 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' (see sections 1.2., 3.3.2. and 3.4.). Pressing 'A' again, the actinic red light is turned off and you can see Ft decreasing.

When you now apply 'F', 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 (see section 1.2.). The minimal fluorescence yield, called Fo, is observed when all PS II reaction centers are open, which is the case after dark-adaptation. By pressing 'Z' this value can be stored in the Fo-field. 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 so-called 'saturation pulses' which are applied by pressing 'M'. Actually, with 'M' both Fo and Fm are determined quickly one after the other (see 3.3.1.1.). At the same time also the value of 'Fv:m' is calculated, corresponding to the ratio Fv:Fm=(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' should amount to approx. 0.8.

Instead of pressing 'M', you can also trigger saturation pulses via 'Y' (Yield) or S (Sat. Pulse). Then with each measurement a new value is entered into the 'Yield'-field. 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 by 'A', you will see the 'Yield'-value first decrease and then rise again, stabilizing in the steady state at a constant value which is characteristic for the photosynthetic performance of the given leaf sample. If you get tired of pressing 'Y' or 'S' you can press 'P' (Pulse Seq.) to trigger a sequence of saturation pulses which will be applied at defined 20 s intervals (Clk-parameter) until you again press 'P' to stop it. While the pulse sequence is still active, you notice that after each pulse not only 'Yield' is up-dated, but also values in a number of other parameter fields, namely No, qP, qN, ETR and Fm' are changing. 'No' simply denotes the current number of a pulse in the sequence. The other parameters relate to different forms of fluorescence quenching, about which you may get informed by reading the corresponding parts in section 3.3.1. or more briefly by consulting the list in section 5.6.

The fluorescence data which you have recorded by activating 'M', 'Y', 'S' and 'P' are not lost. Actually, they were automatically stored in

a so-called 'Report-File' which can be entered by pressing the key combination 'Ctrl E' (see section 3.3.1.17.). You may also want to see the data recorded during the pulse sequence plotted versus time. For that purpose you first return to the 'Parameter Screen' (via 'Esc') and then switch to the 'Kinetic Screen' by pressing 'K' (for details see 3.3.2.).

In the meantime, you not only got started with the PAM-2000 Fluorometer, but also learnt how to perform the basic and most important types of measurements. In the section 3.3.1. (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, the sections 3.2., 3.3.2. and 3.4. are recommended.

3.2. Different modes of data acquisition

Once the Data Acquisition Software DA-2000 is installed, an Autoexecute-Routine initializes the so-called 'Saturation Pulse Mode' when the program is started. This mode of operation is by far the most common with the PAM-2000 Fluorometer, in particular with field measurements, for which this instrument was primarily designed. In addition there are two other modes of operation, namely the 'Continuous Mode' and the 'Triggered Mode'. There is a short mode selection menu which can be called by the key combination 'Alt I'.

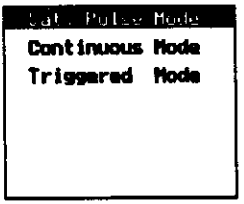


Fig. 8

By pressing the initial characters 'S', 'C' or 'T' the corresponding modes are activated. Alternatively, selection can also be made using the arrow keys and 'Return'.

The choice of the data acquisition mode is determined by the envisaged application:

- **Saturation Pulse Mode**
  For all applications which 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 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 given (see 3.3.1.18.).

- **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 within the PAM-2000 and are transferred to the PC after the recording. In this way much higher time resolution and sampling rates are possible.

Further details on the practical application of these three data acquisition modes are presented in sections 3.3. and 3.4.

3.3. Measurements in the 'Saturation Pulse Mode'

The 'Saturation Pulse Mode' is the most common mode of data acquisition with the PAM-2000 Fluorometer (see 3.2.). It is automatically installed when the DA-2000 program is loaded. With 'Alt I' one can enter the selection menu for the two other modes and also return again to the 'Saturation Pulse Mode'. The recorded data may be displayed in numerical form using the so-called 'Parameter Screen' (see 3.3.1.) or in graphical form using the 'Kinetics Screen' (see 3.3.2.). In addition, all saturation pulse data are stored in a so-called 'Report-file' which is accessible via the 'Ctrl E'-command (see 3.3.1.17.).

3.3.1. Using the 'Parameter Screen'

When the computer is turned on and the DA-2000 program is started, the monitor will show the 'Parameter Screen' (in the 'Saturation Pulse Mode' of data acquisition). In the following illustration the 'Parameter Screen' is displayed with the Standard-settings (except that the meauring light is turned on) which are installed by the '0'-command (see 3.3.1.20.).

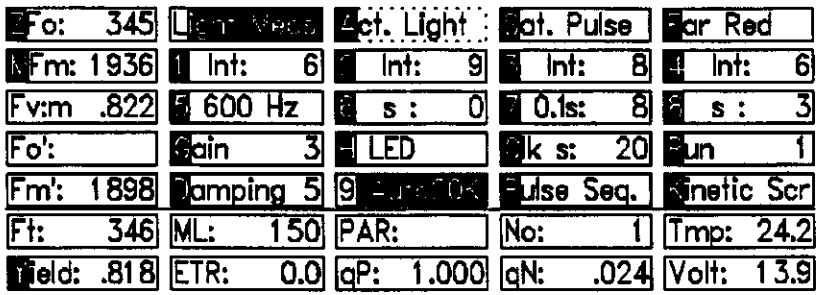


Fig. 9

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 (see 2.1.). The first letters (L, A, S and F) are inverted when the light sources are off. In the given figure, the L-source (measuring light) is switched on. The initial character keys may be visualized as on/off switches. Pressing L, A, S, or F will activitate the corresponding lamp and cause inversion of the letters in the given parameter field. 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 sat. pulse lamp and 3 s with the far-red lamp. The second fields of rows 2-5 pertain to the corresponding light intensities with the function of dial switches. The pre-set values are suitable for standard experiments (see 3.5.). 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). Field selection can also be 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)**. Upon operation of these keys the most relevant determinations of fluorescence and quenching parameters are performed (see corresponding sub-sections). 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 accesses a special 'Report-file' in which the relevant data get stored and which can be edited by the user (see 3.3.1.17.).

In sub-sections 3.3.1.1. to 3.3.1.16. the various functions linked to the different parameter fields are outlined in detail. Each of these sub-sections may be read separately 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 Appendix 5.6.

3.3.1.1. 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 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 (see 1.2.). '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 (see 1.2.), the dark relaxation of which displays several phases with vastly different rates (see 3.5.5.). 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 by pressing 'Z'. If the measuring light (L) is not yet turned on, this will be done automatically before Fo-sampling. When 'M' is activated, there is first Fo- and immediately afterwards Fm-determination by application of a saturation pulse. 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 slightly below 400 by appropriate choice of measuring light intensity (see 3.3.1.8.), Gain (see 3.3.1.9.) or fiberoptics-to-sample distance (see 2.2.). In this way, optimal resolution is provided without the risk of amplifier saturation (see also 3.3.1.4.). With 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, 600 Hz) 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' is pre-set at settings 8 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 'Alt M' command (see 3.3.1.18.).

The values of Fo, Fm and Fv/Fm are written automatically into a 'Report-file' (see 3.3.1.17.) which can be accessed by the 'Ctrl E'-command. When only Fo is determined via 'Z', the measured value is entered in the Ft-column (see 3.3.1.17.).

3.3.1.2. Fo', Ctrl Z and 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:

- **Ctrl Z**

With the Ctrl Z-command a single Fo'-determination is carried out. The determined value of Fo' applies for quenching analysis with the next saturation pulse only. Upon 'Ctr Z' 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, there is averaging of the data points in the five consecutive 1 sec periods of far-red illumination; the lowest of the 5 values is entered as Fo' (in corresponding parameter field and into Report-file (see 3.3.1.17.)).
- 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.

- **Ctrl S**

The 'Ctrl S'-command operates like an on/off switch for a routine to determine Fo' with every application of a saturation pulse via 'S' (on Par. Screen only), 'P' or 'Y'. When 'Ctrl S' is active an asterix appears in the Far Red parameter field and the following sequence of operations is started when a saturation pulse is triggered:

- 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 corresponding parameter field and into Report file (see 3.3.1.17.)).
- 5.5 sec after it was turned on, the far-red light is turned off and simultaneously the actinic light is turned on again.

When Ctrl S is active, the shortest interval between sat. pulses (Clock-parameter, see 3.3.1.11) is 20 s. When Ctrl S is active or after Ctrl Z operation, the quenching coefficients qP and qN are calculated on the basis of Fo' instead of the original Fo (see 3.3.1.7.). Determinations of 'Yield' and 'ETR' are <u>not</u> affected by Fo'-measurements (see 3.3.1.5. and 3.3.1.6.). With the 'M'-command there is normal Fo- and Fm-determination, also when 'Ctrl S' is active.

Fo'-determination is recommended for quenching analysis of samples which have reached steady-state in the light. Steady state fluorescence yield, as indicated by Ft (see 3.3.1.4.) often is close to the original Fo or even below that. In order to determine photochemical and non-photochemical components of overall fluorescence quenching it is then necessary to re-evaluate the true minimal fluorescence yield, Fo'. Depending on conditions, Fo' can be considerably lower than the original Fo (by approx. 35 %). 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 bears consequences on qP- and qN-determination (see 3.3.1.7.). 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 'Ctrl Z' or 'Ctrl S'-function.

Under field conditions, normally actinic illumination is provided by the ambient day light, which cannot be simply turned off as envisaged with the 'Ctrl Z' or 'Ctrl S'-function. 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 care about Fo-quenching and Fo'-determination. With 'Yield' and 'NPQ' two parameters are provided the determination of which does not require knowledge of Fo' (see 3.3.1.5. and 3.3.1.7.). On the other hand, for proper assessment of the proportion of 'open' PS II centers via qP-determination, in most cases determination of Fo' (using Ctrl Z or Ctrl S) is indispensable.

3.3.1.3. Fm'

The parameter Fm' is defined as the maximal fluorescence yield reached in a pulse of saturating light with an illuminated sample. In

green plants, Fm' generally is lower than Fm, which is determined after dark adaptation (see 3.3.1.1.). Per definition, with Fm' as well as with Fm the yield of photochemical energy conversion at PS II centers is zero. The quenching at Fm' with respect to Fm is defined as 'non-photochemical quenching', for which the quenching coefficient qN was introduced (see 3.3.1.7.). The extent of non-photochemical quenching can also be expressed by the so-called 'NPQ'-term (see 3.3.1.7.). After every saturation pulse the current value of Fm' is written into the 'Report-file' along with the on-line calculated parameters (see 3.3.1.17.).

3.3.1.4. Ft

The parameter Ft represents the measured fluorescence yield at any given time. It is determined by redox and energy status of the sample (see 1.2.). For so-called quenching analysis, given Ft-values can be defined as Fo and Fm or Fo' and Fm' by special commands (Z, M, S, Y, Ctrl Z, Ctrl S) (see 3.3.1.1., 3.3.1.2., 3.3.1.3., 3.3.1.5., 3.3.1.7.). With every saturation pulse the current Ft, registered briefly before the pulse, is written into the 'Report-file' along with the on-line calculated parameters (see 3.3.1.17.). An analog signal corresponding to Ft is provided at the 'Output' of the PAM-2000 Fluorometer (see 2.1.). Ft can vary between 0 and 2557, which corresponds 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 and signal amplitude. Hence, this type of noise will be least disturbing the higher the fluorescence signal. On the other hand, reaching 2500 mV should be avoided. In practice, it is recommended to adjust Fo to a value slightly below 400 mV. In recordings involving the 'Kinetics Screen', with the pre-set Y-axis limit at 2 V, the Y-axis is divided into 5xFo and normal induction curves are unlikely to exceed the Y-limit.

3.3.1.5. Yield and Ctrl Y (Averaging)

The 'Yield'-parameter may be considered the most important piece of information obtained with the PAM-2000 Fluorometer. It represents the essence of fluorescence quenching analysis by the saturation pulse method (see section 1.2.). Its information becomes even more instructive when combined with that of effective light intensity and leaf temperature (see 2.5., 3.3.1.6. and 3.3.1.15.). '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 this calculation knowledge of Fo is <u>not</u> required, which is a great practical advantage.

In practice, 'Yield'-determinations are rather simple. After switching on the PAM-2000 Fluorometer and the computer, you only press 'Y' or 'S' (or 'P' for a series of saturation pulses) at the key terminal. The system then automatically turns on the measuring light, samples Ft and immediately afterwards applies a saturation pulse to sample Fm'. If the Leaf-Clip Holder 2030-B is available, 'Yield'-determination can also be started by 'remote control' after activating Run-file 1 (see 3.5.1.) 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 3.3.1.6.).

With every saturation pulse, the measured and the on-line calculated data are written into a so-called 'Report-file' which is accessible via the 'Ctrl E'-command (see 3.3.1.17.). 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 'M'-command (see 3.3.1.1.). Between the last saturation pulse and the 'Ctrl E'-command a few seconds time should have elapsed, to allow entry of the new data.

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 $\Delta F = Fm'-Ft$;
- it decreases with the signal amplitude (see also 3.3.1.1.).

In any case, 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 the 'Ctrl Y'-command. This command functions like an on/off switch. After initialization of the saturation pulse mode the averaging function is off; with 'Ctrl Y' it is switched on and stays on until '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'.

Fig. 10

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' (see 3.3.1.17.), 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. When averaging is started via 'Ctrl Y', the 'No' is reset to 1 upon application of the following saturation pulse. When averaging is stopped via '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	0.592	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			

Fig. 11

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 so-called 'Distance-Clip' which is mounted to the fiberoptics end-piece (see 2.2.) and by use of the Leaf-Clip Holder 2030-B, which is equipped with a remote control button (see 2.5.).

3.3.1.6. ETR, PAR and Alt E

Measurements of the 'ETR'- and 'PAR'-parameters require the Leaf-Clip Holder 2030-B (see 2.5.) or the Micro Quantum/Temp.-Sensor 2060-M (see 2.6.) to be connected to the PAM-2000 Fluorometer. 'ETR' represents the 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:

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

The following assumptions are made:

- 'Yield' represents the overall photochemical quantum yield (see 3.3.1.5.)
- 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 Alt E-command. When 'Alt E' is applied, a dialoge field appears in the center of the monitor screen:

Fig. 12

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 on. The entered coefficient is stored in the Configuration-file (see 3.3.1.22.) when the program is quit via 'Alt X' (see 3.4.1.7.).

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, it is also the topmost layers which normally are 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 signal loss (see 2.5.). 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 (Type LI-190SZ). See section 3.3.1.19. for details on re-calibration and section 3.3.1.20. 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' by operation of 'Y', 'S' (on Par. Screen only) or 'P'. After activation of the 'Ctrl Y'-switch (see 3.3.1.5.) '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 'Ctrl E' command (see 3.3.1.17.).

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 automatic procedure pre-programmed in Run-files 8 and 9 (see 3.5.8. and 3.5.9.).

3.3.1.7. qP, qN, NPQ and Ctrl Q

qP and qN are defined as the coefficients of photochemical and non-photochemical fluorescence quenching, respectively (see also 1.2.):

$$qP = (Fm'-Ft) : (Fm'-Fo) \qquad qN = (Fm-Fm') : (Fm-Fo)$$

These coefficients may vary between 0 and 1. Their on-line calculation requires previous Fo, Fm-determination via the 'M'-command (see 3.3.1.1.). qP and qN are then calculated with every saturation pulse applied via the 'Y', 'S' (on Par. Screen only) or 'P'-commands. The calculated values are written into a 'Report-file' which can be accessed via the 'Ctrl E'-command (see 3.3.1.17.).

The original definition of qP and qN implies that fluorescence quenching affects only the so-called 'variable fluorescence', Fm-Fo, and not to the 'constant fluorescence', Fo. However, it has proven that with qN exceeding 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-2000 offers special procedures for Fo'-determination involving transient darkening and far-red illumination, activated via the 'Ctrl Z'- or 'Ctrl S'-commands (see 3.3.1.2.). When 'Ctrl Z' or 'Ctrl S' are applied, the quenching coefficients are calculated on the basis of Fo' instead of Fo:

$$qP = (Fm'-Ft) : (Fm'-Fo') \qquad qN = (Fm-Fm') : (Fm-Fo')$$

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 the same values.

It should be pointed out that for correct qP and qN-determinations using the 'Ctrl Z'- or 'Ctrl S'-functions 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 activated 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 NPQ-parameter substitutes for the qN-parameter with operation of the 'Ctrl Q'-command. 'Ctrl Q' functions like a switch for selection between qN and NPQ-determination. 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'-determination. On the other hand, NPQ is relatively insensitive to that part of non-photochemical quenching 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.

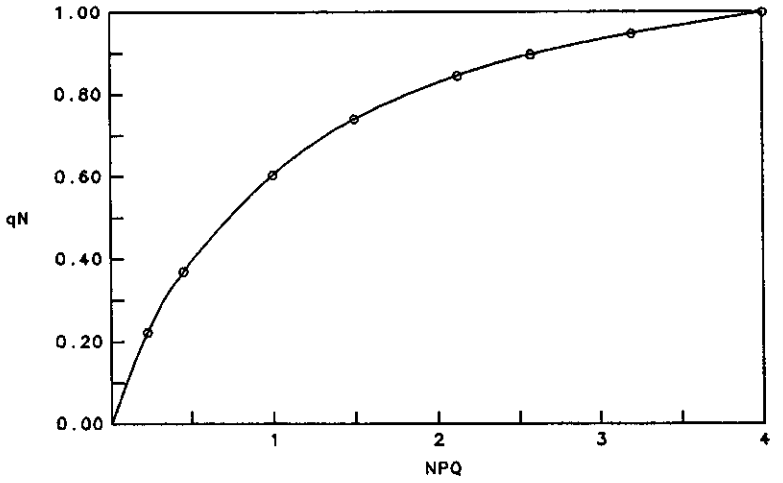


Fig. 13

3.3.1.8. Measuring light parameters

The PAM-2000 Fluorometer operates with a pulse modulated measuring beam and a selective amplifier system which only processes signals arising from fluorescence excited by the measuring light pulses (see 1.3.). After program start and initialization of the 'Saturation Pulse Mode' the parameter fields related to measuring light properties are displayed as follows:

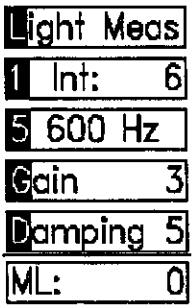


Fig. 14

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:

Light Meas

Via 'L' the measuring light is switched on and off. As long as it is off, the measured fluorescence signal Ft normally is 0. If this is not the case, the displayed value corresponds to an offset intered via the Ctrl O-command (see 3.3.1.20.). When it 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 (see 3.3.1.4.). Measuring light is also automatically activated with a number of commands, the execution of which require measuring light to be on. Such are: 'Z', 'M', 'Y', 'A', 'S' and 'P'. In these cases, a period of 0.6 s is given between onset of measuring light and execution of the given command. After Standard-initialization ('0'-command, see 3.3.1.20.) the measuring light is off.

1 Int: 6

The 'Int'-field is selected by pressing '1'. Alternatively this field (as all parameter fields the status of which can be actively changed) may be selected by cursor (arrow key) operation. The pre-set value can be increased or decreased by operation of 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. To reach the 'super-setting' 11, it is necessary to press '!'. 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 in section 3.5.10.). The absolute measuring light intensity at a given setting depends on the distance between sample and fiberoptics (see illustration in section 2.2.).

5 600 Hz (5 20 KHz) and 9 Auto 20K

Switching from 600 Hz to 20 kHz modulation frequency (and vice versa) is carried out via the '5'-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 (see 3.3.1.3.). 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, couples measuring light frequency with the status of the 'Act. Light' (see 3.3.1.6.). 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 3.3.2.3. and 3.4.).

ML: 0

The ML-value corresponds to the light output of the measuring light LED source (see 2.1.). This is measured within the PAM-2000 housing. The original ML-values at the standard measuring light

intensity 6 should be noted with a new instrument, such that long term loss in measuring LED output can be assessed. It differs between individual instruments and normally amounts to values around 150. 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 also display temperature dependent changes. A temperature increase of 10 °C within the PAM-2000 housing may produce a 10-20 % decrease in measuring light intensity. Actually, this is a most relevant aspect 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 (see 3.3.1.17). 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 apply to all measurements of fluorescence yield ratios (i. e. Yield, Fv:m, qP, qN, NPQ and ETR). They are, however, most relevant for absolute Fo- and Fo'-measurements (see 3.3.1.2.).

3.3.1.9. 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.

Gain 3

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. 300-400 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 (section 3.5.10). By increasing the Gain, not only the signal but also the noise becomes amplified. Therefore, before any Gain-increase, the user should consider whether an equivalent signal increase cannot as well be reached 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.

Damping 5

After selection of the 'Damping'-field via 'D' 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) which is faster than the set time constant.

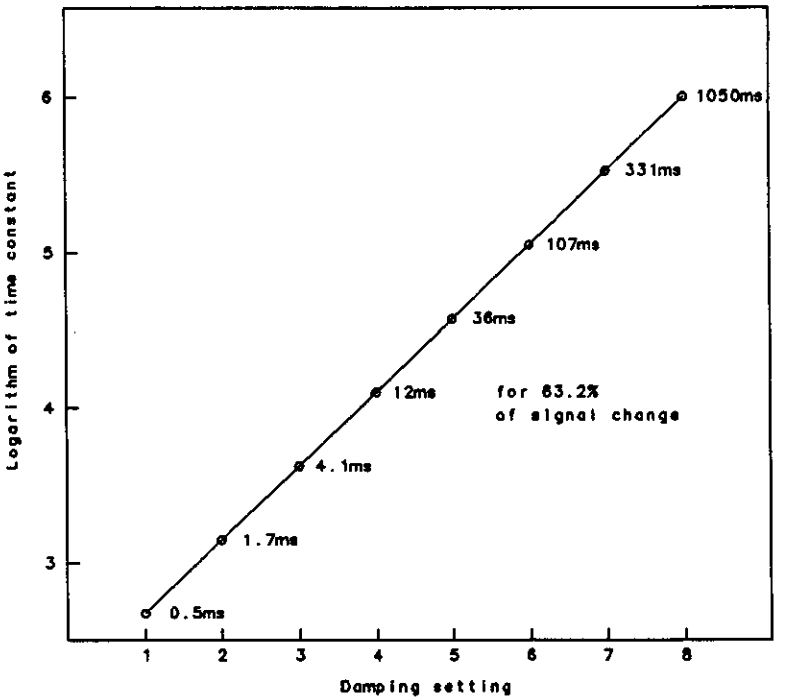


Fig. 15

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 'Sat. 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-2000 software (see 3.4.1.6.).

3.3.1.10. 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:

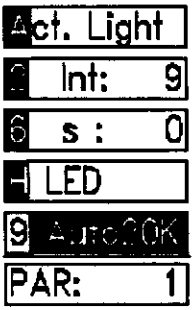
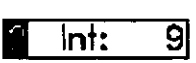


Fig. 16

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:

Via 'A' the actinic light is switched on and off. After Standard-initialization ('0'-command), the LED light source is installed as active lamp. This lamp produces red light peaking around 655 nm (see 2.1.). The on/off characteristics of LEDs are very rapid (in the µs-time range) and well suited for registration of rapid induction kinetics (see 3.4.). The alternative halogen light source, which 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. Normally, with the 'Auto 20 K'-function being active, the frequency of the pulse modulated measuring light automatically is increased from 600 Hz to 20 kHz.



The 'Int'-field for actinic light is selected by pressing '2'. The given setting can be increased or decreased by '+' or '–' operation. The maximal setting obtained in this way is 10. Press '!' to activate the 'super-setting' 11. It is not recommended to operate the LED lamp for longer time periods at this setting. This would cause irreversible loss in LED lamp output. In principle, 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. 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.

Light intensity	1	2	3	4	5	6	7	8	9	10	11
Halogen lamp	46	66	91	148	216	330	491	730	1119	1681	2500
LED lamp	4.2	6.3	9.6	14.1	22.2	32.5	50	74	112	167	250

Fig. 17

The effective 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 (see 3.3.1.6.). At a standard distance of 7 mm and with the fiberoptics fixed at 60° angle, light intensities range from approx. 4 to 250 µmol quanta

$m^{-2}s^{-1}$ with the LED lamp and from approx. 45 to 2500 µmol quanta $m^{-2}s^{-1}$ with the 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.

6 s : 0

This parameter field, which is selected by the '6'-key, refers to the duration of actinic illumination. When 's'-values above 0 are set, actinic light turned on by the 'A'-key is automatically turned off after the given number of seconds. With the pre-set status (denoted with 0), termination of actinic illumination requires manual operation via the 'A'-key. The 's'-settings can be changed by 1 s steps with '+' and '−' operation. Also in the 'Triggered Mode' (see 3.4.) the 's'-settings are effective. Then, however, at setting 0, the duration is automatically adjusted to approx. 2/3 of the total recording time which depends on the sampling rate (see 3.4.1.4.).

H LED or H Halogen

With the 'H'-key one can select between two different actinic light sources, LED lamp and halogen lamp (see 2.1.). 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-2000,
- 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 dissipation, 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-2000 in the vicinity of the halogen lamp exceeds 70 °C (see 2.1.). If longterm illumination with strong white light is essential, e. g. for photoinhibitory treatment, we recommend use of the External Halogen Lamp 2050-H (see 2.7.).

3.3.1.11. 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:

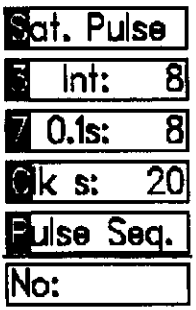


Fig. 18

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:

Sat. Pulse

Single saturation pulses are triggered by operation of the 'S'-key. Alternatively, in the 'Saturation Pulse Mode' single pulses can also be given via the 'Y'- or 'M'-commands (see 3.3.1.5. and 3.3.1.1.). 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 eventualy 'Yield' or 'Fv:m' are calculated. Saturation pulses can also be triggered in the two other modes of data acquisition (see 3.2.). However, then no on-line quenching analysis occurs and the 'Y'-command is blocked. A sequence of saturation pulses can be started by the 'P'-command.

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 3.3.2.3.) where quenching analysis requires 'Y'- or 'P'-operation.

3 Int: 8

The 'Int'-field for saturation pulses is selected by the '3'-key. The pre-set value can be changed by '+' or '–' operation. 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 (see 3.3.1.18.). By assessment of these kinetics the proper setting of pulse intensity and length can be chosen. In section 3.3.1.18. some typical examples

are given for illustration.

7 0.1s: 8

This parameter field, which is selected by the '7'-key, refers to the length of the saturation pulse. The pre-set value can be changed in 0.2 s steps by '+'- and '–'-operation. Maximal value is 1.4 s and minimal value 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 (see 3.3.1.18.).

Clk s: 20

The 'Clock'-parameter field, which is selected by the 'C'-key, displays the time interval between two consecutive saturation pulses triggered by the 'Pulse Seq.' function. The pre-set value can be changed by '+' and '–'-operation first in 10 s steps between 10 and 60 s, then in 1 min steps to 10 min and finally in 10 min steps to 120 min. The Clk-settings cannot be changed while 'Pulse Seq.' is activated. Using the Alt F10-command (see 3.3.1.21.) in conjunction with the 'Pulse seq.'-function (see below), it is also possible to define other units (Act. Light, Far- red light, Runs) to be triggered at the given Clock-intervals.

Pulse Seq.

By the 'P'-command 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 3.3.2.3.) and for automatic averaging of 'Yield'- and 'ETR'-values via the 'Ctrl Y'-command (see 3.3.1.5.). With the Alt F10-command (see 3.3.1.21.) other units (Act. Light, Far-red light, Runs) can be defined to be triggered instead of saturation pulses by the 'Pulse seq.'-function.

Note: A number of commands (like 'C' for changing the clock interval and 'K' for changing over to the Kin. 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 'B' (break) and the pulse sequence stopped by 'P'.

No:

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 (see 3.3.1.1.). When averaging of 'Yield'-and 'ETR'-values is initiated by the 'Ctrl Y'-command (see 3.3.1.5.), 'No' is set to 1 with the first saturation pulse and then corresponds to the number of averages.

3.3.1.12. Far-red light parameters

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

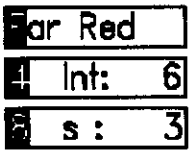


Fig. 19

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:

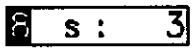
Far Red

Far red illumination is turned on/off by operation of the 'F'-key. A LED with an emission peak around 735 nm is used as far red source (see 2.1.). 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 account of 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. The 'Ctrl Z'- and 'Ctrl S'-commands involve far-red illumination (see 3.3.1.2.). When these commands are activated, an asterix appears in the right hand corner of the Far Red parameter field.

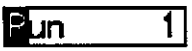
4 Int: 6

The 'Int'-field for far-red light is selected by pressing '4' and the pre-set value can be increased or decreased by '+' or '–' operation. Relatively small intensities already are quite effective. The optimal setting depends on 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, the pre-set value of 3 s can be changed in 1 s-steps with 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-key operates like an on/off switch for manual definition of far-red illumination times.

3.3.1.13. Run



or


The 'Run'-parameter field is selected by the Ctrl R-command or by arrow key operation. One out of 10 settings, which correspond to so-called 'Standard Run-files', can be chosen by '+'/'–' operation. A 'Run-file' represents a pre-programmed sequence of commands which are separated by defined time periods. In addition, for each 'Standard Run-file' specific pre-settings of instrumental parameters (like Gain, Damping, etc.) are defined to provide optimal conditions for the given experiment. These settings can be initialized by the 'I'-command before a particular 'Run' is started via the 'R'-command, 'Return' or by remote control, using the 'remote button' on the Leaf-Clip Holder 2030-B (see 2.5.).

The 10 Standard Run-files represent the most frequent types of measurements carried out with the PAM-2000 Fluorometer. Details on these 'Standard experiments' are given in section 3.5. 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 instrumental parameters even the unexperienced user can make full use of the PAM-2000 measuring capacity.

In addition to the Standard Run-files, also User-Runs can be programmed by the experimenter (see 3.6.). With the cursor position on the Run-field (e. g. after applying Ctrl R) and using the Alt F10-command, a selection menu for write/read Run-files to/from disk is accessible (see 3.6.). When a certain Standard Run is replaced by a User-Run, this is indicated by a (u) in the Run-field.

3.3.1.14. Kin. Scr.

With the 'K'-command the normal 'Parameter Screen' is exchanged against the 'Kinetics Screen'. To return to the 'Parameter Screen' the 'N'-command is used. On the 'Kinetics Screen' the fluorescence data are displayed graphically and a special menu for kinetic data analysis is offered (see 3.3.2.). 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 (see 3.2.) the 'Kinetics Screen' is of major importance. Details on using the 'Kinetics Screen' are presented in sections 3.3.2. and 3.4.

3.3.1.15. 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-2000 (see 2.5.). 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 (see 3.3.1.17.). When 'Yield' and 'ETR' are averaged via the 'Ctrl Y'-command 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. Using the Ctrl 0-command (see 3.3.1.20), a constant offset can be applied to the measured Tmp-value (see also 2.5.).

3.3.1.16. Volt

The 'Volt'-field indicates the given status of internal battery voltage. When this value drops below 11.5 V, there is a warning beep and the message 'Low battery!'. In this situation the PAM-2000 Fluorometer still functions normal. However, with every application of the halogen lamp there is a substantial further drop in voltage. When this reaches about 11.3 V the high currents required for saturation pulse operation cannot be delivered any more and all operations involving saturation pulses become malfunctioning. In this case, there is a warning 'Low battery during saturation pulse'. All other functions are maintained down to about 10 V. With a fully charged battery the displayed voltage is 12.5-12.9 V. When an external DC source is connected, the displayed voltage rises to about 13.8 V, corresponding to the voltage of the internal charging circuitry (see 2.1. and 2.4.). In first approximation, battery voltage can be taken as a measure of remaining battery power. The functional relationship between capacity (Ah) and voltage of a new battery is depicted in 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.

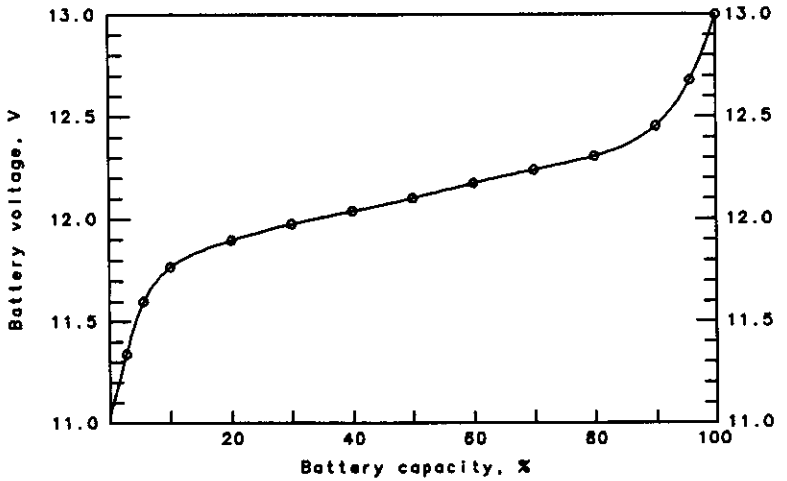


Fig. 20

3.3.1.17. Ctrl E and the Report-file

With the 'Ctrl E'-command the user leaves the 'Parameter Screen' or 'Kinetics Screen' and enters the 'Editor-level' with the so-called 'Report-file'. This file has been automatically installed upon initialization of the 'Saturation Pulse Mode' of data acquisition (see 3.2. and 3.3.). It contains all relevant data measured and calculated in conjunction with the 'M'-, 'Z'-, 'Y'-, 'S'- and 'P'-commands.

The Report-file can be edited by the user, i. e. it may also serve as a convenient 'Notebook' for comments on a particular set of experimental data. For editing similar commands as with 'Wordstar' are effective (see list in section 5.4.). To characterize a particular set of experimental data it is advisable to enter a line of explanatory text before running the experiment.

Demonstration of Report-file information in experiment with spinach leaf

07-JUN-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	93.7	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	0.592	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. 21

A typical print-out shows in the first two lines the information which is stored in the Report-file in conjunction with the 'M'-command. 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 'M'-command was given. Then the actinic light was turned on and first a sequence of 5 saturation pulses was given, before Ctrl S (see 3.3.1.2.) was activated, initiating Fo'-determination with every sat. pulse. Eventually, after having reached a quasi-stationary state, the averaging function was activated by Ctrl Y (see 3.3.1.5.), which is indicated by a line saying "averaging". Averaging is terminated by another 'Ctrl Y'-operation, after which 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 number of saturation pulses applied after Fo, Fm determination via 'M'. The No. is reset to 1 with the start of averaging

(activated via Ctrl Y).

When Fo is determined via the 'Z'-command, i. e. without associated Fm-determination, its value is entered into the column in which Ft is normally listed with saturation pulses (Y, S or P) and Fo with the 'M'-command (see last entrance in Fig. 21).

The Report-file is quit via the 'Esc'-key. In order to store any changes made by editing, the question "save ? (y/n)" should be answered by 'Y'. The Report-file is stored in the directory of the active drive under the name STANDARD.RPT. With the Poqet PC this is drive A while with Desktop PCs this is normally drive C. It is good practice to rename the STANDARD.RPT after accumulation of a certain amount of data. Then, with the next initialization of the Sat. Pulse Mode a new, empty Report-file will be automatically installed (see 3.6.).

The size of the Report-file has an influence on the time required to call the file on the monitor screen after Ctrl E-operation. Also, as new data are written to the end of the file, when this file is very long it may happen that on-line calculated data are not yet entered into the last line, when Ctrl E is applied quickly after the last saturation pulse.

For print-out of a defined section of the Report-file, it is recommended to use the command Ctrl KW to mark begin and end of the desired block and enter 'prn' (see 5.4. for most common editor commands).

The DA-2000 provides the possibility to transform Report-files into WKS-files, such that the data can be further analysed by standard programs like Lotus 1,2,3 or Excel. The transformation is carried out by the RPT2WKS.EXE program. For this purpose, first the corresponding data set in the Report-file is defined by the Ctrl KW-command (block start, block end, Filename NAME.RPT). Then at DOS-level the command RPT2WKS NAME.RPT is given, by which the new file NAME.WKS is created, which can be further processed by programs like Excel.

3.3.1.18. Alt M (Sat. pulse kinetics)

With the 'Alt M'-command the fluorescence kinetics induced by the last saturation pulse are displayed in the right hand corner of the Parameter-Screen. The measured curves are normalized by autoscaling. 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. Dotted horizontal lines represent the levels of Fo and Fm determined by the 'M'-command. Fm-determination can be considered correct, when the Fm-line coincides with a distinct plateau. 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' (see 3.3.2.) is suppressed.

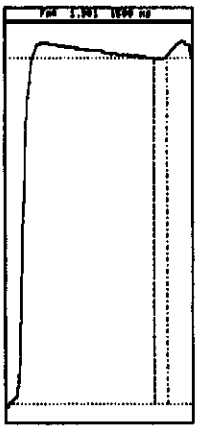
Assessment of the saturation pulse kinetics is important to ascertain that intensity and length of the saturation pulses are appropriate for a given sample under the given conditions. The pre-set standard values of Int:8 and 0.8 s are well suited for most applications. Generally speaking, a plateau should 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 starts dropping before the end of the pulse and/or transiently increases after pulse termination, this is indicative of excessive pulse intensity. Relevant examples are given in the following figure.

Sat. Pulse
Int: 6
0.1s: 4

Fig. 22A

Sat. Pulse
Int: 8
0.1s: 8

Fig. 22B



Sat. Pulse
Int: 10
0.1s: 12

Fig. 22C

In (A) 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. In (B) a correct Fm-determination at the standard settings of 0.8 s pulse length and intensity 8 is shown. In (C) an example is given where the intensity setting 10 is too high and the pulse length 1.2 s is too long.

3.3.1.19. Ctrl C (PAR re-calibration)

With the help of the 'Ctrl C' command the calibration of the micro-quantum-sensor for PAR-measurement can be updated. This function applies only when the Leaf-Clip Holder 2030-B or the Micro

Quantum/Temp.-Sensor 2060-M are connected (see 2.5.). Upon operation of 'Ctrl C' a dialoge-field appears in the center of the monitor screen:

Fig. 23

The current calibration factor is shown (in brackets), which is 1.000 upon instrument delivery. A new calibration factor can be entered and consequently new PAR readings will incorporate this factor. 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 (Type LI-190), 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.

3.3.1.20. Ctrl O (Offset)

With operation of the 'Ctrl O' command a small menu is entered to select fluorescence, PAR or temperature for definition of a certain offset-value.

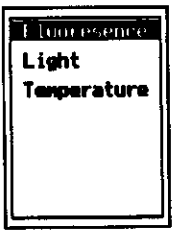


Fig. 24

- Fluorescence

Offset for measured Ft

When this function is selected by 'Return' or 'F', a dialoge-field appears in the center of the monitor screen:

Fig. 25

The given offset is shown (in brackets) which normally is 0. Values between -300 and +300 can be entered. 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.

- PAR

Offset for measured PAR

When this function is selected by 'Return' or 'P', the corresponding dialoge-field appears:

Fig. 26

- Temperature

Offset for measured Tmp

When this function is selected by 'Return' or 'T', the desired offset value can be entered into the corresponding dialoge-field: The offset may be applied to recalibrate 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 (see also 2.5.).

3.3.1.21. Alt F10 (local menu)

The 'Alt F10' command allows to enter 'local menus' related to the definition of instrumental functions. So far, such selection menus are installed for the Pulse Seq.- and the Run-functions. They are accessed by first moving the cursor to the P- or R-parameter field and then applying 'Alt F10'.

- Pulse Seq. and Alt F10

When the cursor is on the P-field and Alt F10 is applied, a selection menu appears in the center of the monitor screen:

Fig. 27

Select unit to be repetitively triggered

One of 4 possible units can be selected by entering the initial letter (or using arrow keys) and 'Return'. The 'Pulse seq.'-function (see 3.3.1.11.) then applies to the selected unit. 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 (see 3.5. and 3.6.), are repetitively carried out by the Pulse Seq.-function.

- Run and Alt F10

With the cursor on the Run-field (e. g. after applying Ctrl R), the 'Alt F10' command allows to enter a short selection menu which is essential for the definition and application of so-called 'User-Runs' (see 3.6.):

Fig. 28

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

Upon start of the DA-2000 program automatically a set of 10 'Standard Runs' is installed (see 3.5.). In addition new sets of 'User Runs' can be defined, which may be derived from the 'Standard Runs' or programmed independently (see 3.6.). The 'User Runs' are stored on disk (normally drive A with Poqet and drive C with desktop PC) under given filenames 'Name. Run'. The 'Standard Runs' or any other set of current Runs can be written to disk using the write command of the Run/Alt F10 selection menu. A name has to be entered which should start with a letter and may contain up to 7 more letters or numbers (e. g. Standard).

Fig. 29

Using the Ctrl E-function (see 3.3.1.17.) the DA-2000 inherent editor can serve to modify given Runs (e. g. Standard Runs) (see 3.6.). A user-defined Run will become active only after reading the corresponding file (Name. Run) from disk via the Run/Alt F10 selection menu. A User-Run is active when for a given Run-number a (u) is displayed in the Run-field.

3.3.1.22. Initialization of instrumental settings and Configuration-file

Three different commands apply to different types of initialization of instrumental settings

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

The '0'-command for initialization of Standard settings is automatically given with every reset (cold or warm start) of the computer. A print-out of the Parameter Screen with Standard settings was already presented at the beginning of section 3.3.1. These settings have proven most useful for common applications. It is good practice to apply '0' before start of an experiment and then 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 whereas it is 5 for Continuous and Sat. Pulse modes. With the I-command the instrumental settings are installed which are appropriate for individual Run-files. Hence, first the desired Run-file is selected (see 3.3.1.13. and 3.5.) and then 'I' is applied. If wished, before the actual start of a Run certain settings still may be changed manually.

With application of the 'U'-command ('up-date'), the user can make sure that the settings displayed in the various parameter fields are indeed effective. Under normal conditions this is not necessary. However, it becomes necessary to apply 'U' after the PAM-2000 was temporarily turned off and 'Low battery!' was displayed.

Normally, when the DA-2000 program is quit via 'Alt X', the current instrumental settings are stored in a 'Configuration-file' (DA-2000.CFG). The same settings are installed, when the program is started again. In this way it is assured that a set of instrumental settings, which has proven useful for a certain type of experiment, is maintained irrespective of turning the measuring system on/off. The Configuration-

file is automatically created upon program installation (see 2.3.).

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 the Configuration-file can be deleted at DOS-level: DEL DA-2000.CFG.

3.3.2. Using the 'Kinetics Screen'

With operation of the 'K'-command, the 'Kinetics Screen' is installed, substituting the 'Parameter Screen'. All instrumental settings and parameter values are maintained when switching between these two screens. Returning to the 'Parameter Screen' proceeds via the 'N'-command. The 'Kinetics Screen' is primarily used in conjunction with the 'Cont. Mode' and 'Trig. Mode' of data acquisition (see 3.2. and 3.4.). In the 'Sat. Pulse Mode' it allows registration of so-called 'saturation pulse induction curves' (see 3.3.2.3.).

In particular with the Poqet PC, after switching from the Par. Screen to the Kin. Screen, it may take a considerable length of time for complete screen installation when the various parameter kinetics from a previous recording are redrawn. This can be avoided by erasing the currently stored kinetic data using the '/'-command.

3.3.2.1. Screen lay-out

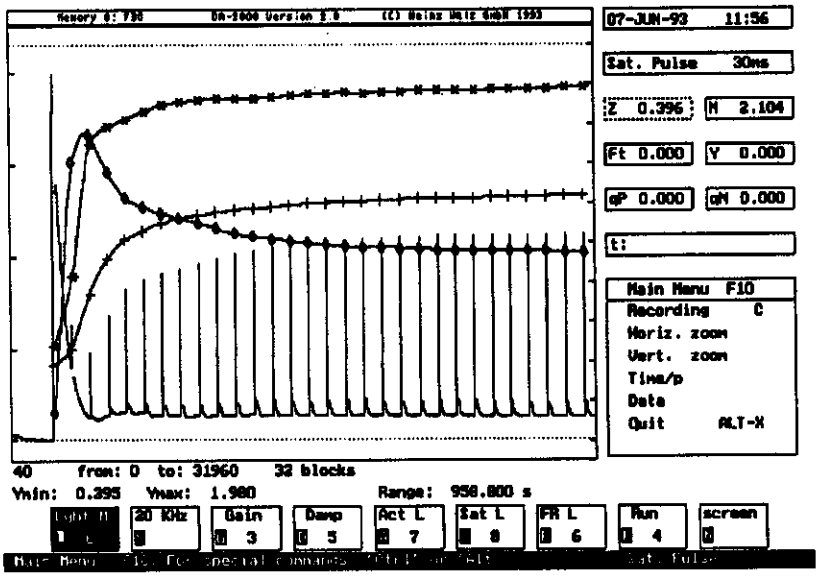


Fig. 30

The 'Kinetics Screen' is divided into four main areas:

- graphics area
- measured parameter area
- menu area
- instrumental parameter area

In the graphics area, the measured curves 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-2000 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 averaged automatically. "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 format when transferred to disk or RAM memory (see 3.7.).

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 (see 3.3.1.1.), 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 'O'-command (see 3.4.1.2.).

The graphics area can be expanded over the full screen via the 'V'-command (Full screen display, see 3.4.1.2.). Hardcopies of either the normal or of the full screen graphics can be obtained with the help of the 'Alt H'-command (see 3.4.1.5.). 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-yield) and M (Fm-yield)
- Ft (fluorescence yield at time t) and Y (overall quantum yield)
- qP and qN (quenching coefficients)
- t (time associated with given fluorescence data point)

Before a kinetic recording is started, normally Fo and Fm are sampled by operation of 'Z' and/or 'M'. With 'M', 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 which is obtained via the 'Alt M'-command when using the 'Parameter Screen' (3.3.1.18.). It is also possible to enter manually values for Fo and Fm. For this purpose, the Fo or Fm field is first selected (which is the case after Z- or M-operation) and then the Del-

command is given.

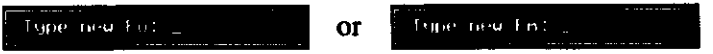


Fig. 31

The new values are entered into the corresponding parameter fields.

Note: The values of Fo and Fm are expressed in Volt, which is in contrast to the Offset-values (see 3.3.1.20.) which are expressed in mV.

The Main Menu for data acquisition and analysis comprises 5 sub-menus in the 'Saturation Pulse Mode', which are detailed in section 3.3.2.4.

The parameter fields representing the major instrumental 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 3.3.2.2.

A so-called '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 are then activated via 'Return'.

At the very bottom of the screen after certain key operations an 'Information Line' appears which presents information on special commands involving the 'Alt'- and 'Ctrl'-keys and which provides a brief description of each menu-point (see 3.3.2.4.).

3.3.2.2. Commands for parameter setting

Poqet PC-version

Desktop-version
Fig. 32

Due to the limited space and for the sake of clarity only 9 out of 20 instrumental parameters are represented in the 'Parameter Line'. Taking account of the small size of the Poqet PC monitor screen, in the Poqet version of the DA-2000 program abbreviations of the involved parameters are displayed in large letters:

L - measuring light
5 - frequency (600 Hz or 20 kHz)
G - gain
D - damping
A - actinic light
S - saturation pulse
F - far-red light
R - run-file
N - normal screen (i. e. 'Parameter Screen')

The first character is identical to the inverted character of the corresponding functions on the 'Parameter Screen'. Operation of this key will carry out the function (as with L, 5, A, S, F and N) or select the function in order to change instrumental settings (as with G, D and R), which are represented by numbers from 1 to 11. Settings are increased by '+' and decreased by '–'. The 'super-setting' 11, possible only with L, A and F, is reached by '!'.

A parameter field is selected by pressing the first character key or by cursor movement using the arrow keys. In the case of L, A, S or F, these fields can be also selected by pressing '1', '2', '3' or '4', respectively, in order to change the corresponding intensities. These are the same keys as used with the 'Parameter Screen' for this purpose. In analogy, although not represented by a parameter-field on the Kinetics Screen, the status of the actinic light source can be switched between LED and Halogen lamp by the 'H'-command.

For more details on the various instrumental settings and functions, please consult the corresponding paragraphs in section 3.3.1.

3.3.2.3. Saturation pulse induction curves

In conjunction with the 'Saturation Pulse Mode' of data acquisition, the 'Kinetics 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. 30 above). 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 on 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 in sections 3.5.3. and 3.5.4. Sampling rate is changed via the Main Menu (see 3.3.2.4.). For quenching analysis, previous determination of Fo and Fm via the M-command is prerequisite (see 1.2. and 3.3.1.1.). On the 'Kinetics Screen' with every operation of 'M' the fluorescence rise kinetics induced by the saturation pulse are displayed. In this way, the correct determination of Fm can be assessed (see 3.3.1.18.). The measured Fm-value is accepted by 'Return'. Then a recording can be started by 'C'. The screen now shows dotted

lines representing Fo and Fm, and a trace of Ft is drawn close to the Fo-line in the absence of actinic illumination. There is autoscaling of the Fm-line to the upper limit of the screen. When actinic light is switched on ('A'), Ft first rapidly increases and then slowly decays again (Kautsky effect, see 1.2.). For quenching analysis, saturation pulses are applied, normally at 20 s intervals, using the 'P'-command for starting the Clock-function (see 3.3.1.11.). The first pulse should be given in the peak of Ft. In this way, the state of minimal photochemical and non-photochemical quenching is assessed. Single pulses can be applied via 'Y' or 'S', but only with 'Y' there is on-line calculation of quenching parameters. If there is on-line quenching analysis, no data points are registered during the saturation pulse and the so-called 'fade-out time' (see 3.3.1.18.), except for a single point marking Fm'. Depending on the 'Join'-status (see 3.4.1.2.) the saturation pulse induced spikes (peaking in Fm') can be made visible or not.

The on-line calculated values of Y, qP and qN are first displayed by different 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 3.3.2.4.) or, more quickly, using the 'O'-command. In particular, when a recording was stopped before reaching the end of the screen, the command sequence 'O' 'Return' 'Return' 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, in order to redraw the screen without the undesired parameter, the command sequence 'O' 'Return' 'Return' is applied. The same procedure is used to restore display of a parameter.

The on-line calculated parameters are automatically written into the Report-file (see 3.3.1.17.).

3.3.2.4. 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 'F10' or by cursor operation, the sub-menus can be selected by cursor movement, using the arrow keys and 'Return', or more quickly by pressing the initial character key. Some menu-points also can be activated directly without entering the menu. 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)
X - Write Mem. 1-4 to disk

In the 'Sat. Pulse Mode', use of the 'Kinetics Screen' and of the Main Menu rather is the exception. The 'Parameter Screen' (see 3.3.1.) in conjunction with the 'Report-file' (see 3.3.1.17.) provides a most convenient framework for data acquisition and analysis in this mode. This is in contrast to the 'Cont. Mode' and 'Trig. Mode' where the 'Kinetics Screen' is mainly used. Hence, a more detailed description of the 'Kinetics Screen' and of 'Menu-guided data acquisition and analysis' is presented in the following section 3.4. which deals with these modes.

3.4. Measurements in the 'Continuous Mode' or 'Triggered Mode'

Upon start of the DA-2000 program the 'Saturation Pulse Mode' is automatically installed, as this mode of data acquisition is most frequently used. In order to select the 'Continuous Mode' or 'Triggered

Mode' the 'Alt I'-command is applied to enter a short selection menu (see 3.2.). These two alternative modes are used for kinetic recordings and, therefore, upon initialization the 'Kinetics Screen' is installed. Details on the layout and use of the 'Kinetics Screen' were already presented in sections 3.3.2.1. and 3.3.2.2.

The 'Parameter Screen' can be installed by the 'N'-command. It is most convenient for changing the pre-set values of instrumental settings. All settings are maintained when returning to the 'Kinetics Screen'. Standard settings can be initialized by the '0'-command. They are identical in the three different modes of data acquisition, except for the value of Damping, which is 2 in the 'Trig. Mode', as compared to 5 in the two other modes. The instrumental settings for Standard Experiments, pre-programmed in the so-called 'Run-files', are initialized via the 'I'-command (see 3.5.). With the 'U'-command all given settings are up-dated. (This becomes necessary when the PAM-2000 is temporarily switched-off while the DA-2000 is still installed). When leaving the program via 'Alt X', all instrumental parameters are stored in a 'Configuration-file' and re-installed when the program is started again (see 3.3.1.22.).
There are some differences between data acquisition in the 'Cont. Mode' and 'Trig. Mode' which shall be briefly outlined:

- **Data recording**
  In the '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.
  In the 'Trig. Mode', the data sampled at high rates are first stored in RAM-memory within the PAM-2000 and only after the actual recording transferred to the PC and displayed on the monitor screen.

- **Sampling rates**
  In 'Cont. Mode' 10 ms/p and 30 ms/p, suited for relatively slow kinetic recordings.

In 'Trig. Mode' 150 µs/p to 3000 µs/p, suited for recordings of rapid induction kinetics.

- **Total number of recorded data points**
  In 'Cont. Mode' 32 000 points
  In 'Trig. Mode' 4 000 points

- **Start of induction curve**
  In 'Cont. Mode' by 'A'-command after recording was already started via 'C'.
  In 'Trig. Mode' start of 'A' is automatically coupled with the 'C'-command.

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

- **'M' and 'Alt M'**
  In 'Cont. Mode' upon application of 'M' and of 'Alt M' the kinetics of the fluorescence rise in the saturation pulse are displayed (see 3.3.1.18.). This is not the case in the 'Trig. Mode'.

The central command with recordings on the 'Kinetics Screen' is 'C', by which the recording process is started. This is also the first point in the Main Menu. A detailed outline of 'Menu-guided data acquisition and analysis' is presented in the following section.

3.4.1. Menu-guided data acquisition and analysis

The DA-2000 offers two levels of system operation and data handling. On one hand, there are immediate commands, like 'C', 'O' or 'W' (see 3.3.2.4.) 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 has the advantage of being quick and, hence, it is advantageous to the experienced user, knowing all commands by heart. The latter can be recommended to beginners, who will profit from the explanatory text accompanying every menu-point.

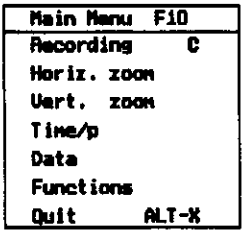


Fig. 33

In the 'Cont. Mode' and 'Trig. Mode' the Main Menu consists of 7 sub-menus. After selection of the Main Menu via 'F10' or by cursor operation, 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' while 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 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, which also appears in the information line at the bottom of the PC monitor screen.

3.4.1.1. Recording C

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)

The only function of this menu-point is to start a kinetic recording. This can also and more easily be achieved without entering the menu by using the 'C'-command. The start of a recording should be preceded by Fo-determination for Y-axis scaling (see 3.4.1.3.) and, at least in the Sat. Pulse Mode, also by Fm-determination. Both Fo and Fm are determined with the 'M'-command. In the 'Trig. Mode', the measured kinetics are displayed some time after the actual recording. In this mode the data are transiently stored within the PAM-2000 Main Control Unit and then transferred via the RS 232 interface to the PC.

3.4.1.2. Horiz. zoom

For horizontal expansion by selecting curve segment and Full Screen display

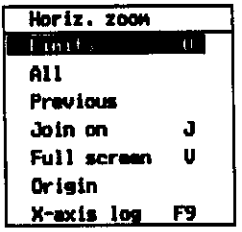


Fig. 34

- **Limits O**

  Define first/last point of curve segment which is to be displayed by zooming

  Select left limit by typing address or using arrow keys

  Select 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 trigger point, which is at address 512

  --- to define a curve segment in block format (multiples of 1000 data points) for storage on disk or RAM (see 3.7.).

  In the 'Trig. Mode' curve limits are maintained for consecutive recordings until they are re-defined. To return to the complete recording, the 'All'-command is used.

  Limits selection is also possible without entering the Main Menu via the 'O'-command.

  When in the Cont. 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 which were obtained by mathematical transformation using the special Function-commands (see 3.4.1.6.). When such a transformed curve is given, upon application of 'J' the original curve or curve segment is redrawn with altered Join-status. Hence, with the 'J'-command a convenient way is given 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 (see 3.3.2.3.).

- **Full screen**
  Display of screen filling curve for high quality print out (Alt H)

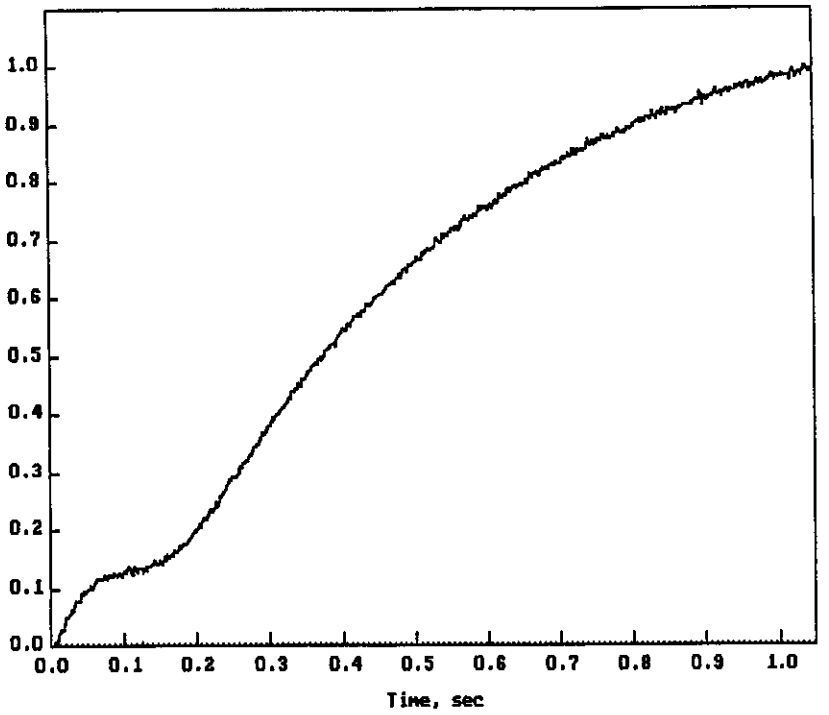


Fig. 35

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 ('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 'Cont. 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 $n \cdot x$ to $n \cdot x^{799}$. 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.

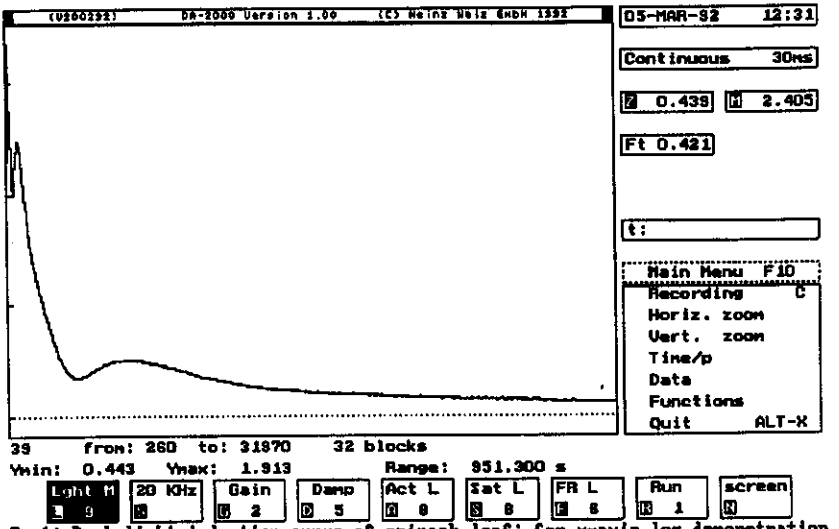


Fig. 36A

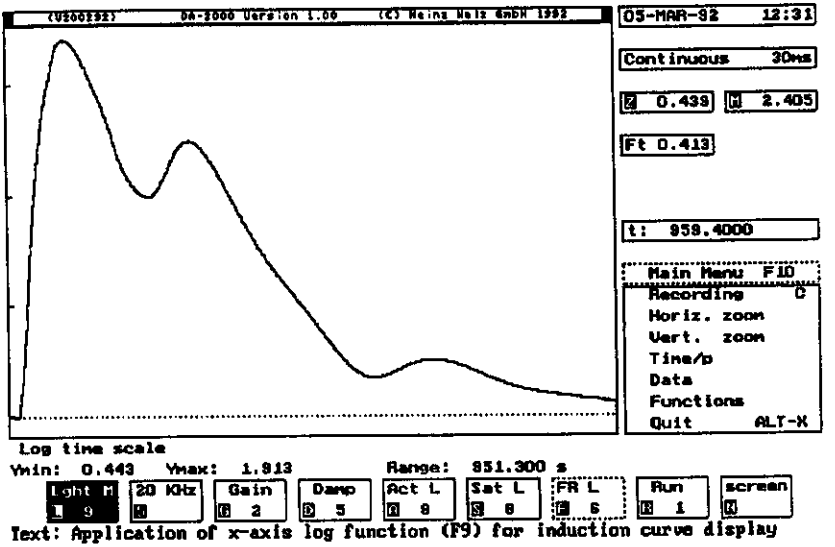


Fig. 36B

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 which 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 3.4.1.2.).

3.4.1.3. Vertical zoom

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

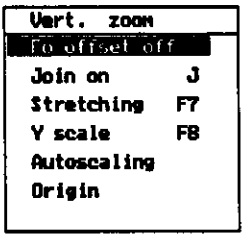


Fig. 37

- **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 Fo' is determined in kinetic recordings (see 3.3.1.2.)

- **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. 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 which were obtained by mathematical transformation using the special Function-commands (see 3.4.1.6.). When such a transformed curve is given, upon application of 'J', the original curve or curve segment is redrawn with altered Join-status. Hence, with the 'J'-command a convenient way is given to return to the original curve or curve segment.

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

Fig. 38

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 which 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 'F7' without entering the Main Menu.

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

Fig. 39

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.

- **Autoscaling**
  For maximal vertical expansion of this and following recordings
  Choosing this function, it is possible to perform autoscaling without entering the brief dialoge involved in F8.

- **Origin**

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

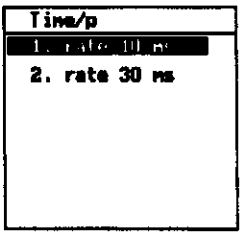
  Select new origin by typing address or using arrow keys

  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.

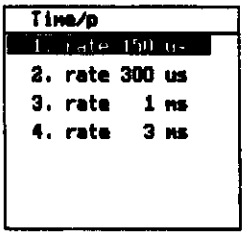
  The new origin will be maintained in consequent recordings until the normal display is re-installed via the All-command ('O' 'A').

3.4.1.4. Rate

Rate of data acquisition in time/data point



Cont. Mode



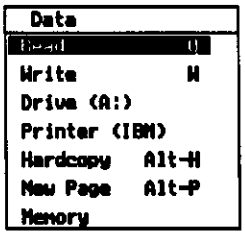
Trig. Mode

Fig. 40

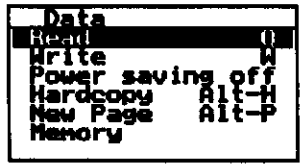
The choice of possible recording rates is different in the Trig. Mode (150, 300, 1000 and 3000 µs/point) and the Cont. Mode/Saturation Pulse Mode (10 and 30 ms/point). In the Trig. Mode, a total of 4000 points are sampled, whereas in the other two modes a full recording consists of 32 000 data points.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

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

3.4.1.5. Data



Desktop version



Poqet PC version

Fig. 41

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

Fig. 42

After selection of 'Read', in the Trig. and Cont. Modes first the Memory to which the file shall be read has to be entered. In the Cont. Mode this is Mem. 0 and in the Trig. Mode one out of Mem. 1-4. Then the desired Filename can be typed. In the bottom information line the names of the stored data files are displayed. With the Poqet PC the active drive for data storage is drive A:, i. e. the RAM card. When a computer with harddisk is used, the active drive should be drive C:. The typed Filename is entered by 'Return'. The Read-function can also be directly called by 'Q', 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. 43

After selection of 'Write', first the memory number has to be entered, which is 0 in the Cont. Mode and 1-4 in the Trig. Mode. 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 one 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, if no name is typed, the name 'Main' is entered. 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.

In the Trig. Mode, the 'X'-command initiates a special routine for writing all Memories 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 by 'W', without entering the menu.

- **Drive (A:)**
  To change disk drive for further Write/Read operations
  (not with the Poqet PC-version)

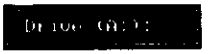


Fig. 44

Upon program initialization disk drive A: is installed as active drive for Write/Read operations. With the Poqet PC, drive A: corresponds to the RAM-card. If data storage on other memory devices is wished, the corresponding drive name must be typed and entered by 'Return' (not with Poqet PC-version). Using a PC with harddisk, it is often appropriate to define drive C: as active drive for Write/Read operations. The current drive definition is stored in the 'Configuration-file' (see 3.3.1.22.) when the program is quit via 'Alt X'.

- **Printer (IBM) or Printer (HP)** (not with Poqet PC-version)
  To change printer type to HP Laserjet for further operations
  To change printer type to 9-pin IBM compatible for further operations

  In the desktop version, the program supports two types of printers: IBM-compatible printers (pre-set status) or HP Laserjet. Printer status can be changed by 'Return' or 'P'. In the Poqet version, the program only supports the Kodak Diconix Printer with serial interface (see 3.7.3.).

- **Hardcopy Alt H**
  To print out copy of screen image

  Hardcopies can be made not only from the 'Kinetics Screen' via the menu, but as well from the 'Parameter Screen' using the 'Alt H'-command. Before giving this command, the user should make sure that the printer is connected and the proper printer-status is activated in the Data-menu. With kinetic recordings, before print-out the user may enter an explanatory text. The print-out is started by 'Return'. When a kinetic recording is displayed in 'Full screen graphics' (see 3.4.1.2.) no text-line is installed. Before the Hardcopy-command is carried out, in the Desktop version of the DA-2000 program the screen is redrawn in black & white. This step can be avoided by installing 'DA-2000 MONO' instead of 'DA-2000' (see 2.3.).
  With Desktop computers DA-2000 MONO must be installed in order to make hardcopy print-outs of superimposed curves (see Memory sub-menu below).

- **New page Alt P**
  To advance printer paper to length of full page

  The normal print-out fills 1/2 of a page with the Desktop and 1/4 of a page with the Poqet version. In order to eject the page, the

'Alt P'-command is given, which operates like the 'Alt H'-command not only on the 'Kinetics Screen' but on the 'Parameter Screen' as well.

- **Power saving off or Power saving on** (only with Poqet PC-version)
  To disable power management
  To enable power management

  With the Poqet PC power consumption is considerably reduced (approx. by a factor of 5) by a special power management. When the DA-2000 is installed, the normal Poqet PC key combination is not active and changes in power management status are performed via the Data sub-menu. When sufficient battery power is available, the user may prefer to disable power management. In the case of Run-files 1 and 2 (see 3.5.), if these are started by the remote control button on the Leaf-Clip Holder 2030-B, the power-down status can cause some delay in triggering of sat. pulses. If this is of concern, power management should be disabled.

- **Memory**

Select, store and overlay memories

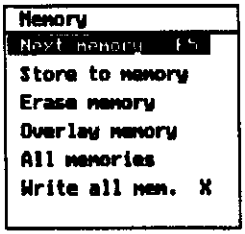


Fig. 45

Kinetic data are first stored in the transient Memory 0 (Main Memory) which is overwritten with each new recording. In the Trig. Mode of data acquisition (see 3.2.) curves or curve segments can be

transferred from Mem. 0 into the RAM Memories 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') is 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' (Memory 0 or Memory 1-4), the data points of which are displayed on the Kin. Screen, are written in the upper left corner of the screen.

- **Next memory F5**
  Select next memory
  With this command, kinetic curves stored in Mem. 0-4 are called on the screen one after the other, such that Mem. 0 is installed again after Mem. 4. 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

 and 

Fig. 46

In the Trig. Mode of data acquisition, curves or curve segments defined by Horizontal Zoom (see 3.4.1.2.) can be saved in Mem. 1-4 via the 'Ctrl M'-command. After typing the desired Memory number and 'Return', one of 'Mem. 1-4' 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 the 'Write'-commands ('W' or 'X', 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' which are involved, are indicated in a line below the Main Menu.

- **Erase memory**
  To clear a memory

Fig. 47

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

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

Fig. 48

With the Overlay-command any curve from Mem. 1-4 can be superimposed on 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 3.4.1.3.) 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.

Note: In order to make hardcopy print-outs of superimposed curves, the monochrome version of the DA-2000 program (DA-2000 MONO) must be installed (see 3.4.1.5.)

- **All memories**

  To show all memories

  Using this command, all occupied Memories will 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, will determine Y-axis scaling. Also, when "Autoscaling" is active (see 3.4.1.3.) this Memory is decisive. In order to return to the display of single Memories, the 'F5'-command is used.

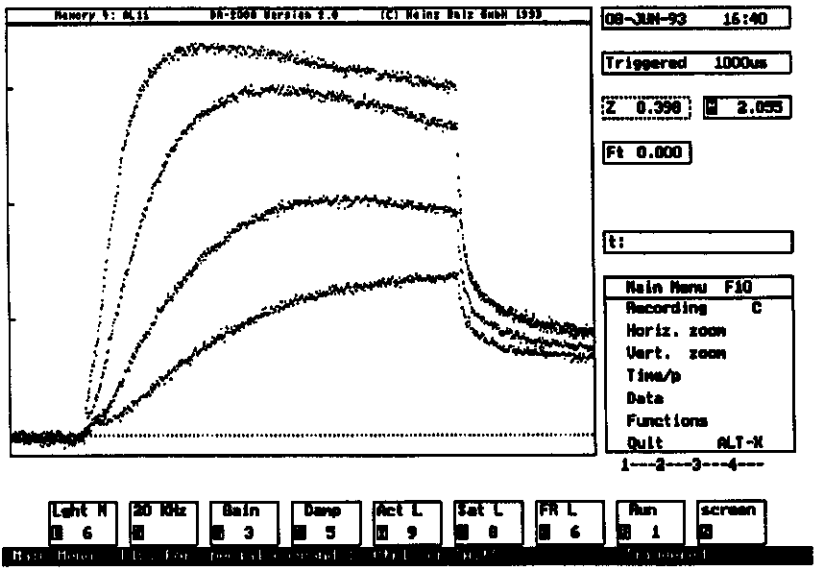


Fig. 49

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 using the 'All memories'-command. 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. 50

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, which can be entered by 'Return', one after the other. The proposed names can be also deleted by 'Esc' and new names can be entered.

3.4.1.6. Functions

Special mathematical transformations of displayed curves or curve segments

All 10 Function-keys are used with the Data Acquisition Program DA-2000. However, only 7 of these are listed in the sub-menu. F10 installs the Main Menu. F5 is reserved for 'memory operations' in the Trig. and Cont. Modes (see 3.4.1.5.) and F9 is listed in the Horiz. zoom sub-menu (see 3.4.1.2.).

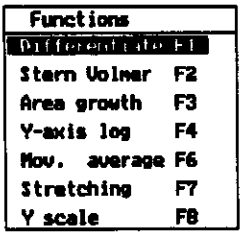


Fig. 51

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.

Note: In order to obtain hardcopies of curves after Special Function application, it may be advantageous to use the 'V'-command for 'Full screen graphics'-display before start of print-out by 'Alt H'.

- **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 by 'F6' is carried out.
- 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. 52

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 3.4.1.2.). The sampling rate (see 3.4.1.4.) of the original recording should match the rate of the transients which are analysed.

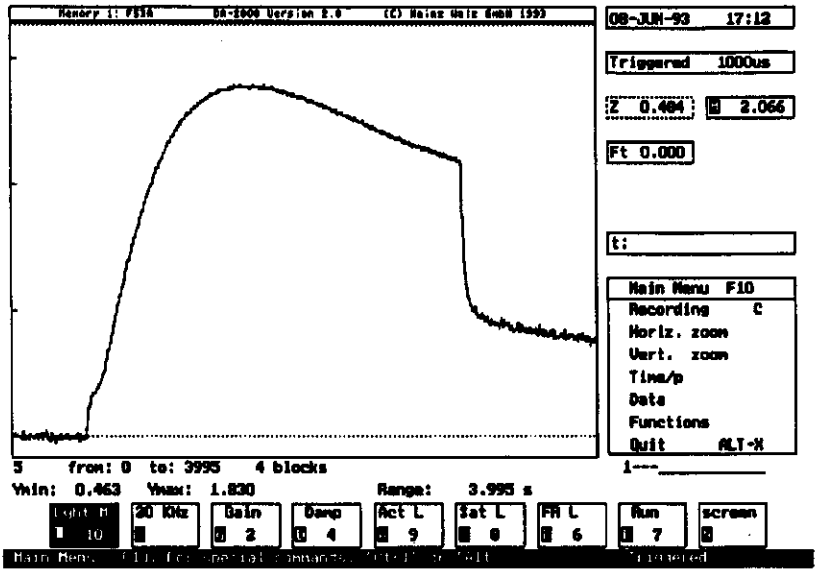


Fig. 53A

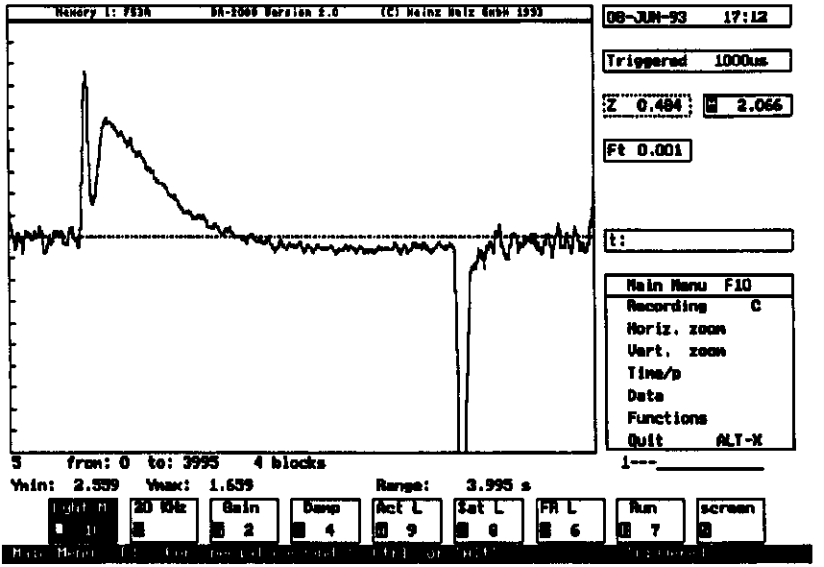


Fig. 53B

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. 54

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.

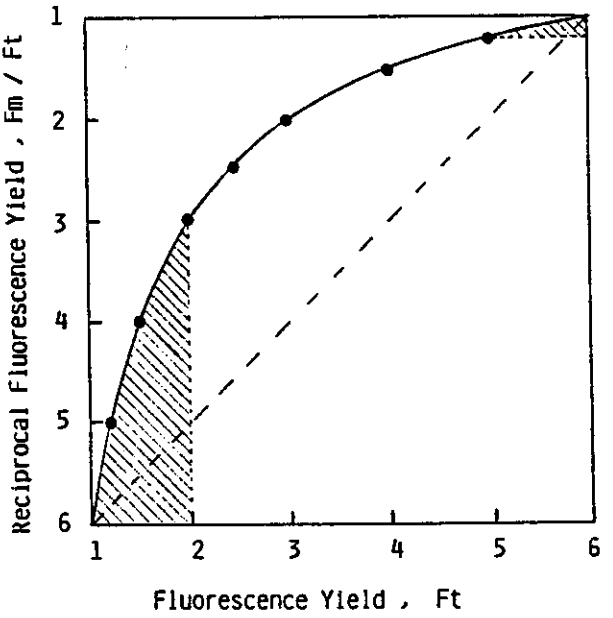


Fig. 55

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'*.

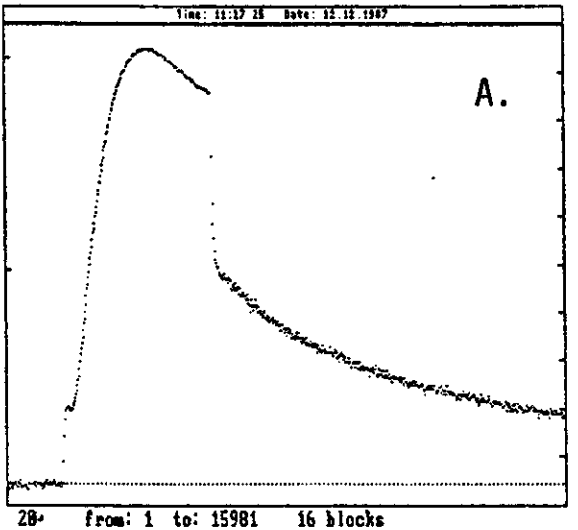


Fig. 56A Text: Original Ft vs. Time curve; spinach leaf; AL11

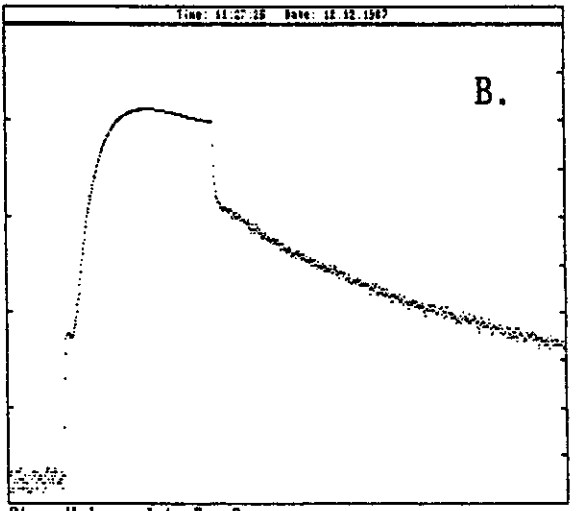


Fig. 56B Text: Assumption of Fm = 5xFo ; Fm/Ft vs. Time

- **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 transiently 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 electrons transported.

Fig. 57

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.

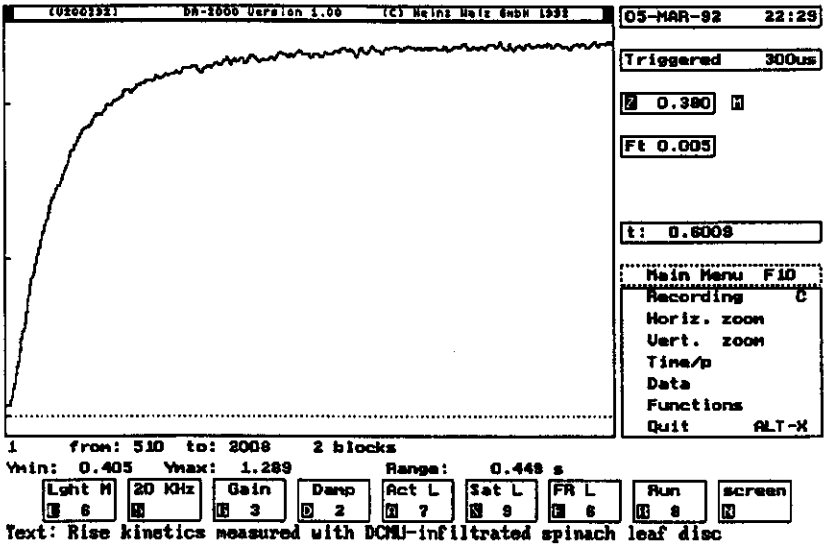


Fig. 58A

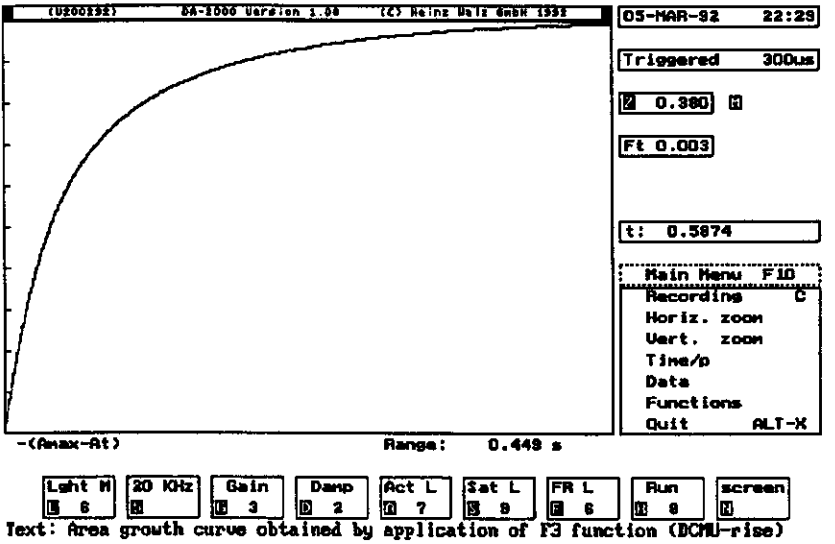


Fig. 58B

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). 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).

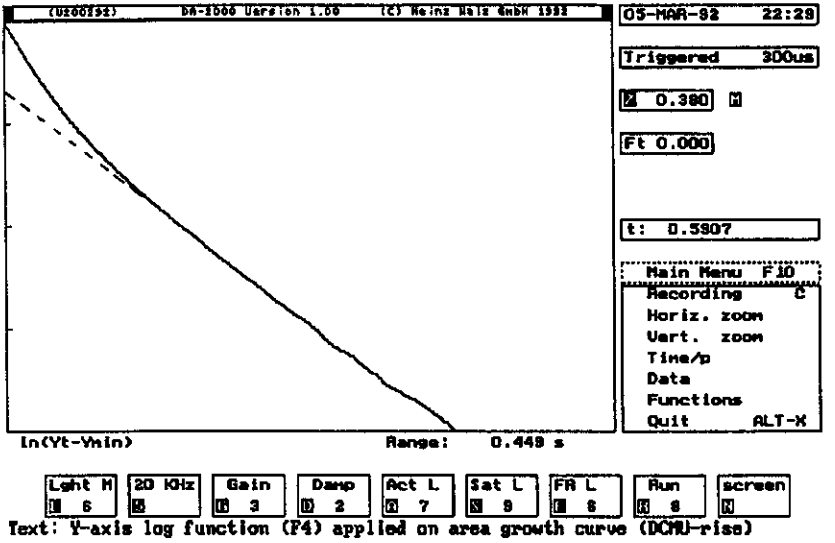


Fig. 59

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 $(Amax)\beta=e^{-1}=0.368$, 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 3.4.1.2.) 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 emphazised.

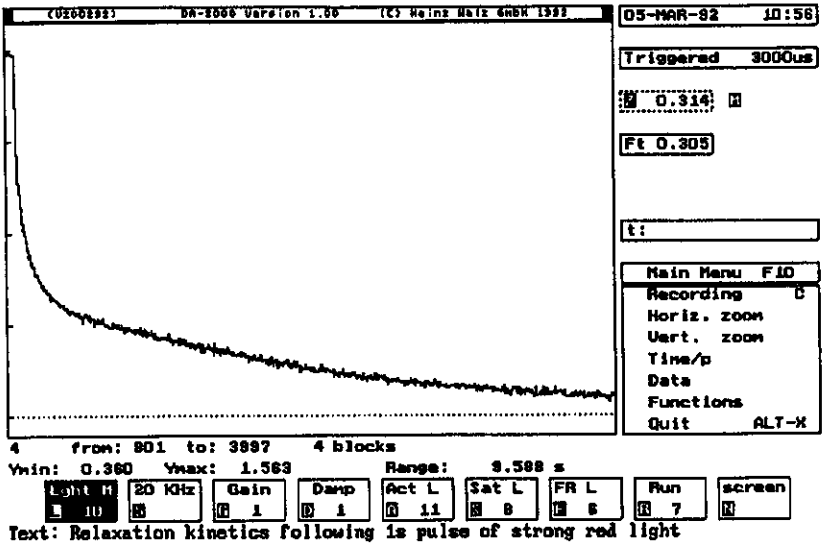


Fig. 60A

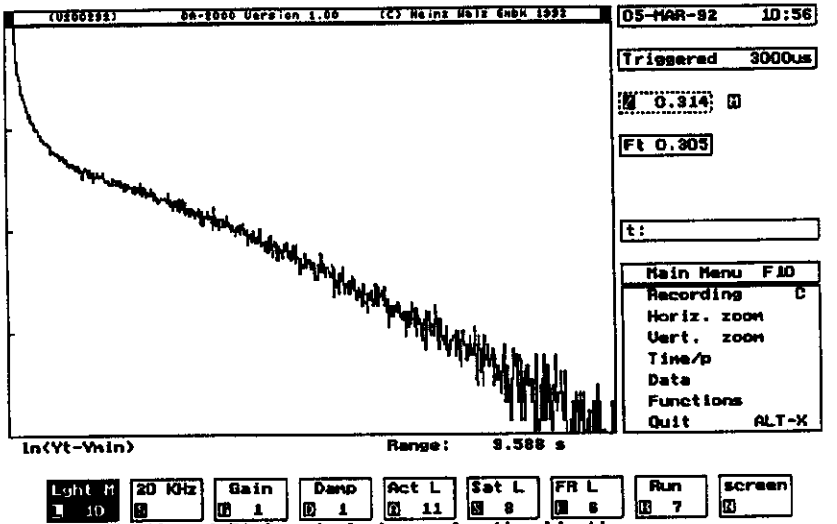


Fig. 60B

- **Next memory F5**
  Select next memory

This function is not shown in the Functions sub-menu, but rather in the Memory sub-menu (see 3.4.1.5.).

- **Mov. average F6**
  Curve smoothing by averaging (4, 8, 16 or 32) neighboring points

In addition to curve smoothing by data point averaging which 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 which 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' 4 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 'Vert. zoom' sub-menu (see 3.4.1.3.).

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 which 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 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 <u>not</u> shown in the Functions sub-menu, but rather in the Hor. zoom sub-menu (see 3.4.1.2.).

3.4.1.7. Quit Alt X

To leave program and return to DOS

By entering this command, the user leaves the DA-2000 program and returns to DOS. Doing so, all instrumental settings are stored in a 'Configuration file' (see 3.3.1.22.) and are re-installed when the DA-2000 is called again. With the Poqet PC it is advisable to quit the DA-2000 program via 'Alt X' before turning-off the computer with the I/O switch. Otherwise, due to a peculiarity of the Poqet PC power management, the computer may switch automatically on again, thus causing uncontrolled power-consumption. This is not the case when turning-off while at the 'Editor-level' which is accessed via the Ctrl E-command (see 3.3.1.17.) or after entering the Main Menu via F10.

3.5. Standard experiments (Run-files)

A 'Run-file' represents a pre-programmed sequence of commands which are separated by defined time periods. In addition, for each Run-file specific instrumental settings are defined to 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-2000 Fluorometer.
- experimental protocols and instrumental settings are strictly reproducible
- running standard experiments, in particular Run-file 10, provides a convenient means of testing proper functioning of the measuring system.

The given Run-files may be modified by the user and completely new 'User-Runs' can be defined (see 3.6.). Before selection of a Run-file (see 3.3.1.13.), the appropriate mode of data acquisition must be installed using the Alt I-command (see 3.2.). For Runs 1-5 and Runs 8-9, this is the 'Sat. Pulse Mode', for Run 3 and 4 optionally also the Cont. Mode, for Runs 6-7 the 'Trig. Mode' and for Run 10 the Cont. Mode. Before starting a Run-file for the first time after mode selection, the Run-file specific instrumental settings are initialized via the 'I'-command. The Run-parameter field can be selected by the Ctrl R-command, then the Run-number is chosen by '+' and '−' operation and the run-specific instrumental settings initialized by 'I'. With the standard experiments a sample-to-fiberoptics distance of 6-12 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' or via the 'R'-key or also by remote control, when the cursor is on the R-parameter field, using the 'remote button' of the Leaf-Clip Holder 2030-B (see 2.5.). 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, instrumenal settings cannot be changed manually. A Run can be interrupted by the 'B'-command (break). In this case a kinetic recording will continue with the given instrumental settings and manual control is re-installed. The recording can be stopped via 'Esc'. It should be avoided to start a Run while a Pulse sequence is activated, because this will result in mulfunctioning (see 3.3.1.11.).

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

Mode: Sat. Pulse Mode
Settings: 1 Int: 9 (Meas. light intensity)
20 kHz is permanently installed
Auto 20K is not active
G 4 (Gain)
3 Int: 9 (Sat. pulse intensity)
Other settings standard
To initialize the Run-specific settings, press the 'I'-key after Run 1 selection.

Run 1 is particularly useful for rapid screening of photosynthetic yield in conjunction with the Leaf-Clip Holder 2030-B (see 2.5.). Then yield-measurements can be triggered by remote control. The on-line calculated fluorescence parameters 'Yield' (ΔF/Fm') and ETR (see 3.3.1.6.) are automatically written into the 'Report-file' (see 3.3.1.17.). For highest accuracy the data from a number of measurements may be averaged (see 3.3.1.5.).

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 3.3.1.4.).

3.5.2. Run 2: Determination of Fv/Fm

Mode: Sat. Pulse Mode
Settings: Standard, except that the measuring light is on
To initialize run-specific settings, press 'I' after Run 2 selection.

Run 2 is particularly useful in conjunction with the Leaf-Clip

Holder 2030-B (see 2.5.). 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 3.3.1.17.).

Run 2 should be used with samples after defined periods of 'dark-adaptation', which does not necessarily involve strict darkness (see 3.3.1.1.). 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 $m^{-2}s^{-1}$ mostly are tolerable. For strict dark-control special leaf-clips are available (see 2.8.), 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 which recovers only slowly (if at all) in the dark (see also 3.5.5.).

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

Mode: Sat. Pulse Mode or Cont. Mode

Settings: Standard

To initialize standard settings press 'I' after Run 3 selection. 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.

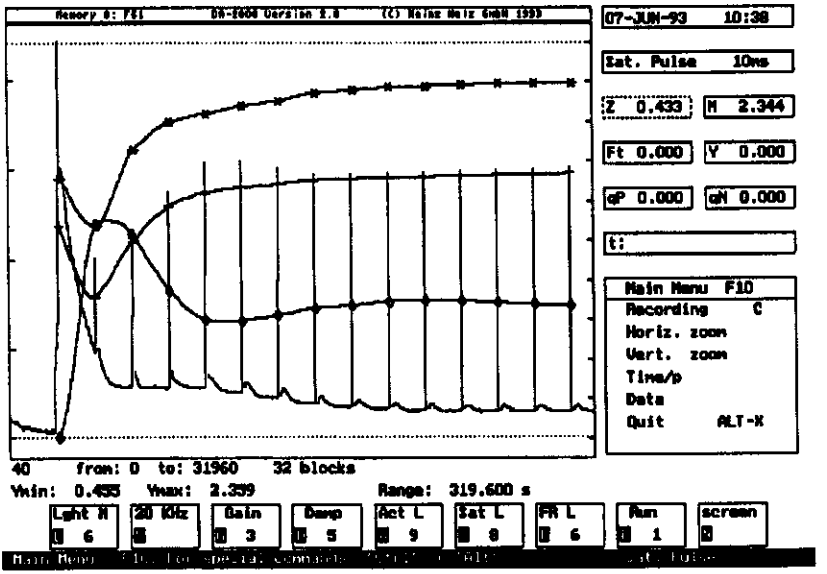


Fig. 61

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 3.3.1.17.).

Run-file 3; Standard Experiment with Nerium oleander leaf

07-JUN-93		ML	Tmp.	PAR	Fo		Fv/Fm			Fm	
10:38:00		120	21.9	0	0.433		0.815			2.344	
Time	No.	ML	Tmp.	PAR	Ft	ETR	Yield	qP	qN	Fm'	Fo'
10:38:32	1	119	22.0	264	1.097	4.3	0.535	0.655	0.002	2.359	
10:38:52	2	120	22.2	263	0.841	2.7	0.358	0.535	0.540	1.311	
10:39:12	3	120	22.4	263	0.683	3.8	0.497	0.729	0.516	1.358	
10:39:32	4	119	22.2	262	0.675	4.4	0.585	0.797	0.375	1.628	
10:39:52	5	119	22.2	262	0.674	4.7	0.620	0.820	0.299	1.771	
10:40:12	6	119	22.2	261	0.650	4.8	0.634	0.838	0.296	1.778	
10:40:32	7	119	22.4	261	0.628	4.8	0.641	0.852	0.311	1.750	
10:40:52	8	119	22.4	261	0.597	4.9	0.652	0.871	0.328	1.716	
10:41:12	9	119	22.2	261	0.584	5.0	0.657	0.881	0.337	1.700	
10:41:32	10	119	22.4	261	0.573	5.0	0.659	0.888	0.346	1.681	
10:41:52	11	119	22.4	261	0.569	5.0	0.660	0.890	0.350	1.674	
10:42:12	12	119	22.4	261	0.565	5.0	0.663	0.894	0.348	1.679	
10:42:33	13	119	22.4	261	0.564	5.0	0.666	0.895	0.344	1.686	
10:42:53	14	119	22.4	261	0.564	5.0	0.668	0.896	0.339	1.696	
10:43:13	15	119	22.4	261	0.563	5.1	0.670	0.898	0.335	1.704	

Fig. 62

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

3.5.4. 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
To initialize standard settings press 'I' after Run 4 selection while on the Par. Screen. 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.

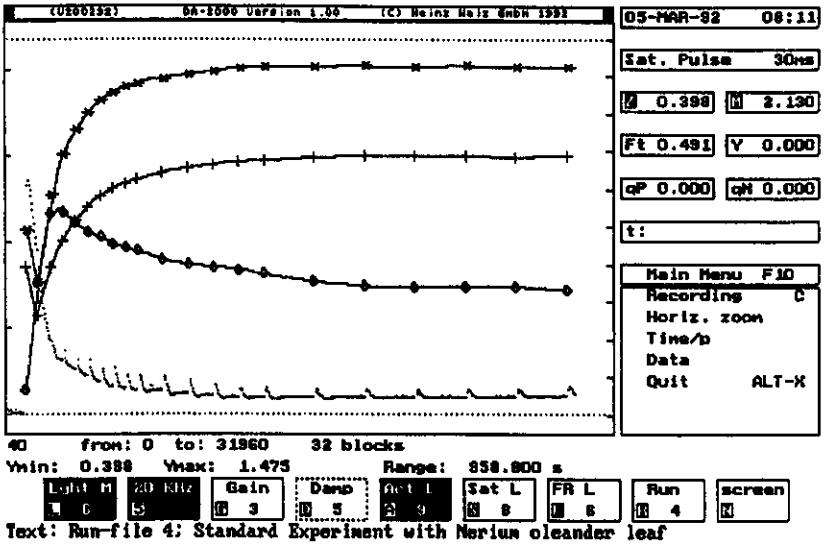


Fig. 63

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 3.3.1.17.).

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.

3.5.5. 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
To initialize run-specific settings press 'I' before start of Run 5.

It may be advantageous, to modify the intensity of the actinic light, to induce different extents 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 by the A-command, after Run 3 or Run 4 is terminated. The kinetic information obtained during Run 3 or 4 may be stored via the Write-function (see 3.4.1.5.) 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 by the M-command with a dark-adapted sample before actinic illumination. Fo and Fm values may also be entered manually using the Del-command (see 3.3.2.1.).

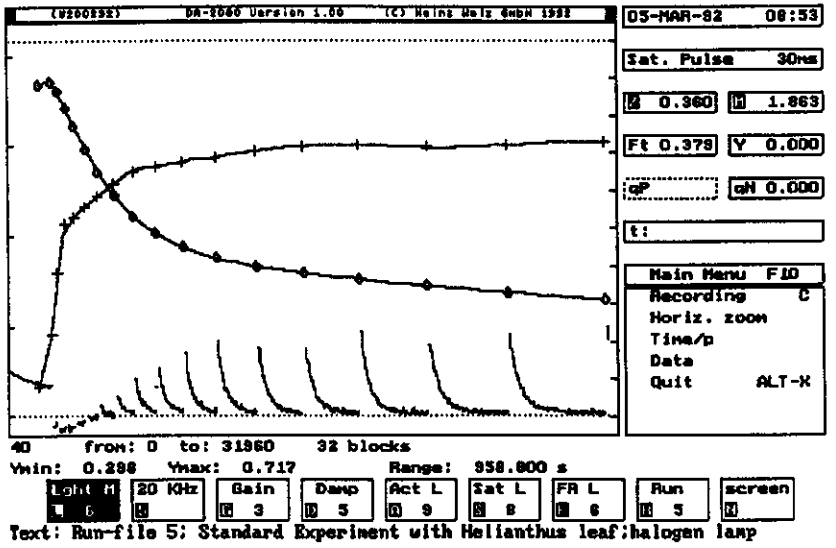


Fig. 64

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:

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

Run 5 is terminated after 16 min. The data are automatically stored in the Report-file (see 3.3.1.17.). On the graphics screen, for each saturation 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 3.3.2.3.). 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 (see 1.2.). 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'-relaxation parallels qN-relaxation (see also 3.3.1.7.).

3.5.6. 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.

To initialize the run-specific settings press 'I' after Run 6 selection.

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.

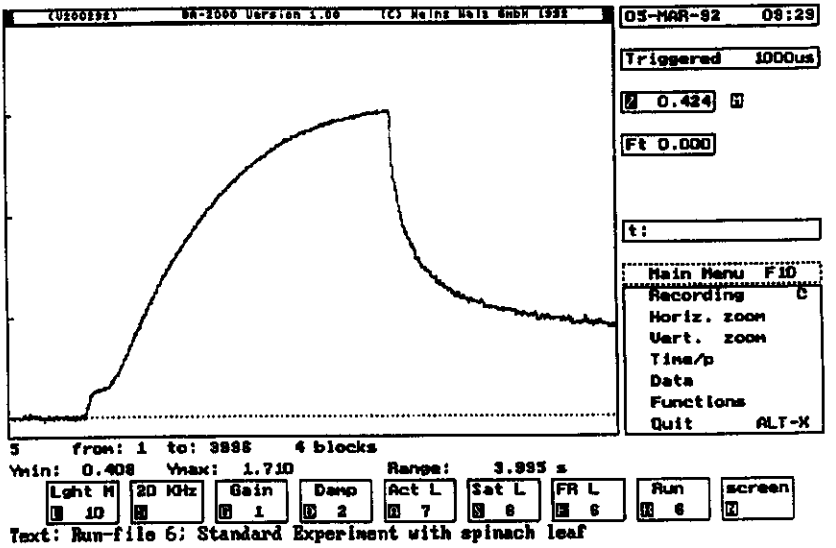


Fig. 65

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 (activation by F-key), reoxidation is accelerated due to electron pumping by PS I.

For further analysis of the rapid induction kinetics special Functions are provided (see 3.4.1.6.).

3.5.7. 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. To initialize the run-specific settings press 'I' after Run 7 selection.

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 DA-2000 program: 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 3.4.1.3.) and definition of the curve origin at the trigger point (see 3.4.1.2.). Furthermore, the curve is redrawn with a logarithmic time scale (see 3.4.1.2. and 3.4.1.6).

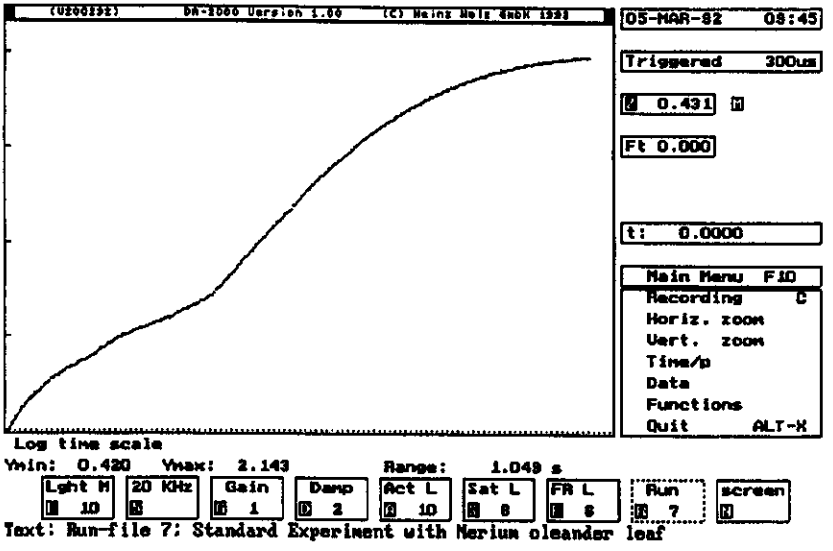


Fig. 66

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 (see 1.2.). 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 3.4.1.2.). Getting back to the log time scale requires the commands 'F 10', 'H' and 'X', which can be given in quick sequence.

3.5.8. Run 8: Actinic light intensity series (running 76 min)

Mode: Sat. Pulse Mode
Settings: 2 Int: 8 (Initial actinic light intensity)

Auto 20K-function off
3 Int: 9 (Sat. pulse intensity)
8 s: 0 (Far-red illumination time)
Other settings standard
To initialize the run-specific settings press 'I' after Run 8 selection.

Run 8 provides a convenient means of assessing 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 PAM-2000 Main Control Unit. Other reasons in favor of the LED actinic source are the much lower power consumption, lesser sample heating and the constancy of spectral output which 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 is too low to saturate photosynthetic electron transport. Hence, this Run may provide only the first, mostly linear part of a light saturation curve.

```
Run-file 8; Standard Experiment with 30 min dark-adapted spinach leaf;
            Before start of Run 8 first 'M' was applied; Ctrl S inactive

08-JUN-93       ML      Tmp.    PAR     Fo              Fv/Fm                   Fm
09:35:52       161      19.2      1   0.406             0.809                2.126

  Time      No.   ML    Tmp.    PAR     Ft     ETR    Yield     qP     qN     Fm'   Fo'
09:54:22     2   160    20.4     12   0.388     3.9   0.774  1.000  0.238  1.718
10:00:52     3   160    20.6     16   0.399     5.3   0.781  1.000  0.176  1.824
10:07:22     4   160    20.6     24   0.396     7.9   0.780  1.000  0.191  1.798
10:13:52     5   160    20.8     34   0.404    11.0   0.774  1.000  0.199  1.784
10:20:22     6   160    20.9     53   0.409    17.1   0.767  0.998  0.217  1.753
10:26:52     7   159    21.0     77   0.411    24.6   0.761  0.996  0.237  1.719
10:33:22     8   159    21.1    117   0.428    36.6   0.745  0.983  0.261  1.678
10:39:52     9   158    21.1    173   0.445    52.8   0.727  0.968  0.288  1.630
10:46:22    10   158    21.3    257   0.461    76.3   0.706  0.953  0.323  1.571
10:52:52    11   156    21.3    378   0.483   106.6   0.671  0.928  0.382  1.469
10:59:22    12   152    21.4    551   0.495   143.6   0.620  0.901  0.478  1.304
```

Fig. 67

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 tabular form. In the given example, before start of the Run Fo and Fm were determined. The correctness of these values depends on the dark-adaptational 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 3.3.2.1.). 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 saturation 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 3.3.1.10.. For correct determination of qP and qN it is necessary to apply 'Ctrl S' before start of Run 8 in order to determine Fo' at each light intensity (see 3.3.1.2.).

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 3.5.9.). There is the possibility to modify the Standard Runs by defining e. g. different adaptation times in so-called 'User-Runs' (see 3.6.).

3.5.9. Run 9: Actinic light intensity series (running 33 min)

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

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 3.3.2.1.). For correct qP and qN determinations Fo-quenching must be considered. In this case, before starting Run 9, 'Ctrl S' should be applied. 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 3.3.1.10.. It is <u>not</u> recommended to use the halogen lamp as actinic light source in this Run, although contrary to Run 8 the heating of the PAM-2000 Main Control Unit is not 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 lesser sample heating and the constancy of spectral output which 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 is too low to saturate photosynthetic electron transport. Hence, this Run may provide only the first, mostly linear part of a light saturation curve.

```
Run-file 9; Standard Experiment with 5 min dark-adapted Saintpaulia leaf
            Before start of Run 9 first 'N' was applied and Ctrl S activated

08-JUN-93       ML    Tmp.   PAR     Fo          Fv/Fm              Fm
11:16:50       120    22.6     0   0.399          0.771           1.741

  Time     No.  ML    Tmp.   PAR     Ft    ETR   Yield    qP     qN     Fm'   Fo'
11:23:45    2  120    22.2    10   0.508   2.9  0.695  0.913  0.059  1.663  0.398
11:26:50    3  120    22.4    16   0.521   4.6  0.683  0.897  0.072  1.644  0.393
11:29:55    4  120    22.5    23   0.540   6.5  0.669  0.880  0.082  1.630  0.391
11:33:00    5  120    22.5    34   0.563   9.3  0.652  0.859  0.092  1.618  0.390
11:36:05    6  121    22.6    53   0.580  14.2  0.639  0.846  0.098  1.609  0.393
11:39:10    7  121    22.5    76   0.621  19.6  0.613  0.811  0.102  1.604  0.393
11:42:15    8  121    22.6   117   0.686  27.7  0.564  0.754  0.125  1.574  0.396
11:45:20    9  120    22.8   173   0.739  36.2  0.498  0.679  0.200  1.471  0.393
11:48:25   10  120    22.8   258   0.748  45.7  0.422  0.602  0.331  1.293  0.388
11:51:30   11  119    22.9   381   0.758  52.2  0.326  0.491  0.453  1.124  0.378
11:54:35   12  116    23.1   557   0.784  51.5  0.220  0.346  0.535  1.005  0.365
```

Fig. 68

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 in tabular form. Starting at intensity 1, the sample is illuminated for 3 min at each intensity setting, at the end of which illumination period 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 3.5.8.). Furthermore, the pre-programmed times may be modified by definition of so-called 'User-Runs' (see 3.6.).

3.5.10. Run 10: Instrumental 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

To initialize the run-specific settings press 'I' after Run 10 selection.

Before Run 10 is started, the following preparations have to be made: The Cont. Mode is selected and a chart recorder may be connected to the analog output. The recorder sensitivity should be 1V full scale; a recording rate of 2 cm/min is appropriate. After selection of Run 10 the run-specific settings should have been initialized by 'I'. 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 3.3.2.1.) and the y-axis limit is set to 1 V using the F8-command (see 3.4.1.3.). Before the Run is started the measuring light should be off.

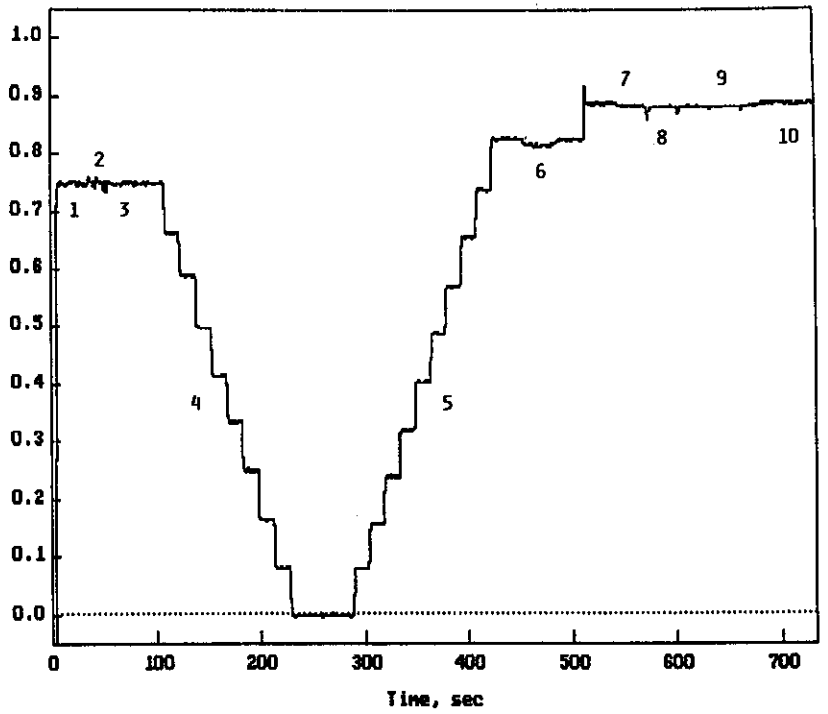


Fig. 69

Run 10 provides a self-test of some essential instrumental 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 3.3.1.8.) and of the fiberoptics (see 2.2.). The former can be more conveniently assessed by the ML-Parameter (see 3.3.1.8.).

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 3.3.1.8.).

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 saturation pulses. The decrease caused by S6 should be less than 1 %. In this order of magnitude the effect on Fm determination may be considered negligable.

10 Lack of an 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.

Run 10 is recorded at the company with every new instrument before its delivery and the recording is provided to the customer with his individual PAM-2000. 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.

3.6. User-Run files

Whereas the standard Run-files (see 3.5.) are incorporated in a fixed form in the DA-2000 program, so-called 'User-Runs' can be programmed by the user, to substitute for the Standard Runs. For this purpose the DA-2000 provides a special routine to Write/Read Run-files to/from disk. Using the 'Alt F10'-command when the cursor is on the Run parameter field (e. g. via Ctrl R), the selection menu for Write/Read Runs is entered (see 3.3.1.21.). The total number of active Runs is always 10, with each number representing either a standard or a user-defined Run. The latter are recognised by the program and indicated by a (u) in the Run parameter field.

3.6.1. 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. For example, Runs involving the Triggered Mode of data acquisition (see 3.2.) should carry the numbers 6 or 7. This has the advantage that the initial instrumental settings, which are installed by the ' I '-command, will be similar to the desired settings.

For modification of a Standard Run at a given mode of data

acquisition, the user may proceed as follows:

1) Enter Write/Read selection menu via Alt F10 (see 3.3.1.21.), while in the relevant mode of data acquisition.
2) Activate 'Write'- and enter the name 'Standard'. Then the given set of Standard Run files which can be used with the given mode of data acquisition is written to the active disk drive (normally A: with Poqet and C: with PC) under the name Standard. Run.
3) Using the DA-2000 inherent editor (entered via the Ctrl E-command, see 3.3.1.17.) the Standard. Run file can be read and modified. At the 'Report-file' level, following the 'Wordstar'-command 'Ctrl KR' (see 5.4.), 'Standard. Run' is entered as the 'File to read'. Then 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.
4) For modification of the given Run-files normal 'Wordstar' editor commands (see 5.4.) and a simple syntax are used (see 3.6.2.).
5) After modification, the relevant part of the file is written to disk, after definition of block start and end (Ctrl KW-command) under its new name, e. g. R3U1.Run (i. e. the first modification of the Standard Run 3). In this way, an almost unlimited number of such user defined Runs can be installed on disk memory.
6) After returning to the Parameter Screen or Kinetic Screen levels, before applying newly defined User Runs, these have to be loaded via the Alt F10 selection menu (see 3.3.1.21.).
7) Substitution of a Standard Run by a User Run is indicated by a (u) in the Run parameter field for the corresponding Run number.

3.6.2. Syntax for User-Runs

User-Runs are defined as ASCII-files (Name.Run). The DA-2000 provides a routine for editing already existing Standard-Runs (see 3.6.1.)

or defining new User-Runs. At the editor-level (e. g. entered via Ctrl E) the following syntax is used:

#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, with the time (in sec following Run Start) preceding the key command, 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 a resolution 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. For example, changing over from the Parameter Screen to the Kin. Screen (K-command) and redrawing previous kinetic recordings may require 15-25-sec with the Poqet PC.

Writing a User-Run program with the routine provided by the DA-2000 is quite simple, if the user is already experienced with the key operations controlling instrumental settings and data analysis (see 3.3.,

3.4. and 5.5.). In order to get acquainted with the syntax and command structure, it is recommended to study the definition of the Standard Runs (see 3.5. and 3.6.1.). 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 (e. g. 8 s with Poqet-PC).

#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 saturation 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 saturation 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
5, 1              ;   type the trigger address 512
5, 2                  (5,"512" would be equivalent)
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, - 1, - 1, - 1, - 1, - 1, - 1, - 1, -	;	lower actinic intensity setting by 7 steps (from 8 to 1) after 10 min illumination at setting 8
970, Y	;	apply saturation pulse for quenching analysis
1000, +	;	increase actinic intensity by one step (from 1 to 2)
1360, Y...4480, Y	;	apply saturation pulses at 390 s intervals with actinic intensity settings being stepwise increased
4510, !	;	increase actinic intensity to setting 11
4870, Y	;	apply last saturation 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 saturation 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, - 1, - 1, - 1, -	;	immediately decrease Damping-setting by four steps (from D7 to D3)
55, 5	;	switch to 20 kHz modulation frequency
85, D	;	move cursor on Damping-field
1, + 1, + 1, + 1, +	;	immediately increase Damping-setting by four steps
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 1, LEFT	;	move cursor two positions to the left to place it on Meas. Light field
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, - 1, - 1, - 1, - 1, -	;	decrease measuring light intensity from setting 11 to 6
1, G 1, +	;	immediately increase Gain-setting by one step
1, D 1, - 1, -	;	immediately decrease Damping-setting by two steps
545, 5	;	switch to 20 kHz
575, S 605, S	;	apply two saturation pulses
1, - 1, - 1, - 1, -	;	decrease Sat. pulse intensity by 4 steps (from 10 to 6)
635, S 665, S	;	apply two saturation pulses
680, 5	;	switch to 600 Hz
710, F	;	turn on far-red light
740, ESC	;	stop recording and redraw kinetic traces
1, F 1, L	;	immediately turn off far-red light and and measuring light
1, 0 1, CR 1, CR	;	apply horizontal zoom by definition of curve limits at first and last point of recording

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 3.6.1.) 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.

3.7. Data storage, transfer and output

3.7.1. Data storage

3.7.1.1. Saturation Pulse Mode

All data which are measured in the Sat. Pulse Mode are automatically stored in a so-called 'Report-file' (see 3.3.1.17.). This file is created upon program installation with the name STANDARD.RPT. With the Poqet PC it is on drive A (RAM card), whereas with other PCs it may be on drive A , B or C (hard disk). It is recommended to 'empty' the Report-file after accumulation of a certain amount of data. This is most conveniently done by the MS-DOS 'Rename'-command. For example, you may decide to rename the content of the Report-file at the end of an experimental series, e. g. proceeding in the following way:

- first leave the program via 'Alt X'
- then enter 'RENAME STANDARD.RPT dd-mm-yy'
- return into program by entering 'DA-2000'

If you then return into the Report-file via 'Ctrl E', you will find it empty and ready for new enterings.

There are numerous ways for editing data stored in the Report-file. For this purpose normal Wordstar-commands are effective (see list in

the Appendix section 5.4.).

With the Poqet PC, the Poqet Write Editor is also available which allows editing text and data files stored in the RAM card (drive A). This provides a comfortable way for further handling of the renamed Report-file data.

It may be noted that with the Poqet Write editor file sizes should not exceed 8 kB (approx. 4 text pages or 6-8 data pages). This should be considered when defining Report-file blocks for later editing with Poqet Write. To enter Poqet Write, first the DA-2000 program must be left via Alt X. Then the Poqet Tools menu is called via the Poqet-Esc key combination. For further details, please consult the Poqet PC User's Guide.

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 'Write'-command (see 3.4.1.5.). 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 a ASP-file is relatively small (approx. 700 kByte). In many cases the user may prefer to erase the CMP-file at DOS-level.

3.7.1.2. Triggered Mode and Continuous Mode

Kinetic data recorded in the Triggered or Continuous Mode are stored via the 'W'- or 'X'-commands (see 3.4.1.5.), by which data-files NAME.CMN are created. 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.

Data which are stored in a CMN-file can be transformed into an ASCII-file by the DOASCII-command with specification of the drive source:

e. g. DOASCII C:

Then a list of the data files in the given drive is displayed, one of which is entered as Filename.

3.7.2. Data transfer from Poqet PC to Desktop PC

A special cable is provided for serial transfer of data from the Poqet PC to a Desktop computer. This so-called Link Cable connects to the RS 232 cable which may remain permanently connected to the expansion port at the back of the Poqet PC. This has the advantage that the delicate 80-pin connector does not need to be disconnected for data transfer. For proper use of the Poqet Link function, the user is referred to the special Poqet Link User's Guide, which is provided together with the Poqet PC, in addition to the general Poqet PC User's Guide.

3.7.3. Data output: Hardcopy print-out

In conjuction with Desktop or Laptop PCs, the DA-2000 supports hardcopy print-outs with IBM compatible printers or with the HP Laserjet printer via the Alt H-command (see 3.4.1.5.). It may be advantageous to install 'DA-2000 MONO' instead of 'DA-2000' for quick hardcopies (see 3.4.1.5.). The MONO version has to be installed for hardcopies of superimposed curves (see Memory sub-menu in section 3.4.1.5.)

With the Poqet PC version, the DA-2000 supports hardcopy print-outs using the battery powered, portable Kodak Diconix 180 si printer,

with serial interface. A special 'Printer Cable PQ-DK' is provided. This cable connects to the RS 232 cable which may remain permanently connected to the Poqet PC. This has the advantage that the delicate 80-pin connector does not need to be disconnected for printing. The same cable also serves for the print-out of text- and data-files, e. g. of the Report-file (see 3.3.1.17.), by IBM or IBM-compatible printers with serial interfaces.

3.8. Program up-dates

The PAM-2000 fluorometer is controlled by software resident in the DA-2000 program (see 2.3.) and in the microcontroller EPROM (see 4.4.) which is located within the PAM-2000 Main Control Unit. Approximately one year after introduction of the PAM-2000 fluorometer, the first updates of the DA-2000 program and of the microcontroller EPROM have become available (Version 2.00). In the update version numerous changes with respect to the original version 1.00 have been made. A considerable number of new commands, functions and routines were included, such that the user is provided not only with new software but in a way also receives a new model of the instrument, with improved performance and properties. Outstanding examples for such improvements in the new Version 2.00 are:

- introduction of a local menu to define other light sources to be triggered by the Clock-function in connection with a pulse sequence (see 3.3.1.21.);

- new possibilities of modifying Standard Runs and programming User-Runs (see 3.6.);

- improvement of the Ctrl S-function, as well as introduction of a Ctrl Z-command, for accurate determination of Fo' (see 3.3.1.2.);

- registration of the ML-Parameter in the Report-file (see 3.3.1.17.);

- provision of the RPT2WKS.EXE program to transform Report-files in Lotus-format (see 3.3.1.17.);

- introduction of a Memory-routine to store kinetic data which were recorded in the Trig. Mode of data acquisition (see 3.4.1.5.);

- provision of new routines to alter Ft, PAR and Tmp readings by defined offset-values (see 3.3.1.20.);

In view of the substantial extensions of the program, together with the DA-2000 Version 2.00 a revised edition of the Manual has been prepared, which is provided free of charge to all owners of the PAM-2000.

Also in the future, there will be further attempts to improve and extend the DA-2000 program. Customers are encouraged to inform us about program errors and to suggest further improvements. There is a wide range of applications for the PAM-2000 Fluorometer and future work is likely to put emphasis on new aspects, to which the system may be adapted. Update-versions of the DA-2000 program and of the microcontroller EPROM will be sent to owners of the PAM-2000 free of charge.

4. MAINTENANCE

Although the PAM-2000 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-2000 Main Control Unit, the Poqet PC 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.
- If possible, keep the custom-made RS 232 cable connected to the expansion port to avoid damage to the delicate contacts.
- Make it a good habit to turn the lamps off when they are not used, thus extending their lifetime.
- Turn the PAM-2000 and the Poqet PC off when no immediate measurements are planned, thus extending battery life time.

4.1. Internal battery and its replacement

The internal battery essentially is 'maintenance free'. However, even when the instrument is switched off, there is some unloading, which is substantially stimulated by elevated temperatures. Therefore, it is good practice to recharge the internal battery every 3 months, using the Battery Charger 2020-L. 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.

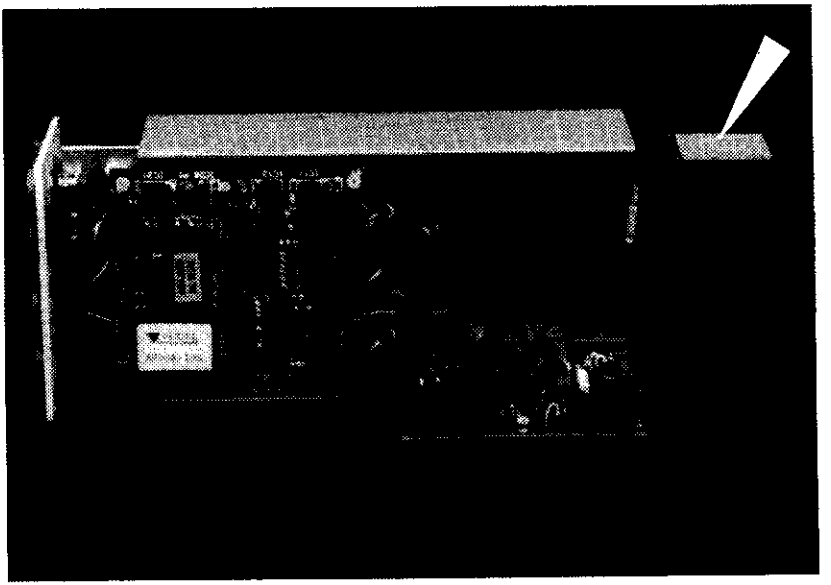


Fig. 70

The above photograph allows a view into the opened PAM-2000 housing for demonstration of battery replacement. For this purpose, the 4 screws at the front-side and the 4 screws at the rear side of the PAM-2000 housing are removed. Make sure that the PAM-2000 power switch is off. When the rear-plate is removed, the circuit-board of the internal charger (on top of battery) can be pulled out to the right. When the main boards are somewhat moved to the left together with the front-plate, the internal battery becomes accessible for replacement. Please note the proper contact polarities (red-positive and black-negative).

4.2. 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 (see 2.1.).

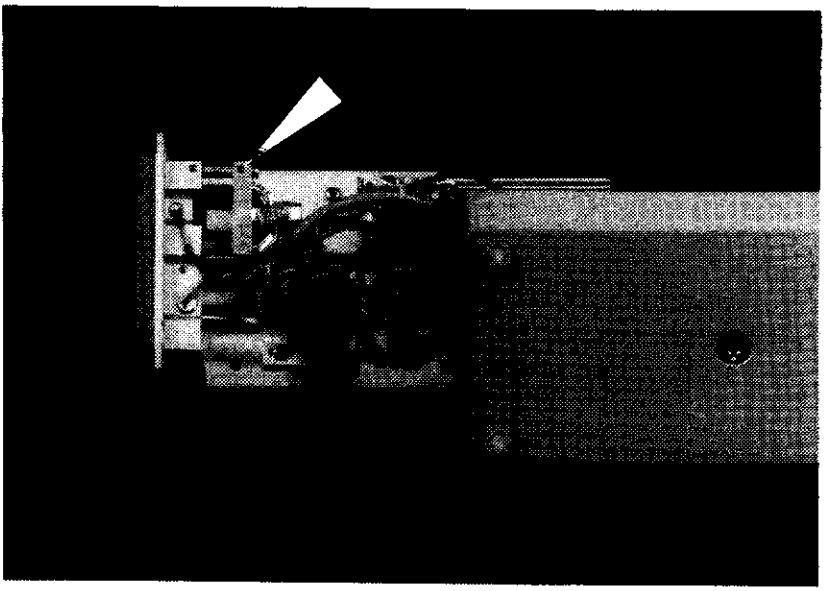


Fig. 71

For halogen lamp replacement, the lid of the housing is lifted after removing the 4 top screws. Also the 2 bottom screws at the front are removed. Then the housing should be turned upside-down. When the front plate is somewhat pulled out, the lamp compartment becomes accessible. Make sure that the PAM-2000 power switch is off. The halogen lamp is held in pre-focused position by an aluminum mounting-frame. Spare halogen lamps also come with this mounting-frame, which is fastened to the housing with 4 screws. These screws have to be removed for lamp replacement. Make sure that the red dots come together.

4.3. Fuse replacement

Two fuses are provided:

- 4 A : for halogen lamp circuit
- 0.5 A : for battery charging circuit.

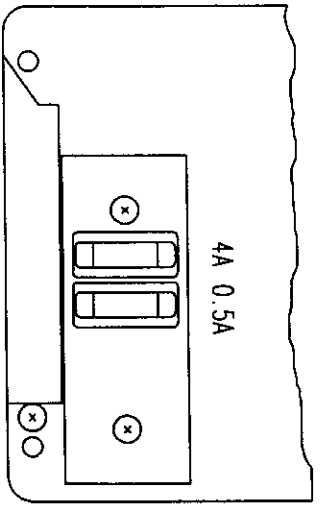


Fig. 72

Both fuses are located on a small circuit board in the back of the halogen lamp. For fuse replacement the same procedure as for halogen lamp replacement can be followed as far as the opening of the PAM-2000 housing is concerned.

4.4. EPROM and its replacement

Besides the various hardware components, the PAM-2000 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-2000 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.

The microcontroller board is located on the bottom side of the main printed circuit board. Before its replacement, first power should be switched off. Then the lid of the housing is lifted after removing the 4 top screws. When you look on the main printed circuit board from the top (front of PAM-2000 to the left), you notice at the right hand side of the board a line of four cable connectors. These need to 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 screws holding the circuit board must be removed before it can be turned around and the microcontroller board becomes accessible.

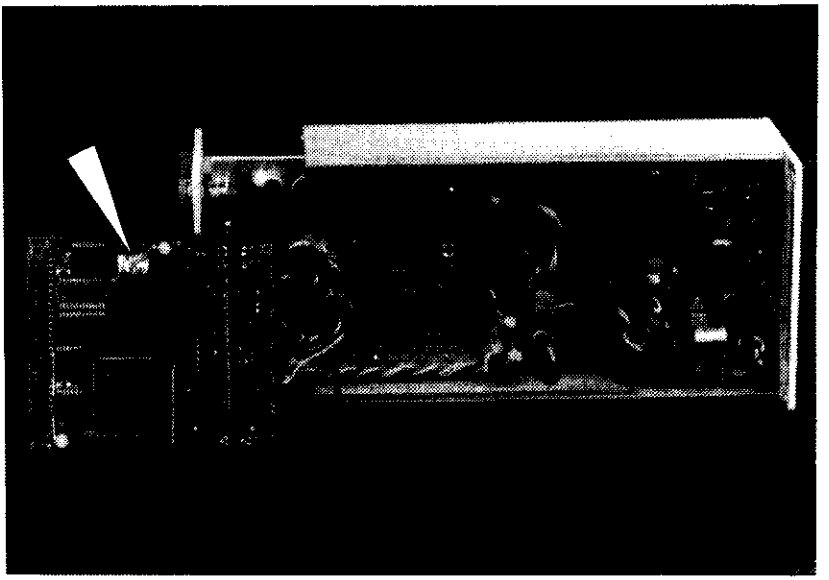


Fig. 73

With the circuit board being up-side down, the EPROM is located in the upper right hand corner of the microcontroller board (see figure). 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.

5. APPENDIX

5.1. Technical specifications

5.1.1. Portable Fluorometer PAM-2000

Measuring light:
Maximum emission: 655 nm
Intensity[1]: 11 levels, standard setting 0.1 µmol quanta $m^{-2}s^{-1}$
Modulation frequency: 600 Hz or 20 kHz, Auto 20 kHz function

Actinic light sources:
LED array:
Maximum emission: 655 nm
Intensity[1]: 11 levels, max. 600 µmol quanta $m^{-2}s^{-1}$

Far red LED:
Maximum emission: 735 nm
Intensity[1]: 11 levels, max. 15 Wm^{-2}

Halogen lamp:
Wave length: <710 nm
Intensity[1]: 11 levels, max. 8500 µmol quanta $m^{-2}s^{-1}$

Saturation pulse light source:
Wave length: <710 nm
Intensity[1]: 10 levels, max. 15000 µmol quanta $m^{-2}s^{-1}$

Signal processing:
Amplification: 10 levels (gain factors from 1 to 10)
Damping: 8 levels with time constants from 0.5 ms to 1 s

1) Intensity at the fiberoptics output (measuring site)

Time resolution:
max. 150 µs/measuring point

Detector:
PIN diode with RG9 filter (>700 nm) and heat protection filter

Microcontroller:
CMOS 80C552

Memory:
Program memory: CMOS EPROM 32 kB
Data buffer: CMOS RAM 32 kB

Power supply:
Internal: battery 12 V/1.2 Ah, capacity sufficient for approx. 500 saturation pulses or approx. 12 hours at a current consumption of 90 mA (basic load)
External: 12 V, e. g. Battery NP-3/12 or Battery Charger 2020-L

Current consumption:
for basic load: 90 mA
with all actinic LED light sources: max. 200 mA

General data:
Analog output: 0 - 2.5 V
Interface: RS 232, 19600 baud
Operation/control: via PC with Data Acquisition Software DA-2000
Measured and calculated values: Fo, Fm, Fv/Fm, Ft, $\Delta F/Fm'$= yield, Fo', qP, qN (or NPQ) and ETR = PAR x yield x 0.5 x 0.84
Permissible ambient temperature: -5 ... +45 °C
Dimensions (LxWxH): 20 cm x 10.5 cm x 8.5 cm
Weight: 2 kg

5.1.2. Data Acquisition Software DA-2000[2)]

Requirements:
Computer: Poqet PC or IBM AT and IBM-compatible
Processor: 80286 or higher
Graphics adapter: Hercules, EGA or VGA
RAM: minimum 640 kB
Diskette drive: 3 1/2" or 5 1/4"
Hard disk: minimum 10 MB
Operating system: MS-DOS Version 3.2 or higher
Interfaces: serial (RS 232) for PAM-2000 and parallel (Centronics) for printer

Printers supported:
IBM and IBM-compatible as well as HP-Laserjet for Desktop PC
Kodak Diconix 180 si, with serial interface in conjunction with Poqet PC

5.1.3. Special Fiberoptics 2010-F

Design: flexible, plastic-sheathed fiberoptics with three-pin "optic connector"
Joint end (measuring site): active dia. 6 mm, outer dia. 8 mm
Length: 100 cm
Weight: 0.3 kg

2) The Poqet PC is supplied with the special DA-2000 software

5.1.4. Battery Charger 2020-L

Design: separate charging outputs for internal battery and external Battery NP-3/12
Charging current: max. 1 A at 12 V
Power supply: 110 V or 220 V, 50/60 Hz
Permissible ambient temperature: -5 ... +45 °C
Dimensions (LxWxH): 15 cm x 9 cm x 7 cm
Weight: 1.1 kg

5.1.5. Transport Case 2040-T

Design: Aluminum frame with plastic sides and foam packing
Dimensions (LxWxH): 52 cm x 33 cm x 21 cm
Weight: 3.1 kg

5.1.6. Carrying bag

Design: nylon bag for carrying the Portable Fluorometer PAM-2000 and the Poqet PC
Dimensions (LxWxH): 25 cm x 16 cm x 15 cm
Weight: 0.49 kg

5.1.7. Hip pack

Design: nylon bag for carrying the Battery NP-3/12
Dimensions (LxWxH): 16 cm x 9 cm x 9 cm
Weight: 80 g

5.1.8. Poqet PC PQ-1024

Design: Standard Poqet PC, additional with 1 MB RAM card for data storage, 512 kB EPROM card with DA-2000 Data Acquisition Software, RS 232 interface cable and Link Cable
Microprocessor: CMOS 80C88, max. 7 MHz
Memory:
CMOS RAM: 640 kB
CMOS ROM: 768 kB
Two PC card drives: for RAM, ROM or EPROM cards
LCD display:
Text mode: with 80 character x 25 lines
Graphic mode: 640 x 200 pixel resolution (CGA)
Keyboard: all functions of an extended PC keyboard
ROM software: MS-DOS 3.3, GW Basic, Poqet BIOS and Poqet Tools
Power supply:
Internal: two AA batteries
External: 5 V (provided by the PAM-2000)
General data:
Permissible ambient temperature: 0 ... +40 °C
Dimensions (LxWxH): 22.3 cm x 10.9 cm x 2.5 cm
Weight: 0.54 kg

5.1.9. Leaf-Clip Holder 2030-B

Micro quantum sensor:
Detector: blue-sensitive photodiode with filter combination for selective PAR measurement (380 - 710 nm)
Optics: micro diffuser (approx. 1 mm^2) and fiberoptics cable
Measuring range: 0 - 20000 µmol quanta $m^{-2}s^{-1}$
Measuring accuracy: ± 5%

Thermocouple:
Type: Ni-CrNi, dia. 0.1 mm
Measuring range: -20 ... +60 °C
Measuring accuracy: ± 0.5 °C

General data:
Power supply: 5 V/4 mA (via PAM-2000)
Analog outputs: 0 - 2.5 V for 0 - 1000 µmol quanta $m^{-2}s^{-1}$ and
0 - 2.5 V for 0 - 20000 µmol quanta $m^{-2}s^{-1}$ as well as 10 mV/°C
(0 mV = -20 °C and 800 mV = +60 °C)
Length of the connecting cable: 100 cm
Permissible ambient temperature: -5 ... +45 °C
Dimensions (LxWxH): 17 cm x 5.7 cm (max.) x 8 cm (max.)
Weight: 0.35 kg

5.1.10. External Halogen Lamp 2050-H

Wavelength: <710 nm
Light intensity: max. 3000 µmol quanta $m^{-2}s^{-1}$, stepless setting
Power supply: 12 V/max. 1.6 A e. g. via Battery NP-3/12
Length of the connecting cable: 120 cm
Permissible ambient temperature: -5 ... +45 °C
Weight: 0.25 kg

5.1.11. Dark Leaf Clip DLC-8

Design: clip made of anodized aluminum with felt contact areas and sliding shutter (closure)
Dimensions (LxWxH): 6.5 cm x 2 cm (max.) x 1.5 cm (max.)
Weight: 3.6 g

5.1.12. Micro Quantum/Temp.-Sensor 2060-M

Micro quantum sensor:
Detector: blue-sensitive photodiode with filter combination for selective PAR measurement (380 - 710 nm)
Optics: micro diffuser (approx. 1 mm^2) and fiberoptics cable
Measuring range: 0 - 20000 µmol quanta $m^{-2}s^{-1}$
Measuring accuracy: ± 5%

Thermocouple:
Type: Ni-CrNi, dia. 0.1 mm
Measuring range: -20 ... +60 °C
Measuring accuracy: ± 0.5 °C

General data:
Power supply: 5 V/4 mA (via PAM-2000)
Analog outputs: 0 - 2.5 V for 0 - 1000 µmol quantum $m^{-2}s^{-1}$ and 0 - 2.5 V for 0 - 20000 mol quantum $m^{-2}s^{-1}$ as well as 10 mV/°C (0 mV = -20 °C and 800 mV = +60 °C)
Length of the sensor cable: 30 cm
Length of the connecting cable: 100 cm
Permissible ambient temperature: -5 ... +45 °C
Dimensions (LxWxH): 16 cm x 3 cm (max.) x 1.7 cm (max.)
Weight: 0.22 kg

5.1.13. Compact Tripod ST-2101

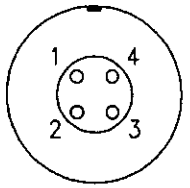
Design: aluminum stand with detachable ball socket and removeable center column
Adjustable height: 43 - 130 cm
Weight: 0.735 kg

5.1.14. Battery NP-3/12

Design: gas-tight, maintenance-free lead acid battery
Rated voltage: 12 V
Rated capacity: 3 Ah
Dimensions (LxWxH): 13.5 cm x 6.7 cm x 6.7 cm
Weight: 1.15 kg

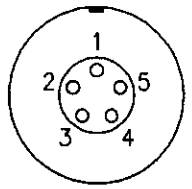
5.2. Pin assignments of PAM-2000 connectors

"EXT.DC"



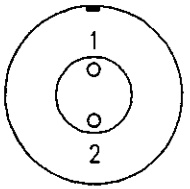
1: +12 V } internal
2: GND } battery
3: Input 12-20 V } charging
4: Input GND } circuit

"RS 232"



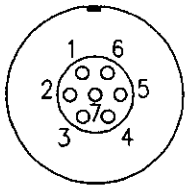
1: +5 V } power supply
5: GND } for Poqet PC
3: TxD } RS 232
4: RxD
2: CTS printer

"OUTPUT"



1: Signal output
2: GND

"LEAF CLIP"



1: +5 V
2: GND
3: } Analog inputs Leaf-Clip
4: } Holder 2030-B
5: }
6: Remote control button
7: not used

5.3. List of warnings and error messages

'No PAM-2000 connected ...'
Either the RS 232 interface cable is not connected or the Power-switch is off.

'Low battery'
Battery voltage has dropped below 11.5 V.

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

'Overload'

Faulty fluorescence readings as the saturation level of 2450 mV was exceeded.

'Attention, low accuracy due to low signal level'

Fm- or Fm'-values measured by a saturation pulse are smaller than 33 times Gain setting; therefore, quenching analysis 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) smaller than Gain-setting; therefore, quenching analysis 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.

'Errror on writing data or disk full'

Either the storage place on the disk is exhausted or the wrong disk drive is installed.

'Write error for report file'

Storage place on disk is exhausted; no more entries into report-file possible.

5.4. List of editor commands

In connection with the 'Report-file' (see 3.3.1.17.) within the DA-2000 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 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

5.5. List of key commands

The following list of commands provides an overview of all commands which are executed by the DA-2000 program. 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 sections 3.3. and 3.4.. If the use of a particular command is restricted to a certain part of the Data Acquisition Program DA-2000, 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 20K 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 instrumental 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 dialoge 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 dialoge for ETR-factor definition

Alt F10: To enter local menus in conjunction with User Runs and Pulse Seq. definition

Alt H : Hardcopy print-out of monitor screen (requires previous selection of printer type)

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 saturation pulse in the right part of the parameter screen

Alt P : Form-feed paper advance with printer

Alt X : To quit the DA-2000 program and to return to MS-DOS

Ctrl C : To enter dialoge 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 dialoge 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 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

5.6. 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. For a more detailed description see section 3.3.1.

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 'L'-command

[I]nt : Change of measuring light intensity between 11 settings; selection via 'I'-command and use of '+'/'–' keys; setting 11 only via 'I'-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 20K : On/off switch for Auto 20K-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, resp.

Column 4

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

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

[7]0.1 : Length of saturation 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
[C]lk m : Time between consecutive saturation pulses in a pulse 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 saturation 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, resp.

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 internal battery; warning 'Low battery!' below 11.5 V

5.7. PAM-2000 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 instrumental functions in the PAM-2000, without involvement of the DA-2000 program. Some of these commands can be also carried out at the Par. Screen level within the DA-2000 program after pressing the T-key, when the 'terminal'-field (T) replaces the normal number-field (No). If, for example, 'a11' is entered the actinic LED light will switch on for 17 sec (as the hexadecimal number 11 corresponds to the decimal number 16+1=17). Before each such command, the T-key has to be pressed. The T-field will be replaced by the No.-field, when the Par. Screen is redrawn (e. g. after '0' or 'I').

The set of commands, which was developed for control of the PAM-2000 by the DA-2000 program, is optimized for a high processing speed. It uses 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.

5.7.1. 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

5.7.2. 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-2000:

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 µsec; 2 = 300 µsec; 3 = 1000 µsec; 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 µE)
7:	Interior temperature

5.8. Literature on chlorophyll fluorescence measurements and quenching analysis with the PAM Fluorometer

The following list of references covers some of the results which so far were obtained with the PAM Fluorometer. This list, which is by far not complete, contains papers published until spring 1993.

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