

# **MULTI-COLOR-PAM**

## **Manual**

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## 2 Safety Instruction

### 2.1 General Safety Instructions

- Read safety instructions and the operating instructions prior to operation of the device.
- Pay attention to all safety warnings.
- Keep device away from water or high moisture areas.
- Keep the device away from dust, sand and dirt.
- Do not put the device near sources of heat.
- Ensure sufficient ventilation.
- Connect the device only to the power source indicated in the operating instructions or on the device. If the device is not in use, remove the mains plug from the socket.
- Ensure that neither liquids nor foreign bodies get inside the device.
- The device should only be repaired by qualified personnel.

## 2.2 Special Safety Instructions

- The MULTI-COLOR-PAM is a highly sensitive instrument which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual in order to avoid potential harm to the user and damage to the instrument.
- The MULTI-COLOR-PAM can emit very strong light! In order to avoid harm to your eyes, never look directly into the emitter unit or Perspex rod connected to the emitter unit.



### 3 Introduction

- The “Multiple Excitation Wavelength Chlorophyll Fluorescence Analyzer MULTI-COLOR-PAM” has been designed for highly sensitive assessment of photosynthesis parameters in dilute suspensions of algae, cyanobacteria and isolated chloroplasts.
- The instrument stands in the tradition of the PAM chlorophyll fluorometers PAM-101, XE-PAM, PHYTO-PAM, DUAL-PAM-100 and PAM-2500. The MULTI-COLOR-PAM combines major virtues of all these instruments in one compact device:
- The MULTI-COLOR-PAM is outstanding by virtue of its different measuring and actinic light colors. Moreover, the instrument offers an especially wide range of measuring light intensities and modulation frequencies.
- Its operating software is based on the PamWin-3 program introduced with the PAM-2500. Major software extensions allow for control of the various colors of measuring light, actinic light, single turnover, and multiple turnover flashes.
- The MULTI-COLOR-PAM is optimized for the study of dilute suspensions of unicellular algae, cyanobacteria and isolated chloroplasts. In addition, the measuring system can be configured to study leaf photosynthesis and epidermal UV-A screening.

### 3.1 Intention of this Manual

The MULTI-COLOR-PAM fluorometer displays a high degree of flexibility in measuring and analyzing fluorescence. Despite its high complexity, first measurements can be started using automated routines for standard measurements. Actually, the "intelligent" central control of all functions by the PamWin-3 software prevents operational mistakes harming the instrument. To make full use of the analytical opportunities offered by the MULTI-COLOR-PAM, however, it is necessary to become acquainted with handling of hardware and software, and to understand the theoretical fundamentals of chlorophyll fluorescence analysis. The present manual provides the information needed to professionally operate the MULTI-COLOR-PAM and also includes links to pertinent publications. The most important chapters of the manual are briefly introduced next:

- Chapter 1, page 11 (Components and Setup) explains hardware setup and software installation required to get started.
- Chapter 4, page 31 (SP-Analysis) introduces standard quenching analysis of chlorophyll fluorescence by the PamWin-3 software for the MULTI-COLOR-PAM.
- Chapter 5, page 79 (Fast Acquisition) deals with rise and decay kinetics of chlorophyll fluorescence as tools to analyze PS II function.
- Chapter 6, page 111 (Script File Window) is devoted to programming the MULTI-COLOR-PAM using the script file option of PamWin-3.

- Chapter 8, page 124 (View Mode) explains how to evaluate saturation pulse and fast kinetics data using PamWin-3.
- Chapter 9, page 131 (Definitions and Equations) provides some background on how fluorescence signals can be used to evaluate photosynthesis analysis by fluorescence signals.
- Sections 9.7 and 9.8 (page 146), and Chapter 1 (page 151) compile pertinent references.

This manual is complemented by information provided in the online help texts of PamWin-3 (see section 5.1, page31).



## **4 Components and Setup**

### **4.1 Extent of Delivery, Basic System**

#### MCP-C Power-and-Control-Unit

MINI-PAM/L charger and power cable

USB cable type A-A

ST-101 Stand for mounting ED 101US/MD

PHYTO-T transport box

#### MCP-D Detector Head

Long-pass filter RG 665

Short-pass filter SP 710

#### MCP-E Multi-Color Emitter Head

#### ED-101US/MD Optical Unit for Suspensions

US-K0 10 x 10 mm Quartz Glass Cuvette

#### Manual and CD Software and Manuals

## 4.2 Basic system

### 4.2.1 Power-and-Control-Unit

The sockets and switches of the Power-and-Control-Unit are outlined and numbered in Fig. 1, their properties and functions are explained on subsequent pages.

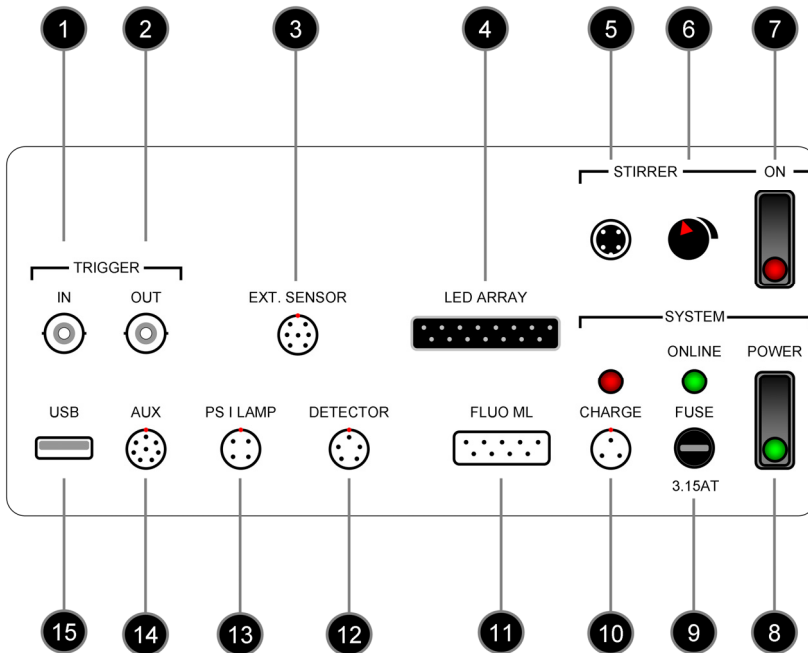


Fig. 1: Front panel of MULTI-COLOR-PAM Power-and-Control-Unit

1

TRIGGER IN BNC socket

Input for external 5V signal to set off the currently active trigger pattern. Triggering requires that external trigger control is activated by the PamWin-3 software.

2

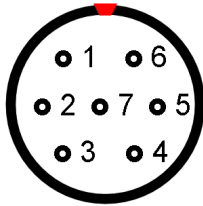
TRIGGER OUT BNC socket

Provides 5 V signals to control external devices.

3

EXT SENSOR (External Sensor Socket)

Socket for light sensors with spherical (US SQS/WB) or flat (US-MQS/WB) diffuser.



(1) +5 V

(2) Ground

(3) Analog input 1  
(0 - 2.5 V)(4) Analog input 2  
(0 - 2.5 V)(5) Analog input 3  
(0 - 2.5 V)

(6) Trigger in (5 V)

(7) -5 V

4

LED ARRAY socket

Socket for cable connecting to the actinic light LEDs in the emitter head (MCP-E).

5

STIRRER socket

To connect the ribbon cable of the stirrer PHYTO-MS.

6

Rotary control for stirrer speed

7

Stirrer switch

In the ON position, the stirrer is on standby, the red LED of the switch is off. To activate stirring, tick the “Stirrer on” checkbox in the “Multi Color” window of PamWin-3. This will also turn on the LED of the stirrer switch.

---

8

Power switch

The integrated green LED lights up when the device is turned on. When switched on, the ONLINE LED starts flashing, and lights continuously after connection between PamWin-3 and MULTI-COLOR-PAM has been established.

---

9

ONLINE LED and FUSE

Continuous light indicates connection between computer and MULTI-COLOR-PAM. The fuse holder contains a 3.15 Ampere slow-blow fuse.

---

10

CHARGE socket and LED

To connect the MINI-PAM/L charging unit. The red LED remains dark in the absence of power supply.

---

11

FLUO ML (Fluorescence measuring light socket)

To connect the LEDs emitting modulated light of the emitter head (MCP-E).

---

12

DETECTOR

To connect the cable of the photodiode detector (MCP-D).

---

13

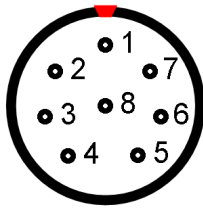
PS I LAMP

For connection of additional light sources, e. g., a high power lamp for specific excitation of PS I (special request).

---



14

AUX (Socket for auxiliary devices)

- |                                |                              |
|--------------------------------|------------------------------|
| (1) DGND<br>(digital ground)   | (5) DGND<br>(digital ground) |
| (2) UBatt<br>(battery voltage) | (6) Trigger Out<br>(5V)      |
| (3) GND (ground)               | (7) +5 V                     |
| (4) DA out<br>(digital output) | (8) -5 V                     |

15

USB socket

Standard USB A socket for connection to computer.

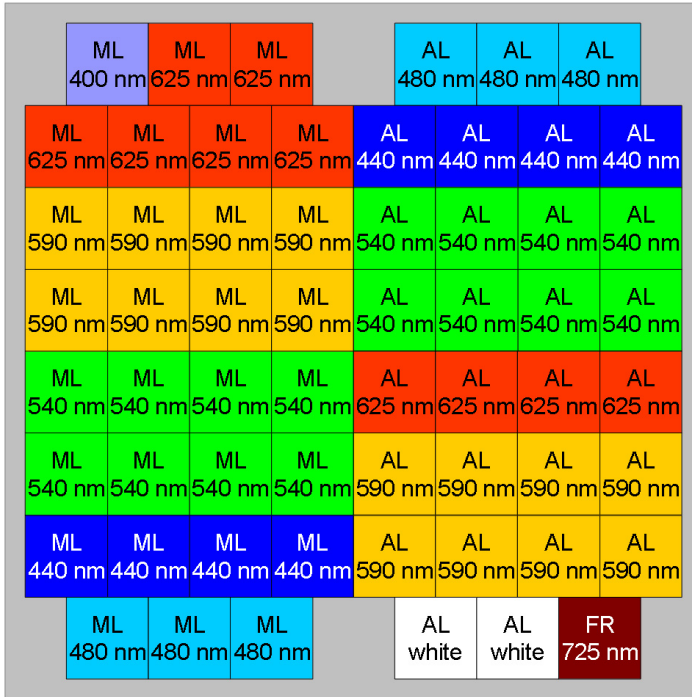
#### 4.2.2 MCP-E Multi-Color Emitter Head

**Note:** Pressure or strong force applied on the Perspex rod might destroy the LED array of the MCP-E Multi-Color Emitter Head

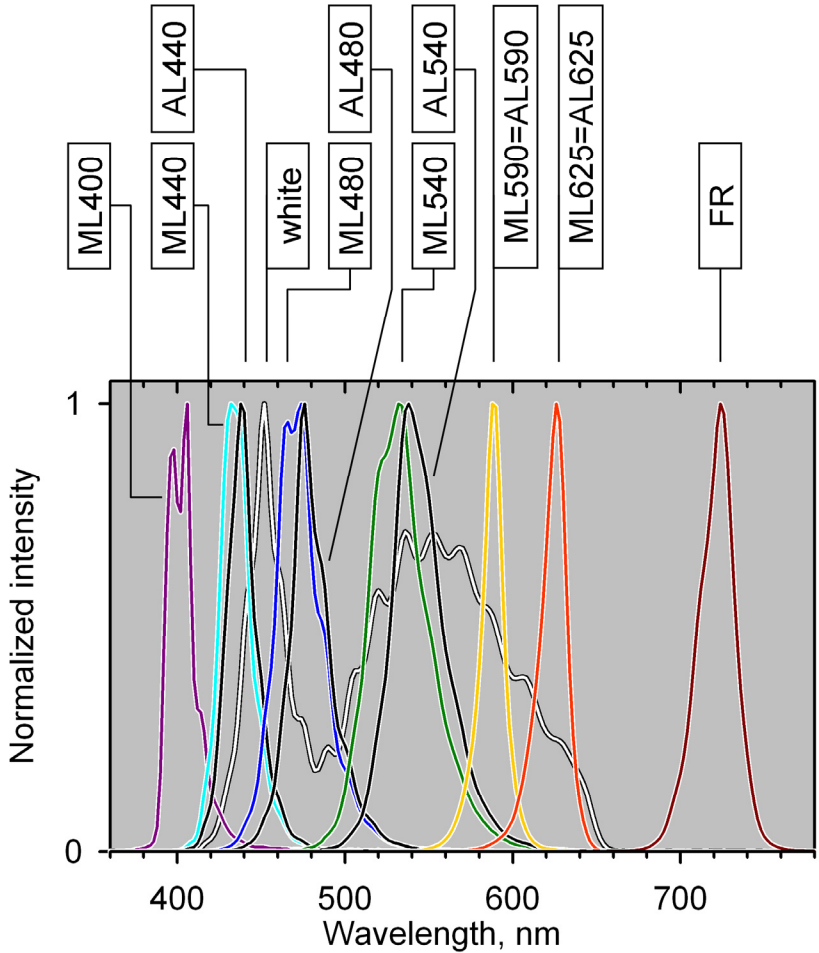
Light sources of the MULTI-COLOR-PAM are light emitting diodes (LED) which are densely arranged on a 10 x 10 mm area (Fig. 2, page 16). In total, the MCP-E head provides 6 differently-colored measuring lights, and 5 spectrally different actinic light sources; the latter are complemented by white and far red LEDs (for spectra see Fig. 3, page 17). To partially compensate for varying brightness of the different types of light emission diodes, more LEDs are used when the intensity of the individual LED is low.

To collect LED radiation efficiently, the distance between LED array and Perspex rod is very small. Therefore, a considerable part of LED emission enters the Perspex rod at low angles re-

sulting in multiple reflection of radiation inside the Perspex rod which creates a rather uniform light field at the opposite end of the rod.



**Fig. 2: Schematic top view of 1 x 1 cm LED board of MCP-E Multi-Color Emitter Head.** AL, actinic light LED. ML, measuring light LED. FR: far red light LED. Wavelengths indicate peak of emission spectra (see Fig. 3, page 17).



**Fig. 3:** Typical normalized emission spectra of MULTI-COLOR-PAM light sources. Spectra are not corrected for spectral response of the spectrometer. ML, modulated measuring light. AL, actinic light. FR, far red light.

### 4.2.3 MCP-D Detector Head

Signal detection in the MCP-D head is by a PIN photodiode with special pulse preamplifier for measuring fluorescence changes with maximum time resolution of 10  $\mu$ s.

The photodiode is protected from modulated excitation light by a RG 665 long pass filter which must be inserted in the filter holder of the MCP-D detector. When the filter SP 710 is placed in the holder as well, fluorescence detection is additionally confined to wavelengths shorter than 700 nm. When the RG 665 and SP 710 are used together, the SP 710 filter should be placed next to the MCP-D head to screen the detector from the weak fluorescence of the RG665 filter.

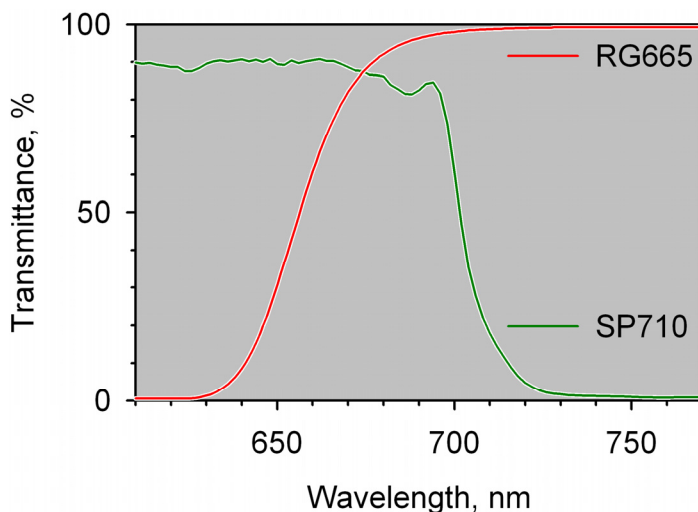
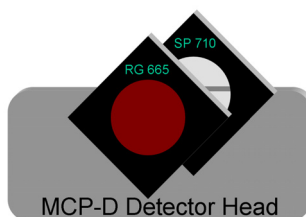


Fig. 4: Transmittance spectra of RG 665 long pass and SP 710 short pass filters.

#### 4.2.4 ED-101US/MD Optical Unit for Suspensions

The optical unit ED-101US/MD consists of a centrally located place for a 10 x 10 mm cuvette and peripherally positioned ports for emitter and detector heads of the MULTI-COLOR-PAM. Perspex rods efficiently guide light from emitters to the sample, and from the sample to the detector. Two plugs are provided to close ports not occupied by emitter or detector heads. These plugs possess a light reflecting and a light trapping end. The reflecting end may be positioned opposite to the fluorescence detector to enhance the fluorescence signal, the light-trapping part can be positioned opposite to the emitter to reduce effects of strayed light on the signal.

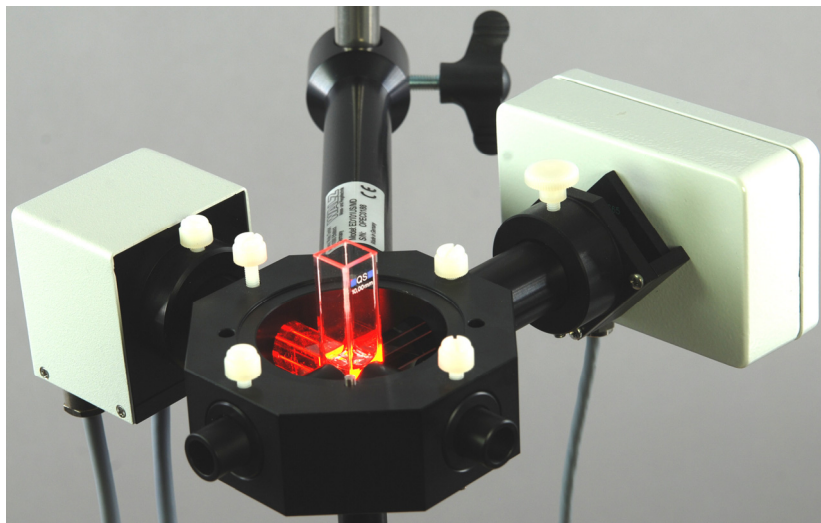
The two-partite standard cover of the unit has a pinhole through which chemicals can be added using a syringe. Another port at the bottom of the ED-101US/MD unit holds the optional PHYTO-MS Magnetic Stirrer.

In standard configuration, a sample volume of 1.25 mL just covers the area illuminated by the MCP-E emitter head. Higher cuvette volumes result in partial illumination of the sample and should be avoided.

### 4.3 Accessories

#### 4.3.1 ED-101US/T Temperature Control Block for Cuvette

The temperature block is firmly screwed on the Optical Unit ED-101US/MD. The temperature is controlled by an external water bath with pump circulator (not included). Efficient heat exchange is achieved by a spring-supported clamping mechanism providing close contact between cuvette and temperature block.



**Fig. 5:** ED-101US/MD Optical Unit with central quartz cuvette. Attached are the MCP-E Multi-Color Emitter Head, and the MCP-D Detector Head. To minimize the background signal, the optical axes of the MCP-E and MCP-D heads are oriented at right angles to each other.

### **4.3.2 US-T Temperature Control Unit**

The US-T unit is placed on top of the ED-101US/MD Optical Unit. The device controls temperature of the cuvette by a Peltier-heat-transfer rod which is immersed in the upper part of the sample suspension. Temperature is measured by a PT-100 sensor which is dipped in the cuvette. Temperatures of 15 K above and below ambient temperature can be achieved.

### **4.3.3 US-SQS/WB Spherical Micro Quantum Sensor**

The US-SQS/WB device consists of a submersible spherical quantum sensor, US-SQS/L, plus a small amplifier unit which is connected to the external sensor socket (EXT. SENSOR) of the central unit. The amplifier is tuned to yield correct PAR readings when a “Calibration factor for PAR” of 1.000 is selected in PamWin-3. For stability of calibration, it is important to keep the diffuser clean. Check calibration regularly by comparison with a standard quantum sensor. A calibration factor substantially greater than 1.000 indicates dirt-deposition on the diffuser. In this case, gently clean the diffuser using a cotton tip applicator moistened with some ethanol.

The 3.7 mm diffusing sphere of the US-SQS/L sensor picks up photosynthetically active radiation (PAR) with an angular response error of less than  $\pm 5\%$  from  $-100^\circ$  to  $100^\circ$  angle. Hence, the sensor is ideally suited to measure light conditions in the suspension cuvette where reflections and scattering results in randomization of light direction. The sensor is an essential for determinations of the optical cross-section of PS II.

The US-SQS/WB includes a hood to mount the sensor on top of the Optical Unit ED-101US/MD. The inner cylinder of the hood must be removed to fit the ED-101US/MD unit. For this purpose, loosen both screws of the hood's top disk, remove inner cylinder, turn top disk by 90° so that the two disk holes become situated above the threaded holes of the hood and fix disk again.

#### **4.3.4 PHYTO-MS Miniature Magnetic Stirrer**

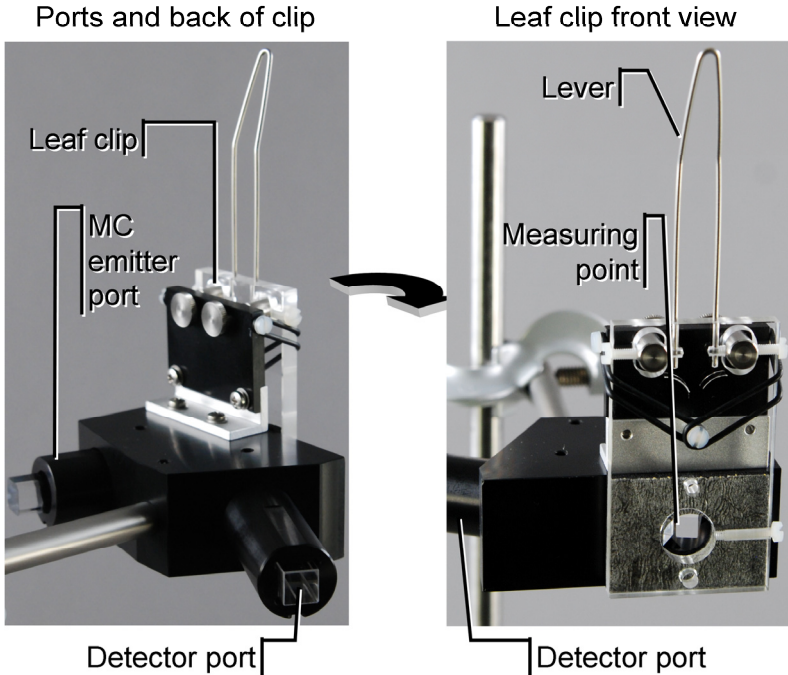
The stirrer has an inductive drive system which results in its compact size and facilitates its integration into the Optical Unit ED-101US/MD. The stirrer moves a miniature stir bar placed inside a cuvette in the optical unit. Stirrer action is software-controlled and stirrer speed is adjusted manually by a control knob at the front panel of the MULTI-COLOR-PAM central unit.

#### **4.3.5 MCP-BK Optical Unit for Leaf Measurements**

The optional MCP-BK optical unit provides optical pathways defined by Perspex rods for efficiently exciting and detecting fluorescence from solid flat samples such as leaves. The Perspex rod for fluorescence excitation points to the leaf almost perpendicularly (12° angle of incidence) and tapers from 10 x 10 mm at the emitter site to 6 x 6 mm at the measuring point (see Fig. 6, page 23). The Perspex rod for fluorescence detection has constant edge lengths of 10 mm and collects fluorescence at a rather large angle of incidence.



### MCP-BK Optical Unit for leaf measurements



**Fig. 6:** MCP-BK Optical Unit. Left, view showing the ports for emitter and detector units. Right, view on leaf clip, rotated clockwise relative to left view.

### Assembly

The MCP-BK Optical Unit is disassembled for shipping. To re-assemble the clip, place screw holes (1, see Fig. 7, page 24) of MCP-BK Clip on top of threads (2) of MCP-BK Optics. On top of each screw hole, place one washer (3). Screw together MCP-BK Clip and MCP-BK Optics using the Phillips screws provided (3).

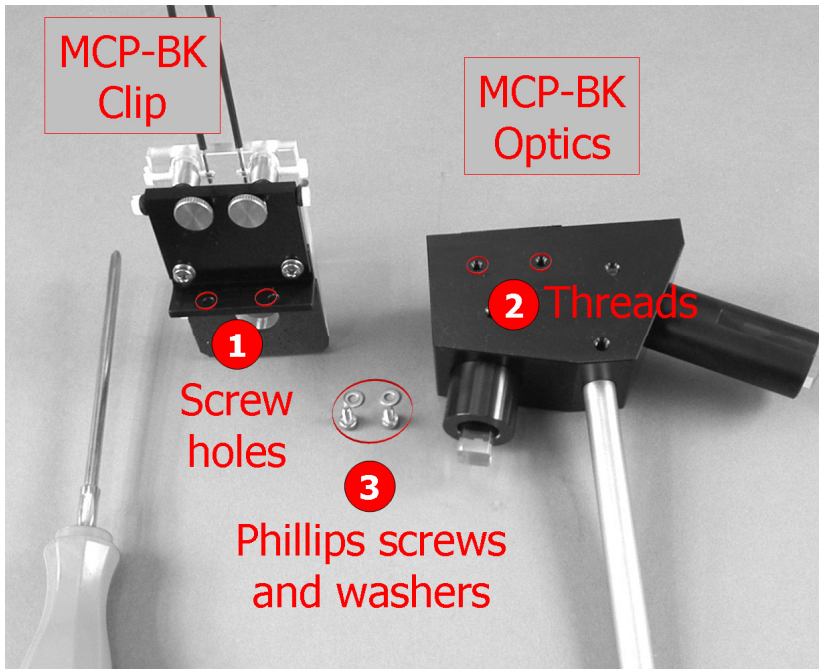


Fig. 7: Assembly of MCP-BK Optical Unit.

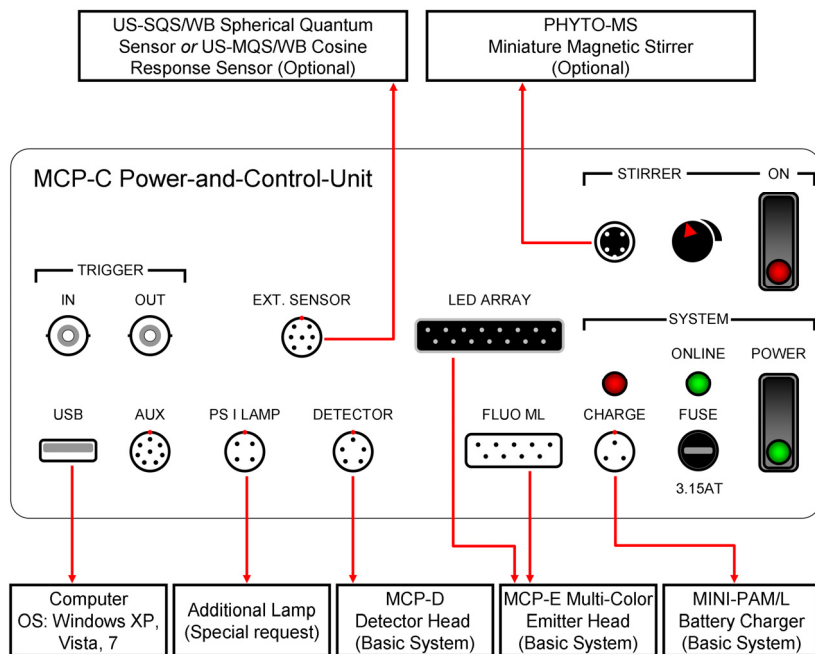
#### 4.3.6 US-MQS/WB Mini Quantum Sensor

The US-MQS/WB combines the cosine response sensor, MQS-B, and a small amplifier unit specifically adjusted to this MQS-B sensor. When connected to the external sensor socket (EXT. SENSOR) of the central unit, the PamWin-3 displays correct PAR readings for a “Calibration factor for PAR” of 1.000. The sensor fits the opening of the measuring point of the MCP-BK Optical Unit.

**Note:** Do not force a plug into the wrong socket. When round sockets are red marked, orientate plug so that the red dot on the plug coincides with the red mark of the socket. Do not try to disconnect a plug by pulling at the cable. Disconnect round plugs by pulling at the rippled metal part.

#### 4.4 Setup of Components

- Choose smooth surface in dry environment and not directly exposed to sun light or strong flickering light sources like fluorescent lamps.
- Assemble and position stand with wooden base plate, mount ED-101US/MD Optical Unit for Suspensions or MCP-BK Optical Unit for Leaf Measurements on stand, carefully attach emitter and detector heads on optical unit. Plastic screws should be fastened finger-tight only.
- Insert appropriate filter in filter holder of detector unit (normally just RG665 for dilute suspensions and RG665+SP710 for leaves or other samples at high Chl content).
- Without exerting force, optimize light paths by minimizing gaps in the light paths defined by Perspex rods.
- Insert miniature magnetic stirrer (PHYTO-MS) via the bottom port.
- Connect peripheral devices to central unit as shown in Fig. 8 (page 26).



**Fig. 8: Connections between central unit and peripheral components of the MULTI-COLOR-PAM.**

## 4.5 PamWin-3 Software Installation

Depending on the type of CD-ROM delivered, start with **a)** or **b)**.

- a) CD contains **only the setup file** (e. g. PamWin\_setup.exe), and the PDF copy of the present manual.

Close all programs, double click on the setup file and follow instructions. The setup routine will create in the C: root directory the folder PamWin\_3 containing the PAM\_WIN.exe program and accessory files. Further, an icon representing a shortcut to PamWin-3 is placed on the computer desktop, and a program group called “PamWin-3” is created in the program list available in the start menu. The PamWin-3 program group includes the uninstall command for PamWin-3.

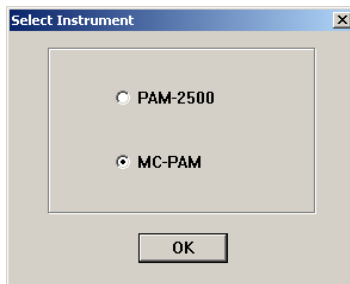
- b) Your CD is entitled “Software & Manuals” and contains a **complete collection of Walz Software & Manuals**. The CD in the CD drive automatically starts the default internet browser of your computer. If automatic browser start fails, double-click on file “index.html” in the root directory of the CD.

The browser loads the Walz homepage from the CD. Click “All Products” in the title bar of the homepage, select PAM-2500 or MULTI-COLOR-PAM (the same software controls both devices).

Close all programs and install PamWin-3 by clicking on “EXE-File”. Alternatively, you can copy the software PamWin-3 to any storage medium and use the copied

file for installation. During the installation process, icons and shortcuts are created as described above.

- c) Connect MULTI-COLOR-PAM to computer via USB-cable, switch Power on and with minimal delay of about 5 s start PamWin-3 by clicking on the “PamWin\_3” icon. If the fluorometer is not switched on or connected, PamWin-3 will ask for the offline mode of operation. To view and analyze data acquired by the MULTI-COLOR-PAM, select “MC-PAM” on the window “Select Instrument”.



#### 4.6 Software Update.

The PamWin-3 software is continuously improved. Therefore, check if the Walz web site provides newer software than that delivered with your instrument. PamWin-3 software is available under the link:

[http://www.walz.com/products/chl\\_p700/pam-2500/downloads.html](http://www.walz.com/products/chl_p700/pam-2500/downloads.html)

#### 4.7 Firmware Update.

After software update, also update firmware. (Firmware is the software residing on instrument RISC processors). The MULTI-COLOR-PAM contains 2 consecutively programmable processors: RISC and TINY, a tiny RISC processor. Proceed as follows:

- Connect MULTI-COLOR-PAM to computer and start PamWin-3.

### RISC processor update

- In the menu bar, select “Service” and “Controller Service”.
- Sequentially click  →  → . The download might require some time.
- The actual process of programming is initiated by clicking the  and then the  key.

**Note:** Stable power supply during firmware update is mandatory. Power failure can lead to malfunction of the processors, which requires re-programming at Walz.

- Finally, terminate the procedure by a mouse click on  in the “Program RISC” window, confirm with  the information on program restart, and once again click  in the Controller Service window.
- Close and restart PamWin-3 software.

### TINY processor update

Programming the TINY processor is similar to the procedure explained for the RISC processor.

- In the menu bar, select “Service” and “Controller Service”.
- Sequentially click on  →  → .

- The actual process of programming is initiated by clicking the  and then the  key.
- Terminate the procedure by a mouse click on  in the “Program RISC” window, confirm with  the information on program restart, and once again click  in the Controller Service window.
- Close and restart PamWin-3 software.



## 5 SP-Analysis

The PamWin-3 software offers two different modes of online operation: “SP-Analysis” and “Fast Acquisition”. In addition, the “VIEW-mode” gives access to all data recorded, including fast kinetics of saturation pulse analysis, and provides various tools for data analysis and file management.

“SP-Analysis” includes classical saturation pulse analysis and routines for medium-term to long-term experiments like dark-light induction curves or light response curves. Emphasis of “Fast Acquisition” is on the recording of kinetic changes controlled by fast trigger files. With the exception of  $F_0$  and  $F_m$  determinations and calculation of  $F_v/F_m$ , “Fast Acquisition” does not automatically determine fluorescence ratio parameters.

### 5.1 Online Help (F1 or @)

All program levels of the PamWin-3 software offer online help texts with information on essentially all active user surface elements. To access the online Help-texts:

- Move mouse cursor on the window element of interest until a small tag (“Tooltip”) appears.
- A “Tooltip” remains visible for 2.5 seconds. During this interval, pressing the **F1** or **@** key results in the display of the relevant help text.
- In some cases extensive help texts can be accessed via special “Help” buttons.

## 5.2 Peripheral Bars

The “General Settings” window appears first after program start. The title, menu, side, and bottom bars shown in Fig. 9 (page 32) are not unique to the window “General Settings” but are present in all windows of PamWin-3. Properties and function these peripheral panels will be described in this Chapter.

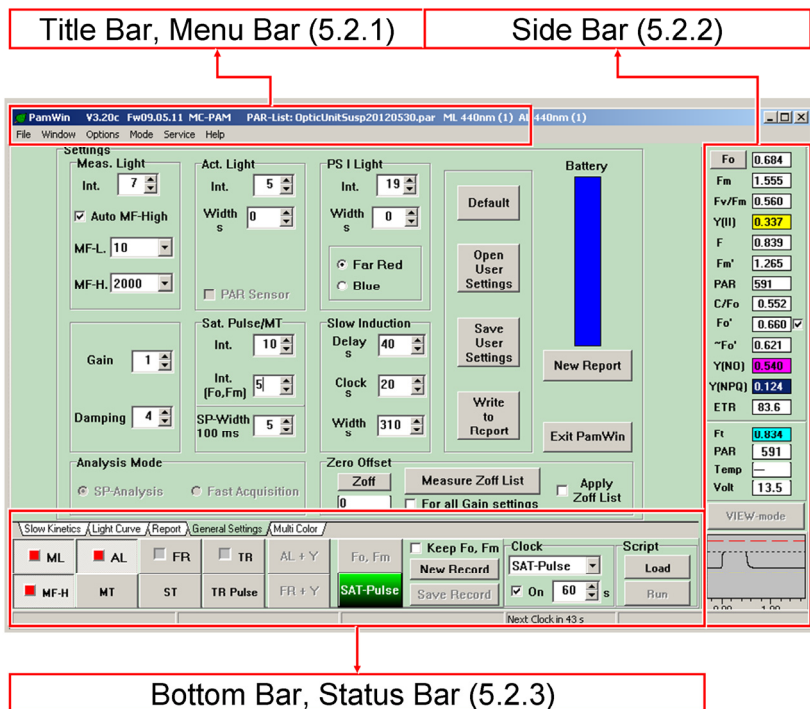
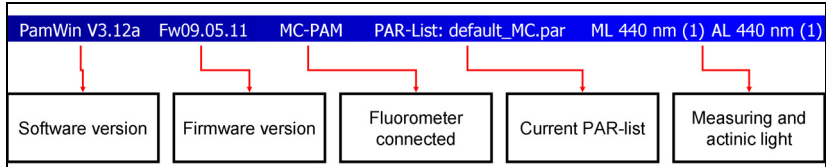


Fig. 9: Peripheral bars.

## 5.2.1 Title Bar, Menu Bar

Information provided by the title bar is sketched below:



The menu bar of the General Settings window contains 6 menus:



The list of items in the File menu varies depending on the window chosen. For example, the “Print Graph” command is only available together with Slow Kinetics or Light Curve windows.

### File Menu

#### New Report



This command creates a new Report and can also be executed via the “New Report” button in the Report window.

A Report contains slow and fast fluorescence kinetics, and comments added by the user. The Report file name is automatically created using the format is YYYYMMDD\_# (Y: year, M: month, D: day, #: order number of Report).

File menu

- New Report
- Import Old Report
- Load Script
- Run Script
- Printer Setup
- Print Graph
- Exit

For example, automatic naming would produce “20120317\_3” for the third Report started on March 17, 2012. The Report “20120317\_3” is stored in the directory named: C:\PamWin\_3\Data\_2500\Report\20120317\_3 which contains the Report data file 20120317\_3.RPT3 together with fluorescence data and comments.

Modifications of a Report name are possible but the names of Report directory must be identical to that of the Report file.

### **Import Old Report**

The command imports data saved in the previous Report file format \*.RPT.

### **Load Script**

This menu item is also accessible via the “Load” button on the bottom bar. The first execution after program start of “Load Script” opens the PamWin-3 default directory for script files from which files can be imported by double-click on file name, or by single-click on file name followed by the  command. A new script file can be started by clicking on  followed by entering the new file name. Second and later executions of “Load Script” open the window for script file editing and script file management.

### **Run Script**

Executes the presently loaded script file. The command can also be triggered by the “Run” button on the Bottom-bar.

### **Printer Setup**

Opens the Windows printer dialogue.

**Print Graph** (only Slow Kinetics and Light Curve windows)

Sends a black and white version of the current graph to the selected printer.

**Print Report** (only Report window)

Prints the current Report on the selected printer

**Exit**

Saves current Report and kinetics data, writes current settings in a configuration file (INI File), and shuts down the PamWin-3 software. The same command can be triggered by the “Exit” button (General Settings) and by the “Close” button in the upper right corner of all PamWin-3 windows.

**Window Menu**

The Window menu lists all windows currently available. The menu items vary depending on the acquisition mode selected (SP-Analysis or Fast Acquisition).

SP-Analysis	Fast Acquisition
Slow Kinetics	Fast Kinetics
Light Curve	Report
Report	Fast Settings
General Settings	General Settings
Multi Color	Multi Color

## Options Menu

---

### L Curve Fit Parameters

This menu item leads to the “Light Curve Fit” window which displays four data:

The **Fv/Fm x ETR factor** is the uppermost value, where Fv/Fm corresponds to the maximal quantum yield of charge separation at

PS II reaction centers and the ETR factor is the fraction of light absorbed by the sample and distributed to PS II (default value for leaves is normally  $0.84/2=0.42$ ), with the assumption that excitation energy is equally distributed between photosystems I and II. For the same sample, the “Fv/Fm x ETR factor” is close to alpha, the initial slope of the light response curve, because

$$\alpha = \frac{ETR}{PAR} = \frac{Y(II) \cdot ETR \text{ factor} \cdot PAR}{PAR} = Y(II) \cdot ETR \text{ factor}$$

and Y(II) approaches Fv/Fm at very low PAR.

The other three parameters (alpha, ETRmax,  $I_k$ ) are so-called cardinal points of a light response curve (see below). These parameters results from fitting a theoretical equation to the measured data. PamWin-3 offers two different model equations for light response curves:

**Model EP.** Eilers PHC, Peeters JCH (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol Model* 42: 199-215

Options Menu

L Curve Fit Parameters

Light calibration

Light offset

Temperature offset

ETR Factor

AL Current/PAR Lists

**Model Platt et al.** Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J Mar Res* 38: 687-701

The MULTI-COLOR-PAM permits light-response curves on the basis of the quantum flux density of illumination, PAR (in units of  $\mu\text{mol photons}/(\text{m}^2 \cdot \text{s})$ ), or on the basis of PAR(II) which is the PAR absorbed by PS II in units of quanta/(PS II·s). When the independent variable is PAR, the units of the dependent variable, ETR, is  $\mu\text{mole electrons}/(\text{m}^2 \cdot \text{s})$ . For PAR(II) as the independent variable, the dependent variable ETR(II) has the unit of  $\mu\text{mole electrons}/(\text{PS II} \cdot \text{s})$ . The units of two of the three cardinal points are affected by the type of X and Y data.

Equation “ $\text{PAR(II)} = \text{Sigma(II)} \cdot \text{L} \cdot \text{PAR}$ ” relates PAR to PAR(II). In the latter equation, Sigma(II) is the wavelength-dependent functional absorption cross-section of PS II, and L stands for the Avogadro constant. The Sigma(II) can be evaluated for the same sample by analysis of the O-I<sub>1</sub> fluorescence rise kinetics in the fast acquisition mode (Section 6.3, page 96).

The three cardinal points of a light response curve are:

**alpha** Initial slope of light response curve which is related to the maximum yield for PS II electron transport. Unit: electrons/quanta

**ETRmax (or ETR(II)max)**

Maximum electron transport rate.

Unit:  $\mu\text{mole electrons}/(\text{m}^2 \cdot \text{s})$  (or electrons/(PS II·s))

**I<sub>k</sub> (or I<sub>k</sub> (II))**

PAR (or PAR(II)) at the point of intersection between the ETRmax line and the extrapolated initial slope.

Computationally, the I<sub>k</sub> results from ETRmax/alpha.

The I<sub>k</sub> may be considered as the PAR (or PAR(II)) at

which light-saturation of photosynthesis sets in.

Unit:  $\mu\text{mol quanta}(\text{m}^2\cdot\text{s})$  (or  $\text{quanta}/(\text{PS II}\cdot\text{s})$ )

### **Light Calibration**

For normal operation of the Walz quantum sensors (the spherical US-SQS/WB or the cosine-response type US-MQS/WB), the default calibration factor for PAR of 1.000 applies. The calibration factor needs to be adjusted for other sensors. Valid factors are in the range from 0.200 to 5.000

### **Light Offset**

The US-SQS/WB and the US-MQS/WB quantum sensors run at two ranges of signal amplification. The Auto Zero function establishes automatically the correct offset values for both ranges. Determination of light offset requires that the PAR sensor is kept in the dark. Also, offset values can be entered manually.

### **Temperature Offset**

“Temperature Offset” adjusts the offset value of an external temperature sensor (offset range is -30 to +30).

### **ETR Factor**

The ETR factor corresponds to the fraction of incident photons absorbed by PS II pigments. The factor is required to derive estimates of electron transport rates from the effective quantum yield of PS II yield,  $Y(\text{II})$ . For leaves, the default value for the ETR factor is  $0.84/2$ . The numerator 0.84 corresponds to the average fraction of absorbed light in the visible range (400-700 nm) of many green leaves, the denominator 2 assumes that absorbed light is equally distributed between PS I and PS II. For suspensions, the leaf ETR factor does not apply, as light absorption depends strongly on cell densities and light



color. Furthermore, PS I/PS II absorption ratios may vary substantially between different types of algae and cyanobacteria.

In dilute suspensions, electron transport rates per PS II can be determined according to Schreiber *et al.* (2011)\* by:

\*<http://www.walz.com/downloads/pan/PAN11001.pdf>

- (1) Deriving PS II specific absorption,  $\text{Sigma(II)}$ , from O-I<sub>1</sub> fluorescence rise kinetics of the dark-acclimated sample.
- (2) Calculating the rate of quanta absorption by PS II,  $\text{PAR(II)}$ , from incident PAR and  $\text{Sigma(II)}$ 

$$\text{PAR(II)} = \text{Sigma(II)} \cdot L \cdot \text{PAR}$$
 where L is the Avogadro constant
- (3) Calculating PS II-specific ETR in the illuminated state by considering lowering of effective quantum yield,  $\text{Y(II)}$ , with respect to the dark  $\text{Fv/Fm}$ :




$$\text{ETR(II)} = \text{PAR(II)} \cdot \text{Y(II)} / (\text{Fv/Fm})$$

Step by step instruction for determination of  $\text{ETR(II)}$  via fast kinetics measurements of the so-called O-I<sub>1</sub> rise and for transformation of PAR into  $\text{PAR(II)}$  are given in the URL above and in the Help texts of PamWin-3 for Calc/O-I<sub>1</sub> rise, Open/Close sigma File and  $\text{PAR(II)}$ .

### **AL Current/PAR Lists**

A full set of PAR lists contains data of photosynthetically active radiation, PAR ( $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), for all colors and intensity settings of actinic illuminations (AL) and multiple turnover flashes (MT), as well as of the blue light from the optional PS I lamp. Far red intensities of the MCP-E emitter head and the optional PS I lamp depend on current values of the same "PS1 far red" list.






Because most of emission of far-red LEDs (Fig. 3 page 17) is outside the photosynthetically active spectral range (~400 nm - ~700 nm), the far red list does not indicate PAR values.

Default PAR lists are provided for setups of suspension and leaf measurements (default\_leaf.par and default\_MC.par, respectively). In the PAR list window, clicking on the comment icon (  ) opens information on the measuring protocol used to create a particular set of PAR list. Sets of PAR lists can be saved as "PAR file" (click: ). A previously stored PAR file can be opened and used as the current set of PAR lists via the "folder icon" (  ).

Each PAR list consists of three columns. The first column, "Settings", is equivalent to the positions of a multistate switch. The setting number does not have a physical meaning. The second column, "current", is a relative scale for the current flow through the LEDs associated with the current PAR list. LED current can be regulated at 8 bit resolution that is the number of 255 corresponds to 100% of the maximum allowed current. The third column, "PAR", lists the PAR measured with the corresponding LED current, except at setting "0" where the actinic current is set to zero, and the PAR corresponds to measuring light intensity at setting 10 and 100 KHz modulation frequency.

When the PAR sensor is not connected to the MULTI-COLOR-PAM, or the PAR sensor is logged off at software level (General Settings), the actual PAR is automatically computed as the sum of actinic PAR plus the PAR of measuring light at the present intensity and frequency settings. The dependence of PAR on setting is described by color-specific 3. order polynoms implicated in the program.

Table 1: Schematic Representation of PAR List Window.

default_MC.par			
			
Open PAR file	Save PAR file	Show comment	Show help text
<b>AL color 1</b>			
PAR Lists - Drop Down Menu			
Setting	Current	PAR	
0	0	577	
1	1	7	
20	255	2765	

Default PAR-lists represent typical data which may deviate from the PAR values of your particular MULTI-COLOR-PAM. The user is responsible for updating the PAR-lists of his instrument which is most conveniently done with a Walz PAR sensor (US-SQS/WB or US-MQS/WB) connected to the MULTI-COLOR-PAM using the “Measure PAR Lists” routine available in the Multi-Color window.

PAR lists can be edited manually using other PAR sensors. Editing of PAR values requires highlighting the field to be edited by mouse click, and typing in the new number. Make sure that PAR of measuring light (the PAR at setting “0”) is determined under standard conditions, that is, intensity setting 10 and 100 KHZ modulation frequency. For further information on

PAR lists, see the Help text under Options → AL Current/PAR Lists.

### S+H-Damping

S+H-Damping allows to adjust the response characteristic of the “sample and hold” circuit of the system. This circuit keeps the original analog signal for a short time for digitization. When “Automatic” is chosen, damping setting D1 is used in the “Fast Kinetics” mode, and damping setting D2 in the “SP-Analysis” mode. The response time of the system depends not only on the damping setting (D1-D4) but also on measuring frequency. Table 2 shows time constants for instrument response after a step input for all S+H damping settings and the three highest modulation frequencies.

**Table 2: Time constants ( $\tau$ ) of system response for sample and hold damping settings D1 to D4, and various modulation frequencies of measuring light (50 to 200 kHz). Damping<sub>General Settings</sub> = 1**

Damping	D1	D2	D3	D4
Frequency	$\tau$	$\tau$	$\tau$	$\tau$
50 kHz	24 $\mu$ s	252 $\mu$ s	809 $\mu$ s	1012 $\mu$ s
100 kHz	18 $\mu$ s	131 $\mu$ s	424 $\mu$ s	520 $\mu$ s
200 kHz	15 $\mu$ s	68 $\mu$ s	213 $\mu$ s	261 $\mu$ s

## **Kinetics auto save**

When “Kinetics auto save” is active, all kinetics acquired in the Fast Acquisition mode are saved to hard disk immediately.

## **Mode Menu**

---

The two items of the menu, MEASURE and VIEW, are equivalent to the buttons  /  in “General Settings”. The button “VIEW-mode” is inactive during slow fluorescence measurements.

## **Service Menu**

---

### **Controller Service**

See Section 4.7, page 28.

### **Read Firmware Version**

Retrieves firmware version. Same information as in title bar (see Section 5.2.1, page 33).

### **Trigger out with SP**

When checked, a 5 Volts trigger pulse is applied to the AUX socket (see Section 4.2.1, page 12). The pulse length is adjusted in the window “Pulse widths”. To access this window, right-click on MT, ST or TR buttons.

## **Help Menu**

---

### **Tooltips**

“Tooltips” (checked by default) enables help tags. Type F1 or @ to read the help text associated with the tag.

### **Info**

Displays version number of current PamWin-3.

## 5.2.2 Side Bar

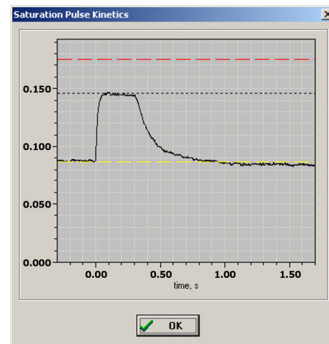
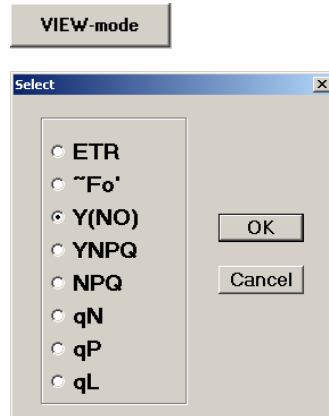
<b>Fo</b>	0.354	<p>The upper 13 types of data (Fo to ETR) are related to saturation pulse analyses, except when a <b>Fo</b> or <b>Fo'</b> measurement is triggered by the respective button. The Fo and Fm fluorescence levels should be measured with a quasi-dark-acclimated sample (i.e. in the case of algae and cyanobacteria preferentially in presence of weak far-red background light), the F, Fm' and Fo' after light exposure of the same sample. The Fo' checkbox must be ticked to activate the Fo' routine consisting of 5 s of far-red illumination and determination of Fo' as the minimum fluorescence during this period. The data of ~Fo' are estimates for Fo' derived from Fo, Fm and Fm' according to Oxborough and Baker (1997, Photosynth Res 54: 135-142). The C/Fo quantifies the contribution of a constant fluorescence component (e.g. phycocyanin and/or PS I fluorescence) to total Fo fluorescence. <math>Fv/Fm=(Fm-Fo)/Fm</math> is the maximum and <math>Y(II)=(Fm'-F)/Fm'</math> is the effective photochemical quantum yield of PS II. Detailed information on fluorescence ratio parameters are compiled (see Section 9.2, page 133).</p>	
<b>Fm</b>	1.674		
<b>Fv/Fm</b>	0.789		
<b>Y(II)</b>	0.229		
<b>F</b>	0.590		
<b>Fm'</b>	0.765		
<b>PAR</b>	224		
<b>C/Fo</b>	0.210		
<b>Fo'</b>	0.313		<input type="checkbox"/>
<b>~Fo'</b>	0.283		
<b>Y(NO)</b>	0.352		
<b>Y(NPQ)</b>	0.419		
<b>ETR</b>	21.5		
<b>Ft</b>	0.638	<p>The lower section of the side bar (Ft to Volt) Reports continuously the Ft (current fluorescence level), the PAR and the battery voltage. In the standard setup of the MULTI-COLOR-PAM, a temperature sensor is not connected.</p>	
<b>PAR</b>	227		
<b>Temp</b>	--		
<b>Volt</b>	13.1		

Note that values of yield ( $Y(II)$ ,  $Y(NO)$  and  $Y(NPQ)$ ) are highlighted to underline that the sum of these three parameters equals unity (Kramer et al. (2004) Photosynth Res 79: 209-218)

The “VIEW-mode” button quits communication with the MULTI-COLOR-PAM and enters the “View-mode” for off-line data analysis.

The type of parameter displayed in the four rows below the  $F_o'$  line can be customized as follows. Double-click in one of the numerical fields to view all 8 parameters available for this section. Select a parameter and click OK.

The fluorescence kinetics triggered by the last saturation pulse is displayed in a miniature panel at the bottom of the side bar. The panel indicates the levels of  $F_m$ ,  $F_m'$  and  $F_o$  fluorescence as dashed red, black and yellow lines, respectively. Double-click on the miniature panel to enlarge the graph. Ordinate of the enlarged graph is original voltage scale of the fluorescence signal.



### 5.2.3 Bottom Bar



**ML**

On/off switch for modulated measuring light, ML. PAM fluorometers measure selectively the modulated fluorescence signal induced by ML.



**ML**

The red square indicates that ML is switched on. Likewise, a red square signifies the on-state for functions MF-H to TR (see below).



**MF-H**

On/off switch for high modulation frequency of measuring light (MF-H). When “Auto MF-High” is active (General Settings window), actinic illumination switches MF-H on automatically.



**AL**

On/off switches for actinic light (AL), far-red light (FR) and 5 V trigger signal applied to AUX socket (TR. See 4.2.1, page 12, for pin assignments). Intensity and duration of illumination is defined in “General Settings”. When the “Width” is set to zero, illumination must be switched off manually. The far red predominantly excites PS I in higher plants and algae. In cyanobacteria, blue is frequently used for PS I-selective excitation. In the presence of an extra blue lamp, right-click on the “FR” button changes to blue as the PS I light.



**FR**



**TR**

**MT**

Trigger for multiple turnover (MT) and single turnover light pulses (ST), as well as for 5V pulses applied to the AUX socket (ST Pulse).

**ST**

**TR Pulse**

Pointing the mouse cursor at one of the buttons displays the current pulse length. The color of MT and ST is identical to the settings for actinic



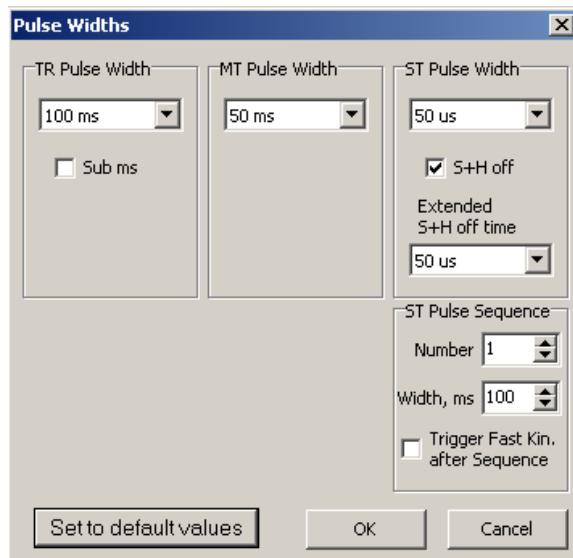
light. MT intensity equals the saturation pulse intensity and ST intensity is always maximal. To edit pulse lengths, open the window “Pulse Widths” by right-click one of the pulse buttons.

---

## TR, MT, ST Pulse Widths

Lengths of trigger, multiple turnover and single turnover pulses can be adjusted via drop down lists. For “TR Pulse Width”, two drop down lists are provided: one offers pulse lengths > 1 ms, the other covers shorter intervals and is available when the “Sub ms” box is checked. The settings for pulse width made here are active in the mode “SP-Analysis”. They do not affect the trigger pattern in the Fast Acquisition mode).

Additional options are available for single turnover flashes. To suppress false signals caused by fluorescence excited by the extremely strong single turnover pulses, the sample and hold circuit is switched off during a flash. Thereafter, the sample



and hold circuit will stay switched off for the time interval selected from the “Extended S+H off time” list when “S+H off” is checked. A prolonged off phase may be useful for removing artifacts in some applications.

Also, sequences of up to 20 single turnover flashes with inter-flash times (widths) up to 500 ms can be specified in the field “ST Pulse Sequence”. Flash sequences are set off in the “Fast Acquisition” mode by mouse click at the ST button. When “Trigger Fast Kinetics after Sequence” is selected, the currently active trigger pattern (FTM-file) is executed after the pulse sequence with a delay corresponding to the width setting.

**AL + Y****AL + F.K.**

The AL + Y command first actinically illuminates the sample and then performs a saturation pulse analysis. Intensity and time interval of actinic illumination is defined under “General Settings”. For intervals < 3 s, the AL + Y command is unavailable. In the mode “Fast Acquisition”, the AL + Y is replaced by the “AL + F.K.” which executes the active trigger pattern (FTM-file) after actinic illumination.

**FR + Y****FR + F.K.****BL + Y****BL + F.K.**

The FR + Y command is in all aspects similar to the AL + Y command except actinic is replaced by far red preillumination (FR), or more generally spoken by PS I light preillumination. In total 4 commands are associated with this button, depending on settings: the status of the FR/Blue switch (see above) determines the light color used for preillumination. The measuring mode, SP-Analysis or Fast Acquisition

tion, determines if saturation pulse analysis or fast kinetics is performed after preillumination. For use of blue PS I light the optional PS I lamp is required.

**Fo, Fm**  
**SAT-Pulse**

The Fo, Fm function measures minimum fluorescence yield (Fo) with quasi-dark-acclimated sample at low measuring light frequency prior to application of a saturation pulse to elicit maximum fluorescence (Fm). From these two parameters, the Fv/Fm (maximum photochemical quantum yield of PS II) is calculated. In algae and cyanobacteria “dark”-acclimation to weak far-red background light is recommended to establish state 1, characterized by maximal values of Fv/Fm.

Sat-Pulse triggers a saturation pulse analysis for light exposed samples. The analysis starts with determination of F, which is the fluorescence level just before Fm' is elicited by a saturation pulse. If Fo' mode on the side bar is checked, the saturation pulse is followed by 5 s of illumination with PS I light (far red) to determine the Fo'. The Fm' and F fluorescence, is sufficient to calculate Y(II), the effective photochemical quantum yield of PS II.

**Script**  
**Load**  
**Run**

The script file button “Load” opens the default directory for PamWin-3 script files. Script files stored in this directory or elsewhere can be opened. Pressing the “Cancel” button leads to the blank script file window in which a new script file can be created. To execute a script

file, close script file window and then click “Run”. Details on script files are outlined (Chapter 1, page 111).

## Status Bar



The status bar displays information on Fast Kinetics trigger patterns (FTM-files), Clock count-down and Script files. The response measured with the last executed FTM-file is displayed in the Fast Acquisition mode under Fast Kinetics. The Clock count-down indicates the time left until the next Clock-triggered event.

## 5.3 General Settings

In the General Settings window, adjustments can be made in different ways:

- The modulation frequency of measuring light is selected from a drop down menu.
- All other parameters can be adjusted by mouse click via the pair of upward/downward arrows. Alternatively (and in most cases preferentially), double-click on the number to be adjusted opens a small window in which the new setting can be typed in.

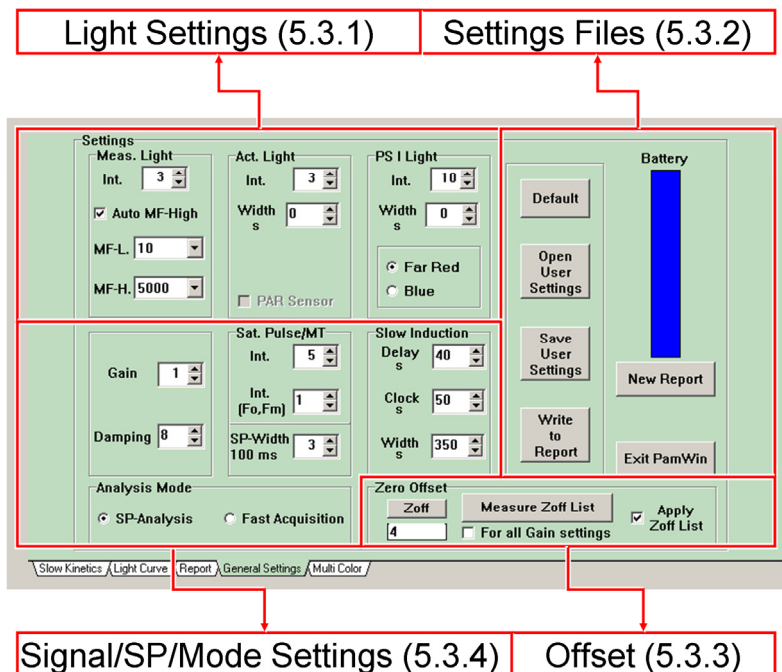


Fig. 10: General Settings.

### 5.3.1 Light Settings

#### Measuring light (Meas. Light)

This section permits adjustments of intensity (Int., 20 levels), low pulse modulation frequency (MF-L., 10 to 5000 Hz) and high pulse modulation frequency (MF-H., 1 kHz to 100 kHz) of measuring light. Additionally, measuring light can be modulated at a frequency of 200 kHz (“MF-max”) in the “Fast Acquisition” mode. Factory values for the intensity of measuring light at intensity level 10 and frequency 100 kHz are written in the

first line of the respective PAR lists (see 4.2.1). When Auto MF-high is selected, high pulse modulation frequency is always active when actinic light is on. Basically, increasing the modulation frequency improves time resolution and signal/noise ratio. At the same time, however, the actinic effect of the ML is increased. Therefore, low frequency measuring light must be used to correctly determine  $F_0$  and  $F_0'$  fluorescence.

### **Actinic light (Act. Light)**

Two parameters define actinic illumination: intensity (Int., 20 levels) 1 to 20) and duration (Width). Allowed widths range from 1 to 900 s. For Width=0, actinic light has to be switched off manually.

### **Actinic light for PS I excitation (PS I Light)**

The PS I light is adjusted as described above for actinic light. For  $F_0'$  detection, samples are illumination for 5 seconds with PS I light. This 5 s interval is not affected the "Width" adjusted here. The option of selecting blue as the PS I light is only available for setups with a special PS I lamp.



### **Read PAR from Sensor (PAR Sensor)**

In case that a PAR sensor is connected to the socket "EXT. SENSOR" of The MULTI-COLOR-PAM, the function "PAR Sensor" is automatically activated (corresponding box is checked = ). With the active PAR sensor, PAR lists are easily measured using the corresponding command in the "Multi Color" window of PamWin-3. Once PAR lists have been measured and saved, "PAR Sensor" can be unchecked and PAR values are then derived from the internal PAR lists.

### 5.3.2 Settings File

<b>Default</b>	<p>The term “settings” means the sum of all current adjustments in PamWin-3. Most of adjustments can be saved by the command Save User Settings. By default, setting files are stored in directory C:\PamWin_3\User Settings, their file name format is Filename.DEF. The Zero offset value is not stored. ETR factor, offset values for light and temperature sensors, and calibration factor for the light sensor are stored in a configuration file which is read at each start of PamWin-3. The command Open User Settings recalls stored settings including the factory default settings for work with suspensions “Walz_MC.DEF”. Factory default settings are also invoked by the Default command. The Write to Report command creates a line describing current settings in the Report file (see overleaf). New Report saves the current Report and associated kinetics and begins a new Report.</p>
<b>Open User Settings</b>	
<b>Save User Settings</b>	
<b>Write to Report</b>	
<b>New Report</b>	

#### Battery

The blue bar indicator displays the state of charge of the MULTI-COLOR-PAM battery.

**Table 3: Documentation of Settings in the Report window**

Date	Time	No.	ML	Temp.	PAR	F	Fm'	Y(II)
06.12.11	15:41:48	FL	MI	G	AI	BI	FR/BL	
		10	3	2	6	4	FR	
		FH	Z	D	FI	SI	AL-Src	ML-Src
		10000	0	4	1	10	440 nm	440 nm
								SI(Fm)
								5
06.12.11	15:41:48	ML	MFH	MFLog	AL	FR	BL	
		On	Off	Off	Off	Off	Off	

**Abbreviations used in Report**

<b>FL</b>	<b>MI</b>	<b>G</b>	<b>AI</b>	<b>BI</b>	<b>FR/BL</b>		
<i>Low frequency of light intensity, Hz</i>	<i>Measuring light intensity</i>	<i>Signal amplification (Gain)</i>	<i>Actinic light intensity</i>	<i>Intensity of optional PS I lamp</i>	<i>PS I light selected</i>		
<b>FH</b>	<b>Z</b>	<b>D</b>	<b>FI</b>	<b>SI</b>	<b>AL-Src</b>	<b>ML-Src</b>	<b>SI(Fm)</b>
<i>High frequency measuring light, Hz</i>	<i>Fluorescence off-set (Zoff)</i>	<i>Signal damping</i>	<i>Far-red light intensity</i>	<i>Saturation pulse intensity</i>	<i>Actinic light source, nm</i>	<i>Measuring light source, nm</i>	<i>Saturation pulse intensity for Fo, Fm determination</i>
<b>ML</b>	<b>MFH</b>	<b>MFLog</b>		<b>AL</b>	<b>FR</b>	<b>BL</b>	
<i>On / Off Information on status of:</i>							
<i>Measuring light</i>	<i>High frequency of measuring light</i>	<i>Logarithmic frequency decrease of measuring light (Fast settings)</i>		<i>Actinic light</i>	<i>Far-red light</i>	<i>Blue light from optional PS I lamp</i>	

**5.3.3 Offset**

The Zoff (Zero offset) and Measure Zoff List commands determine background signals for subtraction from the total signal. Background signals must possess the modulation characteristics of measuring light to be recognized by the differential amplifier. These signals can arise from:



- Fluorescence from suspension media or detector filter excited by measuring light.
- Traces of modulated excitation light transmitted by the detector filter.
- Non-optical modulated "electronic noise".

The background signals are different for the various colors of measuring light, and increase almost linearly with measuring light intensity and gain factor.

**Note:** All measuring conditions must be identical for Zoff determination and sample investigation.

To determine the zero offset for experiments with suspensions, place a cuvette containing pure medium in the optical unit ED-101US/MD, and select one of the three options for zero offset determination (see Table 4, page 55).

**Table 4: Three Levels of Zero Offset Determination for Fluorescence Measurements.**

Command:	Zoff	Measure Zoff List	Measure Zoff List
Measuring Light Intensity	Current	All	All
Measuring Light Color	Current	All	All
Gain	Current	Current	All

The time required for zero offset determination increases with the number of measurements and is highest when all possible adjustments are considered ("Measure Zoff List" + "For all

Gain Settings”). For experiments with constant measuring light and gain, the simple “Zoff” command can be used. The “Zoff” command also allows entering manually a value for zero offset; in this way, the zero offset value can be also reset to zero.

The Zoff Lists are saved as Data\_MC/Zero\_Offset\_Col.ini in folder C:\PamWin\_3\Data\_MC.

Offset lists need to be activated by checking “Apply Zoff List”. Then, the appropriate offset is subtracted from the measured signal. Offset subtraction is inactive when measuring light is switched off.

### **5.3.4 Signal/SP/Mode Settings**

#### **Gain**

The setting of “Gain” determines the amplification factor of the fluorescence signal but also of electronic noise. Therefore, improving the fluorescence signal by increasing measuring light intensity should be considered in case of low signal/noise ratios. Even very high measuring light intensities do not have much actinic effect at modulation frequencies of 100 Hz or lower. Increasing Gain also amplifies the background signal which requires redetermination of zero offset (Zoff) except a complete background determination has been performed.

#### **Damping**

The adjustment of “Damping” is one factor determining the system’s speed of response to fluorescence changes. The overall system response also depends on the setting for the damping of the sample and hold circuit and the modulation frequency of measuring light. Table 5 (page 57) lists response

time measured with sample and hold damping set to D2 (default for slow kinetics when *automatic* sample and hold damping is selected), and 10 and 100 000 Hz pulse modulation frequency.

Table 5 shows that at 10 Hz modulation frequency, response is independent of the damping. The sluggish response is determined by the low modulation frequency. At 100000 Hz, the system response is affected at damping setting 5 and higher.

**Table 5: Gain Factors and Time Constants at Two Modulation Frequencies and S+H-Damping = D2.**

Gain setting	Gain factor	Damping setting	Time constant ( $\tau$ ) at 10 Hz modulation frequency	Time constant ( $\tau$ ) at 100000 Hz modulation frequency
1	1.0	1	1210 ms	0.13 ms
2	2.0	2	1220 ms	0.13 ms
3	2.7	3	1180 ms	0.14 ms
4	3.4	4	1200 ms	0.18 ms
5	4.2	5	1040 ms	0.29 ms
6	5.1	6	1230 ms	0.60 ms
7	5.8	7	1210 ms	1.66 ms
8	7.0	8	1270 ms	4.76 ms
9	7.7			
10	8.6			

### **Sat. Pulse/MT**

Saturation pulses (Sat. Pulse) are applied for quenching analysis and multiple turnover pulses (MT) are typically used to induce fast fluorescence changes in the Fast Acquisition mode. The intensity of both is adjusted in the "Int." field. An exceptional case is the intensity of the saturation pulse used for  $F_o$ ,  $F_m$  determination which is adjusted in the "Int. ( $F_o, F_m$ )" field. The latter feature is useful for samples which exhibit high  $F_m$  fluorescence only at reduced saturation pulse intensities but require strong saturation pulses to elicit  $F_m'$  fluorescence under actinic illumination.

The duration of saturation pulses is adjusted by "SP-Width". Time intervals between 100 and 800 ms are available. The length of multiple turnover pulses is adjusted in the Pulse Width window which opens by right-click on the MT button of the bottom bar.

### **Slow Induction**

PamWin-3 defines the time course of long-term (slow) fluorescence induction curves by three parameters: (1) Delay, (2) Clock, and (3) Width. A slow induction curve is started by selection of Ind.Curve in the drop down menu of the slow kinetics window, followed by pressing the Start button. The definitions of the three parameters are:

Delay, s determines the dark interval between the initial  $F_v/F_m$  determination and onset of actinic illumination. The default interval is 40 s which normally assures that the preillumination effect of the saturation pulse is relaxed.

Clock, s defines the interval between two consecutive saturation pulses during actinic illumination. The default interval is 20 s which normally allows evaluating the kinetic changes of satu-

ration pulse data with negligible effects of the saturation pulses.

Width, s is the total time interval of the slow induction curve. Width assumes only distinct values which are defined by

$$\text{Width} = \text{Delay} + (n \cdot \text{Clock}) + 10$$

where n corresponds to the number of intervals between saturation pulses during actinic illumination. The extra 10 seconds result from 5 s prior to the first saturation pulse after start of illumination plus 5 s at the end of illumination. The total experimental time is 15 minutes longer when monitoring of fluorescence recovery has been activated by selecting the item (Ind.+Rec.) in the drop down menu of the slow kinetics window. Table 6 (page 59) lists the roughly exponentially increasing intervals between pulses during the fluorescence recovery phase.

### Exit PamWin

Saves current Report and kinetics data, writes current settings in a configuration file (INI file), and shuts down the PamWin-3 software.

### Analysis Mode

Field for selection of SP-Analysis or Fast Acquisition modes. The two modes are shortly characterized in Table 7, page 60.

**Table 6: Intervals between Saturation Pulses during Dark Recovery.**

Pulse number	Time interval
1	15 s
2	18 s
3	22 s
4	25 s
5	31 s
6	37 s
7	44 s
8	53 s
9	64 s
10	76 s
11	92 s
12	110 s
13	132 s
14	158 s
$\Sigma$ 877 s	
(15 min)	

**Table 7: Analysis Modes.**

	<b>Analysis Mode</b>	
	<input checked="" type="radio"/> <b>SP-Analysis</b>	<input checked="" type="radio"/> <b>Fast Acquisition</b>
Saturation pulse analysis	Photochemical and non-photochemical fluorescence quenching	Fv/Fm determination
Type of kinetic measurements	Slow (seconds to hours)	Fast ( $\mu$ s to seconds)
Automatic routines	Dark-light fluorescence induction and light response curves	Fast trigger files,
Script files	Yes	Yes

## 5.4 Slow Kinetics Window

This window displays slow fluorescence kinetics recorded in the SP-Analysis mode. View of the graph can be modified in four ways as outlined in Fig. 11 (page 61).

- Zoom in by clicking with the left mouse key on one corner of the rectangular area to be magnified, keeping the key depressed move to the opposite corner of the rectangle, and release key.
- Zoom out by right mouse click anywhere on the chart.
- Shift y-axis by moving the mouse cursor vertically with the shift key depressed. Shift x-axis by moving mouse cursor vertically with the control shift key depressed.

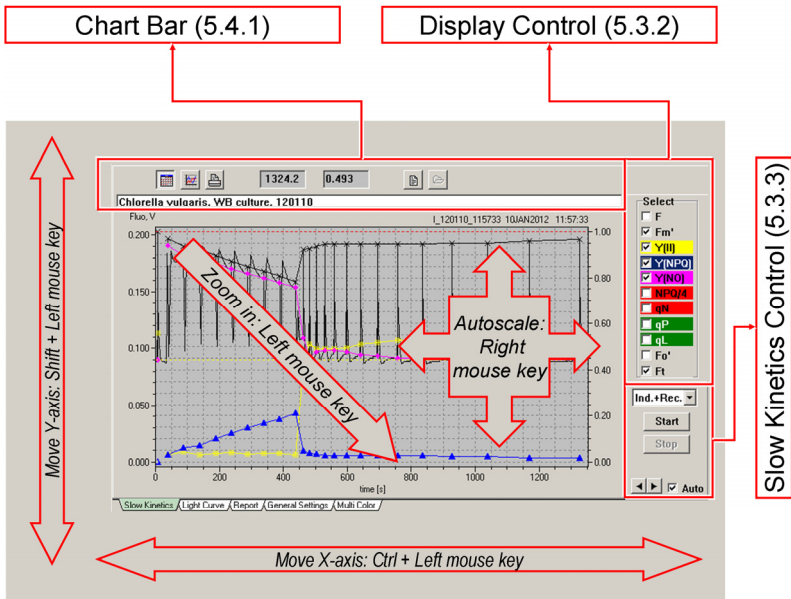







Fig. 11: Slow Kinetics Window.

### 5.4.1 Chart Bar

The chart bar displays the XY coordinates of the current pointer position and contains various buttons with the following functions:

	On/off switch for grid display.
	Zoom out: display of complete graph after zoom in. Equivalent to right mouse click on chart.
	Prints a black/white copy of the graph for documentation.
	Displays notes and comments associated with current slow kinetics.
	Opens a Slow Kinetics file for display. The icon is active only in the VIEW mode.

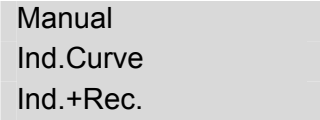
### 5.4.2 Display Control

Eleven different fluorescence parameters can be selected for display. The display is controlled by checkboxes. Data points and background colors of the corresponding checkbox labels have identical colors.



### 5.4.3 Slow Kinetics Control

The “Slow Kinetics Control” (cf. Fig. 11, page 61) provides a drop-down menu consisting of 3 items: “Manual” permits complete manual control of the experiment. “Ind.Curve” corresponds to slow dark-light fluorescence induction curve performed according to the settings in General Settings. When “Ind.+Rec” is selected, the induction curve is followed by a dark recovery phase. During recovery, saturation pulse analysis is conducted at increasing time intervals (see Table 6, page 59).



Manual  
Ind.Curve  
Ind.+Rec.

The  button sets off the selected type of slow kinetics, the  button terminates the experiment.

Recordings of dark-light induction curves automatically start with determination of  $F_0$  and  $F_m$  level fluorescence. Then, the left voltage scale is scaled to fully display the fluorescence signal. All fluorescence ratio parameters are plotted using the right Y axis which ranges from 0 to 1. In case of the non-photochemical quenching, values of NPQ/4 are displayed because the original data can assume values above 1 which would exceed the latter ordinate range. Manual recordings can be carried out without initial  $F_0$ ,  $F_m$  measurements. In this case, automatic scaling according to the  $F_m$  level does not occur.

The arrow keys in “Slow Kinetics Control” (Fig. 11, page 61) increase or decrease the x axis range.



Employing the arrow keys deactivates the “Auto” function which otherwise automatically adjusts the x axis for full display the fluorescence trace.

## 5.5 Light Curve Window

The Light Curve window provides the functional elements for control, display and analysis of light response curves. A light curve consists of a pre-programmed series of illuminations, normally with increasing intensities. At the end of each illumination step, a saturation pulse is applied for quenching analysis. The light curve chart plots fluorescence yields and saturation pulse data against light intensity. In parallel, data are plotted on a time axis in the Slow Kinetics window.

The PAR values used as x-values correspond to those of the active internal PAR list. It is not recommended to measure the PAR online, as the sensor shades part of the sample. If a PAR sensor is connected, it should be removed from the sample and its reading disabled on the General Settings window by removing the checkmark for PAR Sensor.

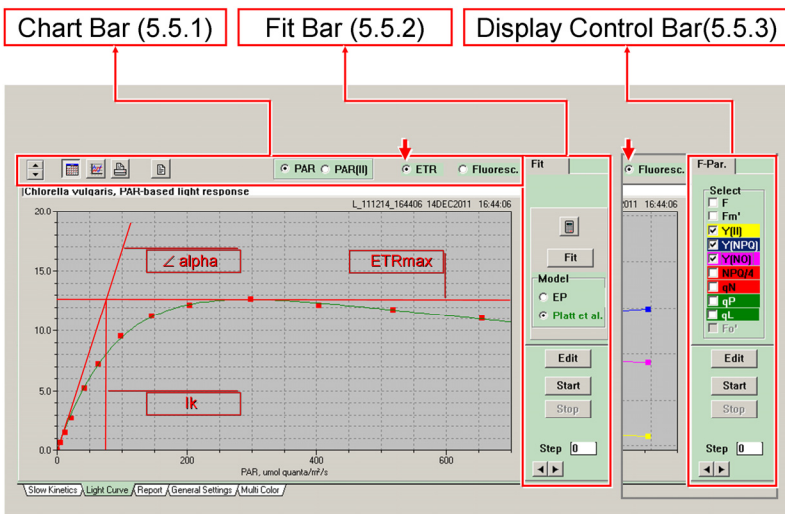



Fig. 12: Light Curve Window.

### 5.5.1 Chart Bar


 The left part of the chart bar provides 4 buttons already known from the Slow Kinetics window. In addition, vertical arrows permit adjustment of X axis scaling. These arrows, together with the horizontal pair of arrows (bottom, right), permit scaling up the initial part of the light curve. Auto scaling of both axes by the right mouse button functions as described for the Slow Kinetics window. The option of zooming in by moving the mouse cursor within the chart is not available.

Different types of Y axes can be selected. To view the relationship between relative photosynthetic rates and light intensities, select "ETR" (electron transport rate). To plot various fluorescence levels ( $F_o'$ ,  $F$ , and  $F_m'$ ) or fluorescence ratio parameters against light intensities, choose "Fluoresc.". If ETR is selected, the side bar provides tools to fit a theoretical function to data of a light response curve ("Fit Bar"). In the other case,

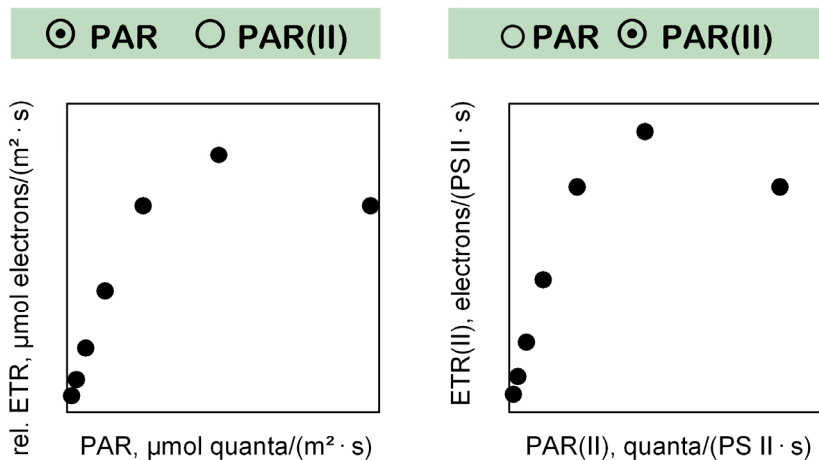


Fig. 13: Two Different Types of Light Curves.

the side bar controls display of fluorescence data (“Display Control Bar”).

Also, different X axes are available (Fig. 13, page 65). Ticking “PAR” uses quantum flux densities of incident radiation as X axis data, when “PAR(II)” is selected, numbers of PS II-specific quantum flux rates are X axis data. In the latter case, ETR(II) in units of electrons per PS II is the dependent variable. The ETR(II) is calculated according to:  $ETR(II) = PAR(II) \cdot Y(II) / (F_v/F_m)$ , where Y(II) and  $F_v/F_m$  are the photochemical yields of PS II in the light-exposed and dark-acclimated state, respectively (see Chapter 9.1, page 131).

Values of PAR(II) correspond to PAR multiplied by the functional absorption cross-section of PS II,  $\Sigma(II)_\lambda$ , and Avogadro’s constant. For determination of  $\Sigma(II)_\lambda$ , PamWin-3 provides a fitting routine which analyzes the fluorescence rise from the O to the  $I_1$  level. This fitting routine will be reviewed in section “Fast Acquisition” (Chapter 1, page 79).

## 5.5.2 Fit Bar/Light Curve Edit


Buttons for the Edit, Start and Stop commands are available on both side bars of the Light Curve window.


The Edit command opens the window for configuration of light curves (Light Curve Edit, Table 8, page 67). The other two commands start and stop a light curve measurement.


Standard lcp-files optimized for various types of samples and colors of illumination are provided with the PamWin-3 Setup program (e.g. Walz\_cyano\_625.lcp and Walz\_leaf\_440.lcp). These light curve programs can be further optimized by the user for particular applications and saved under new names.

**Table 8: Light Curve Program Files (LCP)**

**Light Curve Edit**

  
 Open

  
 Save

 *Open and save commands for LCP (light curve program) files.*

*LCP files contain information on setting of actinic light intensity (“Intens.”) and duration (“Time/10s”) for each of the 20 light steps available. Also, information on all settings affecting the light intensity is stored and automatically installed upon loading an LCP file (actinic light color as well as color, intensity and frequency of measuring light). PAR values result from both the setting programmed for each light step and the various measuring light parameters. Light curve information is linked to the current color of actinic light. Thus, changing actinic light color will change the light curve response.*



Step	PAR	Intens.	Time/10s
1	0	MF1K	3
2	11	MF100K	3
3		1	0
⋮	⋮	⋮	⋮
20	2006	20	

✓ OK

✗ Cancel

Uniform time

Default

 *Editable columns* 

*To edit, click in target field, type in the new data, and click elsewhere in editable columns. Measuring or actinic light LEDs can be used to drive photosynthesis. The strength of measuring light depends on intensity setting and pulse frequency. In light curves, high pulse frequencies are used to cover the lowest PAR range. For example, entering MF100K in the “Intensity” column sets modulation frequency to 100 kHz. All 7 MF-H frequencies can be addressed this way. The actinic light source is controlled by entering a setting number between 0 and 20. The time interval of a step is entered as multiples of 10 seconds in column “Time/10s”. To change the length of all 20 steps at once, check “uniform Time”. A step with time=0 is not executed and terminates the light curve. Before entering 0, disable <Uniform time>.*

After modification of light curve parameters the original name is maintained (unless changed by the user) and the name is marked by an asterisk. An asterisk also is added to the name when the color, ML-intensity, MF-Low and MF-High settings are changed.

Light curve data can be analyzed using the EP model (Eilers and Peeters (1988) *Ecol Model* 42: 199-215) or the [Platt et al. model](#) (Platt T, Gallegos CL, Harrison WG (1980) *J Mar Res* 38: 687-701). The Fit button starts an iterative process during which the free parameters of the selected model equations (EP or Platt et al.) are varied until the best fit between theory and experiment is obtained. Fitting results are represented graphically by a curved line; the three cardinal points of this calculated line are indicated in Fig. 12 (page 64). The values of cardinal points, and an Fv/Fm-derived parameter, are displayed after clicking the calculator button. Table 9 (page 68) summarizes the units of these parameters.

Table 9: LC Fit Parameters.



		PAR	PAR(II)
<b>Fv/Fm x ETR factor</b>	Estimated alpha based on Fv/Fm.		
<b>alpha</b>	Initial slope of light curve.	$\frac{\text{electrons}}{\text{quanta}}$	$\frac{\text{electrons}}{\text{quanta}}$
<b>ETRmax</b>	Maximum electron transport rate	$\frac{\mu\text{mol electrons}}{\text{m}^2 \cdot \text{s}}$	$\frac{\text{electrons}}{\text{PSII} \cdot \text{s}}$
<b>I<sub>k</sub></b>	Photon flux at which light-saturation of photosynthesis sets in.	$\frac{\mu\text{mol quanta}}{\text{m}^2 \cdot \text{s}}$	$\frac{\text{quanta}}{\text{PSII} \cdot \text{s}}$

### 5.5.3 Display Control Bar

To plot fluorescence levels ( $F_o'$ ,  $F$ , and  $F_m'$ ) or fluorescence ratio parameters against light intensities, choose "Fluoresc.". Display of data is controlled by the check boxes on the side bar for display control.

### 5.5.4 Some Comments to Light Curves

Only if illumination steps are long enough to reach steady state of photosynthesis, light curves may contain similar information as classical light response curves (P-I curves). Naturally, any limitation by insufficient  $CO_2$  supply must be avoided during such long-term light curves.

Rapid light curves (RLC) use short illumination steps (down to 10 s) to assess the acclimation state of photosynthesis. In this case it is essential that the sample is quickly transferred from its natural light environment to the sample holder of the PAM fluorometer. Typically, the RLC starts at a PAR value somewhat below that of the natural environment. In the absence of a dark-acclimation period, determinations of  $F_o$  and  $F_m$  level fluorescence is not possible. These parameters, however, are not required for measurements of  $Y(II)$ , on which calculation of ETR is based. Obviously, without  $F_o$ ,  $F_m$  determination the values of all parameters based on  $F_o$  or  $F_m$  (like NPQ and  $qP$ ) are meaningless.

Light curves measured with dilute suspensions of algae and cyanobacteria carry particularly valuable information because PAR gradients are virtually absent.


## 5.6 Report Window


The “Report” documents and saves automatically all key experimental data. The Report view (View Rpt) displays all experiments (“Records”) of the “Report” with information on type of experiment, number of saturation pulse analyses and fast kinetics as well as text written into the diagram headline (see Table 10, page 70).

Table 10: Report Window: **Report View**

20120118_03.RPT3		Rec. 5						
New Report	Report file name	Report comment	Record counter	Record comment	View			
					<input checked="" type="radio"/> Rpt	<input type="radio"/> Rec		
Date	Time	No.	Type	No.SP	NoFK	Comment	Column	
18.01.12	07:02:24	1	ICM			text01	<input type="checkbox"/>	Fo'
18.01.12	07:34:00	2	IC	15		text02	<input type="checkbox"/>	~Fo'
18.01.12	07:41:06	3	IC+	26		text03	<input type="checkbox"/>	Y(II)
18.01.12	08:07:41	4	LC	9		text04	<input type="checkbox"/>	Y(NO)
18.01.12	08:36:51	5		1	5	text05	<input type="checkbox"/>	Y(NPQ)
Record data and time		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	NPQ
Record number							<input type="checkbox"/>	qN
Record type'							<input type="checkbox"/>	qP
ICM	Manual slow kinetics						<input type="checkbox"/>	qL
IC	Induction curve						<input type="checkbox"/>	ETR
IC+	Induction curve with recovery							
LC	Light curve							
no entry	Fast acquisition							
Saturation pulse counter							<input type="checkbox"/>	SP
Fast kinetics counter							<input type="checkbox"/>	Settings
Record comment								



Report comments are entered in a text window which can be opened by clicking on the Report comment icon (). Record titles are written in the head line above the current graph (slow kinetics, light curve, and fast kinetics). The full text window for Record comments becomes available for editing after a slow kinetics experiment.

The View mode (Chapter 8, page 124) is required to access previous Report comments via the Report comment button on top of the Report window (). Also in the View mode, comment lines can be inserted in a Record by the “Insert Comment” command, which is part of a menu evoked by right click on the Report data field.

To view full information of an individual Record, double click on the relevant data line which switches to the Record view mode of the window.

The Record view (View Rec) provides detailed information on an individual experiment (“Record”, see Table 11, page 72). Minimum number of column headlines in the data field is 8 (Date, Time, No., ML, Temp. PAR, F, Fm’). Columns for Fo’ fluorescence and fluorescence ratio parameters can be added by selecting the item on the side bar by ticking checkmarks below “Column”. The values of Fo and Fm are listed in the F and Fm’ columns, respectively (Table 11). They are distinguished from other data by column subtitles Fo and Fm.

The panel “Display” offers options to extend the Record information. Ticking “SP” displays date, time and file name of all saturation pulse kinetics of the Record (Table 11). Checking “Settings” displays all information on instrument settings, the currently active PAR list, and all changes of settings during the experiment. Abbreviations of settings (bordered cells in Table 11) are explained in Table 3 (page 54).

Table 11: Report Window: **Record View**

20120118_03.RPT3 <b>Rec. 5</b>																																																														
<b>New Report</b> Report file name  Report comment  Record counter  Record comment										<b>View</b> <input type="radio"/> Rpt <input checked="" type="radio"/> Rec																																																				
Text line: Enter Record title here																																																														
Date	Time	No.	ML	Temp.	PAR F	Fm'	Y(II)	Y(NPQ)	Column																																																					
18.01.12	14:48:27	Type: ICM (or IC, IC+, LC, no entry)										<input type="checkbox"/>	Fo'																																																	
<div style="border: 1px solid gray; padding: 5px;"> <table border="1"> <tr> <td>18.01.12</td> <td>14:48:28</td> <td>FL</td> <td>MI</td> <td>G</td> <td>AI</td> <td>BI</td> <td colspan="2">FR/BL</td> <td colspan="2"></td> <td><input type="checkbox"/></td> <td>~Fo'</td> </tr> <tr> <td></td> <td>10</td> <td>3</td> <td>2</td> <td></td> <td>4</td> <td colspan="2">FR</td> <td colspan="2"></td> <td><input checked="" type="checkbox"/></td> <td>Y(II)</td> </tr> <tr> <td></td> <td>FH</td> <td>Z</td> <td>D</td> <td>FI</td> <td>SI</td> <td>AL-Src</td> <td>ML-Src</td> <td colspan="2">SI(Fm)</td> <td><input checked="" type="checkbox"/></td> <td>Y(NPQ)</td> </tr> <tr> <td></td> <td>10000</td> <td>0</td> <td>4</td> <td>1</td> <td>10</td> <td>440 nm</td> <td>440 nm</td> <td colspan="2">5</td> <td><input type="checkbox"/></td> <td>Y(NO)</td> </tr> </table> </div>												18.01.12	14:48:28	FL	MI	G	AI	BI	FR/BL				<input type="checkbox"/>	~Fo'		10	3	2		4	FR				<input checked="" type="checkbox"/>	Y(II)		FH	Z	D	FI	SI	AL-Src	ML-Src	SI(Fm)		<input checked="" type="checkbox"/>	Y(NPQ)		10000	0	4	1	10	440 nm	440 nm	5		<input type="checkbox"/>	Y(NO)	<input type="checkbox"/>	NPQ
												18.01.12	14:48:28	FL	MI	G	AI	BI	FR/BL				<input type="checkbox"/>	~Fo'																																						
													10	3	2		4	FR				<input checked="" type="checkbox"/>	Y(II)																																							
													FH	Z	D	FI	SI	AL-Src	ML-Src	SI(Fm)		<input checked="" type="checkbox"/>	Y(NPQ)																																							
	10000	0	4	1	10	440 nm	440 nm	5		<input type="checkbox"/>	Y(NO)																																																			
18.01.12	14:48:28	ML	MFH	MFLog	AL	FR	BL				<input type="checkbox"/>	qN																																																		
		On	Off	Off	Off	Off	Off				<input type="checkbox"/>	qP																																																		
18.01.12	14:48:28	PAR List: default_MC.par										<input type="checkbox"/>	qL																																																	
18.01.12	14:48:27	1	3	0.0	0	Fo	Fm				<input type="checkbox"/>	ETR																																																		
						0.855	1.971				<input type="checkbox"/>																																																			
18.01.12	14:48:27	1	3	0.0	0	0.855	1.971	0.566	0.000	<b>Display</b>																																																				
18.01.12	14:48:26	File: S_120118_144826										<input checked="" type="checkbox"/>	SP																																																	
18.01.12	14:49:06	MF-H: on										<input checked="" type="checkbox"/>	Settings																																																	
18.01.12	14:49:06	Act. Light: on																																																												
18.01.12	14:49:08	2	3	0.0	128	1.405	2.007	0.313	0.000																																																					
18.01.12	14:49:07	File: S_120118_144907																																																												

### 5.6.1 Report Context Menu

Clicking in the Report window (in Record View) with the right mouse key opens a menu with Report-specific commands:

Menu item	Available	Action
Delete Del	Always	Deletes selected row.
Undo Delete File Name Esc	After Delete	Restores last deleted line.
Choose Sigma File Name	Always	Associates functional absorption cross-sections of PS II stored in "Sigma File" with current Report.
Insert Comment Ins	Always	Inserts a line for comment (Cmt. :) below marked line.
Set Settings to Instrument	Measure mode + Settings line <sup>1)</sup> marked	Installs settings of selected line in control unit.
Set Status to Instrument	Measure mode + Status line <sup>2)</sup> marked	Sets control unit to status defined in selected line.
Set Marker in Chart	Protocol line <sup>3)</sup> marked	Writes message of marked line into SlowKinetics graph.

<b>1) Settings line</b>	18.01.12 14:48:28	FL MI G AI BI FR/BL
		10 3 2 4 FR
		FH Z D FI SI AL-Src ML-Src
		10000 0 4 1 10 440 nm 440 nm
<b>2) Status line</b>	18.01.12 14:48:28	ML MFH MFLog AL FR
		On Off Off Off Off
<b>3) Protocol lines</b>	18.01.12 14:53:12	Act. Light: on
	18.01.12 14:54:23	Act. Light: off

## 5.7 Multi Color Window

The major functions of the multi color window are selection of light colors, recording of intensities of internal light sources, and handling of  $\text{Sigma(II)}_{\lambda}$  which is the (wavelength-dependent) functional absorption cross-section of PS II.

### 5.7.1 Light

The same 5 colors are available for measuring and actinic light (Table 12, page 75). These colors are numbered consecutively from 1 to 5. In addition, measuring light peaking at

Light (5.7.1)

Sigma (5.7.2)

Color	Sigma [nm <sup>2</sup> ]	Source Name
440 nm	6.216	F_120120_081959_0
480 nm	4.571	F_120120_082010_0
540 nm	2.903	F_120120_082024_0
590 nm	1.475	F_120120_082035_0
625 nm	1.879	F_120120_082048_0
White		

Fig. 14: Multi-Color Window

400 nm and white actinic light is provided.

Selection of a color for measuring light automatically sets actinic light to the same color, except when 400 nm is chosen. On the other hand, changing the actinic light color does not affect measuring light color so that all combinations of measuring and actinic colors are available.

When Multi Color ST is checked, all actinic LEDs contribute to a single turnover flash which then is particularly strong.

The “Stirrer on” checkbox controls the status of an optional magnetic stirrer connected to the front the control unit. An equivalent command is part of the script file language (Chapter 1, page 111). A break in stirring is required when the moving stir bar disturbs the signal. In most applications, signal disturbance caused by the stirring is negligibly small and continuous stirring is possible.

Note that effective stirring requires close proximity between the inductive drive system of the PHYTO-MS stirrer and the magnetic stir bar. Therefore, the drive system must be moved to the highest position in the bottom port of the optical unit, and the cuvette must be placed at the lowest possible position.

The command Measure PAR Lists is executed only when a

### Measure PAR Lists

PAR sensor is connected. The command starts a routine which measures the PAR values for the currently selected actinic light color. Selecting All Colors will measure PAR for all actinic light colors. For each color, the PAR is measured for all

**Table 12: Light sources**

Measuring light	Actinic light
Peak wavelength, nm	
440 (1)	440 (1)
480 (2)	480 (2)
540 (3)	540 (3)
590 (4)	590 (4)
625 (5)	625 (5)
400	420-645

20 intensity settings of actinic light, for all 20 intensity settings of multiple turnover flashes, and for measuring light at 100 kHz frequency and intensity setting 10. These PAR values are entered in PAR lists that can be accessed via the Options menu. For AL color 1 to AL color 5, the PAR in the first line of the list corresponds to the PAR value established for measuring light. Based on this value, the program calculates the PAR for all combinations of measuring light intensity and frequency.

A PAR list is measured for the actinic light color selected. If one of the colors numbered 1 to 5 is selected (cf. Table 12, page 75), the measuring light color automatically assumes the color of actinic light. If white is selected, each of the 6 measuring light colors can be chosen. The PAR of the selected measuring light source, set to intensity level 10 and 100 kHz frequency, will be written as the intensity representing setting 0 in the PAR list for white actinic light.

Setting 0 of the PAR list for white actinic light corresponds to the intensity of 400 nm measuring light under standard conditions. Therefore, 400 nm measuring light should be selected when PAR lists for white actinic light are measured. The spectral sensitivity of Walz PAR sensors is mostly flat in the range from 400 to 700 nm but steeply sloped around 400 nm. Therefore, sensor calibration is invalid when 400 nm light is measured. In principle, this error can be corrected according to:

$$\text{Setting } 0_{\text{Corrected}} = \frac{\text{Calibration Constant AIR}}{\text{Spectral Sensitivity at 400 nm}} \cdot \text{Setting } 0$$

The “Calibration Constant AIR” and “Spectral Sensitivity at 400 nm” are documented by the calibration certificate of the sensor. Setting 0 is the non-corrected PAR value at position 0 of the PAR list for white actinic light which is then replaced by Setting 0<sub>Corrected</sub>.

Newly measured PAR lists overwrite the current lists. If PAR lists are not recorded for all colors, the current set of PAR lists consists of newly measured and old data. The current set of PAR lists can be saved together with annotations (menu Options → AL Current/PAR Lists).

The PAR acting on a sample is derived from measured PAR lists provided that the PAR sensor is unplugged or inactivated. Then the PAR value is calculated by considering light color and intensity as well as measuring light frequency. Calculation of PAR also works for different colors, e.g., green measuring light and red actinic light.

Correct PAR lists are not only important for PAR measurement as such, but also for assessment of the wavelength- and sample dependent optical cross-section  $\Sigma(\lambda)$  and determination of effective PS II turnover rate  $ETR(\lambda)$ . Therefore, users are encouraged to update the PAR lists of their individual instruments before measuring a new Sigma-file. This should be done under close to identical optical conditions as the Fast Kinetics measurements of the  $O-I_1$  rise (see below).

For measurements using the Optical Unit ED-101US/MD (suspensions), the spherical sensor has to be mounted with the diffuser sphere centered in the 10x10x10mm sample volume. For measurements using the Optical Unit MCP-BK (with leaf clip), the planar sensor has to be fixed in the cylindrical opening of the clip holder, with the diffuser plate in the sample plane (pushed against emitter Perspex rod at 10° angle).

### 5.7.2 Sigma

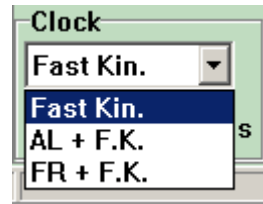
This section is used for managing of Sigma files: existing files can be opened and assigned to the current Report. Also, files can be annotated, saved or removed from the window. Sigma-files contain information on  $\text{Sigma}(\text{II})_{\lambda}$  which is the wavelength-dependent functional absorption cross-section of PS II of a particular sample for up to 6 different colors of light. Values of  $\text{Sigma}(\text{II})_{\lambda}$  are obtained by fitting a theoretical curve to the fluorescence rise from the O to the  $I_1$  level. This procedure will be explained in "Fast Acquisition" (1, page 79). Sigma files are stored in C:\PamWin\_3\Data\_MC\Report\Sigma.



## 6 Fast Acquisition

The fast acquisition mode permits fluorescence kinetics in the sub-msec to sec range at a maximum time resolution of 15  $\mu$ s. Illumination protocols can be programmed at increments of 2.5  $\mu$ s and repeated measurements can be averaged to obtain high signal quality even with weakly fluorescent samples.

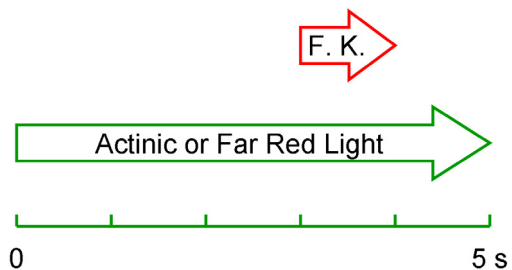
Saturation pulse analysis is limited to Fv/Fm. Events that can be repetitively triggered (Clock) are (1) fast kinetics, (2) fast kinetics with actinic illumination, and (3) fast kinetics with far red illumination.



Manual triggering of these three events is carried out by the buttons (1) , (2)  , and (3)  .

Note that actinic or far red illumination stays on during “fast kinetics with actinic illumination” or “fast kinetics with far red illumination”.

Thus, these protocols differ from pretrigger illumination in Fast Settings which terminates before a trigger pattern is executed.



**Fig. 15:** Exemplary time pattern of “Fast kinetics with actinic illumination” or “Fast Kinetics with far red illumination”. Illumination interval (actinic or far red): 5 s. Fast kinetics starts 2 s before end of illumination.

Fast kinetics with illumination is only available when the respective time interval (actinic or far red light) is greater than 2 s. Then, the fast kinetics will be performed 2 seconds before illumination ends.

## 6.1 Fast Kinetics Window

The display of the fast kinetics graph can be changed as described for the Slow Kinetics window. The new features of the Fast Kinetics window are listed in Table 13, page 81 ff.

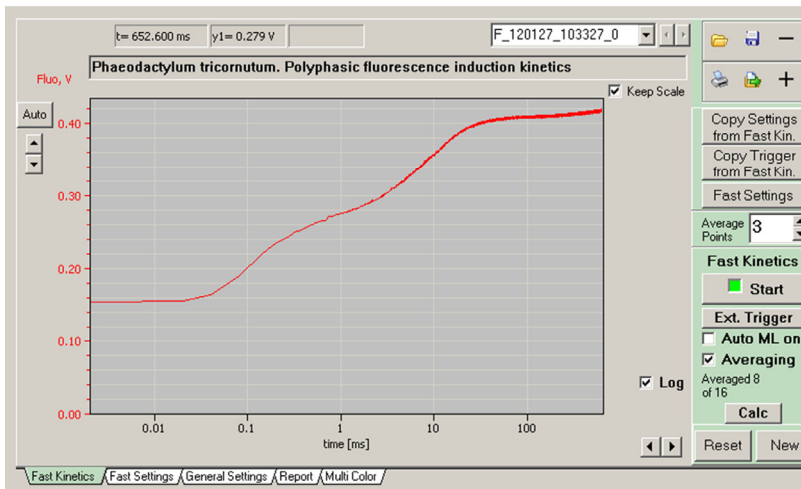




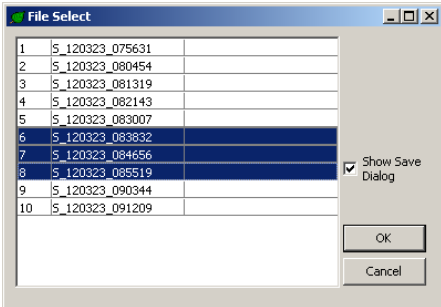

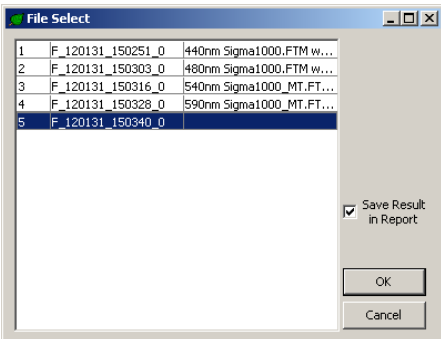



Fig. 16: Fast Kinetics Window.

Table 13: Fast Kinetics Commands

Control	Command
<b>Keep Scale</b>	Keep scaling of fluorescence axis constant, prevents auto scaling of x axis.
<b>Log</b>	Plot fluorescence on a logarithmic time scale.
	<p>Open a PamWin-3 fast kinetic file.</p> <p>Fast kinetics data files contain information on the fluorescence kinetics but also on fast trigger pattern and settings present during recording of the fast kinetics. To reinstall the fast trigger pattern, click "Copy Trigger from Fast Kin." after loading the fast kinetics file, to reinstall settings, click "Copy Settings from Fast Kin."</p> <p>Settings include a) status (on or off) of measuring light, actinic light, far red light, high measuring light frequency, logarithmically decreasing measuring pulse frequency (MF log), b) low and high frequency of measuring light pulses (MF-L and MF-H).</p> <p>Default directory is  C:\PamWin_3\Data_MC\Report\YYYYMMDD_##  (## counter for Reports of same date).</p> <p>File format of saturation pulse kinetics is  S_YYMMTT_HHMMSS.PFK</p> <p>File format of fast kinetics is F_YYMMTT_HHMMSS_#.PFK  (# counter for fast kinetics if the same time).</p>
	<p>Save fast kinetics file.</p> <p>This command can be used to save files which are created by calculations (see below). Original files are saved automatically.</p>
	<p>Print current graph.</p>

Control	Command
	<p>Export selected fast kinetics data as CSV data (comma-separated values).</p> <p>By default, the file name of fast kinetics is used and files are saved to the directory: C:\PamWin_3\Data_MC\Export.</p> <p>If “Show Save Dialog” is checked, different directories can be selected.</p> <p>When only one kinetics is exported, the file name can be edited.</p> 
	<p>Subtract one of the fast kinetics of the Record from the currently displayed fast kinetics.</p> <p>To conduct subtraction, click on minus (-) icon to view the list of fast kinetics, select by mouse click the fast kinetics to be subtracted, and close window by “OK” to display the difference kinetics. To automatically save difference kinetics, check “Save Result in Report” in the window for file selection.</p> 

Control	Command
	<p>Form the average of selected files.</p> <p>To select files, click the plus (+) icon to view the list of fast kinetics. To pick a series of files, click with left mouse key on first file of series, keep mouse button depressed and move to the last file of series, and release. Several non-neighboring files can be selected or deselected by left mouse click on files with the Ctrl key depressed. After picking files for averaging, click “OK” to display the result of averaging. The averaged data is added at the end of the list of fast kinetics and denoted “Manual Average”. Manual Average files are automatically saved provided that “Save Result in Report” has been checked.</p>

### Copy Settings from Fast Kin.

Each fast kinetics is saved with its trigger pattern and instrument settings. These trigger pattern and settings are installed by “Copy Settings from Fast Kin.” command. The settings parameters concerned are summarized in Table 13, page 81. To reproduce the trigger pattern of a previously saved PFK file, switch to View mode, open Report and Record containing the Fast Kinetics of interest, display Fast Kinetics, and click “Copy Settings from Fast Kin.”. Then return to Measure mode.

### Copy Trigger from Fast Kin.

This command works similarly to the previous one except that only the trigger pattern is copied.

### Fast Settings

Opens Fast Settings window.



Point averaging leads to reduction in noise on the cost of time resolution. Hence, point averaging is equivalent to signal damping. In contrast to the analog Damping (General Settings) and the S&H damping (Options), point averaging can be applied after actual measurements, so that an optimal compromise between time resolution and signal/noise ratio may be obtained. Loss of time resolution may be tolerable, however, when original data were recorded at higher time resolution than required for data analysis. The size of exported CSV files decreases with increasing number of averaged points. The command “Averaging” on this side bar starts calculation the mean of repeated fast kinetic curves which results in noise reduction without loss of time resolution.



The Start button on the side panel of the Fast Kinetics window executes the current trigger pattern (Fast Settings window) and records the resulting fluorescence kinetics. The button is equivalent to the Start button on the lower window panel.



The command “External Trigger” opens the window “External Trigger Control” which contains the button “Start External Triggering”. After click on this button, a 5 V DC signal applied to the trigger input socket of the control unit sets off the currently active trigger pattern and recording of fluorescence. To return to the normal state, click “Stop External Triggering” and “Close”.

## Auto ML on

“Auto ML-on” turns on measuring light just before start of a fast kinetics and switches measuring light off immediately thereafter. This function is useful to avoid effects of strong measuring light on the state of the sample. Alternatively, switching-on of the measuring light can be also included in trigger pattern of the fast kinetics.

## Averaging

“Averaging” calculates the arithmetic means of up to “n” consecutively measured fluorescence kinetics. Averaging of kinetics reduces noise without decreasing time resolution. Only the averaged kinetics is saved. Individual kinetics can be triggered by Start buttons (side or bottom bar) or by the clock (bottom bar). The number “n” of individual kinetics is set as “Target Average” on the Fast Settings window. The clock stops automatically after n kinetics are averaged. In the average mode, two additional buttons are available

**Reset**

Stops averaging and deletes averaged kinetics.

**New**

Stops averaging and saves current averaged kinetics. This command is useful when the number of measurements to reach good signal quality is smaller than “Target Average” number.

## Calc

The “Calc” (calculations) button provides access to various methods to quantitatively analyze fluorescence kinetics. These analyses will be reviewed in Section 6.3, page 96.

## 6.2 Fast Settings Window

Timing and light conditions of Fast Kinetics recordings can be adjusted in the Fast Settings window. The window provides various tools to design a particular Fast Kinetics trigger pattern which is graphically outlined on the fast settings chart.

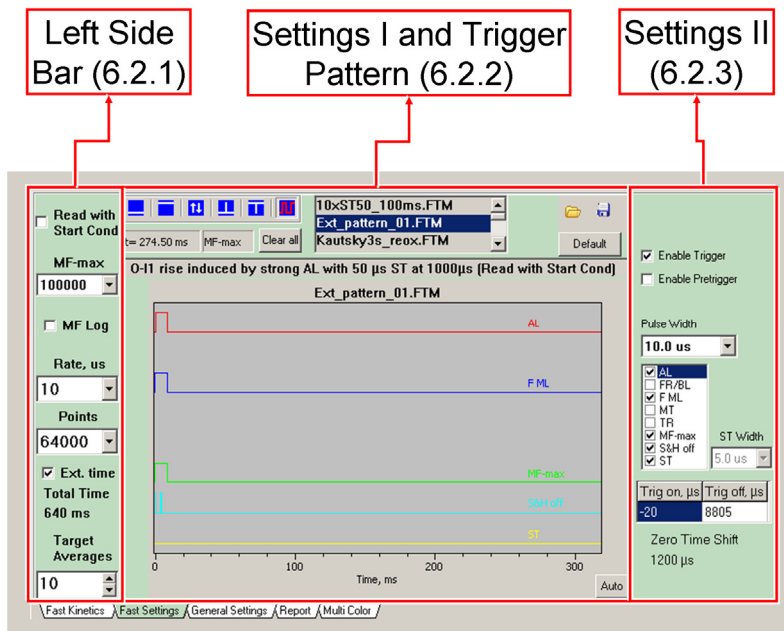


Fig. 17: Fast Settings Window.



## 6.2.1 Left Side Bar

### Read with Start Cond

Similarly as described for fast kinetic files, trigger files contain information on instrument settings (On/off status of measuring light, actinic light, far red light, high measuring light frequency, logarithmically decreasing measuring pulse frequency ,MF log). Further settings saved are low and high frequency of measuring light pulses (MF-L and MF-H.) When “Read with Start Conditions” is enabled, these settings become active.

### MF-max

The available pulse frequencies for MF-max are the same as for MF-H (General Settings) except the highest frequency of 200 000 Hz (providing highest time resolution) which can only be evoked by the MF-max trigger.

### MF-log

The MF-log decreases measuring pulse frequency logarithmically with time. The MF-log is triggered by termination of MF-max. If the tripper pattern does not involve MF-max or MF-max is not terminated within the fast kinetics time, MF-log is not available. The MF-log function minimizes the actinic effect of measuring light, which is particularly important during fluorescence relaxation kinetics following a single turnover flash.

### Rate, us

The “Rate” in  $\mu\text{s}$  corresponds to the time interval between two consecutive measuring light pulses. The total time interval of fluorescence kinetics is the product of rate and “Points”. The maximum number of points for a trigger program is 32 000

(but, see below on “Ext. time”). The overall fluorescence kinetics may comprise up to 128 000 points.

For example, at a rate of 10  $\mu\text{s}$  and 64 000 points, the interval of the trigger pattern is 320 ms followed by another 320 ms for, e.g. recording of fluorescence decay.

### Ext. time

The extended time function is available when the number of data points is  $> 32\,000$ . The function increases total experimental time by increasing the acquisition rate at point 32 001, and, for 128 000 points kinetics, again at point 64 001 (Table 14, page 88).

### Target Averages

“Target Averages” determines the number of fluorescence kinetics to be averaged. Curve averaging is activated in the Fast Kinetics window. The number of kinetics to be averaged can be varied even during curve averaging. Setting a higher number for target averages than used in the previous average process requires pressing the “New” button on the Fast Kinet-

**Table 14: Extended Time (Fast Kinetics).**

Points total	128000	128000	128000
Rate, $\mu\text{s}$ (selected)	2.5	2.5	5
Ext. time OFF ( <input type="checkbox"/> ) or ON ( <input checked="" type="checkbox"/> )	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Rate, $\mu\text{s}$ (true)			
( 1 - 31999 points)	2.5	2.5	5
( 32000 - 63999 points)	2.5	5	10
( 64000 - 128000 points)	2.5	10	10
Total time, ms	320	880	1120

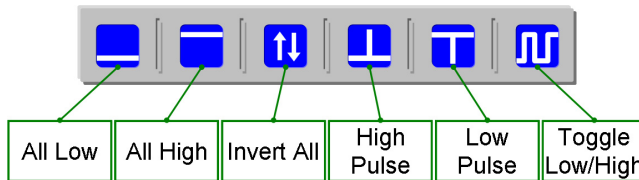
ics window; if “New” is not executed, new data will be added to the existing one until target average is reached.

## 6.2.2 Settings I and Trigger Pattern

A trigger pattern consists of time courses of trigger signals. A trigger signal can be either low or high which corresponds to status off or on, respectively. The switching between on and off is extremely fast and can be programmed with 2.5  $\mu$ s resolution.

- Six different tool icons are provided to configure trigger signals, and 8 events can be triggered.

### 6 Trigger Icons



### 8 Events








- AL
- FR/BL
- F ML
- MT
- TR
- MF-max
- SH off
- ST

- To edit a trigger pattern, check all events which should be triggered. This displays the corresponding trigger lines. “Clear all” sets all triggers to off.
- To enlarge time scale of trigger graph, move mouse pointer with left mouse button depressed parallel to the X axis. Release of the mouse button will enlarge the interval marked. Pressing the right mouse button, enables shifting the x axis by moving the mouse cursor with de-

pressed left key. Another right click returns to the zoom function. The “Auto” button (lower right corner) zooms out to the full scale view.

- Editing trigger signals using icons “All Low”, “All High” and “Invert All” requires selection of the event by clicking on its checkbox label. Selection is indicated by the highlighted checkbox label (white letters on blue background. Compare “AL” below).
- To trigger pulses and change trigger levels, select appropriate trigger icon (red lines in icons indicate selection). Then, events can be selected by mouse click on the trigger line using the instructions of Table 15, page 91. Alternatively, select the event and enter time points in the “Trig on” and “Trig off” fields on the left side bar. Graphical editing of trigger lines is facilitated by the numerical displays of the mouse cursor position (numerical displays below trigger icons).
- The ST (single turnover flash) is unique, as it must be particularly short to turn over PS II reaction centers only once. Therefore, only the “High Pulse” and “All Low” buttons are available for configuring the ST.
- The length of high and low pulses is determined by “Pulse Width” for all events except ST for which “ST Width” applies. Drop down menus for both pulse widths are adapted to the acquisition rate selected.
- Trigger pattern control only the on/off status of an event. Most of events concern internal light sources of the fluorometer. Light properties can be adjusted on settings windows introduced earlier (Fig. 10 page 51) and summarized in (Table 16, page 92).

**Table 15: Trigger - Program Tools**

Action	Icon	Key & mouse combinations	Events
Main Trigger Chart			
High pulse*		<Shift> + <right mouse key>**	All
Low pulse*		<Shift> + <right mouse key>**	All
Switch to high		<Shift> + <left mouse key>**	All but ST
Switch to low		<Shift> + <right mouse key>**	All but ST
All low		First, select event to be triggered. (Checkbox label must be highlighted: white letters on blue background).	All
All high			All but ST
Invert levels			All
Pretrigger Chart			
Toggle between always high and always low		<right mouse key>	All but ST

\* For all events, the length of pulses depends on the time selected from the drop down list "Pulse Width" except ST flashes length which is determined by the time selected for "ST Width".

\*\* Trigger setting *via* these shortcuts does not require selection of a trigger event in the checkbox panel. Simply click on trace to select event.



Trigger files can be saved and recalled. File extension is "FTM, and the standard directory is "C:\PamWin\_3\Fast Kin Trigger".

## Default

“Default” opens FastKin.FTM which triggers a 300 ms polyphasic fluorescence rise typically showing 4 fluorescence levels named O-I<sub>1</sub>-I<sub>2</sub>-P or O-J-I-P. Also, “Default” activates experimental settings for polyphasic rise kinetics, that is, measuring light (ML) switched off and high frequency measuring light (MF-H) enabled.

**Table 16: Triggered Events - Settings**

Label	Event	Settings
<input checked="" type="checkbox"/> AL	Actinic light	Color: Multi Color. Intensity: General Settings.
<input checked="" type="checkbox"/> FR/BL	Far-red or blue light	Color: General Settings or FR/Blue button. Intensity: General Settings.
<input checked="" type="checkbox"/> F ML	Measuring light	Color: Multi-Color. Intensity: General Settings. Frequencies: General Settings.  (MF-L) and (MF-H) on/off buttons define start conditions of kinetics.
<input checked="" type="checkbox"/> MT	Multiple turn-over flash	Color: Multi Color. Intensity: General Settings.
<input checked="" type="checkbox"/> TR	Trigger out	-
<input checked="" type="checkbox"/> MF-max	Maximum frequency of measuring light	Color: Multi-Color. Frequency: MFmax on Fast Settings. Intensity: General Settings.
<input checked="" type="checkbox"/> S&H off	Sample and hold circuit off	-
<input checked="" type="checkbox"/> ST	Single turn-over flash	Color: General settings. Intensity: Always maximal.

### 6.2.3 Settings II

#### Enable Trigger

Should be checked in normal operation. The command is disabled for service purposes only.

#### Enable Pretrigger

The pretrigger feature provides the option to expose samples to preillumination. The length of exposure can be selected from drop down menu which comes available after “Enable Pretrigger” has been checked. Also, a small chart, entitled “Pre”, left of the major trigger graph becomes apparent in which a trigger signal can be toggled on/off by left-clicking on a trigger line. The pretrigger interval is immediately followed by the fast kinetics. Note that the “Clear All” button does not affect the pretrigger settings.

#### Zero Time Shift

To adjust the zero time shift, double-click on the number displayed below the “Zero Time Shift” (e.g. 300  $\mu$ s). Time in  $\mu$ s can be entered manually or by using the pair of vertical arrows beside the number field. Time shift moves the zero point of the time axis so that recording of a fast kinetics starts at negative time values. By keeping the onset of actinic light at point zero, fluorescence kinetics data are plotted against a logarithmic time scale.

### 6.2.4 Example Trigger Pattern

The trigger pattern displayed in Fig. 18 (page 94) drives a fluorescence increase from the O level close to the  $I_1$  level which is probed by a single turnover saturating flash, ST. Specific adjustments were:

- Rate = 2.5  $\mu$ s and points = 1000. The two settings yield a total time interval of 2.5 ms.
- Zero Time shift= 300  $\mu$ s. The x axis starts at -300  $\mu$ s.

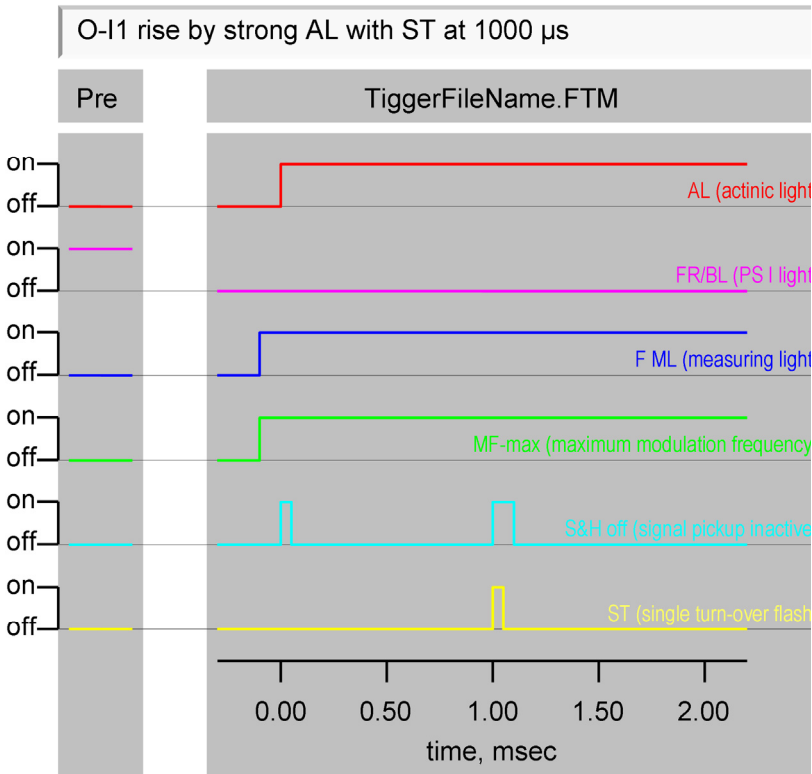


Fig. 18: Example Trigger Pattern.



- Pretrigger enabled. The “Pre” window is visible.
- Only 6 events are triggered, multiple turnover flash (MT) and trigger out (TR) are hidden.
- Other settings which provide suitable conditions for measuring fluorescence induction are:
  - “Act. Light Int.” (actinic light intensity) = 20, to drive fluorescence induction with strong light.
  - ML button off (measuring light switched off) to start fast kinetics without measuring light.
  - Far red selected.

The example trigger pattern has the following characteristics:

### **FR/BR (PS I Light)**

Preillumination with PS I light is terminated at start of the trigger pattern.

### **AL (actinic light)**

The AL to drive fluorescence induction sets in at time zero. (In reality, the “Trig on” time is set to  $-5 \mu\text{s}$  because of a small delay between trigger time point and onset of actinic light. Similarly, a  $5 \mu\text{s}$  shift is needed in the case of a multiple turnover flash.)

### **F ML (measuring light) and MF-max (maximum modulation frequency)**

To measure  $F_0$  level fluorescence, F ML and MF-max are switched on at time  $-100 \mu\text{s}$  that is before onset of actinic light.

### ST (single turnover flash)

To elicit the  $I_1$  level, a 50  $\mu\text{s}$  ST flash (1000 to 1050  $\mu\text{s}$ ) was applied.

### S&H off (signal processing inactivated)

To suppress signal disturbance by sudden increases in absolute fluorescence intensity, the sample-and-hold (S&H) circuit is inactivated from -20 to 5  $\mu\text{s}$  which corresponds to the onset of strong actinic light. Additionally, the S&H was switched off in the interval from 990 to 1085  $\mu\text{s}$ , thus suppressing the disturbing effect of the single turnover flash.

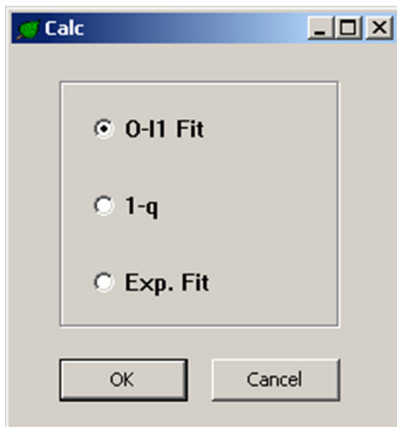
## 6.3 Fast Kinetics Analyses

Data analysis of PamWin-3 is available via the “Calc” button in the Fast Kinetics window.



### 6.3.1 Calc Window

The first step of data analysis is the selection of one out of three different methods. Two of the methods (O- $I_1$  Fit and Exp. Fit) vary the free parameters of theoretical functions until best fit between theory and experiment is obtained. The 1-q method uses



the measured O-I<sub>1</sub> rise kinetics and parameters resulting from the O-I<sub>1</sub> fit procedure to calculate relative amounts of closed PS II reaction centers, q.

Note that the character “O” of O-I<sub>1</sub> Fit symbolizes the minimum fluorescence level of fast fluorescence kinetics which is determined briefly after onset of measuring light during the 20 μs time period preceding onset of actinic illumination. This fluorescence level corresponds to the Fo measured with low frequency measuring light. Therefore, the O and Fo are synonymously used in this section.

### 6.3.2 O-I<sub>1</sub> Fit Window

The O-I<sub>1</sub> Fit window serves for analysis of the O-I<sub>1</sub> rise kinetics by a special fitting routine. The routine was developed for determinations of the functional optical cross-section of PS II, Sigma(II), which is wavelength- and sample-specific. The routine is based on the reversible radical pair model of PS II (Schatz et al., 1988, Biophys J 54: 397-405; Lavergne and Trissl, 1995, Biophys J 68: 2474-2492) and takes into account reoxidation of the primary PS II acceptor Q<sub>A</sub> as well as energy transfer between PS II units (connectivity). Important prerequisites for analysis of the rise kinetics are

- a) Low chlorophyll content (< 300 μg/L for 440 nm excitation) to avoid light gradients.
- b) Oxidized PS II acceptor-side (Q<sub>A</sub> and PQ-pool) achieved by far-red background or preillumination.
- c) Accurate determination of the O- and I<sub>1</sub>-levels. Specifically, the O-level is assessed briefly before onset of actinic illumination and the I<sub>1</sub>-level is the fluorescence level

reached by a single turnover saturating flash (Multi-Color ST) at the end of the illumination period.

The immediate result of an O-I<sub>1</sub> Fit is information on the time constant of the fluorescence rise. With information on the incident PAR driving this rise (saved in the PAR Lists), the functional absorption cross-section of PS II is calculated by the program.

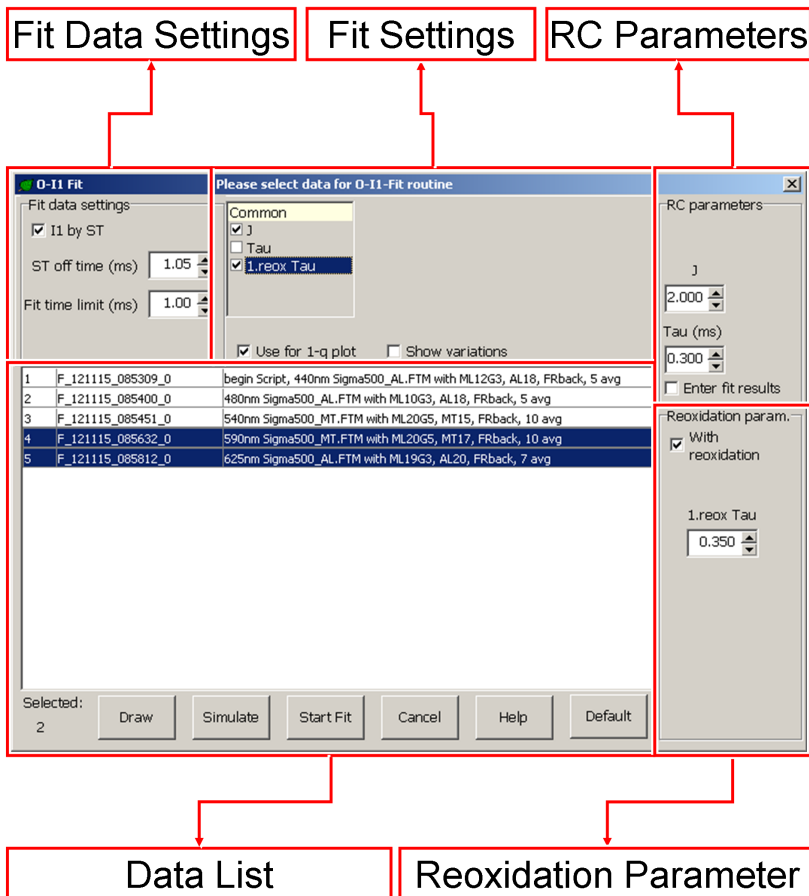


Fig. 19: O-I<sub>1</sub> Fit Window.

Knowledge of  $\Sigma(II)$  permits transformation of incident PAR (in  $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) into the rate of quantum absorption per PS II complex,  $\text{PAR}(II)$  (in  $\text{quanta} \cdot \text{PS II}^{-1} \cdot \text{s}^{-1}$ ). Then, the rate of linear electron transport per PS II reaction center,  $\text{ETR}(II)$  (electrons  $\cdot \text{PS II}^{-1} \cdot \text{s}^{-1}$ ) can be estimated according to  $\text{ETR}(II) = \text{PAR}(II) \cdot Y(II) / (F_v/F_m)$  (see Section 9.5, page 142)

where  $Y(II)$  and  $F_v/F_m$  are the photochemical yields of PS II in the light-exposed and quasi-dark-acclimated state (i.e. with weak far-red background light), respectively. The tools to control the O-I<sub>1</sub> fitting procedure are reviewed subsequently using the grouping outlined in Fig. 19 (page 98).

### Fit Data Settings

The adjustments made in section “Fit Data Settings” provide key information about the measured kinetics for the fitting routine.

---

#### I<sub>1</sub> by ST

“I<sub>1</sub> by ST” must be activated when I<sub>1</sub> level fluorescence is elicited by a single turnover flash. This is the case in many standard trigger files designed to drive O-I<sub>1</sub> rise kinetics. When “I<sub>1</sub> by ST” is disabled, the signal amplitude at the end of the recording is assigned to I<sub>1</sub> (e.g. feasible in presence of DCMU).

---

#### ST off time (ms)

The St off time is the end time point of the single turnover flash applied to determine the I<sub>1</sub> level. For instance, the ST off time is 1.05 ms for the standard trigger files `Sigma1000_AL.FTM` and `Sigma1000_MT.FTM` which both set off a 50  $\mu\text{s}$  ST at time 1000  $\mu\text{s}$ .

---

---

**Fit time limit (ms)**

The time entered defines the upper time limit of the data interval to be considered by the fitting procedure. In case of the standard files Sigma1000\_AL.FTM and Sigma1000\_MT.FTM, normally the time 1.00 ms applies, so that the whole curve up to the ST is fitted; however, also shorter fit limits can be defined.

---

**Fit Settings**

Here, conditions for simultaneous analysis of several curves and handling of fitting results are defined.

---

**Common**

Several fluorescence kinetics can be simultaneously analyzed by curve fitting. To this end, the kinetics of interest have to be marked in the data list of the O-I<sub>1</sub> fit window. Thereafter, the field “Common” is available.

A parameter selected in the Common box will be identical for all marked curves. By assignment of “common” parameters the reliability of the fitting analysis can be improved, if it is unlikely that the enabled parameters vary significantly between measurements. For example, the rate of Q<sub>A</sub><sup>-</sup> reoxidation is not expected to be influenced by the color of light used for driving the O-I<sub>1</sub> rise. Hence, it makes sense to enable the rate constant “1.reox Tau” when fitting O-I<sub>1</sub> rise curves measured with various AL and MT colors (e.g. with the help of the standard Script file Sigma1000Chlor\_10.prg).

---

**Use for 1-q plot**

The values of Fo and I<sub>1</sub>, determined in conjunction with the O-I<sub>1</sub> Fit as well as the estimated parameter J, will be entered in 1-q Fit window (see below). Based on this information, variable fluorescence values can be transformed into 1-q values.

---

**Show variations**

Display in O-I<sub>1</sub> Fit table for all fitted parameters the +/- % signal variations required for +/- 10% changes in fit error. Note that small signal variations are indicative of a reliable fit and normally are written in green. When written in red, a parameter limit was reached during the fitting process.

---

---

## RC Parameters

---

### J

Initial value (at start of O-I<sub>1</sub> Fit) of the free parameter J.

---

### Tau (ms)

Time constant of Q<sub>A</sub><sup>-</sup> formation (Q<sub>A</sub> reduction); initial value (at start of O-I<sub>1</sub> Fit) of free parameter Tau (ms).

---

### Enter fit results

When enabled, the fit results are not only displayed in the table for O-I<sub>1</sub> Fit results, but they are also entered into the fields for initial parameters in the O-I<sub>1</sub> Fit window. "Simulate" (see below) will then calculate theoretical curves with the parameter estimates of previous curve fitting.

---

## Reoxidation parameters

---

### With reoxidation

Reoxidation of Q<sub>A</sub><sup>-</sup> formed during the O-I<sub>1</sub> rise is taken into account. "With reoxidation" should be disabled if Q<sub>A</sub><sup>-</sup> reoxidation cannot occur, e.g. in presence of the PS II inhibitor DCMU.

---

### 1. reox Tau (ms)

Time constant of Q<sub>A</sub><sup>-</sup> reoxidation by Q<sub>B</sub> (first Q<sub>A</sub> reoxidation time constant). The 1. reox Tau is varied during curve fitting

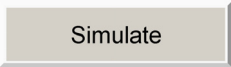
---

## Data List

The O-I<sub>1</sub> Fit window lists all kinetics of the current Record. A simple mouse click selects the curve to be analyzed. To analyze several curves concomitantly, mark the additional curves by mouse click with the Ctrl key depressed. Using the Shift key, a sequence of curves can be selected by mouse click.

A rectangular button with a light gray background and a thin black border. The word "Draw" is centered in a black sans-serif font.

All selected curves are drawn on top of each other for visual comparison of kinetics.

A rectangular button with a light gray background and a thin black border. The word "Simulate" is centered in a black sans-serif font.

“Simulate” calculates model curves using the values of the O-I<sub>1</sub> Fit window. If “Enter fit results” is activated, and a curve fitting has been carried out, simulation starts with the results of the last fitting process. In this case, the effect of parameter variations on goodness of fit can be easily visualized.

A rectangular button with a light gray background and a thin black border. The words "Start Fit" are centered in a black sans-serif font.

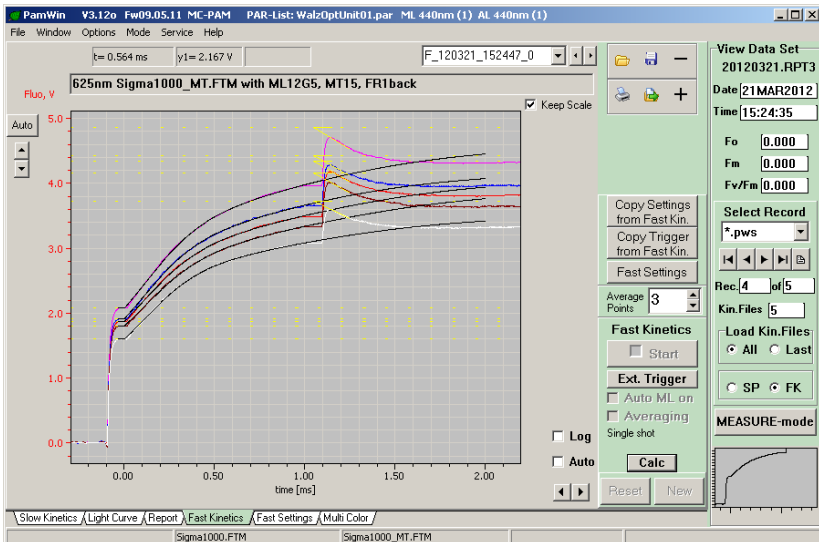
The button starts O-I<sub>1</sub> fitting. The results are compiled in the O-I<sub>1</sub> Fit data table. At the same time, original traces and fitted curves are shown in the Fast Kinetics window. If the experiment used a single turnover flash to elicit I<sub>1</sub> level fluorescence, a yellow sloped line describes the fluorescence decay right after the flash. The I<sub>1</sub> level fluorescence was determined by extrapolating back the sloped line to the end point of the single turnover flash which is 1.05 ms in the sample below. The latter procedure is required because primary data acquisition was switched off during the single turnover flash to avoid signal artifacts.



## O-I1 Fit Table

O-I1 Fit											
Nr.	Filename	Comment	Fo	I1	PAR	p	J	Tau	1.r.Tau	Sigma	Error
			Volt	Volt	$\mu\text{E}/\text{m}^2\text{s}$			ms	ms	$\text{nm}^2$	rel.
1	F_120321_152447_0	440nm Sigma1000.FTM with ML4G3, AL16, FR1back	1.874	4.335	1005	0.625	1.664	0.315	0.456	5.250 @440 nm	10.5
2	F_120321_152458_0	480nm Sigma1000.FTM with ML4G3, AL16, FR1back	2.078	4.865	1343	0.648	1.841	0.301	0.455	4.114 @480 nm	10.7
3	F_120321_152509_0	540nm Sigma1000_MT.FTM with ML20G6, MT16,	1.918	4.419	6307	0.645	1.813	0.287	0.425	0.918 @540 nm	25.4
4	F_120321_152521_0	590nm Sigma1000_MT.FTM with ML20G6, MT20,	1.805	4.158	3975	0.630	1.704	0.325	0.476	1.287 @590 nm	16.3
5	F_120321_152532_0	625nm Sigma1000_MT.FTM with ML12G5, MT15,	1.600	3.721	3002	0.633	1.722	0.288	0.464	1.918 @625 nm	8.1
1	F_120321_152447_0	+ %change at +10% error - %change at +10% error					1.7 -1.7	0.3 -0.3	1.1 -1.1		
2	F_120321_152458_0	+ %change at +10% error - %change at +10% error					1.7 -1.7	0.3 -0.3	1.1 -1.1		
3	F_120321_152509_0	+ %change at +10% error - %change at +10% error					2.7 -2.6	0.4 -0.4	1.8 -1.7		
4	F_120321_152521_0	+ %change at +10% error - %change at +10% error					2.0 -2.1	0.3 -0.3	1.4 -1.3		
5	F_120321_152532_0	+ %change at +10% error - %change at +10% error					1.5 -1.5	0.2 -0.2	1.0 -1.0		

## Fast Kinetics Window: Experimental and Fitted Data





and

**OK**

The export button (left) saves fitting results as CSV data. Export directory is C:\PamWin\_3\Data\_MC\Export. The OK returns to the Fast Kinetics window.

**Help**

The button opens an online-Help-text on the O-I<sub>1</sub> fitting procedure.

**Cancel**

“Cancel” quits the O-I<sub>1</sub> sections and returns to the Fast Kinetics window.

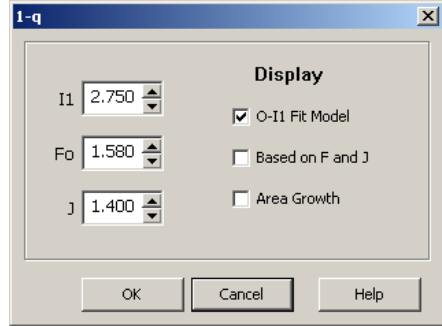
### 6.3.3 1-q

The parameter  $q$  corresponds to the fraction of open reaction centers characterized by the oxidized primary acceptor,  $Q_A$ . Setting the sum of open and closed reactions centers to one, the  $1-q$  is the fraction of closed PS II reaction centers with reduced primary acceptor  $Q_A^-$ .  $1-q$  is non-linearly related to fluorescence yield,  $F$ , due to transfer of excitation energy between PS II units (connectivity). Connectivity explains why  $F$  increases more slowly than  $1-q$  during the initial part of the O-I<sub>1</sub> rise (sigmoidicity).

In the PS II excitation model by Lavergne and Trissl (1995, Biophys J 68: 2474-2492), on which the present O-I<sub>1</sub> analysis is based, the extent of connectivity is described by the parameter  $J$ . This  $J$  is related to the empirical sigmoidicity pa-

parameter  $p$  (Joliot and Joliot, 1964, C R Acad Sci Paris 258: 4622-4625) by the equation  $J = p/(1-p)$ .

Calculations of  $1-q$  require parameters from fitting analysis of the  $O-I_1$  rise, specifically, the  $I_1$ ,  $F_0$  and  $J$ . Therefore, analysis of  $O-I_1$  rise should precede  $1-q$  calculations. Three different  $1-q$  calculations can be performed, based on different models and suited for different applications. The resulting  $1-q$  plots are drawn in different colors.



### **O- $I_1$ Fit Model (displayed in blue)**

Calculation is based on the model underlying  $O-I_1$  Fit. This model takes account of  $Q_A$  reoxidation and, hence, is also valid for analysis of rise kinetics in the absence of PS II inhibitors (like DCMU).

### **Based on F and J (displayed in green)**

Values of  $1-q$  are calculated according to subsequent equation (Lavergne and Trissl, 1995, Biophys J 68: 2474-2492. Joliot and Joliot, 1964, C R Acad Sci Paris 258: 4622-4625):

$$\frac{F(t) - F_0}{I_1 - F_0} = \frac{1 - q}{1 + J \cdot q} \rightarrow q = \frac{I_1 - F(t)}{(F(t) - F_0) \cdot J + (I_1 - F_0)}$$

where  $F(t)$ ,  $F_0$ , and  $I_1$  are the fluorescence curve and its  $O$  and  $I_1$  levels, respectively. This model does not take account of  $Q_A$  reoxidation and, therefore, it is valid only for analysis of rise kinetics in the presence of PS II inhibitors (like DCMU) or at extremely high actinic intensities at which the the rate of PS II charge separation is much higher than the rate of  $Q_A$  reoxida-

tion. In the latter case, however, the quantum efficiency of PS II may be affected (Rappaport et al 2007, *Biochim Biophys Acta* 1767: 56-67).

### **Area Growth (displayed in yellow)**

The 1-q curves are derived by the area growth method (Malin S, Kok B (1966) *Biochim Biophys Acta* 126:413-432. Murata N, Nishimura M, Takamiya A (1966) *Biochim Biophys Acta*. 120:23-33). The method is valid only when reoxidation of reduced primary acceptor  $Q_A$  is prevented, e.g. by presence of DCMU.

### **6.3.4 Exp. Fit**

The “Exp. Fit” routine permits analysis of decay kinetics following termination of actinic illumination (actinic light, multiple turnover or single turnover flashes). Settings and conditions for this fitting procedure are entered into window “Exp. Fit” (Fig. 20, page 108) as explained below.

#### **Fit Data Settings**

---

##### **Light off time (ms)**

Switch-off time of illumination.

---

##### **Fit begin time (ms)**

Lower limit of data considered for curve-fitting.

---

##### **Fit time limit (ms)**

Upper limit of data considered for curve-fitting.

---

---

## Fit Settings

---

### 1-q Fit

This function transforms the fluorescence kinetics into changes of 1-q (cf. Section 6.3.3, page 104) and the kinetic analysis refers to the 1-q relaxation kinetics.

---

### No of Exponentials

The relaxation kinetics can be described by the sum of up to three exponentials. The corresponding free parameters are the amplitudes A1, A2 and A3, and the time constants Tau 1, Tau 2 and Tau 3.

Tau 1 of relaxation kinetics corresponds to 1. reox Tau of the O-I<sub>1</sub> fit, i.e. the time constant of Q<sub>A</sub><sup>-</sup> reoxidation by Q<sub>B</sub>.

Tau 2 of relaxation kinetics corresponds to 2. reox Tau of the O-I<sub>1</sub> fit, i.e. the time constant of Q<sub>A</sub><sup>-</sup> reoxidation by Q<sub>B</sub><sup>-</sup>.

Tau 3 of relaxation kinetics corresponds to the time constant with which double-reduced Q<sub>B</sub><sup>2-</sup> is replaced by fully oxidized Q<sub>B</sub> (involving protonation, release and rebinding of PQ-molecule).

---

### Level

"Level" stands for the additional amplitude A0. A0 corresponds to the fraction of closed reaction centers (1-q) that is not reoxidized during the fit time. A0 is considered to reflect the so-called "inactive PS II" which appears to be blocked in Q<sub>A</sub> to Q<sub>B</sub> electron transport (e.g. due to the Q<sub>B</sub> binding site being vacant). However, as A0 often displays values of 40-50% in perfectly active samples, the real nature of "inactive PS II" remains to be clarified.

---

### I<sub>1</sub>, Fo

Exponential fitting requires the parameters Fo, and I<sub>1</sub>. These are conveniently estimated by recording an O-I<sub>1</sub> rise curve with the same sample under identical measuring light conditions. After O-I<sub>1</sub> fitting analysis, the Fo and I<sub>1</sub> parameters are automatically written into the Exp. Fit window. Alos, both data can be manually entered.

---

## J

The sigmoidicity parameter J is required when “1-q Fit” is active. Similarly as for  $F_0$  and  $I_1$ , the J is taken from a preceding O- $I_1$  curve fit but can be also entered manually.

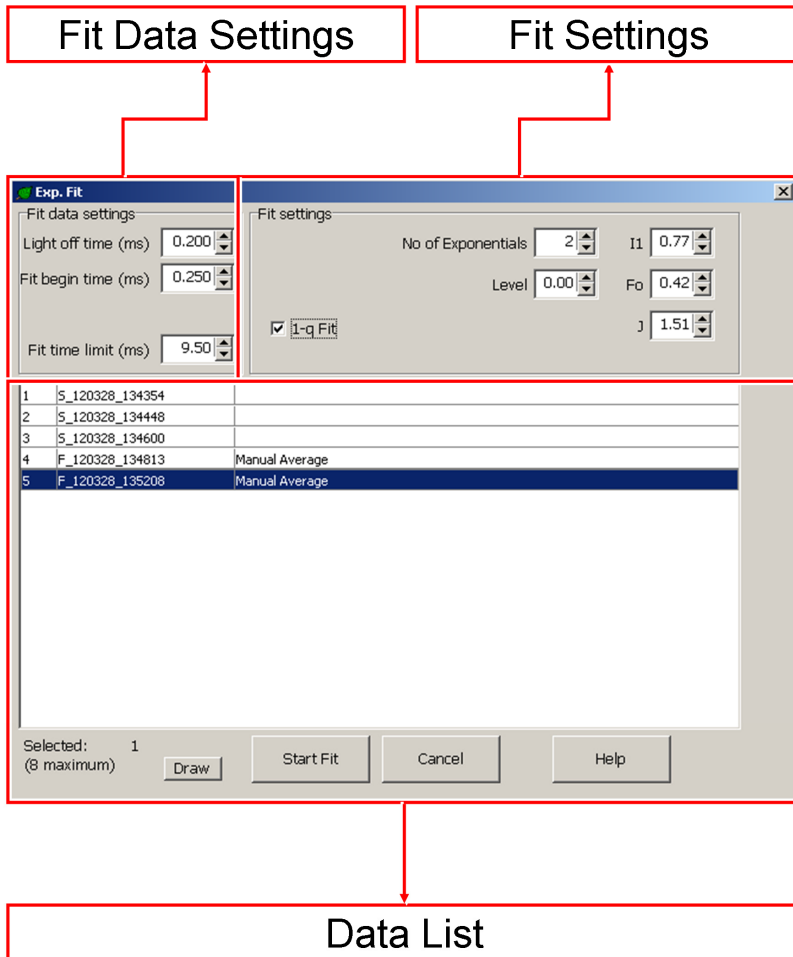


Fig. 20: Exp. Fit Window.

## Data List

The Exp. Fit window lists all kinetics of the current Record. Pick curve for analysis by the mouse cursor. Several curves can be analyzed in parallel: simply mark the curves of interest by mouse click with the Ctrl key depressed; a series curves can be selected using the mouse cursor in combination with the Shift key.



Draw

Draws selected curves in the same Slow Kinetics graph. This function can be also used independently of the Exp.Fit routine for display of superimposed Fast Kinetics.





Start Fit

The button starts fitting exponential curves to the data. The results are compiled in the O-I<sub>1</sub> Fit data table.

### Exp. Fit Table

Nr.	Filename	Comment	Fo	I1	J	PAR	A(tot.)	A0	A1	A2	A3	Tau1	Tau2	Tau3	Error
			Volt	Volt		$\mu\text{E}/\text{m}^2\text{s}$	Volt	%	%	%	%	ms	ms	ms	rel.
1	F_120328_135208	Manual Average	0.420	0.770	1.510	709	0.920	21.3	41.4	37.4		0.840	4.046		17.7

OK 

At the same time, original traces and fitted curves are depicted in the Fast Kinetics window (Fig. 21, page 110). Clicking the export button () saves fitting results as CSV data into C:\PamWin\_3\Data\_MC\Export. The OK returns to the Fast Kinetics window.

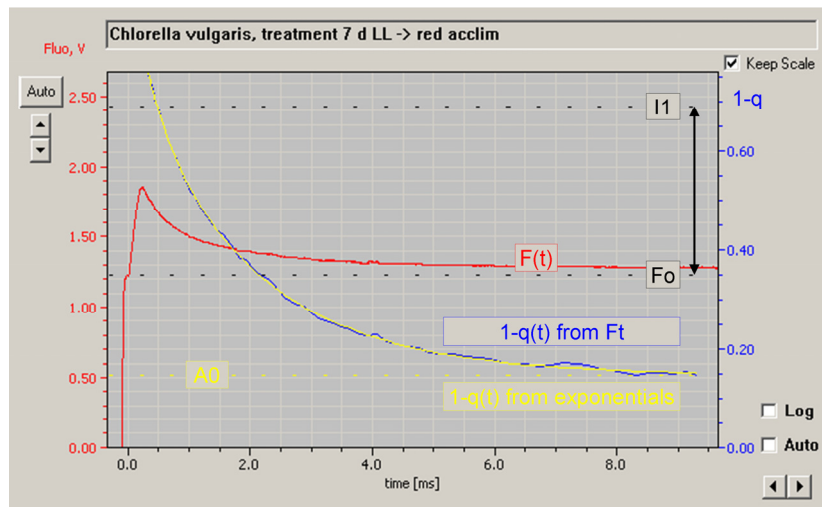


Fig. 21: Fast Kinetics Window: Experimental and Fit Data.



## 7 Script File Window

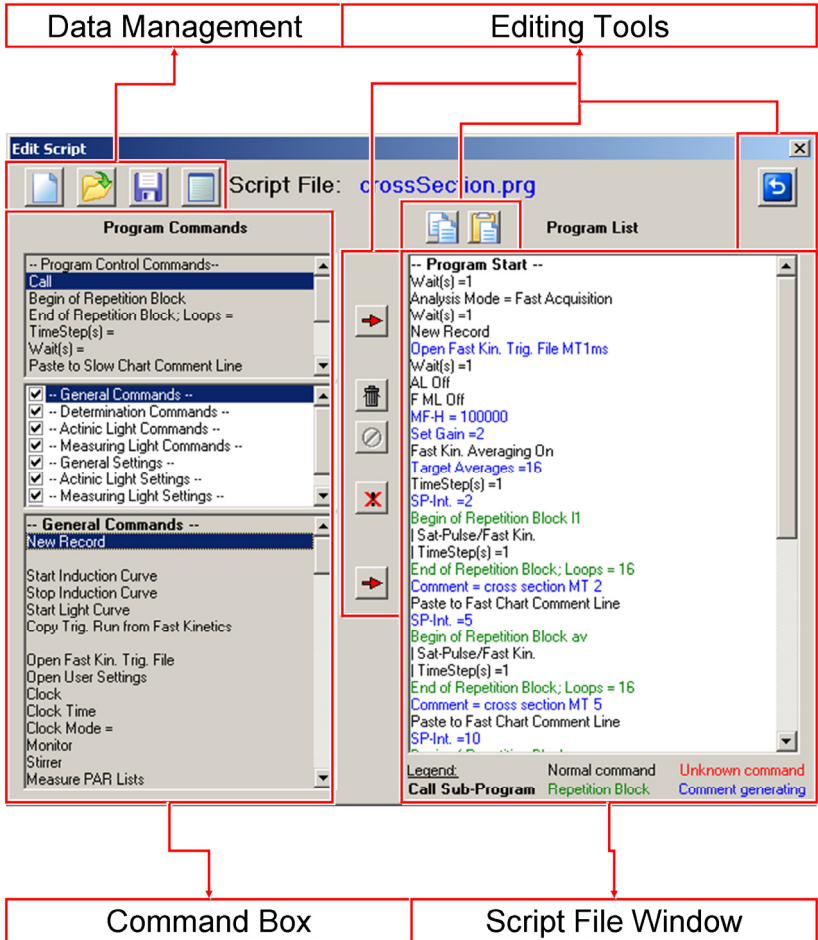


Fig. 22: Script File Window.

Script files are used for automated execution of experimental procedures of various complexities. Particularly, script files are advantageous when the same type of analysis needs to be repeated frequently and when complicated protocols must be exactly reproduced.

Generally, all manual operations in the PamWin-3 software can also be carried out under the control of a Script file. In addition, Script files offer commands for time management, combinations of sub-programs and conditional commands.

To access the script file window, click on the **Load** button on the bottom bar of the PamWin-3 windows. To execute script files, click on the **Run** button below.

## 7.1 Data Management

Four commands are provided for script file management:

---



New Script File. Clears the script file window and prompts for a new script file name.

---



Open Script File. Opens a script file with name format "file-name.PRG". The default directory for script files is C:\PamWin\_3\Script Files. Other directories can be selected..

---



Save Script File. Saves to default or user-defined directory.

---



Script File Comment. Displays a text-window for notes on the current script file. The content of the window is saved as text file with name "filename01.TXT" which is associated with the script file "filename01.PRG".

---

## 7.2 Editing Tools



Copy The command stores one or several lines of the current script file in the clipboard. To execute the copy command, select one or several lines using the mouse cursor (Left-click once to pick one line. Hold down Shift key and select first and last line of a series of script file commands. Hold down Ctrl key to select several scattered lines.) Click Copy icon. The selected commands are now available for pasting within the current or into another script file using the Paste command.



Paste To paste previously copied commands, select a line in the target script file and click the Paste icon. The pasted lines will be written below the selected line.



Insert Generally, “Insert” icons transfer commands from the command box to the script file window. The upper insert icon transfers commands from the box “Program Control Commands”, the lower icon transfers commands from the “General Commands” box. Double click on the command to be transferred is equivalent to the insert icons.

To insert a new command in the program list:

- Select the command to be inserted by mouse cursor in the command box (left text window of Fig. 19, page 110)
- Select by mouse cursor in the script file window (right text window of Fig. 21 (page 110) the line below which the command shall be inserted
- Click Insert button.



Delete Select one or several commands to be deleted (see above) and click icon. The command is equivalent to pressing “delete” on the keyboard.



Undo Delete Reverses the last delete action.




Disable/Enable Disables command lines of the current script file, or enables previously disabled command lines. Disabled lines are printed in grey. To execute the command, select line(s) in script file window and click icon.



**BACK** Closes script file window. In case that a sub-program is displayed, the Back button returns to the script file which calls the sub-program.

### 7.3 Command Box (List of Script File Commands)

The command box of the script file window (Fig. 21, page 110) consists of 3 sections. The upper one contains commands controlling the progress of script files. The middle panel contains the title of the 8 groups of commands which are listed in the bottom panel. Unchecking titles in the middle panels hides the corresponding group of commands in the bottom panel.

PARAMETER	COMMAND, COMMENT	INPUT
<b>Section 1: -- Program Control Commands --</b>		
Call	Execute another PamWin-3 script file as a sub-program. A sub-program can be displayed by double-clicking an existing calling line. Use the Back button (  ) to return to main script file. Sub-programs may call further sub-programs.	Script file name (filename.prg)
Begin of Repetition Block	Mark the beginning of a series of commands (repetition block) which is to be repeated.	Name of repetition block
End of Repetition Block; Loops =	Mark the end of a repetition block. A repetition block may contain other repetition blocks. Repetitions can also be terminated depending on the levels of Ft or temperature (see section "Condition Commands" at the end of this list).	Number of repetitions

---

<b>PARAMETER</b>	<b>COMMAND, COMMENT</b>	<b>INPUT</b>
TimeStep(s) =	Define the time interval between the beginnings of two consecutive events; if the TimeStep command is not preceded by an event, the time interval starts with script file execution. A series of time steps forms a time scale along which actions can be performed with defined time pattern.	Time interval in seconds
Wait(s)	Define the time interval after the last command has been completed and next command is carried out. Wait steps can be placed inside of a TimeStep interval provided that the total time required for command execution plus wait steps is shorter than the time step interval.	Time interval in seconds
Paste to Slow Chart Comment Line	Write the content of the last comment-generating line (printed in blue) into the title line of the Slow Kinetics window. The title line is saved in the "Record Command File" (see Report window). When a script program contains several "Paste to Slow Chart" commands, only the last pasted line will be written to the "Record Command File".	None
Paste to Fast Chart Comment Line	Paste the content of the last comment generating line (typed in blue) into the title line of the Fast Kinetics which can be examined in the "VIEW-mode". Execution of the command requires that a Slow Kinetics has been started.	None

---

PARAMETER	COMMAND, COMMENT	INPUT
Message =	Halt script execution and display a message. Clicking OK on the message window or hitting the Enter key continues execution of the script file.	Message title and message text.
Comment =	Insert a comment line in the script file. A comment line can be written to the slow chart title using the "Paste to Slow Chart" command.	Comment text
Spacer	Insert an empty line after the currently selected line.	None
Exit	Terminate and exit script file.	None
<b>Section: -- General Commands --</b>		
New Record	Start a new Record.	None
Start Induction Curve	Start recording of a slow kinetics: equivalent to the "Start" button on slow kinetics window.	None
Stop Induction Curve	Stop recording of a slow kinetics: equivalent to the "Stop" button on slow kinetics window.	None
Start Light Curve	Start recording of a light curve: equivalent to the "Start" button on the light curve window.	None
Copy Trig. Run from Fast Kinetics	Use the trigger pattern of the Fast Kinetics currently displayed. Note that fast kinetics files contain fluorescence data as well as relevant trigger pattern and instrument settings.	None

<b>PARAMETER</b>	<b>COMMAND, COMMENT</b>	<b>INPUT</b>
Open Fast Kin. Trig. File	Activate trigger pattern of trigger file "filename.FTM" in directory "C:\PamWin_3\Fast Kin Trigger"	File name
Open User Settings	Activate stored instrument settings. File name format: filename.DEF. Default settings: Walz_MC.DEF.	File name
Clock	Start or stop the repetitive trigger.	Check/uncheck
Clock Time	Sets or modifies clock interval.	Clock interval in s (=s). Increment in s to decrease (-s) or increase (+s) the current clock interval
Clock Mode=	Selects the action to be triggered by the clock. Note that the drop-down list for triggered events differ between the SP-Analysis and Fast Acquisition modes.	Select from drop-down list
Monitor	Interrupt/continue transfer of Ft and temperature data.	Check on/off
Stirrer	Switch stirrer on and off. Stirring is activated by the stirrer switch on the front plate of the control unit.	Check on/off
Measure PAR Lists	Measure for all 20 intensity settings and the active measuring light color the PAR of measuring light, actinic light and multiple turnover flashes.	None
Measure Zoff List	Measure zero offset for all measuring light colors at current gain setting.	None
Kinetics auto save	Save kinetics to hard disk immediately after acquisition.	Check on/off

PARAMETER	COMMAND, COMMENT	INPUT
<b>Section 2: -- Determination Commands --</b>		
Fo,Fm	Determine Fo and Fm level fluorescence and calculate Fv/Fm.	None
Sat-Pulse/Fast Kin.	Perform saturation pulse analysis (in SP Analysis Mode). Perform fast kinetics (in Fast Kinetics Mode).	None
Fo	Determine Fo level fluorescence.	None
Fo'	Determine Fo' level fluorescence.	None
FR+Yield/Fast Kin.	Perform far red pre-illumination followed by saturation pulse analysis (in SP Analysis Mode) or followed by fast kinetics (in Fast Kinetics Mode).	None
AL+Yield/Fast Kin.	Perform pre-illumination by actinic light followed by saturation pulse analysis (in SP Analysis Mode) or followed by fast kinetics (in Fast Kinetics Mode).	None
<b>Section 3: -- Actinic Light Commands --</b>		
AL	Switch actinic light on/off.	Check on/off
FR	Switch far red light on/off.	Check on/off
BL	Switch blue light on/off. Command requires separate PS I lamp.	Check on/off
PS I Light =	Switch between far red and blue PS I light. Command requires separate PS I lamp.	Check Far red/Blue
ST	Trigger single turnover flash.	None
MT	Trigger multiple turnover flash.	None
TR Pulse	Apply 5 V trigger pulse at AUX socket.	None



PARAMETER	COMMAND, COMMENT	INPUT
<b>Section 4: -- Measuring Light Commands --</b>		
F ML	Switch measuring light on/off.	Check on/off
MF-H	Switches measuring light frequency between low and high.	Check on/off
<b>Section 5: -- General Settings --</b>		
Analysis Mode	Select between SP-Analysis and Fast Acquisition mode.	Check selection
Recording Mode	Select type of slow kinetics (manual, induction, or induction and recovery).	Drop-down list
Set Gain	Set or modify gain (1 to 10).	Gain setting (= #). Increment for decrease (- #) or increase (+ #)
Set Damping	Set or modify damping (1 to 8).	Damping level (= #). Increment for decrease (- #) or increase (+ #)
Fast Kin. Averaging	Average a series of kinetics.	Checkbox
Target Averages	Set or modify number of fast kinetics to be averaged.	Number of kinetics (= #). Increment number for decrease (- #) or increase (+ #)
New Fast Kin. Average	Stop averaging of fast kinetics and save averaged kinetics.	None

PARAMETER	COMMAND, COMMENT	INPUT
Auto ML on	Turns on measuring light before fast kinetics, and switches measuring light off thereafter.	Checkbox
Keep Fo,Fm	Off: Determine Fo and Fm values at start of each induction or light curve. On: Use the previous Fo and Fm values for all subsequent saturation pulse analyses.	Check on/off
Read with Start Cond	Activate instrument settings of a trigger file when a trigger file is loaded. See also: Copy Settings from Fast Kin.	Check on/off
Apply Zoff List	Activate offset subtraction from fluorescence signal	Check on/off
<b>Section 6: -- Actinic Light Settings --</b>		
AL Color	Select actinic light color.	Check one of 6 colors
Multi Color ST	Use all actinic LEDs for single-turnover pulse.	Check on/off
Stirrer	Switches stirrer on and off, provided stirring is activated by the stirrer switch on the front plate of the control unit.	Check on/off
Intensity levels of:		
AL-Int.	Actinic light,	1 to 20
BL-Int.	Blue light,	1 to 20
FR-Int.	Far red light,	1 to 20
SP-Int.	Saturation pulse.	1 to 20

PARAMETER	COMMAND, COMMENT	INPUT
	Illumination time interval for	
AL Width	Actinic light	0 - 900 s
BL Width	Blue light	0 - 900 s
FR Width	Far red light	0 - 10 s
SP Width	Saturation pulse	0.1 - 0.8 s
	Time interval of	Drop down list
ST Pulse Width =	Single turnover pulse.	5 - 50 $\mu$ s
MT Pulse Width =	Multiple turnover pulse.	1 - 300 ms
TR us-Pulse Width =	Short (sub ms) 5 V trigger pulse.	10 - 250 $\mu$ s
TR ms-Pulse Width =	5 V trigger pulse (sub s).	1 - 1000 ms
	Increase/decrease time interval of:	
ST Pulse Width Step =	Single turnover pulse (Increment = 5 $\mu$ s),	Number of incre- ment to decrease (- #) or to increase (+ #)
MT Pulse Width Step =	Multiple turnover pulse (Increment = 1 ms for width < 10 ms else 10 ms),	
TR us-Pulse Width Step =	Effective for short (sub ms) 5 V trigger pulse (Increment = 10 $\mu$ s),	
TR ms-Pulse Width Step =	Effective for longer (sub s range) 5 V trigger pulse (Increment = 1 ms for width < 10 ms else 10 or 20 ms).	
ST sequence number	Defines the number of single turnover flashes of a flash sequence, or increases or decreases the flash number of a flash sequence.	Number of flashes, number of decrease (- #) or increase (+ #) of flashes. Val- id entries: 1 to 20

PARAMETER	COMMAND, COMMENT	INPUT
ST sequence width	Defines time interval between consecutive single turnover flashes, or increases/decreases time interval.	10 to 500 ms, time interval of decrease (- # ms) or increase (+ #ms)
Fast Kin. After ST sequence	Start fast fluorescence kinetics after sequence of saturation flashes.	Check on/off
<b>Section 7: -- Measuring Light Settings --</b>		
ML Color	Set color of measuring light	Check color
MF-H =	High measuring light frequency (Hz).	1000-100000 (Drop down list)
MF-L =	Low measuring light frequency (Hz).	10-5000 (Drop down list)
MF-H Step	Stepwise increase/decrease of high measuring light frequency (variable increments depending on present frequency).	Number of increment to decrease (- #) or to increase (+ #)
MF-L Step	Stepwise increase/decrease of low measuring light frequency (variable increments depending on present frequency).	Number of increment to decrease (- #) or to increase (+ #)
F ML-Int =	Intensity setting of fluorescence measuring light.	1 - 20
AutoMF_H	Use high measuring light frequency when actinic light is on.	Check on/off
MF Log	Decrease measuring light frequency in logarithmic fashion after termination of MF-max in fast kinetics.	Check on/off

PARAMETER	COMMAND, COMMENT	INPUT
<b>Section 8: -- Condition Commands --</b>		
If Ft	Executes next command if Ft is greater (>) / smaller (<) than the threshold value entered	Ft in Volt
If Temp	Executes next command if temperature is greater (>) / smaller (<) than the threshold value entered	Temperature in °C
Wait until Ft	Interrupt script file execution until Ft is greater (>)/smaller (<) than the threshold value entered	Ft in Volt
Wait until Temp	Interrupt script file execution until temperature is greater (>) /s maller (<) than the threshold value entered	Temperature in °C
End of Rep. Block; Repeat until Ft	Terminates repetition block if Ft is greater (>)/smaller (<) than the threshold value entered	Ft in Volt
End of Rep. Block; Repeat until Temp	Terminates repetition block if temperature is greater (>)/smaller (<) than the threshold value entered	Temperature in °C

## 8 View Mode

The View mode offers six different windows which have been introduced in previous chapters. In the present chapter, additional features provided by the View mode will be explained.

Generally, the View mode of PamWin-3 permits offline analyses of fluorescence measurements. This includes editing of titles of records and measurements, as well as file names. If you want to modify the file name of a Report, however, make sure that Report file name (filename.RPT3) and directory name are identical.

Offline analysis can be performed on computers which are not connected to the MULTI-COLOR-PAM. To analyze Report data on an offline computer, install PamWin-3 on the second computer and copy the Report data folder from the measuring computer (C:\PamWin\_3\Data\_MC\Report\YYYYMMDD) to the corresponding folder on the offline computer.

### View Mode Windows

- Slow Kinetics
- Light Curve
- Report
- Fast Kinetics
- Fast Settings
- Multi Color

### 8.1 Structure of Data

The highest level unit of data in PamWin-3 is called "Record". The Record consists of one or several Reports. A Report file contains experimental parameters like instrument settings and changes of these settings. Further, fluorescence levels and fluorescence ratio parameters of saturation pulse analyses are part of as Report. A Report file refers to other data files, which

can be analyzed in the View mode. Depending on file type, the file name starts with a different character:

First Character of File Name	Type of measurement
F	Fast fluorescence kinetics
I	Dark-light fluorescence induction curve
L	Slow fluorescence kinetics of light curves
M	Manual slow kinetics
S	Saturation pulse-induced fluorescence kinetics

## 8.2 Menu Bar

The file menu of the menu bar (cf. Section 5.2.1, page 33) contains two new items: “Open pws-file” and “Export as CSV file”.

“Open pws-file” is available for the Slow Kinetics and the Light



Curve windows. The corresponding icon is available only in the Slow Kinetics window (see Table below). The command opens a list of all slow kinetics of the current Report. From this list, a slow kinetics can be selected for display.

The second new feature is “Export as CSV file”. The command stores slow kinetics and corresponding Record data as comma-separated values (CSV) in the export folder of PamWin-3 (C:\PamWin\_3\Data\_MC\Export). The export dialogue

### File Menu

VIEW mode	MEASURE mode
New Report	New Report
Import Old Report	Import Old Report
<b>Open pws-file</b>	Load Script
<b>Export as csv file</b>	Run Script
Printer Setup	Printer Setup
Print Graph	Print Graph
Exit	Exit

(but not the export icon) offers the option to export all slow kinetics and Record data of the Report. In this case, data will be stored in a new directory will be created in the export folder. The directory name is that of the current Report.

View Mode	Icon	Command
<b>Window</b>		
Slow Kinetics		Open slow kinetics (pws file)
Slow Kinetics Light Curve Report		Export saturation pulse data of currently selected Record and slow kinetics as CSV file into directory C:\PamWin_3\Data_MC\Export.

## 8.3 Side Bars

### 8.3.1 Record-Associated Side Bar

#### General View Control

Main switch for navigation through data is general view control (Fig. 23, page 127; see also Table 10, page 70). Selecting “Rpt” provides an overview on the Records of the current Report. Each Record is represented by key information such as data and time of measurement and notes; in case of slow kinetic measurements, the type of kinetics and the number of saturation pulse analyses are given.

To view a particular Record, double-click on the corresponding lines of the Record or select Record from the “Select Record”



drop down list on the rightmost side bar (Fig. 23, page 127, “General Side Bar”).

### Fm' Factor

The Fm' factor is a multiplier for Fm' values only; the Fm is not changed by the Fm' factor. This factor can be set to values > 1 if Fm' is underestimated due to measuring artifacts. Indeed, an increase of LED-chip temperature during a saturation pulse can decrease the intensity of pulse-modulated measuring light and, thus, result in Fm' underestimation. In the MULTI-

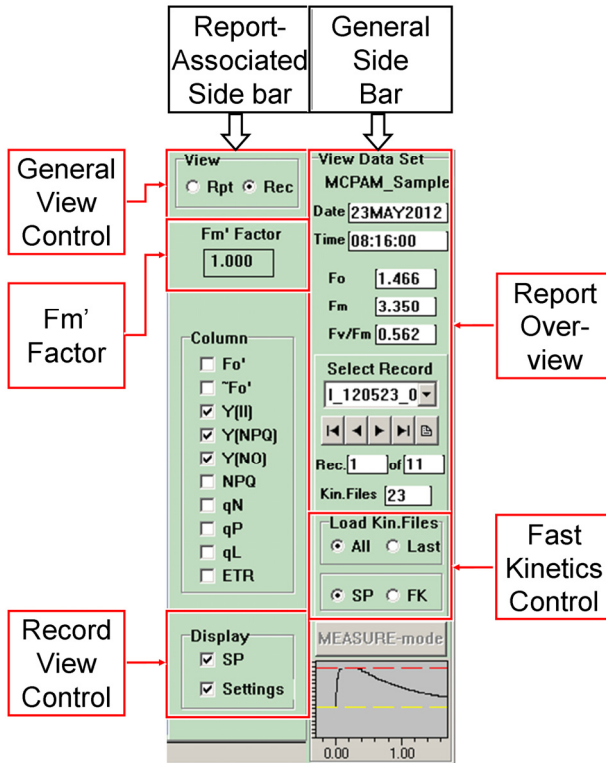


Fig. 23: Side bars in the VIEW mode.

COLOR-PAM, this underestimation is exceptionally small as ML and SP (=AL) LEDs are separated: even with SP-intensity setting of 20 and SP-width of 800 ms, underestimation is smaller than 0.5 % of  $F_m'$  at all colors except for 590 nm where the decrease amounts to about 1 %. Consequently, the default  $F_m'$  factor is 1.000 is appropriate in most cases.

The "SP artifact" can be detected using the plastic fluorescence standard delivered with the instrument. For a correct determination, however, one needs to consider that the standard foil displays a rather small heat capacity and heating of the standard during exposure to the saturation pulse may also cause a small decrease in chlorophyll fluorescence yield. The latter artifact can be minimized by placing a narrow strip of the fluorescence standard in a water-filled cuvette.

The standard foil is a relatively strong fluorescence emitter so that signal saturation can occur. Therefore, if the signal exceeds 6 V, adjust the distance between detector and the fluorescence-guiding Perspex rod appropriately.

Determination of the  $F_m'$  factor should be carried out using the same settings of color, SP intensity and SP-width as used for the physiological measurement. It is important that MF-High is already switched on before the SP is applied, as an increase of ML pulse frequency may also cause a temperature increase and, thus, a decrease in measuring light intensity. The latter artifact does not play a role during actinic illumination when "Auto MF high" is selected because the high measuring light frequency (MF-High), which is automatically invoked, is maintained during the SP.

The SP-induced signal decrease can be determined via a manual slow kinetics recording. The  $F_m'$  values listed in the

Report do not provide the required information, as these values are measured with the help of a peak-detector.

Finally, the  $F_m'$  factor can be calculated as the fluorescence level prior a saturation pulse divided by the fluorescence level during a saturation pulse. An  $F_m'$  factor remains assigned to a Record, unless changed.


### **Record View Control**

This section controls the extent of Record data displayed. Checking SP displays file names of saturation pulse kinetics. Selecting Settings, display initial settings of the Record and changes of settings made during the Record (e.g. actinic light intensity).

## **8.3.2 General Side Bar**

### **Report Overview**

This part of the “General Side Bar” provides tools for scrolling through the current Report.

The downward arrow of the text box “Select Record” opens the list of available PWS files from which a Record file can be selected. Below the text box, four arrow buttons permit selection of a Record (From left to right: first, previous, next, last Record). The  right-most icon opens a new Report file and, thus, is equivalent to the command “Open Report” in the File menu.

Below the arrow-buttons, the number of the currently selected Record and the total number of Records in the current Report are shown. A double click on the field “Rec.” opens a new window. This window permits navigation within the Report by en-

tering the sequence numbers of the Record to be displayed next.

The General Side Bar also shows the total number of Fast Kinetics files of current Record (Kin. Files). Fast Kinetics can be picked from the drop down list of the “Fast Kinetics” window, provided that FK on the “General Side Bar” is checked.

### **Fast Kinetics Control**

This part of the side bar offers two switches. Firstly, switching between All and Last determines whether opening of a Record occurs with loading of all fast kinetic files associated with the Record or with loading of only the last kinetic file of the Record. The latter option has advantages when computer performance limits the speed of data evaluation.

The second switch determines if fluorescence kinetics induced by saturation pulse (SP) or fast kinetics (FK) are displayed in the Fast Kinetics window. Depending on this selection, the drop down list of the Fast Kinetics window lists file names of SP or FK curves.

## 9 Definitions and Equations

### 9.1 Relative Fluorescence Yields

Typically, five different types of fluorescence levels are acquired by Saturation Pulse analyses. In most cases, the PAM fluorescence signal is proportionally related the yield for chlorophyll fluorescence. Therefore, differences between these five fluorescence levels reflect variations in chlorophyll fluorescence yields.

Two of these levels ( $F_o$  and  $F_m$ ) need to be determined with a dark-acclimated sample. The three remaining levels ( $F_o'$ ,  $F$ , and  $F_m'$ ) are repeatedly measured during subsequent sample treatments (e.g., exposure to actinic light; see Fig. 24, page 12).

#### 9.1.1 Measurements with Dark-Acclimated Samples

- F<sub>o</sub>** Minimum fluorescence level excited by very low intensity of measuring light to keep PS II reaction centers open.
- F<sub>m</sub>** Maximum fluorescence level elicited by a pulse of saturating light (Saturation Pulse) which closes all PS II reaction centers.

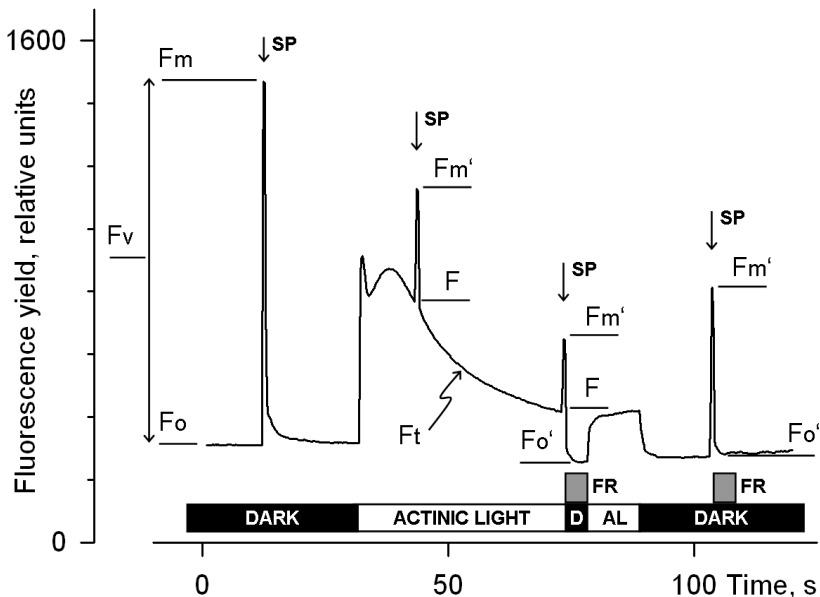


Fig. 24: Measurements for Saturation Pulse Analysis. AL, Actinic Light; D, dark; SP, Saturation Pulse; FR, Far-red illumination.

### 9.1.2 Measurements with Illuminated Samples

**Fo'** Minimum fluorescence level of illuminated sample which is lowered with respect to  $F_o$  by non-photochemical quenching. When the measuring routine for  $F_o'$  is active, the  $F_o'$  level is determined during a dark interval following the Saturation Pulse. In the dark interval, far-red light is applied to selectively drive PS I and to quickly remove electrons accumulated in the intersystem electron transport chain, thus reopening PS II reaction centers (see Fig. 24 (page 132), time point 75 s). Alternatively, the  $F_o'$  can be estimated according to

Oxborough and Baker (1997; List of references at end of Section: 9.8, page 146):

$$F_o' = \frac{1}{\frac{1}{F_o} - \frac{1}{F_m} + \frac{1}{F_m'}}$$

- Fm'** Maximum fluorescence level of illuminated sample as induced by a Saturation Pulse which temporarily closes all PS II reactions centers. Fm' is decreased with respect to Fm by non-photochemical quenching.
- F** The F corresponds to the momentary fluorescence level (Ft) of an illuminated sample measured shortly before application of a Saturation Pulse.

## 9.2 Fluorescence Ratio Parameters

To quantify photochemical use and non-photochemical losses of absorbed light energy, fluorescence ratio expressions have been derived which are based on the relative fluorescence yields introduced above. Table 17 (page 134) compiles the fluorescence ratio parameters available in PamWin-3. Subsequently, these parameters will be briefly explained.

**Fv/Fm and Y(II)** Maximum and effective photochemical quantum yields of PS II

The Fv/Fm and Y(II) estimate the fraction of absorbed quanta used for PS II photochemistry. Fv/Fm corresponds to the maximum Y(II). For measurements of Fv/Fm, it is important that samples are acclimated to darkness or dim light so that all reactions centers are in the open state and non-photochemical dissipation of excitation energy is minimal.

**Table 17: Fluorescence Ratio Parameters.**

Source	Equation
Maximum photochemical quantum yield of PS II (Kitajima and Butler, 1975)	$\frac{F_V}{F_M} = \frac{F_M - F_0}{F_M}$
Effective photochemical quantum yield of PS II (Genty <i>et al.</i> , 1989)	$Y(II) = \frac{F'_M - F}{F'_M}$
Quantum yield of light-induced ( $\Delta$ pH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Genty <i>et al.</i> 1996)*	$Y(NPQ) = \frac{F}{F'_M} - \frac{F}{F_M}$
Quantum yield of non-regulated heat dissipation and fluorescence emission: this type of energy loss does not involve the action of a trans-thylakoid $\Delta$ pH and zeaxanthin (Genty <i>et al.</i> 1996)*	$Y(NO) = \frac{F}{F_M}$
Stern-Volmer type non-photochemical fluorescence quenching (Bilger and Björkman, 1990)	$NPQ = \frac{F_M}{F'_M} - 1$
Coefficient of photochemical fluorescence quenching (Schreiber <i>et al.</i> 1986 as formulated by van Kooten and Snel, 1990)	$q_P = \frac{F'_M - F}{F'_M - F_0}$
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (Kramer <i>et al.</i> 2004)	$q_L = q_P \cdot \frac{F'_0}{F}$
Coefficient of non-photochemical fluorescence quenching (Schreiber <i>et al.</i> 1986 as formulated by van Kooten and Snel, 1990)	$q_N = 1 - \frac{F'_M - F'_0}{F_M - F_0}$

\* Kramer *et al.* (2004) have derived more complex equations for Y(NO) and Y(NPQ). Klughammer and Schreiber (2008) have demonstrated that the equations by Kramer *et al.* (2004) can be transformed into the simple equations of (Genty *et al.* 1996) which are used by PamWin-3 software.



In algae and cyanobacteria, the dark-acclimated state often is not showing maximal PS II quantum yield, as the PS II acceptor pool may be reduced in the dark by stromal reductants and consequently the so-called state 2 is formed exhibiting low PS II quantum yield. In this case, preillumination with moderate far-red light should precede determinations of  $F_o$  and  $F_m$ . The far-red preilluminated quasi-dark state normally serves as reference state with maximal PS II quantum yield for assessment of the functional absorption cross-section of PS II,  $\Sigma(\text{II})$ .

The  $Y(\text{II})$  value estimates the photochemical use of excitation energy in the light. It is lowered with respect to  $F_v/F_m$  by partial closure of PS II centers and various types of nonphotochemical energy losses induced by illumination. To derive from the  $Y(\text{II})$  information on the overall state of photosynthesis, knowledge of the absorbed PAR is essential, as a sample e.g. may be severely damaged in Calvin cycle activity and still show a high value of  $Y(\text{II})$  in weakly absorbed light. This aspect is particularly important in the study of algae and cyanobacteria, which display large wavelength-dependent differences in light absorption.

**$q_P$  and  $q_L$**  Coefficients of photochemical fluorescence quenching.

Both parameters estimate the fraction of open PS II reaction centers. The  $q_P$  is based on the concept of separated PS II antenna units (puddle model), whereas the  $q_L$  assumes interconnected PS II antenna units (lake model) which appears to be the more realistic situation in leaves (*cf.* Kramer *et al.*, 2004). Determinations of  $q_P$  and  $q_L$  do not require fluorescence measurements with the dark-acclimated sample except when  $F_o'$  is not measured but calculated according to Oxborough and Baker (1997).

**q<sub>N</sub> and NPQ** Parameters of non-photochemical quenching

Both parameters are associated with non-photochemical quenching of excitation energy, mainly involving a low thylakoid lumen pH- and a zeaxanthin-dependent quenching mechanism. In contrast to Y(II), qP and qL, calculations of the q<sub>N</sub> and the NPQ parameters always require fluorescence measurements with the sample both in the dark-acclimated and in the light-exposed states (see Table 17, page 134). Calculation of NPQ (or SV<sub>N</sub>; Gilmore and Yamamoto, 1991) corresponds to the Stern-Volmer equation for fluorescence quenching which predicts proportionality between fluorescence quenching (NPQ) and the concentration of fluorescence-quenching centers in the photosynthetic antennae (e.g. zeaxanthin).

**Y(NO), Y(NPQ) and Y(II)** Complementary PS II quantum yields

Genty *et al.* (1996) first presented expressions based on basic fluorescence parameters that describe the partitioning of absorbed excitation energy in PS II between three fundamental pathways, which were further investigated by Klughammer and Schreiber (2004) and expressed in terms of the complementary quantum yields of

Y(NO) sum of non-regulated losses of excitation energy including heat dissipation and fluorescence emission,

Y(NPQ) regulated energy losses of excitation energy *via* heat dissipation involving ΔpH- and zeaxanthin-dependent mechanisms, and

Y(II) use of excitation energy for charge separation.

The yields of photochemical energy conversion and non-photochemical losses sum up to 1:

$$Y(\text{II}) + Y(\text{NPQ}) + Y(\text{NO}) = 1$$

This concept of "complementary PS II quantum yields" is useful to analyze the partitioning of absorbed light energy in photosynthetic organisms. For instance, under high light-conditions, a much higher  $Y(\text{NPQ})$  than  $Y(\text{NO})$  indicates that excess excitation energy is safely dissipated at the antenna level, that is, photosynthetic energy fluxes are well-regulated. In variance, high values of  $Y(\text{NO})$  would signify that excess excitation energy is reaching the reaction centers, resulting in strong reduction of PS II acceptors and photodamage, e.g. via formation of reactive oxygen species.

### 9.3 Constant Fraction of $F_0$ Fluorescence ( $C/F_0$ )

The measured fluorescence signal does not only consist of PS II chlorophyll fluorescence, for which photochemical and non-photochemical quenching mechanisms apply. It is known that PS I chlorophyll contributes significantly to room temperature fluorescence from leaves, algae and cyanobacteria. In cyanobacteria also phycobilin fluorescence has to be considered. The non-PS II fluorescence yield is supposed to be constant during illumination. In leaves, typical values of PS I contribution to total  $F_0$  fluorescence at wavelength  $> 700$  nm are 30% for  $C_3$  and 50% for NADP-ME  $C_4$  plants (e.g., maize) (Genty *et al.* 1990, Pfündel 1998, Agati *et al.* 2000, Peterson *et al.* 2001, Franck *et al.* 2002, Rappaport *et al.* 2007).

The PamWin-3 offers a routine to estimate this contribution, which is expressed by the parameter  $C/F_0$ . The rationale of  $C/F_0$  estimation is as follows:

The original Oxborough & Baker equation is

$$F_o' = \frac{F_o}{\frac{F_m - F_o}{F_m} + \frac{F_o}{F_m'}} \quad (1)$$

which can be transformed into

$$F_o' = \frac{1}{\frac{1}{F_o} - \frac{1}{F_m} + \frac{1}{F_m'}} \quad (2)$$

Equations (1) or (2) would determine  $F_o'$  correctly if the  $F_o$ ,  $F_m$  and  $F_m'$  signals represent pure PS II fluorescence. In reality, there is always a contribution of PS I fluorescence, and often also from other signals (e.g. phycobilin fluorescence). If we assume that a constant signal,  $C$ , is contained in all fluorescence values of the Oxborough-Baker equation, then subtracting the  $C$  from all fluorescence levels results in an equation for pure PS II fluorescence:

$$F_o' - C = \frac{1}{\frac{1}{F_o - C} - \frac{1}{F_m - C} + \frac{1}{F_m' - C}} \quad (3)$$

After rearrangement, one obtains equation (4):

$$C/F_o = \frac{F_o'}{F_o} - \frac{1}{\frac{1}{1 - (C/F_o)} - \frac{F_m}{F_o - (C/F_o)} + \frac{F_m'}{F_o - (C/F_o)}} \quad (4)$$

Except the  $C$ , all fluorescence terms in equations (3) and (4) are measurable. The quotient  $C/F_o$  can be determined iteratively or calculated after solving equation (3) for  $C$ /  $C/F_o$ . Pam-Win-3 uses the latter method (see Pfündel et al. 2013).

In practice, determination of  $C/F_o$  requires:

- Dark-acclimated (in algae and cyanobacteria with far-red background light) sample for accurate determination of  $F_o$  and  $F_m$ .
- Subsequent to  $F_o$ ,  $F_m$  determinations, illumination at a light intensity that induces substantial non-photochemical quenching but does not yet cause appreciable photodamage, in order to measure  $F_o'$  and  $F_m'$ .

When  $C/F_o$  is known, it is possible to subtract  $C$  from all measured fluorescence values, which results in more accurate values saturation pulse analyses (except for the quenching coefficients  $q_P$  and  $q_N$ ). In particular, higher  $Y(II)$  values are obtained.

#### 9.4 Relative Electron Transfer Rate (ETR)

Relative electron transfer rates are calculated according to:

$$\mathbf{ETR = PAR \cdot ETR-Factor \cdot Y(II)}.$$

The basic idea of the ETR equation is to multiply  $Y(II)$ , the effective photochemical quantum yield of PS II, by an estimate for the photon flux density absorbed by PS II. The latter approximation uses two parameters which are explained below:

**PAR**                      Quantum flux density of photosynthetically active radiation

Depending on settings, the PAR values of the active light list or measured data are used.

**ETR-Factor** (Absorptance of photons by PS II)

The ETR-Factor describes the fraction of incident photons absorbed by PS II pigments, which corresponds to overall ab-

sorptance multiplied by the PS II distribution factor ( $P_{PS2}/P_{PPS}$ , see below). The default value for the ETR-Factor is 0.42 which reasonably describes the average PS II absorptance in the visible range (400-700 nm) of many green leaves. Anthocyanins, however, can reduce availability of blue photons for photosynthesis (Pfündel *et al.* 2007) and, hence, lower the ETR-Factor.

In the case of suspensions, of course, total absorptance normally is much lower than 0.84 and PS I/PS II ratios cannot be assumed to be unity, in contrast to leaves. Furthermore, in dilute suspensions of algae and cyanobacteria both overall absorptance and distribution of absorbed quanta to PS II display large differences at different wavelengths. For example, in cyanobacteria at equal quantum flux densities 625 nm light is about 25 times more effective in driving PS II than 480 nm light. To obtain reasonable fluorescence-based estimates of electron transport rates in suspensions, PamWin-3 uses the approach introduced by Schreiber *et al.* (2011) which permits calculation of quanta absorbed by PS II, PAR(II), from incident PAR and Sigma(II), the wavelength-dependent absorption cross-section of PS II.

**$P_{PS2}/P_{PPS}$**  Photons absorbed by PS II relative to photons absorbed by all photosynthetic pigments.

The default value for  $P_{PS2}/P_{PPS}$  for leaves is 0.5. If the PS I/PS II ratio is unity in leaves, as often assumed, this value is reasonable if only linear electron transport is considered, that is, equal electron transfer rates though PS I and PS II, and comparable photochemical quantum yields of PS I and PS II under strongly light-limiting conditions.

The ETR may be compared to the rate of CO<sub>2</sub>-assimilation or of O<sub>2</sub>-evolution. For such comparison the following aspects are relevant:

- 4 e<sup>-</sup> must be transported for every CO<sub>2</sub> assimilated or O<sub>2</sub> evolved
- the value of ETR/4 is not necessarily identical to CO<sub>2</sub>-fixation rate or O<sub>2</sub>-evolution rate; discrepancies e. g. may arise from O<sub>2</sub> dependent electron flow (photorespiration and Mehler-Ascorbate-Peroxidase cycle, nitrite reduction or electron cycling at PS II)
- fluorescence information primarily originates from the top-most chloroplast layers, while gas exchange integrates over all layers; on the other hand, the topmost layers absorb most of the light and, hence, are also responsible for most of the gas exchange, unless photoinhibited.

The combined information of ETR, PAR and Temperature provides 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. In the case of suspensions of algae and cyanobacteria, however, it has to be considered that the obtained values of ETR give a relative measure of photosynthetic electron transport rate only, unless for the same sample and identical color of illumination the absolute rate of quanta absorption by PS II is known. The MULTI-COLOR-PAM provides the means to determine the wavelength-dependent functional absorption cross-section of PS II, Sigma(II), in dilute suspensions, based on which absolute rates of PAR(II) and ETR(II)

can be calculated (for definitions, see the following section).

## 9.5 Parameters and Output of Fo-I<sub>1</sub> Rise Analysis

**Table 18:** Adopted from: Schreiber et al. (2012) Photosynth Res 113: 127-144

Parameters		Definition
<b>Free model parameters</b>		
J	Unit: dimensionless	Sigmoidicity parameter. $J = p/(1-p)$ , $p$ is Joliot's connectivity parameter describing excitation energy flux from a PS II to neighboring PS II.
Tau	Unit: s	Time constant of light-driven Q <sub>A</sub> reduction.
Tau(reox)	Unit: s	Time constant of Q <sub>A</sub> <sup>-</sup> reoxidation.
<b>Data input</b>		
F <sub>0</sub> (or O)	Unit: mV	Fluorescence in the dark-acclimated state. Derived from initial fluorescence level of F <sub>0</sub> → I <sub>1</sub> rise.
I <sub>1</sub>	Unit: mV	Maximum fluorescence elicited by a saturating single turnover flash. Derived from back-extrapolation of fluorescence decay after saturating single turnover flash.
PAR		Photosynthetically active radiation.
Unit: $\mu\text{mol quanta} \cdot (\text{m}^2 \cdot \text{s})^{-1}$		
<b>Derived parameters</b>		
$k(\text{II})=1/\text{Tau}$	Unit: s <sup>-1</sup>	Rate constant of light-driven Q <sub>A</sub> reduction
$\text{Sigma}(\text{II})_{\lambda}=k(\text{II}) \cdot (\text{L} \cdot \text{PAR})^{-1}$		Wavelength-dependent functional cross-section of PS II
Unit: nm <sup>2</sup>		
$\text{PAR}(\text{II})=k(\text{II})=\text{Sigma}(\text{II})_{\lambda} \cdot \text{L} \cdot \text{PAR}$		Rate of quantum absorption of PS II
Unit: quanta · (PS II · s) <sup>-1</sup>		
$\text{ETR}(\text{II})=\text{PAR}(\text{II}) \cdot \text{Y}(\text{II}) \cdot (\text{F}_v/\text{F}_m)^{-1}$		Wavelength-dependent PS II-based electron transport rate
Unit: electrons · (PS II · s) <sup>-1</sup>		
<b>Constant</b>		
$\text{L}=6.022 \cdot 10^{23} \text{ mol}^{-1}$		Avogadro constant



## 9.6 Light Curves

The light curve program of PamWin-3 exposes the sample to increasing intensities of actinic illumination. If the time interval of each intensity level is too short for full equilibration of photosynthetic reactions one refers to “Rapid Light Curves” (RLC). Typically, RLC measurements are carried out with samples in their momentary acclimation status, that is, without intermediate acclimation period. This way, RLC data can provide information on the present acclimation state of photosynthesis.

Measurements of RLC should not be confused with classical photosynthetic light response curves in which photosynthetic rates under steady state conditions are plotted against light intensities. In both cases, plotting ETR versus PAR yields light response curves which are often described by the following three cardinal points.

- $\alpha$  (alpha), electrons/photons: Initial slope of RLC which is related to quantum efficiency of photosynthesis.
- $ETR_{\max}$ ,  $\mu\text{mol electrons}/(\text{m}^2 \cdot \text{s})$ : Maximum electron transport rate.
- $I_K$ ,  $\mu\text{mol photons}/(\text{m}^2 \cdot \text{s})$ : Minimum saturating irradiance.

The EP function used by the PamWin-3 software has been derived by Eilers and Peeters (1988) using a mechanistic model which considers the processes of photosynthesis and photoinhibition. Their final model function:

$$ETR = \frac{PAR}{a \cdot PAR^2 + b \cdot PAR + c}$$

In the above equation, the  $a$ ,  $b$ , and  $c$  are free parameters which are varied by PamWin-3 until best fit between theory and data is achieved.

The cardinal points of a light curve are calculated according to subsequent equations:

$$\alpha = \frac{1}{c}$$

$$ETR_{\max} = \frac{1}{b + 2 \cdot \sqrt{a \cdot c}}$$

$$I_k = \frac{c}{b + 2 \cdot \sqrt{a \cdot c}}$$

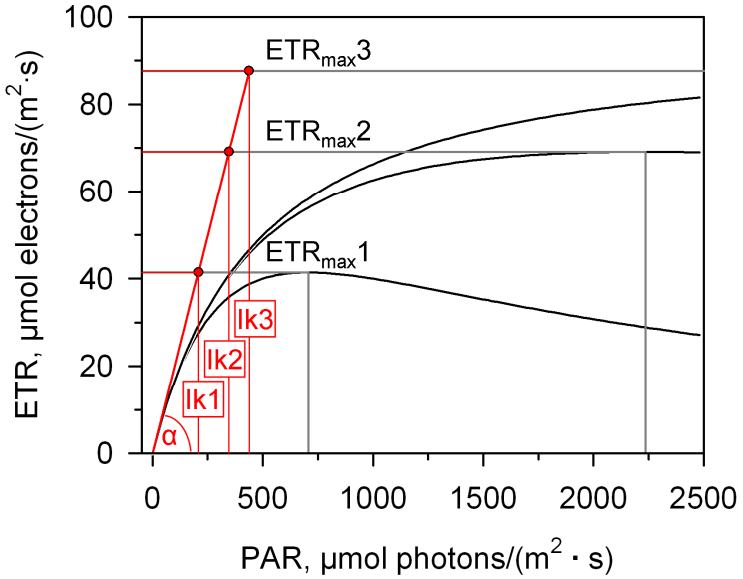
Fig. 25, page 145, illustrates the behavior of the Eilers and Peeters model function for 3 theoretical cases which show identical values of  $\alpha$  but, at high light intensities, different degrees of photoinhibition.

Also, the [Platt \*et al.\* \(1980\)](#) model considers photoinhibition but their equation is not based on mechanistic considerations but on the empirical finding the many light response curves can be described by the product of 2 exponentials:

$$ETR = ETR_{mPot} \cdot \left(1 - e^{-\frac{\alpha \cdot PAR}{ETR_{mPot}}}\right) \cdot e^{-\frac{\beta \cdot PAR}{ETR_{mPot}}}$$

The free parameters in the latter equation are  $ETR_{mPot}$ ,  $\alpha$  and  $\beta$ , where the  $ETR_{mPot}$  is the asymptote value of the rising exponential in the equation above.

The cardinal parameter  $\alpha$  result directly from curve fitting. The two remaining cardinal parameter can be obtained with subsequent equation:



Curve	Parameter					
	a	b	c	$\alpha$	$\text{ETR}_{\text{max}}$	$I_k$
1	$1 \cdot 10^{-5}$	$1 \cdot 10^{-2}$	5	0.2	41	207
2	$1 \cdot 10^{-6}$	$1 \cdot 10^{-2}$	5	0.2	69	345
3	$1 \cdot 10^{-7}$	$1 \cdot 10^{-2}$	5	0.2	88	438

Fig. 25: Exemplary Light Curves.

$$\text{ETR}_m = \text{ETR}_{\text{mPot}} \cdot \left(\frac{\alpha}{\alpha + \beta}\right) \cdot \left(\frac{\beta}{\alpha + \beta}\right)^{\frac{\beta}{\alpha}}$$

$$E_k = \frac{\text{ETR}_m}{\alpha}$$

The model equation of Platt *et al.* (1980) considers photoinhibition by the “photoinhibition parameter”  $\beta$ . Platt *et al.* (1980) introduced the “Photoinhibition Index” ( $I_b$ ) to quantify photoinhibition. The authors defined  $I_b$  as the PAR value required to photoinhibit  $ETR_{mPot}$  by the factor of  $1/e$  according to:

$$I_b = ETR_{mPot} / \beta$$

### 9.7 References (Light Response Curves)

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol Oceanogr* 21: 540-547

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J Mar Res* 38: 687-701

### 9.8 Additional Literature Cited

Agati G, Cerovic ZG, Moya I (2000) The effect of decreasing temperature up to chilling values on the in vivo F685/F735 chlorophyll fluorescence ratio in *Phaseolus vulgaris* and *Pisum sativum*: the role of the Photosystem I contribution to the 735 nm fluorescence band, *Photochem Photobiol* 72: 75–84

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth Res* 25:173-185

Eilers PHC, Peeters JCH (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol Model* 42: 199-215

Franck F, Juneau P, Popovic R (2002) Resolution of the Photosystem I and Photosystem II contributions to chlorophyll fluo-

rescence of intact leaves at room temperature. *Biochim Biophys Acta* 1556: 239–246

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990: 87–92

Genty B, Harbinson J, Cailly AL and Rizza F (1996) Fate of excitation at PS II in leaves: the non-photochemical side. Presented at: The Third BBSRC Robert Hill Symposium on Photosynthesis, March 31 to April 3, 1996, University of Sheffield, Department of Molecular Biology and Biotechnology, Western Bank, Sheffield, UK, Abstract P28

Genty B, Wonders J and Baker NR (1990) Non-photochemical quenching of  $F_0$  in leaves is emission wavelength dependent: Consequences for quenching analysis and its interpretation. *Photosynth Res* 26: 133–139

Gilmore AM, Yamamoto HY (1991) Zeaxanthin formation and energy-dependent fluorescence quenching in pea chloroplasts under artificially mediated linear and cyclic electron transport. *Plant Physiol* 96: 635–643

Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochim Biophys Acta* 376:105-115

Klughammer C and Schreiber U (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Application Notes* 1: 27-35

([http://www.walz.com/e\\_journal/pdfs/PAN078007.pdf](http://www.walz.com/e_journal/pdfs/PAN078007.pdf))

Kramer DM, Johnson G., Kiirats O, Edwards GE (2004) New flux parameters for the determination of  $Q_A$  redox state and excitation fluxes. *Photosynth Res* 79: 209-218

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of  $qP$  and  $F_v'/F_m'$  without measuring  $F_o'$ . *Photosynth Res* 54 135-142

Peterson RB, Oja V, Laisk A (2001) Chlorophyll fluorescence at 680 and 730 nm and leaf photosynthesis. *Photosynth Res* 70: 185–196

Pfündel EE (1998) Estimating the contribution of Photosystem I to total leaf chlorophyll fluorescence. *Photosynth Res* 56: 185–195

Pfündel EE, Ben Ghazlen N, Meyer S, Cerovic ZG (2007) Investigating UV screening in leaves by two different types of portable UV fluorimeters reveals in vivo screening by anthocyanins and carotenoids. *Photosynth Res* 93. 205-221

Pfündel EE, Klughammer C, Meister A, Cerovic ZG (2013) Deriving fluorometer-specific values of relative PSI fluorescence intensity from quenching of  $F_o$  fluorescence in leaves of *Arabidopsis thaliana* and *Zea mays*. *Photosynth. Res.* DOI 10.1007/s11120-012-9788-8

Rappaport F, Béal D, Joliot A, Joliot P (2007) On the advantages of using green light to study fluorescence yield changes in leaves. *Biochim Biophys Acta* 1767: 56–65

Schreiber U, Klughammer C, Kolbowski J (2011) High-end chlorophyll fluorescence analysis with the MULTI-COLOR-PAM. I. Various light qualities and their applications. *PAN* (2011) 1: 1-19

<http://www.walz.com/downloads/pan/PAN11001.pdf>

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res* 10: 51–62

van Kooten O, Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth Res* 25: 147–150





## 10 Some Reviews on Chlorophyll Fluorescence

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu Rev Plant Biol* 59: 89–113

Bernhardt K, Trissl H-W (1999) Theories for kinetics and yields of fluorescence and photochemistry: how, if at all, can different models of antenna organization be distinguished experimentally? *Biochim Biophys Acta* 1409: 125-142

Butler WL (1978) Energy distribution in the photochemical apparatus of photosynthesis. *Annu Rev Plant Physiol* 29:345-378

Dau H (1994) Molecular mechanisms and quantitative models of variable photosystem II fluorescence. *Photochem Photobiol* 60:1-23

Demmig-Adams B and Adams WW, III (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43:599-626

Govindjee (1995) Sixty-three years since Kautsky: Chlorophyll a fluorescence. *Aust J Plant Physiol* 22:131-160

Haldrup A, Jensen PE, Lunde C, Scheller HV (2001) Balance of power: a view of the mechanism of photosynthetic state transitions. *Trends Plant Sci* 6: 301-305

Krause GH and Weis E (1991) Chlorophyll fluorescence and photosynthesis: The basics. *Annu Rev Plant Physiol Plant Mol Biol* 42:313-349

Krause GH, Jahns P (2004) Non-photochemical energy-dissipation determined by chlorophyll fluorescence quenching:

characterization and function. In: Papageorgiou GC, Govindjee (eds.) Chlorophyll a Fluorescence: A Signature of Photosynthesis. Springer, The Netherlands, pp. 463-495

Lazár D (1999) Chlorophyll a fluorescence induction. *Biochim Biophys Acta* 1412: 1-28

Logan BA, Adams III WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. *Funct Plant Biol* 34, 853–859

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *J Exp Bot* 51, 659–668.

Mohammed GH, Binder WD, Gillies SL (1995) Chlorophyll fluorescence: A review of its practical forestry applications and instrumentations. *Scand J Forest Res* 10:383-410

Nedbal L, Koblížek M (2006) Chlorophyll fluorescence as a reporter on *in vivo* electron transport and regulation in plants In: Grimm B, Porra RJ, Rüdiger W, Scheer H (eds) *Advances in Photosynthesis and Respiration, Vol 25, Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications*. Springer, The Netherlands, pp 507-519

Schreiber U (2004) Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. In: Papageorgiou GC, Govindjee (eds.) *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Springer, The Netherlands, pp. 279-319

Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis. *Ecological studies: analysis and synthesis (USA)* 100: 49-70

# 11 Specifications

## MULTI-COLOR-PAM

### 11.1 Basic System

#### 11.1.1 Power-and-Control-Unit MCP-C

**General design:** Microcontroller: 2 x AVR-RISC (8 MHz) + 4 MB SRAM; 256000 data points with 12 bit resolution can be stored

**Sockets:** 2 sockets for measuring light and actinic light of MCP-E Emitter Head, socket for signal detection by MCP-D Detector Head, charge socket or Battery Charger MINI-PAM/L, output socket for PHYTO-MS Miniature Magnetic Stirrer, BNC sockets for 5 V trigger-in and trigger out signals, input socket for US-SQS/WB Spherical Micro Quantum Sensor or US-MQS/WB Mini Quantum Sensor, input socket for auxiliary devices, connector for optional PS I lamp, USB socket

**Communication:** USB 1.1, USB 2.0 and USB 3.0 compatible

**User interface:** Windows computer with PamWin-3 software

**Power supply:** Rechargeable sealed lead-acid battery 12 V/2 Ah; Battery Charger MINI-PAM/L (100 to 240 V AC)

**Dimensions:** 31 cm x 16 cm x 33.5 cm (W x H x D), aluminum housing with carrying handle

**Power consumption:** Basic operation 1.6 W, 8 W with all internal light sources operated at maximum output. Saturation Pulse at maximum intensity, 30 W

**Weight:** 2.5 kg (including battery)

**Operating temperature:** -5 to +40 °C

### 11.1.2 Battery Charger MINI-PAM/L

**Input:** 90 to 264 V AC, 47 to 63 Hz

**Output:** 19 V DC, 3.7 A

**Operating temperature:** 0 to 40 °C

**Dimensions:** 15 cm x 6 cm x 3 cm (L x W x H)

**Weight:** 300 g

### 11.1.3 Emitter Head MCP-E

**Chip-on-board multi-wavelength measuring light LED emitter:** 400, 440, 480, 540, 590, and 625 nm for pulse-modulated measuring light; 20 intensity settings and 14 settings of pulse frequency

**Chip-on-board multi-wavelength actinic LED array:** 440, 480, 540, 590, 625 and 420-640 nm (white) for continuous actinic illumination, max.  $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR; saturating single turnover flashes, max.  $200000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, adjustable between 5 and 50  $\mu\text{s}$ ; multiple turnover flashes, max.  $12000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, adjustable between 1 and 800 ms

**Far-Red LED:** peak wavelength 725nm

**Dimensions:** 10.5 cm x 5.5 cm x 7 cm (L x W x H)

**Weight:** 500 g (incl. cables, 1 m long)

#### 11.1.4 Detector Head MCP-D

**Signal detection:** PIN photodiode with special pulse preamplifier for measuring fluorescence changes with maximum time resolution of 10  $\mu\text{s}$

**Filter box:** For up to 14 mm filter thickness

**Standard detector filter:** long-pass filter > 650 nm (3 mm RG 665) plus short-pass filter SP 710

**Dimensions:** 69.3 cm x 97.7 cm x 64.0 cm (L x W x H)

**Weight:** 355 g (incl. cables, 1 m long)

### 11.1.5 Optical Unit for Suspensions ED-101US/MD

**Design:** Black-anodized aluminum body with central 10 x 10 mm standard glass cuvette; for attachment of Measuring Heads MCP-E and MCP-D, and Miniature Magnetic Stirrer PHYTO-MS; two additional ports for attachment of two additional devices

**Weight:** 750 g

### 11.1.6 Stand ST-101

Stand for mounting the Optical Units ED-101US/MD (suspensions) or MCP BK (leaves)

### 11.1.7 Transport Box 2040-T

**Design:** Aluminum box with custom foam packing for MULTI-COLOR-PAM and accessories

**Dimensions:** 60 cm x 40 cm x 25 cm (L x W x H)

**Weight:** 5 kg

### 11.1.8 System Control and Data Acquisition

**Software:** PamWin-3 System Control and Data Acquisition Program (Windows XP/Vista, Windows 7 32-bit and 64-bit) for operation of measuring system via PC, data acquisition and analysis

## Saturation Pulse Analysis.

Measured:  $F_t$ ,  $F_o$ ,  $F_m$ ,  $F$ ,  $F_o'$  (also calculated),  $F_m'$ . Fast polyphasic rise and decay kinetics (time resolution up to 10  $\mu$ s). PAR using Spherical Micro Quantum Sensor US-SQS/WB or Mini Quantum Sensor US-MQS/WB. Calculated:  $F_o'$  (also measured),  $F_v/F_m$  and  $Y(II)$  (maximum and effective photochemical yield of PS II, respectively),  $q_L$ ,  $q_P$ ,  $q_N$ , NPQ,  $Y(NPQ)$ ,  $Y(NO)$  and ETR (electron transport rate),  $C/F_o$  (constant fraction of  $F_o$  not constituting PS II chlorophyll fluorescence)

## Fitting Routines

Fitting routine for fast fluorescence rise from 0 to the  $I_1$  level to determine functional absorption cross-section of PS II and PS II-specific electron transport rates. Fitting of fluorescence decay after light-to-dark transition by up to three exponentials to estimate primary electron transfer rates. Fitting routine for determination of the cardinal points  $\alpha$ ,  $I_k$  and  $ETR_{max}$  of light curves

## Computer Requirements

Processor, 0.8 GHz. RAM, 512 MB. Screen resolution, 1024 x 600 pixels. Interface, USB 2.0/3.0. Operating system: Microsoft Windows XP SP2/Vista/Windows 7 32 and 64 bit

## **11.2 Accessories for Suspensions**

### **11.2.1 Temperature Control Block for Cuvette ED-101US/T**

Sectioned block with central 10 x 10 mm opening to be mounted on top of the ED-101US/MD unit; to be connected to external flow-through water bath (not included), weight: 250 g

### **11.2.2 Temperature Control Unit US-T**

Consists of Power and Control Unit US-T/DR with separate AC power supply and Peltier-Heat-Transfer Rod US-T/DS. Temperature range: 12 K below ambient temperature, 15 K above ambient temperature. To be mounted on top of 10 x 10 mm cuvette.

### **11.2.3 Spherical Micro Quantum Sensor US-SQS/WB**

3.7 mm diffusing Plexiglas sphere coupled to integrated PAR-sensor via 2 mm fiber, compact amplifier unit and special holder for mounting on Optical Unit ED-101US/MD; to be connected to Power-and-Control-Unit MCP-C.

### **11.2.4 Miniature Magnetic Stirrer PHYTO-MS**

Type Variomag Mini, with adapter for bottom port of Optical Unit ED-101US/MD and connector to plug into



Power-and-Control-Unit MCP-C. Control via MCP-C and PamWin software.

### **11.3 Accessories for Leaves**

#### **11.3.1 Optical Unit for Leaf Measurements MCP-BK**

Featuring optical ports for mounting Measuring Heads MCP-E and MCP-D; including leaf clip holder, with opening for fixing Mini Quantum Sensor US-MQS/WB in the leaf plane. To be mounted on stand.

#### **11.3.2 Mini Quantum Sensor US-MQS/WB**

Consisting of cosine-corrected PAR-sensor (housing: diameter 14 mm, height 16 mm; diffuser diameter 5.5 mm) with cable 3 m long (BNC connector), compact amplifier unit and adapter set. To be connected to the Power-and-Control-Unit MCP-C. For measuring incident PAR, when using the Optical Unit for Leaf Measurements MCP-BK.

Subject to change without prior notice



## 12 Warranty

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

### 12.1 Conditions

This warranty applies if the defects are called to the attention of Heinz Walz GmbH, Germany, in writing within one year (1) of the shipping date of the product.

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

## 12.2 Instructions

**To obtain warranty service, please follow the instructions below:**

1. The Warranty Registration form must be completed and returned to Heinz Walz GmbH, Germany.
2. The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, custom duties, and/or shipping costs incurred in returning equipment for warranty service are at customer expense. Accompany shipment by the Walz Service and Repair form:  
<http://www.walz.com/downloads/global/Service03.pdf>
3. All products being returned for warranty service must be carefully packed and sent freight prepaid.
4. Heinz Walz GmbH, Germany is not responsible or liable, for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

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