DUAL-PAM-100
DUAL-PAM/F

MANUAL

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1 Contents

2 Safety Instructions

2.1 General Safety Instructions

- Read safety instructions and the operating instructions prior to operation of the device and its accessories.
- Pay attention to all safety warnings.
- Keep device and its accessories away from water or high moisture areas.
- Keep the device and its accessories away from dust, sand, and dirt.
- Do not put the device and its accessories near sources of heat.
- Ensure that neither liquids nor foreign bodies get inside the device or its accessories.
- 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.
- The device and its accessories should only be repaired by qualified personnel.

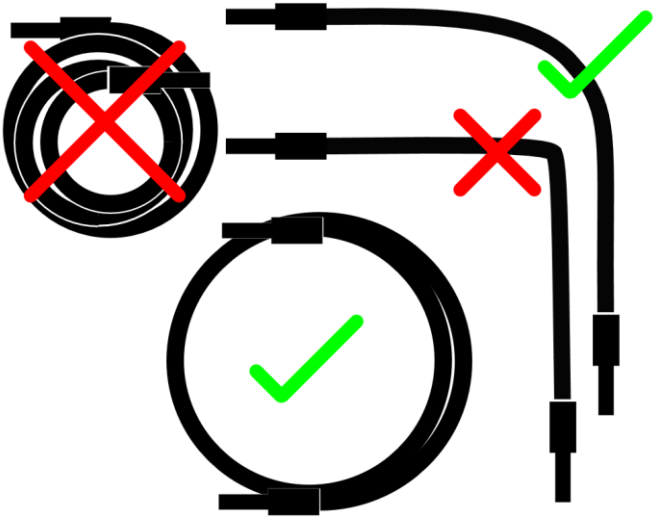
2.2 Special Safety Instructions

- The DUAL-PAM-100 and the DUAL-PAM/F are a highly sensitive instruments which should be only used for research purposes, as specified in this manual. Follow the instructions of this manual to avoid potential harm to the user and damage to the instrument.

- The DUAL-PAM-100 and the DUAL-PAM/F can emit very strong light! To avoid harm to your eyes, never look directly on the light exits of the DUAL-PAM-100 measuring heads or the DUAL-PAM/F fiberoptics.
- Handle measuring heads carefully. Avoid any impact or force acting on the Perspex light guides of the measuring heads.

2.3 Handling of Fiberoptics

The Special Fiberoptics 2010-F of the device DUAL-PAM/F is made of delicate glass fibers. Each glass fiber extents over the entire length of the fiber optics. Do not sharply bend, stretch, or crush the fiber optics as this can break the glass fibers. Each broken fiber reduces light transmission by the fiber optics. If many fibers are broken, the fiber optics must be replaced. Place protection caps on fiber ends when the fiber optics is not in use.



3 Introduction

3.1 DUAL-PAM Systems

The DUAL-PAM-100 and its fiber version, the DUAL-PAM/F, simultaneously analyze the state and performance of photosystem I and photosystem II. Photosystem I is analyzed by an absorption spectroscopic method. This procedure measures the absorption change associated with the oxidation (closure) of the photosystem I reaction center. As for photosystem II, saturation pulses are employed to generate maximum closure of photosystem I reaction centers. For photosystem II, the established combination of pulse-amplitude modulated and saturation pulse analysis is employed.

For photosystem I analysis, DUAL-PAM systems use two wavelengths (sample and reference) to reduce signal noise and baseline drift. The further advanced DUAL-KLAS-NIR employs four wavelength pairs to monitor the redox state of plastocyanin and ferredoxin, in addition to the photosystem I reaction center.

DUAL-KLAS-NIR: https://www.walz.com/products/chl_p700/dual-klas-nir/introduction.html)

The earlier established DUAL-PAM system has introduced features which are in the process of being added to the DUAL-KLAS-NIR. Examples are the modules to measure the electrochromic band shift, NADPH formation, or pH sensitive dyes (see Section 5, page 29), but also the flux method to quantitatively analyze the electron flux through photosystem I. Only the DUAL-PAM system is available as fiber version.

3.2 Comment to the Manual

This manual deals with the standard configuration of DUAL-PAM systems, that is the combined measurement of photosystem I and II. During the past years, the options and features of the DualPAM software were considerably extended. The manual has grown correspondingly. Navigation in the electronic manual is particularly easy because its hyperlinks lead by mouse click to the cited place. Download here: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html

The current manual provides information for setting up a system and installing software (Chapter 4). A full list of accessories gives Chapter 5.

The description of the software (Chapter 6) forms the main part of the manual. The various settings and options are described in Sections 6.1 and 6.2. Section 6.3 deals with the SP Analysis Mode of the software and Section 6.4 explains the Fast Acquisition Mode. Section 6.5 section introduces script file programming of experiments.

Chapter 7 is a short introduction into saturation pulse analysis and Chapter 8 includes subjects such as general hints, advice for trouble shooting and maintenance. Specifications for DUAL-PAM systems and accessories are provided in Chapter 9.

The manual closes with Chapter 10 (Guarantee), Chapter 11 (Index), Chapter 12 (List of Figures), and Chapter 13 (List of Tables).

I have designed a number of figures and tables to facilitate the understanding of the sometimes complex matter. I hope that these elements are helpful.

4 Basic Systems

4.1 MODULAR VERSION

4.1.1 Extent of Delivery (Basic System MODULAR)

Table 1: Extent of delivery MODULAR Version

Power-and-Control-Unit	DUAL-C
Measuring Head with P700 NIR Emitter	DUAL-E
Measuring Head with blue Fluorescence Measuring Light, or Measuring Head with red Fluorescence Measuring Light	DUAL-DB or DUAL-DR
Charging unit	MINI-PAM/L
Mounting for measuring heads	DUAL-B
Stand with clamp holders	ST-101
Adapter for one-sided illumination	DUAL-TW
USB type A to A cable	
Fluorescence standard foil (see 4.1.3, page 14)	
Manual	
WALZ Manuals & Software CD	
Transport box	PHYTO-T

4.1.2 Control Unit

All connections and control elements of the DUAL-PAM-II control unit are described in Fig. 1 and Table 2. How detector and emitter measuring heads are connected is illustrated in Fig. 4.

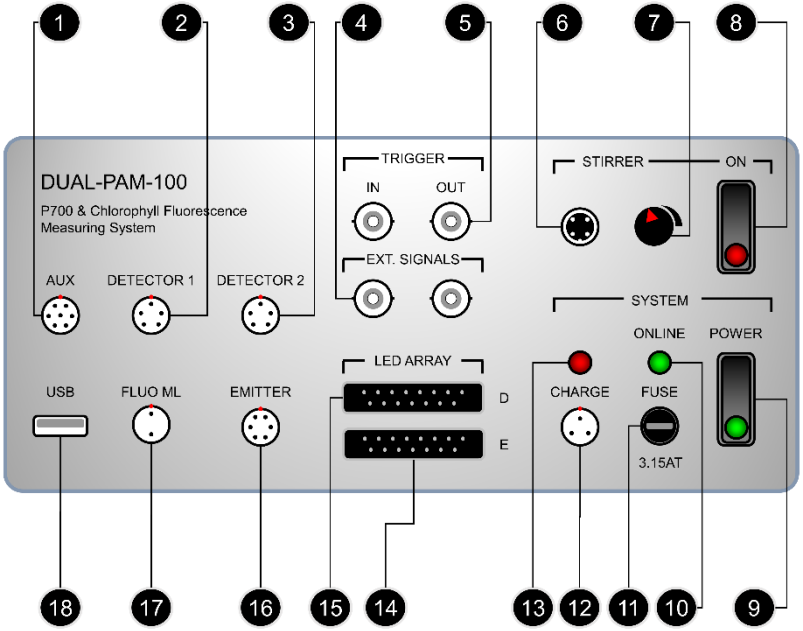


Fig. 1: Front panel of Control-Unit (MODULAR Version)

Fig. 2: Self-locking Connector (MODULAR Version)

To lock connection, align red dots of plug and socket and push in. To disconnect, hold the knurled sleeve and pull out. Applies for connection numbers 1-3, 12, 16, and 17 in Fig. 1.

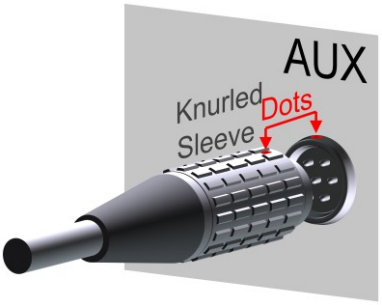
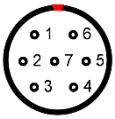


Table 2: Front Panel of MODULAR Version

Number in Fig. 1, page 12	Item	Function
1	AUX	Connection for light sensor US-SQS/WB or 2030-B leaf clip.  (1) +5 V, (2) Ground, (3) to (5) Analog inputs (0 - 2.5 V), (6) Trigger in (5 V), (7) -5 V.
2	DETECTOR 1	Connection for DETECTOR cable of DUAL-DB or DUAL-DR measuring unit
3	DETECTOR 2	Not used for standard fluorescence/P700 measurements
4	EXT. SIGNALS	Two BNC sockets to connect external DC signals, 0 - 2.5 V. Possible combinations: Left BNC + P700, right BNC + fluorescence, left and right BNC
5	TRIGGER	IN: Input for a 5 V trigger pulse to start a fast kinetics. OUT: Output for a 5 V trigger pulse to start an external event
6	STIRRER (Socket)	Connection for stirrer cable
7	STIRRER Rotary button	Control for rotational speed
8	STIRRER ON/OFF switch	ON puts the stirrer in the standby. Activate stirring by the “Trigger” command of the software
9	SYSTEM POWER switch	The inbuilt LED shines green when the unit is switched on
10	SYSTEM ONLINE LED	Green shining LED indicates communication with the DualPAM software
11	SYSTEM FUSE	Fuse holder for a 3.15 Ampere slow-blow fuse

12	SYSTEM CHARGE Socket	Connection for charger
13	SYSTEM CHARGE LED	The red LED flashes when the device is charged
14	LED ARRAY E	Connection for LED ARRAY cable of DUAL-E measuring unit. (Connecting the LED ARRAY cable via the DUAL-TW adapter inactivates the red actinic light of the DUAL-E measuring unit. P700 measuring light and far-red light are not affected)
15	LED ARRAY D	Connection for LED ARRAY cable of DUAL-DB or DUAL-DR measuring unit
16	EMITTER	Connection for EMITTER cable of DUAL-E measuring unit
17	FLUO ML	Connection for FLUO ML cable of DUAL-DB or DUAL-DR measuring unit
18	USB	Connection for USB cable

4.1.3 Setup Leaf

Installation site

Set up the system at a place which is not exposed to sunlight or frequency flickering light sources. If possible select a dim environment. Keep away from heat sources and humidity. The location must be free of vibrations which interfere with P700 measurements.

Measuring heads

The setup of measuring heads is illustrated in Fig. 3: Assemble stand and attach with clamp holder the mounting for measuring heads. Adjust guide pins inside of guide rings so that they extent into the lower grove of the black front tube of the measuring heads.

The guide pins and locking screws of the guide rings must not obstruct back and forth motion of measuring heads. Insert measuring heads in guide rings. Leave space between the two Perspex cuboids for mounting the leaf adapter rings. Secure measuring heads with locking screw. Push on leaf adapter rings and secure by their locking screw (see ↓ in Fig. 3). Place sample between leaf adapter rings. Loosen locking screws of guide rings and push together measuring heads. Do not squeeze the sample.

Connect measuring heads to main unit as shown in Fig. 4.

Fluorescence Standard Foil

The fluorescence standard has a metallic-shiny face and a dark-violet face. The violet color originates from a fluorescent dye. The fluorescence standard can be employed to test the fluorometer function.

Applying a saturation pulse to the fluorescence standard does not affect the PAM fluorescence signal because the fluorescence yield does not respond to strong light and remains constant. Different from that, a saturation pulse increases the PAM fluorescence signal of a leaf because the leaf increases the yield for fluorescence in response to strong light.

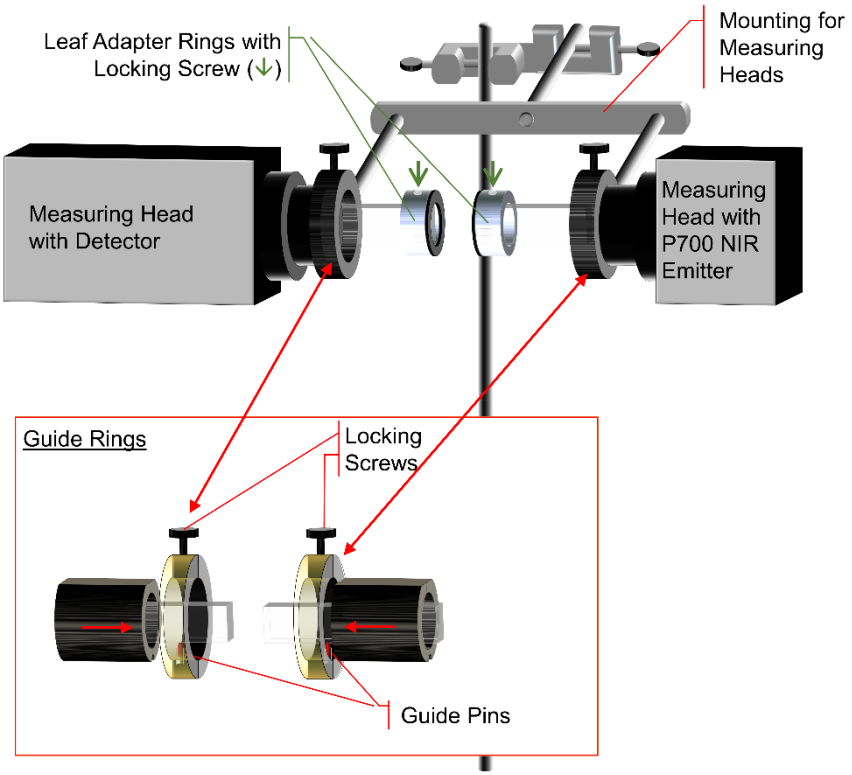


Fig. 3: Setup for Leaves

Table 3: Measuring Heads

Measuring Head with Detector	Measuring Head with P700 NIR Emitter
PIN photodiode detector	
Blue (DUAL-DB) or red (DUAL-DB) fluorescence measuring light	P700 measuring light (Sample wavelength 830 nm, reference wavelength, 870 nm)
Red actinic light	Red actinic light. (Inactivated when the DUAL-TW adapter is interposed)
Blue actinic light	Far-red light

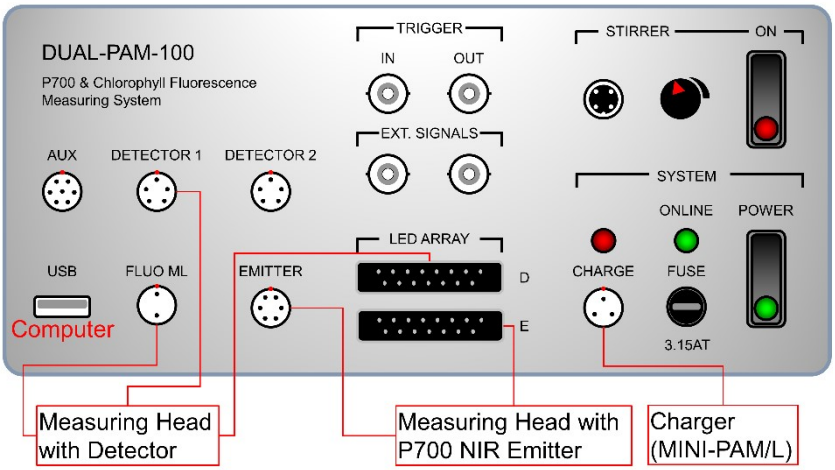


Fig. 4: Connections MODULAR Version

4.1.4 Setup Suspension

Mount optical unit on lab stand (Fig. 5A). Detector and emitter unit must be positioned opposite to each other. Make sure that the ports for measuring heads are not obstructed by the locking screw or the guide pin (Fig. 5B). The guide pin is only to prevent turning of measuring head. The measuring head is fixed by the locking screw.

Place cuvette in the center of the optical unit. Carefully slide in both measuring heads until they touch the cuvette. Fix by locking screw. Insert the stoppers of the optical unit through the open ports until they touch the cuvette (Fig. 5D). Fix by locking screw. The stoppers require an adapter ring to properly fit into a port of the optical unit. When inserting the adapter ring, position opening on top so that the locking screw can reach the stopper (Fig. 5C).

The stirrer is inserted from the bottom and fixed by a locking screw (Fig. 5C). To efficiently rotate the stir bar inside the cuvette, the

stirrer top must be positioned directly below the cuvette (Fig. 5D). A sample volume of maximum 1.5 mL is illuminated by the measuring heads. Larger volumes are not fully illuminated. Smaller volumes result in noisy signals because the turbulent surface is inside the measuring beam and, hence, disturbs the signal.

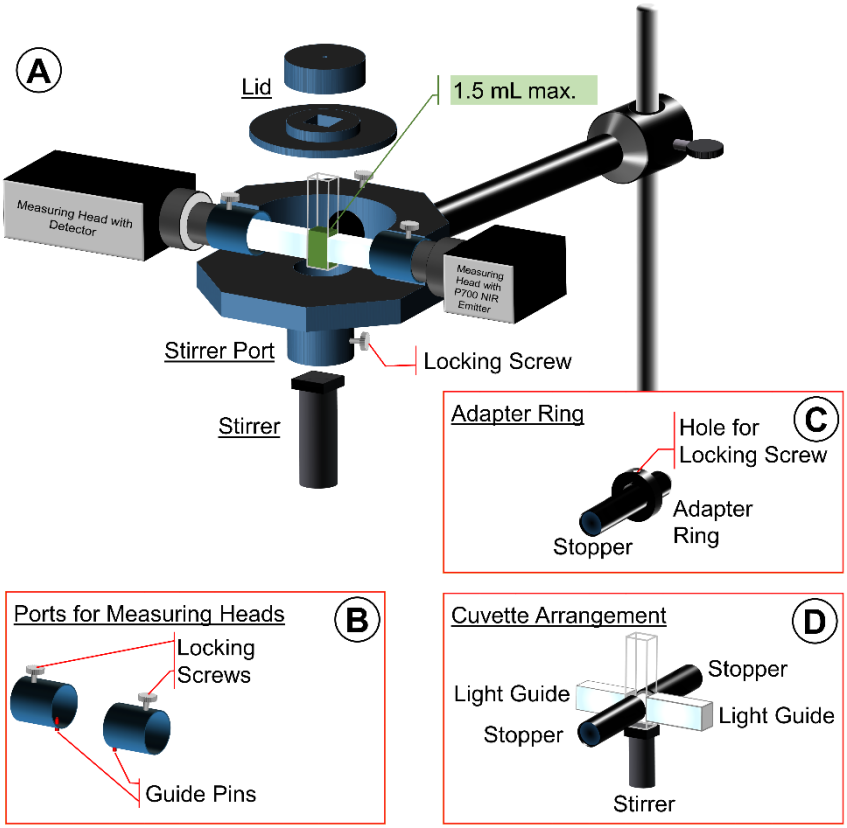


Fig. 5: Setup for Suspensions

A, overview. B, elements extending into the measuring head port (locking screw and guide pin). C, adapter ring for stopper of optical unit (position of hole for locking screw indicated). D, elements touching the cuvette (sideways: Perspex light guides and stoppers; from below. stirrer).

The Special Covering Plug (Fig. 6) separates the suspension bubble-free from the air space. The plug serves as auxiliary means to homogeneously illuminate the sample. Using the plug is not mandatory, provided that the sample volume is exactly 1.5 mL.

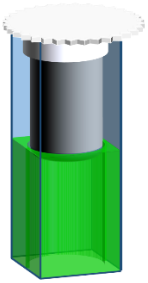


Fig. 6: Special Covering Plug
Special Covering Plug inserted in 10 x 10 mm cuvette. The liquid level (green) should allow the plug to be slightly submerged.

4.2 FIBER VERSION

The fiberoptics version Dual-PAM-100/F is functionally fully equivalent to the DUAL-PAM-100. The control unit of the FIBER version integrates all optical components (Table 5). The fiberoptics consist of three mixed fiber bundles corresponding to the three ports of the fiberoptics connection (#9 in Fig. 7). The P700 is measured in the "remission mode", that is, the P700 measuring light is picked up by the fiberoptics after partial absorption by the sample.

4.2.1 Extent of Delivery

Table 4: Extent of delivery FIBER

Optoelectronic-, Power and Control Unit	DUAL-PAM/F
Fiberoptics	2010-F
DUAL-PAM Leaf Clip	DUAL-LC
Adapter for fiberoptics	DUAL-A
Charging unit	MINI-PAM/L
Lab stand with fiber holder	ST-2500
USB type A to A cable	
Fluorescence standard foil	
Manual	
WALZ Manuals & Software CD	
Transport box	PHYTO-T

4.2.2 Control Unit

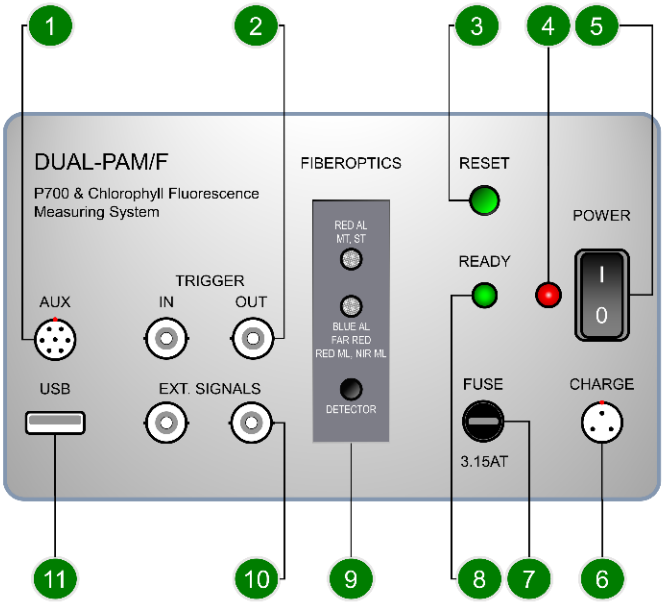


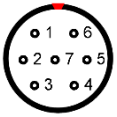
Fig. 7: **Front panel of Control-Unit (FIBER Version)**

All elements marked by numbers are explained in Table 6, page 22.

Table 5: Control Unit FIBER, Components of the Measuring System

DUAL-PAM/F	
PIN photodiode detector	
Red fluorescence measuring light.	Red actinic light.
P700 measuring light (Sample wavelength 830 nm, reference wavelength, 870 nm)	Blue actinic light Far-red light

Table 6: Front Panel of FIBER Version

Number in Fig. 7, page 21	Item	Function
1	AUX	Connection for US-MQS/WB light sensor or 2030-B leaf clip.  (1) +5 V, (2) Ground, (3) to (5) Analog inputs (0 - 2.5 V), (6) Trigger in (5 V), (7) -5 V.
2	TRIGGER	IN: Input for a 5 V trigger pulse to start a fast kinetics. OUT: Output for a 5 V trigger pulse to start an external event
3	RESET	Interrupts communication between device and computer. Equivalent to the disconnecting the device by the LED symbol of the DualPAM software.
4	POWER LED	When power switch in I (on) position, the continuously shining LED indicates “device switched on”. When power switch in O (off) position, the LED flashes when the device is charged.
5	POWER switch	Control of power supply
6	CHARGE	Connection for charger
7	FUSE	Fuse holder for a 3.15 Ampere slow-blow fuse
8	READY	Green shining LED indicates communication with the DualPAM software
9	FIBEROPTICS	Connection for fiberoptics
10	EXT. SIGNALS	Two BNC sockets to connect external DC signals, 0 - 2.5 V. Possible combinations: Left BNC + P700, right BNC + fluorescence, left and right BNC
11	USB	Connection for USB cable

4.2.3 Setup Leaf

Installation site: See 4.1.3, page 14.

The system is optimized for 1 mm distance between end of fiber optics and sample. This distance is met by the DUAL-LC Leaf Clip when the fiberoptics is fully inserted in the fiber port and a leaf sample is positioned in the closed clip (Fig. 8). The lower part of the clip reflects light which improves the P700 signal quality. The 1 mm distance is also met by DUAL-A Adapter when the fiberoptics is fully inserted in the fiber port and the adapter is placed on a (bulky) sample.

With increasing distance, the strengths of the fluorescence and P700 signals decrease. With high chlorophyll contents, satisfactory measurements can be carried out up to 15 mm distance. Signal amplitudes can be increased by placing the sample on a reflecting surface.

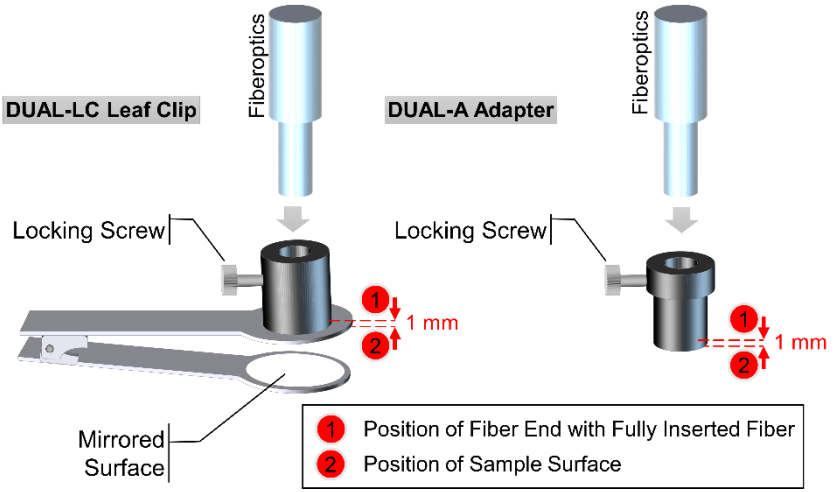


Fig. 8: Leaf Clip and Adapter

4.2.4 Setup Suspension

The Suspension Cuvette KS-2500 permits measuring suspensions of microalgae and isolated chloroplasts (see Section 5.2.2.1 and 5.2.2.2, page 41).

4.3 Software Installation

The DualPAM software supplied with the Software and Manuals CD. The DualPAM software is continuously improved. Check the Walz website for the most recent version:

www.walz.com→Downloads→DUAL-PAM-100

Installation

Back up existing DUAL-PAM data, close all programs and execute DualPAM setup.exe.

The welcome window must identify the Heinz Walz GmbH as Verified Publisher. The installation dialogue is mostly self-explaining. The option “Register DualPAM.exe for ActiveX?” prepares the software for access by other Windows software. Allow installation of the USB driver as it is indispensable for communication with the DUAL-PAM machine.

Connect and switch on DUAL-PAM-100. Proper connection is indicated in the device manager by an icon labelled “USB Serial Port (COM#)” in the device group Ports (COM & LPT; Table 7). (Note that the USB Serial Port is also displayed when the power switch is in the OFF position. Operation of the DUAL-PAM machine requires the ON position of the switch.)

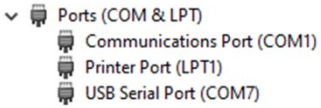
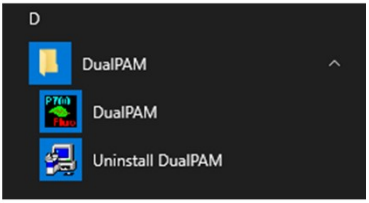
The DualPAM software can be started by double click on the desktop icon or from the program submenu on the start menu. Note:

Run DualPAM as administrator to allow the software writing data and settings to its folder.

DUAL-PAM-100 users (MODULAR version): Apply user settings “Walz.DEF” for measurements.

DUAL-PAM/F users (FIBER version): Apply user settings “Walz_Fiber.DEF” for measurements.

Table 7: Features after DualPAM Installation

Device Manager	Desktop Icon	Start Program Submenu
		

4.3.1 Firmware Update

The DUAL-PAM machine contains two Reduced Instruction Set Computer (RISC) processors for fast execution of commands and data management. Firmware is a specific software running on the RISC processors. The most recent firmware is contained in the DualPAM software and must be uploaded to the RISC processors. The system may not work properly with outdated firmware. Proceed as follows:

- Start DualPAM software.
- Open drop-down menu “Firmware Update” (Upper left margin of DualPAM software interface.)
- Click “Program MEGA RISC”.
- Click “Read HexFile” and “Download HexFile”, wait for completion of download.
- Click “Program RISC”, read warning and proceed by “yes”.
- Reestablish connection with the DUAL-PAM machine by clicking the status LED (top left corner).
- Click “Program TINY RISC” menu “Firmware Update”.
- Proceed as described for the MEGA RISC processor.
- Close DualPAM software and restart.

4.3.2 DualPAM Data Files

DualPAM software version 3.10 and newer saves report data (saturation pulse data) and kinetic data (fast kinetics either induced by saturation pulses and by a fast trigger) in the same folder. All Report folders are located in the folder “DualPamReport” (see Fig. 9). The default name of each Report folder contains the year, month and day when the Report was created in the form <YYYYMMDD>”. When several Reports are created at the same day, the folders are numbered consecutively.

Software version 1.9 and older stored report date in the folder “Report” using the name format Report_YYMMDD_#.cds. The fast kinetics of this Report are stored in:
Report\Kinetics\Report_YYMMDD_#.

When new software is installed, old data remain unchanged. To open data obtained with previous software, disconnect the DUAL-PAM-100 system by clicking on the green LED and select “Read CDS-Reports” from the Options menu (Table 8, page 46 and Table 10, page 47).

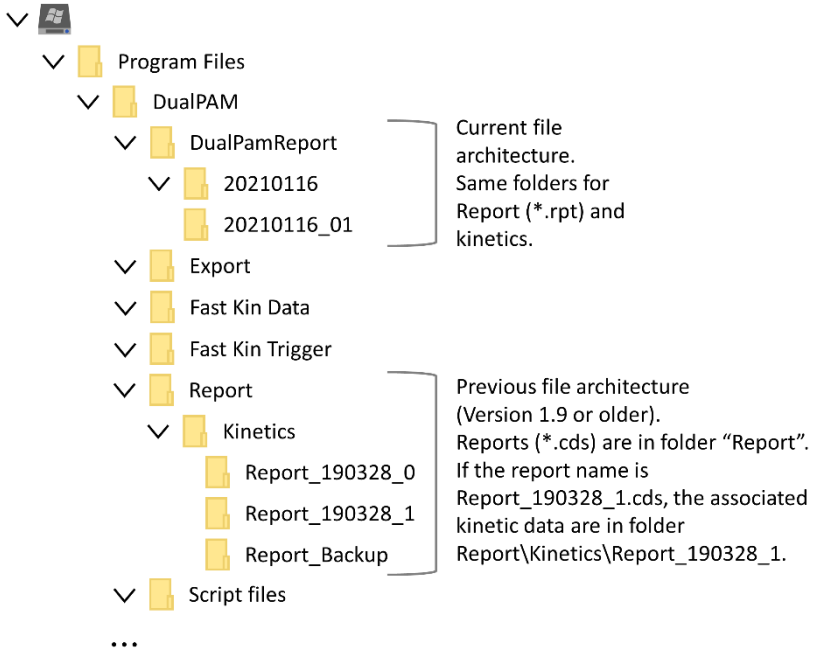


Fig. 9: DualPAM Data Files

5 Accessories

5.1 MODULAR VERSION

5.1.1 Leaves

5.1.1.1 90 Degree Measuring Head Holder DUAL-H90

For fluorescence measurements with leaves. The holder positions two measuring heads so that their optical axes are at right angles to each other. Fluorescence excitation and detection is at an angle of 45 degrees. Designed for simultaneously recording short and long wavelength fluorescence, and for assessment of epidermal UV-A screening (MULTI-COLOR-PAM application).

Paper: https://rdcu.be/ceFHU

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

Fig. 10: 90 Degree Measuring Head Holder DUAL-H90

5.1.1.2 Linear Positioning System 3010-DUAL/B

For measurements of leaves or suspensions using the low-drift accessory for DUAL-K25. Employing a high-quality rack and pinion drive, the system adjusts precisely and smoothly the distance between two measuring heads, for example, measuring heads DUAL-DB(-DR) and DUAL-E, or measuring heads DUAL-DP515 and DUAL-EP515.

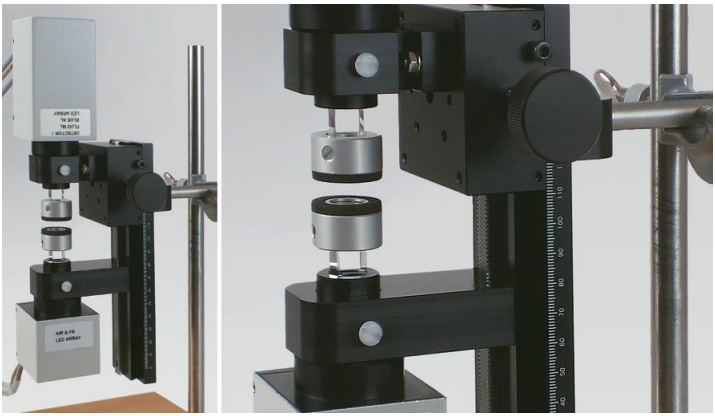


Fig. 11: Linear Positioning System 3010-DUAL/B

5.1.1.3 Optical Pinholes DUAL-OP

When leaf samples are smaller than the cross-sectional area of the measuring beam, the measuring light bypassing the leaf diminishes the P700 signal quality. To prevent the negative effects of bypassing measuring light, we offer a set of optical pinholes (DUAL-OP) to adjust the cross-sectional area of the measuring beam to the sample area.

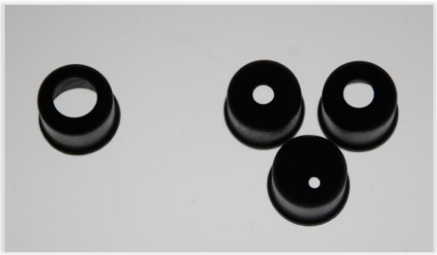


Fig. 12: Optical Pinholes DUAL-OP

5.1.1.4 Two-way Adapter for Unilateral Actinic Illumination DUAL-TW

Usually, the MODULAR version of the DUAL-PAM-100 illuminates the sample from two sides (Table 3, page 16). When the cable of the P700 emitter DUAL-E is connected via the adapter DUAL-TW to the Power-and-Control-Unit DUAL-C, the actinic light of the DUAL-E head is inactivated. The far red is still working.

Fig. 13: Two-way Adapter for Unilateral Actinic Illumination DUAL-TW

5.1.1.5 P515/535 Emitter-detector Module

The module simultaneously measures the 515-520 nm ("P515") and 535 nm ("scattering") absorbance changes.

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

Fig. 14: P515/535 Emitter-detector Module

5.1.1.6 DUAL-PAM-100 Gas-Exchange Cuvette 3010-DUAL

To combine chlorophyll fluorescence and P700 measurements with gas exchange measurements using the Walz GFS-3000 gas exchange system, we have developed the DUAL-PAM-100 gas-exchange cuvette (3010-DUAL).

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

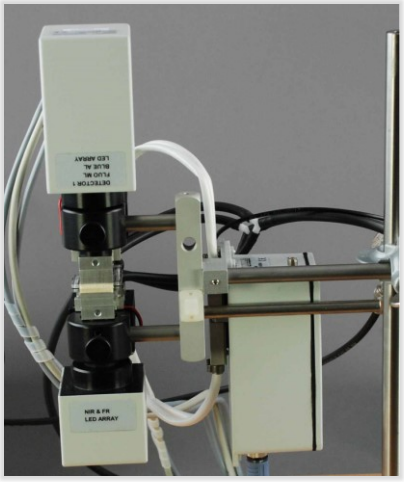


Fig. 15: DUAL-PAM-100 Gas-Exchange Cuvette 3010-DUAL

5.1.2 Suspensions

5.1.2.1 Optical Unit ED-101US/MD

For measurements with suspensions using 10 x 10 mm fluorescence cuvettes, we provide a black aluminum unit (ED-101US/MD). The unit holds the fluorescence cuvette in its center and has four light ports to connect standard measuring heads of the DUAL-PAM-100 or alternative fluorescence detectors. A two-part black cover of the cuvette compartment with syringe port is provided.

Fig. 16: Optical Unit ED-101US/MD

5.1.2.2 Miniature Magnetic Stirrer PHYTO-MS

Settling of particles is prevented by using a miniature magnetic stirrer (US-MS). The stirrer is mounted directly beneath the sample cuvette (Fig. 5, page 18). A rotating magnetic field created by the stirrer tip moves a miniature magnetic stir bar in the cuvette. The stirrer is connected to the DUAL-PAM-100 control unit (DUAL-C). Stirring can be switched on and off by the DualPAM software.

Fig. 17: Miniature Magnetic Stirrer PHYTO-MS

5.1.2.3 Photodiode-Detector Unit DUAL-DPD

The DUAL-DPD is used when fluorescence levels of suspensions are too low to be accurately detected by the DUAL-DB or DUAL-DR heads. Compared to the two latter units, the DUAL-DPD detector is about tenfold more sensitive permitting measurements down to concentrations of 5 µg Chl/L using the blue modulated excitation light of the DUAL-DB head. Normally, the DUAL-DPD is mounted on the ED-101US/MD optical compartment right-angled to the DUAL-DB or DUAL-DR head.

Fig. 18: Photodiode-Detector Unit DUAL-DPD

5.1.2.4 Photomultiplier-Detector Unit DUAL-DPM

The outstanding sensitivity of the photomultiplier DUAL-DPM exceeds the performance of the DUAL-DPD photodiode. Using the DUAL-DPM in conjunction with the DUAL-DB unit, which provides blue modulated for fluorescence excitation, allows reliable measurements of suspensions with chlorophyll concentrations down to 0.5 µg/L. Like the DUAL-DPD photodiode, the DUAL-DPM is

mounted on the ED-101US/MD optical compartment right-angled to the DUAL-DB or DUAL-DR head.

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

Fig. 19: Photomultiplier-Detector Unit DUAL-DPM

5.1.2.5 Temperature Control Unit US-T Optical Unit ED-101US/MD

The US-T unit consists of a heat-transfer head with a cooling/heating Peltier element, and a separate power-and-control unit. The heat-transfer head is mounted on top of a Walz optical unit (ED-101US-type) so that the tip of the rod is in touch with the suspension being investigated. The maximum temperature difference relative to the ambient temperature is -12 K and +15 K, respectively.

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

Fig. 20: Temperature Control Unit US-T Optical Unit ED-101US/MD

5.1.2.6 Temperature Control Block ED-101US/T

For measurements under defined temperatures, a temperature control block (ED- 101US/T) can be mounted on the optical unit (ED-101US/MD). The block consists of an inner flow-through metal part which is slightly pressed on the sample cuvette by a spring mechanism, and an external foam part for temperature insulation. Temperature control is achieved by an external flow-through water bath (not included) connected to the temperature block.

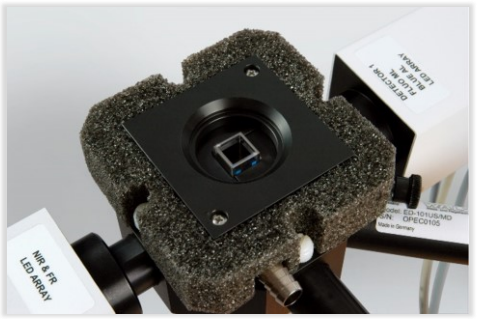


Fig. 21: Temperature Control Block ED-101US/T

5.1.2.7 Low-Drift Cuvette DUAL-K25

By employing a vertical optical pathway the DUAL-K25 quartz glass cuvette reduces baseline drifts caused by particle settling in suspensions of isolated chloroplasts, unicellular algae and cyanobacteria.

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

Fig. 22: Low-Drift Cuvette DUAL-K25

5.1.2.8 Acridine Orange/Yellow Fluorescence Emitter-detector Mod.

Excitation and detection wavelengths of the Acridine Orange/Yellow fluorescence emitter-detector module are optimized for fluorescence measurements of the dyes acridine.

Manual: https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

Fig. 23: Acridine Orange/Yellow Fluorescence Emitter-detector Module

5.1.2.9 NADPH/9-AA Module

Excitation and detection wavelengths of the NADPH/9-AA Emitter-detector Module are optimized for fluorometric NADPH determinations. The module's excitation and detection wavelengths are also suited to measure 9-amino acridine fluorescence.

Manual:

https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

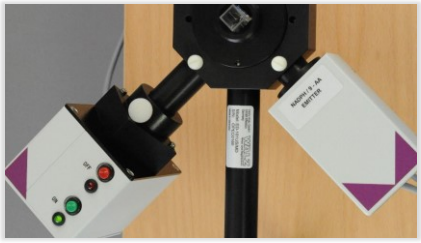


Fig. 24: NADPH/9-AA module

5.2 FIBER VERSION

5.2.1 Leaves

5.2.1.1 DUAL-BA Leaf Adapter

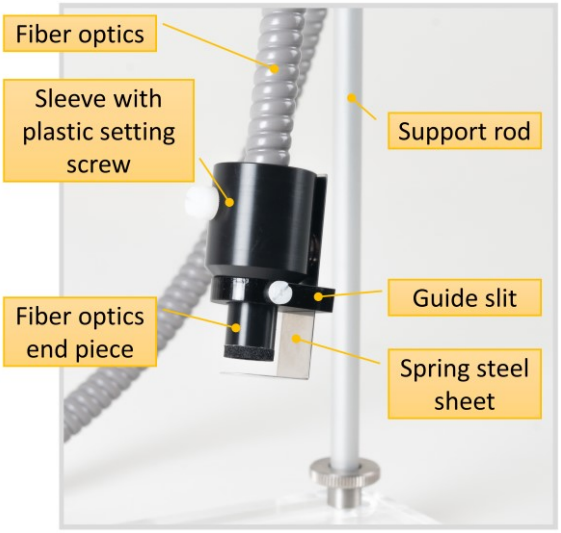


Fig. 25: DUAL-BA Leaf Adapter

The DUAL-BA has been designed for high throughput measurements of leaves. The DUAL-BA consists of a sleeve in which the fiberoptics is inserted. Sideward located is a guide slit for a rectangular bended spring steel. The longer part of the spring steel is inserted into the guide slit. The shorter part of the spring steel is positioned in front of the fiberoptics. A neodymium magnet located behind the guide slit fastens the sleeve. The sample is positioned between the shorter part of the spring steel and the end piece of the fiberoptics. Samples can be quickly changed by sliding the spring steel. The DUAL-BA includes a stand with fiberoptics guide.

Manual:

https://www.walz.com/products/chl_p700/dual-pam-100/downloads.html Manuals & Documentation

5.2.1.2 2030-B Leaf-Clip Holder

The clip measures ambient light and temperature. Saturation pulse measurements can be triggered by pushing the control button of the clip.

Fig. 26: 2030-B Leaf-Clip Holder

5.2.2 Suspensions

5.2.2.1 Suspension Cuvette KS-2500

The suspension cuvette includes a 400 µl sample compartment made of stainless steel with POM exterior. The cuvette is

equipped with a 7 mm fiberoptics window adapter, an injection port for microliter syringes, and nozzles for connecting an external flow-through water-bath for temperature control.

5.2.2.2 Magnetic Stirrer with Fiberoptics Holder MKS-2500

The device is equipped with a specially modified stirrer plate to center and hold the KS-2500 Suspension Cuvette. The MKS-2500 Magnetic Stirrer comes with a Perspex base plate with stand bar for mounting fiberoptics on top of cuvette.

Fig. 27: KS-2500 Suspension Cuvette

5.3 LIGHT SENSORS

5.3.1 Micro Quantum Sensor US-SQS/WB

Especially under water or when working with suspensions, the amount of scattered light is very high, so that a spherical sensor should be used. The US-SQS sensor uses a plastic diffuser to obtain an angular response error of less than ± 5% from -100° to 100° angle. The US-SQS/WB comprises the US-SQS/L sensor, a black hood, and an amplifier box. The hood fits on mountings for suspension cuvettes. Technically the connection for the US-SQS/WB is the same as for the Leaf Clip Holder 2030-B.

Fig. 28: Micro Quantum Sensor US-SQS/WB

5.3.2 Cosine Corrected Mini Quantum Sensor US-MQS/WB

The Cosine Corrected Mini Quantum Sensor MQS-B is designed for light measurements in units relevant for plant leaves. The US-MQS/WB comprises the sensor MQS-B and an amplifier box. Technically the connection for the US-MQS/WB is the same as for the Leaf Clip Holder 2030-B.

Fig. 29: Cosine Corrected Mini Quantum Sensor US-MQS/WB

6 DualPAM Software

6.1 SETTINGS

This section considers the Dual Channel mode “Fluor + P700” (6.1.6, page 52). Some elements or functions are absent in the Single Channel modes.

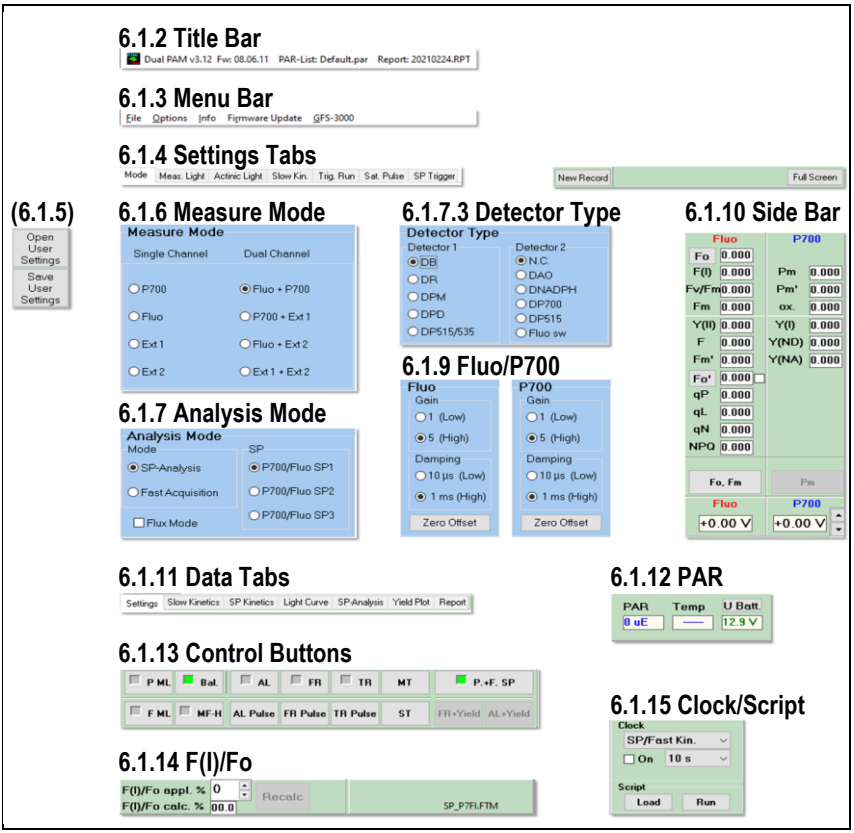


Fig. 30: Mode Window

6.1.1 Mode (Settings)

Click “Settings” in the lower row of tabs (data tabs) to access all settings of the DUAL-PAM system. The basic configuration of the system is made in “Mode” window. This section describes the Mode window plus the top and side panels which are also present in other windows. A segmented overall view links the control elements to the subsections (Fig. 30, page 45).

6.1.2 Title Bar

The “Title Bar” displays the software version, firmware creation date, currently valid light intensities of internal light sources (PAR list), and the file name of the current Report. The firmware creation date applies to the main RISC processor of the DUAL-PAM system (MEGA RISC) but firmware update mostly concerns the ancillary processor (TINY RISC).

6.1.3 Menu Bar

Table 8: Menu Bar

Menu	File	Options	Info	Firmware Update	GFS-3000
Shortcut	Alt+f	Alt+o	Alt+i	Alt+r	Alt+g
Content	Data management	Software behavior and PAR lists	Software information	Firmware update	Communication with GFS-3000

6.1.3.1 File

Table 9: File Menu	
New, Open, Save Report	Handling of Reports (sat. pulse data, slow + fast kinetics).
Export (Report)	Export Report as csv (comma-separated values).
Print, Printer Setup	Printer handling.
Load, Run Script	Script file handling. Equivalent to 6.1.15.
Exit	

6.1.3.2 Options

Table 10: Options		
✓	Show Hints	Small text boxes pop up depending on the position of the mouse pointer. Pressing "F1" displays the help text for the interface element on which the mouse pointer is located.
	Auto Report Backup off	The software automatically saves data during measurements which can reduce system performance. Checking switches off this feature.
✓	Automatic Signal Zero	Sets signals to zero at system start. Switch off only for diagnostic purposes.
	Keep Scale of >	✓ Slow Kinetics ✓ Fast Kinetics. Maintains y axis scaling when scrolling through a series of fast or low kinetics.
	Off with Screensaver	Stops recording with start of screen saver.
	Current/PAR Lists	Opens list of intensities of light sources (see below)
✓	Show both PAR	Requires plugged-in PAR sensor. When active, the two levels of amplification of the PAR sensor are displaced on the lower edge of the software interface.
	Warning when Decimal-Separator >	 Compares decimal symbol of computer setting with that of the DualPAM software. Warning appears at software start. Activate feature in the online mode.

Table 10: Options

Read CDS-Reports		 Only available when communication to DUAL-PAM machine is off. Allows opening of data acquired with DualPAM version 1.9 or older.
Color	>	Changes background color of charts
USB Thread Priority	>	Determines the rank of Dual-PAM communication relative to other CPU duties. Default is 3.
High Resolution Trig. Run Option		Performs triggered runs with highest timing precision. The number of data points is limited to 32000. Works only in Fast Acquisition mode. See 6.2.4, page 90.
Clock frequency x 10		Permits clock frequencies up to 10 Hz.
Trigger SP by		✓ Ext. 1 ✓ Ext. 2 Enables external triggering of saturation pulses or fast kinetics by voltage pulses of 1 s or longer applied to input External Signal 1 or 2 (see 4.1.2, 4.2.2). The available input (Ext. 1 or Ext. 2) depends on the signal measured as specified below.

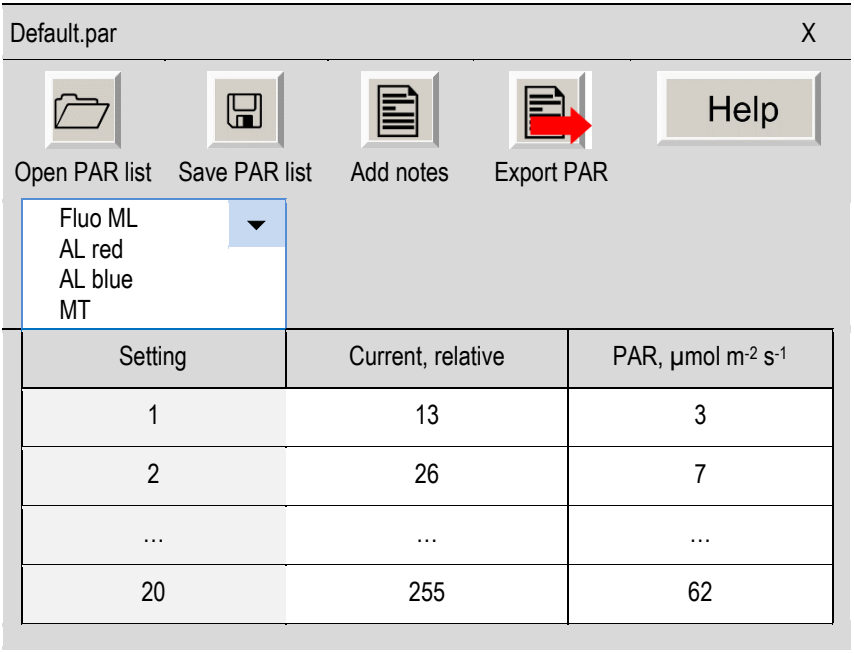
Measured signal	Available Trigger Input
Fluo	Ext. 1
P700	Ext. 2
Fluo + P700	Not available
Fluo+Ext. 2	Ext. 2
P700+Ext. 1	Ext. 1
Ext. 1	Ext. 1 or Ext. 2
Ext. 2	Ext. 1 or Ext. 2
Ext. 1+Ext 2	Ext .1 or Ext. 2

AL Current/PAR Lists

The PAR (unit: µmol m^{-2} s^{-1}) is listed for three actinic light sources and for saturation pulses. The four lists can be selected in the drop-down menu of the PAR list window (Table 11). A PAR list for far-red light is not available because most of the far-red emission is outside the wavelength range of PAR.

Each PAR list consists of three columns (Table 11). The column "Settings" refers to the 20 adjustable intensity levels. The column "Current" shows relative values of the current flow through the LEDs, where the number of 255 corresponds to the maximum current. The column "PAR" lists the PAR values in µmol m^{-2} s^{-1}. Both current and PAR values can be edited manually by double-click in the numerical field. After changing the current value of particular setting, the PAR is this setting must be newly measured. Changing the PAR value does not affect light intensity.

Table 11: PAR Lists



Setting	Current, relative	PAR, µmol m^{-2} s^{-1}
1	13	3
2	26	7
…	…	…
20	255	62

The DualPAM software provides five default PAR lists (Table 12). These lists represent factory values measured under different conditions. The PAR lists 1 to 4 in Table 12 were measured with recently built systems, the PAR list 5 was recorded with a device

fabricated before the year of 2010 when the LED power was lower than today. Illumination with the detector head only (PAR lists 1 and 2 in Table 12) represents a setup, which is frequently employed with leaves. Two-sided illumination (PAR lists 3 and 4 in Table 12) is more common with suspensions.

The default PAR values of Table 12 are not generally valid. The light output for each DUAL-PAM system should be quantified. The PAR can be measured with any calibrated PAR sensor. The measured PAR can then be entered in the PAR column as described in Section 6.2.281. With a Walz PAR sensor US-SQS/WB (spherical) or US-MQS/WB (planar) connected to the AUX port (Fig. 1, Fig. 7), the DualPAM software creates PAR lists automatically (see Table 13).

Table 12: PAR Lists Default

Number	File name	Measuring Light and Condition		Illumination	Sensor
1	DefaultAirBlueML.par	Blue	Air	One-sided	Planar
2	DefaultAirRedML.par	Red	Air	One-sided	Planar
3	DefaultWaterBlueML.par	Blue	H_2O	Two-sided	Spherical
4	DefaultWaterRedML.par	Red	H_2O	Two-sided	Spherical
5	DefaultOld.par	Red	Air	One-sided	Planar

Table 13: Measuring PAR Lists

Light source	Entire List	Current PAR
Fluo ML PAM fluorescence measuring light	Script file Get_FML_Intensities.prg	Set MF-high = 10 000 Hz, Switch ON MF-H. Click "Get PAR" in Window "Meas. Light" settings.
Note that intensities of PAM fluorescence measuring light apply for the measuring light frequency of 10 000 Hz. The actual intensity is the listed PAR value times the active measuring light frequency divided by 10 000. The software calculates PAR values as the sum of actinic PAR plus the measuring light PAR for the used frequency.		
AL red Red actinic light	Script file Get_AL_Intensities.prg	Command "Get PAR" in Act. Red Light section of settings for actinic light (6.2.2, page 81).
AL blue Blue actinic light	Script file Get_BL_Intensities.prg	Command "Get PAR" in Act. Blue Light section of settings for actinic light. (6.2.2, page 81).
MT Multiple turnover pulse, Saturation pulse	Command "Get MT PAR" in Settings Fast Acquisition.	
Note: The PAR sensor may saturate at higher settings. Establish full MT PAR list by extrapolating the linear relationship obtained for low intensity settings."		

6.1.3.3 Info

Information on software version and copyright.

6.1.3.4 Firmware Update

See 4.3.1, page 25.

6.1.3.5 Communication with GFS-3000

Controls communication between the system combination “GFS-3000 & DUAL-PAM-100”.

6.1.4 Settings Tabs

Tabs for all available settings (see Section 6.2, page 72).

6.1.5 User Settings

Buttons to store and retrieve settings of the DUAL-PAM machine. Settings include light lists, light curve configuration, mode settings, light intensities and fast trigger pattern. Universal settings for the DUAL-PAM-100 (MODULAR) and the DUAL-PAM/F (FIBER) are Walz.DEF and Walz_Fiber.DEF, respectively. Settings files are stored in a separate folder in the DualPAM directory.

Current settings are saved when the program is terminated in the file DualPAM.INI (located in C:\Program Files\DualPAM). These settings will be loaded the next time the program is started. Current settings are saved only when at least one measurement was carried out.

6.1.6 Measure Mode

In the basic configuration if the DUAL-PAM-100 (MODULAR), and always in case of the FIBER version, three measure modes are available. (i) Fluo, fluorescence analysis of photosystem II. (ii) P700 absorption measurement to analyze photosystem I. (iii) Simultaneous Fluor and P700 measurement.

6.1.7 Analysis Mode

6.1.7.1 Mode

"SP-Analysis" applies the classical saturation pulse analysis to evaluate photosynthesis. "Fast Acquisition" offers high time resolution and fast trigger programming to conduct experiments. The number of saturation pulse parameters is reduced.

6.1.7.2 Flux Mode

Flux Mode

The flux mode measures the photosynthetic electron transport of photosystem I by modulation of the actinic light intensity. The P700 signal meets the essential requirements of the flux method which are:

- The signal is a linear measure of changes of electric charge.
- The signal is generated directly by reaction centers.

The actinic light regime consists of alternating light and dark periods of equal duration (Fig. 31). The acquisition rate set in Slow Kinetic settings determines the duration. The dark-light changes reduce the average actinic PAR to half of the PAR list value which is automatically taken into account by the software.

The rationale of the flux method is that the rate of P700 reduction right after a light to dark transition corresponds to the electron flow into photosystem I. The flux method integrates linear and cyclic electron flow and may also contain charge recombination within photosystem I.

Ideally, the P700 signal decreases linearly with time during a short dark phase as is the case for an acquisition rate of 5 ms (Fig. 31A). With increasing acquisition time, P700 reduction, deviations from linearity and can compromise flux results (compare Fig. 31 A and

B). Shorter acquisition rates increase signal noise because the amplitude of ΔP700 decreases linearly with decreasing Δt. The noise can partially be compensated by averaging data points. In the steady state, the amplitude of P700 oxidation in the next light phase is equal to the amplitude of P700 reduction of the dark phase. That P700 oxidation is curvilinearly linked to time does not affect the amplitude of P700 oxidation which is evaluated by the flux method.

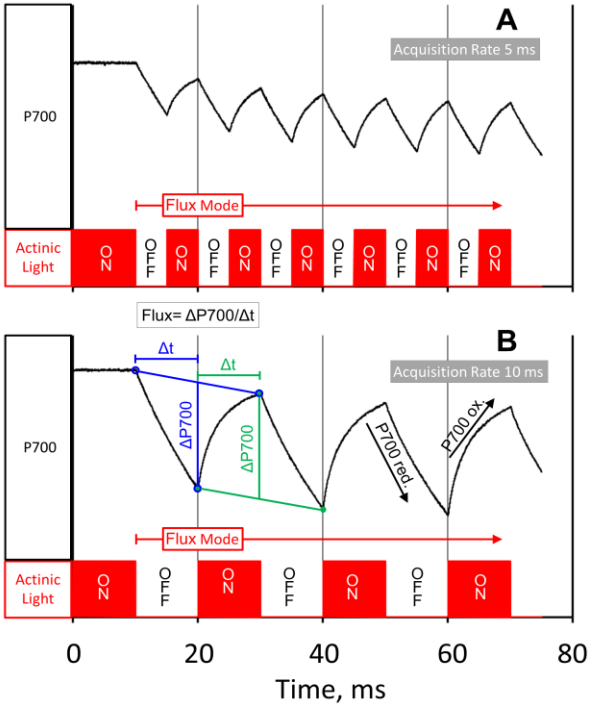


Fig. 31: Flux Mode

Illustration of the Flux Mode for acquisition rates of 5 ms (A) and 10 ms (B). Calculation of the flux signal is outlined in blue and green in panel B. P700 kinetics were recorded in the Fast Kinetics mode, the kinetics is temporarily not resolved in the Flux Mode.

The flux signal is calculated as ΔP700/Δt (Fig. 31). The ΔP700 is the absolute value of the difference between the P700 level at the end of a dark or light period and the corresponding baseline level at the same time point. The Δt is the period's length of time. The baseline is a straight line running through the P700 level at start of the dark or light phase and the P700 level at the end of the next phase (Fig. 31). The baseline minimizes effects of long-term changes of the P700 signal on flux evaluations.

Flux calibration.

The Pm measurement (maximum amplitude of P700) can calibrate the flux signal to yield electrons per photosystem I (PSI) and time. The Pm absorption change corresponds to the conversion of all PSI reaction centers from the open state (P700) to the closed, oxidized state ($P700^+$) in a one electron process. Thus, the Pm amplitude corresponds to 1 electron per PSI.

The Pm amplitude contains minor plastocyanin absorption changes. Therefore, the true absorption change for 1 electron per PSI is somewhat smaller. The contribution of plastocyanin to the flux signal itself should be minimal due to its slower response to light modulation.

To calibrate the flux signal, the kinetics must be exported. The exported column entitled "PSI Flux x Number" contains the flux signal, where "number" is the "Flux Multiplier" set in the Slow Kinetics window (Fig. 58, page 112). The units of the exported flux signal differ:

Case 1: When the flux signal is measured in Volt, that is, the voltage scale was not converted in a ΔI/I scale using the "Calib." command, the unit is V/ms.

Calibrate using the following equation:

$$Flux\left(\frac{e^-}{PSI \cdot s}\right) = \frac{Flux}{Flux\ \ Multiplier}\left(\frac{V}{ms}\right) \cdot 1000\ \left(\frac{ms}{s}\right) \cdot \frac{1}{P_M}\left(\frac{\frac{e^-}{PSI}}{V}\right)$$

where

Flux (V/ms) Exported flux data

Flux Multiplier Factor indicated in column title

P_M(V/(e-/PSI)) P_M value in Report

Case 2: When the “Calib.” command was performed prior to the flux measurement, the exported flux data are non-dimensional ΔI/I values per ms multiplied by 1000. However, the P_M value in the Report is still given in units of V. To arrive at the quantity of ΔI/I, the P_M, corresponding to a signal change ΔI_{PM}, must be divided by $I_{Calib.}$, which is the absolute signal value shown in the Calib. diagram (Fig. 32). The $\Delta I_{PM}/I_{Calib.}$ must be multiplied by 1000 to match with the exported ΔI/I data. The following equation applies:

$$Flux\left(\frac{e^-}{PSI \cdot s}\right) = \frac{\frac{\Delta I}{I}}{Flux\ \ Multiplier}\left(\frac{1}{ms}\right) \cdot 1000\left(\frac{ms}{s}\right) \cdot \frac{1}{1000} \cdot \frac{I_{Calib.}}{\Delta I_{P_M}} \cdot \left(\frac{e^-}{PSI}\right)$$

$$= \frac{\frac{\Delta I}{I}}{Flux\ \ Multiplier} \cdot \frac{I_{Calib.}}{\Delta I_{P_M}}$$

Typically, the rates of electrons per PSI and second are in the two-digit range where highest values are measured with sun-acclimated leaves and saturating light intensities.

The P700-flux method has been introduced by Christof Klughammer:

Klughammer C (1992) Entwicklung und Anwendung neuer absorptionsspektroskopischer Methoden zur Charakterisierung des photosynthetischen Elektronentransports in isolierten Chloroplasten und intakten Blättern. Ph.D. Thesis, University of Würzburg

The flux method can also be applied to the electrochromic absorption change of photosynthetic pigments (P515) using the P515/535 Emitter-detector Module of the DUAL-PAM-100 system. See:

Klughammer C, Siebke K, Schreiber U (2013) Continuous ECS-indicated recording of the proton-motive charge flux in leaves. Photosynth Res 117: 471–487. https://doi.org/10.1007/s11120-013-9884-4

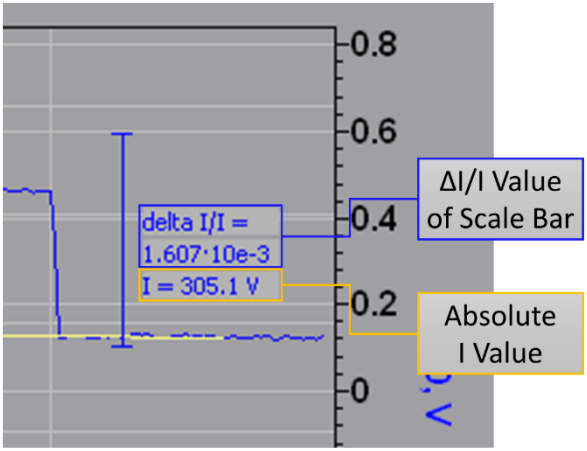


Fig. 32: P700 Calibration

6.1.7.3 SP-Analysis

For the mode “SP-Analysis” in combination with “P700” or “Fluo + P700” measuring mode, three types of trigger files for saturation pulses are available. The pulse types P700 SP1 (P700) or P700/Fluo SP1 (Fluor + P700) cover almost all applications. The SP2 and SP3 pulse types differ mainly in their longer dark phase after the saturation pulse.

In rare cases, the $P700^{+}$ reduction after the saturation pulse is very slow and may not reach the maximum reduction level within the

dark phase (1.2 s) of P700 SP1 or P700/Fluo SP1 P700 kinetics. In such cases, the SP2 and SP3 pulse types can be applied.

6.1.8 Detector Type

In the basic configuration, either a detector with blue (DB) or with red measuring light (DR) is available which must be connected to the "DETECTOR 1" socket (Fig. 1, page 12). On the mode window, chose DB or DR as "Detector 1". Detector 2 is absent, and nothing connected (N.C.) must be checked.

(This paragraph does not apply to the FIBER version.)

6.1.9 Fluo/P700

Both Fluo and P700 are visible when the measure mode "Fluo + P700" is active. In the P700 or Fluo Single Channel mode, only one of the boxes is available.

Gain is set by default to "High". Low gain in the Fluo channel is required only for very strongly fluorescing samples.

In the SP-Analysis mode, the default setting for signal damping is high in the Fluo and P700 channels. However, when the flux mode is activated, the damping for P700 switches automatically to low. The flux mode requires low damping to record the fast P700 signal changes (Fig. 31). In the Fast Acquisition mode, low damping is required. The software automatically switches damping to low in the Fast Acquisition mode.

6.1.10 Side Bar

Table 14: Side Bar

	Fluorescence level and ratio parameters			NIR absorption difference and ratio parameters		
	Fluo			**P700**		
F_0 and F_0 command (dark-acclimated sample)	**Fo**	0.603				
Absolute value of currently used photosystem I (non-variable) fluorescence	**F(I)**	0.000		**Pm**	2.439	Maximum absorption difference (dark-acclimated sample)
F_M and maximum photosystem II yield, F_V/F_M (dark-acclimated sample)	**Fv/Fm**	0.651		**Pm'**	2.260	Absorption difference and pre-saturation pulse level, ox. (light-exposed sample)
	Fm	1.728		**ox.**	1.870	
Effective photosystem II yield and fluorescence levels to calculate quenching parameters (light-exposed sample)	**Y(II)**	0.051		**Y(I)**	0.160	Effective photosystem I yield and donor/acceptor limitations (light-exposed sample)
	F	0.449		**Y(ND)**	0.767	
	Fm'	0.474		**Y(NA)**	0.073	
	Fo'	0.341	✓			
Current photochemical quenching	**qP**	0.184				
	qL	0.140				
Current non-photochemical quenching	**qN**	0.882				
	NPQ	2.647				
Command to determine F_V/F_M (right-click: F_M only)	**Fo, Fm**			**Pm**		Command to determine P_M
	Fluo			**P700**		
Current fluorescence level	0.51 V			-0.32 V		Current P700 level and fine tuning of P700

↑ Checkbox to activate F_0' mode (see Fig. 34)

Table 14 outlines elements and functions of the side bar. The side bar is split into a left side showing fluorescence data (Fluo), and a right side showing P700 data. Both sides are present when the mode “Fluo + P700” is active. The parameters are explained in Chapter 7 (page 161).

The side bar provides buttons to measure fluorescence or P700 properties of dark-acclimated samples: Fo takes the current fluorescence level as the minimum fluorescence, the F_0 value. Fo, Fm determines the F_0 fluorescence and then takes the maximum fluorescence induced by a saturation flash as the F_M level. Right click converts the Fo, Fm button to Fm which triggers only an F_M determination.

The button Pm starts an illumination sequence to measure the maximum P700 absorption change of the sample (Fig. 33, page 62, see also Section 7.2, page 172). The Pm command is not available (greyed out) when P700 measuring light is off or not balanced, or when the signal is very noisy as can be the case with stirred suspensions.

The sequence starts with several seconds of far-red illumination. The far-red is absorbed by photosystem I but photosystem II absorption is negligible. Thus, the far-red-driven charge separation in photosystem I reaction centers accumulate the $P700^+$ state because of the lack electrons donated by photosystem II. This “donor limitation” oxidizes not all photosystem I reaction centers because of electron supply independent of photosystem II turnover (e.g. electron donation from stromal reductants).

In this donor-limited situation, a red saturation pulse is given. The strong light induces a high rate of charge separations in PS I which exceeds the rate of electron supply by sources independent of photosystem II. Thus, the remaining P700 is converted into $P700^+$.

Full oxidation of the photosystem I reaction center exists only at the very beginning of the saturation pulse when the P700 signal jumps up rapidly. Because the saturation pulse also excites photosystem II, the donor limitation established by far-red is quickly removed. Therefore, the P700 signal starts dropping immediately after the initial rise (Fig. 33).

Because of the high damping in the SP Analysis mode, the P700 signal level corresponding to oxidation of all photosystem I reaction centers is not fully reached. Therefore, this signal level is estimated by back-extrapolating the P700 decay curve to the beginning of the saturation pulse (Fig. 33). The time intervals used for extrapolation can be defined in the Sat. Pulse settings.

In the dark phase following the saturation pulse, all $P700^+$ is rapidly reduced. The P700, however, frequently shows an undershoot probably caused by ferredoxin redox changes. The signal level corresponding to the fully reduced P700 is measured after the undershoot. The P_M value is the difference of the extrapolated maximum signal minus the dark signal (Fig. 33).

The complete method of saturation pulse analysis of photosystem I is described in:

Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via $P700^+$-absorbance changes at 830 nm. Planta 192: 261–268. https://doi.org/10.1007/BF00194461

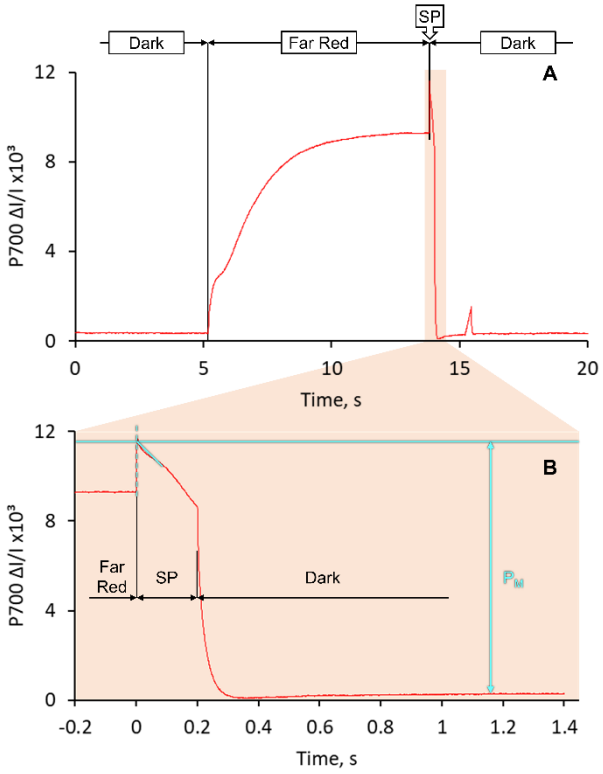


Fig. 33: P_M Determination

A: Far-red preillumination and saturation flash (SP). Panel **B** zooms in to the light-orange zone of panel A. Sky blue lines show the geometrical construction used for P_M determination: Dashed line, onset of SP. Sloped line, linear regression line to data interval defined in Sat. Pulse settings. The intersection of dashed and sloped lines corresponds to maximum oxidation of P700.

The fluorescence level F_0' is a property of the light-exposed sample (Fig. 72, page 163). The button Fo' determines this fluorescence level by replacing for a period of 5 s the actinic illumination by far-red light. The minimum fluorescence level during this period is taken as the F_0' value. Checking the box located right of the Fo' button, automatically starts far-red illumination without

actinic light after each measurement of maximum fluorescence (F_M'; Fig. 34, page 63)). The duration of 5 s is fixed and not affected by the Width setting for far-red light in the window for Actinic Light settings.

Under far-red illumination, only photosystem I operates and takes up electrons of the intersystem electron transport chain entailing opening (oxidation) of photosystem II reaction center. This situation is comparable to the dark state of leaves in which photosystem II reaction centers are open, too. A lower F_0' than F_0 level is traditionally viewed as indicating non-photochemical quenching in the light harvesting antennae.

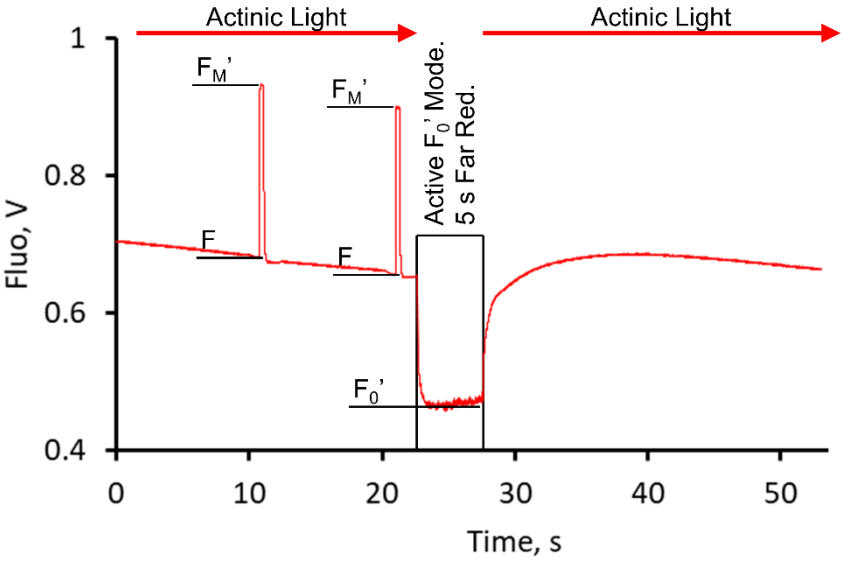


Fig. 34: F_0' Mode

When the box for automatic F_0' evaluation is unticked, the F_0' is calculated with F_0, F_M and F_M' according to:

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54, 135–142. https://doi.org/10.1023/A:1005936823310

See also Section 7.1.2, page 164.

6.1.11 Data Tabs

Tabs for all graphical display and alphanumerical windows.

6.1.12 PAR/Temp/U Batt

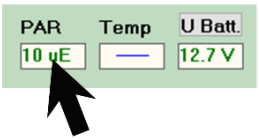
The bottom section of the side bar displays PAR, temperature, and battery voltage (Table 15).

Table 15: PAR/Temp/U-Batt

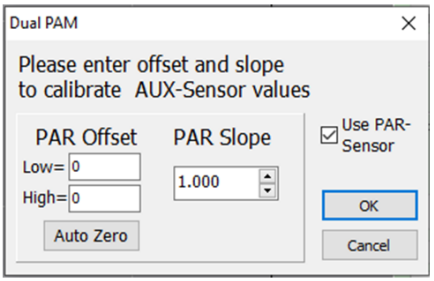
PAR	Temp.	U Batt.
401 uE	——	14.0 V
Current PAR (µmol m^{-2} s^{-1}, see Table 16)	Temperature (when 2030-B clip is connected)	Battery voltage (see below)

To measure PAR, plug in light sensor into AUX port and right-click on the alphanumeric PAR field (Table 16). When the light sensor is plugged in, the software automatically uses the actually measured PAR, that is, the option "Use PAR-Sensor" is active (Table 16).

Table 16: PAR



- Connect light sensor.
- Right-click into alphanumeric PAR field



Sensor	PAR Slope WATER	PAR Slope AIR	Immersion Factor $\left(\frac{\text{Sensitivity AIR}}{\text{Sensitivity WATER}}\right)$
Spherical US-SQS/WB	1.000	0.581	1.72
Planar US-MQS/WB	1.15	1.000	1.15
Leaf Clip 2030-B	-	1.000	-

The default gain of 1.000 is correct when the medium of the current measurement is the same as that used for calibration. This applies, for instance, to PAR measurements with the spherical US-SQS/WB in aqueous environment, because the sensor was calibrated in water. When the US-SQS/WB is used in air, Gain=1.000 is invalid, and the immersion factor must be considered (Table 16).

After adjusting the gain factor, keep sensor dark and click the Auto Zero button (Table 16). The software determines and automatically subtracts from future PAR measurements the dark signal. The “Low” and “High” values are the offset levels of the two different gain levels of the amplifier.

Right of the PAR field, the temperature indicator is active when the 2030-B leaf clip is connected. The temperature sensor does not require adjustments.

The button U Batt starts a test method for the DUAL-PAM battery. Instructions are given in Fig. 35. The U Batt. command triggers a saturation pulse. Typically, the pulse decreases battery voltage by about 0.1 V. Larger drops may indicate low battery capacity. The DUAL-PAM system switches off when the voltage drops below 11.5 V. The battery voltage must exceed a threshold value (between 12.0 and 12.5 V) before the DUAL-PAM system can be started again.

Carry out battery test without charger and with both measuring heads connected.

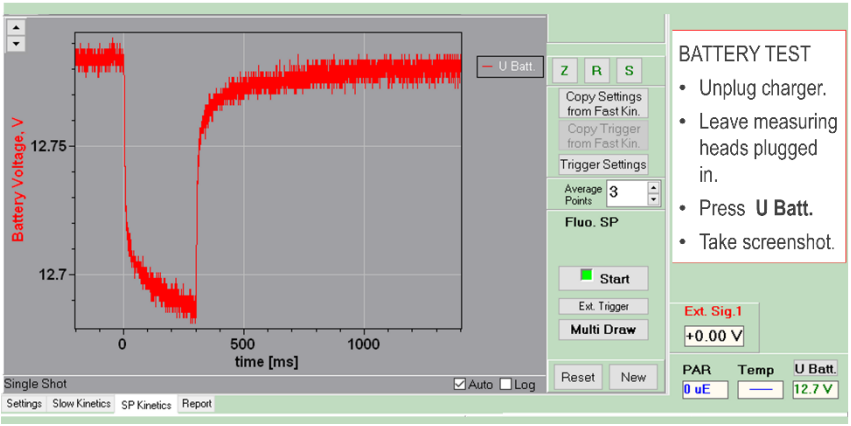
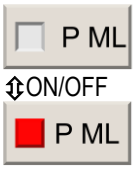
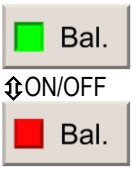
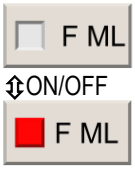
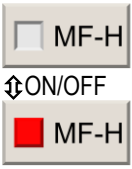


Fig. 35: Battery Test

6.1.13 Control Buttons

The functions of control buttons include measuring and actinic light control plus the command for saturation pulse analysis with light-exposed samples. The four leftmost buttons control measuring light as is indicated in Table 17.

Table 17: Measuring Light

P700 measuring light			
	Measuring light, switch and indicator		Dual function: measuring light ON switch and balance. When measuring light is on, balance only
Fluorescence measuring light			
	Measuring light, switch and indicator		Permeant activation of high measuring light frequency. Indicator of high measuring light frequency

The middle area of the field Control Buttons (compare Fig. 30, page 45) has seven functions to operate the actinic light sources, and one function, TR, to operate the voltage of the TRIGGER OUT socket at the front of the housing (4.1.2, page 12, and 4.2.2, page 21). The TR also activates stirring when the stirrer switch is in the ON position.

Table 18 summarizes the options for illumination. Samples can be exposed to red or blue light. The light may either be manually switched on and off, or illumination is for a defined time interval. Illumination intervals range from 5 µs to 60 min.

The duration of short light pulses is selected in a special window (Table 19, page 69). In this window, sequences of very short (single turnover) pulses can be defined. These sequences are used to achieve certain S-states of the oxygen evolving complex of photosystem II.

Table 18: Actinic Light and Pulses

Always, the light intensities of the respective settings windows apply, except the ST.

<table>
<tr><td colspan="4">Define continuous illumination or exposure time in settings windows for Actinic Light or Saturation pulses.</td></tr>
<tr><td>AL

Actinic light switch.</td><td>FR/BL

Far-red/Blue actinic light switch. Right-click toggles between far-red and blue. Note: the setting also applies to FR/BL pulse, FR/BL + yield, and the F_0' mode.</td><td>TR

Trigger switch. The trigger affects both TRIGGER OUT socket (4.1.2, page 12 and 4.2.2, page 21) and the stirrer. Right-click changes to “Stir”

Stir</td><td>MT

Multiple turnover pulse. (Length permits several photochemical turnover of photosystem II.)</td></tr>
<tr><td>AL Pulse

Actinic light pulse.</td><td>FR/BL Pulse

Far-red/Blue actinic light pulse.</td><td>TR Pulse

Trigger pulse.</td><td>ST

Single turnover pulse. (Length permits one photochemical turnover of photosystem II.)</td></tr>
<tr><td colspan="4">To define pulse lengths, right-click on button to open “Pulse Widths” window. These pulses do not affect the measuring light frequency, but maximum measuring light frequency is automatically employed in saturation pulse analyses.</td></tr>
</table>

Table 19: Pulse Configuration

Pulse Widths					
	AL Pulse Width	FR Pulse Width	TR Pulse Width	MT Pulse Width	ST Pulse Width
Range	1 - 1000 ms	1 - 1000 ms	1 - 1000 ms 10 – 250 µs	1 - 300 ms	5 – 50 µs
ST Pulse Applications					
Feature	☑ S+H off	Extended S+H off time	ST Pulse sequence	☑ Trigger Fast Kin. after Sequence	
Comment	When checked the Sample and Hold amplifier is switched off during the ST to prevent artefactual signals.	Additional period after the ST in which the Sample and Hold amplifier is switched off (10 – 100 µs).	A sequence is defined by the number of ST pulses (1 -20) and the time between two ST pulses (width 20 – 500 ms). To execute an ST pulse ST Flash sequence (i) Select "Fast Acquisition Analysis Mode", (ii) open "Fast Kinetics" window, and (iii) click the ST button. When checked, the currently loaded "Fast Trigger File" will be executed after the ST sequence.		

The rightmost control buttons trigger saturation pulse analysis with light-exposed samples (Table 20). Several saturation pulse routines are preprogrammed (6.1.7.3, page 57). For analysis of P700, saturation pulses are followed by a short dark phase to determine the signal when all P700 is reduced. The predefined routines can be edited in the settings window "Sat. Pulse". When activated, the F_0' mode assesses the F_0' level after the saturation pulse (Fig. 34, page 63).

Table 20: SP Buttons

	Trigger button for saturation pulse analysis with light exposed samples.
	Same as above plus preceding illumination period with blue, red, or far-red light. These buttons are inactive (greyed-out) when in Actinic Light" settings the width is set to manual.

6.1.14 F(I)/Fo

The F(I)/Fo field addresses fluorescence not emanated by photosystem II. Different from photosystem II fluorescence, this fluorescence is constant. In higher plants, the constant fluorescence is principally attributed to photosystem I.

The amount of constant fluorescence is automatically calculated and displayed as the percentage of constant fluorescence relative to total F_0 fluorescence: “F(I)/Fo calc. %” (Table 21). These relative numbers are logged in the Report table in column “F(I)/Fo calc”.

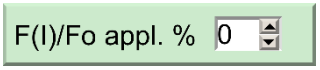
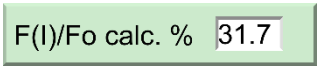
Calculation of constant fluorescence requires that the F_0’ mode is active and that the F_0’ is smaller than the F_0 (see Chapter 7, page 161 for definitions). The equation used has been derived in:

Pfündel EE, Klughammer C, Meister A, Cerovic ZG (2013) Deriving fluorometer-specific values of relative photosystem I fluorescence intensity from quenching of F_0 fluorescence in leaves of *Arabidopsis thaliana* and *Zea mays*. Photosynth Res 114:189–206. https://doi.org/10.1007/s11120-012-9788-8

A reliable estimation of F(I)/Fo is most successful when samples are slowly acclimated to light. This is achieved in light curve experiments with individual light steps of three minutes or longer.

To correct the fluorescence signal for constant fluorescence, a percentage value must be entered in the field “F(I)/Fo appl. %” (Table 21). The command Recalc. subtracts the constant part from total fluorescence for all fluorescence levels and newly calculates fluorescence ratio parameters (F_V/F_M, Y(II) NPQ …). The absolute value subtracted is listed in column F(I) of the Report table. The correction is made only for the currently selected Record. To enable Recalc., save Report and open it again.

Table 21: F(I) Fluorescence

	Manual input field. Relative unit: photosystem I fluorescence or constant fluorescence per F_0 fluorescence.
	The command Recalc subtracts the value entered in the input field described above from all fluorescence levels, and then newly calculates all fluorescence ratio parameters.
	Photosystem I fluorescence or constant fluorescence per F_0 fluorescence. The value is automatically calculated if F_0'(measured)<F_0.

6.1.15 Clock/Script

The clock function repetitively triggers an event. The number of repetitions is defined in "Slow Kin." Setting (Section 6.2.3.1, page 87). The clock is manually switched off for the setting "infinite". The clock interval must be longer than the time of the event selected.

Table 22: Clock

Events		**Standard Intervals**
SP/Fast Kinetics ST MT AL Pulse FR Pulse TR Pulse AL+Yield FR+Yield	Fast Kinetics is executed in the mode "Fast Acquisition". All other events are explained in 6.1.13 (page 66).	1 s to 60 min **Shortened intervals** 0.1 s to 6 min Applies when "Clock frequency x 10" in the Options menu is active. Note: Standard intervals are still displayed and counter is inactive. **Counter** Counts down until the next event

The buttons below the Clock field give access to the script file window. Script files permit automatic runs of experiments (6.5, page 144).

6.2 FURTHER SETTINGS

In addition to Mode settings, six additional settings windows exist. Their tab title and section number in this manual are listed in Table 23.

Table 23: Settings Tabs

Tab Title	Meas. Light	Actinic Light	Slow Kin.	Trig. Run	Sat. Pulse	SP Trigger
					In Fast Acquisition Mode: Fast Acqui.	In Fast Acquisition Mode: Fast Trigger
Section	6.2.1, page 72	6.2.2, page 81	6.2.3, page 87	6.2.4, page 90	6.2.6, page 97	6.2.7, page 100

6.2.1 Measuring Light

6.2.1.1 Intensity

Measuring light intensities are adjusted in the “Int.” boxes of the fluorescence and P700 fields. Intensities are adjustable in 20 steps. For fluorescence measuring light, the column entitled “uE” gives the photosynthetically active radiation (PAR, µmol s^{-1} m^{-2}) for each step. The column “Value” contains the corresponding relative current through LEDs (see also Table 11, page 49 and Section 6.1.12, page 64). P700 measuring light intensities are not listed because the near infrared (NIR) radiation is photosynthetically inactive.

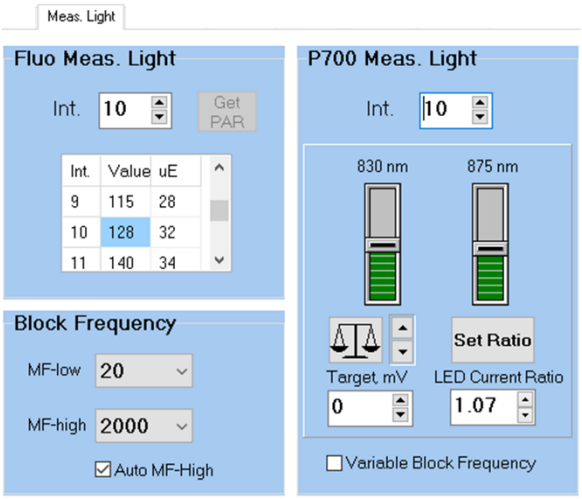


Fig. 36: Measuring Light Settings

Default settings after loading Walz.DEF.

Get PAR The button initiates the measurement of the PAR of the current fluorescence measuring light and writes the PAR for the condition of 10 000 Hz block frequency in the PAR list. For setting “block frequency = 10 000 Hz”, the measured PAR is directly used. For lower measuring light frequencies, the measured PAR will automatically be upscaled to the PAR at 10 000 Hz block frequency.

To Get PAR, connect light sensor, adjust Zero offset and gain (see Section 6.1.12, page 64), switch on measuring light an choose high frequency measuring light (MF-H).

6.2.1.2 Block Frequency

DUAL-PAM machines use “blocks” of fluorescence and measuring light pulses except for MF-max (Fig. 38B, page 77). The MF-max

is available only for fast kinetics in the Single Channel mode. A block consists of 14 measuring pulses and a dark phase (Fig. 37A).

MF-low is the block frequency used for F_0 determination with dark-acclimated samples. The default value is 20 Hz. With increasing frequency, the time resolution increases but also the integrated light intensity. Increased measuring light intensities can partially close the photosystem II reaction centers which elevates the measured fluorescence and makes F_0 measurements incorrect.

When the measuring light is switched on, the sample fluorescence should jump to the F_0 level and stay there. When the initial jump is followed by a slowly rising signal, the frequency or intensity (or both) of the measuring light should be reduced.

By default, the fluorescence option “Auto MF-high” is active. This means that the block frequency selected as MF-low is active when actinic light is off. Switching on actinic light automatically activates block frequency MF-high (Fig. 37, page 75 and Fig. 39, page 78). Hence, the Auto MF-high applies very low integrated measuring light intensities for correct F_0 determination in the dark, but automatically increases time resolution in light-exposed samples when increased integrated measuring light intensities are acceptable.

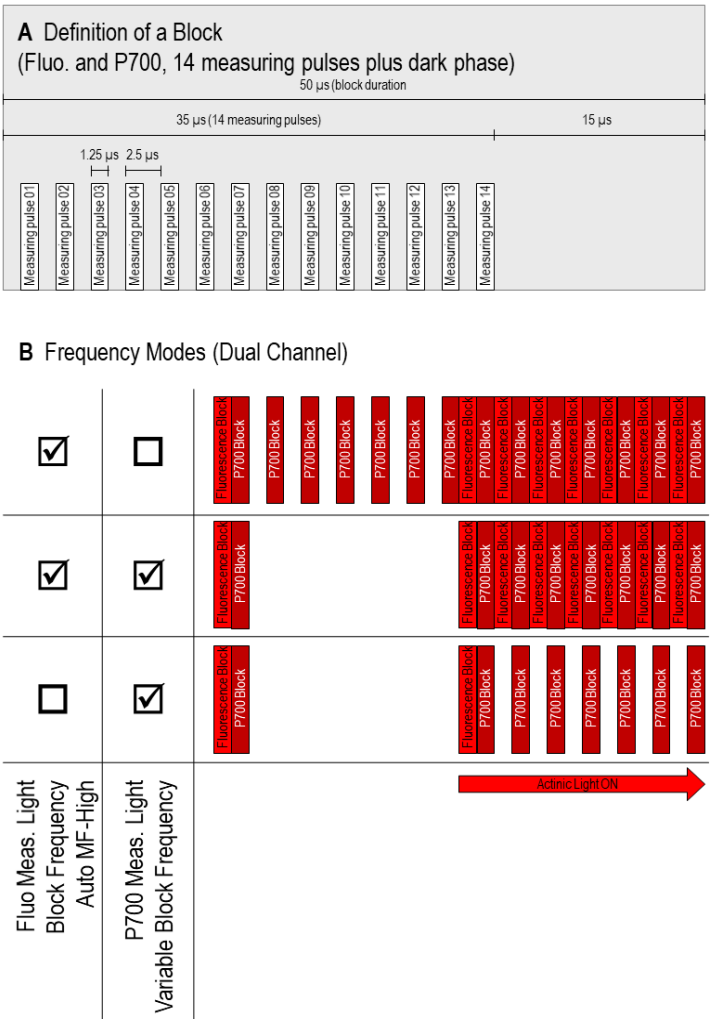


Fig. 37: Block Modes

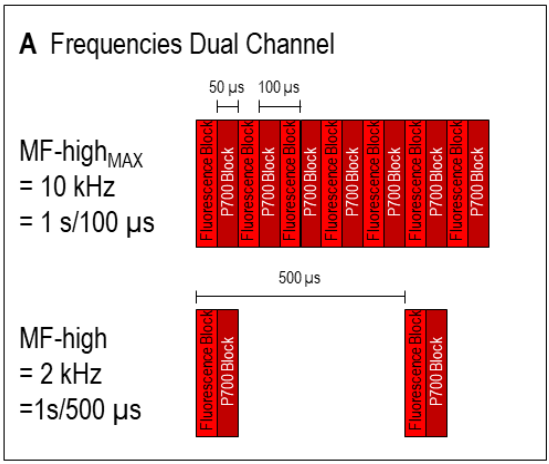
A: Outline of a fluorescence or P700 measuring light block. **B**: Block settings. Upper row is default. Middle row, low P700 measuring light frequency in the dark, applicable when P515 instead of P700 is measured. Lower row, Auto MF-high off to keep measuring light intensity low.

Also during the far-red phase for F_0' measurement, the function "Auto MF-high" switches the MF-high to MF-low (Fig. 39, page 78). The low measuring light intensity of MF-low is photosynthetically insignificant and supports the F_0' determination (see Section 6.1.10, page 59). Increased light intensities can drive charge separation of photosystem II reaction centers at rates competing with the drain off of electrons by photosystem I.

Inactivating "Auto MF-high" is useful when actinic and measuring light differ and the influence of the measuring light color must be kept low.

The P700 option "Variable Block Frequency" is off by default. This sets the P700 block frequency always to MF-high (Fig. 37, page 75). Because the P700 measuring light is not photosynthetically active, the high measuring light frequency does not disturb the dark acclimation status of a sample. The function "Variable Block Frequency" is important for P515 measurements in which measuring light is absorbed by photosynthetic pigments.

Always, the highest possible measuring frequency is applied in saturation pulse kinetics. This frequency is 10 kHz in the Dual Channel mode and 400 kHz in the Single Channel mode (Fig. 38, page 77 and Fig. 39, page 78).



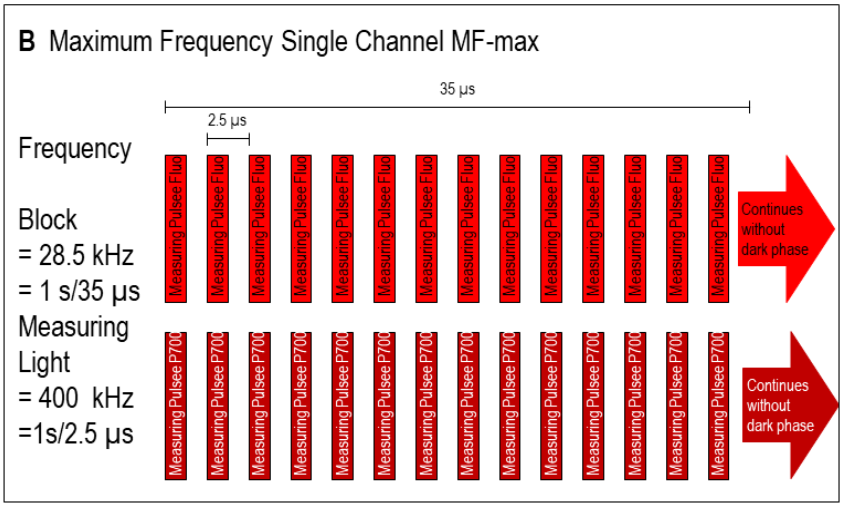


Fig. 38: Measuring Light Frequency

A: Dual Channel mode. Alternating blocks of fluorescence and P700 measuring light. Maximum frequency, 10 Khz. Default frequency, 2 kHz. **B**: Single Channel mode. Maximum frequency is determined by measuring light pulses not by blocks. Only available for fast kinetics.

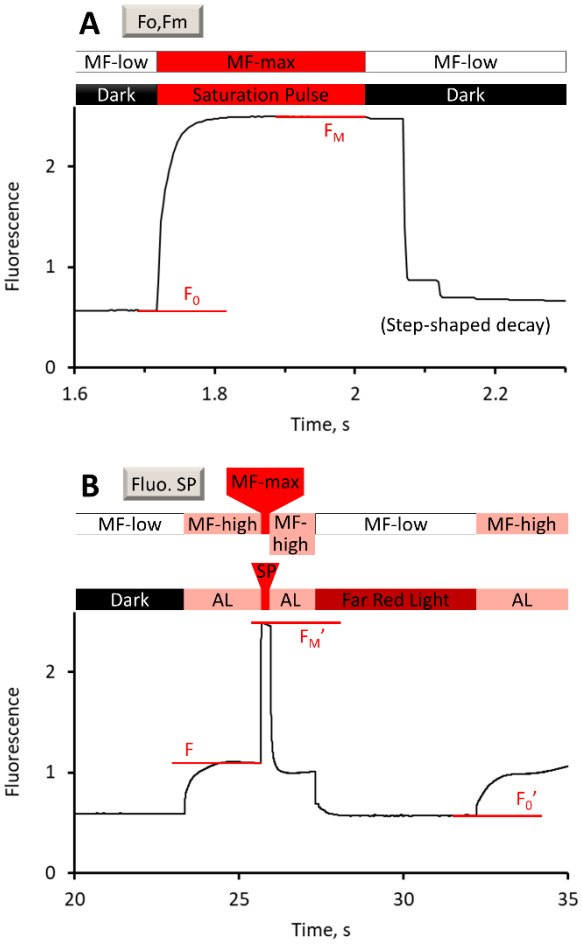


Fig. 39: Auto MF-high

Measuring light frequencies when "Auto MF-high" is active. Mode, SP-Analysis and Single Channel Fluo. A: F_0, F_M determination. Saturation pulse analysis always switches measuring light frequency to MF-max, irrespective of whether or not the Auto MF-high is active. The step-shaped decay is due to the low measuring light frequency (MF-low) in the dark. B: First saturation pulse of an induction curve. Actinic light (AL) switches measuring light frequency from MF-low to MF-high.

6.2.1.3 P700 Meas. Light

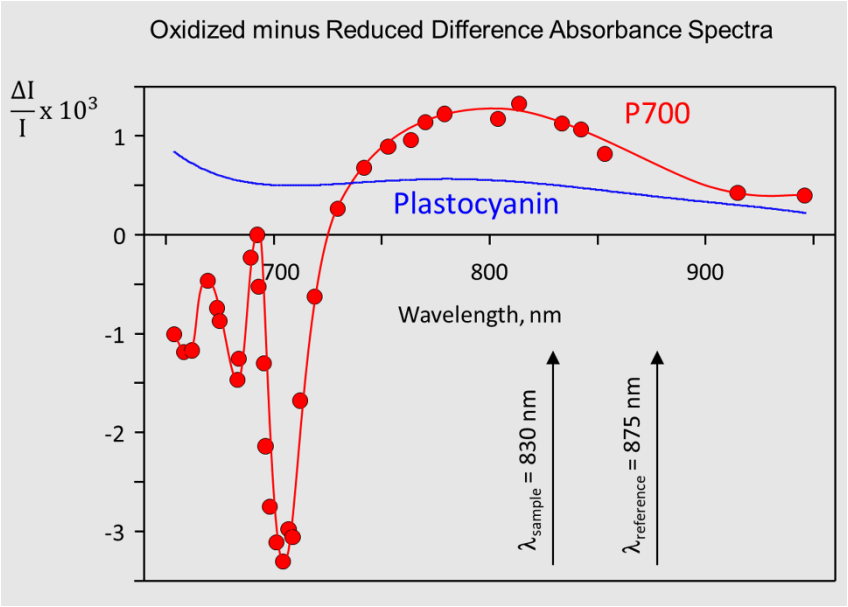


Fig. 40: P700 Difference Spectrum

Spectra redrawn from Klughammer C, Schreiber U (1991) Analysis of light-induced absorbance changes in the near-infrared spectral region I. Characterization of various components in isolated chloroplasts. Z Naturforsch 46c: 233-244. https://doi.org/10.1515/znc-1991-3-413. Vertical arrows indicate the wavelengths of P700 measuring light.

The oxidation of the photosystem I reaction center P700 to $P700^+$ causes a narrow negative absorbance peak at 700 nm and a broad positive absorbance band centered at around 800 nm (Fig. 40, page 79). The absorbance change at 700 nm is larger than at 800 nm. Still, the band in the near infrared (NIR) is used to evaluate the P700 state. The reason is that the photosynthetic samples are transparent in the NIR which avoids interferences by photosynthetic pigments.

The P700 is probed by a two-wavelength absorption method. The sample wavelength is positioned near the 800 nm maximum and the reference wavelength at its long wavelength edge (Fig. 40, page 79). The method only requires that the P700 absorption change is greater at the sample than at the reference wavelength. The wavelengths of sample and maximum absorbance change do not have to be identical, and the reference wavelength may lie in the region of P700 absorption changes.

The reference measurement corrects the sample measurement for nonspecific absorption changes. The correction works perfectly when a non-specific absorption change has the same extent at sample and reference wavelengths. Oxidation of plastocyanin results in a difference absorption spectrum which is relative featureless but shows higher values at sample compared to the reference wavelength (Fig. 40, page 79). Therefore, plastocyanin absorption changes are not fully corrected by the two-wavelength method.

Table 24: P700 Settings

	The P700 signal is the difference between the signal measured at 830 nm minus the reference signal at 870 nm. The “Balance” button automatically adjusts the LED currents so that the signal difference is close to zero (Target = 0). The balance button is equivalent to the “Bal.” button of the Control Buttons (Section 6.1.13, page 66). Balancing must be carried out with a sample in place. A stable baseline requires that the measuring light LEDs have warmed up for at least 30 seconds.
	Manually setting the current ratio (Ratio) is usually not required for P700 measurements.

6.2.2 Actinic Light

6.2.2.1 Intensity

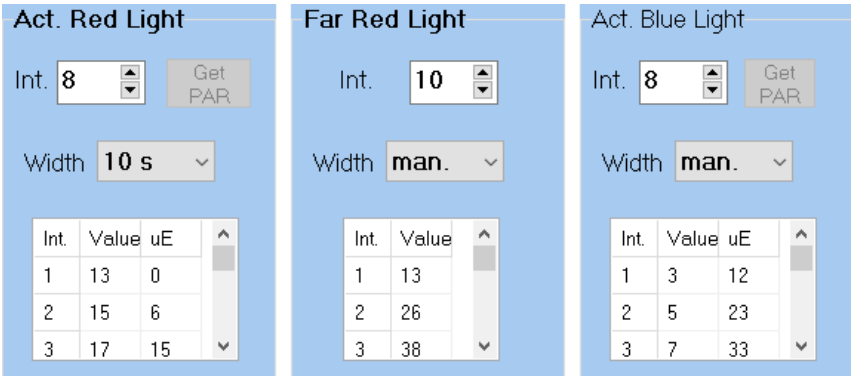


Fig. 41: Actinic Light Intensities

In contrast to measuring light, actinic light is employed to drive photosynthesis. The three actinic light sources are red, blue, and far-red. 20 intensity levels are available. The intensity level is adjusted by the up/down arrows of the “Int.” field (Fig. 41), or by a left-click and typing in the intensity level.

The 20 intensity levels form the (“Int.”) column of the actinic light tables. For each level, the relative LED current (“Value”), and the PAR in µmol m^{-2} s^{-1} is given, except for far-red. Far-red intensity information is omitted because most of this radiation is not absorbed by photosynthetic pigments and, thus, intensity data are potentially misleading.

The LED current (“Value”) can be edited to create a new PAR list. After having changed the LED current, the PAR must be newly measured. See Table 11, page 49 for information on PAR lists and Table 13, page 51 for information on the command “Get PAR” and PAR measurements.

All actinic light sources can be operated manually by selecting “man.” in the respective drop-down menu for “Width”. Alternatively, fixed illumination intervals from 1 s to 1 h can be set. The actinic light source is automatically terminated when the interval has elapsed. A fixed illumination interval can be switched off manually.

Default

The Default command resets all actinic light and trigger settings to their default value. Default also selects far-red as second actinic light source.

6.2.2.2 Sine Mode

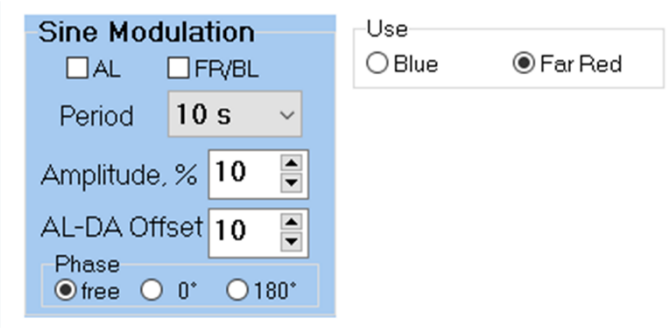


Fig. 42: Sine Settings

All three actinic light sources (red, far-red, and blue) can be sine modulated. Sine modulation is active only when a “Slow Kinetics” runs in the “Manual” mode, or in the “Triggered Run” mode.

Select sine modulation for a single light source by ticking the "AL" or “FR/BL” check box. Tick both check boxes to simultaneously sine modulate red (AL) and blue light, or red and far-red light. Blue and far-red are mutually exclusive. Select blue or far-red radiation

by clicking the “Blue or “Far-red” radio button (Fig. 42), or by right click on the FR/BL area of the Control Buttons (6.1.13, page 66).

The “Period” defines the time between two maxima. Periods ranging from 1 s to 24 h can be chosen from the drop-down menu.

The “Amplitude” is the half distance between maximum and minimum of the sine wave (Fig. 43A). The amplitude settings range from 10 % to 100 % at increments of 10 %. The intensity variation by sine modulation varies from maximum to minimum of the sine wave. The sine wave maxima coincide with the respective setting of the actinic light intensity.

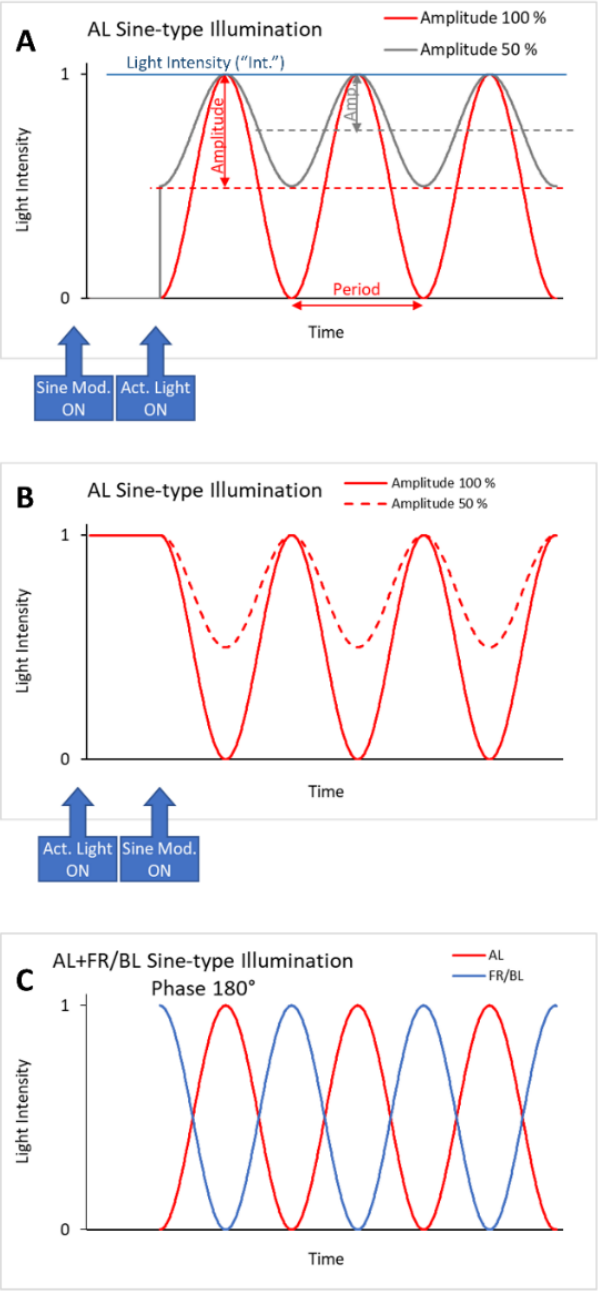
Fig. 43 (page 84) gives examples for sine modulation. Panel A: For amplitude=100 %, the light intensity ranges from zero to the intensity set for the actinic light source; for amplitude=50 %, the intensity varies between half and full intensity.

Fig. 43A and B: When the sine mode is selected first, followed by switching on actinic light, the intensity starts at 0 for amplitude=100 %, and it starts at 50% of the actinic light intensity setting for amplitude=50 %. When actinic light is switched on first followed by activation of sine modulation, sine modulation starts with the actinic light intensity setting which varies as in panel A.

The numbers in the “AL-DA Offset” box are relative current values, as are the data in the “Value” column of light lists. The entire sine illumination is increased by the AL-DA Offset value selected. The AL-DA Offset is employed for sine modulations with 100 % amplitude value. At the minimum of these sine curves, the relative current value drops to zero. Already before reaching zero, the voltage applied can be lower than the breakdown voltage of the actinic LEDs, that is, the LEDs switch off. This results in a sine illumination that suddenly drops to zero before the actual minimum is reached. Using an appropriate AL-DA Offset prevents this behavior.

Fig. 43: Sine Illumination

Time courses of sine illumination. **A** Sine mode is activated first, followed by switching on actinic light (see blue boxes). Red curve, amplitude setting 100 %; gray curve, amplitude setting 50 % (see double arrowed lines). Blue horizontal line on top, AL intensity setting. Bottom double arrowed horizontal line, period. **B**, Actinic light is switched on first, followed by activation of sine mode for AL (see blue boxes). **C**, Simultaneous illumination with red and blue or far-red light. Phase 180°, amplitude 100%.



The intensity of high frequency measuring light can interfere with sine modulation when a 100°% amplitude value is used: the effective illumination does not become zero at the minimum of the sine wave. The effect of measuring light can be reduced by setting MF-high to lower frequencies or switching off Auto MF-high (see 6.2.1.2, page 73).

The “Phase” setting applies for sine modulation of two actinic light sources. Both light sources change synchronously when “0°” is ticked. Maximum intensity of one light source occurs together with minimum intensity of the other light source when “180°” is selected (Fig. 43C). The setting “free” starts sine modulation of the second light source when it is actually switched on. Use a Triggered Run for exact timing of the free phase shift.

6.2.2.3 Blue and Red Light

Choose between blue and far-red radiation using the respective radio buttons (Fig. 44) or the FR/BL button. Selecting blue replaces the far-red phase of the F_0’ mode by a blue phase (cf. Fig. 34, page 63). To replace far-red of the preillumination phase for Pm determination by blue (cf. Fig. 33, page 62), check “Use for Pm-Determination” (Fig. 44). Applying blue light can be useful for work with cyanobacteria.

The box “Use as actinic light” applies for induction and light curves. In these experimental routines, the red actinic light is replaced by blue light (check “Blue”), or blue light is added to red light (Check “Red and Blue”, Fig. 44). For induction curves, the intensity settings for actinic light apply. For light curves, the same light intensity steps apply for red and blue. To modify red/blue intensity ratios, edit the LED current values and measure PAR.

Fig. 44: Blue and Red Light

6.2.2.4 Trigger and Pulse

The trigger “Width” works as described for actinic light (Section 6.2.2.1, page 81). The trigger applies a 5 V signal to the Trigger OUT channel of the control unit and activates the stirrer when switched on (see Chapter 4, page 11). The button Pulse Widths opens the “Pulse Widths” window (Table 19, page 69).

Fig. 45: Trigger and Pulse

6.2.3 Slow Kinetics

6.2.3.1 Acquisition and Clock

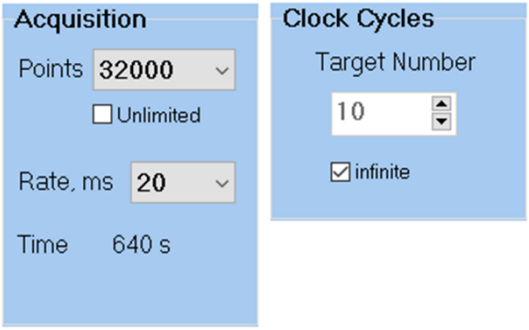


Fig. 46: Acquisition and Clock

The product of “Points” times “Rate” in the “Acquisition” box determines the length of a slow kinetics. The range of points extents from 1000 to 1024000 and the rate extents from 1 ms to 100 ms (see the drop-down menus). Thus, the total acquisition time can be as short as 1 s and as long as 28 h. (Note that the rate is defined in the DualPAM software in a rather unusual way as the time interval between two measurement points.)

Set the acquisition time longer than the duration of an automatic experimental sequence. Induction and light curves will not start if the time of the sequence is longer than the acquisition time. Long acquisition times can be terminated manually. Experiments run until acquisition is manually terminated when “Unlimited” is checked.

The “Rate” affects the behavior of the flux method (see 6.1.7.2, page 53). Acquiring slow kinetics with a small acquisition rate (e.g., 1 point per ms) permits data smoothing by averaging without apparent loss of time resolution.

In the “Clock” box, the “Target Number” is the number of clock-triggered events. After manual clock start (Section 6.1.15, page 71), the clock stops automatically after the specified number of events. Checking “infinite” renders the function “Target Number” inactive. In this case, the clock runs until terminated manually.

6.2.3.2 Slow Induction Curve

The box “Slow Induction Curve” (Fig. 47A) configures an experiment in which a dark-acclimated sample is exposed to light for a certain period of time. The exposure experiment can be followed by recording the fluorescence in the dark (recovery curve). Recording the recovery process is activated in the drop-down menu for recording modes on the Slow Kinetics window. All configuration parameters are illustrated in Fig. 47B-D.

The “Delay” specifies time interval between the first saturation pulse of the experiment (F_V/F_M determination) and onset of actinic light (Fig. 47C). The “SP2 Delay” defines the time lag after onset of actinic light of the first saturation pulse of the light period (Fig. 47D). The “Clock” specifies the time interval between neighboring saturation pulses in the light period (Fig. 47B). “AL-Width” and “Recovery Time” correspond to the duration of light exposure and measuring time in the subsequent dark period, respectively. All time units are seconds except the SP2 Delay which has to be entered in milliseconds.

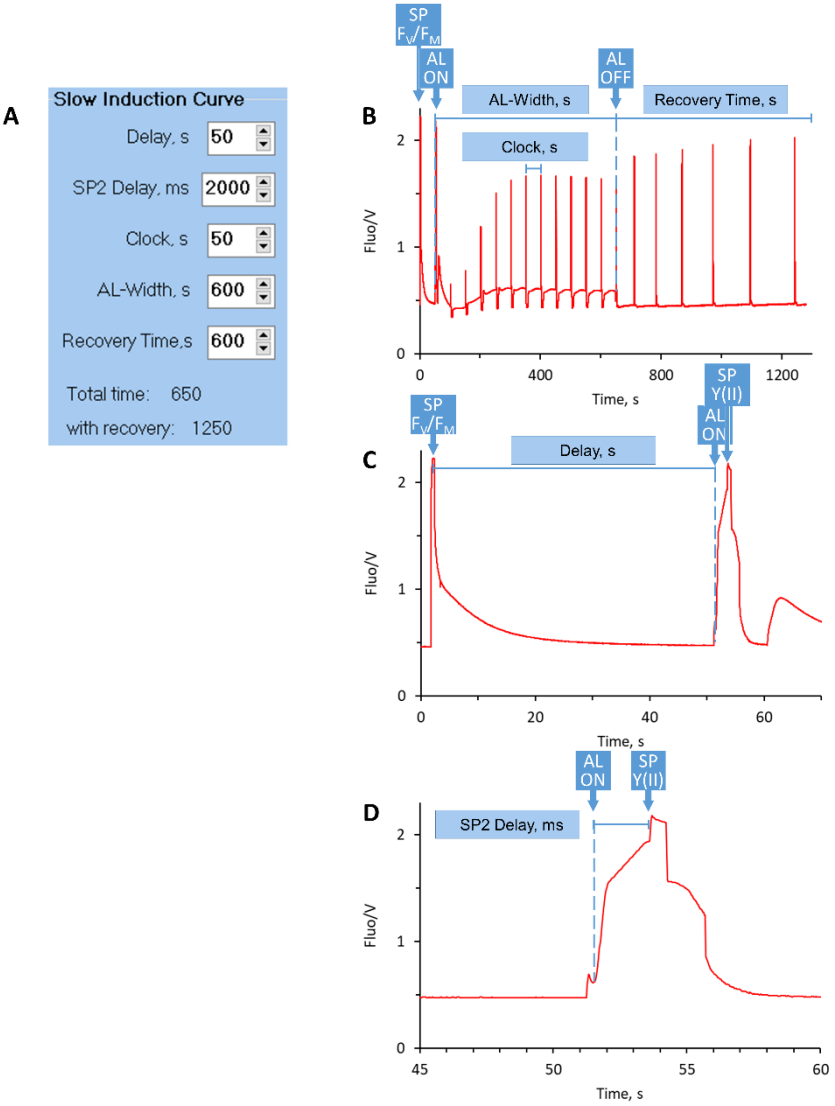


Fig. 47: Slow Induction Configuration

6.2.4 Triggered Run

A triggered run performs experiments automatically. A triggered run can repeat exactly a previously recorded experiment.

A “standard” trigger table can be built by copying all actions of a manually operated slow kinetics. After the slow kinetics has been executed, click Copy Trig. Run from Man. Rec. The button Copy Settings from Man. Rec. adds the current device settings to triggered run. The trigger table including settings can be stored and recalled. The triggered run is executed by running a slow kinetics in the mode “Trig. Run” (see drop-down menu in Slow Kinetics window).

Lines of a trigger table are edited by a menu popping up after right click on a line number of the trigger table Fig. 48 (page 91). In this menu, the commands New/Delete/Clear Line are self-explaining. The command “Copy Line” reproduces the line selected. The number of copies is chosen from a list which appears when the Copy Line command is executed.

“Set Copy width” defines the time shift of a copied line relative to its origin, or relative to the previous line when several copies are made. The selected line plus successive lines can be copied as a group. The number of lines of the group is specified via the menu item “Set Copy Lines”.

A trigger table consists of maximally nine columns (Fig. 48). The first one, the “Time” column, determines the time point in ms of the action defined in the same line. The time point can be easily edited by clicking into the time field.

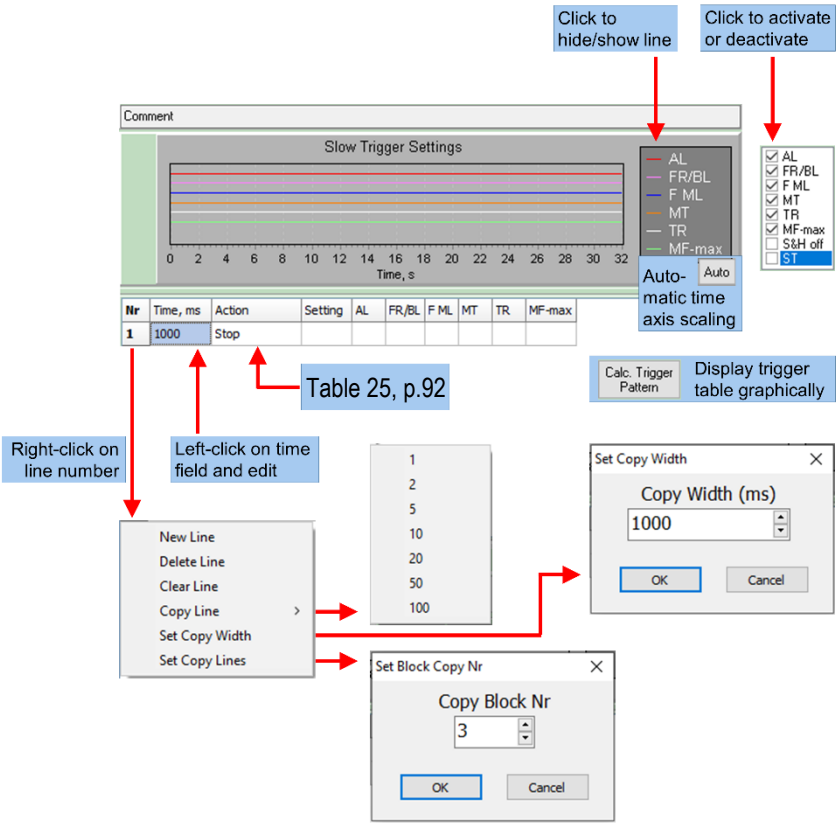


Fig. 48: Trigger Table Line Editing

The second column “Action” contains the commands to be executed. Each command is picked from a drop-down list which opens by three clicks into any action field. Table 25 compiles all commands.

Several types of commands are available. Each command affecting settings is accompanied by a drop-down menu in the neighboring “Settings” field (column number 3) from which the setting is chosen. Examples are “AL-Int.” or “MF-L Change”, see Table 25.

Actions like “FoFm” or “Pm “are executed with the present settings.

The command “Trig. Out” is particular as it controls the switch-on and switch-off times of 6 different channels. How to set trigger on and off switches is illustrated in Fig. 49.

Table 25: Triggered Run Commands

Open a drop-down list of actions by three clicks (mark “Action” field, mark command, open drop-down list). Quickly find a particular action by typing the first letter of the command. Pressing the key twice in rapid succession leads to the second command in the list with the same first letter, and so on.

	Action	Meaning	Comment
1	Stop	Stop acquisition	
2	Fluo. SP/Fast Kin.	In mode SP-Analysis: Execute saturation pulse analysis. In mode Fast Acquisition: Execute current fast trigger pattern.	
3	Fo	Measure minimum fluorescence of dark-acclimated sample.	
4	Fo’	Measure minimum fluorescence of light-exposed sample	
5	FoFm	Saturation pulse analysis with dark-acclimated sample	
6	Pm	Pm determination (dark-acclimated sample)	
7	ST	Single turnover flash	Only high resolution triggered run

Table 25: Triggered Run Commands

Open a drop-down list of actions by three clicks (mark "Action" field, mark command, open drop-down list). Quickly find a particular action by typing the first letter of the command. Pressing the key twice in rapid succession leads to the second command in the list with the same first letter, and so on.

	Action	Meaning	Comment
8	MT	Multiple turnover flash	The pulse width of the Pulse Width table and the intensity of the Fluo-SP (SP-Analysis) or MT (Fast Acquisition) apply. Open Pulse Width table by right-click on MT button.
9	**Trig. Out**	On/Off (0/1) switch for AL (actinic red light), FR/BL (far-red or blue), F ML (fluorescence measuring light), MT (multiple turnover pulse), TR (trigger out), and MF-max (maximum measuring light frequency).	Beginning and end of the MT must be entered (compare with the action "MT", above). MT lengths > 0.8 s will be automatically terminated to prevent LED damage.
10	MF-L	Use low frequency measuring light	The measuring light settings apply.
11	MF-H	Use high frequency measuring light	As stated above.
12	MF-L Change	Set value for low measuring light frequency.	Choose value from drop-down menu in the column "Setting".
13	MF-H Change	Set value for high measuring light frequency.	As stated above.
14	XML on	P700 measuring light on	
15	XML off	P700 measuring light off	
16	AL Pulse	Execute red actinic light pulse.	The width of the Pulse Width table and the AL intensity setting apply.

Table 25: Triggered Run Commands

Open a drop-down list of actions by three clicks (mark "Action" field, mark command, open drop-down list). Quickly find a particular action by typing the first letter of the command. Pressing the key twice in rapid succession leads to the second command in the list with the same first letter, and so on.

	Action	Meaning	Comment
17	FR Pulse	Execute far-red pulse.	The width of the Pulse Width table and the FR intensity setting apply.
18	TR Pulse	Execute trigger pulse.	The width of the Pulse Width table applies.
19	AL-Int.	Set red actinic light intensity.	Setting is 1 to 20. Double-click slowly on "Setting" field, type in number or use up/down arrows.
20	FR-Int.	Set far-red intensity.	As stated above.
21	BL-Int.	Set blue actinic light intensity.	As stated above.
22	SP-Int	Set intensity for saturation pulse (SP-Analysis) or multiple turnover pulse (MT, Fast Acquisition).	As stated above.
23	FML-Int.	Set measuring light intensity.	As stated above.
24	Toggle FR/BL	Select blue or far-red.	
25	Mod. Period	Period of sine modulation of actinic light.	Select from drop-down menu
26	AL-Mod on	Activate sine modulation of red actinic light.	
27	AL-Mod off	Terminate sine modulation of red actinic light.	
28	FR/BL-Mod. on	Activate sine modulation of far-red or blue actinic light.	

Table 25: Triggered Run Commands

Open a drop-down list of actions by three clicks (mark "Action" field, mark command, open drop-down list). Quickly find a particular action by typing the first letter of the command. Pressing the key twice in rapid succession leads to the second command in the list with the same first letter, and so on.

	Action	Meaning	Comment
29	FR/BL-Mod. off	Terminate sine modulation of far-red or blue actinic light.	

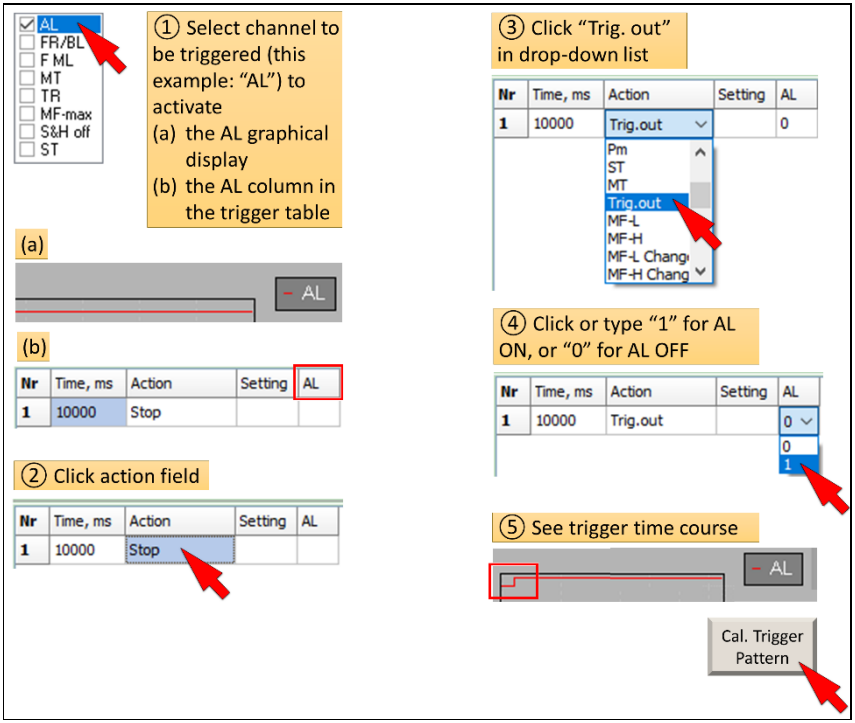


Fig. 49: Setting Trigger Commands

6.2.5 High Resolution Triggered Run

The time point of an event of a triggered run may differ by several seconds from experiment to experiment. Time shifts in successively triggered runs can interfere with curve averaging. This problem is avoided by the “high resolution” triggered run, which executes events at exactly the same time.

The high resolution triggered run controls 8 channels by the “Trig. Out” command (Fig. 49). The single turnover flash and the “S&H off” commands are only available in the high resolution mode and they are executed with the current Width settings. The action commands of the standard triggered run (Table 25) are not available. Table 26 compares the standard and high resolution triggered runs. Several events can be triggered at the same time.

Table 26: Triggered Run Types

Triggered Run Type	Analysis Mode	Time Precision	Commands	Number of data points	Simplified programming
Standard	SP-Analysis and Fast Acquisition	The same event in repeated runs can be time-shifted	30 actions plus trigger for 6 channels	Not limited	Transfer of commands from manual slow kinetics
High Resolution	Fast Acquisition only	No time shift	Trigger for 8 channels	32000	No

To enter the mode “high resolution triggered run”, check “High Resolution Trig. Run Option” in the menu “Options” (see Section 6.1.3.2, page 47) and select the analysis mode “Fast Acquisition” (see Section 6.1.7.1, page 53). The maximum number of data points of a high resolution triggered run is 32000. The maximum total time depends on the rate of data acquisition defined in the slow kinetics window (Section 6.2.3, page 87).

The high resolution mode offers the option to expose a sample to internal light prior to the triggered phase. How to activate and configure the pretrigger function is illustrated in Fig. 50.

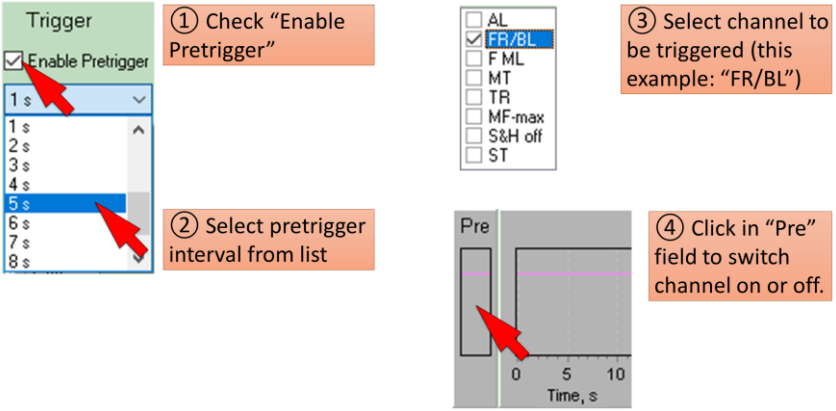


Fig. 50: Pretrigger Period

6.2.6 Sat. Pulse and SP Trigger

The window "Sat. Pulse" is present only in the mode "SP-Analysis". This window contains the settings for the saturation pulse for "saturation pulse analysis". In the mode "Fast Acquisition", the "Sat. Pulse" window is replaced by the "Fast Acqui." Window. The Sat. Pulse and Fast Acqui. windows are compared in Fig. 51.

In the window "Sat. Pulse", intensity and width of saturation pulses are adjusted. The setting of "Fm Intensity" applies only for Fo, Fm determinations with dark-acclimated samples, the setting of "Intensity" applies for all other saturation pulse analyses. The reason for two different saturation pulse intensities is that sometimes samples exhibit maximum F_V/F_M values at lower saturation pulse intensities than the saturation pulse intensity required to induce full variable fluorescence in illuminated samples.

The duration of a saturation pulse (Width) is picked from the drop-down list extending from 1 ms to 1 s. However, the effective maximum width cannot exceed 0.8 s because a safety shutdown prevents widths above this interval.

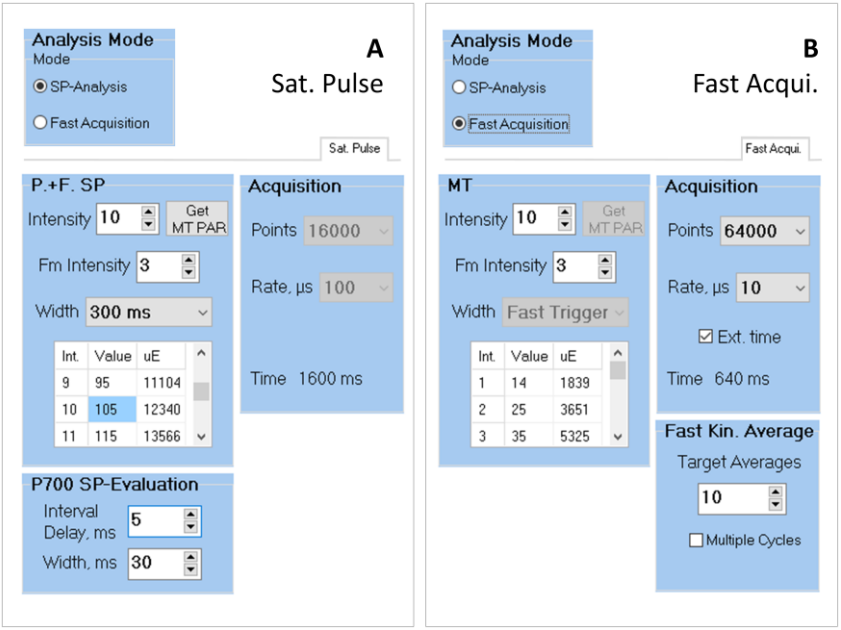


Fig. 51: Sat. Pulse/Fast Acqui.

The window “SP Trigger” displays the trigger pattern of the currently active saturation pulse. Changing in window “Sat. Pulse” the parameter “Width” modifies the trigger pattern in the window “SP Trigger”. Set to default values restores the original saturation pulse pattern.

The number of acquisition points and the acquisition rate of the fast kinetics cannot be edited. These two parameters depend on the type of saturation pulse (Section 6.1.7.3, page 57). For width values < 100 ms, the acquisition rate switches automatically from 100 µs/point to 10 µs/point, which reduces total acquisition time of

a fast kinetics from 1600 ms to 160 ms. The software displays a warning when the width of the saturation pulse is shorter than the interval of P700 SP evaluation (Fig. 52).

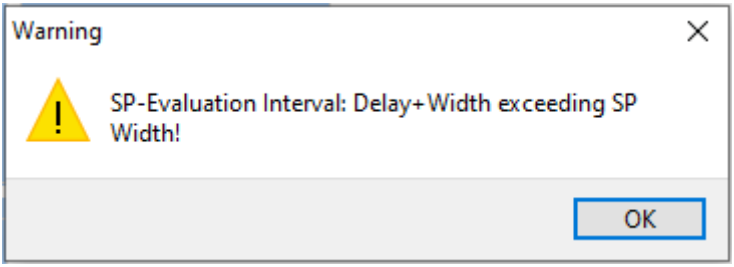


Fig. 52: P700 SP-Warning

The button Get MT PAR measures the saturation pulse intensities for all settings (see Table 13, page 51). The button is active only when a light sensor is connected.

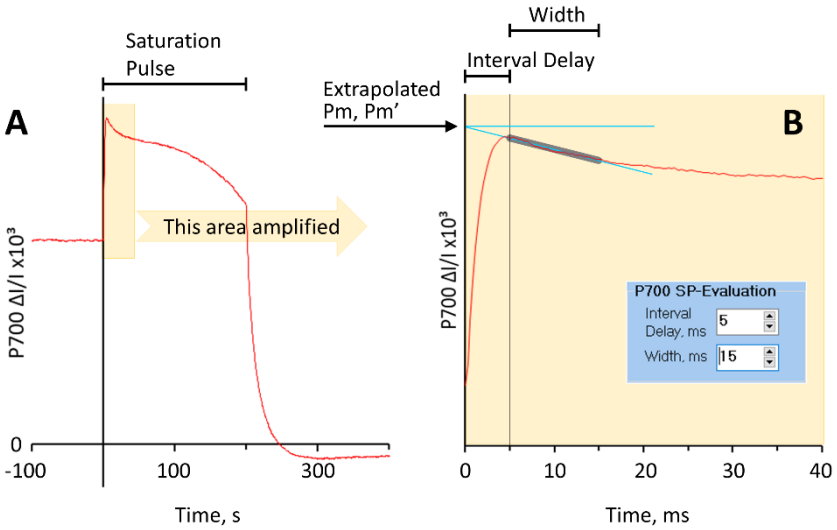


Fig. 53: P700 SP-Evaluation

The settings in input field “P700 SP-Evaluation” determine how the Pm or Pm’ is evaluated. The values are obtained by extrapolating to time zero the P700 decay right after onset of the saturation pulse (Fig. 53). The P700 at beginning of the saturation pulse cannot be measured because signal detection is switched off for the first 10 µs of the saturation pulse to avoid artefactual signals.

6.2.7 Fast Acquisition/Fast Trigger

In the mode “Fast Acquisition”, the windows “Fast Acquisition” and “Fast Trigger” replace the windows Sat. Pulse and SP Trigger.

The number of points and the rate of data acquisition is adjusted in the window Fast Acquisition. The width of the MT (multiple turnover) pulse is set in the window Fast Trigger, which also displays the currently active trigger pattern.

The maximum number of points covered by a trigger pattern is 32000. Keeping the number of acquisition points constant and increasing the acquisition rate increases the total time covered by the trigger pattern. Similarly, decreasing the acquisition rate shortens the time span of the trigger pattern.

The number of points selected in the Fast Acquisition window can be greater than 32000 (64000 or 128000, see drop-down menu). In this case, data acquisition continues after the 32000 points of the fast kinetics trigger until the total number of data points is reached.

The option extended time (☑ Ext. time) is available when 64000 or 128000 points are selected. The extended time function further prolongs total measuring time by increasing the acquisition rate at point 32001. When 128 000 points is selected, the acquisition rate is increased again at point 64 001 (see Table 27, page 101).

Table 27: Extended Time

The function “Extended time” increases the acquisition rate twice when 128000 points and 2.5 µs acquisition rate are selected.

Points total	128000	128000	128000
Rate, µs (selected)	2.5	2.5	5
Ext. time OFF ( ) or ON (✓)		✓	✓
Rate, µs (true)			
(1 - 32000 points)	2.5	2.5	5
(32001 - 64000 points)	2.5	5	10
(64001 - 128000 points)	2.5	10	10
Total time, ms	320	880	1120

Signal averaging by repeated recording of the same kinetics improves the signal to noise ratio by decreasing noise and, thus, it reduces the detection limit. As a rule of thumb, the noise approximately decreases by the square root of the number of measurements averaged. Signal averaging improves the signal without compromising the response time. Signal averaging requires a sequence of identical kinetics. Choose the interval between individual measurements so that a measurement is not affected by the previous one.

To improve a very noisy kinetics, a larger number of measurements must be averaged than for a low-noise signal. The value entered in the field “Fast Kinetics Average” determines the number of consecutive recordings to be averaged. The individual kinetics used for averaging are not stored. To keep original data, record individual kinetics and average kinetics using the average function (+). The plus button is available in both the window SP Kinetics (SP Analysis mode) and the window Fast Kinetics (Fast Acquisition mode).

Sequences of kinetics can be triggered manually, by the Multi-Start button (Fig. 65, page 129) or by the clock function (6.1.15, page 71). The clock stops after having triggered the number of kinetics to be averaged. When “Multiple Cycles” is checked, the clock continues triggering fast kinetics to form the next average of kinetics.

A sequence of fast kinetics can be aborted by the buttons Reset or New. “Reset” deletes the kinetics recorded so far and sets the counter for averaging to zero. “New” keeps the kinetics and calculates the average with the incomplete sequence of kinetics. Typically, the “New” button is employed when the signal quality is satisfactory before the average number is reached.

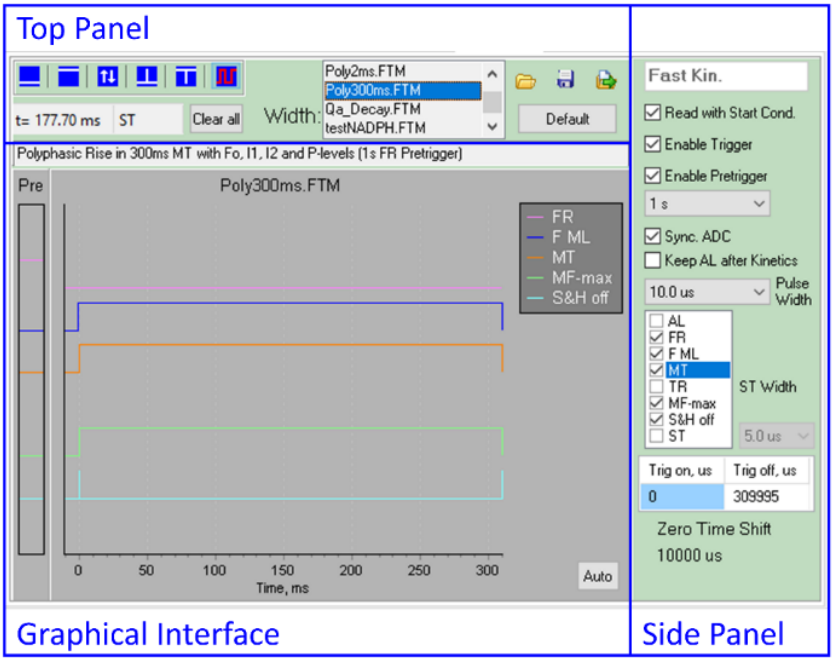
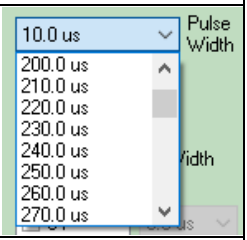
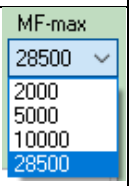
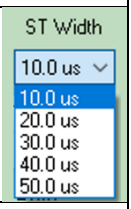


Fig. 54: Fast Trigger Window

Overview of Fast Trigger Window with its three functional sections: Top panel, graphical interface, and side panel.

6.2.8 Programming Fast Trigger Pattern

Table 28: Triggered Events		
Abbreviation	**Event***	**Settings**
☑ AL	Actinic light	Intensity (6.2.2.1, page 81).
☑ FR/BL	Far-red, blue light	Intensity (6.2.2.1, page 81). Far-red or blue (6.2.2.3, page 85).
☑ F ML	Measuring light	Intensity and frequency (6.2.1, page 72). The button ■ MF-H (Table 17, page 67) determines whether low or high measuring light frequency is applied.
☑ MT	Multiple turn-over pulse	Intensity (Fig. 51, page 98). Width (Side Panel). 
☑ TR	Trigger out	Width (as described for MT)
☑ MF-max	Maximum frequency of measuring light	Intensity (6.2.1, page 72). Frequency (Side Panel, when measure mode =“Single Channel Fluo” (cf. Fig. 30, page 45), else always 28500 Hz for Single Channel + P700 mode, and 10000 Hz for Dual Channel mode). 
☑ S&H off	Sample and hold circuit off	Width as described for MT.
☑ ST	Single turn-over flash	Intensity is always maximal. Width (Side panel). 
* Except ST, events can be switched on and off and they can be given as pulses.		

The window “Fast Trigger” consists of three functional sections: top panel, graphical interface, and side panel (Fig. 54). The window allows simultaneous timing of eight events at maximum resolution of 2.5 µs. Six of the 8 events affect either actinic or measuring light. Table 28 lists all eight events together with the settings for each event.

Top Panel

The six blue control buttons of the top panel are used to switch events (Table 29). Select an event by clicking on the respective button. Only the “Always off” and “On Pulse” buttons are available for the single turnover (ST) event.

Table 29: Fast Trigger Command Buttons

 Always off	 Always on	 Invert on/off	 On pulse	 Off pulse	 Switch on/off

The two numerical fields below the blue control buttons show the cursor position inside the graphical interface. The Clear all sets all triggers to zero.

The top panel further provides the options to save and load fast trigger files by using the folder and disk icon, respectively (Table 30). Fast trigger files have the extension “FTM" and are stored by default in the directory C:\Program Files\DualPAM\Fast Kin Trigger (see Fig. 9, page 27). You can export the trigger data as text file using the folder/arrow icon (Table 30).

Table 30: Fast Trigger File Handling

 Open Fast Kinetics Trigger File	 Save Fast Kinetics Trigger File	 Export trigger information as text file (rename export file to avoid overwriting).

The Default button opens FastKin.FTM which triggers a 300 ms poly-phasic fluorescence transient. When “Read with Start Cond.” on the side panel is checked, opening FastKin.FTM installs the settings that were active when the FastKin.FTM was saved, that is, measuring light (ML) is switched off and measuring light frequency is set to high (MF-H).

Papers describing the poly-phasic fluorescence transient are:

Schreiber U, Hormann H, Neubauer C, Klughammer C (1995) Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. Aust J Plant Physiol 22:209–220. https://doi.org/10.1071/PP9950209

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll *a* fluorescence transients in plants and cyanobacteria. Photochem Photobiol 61:32–42. https://doi.org/10.1111/j.1751-1097.1995.tb09240.x

The file list of the top panel contains the Qa_Decay.FTM fast trigger pattern. This file closes reaction centers by a single turnover flash and monitors reopening of reaction centers by measuring the fluorescence decay kinetics. In many cases, this decay kinetics can be described by the sum of two exponential functions, which might reflect electron transfer to Q_B and Q_B^-, respectively. Pertinent papers on this issue are:

Bowes JM, Crofts AR (1980) Binary oscillations in the rate of reoxidation of the primary acceptor of Photosystem II. Biochim Biophys Acta 590:373–384. https://doi.org/10.1016/0005-2728(80)90208-x

Robinson HH, Crofts AR (1983) Kinetics of the oxidation reduction reactions of the photosystem II quinone acceptor complex and the path for deactivation. FEBS Lett 151:221–226. https://doi.org/10.1016/0014-5793(83)80152-5

Side panel (see Fig. 54, page 102)

Read with start cond. Explained above.

Enable Trigger should be checked during normal operation. The command is disabled for service purposes only.

Enable Pretrigger: The pretrigger feature provides the option to preilluminate samples. Activate pretrigger by clicking the check-box. When activated, a drop-down menu containing a list of preexposure intervals is available below the check box. Furthermore, a chart, entitled “Pre”, left of the major trigger graph is visible. In this pretrigger chart, an event can be toggled on/off by clicking on the respective trigger line. Preexposure is immediately followed by the fast kinetics. Note that the “Clear All” button does not affect the pretrigger settings.

Sync. ADC synchronizes signal sampling and analog-to-digital conversion (ADC). The synchronization is on during normal operation. Sync. ADC can cause small artefactual signals which become noticeable when a large number of fast kinetics is averaged. In this case, the Sync. ADC should be switched off.

Keep AL after Kinetics continues illumination by actinic light after the fast kinetics. When this function is not active, actinic light will be automatically turned off at end of the fast kinetics.

Located on the side panel is the drop-down menu ST Width. The menu lists the available time intervals for single turnover flashes. The drop-down menu Pulse Width lists the time intervals of pulses for all other events.

The drop-down menu MF-max (only Mode: Single Channel + Fluo) permits selection of various measuring light frequencies (unit Hz) for the event MF-max (see figures in section 6.2.1.2, page 73). Lower frequencies than the maximal possible value of 28500 Hz reduce the action of measuring light on the sample. However,

lower measuring light frequency is associated with lower time resolution.

The measuring mode “Single Channel + P700” always uses 28500 Hz measuring light frequency. MF-max = 10000 Hz in the Dual Channel mode.

MF Log. decreases measuring light frequency during fast kinetics in a logarithmic fashion. This feature is useful for fluorescence decay kinetics when the effective measuring light intensity should be low. MF-max cannot be selected when MF log. is active.

Trig on/Trig off are numerical fields displaying the time points at which the trigger is switched on and off, respectively. These numerical fields and the box for event selection are explained in Fig. 55A.

Zero Time Shift moves the time point zero inside the trigger pattern. To edit the zero-time shift, double click on the numerical field. Placing the time point zero at start of a fluorescence transient permits viewing the entire transient on a logarithmic time scale. See e.g., the default polyphasic rise kinetics.

6.2.8.1 Examples

Using the example of switching actinic light (AL) on and off, Fig. 55A illustrates how trigger points are set by entering data in the numerical fields of the side panel, and Fig. 55B shows how a trigger pattern is created in the graphical interface by the mouse cursor. AL can also be given as a pulse. In this case, the time for switching off the AL is determined by the pulse width selected on the side panel. A trigger pattern may consist of several switch on and off processes and several pulses.

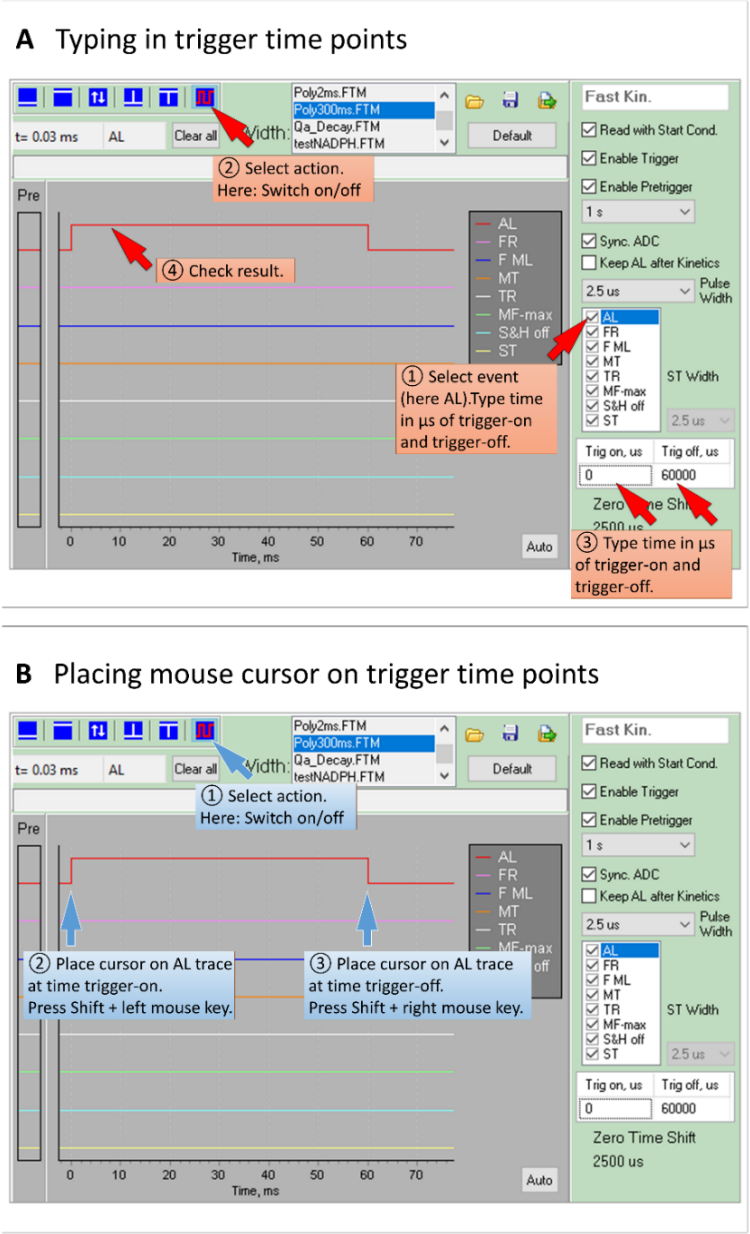


Fig. 55: Switching AL

A and B: Setting the switching times with keyboard and mouse cursor, respectively.

The trigger pattern shown in Fig. 56 increases fluorescence from the O (F_0) level close to the I_1 level (details in references above). The true I_1 level fluorescence is elicited by a single turnover saturating flash, ST, at time 1.00 msec.

Settings: Rate = 2.5 µs and points = 1000 yielding a total time interval of 2.5 ms. Zero Time shift= 300 µs. Pretrigger enabled ("Pre" window visible). Only 6 events are triggered, multiple turnover flash (MT) and trigger out (TR) are hidden.

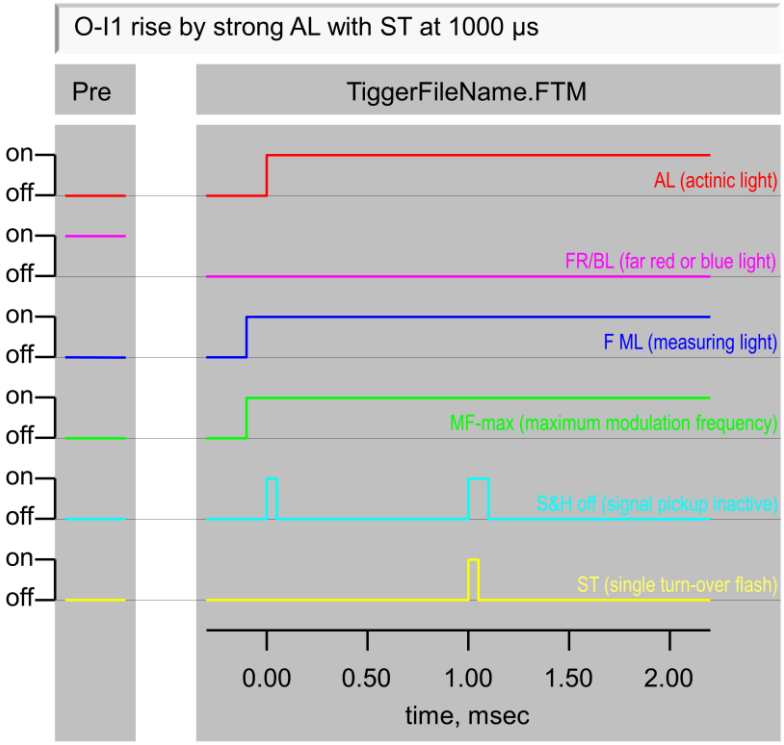


Fig. 56: Trigger Pattern (Example)

The example trigger pattern in Fig. 56 has the following characteristics:

AL (actinic light). The AL sets in at time zero.

FR/BL (far-red or blue light). Select FR or BL by control button, see Table 18, page 68). Preillumination with FR/BL light.

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 µs that is before onset of actinic light.

ST (single turnover flash). To arrive at the I_1 level, a 50 µs ST flash (1000 to 1050 µs) is applied.

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

6.3 SP-ANALYSIS MODE

6.3.1 Chart tools

Chart tools modify the visible area according to your interest by changing axes scaling and position. The options available are summarized in Fig. 57 using the Slow Kinetics chart. These options also apply to the Fast Kinetics, the Light Curve and the SP Analysis windows.

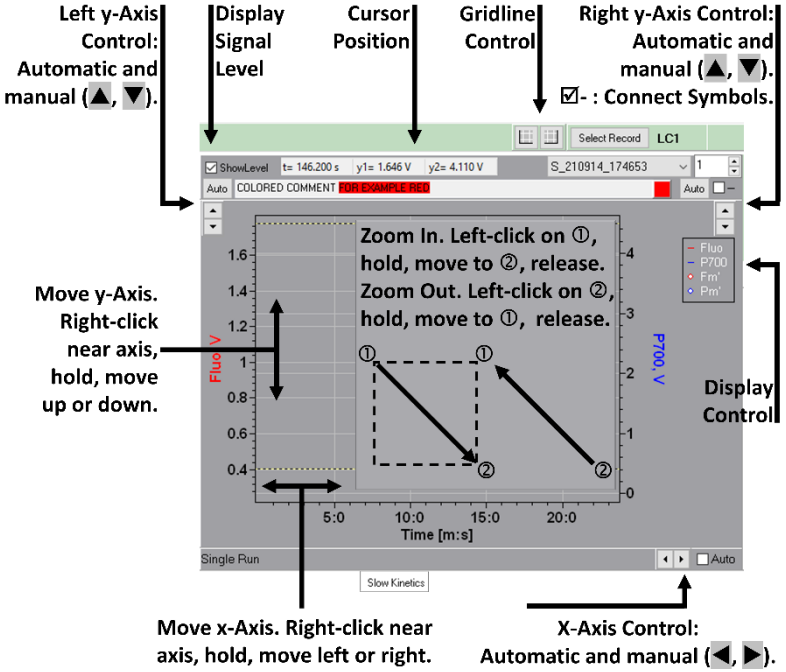


Fig. 57: Chart Tools

6.3.2 SLOW KINETICS CHART

This section deals with the Slow Kinetics sidebar (Fig. 58). For other areas visible, see 6.1.9 Fluo/P700, page 58 and following.

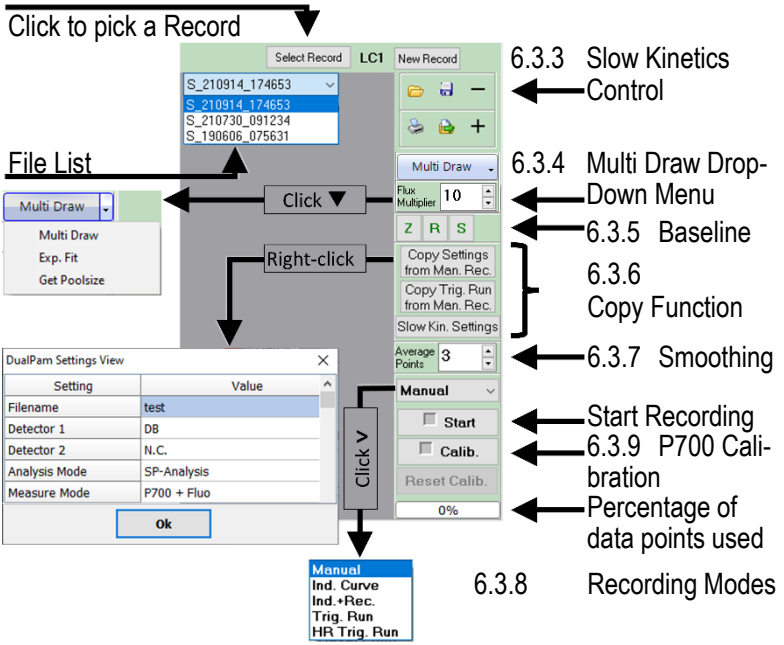


Fig. 58: Slow Kinetics sidebar

Features of the sidebar of the slow kinetics window in the SP-Analysis mode are highlighted. When available, section numbers are indicated.

6.3.3 Slow Kinetics Control

In total six commands are available under Slow Kinetics Control (Table 31). The same six commands are also available in the SP kinetics window.

Table 31: Slow Kinetics Control		
 Add previously recorded Slow Kinetics to current Report. Default source folder is C:\Program Files (x86)\DualPAM\Slow Kin Data. Kinetics and settings are loaded. To view settings, right-click on "Copy Settings from Man. Rec.".	 Save currently displayed Slow Kinetics with current settings. Default target folder is "C:\Program Files (x86)\DualPAM\Slow Kin Data". Note that "Save Report" saves all data including slow and fast kinetics, saturation pulse analyses and settings.	 Subtract Slow Kinetics. For the operation: Curve A minus Curve B: - Display A on chart. - Click - and select B from list. - Click Ok. The difference is displayed.
 Print Slow Kinetics window. The command prints immediately without printer dialogue.	 Export Slow Kinetics as csv file. Select one or several Slow Kinetics. Default directory is "C:\Program Files (x86)\DualPAM\Export"	 Average Slow Kinetics. Select Slow Kinetics to be averaged and click ok.
* Pick multiple files using the Ctrl or Shift key.		

6.3.4 Multi Draw Drop-Down Menu/Flux

The Multi Draw menu includes three items: the name-giving "Multi Draw" option which allows to draw several kinetics on top of each other, plus analysis of decay curves by curve fitting, and a special routine assessing the pool size of electrons of the intersystem electron transport chain.

The see the menu list, click the downward arrow right of "Multi Draw", and select an item. Click on selected menu item to start its function.

6.3.4.1 Multi Draw

To draw several kinetics, select data in Multi Draw window by Ctrl or shift key. All kinetics displayed are listed in the graph's legend box. Clicking on a legend line hides the associated graph.

The Multi Draw features a Zero Offset function (☑ Zero Offset, positioned top right in Multi Draw window). When Zero Offset is active, the interval to be considered for the Zero Offset operation can be defined.

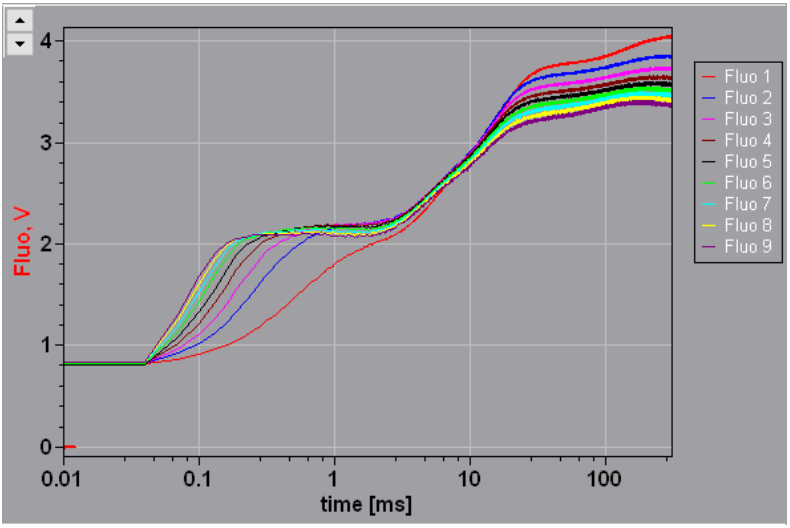


Fig. 59: Multi Draw
The effect of light intensity on the polyphasic fluorescence rise O-I1-I2-P or O-J-I-P.

6.3.4.2 Exp. Fit

The option Experimental Fit (Exp. Fit) fits up to three exponentials to a monotonically increasing or decreasing kinetics. The fitting procedure can be applied to several kinetics simultaneously.

Here, the Experimental Fit is explained using a P700 decay kinetics as the example (Fig. 60). In the example, a leaf was illuminated from 5 to 20 seconds after experiment start. The signal decay was monitored for further 80 seconds.

For the fitting procedure, the following values were entered in the input window (note that the unit of time is milliseconds):

Signal range considered. Begin of interval = 20000, because at 20 seconds the far-red was switched off and the decay kinetics started. The Width of interval was set to 80000 to consider the entire decay kinetics.

The “Initial Level” was calculated as the average of data in the interval from 5 seconds before start of the decay kinetics to begin of the decay kinetics. That is, the radio button “⦿ average” was checked, the begin and width of interval was -5000 and 5000, respectively.

Alternative ways to determine the initial level are:

⦿ extrapolate. Calculate line of best fit for the interval defined as “Initial Level”. Extrapolate the straight line to “Begin of interval” and use this value as Initial Level” for the decay kinetics.

⦿ maximum or minimum. Use maximum or minimum single value within the interval specified.

The “End Level” is the asymptote of the decay kinetics. The end level is calculated as the average of data in the interval specified by “Begin of interval” and “Width of interval”. In the present case, the last three seconds of the decay kinetics are to be used to calculate the End Level”. Therefore, Begin of interval = 77000 and Width of interval = 3000. Like the Initial Level, the End Level can be set manually.

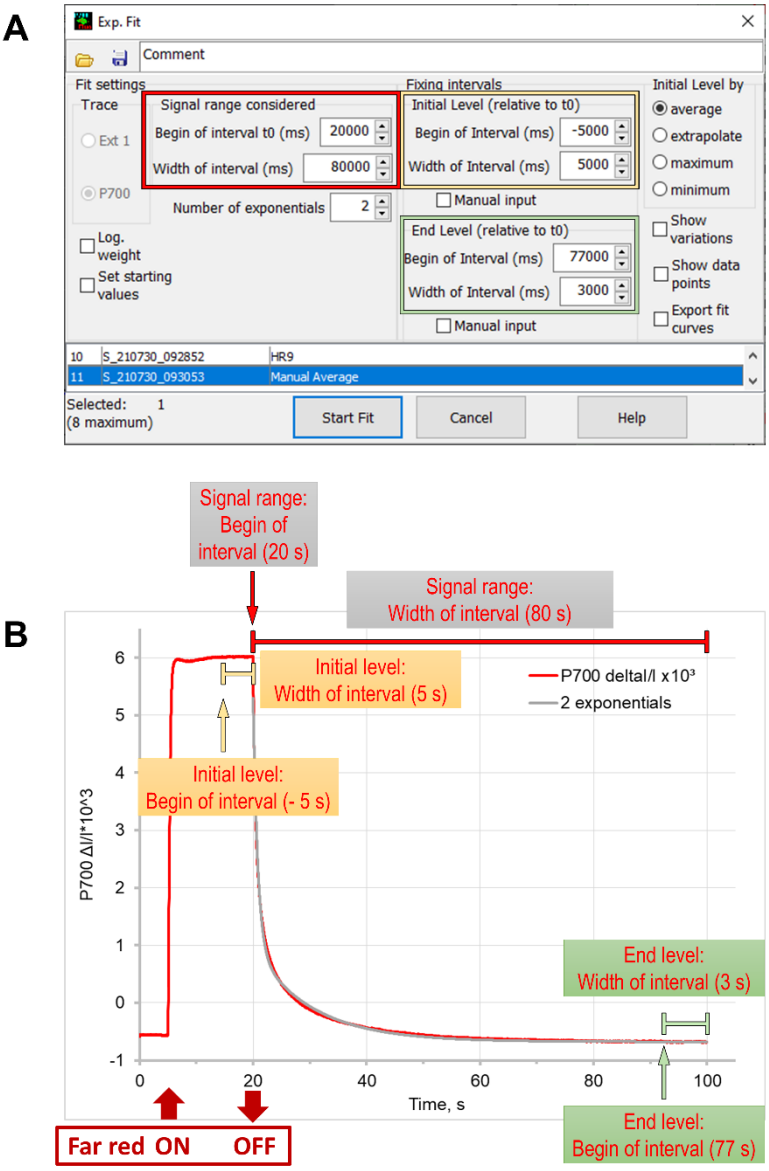


Fig. 60: Exp. Fit: Ranges

A: Input window. **B:** Input values shown on a sample graph.

☑ Manual input. Both the initial and the end level can be entered manually as well.

Number of exponentials. One to three exponential decay functions can be fitted to the experimental curve. In the present example, the number of exponentials was 2.

Logarithmic weight of data points (Log. weight). This function puts emphasis on the initial part of the decay kinetics. Use "☑ Show data points" to indicate the data actually considered by the fitting process.

An exponential decay function is given by:

$$A(t) = A \cdot e^{-\frac{t}{\tau}}$$

where A(t) is the quantity of A at time t, A is the initial quantity of A, and τ is the lifetime of A. In case of three exponentials, the A and the τ are consecutively numbered (A1 to A3, and τ1 to τ3, denoted as Tau1 to Tau3 in input windows of Exp. Fit).

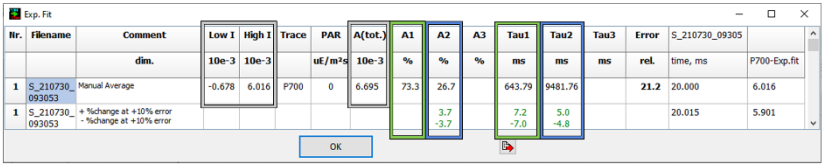
The fitting routine varies the A and τ until the best fit between theory and measurement is reached. The fitting routine requires starting values for A and τ. By default, the start values are set by the software. Only for τ, start values can be entered manually using the option "☑ Set starting values".

The following commands affect the output of the fitting procedure (Fig. 61A): "☑ Show variations" evaluates the percentage change required to produce a 10 % increase in the "Error". The "Error" is proportional to the sum of squared deviations.

"☑ Export fit curves" includes the best fit curve when data are exported. The last two columns of the results table (Fig. 61A) show the data of the fitted curve.

From left to right, the results table (Fig. 61A) displays the measured “Initial Level” and “End Level” (High I and Low I, respectively) of the decay kinetics, the total amplitude (A(tot.)) derived therefrom, the fitted parameters A1 and A2 in %, and the τ1 and τ2 in milliseconds.

A



B

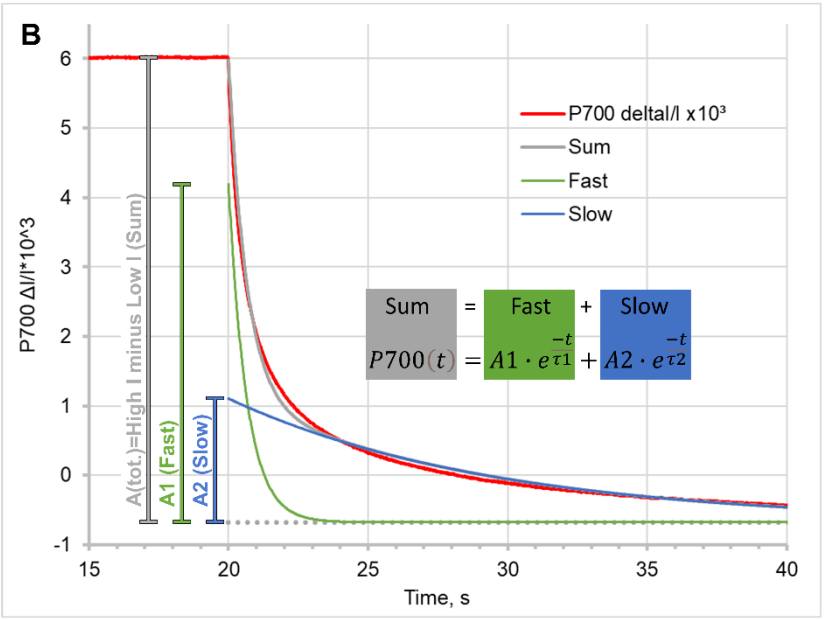


Fig. 61: Exp. Fit: Results

A: Table of fitting results. **B:** Fitting results illustrated on a sample graph.

Statistical information is given for A2, τ1, and τ2 (Fig. 61A). The numbers correspond to the percentage increase or decrease of the parameter required to cause a 10% increase in the "Error" (see above). The sum of A1 +A2 is given by the measured A(tot.). Hence, variations in A2 must be counterbalanced A1, and approximately the ± change calculated for the A2 and multiplied by the ratio A2/A1 applies for the A1.

6.3.4.3 Pool Size

"Get Poolsize" is a method to roughly approximate the maximum number of electrons per photosystem II in electron transport carriers between the photosystems that can reduce P700. Most of these electrons are located in the plastoquinone pool. Pertinent papers are:

Schreiber U, Klughammer C, Neubauer C (1988) Measuring P700 absorbance changes around 830 nm with a new type of pulse modulation system. Z Naturforsch 43c: 686-698. https://doi.org/10.1515/znc-1988-9-1010

Asada K, Heber U, Schreiber U (1993) Electron flow to the intersystem chain from stromal components and cyclic electron flow in maize chloroplasts, as detected in intact leaves by monitoring redox change of P700 and chlorophyll fluorescence. Plant Cell Physiol 34: 39–50. https://doi.org/10.1093/oxfordjournals.pcp.a078398

The procedure requires far-red illumination. In the absence of other light sources, the far-red oxidizes P700 to a large part because electrons are not supplied by photosystem II (compare Fig. 33, page 62). Adding visible light starts electron transport from photosystem II to I. The electron flow reduces P700. The degree of P700 reduction increases with increasing electron flow.

The far-red predominantly excites photosystem I. The photosystem II excitation rate under far-red light is low but not zero and its

reaction centers are thought to exist as mixed population with oxidized plastoquinone Q_B or single reduced plastosemiquinone Q_B^-.

One charge separation triggered by a single turnover flash (ST) converts all semiquinones into plastoquinols Q_BH_2, which unbind from the reaction center and add two electrons to the intersystem electron transport chain. Assuming that 50% of photosystem II reaction centers possess a semiquinone, the single turnover flash releases on average one electron per photosystem II reaction center.

As a result, the P700 oxidized by far-red will temporarily be reduced, which is visible by a corresponding drop of the P700 signal. The area enclosed by the reduction/oxidation trace, and a straight line drawn through the P700 signal before and after the reduction/oxidation event, represents 1 electron per photosystem II reaction center.

A multiple turnover flash (MT) triggers many charge separations. Width and intensity of the MT is chosen to fully reduce the intersystem electron transport carriers and P700. The resulting fast drop of the P700 signal is followed by a slow return to the P700 level before the MT. The area of the reduction/oxidation transient, calculated as described above, represents the pool of electrons of the intersystem electron transport chain.

Dividing the MT area by the ST area yields the number of electrons of the intersystem electron transport chain per photosystem II. This number is called functional pool size of electrons.

Because this number includes electrons of all intersystem carriers plus P700, it is difficult to derive the relationship between a particular carrier and photosystem II (e.g. the plastoquinol/ photosystem II ratio).

Procedure

The following procedure works with most green leaves.

- Use freshly harvested or attached leaf. Avoid long dark acclimation. Preilluminate with weak to moderate light and keep dark for some minutes or less. Typical pool size data for C_3 plants range between 5 and 15 depending on light acclimation status.
- Switch on P700 measuring light (see 6.1.13, page 66). Let LEDs warm up for at least 30 s. Balance P700 measuring light.
- Select measure mode “Single Channel” and “P700” (see 6.1.6, page 52).
- Open triggered run “Poolsize_Determination.STM” (see 6.2.4, page 90). An acquisition rate of 1 ms is automatically set when opening this triggered run file.
- Switch on far-red light, set MT width to 50 ms, and ST width to 50 µs (see Table 18, page 68). Wait 10 s.
- In slow kinetic window, select “Trig. Run” as the operating mode and start experiment. The triggered run executes an ST→MT→ST sequence (Fig. 62).
- On slow kinetics side panel, click on downward arrow to the right of the “Multi Draw” command button. Select “Get Poolsize” and click “Get Poolsize”.
- In the now appearing Poolsize window, enter the starting time of the ST and MT pulses. Enter “Interval Width” (see Fig. 62).
- The “Interval Width” determines a time range before each pulse. For each interval, the y data are averaged. The

straight lines, required to calculate the area of a reduction/oxidation event, are constructed using these mean values. For example, the straight line for the first ST area goes through the mean prior to the ST and the mean prior to the MT.

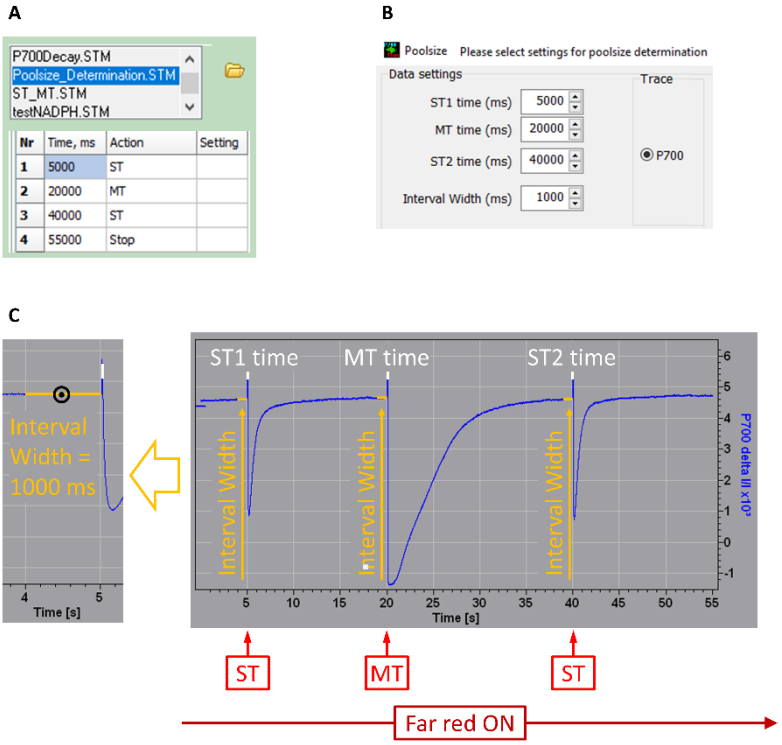


Fig. 62: Electron Pool Size: Terms

A: The triggered run "Poolsize_Determination.STM" was used to carry out the experiment in this and the next figure. **B:** Poolsize window to enter time points of flashes and the intervals used for construction of baselines. **C:** Experiment with explanation of input parameters and light regime (bottom of C).

For each reduction/oxidation event, a separate line is calculated (Fig. 63, page 123). For the second ST, the starting point of the straight line is the mean value before the ST. The time position of

the end point is the time point of the second ST plus the distance between the first ST and the MT pulse.

- After all data are entered, click “Calculate”.
- The results are displayed in the window for fitting results. The table lists the signal levels used to construct straight lines, the areas of each reduction/oxidation event, and the poolsize (Fig. 63).

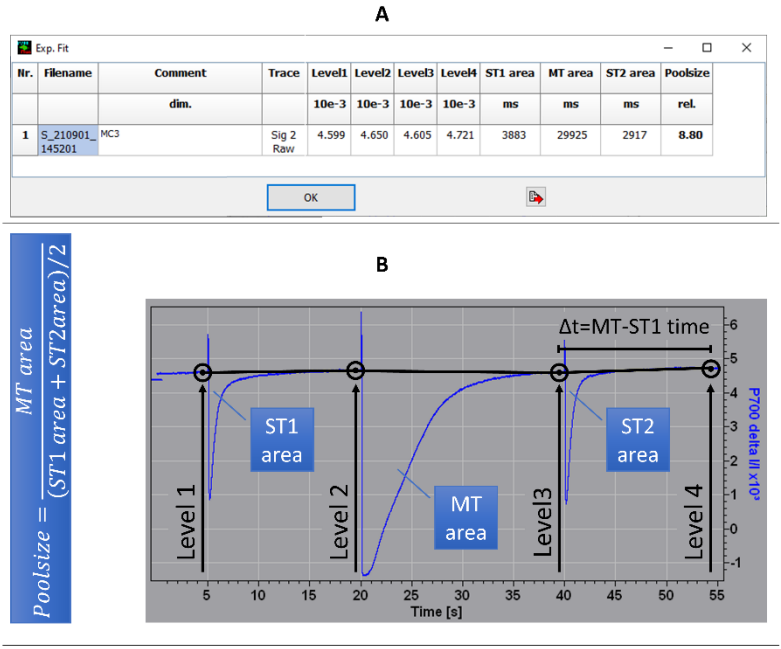


Fig. 63: Electron Pool Size: Evaluation

A: Results of poolsize determination. Level 1 to 4 are graphically depicted in B as circles with central dot. The position of the last level (Level4) is determined by the time of ST2 plus the time difference MT minus ST1. Areas (St1, Mt, and St2) are indicated by callouts in B. The poolsize is calculated by the equation shown in B. **B:** Experimental trace with results of poolsize determination indicated.

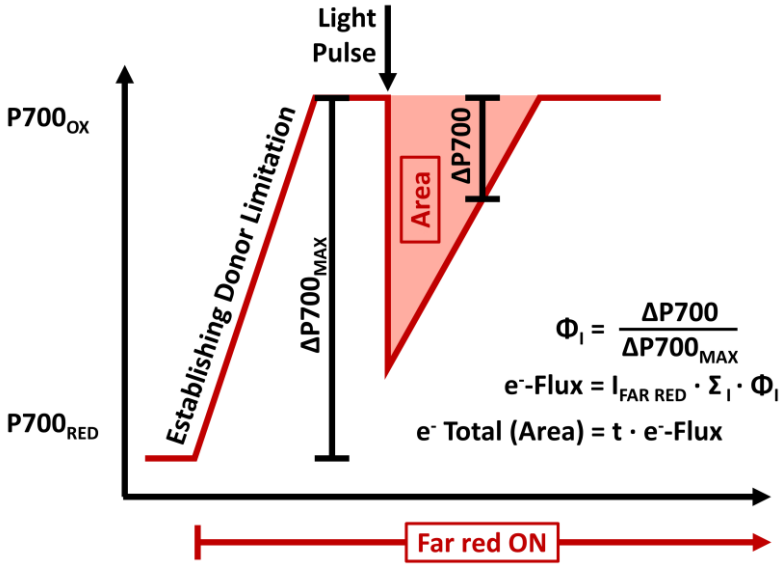


Fig. 64: Electron Pool Size: Theory

Y axis, reduction state of photosystem I reaction center, P700. X axis, reaction time. From left to right: far-red illumination predominantly drives photosystem I. The shortage of photosystem II-derived electrons creates a state of P700 donor limitation. Donor limitation results in oxidation (that is, closure) of most of P700. The maximum signal obtained by far-red preillumination minus the initial signal is denoted $\Delta P700_{MAX}$. (That the $\Delta P700_{MAX}$ is smaller than the true maximum P700 amplitude, P_M, is disregarded here.) A light pulse temporarily reduces P700 resulting in a sudden signal drop followed by a signal increase until the level $\Delta P700_{MAX}$ is reached again. At each point of the curve, the difference of $\Delta P700_{MAX}$ minus the actual signal is denoted ΔP700. Since the $\Delta P700_{MAX}$ represents all photosystem I reaction centers, the ratio of $\Delta P700/\Delta P700_{MAX}$ represents the relative amount of open photosystem I reaction centers. This ratio can be viewed as the current quantum yield of photosystem I, Φ_I (cf. Klughammer C, Schreiber U (1994) Planta 192: 261-268). The fraction of open photosystem I reaction centers varies with time because electron supply is variable: it is maximal right after the light pulse and then slowly returns to the original donor-limited situation. For each point, the product of Φ_I ($\Delta P700/\Delta P700_{MAX}$) times the (constant) quantum flux of far-red radiation, $I_{FAR-RED}$, times the (constant) absorption cross section of photosystem I for far-red radiation, Σ_I, results in the electron flux through photosystem I, e^--Flux. Integrating the e^--Flux yields the total number of electrons flowing through photosystem I caused by the light pulse. In practice, the area of $\Delta P700/\Delta P700_{MAX}$ is considered a proportional to the light pulse-induced total number of electrons flowing through photosystem I.

Notes

Theory of pool size analysis

Fig. 64 outlines why the area enclosed by baseline and the flash-induced P700 signal (see Fig. 63) can be viewed as proportional to the total electron flow through photosystem I caused by this flash.

Travel time of electrons from photosystem II to photosystem I

Inspection at high time resolution of the P700 trace right after the MT (cf. Fig. 62) shows a lag phase of several ms prior to P700 reduction. The lag phase is related to the travel time of electrons from photosystem II to photosystem I. This travel time is studied best using fast kinetics in the mode “Fast Acquisition”.

6.3.4.4 Flux Multiplier

See Section 6.1.7.2, page 53.

6.3.5 Baseline

The commands under “Baseline” can shift the entire kinetics parallel to the y-axis or subtract a sloped line from the kinetics. Baseline correction applies only to absorption measurements like P700 or P535, and it does not change the original data. Table 32 outlines the properties of baseline correction which differ in the window Slow Kinetics and SP Kinetics.

Table 32: Baseline Correction		
Button/Correction	Mouse Key	Range considered
Slow Kinetics		
Z /Zero Offset	Left	Initial part until signal change.
S /Slope	Left	Initial course of signal
S /Slope	Right	Last 10% of kinetics
Fast Kinetics		
Z / Zero Offset	Left	User defined interval
Z /Offset and Slope	Right	Input window for user-defined interval.
S /Slope	Left	User defined interval
S /Slope	Right	User defined interval plus last 2% of kinetics

6.3.6 Copy Function

Three commands are summarized under the term “Copy Function”: two actual copy commands and one command opening the window “Slow Kinetic Settings”. The two copy commands transfer the timing of actions and settings, respectively, of the current slow kinetics to the Triggered Run table (see Section 6.2.4, page 90).The copy commands are convenient tools to create Triggered Run tables, that is, automatically executable experiments. Triggered Run tables can be created from a Manual Record that was just executed (Section 6.3.8, page 127) or from earlier executed and stored Slow Kinetics. Note that a Slow Kinetics file contains the settings with which it was recorded.

6.3.7 Smoothing

The smoothing function (“Average point” plus numerical field, Fig. 58, page 112) reduces high frequency signal noise. The value entered in the average points field determines the number of neighboring y-data to be averaged. The result is positioned at the central time of the averaged data. This operation is repeated point by point through the Slow Kinetics. Smoothing does not affect the original data. However, when a Slow Kinetics is exported as csv data, smoothing reduces the number of data points. Always watch if the smoothing number applied noticeably reduces time response of the signal.

6.3.8 Recording Modes

Five recording modes are available:

Manual: All actions are manually controlled.

Ind. Curve: Slow Kinetics routine (induction curve) as described in 6.2.3.2 (page 88).

Ind. + Rec.: Induction curve plus dark recovery period.

Trig. Run: User-defined automatically executed Slow Kinetics (Triggered Run, see 6.2.4, page 90).

HR Trig. Run: User-defined automatically executed Slow Kinetics (High Resolution Triggered Run, see 6.2.5, page 96).

6.3.9 P700 Calibration

The Calib. (calibration) command turns the P700 voltage scale into a P700 absorption scale. Calibration must be done with the sample in place. Calibration is sample-specific. For calibration, a defined positive absorption change is simulated by varying the intensity relation of the P700 measuring beams (Fig. 32, page 57).

The signal change for calibration amounts to two steps on a 4096-part scale for the measuring light intensity, where the value of 4096 corresponds to the measuring light intensity 20. For measuring light intensity 10 (digital value 4096/2=2048), the simulated change is:

$$\frac{\Delta I}{I} = \frac{2}{2048} = 0.977 \cdot 10^{-3}$$

The simulated absorption change and a scale bar displaying the ΔI/I change is displayed on a voltage scale. The length of the scale bar is kept constant when the scale of the voltage axis is varied and, thus, the ΔI/I changes accordingly.

Calibration further calculates the absolute value for the I. The I value decreases with increasing attenuation of P700 measuring light by the sample, and it increases with increasing measuring light intensity. Therefore, the same two-digit variation can result in different ΔI/I calibration.

6.3.10 SP Kinetics Chart

Similar features of the sidebars of the SP Kinetics and Slow Kinetics side bar are the control icons (Table 31, page 113), the button to access the corresponding settings window, and point averaging. The new features are highlighted in Fig. 65 and explained subsequently.

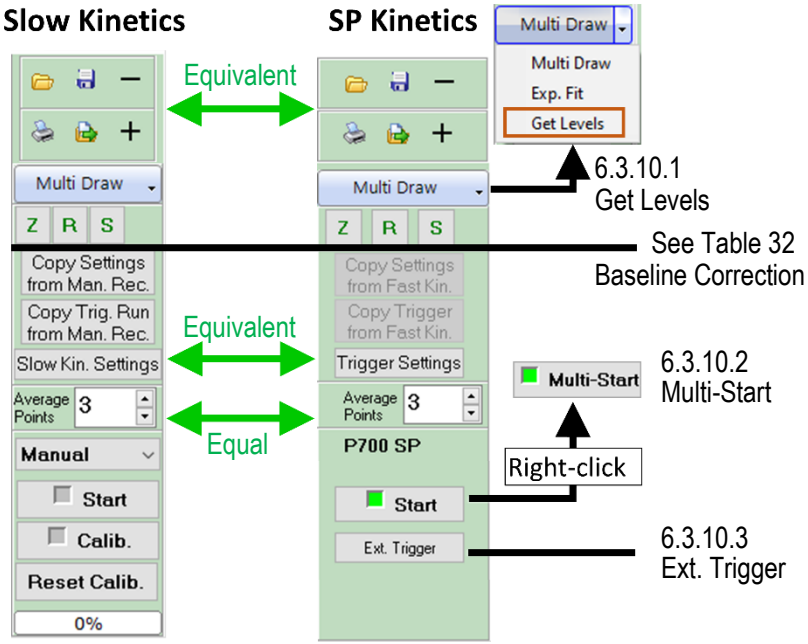


Fig. 65: SP Kinetics Sidebar

Features present in the both the Slow Kinetics (**Fig. 58**) and the SP Kinetics sidebar are highlighted by green double arrows. The buttons for baseline correction have different functions on the Slow Kinetics and SP Kinetics Window as summarized in Table 32. New features of the SP Kinetics sidebar ("Get Levels", Multi-Start, and Ext. Trigger) are indicated with their section number.

6.3.10.1 Get Levels

“Get Levels” is part of the Multi Draw menu of the SP Kinetics window. The function analyzes the signal levels at three adjustable time points of a fast kinetics. When a series of fast kinetics with different PAR values is analyzed, Get Levels plots the three signal levels as a function of time.

Note that at the time of writing this manual, only the PAR of actinic illumination before and continuing during the Fast Kinetics is used as x-axis data.

6.3.10.2 Multi-Start

“Multi-Start” triggers repetitively the currently active trigger pattern at the highest possible frequency. The number of trigger events is determined by the number for Fast Kinetics Average in the window Fast Acquisition (6.2.7, page 100).

6.3.10.3 Ext. Trigger

The button Ext. Trigger allows an external device to control the timing of saturation pulse analysis, or generally spoken, of the currently active trigger pattern. After clicking “Ext. Trigger” and “Start External Triggering”, the function is active. The trigger signal is a 5 V DC signal applied to the “Trigger In” port of the control unit (Modular Version: 4.1.2, page 12. Fiber Version: 4.2.2, page 21).

6.3.11 Light Curve Chart

A light curve (light response curve) consists of a series of illumination steps, normally with increasing intensities. At the end of each illumination step, a saturation pulse analysis is carried out. Only saturation pulse data are recorded, when “Light Curve with Slow Kinetics” is unchecked.

Light curves are configured in the Edit window (Table 33). A light curve can consist of up to 20 illumination steps. The PAR for each step is adjusted in the column “Intensity” (Intens.). 20 intensity levels are available. The PAR corresponding to an intensity setting is automatically taken from the Current/PAR list (Table 11, page 49).

The PAR of the default light list is exponentially related to the intensity setting. A custom PAR list is created be changing the value of the LED current in window “Actinic Light”, followed by entering the PAR measured for the new current setting (6.2.2.1, page 81). The light curve configuration is part of the user settings (6.1.5, page 52). Thus, the commands Save/Open User settings are used to save and recall a light curve configuration.

All the parameters of saturation pulse analysis shown on the Fluo and P700 sidebars plus the fluorescence fractions F/F_M, F_M'/F_M, F_0'/F_0 can be plotted as a function of PAR. Additionally, the photosystem I und photosystem II electron transport rates (ETR(I) and ETR(II)) can be graphically represented using the y-axis on the right side of the graph as scale.

By fitting a model function to the experimental data (click Fit), the ETR data are analyzed. Two different model functions are available (Table 34). The outcome of the fitting process are the so-called cardinal parameters (Table 34).

Click the calculator button to display these parameters in the window “DualPAM Light Curve Fit” (Fig. 66). A button to export fitting parameters as csv data is part of this window. In addition to the cardinal parameters introduced in Table 34, the window “DualPAM Light Curve Fit” displays estimates for α, which are derived from the maximum quantum yields of photosystem I and II (topmost data in Fig. 66).

In case of photosystem II, estimation of α considers the maximum efficiency of absorbed photons for stabile charge separation, F_V/F_M. The F_V/F_M is multiplied by the ETR-factor which corrects for the fraction of incident light that is not absorbed. The default ETR-factor is 0.84 meaning that 84% of incoming light is absorbed. The final result is obtained by dividing the corrected F_V/F_M by 2, which assumes that absorbed energy is roughly evenly distributed between the two photosystems. The same concept is applied to photosystem I, except that the maximum efficiency of absorbed photons for stable charge separation is set to 1.

Table 33: Light Curve Edit

Total time = 60 s (Calculated automatically.)		☑ Uniform Time (Editing tool. Check to assign the same length to all light steps. Then enter length in any field and hit enter.)	
Step	**PAR** (Taken over from PAR list, see Table 11, page 49.)	**Intensity** (Select setting 1 to 20)	**Time/10s**
1	15	**2**	**3** (30 s step)
2	52	**4**	**3** (30 s step)
3	107	**6**	**0** (end of light curve)
20	1386	**16**	**3** (30 s step)

Table 34: Light Curve Model Functions

Abbreviation and Source	Equations
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. https://doi.org/10.1016/0304-3800(88)90057-9	$ETR_{PAR} = \frac{PAR}{a \cdot PAR^2 + b \cdot PAR + c}$ $\alpha = \frac{1}{c}$ $ETR_{max} = \frac{1}{b + 2 \cdot \sqrt{a \cdot c}}$ $I_K = \frac{c}{b + 2 \cdot \sqrt{a \cdot c}} = \frac{ETR_{max}}{\alpha}$
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. https://images.peabody.yale.edu/publications/jmr/jmr38-04-06.pdf	$ETR_{PAR} = ETR_{mPot} \cdot (1 - e^{-\frac{\alpha \cdot PAR}{ETR_{mPot}}}) \cdot e^{-\frac{\beta \cdot PAR}{ETR_{mPot}}}$ $ETR_{max} = ETR_{mPot} \cdot (\frac{\alpha}{\alpha + \beta}) \cdot (\frac{\beta}{\alpha + \beta})^{\frac{\beta}{\alpha}}$ $I_K = \frac{ETR_{max}}{\alpha}$ $I_b = \frac{ETR_{mPot}}{\beta}$

Cardinal parameters of light curves

α, electrons/photons	Initial slope of RLC, related to quantum efficiency of photosynthesis
ETR_{max}, µmol e^-/(m² · s):	Maximum electron transport rate.
I_K, µmol photons/(m² · s)	Transition point between light limiting and light saturating phases.

Other Abbreviations

I_b	Photoinhibition Index: PAR at which photoinhibition reduces ETR_{mPot} by the factor of 1/e.
ETR_{mPot}	Maximum electron transport rate in the absence of photoinhibition.

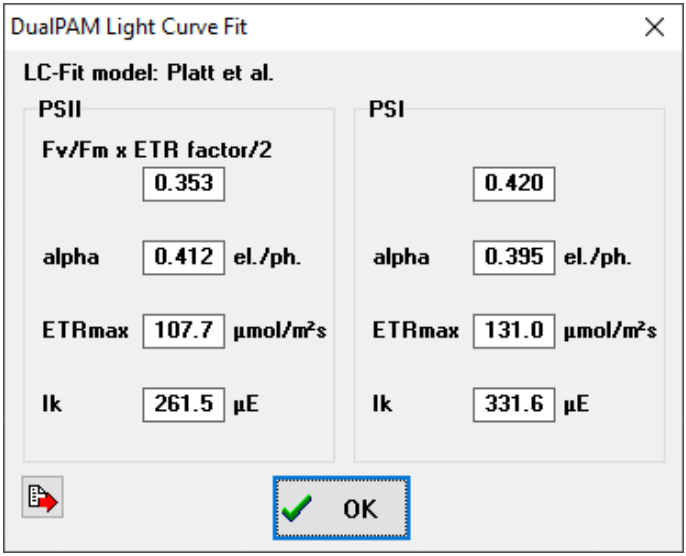


Fig. 66: DualPAM Light Curve Fit

6.3.12 SP-Analysis Chart

The fluorescence ratio parameters of the Light Curve chart are also available for the SP-Analysis chart. Additionally, the continuously recorded fluorescence can be displayed. Different from the Light Curve chart, data are plotted against time (Fig. 67).

Recalc. with ⦿ Fm O max. Fm'

The level of maximum fluorescence measured with a fully dark-acclimated sample is called F_M. The F_M is required to calculate the quotients F_V/F_M, NPQ, Y(NO), or q_N. Sometimes, the maximum fluorescence in the light (F_M') is higher than the F_M. This can happen right after onset of illumination. In this case, the fluorescence quotients can be recalculated with the maximum value for F_M' (select ⦿ max. Fm').

F_M' can be higher than F_M (i) when light increases the photosystem II antenna size due to a state 2 – state 1 transition, like many algae and cyanobacteria, (ii) when the first saturation pulse does not fully reduce the PQ pool or (iii) when the dark measurement was carried out before complete relaxation of non-photochemical quenching.

Start Man. Record

The Manual Record (MR) contains only saturation pulse data. Continuous signal recording, as in manual curves (MC), is off.

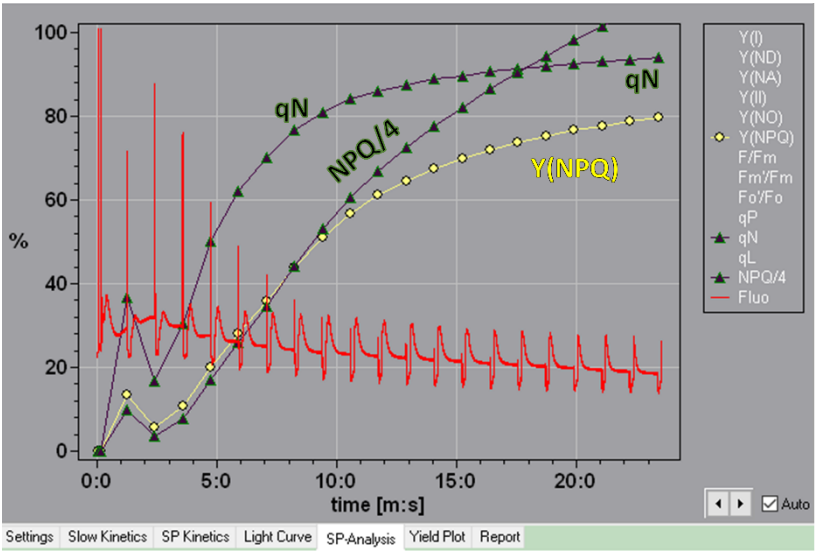


Fig. 67: SP-Analysis Chart

Chart with fluorescence trace and three types of information on non-photochemical quenching: qN, NPQ/4, and Y(NPQ) (details in Table 37, page 170). The SP-Analysis chart draws all data on a 0 to 100% scale. The fluorescence trace (red line) is normalized so that F_M = 100%. By definition, the parameters qN and Y(NPQ) always stay within the Y-axis range. NPQ/4 values up to 1 can be plotted. In fact, the NPQ does not exceed the value of 4, in most cases. Hence, plotting one fourth of the true NPQ value keeps the NPQ inside the y-axis range. In the present case, the NPQ is greater than 4 and exceeds the y-axis range.

6.3.13 Yield Plot

The Yield-Plot draws the photochemical quantum yield of photosystem I, Y(I), against that of photosystem II, Y(II). Often, straight relationships between the two parameters are observed in slow light curves (see 6.3.11, page 131). Slow means that steady state is reached at the end of each light step which usually is the case for light steps of 10 minutes.

In such plots, the Y(II) is often lower than the corresponding Y(I) (Fig. 68A), suggesting the photosystem II continuously operates with lower quantum yield than photosystem I. However, the presence of constant background fluorescence from photosystem I results in an underestimation of Y(II).

In the numerical field F(I)/Fo appl. an estimated value for photosystem I fluorescence can be entered. The value corresponds to the level of photosystem I fluorescence relative to the level of total F_0 fluorescence. In the example of Fig. 68, the value of 30% for F(I)/Fo appl. was empirically found to move the data points on the diagonal 1:1 line (Fig. 68B). Thus, when photosystem I is considered, the two photosystems work with the same quantum yields.

Finding the value for F(I)/Fo appl. which moved data points on the diagonal 1:1 line can be employed to empirically determine the amount of photosystem I fluorescence emitted by a sample.

Papers dealing with photosystem I fluorescence are:

Pfündel EE (1998) Estimating the contribution of Photosystem I to total leaf chlorophyll fluorescence. Photosynth Res 56, 185–195 (1998). https://doi.org/10.1023/A:1006032804606

Pfündel EE, Klughammer C, Meister A, Cerovic ZG (2013) Deriving fluorometer-specific values of relative PSI fluorescence intensity from quenching of F_0 fluorescence in

leaves of *Arabidopsis thaliana* and *Zea mays*. Photosynth Res 114:189–206. https://doi.org/10.1023/A:1006032804606

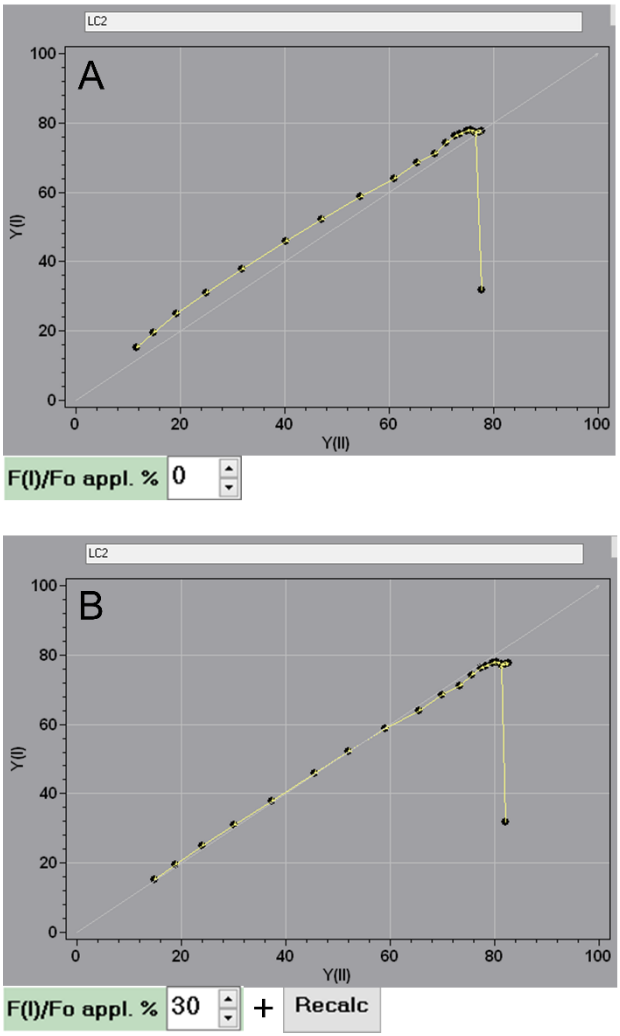


Fig. 68: Yield Plot

Yield Plot of a light curve. A: Original data. B: Recalculated data assuming a constant photosystem I fluorescence amounting 30% of the F_0 fluorescence level.

6.3.14 Report

6.3.14.1 Report View

A data file created by the DualPAM software is called Report. A Report is divided into Records. Typically, each Record contains an independent experiment. A new Record is automatically created when an Induction Curve or a Light Curve is started. Records can be manually created by the command New Record. The elements of the Report window are outlined in Table 35.

Table 35: Report Window 1

Elements that are only used in the Record view (Table 36) are not shown. Printed in orange are comments. The relative position of elements differ from the software interface. Column headlines of the Report table are shown on blue background).

View ◉ Rpt ○ Rec View Report or Records	Manual start of Record New Record
Records # Total number of Records	Curr. # Currently selected Record
Delete Open Save Print Export Note	
YYYYMMDD.RPT Current Report file name (year, month, day).	

Date	Time	No.	Type	Name	No.SK	No.SP	No.FK	Comment
			Acronym	Acronym plus Counter	Number of Slow Kinetics	Number of Saturation Pulses	Number of Fast Kinetics	Automatically Created Comment
		1	MR	MR1	1	7	0	Manual Record
		2	MR	MR2	1	14	0	Manual Record
		3	IC+	IC+1	1	20	0	Induction Curve plus recovery
		4	LC	LC1	1	7	0	Light Curve
		5	FLC	FLC1	0	0	42	Fast Kinetics Light Curve

6.3.14.2 Record View

The basic properties of a Record table are shown in Table 36. The table shows entries of a light curve experiment carried out in the measuring mode Dual Channel and the analysis mode SP-Analysis (see 6.1.6 and 6.1.7, page 53 and subsequent pages).

A line in the Record table is created at start and another one at the end of Record. For example, the beginning of a light curve creates a line with “LC-start” typed in the Action column, LC-Stop marks the end of a light curve. Further, a Record line is created for each determination of a signal level for saturation pulse analysis (fluorescence and P700) and for each fast kinetics. Each line includes a so-called “Memo” text field located above the column titles (Table 36). Comments can be added to the Memo text created by the software. The command Save Memo stores the newly entered text.

A Record table can also display changes made in the Mode window (check “Display Settings”) and information on the Fast Kinetics of the Report (check “Display Fast Kinetics”).

A full saturation pulse analysis can comprise two lines. An example is the ratio F_V/F_M which is derived from the F_0 level before the saturation pulse (first line), and the F_M level induced by the saturation pulse (second line). Two lines per saturation pulse analysis also exist when the F_0’ mode is active. The first line displays the calculated F_0’ value in the column “Fo,Fo'” (for the equation see Section 7.1.2, page 164), the second line shows the measured F_0’ value. The photosystem I fluorescence is derived from the difference between measured and calculated F_0’ (see 6.1.14, page 70).

In both examples, fluorescence levels of the first line are repeated in the second line except those levels which were newly determined for the second line. In the latter case, the first and second

line contain different fluorescence ratio parameters when the formula contains F_0' fluorescence, that is, parameters are obtained with the calculated F_0' in the first line and with the measured F_0' in the second line.

Table 36: Report Window 2

Only elements used in the Record view are shown. Printed in orange are comments. The relative position of elements differs from the software interface. Column headlines of the Record table are shown on blue background).

View ○ Rpt ◉ Rec

View Report or Records

Save Memo — Save comment in Memo field.

Display: ☑ Fast Kinetics, ☑ Settings — List information of Fast Kinetics and Settings in Record.

Parameters: ☑ Fluo, ☑ P700 — Select/deselect all fluorescence and/or P700 parameters.

Memo Window: A "Memo" is associated with each line of a Record

Action	ID	Name	F(I)/Fo calc (see 6.1.14, page 70).	Fo,Fo'	Fm,Fm'	F	Y(II)
LC-Start Licht Curve Experiment	LCB	LC1					
Manual	SK	S_210914_174653 Slow Kinetics File					
Dark Sample							
Fo-Det.	Fo			0.4042			
Fm-Det. uncalibrated Saturation Pulse. ("Uncalibrated" refers to Pm.-Det..)	SP	F_210914_174655_0 Fast Kinetics File (Saturation Pulse)		0.4042	1.7777	(0.4344)	0.773
				F_V/F_M determination			
Pm.-Det. (See Fig. 33, page 62)	SP	F_210914_174703_0 Fast Kinetics File (Saturation Pulse)		0.4044	1.7809	0.4305	0.758
Light Exposed Sample							
P.+F. SP	SP	F_210914_174807_0 Fast Kinetics File (Saturation Pulse)		0.327 F_0 calculated	0.872	0.4575	0.475
Fo'-Det. F_0' determination (see Fig. 34, page 63).	SP+		0.243	0.350 F_0 measured	0.872	0.4575	0.475

6.4 FAST ACQUISITION MODE

The Fast Acquisition focuses on recording of fast signal changes. Automatic evaluation of saturation pulse analyses is restricted to F_V/F_M and P_M. Damping is automatically set to low.

6.4.1 Slow Kinetics

Only the saturation pulse commands Fo, Fm and Pm are active. Both commands must be executed prior to the start of a Slow Kinetics. The commands execute the trigger pattern for saturation pulse analysis (6.2.6 and 6.2.7, page 97 and following pages). During a Slow Kinetics experiment, only the currently active fast trigger pattern can be executed: the fast trigger pattern cannot be changed.

The Slow Kinetics windows shows the F_V/F_M derived from the F_0 and F_M measurement. From the P_M kinetics, the signal levels reached by far-red preillumination (ox.) and the total signal change (Pm) are derived. Then, two parameters for photosystem I are calculated (i) the photochemical yield, $Y(I)=(P_M\text{-ox.})/P_M$, and (ii) the limitation of photosystem I turnover by shortage of electrons, $Y(ND)=ox./P_M$. The limitation of photosystem I turnover by shortage of electron acceptors is assumed to be negligible and Y(NA) is set to 0. See Section 7.2, page 172, for definitions of the yield values of photosystem I.

The Y(I) measured here must not be confused with the maximum photochemical quantum yield of photosystem I which is close to unity.

The side panel elements function as described before (6.3.3, page 112 and following pages). All recording modes (6.3.8, page 127),

however, execute the currently loaded Fast Kinetics trigger pattern instead of the trigger pattern used for saturation pulse analysis.

6.4.2 Fast Kinetics

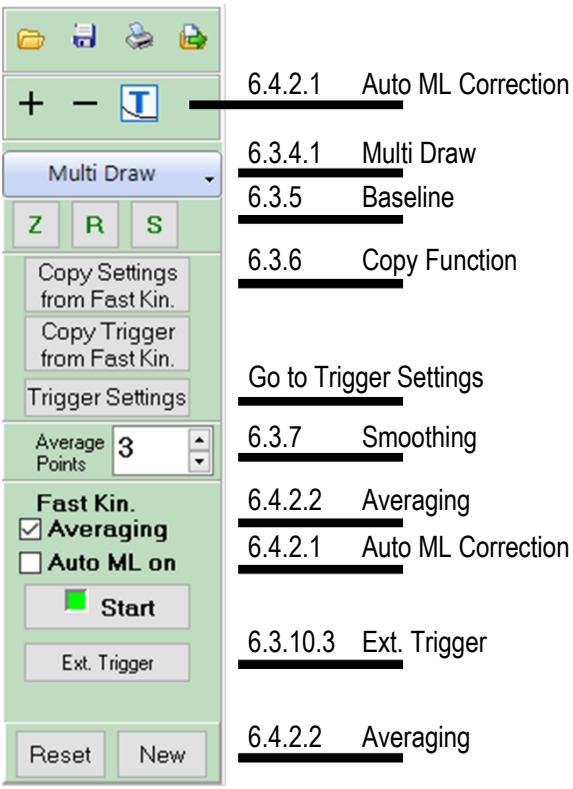


Fig. 69: Fast Kinetics Sidebar
The numbers of sections explaining the various side bar function are indicated. Functions not introduced above are Auto ML Correction and Averaging.

6.4.2.1 Auto ML Correction

The feature Auto ML Correction has been designed for experiments with measuring light that potentially can drive photosynthesis (actinic action of measuring light). Actinic action must be considered for the green measuring lights of the P515/535 Emitter-detector Module but in most cases not for the P700 measuring light (compare Section 6.2.1.3, page 79).

To minimize actinic effects by measuring light, the command ☑ **Auto ML on** switches measuring light on shortly before start of a fast kinetics, and switches it off at end of the fast kinetics. As a result, the warming up measuring light LEDs cause signal drift. This section explains how this signal drift can be measured and then used to correct subsequent "Auto ML on" measurements.

Procedure

- Select Fast Acquisition analysis mode.
- Open the Fast Trigger file of interest and inactivate all events except measuring light.
- Only prior to the first execution of Auto ML Correction, manually switch on measuring light, and balance P700 or P515 measuring beams.
- Switch off measuring light. Check Auto ML Correction. Execute Fast Kinetics.
- Subject the kinetics to Exp. Fit (6.3.4.2, page 114). Check the option ☑ Use for correction that is located at the lower right edge of the results window of Exp. Fit.
- Open original Fast Trigger file of interest. Carry out measurements.

- Select Fast Kinetics recorded with measuring light only (first measurements). Click T. Select all Fast Kinetics to which baseline correction should be applied.
- Apply baseline correction by clicking "☑ Show corrected" located below the Fast Kinetics chart.

The baseline can be saved and recalled (see 6.3.3, page 112). Thus, a baseline is available for other Fast Kinetics using the same trigger pattern.

6.4.2.2 Averaging

☑ Averaging calculates the mean of several identical kinetics to improve the signal to noise ratio. For details, see Section 6.2.7 (page 100).

6.5 SCRIPT FILES

Script files are used for automated execution of experimental procedures of various complexities. Script files are advantageous when the same type of analysis is repeated frequently or for complex experimental protocols.

Almost all keyboard and mouse key entries in the DualPAM software can be carried out by Script files. In addition, Script files offer commands for time management, combinations of sub-programs and conditional commands.

To access the script file window, click the Load button on the bottom bar of the DualPAM interface, the neighboring Run button executes the script file currently loaded.

Script Files are created in the Script File window. Fig. 70 outlines the functional groups of the Script File window.

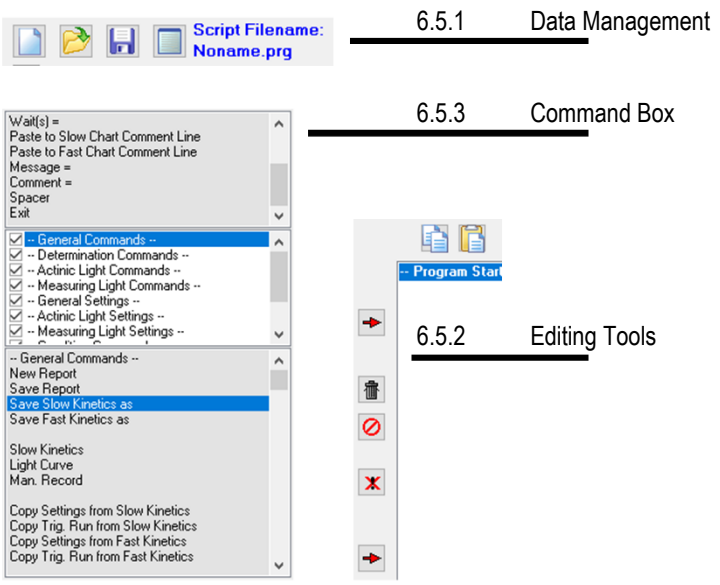


Fig. 70: Script File Window

Overview of Script File window. Functional groups are indicated with section numbers.

6.5.1 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 file extension PRG). The default directory for script files is C:\Program Files\DualPAM\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 comments. Comments are saved as text file using the file name of the script file (e.g. comment file: MyScript.TXT. Script file: MyScript.PRG).

6.5.2 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 into 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 applies to commands in the box “Program Control Commands”, the lower icon applies to commands of 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. 70)
- Select by mouse cursor in the script file window (right text window of Fig. 70) 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 called the sub-program.

6.5.3 Command Box

Export all Commands Creates a file listing all script file commands and their settings.

The command box of the script file window (Fig. 70, page 145) consists of 3 sections. The upper section contains commands controlling the progress of script files and editing tools. The middle panel contains the title of the 11 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.

6.5.3.1 Program Control Commands

COMMAND	COMMENT	INPUT
Call	Executes another DualPAM 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 (file-name.prg)
Begin of Repetition Block	Marks the beginning of a series of commands (repetition block) which is to be repeated.	Name of repetition block
End of Repetition Block; Loops =	Marks the end of a repetition block. A repetition block may contain other repetition blocks. Repetitions can also be terminated when a certain level of fluorescence, P700 or temperature is reached (see section “Condition Commands” at the end of this list).	Number of repetitions. Maximum 32000.
TimeStep(s) =	Defines 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. Maximum 32000 s.

COMMAND	COMMENT	INPUT
Wait(s)	Defines the time interval between completion of the last command and start of the next command. 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. Maximum 32000 s.
Paste to Slow Chart Comment Line	Writes the content of the last comment-generating line (printed in blue) into the title line of the Slow Kinetics window. When a script program contains several “Paste to Slow Chart” commands, only the last pasted line will be written to the “Record Commend File”.	None
Paste to Fast Chart Comment Line	Pastes the content of the last comment generating line (printed 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
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 =	Inserts 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	Inserts an empty line after the currently selected line.	None
Exit	Terminates and exits script file.	None

6.5.3.2 General Commands

COMMAND	COMMENT	INPUT
New Report	Starts a new Report (see 6.3.14, page 138).	None

COMMAND	COMMENT	INPUT
Save Report	Saves current Report (see 6.3.14, page 138).	None
Save Slow Kinetics as	Saves current Slow Kinetics.	File name
Save Fast Kinetics as	Saves current Fast Kinetics.	File name
Slow Kinetics	Starts or ends a slow kinetics: equivalent to the “Start/Stop” button on slow kinetics window.	On/Off
Light Curve	Starts or ends a light curve: equivalent to the “Start/Stop” button on the light curve window.	On/Off
Man. Record	Requires SP-Analysis mode. Starts/stops Manual Record on window “SP Analysis”.	On/Off
Copy Settings from Slow Kinetics	Adds settings of the current Slow Kinetics to the current Triggered Run.	None
Copy Trig. Run from Slow Kinetics	Creates a Triggered Run based on current Slow Kinetics (see 6.2.4, page 90).	None
Copy Settings from Fast Kinetics	Adds settings of the current Fast Kinetics to the current Fast Kinetics trigger pattern.	None
Copy Trig. Run from Fast Kinetics	Creates Fast Kinetics trigger pattern based on current Fast Kinetics (see 6.2.7, page 100).	None
Open Fast Kin. Trig. File	Loads Fast Kinetics trigger pattern (filename.FTM) located in directory C:\Program Files\DualPAM\Fast Kin Trigger.	File name
Open Slow Kin. Trig. File	Loads Triggered Run (filename.STM) located in directory C:\Program Files\DualPAM\Slow Kin Trigger.	File name

COMMAND	COMMENT	INPUT
Select Record	Opens the list of Records in the current Report	None
Clock	Starts or stops the repetitive trigger.	On/Off
Clock Time	Sets or modifies clock interval (see 6.1.15, page 71).	Set interval in s by equal sign s (=). Increase current interval by plus sign (+), or decrease current interval by minus sign (-)
Clock Mode=	Selects the action to be triggered by the clock.	Select from drop-down list
Clock Target Number	Sets number of trigger actions.	Number, maximum 10000
Infinite Clock Cycles	Trigger action not limited, standard setting.	On/Off
ST Flash Target Number	Inactive command.	Does not apply
ST Flash Rep.Time	Inactive command.	Does not apply
Stirrer	Stirrer control (see 4.1.2, page 12 and 6.1.13, page 66).	On/Off

6.5.3.3 Determination Commands

COMMAND	COMMENT	INPUT
Sat-Pulse/Fast Kin.	Carries out saturation pulse kinetics or fast kinetics dependent on software settings.	None
Switch to Multi FK	Activates the Multi-Start Option (see 6.3.10.2, page 130).	None
Switch to FK	Returns from Multi-Start to single start.	None

COMMAND	COMMENT	INPUT
Multi-Start	Triggers a sequence of fast kinetics (see 6.3.10.2, page 130).	None
Fo	Determines F_0 fluorescence	None
Fo'	Determines F_0' fluorescence (see Fig. 34, page 63).	None
Fo,Fm	Determines F_0 and F_M fluorescence.	None
Calibration	Calibrates the P700 signal (see 6.3.9, page 128).	None
Pm	Determines P_M (see Fig. 33, page 62).	None
Auto FoFm/Pm	Ignores question "Keep previous Fo, Fm determination" to avoid stop of script execution.	On/Off
FR+Yield/Fast Kin.	Far-red exposure followed by saturation pulses analysis or fast kinetics (see Table 20, page 69).	None
AL+Yield/Fast Kin.	Actinic light exposure followed by saturation pulses analysis or fast kinetics (see Table 20, page 69).	None
Ext. Trigger open	Puts external triggering of Fast Kinetics from off to standby (see 6.3.10.3, page 130).	None
Ext. Trigger close	Terminates external triggering of Fast Kinetics.	None
Ext. Trigger	Follows the command "Ext. Trigger open" and activates triggering of Fast Kinetics by 5 V pulse.	On/Off
Trig. SP by Ext.1	Enables external triggering of saturation pulses by voltages pulse applied to sockets External Signal 1. See Table 10, page 47.	On/Off

COMMAND	COMMENT	INPUT
Trig. SP by Ext.2	Enables external triggering of saturation pulses by voltage pulse applied to sockets External Signal 2. See Table 10, page 47.	On/Off

6.5.3.4 Actinic Light Commands

COMMAND	COMMENT	INPUT
AL	Actinic light switch.	On/Off
TR	Switch for trigger output (see 4.1.2, page 12, and 4.2.2, page 21).	On/Off
FR/BL	Switch to far-red or blue light	On/Off
Toggle FR/BL	Toggles between far-red and blue light (see 6.2.2, page 81).	None
Select FR/BL	Selects either far-red or blue light (see 6.2.2, page 81).	Blue/Far-red
Select Act. Light	Selects actinic light color (see 6.2.2.3, page 85).	Blue, Red, Red and Blue
ST	Triggers single turnover flash (individual or sequence, see Table 19, page 69).	None
MT	Triggers multiple turnover flash.	None
AL Pulse	Triggers a pulse of actinic light (see 6.2.2).	None
FR/BL Pulse	Triggers a pulse of far-red or blue light.	None
TR Pulse	Applies a 5 V trigger pulse to the port “trigger out”, and the stirrer.	None

6.5.3.5 Measuring Light Commands

COMMAND	COMMENT	INPUT
F ML	Switch for fluorescence measuring light.	On/Off
P ML	Switch for P700 measuring light.	On/Off
Balance	Balances the P700 sample and reference beams.	None
P700 Sig. Fine Up	Increases the difference signal by about 0.15 V.	None
P700 Sig. Fine Down	Decreases the difference signal by about 0.15 V.	None
MF-H	Activates high measuring light frequency (see 6.2.1, page 72).	On/Off

6.5.3.6 General Settings

COMMAND	COMMENT	INPUT
Open User Settings	Activates previously defined software settings.	File name.
Detector 1	Defines detector 1 (see 6.1.1, page 46).	Pick from selection.
Detector 2	Defines detector 2 (not present in standard configuration fluorescence and P700).	Pick from selection.
Measure Mode	Defines measure mode (see 6.1.1, page 46).	Pick from selection.
Analysis Mode	Defines analysis mode (see 6.1.7, page 53).	Pick from selection
Recording Mode	Defines recording mode (see 6.3.8, page 127).	E.g., manual, ind. Curve, …
Gain Ch1	Amplification of fluorescence signal	Low/High
Gain Ch2	Amplification of P700 signal	Low/High
Damping Ch1	Damping of fluorescence signal.	Low/High
Damping Ch2	Damping of P700 Signal.	Low/High

COMMAND	COMMENT	INPUT
Zero Offset Ch1	Offset compensation of fluorescence signal.	None
Zero Offset Ch2	Offset compensation of P700 signal.	None
Slow Kin. Acquisition Points	Number of data of slow kinetics (see 6.2.3.1, page 87).	Number
Slow Kin. Acquisition Rate	Defines interval between two consecutive measurements (see 6.2.3.1, page 87).	Time in ms
Target Averages	Number of measurements to be averaged (see 6.2.7, page 100).	Number, maximum 10000
Multiple Average Cycles	Runs several series of fast kinetics for averaging.	On/Off
Fast Kin. Averaging	Calculates the average of a series of consecutive fast kinetics.	On/Off
Auto ML on	Switches measuring light on shortly before start of a fast kinetics (see 6.4.2.1, page 143).	On/Off
Read with Start Cond	Uses the settings of a stored Fast Trigger pattern (see 6.2.8, page 103).	On/Off
Auto Fo'	Activates F_0' mode (see Fig. 34, page 63).	On/Off
PAR Sensor	Prevents/allows reading PAR from connected sensor.	On/Off
SP-Evaluation Int.Delay	Delay parameter to evaluate P700 SP kinetics (see Fig. 53, page 99).	Number, ms
SP-Evaluation Int.Width	Width parameter to evaluate P700 SP kinetics (see Fig. 53, page 99).	Number, ms

6.5.3.7 Actinic Light Settings

COMMAND	COMMENT	INPUT
AL-Int. BL-Int. FR-Int. SP-Int. Fm-Int.	Sets or changes intensity of red, blue, far-red light, as well as of saturation pulses (See 6.2.2.1, page 81, and 6.2.6, page 97.)	Absolute setting or change of setting
AL Width FR Width BL Width	Length of exposure with red, blue, far-red light (see 6.2.2.1, page 81).	Pick from drop-down list
SP Width	Length of saturation pulse (see 6.2.6, page 97).	
AL Pulse Width FR Pulse Width ST Pulse Width MT Pulse Width TR us-Pulse Width TR ms-Pulse Width	Length of pulse widths (see Table 19, page 69).	
ST sequence number	Number of Single Turnover flashes of a sequence (see Table 19, page 69)	Number of flashes. Maximum = 20
ST sequence width	Interval between Single Turnover flashes.	Time in ms. Maximum = 500
Fast Kin. after ST sequence	Carries out a Fast Kinetics after a sequence of single turnover flashes (see Table 19, page 69)	On/Off

COMMAND	COMMENT	INPUT
SP Width Step AL Pulse Width Step FR Pulse Width Step ST Pulse Width Step MT Pulse Width Step TR us-Pulse Width Step TR ms-Pulse Width Step	Change pulse width stepwise. The step size is picked from the corresponding dropdown menu.	Number of steps, 5 = maximum
Get AL PAR Get BL PAR	Measures the current PAR (see 6.2.2.1, page 81).	None
Sinus mod period Sinus mod amplitude AL sinus mod FR/BL sinus mod Sinus mod phase	See 6.2.2.2, page 82.	See 6.2.2.2, page 82.

6.5.3.8 Measuring Light Settings

COMMAND	COMMENT	INPUT
MF-H MF-L MF-M	Defines high/low/maximum measuring light frequency (see).	Select from dropdown list
MF-H Step MF-L Step	Changes high/low measuring light frequency. Step size is defined by the values of the corresponding dropdown menu.	Number of steps, Maximum = 4
F ML-Int.	Sets or changes fluorescence measuring light intensity.	Number of steps, Maximum = 20
P ML-Int.	Sets or changes P700 measuring light intensity.	Number of steps, Maximum = 20

COMMAND	COMMENT	INPUT
AutoMF_H	Activates automatic increase of measuring light frequency when actinic light is switched on (see 6.2.1.2, page 73).	On/Off
MF Log.	Logarithmically decreases measuring light frequency of fast kinetics (see 6.2.8, page 103).	On/Off
Variable Block Frequency	See 6.2.1.2, page 73.	On/Off
Get FML PAR	Measures the current fluorescence measuring light intensity (see 6.2.8, page 103).	None

6.5.3.9 Condition Commands

COMMAND	COMMENT	INPUT
If Sig.1(Fluo) If Sig.2(P700) If Temperature	Executes next command if the signal (fluorescence, P700, or temperature) is greater (>) / smaller (<) than the threshold value entered.	Ft or P700 in Volt, or Temperature in °C
else	Follows an „if“ command and executes the next command when the “if condition” is not true	None
Wait until Sig.1(Fluo) Wait until Sig.2(P700) Wait until Temperature	Interrupt script file execution until signal (fluorescence, P700, or temperature) is greater (>)/smaller (<) than the threshold value entered	Ft or P700 in Volt, or Temperature in °C
End of Rep. Block; Repeat until Sig.1(Fluo) End of Rep. Block; Repeat until Sig.2(P700)	Continues loop execution until signal (fluorescence or P700) is greater (>)/smaller (<) than the threshold value entered.	Ft or P700 in Volt
Wait until Clock Counter	Interrupt script file execution until clock counter is greater (>)/smaller (<)/equal (=) than the number entered.	Number

6.5.3.10 Display Commands

COMMAND	COMMENT	INPUT
Visible Page	Selects a graphical or numerical display window.	Pick from selection
Visible Settings Page	Selects a settings window.	Pick from selection
Slow Kinetics Auto Scaling Left Slow Kinetics Auto Scaling Right Slow Kinetics Auto Scaling Horizontal	Activates automatic axis scaling.	None or On/Off
Slow Kinetics Average Points	Configures the running average of slow kinetics (see 6.3.7, page 127).	Number, maximum = 1000
Fast Kinetics Auto Scaling Left Fast Kinetics Auto Scaling Right Fast Kinetics Auto Scaling Horizontal Fast Kinetics Horizontal Log.	Activates automatic axis scaling, or switches to logarithmic time axis.	None or On/Off
Fast Kinetics Average Points	Configures the running average of fast kinetics (see 6.3.7, page 127).	Number, maximum = 1000
Fast Kinetics File Inc	Displays next Fast Kinetics.	None
Fast Kinetics File Dec	Displays previous Fast kinetics.	None

6.5.3.11 GFS-3000 Commands

GFS Connect	GFS CO2 Control off
GFS Disconnect	GFS H2O Control off
Send Header to GFS	GFS TempControl off
Send Data to GFS	GFS Auto ZP
GFS Show Time	GFS Set Tcuv
GFS New Record	GFS Set Tleaf
GFS Comment	GFS Area/Weight
GFS Count up Object No	GFS CO2 push valve
GFS Storing Interval	GFS wait until ready
GFS Store MP ZP	GFS wait until steady
GFS Save Chart	GFS wait until steady, max
GFS Mode	
GFS Set Flow	See the manual for Portable Gas Exchange Fluorescence System GFS-3000: https://www.walz.com/products/gas_exchange/gfs-3000/downloads.html
GFS Set CO2	
GFS Set H2O	

7 Saturation Pulse Analysis

7.1 Photosystem II

7.1.1 Pulse-amplitude Modulated (PAM) Fluorescence

The PAM principle is illustrated by the original drawing of Dr. Ulrich Schreiber (Fig. 71). The top part shows the total fluorescence of a sample. µs-measuring flashes are given throughout the experiment starting with “Pulse on”. These flashes cause the spikes in the fluorescence trace. From left to right, an external effect induces a “False Signal” of continuous fluorescence in the darkened sample. Then the sample is exposed to a period of actinic illumination (“Actinic on” and “Actinic off”), and, finally, the sample is kept in the dark again.

During actinic illumination, an effect of stray light on the fluorescence signal is additionally assumed. The fluorescence level at onset of stray light plus actinic light is denoted “Actinic F_0“. The further increase of continuous fluorescence during illumination is denoted “Actinic F_V”, where the V stands for variable fluorescence. The “Actinic F_V“ reflects changes of the fluorescence yield in the sample because stray light and actinic light are constant during the illumination period.

In Fig. 71, not only continuous fluorescence varies but also the amplitude of fluorescence spikes. PAM fluorometers ignore the changes of continuous fluorescence and measure only the amplitude of fluorescence spikes. This is achieved by subtracting the fluorescence level just before the µs-measuring flash from the fluorescence level at the µs-measuring flash. In Fig. 71, the PAM fluorescence amplitude during the initial dark phase is denoted

"Pulsed F_0", and the maximum variable fluorescence at the end of actinic illumination is denoted "Pulsed F_V".

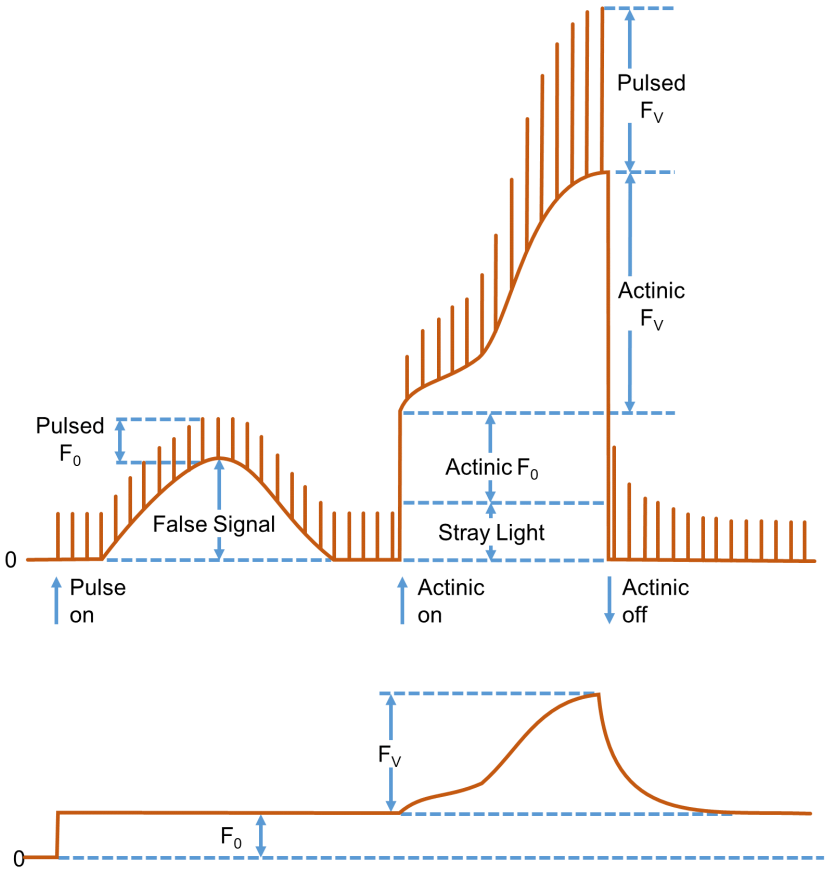


Fig. 71: Illustration of the PAM Measurement Principle

Figure redrawn for Dr. Ulrich Schreiber, Manual for PAM-101.

Because the µs-measuring flashes have constant amplitude, the varying amplitudes of fluorescence spikes is a measure of how efficient excitation light is converted into fluorescence. In other words, PAM fluorescence is proportional to the fluorescence yield.

The lower trace in Fig. 71 outlines the PAM fluorescence trace. Obviously, PAM fluorescence ignores the “False Signal” of total fluorescence at the beginning of the experiment, and also the fluorescence jumps when actinic light is switched on and off. The course of continuous fluorescence within the range “Actinic FV“ resembles the corresponding trace of PAM fluorescence, because both measuring light and actinic illumination are constant.

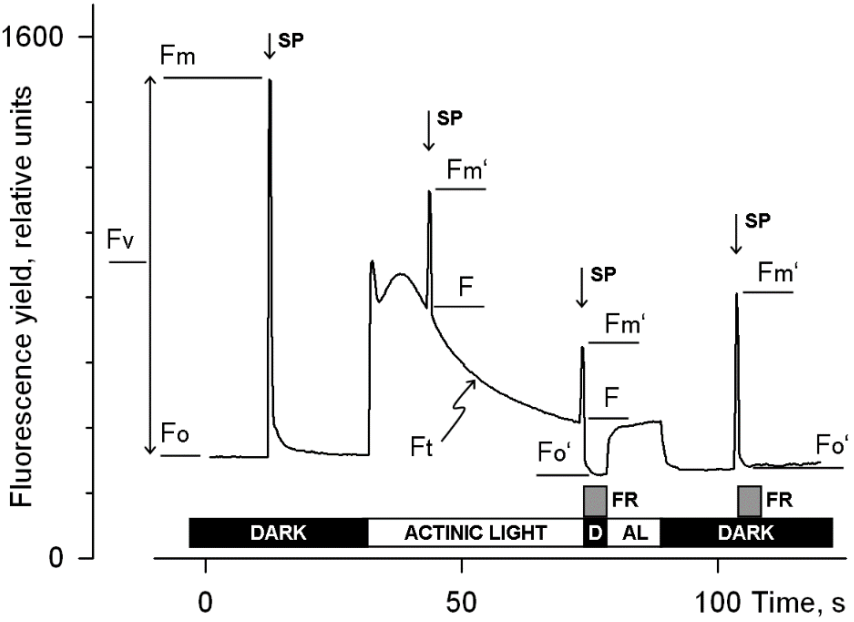


Fig. 72: Fluorescence Levels of Saturation Pulse Analysis

Y-axis (Fluorescence yield) corresponds to PAM fluorescence, see 7.1.1. AL, Actinic Light; D, dark; SP, Saturation Pulse; Ft, continuously recorded PAM fluorescence; FR, far-red illumination.

7.1.2 Saturation Pulse Analysis

The five principal levels of PAM fluorescence which are used for saturation pulse analysis are shown in Fig. 72. Two of these levels (F_0 and F_M) must be measured with the dark-acclimated sample. The three other levels (F_0', F, and F_M') are measured with the actinic light-exposed sample or in a dark period following this light treatment. Some parameters of saturation pulse analysis require fluorescence measurement of the same sample in both the dark-acclimated and light-exposed state (Table 37, page 170).

Because PAM fluorescence is excited by µs pulses of constant amplitude, variations between fluorescence levels are usually interpreted as variation in chlorophyll fluorescence yield. This applies for variations between different types of fluorescence levels (e.g. between F_0 and F_M) and for variations of the same type of fluorescence level (e.g. the change of F_M' during a fluorescence induction curve).

Measurements with Dark-Acclimated Samples

F_0 Minimum fluorescence level excited by very low integrated intensity of measuring light to keep photosystem II reaction centers open.

F_M Maximum fluorescence level in the presence of a pulse of saturating light (Saturation Pulse) which closes all photosystem II reaction centers.

Measurements with Illuminated Samples

F_0' Minimum fluorescence level of illuminated sample. The F_0' is lowered relative to F_0 by non-photochemical quenching. The measuring routine for F_0' (see Fig. 34, page 63) determines the F_0' level during a dark interval following a Saturation Pulse. In this dark interval, far-red

light is applied which selectively drives photosystem I. As a consequence, electrons are removed from the intersystem electron transport chain and opening of photosystem II reaction centers is efficiently accelerated

If the F_0' Mode is switched off, the F_0' will be calculated according to Oxborough and Baker:

$$F_0' = \frac{1}{\frac{1}{F_0} - \frac{1}{F_M} + \frac{1}{F_M'}}$$

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54 135-142. https://doi.org/10.1023/A:1005936823310

In the Record table, the calculated value F_0' is preceded by a tilde sign (~).

F_M' Maximum fluorescence level of the illuminated sample. The F_M' is induced by a Saturation Pulse which temporarily closes all photosystem II reactions centers. F_M' is decreased relative to F_M by non-photochemical quenching.

F The F corresponds to the level of continuously recorded fluorescence (Ft) of an illuminated sample shortly before application of a Saturation Pulse.

To quantify photochemical use and non-photochemical losses of absorbed light energy, fluorescence ratio expressions have been derived which use two or more of the five relative fluorescence yields introduced above. Table 37 (page 170) compiles the fluorescence ratio parameters available in the DualPAM software. Below, these parameters will be explained briefly.

F_V/F_M and Y(II) Maximum and effective photochemical quantum yields of photosystem II

The F_V/F_M and Y(II) estimate the fraction of absorbed quanta used for photosystem II photochemistry. F_V/F_M corresponds to the maximum photochemical yield of photosystem II, Y(II) is the effective photochemical yield of photosystem II. Measurements of F_V/F_M require 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.

In algae and cyanobacteria, however, the dark-acclimated state often is not showing maximal photosystem II quantum yield, as the photosystem II acceptor pool may be reduced in the dark by stromal reductants and, consequently, the so-called State 2 is formed exhibiting low photosystem II quantum yield. In this case, preillumination with moderate far-red light should precede determinations of F_0 and F_M.

The Y(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 photosystem II centers and various types of non-photochemical energy losses induced by illumination.

q_P and q_L Coefficients of photochemical fluorescence quenching

Both parameters estimate the fraction of open photosystem II reaction centers. The q_P is based on the concept of separated photosystem II antenna units (puddle model), whereas the q_L assumes interconnected photosystem II antenna units (lake model) which was assumed to be present in leaves (*cf.* Kramer *et al.*, 2004). When the F_0' mode is switched on, calculations of q_P an q_L do not require an F_0 measurement. When the F_0' mode is switched off, the F_0' must be calculated according to Oxborough and Baker (1997) which requires an F_0 measurement.

q_N 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. The qN and the NPQ parameters require fluorescence measurements with the sample in the dark-acclimated and in the light-exposed states (cf. Table 37, page 170).

Calculation of NPQ (or SV_N; 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 photosystem II yields

Genty *et al.* (1996) and Kramer *et al*. 2004 have presented expressions describing the partitioning of absorbed excitation energy in photosystem II between three fundamental pathways the sum of which adds up to one:

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

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

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

This concept of "complementary photosystem II quantum yields" is useful to analyze the partitioning of absorbed light energy in photosynthetic organisms. For instance, in the presence of strong light, a much higher Y(NPQ) than Y(NO) indicates that excess excitation energy is safely dissipated at the antenna level and that photosynthetic energy fluxes are well-regulated.

In contrast, high values of Y(NO) would signify that excess excitation energy is reaching the reaction centers, resulting in strong reduction of photosystem II acceptors and photodamage, e.g. via formation of reactive oxygen species.

7.1.3 Relative Electron Transfer Rate (ETR)

Relative electron transfer rates for photosystem II are calculated according to:

$ETR(II) = PAR \cdot ETR\text{-}Factor \cdot P_{PS2}/P_{PS1+2} \cdot Y(II)$.

The basic idea of the ETR equation is to multiply Y(II), the effective photochemical quantum yield of photosystem II, by an estimate for the photon flux density absorbed by all photosystem II in the sample. The latter estimate is derived from three numbers:

(1) PAR Quantum flux density of photosynthetically active radiation (PAR) impinging on the sample.

(2) ETR-Factor Sample absorptance (= 1 – transmittance)

The ETR-Factor describes the fraction of incident photons absorbed by the sample. The most frequently used default value for green leaves is 0.84 meaning that 84% of incoming light is absorbed. The ETR-Factor can be lower in bleached leaves or leaves containing considerable amounts of non-photosynthetic pigments like anthocyanins.

(3) P_{PS2}/P_{PS1+2} Relative distribution of absorbed PAR to photosystem II

The default P_{PS2}/P_{PS1+2} is 0.5 which assumes that the photosystem II contributes 50% to total sample absorptance. The P_{PS2}/P_{PS1+2} may deviate from the idealized factor of 0.5 depending on wavelength of light and acclimation status of the sample.

The same concept is used to calculate electron transport rates for photosystem I. The following equation is used

$ETR(I) = PAR \cdot ETR\text{-}Factor \cdot P_{PS2}/P_{PS1+2} \cdot Y(I)$.

For a definition of Y(I) see Section 7.2, page 172)

7.1.4 Reviews on Saturation Pulse Analysis of Photosystem II

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59: 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M, Bussotti F, Calatayud A, Dąbrowski P, Elsheery NI, Ferroni L, Guidi L, Hogewoning SW, Jajoo A, Misra AN, Nebauer SG, Pancaldi S, Penella C, Poli DB, Pollastrini M, Romanowska-Duda ZB, Rutkowska B, Serôdio J, Suresh K, Szulc W, Tambussi E, Yanniccari M, Zivcak M (2014) Frequently asked questions about *in vivo* chlorophyll fluorescence: practical issues. Photosynth Res 122: 121-158. https://doi.org/10.1007/s11120-014-0024-6

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42: 313-349. https://doi.org/10.1146/annurev.pp.42.060191.001525

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. J Exp Bot 51: 659-668 https://doi.org/10.1093/jexbot/51.345.659

Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 64: 3983-3998. https://doi.org/10.1093/jxb/ert208

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, Dordrecht, pp 279-319. https://doi.org/10.1007/978-1-4020-3218-9_11

Table 37: Fluorescence Ratio Parameters.

Source	Equation	Sample State	Range [Theory] [Experiment]
Maximum photochemical quantum yield of PS II (Kitajima and Butler, 1975)	$\frac{F_V}{F_M} = \frac{F_M - F_0}{F_M}$	Dark	[0, 1] [0, ~0.84]
Effective photochemical quantum yield of PS II (Genty *et al.*, 1989)	$Y(II) = \frac{F'_M - F}{F'_M}$	Light	[0, 1] [0,~ 0.84]
Quantum yield of light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Genty *et al*. 1996, Kramer *et al*. 2004)*	$Y(NPQ) = \frac{F}{F'_M} - \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Quantum yield of non-regulated heat dissipation and fluorescence emission: this type of energy loss does not involve the action of a trans-thylakoid ΔpH and zeaxanthin (Genty *et al*. 1996, Kramer *et al*. 2004)*	$Y(NO) = \frac{F}{F_M}$	Dark and Light	[0, 1] [0, ~ 0.9]
Stern-Volmer type non-photochemical fluorescence quenching (Bilger and Björkman, 1990; Gilmore and Yamamoto, 1991))	$NPQ = \frac{F_M}{F'_M} - 1$	Dark and Light	[0, ∞] [0, ~4]
Coefficient of photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_P = \frac{F'_M - F}{F'_M - F'_0}$	Light. If F_0' calculated, Dark and Light	[0, 1] [0, 1]
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (Kramer *et al.* 2004)	$q_L = q_P \cdot \frac{F'_0}{F}$	As $q_{P'}$.	[0, 1] [0, 1]
Coefficient of non-photochemical fluorescence quenching (Schreiber *et al.* 1986 as formulated by van Kooten and Snel, 1990)	$q_N = 1 - \frac{F'_M - F'_0}{F_M - F_0}$	Dark and Light	[0, 1] [0, ~0.95]

* Kramer *et al.* (2004) have derived more complex equations for Y(NO) and Y(NPQ). Klughammer and Schreiber (2008) have transformed the equations by Kramer *et al.* (2004) into the simple equations of Genty *et al*. (1996).

Table 38: References Cited in Table 37

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. https://doi.org/10.1007/BF00033159

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. https://doi.org/10.1016/S0304-4165(89)80016-9

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

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. https://doi.org/10.1104/pp.96.2.635

Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376:105-115. https://doi.org/10.1016/0005-2728(75)90209-1

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 https://www.walz.com/files/downloads/pan/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. https://doi.org/10.1023/B:PRES.0000015391.99477.0d

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. https://doi.org/10.1007/BF00024185

van Kooten O, Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147–150. https://doi.org/10.1007/BF00033156

7.2 Photosystem I

The DUAL-PAM systems employ the method by Christof Klughammer and Ulrich Schreiber to analyze photosystem I:

Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via $P700^+$-absorbance changes at 830 nm. Planta 192: 261-268. https://doi.org/10.1007/BF01089043

Klughammer C, Schreiber U (2008) Saturation Pulse method for assessment of energy conversion in PS I. PAM Application Notes (2008) 1: 11-14. https://www.walz.com/files/downloads/pan/PAN07002.pdf

Photochemistry of the photosystem I reaction center is defined as the conversion of the reaction center chlorophyll *a* (P700) in its oxidized form ($P700^+$), which goes along with the reduction of an electron acceptor ($A \rightarrow A^-$).

The transition $P700 \rightarrow P700^+$ causes typical absorption changes (Fig. 40, page 79). The DUAL-PAM systems measure the absorbance change in the near-infrared region (NIR). The advantage of NIR measuring light is that it is not absorbed by light-harvesting pigments. Consequently, the NIR method does not influence photosynthesis, and high measuring light intensities reach the detector resulting in excellent signal quality.

The maximum absorption change of a sample is denoted P_M. The P_M level is reached by far-red preillumination which is followed by a saturation pulse (see Fig. 33, page 62). The far-red oxidizes P700 because it is predominantly absorbed by photosystem I, that is, the photosystem I reaction center separates charges but electron supply by photosystem II is nearly absent.

Far-red illumination does not fully oxidize all reaction centers because electrons flowing from non-photosynthetic sources to photosystem I compete with the rather moderate turnover rates of photosystem I under far-red illumination. However, the strong excitation by a saturation pulse is capable of (at least temporarily) completely oxidizing all P700.

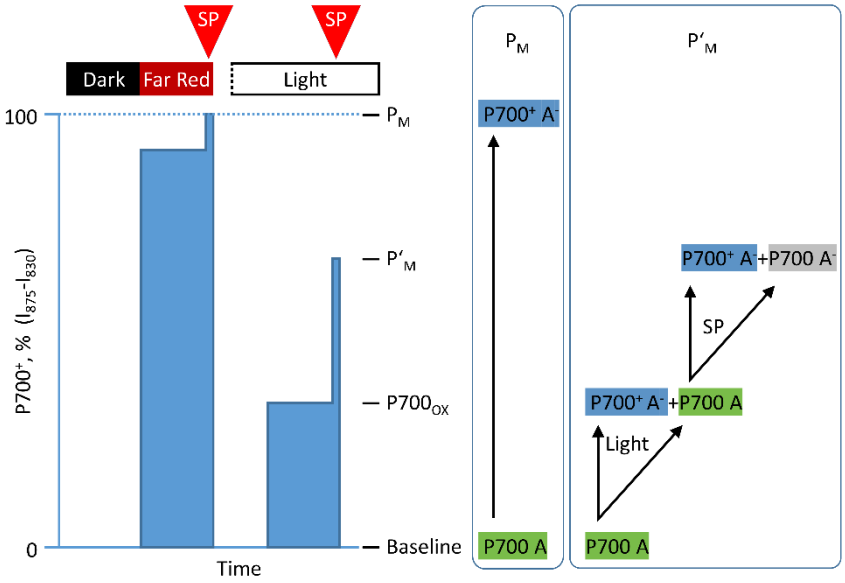
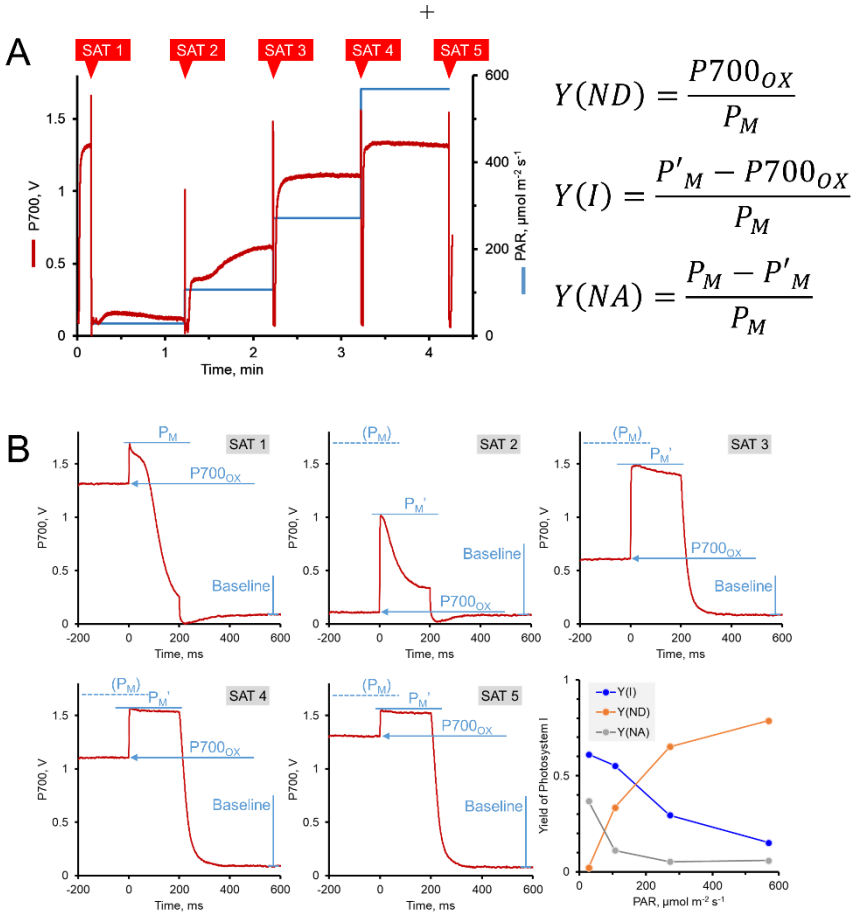


Fig. 73: P700 Method

Y-axis, oxidation of the chlorophyll *a* of the photosystem I reaction center as measured by NIR absorption. P_M, maximum absorption change caused by a saturation pulse (SP) of a (Dark) sample preilluminated by far-red light; $P700_{OX}$, absorption change under actinic illumination (Light); P'_M, maximum absorption change caused by an SP applied to the illuminated sample. P700 A, open photosystem I reaction center with P700 and acceptor in the neutral state; $P700^+ A^-$, closed photosystem I reaction center with oxidized P700 and reduced acceptor; P700 A^- closed photosystem I reaction center with P700 in the neutral state and reduced acceptor. After Klughammer and Schreiber, 1994, modified.



$$Y(ND) = \frac{P700_{OX}}{P_M}$$

$$Y(I) = \frac{P'_M - P700_{OX}}{P_M}$$

$$Y(NA) = \frac{P_M - P'_M}{P_M}$$

Fig. 74: P700 Light Curve

A: Light curve with 4 light intensity steps. Blue trace, light intensity; red trace, NIR absorption of P700; SAT1 to SAT 5, saturation pulse number 1 to 5. Equations: *Y(ND)*, percentage of photosystem I reaction centers which are closed because of lacking electron donation; *Y(NA)*, percentage of photosystem I reaction centers which are closed because of lacking electron acceptors; *Y(I)*, percentage of open photosystem I reaction centers. The three Y-terms add up to 1. **B:** Fast kinetics of SAT1 to SAT 5 and Y terms plotted against light intensity (photosynthetically active radiation, PAR).

The P_M is the maximum NIR absorption change of the sample's photosystem I population, because all photosystem I reaction centers underwent the transition P700 A → $P700^+$+A^-. Each transition occurred with the quantum yield Φ_I. Therefore, P_M is a measure for Φ_I of the entire photosystem I population, which is commonly assumed to be close to 1.

Y(I), see equation in Fig. 74

Light exposure reduces the number of photosystem I reaction centers which can be oxidized. This reduced number is measured by the NIR absorption increase induced by a saturation flash. The NIR absorption increase is calculated as P'_M – $P700_{OX}$ (Fig. 73). Relating this difference to P_M gives the effective quantum yield of photosystem I (Fig. 74).

Y(ND), see equation in Fig. 74

The shortage of electrons to reduce $P700^+$ to P700 is one mechanism controlling Y(I). This shortage is called "donor limitation". Donor limitation forms under light exposure, and it increases the continuously recorded NIR level above the baseline ($P700_{OX}$, Fig. 73). Donor limitation mounts up with increasing light intensity (Fig. 74). This relationship is governed by a rate-limiting step of electron transport which is slowed down by high light intensities. The percentage of donor-limited photosystem I reaction centers (Y(ND)) is $P700_{OX}$ divided by P_M. Fig. 74 shows that under high light, donor limitation is the main factor regulating photosystem I activity.

Y(NA), see equation in Fig. 74

Another factor controlling Y(I) is acceptor limitation meaning that the P700 → $P700^+$ transition cannot take place because the acceptor is already reduced. Acceptor limitation is the reason why the P'_M level is lower than the P_M level. Thus, the fraction of ac-

ceptor limited photosystem I reaction centers (Y(NA)) is the difference P_M minus P'_M divided by P_M (Fig. 73). Mostly, acceptor limitation play a role at low light intensities but is insignificant at high light intensities (Fig. 74).

8 Hints, Troubleshooting, Maintenance

8.1 Hints

8.1.1 Default settings

For fluorescence measurements with most healthy green leaves, default settings are fine. Users of the MODULAR version start with default settings Walz.DEF, users of the Fiber version start with default settings the Walz_Fiber.DEF (see 4.3, page 24).

8.1.2 Baseline

P700

The P700 baseline needs at least 30 s to stabilize. The LEDs need this time to warm up. Test baseline with a sclerophyllous leaf which is well-acclimated to the environment of the DUAL-PAM system.

Detached Leaf

Delicate leaves will change their water status when detached. This results in changes in leaf optical properties which can cause steep baseline drifts.

Suspensions

Baseline drift may be caused by particle settling. Check stir bar.

8.1.3 Signal Noise

P700 signal noise can be a problem with suspensions. Use bubble and flake free suspensions. Stop stirring about 10 s prior to saturation pulse analysis (see 6.2.2.4, page 86).

Shield system from fluctuating light sources (fluorescent tubes, computer screens).

8.1.4 F_0 Fluorescence

F_0 fluorescence should be around 500 mV. If F_V/F_M is 0.84 and signal saturation occurs at 4000 mV, then F_0 should be below 640 mV to avoid signal saturation at F_M level fluorescence (see Table 39).

Table 39: Maximum F_0 of a Dark-acclimated Leaf

$\left(\frac{F_V}{F_M}\right)_{Max} = \frac{(F_M)_{Max} - (F_0)_{Max}}{(F_M)_{Max}}$ $with\ (F_V/F_M)_{Max} = 0.84$ $(F_0)_{Max} = 640$	$(F_M)_{Max}$, maximum possible F_M value = 4000. $(F_0)_{MAX}$, unknown maximum F_0 value (the F_M associated with this F_0, or with smaller F_0, is not saturating). $(F_V/F_M)_{Max}$, assumed maximum possible photosystem II photochemical yield.

At low signal levels, signal height can be increased by increasing measuring light intensity. At too high intensities, the measuring light might drive photosynthesis to some degree. Therefore, test if switching on measuring light results in a stable signal or if any signal increase occurs. In the latter case, the effective measuring light intensity must be decreased either by reducing the amplitude of measuring light (measuring light intensity) or by reducing measuring light frequency or both.

8.1.5 F_M Fluorescence

The F_M and F_M' levels are determined as the maximum fluorescence signal induced by a saturation pulse. Using default settings, the fluorescence rise induced by a saturation pulse reaches a plateau with most leaves (Fig. 75A). When a plateau is not reached (Fig. 75B), increase saturation pulse intensity or/and width. On the other hand, fluorescence kinetics can reach its maximum clearly before end of the saturation pulse (Fig. 75C). This does not result in erroneous F_M or F_M' values which is determined as the maximum of a fast kinetics. However, in such cases the saturation pulse width can be reduced. In case of Fig. 75C, saturation pulse intensity or/and length might also be decreased.

Some samples, particularly low light grown or senescing plants, do not exhibit the true F_V/F_M values with standard settings. Instead, F_V/F_M increases when a saturation pulse intensity lower than the default setting is chosen. Therefore, testing the F_V/F_M at various saturation pulse intensities is important to optimize your saturation pulse settings.

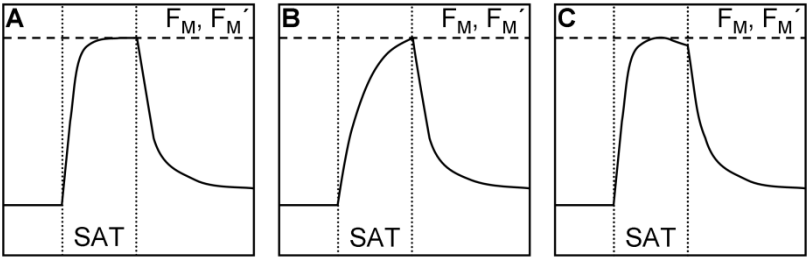


Fig. 75: Fluorescence Kinetics Induced by a Saturating Pulse

8.2 Troubleshooting

8.2.1 Instrument not switched on

In rare cases, starting the DualPAM software connects a DUAL-PAM system although the machine is switched off. This malfunction triggers the error message “Instrument not switched on or battery low”. For normal system start, close software, switch on fluorometer (see Section 4.1.2, page 12 or Section 4.2.2, page 21), and start DualPAM software again.

After about three years of use, the lead acid battery inside DUAL-PAM systems shows loss of capacity. At a certain state of decay, the battery support required at system start up and for saturation flashes becomes insufficient. This state triggers the error message mentioned above. The power provided by the charger is not sufficient to cover the peak demands of DUAL-PAM systems. Normal function is restored by battery change (Section 8.3.1, page 181).

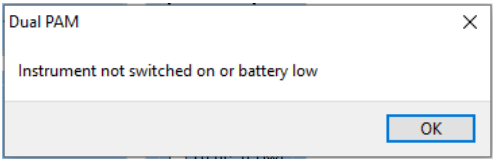


Fig. 76: Error Message

8.2.2 Software Failure

General. When the DualPAM software hangs or behaves strangely, close software, switch off machine, and unplug USB cable from computer. Restart. When the DualPAM software does not respond at all, close program in Task-Manager.

Script File. A software crash during execution of a script file indicates the presence of a forbidden command sequence. Check the step of the script file at which software crashes.

New Installation. In some cases, normal function of the DualPAM software requires software uninstallation, deletion of all files in the DualPAM directory (see Fig. 9, page 27), and new software installation. Backup data before software uninstallation.

8.2.3 P700 does not Balance

When balancing of NIR measuring beams fails, reduce measuring light intensity from setting 10 (default) to 6 and balance again. After successful balancing, you can return to the default intensity setting for P700 measuring light.

8.3 Maintenance

8.3.1 Battery Change

The DUAL-PAM-100 battery is a commercially available MP2-12SL – 12V/2 Ah battery with the dimensions 150 x 20 x 89 mm. The production of the YUASA battery as shown in Fig. 78 has been discontinued. Check availability of the MP2-12SL in your country. The dimensions indicated may slightly vary between suppliers. When not available, order with Walz (order number is 00160101312).

To change battery, unscrew the four screws on the rear side and remove back panel of DUAL-PAM control unit (Fig. 77). Pull out battery plugs. Lift battery with a screwdriver (the battery is fastened to the back side by a double-sided tape). Remove battery.

Position and attach new battery using double-sided tape. Connect the red plug to the plus (+) pole, and the black plug to the minus (-) pole of battery. When mounting the back panel, control position of battery cables to avoid jamming of these cables.

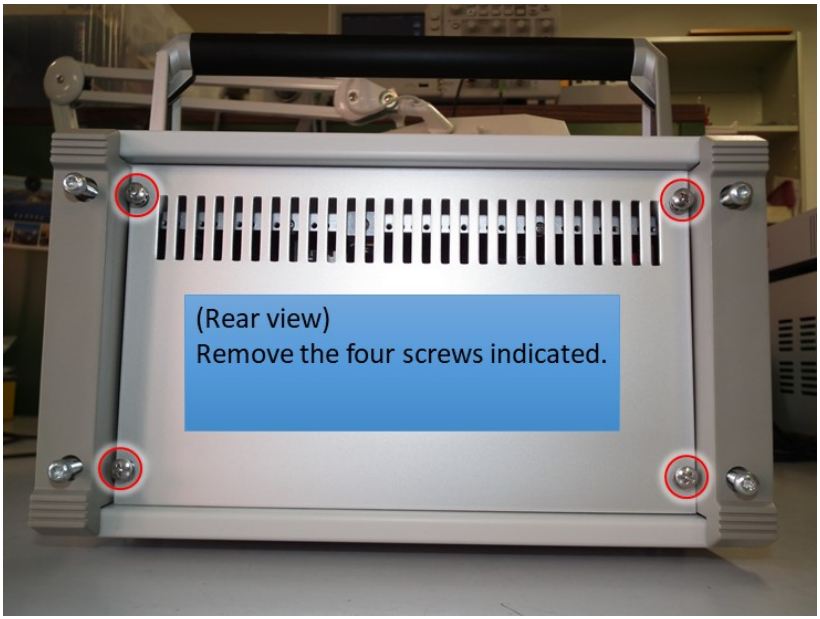


Fig. 77: Open DUAL-PAM

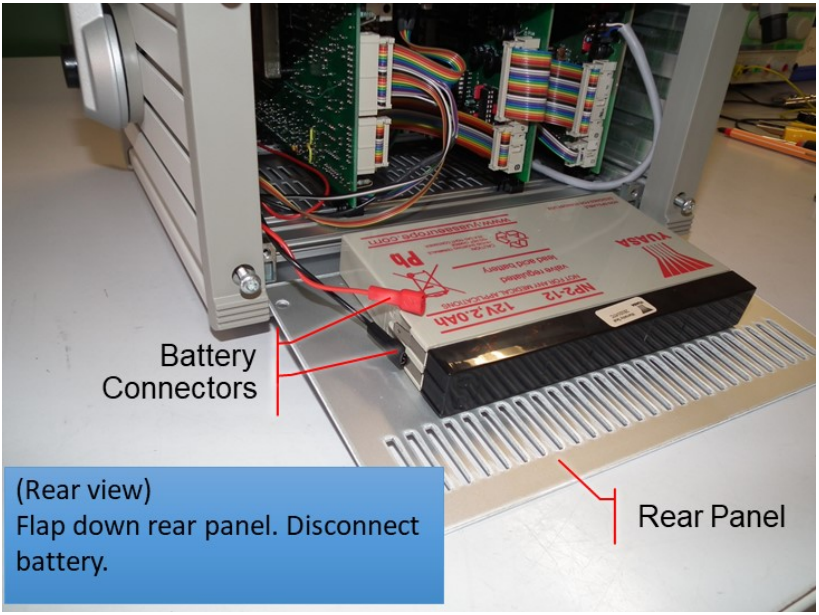


Fig. 78: Change battery

Note that the battery is fastened by a double-sided adhesive tape. When reassembling, take extreme care not to squeeze a ribbon cable.

9 Specifications DUAL-PAM

9.1 MODULAR Version

9.1.1 Basic System

9.1.1.1 Power-and-Control-Unit DUAL-C

General design: 2 x AVR-RISC microcontroller (8 MHz) + 4 MB SRAM; 256 000 data points with 12 bit resolution can be stored

PC interface: USB 1.1, 2.0 and 3.0 compatible

User interface: Windows computer with DualPAM software

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

Power consumption: During basic operation 160 mA

Sockets: 6 ports for measuring heads (power for single wavelength and double wavelength modulated measuring light, power for 2 LED arrays, input for 2 detectors), socket for stirrer (plus speed controller and standby switch), AUX (for Leaf Clip 2030-B or Spherical Micro Quantum Sensor US-SQS/WB or Cosine-Corrected Mini Quantum Sensor US-MQS/WB), USB (for USB cable), TRIGGER IN (input for 5 V rectangular signals to trigger fast kinetics externally), TRIGGER OUT (output of 5 V rectangular signals to trigger external devices), 2 EXT. SIGNALS (input for external DC signals. Range 0 - 1V or 0 - 5 V), and CHARGE (for MINI-PAM/L charger)

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

Weight: 4.5 kg

9.1.1.2 Measuring Head with P700 NIR Emitter DUAL-E

Measuring light: P700 dual-wavelength emitter. Sample wavelength 830 nm, reference wavelength 870 nm

Actinic light: Far-red LED lamp: 720 nm. Chip-on-board (COB) LED array: 635 nm for continuous actinic illumination, maximum 3000 µmol m^{-2} s^{-1} PAR. Saturating single turnover flashes, maximal 200 000 µmol m^{-2} s^{-1} PAR, adjustable between 5 and 50 µs. Multiple turnover flashes, maximal 30 000 µmol m^{-2} s^{-1} PAR, adjustable between 1 and 1000 ms

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

Weight: 400 g (incl. cables, 1 m long)

9.1.1.3 Measuring Head with Detector, DUAL-DB (Blue) or DUAL-DR (Red)

Fluorescence-measuring light: 460 nm (DUAL-DB) or 620 nm (DUAL-DR)

Actinic light: Blue (460 nm) LED lamp for continuous actinic illumination, maximum PAR 1100 µmol m^{-2} s^{-1}. Chip-on-board LED array identical to that of DUAL-E measuring head

Signal detection: PIN photodiode with special pulse preamplifier for measuring P700 and fluorescence changes with maximal time resolution of 30 µs. Fluorescence is detected at wavelengths longer than 700 nm

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 500 g (including cables, 1 m long)

9.1.1.4 Transport Box

Design: Aluminum box with custom foam packing for DUAL-PAM-100 and accessories

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

Weight: 5 kg

9.1.1.5 Computer Requirements

1 free USB socket; 500 or more MB RAM; operating system: Microsoft Windows XP/Vista/7/8/10

9.1.2 Accessories Leaves

9.1.2.1 90 Degree Measuring Head Holder DUAL-H90

Design: Right angle bracket made of black anodized aluminum, with metal rod for attachment to a laboratory stand, each bracket arm with special adapter made of Polyoxymethylene (POM) to position a measuring head. Including a laboratory scissor jack and non-fluorescent rubber foam mat

Dimensions: Holder, 10.0 cm x 4.0 cm x 5.5 cm (W x D x H). Laboratory scissor jack, 14.0 cm x 12.0 cm x 6.0 cm (W x D x H)

Weight: Holder, 175 g. Laboratory scissor jack, 1370 g

9.1.2.2 Measuring Head with Blue Measuring Light and Far-Red Illumination DUAL-DB/FR

All specifications identical to Measuring Head with Detector DUAL-DB, except double intensity of blue (460 nm) fluorescence measuring light, and blue actinic light replaced by far-red light. (When operated together with a DUAL-E unit, the far-red light of the DUAL-DB/FR is inactivated unless the LED array cable of the DUAL-E unit is unplugged.)

9.1.2.3 Linear Positioning System 3010-DUAL/B

Design: Consisting of black anodized aluminum baseplate with gear rack on which one measuring head holder is fixed and another measuring head holder is mounted on a movable stage which can be precisely positioned along the gear rack by a lateral adjustment knop. Includes a 13 cm lab stand rod which can be screwed-on to the bottom of the baseplate and a 3 mm Allen wrench

Dimensions: 18.5 cm x 11.5 cm x 12 cm (L x W x H, max. without lab stand rod)

Weight: 1050 g

9.1.2.4 Optical Pinholes DUAL-OP

Design: Set of 4 non-fluorescent rubber caps, 3 with 1 central hole of varying diameters (10 mm, 6 mm, and 4 mm), the fourth positioned closed to the rim with 2 mm diameter

Dimensions: H: 14 mm, ø 17 mm

9.1.2.5 P515/535 Emitter-detector Module

P515/535 Emitter Head DUAL-EP515

Measuring light: 520 and 550 nm dual wavelength pair, 535 nm single wavelength

Actinic light: Identical to that of Measuring Head DUAL-E

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 400 g (incl. cable, 1 m long)

P515/535 Detector Head DUAL-DP515

Signal detection: PIN photodiode with special pulse preamplifier. Detection window, 400 nm – 580 nm

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 400 g (incl. cable, 1 m long)

9.1.2.6 DUAL-PAM-100 Gas-Exchange Cuvette 3010-DUAL

Design: Cuvette consisting of a sandwich of two 2 x 2 cm aluminum frames, each holding the end part of a Walz standard Perspex rod to connect various measuring heads of the DUAL-PAM-100. Sealing material between frames and leaf: silicon foam gasket. Distance between Perspex rod and leaf: ca. 1 mm on each leaf side. Pneumatically separated upper and lower cuvette halves, controlled by a regulator unit with sockets for cable connections to the 3000 C control unit of the GFS-3000. Leaf area examined: 1.3 cm2. Leaf temperature measurement: thermocouple, range -10 to +50 °C, accuracy ±0.2 °C. External cosine-corrected Micro Quantum Sensor MQS/A for PAR measurements ranging from 0 to 2500 µmol m^{-2} s^{-1}, accuracy ±5 %

Operating temperature: -5 to +45 °C

Dimensions: Assembled cuvette: 10 cm x 4 cm x 12 cm (L x W x H), electronic box: 7 cm x 7 cm x 15 cm (L x W x H)

Weight: Cuvette, regulator unit, cables, and mounting frame: 1.7 kg; mounting stand ST-101: 2 kg

9.1.3 Accessories Suspensions

9.1.3.1 Optical Unit ED-101US/MD

Design: Black-anodized aluminum body with central 10 x 10 mm standard glass cuvette; for attachment of Measuring Heads DUAL-DB (or DUAL-DR) and DUAL-E and Miniature Magnetic Stirrer PHYTO-MS; additional ports for attachment of two additional measuring heads (e.g. acridine orange, and NADPH fluorescence)

Weight: 750 g

9.1.3.2 Miniature Magnetic Stirrer PHYTO-MS

Based on device manufactured by h+p (type Variomag-Mini); featuring adapter to be mounted in bottom port of the Optical Unit ED-101US/MD; powered and controlled by the Power-and-Control-Unit DUAL-C

9.1.3.3 Photodiode-Detector Unit DUAL-DPD

Signal detection: PIN photodiode with special pulse preamplifier. Fluorescence is detected at wavelengths longer than 650 nm

Filter holder: For optical filters (standard 30 x 30 mm), up to 15 mm thick

Dimensions: 9.7 cm x 7.1 cm x 7.8 cm (L x W x H)

Weight: 350 g

9.1.3.4 Photomultiplier-Detector Unit DUAL-DPM

Signal detection: 8 mm diameter side-on photomultiplier tube with a high voltage power supply assembled in a compact aluminum housing (Hamamatsu H6779). Two filters are provided for fluorescence detection at wavelengths > 650 nm or > 700 nm

Filter holder: With cover. For optical filters (standard 30 x 30 mm), up to 15 mm thick

Dimensions: 100 mm x 66 mm x 108 mm (L x W x H)

Weight: 490 g (incl. cable, 1.5 m long)

Amplifier Box PM-101/N

Included in extent of delivery of NADPH/9-AA Photomultiplier Detector Unit or Photomultiplier Detector DUAL-DPM

Design: Aluminum chassis with texture paint. Line input 115/230 V AC, 50-60 Hz, 0.04/0.02 A. Two rotary buttons permit selection of 6 coarse amplification factors which 11 subdivisions

Dimensions: 11 cm x 11 cm x 7 cm (L x W x H)

Weight: 700 g

9.1.3.5 Temperature Control Unit US-T Optical Unit ED-101US/MD

Power-and-Control Unit US-T/DR

Display: Three line LCD display

Control range: 0 °C to 50 °C at 0.1 K steps

Operating voltage: 11 V - 14 V DC

Maximum Peltier current: 1 A

Size: 105 mm x 90 mm x 130 mm (W x H x D)

Weight: 0.57 kg

Peltier Heat-Transfer Head US-T/DS

Achievable temperatures: 12 K below ambient temperature, 15 K above ambient temperature (Quartz cuvette placed in Optical Unit for Suspensions ED-101US/MD with 1.5 mL water and stirrer PHYTO-MS on)

Size: ⌀ 55 mm, 110 mm height

Cable length: 130 cm

Weight: 0.29 kg (including cable)

AC Adapter

Input: 100 V - 240 V AC 1.5 A 50-60 Hz

Output: 12 V DC 5.5 A

Size: 130 mm x 56 mm x 30 mm (L x W x H)

Weight: 0.50 kg (including cable)

9.1.3.6 Temperature Control Block 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

9.1.3.7 Low-Drift Cuvette DUAL-K25

Design: Quartz glass cuvette, cross section: 10 mm x 10 mm, external dimensions: 12.5 mm x 12.5 mm x 26 mm (L x W x H). Special cuvette holder to position the cuvette between two measuring heads. U-shaped black-anodized aluminum shields to screen out external light. Three sealing gaskets to protect lower measuring head from spills

9.1.3.8 Acridine Orange/Yellow fluorescence emitter-detector mod.

Acridine Orange Emitter Head DUAL-EAO

AO measuring light: 455 nm

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

Weight: 400 g (incl. cables, 1 m long)

Acridine Orange Detector Head DUAL-DAO

Signal detection: PIN photodiode with special pulse preamplifier. Detection window, 500 nm – 580 nm

Chlorophyll fluorescence measuring light: Modulated excitation at 620 nm

Actinic light: Blue (460 nm) LED lamp for continuous actinic illumination, maximum PAR 700 µmol m^{-2} s^{-1}. Chip-on-board LED array identical to that of DUAL-E measuring head

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

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

9.1.3.9 NADPH/9-AA module

Emitter Head DUAL-ENADPH

NADPH measuring light: 365 nm

Chlorophyll fluorescence measuring light: Modulated excitation 620 nm

Actinic light: Far-red LED lamp: 740 nm. Chip-on-board LED array identical to that of DUAL-E

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 500 g

Detector Head DUAL-DNADPH

Signal detection: Blue-sensitive photomultiplier with filter sandwich transmitting from 420-550 nm

Dimensions: 10 cm x 6.5 cm x 10.5 cm (L x W x H)

Weight: 460 g (incl. cable, 1 m long)

9.2 FIBER Version

9.2.1 Basic System

9.2.1.1 Power-and-Control-Unit DUAL-PAM/F

General design: Microcontroller: 2 x AVR-RISC (8 MHz) + 4 MB SRAM; 256 000 data points with 12 bit resolution can be stored

Measuring light: P700-dual-wavelength-emitter: Sample wavelength 830 nm, reference wavelength 870 nm. Fluorescence emitter: 620 nm

Actinic red light: Far-red LED lamp: 720 nm. Chip-on-board (COB) LED array: 635 nm for continuous actinic illumination, maximum 4000 µmol m^{-2} s^{-1} PAR. Saturating single turnover flashes, maximal 200 000 µmol m^{-2} s^{-1} PAR, adjustable between 5 and 50 µs. Multiple turnover flashes, maximal 20 000 µmol m^{-2} s^{-1} PAR, adjustable between 1 and 1000 ms.

Actinic blue light: Blue LED lamp: 460 nm for continuous actinic illumination, maximal 500 µmol m^{-2} s^{-1} PAR.

Signal detection: PIN photodiode with special pulse preamplifier for measuring P700 and fluorescence changes with maximal time resolution of 10 µs

Communication: PC interface: USB 1.1, 2.0 and 3.0 compatible

User interface: Windows computer with DualPAM software

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

Power consumption: During basic operation 160 mA

Sockets: AUX (for Leaf Clip 2030-B or Cosine-Corrected Mini Quantum Sensor US-MQS/WB), USB (for USB cable), TRIGGER IN (input for 5 V rectangular signals to trigger fast kinetics externally), TRIGGER OUT (output of 5 V rectangular signals to trigger external devices), 2 EXT. SIGNALS (input for external DC signals. Range 0 - 1V or 0 - 5 V), and CHARGE (for MINI-PAM/L charger).

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

Weight: 4.5 kg

9.2.1.2 Special Fiberoptics 2010-F

Design: Flexible, steel-spiral, plastic-covered bundle with three-pin optical connector

Joint end (measuring site): Active diameter 6 mm, outer diameter 8 mm

Length: 100 cm

Weight: 300 g

9.2.1.3 Transport Box Phyto-T

Design: Aluminum box with custom foam packing for DUAL-PAM-100 and accessories

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

Weight: 5 kg

9.2.1.4 Computer Requirements

1 free USB socket; 500 or more MB RAM; operating system: Microsoft Windows 10

9.2.2 Accessories Suspensions

9.2.2.1 Suspension Cuvette KS-2500 and Stirrer MKS-2500

Cuvette: Round stainless-steel cuvette (7.5 mm wide, 9.0 mm deep) with top window adapter for connecting the fiberoptics; embedded in PVC body with injection port for Hamilton syringes and hose nozzles for connecting an external flow-through water bath (not included). Including three 6.0 x 1.5 mm magnetic stir bars

Magnetic stirrer: To drive the magnetic stir bar in the Suspension Cuvette KS-2500; with PVC ring for centering the cuvette and miniature stand to fix the fiberoptics on top of the cuvette

9.2.2.2 DUAL-BA Leaf Adapter

Design: Tube-shaped fiber tip holder composed of polyoxymethylene (POM; 4.5 cm x 2.5 cm, L x D max,) with recessed permanent neodymium magnet. Spring steel band (0.3 mm thick, 1.5 cm wide) consisting of two arms (2 cm and 5 cm, respectively) which form a right angle. The leaf is positioned between the 2 cm arm and the fiber optics tip. The 5 cm arm is attached to the fiber tip holder by the magnet. The 5 cm arm runs in a slit guide. Including a stand with fiberoptics guide

9.2.2.3 2030-B Leaf-Clip Holder

Mini quantum sensor: Magnetically attached, swivel-mounted sensor, selective PAR measurement, 0 to 20000 µmol m^{-2} s^{-1} PAR

Thermocouple: Ni-CrNi, diameter 0.1 mm, -20 to +60 °C

Output: PAR, high sensitivity range: 0 to 1000 µmol m^{-2} s^{-1}; normal sensitivity range: 0 to 20000 µmol m^{-2} s^{-1} (output 0 to 2.5 V for each range). Leaf temperature, -20 to +60 °C (0 to 0.8 V). Remote trigger button, signal line connected to ground

Power supply: AUX socket (5 V/4 mA)

Cable length: 100 cm

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

Weight: 310 g

9.3 Light Sensors

9.3.1 Micro Quantum Sensor US-SQS/WB

Application: Suspension cuvette

Connects to: WATER-PAM, DUAL-PAM-100, ULM-500 (AUX), PAM-2500 and MINI-PAM (instead of Leaf Clip Holder 2030-B or 2060 M)

Connector: Same as Leaf Clip Holder 2030-B Includes hood for suspension cuvette, amplifier for aux input of PAM Control

Signal output: 0…2.5 V DC / 0…1000 µmol m^{-2} s^{-1} or 0…2.5 V DC / 0…20.000 µmol m^{-2} s^{-1} Power provided by connected instrument

Cable length: 3 m + 0.5 m

Size: Sensor: Diameter 1 cm, length: 11 cm; Hood: Diameter 3.4 cm height: 3.2 cm; Amplifier: 5 x 5 x 5 cm (W x L x H)

Weight: 175 g

9.3.2 Cosine Corrected Mini Quantum Sensor US-MQS/WB

Design of sensor: Mini quantum sensor for selective PAR (photosynthetically active radiation) measurement, cosine corrected for PPFD (photosynthetic photon flux density) measurement.

Sensor housing: Black anodized aluminum housing

Diffuser material: Perspex

Signal detection: High stability silicone photovoltaic detector with filter set for PAR correction (to learn more about the typical sensitivity see “General Features”). Signal output typically -2 µA / (1000 µmol m^{-2} s^{-1})

Temperature coefficient of photodiode: 0.01 %/K

Absolute calibration: ± 5 %

Angular dependence: error < 4 % between angles from -80° to +80° from normal axis

Immersion coefficient: Typically 1.32

Operating temperature: - 5 °C … + 45 °C

Cable length: 3 m

Connector: BNC

Power supply: Not required

Size: Height: 16 mm; diameter: 14 mm; Diffuser diameter: 5.5 mm
Weight: 32 g

Subject to change without prior notice

10 Guarantee

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 two (2) years from the shipping date (date on invoice).

10.1 Manufacturer's Guarantee

Under this Manufacturer's Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product's features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product's respective brochure, data sheet, or respective tab on the Manufacturer's website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer's option, and this Guarantee does not include any other rights or remedies.

10.2 Conditions

This Guarantee 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.

- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.

- Damage caused from improper packaging during shipment or any acts of God.

- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps (halogen, LED), thermocouples, and underwater cables.

- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.

- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

10.3 Instructions

- To obtain guarantee service, please follow the instructions below:

- The Walz Service Information Form available at https://www.walz.com/support/repair_service.html must be completed and returned to Heinz Walz GmbH, Germany.

- 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, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.

- All products being returned for guarantee service must be carefully packed and sent freight prepaid.

- 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.

10.4 Applicable law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.

11 Index

12 List of Figures

13 List of Tables