JUNIOR-PAM

Teaching Chlorophyll Fluorometer

Manual

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Contents

1 Safety Instructions

1.1 General Safety Instructions

- Read all the safety instructions and operating instructions thoroughly before using the device for the first time. Keep these safety instructions and operating instructions safe in case you need to refer to them in the future.
- Observe the safety warnings on the device and in the operating instructions. Follow the instructions for operation and use of the device in all respects.
- Connect the device only to the 5 Volt power source of a USB-A socket of a computer using the USB cable supplied with the device. Run the USB cable so that no one can step on it and nothing can rest on or against it.
- Do not install near heat sources such as radiators, hair dryers, stoves, etc. Do not place naked flame sources, such as lighted candles or Bunsen burners, on or near the unit.
- Do not expose this unit to rain or moisture. Do not expose the apparatus to dripping or splashing and do not place objects filled with liquids, such as drinking vessels, beakers or test tubes, on or near the apparatus. Never use the machine near water, for example near a sink.
- Do not expose the device to dust, sand, and dirt.
- Do not allow liquids or foreign objects to enter the unit. The device may only be repaired by the manufacturer.

1.2 Special Safety Instructions

- The JUNIOR-PAM is a sensitive instrument designed for teaching purposes, as described in this manual. Follow the instructions in this manual to avoid potential harm to the user and damage to the instrument.
- The JUNIOR-PAM can emit strong light! To avoid damage to your eyes, never look directly into the light port of the JUNIOR-PAM-II or its fiberoptics.

1.3 Correct Handling of the Fiberoptics

The JUNIOR-PAM fiberoptic consists of a plastic core surrounded by a cladding which has a lower refractive index than the core. Total reflection occurs at the interface between the core and cladding, keeping the light inside the fiberoptics. Do not scratch the surface of the fiberoptics. Scratches will destroy the cladding and cause light loss. To avoid fiberoptics breakage, do not bend the fiberoptics sharply. Broken fiberoptics ends result in significant loss of signal.

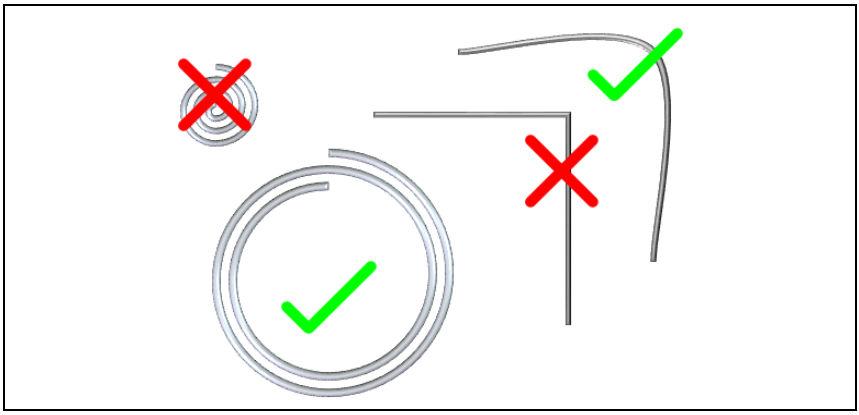


Fig. 1: Fiberoptics Handling

2 Introduction

2.1 Features JUNIOR-PAM

- In the 1990s, the Walz Teaching-PAM fluorometer (PAM-200) brought PAM fluorometry into university classrooms. Since then, there has been considerable progress in LED and PC technology, enabling the development of a powerful and versatile, and yet compact teaching fluorometer: the JUNIOR-PAM.
- The JUNIOR-PAM is manufactured with state-of-the-art optical and electronic components. It is available as version with blue or with red measuring and actinic light. Both versions possess a far-red LED for selective excitation of photosystem I to determine F_0' level fluorescence.
- The fluorometer is controlled by the software WinControl-3, which is also employed by Walz research fluorometers.
- Two different types of leaf-clips are provided. Measurements in ambient light are performed with a 60° leaf clip. A magnetic leaf clip is provided for experiments requiring dark-acclimated samples, that is for measuring of the maximum photochemical yield of photosystem II (F_V/F_M).
- The JUNIOR-PAM uses fiberoptics with an optically active cross section of 1.8 mm^2. Despite this relatively small cross section, the signal is perfect for teaching purposes. For comparison, the optically active cross section of fiberoptics of Walz research PAM fluorometers (MINI-PAM, PAM-2500) is more than 12-fold higher.

- When equipped with the optional accessory Monitoring Leaf-Clip JUNIOR-BD, the JUNIOR-PAM measures photosynthetically active radiation, and can calculate electron transport rates driven by external light sources including sun light.

2.2 Structure of Manual

Prior to working with the JUNIOR-PAM, read the Safety Instructions (Chapter 1, page 1).

The parts delivered with the JUNIOR-PAM and optional accessories are listed in Chapter 3 (page 5) and Chapter 4 (page 11), respectively. How to set up the system describe Chapter 5 (page 15) and Chapter 6 (page 17).

The JUNIOR-PAM can be operated with the simplified Teaching Edition of WinControl-3, or with the Standard Mode of WinControl-3. The Teaching Edition is introduced in Chapter 1 (page 23). This chapter also contains a simple introduction into PAM fluorescence measurements, and three experiments to get to know the instrument. All the functions of WinControl-3 (Standard Mode) are explained in Chapter 1 (page 43), which is followed by Chapter 9 (page 81) which contains some hints on optimizing settings.

The JUNIOR-PAM fluorometer provides a vast range of settings and protocols. To optimize your experiment, become acquainted with terminology and principles of saturation pulse analysis (see Chapter 10, page 85). Section 10.4 (page 93) provides a list of review papers on PAM chlorophyll fluorescence and saturation pulse analysis.

Further, technical information (Chapter 11, page 97) and guaranty conditions (Chapter 12, page 101) are provided.

This manual ends with lists of keywords (Chapter 13, page 105) figures (Chapter 14, page 109) and tables (Chapter 15, page 111).

3 Components

3.1 Extent of Delivery

JUNIOR-PAM or JUNIOR-PAM/W Teaching Chlorophyll Fluorometer (see Section 3.1.1).

Fiberoptics 000 245 100 714 (500 mm x 1.5 mm, L x Ø).

Magnetic Leaf Clip JUNIOR-PAM/MLC.

Open Leaf Clip 60° JUNIOR-PAM/A.

Fluorescence Standard Foil.

USB Cable A Male to B Male (to connect the JUNIOR-PAM fluorometer to a computer).

WinControl-3 Software on USB Key.

Printed JUNIOR-PAM Manual.

Transport Case.

3.1.1 BLUE & WHITE Version

The two versions of the JUNIOR-PAM fluorometer provide visible light of different spectral composition: the BLUE version (JUNIOR-PAM) emits between 400 and 500 nm with a maximum around 445 nm. The WHITE version (JUNIOR-PAM/W) shows a narrow-band blue emission peaking around 445 nm plus a broad band emission ranging from 475 to 650 nm (Fig. 2). Both versions are equipped with a far-red LED with maximal emission around 745 nm and an emission range from 675 to 800 nm.

In addition, the spectral window for fluorescence detection distinguishes the two versions. The BLUE version detects fluorescence at wavelengths > 630 nm and the WHITE version at wavelengths > 650 nm (Fig. 3).

The BLUE version is the standard type of the JUNIOR-PAM and suitable for fluorescence measurements with most eukaryotic algae and higher plants. For samples absorbing poorly in the blue, as can be the case in species of the phylum cyanobacteria, the WHITE version yields significantly better fluorescence signals than the BLUE version.

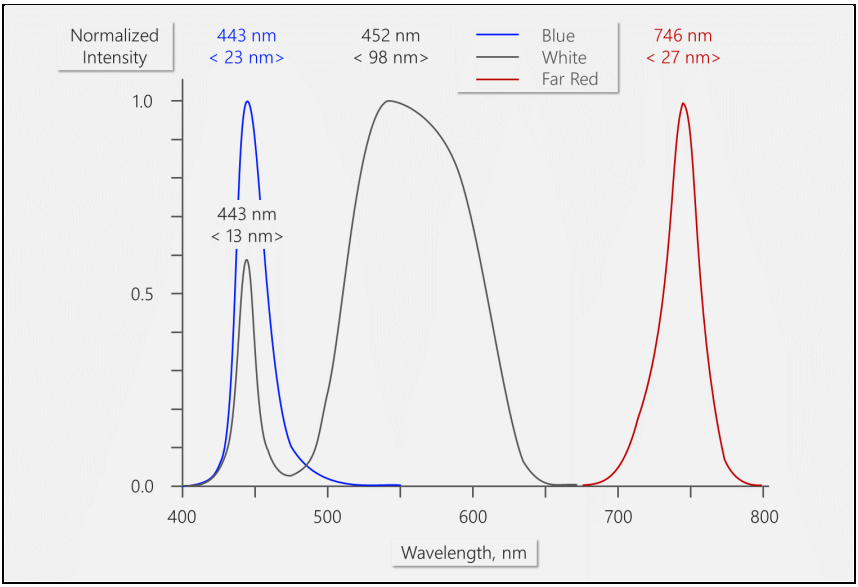


Fig. 2: JUNIOR-PAM LED Spectra

Typical emission spectra of JUNIOR-PAM LEDs normalized to their maximum are shown. Blue line, blue LED of JUNIOR-PAM. Grey line, white LED of JUNIOR-PAM/W. Dark red line, far red LED of JUNIOR-PAM and JUNIOR-PAM/W. For each peak, maximum and full width at half maximum (FWHM, in brackets) is indicated in nm.

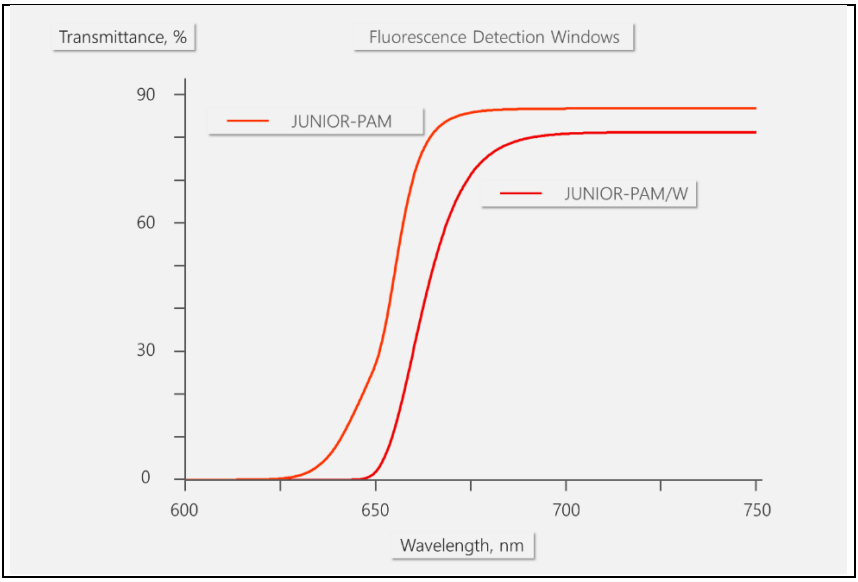


Fig. 3: JUNIOR-PAM Fluorescence Detection Windows

Transmittance spectra of red filters positioned in front of the fluorescence detector. Orange-red curve, JUNIOR-PAM. Red curve, JUNIOR-PAM/W.

3.2 Magnetic Leaf Clip JUNIOR-PAM/MLC

The magnetic leaf clip (Fig. 4) is suited for dark-acclimation of samples prior to measurements of F_0 and F_M fluorescence (for definition see Section 10.2, page 88). The clip consists of two magnets. In case of delicate samples, the magnetic force might be too strong and can squeeze the sample. To avoid damage, the lower magnet can be replaced by a washer. Samples can also be placed on the sample support (Fig. 4) and held by the upper part of the magnetic leaf clip. See Fig. 13, page 28 for comments on PAR and the JUNIOR-PAM/MLC.

Fig. 4: Magnetic Leaf Clip

3.3 Open Leaf Clip 60° JUNIOR-PAM/A

The 60° leaf clip is suited for measurements in ambient light. the sample is held by the spring steel clip. In case of relative thick leaves, or when lichens and mosses are examined, the sample may be placed below the hole of the clip.

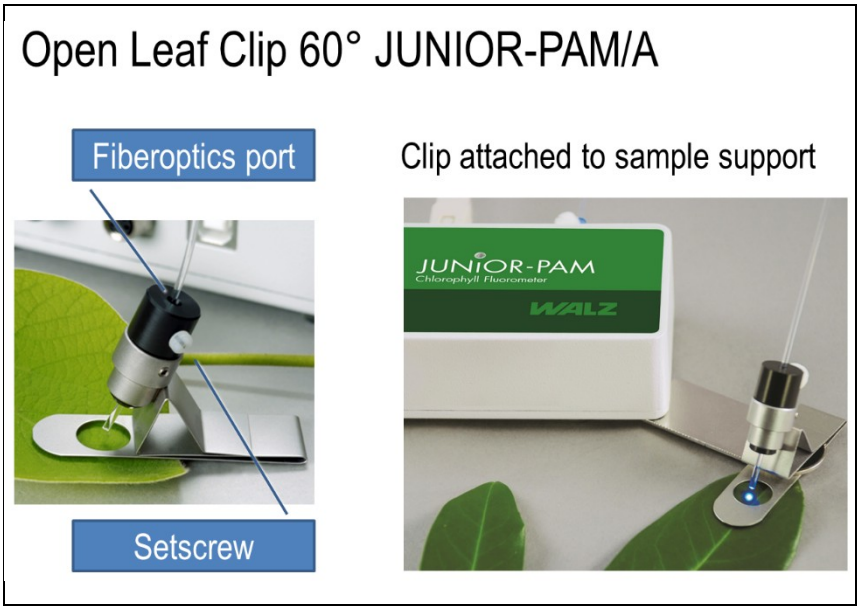


Fig. 5: Open Leaf Clip 60°

3.4 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 (Fig. 6). 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.

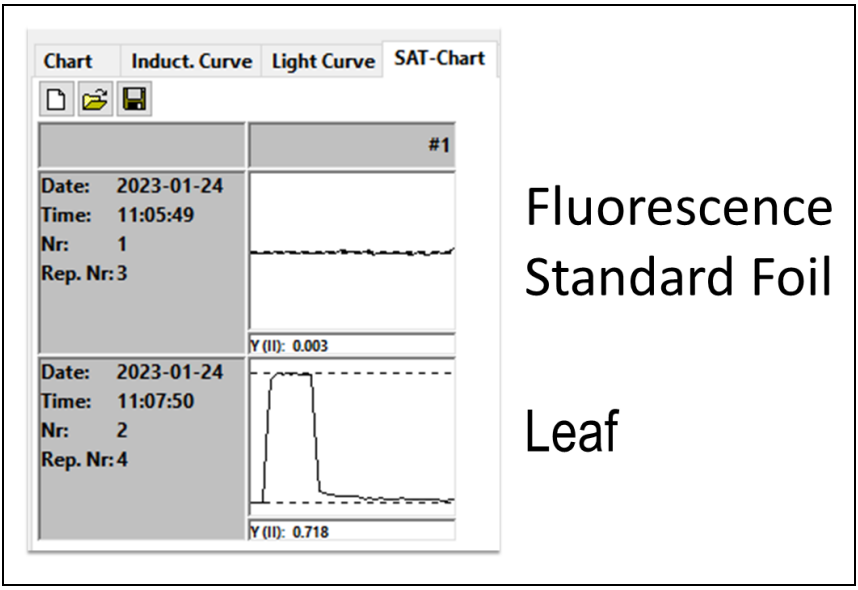


Fig. 6: Fluorescence Standard Foil

Effect of a saturation pulse on fluorescence kinetics. Top, fluorescence standard foil. Bottom, leaf.

4 Accessories

4.1 Monitoring Leaf-Clip JUNIOR-BD

The JUNIOR-BD leaf clip (Fig. 7) adds measurements of ambient light intensity and leaf temperature as complementary information to fluorescence data. The clip holds the leaf at constant distance to the fiber tip and at an angle of 60° between fiber axis and leaf plane.

Photosynthetically active radiation, PAR, in µmol m^{-2} s^{-1} is measured by a micro quantum sensor mounted at sample level. With the parameters PAR and photosystem II yield (Y(II)), electron transport rates (ETR) are automatically calculated by the software as described in Section 10.3 (page 92).

Note that PAR readings are only valid when the diffusing disk of the PAR sensor is uniformly illuminated (e.g., by sunlight). PAR readings are invalid when the diffusing disk is not homogeneously or partially illuminated, as is the case for illumination at narrow distance with the fiberoptics of the JUNIOR-PAM. However, PAR measurements of small light flecks are feasible using the "MQS-B/A Adapter Set for Thin Fiberoptics" plus an MQS-B quantum sensor.

Temperature is recorded by a Ni-CrNi thermocouple which touches the lower leaf surface (Fig. 7). Temperature is an important environmental factor which can favor photosynthesis but also act as a significant stress factor.

Compared to its predecessor (JUNIOR-B), the JUNIOR-BD clip provides advanced digital features: the clip converts primary PAR and temperature signals into digital information and, thus, transfers signals without loss to the JUNIOR-PAM. The calibration data

of PAR and temperature sensors are stored on the clip's flash memory. These calibration data are automatically activated when the JUNIOR-BD leaf clip is connected to the fluorometer (Table 1).

The JUNIOR-BD clip is only compatible with newer types of JUNIOR-PAM fluorometers. The predecessor leaf clip, however, is still available. When reordering a leaf clip, please provide serial number of your JUNIOR-PAM so that we can select the proper accessory for you.

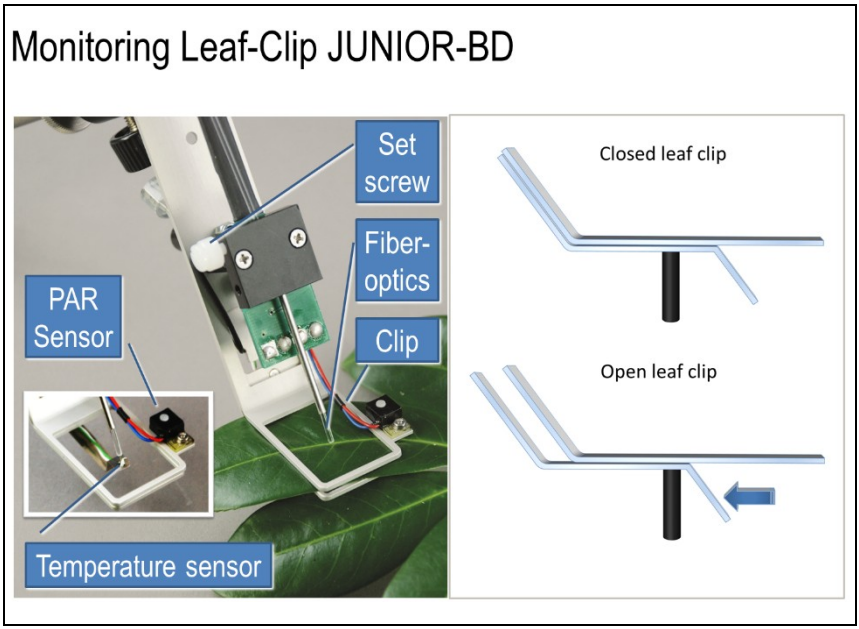


Fig. 7: Monitoring Leaf Clip

Left - PAR and temperature sensors of Monitoring Leaf Clip JUNIOR-BD. Right – To insert a sample, slide the lower metal rail by pressing the bent part against the black rod.

Table 1: Calibration Information.

JUNIOR-BD	**JUNIOR-B**
(Digital version. After April 2015.)	(Analog version. April 2015 and earlier).
Calibration factors are stored on JUNIOR-BD flash memory and automatically activated when the leaf clip is connected to the JUNIOR-PAM.	Enter constants Gain and Ext. Temp. Offset manually.
	Tag on cable
	Light Sensor LS-B S/N: **LSTB0118** Cal. Const.: **5.616** µA per 1000 µmol s^{-1} m^{-2} Cal. Mult.: **-178.06** µmol s^{-1} m^{-2} per µA Gain (WinControl): **178** Ext. Temp. Offset (WinControl): **4.4**

4.2 Extra-long fiberoptics

The fiberoptics with 100 cm length is suited for investigations of samples which are difficult to access, and for submerged samples.

5 Setup

The JUNIOR-PAM fluorometer has three ports (Fig. 8). The two outer ports are used for the basic system. The central port is available for the Monitoring Leaf-Clip JUNIOR-BD.

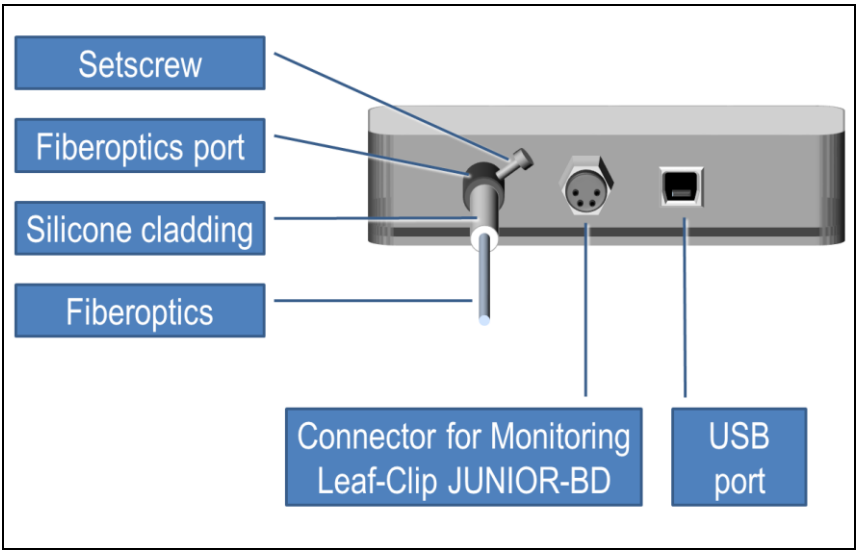


Fig. 8: JUNIOR-PAM Ports

Fiberoptics

Unpack fiberoptics. Carefully insert the fiber end with the silicone sleeve into the fiber port of the JUNIOR-PAM (Fig. 8). The fiber is positioned correctly when silicone sleeve touches the fiber port.

The fiber can encounter resistance when inserted. If this is the case, remove the fiber, carefully straighten the fiber by hand, and insert again. Do not forcible overcome the resistance. Finger-tighten the setscrew of the fiber port.

Connect free fiber end to any of the three leaf clips.

USB Cable

Connect USB cable to JUNIOR-PAM and computer with Windows 10/11 operating systems. Now, the signal LED on top of the JUNIOR-PAM should flash green at a frequency of 1 Hz, and at the end of the fiber, blue measuring light should be visible.

Monitoring Leaf Clip JUNIOR-BD

Align pins of cable plug with holes of connector (compare Fig. 8, page 15). Press cable plug slightly on connector. Screw the plug tight. Slide in fiberoptics until the fluorescence readout of a dark-acclimated sample is between 300 and 600 (compare Table 26, page 82). Fasten setscrew.

Magnetic Leaf Clip JUNIOR-PAM/MLC

Insert the fiberoptics into the fiberoptics port of the of the magnetic leaf clip (Fig. 4). Slide in fiberoptics until its tip is flush with the end of the fiberoptics port. Finger-tighten the setscrew.

The distance between fiber tip and sample level is now 1 mm. The actinic light list of the JUNIOR-PAM is valid for 1 mm distance between fiberoptics tip and sample surface. The light list was established at the factory with the upper part of the Magnetic Leaf Clip placed on the MQS-B PAR sensor.

Open Leaf Clip 60° JUNIOR-PAM/A

Insert fiberoptics in fiberoptics port of the 60° clip (Fig. 5, page 9). Slide in fiberoptics until the fluorescence readout of a dark-acclimated sample is between 300 and 600 (compare Table 26, page 82). Fasten setscrew.

6 WinControl-3 Installation

The WinControl-3 software is provided on a Walz USB flash drive. The WinControl-3 software is regularly improved. The latest software version is available on the Walz website:

https://www.walz.com/products/chl_p700/junior-pam/downloads.html

WinControl-3 can be installed from the Walz USB flash drive or using the setup software downloaded from the Walz website.

6.1 Installation process

Installation of WinControl-3 is mostly automatic. Dialog boxes appearing during setup provide advice or allow configuration of WinControl-3. To install WinControl-3, proceed as follows:

- Close other programs as advised by the setup wizard.
- Execute setup file: double-click on file or right-click on file and choose “run” from context menu.
- A pop-up windows appears which must identify the Heinz Walz GmbH as verified publisher. Allow program to make changes to your computer.
- Accept default folder for program installation or choose different folder after clicking Browse… .
- Select “Standard” Installation or “Standard plus JUNIOR-PAM Teaching Edition”.
- Install USB driver and select optional WinControl-3 links (icon or shortcut). When “Standard plus JUNIOR-PAM Teaching Edition” is installed, an icon for each is version is placed on the desktop.

- If JUNIOR-PAM is connected to the computer, run PAM Firmware Update. If the current firmware* is outdated, PAM Firmware Update will automatically replace it by the recent version. Running PAM Firmware Update after installation of WinControl-3 is important because new software properties may function only in the presence of the latest firmware.

  *The term firmware denotes a piece of software residing on a flash memory of the JUNIOR-PAM. The firmware is included in the WinControl-3 software.

6.2 WinControl-3 Program Group

The setup of WinControl-3 creates the WinControl-3 program group in the Windows Start menu (Table 2). The WinControl-3 program group consists of 6 items. The items are introduced in sections 6.2.1 through 6.2.6.

Table 2: WinControl-3 in Windows Start Menu

WinControl-3
PAM Firmware Update
Uninstall WinControl-3
WinControl-3
WinControl-3 – Network Mode
WinControl-3 – Offline Mode
WinControl-3 – Teaching Edition

6.2.1 PAM Firmware Update

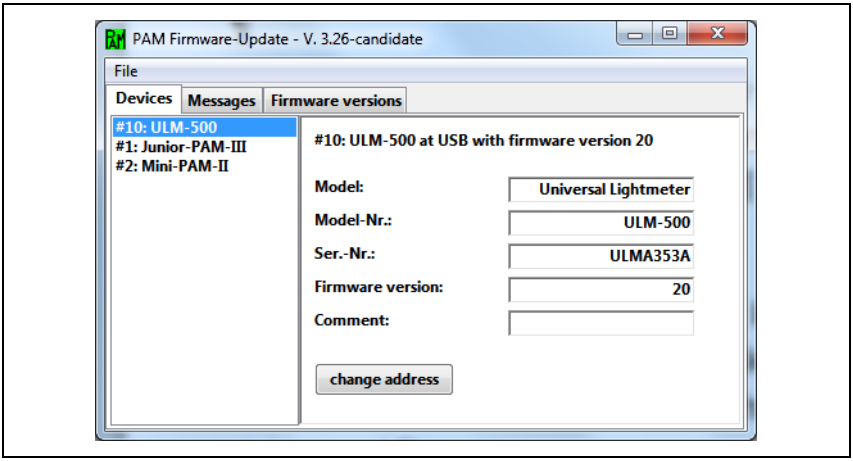


Fig. 9: PAM Firmware Update
Several devices connected to the same computer must have different addresses (channel numbers).

Initialization of PAM Firmware Update triggers a search for PAM devices connected to the computer. The result is displayed in the right panel of window “Devices” (Fig. 9, page 19). Each device name is preceded by its address number (between hash and colon).

“PAM Firmware Update” compares the firmware in the device with the firmware included in the WinControl-3 software. If WinControl-3 includes newer firmware, the device is automatically updated.

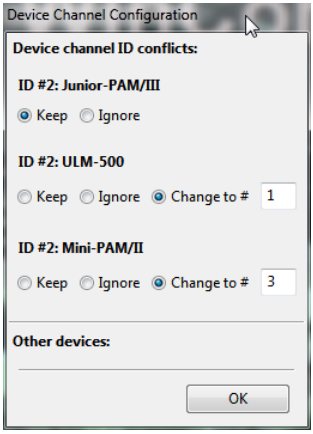
“PAM Firmware Update” cannot update firmware of first-generation devices (DIVING-PAM, MICROFIBER-PAM, MICROSCOPY-PAM, MINI-PAM, WATER-PAM). In these devices, firmware resides on an EPROM chip and firmware update requires exchange of this chip.

A device can be selected by mouse click. The currently selected

device is highlighted (white letters on blue background). The main panel of the window shows information on the device selected. The first four information lines define the hardware and software state of the device. The last line displays a comment associated with the device and entered in the WinControl-3 software.

Devices with identical addresses cannot be operated simultaneously. If WinControl-3 detects identical addresses, the window “Device Channel Configuration” (Fig. 10, page 20) pops up, offering a working address configuration and the option to change addresses manually. Note that address number is synonymous to channel number in the software WinControl-3.

Fig. 10: Device Channel Configuration



Address numbers can be changed manually via the button change address. Then, determine new address by picking a number from the drop-down list “New address:”. If several devices are connected, the drop-down list offers only unused address numbers. The window “Messages” displays the protocol of activities including firmware update of devices. The window “Firmware Versions” compiles all software version provided by PAM Firmware Update.

6.2.2 Uninstall WinControl-3

This program removes WinControl-3 and all its links. It does not remove the USB driver software.

6.2.3 WinControl-3

This command starts WinControl-3 in the default mode. When devices which are compatible with the WinControl-3-type software are detected, WinControl-3 enters the measure mode. Clicking Offline-Mode interrupts the search process and WinControl-3 starts in the offline mode. When the search process fails to find compatible devices, a pop-up window provides three options: Yes triggers another search for devices, No launches the offline mode of WinControl-3, and Cancel ends the whole process. Measuring mode plus several instances of WinControl-3 in the offline mode, can run in parallel.

After detection of the JUNIOR-PAM-II, measuring of fluorescence starts. With a green leaf in the Magnetic Leaf Clip JUNIOR-PAM/MLC (Section 3.2, page 7), fluorescence values of 400 to 600 should be observed (Ft value, see right panel of Teaching Version and bottom of window of Standard version). The Teaching Version displays the Ft on the chart right from the start. In the Standard version, Check “Rec. Online” or click Start Online to display continuously the Ft. If the Ft is much lower than 400, make sure that the fiberoptics is properly inserted. Click Autoscale if data are not visible. Trigger saturation pulse analyses by pressing Fo, Fm or SAT. A healthy leaf, which was kept dark before, should show a value for F_V/F_M of 0.8 or higher.

6.2.4 WinControl-3 Network Mode

This mode allows connecting WinControl-3 compatible devices to an Ethernet network. Does not apply for the JUNIOR-PAM.

6.2.5 WinControl-3 Offline Mode

This command launches Wincontrol-3 without the initial search for available PAM devices.

6.2.6 WinControl-3 Teaching Edition

The Teaching Edition is designed for beginners in the field of PAM fluorescence and saturation pulse analysis. The user interface of the Teaching Edition exhibits reduced options for device configuration and a limited number of saturation pulse parameters. You can easily switch to the full version of WinControl-3 by clicking the icon Advanced.

7 Teaching Edition WinControl-3

The Teaching Edition is designed to explore photosynthesis through simple experiments. Compared to the standard mode of WinControl-3, the options to modify fluorometer settings are reduced. Also, the range of data displayed is reduced.

Fig. 11: User Interface Teaching

7.1 User Interface

The data measured by the JUNIOR-PAM fluorometer is displayed graphically on the Chart and numerically in the Results section (Fig. 11, page 23). Further elements of the user interface are a group of buttons for data handling and changing chart properties, plus the Status bar on the bottom of the window.

7.1.1.1 Buttons

The function of all buttons on top of the user interface are compiled and explained in Table 3.

Table 3: Buttons

Icon	Meaning	Comment
	Delete	Deletes all current data.
	Load	Same function as “Load Data” in the File above the row of buttons.
	Save	Same function as “Save” in the File menu above the row of buttons.
	Print	Prints current chart view.
	Grid on/off	Controls display of chart grid.
	Lines	Connects data points with lines.
	Symbols	Controls the display of symbols.
Options		See Table 13.
Or right-click on chart.		

7.1.2 Chart

Right after start of the Teaching Edition, the currently measured fluorescence intensity (Ft) is measured and plotted as continuous line on the chart. The initial signal level should be around 500. If the signal is much lower, decrease the distance between fiber and sample.

Pressing Fo, Fm starts a new Record and minimum and maximum fluorescence levels (F_0 and F_M, respectively) are measured. Both parameters are shown as horizontal dashed lines (Fig. 16, page 38). All other data are represented by symbols differing in shape and color (see Table 4). The data to be displayed is selected on the bar to the right of the chart.

Table 4: Parameters Displayed on Chart

Parameter and representation		Condition	Signal Type
Ft	——	For all lighting conditions	PAM fluorescence, continuously recorded
F_0	- - -	Pre-darkened	Fluorescence intensity used for saturation pulse analysis
F_m	- - -	Pre-darkened + sat. pulse	
F	**+**	Light, prior to saturation pulse	
F_m'	**x**	Light + Saturation Pulse	
Y(II)	Y	Light + Saturation Pulse (Same symbol for $F_V/F_M=(F_M-F_0)/F_M$)	Fluorescence ratio
Y(NO)	□	Light + Saturation Pulse	Fluorescence ratio
Y(NPQ)	■	Light + Saturation Pulse	Fluorescence ratio
ETR	●	Light + Saturation Pulse	Result of ETR equation

Fig. 12 (page 26) outlines the tools to adjust the chart. The scale of y- and x-axis can be manually changed: a vertical pair of arrows affects the y axis, and a horizontal pair of arrows adjusts the x-axis (see lower border of Fig. 12). y-axis scaling affects only the active y-axis unit. The active y-axis unit can be picked from a drop-down menu located in the upper left corner of the chart. The active y-axis unit also determines the y-data of the cursor position on the chart.

Right of the horizontal pair of arrows is the Autoscale button which fits all data of the current record into the chart area. To zoom into a particular x-axis interval, mark the x-interval of interest (see Fig. 12), open the context menu by right-click on chart, and select the command “Zoom to Selection”.

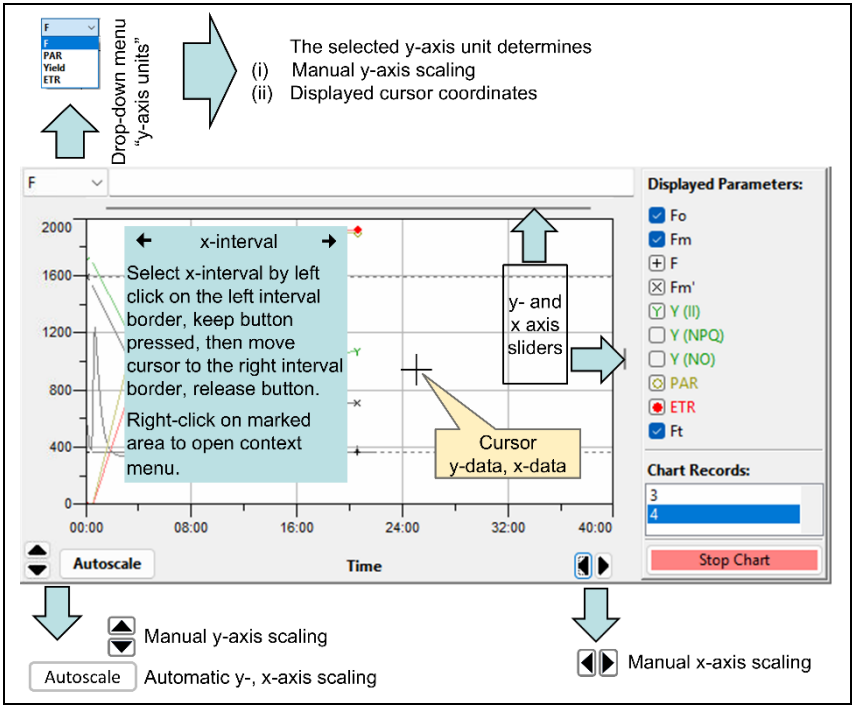


Fig. 12: Chart Tools

7.1.3 Current Data

The Current Data panel lists the numerical data of the experiment (Table 6). Data that must be measured with a fully dark-acclimated sample are printed on blue background. Data of the light-exposes sample are printed on green background. In the default mode of the Teaching version, PAR (photosynthetically active radiation) is taken from the light list established in the factory, even if a Monitoring Leaf-Clip JUNIOR-BD is connected.

7.1.3.1 PAR

The actinic light intensity (Table 5, photosynthetically active radiation, PAR) is established at the factory. The PAR values are valid when the fiberoptics directs vertically to the sample surface and the distance between fiber and sample is 1 mm.

The JUNIOR-PAM/MLC Magnet Leaf Clip is designed for illumination at 1 mm distance. Simply insert fiberoptics until flush with the inner surface of the upper clip half, and place sample between upper and lower clip half (Fig. 13, page 28).

Table 5: PAR Values

Factory PAR values valid for 1 mm distance between fiber end and sample level.

Setting	PAR, µmol m^{-2} s^{-1}	Setting	PAR, µmol m^{-2} s^{-1}
1	25	7	285
2	45	8	420
3	65	9	625
4	90	10	820
5	125	11	1150
6	190	12	1500

The factory PAR list ranges from 25 to 1500 µmol m^{-2} s^{-1}. For comparison, the midday PAR in equatorial regions is about 2000 µmol m^{-2} s^{-1}. In the factory PAR list, the increase in PAR is greater when switching from level 2 to 3 compared to switching from level 1 to 2. This trend continues throughout the entire list. Smaller increments at low PAR values and higher increments at higher PAR values are favorable for light curve experiments.

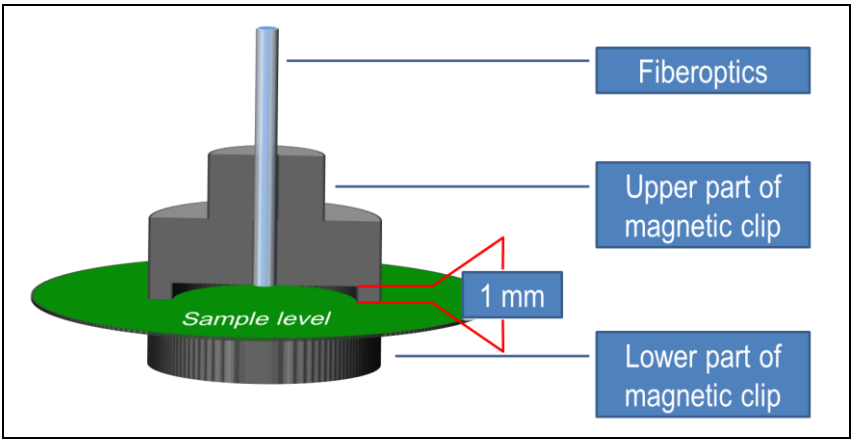


Fig. 13: Fiberoptics and Magnetic Leaf Clip

One mm distance between fiberoptics and sample is achieved when the fiberoptics is flush with the surface of the upper part of the magnetic leaf clip. For 1 mm distance, the factory PAR list is valid.

7.1.3.2 Fluorescence

Fluorescence data fall into two categories: fluorescence intensities and ratios of fluorescence intensities. The latter are represented by F_V/F_M, and three additional quantum yield parameters (Y(II), Y(NPQ), and Y(NO), see Table 6). How to calculate these four parameters is described in Table 27 (page 94).

Table 6: Current Data

Parameter	Sample Data	Comment
Fo	441	Minimum and maximum fluorescence of the dark-acclimated sample.
Fm	1818	
Fv/Fm	0.757	Maximum photochemical quantum yield of Photosystem II.
F	506	F, Ft shortly before determination of maximum fluorescence (Fm') of the light-exposed sample
Fm'	566	
Y(II)	0.106	Quantum yields of Photosystem II of the light-exposed sample. Y(II), Effective photochemical quantum yield of Photosystem II.
Y(NPQ)	0.616	
Y(NO)	0.278	
PAR	285	Actinic light intensity, µmol photons m^{-2} s^{-1}.
ETR	12.7	Electron transport rate, µmol electrons m^{-2} s^{-1}.
Online		
Ft	482	Current fluorescence.

To calculate the four fluorescence ratios, only four fluorescence measurements are needed: F_0, F_M, F, and F'_M. The results of fluorescence measurements with the JUNIOR-PAM are dimensionless. Fig. 14 (page 30) illustrates the four different states of Photosystem II associated with the four fluorescence measurements.

The intensity of the measuring light is constant in all 4 of these states. The absorption of measuring light produces excitation energy in Photosystem II. Hence, the formation of excitation energy by measuring light is also constant for all four states.

ML	Measuring light (consisting of a train of µs pulses)
PS II	Photosystem II
P	Use of excitation energy for photosynthesis
H	Dissipating excitation energy as heat
F	Dissipating excitation energy as fluorescence
SAT	Strong light pulse of several hundreds of ms
AL	Actinic light driving photosynthesis

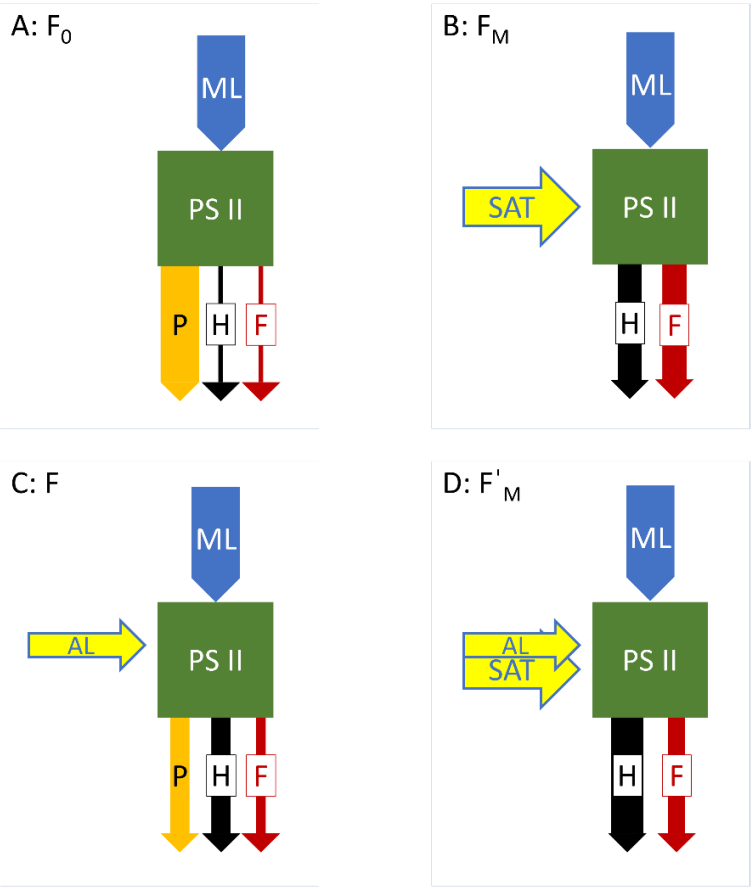


Fig. 14: Fluorescence Levels and Saturation Pulse Analysis

Eventually, the measuring light-induced excitation energy leaves Photosystem II. Three outward routes are considered here: (1) conversion to chemical energy by the reaction center, (2) dissipation as heat, or (3) fluorescence emission. The sum of the three outward routes must be constant because the energy influx by measuring light is constant. Therefore, variations in the measured intensity of fluorescence (route 3) contain information about variations in the energy flow through routes (1) and (2).

The F_0 state. In a dark-acclimated sample, and in the presence of very weak measuring light, virtually all reaction centers are eager to catch excitation energy to perform chemistry. That means that the route to the reaction center is dominating, and, consequently, heat dissipation and fluorescence intensity are low (Fig. 14A).

The F_M state. The state is reached by exposing the dark-acclimated sample to a strong light pulse. In the presence of the strong light, all reaction centers become engaged in the conversion of excitation energy to chemical energy and cannot uptake further excitation energy. This virtually eliminates the pathway to the reaction center, which in turn increases the fluorescence to F_M level (Fig. 14B).

Be aware that here and in the cases below, the intense fluorescence excited by the strong light pulse is not discussed because it is not measured by the PAM fluorometer. This also applies to fluorescence induced by actinic light.

The F state. The F level fluorescence is the fluorescence intensity measured when the sample is exposed to light intensities driving photosynthesis at noticeable rate (actinic light). Compared to the F_0 state, the route of excitation energy to the reaction center is narrower, which would raise the fluorescence level. At the same time, heat dissipation gains importance, which would lower the fluorescence level.

Often, the decrease in fluorescence due to heat dissipation does not compensate for the increase in fluorescence due to reduced energy flux to the reaction center. Therefore, F fluorescence is greater than F_0 fluorescence, in most cases (Fig. 14C).

The F'_M state.

Similarly, as for F_M, a strong light pulse converts the F to the F_M state. Closing the route for excitation energy to the reaction center increases fluorescence. Because heat dissipation is more prominent than in the dark state, F_M' is smaller than F_M (Fig. 14D).

7.1.3.3 Yield (Fluorescence Ratios) and ETR

F_V/F_M and Y(II)

Both fluorescence ratios are photochemical quantum yields of Photosystem II. In other words, each parameter indicates the fraction of photons that are converted into chemical energy relative to all photons absorbed by Photosystem II. For example, when 100 absorbed photons cause 50 charge separations in the reaction center, the photochemical quantum yield is 0.5.

The F_V/F_M is the maximum photochemical quantum yield of photosystem II and must be measured with a fully dark-acclimated sample. Dark-acclimation times are often between 20 and 120 minutes. Normally, the F_V/F_M is not higher than 0.835. Values below 0.75 may indicate damage to Photosystem II or incomplete dark-acclimation.

The Y(II) (also Φ_{II}) is the effective photochemical which is lower than F_V/F_M. A low value for Y(II) does not indicate that the rate of photosynthesis is low. Simple calculations demonstrate that the photosynthetic electron transport rate (ETR) can increase although Y(II) decreases (Table 7).

The ETR is the product of Y(II) times PAR, multiplied by two constants. Constant #1 equals 0.84 and corresponds to the number of absorbed photons relative to the number of photons arriving on the sample. Constant #2 equals 0.5 and means that absorbed photons are equally distributed between Photosystem I and Photosystem II. Clearly, low efficiency in using light for chemistry in combination with high light intensity results in a strong ETR. Conversely, a high efficiency of using light for chemistry will not result in a high ETR if light is scarce.

Table 7: Electron Transport Rate

Theoretical values assuming a decrease of Y(II) by 80%.

$$ETR = Y(II) \cdot PAR \cdot 0.84 \cdot 0.5$$

ETR $\frac{\mu mol\ e^-}{m^2 \cdot s}$	Y(II)	PAR $\frac{\mu mol\ photons}{m^2 \cdot s}$	Fraction of photons absorbed	Fraction of absorbed photons directed to Photosystem II
21	0.5	100	0.84	0.5
42	0.1	1000	0.84	0.5

Y(II), Y(NPQ), and Y(NO)

The yields Y(NPQ) and Y(NO) are analogously defined as Y(II) above. The three yields add up to one:

Y(II) + Y(NPQ) + Y(NO) = 1

The NPQ in Y(NPQ) stands for “non-photochemical quenching”. The term includes mechanisms for safely deactivating excitation energy when it is present in excess. Excess excitation energy can have toxic effects on Photosystem II.

The NO in Y(NO) stands for non-organized. The term non-organized indicates that the Y(NO) relates to energy leakages which are not as controlled and regulated as safe energy dissipation via NPQ pathways.

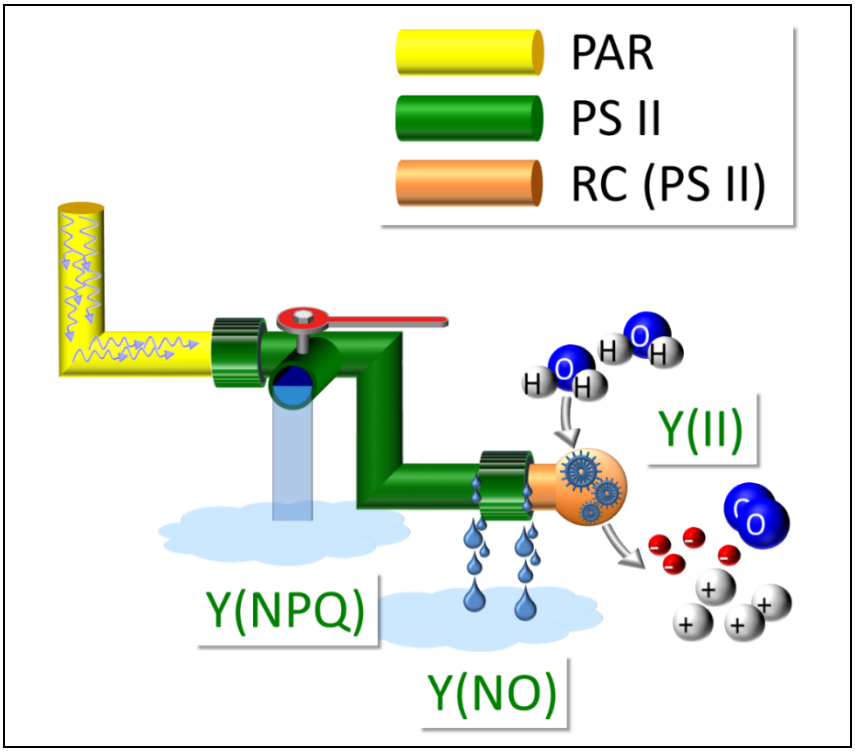


Fig. 15: Y(II), Y(NPQ), and Y(NO): The Plumber's Model

The plumber's model for Photosystem II is suitable for illustrating Y(NO) (Fig. 15). The model represents Photosystem II as green-colored water pipe with pipe connectors. The reaction center is positioned at one end of the pipe. One of the pipe connectors is leaky. Similarly, Photosystem II is "leaky", that is, it loses excitation energy even under F_0 conditions (see above). If it did not lose excitation energy, the F_V/F_M would be one and F_0 fluorescence would be zero.

In the plumber's view, the amount of loss through the leak automatically increases with "water pressure". In reality, the degree of a surplus in excitation energy mounts when energy input by light absorption exceeds energy outflow through the Y(II), Y(NPQ), and Y(NO) pathways.

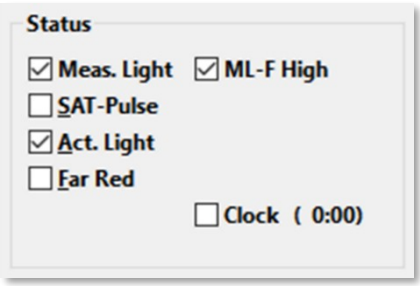
Typically, right after the transition from dark to light, the Y(II) drops because the reaction center is not yet running smoothly, and the Y(NPQ) is still small. Hence, the Y(NO) pathway is left for energy outflow. Therefore, high Y(NO) values are usually observed at the beginning of fluorescence induction experiments. Samples with persistently high Y(NO) during the rest of the experiment suffer from light stress.

7.1.4 Status

All properties of the status bar are compiled in Table 8.

Table 8: Status Bar

Status Panel



☑, ☐ Checkboxes:

Checkboxes have a dual function. They act as On/Off switches, and they indicate the active state of a function.

Meas. Light:

☑ indicates that PAM measuring light of low frequency is on.

ML-F High: ☑ indicates that measuring light is given at high frequency, when measuring light is on. Two cases must be distinguished. Case 1: ML-F High is not selected. In this case, the measuring light automatically changes to high frequency when the actinic light is switched on. Case 2. ML-F High is selected. In this case the measuring light is always at high frequency.

Note that for proper measurements of the minimum fluorescence (F_0), ML-F High must not be selected.

SAT-Pulse: Saturation pulse analysis to determine Y(II).

Act. Light: Actinic light to drive photosynthesis.

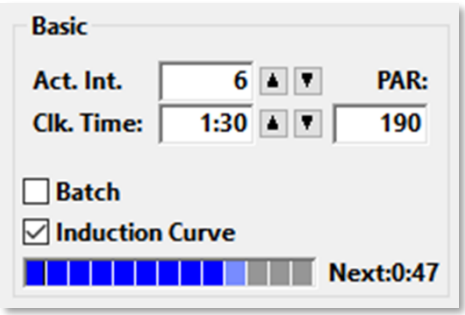
Far-red: Light at wavelengths > 700 nm.

Clock: Repetitive trigger for saturation pulses.

Continued next page.

Table 8: Status Bar

Basic Panel



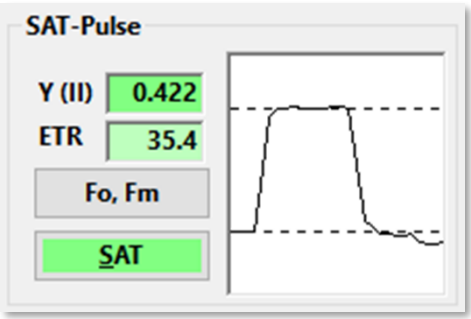
Act. Int. and **PAR:** Setting and intensity (in µmol m^{-2} s^{-1}), respectively, of light driving photosynthesis (actinic light).

Clk. Time: Time interval (minute:second) between automatically triggered (clock-triggered) events.

Batch: Execution of batch file program.

Induct. Curve: F_0, F_M determination and repeated saturation pulse analysis in a subsequent illumination period.

SAT-Pulse Panel



Y(II): Effective photochemical quantum yield of Photosystem II, or maximum quantum yield of Photosystem II when Fo, Fm was pressed.

ETR: Electron Transport Rate

Fo, Fm: Saturation pulse analysis to determine F_0, F_M, and F_V/F_M.

SAT: Saturation pulse analysis to determine F, F'_M, and Y(II).

7.2 Simple Experiments

This section introduces three experiments demonstrating how to get information about photosynthesis by saturation pulse analysis. More experiments are described in the Walz PAM Tutorial "Chlorophyll Fluorescence and Photosynthesis: Simple Experiments with the JUNIOR-PAM Chlorophyll Fluorometer", which can be downloaded from the Walz website:

https://www.walz.com/products/chl_p700/junior-pam/downloads.html

7.2.1 Getting Acquainted

Preparations:

Take a leathery leaf from a garden plant, e.g., ivy, holly, or cherry laurel. Leathery leaves can be used detached as they do not wilt easily. Garden plants are better than indoor plants which often show odd behavior.

Keep in darkness for at least 20 minutes.

Set up JUNIOR-PAM as described in Chapter 5 (page 15). Connect fiberoptics to magnetic leaf clip (see Fig. 13).

Experiment:

Place sample in magnetic leaf clip. If dark acclimation was not done in the magnetic leaf clip, transfer leaf to clip in dim light. The Ft should not show a trend.

Press Fo, Fm to start a new chart and to measure F_V/F_M. The effect of the saturation pulse is shown in several ways (Fig. 16A, page 38). On the chart, the Ft trace rises and decays in the dark period. Also, horizontal lines show F_0 and F_M. Further, the fluorescence kinetics is shown on a separate graph with increased time resolution.

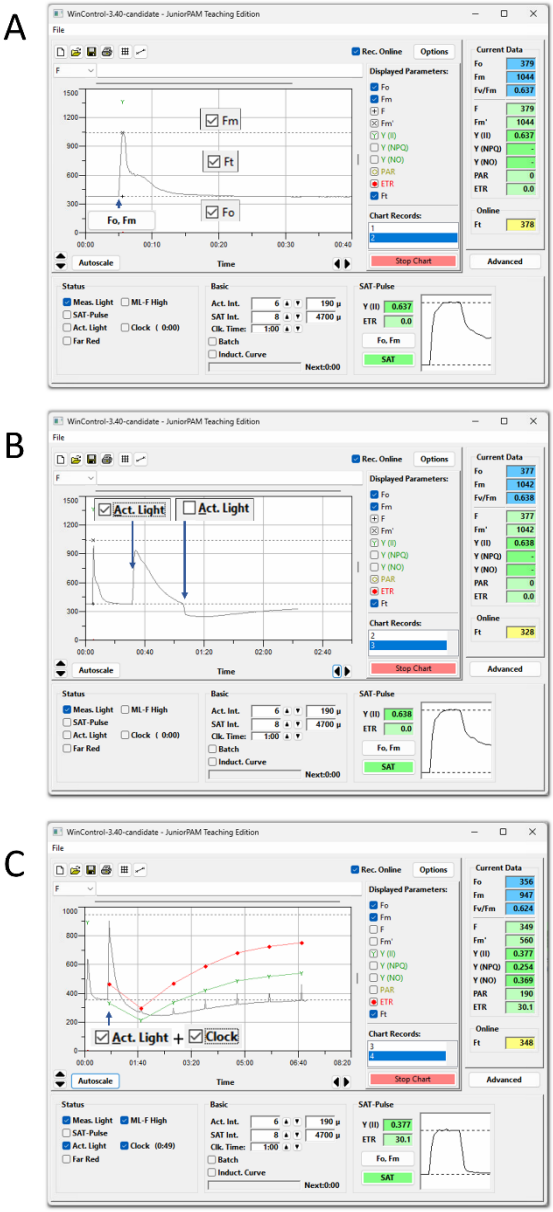


Fig. 16: Getting Acquainted

On the results sidebar, F_0, F_M, and F_V/F_M are numerically displayed. Since Y(II) was not measured yet, the values for F_0, F_M, and F_V/F_M are also displayed in the fields for F, F'_M, and Y(II), respectively.

Switch on actinic light for a minute. In the light, Ft first rises and then falls, suggesting the onset of photochemistry and later the development of non-photochemical quenching (NPQ) (Fig. 16B, page 38).

Switch off actinic light. Wait until Ft has reached the initial level. Set clock time to 1 min. Switch on actinic light and then the clock. Now the fields for F, F'_M, and Y(II) display the data of the current saturation pulse analysis. You can follow the fluorescence levels and yield values with a time resolution of 1 minute (Fig. 16C, page 38).

7.2.2 Induction Curve

Preparations:

See Section 7.2.1, page 37.

Experiment:

Set clock time to 30 s. Start routine “Induction Curve”. Typically, the fluorescence F and F'_M show peak values right after onset of actinic light. Then, F and F'_M reach a valley about 1 minute after light onset. At this time, the variable fluorescence induced by saturation pulses also reaches a minimum. Eventually, fluorescence levels and variable fluorescence arrive at values intermediate between the initial measurements (Fig. 17A, page 40).

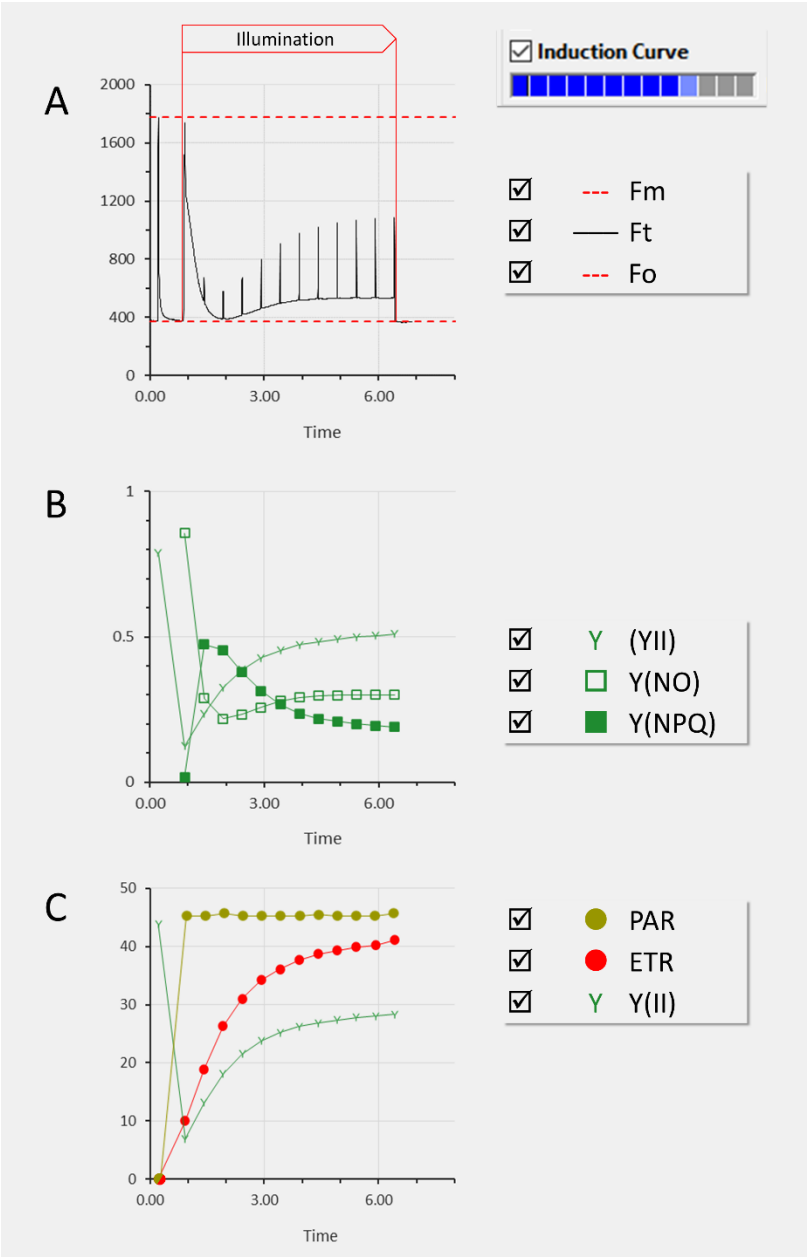


Fig. 17: Induction Curve

Frequently, the initial peak is explained by incomplete activation of primary photosynthetic reactions. In fact, the Y(II) is low at the beginning of illumination (Fig. 17B). The situation can be compared to the F_M case in Fig. 14 (page 30).

The trough is related to full functioning of primary photosynthetic reactions but incomplete activation of secondary photosynthetic reactions (CO_2 fixation). In this condition, a backlog of products of primary photosynthetic reactions (ATP, NADPH) curbs energy-processing by the reaction center and promotes non-photochemical quenching. The Y(NPQ) peaks in this region (Fig. 17B).

The final recovery phase is attributed to fully functioning of both primary and secondary photosynthetic reactions. After the activation phase and under favorable conditions, a significant part of the excitation energy is used for photochemistry and non-photochemical quenching shows moderate values (Fig. 17B).

7.2.3 Lichen Resurrection

A number of photosynthetic organisms can dry out completely, and regain full photosynthetic function when watered. Survival of complete dryness is also a property of lichens, which consist of a photosynthesizing part (green alga or cyanobacterium) and a fungus.

Preparations:

Collect lichen from the outside. Make sure that the lichen is completely dry, or let dry out indoors.

Depending on shape, place lichen in the Magnetic Leaf Clip or the Open Leaf Clip 60°. Dark-acclimation is not needed. To probe lichens growing on stone, use adhesive tape to attach the Open Leaf Clip 60° to the stone.

Experiment:

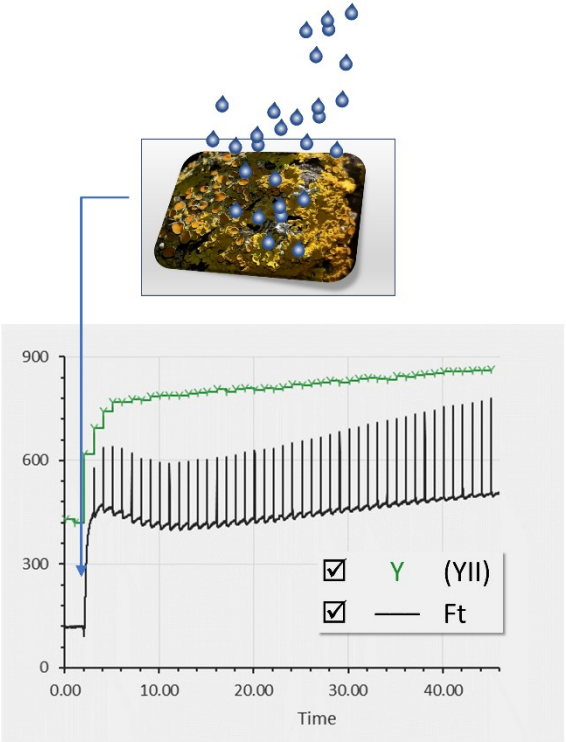
Set clock time to 1 minute and switch on clock. Saturation pulses do not induce variable fluorescence indicating that the reaction center of Photosystem II is inactive.

Add some water (tap or deionized). Within seconds of wetting, the fluorescence increases, suggesting the disappearance of a strong energy quenching factor (Fig. 18, page 42).

Already after 1 minute of wettings, variable fluorescence appears, indicating that the reaction center became activated. Still discussed are the mechanisms capable of setting Photosystem II in a resting state in which it is protected from photodestruction by a strong energy quencher.

Fig. 18: Resurrection of Lichen

Compare, e.g., Veerman J, Vasil'ev S, Paton GD, Ramanauskas J, Bruce D (2007) Photoprotection in the Lichen *Parmelia sulcata*: The origins of desiccation-induced fluorescence quenching. Plant Physiol 145: 997–1005, https://doi.org/10.1104/pp.107.106872



8 WinControl-3 Standard Mode

The WinControl-3 user interface consists of 7 Windows (Table 9). At software start, the Chart window appears. The additional window “Moni-Bus” shows up when more than one device is connected to the computer.

Table 9: Windows of WinControl-3

	Window	Availability	Main panel	Content
1	Chart	Online and Offline	Graphics	Data versus time of all experiments
2	Induct. Curve	Online and Offline*	Graphics	Data versus time of fluorescence induction curves
3	Light Curve	Online and Offline*	Graphics	Data versus time of light response curves
4	SAT-Chart	Online and Offline*	Graphics	Saturation pulse kinetics
5	Report	Online and Offline	Alphanumeric	Saturation pulse data and data collected at the same time
6	Batch	Online and Offline	Commands	Site for automatic execution of experiments
7	Settings	Online only	Settings	Device settings

* When data loaded

8.1 Chart Window

Fig. 19 divides the Chart window into six areas which are explained in Sections 8.1.1 to 8.1.6, respectively. In the manual's electronic form, click on the section numbers in Fig. 19 to go to the appropriate section in the text.

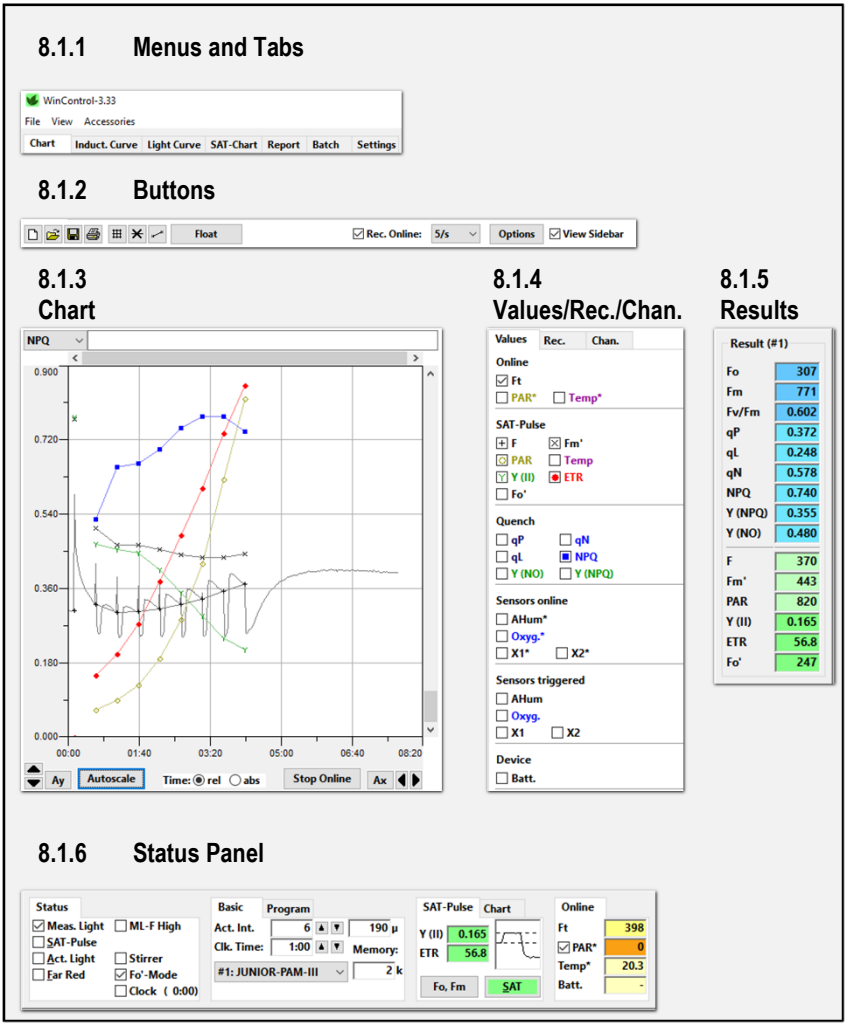


Fig. 19: Chart Window

8.1.1 Menus and Tabs

The top bar includes three menus (File, View, and Accessories), and all seven window tabs (compare Table 9, page 43). The three menus are explained in Table 10.

Table 10: Menu Overview

Menu	Item	Comment
File	Load Data	Opens WinControl-3 files. Save current data first and switch off online recording of data.
	Save Data	Saves present data in WinControl-3 format.
	Save Settings	Saves all current instrument settings in a batch file. The settings can be restored by executing this batch file.
	Export Report	Exports the data displayed on Report window. For information on configuration of export data see Table 11 (page 46).
	Export Chart Record	Exports the data displayed on Chart.
	Quit	Exit WinControl-3.
View	Results Panel	Switches Results panel (Section 8.1.3) on or off.
	Status Panel	Switches Status panel (Section 8.1.6) on or off.
	Warnings	Prompts the display of the 'program starting time' and non-critical s.
	Batch Window	Switches Batch File window on or off.
	Control Panel	Does not apply to JUNIOR-PAM.
	Sensor Panel	Does not apply to JUNIOR-PAM.
Accessories	Temperature Units	Toggles between °Celsius and °Fahrenheit. The temperature unit affects only the numerical display on the Status Panel (8.1.6, p.54).
	Plugins	Does not apply to JUNIOR-PAM.
	Record File	Saves data continuously to reduce data loss in case of program failure. The command prompts for a folder in which the file should be saved. The file name is created automatically using data and time of record start: WinControl-Record-YYYY-MM-DD-hh_mm_ss.pam.

Table 11: Export Format for Reports

Parameter (compare Fig. 20)	Action when checked
☑ Tabular measurement data	Exports data of saturation pulse analysis and all other data recorded at the same time.
☑ Headers	Exports column headers.
☑ Comments and metadata	Exports user and software comments (=”metadata”, e.g., device information).
☑ Regression data	Exports the cardinal numbers of light curves.
☑ Start/end marks	
⦿ Separate Lines	Writes start and end of an experiment in separate lines.
○ Marking Column	Writes start and end of an experiment in a separate column.
☑ Datetime format	Exports date and time. Format: day/month/year hours:minutes:seconds.ms. You may need to custom-format the datetime cell by entering dd/mm/yyyy hh:mm:ss.000 in the Type line.
☑ Timestamp	Export UTC time in ms (Timestamp 0 corresponds to 1970/01/01 00:00:00).
☑ Relative time in ms	Export time of experiment in ms.
☑ Remove “~” marks	Remove tilde (swung dash) signs. WinControl-3 marks calculated F'_0 values by a tilde.
⦿ File (semicolon separated)	Creates a file in which individual data are separated by semicolons.
○ Clipboard (tab separated)	Copies the data to the clipboard. Data are separated by tab characters. Use the paste command to copy the data into a spread sheet program.

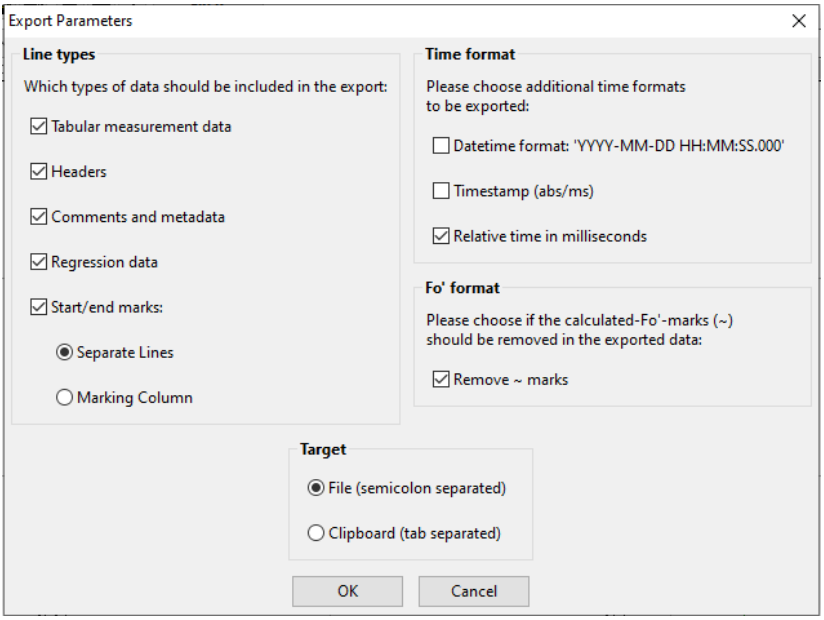


Fig. 20: Export Format for Reports

8.1.2 Buttons

The elements of the buttons bar are introduced in Table 12 and Table 13.

Table 12: Buttons

Icon	Meaning	Comment
	Delete	Deletes all current data.
	Load	Same function as “Load Data” in the File menu (Section 8.1.1.).
	Save	Same function as “Save” in the File menu (Section 8.1.1.).
	Print	Prints current chart view.
	Grid on/off	Controls display of chart grid.
	Lines	Connects data points with lines.
	Symbols	Controls the display of symbols.
Float	Add Chart	Creates an additional chart window with separate view settings.
☑ Rec. Online	Continuous recording	Controls continuous recording of fluorescence, PAR, and temperature.
5/s ▼	Sampling frequency	Sets sampling frequency for online data. Open drop-down menu by the downward arrow. Available sampling frequencies are 5/s, 1/s, and 1/10s. 5/s is available only for fluorescence.
Options		See Table 13.
Or right-click on chart.		
☑ View Sidebar		Controls display of side bar (see 8.1.4, Values/Rec./Chan.)

Table 13: Options

Menu Item	Present	Comment
Export Record	Always	Exports the data displayed.
Select current record	Always	Highlights data of a Record on the chart, and also on windows SAT-chart and Report.
Split Selection	After selection of interval.*	Put selection in separate record.
Zoom to Selection	After selection of interval.*	Displays the interval enlarged.
Export Selection	After selection of interval.*	Exports the data selected.

* How to select an interval on the chart: keep the left-hand mouse button pressed, move cursor across x-interval and release.

8.1.3 Chart

Fig. 21 outlines the tools to adjust the chart. Section 8.1.4 explains how to select data for display.

The scale of y- and x-axis can be manually changed: a vertical pair of arrows affects the y axis, and a horizontal pair of arrows adjusts the x-axis (see lower border of Fig. 21). Right next the two pairs of arrows are buttons for automatic axis scaling.

Manual and automatic y-axis scaling affects only the currently active y-axis unit. In contrast, the command Autoscale adjusts y- and x-axis so that all data fit in the chart area.

The y-axis unit can be picked from a drop-down menu located in the upper left corner of the chart. The active y-axis unit also determines the y-data of the cursor position on the chart.

A way to zoom in to a particular x-axis interval is to mark the x-interval of interest (see Fig. 21), open a context menu by right-click on the chart area, and select the command “Zoom to Selection”.

The selection of data on the chart results in highlighting the corresponding saturation pulse kinetics in the window SAT-Chart and the corresponding data lines in the window Report.

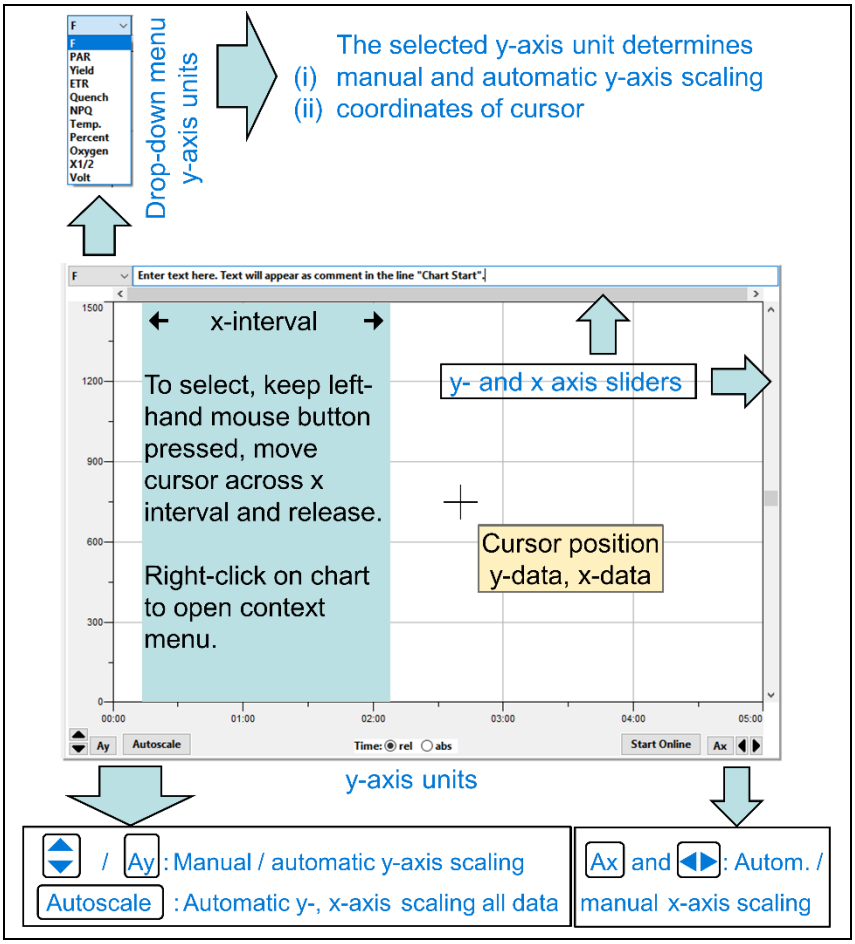


Fig. 21: Chart Tools

8.1.4 Values/Rec./Chan.

The field “Values Rec. Chan.” includes three different sidebars (Fig. 22). Data which should be displayed on the chart are selected on the Values sidebar. The Values sidebar distinguishes continuously recorded data (data groups: Online and Sensors Online) from saturation pulse data and data taken at the time of a saturation pulse (data groups SAT Pulse, Quench, and Sensors Triggered).

The Rec. (= Record) sidebar lists the number of individual charts of the present data file (= Report). Additional Records can be started by the button New Record and existing Records can be deleted by Delete Record. The latest Record, or the Record selected by mouse click, will be displayed on chart. The windows “Induction Curve” and “Light Curve” also possess the Rec. sidebar but in these cases the Induction Curve or Light Curve experiments, respectively, are listed.

The Chan. sidebar displays all devices detected by the WinControl-3 software. Only a JUNIOR-PAM was connected in Fig. 22.

On the Values sidebar, the Ft represents a continuously recorded PAM fluorescence signal (online signal). The fluorescence level “F” is the corresponding saturation pulse signal (F equals the Ft measured right before the saturation pulse).

PAR* (photosynthetic active radiation, µmol photons $m^{-2} \cdot s^{-1}$) and Temp* (Temperature, °C) are two further online signal. They are distinguished by a superscript asterisk from their pendants which are measured together with saturation pulses. In the same way, data of additional sensors are marked (Fig. 22). By default, additional sensors are not available for the JUNIOR-PAM.

Besides F level fluorescence, the SAT-Pulse fluorescence levels are maximum fluorescence F_M' (including F_M) and minimum fluorescence F_0' (F_0). Derived from these fluorescence levels is the photochemical quantum yield of photosystem II, Y(II) (including F_V/F_M). The electron transport rate (ETR, µmol electrons/($m^2 \cdot s$) is calculated with Y(II) and PAR. The section “Quench” of the Values sidebar includes six more saturation pulse parameters. All fluorescence parameters are explained in Chapter 10 (page 85).

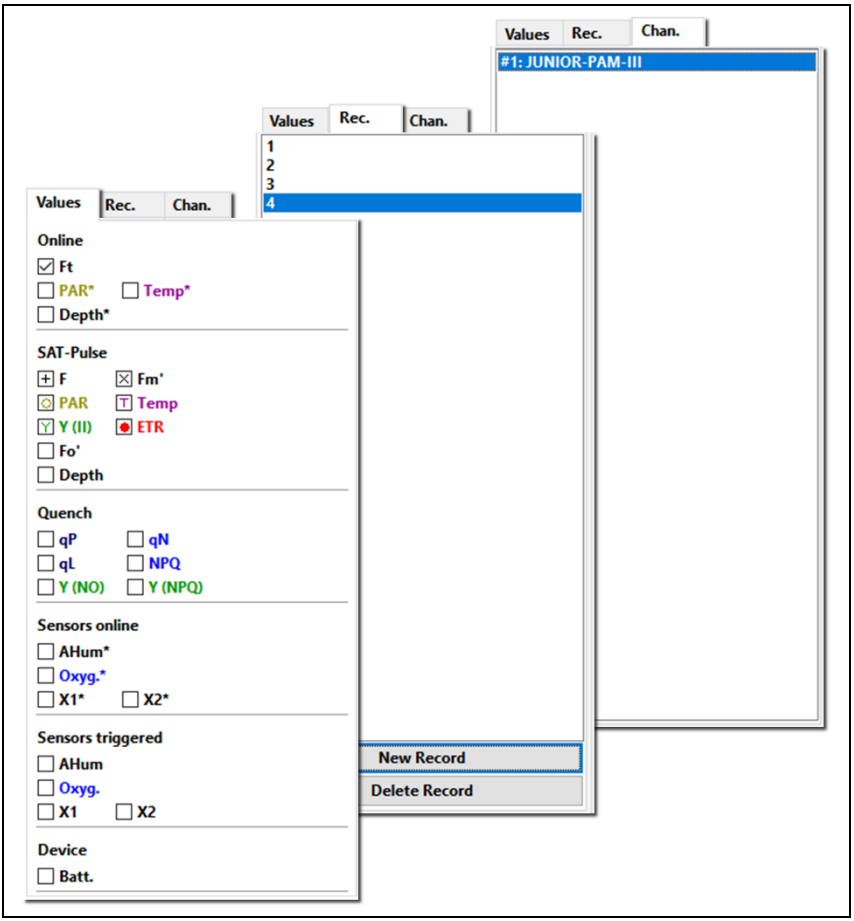


Fig. 22: Values Sidebar

8.1.5 Results

The Results sidebar (Table 14) shows numerical values of the current experiment. The upper part of the sidebar displays data of the Fo, Fm determination. These data are maintained throughout the current experiment. All other data are updated with each saturation pulse analysis. All fluorescence levels and ratios are explained in Chapter 10 (page 85).

Table 14: Sidebar

Parameter	Sample Data	Comment
Fo	441	Fluorescence properties of the dark-acclimated sample.
Fm	1818	
Fv/Fm	0.757	
qP	0.237	Fluorescence quotients describing the state of the light acclimated sample.
qL	0.147	
qN	0.816	
NPQ	2.212	
Y(NPQ)	0.616	
Y(NO)	0.278	
F	506	Fluorescence properties of the light-acclimated sample.
Fm'	566	
PAR	285	Actinic light intensity, µmol m^{-2} s^{-1}.
Y(II)	0.106	Fluorescence property of the light-acclimated sample.
ETR	12.7	Relative electron transport rate, µmol m^{-2} s^{-1}.
Fo'	313	Fluorescence property of the light-acclimated sample.

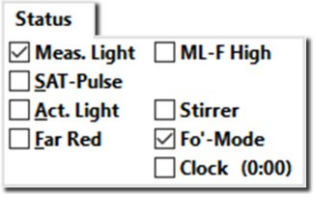
8.1.6 Status Panel

The “Status” field indicates the state of light sources and special functions (Table 15). The checkboxes act as both indicators and on/off switches.

The “Basic” and the “Program” fields are stacked and can be selected by tabs. Actinic light and the clock frequency is adjusted on the Basic field, all automatic routines of the WinControl-3 software can be started in the Program field (Table 15).

The stacked “SAT-Pulse” and “Chart” field provide buttons to trigger F_V/F_M or Y(II) analyses, and graphic areas displaying fluorescence kinetics induced by saturation pulses. The “Online” field display live data numerically.

Table 15: Status Bar



Meas. Light: Low frequency PAM measuring light.

ML-F high: High frequency measuring light. Measuring light changes automatically to high frequency when actinic light is switched on.

SAT-Pulse: Saturation pulse analysis to determine Y(II); equivalent to SAT button (see below).

Act. Light: Actinic light to drive photosynthesis.

Far-red: Light at wavelengths > 700 nm.

Stirrer: Does not apply for the JUNIOR-PAM-II.

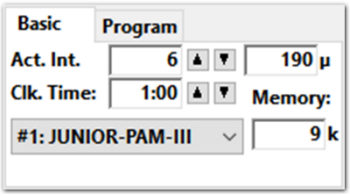
F_0'-Mode: Automatically takes as F_0' fluorescence the minimum fluorescence in a period of far-red-light illumination following a saturation pulse.

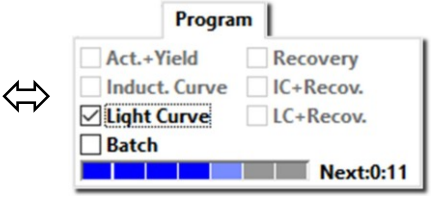
Clock: Repetitive trigger of the event specified on

Settings Window (Section 8.6, page 71). See below on how to adjust the interval between trigger events.

(Table continues on next page.)

Table 15: Status Bar





Act. Int.: Setting and intensity of JUNIOR-PAM-II light source in µmol m^{-2} s^{-1}.

Clk. Time: Time interval between automatically triggered events in minutes : seconds.

#1: JUNIOR-PAM: Device connected (channel).

Memory: Current file size in kilobytes (k).

Experimental Routines

Experimental routines are defined on the Settings Window (Section 8.6, page 71). Most routines can be triggered by the clock.

Act.+Yield: Period of actinic illumination terminated by saturation pulse analysis.

Recovery: Dark phase with saturation pulse analyses performed at increasing intervals.

Induct. Curve: F_0, F_M determination followed by illumination by actinic light with repeated saturation pulse analysis.

IC+Recov.: Induction curve plus dark phase with saturation pulse analyses performed at increasing intervals.

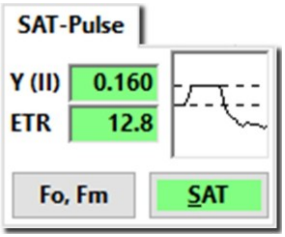
Light Curve: F_0, F_M determination followed by illumination with stepwise increasing light intensities where each step is terminated by a saturatin pulse analysis.

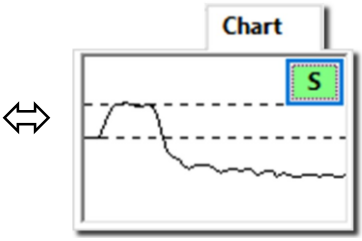
LC+Recov.: Light Curve followed by a dark phase with saturation pulse analyses.

Batch: Execution of batch file program.

(Table continues on next page.)

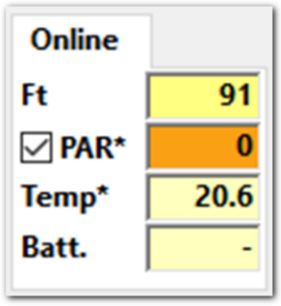
Table 15: Status Bar





Fo, Fm: Saturation pulse analysis to determine F_0, F_M, and F_V/F_M.

SAT = S: Saturation pulse analysis to determine F, F'_0, F'_M, and Y(II).



Online data are continuously recorded with the frequency selected defined in the frequency drop-down menu (see Table 12, page 48).

Ft, PAR* and Temp*: see 8.1.4, page 51. When PAR* is unchecked, the intensity is taken from the internal PAR list (Section 8.6, page 71); when checked, the actually measured value is displayed. Battery voltage does not apply to the JUNIOR-PAM.

8.2 Induct. Curve/Light Curve Windows

The icons and bars of the Chart window introduced above are also present in the Induction Curve and Light Curve windows. In contrast to the Chart window, which displays all data of a Record, the Induction Curve window displays individual induction curves, and the Light Curve window displays individual Light Curves. The same Record can include several Induction and Light Curves. Use up and down arrow keys to scroll through the list of curves.

Table 16 summarizes further differences between the three windows. The windows have different start buttons and only the Light Curve windows possesses PAR as x-axis unit. The button Start IC starts an experiment in which a sample is exposed

to a single defined light intensity, the button Start LC initiates a routine in which a sample is exposed to incrementally increasing light intensities. The x-axis radio button “Time” of the Light Curve window corresponds to the radio button “rel” (relative time) in the two other windows.

The Light Curve window allows fitting model functions to experimental data. The experimental data are the electron transport rates (ETR) plotted against the photon flux density, PAR. WinControl-3 provides two model functions called REG1 and REG2 which are introduced in Fig. 23 and Fig. 24, respectively. The function REG1 can decrease at high PAR values. Therefore, REG1 can consider photoinhibition of photosynthesis, where β is a photoinhibition parameter. In contrast, REG2 is a rectangular hyperbola which cannot describe photoinhibition.

Both models calculate the three cardinal parameters of a light curve:

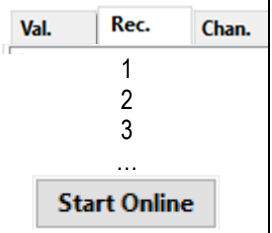
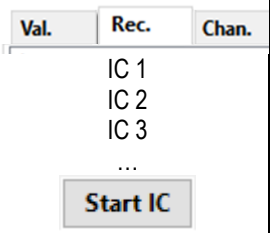
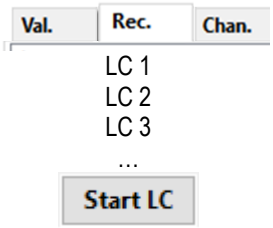
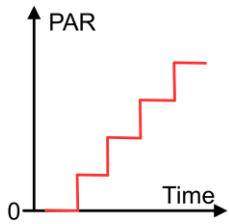
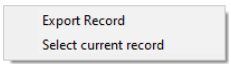
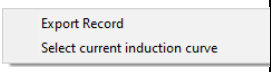
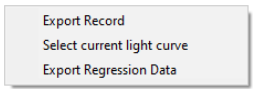
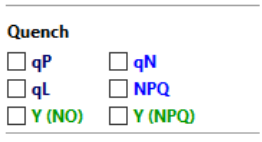
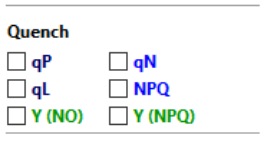
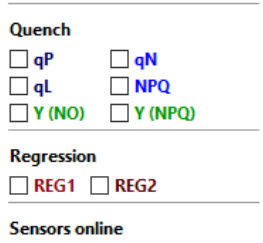
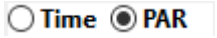
(i) α, electrons/photons: Initial slope of RLC which is related to the quantum efficiency of photosynthesis.

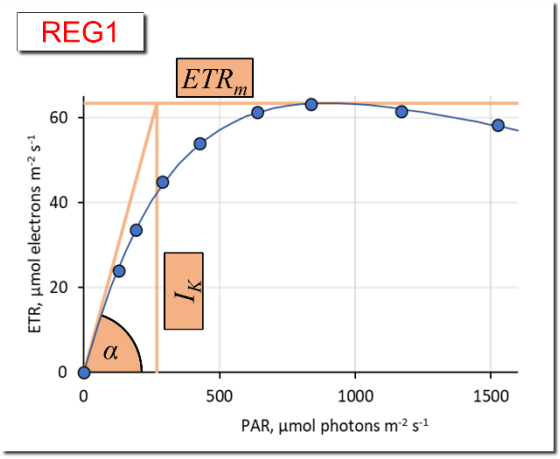
(ii) ETR_m, µmol electrons $m^{-2} \cdot s^{-1}$: Maximum electron transport rate.

(iii) I_K, µmol photons $m^{-2} \cdot s^{-1}$: Idealized PAR value at which light-limited photosynthesis becomes light-limited.

The cardinal parameters are written into the Report. To export cardinal parameters separately, right click on the chart of the Light Curve window and select from the menu “Export Regression Data” (see Table 16). The item “Select current light curve” in the same menu highlights the data of the currently displayed light curve in the Chart and Report windows. A similar function is available for the window Induction Curve.

Table 16: Three Graphics Windows

Chart Window	Induct. Curve Window	Light Curve Window
Rec. Sidebar 	Rec. Sidebar 	Rec. Sidebar 
Lighting 	Lighting 	Lighting 
Right click on chart 	Right click on chart 	Right click on chart 
Values Sidebar 	Values Sidebar 	Values Sidebar 
x-axis units 	x-axis units 	x-axis units 



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

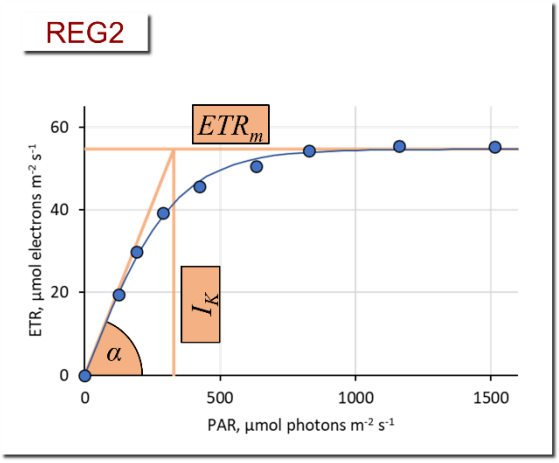
$$ETR_m = ETR_{mPot} \cdot (\frac{\alpha}{\alpha + \beta}) \cdot (\frac{\beta}{\alpha + \beta})^{\frac{\beta}{\alpha}}$$

$$I_K = \frac{ETR_m}{\alpha}$$

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

Fig. 23: Model Function REG1

The three cardinal points of the light curve are indicated (α, ETR_m, and I_K). The decrease at PAR>1000 µmol m^{-2} s^{-1} is frequently attributed to photoinhibition of photosystem II by strong light. I_b is the theoretical PAR at which the light curve reaches 1/e of ETR_{mPot}. ETR_{mPot} is the ETR_m in the absence of photoinhibition. According to: Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38: 687-701



$$ETR = ETR_m \cdot tanh(\frac{\alpha \cdot PAR}{ETR_m})$$

$$I_K = \frac{ETR_m}{\alpha}$$

Fig. 24: Model Function REG2

The three cardinal points of the light curve are indicated (α, ETR_m, and I_K). According to Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21: 540-547.

8.3 SAT-Chart Window

The SAT-Chart window displays saturation pulse kinetics of fluorescence (Fig. 25). The automatically determined values of F and F_M' are shown as dashed horizontal lines. Fluorescence traces are automatically scaled to fit into the coordinate system. The x-axis covers about 2 s, the saturation pulse starts at 200 ms, and the distance between data points is 50 ms. The mouse wheel scrolls through graphs.

Each graph is accompanied by a protocol panel. The protocol panel lists the pulse number (Nr) and the line number of the Report (Rep. Nr). Select a fluorescence trace by double-click in the protocol window. The selected graph automatically moves to the top of the window.

The Options menu (upper right corner of the SAT-Chart window) links the actual view of saturation pulse kinetics to the saturation pulse events selected in one of the three previous windows. The option “Follow Selection” automatically displays on top of the window the saturation pulse kinetics of marked events. When Follow Selection” is inactive, the option “Jump to Selection” brings the selected kinetics into view.

The Values sidebar is similarly configured as described in Section 8.1.4 (page 51), except “Online Data” are omitted and the electron transport factor (ETR-F.) is added. The ETR-F. is the fraction of absorbed photons by the sample relative the PAR to which the sample is exposed. The ETR-F. is a factor of the equation for electron transport (Chapter 10, page 85). Each item selected on the Values sidebar is numerically displayed below the corresponding saturation pulse kinetics.

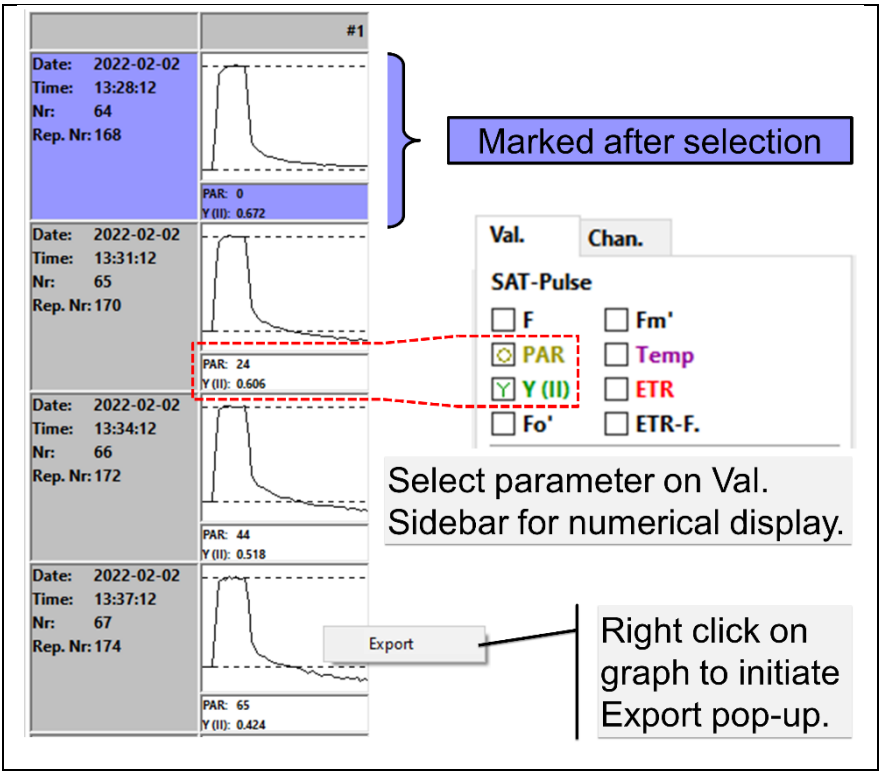


Fig. 25: SAT-Chart Window

Table 17: SAT Kinetics in Raw File

To extract a series of saturation pulse kinetics, open PAM file with spread sheet program, sort by SG and copy data right of SG.

	A	B	C	D	…	AD	AE	AF	AG	AH
1	Date	Datetime	Type	No.						
2	08/03/2022	08/03/2022 09:17:42.997	F	2	…	**SG**	50	419	419	…
3	08/03/2022	08/03/2022 09:17:44.389	F	3	…	**SG**	50	383	383	…
						SAT Graph Label	Time interval in ms	Data of Saturation pulse kinetics		

Single saturation pulse kinetics can be exported by right-click in its chart field. To export a series of saturation pulse kinetics, open the PAM file with a spread sheet program. In the text file, saturation pulse kinetics are preceded by an SG (Table 17). Sort the lines of the file by SG. Create a time scale by considering the neighboring points are spaced apart by 50 ms.

8.4 Report Window

The Report window lists all data associated with saturation pulse analyses. Continuously recorded signals, saturation pulse kinetics and spectra are not listed. The fact that a spectrum was recorded is indicated by “SPEC” in the column “Type” (Table 18). The Report window also displays start and end of induction and light curves, as well as the cardinal points of light curves (Table 18).

The types of data displayed is controlled by the Values side bar, which is configured as described for the SAT-CHART window (Section 8.3, page 61). Only data displayed on the Report table are exported.

The Options menu of the Report window can be opened by clicking the Options button or by right-click in the Report field. The options menu contains various tools for handling the Report table. All menu items and their function are summarized in Table 19.

The Command “Insert Settings” of the options menu writes the current device settings in the report. The abbreviations used for the various settings are compiled in Table 20.

Table 18: Report Table

Type: D, device. FO, F_0 and F_M determination. F, determination of F, F_0', and F_M'. SPEC, spectrum. SICWS and SICSE, induction curve start and end, respectively. SLCS and SLCE, light curve start and end, respectively. REG1 and REG2, cardinal parameters of regression analysis 1 and 2, respectively (see 8.2). The JUNIOR-PAM has the channel number #1. "#1" is preceding the cardinal values of light curve analysis, REG1 and REG2.

	Date	Time	Type	No.	1:F	1:Fm'	1:PAR	1:Y (II)	1:ETR
1			D		Device Nr: #2, JUNIOR-PAM-III (CFMG1166B)				
2	2022-03-08	09:07:11	SCHS						
3	2022-03-08	09:16:20	FO	1	461	1932	6	0.761	1.9
4	2022-03-08	09:16:50	F	2	680	774	6	0.121	0.3
5	2022-03-08	09:17:24	SPEC		PAR: 12; Temp.: 21.2				
6	2022-03-08	09:17:42	SICS		Induction Curve start				
7	2022-03-08	09:17:48	SICE		Induction Curve end				
8	2022-03-08	09:17:59	SLCS		Light Curve start				
9	2022-03-08	09:18:07	REG1		#1: alpha: 0.226, ETRm: 51.750, Ik: 228.970 (beta: 0.027, ETRmPot: 75.879) (Platt et al. 1980)				
10	2022-03-08	09:18:07	REG2		#1: alpha: 0.193, ETRm: 49.077, Ik: 254.690 (Jassby and Platt 1976)				
11	2022-03-08	09:18:05	SLCE		Light Curve end				

Table 19: Options Menu

Options Menu Item	Comment
	General Report Management
Follow Selection	Automatically displays the data associated with events marked in other windows
Show Mark	Display marker letters (see "Mark" in Table 18)
Insert Settings	Writes settings of JUNIOR-PAM in Report table (see Table 20)

(Table continues on next page.)

Table 19: Options Menu

Options Menu Item	Comment
	Manipulation of All Data
Export All	For details see Section 8.1.1, page 45.
Delete All Measure Data	Self-explaining
Page Setup for Printing	Basic configuration of print layout. Checks if output fits on page
Preview Printing	Self-explaining
Print Report	Selects and configures printer, prints current Report
	Manipulation of Selected Data
	Requires that saturation pulse events on a chart or lines of the Report have been selected. To select, move the cursor with left mouse key pressed over events
Export Selected Lines	Confines export to lines of interest
Jump to Selection	Brings data into view, when “Follow Selection” is off
Delete Selected Data	As above. Affects only selected lines.
Preview Print Selection	As above. Affects only selected lines.
Print Selected Data	As above. Affects only selected lines.
Mark as Light Curve	Combines a series of saturation pulse analysis into a light curve and performs regression analysis REG1 and REG2. The series must not contain start or end marks of previous light or induction curves.

Table 20: Abbreviations for Settings

At the time of the printing of this manual, the range of settings and naming of abbreviations is under review. Future information on settings may differ from the list below. For the updated manual, visit: https://www.walz.com/products/chl_p700/junior-pam/downloads.html	
MEA	Measuring Light Intensity
MI	Measuring Light Frequency
DI	Damping
GA	Gain
EF	ETR-Factor
FZ	F Offset
CW	Clock Interval
SI	Saturation Pulse Intensity
SW	Saturation Pulse Width
AI	Actinic Light Intensity
AF	Actinic factor
FRI	Far-red Intensity
FRW	Far-red Width
AW	Length of exposure of “Act. + Yield” routine
ICD	Delay of Induction Curve
ICW	Interval Between Saturation Pulses of Induction Curve
ICL	Length of Induction Curve
LCW	Step Length of Light Curve
LCI	Initial Intensity Setting of Light Curve
LCL	Length of Light Curve
LO	Offset of External PAR Sensor
LG	Calibration Factor of External PAR Sensor
LO2	Offset of External PAR Sensor 2
LG2	Calibration Factor of External PAR Sensor 2
TO	Offset of External Temperature Sensor
TG	Gain of External Temperature Sensor
ILO	Offset of Internal PAR Sensor
ILG	Gain of Internal PAR Sensor
PARGAIN_RED	Calibration Factor for Red LED of 2054-L
PARGAIN_GREEN	Calibration Factor for Green LED of 2054-L
PARGAIN_BLUE	Calibration Factor for Blue LED of 2054-L
PARGAIN_WHITE	Calibration Factor for White LED of 2054-L
PARGAIN_CUSTOM	Calibration Factor for Other light Sources
ITO	Offset of Internal Temperature Sensor
ITG	Gain of Internal Temperature Sensor
MLC	Fm Correction Factor 1
MLC2	Fm Correction Factor 2
TRM	Trim Value for Measuring Light
TRA	Trim Value for Actinic Light
TRSA	Trim Value for Saturation Pulse
TRFR	Trim Values for Far-red Light

8.5 Batch Window

Batch files automatically execute experimental procedures. To activate the Batch window, click icon new batch or open existing batch file (Fig. 26, “Batch Start Buttons”). The click Edit and choose between “Add command” and “Record Macro”. The command “Update indentation” is an automatic editing tool to improve readability.

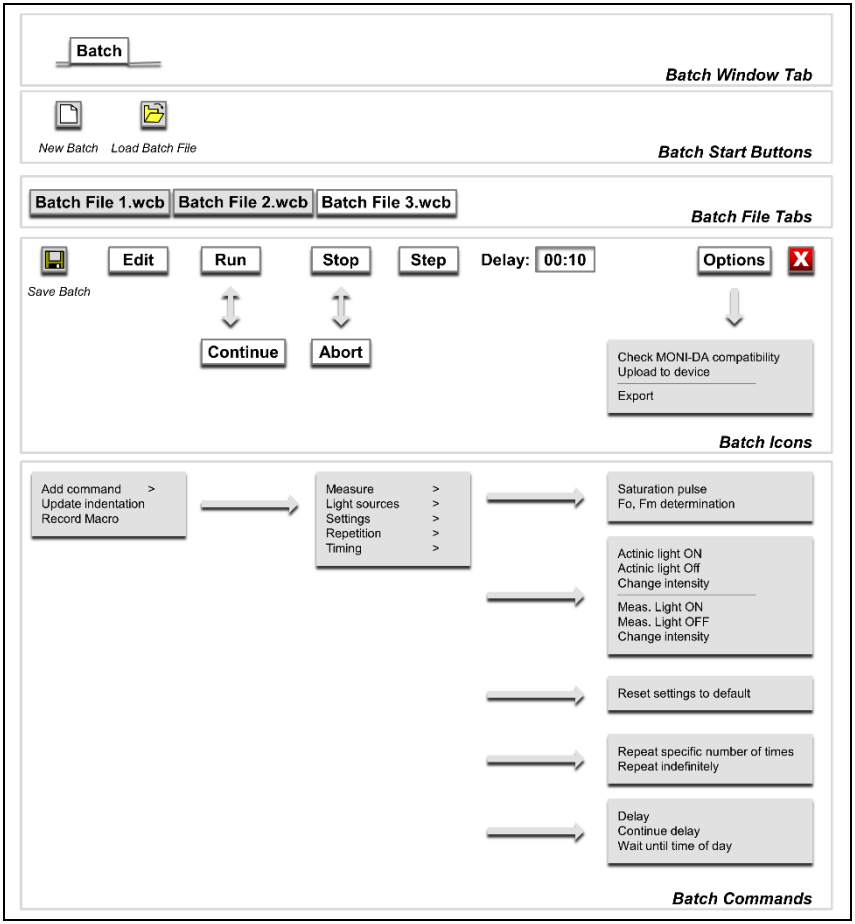


Fig. 26: Batch Window Overview

8.5.1 Add Command

Add command leads to five groups of commands (Fig. 26). A command can be inserted into a batch file by left click.

Measure commands

Saturation pulse	Saturation pulse analysis of light-exposed sample
F_0, F_M determination	Saturation pulse analysis of dark acclimated sample

Light Sources commands

Controls for actinic and measuring light. The light ON command asks for the light intensity: simple enter the light intensity setting in the grey-shaded field. New intensity settings can be chosen using the commend “Change intensity”.

Settings commands

“Reset settings to default” installs factory settings.

Repetition commands

The group contains two commands. “Repeat specific number of times” and “Repeat indefinitely”. Both commands write two lines on the batch file sheet. In case of the first command, these lines are:

Line 1:

```
for $loopvar = 1 to count // Start repetition block with specified number of repetitions
```

Line 2:

```
next // End of repetition block
```

Write commands to be repeated between Line 1 and 2. Define how often the commands should be repeated by the number entered in field count.

The second command (Repeat indefinitely) repeats the commands placed between the two lines until the batch program is stopped manually.

Line 1:
```
while 1    // Start block repeating indefinitely
```

Line 2:
```
wend       // End of repetition block
```

Timing

The command “Delay” inserts a time interval after the previous command has been terminated. The next command is executed when the time interval ends.

The command “Continue Delay” takes the end of the previous delay phase as starting point. Actions within this time interval are performed without affecting the interval defined for Continue Delay. A continuous time scale can be built by a series of Continue Delay commands.

“Wait until time of day” delays start of the batch program until the time specified.

8.5.2 Record Macro

The function “Record Macro” converts your manually entered commands into batch file lines. Simply click “Record Macro”, perform experiment, and click “Record Macro” again.

8.5.3 Options

All items of the options menu of the Batch window are related to the MONI-DA data acquisition system.

Check MONI-DA Compatibility

Some batch file commands cannot be executed by the MONI-DA. This command searches for such incompatibilities.

Upload to Device

Transfers a batch file to the MONI-DA memory.

Export

Export the current batch file as “WinControl-3 Compiled Batch File” (*.wccb). This file format is for future use. Upcoming MONI-DA versions will be enabled to directly download wccb files, that is, without being connected to WinControl-3. The final goal is to install batch files by remote control.

8.6 Settings Window

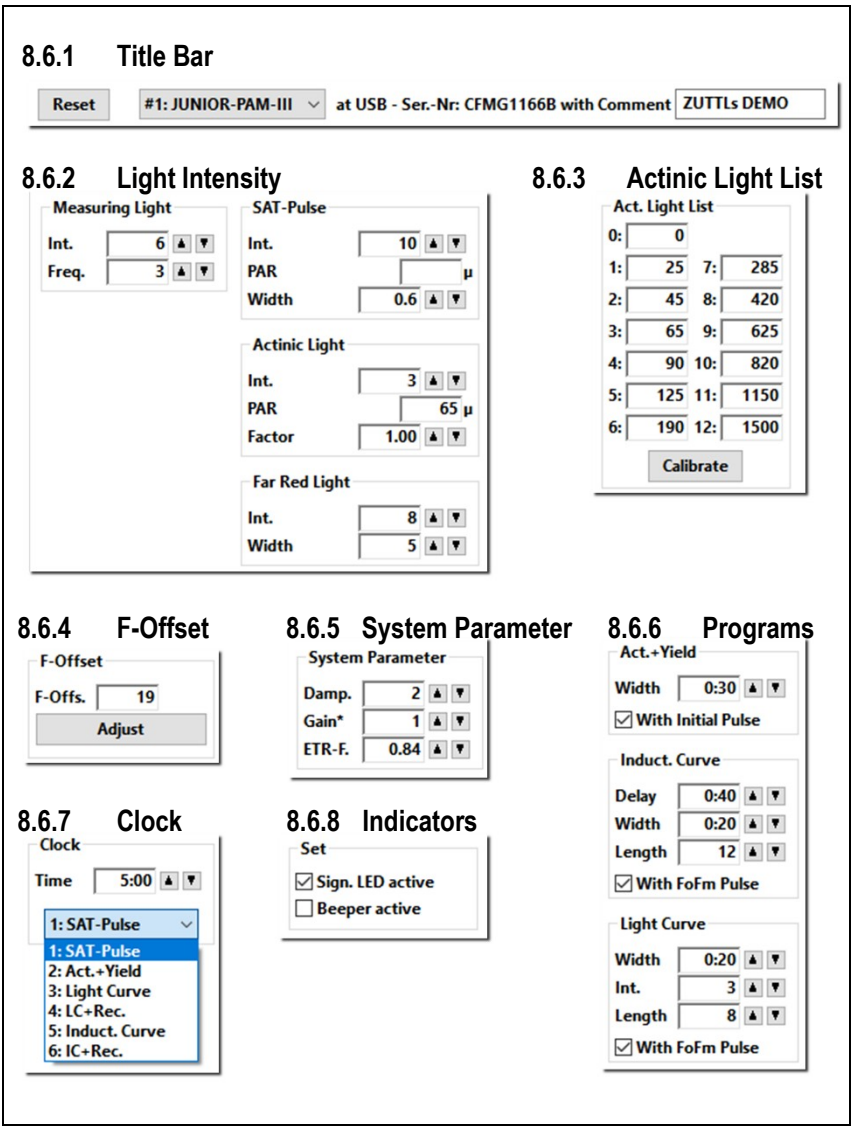


Fig. 27: Settings Window

8.6.1 Title Bar

The Reset button restores factory values for all settings of the current window. Located right of the Reset button is a drop-down menu listing all devices connected. The settings displayed on the current window, and the serial number displayed right of the drop-down menu, belong to the device selected. In the text box right of the serial number, up to 20 characters can be entered. Serial number and text box content are written in the first line of each record file. System information is displayed on the bottom of the Settings and the System Settings windows (see Table 24, page 77)

8.6.2 Light Intensity

When actinic light is switched off, µs measuring pulses are delivered at 5 to 25 Hz depending on frequency setting “Freq.“ (Table 21, page 73). Switching on actinic light automatically increases the measuring light frequency to 100 Hz. The measuring light frequency can also be manually set to 100 Hz by checking “MF-F High” on the Status Bar (Table 15, page 54).

The intensity setting “Int.” adjusts measuring light intensity. Twelve intensity levels are available. The numerical value of the setting is proportional to the measuring light intensity. How to estimate the integrated measuring light intensity for the frequency and intensity settings used is described in Table 21.

Note that increasing measuring light intensity increases all fluorescence levels proportionally. An increase in measuring light frequency increases the fluorescence level only when it is strong enough to drive significant rates of photosynthesis, e.g., by reaction center closure. If F_0 fluorescence is increased by measuring light, reduce intensity or frequency or both.

Like measuring light, the intensity of saturation pulses, actinic light and far-red light can be adjusted in 12 levels. The PAR of saturation pulses is indicated in newer versions of WinControl-3. The PAR of actinic light is taken from the light list (see below). Most of far-red emission is not absorbed by chlorophylls. Therefore, intensity data for far-red radiation (PAR or photon flux density) is not given as it would strongly overestimate its actual actinic effect.

Table 21: Calculating Light Intensity

Setting	Frequency, *f*	Intensity setting	Intensity at 1 mm distance from fiberoptics
1	5 Hz	3 / 6 / 12	0.01 / 0.03 / 0.06 µmol m^{-2} s^{-1}
2	10 Hz	3 / 6 / 12	0.03 / 0.06 / 0.11 µmol m^{-2} s^{-1}
3	15 Hz	3 / 6 / 12	0.04 / 0.08 / 0.17 µmol m^{-2} s^{-1}
4	20 Hz	3 / 6 / 12	0.06 / 0.11 / 0.22 µmol m^{-2} s^{-1}
5	25 Hz	3 / 6 / 12	0.07 / 0.14 / 0.28 µmol m^{-2} s^{-1}
high	100 Hz	3 / 6 / 12	0.28 / 0.55 / 1.10 µmol m^{-2} s^{-1}

Calculation

$$I_{ML}(\frac{\mu mol}{m^2 \cdot s}) = I_{ML}max\ (\frac{\mu mol}{m^2 \cdot s}) \cdot \frac{f(Hz)}{100(Hz)} \cdot \frac{Int.\ Sett.}{12}$$

where

$I_{ML}max$ = 1.1 µmol m^{-2} s^{-1}	Maximum PAR of measuring light (frequency = 100 Hz, intensity setting = 12). Measured at sample level of the Magnetic Leaf Clip JUNIOR-PAM/MLC with fiberoptics tip flush with the lower face of the upper part of the clip (see 3.2, page 7): fiberoptics tip at 1 mm distance from sample level.
f	Measuring light frequency in Hz
Int. Sett.	Setting of measuring light intensity on Settings window. The intensity of measuring light is proportional to the setting number.

8.6.3 Actinic Light List

Twelve actinic light levels are listed. These values are target PAR values. As delivered from the factory, the light emission of the LED is adjusted so that these target values are present at the sample level of a 2035-B Leaf Clip Holder. The target values can be varied by changing the “Factor” in the field “Actinic Light” (Section 8.6.2).

8.6.4 F-Offset

The F-Offset is the background signal contributing to the total signal measured. Background signals must fully or partly possess the modulation characteristics of measuring light to be recognized by a PAM fluorometer. These signals can arise from:

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

Usually, the background signal increases with measuring light intensity and signal amplification (gain). Therefore, the Adjust F-Offset command determines the background signal for all measuring light intensities and all gain settings. The currently active offset is displayed in the bottom line of the PAM Settings window (Fig. 27, page 71).

The “Adjust F-Offset” command determines the background signal which is subtracted from the total signal.

Procedure

- Choose dim environment.
- Switch off any flickering light sources like fluorescent lamps or computer screens.
- Point fiber tip away from any objects, keep fiber tip clear.
- Run “Adjust F-Offset”.

8.6.5 System Parameter

Damping (Damp.) Function for data smoothing. The smoothing effect increases with increasing numerical value, at the same time, but instrument response decreases.

Gain Factor (Gain). The Gain factor amplifies the signal including its noise. At low signal levels, increasing the gain improves digital resolution.

ETR-Factor (ETR-F.). The ETR-Factor is the percentage of light absorbed by the sample and is a parameter for calculating the electron transport rate (Section 10.3, page 92).

8.6.6 Programs

Act. + Yield: Light exposure followed by saturation pulse analysis

“Width” is the duration of light exposure. The actinic light intensity adjusted in the field “Actinic Light” applies (Section 8.6.2, page 72). A saturation pulse analysis is always carried out at end of illumination. Checking “With Initial Pulse” places an additional saturation pulse analyses before illumination. The fluorescence levels of the initial saturation pulse analysis are defined as F_0 and F_M. All fluorescence ratio parameters requiring these two fluorescence

levels (Table 27, page 94) are only available with initial saturation pulse.

Induct. Curve: Fluorescence curve following the onset of light

Three parameters determine the sequence of events. “Delay” indicates the dark time interval between F_0 and F_M determination and onset of light. The delay time can be adjusted between 5 seconds and 10 minutes. Default value is 40 seconds. “Width” is the time interval between neighboring saturation pulses in the light period. Length is the number of saturation pulse analyses in the light period. Hence, total time of actinic illumination is approximately the product of “Width” times “Length”. A dark period (“recovery”) can be appended to an induction experiment (Table 22).

Light Curve: Saturation pulse analyses at end of exposure to different light intensities

The “Light Curve” program exposes a sample to consecutively increasing actinic light intensities. Three input values define the procedure. “Width” is the duration of exposure to a particular light intensity. “Int.” is the first light level of the light curve. “Length” is the number of light steps. The duration of a light curve is determined by “Width” times “Length”. A dark period can be appended to illumination (see above).

☑ **With FoFm pulse**: Both Induction and Light Curves can be performed without initial saturation pulse (F_0 and F_M measurement).

Table 22: Time Points for Recovery Curves

SAT number	Time in darkness, min
1	0.00
2	0:30
3	1:30
4	4:00
5	9:00
6	19:00
7	39:00

8.6.7 Clock

The clock utility repetitively triggers one out of 6 different measuring routines: Saturation pulse analysis, Act.+Yield, Induct. Curve, IC+Recov., Light Curve and LC+Recov (see Above). The interval time between two events (Clk. Time) can be adjusted between 10 s and 50 min. The time interval must be longer than the time required by the triggered event.

8.6.8 Indicators

The signal LED located above the touchscreen indicates the operational state of the JUNIOR-PAM as listed in Table 23. The beeper acoustically marks begin and end of a saturation pulse analysis.

Table 23: LED Signal Code

LED Color	Frequency	Process
Measuring	**mode**	
Green	1 Hz	Normal operation
Green double flash	1 Hz	Normal operation and clock running
Green	continuous	Normal operation and saturation flash
Additional codes during firmware update		
Green/red alternating	high	Waiting for software
Red	continuous	Update running

Table 24: System Information

System information displayed on bottom of Systems and Systems Settings window.

Headline	Model	Model Number	Serial Number
Display	JUNIOR-PAM (blue) or JUNIOR-PAM (white)	JUNIOR-PAM or JUNIOR-PAM/W	CFMG####B or CFMN####B
Information	Color version	Order code	S/N convention

8.7 System Settings Window

Different from other windows of WinControl-3, the window System Settings is not represented in the row of tabs. To access system settings, open Settings window and click “System Settings” (Fig. 28).

The System Settings window is divided into functional entities in Fig. 29 (page 79). Each entity is headed by number and title of the corresponding explaining section. together with their section numbers. In electronic manuals, clicking on the title automatically jumps to the corresponding text.

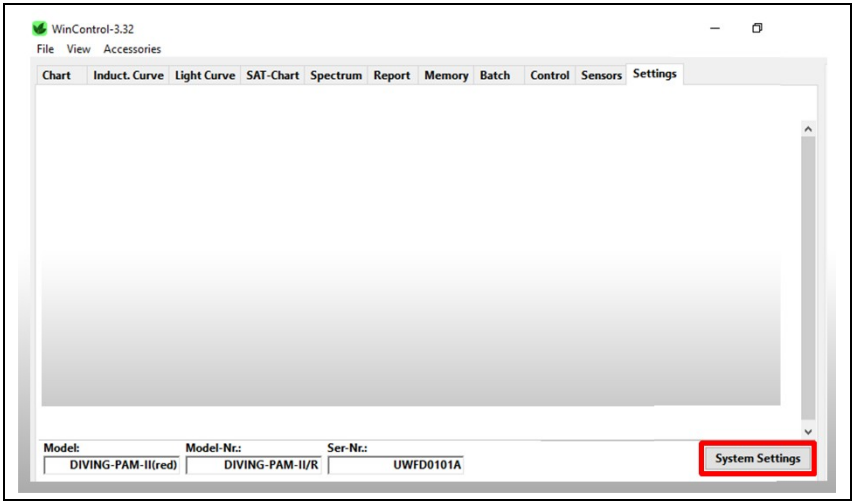


Fig. 28: System Settings Button

Click to open System Settings window.

8.7.1 Title Bar

The Reset button of the System Settings page recalls all calibration data established at the factory. This information is taken from the flash memories of the JUNIOR-PAM and of the Monitoring Leaf-Clip JUNIOR-BD. These original calibration data cannot be changed by the user. The other elements of the Title Bar are described in Section 8.6.1, page 72.

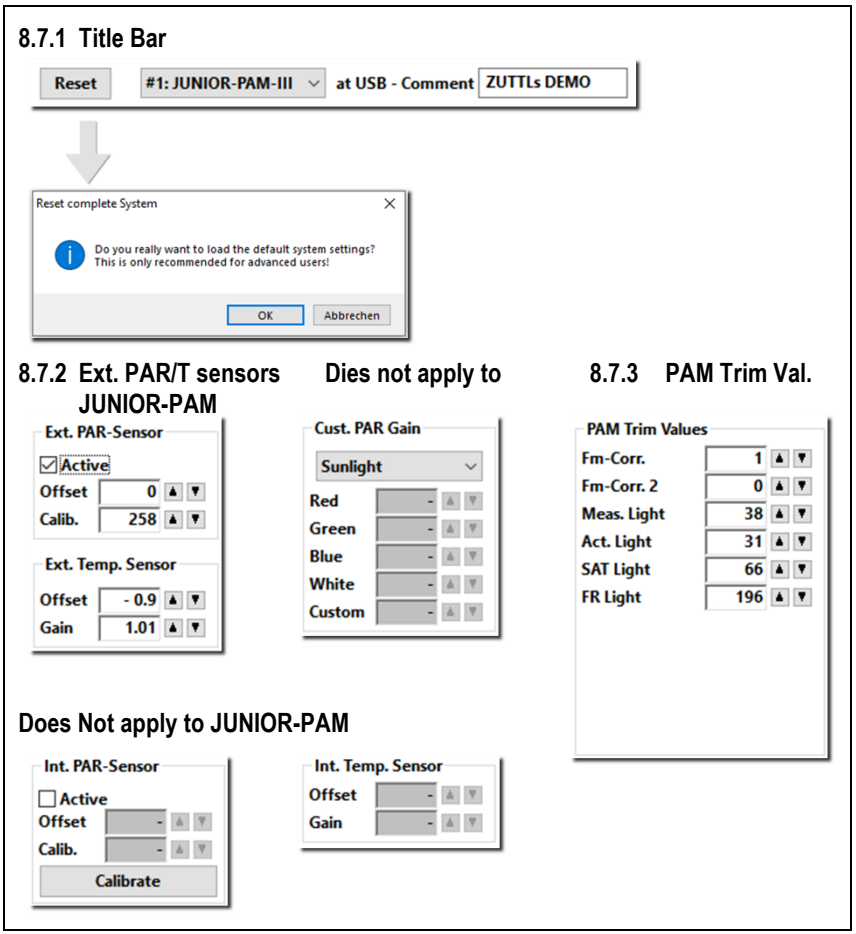


Fig. 29: System Settings

8.7.2 Ext. PAR/T sensors

The boxes “Ext. PAR Sensor” and “Ext. Temp. Sensor” (Fig. 29) display the factory-established calibration factors of the Monitoring Leaf-Clip JUNIOR-BD. Calibration factors must be manually entered when a PAR sensor does not support automatic installation of its calibration data.

8.7.3 PAM Trim Val.

Trim values have been set at the factory so that the JUNIOR-PAMI meets its specifications.

Table 25: PAM Trim Values

The table compiles the types of gain factors with their application, which are available for the PAR sensor of the 2035-B leaf clip or the PAR sensor of the 2065-M device.

Type	Application
Fm-Corr.	Factor to compensate a signal decrease during a saturation pulse. The factor is determined with a Walz fluorescence standard foil. Signal compensation is obsolete (value=0) for the latest generation of LEDs.
Fm-Corr.2	Second factor to compensate a signal decrease during a saturation pulse. Two factors are required when a biphasic signal decrease occurs. Signal compensation is obsolete (value=0) for the latest generation of LEDs.
Meas. Light Act. Light SAT Light FR Light	Factors adjusting the intensities of measuring light/actinic light/saturation pulse light/far-red light to meet the respective specification.

9 Hints

9.1 Instrument Settings

The fluorescence offset (F-Offset) of your system has been measured and was saved on the JUNIOR-PAM memory. That means that the JUNIOR-PAM should show a fluorescence value close to zero in the absence of a sample. If this signal deviates clearly from zero, newly adjust F-Offset (see Section 8.6.4, page 74).

9.2 Default settings

For fluorescence measurements with green leaves, the default settings for measuring light and saturation pulses are well suited. Some samples require special settings. The following sections give some hints to adjust the settings properly.

9.3 F_0 Fluorescence

Usually, measuring light intensity is adjusted to reach F_0 fluorescence levels around 500 mV (for a definition of F_0 see Section 10.2, page 88). Theoretically, the F_0 should stay below 640 mV. The latter upper value is derived from the assumption that the maximum recorded F_V/F_M is 0.84, and from the fact that signal saturation occurs at 4000 mV (see equation below, Table 26). If required, measuring light can be adjusted (Section 8.6.2, page 72).

Table 26: 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 PS 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, average 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.

9.4 F_M Fluorescence

The F_M and F_M' levels are determined as the maximum of the fluorescence signal induced by a saturation pulse. Factory settings of saturation pulse width and intensity are adjusted to arrive at a plateau with green leaves (Fig. 30A). Some high light grown samples do not reach a plateau with standards settings (Fig. 30B). In this case, saturation pulse intensity or/and length should be increased. Also, fluorescence kinetics can reach its maximum clearly before end of the saturation pulse (Fig. 30C). The latter does not result in erroneous F_M or F_M' values because these values correspond to the maximum of fluorescence kinetics. In case of Fig. 30C, saturation pulse intensity or/and length might be decreased.

Some samples, particularly those grown in low light conditions, or senescent plants, show slightly reduced F_V/F_M values under default conditions. These samples increase the F_V/F_M with decreasing saturation pulse intensity. Therefore, testing the F_V/F_M at various saturation pulse intensities is important to optimize saturation pulse settings.

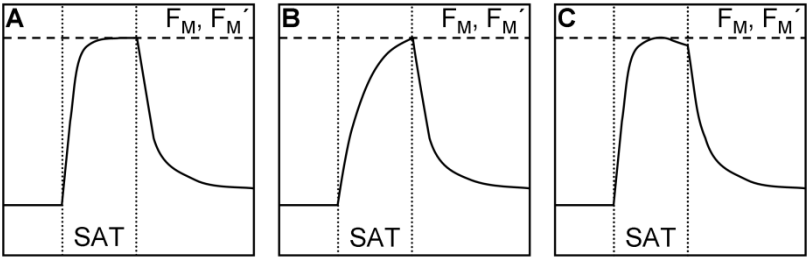


Fig. 30: Fluorescence Kinetics Induced by a Saturation Pulse

10 Saturation Pulse Analysis

10.1 Pulse-amplitude Modulated (PAM) Fluorescence

The PAM principle is illustrated by Fig. 31. 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. 31, 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. 31, 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”.

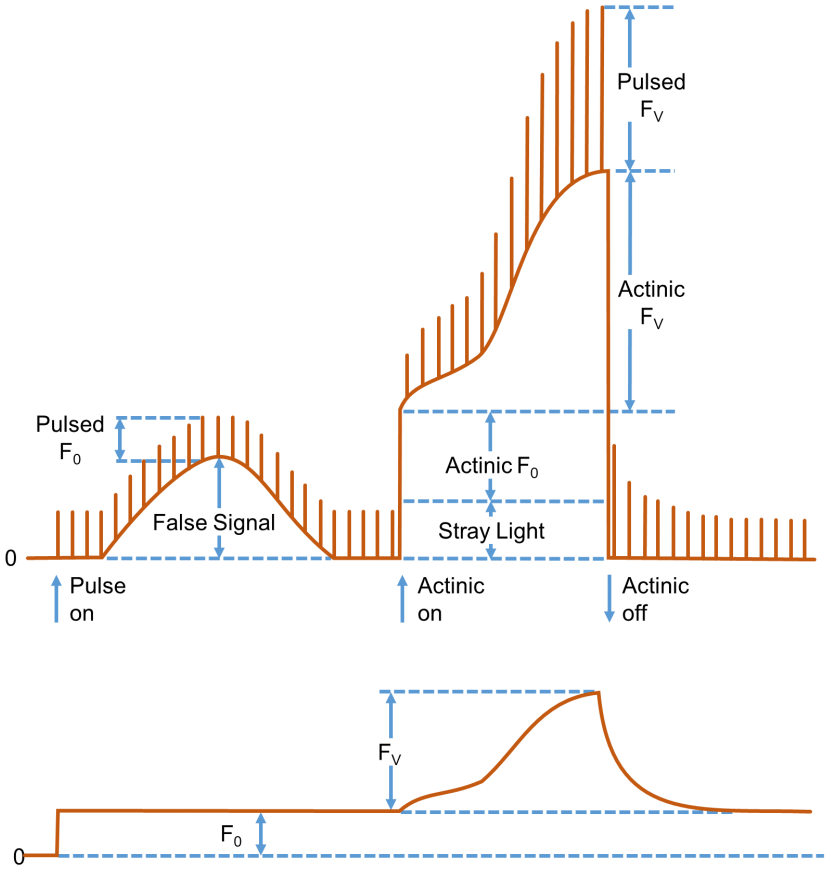


Fig. 31: **Illustration of the PAM measurement principle**
Figure redrawn from Dr. Ulrich Schreiber, Manual for PAM-101.

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. 31 outlines the PAM fluorescence trace. Obviously, PAM fluorescence irons out 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 F_V" resembles the corresponding trace of PAM fluorescence, because both measuring light and actinic illumination are constant.

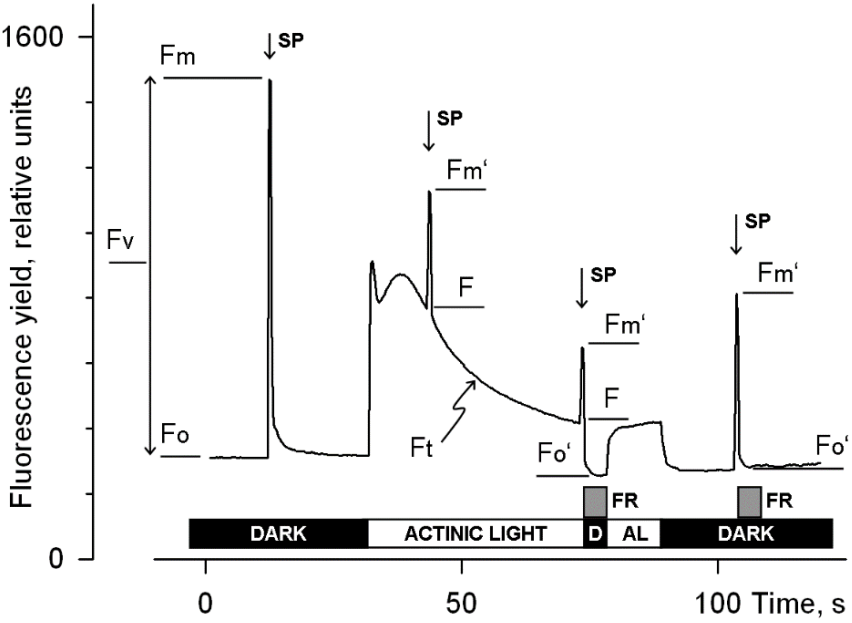


Fig. 32: Fluorescence Levels of Saturation Pulse Analysis

Y-axis (Fluorescence yield) corresponds to PAM fluorescence, see 10.1. AL, Actinic Light; D, dark; SP, Saturation Pulse; Ft, continuously recorded PAM fluorescence; FR, far-red illumination.

10.2 Saturation Pulse Analysis

The five principal levels of PAM fluorescence which are used for saturation pulse analysis are shown in Fig. 32. 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 27, page 94).

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 intensity of measuring light to keep photosystem II reaction centers open.

F_M Maximum fluorescence level elicited by 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. 32, page 87) 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 momentary fluorescence level (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 27 (page 94) 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). Determinations of q_P an q_L do not require fluorescence measurements with the dark-acclimated sample, except the F_0' mode is switched of and F_0' is calculated according to Oxborough and Baker (1997).

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 27, page 94).

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 variance, 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.

See also “The Plumber’s Photosystem II Model” (Fig. 15, page 34).

10.3 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 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.

10.4 Reviews on Saturation Pulse Analysis of Photosystem II

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Table 27: 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 28: References Cited in Table 27

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11 Specifications

11.1 Basic System

11.1.1 Optoelectronic Unit JUNIOR-PAM (BLUE Version)

Modulated fluorescence excitation: Blue LED (wavelength of maximum emission: 445 nm). Modulation frequencies 5 to 25 Hz adjustable in increments of 5 Hz, and 100 Hz. Fluorescence at wavelengths greater than 630 nm is measured

Actinic light: Same LED as used for modulated light. Actinic PAR, 25 to 1500 µmol µmol m^{-2} s^{-1}; maximum PAR of saturation pulses, 7000 µmol m^{-2} s^{-1}. All data valid for 1 mm distance from the tip of the 50 cm JUNIOR-PAM light guide

11.1.2 Optoelectronic Unit JUNIOR-PAM/W (WHITE Version)

Modulated fluorescence excitation: White LED (wavelengths of maximum emission: 445 and 545 nm). Modulation frequencies 5 to 25 Hz adjustable in increments of 5 Hz, and 100 Hz. Fluorescence at wavelengths greater than 650 nm is measured

Actinic light: Same LED as used for modulated light. PAR at 1 mm distance from the tip of the 50 cm JUNIOR-PAM light guide: between 25 and 1500 µmol m^{-2} s^{-1}. Maximum PAR of saturation pulses, 7000 µmol m^{-2} s^{-1}

11.1.3 Optoelectronic Unit JUNIOR-PAM and JUNIOR-PAM/W

Housing: Aluminum housing with USB type B socket, M8 4-pole socket to connect the JUNIOR-PAM monitoring leaf clip (accessory), port for JUNIOR-PAM light guide, and swivel-mounted sample support

Far-red light: LED with 745 nm maximum emission wavelength for selective excitation of photosystem I

Fluorescence detection: PIN photodiode protected by long-pass filter. Selective window amplifier to measure pulse-amplitude modulated (PAM) fluorescence

Communication: Standard USB communication via 1.2 m USB-cable type A-B

Power supply: 5 V DC supplied by the USB-cable type A-B used for communication

Power consumption: 100 mW at normal operation and 500 mW during saturation pulse

Dimensions: 11.5 cm x 6.5 cm x 3 cm (L x W x H)

Weight: 200 g

Operating temperature: 0 to + 40 °C

Operating humidity range: 35 to 85% RH (to avoid condensation)

11.1.4 Software WinControl-3

Program: WinControl-3 System Control and Data Acquisition Program (Microsoft Windows 10 and 11) for operation of measuring system via PC, data acquisition and analysis. Not compatible with Windows 10 on ARM

Saturation Pulse Analysis: Measured: F(t), F_0, F_M, F, F'_0 (also calculated), F'_M. PAR and leaf temperature when Monitoring Leaf-Clip JUNIOR-BD is connected. Calculated: F_0' (also measured), F_V/F_M and Y(II) (maximum and effective photochemical yield of PS II, respectively), q_L, q_P, q_N, NPQ, Y(NPQ), Y(NO) and ETR (electron transport rate)

Fitting Routines: Two routines for determination of the cardinal points α, I_k and ETRmax of light curves

Programmability: Fully programmable using batch file language

Computer Requirements: 1 free USB socket. Processor, 1 GHz. RAM, 512 MB. Screen resolution, 1024 x 600 pixels. Interface, USB 2.0/3.0

11.1.5 Miscellaneous items included

Light guide: 50 cm x 1.5 mm (length x diameter) plastic fiber

Sample clips: Open Leaf Clip 60° (angle between incident radiation from JUNIOR-PAM and leaf surface, 60°) and Magnetic Leaf Clip (angle between incident radiation from JUNIOR-PAM and leaf surface, 90°)

Fluorescence standard: 3 cm x 2 cm fluorescence foil

Polishing set: Set for fiber tips including various polishing pads

USB cable: A-B USB cable

Software on USB memory key: Including latest WinControl-3 software

Transport Case JUNIOR-T: Grey plastic box with handle. Interior: convoluted foam padding. Dimensions: 29 cm x 25 cm x 6 cm (L x W x H). Weight: 510 g

11.2 Accessories

11.2.1 Monitoring Leaf-Clip JUNIOR-BD

Micro quantum sensor: Selective PAR measurement, 0 to 2500 µmol m^{-2} s^{-1} PAR

Thermocouple: Ni-CrNi, -20 to +60 °C

Leaf temperature: -20 to +60 °C

Power supply: JUNIOR-PAM special socket

Cable length: 100 cm

Dimensions: 13 cm x 2.5 cm (max.) x 5.5 cm (max.) (L x W x H)

Weight: 162 g (including cable)

11.2.2 Extra-long Light-Guide

100 cm x 1.5 mm (length x diameter) plastic fiber for difficult to access samples or underwater measurements

Subject to change without prior notice

12 Guarantee

12.1 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.

12.2 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, 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.

12.3 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.

12.4 Applicable law

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

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