Instruction Guide

PHOTON SYSTEMS INSTRUMENTS

AquaPen-C AP 110-C
AquaPen-P AP 110-P

Please read the Guide before operating this product

Manual Version: 2023/07

www.psi.cz

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1 INFORMATION BEFORE USING AQUAPEN DEVICE

Read this manual carefully before operating the device. If you are not sure about something in the manual, contact the manufacturer for clarification.

	By accepting the device, the customer agrees to follow the instructions in this guide.

Always follow corresponding manuals while working with the AquaPen device or doing the maintenance.
It is forbidden to interfere with the hardware or software of the AquaPen device in any way without previous agreement with the manufacturer.

The following table presents basic highlight symbols used in this manual:

Symbol	Description
	Important information, read carefully.
	Complementary and additional information.

Tab. 1 Used symbols.

2 General Description

AquaPen (AP) is a lightweight, hand-held fluorometer intended for quick and reliable measurements of photosynthetic activity in algal, cyanobacterial or plant cell suspensions. The photosynthetic activity is derived from the chlorophyll fluorescence (ChlF) kinetics. ChlF is determined based on a Pulse Amplitude Modulated technique (PAM). For user convenience, all illumination protocols are predefined and AP offers a set of illumination protocols (more in chapter 5.1) to determined fast fluorescence kinetics known as OJIP-test as well as slow ChlF kinetics such as quenching analysis or Light response curve.

AquaPen is available in two versions: AquaPen-C AP 110-C and AquaPen-P AP 110-P.

AquaPen AP 110-C, cuvette version is equipped with blue (455 nm) and red (630 nm) LED emitters whereas AquaPen AP 110-P, probe version incorporates just blue (470 nm) LED emitter. These are optically filtered and precisely focused to deliver PAR values of up to 3,000 $\mu mol.m^{-2}.s^{-1}$ to measured volume. Blue excitation light is intended for excitation of chlorophylls and thus for measurements of algal cultures and plant cell suspensions. Red-orange excitation light is suitable for measurements of cyanobacteria which tend to absorb inefficiently the blue light.

AquaPen-P AP 110-P is a probe version, which allows detection of chlorophyll fluorescence in liquid samples by directly submersing the probe in the suspension medium. It is designed for laboratory measurement and for field studies (in ponds and natural bodies of water). This AquaPen version is supplied with single blue LED emitter (optionally red or white).

AquaPen-C AP 110-C is a cuvette version of the fluorometer. The sample is measured in a plastic cuvette inserted into an optical holder with a lid. This version of the AP can also be used in laboratory conditions or field studies where samples of suspension may be obtained and placed in the AP. The AP 110-C contains a built-in turbidity meter for measurements of optical densities in addition to chlorophyll fluorescence. The AP 110-C also contains two LED emitters, blue and red.

Both AP versions have ultra-high sensitivity to chlorophyll with detections of up to 0.5 µg Chl/l – therefore natural water samples containing very low concentrations of phytoplankton can be measured.

AP can be operated as a stand-alone instrument. Measured data are sequentially stored in the internal AquaPen memory. Data transfer is via USB and Bluetooth communication. Comprehensive FluorPen 1.1 software provides data transfer routines and many additional features for data viewing in tables and graphs.

	AP 110-P does not measure Optical Density.

2.1 TECHNICAL SPECIFICATION

AquaPen	
Protocols	F_t – instantaneous chlorophyll fluorescence Quantum Yield OJIP Non-photochemical quenching Light curve Optical density at 680 and 720 nm (AP 110-C only)
LED emitter	AP 110-C: Red-orange (630 nm) and blue (455 nm) AP 110-P: Blue (470 nm), other wavelengths on request
Saturating pulse Illumination	Up to 3,000 $\mu mol.m^{-2}.s^{-1}$ (adjustable from 10 to 100 %)
Actinic Illumination	Adjustable from 10 to 1,000 $\mu mol.m^{-2}.s^{-1}$
Measuring Illumination	Up to 0.09 $\mu mol.m^{-2}.s^{-1}$ per pulse (adjustable from 10 to 100 %)
Detector	PIN photodiode with bandpass filters Wavelength range from 667 to 750 nm
Internal memory capacity	Up to 16 Mb
Internal data logging	Up to 149,000 measurements (depending on protocol)
Data transfer	USB cable Bluetooth (transfer up to 3Mbps for distance up to 20 m)
PC software	FluorPen 1.1 (Windows 7 and higher)
Battery	Li-Ion rechargeable battery Capacity 2000 mAh Max. charging current 0.5 A Charging via USB port - PC, power bank, USB charger, etc. 48 hours typical with full operation Low battery indicator
Sample holder	AP 110-C: 4 ml cuvette AP 110-P: Submersible optical probe
Display	Graphical display
Keypad	Sealed, 2-key tactile response Turns off after 5 minutes of no use
Built in GPS module	Ultra-high sensitivity down to -165dBm High accuracy of <1.5 m in 50 % of trials
Size	165 x 65 x 55 mm
Weight	290 g
Operating conditions	Temperature: 0 to +55 °C Relative humidity: 0 to 95 % (non-condensing)
Storage conditions	Temperature: -10 to +60 °C Relative humidity: 0 to 95 % (non-condensing)
Warranty	1-year parts and labor

Bluetooth module compliance data		
Category	Country	Standard
Radio	USA	FCC Part 15 Subpart B: 2008 Class B FCC CRF Title 47 Part 15 Subpart C
	FCC ID:	T9J-RN42
	Europe	ETSI EN 301 489-1 V1.8.1 ETSI EN 301 489-17 V2.1.1 ETSI EN 300 328 V1.7.1
	Canada	IC RSS-210 low power comm. device
	Certification number:	6514A-RN42
EMC	USA	FCC CFR47 Part 15 subclass B
	Europe	EN 55022 Class B radiated EN61000-4-2 ESD immunity EN61000-4-3 radiated field EN61000-4-6 RF immunity EN61000-4-8 power magnetic immunity

3 Device Description

Fig. 1 Device description.

3.1 LIST OF EQUIPMENT AND CUSTOMER INFORMATION

Standard version of the AquaPen device package consists:

- AquaPen-C AP 110-C or AquaPen-P AP 110-P
- Carrying Case
- pieces of 4 ml volume plastic cuvette with stopper (AquaPen-C only)
- FluorPen software and driver (on a USB flash disc)
- Operation Manual (PDF on a USB flash disc)
- USB Cable
- Other Accessories or Optional Features (according to your specific order)

	For data download via USB connection, the USB driver needs to be installed on the PC. It can be found on the installation disk (USB driver folder). If any item is missing, please, contact the manufacturer. Also check the carton for any visible external damage. If any damage is found, notify the carrier and the manufacturer immediately. The carton and all packing materials should be retained for inspection by the carrier or insurer. For customer support, please write to: support@psi.cz

3.2 CARE AND MAINTENANCE

AquaPen-P AP 110-P

- Never submerge the whole device in the liquid!
- Only the optical tip can be submerged!
- Rinse the optical tip of the AquaPen-P in freshwater after each use.
- Inspect visually the optical window after each use. If cleaning is needed, use soapy water and soft, non-abrasive tissue for cleaning the optical part.
- The device should not come in contact with any organic solvents, strong acids or bases.

AquaPen-C AP 110-C

- Never submerge the device in water!
- Keep the optical part clean and dry. If cleaning is needed, use soft, non-abrasive tissue.
- The device should not come in contact with any organic solvents, strong acids or bases.
- To measure samples, use a standard 4 ml volume cuvette (plastic cuvettes with 4 clear faces for visible range from Kartell are recommended). Fill the cuvette with 3 ml of the sample. Minimal volume for accurate measurements is 2 ml.
- Clean the cuvettes with distilled water, avoid contact with alcohol and solvents.

Li-ion battery

- Avoid fully discharging of the battery.
- Do not keep the battery at full charge for all the time.
- Keeping at high temperatures shortens battery life.

4 PRINCIPLE OF MEASUREMENT

AquaPen is a fluorometer adapted for measurements of chlorophyll fluorescence parameters in liquid suspensions of algae, cyanobacteria and isolated plant cells. Two versions of the AquaPen are available, the cuvette version (AP-C) and the probe version (AP-P). Both versions are equipped with a **blue LED emitter** (455 nm for AP-C, 470 nm for AP-P)**. The cuvette version of the AquaPen also has a red LED emitter (Fig. 3A).** These are optically filtered and precisely focused to deliver light intensities of up to 3,000 µmol.m^{-2}.s^{-1}. **Blue excitation light is intended for chlorophyll excitation, i.e., for measuring chlorophyll fluorescence in algal cultures and plant cell suspensions. Red-orange excitation light is intended for excitation of phycobilins and is suitable for measuring in cyanobacterial cultures.** The AquaPen **can detect chlorophyll levels down to 0.5 µg Chl/l.** Because of this high sensitivity it can be used for measurements of natural water samples containing low concentrations of phytoplankton.
Chlorophyll fluorescence parameters measured by both versions of the AquaPen are F_t, QY, NPQ, OJIP Analysis, Light Curve response of QY. The cuvette version of the AquaPen (AP 110-C) also measures optical density at 680 and 720 nm.
To use measurements of chlorophyll fluorescence to analyze photosynthesis, researchers must distinguish between **photochemical quenching** and **non-photochemical quenching** (heat dissipation). This is achieved by stopping photochemistry, which allows measurements of fluorescence in the presence of non-photochemical quenching alone. To reduce photochemical quenching to negligible levels, a high intensity, short flash of light is applied to the sample. This transiently closes all PSII reaction centers, and prevents energy of PSII being passed to downstream electron carriers. Non-photochemical quenching will not be affected if the flash is short. During the flash, the fluorescence reaches the high level in the absence of any photochemical quenching, known as **maximum fluorescence F_m**. The efficiency of photochemical quenching (which is a proxy of the efficiency of PSII) can be estimated by comparing F_m to the **steady yield of fluorescence in the light F_t** and the yield of fluorescence in the **absence of photosynthetic light F_0**. The efficiency of non-photochemical quenching is altered by various internal and external factors. Alterations in heat dissipation mean changes in F_m. Heat dissipation cannot be totally stopped, so the yield of chlorophyll fluorescence in the absence of non-photochemical quenching cannot be measured. See picture below.

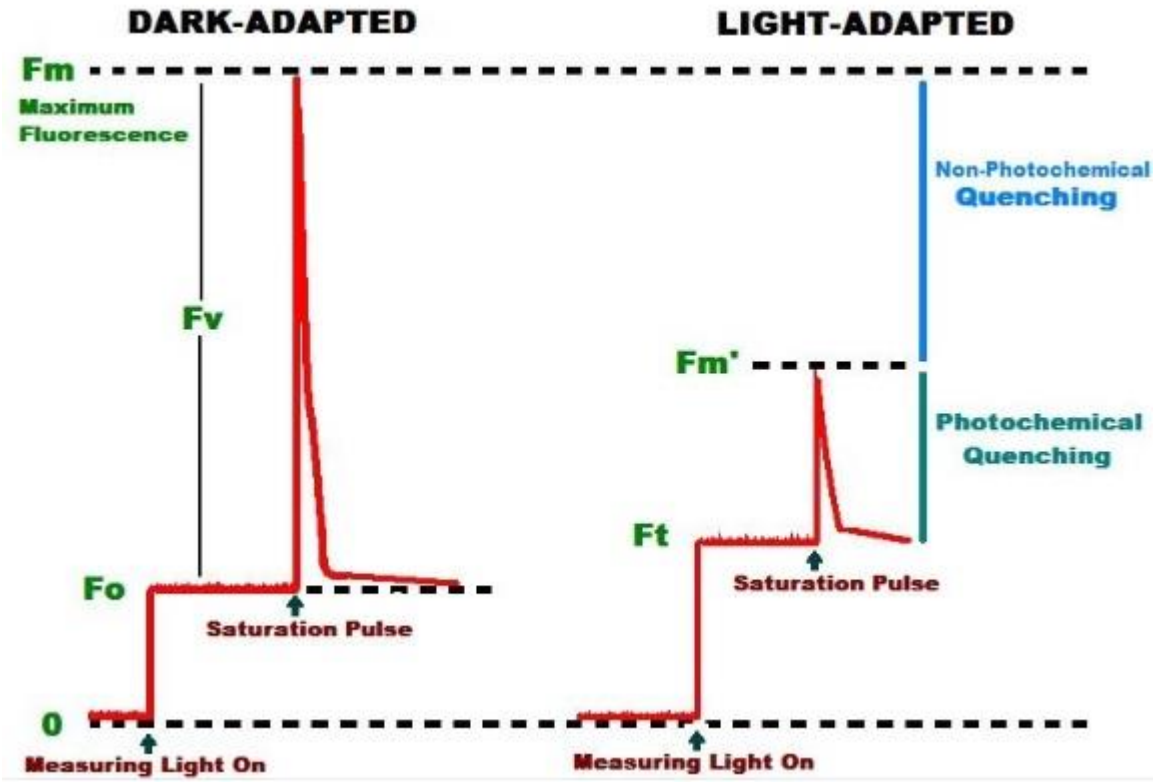


Fig. 2 Chlorophyll fluorescence.

Measuring the optical density of growing cultures is a common method to quantify various important culture parameters like cell concentration, biomass production or changes in the cell morphology. The cuvette version of the AquaPen measures OD at two wavelengths 680 and 720 nm.

AquaPen measures:

Protocol	Description
F_t - Instantaneous Chlorophyll Fluorescence	Ft is equivalent to F0 if the sample is dark-adapted.
QY - Quantum Yield	QY is a measure of the Photosystem II efficiency. QY is equivalent to Fv/Fm in dark-adapted samples and to Fv'/Fm' in light-adapted samples.
OJIP - Chlorophyll Fluorescence Induction Kinetics	The OJIP curves show major changes that occur during exposure of a sample to high irradiance (see more in chapter 5.1.2).
NPQ - Non-Photochemical Quenching	The NPQ protocol is used to quantify photochemical and non-photochemical quenching. The measurement should be performed with a dark-adapted sample (see More in chapter 5.1.3).
LC - Light Curve	Photosystem II Quantum Yield estimated from fluorescence that is measured sequentially at several different light levels. More in chapter 5.1.4.
OD - Optical Density* at 680 nm and 720 nm. (AP-C only)	Optical density at 680 nm represents light scattering and chlorophyll absorption. Optical density at 720 nm represents light scattering that corresponds to cell density. More in chapter 5.2.

*Optical density is defined as **OD = -Log(I/Io)**

Io is the irradiance that is transmitted through the cuvette filled with medium without algae or cyanobacteria. This quantity must be measured as the reference.

I is the irradiance transmitted through the cuvette with algal or cyanobacterial suspension in which the OD is measured.

Log is the decadic logarithm of the I/Io ratio. Thus, the optical density OD=1 means that the light at the respective wavelength is attenuated by the algae or cyanobacteria 10 times relative to the reference. With OD=2, the attenuation relative to the reference is 100 times.

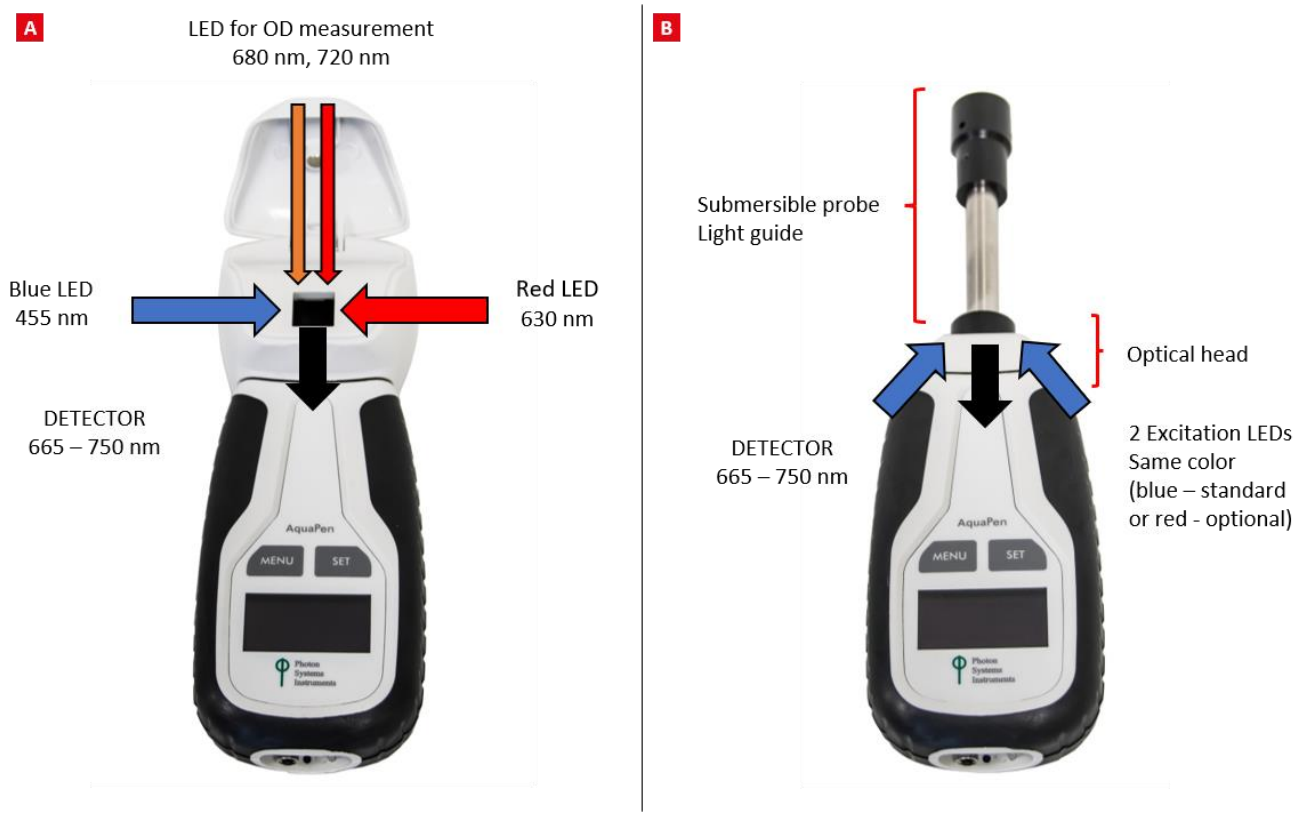


Fig. 3 A) AquaPen-C AP 110-C. B) AquaPen-P AP 110-P.

5 GETTING STARTED

For more detailed information on steps of AquaPen operation please refer to chapter 6.
The device is powered by built in Li-Ion battery. Ensure that the battery if fully charged by plugging it into a PC via USB cable or the AP outlet via USB adapter (not included) and the cable.

The AquaPen is controlled using two buttons:

- Use the **MENU** key to scroll through sequential menu options on the digital display and to turn the device off (hold for 3 sec).
- Use the **SET** key to turn the device on (hold for 3 sec) and select a menu option based on cursor (>) position.

5.1 MEASUREMENTS BASED ON FLUORESCENCE

5.1.1 PULSES DESCRIPTION AND SETTING

Flash pulse
This function serves for setting of measuring pulses intensity. The measuring pulses are weak light pulses, which are able to induce the minimal chlorophyll fluorescence (F_0 or F_t). It takes only 30 µs and the maximum intensity is 3,000 µmol.m^{-2}.s^{-1}. It means 30 µs * 3,000 µmol.m^{-2}.s^{-1} = 0.09 µmol.m^{-2} per pulse is the maximal intensity of the flash pulse.

Super pulse
This function serves for setting intensity of the saturating pulse. Saturating light pulse is able to induce maximum chlorophyll fluorescence (F_m). 100% of intensity represents approximately 3,000 µmol.m^{-2}.s^{-1}.

Actinic pulse
This function serves for setting intensity of actinic light. It is the ambient light in which the algae are growing. 100 % of intensity equals approximately 1,000 µmol.m^{-2}.s^{-1}.

Pulses used in predefined protocols:

Measurements based on fluorescence	Used pulses
F_t	Flash pulse
QY	Flash pulse, Super pulse
OJIP	Super pulse
NPQ protocols	Flash pulse, Super pulse, Actinic pulse
Light curves	Flash pulse, Super pulse (Actinic pulse is preset)

Default setting of light color and intensities in AquaPen firmware. These may be changed according to user requirements and algal growth conditions:

Measuring color	455 (470, respectively) nm
Flash pulse 30 %	Measuring flash pulse
Super pulse 50 %	Saturating pulse
Actinic pulse 100 µmol.m^{-2}.s^{-1} (10 %)	Actinic light

	Please note that those parameters are recommended by manufacturer and can be changed according to user needs.

Setting of optimal intensities of pulses:

Flash pulse setting

595	596	597
15:17:42 19.7.2016	15:19:01 19.7.2016	15:20:03 19.7.2016
QY	QY	QY
0.71	**0.69**	**0.68**
Fo Backgr 289	Fo Backgr 289	Fo Backgr 390
Fo Flash 2552	Fo Flash 4426	Fo Flash 8875
Fm Backgr 309	Fm Backgr 269	Fm Backgr 390
Fm Flash 7995	Fm Flash 13419	Fm Flash 26659
30% f_pulse	50% f_pulse	100 % f_pulse

Fig. 4 Optimization of Flash pulse intensity – QY data.
QY measurement performed with different intensities of Flash pulse. Optimal setting is highlighted in red rectangle.

The optimum value of Flash pulse can be identified during QY measurement as shown in Fig. 4 below. Before performing QY measurement it is recommended to set the pulse color according to culture used (blue for algae and red for cyanobacteria) and intensity of Super pulse to 70 %.
Please note that QY measurement should be performed with dark adapted suspension. following the first exposure to flash pulse (during QY measurement) the sample may be inhibited and it is recommended to use a new dark-adapted sample for future measurements or allow sufficient time to re-adapt the sample in the dark.
F_0 increases linearly with growing intensity of the Flash pulse. The Flash pulse setting recommended by manufacturer is 30 %. This intensity of Flash pulse may be increased if the culture is very dilute. Please note that high intensities of Flash pulse can cause undesirable "actinic effect" as a result of initiated photochemistry. These effects may lower F_0 and the QY values.

The optimal Flash pulse intensity is that at which the highest value of QY is reached. This can be determined by measuring QY at different flash pulse intensities using fresh dark-adapted suspensions of the same culture (Fig. 4). In this example the optimal flash pulse setting is 30 %.

Super pulse setting

To determine the optimal intensity of the Super pulse is to perform OJIP measurement with different suspensions of the same culture at different Super Pulse settings.
Please note that OJIP measurement should be performed with dark adapted culture. Similarly, as for QY measurements, new sample should be used for subsequent measurements of OJIP or sufficient time should be allowed for the sample to be dark adapted again.
The Super pulse setting recommended by manufacturer is 80 %.
When performing the OJIP measurement with different intensities of Super pulse the value of F_v/F_m will stop increasing with subsequent increases in Super pulse intensities. When that occurs, the Super pulse intensity is optimal for the culture (Fig. 5 and Fig. 6).

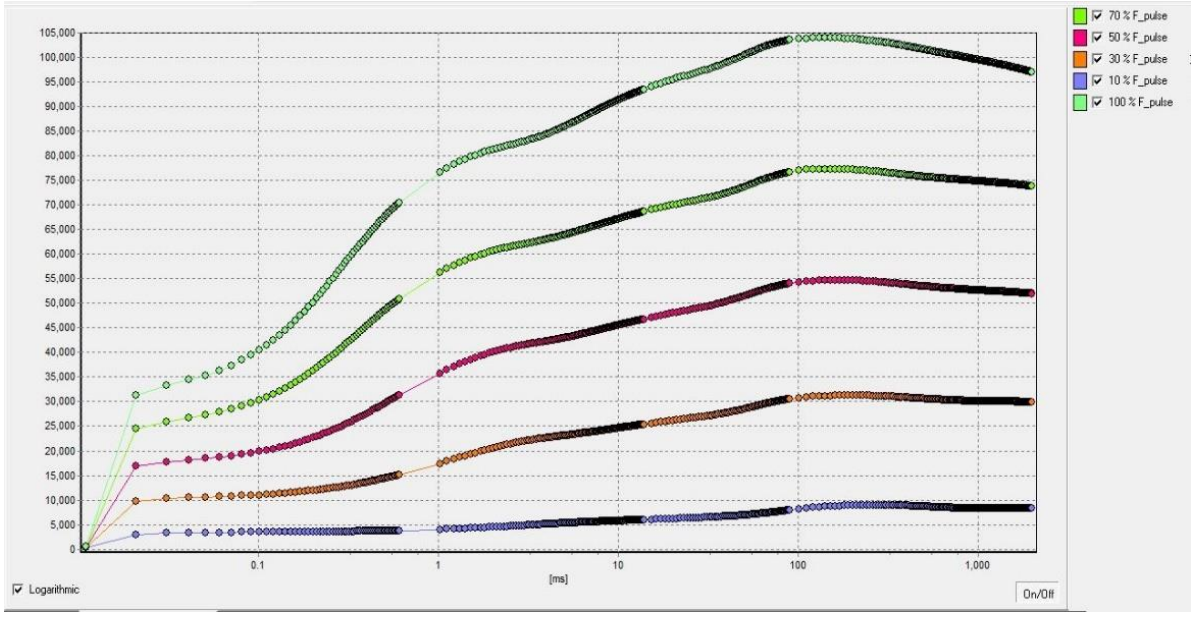
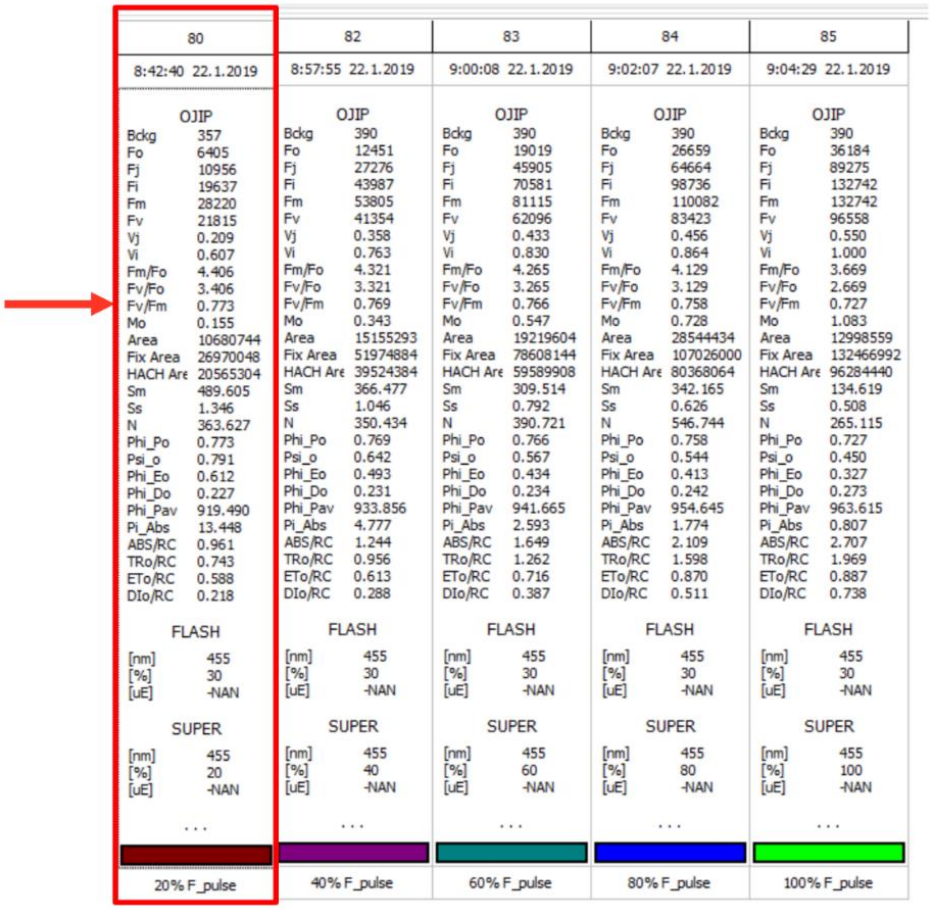


Fig. 5 Optimization of Super pulse intensity – OJIP curve.

OJIP curves - measurement performed with different intensities of Super pulse.



80	82	83	84	85
8:42:40 22.1.2019	8:57:55 22.1.2019	9:00:08 22.1.2019	9:02:07 22.1.2019	9:04:29 22.1.2019
OJIP				
Bckg 357	Bckg 390	Bckg 390	Bckg 390	Bckg 390
Fo 6405	Fo 12451	Fo 19019	Fo 26659	Fo 36184
Fj 10956	Fj 27276	Fj 45905	Fj 64664	Fj 89275
Fi 19637	Fi 43987	Fi 70581	Fi 98736	Fi 132742
Fm 28220	Fm 53805	Fm 81115	Fm 110082	Fm 132742
Fv 21815	Fv 41354	Fv 62096	Fv 83423	Fv 96558
Vj 0.209	Vj 0.358	Vj 0.433	Vj 0.456	Vj 0.550
Vi 0.607	Vi 0.763	Vi 0.830	Vi 0.864	Vi 1.000
Fm/Fo 4.406	Fm/Fo 4.321	Fm/Fo 4.265	Fm/Fo 4.129	Fm/Fo 3.669
Fv/Fo 3.406	Fv/Fo 3.321	Fv/Fo 3.265	Fv/Fo 3.129	Fv/Fo 2.669
Fv/Fm 0.773	Fv/Fm 0.769	Fv/Fm 0.766	Fv/Fm 0.758	Fv/Fm 0.727
Mo 0.155	Mo 0.343	Mo 0.547	Mo 0.728	Mo 1.083
Area 10680744	Area 15155293	Area 19219604	Area 28544434	Area 12998559
Fix Area 26970048	Fix Area 51974884	Fix Area 78608144	Fix Area 107026000	Fix Area 132466992
HACH Are 20565304	HACH Are 39524384	HACH Are 59589908	HACH Are 80368064	HACH Are 96284440
Sm 489.605	Sm 366.477	Sm 309.514	Sm 342.165	Sm 134.619
Ss 1.346	Ss 1.046	Ss 0.792	Ss 0.626	Ss 0.508
N 363.627	N 350.434	N 390.721	N 546.744	N 265.115
Phi_Po 0.773	Phi_Po 0.769	Phi_Po 0.766	Phi_Po 0.758	Phi_Po 0.727
Psi_o 0.791	Psi_o 0.642	Psi_o 0.567	Psi_o 0.544	Psi_o 0.450
Phi_Eo 0.612	Phi_Eo 0.493	Phi_Eo 0.434	Phi_Eo 0.413	Phi_Eo 0.327
Phi_Do 0.227	Phi_Do 0.231	Phi_Do 0.234	Phi_Do 0.242	Phi_Do 0.273
Phi_Pav 919.490	Phi_Pav 933.856	Phi_Pav 941.665	Phi_Pav 954.645	Phi_Pav 963.615
Pi_Abs 13.448	Pi_Abs 4.777	Pi_Abs 2.593	Pi_Abs 1.774	Pi_Abs 0.807
ABS/RC 0.961	ABS/RC 1.244	ABS/RC 1.649	ABS/RC 2.109	ABS/RC 2.707
TRo/RC 0.743	TRo/RC 0.956	TRo/RC 1.262	TRo/RC 1.598	TRo/RC 1.969
ETo/RC 0.588	ETo/RC 0.613	ETo/RC 0.716	ETo/RC 0.870	ETo/RC 0.887
DIo/RC 0.218	DIo/RC 0.288	DIo/RC 0.387	DIo/RC 0.511	DIo/RC 0.738
FLASH				
[nm] 455	[nm] 455	[nm] 455	[nm] 455	[nm] 455
[%] 30	[%] 30	[%] 30	[%] 30	[%] 30
[uE] -NAN	[uE] -NAN	[uE] -NAN	[uE] -NAN	[uE] -NAN
SUPER				
[nm] 455	[nm] 455	[nm] 455	[nm] 455	[nm] 455
[%] 20	[%] 40	[%] 60	[%] 80	[%] 100
[uE] -NAN	[uE] -NAN	[uE] -NAN	[uE] -NAN	[uE] -NAN
...	...	...	...	...
20% F_pulse	40% F_pulse	60% F_pulse	80% F_pulse	100% F_pulse

Fig. 6 Optimization of Super pulse intensity – OJIP data.

OJIP data - measurement performed with different intensities of Super pulse. The highest F_v/F_m value indicates the optimal intensity of Super pulse (20 % in this case).

Actinic pulse setting

Intensity of Actinic pulse should correspond with cultivation light intensity or should be set according to application.

	If Overflow is displayed during the measurement, there are two ways to solve this problem - dilute the sample or decrease the pulse intensity. If Low is displayed during the measurement, there are two options - concentrate the sample or increase the pulse intensity.

MEASUREMENT

No device calibration is required before making chlorophyll fluorescence measurement. Results of fluorescence measurement are affected by the device settings and the physiology of the sample.

Steps for Chlorophyll Fluorescence measurements with AquaPen-C:

- Fill the cuvette with the sample of algae or cyanobacteria and close the cuvette with the stopper. Fill the cuvette with 3 ml of the sample. Minimal volume for accurate measurements is 2 ml.
- Place the cuvette with the sample inside the AquaPen cuvette holder and close the cover to allow dark adaptation.
- Dark adaptation of the sample is required prior to the following measurements: F_0, QY, NPQ, LC. Duration of dark-adaptation period depends on species but mostly varies between 5 and 15 minutes.
- Mix the sample to avoid sedimentation by holding the AquaPen cover and turning over a few times. This is essential to prevent inaccurate readings.
- Turn ON the device – hold SET button for 1 sec.
- Select Measurement and press SET > select required parameter for example - F_t.
- Press SET to start the measurement.
- AquaPen will measure the parameter. If a protocol was selected such as OJIP, LC or NPQ the display will only show the progress of the measurements in % but no data will be visible.
- When measuring F_t and QY the value of the parameter will appear on the display after completion of the measurement. To visualize the data obtained with OJIP, NPQ or LC protocol recorded data has to be download from the AquaPen to the PC computer via USB cable or the Bluetooth connection using FluorPen Software (page 41).
- All measured data are stored in the device memory and can be downloaded to PC after completion of the experiment.

Steps for Chlorophyll Fluorescence measurement with AquaPen-P:

- For measurements of F_t, QY, NPQ, LC the sample requires dark adaptation period of 5-15 min (this varies with species). Place the sample in the dark to achieve this. Turn ON the device – hold SET button for 1 sec.
- Select Measurement and press SET > select required parameter for example - F_t.
- Submerge the probe in the sample and ensure that no air bubbles get trapped inside the probe.
- Press SET to start measurements.
- AquaPen will measure the parameter. If a protocol was selected such as OJIP, LC or NPQ the display will only show the progress of the measurements in % but no data will be visible.
- When measuring Ft and QY the value of the parameter will appear on the display after completion of the measurement. To visualize the data obtained with OJIP, NPQ or LC protocol, recorded data has to be download from the AquaPen to the PC computer via USB cable or the Bluetooth connection using FluorPen Software (page 41).
- All measured data are stored in the device memory and can be downloaded to PC after completion of the experiment.

5.1.2 OJIP PROTOCOL

The AquaPen device offers the protocol to capture rapid fluorescence transient – OJIP, which occurs during exposure of photosynthetic organisms to high irradiance. The FluorPen software enables data downloading to a PC and subsequent OJIP visualization of the analyzed data in a graphical and tabular format.

The OJIP protocol includes the following measured and calculated parameters:

Abbreviation	Explanation
Bckg	Background
F_0	$F_0 = F_{50\mu s}$, fluorescence intensity at 50 µs
F_j	F_j = fluorescence intensity at J-step (at 2 ms)
F_i	F_i = fluorescence intensity at i-step (at 30 ms)
F_m	F_m = maximal fluorescence intensity
F_v	$F_v = F_m - F_0$ (maximal variable fluorescence)
V_j	$V_j = (F_j - F_0) / (F_m - F_0)$
V_i	$V_i = (F_i - F_0) / (F_m - F_0)$
F_m / F_0	
F_v / F_0	
F_v / F_m	
M_0 or $(dV/dt)_0$	$M_0 = TR_0 / RC - ET_0 / RC = 4 (F_{300} - F_0) / (F_m - F_0)$
Area	Area between fluorescence curve and F_m (background subtracted)
Fix Area	Area below the fluorescence curve between $F_{40\mu s}$ and F_{1s} (background subtracted)
S_M	S_M = Area / ($F_m - F_0$) (multiple turn-over)
S_S	S_S = the smallest S_M turn-over (single turn-over)
N	$N = S_M . M_0 . (1 / V_J)$ turn-over number Q_A
Phi_P_0	Phi_$P_0 = 1 – (F_0 / F_m)$ (or F_v / F_m)
Psi_$_0$	Psi_$_0 = 1 - V_J$
Phi_E_0	Phi_$E_0 = (1 – (F_0 / F_M)) . Psi_{_0}$
Phi_D_0	Phi_$D_0 = 1 –$ Phi_$P_0 = (F_0 / F_m)$
Phi_Pav	Phi_Pav = Phi_P_0 (S_M / t_{Fm}) t_{Fm} = time to reach F_m (in ms)
ABS / RC	$ABS / RC = M_0 . (1 / V_J) . (1 /$ Phi_$P_0)$
TR_0 / RC	$TR_0 / RC = M_0 . (1 / V_J)$
ET_0 / RC	$ET_0 / RC = M_0 . (1 / V_J) .$ Psi_$_0$
DI_0 / RC	$DI_0 / RC = (ABS / RC) – (TR_0 / RC)$

Tab. 2 OJIP Protocol – measured and calculated parameters.

Formulas derived from: R.J. Strasser, A. Srivastava and M. Tsimilli-Michael (2000): The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Probing Photosynthesis: Mechanism, Regulation and Adaptation (M. Yunus, U. Pathre and P. Mohanty, eds.), Taylor and Francis, UK, Chapter 25, pp 445-483.

5.1.3 NON-PHOTOCHEMICAL QUENCHING (NPQ) PROTOCOLS

The NPQ protocol is used to quantify photochemical and non-photochemical quenching. It should be performed with dark-adapted samples. The NPQ protocol starts with a measurement of minimal level of fluorescence F_0 during a dark period. A short saturating flash of light is then applied to reduce the plastoquinone pool and measure maximum fluorescence in the dark-adapted state, F_m. After a short dark relaxation, the sample is exposed to actinic irradiance for tens to hundreds of seconds to elicit a transient called the Kautsky effect. A sequence of saturating flashes is then applied during the exposure to the actinic light to probe the non-photochemical quenching NPQ and effective quantum yield of photosynthesis *QY* in light adapted state. After exposure to continuous illumination, the relaxation of non-photochemical quenching is determined by means of saturating pulses applied in dark. This sequence of the protocol is illustrated in Fig. 7.

The AquaPen comes with three predefined NPQ protocols, NPQ1, NPQ2 and NPQ3. The protocols differ in the duration of the light exposure and the dark recovery phase, in the number and interval between pulses. See Tab. 3.

	Phase	Duration	# of pulses	1st pulse	Pulse interval
NPQ1	Light	60 s	5	7 s	12 s
	Dark recovery	88 s	3	11 s	26 s
NPQ2	Light	200 s	10	10 s	20 s
	Dark recovery	390 s	7	20 s	60 s
NPQ3	Light	200 s	10	11 s	21 s
	Dark recovery	60 s	2	20 s	21 s

Tab. 3 NPQ Protocols.

The NPQ protocols include the following measured and calculated parameters (Tab. 4):

Abbreviation	Explanation
F_0	minimum fluorescence in dark-adapted state
F_m	maximum fluorescence in dark-adapted state, measured during the first saturation flash after dark adaptation
F_p	fluorescence in the peak of fast Kautsky induction
Fm_Ln, Lss, D, Dn[1]	maximum fluorescence
QYmax[2]	maximum quantum yield of PSII in dark-adapted state - F_v/F_m
QY_Ln, Lss, D, Dn[1,3]	effective quantum yield of PSII
NPQ_Ln, Lss, D, Dn[1,4]	non-photochemical chlorophyll fluorescence quenching
Qp_Ln, Lss, D, Dn[1,5]	coefficient of photochemical quenching, an estimate of open PSII reaction centers

Tab. 4 NPQ protocols - measured and calculated parameters.

[1] *L* - indicates light adapted parameters; *D* - refers to dark recovery phase after switching of the actinic illumination; *n* - represents a sequential number of light phases; *ss* - steady state
[2] Calculated as $(F_m - F_0) / F_m$
[3] Calculated as $(F_m\_Ln - F_t\_Ln) / F_m\_Ln$ or of corresponding steady state or dark recovery parameters
[4] Calculated as $(F_m - F_m\_Ln) / F_m\_Ln$ or of corresponding ss, Dn or Dss parameters
[5] Calculated as $(F_m\_Ln - F_t\_Ln) / (F_m\_Ln - F_0\_Ln)$ or of corresponding ss, Dn or Dss parameters; F_0_Ln is calculated as $F_0 / ((F_m - F_0) / F_m + F_0 / F_m\_Ln)$.

For more details, please refer to: Oxborough K., Baker N.R. (1997): Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components: calculation of qP and Fv'/Fm' without measuring F0'. Photosynthesis Research 54: 135-142.

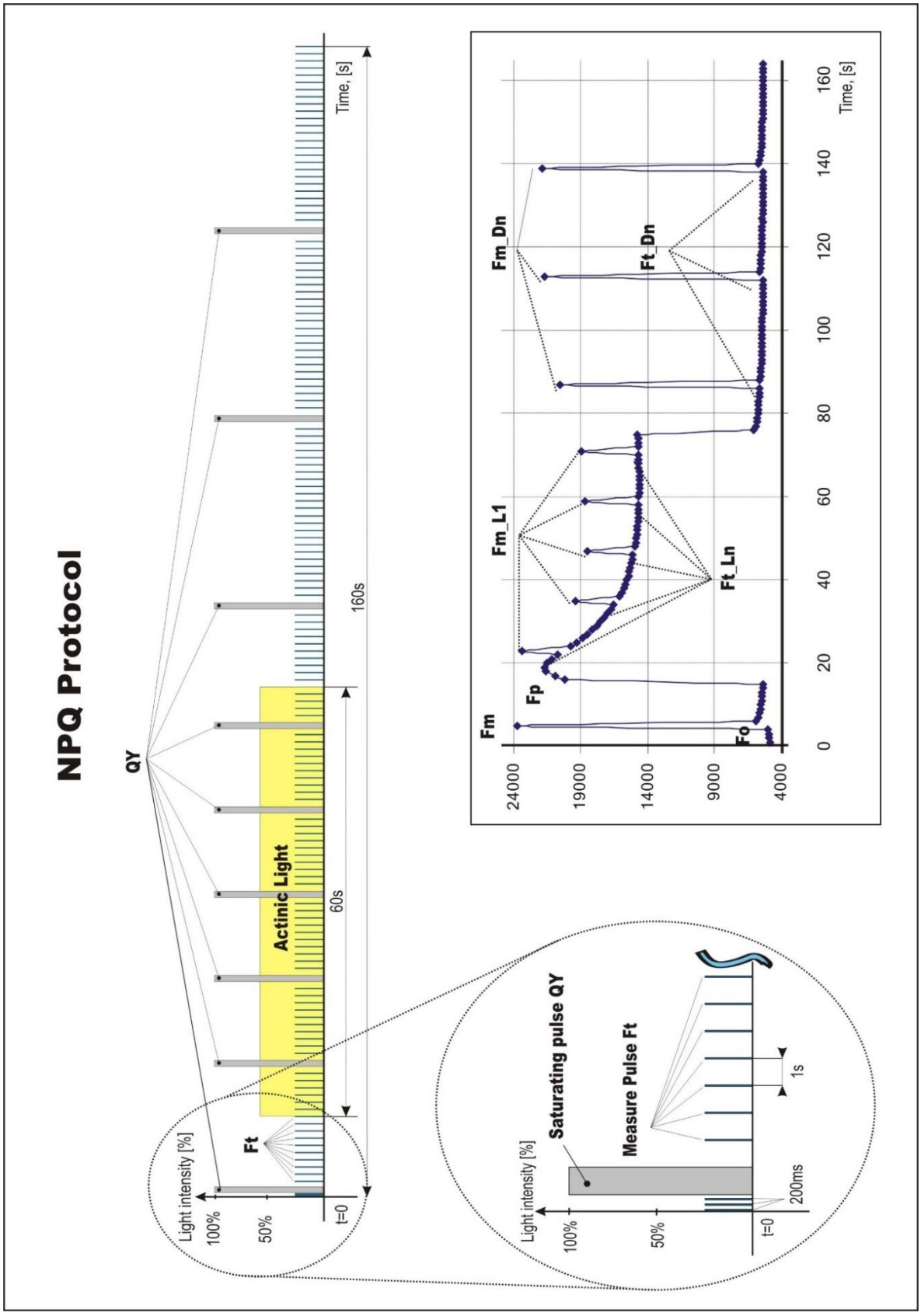


Fig. 7 NPQ1 Protocol.

5.1.4 LIGHT CURVE (LC) PROTOCOLS

The protocols called Light Curve (LC) were designed to acquire parameters for construction of Light Response Curve relating the rate of photosynthesis to photon flux density. The method is based on successive measurements of the sample exposed to a stepwise increase of light intensity. The effective quantum yields of photosynthesis are determined under various light intensities of continuous illumination. Measurement is based on pulse modulated fluorometry (PAM).

Three predefined LC protocols are available. These differ in number and duration of individual light phases and light intensities as shown in Tab. 3 below. The visual representation of the LC1 and LC2 protocols is shown in Fig. 8 and below Fig. 9.

	# of phases	Phase duration	Light intensities [µmol.m^{-2}.s^{-1}]
LC1	6	60 s	10; 20; 50; 100; 300; 500
LC2	5	30 s	100; 200; 300; 500; 1000
LC3	7	60 s	10; 20; 50; 100; 300; 500; 1000

Tab. 5 LC protocols.

The LC protocols include the following measured and calculated parameters:

Abbreviation	Explanation
F_0	minimum fluorescence in dark-adapted state
F_m	maximum fluorescence in dark-adapted state
F_m_Ln[1]	maximum fluorescence in light adaptation state
F_t_Ln[1]	instantaneous fluorescence during light adaptation
Qymax[2]	maximum quantum yield of PSII in dark-adapted state - F_v/F_m
QY_Ln[1, 3]	instantaneous PSII quantum yield induced in light

Tab. 6 LC protocols – measured and calculated parameters.

[1] *L* - indicates light adapted parameters; *n* - represents a sequential number of light phases
[2] Calculated as $(F_m - F_0) / F_m$
[3] Calculated as $(F_m\_Lx - F_t\_Lx) / F_m\_Lx$

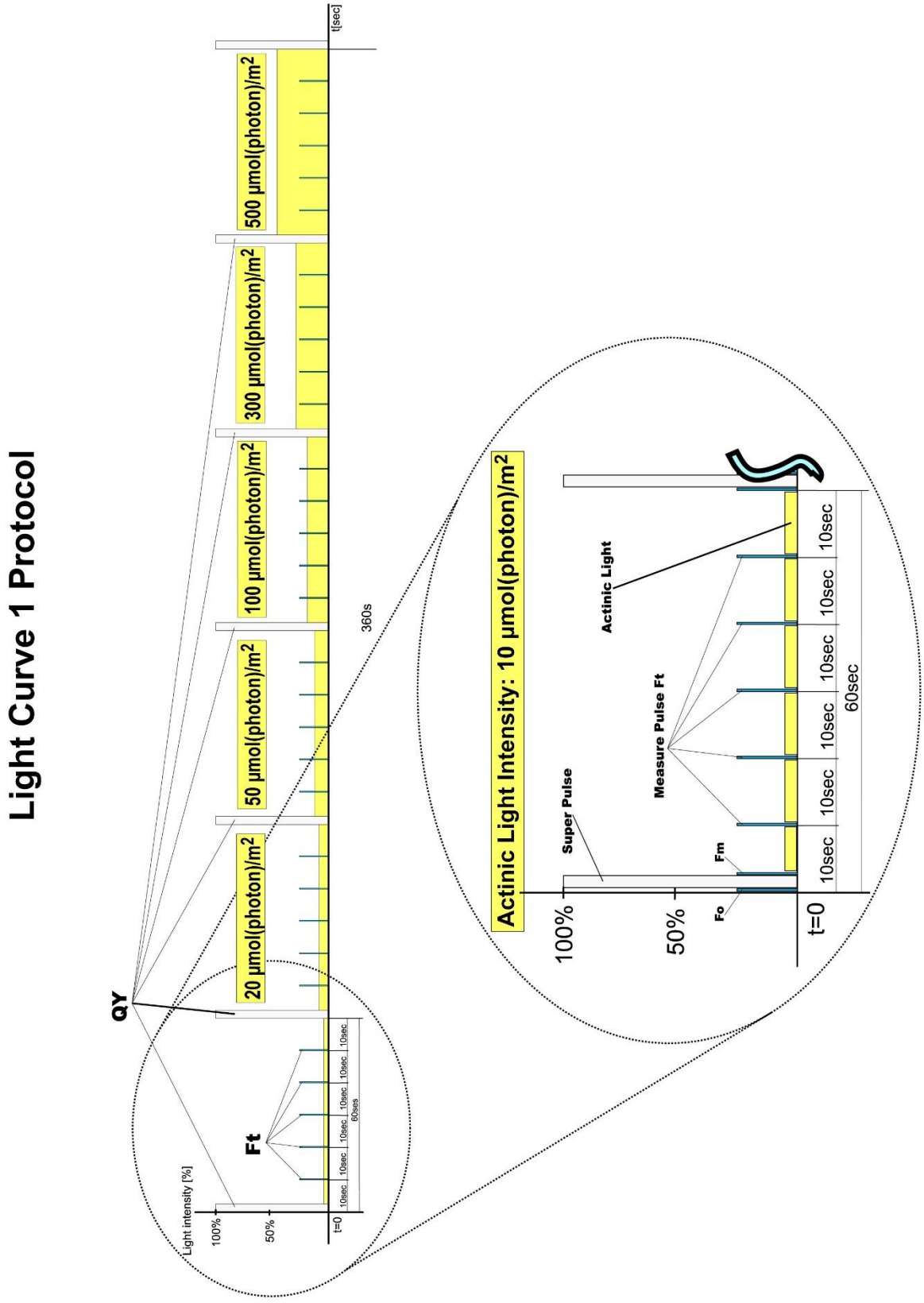


Fig. 8 LC1 Protocol.

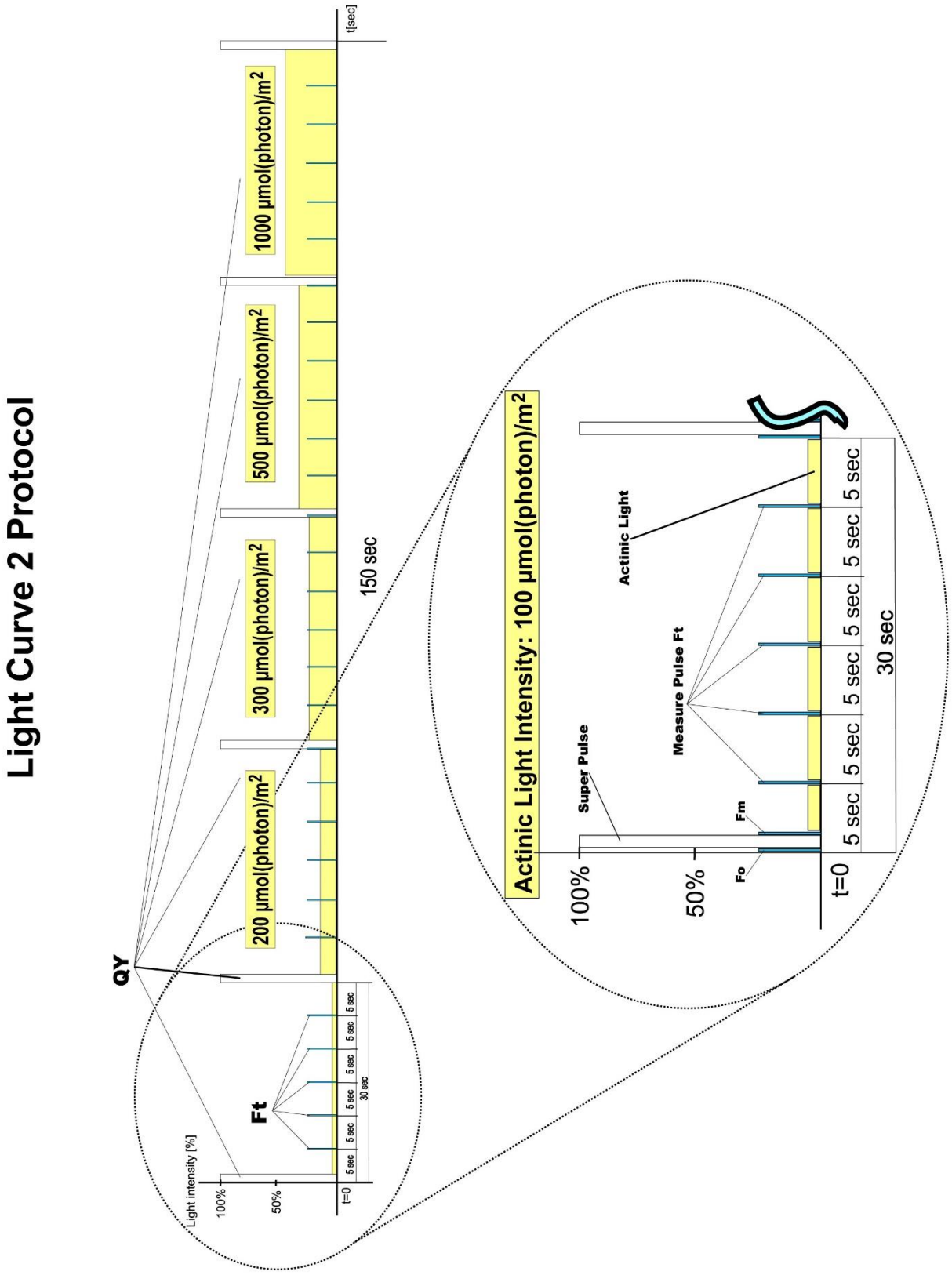


Fig. 9 LC2 Protocol.

5.2 Optical density measurement (AquaPen-C only)

5.2.1 Calibration

Calibration of the AquaPen is required before measuring OD. It can be done with either plain water or ideally, culture medium placed in a cuvette and the AquaPen. Calibration assures accurate OD measurements and it is recommended before every set of samples.

	It is highly recommended to calibrate AquaPen for OD measurements every time the instrument is switched ON.

Steps for calibration of the AquaPen for OD measurements:

- Use the standard 4 ml cuvette.
- Clean the cuvette with distilled water and paper tissue.
- As a calibration standard use cultivation medium (BBM, BG11 etc.) or distilled water.
- Put cuvette with medium (optimal volume 3 ml) into the AquaPen cuvette holder.
- Turn ON the device – hold SET button for 1 sec.
- select Measurement > OD > Calibration.
- Press SET button to start the calibration.
- To check the validity of the calibration, select Measurement > OD > OD680nm (or OD720nm) with the blank cuvette in the AquaPen.
- Press SET to do the measurement.
- The display should show value of 0.000.
- If the OD value is different than 0.000 repeat OD calibration again.
- Remember that the calibration is specific to a particular cuvette. New calibration should be performed for a new cuvette.
- Calibration is automatically stored in the device memory and is saved until the device is turn OFF.

	Please remember or mark the orientation of the cuvette when placed in the device. For repeated measurements it is recommended to position the cuvette always in the same orientation in the AP cuvette holder.

5.2.2 Measurement

- Fill the cuvette with a sample of algae or cyanobacteria and close the cuvette with the stopper. Minimal volume of sample is 2 ml.
- Place the cuvette with sample inside the AquaPen cuvette holder.
- Close the cover.
- Turn the device on by pressing SET key for 1 sec
- Select from the menu Measurement > OD680nm or OD720nm.
- Press SET to start the measurement.
- Value of the measured parameter will appear on the display. All measured data are stored in the AquaPen memory and can be downloaded to a PC via USB connection or Bluetooth connection using FluorPen software (page 41).

5.3 MULTIPLE MEASUREMENT

In addition to the single measurement of each available protocol, it is possible to perform also the multiple measurements of the same protocol over a period of time. The AquaPen may be set up to perform repeated measurements of the same parameter or protocol by selecting **Settings > Multi** (see Menu tree, page 25):
Multi type – select the required parameter or protocol - F_t, QY, OJIP...
Multi interval – set the time interval between measurements (the interval represents period from the protocol beginning to the next protocol beginning and so, the interval should be longer than the duration of the selected protocol)
Multi repeats – set the number of measurements

- Prepare the sample for measurement as described above.
- Select in the display menu: Measurement > Multi.
- Press SET to start the measurements.
- Values of measured parameters (F_t, QY) will appear on the AquaPen display after each measurement repetition and will be stored to the device memory automatically. If protocol (OJIP, NPQ, LC) was used all data will be saved to the device memory and visualization will be possible after the data download to the PC (page 41).

Modes of Multiple measurement:

There are two modes of the multiple measurements in the AquaPen.

The device is connected via USB to the computer.
The device measures according to the predefined protocol, interval and repeats. The device does not switch off between measurements and display a progress on the front control display. After reaching of the predefined number of repeats, the device turns off the Multiple measurement automatically.

AquaPen is not connected to the computer.
The device measures according to the predefined protocol and interval but it doesn't stop the Multiple measurement after reaching of the predefined repetitions: the device measures continuously as long as it is stopped manually (excepting the AP-C version that follows the set number of repetitions). Also, the device turns on and off between the measurements automatically (excepting the AP-C version that doesn't turn off during the measurements).

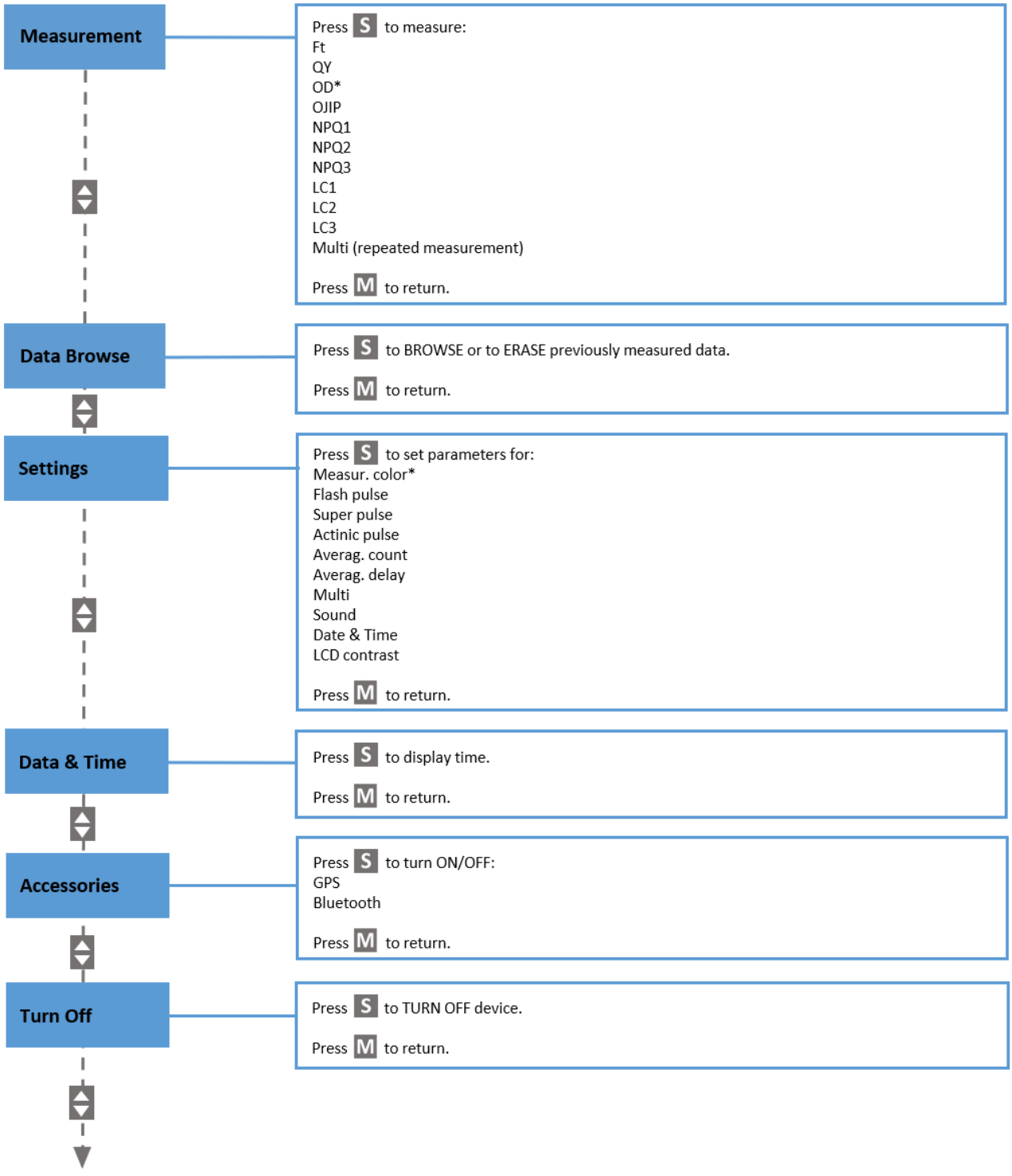
The Multiple measurement can be stopped manually by switching on the device (via the SET button) during the switched off period. Should the device be in the measuring mode, then the measurement needs to be interrupted via a long press of the MENU button and after that the Multiple measurement needs to be stopped by switching on the device via the SET button.

6 CONTROL MENU TREE

The next few pages of this manual show the structure of the firmware menu on the AquaPen device, and explain in a schematic way the operation of the AquaPen. The schematic shows the Main Menu, first-level Sub-Menus and second-level Sub-Menus.

- The blue color represents the Main Menu and its Options.
- The yellow color represents the first-level Sub-Menus and their Options.
- The green color represents the second-level Sub-Menus and their Options.
- Full-line arrows are used to indicate the **SET** key operations.
- Dashed-line arrows are used to indicate the **MENU** key operations.

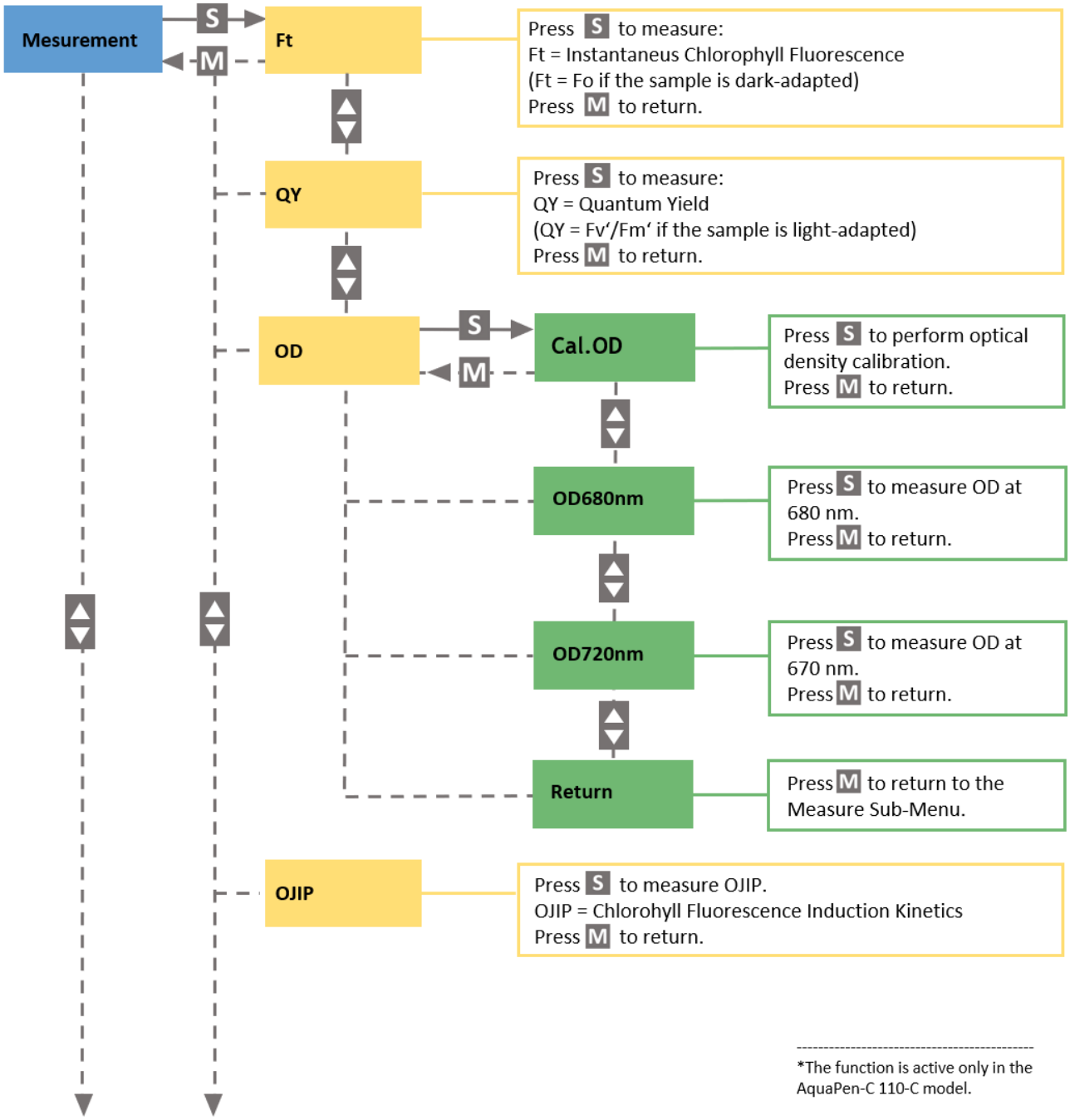
Menu Tree – Main



*The function is active only in the AquaPen-C 110-C model.

Measure Sub-Menu – Part 1

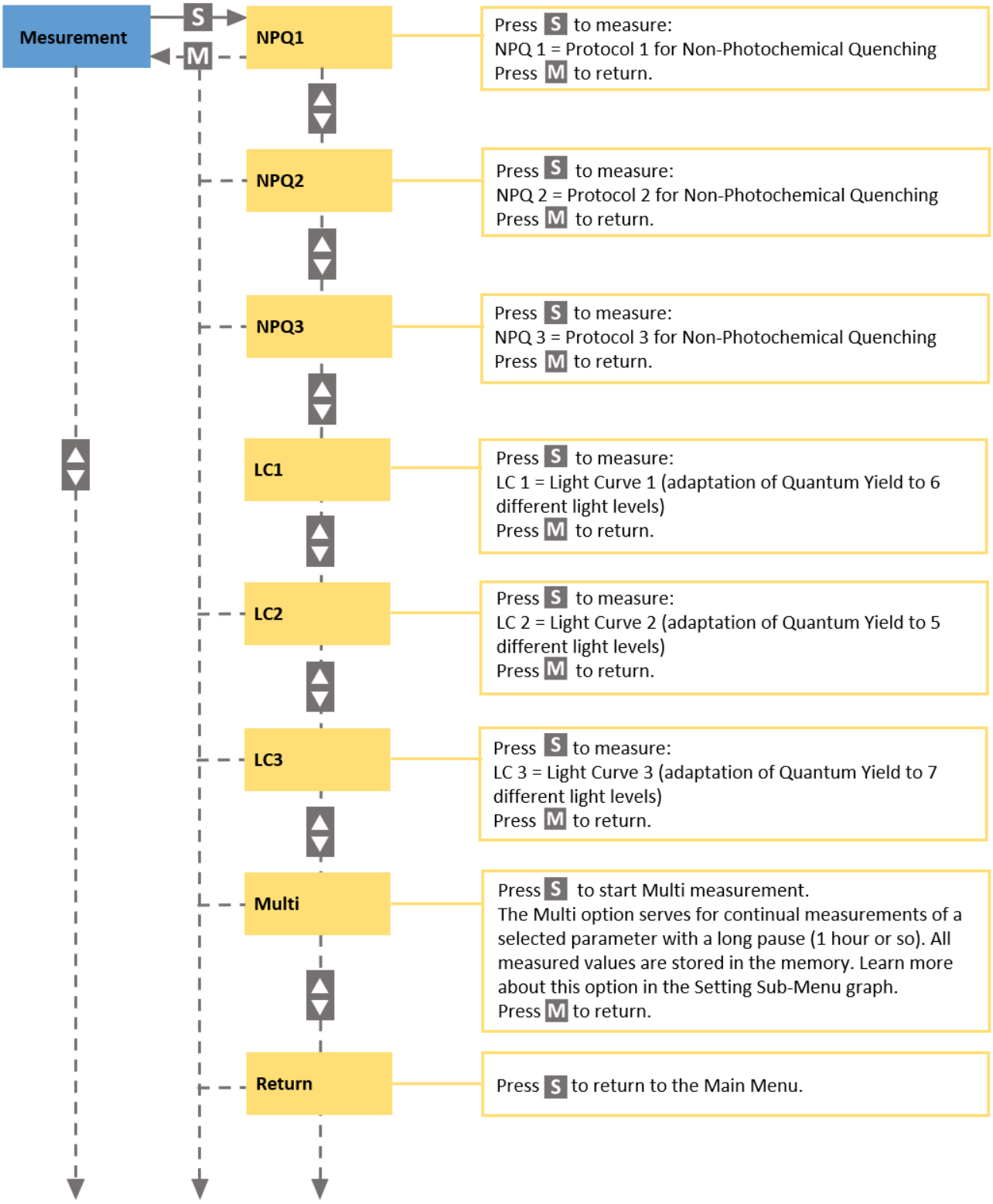
Use the Measure Sub-Menu when measuring selected parameters.



*The function is active only in the AquaPen-C 110-C model.

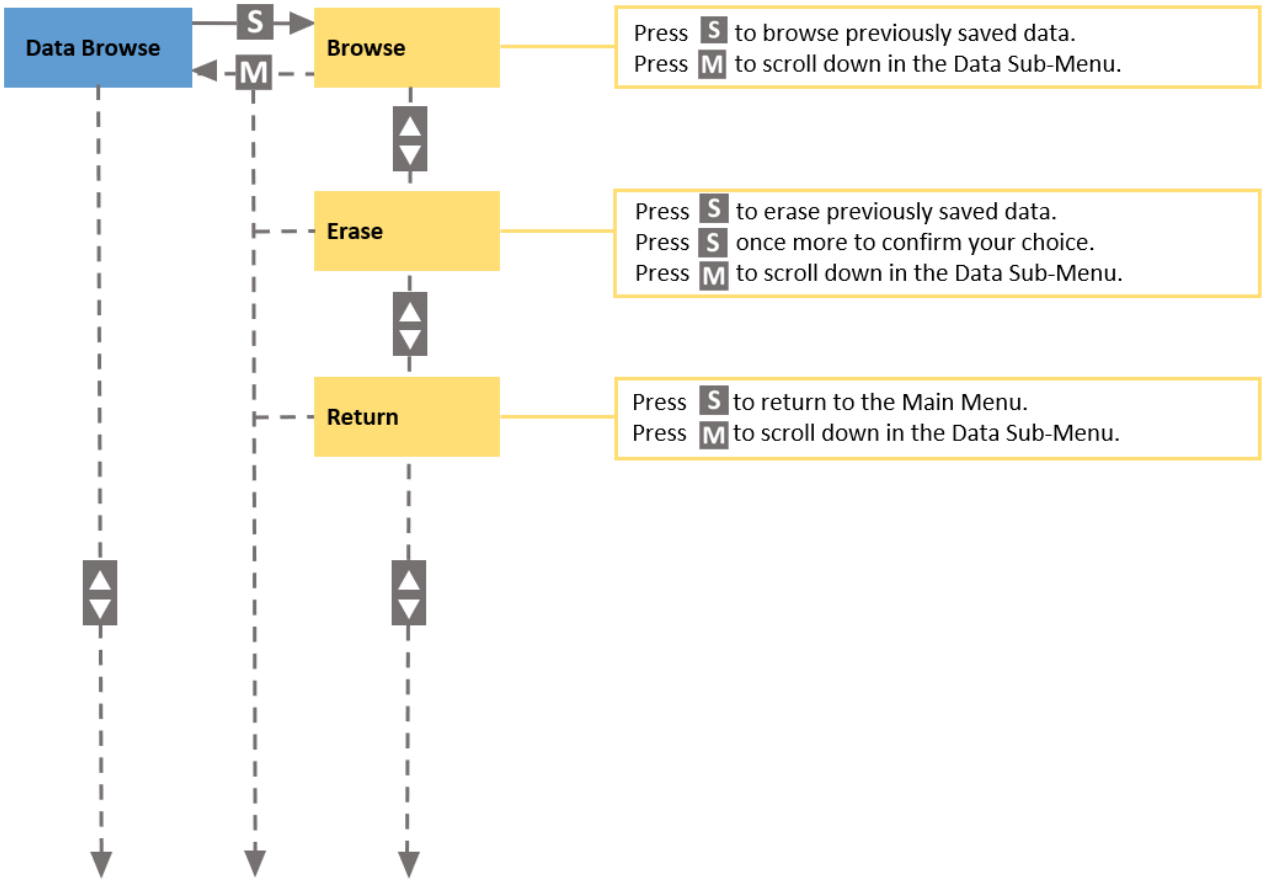
Measure Sub-Menu – Part 2

Use the Measure Sub-Menu when measuring selected parameters.



Data Sub-Menu

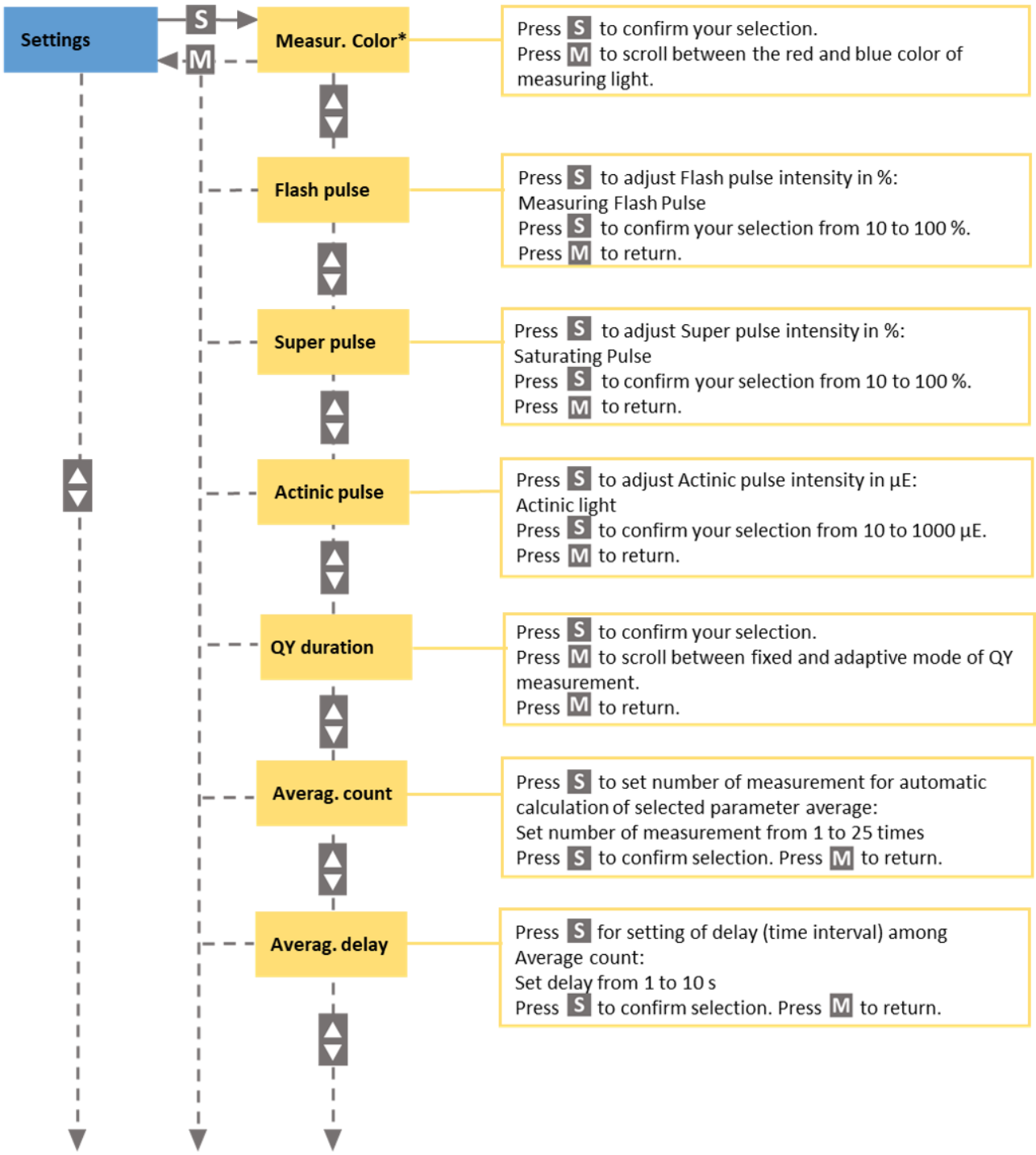
Use the Data Sub-Menu when browsing or erasing previously measured data.



IMPORTANT NOTE: Be aware that it is not possible to erase single data. **All stored data are erased!**

Setting Sub-Menu – Part 1

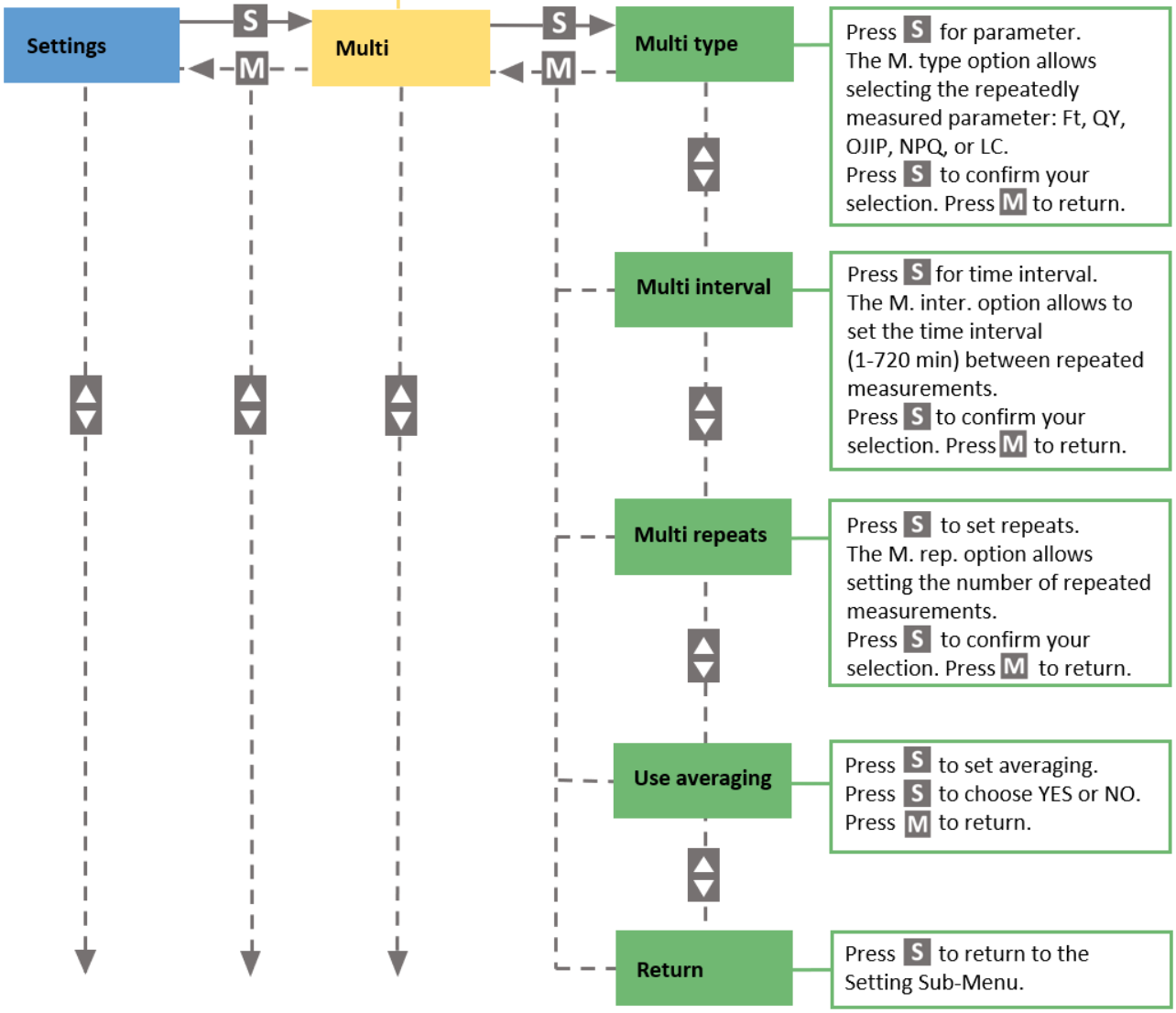
Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.



Setting Sub-Menu – Part 2

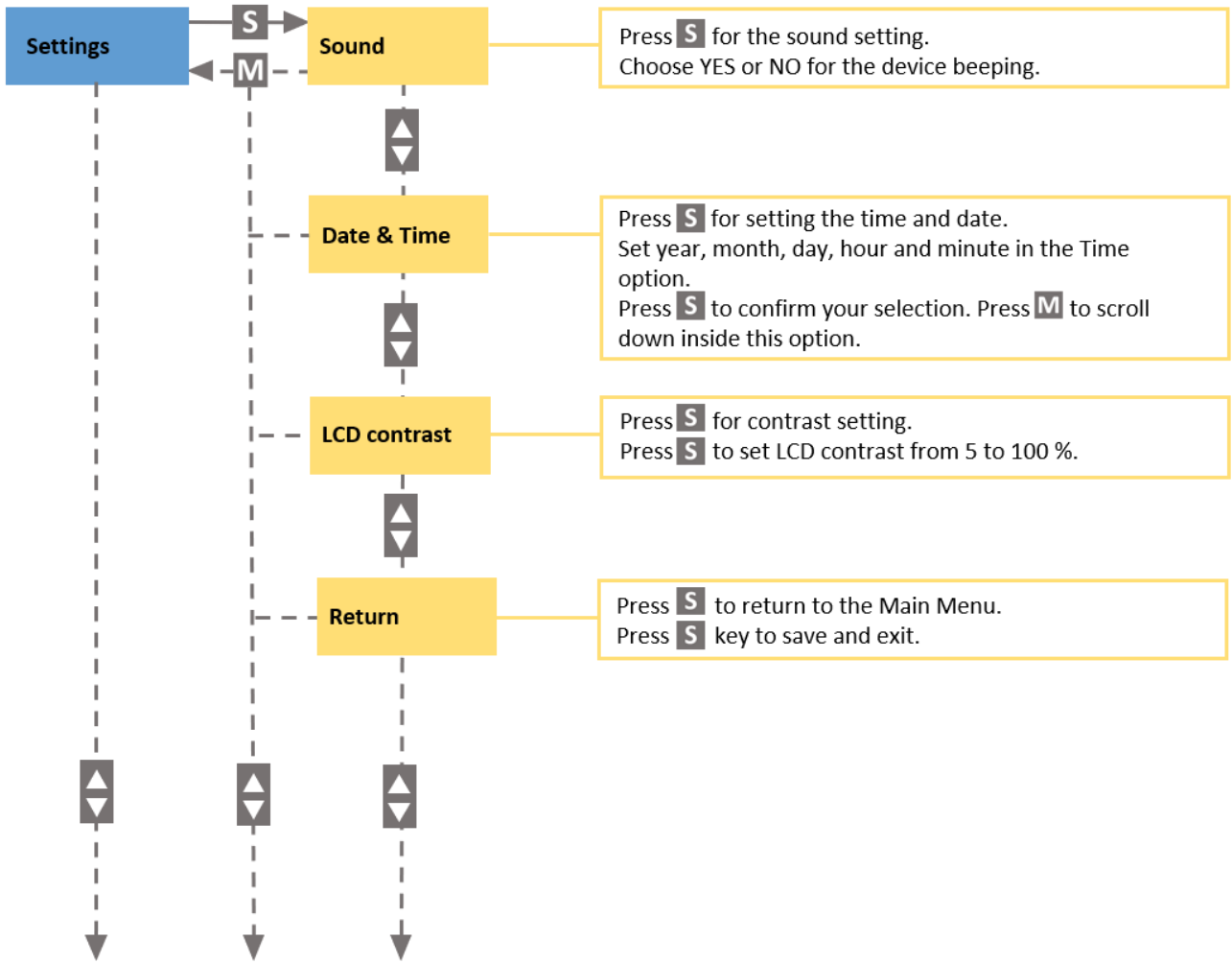
Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.

Press S for setting the Multi Option. The Multi Option is used for multiple (automatically repeated) measurements of a selected parameter: Ft, QY, OJIP, NPQ, or LC.



Setting Sub-Menu – Part 3

Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.



7 USB CONNECTION

AquaPen comes with the USB cable that is required for charging of the Li-ion battery and can also be used for data transfer to the PC after completion of measurements. To connect the USB cable with the AquaPen device Follow the picture instructions below. Please note that a lock in system is used to secure the USB cable to the AquaPen and extreme caution has to be used when setting up this connection to avoid damage to the cable pins.

⚠	When connecting the USB cable take extra caution to prevent damage to the cable connector pins. Ensure correct orientation of the cable as shown in the pictures below so the circled portion of the plug and the cable in photo A and B are perfectly lined up prior to pushing them together. Once this connection is achieved the cable may be secured in position by turning the metal cover of the cable and locking the cable in position.

To connect AquaPen with your computer please follow steps below in Fig. 10:

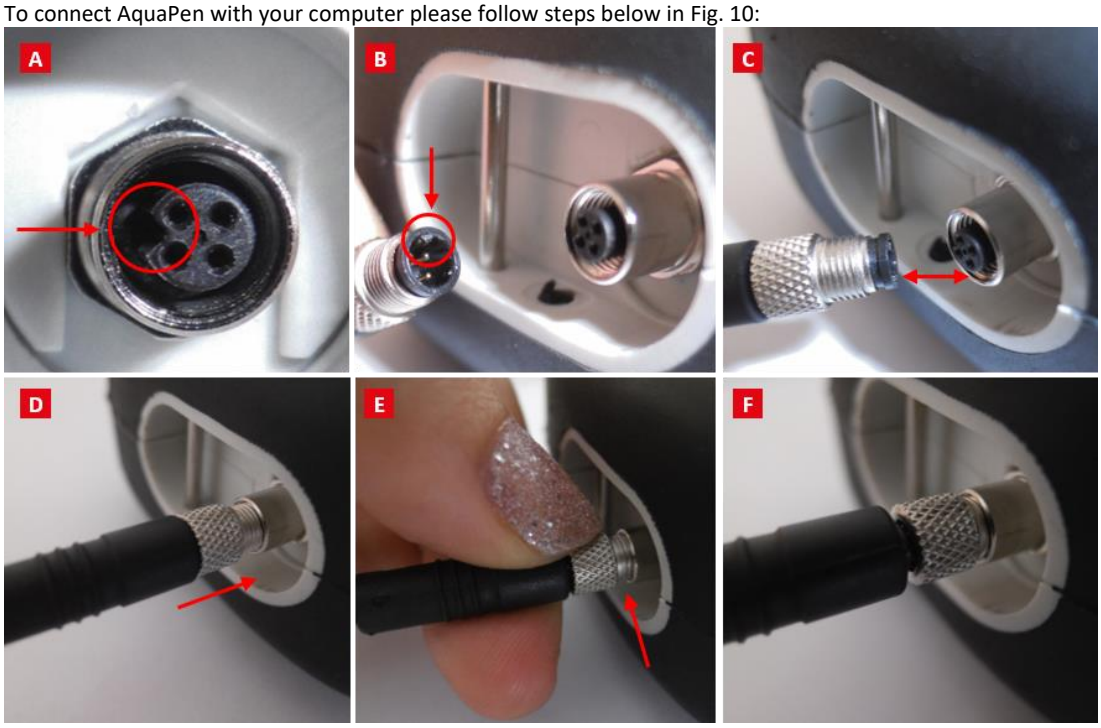


Fig. 10 AquaPen connection with PC.

A) Connector of the AquaPen device. B) The USB cable connector with pins. C – E) Place the cable horizontally and line up the red circled parts of the cable and the connector, plug in the inlet and screw the securing screw. F) Correct connection of the USB cable and Pen device.

Once the cable is securely attached to the AquaPen the other end may be connected to the USB port on a PC. The AquaPen **switches ON** automatically after connecting the cable to the PC. For the USB connection to be successful the USB driver and the FluorPen software need to be installed on the PC. Both may be found on the installation disk (USB driver folder) delivered with the device. Once the USB driver is installed the Device Manager in Windows will list the USB serial port in the device tree. The USB driver may also be downloaded from PSI websites www.psi.cz . Once the driver is installed correctly the connection between the AquaPen device and the computer is initiated by selecting in the software on the computer **Setup > Device ID.**
For more information about FluorPen software see chapter 9.

8 BLUETOOTH CONNECTION

In addition to data transfer via USB, the AquaPen may be connected to the software via Bluetooth for data transfer. Before setting up the Bluetooth connection between the FluorPen and the PC, ensure the following components are in place:

Bluetooth hardware installed on the computer
The PC must have Bluetooth wireless technology, either built-in or through a Bluetooth card. Ensure that the PC's Bluetooth setting is in "discoverable" mode (meaning that it shows up when other devices search for nearby Bluetooth connections). Consult the user guide for the PC or Bluetooth card to learn how to do this.

Bluetooth configuration software properly set up on the PC
The Bluetooth software that came with the PC or the PC's Bluetooth card needs to be activated. This software varies by manufacturer. Please consult the PC's Bluetooth documentation for more information.

Bluetooth must be switched on and be visible on both devices
To pair the AquaPen with another Bluetooth device, such as a computer, ensure that Bluetooth is switched on visible on both devices.

8.1 BLUETOOTH PAIRING

Enabling Bluetooth in the AquaPen

- Switch **ON** the AquaPen (press and hold the **SET** key for 1 s).
- Scroll to the Accessories menu (press the **MENU** key) and select Accessories by pressing the **SET** key.
- Select Bluetooth On (press the **MENU** key, then turn it **ON** by pressings the **SET** key.

⚠	Keep in mind that the AquaPen turns off automatically after about 8 minutes of no action. Turning off the AquaPen always turns Bluetooth off.

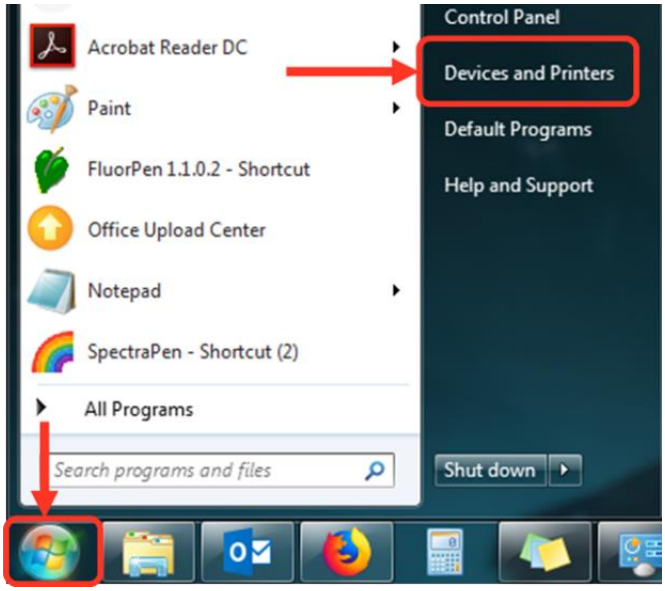


Fig. 11 Bluetooth application - start.

Starting Bluetooth Application on the PC
The following description of how to set up the Bluetooth connection between the PC and the device is for Windows 7; some of the steps may be different with different version of Windows.

- Select: Start > Devices and Printers (Fig. 11).
- You may also start your Bluetooth application via the Control Panel: Start > Control Panel > Hardware and Sound > Devices and Printers.

Fig. 12 Bluetooth – add a device.

Adding Bluetooth device to computer

- Select: "**Add a device**" to start searching for the new Bluetooth device. Be sure that the AquaPen is in discoverable mode).

- Select: PSI AquaPen icon.
- Click: Next (Fig. 13).

Fig. 13 Bluetooth – AquaPen selection.

Starting the Pairing Process

This step differs for old and new version of the AquaPen (AP-100 x AP-110) that are equipped with various Bluetooth module.

Old version of AquaPen AP-100:

The Bluetooth Pairing Code is 0000.

- Select: "Enter the device's pairing code".
- Enter: 0000 (four digits).
- Select: Next (Fig. 14).

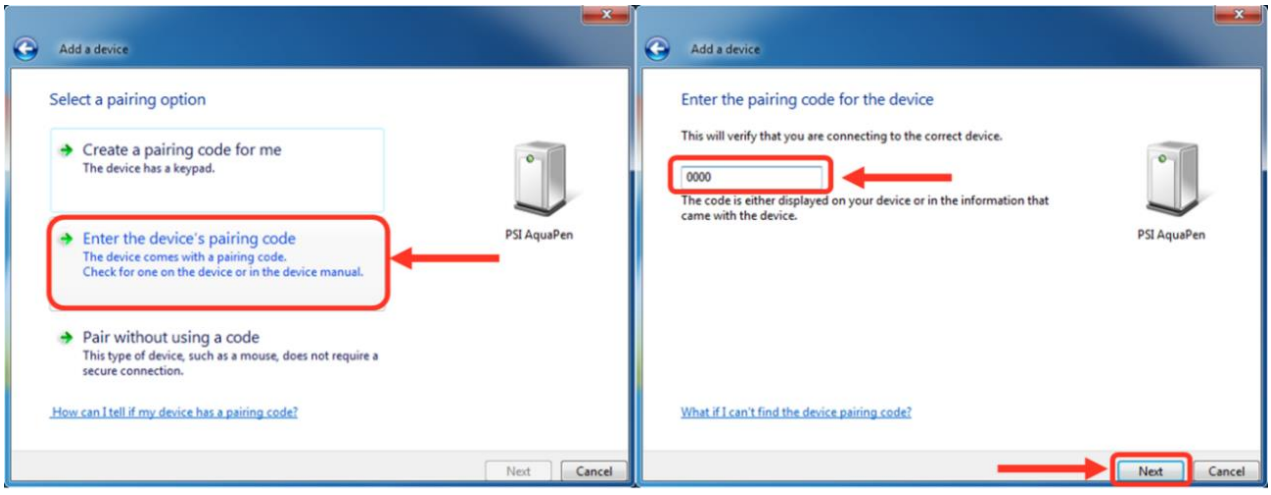


Fig. 14 Bluetooth – pairing process.

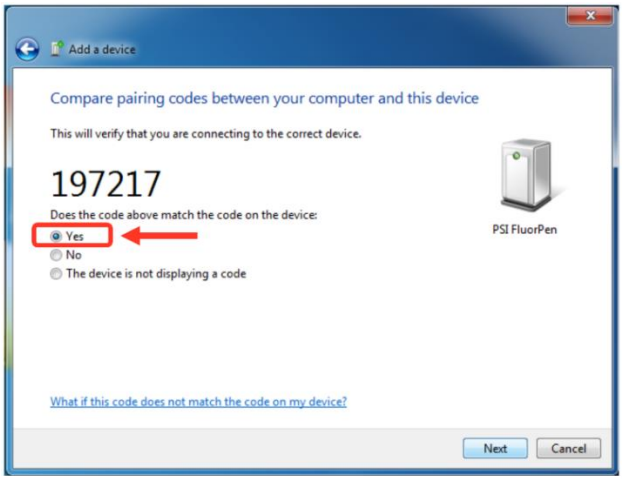


Fig. 15 Bluetooth – pairing verification.

New version of AquaPen AP-110:

- Select: Yes (Fig. 15).
  Please note that the FluorPen device does not display the verification number. The verification code is not important for the BT connection.
- Select: Next.

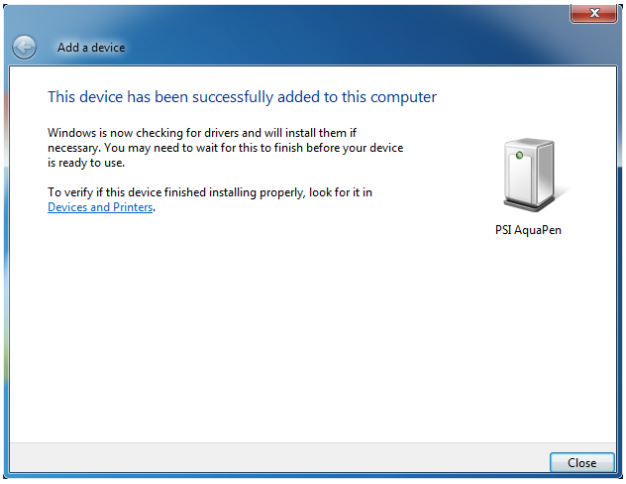


Fig. 16 Bluetooth – pairing completion.

Completing the FluorPen Pairing

- Select: Close (Fig. 16).

The Bluetooth pairing is now complete, and the next step is to open the FluorPen software (included on the delivered USB flash disk). For more information about FluorPen software see chapter 9.

9 FLUORPEN SOFTWARE

9.1 SOFTWARE INSTALLATION

1. Copy the FluorPen software provided on the USB flash disk to your computer and launch the FluorPen program.
2. To connect and recognize the AquaPen device in the FluorPen software, proceeds first with the registration of the FluorPen software (Fig. 17).

- Select: **Help > Register**
- Enter: your serial registration number (found in a text file on the USB flash disk drive included with the device).
- Select: OK

Fig. 17 Software registration.

	Please note that the serial (registration) number for the AquaPen may be found in the file SN.txt, which is included on the enclosed USB flash disk. Please Note: it is not possible to download data from the AquaPen device without software registration.

3. Switch on the FluorPen and enable Bluetooth or connect USB cable to the PC.
4. Ensure the PC and the AquaPen are properly paired (see chapter 7 and 8 for complete information on USB and Bluetooth pairing).
5. In the software select: **Setup > Device ID (Ctrl+I).** If properly connected, the message "Device: AquaPen" appears in the bottom part of the screen (Fig. 18). If the connection is not successful then message "Device not found" will appear. In the latter case check all the connections (USB) and Bluetooth pairing.

Fig. 18 Connecting AquaPen to software.

9.2 MENU AND ICONS EXPLANATION

9.2.1 MAIN MENU

MENU: File

Load	Loads previously saved data files.
Save	Saves data to hard disc.
Export	Exports data in .txt format.
Export to JSON	Exports data in JavaScript Object Notation.
Close	Closes the current experiment.
Close All	Closes all running experiments.
Exit	Exits the program.

Fig. 19 Menu File.

MENU: Device

Download	Downloads data from the AquaPen to your PC.
Erase Memory	Erases data from the AquaPen memory.
Online Control	Online control of AP device.
Attach GPS File	Used for download data from GPS module (active only in AquaPen version AP 100).

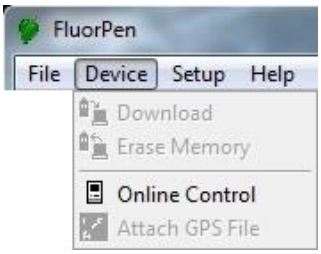


Fig. 20 Menu Device.

MENU: Setup

Device ID	Detects the connected device.
Update Firmware	Used for firmware updates.
Settings	Used for modification of the program settings.

Fig. 21 Menu Setup.

MENU: Help

About	Offers basic information about the program.
Register	Used for the FluorPen software registration.

Fig. 22 Menu Help.

Icon Explanation:

Download	Downloads data from the AquaPen to PC.
Load	Loads (opens) previously saved data files.
Save	Saves data to hard disc.
Export	Exports data in .txt format.

9.2.2 MENU SETTINGS

MENU > Setup > Settings (Fig. 23)

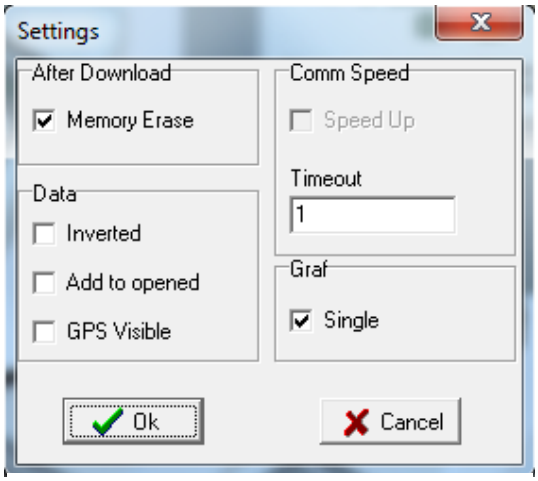


Fig. 23 Settings.

After Download – Memory Erase
If the box is checked the AquaPen memory is erased after each data download.

Data – Inverted
If the box is checked the polarity of data is inverted, e.g., multiplied by -1. This feature can be helpful for a certain type of experiment when the measured data are undesirably interpreted as negative values.

Data – Add to opened
If the box is checked the downloaded data are added to that of the current opened experiment.

Data – GPS Visible
This option is active only in older AquaPen version AP 100. In new version AP 110 the GPS data are automatically downloaded and paired with protocol measurements.

Graf – Single
If the box is checked all measured data are visualized in one graph, i.e., the value of each new measurement is added to the currently used graph window. If the box is not checked a new graph is opened for every new measurement.

9.2.3 MENU ONLINE CONTROL

This function can be used for Online Control the AquaPen device after connection with the PC.

MENU > Device > Online Control > Switches (Fig. 24)

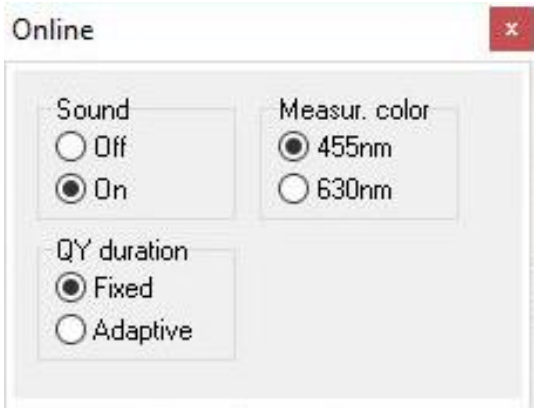


Fig. 24 Online control – Switches.

Sound On/Off
This option switches the device beeping on/off when the MENU and SET buttons are pressed.

Measuring color
Choose 455 nm or 630 nm for measuring fluorescence protocols. This function is active only in the AquaPen-C 110-C model.

QY duration
Fixed: the super pulse used for each QY determination (also within NPQ and LC) lasts 1 second, i.e. the F_m for the QY calculation is determined after a 1 second super pulse has been applied.
Adaptive: the super pulse lasts until the maximum fluorescence yield is reached, at which point it stops and the F_m is determined to calculate the QY. The super pulse duration can be shorter than 1 s, but the maximum length is always 1 s.

MENU > Device > Online Control > Values (Fig. 25)

The tab Values allows to set the measuring characteristics of light pulses, Averaging and Multiple measurement.

⚠	Please remember that each set value needs to be confirmed either by pressing the ENTER key on the keyboard or leaving the box.

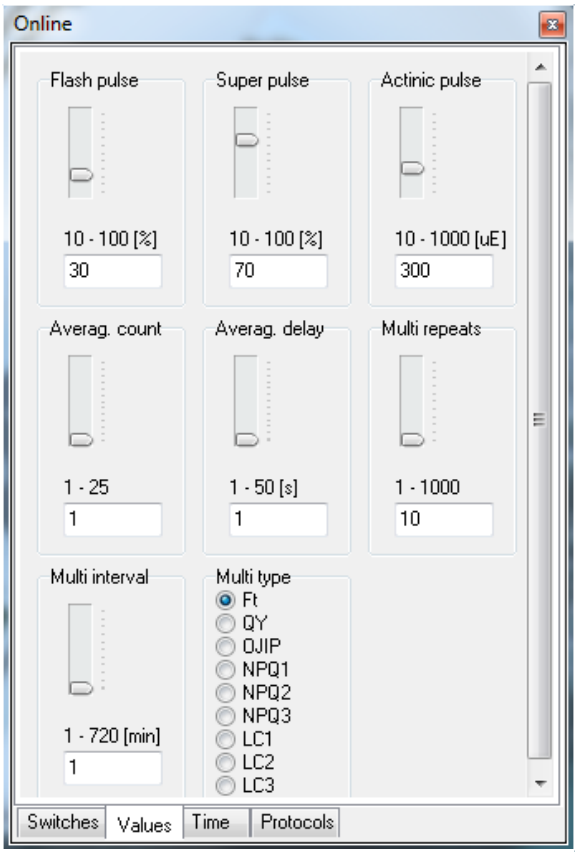


Fig. 25 Online control – Values.

The intensities of Actinic, Super and **Flash Pulse lights** can be set in this window.

Averaging enables to measure specific consecutive protocols and to calculate arithmetic mean of the measurements. The measurement result is represented by one average value or graph.
The number of the measurement (**Averag. Count**) and the delay between the individual measurements (**Averag. delay**) need to be set. The averaging is not available for the QY measurement.
Please remember, that this function is not suitable for the dark-adapted samples (excepting the Ft measurement) as the sample is illuminated by the appropriate light pulses during the first measurement.

Multiple measurement is automatically repeated measurement of one specific protocol over a time period. The measurement result is represented by the values or graphs obtained in the specific data points.
The **Multi interval** represents the time between the beginnings of the single measurements and so, the Multi interval should be longer than the duration of the selected protocol. The number of **Multi repeats** from 1-1000 can be set in this window. Finally, the type of Protocol selected for Multiple measurements (**Multi type**) is also set in this window (see Fig. 25).
Please note that the Multi measurements have to be started from the device or by clicking on the **Multi** button in the "Protocols" tab of the Online Control window (see **Error! Reference source not found.**).

MENU > Device > Online Control > Time (Fig. 26)

The AquaPen time and date can be set in this window. The time and date can be edited either manually by setting and saving it or synchronized with the computer automatically by ticking the box. The synchronization is performed just at once (i.e., the option doesn't synchronize the time continuously). This is essential for correct GPS data acquisition and therefore recommended.

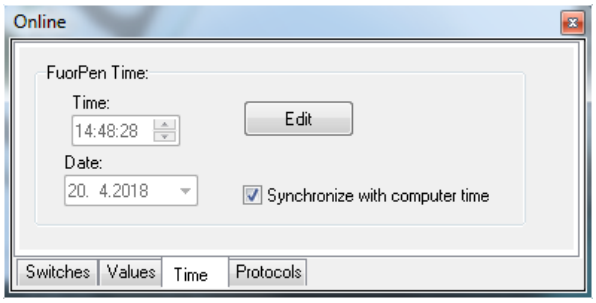


Fig. 26 Online control – Time.

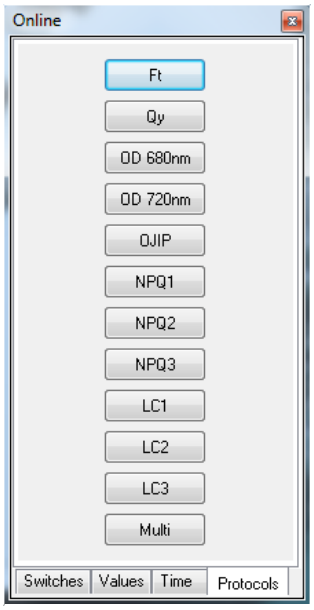


Fig. 27 Online control – Switches.

MENU > Device > Online Control > Protocols (Error! Reference source not found.**)**
The requested Protocol using the previous settings can be started in this window. Once the measurement is completed the data is saved to the device and can be downloaded to the PC later. Measuring of OD680 nm and OD720 nm is active only in the AquaPen AP 110-C model.

9.3 DATA TRANSFER AND VISUALIZATION

1. Once kinetic protocols data (OJIP, NPQ, LC) have been collected with the AquaPen to visualize the data it needs to be downloaded to the PC first via FluorPen software. Before data transfer can occur a successful connection between the AquaPen and the PC needs to be established via USB cable or Bluetooth module (see chapter 7 and 8 for details).
2. Click the **Download** icon or select **Device > Download**.
3. Once the download is complete the Data can be visualized in a table shown below (Fig. 28).

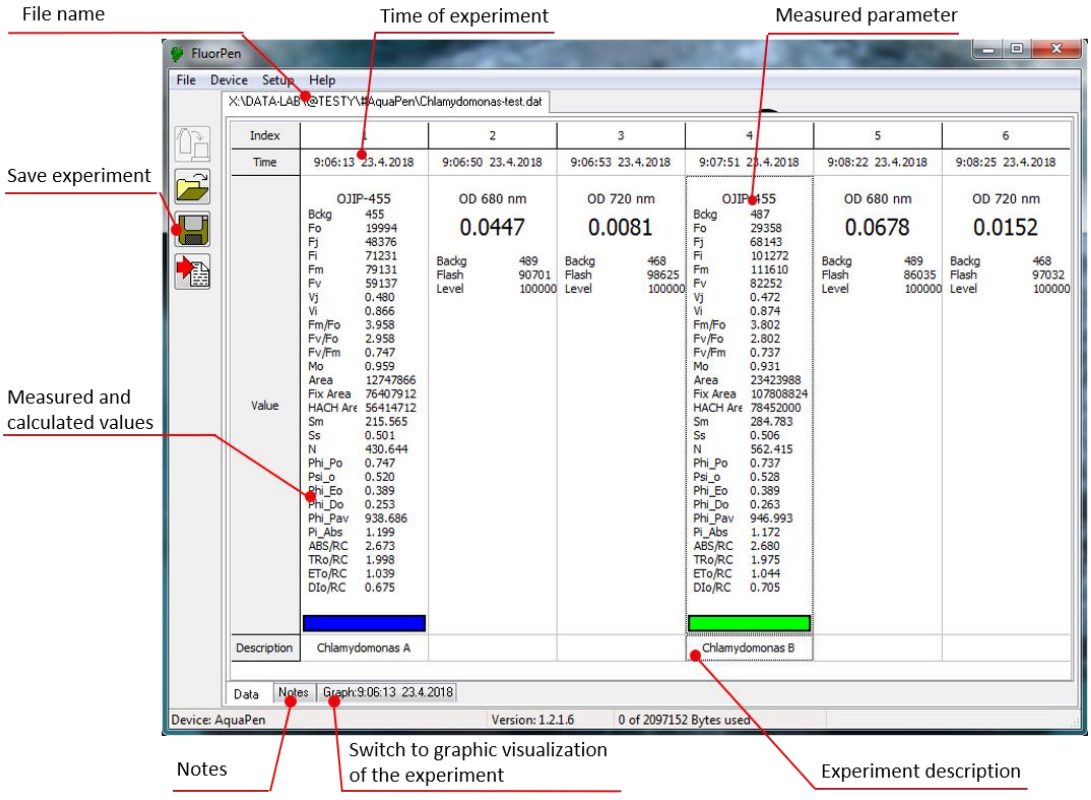


Fig. 28 Data visualization.

4. To visualize the data in the graph mode, click the **Graph** field in the bottom bar.
5. The selected set of data will be shown on the Graph (Fig. 29).

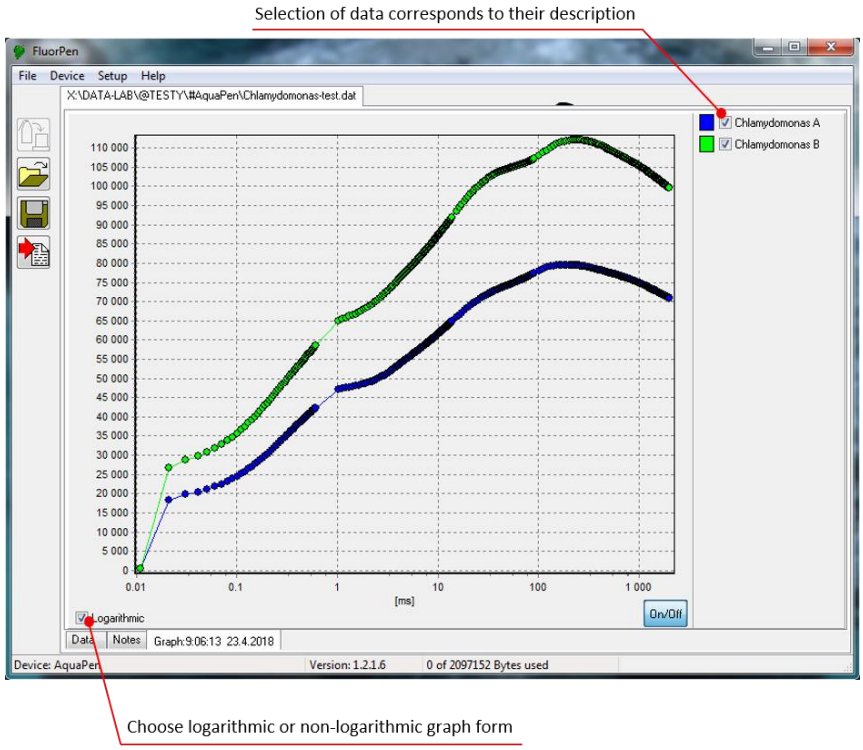


Fig. 29 Graph visualization.

6. To **export** data from the FluorPen software select **File > Export** or **Export** icon. Select data type to export (Ft, QY, OJIP…) -Fig. 30.
   Selected only – exports only one measurement that is selected by mouse, otherwise it will export everything.
   Source data – exports raw data, in case of OJIP: points of the curve.
   Description – exports the data description if any.
   Computed values – export calculated data, in case of OJIP: F_0, F_i, F_j...

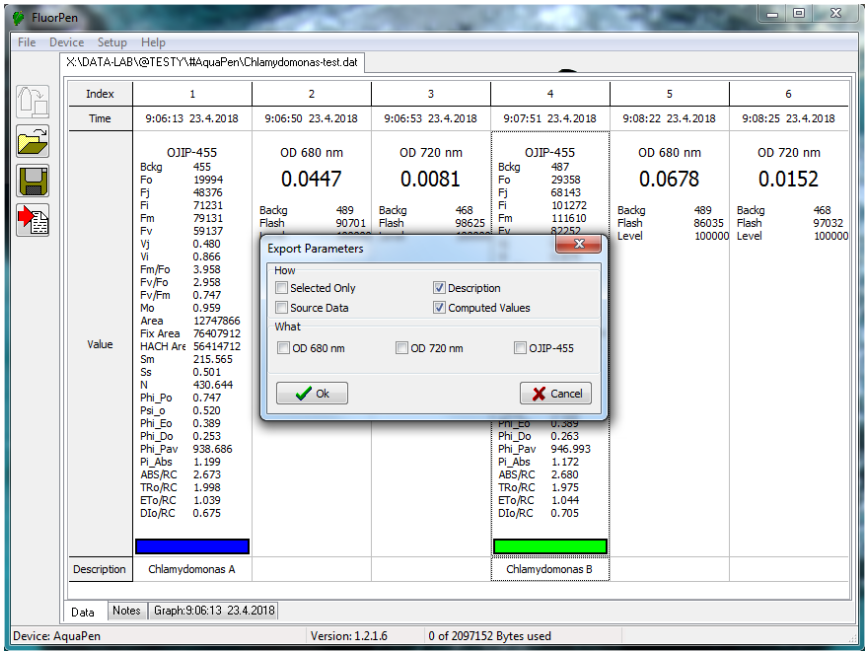


Fig. 30 Data export.

9.4 FIRMWARE UPDATE

⚠	All data in the AquaPen memory are erased during the firmware update! Before starting any firmware update, download all your data from the AquaPen memory to the computer!

1. Starting Update: Select **Setup > Update Firmware From File** (Fig. 31).

Fig. 31 Firmware update.

2. Warning: Select **OK** to start update (Fig. 32)

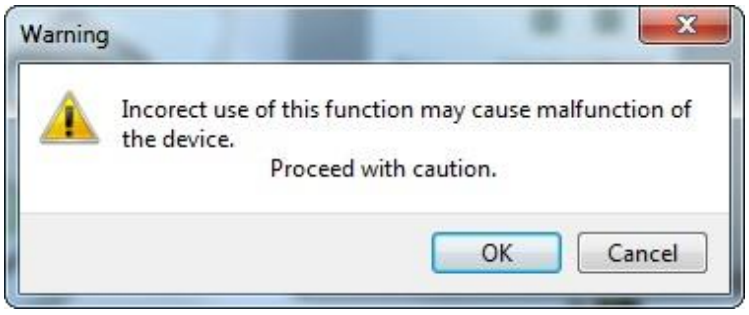


Fig. 32 Firmware update - warning.

3. Selecting **.bxn file**

Find firmware update file (a binary file with the extension .bxn provided from PSI) and select **Open** (Fig. 33).

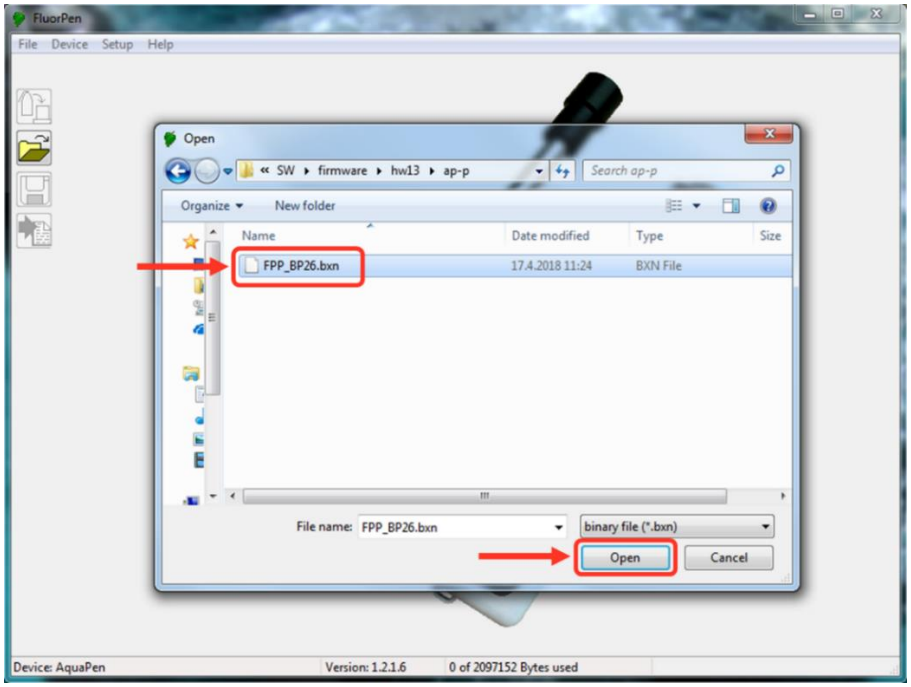


Fig. 33 Selection of the firmware file.

4. Finishing Upload

Select **OK** to start uploading the update (Fig. 34).

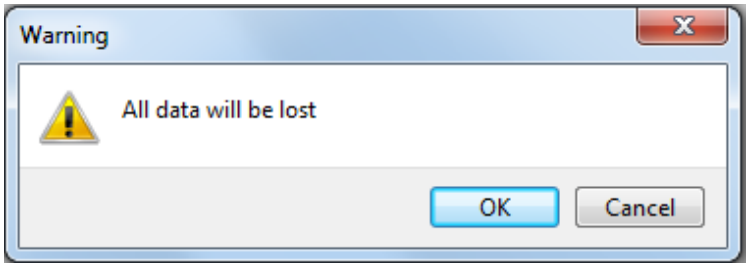


Fig. 34 Data loss warning.

The bottom bar indicates the upload progress (Fig. 35).

Fig. 35 Firmware upload progress.

Press: **OK** to finish upload (Fig. 36).

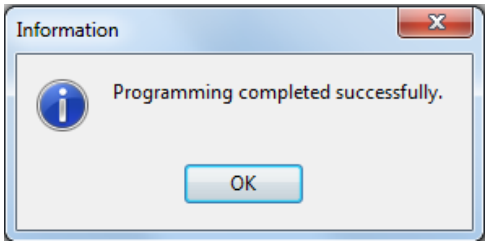


Fig. 36 Firmware upload finishing.

10 GPS MODULE

The new versions of the AquaPen device (AP 110) have integrated GPS module which can be turned on during the measurements. When GPS module is turned on the map coordinates will be automatically saved with all collected data and will be downloaded during data download.

	For proper GPS reading, the time in your AquaPen and in your computer must be synchronized. Preset time and time zone must correspond to GPS time (time zone) in your location.

10.1 GPS/AQUAPEN OPERATION

1. Check the time setting on the AquaPen device: **Settings > Date & Time**
2. Switch the GPS module "ON" on the AquaPen device by following these steps in the menu:

- Select: **Accessories > GPS**
- Press **SET** to confirm.
- Wait until the GPS position is found – **"Starting GPS".**
- The GPS module is ready when the icon in upper panel changes as shown on Fig. 37.

Fig. 37 GPS icons.

3. If the picture on the display of the device does not change then proceed to **Accessories > GPS > Location** selection in the menu and manually map the GPS by pressing SET. **"GPS Acquisition**" message will appear followed by coordinate. If the GPS module has difficulties mapping the coordinates, a message stating **"GPS not locked"** will appear on the display. It may be necessary to take the device outside into a location that is easily accessible by the satellite (clear sky view) and repeat the process of mapping.
4. Once the GPS has been turned on and successfully activated proceed to **Measurement** and select required protocol.

	For prompt determination of the coordinates use the option Accessories > GPS > Location.

	The device may need a clear view of the sky to acquire satellite signal. Keep in mind that the AquaPen turns off automatically after about 8 minutes of no action. Turning off the AquaPen always turns off GPS module.

10.2 DATA DOWNLOAD

1. **Enabling Communication:**
- Switch on the computer and the FluorPen software.
- Set your computer to AquaPen communication: enable Bluetooth or connect to USB port (see instructions on pg. 33).

2. **Downloading Data** from the AquaPen:
- Start FluorPen program.
- Connect AquaPen device: Setup > Device ID (Ctrl+I)
- Download measured data from the AquaPen to your PC by clicking the download icon (top icon). Data measured with activated GPS module are downloaded with GPS coordinates (Fig. 38).

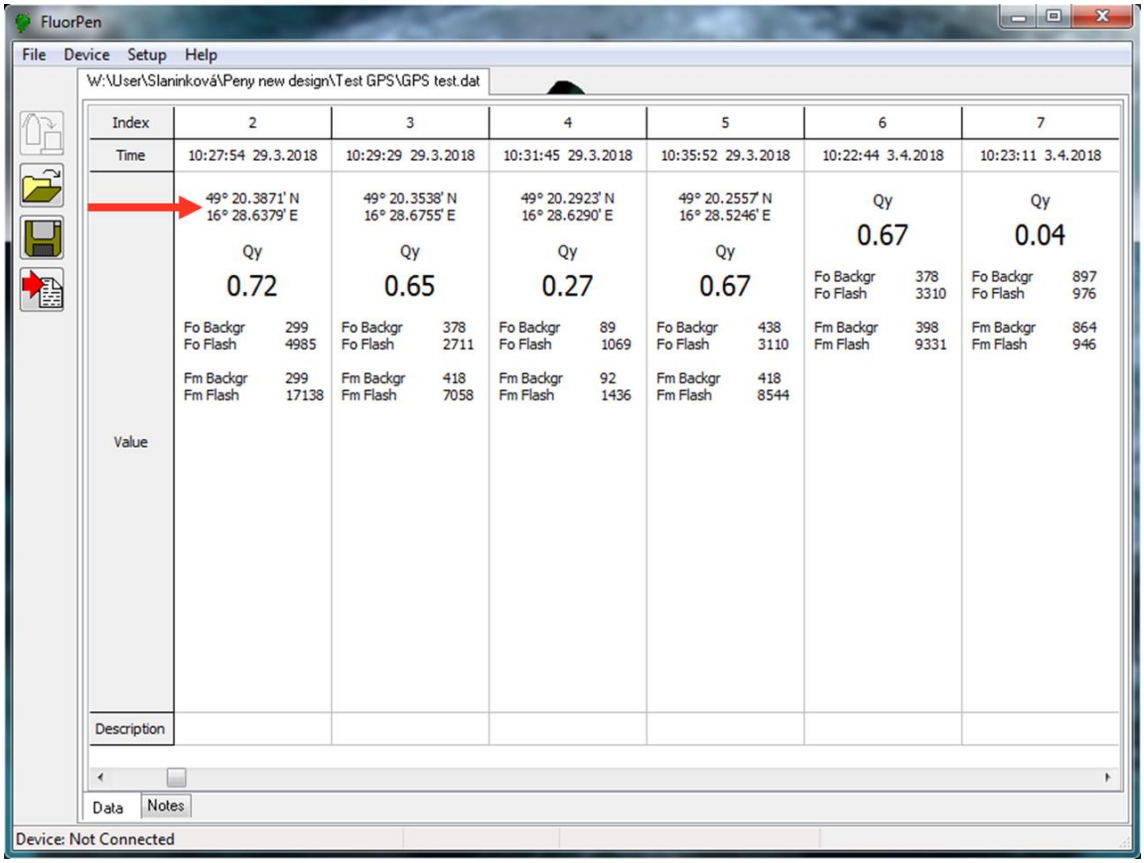


Fig. 38 GPS coordinates.

11 WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the FluorPen device. It is valid for one year from the date of shipment.
- If at any time within this warranty period the instrument does not function as warranted, return it and the manufacturer will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. The manufacturer is responsible for shipping and insurance on return of the instrument to the customer.
- No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by the manufacturer; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by the manufacturer.
- The warranty is return-to-base only and does not include on-site repair charges such as labor, travel, or other expenses associated with the repair or installation of replacement parts at the customer's site.
- The manufacturer repairs or replaces faulty instruments as quickly as possible; the maximum time is one month.
- The manufacturer will keep spare parts or their adequate substitutes for a period of at least five years.
- Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.
- PSI also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.
- Wear & Tear Items (such as sealing, tubing, padding, etc.) are excluded from this warranty. The term Wear & Tear denotes the damage that naturally and inevitably occurs as a result of normal use or aging even when an item is used competently and with care and proper maintenance.

12 TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of problems with the FluorPen visit **FAQ** on our websites (http://psi.cz/support/faq) or contact customer support by email to support@psi.cz, or contact your local distributor.

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14 List Of Tables