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

Manual and User Guide

Please read this manual before operating this product

Photon Systems Instruments

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The contents of this manual have been verified to correspond to the specifications of the device. However, deviations cannot be ruled out. Therefore, a complete correspondence between the manual and the real device cannot be guaranteed. The information in this manual is regularly checked, and corrections may be made in subsequent versions.

The visualizations shown in this manual are only illustrative.

This manual is an integral part of the purchase and delivery of equipment and its accessories and both Parties must abide by it.

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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 TECHNICAL SPECIFICATION

Protocols	
AquaPen AP 110-C	- Ft – instantaneous chlorophyll fluorescence - Quantum Yield - OJIP - Non-photochemical quenching - Light curve - **Optical density at 680 and 720 nm**
AquaPen AP 110-P	- Ft – instantaneous chlorophyll fluorescence - Quantum Yield - OJIP - Non-photochemical quenching - Light curve
LED lighting	
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 µmol(photon).m^{-2}.s^{-1} (adjustable from 10 to 100%)
Actinic Illumination	Adjustable from 10 to 1,000 µmol(photon).m^{-2}.s^{-1}
Measuring Illumination	Up to 0,09 µmol(photon).m^{-2}.s^{-1} per pulse (adjustable from 10 to 100%)
Detector	
Type	PIN photodiode with bandpass filters
Wavelength range	From 667 to 750 nm
Data storage and transfer	
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 20m)
PC software	FluorPen 1.1 (Windows 7 and higher)
Battery	
Type	Li-Ion rechargeable battery
Capacity	2000 mAh
Max. charging current	0.5 A
Charging	Via USB port - PC, power bank, USB charger, etc.
Battery life	48 hours typical with full operation Low battery indicator
Other	
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 GENERAL INFORMATION

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 7.1) 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 AP110-C** and **AquaPen-P AP110-P**.

AquaPen AP110-C, cuvette version is equipped with blue (455 nm) and red (630 nm) LED emitters whereas AquaPen AP110-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.

3.1 DEVICE DESCRIPTION

Fig. 1 Device description.

4 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**
- **3 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**

5 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.

6 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** (**Error! Reference source not found.**, **Error! Reference source not fo und.**). 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*

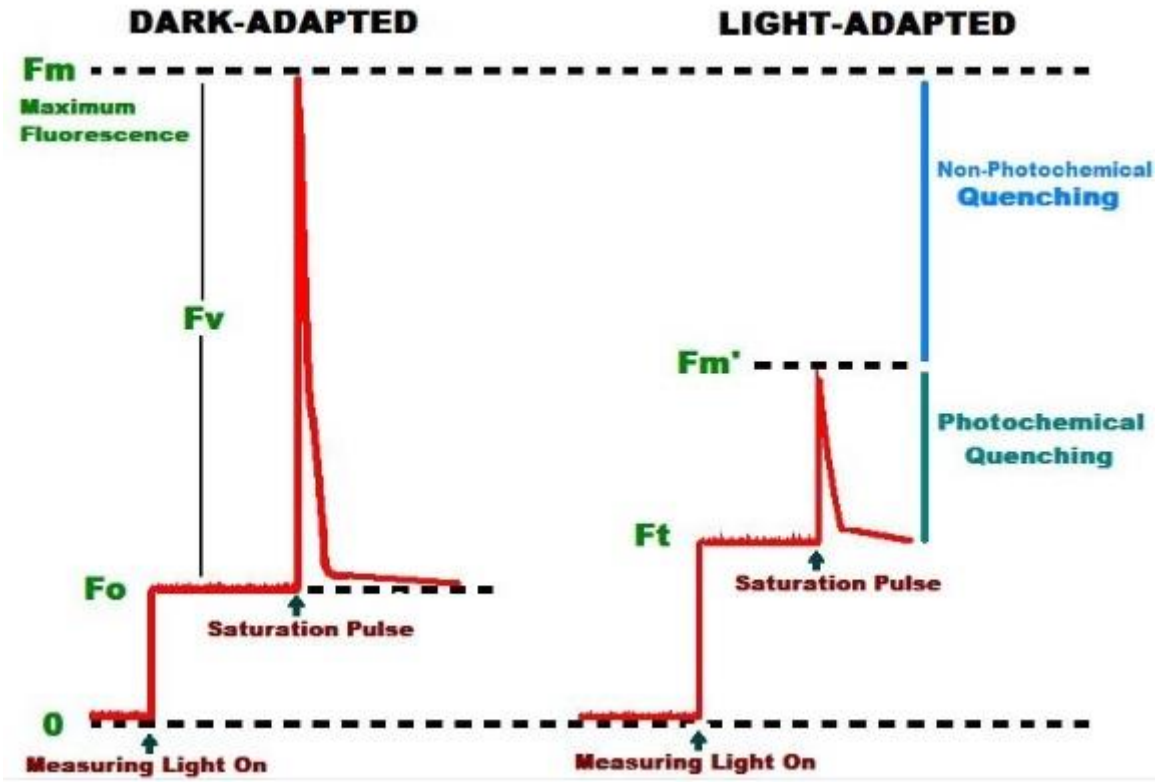


Fig. 2 Chlorophyll fluorescence.

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 720nm.

AquaPen measures:

F_T - Instantaneous Chlorophyll Fluorescence F_t is equivalent to F_0 if the sample is dark-adapted.
QY - Quantum Yield QY is a measure of the Photosystem II efficiency. QY is equivalent to F_v/F_m in dark-adapted samples and to F_v'/F_m' 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 0).
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 7.1.4).
LC - Light Curve Photosystem II Quantum Yield estimated from fluorescence that is measured sequentially at several different light levels. More in chapter 7.1.5.
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 7.2.

**Optical density is defined as*

***OD = -Log(I/Io)** - where "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.*

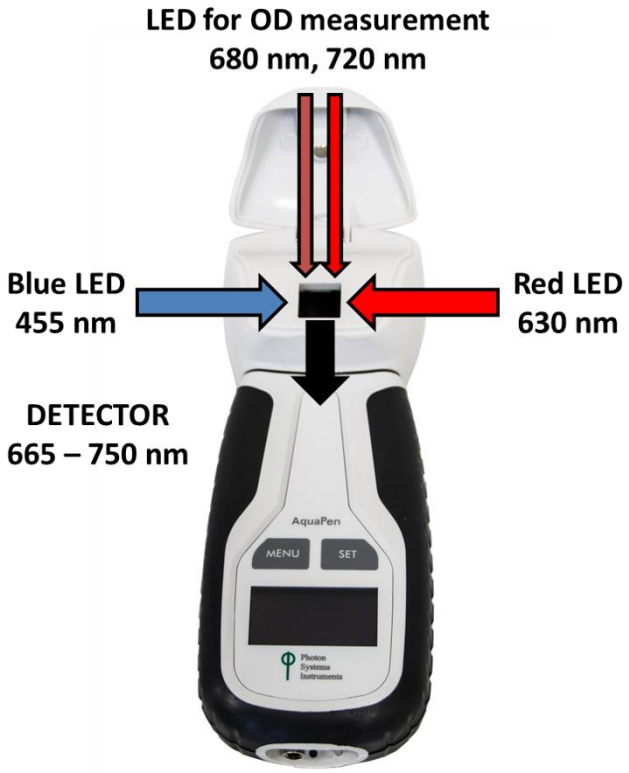


Fig. 3 AquaPen-C AP 110-C

Fig. 4 AquaPen-P AP 110-P

7 GETTING STARTED

For more detailed information on steps of AquaPen operation please refer to chapter 8.

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 AC 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 shown 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.

7.1 MEASUREMENTS BASED ON FLUORESCENCE

7.1.1 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 measuring pulses. Actinic light 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
Ft	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 70% = Saturating pulse

Actinic pulse 300 µmol.m^{-2}.s^{-1} (30 %) = Actinic light

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

Setting of optimal intensities of pulses:

Flash pulse setting

The optimum value of Flash pulse can be identified during QY measurement as shown in Fig. 5 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. 5). In this example the optimal flash pulse setting is 30%.

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 Flash 2552	Fo Backgr 289 Fo Flash 4426	Fo Backgr 390 Fo Flash 8875
Fm Backgr 309 Fm Flash 7995	Fm Backgr 269 Fm Flash 13419	Fm Backgr 390 Fm Flash 26659
30% f_pulse	50% f_pulse	100 % f_pulse

Fig. 5 QY measurement performed with different intensities of Flash pulse. Optimal setting is highlighted in red rectangle.

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. 6 and Fig. 7).

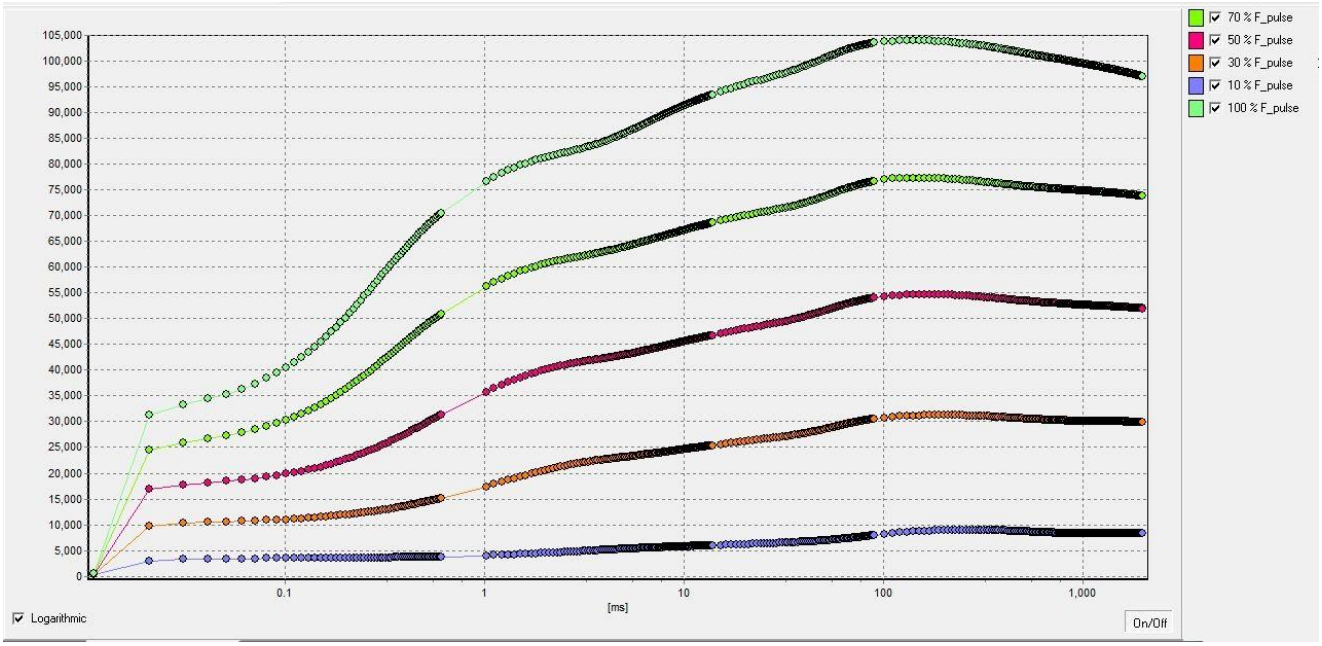


Fig. 6 OJIP curves - measurement performed with different intensities of Super pulse.

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

Fig. 7 OJIP data - measurement performed with different intensities of Super pulse. The highest Fv/Fm 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 observed on display during measurement there are two options to resolve this problem - to dilute the sample or lower the pulse intensity. In case of **Low value** on display during measurement there are two options - concentrate the sample or increase the pulse intensity.

7.1.2 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 - Ft.
- 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 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 47).
- 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 Ft, 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 - **Ft**.
- 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 47).
- All measured data are stored in the device memory and can be downloaded to PC after completion of the experiment.

7.1.3 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	$DI_0 / RC = (ABS / RC) – (TR_0 / RC)$

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.

7.1.4 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. 8.

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 Table. 2:

	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

Table. 2 NPQ Protocols.

The NPQ 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, measured during the first saturation flash after dark adaptation
F_p	fluorescence in the peak of fast Kautsky induction
F_m_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

[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 F_v'/F_m' without measuring F_0'. Photosynthesis Research 54: 135-142.

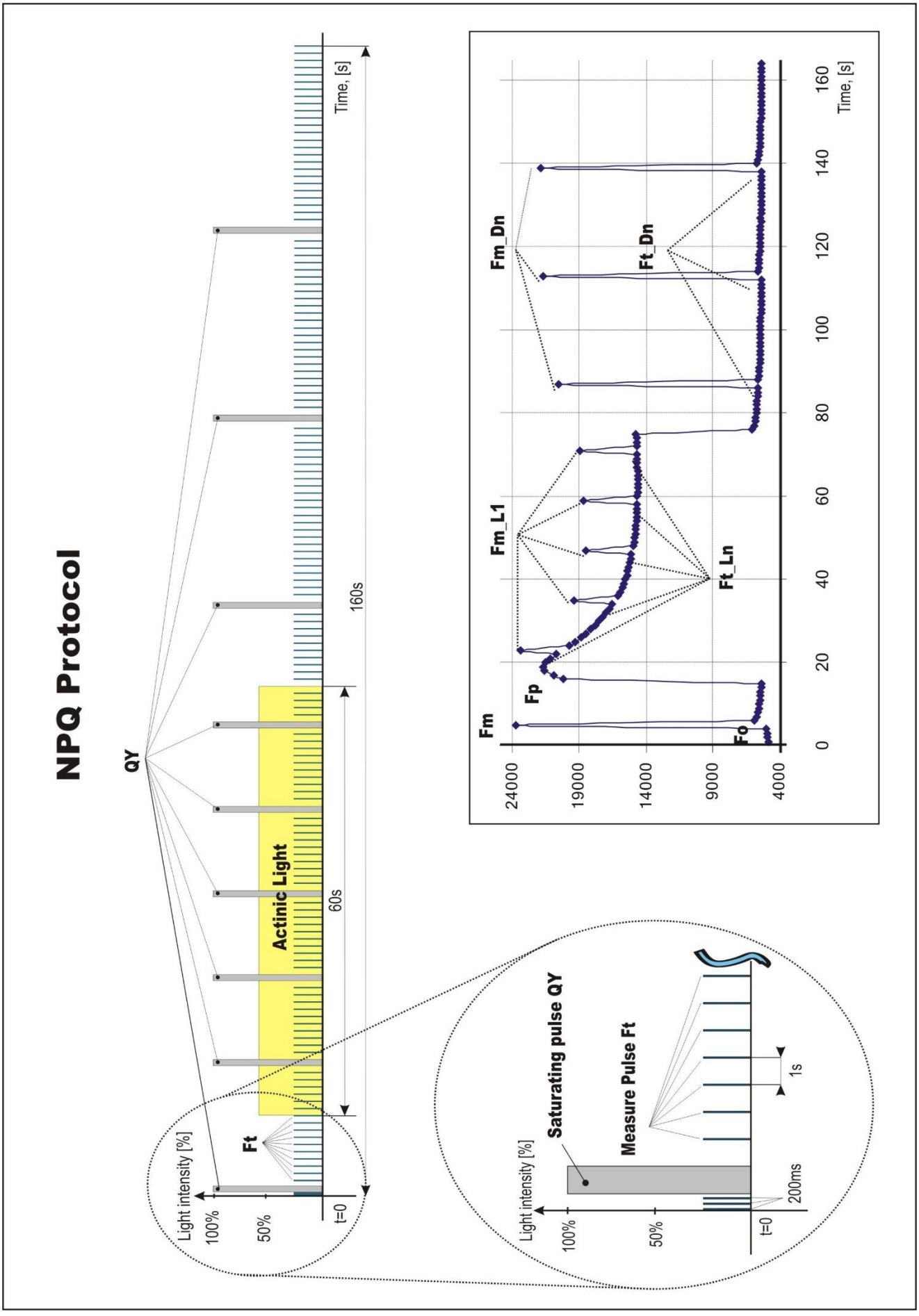


Fig. 8 NPQ Protocol.

7.1.5 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 Table 3 below. The visual representation of the LC1 and LC2 protocols is shown in Figs. 8 and 9.

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

Table. 3 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‡	maximum fluorescence in light adaptation state
F_t_Ln‡	instantaneous fluorescence during light adaptation
QYmax*	maximum quantum yield of PSII in dark-adapted state - Fv/Fm
QY_Ln‡**	instantaneous PSII quantum yield induced in light

‡ n represents a sequential number of light phases

*Calculated as (F_m – F_0) / F_m

** Calculated as (F_m_Lx – F_t_Lx) / F_m_Lx

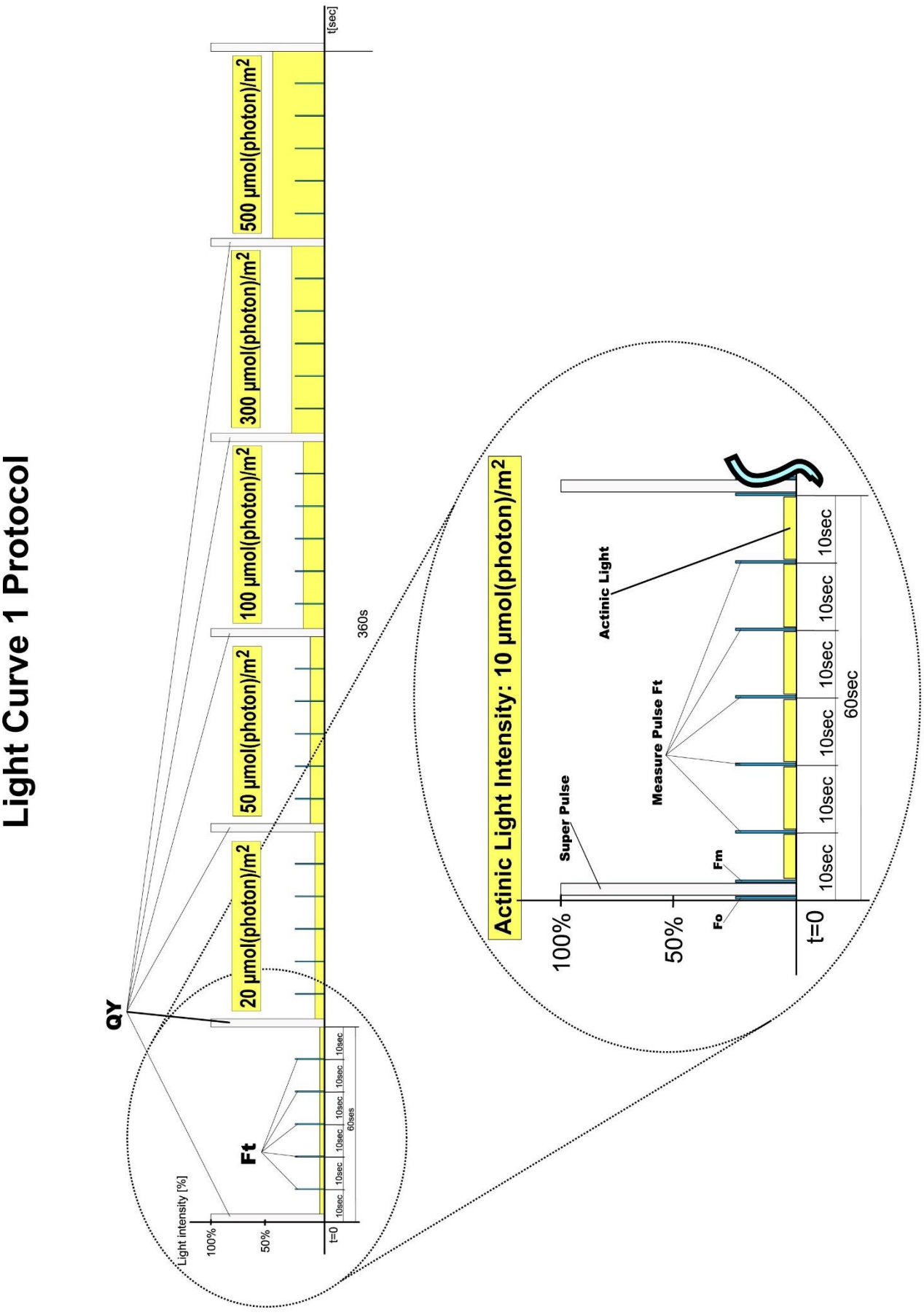


Fig. 9 LC1 Protocol.

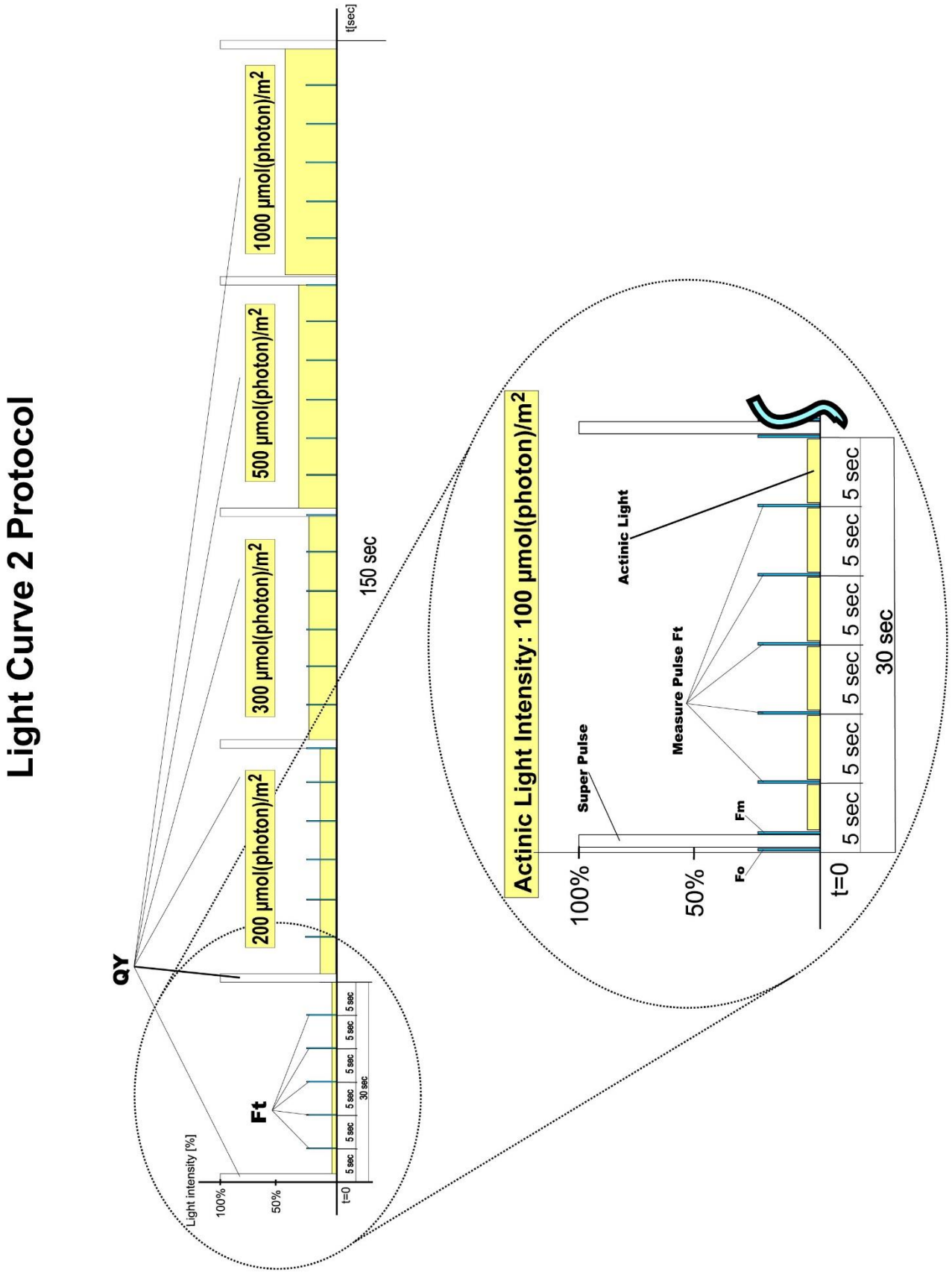


Fig. 10 LC2 Protocol.

7.2 OPTICAL DENSITY MEASUREMENT (AQUAPEN-C ONLY)

7.2.1 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.

7.2.2 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 47).

7.3 MULTIPLE MEASUREMENT

In addition to **single** measurement with each available protocol it is possible to perform **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 in **Settings > Multi** appropriate parameter or protocol (see Menu tree, page 29)

Multi type – select the required parameter - Ft, QY, OD....

Multi interval – set the time interval between measurements

Multi repeats – set the number of repeated measurements

Use averaging – result of some protocols can be calculated as average of multiple measurements– select YES or NO. Recommended primarily for Ft protocol.

- Prepare the sample for measurement as described above.
- Select **Measurement > Multi**.
- Press **SET** to start.
- Values of measured parameter will appear on the AquaPen display after each repeat of measurement and will automatically be stored to the device memory. If protocol (OJIP, NPQ, LC) was used all data will be saved to the device memory and visualization will be possible after the download of the data to the PC (page 47).

Modes of Multiple measurement:

There are two modes of multiple measurements with the AquaPen.

1. **The device is connected via USB to the computer.**

The device performs predefined number of measurements and does not switch off between measurements. Progress of the measurement is displayed in percentage in the software on the computer.

1. **AquaPen is not connected to the computer.**

The device measures continuously according to predefined protocol and interval. The device turns on and off between measurements automatically. The multiple measurement is interrupted only by manual switching MENU of the device.

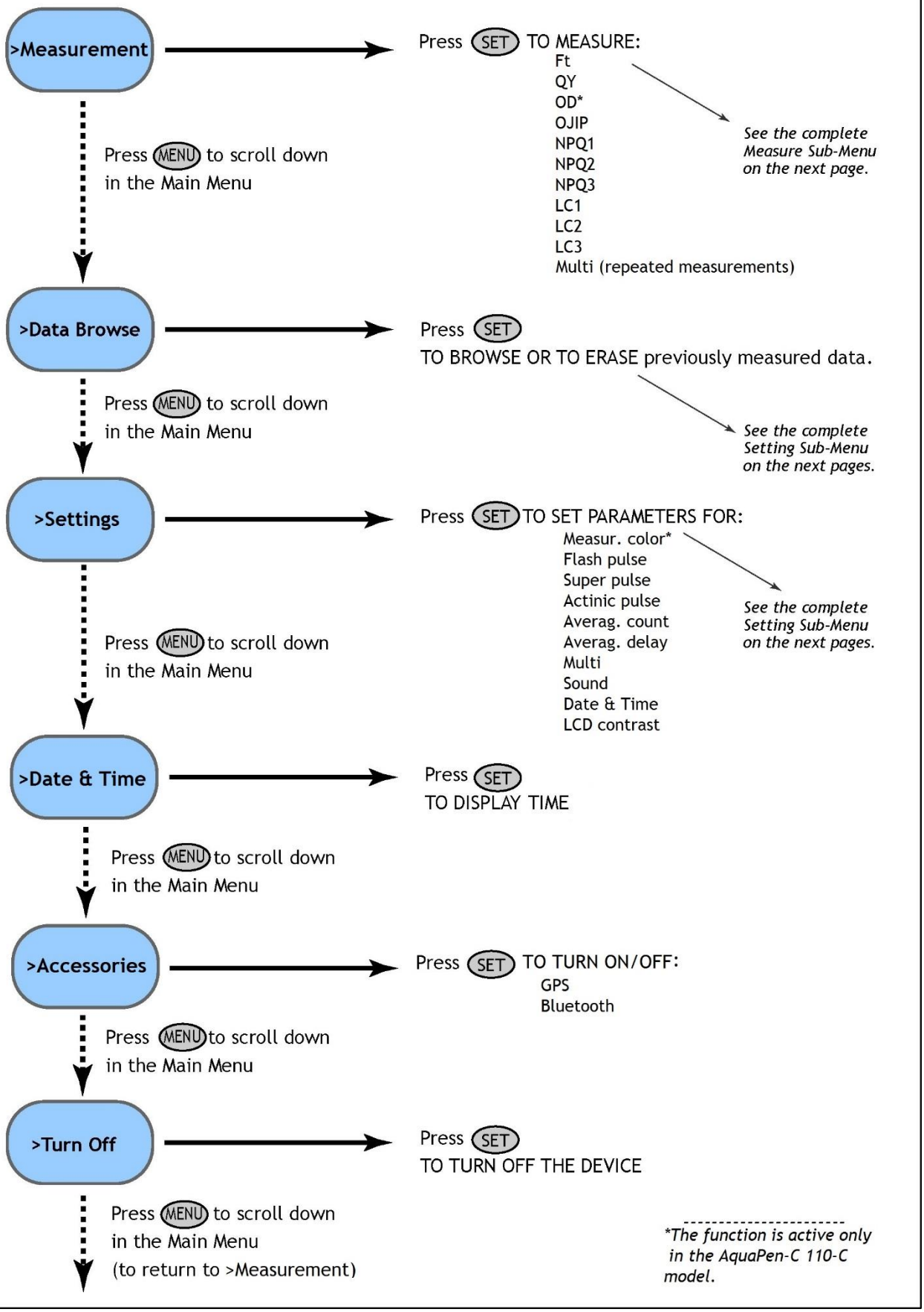
8 CONTROL MENU TREE

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.

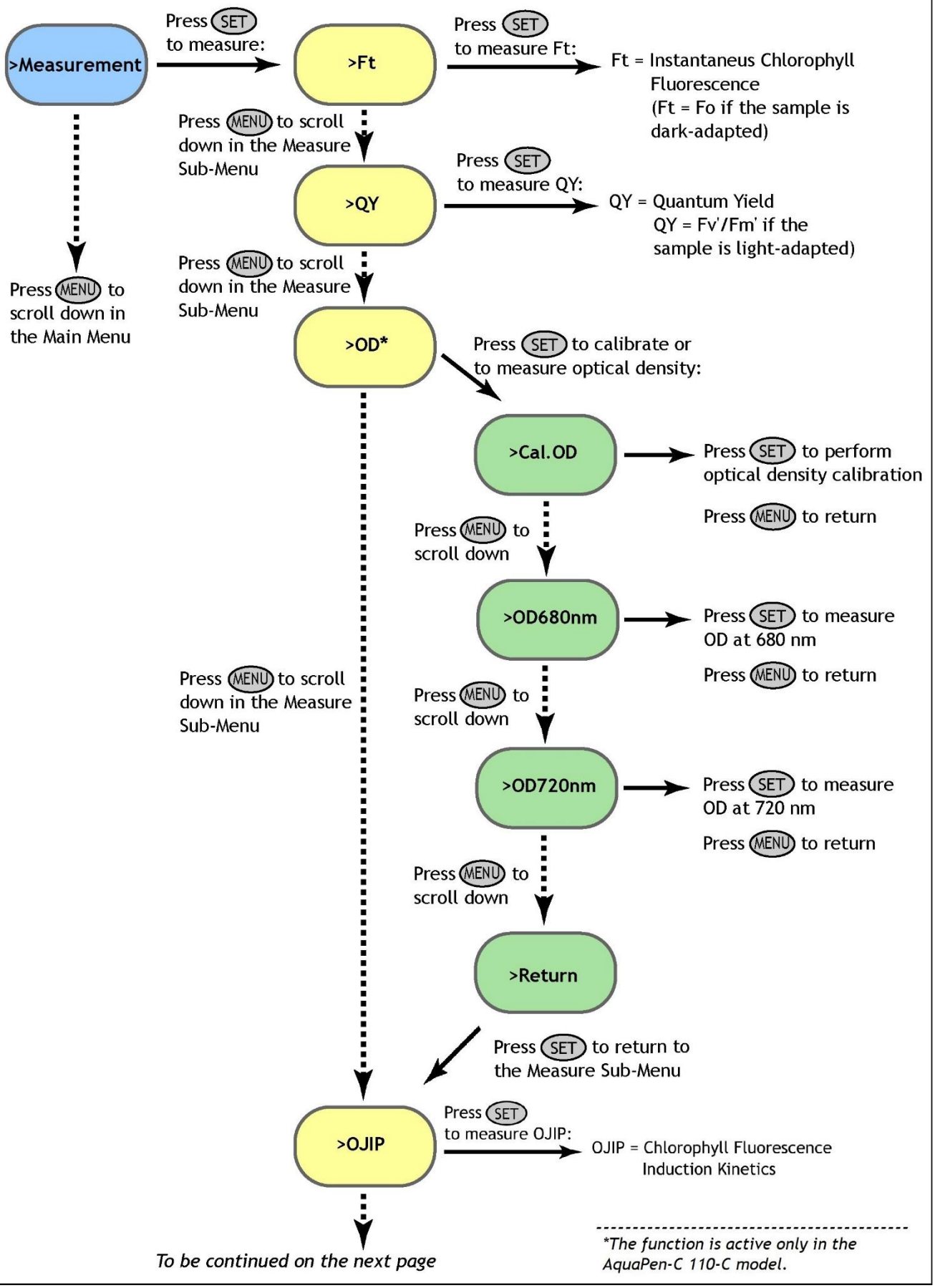
Main Menu

To start hold the SET key for 1 second.



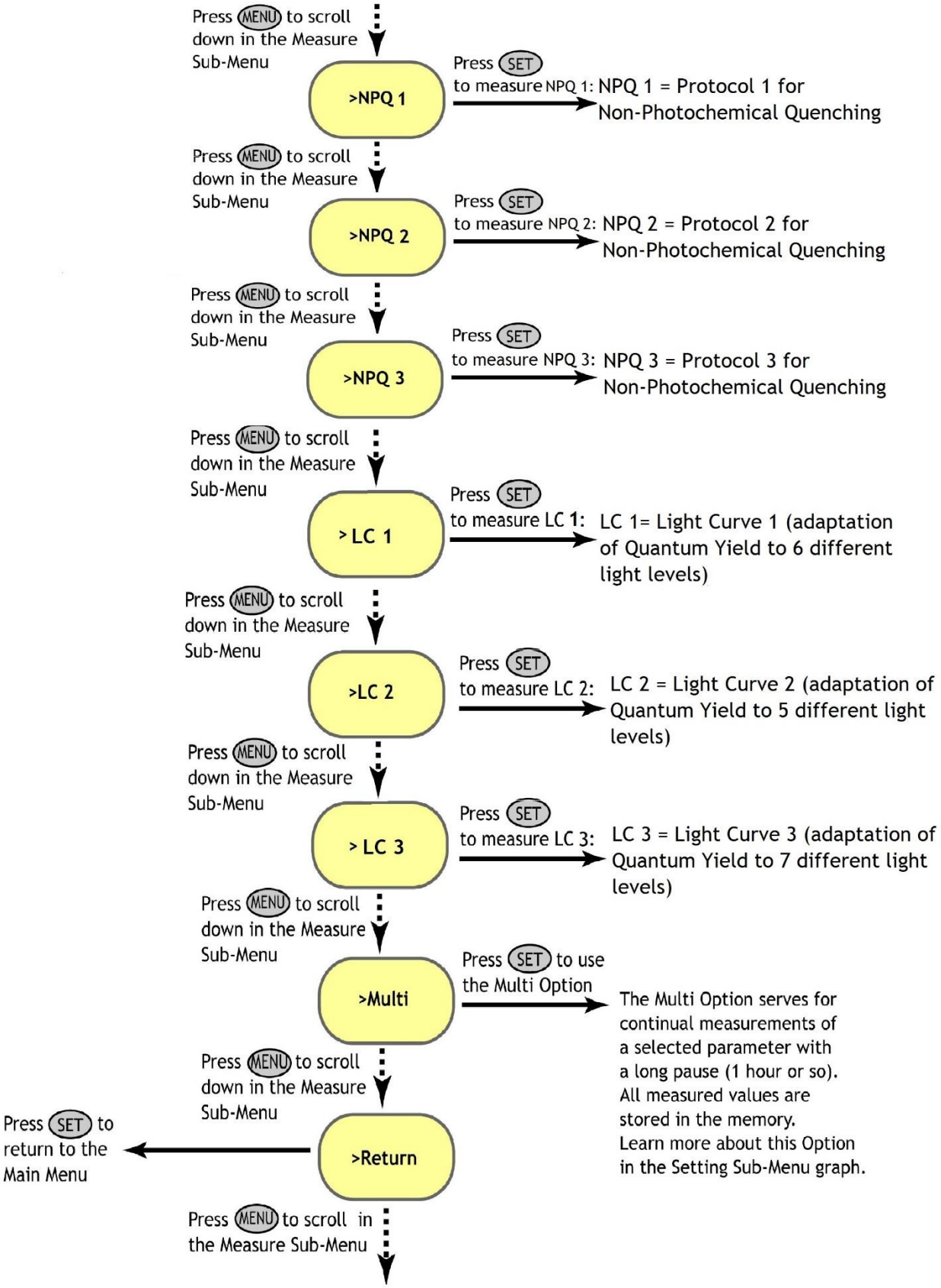
Measure Sub-Menu - Part 1

Use the Measure Sub-Menu when measuring selected parameters.



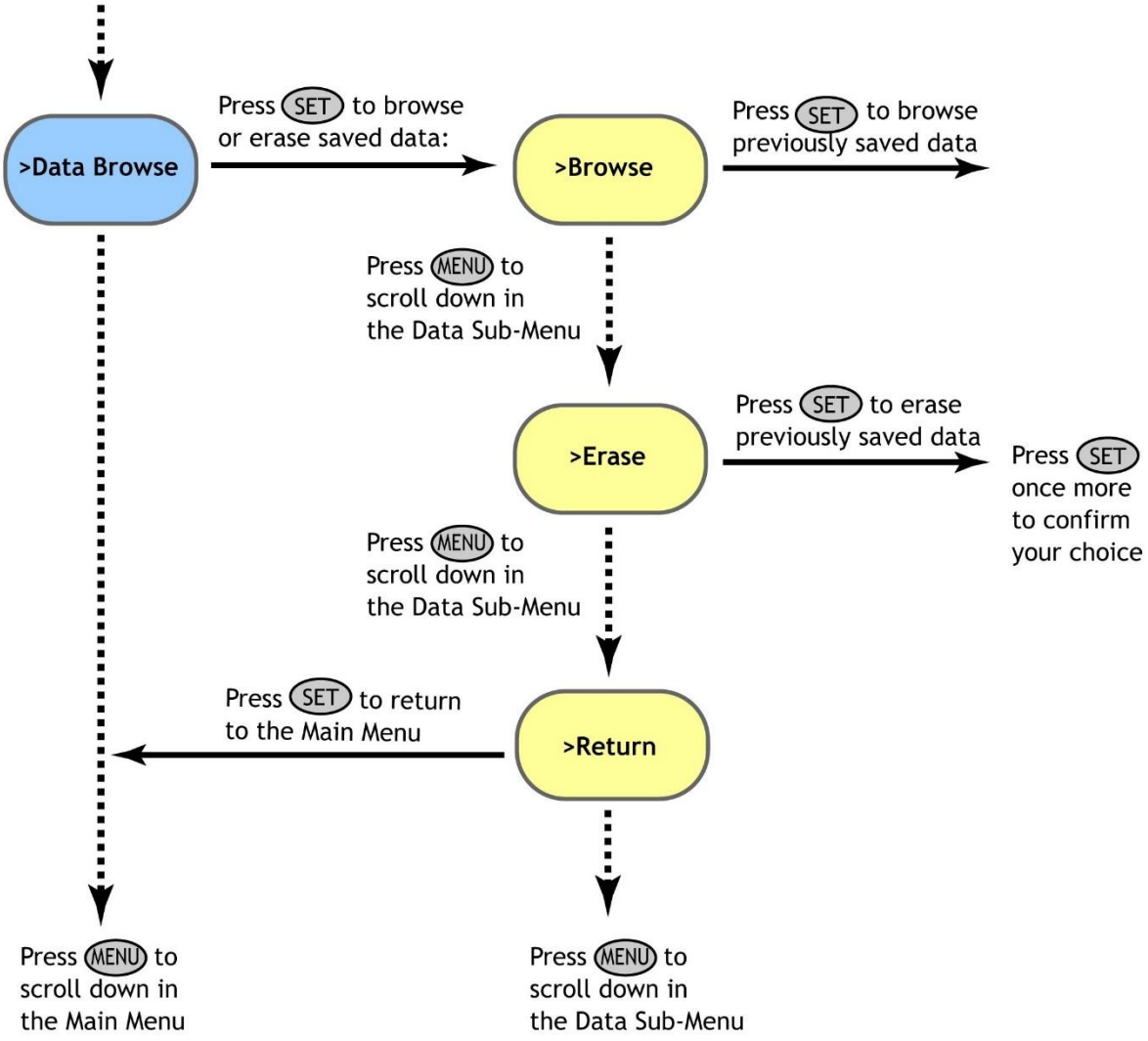
Measure Sub-Menu - Part 2

Use the Measure Sub-Menu when measuring selected parameters.



Data Sub-Menu

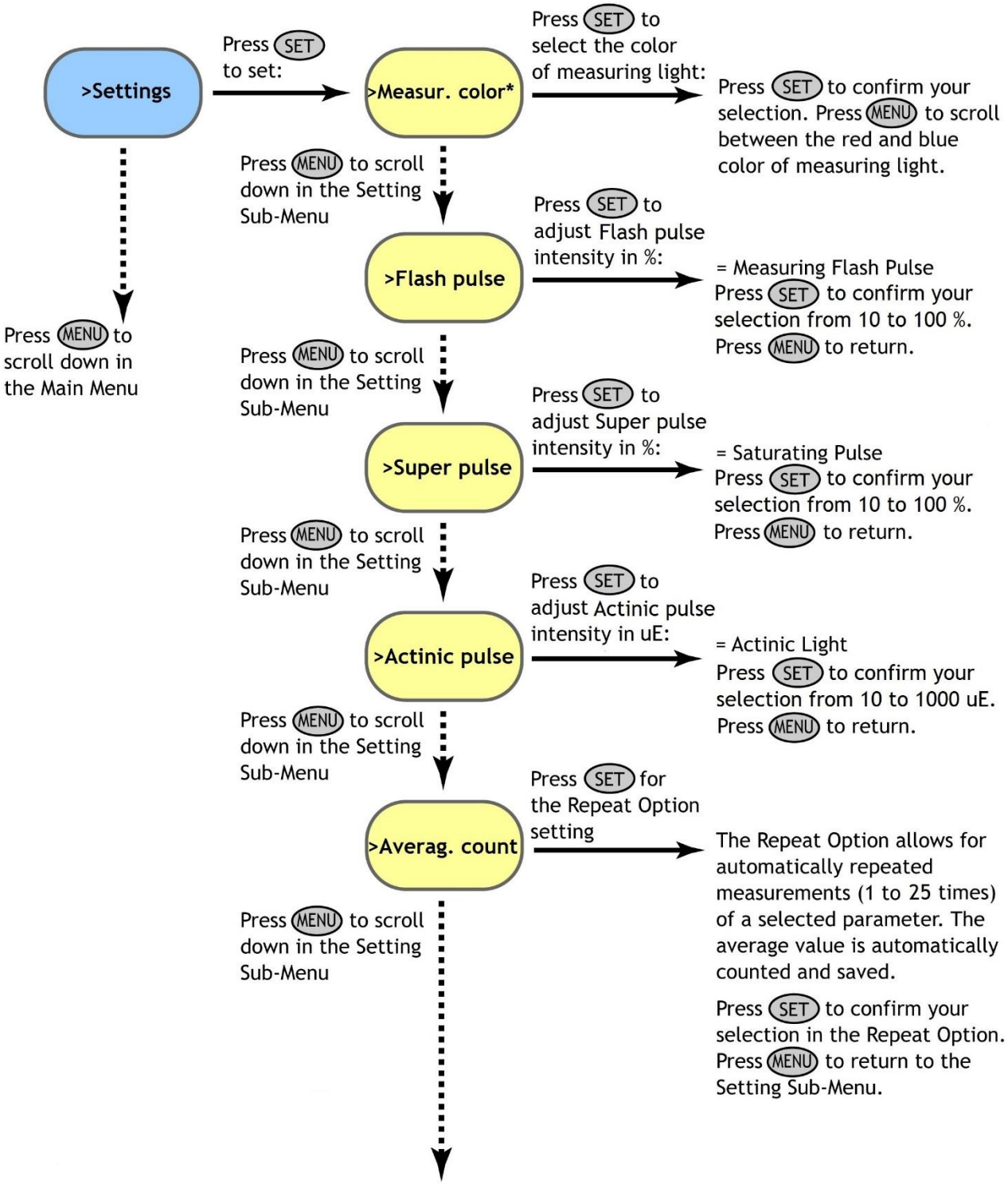
Use the Data Sub-Menu when browsing or erasing previously measured data.



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.

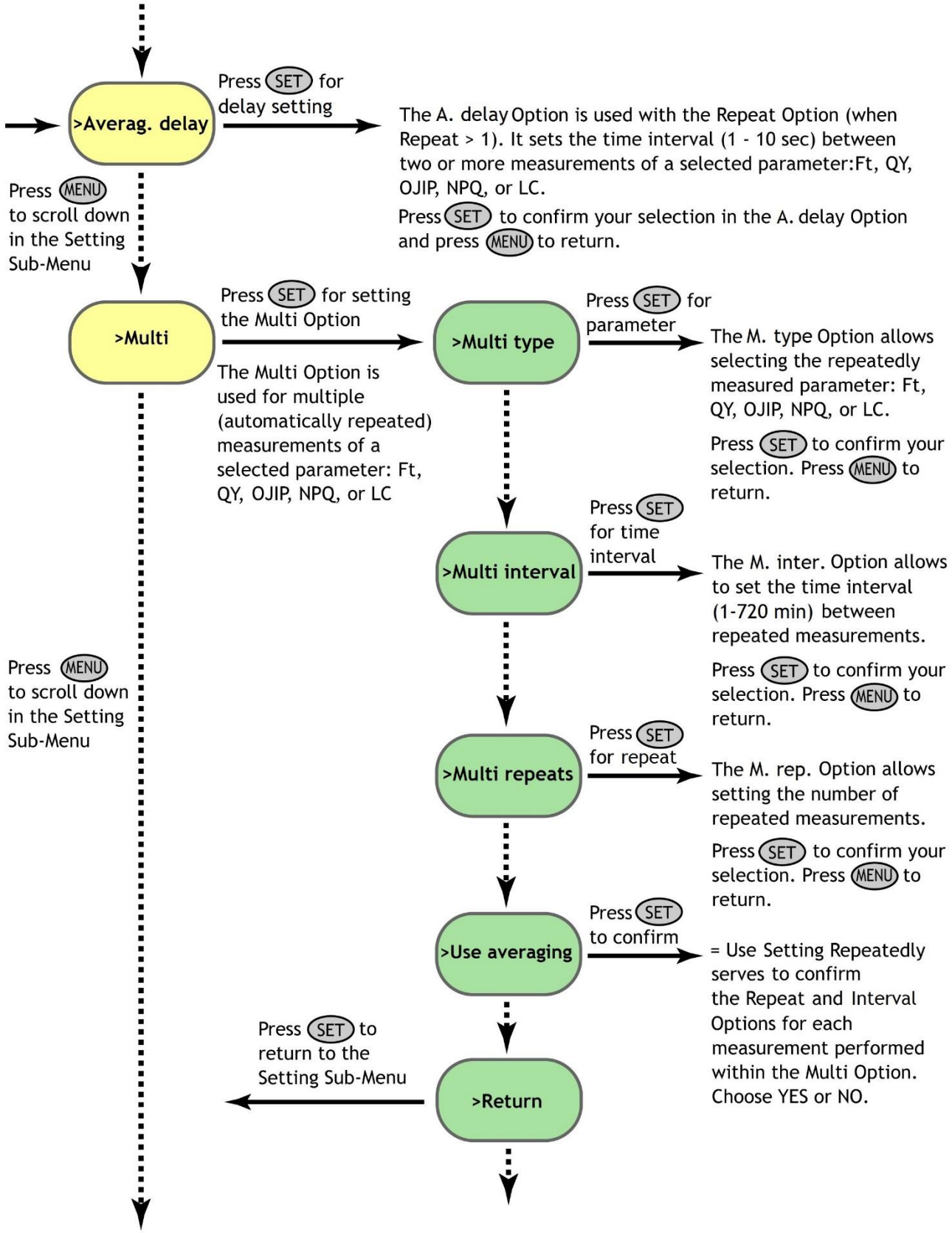


To be continued on the next page

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

Setting Sub-Menu - Part 2

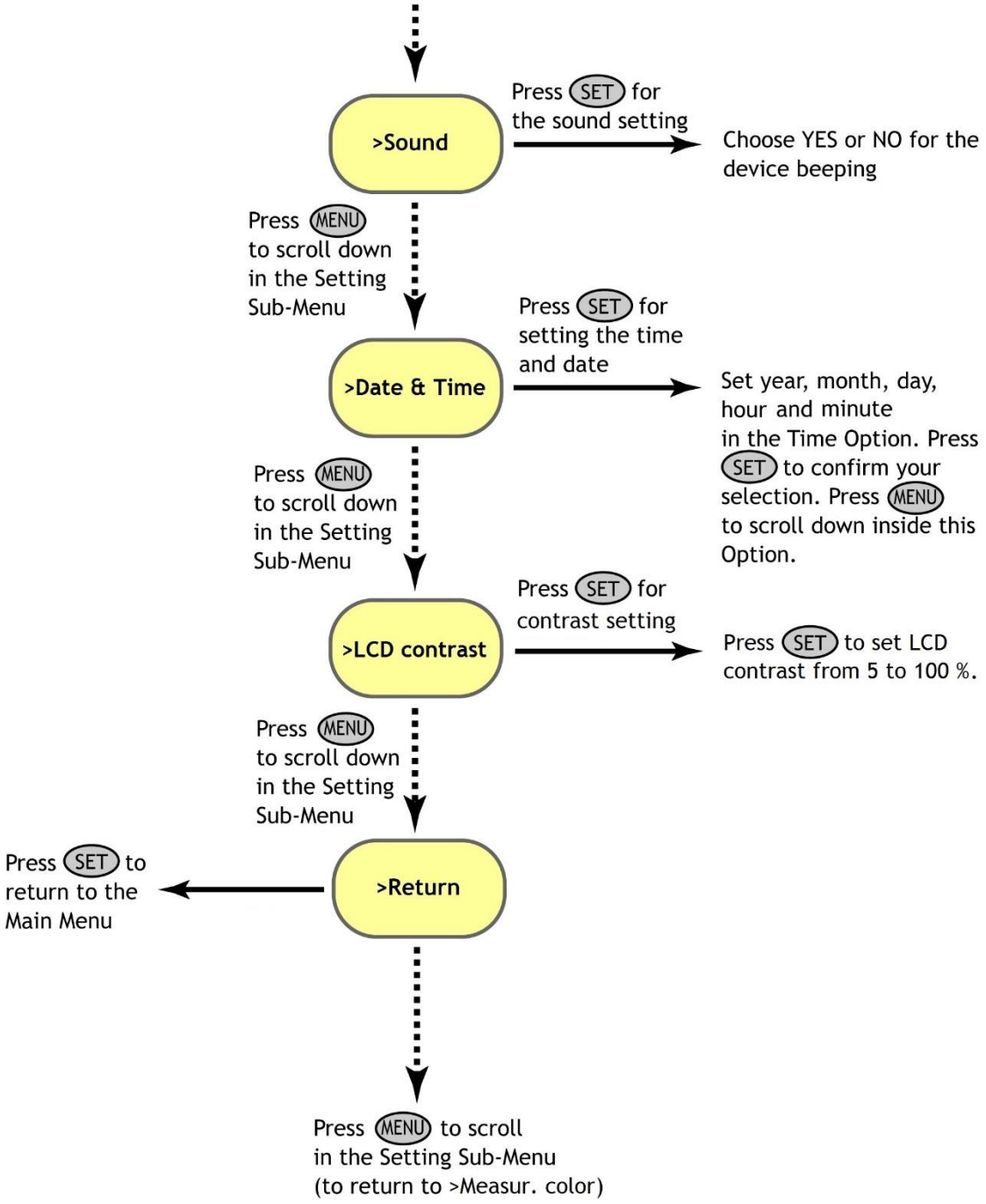
Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.



To be continued on the next page

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.



9 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. 11:

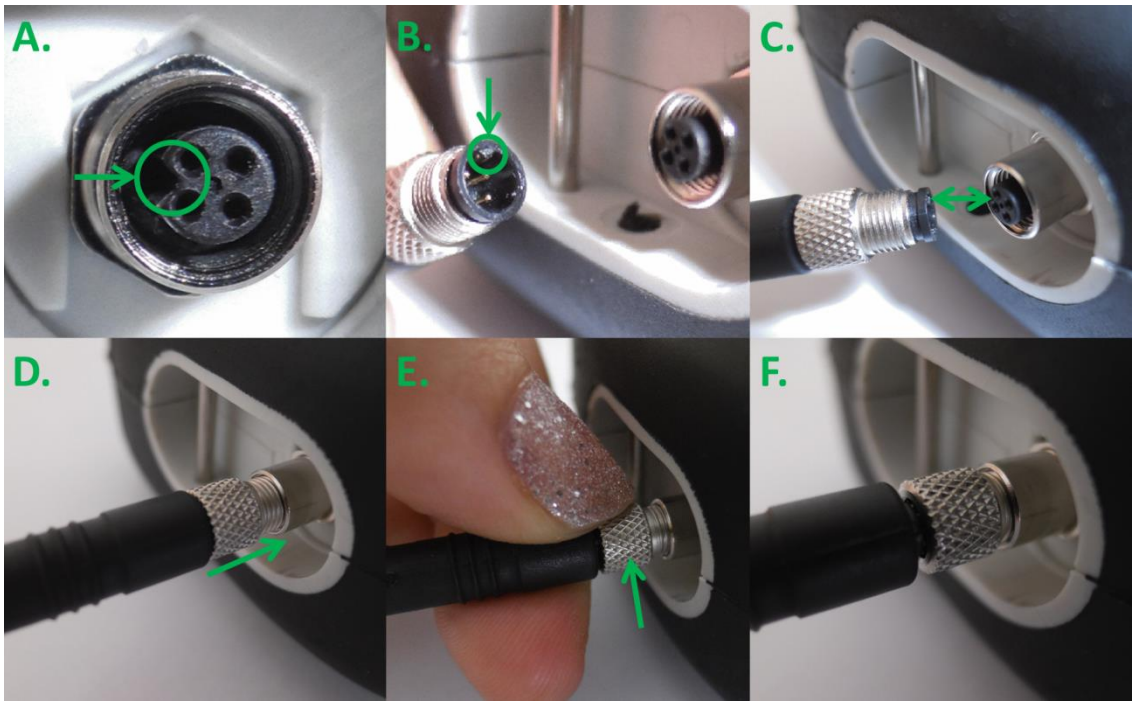


Fig. 11 How to connect AquaPen with PC.

A) connector on the AquaPen device. B) Portion of the USB cable with pins. C – E) Position the cable horizontally and line up the green 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 11.

10 BLUETOOTH CONNECTION

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

1. **Bluetooth enabled PC**

The PC must have Bluetooth wireless technology, either built-in or through a Bluetooth card. En sure 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.

2. **Bluetooth configuration software properly set up on the PC**

Before you connecting the AquPen to the PC and downloading data files 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.

3. **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.

10.1 BLUETOOTH PAIRING

1. **Enabling Bluetooth on 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.

2. **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. 12).

  You may also start your Bluetooth application via the Control Panel: **Start > Control Panel > Hardware and Sound > Devices and Printers**.

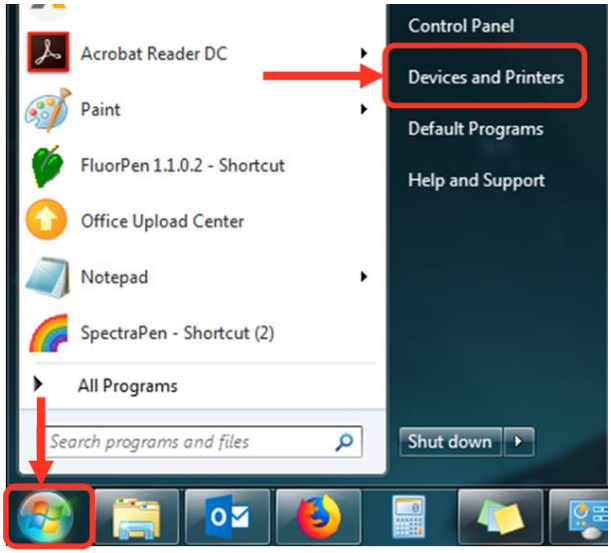


Fig. 12 Start Bluetooth Application.

3. Opening the Add Bluetooth Device Application

- Select: "Add a device" to start searching for the new Bluetooth device. Be sure that the AquaPen is in discoverable mode (see step 1).

Fig. 13 Add a device.

4. Selecting the AquaPen

- Select: PSI AquaPen icon.
- Click: Next (Fig. 14).

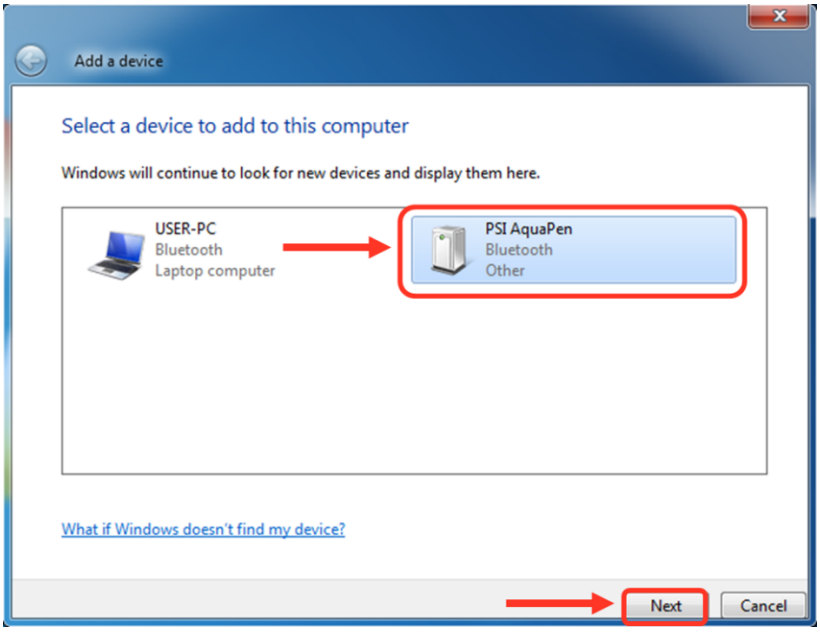


Fig. 14 Select the AquaPen.

5. Starting the Pairing Process

This step is different for old and new version of the AquaPen, that are equipped with disparate Bluetooth module.

Old version of AquaPen (AP-100):

Your Bluetooth Pairing Code is: 0000

- Select: “Enter the device’s pairing code”.
- Enter: **0000** (four digits).
- Select: Next (Fig. 15).

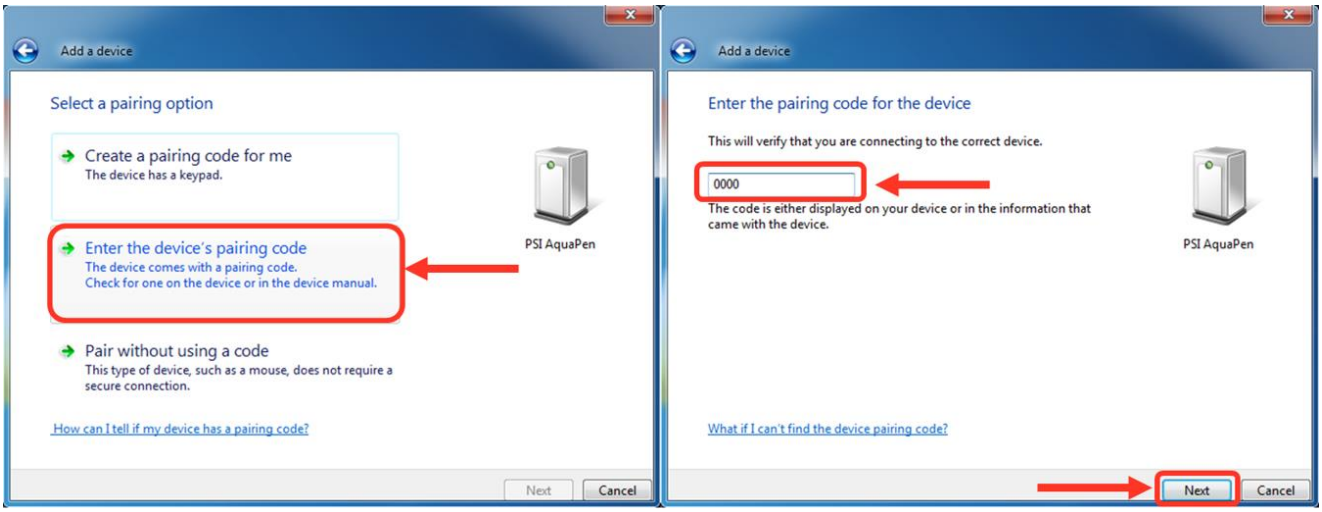


Fig. 15 Pairing process.

New version of AquaPen AP-110:

- Select: Yes (Fig. 16). **Please note that the AquaPen device does not display the verification number. The verification code is not important for the BT connection.**
- Select: Next.

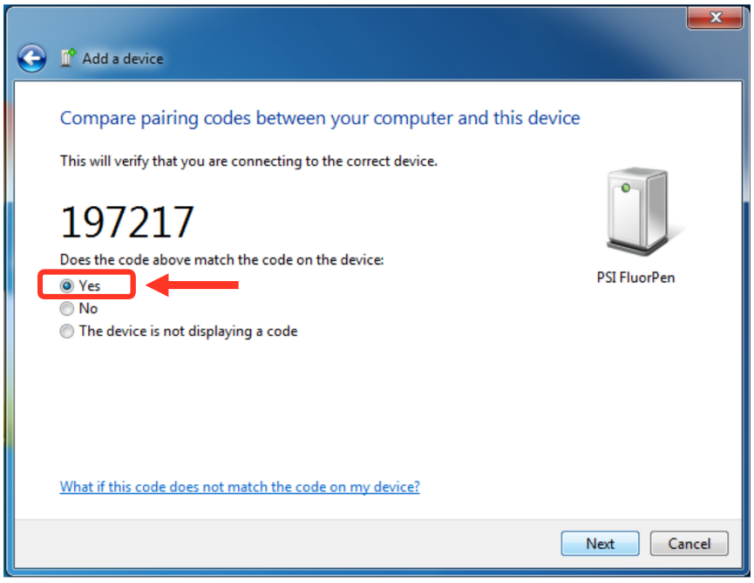


Fig. 16 Verifying of the BT pairing.

6. Completing the AquaPen Pairing

- Select: Close (Fig. 17).

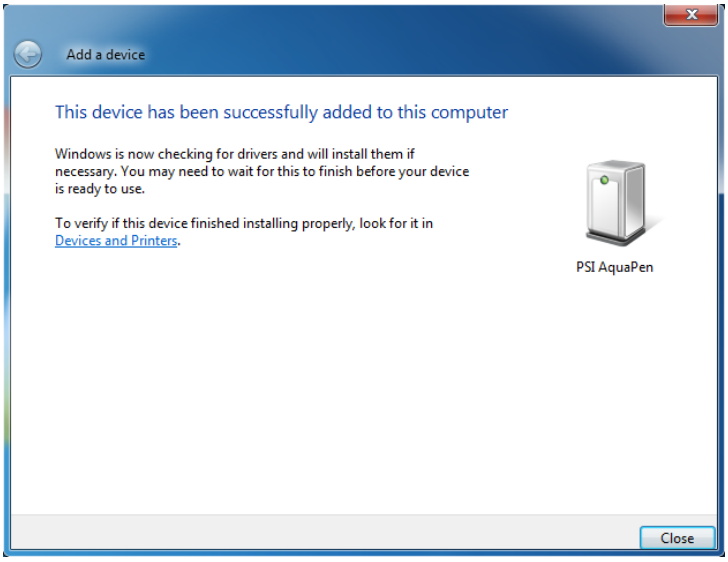


Fig. 17 Finishing.

The Bluetooth pairing is now complete, and the next step is to open the program FluorPen 1.1 (included on the USB flash disk) For more information about FluorPen software see chapter 11.

11 FLUORPEN SOFTWARE

11.1 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. 18).
   - 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

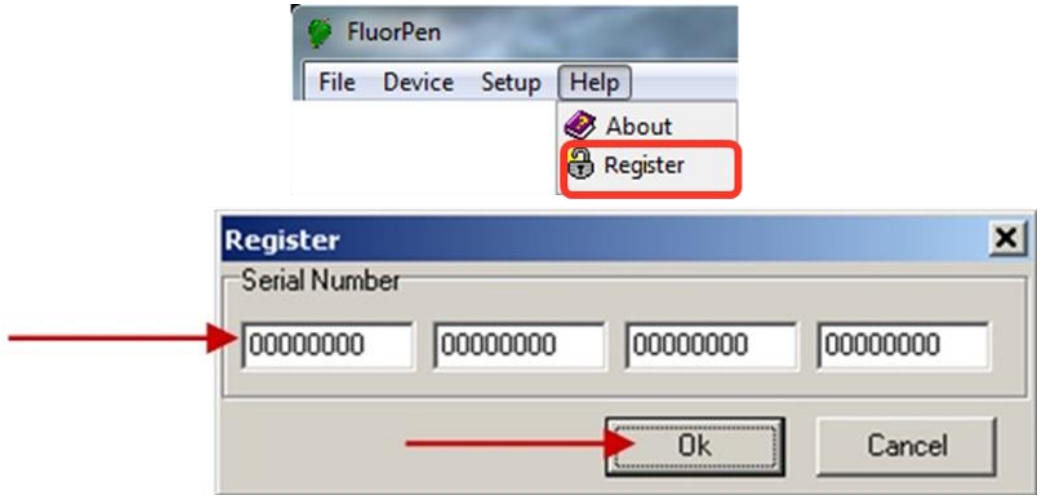


Fig. 18 Software registration.

	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 AquaPen and enable Bluetooth or connect USB cable to the PC.
4. Ensure the PC and the AquaPen are properly paired (see chapter 9 and 10 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. 19). 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

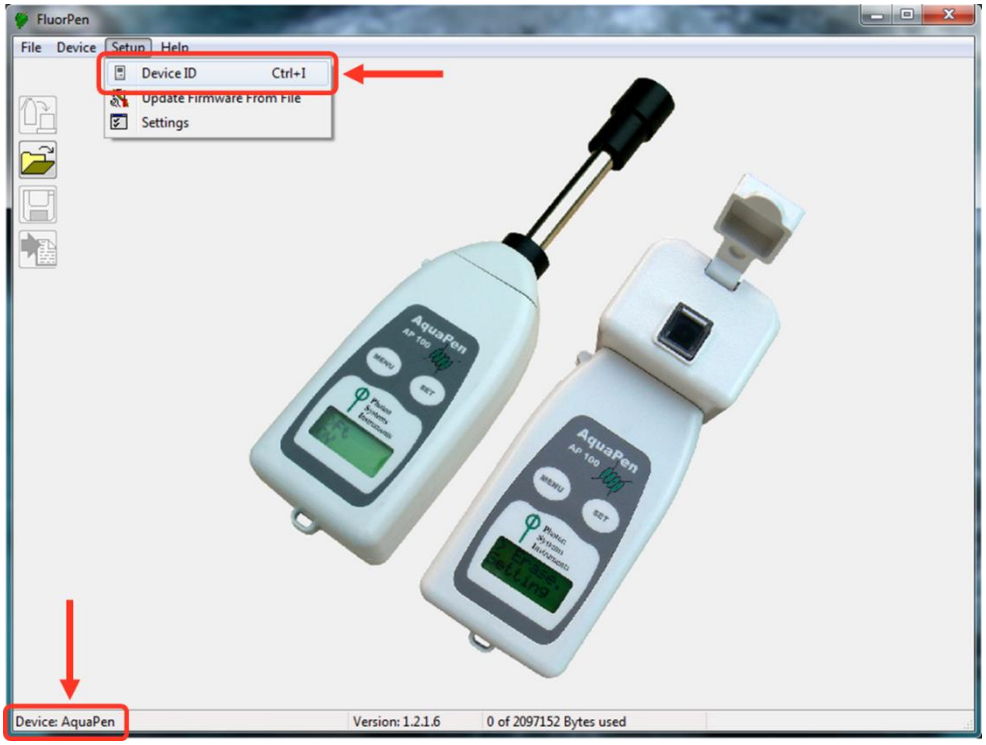


Fig. 19 Connecting AquaPen with Software.

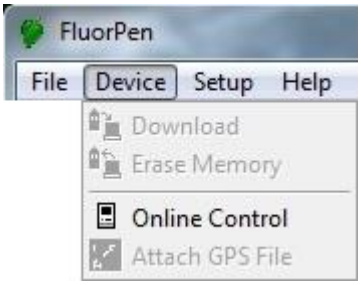
11.2 MENU AND ICON EXPLANATION

11.2.1 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.

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).

MENU: Setup

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

MENU: Help

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

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.

11.2.2 MENU SETTINGS

MENU > Setup > Settings

After Download – Memory Erase

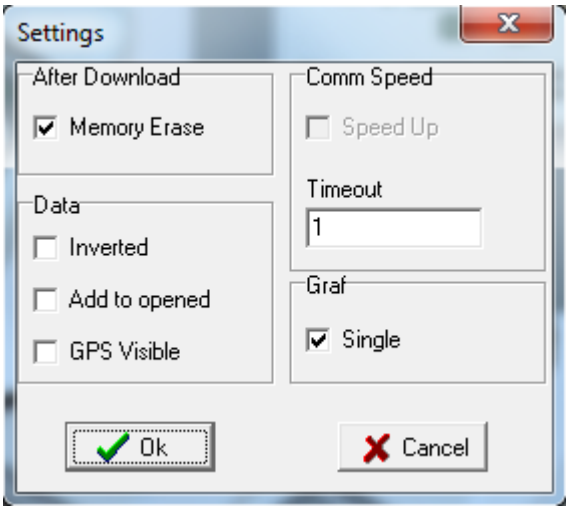
If the box is checked the AquaPen memory is erased after each data download.

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.

11.2.3 MENU ONLINE CONTROL

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

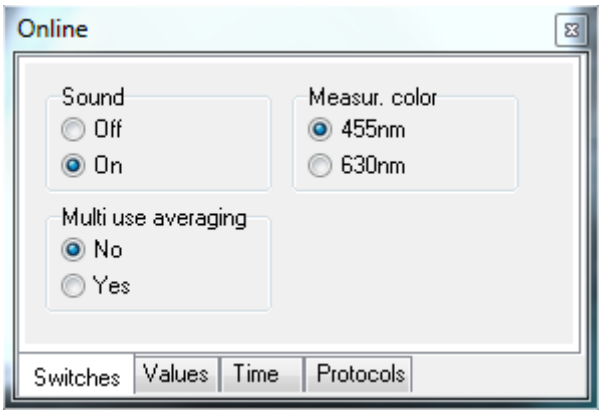
- Select: **Menu > Device > Online Control**

Online Control – Switches

Sound On/Off – select presence of sound - device beeping when pressing MENU and SET keys.

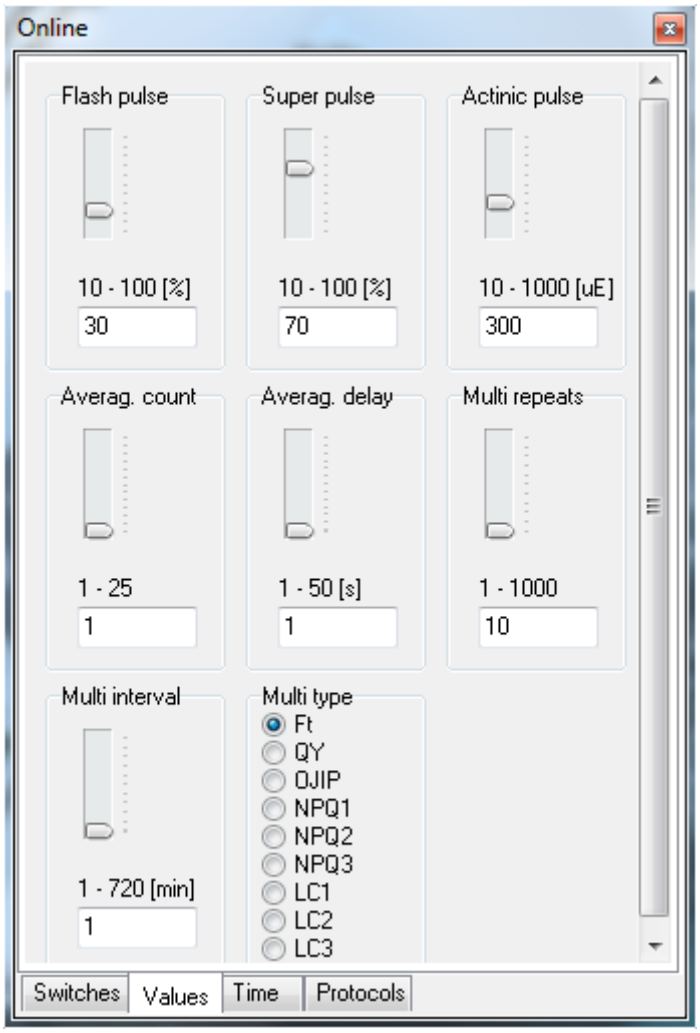
Measuring color – choose 455nm or 630nm for measuring fluorescence protocols. This function is active only in the AquaPen-C 110-C model.

Multi use averaging (YES/NO) – serves to confirm **Repeat** (number of repetitions) and **Interval** (time between measurements). Settings for each measurement within **Multi** Option preset by the user on the AquaPen device or in the software under Values tab (see below)– select YES or NO.



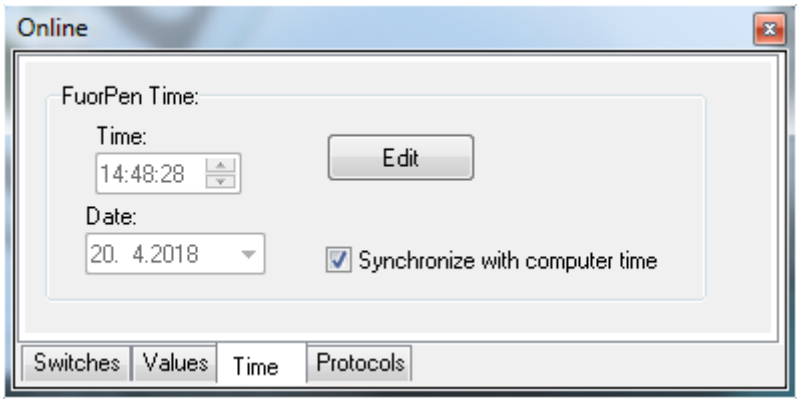
Online Control – Values

One can change settings of Actinic, Super or Flash Pulse light in this window. Here is where averaging of measured parameters (**Averag. cou**nt and **Averag. delay**) is also set up. The time between measurements (**Multi-Interval**) and the number of measurements (**Multi-repeats**) from 1-1000 can be set in this window. Finally, the type of Protocol selected (**Multi-type**) for Multiple measurements is also set in this window (see picture below). 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 window (see the image of the window below on pg. 47).



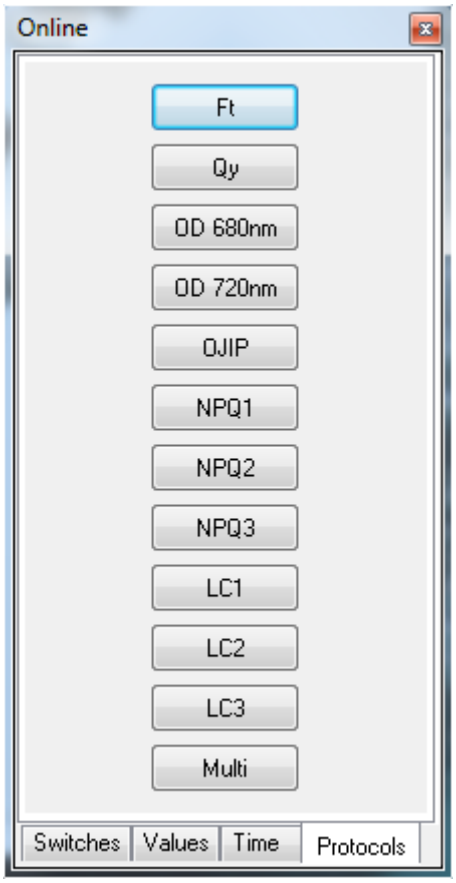
Online Control – Time

In this window the AquaPen time and date. One can also synchronize time of AquaPen device with computer time. This is essential for correct GPS data acquisition and therefore recommended



Online Control – Protocols

Selection of the protocol for single measurements may be done under this tab in the software. Once the measurement is completed the data is saved to the device and can be downloaded to the PC later. Also, by pressing the Multi button in this window the Multiple measurements can be started remotely. Measuring of OD 680nm and OD 720nm is active only in the AquaPen-C 110-C model.



11.3 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 9 and 10 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. 20).

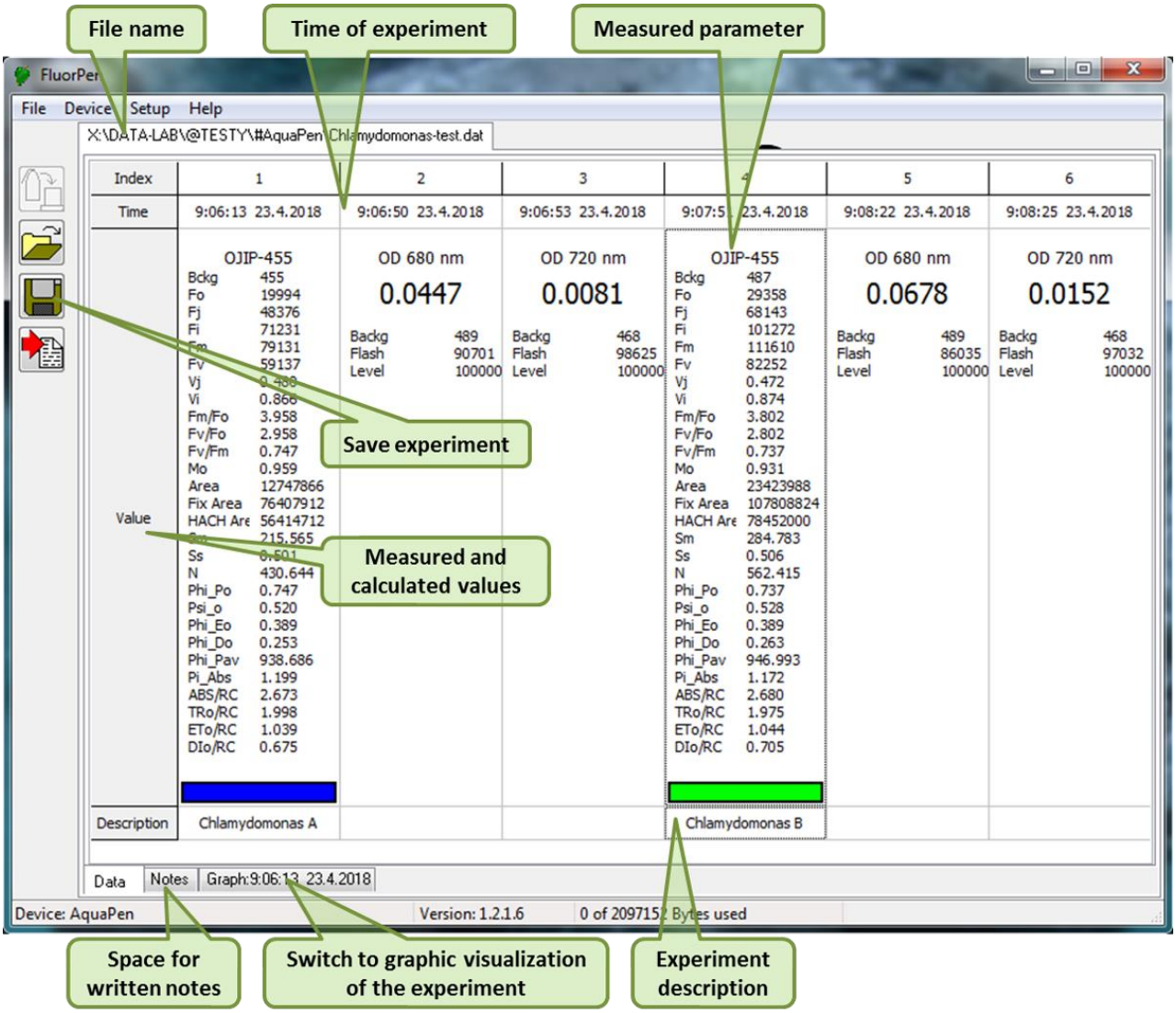


Fig. 20 Example of Data Transfer and Visualization.

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. 21).

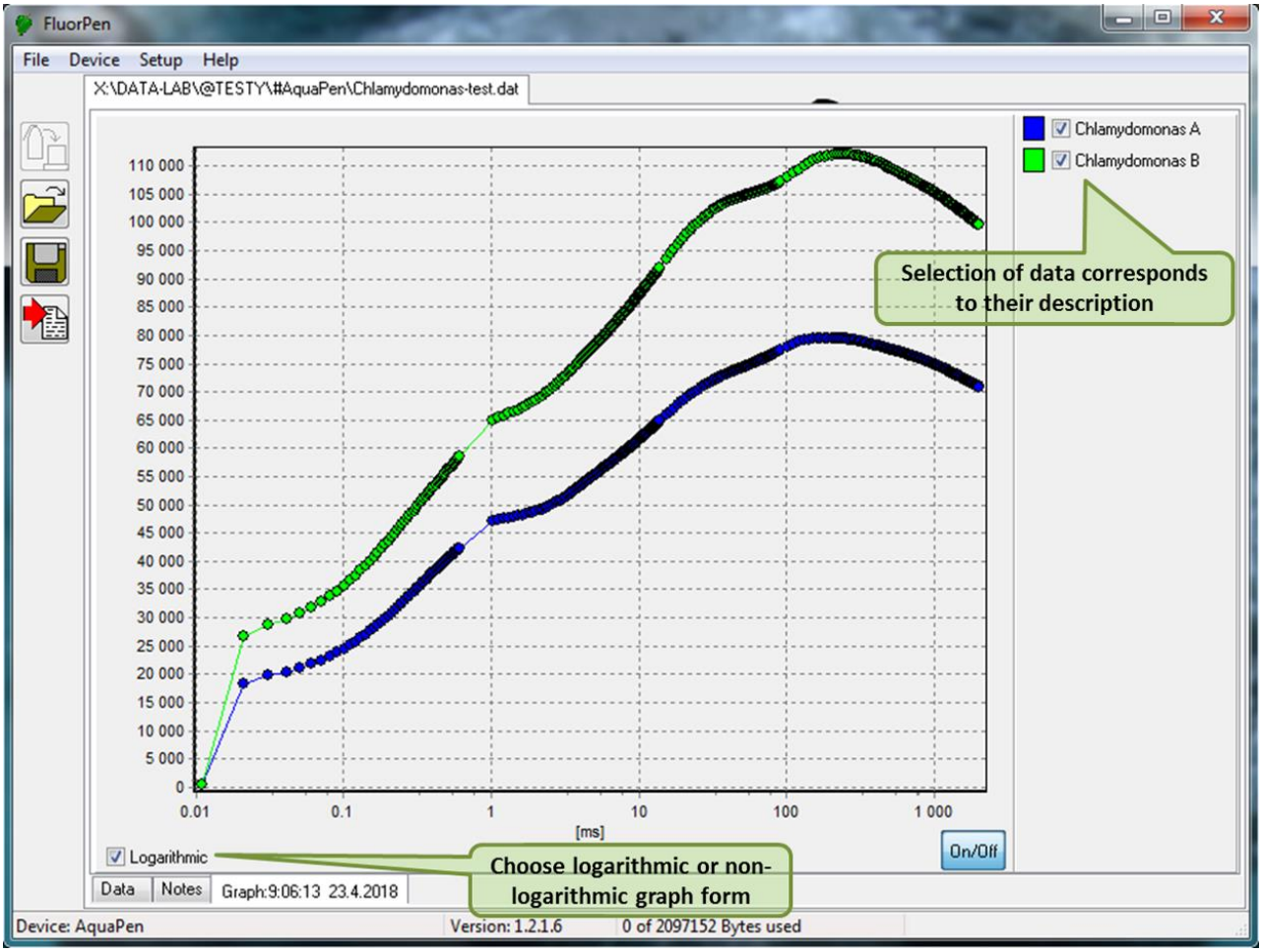


Fig. 21 Graphic visualization of experiment.

6. To **export** data from the FluorPen software select **File > Export** or **Export** icon. Select data type to export (Ft, QY, OJIP…) - Fig. 22.

   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...

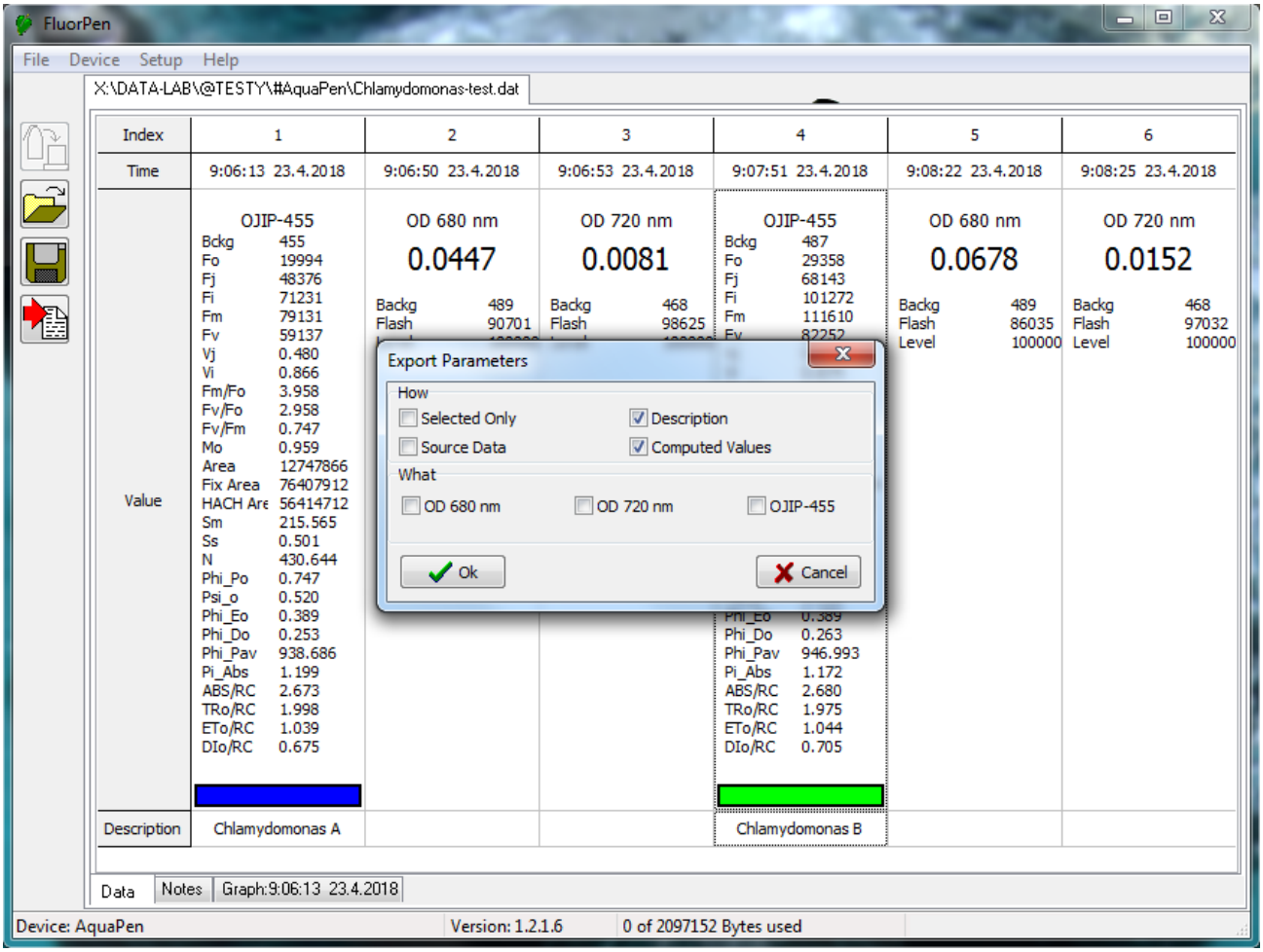


Fig. 22 Export data.

11.4 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. 23).

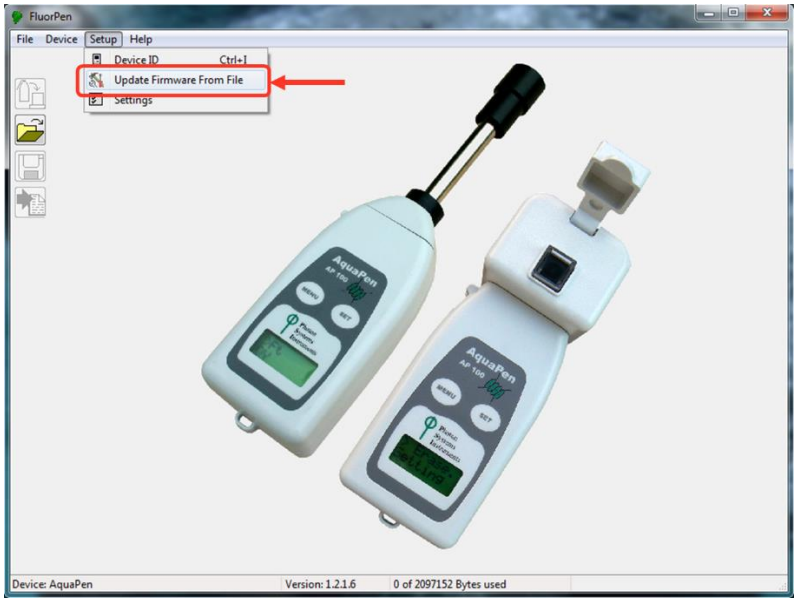


Fig. 23 Update Firmware.

2. Warning

- Select: **OK** to start update (Fig. 24)

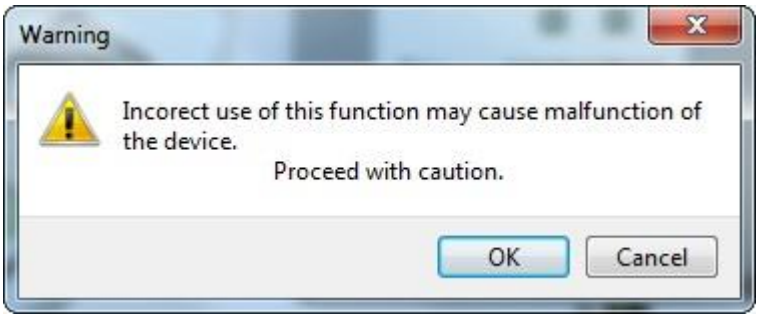


Fig. 24 Warning.

3. Selecting .bxn file

- Find: firmware update file: Binary file (with the extension .bxn) (Fig. 25).
- Select: Open.

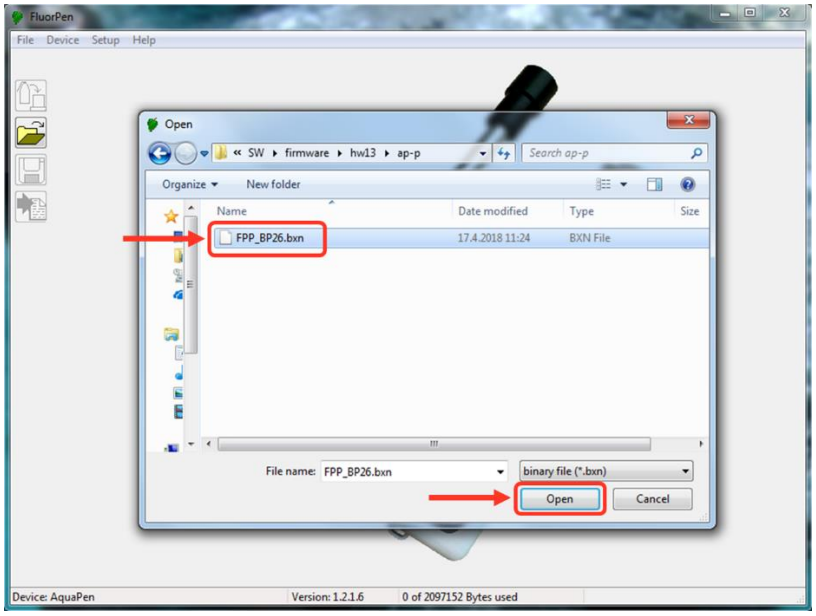


Fig. 25 Select .bxn file.

4. Finishing Upload

- Select: **OK** to start uploading of the update (Fig. 26).

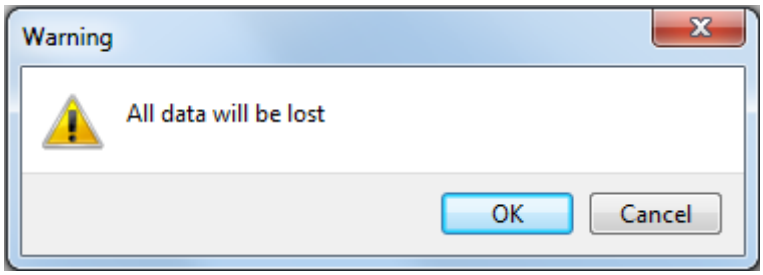


Fig. 26 Data loss warning.

- The bottom bar indicates the upload progress (Fig. 27).

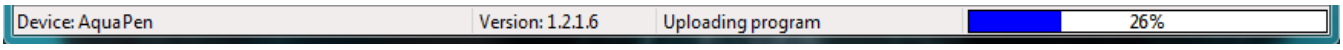


Fig. 27 Upload progress.

- Press: OK to finish upload (Fig. 28).

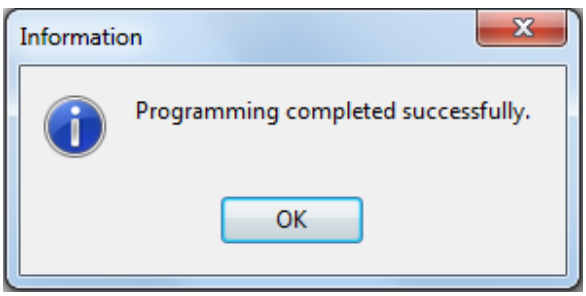


Fig. 28 Finish upload.

12 GPS MODULE

The new versions of the AquaPen device (AP110) 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.

12.1 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. 29.

Fig. 29 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.

12.2 DATA DOWNLOAD

1. Enabling Communication:
   - Switch on the computer and the AquaPen. Set your computer to AquaPen communication: enable Bluetooth or connect to USB port (see instructions on pg. 37).
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 down lad icon (top icon). Data measured with activated GPS module are downloaded with GPS coordinates (Fig. 30).

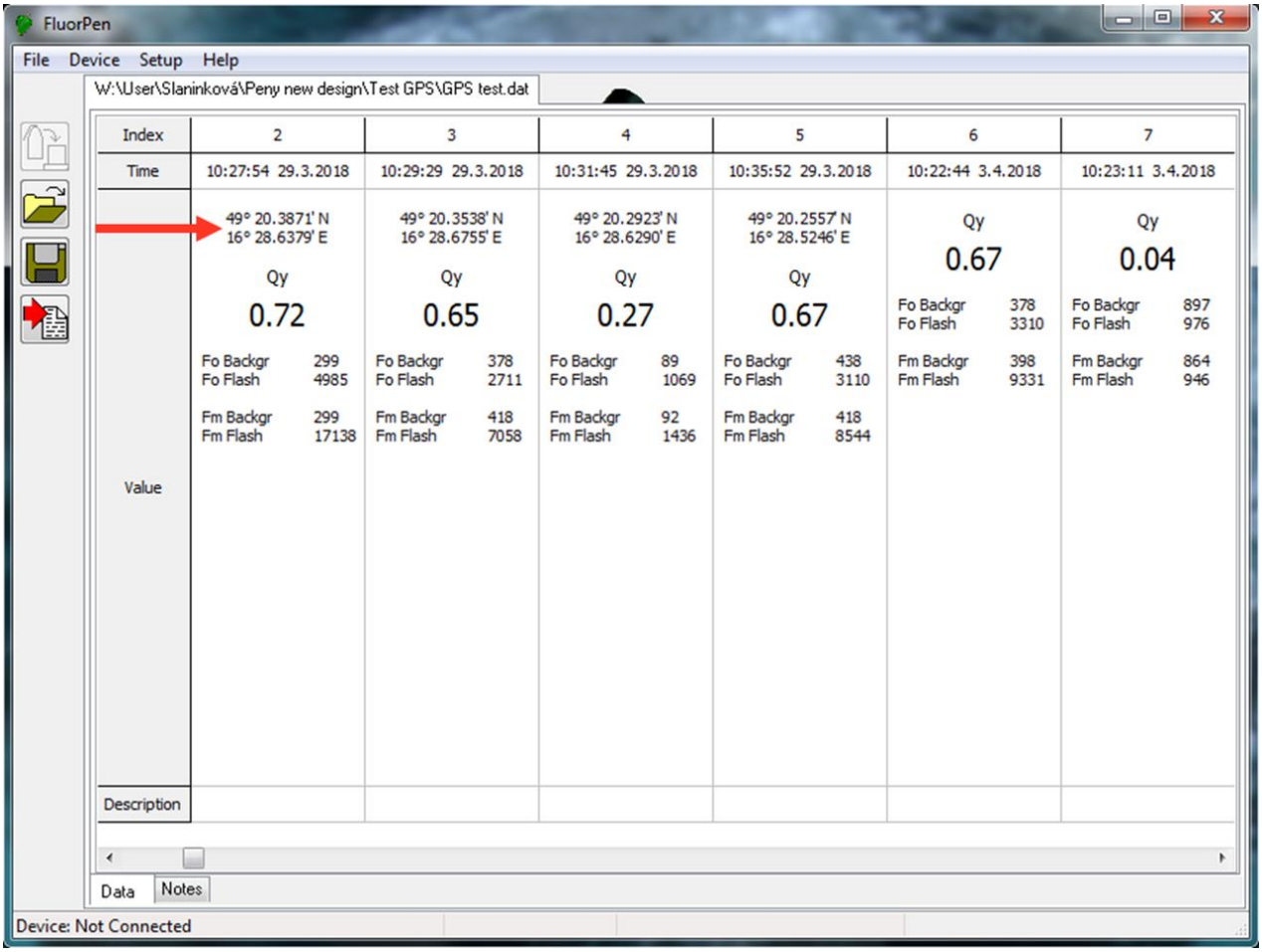


Fig. 30 GPS coordinates.

13 WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the AquaPen 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.

14 TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of problems with the AquaPen 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.