FluorPen FP 110
PAR-FluorPen FP 110
Monitoring Pen MP 100

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 FLUORPEN DEVICE

Read this manual carefully before operating the device. If you are not sure about anything 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 manual while working with the FluorPen device or doing the maintenance.

It is forbidden to interfere with the hardware or software of the FluorPen 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.
ℹ	Additional information.

Tab. 1 Used symbols.

2 TECHNICAL SPECIFICATION

PAR-FluorPen & FluorPen

Protocols	
PAR-FluorPen FP 110	- Ft – instantaneous chlorophyll fluorescence - Quantum Yield - OJIP - Non-photochemical quenching - Light curve - **Photosynthetically Active Radiation (measured as PPFD)**
FluorPen FP 110	- Ft – instantaneous chlorophyll fluorescence - Quantum Yield - OJIP - Non-photochemical quenching - Light curve
LED lighting	
LED emitter	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	Standard leaf-clip (FP 110/S) Detachable leaf-clip (FP 110/D) Probe (FP 110/P)
PAR sensor cosine correction	Cosine corrected up to 80° angle of incidence
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	134 x 65 x 33 mm
Weight	188 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

Monitoring Pen

Protocols	
- Ft – instantaneous chlorophyll fluorescence - Quantum Yield - OJIP - Non-photochemical quenching - Light curve	
LED lighting	
LED emitter	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	Serial cable
PC software	FluorPen 1.1 (Windows 7 and higher)
Battery	
External battery	Standard battery pack - operating temperature from 10 to 40 °C - rechargeable
	Extended temperature range battery pack - operating temperature from -40 to 60 °C - non-rechargeable (spare battery)
Capacity	12Ah
Battery life	Up to 2 years of operation (1 QY measurement per hour)
Other	
Sample holder	Probe
Display	2 x 8 characters LC display
Keypad	Sealed, 2-key tactile response Turns off after 5 minutes of no use
Size	134 x 65 x 33 mm
Weight	188 g
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

FluorPen FP 110 is a portable, battery-powered fluorometer that enables quick and precise measurement of chlorophyll fluorescence parameters (Ft, QY, NPQ, OJIP, and LC of (QY) in plants. The FluorPen can be used in the laboratory, greenhouse, or in the field, where data can be mapped precisely to the location with the built in GPS module. With the built-in GPS module, the FluorPen is a great device for studying photosynthetic activity, stress detection, herbicide testing, or mutant/variety/transgenic screening in the field. Affordable price and user-friendly two-button operation make the FluorPen a perfect tool for teaching photosynthesis. Because of its rapid measurement capability and large internal memory, the FluorPen is also an invaluable tool for large plant-screening programs.

PAR-FluorPen FP 110 includes all features of the FluorPen FP 110, and measures the same chlorophyll fluorescence parameters as FP110, but in addition, the PAR-FluorPen has an integrated Light Meter for direct digital readouts of Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm. PAR is measured as Photosynthetic Photon Flux Density (PPFD), which is indicated by units of quanta (photons) per unit time per unit surface area. The sensor has a uniform response to photons in the 400-700 range. Instant readouts are provided as average values of 20 measurements. It is recommended to recalibrate the PAR sensor every 2 years.

Monitoring Pen MP 100 is a lightweight, portable and a more durable version of the FluorPen. It is designed for long-term, unattended monitoring of chlorophyll fluorescence parameters in the field or lab experiments. It features weatherproof construction for use even in adverse environmental conditions, or in the laboratory /greenhouse long term experiments. It is battery operated (internal or external battery) and as an option may be used with a solar panel as a power supply.

All measured data are sequentially stored in the internal memory of the FluorPen, PAR-FluorPen or Monitoring Pen all collected data can be transferred from the devices to the PC computer via both USB and Bluetooth communication -. Comprehensive FluorPen 1.1 software, included with the device provides data transfer, and visualization protocols.

Unless stated otherwise, the information regarding the FluorPen FP 110 is relevant also to PAR FluorpenFP110 and the Monitoring Pen MP 100.

FluorPen versions:

FluorPen FP 110/S
Equipped with a standard attached leaf-clip.

FluorPen FP 110/D
Adapted for use with detachable leaf-clips; leaf clips sold separately in sets of 10.

FluorPen FP 110/P (Fig. 1c)
Intended for autonomous use in indoor conditions (previously Monitoring Pen-S). It features a plastic case, measuring probe and thread for tripod attachment.

FluorPen FP 110/X
The “X“ version is mounted with custom-made leaf-clip.

PAR-FluorPen FP 110/S (Fig. 1a)
Same features as the standard FluorPen FP 110/S plus Photosynthetically Active Radiation (PAR) meter in the range from 400 to 700 nm. Equipped with a standard leaf-clip.

PAR-FluorPen FP 110/D (Fig. 1b)
Same features as the FluorPen FP 110/D adapted for use with detachable leaf clips plus Photosynthetically Active Radiation (PAR) meter in the range from 400 to 700 nm. Leaf clips sold separately.

PAR-FluorPen FP 110/X
Same features as the PAR-FluorPen FP 110/D but the "X" version is mounted with custom-made leaf-clip.

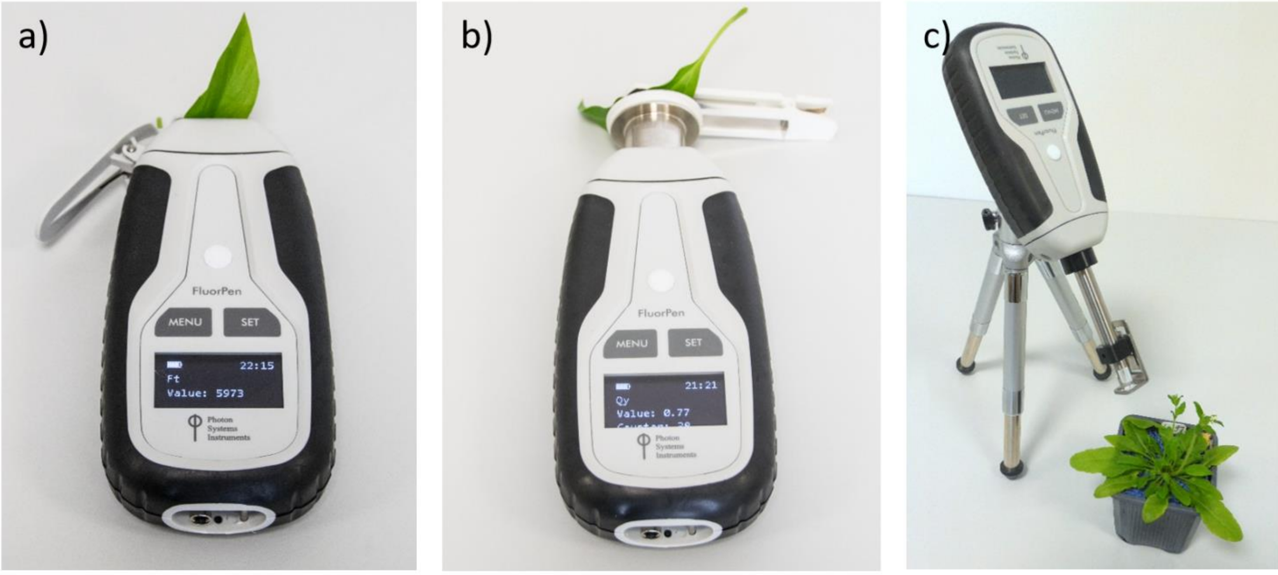


Fig. 1a) PAR-FluorPen FP 110/S. b) PAR-FluorPen FP 110/D. c) FluorPen FP 110/P.

Monitoring Pen versions:

Monitoring Pen MP 100-E (Fig. 2a)

Monitoring Pen MP 100-E is a modified FluorPen designed for extra durability, battery-powered and intended for autonomous use in field conditions during extended experiments. It features waterproof metal case, measuring probe, thread for tripod attachment, external pack with batteries and the FluorPen 1.1 software for data collection and processing.

Monitoring Pen MP 100-A (Fig. 2b)

This is a submersible, battery-powered FluorPen intended for underwater measurements of chlorophyll fluorescence parameters (also autonomous). It features a waterproof case, measuring probe, and the FluorPen 1.1 software for data collection and processing. External battery pack with batteries is sold separately. This aquatic version of the monitoring pen is intended for use at maximum water depth of 2 meters. The device is equipped with two buttons that allow direct control of the device (even under water). A customized Version B of this instrument is also available for use in deeper water (maximum 10 m). There are no control buttons on this version of the device. Version B is controlled via software and a PC (placed above water). The device is usually fixed in static position under water.

Fig. 2a) Monitoring Pen MP 100-E. b) Monitoring Pen MP 100-A.

3.1 DEVICE DESCRIPTION

Fig. 3 Device description.

4 LIST OF EQUIPMENT

Carefully unpack the carton. You should have received the following items:

- **FluorPen/Monitoring Pen**
- **Carrying Case**
- **Textile Strap for Comfortable Wearing**
- **FluorPen Operating Manual** (on a USB flash disc)
- **FluorPen software and driver** (on a USB flash disc)
- **USB cable**
- **Self-Adhesive Rubber Pads for Optics Protection** (FP 110/S only)
- **Detachable Leaf-clips** (FP 110/D only and sold separately)

Other Accessories or Optional Features (according to your specific order)

	For USB connection you need to have the USB driver installed on the PC. The driver can be found on the installation disk (USB driver folder). If any item is missing, please, contact PSI. Also check the carton for any visible external damage. If you find any damage, notify the carrier and PSI 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

FluorPen and Monitoring Pen

- Never submerge the device in water! (except Monitoring Pen MP 100-A).
- The device should not come in contact with any organic solvents, strong acids or bases.
- Keep the optical part clean and dry. If cleaning is needed, use soft, non-abrasive tissue.
- Battery charge lasts approximately 48 hours when the FluorPen is operated continuously.
- If the battery can no longer be charged please contact PSI for replacement battery and installation instructions.

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

FluorPen is a chlorophyll fluorometer and is used to measure different photosynthetic parameters in plants. It is equipped with a **blue LED emitter (470 nm),** optically filtered and precisely focused to deliver light intensities of up to 3,000 $\mu mol.m^{-2}.s^{-1}$ to measured plant tissue (Fig. 4).

Fig. 4 Fluor Pen FP 110/S.

When studying photosynthesis using chlorophyll fluorescence, researchers must distinguish between **photochemical quenching** and **non-photochemical quenching** (heat dissipation). This is achieved by stopping photochemistry, which allows researchers to measure 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 leaf. This transiently closes all PSII reaction centers, which 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 level obtained 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 result in 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. 5). When measuring Fm it is important to dark adapt the samples. This can be achieved by placing the sample in the dark for few minutes (the time varies with conditions) or by using the FP110/D version of the FluorPen that has been adapted for detachable leaf clips. The leaf clips may be placed on the leaf ahead of the measurements and once dark adaptation has been achieved the FP-110/D may be attached to the leaf clip without exposing the leaf to light.

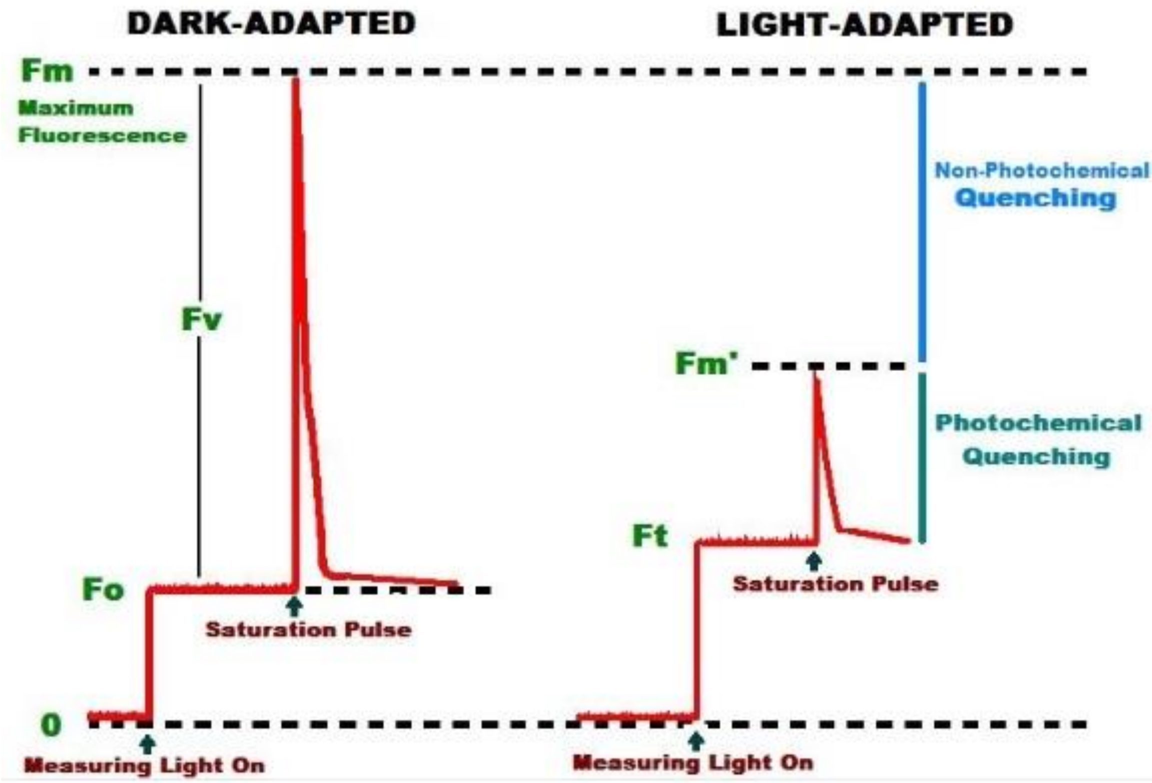


Fig. 5 Chlorophyll fluorescence.

Parameters measured by the FluorPen:

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 7.3.1).
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 0).
LC - Light Curve Photosystem II Quantum Yield estimated from fluorescence that is measured sequentially at several different light levels (see more in Chapter 7.3.3).
PAR* - Photosynthetically Active Radiation Photosynthetically Active Radiation measured as Photosynthetic Photon Flux Density (PPFD).

**** Only in PAR-FluorPen FP 110.***

7 GETTING STARTED

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

The device can is powered with built in Li-Ion battery. Ensure the battery is fully charged by plugging it into a PC via USB cable or the AC outlet via the USB cable and a USB adaptor (not included). Monitoring Pen can be powered from an optional battery pack (see more in chapter 15.1).

The FluorPen 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 1s).
- Use the **SET** key to turn the device on (hold for 1 sec) and select a menu option based on cursor (>) position.

7.1 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 light pulse. Saturating pulse induces maximum chlorophyll fluorescence (F_m). 100 % of intensity equals 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
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 intensities in the FluorPen firmware. These may be changed according to user requirements and algal growth conditions:

Flash pulse 30 % = Measuring flash pulse

Super pulse 80 % = Saturating pulse

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

Please note that those parameters are recommended by the manufacturer but can be changed by the user according to requirements.

Setting the optimal intensities of pulses:

Flash Pulse setting

The optimum value of Flash pulse can be determined with QY measurement as shown in Fig.6 below. Before performing QY measurement it is recommended to set the intensity of Super pulse to 80 %.

Please note that QY measurement should be performed with dark adapted sample, therefore the same sample (position on the leaf) should not be used more than once unless dark adaptation follows the first measurement. The recommendation is to use a new sample (new area on the leaf) for each QY measurements.

F_0 increases linearly with growing intensity of the Flash pulse.

The Flash pulse setting recommended by manufacturer is 30 %. One can increase the intensity of Flash pulse for samples with very low chlorophyll density. However, it should be noted that high intensities of Flash pulse can cause undesirable “actinic effect” as higher intensity High Flash pulse will initiate the photochemistry. Changes in the Flash pulse will affect F_0 and the QY value will be lower.

The optimal Flash pulse intensity is that at which the highest value of QY is reached. This can be easily determined on one leaf by measuring QY in few different spots with different flash pulse settings. See (Fig. 6) below. 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 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. 6 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 Super pulse is to perform OJIP measurement with different Super pulse settings.

Please note that OJIP measurement should be performed with dark adapted sample. New sample (new section of the same leaf) should be used for every measurement as exposure to super pulse will change photochemistry of the leaf in that section.

The Super pulse setting recommended by manufacturer is 80 %.

When performing the OJIP measurement with different intensities of Super pulse the F_v/F_m value will stop increasing when the optimal level has been reached for the samples used (Fig. 7 and Fig. 8).

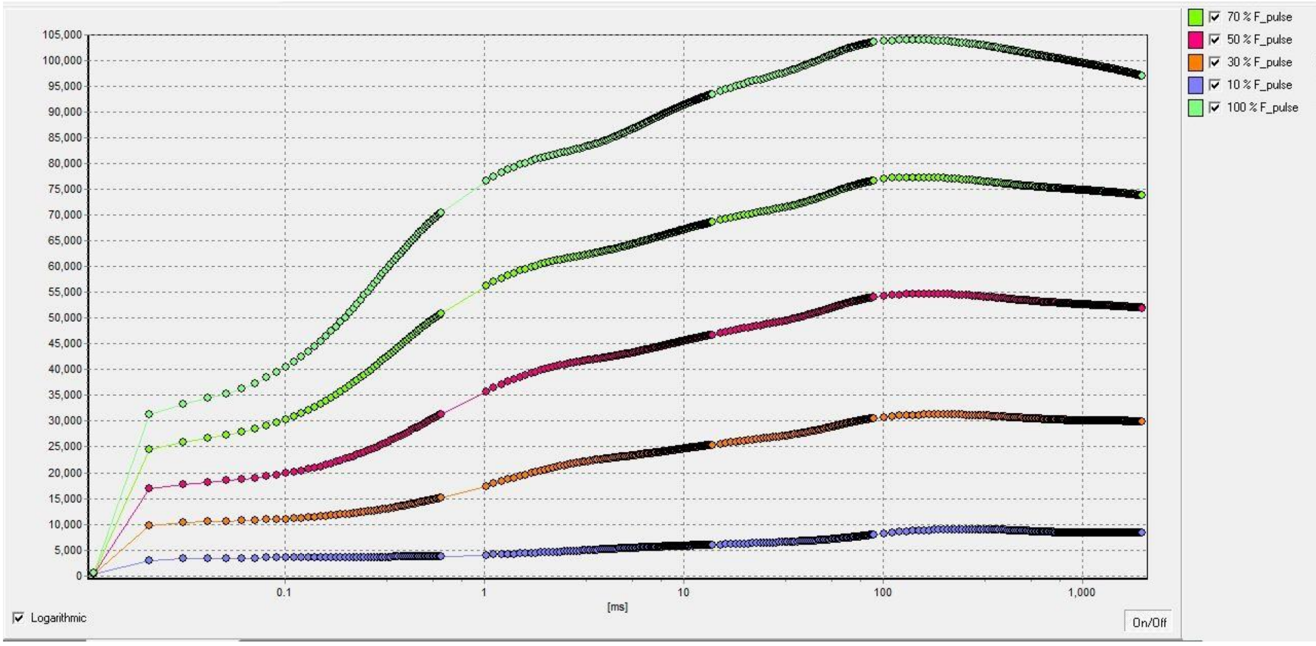


Fig. 7 OJIP measurement performed with different intensities of Super pulse.

	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	OJIP	OJIP	OJIP	OJIP	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	FLASH	FLASH	FLASH	FLASH	FLASH
[nm]	455	455	455	455	455
[%]	30	30	30	30	30
[uE]	-NAN	-NAN	-NAN	-NAN	-NAN
SUPER	SUPER	SUPER	SUPER	SUPER	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. 8 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.

	Should **Overflow** be observed on the display during the measurement, lower the intensity of the used pulses. In case of **Low value** on display during measurement, increase the intensity of the used pulses.

7.2 MEASUREMENT

No device calibration is needed before chlorophyll fluorescence measurements are made. Results of fluorescence measurement depend on device settings and the samples.

How to perform Chlorophyll Fluorescence measurement with FluorPen:

- Prepare dark adapted sample first (prior to F_0, QY, NPQ, LC measurements) by placing the sample for at least 10-15 min in the dark. Alternatively, dark adaptation can be easily achieved by placing the detached leaf clips in closed position on the leaf ahead of the measurements. Only the FP110/D or PAR-FP110/D is designed for use with the detachable leaf clips. The duration of dark-adaptation period depends on plant species and growth conditions.
- For light adapted measurements no dark adaptation of the sample is required.
- Turn ON the device by holding the **SET** button for 1 sec.
- Place the dark-adapted leaf in the leaf-clip (FP110/S or PAR-FP110/S) or in case of detachable leaf clips place the leaf clip on the optical probe of the FluorPen and slide open the screen of the leaf clip to expose the leaf to the optical probe.
- Select **Measure > from the menu and** select required parameter for example – **QY** (press SET as Enter button when making selections).
- Press **SET** to start the measurements.
- When **OJIP**, **LC** or **NPQ** are being measured the display on the device shows the progress of the measurement as percentage.
- When Ft or QY are measured the values appear on the device display. The result of OJIP, NPQ or LC protocol are not visible on the display of the device and need to be download to PC computer (via USB cable or BT connection, see instructions on pg. 35, Chapter 8 and 9) using FluorPen Software (downloaded to PC earlier).
- All measured data are stored in the device memory and can be downloaded to PC computer after completion of the experiment.

7.3 PROTOCOLS EXPLANATION

7.3.1 OJIP PROTOCOL

The FluorPen 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 curve and calculated data visualization.

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.

7.3.2 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 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 exposure to 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. 9.

The FluorPen device 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, and in the number and interval between pulses. See table below:

	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. 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.

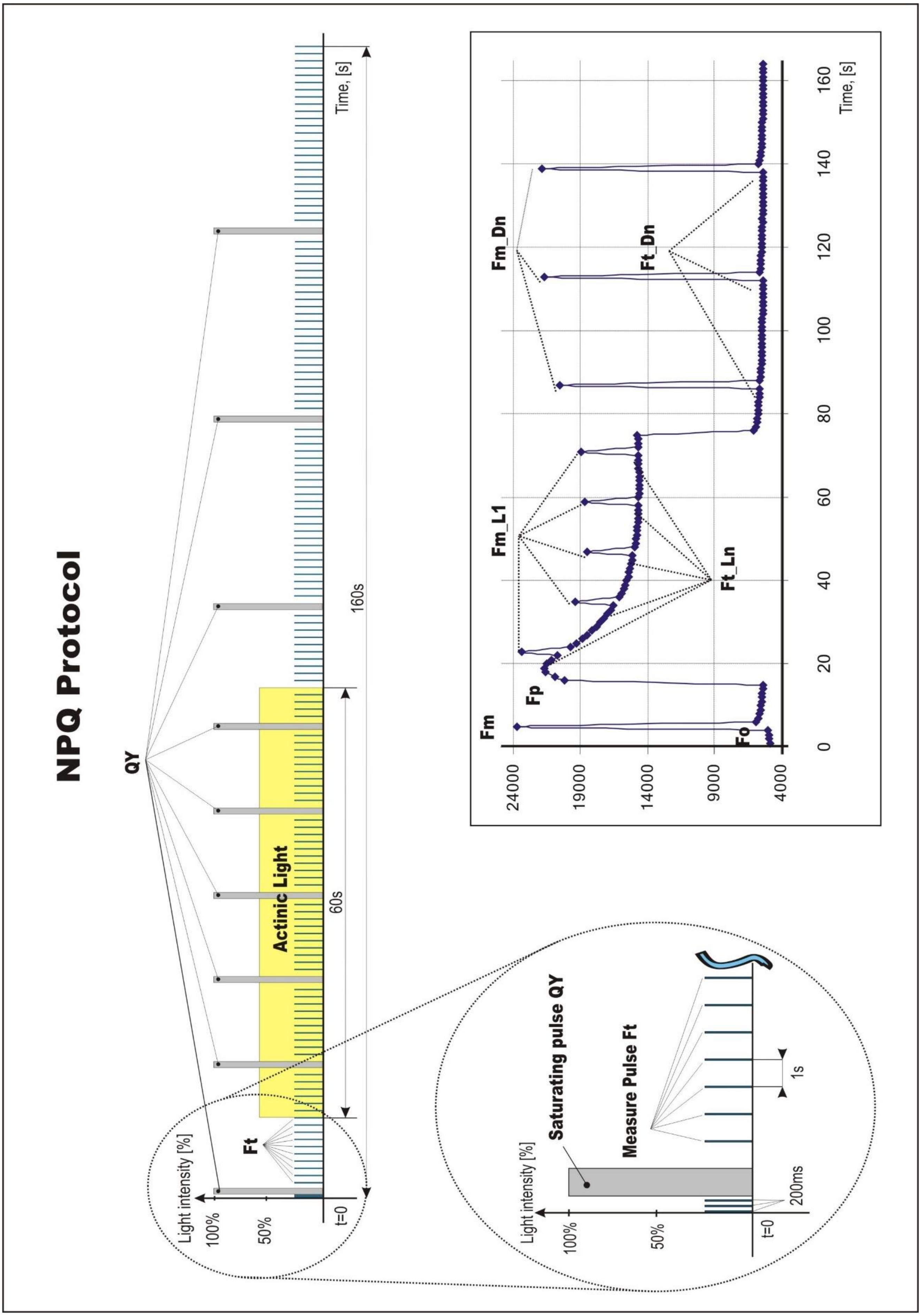


Fig. 9 NPQ Protocol.

7.3.3 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 predetermined 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 Fig. 10 and Fig. 11 below.

	# 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

Tab. 3 LC Protocols.

The LC protocol includes 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 $(F_m\_Lx - F_t\_Lx) / F_m\_Lx$

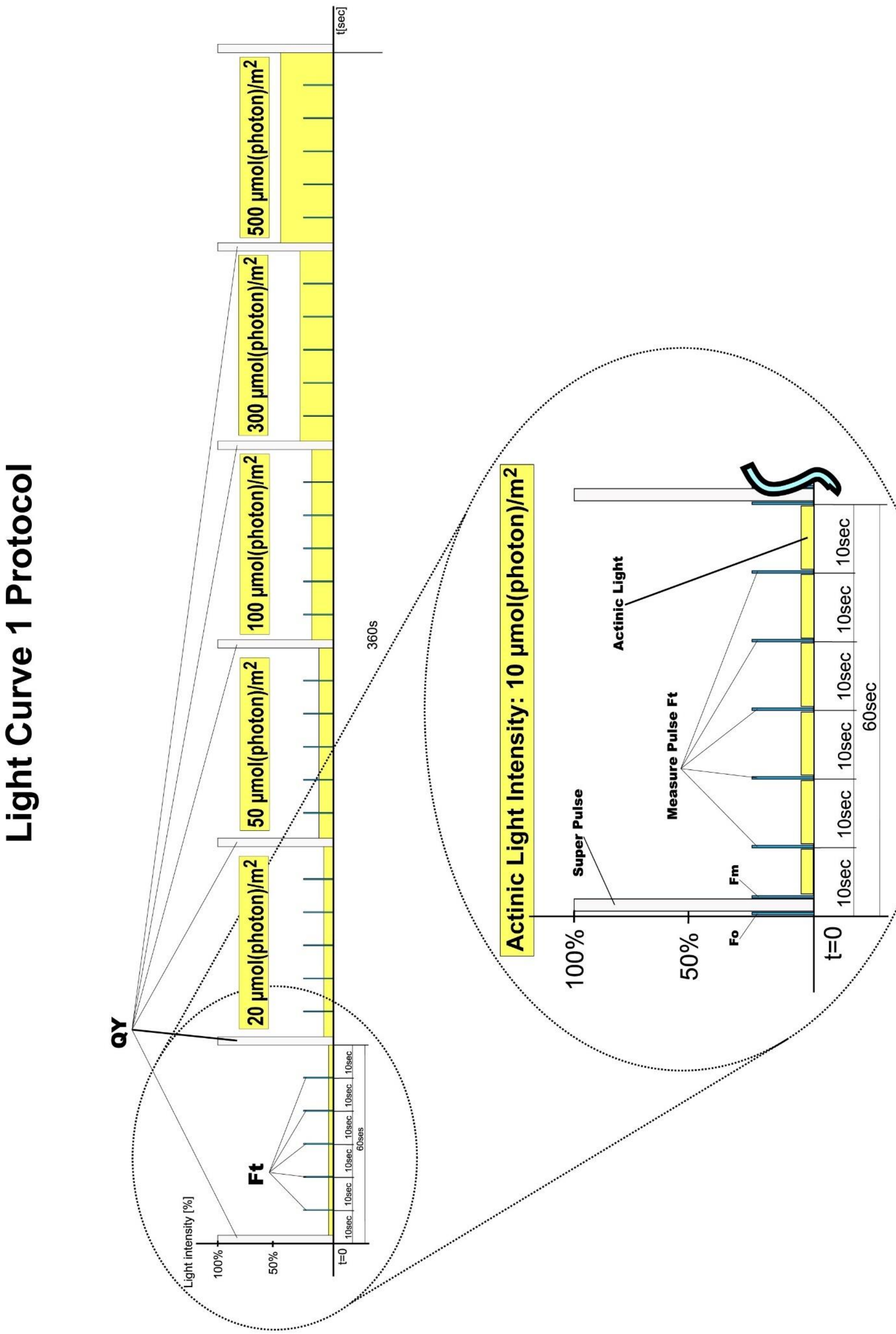


Fig. 10 LC1 Protocol.

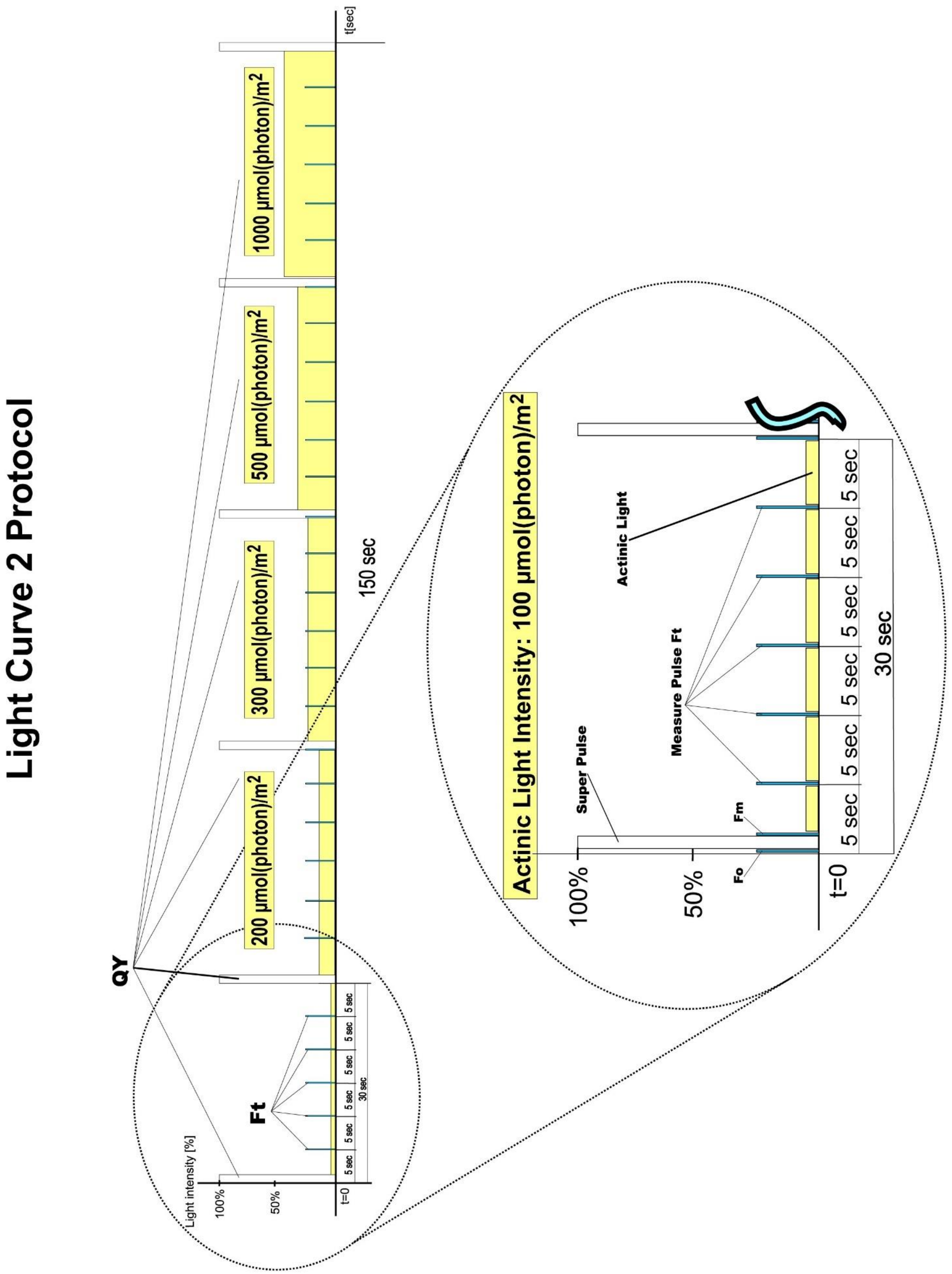


Fig. 11 LC2 Protocol.

7.4 MULTIPLE MEASUREMENT

In addition to a **single** measurement with each of the available protocols, it is possible to perform **multiple** measurements of the same protocol over a period of time. The FluorPen may be setup to make multiple measurements by selecting in the **Settings > Multi,** appropriate parameter/protocol (see Menu tree, page 28)

Multi type – choose your required parameter - Ft, QY, OJIP....

Multi interval – set the time interval between measurements

Multi repeats – set the number of repeated measurements

Use averaging – serves to confirm Repeat and Interval Options for each measurement within Multi Option – select YES or NO.

- Prepare the sample as for a **single** measurement.
- Select in the menu: **Measurement > Multi**.
- Press **SET** to confirm and start the measurements.
- Values appear on display after each repeat of measurement and are automatically stored to the device memory. If protocol (OJIP, NPQ, LC) was used, no data will be visible on the display. Data will need to be download from the device to a PC first to visualize it (page 45).

Modes of Multiple measurement:

1. **FluorPen is connected via USB to computer**
   The device performs preset number of repeated measurements and does not switch off between measurements. Progress of the measurement is displayed in percentage on the computer.

2. **FluorPen is not connected to the computer**
   The device measures continuously according to predefined protocol and interval. The multiple measurement is interrupted only by manual switching „MENU“ of the device. The device turns off between measurements.

8 CONTROL MENU TREE

The next few pages of this manual show the structure of the menu and explain in a schematic way the operation of the Fluor Pen. The schematic diagrams show 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 **SET** key operations.
- Dashed-line arrows are used to indicate **MENU** key operations.

Main Menu

To start hold the SET key for 1 second.

>Measure

Press SET TO MEASURE:
Ft
QY
PAR*
OJIP
NPQ 1
NPQ 2
NPQ 3
LC 1
LC 2
LC 3
Multi (repeated measurements)

See the complete Measure Sub-Menu on the next page.

Press MENU to scroll down in the Main Menu

>Data Browse

Press SET
TO BROWSE OR TO ERASE previously measured data.

See the complete Setting Sub-Menu on the next pages.

Press MENU to scroll down in the Main Menu

>Settings

Press SET TO SET PARAMETERS FOR:
Measur. color
Flash pulse
Super pulse
Actinic pulse
PAR Calibration*
Averag. count
Averag. delay
Multi
Sound
Date & Time
LCD contrast

See the complete Setting Sub-Menu on the next pages.

Press MENU to scroll down in the Main Menu

>Date & Time

Press SET
TO DISPLAY TIME

Press MENU to scroll down in the Main Menu

>Accessories

Press SET TO TURN ON/OFF:
GPS
Bluetooth

Press MENU to scroll down in the Main Menu

>Turn Off

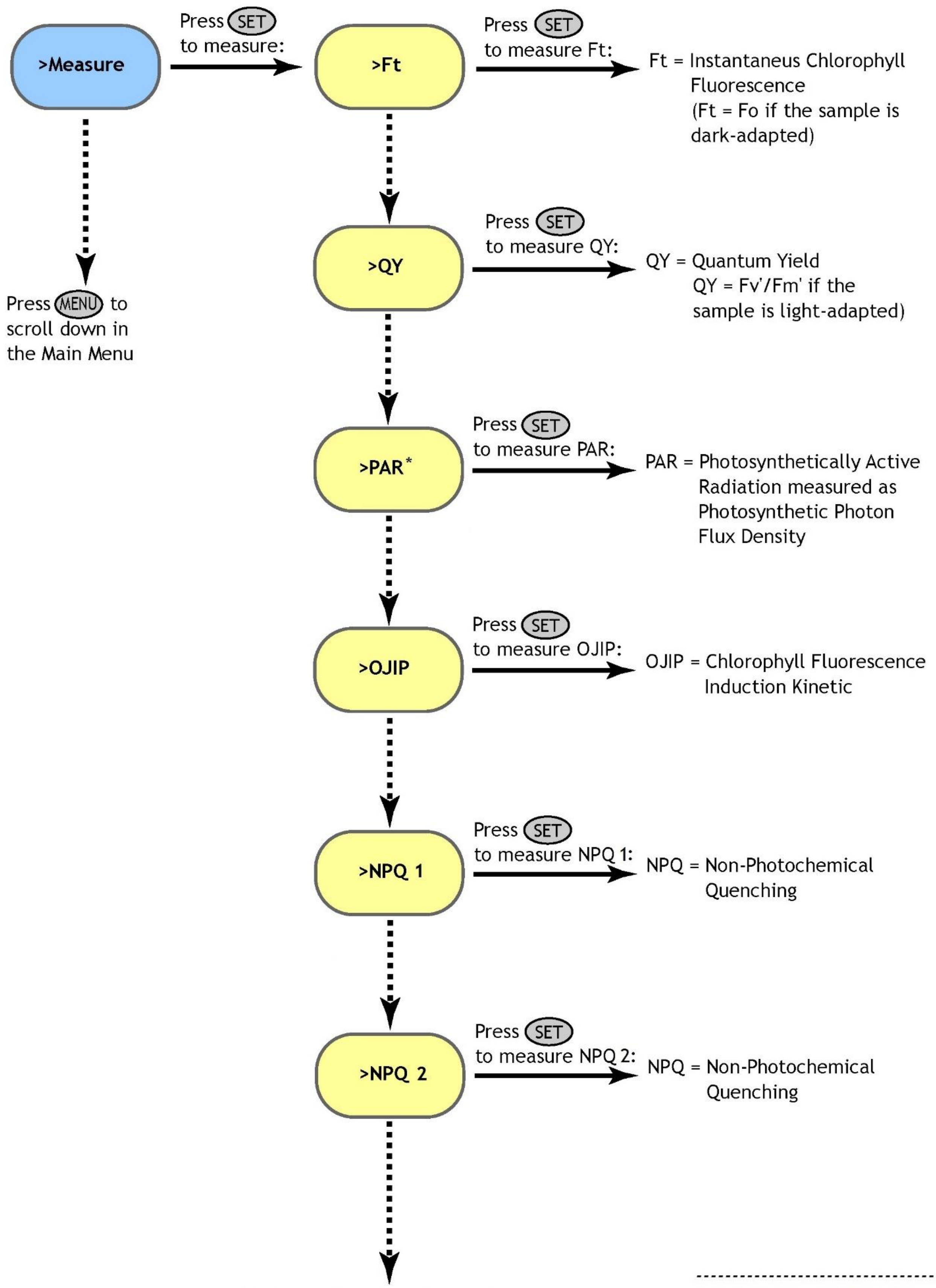
Press SET
TO TURN OFF THE DEVICE

Press MENU to scroll down in the Main Menu (to return to >Measurement)

** Only in the PAR-FluorPen FP 110*

Measure Sub-Menu - Part 1

Use the Measure Sub-Menu when measuring selected parameters.

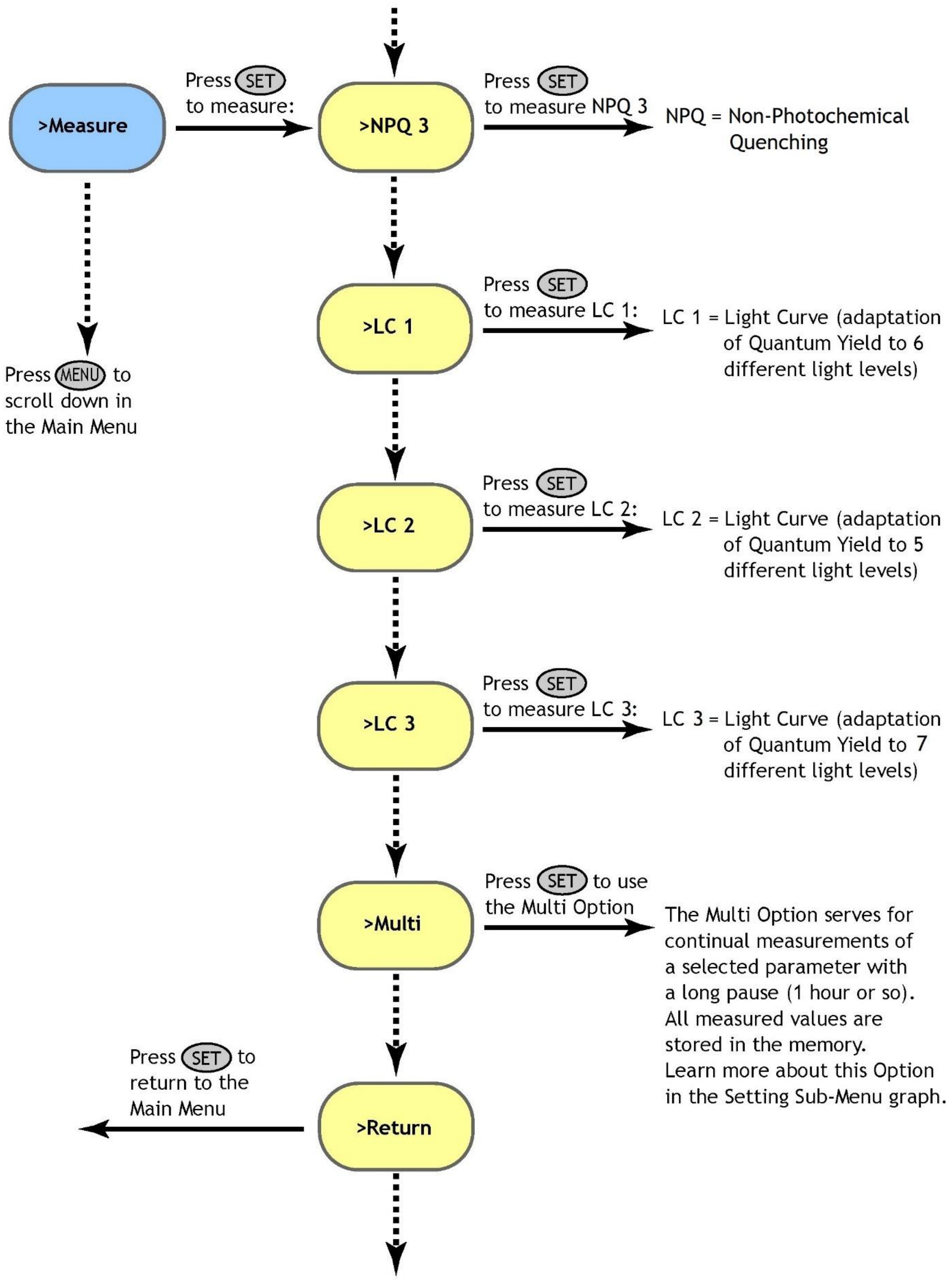


To be continued on the next page

** Only in the PAR-FluorPen FP 110*

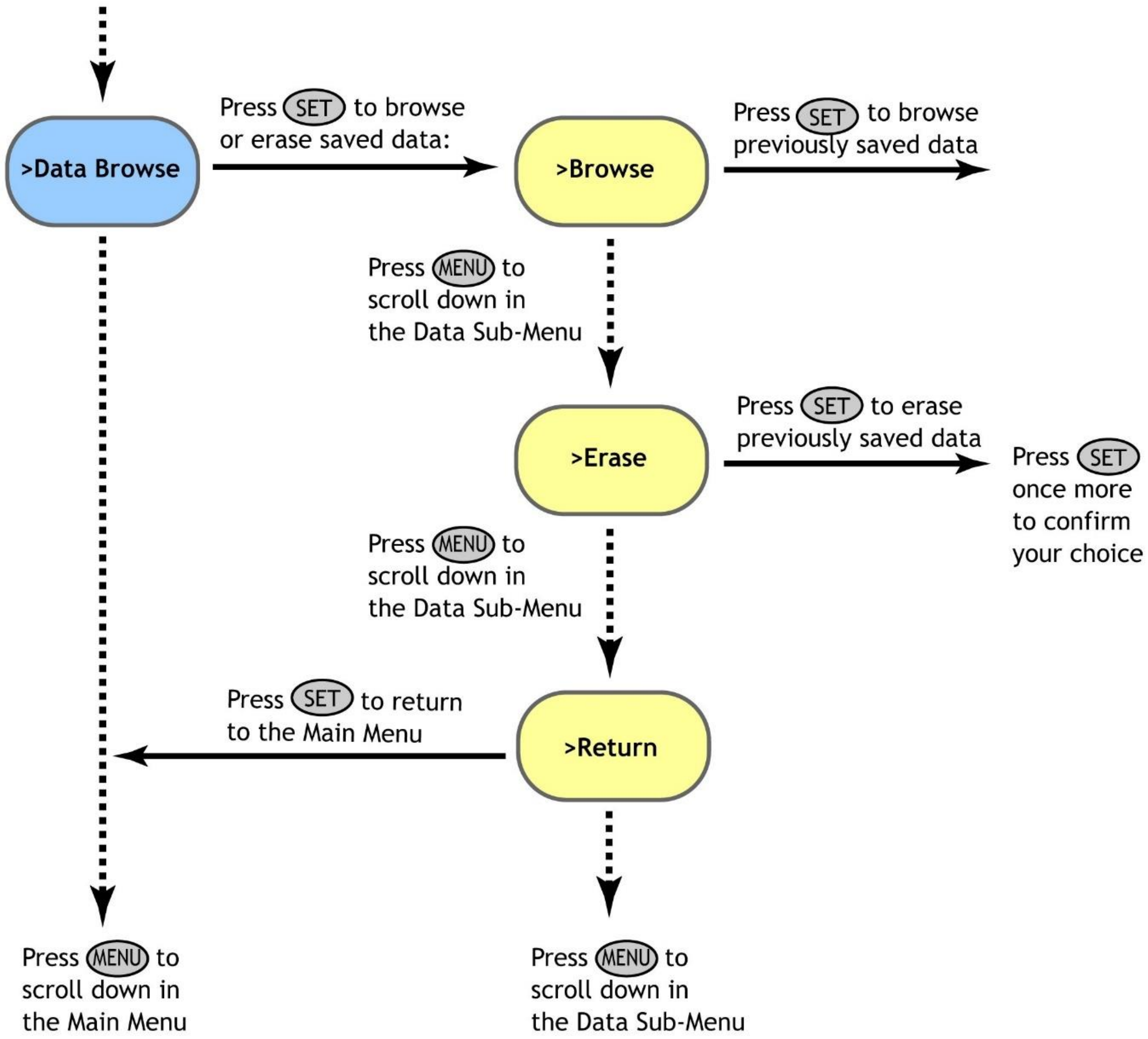
Measure Sub-Menu - Part 2

Use the Measure Sub-Menu when measuring selected parameters.



Data Sub-Menu

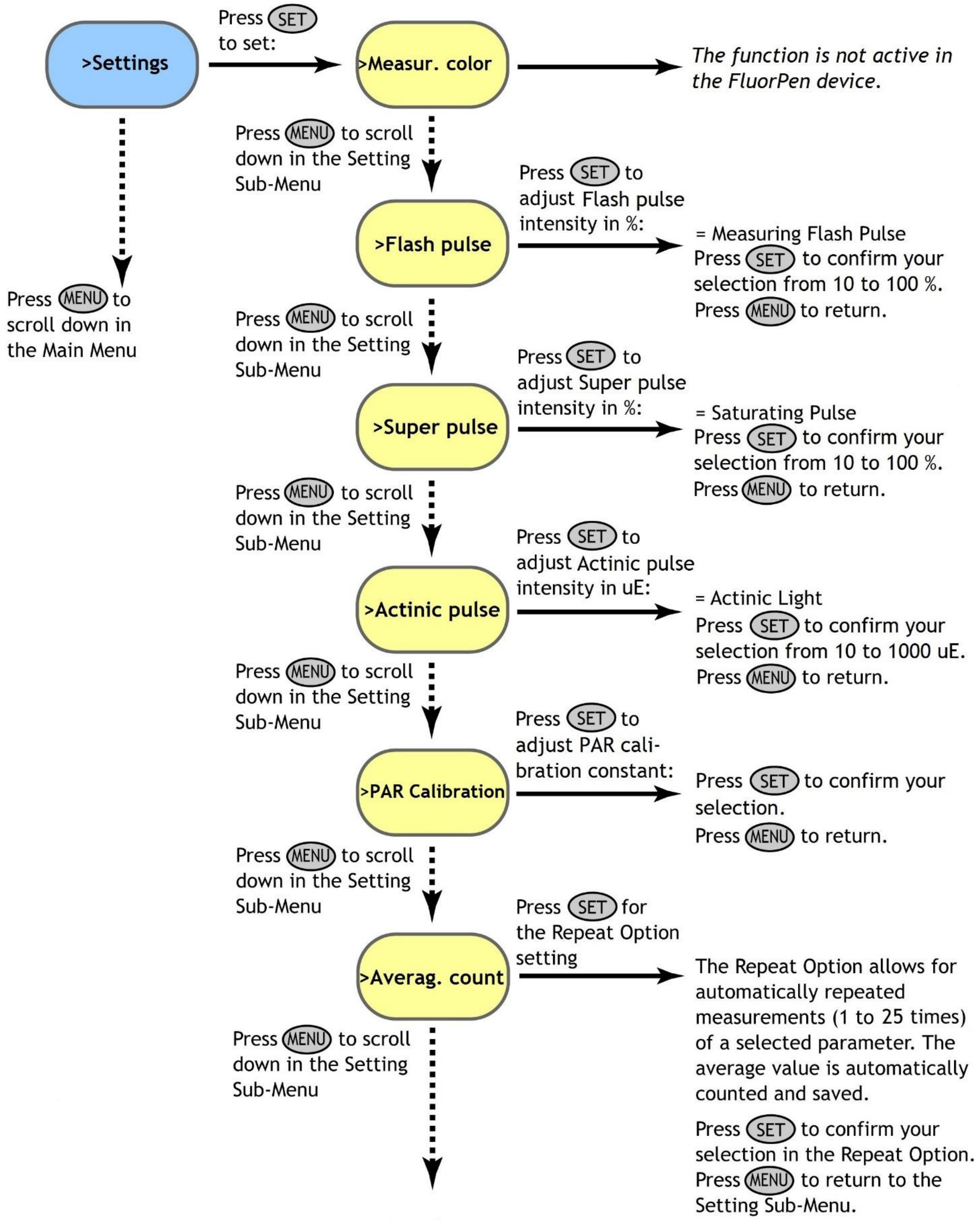
Use the Data Sub-Menu when browsing or erasing previously measured data.



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

Setting Sub-Menu - Part 1

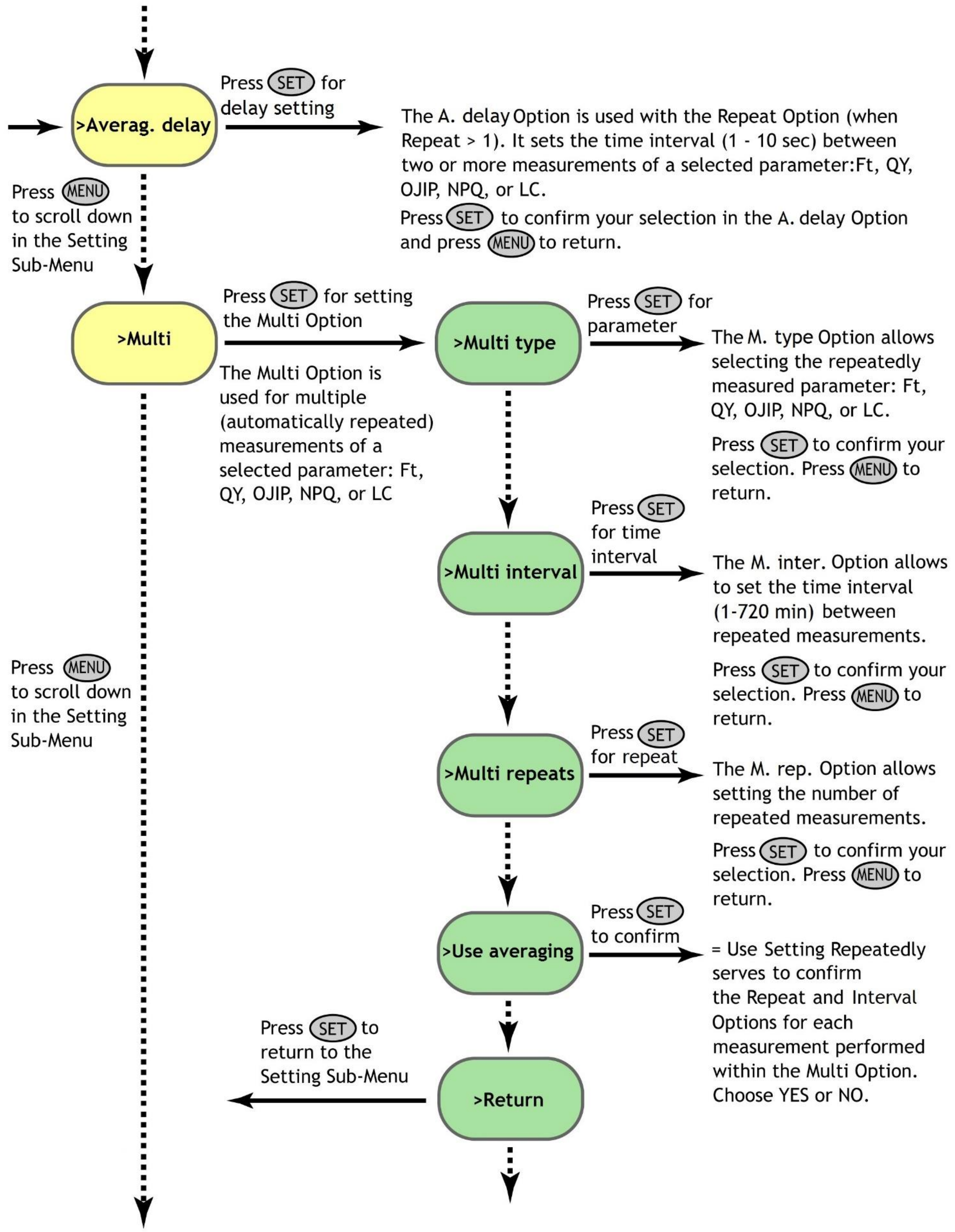
Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.



To be continued on the next page

Setting Sub-Menu - Part 2

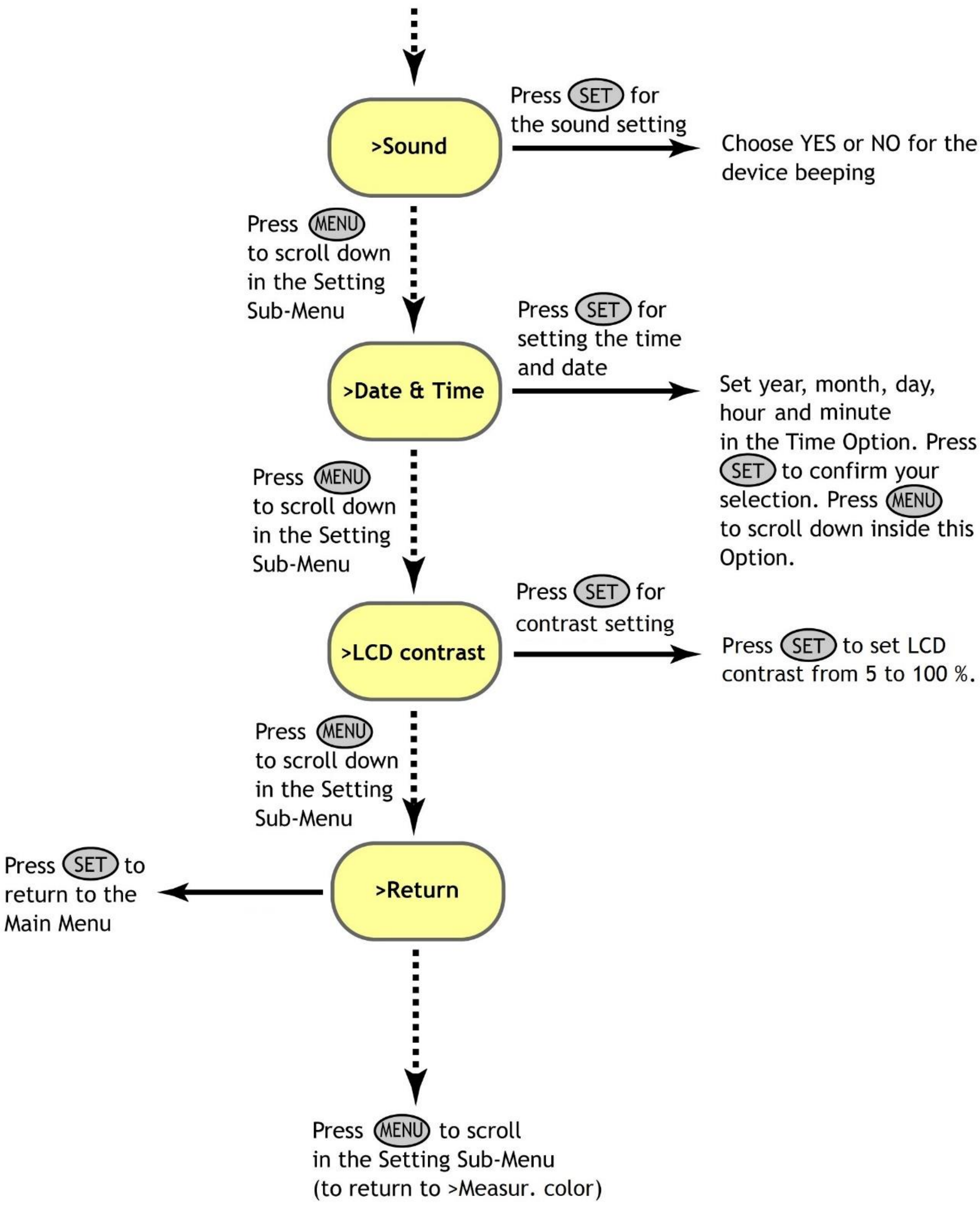
Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.



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

All FluorPens come with USB cable that is required for charging of the Li battery and can be also used for data transfer. To connect the USB cable with the FluorPen device Follow the picture instructions below. Please note that a lock-in system is used to secure the USB cable to the FluorPen and extreme caution has to be used when setting up the connection. Otherwise damage to the cable pins may occur.

⚠	When connecting the USB cable take extra caution to prevent damage to cable connector pins. Ensure that the cable is oriented correctly as shown in the photos below so the circled portion of the plug and the cable in photo A and B are perfectly lined up prior to pushing the cable into the device plug. 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 FluorPen with your computer please follow steps below in (Fig. 12).

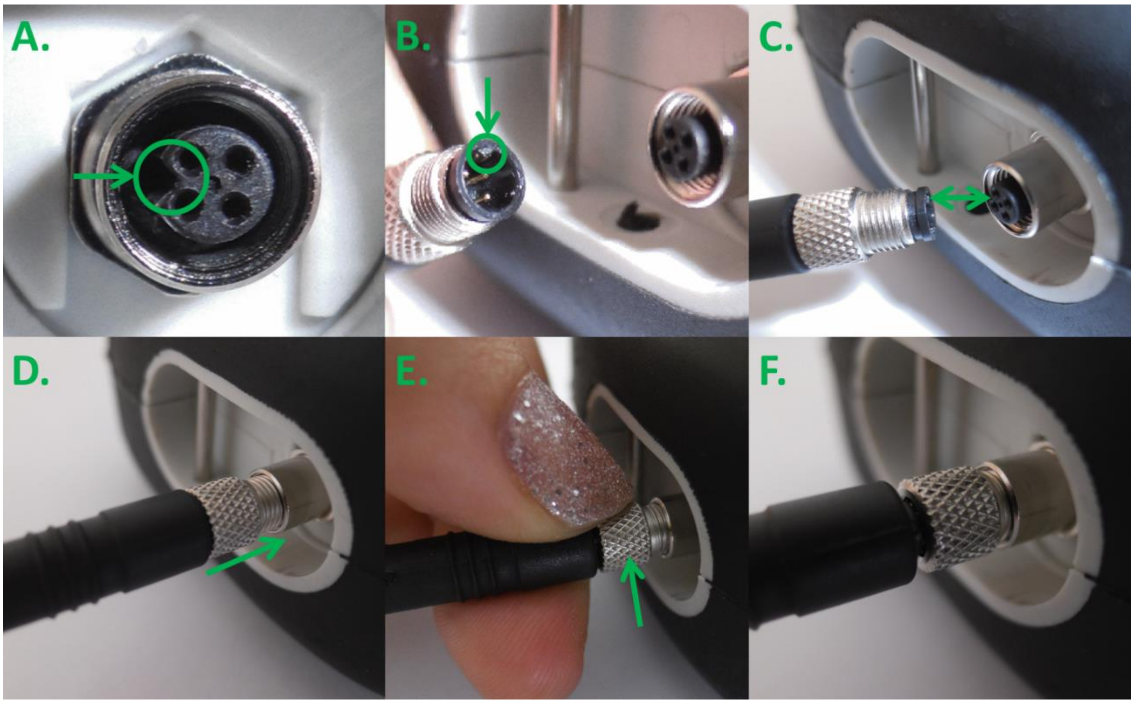


Fig. 12 How to connect FluorPen with USB cable.

A) Connector on FluorPen device. B) Portion of the USB cable with pins. C – E) Position the cable horizontally, 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 FluorPen device the other end may be Connected to the USB port on a PC. The FluorPen **switches ON** automatically after connecting the cable to the PC. For USB connection to be successful the USB driver and the Fluor Pen software, included on the USB disk, need to be first installed on the PC. Once the USB driver is installed the Device Manager in Windows will list the USB serial port in the device tree. In case this driver installation is not successful the driver may be downloaded from PSI websites www.psi.cz. When the driver is installed correctly the connection between the FluorPen and the PC computer is initiated by selecting in the software on the computer **Setup > Device ID.**

For more information about FluorPen software see chapter 11.

ℹ	Monitoring Pen device equipped with Battery Pack has to be connected to PC through Battery Pack.

10 BLUETOOTH CONNECTION

In addition to data transfer via USB the FluorPen may be connected to the software via Bluetooth. Before setting up the Bluetooth connection between the FluorPen 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. 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.

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

Before connecting the device to the PC and downloading data files the Bluetooth software that came with the PC, or the PC Bluetooth card Is activated. This software varies by manufacturer. Please consult the documentation that came with the PC of card for more information.

3. **Bluetooth must be switched on and be visible on both devices**

To pair the FluorPen with another Bluetooth device, such as a PC, ensure that Bluetooth is switched on and visible on both devices.

10.1 BLUETOOTH PAIRING

1. **Enabling Bluetooth on the FluorPen**

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

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

2. **Starting Bluetooth Application on Your PC**

The following description of how to set up the Bluetooth connection between the computer and the device is for Windows 7; some of the steps may be different if different version of Windows is used.

- Select: **Start > Devices and Printers** (Fig. 13).

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

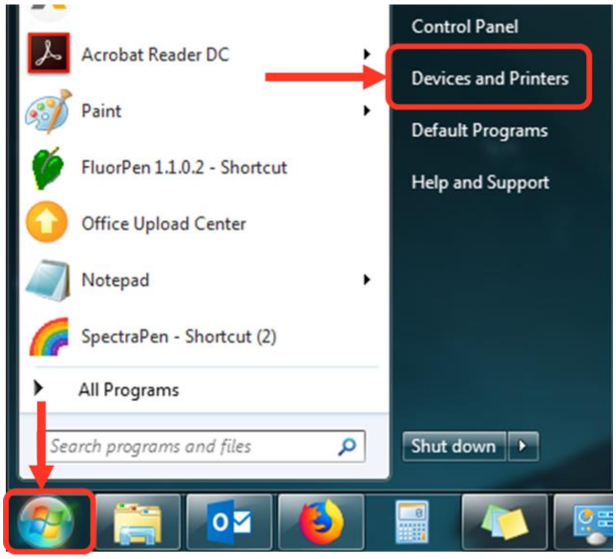


Fig. 13 Start Bluetooth Application.

3. Opening the Add Bluetooth Device Application

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

Fig. 14 Add a device.

4. Selecting the FluorPen

- Select: PSI FluorPen icon.
- Select: Next (Fig. 15).

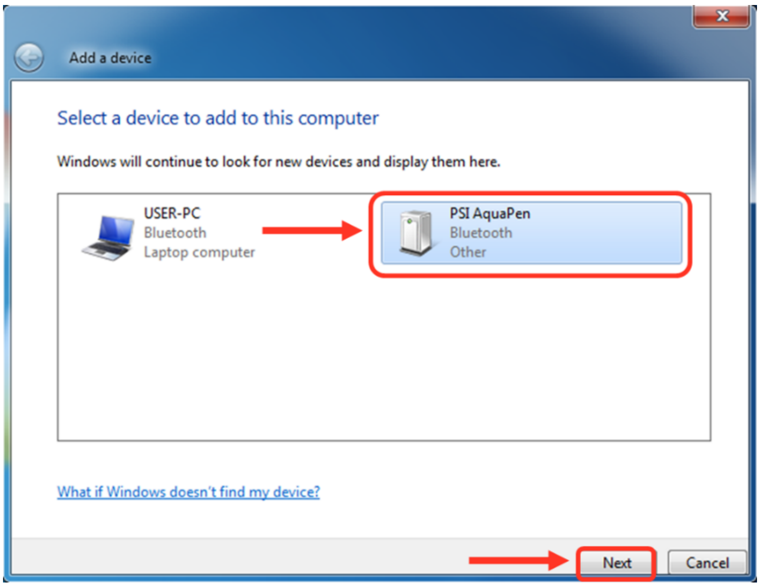


Fig. 15 Select the FluorPen.

5. Starting the Pairing Process

This step is different for old (FP100) and new version (FP110) of the FluorPen.

The old version FP100:

The Bluetooth Pairing Code is: 0000

- Select: “Enter the device’s pairing code”.
- Enter: **0000** (four digits).
- Select: Next (Fig. 16).

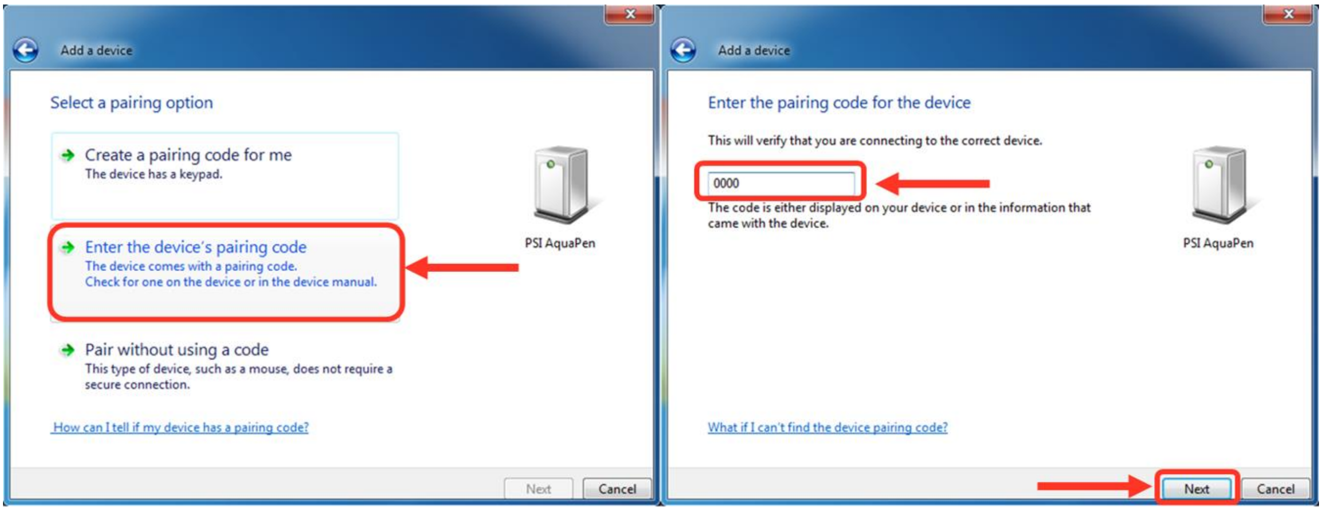


Fig. 16 Pairing process.

The new version of FP110:

- Select: Yes (Fig. 17). **Please note that the FluorPen device does not display the verification number. The verification code is not required for the BT connection.**
- Select: Next.

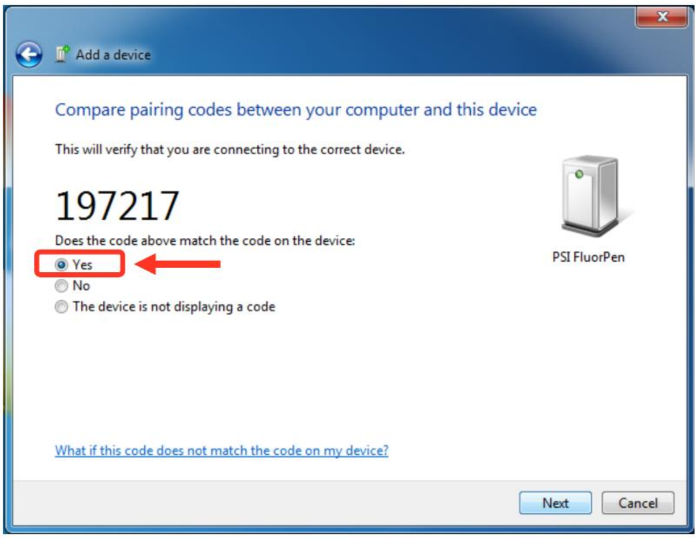


Fig. 17 Verifying of the BT pairing.

6. Completing the FluorPen Pairing

- Select: Close (Fig. 18).

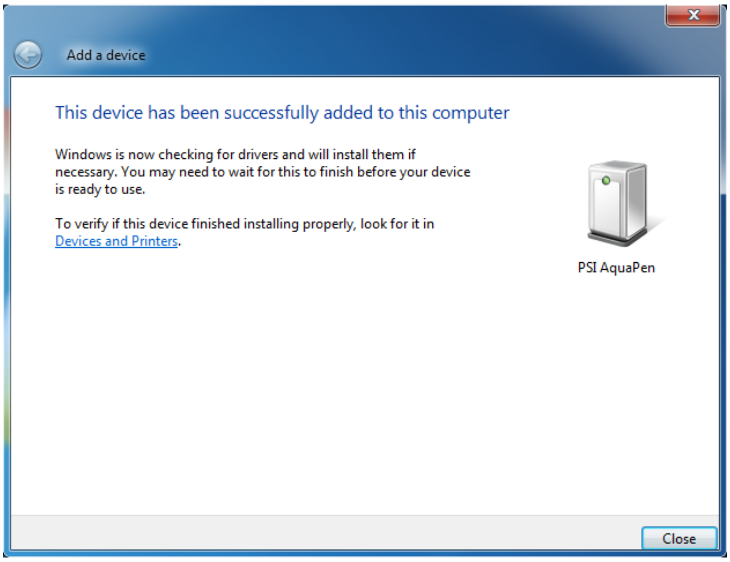


Fig. 18 Finishing.

The Bluetooth pairing is now complete and the next step is to open the Fluor Pen 1.1 software (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 launches the FluorPen program.
2. To connect and recognize your FluorPen device in the FluorPen software, proceed first with the registration of your FluorPen software (Fig. 19).
   - Select: **Help > Register**
   - Enter: your serial registration number (found in a text file SN.txt on the USB flash disk drive included with the device).
   - Select: OK

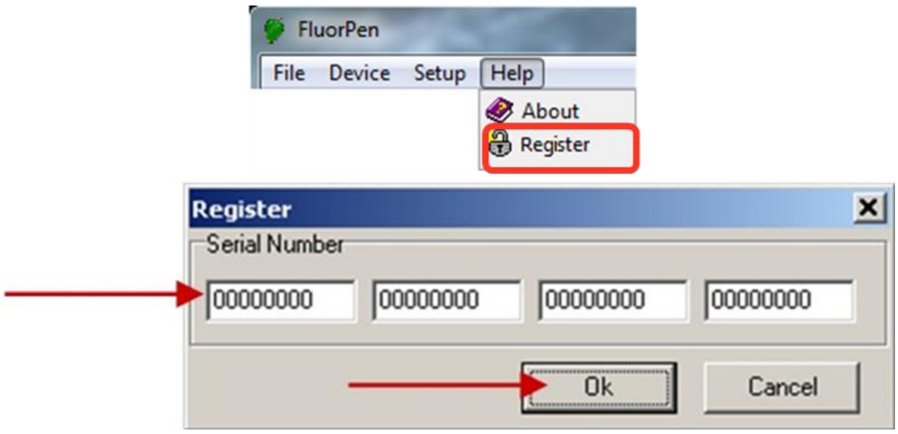


Fig. 19 Software registration.

	Please note that the serial registration number for the FluorPen 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 FluorPen device without software registration.

3. Switch on the FluorPen and enable Bluetooth or connect USB cable to the PC.
4. Ensure that the PC and the FluorPen are properly paired (see chapter 9 and 10 for complete information on USB connection and Bluetooth pairing).
5. In the software select: **Setup > Device ID (Ctrl+I)**. If properly connected, the message “Device: FluorPen” appears in the bottom left portion of the screen (Fig. 20). If the connection is not successful the message “Device not found” will appear. In the latter case check all the physical connections for USB cable and for Bluetooth repeat steps in chapter 10 on Bluetooth pairing.

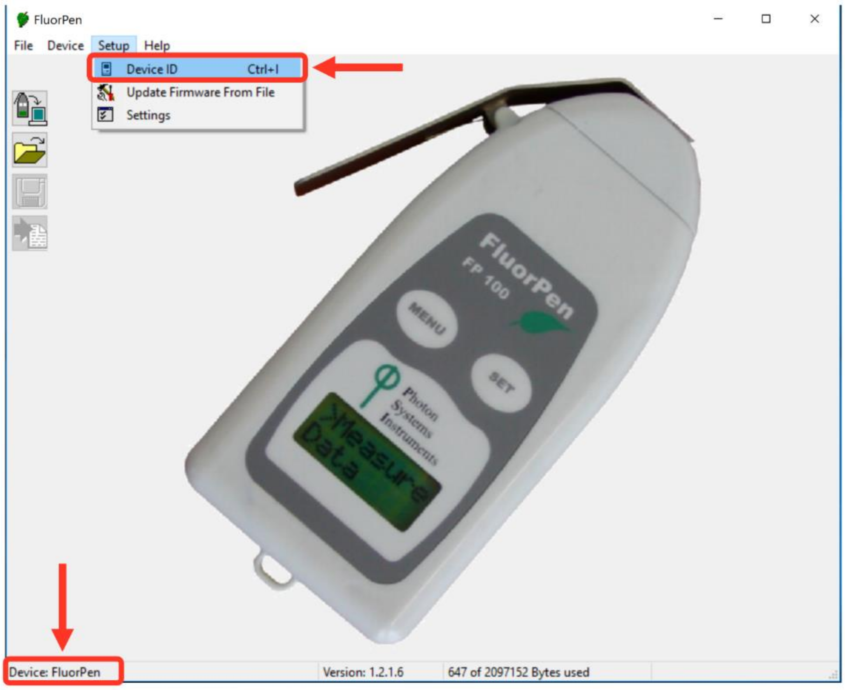


Fig. 20 Connecting FluorPen device with Software.

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 FluorPen to the PC.

Erase Memory Erases data from the FluorPen memory.

Online Control Online control of FP device.

Attach GPS File Used to download data from the GPS module of the old version of the FluorPen - FP 100 and Monitoring Pen MP 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 FluorPen 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

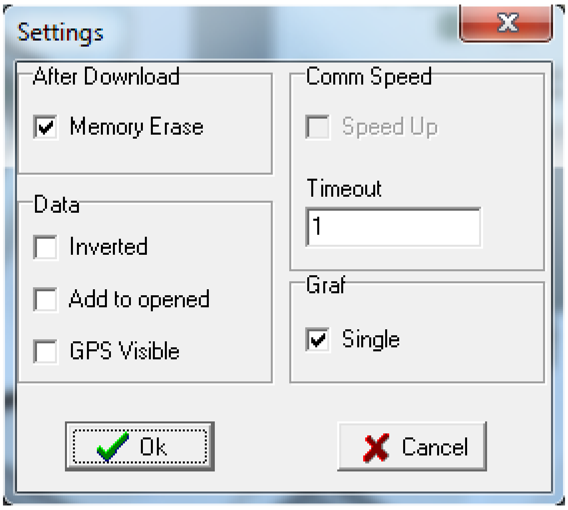
If the box is checked the FluorPen memory is erased after each data download.

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 version of the FluorPen - FP 100 and Monitoring Pen MP 100. In new versions of FP 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

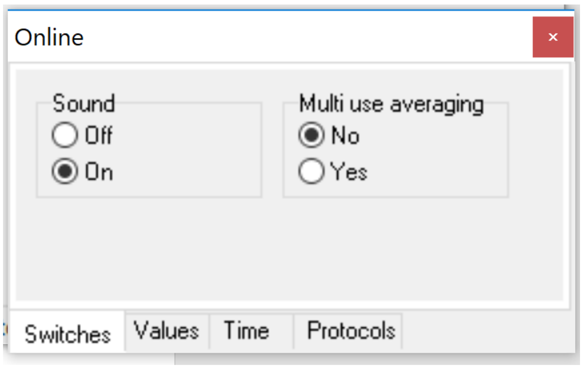
This function can be used for remote - online control of the FluorPen device after connection with the PC. Here is where changes to FP settings can be made via the software rather than the device itself and the multi measurements can also be set up.

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

Online Control – Switches

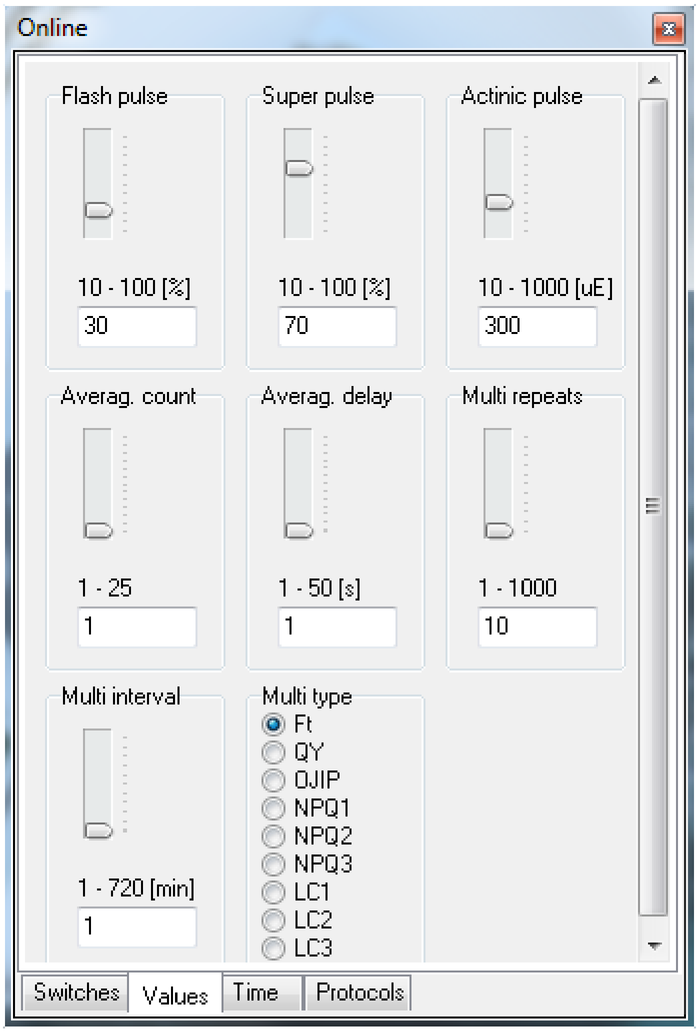
Sound (On/Off)- select presence of sound - device beeping when pressing the MENU and SET keys.

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 FP device or in the software under **Values** tab (see below)– select YES or NO. See picture below.



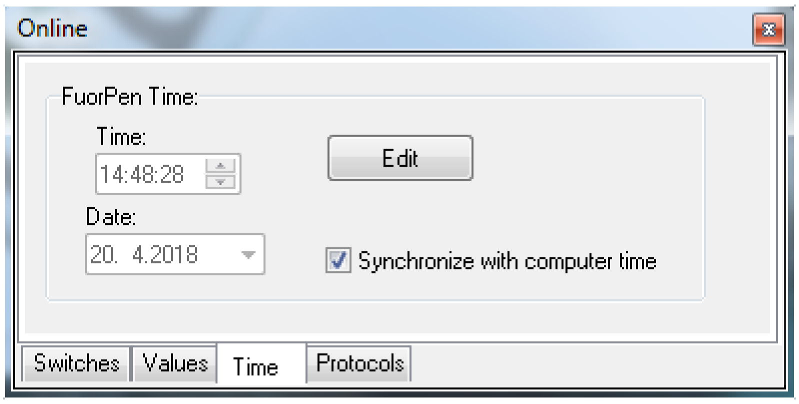
Online Control – Values

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. 45).



Online Control – Time

In this window the FluorPen time and date are set. You can also synchronize the time of the FluorPen device with the 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 at a later time. Measuring of PAR is active only in the PAR-FluorPen FP 110 (see picture below). Also, by pressing the Multi button in this window the Multiple measurements can be started remotely.

11.3 DATA TRANSFER AND VISUALIZATION

1. Once kinetics protocol data (OJIP, NPQ, LC) has been collected by the FluorPen it needs to be downloaded to PC to be visualized. Fig. 21 below shows example of an OJIP and NPQ protocol data.
2. Click the **Download** icon or select **Device > Download**.
3. Once download is complete the Data table appears as shown below in Fig 21.

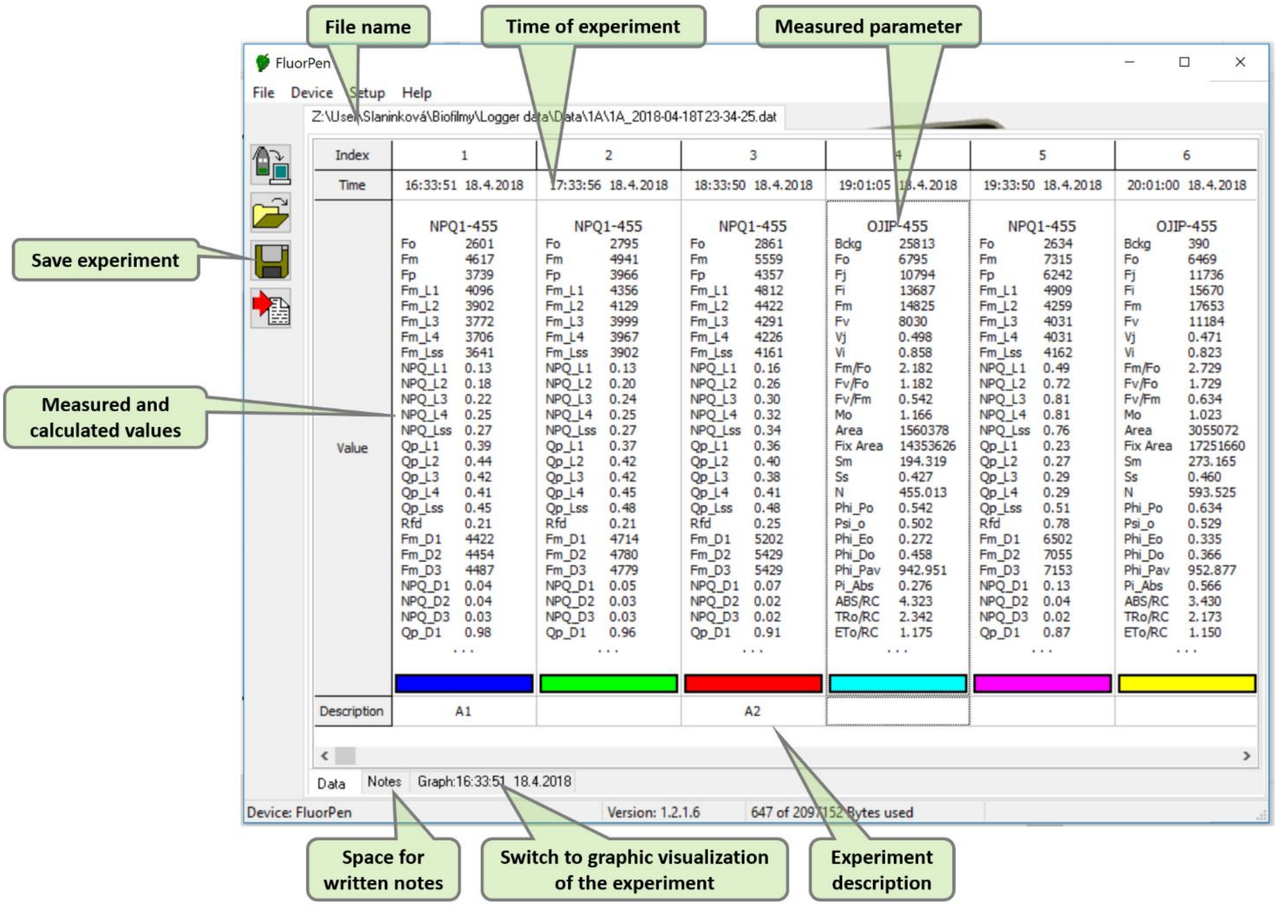


Fig. 21 Example of Data Transfer and Visualization.

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. 22).

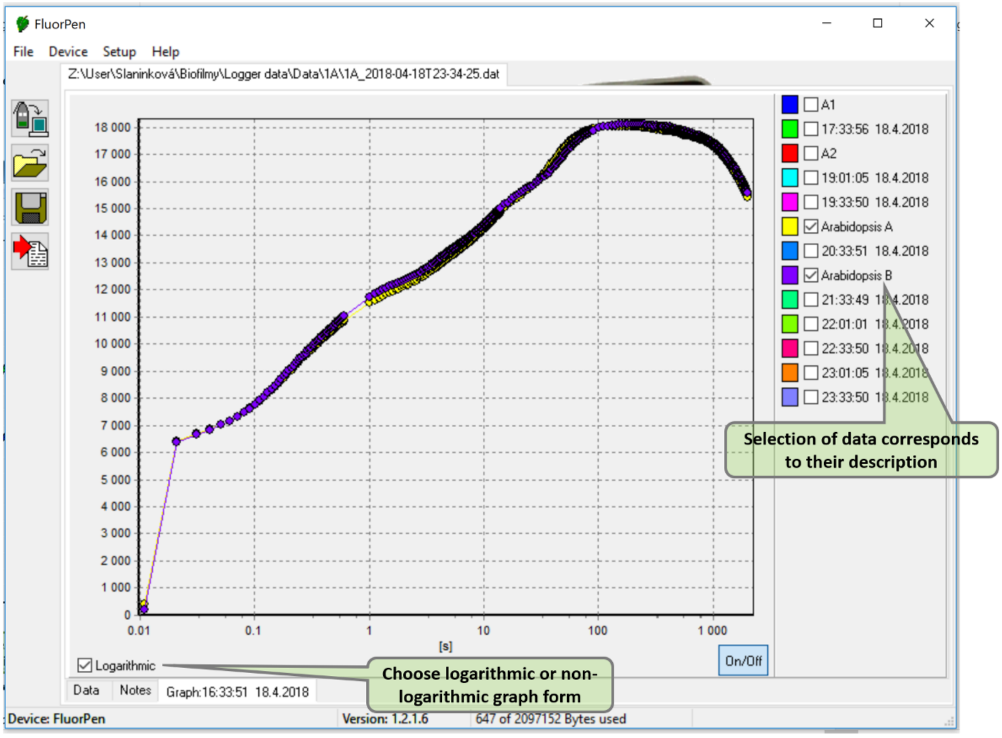


Fig. 22 Graphic visualization of experiment.

6. To **export** data from FluorPen software select **File > Export** or **Export** icon. Select the type of data to export (Ft, QY, OJIP…) (Fig. 23).

   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...

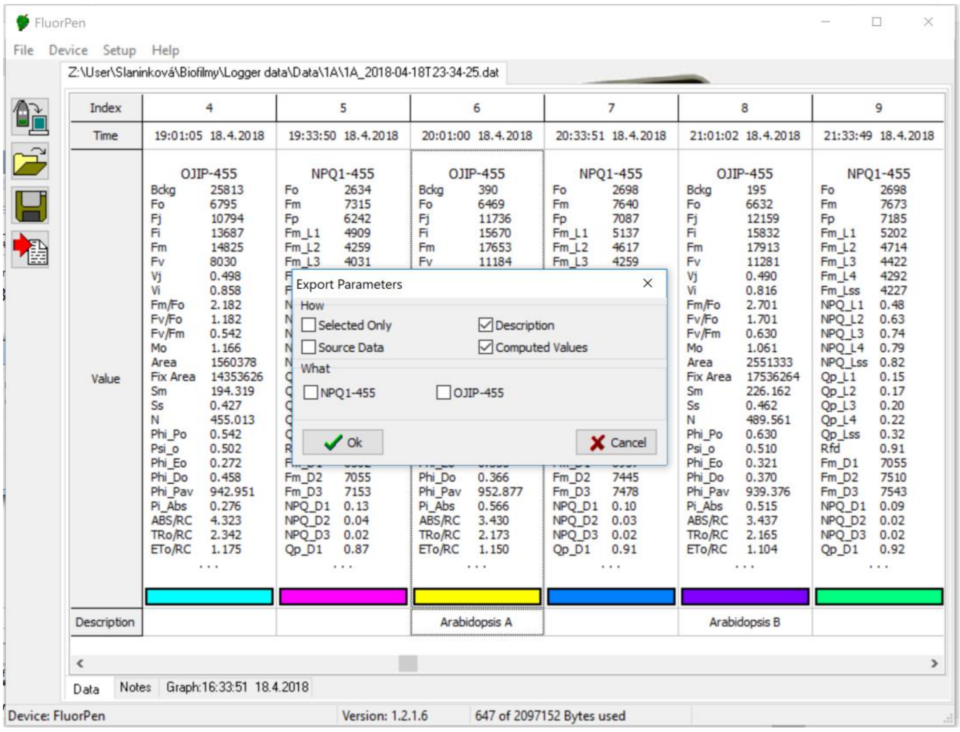


Fig. 23 Export data window.

11.4 FIRMWARE UPDATE

All data in the FluorPen memory are erased during the firmware update!

Before starting any firmware update, download all your data from the FluorPen memory to the computer and save!

As changes to the firmware of the device become available these may be applied by doing a firmware update of the device. This requires a firmware update file (with .bxn extension) which may be obtained from the manufacturer.

1. **Starting Update**

   - Select: **Setup > Update Firmware From File** (Fig. 24).

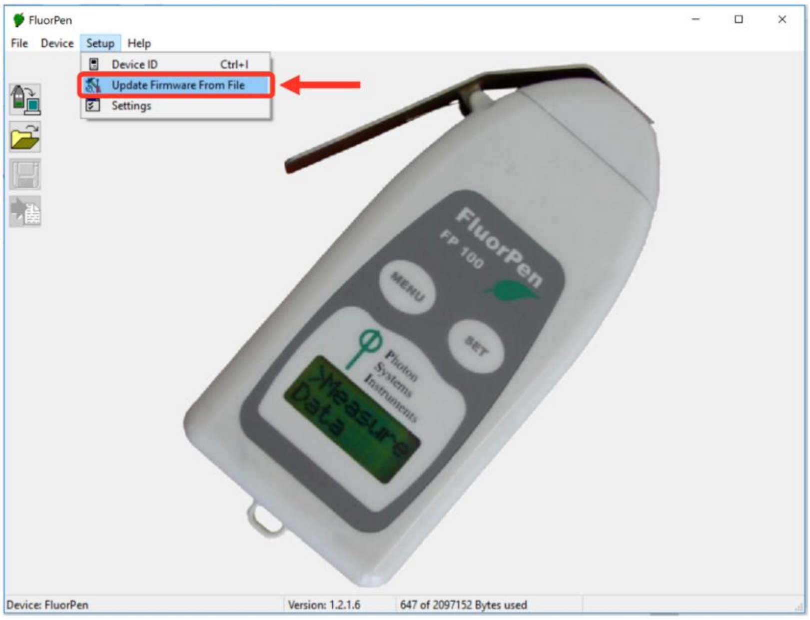


Fig. 24 Update Firmware.

2. Warning

- Select: **OK** to start update (Fig. 25).

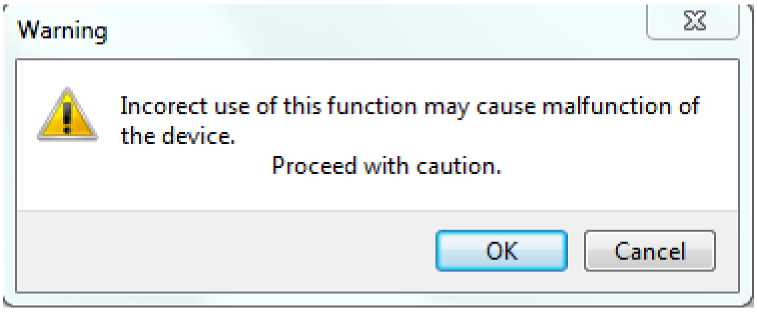


Fig. 25 Warning.

3. Selecting .bxn file

- Find firmware update file: Binary file (with the extension .bxn) (Fig. 26).
- Select: Open.

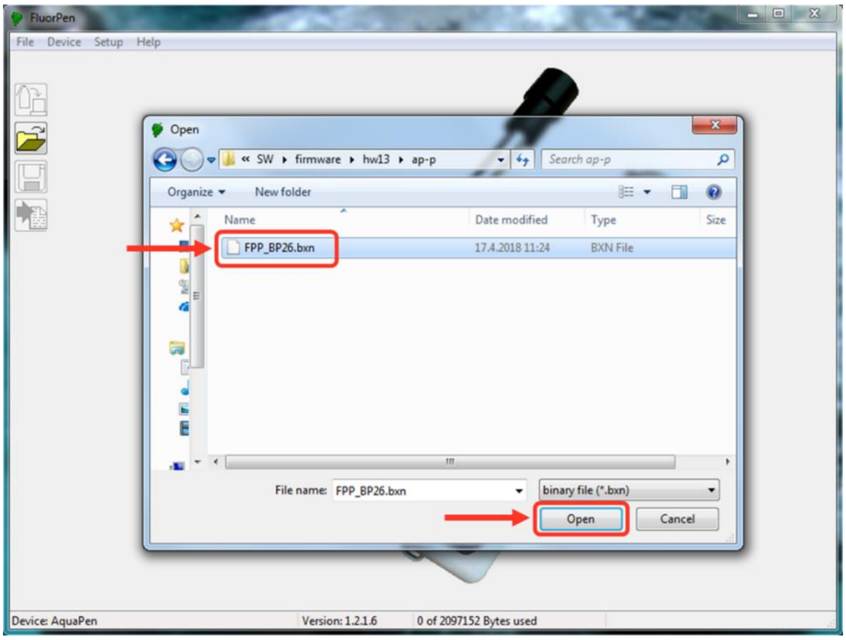


Fig. 26 Select .bxn file.

4. Finishing Upload

- Select: **OK** to start uploading of the update (Fig. 27).

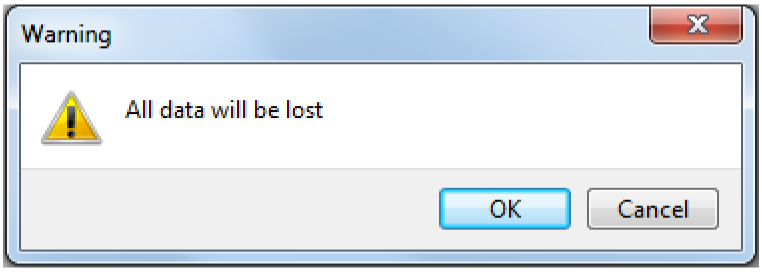


Fig. 27 Data loss warning.

- The bottom bar indicates the upload progress (Fig. 28).

Fig. 28 Upload progress.

- Press: OK to finish upload (Fig. 29).

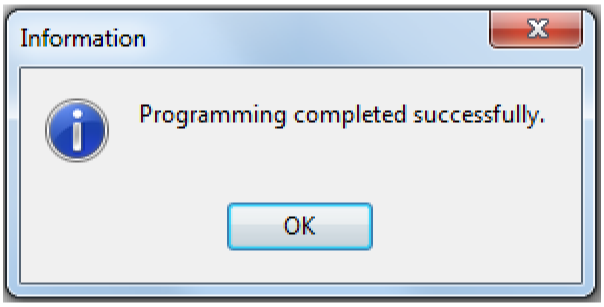


Fig. 29 Finish upload.

12 GPS MODULE

All new versions of the FluorPen devices FP110 have integrated GPS module which may be turned on during the measurement for mapping of the collected data to specific filed position. 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 FluorPen 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 / FLUORPEN OPERATION

1. Check the time setting on the FluorPen device: **Settings > Date & Time**
2. Switch the GPS module "ON" on the FP device by following these steps in the FluporPen menu:
   - Select: **Accessories > GPS**
   - Press **SET** to turn it on.
   - Wait until the GPS position is found – "**Starting GPS**".
   - The GPS module is ready when the icon in upper left side of the display changes as shown on the picture below – see on Fig. 30.

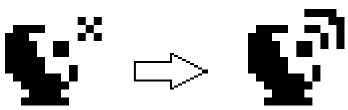


Fig. 30 GPS icons.

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 FluorPen turns off automatically after about 8 minutes of no action. Turning off the FluorPen always turns off GPS module.

12.2 DATA DOWNLOAD

1. Enabling Communication:
   - Switch on the computer and the FluorPen device. Set the computer to FluorPen communication: enable Bluetooth or connect to USB port (see instructions above in chapter 9 and 10).
2. Downloading Data from the FluorPen
   - Start FluorPen program.
   - Connect FluorPen device: **Setup > Device ID (Ctrl+I)**
   - Download measured data from the FluorPen to your PC. Data measured with activated GPS module are downloaded with GPS coordinates (Fig. 31).

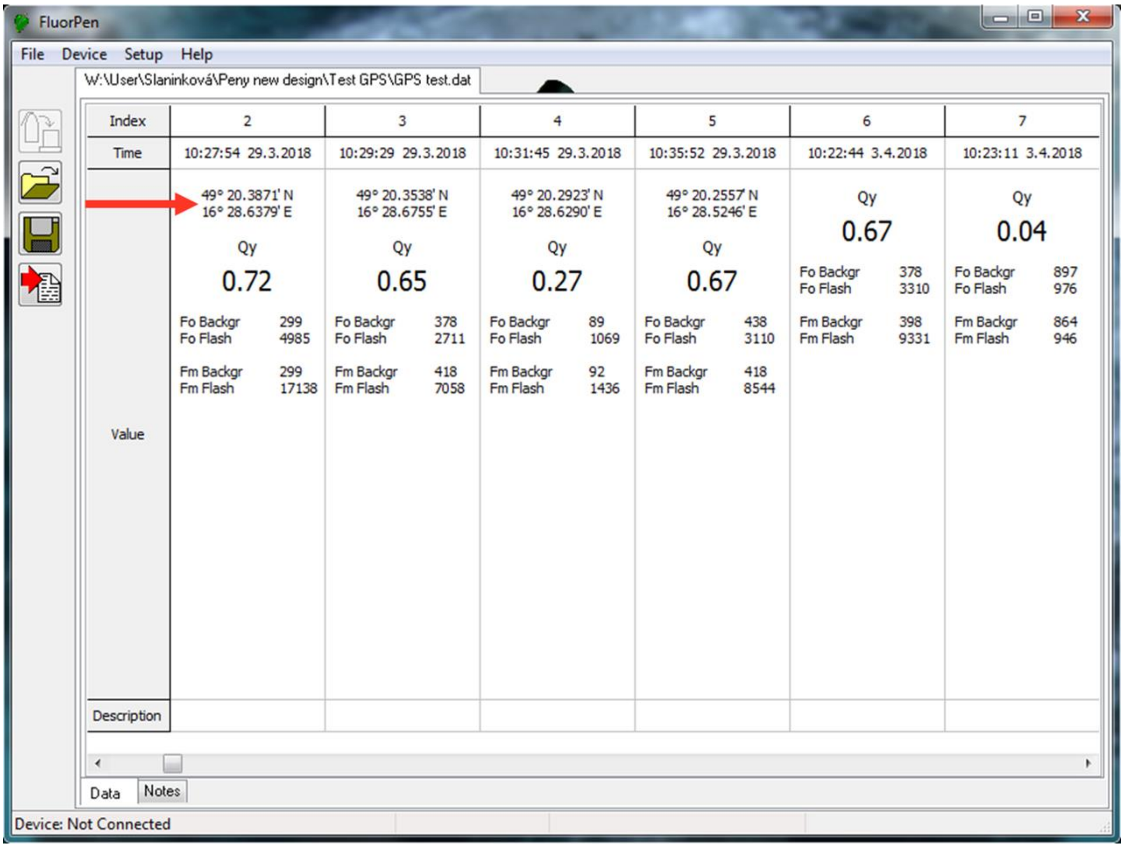


Fig. 31 GPS coordinates.

13 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.

14 TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of troubles and for customer support, please, visit **FAQ** on our websites, write to **support@psi.cz** or contact your local distributor.

15 APPENDIX

15.1 BATTERY PACK FOR MONITORING PEN

Battery pack serves as an external power source for Monitoring Pen devices. The external battery provides power during long-term experiments.

	Please note that Monitoring Pen devices equipped with battery pack do not have internal battery, therefore it is not possible to use them without the battery pack.

15.1.1 STANDARD BATTERY PACK

Standard battery pack (Fig. 32) is intended for the operation within temperature range from +10 ºC to +40 ºC. The operating time is up to 2 years (QY measurement every 1 hour). The pack includes battery case with rechargeable sealed lead acid battery (12Ah), charger, two types of cables (serial and device) and serial convertor.

Fig. 32 Standard Battery Pack.

Connectors of Battery Pack (Fig. 33):

FluorPen - connects the Monitoring Pen to the battery. This connection is necessary for Monitoring Pen operation and data download, it provides power to the Monitoring Pen.

Serial - enables communication between Monitoring Pen and PC for control and data download.

Charger - allows for charging of the battery.

Fig. 33 Connectors of Standard Battery Pack.

Replacement of the Battery:

If the battery needs to be changed follow these steps:

1. Unscrew 4 screws (in each corner of the battery casing) and remove the battery pack cover (Fig. 34a).
2. Disconnect the internal battery from the cables.
3. Place new battery inside the casing, connect it with the cables – red cable with red and black cable with black marked connector (Fig. 34 b, c). Replace the cover.

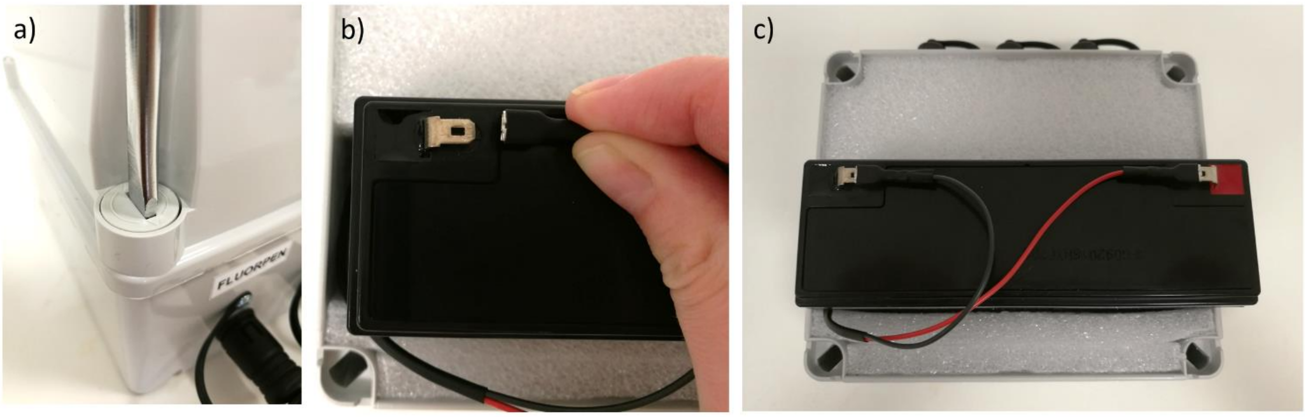


Fig. 34 Replacement of battery in a Standard Battery Pack.

15.1.2 EXTENDED TEMPERATURE RANGE BATTERY PACK

Extended temperature range battery pack (Fig. 35) is intended for operation within temperature range from -40 ºC to +60 ºC. Operating time is up to 2 years (QY measurement every 1 hour). The pack includes battery case with non-rechargeable Li-SOCl2 battery (5.5Ah), two types of cables (serial and device) and serial convertor.

	Extended temperature range battery pack cannot be recharged. Spare battery is offered as additional accessory.

Fig. 35 Monitoring Pen MP 100-A charged via Extended Temperature Range Battery Pack.

Connectors of Battery Pack:

Device - provides power connection between Monitoring Pen and the battery. This connection is necessary for Pen operation and data download.

Serial - enables communication between Monitoring Pen and the PC for control and data transfer.

Replacement of the Battery:

Follow these steps to replace the battery:

1. Remove 4 screws in the corner of the case and remove the battery pack cover (Fig. 36 a, b).
2. Disconnect the internal battery from the cable (Fig. 36 c).
3. Place the new battery inside the casing, connect it with the cable and replace the cover.

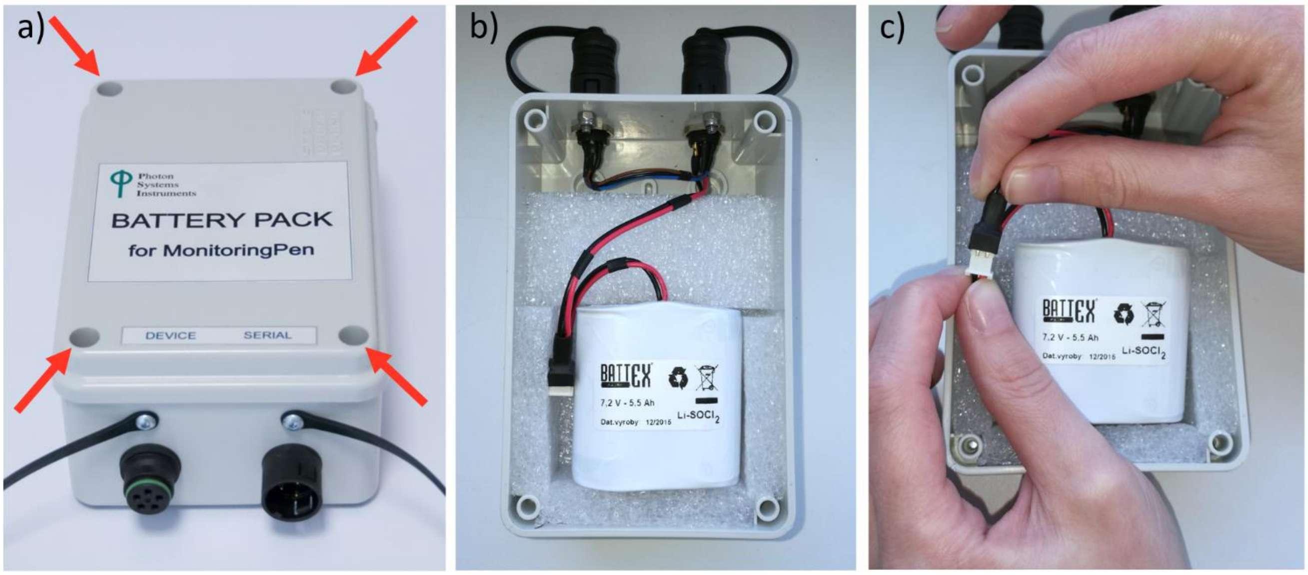


Fig. 36 Battery replacement for Extended Temperature Range Battery Pack.

15.2 INSTALLATION AND OPERATION OF THE MONITORING PEN MP 100-A/B

15.2.1 DEVICE CONTROL

To **turn on** the device connect the serial cable to the battery pack and pc.

The device **stays on** when the serial cable is connected.

The Monitoring Pen automatically **turn off** after serial cable disconnection.

Protocol setting is possible only using Online control in the FluorPen software.

Multiprotocol serves for automated measurement of predefined protocols in predefined time interval. After setting of multiprotocol disconnect the serial cable. The device automatically switches to standby mode, which saves the battery, and measures according to predefined setting. More information about the Multiprotocol are mentioned in chapter 11.2.3.

Online control enables checking of **battery status** (Fig. 37). The current battery voltage is shown as measured data in FluorPen software (Fig. 38).

Fig. 37 Online control window enables checking of battery status.

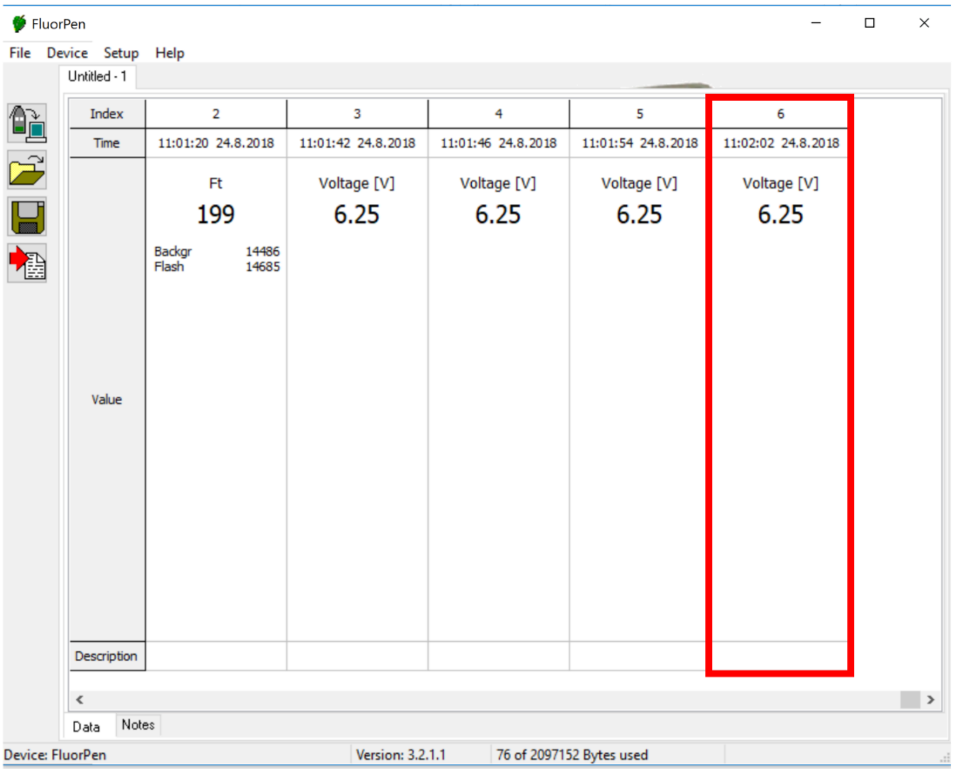


Fig. 38 Battery status.

⚠	The minimal battery voltage is 4.9 V. The device does not work at lower voltage. Please find more information about the battery pack in chapter 15.1.

15.2.2 DEVICE INSTALLATION

1. Connect the Monitoring Pen to the cable.
2. Place the Monitoring Pen under the water.
3. Connect the device cable to the battery pack.
4. For single measurement and multiprotocol setting connect the serial cable to the battery pack and pc.
5. Open the FluorPen software and connect the device (more details in chapter 11).
6. Set the protocol using the Online control and start the protocol.
7. Disconnect the serial cable. The device switches automatically to standby mode and measures according to preset protocol.
8. For data download connect the serial cable. Connection of serial cable cancels the multiprotocol measurement. Start the multiprotocol again for following measurement.

⚠	Please note that only the Monitoring Pen and device cable are submersible.

15.3 DETACHABLE LEAF CLIPS

Detachable leaf clips are used with the FluoPen FP 110/D and PAR-FluorPen FP110/D for dark adaptation of the leaf before measurements of chlorophyll fluorescence. Multiple leaf clips may be placed on leaves in a closed position ahead of time to allow dark adaptation while measurements of other leaves proceed. Start with the clip in a closed position (metal screen covering the leaf). Attach the FluorPen probe to the clip by pressing it into the clip. Once the connection is secure, slide the metal screen to expose the leaf to the FluorPen's optical probe. Proceed with the measurements. See Fig. 39 for visual of the leaf clip in a closed and open position. Detachable leaf clips may be purchased in sets of 10.

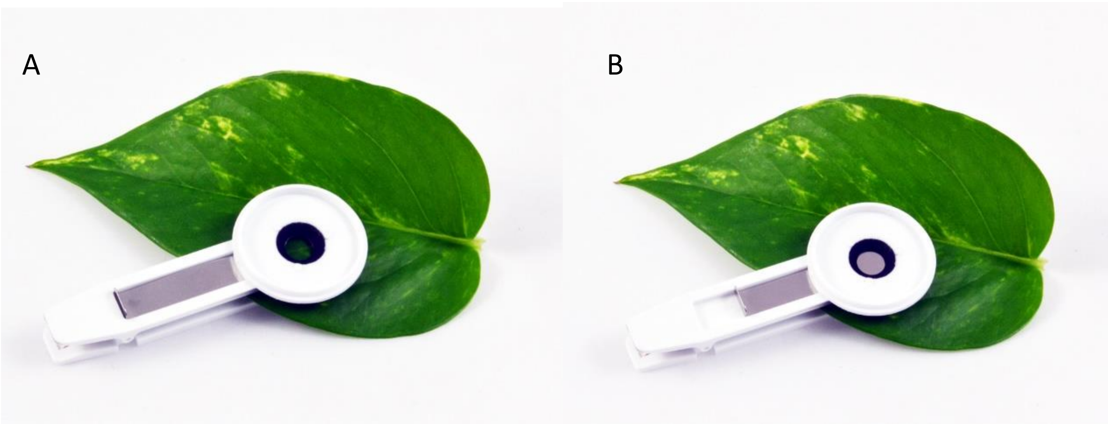


Fig. 39 The detachable leaf clip in open (A) and closed (B) position.