# **FluorPen FP 100**

# **Series**

# **Operation Manual**

For: FluorPen FP 100

FluorPen FP 100-MAX

PAR-FluorPen FP 100-MAX-LM

FluorPen FP 100-B

FluorPen FP 100-U

FluorPen or PAR-FluorPen with detachable leafclips



PSI (Photon Systems Instruments), spol. s r.o. Drasov 470, 664 24 Drasov, Czech Republic http://www.psi.cz

# **Table of Contents**

1. Technical Specification	3
2. General Information	5
3. List of Equipment	8
4. Physical Features	9
5. Accessories	10
6. Operation Instructions	12
7. Bluetooth Pairing and Connecting	20
7.A. General Information	20
7.B. Bluetooth Pairing	21
7.C. Bluetooth Connection	24
8. USB Pairing and Connecting	26
8.A. General Information	26
8.B. USB Pairing	26
9. FluorPen Software	27
9.A. Starting up	27
9.B. Menu and Icon Explanation	28
9.C. Example of Data Transfer and Visualization	30
9.D. Explanation of OJIP Parameters	33
9.E. Non-Photochemical Quenching (NPQ) Protocol	35
9.F. Light Curve (LC) Protocol	38
9.G. Software Update	43
10. GPS Module	45
10.A. GPS Module Description	45
10.B. First Time Installation of the GPS Module	46
10.C. GPS / FluorPen Operation	47
10.D. Data Download	48
11. Statement of Limited Warranty	50

# **1. Technical Specification**

Measured and Calculated Parameters: Fo, Ft, Fm, Fm', QY, NPQ 1*, NPQ 2*, OJIP*, LC 1*, LC 2*, PAR**
Cosine Correction**: Cosine corrected up to 80º angle of incidence
Linearity**: Maximum deviation of 1 % up to per 10,000 µmol s <sup>.1</sup> m <sup>.2</sup>
Saturating Light: Adjustable from 0 to 3,000 μmol(photons)/m².s (0 to 100 %)
Actinic Light: Adjustable from 0 to 1,000 μmol(photons)/m².s (0 to 100 %)
Measuring Light: Adjustable from 0 to 3,000 μmol(photons)/m².s (0 to 100 %)
Detector Wavelength Range: PIN photodiode with 697 to 750 nm bandpass filters
Communication: Bluetooth, USB, or serial (not included in the FluorPen FP 100)
FluorPen 1.0 Software: Windows 2000, XP, or higher compatible***
Memory Capacity: Up to 4 Mb
Internal Data Logging: Up to 100,000 data points
Display: 2 x 8 characters LC display
Keypad: Sealed, 2-key tactile response
Keypad Escape Time: Turns off after 3 minutes of no use
Power Supply: 4 AAA alkaline batteries (single use or rechargeable)
Battery Life: 48 hours typical with full operation
Low Battery Detection: Low battery indication displayed
Size: 120 mm x 57 mm x 30 mm 4.7" x 2.2" x 1.2"
Weight: 180 g, 6.5 oz
Sample Holder: Mechanical leaf clip (non-destructive for plants, effective for sample pre-darkening)
<mark>Operating Conditions:</mark> Temperature: 0 to 55 ºC; 32 to 130 ºF Relative humidity: 0 to 95 % (non-condensing)
Storage Conditions: Temperature: -10 to +60 ºC; 14 to 140 ºF Relative humidity: 0 to 95 % (non-condensing)

# Warranty:

1 year parts and labor (see the last page of this Operation Manual for precise conditions)

\* Only in the FluorPen FP 100-MAX or PAR-FluorPen FP100-MAX-LM.

- \*\* Only in the PAR-FluorPen FP100-MAX-LM.
- \*\*\* Windows is a registered trademark of Microsoft Corporation.

The Bluetoothmodule BlueNiceCom III is endowed with a declaration of conformity with the following norms: EN 300 328 V1.6.1 (2004-11) EN 301 489-1, -3 V1.5.1 (2003-12) EN 50371 December 2002 EN60650 FCC Part 15.247 FCC Grantee Code: R7T Bluotooth Qualified Product Notice: GRA\_013\_04 Bluetooth Listing Identifier: B01572

# **2. General Information**

**FluorPen** is a portable, battery-powered fluorometer that enables quick and precise measurement of chlorophyll fluorescence parameters in the laboratory, greenhouse, or in the field. It can be effectively used for studying photosynthetic activity, stress detection, herbicide testing, or mutant screening. Affordable price and straight-forward two-button operation makes 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 100-MAX-LM** includes all features of the FluorPen FP 100-MAX, i.e., it measures chlorophyll fluorescence parameters Ft, QY, NPQ, OJIP, and Light Curve (QY).

Besides that, the PAR-FluorPen incorporates an integrated **Light Meter** for direct digital readouts of Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm, the span in which plants use energy during photosynthesis. 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 withing the 400-700 nm waveband. Instant readouts are provided as average values of 20 measurements.

Measured data are sequentially stored in the internal FluorPen or PAR-FluorPen memory. Data transfer to a PC is via USB or Bluetooth communication. Comprehensive FluorPen 1.0 software provides data transfer routines and many additional features for data presentation in tables and graphs.

#### FluorPen versions:

#### FluorPen FP 100-MAX

Includes one of the communication modules (Bluetooth, USB, or serial), FluorPen 1.0 software, protocol update, user's guide. Measures Ft, QY, NPQ, OJIP, and Light Curve. Equipped with a standard leaf-clip.

#### FluorPen FP 100-MAX-D

Includes one of the communication modules (Bluetooth, USB, or serial), FluorPen 1.0 software, protocol update, user's guide. Measures Ft, QY, NPQ, OJIP, and Light Curve. Adapted for use with detachable leafclips; leaf clips sold separately.

#### FluorPen FP 100-MAX-W

This version of the FluorPen FP 100-MAX is equipped with a special open leaf-clip that enables measurements in ambient light. The open leaf-clip may is supplied with a cover for short term dark adaptation of measured samples.

#### FluorPen FP 100

Standard FluorPen version with no PC communication. Data are saved to the device internal memory only.

#### FluorPen FP 100-B

Standard FluorPen version supplemented with Bluetooth communication module and FluorPen 1.0 software for data transfer to a PC.

#### FluorPen FP100-U

Standard FluorPen version supplemented with USB communication module and FluorPen 1.0 software for data transfer to a PC.

#### PAR-FluorPen FP 100-MAX-LM

Includes an integrated Light Meter, one of the communication modules (Bluetooth, USB, or serial), FluorPen 1.0 software, protocol update, and user's guide. Measures Ft, QY, NPQ, OJIP, Light Curve, and Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm. Equipped with a standard leaf-clip.



#### PAR-FluorPen FP 100-MAX-LM-D

Includes an integrated Light Meter, one of the communication modules (Bluetooth, USB, or serial), FluorPen 1.0 software, protocol update, user's guide. Measures Ft, QY, NPQ, OJIP, Light Curve and Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm. Adapted for use with detachable leaf-clips; leaf clips sold separately.



#### FluorPen measures the following parameters:

#### **Ft - Instantaneous Chlorophyll Fluorescence**

Ft is equivalent to Fo if the leaf sample is dark-adapted.

#### QY - Quantum Yield

QY is a measure of the Photosystem II efficiency. In a dark-adapted leaf this is equivalent to Fv/Fm. In a light-adapted leaf it is equivalent to Fv'/Fm'.

#### **NPQ\* - Non-Photochemical Quenching**

The NPQ protocol is the most typically used measuring approach to quantify photochemical and non-photochemical quenching. The measurement should be performed with a dark-adapted sample (see more in Chapter 8.E. of this Manual).

# **OJIP\* - Chlorophyll Fluorescence Induction Kinetics**

The OJIP curves enable observing major changes that occur during exposure of plants to high irradiance (see more in Chapter 8.D. of this Manual).

# Light Curve\*

The LC 1 and LC 2 protocols serve to describe adaptation of Quantum Yield to six or five different light levels (see more in Chapter 8.F. of this Manual).

# PAR\*\* - Photosynthetically Active Radiation

Photosynthetically Active Radiation measured as Photosynthetic Photon Flux Density (PPFD).

\* Only in the FluorPen FP 100-MAX, PAR-FluorPen FP100-MAX-LM or in the FluorPen models including the protocol update (FluorPen FP 100-B, FP 100-U).

\*\* Only in the PAR-FluorPen FP100-MAX-LM.

# 3. List of Equipment

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

- FluorPen
- 4 AAA Alkaline Batteries
- Carrying Case
- Textile Strap for Comfortable Wearing
- Self-Adhesive Rubber Pads for Optics Protection (FP 100, FP 100-B, FP 100-U, FP 100-MAX or FP 100-MAX-W only)
- FluorPen Quick Guide
- This Owner's Manual (on a USB flash disc)
- Install USB flash disc with FluorPen software (FluorPens with communication only)
- Bluetooth Communication Module (FluorPen FP 100-B or FP 100-MAX\* only)
- USB Communication Module (FluorPen FP 100-U or FP 100-MAX\* only)
- Other Accessories or Optional Features (according to your specific order)

\* FluorPen FP 100-MAX includes only one communication module (Bluetooth or USB).



#### Note:

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.

# **4. Physical Features**



\* Only in the PAR-FluorPen FP 100-MAX-LM \*\* Only in the FluorPen FP 100-MAX-W (supplied with a special "open-window" leaf clip).

# **5. Accessories**

#### **Carrying Case**

The FluorPen is supplied with a carrying case which is padded to protect the instrument during transportation. You can either carry the FluorPen case over the shoulder or hang it on your belt.



#### **Protective Rubber Pad**

The optical part of the FluorPen is covered with a self-adhesive rubber pad that protects the optics from dirt or moisture. The damaged or dirty pad can be easily removed by tearing it off from the surface. To affix a new one, just remove the sheeting and stick the pad on cleaned and dried surface. Be sure that you placed the pad properly and that you did not cover the optical window.



#### **Batteries**

The FluorPen operates from four AAA single-use or rechargeable batteries. They may be easily replaced by unscrewing the cover of the battery holder on the rear of the instrument. Battery life is approximately 48 hours when the FluorPen is operated continuously.



# **Detachable leaf-clip**

Supplementary detachable leaf-clips for use with the FluorPen FP 100-MAX-D and PAR-FluorPen FP 100-MAX-LM-D. Sample holder is a clip for gentle fixing of a leaf sample and its short duration dark adaptation. "D" version of the PAR-FluorPen with detachable leaf-clips is suitable for experiments where long term dark adaptation is needed.



# **6. Operation Instructions**

The next six pages explain the structure of the Main Menu and three Sub-Menus with all their options.

- 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 for the **SET** key.
- Dashed-line arrows are used for the **MENU** key.

 $\longrightarrow$ 

In general:

- Use the **MENU** key to scroll through sequential menu options on the digital display.
- Use the **SET** key to select a menu option based on cursor (>) position.















This Chapter applies to users of the FluorPens with an incorporated Bluetooth communication module.

# 7.A. General Information

#### What you will need first:

Before you set up the Bluetooth connection between the FluorPen and PC, make sure you have these components:

#### 1. Bluetooth enabled FluorPen

FluorPen FP 100-MAX and FluorPen FP 100-B include built-in Bluetooth capabilities.

#### 2. Bluetooth enabled PC

The PC with which you connect must have Bluetooth wireless technology, either built-in or through a Bluetooth card. Make sure that the PC's Bluetooth setting is "discoverable" (meaning that it shows up when other devices search for nearby Bluetooth connections). Consult the user guide for your PC or Bluetooth card to learn how to do this.

#### 3. Bluetooth configuration software properly set up on PC

Before you can exchange files with your PC, you will need to set up the Bluetooth software that came with your PC, or your PC's Bluetooth card. This software varies by manufacturer. Please consult your PC's Bluetooth documentation for more information.

#### 4. Bluetooth must be switched on visible on both devices

To pair the FluorPen with another Bluetooth device, such as a computer, you will need to ensure that Bluetooth is switched on visible on both devices.

# 7.B. Bluetooth Pairing

# Step 1: Enabling Bluetooth in the FluorPen Switch on the FluorPen (press and hold the SET key). Scroll to the "Setting" menu (press the MENU key twice, then press the SET key once). Select "BT\_On" to enable Bluetooth (press the SET key). \* Keep in mind that the FluorPen turns off automatically after about 3 minutes of no action. Turning off the FluorPen always turns off Bluetooth.

# **Step 2: Starting the Bluetooth Application on Your PC**

Be aware that this description is a general example; some of the steps may be different on your PC.



You may also start your Bluetooth application via the Control Panel:

**Select:** Start>Settings>Control Panel>Bluetooth Devices.



#### Step 3: Opening the Add Bluetooth Device Wizard



#### Step 4: Searching for a New Bluetooth Device

Add Bluetooth Device Wizard × Welcome to the Add Bluetooth ® Mark the following box: "My device is set up and Device Wizard ready to be found". Before proceeding, refer to the "Bluetooth" section of the device documentation. Then set up your device so that your computer can find it: Select: Next. Turn it on - Make it discoverable (visible) - Give it a name (optional) Press the button on the bottom of the device (keyboards and mice only) My device is set up and ready to be found. Add only Bluetooth devices that you trust. ٦ <u>N</u>ext > Cancel



#### **Step 6: Starting the Pairing Process** Add Bluetooth Device Wizard Do you need a passkey to add your device? ∦ Your Bluetooth Pairing Passkey is: To answer this question, refer to the "Bluetooth" section of the documentation that came with your device. If the documentation specifies a passkey, use that one. 0000 C Choose a passkey for me Select: "Let me choose my own passkey." O Use the passkey found in the documentation: Enter: 0000 (four digits). Itet me choose my own passkey: 0000 Select: Next. O Don't use a passkey You should always use a <u>passkey</u>, unless your device does not support one. We recommend using a passkey that is 8 to 16 digits long. The longer the passkey, the more secure it will be. < Baci <u>N</u>ext > Cancel

# **Step 7: Completing the FluorPen Pairing**

Select: Finish.



# 7.C. Bluetooth Connection

# Step 1: Installing the FluorPen Software

Install the FluorPen software to your PC. Use the CD that came enclosed with your FluorPen.\*

\* See Chapter 8 of this Operation Manual for complete information on FluorPen software.

# Step 2: Enabling Bluetooth in the FluorPen

Switch on the FluorPen (press and hold the SET key).Scroll to the "Setting menu" (press the MENU key twice, then press the SET key once).

Select "BT\_On" to enable Bluetooth (press the SET key).\*

\* Keep in mind that the FluorPen turns off automatically after about 3 minutes of no action. Turning off the FluorPen always turns off Bluetooth.





# Step 4: Registering the FluorPen Software

Select: Help>Register. Enter your serial (registration) number.* Select: OK.	Register           Serial Number           00000000           00000000           00000000	×
* You will find your serial (registration)	Ok Cancel	

number in the file **SN.txt on the enclosed CD**.

#### **Important Note: How to Reconnect Bluetooth**

Disconnection can occur, either when the Bluetooth feature has been turned off in one or both of the devices, or when the units move outside their operating range.

If the devices have been turned off, simply turn them on and enable Bluetooth in the FluorPen again.

If the FluorPen has been moved outside the Bluetooth operational range, bringing it back into range within 90 seconds will allow it to reconnect automatically. If more time elapses, simply turn the FluorPen on and enable Bluetooth again.

# 8.A. General Information

#### What you will need first:

Before you set up the USB connection between the FluorPen and PC, make sure you have these components:

#### 1. USB enabled FluorPen

FluorPen FP 100-MAX and FluorPen FP 100-U include connector for communication by USB cable.

#### 2. USB cable



# 8.B. USB Pairing

Connect the USB cable to a computer and FluorPen as shown below. Then **Switch on** the FluorPen (press and hold the SET key). On computer run the program Fluorpen 1.0. Then folow chapter 9. – FluorPen software.





This Chapter applies to users of the FluorPen FP 100-MAX, PAR-FluorPen FP 100-MAX-LM and to users of the FluorPen FP 100-B and FP 100-U.

# 9.A. Starting up

#### **Starting up:**

- 1. Switch on the computer.
- 2. Switch on the FluorPen and enable Bluetooth.\*
- 3. Make sure that your PC and the FluorPen are properly paired.\*
- 4. For FluorPens supplied with the USB communication module, use provided USB cable to connect your device to a PC. Please note that FluorPens battery is automatically re-charged when connected to the PC.
- 5. Start the FluorPen program.

Please note that the device always comes with just one communication module - either USB or Bluetooth. For USB connection you need to have the USB driver installed in your PC. You find the driver on the installation disk (USB driver folder). If you check the Device Manager in Windows you should see the USB serial port in the device tree. In case of missing driver you may download it from the following link: http://www.psi.cz/ftp/FluorPen/USB\_Driver\_Setup.exe. When the driver is installed correctly you should be able to connect to the device in the FluorPen software menu Setup->Device ID.

\* Points 2 and 3 apply only to users of the FluorPen FP 100-MAX and FluorPen FP 100-B. See Chapter 7 of this Operation Manual for complete information on Bluetooth pairing and connecting.

Connecting:	FloorPen
Select: Setup>Device ID (Ctrl+I).	Update Firmware Settings
If properly connected, the message "Device: FluorPen" appears in the bottom part of the screen.	
	Device: FluorPen Version: 1.0.1.0 3318 of 524288 Bytes used

# 9.B. Menu and Icon Explanation

# 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		🌾 Fi	luorPen		
		File	Device S	etup Help	p
Download	Downloads data from the FluorPen to your PC.		Downlo	ad	
Erase Memory	Erases data from the FluorPen memory.		Erase N	lemory	
Online Control	Settings sound and time.		Online	Control	
Attach GPS File	Used for download data from GPS module.		💒 Attach	GPS File	
_					

# Menu: Setup

Menu: Setup		🍯 FluorPen					
Device ID	Detects the Bluetooth	File	Device	Setup Help			
	connected device.			Device ID	Ctrl+I		
Update Firmware	Used for software updates.*			<table-of-contents> Update Firmware From I</table-of-contents>	File		
Settings	Used for modification of the program settings.**			Settings			

\* For more information on software updating, see Chapter 9G of this Operation Manual.

*\*\* See more information on the next page.* 

Menu: Help		🍯 Fli	uorPen	23	
About Register	Offers basic information about the program. Used for the FluorPen software registration.*	File	Device	Setup	Help About Register

\* See Chapter 7C of this Operation Manual for more information on FluorPen software registration.

Menu: Settings After Download - Memory Erase	Settings	×
If the box is checked, the FluorPen memory is erased after each data download.	After Download	Comm Speed
<b>Data - Inverted</b> If the box is checked, the polarity of data is inverted, e.g., multiplied by -1.*	Data Inverted	Timeout 1 Graf
<b>Data - Add to Opened</b> If the box is checked, the downloaded data are added to that of the current opened experiment.	GPS Visible	Single
Graf - Single		and the second se

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.

\* This feature can be helpful for a certain type of experiment when the measured data are undesirably interpreted as negative values.

# **Icon Explanation:**

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

# 9.C. Example of Data Transfer and Visualization

**Step 1:** Do a measurement with your FluorPen. (Here, we did an OJIP measurement.)

**Step 2:** Click the "Download" icon or select Device>Download.

**Step 3:** The Data table appears.

<b>File name</b>	arameter						
<b>FluorPen</b> File Device Setup Help T:\Final\FluorPen\Test	_Data\data.dat	1					
Time 6:26	3 2.1 2007	6:26:8 2.1 2007	V 6:26:32 2.1 2007	6:27:25 2.1 2007	6:4 9 2.1 2007		
	QY	OJIP	Ft	QY	V <sub>OJIP</sub>		
Fo Back Fo Flash Fm Bac Fm Flas Save experime Value Measured and calculated values	0.64	Fo         618           Fi         1422           Fi         2149           Fm         2423           Fv         1805           Vi         0.445           Vi         0.848           Fm/Fo         3.921           Fv/Fo         2.921           Fv/Fo         2.921           Fv/Fm         0.745           Mo         0.911           Area         4786890           Sm         2652.017           Ss         0.489           N         5422.780           Phi_Po         0.745           Psi_o         0.555           Phi_Eo         0.413           Phi_Do         0.255           Phi_Eo         2.7311           ABS/RC         2.745           TRo/RC         2.045           ETo/RC         1.134           Dlo/RC         0.700	<b>355</b> Backgr 1 Flash 356	0.71 Fo Backgr 1 Fo Flash 275 Fm Backgr 1 Fm Flash 942	Fo         646           Fi         1491           Fi         2438           Fm         2639           Fv         1993           Vi         0.424           Vi         0.899           Fm/Fo         4.085           Fv/Fo         3.085           Fv/Fo         3.085           Fv/Fo         3.085           Fv/Fo         3.085           Fv/Fm         0.755           Mo         0.831           Area         5229888           Sm         2624.129           Ss         0.510           N         5142.671           Phi_Po         0.755           Psi_o         0.576           Phi_Eo         0.435           Phi_Do         0.245           Phi_Pav         2908.289           ABS/RC         2.595           TRo/RC         1.960           ETo/RC         1.129           Dlo/RC         0.635		
Description		Arabidopsis A			Arabidopsis B		
Data     Notes     Graph:6:26:8 2.1,2007       Device: FluorPen     Version: 1.0.1.0     0 of 524288 Bytes used       Space for written notes     Switch to graphic visualization of the     Experiment description							

**Step 4:** To visualize measurement in the graphic mode, click the "Graph" field in the bottom bar.

**Step 5:** The colored Graph of measured data appears.



**Step 6:** For **export** press File>Export or "Export" icon, select what you want to export (Ft, QY, Measuring by protocol...). Measuring by protocol export all protocols at once (OJIP, NPQ, LC...).

**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

Computed values - export calculated data, in case of OJIP: Fo, Fi, Fj...

Description - exports the data description if any

🍯 FluorP	Pen	100	1		100	52	-	2	100	1.0	_	
File De	vice Setup	Help		P.Q.L.1		a maal						
	W:\Laborka\L	DATA-LAB (@D	UNUMENT	i i Manuaj	YAP, FPADAT	A-FP.dat				1		1
	Index	1			2		3		4		5	E
	Time	21:04:12 19	3.2.2000	19:31:57	12.3.2000	19:32:15	5 12.3.2000	19:35:16	6 12.3.2000	19:41:37	7 12.3.2000	19:47:58
	GPS											
	ID	OJIP-4	55	Export					× 1	LC	1-455	LC1-
	Value	Bekg         8           Fo         20           Fi         19           Fm         19           Fw         -4           Vi         0.7           Fm/Fo         0.9           Fv/Fo         -0.0           Fv/Fo         -0.0           Area         37           Fix Area         19           HACH Area-76         Sm           Ss         0.0           Phi_PO         -0.           Psi_po         -0.7	00 19 17 16 250 750 980 0.20 0.20 0.20 0.20 12308 684 333, 82 12308 684 333, 82 12308 684 000 000 000 000 000 000 000 0	How ✓ Selecte ✓ Source ✓ Comput What ← Ft ← Qy ← Measur ← NDVI ← PRI ← MURPI ← OI N Phi_Po Psi_o	d Only Data ted Values ing By Protoco 40.326 0.657 0.453	N Phi_Po Psi_o	Description OD N PAR ALAI BWI PAR-ID 1899.346 0.606 0.516	¥ Can Qp_Lss Rfd Fm_D1	0 3 5 9 6 6 2el 1.22 12322	Fo Fm_L1 Fm_L2 Fm_L3 Fm_L4 Fm_L5 Fm_L6 Ft_L1 Ft_L2 Ft_L3 Ft_L4 Ft_L5 Ft_L6 QY_max QY_L1 QY_L2 QY_L2 QY_L4 QY_L5	5364 18954 19116 17588 15865 12192 10371 10078 7087 7900 7738 7933 9428 9753 0.72 0.63 0.72 0.63 0.55 0.51 0.35 0.09	Fo Fm_L1 Fm_L2 Fm_L3 Fm_L4 Fm_L5 Fm_L6 Ft_L1 Ft_L2 Ft_L3 Ft_L4 Ft_L5 Ft_L6 QY_L4 QY_L1 QY_L2 QY_L3 QY_L3 QY_L5
	Description	OJIP	•	(	)JIP	(	JIP	N	IPQ1		LC1	LC
	Data Note	e Graph:21-0	14/12/19/2	2000								•
Device: N	ot Connected		04.12 13.2	.2000								
Device, N	or connected						]					11.

# 9.D. Explanation of OJIP Parameters

The FluorPen also offers the possibility to capture rapid fluorescence transient – OJIP, which occurs during exposure of plants to high irradiance. The FluorPen software enables data downloading to a personal computer and subsequent OJIP analysis. The OJIP protocol includes the following extracted and technical parameters\*:

Formula Abbreviation	Formula Explanation
Bckg	Background
F <sub>0</sub>	$F_0 = F_{50\mu s}$ , fluorescence intensity at 50 µs
FJ	F <sub>J</sub> = fluorescence intensity at J-step (at 2 ms)
Fi	F <sub>i</sub> = fluorescence intensity at i-step (at 60 ms)
F <sub>M</sub>	F <sub>M</sub> = maximal fluorescence intensity
Fv	F <sub>V</sub> = F <sub>M</sub> - F <sub>0</sub> (maximal variable fluorescence)
VJ	$V_{J} = (F_{J} - F_{0}) / (F_{M} - F_{0})$
Vi	$V_i = (F_i - F_0) / (F_M - F_0)$
<b>F</b> <sub>M</sub> / <b>F</b> <sub>0</sub>	
<b>F</b> <sub>V</sub> / <b>F</b> <sub>0</sub>	
Fv / F <sub>M</sub>	
$M_0$ 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 $F_{\ensuremath{M}}$ (background subtracted)
Fix Area	Area below the fluorescence curve between $F_{40\mu s}$ and $F_{1s}$ (background subtracted)
S <sub>M</sub>	$S_M = Area / (F_M - F_0)$ (multiple turn-over)
Ss	$S_S$ = the smallest $S_M$ turn-over (single turn-over)
Ν	$N = S_M \cdot M_0 \cdot (1 / V_J)$ turn-over number $Q_A$
Phi_P <sub>0</sub>	$Phi_P_0 = 1 - (F_0 / F_M) (or F_V / F_M)$
Psi_0	$Psi_{0} = 1 - V_{J}$
Phi_E <sub>0</sub>	$Phi_{E_0} = (1 - (F_0 / F_M)) \cdot Psi_0$
Phi_D <sub>0</sub>	$Phi_D_0 = 1 - Phi_P_0 - (F_0 / F_M)$
Phi_Pav	Phi_Pav = Phi_P <sub>0</sub> ( $S_M / t_{FM}$ ) $t_{FM}$ = time to reach $F_M$ (in ms)
ABS / RC	ABS / RC = $M_0$ . (1 / $V_J$ ). (1 / $Phi_P_0$ )
TR <sub>0</sub> / RC	$TR_0 / RC = M_0 . (1 / V_J)$
ET <sub>0</sub> / RC	$ET_0 / RC = M_0 . (1 / V_J) . Psi_0$
DI <sub>0</sub> / 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.

# Example of OJIP protocol



The NPQ protocol is the most typically used measuring approach to quantify photochemical and nonphotochemical quenching. The measurement should be performed with a dark-adapted sample. Thereby, it may not be appropriate under field conditions.

The NPQ protocol starts by giving a measuring light to acquire minimal level of fluorescence *Fo.* A short saturating flash of light is then applied to reduce the plastoquinone pool and measure maximum fluorescence in the dark adapted state, *Fm.* After a short dark relaxation, the sample is exposed to actinic irradiance for tens to hundreds of seconds to elicit a transient of the Kautsky effect. Moreover, a sequence of saturating flashes is applied on top of 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.

Two NPQ protocols, NPQ1 and NPQ2 are predefined. The protocols differ in the duration of the light exposure and the dark recovery phase, in the number and interval between pulses. See table below.

	Phase	Duration	# of pulses	1st pulse	Pulse interval		
NDO1	Light	60s	5	7s	12s		
NPQI	Dark recovery	88s	3	11s	26s		
NDO2	Light	200s	10	10s	20s		
NPQ2	Dark recovery	390s	7	20s	60s		

Abbreviation	Explanation
Fo	minimum fluorescence in dark-adapted state
Fm	maximum fluorescence in dark-adapted state, measured during the first saturation flash after dark adaptation
Fp	fluorescence in the peak of fast Kautsky induction
Fm_L, Lss, D, Dss <sup>1</sup>	maximum fluorescence
QYmax <sup>2</sup>	maximum quantum yield of PSII in dark-adapted state - Fv/Fm
QY_L, Lss, D, Dss <sup>1,3</sup>	effective quantum yield of PSII
NPQ_L, Lss, D, Dss <sup>1,4</sup>	non-photochemical chlorophyll fluorescence quenching
Qp_L, Lss, D, Dss <sup>1,5</sup>	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 phase; ss - steady state

<sup>2</sup> Calculated as (*Fm* – *Fo*) / *Fm* 

<sup>3</sup> Calculated as (*Fm\_Ln – Ft\_Ln*) / *Fm\_Ln* or of corresponding steady state or dark recovery parameters

<sup>4</sup> Calculated as (Fm – Fm\_Ln) / Fm\_Ln or of corresponding ss, Dn or Dss parameters

<sup>5</sup> Calculated as (*Fm\_Ln – Ft\_Ln*) / (*Fm\_Ln – Fo\_Ln*) or of corresponding ss, Dn or Dss parameters



# Example of NPQ1 protocol



# Example of NPQ2 protocol



# 9.F. Light Curve (LC) Protocol

The protocol called Light Curve (LC) was 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).

Several LC protocols are predefined in FP. These differ in number and duration of individual light phases and light intensities.

	# of phases	Phase duration	Light intensities, [µmol m <sup>-2</sup> s <sup>-1</sup> ]
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

The protocol includes following measured and calculated parameters:

Abbreviation	Explanation				
Fo	minimum fluorescence in dark-adapted state				
Fm	maximum fluorescence in dark-adapted state				
Fm_Ln <sup>‡</sup>	maximum fluorescence in light adaptation state				
Ft_Ln <sup>‡</sup>	n <sup>‡</sup> instantaneous fluorescence during light adaptation				
QYmax*	maximum quantum yield of PSII in dark-adapted state - Fv/Fm				
QY_Ln <sup>‡**</sup>	instantaneous PSII quantum yield induced in light				

<sup>‡</sup> n represents a sequential number of light phase

\* Calculated as (Fm - Fo) / Fm

\*\* Calculated as (Fm\_Lx - Ft\_Lx) / Fm\_Lx





# Example of LC1 protocol



# Example of LC2 protocol



# Example of LC3 protocol



# 9.G. Software Update

#### **Very important!**

The FluorPen memory is erased during the software update!

Before starting any software update, export all your data from the FluorPen memory into your computer!

# Step 1: Starting Update





# **Step 3: Finishing Upload**

**Select:** "OK" to start uploading of the update.



The bottom bar indicates the upload progress.

Press: "OK" to finish upload.



# **10. GPS Module**

For all devices in FluorPen Series GMS module might be connected to the device.

# **IMPORTANT INFORMATION:**

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.

# 10.A. GPS Module Description



- Zoom keys
- ② Back key
- ③ Thumb Stick<sup>™</sup>
- Menu key
   Menu key
- (b)/Backlight key



- Mini-USB port (under weather cap)
- Battery cover
- Battery cover locking ring
- Mounting spine

#### **1. Installing AA Batteries**

The device operates on two AA batteries. You can use alkaline, NiMH or lithium batteries. Use NiMH of lithium batteries for best results.

- 1. Turn the D-ring counter clockwise and pull up to remove the cover.
- 2. Insert the batteries, observing polarity.
- 3. Replace the battery cover and turn the D-ring clockwise.
- 4. Hold 🙂
- 5. Select Setup->System->Battery Type
- 6. Select Alkaline, Lithium or Rechargeable NiMH

# 2. Device settings



- 2. After the device is on, it begins acquiring satellite signals. The device may need a clear view of the sky to acquire satellite signals.
- 3. You can check current position and accuracy in Satellite Page
- For better precision you can enable GPS+GLONASS mode in Setup->System
   ->Satellite System->GPS+GLONASS and turn WAAS/EGNOS On.
- 5. Make sure that USB Mode is set to Mass Storage (Setup->System->USB Mode)

For more information please check Garmin eTrex manual.

# 10.B. First Time Installation of the GPS Module

#### Step 1: Enabling GPS Module in the FluorPen Software

Select: Setup>Settings



<b>x</b>	After Download	Comm Speed
	Memory Erase	Commispeed Up
	Data	Graf
$\sim$	☐ Inverted	🔽 Single
	Add to opened	
	GPS Visible	

# **Step 2: Accepting GPS Setting**

Close and restart the FluorPen program to accept GPS setting.

# 10.C. GPS / FluorPen Operation

# **Step 1: Time Synchronization**

Synchronize the FluorPen time with the time of your PC. Time must be set correctly witch respect to your time zone.

# **Step 2: GPS Positioning**

Switch the GPS module on and wait until the GPS position is fixed (GPS green LED indicator flashes 1s ON and 2s OFF while tracking the position).

#### **Step 3: Operation**

Be aware that while performing field experiments, the FluorPen and the GPS module must be kept close to each other.

# 10.D. Data Download

# **Step 1: Enabling Communication**

Switch on the computer, FluorPen, and GPS module and set your computer to FluorPen communication (enable Bluetooth or connect to serial or USB port).

# Step 2: Downloading FluorPen Data



· · · · · · · · · · · · · · · · · · ·	FluorPe	en								
	ile Device	Setup H	elp							1
Be aware that no GPS coordinates are	U	ntitled - 1								
visible at this moment.		Index		1		2		3		(
		Time	16:13:03	13.5.2009	16:13:40	13.5.2009	16:14:4	7 13.5.2009		
<u>[</u>	2	GPS								
		ID	C	JIP	(	JJIP		DJIP		
		Value	Bokg Fo Fi Fm Fw Fw/Fo Fv/Fo Fv/Fm Mo Area Fix Area Sm Ss N Phi_Po Phi_Eo Phi_Eo Phi_Do	59 3370 5135 5972 6212 2842 0.621 0.916 0.843 0.843 0.843 0.843 0.843 0.826 2640077 6177313 931.062 0.671 1388.416 0.458 0.379 0.173 0.173 0.542	Bokg Fo Fi Fin Fw Fv Vi Vi Vi Fw/Fo Fv/Fo Fv/Fo Fv/Fo Fv/Fo Fv/Fm Mo Area Sm Ss Ss Ss N Phi_Po Phi_Do Phi_Do	97 10956 19897 22385 23928 12972 0.689 0.927 2184 1.184 0.542 23695322 381.050 0.474 803.610 0.542 0.347 0.542 0.311 0.168 0.458	Bokg Fo Fi Fi Fw Fv Vi Fv/Fo Fv/Fo Fv/Fo Fv/Fo Area Si Ss Ss N Phi_Po Phi_Eo Phi_Do	99 3201 4576 5254 5544 0.587 0.732 0.732 0.732 0.732 0.732 0.423 0.732 5467296 674.411 0.735 918.180 0.423 0.413 0.413 0.413 0.577		
	[	Description								
	F	ata Note	s Graph:	16:13:03 13!	5.2009					
								0 -6 504000 5	D. Associated	 
De	evice: Fluori	ren				version: 1.2.	1.1	U OF 524288 E	Bytes used	10

48

# **Step 3: Connecting GPS Module**

Connect the GPS Module to your PC. Communication is set properly if the hardware is recognized by your PC.

# **Step 4: Downloading GPS Data**

Select: Device>Attach GPS file to download data from the GPS	File Devi File Devi Control	Pen Ce Setup H Download Crase Memory Dnline Control	ielp	1 3:03 13:5:2009	16:13:4	2 0 13.5.2009	16:14:4	3 7 13.5.2009			
module.		Value	Bckg Fo Fi Fi Frw Vi Vi Frv/Fo Fv/Fo Fv/Fm Mo Ss Ss Ss N Phi_Pi Phi_Di	CUIP 59 3370 5135 5972 2842 0.621 0.843 0.458 0.325 264077 3617731 381.052 0.677 1.388.416 0.458 0.325 0.458 0.325 0.457 0.338 0.458 0.325 0.457 0.458 0.325 0.458 0.458 0.325 0.4588 0.4588 0.4588 0.4588 0.4588 0.4588 0.4588 0.4588	Bokg Fo Fi Fi Fi Fw Fv/Fm Mo Area Fr/Fo Fr/Fo Phi/Po Phi_Do Phi_Do	DJIP 97 10956 19897 22995 23928 12972 0.827 2.184 0.542 1.184 0.542 1.184 4342979 2369522 381.050 0.474 800.3610 0.542 0.341 0.542 0.542 0.341 0.542 0.341 0.542 0.341 0.542 0.341 0.542 0.542 0.341 0.542 0.341 0.542 0.341 0.542 0.341 0.542 0.542 0.542 0.542 0.341 0.542 0.552 0.555 0.555 0.552 0.555 0.555 0.555 0.555 0.555 0.555 0.55	Bokg Fo Fi Fi Fi Fw Fw/Fm Mo Area Fix/Rea Sm Ss N Phi_Po Phi_Do Phi_Do	DJIP 99 3201 4576 5254 4576 5254 2343 0.876 1.732 0.423 0.876 1.732 0.732 0.732 0.739 1580145 5467291 0.735 918.180 0.423 0.413 0.413 0.577			
	-	Data Note	es j Gira	aph: 16:13:03-13.5	5.2009 J			-		 	
	Device: Flu	orPen			_	Version: 1.2	1.1	0 of 524288	lytes used		11.

# **Step 5: Completing the Download**

Successfully downloaded GPS coordinates paired with FluorPen data.

Index	1	2	3	
The	16:13:03 13.5.2009	16:13:40 13:5.2009	16:14:47 13:5.2009	
GPS	49° 13.7739' N 16° 36.2356' E	49° 13.7585' N 16° 36.2354' E	49* 13.7949' N 16* 36.2300' E	
ID	OJIP	OJIP	OJIP	
Value	Bckg         59           Fo         3370           Fi         5135           Fi         5972           Fr         5972           Fr         5972           Fr         2842           Vi         0.621           Vi         0.916           Fw/Fo         1.843           Fv/Fo         0.843           Fv/Fo         0.843           Fv/Fo         0.826           Area         2646077           Sa         0.671           N         1308.416           Phi_Do         0.458           Phi_E.co         0.173           Phi_E.co         0.379           Phi_E.co         0.172           Phi_E.co         0.542	Bckg         97           Fo         10956           Fi         19897           Fi         22985           Fi         22985           Fw         12922           Fv         12922           Vi         0.65           Fir/Fo         1.16           Fv/Fo         1.16           Fv/Fo         1.16           Fv/Fo         1.16           Fv/Fo         381           Sm         381           Sa         0.474           N         803.610           Phi_Do         0.458	Bckg 99 Fo 3201 Fi 4576 Fi 5254 Fw 5254 Fv 2343 GPS information adde CK Sm 674.411 St 0.735 N 918.180 Phi_Po 0.423 Phi_Eo 0.113 Phi_Eo 0.137 Phi_Eo 0.577	
escription				

- This Limited Warranty applies only to the FluorPen and its accessories (excluding any batteries). It is valid 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 PSI will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. PSI 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 PSI; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by PSI.
- 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.
- PSI repairs or replaces faulty instruments as quickly as possible; the maximum time is one month.
- PSI 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-ondelivery 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.

For customer support, please write to: <a href="mailto:support@psi.cz">support@psi.cz</a>

Copyright © Photon Systems Instruments, 2014-08