



PSI Fluorpen

## Part 2 – Chlorophyll fluorescence

### PAR-FluorPen FP 100-MAX-LN

Erik Murchie

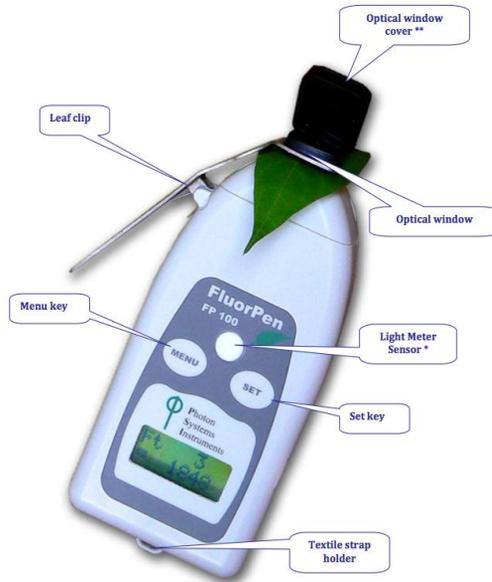


PSI Fluorpen



- When Time of day ?  
How many replicates per plant ?  
How many replicates per plot ?
- What  $F_v / F_m$  (dark adapted) ?  
 $\Delta F / F_m'$  in the light ?
- How How long to dark adapt ?  
How should I dark adapt ?  
Details of using the device.  
Do I store the data or manually record it ?

## PSI Fluorpen



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**Measured and Calculated Parameters:**

F<sub>o</sub>, F<sub>t</sub>, F<sub>m</sub>, F<sub>m</sub>' , QY, NPQ 1\*, NPQ 2\*, OJIP\*, LC 1\*, LC 2\*, PAR\*\*

**Saturating Light:**

Adjustable from 0 to 3,000  $\mu\text{mol}(\text{photons})/\text{m}^2.\text{s}$  (0 to 100 %)

**Actinic Light:**

Adjustable from 0 to 1,000  $\mu\text{mol}(\text{photons})/\text{m}^2.\text{s}$  (0 to 100 %)

**Measuring Light:**

Adjustable from 0 to 3,000  $\mu\text{mol}(\text{photons})/\text{m}^2.\text{s}$  (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



PSI Fluorpen

**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



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## Measurements of $F_v / F_m$

Over view of protocol

1. Dark adapt the leaf for a minimum of 20 minutes
2. Insert the leaf into the leaf-holder without exposing the leaf area you want to measure to any light (this takes some practice!).
3. Measure  $F_v / F_m$ . This takes place in darkness and will measure  $F_o$  and then  $F_m$  with a saturating flash (over  $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ )
4. Data can be recorded manually ( $F_v / F_m$  only, not  $F_o, F_m$ ) or on the device for download later (all parameters).

## PSI Fluorpen

1. Around mid- day is best to maximise chances of photoinhibition (11 am – 3 pm)
2. Use dark adaptation clips if available, or aluminium foil. Make sure the target leaf area is fully covered.
3. 20 minutes minimum. Be relatively precise and consistent i.e. within 5 minutes error each time.
4. **Do not expose the leaf to light when removing the foil.** Try 'clamping' the leaf with the Fluorpen and sliding the foil out.
5. Leave 5 – 10 seconds in the clip , in the dark , before making the measurement
6. To test for severe stress, try a pre-dawn measurement !

## Dark Adaptation



Figure 13.3. A self-made dark adaptation leaf clip using aluminum foil.

## PSI Fluorpen

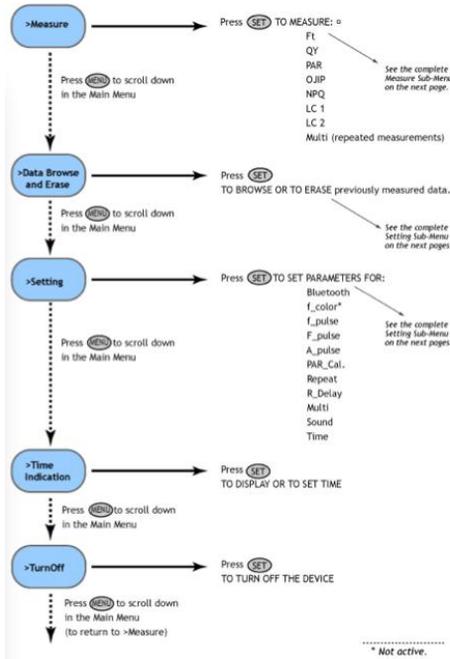
## Protocol

1. Leave for 5 – 10 minutes to attain ambient temperature
2. Switch on by pressing 'Set'
3. Use 'menu' to select correct option and then 'set' to select.
4. It gives the 'real – time' value of fluorescence.
5. Selecting QY or OJIP protocol will give you values of  $F_0$ ,  $F_m$  and  $F_v / F_m$  . OJIP will consume more memory .
6. You can track measurements by the 'record number'.



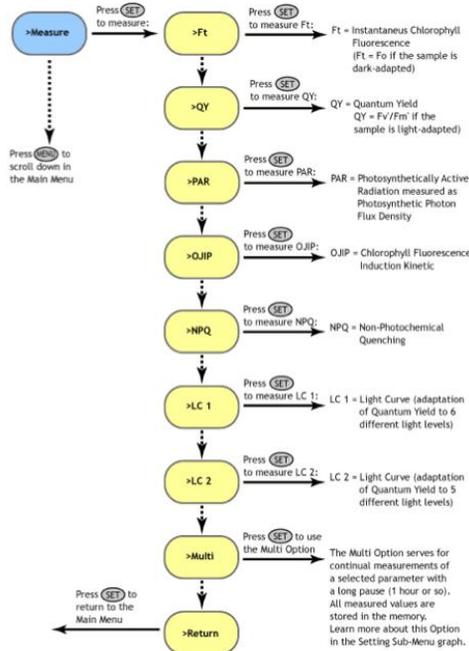
### Main Menu

To start hold the SET key for 1 second.



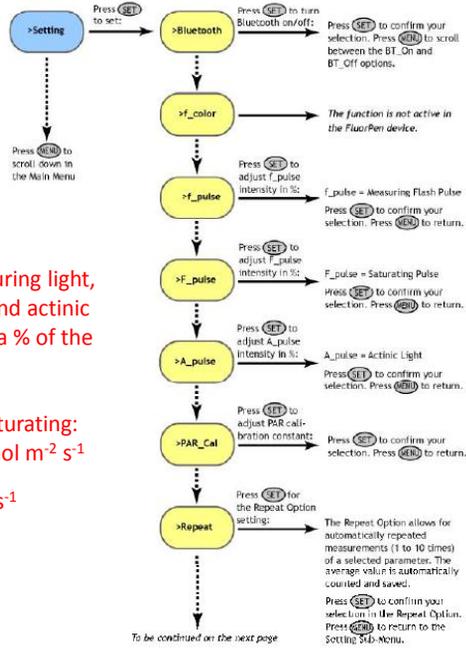
### Measure Sub-Menu

Use the Measure Sub-Menu when measuring selected parameters.



### 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 made.



Settings for measuring light, saturating pulse and actinic light are made as a % of the maximum:

Measuring and saturating:  
Range 0- 3000  $\mu\text{mol m}^{-2} \text{s}^{-1}$

Actinic  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
Range 0-1000

➡ Measuring light (pulse) – should be low %

➡ Saturating pulse – should be high %

➡ Actinic not needed for Fv / Fm

'R\_Delay' and 'Multi' can be used to set the Fluorpen to automatic, repetitive logging



PSI Fluorpen

Leaf clip gaskets

Keep in good condition and replace when necessary



Replace batteries by removing the back cover



Software is easily installed (download free from [www.psi.cz](http://www.psi.cz)) on Windows XP upwards.

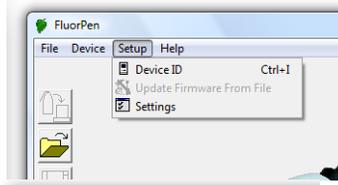
Copy all folders to your PC

Open the software Desktop icon.



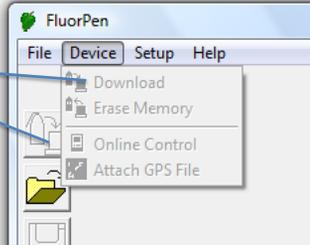
-  **Download** Downloads data from the FluorPen to PC.
-  **Load** Loads (opens) previously saved data files.
-  **Save** Saves data to hard disc.
-  **Export** Exports data in .txt format.

Connect the Fluorpen via USB.  
 'Setup', Device ID, will connect the Fluorpen.

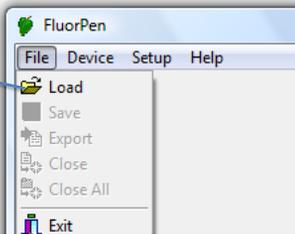


You may need to enter serial number.

Download data from the Fluorpen



Open an existing file here



Downloaded QY data looks like this.

QY becomes Fv/Fm when measured in the dark

Save data here

Export data  
 Here

When exporting,  
 Select 'QY' from  
 The list

Exports as  
 ASCII file

Open in Excel, use  
 Import wizard

Index	544	545	546	547	548	549
Time	14:12:51 17.11.2013	14:13:32 17.11.2013	14:13:37 17.11.2013	14:13:42 17.11.2013	14:15:31 17.11.2013	14:15:40 17.11.2013
ID	QY	QY	QY	QY	QY	QY
Value	0.73	0.72	0.72	0.76	0.73	0.73
Fv Backgr	149	109	99	119	119	119
Fv Flash	5503	4655	4606	4326	4566	4566
Fm Backgr	189	129	139	159	149	149
Fm Flash	20059	16261	16171	17457	16480	16480



- When Time of day ?  
 How many replicates per plant ? **One per plant**  
 How many replicates per plot ? **3 – 5 plants per plot**
- What  $F_v / F_m$  (dark adapted) ? **Yes**  
 $\Delta F / F_m'$  in the light ?
- How How long to dark adapt ? **At least 20 minutes**  
 How should I dark adapt ? **Clips or aluminium foil**  
 Details of using the device.  
 Do I store the data or manually record it ? **Try both at first**