Dual -Modulation Kinetic Fluorometer FL 6000
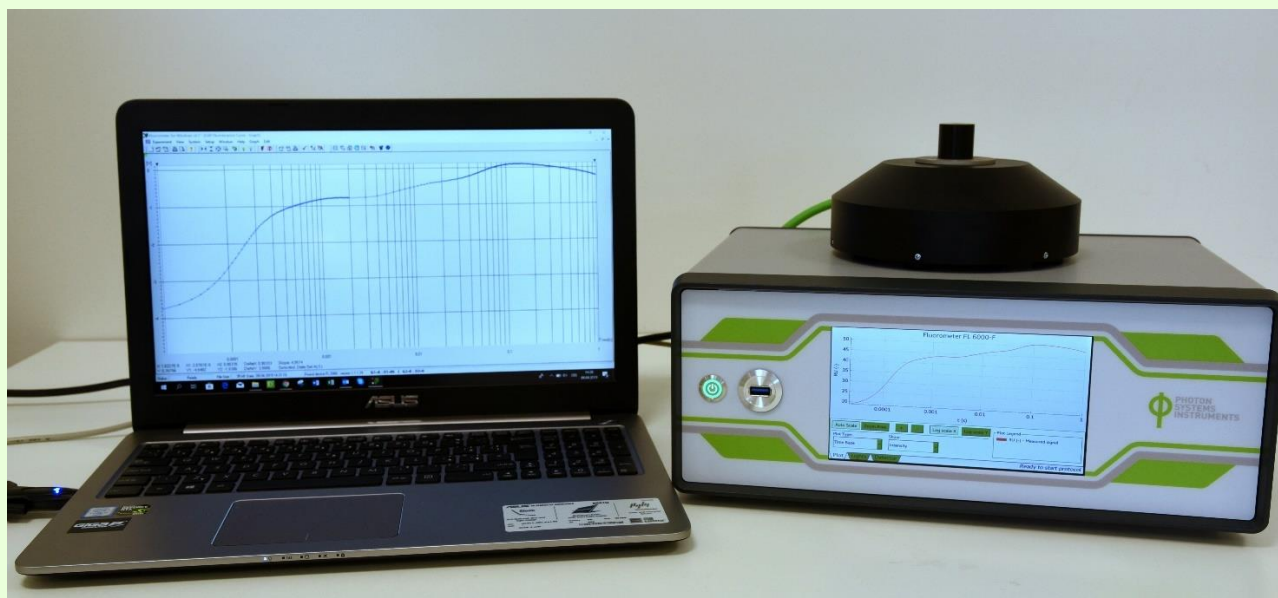
Manual and User Guide

Please read this manual before operating this product

TABLE OF CONTENTS

1   INFORMATION BEFORE USING FLUOROMETER

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 Fluorometer device or doing the maintenance.

It is forbidden to interfere with the hardware or software of the Fluorometer 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.
ℹ️	Additional information.

Tab. 1 Used symbols.

2   TECHNICAL SPECIFICATION

Protocols	
FL 6000-S	- Instantaneous fluorescence - QA- reoxidation kinetics - Kautsky effect - Quenching parameters analysis - Fast OJIP transient - S-states
FL 6000-F	- Instantaneous fluorescence - QA- reoxidation kinetics - Kautsky effect - Quenching parameters analysis - Fast OJIP transient - S-states - **Flash Fluorescence Induction (FFI)**
Custom defined protocols	variable timing, special language and scripts
Illumination – LED lighting	
Light source	623 nm and 460 nm; other wavelengths available on request
Single turnover flash	Up to 170,000 μmol(photon).m^{-2}.s^{-1}
Actinic light	Up to 3,500 μmol(photon).m^{-2}.s^{-1}
Detector	
Type	PIN photodiode
Time resolution	4 μs (FL 6000-S) 1 μs (FL 6000-F)
Detection limit	100 ng Chl-a/l (FL 6000-S) 1 μg Chl-a/l (FL 6000-F)
A/D resolution	16 bit
Software	
Type	FluorWin 3.8
Control	Predefined protocol as well as custom defined protocols with variable timing, special language and scripts
Communication	RS232/USB
Accessories (optional)	
Thermoregulator TR 6000	Precise temperature control of the sample in range from +5 to +60 °C; magnetic stirrer highly recommended
Oxygen detector module	Oxygen evolution detection; consists of convertor and electrode; magnetic stirrer highly recommended
Magnetic stirrer	Homogenization of the sample during measurement
Infra-Red LED unit	Measuring of F_0' and PAR absorbance
PC with preinstalled software	Notebook with preinstalled software
Additional measuring unit	Measuring unit constructed with respect to customer's experimental needs: specific LED colors (Blue, Cyan, Amber) and detection bands (Chl-a, Chl-b)
Other	
Control unit dimension	365 x 275 x 150 mm
Measuring unit dimension	Diameter 160 x 60 mm
Total weight	Appr. 5 kg
Electrical	90 VAC – 240 VAC, 50 Hz – 60 Hz

3 GENERAL INFORMATION

In fluorescence experiments light serves to drive photosynthetic reactions and, at the same time, to excite fluorescence emission. To separate these roles, two different light sources are frequently used. The actinic light delivers power that is sufficient to induce substantial change in the state of photosynthetic apparatus. On the contrary, the mean power of the measuring light is so low that the impact of the induced photosynthetic reactions can be neglected. In most of the presently used fluorometers, either the actinic or the measuring light is modulated, so that the detection system can separate the fluorescence excited by the measuring light.

Here, we present fluorometer **FL 6000** that is based on a non-periodic, user-definable modulation of both, the Actinic (F1) and Measuring Flashes (f): **double-modulation**. In addition, continuous Actinic Light (A1, optionally A2) supplied by separate sets of LEDs is available. The wide dynamic range of the instrument is achieved primarily by the programmable timing of the measuring flashes. In one transient, the fast processes can be covered by a sampling on the microsecond time-scale while the slow processes requiring minimal disturbance by the measuring light can be monitored by weak measuring flashes spaced several minutes apart.

The **software FluorWin** includes a Wizard with frequently used experimental protocols that allows an easy operation. For an experienced professional, the instrument offers a sophisticated programming language that can be used to design novel timing and measuring sequences.

Predefined protocols measured by the Fluorometer:

Meter
Serves to measure of Instantaneous Chlorophyll Fluorescence. F_t is equivalent to F_0 if the sample is dark-adapted.
QA-Reoxidation
The Protocol measures F_0, executes a single-turnover flash to reduce Q_A acceptor and follows the subsequent decline of fluorescence that reflects the reoxidation kinetics.
OJIP
The OJIP curves enable observing major changes that occur during exposure of a sample to high irradiance.
Kautsky Effect
The Protocol monitors the typical behavior of a chlorophyll fluorescence when the photoautotrophic organism is exposed to light. The Protocol measures F_0 and switches on Actinic Light that elicits the induction.
Quenching Analysis
The analysis of fluorescence quenching is usually based on a comparison of the maximal fluorescence emission (F_{max}, $F_{max'}$) before and during an exposure to Actinic Light. This protocol is the most typically used measuring approach to quantify photochemical and non-photochemical quenching.
S-States
The protocol allows monitor states of the Oxygen-Evolution Complex (S-states).
Flash Fluorescence Induction*
Measures Photosystem II antenna heterogeneity and the antenna size.

* Valid only for fast version FL 6000-F

Fluorometer versions:

Standard Fluorometer FL 6000-S

The standard version can measure Chlorophyll fluorescence signal with **time resolution up to 4 µs.**

Fast Fluorometer FL 6000-F

The fast version of the instrument (16 bit, 1MHz) allows measuring the fluorescence induction during a single-turnover saturating flash with **time resolution up to 1 µs**. This technique using the Flash fluorescence induction protocol makes possible to assess the Photosystem II antenna heterogeneity, the antenna size and the connectivity without disturbing the measured structure by herbicides as DCMU or atrazine (Nedbal et al., J.Photochem.Photobiol.1998).

4 CARE AND MAINTENANCE

- Never submerge the device in water

- 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.

- Avoid to use sharp objects for touch screen of the control unit

5   DEVICE DESCRIPTION

A typical configuration of the Fluorometer is shown in the Fig. 1. The device consists of Control unit with touch screen and measuring unit. Individual part are described bellow.
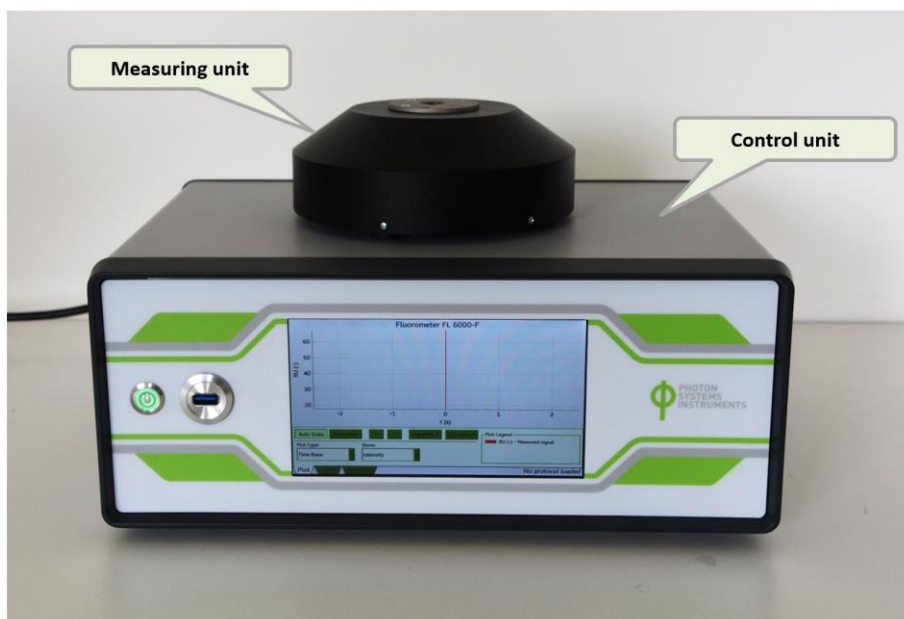

Fig. 1 Fluorometer FL 6000.

Measuring unit

Main part of the measuring unit is the measuring chamber indended for 4 ml cuvettes. The measuring unit is primarily designed for liquid samples. The chamber is equipped with LED lights and PIN detector with optical filters with band pass 690 – 730 nm, RG695.

Standard version of the measuring unit includes (Fig. 2):

| Name	Colour	Number	Used in protocols
Measuring light 1 (M)	Red (620 nm)	1 pc	Meter, QA reoxidation, Kautsky Effect, Quenching Analysis, S-States
Measuring light 2 (M)	Blue (460 nm)	3 pcs	Meter, QA reoxidation, Kautsky Effect, Quenching Analysis, S-States
Single turnover flash (F)	Red (620 nm)	6 pcs	QA reoxidation, S-States, Flash Fluorescence Induction
Actinic light (A)	Red (620 nm)	6 pcs	Kautsky Effect, Quenching Analysis, OJIP
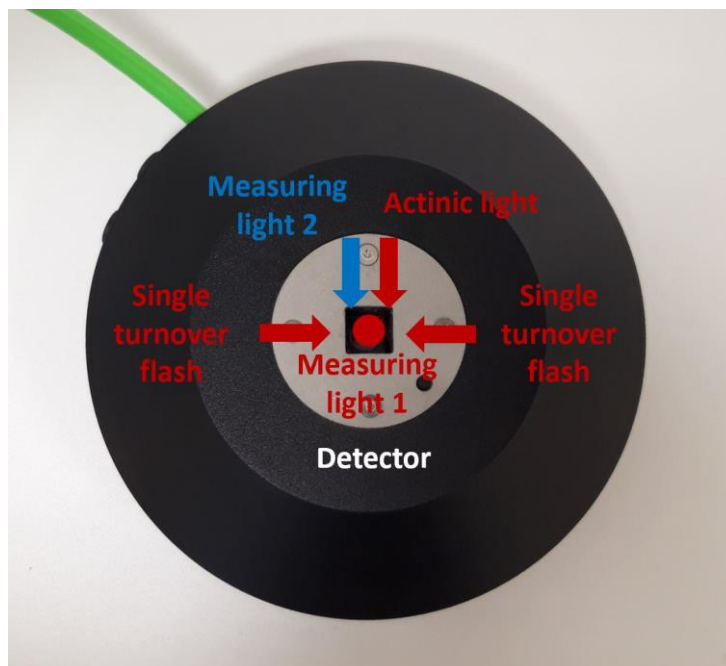
Fig. 2 Measuring unit.

The measuring unit is powered by the green cable, which is plugged into the "Measuring CH1" connector placed on the back side of the control unit. The 4-pin female connector is intended for additional Infra-red LED unit. The 2-pin female connector serves for connection of the magnetic stirrer (Fig. 3).

Fig. 3 Connectors of measuring unit.

Control unit

The controlling electronics for FluoroMeter and additional devices is integrated in compact Control Unit equipped with touch screen displey. The display enables basic settings of the device and realtime data reading during the measurement. Except the display the front panel contains the Power button and USB connector, which serves for firmware upload (Fig. 4).

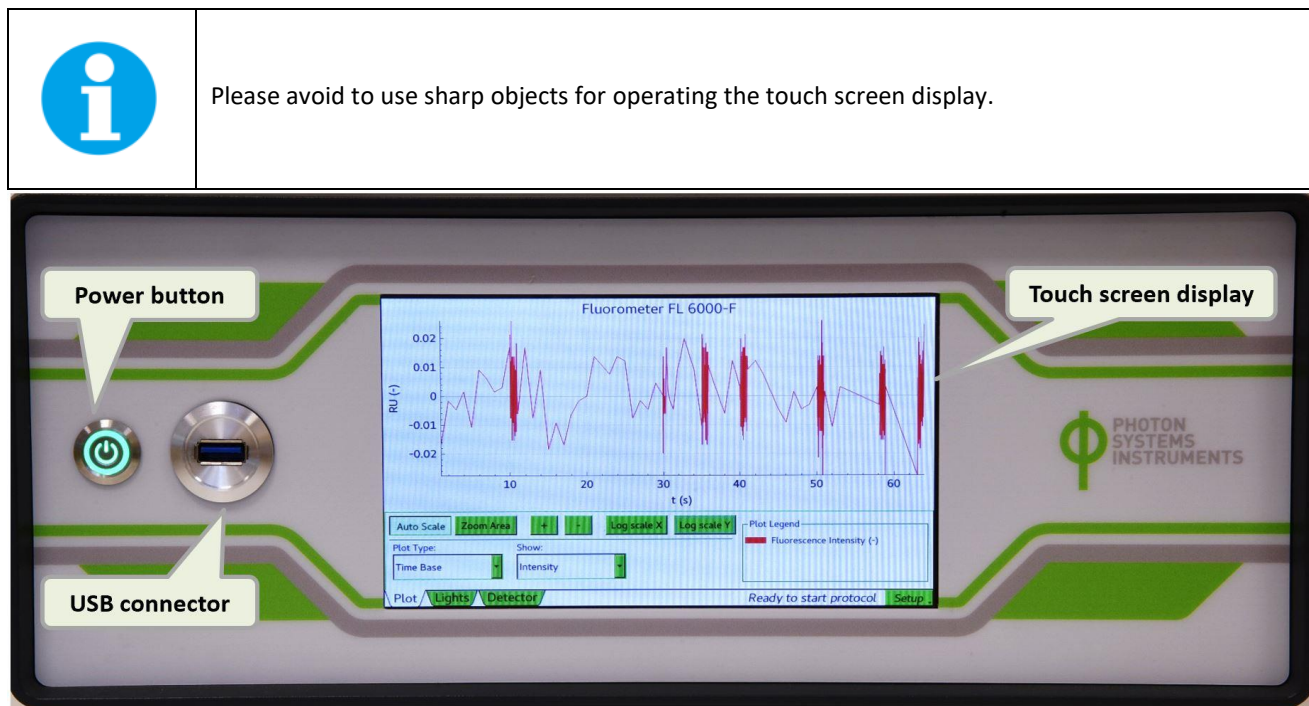|	
ℹ️	Please avoid to use sharp objects for operating the touch screen display.

Fig. 4 Front panel of the control unit.

The back side of the Fluorometer contains connectors for standard and additional parts of the device and the main power button (Fig. 5).
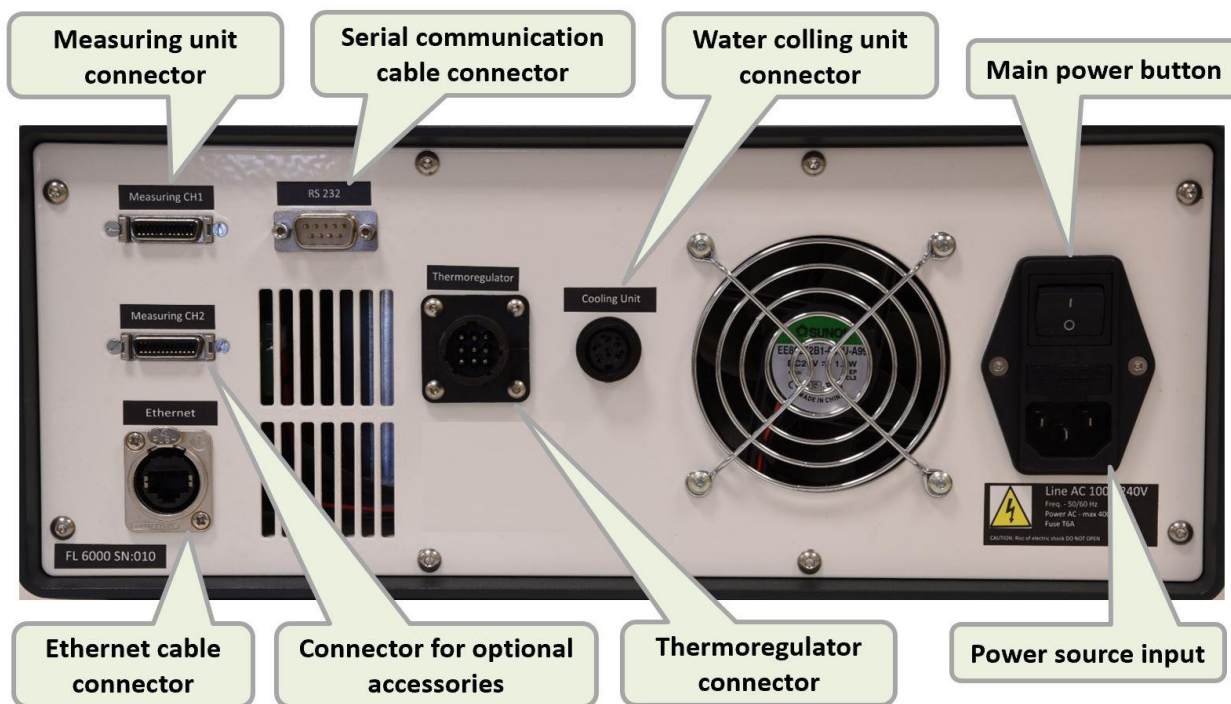

Fig. 5 Back side of the control unit.

5.1   LIST OF EQUIPMENT

Except control and measuring unit you should have received the following items:

- **Power supply 12V for Control Unit**

- **Serial cable connecting computer and Control unit**

- **Serial – USB Converter**

- **Plastic cuvettes – 10 pieces**

- **Cuvette stopper**

- **FluorWin installation flash drive** (on a USB flash disc)

- **User's Guide** (on a USB flash disc)

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

ℹ️	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**

5.2   OPTIONAL ACCESSORIES

Additional measuring head

Fluorometer standard configuration can be enhanced by adding a second Measuring unit. This Measuring unit can be constructed with respect to customer's experimental needs: specific LED colours (Blue, Cyan, Amber) and detection bands (ChlA, ChlB).

Additional Actinic light 2

Build-in light source available in three wavelengths: blue (455 nm), cyan (505 nm), amber (590 nm).

Infra-Red LED unit

Additional external light source with wavelength 730 nm allows measuring Fo' and PAR absorbance. It is also used during quenching analysis protocol.

Thermoregulator TR 6000

Provides precise temperature control in the range of 5 °C to +60 °C with an accuracy of 0.1 °C. TR 6000 can work in two modes: (i) constant mode, (ii) temperature ramp mode. In the constant mode, the instrument maintains a constant temperature of the measured sample. The temperature ramp mode enables linear changing of the sample temperature with a rate ranging from 0.1 °C/sec to 1 °C/sec. It is highly recommended to use the Thermoregulator with the magnetic stirrer.

⚠️	Only version TR 6000 is suitable for Fluorormeter FL 6000. Previous version (TR 2000) is not compatible with FL 6000 and causes irreversible damage of the device.

Oxygen detector

Serves for oxygen evolution detection based on dynamic quenching of fluorescence (electrode sold separately). It is highly recommended to use the oxygen detector module with the magnetic stirrer.

Magnetic stirrer with magnetic bars

Magnetic Stirrer is designed to provide continuous stirring with little speed deviation and minimum heat build-up. Continuous, uniform stirring is essential for keeping a constant temperature within the entire sample volume when the temperature control is applied. The rate of stirring is set by a knob on the front panel of the device. Magnetic Stirrer is a handy accessory to Fluorometers: it can be connected to the Control Unit and controlled by it (switched on and off).

PC with preinstalled control software

Notebook PC with preinstalled software.

6   CONTROL UNIT TOUCH SCREEN OPERATION

The touch screen of the control unit enables real time data reading during the measurement as well as graph and device setting. In the upper part is placed the name of connected device (fluorometer, thermoregulator, oxygen module). The main section of the screen is the graph. The data in the graph are shown in real time. In the graph you can move using swiping. In the lower right corner the status of the measurement is shown. Touch screen includes these options:

Plot (Fig. 6)

Below the graph there are following visualization settings:

|	
Auto Scale	enables auto scaling of the graph
Zoom Area	serves for zooming of selected area in the graph
+ and - mark	zoom in and out in the graph without area selection
Log Scale X	logarithmic view of X axis, used in OJIP or QA reoxidation protocol
Log Scale Y	logarithmic view of Y axis
Plot type	there is only option time base for fluorometer device
Show	fluorescence signal (intensity) is usually shown in the graph, temperature or dissolved oxygen can be displayed when using additional accessories
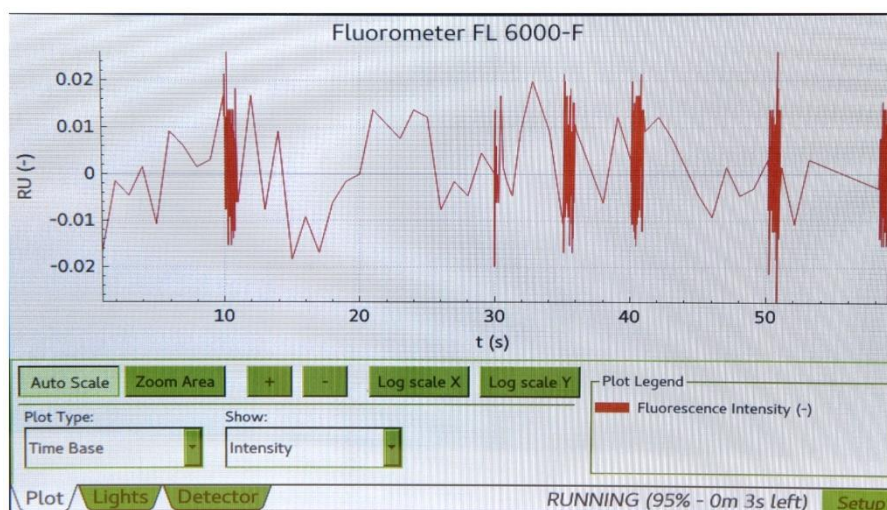
Fig. 6 Control unit display - Plot window.

Lights (Fig. 7)

This option informs the user about the lights status and setting. The changes in lights setting can be done only via the FluorWin software.
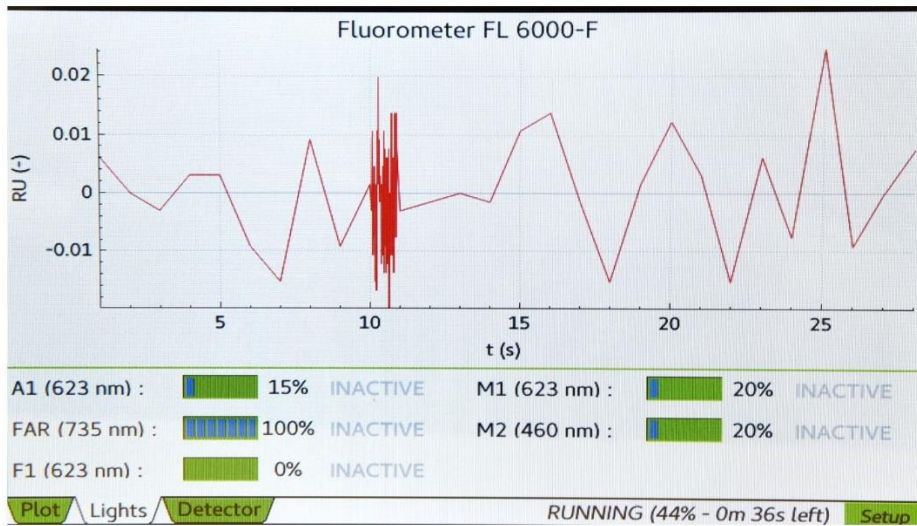
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Fig. 7 Control unit display – Lights window.

Detector

Detector setting can be defined in right side of this menu. Detector **Gain** is set using the slider. For precise adjustment use the – and + marks. Function **Auto offset** (Fig. 8) tune the offset of the detector automatically before each measurement. It is done based on chlorophyll concentration in the sample. If the Auto offset is switched off, the **Offset** can be adjusted manually with slider (Setup > Offset) (Fig. 9).

⚠	When adjusting the offset manually check the actual signal level. When the level is set to 0%, no signal is detected.

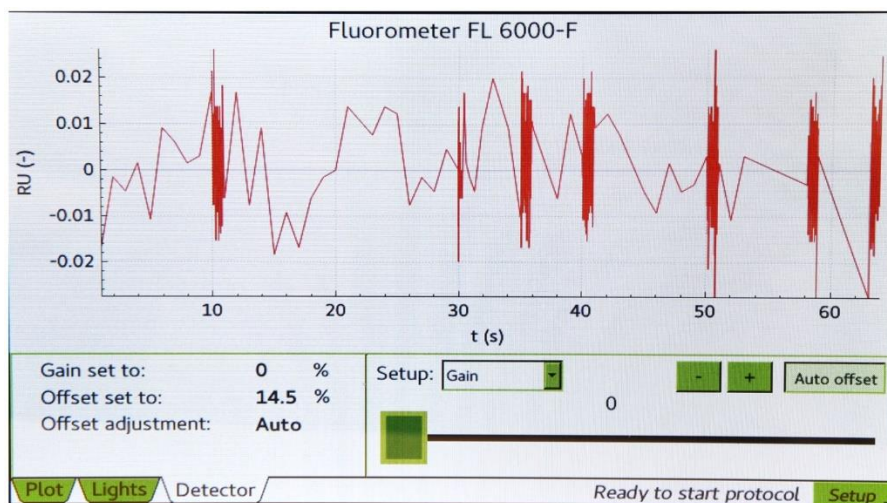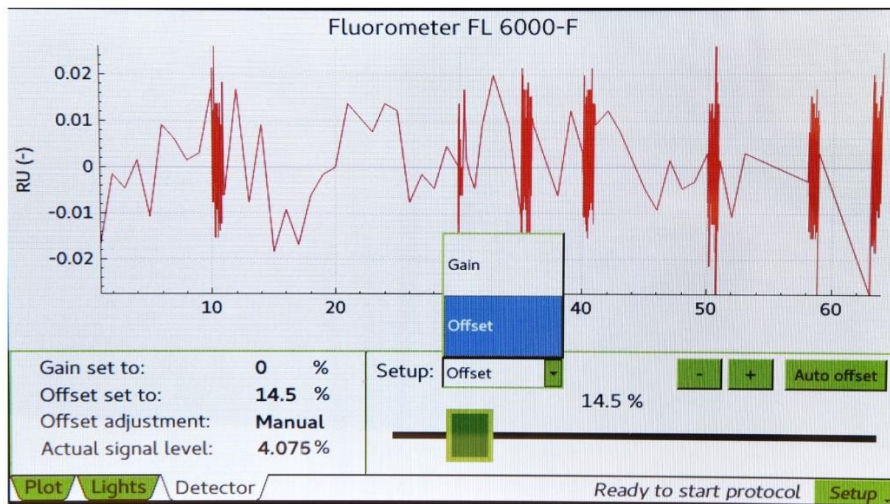| ⚠	Please note that Detector can be adjusted also via the FluorWin software. Detector functions are more described in chapter 0. The setting is linked between the touch screen and the software. **Auto offset** function is available only on touch screen.

Fig. 8 Control unit display - detector window - Auto offset.

Fig. 9 Control unit display - detector window - manual offset.

Setup (Fig. 10)

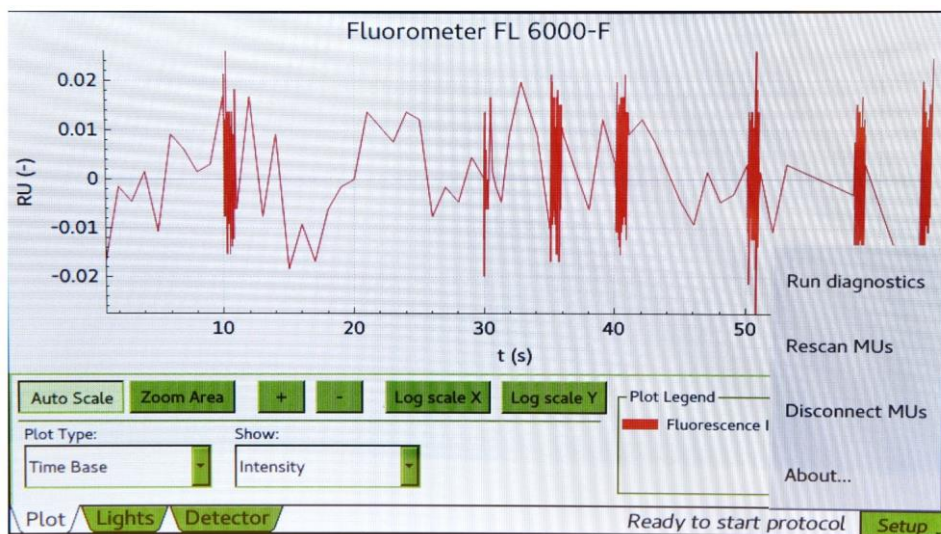|	
Run diagnostics	in case of malfunction, the user can be asked for run the diagnostics test, which is send automatically to the manufacturer
Rescan MUs	rescanning of control unit ports in case of connection new device (e.g. thermoregulator)
Disconnect MUs	disconnection of all current connected devices
About…	information about the device (Fig. 11)

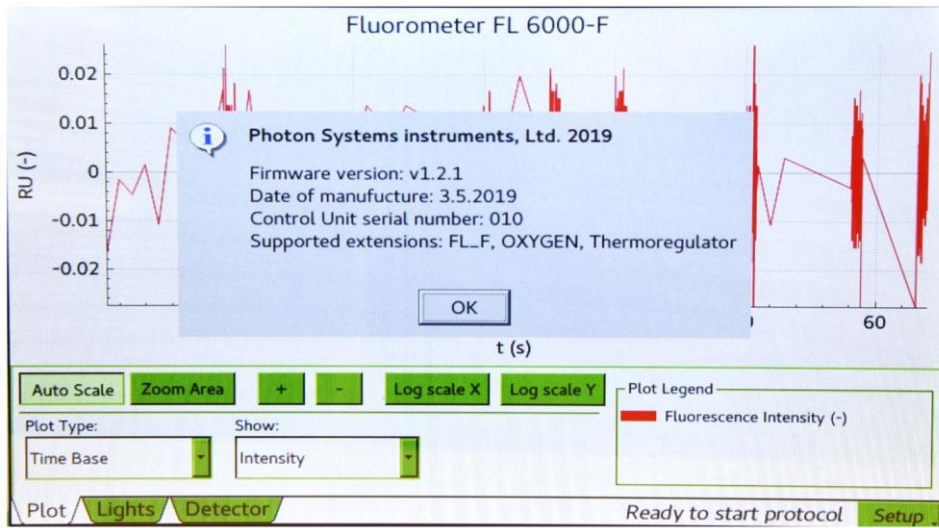*Fig. 10 Control unit display – setup.*

Fig. 11 Control unit display - About.

7 FLUORWIN SOFTWARE

The FluorWin software is designed for protocol generating and modification as well as for data visualization and export.

ℹ️	The Fluorometer is not able to operate without the software.

7.1 GETTING STARTED

- Assembly the device, plug it into an outlet and plug the serial cable with USB converter to pc.
- **Switch ON the device** using the main power button on the back side of the control unit and then using the power button on the front side, the front button turns green. It takes few seconds to turns on the touch screen.
- Copy the FluorWin software to your pc.
- Check the COM port number of the Fluorometer in your pc (Device manager > Ports).

⚠️	The COM port number has to be lower than 10, otherwise the device will not connect. If necessary, change the COM port number (select the Fluorometer COM port > Port settings > Advanced > COM port number).

- **Run the FluorWin** software.
- Go to the top line menu and select **Setup > Communication**... Program will start scanning all installed ports on your computer. Not used ports are disabled. Select the COM port (i.e. COM1) to which you have connected the Fluorometer and click OK (Fig. 12).
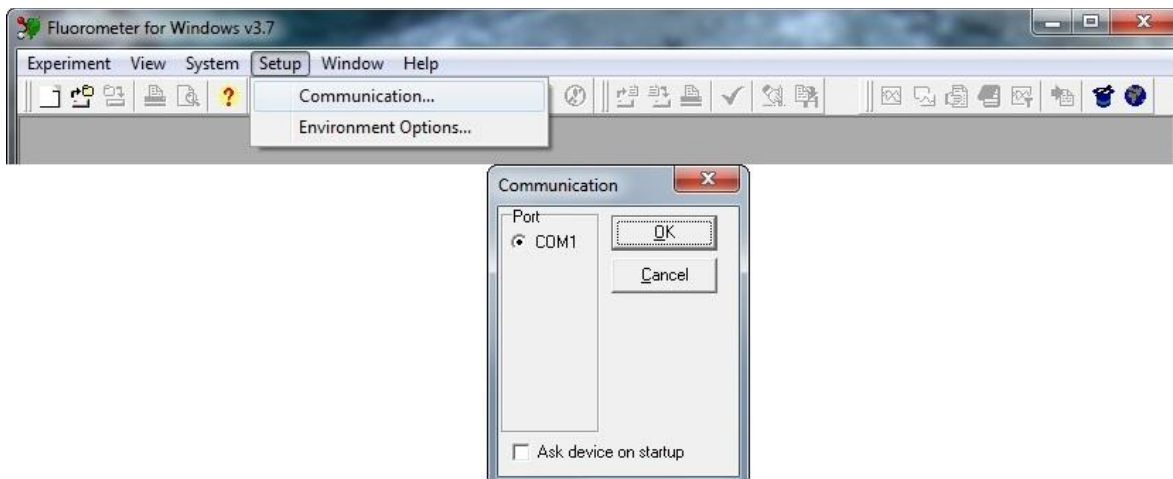

Fig. 12 Communication setting.

- Select **System > Device ID** from the menu (Ctrl-I Shortcut). Program initiates to searching the connected devices (Fluorometer in this case) on the previously selected COM port. Message "Searching on COM …" is displayed in the "Device Info" array of the Status bar. This Status bar is located at the bottom of the FluorWin window. "Found device …" message is displayed here if searching of the Control unit was successful. The Device Status array presents "Ready" and the Device Info shows the instrument bios name and version (Fig. 13). If there is not a device connected to selected COM port, "Device not found" message is displayed.

Fig. 13 Status bar.

- Open the **Wizard** menu using the blue hat on the upper bar or go to Window > Protocol Wizard (Fig. 14).

Fig. 14 Starting Wizard menu.

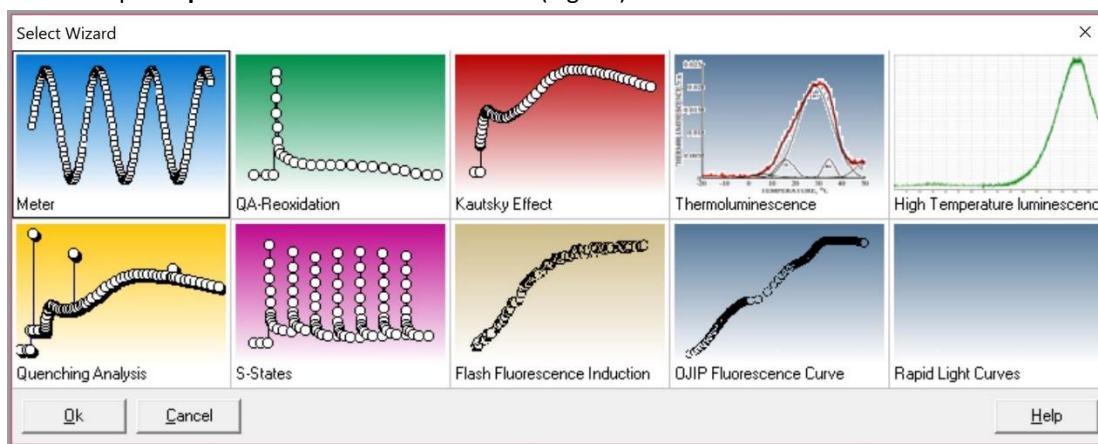- **Select** the required **protocol** from the Wizard menu (Fig. 15).

Fig. 15 Wizard menu.

- Selected protocol can be started with **Start** icon (red flash). If the Start icon is grey, it signals that the instrument is not connected. To stop already started protocol is possible with **Abort** icon (Fig. 16).

Fig. 16 Experiment start and abort.

If no protocol has been started, there is notice Empty in the left bottom corner of Status bar. The progress of the experiment (in percent) is indicated here during the measurements. When 100 % is reached, data are automatically downloaded from the Control Unit to the computer (downloading is also presented in Status Bar). After successful download Ready notification appears.
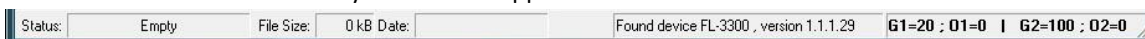

Fig. 17 Status bar - empty.

7.2 SOFTWARE FEATURES

- The software can open more experiments simultaneously. Only one of these opened files can be active. This active experiment is marked with **green square in the left upper corner** in DataSet table window or Graph window (Fig. 18). If the active experiment was closed or is required to be changed, use menu Window > Make Active. This command will activate the experiment which is displayed on the screen at the moment. The green square will appear in its window subsequently. After start of the active experiment a **red square** appears next to green one to indicate Busy state.
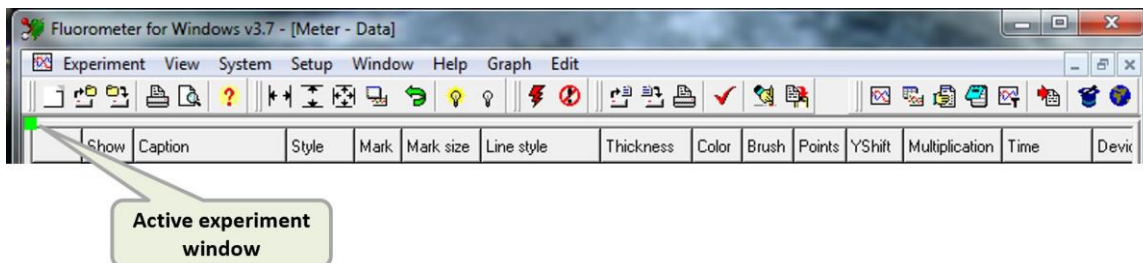
Fig. 18 Active experiment window.

- Each Fluorometer experiment consists of 4 information blocks: **Graph, DataSet table, Protocol and Notes**. For some types of devices such as Thermoluminescence, fifth block called T-Graph is provided.

Fig. 19 Icon bar - experiment.

7.2.1 GRAPH (F5)

- The Graph window presents the experimental data. The graphic presentation can be adjusted by **Graph menu** on the upper bar (Fig. 20).
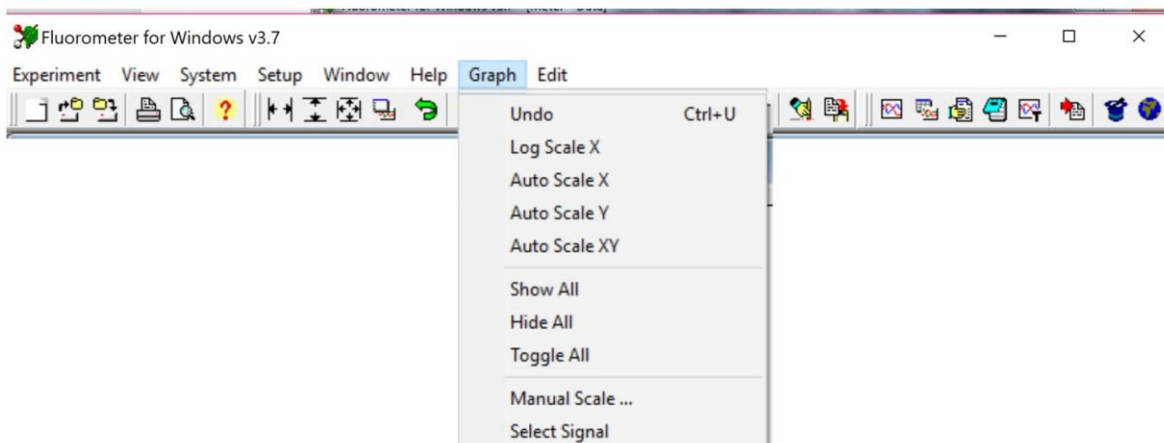
Fig. 20 Graph menu.

Basic options of Graph menu are placed directly on the upper bar (Fig. 21).
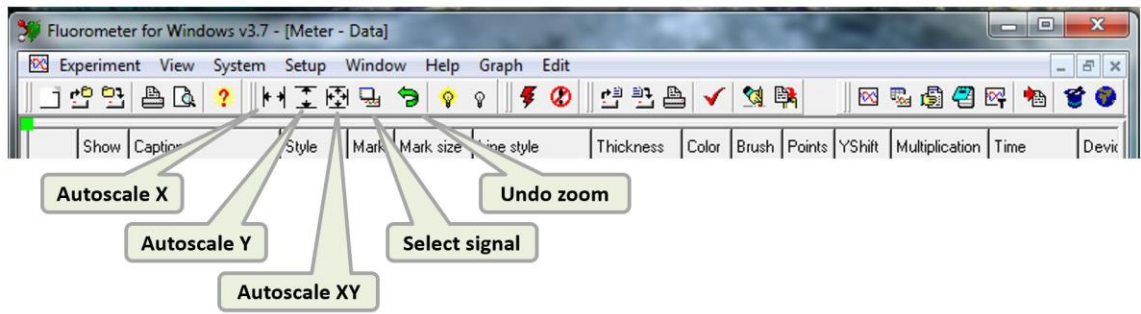
Fig. 21 Graph option on upper bar.
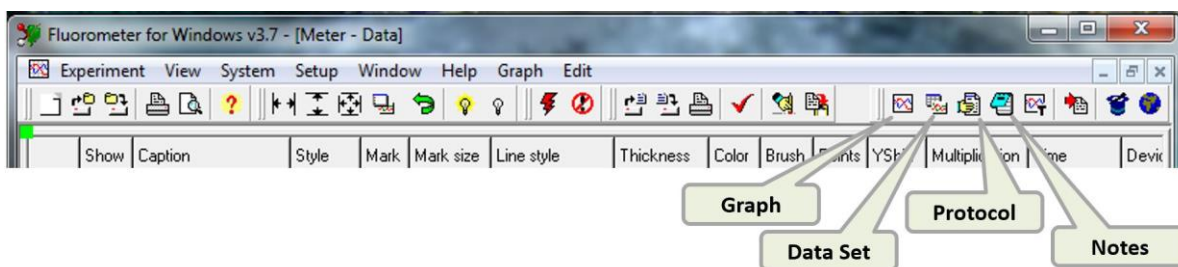
- To **zoom in** on the details of the graph press the left mouse button and move the cursor on the screen to define the rectangular area for zoom. Any action can be corrected by the Undo icon on the menu bar. Use left mouse button to zoom in (Fig. 22).
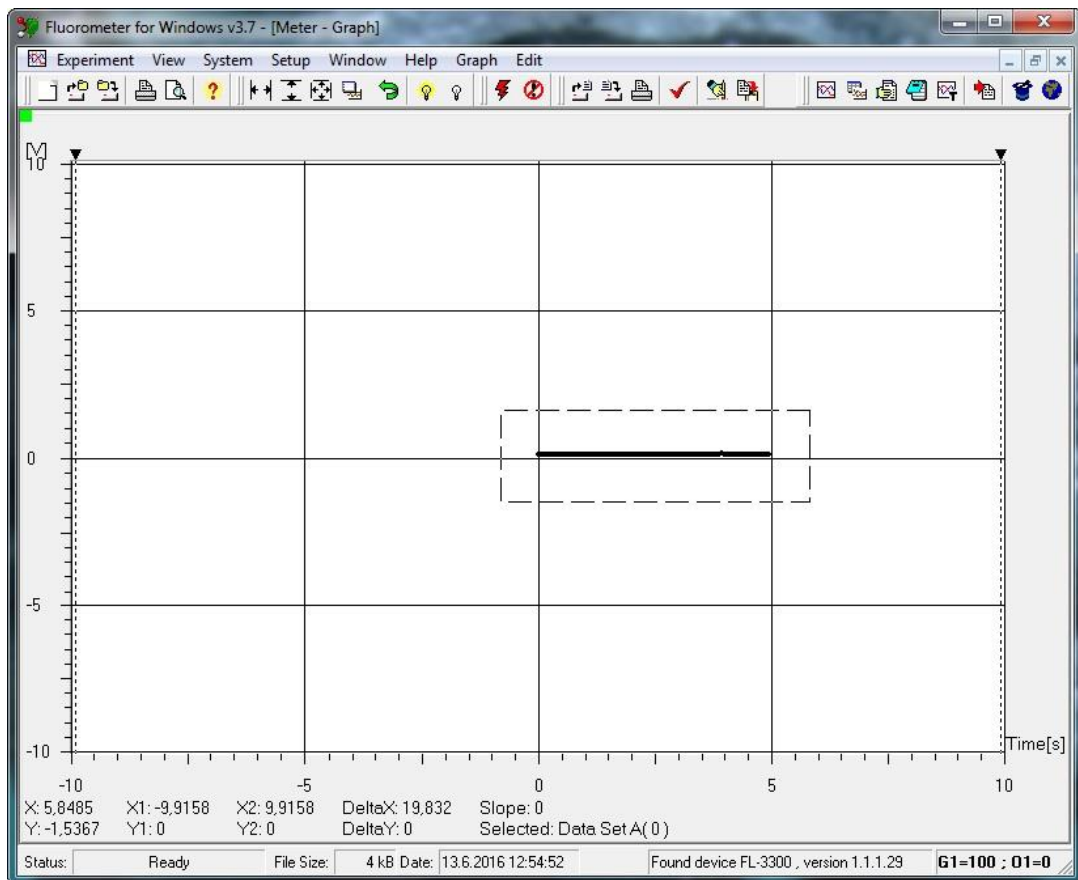
Fig. 22 Zoom in.

- For **zoom out** use right mouse button or autoscale functions.
- Visualisation of Reoxidation kinetics and OJIP curve require **logarithmic scaling** of the time axis. Switching between logarithmic and non-logarithmic presentation is possible in menu Graph > Log Scale X (Fig. 23).
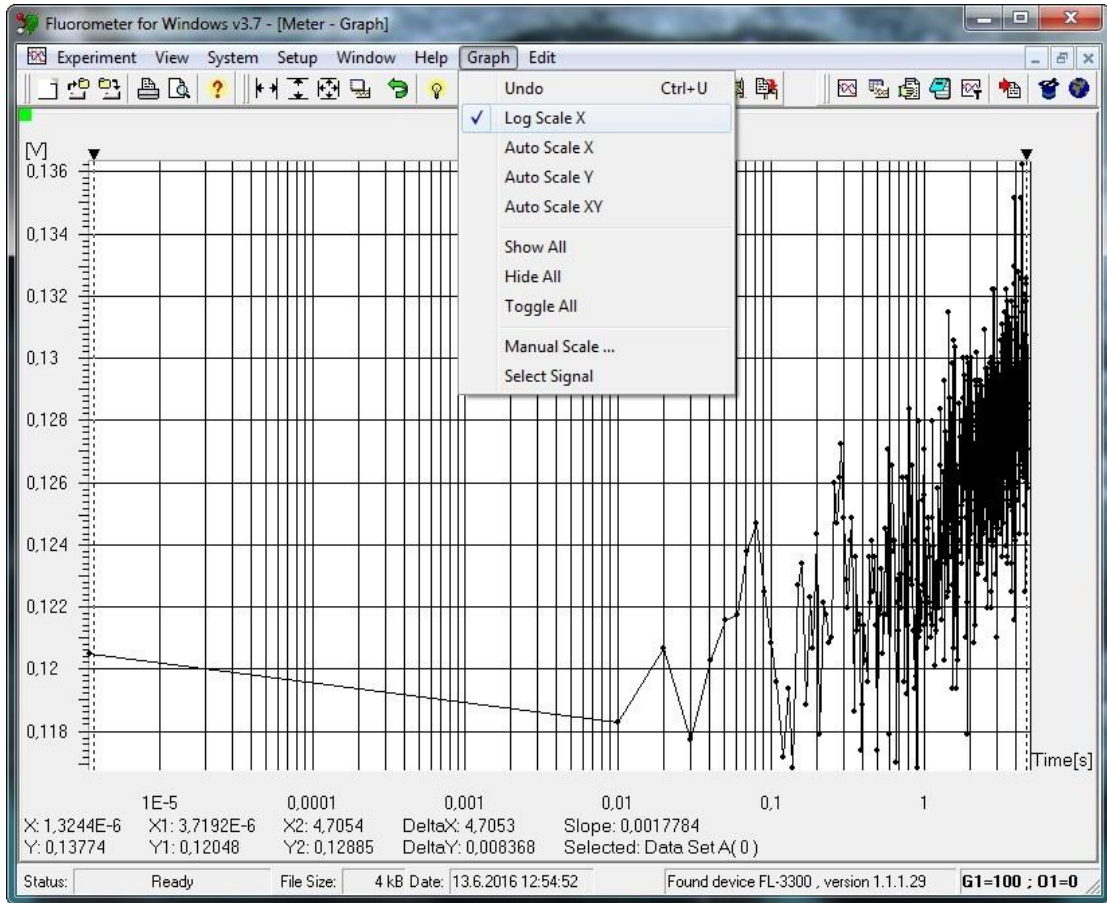
Fig. 23 Logarithmic scaling of X axis.

- There are two **cursors in the graph**. Their position X difference, Y difference and Slope are displayed below the graph. Required DataSet curve for cursor positions can be selected by icon Select Signal (Fig. 24) or left mouse button/ single click on the yellow rectangle with the name of the data set.
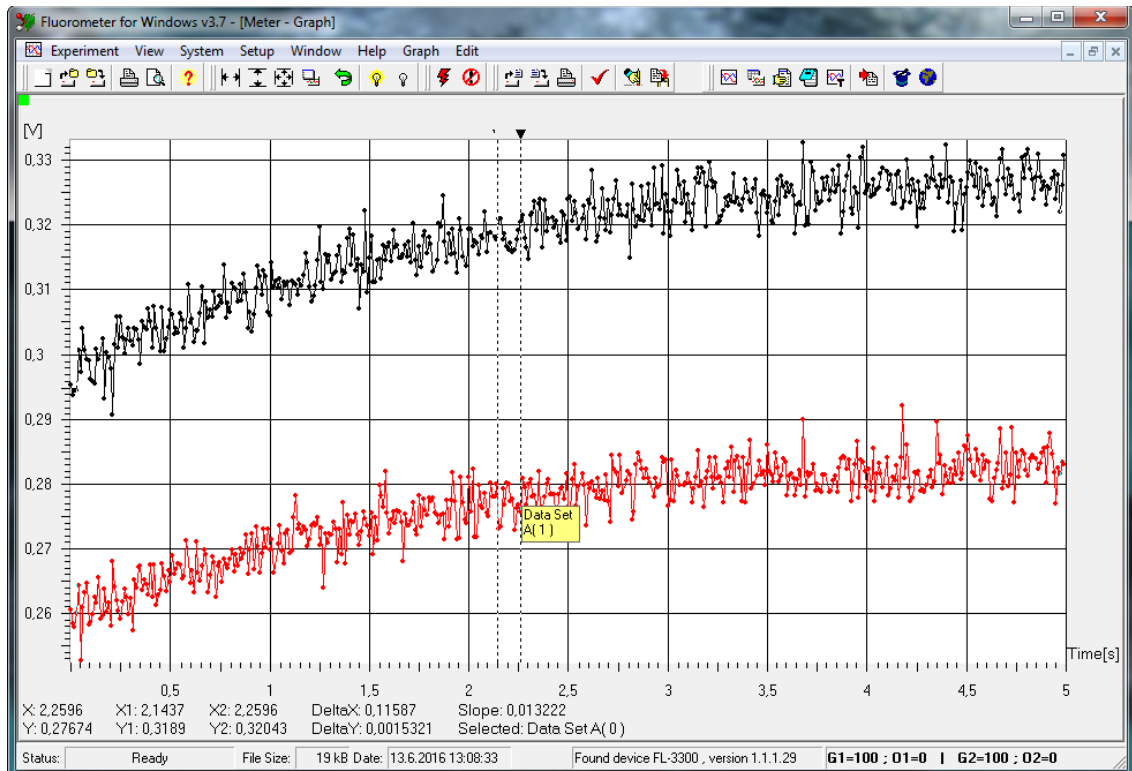
Fig. 24 Graph cursors.

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- All data displayed in the graph are shown in the table Graph > Select signal (Fig. 25). DataSet is selected by the left mouse button double-click. Name of the selected DataSet will appear under the graph.
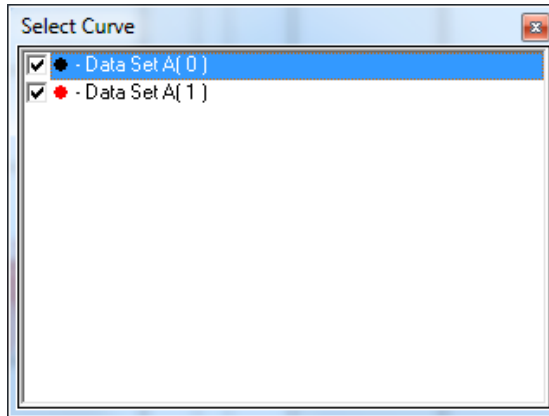
Fig. 25 Data displayed in the graph.

7.2.2   DATASET TABLE (F6)

The DataSet table window presents table of measured data. Each line in the chart corresponds to measuring with one of the two channels. In the case of experiment contains measurements on channel 1 and 2 at one time (such as measuring temperature on channel 2 and ChlA signal on channel 1), two lines are added into the table after the experiment is done – one line for the channel 1 (with default name DataSet A(x)), the second for channel 2 (DataSet B (x)).
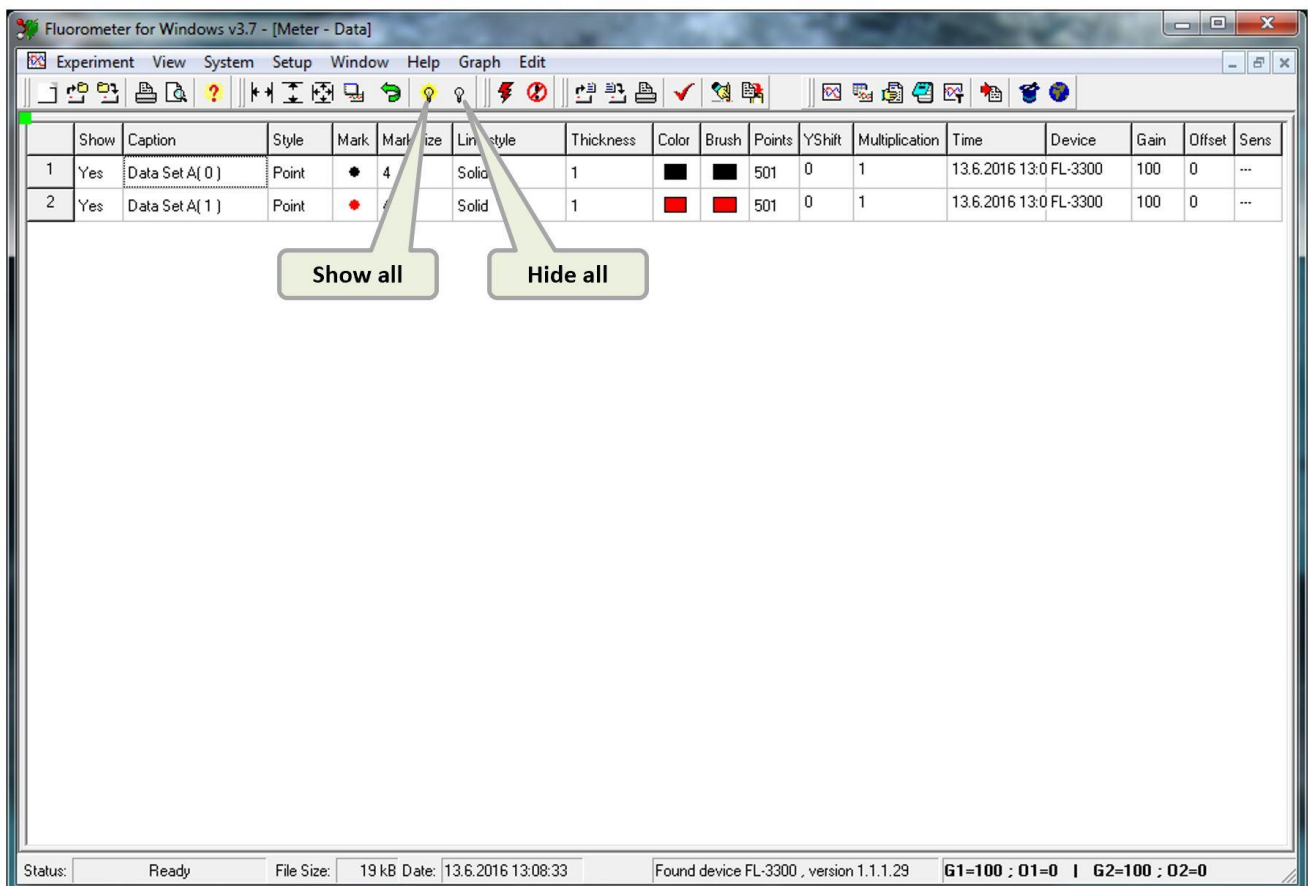
Fig. 26 DataSet table.

Individual DataSets can be deleted by pressing Delete key on a chosen line. This line contains the highlighted box. Confirmation dialog box must be accepted for applying this delete.

Maximum number of DataSets in the experiment is restricted in FluorWin.ini for 80.

Most of the parameters of DataSet can be edited by the left mouse double-click. Description of columns is as follows:

- **Show** is used to show/hide DataSet in the graph individually. State of this parameter in all DataSets may be changed by Show all icon or Hide all respectively.

- **Caption** enables name the DataSet by user defined name. Using own captions is recommended for better orientation in experiments. All ASCII characters are allowed including spaces, dots, commas, etc. Caption can have maximally 255 characters. To change the width of this column, drag the boundary on the right side of the Caption column heading until the column is the width that you want.

- **Style** is used to set Draw Style (Fig. 27).

Fig. 27 Draw style.

- **Mark** is used to change the look of the data marker style of the DataSet in graph (Fig. 28).

Fig. 28 Point style.

- **Mark Size** is used to change the data marker size of the DataSet in the graph.

- **Line Style** is used to change the line style of the DataSet in the graph (Fig. 29).

Fig. 29 Line style.

- **Thickness** is used to change the line weight of the DataSet in the graph.

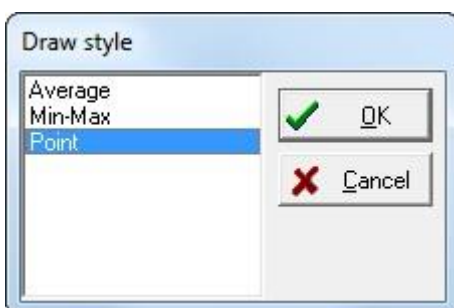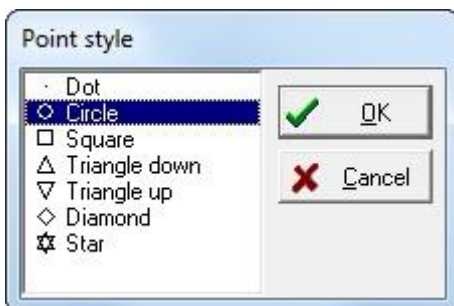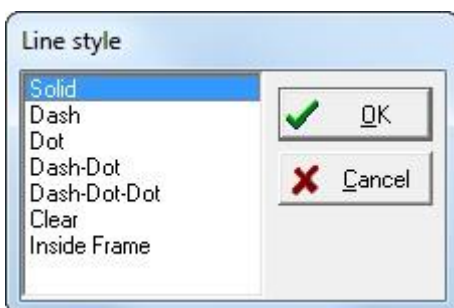- **Color** is used to change the line and data marker outline color of the DataSet in the graph (Fig. 30).

Fig. 30 Color of the DataSet.

- **Brush** is used to change the data marker fill color of the DataSet in the graph.

- **Points** informs about the number of sampled points in the DataSet. This column is a read-only parameter.

- **Yshift** and **Multiplication** can be edited with left mouse single-click. Changing of these columns enables linear arithmetic operation on acquired DataSet points. This function may be used for data calibration purposes or for manual normalization of the graph results. Comma style for decimal numbers is used from the Windows regional settings.

  Example of the normalization in Fmax time:

  Maximal y-axis coordinate (Fmax) of the measured curve is 0,4. "Blank" signal (the same experiment with cuvette with medium only inserted in the device) was -0,1 at this time (value taken from the graph with Yshift=0 and Multiplication = 1,0). As the result we want to have blank signal at 0.0 and Fmax at 1,0. Let's change the Multiplication = 1,0/(0,4 – (-0,1)) = 2. Than check the "blank" data point in Fmax time and it is now -0,2. Let's change the Yshift = -(-0,2) = 0,2. Data in the graph are now normalized with "blank" signal having 0.0 y-axis coordinate in Fmax time and sample signal having 1,0 y-axis coordinate in Fmax time. See the chapter about QA reoxidation for next explanation.

- **Time** refers to system time captured at time of experiment start. This parameter is read-only.

- **Device** refers to a type of device used for measuring the experiment. The actual device name is presented in Status Bar. This parameter is read-only.

- **Gain** refers to electronic gain of the detector. This parameter is read-only.

- **Offset** refers to electronic offset of the detector. This parameter is read-only.

- **Sens** refers to sensitivity of the detector. This function is not available for Fluorometer and "---" is displayed in this case. This parameter is read-only.

7.2.3 PROTOCOL (F7)

The Protocol window consists of tabs, which contain protocol scripts used for each experiment run.

- **Current tab** is the only editable tab and contains protocol script, which will be used for the next experiment start. Other tabs with already measured scripts are read-only tabs. These tabs are named according to the DataSet Caption in the DataSet table. This means that each DataSet is saved together with the protocol script, which defines experiment settings.

- Icon **Copy to current** may be used for repeating the previously measured experiment with the same settings.
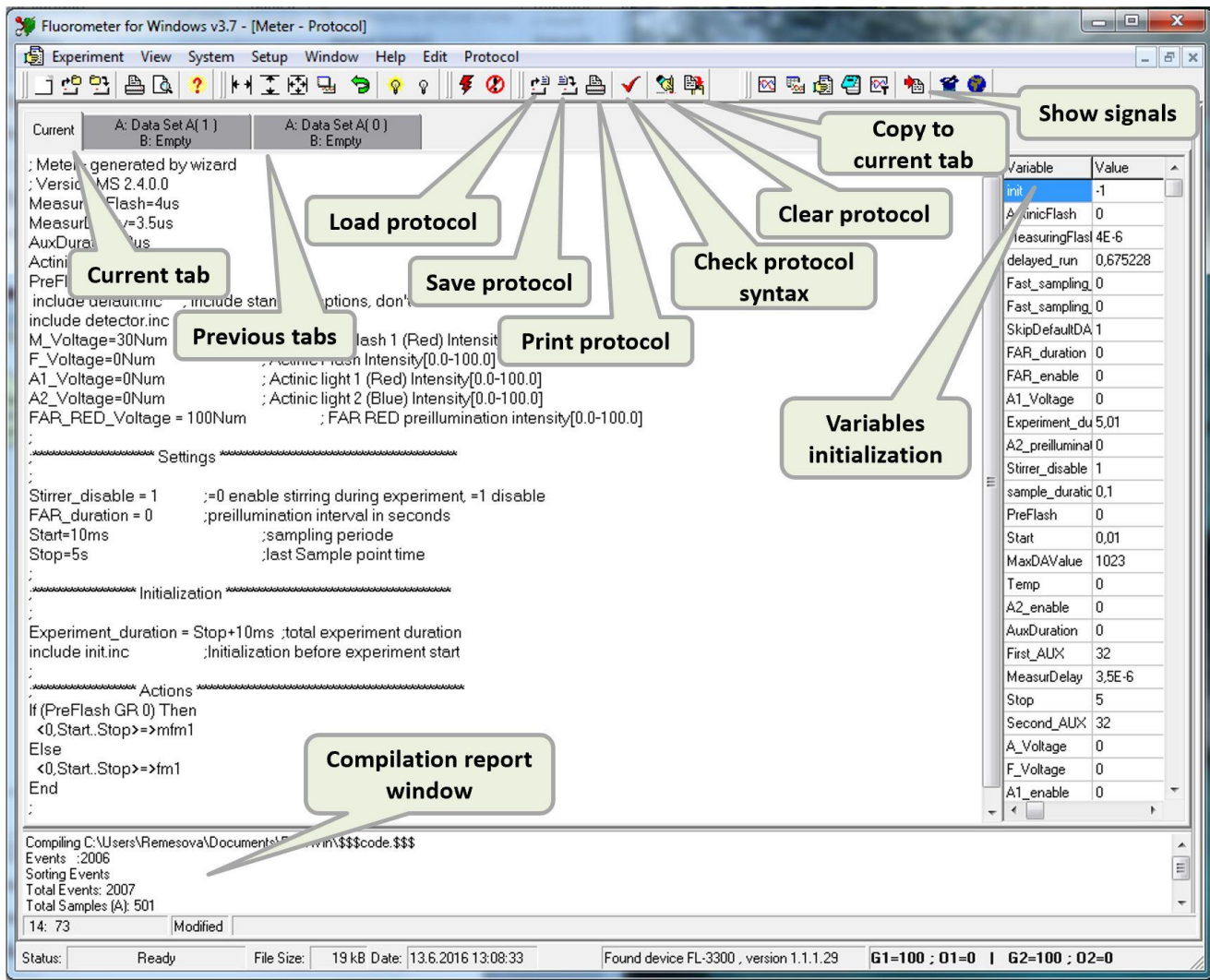
Fig. 31 Protocol window - options.

- Current protocol syntax can be saved as a text file with *.p extension by clicking the **Save protocol** icon.

- Saved protocols in *.p format can be subsequently **Load** and used for next measurement.

- Click on the **Clear Protocol** icon rewrites the Current protocol with header template. Individual items of this header are described in respective wizards.

- Click on the **Print Protocol** icon runs dialog box for printing the protocol script.

The protocol script consists of three mean parts: Protocol header, Experiment settings and Experiment body (Fig. 32). User can affect settings of the experiment by rewriting protocol script. Changes in the **Header** section and in the section **Settings** are allowed for users without protocol syntax knowledge. Editing in the **Experiment body** section is for more experienced users.

ⓘ	It is not recommended to make changes in Experiment body without prior study of protocol syntax.
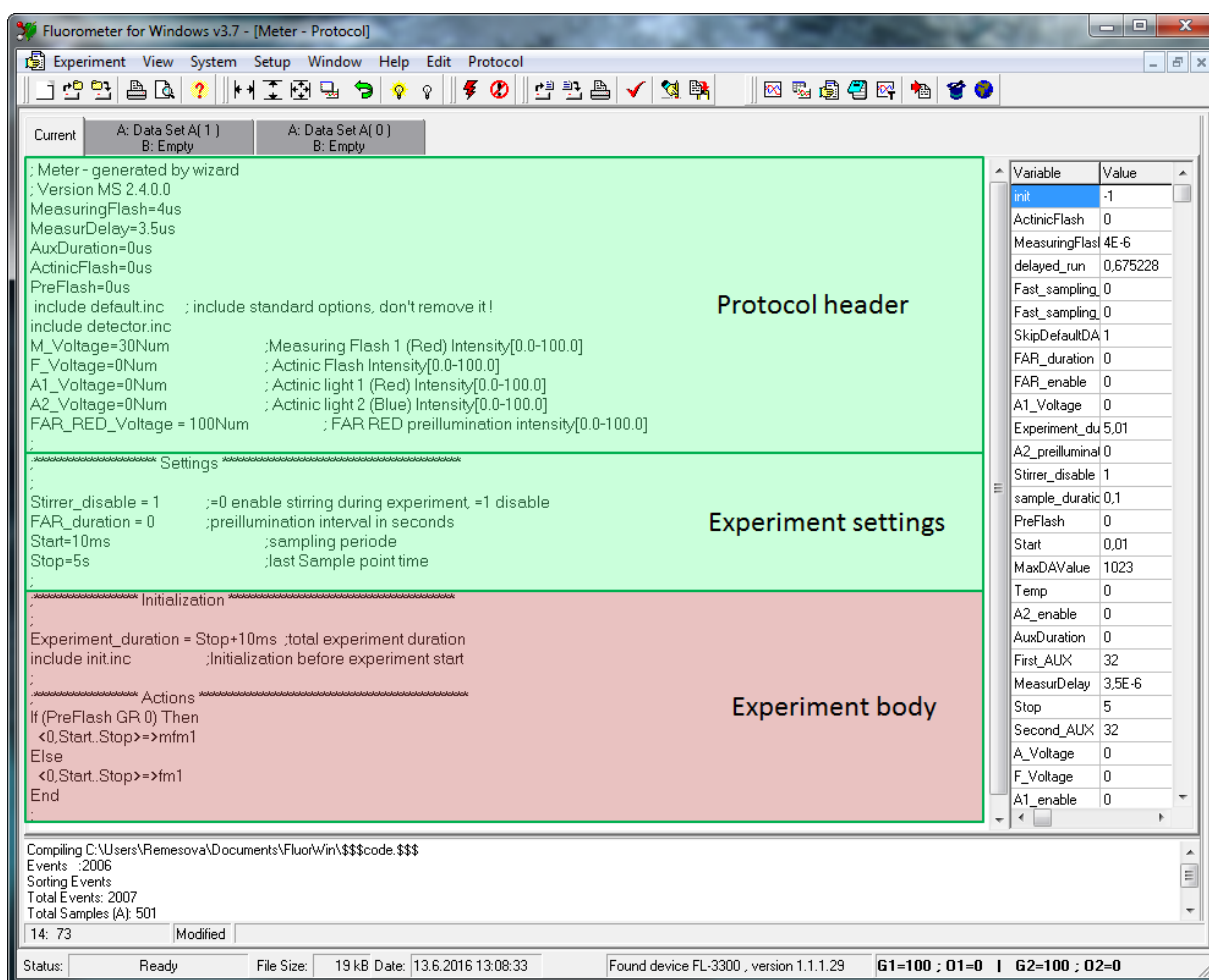
Fig. 32 Protocol description.

- Syntax of the Current protocol may be checked by the icon **Check syntax**. This executes the built in compilation program. Information about the compilation results are displayed in the **Compilation report window** below the Current tab. In the case the syntax check was successful, "**Done**" is displayed at the last line of this window. Warnings may announce non-critical errors in the protocol syntax. Compilation error is announced by Compilation Abort message window. Error line number together with the error description is presented in the report window than. Actual position (row: column) of the cursor in the Current protocol script is displayed at the bottom line of the Compilation report window.

  Successful compilation process initialize variables declared in Current protocol. Each variable name gets the initial value according to the protocol definitions. The list of all declared variables with initial values in actually displayed protocol script is displayed in the Variable initializations window.

- Each successfully compiled Current protocol script can be visualized using the **Show signals** icon (F4). Graph and DataSet table icons behave than in "Show signals" mode. DataSet table shows the list of accessible TTL signals, which can be displayed in the graph window (with the help of Graph icon now). Each row has predefined Caption with the respect of the protocol script commands (Fig. 33).
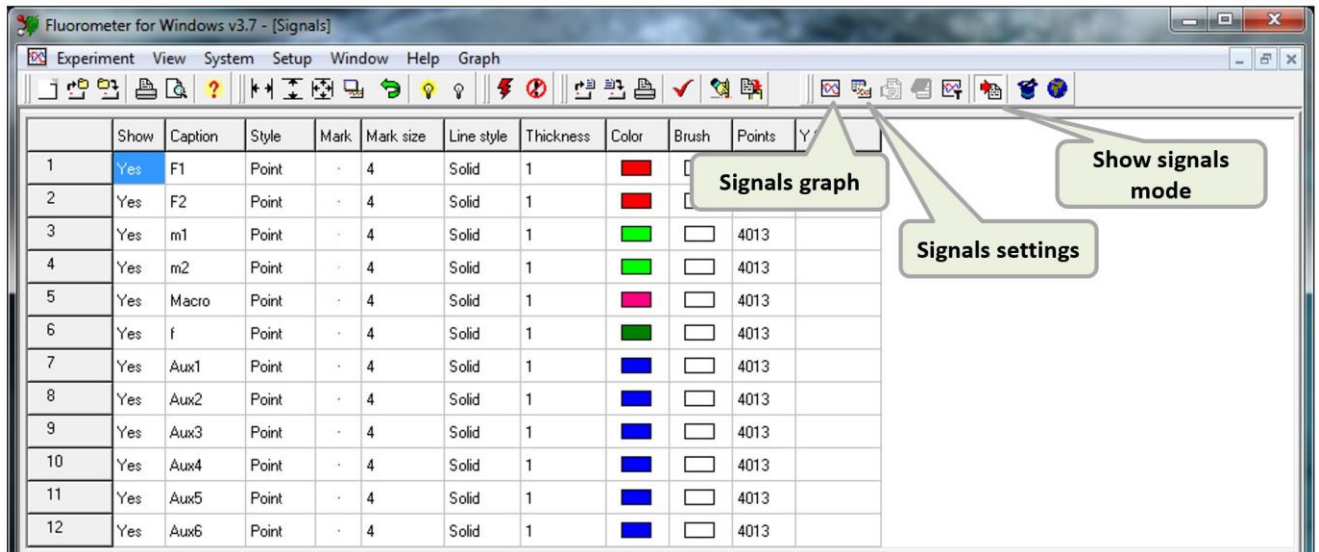
Fig. 33 Signals setting.

- **Showed signals:**

  Aux1 and Aux2 - actinic light A1 and A2 triggers

  Aux3 (A3 command in the protocol syntax) - Infra-Red LED trigger

  Aux4 and Aux5 - not used in predefined experiments

  Aux6 (A6) - "Stirrer disable" trigger

  Macro - trigger for the starting of Complicated actions

  m1 and m2 - trigger of the sampling on the channel 1 resp. channel 2

  F1 - Actinic Flash trigger

   f - measuring light 1 trigger

  F2 - measuring light 2 trigger

  All these signals may be displayed on the Graph at predefined position (Fig. 34). Caption of the TTL signal can be shown by holding the mouse cursor on the respective trigger signal line.
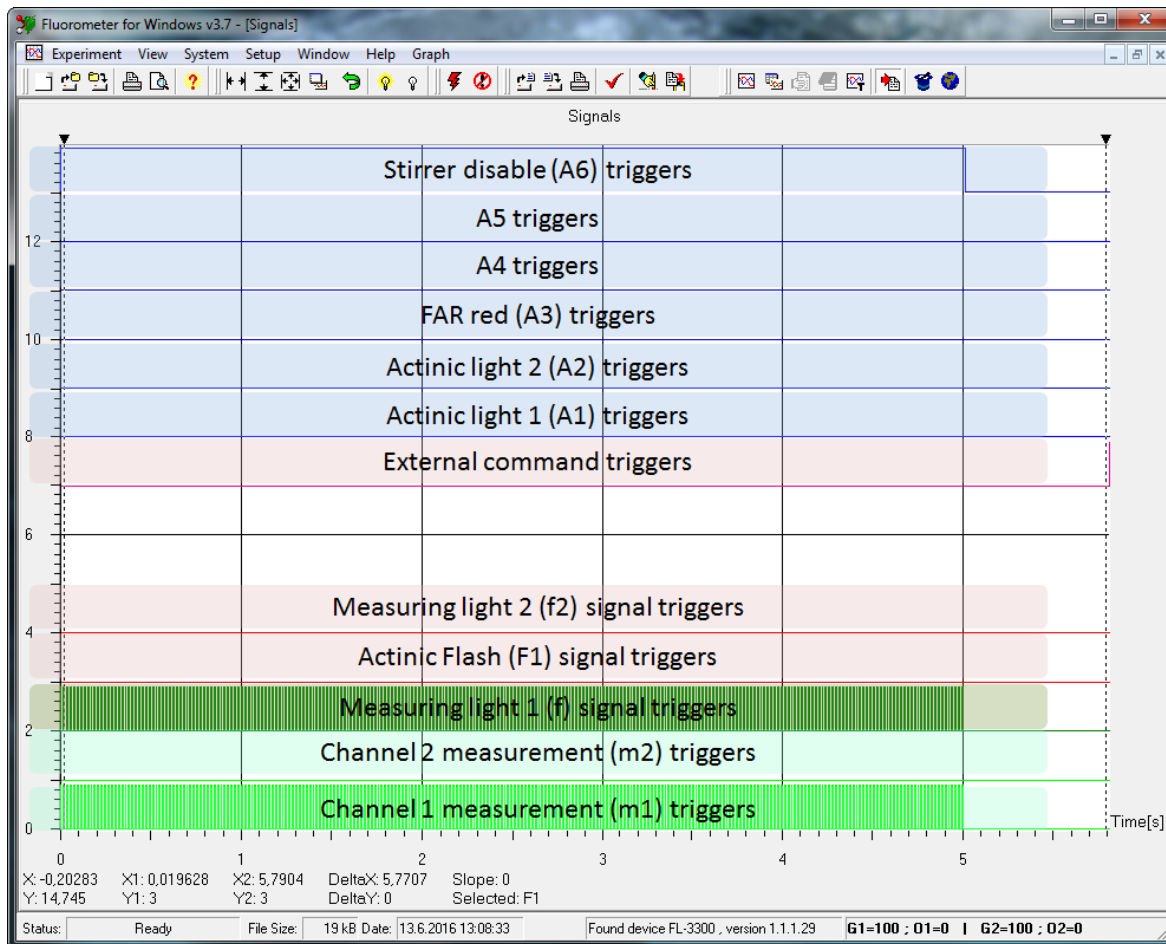
Fig. 34 Signals graph.

- Each line refers to one TTL signal which can be controlled in the protocol script by appropriate command. The left mouse button can be used to zoom in the Signals graph window. The detail shows timing of a single mfm1sub command.

  Example on PAM based instantaneous fluorescence measurement:

  At the time 1 s, the first measurement (background) is executed. Signal is sampled just on the rising edge of m1 trigger. With a delay of 10 µs, the measuring flash lasting 4 µs is triggered. 3.5 µs later, the second measurement (fluorescence + background) is executed. The two measurements are subtracted to get the fluorescence signal free of the background.

- Left mouse click on the Show signal icon **exits the "Show signal"** mode.

7.2.4   NOTES (F8)

The **Notes** window allows to make notes about experiments and to save them with the experiment data (Fig. 35). This window works as a simple text processor. The Title of the Experiment specified in the Notes shows up also in the Graph window as a Title of the graph.

Fig. 35 Experiment notes.

7.2.5 T-GRAPH WINDOW

The T-Graph window is a graph window used for presenting results of **Thermoluminescence** experiment (Fig. 36).

Fig. 36 T-graph.

Please note that T-graph option is not active for Fluorometer devices.

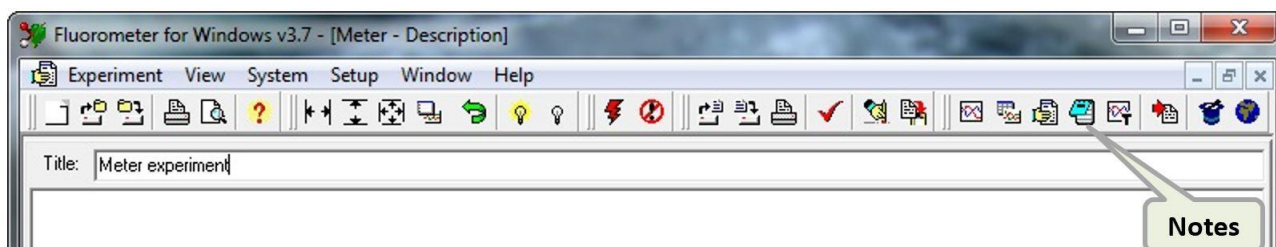### 7.2.6 ENVIRONMENT OPTIONS

Setting the Fluorwin program behavior may be affected in the Environment option menu. User can find it in Setup>Environment options menu (Fig. 37).

Fig. 37 Environment options.

Menu option:

- **Create blank experiment on startup** – after starting FluorWin program a new experiment is automatically opened with protocol script header. Because of this header doesn't contain any actions, it is called blank experiment. User must write Load a protocol or write its own for starting the experiment.

- **Show wizard on startup** – after starting FluorWin program a Wizard window automatically opens and user can choose experiment to measure.

- **Auto scale after measurement** – after the measurement is done and data are downloaded from the FL3500 control unit, automatic XY scaling of the result is processed on the graph.

- **Exclude sets without data** – in case of there hasn't been defined measurement on one of the channels, data from this channel are not presented in the DataSet table. This setting is default checked.

- **Auto reconnect control unit** – FluorWin program tries to connect with the Control unit permanently for checking whether it is connected to a PC.

- **Confirm actions** – prevents possible loss of unsaved data. If checked, an information window asking about experiment saving displays before experiment is closed. Checking of this checkbox is recommended.

- **Data checklist box** – settings of the DataSet printing options (This print can be found in the Experiment/Print menu).

- **Print Notes** – checking of this checkbox sets the printing of text array from Notes window.

- **Ordering windows at startup** – default mode for organizing windows after program starts.
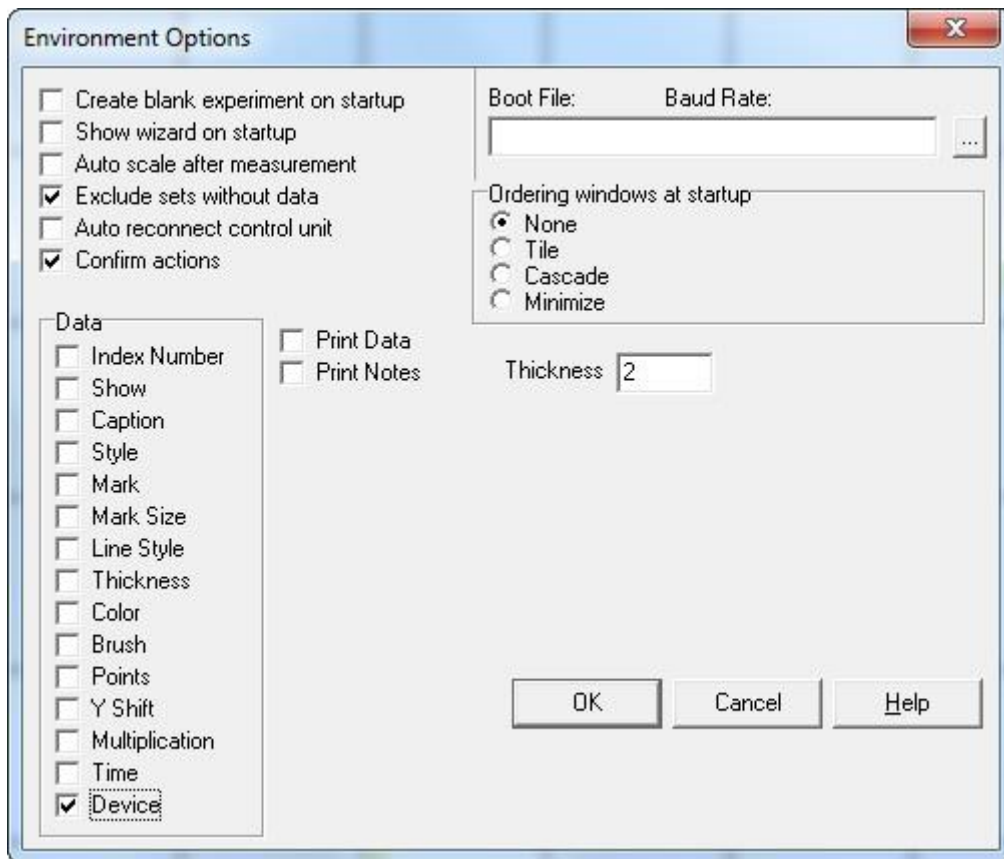
Fig. 38 Environment option menu.

7.3   DETECTOR SETTING

System Monitor

This window serves for setting of detector or checking the detector functionality. It can be accessed via Navigator, via the menu System > System monitor item or using Shortcut Ctrl+M.

Signal acquired by detectors connected to Channel 1 and Channel 2 is presented on indicators. Sampling period is set to 1 s. In case the Measuring unit works correctly and is properly connected to Channel 1 connector, noise level values around 20 mV are presented. Checkbox *Measuring Flash* enables synchronized trigger of the Measuring flash 1 pulse. Intensity of this pulse must be set in the *Test* Keys Window. Indicator shows than signal acquired during this Measuring pulse (named as Ft value in the literature).

Measuring unit of Standard and Fast version of the FL6000 is always connected to Channel 1. Left part of the System Monitor window belongs to Channel 1 (Fig. 39). Channel 2 serves for connecting of additional devices, e.g. Thermoregulator TR2000. Channel 2 is presented in the right part of the System Monitor window, the fuction Gain and Offset is no active for Channel 2.
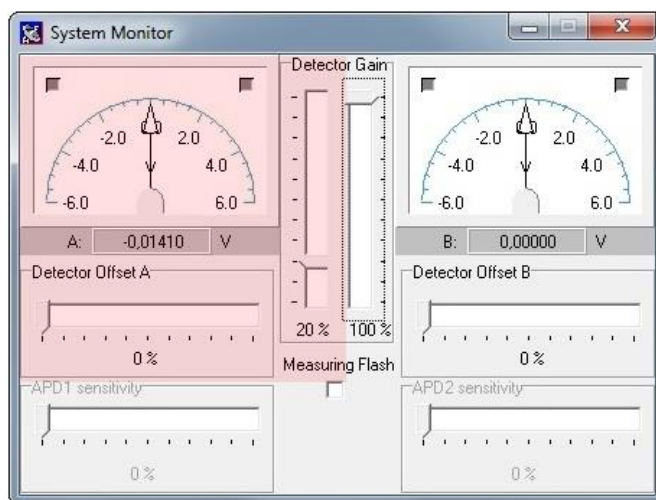
Fig. 39 System Monitor window (highlighted part belongs to Measuring unit detector).

Gain

Gain determines amplification of a photocurrent elicited by luminescent photons falling to detector. Gain moves in the range from 1 to 40 (Gain = 0 % => no gain, Gain = 100 % => gain approximately 40 times, **Error! Reference source not found.**). See the table in Fig. 41 for the gain and time response dependence.

(i)	Please note that Gain setting in percentage is not linear with corresponding voltage on the detector (see Y axis in Fig. 40)

Fig. 40 Effect of different gain setting on fluorescence signal.

Gain (%)	Time response 90% (us)	Signal
0	0,7	1,0
5	0,7	2,2
10	0,8	4,3
20	1	8,5
30	1,4	12,8
40	1,8	16,9
50	2,1	21,3
60	2,5	25,4
70	2,7	29,7
80	3	33,9
90	3,3	38,0
100	3,5	42,3
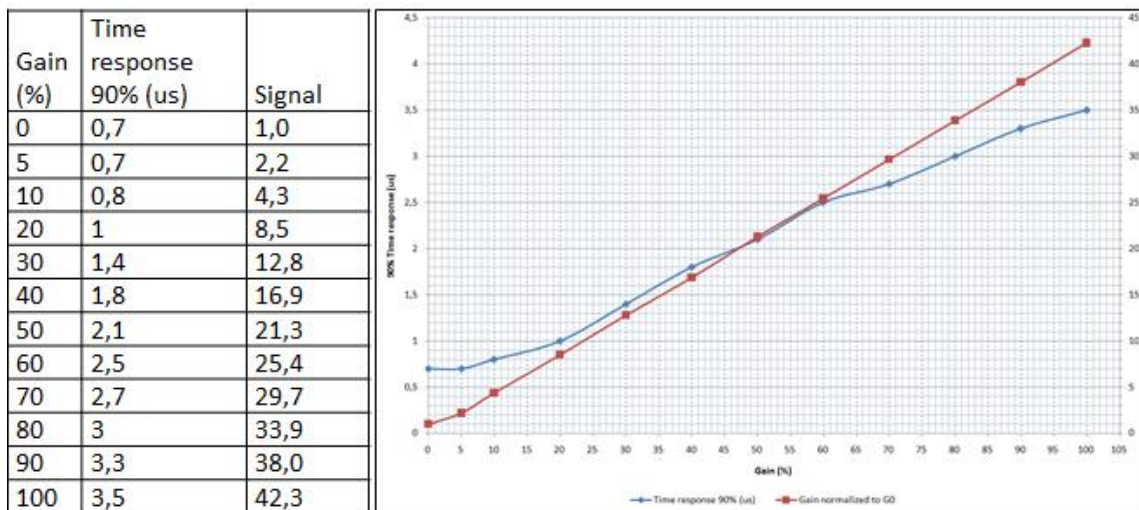
Fig. 41 Time response of the fast detector.

Offset

Offset moves signal electronically towards minus values and increase the dynamic range of the measurement. It does not affect gain. The detector range is from -5.99 V to +5.99 V. Converter resolution is 16 bits.
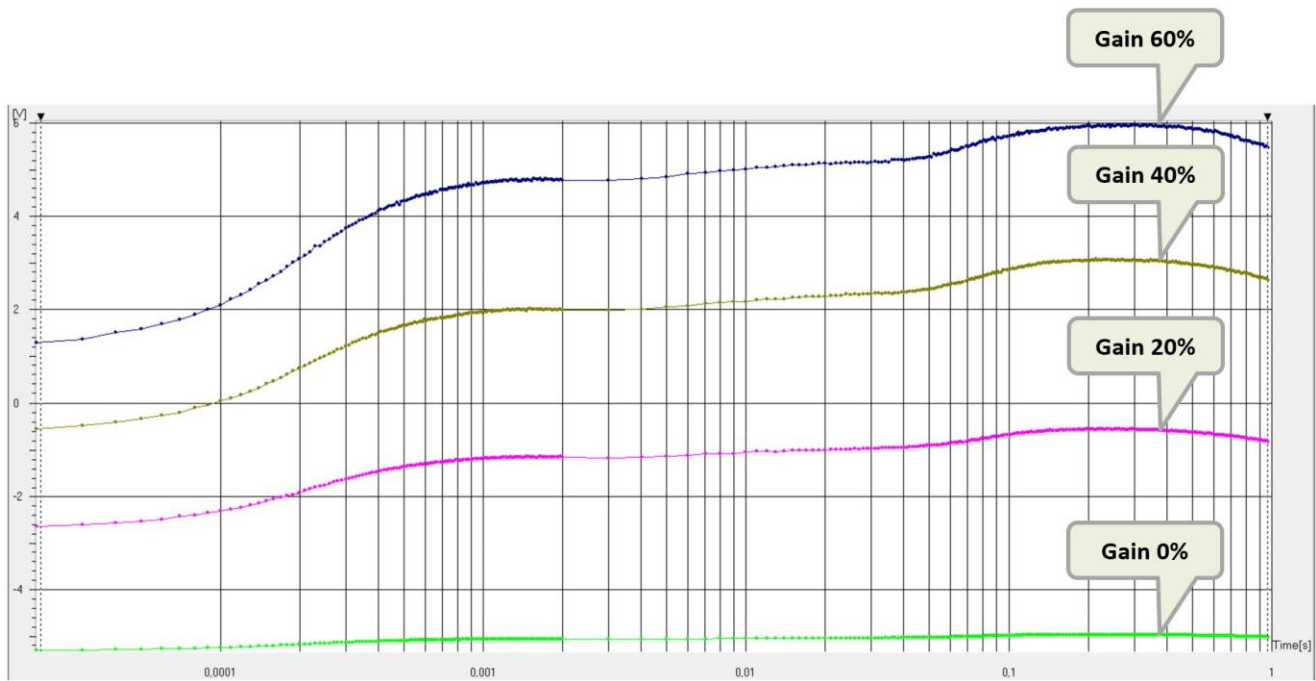
Do not use the limit value -5.999 and +5,999 V, because this setting causes oversaturation of the detector, so the data will be out of the measurement range and will be lost. These oversaturated points are displayed as maximal measurable values +5.999 V or -5.999 V in the graph. Using mfmsub command (Pre-Flash parameter is set) induce presenting the already subtracted value on graph. Wrong value is displayed than if the second sample is oversaturated. In such case, user is recommended to lower electrical Gain of the detector (*System/System Monitor*), or to decrease detector Offset (*System/System monitor*) leading to increase in dynamic scope of the detector.
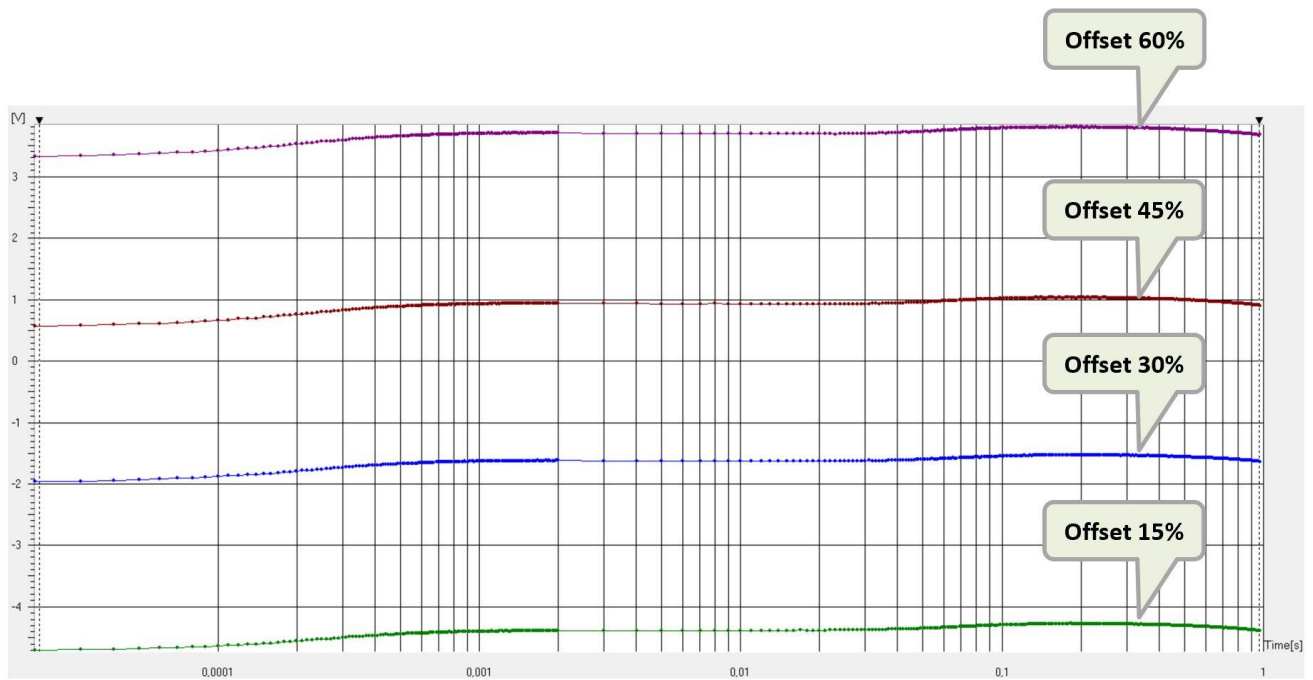
Fig. 42 Effect of different offset setting on fluorescence signal.

ℹ️	Please note that last Gain and Offset values pre-set via the **System Monitor** or **touch screen display** are stored in FluorWin memory. They are recovered after Software start/restart. System Monitor setting is not changed by the protocol. This setting must be reconfigured again with loading the new protocol based on species and various content of chlorophyll.
⚠️	Please note that the detector setting can be done also via touch screen display. The setting is linked between the touch screen and the software. It is recommended to use the touch screen display for apply **Auto offset function** (available only on touch screen), which makes the detector setting easier.

7.4   PREDEFINED PROTOCOLS - WIZARDS

7.4.1   METER

The Meter protocol serves for measuring of **instantaneous chlorophyll fluorescence** (ChlF) in dark adapted (F_0) or light adapted state (F_t). The ChlF in this protocol is measured using very weak and short (typically 5 – 50 µs) measuring light pulses, that do not induce the electron transport in Photosystem II (PSII) and do not affect the PSII photochemistry. The ChlF signal is proportional to measuring light power (Fig. 43).

Desired result of this measurement is **flat line** indicating the current level of ChlF (Fig. 43 black line). High intensity of measuring light causes undesirable "actinic affect" (Fig. 43 blue and red line). It means that the measuring light starts the electron transport in PSII. In this case it is recommended to lower the light intensity.

|	
⚠️	In case of "actinic effect" please lower the measuring light intensity or shorten the pulses duration. The light intensity and duration should be optimized for each single organism and measurement.
ℹ️	The Meter protocol is among others suitable for determination of optimal intensity of measuring light before running other protocols, where this light is used (see more in chapter 5). Suboptimal setting of measuring light strongly affects experimental results.
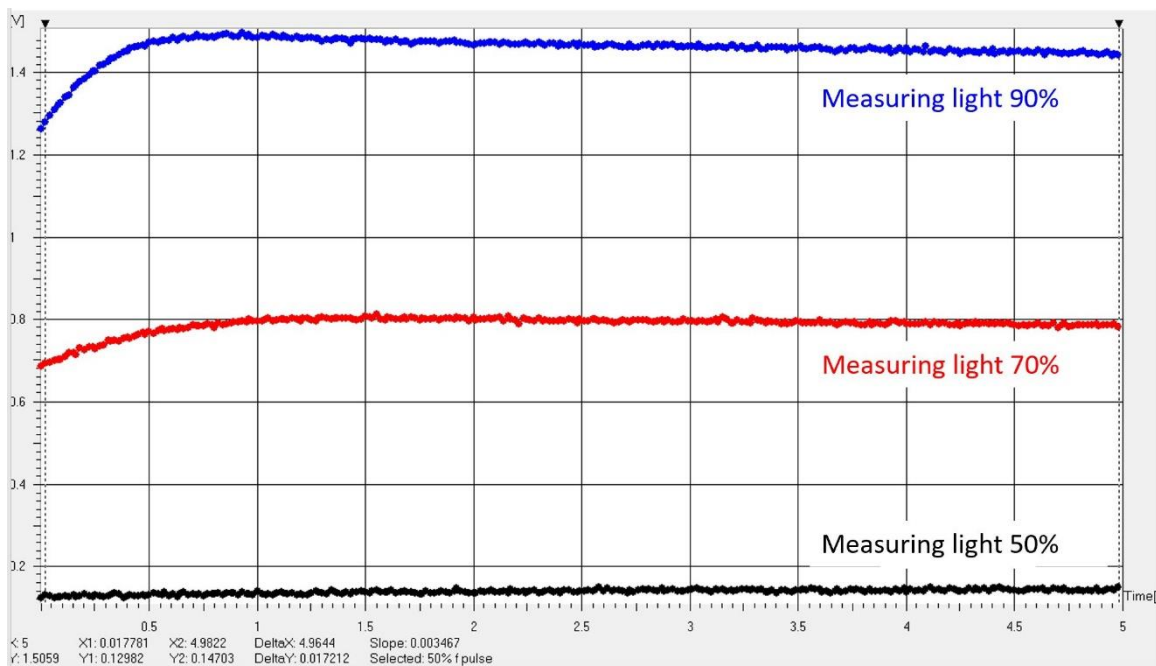
Fig. 43 Meter measurement on Chlorella vulgaris.

Meter Wizard

The wizard enables to set parameters for the measurement. The most important are color of measuring light (Use Measuring Flashes 1 or 2), measuring light voltage and duration. The protocol is created based on values in the wizard.

Meter	×

Parameters

Experiment duration (s) `5` Duration of the measurement

☑ Use Measuring Flashes 1 Selection of measuring light color (usually 1 – red and 2 – blue)

☐ Use Measuring Flashes 2

M. Flash Voltage (%) `30` Measuring light intensity

M. Flash Duration (us) `4` Duration of measuring light flashes

M. Delay (us) `3,5` Delay between measuring flash initiation and the A/D integration

M. Flash Period (ms) `10` Time period between measuring light flashes

Pre-Flash Measurement (us) `0` Background measurement before each measuring light flash (Fig. 44), if 0 no background is measured

Optional parameters

FAR-RED Preillumination (s) `0` Illumination using the optional Infra-Red LED unit before the measurement

☐ Measure temperature on channel 2 Activation of sample temperature measurement (for TR only)

☑ Stirrer OFF during Experiment Disabling of the stirrer during the measurement

OK   Cancel
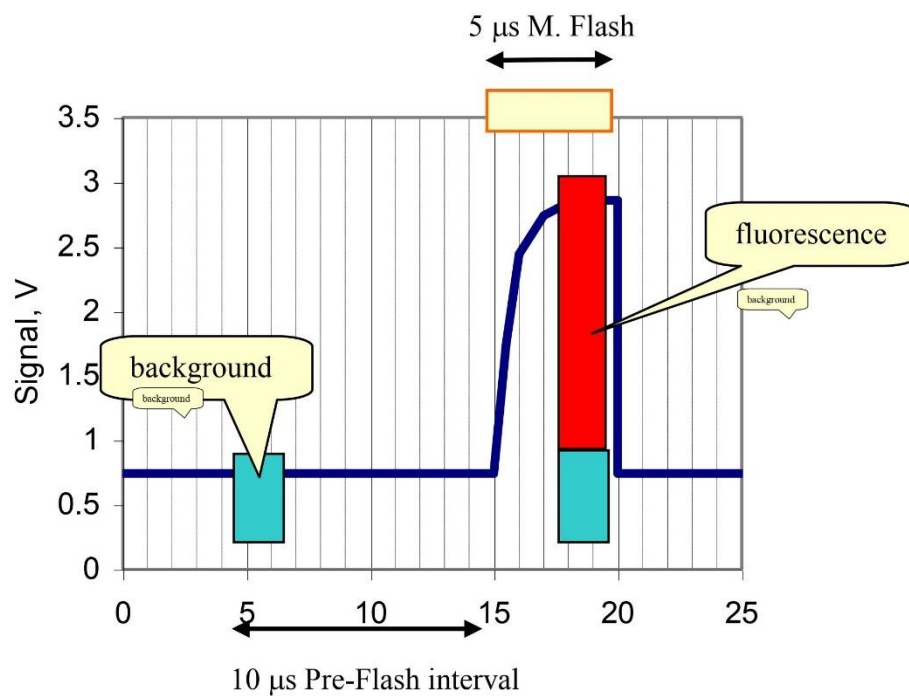
Fig. 44 Illustration of Pre-flash measurement set to 10 μs.

7.4.2   QA REOXIDATION

The protocol records electron transport from primary electron acceptor Q_A to secondary electron acceptor Q_B. At first F_0 is measured, executes a single-turnover flash to reduce Q_A acceptor and follows the subsequent decline of ChlF that reflects the reoxidation kinetics. Fig. 45 shows typical Q_A reoxidation experiment measured on *Chlorella vulgaris* control sample and *Chlorella vulgaris* treated with DCMU. DCMU inhibits the electron flow from photosystem II to plastoquinone and thus reduces the ability of the photosynthetic organism to turn light energy into chemical energy.

Fig. 45 Q_A reoxidation kinetic measured on Chlorella vulgaris control (black) vs. DCMU treated (blue).

QA reoxidation Wizard

Experiment Duration defines how long the measurement will last.
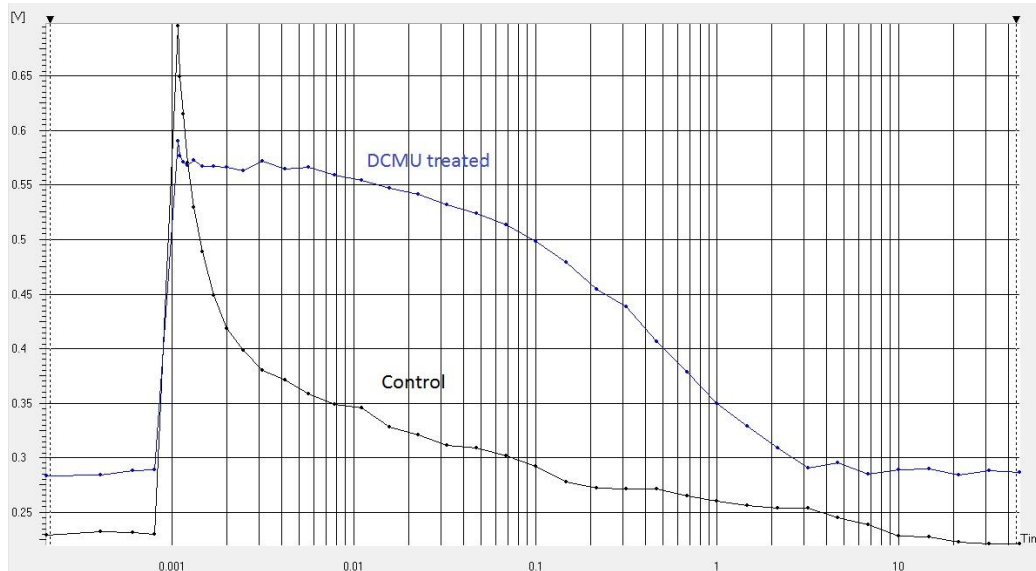
No. DataPoints / Decade: The reoxidation is usually a multiexponential process with components as fast as hundreds of microseconds and as slow as many seconds. This dynamic range is well covered by logarithmic timing series that is defined by a constant number of datapoints per decade. This series always includes timing in decades (10i) and a constant number of measurements in each decade. The data looks equidistant on logarithmic time-axis. For example: with 4 datapoints per decade, the series may look like ….1, 1.78, 3.16, 5.62, 10, 17.8, 31.6, 56.2, 100 …. (= 10 0, 10 1 / 4, 10 2 / 4, 10 3 / 4, 10 4 / 4 ….)

FirstDataPoint After F: defines an approximate starting point for the logarithmic series used to measure reoxidation. The default value of 50 µs means that the first measurement will not be taken earlier than 50 µs after the Actinic Flash trigger. In case that 4 datapoints/decade are required, the first datapoint will be taken 56.2µs after the Actinic Flash trigger. Please, note that for a 30µs-long Actinic Flash the interval between its end and the first measuring flash is only ca. 26.2 µs.

A.Flash Voltage set the intensity of the Actinic saturating light.

A.Flash Duration defines the duration of the Actinic saturating light.

Use Measuring Flashes 1 or 2 selects measuring light color (usually 1 – red and 2 – blue).

M.Flash Voltage defines the relative power of the Measuring Flashes. Use the Meter protocol to determine the optimal M.Flash Voltage. Care must be taken to avoid actinic effects of the Measuring Flashes.

Pre-Flash Measurement has the same function as in the Meter Wizard. Here it may be considered for reduction of flash artefacts in case of long-tail flashes.

Use **Pre-flash measurement** (1 µs recommended) in case of saturating flash artefacts were detected by baseline measurement. This phenomenon is common for standard version FL 6000-S, which is equipped with high sensitivity detector.

7.4.3   KAUTSKY EFFECT

Kautsky effect is phenomenon concerning the characteristic behaviour of ChlF, when dark-adapted photosynthetic organism is exposed to light. The Protocol measures F_o and switches on Actinic Light that elicits the ChlF induction (Fig. 46). The induction is displayed in the logarithmic timing. The fast processes at the beginning of the induction are measured in detail while the later slower processes are measured much less frequently.
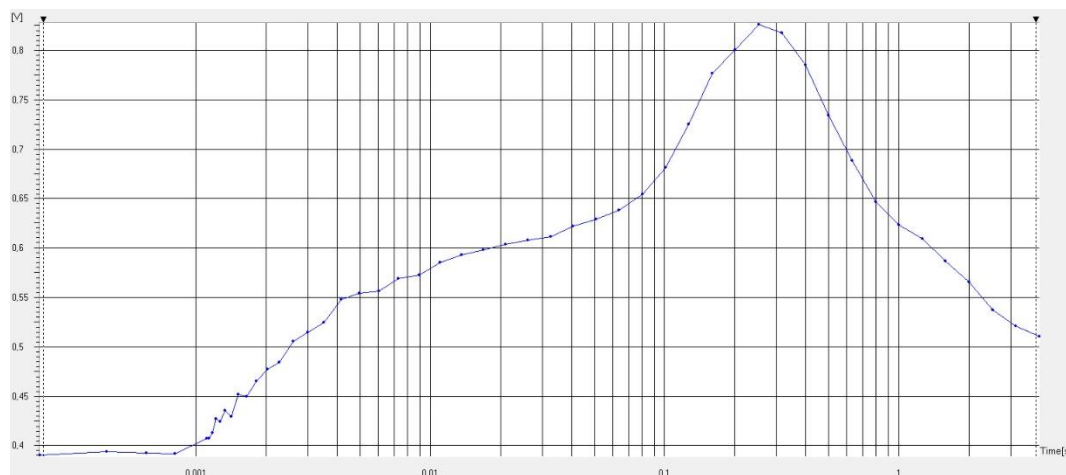

Fig. 46 Kautsky effect measured on Chlamydomonas reinhardtii culture.

Kautsky effect Wirazd

Most of the parameters have the same meaning as in the QA- Wizard window (see chapter 7.4.2). Actinic Light Interval can be shorter than Experiment duration when dark relaxation after the induction is studied.

7.4.4   QUENCHING ANALYSIS

The analysis of fluorescence quenching is usually based on a comparison of the maximal fluorescence emission (F_{max}, F_{max}') before and during an exposure to Actinic Light. The F_{max} (F_{max}') is measured as fluorescence emission that corresponds to the reduction of the primary quinone acceptor in all PSII reaction centers. This can be achieved in several ways:

a)   With added herbicide (e.g. DCMU), the F_{max}DCMU can be reached even in moderate light or in a short flash. The PSII reaction centers are in the state [QA- / herbicide / PQ oxidized state / S-states advanced by 1].

b)   In a single-turnover saturating flash (F_{max}flash), the PSII reaction centers are in the state [QA- / PQoxidized / S-states advanced by 1].

c)   Frequently a long pulse (F_{max}long) of continuous light is used to bring the reaction centers to the state [QA- / PQreduced state / S-states scrambled].

The F_{max}DCMU, F_{max}long and F_{max}flash parameters are measured in different states of the photosynthetic apparatus and are not necessarily equal. It is up to the user to decide on which parameter the quenching analysis must be based.

The Quenching analysis Protocol is based on the Kautsky effect Protocol. The difference is only in added F_{max} (F_{max}') measurements. Prior to the Actinic Light period, the F_{max} is measured in a short series of single-turnover saturating flashes. The measurement of F_{max}' is repeated during the Actinic Light period when various quenching mechanisms are becoming effective. Fig. 47 shows typical shape of the Quenching curve measured on green alga Chlamydomonas reinhardtii. For detailed timing description of the protocol see Fig. 48.
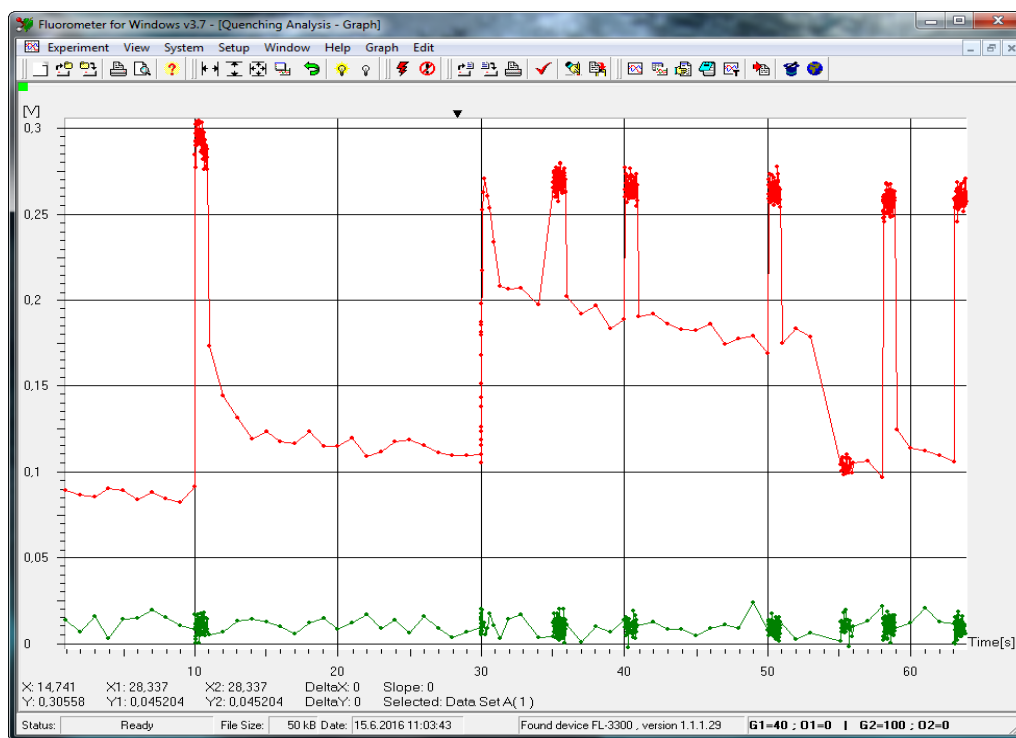

Fig. 47 Quenching analysis measured on Chlamydomonas reinhardtii culture (red) and base line (green).

Quenching analysis Wizard

The Wizard definition window allows to define experimental parameters similar to the other Wizard windows. In addition to parameters of the Kautsky effect Wizard, the Actinic Flash power is requested for the F_{max} measurements.

41

Quenching Analysis

Parameters

Act. Light Voltage (%)	15
Sat. Pulse Voltage (%)	80
Actinic light exposure duration (s)	30
Dark relaxation duration (s)	20
☑ Use Measuring Flashes 1	
☐ Use Measuring Flashes 2	
M. Flash Voltage (%)	20

Optional parameters

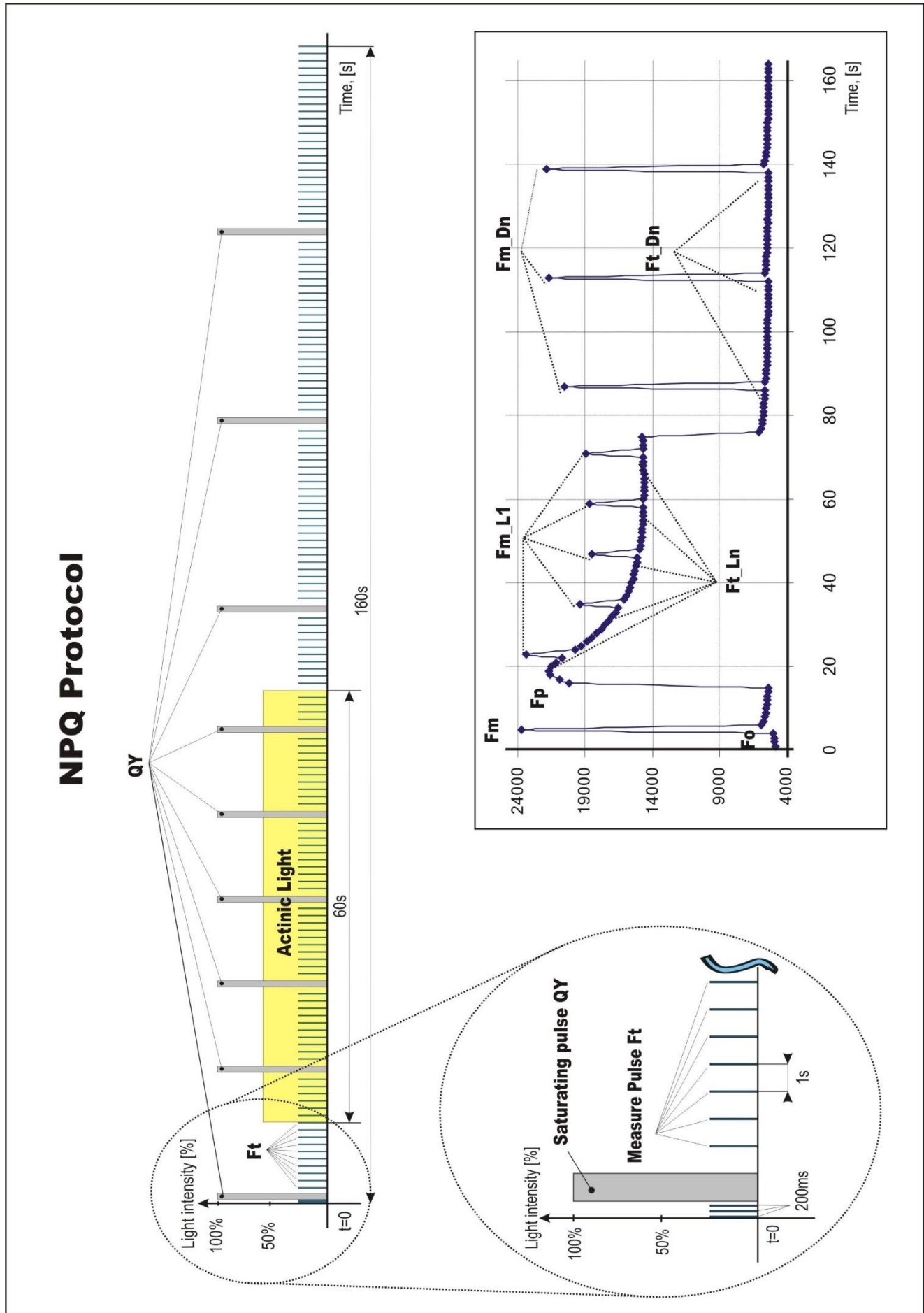|	
Act. Light 2 Voltage (%)	0
Sat. Pulse 2 Voltage (%)	0
FAR-RED Preillumination (s)	0
☑ Use FAR-RED for Fo_Lss	
☐ Measure temperature on channel 2	
☑ Stirrer OFF during experiment	

OK   Cancel

Fig. 48 Quenching analysis protocol schema.

7.4.5 S-STATES

The fluorescence emission is dependent not only on Q_A redox state but, among other factors, also on the state of the Oxygen-Evolution Complex (S-states). There are five S-states: $S_0 - S_4$. A dark adapted organism has the PSII RCs statistically in a mixture of S_0 and S_1 states (S_0 / S_1). By Actinic Flashes the S-states are advanced through S_1 / S_2 (1st flash), S_2 / S_3 (2nd flash), $S_3 / S_4 \rightarrow S_0$ (3rd flash), $S_4 \rightarrow S_0 / S_1$ (4th flash). This cycle explains periodicity of four in the oxygen evolution. A corresponding periodicity is observed also in the fluorescence quantum yield.

The F_0 fluorescence is measured during the first 1 ms. 10 Actinic Flashes are fired 100 ms apart to advance the S-states. After each Actinic Flash, the fluorescence decay is measured. The fluorescence emission at the end of each 100 ms period is somewhat higher than F_0 because a fraction of PSII reaction centers is unable to re-oxidize completely Q_{A-} (non-periodic difference) and because the F_0 quantum yield is S-states dependent. The S-states modulate the signal with the periodicity of 4 (Fig. 49).
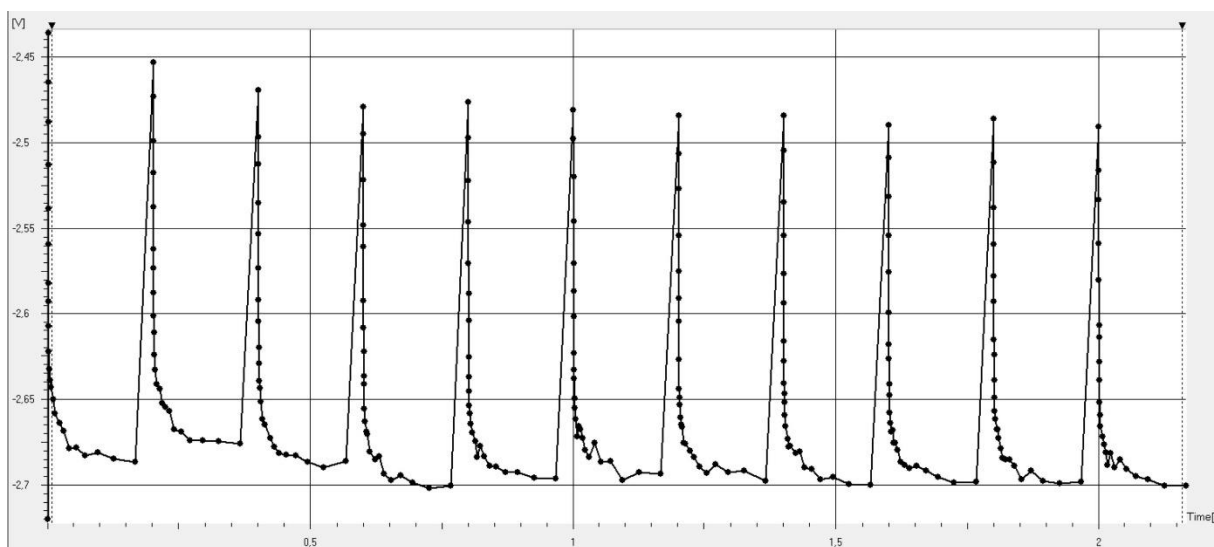

Fig. 49 S-states protocol measured on Chlorella vulgaris culture.

S-states Wizard

7.4.6   FLASH FLUORESCENCE INDUCTION

This protocol is based on measurement of **ChlF induction during a single-turnover**, saturating flash (Fig. 50). It allows investigation of Light harvesting system of PSII, specifically antenna size and heterogeneity. Details are described in Nedbal et al. J. Photochem. Photobiol. B: Biol. 48 (1999)

(i)	Please note that only Fluorometer FL6000-F with time resolution up to 1 µs enables measurement of Flash Fluorescence Induction protocol.
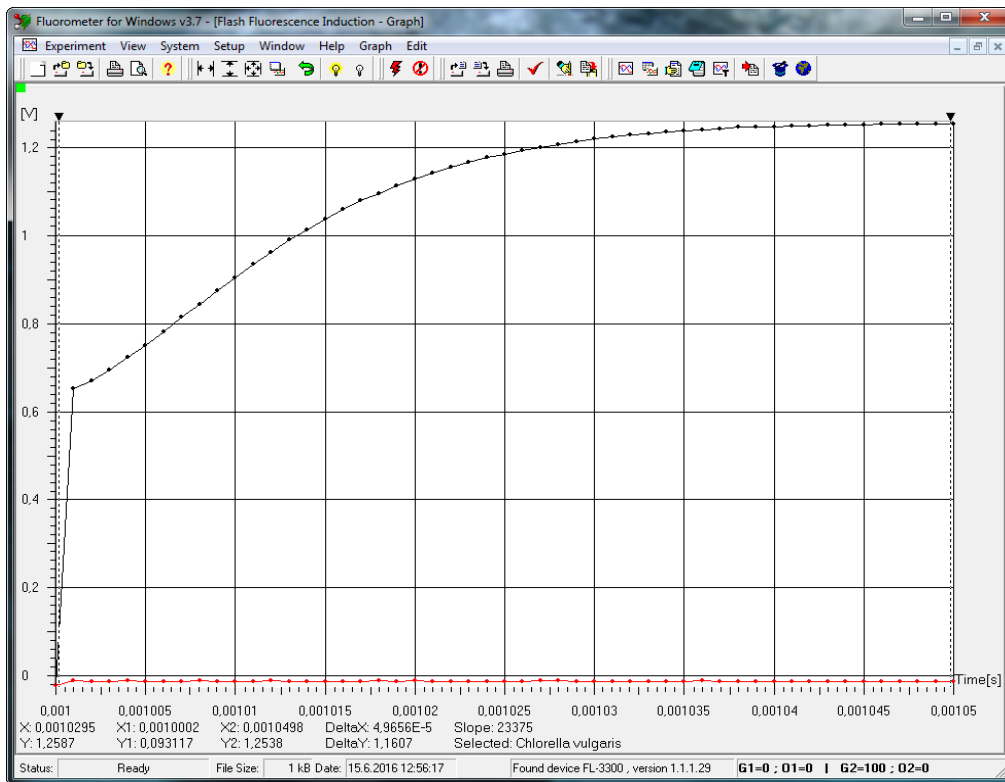
Fig. 50 Flash fluorescence induction measured on Chlamydomonas reinhardtii.

Flash fluorescence induction Wizard

7.4.7   OJIP

This type of experiment offers the possibility to capture rapid fluorescence transient – OJIP, which occurs during exposure of photosynthesizing organisms to high irradiance (Fig. 51). O, J, I, and P steps correspond to the redox states of PS II and PS I and to the efficiencies of electron transfer through the intersystem chain to the end electron acceptors at the PSI acceptor side (Strasser et al., 2000, 2004).

Fig. 51 OJIP curve measured on Chlamydomonas reinhardtii.

OJIP Wizard

7.5 PROTOCOL SYNTAX

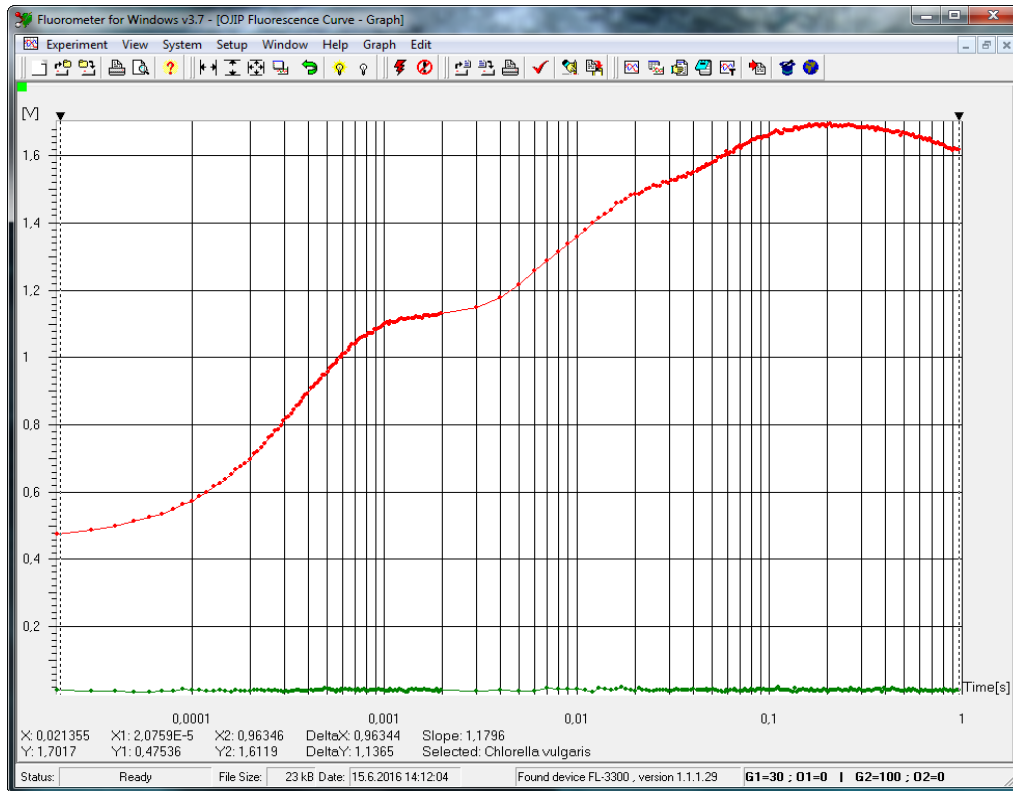### 7.5.1 SUMMARY OF THE PROTOCOL HEADER SYNTAX

	Interpretation	Typical	Units
MeasuringFlash=	Duration of the Measuring Flash (f)	1 – 10 µs	µs
ActinicFlash=	Duration of the Actinic Flash (F1)	15 – 100 µs	µs
MeasurDelay=	Delay of the measurement after the Measuring Flash trigger	1 – 10 µs	ns, µs
AuxDuration=	Duration of the Actinic Light (A1, A2)	Seconds – minutes	ms, s, min, h
PreFlash=	Interval separating the Measuring Flash from the preceding Background Measuremnt	0 – 20 µs	µs
include default.inc include default1.inc	Default setting file for red/blue measuring pulses		
include detector.inc	Default setting file for detector control		
include thermoregulator.inc	Default setting file for thermoregulator TR2000 control		
M_Voltage=	Relative power of the Measuring Flashes	10 – 30 % for red 50 – 70 % for blue	
F_Voltage=	Relative power of the Actinic Flashes	40 – 100 %	
A1_Voltage=	Relative power of the red Actinic Light	5 – 100 %	
A2_Voltage=	Relative power of the blue Actinic Light	5 – 100 %	
FAR_RED_Voltage=	Relative power of the FAR-RED light	50 – 100 %	
Stirrer_disable=	Switch OFF stirrer during experiment	1; 0	Num
FAR_duration=	Duration of the FAR preillumination (IR)	0 – 20 s	ms, s

7.5.2 TIMING OF PROTOCOL SYNTAX

Timing unit declaration:

Time unit ns (µs, ms, s, min, hour)

The timing unit can be changed several times in any Protocol. The declared time unit is attributed to any constant or variable of Value type that is without locally specified time unit and that is used after the present time unit declaration and before subsequent time unit declaration.

Example:

time unit µs   ; first declaration

<100> => fm   ; timing is 100µs for flash-measure (fm) Action.

<200> => fm   ; timing is 200µs

<1ms> => F1   ; locally defined (ms) timing for Actinic Flash overrides the previously declared timing (µs)

time unit hour   ; second declaration changing the time unit for subsequent part of the Protocol (not shown here)

Definition of a single timing variable:

name = <value of implicit or explicit timing unit>

Example:

n = <100µs>   ; a new variable named n is introduced and set to 100µs

Definition of an arithmetic timing series:

name = <first,second..last>

Example:

k = <100µs,200µs..0.5ms>   ; an arithmetic timing series named k is introduced including 100µs, 200µs, 300µs, 400µs and 500µs.

Definition of a logarithmic timing series:

name = [first,second..last]

Example:

j = [10 µs,100 µs..10s]   ; a logarithmic timing series named j is introduced including 10 µs

Combination of two timing series:

Name1|Name2

Example:

k|j   ; generates a new timing series combining all element of series k and all ellements of series j.

Cartesian multiplication of two timing series:

Name1#Name2

Example:

k#j ; generates a new timing series by Cartesian multiplication: e.g., for k=<1s,2s> and j=<10ms,20ms>, k#j=<1.01s,1.02s,2.01s,2.02s>.

Execution of an Action:

Name => ActionName

<Timing> => ActionName

Examples:

k => fm1 ; Action fm1 (flash-and-measure Channel 1) is executed at the timing defined by the variable k or by the timing series value k that were defined with parenthesis by k=<..> or k=[..] commands. For other ActionNames see section 7.

<i> => F1 ; Action F1 (Actinic Flash F1) is executed at the timing defined by the variable i that was set in a format without parenthesis, e.g. i=1ms.

<1ms> => A1(10s) ; Action A1 (Actinic Light A1) will be switched on 1 ms after the start of the experiment for a period of 10s.

7.6   SHORTCUT KEYS AND KEY-CONTROLED ACTIONS

Alt + Backspace – Undo last Protocol modification

Alt + X – Exit FluorWin

Ctrl + 1 – Trigger Aux1 TTL (A1) manually – usually Actinic Light 1

Ctrl + 2 – Trigger Aux2 TTL (A2) manually – usually Actinic Light 2

Ctrl + 3 to 6 – Trigger Aux3 to Aux6 TTL (A3 to A6) manually

Ctrl + F1 – Trigger Actinic Flash (F1) manually

Ctrl + F2 – Trigger Actinic Flash (F2) manually

Ctrl + Ins – Copy in Protocol & Notes

Ctrl + Del – Delete in Protocol & Notes

Ctrl + M – Trigger Measuring Flash (f) manually

Ctrl + T – Test Keys window

Ctrl + U – Undo last Graph modification

Ctrl + I – Device ID. May be used to recover communication with the Control Unit

Delete with selected DataSet in Graph – removes irreversibly the Data Set.

F2 – Save experiment file

F3 – Open existing experiment file

F4 – Signals

F5 – Graph

F6 – Data

F7 – Protocol

F8 – Notes

F9 – Start experiment

F11 – Navigator

Ctrl + M – System monitor

Shift + Del – Cut in Protocol & Notes

Shift + Ins – Insert clipboard in Protocol & Notes

7.7 LIGHTS TESTING

Use Test Keys if the verification of functionality of LEDs in the measuring unit is required.

- Open the Navigator window (the globe button 🌐 at the right edge of the icon bar) and select **Test Keys** or press shortcut *Ctrl+T*.

Fig. 52 Navigator.

- The Test Keys Window (Fig. 53) sliders allow manual setting of the voltage for Measuring Flashes, Actinic Flash, Actinic Lights and IR light. Pressing Signals buttons activates LEDs for default duration (15 µs for the Measuring flash, 1 s for the Actinic light and IR Light). For checking the LED function move the Voltage slider of the tested light on 60 %. Than look from 20 cm distance into the chamber of the Measuring unit, press respective button and check visually correct functioning of particular LED.
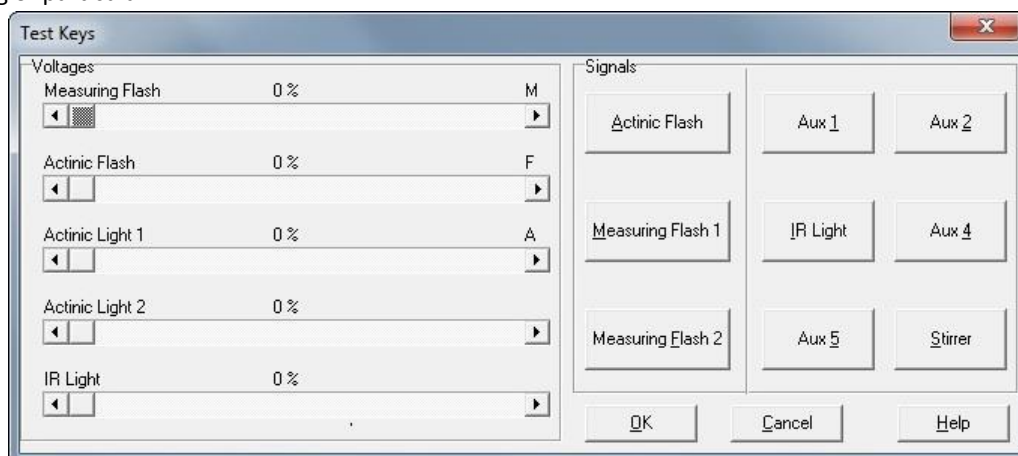

Fig. 53 Test Keys window.

- Set all sliders to 0% after the light test.

8   WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the SpectraPen 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.

9   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.