

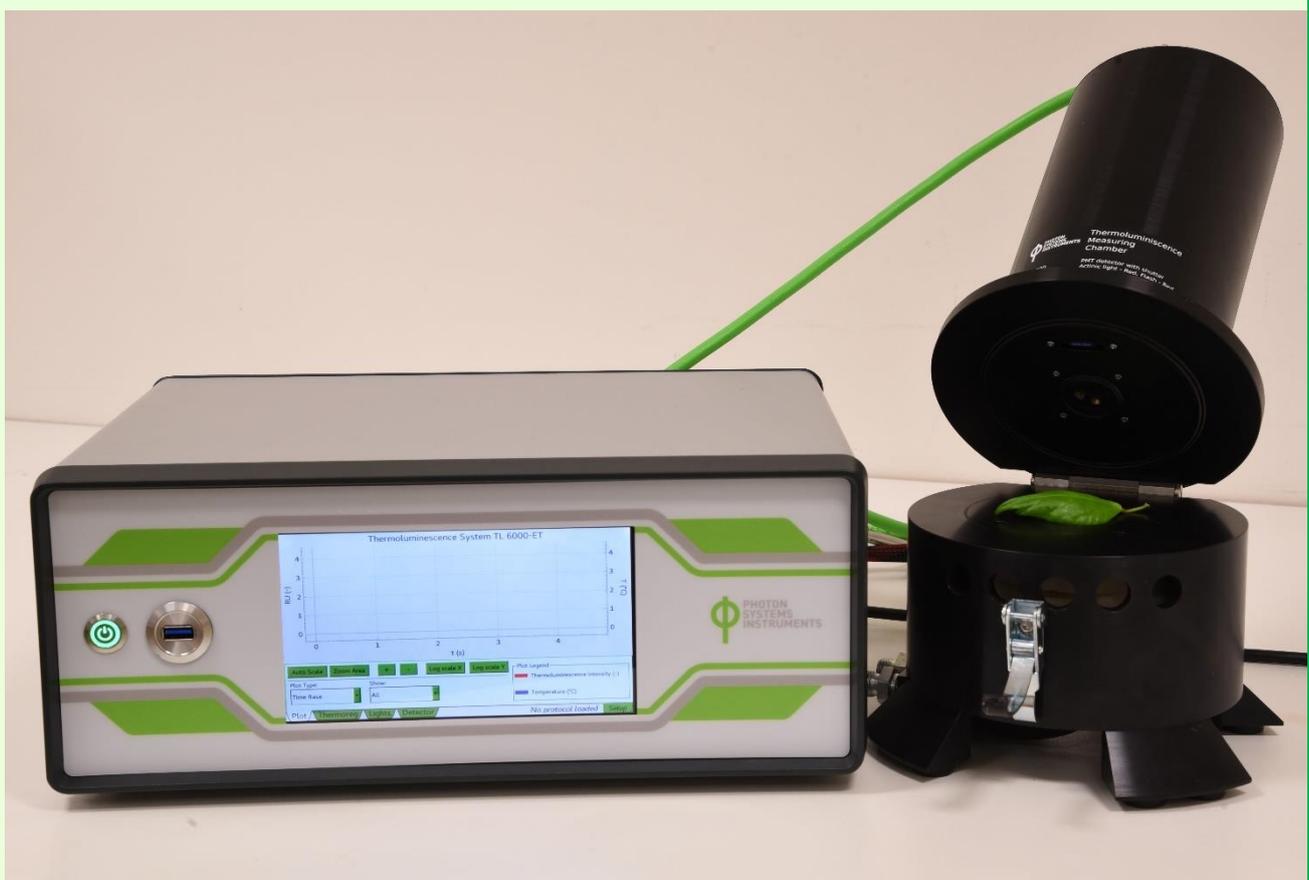
# Thermoluminescence

## TL 6000/ST

## TL 6000/ET

### Manual and User Guide

Please read this manual before operating this product



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

*The visualizations shown in this manual are only illustrative.*

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

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## 1 INFORMATION BEFORE USING THERMOLUMINESCENCE

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.
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Always follow corresponding manual while working with the Thermoluminescence device or doing the maintenance.

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

*Tab. 1 Used symbols.*

## 2 TECHNICAL SPECIFICATION

<b>Thermoregulation</b>	
Thermoregulation system	Peltier element and water cooling unit (TL 6000/ST) Nitrogen tank and resistance heater (TL 6000/ET)
Temperature range	-25 – 70 °C (TL 6000/ST) -100 – 200 °C (TL 6000/ET)
Maximal linear heating rate	1.5 °C/s (TL 6000/ST) 1.8 °C/s (TL 6000/ET)
Temperature control	Manual (constant temperature) Protocol defined temperature profiles
Overheating protection	Yes
<b>Illumination – LED lighting</b>	
Light source	623 nm
Saturating pulse	Up to 300,000 $\mu\text{mol}(\text{photon}).\text{m}^{-2}.\text{s}^{-1}$
Actinic light	Up to 2,000 $\mu\text{mol}(\text{photon}).\text{m}^{-2}.\text{s}^{-1}$
<b>Detector</b>	
Type	Photomultiplier with sensitivity software control
Spectral response	300 nm - 900 nm
Minimum sampling period	100 ms
Switch-On delay	100 ms
Ambient light protection	Yes
<b>Sample disc</b>	
Material	Gold-plated copper
Diameter	14 nm (TL 6000/ST) 22 nm (TL 6000/ET)
Typical samples	Algal and cyanobacterial suspensions Leaf segments
<b>Software</b>	
Type	FluorWin 3.8
Control	Predefined protocol as well as custom defined protocols with variable timing, special language and scripts
Communication	RS232/USB
<b>Other</b>	
Control unit dimension	365 x 275 x 150 mm
Measuring unit dimension	310 x 200 x 200 mm
Electrical	90 VAC – 240 VAC, 50 Hz – 60 Hz
Warranty	1 year parts and labor (see the last page of this Operation Manual for precise warranty conditions)

### 3 GENERAL INFORMATION

The **Thermoluminescence System TL 6000** is designed to investigate structure of energetic levels in the Photosystem II noninvasively. Light-induced charge separation in the Photosystem II reaction centres results in accumulation of radical pairs that store the absorbed light energy. Heating induces recombination of these radical pairs and it triggers light emission and formation of characteristic thermoluminescence glow curves. The shape and the peak position of the different thermoluminescence bands provide valuable information about the energetic stability of the respective radical pairs as well as about the functioning of the Photosystem II reaction centres. Interpretation of the obtained data requires a thorough understanding of the charge pairs that are responsible for generating different thermoluminescence bands. High-temperature thermoluminescence appears as a result of accumulation of lipid peroxides and can be used as a simple and efficient tool to monitor oxidative stress in photosynthetic organisms.

Using thermoluminescence is possible to measure wide spectrum of samples – plant segments, thylakoids, PSII particles, lichens, algal, cyanobacterial, bacterial and plant cell cultures.

#### **Thermoluminescence versions:**

##### **TL 6000/ST**

The standard version works in temperature range from -25 to +70 °C and enables investigations of Q, A, B1, B2, C, and AG glow curve peaks. The temperature regulation is provided by Peltier element and water cooling unit.

##### **TL 6000/ET**

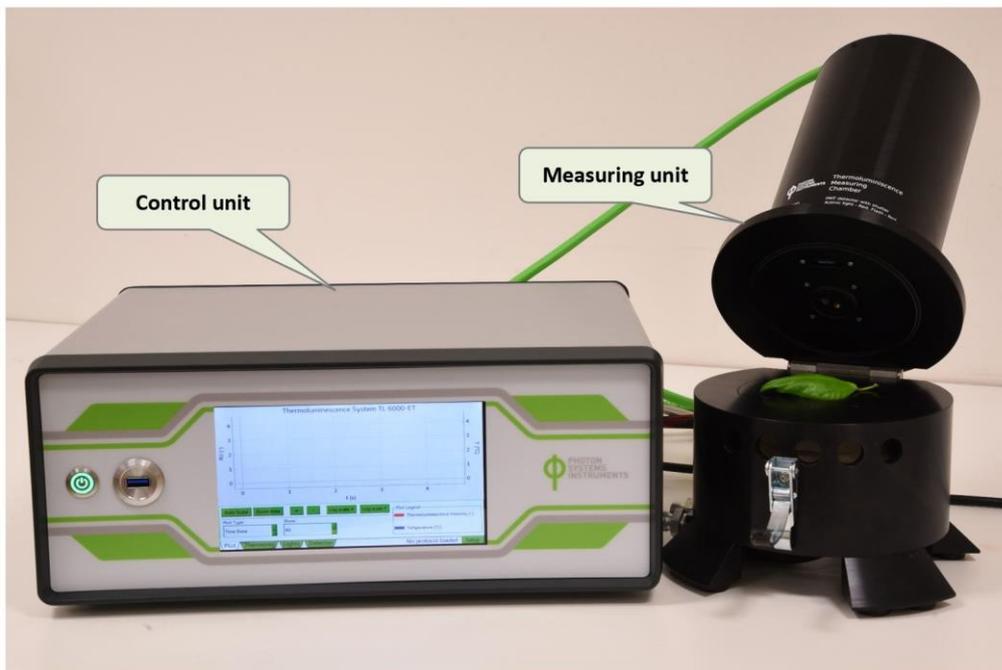
The extended version operates in temperature range from -100 to +200 °C. Wide temperature range allows to monitor Z1 glow curve peak and lipid peroxidation. The extended version is equipped with liquid nitrogen tank (included in the price) and resistance heater.

## 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

Standard components of the Thermoluminescence are shown in the Fig. 1. The device consists of control unit with touch screen and measuring unit. Individual parts are described below.



*Fig. 1 Thermoluminescence TL 6000.*

Other mandatory parts depend on version of the device. The standard version **TL 6000/ST** is supplied with **water cooling unit**. The extended version **TL 6000/ET** is equipped with **liquid nitrogen tank**.

### 5.1 MEASURING UNIT

The body of the TL 6000 Measuring unit (MU) is composed of two mechanical sub-units: top, optical part and bottom thermo-regulating segment (Fig. 2 and Fig. 3).

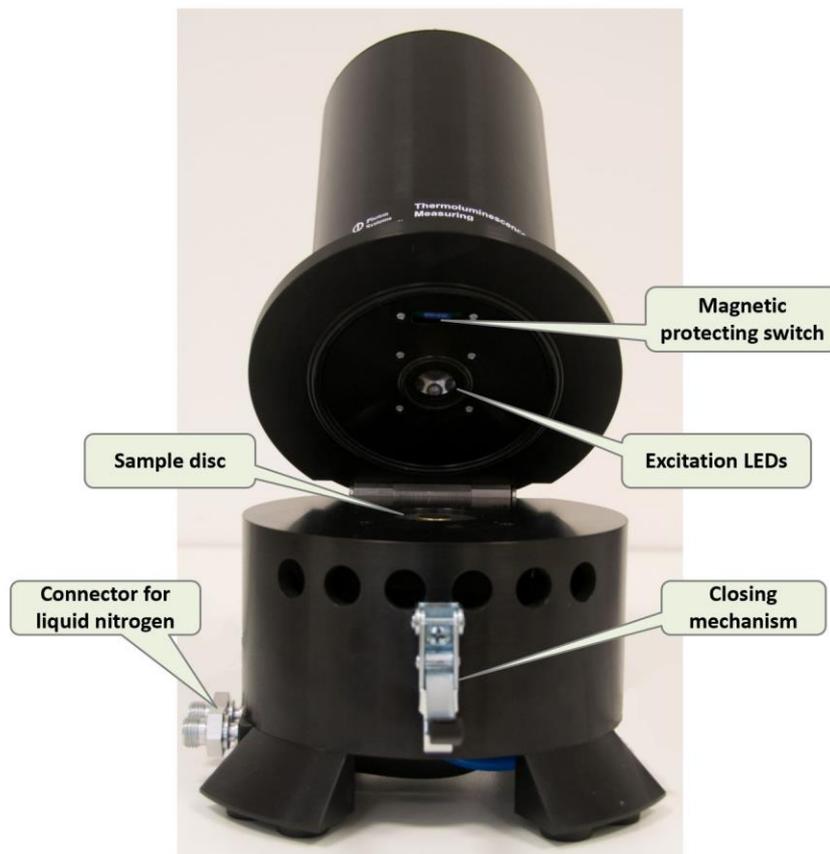


Fig. 2 TL 6000 measuring unit.

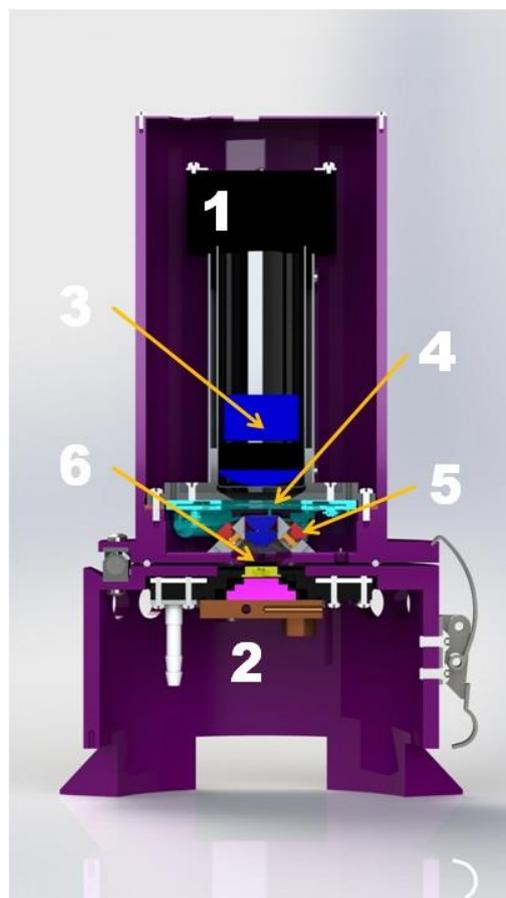


Fig. 3 The transverse section of the measuring unit: (1) PMT, (2) Thermoregulating block, (3) PMT optics, (4) Mechanical shutter, (5) Excitation LEDs with optics, (6) Thermoregulated measuring disc in the measuring chamber.

### 5.1.1 OPTICAL PART

The optical part contains detector: a sensitive Photomultiplier (PMT) with the collimating optics and illumination head with a ring of excitation LEDs.

#### The Photomultiplier

The PMT is protected by a software controlled mechanical shutter against unwanted illumination. The shutter is automatically closed when MU opens. Opening the shutter is possible just by the FluorWin software. In predefined thermoluminescence protocols, the shutter is open 100 ms after applied excitation light flash for the detection of the delayed luminescence. The PMT detects photons in the wavelength range of 300 to 900 nm.

Setting of detector gain and offset can be done by FluorWin software using System Monitor window (described in chapter 7.4) or by touchscreen display of control unit (described in chapter 6)

	<p>Measuring chamber must be accurately closed by the closing mechanism during the measurements. Ambient light can cause irreversible damage of the PMT.</p>
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	<p>When the Measuring unit is open, the PMT is automatically switched to a power-down mode and the shutter is automatically closed. No signal is detected.</p>
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#### Excitation LEDs

The excitation light source is based on LEDs arranged in circular geometry the way that the illumination is focused on the sample disc. The standard peak wavelength of LEDs is 623 nm. The light source enables illumination in two modes: 2 LEDs provide continuous actinic illumination and another 6 LEDs single turnover saturating flashes (STF). Light duration and intensity can be defined in protocols. The maximum intensity of the actinic light is around  $2,000 \mu\text{mol (photons).m}^{-2}.\text{s}^{-1}$ . The intensity of STF is around  $300,000 \mu\text{mol (photons).m}^{-2}.\text{s}^{-1}$ . The maximum flash duration is limited to 150  $\mu\text{s}$ .



Fig. 4 Ring of excitation LEDs in optical part of MU.

	<p>The optics and electronics are protected against the water vapor by the water proof and sealed glass window. Be careful when cleaning the protective glass, it is fragile.</p>
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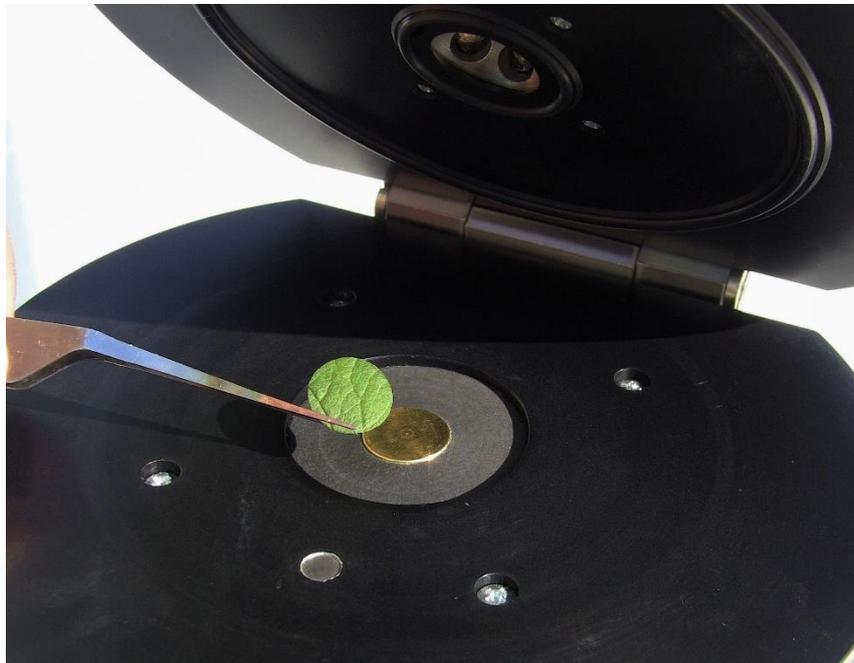
### 5.1.2 THERMOREGULATING PART

The thermoregulating elements are installed in the base of MU. They comprise the Peltier element and water cooling circuit in case of standard version or eventually resistance heater and liquid nitrogen cooling circuit for extended version.

The core is the **Sample disc** made of heat conductive copper with the chemically resistant gold-plated surface. Its temperature is precisely regulated via the temperature sensor mounted under the surface of the disc. The sensor is calibrated in the range of -100 °C to +200 °C with an absolute accuracy of 0.1 °C. The diameter of the Sample disc is 14 mm (ST version) or 22 mm (ET version). It is recommended to fix the sample on the disc using the Teflon ring of the same diameter as a sample disc to ensure good sample to pan contact.



It is recommended to fix leaf samples in the disc using teflon ring and grid to ensure optimal heat exchange between the sample and the disc. For description of samples preparation please follow the chapter 8.



*Fig. 5 Sample disc.*

## 5.2 CONTROL UNIT

The controlling electronics for Thermoluminescence and additional equipment is integrated in compact Control Unit equipped with touch screen display. 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. 6).



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

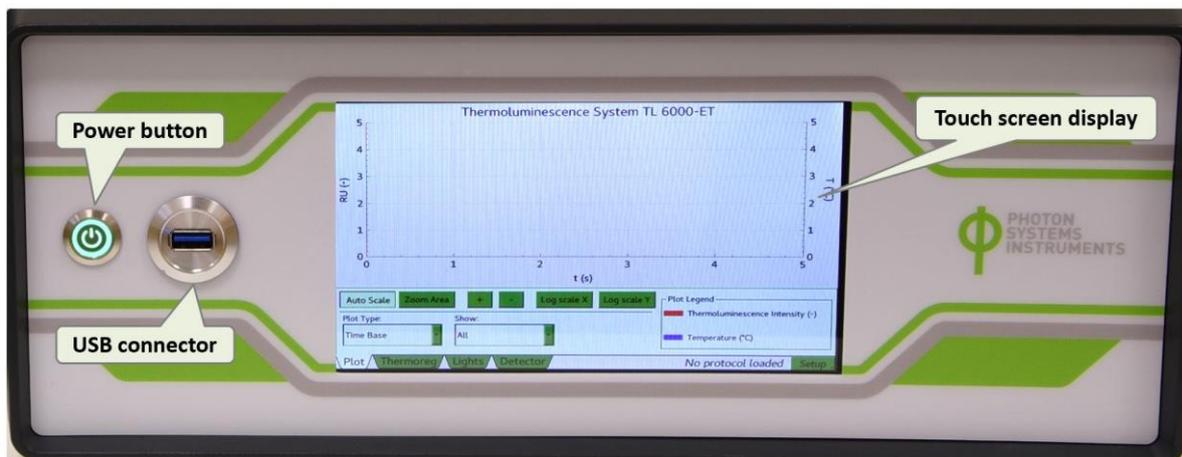


Fig. 6 Front panel of the control unit.

The back side of the TL control unit contains connectors and the main power button (Fig. 7 Fig. 7).

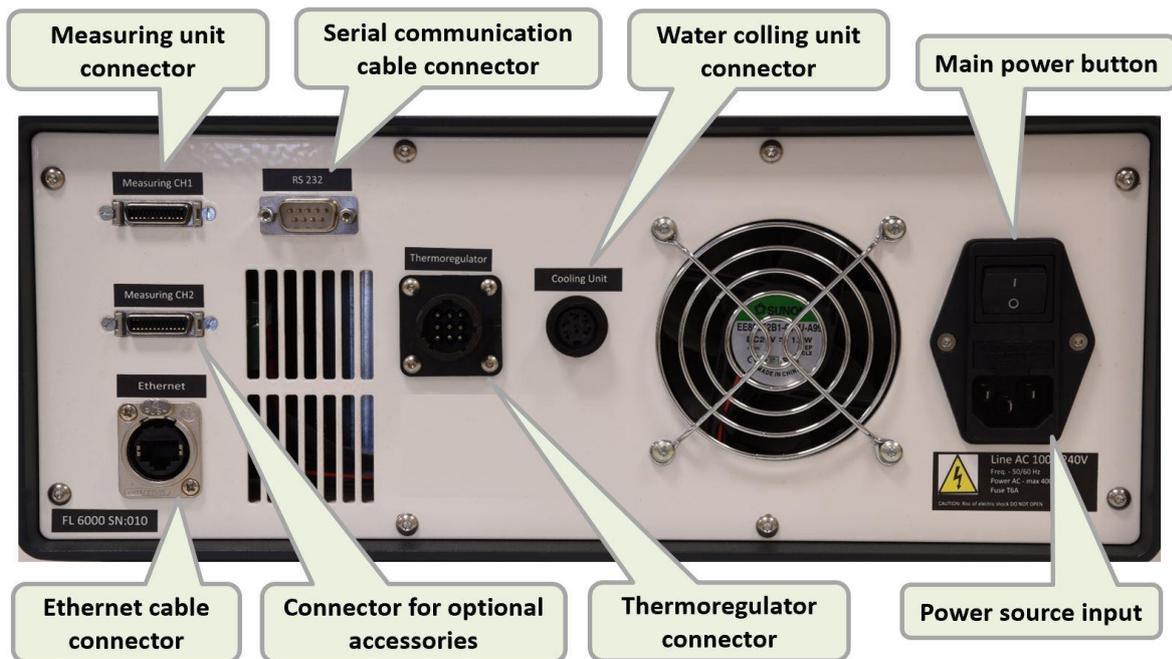


Fig. 7 Back side of the control unit.

## 5.3 COOLING UNITS

### 5.3.1 WATER COOLING UNIT

The water cooling unit, standalone chiller AC-700, supplies the cooling medium for supplementary water cooling circuit required for proper operation of standard version TL 6000. The reason is that the Peltier element itself would not be able to cool the measuring pan down to  $-25\text{ }^{\circ}\text{C}$ .



The deeper cooling, down to  $-70\text{ }^{\circ}\text{C}$  is possible only with additional Liquid Nitrogen Cooling unit.



*Fig. 8 Water cooling unit AC-700.*

Cooling Unit AC-700 package consists of:

- AC-700 water pump
- Hailea HC-130A water chiller
- One piece of power cable
- One piece of AUX cable
- One piece of elastic silicone tube 8/6 mm – 5 m length
- Rubber seals 2mm/3mm



The AC-700 cooling kit is supplied in two versions – for 210-240V AC and 110V AC power line.

### Water cooling unit installation

Let the TL device switch OFF during the AC-700 cooling unit installation.



Place the Cooling Unit AC-700 on a flat, firm and dry surface! Let it stand in upright position for at least **12 hours before plugging it into power supply!**

1. Place two circular rubber seals around the outlets on the top of the Hailea water chiller (Fig. 9).

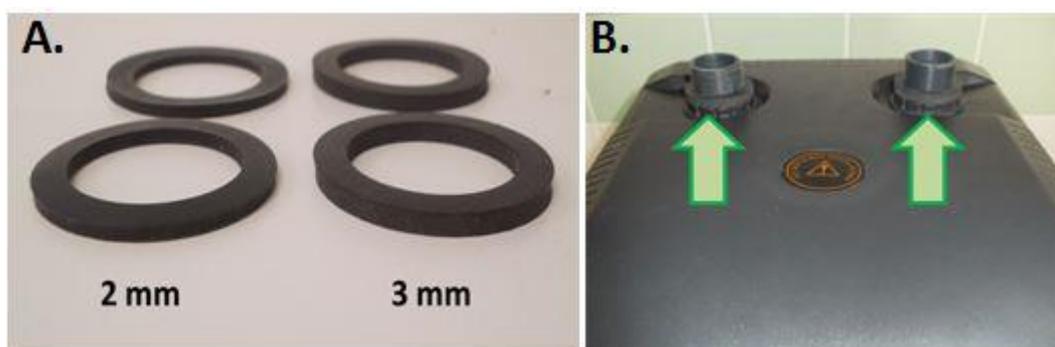


Fig. 9 A) Rubber seals. B) Hailea outlets.

2. Put the water pump on the top of the water chiller (Fig. 10A) and place the other two seals around the outlets of Hailea water chiller (Fig. 10B).

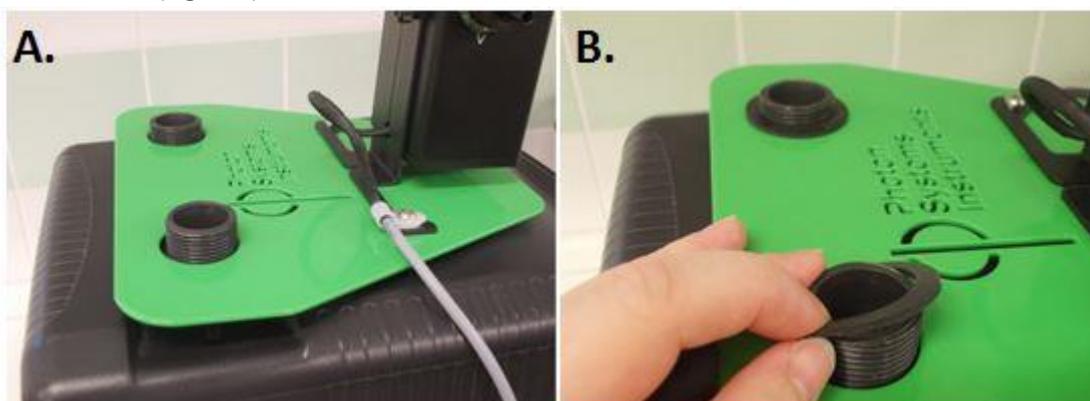


Fig. 10 A) Water pump on the top of the chiller. B) Seals around the outlets of Hailea.

3. Finally, fix the water pump to the water chiller with screws (Fig. 11).

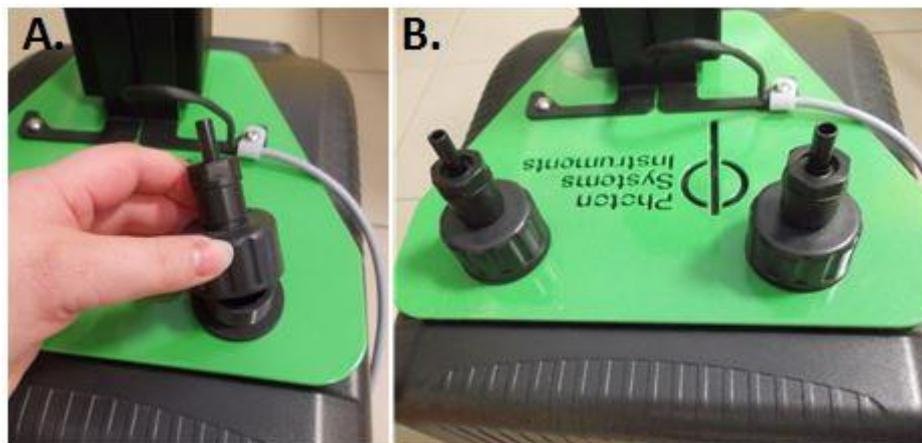


Fig. 11 Fixing of the pump with screws.

4. Connect the short 20 cm silicon hose to the **lower port** (Fig. 12A1) on the right side of the water pump. Connect the second end of the 20 cm tubing to the right top input of the water chiller (Fig. 12A2).
5. Connect long silicon hose to the **upper port** (Fig. 12A3) on the right side of the water pump. Same long silicon hose connect to the **Hailea port** (Fig. 12A4)
6. Interconnect the tubing of the cooling unit and the MU of thermoluminescence using Luer-Lock fittings (Fig. 12B).

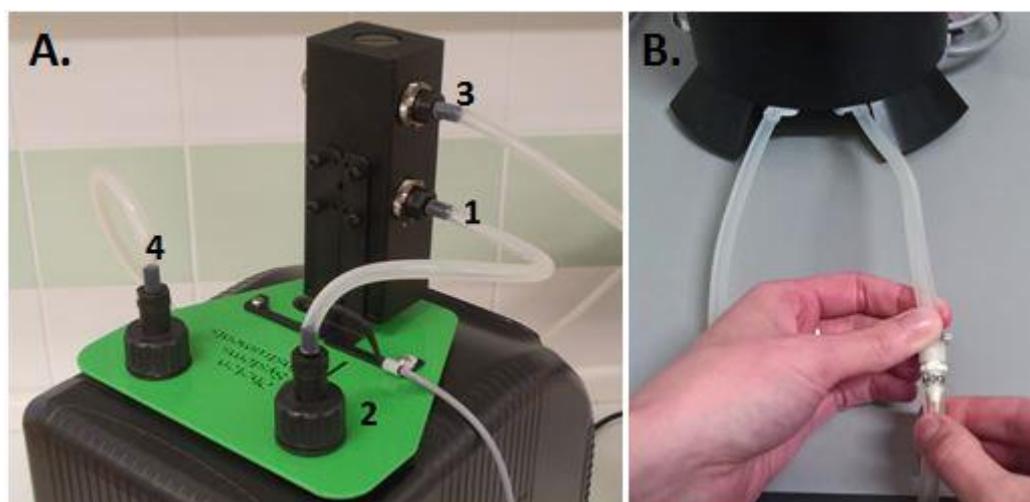


Fig. 12 A) Connection of cooling unit tubing. B) Interconnection of cooling unit and TL measuring unit.

7. Plug the AC-700 water pump AUX cable into the Cooling unit output on the back panel of the control unit. This connection provides the powering of the pump as well as controls its function in remote.
8. Plug the Hailea HC-130A water chiller in AC electricity.
9. Switch **ON** the HC-130A water chiller. Front display shows the actual temperature in the small water reservoir positioned inside of the HC-130A.
10. Unscrew the top cover of the AC-700 water pump. This way you access the filling tank of the water circuit (Fig. 13A).
11. Switch **ON** the TL control unit.
12. Pour carefully distilled water in the water pump reservoir (Fig. 13B).
13. Screw back the top cover of the AC-700 water pump.

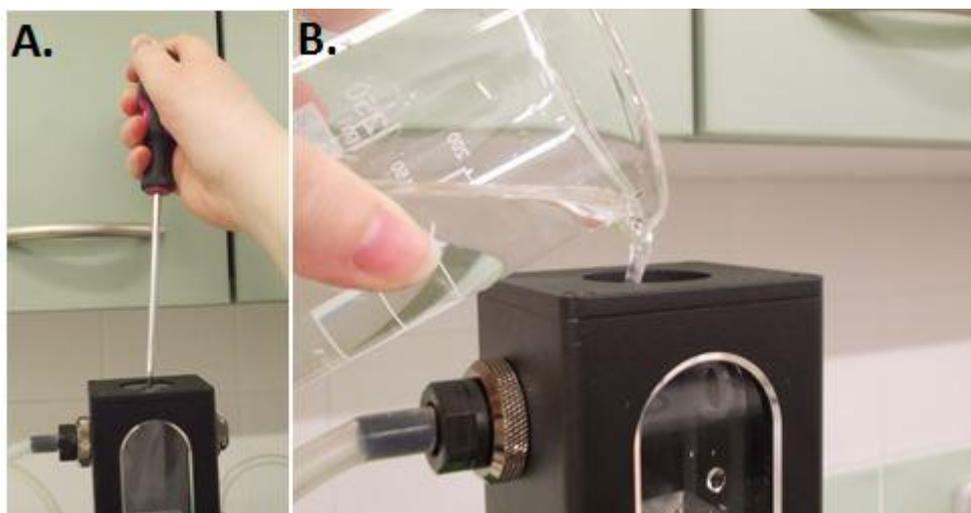


Fig. 13 A) Unscrewing of water pump top cover. B) Filling of water pump.

### 5.3.2 LIQUID NITROGEN COOLING UNIT

For cooling temperature reaching down to  $-100\text{ }^{\circ}\text{C}$ , the Liquid nitrogen cooling unit must be used. This option is a part of extended version TL 6000.

The container consists of an inner stainless steel cylinder securely supported in an outer jacket shell. The space between the inner and outer vessels contains a highly efficient insulation material and is evacuated.



The container needs to be filled by specialized company or competent person.

#### Installation

1. Connect the Liquid Nitrogen Unit to the Measuring unit. First, connect the inlet pipe to the Measuring unit (Fig. 14A). Then connect the outlet pipe. Tighten both connections well; use the supplied wrenches No. 19 (Fig. 14B).



The outlet pipe must be positioned so as to vent escaping vapors into well ventilated space (open window, for instance). The outlet pipe should be fixed on its end because the escaping vapors cause the movement of the tubing.

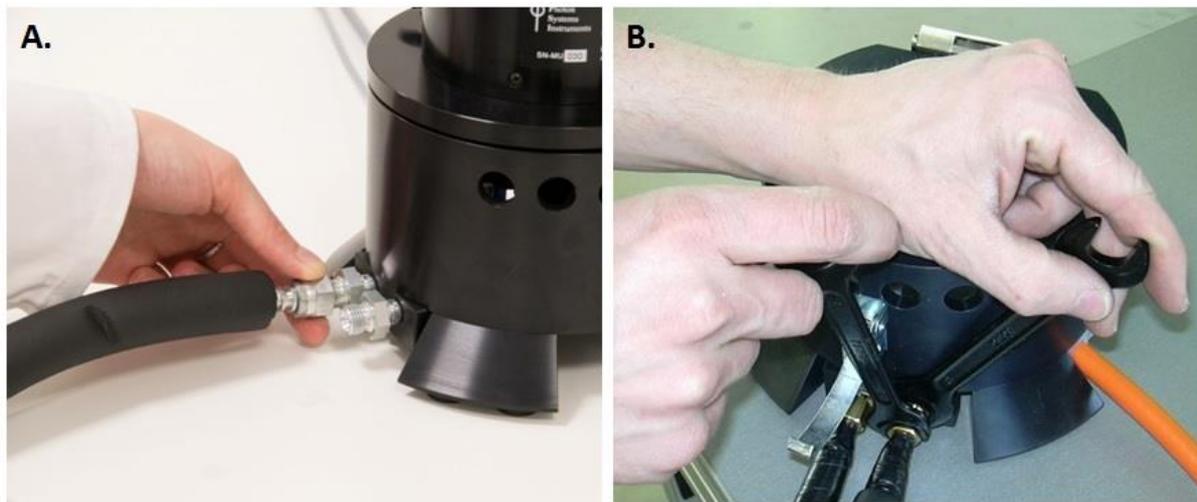


Fig. 14 A) Connecting of inlet pipe to the measuring chamber. B) Tightening of the connection using wrenches.

2. Connect the Nitrogen cooling unit solenoid valve to the control unit (Fig. 15).



Fig. 15 Connector for Nitrogen cooling unit.

### Safety Devices

A relief valve (Fig. 16C) and a rupture disc (Fig. 16B) protect the inner liquid reservoir, both located on the manifold.

A combination evacuation valve and relief valve is provided to service the vacuum space. This protects the container in the event of a leak in the inner reservoir.

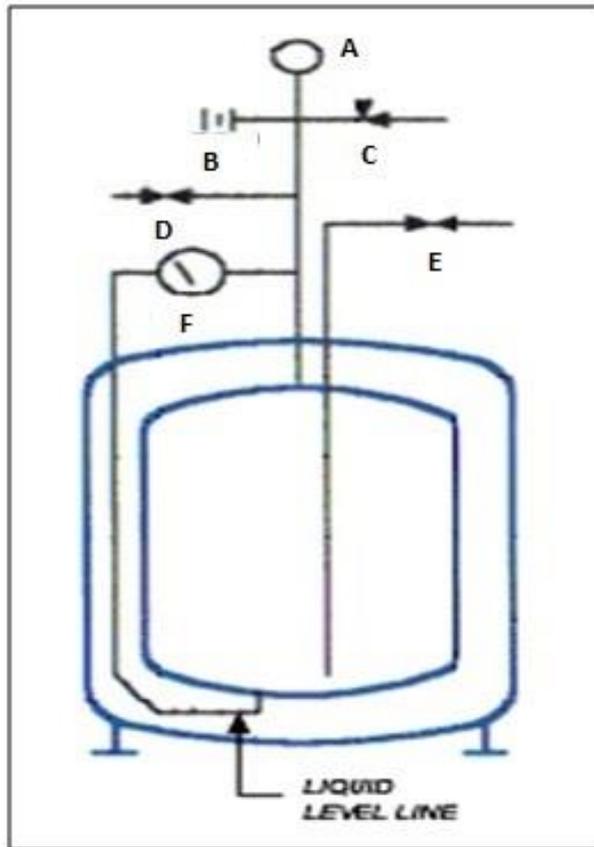


Fig. 16 Scheme of Nitrogen Cooling Unit: A) Pressure gauge. B) Rupture disc. C) Relief valve. D) Vent Valve. E) Liquid fill/withdrawal. F) Liquid level gauge.



If this device leaks, contact PSI. Do not attempt to use the container or re-evacuate the insulation space.

### Gauges

A pressure gauge (Fig. 17A) is provided indicating inner vessel pressure. A liquid level gauge (Fig. 17B) is provided to indicate approximate container contents.

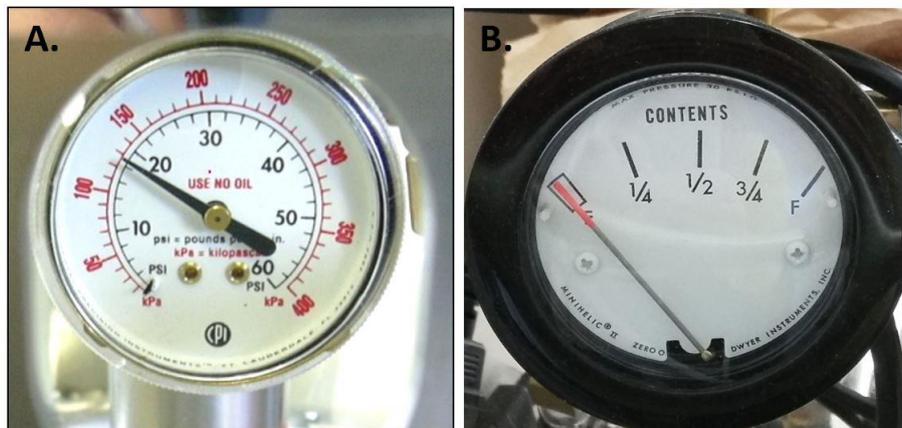


Fig. 17 A) Pressure gauge, B) Liquid level gauge.

## Handling

These containers are designed for use in the upright position and should not be laid on their side. If a container must be lifted, use a forklift or similar device beneath the base, or hoist by means of the lifting slots in the halo ring struts. Do not attempt to lift by the handles or by means of slings around the shell.

## Withdrawal

Before liquid transfer, be sure the vent valve is closed and check the pressure gauge to see that the vessel pressure is adequate for the intended application. If additional pressurization is required introduce room temperature gas, of the same composition as the contents, through the vent valve. The gas should be supplied at a pressure no greater than the relief valve setting.

Attach a suitable transfer line to the fill/withdrawal valve. Open the fill/withdrawal valve as far as necessary to obtain the desired flow rate. When the transfer is complete, close the fill/withdrawal valve and disconnect the transfer line.

## Changing service

Although these containers can be supplied from the factory for LN<sub>2</sub>, LARG or LOX service, do not attempt to change the service for which the container was originally supplied without consulting the factory.

## General

To insure safe control of Liquid nitrogen in laboratories, all orders for these materials should be cleared through a responsible person. This individual will insure that the potential user is aware of the danger involved and will follow recommended procedures.

Liquid nitrogen should never be used in combination with other substances without knowing what the result may be. When in doubt, consult a competent authority.

## Purity

Liquid nitrogen, when placed in the container at the manufacturer's plant is of a definite purity but this purity is subject to change since the nitrogen evaporates in preference to the very small oxygen impurity. If liquid nitrogen remains in the container until a large portion of the liquid is evaporated, an analysis of the remaining liquid should be made before it is used for any purpose where a high oxygen impurity or high oxygen content would be dangerous.

## Toxicity

Nitrogen is nontoxic, but if allowed to accumulate in sufficient quantity it may act as asphyxiate. This is because it lowers the concentration of oxygen that is normally present in the air.



Liquid nitrogen should never be stored or used in small closed compartments, rooms or excavations without added ventilation. Well-ventilated storage and working space should be provided

## Pressure buildup

The heats of vaporization of most Liquid gases are low. In addition, a small quantity of liquid produces a large volume of gas at atmospheric pressure. Small heat flow from the atmosphere into the liquid, therefore, will produce an appreciable volume of gas. For this reason, all storage vessels should be provided with pressure relief devices unless the container is vented properly to provide escape of evaporating gases. All lines and vessels in which the liquid may be trapped between closed valves should be equipped with pressure relief valves. If there is any likelihood that the relief valve may freeze, as for instance, from ice formed from dripping water or condensed moisture, such vessels and lines should be equipped with rupture discs. Both pressure relief valves and rupture discs should be placed and protected so that water cannot splash or condense upon them. In addition, it is desirable and sometimes necessary, to vent relief valves and rupture discs to the outside atmosphere.

Liquid nitrogen should be transported only in suitable insulated containers that provide means for the escape of gas as liquid evaporates. Never cork or plug the outlet to such containers.

### **Liquid nitrogen handling**

Personnel handling liquid nitrogen should be thoroughly instructed as to the nature of the materials. Training is essential to minimize accidental spilling.

Small amounts of liquid nitrogen are frequently handled in glass Dewar flasks which occasionally collapse. Therefore these flasks should always be kept behind protective shields while in use.

Liquid nitrogen, because of its extremely low temperature, will "burn" the skin like hot liquid. Never permit Liquid nitrogen to come into contact with the skin or allow it to soak clothing. Serious burns may result from careless handling.

When personnel are handling Liquid nitrogen, they are advised to protect themselves by wearing goggles or face shields and leather gloves large enough to allow quick removal. Rubber aprons and high-topped shoes worn with trouser legs outside the tops are also desirable.

When pouring Liquid nitrogen from one container to another, the receiving container should be cooled gradually to prevent thermal shock. The liquid should be poured slowly to avoid spattering. The receiving vessel should always be vented to the atmosphere and high concentrations of gaseous nitrogen should not be allowed to collect.

Introduction of a substance that is at normal room temperature into a Liquid gas is always somewhat hazardous. There is a violent evolution of gas, and there is likely to be considerable splashing of the liquid. Personnel doing this work should be instructed of the hazard and should always wear full-face shield and protective clothing.

In the event a person is burned by Liquid gas, the following first aid treatment should be given pending the arrival and care of a physician:

1. If any liquid gas contacts the skin or eyes, immediately flood that area of the body with large amounts of unheated water and then protect frozen parts with loose, bulky, dry, sterile dressings.
2. If the skin is blistered or there is any chance that the eyes have been affected, get the patient immediately to a physician for treatment.

### **Material limitations**

The physical properties of many materials at extremely low temperatures may be quite different from the properties of the same materials at normal temperatures. Therefore, materials that have been cooled to the temperatures of liquid nitrogen should be carefully handled until their properties, under these conditions, are known.

Metals to be used for equipment in liquid nitrogen, must possess satisfactory physical properties at the low operating temperatures. Since ordinary carbon steels, and to a lesser extent most alloy steels, lose their ductility when subjected to the low temperatures of liquid nitrogen, they are considered unsatisfactory for such service. The austenitic nickel-chromium alloys have good ductility at the low service temperatures under consideration, and the most widely known is 18-8 stainless steel. Cooper, monel, brass and aluminum are also considered satisfactory materials for low temperature use.

Each new use for these liquids should be carefully considered before it is instituted and safety precautions should be completely outlined.

**Technical specification**

Net Liter Capacity	25
Gross Liter Capacity	28
Outside Diameter	41 cm (16")
Height	81 cm (32")
Empty Weight	28 kg (62 lbs)
Full Weight LN2	50 kg (110 lbs)
N.E.R. Lt/Day	1
M.A.W.P.	25
Secondary Relief (PSIG)	50
Caster Size	3"
Liquid Flow 22 PSIG	TBD
Liquid Fitting	3/8 NPT (or CGA 295 1/2" 45° Male Flare)
Vent Fitting	3/8 NPT
Material of Construction	304

**5.4 LIST OF OTHER EQUIPMENT**

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

- **Data cable for measuring unit**
- **Power cable for control unit**
- **Serial cable connecting computer and control unit**
- **Serial – USB Converter**
- **FluorWin installation flash drive** (on a USB flash disc)
- **User's Guide** (on a USB flash disc)
- **Hole punches set**
- **Teflon rings**
- **Metal grid**
- **Other Accessories or Optional Features** (according to your specific order)



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](mailto:support@psi.cz)

## 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. 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. 18)

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	time base or temperature base for thermoluminescence data
Show	both, thermoluminescence signal and temperature are shown as default in the graph, this option enables to display them individually

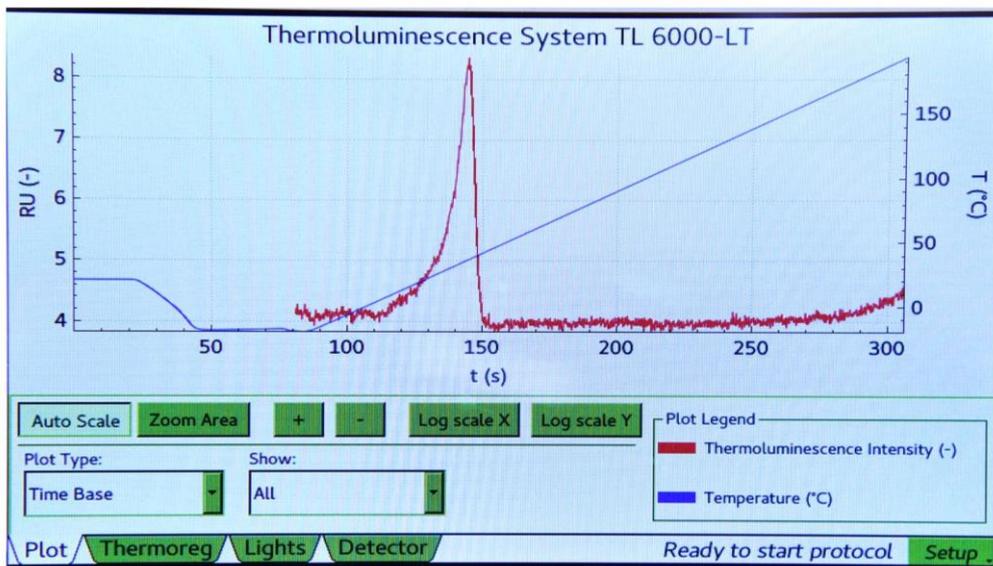


Fig. 18 Control unit display – Plot.

### Thermoregulation (Fig. 19)

Thermoregulation panel shows the Actual temperature of the sample disc, Set temperature based on the protocol and status (cooling/heating). Stirrer is not active for Thermoluminescence device.

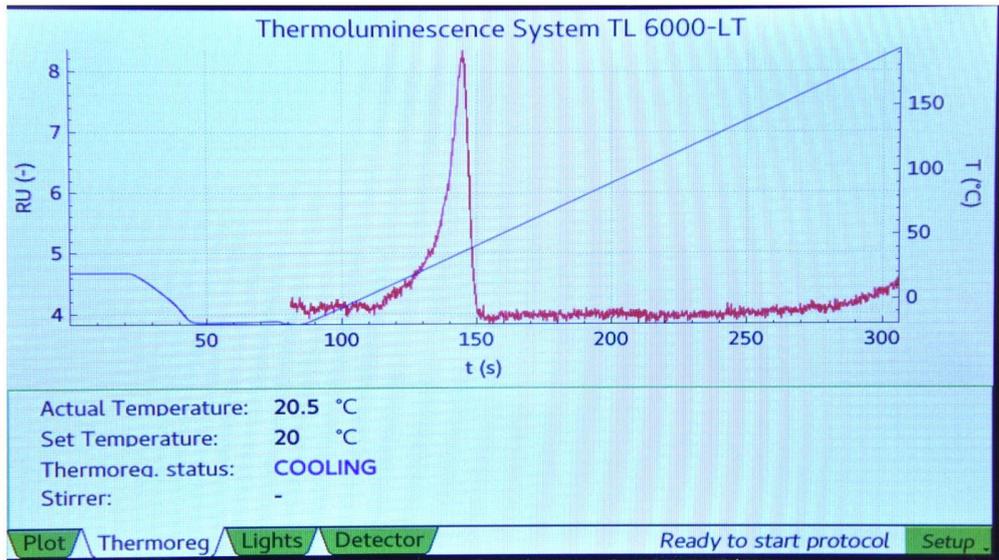


Fig. 19 Control unit display – Thermoregulation.

### Lights (Fig. 20)

This option informs the user about the lights status and setting. The changes in lights setting can be done only via the FluorWin software.

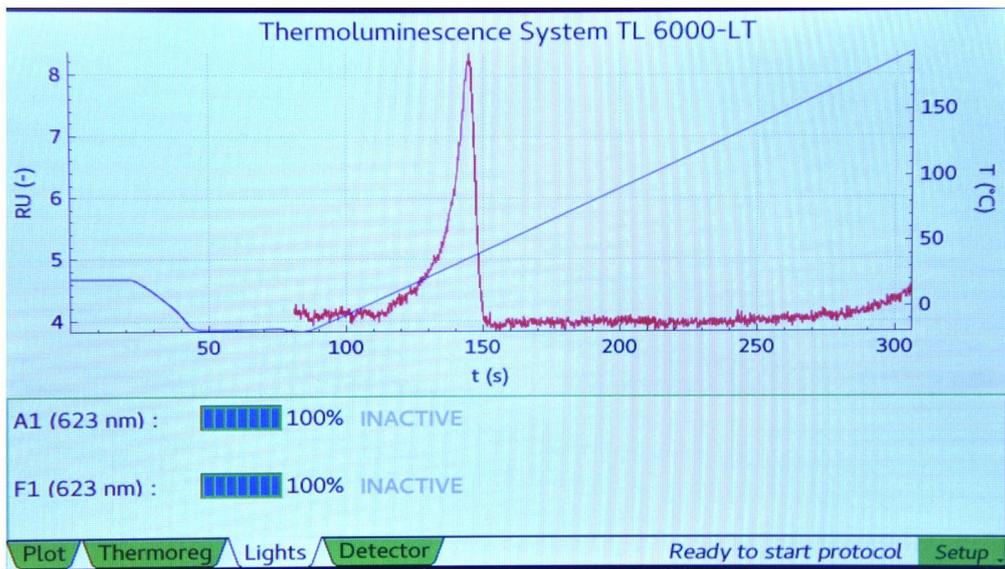


Fig. 20 Control unit display - Lights.

### 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. 21) 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).

	<p>When adjusting the offset manually check the actual signal level. When the level is set to 0%, no signal is detected.</p>
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	<p>Please note that Detector can be adjusted also via the FluorWin software. Detector functions are more described in chapter 7.4. The setting is linked between the touch screen and the software.</p> <p><b>Auto offset</b> function is available only on touch screen.</p>
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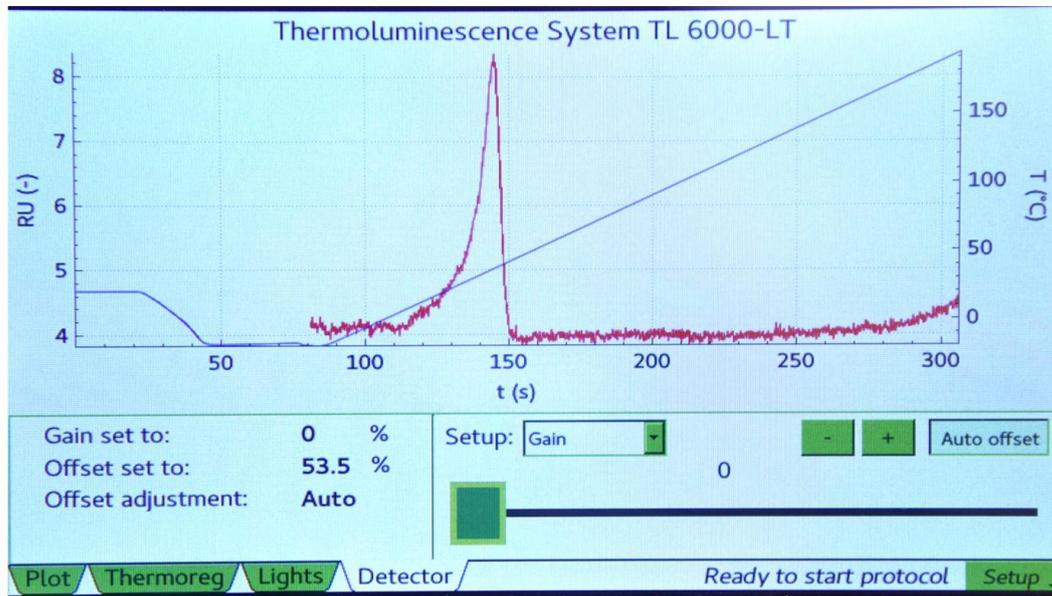


Fig. 21 Control unit display - Detector.

**Setup** (Fig. 22)

- 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. cooling unit)
- Disconnect MUs            disconnection of all current connected devices
- About...                    information about the device (Fig. 23)

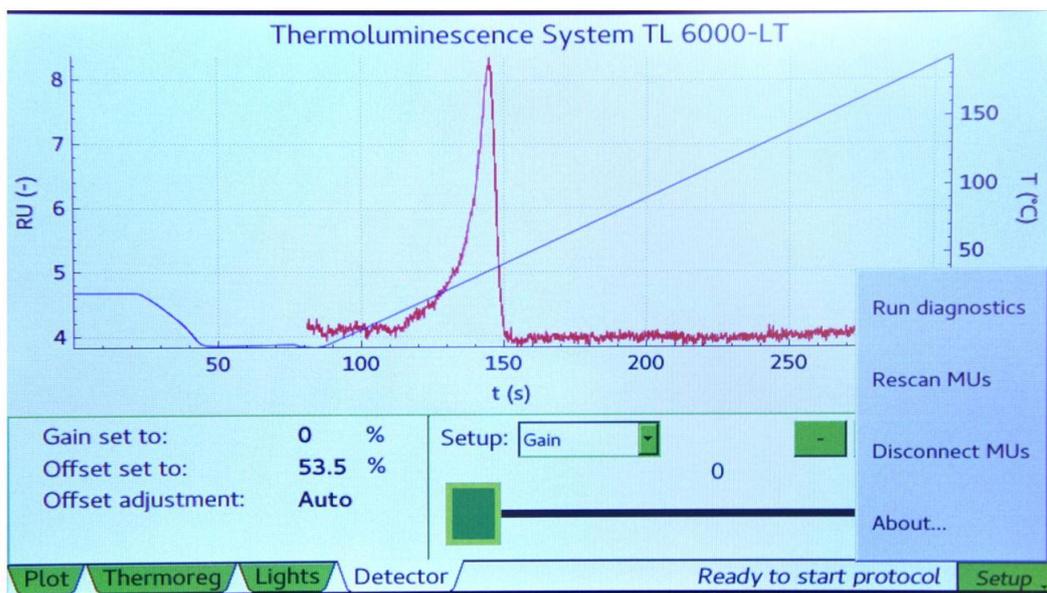


Fig. 22 Control unit display - Setup.

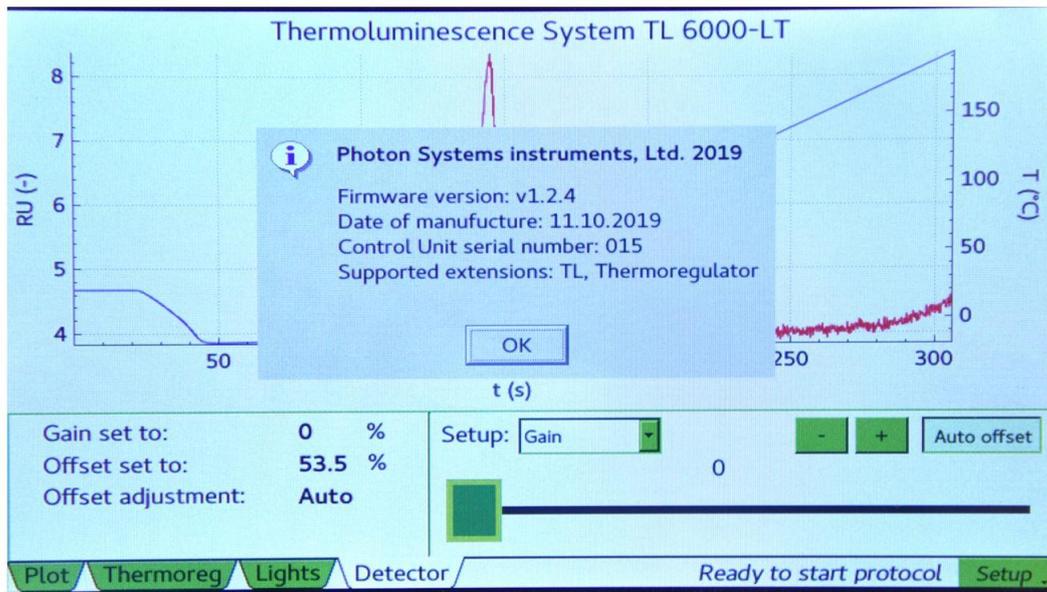


Fig. 23 Control unit display - About.

## 7 FLUORWIN SOFTWARE

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

	<p>The Thermoluminescence is not able to operate without the software.</p>
---	--

### 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 Thermoluminescence in your pc (Device manager > Ports).

	<p>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 Thermoluminescence COM port &gt; Port settings &gt; Advanced &gt; COM port number).</p>
---	---

- **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 Thermoluminescence and click OK (Fig. 24).

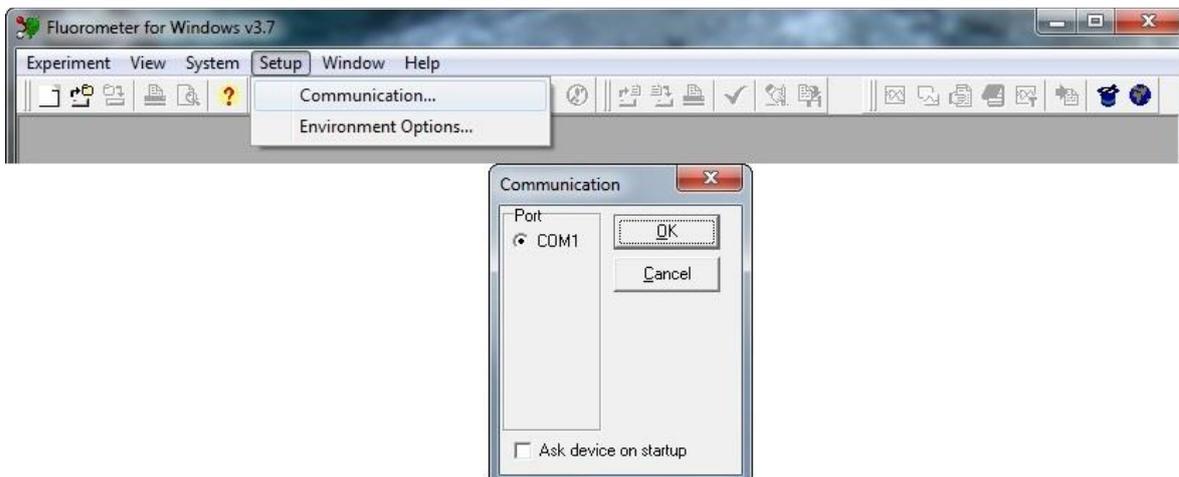


Fig. 24 Communication setting.

- Select **System > Device ID** from the menu (Ctrl-I Shortcut). Program initiates to searching the connected devices (Thermoluminescence 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. 25). If there is not a device connected to selected COM port, “Device not found” message is displayed.



Fig. 25 Status bar.

## 7.2 THERMOLUMINESCENCE WIZARDS

Wizard is a program tool which guides a user writing self experimental protocols.

- Open the **Wizard** menu using the blue hat on the upper bar or go to Window > Protocol Wizard (Fig. 26).



Fig. 26 Starting Wizard menu.

- Select the required **thermoluminescence protocol** from the Wizard menu (Fig. 27).

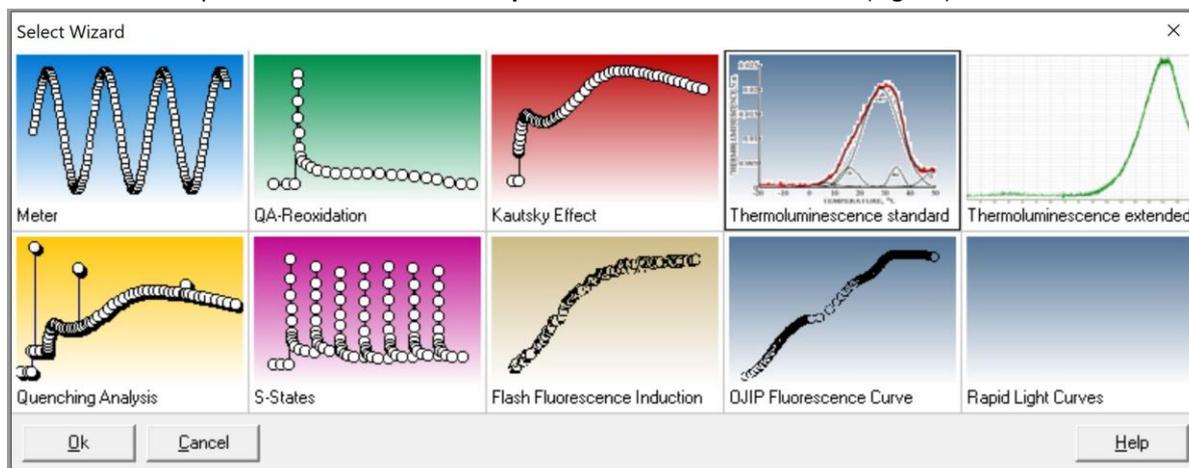


Fig. 27 Wizard menu.

- 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. 28).

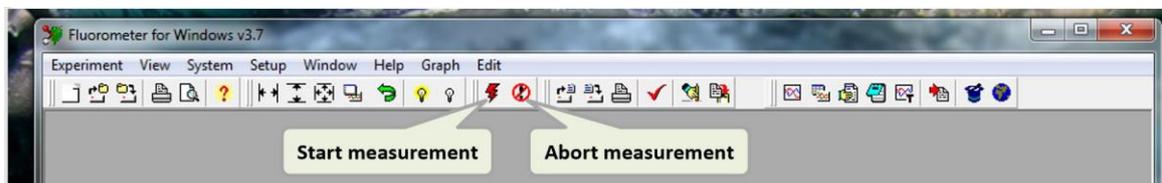


Fig. 28 Experiment start and abort.

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.

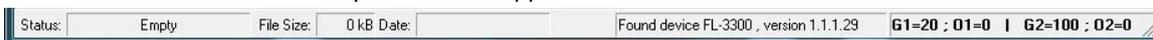


Fig. 29 Status bar - empty.

## 7.2.1 WIZARD FOR STANDARD VERSION

Fig. 30 Thermoluminescence standard wizard.

**Basic settings**

- *Dark adaptation temperature (°C)* - temperature at which dark adaptation of the sample is performed
- *Dark adaptation time (s)* - the length of dark adaptation period in seconds
- *Measurement periode (ms)* - sampling period for PMT and Thermoregulator. Minimum is 100 ms.

**Cooling settings**

- *Cooling temperature (°C)* – the desired low temperature for TL excitation; range from -25 to 0 °C
- *Cooling time (s)* - the time period intended to reach desired freezing temperature

**Heating definitions for thermoluminescence**

- *Heating rate for thermoluminescence (°C/s)* – the heating rate can be set from 0.1 to 1.5 °C/s; heating rate affects the thermoluminescence intensity (Fig. 31)
- *Final temperature (°C)* – maximal heating temperature 70 °C

**Actinic flash definition**

- *Flash time from start experiment (s)* - the time at which STF is applied, it can be anytime during Dark adaptation or Cooling period, but latest 1s before start of Heating period, resp. measuring period; software protected
- *Actinic flash intensity (%)* - intensity of Single Turnover Flash, 1 – 100 %, recommended 100 %
- *Flash duration (us)* - the length of STF, recommended 50  $\mu$ s
- *Amount of flashes* - number of applied STFs
- *Flash period (ms)* - period of application individual STFs

**Actinic light definition** – actinic pre-illumination before STF

- *Actinic light intensity (%)* – intensity of Actinic light, 1 – 100%
- *Actinic light duration (s)* – definition of Actinic light period

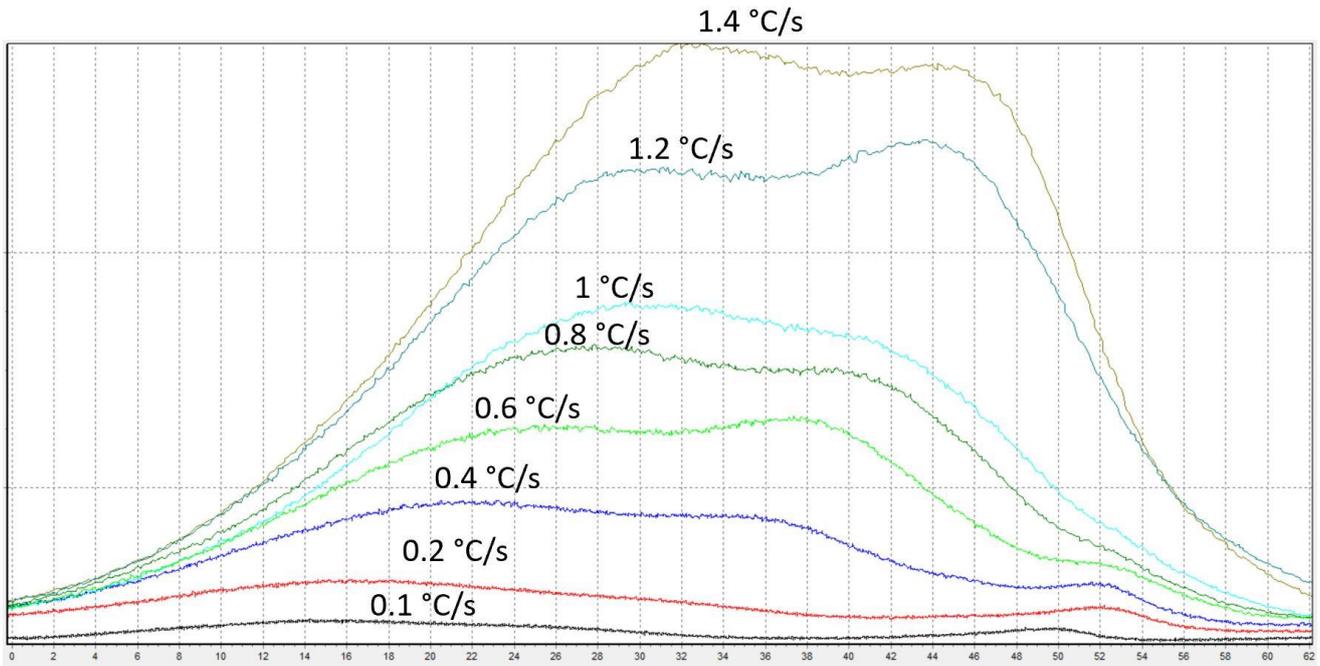


Fig. 31 Effect of heating rate on thermoluminescence signal.

7.2.2 WIZARD FOR EXTENDED VERSION

Thermoluminescence extended ×

<p><b>Basic settings</b></p> <p>Dark adaptation temperature (°C) <input style="width: 100px;" type="text" value="20"/></p> <p>Cooling temperature (°C) <input style="width: 100px;" type="text" value="-100"/></p> <p>Drying temperature (°C) <input style="width: 100px;" type="text" value="70"/></p> <p>Dark adaptation time (s) <input style="width: 100px;" type="text" value="120"/></p> <p>Drying time (s) <input style="width: 100px;" type="text" value="120"/></p> <p>Measurement periode (ms) <input style="width: 100px;" type="text" value="100"/></p> <p><b>Heating definitions for thermoluminescence</b></p> <p>Heating rate for thermoluminescence (°C/s) <input style="width: 100px;" type="text" value="1"/></p> <p><b>Heating definitions for chemiluminescence</b></p> <p>Heating rate for chemiluminescence (°C/s) <input style="width: 100px;" type="text" value="1"/></p> <p>Final temperature chemiluminescence (°C) <input style="width: 100px;" type="text" value="200"/></p>	<p><b>Flash definition</b></p> <p>Actinic flash voltage (%) <input style="width: 100px;" type="text" value="100"/></p> <p>Flash duration (us) <input style="width: 100px;" type="text" value="50"/></p> <p>Amount of flashes <input style="width: 100px;" type="text" value="1"/></p> <p>Flash period (ms) <input style="width: 100px;" type="text" value="200"/></p> <p>Flash before warm (s) <input style="width: 100px;" type="text" value="10"/></p> <p><b>Actinic Light definition</b></p> <p>Actinic light Intensity (%) <input style="width: 100px;" type="text" value="100"/></p> <p>Actinic light duration (s) <input style="width: 100px;" type="text" value="0"/></p>
--	--

Fig. 32 Thermoluminescence extended wizard.

## Basic settings

- *Dark adaptation temperature (°C)* - temperature at which dark adaptation of the sample is performed
- *Cooling temperature (°C)* – desired freezing temperature; range from -100 to 0 °C
- *Drying temperature (°C)* - temperature at which the sample is incubated for certain time to get rid of water because water vapour could cause unwanted effects at higher temperatures, recommended +70 °C
- *Dark adaptation time (s)* - the length of dark adaptation period in seconds
- *Drying time (s)* – time period dedicated form sample drying; usually between thermoluminescence and chemiluminescence heating
- *Measurement periode (ms)* - sampling period for PMT and Thermoregulator. Minimum is 100 ms.

## Heating definitions for thermoluminescence

- *Heating rate for thermoluminescence (°C/s)* – the heating rate can be set from 0.1 to 1.8 °C/s; heating rate affects the thermoluminescence intensity (Fig. 31)

## Heating definitions for chemiluminescence

- *Heating rate for chemiluminescence (°C/s)* – the heating rate can be set from 0.1 to 1.8 °C/s; heating rate affects the chemiluminescence intensity
- *Final temperature chemiluminescence (°C)* – maximal heating temperature 200 °C

## Flash definition

- *Actinic flash voltage (%)* - intensity of Single Turnover Flash, 1 – 100 %, recommended 100 %
- *Flash duration (us)* - the length of STF, recommended 50 μs
- *Amount of flashes* - number of applied STFs
- *Flash period (ms)* - period of application individual STFs
- *Flash before warm (s)* - the time at which STF is applied, it can be anytime during Dark adaptation or Cooling period, but latest 1s before start of Heating period, resp. measuring period; software protected

## Actinic light definition – actinic pre-illumination before STF

- *Actinic light intensity (%)* – intensity of Actinic light, 1 – 100%
- *Actinic light duration (s)* – definition of Actinic light period

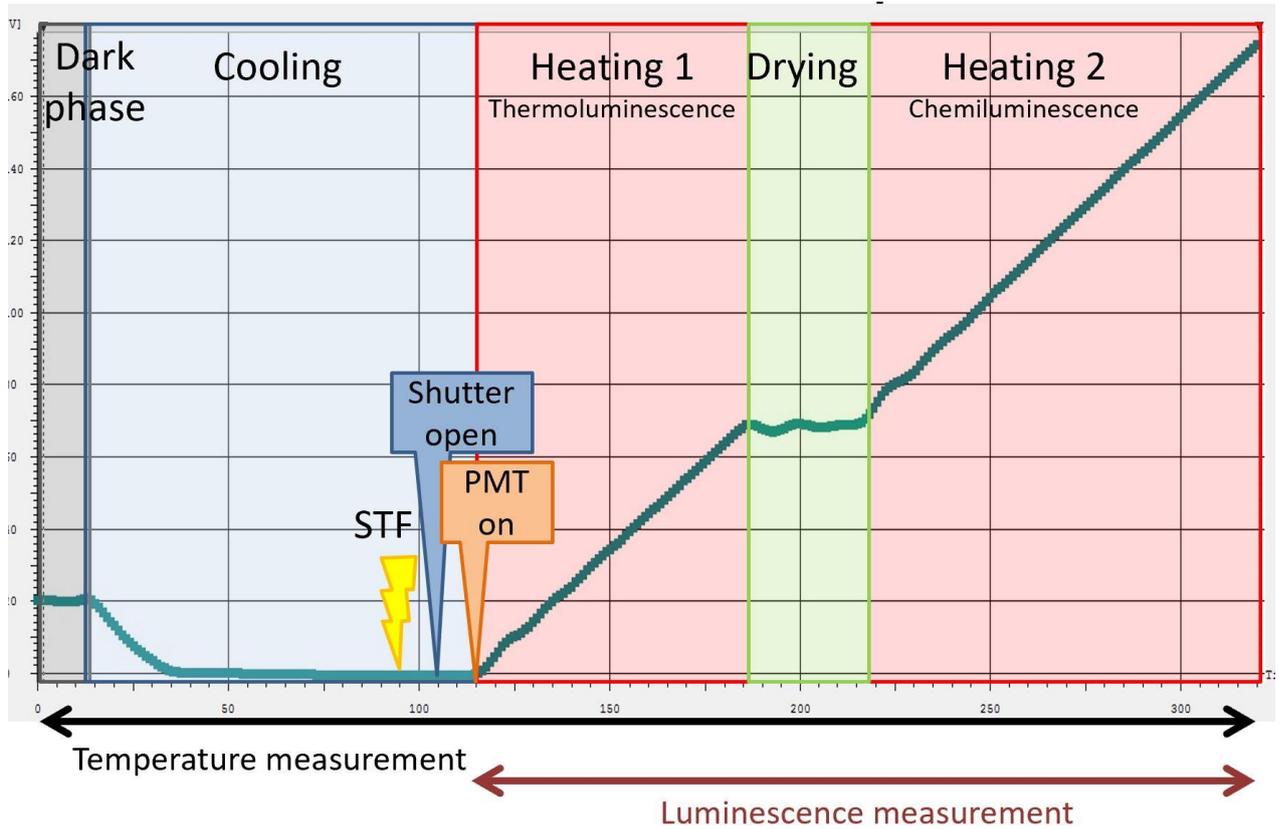


Fig. 33 Visualization of protocol for extended version.

### Typical Thermoluminescence experiment signals description

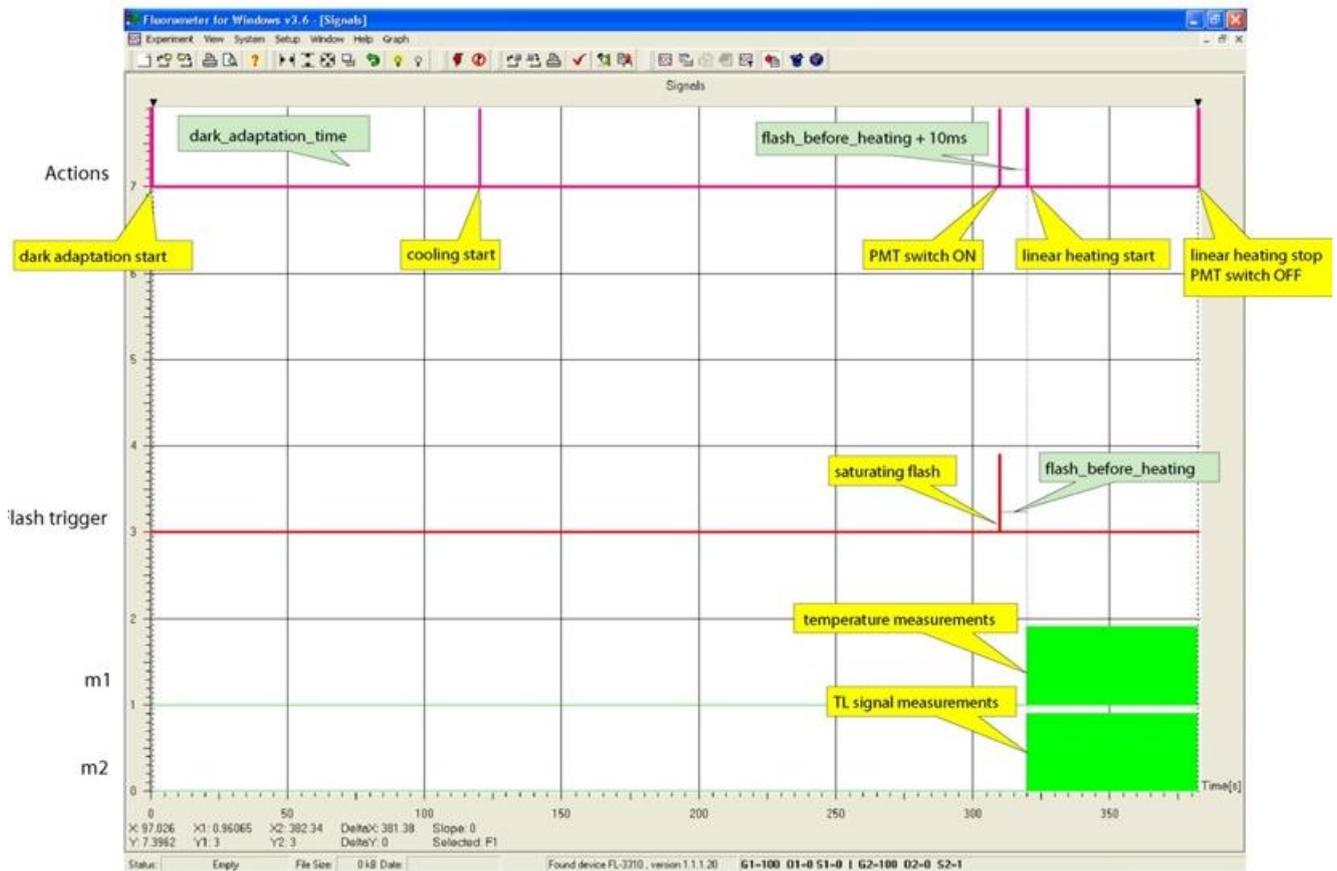


Fig. 34 Description of typical thermoluminescence experiment signals.

### 7.3 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. 35). 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.

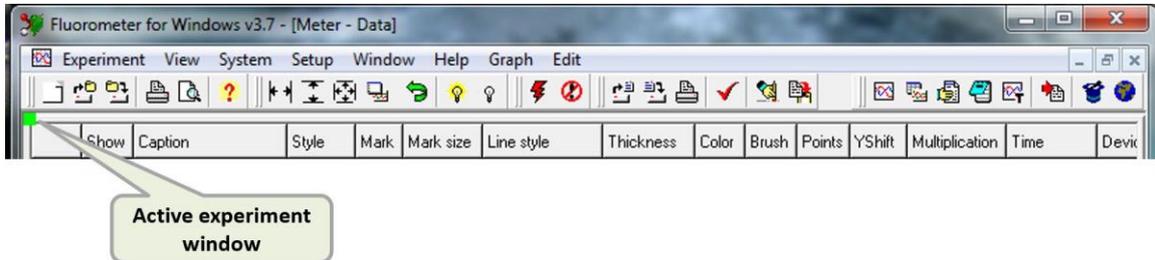


Fig. 35 Active experiment window.

- Each Thermoluminescence experiment consists of 5 information blocks: **Graph, DataSet table, Protocol, Notes and T-Graph.**

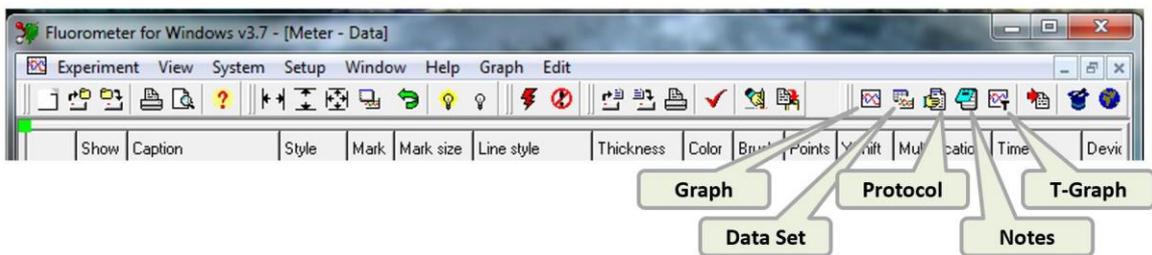


Fig. 36 Icon bar - experiment.

#### 7.3.1 GRAPH (F5)

- The Graph window presents the experimental data (Fig. 37).

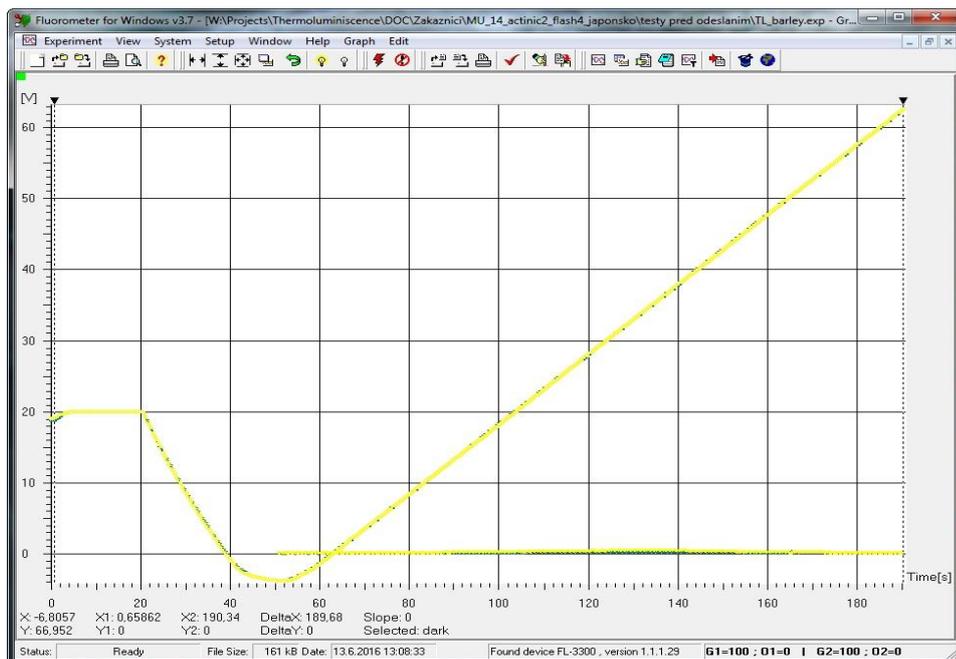


Fig. 37 Graph showing the experimental data – temperature and thermoluminescence curve.

- The graphic presentation can be adjusted by **Graph menu** on the upper bar (Fig. 38).

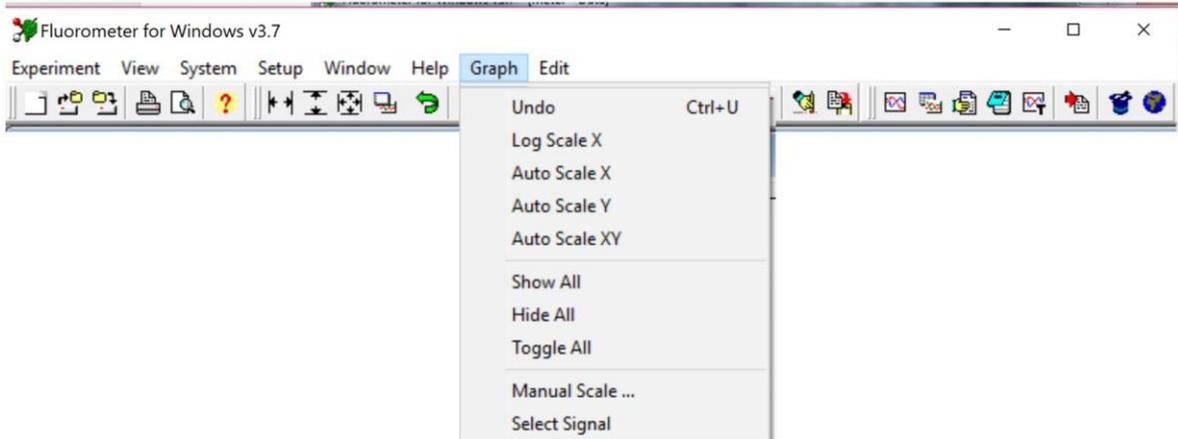


Fig. 38 Graph menu.

Basic options of Graph menu are placed directly on the upper bar (Fig. 39).

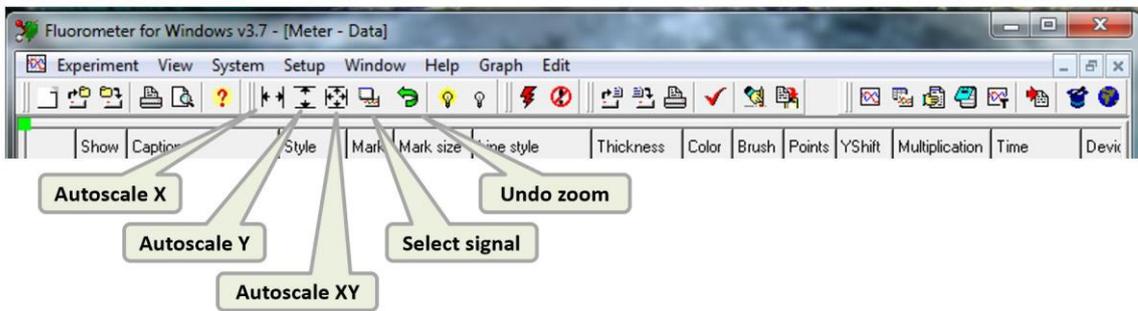


Fig. 39 Graph option on upper bar.

- 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. 40).

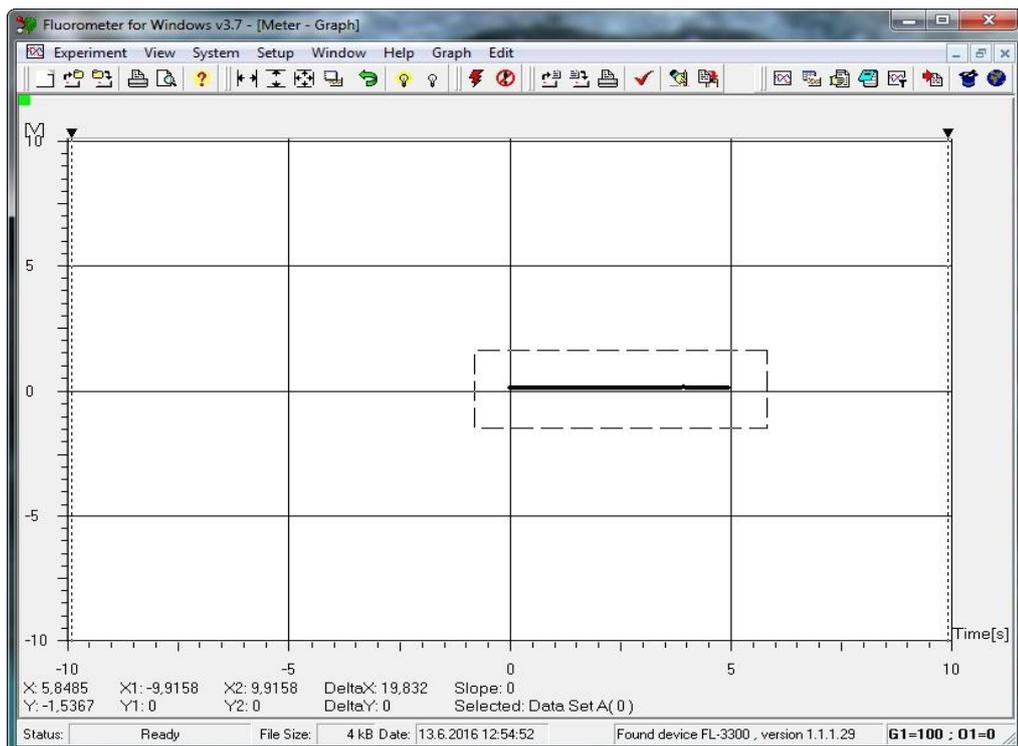


Fig. 40 Zoom in.

- For **zoom out** use right mouse button or autoscale functions.
- 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. 41) or left mouse button/ single click on the yellow rectangle with the name of the data set.

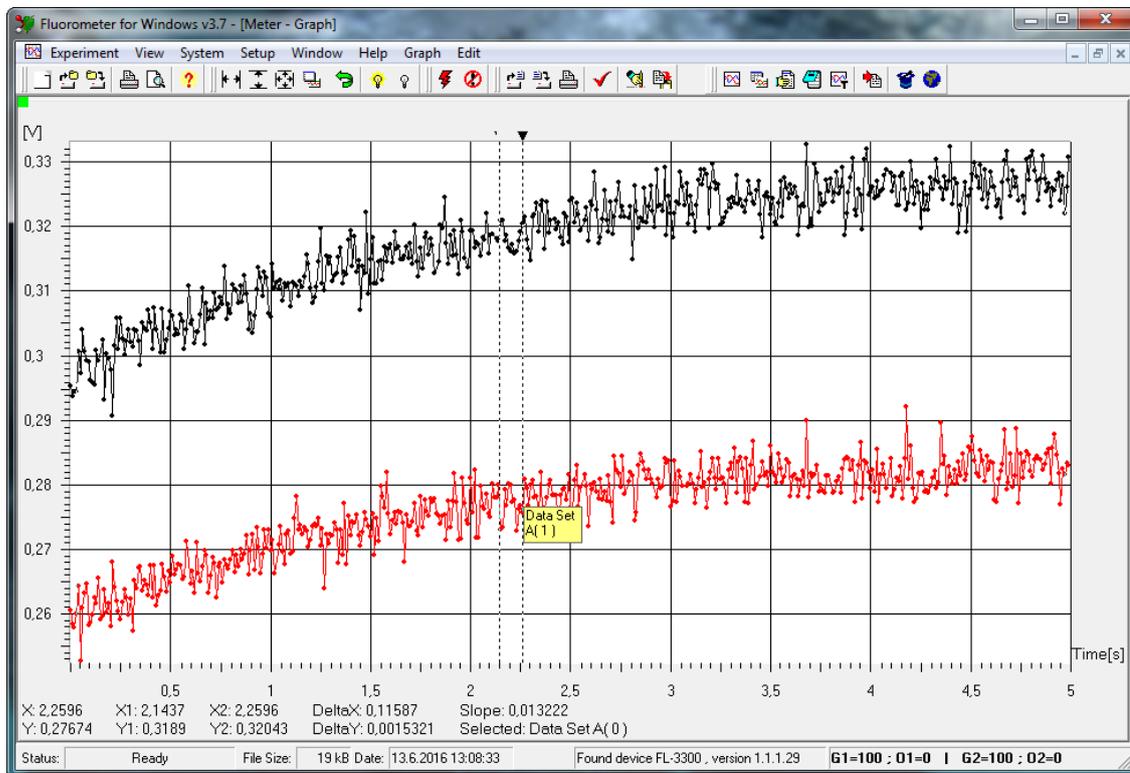


Fig. 41 Graph cursors.

- All data displayed in the graph are shown in the table Graph > Select signal (Fig. 42). DataSet is selected by the left mouse button double-click. Name of the selected DataSet will appear under the graph.

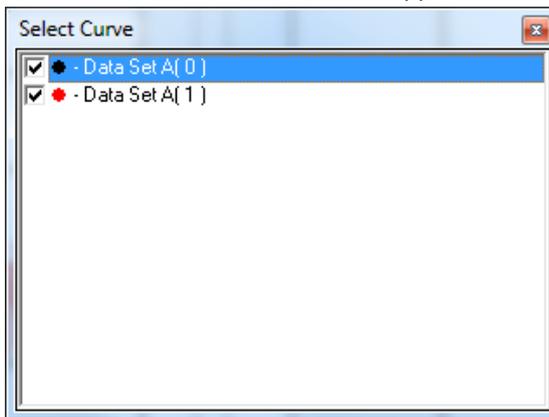


Fig. 42 Data displayed in the graph.

### 7.3.2 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 luminescence 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)).

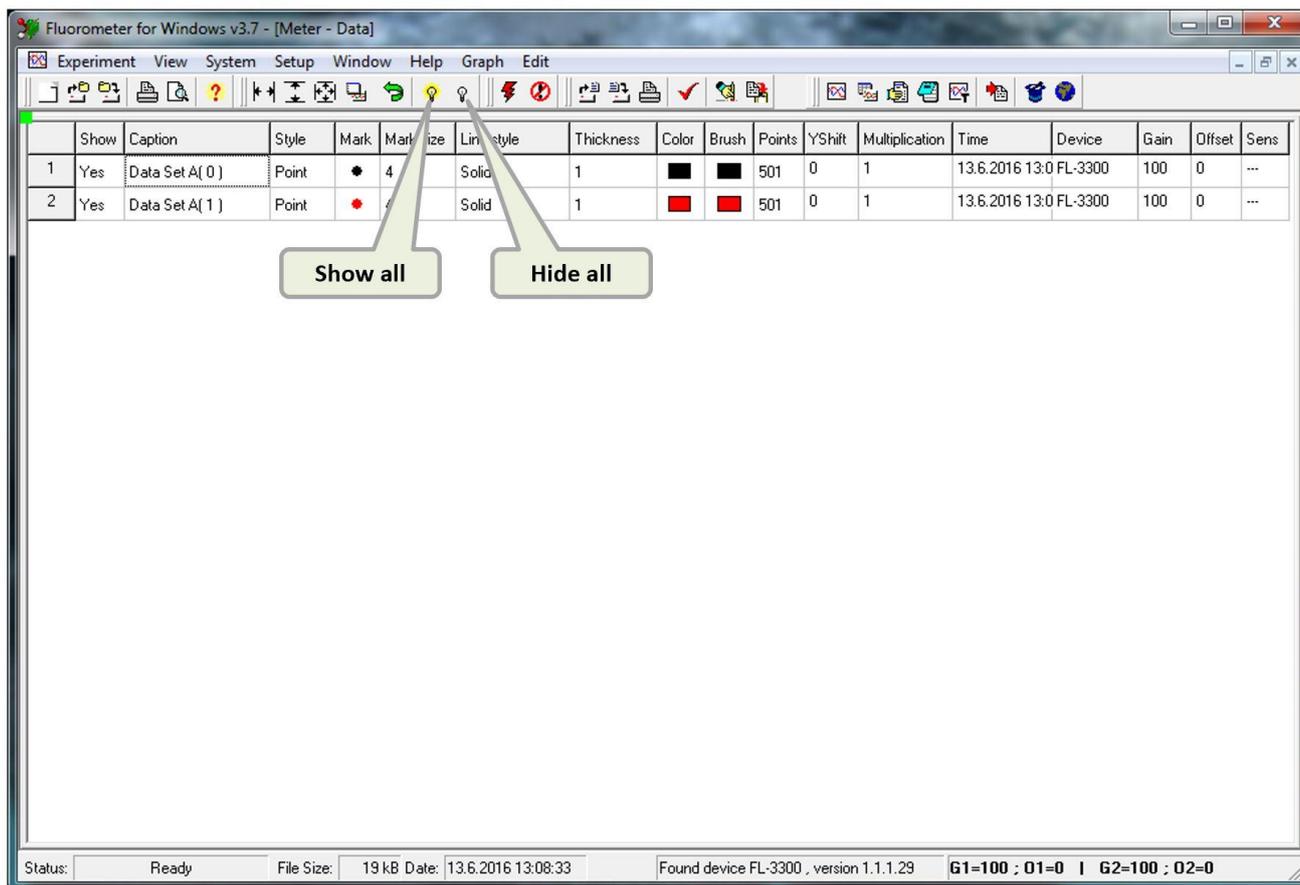


Fig. 43 DataSet table.

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.

	<p>Maximum number of DataSets in the experiment is restricted in FluorWin.ini for 80.</p>
---	---

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. 44).

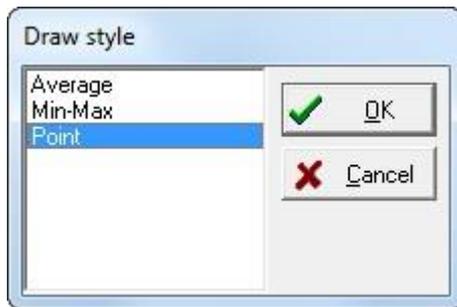


Fig. 44 Draw style.

- **Mark** is used to change the look of the data marker style of the DataSet in graph (Fig. 45).

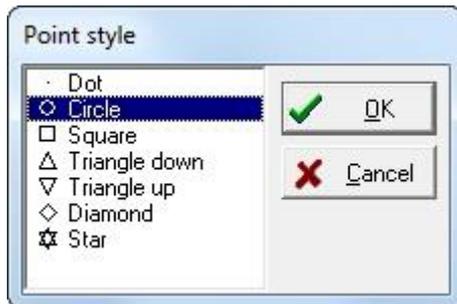


Fig. 45 Point style.

- **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. 46).

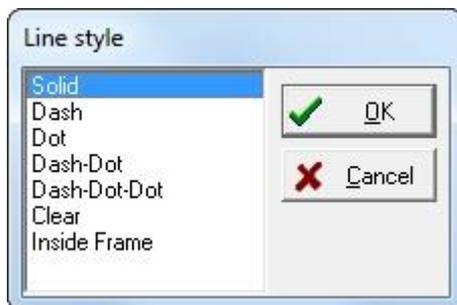


Fig. 46 Line style.

- **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. 47).

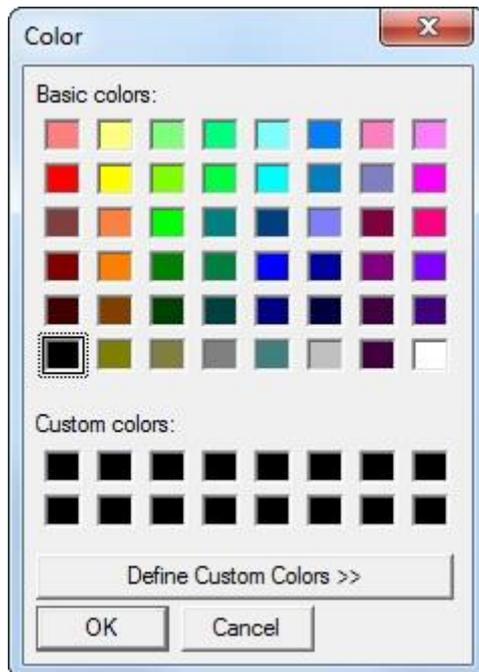


Fig. 47 Color of the DataSet.

- **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.

### 7.3.3 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.

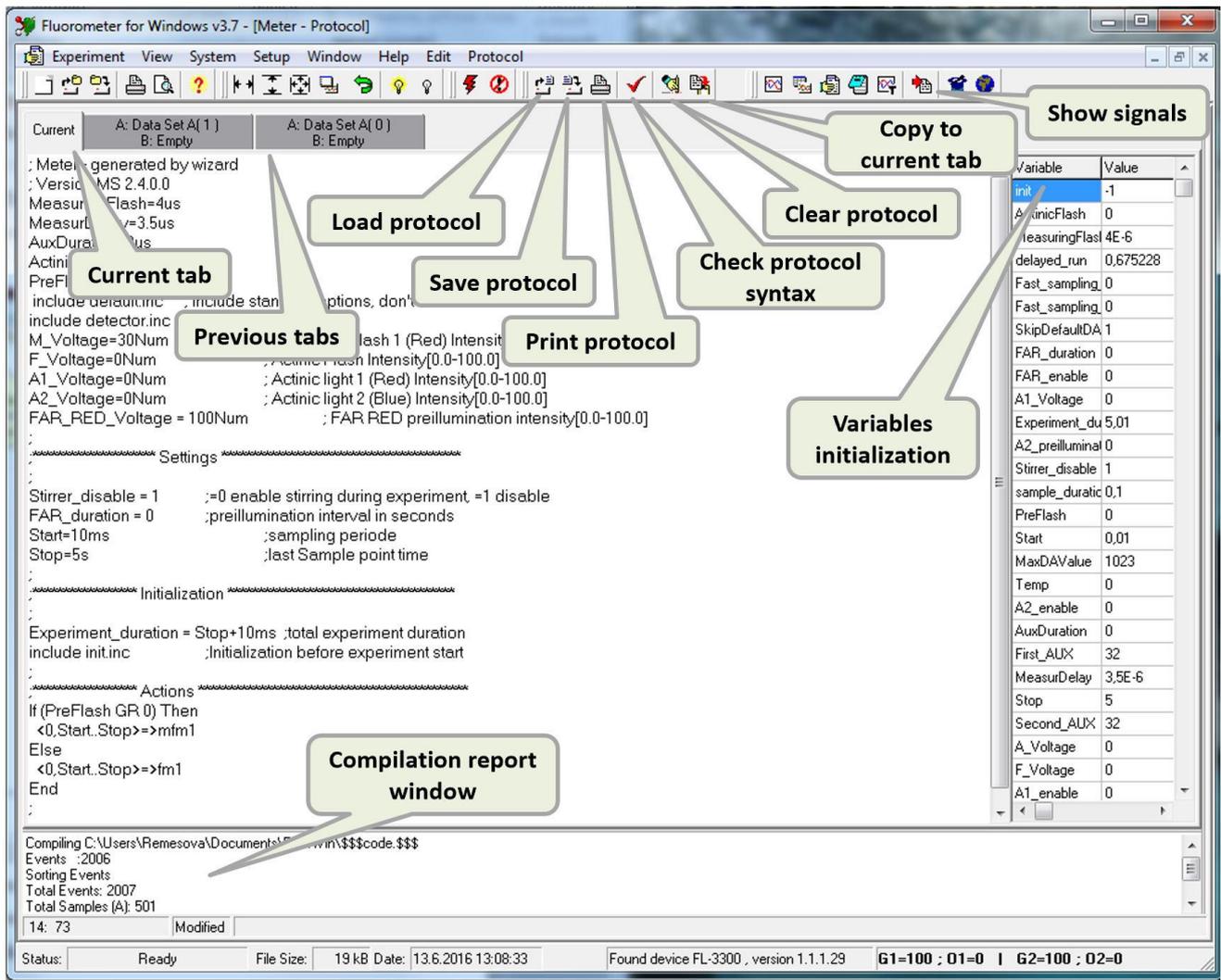


Fig. 48 Protocol window - options.

- 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 main parts: Protocol header, Experiment settings and Experiment body (Fig. 49). 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.

	<p>It is not recommended to make changes in Experiment body without prior study of protocol syntax.</p>
---	---

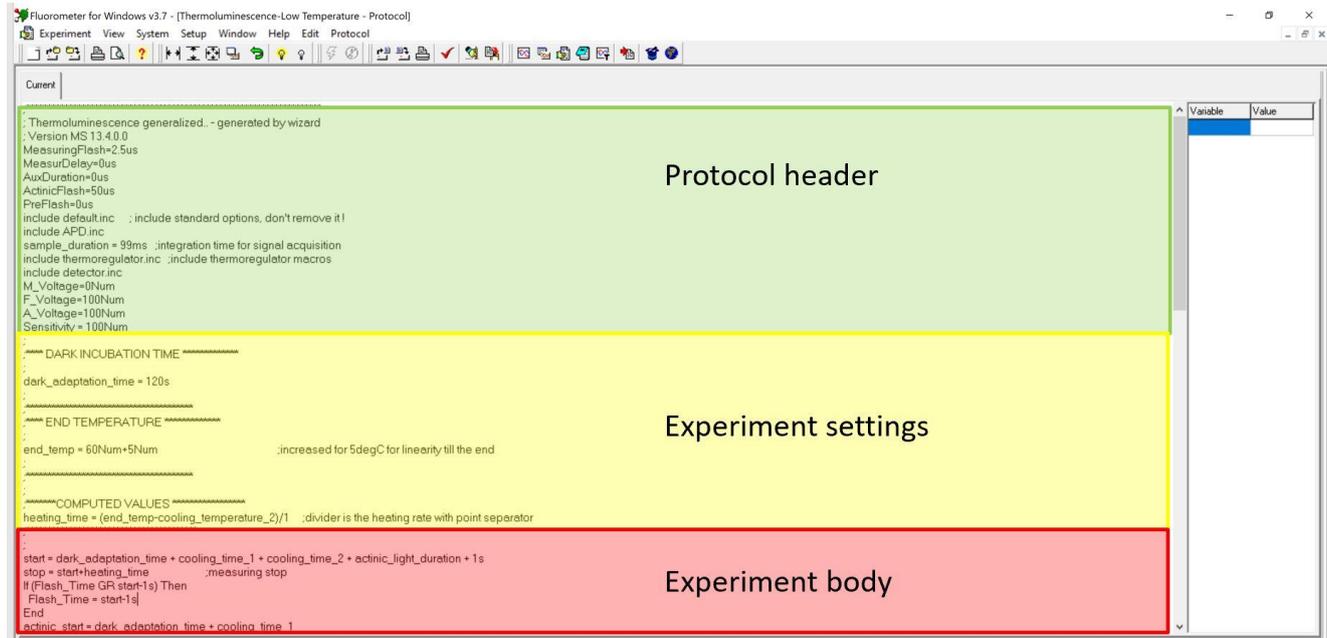


Fig. 49 Protocol description.

- 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.

### 7.3.4 NOTES (F8)

The **Notes** window allows to make notes about experiments and to save them with the experiment data (Fig. 50). 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. 50 Experiment notes.

### 7.3.5 T-GRAPH WINDOW

The T-Graph window is a graph window used for presenting results of **Thermoluminescence** experiment (Fig. 51, Fig. 52).



Fig. 51 T-graph.

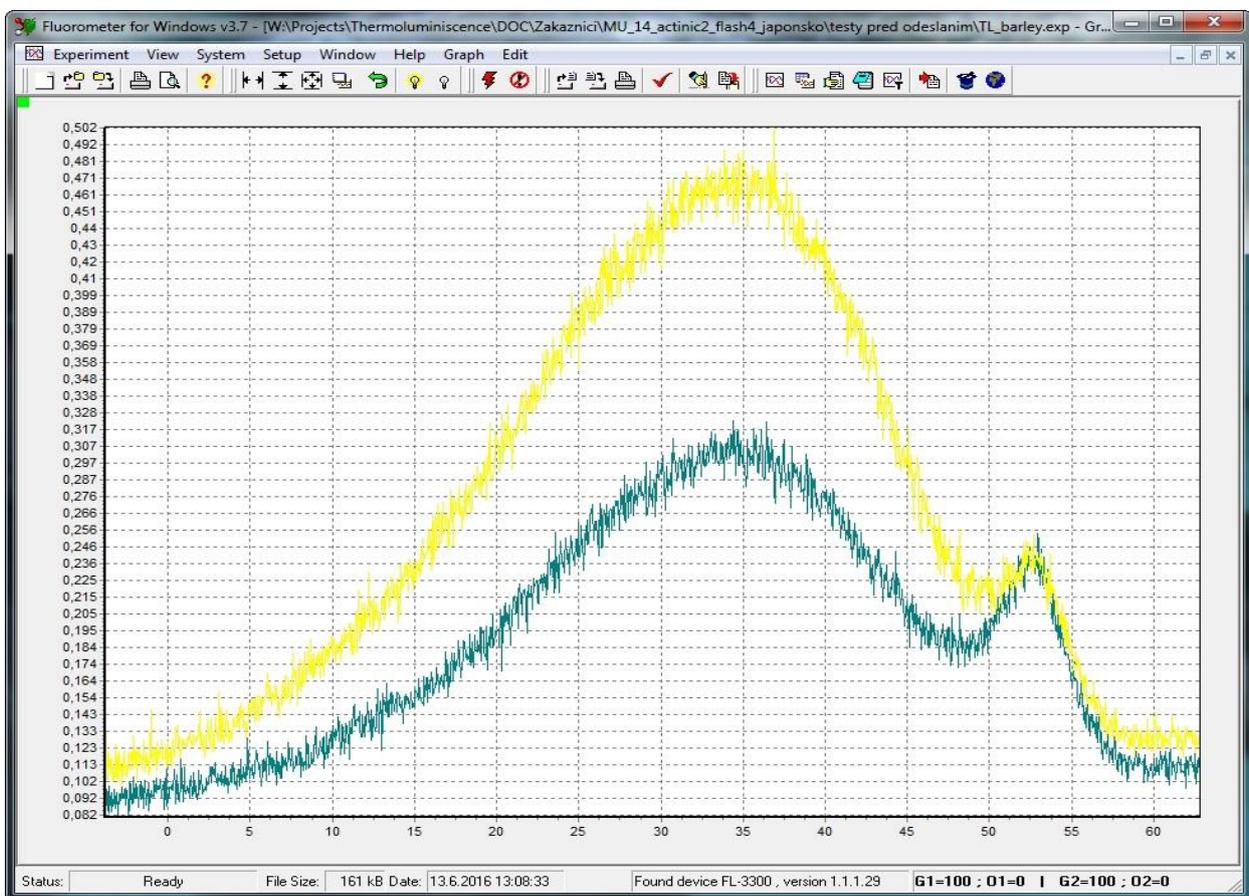


Fig. 52 T-graph showing thermoluminescence signal depending on the temperature.

### 7.3.6 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. 53).



Fig. 53 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.

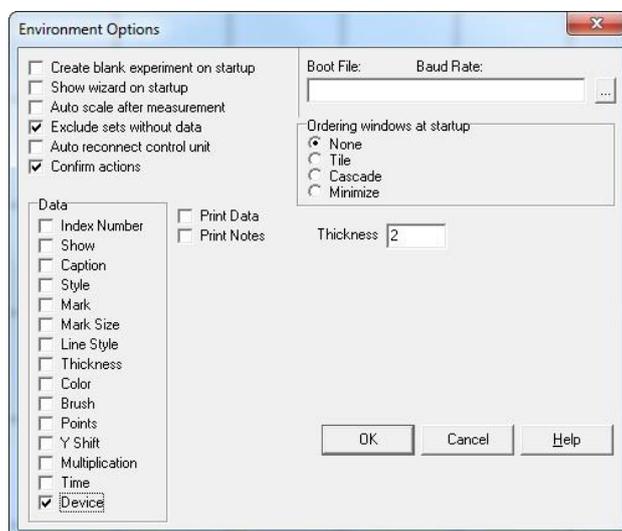


Fig. 54 Environment option menu.

## 7.4 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.

Thermoluminescence measuring unit is always connected to Channel 1. Left part of the System Monitor window belongs to Channel 1 (Fig. 55). Channel 2 is no active.

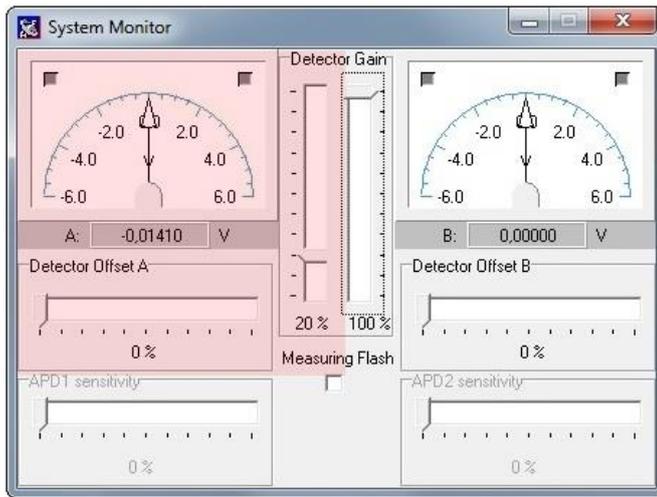


Fig. 55 System Monitor window (highlighted part belongs to Measuring unit detector).

### 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). Gain function is shown in Fig. 56 on fluorescence data measured using Fluorometer, the principle is the same for thermoluminescence data.

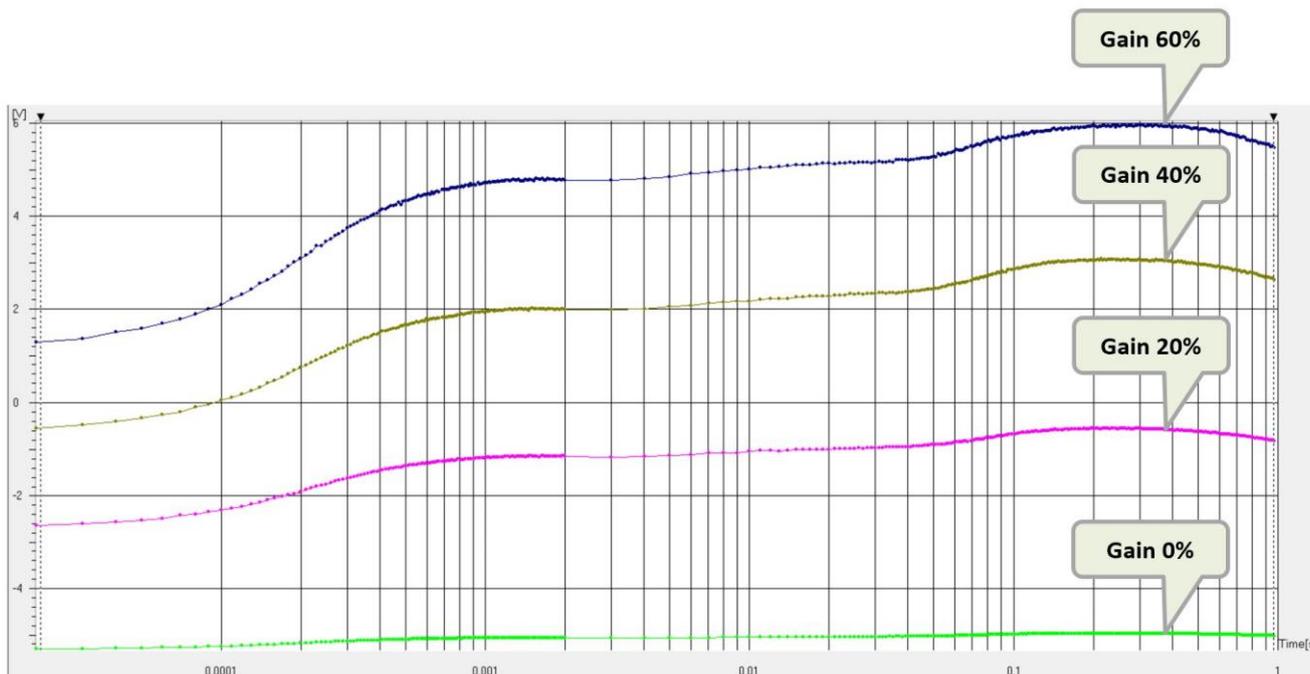


Fig. 56 Effect of different gain setting on fluorescence data (Fluorometer).



Please note that Gain setting in percentage is not linear with corresponding voltage on the 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. Example of offset function is presented in Fig. 57 on fluorescence data measured using Fluorometer, the principle is the same for thermoluminescence data.

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.

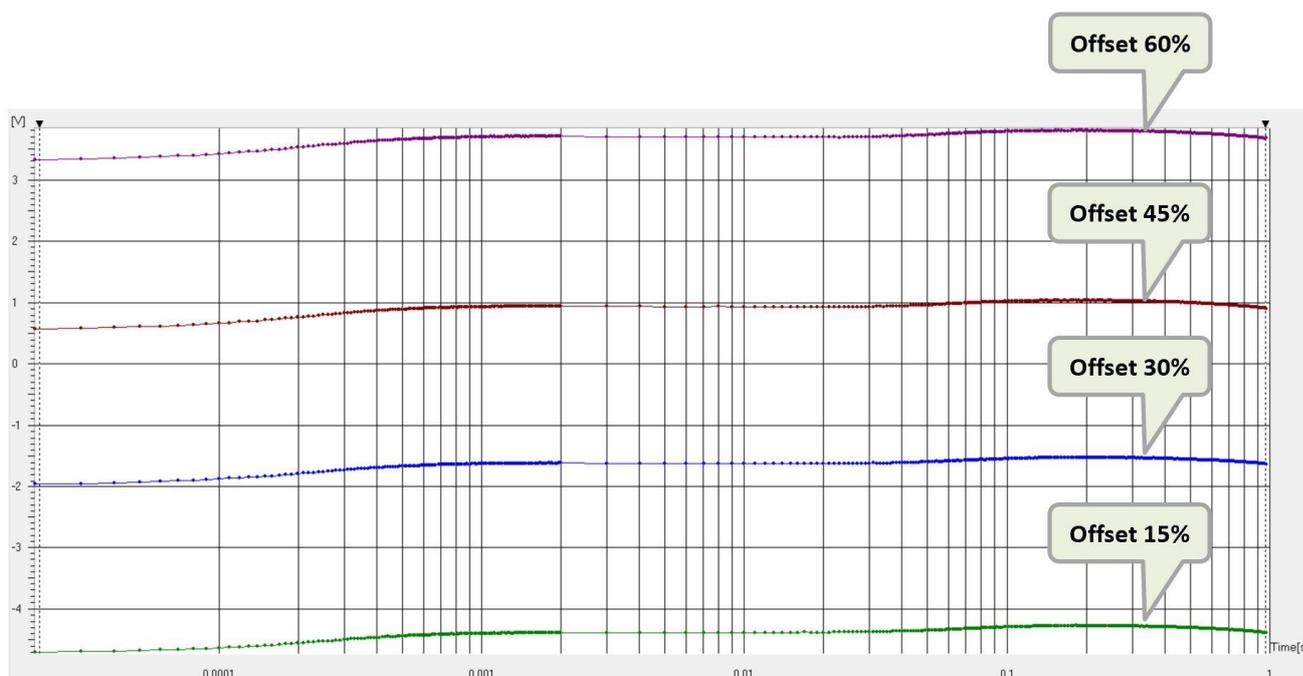


Fig. 57 Effect of different offset setting presented on OJIP curves (Fluorometer).



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.

## 7.5 PROTOCOL SYNTAX

### 7.5.1 SUMMARY OF THE PROTOCOL HEADER SYNTAX

	<b>Interpretation</b>	<b>Typical</b>	<b>Units</b>
ActinicFlash=	Duration of the Actinic Flash (F1)	15 – 100 $\mu$ s	$\mu$ s
include default.inc include default1.inc	Default setting file for red/blue measuring pulses		
include APD.inc			
sample_duration	Integration time for signal acquisition		ms
include thermoregulator.inc	Default setting file for thermoregulator		
include detector.inc	Default setting file for detector		
F_Voltage=	Relative power of the Actinic Flashes	40 – 100 %	
A_Voltage=	Relative power of the red Actinic Light	5 – 100 %	
Sensitivity=	Sensitivity of the detector	0 – 100%	



Variables in header syntax that are not mentioned are not active for Thermoluminescence device.

## 7.5.2 TIMING OF PROTOCOL SYNTAX

### Timing unit declaration:

Time unit ns ( $\mu$ s, 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  $\mu$ s           ; first declaration
<100> => m2           ; timing is 100 $\mu$ s for temperature measurement Action.
<200> => m2           ; timing is 200 $\mu$ s
<1ms> => F1           ; locally defined (ms) timing for Actinic Flash overrides the previously declared timing ( $\mu$ s)
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 = <100 $\mu$ s>           ; a new variable named n is introduced and set to 100 $\mu$ s
```

### Definition of an arithmetic timing series:

name = <first,second..last>

Example:

```
k = <100 $\mu$ s,200 $\mu$ s..0.5ms> ; an arithmetic timing series named k is introduced including 100 $\mu$ s, 200 $\mu$ s, 300 $\mu$ s, 400 $\mu$ s and 500 $\mu$ s.
```

### Definition of a logarithmic timing series:

name = [first,second..last]

Example:

```
j = [10  $\mu$ s,100  $\mu$ s..10s] ; a logarithmic timing series named j is introduced including 10  $\mu$ s
```

*Combination of two timing series:*

Name1|Name2

Example:

```
k|j ; generates a new timing series combining all element of series k and all elements 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 => m1 ; Action m1 (measure signal on 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-CONTROLLED 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. 58 Navigator.

- The Test Keys Window (Fig. 59) sliders allow manual setting of the voltage for Actinic Flash and Actinic Light. Pressing Signals buttons activates LEDs for default duration. For checking the LED function move the Voltage slider of the tested light at least on 60 %. Then look from 20 cm distance into the open measuring unit, press respective button and check visually correct functioning of particular LED.

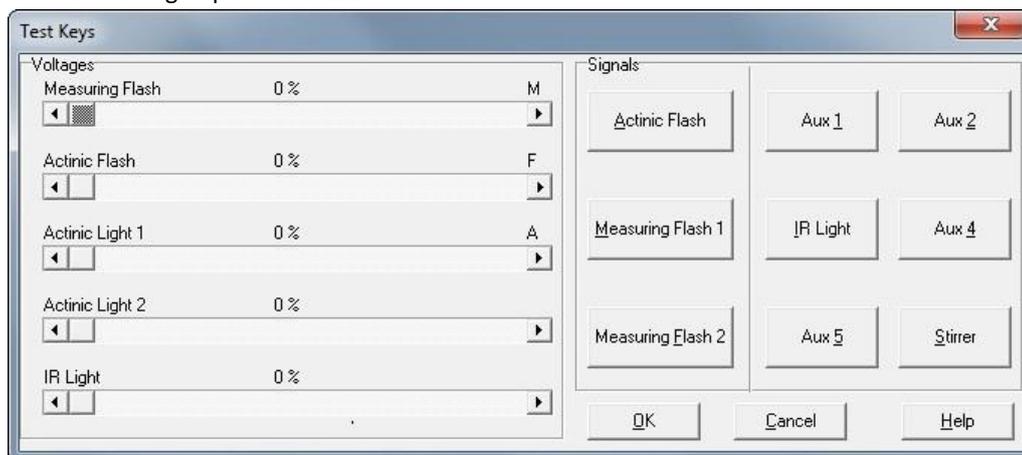


Fig. 59 Test Keys window.

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

## 8 SAMPLE PREPARATION

### Suitable samples for Thermoluminescence measurement

- Cell cultures (algal, cyanobacterial, bacterial suspensions)
- Plant (leaf) segment
- Isolated components of photosynthetic apparatus (PSII-particles-BBY preparation, reaction centers from photosynthetic bacteria, liposomes, etc.)

### Liquid samples

1. Before measurement concentrate the sample using gently centrifugation, sedimentation, etc. The thermoluminescence signal is very weak, therefore is necessary to measure highly concentrated samples optimal with OD<sub>680</sub> in range 10 - 15.



Please note that the centrifugation can cause cell damage, that affects results of the measurement. Concentrate the sample based on the species requirements.

2. It's recommended to dark-adapt the sample before measurement.
3. Use two pieces of microscopic cover-glass. Apply 8 -16  $\mu$ l of sample on first cover-glass (Fig. 60A) and cover it with the second cover-glass (Fig. 60B). Cover-glasses ensure homogeneous distribution of the sample and simpler exchange of samples between measurements without sample disc cleaning.

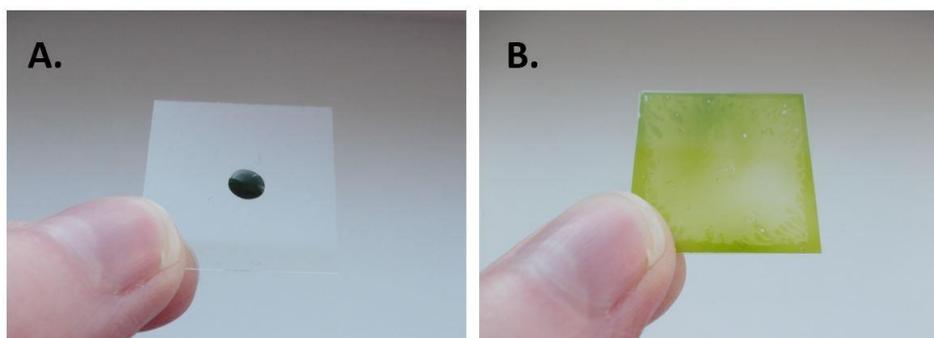


Fig. 60 Liquid sample preparation.

### Solid samples

1. For measuring of leaves or parts of plants, is recommended to avoid parts with vessels. To get reproducible results it is highly important to be in the tight contact with the sample disc, and thus ensure homogeneity of the sample heating.
2. Every leaf sample is producing different signal intensity and thus the setting of the sample size and setting of detector sensitivity are highly important. Smaller pieces of leaf samples are better, because the tight contact with the sample disc is ensured, and amount of vessels is minimized. To produce the optimal plant disc size use the hollow punch set (Fig. 61A).
3. To fasten the sample to the sample disc, use the metal grid and the teflon ring (Fig. 61B).



Please note that leaf waxes and trichomes can disturb result of the measurement.

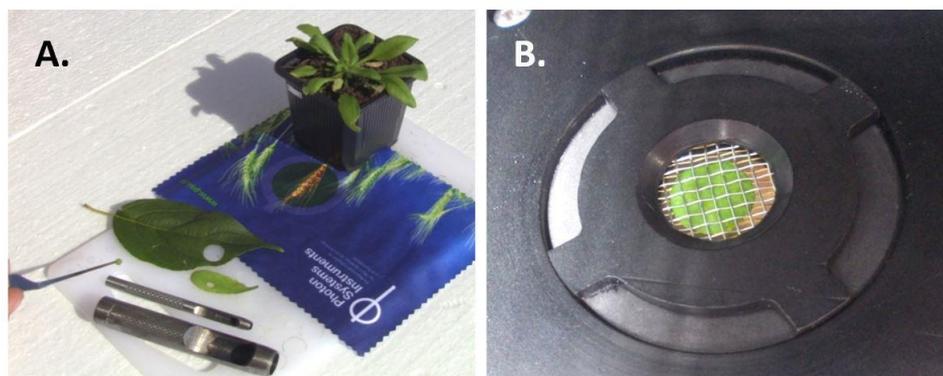


Fig. 61 Leaf sample preparation.



Above mentioned methods for samples preparation are general recommendations. Follow your own experience with the organism you are working with.

#### Important notes

- Before each TL experiment, clean the measuring surface using distilled water and dry it carefully.
- Before performing the experiment make appropriate measurement using water/medium/buffer to make sure that there are not contaminants on the measuring surface which could affect the TL signal.
- To get repeatable results, perform all your TL measurements in same conditions (dim light, same volumes of samples, time of centrifugation, etc.).
- All the FluorWin protocols entire the dark adaptation step. Nevertheless it is recommended to keep your samples in darkness also before measurement.

## 9 WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the Thermoluminescence 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.

## 10 TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of troubles and for customer support, please, visit [FAQ](#) on our websites, write to [support@psi.cz](mailto:support@psi.cz) or contact your local distributor.