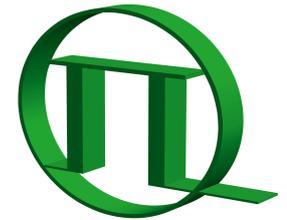


Setup Manual



PICOQUANT

Automated, Compact, High Performance
Fluorescence Lifetime Spectrometer

Unpacking and setting up the hardware



Setup Manual

Document version 1.0.2

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Dear customer,

Remote Support Sessions

Thank you very much for purchasing a FluoTime 250 lifetime spectrometer. We would like to offer you a special service: **remote support**. In order to arrange for a live remote session, please contact the PicoQuant Support Team at:

<https://support.picoquant.com>

or

support@picoquant.com

Contacting us via one of these channels will allow us planning and organizing remote support sessions together with you. Thank you again for choosing a PicoQuant product.

Sincerely, the PicoQuant Support Team.

Included Documentation

Your FluoTime 250 is delivered with an extensive documentation including the following items, ranked in the order in which you should consult them:

1. Setup Manual for the FluoTime 250

The printed Setup Manual describes how to unpack and set up the FluoTime 250. Please keep the Setup Manual in a safe place along with the packaging material in case you need to ship the FluoTime 250 somewhere.

2. User's Manual

The document you are currently reading. Besides containing a detailed description of the FluoTime 250 system, its hardware components, this manual includes the wiring scheme, laser and electrical safety information as well as a troubleshooting guide. It is available both in printed and digital form (PDF document).

3. Additional components manuals

Additional manuals for individual components such as laser driver and TCSPC electronics are also available in printed as well as PDF format.

4. Online FluoTime 250 tutorials

A collection of interactive tutorials can be found under <https://fluotime250.picoquant.com>. To log into the web site, please use the user name and password provided in the "README.TXT" that can be found on the USB thumb drive that was delivered with your system. If the USB thumb drive was lost, you can contact PicoQuant's support team at support@picoquant.com. Note that the online tutorials can be downloaded for offline use. To do so, use the "save page as..." command (CTRL + S) of your browser (Mozilla FireFox or Google Chrome; does not work with Microsoft Edge) and save the desired pages as a HTML file.

5. USB thumb drive

A USB thumb drive is included which contains all manuals in PDF format as well as a collection of scripts for carrying out performance checks of the FluoTime 250. The USB thumb drive also includes a "README.TXT" file that will provide you with the login credentials (user name and password) for the collection of online tutorials for the FluoTime 250.

6. EasyTau 2

The system and analysis software EasyTau 2 features an extensive, integrated help function that can be accessed by pressing the F1 key. Additionally, useful tool tips for all controls appear when you hover the mouse over the control element for a while.

7. Maintenance Manual

This manual describes how to handle technical difficulties and how to prepare the FluoTime 250 for shipping.

1. General safety information



CAUTION!

Before using this device, make sure that you have read and understood the content of this setup manual. Store this document in a safe and easily accessible place for future reference.

Incorrect handling of this product may result in personal injury or physical damage. The manufacturer assumes no responsibility and cannot be held liable for any injury / damages resulting from operating the device outside of the normal usage defined in this manual.

To unpack the crate in which the FluoTime 250 was shipped always use protective gloves and strictly follow the instructions given in this manual.

1.1. Warning Symbols and Conventions

The following symbols and conventions will be used throughout this manual. Please take time to familiarize yourself with their meaning before proceeding.

	The general safety alert symbol is used to alert you to hazards that may lead to personal injury or physical damage. Follow all associated safety instructions to avoid possible injury or death.
	A high voltage warning symbol is used to indicate the presence of un-insulated, dangerous voltage inside the enclosure. Note that this voltage may be sufficient to constitute a risk of shock.
	The laser radiation warning symbol alerts you that the device can generate laser radiation. Follow all applicable laser safety instructions to avoid injury or damages.
	The device's susceptibility to electrostatic discharge (ESD) is indicated by the ESD warning symbol . Ensure that you follow proper ESD protection rules to avoid damaging the device.
CAUTION!	Make sure to follow any instructions prefaced with " CAUTION! " to avoid personal injury or damaging the device.
WARNING!	The " WARNING! " label prefaced any instructions that shall be followed to avoid severe injury or death.
NOTICE	Important tips and information for device operation that do not include a risk of injury or damage are prefaced with the " NOTICE " label.
	This symbol indicates that an earth terminal shall to be connected to the ground (to avoid risks of electrical shock).

1.2. Electrical Safety Instructions



WARNING! The connection of all conductive housings, electrical equipment with a grounded protective conductor and with the main earthing bar is the basis for protection against electric shock (*protective earthing*). Therefore all housings and power sockets must be grounded according to the standards **IEC 60364-4-41:2005** and **DIN VDE 0100-410:2007-06** (for Germany).

The technical design for the equipotential grounding, the dimensioning of the cross-sections and the standardized terms are given in the international standard **IEC 60364-5-54: 2011** and the **DIN VDE 0100-540: 2012-06** (for Germany).

NOTICE To realize a *functional earthing* between all components, the FluoTime 250 must be grounded together with the PC, the PDL 820 laser driver as well as the PicoHarp 300 TCSPC unit (if present). If your lab supplies a functional earthing connection following the standards mentioned above, we recommend to connect the FluoTime 250 to it via the PDL 820 laser driver grounding pin. Please contact your electrical safety officer / facility staff in charge of electrical installations to ensure that equipotential grounding according to the standards mentioned above has been achieved before operating the FluoTime 250.

Never connect or disconnect any cable while the system is powered ON. Charged signal cables can damage electronic devices!

This device contains electrical components that are not user serviceable. Servicing of these internal electrical components is restricted to qualified personnel.



Disconnect the power cord from the electrical outlet before performing any maintenance.

1.3. Laser Safety Instructions



WARNING! Visible and invisible laser radiation

The FluoTime 250 can be used with laser diode heads that can emit visible, infrared, or UV light. Infrared or UV light is not visible to the eye! **These diode lasers can emit laser light of up to class 3b / IIIb.** Please refer to the labels affixed to the laser head for information on classification.

Lasers can be hazardous and have unique safety requirements. Permanent eye injury and blindness is possible if lasers are used incorrectly. Pay close attention to each safety related CAUTION and WARNING statement in the user manual. Read all instructions carefully BEFORE operating this device.

The laser diode heads of the LDH Series are manufactured according to the International Laser Safety Standard IEC 60825-1:2014 and comply with the US law 21 CFR §1040.10 and §1040.11.

Required Laser Safety Measures

Please observe the laser safety measures for class 3b / IIIb lasers in accordance with applicable national and federal regulations. The owner / operator is responsible for observing the laser safety regulations.

What does the owner / operator have to observe?

- The owner / operator of this product is responsible for proper and safe operation and for following all applicable safety regulations.

- The owner / operator is fully liable for all consequences resulting from the use of the laser for any purposes other than those listed in the operating manual. The laser may be operated only by persons who have been instructed in the use of this laser and the potential hazards of laser radiation.
- The owner / operator is responsible for performing and monitoring suitable safety measures (according to IEC/EN 60825-1 and the corresponding national regulations).
- The owner / operator is also responsible for naming a laser safety officer or a laser protection adviser (according to the standard IEC/EN 60825-1: "Safety of laser products, Part 1: Classification of systems, requirements and user guidelines" and the respective national regulations).

The following security instructions must be followed at all times.

General Safety Instructions for Operation

- Never look directly into a laser beam or a reflection of the laser beam. Avoid all contact with the laser beam.
- Do not introduce any reflective objects into the laser beam path. This includes jewelry, watches, etc.
- Every person involved with the installation and operation of this device has to:
 - Be qualified
 - Follow the instructions of this manual
- As it is impossible to anticipate every potential hazard, please be careful and apply common sense when operating the laser diode heads and associated driver unit. Observe all safety precautions relevant to Class 3b / IIIb lasers.
- Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

1.4. Laser Safety Labels

On the Laser Device

The safety labels are affixed to the laser head housings, with an arrow pointing towards the laser emission aperture (see Fig. 1 for some examples). The label states the laser product classification, certification, power, and wavelength range relevant for the classification.

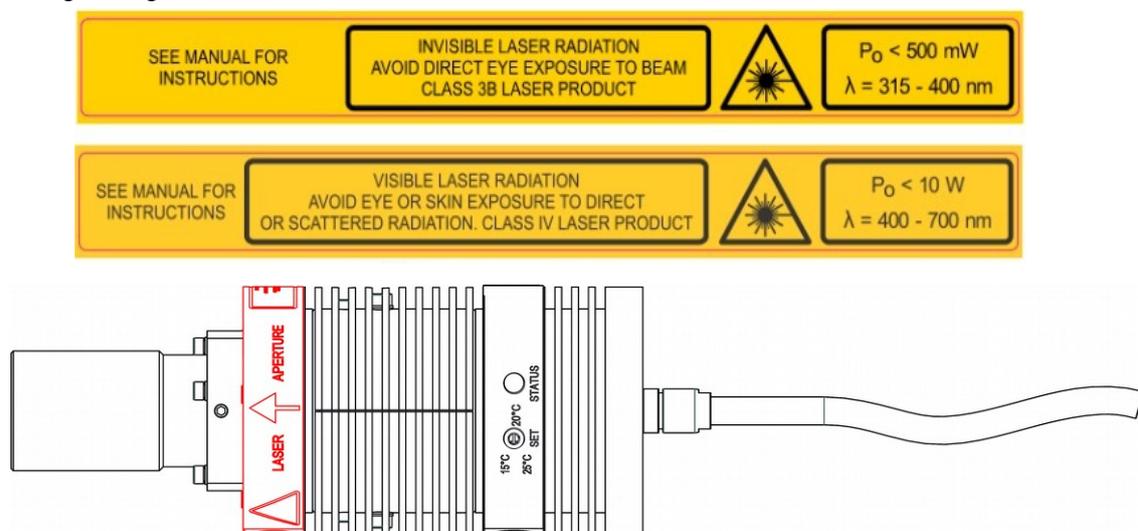


Fig. 1: Example of laser warning, certification, and classification label (top and middle) and location of said label on laser heads from the LDH Series (bottom, highlighted in red color).

Please refer to the *laser delivery report* in the Appendix of the FluoTime 250 User's Manual for information on the central emission wavelength, maximum achievable optical output power, and pulse shape of the delivered LDH laser heads.

On the FluoTime 250

Several laser safety labels are affixed to the housing of the FluoTime 250, which are highlighted in red (Fig. 2).

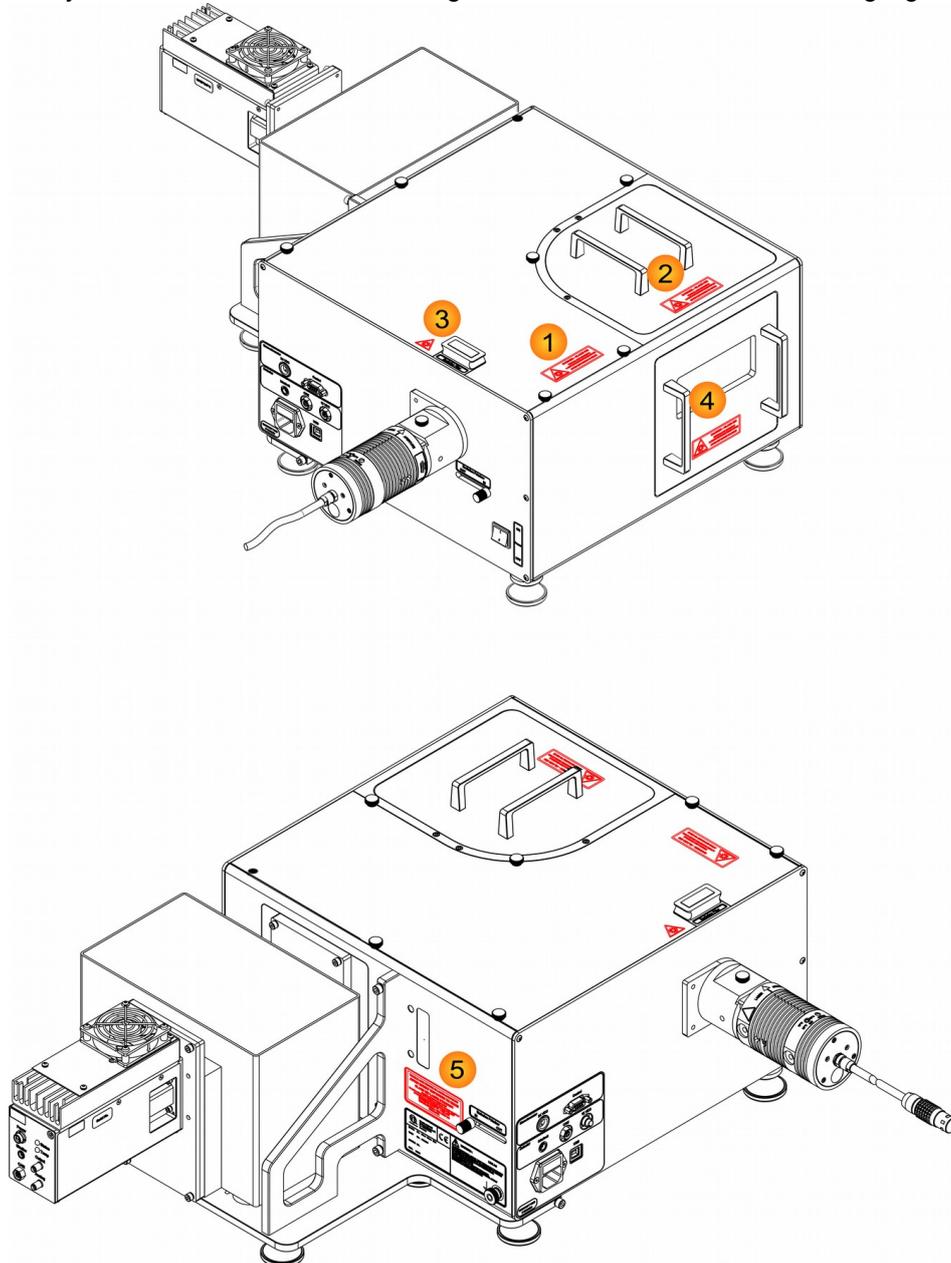


Fig. 2: Location of the laser safety labels on the FluoTime 250.

The system is labeled in 4 positions and the signs indicate that access to a class 3B / IIIB laser beam when:

- 1 The cover to the sample chamber is open
- 2 The holder for the attenuation holders is removed
- 3 The upper cover of the system has been removed
- 4 The sample mounting unit has been removed from the FluoTime 250

An additional label (5) is located at the side of the FluoTime 250.

1.5. Remote Interlock Connector

A hardware lock as well as a remote interlock connector are part of the laser driver that is included with your FluoTime 250. Removing the connector or breaking the interlock circuit will immediately deactivate the power supply of the laser.

In order to meet laser safety regulations, you may need to install a remote interlock, e.g., a door switch, to deactivate the power to the laser when the door to the laser area is opened.

Please refer to the corresponding laser driver manual for further details on the remote interlock connector.

1.6. Third-party lasers

PicoQuant does **NOT** endorse the use of non-approved third-party laser devices with the FluoTime 250 system. The end-user should not connect non-approved laser devices to or operate the FluoTime 250 with them.

PicoQuant declines any responsibility and cannot be held liable for any direct or indirect damages to the users and/or instrumentation resulting from the use of non-approved third-party laser devices by the end-user.

CAUTION! Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure!

2. Unpacking the FluoTime 250

If you need to store the FluoTime 250 before installation, make sure that the closed crate is **stored in a dry place (<70% rel. humidity) and at temperatures between +15° and +30 °C.**

Before installing the system, we recommend to let the crate (with its content still packed) stand in the room where the system will be finally located for at least 1 day.

2.1. Necessary Tools

Use the following tools (shown in Fig. 3) to unpack the FluoTime 250:

- 2 pairs protective gloves, as 2 people are required to lift the FluoTime 250 from the crate
- One utility knife with automatically retracting blade
- The special lever fixed to the outer wall of the wooden shipping crate. This tool is needed to remove the metal brackets holding the crate's walls together. This lever can be removed with a Phillips screwdriver.



Fig. 3: Tools for unpacking the packaging box in which the FluoTime 250 was delivered.

CAUTION! Please note that 2 persons are required to lift the instrument out of the crate!

2.2. Before Unpacking

The FluoTime 250 system arrives in a wooden shipping crate. Upon receipt, check the crate for any visible damage. The crate is also equipped with shockwatch sensors (Fig. 4). Please check whether the shockwatch sensors have been “activated”. An undamaged shockwatch is shown in Fig. 4. If any sensor has been triggered or the crate is damaged, document this immediately by taking photos and contact PicoQuant or your local distributor as well as the freight company. Make sure that the delivery agent / driver sees the damage. Write it down on the shipping documents and let the driver acknowledge it by having them sign the delivery report.



Fig. 4: Shockwatch sensor.

The delivery note is attached to the top lid and contains a list of all delivered parts. You can use it to check whether all ordered parts have actually been delivered. Please note that some parts (e.g., filters or polarizers) are already mounted into the FluoTime 250 system.

2.3. Opening of the wooden shipping crate

Please follow the procedure outlined below to unpack the FluoTime 250 system:

- Wear protective gloves
- Cut the strips and tape around the crate
- Use the lever to remove the metal brackets. Start by removing the brackets of the lid as indicated in Fig. 5:



Fig. 5: Removing the metal brackets using the lever. Start by sliding the lever under one of the metal bracket's grooves and lift it away from the wooden wall while holding the top of the bracket (1). Remove the bracket as shown in (2)

- Lift the lid off.
- Remove the brackets holding the side walls in place using the same procedure as shown above.
- Remove all four side walls.
- Carefully lift the different items out of the box. **Since the FluoTime 250 itself is rather heavy (ca. 50 kg), it needs be lifted out of the box by 2 people.** Please check and observe local safety guidelines regarding lifting heavy items.
- Remove the FluoTime 250 as well as other parts of the spectrometer (e.g., laser driver PDL 820, computer, laser heads etc.) from their cardboard boxes.
- Inspect all parts for damages. If any part shows external damage, take photos of the damage and inform PicoQuant or your local distributor immediately.

3. Setting up the FluoTime 250

Here we assume that all parts of the FluoTime 250 spectrometer have been removed from their respective boxes, had all packaging materials removed (e.g., bubble wrap), and properly identified. Place the FluoTime 250 in its final position. Place the computer and monitor next to it. Leave some space for the PDL 820 laser driver close to the laser entrance port of the FluoTime 250. If you have a PicoHarp 300 TCSPC unit, place it within (cable) reach of the computer connected to the FluoTime 250. The PicoHarp 300 can be stored on a shelf under the table, for example.

3.1. Functional Grounding of the FluoTime 250 spectrometer

CAUTION! *The functional grounding of the FluoTime 250 has to be done before connecting any other cables to the system!*
If your lab supplies a functional earthing connection according to the standards mentioned below, we recommend connecting the FluoTime 250 to it via the PDL 820 laser driver. Please check with your electrical safety officer or facility staff in charge of electrical installations to ensure that the grounding has been done according to the standards cited below (IEC 60364-5-54: 2011 and/or DIN VDE 0100-540: 2012-06).

Please refer to the cabling scheme provided in the Appendix to the FluoTime 250 User's Manual delivered with your system as well as to the grounding schemes given in Fig. 6 and Fig. 7.

- The system grounding cables (yellow with green lengthwise stripes) delivered with your FluoTime 250 should be connected to the corresponding grounding pins of the FluoTime 250, computer, PicoHarp 300 (if present) and PDL 820 laser driver.
- The technical design for the equipotential grounding, dimensioning of the cross-sections and standardized terms are given in the international standard **IEC 60364-5-54: 2011** as well as the German standard **DIN VDE 0100-540: 2012-06**.

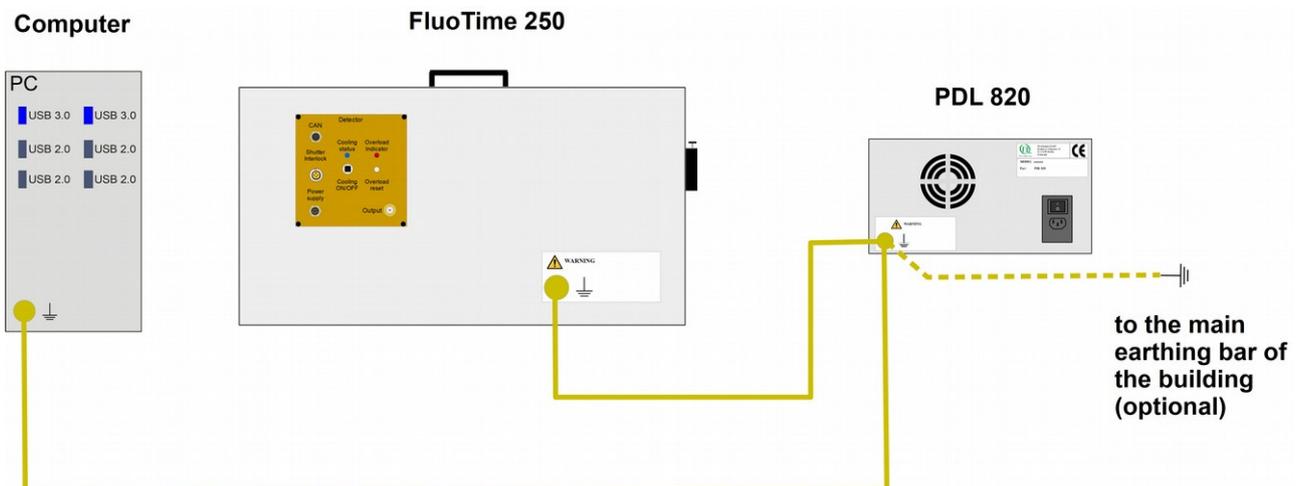


Fig. 6: The grounding scheme for the FluoTime 250 connected to a PDL 820 and computer equipped with a TimeHarp 260 Pico TCSPC card.

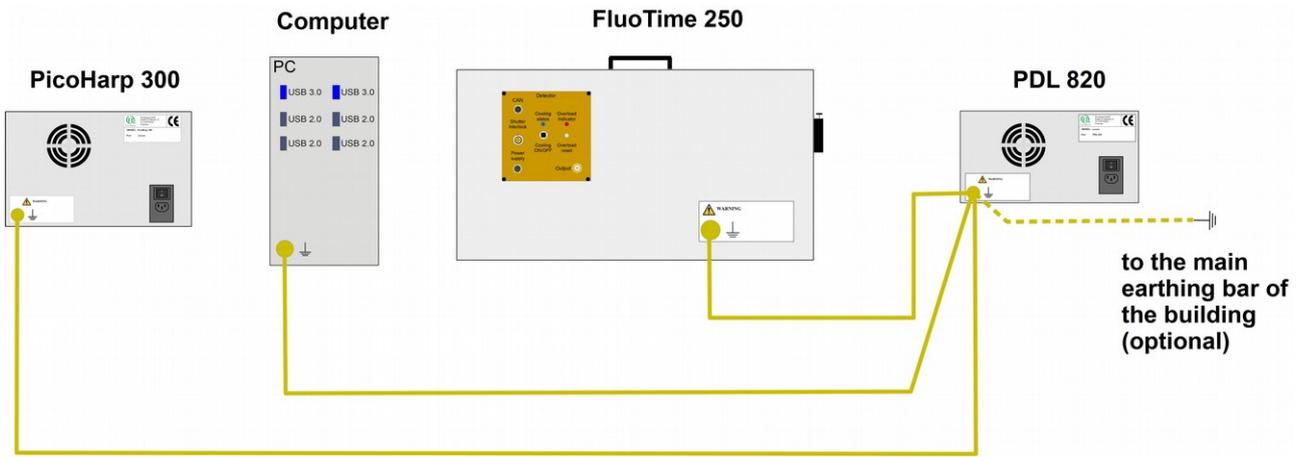


Fig. 7: The grounding scheme of the FluoTime 250 connected to a PDL 820, a PicoHarp 300 TCSPC unit and a computer.

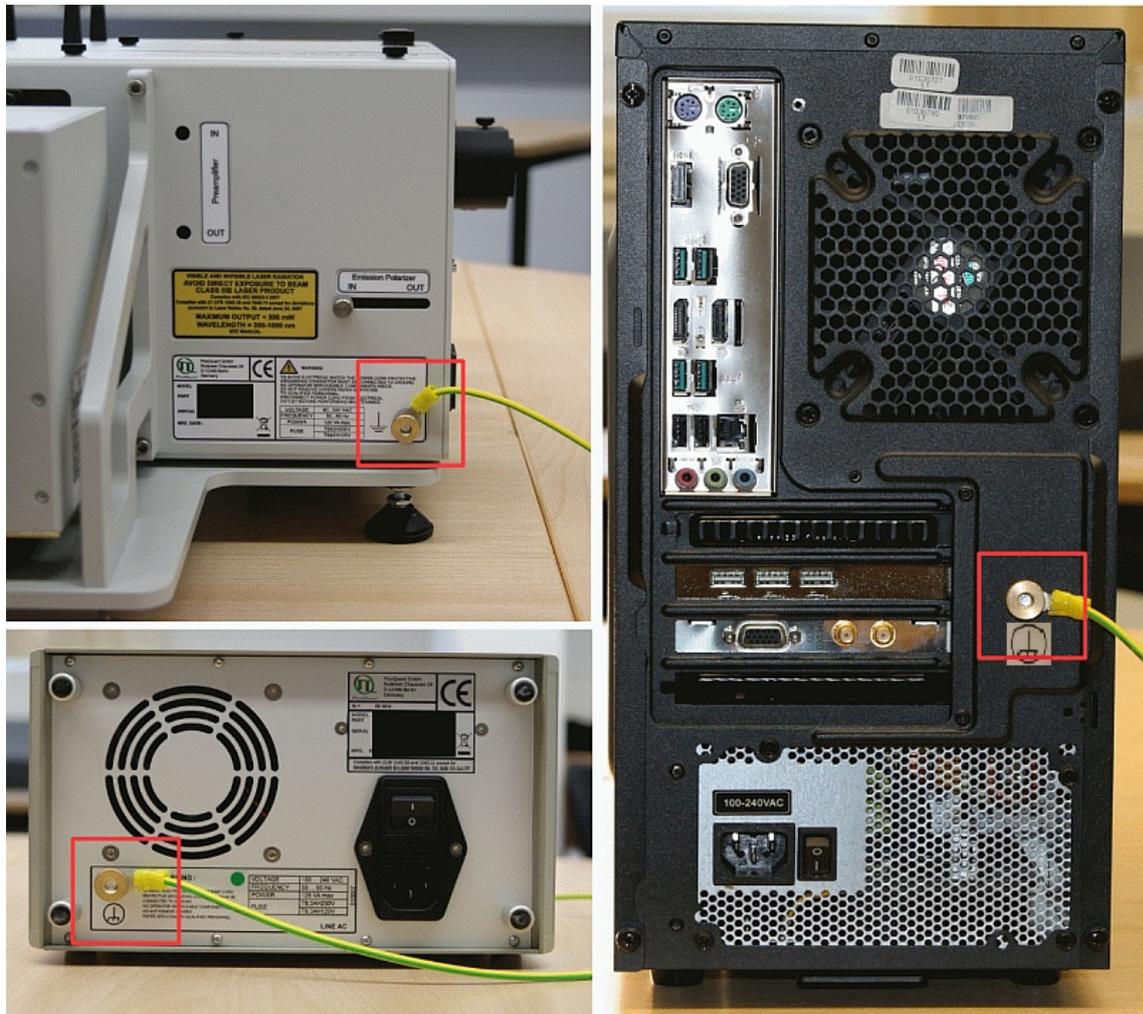


Fig. 8: Location of the grounding pins (indicated by red squares). Top left: on the FluoTime 250. Bottom left: on the back of the PDL 820 laser driver. Right: on the back of the PC.

3.2. Wiring of the FluoTime 250 spectrometer

The detailed cabling plan for your FluoTime 250 spectrometer can be found in the appendix of the user's manual delivered with your system. **All included cables are labeled on both ends.**

NOTICE The colors used in the depiction of cables in all wiring schemes are for illustrative purposes only. The actual color of the cables will be different. Please refer to the cable labels for proper identification.

3.2.1. Power and laser trigger cables

- After the system has been properly grounded (as per section 3.1), connect the power cables of the FluoTime 250, computer, PDL 820 laser driver and PicoHarp 300 to the provided power strip.
- Plug the power strip into a grounded outlet
- Connect the red trigger cable to the PDL 820 as shown in Fig. 9.



Fig. 9: Trigger cable at the PDL 820.

3.2.2. Connecting the detector

How to wire your detector to the FluoTime 250's terminal panel depends on the type of detector you bought. Check which detector type you have and refer to the appropriate sub-section below.

PMA and PMA Hybrid Series

Connect the Power, Shutter, and CAN connectors of the detector to the FluoTime 250 terminal panel. For a FluoTime 250 system equipped with a PMA detector, refer to Fig. 10. For a system using a PMA Hybrid detector, check Fig. 11.

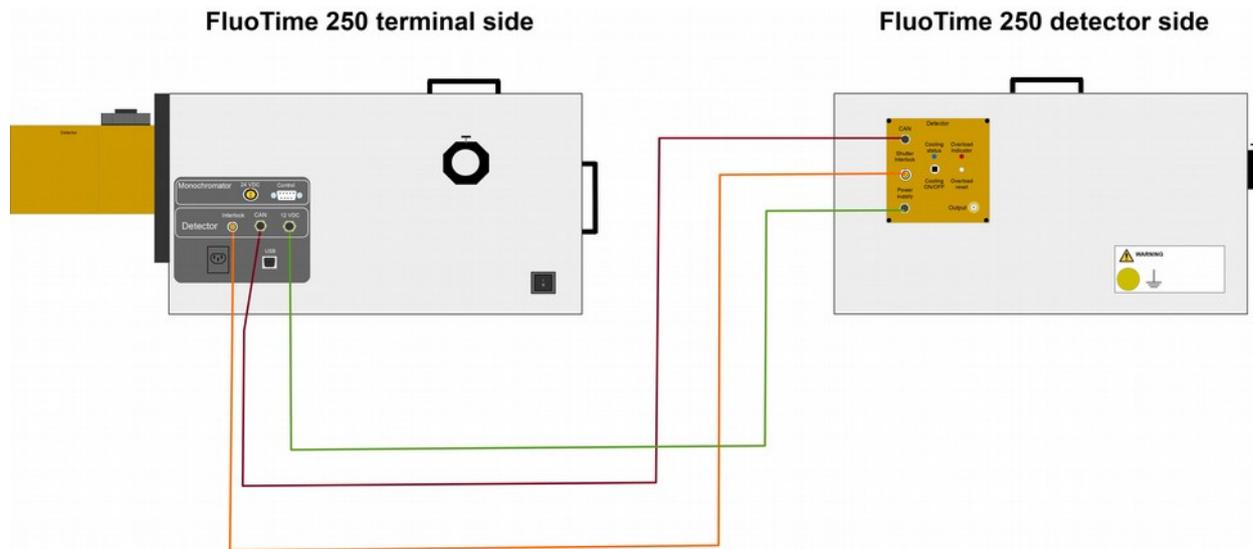


Fig. 10: Wiring of the PMA detector with the FluoTime 250 terminal. Green: power cable, dark red: CAN cable, orange: shutter cable.

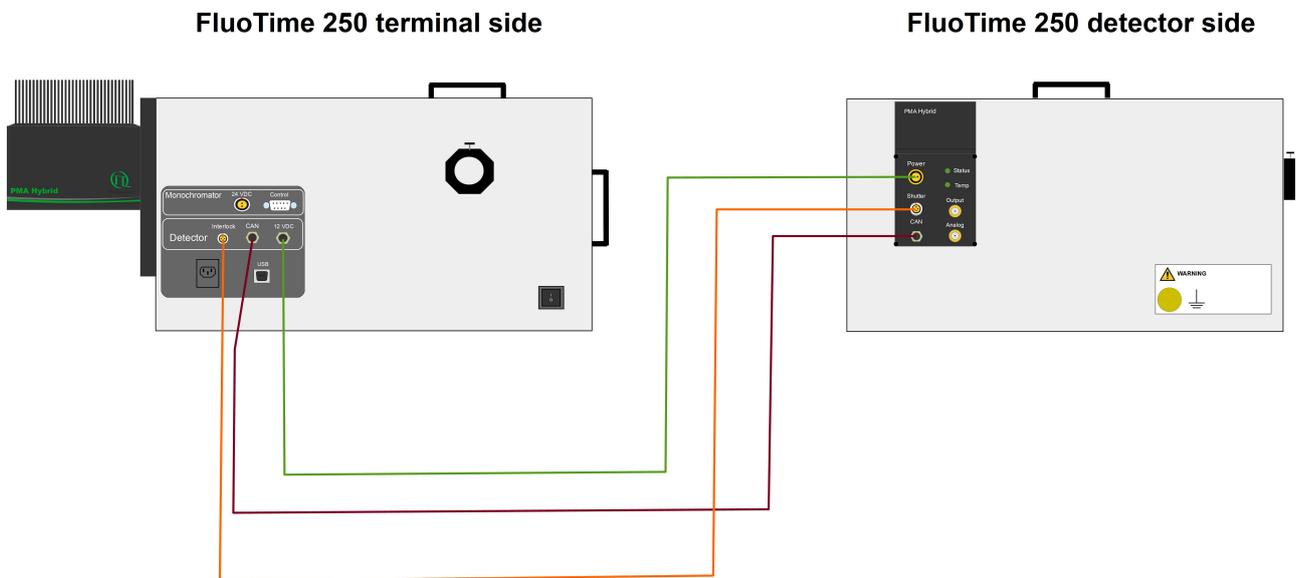


Fig. 11: Wiring of the PMA Hybrid detector with the FluoTime 250 terminal. Green: power cable, dark red: CAN cable, orange: shutter cable.

NIR-PMA detector

If your FluoTime 250 system is equipped with an **NIR-PMA detector**, lock the Interlock connector at the FluoTime 250 terminal using the corresponding cable (see Fig. 12). Connect the **Input** connector of the NIR-PMA detector to the **Output** connector at the NIR-PMA controller (see Fig. 12). Connect the controller connector of the NIR-PMA detector to the corresponding controller connector of the NIR-PMA controller (Fig. 12, right).

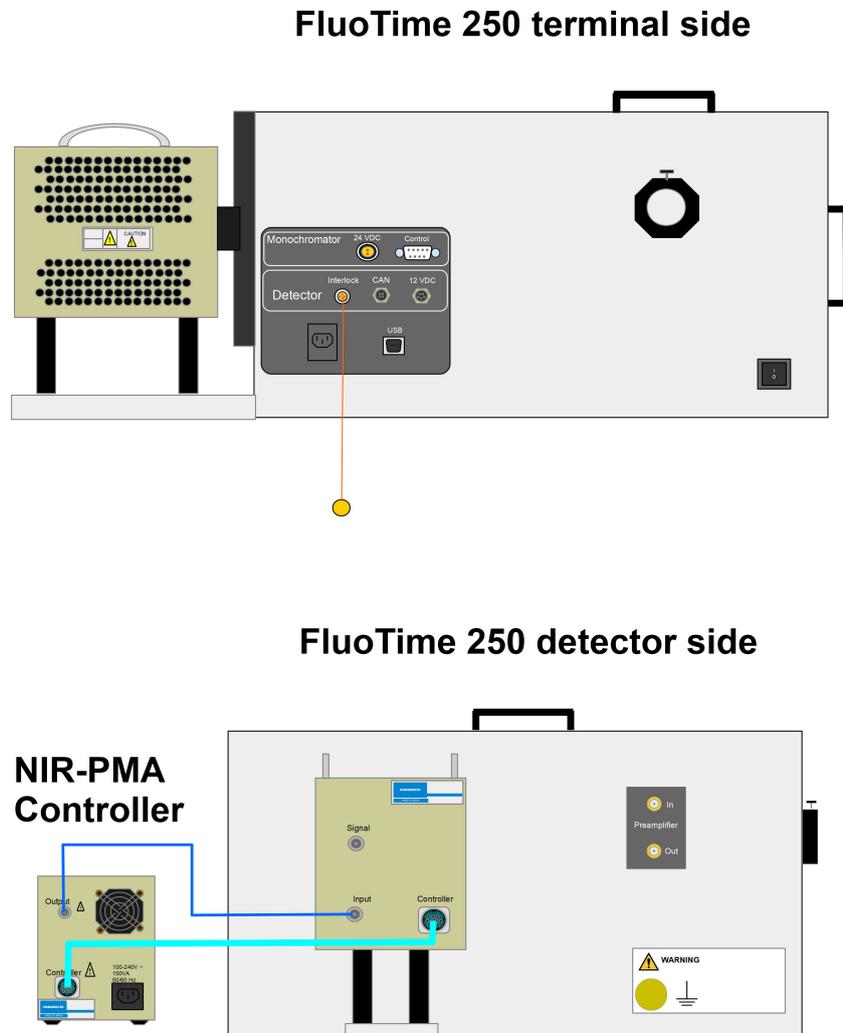


Fig. 12: Connecting an NIR-PMA detector to an NIR-PMA controller. Orange: shutter cable, dark blue: input/output cable, light blue: controller cable

3.2.3. Connecting the (optional) monochromator

If you purchased a FluoTime 250 equipped with the optional **monochromator**, please connect the power cable to the **24 VDC** and **Control** connectors of the FluoTime 250 terminal panel to the **Power** and **Signal** connectors at the back of the monochromator (see Fig. 13).

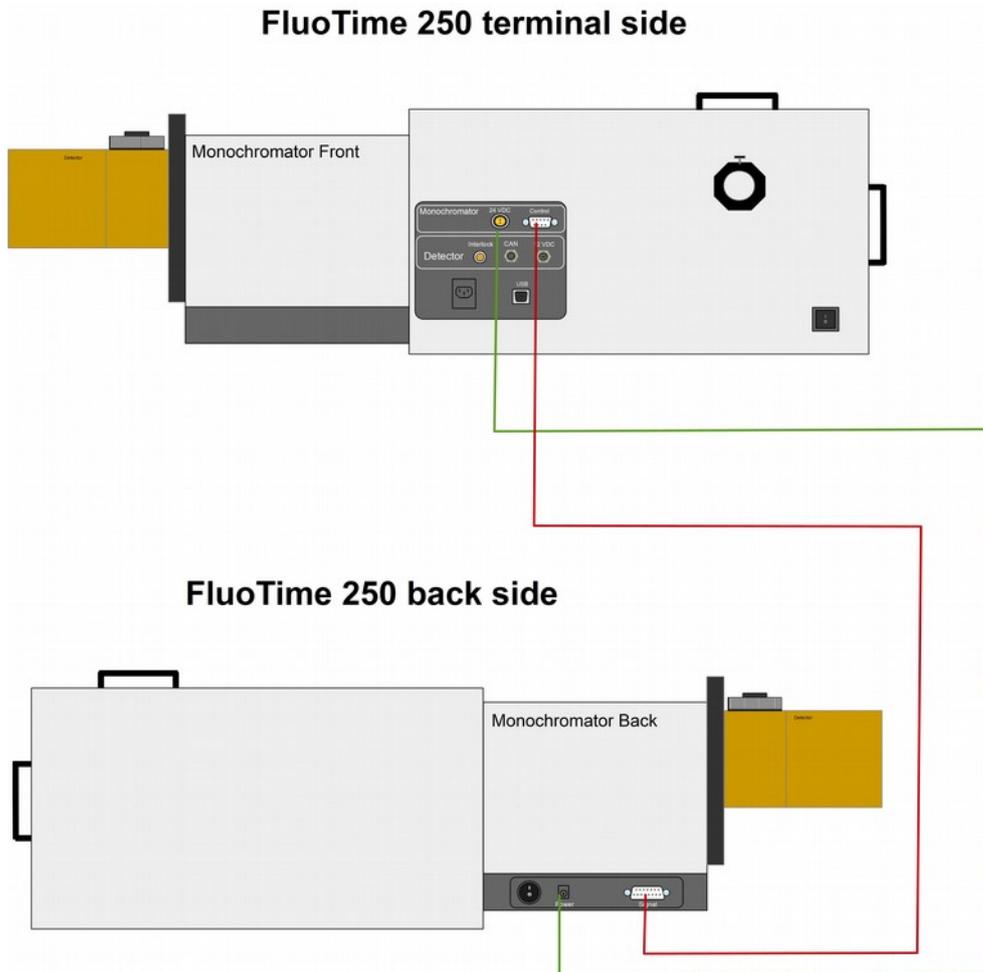


Fig. 13: Monochromator at the FluoTime 250. Green: power cable, dark red: signal cable.

3.2.4. Connecting the detector signal cable to the TCSPC unit

Use the signal cable to connect the signal output connector of the detector to the signal input connector of the TCSPC electronics. The proper wiring scheme depends on both detector type and TCSPC unit. Check the sub-sections below to find your configuration.

PMA or PMA Hybrid Series detector with TimeHarp 260 Pico

If your FluoTime 250 system is equipped with **TimeHarp 260 Pico** TCSPC card (which is already installed in the FluoTime 250 computer) and a **PMA** or **PMA Hybrid** detector, refer to the wiring scheme shown in Fig. 14. Please note that the signal input connector on the TimeHarp 260 Pico is marked as **1**.

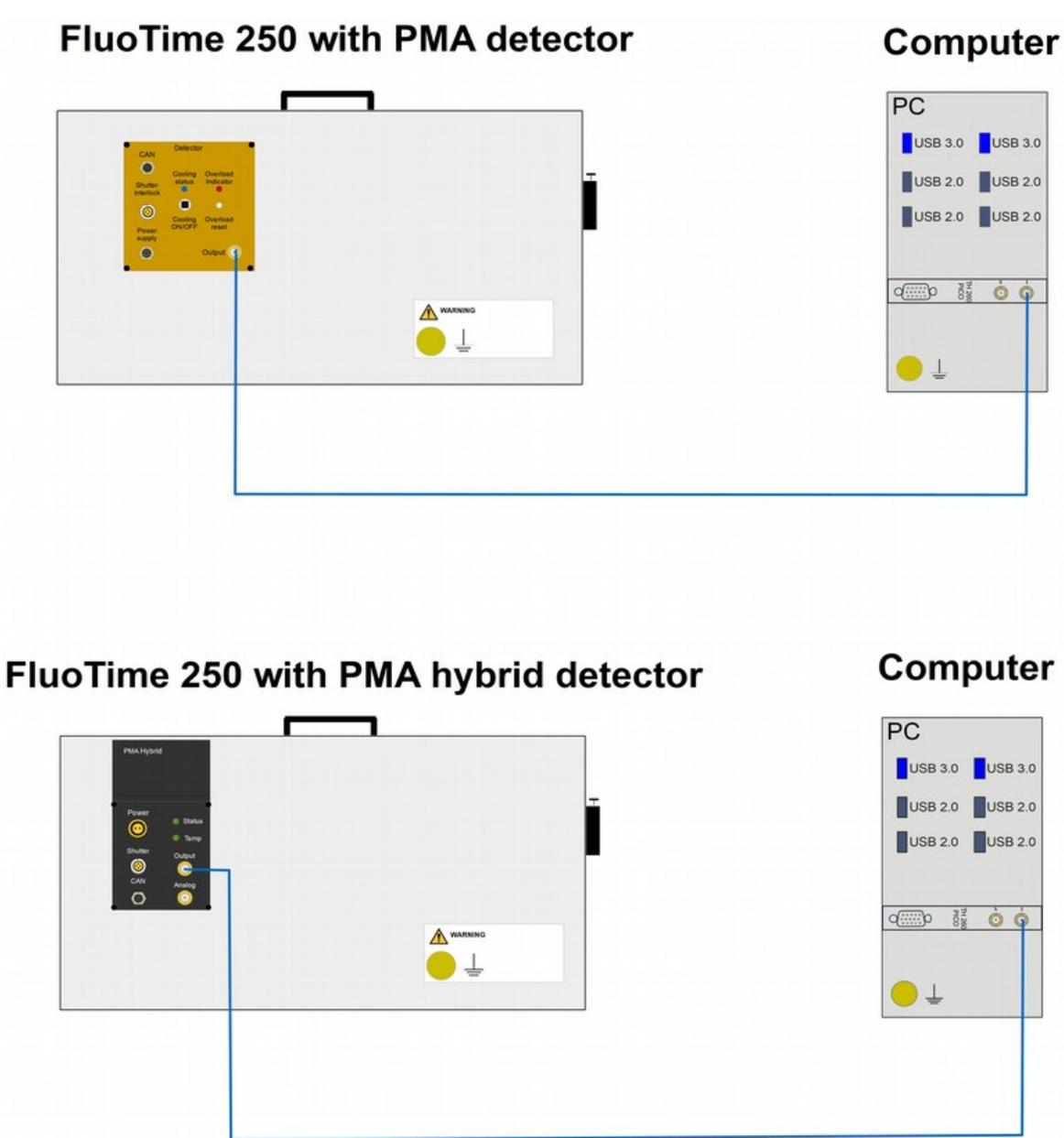
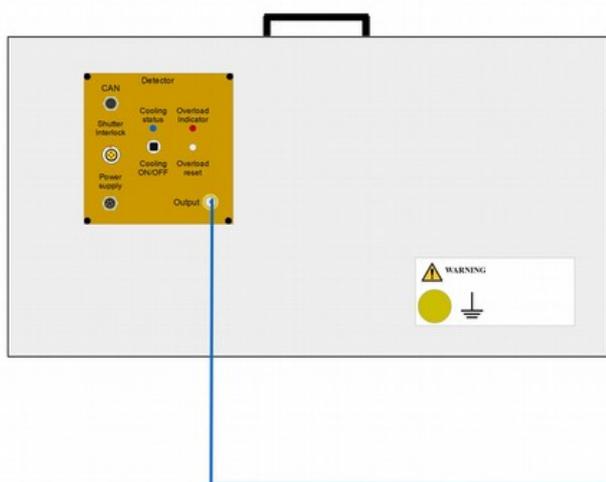


Fig. 14: Wiring scheme for connecting a FluoTime250 system with a TimeHarp 260 Pico TCSPC card and a PMA (top) or PMA Hybrid (bottom) detector.

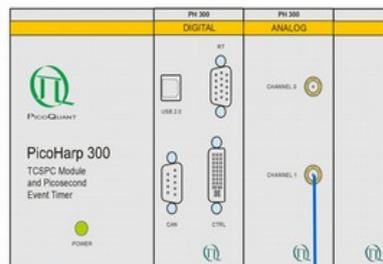
PMA or PMA Hybrid Series detector with PicoHarp 300

If your FluoTime 250 was delivered with a **PicoHarp 300** TCSPC unit, connect the signal output connector on the detector to the **Channel 1** input connector on the PicoHarp 300 (see Fig. 15).

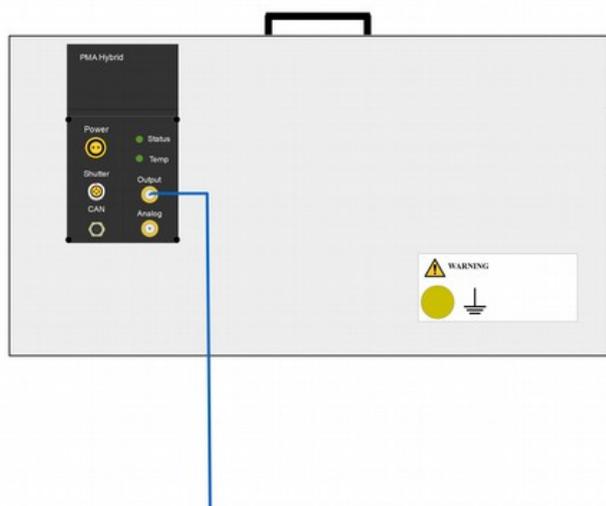
FluoTime 250 with PMA detector



PicoHarp 300



FluoTime 250 with PMA-Hybrid detector



PicoHarp 300

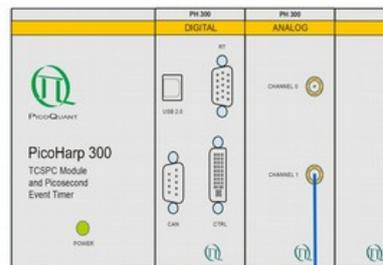


Fig. 15: Wiring scheme for the signal cable connecting the FluoTime 250 system to a PicoHarp 300 and a PMA (left) or PMA Hybrid (right) detector.

NIR-PMA detector with TimeHarp 260 Pico

If your FluoTime 250 system is equipped with the **NIR-PMA** detector, please connect the signal output connector on the detector to the IN connector of the preamplifier, which is located on the same side of the FluoTime 250 as the detector (see Fig. 16). Also connect the OUT connector on the preamplifier to the signal input connector of the TimeHarp 260 PICO TCSPC card installed in the computer (Fig. 16). Please note that the signal input connector on the Time Harp 260 Pico is marked as **1**.

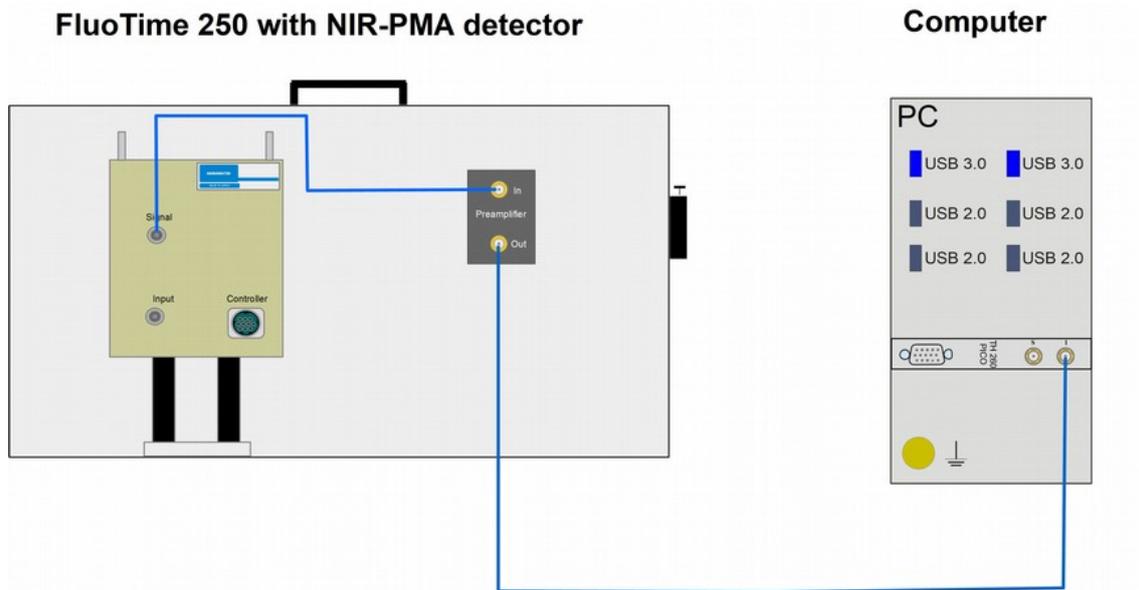


Fig. 16: Wiring scheme for the signal cable connecting the FluoTime 250 system with a TimeHarp 260 Pico TCSPC card and an NIR-PMA detector.

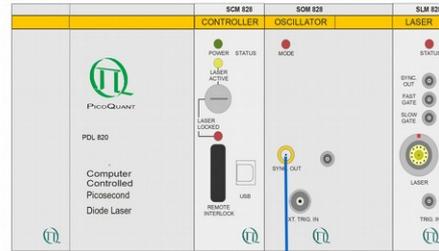
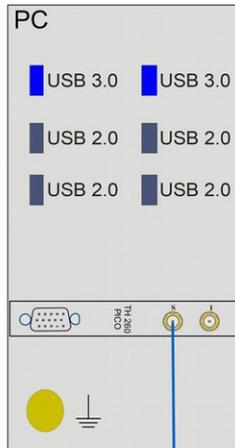
3.2.5. Connecting the trigger cable

The next step is to connect the trigger cable to the SYNC OUT connector of the PDL 820 laser driver and to the trigger input connector on the TCSPC electronics (Fig. 17).

NOTICE The trigger input connector on the TimeHarp 260 PICO is labeled with an S, while the connector on the PicoHarp 300 is called “Channel 0”.

Computer with TimeHarp 260 Pico

PDL 820



PicoHarp300

PDL 820

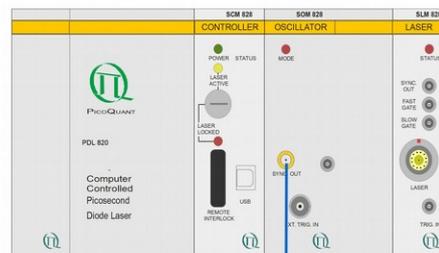
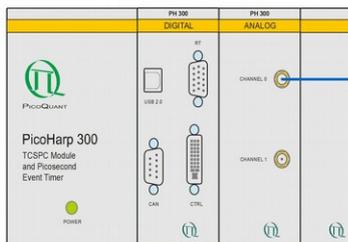


Fig. 17: Wiring schema of the trigger cable for the FluoTime 250 system with the TimeHarp 260 PICO (left) and PicoHarp 300 (right) TCSPC electronics.

3.2.6. Setting up the TLC50 sample mounting unit (optional component)

If you ordered the **Peltier cooled TLC50 sample mounting unit**, please plug it to the corresponding temperature controller according to the wiring scheme shown in Fig. 18. Connect a coolant source (water bath BATH100) to the water connections on the TLC50 sample mounting unit (see Fig. 19) by using the tubes delivered with your FluoTime 250. Attach one tube to the brass fitting on the top of the submersible pump and insert the other into the water tank (Fig. 19). Fill water into the water tank until the pump is covered. Plug the power cable of the submersible pump to the **"Pump"** connector on the back side of the temperature controller.

If needed, connect a source of dry gas (typically dry nitrogen) to the "dry gas" connector in front of the TLC50 sample mounting unit. A continuous flow of dry gas must be used to prevent condensation on the cuvette windows when working below the dew point of water. For detailed instructions, refer to the corresponding TLC50 manual included with your FluoTime 250 system.

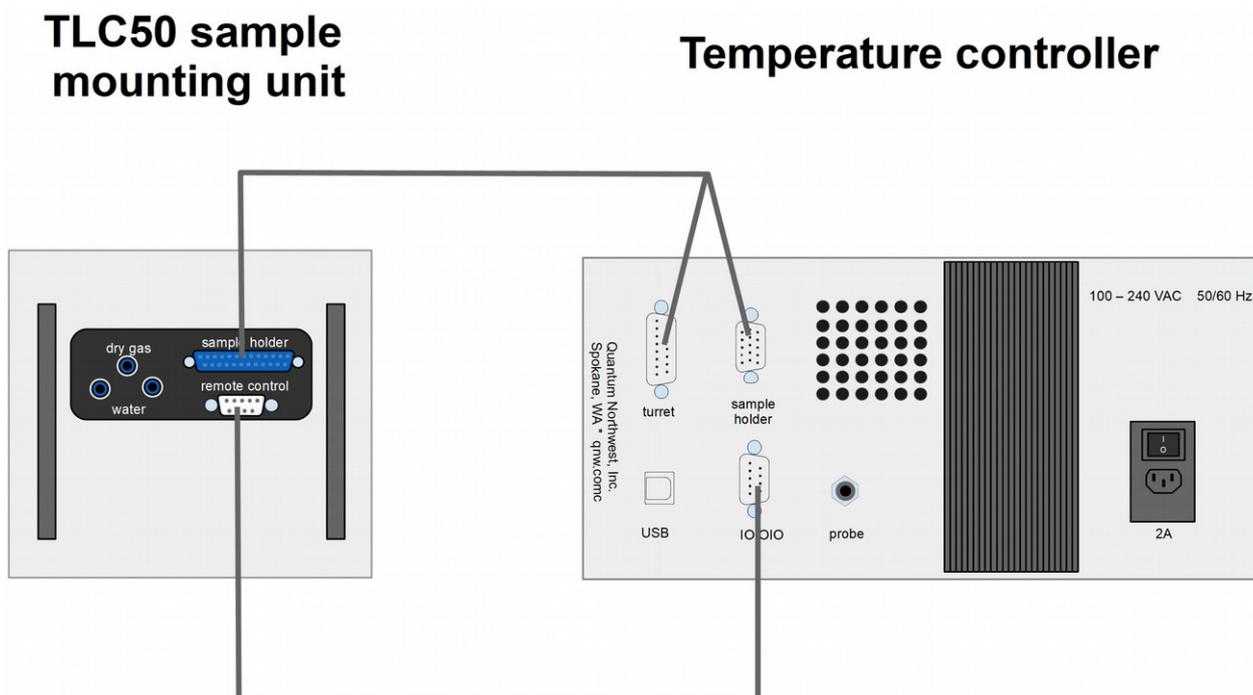


Fig. 18: Wiring scheme for the TLC50 sample mounting unit.

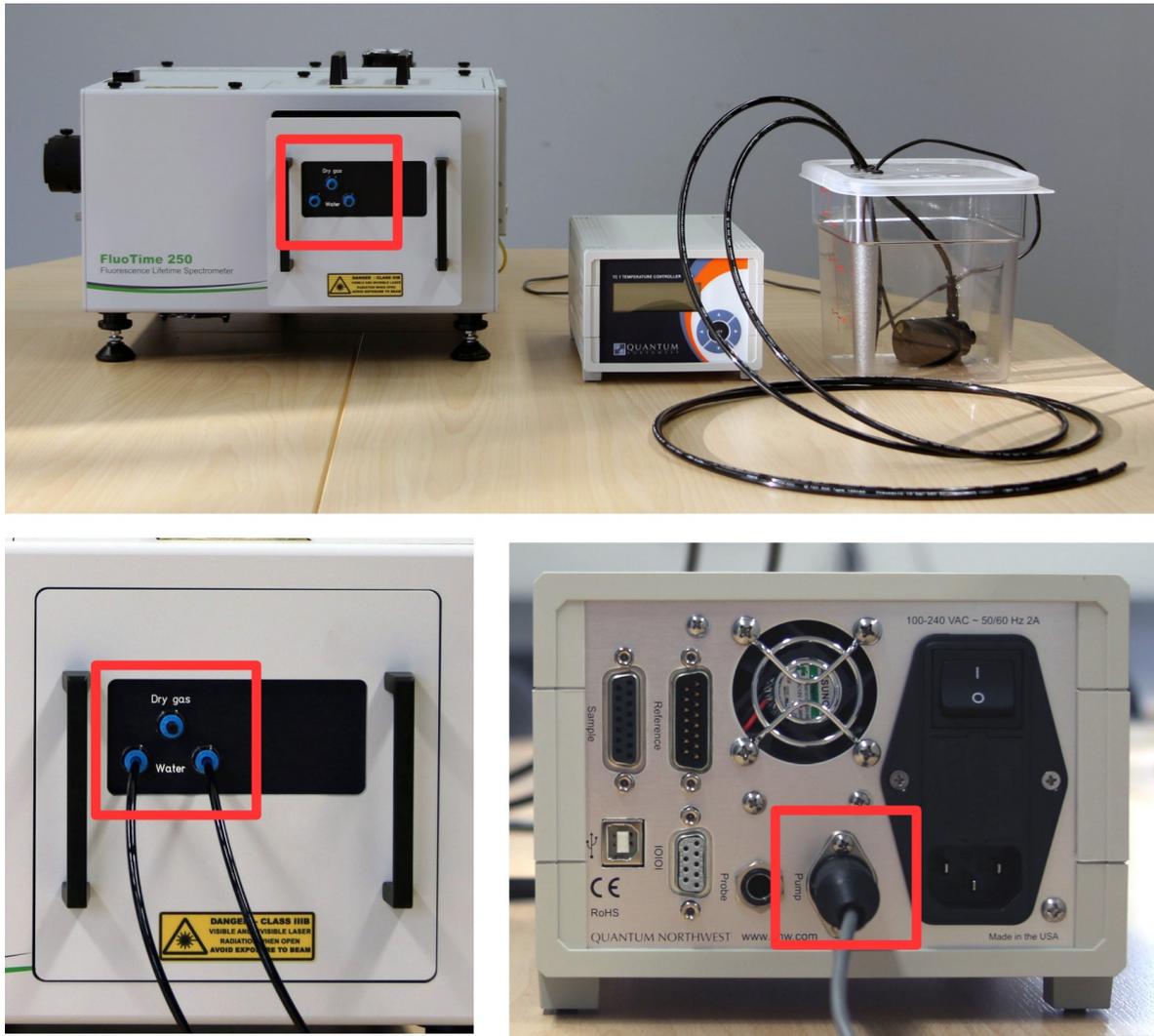


Fig. 19: Coupling of the coolant water tubes to the TLC50 sample mounting unit and Bath100. Top (from left to right): FluoTime 250 with sample mounting unit, bath controller unit and water tank with pump and tubing. Bottom left: connect tubing after removing the corresponding glands. Bottom right: connect the pump cable to the back panel of the controller as shown.

3.2.7. USB connections

Connect each component (FluoTime 250, PDL 820 laser driver and – if present – PicoHarp 300) to separate USB ports of the FluoTime 250 computer. Refer to Fig. 20 or Fig. 21, depending on your configuration.

The PicoHarp 300 is ideally connected to a USB 3.0 port, while the FluoTime 250 and the PDL 820 laser driver should be connected to the ports on the additional USB-board that is already installed in the FluoTime 250 computer.

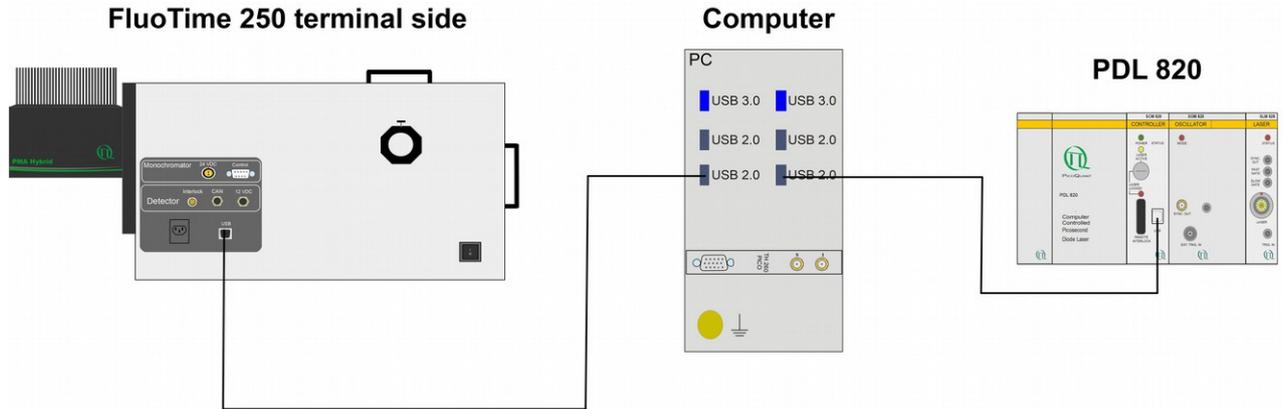


Fig. 20: USB wiring scheme for the FluoTime 250 spectrometer with a TimeHarp 260 PICO.

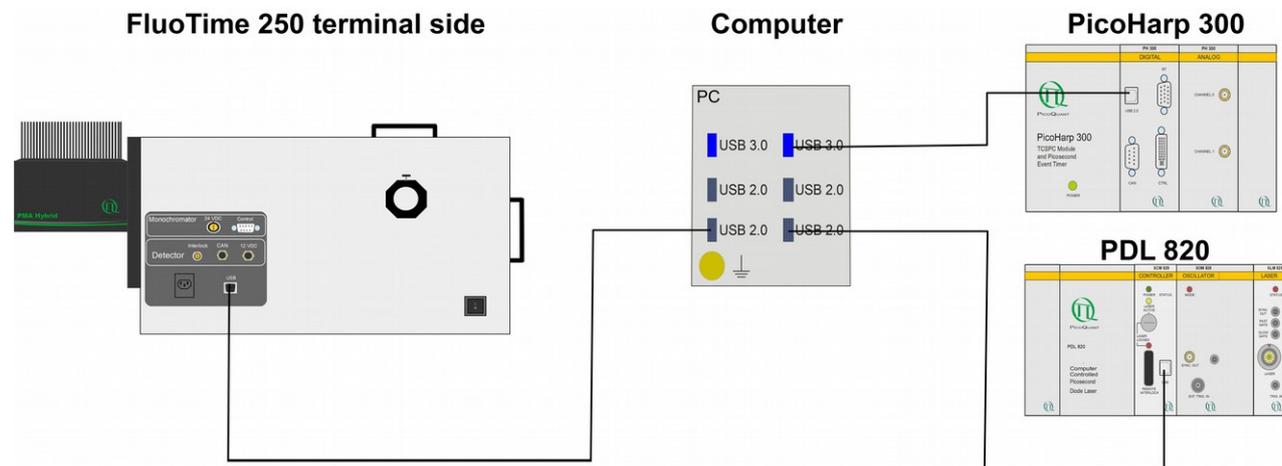


Fig. 21: USB wiring scheme for the FluoTime 250 spectrometer with a PicoHarp 300.

3.2.8. Attaching a light source

Insert a laser head or PLS into the laser coupling port of the FluoTime 250 and fix it with the holding screw (Fig. 22).

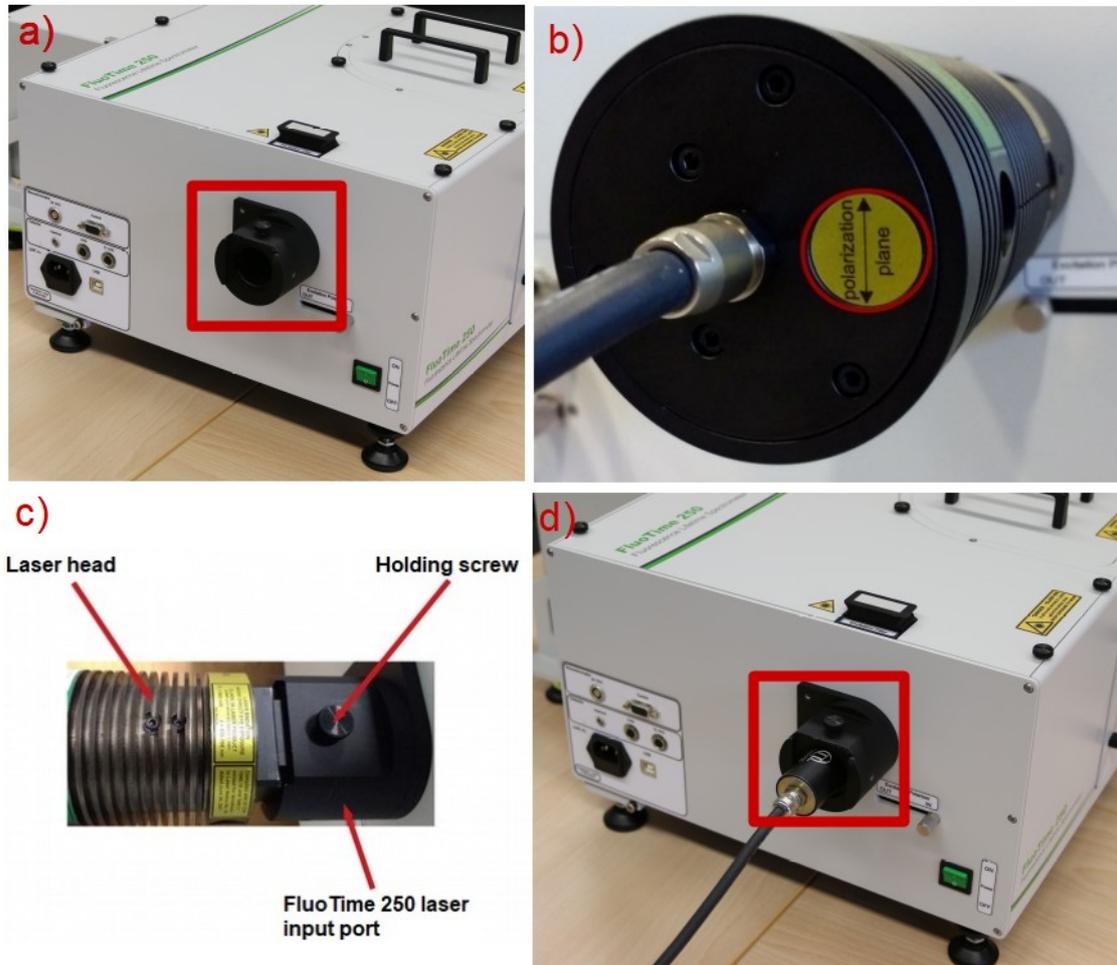


Fig. 22: a) laser input port of the FluoTime 250 (highlighted with a red box. b) label indicating the orientation of the laser heads polarization plane. c) laser head attached at the laser coupling port of the FluoTime 250. d) an LED head from the PLS Series connected to the FluoTime 250.

- Plug the laser (or PLS) cable into the PDL 820 laser driver (Fig. 23).



Fig. 23: Connection of the light source to the PDL 820 laser driver.

3.2.9. Inserting a sample mounting unit

Insert the desired sample mounting unit by sliding it into the sample chamber as shown in Fig. 24. Make sure that the sample mounting unit is fully pushed in before powering up the system.



Fig. 24: Sliding a sample mounting unit into the FluoTime 250

Make sure that the sample chamber lid is closed (Fig. 25).



Fig. 25: Sample chamber lid of the FluoTime 250

Your FluoTime 250 system is now ready for use and can be turned on (see next section).

4. Starting the FluoTime 250

- Start by turning on all PicoQuant devices of the system such as the PicoHarp 300 TCSPC module (if present) and the laser driver. Refer to their dedicated manuals for further details.
- If present, turn on the temperature controller of the sample holder
- Make sure that the hardlock USB dongle is connected to the computer
- Next, switch on any other external component (if applicable) like high voltage supply for the PMT, stand-alone detector controller, temperature controller unit etc. Refer to their respective manuals for additional details.
- Make sure that the correct sample mounting unit is inserted into the instrument and that the instrument top cover as well as the sample chamber lid are closed.
- Check that the appropriate laser head or PLS is inserted into the laser coupling port and is properly secured by means of the fixing screw.
- Turn on the FluoTime 250 using the main switch (Fig. 26).



Fig. 26: Turning the FluoTime 250 on.

NOTICE

The optional monochromator has its own rocker switch. This switch should **NOT** be used to turn the monochromator on or off. This switch should always stay in the **ON** position.

- Start the FluoTime 250 computer.
- Start the EasyTau2 software by double clicking on the corresponding icon (Fig. 27) on the computer desktop. Alternatively, launch the software from the Windows Start menu.



Fig. 27: EasyTau2 icon

- Insert a sample into the sample holder and close the sample chamber lid.

- Activate the laser by turning the key on the PDL 820 laser driver front panel to the “**laser active**” position (Fig. 28).

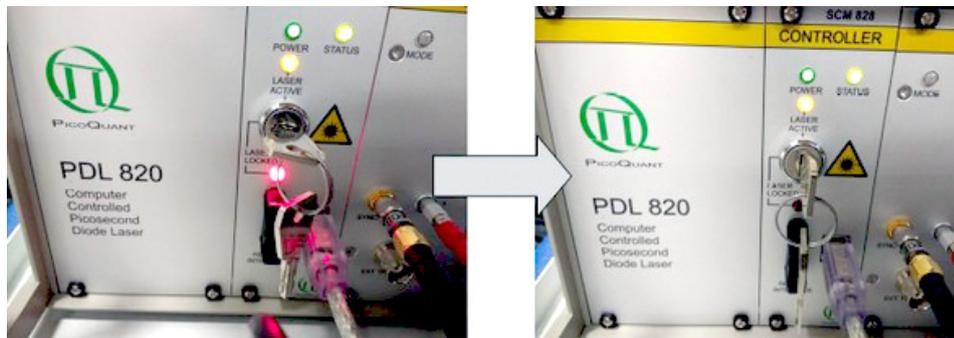


Fig. 28: Turning the laser on.

- Finally select the desired measurement conditions and start the measurement. For using the Easy-Tau2 software and performing measurements with the FluoTime 250, please consult the video and written tutorials stored on the delivered FluoTime 250 computer. The EasyTau2 software has an extensive online help function that can be opened by pressing the F1 key.

5. Performance Check

5.1. Check the FluoTime 250 configuration

- Switch on the laser driver, PicoHarp 300 (if available), and the FluoTime 250 spectrometer
- Turn on the PC and start EasyTau2 software. Refer to section 4 Starting the FluoTime 250.
- Select *Customized Measurement* mode
- Check that the block diagram in the *System status* window matches the block diagram given in the Appendix (Fig. 29 is provided as an example)

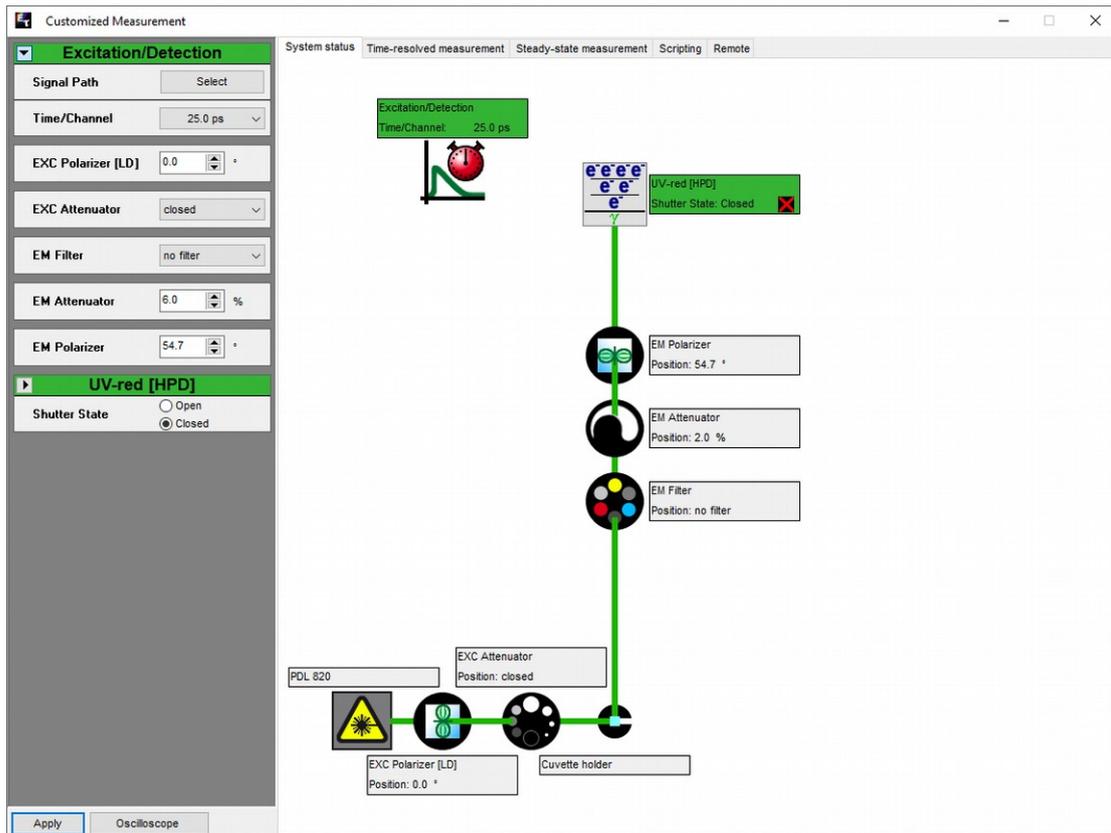


Fig. 29: Example of the block diagram of the FluoTime 250.

- If the layouts match, then the system is wired up properly
- Should this not be the case please first check, whether all cables are properly connected (as per the corresponding wiring scheme provided in the Appendix)
 - Close EasyTau2 software, switch all FluoTime 250 devices off and on again, start EasyTau2 software and check the block diagram in the Customized measurements menu.
 - If this diagram is still differ from that one given in Appendix, please contact us at: <https://support.picoquant.com> or support@picoquant.com. Please include the serial number of your device.

5.2. Measure an instrument response function (IRF) of a connected laser

- Couple the corresponding laser head to the laser driver and FluoTime 250 spectrometer.
- Switch the laser driver, FluoTime 250 and computer on
- Fill ca. 3 ml of the Ludox solution that was delivered with your system in a 1 x 1 cm quartz cuvette (if excitation wavelength is below 350 nm) or in a plastic cuvette
- Insert this cuvette into the sample holder of the FluoTime 250 spectrometer
- Activate the laser by turning the key on the laser driver front panel to the “**laser active**” position
- Start the EasyTau2 software
- Create new workspace (*File* → *New Workspace*)
- In the EasyTau2 software go to the *Customized measurements* mode
- Press “Signal path” button

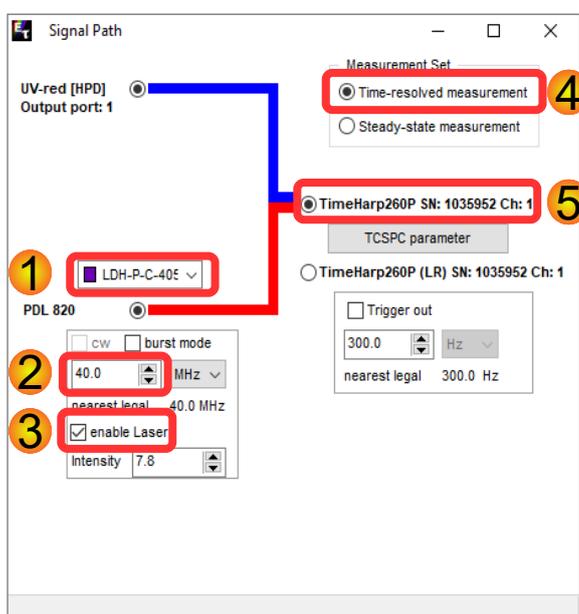


Fig. 30: Signal Path of the FluoTime 250.

- Make sure that the right laser is selected (1, Fig. 30). If not, please choose the laser from the drop down menu
- Set the repetition rate of the laser to 40 MHz (2, Fig. 30)
- Tick the “enable Laser” checkbox (3, Fig. 30)
- Click on the radio buttons “Time-resolved measurement” and “TimeHarp260P” (1 and 1, Fig. 30).
- Close this window
- Choose *Time-resolved measurement* (1, Fig. 31)
- Enter the laser name (e.g., LDH-D-C-440) into the “Sample Name” field (2, Fig. 31)
- Press “Prepare for IRF” button (3, Fig. 31) and then OK

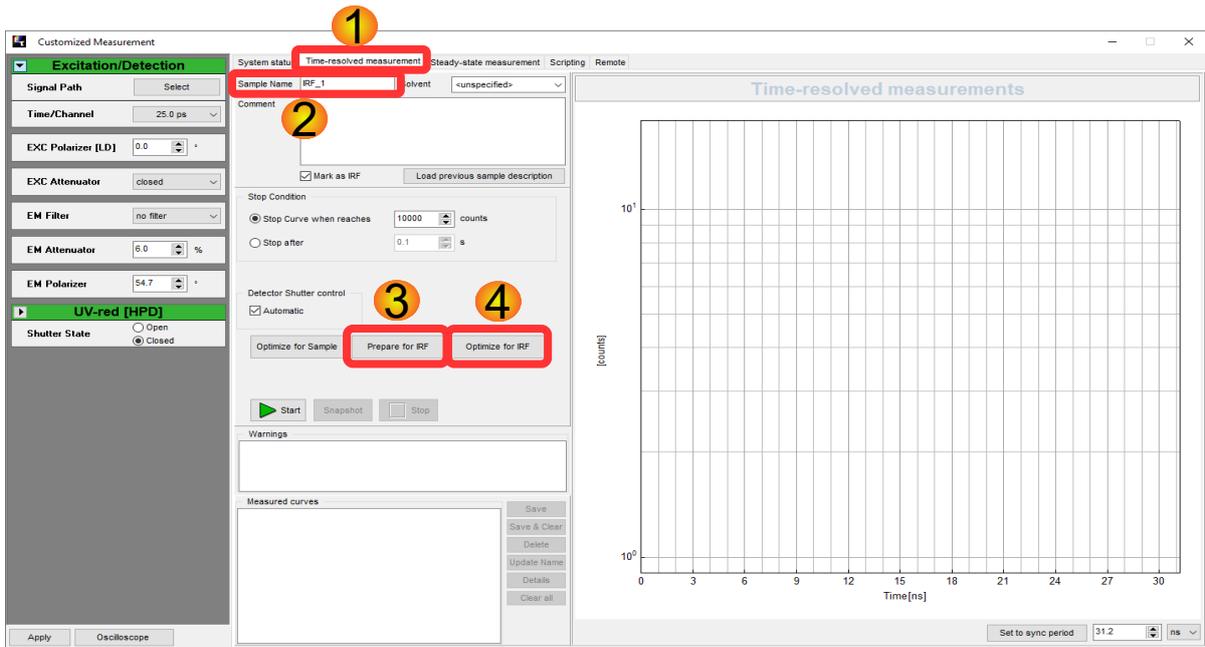


Fig. 31: Time-resolved measurement mode.

- Press “Optimize for IRF” button (4, Fig. 31). The following window will appear (Fig. 32):

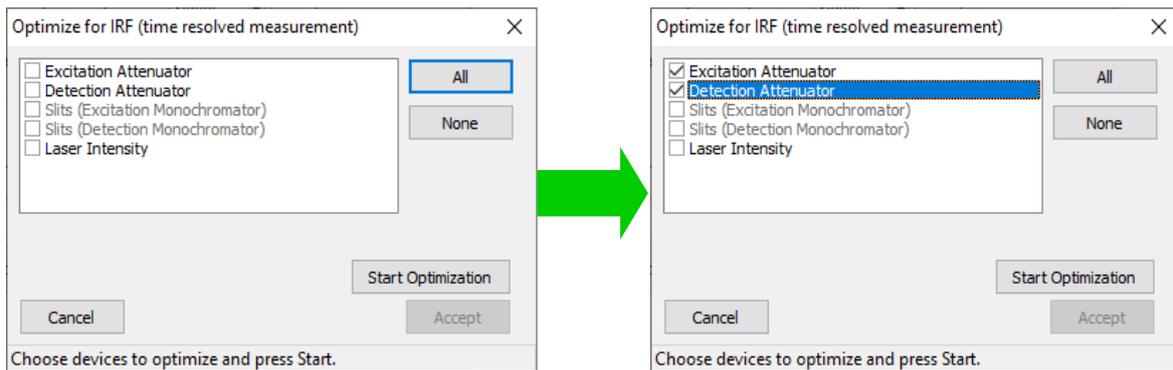


Fig. 32: Optimize for IRF window.

- Tick “Excitation Attenuator” and “Detection Attenuator” check boxes
- Press “Start Optimization” button. The software starts to optimize both excitation and emission attenuators in order to reach optimal signal level. When optimization is successfully finished (signal level is in the green area, see Fig. 33), press “Accept” button.

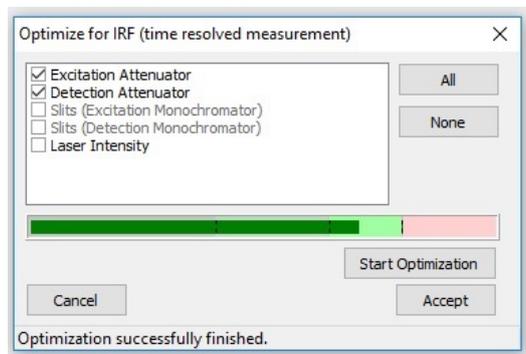


Fig. 33: IRF optimization.

- Press “Start” button and collect IRF signal (Fig. 34).

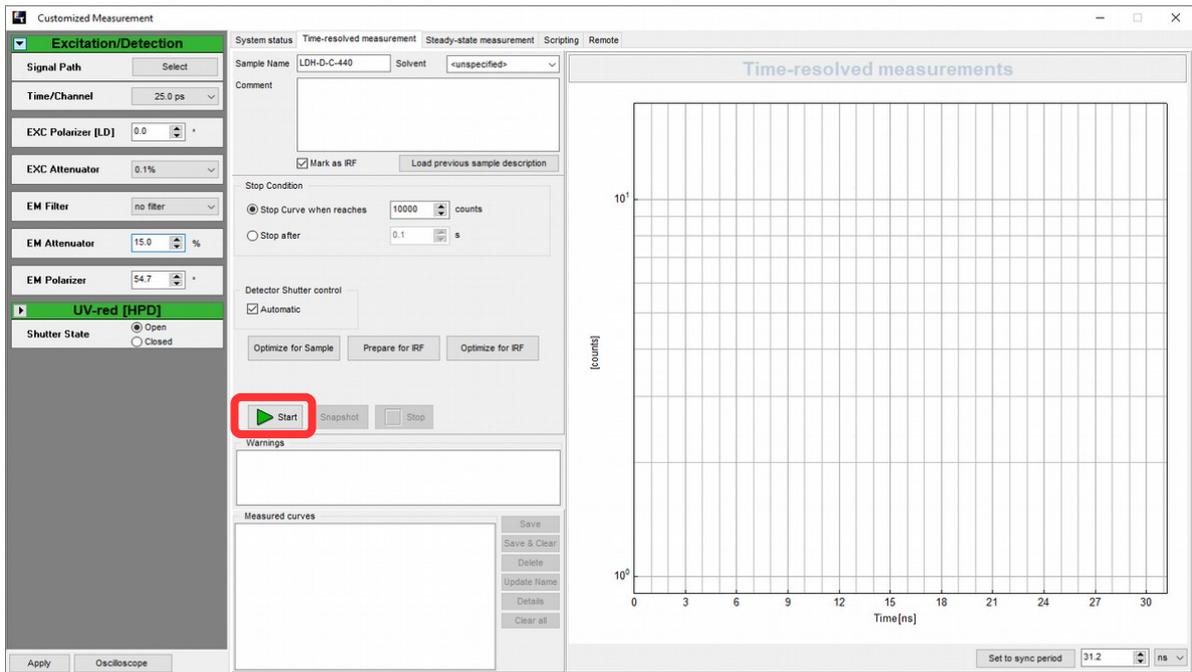


Fig. 34: Start measuring the IRF.

- When the measurement has finished, press the “Save” button (Fig. 35). The data will be saved in the currently open workspace.

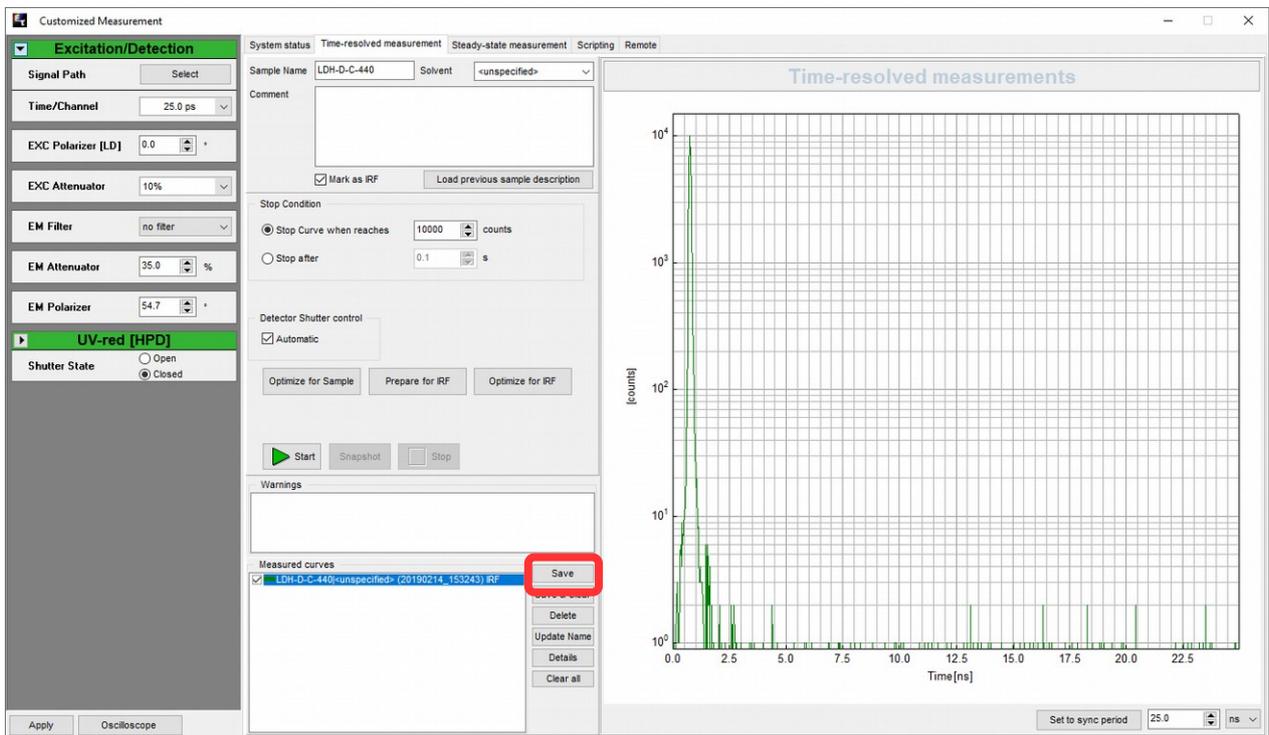


Fig. 35: Saving the measured IRF.

- Plot the IRF by clicking on the corresponding “Plot data” button in the toolbar menu of the Easy-Tau2 software. A window as shown below will appear (Fig. 36):

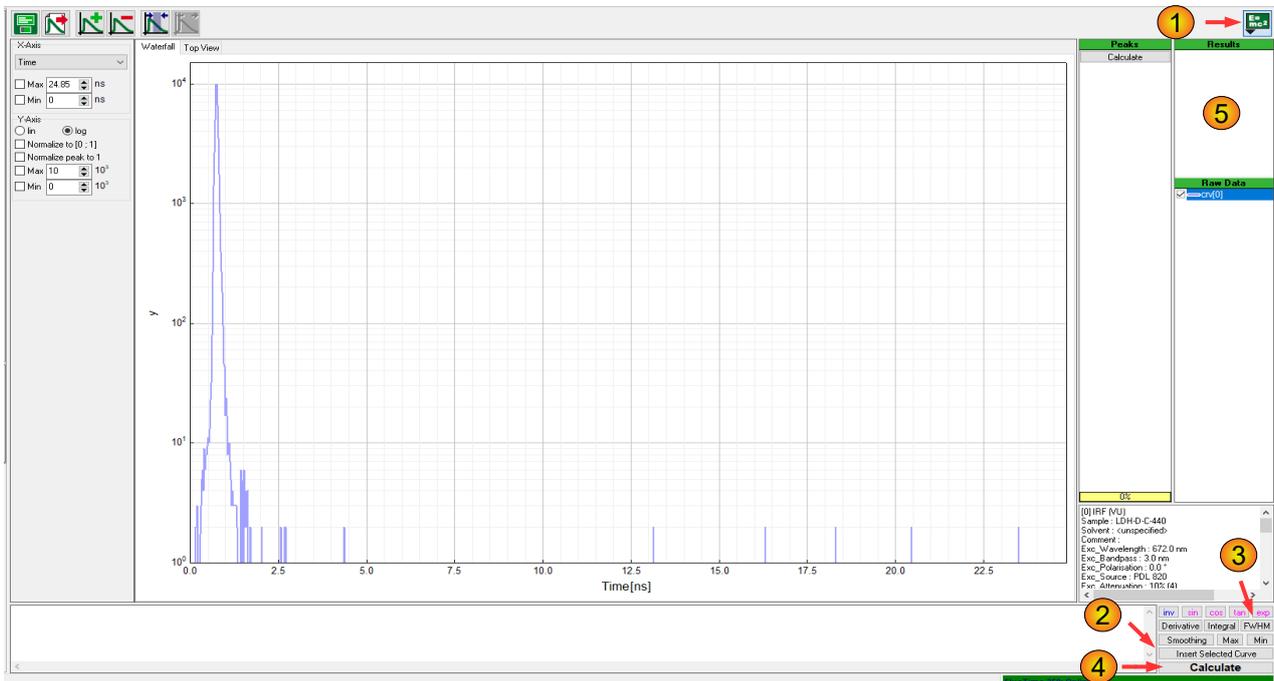


Fig. 36: Plotting of the measured IRF and calculation of the FWHM of the IRF.

- Click on the “E = mc²” button (1 in Fig. 36) to open the scripting window below the plot
- Press “Insert Selected Curve” button (2 in Fig. 36), then “FWHM” button (3 in Fig. 36) and finally “Calculate” button (4 in Fig. 36). The FWHM of the IRF will be calculated and the result (in nanoseconds) is shown in Results window (5 in Fig. 36).
- Compare this value to the one given in the Appendix. They should be the same (± 25 ps).

5.3. Fluorescence decay measurement of the reference polymer block (if the reference polymer block was ordered)

The performance of the FluoTime 250 spectrometer was checked by PicoQuant using the reference fluorescence polymer block delivered with your FluoTime 250 spectrometer. The corresponding data as well as measurement settings are listed in the Appendix. Moreover, a measurement script is included on the USB thumb drive delivered with the FluoTime 250 in the “**Scripts**” folder under the name: **Ref_x_LDH-x-x-xxx_Filter-x** (where Ref_x is the name of the reference fluorescence polymer block, LDH-x-x-xxx is the laser head used for the measurement, Filter-x is the long-pass emission filter used).

- Connect the corresponding laser head to the laser driver as well as to the FluoTime 250 spectrometer
- Activate the laser by turning the key on the laser driver front panel to the “**laser active**” position
- Create new workspace (*File* → *New Workspace*)
- Plug the PicoQuant USB thumb drive into the computer
- Insert the reference polymer block in the sample holder. **CAUTION:** use gloves to prevent leaving fingerprints on the sample!
- In the EasyTau2 software, go to the *Customized measurements* mode
- Select “Scripting” measurement mode (Fig. 37)

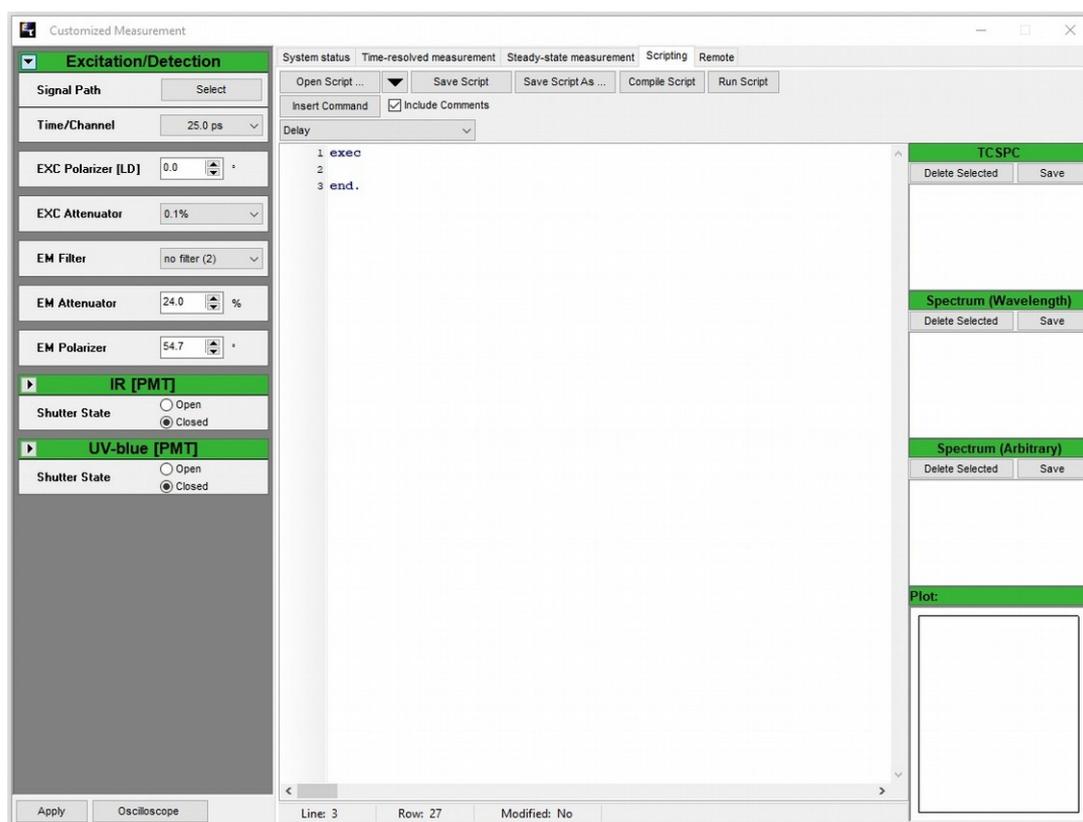


Fig. 37: Measurement in scripting mode.

- Press “Open Script...” button and open the corresponding script-file from the USB thumb drive (Fig. 38).
- Press “Run Script” button to execute the script.
- After finishing the measurement, perform an analysis of the measured decay (refer to the tutorial “Measuring Fluorescence Decays” for more details) and compare the obtained fluorescence decay time with the one given in the Appendix. Both values should match each other within ± 0.1 ns.

The screenshot displays the 'Customized Measurement' window in the PicoQuant software. The interface is divided into several sections:

- Excitation/Detection:** A sidebar on the left containing controls for Signal Path, Time/Channel (25.0 ps), EXC Polarizer (LD) (0.0), EXC Attenuator (0.1%), EM Filter (no filter (2)), EM Attenuator (24.0 %), EM Polarizer (54.7), and two PMT sections (IR and UV-blue) with Shutter State (Open/Closed) options.
- Scripting:** A central text area containing a script. The script starts with a comment: 'result of a Wizard run on 2018-09-20 17:31'. It includes instructions to read lines carefully and lists prerequisites for running the script, such as sample and laser setup. The script defines parameters like MaxCounts (10000) and IRFDecay (Curve), and sets various ACTPos values for detection filter, attenuator, excitation attenuator, and polarizer.
- TCSPC:** A panel on the right for Time-Correlated Single Photon Counting, showing Curve 1 and Curve 2.
- Spectrum (Wavelength) and Spectrum (Arbitrary):** Two panels for spectral analysis, each with Delete Selected and Save buttons.
- Plot:** A panel at the bottom right for displaying the measurement results.

Buttons for 'Open Script...', 'Save Script', 'Save Script As...', 'Compile Script', and 'Run Script' are visible at the top of the Scripting section. The script text is as follows:

```
1 { Decay
2 result of a Wizard run on 2018-09-20 17:31
3
4 READ the following lines carefully.
5
6 Before running the script please ensure that:
7
8 - the sample "SB_B_LDH-D-C-375 | <unspecified>" is inserted
9 - the sample for IRF measurement is available
10 - the laser "LDH-D-C-375 (01021473)" is attached vertically
11 - Detector "UV-blue [PMT]" is choosen as active detector
12 - the detection filter used last time is inserted
13 - TimeHarp260P is choosen as active measurement device
14
15 }
16 def
17 MaxCounts: Int;
18 IRFDecay: Curve;
19 exec { Start of script }
20 { Start Measurement parameters }
21 Sample = "SB_B_LDH-D-C-375";
22 Solvent = "<unspecified>";
23 MaxCounts = 10000; // Measure until maximum of histogram reaches MaxCo
24 { End Measurement parameters }
25 DeleteAllCurves;
26
27 { Detection Filter settings }
28 DETECTION_FILTER.ACTPos = 2; // FGL400 (3)
29 DisplayStatus("Detection filter set.");
30
31 { Detection Attenuator settings }
32 DETECTION_ATTENUATOR.ACTPos = 58; // 58 %
33 DisplayStatus("Detection attenuator set.");
34
35 { Excitation Attenuator settings }
36 EXCITATION_ATTENUATOR.ACTPos = 3; // 1%
37 DisplayStatus("Excitation attenuator set.");
38
39 { Excitation Polarizer settings }
40 EXCITATION_POLARIZER_LEFT.ACTPos = 0.0; // 0.0°
41 DisplayStatus("Excitation polarizer set.");
42
43 { Detection Polarizer settings }
44 DETECTION_POLARIZER.ACTPos = 54.7; // 54.7°
```

Fig. 38: Script for measurement of the reference fluorescent polymer block.

6. Turning off the FluoTime 250

- Shut down the EasyTau2 software in order to avoid data loss.
- Switch the laser driver into its stand-by mode before turning it completely off.
- Turn off the FluoTime 250 via the main switch (Fig. Fehler: Verweis nicht gefunden).

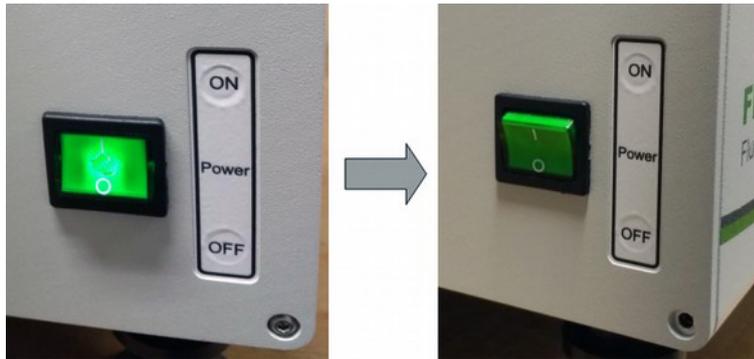


Fig. 39: Turning the FluoTime250 off.

NOTICE

If the optional monochromator is available, leave the monochromator rocker switch in the ON position!

- Shut down the remaining devices (no specific order required).

7. Support

7.1. Returning Products for Repair

Should you encounter serious problems that require sending the device in for inspection / repair, please contact us first at: <https://support.picoquant.com> or support@picoquant.com and request an RMA number before shipping the device. Observe precautions against static discharge under all circumstances during handling, packaging and shipping. Use original or equally protective packaging material. Inappropriate packaging voids any warranty.

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