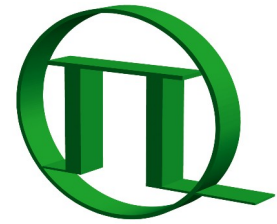


Single Channel PMA Hybrid Detection Unit



PICOQUANT

External Detection Unit for Laser
Scanning Microscopes



User's Hardware Manual and Technical Data

Version 1.4

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1. Safety Instructions

LASER Warning!

Lasers for two photon excitation deliver infra red emission which is in most cases not visible for the eye. These lasers emit light of laser class 4 / IV. Laser class 3B / IIIb and 4 / IV requires to wear special laser safety glasses. **Do not remove or change any items of the Single Channel Detection Unit, especially the filter, before switching the microscope and laser completely off (see Fig. Fehler: Verweis nicht gefunden).** The installation room of the LSM Upgrade kit must be labeled as laser area. For class 3B / IIIb and 4 / IV lasers, a laser safety officer has to be announced to meet the laser safety regulations.

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

Required Laser Safety Measures

Please observe the laser safety measures for laser class 3B / IIIb (VIS and UV systems) and laser class 4 / IV 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 safe maintenance of the system and for following all applicable safety regulations.
- The owner/operator is fully liable for all consequences resulting from the use of the system 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 the system 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 advisor (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).

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.
- Switch off all lasers before removing or changing the filter (see Fig. Fehler: Verweis nicht gefunden)
- Do not introduce any reflective objects into the opening which remains open after removing the filter holder (see Fig. Fehler: Verweis nicht gefunden)
- Every person involved with installation and maintenance 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 this product. Observe all safety precautions relevant to Class 3B / IIIb lasers and Class 4 / IV lasers.
- Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.
- The laser power levels accessible if the unit is opened can cause instant blindness, skin burns and fires. Class 3B / IIIb or 4 / IV lasers can present a major hazard through exposure to the direct (intra-beam) or reflected (specular or diffuse) laser beams when the laser is inadvertently "on" and there is a direct line-of-sight path to the laser beam or its reflection.



Laser light of class 3B / 4 lasers may be delivered via the multimode fiber or light guide into the Single - Channel PMA Hybrid Detection Unit. Therefore, the multimode fiber or light guide must not be removed from the detection unit. Do not remove any items inside the unit.



The LSM upgrade kit is fitted with one or more diode lasers. To avoid hazardous radiation exposure you should carefully obey the safety instructions that are provided with your diode laser operation manual. If your instrument uses another excitation system, follow the safety instructions of the relevant manual.

The delivered instruments are pre-set by PicoQuant to operate on the power outlet line voltage for the country of delivery. Nevertheless, please check that the actual line voltage corresponds to the value set on these instruments!

Never connect or disconnect any cable while the data acquisition and control electronics are ON. Charged signal cables can destroy the devices!

Protect the photon detectors (PMA Hybrid) as much as possible, particularly from excessive light intensities, e.g. microscope illumination lamp, unattenuated backscattered excitation, etc.

To avoid hazardous radiation exposure you should carefully obey the safety instructions that are provided with your laser scanning microscope and laser manual. If your instrument uses another excitation system, follow the safety instructions of the relevant manual.

The delivered instruments are pre-set by PicoQuant to operate on the power outlet line voltage for the country of delivery. Nevertheless, please check that the actual line voltage corresponds to the value set on these instruments!

Never connect or disconnect any cable while the data acquisition and control electronics are ON. Charged signal cables can destroy the devices!

Protect the photon detectors (PMA, PMA Hybrid) as much as possible, particularly from excessive light intensities, e.g. room light, microscope illumination lamp, unattenuated backscattered excitation, etc.

1.1. Safety labels on the system

The corresponding safety labels are selected dependent on the laser configuration (VIS, UV, IR) and attached in the following locations.

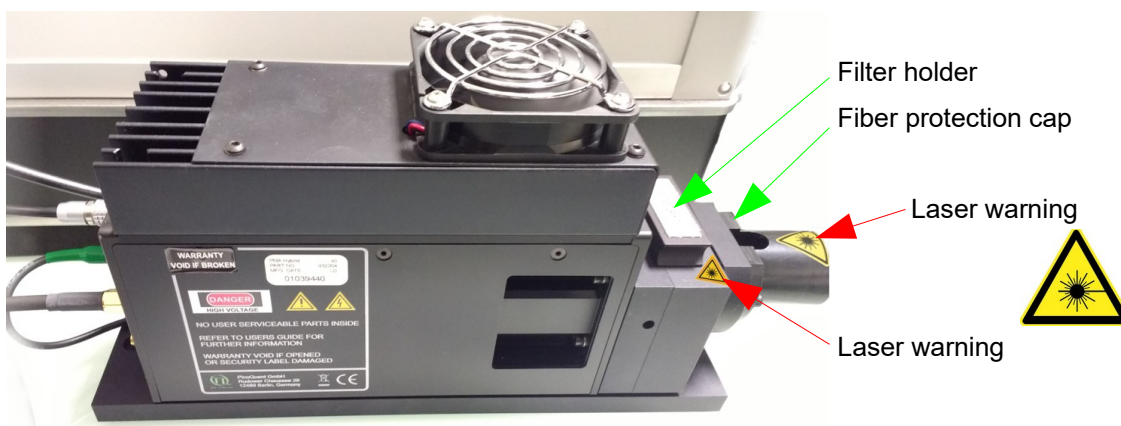


Fig. 1.1: Safety label on top of the Single Channel Detection Unit. Please be careful when removing the filter holder. Inside the space occupied by the filter holder there might be laser radiation. Never enter this space with a tool. Never remove the fiber protection cap. Laser radiation might exit from the fiber or lightguide.



Take care to obey all safety regulations. Before removing the filter holder, stop all measurements or scans of the LSM. Never remove any item of light guiding fibers / lightguides. Keep away from the inner part of the multi detection unit since visible or invisible laser CLASS 3B radiation can occur inside the housing.

2. Introduction

This manual describes the basic components of the single channel PMA Hybrid detection unit, as well as operation, maintenance and adjustment of the detectors .

The Single Channel Detection Unit is an external detection unit for standard confocal Laser Scanning Microscopes (LSMs). The detection unit is connected to the exit port of the LSM via a multi-mode fiber and for NDD using a light guide. The PMA Hybrid detector allows time-resolved measurements with high sensitivity. This feature cannot only be used in FLIM applications, but also allows a broad range of FCS measurements. Possible applications are:

- FLIM and FLIM - FRET measurements with single molecule sensitivity
- FCS measurements (autocorrelation)

Look at PicoQuant's website to download Technotes (<http://www.picoquant.com/appnotes.htm>) regarding different techniques. Refer to your LSM manual for instructions on how to perform different measurements with your confocal microscope.

The PMA Hybrid detector has an internal over - illumination shut down. When too high count rates are reached (typically above ~5 Mcps), the shutter in front of the PMA Hybrid is closed for some seconds and a beep sound is emitted. Still, illumination levels of the PMA Hybrid detector should be kept as low as possible, because too extensive light can destroy the detector or accelerate the ageing process which leads to a decrease in sensitivity.



All abbreviations are explained at the end of the manual.

3. Physical Properties and Installation Requirements

SIZE [CM] (WIDTH × DEPTH × HEIGHT)	6 × 24 × 14
WEIGHT [KG]	5 KG
OPERATION VOLTAGE	115V - 230 V AC
FIBER CONNECTOR	FC-APC
ROOM REQUIREMENTS	TEMPERATURE: < 30°C REL. HUMIDITY: < 60%
TEMPERATURE VARIATION	SHOULD BE KEPT WITHIN 1°C, OTHERWISE DEADJUSTMENT OF THE OPTICAL ELEMENTS CANNOT BE EXCLUDED

4. DETECTOR TYPE

PMA Hybrid

- Suitable for the **wavelength range** from **300 nm to 720 nm**
- Provides high **detection efficiency** (typical 40% at 500 nm)
- **photosensitive area** with **3 mm** diameter (article no. 932200) and **5 mm** diameter (article no. 932203)
- **Dark count rate** < **1000 cps** (article no. 932200) and < **8000 cps** (article no. 932203)
- **NIM signal** output
- **Instrument response function** < **150 ps FWHM**
- Built-in **thermoelectrical cooler** with controller and signal amplifier

Please read the PMA Hybrid manual for details of use.

5. Set up of the Single Channel Detection Unit

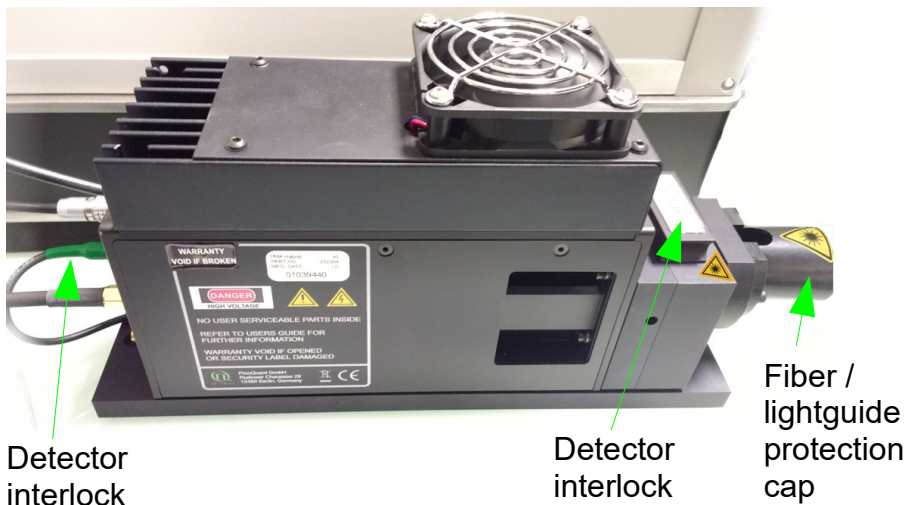


Fig. 1.2: Description of the Single Channel Detector

- In front of the PMA Hybrid, a **filter** can be placed into the filter holder (25 mm or 1 inch diameter).



Do not remove or change the filter of the Single Channel Detection Unit, before switching the microscope and laser completely off (see Fig. Fehler: Verweis nicht gefunden)

- A detector shutter **closes automatically** when the a filter is removed from the holder. This shutter protects the detector from overillumination. **It does not switch off the laser radiation** entering the Single Detection Unit.
- The connection for interlock should be always connected to the plug labeled with "Shutter" (otherwise the shutter does not open).



The built in overload protection has an approximately 1 second latency and is meant as a last rescue only. Protect the detector against over-illumination as much as possible. When the detector goes into the overload protection, a beep sound is emitted. The detector returns from overload into the normal status after some seconds.

- **Power supply:**
The detector can be switched on and off using the power switch at the power distribution board (Fig. 7.1).
- The detector signals are fed into the **TCSPC unit** . Look at the system specifications of your system (part of your LSM manuals) to ensure correct cabling.
- For correct operation, the CFD threshold setting has to be set correctly. For complete systems, this is done at the factory. In case you bought the PMA Hybrid without a complete system, the CFD threshold setting must be adjusted.

6. Adjusting the CFD threshold setting

In case you bought the PMA Hybrid without a complete system, the CFD threshold setting must be adjusted. This setting can be adjusted in the SymPhoTime64 software. Please make sure to have administrator rights and start the software in the "configuration" mode from the start menu. The measurement for the setting is done in the "Test" Tab using the "TCSPC Settings" drop down window (Fig. 6.1).

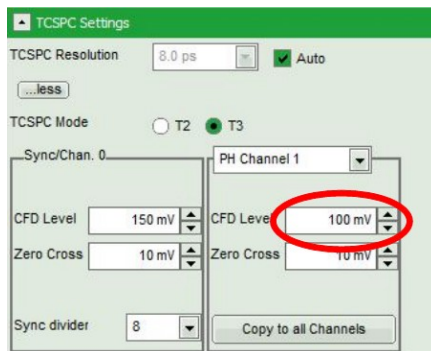


Fig. 6.1: Adjustment of the **CFD threshold level** by using the SmyPhoTime64 software

- The optical fiber or the light guide should be attached to the microscope. No light should enter the fiber or light guide for NDD. In case of a light guide, switch the room light off. The room must be completely dark.
- Please make sure that the detector is switched on and a filter is inserted into the filter holder in front of the PMA Hybrid (see Fig. Fehler: Verweis nicht gefunden). While switching the PMA Hybrid on, both lamps at the rear side should be on. They should switch off after some time. Now the detector is ready.
- Start a time trace measurement.
- HydraHarp and PicoHarp TCSPC: Set the CFD level to 100 mV, as displayed in Fig. 6.1.
- TimeHarp and MultiHarp TCSPC: Set the CFD level to - 100 mV.
- The final setting should be 10 mV higher compared to the setting, at which the count rate starts to strongly increase.
- Please save this setting and restart the software in the standard user mode.

7. Operation

7.1. Switching Your Single Channel Detection Unit On

1. Make sure that all **cables** are **connected correctly** according to the labeling scheme in the "system specifications".
2. Make sure that the **correct filter** is in the filter holder (see Fig. Fehler: Verweis nicht gefunden).
3. **Switch all components** of the system **on** (we recommend powering all devices by one central power switch).
4. Switch the detector on at the power distribution board (Fig. 7.1).



Fig. 7.1: Main power switch at the power distribution board

5. Using your LSM, **place** your **sample** into the focus and **take** an **image** with correct filter settings using internal LSM detectors.
6. Guide the **detection light** into the **fiber exit port** of the LSM.
7. Now the system is ready to take FLIM images or to make point measurements. For detailed description about how to perform individual measurements, follow the instructions in the LSM manual.

7.2. Switching Off Your System

1. Switch the detector off at the power distribution board (**Fig. 7.1**).
2. Turn off all other equipment, which is no further needed.

7.3. Checking Instrument Performance

1. To check the general performance, **acquire images** with the **normal LSM detectors** and afterwards using the **PicoQuant detectors**. Thus a drop in performance can be easily recognized. In the following description we assume that LSM images using the standard LSM detectors can be obtained without problems, using reasonable settings.
2. FCS setup only: A good way to check the performance of the detection unit is the acquisition of FCS-traces of a nM solution of a dye that can be effectively excited (see LSM manual, only for FCS systems). Acquire such traces with a standard solution from time to time using always the same settings (laser intensity, filters, pinhole size, objective, and the distance from the focus and the cover slide). If you are using a water objective, make sure the the cover slide correction ring is always at the same position. The count rate should not exceed 500 kHz, as the detector response becomes non linear at higher count rates. For FCS measurements, also cw lasers from your LSM can be used. If the laser power needs to be increased significantly in order to reach the same intensity values on the objective, the fiber coupling unit (LCU) must be optimized (see LCU manual).
3. FCS setup only: Calculate the **molecular brightness** of your dye by dividing the count rate of your detector by the number of molecules in the focus provided by FCS fitting (see SymPhoTime online help). Suited dyes for different wavelengths are: ATTO655 for 635 nm, Rhodamine 6G for 532 nm or ATTO488 for 485 nm excitation. The molecular brightness is not too sensitive to concentration changes. In general, a nM solution should be used. A more detailed description for acquiring FCS measurements is given in the LSM manual. If the molecular brightness has dropped significantly, the pinhole setting needs to be adjusted.
4. PMA detectors: The detector can decrease its sensitivity with time. At normal operation conditions with count rates below 5 Mcps the decrease is very slow. However, by exposing the detector to too much light (e.g. switching the room light on by an operating a non - shielded NDD detector) the ageing process can be strongly enhanced.

8. Further Reading

8.1. PicoQuant Bibliography

PicoQuant maintains a database of publications mentioning PicoQuant devices. It can be found at our website <http://www.picoquant.com/biblio.php>. It is a valuable source if you would like to know which laboratories are using PicoQuant products or how broad the field of various applications is.

8.2. Download of Technical Notes / Application Notes

PicoQuant, along with our customers, continuously writes and publishes short documents about techniques, methods and applications that are possible with our hardware or software. The download section can be found at <http://www.picoquant.com/apnotes.htm>

8.3. Recommended Websites

Optical filter manufacturers:

- <http://www.chroma.com>
- <http://www.omegafilters.com>
- <http://www.semrock.com>
- <http://www.lambda.cc>

Fluorescence dyes, probes and labels:

- <http://www.probes.com> (Alexa dyes)
- <http://www.amershambiosciences.com> (Cy dyes)
- <http://www.exciton.com>
- <http://www.eurogentec.com>
- <http://www.sigmaaldrich.com>
- <http://www.atto-tec.com> (Atto-dyes)
- <http://www.dyomics.com> (DY-dyes)

9. Abbreviations

BNC	British Naval Connector or Bayonet Nut Connector or Bayonet Neill Concelman
CCD	Charge-Coupled Device
CFD	Constant Fraction Discriminator
cps	Counts per Second
cw	Continuous wave (not pulsed)
FCS	Fluorescence Correlation Spectroscopy
LCU	Laser Coupling Unit
FIFO	First In, First Out (buffer type)
FLIM	Fluorescence Lifetime Imaging
FRET	Förster Resonance Energy Transfer
FWHM	Full-Width at Half-Maximum
IO	Input-Output
IRF	Instrument Response Function
LED	Light Emitting Diode
LSM	Laser Scanning Microscope
MCS	Multichannel Scaling
NDD	Non Descanned Detection
OD	Optical Density
PC	Personal Computer
PCI	Peripheral Component Interface
PIE	Pulsed Interleaved Excitation
PMT	Photomultiplier Tube
RGB	Red-Green-Blue (colour scheme)
ROI	Region of Interest
SMA	SubMiniature version A (connector type)
SMD	Single Molecule Detection
PMA	Photo-Multiplier Assambly
SYNC	Synchronization (signal)
TCSPC	Time-Correlated Single Photon Counting
TTL	Transistor-Transistor Logic
TTTR	Time-Tagged Time-Resolved

10. Support

10.1. Returning Products for Repair

If you have serious problems that require the device to be sent in for inspection / repair, please contact us at: info@picoquant.com and request a RMA number before shipping the device. Observe precautions against static discharge under all circumstances in handling, packaging and shipping. Use original or equally protective packaging material. Inappropriate packaging voids any warranty.

10.2. PicoQuant Community Forum

This forum is intended as a platform for users of PicoQuant's systems, components and software packages. It is not strictly limited to software related questions. As PicoQuant products cover a wide range of applications from single molecule experiments to life sciences and material science, discussions of the scientific background are of course welcome. The forum can be found at <http://forum.picoquant.com>

10.3. Problems and solutions

If you observe any errors or bugs, please try to find a reproducible error situation. E-mail a detailed description of the problem and relevant circumstances to info@picoquant.com. Your feedback will help us to improve the product and documentation.

In any case, we would like to offer you our complete support. Please do not hesitate to contact PicoQuant if you would like to have assistance with your system.

Of course, we also appreciate good news: If you have obtained exciting results with the LSM Upgrade Kit or published scientific papers, we would also like to know!

Please send us an e-mail to info@picoquant.com containing the appropriate citation. Gain additional publicity! PicoQuant maintains a database of publications mentioning PicoQuant devices and/or written by us. It can be found at our website at http://www.picoquant.com/_scientific.htm. It is a valuable source if you want to know which laboratories are using PicoQuant products or how broad the field of various applications is.

Thank you very much in advance for your kind cooperation!

11. Legal Terms

11.1. Disclaimer


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<p>Retraction of old devices</p> <p>Waste electrical products must not be disposed of with household waste. This equipment should be taken to your local recycling centre for safe treatment.</p> <p>WEEE-Reg.-Nr. DE 96457402</p>	
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