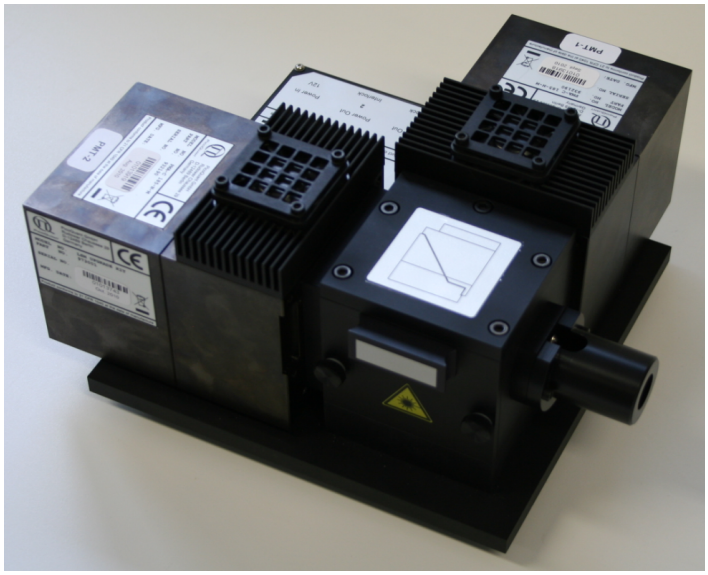
Dual Channel PMT Detection Unit

PicoQuant GmbH
Unternehmen für optoelektronische Forschung und Entwicklung

External Detection Unit for Laser Scanning Microscopes



User's Hardware Manual and Technical Data

Version 1.2

Table of Contents

Safety Instructions

LASER Warning!

Laser light of class 3B lasers may be delivered via the multimode fiber into the dual channel PMT Detection Unit. Therefore, the multimode fiber must not be removed from the detection unit. Do not remove any items inside the unit. The installation room of the LSM Upgrade kit has to be labeled as laser area. For class 3B lasers, a laser safety officer has to be announced to meet the laser safety regulations.

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 PDL 800-B or PDL 808 „Sepia“ 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 as much as possible, particularly from excessive light intensities, e.g. microscope illumination lamp, unattenuated backscattered excitation, etc.

1. Introduction

This manual describes the basic components of the dual channel PMT detection unit, as well as operation, maintenance and adjustment of the detectors .

The dual 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. Two single photon photomultipliers (PMT) as detectors allow time-resolved measurements on two different channels simultaneously. Possible applications are:

- Fluorescence lifetime imaging (FLIM)
- FLIM and FRET-FLIM measurements with 2 spectrally separated detectors

Look at PicoQuant's website to download technotes regarding different techniques and your LSM manual for instruction on how to perform different measurements on your confocal LSM.

The dual channel detection unit needs to be completed with a PMT router, which allows correct assignment of the detected photon events to the two detectors. For correct timing information, the constant fraction discriminator (CFD) must be set for each detector separately. This setting is done in the SymPhoTime software.

See the corresponding manuals for further information.

All abbreviations are explained at the end of the manual.

2. Physical Properties and Installation Requirements

Size [cm] (Width × Depth × Height)	19 × 19 × 11
Weight [kg]	6 kg
Operation Voltage	115V - 230 V AC
Fiber connector	FC-APC
room requirements	clean and dry
Temperature variation	should be kept within 3°C, otherwise de-adjustment of the detection optics cannot be excluded

3. PMT Detectors

The PMA type detector is a single photon sensitive, fully integrated, fast time response and low noise photon sensor from PicoQuant. The PMA integrates a fast photomultiplier tube (PMT), a high voltage power supply and a pre-amplifier. These devices are built in a gold plated iron housing to achieve the highest level of RF and magnetic shielding and protection against interferences with other devices.

The typically chosen PMA(-C) 185 module with an extended red cathode covers a wavelengths range from 300 nm to 850 nm. The dark count rate is around 2000 (300 cooled version) counts per second. The time resolution accounts typically to 220 ps FWHM. Around 10% detection quantum efficiency can be measured in the wavelengths range from 400 nm to 600 nm.

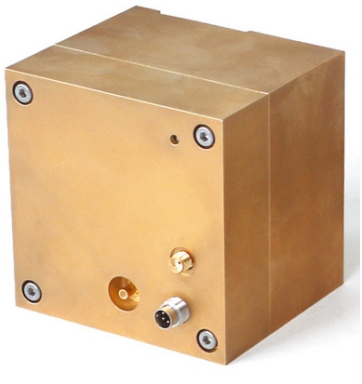


Fig. 3.1: PicoQuant PMA module

Fig. 3.2: PicoQuant PMA-C cooled module

4. Setup of the Dual Channel Detection Unit

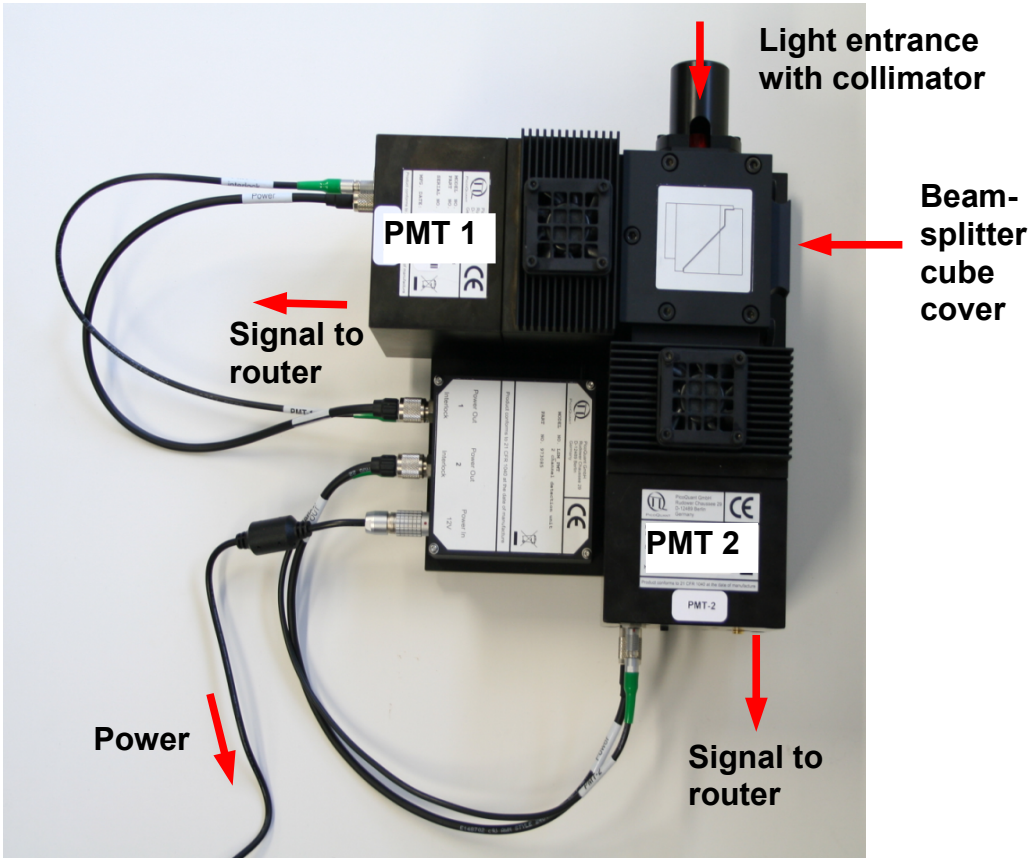


Fig. 4.1: Dual channel PMT detection unit

The dual channel detection unit consists of a fiber output collimator, a light tight housing for a beamsplitter cube and two PMA type PMT detectors with inbuilt shutters (see (Fig. 4.1)

- Shutters in front of each PMT **close automatically** when the cover for the filter cube is removed.
- The **beam splitter cube** (U-MF2 from Olympus)
  can hold one dichroic and two band pass filters (Fig. 4.2)
  - The dichroic should have a size of (25.5 x 36 x 1) mm rectangular. In order to exchange the dichroic, the two screws (arrows (1) in Fig. 4.2, left) should be removed.
  - The band pass detection filters should have a diameter of 25 mm or 1 inch. Use the tool delivered with the cube to mount the filters.
  - In order to remove the complete filter cube from the holder, loosen the screw indicated by arrow (2) in Fig. 4.2 (left).

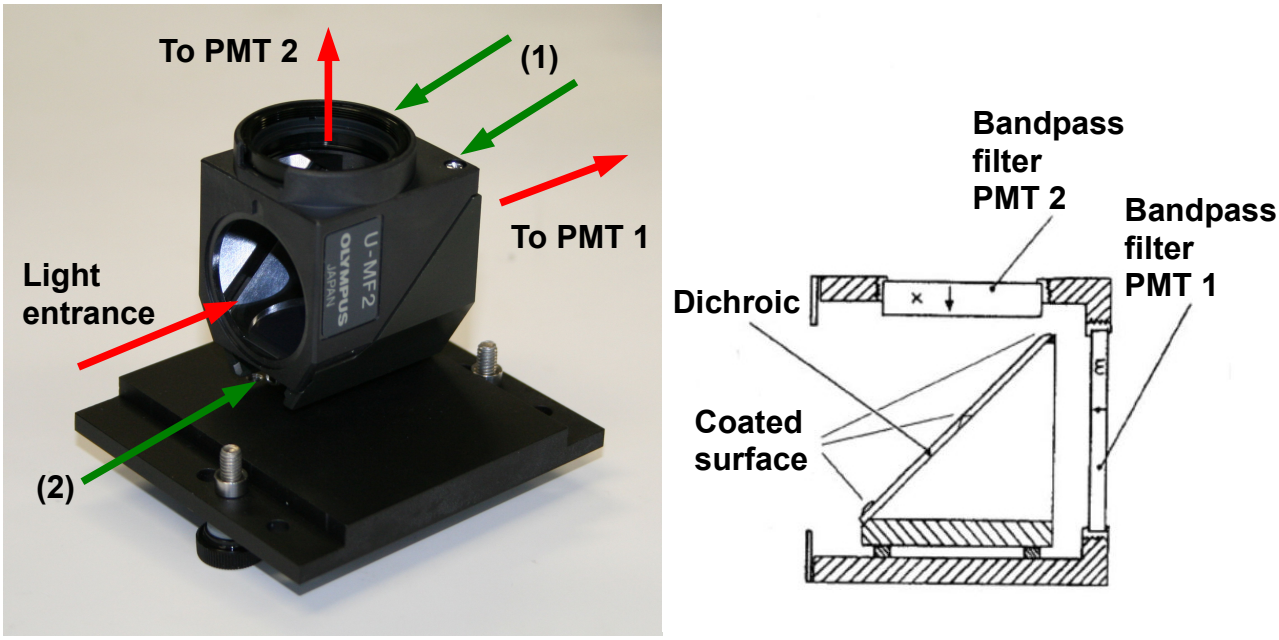


Fig. 4.2: The beam splitter cube. Left: Filter cube mounted on the dove tail. Right: Mounting of the optical elements: dichroic and bandpass filters.

- The PMT detector signals are fed into a **router**. The **router unit** permits in general up to **four PMT detectors** to operate quasi in parallel on the TimeHarp 200 or PicoHarp 300 board. Its role is to feed the detectors' signals into the single start input of the TCSPC device, simultaneously generating routing information (i.e. detector number) for each detected photon event.
- **Detailed information** about the routers can be found in the Router, TimeHarp 200 or PicoHarp 300 **manual**. Note that as the PH 300 and the TH 200 require different electronic signals, a router designed for the PH 300 cannot be used together with the TH 200 and vice versa.
- In order to perform fluorescence lifetime imaging, the **spatial origin** of the photons must be recovered as well. The TTTR file generated by the TimeHarp or PicoHarp then contain **markers**, e.g. synchronization information derived from the LSM scan-controller. This enables to reconstruct the 2D image from the stream of TTTR records, since the relevant XY-position of the scanner can be determined during the data analysis.
- Since **all three markers** are **already used** for the scanner synchronization, no additional marker inputs are free to be used for custom purposes.
- For **IRF** measurements it is recommended to use attenuation filters with an attenuation of 3 orders of magnitude (OD3 – filters) at the place of the bandpass filters and a (50% / 50%) transmission / reflection beamsplitter plate at the place of the dichroic.

5. Operation

5.1. Switching On your Dual Channel Detection Unit

1. Make sure that all **cables** are **connected correctly** according to the label scheme in your LSM upgrade appendix.
2. Make sure that the **correct filters** and **dichroic** are in the beamsplitter cube.
3. Make sure that the power supply of the dual channel PMT unit is plugged in.
4. **Switch all components** of the system **on** (we recommend powering all devices by one central power switch).
5. Using your LSM, place your sample into the focus and take an image with correct filter settings using the internal LSM detectors.
6. Guide the **fluorescence 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 a detailed description about how to perform individual measurements, follow the instructions in the LSM manual.

5.2. Switching Off your System

1. **Switch off all components** by switching off all components separately or use switchable power distribution block.

6. Recommended Literature

Publications related to the LSM Upgrade Kit hardware, software, and underlying key technologies:

Wahl M., Koberling F., Patting M., Rahn H., Erdmann R.: *Time-resolved confocal fluorescence imaging and spectroscopy system with single molecule sensitivity and sub-micrometer resolution.* Current Pharmaceutical Biotechnology, Vol.05, p.299-308 (2004) Koberling F., Wahl M., Patting M., Rahn H.-J., Kapusta P., Erdmann R.: *Two-channel fluorescence lifetime microscope with two colour laser excitation, single-molecule sensitivity, and submicrometer resolution.* Proceedings of SPIE, Vol.5143, p.181-192 (2003)

Ortmann U., Dertinger T., Wahl M., Rahn H., Patting M., Erdmann R.: *Compact TCSPC upgrade package for laser scanning microscopes based on 375 to 470 nm picosecond diode lasers* Proceedings of SPIE, Vol.5325, p.179 (2004)

Benda A., Hof. M., Wahl M., Patting M., Erdmann R., Kapusta P.: *TCSPC upgrade of a confocal FCS microscope.* Review of Scientific Instruments, Vol.76, 033106 (2005)

The following application and technical notes are available from PicoQuant upon request:

Koberling F., Schuler B.: *FRET analysis of freely diffusing molecules using the MicroTime 200*

Krämer B., Koberling F.: *Lifetime based hydrophobicity analysis of hepatocytes using the MicroTime 200*

Krämer B., Koberling F., Tannert A., Korte T., Hermann A.: *Lifetime based analysis of lipid organization in hepatocytes using the MicroTime 200*

Ortmann U., Dertinger T., Wahl M., Bülter A., Erdmann R., Kahl H.: *Compact FLIM and FCS upgrade kit for Olympus FV 300 and FV 1000 laser scanning microscopes*

Wahl M.: *Time-correlated single photon counting in fluorescence lifetime analysis*

Wahl M.: *Time tagged time resolved fluorescence data collection*

Numerous measurement examples are published on the PicoQuant website. Please visit the LSM Upgrade Kit section of `http://www.picoquant.com/_systems.htm`.

This manual frequently refers to information in additional PicoQuant manuals, which also belong to the LSM Upgrade Kit documentation:

- LSM Upgrade Kit Manual
- FCU Manual
- PDL 800-B – User's Manual
- PDL 808 "Sepia" – User's Manual
- TimeHarp 200 – User's Manual and Technical Data (this manual also contains an introduction into Time Correlated Single Photon Counting)
- PicoHarp 300 – User's Manual and Technical Data (this manual also contains an introduction into Time Correlated Single Photon Counting)
- SymPhoTime – User's Manual and Technical Data

7. Useful WWW Resources

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)

8. 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
FCU	Fiber 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
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
SPAD	Single Photon Avalanche Diode
SYNC	Synchronization (signal)
TCSPC	Time-Correlated Single Photon Counting
TTL	Transistor-Transistor Logic
TTTR	Time-Tagged Time-Resolved

9. Support

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!

All information given here is reliable to the best of our knowledge. However, no responsibility is assumed for possible inaccuracies or omissions. Specifications and external appearance are subject to change without notice.

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

PicoQuant GmbH
Unternehmen für optoelektronische Forschung und Entwicklung
Rudower Chaussee 29 (IGZ), 12489 Berlin, Germany

Telephone: +49 / (0)30 / 1208820-0
Fax: +49 / (0)30 / 1208820-90
e-mail: info@picoquant.com
www: http://www.picoquant.com