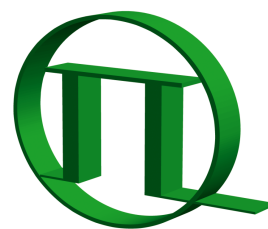


Single Channel SPAD Detection Unit



PICOQUANT

External Detection Unit for Laser
Scanning Microscopes



User's Hardware Manual and Technical Data

Version 3.1

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Safety Instructions



LASER Warning!

Laser light of class 3B lasers may be delivered via the multimode fiber into the Dual Channel SPAD 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 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 (SPAD or PMT) as much as possible, particularly from excessive light intensities, e.g. microscope illumination lamp, unattenuated backscattered excitation, etc. In case of operation with the DSN 102, the SPAD detector(s) must be switched off using the DSN 102 power supply unit before switching off the system by the main power button.

1. Introduction

This manual describes the basic components of the single channel SPAD 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. A single photon avalanche diode (SPAD) as detector allows time-resolved measurements with high sensitivity. These features cannot only be used in FLIM applications, but also allow a broad range of FCS measurements. Possible applications are:

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

Look at PicoQuant's website to download Technotes regarding different techniques and your LSM manual for instructions on how to perform different measurements with your confocal microscope.

Depending on the application, the unit can be equipped with two types of detectors. PicoQuant's τ -SPAD has a high sensitivity, especially in the spectral region above 600 nm, while the PDM Series by MPD have a significantly better time resolution, making them superior in FLIM-applications.

The Single Channel detection unit in conjunction with a PicoQuant τ -SPAD detector has to be completed with a DSN 102 module, a stand-alone device, which provides the power supply of the detector, but also protects the SPAD against hazardous over-illumination. Furthermore the device is equipped with a count rate meter. Detectors manufactured by MPD have a build-in over-illumination protection. Still, illumination levels of all SPAD detectors should be kept as low as possible. If your system is equipped with a DSN 102, look at its manual for further information.



All abbreviations are explained at the end of the manual.

2. Physical Properties and Installation Requirements

Size [cm] (Width × Depth × Height)	13 × 33 × 13
Weight [kg]	5.1 kg
Operation Voltage	115V - 230 V AC
Fiber connector	FC-APC
room requirements	clean and dry
Temperature variation	should be kept within 1°C, otherwise deadadjustment of the optical elements cannot be excluded

3. Detector Types

τ-SPAD Single Photon Avalanche Diode from PicoQuant

- Suitable for the **wavelength range** from **400 nm to 1100 nm**
- Provides high **detection efficiency** (typical 45% at 470 nm and 75% at 670 nm)
- Small **photosensitive area** with **0.15 mm** diameter
- **Dark count rate < 100 cps**
- **TTL and NIM signal** output
- **Instrument response function between 350 and 800 ps FWHM** dependent on module, wavelength and signal rate
- Built-in **thermoelectrical cooler** with controller, signal amplifier and discriminator
- Power supplied by the PicoQuant **DSN 102** module



Fig. 3.1: τ-SPAD detector module from PicoQuant

PDM Series Single Photon Avalanche Diode from MPD (MPD SPAD)

- Highest **detection efficiency** of **45 %** at 530 nm
- The **active area** in a mounted module has **100 μm diameter**.
- The **dark count rate** of a module in its cooled version is **<250 cps**.
- The **instrument response function** accounts to **50 ps FWHM**, an overall instrument response function below 150 ps can be obtained using a PicoHarp 300 TCSPC device together with a pulsed diode laser.
- The **instrument response function** does **not shift** with high count rates, so that lifetime measurements can be reliably performed with count rates up to more than 1 Mhz.
- The PDM series offer a **TTL and a NIM output**. The NIM output has a better timing response and is used in conjunction with the PicoHarp 300 device, while the TTR output is used for feeding the DSN102 for count rate display.



Fig. 3.2: MPD PDM series SPAD module

4. Set up of the Single Channel Detection Unit

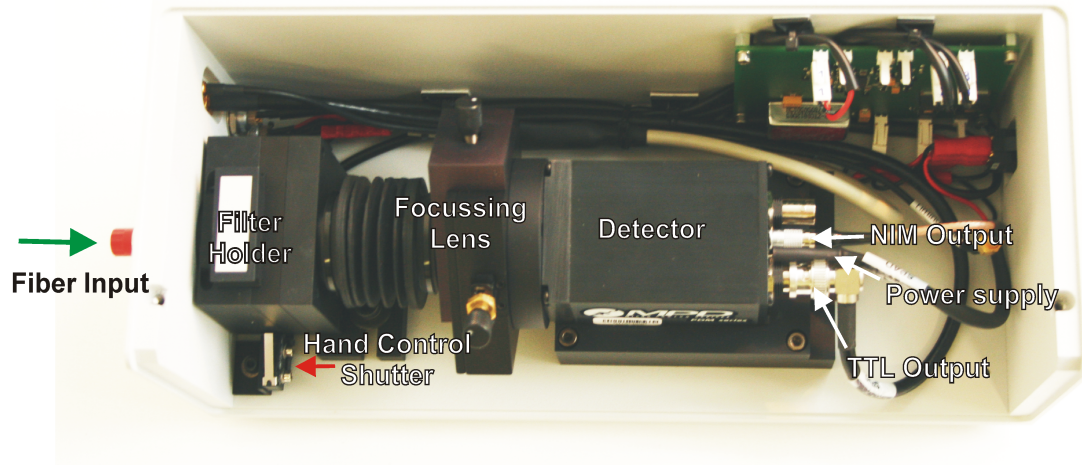


Fig. 4.1: Single SPAD detector unit, equipped with a MPD SPAD detector

- In front of the SPAD, a **filter** can be placed into the filter holder (25 mm diameter).
 - A shutter **closes automatically** when the a filter is removed from the holder.
 - The **shutter** can be **manually opened** by pressing the hand control (Fig. 4.1).
 - **DSN 102 Power supply (optional):**
 The **Power Supply** of the detector is provided by a **DSN 102** module. The **TTL output** of the detectors has to be connected with the DSN 102. The complete cable scheme of your setup is part of the system specifications to your system.
 - ➔ The DSN 102 can control and monitor up to **two SPAD detectors** at the same time, displaying the count rate of one selected module.
 - ➔ Power connectors are provided for τ -SPAD and MPD module types. Contact PicoQuant, if you want to change the detector type in your system.
 - ➔ Because excessive light exposure will damage the SPAD module, we have equipped the DSN with **automatic protection circuitry**. The built-in logic protects the SPAD modules by shutting down the supply voltage when the signal count rate exceeds 8 000 000 cps. Shutting down a SPAD module will be signalled with a warning beep and a message **Err** will appear on the display. The affected detector must then be powered up again manually.
- ⚠ This 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.
- ⚠ Do not turn off the main power before switching off the SPADs via the DSN.
- ⚠ The SPAD module must be connected **before** you switch on the DSN 102.



Fig. 4.2: DSN 102 dual SPAD power supply (optional)

- At the DSN 102 (optional) select the appropriate input **resistance** (impedance) for the signal input with the input termination switch. Choose **1 MOhm** if the **signal is fed through** this device to another device with an appropriate 50 Ohm termination. **Choose 50 Ohm** termination only if there is **no termination** in the subsequent signal chain, for example when no cables are connected to *TTL OUT* sockets as shown (Fig. 4.2).



Connecting the signal output with two shunting 50 Ohm terminations may damage the attached SPAD module(s).

- System without a DSN 102 power supply:**
If the system is **not equipped with a DSN 102 module**, the detector can be switched on and off using the power switch at the rear side of the module (Fig. 5.1). The power switch will light green when the detector is switched on. **Keep care not to damage the detector by too high light intensities (count rates beyond 8 Mcps).**
- The detector signals are fed into a **photon counting board** and may have to be attenuated or inverted. Look at the Appendix of your system (part of your LSM manual) to ensure correct cabling. In case of MPD detectors, the NIM signal of those detectors is used due to the better time resolution, in case of PE - detectors, the TTL signals are fed into the board via the DSN. Due to the better time resolution, we recommend usage of a PH 300 when MPD detectors are attached to the system.

5. Operation

5.1. Switching Your Single Channel Detection Unit On

- Make sure that all **cables** are **connected correctly** according to the labeling scheme in the appendix.
- Make sure that the **correct filter** is in the filter holder.
- Switch all components** of the system **on** (we recommend powering all devices by one central power switch).
- System without a DSN 102 power supply:** Switch the detector on at the main switch of your Single Channel Detection Unit (Fig. 5.1).



Fig. 5.1: Main power switch of the Single Channel Detection Unit

5. **System with a DSN 102 power supply:**

If you have a DSN 102, **switch the detector on**, make sure that the count rate is sufficiently low. Using the DSN 102, the main power switch of the detector module (Fig. 5.1) has no function.

6. Using your LSM, **place your sample** into the focus and **take an image** with correct filter settings using internal LSM detectors.
7. Guide the **detection light** into the **fiber exit port** of the LSM.
8. 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.

5.2. Switching Off Your System


1. If your system is equipped with a DSN 102, turn off the detector at the DSN 102 **first**.
2. Turn off all other equipment, which is no further needed.

5.3. 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. 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). 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 (FCU) must be optimized (see FCU manual).
3. 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 manual). Suited dyes for different wavelengths are: ATTO655 for 635 nm, Rhodamine 6G for 532 nm or Fluorescein (in a solution with high pH) for 470 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 detector needs to be adjusted.

5.4. Detector Unit Adjustment

5.4.1. Standard Alignment Procedure

1. Place an **empty cover slide** on the microscope stage.
2. Place an **OD3 light attenuator** at the filter holder in front of the SPAD detector. The filter is placed into the filter holder shown in Fig. 4.1.
3. **Switch** the corresponding **pulsed laser on** and lift the lever at the FCU to **direct pulsed laser light** through the objective (see FCU manual).
4. Select the settings in the LSM in a way, that the **back reflection** of the cover slide can be **monitored** by the LSM detectors (e.g. if a 470 nm laser diode is used for the adjustment, select the appropriate beamsplitter and an emission filter that is transmitting at 470 nm. Guide the reflection light to the fiber exit port where the detection unit is attached). As the pulsed lasers do not pass an acousto - optical modulator that controls the Olympus cw - lasers, light should be visible already on the microscope objective.
5. Activate the **oscilloscope window** in the SymPhoTime software () and select the Time Trace - tab with settings shown in Fig. 5.2. Important is the setting of a **linear count axis**.
6. Start the Oscilloscope measurement by clicking the Start - icon. The amplitude axis can be adapted by scrolling with the mouse wheel button while the arrow is placed over the window.
7. **Remove** the **cover** from the Single Channel SPAD Detection Unit.
8. Adjust the laser settings and the axial position of the objective on the microscope until you detect a reasonable count rate (e.g. 50000 cps)



Do not remove any filter from a Single Channel detection unit, while the manual shutter control is pressed.

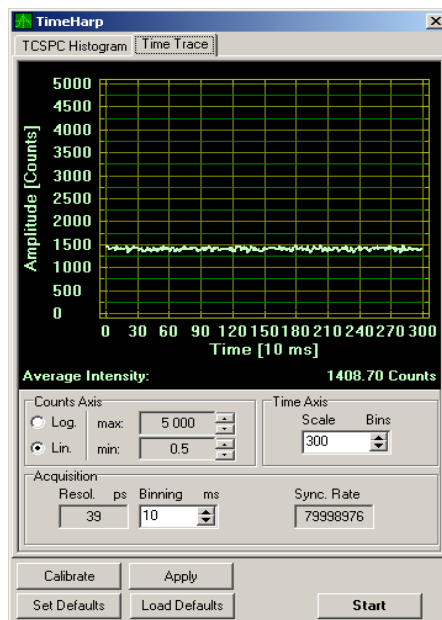


Fig. 5.2: The "Time Trace" tab from the Oscilloscope window

9. **Shift** the **focussing lens** in front of the detector by turning the **adjustment screws** indicated by the arrows in Fig. 5.3. Turning one screw moves the lens in one single direction. In the oscilloscope window, the count rate should directly react to the lens movement.

If you shift the lens back and forth around the optimal position with one screw, the time trace should show a **plateau** (Fig. 5.4).

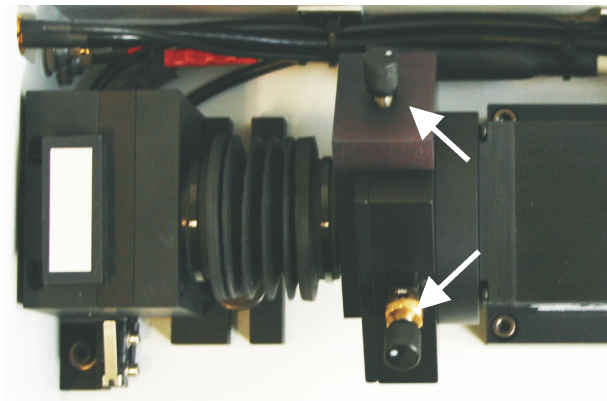


Fig. 5.3: Adjustment screws for the focussing lens in front of the SPAD detector.

10. The adjustment for a MPD SPAD is finished, when the lens is directed to the **center** of the **plateau**, indicating maximum count rate.

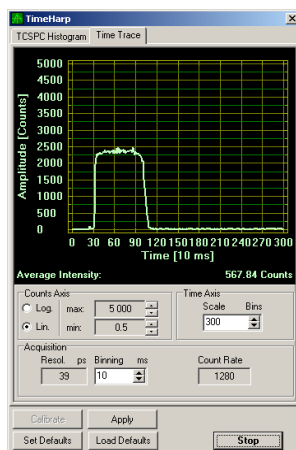


Fig. 5.4: Response of a τ -SPAD detector upon scanning a fluorescence beam over the detection area by moving the focussing lens.

11. For an τ -SPAD detector from PicoQuant however, the maximum signal rate is only one of the criteria to optimize the lens position. In a second step, you have to position the focus close to the center of the active area of the SPAD to ensure the narrowest IRF (i.e. the best time resolution) of the detector. Switch to TCSPC histogramming mode (by selecting the *TCSPC Histogram* tab) to constantly monitor the width and the time-position of the detector's IRF, next to the average signal count rate. Fine tune now the position of the focus on the active area by moving the focussing lens again, and watch the width of IRF.

As a rule of thumb, try to move the IRF to its leftmost position. For PicoQuant τ -SPAD modules, this position corresponds directly to the shortest IRF (Fig. 5.5). For MPD modules, this alignment step is not necessary.

12. When finished, lower the objective and remove the test sample. The system is now **ready** to perform measurements.

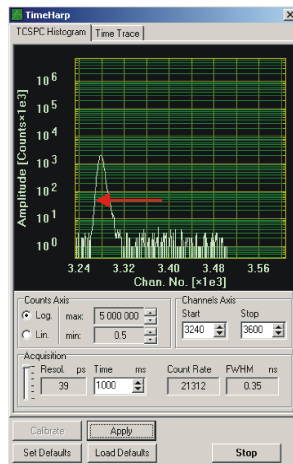


Fig. 5.5: IRF of a PicoQuant τ -SPAD module in the oscilloscope window. The shortest IRF is obtained when the pulse peak is shifted maximally to the left. The width of the IRF is given by the FWHM value

5.4.2. Fundamental Alignment of the Single Channel SPAD Detection Unit

If adjustment of the focussing lens does not lead to a satisfactory result, a fundamental alignment is necessary.

Switch the SPAD detector off before performing the alignment steps!



LASER Warning!

Laser light of class 3B lasers may be delivered via the multimode fiber into the Single Channel SPAD Detection Unit. Therefore, the multimode fiber must not be removed from the detection unit. As during the alignment, items within the Dual Channel Detection Unit will have to be removed, follow the laser safety rules for treating open laser beams. Do not look into the beam. Do not insert reflecting items (e.g. mirrors, rings) into the beam.



Generally, this alignment procedure should be a rare event. During the installation of the system by PicoQuant, the optics is aligned and then remains stable for a long time. Re-alignment is necessary when the LSM Upgrade Kit detector unit is disassembled, or when its optical design is changed. Carrying out such a procedure requires considerable experience. Before proceeding with the alignment, make sure that the problem is not in the excitation path and that the alignment cannot be done with the ordinary procedure.

Proceed the following steps:

Switch the SPAD detector off before performing the alignment steps!

1. Prepare the **alignment tool** (Fig. 5.6) delivered with the instrument.



Fig. 5.6: Alignment tool for detector unit alignment

2. Detach the two screws at the cover of the Single SPAD Detection Unit and remove it. Make sure that the **detector is switched off with the switch of at the Single Channel detection Unit**. To keep the shutter open, the power supply cable should not be unplugged.
3. Use the **back reflection** of a **mirror** or the lower side of the sample holder plate. Increase the laser power of all lasers to the maximum.
4. **Remove the tubing** between the focussing length and the shutter (indicated by the white arrow in Fig. 5.7). The tubing is screwed into the focussing lens holder, the direction to unscrew the tubing is indicated by the green arrow. Also, remove any filter from the filter holder and put an empty filter holder. Alternatively, the filter holder can be removed and the hand control button can be pressed to open the shutter (Fig. 4.1)

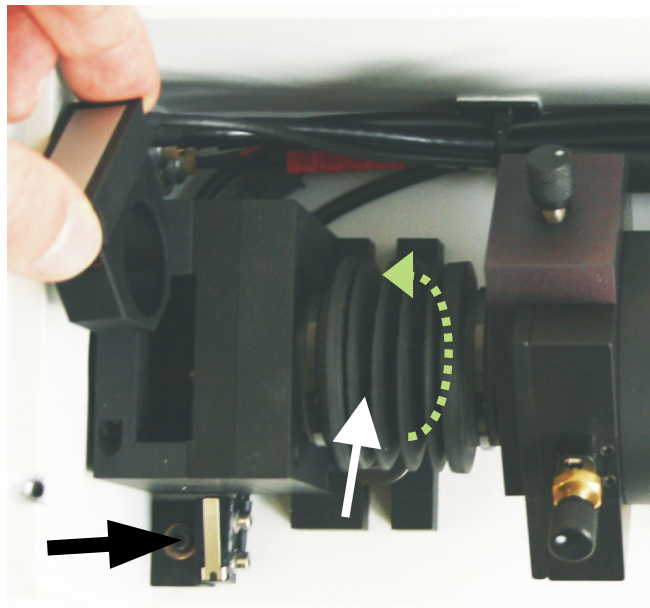


Fig. 5.7: Single Channel SPAD detection unit with empty filter holder being placed in the appropriate position. For fundamental alignment, the tubing indicated by the white arrow has to be removed. The hand control button (black arrow) can be pressed to open the shutter.

5. Place the adjustment plate (Fig. 5.6) before the detector as shown in Fig. 5.8. In case of correct adjustment, the beam is placed at the center of the adjustment plate. Otherwise the beam needs to be directed to the center of the adjustment plate by adjusting the screws at the fiber coupler (Fig. 5.9). To access the fiber coupler screws, remove the protection tube from the coupler. Adjust the beam the screws indicated in green in Fig. 5.9; if necessary, loose the counter screws indicated in yellow in Fig. 5.1. The counter screws must be tight after alignment.

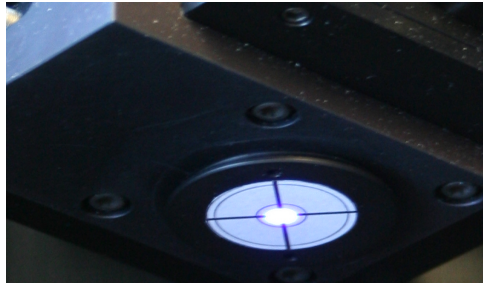


Fig. 5.8: SPAD with removed tubing and attached beam alignment tool.

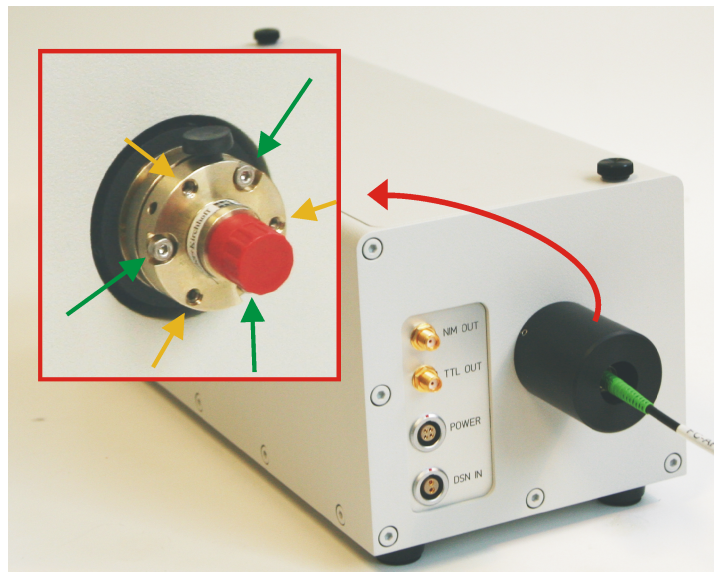


Fig. 5.9: Fiber outcoupling of the Single SPAD Detection Unit with protection cap and coupler without protection cap (inset). The adjustment screws for tilting the beam are indicated in green, the counter screws for stabilizing the coupler position are indicated in yellow. Unlike in the real instrument, no fiber is attached to the coupler in the inset.

6. When the beam is aligned, insert the tubing and place the the protection cap again. Finally, the lens position needs to be optimized using a scattering or reflecting test sample as described in the standard alignment procedure.

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:

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., Bültner 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 photonics@pq.fta-berlin.de 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.

<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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