# Multi Channel Detection Unit



External Detection Unit for Zeiss Laser Scanning Microscopes



User's Hardware Manual and Technical Data

Version 4.1

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# 1. Safety Instructions

# 1.1. General safety information





Before using this device, make sure that you have read and understood the content of this user manual. Store this documentation 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.

# 1.2. 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 <b>general safety alert symbol</b> 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.
4	A <b>high voltage warning symbol</b> 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 <b>laser radiation warning symbol</b> 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 <b>ESD</b> 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 prefaces any instructions that have to be followed to avoid sever 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 " <b>NOTICE</b> " label.
	This symbol indicates that an earth terminal needs to be connected to the ground (to avoid risks of electrical shock).

# 1.3. Laser Radiation Safety Instructions



## **WARNING!** Visible and invisible laser radiation

Laser light of class 3B laser radiation may be delivered via the optical connections into the Multi Channel Detection Unit. Therefore, the fibers and light guides should not be removed from the detection unit. Inside the Multi Detection Unit laser radiation of up to class 3B can occur.

Laser class 3B / IIIB require to wear special laser safety glasses. The installation room of the LSM Upgrade kit must be labeled as laser area.

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 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**

- Please stop any measurement or scan at the Zeiss LSM before opening the filter holder.
- Once the filter holder is removed from the multi detection unit, an interlock system switches the laser radiation off.
- However, if the interlock is defeated, the laser beam inside the housing is freely accessible.
- 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.
- 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.
- 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 eye injury. Class 3B / IIIB 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. If your instrument uses another excitation system, follow the safety instructions of the relevant manual.
- Never remove the optical fiber from the system when the lasers are powered.
- · Do not disable the interlocks on the Multi Detection Unit
- 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, PMA, PMA Hybrid) as much as possible, particularly from
  excessive light intensities, e.g. room light, a microscope illumination lamp, unattenuated
  backscattered excitation light, etc. The SPAD detector(s) must be switched off using the remote
  control unit BEFORE switching off the system by the main power button.



- To avoid hazardous radiation exposure you should carefully obey the safety instructions that are provided with your laser scanning microscope and laser manual. 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, PMA Hybrid, SPAD) as much as possible, particularly from excessive light intensities, e.g. room light, microscope illumination lamp, unattenuated backscattered excitation, etc.

# 1.4. 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: Laser radiation safety label on the filter holder of the Multi Channel Detection Unit. Do not remove the fiber protection cup or fiber.



Fig. 1.2: Safety label of the Multi Channel Detection Unit after removing the filter holder. Once the filter holder is removed, laser radiation of laser class 3B can occur inside the housing when interlock defeated.

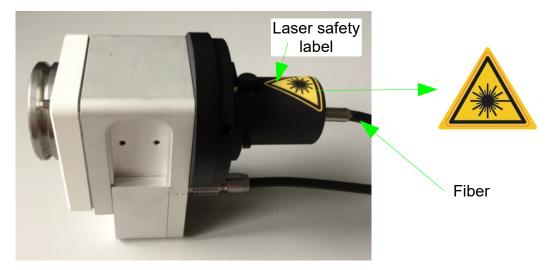


Fig. 1.4: Laser radiation safety label on the fiber protection cup at the Fiber coupler (confocal setup). Do not remove the fiber coupler, do not remove the fiber protection cup or fiber.

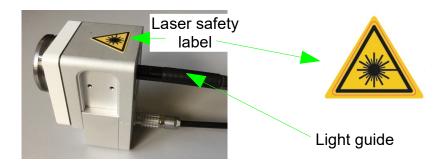


Fig. 1.5: Laser radiation safety label on the light guide at the NDD coupler (NDD = Non-Descanned-Detection). Do not remove the light guide or NDD coupler!



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 when interlock is defeated.

## 2. Introduction

This manual describes the basic components of the Multi Channel Detection Unit, as well as operation, maintenance and adjustment of the detectors.

The Multi 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 or light guide. The detection unit is mainly used in conjunction with PMA Hybrid detectors, but also SPAD detectors can be used. The PMA Hybrid detector allows time-resolved measurements with high sensitivity and precision. The SPAD detector with a dark count rate < 100cps provides a high detection efficiency especially in the red range. 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 (auto- and crosscorrelation)

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

The PMA-Hybrid and the SPAD detector have an over - illumination shut down. When too high count rates are reached, 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.

The SPAD detection device is equipped with a DSN power supply which switches the SPAD off, if the detection count rate exceeds 8 Mcps. The DSN displays the count rate for fast visual inspection.

# 3. Detector Types

#### **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. 932204) and 5 mm diameter (article no. 932207)
- Dark count rate < 1000 cps (article no. 932204) and < 8000 cps (article no. 932207)</li>
- Negative polarity signal output
- **Instrument response function < 120 ps FWHM** (only detector contribution, the measured instrument response function might be broader due to laser, electronics and optics)
- Built-in thermoelectrical cooler with temperature controller and signal amplifier

#### PMA (photomultiplier tube)

- Spectral **wavelength range** from **185 nm to 900 nm** (depending on PMA type, note that the wavelength range is also restricted by the optical fiber and the LSM, typically to wavelength above 370 nm)
- Provides high **detection efficiency** (typical max. 40% at 400 nm, PMA-175, 20% at 400 nm, PMA-182, 18% at 470nm, PMA-192, see detector data sheet for the spectral response curves of your detector)
- Photosensitive area with 8 mm diameter
- Dark count rate <50 cps (PMA-175), < 900 cps (PMA-182) and < 1500 cps (PMA192, cooled)</li>
- Negative polarity signal output
- **Instrument response function < 180 ps FWHM** (only detector contribution, the measured instrument response function might be broader due to laser, electronics and optics)
- Built-in cooler for red-sensitive PMAs

## SPAD (Single Photon Avalanche Diode)

- suitable to detect single photons in the spectral range between 400 nm and 1100 nm.
- provides high detection efficiency especially in the red range (around 70% at 700 nm)
- Photosensitive area with 180 µm diameter
- Dark count rate typical 100 cps
- generates a TTL output designed for a 50 Ohm load

The TTL signal output is connected to the DSN for count rate monitoring, the TCSPC module is connected to the signal output connector of the DSN. In case the TCSPC unit requires a NIM signal, the TTL to NIM converter SIA400 is used.

- Instrument response function typically 350 ps 800 ps FWHM, depending on model, wavelength and signal rate
- SPAD problems:

single photon timing resolution of the SPAD depends on three factors and is generally different for every singe module (please see the test report of your SPAD for details):

- IRF shift at high count rates:
   the FWHM of the photon timing resolution increases at high count rates. For FLIM applications is recommended to work with count rates below 2 Mcps.
- IRF change at different detection wavelengths:
   the best photon timing resolution (i.e smallest FWHM) is generally achieved at around 680 nm.
   The FWHM increases towards lower detection wavelengths.
- Afterpulsing is a detector artefact. With a probability of around 1%, a detected photon is followed by an additional detector pulse which does not have its origin in a detected photon.

Please read the PMA Hybrid / PMA manual for details of use.

# 4. Set up of the Multi Channel Detection Unit



Fig. 4.1: Multi Channel Detection Unit for two detection channels. In case more than 2 detectors are present, additional beam splitter towers are mounted.

- The Multi Channel PMA Hybrid Detection Unit can be equipped with up to 4 PMA Hybrid, PMA detectors or SPCM-SPADs.
- Via an optical fiber, the unit can be connected with the LSM in order to measure FLIM using descanned detection.
- Additionally, the unit can be used for NDD imaging (In systems equipped with max. 2 detectors or using
  only a single detector) via connecting the unit with a liquid lightguide to the LSM. This configuration is
  described in a dedicated manual and is only available with PMA Hybrid detectors.
- Descanned and non-descanned detection can be combined using different input ports at the Multi Channel PMA Hybrid Detection Unit.
- In systems equipped with a polarizing beam splitter at the LSM, the unit can also be configured for anisotropy imaging using two fiber inputs.
- In order to split the fluorescence, the filter holder of the Multi Channel Detection Unit can be equipped with emission filters for each channel as well as a dichroic. All filters should have standard size (25 mm or 1 inch diameter for the emission filters and 36 x 25.5 x 1 mm for the dichroic).

An emission filter can be easily exchanged by opening the screw holding the filter.



Please make sure that before removing the filter holder, stop all measurements or scans of the LSM.

Please be avare that removing the filter holder or switching off the Multi Detection Unit switches off all lasers, also for standard LSM measurements.

- A dichroic can be easily replaced by sliding it out and after replacement in again.
- Please be aware that each filter holder has a specific alignment for the respective tower as indicated on
  the filter holder. For example a filter holder for splitting the fluorescence between detector 1 and 3 in the
  first tower can not be used for the splitting between detector 2 and 3 in the second tower (while having a
  three channel setup). In addition, the filter holders for SPAD detection differ from the holders used with
  PMA detectors. They are equipped with a fine-adjustment for the horizontal turning of the dichroic
  element.
- All detector shutters **close automatically** when a filter holder is removed from the Multi Channel Detection Unit.
- The connections for the interlock should be always connected (otherwise the shutter does not open). In case of a PMA detector the plug is labeled with "Shutter".



PMA Hybrid detectors: The built in overload protection has an approximately 1 second latency and is meant as a last rescue only. Protect the detectors 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.

- The detector can be switched on and off using the power switch at the Multi Channel Detection Unit (Fig. 4.1). Switching off the device disables laser radiation of the laser scanning microscope, also for standard operation.
- System with fiber coupled descanned detection in addition to non-descanned detection (NDD with light guide coupling): While using standard descanned detection, the NDD port must be covered with special beam stops which have to be inserted into the filter holder at the side where the NDD light enters the Multi Channel Detection Unit (compare Fig. 4.1).
- 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 Multi Channel Detection Unit without a complete system, the CFD threshold setting must be adjusted.

# 5. Setup of Multi Channel Detection Unit with SPADs

• When using the Multi Channel Detection Unit with SPAD detectors, special filter holders for SPADs are used for the corresponding tower. Which filter holders can be used for which tower is indicated on the tower and at the filter holders by specific numbers (see Fig. 5.1).



Fig. 5.1: Correct filterholders in corresponding towers are indicated by numbers

• The SPADs are connected with a DSN-Module. The TTL signal output of the SPAD is connected to the DSN for count rate monitoring. The DSN controls the count rate of the SPAD and prevents over-illumination. The power of the SPAD is switched off, if count rates larger then 8 Mcps are reached. In addition, the SPAD will switch off, if a detection filter holder is removed. This prevents the SPAD from detecting room light. For this reason, the interlock device TMF 400 is connected to the Gate input of the SPAD. The input of the TMF 400 is connected to the SPAD interlock output at tower number 1.

# 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).

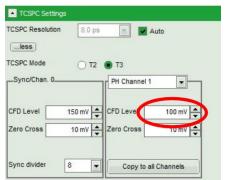


Fig. 6.1: Adjustment of the CFD threshold level by using the SmyPhoTime64 software

- 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 detector.
  - PMA hybrid: 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. Please remove now the filter in front of the PMA Hybrid in order to close the shutter of the detector.
- Start a time trace measurement, using a reflective sample or a strongly quenched dye (e.g. concentrated Erythrosin B in saturated KI solution). Ideally, do a point measurement. If this in not available, maximize the zoom to get an almost point like region and a stable countrate.
  - PMA hybrid: Set the CFD level to 100 mV, as displayed in Fig. 6.1.

- PMA: Set the CFD level to 10 mV, as displayed in Fig. 6.1. Please write down the count rate. Now go in steps of 10 mV higher and write down the count rate at each step. You will find that the count rate decreases when increasing the CFD-level. Stop when the countrate has dropped to 60 70 % of the max. value. Now, monitor the TCSPC histogram. Set the Zero Cross level to 10 mV and vary until you get the smallest peak FWHM.
- Please save this setting. Therefore, press "Save Settings" in the drop down menu, then go to Settings→Save factory configuration or save user configuration and restart the software in the standard user mode.
- If the system has been installed by PicoQuant, settings are already set correctly and saved. The factory configuration set by PicoQuant is additionally stored on an USB memory device in case that the software needs to be re-installed.

# 7. Operation

# 7.1. Switching Your Multi 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 filters and dichroics** are in the filter holder.



Please make sure that before removing the filter holder, stop all measurements or scans of the LSM.

- 3. **Switch all components** of the system **on** (we recommend powering all devices by one central power switch located at the power distribution unit).
- 4. Start the SymPhoTime 64 software.
- 5. Switch the detector on at the Multi Channel Detection Unit (Fig. 4.1). In the SymPhoTime 64 software, the dark count rate can now be monitored.
- 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** to the **fiber exit port** of the LSM or switch to NDD detection.
- 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.

# 7.2. Switching Off Your System

- 1. Switch the detector off at the Multi Channel Detection Unit (Fig. 4.1).
- 2. Turn off all other equipment, which is no further needed (we recommend powering off all devices by one central power switch located at the power distribution unit). Please be avare that removing the filter holder or switching off the Multi Detection Unit switches off all lasers, also for standard LSM measurements.

# 7.3. Checking Instrument Performance

 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 that the cover slide correction ring is always at the same position and always use cover slides with the same thickness. The count rate should not exceed 2 Mcps, as the detector response becomes non linear at higher count rates. For FCS measurements, also cw lasers from your LSM can be used.
- 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 only slightly 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.

# 7.4. Detector Unit Adjustment

## **General Remark:**

In case you moved one adjustment element and lost the signal <u>NEVER</u> turn a different adjustment element. Always make sure to readjust the element you just moved. 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 and should be performed by the PicoQuant service team.

## 7.4.1. Alignment of SPAD modules

For alignment of the XY lens in front of the SPAD detector modules the following steps should be performed:

- Prepare a ~20 μM solution of fluorescent dye for the selected laser wavelength (the color of the solution should be clearly visible)
- 2. Clean the objective and apply immersion medium
- 3. Mount a cover glass in the sample holder and put a drop of the dye solution on it
- 4. Place a suitable fluorescence filter in front of one detector
- 5. Reduce the laser power (select OD2 or OD3 on LCU) to prevent over-illumination of the detector
- 6. Start a point measurement and switch to the time trace view
- 7. Raise the objective so that the focus is 1µm inside the solution
- 8. Set the count rate to 2 Mcps by adjusting the laser intensity
- 9. Turn one of the XY SPAD lens position knobs back and forth. You should observe an intensity plateau with steep edges (SPCM-SPAD modules have a large active area), see Fig. 7.1

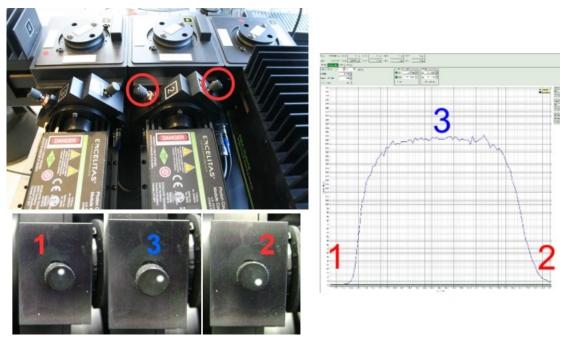


Fig. 7.1: Different positions of the SPAD-XY lens position knobs (displayed on the left) result in an intensity plateau of the active detector area

- 10. Adjust the XY SPAD lens position inside the plateau range or on the maximum respectively by iteratively turning both knobs
- Switch to the TCSPC histogram view.
   Set the y axis to linear and magnify the rising edge of the time response curve.
- 12. Adjust both XY SPAD lens position knobs so that the rising edge of the TCSPC curve moves as much as possible to shorter times (refer to Fehler: Verweis nicht gefunden).

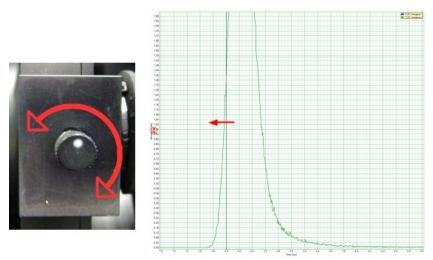


Fig. 7.2: Fine tuning the SPAD lens alignment for a SPAD: Turn both adjustment knobs of the SPAD module in order to move the rising edge oft the TCSPC diagram as much as possible to the left. This corresponds to the sharpest IRF. For your convenience click on the "Search Edges" button on the top of the graph.

13. Repeat the lens position optimization procedure for every SPAD.

## 7.4.2. Alignment of the PMA-Hybrid Filter Holder

Under standard conditions, no alignment is necessary. However, in case you want to align the Multi Channel Detection Unit here you will find some alignment steps. Please contact PicoQuant beforehand to make sure that you really need to perform these steps. They are only advised for people trained in optical alignment and laser handling.

After aligning the fiber incoupling (refer to previous paragraph) the alignment of the filter holder is in most cases necessary.



## **LASER Warning!**

Laser light of class 3B laser radiation may be delivered via the optical connections into the Multi Channel Detection Unit. Therefore, the fibers and light guides should not be removed from the detection unit. When perfoming the alignment, always keep the filter holder on the Multi Detection Unit



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 with the following steps:

- 1. Please note that an alignment of the Filter Holder effects only the detector which gets the light deflected by the dichroic inside the Filter Holder (see red arrow in Fig. 7.3).
- Place a homogeneous sample (dye solution of some μM concentration or fluorescent plastic slide) on the microscope.
- 3. Apply a filter holder with an appropriate dichroic for the sample used.
- 4. Set everything up to detect a constant count rate of 100 kcps at the detector, which gets the light deflected by the dichroic inside the Filter Holder (see red arrow in Fig. 7.3).
- 5. Display the count rate with the time trace in the "Test" tab.
- 6. Alignment without a count rate of around 100 kcps leads to a complete misalignment of the unit.

  Please contact PicoQuant in this case. Do not proceed with any alignment!



Fig. 7.3: Alignment of the filter holder

## 7. Dichroic angle adjustment:

The two angle alignment screws indicated with the green arrows in Fig. 7.3 press from both sides against the plate which is holding the dichroic. By fastening one screw while loosening the other the angle of the dichroic can be changed.

- 8. Try first by fastening the first screw just a bit while loosening the other slightly. Does the count rate increase? Then proceed with this procedure until a maximum in the count rate is reached.
- 9. If the count rate decreases, then loosen the first screw a bit while fastening the other slightly.
- 10. Proceed until you reach a maximum in count rate.
- 11. Do not proceed with the next step until the maximum of the count rate is reached.

## 12. Alignment of the dichroic through turning:

The big round knob at the filter holder can be turned when loosening the fixing screws marked by the white arrows in Fig. 7.3.

- 13. Mark now the present position with some marker as indicated by the blue line in Fig. 7.3. By this means the present position can be easily recovered.
- 14. Now loosen the two screws marked by the white arrows in Fig. 7.3 slightly. Now you can turn the big round knob a few degrees and optimize the count rate.
- 15. If the count rate is maximized you can fix the two screws marked by the white arrows in Fig. 7.3.
- 16. The alignment is now ready!

# 8. Technical Data / Specifications

## **Connectors**

Fiber Connector	FC-AFC			
Detector Signals	SMA			
Operation Voltage (external power supply)	115V - 230 V AC, 50/60 Hz, max. 70 W			
Dimensions (Depth x Width x Height, in mm)	_280 x 310 x 180			
Temperature Range	15°C - 30°C			
Temperature Variation	_should be kept within ±3°C			
otherwise deadjustment of the optical elements cannot be excluded				
Humidity	< 60% rel. humidity			
Location Hight	_max. 2000 m over sea level			
Weight	~ 10 kg			

## **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



# 9. Further Reading

## 9.1. PicoQuant Bibliography

PicoQuant maintains a database of publications mentioning PicoQuant devices. It can be found at our website <a href="https://www.picoquant.com/scientific/references">https://www.picoquant.com/scientific/references</a>. 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.

## 9.2. 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 <a href="https://www.picoquant.com/scientific/technical-and-application-notes">https://www.picoquant.com/scientific/technical-and-application-notes</a>

## 9.3. 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:

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

## 10. 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 Continous 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

NIM Nuclear Instrumentation Module

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 Assembly
SYNC Synchronization (signal)

TCSPC Time-Correlated Single Photon Counting

TTL Transistor-Transistor Logic
TTTR Time-Tagged Time-Resolved

# 11. Support

## 11.1. Returning Products for Repair

If you have serious problems that require the device to be sent in for inspection / repair, please contact us at: <a href="mailto:info@picoquant.com">info@picoquant.com</a> 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.

## 11.2. 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!

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