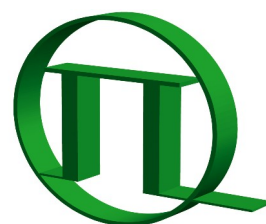


NDD Adapter for Upright Nikon Microscopes



PICOQUANT

External NDD Adapter for Laser Scanning Microscopes



User's Hardware Manual and Technical Data

Version 2.2

Table of Contents

1. Safety Instructions.....	3
1.1. General Safety Instructions.....	3
1.2. Safety Labels on the System.....	4
2. Introduction.....	6
3. Physical Properties and Installation Requirements.....	6
4. Detector Type.....	7
5. Set up of the NDD Adapter.....	7
6. Operation.....	9
6.1. Performing a Measurement with Your NDD Adapter.....	9
6.2. Switching Your System Off.....	9
6.3. Alignment.....	9
7. Further Reading.....	10
7.1. PicoQuant Bibliography.....	10
7.2. Download of Technical Notes / Application Notes.....	10
7.3. Recommended Websites.....	10
8. Abbreviations.....	11
9. Support.....	12
9.1. Returning Products for Repair.....	12
9.2. Problems and Solutions.....	12
10. Legal Terms.....	13
10.1. Disclaimer.....	13
10.2. Copyright.....	13
10.3. Trademarks.....	13

1. Safety Instructions

1.1. General Safety Instructions



LASER Warning!

Lasers for two photon excitation deliver infrared 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 you to wear special laser safety glasses. **Do not remove any items of the NDD adapter before switching the microscope and laser completely off.** The installation room of the LSM Upgrade kit must be labeled as a laser area. For class 4 / IV lasers, a laser safety officer has to be named 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.
- 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 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.



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!

Never remove any item of the NDD adapter as long as laser and microscope are powered ON!

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.

1.2. Safety Labels on the System

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



Fig. 1.1: Safety labels:
LASER APERTURE and Laser safety labels.

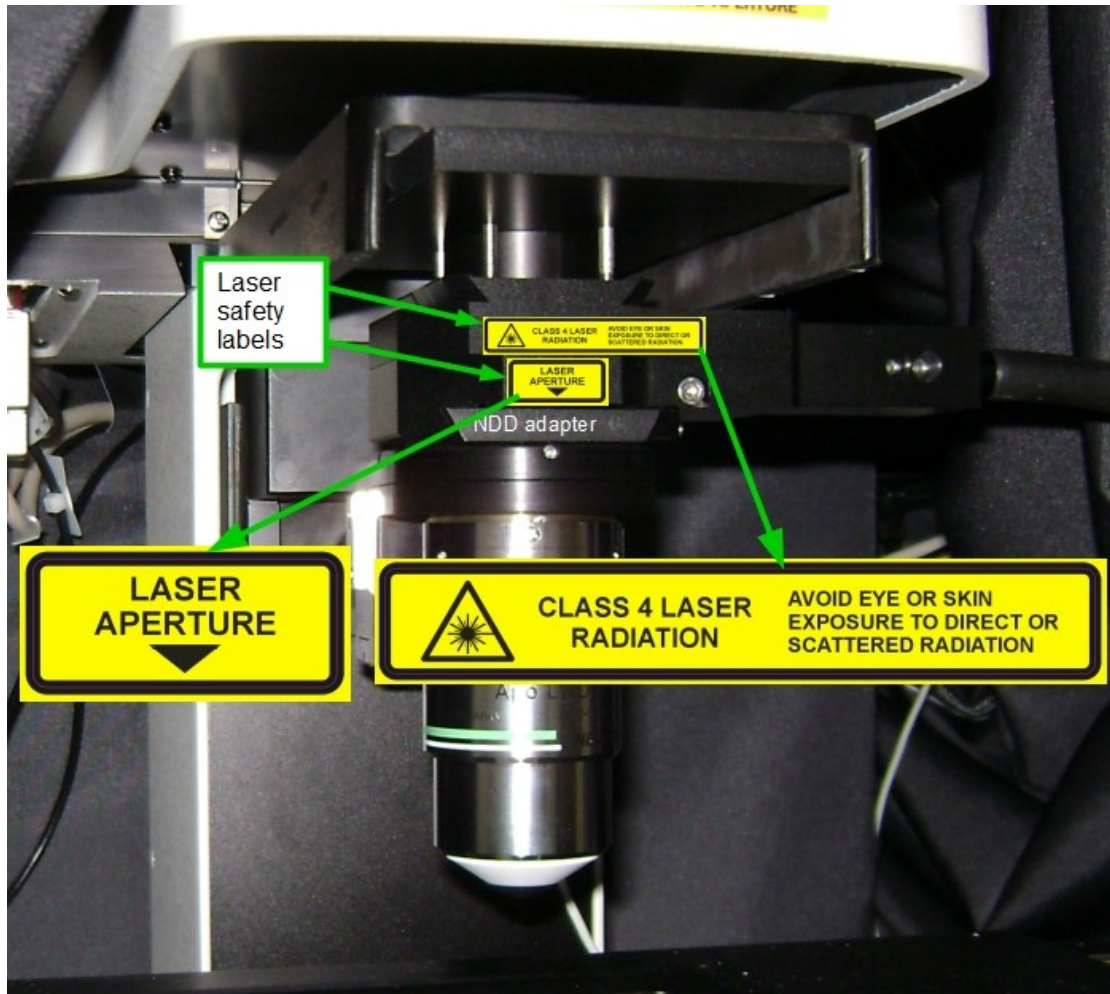


Fig. 1.2: Safety labels: CLASS 4 LASER RADIATION and LASER APERTURE Labels.



Fig. 1.3: Safety labels on light guide connecting the NDD adapter with the detection unit: LASER APERTURE and Laser safety label.



Take care to obey all safety regulations. Never remove any item of the NDD adapter as long as laser and microscope are powered on! Never remove the liquid light guide from the NDD adapter or detector.

2. Introduction

This manual describes the basic components of the NDD adapter, as well as operation, maintenance and adjustment of the detectors. NDD means Non Descanned Detection which can be only used in conjunction with two-photon excitation (TPE) with extremely short pulses of pico or femtosecond duration. The advantage of this NDD adapter is that it collects the light directly at the objective. Deep tissue imaging works better the nearer the fluorescence light is collected in regard to the microscope objective. Photons which are coming from deep places inside the specimen and are scattered on their way are collected by the objective and guided to the detection unit using the NDD adapter.

The NDD adapter is mounted on an upright Nikon microscope for the aim of time resolved NDD measurements with confocal Laser Scanning Microscopes (LSMs) which are equipped with two photon excitation. The fluorescence light is collected directly at the objective using a short pass dichroic with a splitting wavelength of 680 nm. All fluorescence between 400 nm and 680 nm is focussed into a light guide. The light guide transmits the fluorescence with a transmission of 80% to the FLIM detection unit. The detection unit is mainly used in conjunction with PMA (Photo Multiplier Assembly) Hybrid detectors. The PMA Hybrid detector allows time-resolved measurements with high sensitivity. This feature can be used not only in FLIM applications, but also in FCS measurements. For all applications the area around the NDD adapter must be completely dark since ambient light can enter easily the detectors and will lead to a strong background in the measurement.

Possible applications are:

- NDD FLIM and FLIM - FRET measurements
- TPE imaging with very high sensitivity
- FCS measurements

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 and accelerate the ageing process which leads to a decrease in sensitivity.



Switch the detector on only if the surrounding area of the NDD adapter is completely dark! Switch either the room light off and dim the monitors or set the microscope in a light shielding box. Switch the detector off after finishing the experiments or when leaving the room!

3. Physical Properties and Installation Requirements

Size [mm] (height), without extension	47
Size [mm] (height), with one or two extensions	57 or 67

4. Detector Type

The NDD adapter works with one or two channels of a PMA or PMA Hybrid based detection unit. Please refer to the detection unit manual.

5. Set up of the NDD Adapter

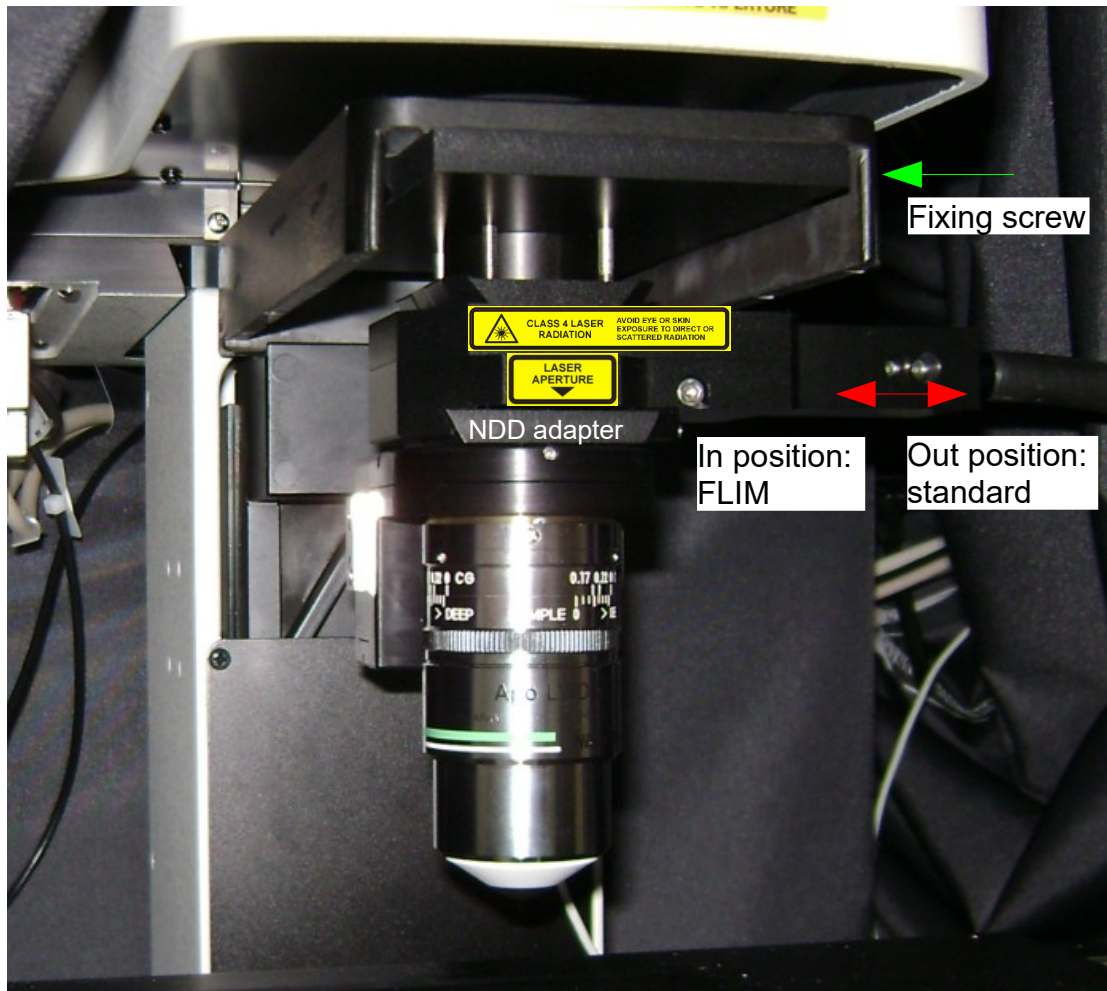


Fig. 5.1: NDD adapter mounted at the pigtail holder of the microscope stand. The NDD adapter is fixed by a screw which can be reached from the right side (green arrow). The inset can be moved in (to the left) for FLIM imaging and out (to the right) for standard imaging (red arrow).

- **⚠ Before mounting the NDD adapter make sure that the FLIM detector unit, microscope and all lasers are switched off. Otherwise a reflection of the invisible laser light can lead to severe eye injury.**
- Never remove the light guide from the NDD adapter or loosen the screws fixing the light guide (green arrows in Fig. 5.2). **Otherwise exiting invisible laser light can lead to severe eye injury.**
- Please make sure not to bend or to squeeze the light guide. Do not exert force to it. It might be damaged.
- The adapter has to be mounted between objective and the pigtail mount of the objective holder (Fig. 5.1).
- The height of the NDD adapter (47 mm) corresponds roughly to the height of the standard Nikon objective mount. Also a PIFOC for fine tuning of the z distance can be employed.

- The NDD adapter can be extended for certain applications to achieve heights of 57 or 67 mm correspondingly (Fig. 5.2 and Fig. 5.3).
- The NDD adapter has a pigtail connector on the lower side so that different thread types of objectives can be mounted. Adapters are delivered for the 60 mm and 75 mm objective heights.
- If the NDD adapter is in FLIM position, a short pass dichroic splitting at 680 nm guides the light towards the light guide.



Protect the detectors against over-illumination as much as possible. Make sure that the NDD adapter is always in complete darkness during the operation of the FLIM detection unit.



Detector overload will decrease the sensitivity of the detector. Switch the detector always off if not needed or if you dismount the NDD adapter. Please refer to the detector unit manual.



PMA Hybrid detectors: The built in overload protection has an approximately 1 second latency and is meant as a last rescue only. When the detector goes into the overload protection, a beep sound is emitted. The detector returns from overload into the normal status after a few seconds.

- The FLIM detector unit can be switched on and off using the its own power switch (please refer to the detection unit manual).
- The security shutter of the detection unit can be closed by removing the optical filter. Optimally mount the filter only a short time before the measurement takes place so that the security shutter is closed as long as possible.
- Choose an adequate optical detection filter for the detection unit

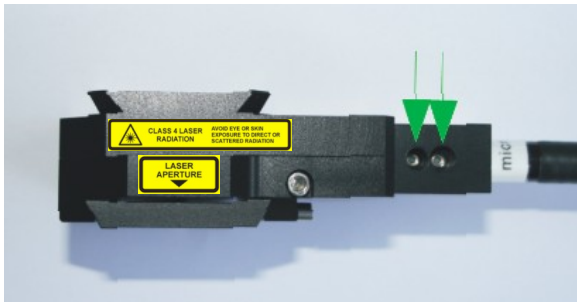


Fig. 5.2: NDD adapter without extension. The additional height is 47 mm.



Fig. 5.3: NDD adapter with two extension tubes, each adding 10 mm. The additional height is now 67 mm.

- Make sure that the other end of the light guide is mounted correctly at the FLIM detection unit (Fig. 5.4).

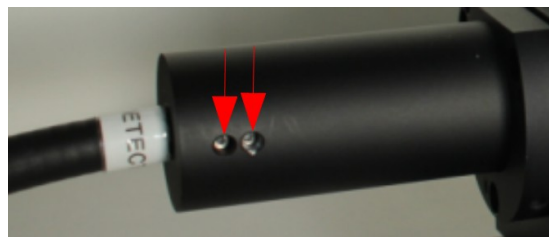


Fig. 5.4: NDD detection unit adapter. The light guide should never be removed or the screws (red arrows) loosened.

6. Operation

6.1. Performing a Measurement with Your NDD Adapter

1. Make sure that the NDD detector is surrounded by complete darkness.
2. Make sure that all **cables** are **connected correctly** according to the labeling scheme in the "system specifications".
3. Make sure that you have chosen the correct optical filters. **Leave the optical filter unmounted so that the shutter of the FLIM detection unit will be closed.**
4. Set the NDD adapter into normal operation mode (insert moved out as shown in Fig. 5.1).
5. Switch the microscope and lasers on.
6. **Switch all FLIM components on** (we recommend powering all devices by one central power switch).
7. Optimize the sample and microscope settings with the standard NDD detectors of the system. Record an image.
8. Set the NDD adapter into FLIM operation mode (insert moved in, as shown in Fig. 5.1)
9. **Insert the optical filter** so that the shutter of the detection unit **opens**.
10. Now the system is ready to take FLIM images or to make FCS measurements. For detailed description about how to perform individual measurements, follow the instructions in the LSM manual.
11. After the measurement, remove the optical filter of the FLIM detection unit so that the security shutter will close.

6.2. Switching Your System Off

1. Switch off the FLIM detection unit.
2. Turn off all other equipment, which is no further needed.

6.3. Alignment

- There is no alignment needed on the system.

7. Further Reading

7.1. PicoQuant Bibliography

PicoQuant maintains a database of publications mentioning PicoQuant devices. It can be found at our website <https://www.picoquant.com/scientific/technical-and-application-notes>. 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.

7.2. 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 <https://www.picoquant.com/scientific/references>

7.3. 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)

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
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 (only for two photon excitation, TPE)
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
TPE	Two Photon Excitation
TTL	Transistor-Transistor Logic
TTTR	Time-Tagged Time-Resolved

9. Support

9.1. 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.

9.2. 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!

10. Legal Terms

10.1. Disclaimer


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.

10.2. Copyright

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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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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>