LSM Upgrade Kit

for FLIM and FCS on Zeiss LSM 990
confocal microscope system

PicoQuant

Preinstallation requirements

Document version 01

Check list

Please use the following check list to make sure that you meet all requirements for the installation of the system you ordered, ensuring a smooth installation process. Questions? Contact us at support@picoquant.com

Prior to delivery	**Check**
Read carefully the attached “Preinstallation Requirements”	☐
Double check the quotation: • Is the right mains voltage specified? • Are all components you wish to order from PicoQuant included?	 ☐ ☐
Does your Zeiss LSM990 meet the configuration requirements? • Requires module license for ZEN blue: 410136-1111-300 ZEN blue FLIM Handshake • ZEN blue software license must include the ZEN Module Macro Environment The order numbers of the Zeiss DC adaptation for BiG are: • LSM 990 without AiryScan detector: ◦ 000000-2074-962 Secondary bBamsplitter filter wheel (standard configuration with Airyscan) ◦ 000000-2014-998 DC adaptation for BiG • LSM 990 with AiryScan detector: ◦ 000000-2074-962 Secondary Beamsplitter filter wheel ◦ 000000-2014-998 DC adaptation for BiG ◦ 000000-2078-390 T-switch “Connection for BiG.2 on LSM scan module with Airyscan” • FLCS requires a special MBS which excludes the 405nm laser line	 ☐ ☐ ☐ ☐ ☐ ☐ ☐
Is the room in which the system will be installed ready? • Does it meet the environmental requirements (section 2.1)? • Is enough space available (section 2.2)? • Are enough tables / optical benches present? • Does the room meet the power requirements (section 2.3)? • Have you ensured compliance with all applicable laser safety regulations (section 2.4)? • Make sure that all applicable requirements from section 3 are met • Are all required third-party components present and working properly (only for specific systems, see section 3)?	 ☐ ☐ ☐ ☐ ☐ ☐ ☐
Is a suitable storage location available (if needed, section 1.3)?	☐
Can the shipping crates be moved to the installation or storage room(s)? • see section 1.1 for sizes; make sure that crates can pass all doors, stairwells, elevators, and corridors on the way to the installation / storage rooms	☐
Upon delivery	**Check**
Check shipping boxes / crates for transport damage: • report any damage and shockwatch status (if present) to PicoQuant	☐
After installation	**Check**
Together with your laser safety officer: check that your system complies with all applicable laser safety regulations	☐

Table of Contents

1. Shipping and storage

1.1. Upon delivery

Please check the cases immediately after arrival for any visible signs of external damage and report the results without delays to PicoQuant at support@picoquant.com. Also check the attached shockwatch sensors (if present) and report their status.

1.2. Storing the shipment

The boxes should be stored at a dry (< 40% rel. hum.) and temperate (15 to 25 °C) place until the system is installed by a PicoQuant specialist. **Do not open the cases by yourself!**

2. Room requirements

2.1. Environmental conditions

The room where the upgrade kit is installed must be clean and dry. The room should be air-conditioned with no more than 1 °C temperature variance to prevent loss of adjustment due to thermal drifts. For FCS measurements, the microscope should be placed on a table with vibration isolation.

2.2. Space considerations

Depending on your specific configuration, the LSM Upgrade Kit requires a significant amount of space in close proximity to the LSM for the additional equipment. General space requirements for the individual components can be found in the table below:

Component	**Size [cm]** (width × depth × height)	**Weight [kg]**
Pulsed laser without fiber coupling unit *Free fiber length: 3 m*	30 x 10 x 15	2
Laser coupling unit (LCU) *Free fiber length: 2.5 m*	40 × 60 × 25	40
Laser driver (PDL800-D or SEPIA II with 2 [4] laser channels *Max. distance from lasers: 1 m*	23 × 30 × 12 24 [45] × 31 × 14	2 6 [10]
Detection unit *Free fiber length: 3 m*	25 × 51 × 26 (2 detectors) 13 × 13 × 33 (1 detector)	19 5
Detector power supply DSN	24 × 31 × 14	4
MultiHarp 150	30.5 × 24 × 9.5	4
PC	20 × 45 × 50	10
1 flat screen monitor	83 × 35 × 50	25

2.3. Electrical requirements

The system is shipped together with all necessary power distribution blocks and has therefore a single IEC 320-A14 type male power inlet. The total current consumption of a standard system is 4 A at 220 V AC, 8 A at 120 V AC. Note that special rules apply to upgrade kits shipped to Canada.

2.4. Laser safety considerations

WARNING! Visible and invisible laser radiation

The LSM Upgrade Kit may be equipped with laser diode heads that can emit visible, infrared, or UV light. Infrared or UV light is not visible to the eye! **These diode lasers can emit laser light of up to class 3b / IIIb.** Please refer to the labels affixed to the laser head for information on classification.

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

All of PicoQuant's laser diode heads and drivers are manufactured according to the International Laser Safety Standard IEC 60825-1:2014 and comply with the US law 21 CFR §1040.10 and §1040.11.

Required Laser Safety Measures

Please observe the laser safety measures for **class 3b / IIIb** lasers in accordance with applicable national and federal regulations. The owner / operator is responsible for observing the laser safety regulations.

CAUTION! Please note that it may happen that the microscope system incorporates lasers of class 4 / IV. In this case, all safety requirements for this laser class must be guaranteed by the owner / operator.

What does the owner / operator have to observe?

- The owner / operator of this product is responsible for proper and safe operation and for following all applicable safety regulations.
- The owner / operator is fully liable for all consequences resulting from the use of the laser 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 this laser 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 adviser (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).

The following security instructions must be followed at all times.

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. This includes jewelry, watches, etc.
- Every person involved with the installation and operation 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 the laser diode heads and associated driver unit. 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.

Do not open the housing of either LCU (Laser Combining Unit), laser head or driver unit under any circumstances! There are no user serviceable parts inside. The only maintenance needed is the visual inspection for external damage and cleaning of the housing.

3. Installation Requirements

Please make sure that the Zeiss LSM 990 is equipped with all parts listed below prior to installing the LSM Upgrade Kit. Should you have any questions, then please contact PicoQuant at info@picoquant.com.

3.1. General Remark

PicoQuant upgrade for FLIM can be added to inverted and upright microscope systems LSM 990.

3.2. Zeiss Software

For LSM 990 using the ZEN Blue software, the additional "Zeiss PicoQuant Application" tool must be ordered in order to ensure seamless integration of Zeiss ZEN with PicoQuant's SymPhoTime 64 software.

This tools allows starting FLIM, FLIM FRET, FLIM Pattern Matching, z-stack and time laps measurements directly in the ZEN software. The "Zeiss PicoQuant Application" software tool has the following order number:

- Modul license for ZEN blue: **410136-1111-300** ZEN blue FLIM Handshake.

Additionally, the ZEN blue software license must include the **ZEN Module Macro Environment**.

3.3. Pulsed diode lasers from PicoQuant

- Pulsed diode lasers with the following wavelengths ae available from PicoQuant: 440, 470, 485, 510, 530, 560, 595 and 640 nm
- Pulsed diode lasers from PicoQuant can be operated at repetition rates ranging from 250 kHz to 40 MHz.
- Lasers of different wavelengths can be used at the same time. Sequences of alternating laser pulses with different wavelength (I.e. pulsed interleaved excitation, PIE) can be setup by using the PDL 828 laser driver.
- The lasers can be switched on or off with the ZEN software using a special shutter from Zeiss. This shutter will be integrated into PicoQuant's Laser combining Unit. (LCU)
- Lasers from PicoQuant are coupled into the InVIS port of the system. Therefore, one port must be free. In case the system is already equipped with a **440 nm laser** which blocks this port, the laser **must be removed**. The required, special optical fiber must be purchased from Zeiss.
- When integrating PicoQuant lasers into the system, the LSM 990 must be equipped with dichroics suitable for the chosen wavelengths.
- During the installation, a Zeiss technician should be present to couple the fiber(s) of the PicoQuant lasers into the LSM.

3.4. Descanned Confocal Detection: exit port

In order to connect the PicoQuant detectors, the LSM system should have a free and configured DC port with adaptation for BiG. **No BiG detector should block this port.** Also switching between PicoQuant detectors and BiG detector is not possible.

The order numbers of the Zeiss DC adaptation for BiG are:

- LSM 990 without AiryScan detector:
  - 000000-2074-962 Secondary bBamsplitter filter wheel (standard configuration with Airyscan)
  - 000000-2014-998 DC adaptation for BiG
- LSM 990 with AiryScan detector:
  - 000000-2074-962 Secondary Beamsplitter filter wheel
  - 000000-2014-998 DC adaptation for BiG
  - 000000-2078-390 T-switch "Connection for BiG.2 on LSM scan module with Airyscan"

It is possible to use the Zeiss BiG.2 detector for time-resolved measurements. The BiG.2 can be directly connected to PicoQuant´s TCSPC electronics such as the MultiHarp 150. In this case, a Zeiss ZEN “Photon Counting” license is necessary.

3.5. Two Photon Excitation (NLO)

For Two Photon Excitation, a femtosecond laser (e.g., Ti:Sa) must be present at the system.

The internal trigger diode of the femtosecond laser has to be used to recieve a synchronization signal for the TCSPC unit. However, it might be possible that no stable triggering signal can be achieved when operating the femtosecond laser at the edges of its wavelength range (depending on the internal trigger diode of the femtosecond laser).

The repetition rate of the femtosecond laser should not exceed 100 MHz.

3.6. Non-Descanned Detection (with Two Photon Excitation only)

NDD (Non-Descanned Detection) is possible for both inverted and upright microscopes. The fluorescence light is collected with a special adapter at the end of the Zeiss NDD detector(s) and guided via a liquid light guide to the PMA Hybrid detector unit. NDD can be used with a maximum of two detection channels.

Zeiss offers adapters to allow attachment of non-Zeiss detectors (“T-adapters”). With such a T-adapter, the NDD FLIM detector can be used without removing the Zeiss NDD detectors. Filter sets for these adapters, which are also suitable in combination with detectors from PicoQuant, can be purchased from Zeiss. Zeiss filter cubes can be equipped with standard filters (Ø = 25 mm, d = 3 mm) and dichroic mirrors (36 x 26 x 1 mm). The T-adapter has the order number “SCM 2298 1962-186”.

CAUTION! **For laser safety reasons the system must be fully powered down before disassembling the adapter and detector!**

Slight back-reflections originating from the excitation may occur and be visible in the TCSPC histogram as an additional peak.

For NDD FLIM measurements, the microscope stand must be shielded against ambient light.

3.7. Optical Filters

Filters and filter sets are specifically listed in the quotation. Make sure that the quotation contains the correct filters for your applications. By default, filters are only quoted when pulsed lasers from PicoQuant are purchased.

3.8. Line and Frame Markers

The system should be operated with the current Zeiss software. Older systems may still run with a software version that does not provide the necessary marker signals. If you have an older system, please contact Zeiss to confirm that the installed database is compatible with the PicoQuant upgrade.

3.9. Optical Filters

Filters and filter sets are specifically listed in the quotation. Make sure that the quotation contains the correct filters for your applications. By default, filters are only quoted when pulsed lasers from PicoQuant are purchased.

3.10. FCS Measurements

For FCS Measurements the following triple band Main Beam Splitter (MBS) is needed:
MBS 485/561/639 , SCS order number: 000000-2243-672

For FCS measurements we recommend:

- Apochromatic corrected objective, N.A. 1.2, water immersion for measurements using water-rich samples (e.g., biological cells)

- Excitation wavelength: > 440 nm (shorter wavelengths lead to strong photo-bleaching and auto-fluorescence)
- Using Multi-Photon Excitation (MPE) for FCS is very difficult due to strong fluorophore bleaching under MPE. Also, the alignment of the MPE beam for FCS plays a critical role.
- The microscope should be placed on a table with vibration isolation.

3.11. General Requirements

During the installation of an upgrade kit with PicoQuant lasers a Zeiss technician must be on site for one day. Please arrange this with Zeiss accordingly.

It is recommended to allow for about 0.5 m free space around all sides of the microscope system for convenient access, as the user needs to interact with all parts. We recommend an additional table close to the LSM for all components of the PicoQuant upgrade kit. The maximum distances between the A1 controller and the additional PicoQuant components are limited to about 2.5 m by the cabling.

We strongly recommend using a PC pretested by PicoQuant. The PCs provided by PicoQuant come in tower cases and use Microsoft Windows 10 (64 bit) as operating system.

Please note that PC security settings and anti.virus software should be set and installed by the user, if necessary. Note that the LAN connection between the FLIM / FCS computer and the LSM computer should not be blocked by security settings.

If you have **not** ordered a PC monitor for the system, please make sure that your monitor(s) fulfill the following specifications:

- Must have a Display Port connector
- For a single monitor set-up for the FLIM / FCS system: one display with a diagonal of at least 27” and a resolution of at least 2500 x 1400 pixels

Depending on your specific configuration, the LSM upgrade kit requires a significant amount of space to install the additional equipment in close proximity to the LSM. General space requirements of the individual components are given in the chapter 2.2 Space considerations.

4. Legal Terms

4.1. Copyright

4.2. Trademarks

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