LSM Upgrade Kit

for FLIM and FCS on Evident FV5000
and FV5000MP 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 PicoQuant system you ordered. Ensuring that everything is properly prepared and ready before the PicoQuant specialists arrive will lead to a smooth installation process.

If you have any questions, please contact PicoQuant at support@picoquant.com

Prior to delivery	Check
Read carefully the attached “Preinstallation Requirements”	☐
Does your Evident FV5000 system have the required configuration? • I/O interface Box FV30-ANALOG (required for lifetime imaging) for FV5000 / FV5000MP • Evident FV31-BCOMB (required for PicoQuant laser incoupling) for FV5000 • Fiber exit port must be free for FV5000	☐ ☐ ☐
Double check the quotation: • Is the right mains voltage specified? • Are all components you wish to order from PicoQuant included?	☐ ☐
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]
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. 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 Evident FV5000 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. Evident Controller and Software

The FV5000 must be equipped with the **I/O interface Box FV30-ANALOG** (article no. N5169100) from Evident for scanning synchronization and laser switching.

3.2. Pulsed Lasers (405-640 nm)

The Evident laser combiner **FV40-BCOMB** (article no. N6422800) is necessary to use PicoQuant's pulsed diode lasers. The laser combiner adds an additional laser input port to the FV5000.

In addition, the polarizing beamsplitter **FV31-PBSC** (article no. N5663400) is needed which will be built into the FV40-BCOMB combiner.

The beams from the pulsed lasers are coupled into the FV5000 scan head using the FV31-BCOMB. The FV41-BCOMB must be installed on the system prior to the installation of the FCS / FLIM system by PicoQuant (Fig. 1).

The pulsed laser beams are overlaid inside the FV31-BCOMB with the cw lasers beams using a polarizing beamsplitter. In this way the combination is wavelength independent.

Custom dichroics for the FV5000 dichroic filter wheel have to be supplied by Evident in order to reflect the light from all selected PicoQuant pulsed lasers towards the sample.

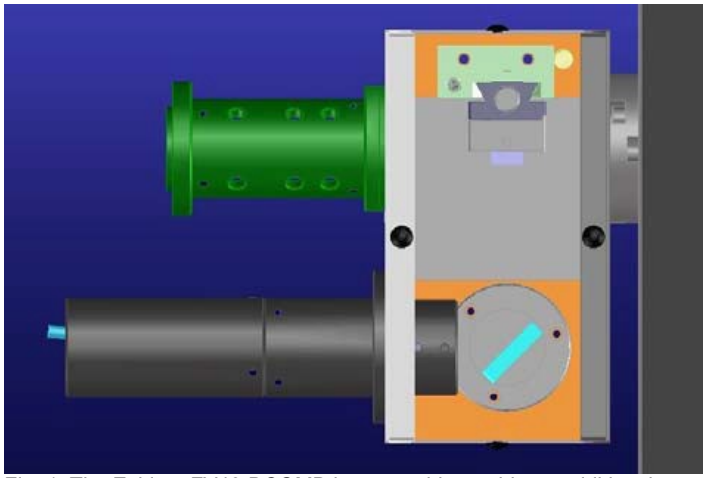

Fig. 1: The Evident FV40-BCOMB laser combiner adds an additional laser input port for PicoQuant's pulsed lasers.

3.3. Pulsed UV Laser 375 nm

A 375 nm laser can **not** be connected to the FV5000 system.

3.4. ROI Scanning

Region of Interest (ROI) scanning is possible using one PicoQuant laser in pulsed mode. During ROI scanning, the laser is only switched on in a pre-defined ROI in the image. ROI scanning can be used for photobleaching or photo-activation. In order to perform ROI scanning, the analog output connector unit FV31-AUX-OUT from Evident is necessary. It has to be ordered from and installed by Evident.

3.5. Two Photon Excitation

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

For upright microscopes, the NDD light collection unit is located above the objective. This leads to a lowering of the objective by 35 mm. Please make sure that the 35 mm can be compensated for (e.g., by lowering the sample stage).

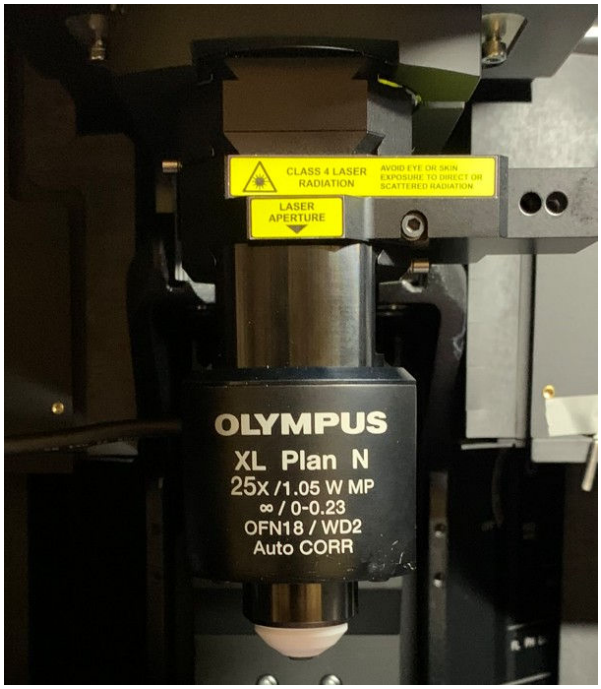

Fig. 2: Nosepiece adapter (with yellow laser warnings) for NDD FLIM measurements on upright microscopes

The femtosecond laser repetition rate should not exceed 84 MHz and must provide a suitable trigger output (SYNC) signal for timing synchronization. Generally, this trigger output will be used for the FLIM integration.

For NDD measurements, the room must be completely dark.

Descanned detection works fine for imaging areas not too deep inside of the specimen. However, after each excitation wavelength change the customer has to perform a special alignment procedure to ensure an exact overlay of the IR laser beam with the VIS lasers.

Combining two photon excitation with pulsed excitation from PicoQuant lasers is quite challenging. Please ask PicoQuant for advice in such a case.

3.6. Exit port

The fiber exit port (on top of the scan head of the FV5000) must be free.

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 application. By default, filters are only quoted when pulsed lasers from PicoQuant are purchased.

3.8. General Requirements

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 Evident 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.

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.

4. Legal Terms

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