

# Coupling of Pulsed Laser Sources into the Olympus FluoView FV300 / FV1000 via the IR Port Using a Polarization Beam Splitter



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## Introduction

Fluorescence Lifetime Imaging (FLIM) using a Laser Scanning Microscope such as the Olympus FluoView FV300 or FluoView FV1000 from Olympus requires to use pulsed excitation sources at excitation wavelengths typically ranging from 375 nm up to 640 nm. For the ease-of-use and also for more advanced measurements the (simultaneous) usage of pulsed and continuous wave (cw) lasers should be possible without need for readjustments or realignments from the user.

The problematic issue is that the FV300 and FV1000 only have a limited number of fibre connection ports, which are partly occupied by the cw lasers from the LSM laser combiner. A combination of pulsed and cw lasers into one fibre is in principle possible, but leads to noticeable losses in laser intensity. To overcome this limitation a new combining scheme for pulsed and cw lasers was developed by PicoQuant and Olympus.

## Technical realisation

The new combination scheme is based on combining the laser beams by means of a polarizing beam splitter, which is inserted in the very often unused fibre coupling port for IR lasers. The scheme requires that the polarisation of the pulsed lasers is orthogonal to the polarisation of the cw lasers, which is achieved by rotating the polarisation of the pulsed lasers accordingly.

The polarizing beam splitter is mounted in the scan head instead of the standard dichroic mirror of the IR port. PicoQuant has developed a beam splitter holder which is similar to the standard dichroic holder but equipped with a polarizing beam splitter. The reflecting surface is placed at exactly the same position as the standard dichroic mirror, which allows for an easy and fast initial adjustment using standard procedures. Measurements performed at the Olympus Europe headquarter in Hamburg, Germany, demonstrated clearly the feasibility of this approach. Pulsed and cw excitation sources could be superimposed nearly without power losses (<10%) and used for imaging or FCS measurements at the same time.

The only disadvantage of this approach is,

however, that DIC measurements with pulsed excitation are not possible, since the polarization is perpendicular to that needed for DIC. It is of course also not feasible if the IR port is blocked for two photon excitation – in that case the combination of pulsed and cw lasers into one fibre might still be an option (depending on the system configuration).

## Setup for the FV1000

The FV1000 has three fibre connection ports and the cw lasers are usually connected to the VIS port.

The pulsed VIS lasers with wavelengths ranging from 405 nm up to 640 nm are combined using the Laser Coupling Unit LCU from PicoQuant (Fig. 3) and coupled in a polarisation maintaining single mode fiber. The fibre is then connected to the modified IR port using the standard VIS collimator from Olympus (see Fig. 1).

A pulsed laser at 375 nm is directly coupled to a polarisation maintaining single mode fiber and connected to the UV port using the the standard UV collimator from Olympus.

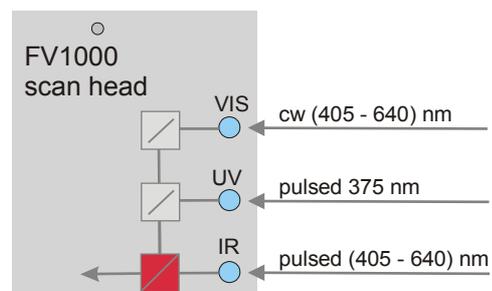


Fig. 1: Coupling of pulsed lasers into the FV1000 scan head. The polarising beam splitter is shown in red. If the UV / IR port is blocked by a different laser, please ask PicoQuant for advice.

## Setup for the FV300

The FV300 has two fibre connection ports and the cw lasers are usually connected to the VIS port. The second fibre connection port can be adapted to couple either UV (375 nm to 440 nm) or VIS laser light (405 nm up to 640 nm). The corresponding pulsed lasers are combined using the LCU and connected to the fibre port using the appropriate collimator from Olympus (see Fig. 2).

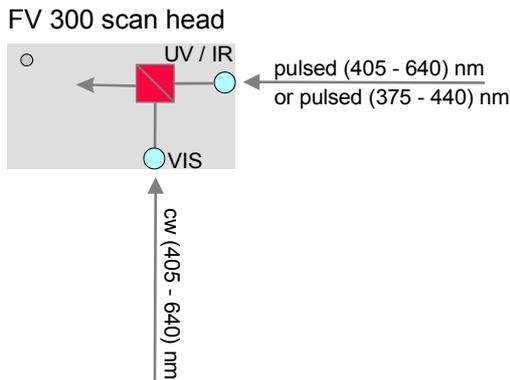


Fig. 2: Coupling of pulsed lasers into the FV300 scan head. The polarising beam splitter is shown in red. The UV / IR port accepts either the wavelengths range (405 – 640) nm or (375 - 440) nm, because different collimators have to be utilized for the two wavelengths ranges. If the UV / IR port is blocked by a different laser, please ask PicoQuant for advice.

### Conclusion

The new setup provides an excellent flexibility for the use of the confocal microscope. Due to the polarization beam splitter many different wavelengths from pulsed and cw excitation can be combined and utilized at the same time. The polarizing beam splitter combines both beams with nearly no intensity loss. The spatial overlay of both laser sources is extremely stable over time and maintenance free. The necessary changes including complete adjustments can be done within a short time.

Due to the different time pattern of pulsed and cw lasers, these can be differentiated during analysis in the PicoQuant SymPhoTime software using time gating in the case of fluorescence lifetime imaging (FLIM) and applying fluorescence lifetime correlation spectroscopy (FLCS).



Fig. 4: Guiding cw and pulsed light into the FV1000 scan head: cw light delivery fiber with collimator (1), pulsed light delivery fiber with collimator (2), polarizing beamsplitter holder (3), fluorescence exit port with fiber coupling for external detector for FLIM, FCS, etc..(4)

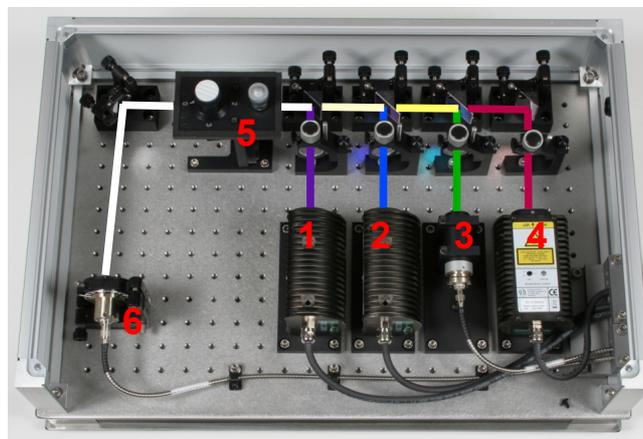


Fig. 3: Laser coupling unit (LCU) with three pulsed diode lasers (LDH): 405 nm (1), 470 nm (2), 640 nm (4). The pulsed laser light from the (physically larger) 530 nm laser is guided with a fiber into the LCUI (3). The laser light is combined by dichroic beam splitters and can be attenuated by means of scaffold and OD filters (5). A fiber coupler couples the laser light into a single mode polarization maintaining fiber (6). Up to four wavelengths can be combined in the LCU ranging from 375 nm up to 640 nm.

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