

## FLIM/FFS and Nanoimaging Upgrade of Olympus confocal microscopes

Shih-Chu Liao, Yuansheng Sun, Ulas Coskun  
ISS, Inc.

### 1 Introduction

The FLIM and FFS Upgrade Package for the Olympus confocal microscopes (FV1000, FV1200, FV3000, and FVMPE-RS) incorporates the acquisition of Fluorescence Lifetime Imaging (FLIM) while introducing an additional measurement capability for the Fluorescence Fluctuations Spectroscopy (FFS) family.

The Upgrade Package does not reduce any of the microscope original operations to the standard measurements; in contrast, it provides the following additional measurements:

<b>Fluorescence Lifetime imaging</b> <i>Measurements can be carried out in a combination of X, Y, Z, and t dimensions</i>	
Digital frequency-domain (DFD) FLIM	<ul style="list-style-type: none"> <li>Acquired by FastFLIM.</li> </ul>
Time-domain FLIM	<ul style="list-style-type: none"> <li>Acquired in time-correlated single photon counting (TCSPC)</li> </ul>

<b>Fluorescence fluctuation spectroscopy utilities</b> <i>Data can be acquired in the counts, the time tagged or the time tagged time resolved (TTTR) mode</i>	
Autocorrelation (FCS)	<ul style="list-style-type: none"> <li>The FCS function gives the temporal correlation of the fluctuations</li> </ul>
Cross-correlation (FCCS)	<ul style="list-style-type: none"> <li>The FCCS function provides the temporal correlation of the fluctuations related to events occurring simultaneously on two or three channels.</li> </ul>
Photon Counting Histogram (PCH)	<ul style="list-style-type: none"> <li>The PCH function plots the distribution of photon counts at the specified time interval</li> </ul>
FFS measurement at target XYZ locations in an image	<ul style="list-style-type: none"> <li>The user selects the XYZ location by moving the cursor or entering the values in the software.</li> </ul>
FLCS, fluorescence lifetime correlation spectroscopy	<ul style="list-style-type: none"> <li>The FCS data points are acquired in the Time-Tagged-Time-Resolved mode, allowing both FCS and FLIM analysis.</li> </ul>

Number & Brightness (N&B)	<ul style="list-style-type: none"> <li>• A series of raster images acquired in fast way and in photon counting mode. It separates the mobile from the immobile fluorophores; it separates monomers from dimers.</li> </ul>
Anisotropy	<ul style="list-style-type: none"> <li>• Anisotropy measurements provide information about orientation and rotation information thru polarization measurements</li> </ul>

<b><i>Nanoimaging and Orbital Particle Tracking</i></b>	
Particle tracking	<ul style="list-style-type: none"> <li>• Tracks isolated particle</li> <li>• Tracks particle with nanometer resolution</li> <li>• Analyze the dynamics of the system like motion characteristics (ballistic, diffusion, trapped, etc), lifetime, brightness along the path</li> </ul>
Edge Tracking	<ul style="list-style-type: none"> <li>• Studies dynamics on interface surfaces like cell membrane</li> </ul>
Nanoimaging	<ul style="list-style-type: none"> <li>• Provides image for a micrometer size extrusions with nanometer diameter like microtubules</li> </ul>



**Figure 1.1** Model FV1200 confocal microscope

The upgrade package requires an attentive selection of four key elements:

- The wavelengths required for single-photon excitation
- The way to carry out the upgrade (use of internal detectors, or addition of external detectors)

- The type of light detectors to be utilized for the acquisition (GaAs, hybrid detectors)
- The FLIM/FFS data acquisition modality (FastFLIM or TCSPC)

## 2 Different ways to carry out the upgrade

The upgrade of the Olympus systems can be achieved in three different ways:

- Using the internal light detectors of the FV1000 LSM system; or the detectors provided by Olympus in the FV1200; or the detectors mounted on the descanned or non-descanned NDD port.
- Collecting the fluorescence signal from the fiber port of the confocal head and diverting it to the 2-detector unit (made by ISS). The unit, controlled through the USB port, features an automated filterwheel holding the dichroics, automated filterwheels in front of each detector, and automated shutters.
- Adding two detectors (mini TDU, made by ISS) to the fiber port of the microscope; additional detectors can be cascaded for more acquisition channels. This option offers the maximum sensitivity.

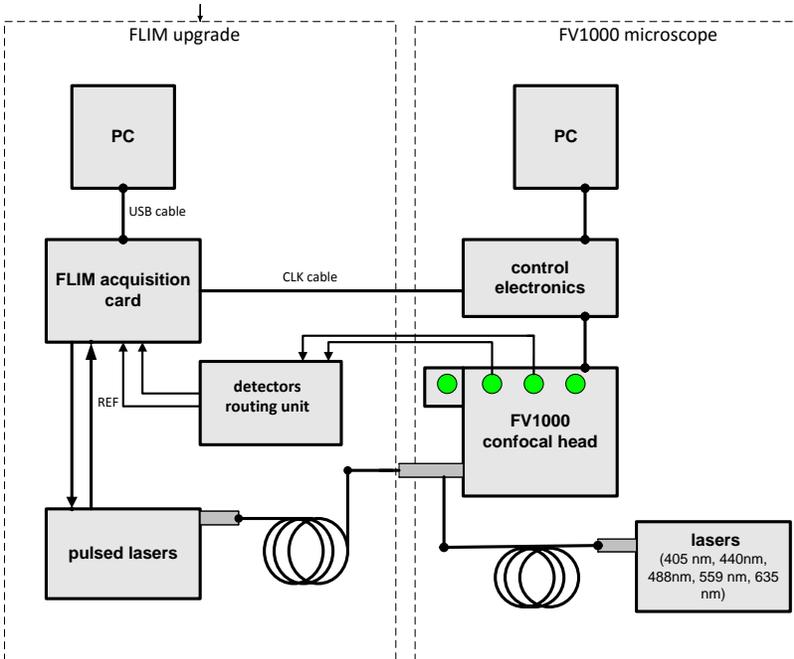
The upgrade works for both single-photon laser excitation and multiphoton laser excitation. When the single-photon excitation option is used, the port for multiphoton excitation is left untouched (that is, a multiphoton laser can still be connected to it), a feature not available with other commercial upgrade options. The upgrade package does not modify in any ways the operations of the instrument as originally installed by Olympus. The single-photon light beam will be injected into the scanner head utilizing the VIS port and a laser combiner unit that merges the beam from the Olympus laser launcher with the beam from the ISS laser launcher.

### Comparison Table

	<i>Features</i>	<i>Disadvantages</i>
Using internal detectors	Economical upgrade package	Sensitivity and response time for FLIM is limited by the detectors
Using the fiber port	The 2-detector unit features automated filter wheels in front of each detector.	15% loss of signal
Using the 2-detector unit connected to the output port of the microscope	Highest sensitivity Anisotropy measurement capability can be added.	Filters and dichroic have to be inserted and replaced manually by the operator

## 2.1 Using the internal detectors of the Olympus LSM microscope

Figure 1 below is a schematic of the upgrade using the internal detectors of the Olympus FV1000 system. With this upgrade the ISS technical personnel accesses the internal detectors and adds a routing system that diverts the signal to the data acquisition card. For the FV1200, Olympus may provide two external detectors (GaAs); the signal can be collected at the detectors directly.



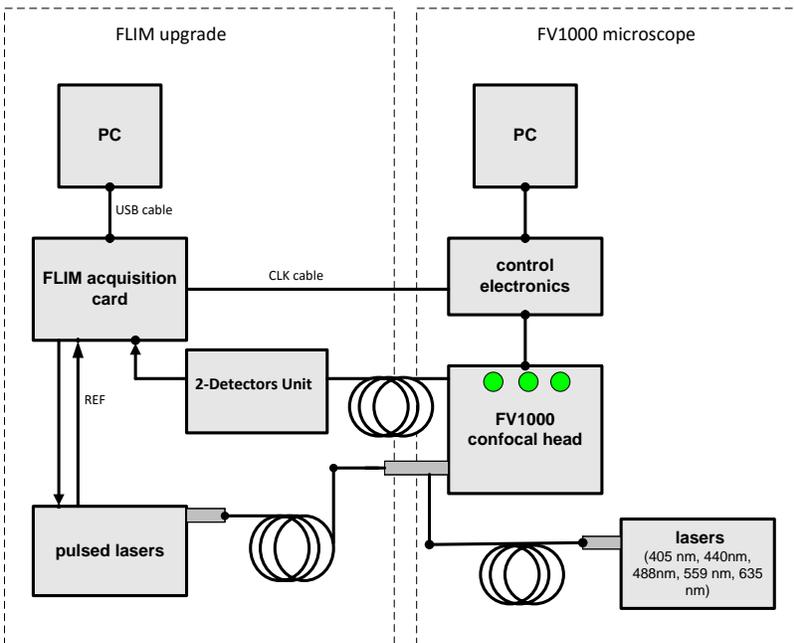
**Figure 2.2.1** Schematics of the FLIM Upgrade using the internal PMTs of the Olympus FV1000. The parts to the right include the instrument components (PC, control electronics, scanner and laser launcher). The left part of the schematics includes the components provided by ISS with the upgrade package. In this schematics the lasers for FLIM are delivered through a fiber that joins the fiber from the Olympus lasers using the laser combiner.



**Figure 2.2** Confocal head of the FV1000 with the ISS panel for the Output signals of the internal detectors.

## 2.2 Collecting the fluorescence signal from the fiber port and diverting to the 2-detector unit by ISS

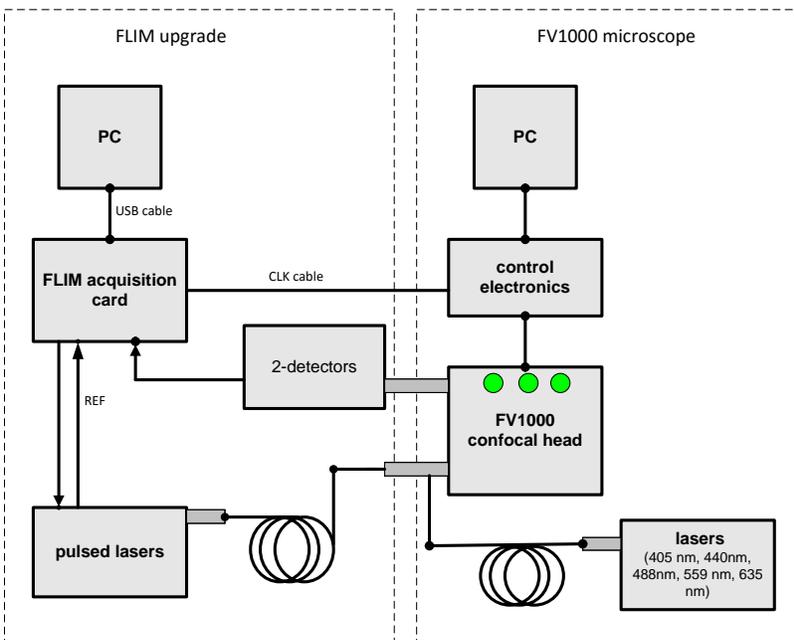
The Figure below shows the upgrade package where the fluorescence light is collected by the fiber port of the confocal head.



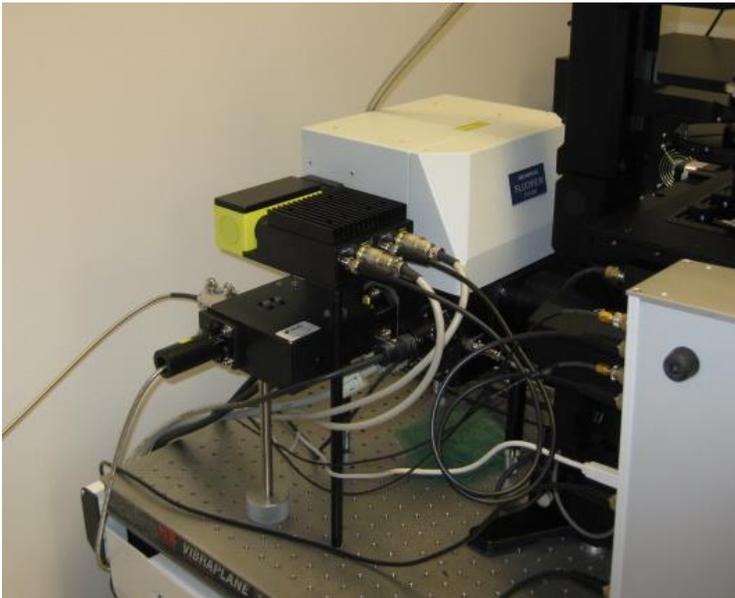
**Figure 2.3** FLIM Upgrade using the fiber port on the confocal head of the Olympus FV1000. The parts to the right include the instrument components (PC, control electronics, scanner and laser launcher). The left part of the schematics includes the components provided by ISS with the upgrade package. In this schematics the lasers for FLIM are delivered through a fiber that joins the fiber from the Olympus lasers using the laser combiner.

## 2.3 Adding two detectors (mini TDU) to the output port of the microscope

A schematics of the two-detectors added to the confocal head of the Olympus is shown below. The two-detectors feature a dichroic filter and separate filters inserted manually by the user.



**Figure 2.4** FLIM Upgrade where the two detectors are connected to the confocal head of the Olympus FV1000. The parts to the right include the instrument components (PC, control electronics, scanner and laser launcher). The left part of the schematics includes the components provided by ISS with the upgrade package. In this schematics the lasers for FLIM are delivered through a fiber that joins the fiber from the Olympus lasers using the laser combiner.



**Figure 2.5** The two GaAs detectors (upper deck with yellow housing) and ISS beam combiner (lower deck with white ISS sticker) are coupled directly on the confocal head of the FV1000.

### 3 FLIM in frequency-domain (FastFLIM) or in time-domain (TCSPC)

FLIM acquisition is available from ISS in either modality:

- frequency domain (FastFLIM); or,
- time-domain (time correlated single photon counting, TCSPC)

The choice is left to the customer and, in fact, if required, both acquisition modalities can be implemented on the same instrument. FastFLIM is more immune the pile-up effect compare to the TCSPC as the dead time is much less and the duty cycle is higher; FastFLIM is suggested when measuring FRET in live cells or with bright isolated species.

	FastFLIM	TCSPC
Number of input channels	4 parallel channels	1 channel Additional cards can be used in parallel; or a router can be utilized for 4 channel
Dead time	3.125 ns	125 ns
Max signal count	13,000,000 counts/sec	1,000,000 counts/sec
Min time bin	20 ps	813 fs
Architecture	USB2	PCI bus

**Table I.** Comparison of FastFLIM and TCSPC acquisition

#### 4 The detectors

The detectors utilized by ISS, made by Hamamatsu, are

- H7422P (GaAs)
- R10467 (hybrid)



**Figure 4.1** Dual-in line H7422P PMT; the unit includes a dichroic for the separation of the incoming beam and filters in front of each detector.

The sensitivity region of the detectors has to match the wavelength acquisition range of the measurements; contact ISS for recommendations and selection.

## 5 The laser launcher

In multiphoton system, ISS uses the laser originally installed with the instrument. For single photon excitation, ISS provides lasers for the FLIM/FFS upgrade. The wavelengths available are:

Laser diodes	375, 405, 440, 473, 488, 514, 530, 635 nm
DSSP laser	561, 588, 598 nm

ISS laser launchers can accommodate 3-, 4- and 6-lasers. The laser launcher can accommodate lasers made by ISS, B&H, and Coherent.

The laser launcher is controlled through the USB port of the computer by the ISS VistaVision software. Each laser beam can be ON/OFF; also the intensity can be controlled independently. The lasers beams are superimposed in the unit and delivered to the laser combiner by using a single-mode fiber.



**Figure 5.1** Three-laser launcher. The unit can mount different lasers; the beams are superimposed inside the unit and the light is delivered to the confocal head by the fiber optic cable.



**Figure 5.2** Four-laser launcher. The lasers are mounted inside the unit.

The lasers are housed in the laser launcher where the lasers beams are superimposed and focused into a single-mode fiber; the fiber delivers the beam into the laser combiner connected to the VIS port (the multiphoton port is not used). Lasers made by ISS, B&H and Coherent can be accommodated in the unit.

The laser combiner superimposes the beams coming from the ISS laser launcher and from the Olympus laser launcher with a polarizer to minimize the coupling loss. The ISS beam combiner can accept OZ optics, QI-Optics, and Olympus fiber couplers.



**Figure 5.1** Laser combiner. The adapter from the Olympus laser launcher is connected to the left (point A); the adapter from the ISS laser launcher is connected to the top. The brass thumbscrew (C) allows for selection either laser launcher.

## 6 Summary

The FLIM/FFS upgrade of your Olympus Laser Scanning Microscope can be done in several ways. Contact ISS for discussing your intended application; ISS technical personnel is available for answering your questions and discuss the best way to achieve your goals.

For more information please call (217) 359-8681  
or visit our website at [www.iss.com](http://www.iss.com)



**ISS**<sup>™</sup>

1602 Newton Drive  
Champaign, Illinois 61822 USA  
Telephone: (217) 359-8681  
Fax: (217) 359-7879  
Email: [iss@iss.com](mailto:iss@iss.com)

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