FLIM/FFS Upgrade Kits for Zeiss LSM systems

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1 Introduction

The FLIM and FFS Upgrade Package for the Zeiss confocal microscopes (LSM780, LSM880) incorporates the acquisition of Fluorescence Lifetime Imaging (FLIM) and additional measurement capabilities for the Fluorescence Fluctuations Spectroscopy (FFS). The Upgrade Package does not reduce any of the microscope original operations to the standard measurements; in contrast, it provides the following additional measurements:

Fluorescence Lifetime imaging ***Measurements can be carried out in a combination of X,Y, Z, and t dimensions***	
Digital frequency-domain (DFD) FLIM	• Acquired by FastFLIM.
Time-domain FLIM	• Acquired in time-correlated single photon counting (TCSPC)

Fluorescence fluctuation spectroscopy utilities ***Data can be acquired in the counts, the time tagged or the time tagged time resolved (TTTR) mode***	
Autocorrelation (FCS)	• The FCS function gives the temporal correlation of the fluctuations
Cross-correlation (FCCS)	• The FCCS function provides the temporal correlation of the fluctuations related to events occurring simultaneously on two or three channels.
Photon Counting Histogram (PCH)	• The PCH function plots the distribution of photon counts at the specified time interval
FFS measurement at target XYZ locations in an image	• The user selects the XYZ location by moving the cursor or entering the values in the software.
FLCS, fluorescence lifetime correlation spectroscopy	• The FCS data points are acquired in the Time-Tagged-Time-Resolved mode, allowing both FCS and FLIM analysis.
Number & Brightness (N&B)	• 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 form dimers.

Figure 1.1 Model LSM780 confocal microscope. The ISS detector(featured on the right section / yellow color) is added to the NDD port

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

- The way to carry out the upgrade (use of descanned or non-descanned port)
- The wavelengths required for single-photon excitation.
- The type of light detectors to be utilized for the acquisition (GaAs, hybrid detectors)
- The FLIM/FFS data acquisition modality (FastFLIM or TCSPC)

2 Descanned and non-descanned detection ports

The signal from the Zeiss LSM system can be collected either from the descanned or the non-descanned detection (NDD) port; the latter is used when the excitation light is a multiphoton laser. The ISS detectors are mounted in a parallel (the detectors are mounted parallel to each other) or in a perpendicular geometry (the detectors are mounted in perpendicular directions to each other). While the perpendicular geometry can be used on either port, the parallel geometry can only be used on the NDD port.
The customer should request the implementation of the descanned port when the order is placed, otherwise it will require retrofitting.

2.1 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 Zeiss is shown below. The two-detectors feature a dichroic and separate filters (inserted manually by the user).

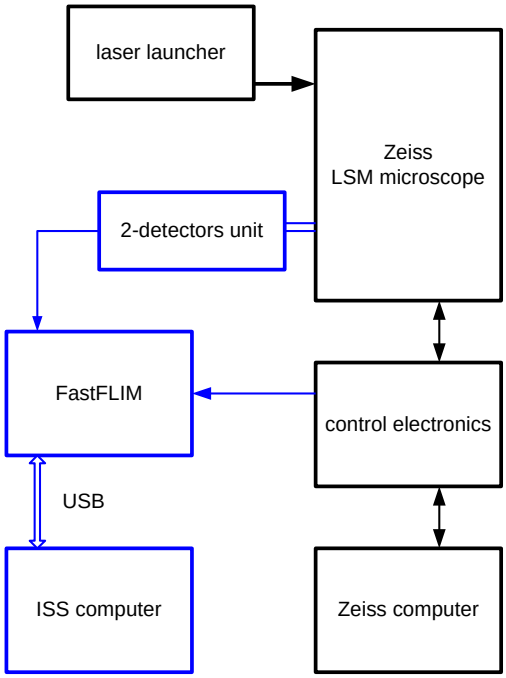
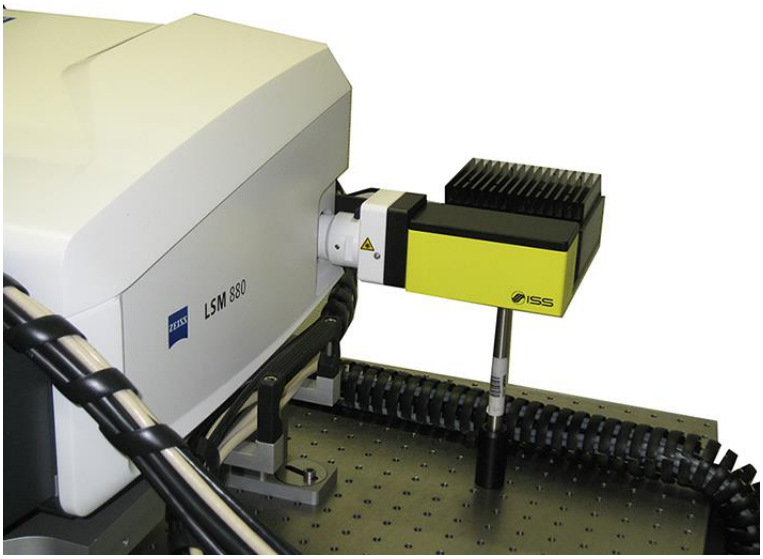


Figure 2.1 FLIM/FFS Upgrade where the two detectors are connected to the confocal head of the Zeiss LSM system.
The parts to the right include the instrument components (PC, control electronics, scanner and laser launcher). The left part of the schematics includes the components provided by ISS with the upgrade package.

Two-detector units (PMT Model H7422P-40) coupled to the descanned (DD) port of the LSM780 system.

In the background, the FastFLIM, the external power supplies for the detectors and the Signal Conditioning Unit for the multiphoton laser.

Two-detector units (PMT Model R10467U) coupled to the non-descanned detection (NDD) port of the LSM 880 system. The two detectors are positioned parallel with respect to each other.



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 compared to the TCSPC as the dead time is lower 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

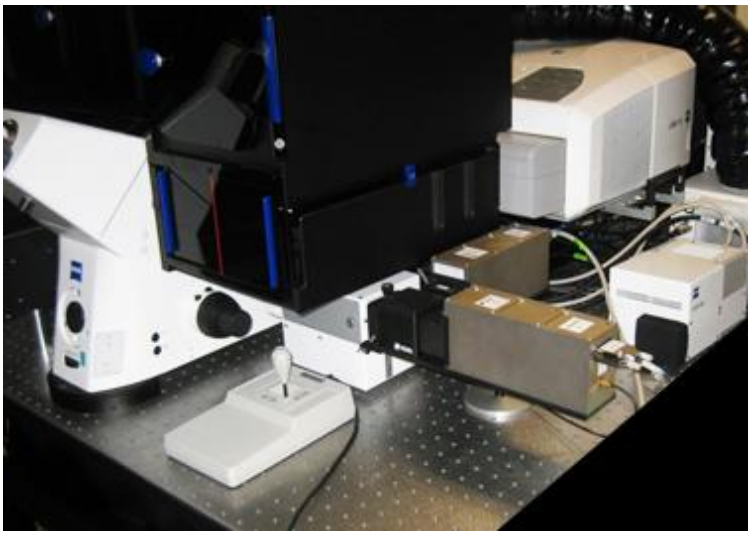
4 The detectors

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.

Figure 4.2 Two GaAs PMTs Model H7422P-40 installed on the NDD port of the LSM 780 microscope. The unit includes a dichroic for the separation of the incoming beam and filters in front of each detector.



Two-detector units (PMT Model R10467U) coupled to the non-descanned detection (NDD) port of the LSM 780 system. The two detectors are positioned perpendicularly with respect to each other.

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

5 The lasers

The Zeiss LSM system is equipped most of the times with the InTune laser, single-photon lasers, or multiphoton lasers. The FLIM/FFS Upgrade kit package works with any laser.

6 Summary

The FLIM/FFS upgrade of your Zeiss LSM adds new functionality to the confocal microscope. 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

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