fastFLIM™

FOR ZEISS
LSM 510/710/780

fastFLIM is a digital frequency domain(DFD) approach to FLIM measurements

Its advantages, when compared to other established FLIM techniques, are twofold:

- The short time required for data acquisition, which increases the photo-stability of the sample
- The higher sensitivity of the technique due to the 100% duty-cycle

The upgrade package is installed on-site by ISS technical personnel: the user does not ship back to ISS any components. Five laser wavelengths are available: 370, 405, 473, 635 nm laser diodes.The beam is delivered to the microscope through single-mode optical fiber.

The FastFLIM package can also be installed on systems using a supercontinuum laser source or multiphoton excitation Ti:Sapphire laser.

ISS™

The Zeiss Upgrade Package

The package includes the following items:

FastFLIM unit	It accepts the output (via BNC) from one PMT of the confocal unit. The synchronization signal from the Zeiss confocal head is connected to it.
Two-detector unit	Coupled to the output port of the Zeiss microscope. The detectors are GaAs PMTs or SPADs.
Laser launcher w/at least one laser diode	The output of the laser is connected to the Zeiss confocal head by using a fiber optic with SMA connector
Computer running VistaVision by ISS	A separate computer, with a 27" flat monitor

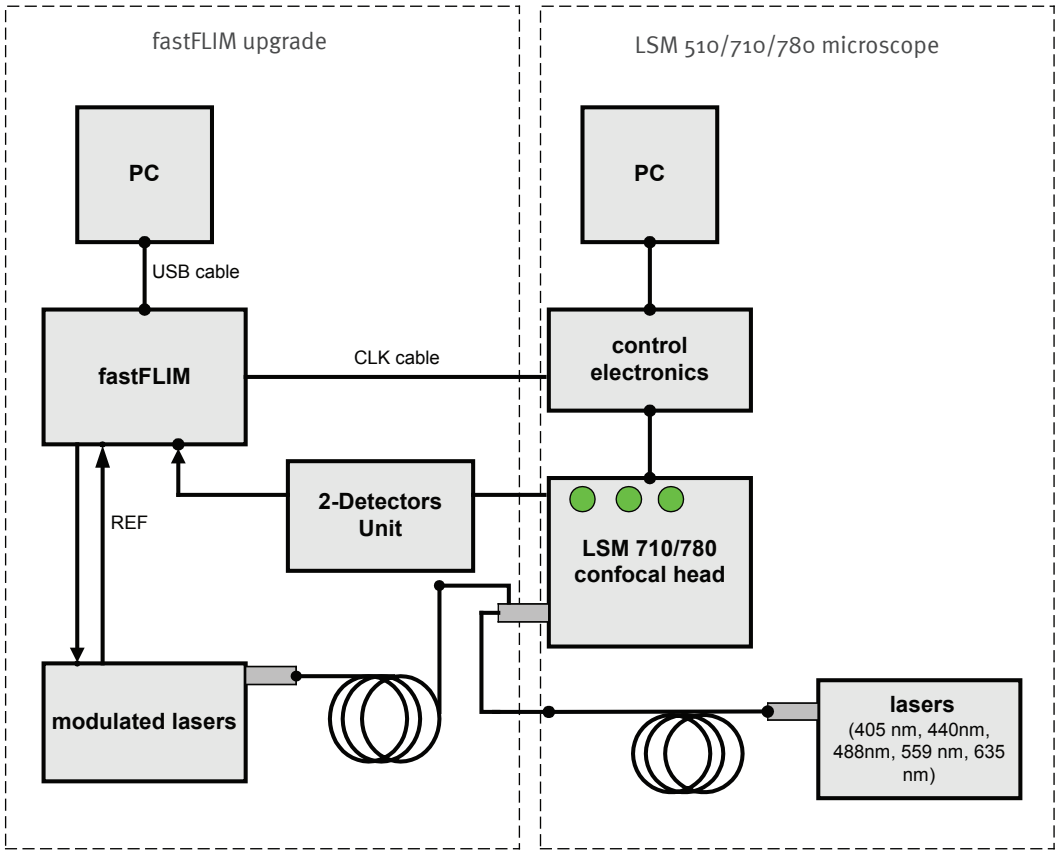


Figure 1. Schematics of the upgrade package for the Zeiss LSM instrument. The part to the right includes the instrument components (PC , control electronics, scanner and laser launcher).The left part of the schematics includes the components provided by ISS with the upgrade package.

Acquisition and Processing Software

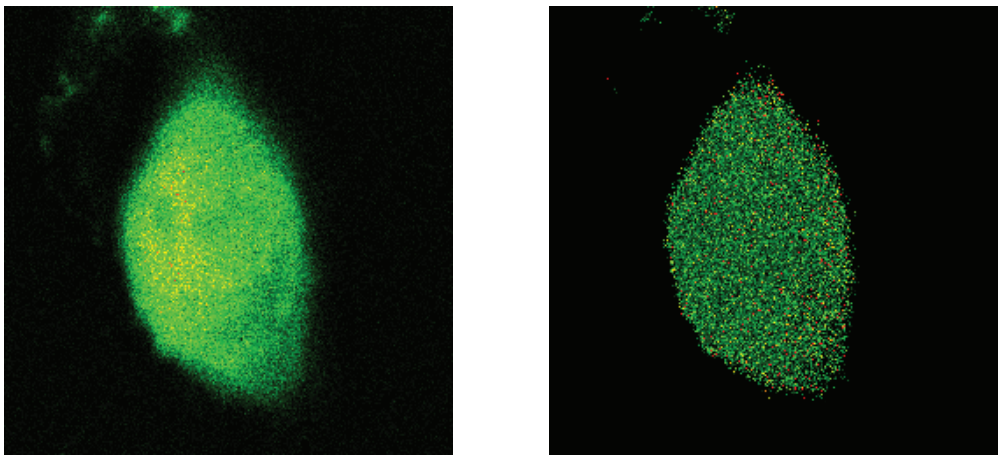
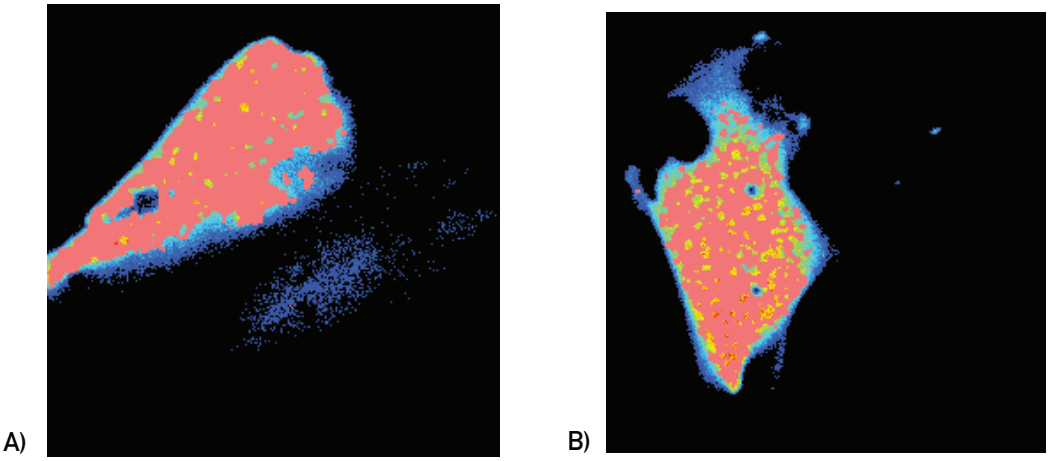


Figure 4. Measurements were acquired with excitation at 440 nm.On the left, fluorescence intensity of a cell expressed with GFP. Decay time is 2.6 ns. On the right, fluorescence lifetime of GFP in presence of YFP. Decay time is 1.7 ns; the decrease in the decay time is due to FRET occurring between CFP and YFP *(courtesy of Laboratory for Fluorescence Dynamics, University of California at Irvine)*.

The data analysis software is VistaVision by ISS which allows data representation in the phasor plot for easy interpretation of FRET processes and ultrafast processing.



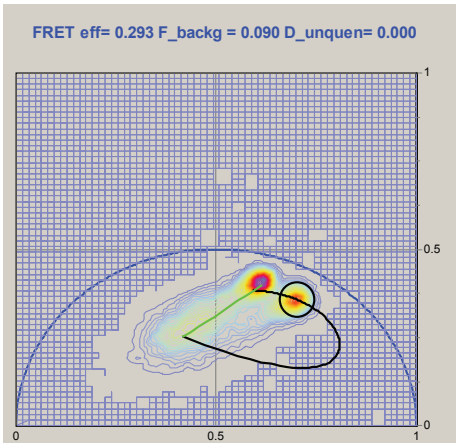


Figure 5.
Cells with FRET pair cerulean-venus construct. Cell in A has only the cerulean protein while cell in B has the cerulean-venus construct. The lifetime of the cerulean-venus is shortened due to FRET in B.
The FRET calculator evaluates the FRET efficiency taking into account cell autofluorescence and the fraction of donors without acceptors. For this cell, the FRET efficiency was 0.29. As the cursor moves along the FRET trajectory in the phasor plot the two different cells are selected (*courtesy of Laboratory for Fluorescence Dynamics, University of California at Irvine)* .

Specifications

Unit	Description
Laser light source	Laser diodes: 370, 405, 440, 473, 635 nm. Ti:Sapphire laser. Supercontinuum laser.
Laser launcher	Up to 6 laser diodes. Light is delivered to the microscope through a single-mode fiber optic.
Input Channels	Two
Detectors	PMT GaAs Model H7422P
Clock	Pixel, line, frame
FLIM image data acquisition dwell time	6 µs/pixel or better
Unit Control	Through USB port
Computer	3 GHz, 4GB RAM, 200 GB hard drive, and 27" color monitor; (minimum specifications shown).
Acquisition and analysis software	VistaVision by ISS and SimFCS by LFD

FastFLIM is covered by US Patent 8,330,123; other patents are pending.

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