NIS-Elements PicoQuant FLIM & FCS Software Control Plug-in

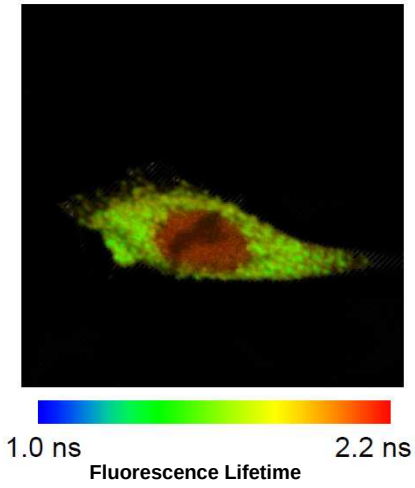
Introduction

The PicoQuant LSM Upgrade Kit for the Nikon Confocals (A1/C2) adds high quality FLIM and FCS detection to the Nikon confocal systems.

The NIS-Elements plug-in for communication with the PicoQuant SymPhoTime 64 software is distributed with the LSM Upgrade Kit. The plug-in is developed by Nikon in close collaboration with PicoQuant and ensures a smooth workflow during FLIM and FCS experiments.

FLIM

Combining the FLIM pulsed excitation and single photon detection system of PicoQuant with a Nikon confocal allows for fluorescence lifetime imaging (FLIM). Fluorescence lifetime is the time in which the fluorescence emission intensity decays after a short excitation pulse. The lifetime is a molecular property that depends on dye properties and the molecular environment. Because fluorescence lifetime is not affected by the concentration of the dye, it allows for absolute measurements of e.g. ion concentrations and Förster Resonance Energy Transfer (FRET).

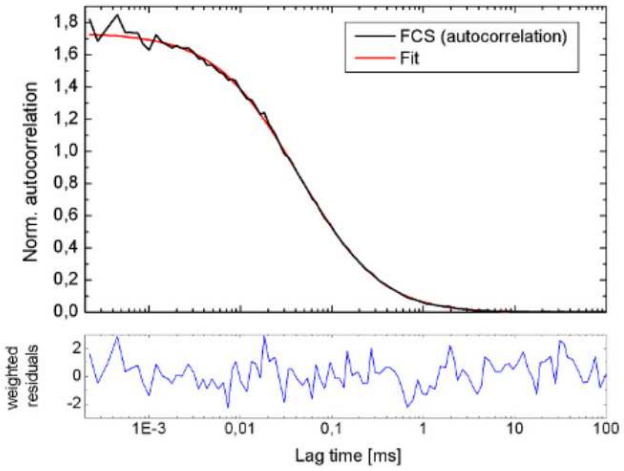


FLIM-FRET analysis allows protein binding sites to be identified. To monitor protein interactions in filopodia and vesicle formation, cells expressing GFP-N-WASP (donor) and mRFP-TOCA-1 (acceptor) were analysed. Cytoplasmic and cellular regions show pronounced lifetime differences in the FLIM image providing information about protein interactions at specific subcellular sites. A two component analysis revealed quenching of the GFP-NWASP lifetime from 2.1 ns to 1.1 ns due to FRET. From the FLIM image we can see that the binding of GFP-N-WASP to mRFPTOCA- 1 takes place in cytoplasmic vesicles but not in other cellular regions.

Sample courtesy of S. Ahmed, T. Sudhaharan, Institute of Medical Biology, Singapore

FCS

Fluorescence Correlation Spectroscopy is used to measure single molecule properties such as molecular brightness, diffusion coefficients and absolute concentrations. Adding the FCS detection system of PicoQuant to a Nikon Confocal allows for an easy workflow of doing FCS experiments at multiple points in the field-of-view.



Details

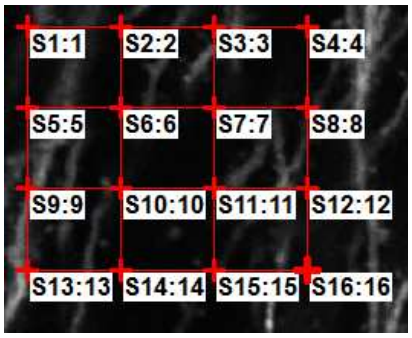
For single FCS/FLIM measurements, the TCSPC module is synchronized with the Nikon Confocal controllers to capture FLIM images and FCS curves. The NIS-Elements plug-in implements software synchronization for more complex acquisition schemes such as multi-point, time-series and z-stacks.

Screenshots

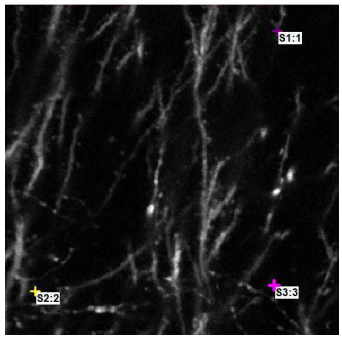
One panel allows to setup and control confocal, FLIM and FCS basic and complex experiments (picture on the right). For FCS multi-point operation two modes are available: the Grid mode on which the measurement points are located on a regular grid pattern and the Free mode in which the points can be set manually.

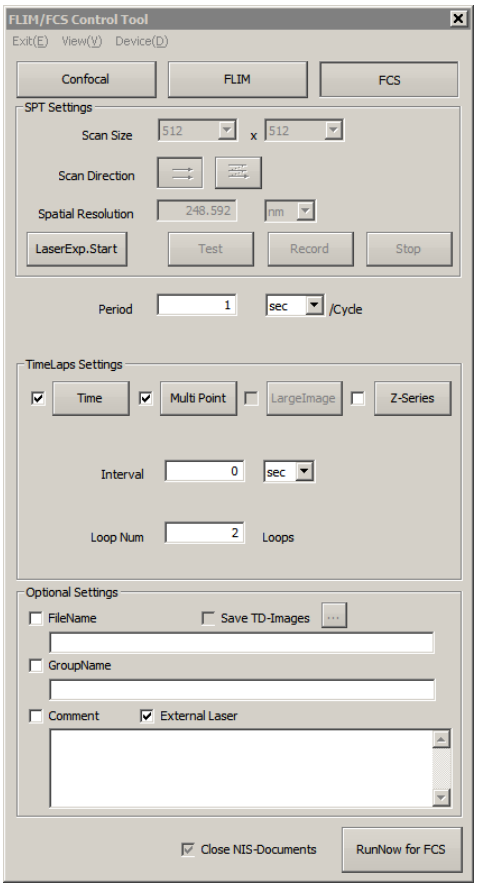
The Time-Series, Multi-Point and Z-Series acquisition modes can be all combined in one experiment.

Grid FCS multi-point



Free FCS multi-point





Information

Product Code:	this plugin is distributed by PicoQuant
Required Hardware:	Nikon Confocal (A1/C2) PicoQuant LSM Upgrade Kit
Plug-in for:	NIS-C
Related Plug-ins:	none
Required Plug-ins:	none

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NIS_PI_PicoQuant_FLIM_FCS_Control_Plugin_E04