FastFLIM STED

MICROSCOPY MODULE

Stimulated Emission Depletion (STED) is a powerful microscopy technique that allows for the observation of macromolecular complexes and sub-cellular structures with spatial resolution below the diffraction limit. The ISS module, developed for Alba v5, uses the pulsed excitation and pulsed depletion approach (pSTED) in combination with the digital frequency domain fluorescence lifetime imaging (FastFLIM) to record the time-resolved photons which allows for an increase in the image resolution and the separation of two labels with the same excitation wavelength.

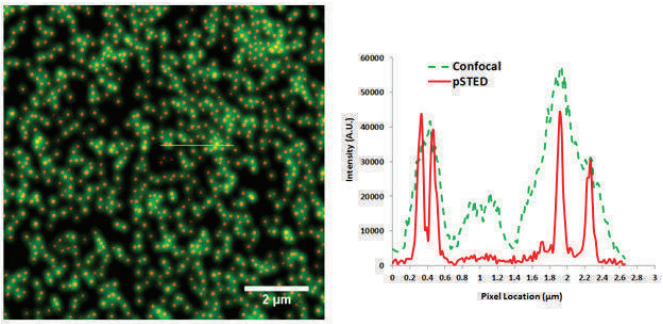
Key Features

- pSTED (Pulsed excitation and pulsed STED)
- FastFLIM for time-resolved pSTED acquisition
- Improved image resolution using the phasor plot
- Dual-label STED
- Fast image acquisition
  (5 frames/second at 256x256 pixels)
- High dynamic range (signal up to 13 million counts/s per channel)

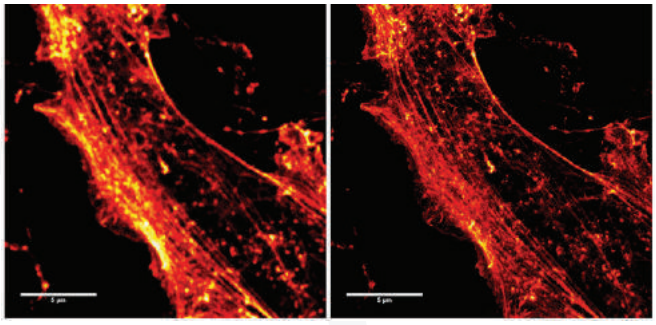
FastFLIM for the Time-resolved pSTED Acquisition

FastFLIM is the data acquisition card for your FLIM acquisition when acquisition speed is of the essence. The card is based upon the Digital Frequency Domain (DFD) technique that allows for the acquisition of Time-Tagged-Time-Resolved data without the dead time typical of TCSPC approach. The card features an extremely high dynamic range: signals of up to 13 million counts/sec when using the appropriate detector can be recorded (versus the about 100,000 counts/sec typical of TCSPC).

The 4 independent input channels can be configured for accepting signals from PMTs, APDs with TTL output, or a combination of the two types of detectors. Decay times from 1 second to 50 picoseconds can be resolved.The card is supported by Windows 10 operating system and the connection to the computer is through USB2.



Confocal (green) vs. pSTED (red) images of 60-nm fluorescent beads, acquired by FastFLIM.



Confocal (left) and pSTED (right) images of the actin labeled with the SiR dye in fixed glia cells, acquired by FastFLIM.

FastFLIM and the Phasor Plot

The acquisition of time-resolved information allows for the separation of molecules that have been excited only by the excitation laser from the molecules that have been excited by both the excitation and depletion lasers. The separation is visualized in the Phasor Plot because of the different decay times of the molecules. The final result is an image featuring a higher resolution that the standard STED image.

The FastFLIM pSTED introduces several features:

- Lower STED power laser to reduce photo-toxicity
- Improvement of the image resolution by using time-resolved information
- Capability to do dual-label STED by a single pair of excitation and depletion lasers.

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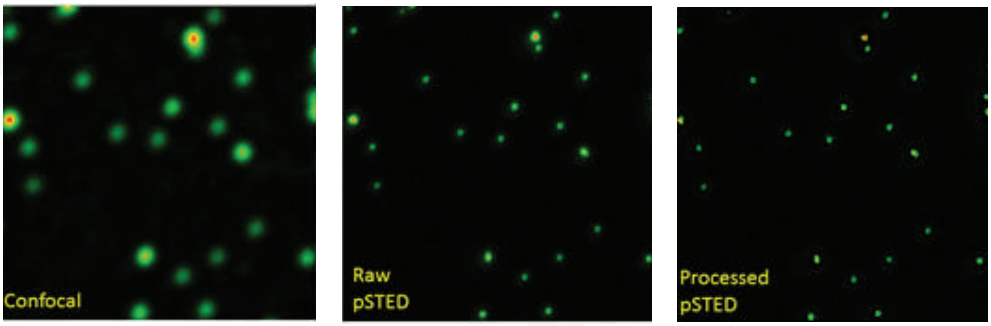
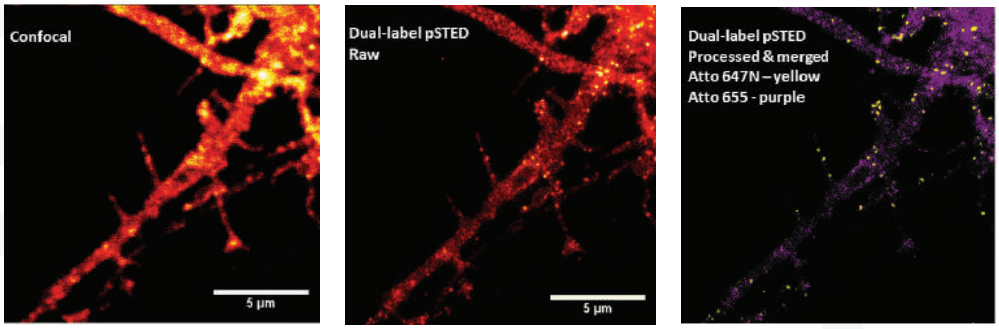
FastFLIM STED Specifications



Confocal, Raw pSTED and Processed pSTED images.



Dual-label (Atto 647N and Atto 655) pSTED image acquired by FastFLIM. The two dyes are first separated from the phasor plots, and then assigned with two different false colors (Atto 647N – yellow, Atto 655 - purple) to produce the processed and merged pSTED image of the two labels.

Feature	Description
Excitation laser	Pulsed, 640 nm Pulsewidth (at medium power): 40-90 ps Repetition rate: 20, 50, 80 MHz; or Ext CLK Power (at 50 MHz): up to 5mW
STED laser	Pulsed, 775nm Avg. output power: 1 W Pulsewidth: about 600 ps Repetition rate: 0-100 MHz; or EXT CLK Beam quality: M2 <1.1, TEM00 Amplitude noise: < 4.0% rms
Light detectors	Hybrid PMTs GaAs PMTs SPADs
FastFLIM	4 separate input signal Dynamic range: up to 13 million counts/s per channel Lifetime range; from picosecond to second
Image acquisition	FLIM acquisition: 5 frames/sec (256 x 256 pixels)
Software	VistaVision 64 bit, Windows 10 OS Pro

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

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