SPECIFICATIONS

FastFLIM

FastFLIM is the data acquisition card for your FLIM acquisition when acquisition time is of the essence. The card has been developed using Digital Frequency Domain (DFD) technique that allows for the acquisition of Time-Tagged-Time-Resolved (TTTR) data without the dead time typical of TCSPC approach. The 4 independent input channels can be configured for accepting signals from PMTs and/or APDs. The design allows for maximum FLIM data acquisition of up to $80x10^6$ counts/second/channel for two channels simultaneously, or $40x10^6$ counts/second/channel for four channels simultaneously. Decay times from the picosecond to the second time scales can be resolved (FLIM and PLIM). In addition to the fitting analysis, the FLIM data acquired by FastFLIM can be directly used for phasor plots without any distortion. The card is supported by drivers in Windows 7/10/11, 64-bit, through the USB connection.

Measurements:

- Time-Tagged-Phase-Resolved lifetime measurements
- Single-wavelength and multi-wavelength FLIM/PLIM
- Confocal images
- Anisotropy measurements (steady-state and time-resolved)
- FCS, FCCS, PCH
- Scanning FCS, RICS, N&B
- Stoichiometry
- Single Molecule FRET
- PIE measurements

Features:

- 4-channel simultaneous acquisition
- Direct input from PMTs and APDs
- Photon count rate up to 10^9 counts per second
- Dead time 1.5625 ns
- Trigger out to synchronize external devices
- Trigger input from external source
- Line and Frame CLK synchronization
- USB 3.0 communication
- Drivers for Windows 7/10/11, 64-bit, OS

Features

FLIM: Measurements of short decay times

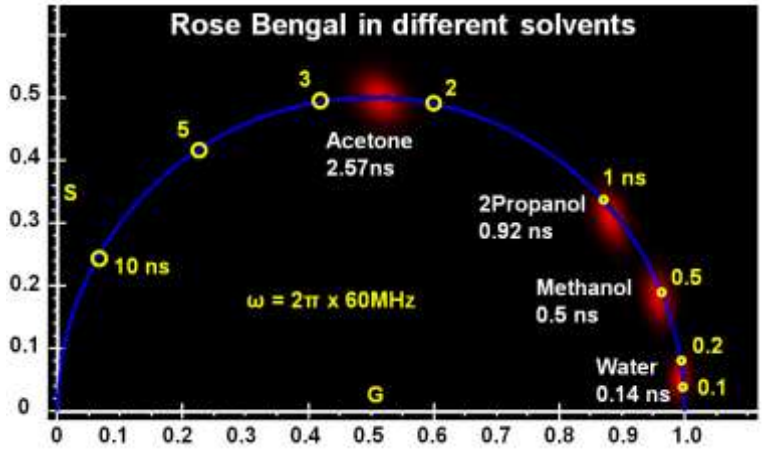


Figure 1. Measurements of Rose Bengal in different solvents reported on the phasor plot. Decay times from 140 ps to 10 ns are displayed. *(courtesy of Dr. A. Periasamy, University of Virginia*)

PLIM: Measurements of long decay times

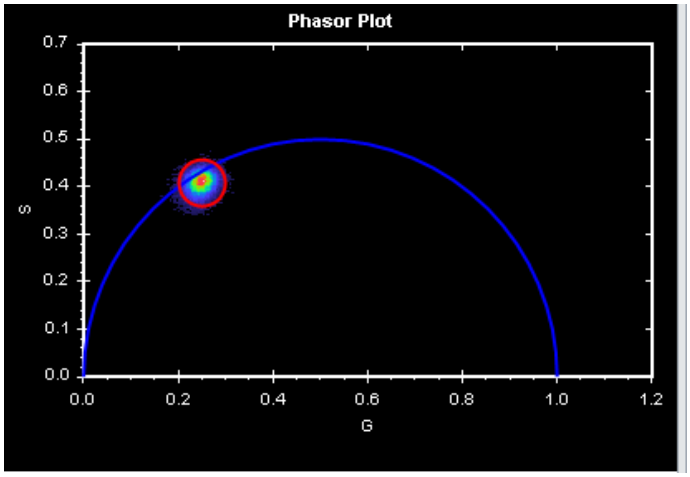


Figure 2. Upconversion nanoparticles sample of ncx-ucnp (negative control) and channelrhodopsin-ucnp (positive control) are used to measure the PLIM FRET. The excitation wavelength of ucnp is 980nm and the emission is at 453nm and 474nm. The measured decay time is 272 µs. *(courtesy of Dr. Hsien-Ming Lee , Academia Sinica, Taipei, Taiwan ROC*)

Pulsed Interleaved Excitation (PIE)

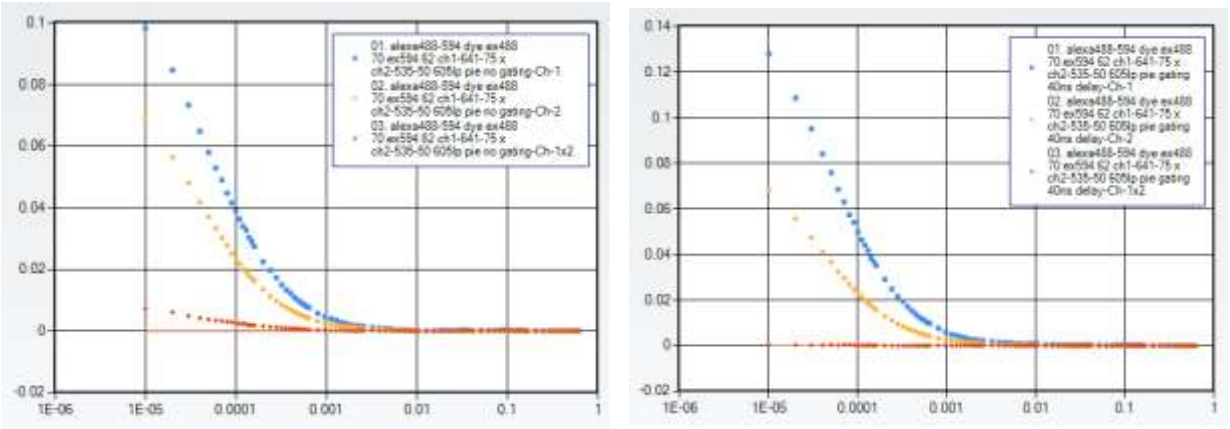
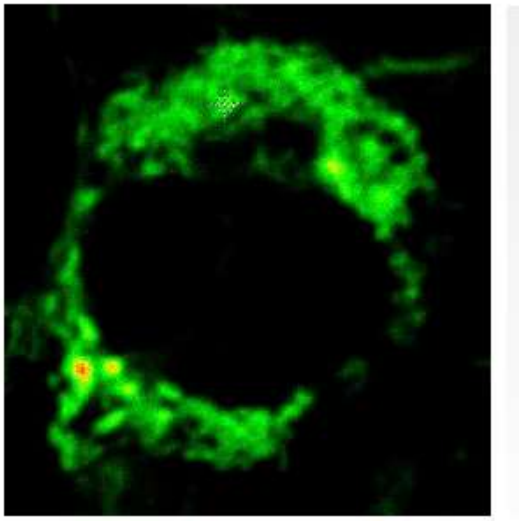


Figure 3. Comparing FCCS measurements of a mixture of Alexa488 and Alexa594 free dyes in solution by both 488-nm and 594-nm lasers with (left) and without (right) PIE.

Applications

Cell Biology



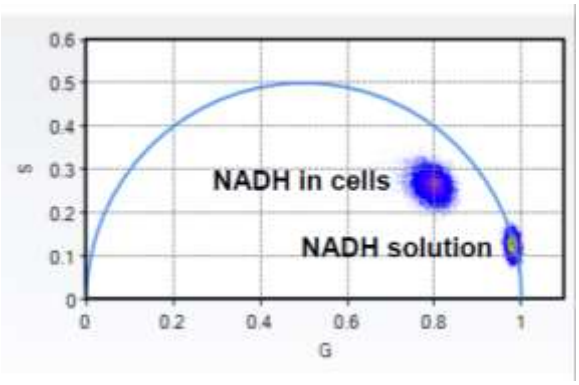
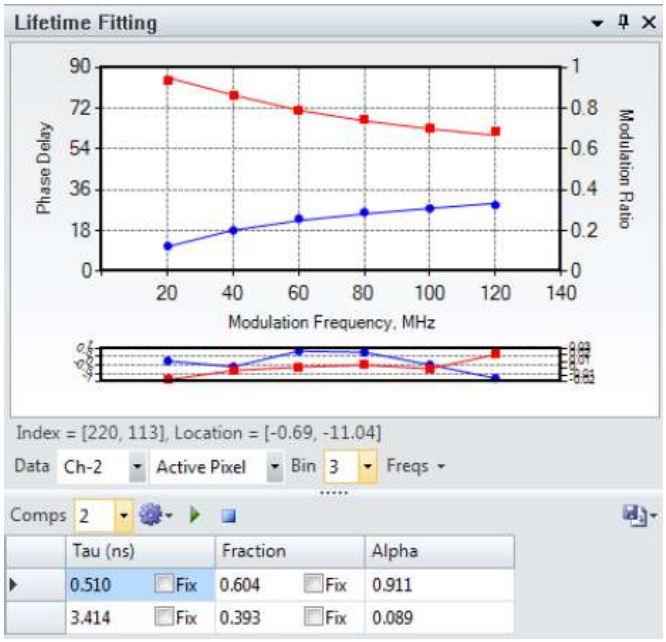


Figure 4. The autofluorescence from NADH is resolved and compared in the phasor plot with the decay time of NADH in vitro. FastFLIM time-resolved data are represented in phase and modulation plot (bottom right) and phasor plot (bottom left).
(*courtesy of Dr. A. Periasamy, University of Virginia*)



Single molecule FRET

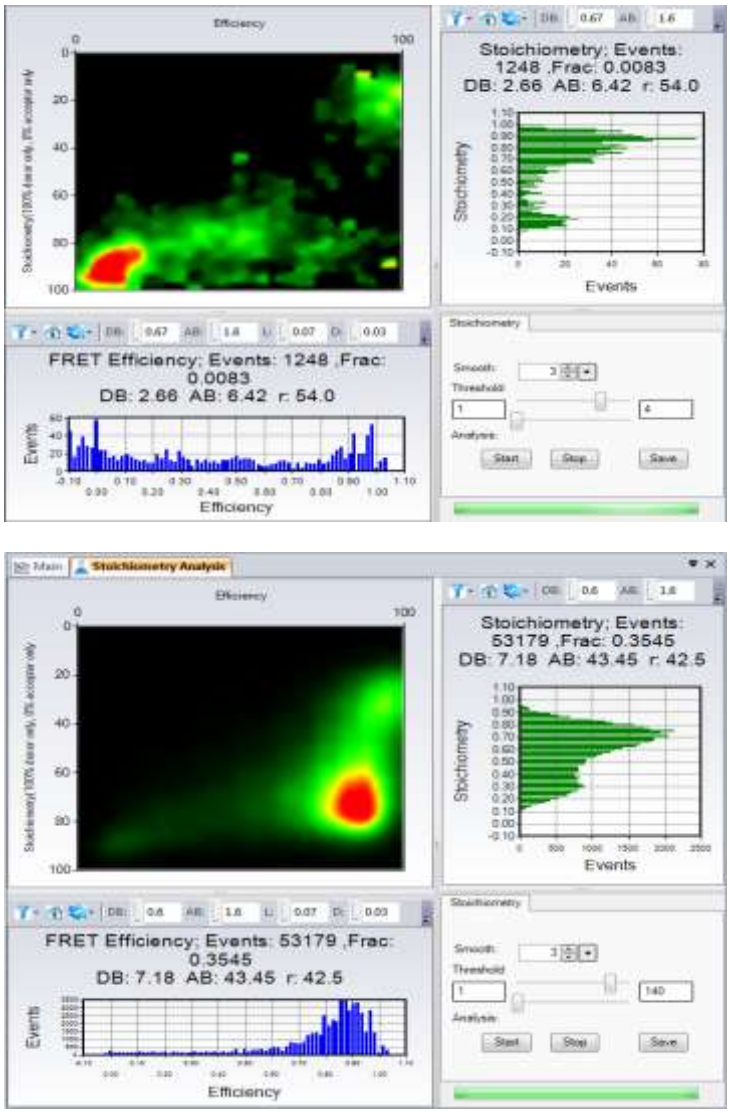


Figure 5. Folding of α-synuclein and conformational switching at different environment: (left) buffer only, (right) with SDS. In the right panel, the events happen mostly in high FRET efficiency, when donor is more abundant.
(courtesy of Dr. A. Ferreon; Baylor College of Medicine, Houston, TX)

STED FLIM

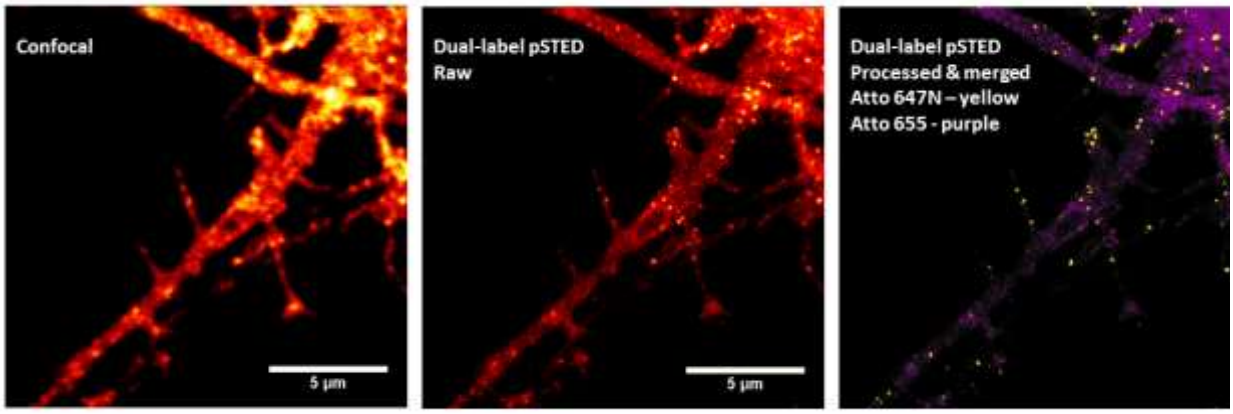


Figure 6. Images of neurons dissociated from primary hippocampal and glia cultures prepared from E 18.5 Sprague–Dawley rat embryos. For dual labeling of membrane and AMPA receptor, neurons expressing GPI-RFP, biotinylated GluR2 and Homer1-mGeos-M were first incubated in 3 nM streptavidin-Atto647N diluted in imaging buffer with 0.6% BSA for 5 min. Subsequently, cells were incubated in 50 nM RBP-Atto655 (RFP-booster, Chromotek) diluted in imaging buffer with 0.6% BSA for 10 min. The dual-label was excited by pSTED using a single excitation / depletion laser combination and data were acquired by FastFLIM. Using the time-resolved information in the phasor plot, the location of Atto 647N (yellow) and Atto 655 (purple) are shown.

Specifications

Feature	Description
Architecture	USB 3.0
CLK frequency	640 MHz
No. of INPUT channels	4 independent channels
Input voltage range	From PMTs and/or APDs (-1V ~ +5V, 50Ω)
Decay times measurement	PLIM and FLIM: 100 picoseconds to 100 milliseconds
Dead Time	1.56 ns
External CLK IN	10 ~ 80 MHz (LVTTL / TTL, 50Ω)
Reference CLK OUT	0.0596 Hz – 80 MHz, Amplitude: + 1.2 & 1.8 V (50Ω)
Reference SYNC OUT	20 MHz 50% duty cycle, Amplitude: + 2.5 V (50Ω)
LINE/FRAME/PIXEL Scan	Synchronization w/ scanner
Data handling and storage	Acquisition of raw data for FCS, FCCS, PCH, smFRET, burst. On-line processing or post-processing
Raw data size	32 bits
Raw data file structure	Binary File with a header of 256 bytes
Max signal in Counts (steady-state intensity only)	10^9 counts / second (250×10^6 counts/sec per channel for 4 channels simultaneously)
Max signal in FCS & FLIM (time tagged & time tagged time resolved – TTTR)	160×10^6 counts / second (80×10^6 counts/sec per channel for 2 channels simultaneously) (40×10^6 counts/sec per channel for 4 channels simultaneously)
Mechanical & Electrical	
Power	120/240 V, 50/60 Hz, 40 W
Dimensions (cm)	42.5 (W) x 36 (D) x 10 (H)

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

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