Q2

Laser Scanning Nanoscope

The compact instrument for quantitative cell biology with single-molecule detection

Q2 is a laser scanning nanoscope (LSN) that incorporates several measurement modalities for experimental quantitative biology and material sciences applications requiring the single molecule detection sensitivity.

Table of Contents

Why the Q2 Nanoscope? **3**

The Q2 design **4**
- Easy of Use and Simplicity of Design 4
- Innovation 4

Q2 System Configuration **5**
- Microscope 5
- Laser ports and laser launchers 5
- The detectors 6
- Imaging modality 6

All in One with the VistaVision software **7**
- VistaVision Instrument Control module 7
- VistaVision Imaging module 7
- VistaVision FLIM module – Time and Frequency 7
- VistaVision Anisotropy module – steady state and time resolved 8
- VistaVision Fluorescence Fluctuations Spectroscopy module 8
- VistaVision Single Molecule module 8
- VistaVision Particle Tracking and Nanoimaging module 8

Measurements **9**
- 1p and 2p Confocal Imaging 9
- Colocalization in Confocal Imaging 10
- Fluorescence Lifetime Imaging (FLIM) 11
- Fluorescence Lifetime Imaging - TCSPC 13
- Fluorescence Fluctuation Spectroscopy (FFS) 16
- 2p FCS 17
- 1p FCS 18
- FCCS with Pulsed Interleaved Excitation (PIE) 19
- Scanning FCS 20
- Polarization Anisotropy images 21
- Single molecule imaging and FRET 22

Technical Specifications **23**
- Hardware 23
- Software 24

Why the Q2 Nanoscope?

We have entered the era of quantitative live cell biology; the dynamics of single proteins within the cell are measured and new insights about the cellular environment and inner machinery are provided. The Q2 is a compact and fast multi-channel multiphoton confocal microscope for quantitative live cell biology applications. The standard configuration includes two detection channels and a multiphoton laser emitting at 780 nm although a Ti:Sapphire laser, the supercontinuum laser and single-photon lasers can be coupled to the instrument too. Detectors are either GaAs PMT, hybrid PMT, or APD.

The Q2 allows for the acquisition of confocal images, FLIM images and all of the Fluorescence Fluctuations Spectroscopy measurements (autocorrelation, cross-correlation, photon counting histogram, scanning FCS, RICS, and Number & Brightness). The instrument is powered by the 64-bit VistaVision software by ISS. When equipped with the nanoimaging option it allows for the reconstruction of images of dynamic structures with a resolution of 20 nanometers.

In one integrated unit, Q2 provides all of the measurements for quantitative biology. Routine applications by Q2 are listed in Table I.

Intensity and Lifetime Imaging:

- 1p or 2p confocal imaging in x, y, z and t
- FLIM in frequency-domain (FastFLIM) or in TCSPC
- Phosphorescence Lifetime Imaging (PLIM)
- Steady-state and time-resolved anisotropy imaging

Fluorescence Fluctuations Spectroscopy:

- Fluorescence Correlation Spectroscopy (FCS)
- Fluorescence Cross-Correlation Spectroscopy (FCCS) with pulse interleaved excitations and synchronized gating detections
- Photon Counting Histogram (PCH)
- Fluorescence Lifetime Correlation Spectroscopy (FLCS)
- Scanning FCS by orbit scanning
- Number & Brightness (N&B)
- RICS (raster imaging correlation spectroscopy)

Single Molecule Imaging

- Burst Analysis
- FRET efficiency determination
- Stoichiometry determination with pulse interleaved excitations and synchronized gating detections

3D Particle Tracking and Nanoimaging

- 3D particle tracking trajectories
- Nanoimaging reconstruction with 20 nm resolution

Table I: Routine Applications for quantitative biology by Q2

The Q2 design

Easy of Use and Simplicity of Design

Compact design. Figure 1 shows a typical configuration of Q2, equipped with a Toptica FemtoFiber Pro multiphoton laser, an Olympus IX71 microscope, and two hybrid PMT detection channels. The entire system can be easily fit onto a 0.6m x 1.2m table.

Expandability. To name a few marks, it provides three laser input ports to cover the range from UV to VIS to NIR; it mounts high quality and tunable polarization optics built-in as an option to work for the polarization specified applications regardless of the input laser polarization; it allows multiple dichroics for various laser lines on a computer controller motorized wheel for a click-and-select operation.

Reliability and quality design to maximize the system sensitivity, because quantitative imaging and analysis is our business where every single fluorescent photon is precious. All the optics equipped in Q2 are aligned and tuned by ISS engineers by using tools for beam positioning and diagnosis. The quality design of the system makes these alignments robust and stable, and also makes the system easy for the user to operate.

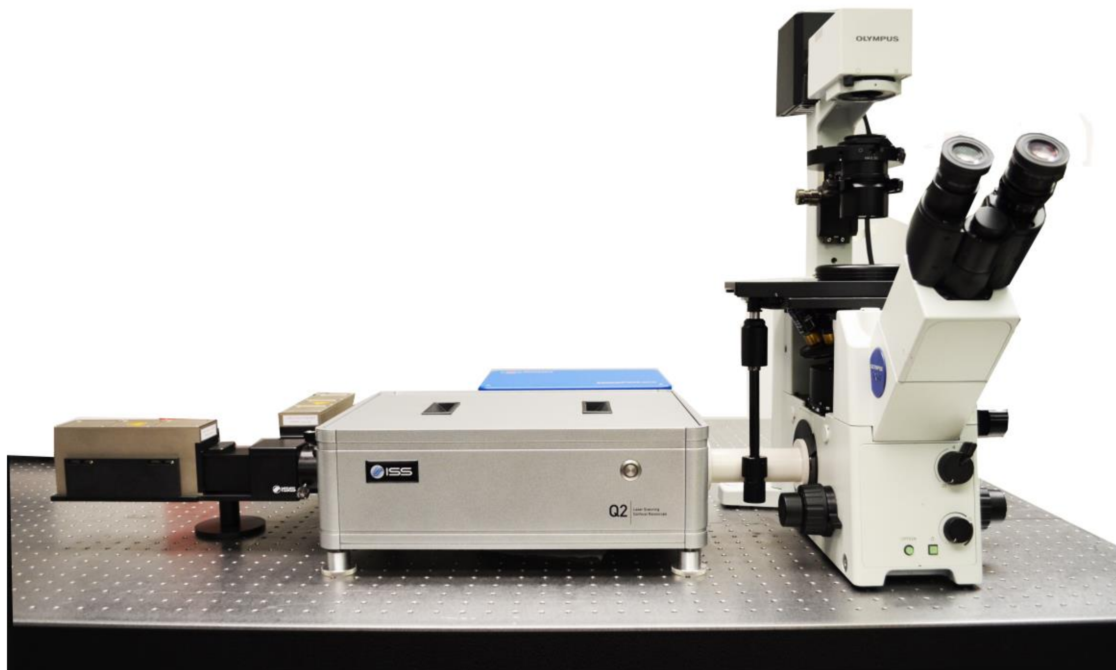


Figure 1. Q2 coupled to an inverted microscope (Model IX71 by Olympus)

Innovation

Q2 incorporates innovative technology (the latest light detectors, the capability of acquiring fluorescence lifetime imaging (FLIM) data either in time-domain (TCSPC) or digital frequency-domain (FastFLIM); the data analysis using both the standard fitting algorithm and the phasor plots).
Moreover, the Q2 incorporates innovative acquisition techniques tailored for quantitative cell biology studies (scanning FCS, raster image correlation spectroscopy, number & brightness, single molecule particle tracking, nanoimaging with 20 nm resolution).

Q2 System Configuration

Microscope and Coupling	• Olympus, Nikon, Zeiss, Leica or customized • Inverted and upright • Left side port and back port
Laser Sources	**1p Excitation**: • ISS laser launcher (models for 3, 4 and 6 laser diodes), wavelengths available from 375 nm to 640 nm; • pulse interleave excitation (PIE). • Super-continuum lasers, wavelength from 400 to 700 nm; **2p Excitation**: • Ultrafast femtosecond pulse Ti:Sapphire lasers • Ultrafast femtosecond pulse fiber lasers
Data Acquisition Unit	• FastFLIM (Digital Frequency domain FLIM) • TCSPC card (Time domain FLIM) • IO Tech DAQ card (required for particle tracking and nanoimaging)
Detectors	• GaAs PMT (Hamamatsu H7421 and H7422P models) • Hybrid PMTs (Hamamatsu R10467U models) • APDs
Positioning controls	• ISS 3-axis control unit • ISS XY galvo scanning mirrors control unit • ISS Z-piezo control unit • Microscope built-in focusing control module • Automatic XY stage
Software	VistaVision 64bit
Computer & Monitor	• 3GHz Processor, 16GB RAM, 500GB Hard drive for 64-bit VistaVision • 27” 1280 x 1024 resolution monitor • Windows 7 or 10 64 bit professional

Microscope

Q2 can be utilized with most commercial epifluorescence inverted and upright research microscopes. The microscope is not altered; it maintains all of the original functionality and upgrades capabilities. Moreover, some automated microscopes can be controlled by the ISS VistaVision software resulting in an all integrated unit.

Laser ports and laser launchers

Q2 is equipped with three laser ports, each of which has an option for mounting a mechanical shutter. Both single-photon and multi-photon lasers can be aligned and coupled to the instrument; the selection of the laser source is done through the software.
The multiphoton laser is delivered to Q2 in free air. Before entering the unit, it passes through an intensity control unit that allows for the user to select and control the excitation intensity.
Single photon lasers are accommodated into a laser launcher; the beam is delivered to Q2 by using a single-mode polarization maintained fiber. The ISS laser launchers are designed to accommodate a

variety of lasers, either continuous wave (cw) or pulsed. The intensity of each laser is controlled by a variable density filter; a shutter allows the selection or blockage of each individual laser beam.
Q2 was fully evaluated and validated for using ISS laser launcher, a supercontinuum laser (Model SC-400 by Fianium) and a fiber laser (Model FemtoFiber Pro 2p laser by Toptica).

Figure 2. 4-diode Laser launcher

The detectors

Three types of detectors are routinely utilized in Q2. Table II reports the detectors recommended for specific applications.

Application	APD	GaAs PMT	Hybrid PMTs
FCS, FCCS, PCH	●	●	●
FLIM		●	●
Scanning FCS, N&B, RICS	●	●	●
Single molecule spectroscopy	●		

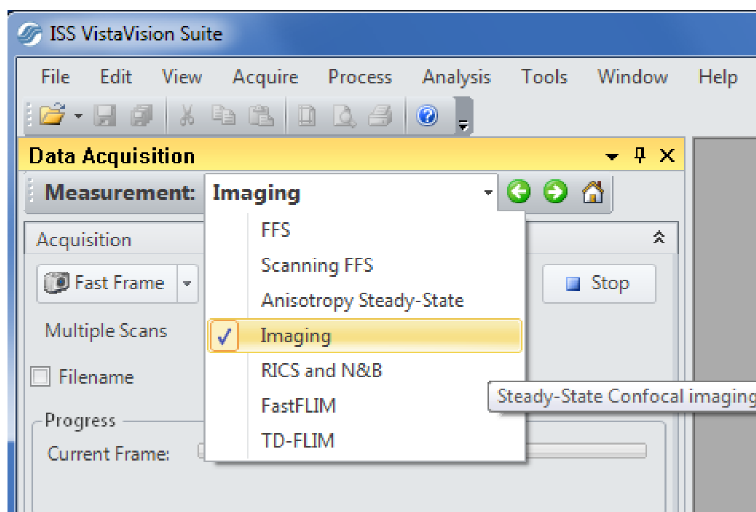
Table II: Typical detectors used in Q2

Imaging modality

The Q2 is a laser scanning instrument**:** the laser beam is scanned over the sample following a predetermined pattern. This is achieved by using fast galvo-controlled mirrors that scan the beam on the XY-plane of a surface area of about 200 µm in diameter (using a 60X objective) with no optical distortions in the image. Image acquisition is fast (up to 4 µs dwell time per pixel). Galvo-controlled scanning mirrors offer the best solution when fast imaging acquisition is required. This option is utilized for fast imaging acquisition, scanning FCS, RICS and N&B acquisition, and particle tracking.
The optical sectioning (z-axis scan) is achieved by mounting the objective on a piezo-stage, or using a stage or an objective turret with built-in z-axis control.
Q2 powered by the ISS VistaVision software provides utilities for measurements in spectroscopy mode (at a single point), raster or orbit scan mode (2D XY), optical sectioning mode (3D), time-lapse mode, stage scan mode for multi wells, or combination of them.

All in One with the VistaVision software

VistaVision (Windows 7 or 10 64-bit) is a complete software package for instrument control, data acquisition, data processing and analysis. VistaVision enables control of the automated devices on the Q2 instruments including shutters, filterwheels, XY stages and light detectors. The user selects the measurement modality and can switch to a different measurement by simply opening a menu.



VistaVision Instrument Control module

Includes the routines for instrument control (automatic instrument alignment of pinhole, shutter control, selection of the light detector gain/bias control); control of the galvo-mirrors and microscope stages); laser launcher (laser intensity, laser modulation); and control of microscope automation features.

VistaVision Imaging module

Includes routines for image acquisition, image processing and image display that allows for the user to acquire single-point data (intensity, kinetics, polarization, lifetime); line data; and images.
The user interface includes setting/adjusting the acquisition parameters (pixel dwell time, image size, and the image resolution) and the selection of image type (polarization, FLIM, N&B, RICS).
Images stacks can be acquired in different direction (XYZ, XZY). An array of time series is available (t, Xt, XYt, XZt) for both steady-state images and FLIM.
Images can be exported to ImageJ and MetaMorph; plots are exported to popular formats (png, jpeg, gif, tiff, bitmap, metafile). Movies are produced in avi format. The software includes operations between images, smoothing, filtering, rotation, zooming, scaling and automatic threshold setting for image contrast enhancement, profile and histogram plots, ratiometric and advanced mathematical calculations, etc.

VistaVision FLIM module – Time and Frequency

ISS provides two options for fluorescence lifetime imaging (FLIM) as well as phosphorescence lifetime imaging (PLIM) applications - both the digital frequency-domain (FastFLIM) and the time-domain (TCSPC) solutions (see Note 1). The range of lifetimes is measured from ps to ms.
Both TCSPC and DFD FLIM data can be analyzed using the lifetime fitting (Marquardt-Levenberg minimization algorithm) and the phasor plots. Analyzed FLIM results can be exported as lifetime images, images of pre-exponential factors, images of fractional contributions.

VistaVision Anisotropy module – steady state and time resolved

The combination of a halfware plate and a linear polarizer is used in the exciation path to allow an easy set of the excitation polarization at an extinction ratio above 1000:1 regardless of the input laser polarization. A polarization beam splitter with a contrast ratio above 1000:1 is used in the emission path to separate the emission signals into the parallel and the perpendicular channels.
VistaVision includes routines to calculate the steady-state anisotropy map as well as to resolve both rotation times and lifetimes for time resolved anisotropy measurements.

VistaVision Fluorescence Fluctuations Spectroscopy module

Includes routines for multi-channel data acquisition and data processing of up to 3 components. Data are acquired in photon counts mode, photon time-tag mode, or photon time-tag time-resolved (TTTR) mode. VistaVision features a real-time display of the auto correlation function, G(τ) - apart from a nominal delay (less than one second) required for the computation of the function. A sequence of multiple data acquisition files can be acquired (for instance, when using a microwell plate on a computer-controlled XY stage) and displayed and stored automatically. Several analysis models are included for both single-photon and multi-photon excitation; custom models can be entered too thus allowing the researcher complete freedom over the data modeling.

VistaVision Single Molecule module

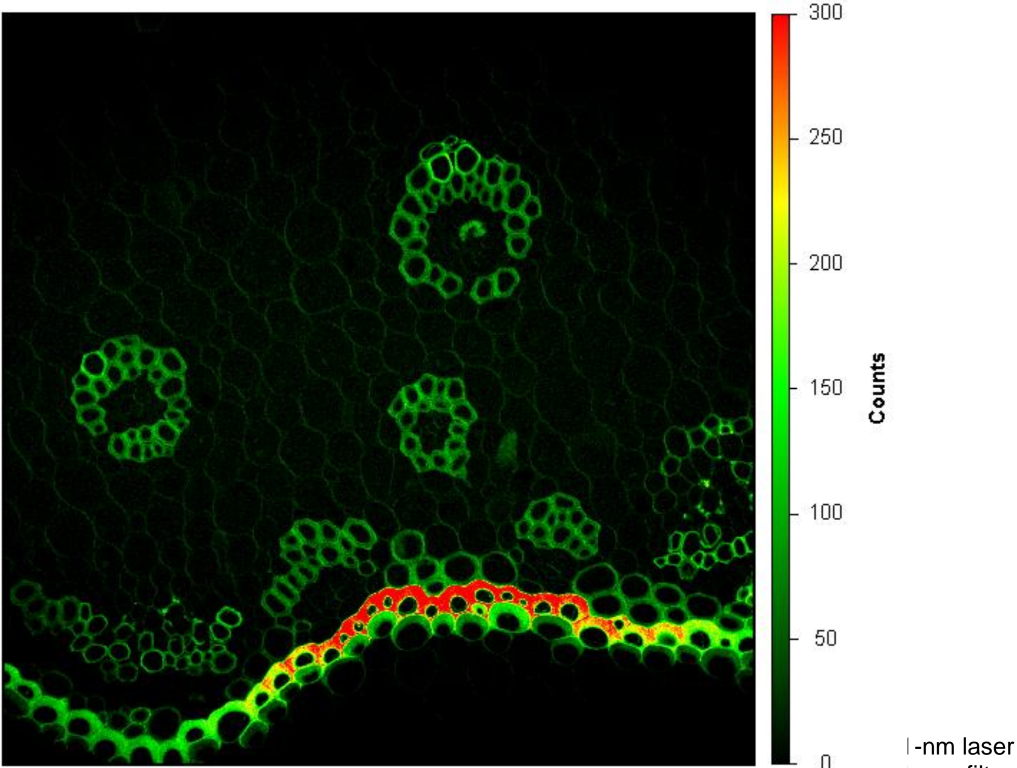
Includes routines for the Burst Analysis and for the Single Molecule Fluorescence Resonance Energy Transfer and Correlation Methods showing the quantity of Donor and Acceptor species in the specific FRET efficiency process.

VistaVision Particle Tracking and Nanoimaging module

The module allows for the 3D tracking of molecules and the reconstruction of imaging structures with 20 nm resolution.

Measurements

1p and 2p Confocal Imaging



-nm laser
pass filter.
Objective: Olympus 20X/0.54 NA. Size: 1024x1024 pixels (600µm x 600µm).

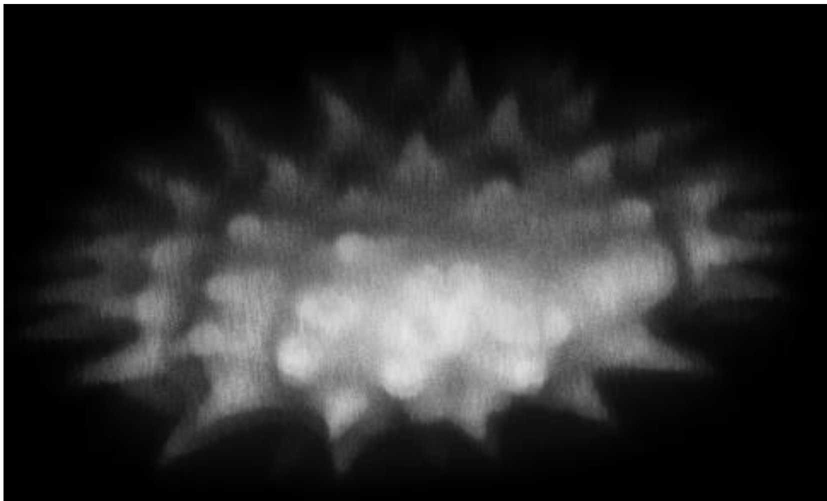


Figure 4. The 3D volume rendering from the optical sections of a pollen grain. The z-stack images were acquired using the 780-nm 2p excitation wavelength (FemtoFiber Pro, by Toptica) and a 575/150-nm bandpass filter. Objective: Nikon 60X/1.2NA water. Size: 512x512x32 (42.8μm x 42.8μm x 32μm).

Colocalization in Confocal Imaging

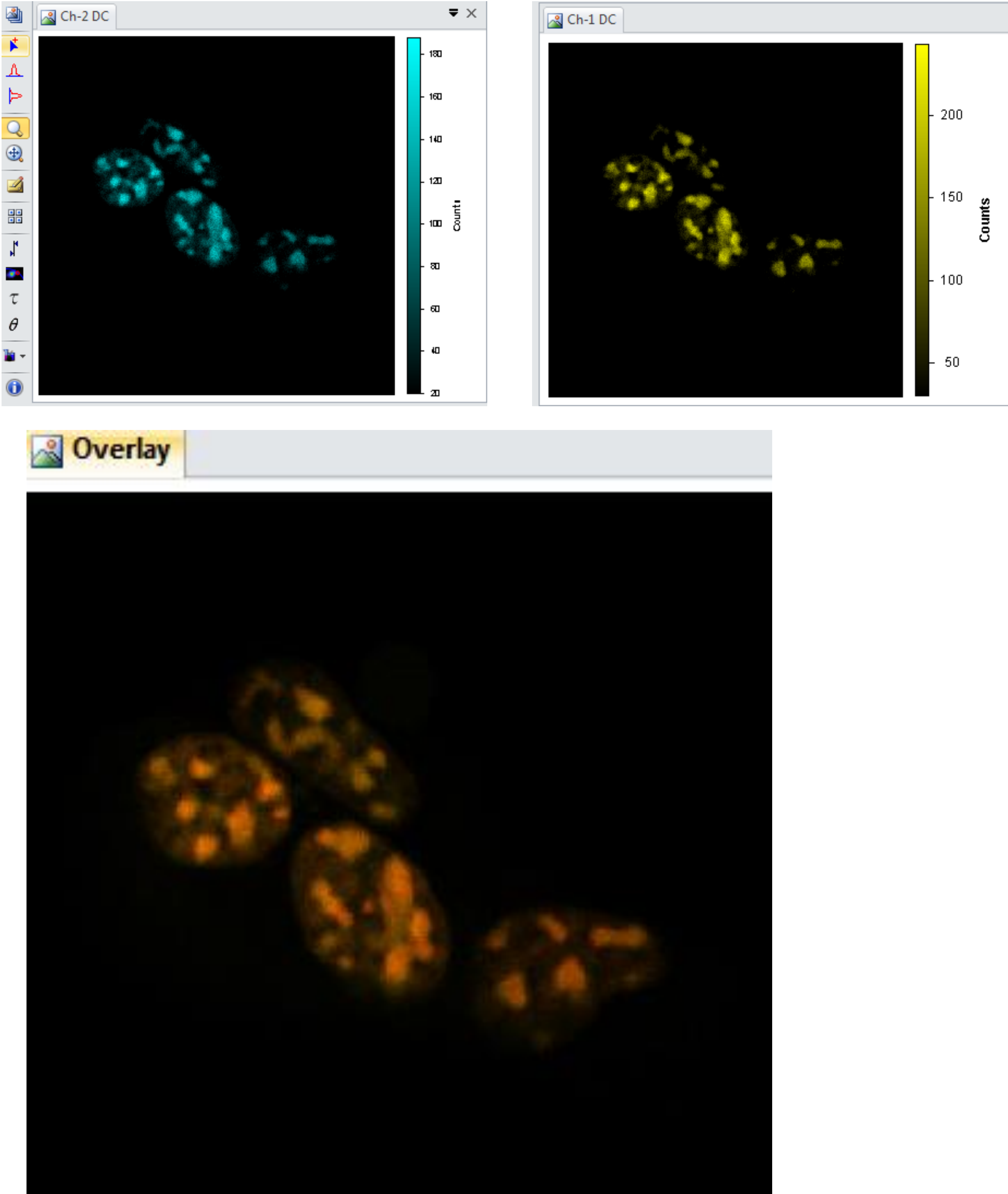


Figure 5. Measurements were acquired from live cells co-expressing Cerulean-CEBP/α and Venus-CEBP/α excited by using both ISS 440-nm and 514-nm diode laser excitation wavelengths in a PIE manner. The detection channels (Ch1: 550/49-nm for Venus and Ch2: 480/30 for Cerulean) are synchronized with the respective laser excitation to eliminate the cross talks. Objective: Nikon 60X / 1.2NA water.

Fluorescence Lifetime Imaging (FLIM) – two options: digital frequency-domain (FastFLIM) and time-domain (TCSPC) **<see Note 1>**

FLIM is a useful technique that provides, with its peculiar selectivity of fluorophores, a higher contrast of confocal images; moreover, it is used for providing quantitative information of the cell environment (ions, pH, oxygen content, electrical signals, index of refraction, etc.). When used in Förster Resonance Energy Transfer (FRET) applications, the measurement of the fluorescence decay kinetics provides indirect information about the proximity of target fluorophores within 10 nm. FLIM is now routinely used for dynamic measurements of signaling events inside living cells.

ISS provides both frequency-domain (FastFLIM) and time-domain (TCSPC) solutions for FLIM applications. The VistaVision software contains the traditional routines developed based on the least-square fitting to analyze both FastFLIM and TCSPC images. It also provides the phasor plot approach for a simple interface to analyze the raw FLIM data quantitatively. A summary of the merits of the phasor plot given by Dr. Enrico Gratton at LFD, UC Irvine are - “No expertise necessary; Instantaneous results; Independent of initial choices; Quantitative results; and Intuitive simple interface”.

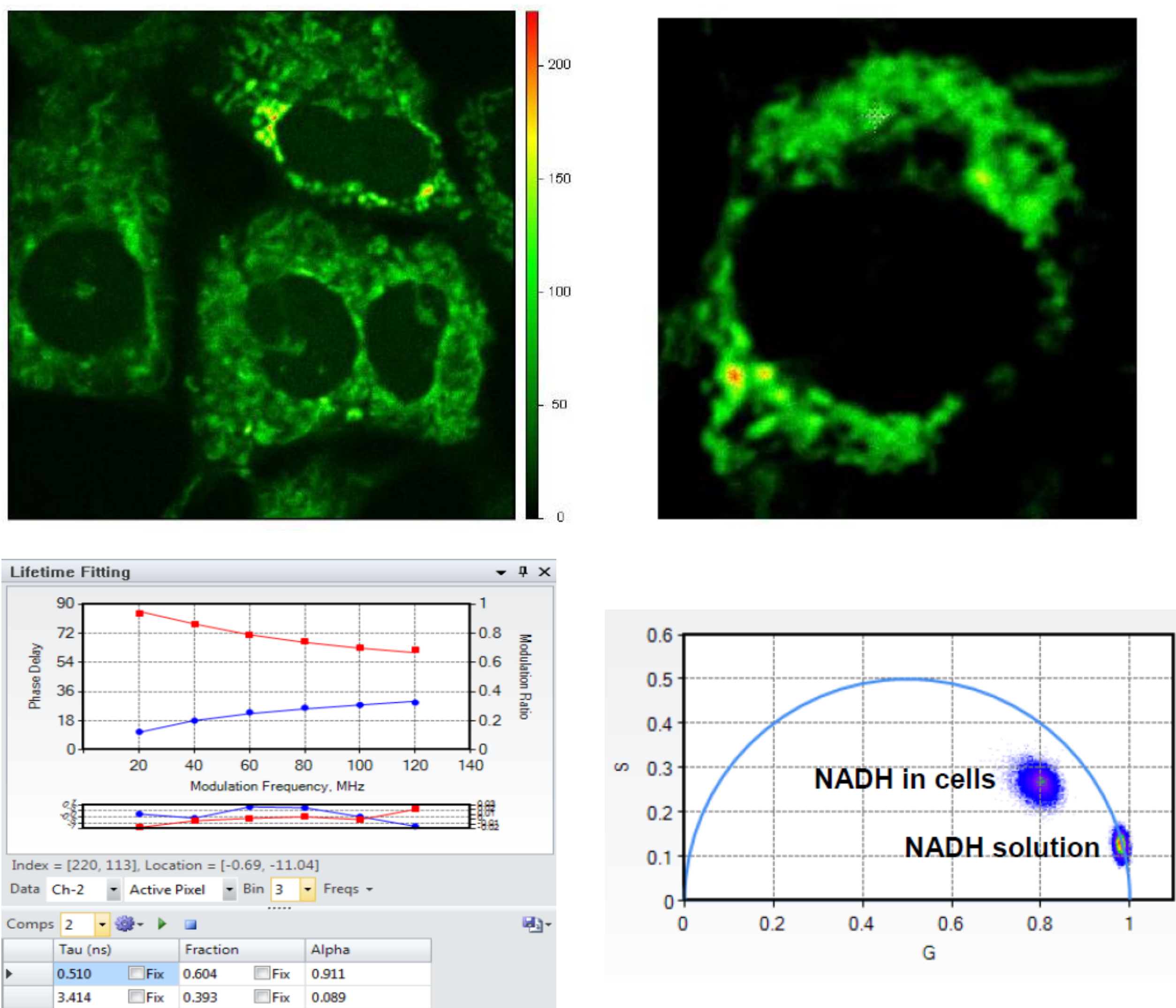


Figure 6. NADH in live cells. Analysis is conducted using the fitting algorithm (left), which provides two decay times, 510 ps (60%, free) and 3.4 ns (40%, bound). The phasor plot (right) displays the NADH in solution (single exponential, 450 ps) and in the cell.

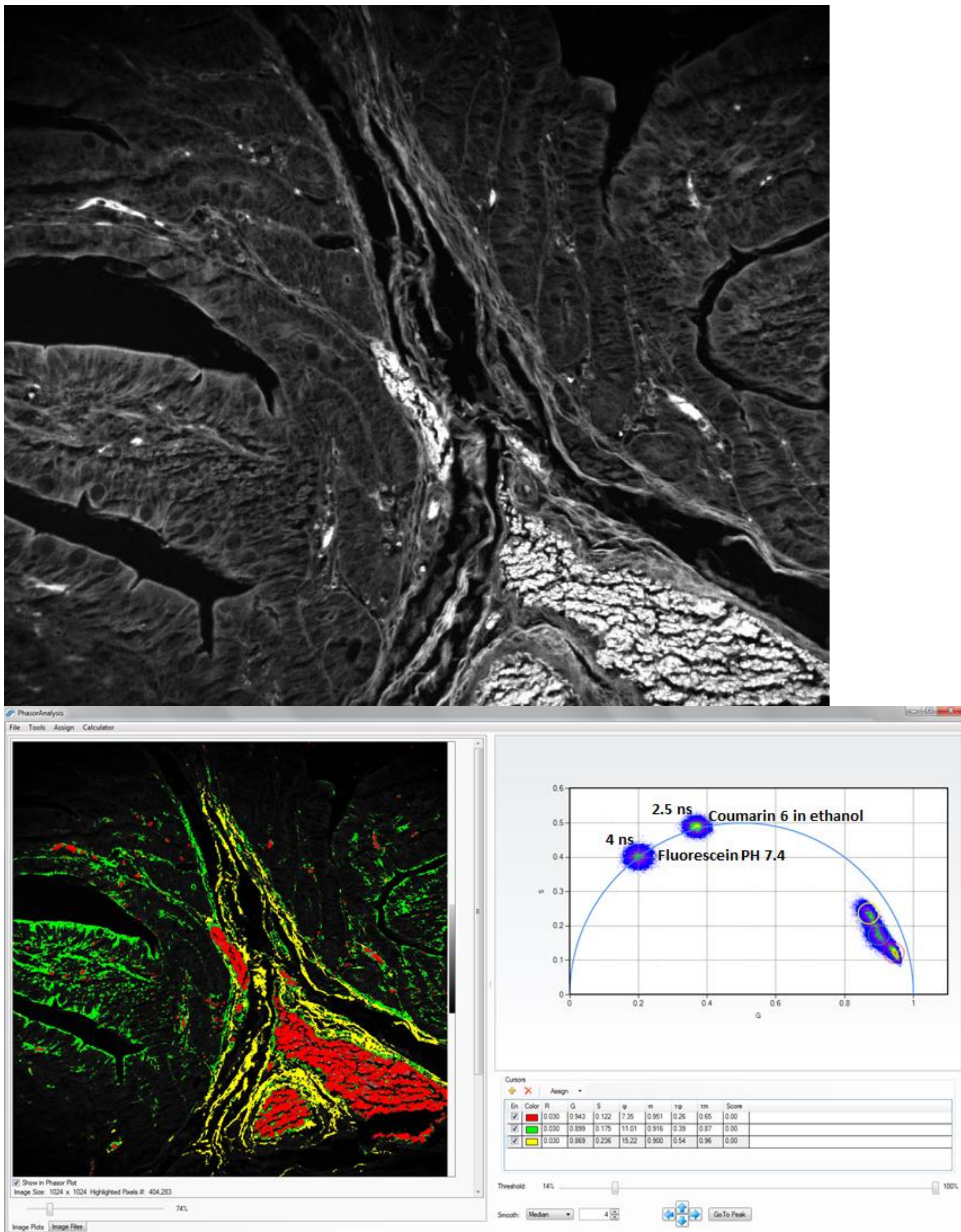
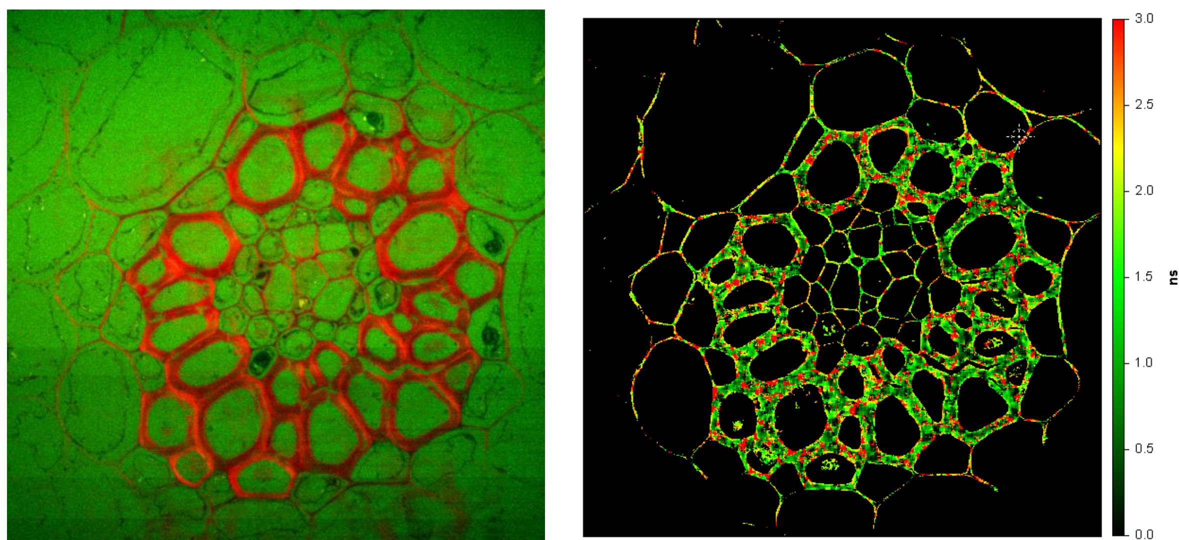


Figure 7. 2p fluorescence lifetime imaging of the HE stained pig intestine tissue. Top: intensity image. Bottom: Segmentation of the tissue based on the lifetime distribution on the phasor plot. The phasor plot reports the decay times of the fluorophores in solution on the circle (single exponentials) and the decay times measured in the cell. Laser excitation: 780 nm, 80 MHz repetition rate. Emission: 500 – 650 nm. Objective: Olympus 20X / 0.54NA. Image Size: 1024x1024 (200 µm x 200 µm).

Fluorescence Lifetime Imaging - TCSPC



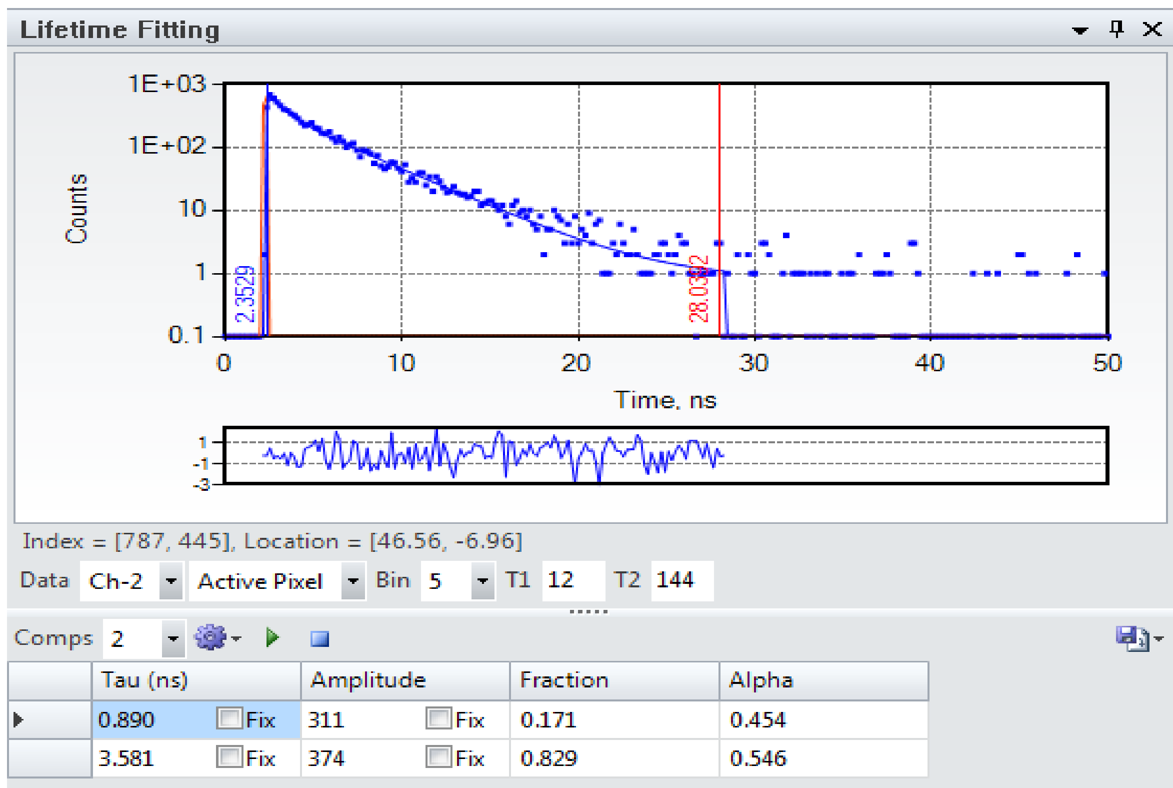


Figure 8: The sample is Convallaria (Lily of the valley). Top: intensity and lifetime images; Bottom: analysis of the data at one ROI - Two decay times are measured (890 ps (17%) and 3.6 ns (83%). The laser excitation wavelength is 488 nm from a supercontinuum laser (20 MHz repetition rate, 6-ps pulse width). The emission on Ch1 is acquired through a 600/37 nm filter; on Ch2 the emission is acquired through a 525/50nm filter. The objective is Olympus 60X (water immersion; NA = 1.35).

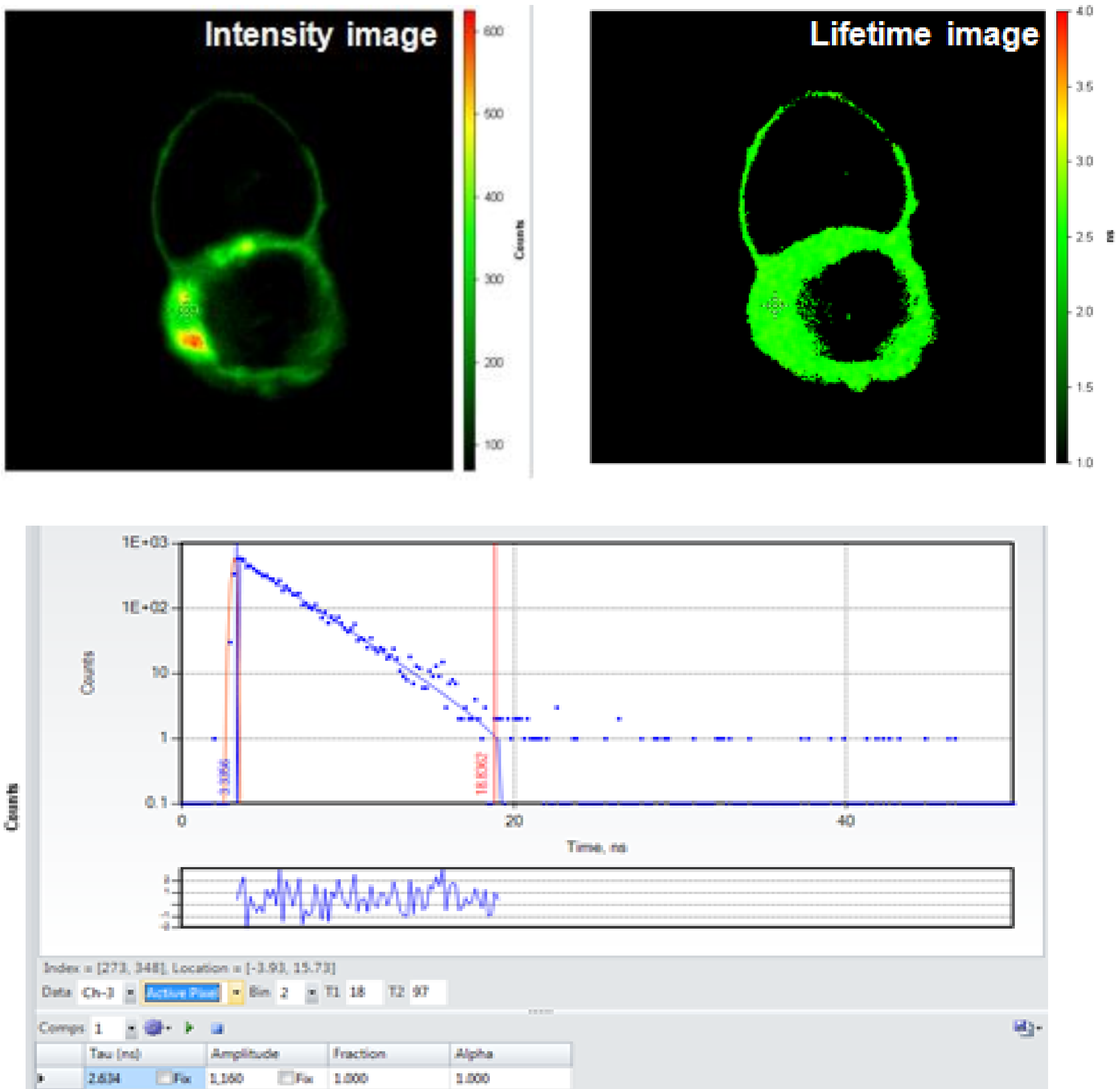


Figure 9: The fluorescence lifetime of GFP expressed in live cell membrane was measured by confocal TCSPC FLIM. A single decay time (2.6 ns) is determined.

FLIM-FRET: Efficiency Measurements are Easy using the Phasor Plots

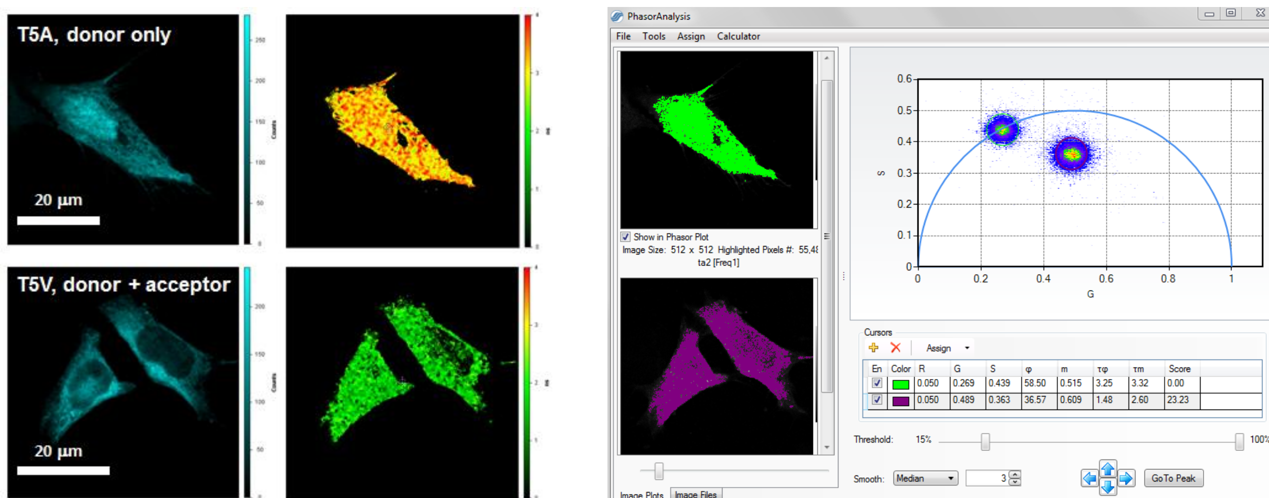


Figure 10: 2-photon FLIM measurements of cells expressing mTurquoise-5aa-Amber (T5A, donor-alone control) and mTurquoise-5aa-Venus (T5V, FRET standard) and the phasor plots analysis.Laser excitation: 780nm, 80MHz repetition rate; Emission: donor channel: 475/35nm. Objective: Olympus 60X / 1.35NA.

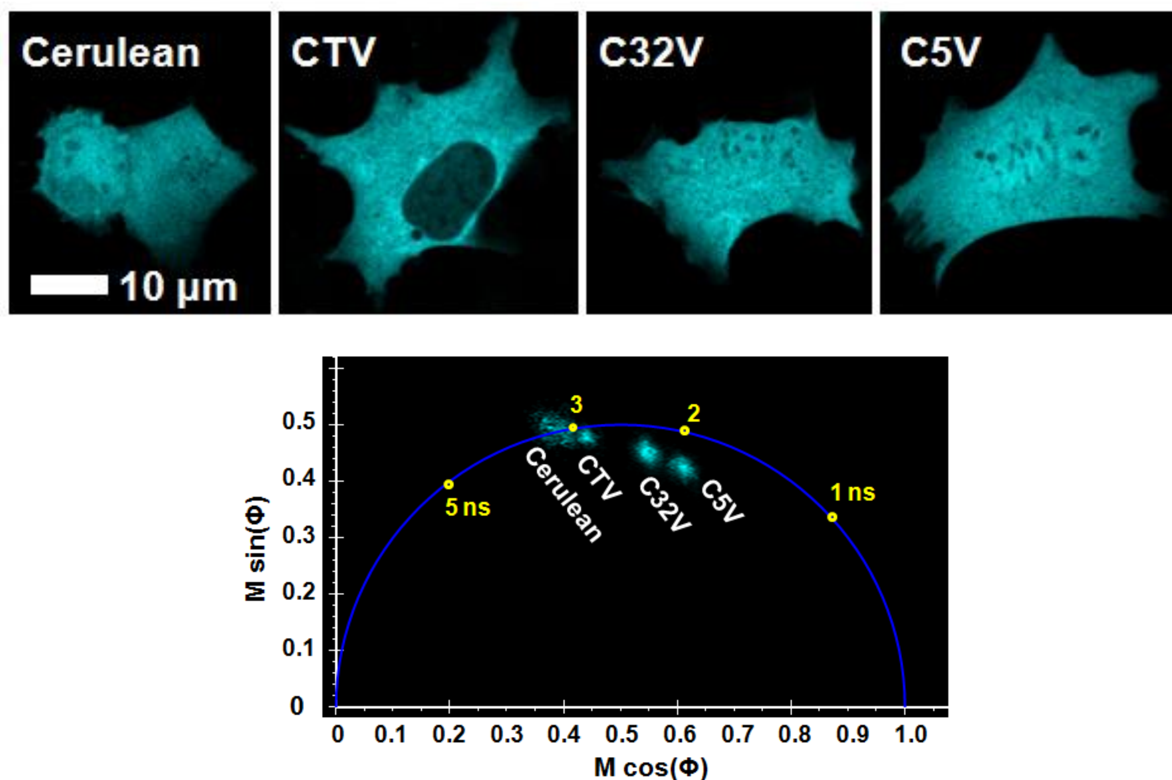


Figure 11: Measuring a series of FRET-standard constructs expressed in live cells. The phasor plot reports the measurements; the yellow dots indicate the location of single exponential decays, respectively 1, 2, 3 and 5 ns.

Fluorescence Fluctuation Spectroscopy (FFS)

FFS is utilized to measure translational and rotational diffusion coefficients, kinetic rate constants, molecular aggregation, polydispersity, and molecular weights. Measurements can be acquired in solutions or in living cells. In a cellular environment, the technique allows for the measurements of molecular dynamics parameters in different compartments of a cell (cytoplasm, nucleus, membrane). A variety of application benefits from the measurements of molecular dynamics parameters:

- *Kinetics rate constants*
- *Antibody-antigen interactions*
- *Receptor-Ligand Interactions*
- *DNA/Protein Hybridization*
- *Nucleic Acid/Nucleic Acid Interactions*
- *Enzymes Activity*
- *Protein-protein interactions*
- *Molecular aggregation, polydispersity, and molecular weights*
- *Properties of viruses*

FFS comprises a whole family of application tools that reveal the inner molecular dynamics upon the detection of fluctuations of molecules due to thermal motion. They include

- FCS, Fluorescence correlation spectroscopy
- FCCS Fluorescence cross-correlation spectroscopy
- PCH, photon counting histogram

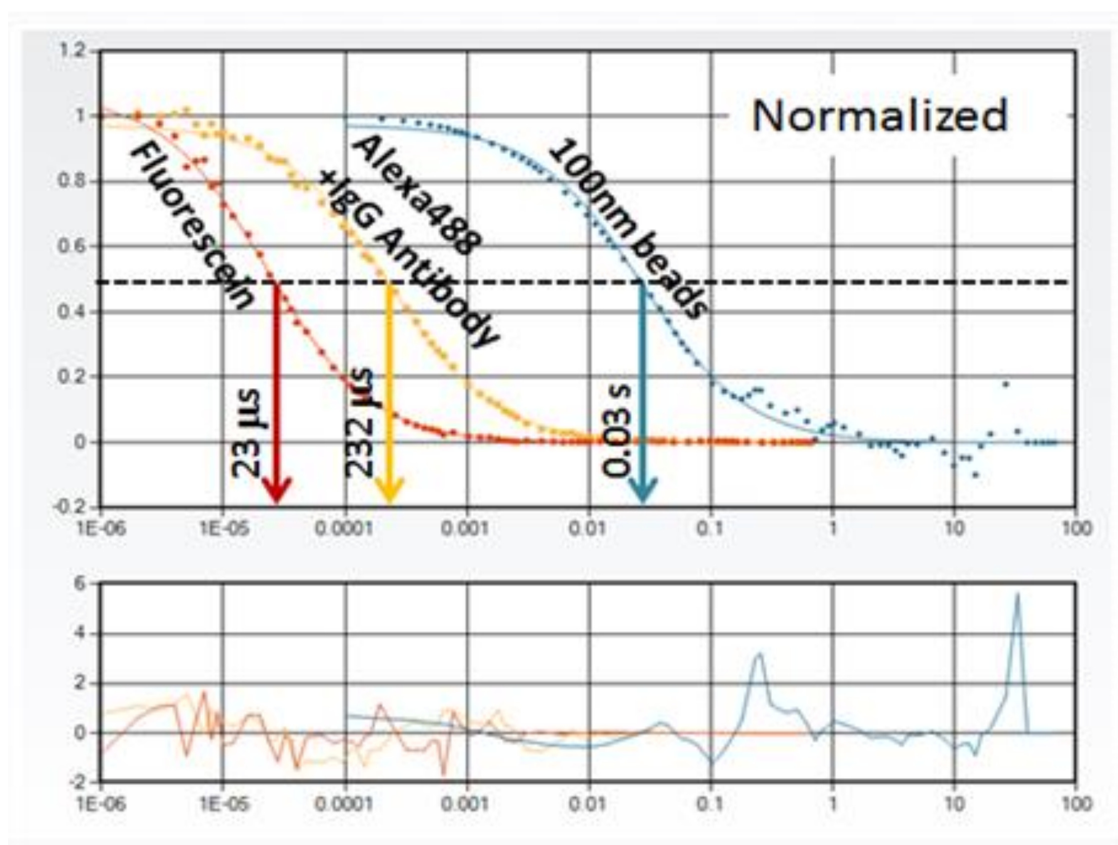


Figure 12. Measuring mobility with FCS

2p FCS

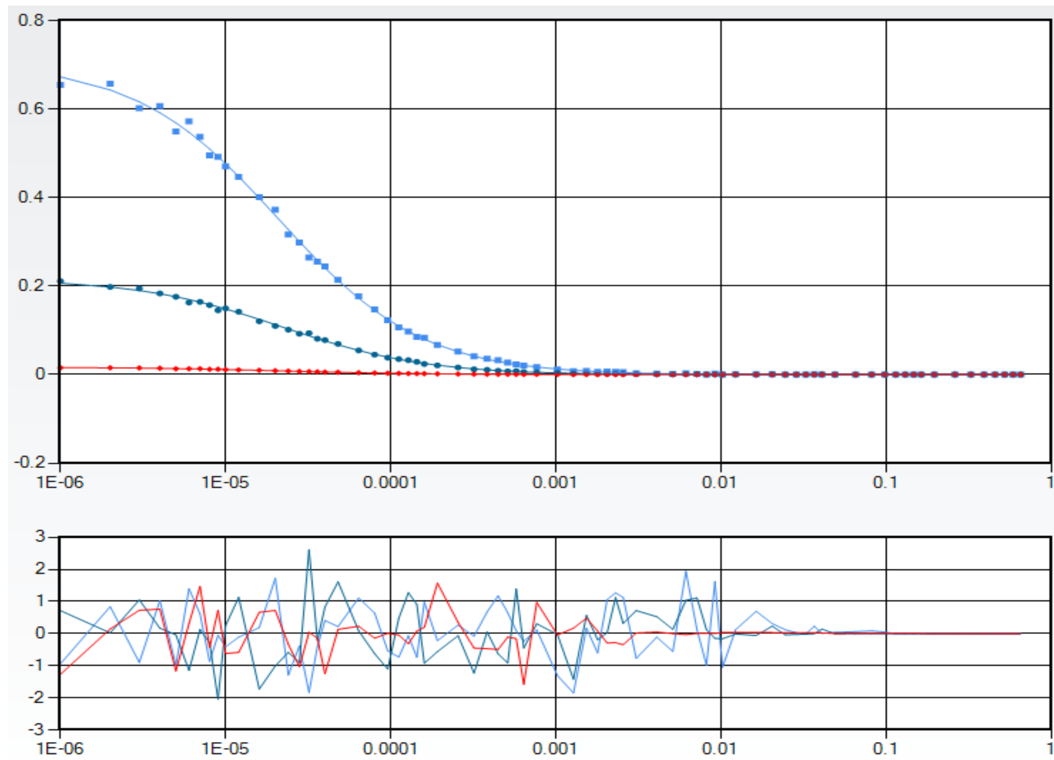


Figure 13. Fluorescein in HPLC water pH 7.4

1p FCS

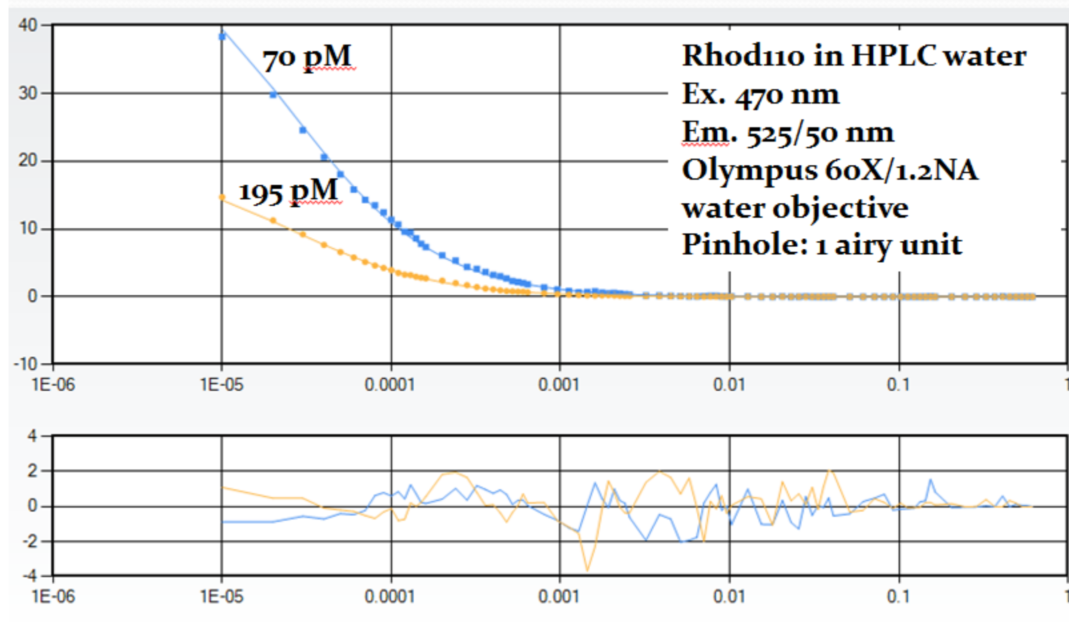


Figure 14. FCS at the single molecule level

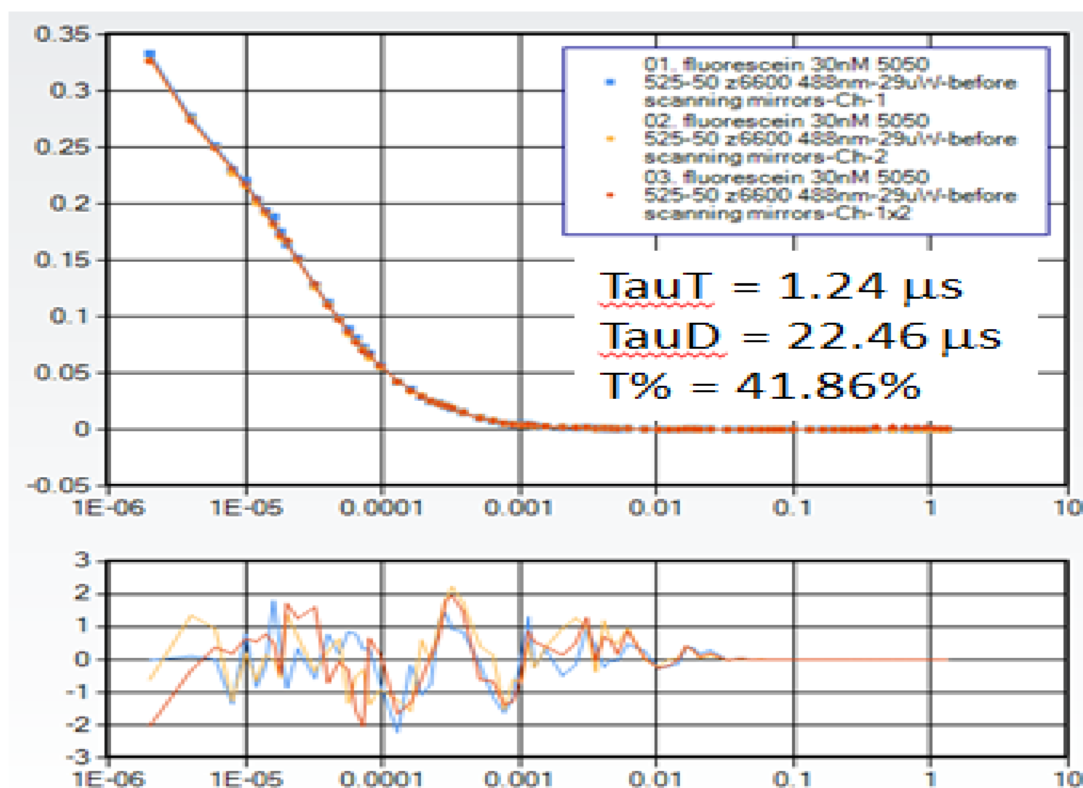


Figure 15. Measuring the triplet state with FCS

FCCS with Pulsed Interleaved Excitation (PIE)

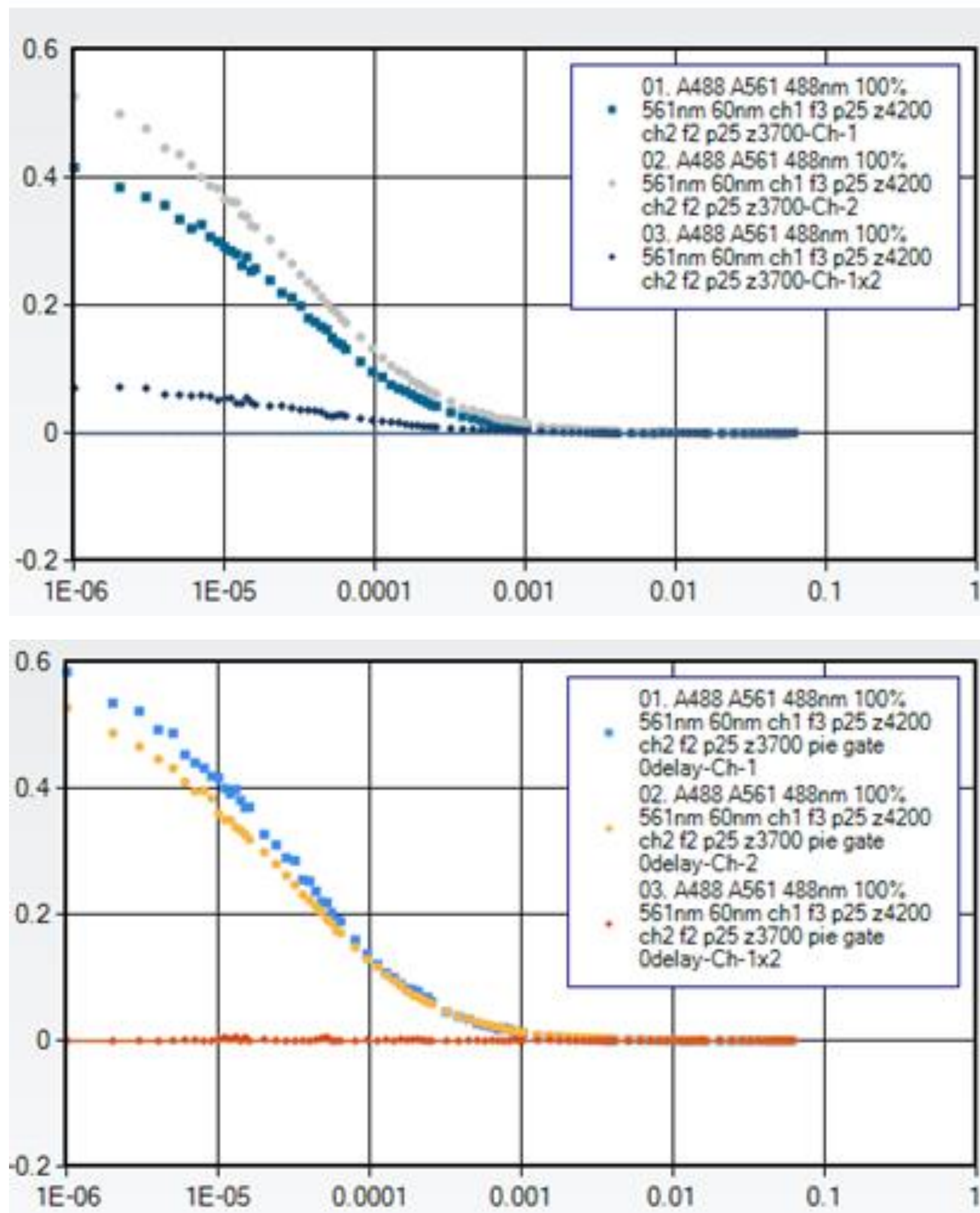


Figure 16. PIE effectively removes the false correlation due to the bleedthrough

Scanning FCS

The imaging counterparts of the FFS measurements include:

- RICS, raster image correlation spectroscopy
- N&B, number and brightness
- Scanning FCS

All of these measurements modalities are feasible with the Q2 and provide the researcher with an unparalled amount of information about the dynamic cellular environment.

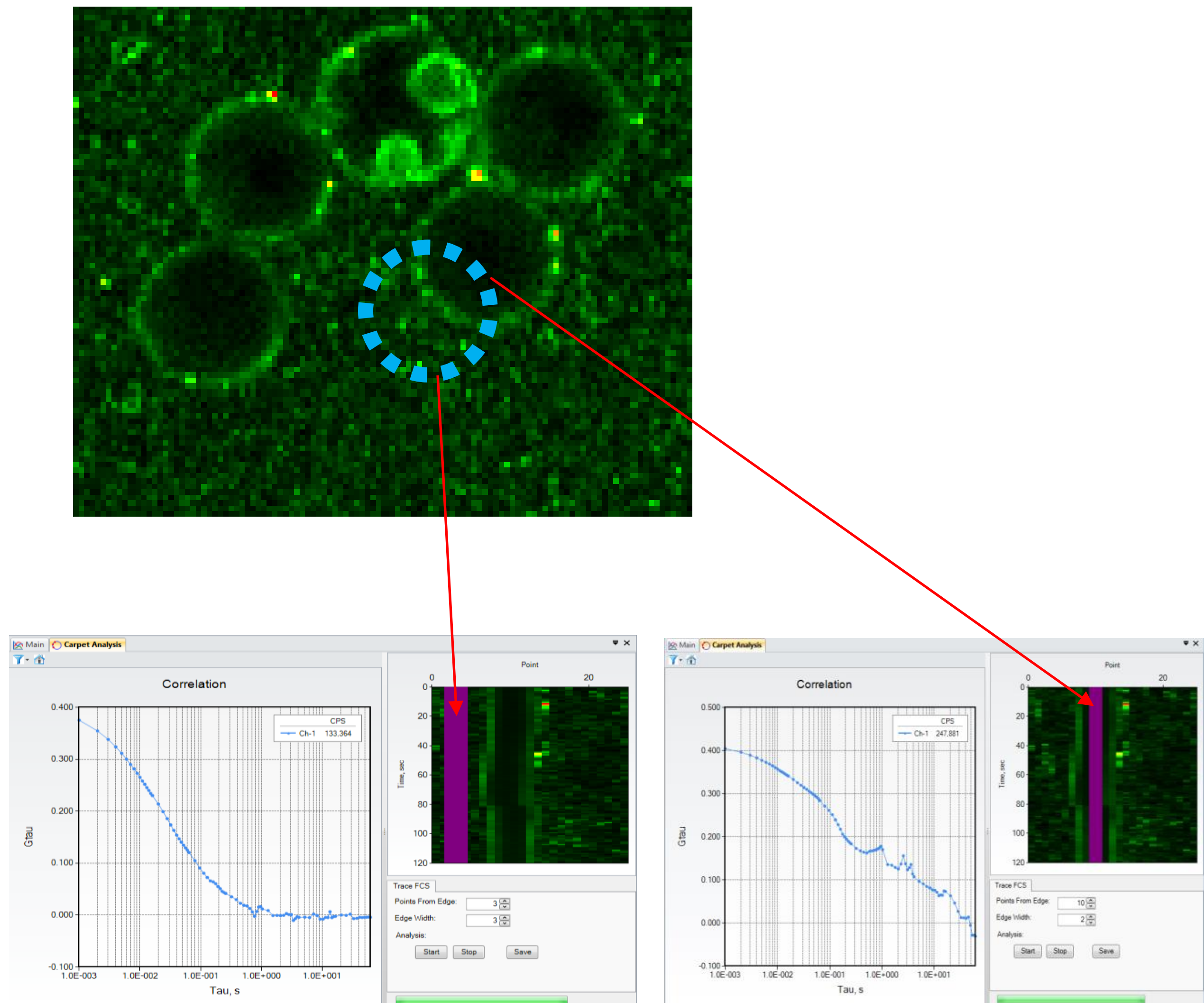


Figure 17. The solution contains beads of 10µm-diameter and 100µm-diameter. The orbit interjects one bead of 10µm. Data are acquired every 5 degrees and the carpet analysis provides the diffusion coefficients for the two samples.

Polarization Anisotropy images

For anisotropy measurements, a beam-splitter polarizer is installed in D3; the images collected by channel 1 and channel 2 are polarized in the (V)ertical and (H)orizontal plane, respectively. Either the polarization or anisotropy image can be reconstructed by the software upon introducing the proper corrections due to the NA of the objective.

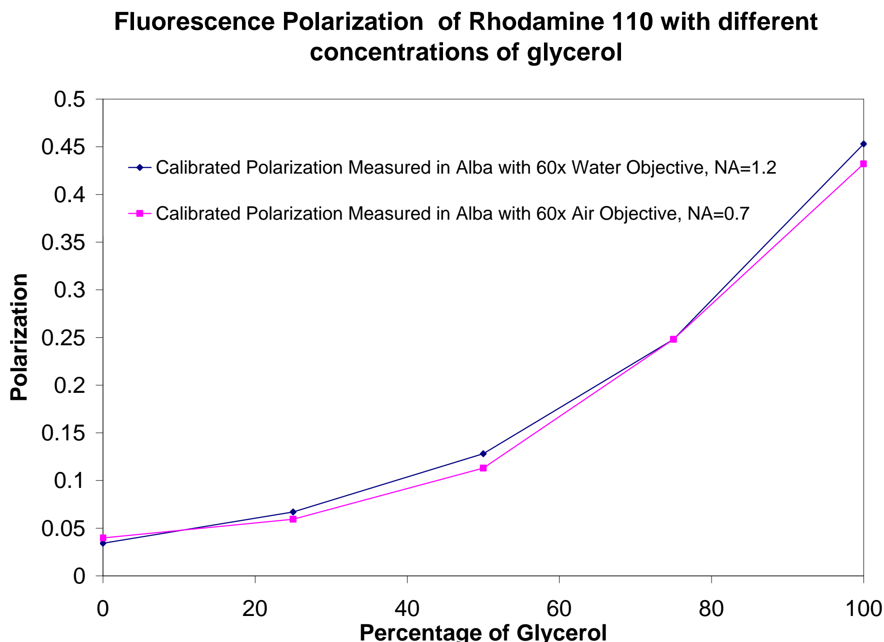


Figure 18. Data of Table I and II above. Data have been calibrated using the routine implemented in the Vista software. The polarization values obtained with the two objectives are similar.

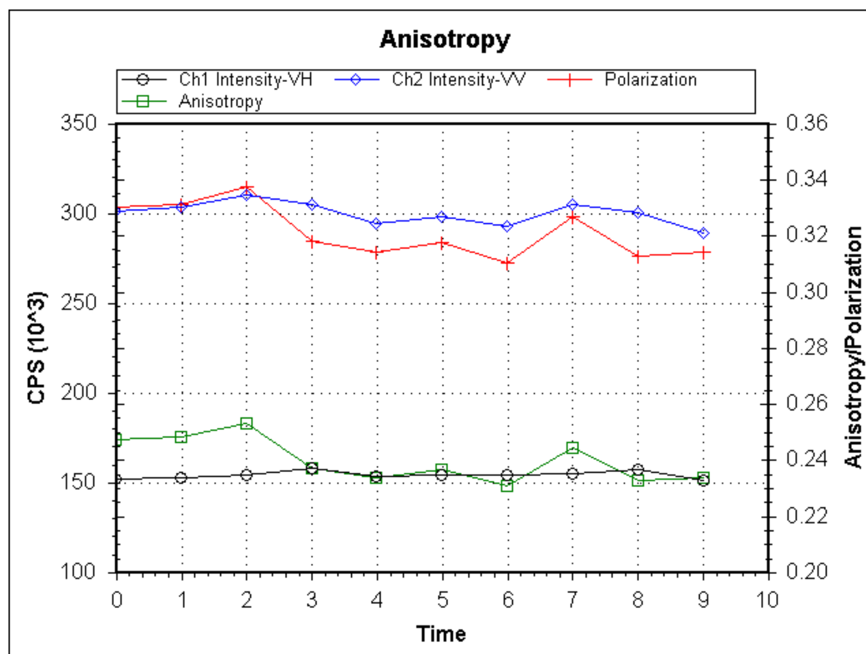


Figure 19. Anisotropy measurements of a solution of 30nM Alexa 633 dispersed in blood plasma.

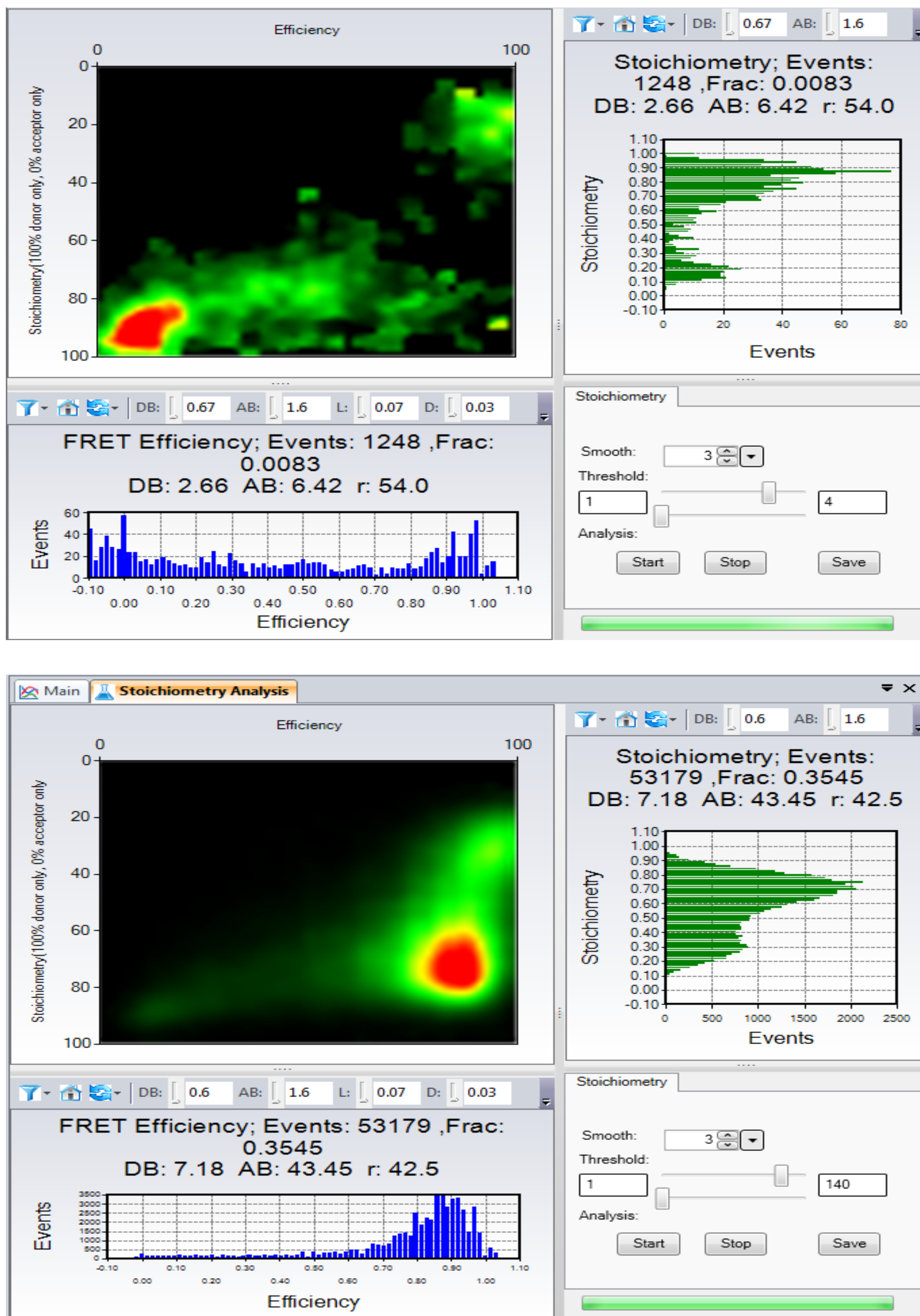


Figure 20. α-synuclein folding and conformational switching at different environment: (Top) buffer only, (bottom) with SDS. In (bottom) we can see the events happen mostly in high FRET efficiency, when the acceptor is more abundant.

Technical Specifications

Hardware

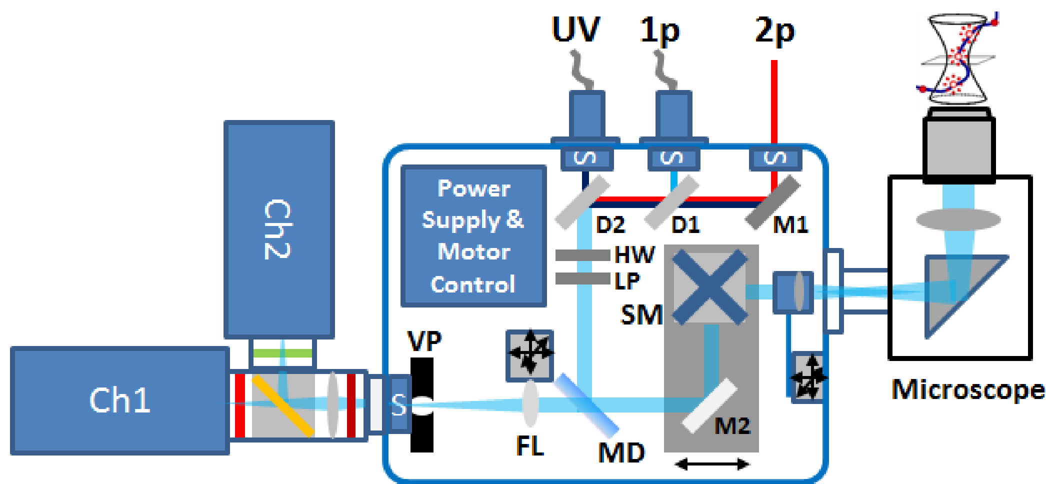


Figure 21. Schematics of a Q2 system with two detections channels.

Q2 Specifications	
Dimensions (H x L x W)	***5.1" (130mm) x 16.8" (427mm) x 13.2" (335mm)***
Weight	***40Kg***
Laser entry ports	***3, designed to couple UV-VIS-NIR lasers in free space or via single mode fiber coupling***
Detection unit coupling	***MiniTDU, coupled by a rigid connector Motorized TDU, coupled via a multimode fiber***
Mechanical shutters	***Motorized and installed at the detection port and optionally at each laser entry port***
Galvo scanning module	***Protected silver coating for both XY mirrors Clear optical surface: 3mm or 5mm versions Maximum scan rate: 5KHz for 3mm and 1KHz for 5mm***
Variable pinhole	***Motorized and tunable from 20 μm to 1 mm***
Internal magnification	***2***
Power requirements	***100 – 240 V, 50-60Hz, 1A @ 110V input***
Computer connection	***USB 2.0 (only for motor controls)***

Software

General Features		
	Operating system	Windows7 or 10 professional, 64-bit
	Computer (minimum specifications)	64-bit computer workstation with 8GB Ram 1- or 2-monitors operations

Measurements Modules		
	FFS module	• Fluorescence Correlation Spectroscopy (single channel and cross-correlation) - Data are acquired in photon counts mode, photon time-tag mode, or photon time-tag time-resolved (TTTR) mode • Photon Counting Histogram (PCH) (ISS Patent)
	Confocal Imaging module	• Confocal images • Fluorescence Lifetime Images (FLIM)
	Polarization module	Polarization measurements
	Measurements requiring Imaging and FFS modules	• Scanning FCS • Raster Imaging Correlation Spectroscopy (RICS) • N&B

Confocal Imaging Module		
	FLIM modality	Frequency-domain Time-domain
	FLIM time-resolution	sub 100 ps – sub ms
	Raster Scan	Resolution: up to 1.5 nm Pixels number: user selectable from 2 to 8192 Max line frequency: 5 KHz (on 20 points) Min line frequency: 0.01 Hz Max frame rate 512x512: 3 sec Max frame rate 512x16: 25 Hz Beam park & Panning
	Scan Modes	X, Y, Z, t, and their combinations
	Image Formats	Export to: ImageJ, MetaMorph Plots can be saved and exported to: GIF, TIFF, JPEG, PNG, Bitmap and Metafile formats
	2D visualization and operations	Rotation Histogram based colocalization Zooming Scaling Arithmetic Smoothing

Fluorescence Fluctuations Spectroscopy (FFS) Module		
	Parameters determined by the FFS software module	• When using autocorrelation and cross-correlation functions: One or two species using: Diffusion coefficient Diffusion time Concentration Triplet state decay time constant Triplet function Flow rate Size of excitation volume Number of molecules • When using photon counting histogram (PCH): One or two species using: Number of molecules Molecular brightness
	Number of channels acquired simultaneously	Two
	Modeling of laser beam PSF	Single photon Multi-photon
	Statistical functions utilized for data analysis	Autocorrelation function (FCS) Cross-correlation Photon Counting Histogram (PCH)
	Single set and Global fitting models available in the FCS software	• When using autocorrelation and cross-correlation functions: One or two species, with 1- or 2-photon excitation, using: 2D- or 3D-Gaussian PSF 2D- or 3D-Gaussian PSF triplet state 3D-Gaussian-Lorentzian PSF presence of flow Input of user-defined equation • When using photon counting histogram (PCH): One or two species, with 1- or 2-photon excitation, using: 2D- or 3D-Uniform 3D-Gaussian-Lorentzian PSF • Input of user-defined equation
	Minimization routine	Marquardt-Levenberg algorithm
	Scanning FCS	User defined area

Focus and Discovery

For more information please call (217) 359-8681 or visit our website at **www.iss.com**

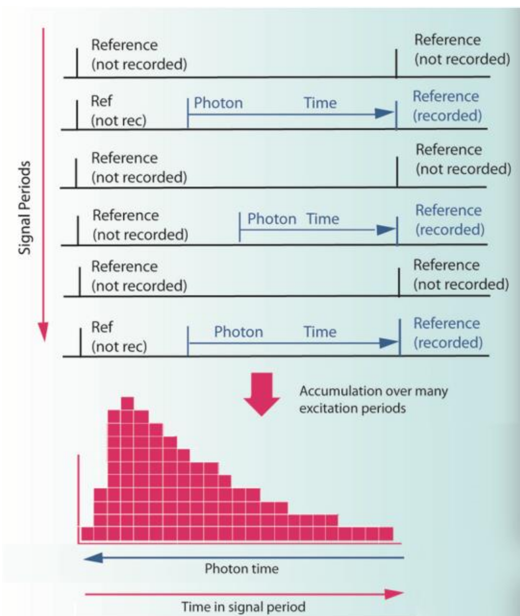
1602 Newton Drive
Champaign, Illinois 61822 USA
Telephone: (217) 359-8681
Telefax: (217) 359-7879
Email: iss@iss.com

Copyright ©2015 ISS, Inc. All Rights Reserved.

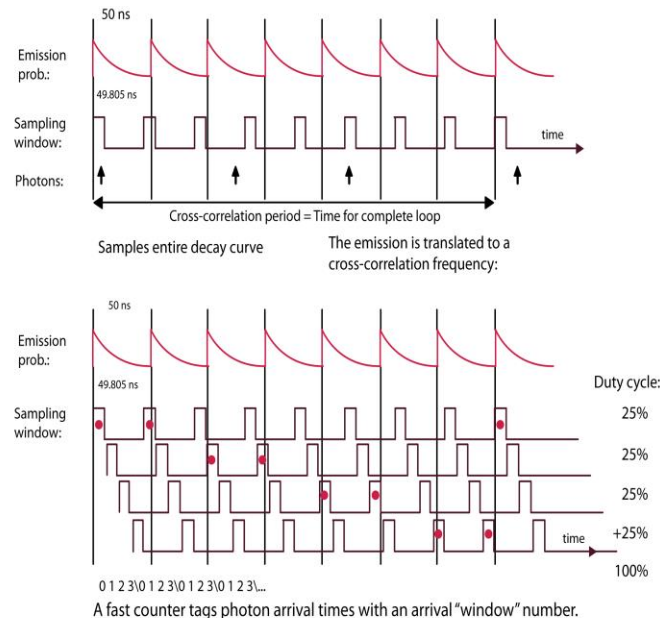
Note 1: Time-domain or Frequency-domain?

Time-domain data are mostly collected using the time-correlated single photon counting (TCSPC). A pulsed laser is used for the measurement. When a fluorescence photon is recorded by the detector, the instrument measures the time between its arrival and the arrival of a subsequent pulse from the laser; the process is repeated for several thousand photons until a histogram of the arrival time of single photons is built. The histogram is fitted using a decay model that provides the decay times of the fluorophores.

In digital frequency-domain the photons emitted by the fluorophore upon excitation are collected into bins that separate them by the respective phase shifts. The bins are generated by a sampling window that restart after a time given by the inverse of the cross-correlation of the arriving photons are measured and a histogram is built; the decay time is determined by processing these phase shift. Typically frequency-domain data acquisition is faster than time-domain because of the reduction or lack of dead time in the acquisition electronics.



TCSPC



DFD

A unique feature of the ISS FLIM/FFS instrument is that it can provide both DFD and TCSPC solutions on the same instrument and driver by the same software – VistaVision 64-bit.

FastFLIM exclusively by ISS is a recent development based on the DFD technique; when compared to other established FLIM techniques, it has two main advantages:

- The device is easily programmable to fit into different applications, such as measuring lifetimes from sub nanoseconds to microseconds; ISS made this simple and straightforward for users by developing many firmware for various applications.
- This digital device has no dead time, resulting in high photon collection efficiency and in turns the short time required for data acquisition.

Becker & Hickl SPC-150 card is a classical state-of-the-art electronical design for the highest time resolution in the TCSPC applications, but the electronics suffer from the long dead time, and thus limiting its data collection efficiency.

Both techniques have their plus and minus points; a brief comparison on several key points are given in the table below.

Data Acquisition Unit	DFD FLIM by ISS FastFLIM unit	TCSPC FLIM by BH SPC-150 card
Method and Measurements	Frequency domain: phase delay and modulation ratio	Time domain: decay histogram
Input Channels	4 channels simultaneously	1 channel on the card, compatible with 4 channels when using a router device
Time Resolution	NA	Minimal 813 fs / time bin FWHM 7ps, RMS 3ps
Dead time	10 ns (due to the CFD used to convert the PMT output to the TTL input given to the FPGA)	100 ~ 550 ns depending upon the TAC range (mostly due to the TAC circuit)
Count rate	> 10 million counts per second (CPS) at each channel	Typically < 1 million CPS at the SPC card input to avoid the pile-up effect for the 80MHz laser repetition rate
Data analysis	Non-linear least square fitting Phasor plots on the fly	Non-linear least square fitting Phasor plots after Fourier transform
Computer Interface	USB 2.0	PCI