DESCRIPTION

Alba v5

Laser Scanning Microscope

The instrument for quantitative cell biology at single-molecule detection

Alba is a laser scanning microscope that incorporates several measurement modalities for experimental quantitative biology and material sciences applications requiring the single molecule detection sensitivity.

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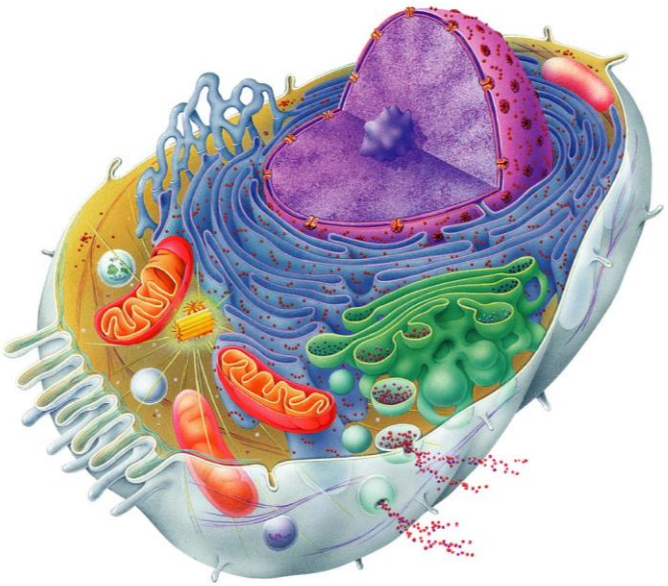
Why Alba?

The revolution in quantitative cell biology is here. In the past few years the application of functional genomics, proteomics and metabolomics to the single cell system has allowed the analysis of collectives of molecules and the structures they form within the single cell.
Alba is the laser scanning confocal microscope that incorporates several of the tools required by quantitative cell biology to identify and clarify the molecular dynamics processes and molecular interactions within the cell at the single molecule level sensitivity.



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The Alba design

Flexibility

Alba unique design is based upon an open architecture allowing for the researcher to accommodate any research requirements that may arise: at any time, the user can replace or mount filters and dichroic on the various automated filter wheels without any direct intervention of the factory; and at any time, lasers can be added when the research projects require new wavelengths.

Alba can start with the basic configuration and grow with the researcher and with the changing requirements of the research laboratory:

- The basic unit features a 2-channel acquisition that can be upgraded to a 4-channel unit.
- An additional external port is available on the unit. The port is ready for mounting a camera for spectral FLIM images.
- Both single-photon and multi-photon lasers can be coupled simultaneously to the instrument and their operations controlled through the VistaVision software.
- Each channel can be dedicated to a measurement modality (FFS, FLIM, spectra acquisition) by optimizing the choice of the detectors.

Innovation

Alba makes use of 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, Alba 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). The innovative design of Alba encompasses several areas:

1. Using a pinhole on each acquisition channel for precise FCS measurements.

2. The movement of the acquisition channels along the optical axis for allowing the positioning of each individual pinhole on the image plane of a selected wavelength range.

3. The use of the dichroic for maximizing the sensitivity, where the fluorescence if reflected while the excitation light is transmitted.

4. The choice of the light detectors for the optimal acquisition of a specific measurement.

5. The integration of several measurement modalities in the same unit.

Sensitivity

The simple optical design and the minimization of the number of optical surfaces provide the researcher an instrument with the sensitivity of single-molecule acquisition. FCS data have been acquired on a 15pM solution of Rhodamine 110 [see *Zeno Földes-Papp et al.; Current Pharmaceutical Biotechnology 11 (2010) 639-653*].

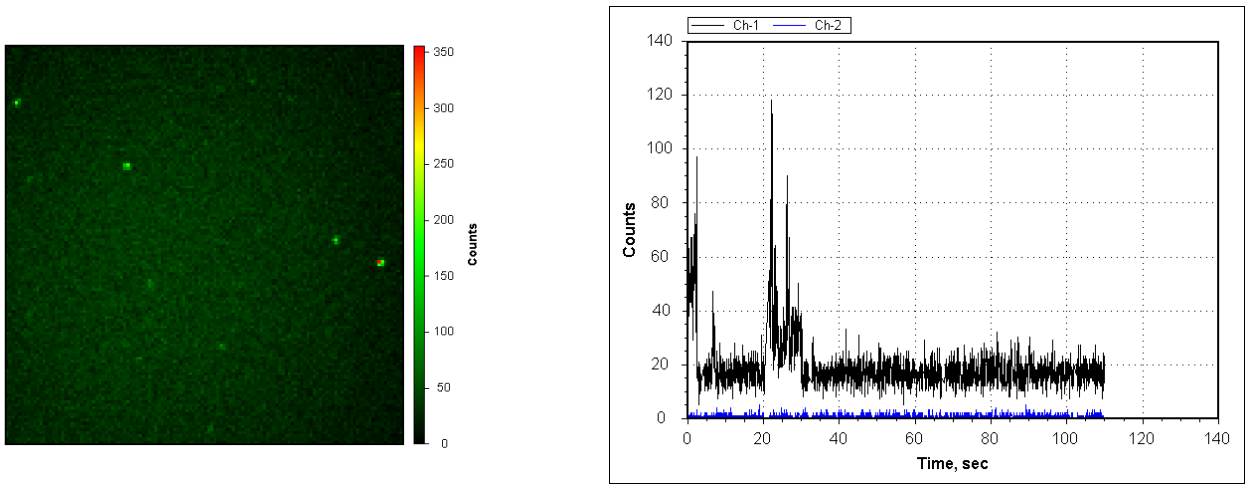
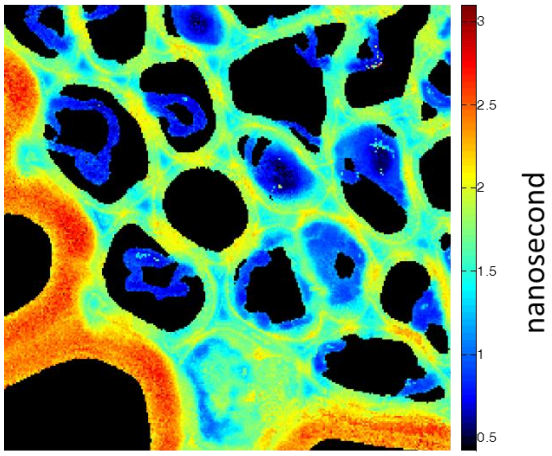
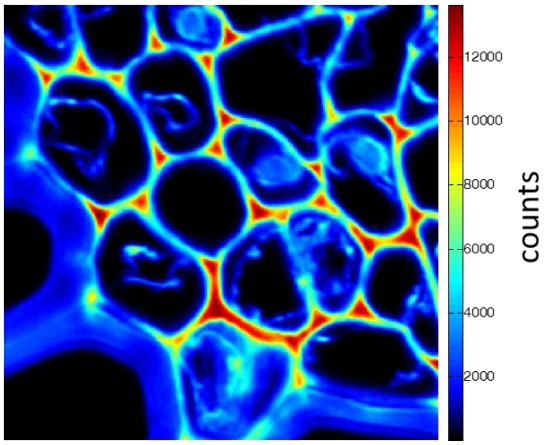


Figure 1. Detection of single Cy5 molecules (left) and their photobleaching response (right).



Lifetime image



Fluorescence intensity image

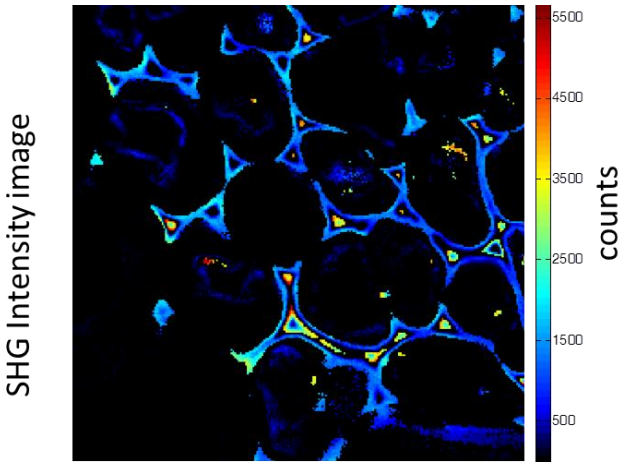


Figure 2. FLIM images of a cross section of the rhizome of Convallaria (Lily of the Valley). The image data was taken using a multiphoton fluorescence confocal microscope with the two-photon excitation at 800 nm of the pulsed laser beam. One is the fluorescence intensity image and the other is the fluorescence lifetime image, both which has 256×256 pixels (40×40 square microns).
(*courtesy of Dr. Zhang, Beckman Institute, Urbana, IL*)

Alba: Optical and Mechanical Components

A schematic of the optical layout of the instrument is shown in Figure 3.

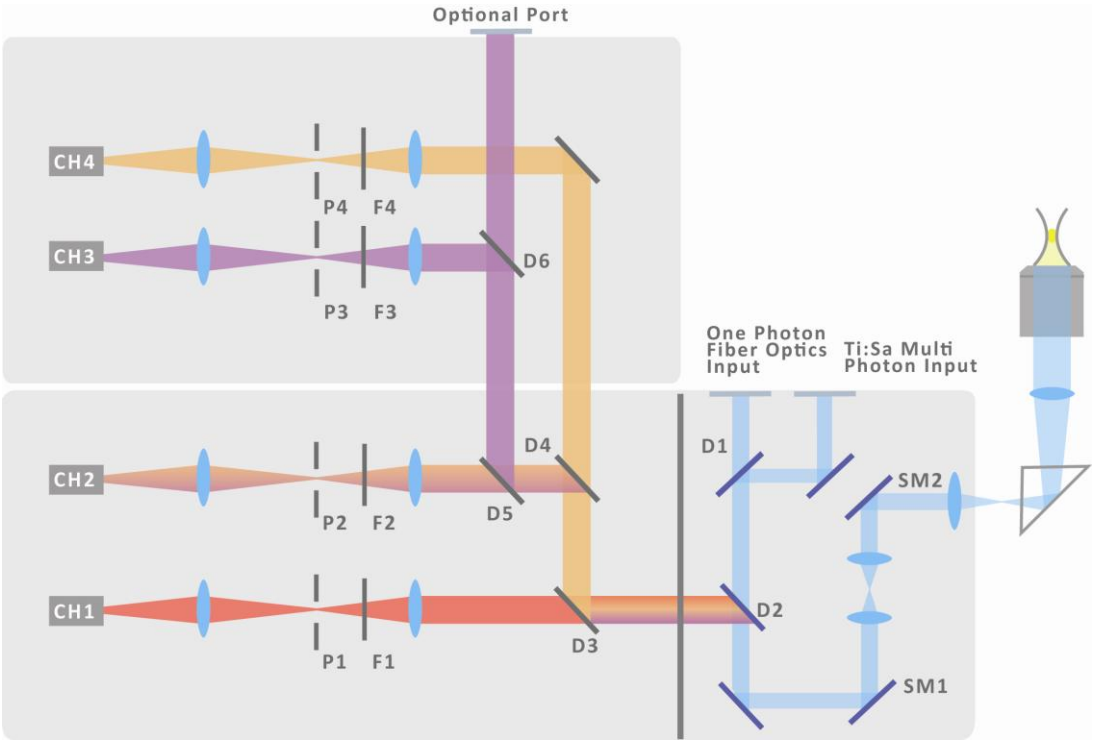


Figure 3. Schematics of the 4-channel unit. A camera for spectral FLIM can be mounted on the AUX port.

Single photon and multiphoton lasers beams enter in different ports and their optical paths superimpose after the dichroic D1. The beam travels through the dichroic D2 and is reflected by the corner mirror. The fast scanning mirrors SM1 and SM2 generate the raster scan; after the second mirror the beam goes through the descanning lens and enters into the microscope. The fluorescence signal follows the same travel path: from the microscope, it goes through the descanning lens, the scanning mirrors, the corner mirror and the dichroic D2, where it is reflected into the detection unit. In the detection unit the dichroics D3-D6 separate the wavelength ranges to be detected by the four acquisition channels. Each acquisition channel comprises a filterwheel F and a pinhole P; each detector mounts an automated shutter. The Auxiliary Port is used to connect a camera for spectral FLIM images acquisition.

Microscope

The Alba is a confocal detection unit that can be utilized with most commercial epifluorescence 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.

Figure 4. Alba coupled to an inverted microscope (Model Ti by Nikon)

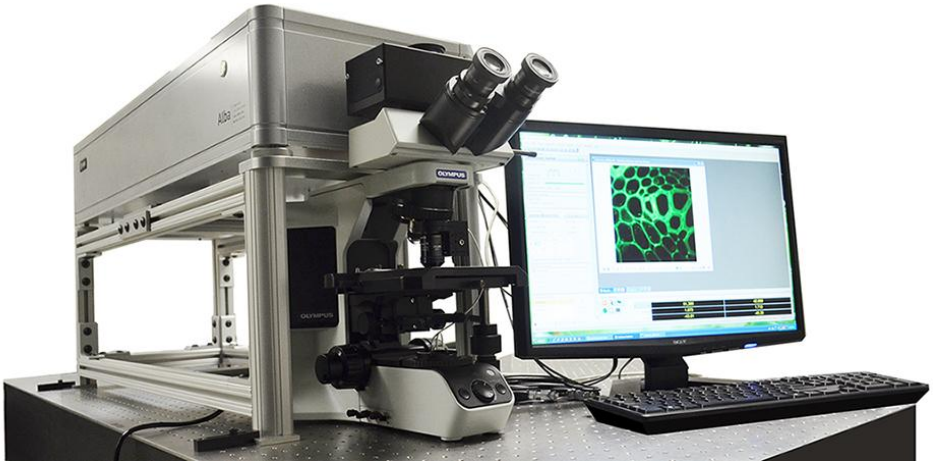


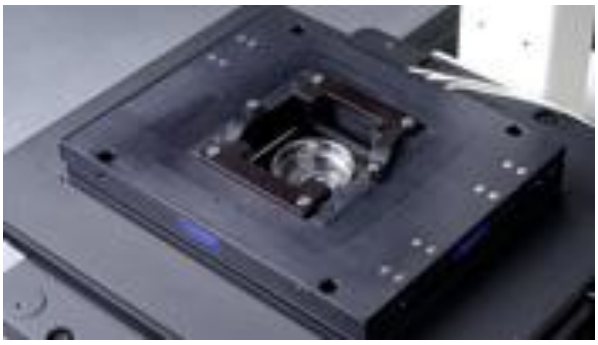
Figure 5. Alba coupled to an upright microscope (Model BX53 by Olympus)

Imaging modalities

Two options are available for image acquisition using Alba. The user can select one of the two, or they can be implemented at the same time on the instrument.

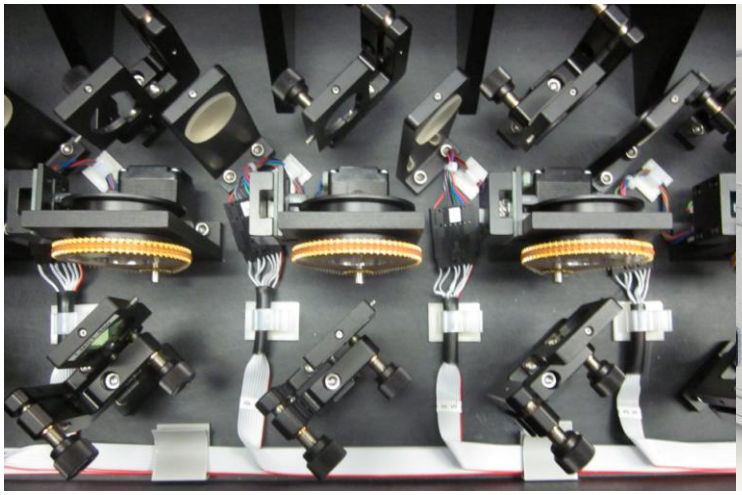
- **Using galvo-controlled mirrors:** the laser beam is scanned over the sample following a predetermined pattern (laser scanning microscope). This is achieved by using galvo-controlled mirrors that scan the beam on the XY-plane a surface area of about 200 µm in diameter with no optical distortions in the image. Image acquisition is fast (up to 4 µs dwell time per pixel). The z-axis change is achieved by mounting the objective on a piezo-stage, or using a stage with z-axis control.
  Galvo-controlled scanning mirrors offer the best solution when fast imaging acquisition is required. This option is utilized for fast imaging acquisition, fast scanning FCS, particle tracking and RICS acquisition.

- **Using a XYZ piezo-controlled stage.** When using this option the beam is set at a position while the sample is moved over the beam (stage moving microscope). The XYZ PZT is an actuated linear nanopositioning stage of exceptional resolution and stability. With its large distance of travel and high stability, the PZT is ideal for the most challenging microscopy and positioning applications where acquisition speed is not a requirement.



Laser launchers

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. Laser beams are superimposed using dichroic and focused onto a single mode fiber that delivers the beam to the Alba unit.
The multiphoton laser is delivered to the Alba 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.
Upon entering the Alba, both beams are superimposed by a dichroic mirror and the user can select either one through the software.



The acquisition channels

The complete instrument features four acquisition channel and an additional auxiliary output channel (AUX port). Each acquisition channel comprises a filterwheel F, a pinhole P, the focusing lens and the light detector. Each channel is movable along the optical axis for positioning the pinhole in the image plane of a selected wavelength for precise fluorescence correlation spectroscopy measurements.
The AUX port accommodates a camera or a dispersive device for the acquisition of spectral information of the fluorescence.

The detectors

Three types of detectors are routinely utilized in Alba. Each acquisition channel can be fitted with a specific detector; or, detectors are used in pair; channels 1-2 and channels 3-4 mount different detectors.

application	**APD**	**GaAs PMT**	**Hybrid PMTs**
FCS, FCCS, PCH	●	●	●
FLIM	●	●	●
Scanning FCS, N&B, RICS	●	●	

The VistaVision software

VistaVision (Windows XP, Windows 7 and Windows 8) is a complete software package for instrument control, data acquisition, data processing and analysis. VistaVision enables control of the automated devices on all Alba instruments including shutters, filterwheels, XY stages and light detectors. A convenient signal monitor displays the signal intensity from each channel in real time, and it is utilized during instrument alignment. The software has been developed in modular components that can be flexibly configured when constructing a custom-built instrument that uses ISS modular components.

VistaVision Instrument Control module

Includes the routines for instrument control (automatic instrument alignment of pinholes and lens positions, shutter control, selection of the light detector gain/bias control, overload protection, etc.); control of the Imaging Devices (galvo-mirrors, piezo-controlled stages; stepper-motor controlled 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. FLIM images are acquired using either the frequency-domain (FastFLIM) technique or time-domain (TCSPC); both acquisition modalities can be implemented on the same instrument. 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. The software includes operations between images, smoothing, filtering, rotation, zooming, scaling and automatic threshold setting for image contrast enhancement. 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.

VistaVision Fluorescence Fluctuations Spectroscopy module

Includes routines for multi-channel (up to 4) 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, $G(\tau)$ - 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.

All of the measurements for quantitative biology in one integrated unit

Alba is capable of the following measurements:

Confocal images
FLIM spectra
FLIM TCSPC
FLIM digital frequency domain (FastFLIM)
FCS, FCCS, PCH (single point)
Scanning FCS
Number & Brightness (N&B)
RICS (raster imaging correlation spectroscopy)
Polarization images
Particle tracking and superresolution

Fluorescence Lifetime Imaging (FLIM) for FRET

Data Acquisition: Digital Frequency-domain or Time-domain (TCSPC)
Alba can acquire FLIM data using the digital frequency domain (DFD) or the time-domain (TCSPC, time correlated single photon counting): the user selects the modality of preference for the instrument, or may decide to have both on the same instrument.

When acquiring a FLIM image in DFD, the image includes the steady-state information as well. Also, if the dwell time is selected properly, the image includes the information for RICS analysis and N&B analysis. All the information in the same image!

When acquiring a FLIM image in TCSPC, the image includes at each pixel the FCS information as well (TTTR format). That is, at each pixel, the FCS curve can be determined in addition to the decay time curve.

Data Analysis: Fitting curves and Phasor Plots
Data analysis has been traditionally carried out at each pixel (or group of adjacent pixels) using the least-square minimization routine (Marquardt-Levenberg algorithm). This modality is available for both DFD and TCSPC data (Figure 7).

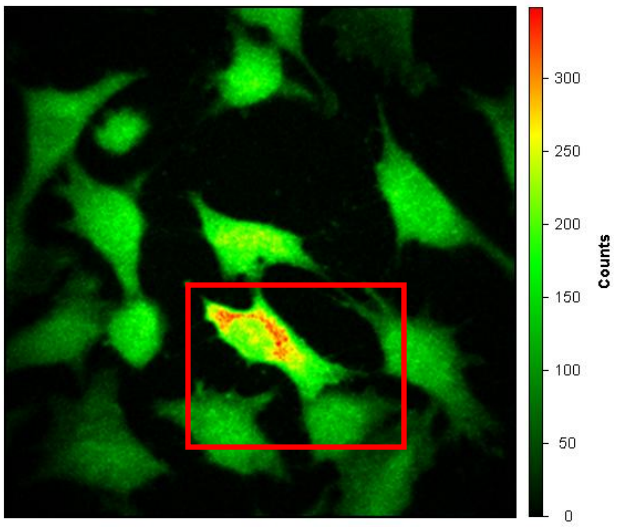


Figure 6. FLIM data were acquired for the cell in the red rectangle using FastFLIM (frequency-domain) and TCSPC (time-domain).

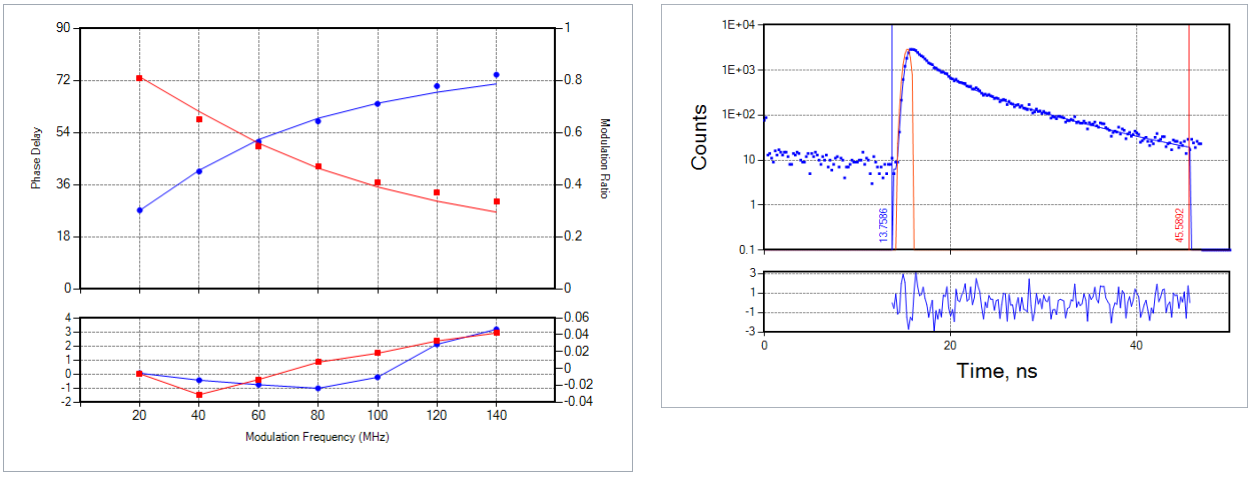
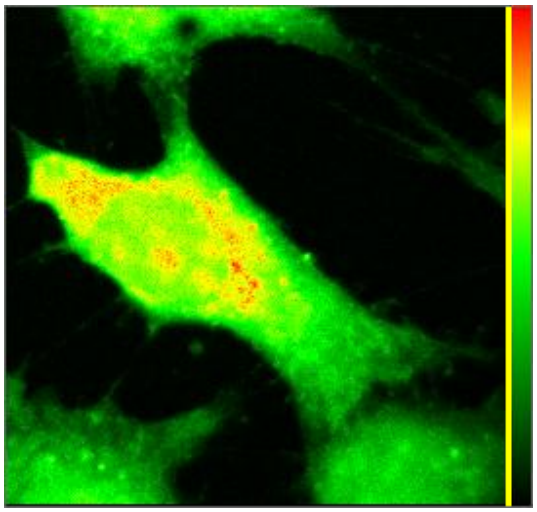


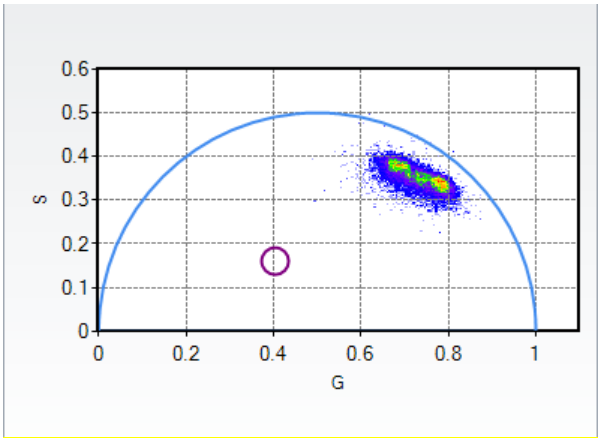
Figure 7. Fitting analysis of frequency-domain data (left) and time-domain data (right) for the cell of Figure 6. Two decay times were determined, 7.5 ns and 2.0 ns (with fractional contributions of 48% and 52% respectively).

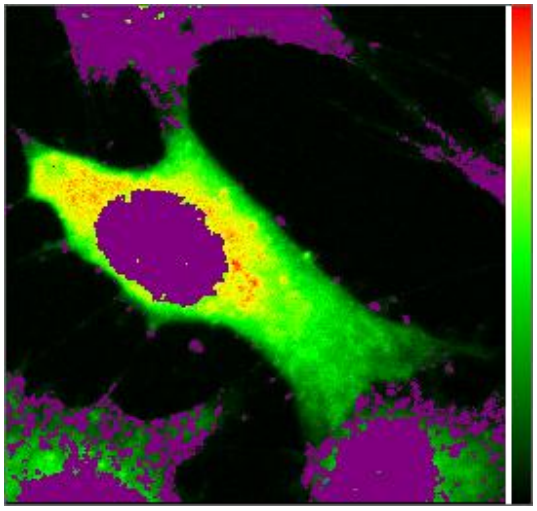
In addition, the VistaVision software allows for the FLIM analysis using the phasors plots, which apply to both time-domain and frequency-domain data files.

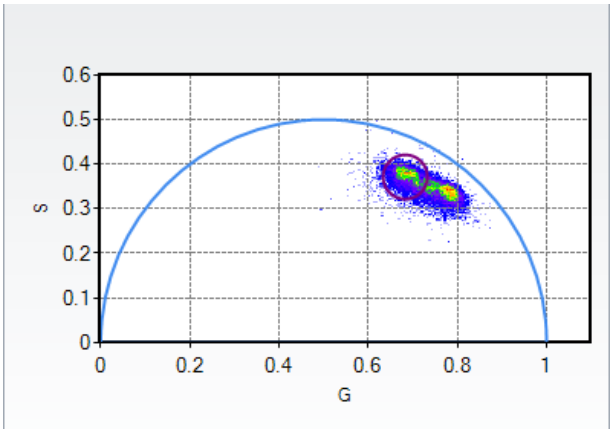
As a further example, we study the case of a cell where there are two exponential decays. In the image below, we select the cell framed by the red lines.

When applying the phasor plot analysis, one sees right away that more than one decay is present in the system. In the phasor plot the pixels are clustered along one line, featuring at least two major points of accumulation.









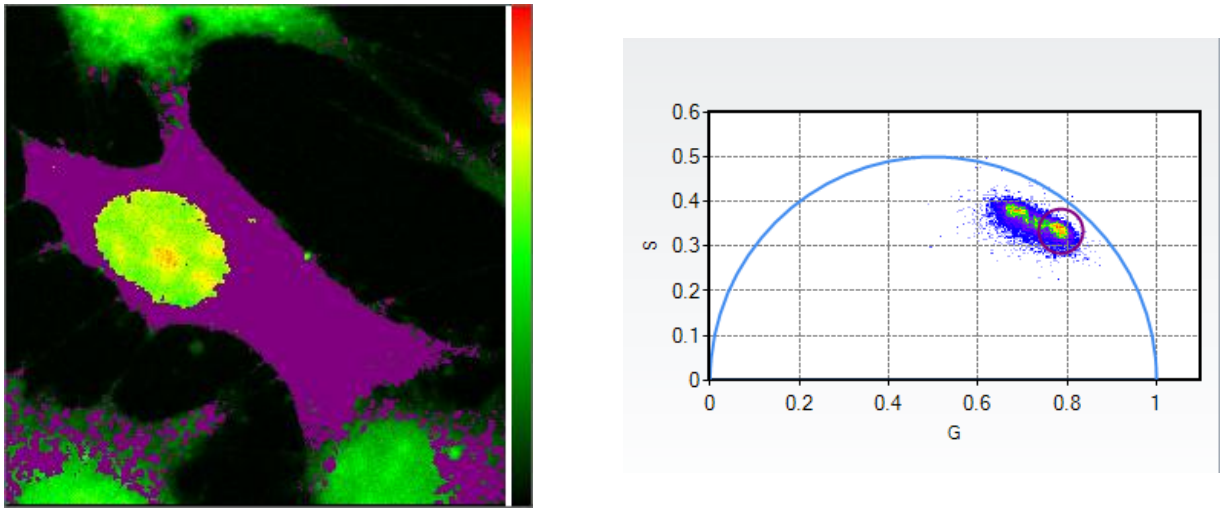


Figure 8. When the cursor is positioned on the top of the first accumulation point, the pixels corresponding to the nucleus of the cell are outlined, as well as pixels located outside the cell. The second area of accumulation outlines the pixels correspondent to the citoplasma.

Using the FRET calculator of the VistaVision software, we assign the first group to the Donor and the outside to the background. By moving the <efficiency> slidebar (Figure 8.), the cursor is positioned onto the second accumulation point when the efficiency is at about 26%.

The phasor plot of the donor in the absence of the acceptor is obtained from an independent measurement in which the acceptor is absent. The realizations of all possible phasors that are quenched with different efficiencies describe a curved trajectory in the phasor plot. The experimental position of the phasor of a given pixel along the trajectory determines the amount of quenching and therefore the FRET efficiency.

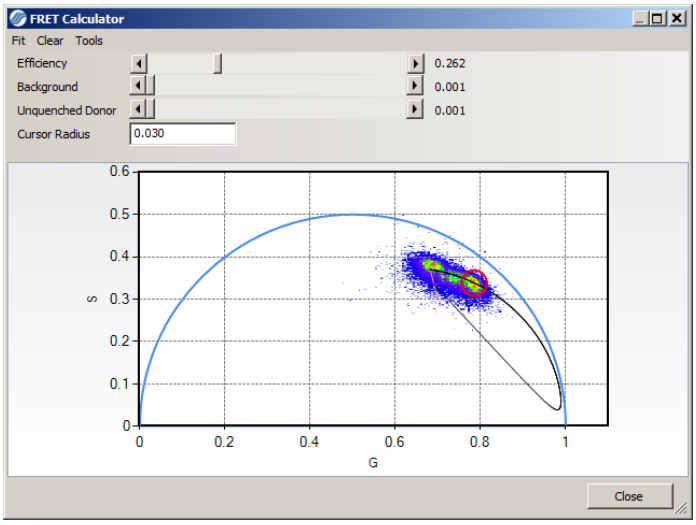


Figure 9. FRET calculator: the tools allows for the determination of the FRET efficiency without determining the decay time of the Donor and the Donor in presence of the Acceptor.

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*

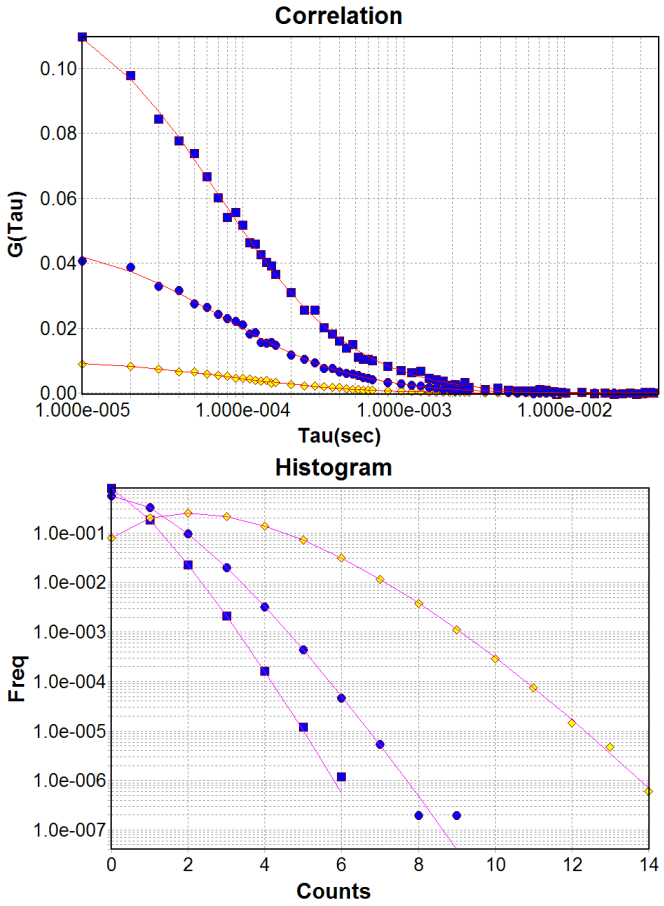


Figure 10. FCS data (top) and PCH data (bottom) for a solution of Rhodamine110 at three different concentrations: 2.6 nM, 6.4 nM and 32 nM, respectively.

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

RICS and N&B

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 Alba and provide the researcher with an unparalled amount of information about the dynamic cellular environment.

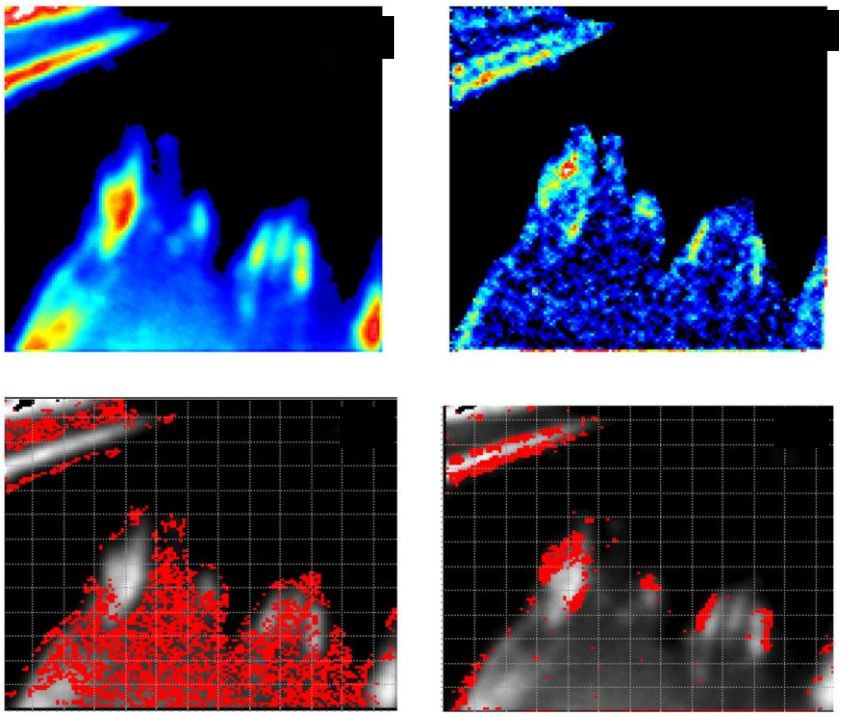


Figure 11. CHO-K1 cell expressing paxillin-EGFP. This protein is monomer in the cytosol and resides in complexes in portions of some adhesions. In C thpoint with brightness 1150 csm are selected (monomer in the cytosol). In D points with 11500 csm are selected. These pixels accumulate at the border of the adhesions. (*courtesy of E. Gratton, University of California at Irvine*).

Anisotropy (polarization) 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.

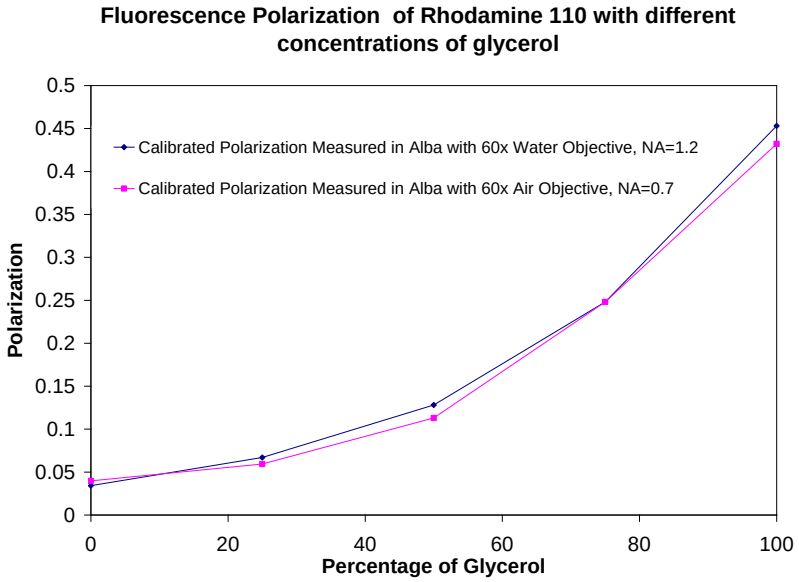


Figure 12. Data of Table I and II above. Data have been calibrated using the routine implemented in the Vista software. The polarization values obtained with the two objectives are similar.

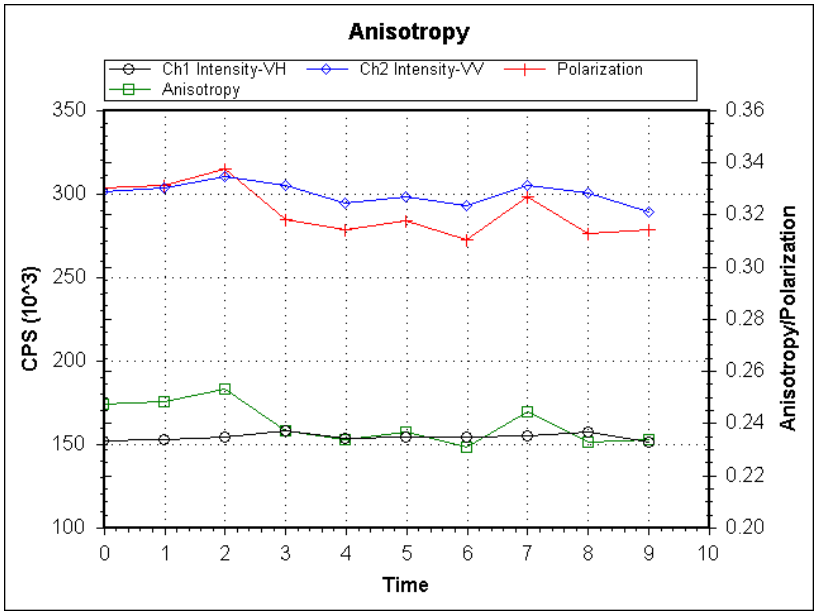


Figure 13. Anisotropy (polarization) measurements of a solution of 30nM Alexa 633 dispersed in blood plasma.

3D NanoImaging and Particle Tracking

With the Single Molecule Tracking (SMT) NanoImaging approach to super-resolution, the laser beam does not scan the sample following a predetermined pattern as is the case in raster images. Instead, the laser scanning imaging is based upon a feedback algorithm where the path followed by the laser beam is continuously adjusted and decided during the scan according to the shape of the object to be imaged. The algorithm moves the laser spot surface are known parameters, they are utilized to reconstruct the shape of the object. 3D cellular structures can be resolved down to 20-40 nm with a precision of 2 nm in a matter of a few seconds.

Principle of operation: the modulation tracking algorithm

The sequence of operations for using the SMT NanoImaging is straightforward: firstly, a confocal image of the area of interest is acquired; then, the object to be imaged is identified by the user. The SMT NanoImaging is activated through the switch and the laser beam is positioned at a distance of 100-200 nm from the center of the object. As the laser spot approaches the surface to be imaged, the amount of fluorescence increases. Yet, the increase in fluorescence depends upon the distance as well as upon the concentration of the fluorophores and their respective quantum yield. In order to separate the effect of the distance from the effect due to the concentration, the position of the spot is forced to oscillate perpendicularly to the surface. That is, the intensity of the fluorescence changes during the oscillation (Figure 14).

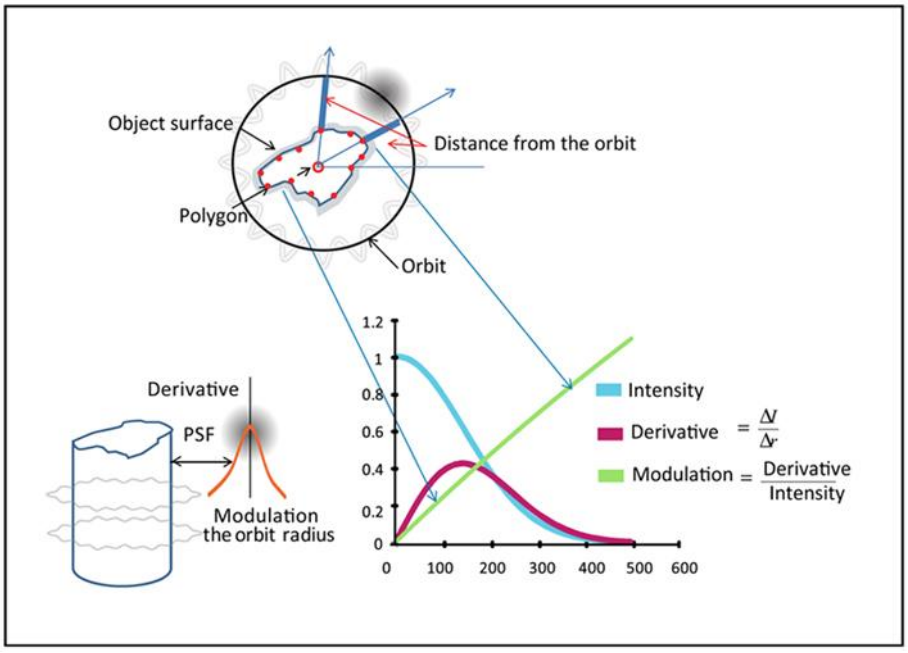


Figure 14. Schematics of the modulation tracking technique. The beam spot travels in a circular orbit around the object and its distance from the object's surface is varied periodically at a set frequency; typically, for each orbit the number of oscillations is between 8 and 32 depending upon the size of the object. These small oscillations of the radius are used to calculate the modulation function of the orbit, from which the distance of the spot from the surface is determined.
The modulation function is defined as the ratio between the alternating part and the average part due to the local fluorescence of the surface. Practically, the modulation is the ratio between the spatial derivative of the PSF and the intensity. The modulation function increases quasi linearly as a function of the distance from the surface and this feature allows for its use in determining the distance of the laser spot from the surface along the orbit. In this way, the transversal shape of the object is calculated and reconstructed.

Acquisition and Processing Software

Instrument control and analysis software are provided by the SimFCS software (by Globals Unlimited). Once the confocal image has been acquired and the orbit location is selected, the user selects the initial orbit coordinates (radius) and the number of oscillations per orbit. Along an orbit data are acquired at 8-32 oscillations; a linear interpolation at 128 points is used to reconstruct the geometrical shape of the orbit. The operation is repeated at different values of the z-position; eventually a 3D mesh reconstruction of the object is achieved using the stack of images at different z-planes of the orbit. The final touch is given by covering the mesh with a "texture" given by the specific quantities acquired such as the fluorescence intensity at each point.

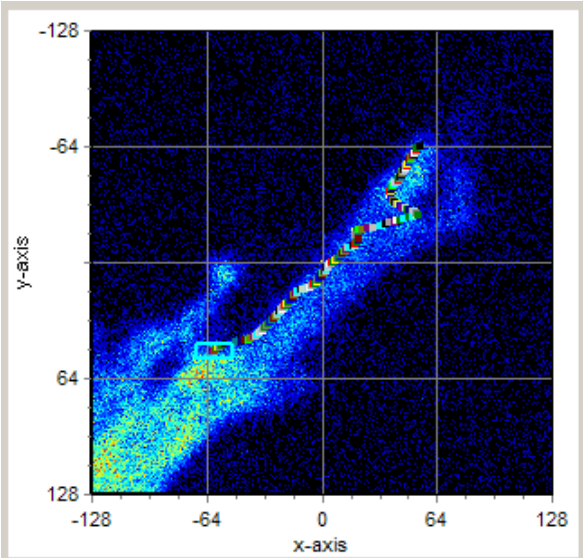
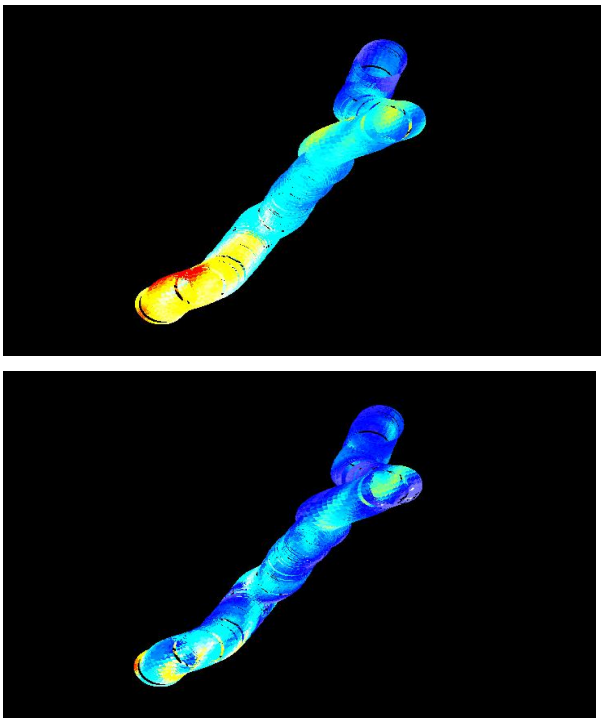


Figure 15. 3D raster scan image of a protrusion of MB231 cell growing in a 3D collagen matrix expressing actin-EGFP
(courtesy of Laboratory for Fluorescence Dynamics, University of California at Irvine).



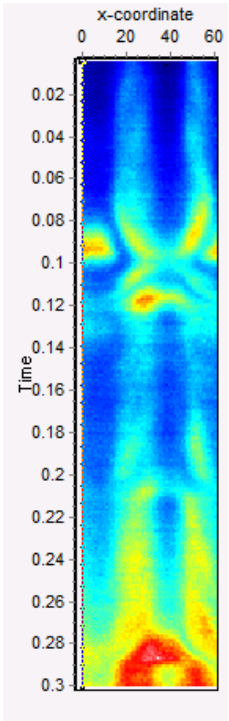


Figure 16. MT image of the rectangular portion of the protrusion indicate in Figure 3 where in Channel 1 (top figure) the Actin-EGFP was tracked while in Channel 2 (bottom figure) the SHG signal of collagen was acquired. The diameter of the protrusion changes along the filopodium. The fluorescence is not uniform on the cell surface but clusters at specific direction where contacts are made with the collagen matrix.
(courtesy of Laboratory for Fluorescence Dynamics, University of California at Irvine).

Technical Specifications

Hardware

Instrument Features

- Up to 4 channel acquisition; a 5th port is available
- Separate pinholes for each channel for higher resolution
- Computer-controlled alignment of the confocal pinhole and optics
- Choice between scanning mirrors or piezo-controlled XYZ stage
- Single- and multi-photon excitation
- Powered by VistaVision, a user-friendly software package for the acquisition of FLIM, FRET, FFS, RICS, scanning FCS, N&B

Image Parameters Acquired by Alba:

- Pixels numbers: user selectable from 20 to 4096
- Max line frequency: 4 KHz (on 20 points)
- XY and XZ sections

Optical Unit

- **Light Sources:**
  - Single photon lasers housed in a laser launcher with control of each laser intensity and shutter; or,
  - Multi-photon excitation with laser intensity control and shutter

- **Optics:**
  - **Microscopes:** Inverted and upright microscopes (Nikon, Olympus, Leica, Zeiss)
  - **Objectives:**
    - Air objectives with 20X, 40X, 60X magnification and 1.5-8.1 working distances
    - Oil immersion objectives, 1.4 NA and 60X (standard); other apertures available
    - Water immersion objectives,1.2 NA 60X (standard), with coverslip correction (for 0.15-0.18 coverslip); other apertures available
  - **Dichroic Filters:**
    - 25mm-diameter
  - **Polarizer:**
    - Cube beam splitter, wavelength range: 450-1100; extinction ratio: 10,000:1 at ±3 degrees

- **Imaging Stage:**
  - XYZ piezo-controlled stage, 100x100x50 µm with 5 nm step resolution.
  - Optional Coarse Microscope Stage:
    - Manual stage; or
    - Stepper motor controlled XYZ stage (100x100x10 mm)

- **Light Detectors:**
  - Fast photomultiplier tubes (PMTs)
  - Avalanche Photodiodes (APDs) detectors for FFS
  - Hybrid detectors

- **Laser Scanner Unit:**
  - Galvo-controlled mirrors

Data Acquisition

- **Data acquisition board:**Proprietary, 8-channel card.
- **Sampling rate:** 200 MHz
- **Input/Output triggers:** For monitoring temperature, pH, laser light source fluctuation and starting measurement.

Software: *VistaVision -FFS and FLIM*

- **Operating system**
  - Windows7, 64-bit
- **Image format**
  - Export to ImageJ, MetaMorph
  - Plots export to png, gif, jpeg, bitmap formats

Instrument

- **Computer**
  - Intel-type CPU, Windows7 operating system, 64-bit
- **Power Requirements**
  - Universal power input: 110-240 V, 50/60 Hz, 400 VAC
- **Dimensions**
  - 538 mm (L) x 563 mm (W) x 205 mm (H)
- **Weight:** 40 Kg

Software

General Features	Operating system	Windows7, 64-bit
	Computer (minimum specifications)	Intel CPU, 1- and 2-monitors operations

Measurements Modules	FFS module	• Fluorescence Correlation Spectroscopy (single channel and cross-correlation) • 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)

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
	Data acquisition modes	Time mode Photon mode
	Number of channels acquired simultaneously	Up to 4
	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 Report of fitted curves
	Scanning FCS	User defined area
	Raster Image Correlation Spectroscopy (RICS)	Up to 3 KHz

Confocal Imaging Module	FLIM modality	Frequency-domain Time-domain
	FLIM time-resolution	100 ps – 100 µs
	Raster Scan	Resolution: up to 1.5 nm Pixels number: user selectable from 2 to 8192 Max line frequency: 4 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 Field rotation: 200^{o} optical Field diameter: 18 mm
	Scan Modes	Kinetic studies: t, Xt, XYt, XZ, XYZ and XZt Optical sectioning: XZ, XYZ) of specimens
	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
	Input/Output	2 channels input 8 channels output

Measurements

	Channels 1-2	Channels 3-4	Aux port	Non descanned port
Confocal images	●	●		●
FLIM spectra			●	
FLIM TCSPC	●	●	●	●
FLIM digital frequency domain	●	●		●
FCS, FCCS, PCH	●	●		●
Scanning FCS	●	●		
Number & Brightness	●	●		
RICS	●	●		
Polarization images	●	●		
Particle tracking	●	●		

Focus and Discovery

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