VistaVision

Confocal Imaging and Spectroscopy 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, filter wheels, 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.

A)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.

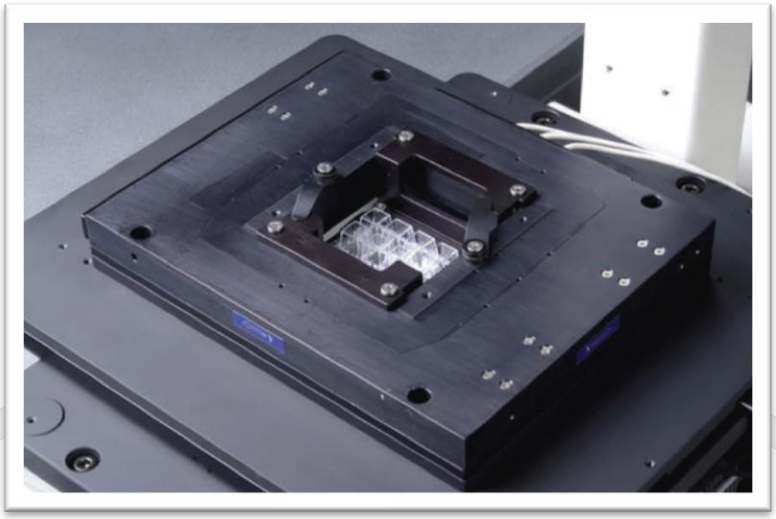
B)Imaging module

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 (DFD) 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.

C)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(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.

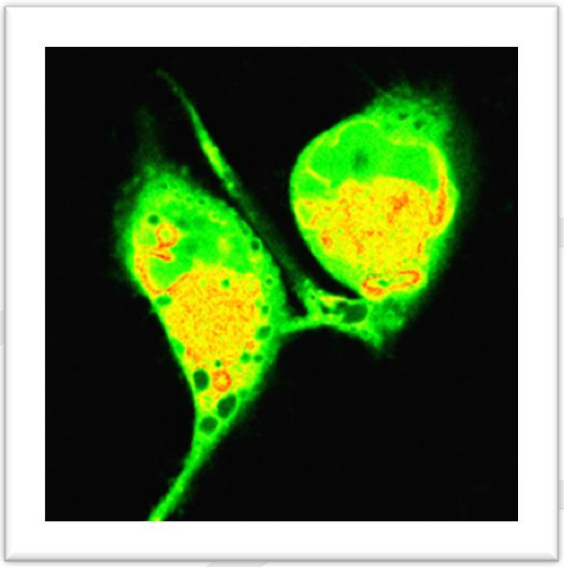
Instrument Control Module

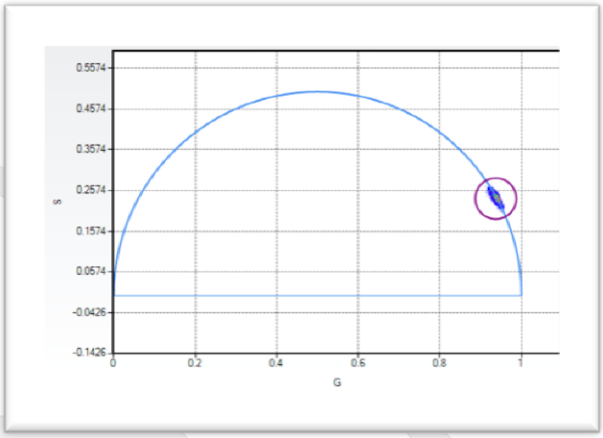


VistaVision uses a flexible hierarchical configuration system that allows the user to select the proper hardware components and to conveniently set parameters for the selected components.

Data Acquisition Cards	• ISS FCS card (for FFS data acquisition) • ISS 3-Axis DAC card (for controlling imaging devices) • ISS FastFLIM card (for digital frequency-domain FLIM and FFS) • NI 6052E, ISS A2D200K card for analog frequency-domain FLIM B&H SPC-830, SPC-150 cards (for TCSPC FLIM and FFS)
Scanning Mirrors Modules	• ISS scanning mirrors module • Cambridge Technology Models
Piezo-controlled Stages	• ISS XYZ piezo-controlled stage • MadCity Models Nanoposition • PI Models PiezoNano • Nanomotion, Model SC-AT
Automated microscopes	• Nikon Models TE2000-E, Ti-E
Microscope stages	• ASI Model 2000 • Prior Scientific Model H117P2IX
Laser Launchers	• ISS Laser Launchers Series • Intensity control unit for Ti:Sapphire laser
Two-detector unit	• Detector unit including two shutters, two filter wheels and one dichroic wheel used for LSM upgrade packages (Olympus, Leica, Nikon, Zeiss)

Imaging Module





Cell expressed with Cerulean. Excitation wavelength was 488 nm from a pulsed laser diode. The phasor plot collects the pixels of the image; a decay time of 4 ns is measured.

Data Acquisition

Single point measurements	• Intensity • Polarization • Kinetics • Lifetime
Line measurements	• Line acquisition • Profile acquisition
Steady-state images (single plane and z-stack)	• Polarization • Ratiometric • Kinetics • Time-lapse recording
FLIM images (digital frequency-domain) (single plane and z-stack)	• Acquired in digital frequency-domain (DFD). The routine acquires simultaneously a FLIM image and a steady-state image.
FLIM images time-domain (single plane and z-stack)	• Acquired in time-correlated single photon counting (TCSPC)
Raster Image Correlation Spectroscopy (RICS)	Acquires an image with a dwell time suitable for the molecular dynamics to be resolved. The technique for measuring molecular dynamics and concentrations from fluorescence confocal images

Scanning FC(requires FFS module)	The laser beam rotates on a circle with diameter 100-200 nm (user determined) and FFS data are acquired at set angles. At each angle an FCS curve is reconstructed.
Number and Brightness (N&B)	Acquires an image with a dwell time suitable for the acquisition of local fluctuations. The measurement provides the presence of clusters and monomers/dimers.

Image Acquisition (Raster Scan)

Min dwell time	• 4 µs
Pixel number	• User selectable from 2 to 4096 (for steady-state images) • User selectable from 2 to 2048 (for FLIM images)
Max line frequency	• 12 KHz (on 20 points)
Min line frequency	• 0.01 Hz
Max frame rate	• 512x512: 1 second
Beam park	• The beam can be parked at any position for the acquisition of FFS data
Scan modes	• For kinetics studies: t, Xt, XYt, XZt, XYZt, XZt • For optical sectioning: XZ, XYZ

Image Processing and Analysis

Operations on images	• Scaling • Arithmetic • Smoothing • Zooming • Rotation
FLIM processing	• Lifetime fitting (Marquardt-Levenberg minimization algorithm) • Phasor plots
FLIM display	• Lifetime image • Fractional contributions image • Pre-exponential factors image
Image export format	• Export to ImageJ, MetaMorph
Plots export format	• Gif, tiff, jpeg, png, bitmap, metafile
Movies export format	• avi

Data Files Formats for Imaging Module

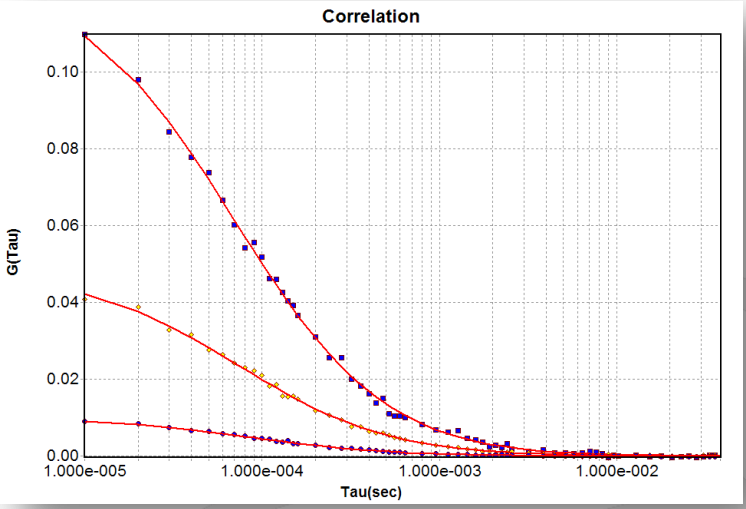
Data files are stored in several formats:

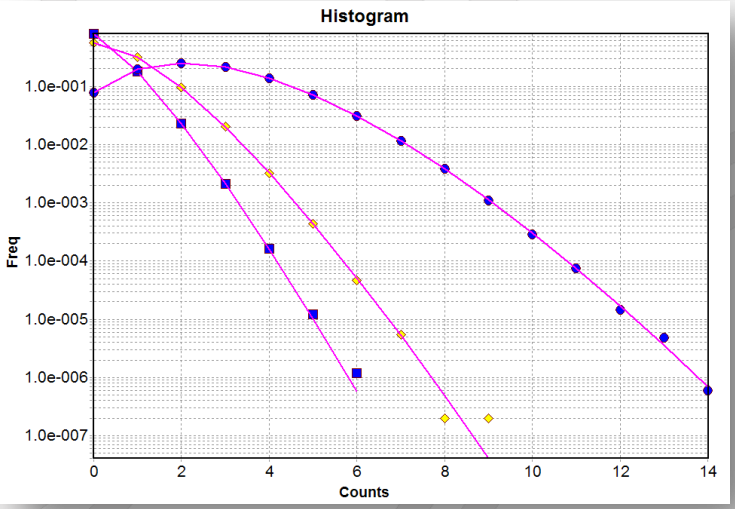
• VistaVision acquires and stores raw data files, which can be opened at a later time and analyzed for FLIM, RICS and N&B.

• The header includes the relevant information about the acquisition.

• The Time Tagged Time Resolved (TTTR) format is available when FFS data are to be provided along with lifetime data (FLIM).

Extension	Format	Description/application
ifi	binary, 32-bit floating	Confocal images (steady-state) [File header includes: image size, pixel time, intensity, etc.]
ifli	binary, 32-bit floating	DFD (FastFLIM) AFD (analog FD) [Includes: DC, phasor, G, S. The file header includes the image size, pixel time, etc.. The file is already corrected with reference.]
fbd	binary,16-bit	DFD (FastFLIM) raw data (FIFO data); no correction applied to the data [This file is saved simultaneously to the .ifli-format file. No header, image size, pixel time are not stored]
fbs	text	Experiment Info Header for the fbd-format [file header includes: image size, dwell time, padding info]
tif	TIFF tagged images	Confocal images (steady-state)
bin	binary, 16-bit	LFD image file or FCS in time-mode Confocal images (steady-state) RICS
int	Binary, 32-bit floating	LFD image file
ref	binary	LFD ref file, used for DFD (FastFLIM), 256 x256 pixels already corrected with reference file
spc	binary, 32-bit	B&H raw data (FIFO Data), used with TCSPC TTTR, time tagged time resolved
set	text	B&H, used as Header for the spc-format [file header includes: image size, dwell time, padding info]





Autocorrelation curves (top) and photon counting histogram (bottom) for a solution of Rhodamine110 at three different concentrations, 2.6 nM, 6.4 nM and 32 nM.

FFS Data Acquisition

Autocorrelation (FCS)	The FCS function gives the temporal correlation of the fluctuations
Cross-correlation (FCCS)	The FCCS function provides the temporal correlation of the fluctuations related to events occurring simultaneously on two or three channels.
Photon Counting Histogram (PCH)	The PCH function plots the distribution of photon counts at the specified time interval
FFS measurement at target XYZ locations in an image	The user selects the XYZ locations by moving the cursor or entering the values in the software. The laser beam moves sequentially to each location to acquire FFS data that are then analyzed.
FLCS, fluorescence lifetime correlation spectroscopy	The user selects the XYZ locations by moving the cursor or entering the values in the software. The laser beam moves sequentially to each location to acquire FFS data that are then analyzed.
Scanning FCS	The laser beam rotates on a circle with diameter 100-200 nm (user determined) and FFS data are acquired at set angles. At each angle an FCS curve is reconstructed.

FFS Data Analysis

Statistical function utilized for FFS data analysis	Single set and Global fitting models available in the FFS module	Parameters determined by the FFS module
Autocorrelation (FCS) Cross-correlation (FCCS)	• One or two species using: ○ 2D- or 3D-Gaussian PSF ○ 3D-Gaussian-Lorentzian PSF ○ one-photon excitation ○ two-photon excitation ○ presence of flow • Global analysis fitting.	• One or two species using: ○ Diffusion coefficient ○ Concentration ○ Triplet state decay time constant ○ Triplet function ○ Flow rate ○ Size of excitation volume
User Defined Equation	• Up to 50 different user defined equations kept in the panel list for user to choose. • Equation could include Sin, Cos, Exponential, etc. Except the integral unclosed form equation. • Global analysis fitting.	• Up to 30 parameters allowed in the equation.

Data files formats for FFS module

Data files are stored in several formats:

• VistaVision acquires and stores raw data files, which can be opened at a later time and re-correlated; sections of each raw data file can be purged from the presence of impurities (for instance, when an impurity traverses the observation volume) and re-analyzed.

• The software also stores the autocorrelation function, the cross-correlation function and the photon-counting histogram data files.

• The Time Tagged Time Resolved (TTTR) format is available when FFS data are acquired along with lifetime data (FLIM).

extension	format	description/application
fcs	binary format	FFS raw data file
csv	ASCII format (csv)	FCS (correlation and autocorrelation function)
hst	ASCII format (csv)	PCH photon counting histogram
nts	text file	Notes on FFS files

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