

Confocal * Widefield * Multi photon * Nipkow disk

Large server * PSF distiller * SFP volume visualization

From 2D to multi channel 3D-time images

Huygens Pro specifications

Image restoration functions

- Accelerated Maximum Likelihood Estimation (MLE) restoration algorithm, optimized for low light level images
- Quick MLE restoration algorithm
- Quick and Iterative Constrained Tikhonov-Miller restoration algorithms
- Point Spread Function (PSF) measurement tool box, to derive a microscopic PSF from finite sized micro bead images (with automatic alignment and averaging to combine many beads, and finite size correction)
- Theoretical PSF generation
- Supports Widefield, Confocal, Nipkow, 4Pi and two-photon microscopes
- · Based on electromagnetic diffraction theory
- · Takes into account spherical aberration
- Automatic bleaching correction of 3D and 4D Widefield images, and of 4D confocal and multi-photon images
- · Z-drift correction for time series

Basic image processing capabilities

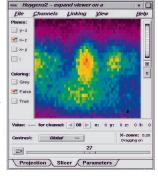
- · Capability to handle multiple images simultaneously
- Time series support
- Multi parameter (multi channel) image elements (stacked or packed)
- Basic data types: unsigned byte, 16 bit signed integer, 32 bit float, 2 x 32 bit complex
- Per image undo/redo capabilities
- Scripting and batch processing environment based on Tcl (Tool Command Language)

Core image processing functions

- Create, destroy, copy, copy block, convert, split, join, zoom, rotate, iso-sample, shift, replicate image
- Add/remove border, shift to sub-pixel accuracy, mirror image, swap image octants
- Arithmetic operations on two image operands, one image operand and a scalar, mathematical functions on one image operand, soft clipping & thresholding
- 4D Gaussian filter of arbitrary widths, 4D Laplacian fil-
- Generate solid and hollow bandlimited spheres, generate Poisson and Gaussian noise
- Real and complex 4D Fast Fourier transforms

Reporting & display operations

- Image statistics
- Report sampling density with respect to Nyquist rate
- Image histograms of images with up to three channels
- Plots of Energy Flux as function of time and axial posi-



Actin filaments in twophoton fluorescence excitation microscopy. The deconvolution result, shown in the XZ mode of the Huygens' Expand Viewer, demonstrates effective noise reduction and three- to four-fold resolution gain in the axial direction.

Image I/O file formats

- Read/write ICS (Image Cytometry Standard), Nikon-ICS, Leica style numbered TIFF, Biorad 'pic' and Imaris classic images
- Read Zeiss 'Lsm5', Metamorph 'stk', 'MRC', Olympus 'Fluoview', and DeltaVision 'IMSubs'
- Read/write a single or numbered series TIFF images into/from 3D volume image
- 4D support: ICS, TIFF series, Biorad, numbered 'stk'.

Analysis functions

- Threshold and label 3D image
- Analyze labelled objects: compute centre of mass, volume and integrated intensity
- Estimate background
- · Measure distance
- Compute image ratio
- Co-occurrence matrix
- Co-localization (Pearson and Manders coefficients)

Visualization

- · Thumbnail images
- Multiple Expand viewers on one or many multi channel images. Each Expand viewer is able to
- Show x-y, x-z or y-z slices for selectable points in time while optimizing contrast on a global or perplane basis
- Show Sum or MIP projection, animate projections of time series
- Report individual pixel/voxel positions and values
- Swing through planes or time
- Slicing positions in expand viewers can be dynamically linked for easy image comparison
- Volume rendering by Simulated Fluorescence Process

Scripting environment

To facilitate execution of scripts in the background or batch processing on servers a light weight Huygens scripting environment is available.

Supported platforms

□ x-y

Huygens Pro

- All SGI® platforms running Irix 6.5 including SGI's 64bit multiprocessor systems
- Linux: Red Hat 7.2 and SuSE 7.3 or higher

Huygens Scripting

- 64-bit multiprocessors running Irix 6.5 or IBM AIX 5.1
- Windows NT, 2000, XP, Mac OS X 10.2, Linux Red Hat 7.2 and SuSE 7.3 or higher

Cover illustration: Macrophage fluorescently stained for tubulin (yellow/green), actin (red) and the nucleus (DAPI, blue). Recorded by Dr. James Evans, Whitehead Institute, MIT, Boston MA, USA, using widefield microscopy. Top image: left part: original data; right part: as deconvolved with the Huygens System.

Bottom image: Image of a 25 µm pollen grain Spathipyllum recorded by Dr. Erik Manders, Swammerdam Institute for Life Sciences, Faculty of Science, University of Amsterdam, The Netherlands using a Perkin-Elmer Scanning Disk Confocal Microscope. All images show deconvolved data. Visualization by FluVR's spectral fluorescence volume renderer.



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