



Biological Image Processing and Analysis in *Imaris*

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Outline

- Introduction to Imaris
- Getting Started
- Data Types- 3D and 4D, file formats
- Image processing
- Visualization methods
- Segmentation/Measurements
- Tracking
- Colocalization
- Animations



Where do I access Imaris

Imaris is available on PCs at

- Basic Sciences Computational Laboratory (BSCL),
1-280 BSBE
- Scientific Development and Visualization Laboratory
(SDVL),
575 Walter Library
- SDVLApp1- remote windows application server, which can
be connected to via Citrix



Supported File Formats

Applied Precision, Inc: DeltaVision (*.i3d, *.dv)
Biorad: MRC-600, MRC-1024 (*.pic)
Bitplane: Imaris Series (*.ims)
Bitplane: Imaris Classic/Old (*.ims)
Bitplane: Imaris Scene File (*.imx)
Carl Zeiss: LSM 510 (*.lsm)
Carl Zeiss: LSM 410, LSM 310 (*.tif, *.tiff)
Carl Zeiss: Axiovision (*.zvi)
Image Cytometry Standard: ICS - used by Nikon, and others (*.ics, *.ids)
Leica: TCS-NT (*.tif, *.tiff)
Leica: LCS (*.lei, *.raw, *.tif, *.tiff)
Leica: series (*.inf, *.info, *.tif, *.tiff)
Olympus: FluoView TIFF (*.tif, *.tiff)
Olympus: FluoView 1000 OIF (*.oif)
Olympus: FluoView 1000 OIB (*.oib)
Olympus: Cell^R 1.1/standard (*.tif, *.tiff)
Open Microscopy Environment XML (*.ome)
Scanalytics: IPLab (*.ipl)
TILL Photonics: TILLvisION (*.rbinf)
Universal Imaging: Metamorph STK (series) (*.stk)
Also supported are basic numbered tiff image series.



Image sequences

Working with Series:

- A single 2d image xy dimensions
- A stack of 2d images x,y,z forming a volume
- Multiple stacks of the same sample, but depicting collected at different frequencies (multiple channels).
- Time varying series(the 4th dimension).

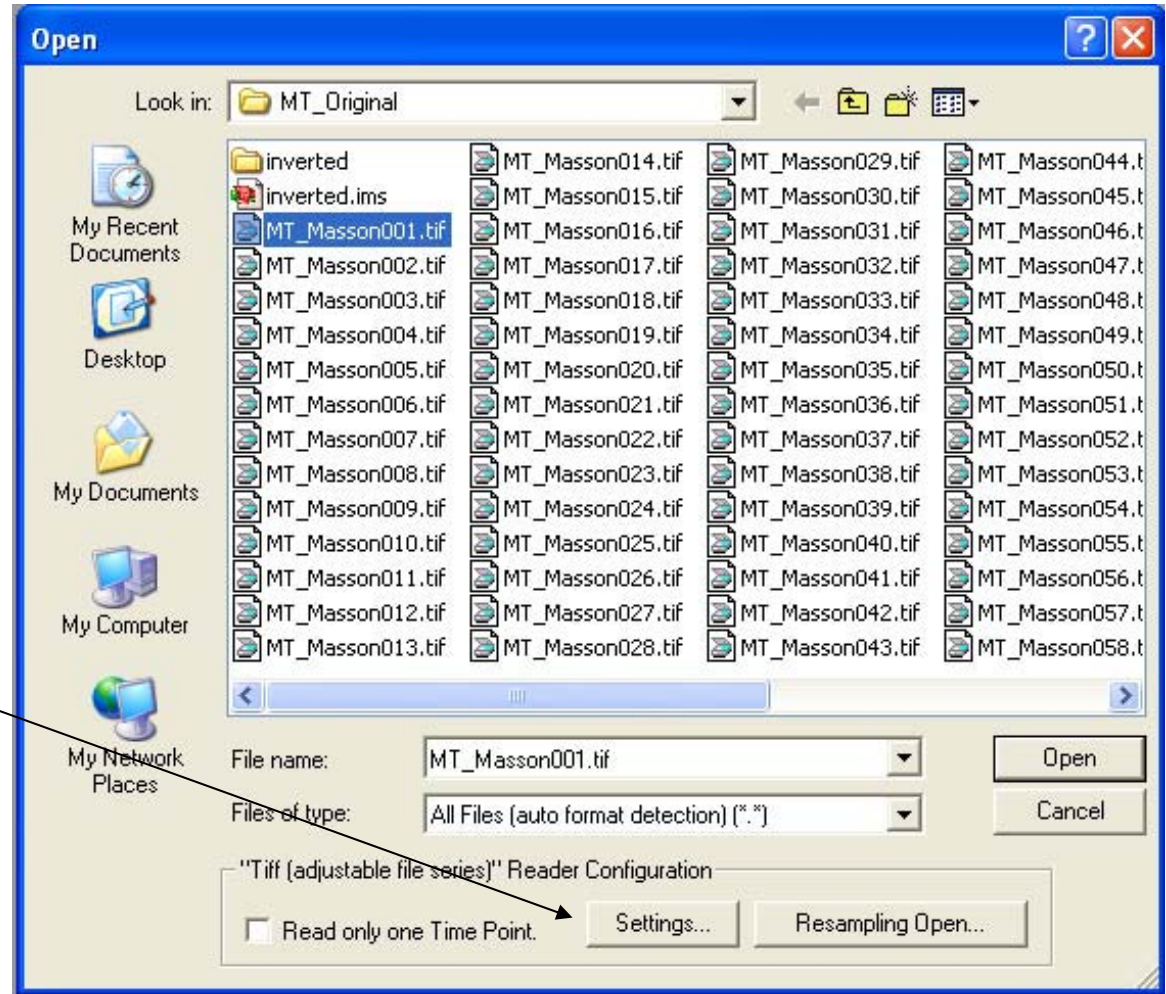
eg. xyz-ch1-t1, xyz-ch2-t1, xyz-ch1-t2, xyz-ch2-t2....and so on.

Loading Images in Imaris

File->Open->Select file

Reading Tiff Series:

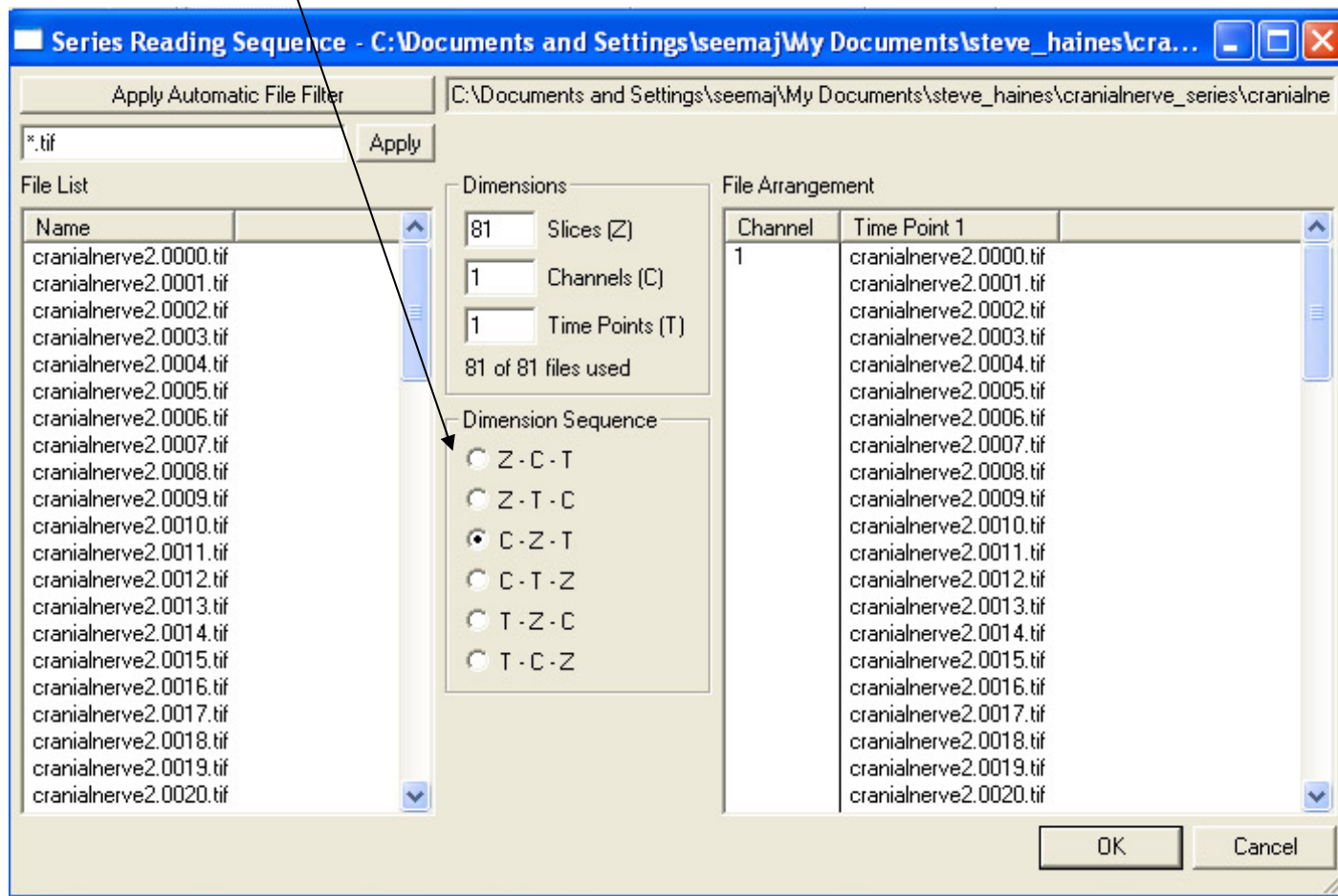
- When working with a 2d tiff image series, select the first file, and Click 'Settings' in the dialog box.



Loading Images in Imaris

Reading Tiff Series

- Choose the order of reading the series.
- Choose the Dimension Sequence for Slices, Channels and Time ports





Loading Images in Imaris

Reading multiple channels:

Note 1 :

If different channels are saved in multiple files with sequentially numbered filenames, Imaris will automatically load all of them.

Otherwise, for additional channels-

Edit->AddChannels->Select file

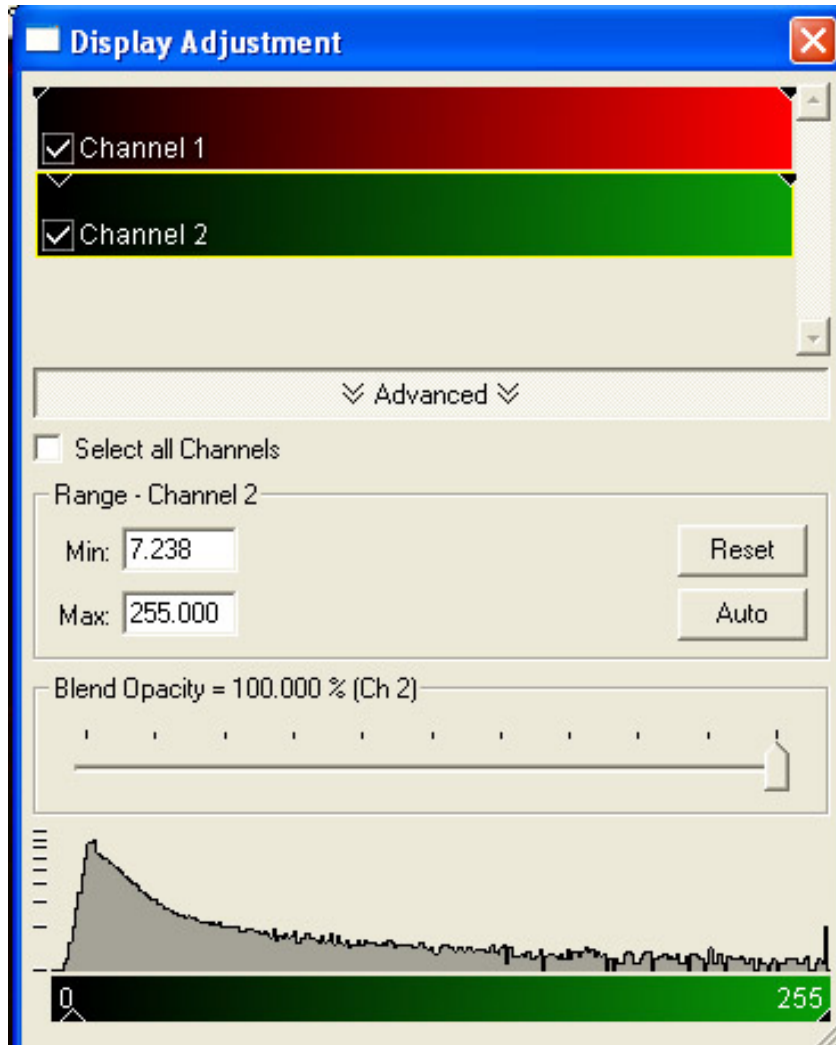
Note 2:

Imaris does not support 16-bit tiff images.

- Save your images in 16-bit 'unsigned' format, or convert them to 8-bit first and then load Imaris

Display Properties

Image Processing



- Changing display properties for brightness/contrast/opacity is not permanent
- However, using the image processing functions change the original dataset. They should be used only to remove noise.

Image Properties

Image Processing

Image Properties

- Geometry
- Data Set
- Channels
 - Channel 1
 - Channel 2
- Thumbnail
- Parameters

Geometry

Type: 8 bit (unsigned)

Size

	X	Y	Z	T
Size	512	512	98	1

Resample 3D...

Coordinates

	X	Y	Z
Voxel Size	1.001957	1.001957	1.001000
Min	0.000000	0.000000	0.000000
Max	512.000000	512.000000	97.097008

Unit: unknown

Time Point: 1

Date [YYYY-MM-DD]: 2005-03-29

Time [HH:MM:SS.SSS]: 00:00:00.000

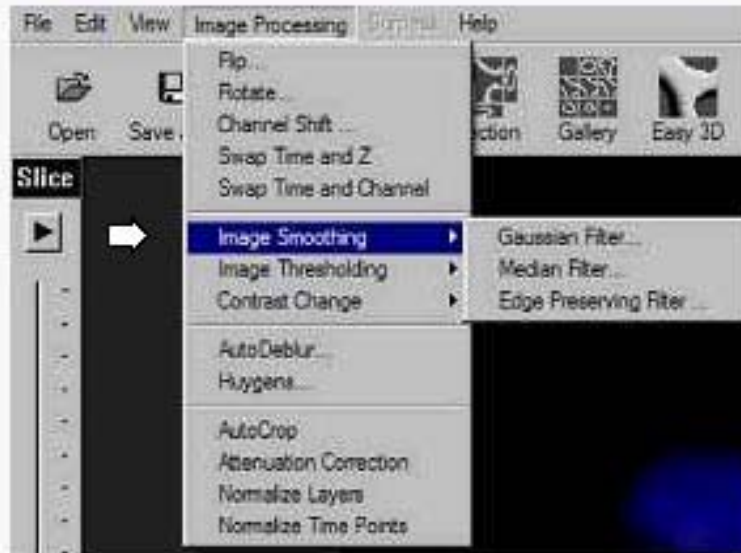
All Equidistant...

OK Cancel

- **Edit->ImageProperties**
- Change base colors for each channel
- change dataset geometry- resampling and voxel sizes

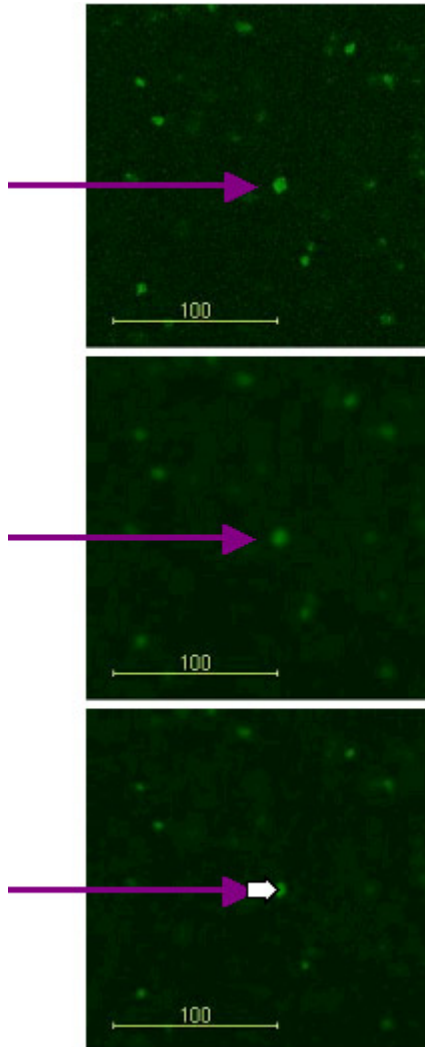
Smoothing

Image Processing



- Gaussian. One voxel's intensity is replaced by the weighted sum of the intensities of its neighbours. The weights in any direction correspond to a bell curve centered at the voxel's position.
- Median. One voxel's intensity is replaced by the median intensity of its neighbours.
- Edge Preserving. Similar to Gaussian but instead of filtering all directions it only filters in directions without gradient.

Edge Preserving Noise filter



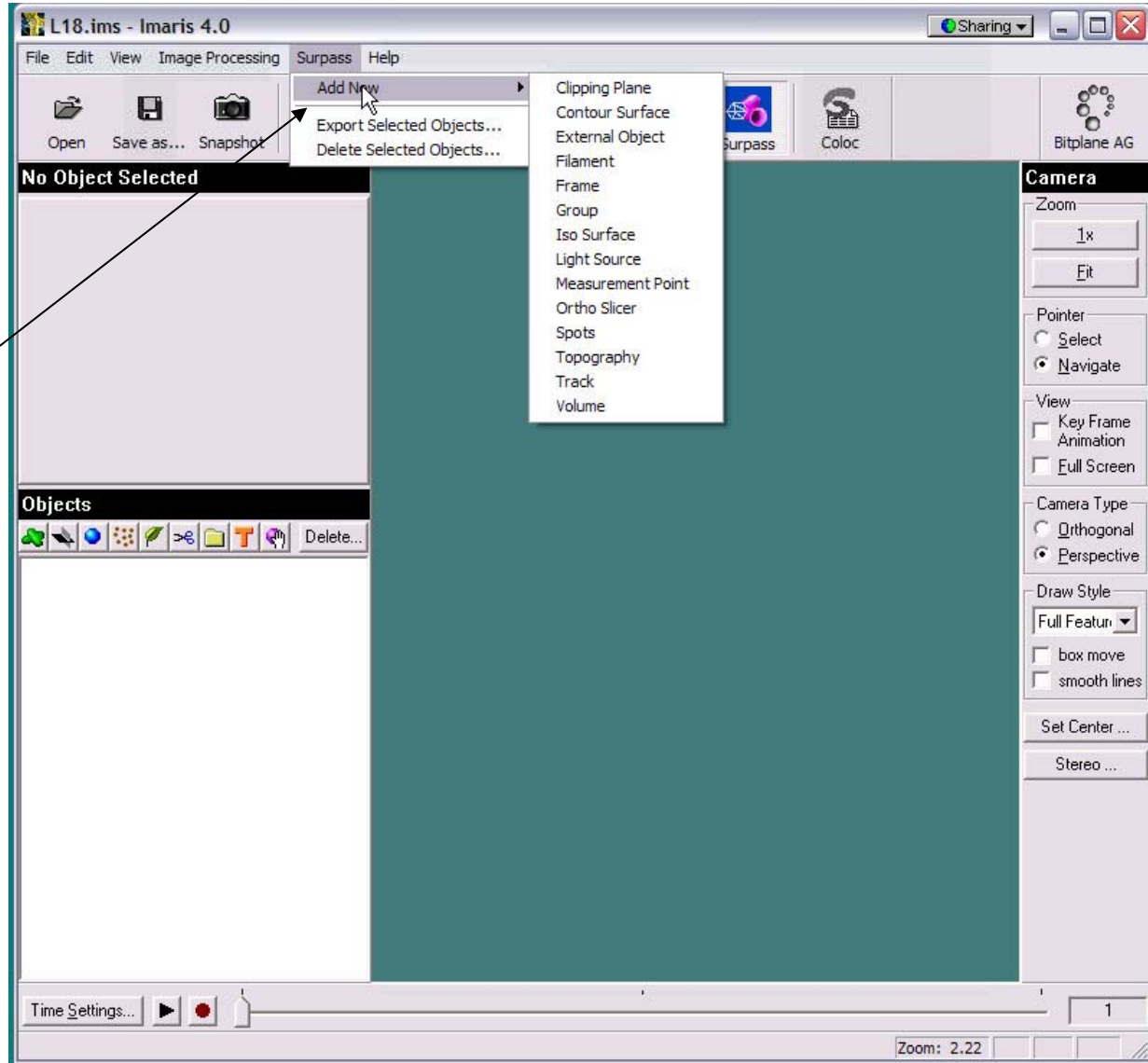
- Top: Original single section.
- Middle: Image filtered with Gaussian filter.
- Bottom: Image filtered with edge preserving filter (EPF) using same filter width than for Gaussian filter.
- The EPF is not a standard kernel operation. Instead the filter kernel is computed newly for each voxel position to avoid filtering across edges.
- Benefit: While the noise removal effect (smoothing) of the EPF is similar in areas of more homogenous intensities it does not filter across intensity gradients (edges).
- This filter preserves the overall intensity in the image. The integral of all voxel intensities remains constant.



Undo Functions in Imaris

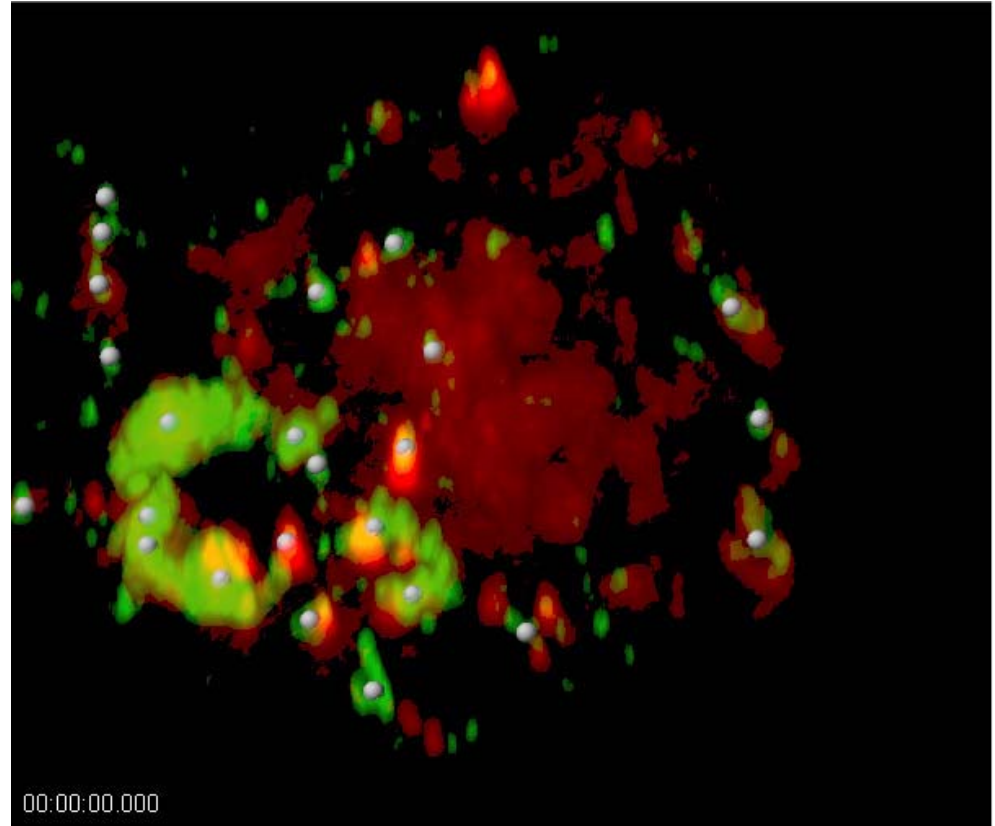
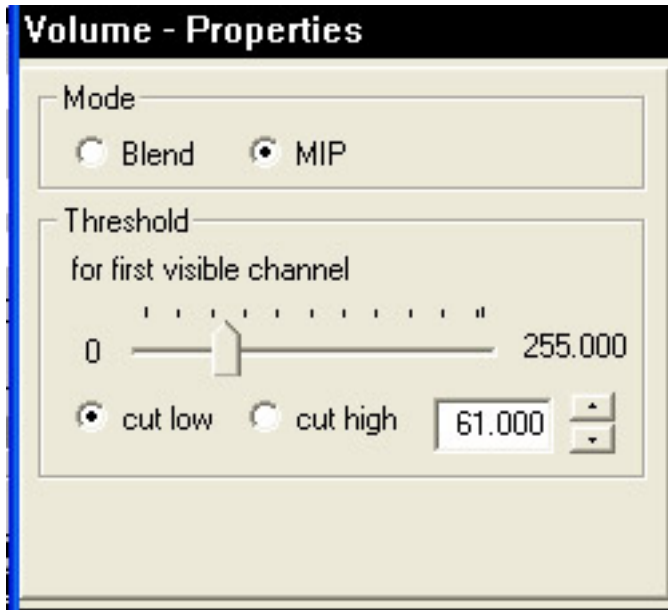
- Before Imaris activates an image processing function it stores the current image for an eventual subsequent undo operation.
- The number of undo steps can be customized using the Preferences dialog of the Edit menu.
- Increase the number for „Maximum Number of Commands in History“ to be able to undo more than just the last operation. Keep in mind that each undo step require a full copy of all channels, layers, and time points and adjust accordingly.

Surpass tools



Existing palette and additional tools in the surpass menu

Volume Visualization contd.



- Choose between MIP and Blend modes
- Cutoff threshold values to remove 'black voxels' from rendering



Volume Visualization contd.

Imaris needs three textures for a good volume visualisation, one for each direction in space. The size of voxels should be the same in all x, y, z directions. To do this:

- Find voxel sizes . Z size is often bigger than X and Y size.

Edit - >Image Properties - >Geometry->Size Coordinates

- Use this formula to calculate the new number of Z layers to get the desired Z voxelsize:

$(\text{Voxelsize Z} / \text{Voxelsize Y}) * \text{Number of Z-Layers} = \text{Number of desired Z-Layers}$

- Change Z to the result of the formula.

Edit - >Image Properties - >Size - Resample 3D

Example: Original Voxelsize X: 0.164600 Y: 0.164600 Z: 0.200000

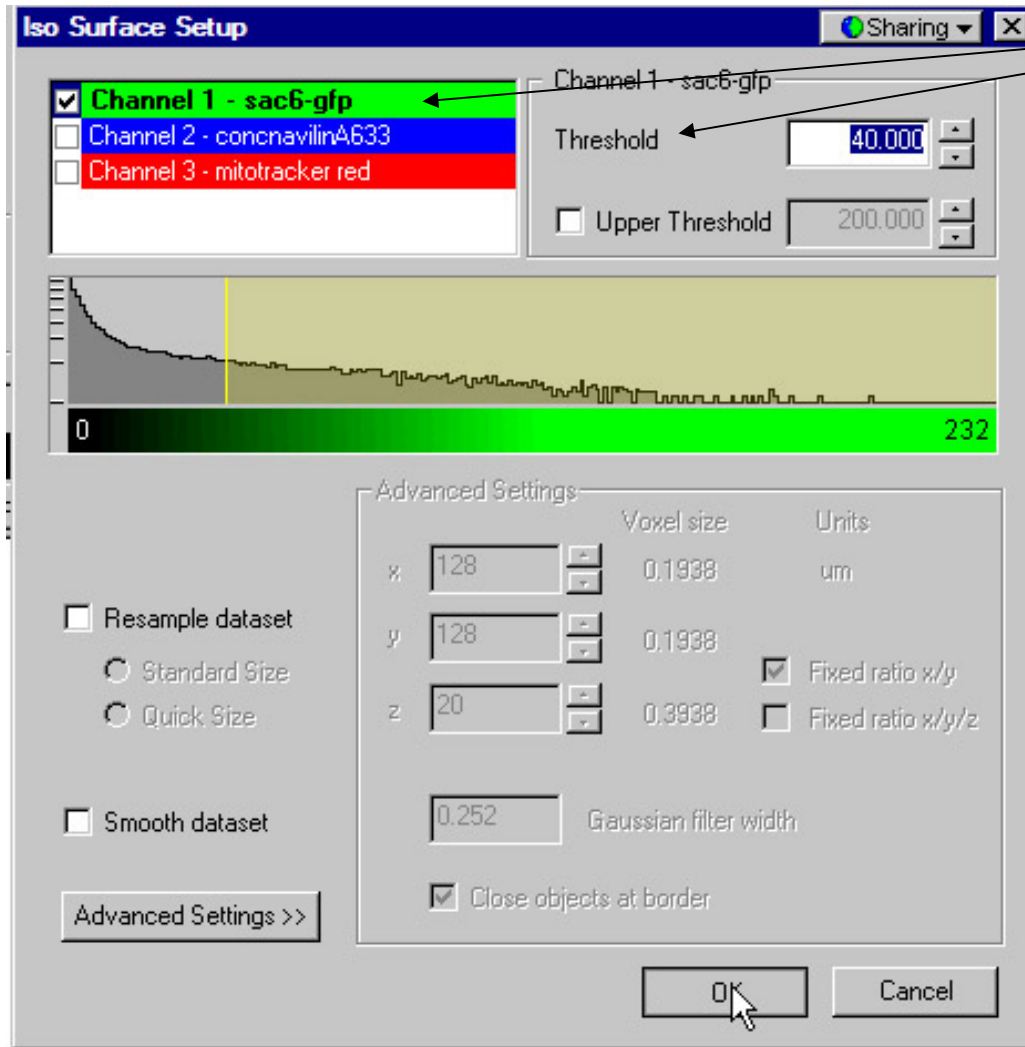
Original Amount of Z-Layers 64

Apply formula: $0.2 / 0.164600 \times 64 = 77,76$

Resample Z to 78

New Voxelsize: X: 0.164600 Y: 0.164600 Z: 0.163636

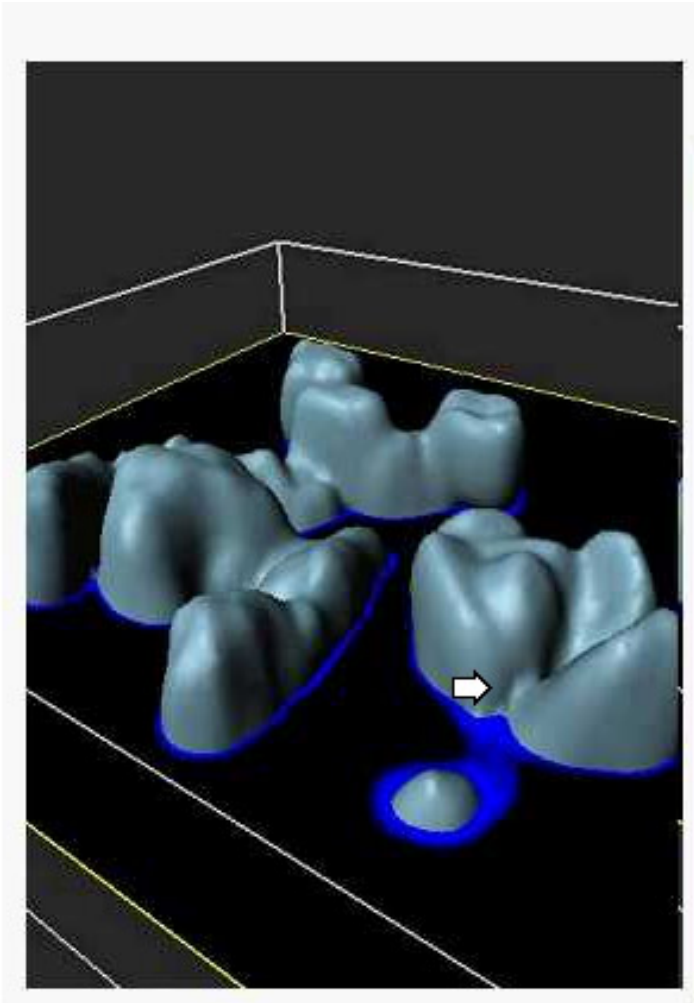
Isosurfaces



Imaris automatically finds and sets a threshold value for each channel, but it can be modified.

Isosurfaces

Image Segmentation



Tip: Try superimposing the isosurface with slice-

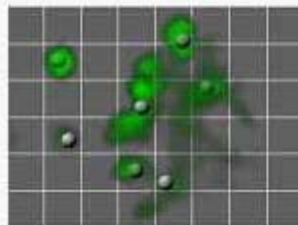
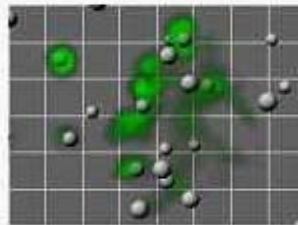
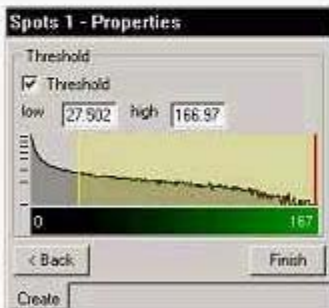
This helps to test whether the chosen threshold level is good or not

Tip: If you are unable to get an isosurface without getting too much noise or losing too much information, then try Connective baseline filter and create isosurfaces again

Isosurface can also be used to detect spots.

Tip: Split All spots to create individual objects. Collect statistics from Isosurface group object

Spot Detection (from volume rendering)



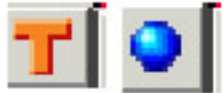
- Display image as a volume component in Surpass to verify the spot creation process.
- Add a spot component.
- Choose the channel in which spots are to be detected.
- Choose the approximate spot diameter.
- Define minimal brightness of the spot's intensity maxima and see the disappearance of weak spots.

Tracking Objects

Image measurements

- Where do cells migrate?
- Are all cells moving with the same speed?
- Are all organelles moving in the same direction?
- Are there any dividing cells?
- Are vesicle growing over time?
- Who does the time series look with system drift corrected?
- How do I display and show the behaviour of hundreds of tracks?

Tracking Methods in Imaris



- The surface tracking system tracks SurfaceObjects from one time point to the next.
- Objects can merge and split.
- Volume/intensity changes are automatically computed, displayed, and reported.
- The surfaces of one object in two consecutive time points must overlap for automatic tracking.



- The spot tracking identifies „relatively“ bright spots in a local surrounding.
- The tracking subsystem makes assumption on the future location of the object based on its past movement.
- Objects can be tracked even though they are not overlapping in two consecutive time points.
- Spot tracking works well on objects touching each other.



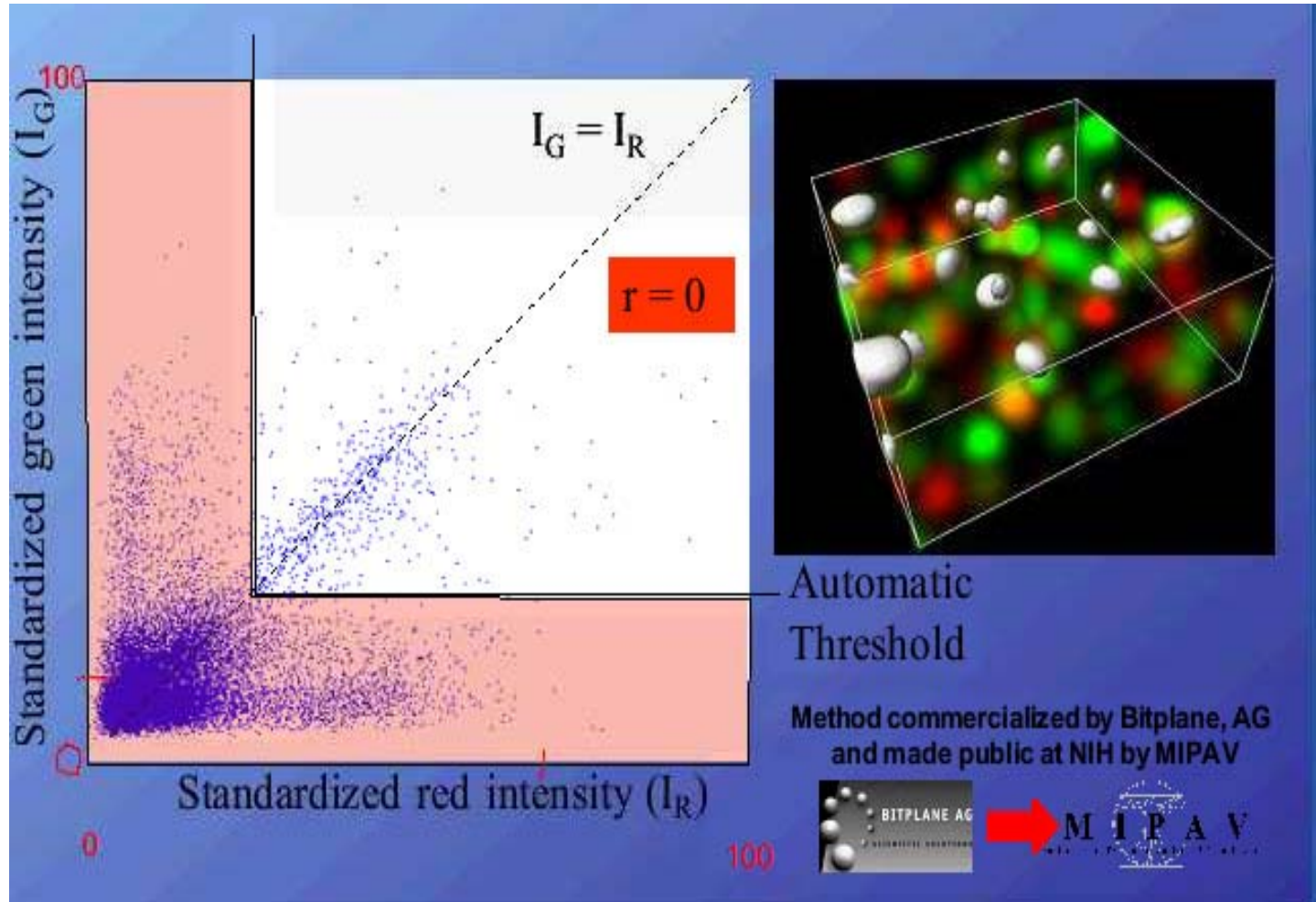
Colocalization

Simple Qualitative Colocalization

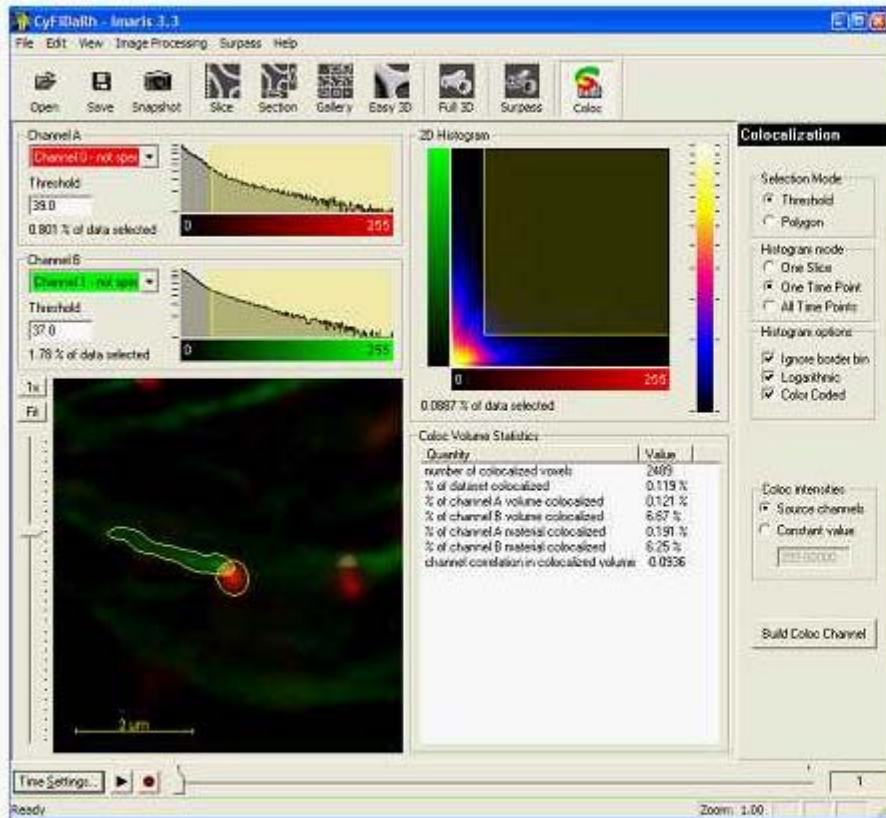
- Stain two objects of interest that interact *in-vitro*
- Manually adjust brightness contrast values and view overlapping regions as colocalised.

This method is random and does not give statistical proof

Colocalization



Courtesy: Bitplane Inc.



- Automated, standardized determination of colocalized voxels.
- Manual selection of thresholds on histogram or on image.
- Full integration with other Imapris modules.
- Processes 2D, 3D, and 4D images (with ImaprisTime).
- Histograms of 2D, 3D (and 4D with ImaprisTime).
- Real-time feedback on changes in selection.
- Display of colocalized voxels with original channels as defined in channel visibility editor.
- Advanced visualization in combination with Surpass.



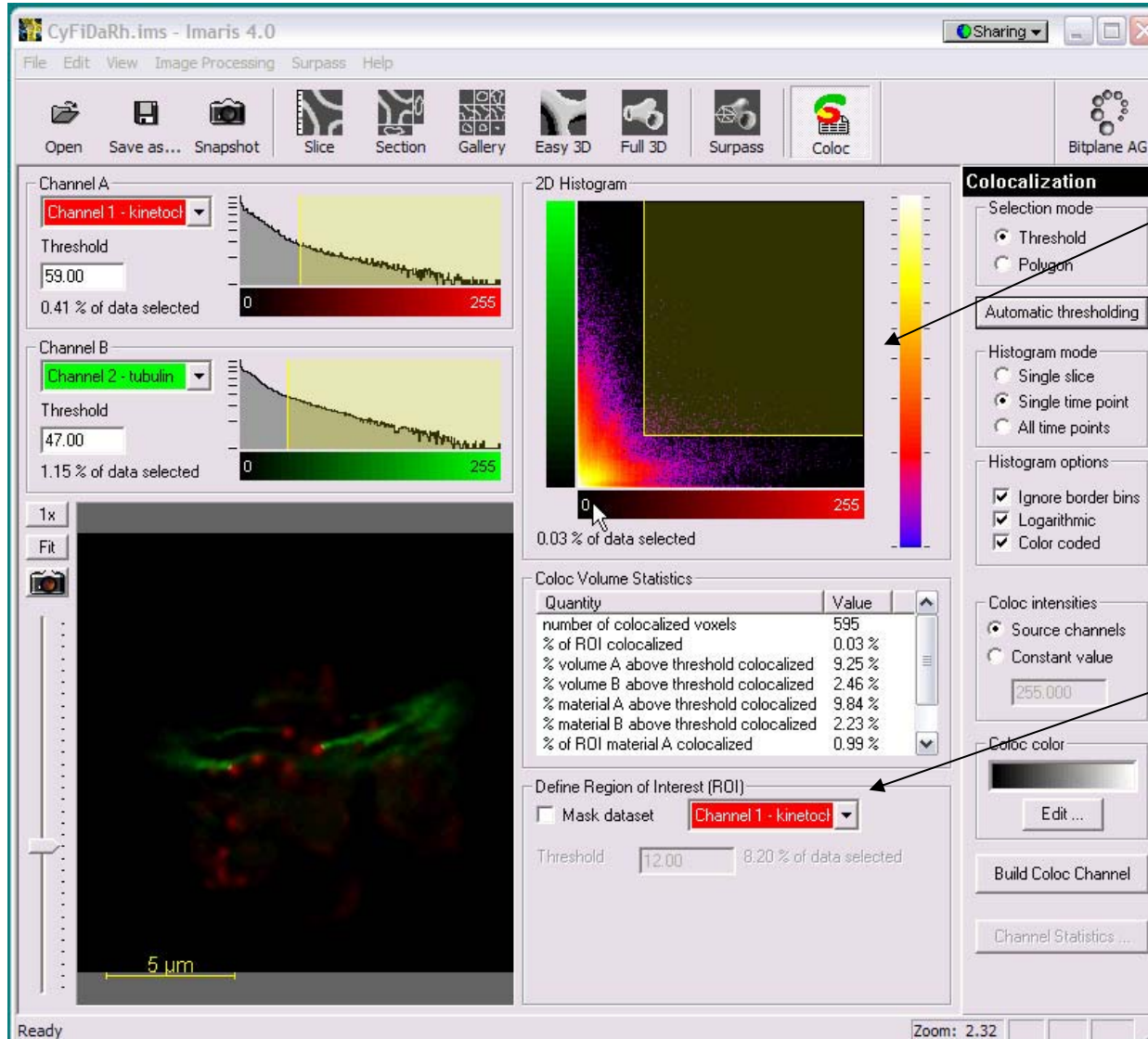
Automatic Thresholding

Colocalization

- Exclude unspecific staining- Set a threshold value in one channel to mask the entire image, prior to automatic thresholding
- Click Automatic Thresholding
- The software analyzes a pair of thresholds just that voxels above this show a positive spatial correlation. i.e. $r=0$ at this pair. This mean that's voxels above this pair stain the same structure.

Automatic Thresholding

Colocalization



The screenshot shows the Imaris 4.0 software interface with the following components:

- Channel A:** Channel 1 - kinetocyl. Threshold: 59.00. 0.41 % of data selected.
- Channel B:** Channel 2 - tubulin. Threshold: 47.00. 1.15 % of data selected.
- 2D Histogram:** Shows a 2D plot of the two channels. A yellow box highlights a region. 0.03 % of data selected.
- Colocalization Panel:**
 - Selection mode: Threshold, Polygon
 - Automatic thresholding:
 - Histogram mode: Single slice, Single time point, All time points
 - Histogram options: Ignore border bins, Logarithmic, Color coded
 - Coloc intensities: Source channels, Constant value (255.000)
 - Coloc color: Edit ...
 - Build Coloc Channel
 - Channel Statistics ...
- Coloc Volume Statistics Table:**

Quantity	Value
number of colocalized voxels	595
% of ROI colocalized	0.03 %
% volume A above threshold colocalized	9.25 %
% volume B above threshold colocalized	2.46 %
% material A above threshold colocalized	9.84 %
% material B above threshold colocalized	2.23 %
% of ROI material A colocalized	0.99 %
- Define Region of Interest (ROI):**
 - Mask dataset
 - Channel 1 - kinetocyl
 - Threshold: 12.00 (8.20 % of data selected)
- Main View:** 3D visualization of the data with a 5 µm scale bar.

2d histogram adjustment

Masking irrelevant thresholds to reduce subset

Image Properties

- Geometry
- Data Set
- Channels
 - Channel 1
 - Channel 2
 - Channel 3
 - Channel 4
 - Channel 5
- Thumbnail
- Parameters

Channel 5

Name: Colocalization Result

Description: Source Channels: 1 & 2
Selection Method: Threshold
Threshold A: 40.00 Threshold B: 43.00

Base color | Mapped color | Coloc Statistics

time frame	1
number of colocalized voxels	1739
% of dataset colocalized	0.08
% of ROI colocalized	0.08
% of volume A above threshold colocalized	11.00
% of volume B above threshold colocalized	6.05
% of material A above threshold colocalized	11.51
% of material B above threshold colocalized	5.66
% of ROI material A colocalized	2.07
% of ROI material B colocalized	1.59
channel correlation in dataset volume	0.3811
channel correlation in ROI volume	0.3811

Export...

OK Cancel



Measurements in Imaris

Measurements

- Measurements are integrated at several places in Imaris
- Mostly found as a 'Statistics' tab in
 - Spots(count, radius, position),
 - Tracks(length, no of tracks),
 - Surfaces(surface areas, counts)
- Measure distances by selecting points on surfaces or slice
- Export statistics to excel or save as tab delimited text for further analysis
- Use programs like Tecplot, Matlab, SigmaPlot
- Click open Excel Tab to get Pivot Tables



Saving your work and Graphics

- It is as simple as taking a snapshot
- Collect images from Slice, Easy3d or surpas svIEWS
- Make sure that Offscreen rendering is in your display preferences.

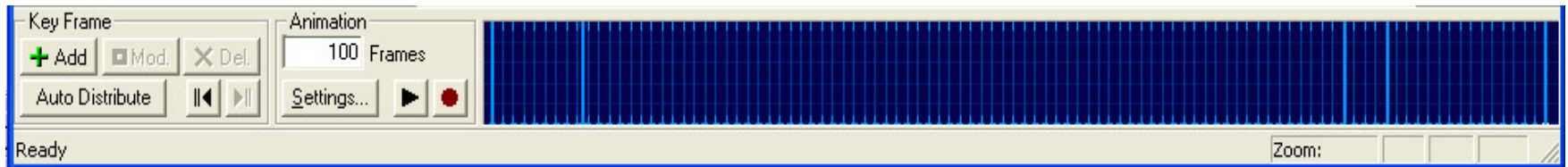
Edit->Preferences->Display

- Save images in tiff format for higher quality
- If you have made changes to your original set, like filtering etc.- you can save the modified set. File->Save As- > Select any image format, preferably tiff or pic
- To save the scene, File-> ExportScene
- Retrieve the scene by File->Load Scene
- Retrieve the Image file by File->Open



Creating Movies

- Select the Animation module
- A timeline appears the bottom.
- Set number of frames depending on the duration of your movie
- Click Settings to change your rendering preferences- this is only to optimize memory and refresh rates; not the actual rates
- Navigate in the viewer, move to frame position in the timeline and add keyframe
- Keep adding keyframes until you have finished the 'choreography'
- You can add, delete or slide keyframe by selecting in the timeline
- When done, hit the red record button. Save movie





More in Imaris

ImarisXT allows an eXTension of Imaris
Can be used to write one's own modules as plugins
Interfaces with Matlab- The modules can be written in
Matlab and brought in Imaris



References/Help

www.bitplane.com

Bitplane's FAQ server

<http://flash.bitplane.com/support/faqs/faqs.cfm>

Advanced Imaging Centers- NAAIC

<http://www.advanced-imaging-center.org/naaic.html>

Help at MSI-

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