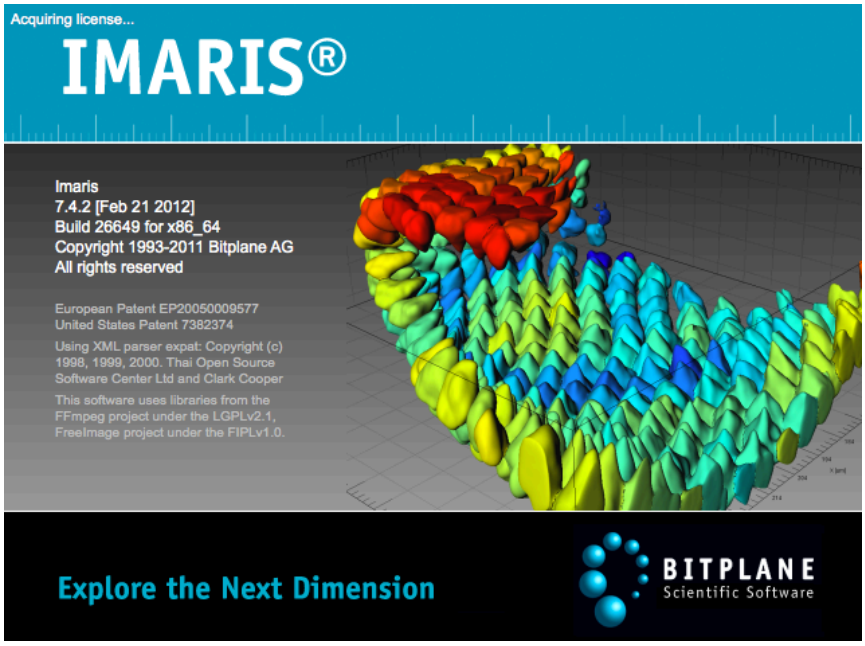
Imaris Course

Imaging Core Facility / Biozentrum
Oliver Biehlmaier, Alexia Ferrand,
Niko Ehrenfeuchter



Course structure

MORNING

09.30-10.30 Section 1. Basic Imaris functionalities (OB)

10.45-12.30 Section 2. Basic Imaris hands-on (OB, AF, NE)

AFTERNOON

13.30-14.30 Section 3. Advanced Imaris functionalities (OB)

14.45-17.00 Section 4. Advanced Imaris hands-on, examples and own data (OB, AF, NE)

The aim is that at the end of the course you become semi expert **IMARIS USER** and are able to work alone with the system.

Imaging Basics – bit depth

Bit depth	Number of colors/shades	Approximate file size
2^1	2	0.125 MB
2^4	32 *human sensitivity*	0.5 MB
2^8	256	1.0 MB
2^{12}	4096	1.5 MB
2^{16}	65,536	2.0 MB
2^{24}	16,777,216	3.0 MB

Bit-depth relates to the number of colors that can be displayed in the image.

- Images with only two colors are binary, the pixels are either black or white.
- Monitors and imaging hardware are typically limited to displaying grayscale images in 8-bit mode (256 shades of gray).
- Most monitors can only display color images in a maximum of 24-bit mode (true color), due to the limitations of the electronics in the cathode ray tube.
- Even these ranges are greater than the sensitivity of the human eye, which is often stated as only being able to detect 16-32 shades of gray and roughly 2,000,000 colors.
- Larger bit depth only if needed for intensity measurements/analysis

File formats - standard

- **JPEG** –
  joint photographic experts group, supports 24-bit color, uses a lossy compression technique (discrete cosine function), most often used on web pages, not suitable for most scientific images.
- **PNG** –
  portable network graphics, supports 48-bit color and 16 grayscale, lossless compression, a relatively new format that is not widely supported yet
- **TIFF** –
  tagged image file format, supports paletted images (up to 8 bit), 8 & (in some programs) 16 bit grayscale as well as 24 bit color, this is probably the most commonly used file format for scientific images, supports lossless LZW compression (although not all programs can open compressed

File formats - proprietory

Supported files

- Andor: Multi-Tiff (Series) (*.tiff, *.tif)
- Andor: iQ ImageDisk (*.kinetic)
- API DeltaVision (*.r3d, *.d3d, *.dv)
- Biorad MRC 1024, 600 Series (*.pic)
- Biovision: Ivision (.ipm)
- Bitplane Scene File (*.imx)
- BMP (adjustable file series) (*.bmp)
- Format b
- Huygens and Huygens Compatible Nikon
- ICS File (*.ics, *.ids)
- ICS/IDS
- Imaris 2.7, Imaris 3, and Imaris 5.5 (*.ims)
- IMOD binary file (*.imod, *.mod), object scene file
- Leica Image Format LIF (*.lif)
- Leica LCS (*.tif, *.tiff, *.lei, *.raw)
- Leica Series (*.tif, *.tiff, *.inf, *info)
- Leica TCS-NT (*.tif, *.tiff)
- Molecular Devices (Formerly Universal Imaging now MDS Analytical Technologies) Metamorph Stack (*.stk)
- MRC (*.mrc, *.st, *.rec)
- Nikon ND2 (*.ND2)
- Olympus Cell^R 1.1 (*.tif, *.tiff)
- Olympus FluoView (*.tif, *.tiff)TIFF
- Olympus OIB (PC Only) (*.oib)
- Olympus OIF (*.oif)
- Open Microscopy Environment Tiff (*.tiff, *.tif)
- Open Microscopy Environment XML (*.ome)
- OpenLab LIFF (*.liff)
- OpenLab Raw (*.raw)
- Perkin Elmer: Ultraview (*.tim, *.zpo)
- Scanalytics: IPLAB (*.ipl)
- TIFF (adjustable file series) (*.tiff)
- TILLvisION (*.rbinf)
- Zeiss Axiovision (*.zvi) (PC Only)
- Zeiss LSM410, LSM310 (*.tif, *.tiff)
- Zeiss LSM510, LSM 710 (*.lsm)

Metadata

Defined as **data providing information about one or more aspects of the data**, such as:

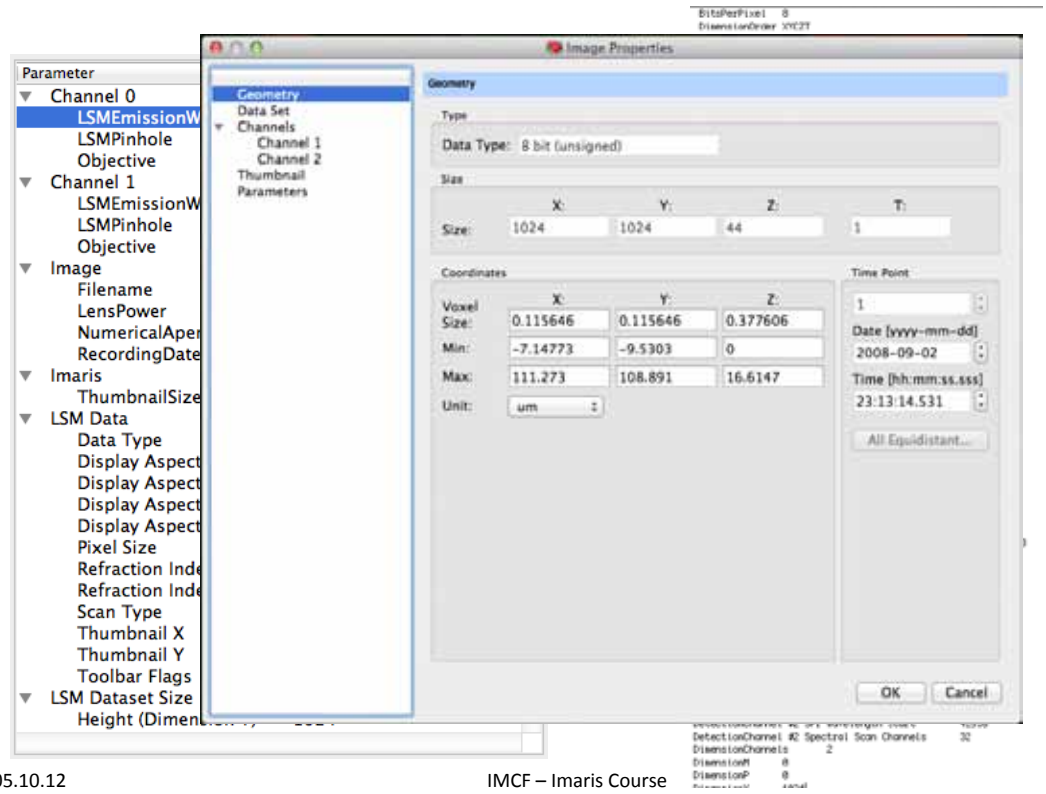
- Means of creation of the data
- Purpose of the data
- Time and date of creation
- Creator or author of data
- Location on a computer network where the data was created
- Standards used
- The song title of a piece of music

For example, a digital image may include metadata that describes how large the picture is, the color depth, the image resolution, when the image was created, and other data. A text document's metadata may contain information about how long the document is, who the author is, when the document was written, and a short summary of the document.

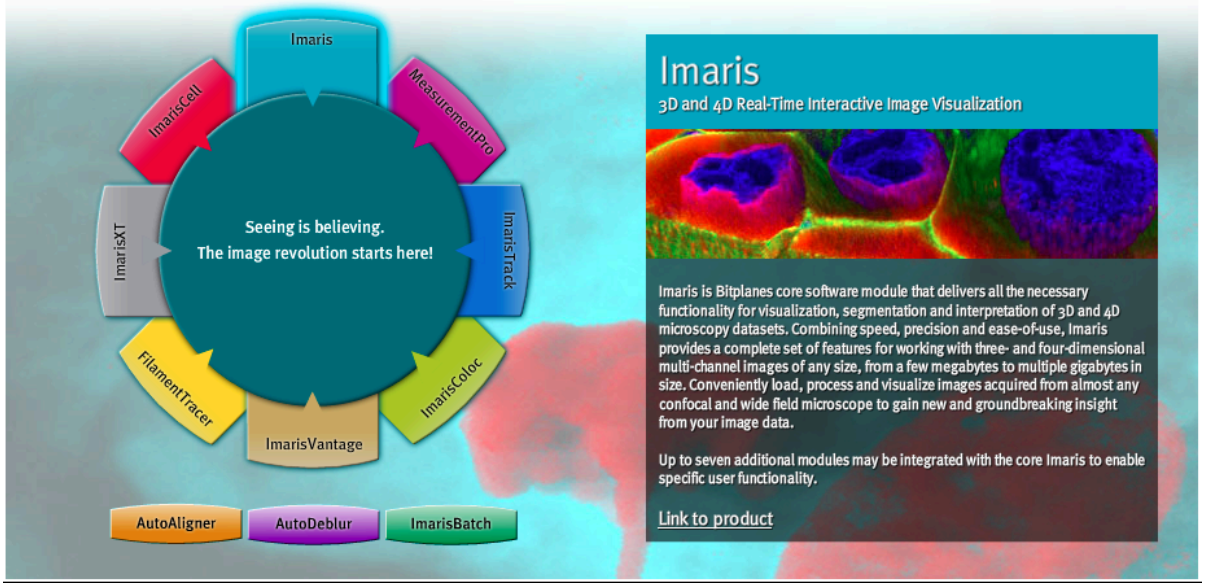
Metadata found in

- **Photographs / Video –** *EXIF, geo tags, etc.*
- **Web pages -** *meta tags*
- **ebooks -** *ISBN, author, tags, etc.*
- **Microscopy data:**
  objective magnification and numerical aperture, exposure time, excitation wavelength, emission wavelength, x- and y-resolution, z-resolution, …

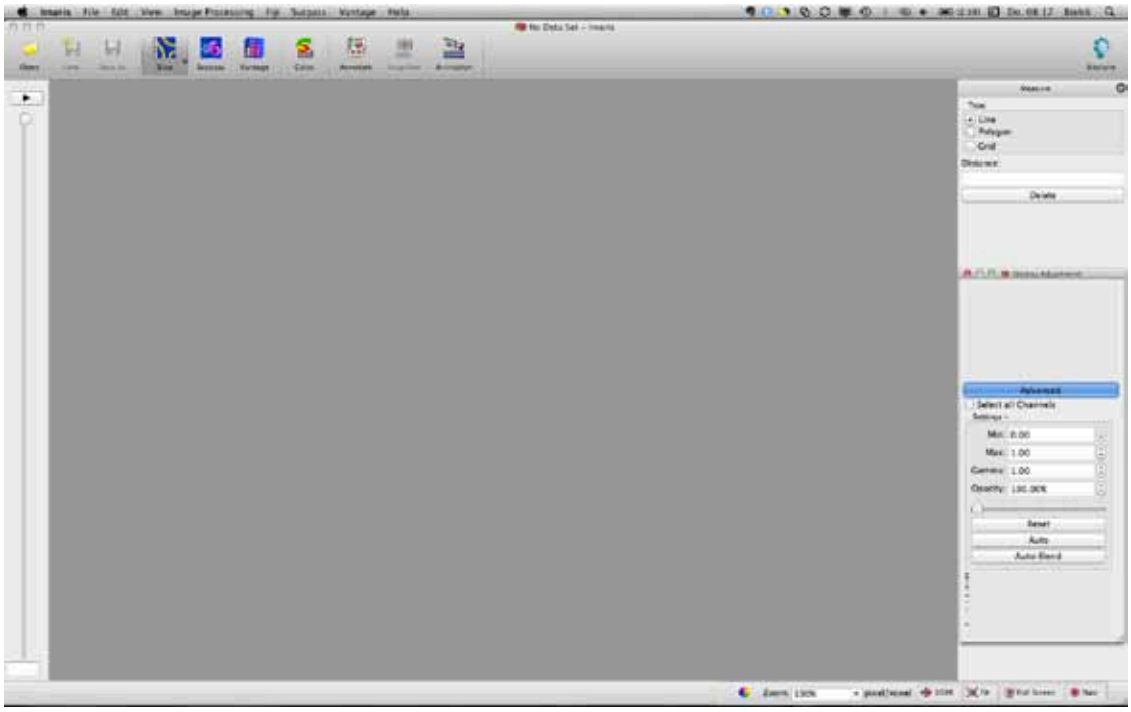
Metadata - examples



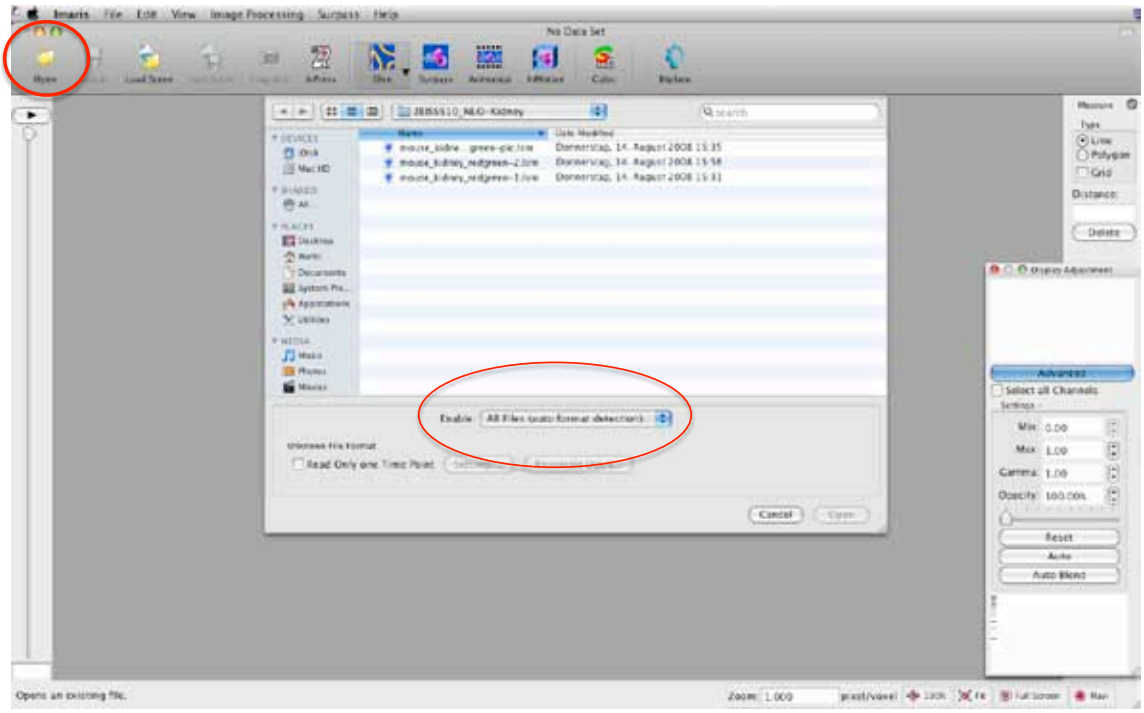
How to use IMARIS



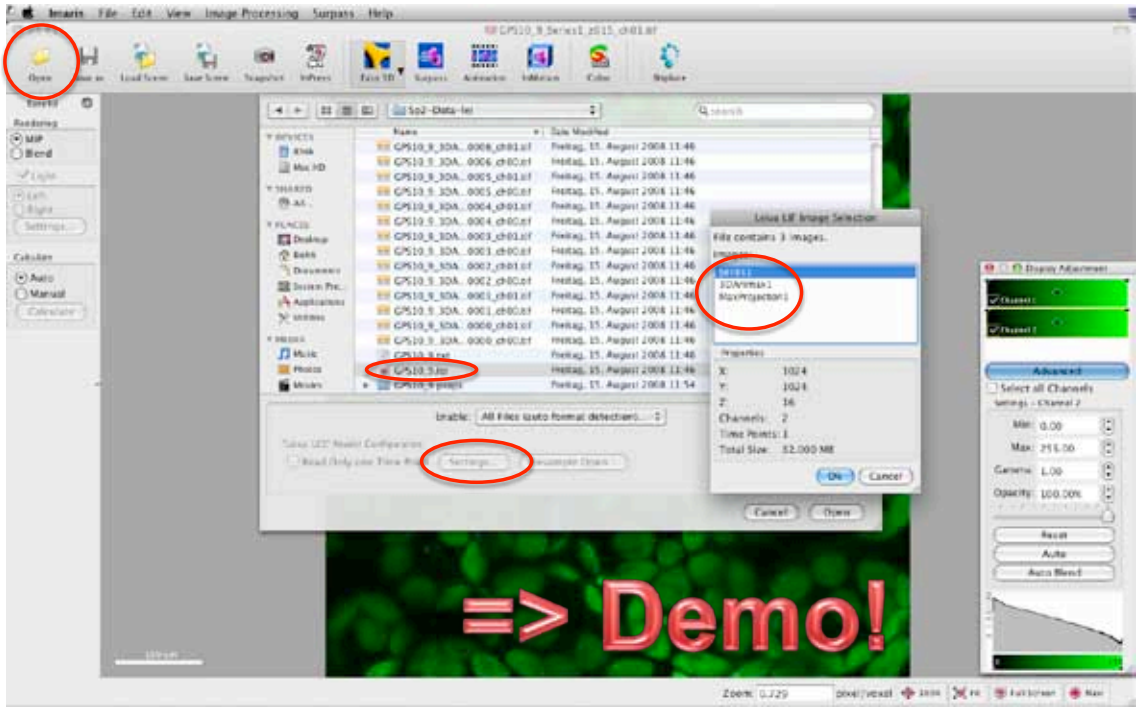
The Imaris program surface



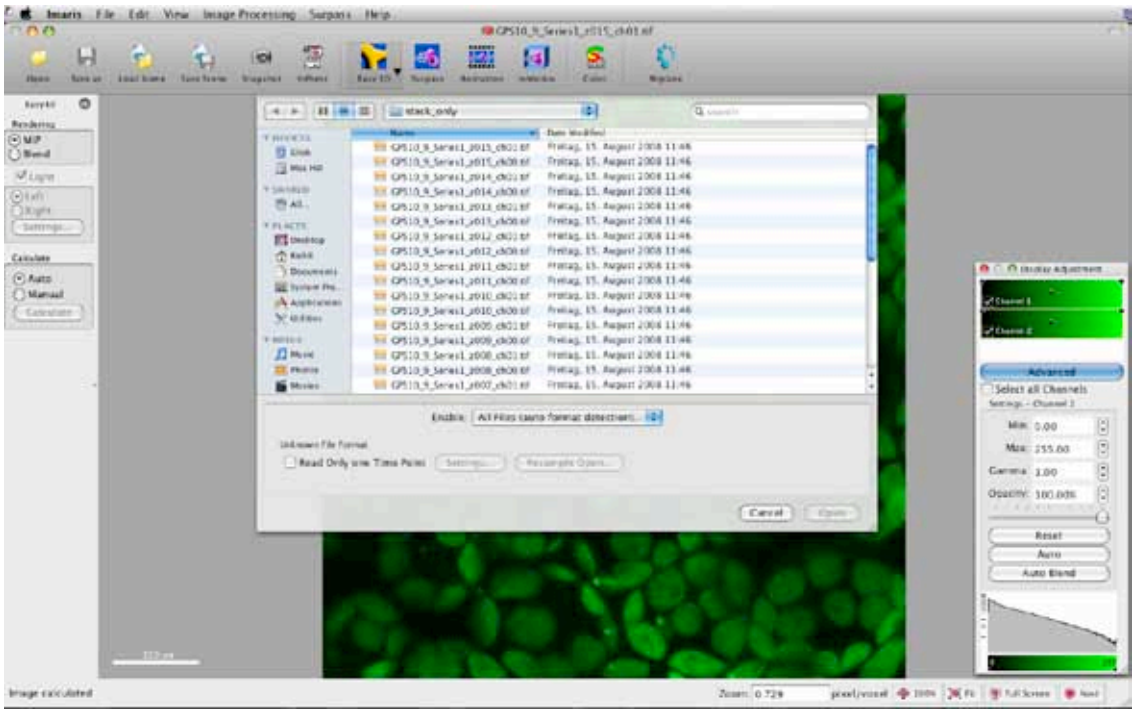
Loading 3D images (xyz) – from native mic. File



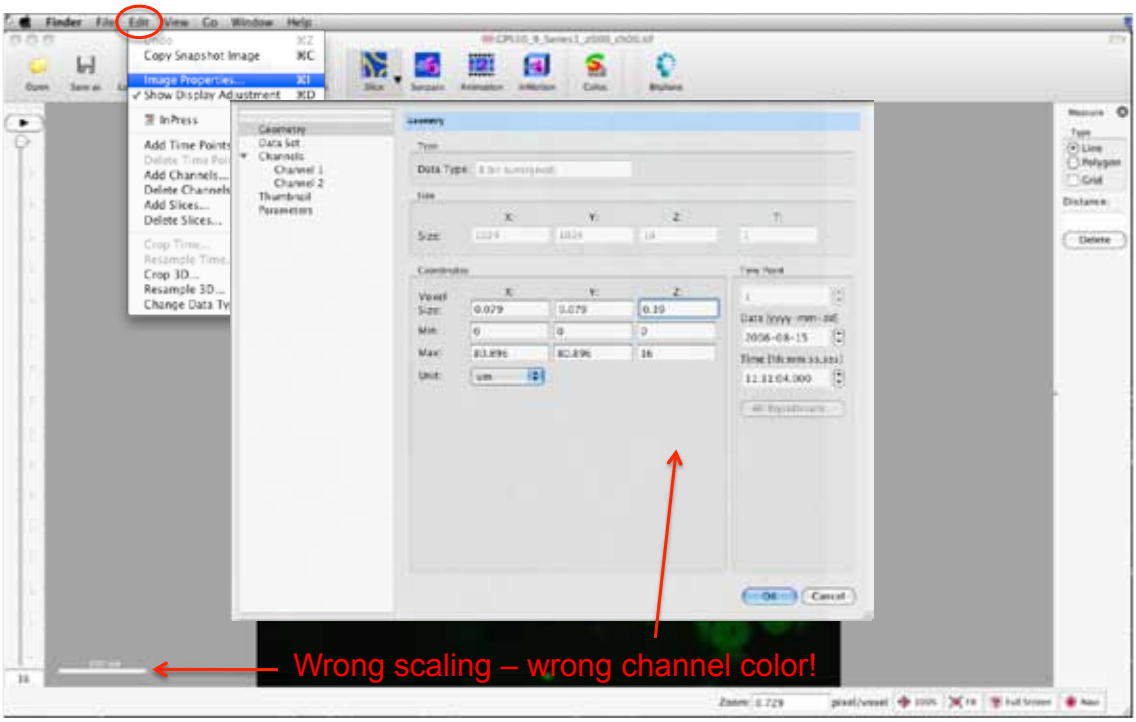
Loading images – from native mic. files (settings)



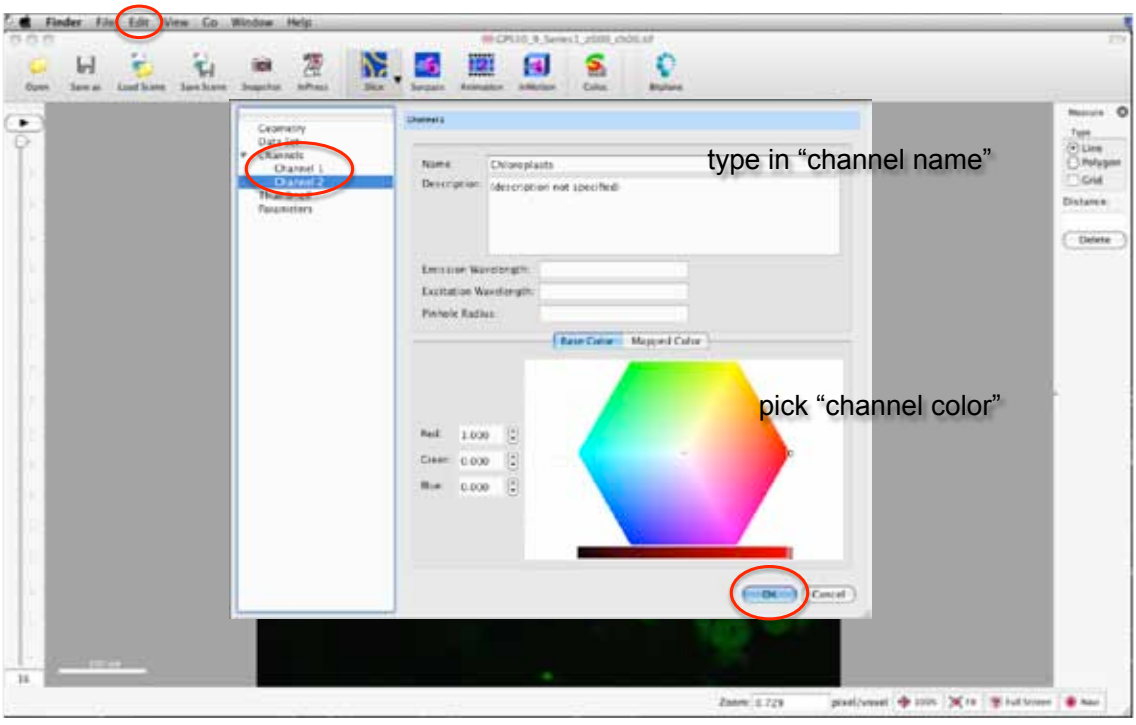
Loading 3D images (xyz) – from stacks (tif or jpg)



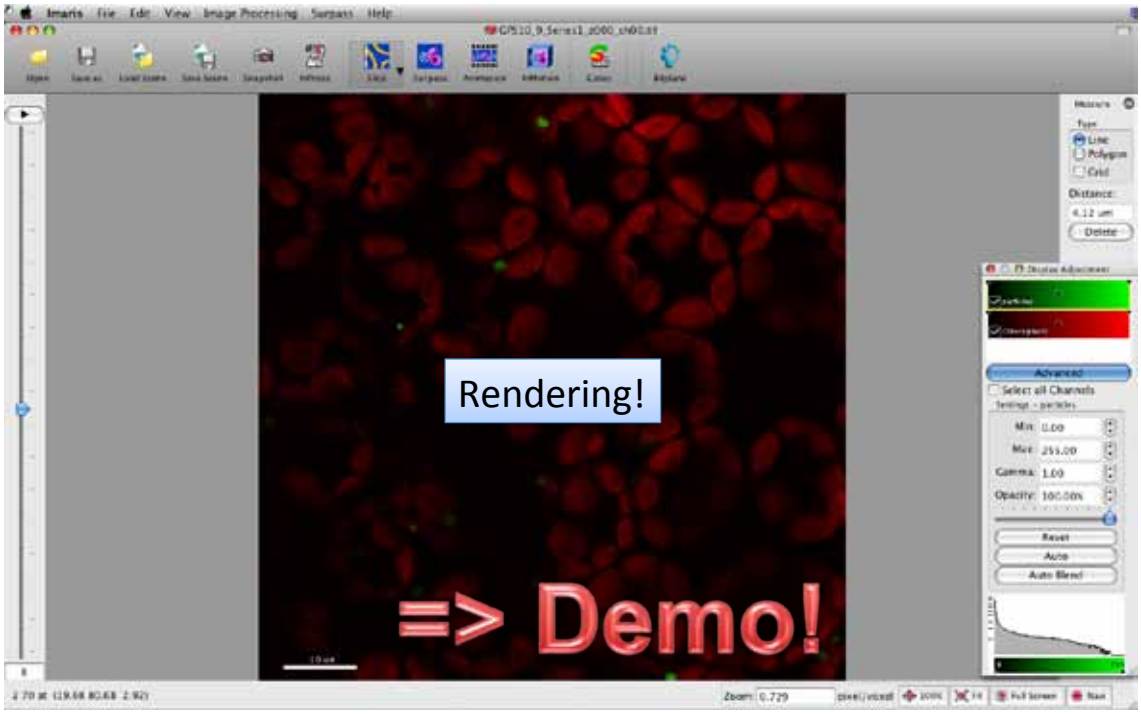
Edit image scaling



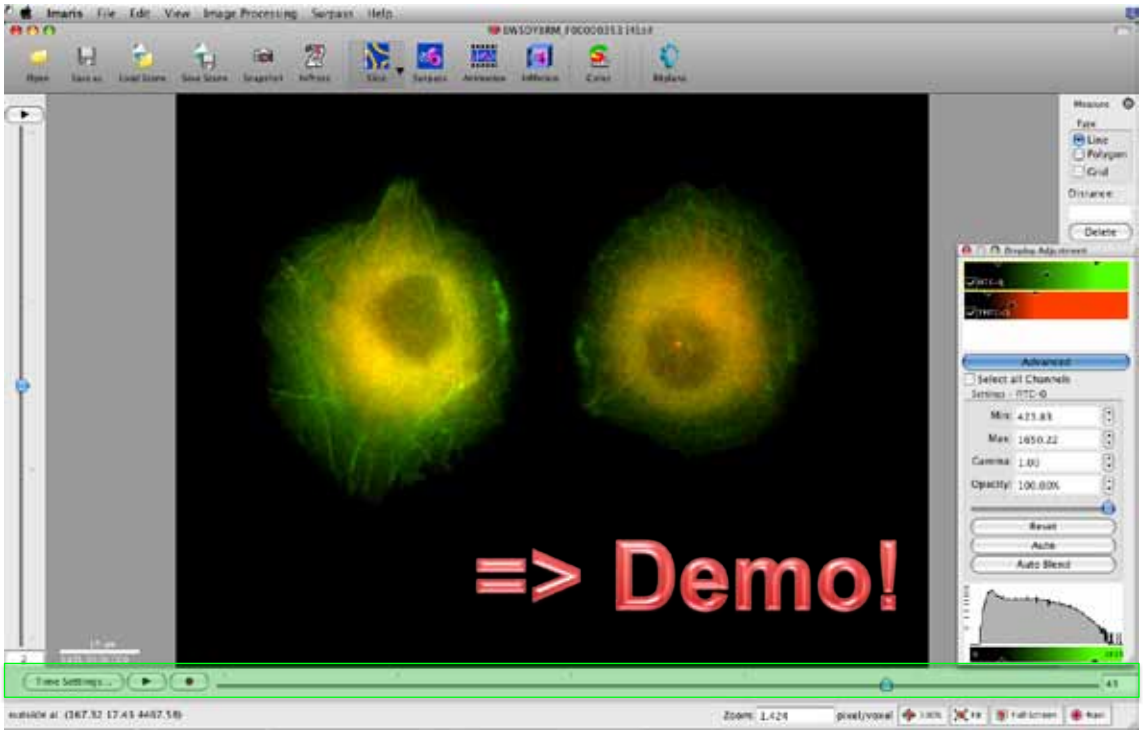
Edit image color



Edit - resample

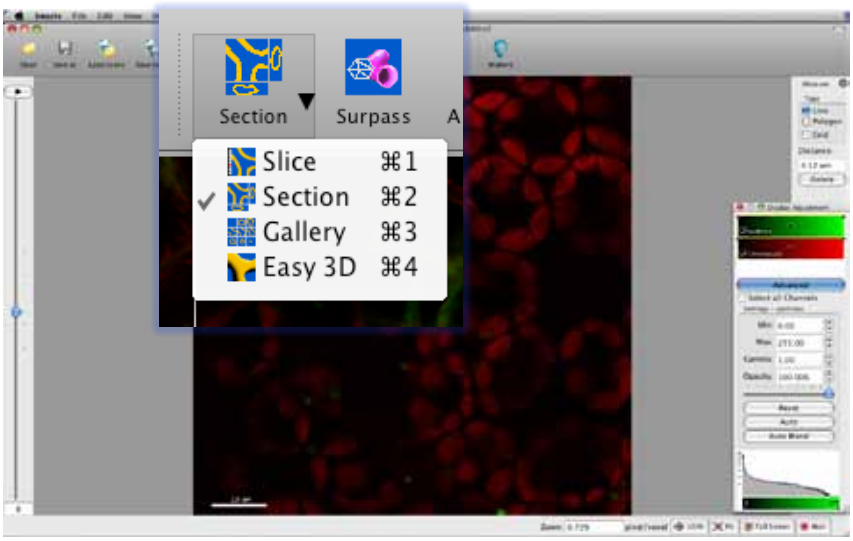


Loading 4D images (xyzt)

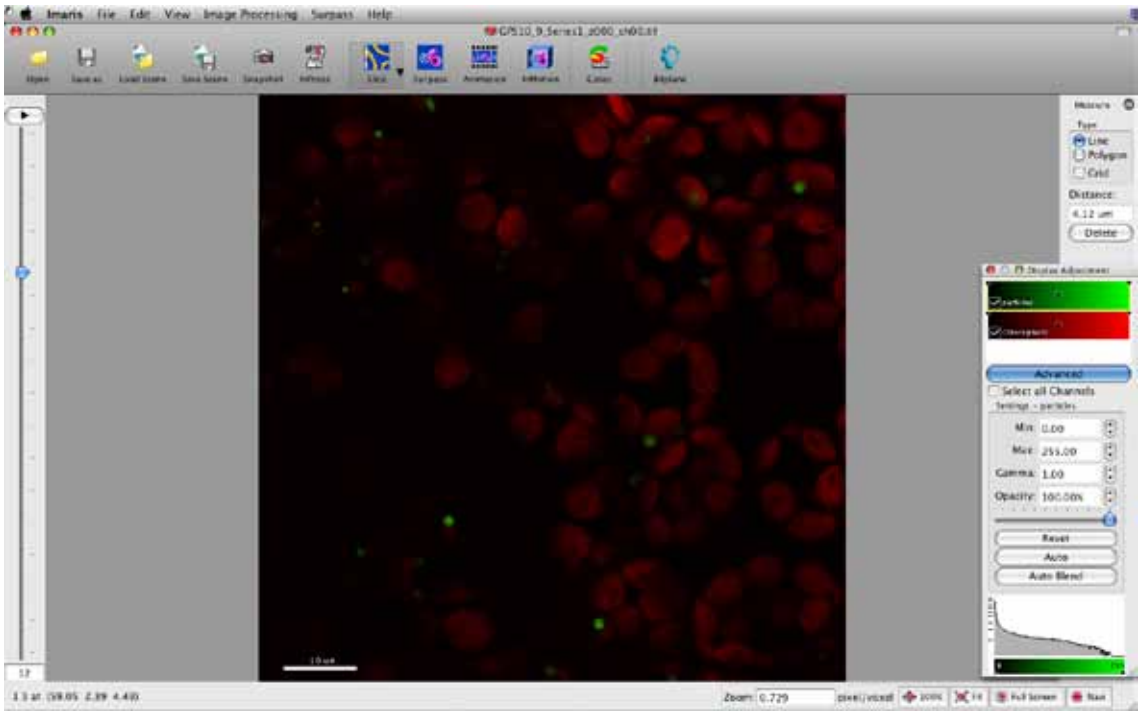


Basic visualization features

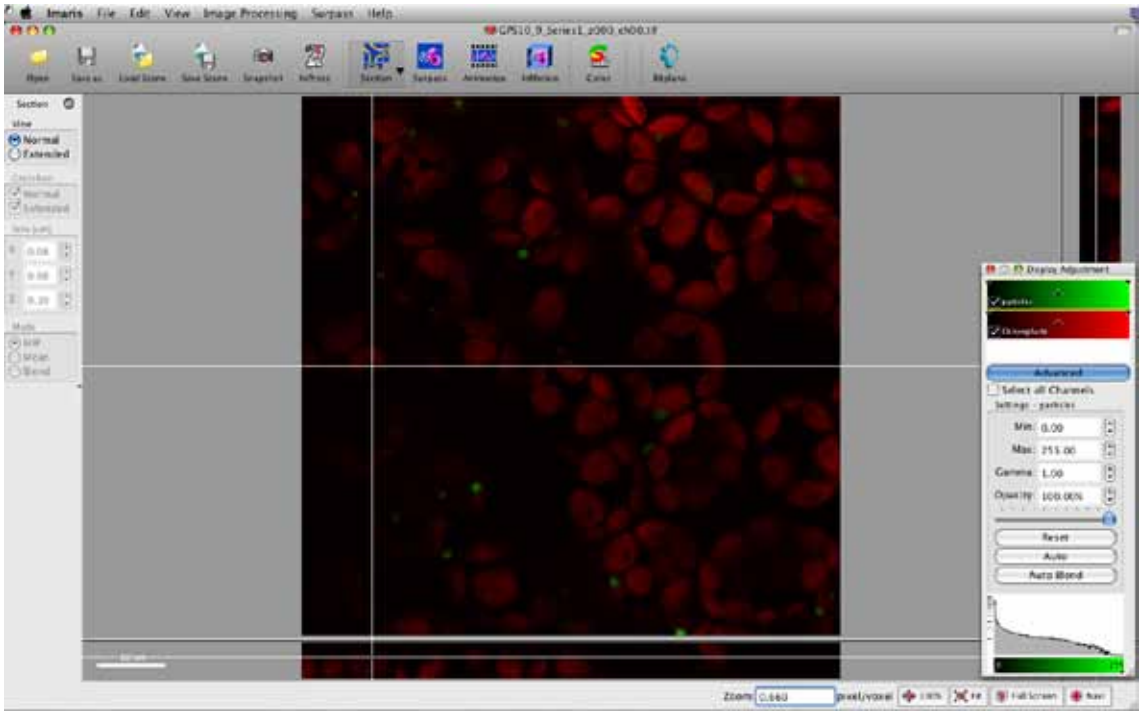
- “Slice”
- “Section”
- “Gallery”
- “Easy-3D”



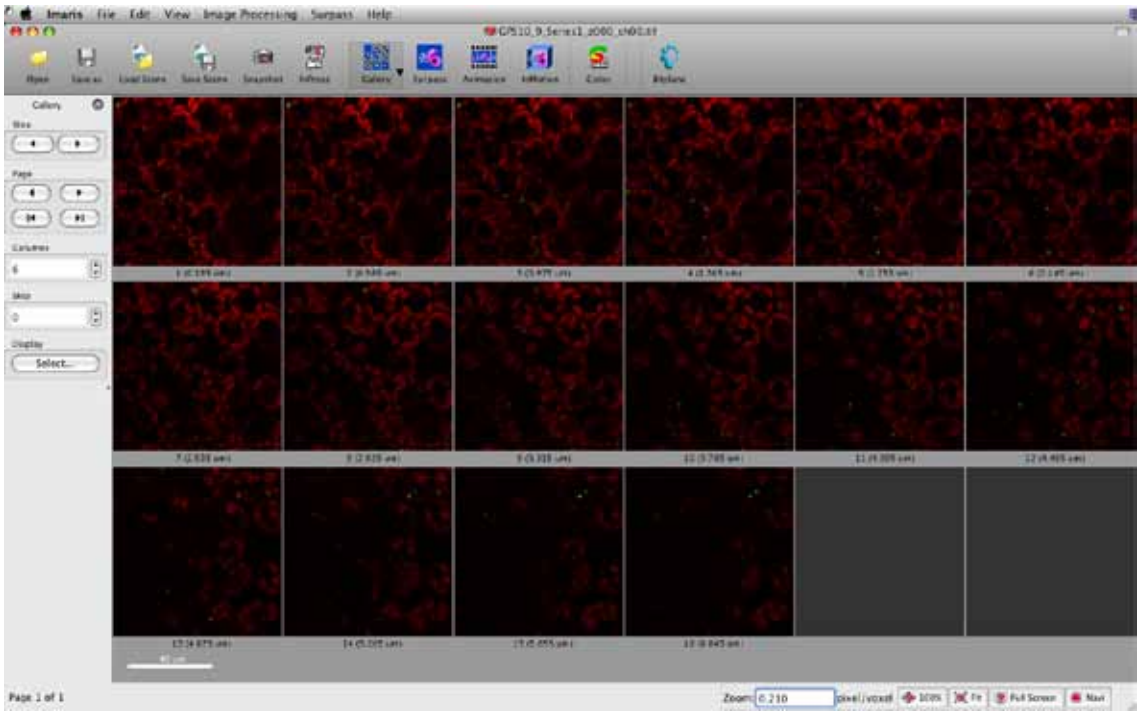
“Slice” mode



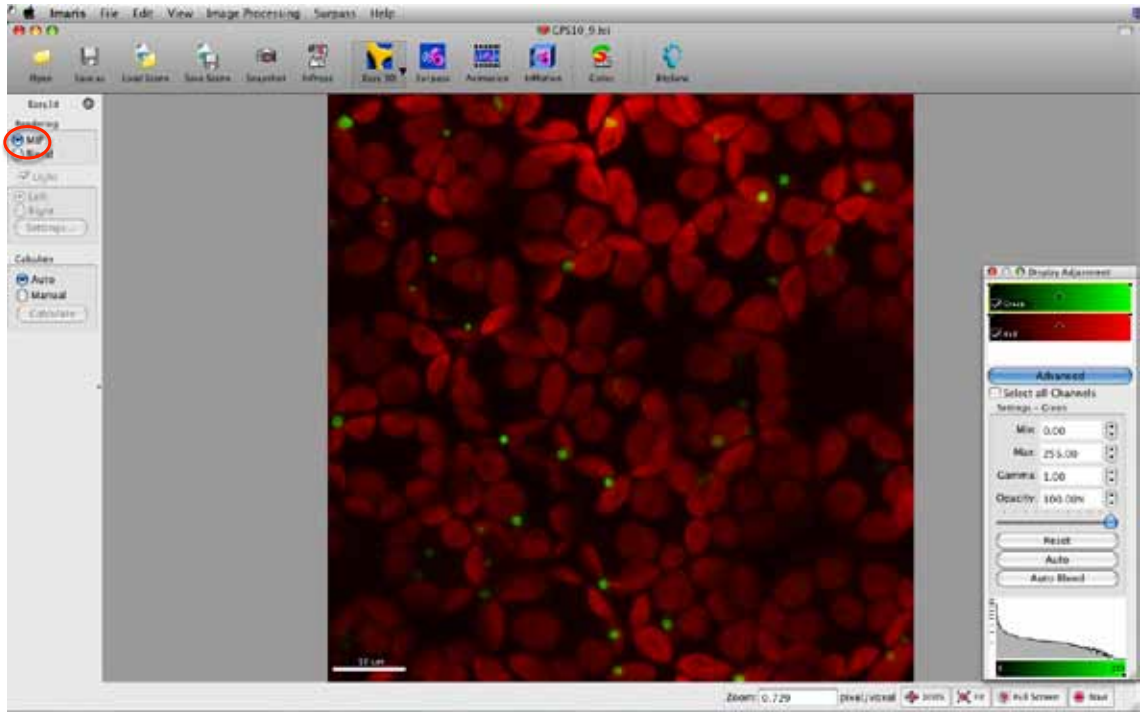
“Section” mode



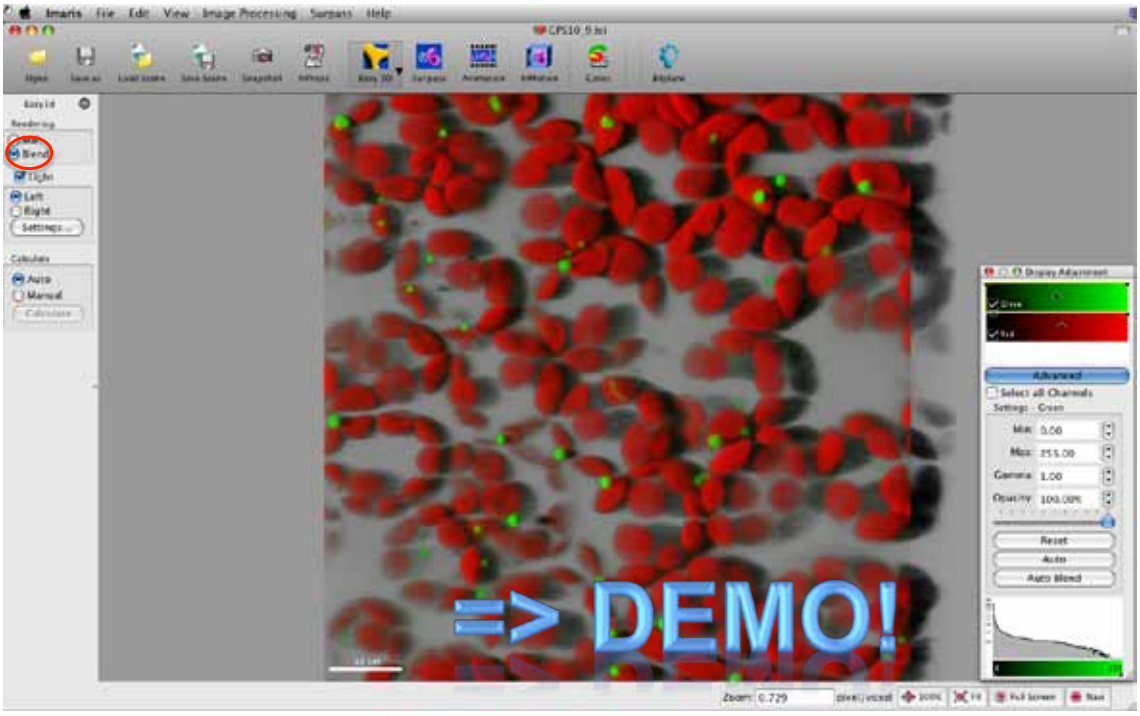
“Gallery” mode



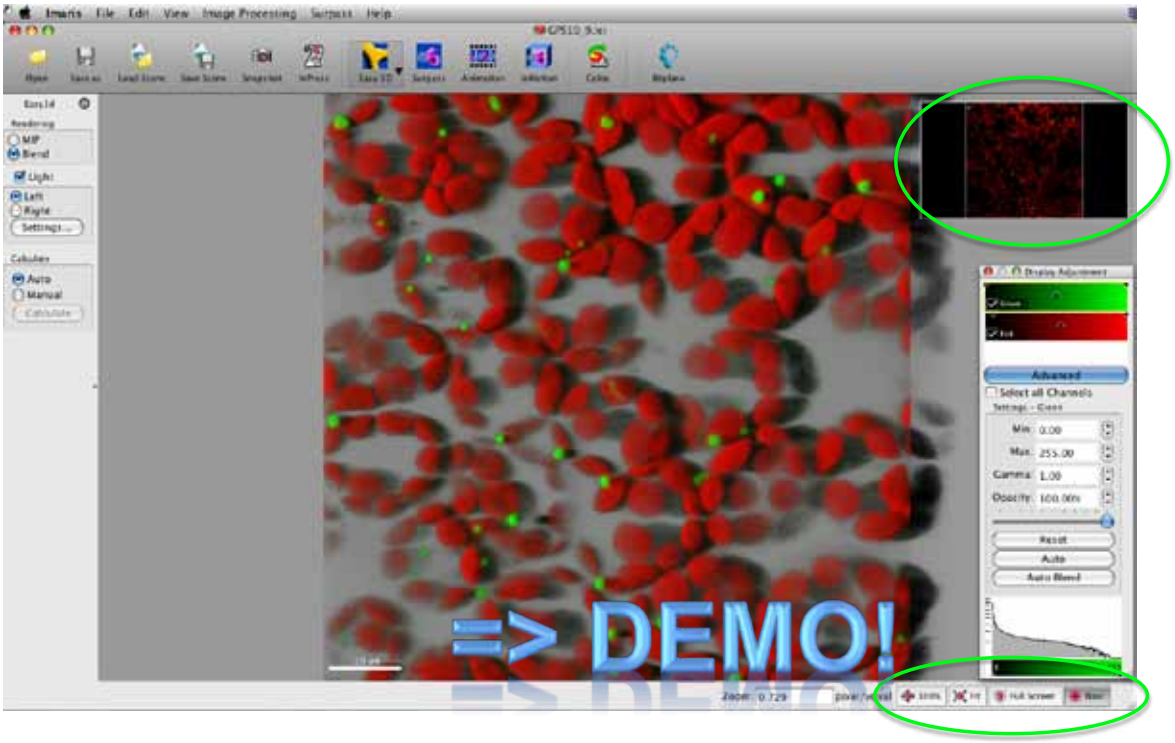
“Easy-3D” mode – MIP
(Maximum Intensity Projection)



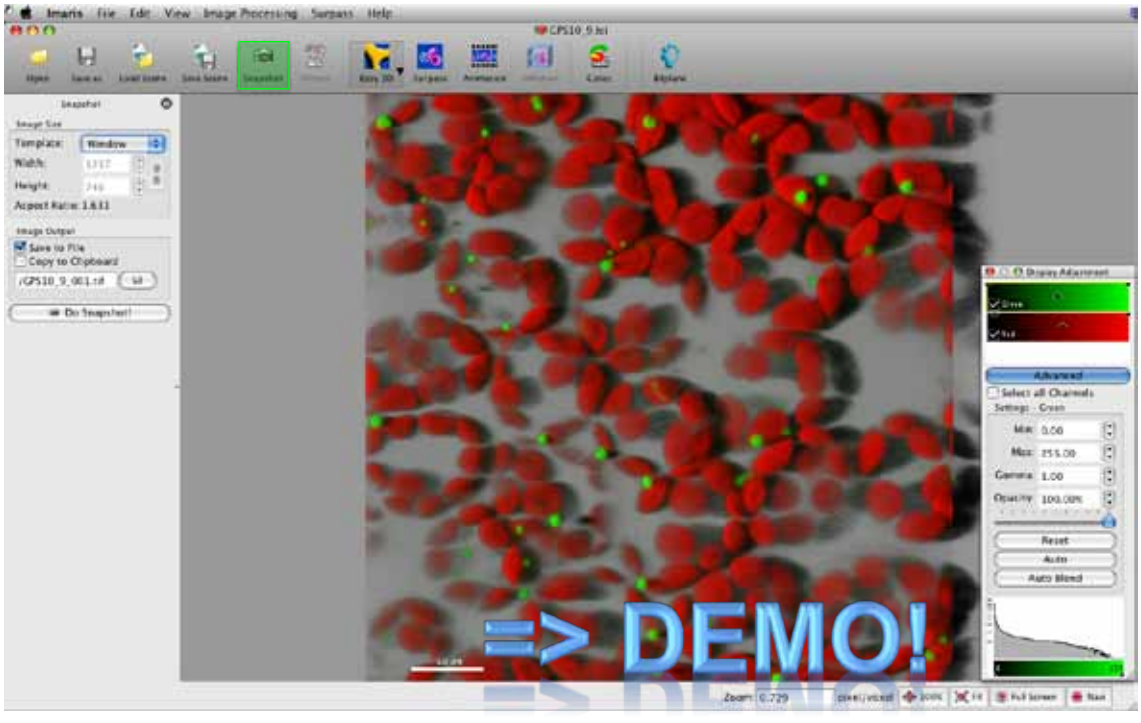
“Easy-3D” mode – Blend



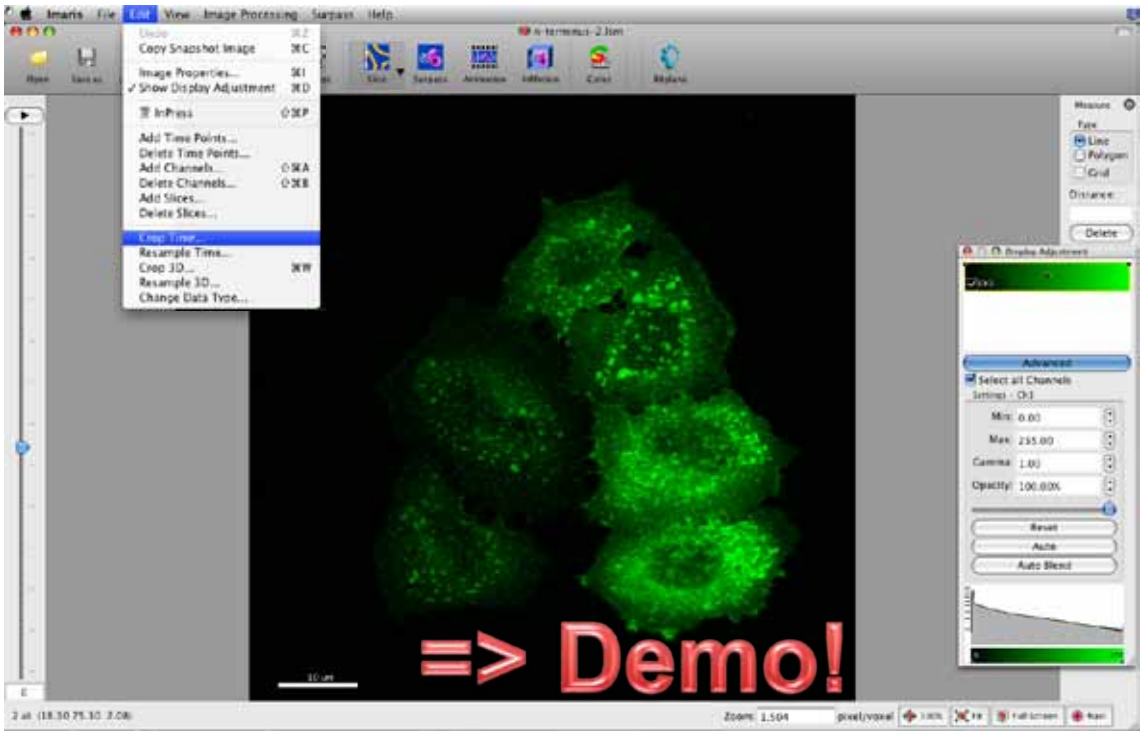
Display settings



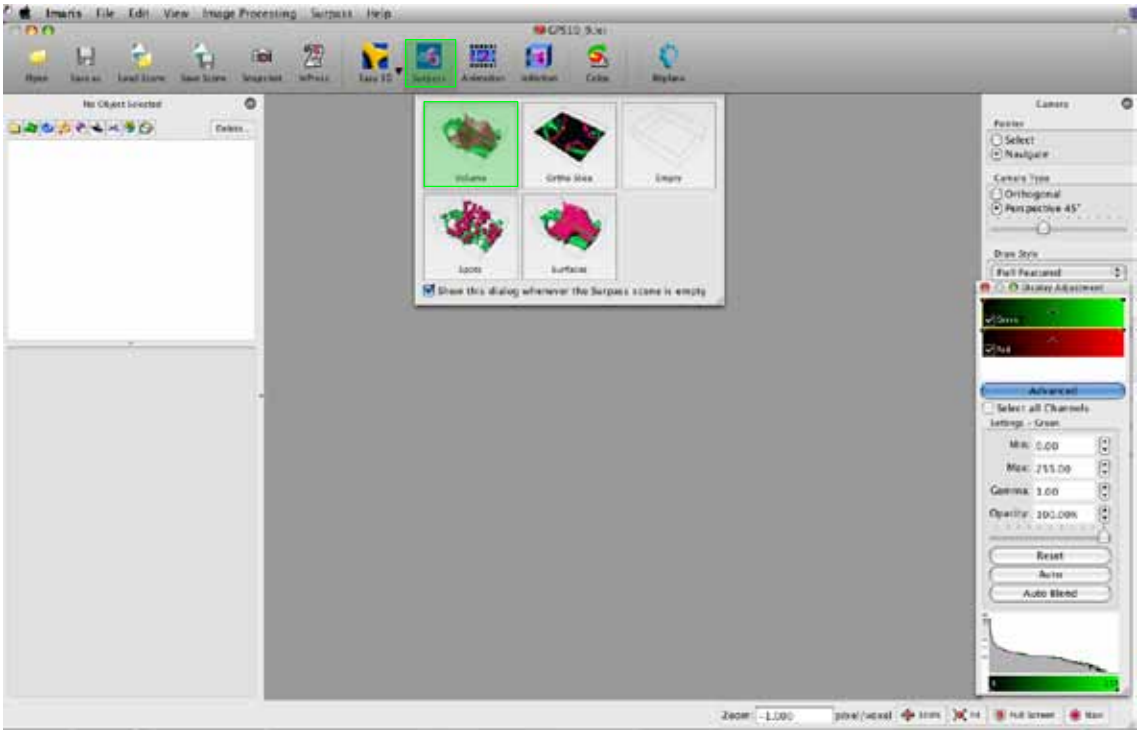
“Do a snapshot”



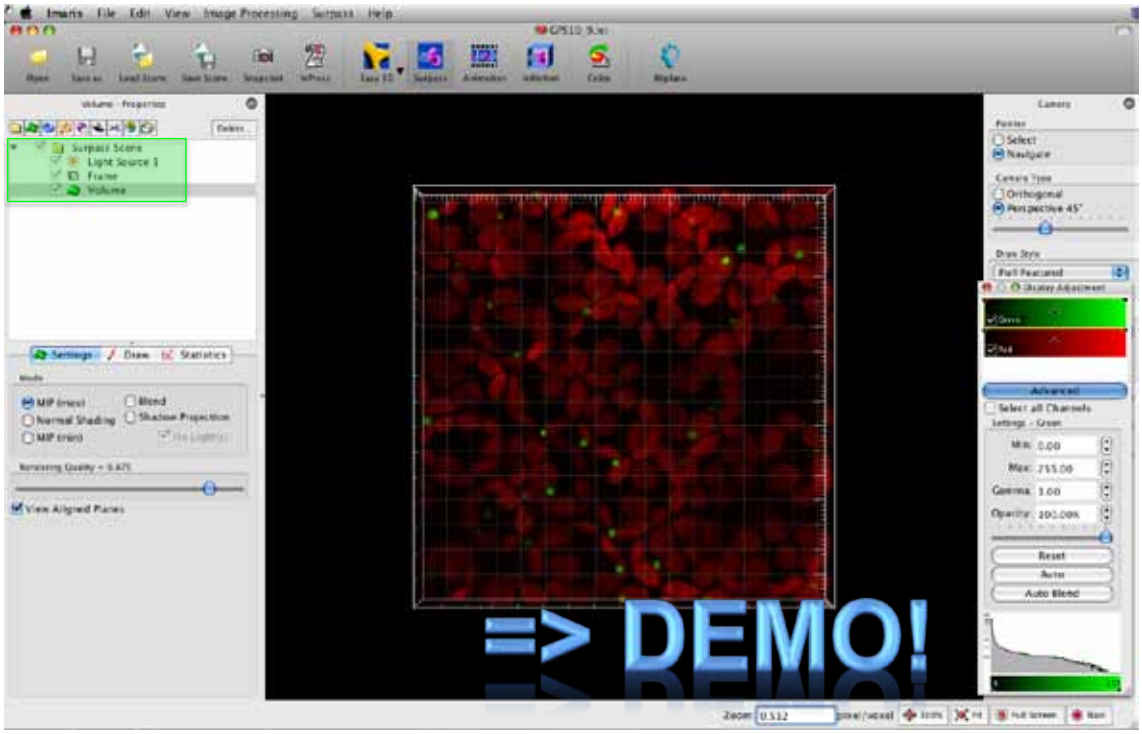
Crop an image (3D and time)



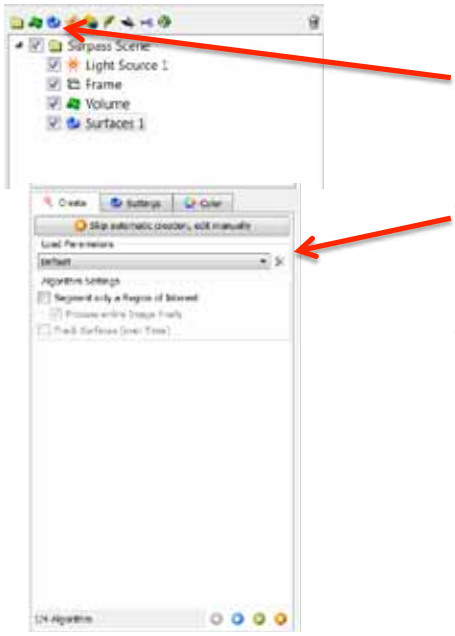
Surpass



Surpass - properties

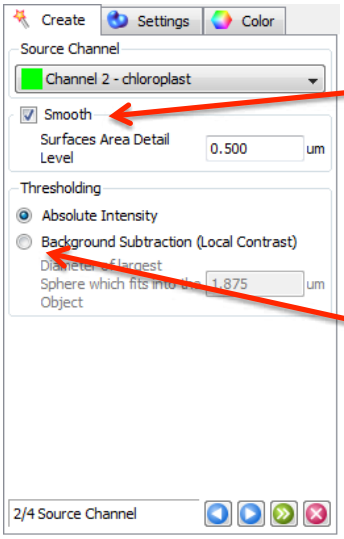


Surpass – surface rendering



- Press the surfaces button to initiate the wizard
- Choose the algorithm settings and click next
- If you want to skip the automatic creation and immediately work manually press the edit button.

Surpass – surface creation wizard



- Smoothing applies a Gaussian filter to the data set, most often to reduce noise.

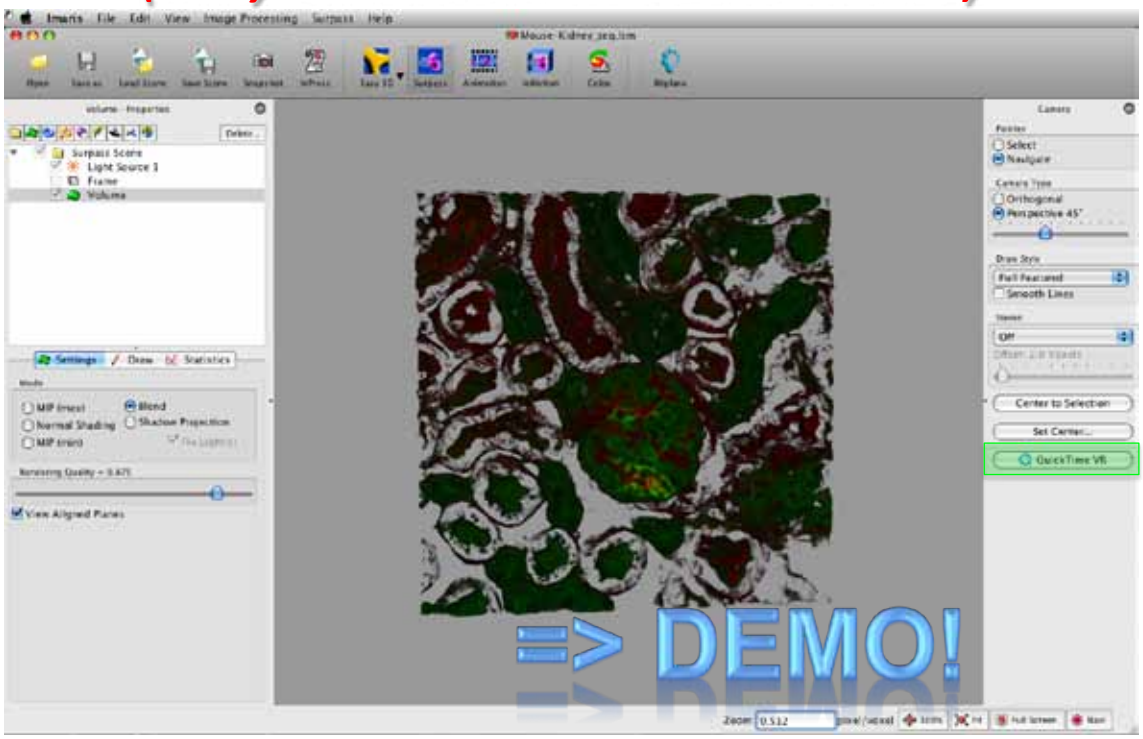
  Smoothing can result in the loss of small objects/details, so reduce the value or disable if the dataset contains very small features

- Use background substraction, when a single absolute threshold will not segment all objects.

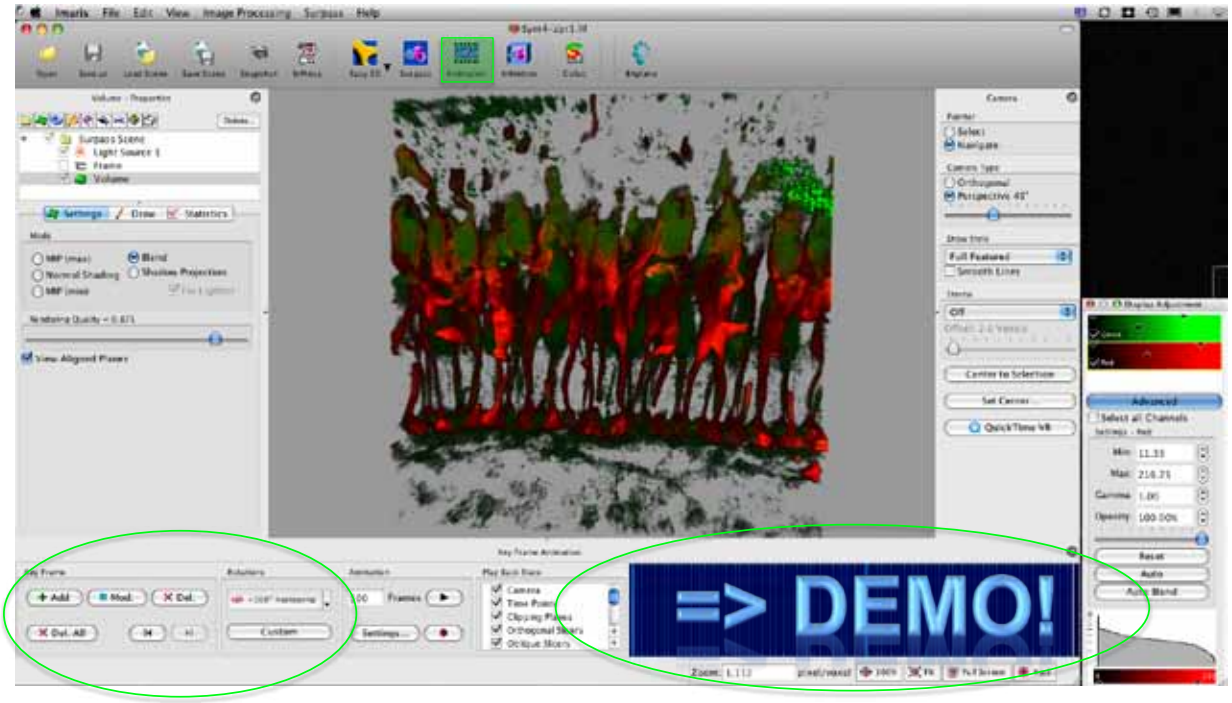
=> DEMO!

Surpass – *create a VR-file*

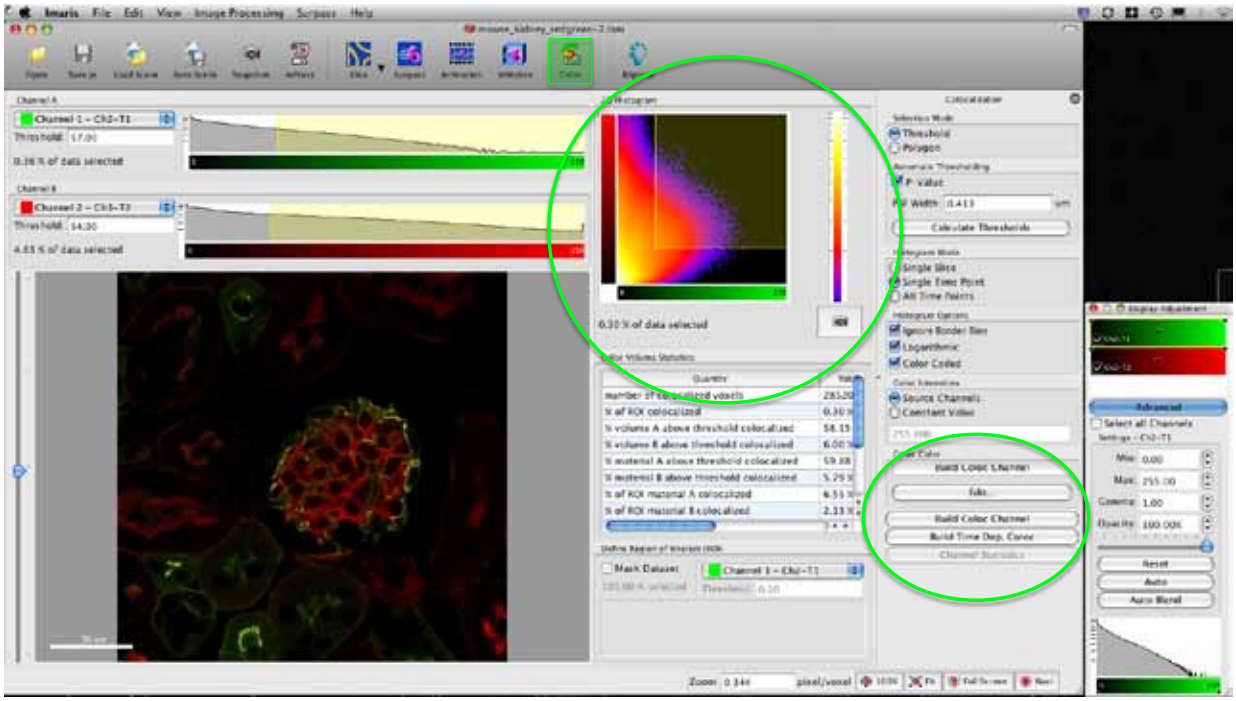
(only available in 32bit-mode)



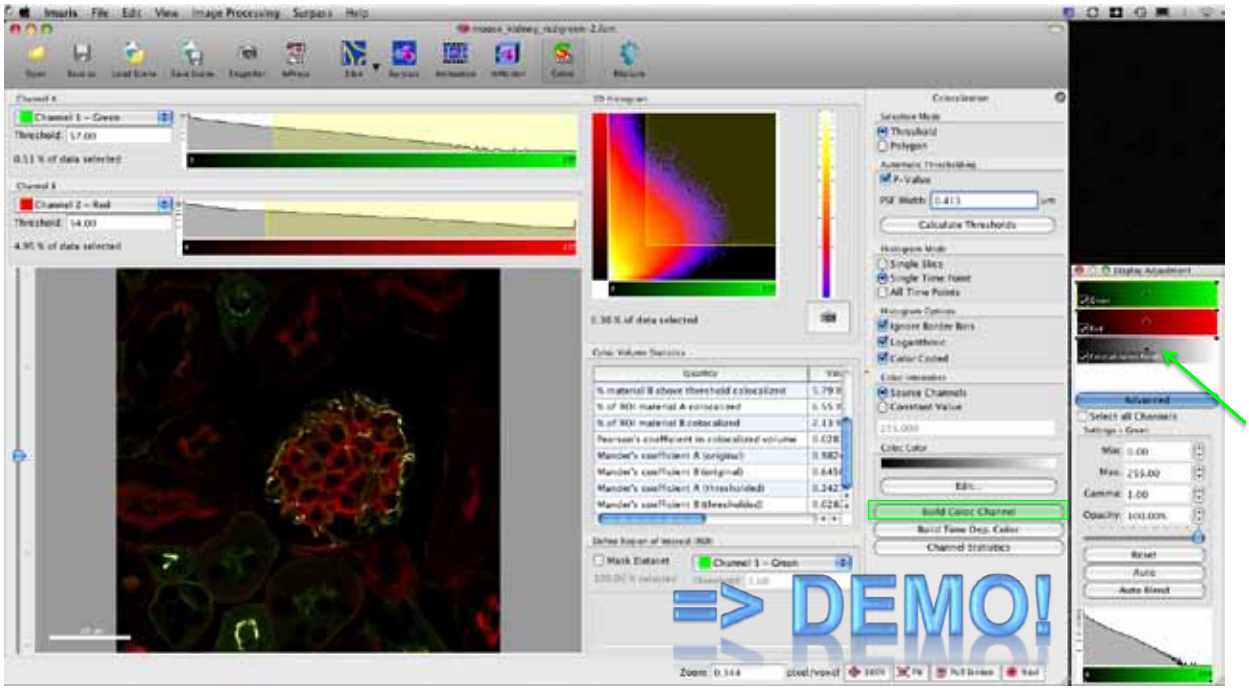
Animation



Colocalization *(quick-and-dirty)*



Generate colocalization channel



Hands-On

Imaging Core Facility

08.03.2012

Imaris course
by Oliver Biehlmaier, Alexia Ferrand, Niko Ehrenfeuchter

Basic Imaris functions – hands-on:

1) Loading images – from native mic. Files
use files from folders:
   - *"Raw-Data/SP2_Chororphyll_GB/Sp2-Data-lei/GPS10_9.lei"*
   - *"Raw-Data/SP5_Synt4-zpr1-ad/Synt4-zpr1.lif"*
   - *"Raw-Data/ZEISS510_NLO-Kidney/Mouse-Kidney_seq.lsm"*

2) Loading images – from stacks (tif or jpg) and use "Edit" to alter scaling and channel color
Use:
   - *"Raw-Data/SP2_Chororphyll_GB/stack_only"*
   - *"Raw-Data/MM_SpinDisk/SpinningDisk-kidney"*

3) Loading time lapse images and do a time lapse movie
Use:
   - *"Raw-Data/Timelapse_Cell^R/Alexas_cells.tif"*

4) Use the basic visualization features ("Slice", "Section" "Gallery", "Easy-3D") on the example files in the Raw-Data-Folder

5) "Save scene" and "do a snapshot"

6) Load a 4D file and crop 3D and time
Use:
   - *"Raw-Data/Timelapse_Cell^R/Alexas_cells.tif"*

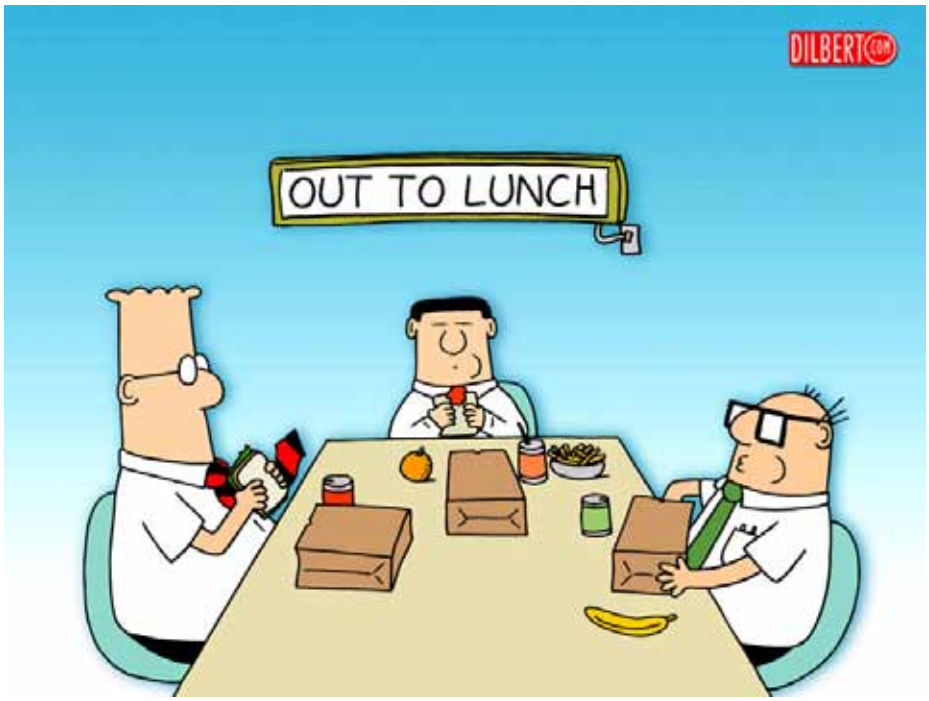
7) Use "surpass - volume" to do volume rendering on different raw data files on the course DVD and do snapshots.
Is there a difference to the snapshots you did before?

8) Do "Animation" on the "surpass-images" and export/save them in different formats (avi, mov. Vr, etc.)

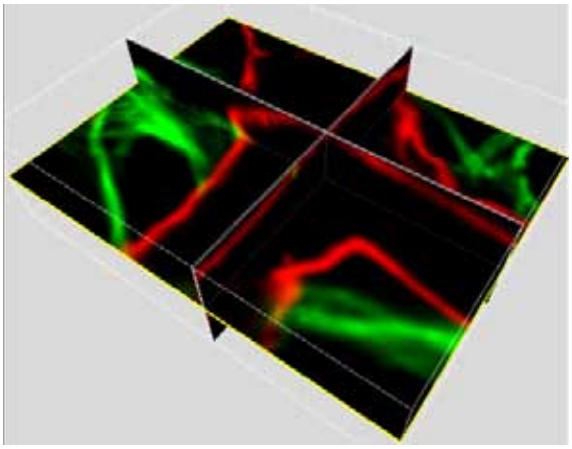
9) Load a file that contains probable colocalization and use Imaris' "colocalization tool" on the dataset.

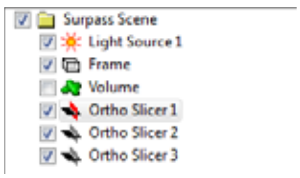
10) Analyze your own data by using the above features.

Lunchbreak (until 13:30)

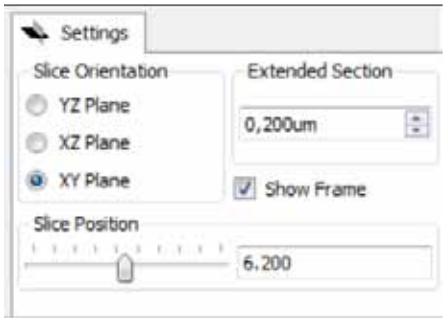


Ortho Slicer

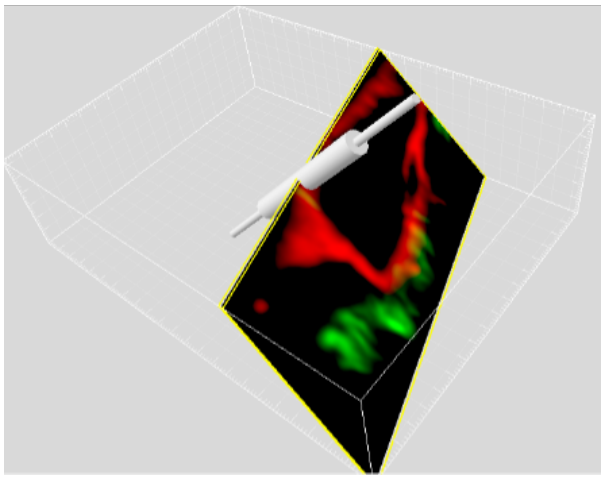




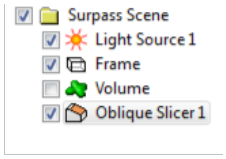
- Orthogonal plane on which original data are projected
- Can be moved within the dataset
- "Extended section" adjusts slices thickness

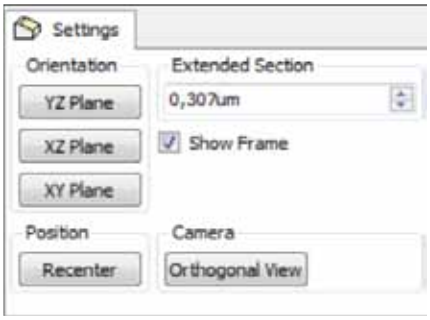


Oblique Slicer

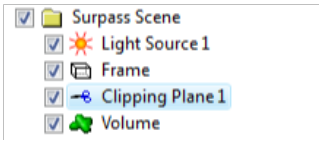


- Plane on which original data are projected
- Can be freely moved and rotated within the dataset (similar to Clipping Plane)
- “Extended section” adjusts slices thickness

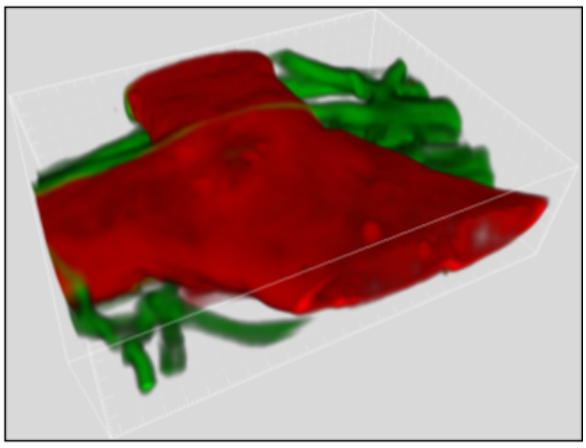


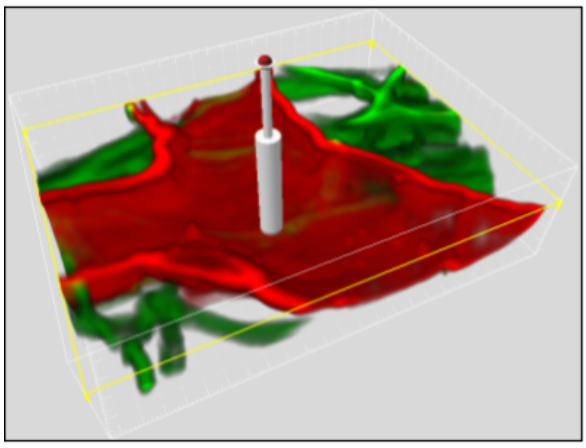


Clipping plane

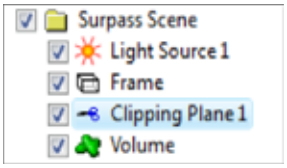


- Cuts away objects on one side of the plane
- Allows you to look inside any object
- Can be freely rotated

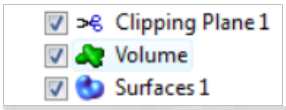


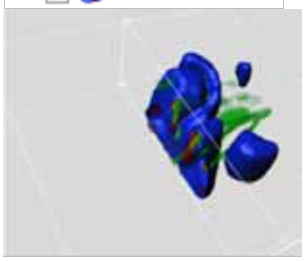


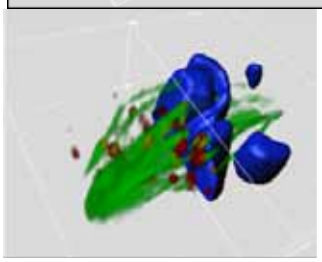
Clipping Plane

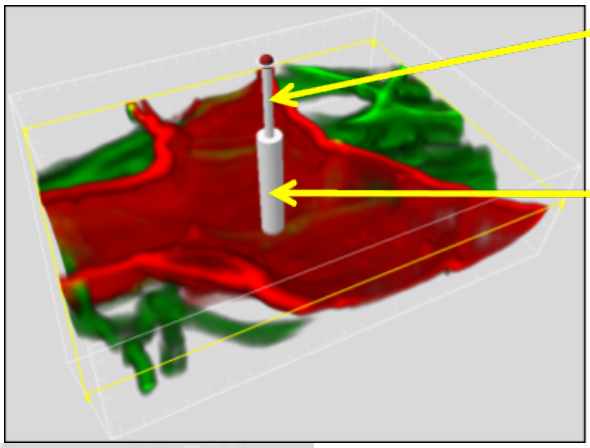


Only cuts objects lower in Imaris tree





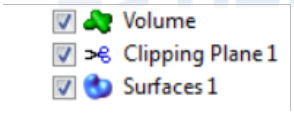




Use this rod to freely rotate the plane

Use this rod to change the position of the plane

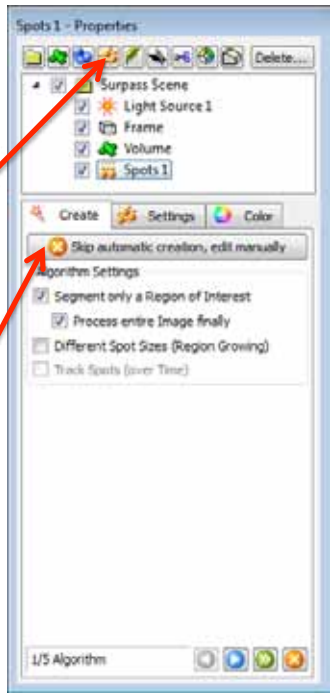
=> DEMO!



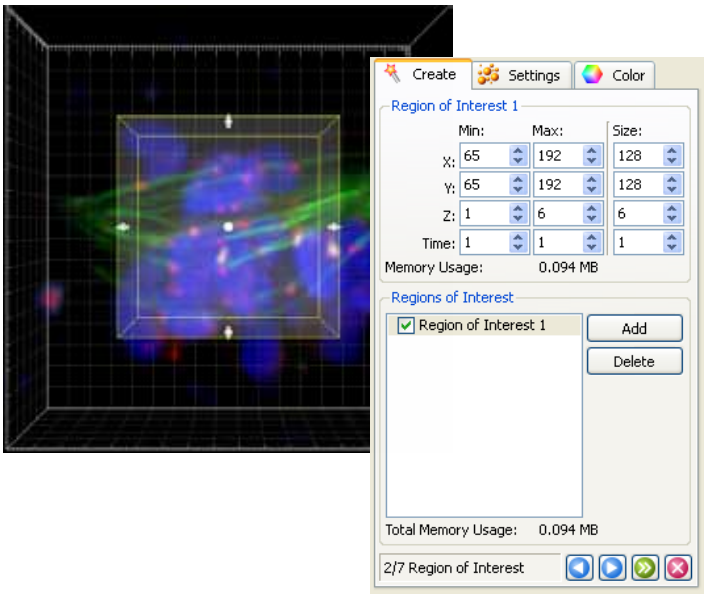
Spot Detection

For automatic segmentation of objects, imaris uses creation wizards which guide you through all the steps of the process and and facilitate the use of complex functionality

- Press the spots button to initiate the wizard
- Choose the algorithm settings
- If you want to skip the automatic creation and immediately work manually press the edit button.



Sub Region Processing

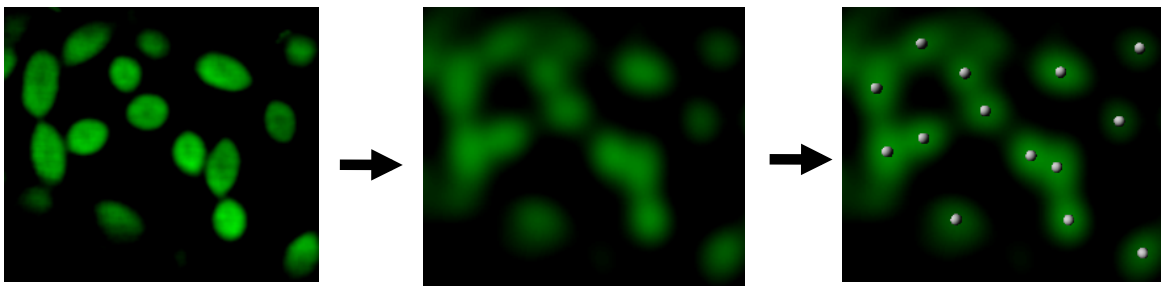


- Position region of interest (ROI)
  - Pointer must be in Select mode to drag the box or arrows
- Option of applying parameters to multiple ROIs with “Add” button.

Spot Detection / Calculation

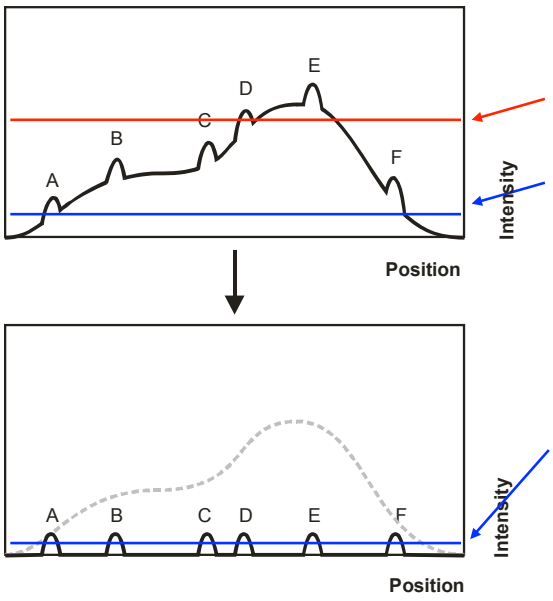
Spot Detection works as follows:

1. Filters (smooth) the image with the Gaussian or Mexican Hat** filter.
2. Spots are located at local extrema of the filtered image.



**often used technical term for what is called *Background Subtraction* or *Local Contrast* in Imaris

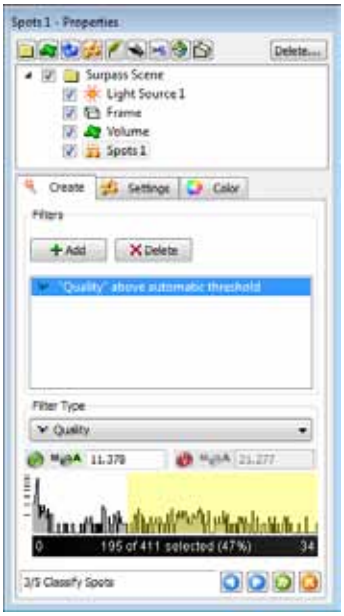
Mexican Hat Effect



- Often the objects in the image do not have the same intensity
- Threshold too high: dim objects will be missed
- Threshold too low: too much background is above the threshold

- Background is now lower than all the object peaks
- A single threshold height could now include all the objects, and exclude all the background

Spot Detection / Classification



- The initial threshold is intelligently set by Imaris based on your data
- It is calculated from all spots based on k-means statistical method.
- Increasing or decreasing the threshold value will allow you to include or exclude spots in the image
- As you drag the threshold value, the image will change interactively as spots appear and disappear
- When satisfied with the Spots, click Next.

=> DEMO!

Tracking

1) Select from 4 Algorithms

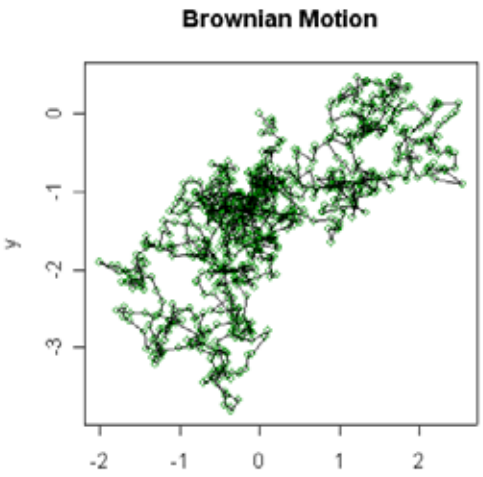
- **Brownian Motion**
- **Autoregressive Motion**
- **Autoregressive Motion Expert**
- **Connected Components**

2) Specify Tracking Parameters

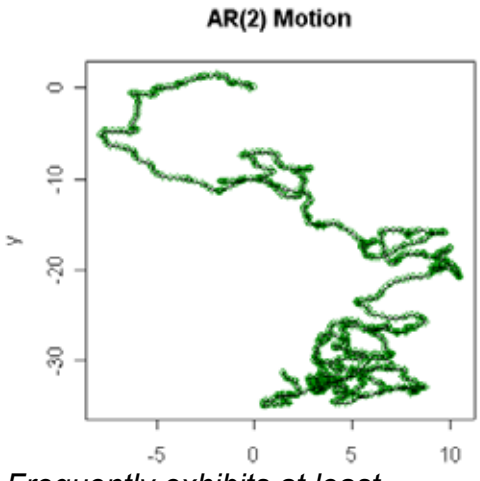
- "maximum distance"
- "gap size"

3) Create Track

Tracking Motion Models

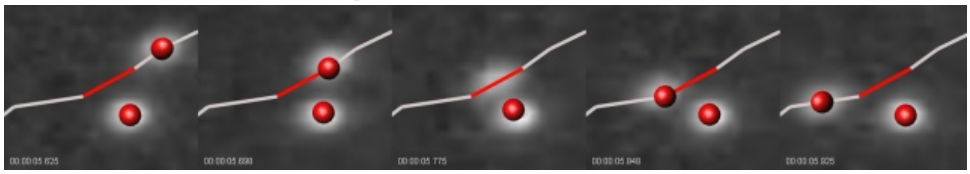


Very rarely exhibits momentum / direction



Frequently exhibits at least temporary momentum / direction

Tracking – Max Gap size



Gap Problem:

•Object segmentation can fail at certain time points. (Two 'spatially close' objects will sometimes not be recognized as two distinct bodies in the image).

Resulting Issues with Tracks:

•Single object is separated into two or more tracks which leads to:
- Invalid Statistics
- Manual track correction (extra unwanted work)

=> Demo!

To Correct This Issue:

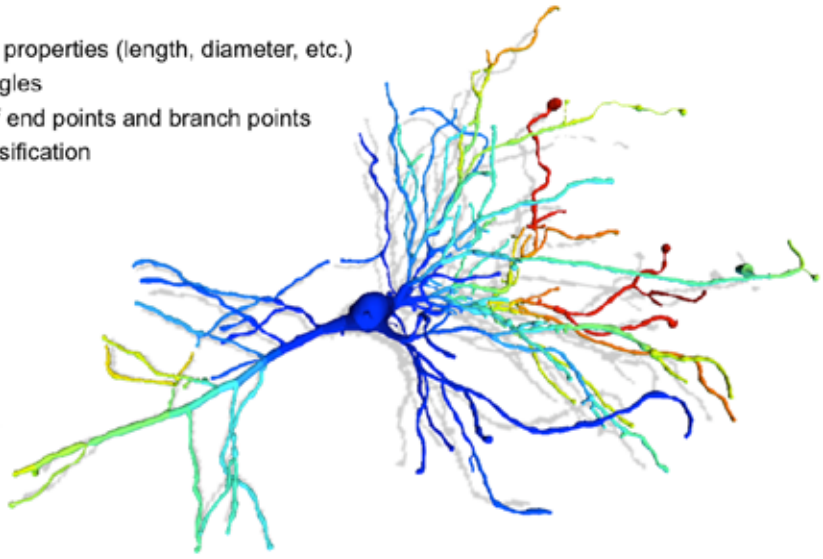
•*Gap Close to the rescue!*

The gap closing algorithm allows the unrecognised objects to reconnect with their original track, should it reappear at a future time point

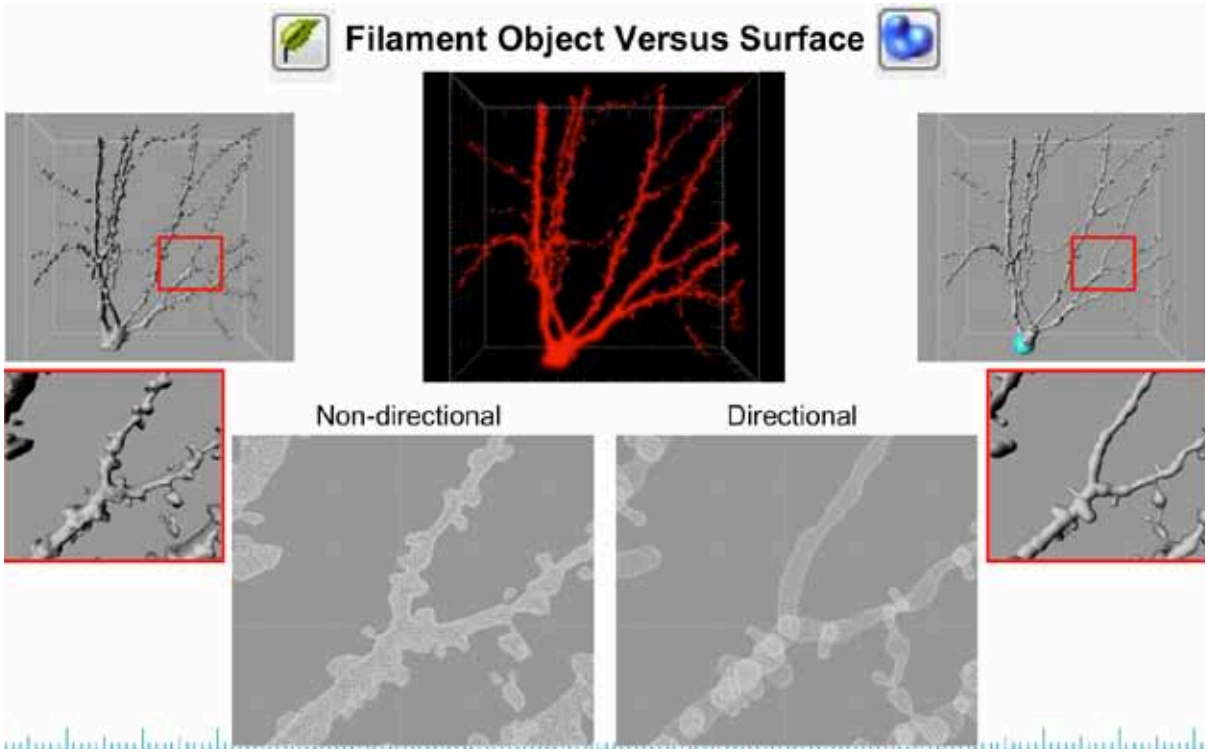
Filament tracer

Allows for measurements of neuronal or other branching structures (eg. Mycorrhiza, blood vessels, mitochondria network etc.)

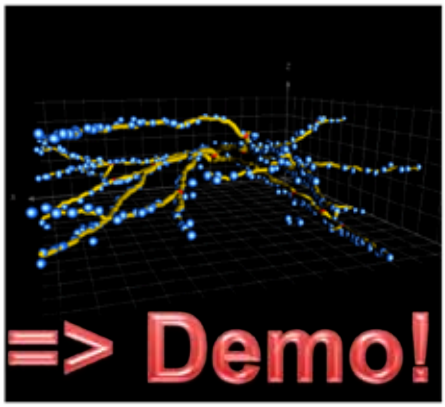
- Segement properties (length, diameter, etc.)
- Branch Angles
- Number of end points and branch points
- Spine classification
- Etc.



Filament tracer



Filament tracer – tracing methods



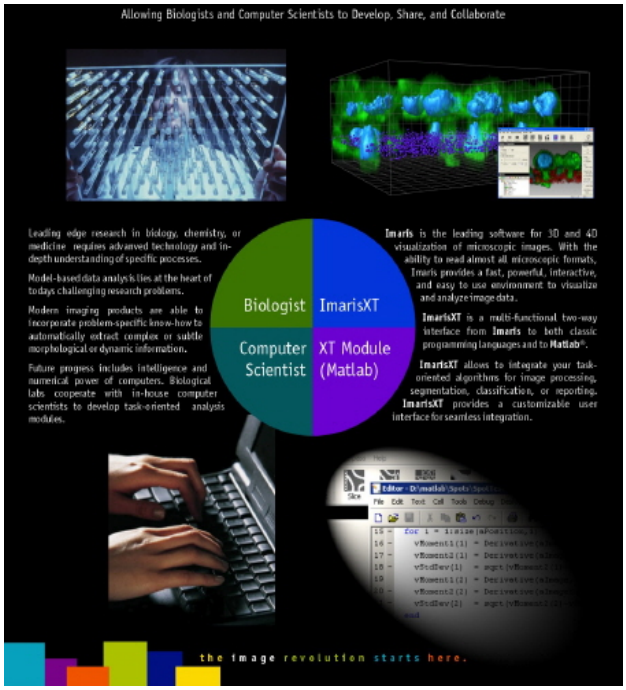
Multiple tracing methods to ensure accurate results:

1. Automatic detection
   a) Fully automatic detection based on intensity threhold (may have loops)
   b) Full automatic (creates tree without loops)
   c) Iterative Tracing
   d) Region of Interst (ROI) Tracing
2. Semi-Automatic - AutoPath
3. Semi-manual tracing - Autodepth
4. Manual with automatic Z placement

Unique ability to create and edit filaments using any combination of tracing modes + simultaneous visualization of tracing and raw data

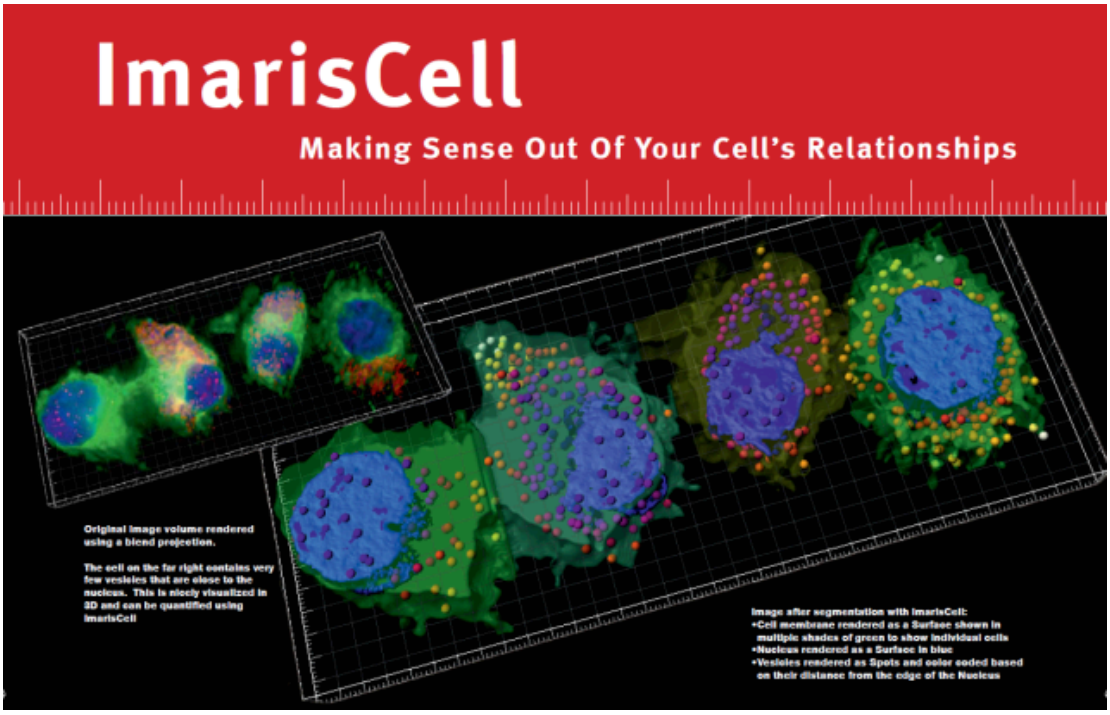
→Accurate and consistent tracing results

Imaris XT

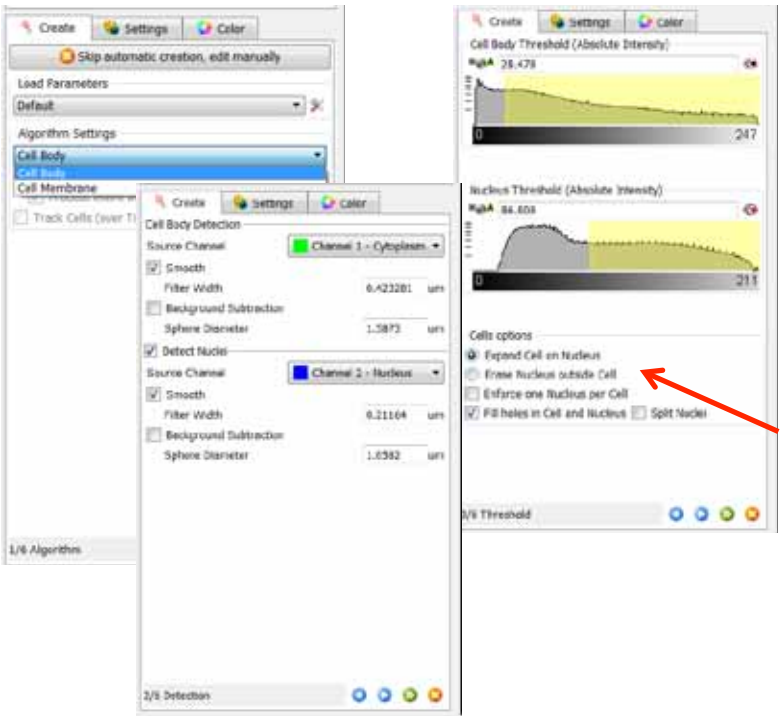


- Special module to exchange data between IMARIS and software used for image processing like:
  - MatLab
  - ImageJ

Imaris Cell

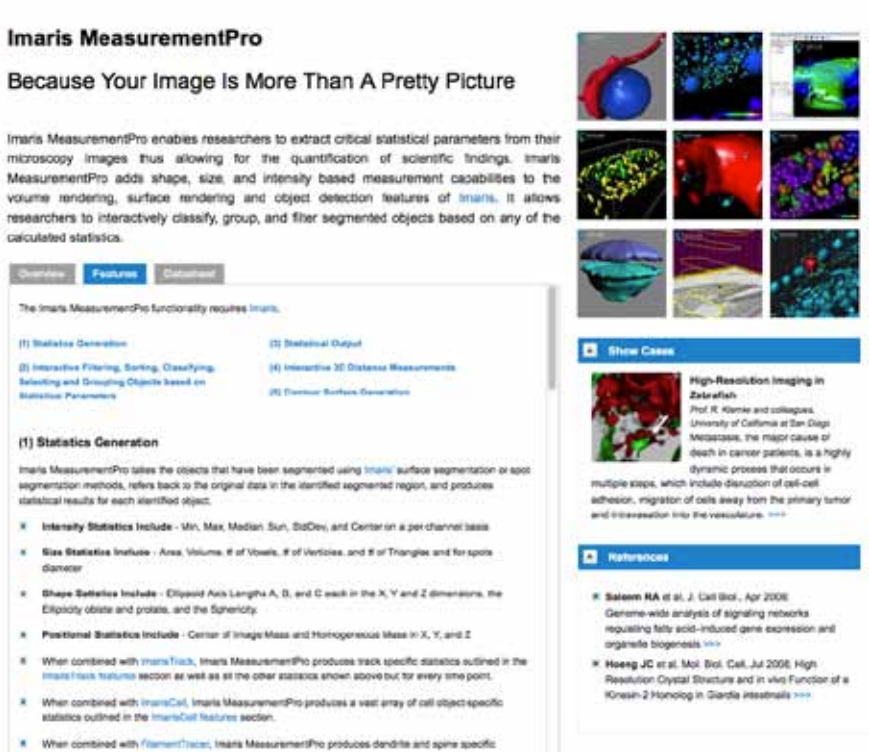


Imaris Cell



- The employment of wizards results in a massive reduction of interaction steps
- Most of the segmentation options will be familiar from Surfaces and Spots creation
- Simultaneous detection enables use of biological constraints (delete nuclei outside of cells, force one nucleus per cell).

Imaris Measurement Pro



Hands-On

- Use the following modules on the given examples or on your own data:
  - Slicers & clipping plane
  - Spot detection
  - Spot or surface tracking
  - Filament tracer
  - All other modules…

The End

We hope that you are all experts by now!

Questions?

Thank you!