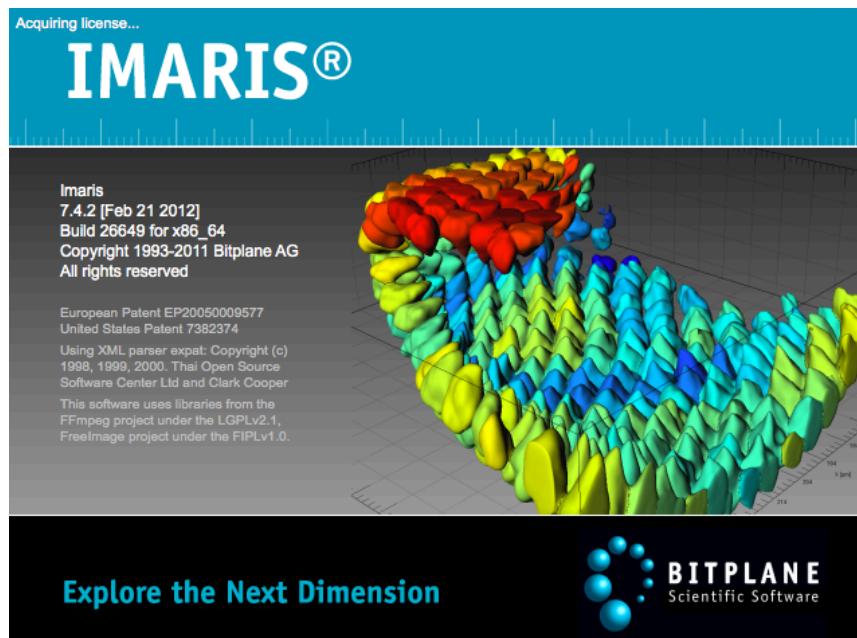


# Imaris Course

**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     |
| $2^8$     | 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 palettized 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 - proprietary

## 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)



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# Metadata

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

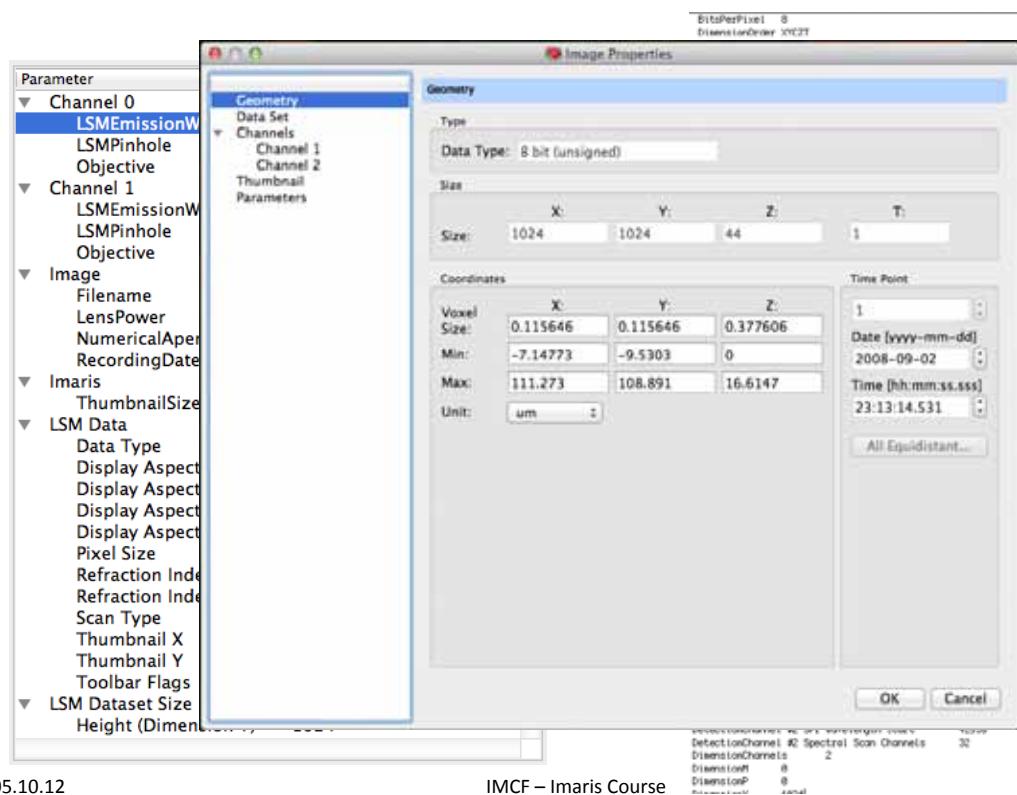
- Means of creation of the data
- Purpose of the data
- Time and date of creation
- Creator or author of data
- Location on a computer network where the data was created
- Standards used
- The song title of a piece of music

*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

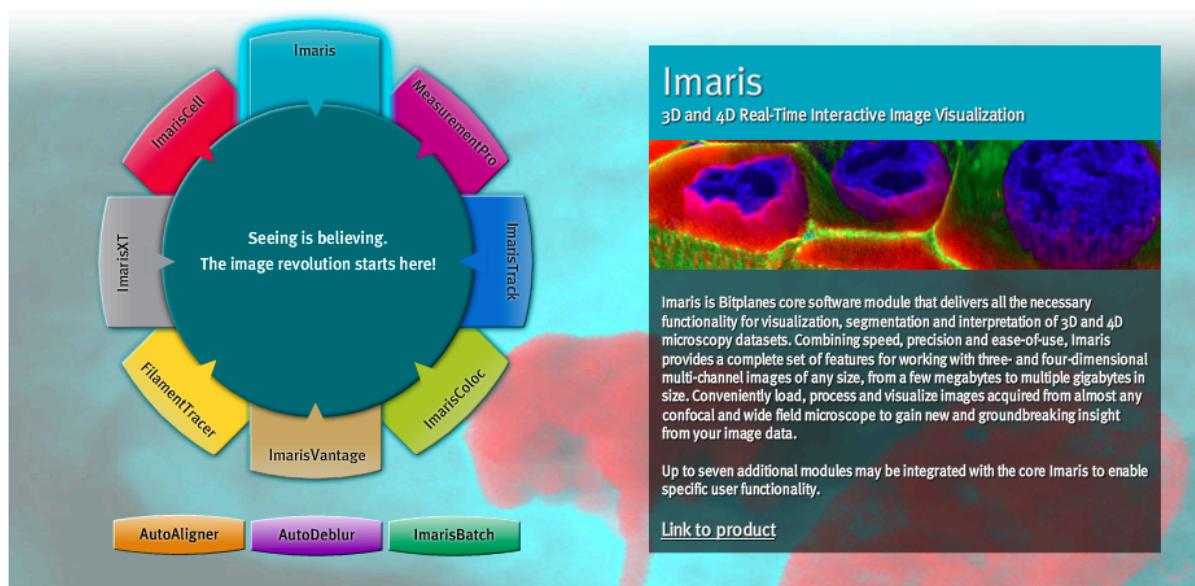


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# How to use IMARIS



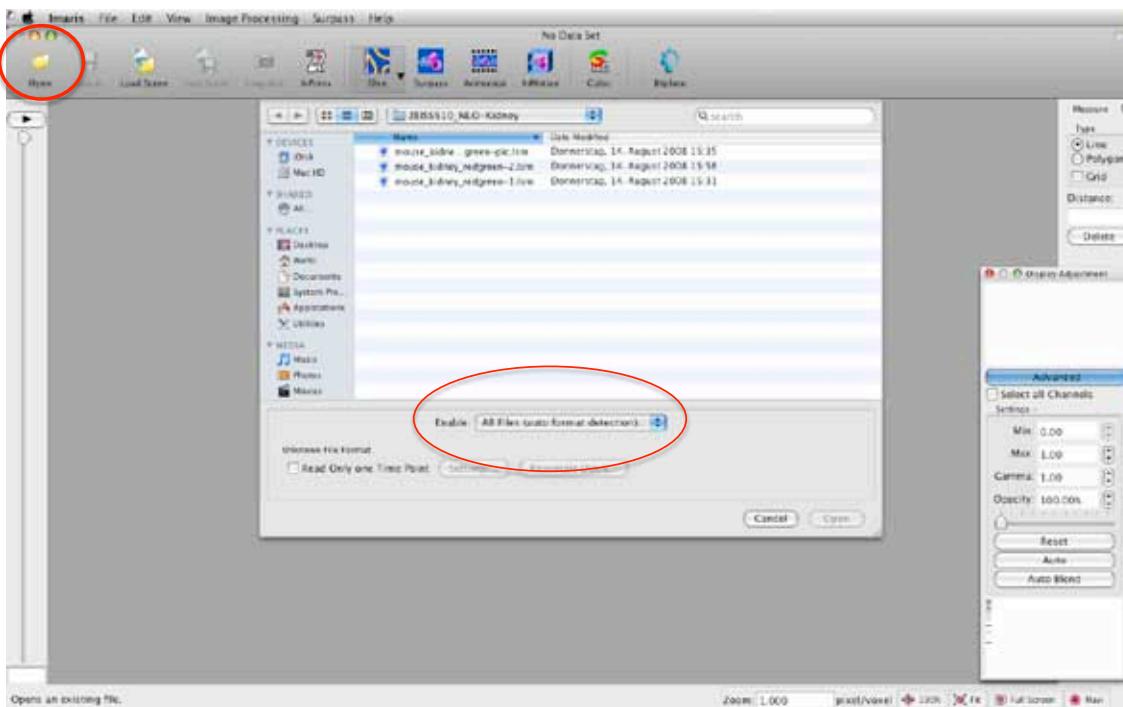
# The Imaris program surface



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



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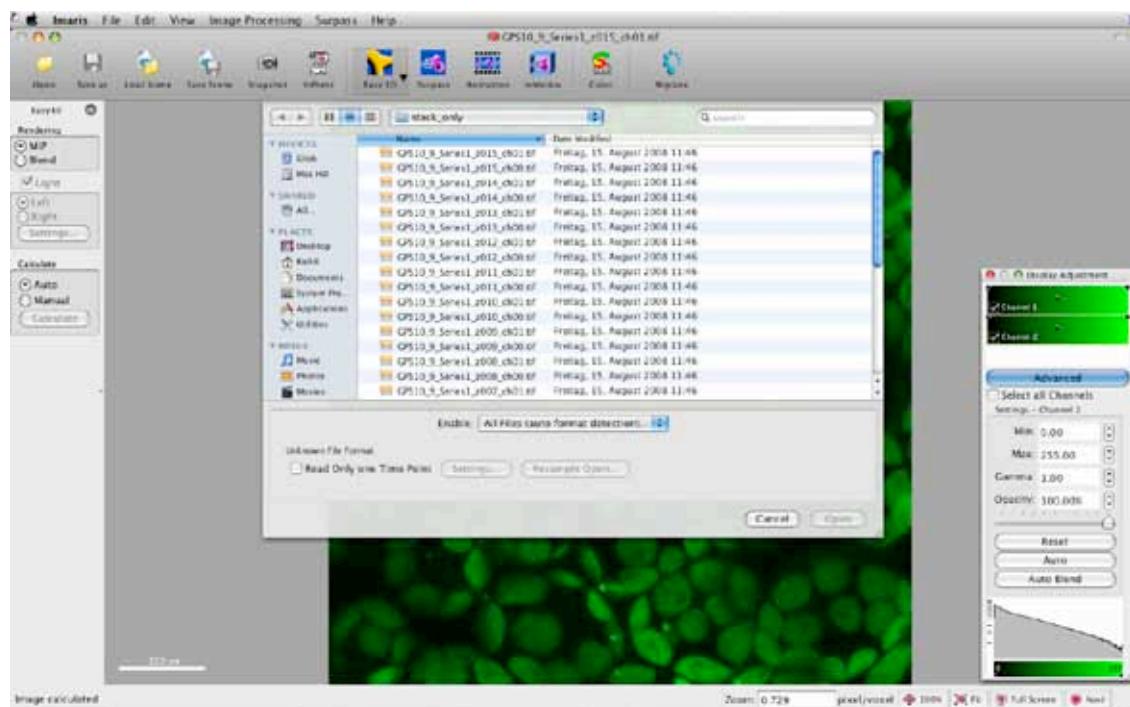
## Loading images – from native mic. files (settings)



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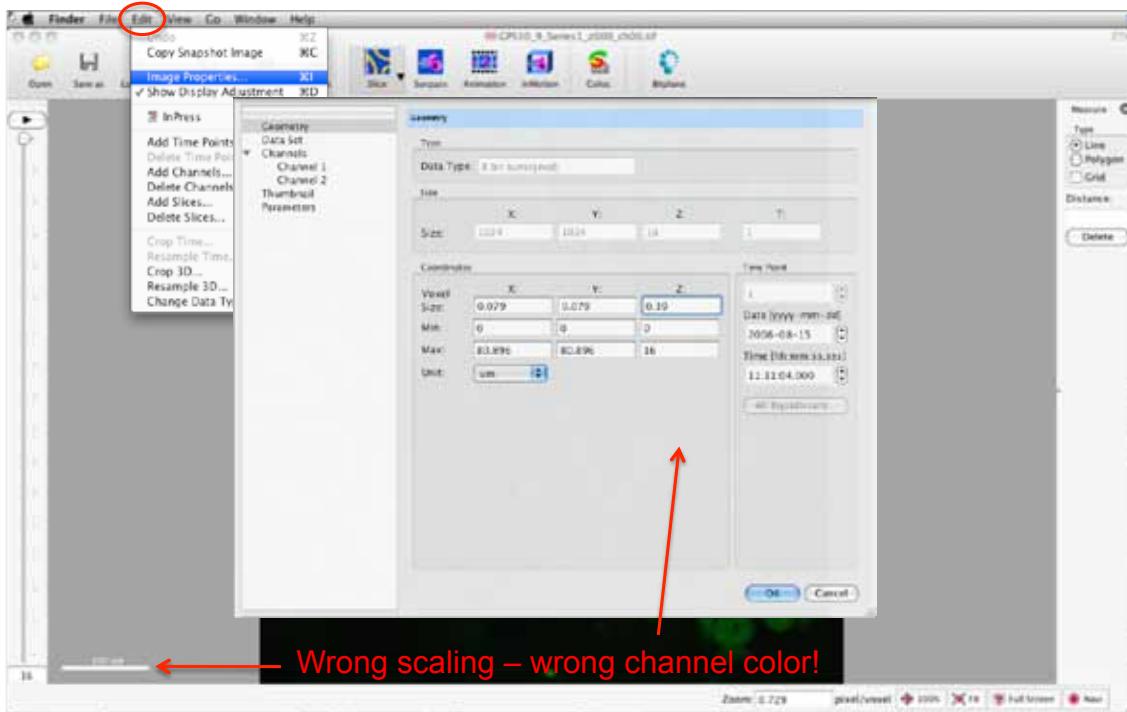
## Loading 3D images (xyz) – from stacks (tif or jpg)



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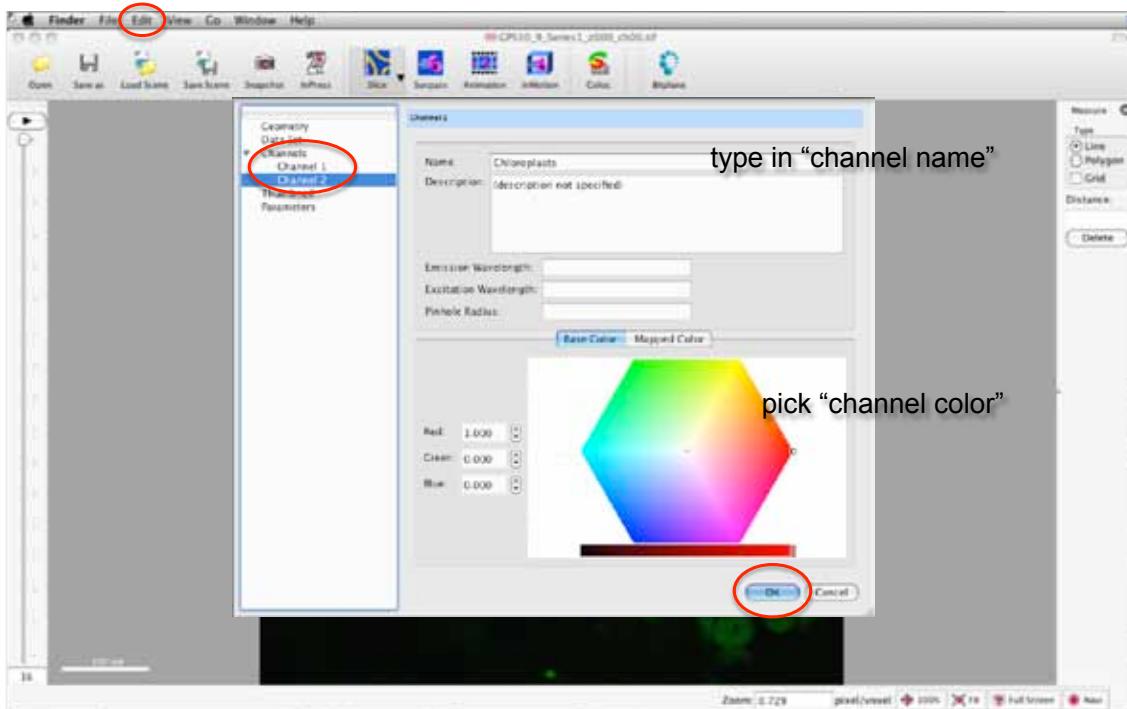
# Edit image scaling



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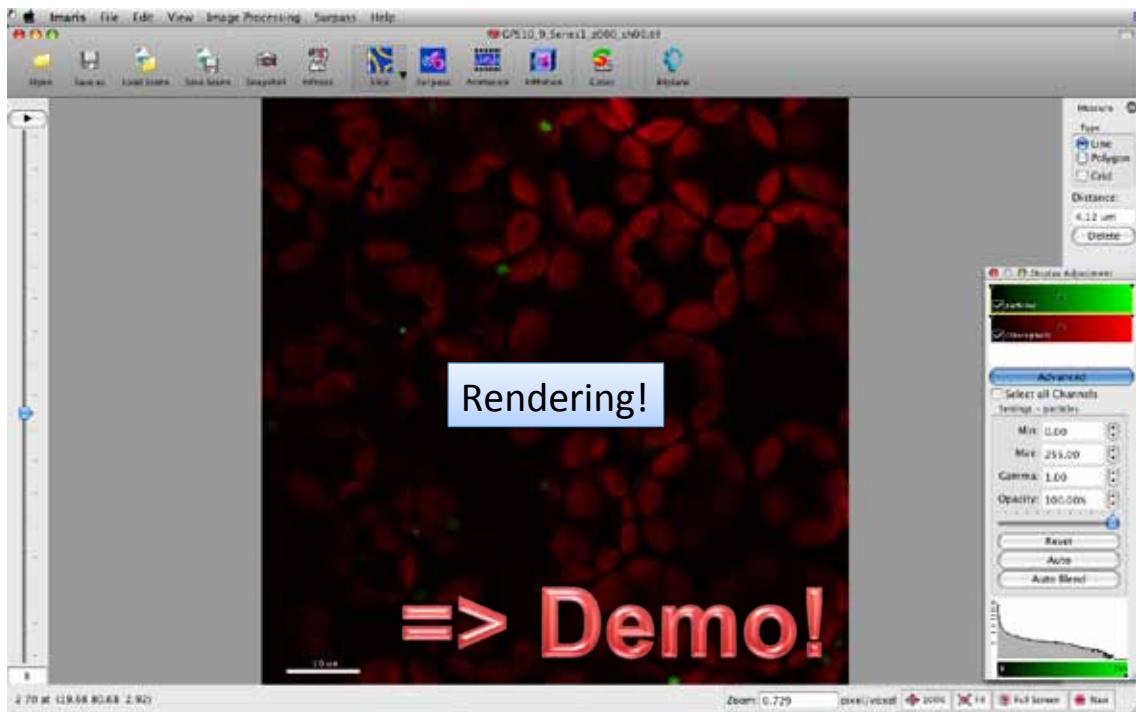
# Edit image color



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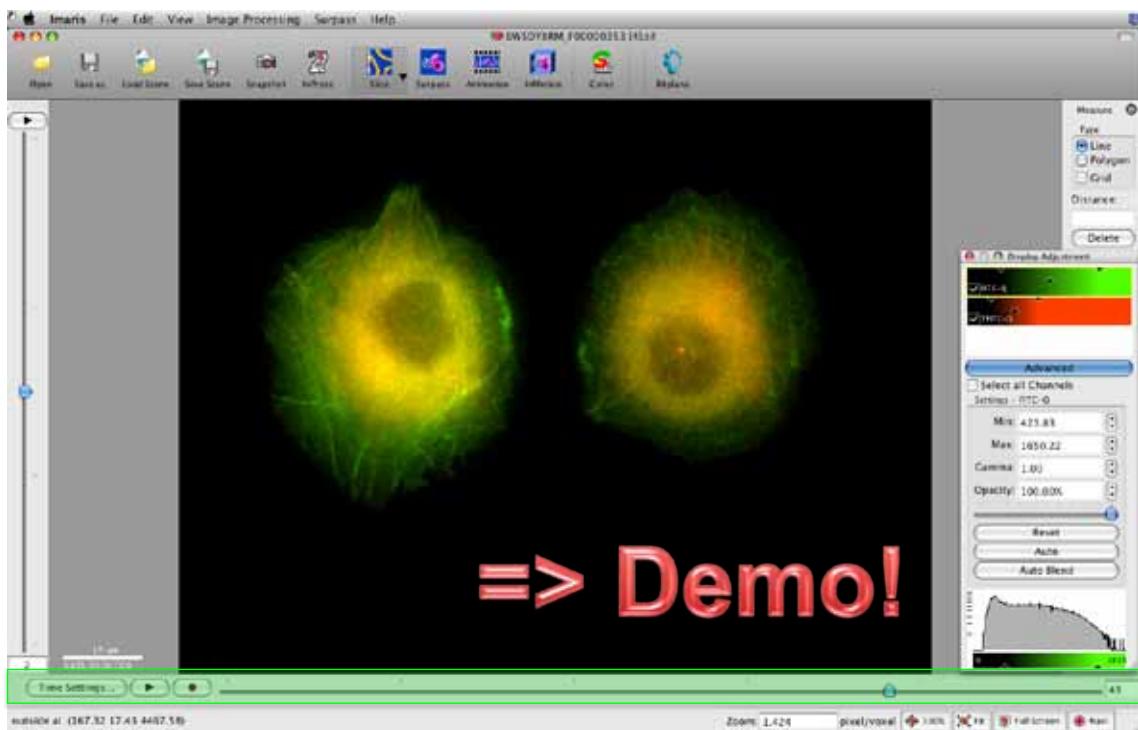
# Edit - resample



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# Loading 4D images (xyzt)

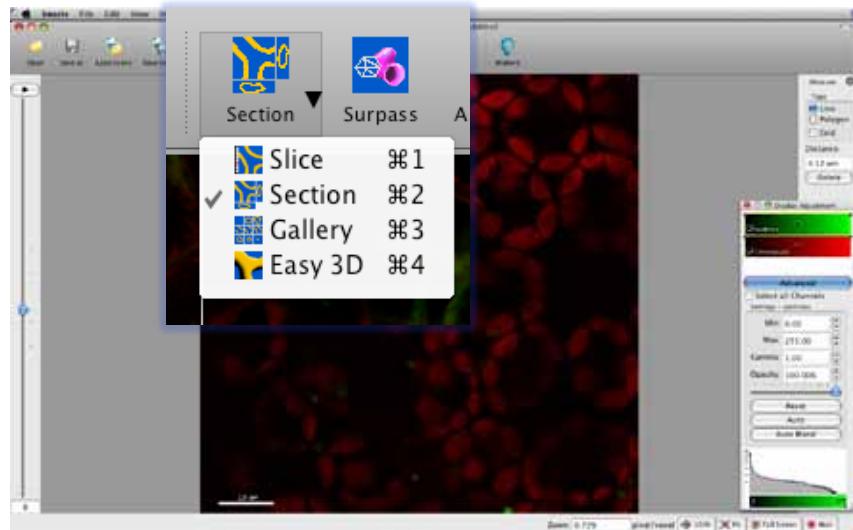


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# Basic visualization features

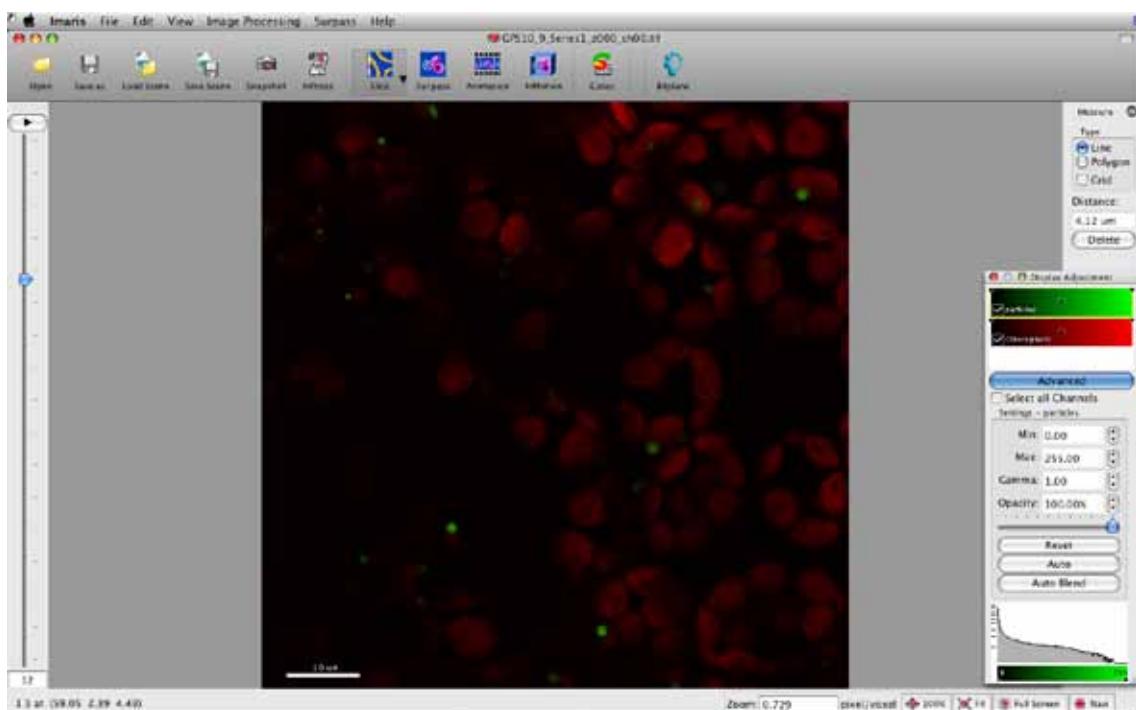
- “Slice”
- “Section”
- “Gallery”
- “Easy-3D”



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## “Slice” mode

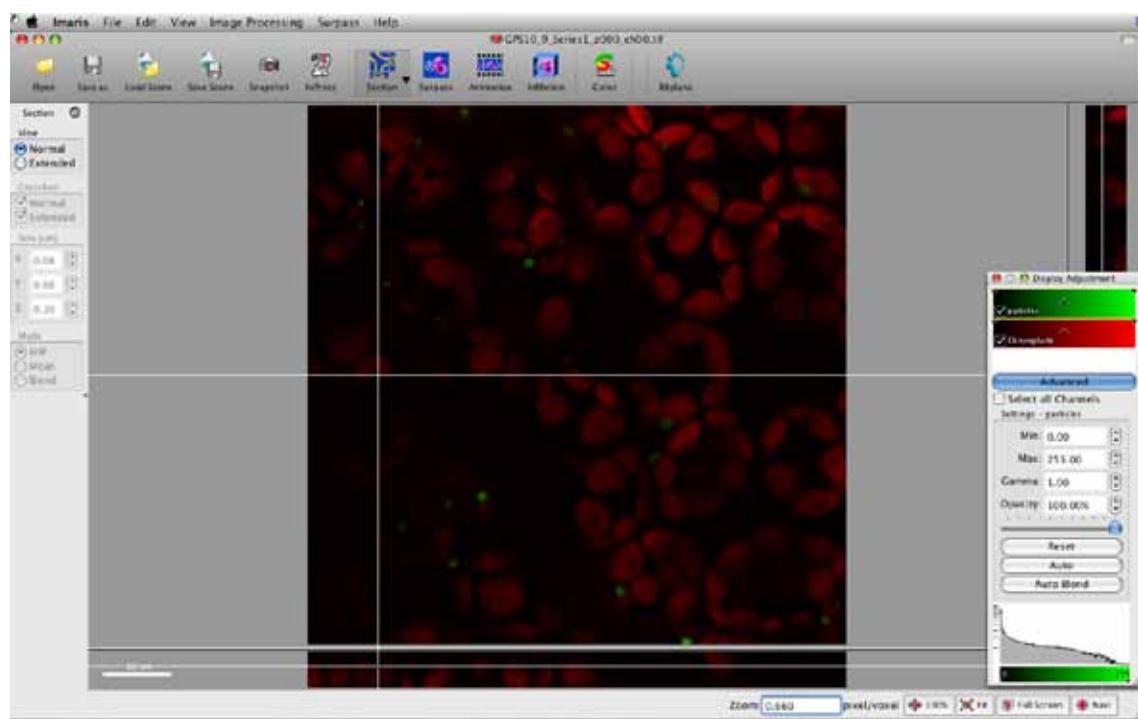


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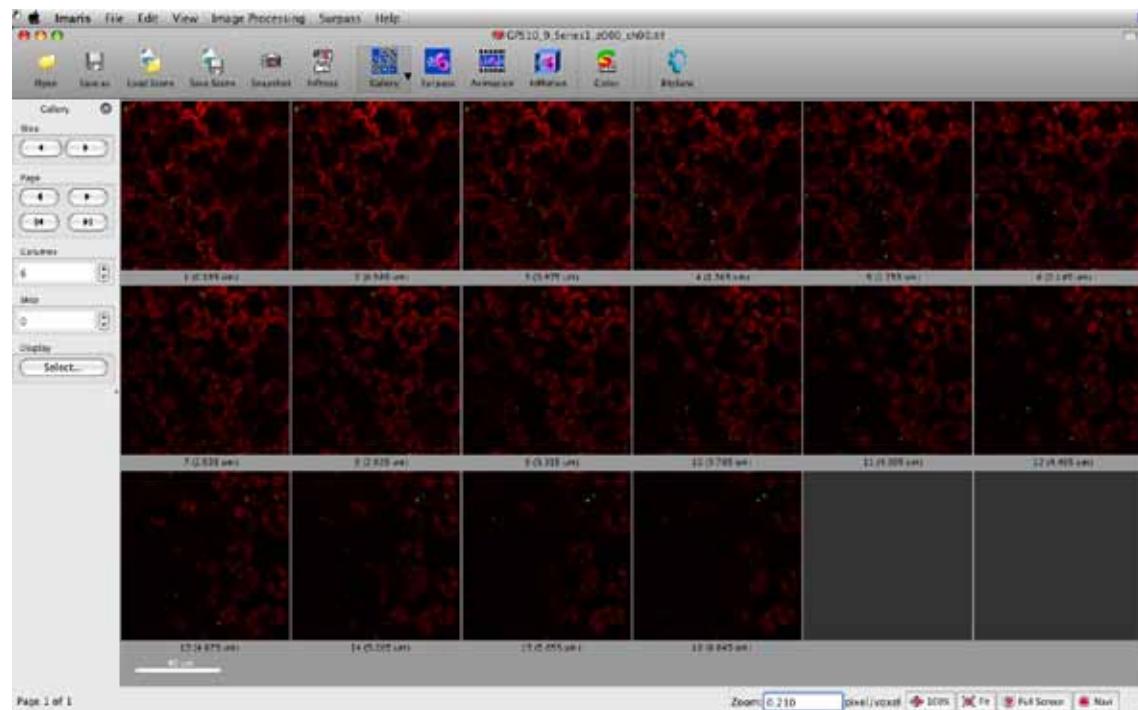
# “Section” mode



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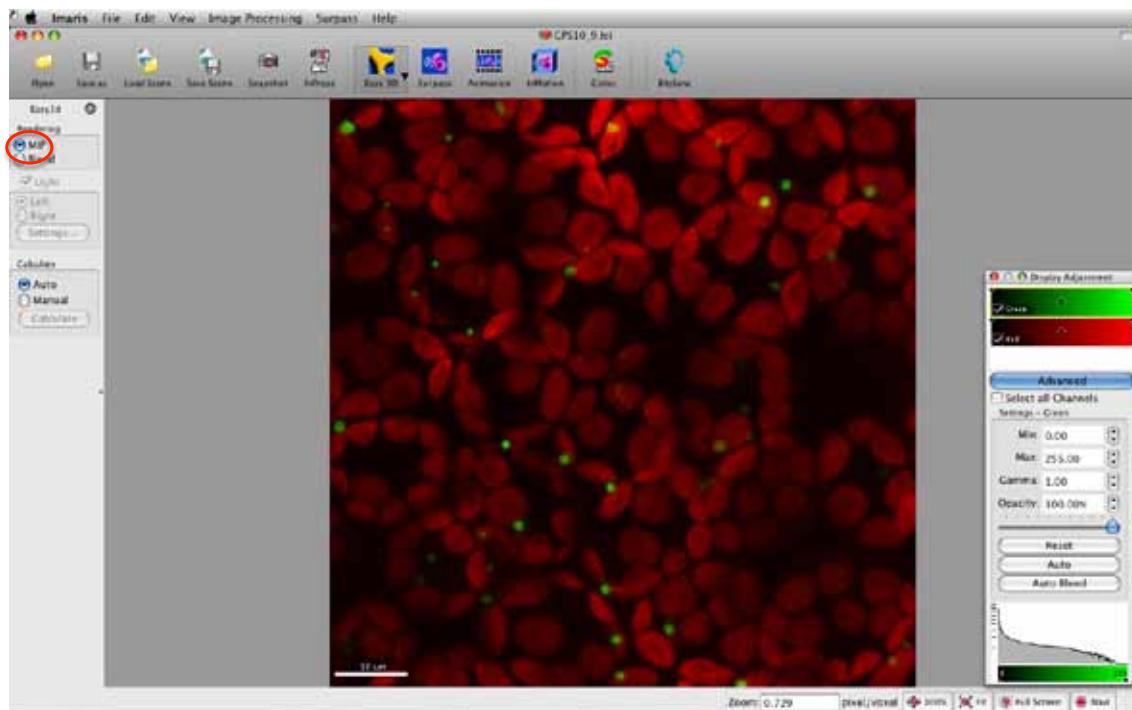
# “Gallery” mode



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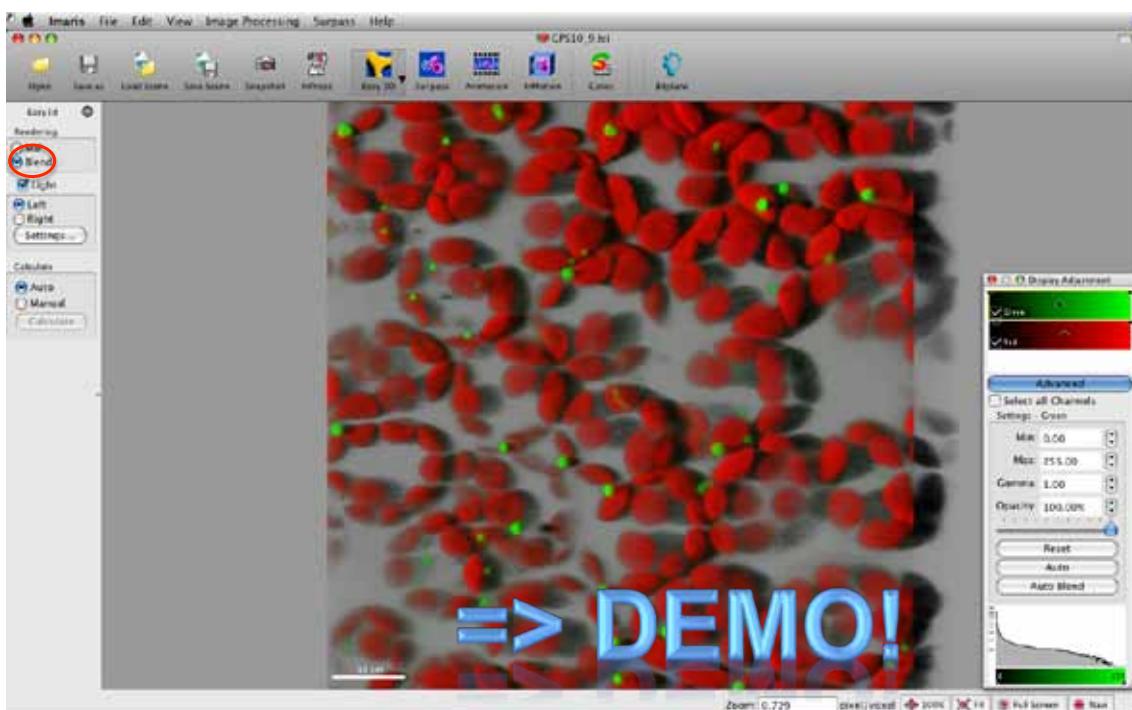
## “Easy-3D” mode – MIP (Maximum Intensity Projection)



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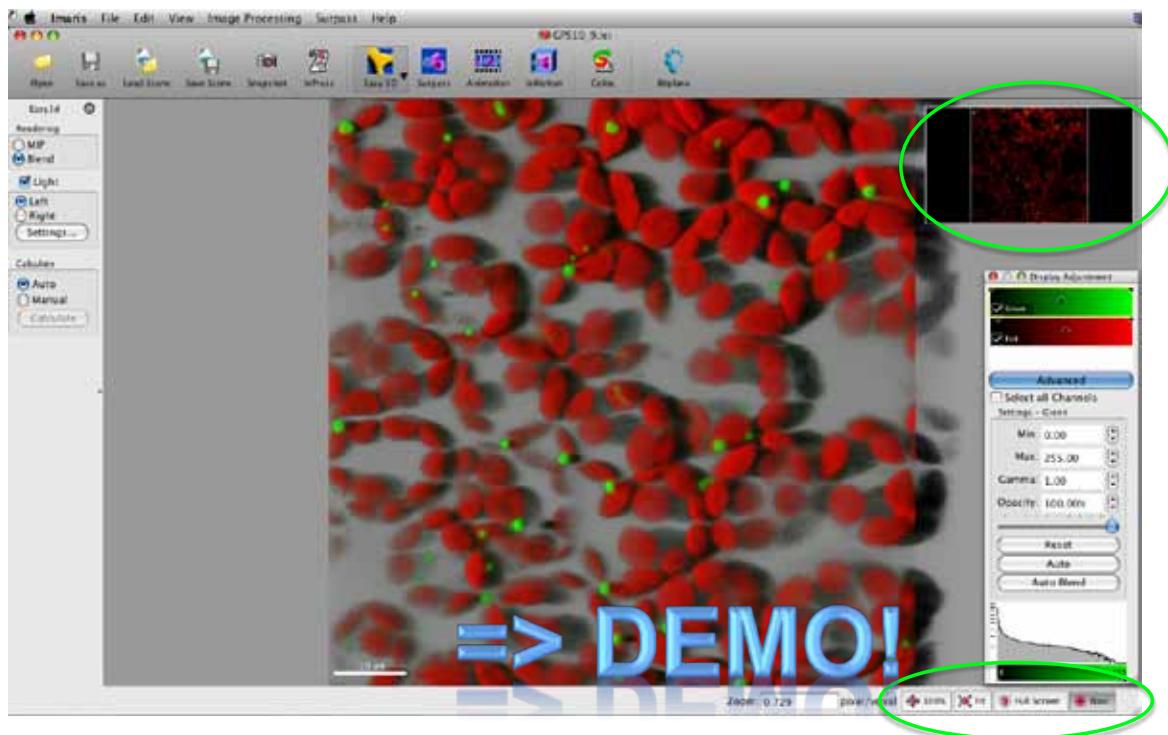
## “Easy-3D” mode – Blend



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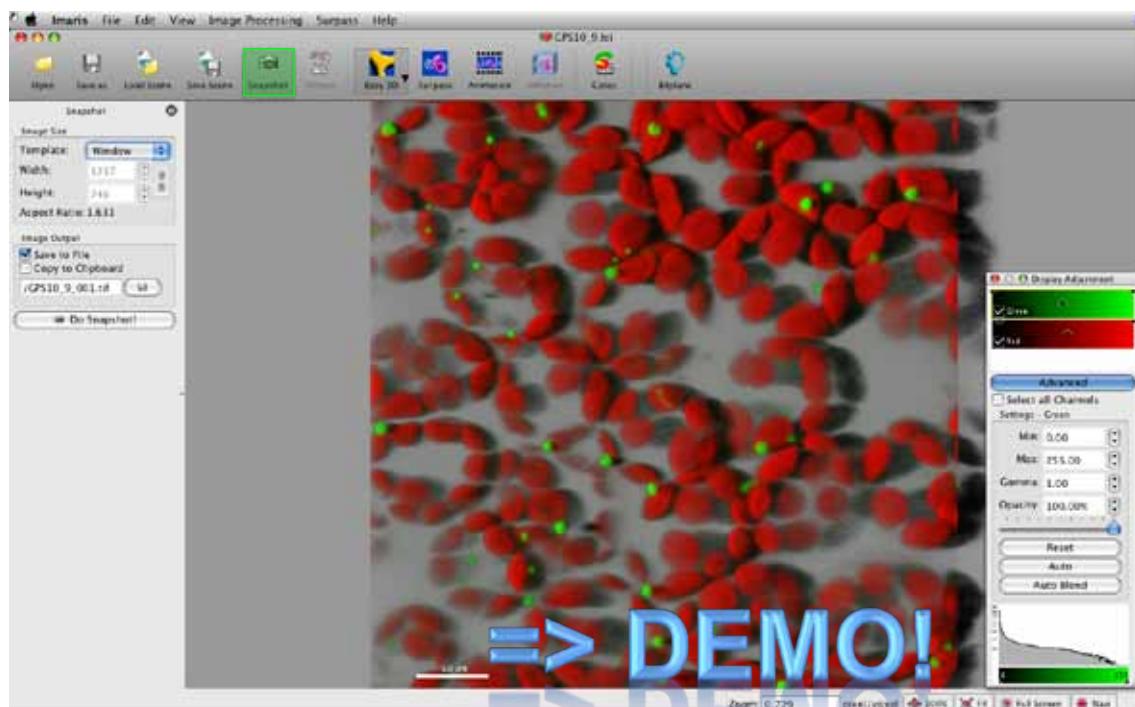
# Display settings



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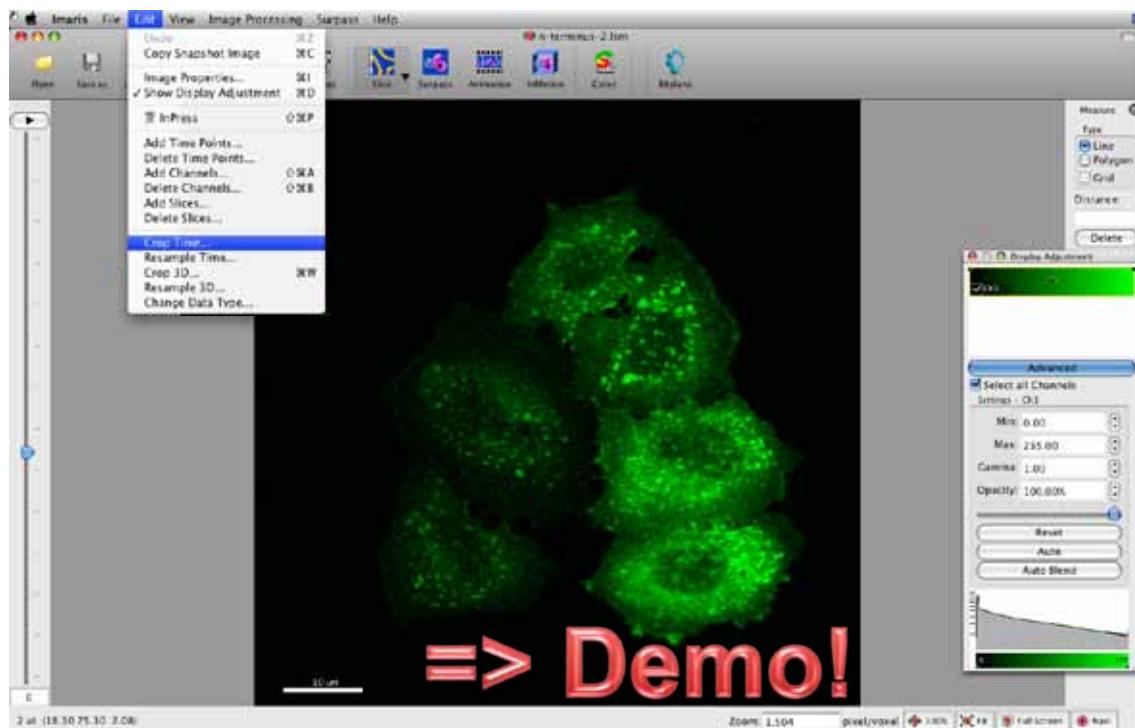
“Do a snapshot”



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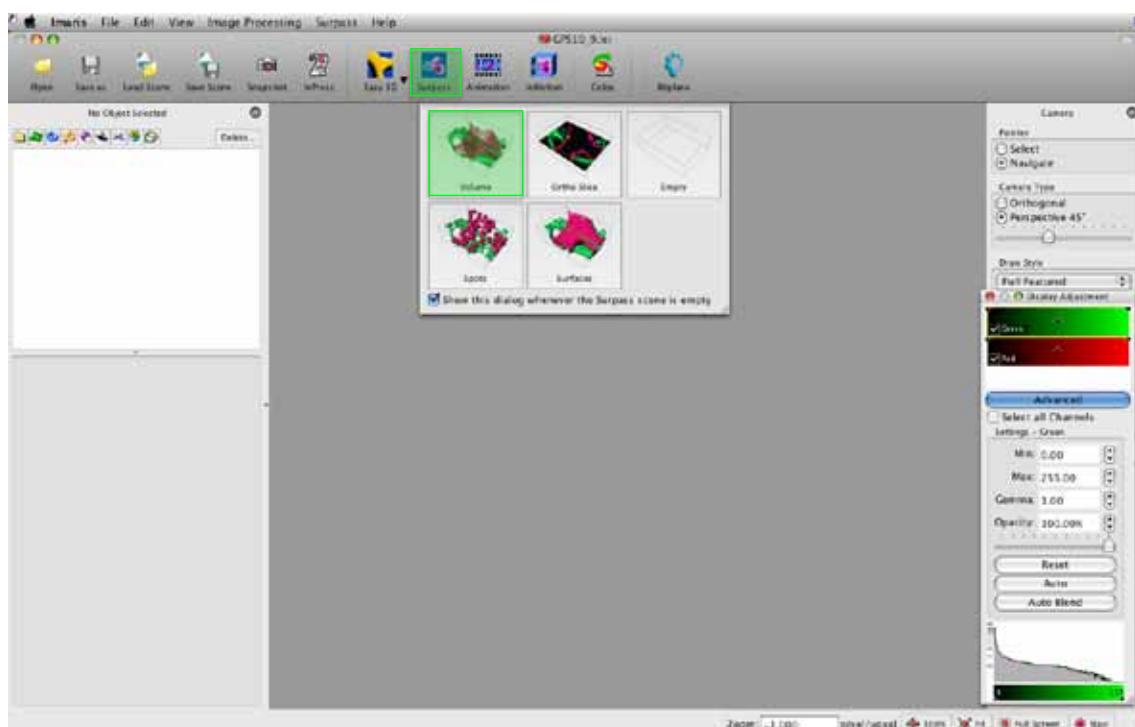
# Crop an image (3D and time)



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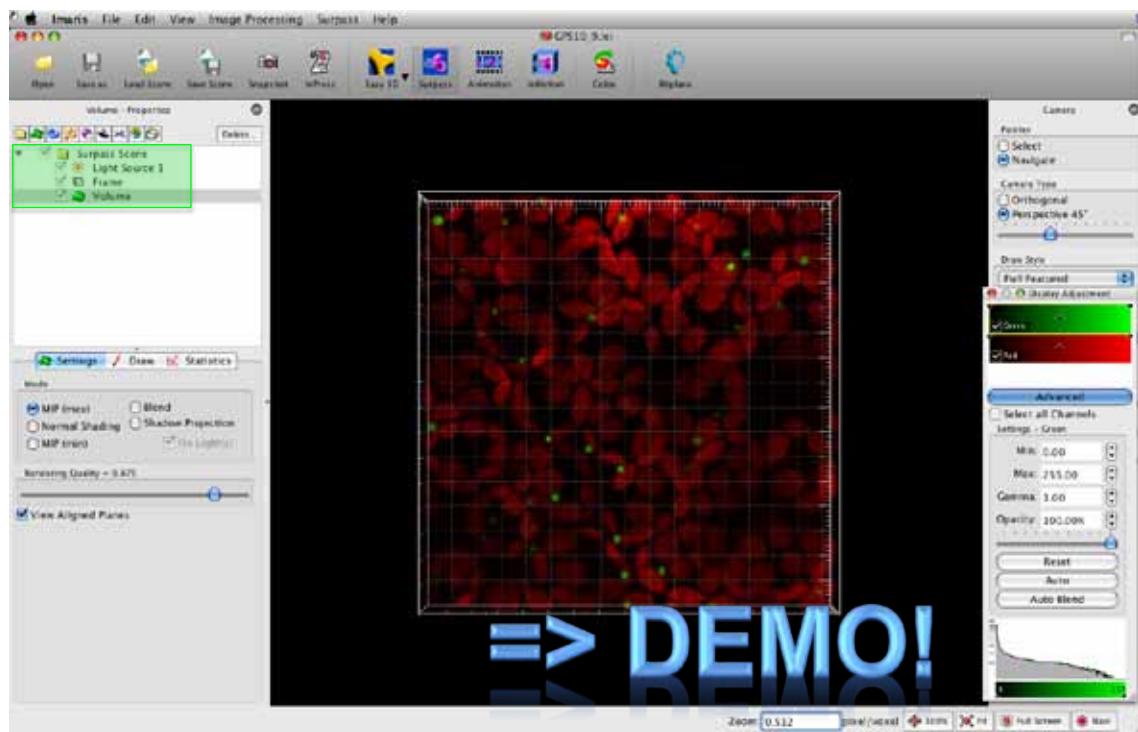
# Surpass



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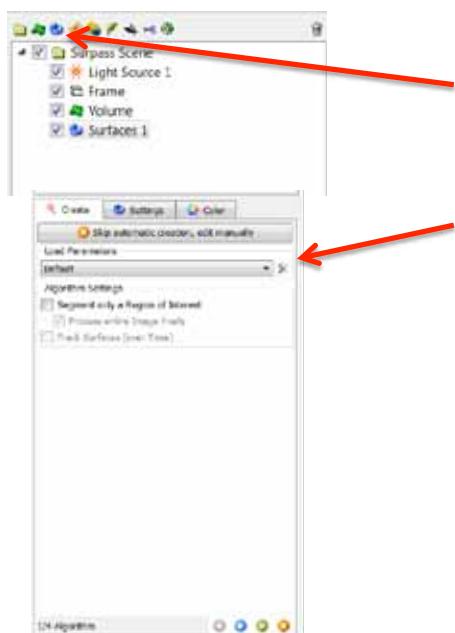
# Surpass - properties



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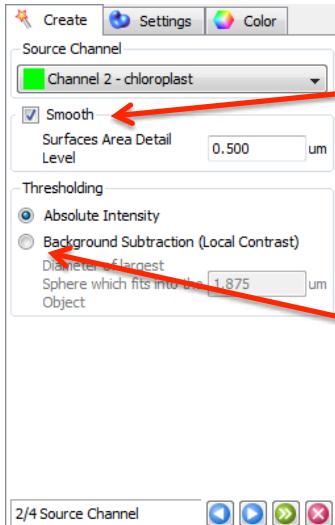
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# Surpass – surface rendering



- 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 subtraction, when a single absolute threshold will not segment all objects.

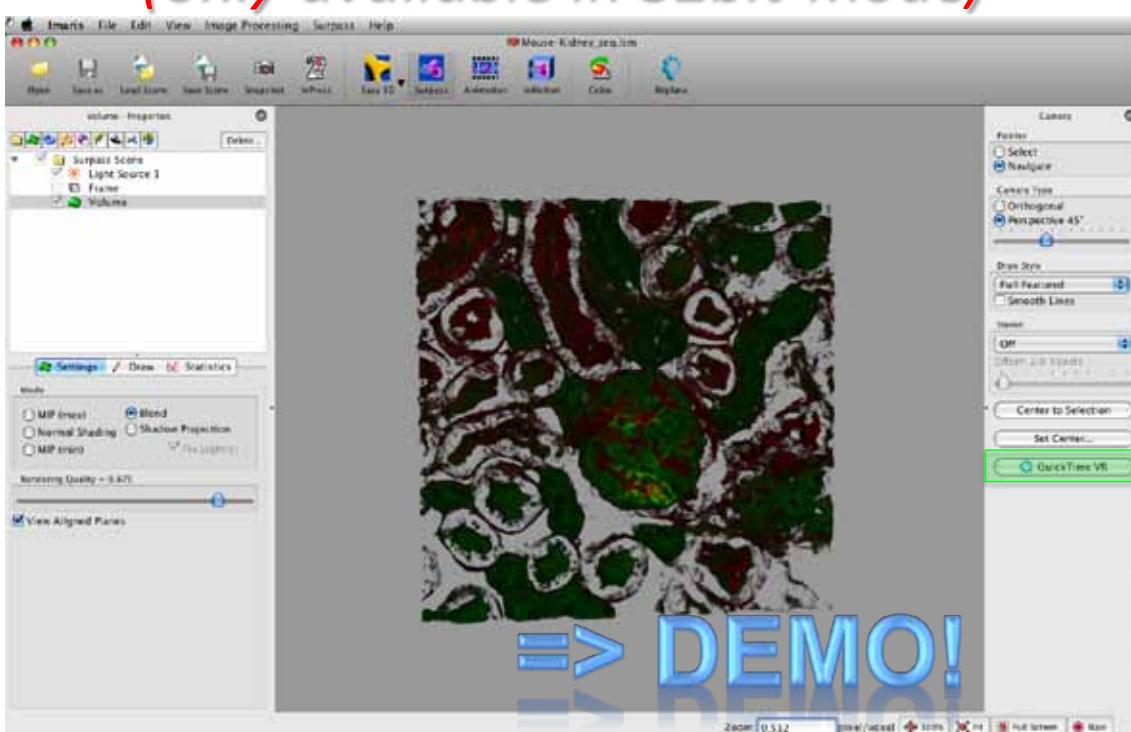
=> DEMO!

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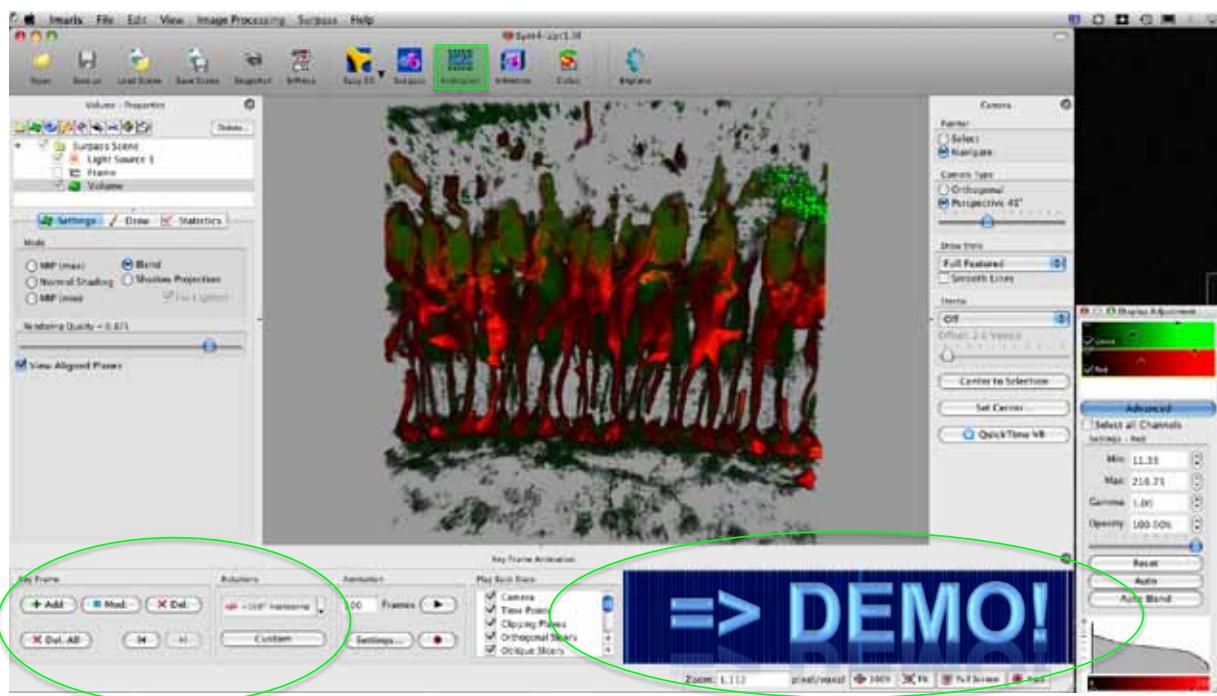
## Surpass – *create a VR-file* *(only available in 32bit-mode)*



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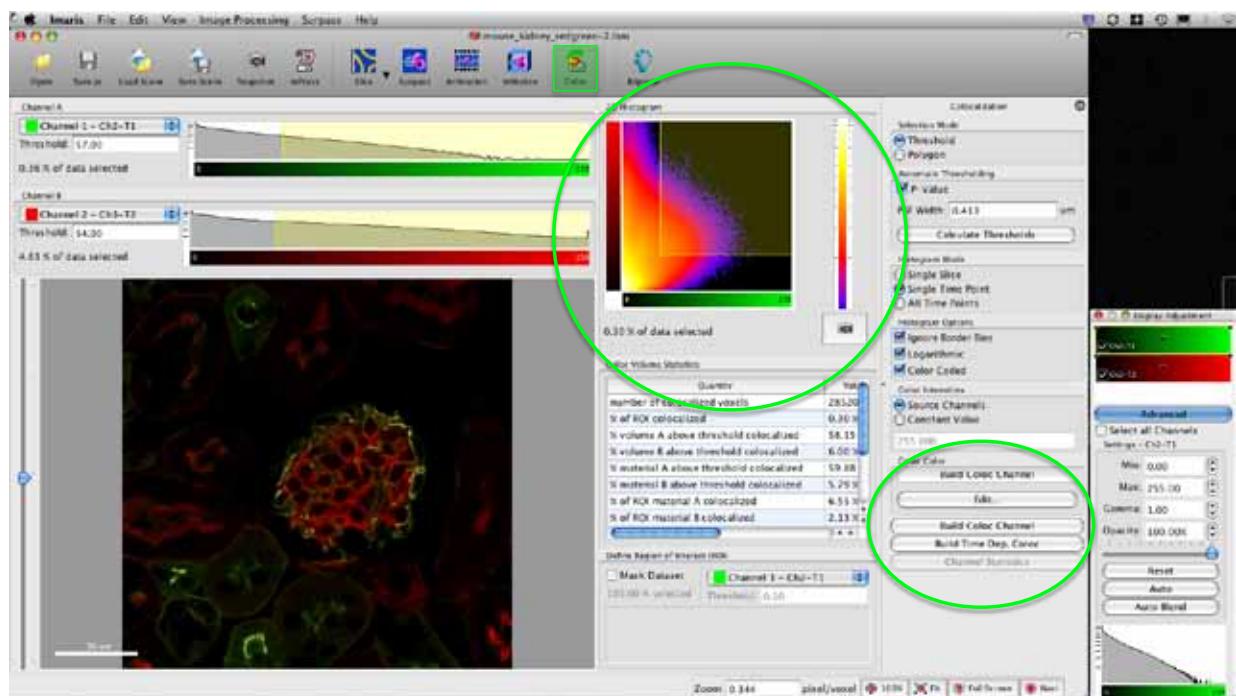
# Animation



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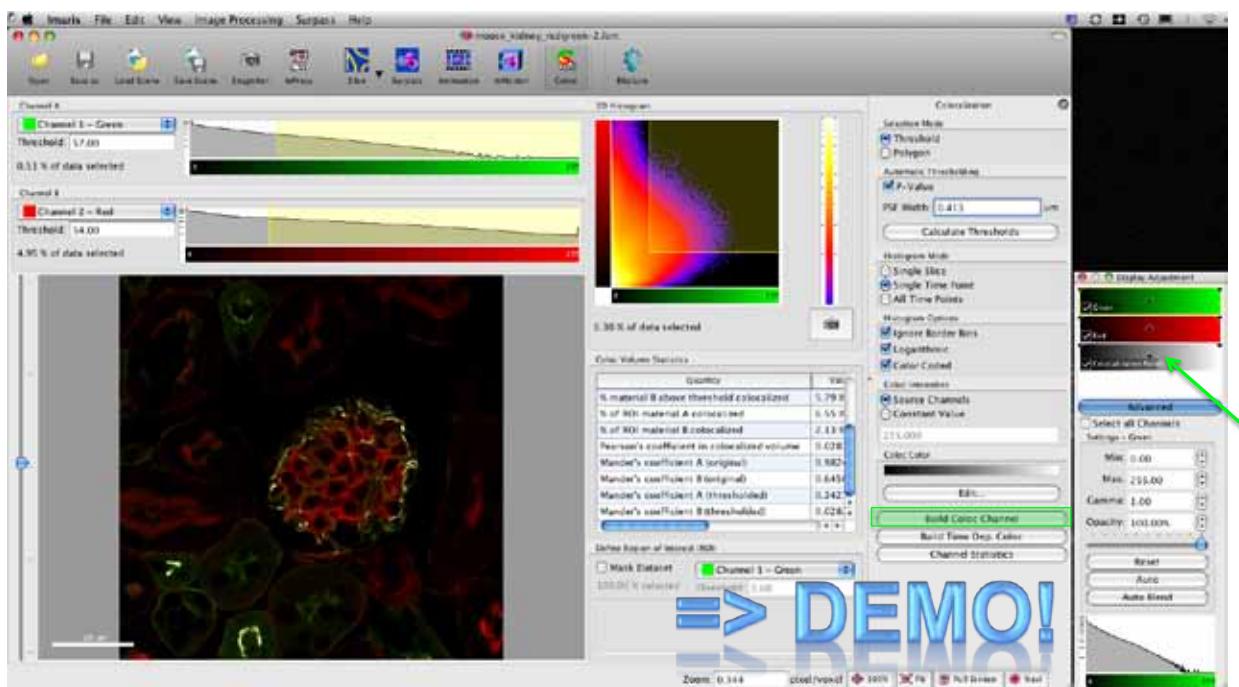
## Colocalization (quick-and-dirty)



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# Generate colocalization channel



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## Hands-On

Imaging Core Facility

08.03.2012

### Imaris course

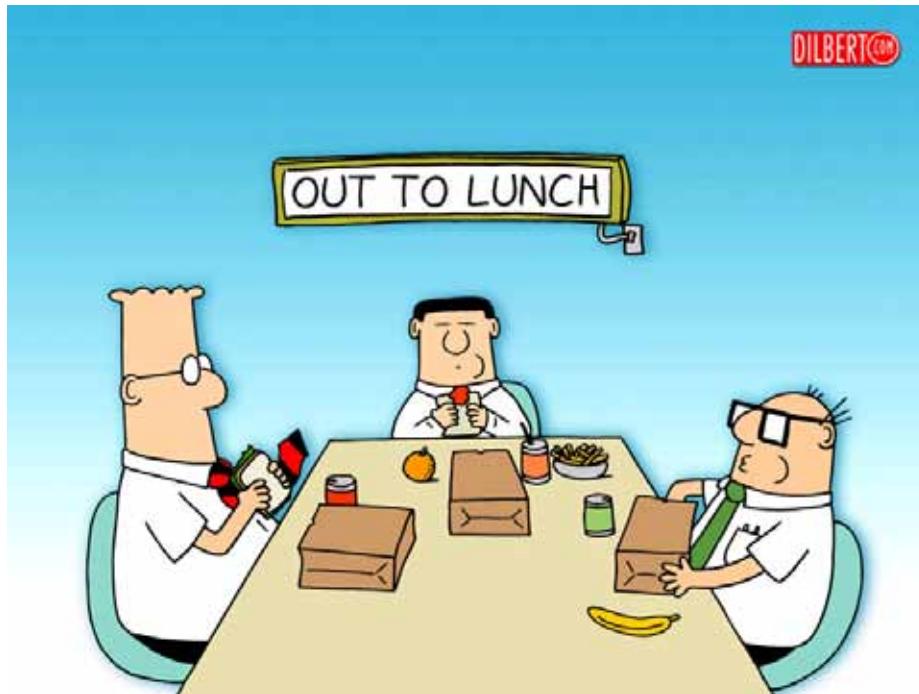
by Oliver Biehlmaier, Alexia Ferrand, Niko Ehrenfeuchter

#### Basic Imaris functions – hands-on:

- 1) Loading images – from native mic. Files  
use files from folders:
  - "Raw-Data/SP2\_Chlorophyll\_GB/Sp2-Data-lei/GPS10\_9.lei"
  - "Raw-Data/SPS\_Synt4-zpr1-ad/Synt4-zpr1.tif"
  - "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\_Chlorophyll\_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)

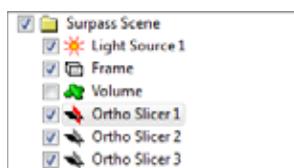
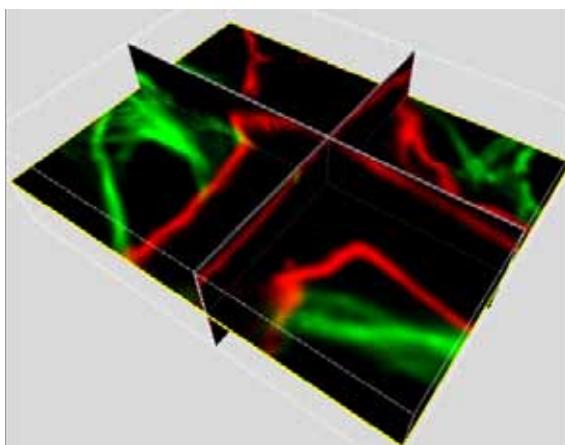


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## Ortho Slicer



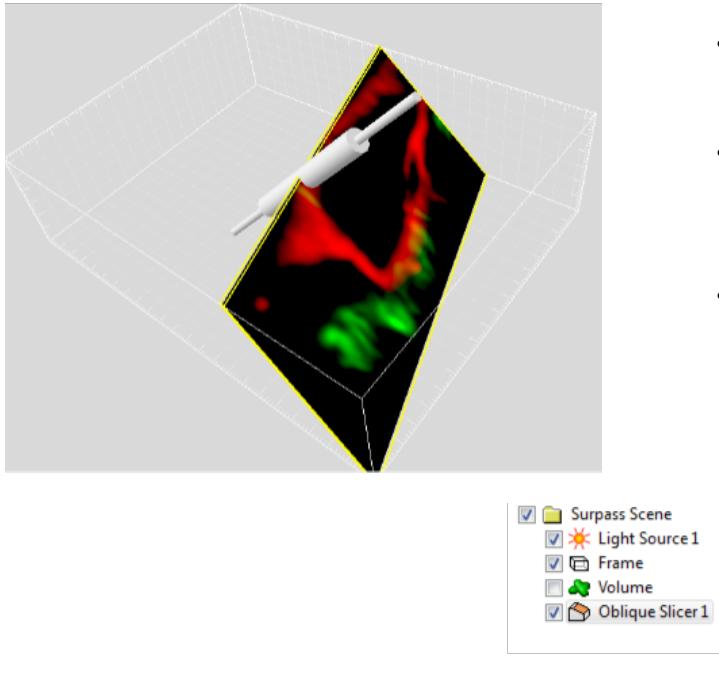
- Orthogonal plane on which original data are projected
- Can be moved within the dataset
- “Extended section” adjusts slices thickness

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# Oblique Slicer

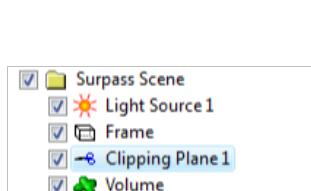


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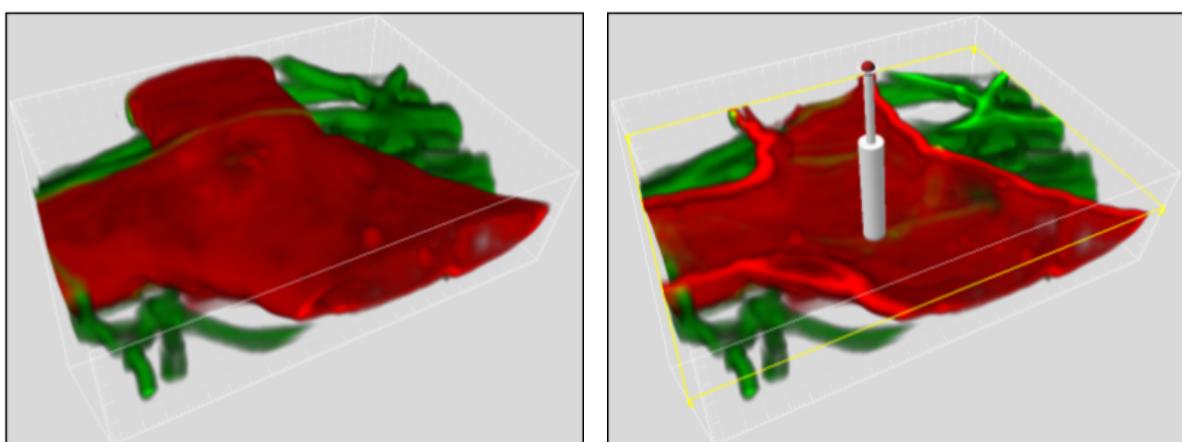
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- Plane on which original data are projected
- Can be freely moved and rotated within the dataset (similar to Clipping Plane)
- “Extended section” adjusts slices thickness



## Clipping plane

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

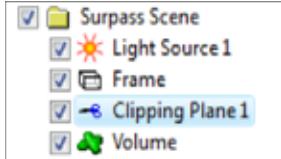


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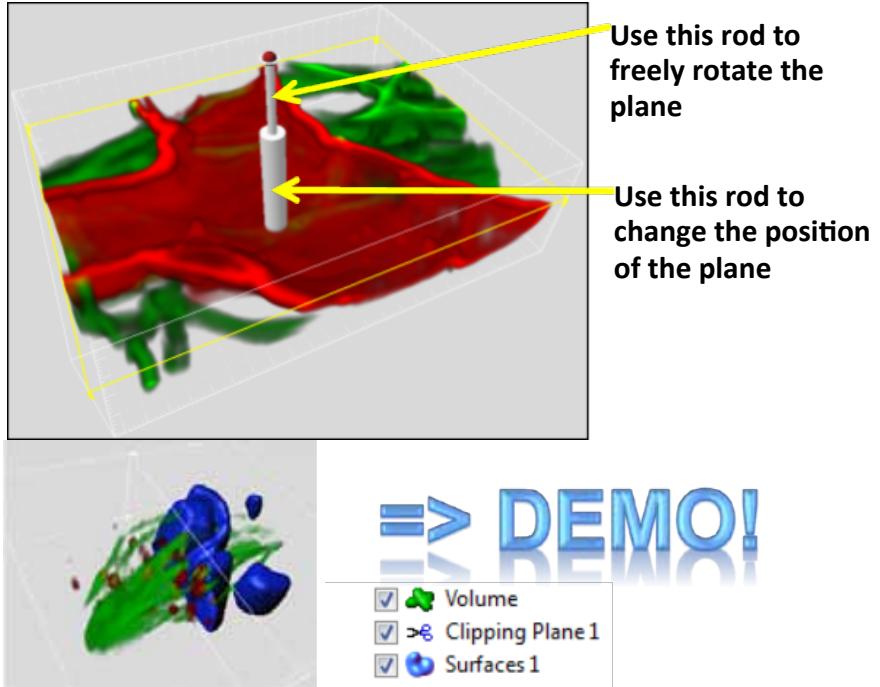
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# Clipping Plane



Only cuts objects lower  
in Imaris tree



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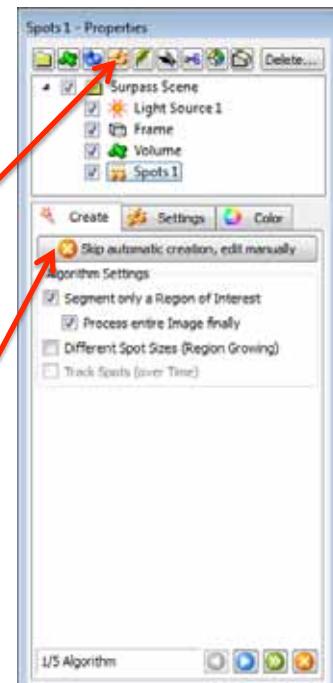
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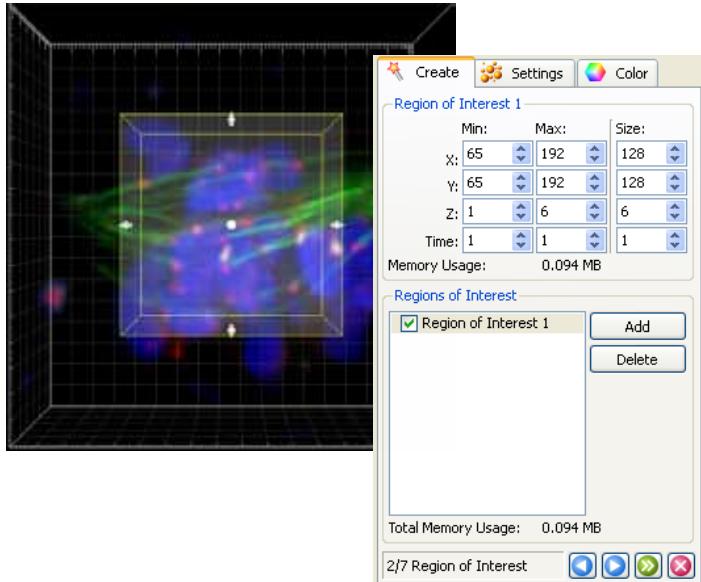
# Spot Detection

For automatic segmentation of objects, imaris uses creation wizards which guide you through all the steps of the process 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

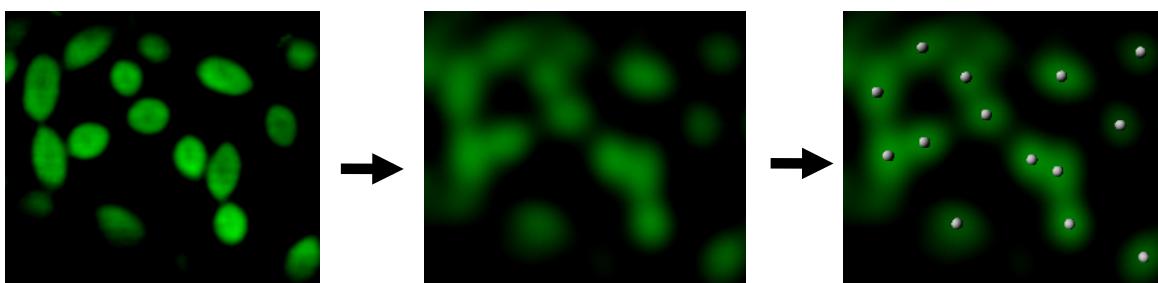


- Position region of interest (ROI)
  - Pointer must be in Select mode to drag the box or arrows
- Option of applying parameters to multiple ROIs with “Add” button.

## Spot Detection / Calculation

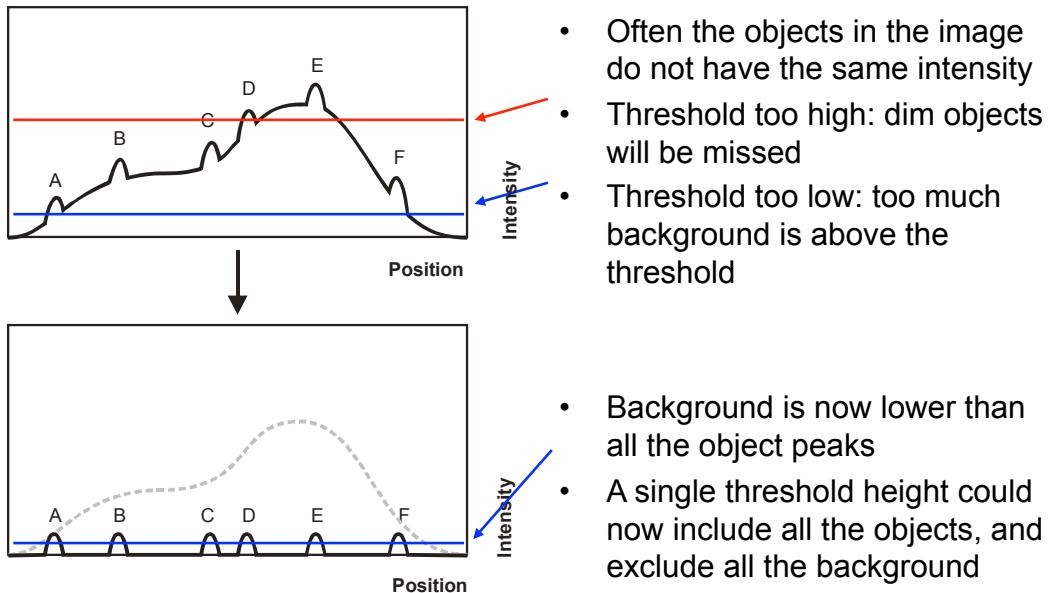
Spot Detection works as follows:

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



## 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

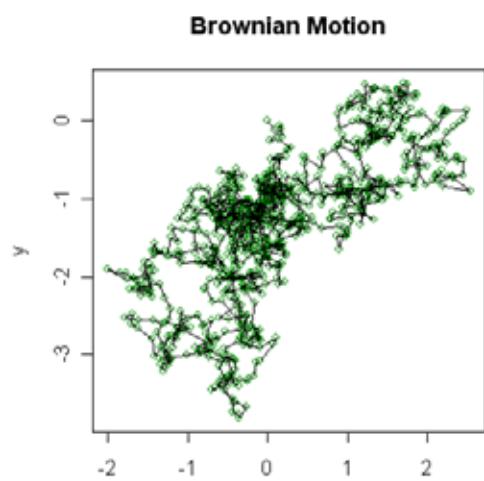
- Brownian Motion
- Autoregressive Motion
- Autoregressive Motion Expert
- Connected Components

## 2) Specify Tracking Parameters

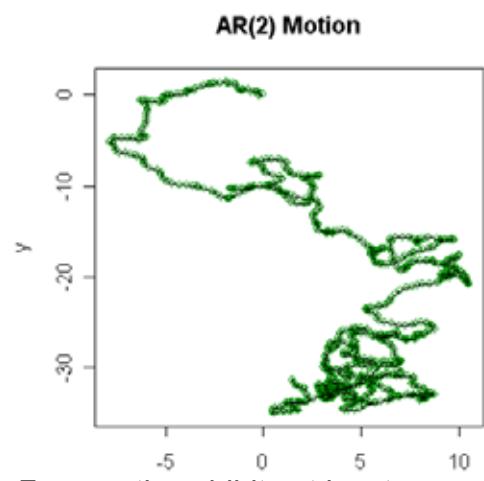
- “maximum distance”
- “gap size”

## 3) Create Track

# Tracking Motion Models

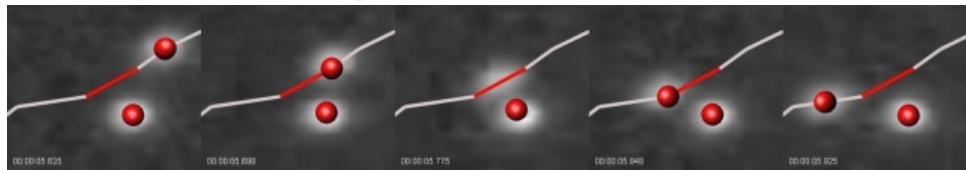


*Very rarely exhibits momentum / direction*



*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:**

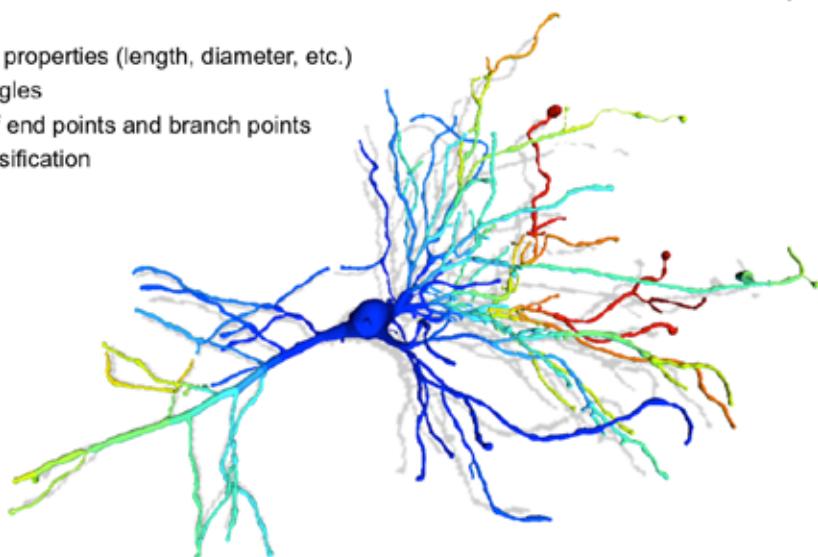
- *Gap Close to the rescue!*

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

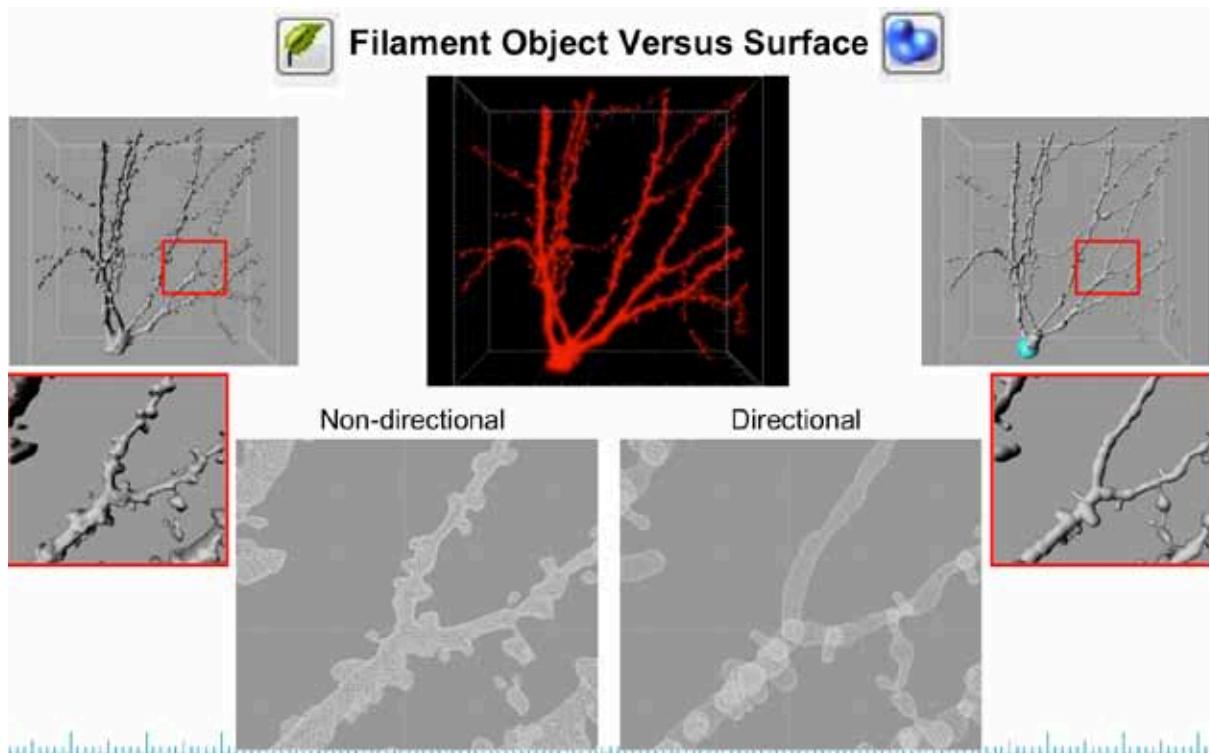
# Filament tracer

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

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



# Filament tracer

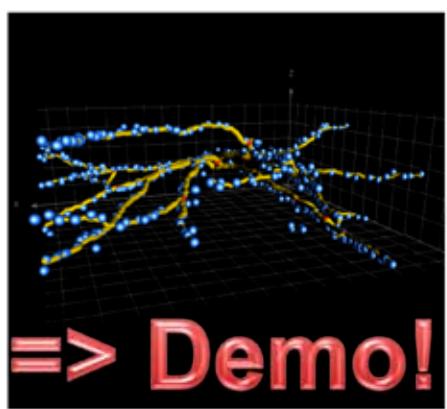


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## Filament tracer – tracing methods



Multiple tracing methods to ensure accurate results:

1. Automatic detection
  - a) Fully automatic detection based on intensity threshold (may have loops)
  - b) Full automatic (creates tree without loops)
  - c) Iterative Tracing
  - d) Region of Interest (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

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# Imaris XT

Allowing Biologists and Computer Scientists to Develop, Share, and Collaborate

Leading edge research in biology, chemistry, or medicine requires advanced technology and in-depth understanding of specific processes.

Model-based data analysis lies at the heart of today's challenging research problems.

Modern imaging products are able to incorporate problem-specific know-how to automatically extract complex or subtle morphological or dynamic information.

Future progress includes intelligence and numerical power of computers. Biological labs cooperate with in-house computer scientists to develop task-oriented analysis modules.

Imaris is the leading software for 3D and 4D visualization of microscopic images. With the ability to read almost all microscopic formats, Imaris provides a fast, powerful, interactive, and easy-to-use environment to visualize and analyze images.

ImarisXT is a multi-functional two-way interface from Imaris to both classic programming languages and to Matlab®.

ImarisXT allows to integrate your task-oriented algorithms for image processing, segmentation, classification, or reporting. ImarisXT provides a customizable user interface for seamless integration.

the image revolution starts here.

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

– MatLab

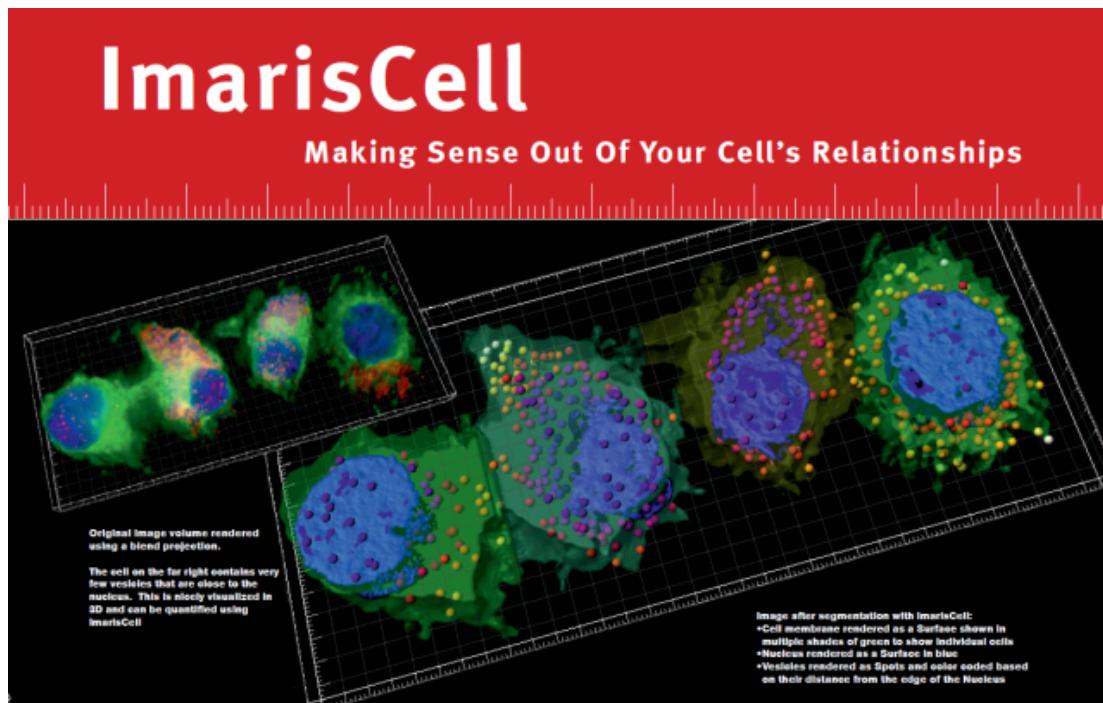
– ImageJ

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# Imaris Cell

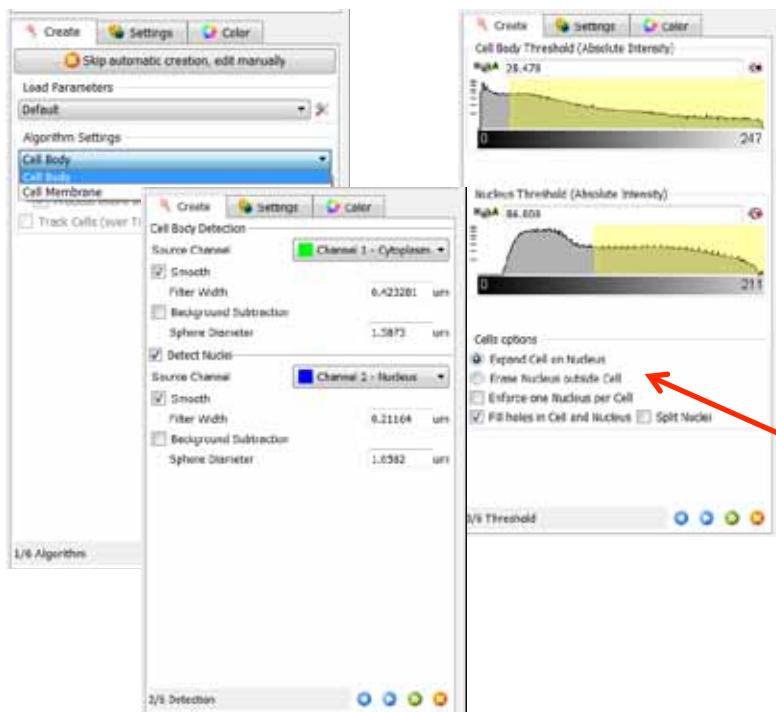


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# Imaris Cell



- 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).

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# Imaris Measurement Pro

## Imaris MeasurementPro

Because Your Image Is More Than A Pretty Picture

Imaris MeasurementPro enables researchers to extract critical statistical parameters from their microscopy images thus allowing for the quantification of scientific findings. Imaris MeasurementPro adds shape, size, and intensity based measurement capabilities to the volume rendering, surface rendering and object detection features of Imaris. It allows researchers to interactively classify, group, and filter segmented objects based on any of the calculated statistics.

**Overview**   **Features**   **Download**

The Imaris MeasurementPro functionality requires Imaris.

**(1) Statistics Generation**

Imaris MeasurementPro takes the objects that have been segmented using Imaris' surface segmentation or spot segmentation methods, refers back to the original data in the identified segmented region, and produces statistical results for each identified object.

- **Intensity Statistics Include** - Min, Max, Median, Sum, StdDev, and Center on a per-channel basis
- **Size Statistics Include** - Area, Volume, # of Vertices, # of Vertices, and # of Triangles and for spot diameter
- **Shape Statistics Include** - Ellipsoid Axis Lengths A, B, and C each in the X, Y and Z dimensions, the Ellipticity oblate and prolate, and the Sphericity
- **Positional Statistics Include** - Center of Image Mass and Homogeneous Mass in X, Y, and Z
- When combined with **ImarisTrack**, Imaris MeasurementPro produces track specific statistics outlined in the **Imaris Track Features** section as well as all the other statistics shown above but for every time point.
- When combined with **ImarisCell**, Imaris MeasurementPro produces a vast array of cell object specific statistics outlined in the **ImarisCell Features** section.
- When combined with **ImarisTracer**, Imaris MeasurementPro produces dendrite and spine specific

**(2) Statistical Output**

**(3) Interactive 3D Distance Measurements**

**(4) Interactive Surface Generation**

**Show Cases**

**High-Resolution Imaging in Zebrafish**  
Prof. R. Klemke and colleagues, University of California at San Diego. Metastasis, the major cause of death in cancer patients, is a highly dynamic process which include disruption of cell-cell adhesion, migration of cells away from the primary tumor and invasion into the vasculature. >>>

**References**

- Salama HA et al. J. Cell Biol., Apr 2006. Genome-wide analysis of signaling networks regulating fatty acid-induced gene expression and organelle biogenesis. >>>
- Hoepf JC et al. Mol Biol Cell, Jul 2006. High Resolution Crystal Structure and *in vivo* Function of a Kinase-2 Homolog in Glandia Insularis. >>>

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# 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!