

# Multidimensional Bioimage Analysis

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30<sup>th</sup> June, 2016



EXCELENCIA  
MARÍA  
DE MAEZTU

# Overview

- Multidimensional image
- Fiji: Stack manipulation
- Fiji: 3D image reconstruction and visualization  
(volume & surface rendering)
- Fiji: An application study: 3D image analysis (+ macro scripting)

# Images can have many dimensions

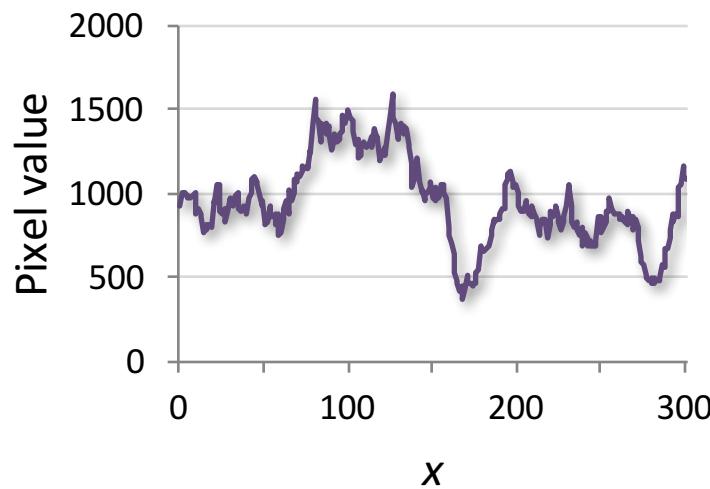
The number of dimensions is the number of things you need to know to find each pixel

17

***To find a pixel, you need:***

0 dimensions  
(just a single pixel)

# Images can have many dimensions

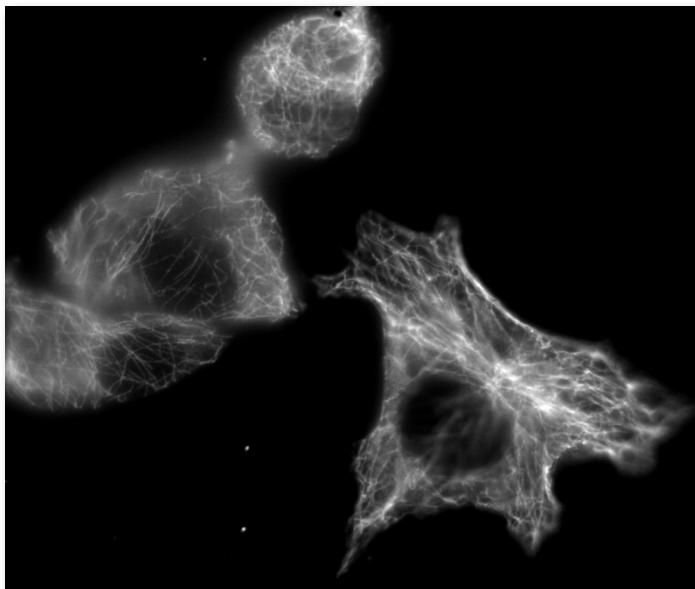


1 dimension  
(can be plotted as a  
graph)

The number of dimensions is the number of things you need to know to find each pixel

**To find a pixel, you need:**  
*x coordinate*  
*(x)*

# Images can have many dimensions

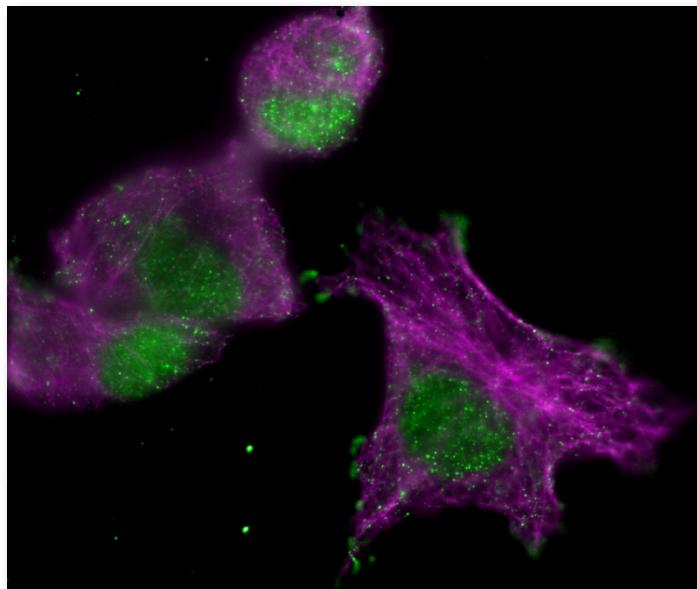


2 dimensions

The number of dimensions is the number of things you need to know to find each pixel

***To find a pixel, you need:***  
*x coordinate*  
*y coordinate*  
*(xy)*

# Images can have many dimensions

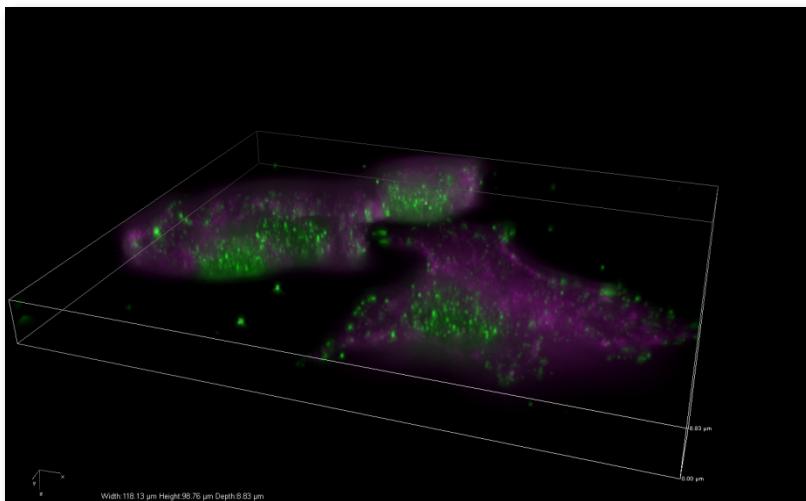


3 dimensions

The number of dimensions is the number of things you need to know to find each pixel

***To find a pixel, you need:***  
*x coordinate*  
*y coordinate*  
*Colour channel*  
*(xyc or xyz for 3D)*

# Images can have many dimensions

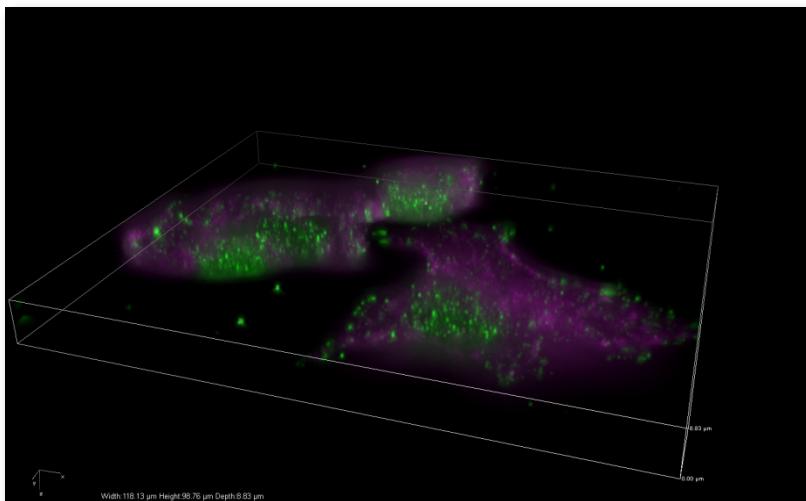


4 dimensions

The number of dimensions is the number of things you need to know to find each pixel

**To find a pixel, you need:**  
*x coordinate*  
*y coordinate*  
*Colour channel*  
*z plane*  
*(xyzc or xycz)*

# Images can have many dimensions



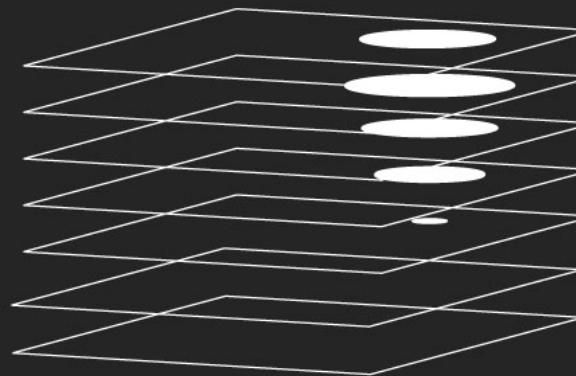
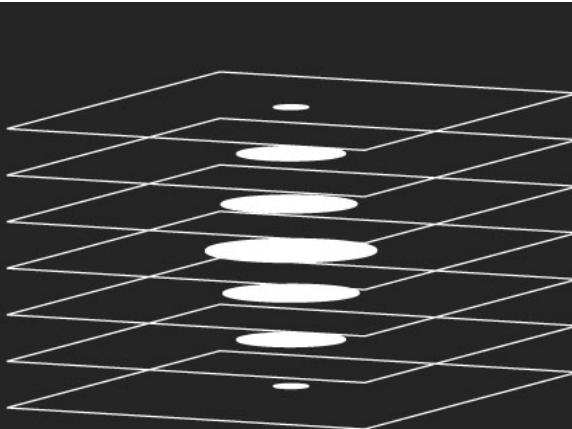
5 dimensions

The number of dimensions is the number of things you need to know to find each pixel

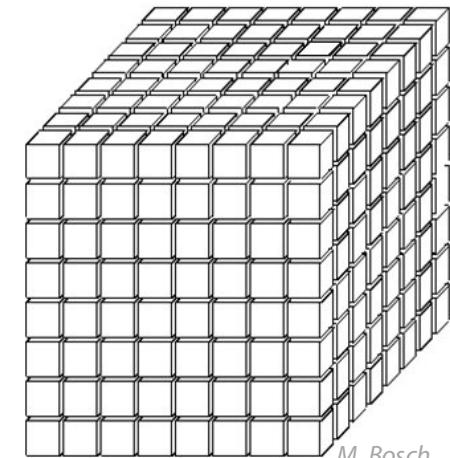
***To find a pixel, you need:***

- x coordinate*
- y coordinate*
- Colour channel*
- z plane*
- Time point*
- (xyczt or other ordering)*

# Stack & Hyperstack



K. Miura



M. Bosch

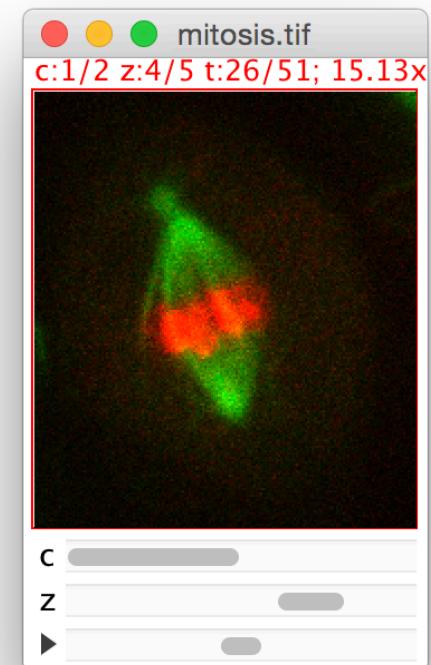
**Stack:** a set of related images of the same size and bit depth (*xyz* or *xyt*).

**Slices:** the images that make up a stack

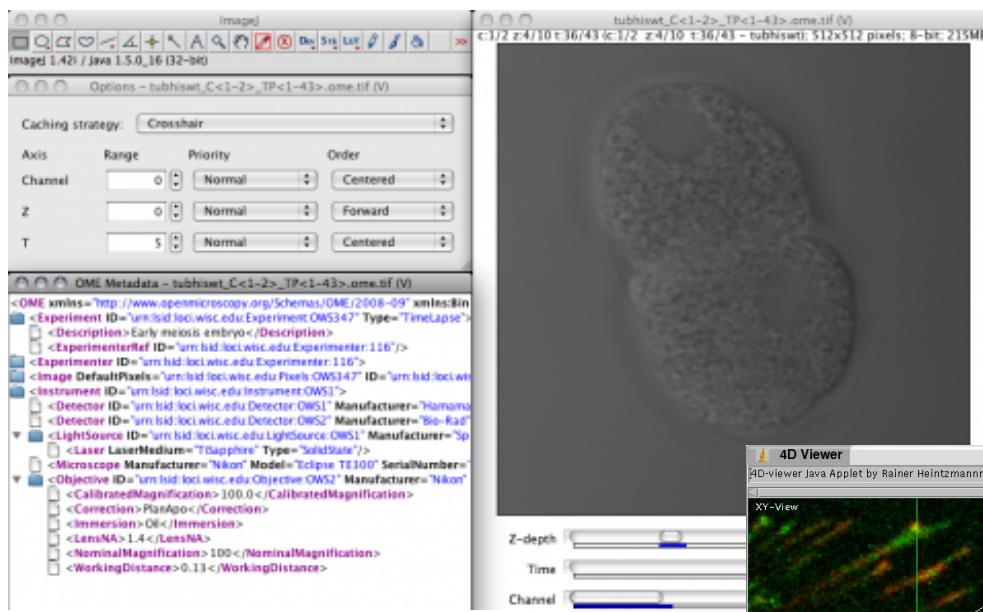
**Voxel:** volumetric image element

**Hyperstack:** When more dimensions are combined into a stack, i.e. four (4D) or five (5D) dimensions: *x* (width), *y* (height), *z* (slices), *c* (channels or wavelengths) and *t* (time frames).

In Fiji, hyperstacks are commonly displayed in a window with up to three scrollbars.



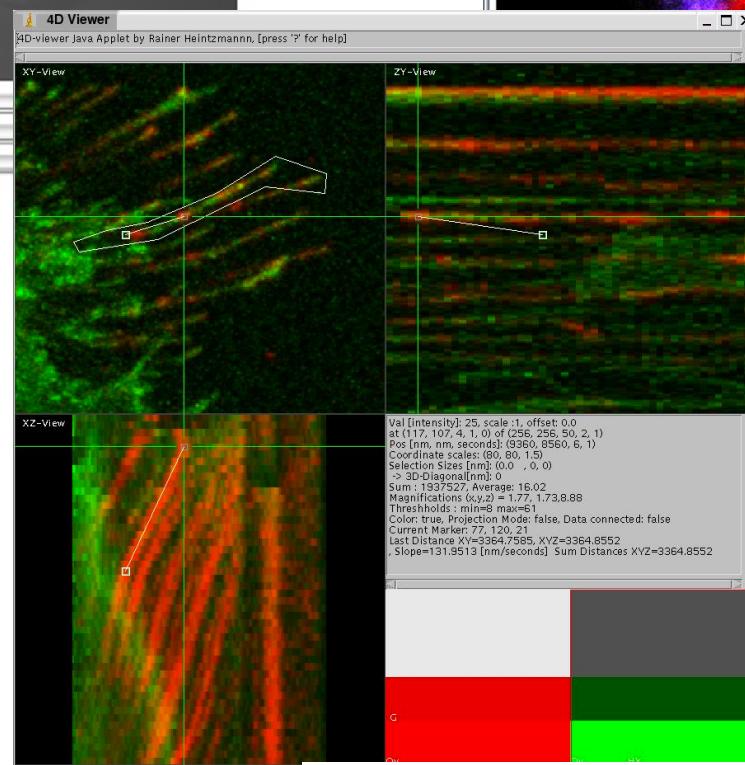
# Other Fiji plugin (hyperstack) viewers



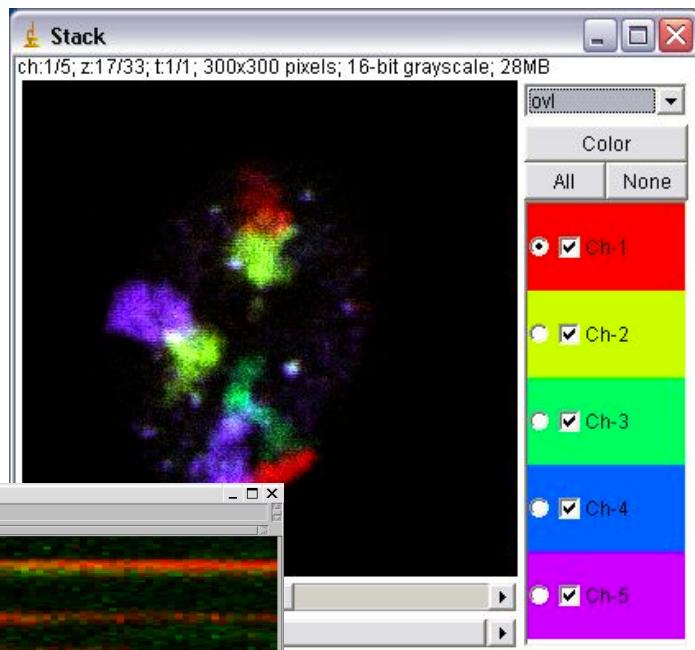
<http://loci.wisc.edu/software/data-browser>

## LOCI Data Browser

View5D



<http://www.nanoimaging.de/View5D>

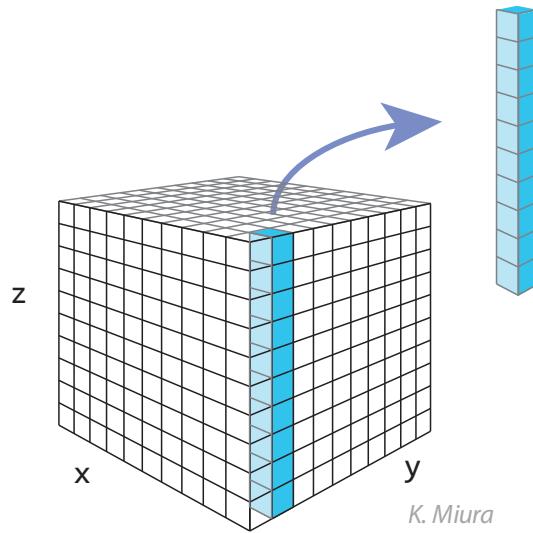


<http://imagej.net/Image5D>

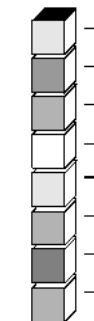
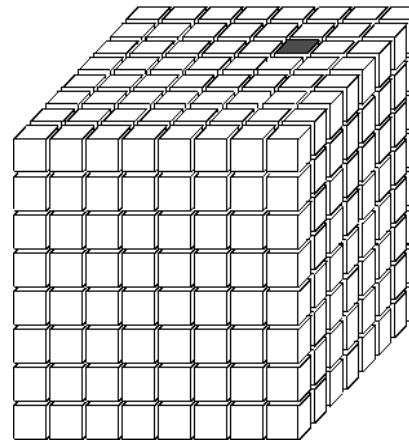
## Image5D

# Projections: "flatten" high dimensional stack

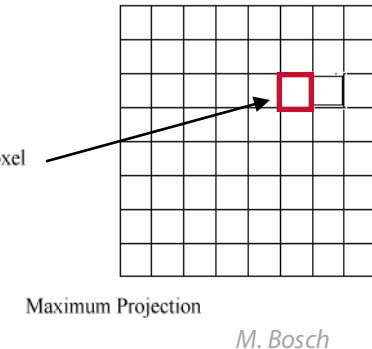
**Projections:** output a single image that results from a calculation at each pixel location over all images in the stack



Stack of images



Projected image



[Image > Stacks > Z Project...]

**Average:** each pixel stores the average intensity over all slices at the particular location

**Maximum/Minimum:** each pixel stores the maximum/minimum value over all slices at the particular location

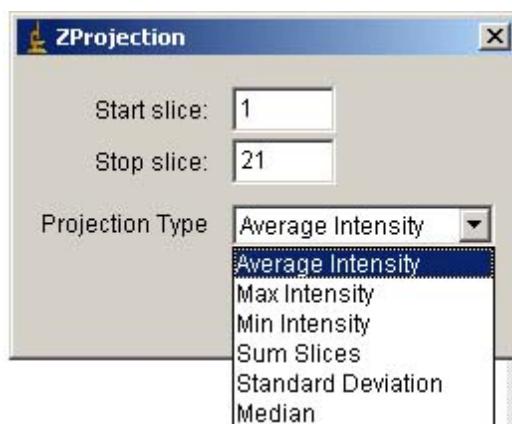
**Sum Slices:** sum all the slices in the stack

**Standard deviation:** standard deviation of all the slices in the stack

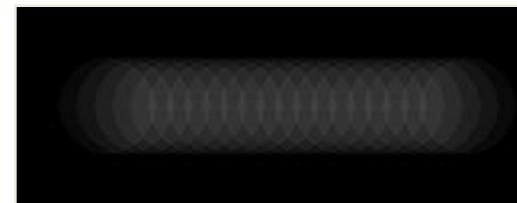
**Median:** each pixel stores the median intensity over all slices at the particular location

# Exercise - Projections: "flatten" 3D stack

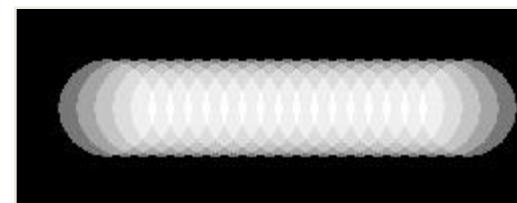
1. [File > Open > moving\_dot.tif ]
2. [Image > Stacks > Z Project]



Average



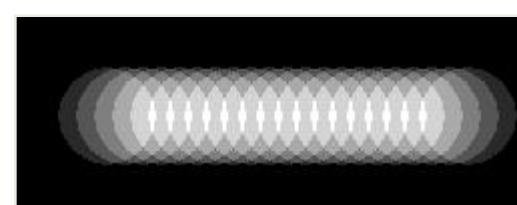
Standard Deviation



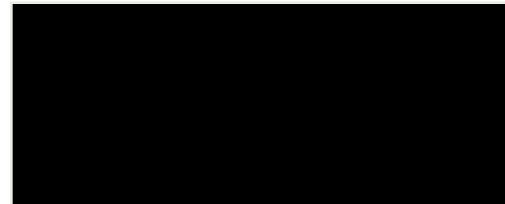
Maximum



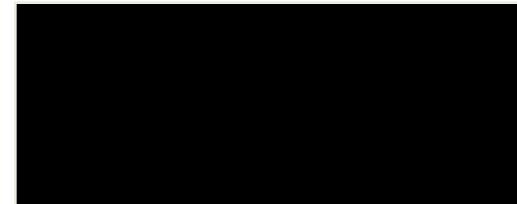
Sum



Minimum



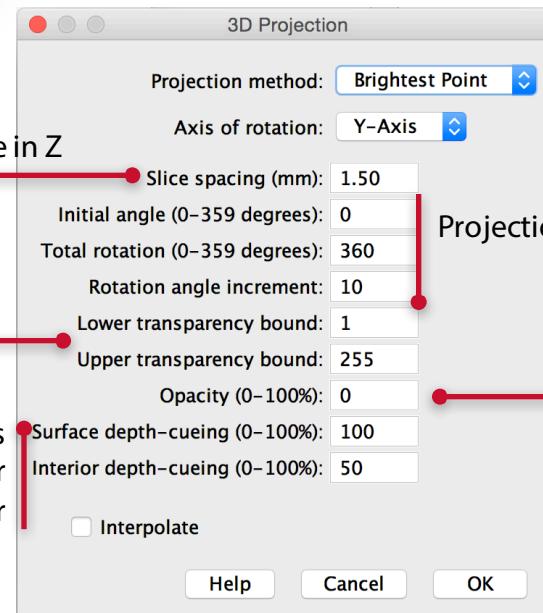
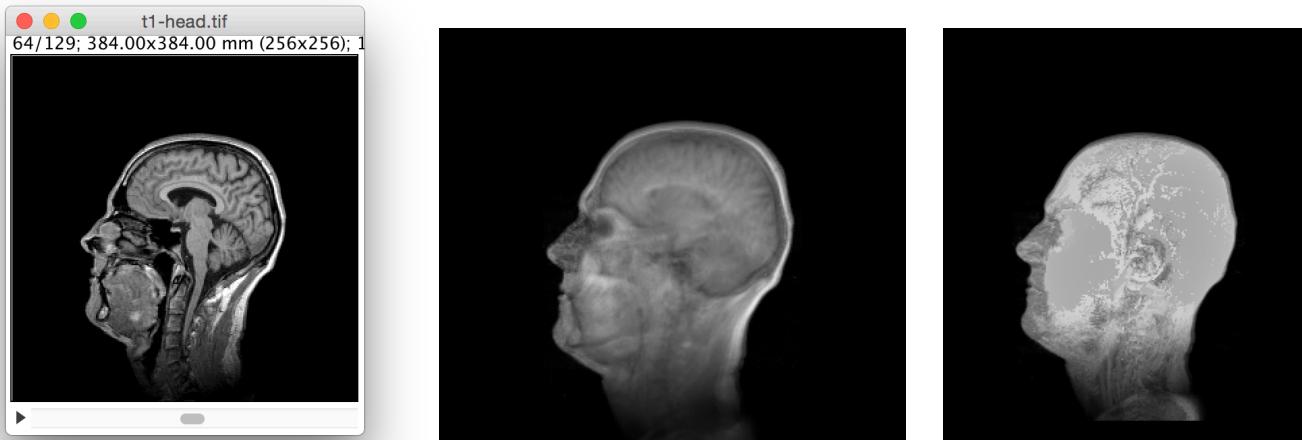
Median



# 3D Projection (for rendering)

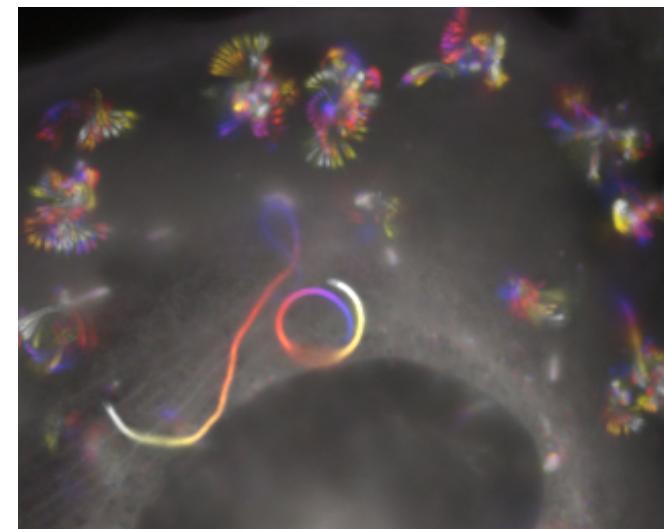
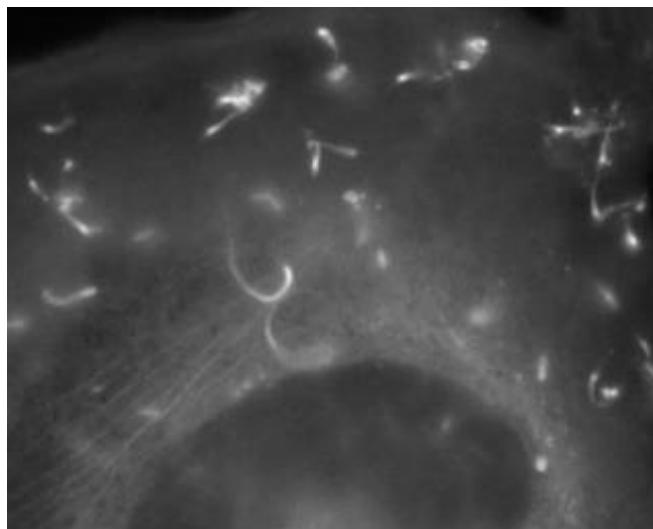
Add 3D labelled  
image,binary  
image,etc.

1. [File > Open Samples > T1 Head]
2. [Image > Stacks > 3D Project...]



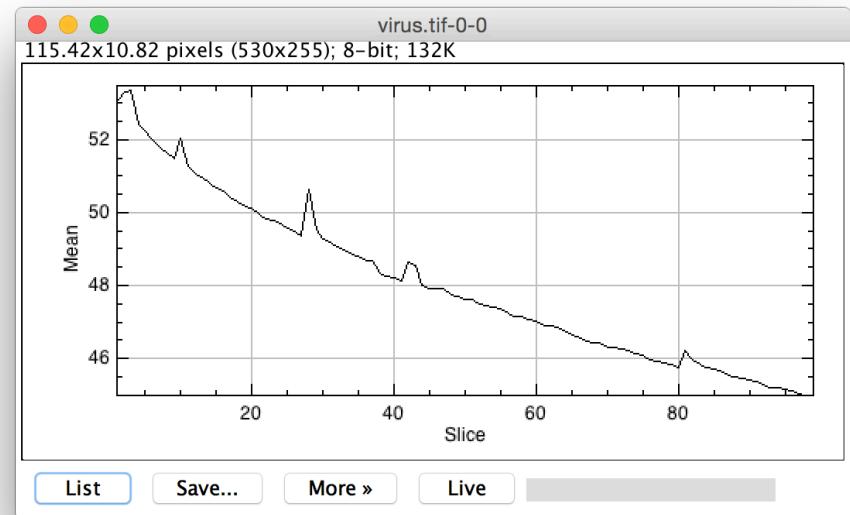
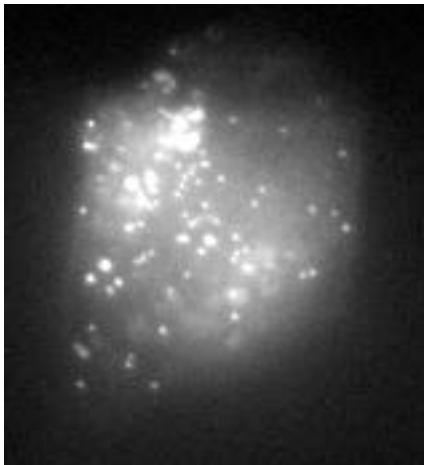
# Color coding – temporal/depth coding

1. [EMBL > Samples > Listeriacells.tif]
2. [Image > Hyperstack > Temporal Color Code]



# Plot Mean grey value vs Slice number

1. [EMBL > Samples > virus]
2. [Image > Stack > Plot Z-axis Profile]

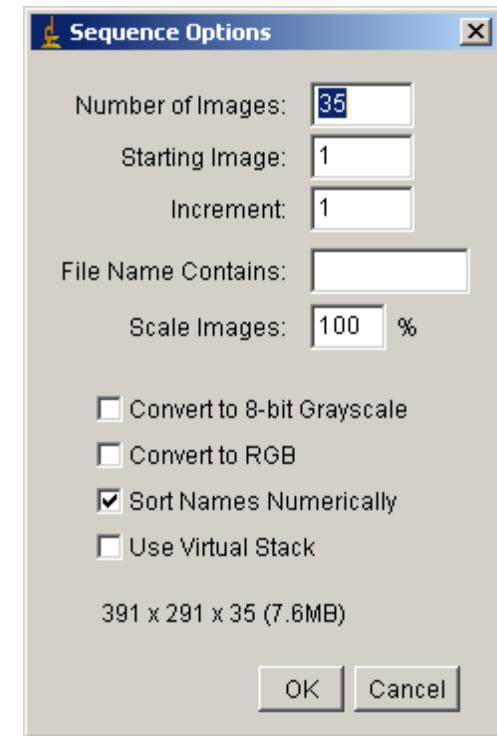
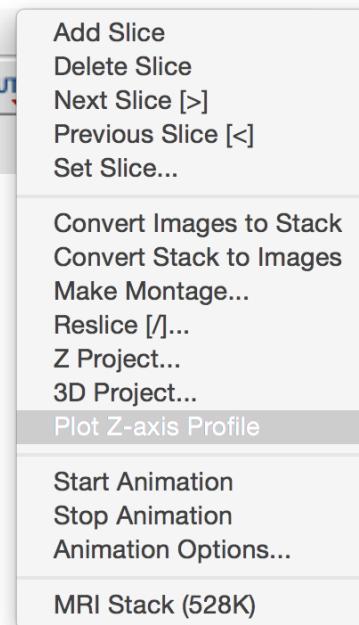


# Open/Import stacks

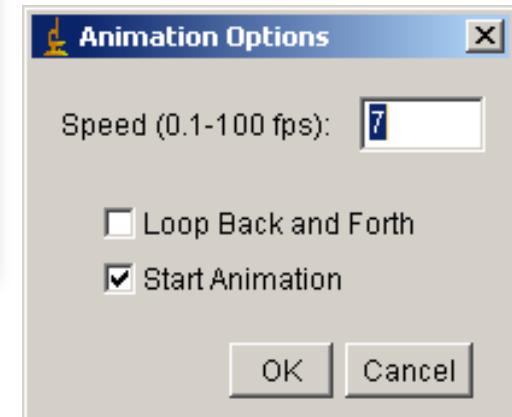
[File > Import > Image Sequence...]

or

[File > Import > Bio-Formats]

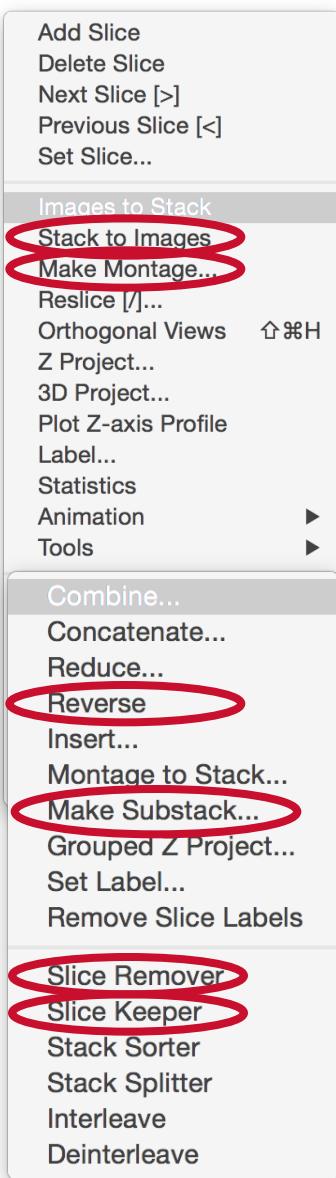


[Image > Stacks > Animation > animation options]



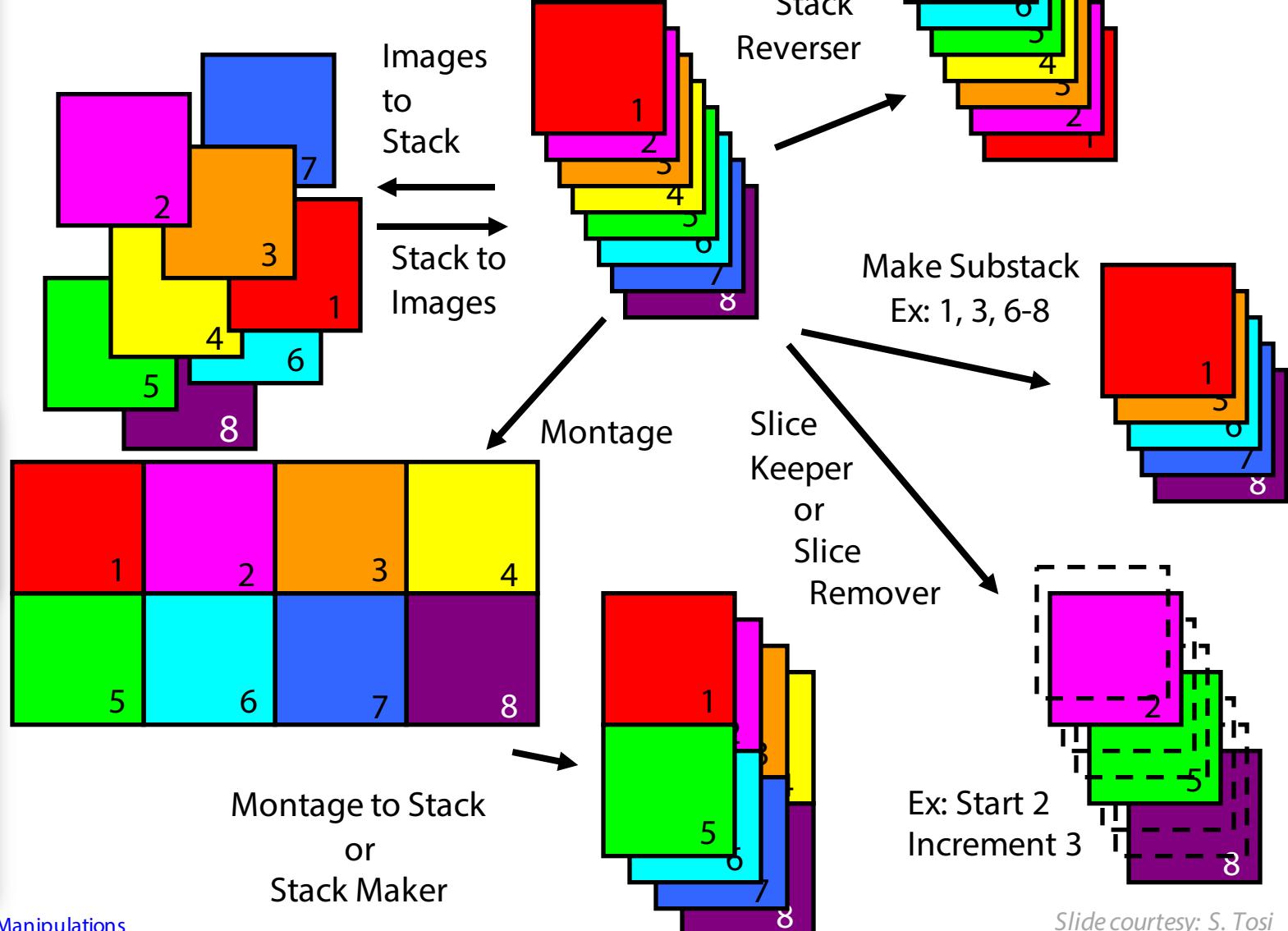
Open example image at [Stk > MRI stack] to play a bit with the functionalities available

# Stack-Slice Manipulation



[ Image > Stacks ]

[ Image > Stacks > Tools ]

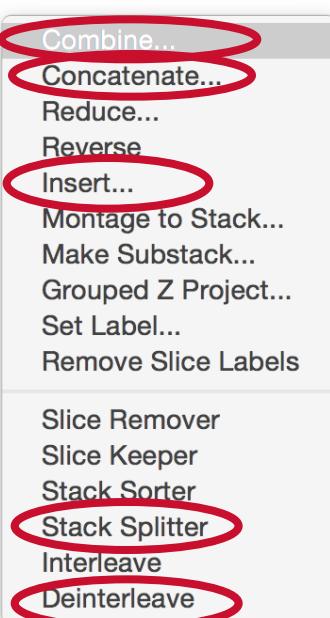


Slide courtesy: S. Tosi

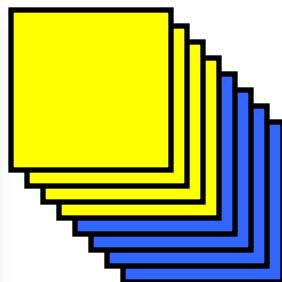
# Stack-Slice Manipulation

[ Image > Stacks ]

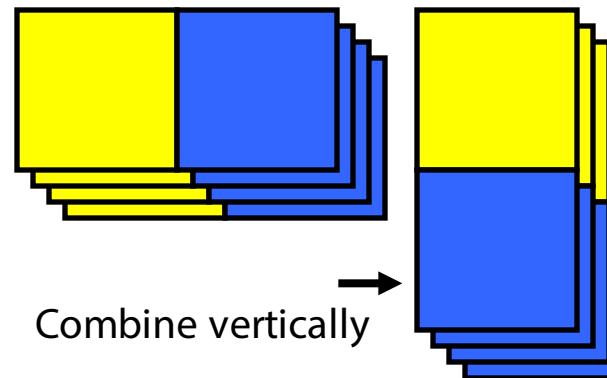
[ Image > Stacks > Tools ]



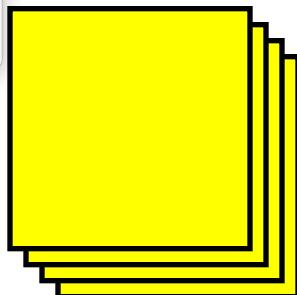
Concatenate



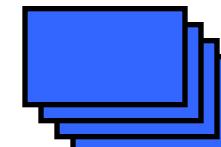
Combine



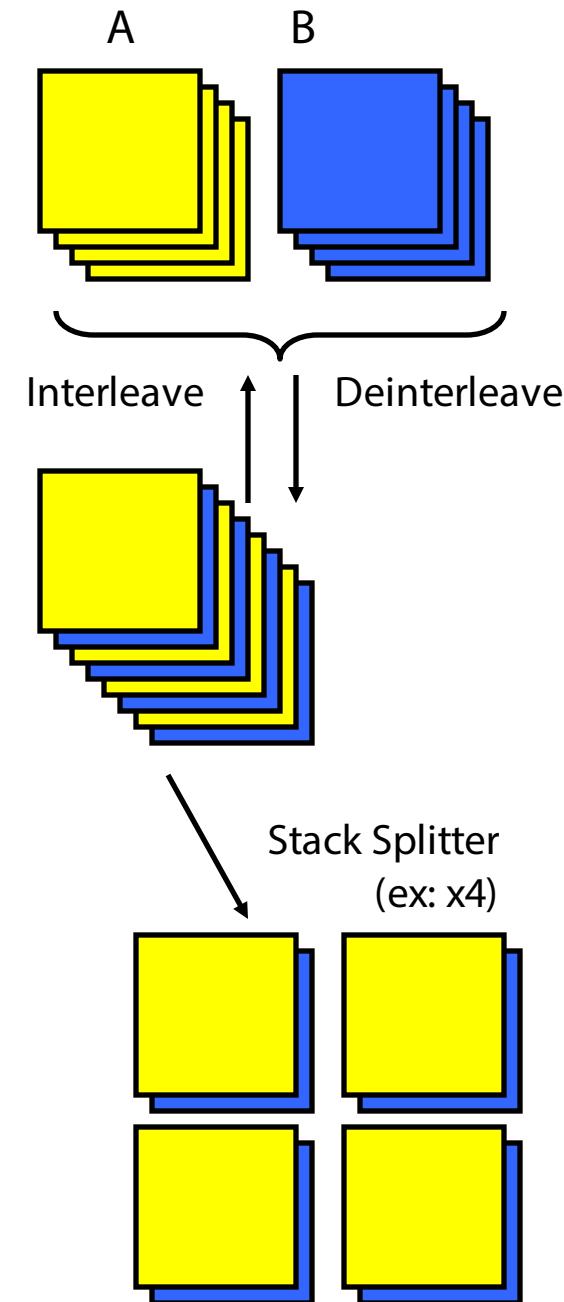
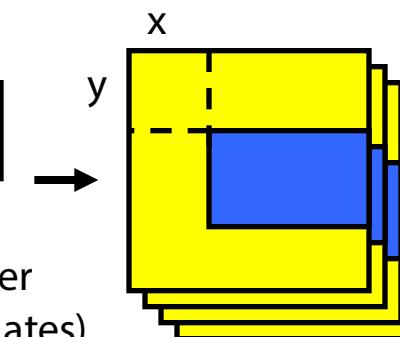
Destination



Source



Stack Inserter  
(x,y coordinates)



# Exercise - Stack-Slice Manipulation

## 1. [File > New > Image...]

- Type: 8-bit
- Fill with: Black
- Width: 200
- Height: 200
- Slices: 10

## 2. [Image > Stacks > Time Stamper]

- Play with X/Y location & font size (70, 90, 30)

## 3. [Image > Stacks > Make Montage...]

- |                |    |
|----------------|----|
| • Columns      | 5  |
| • Rows         | 2  |
| • Border Width | 1  |
| • Label Slices | On |

0 sec	1 sec	2 sec	3 sec	4 sec
1	2	3	4	5
5 sec	6 sec	7 sec	8 sec	9 sec
6	7	8	9	10

K. Miura

## 4. Deleting every second frame

[Image > Stacks > Tools >  
Slice Remover ]  
First/Last slice,  
increment: 2,10,2

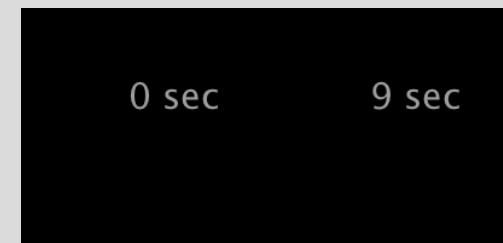


## 5. Duplicate the stack, Invert the stack order and combine them side by side to make a single stack.

[Image > Stacks > Tools > Reverse]

[Image > Stacks > Tools > Combine...]

- Select the two stacks
- untick Combine vertically



# Exercise – 3D reconstruction

1. [EMBL > Samples >  
Mitosis\_4D.tif]

- Find out hyperstack dimensions

2. [Image > Hyperstacks > Re-order  
Hyperstacks...]

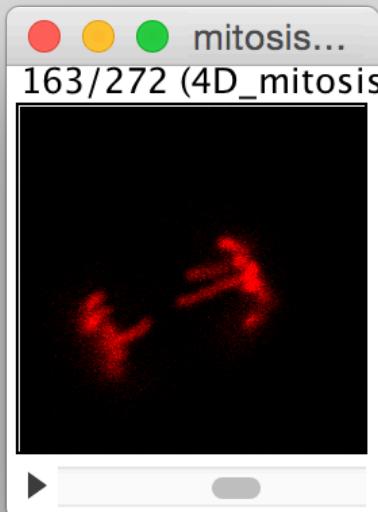
3. Extract substack of 3D anaphase  
at time point 11

[Image > Stacks > Tools > Make Substack...]

Slices: 1-16, Frames: 11

Or from original hyperstack:

Slices: 161-176-1



4. Change voxel depth to 5

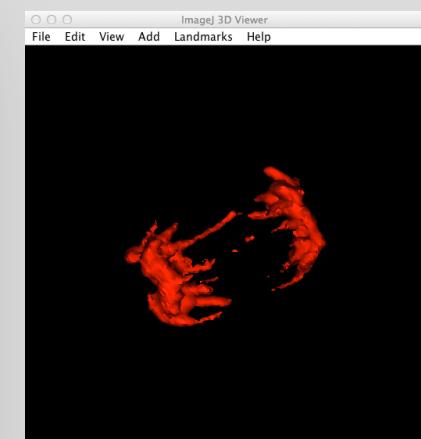
[Image > Properties...]

Voxel depth: 5

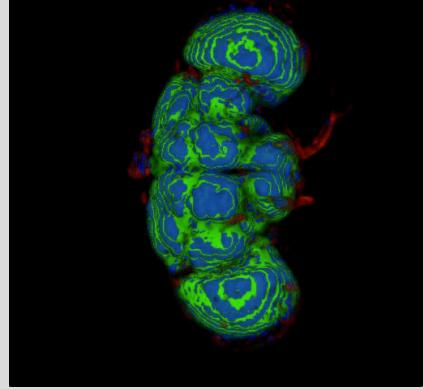
5. [Plugins > 3D Viewer]

- Display as: Surface
- Color: Red
- Threshold: 41
- Resampling factor: 1

6. Try Mitosis\_4D.tif to view a  
time series in the 3D viewer



# Exercise – 3D Viewer



1. [File > Open Samples > flybrain.tif]
2. Split channels, and save the red and green channels
3. [Plugin > 3D Viewer]
  - Image: flybrain.tif (green)
  - Display as: volume
  - Color: green
4. [Add > From Image]
  - Image: flybrain.tif (green)
  - Display as: surface
  - Name: mesh
  - Color: white
  - Threshold: 100
5. [Edit > Select > flybrain.tif (green)], rotate/translate it  
[Edit > Select > flybrain.tif (green)], rotate/translate it  
select each object, do [Edit > Transformation > Reset Transformation]  
unselect any object
6. Select flybrain.tif (green), [Edit > Change Transparency], play till satisfied  
Select mesh, [Edit > Change Color] to blue, and play with transparency till  
satisfied, you can also [Edit > Adjust threshold]
7. [Add > From Image]
  - Image: flybrain.tif (red)
  - Display as: volume
  - Color: red

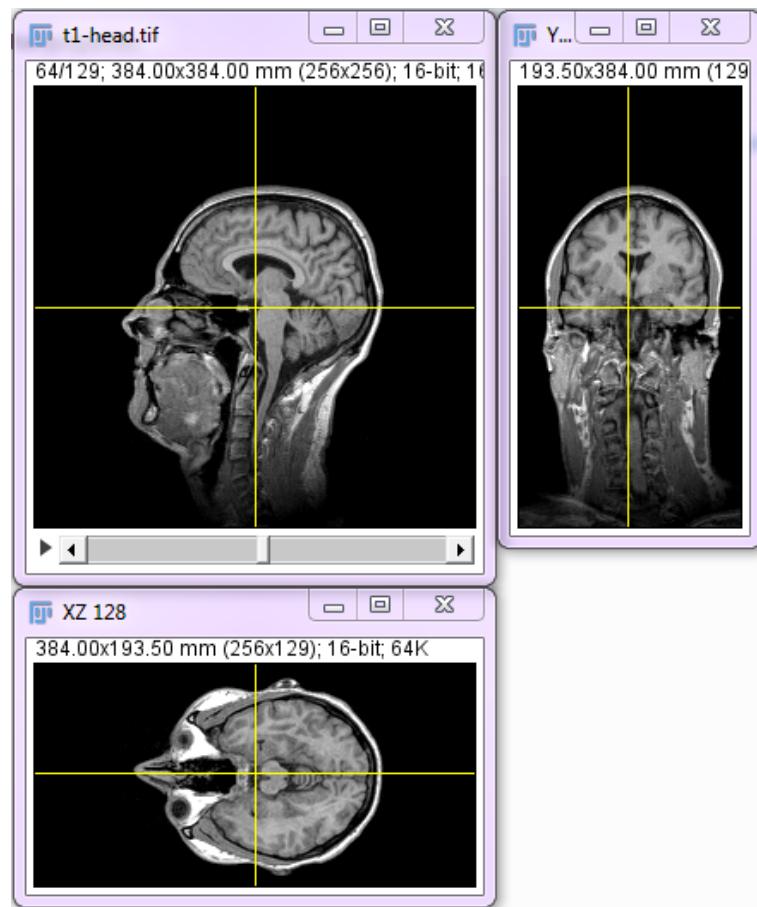
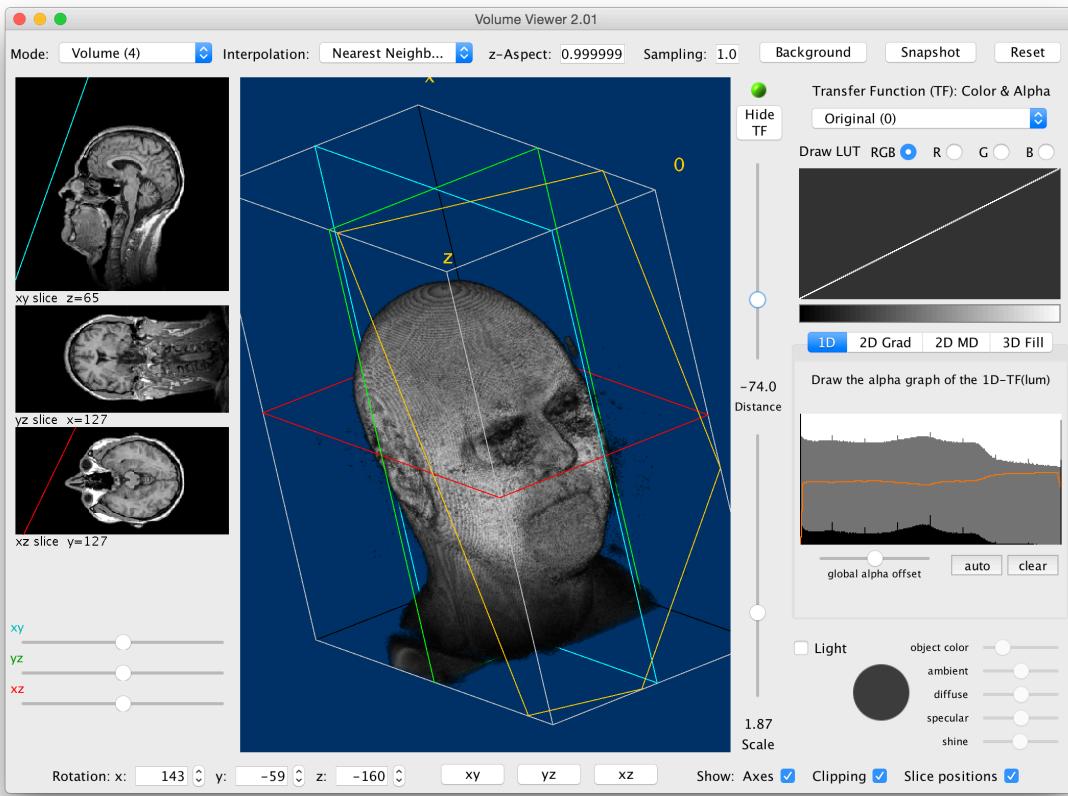
[View > Change animation options], rotate around Y-axis, rotation interval: 5 degrees

[View > Record 360 degree animation] or [View > Start freehand recording] then stop  
change the animation speed to 15 fps
8. Select 3D Viewer, select mesh, [Edit > Delete]

If you want, they can be recorded as macro!

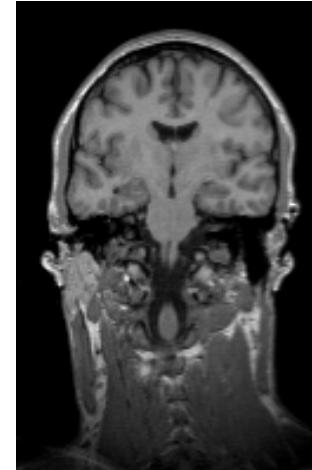
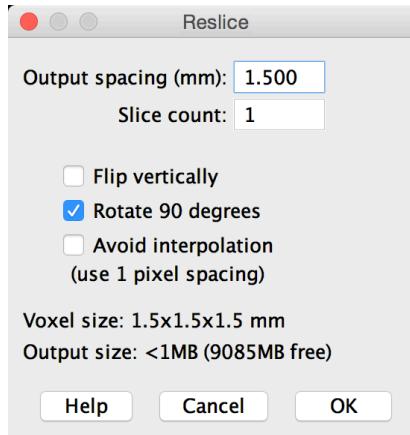
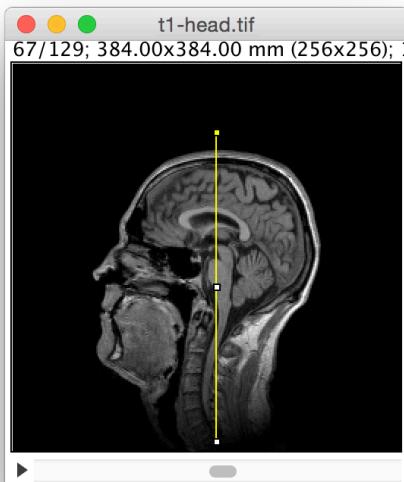
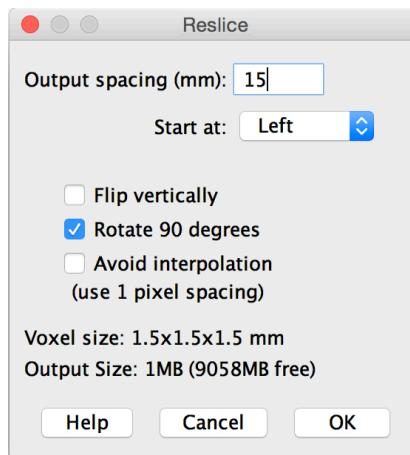
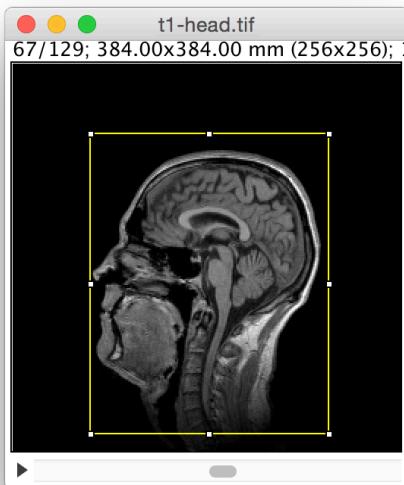
# Orthogonal Views

1. [File > Open Samples > T1 Head]
2. [Image > Stacks > Orthogonal views]
  
3. [Plugins > Volume Viewer]



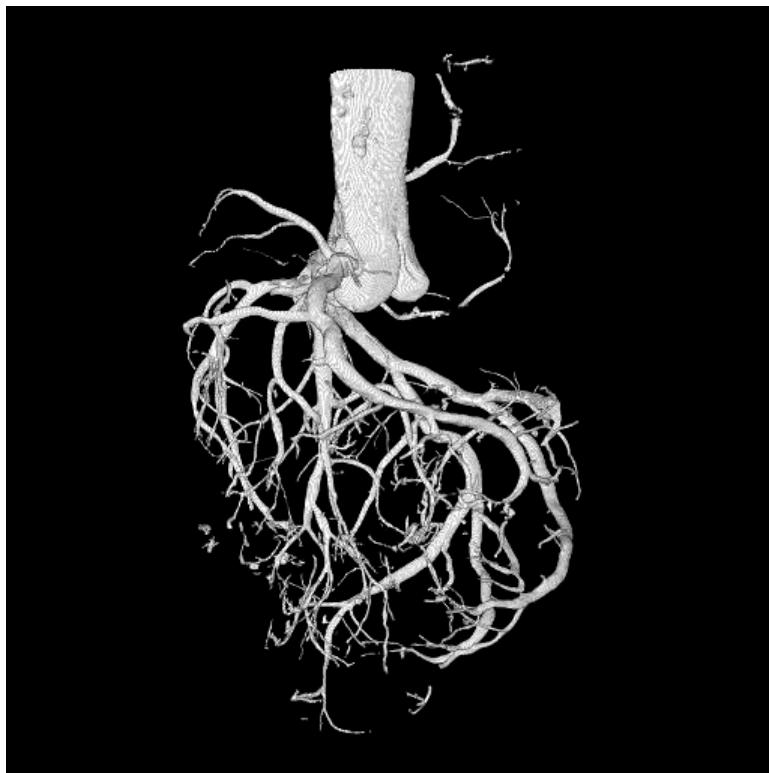
# Reslice

1. [File > Open Samples > T1 Head]
2. [Image > Stacks > Reslice]



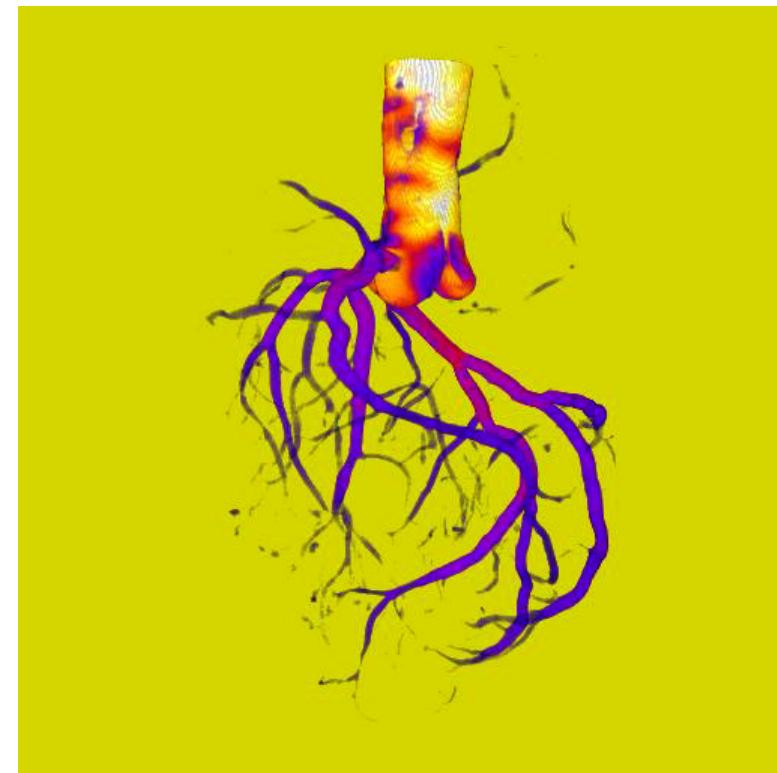
# Volume rendering – color coding

(Binary) volume



Local thickness estimate

[Analyze > Local Thickness]



116  
0

# 3D visualization tools

## Proprietary software:

Imaris (Bitplane), VoloCity (Perkin Elmer), Amira (Visualization Sciences Group), Huygens (Scientific Volume Imaging), ...

## Free software:

ImageJ, Fiji, Icy, Vaa3D, ImageSurfer, BioImageXD, Paraview, Volview, ...

## ImageJ / Fiji plugins:

3D Viewer, Volume viewer, VolumeJ

# Exercise – Segment 3D spots

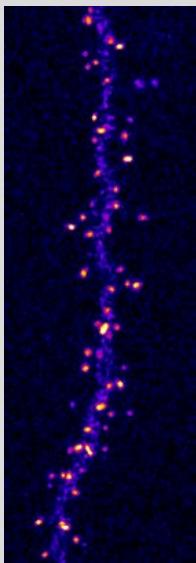


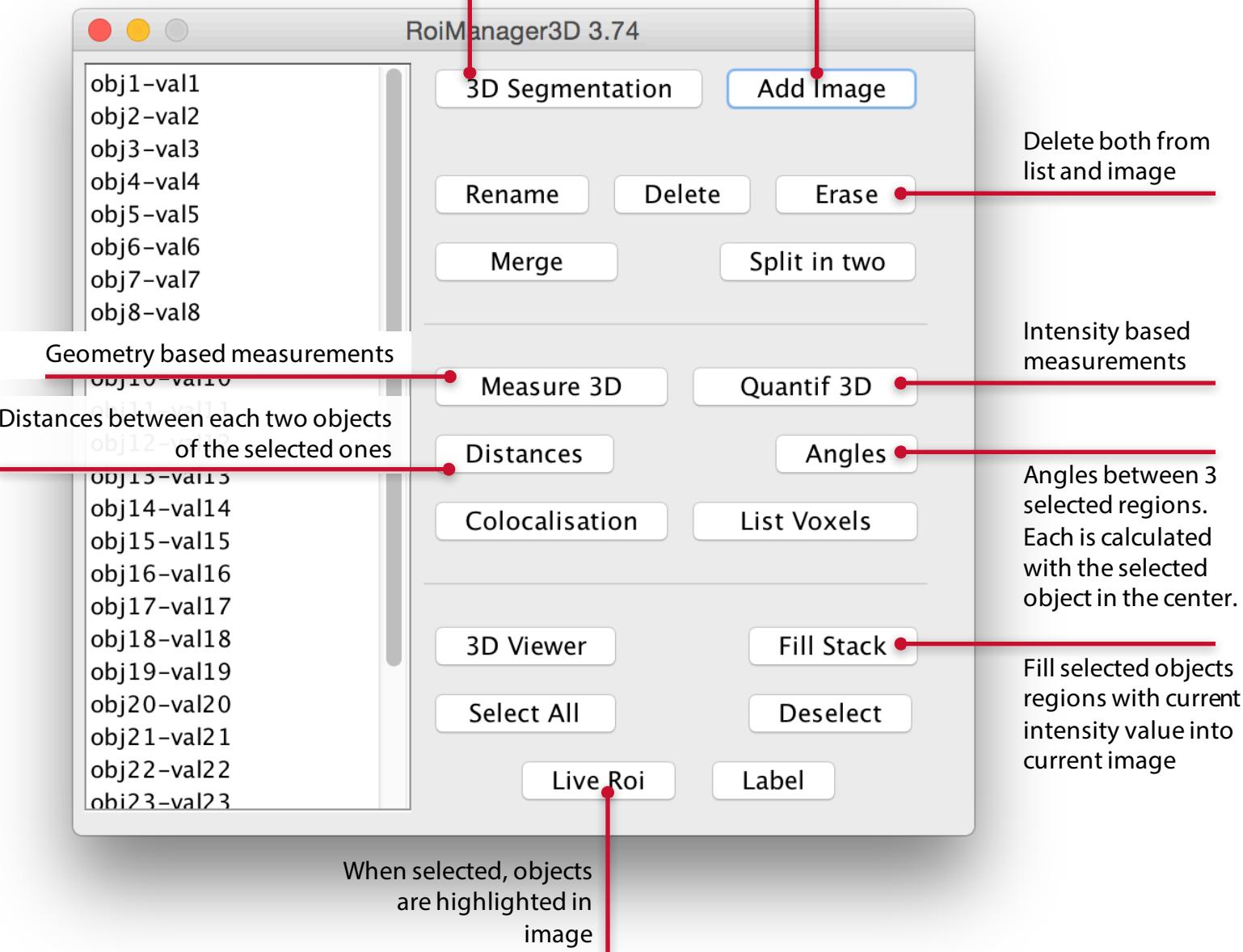
Image courtesy of:

Dr. Sebastiane Tosi  
Institute for Research in  
Biomedicine



0. ([ Plugins > Macros > Record ])
1. [ Open > GFP neurons spines.tif ]
2. [ Plugins > Feature Extraction > FeatureJ > FeatureJ Laplacian ]
  - apply the filter with a smoothing scale of 2
3. On the filtered image: [ Image > Adjust > Threshold ]
  - un-tick set background pixels to NaN
  - un-tick "calculate threshold for each image"
  - if wish background as white, un-tick "black background"
  - apply the threshold
4. [ Process > Binary > Fill holes ]
5. [ Analyze > 3D OC Options ]
  - tick the interested measurements.
  - tick "show masked image" & select the original stack in the "redirect to" drop down menu.
  - untick "show numbers".
6. [ Analyze > 3D objects counter ]
  - threshold: 128; minimum size filter: 5 voxels
  - tick objects, statistics and summary
7. ([ Analyze > Tools > Sync windows ])
  - Explore objects map stack (1 color per object) together with the original image

# 3D Manager



# 3D processing/analysis filters



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You are here: Welcome to the ImageJ Information and Documentation Portal » Plugins » [stacks](#) » 3D ImageJ Suite

## 3D ImageJ Suite

This "suite" provides plugins to enhance 3D capabilities of ImageJ.

### Author

Thomas Boudier (thomas dot boudier at snv dot jussieu dot fr)  Cellular Modeling and Biological Imaging.

With many contributions from J. Ollion (MNHN USM0503).

### Features

This "suite" is composed of :

- 3D Filters (mean, median, max, min, tophat, max local, ...)
- 3D Segmentation (hysteresis thresholding, spots segmentation, watershed, ...)
- 3D Mathematical Morphology tools (fill holes, binary closing, distance map, ...)
- 3D ROIManager (3D display and analysis of 3D objects)
- 3D Analysis (Geometrical measurements, Mesh measurements, Convex hull, ...)
- 3D MereoTopology (Relationship between objects)
- 3D Tools (Drawing ellipsoids and lines, cropping, ...)

A 2D/3D spatial statistics plugin is also available.

### Installation

Download and copy the following jars in your plugins folder or alternatively [download](#) the  bundle 2.6 and unzip it in your plugins folder. The various plugin jars are:

You have also to manually download and copy into your plugins directory the **imageScience.jar** library from :  <http://www.imagescience.org/meijering/software/>

- 3D Fast Filters
- 3D Edge and Symmetry Filter
- 3D Hysteresis Thresholding
- 3D Simple Segmentation
- 3D Maxima Finder
- 3D Spot Segmentation
- 3D Iterative Thresholding
- Manual Spot Segmentation
  
- 3D Fill Holes
- 3D Binary Close Labels
- 3D Distance Map
- 3D Watershed
- 3D Watershed Split
- 3D Watershed Voronoi
  
- 3D Manager
- 3D Manager Options
- 3D Geometrical Measure
- 3D Shape Measure
- 3D Intensity Measure
- 3D Mesh Measure (slow)
- 3D MereoTopology
- 3D Exclude Borders
  
- 3D Radial Distribution
- 3D Ellipsoid Fitting
- 3D Convex Hull
  
- 3D Draw Shape
- 3D Draw Line
- 3D Crop
- 3D Crop All
- 3D Binary Interpolate
- 3D Draw Rois

# Exercise – 3D quantification with macro

To write a script using this plugin we need to look at its own macros functions.

AddImage	Adds the objects in the current labeled image to the list			Ext.Manager3D_AddImage();
Count	Get the number of objects		Number of objects	Ext.Manager3D_Count(nb_obj); print("number of objects",nb_obj);
Select	Select an object (behavior depends on select mode, see monoselect and multiselect)	The object number		object=0 // 0 = first object Ext.Manager3D_Select(object);

Write a script to colorize the objects based on their volume  
 Remember, you can use the macro recorder

```

1 waitForUser("Select the image with the objects to colorize");
2 w=getWidth();
3 h=getHeight();
4 s=nSlices();
5 run("3D Manager");
6 Ext.Manager3D_AddImage();
7 run("3D Manager Options", "volume compactness mean_grey_value std_c");
8 Ext.Manager3D_Measure();
9 selectWindow("3D Measure");
10 IJ.renameResults("3D Measure", "Results");
11 newImage("color size", "8-bit black", w, h, s);
12 selectWindow("color size");
13 vtab=newArray(nResults);
14 for (i=0;i<nResults;i++)
15   vtab[i]=getResult("Vol (unit)", i);
16 Array.getStatistics(vtab, vmin, vmax, vmean, vstd);
17 for (i=0;i<nResults;i++) {
18   Ext.Manager3D_Select(i);
19   v=getResult("Vol (unit)", i);
20   r=(v-vmin)/(vmax-vmin);
21   Ext.Manager3D_FillStack(255*r, 255*r, 255*r);
22 }
23 run("Fire");
24 setSlice(s/2+1);
25 run("Enhance Contrast", "saturated=0.35");
26 print("finished!");
27
  
```

ImageJ macro functions related to results in a table only work if the table is called "Results"

Storing all volume measurements in an array allows us to later obtain the statistics

Calculate normalized voxel size, and store it to each object in the new stack

Image courtesy of:

Dr. Sebastianne Tosi  
 Institute for Research in Biomedicine



September 12, 13 morning (optional)

ilastik (<http://ilastik.org>)

simple, user-friendly **interactive** tool  
image classification  
**segmentation** up to **5D**  
**track** animals and dividing objects  
**count** without detection

Workshop page: <http://bit.ly/292q8TR>  
Sign up for **FREE**: <http://bit.ly/292O36v>  
Agenda: <http://bit.ly/292ubQy>

# ILASTIK WORKSHOP

## MACHINE LEARNING FOR EVERYONE

September 12, 2016  
10.00 a.m. - 06.00 p.m.  
Roc Boronat building  
— 54.030

### Beginner?

Heard about ilastik, but never tried it?  
Never heard about ilastik, but would like to try a tool for interactive classification, segmentation, counting and tracking?

### Advanced users?

Want to know about new features? Tried ilastik already, but it didn't work for your data? It worked, but you think we could make it work better?

Use ilastik together with other tools?  
Developed your own ilastik extension?  
Run ilastik on a cluster?

**Sign up here!**  
<http://bit.ly/292036v>

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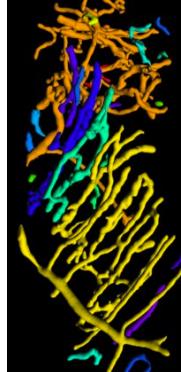


Universitat  
Pompeu Fabra  
Barcelona

Department of Information  
and Communication Technologies

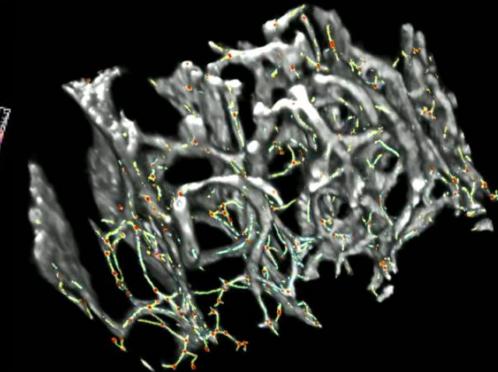
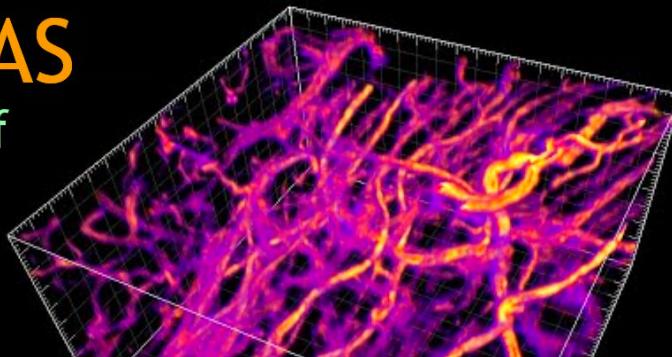


BIOIMAGE  
ANALYSIS



# NEUBIAS

## Network of European Biolimage Analysts



### Day 1 (Sep 13th, Tue)

- Introduction & workflows in Biolimage analysis
- Introduction to ImageJ macro language
- Segmentation, operations with binary images, task automation
- Image restoration (background subtraction, denoising, deconvolution...)

### Day 2 (Sep 14th, Wed)

- 3D Colocalization analysis
- 3D Blood vessels segmentation, network analysis and measurements
- *Meet the taggers 1:* opportunity to meet bioimage analysts working to build new NEUBIAS resources (see Taggathon)
- Social dinner with taggers

### Day 3 (Sept 15th, Thu)

- Introduction to Matlab
- Image processing and data analysis with Matlab
- *Meet the taggers 2:* opportunity to meet bioimage analysts working to build new NEUBIAS resources (see Taggathon)
- Social dinner with taggers

### Day 4 (Sept 16th, Fri)

- Directionality analysis of EB1 movement along microtubules (ImageJ + Matlab)
- Advanced visualization and registration of large data, ImageJ BigData Viewer
- Open session: *Present your problem, code your workflow, get help from trainers*

September 13-16

NEUBIAS training school for facility staff

Application deadline: July 15!

Application: <http://bit.ly/294a0Cq>



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Barcelona



EXCELENCIA  
MARÍA  
DE MAEZTU

# Thank You!

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30<sup>th</sup> June, 2016

Slides courtesy:



Pete Bankhead  
*Queen's University  
Belfast*

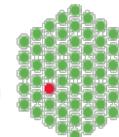


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