

USING FIJI TO

EXPLORE IMAGE STACKS

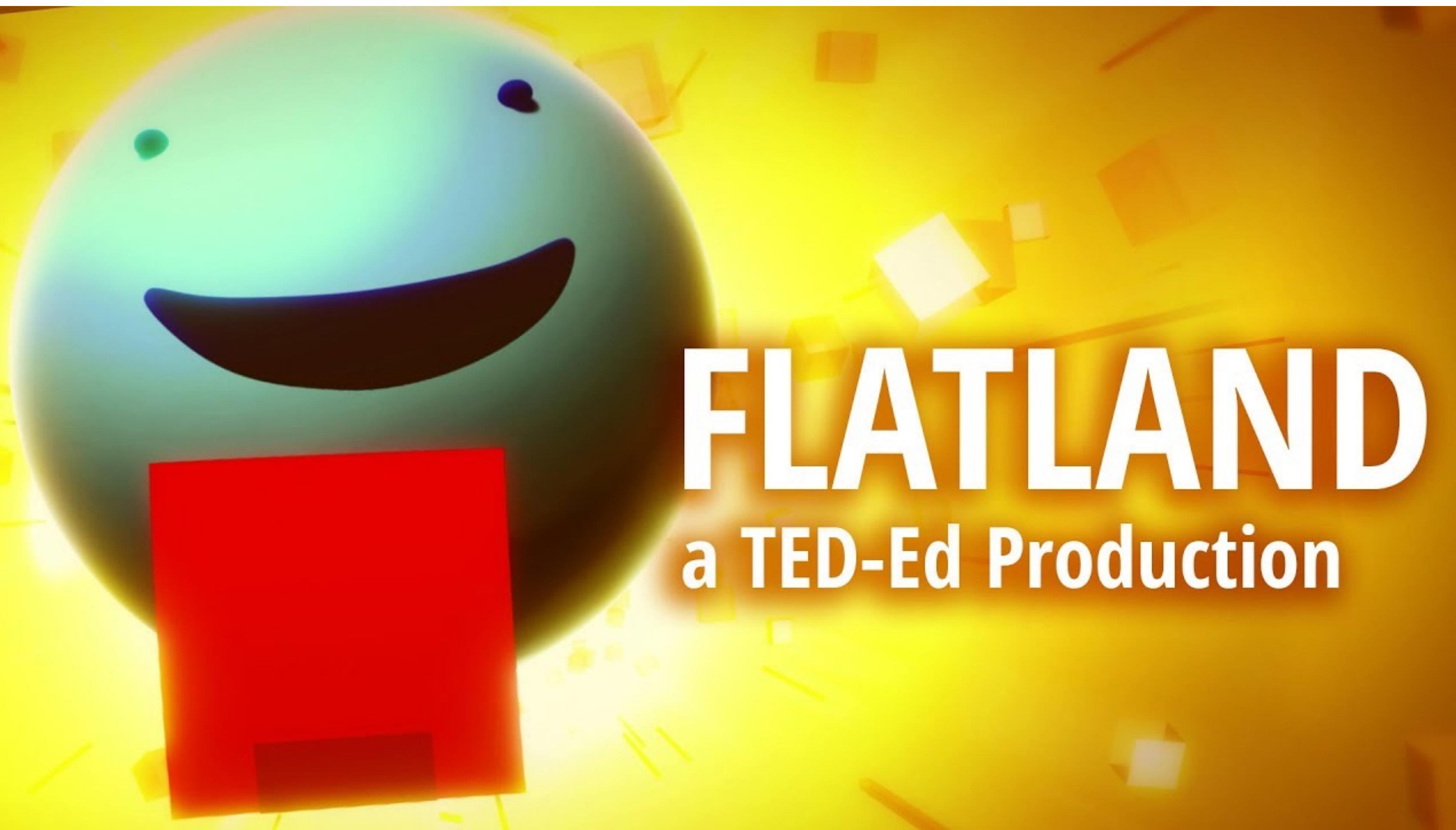
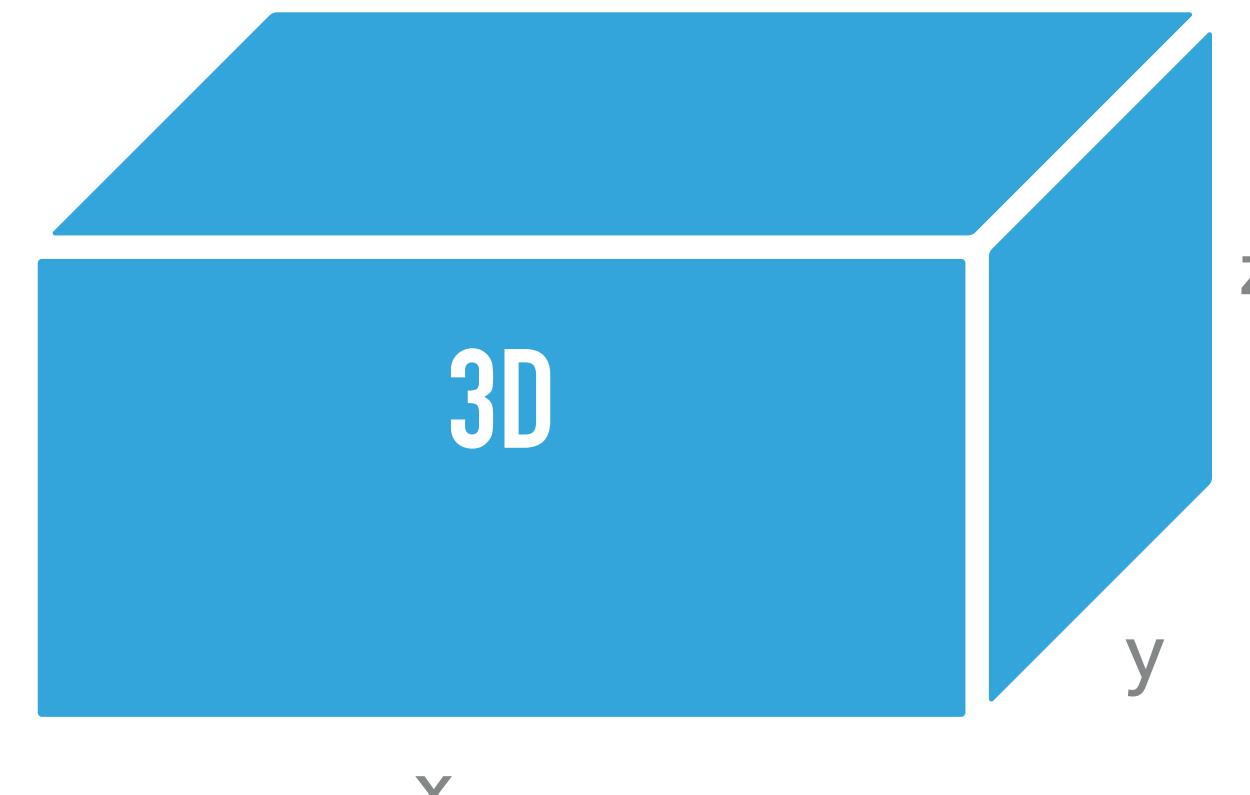
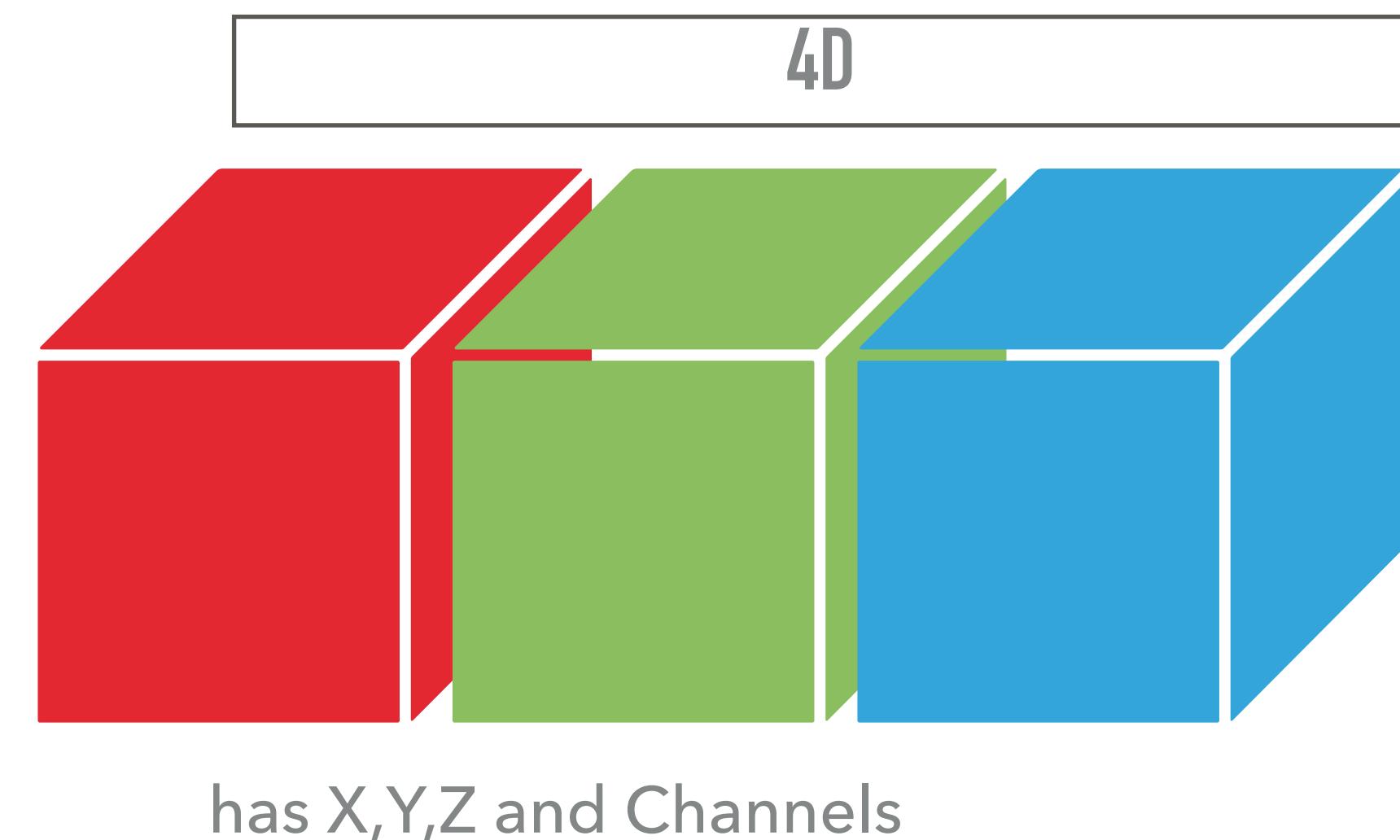


IMAGE STACKS

3D AND BEYOND

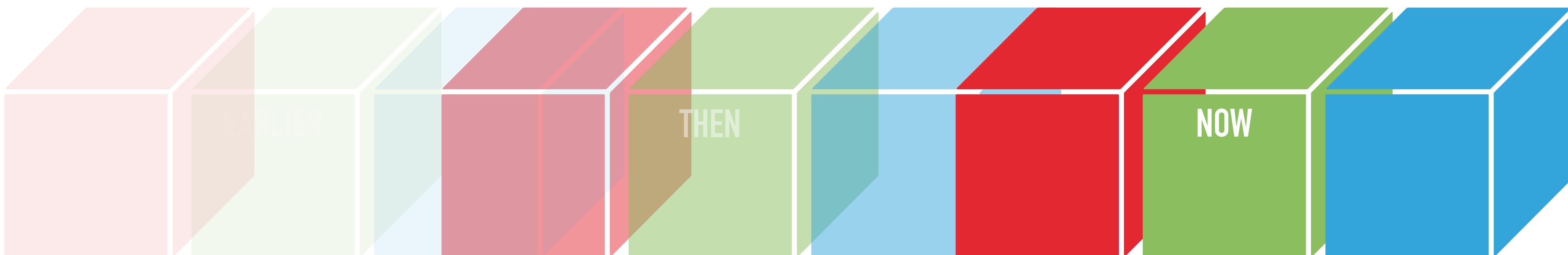


has X,Y,Z



has X,Y,Z and Channels

5D

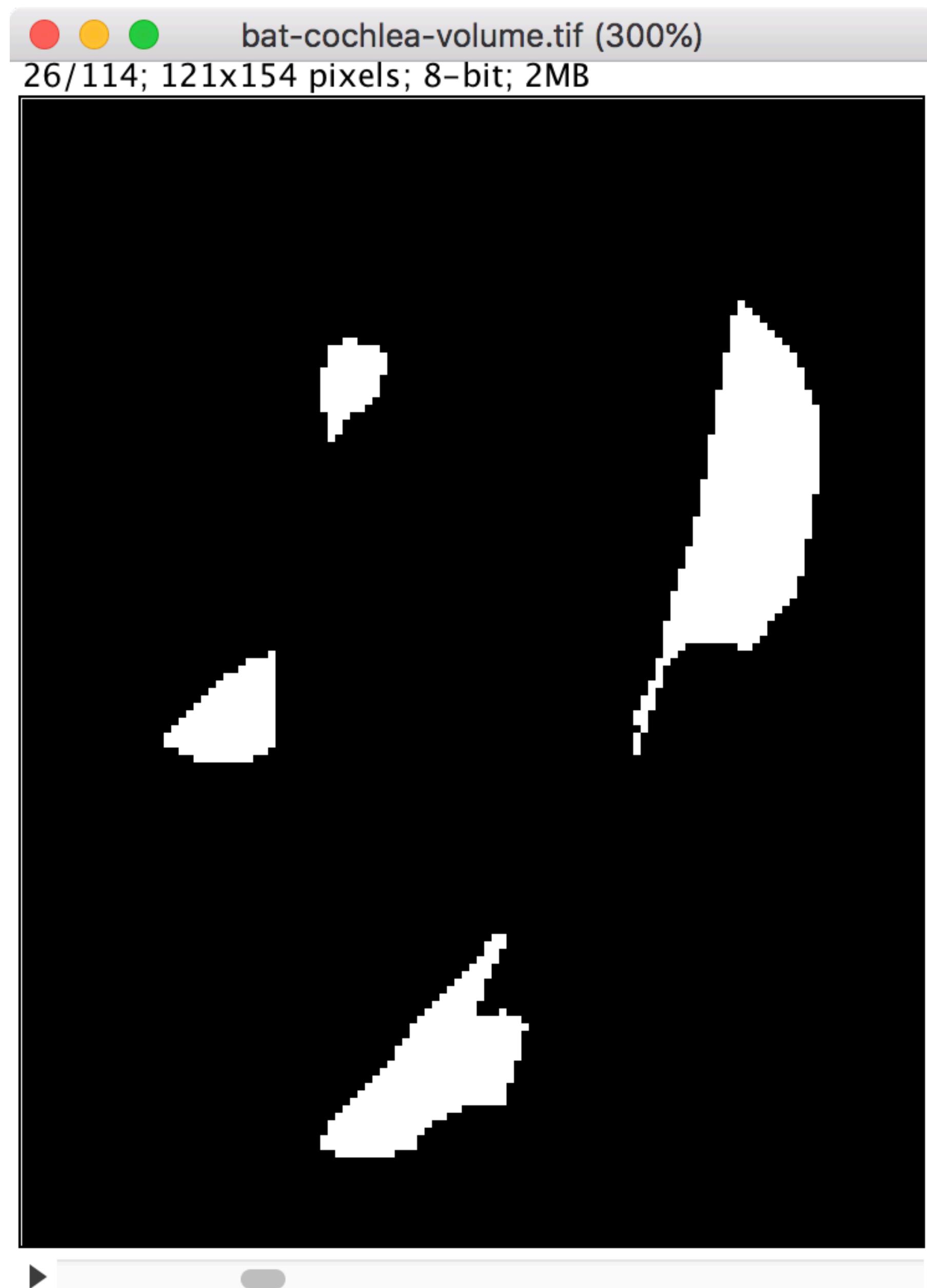
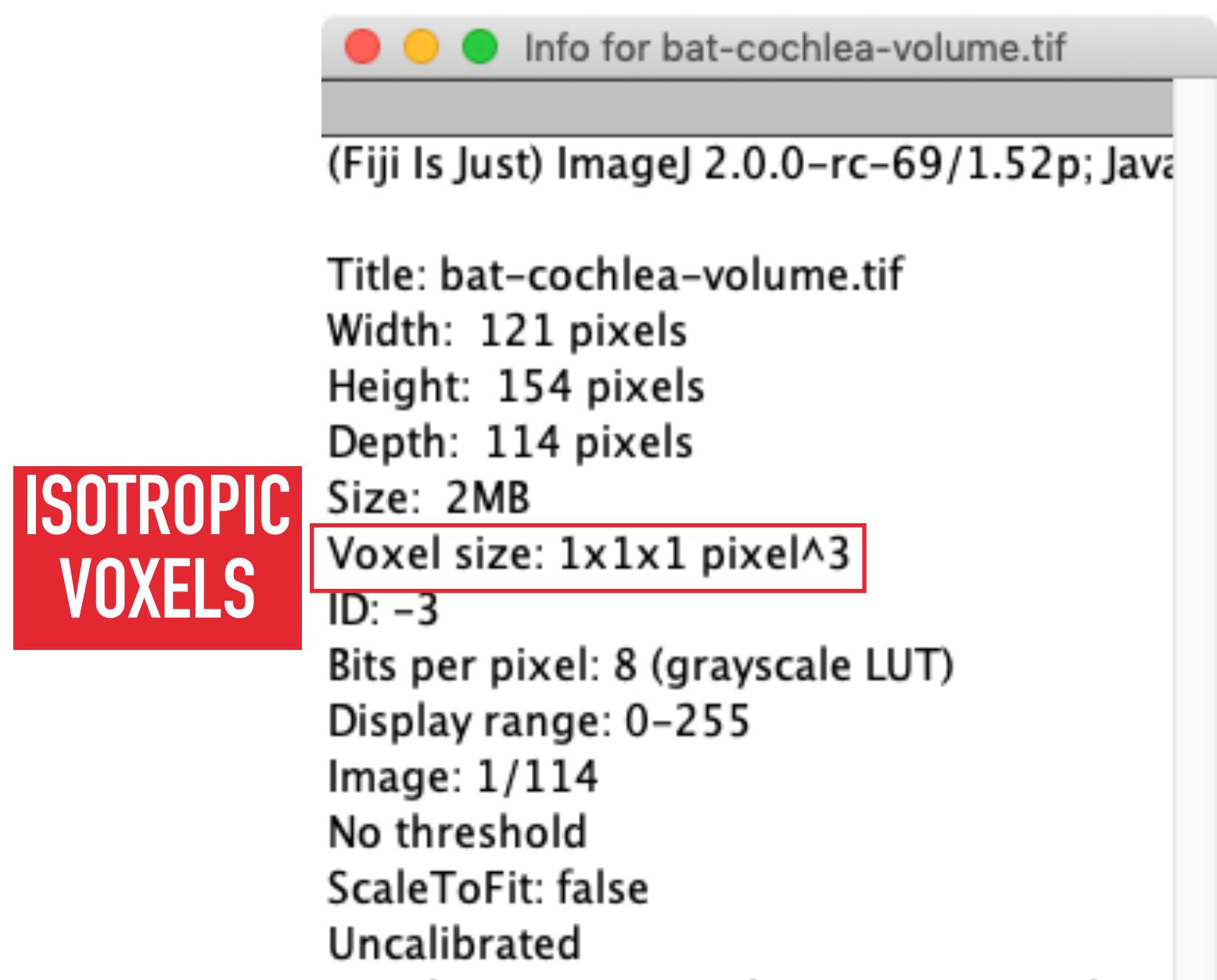


has X,Y,Z , Channels, and Time

3D STACK

BAT COCHLEA VOLUME

- ▶ A 1-Channel, 114 slice 3D stack
 - ▶ 114 images in total
- ▶ Notice that there is only one scroll bar at the bottom



3D STACK - BAT COCHLEA

MAKE A MONTAGE

IMAGES:STACKS

Add Slice
Delete Slice
Next Slice [>]
Previous Slice [<]
Set Slice...

Images to Stack
Stack to Images

Make Montage...

Reslice [/]...

Orthogonal Views

Z Project...

3D Project...

Plot XY Profile

Plot Z-axis Profile

Label...

Statistics

Animation

Tools

Radial Reslice

Dynamic Reslice

Series Labeler

Kalman Stack Filter

Time Stamper

Reslice Z

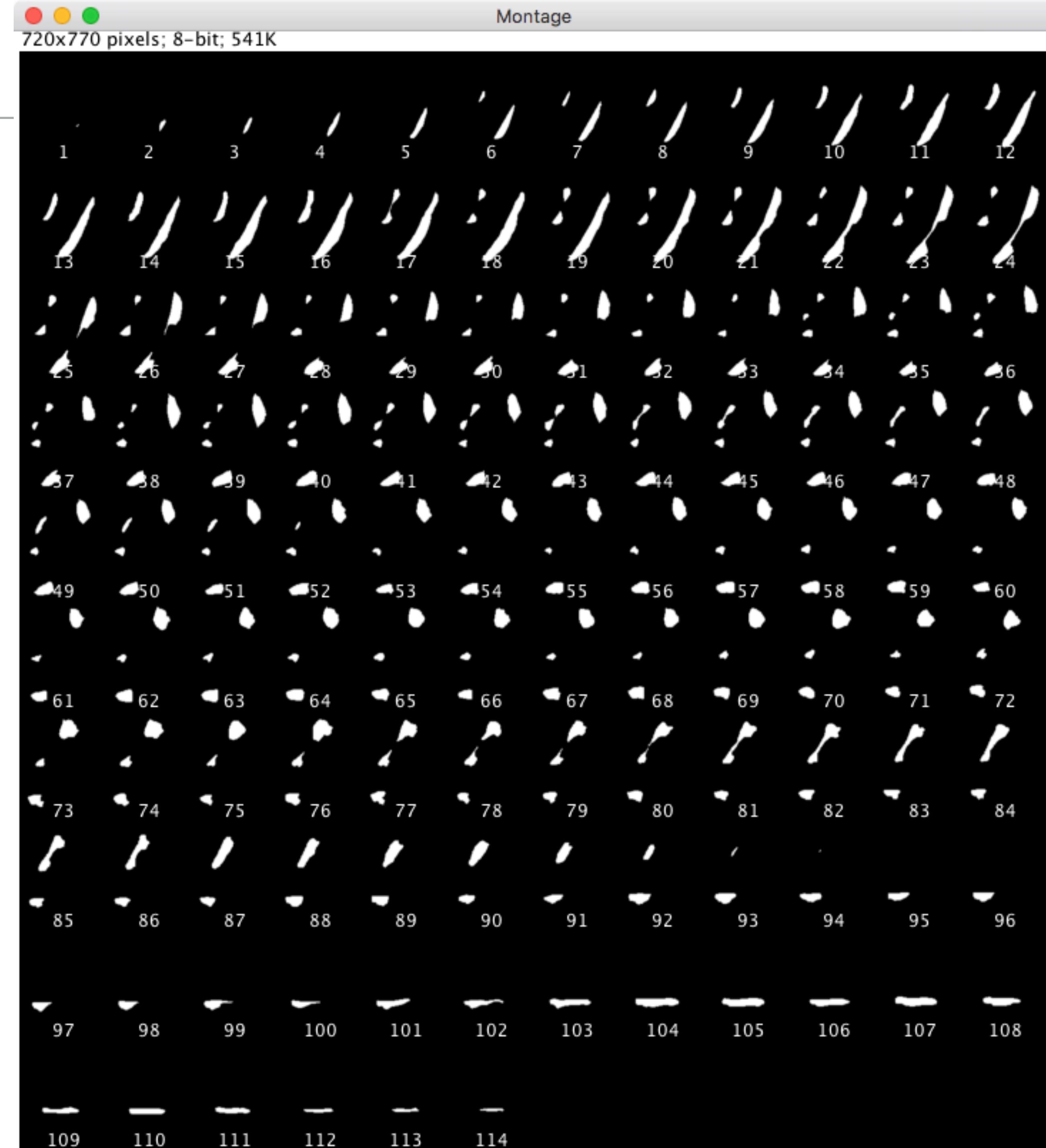
View5D

Make Montage

Columns:	12
Rows:	10
Scale factor:	0.50
First slice:	1
Last slice:	114
Increment:	1
Border width:	0
Font size:	12

Label slices
 Use foreground color

Cancel **OK**



3D STACK - BAT COCHLEA

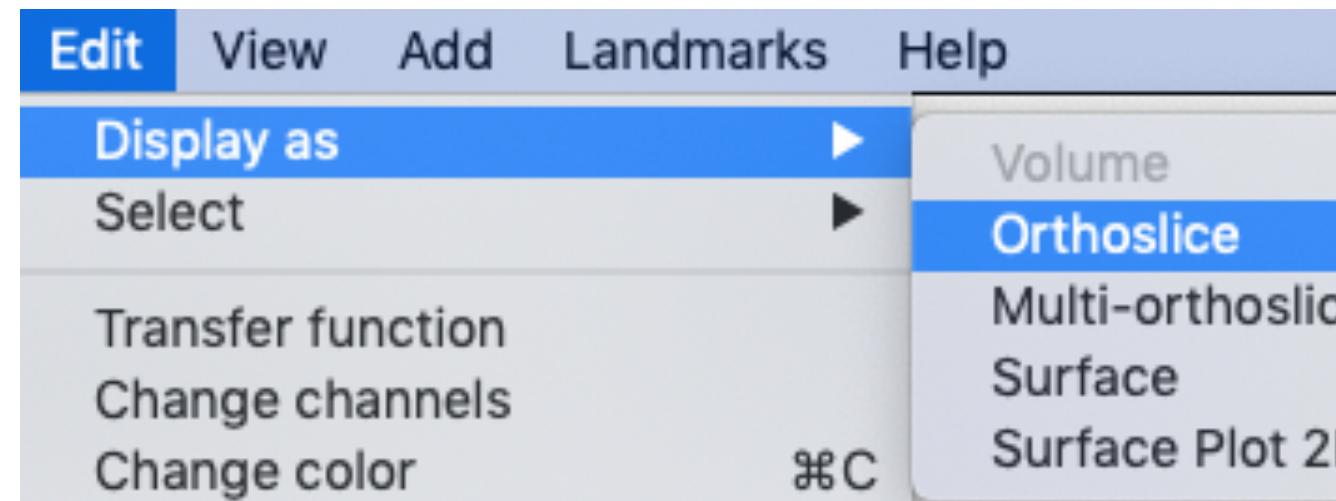
3D VIEW MENU

1

EDIT

- Display as ►
- Select ►
- Transfer function
- Change channels
- Change color ⌘C**
- Change transparency ⌘T
- Adjust threshold
- ✓ Show content
- Show coordinate system
- Show bounding box
- ✓ Show all coordinate systems
- Delete ✕
- Object Properties
- Transformation ►
- Keyboard shortcuts
- View Preferences

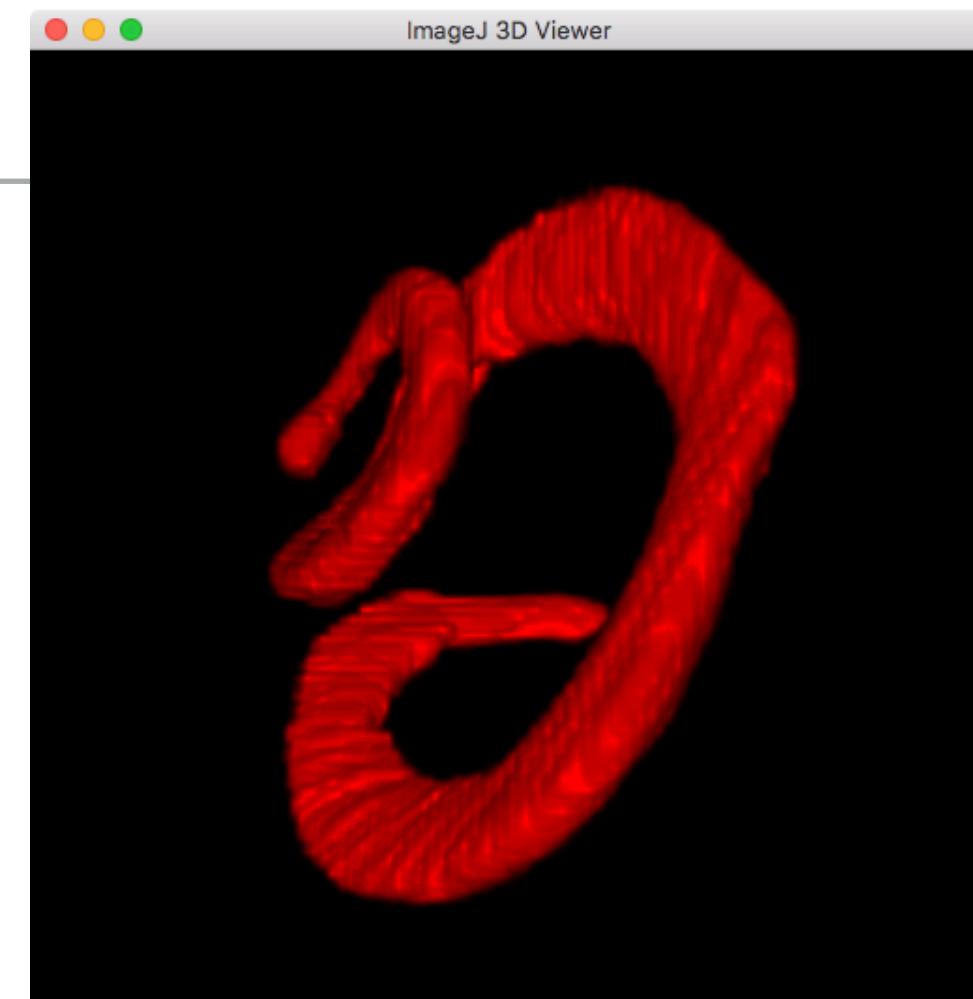
2



3

VIEW

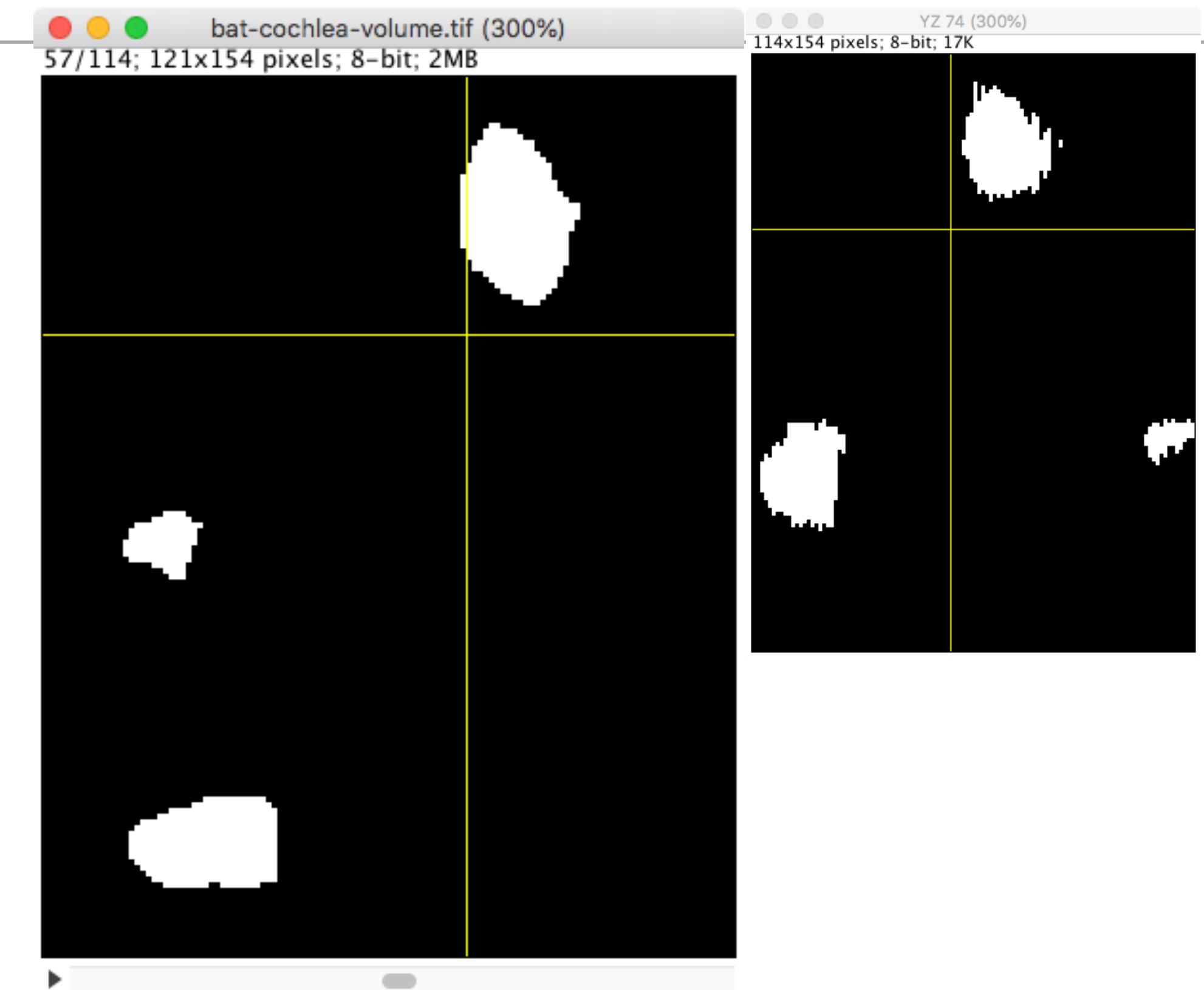
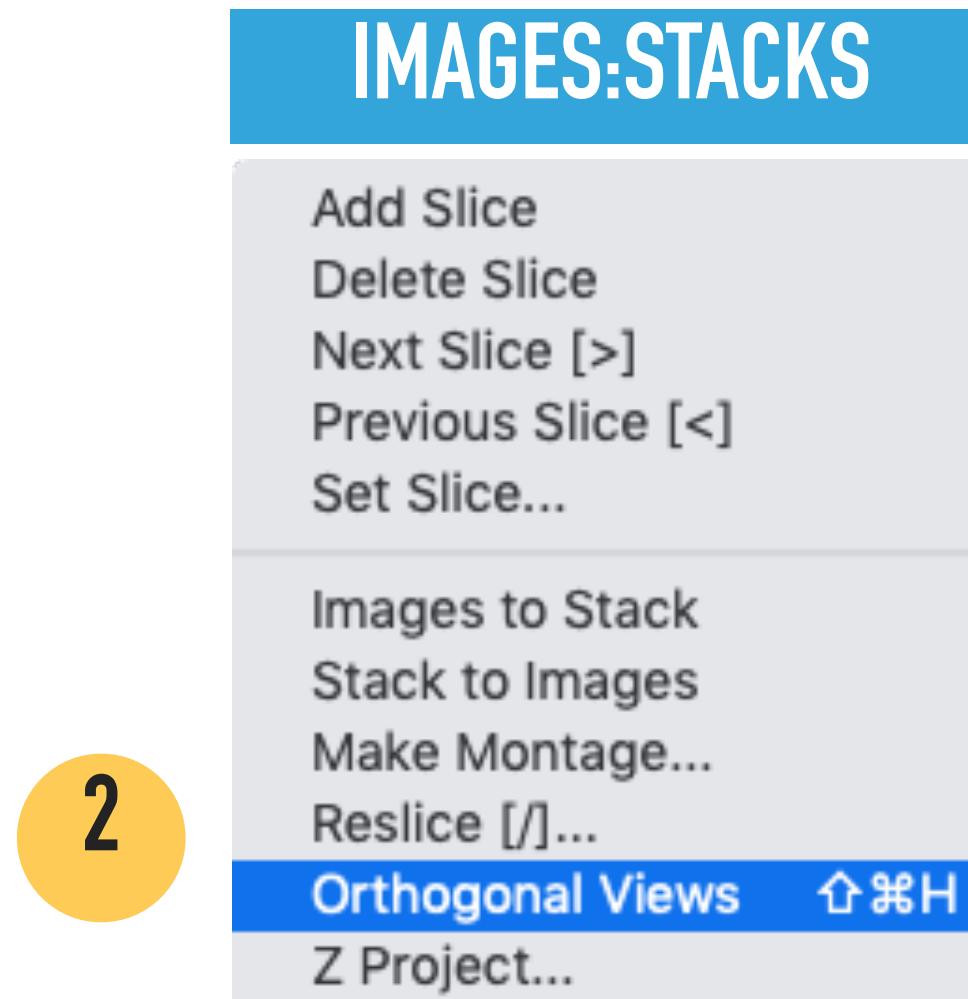
- Reset view ⌘H
- Center ►
- Fit view to ►
- Set view ►
- Take snapshot
- Record 360 deg rotation**
- Start freehand recording
- Stop freehand recording
- Start animation
- Stop animation
- Change animation options
- ✓ Sync view
- Edit Scalebar
- Adjust light
- Change background color
- Fullscreen ⌘F



3D STACK - BAT COCHLEA

ORTHOGONAL VIEWS

1 activate volume



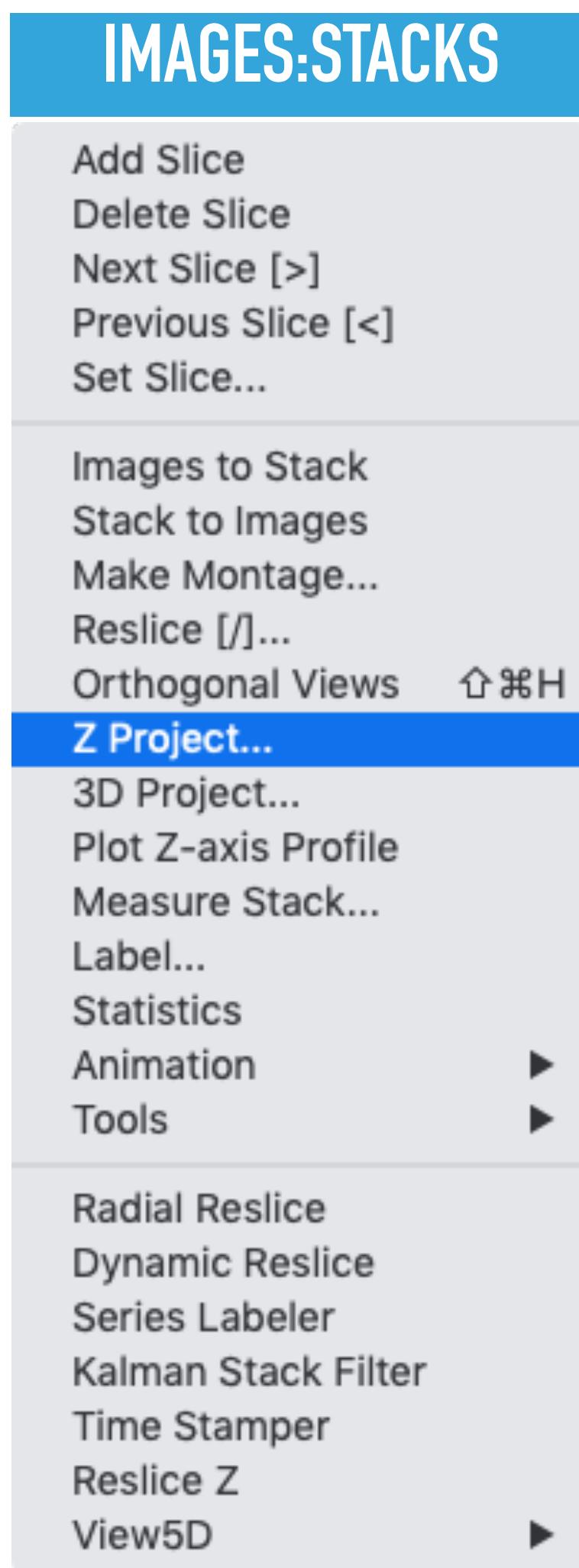
3 click on yellow lines to move through volume

3D STACK - BAT COCHLEA

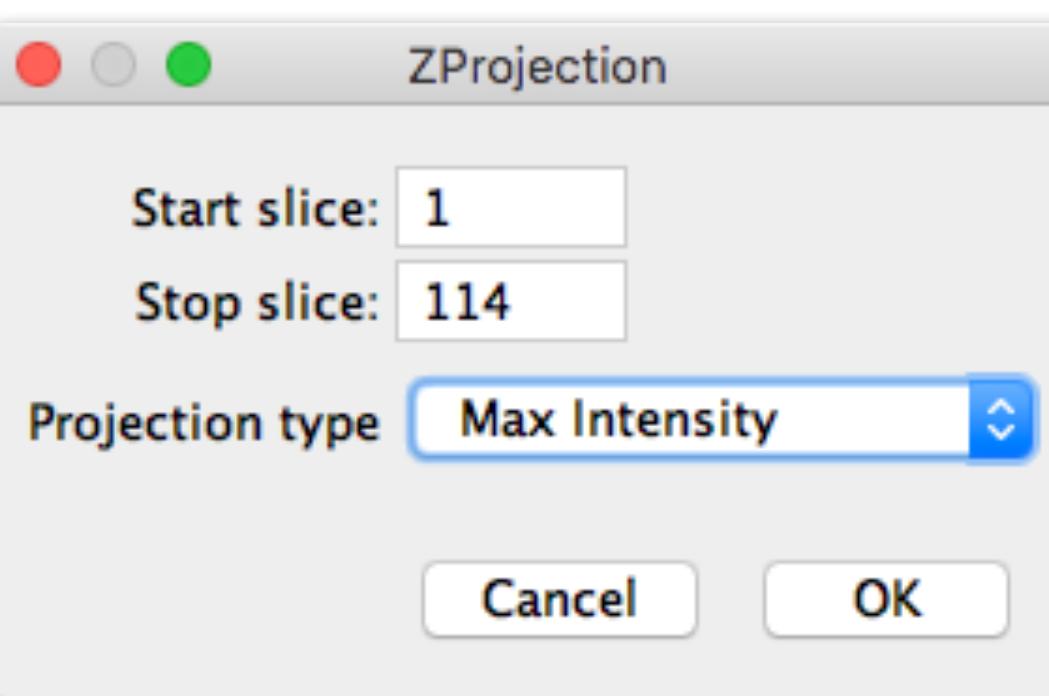
Z PROJECTION

1

activate volume (click on window)



2

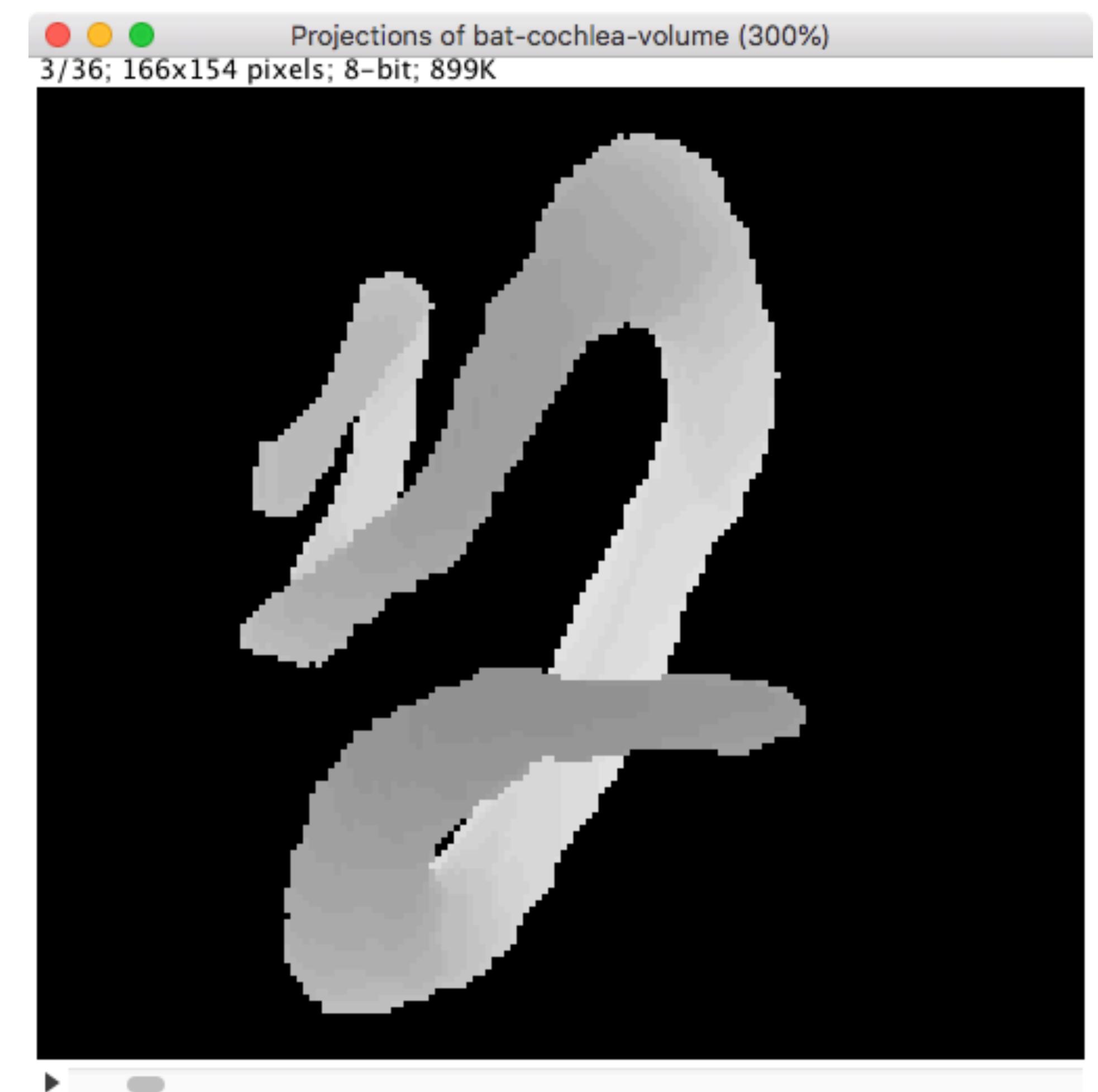
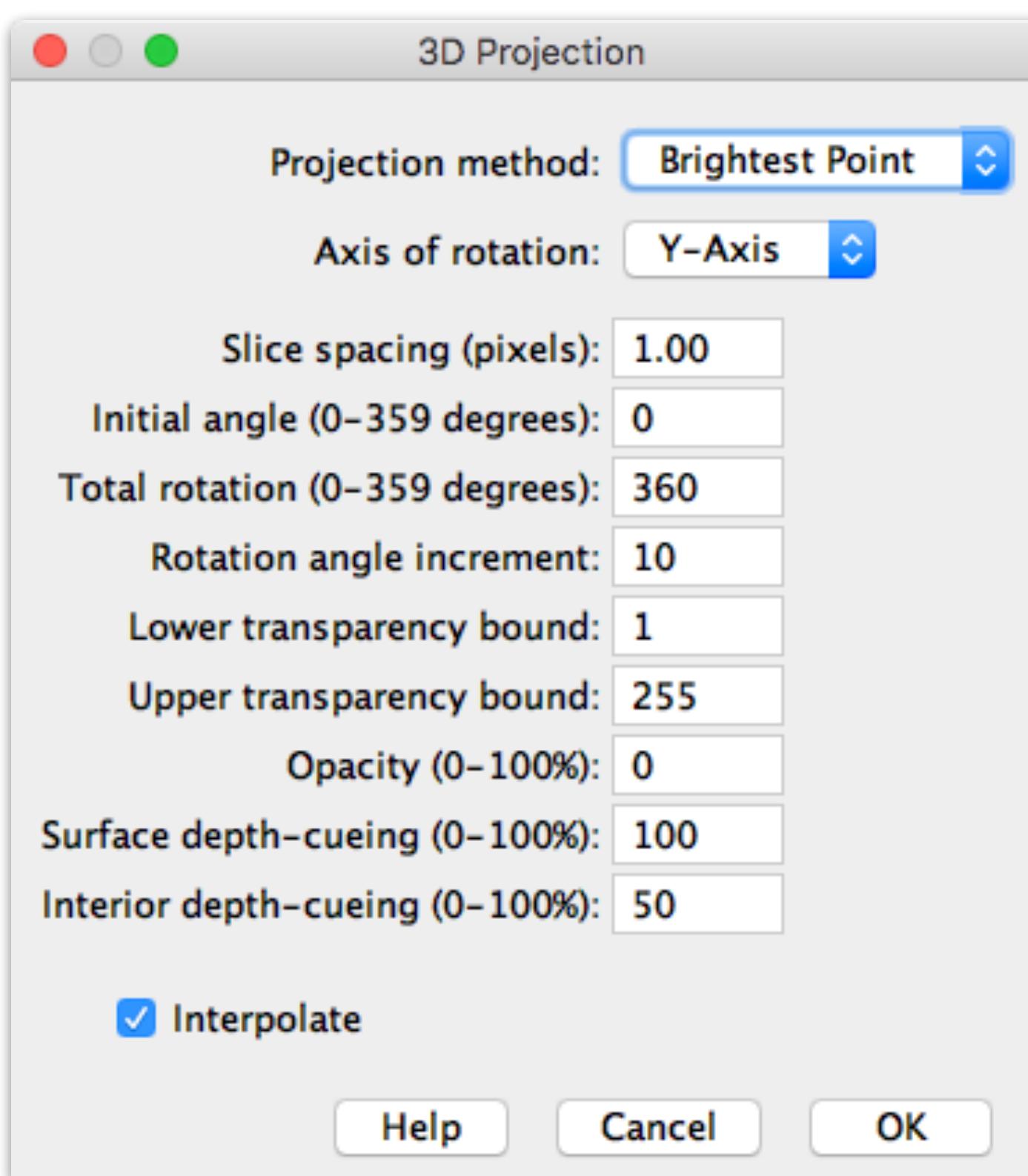


3D STACK - BAT COCHLEA

3D PROJECT...

IMAGES:STACKS

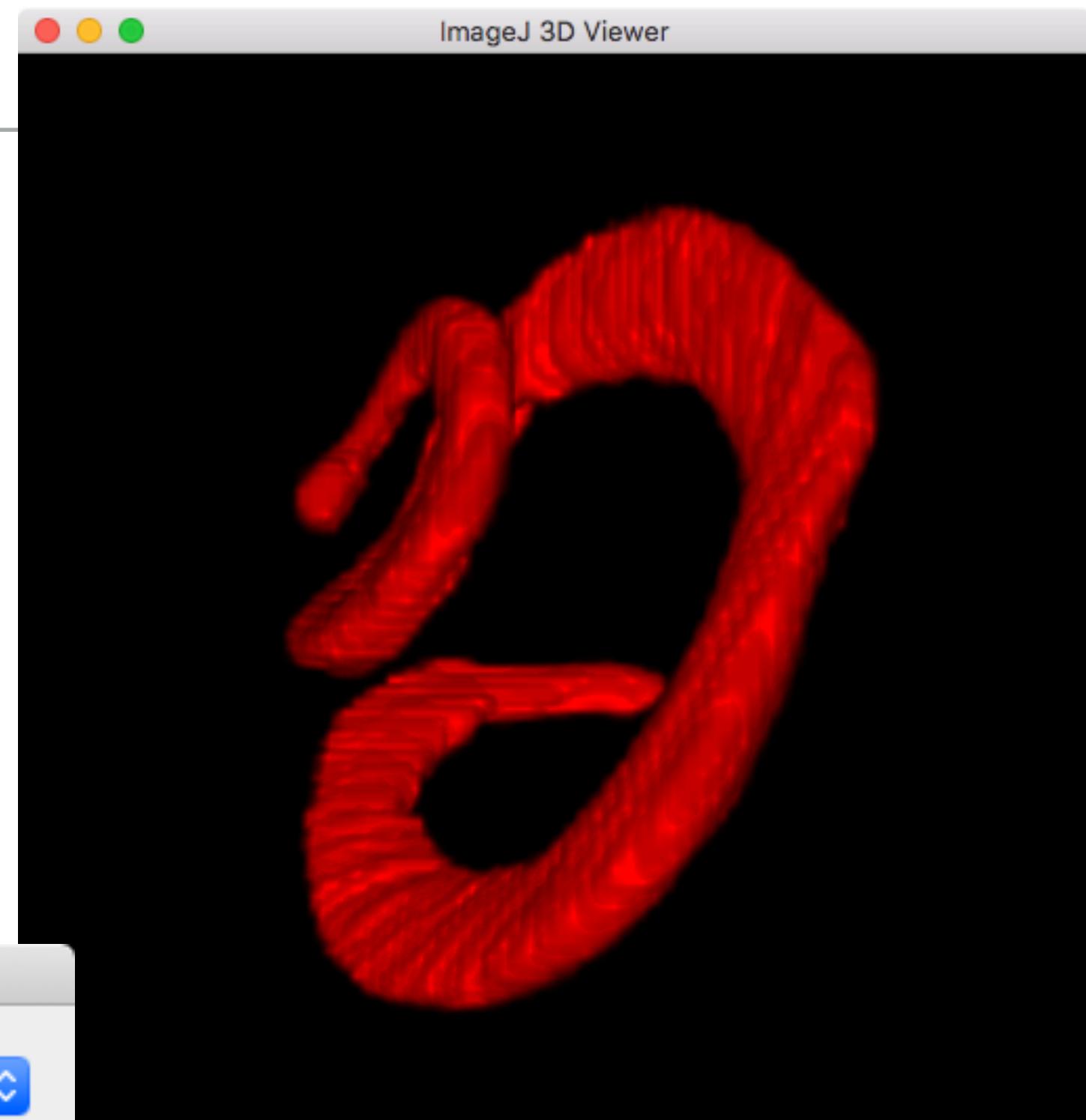
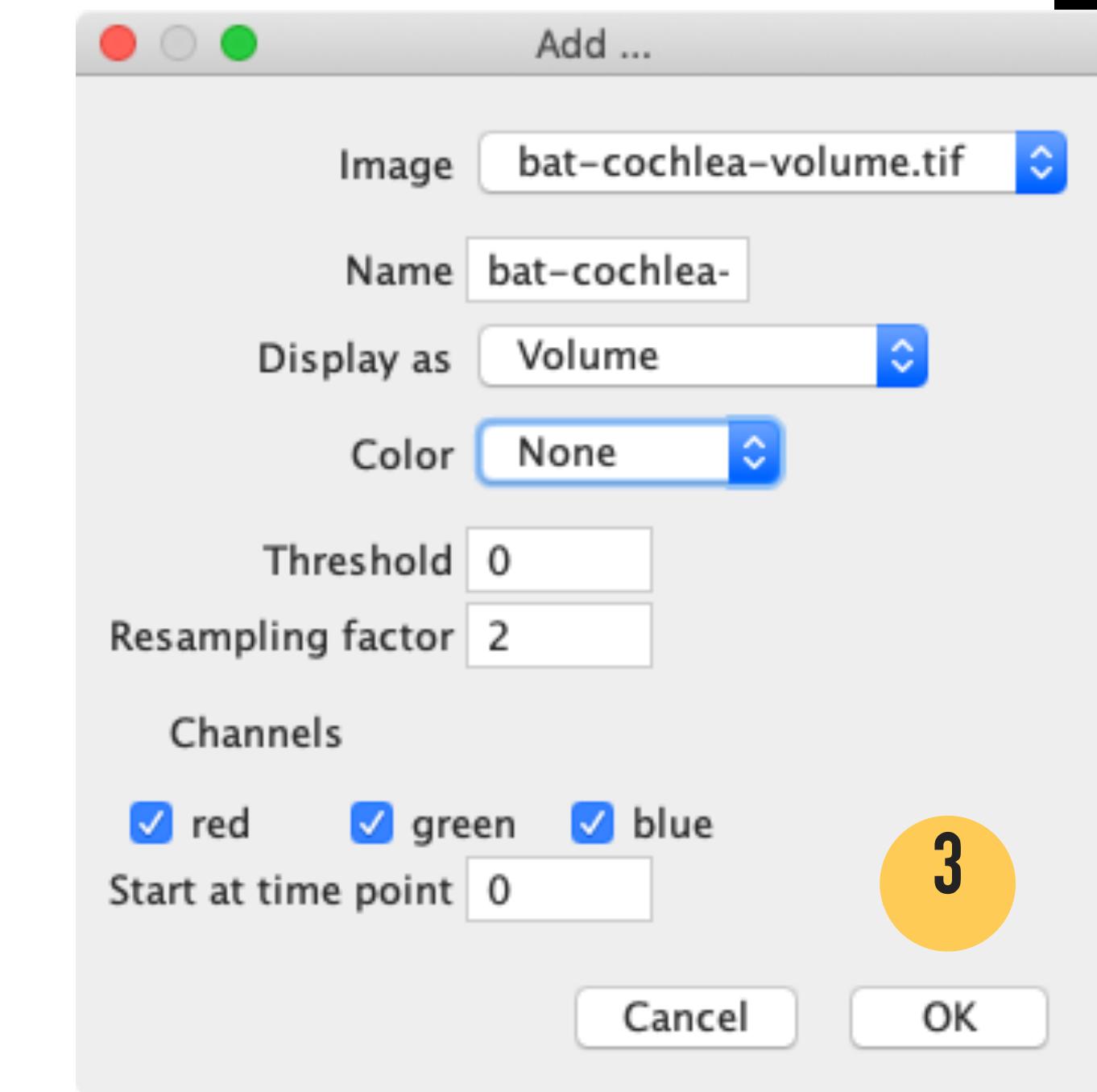
- Images to Stack
- Stack to Images
- Make Montage...
- Reslice [/]...
- Orthogonal Views ⌘⌘H
- Z Project...
- 3D Project...**
- Plot XY Profile
- Plot Z-axis Profile
- Label...
- Statistics
- Animation
- Tools ▶



3D STACK - BAT COCHLEA

3D VIEW

1 activate bat volume

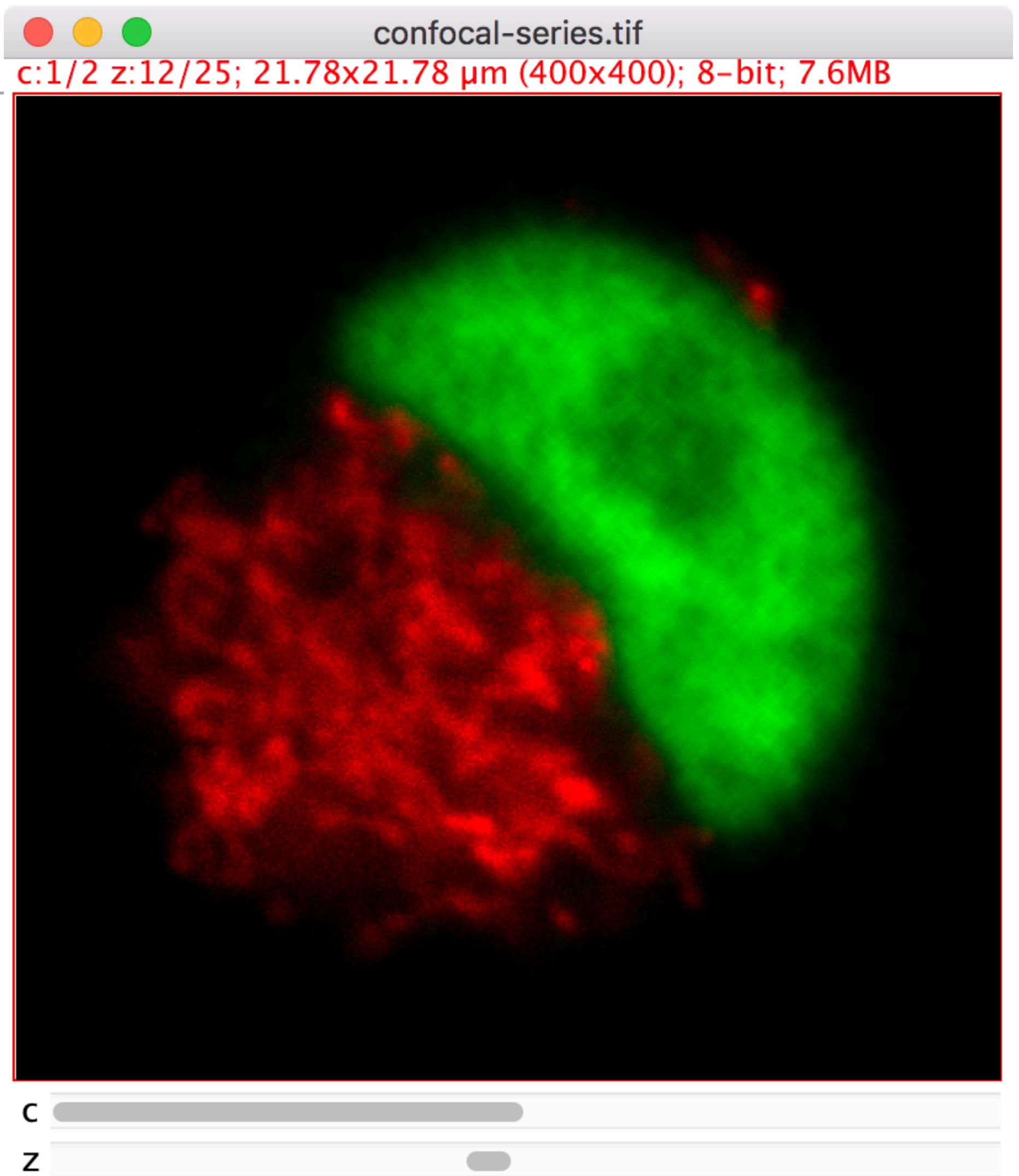


4D

4D STACK

CONFOCAL SERIES

- ▶ A 2-Channel, 25 slice 4D stack
 - ▶ $2 \times 25 = 50$ images in total
- ▶ Notice that there are now 2 scroll bars at the bottom:
 - ▶ C and Z
- ▶ 8-bits per channel
- ▶ Voxel size = $0.05 \times 0.05 \times 0.5 \mu\text{m}^3$
 - ▶ isotropic voxels (little cubes)

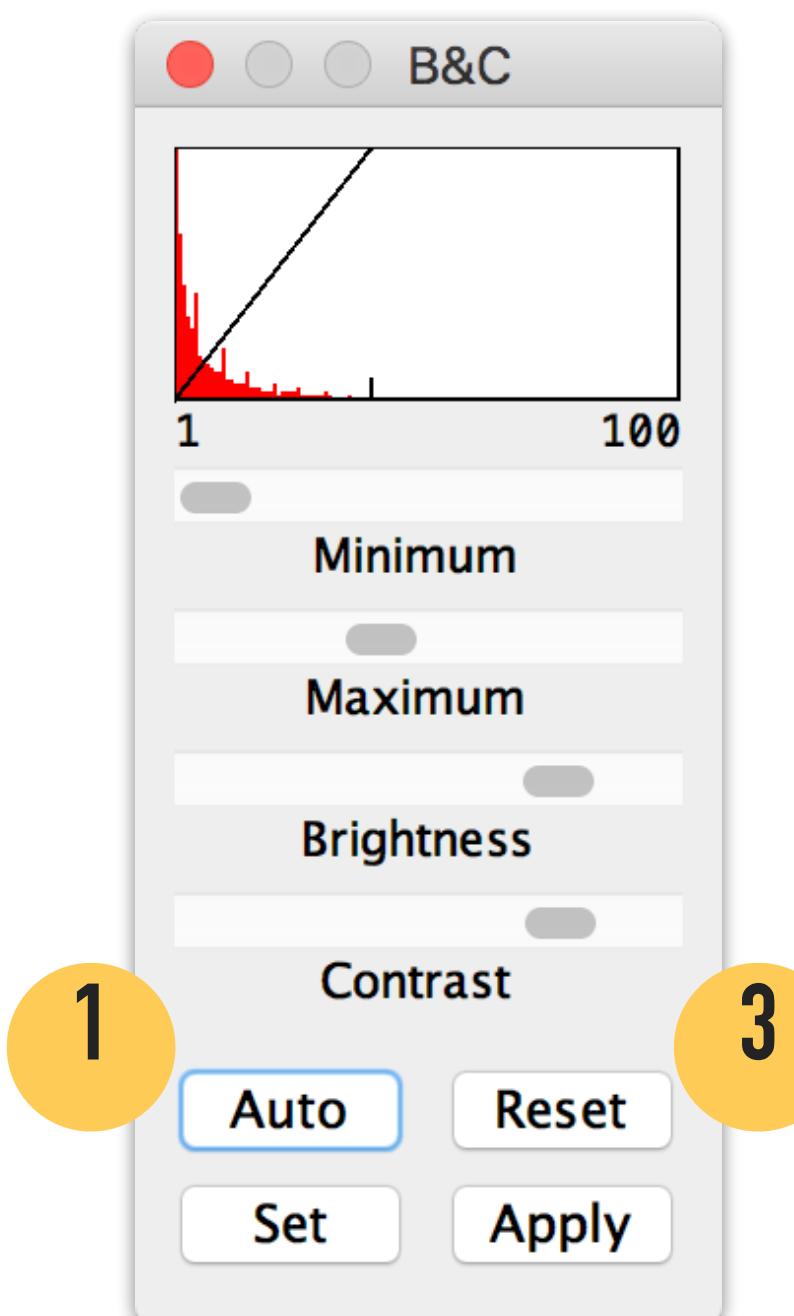
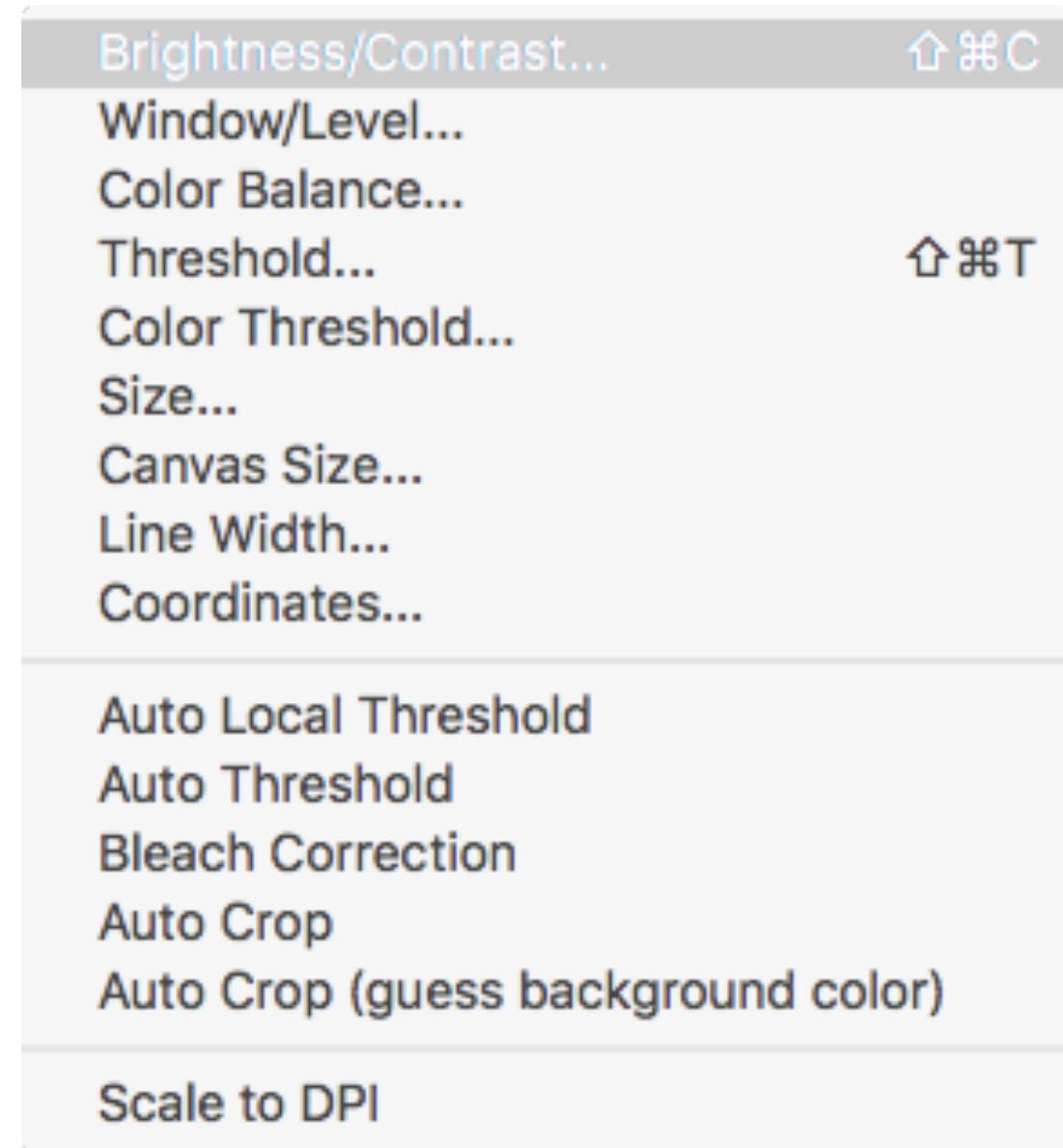


Cells double labeled with P53-YFP and Mito-Red imaged on a Leica SP1 confocal microscope. Original series cropped from 512x512 pixels to 400x400. The green channel was subjected to one round of smoothing.

4D STACK - CONFOCAL SERIES

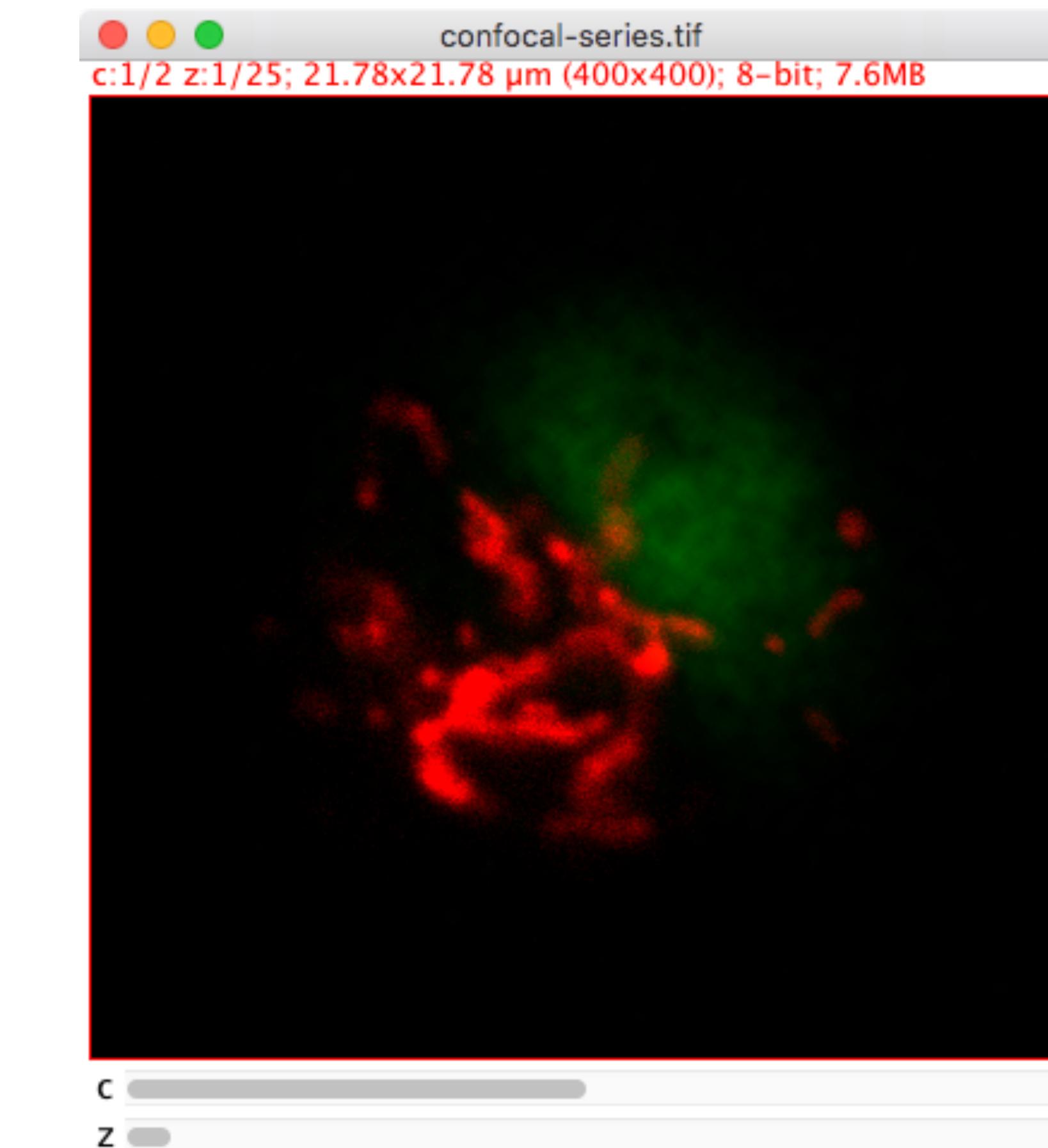
ADJUST STACK

IMAGES:ADJUST



1

3

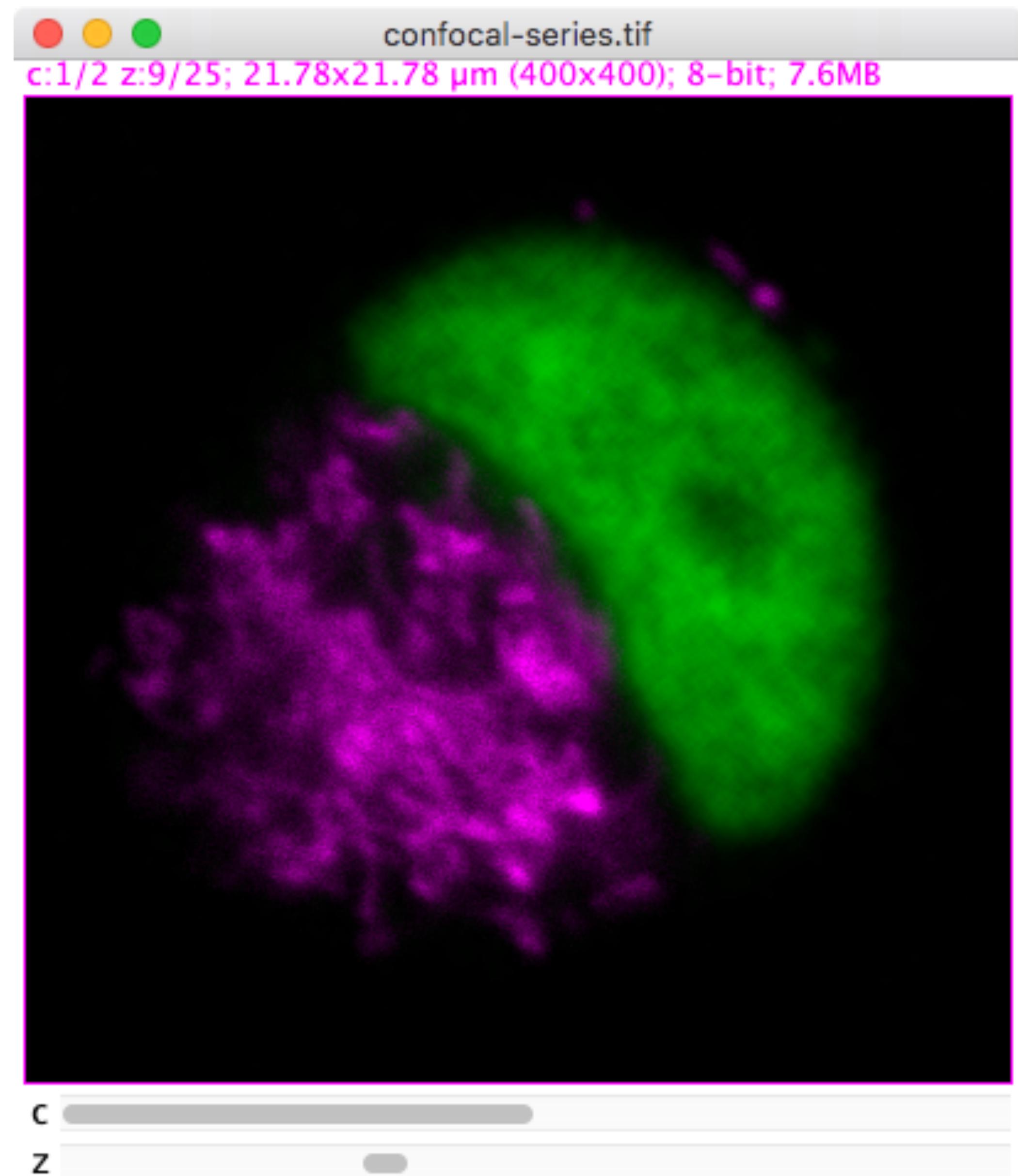
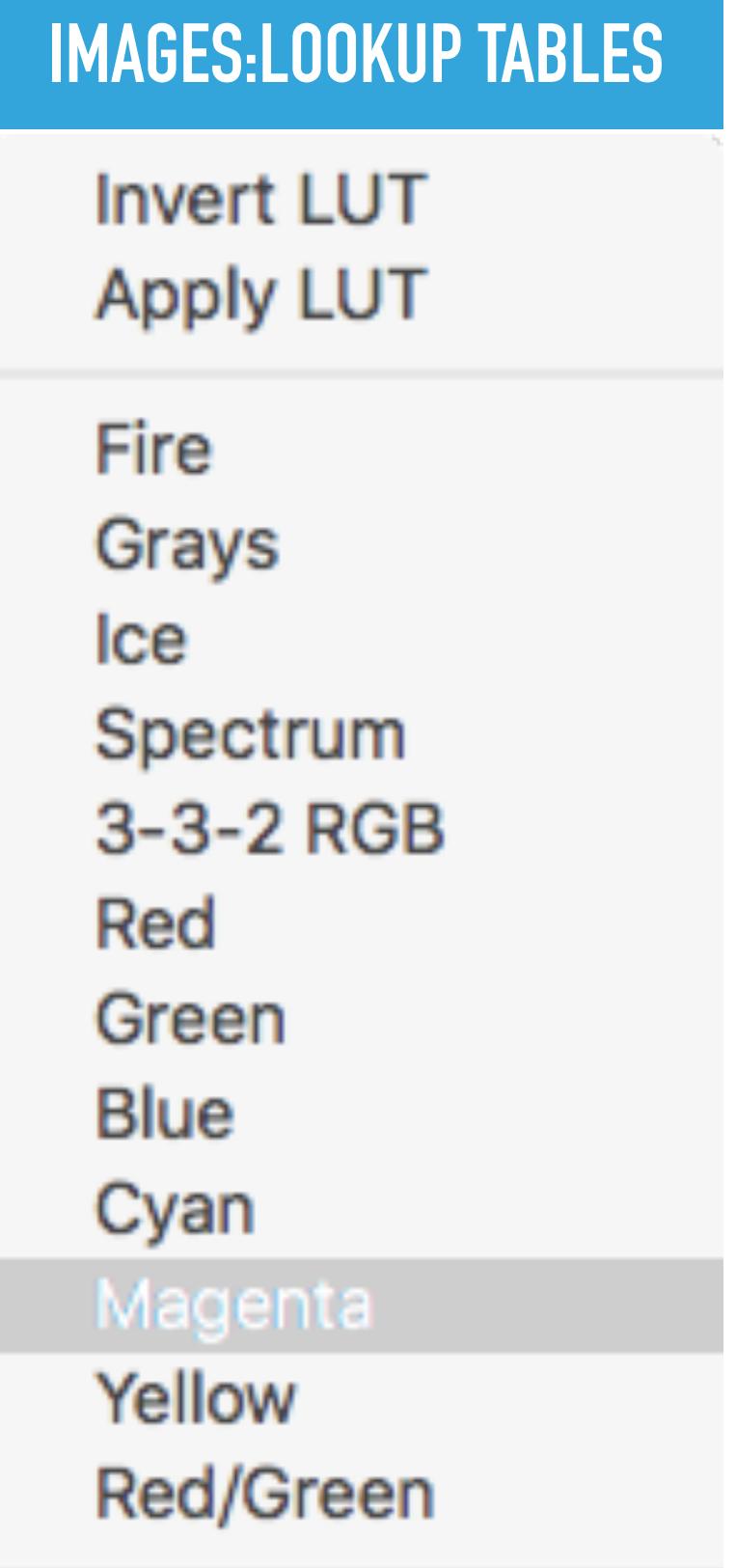


2

Scrub through z slices - adjustment affects ALL slices in the current channel

4D STACK -CONFOCAL SERIES

CHANGE LOOKUP TABLE

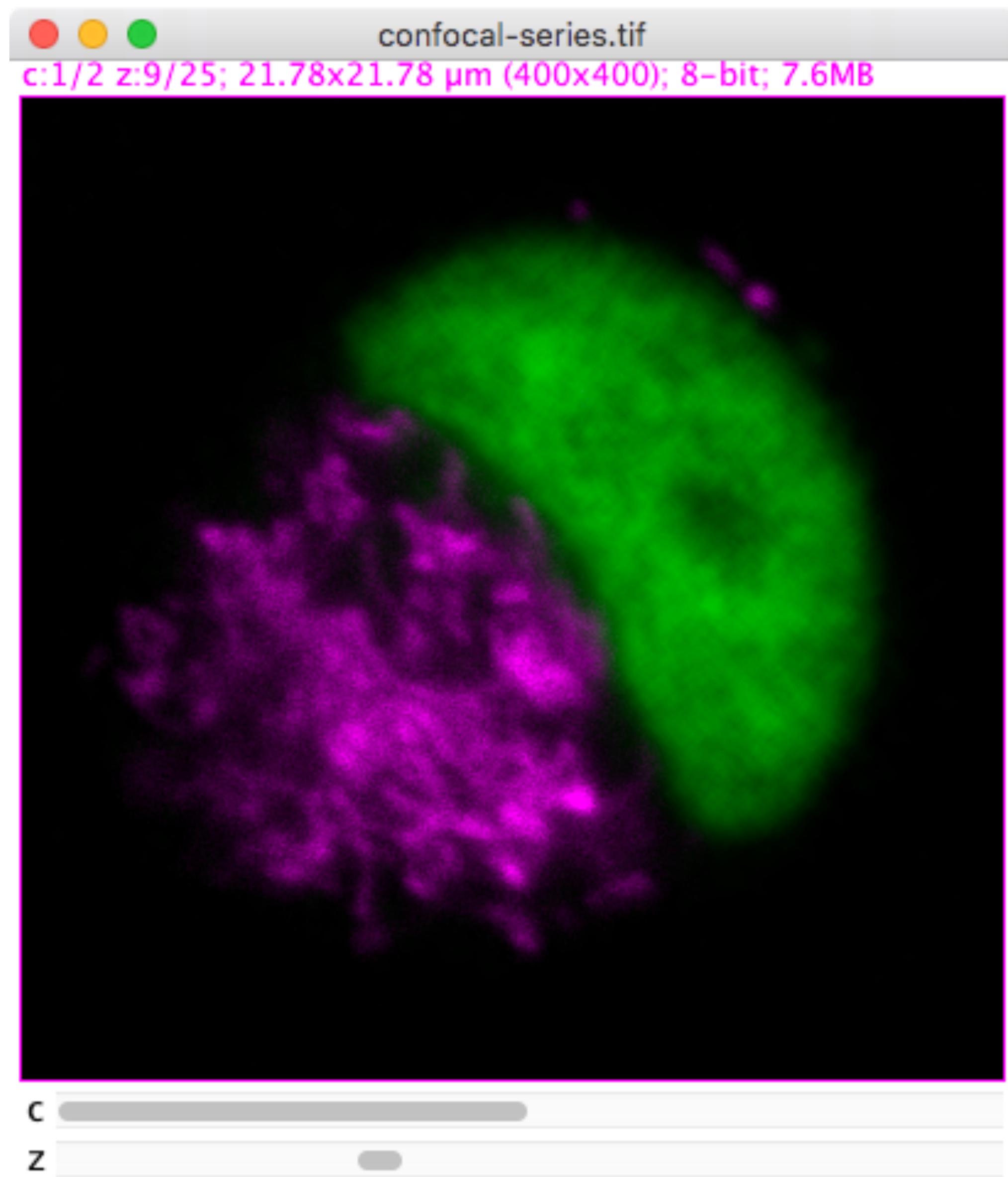
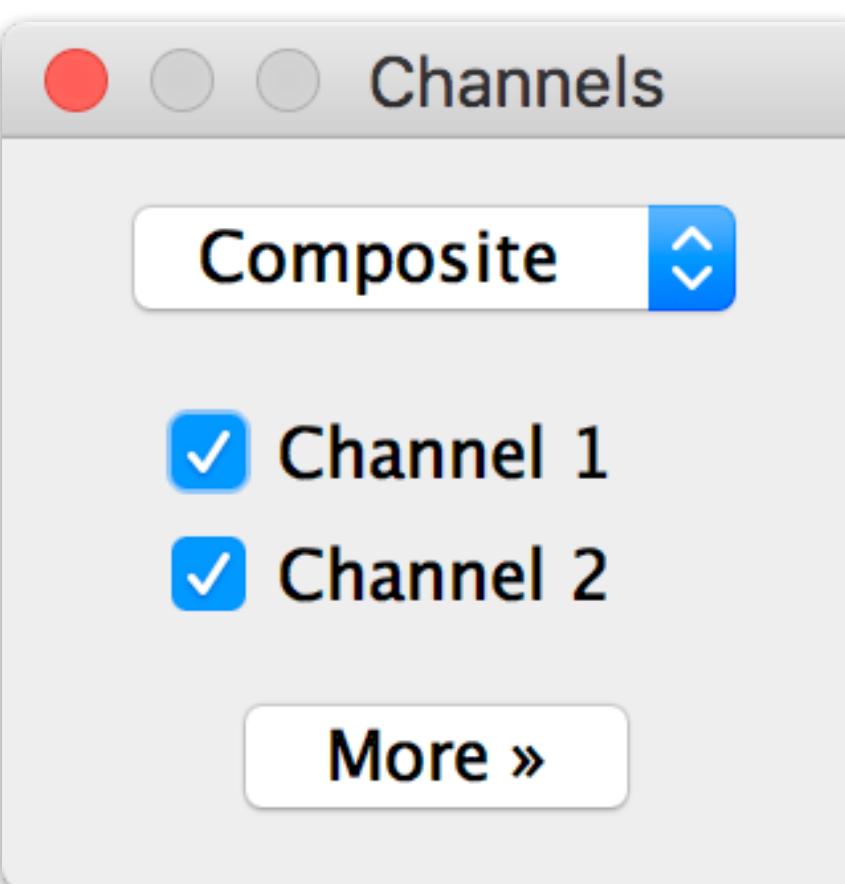


4D STACK -CONFOCAL SERIES

DISPLAY CHANNELS TOOL

IMAGE:COLOR

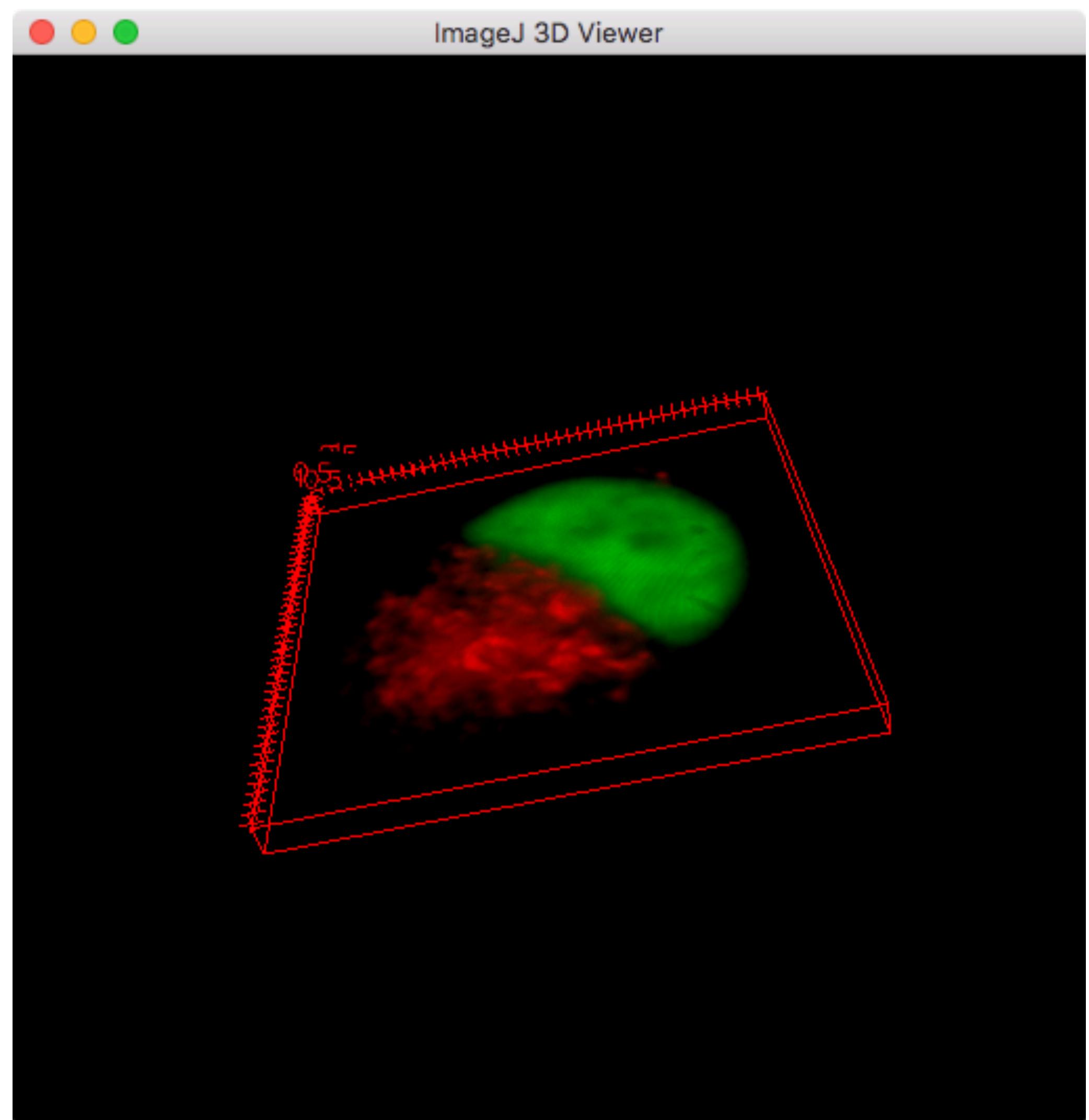
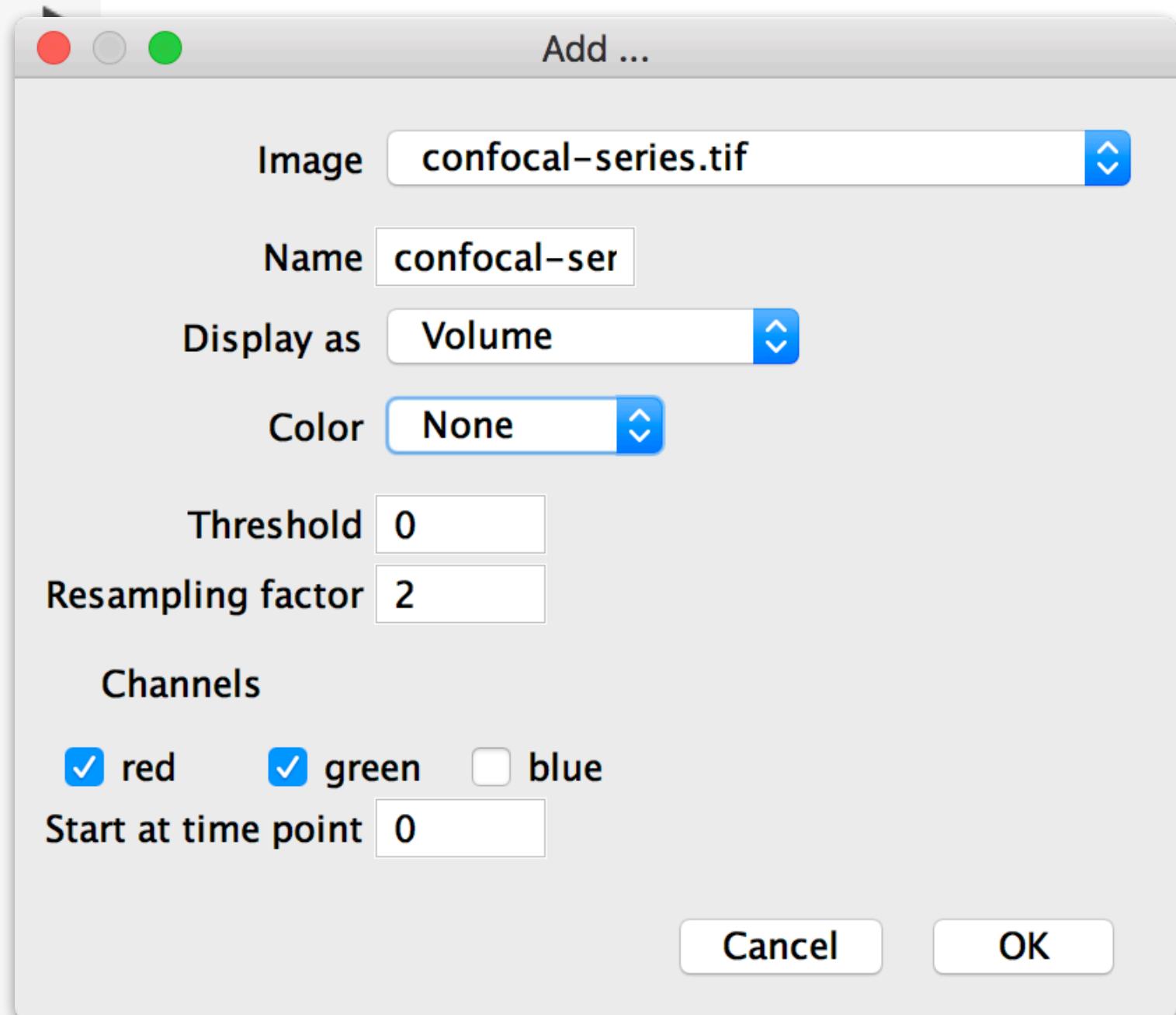
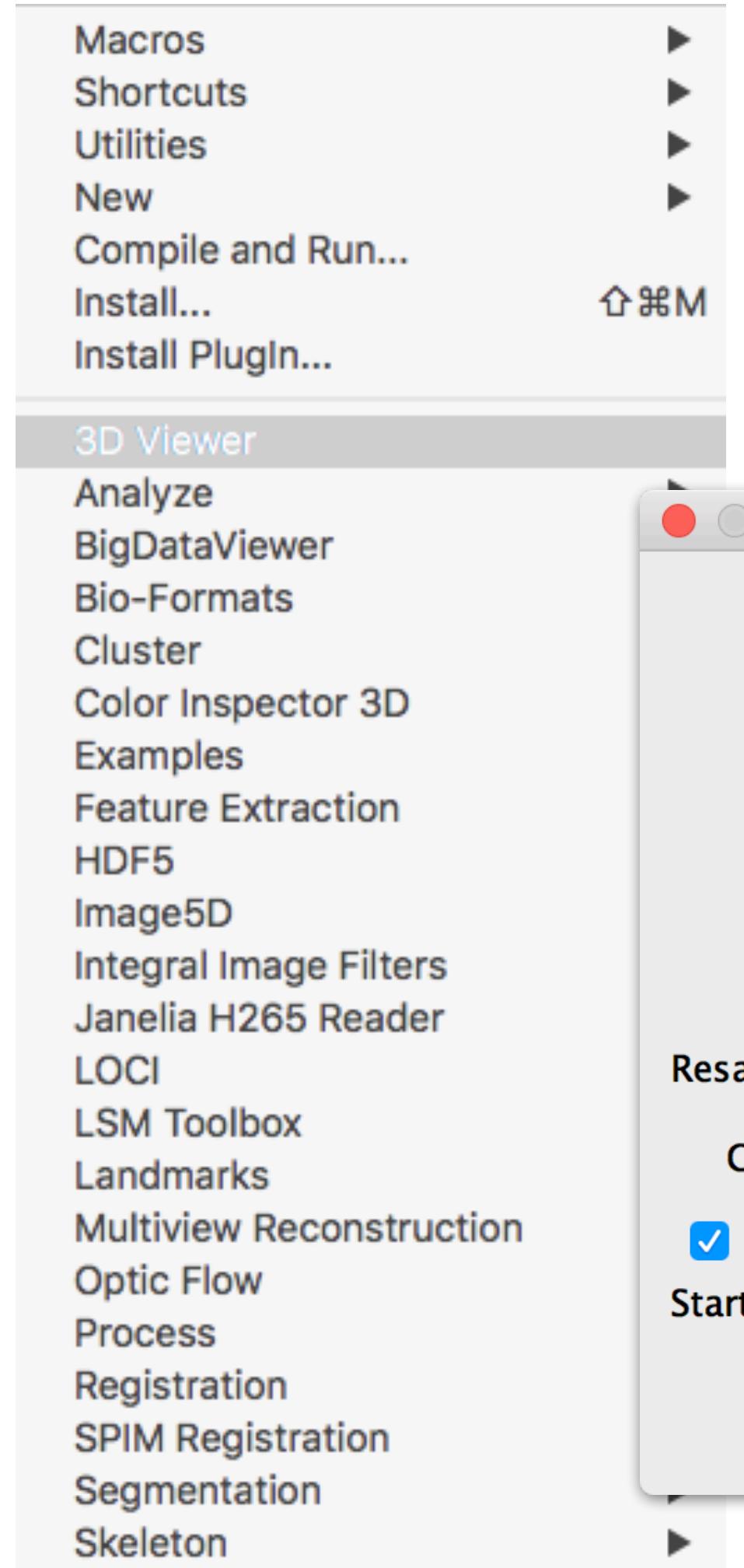
- Split Channels
- Merge Channels...
- Arrange Channels...
- Channels Tool...** ⌘⌘Z
- Stack to RGB
- Make Composite
- Show LUT
- Display LUTs
- Edit LUT...
- Color Picker... ⌘⌘K
- Colour Deconvolution
- Dichromacy
- Simulate Color Blindness
- Retinex
- Replace Red with Magenta
- Replace Red with Magenta (system clipboard)
- Average Color
- RGB to CIELAB
- RGB to Luminance
- Set Color By Wavelength



4D STACK - CONFOCAL SERIES

3D VIEW

PLUGINS



Notice how thin the stack is

4D STACK - CONFOCAL SERIES

MAKE A MONTAGE

IMAGES:STACKS

Add Slice
Delete Slice
Next Slice [>]
Previous Slice [<]
Set Slice...

Images to Stack
Stack to Images
Make Montage...

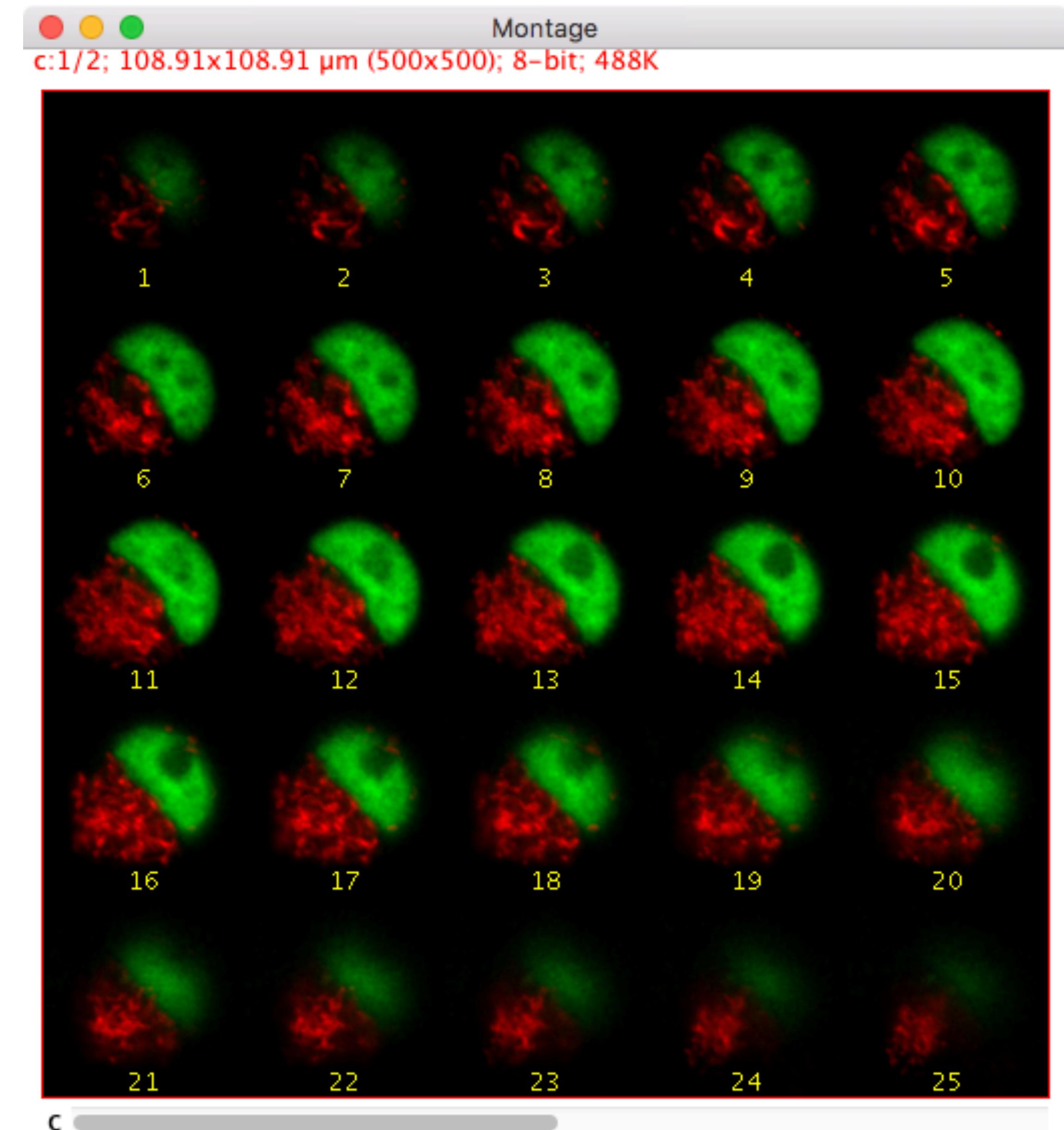
Reslice [/]...
Orthogonal Views ⌘⌘H
Z Project...
3D Project...
Plot Z-axis Profile
Measure Stack...
Label...
Statistics
Animation
Tools ►

Make Montage

Columns:	5
Rows:	5
Scale factor:	0.25
Increment:	1
Border width:	0
Font size:	12

Label slices
 Use foreground color

Cancel **OK**



4D STACK - CONFOCAL SERIES

ORTHOGONAL VIEWS

IMAGES:STACKS

- Add Slice
- Delete Slice
- Next Slice [>]
- Previous Slice [<]
- Set Slice...

- Images to Stack
- Stack to Images
- Make Montage...
- Reslice [/]...

Orthogonal Views ⌘⌘H

- Z Project...

- 3D Project...

- Plot XY Profile

- Plot Z-axis Profile

- Label...

- Statistics

- Animation

- Tools ►

- Radial Reslice

- Dynamic Reslice

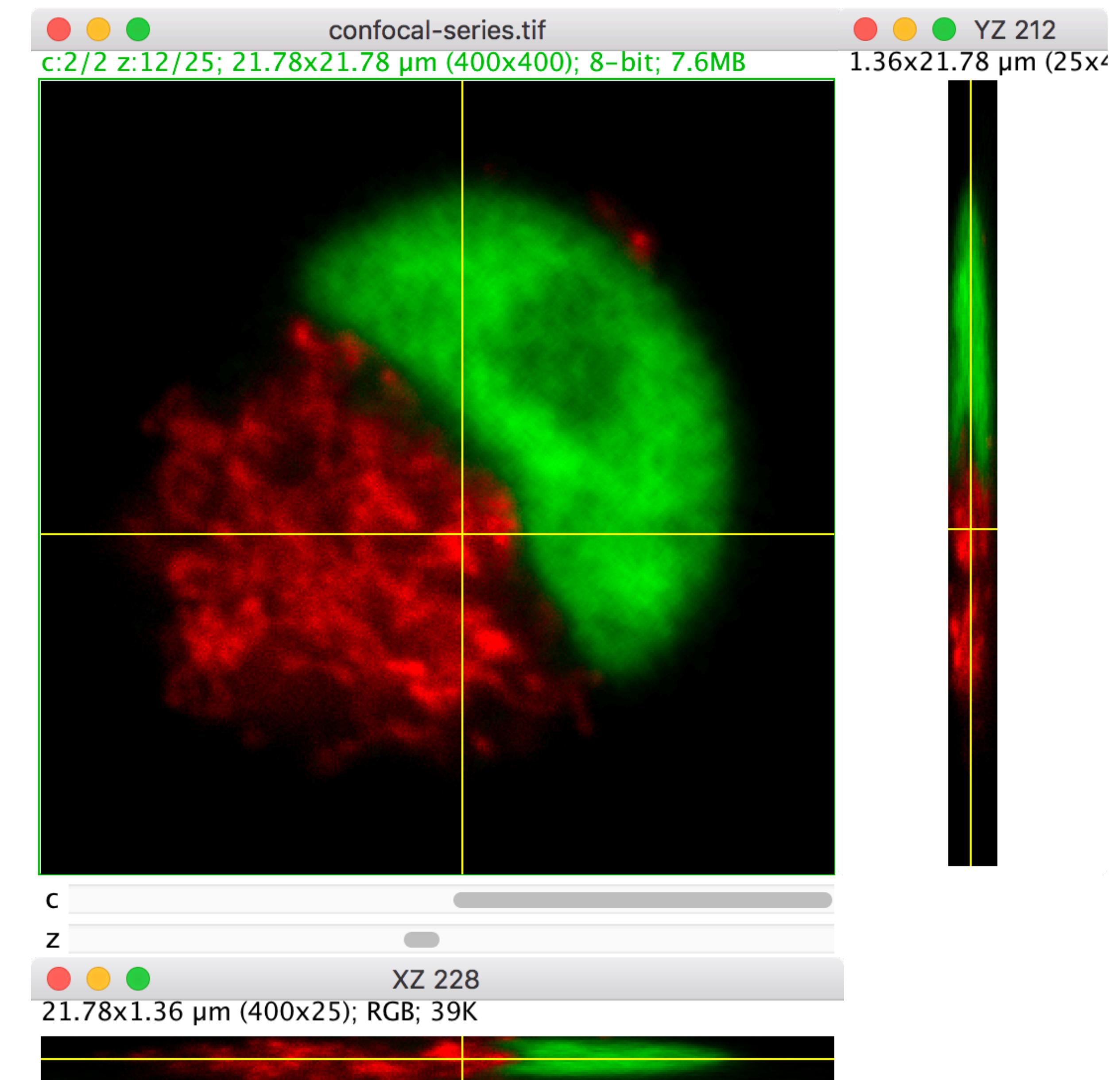
- Series Labeler

- Kalman Stack Filter

- Time Stamper

- Reslice Z

- View5D ►



4D STACK - CONFOCAL SERIES

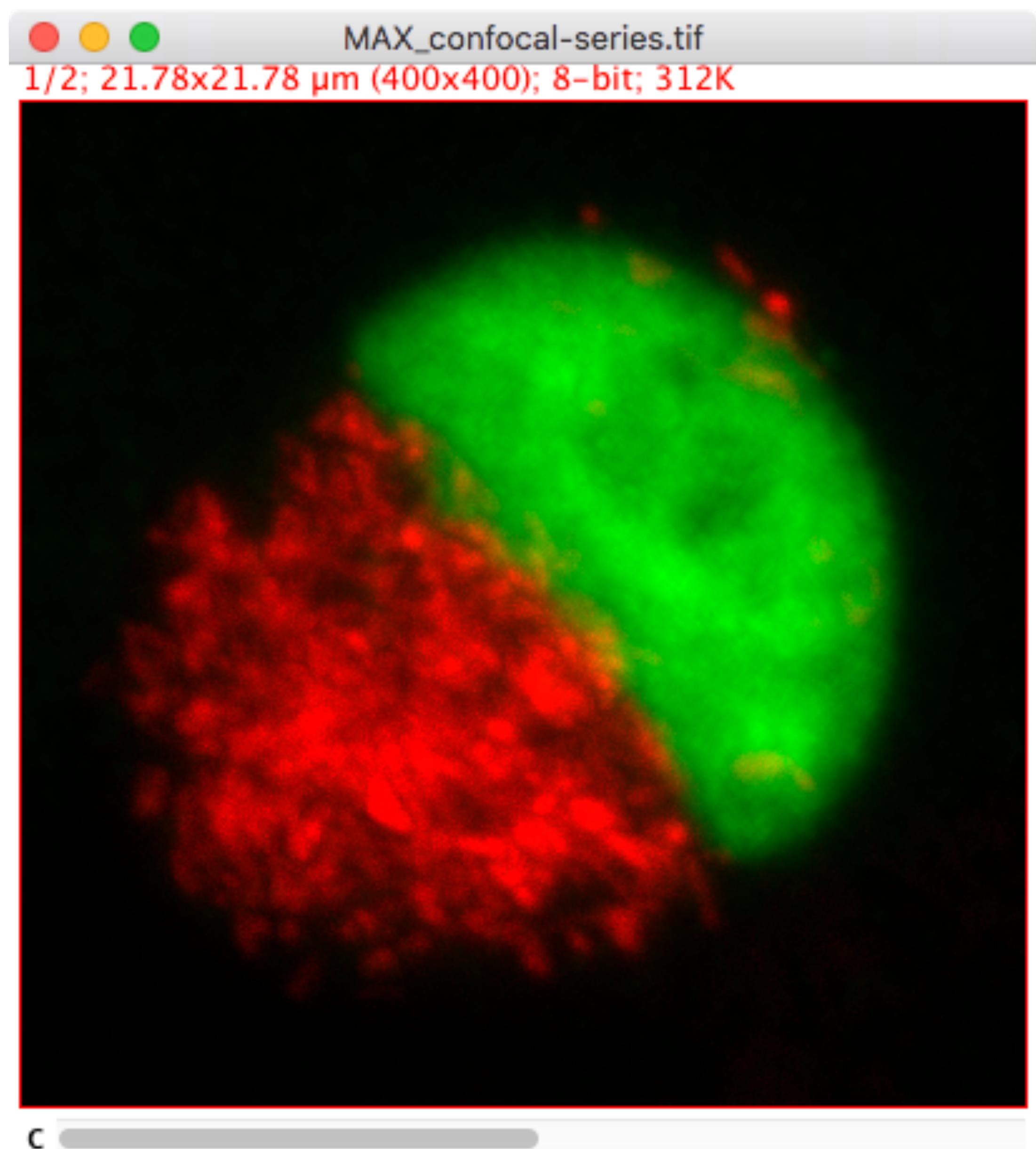
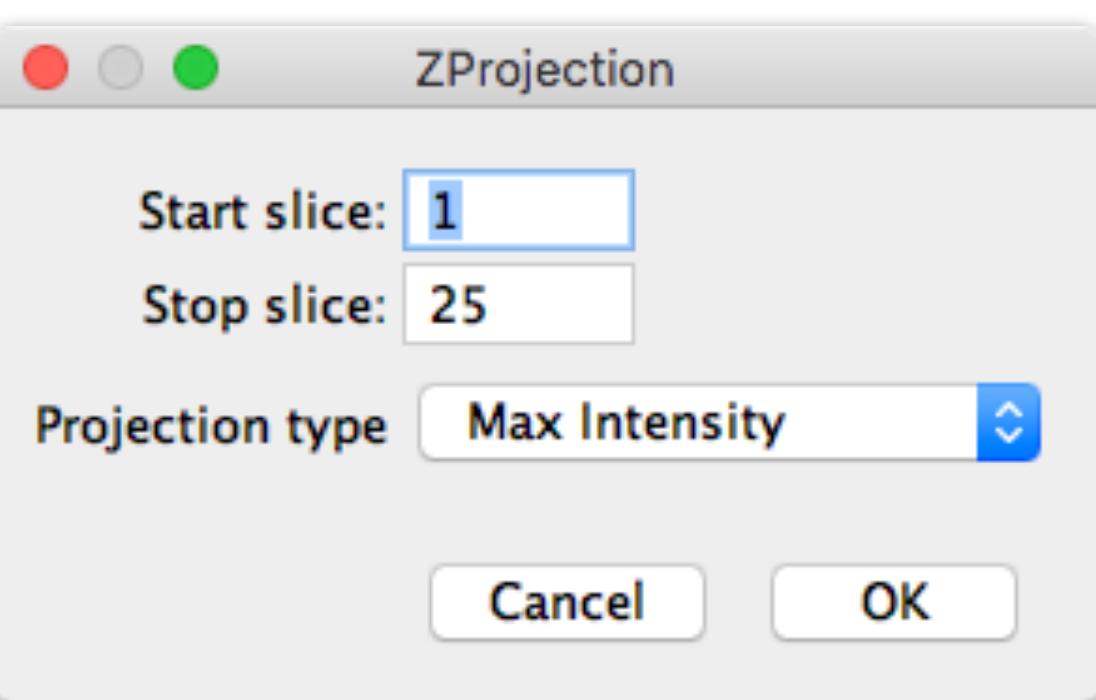
Z PROJECTION

IMAGES:STACKS

- Add Slice
- Delete Slice
- Next Slice [>]
- Previous Slice [<]
- Set Slice...

- Images to Stack
- Stack to Images
- Make Montage...
- Reslice [/]...
- Orthogonal Views ⌘H
- Z Project...**
- 3D Project...
- Plot XY Profile
- Plot Z-axis Profile
- Label...
- Statistics
- Animation
- Tools ►

- Radial Reslice
- Dynamic Reslice
- Series Labeler
- Kalman Stack Filter
- Time Stamper
- Reslice Z
- View5D ►

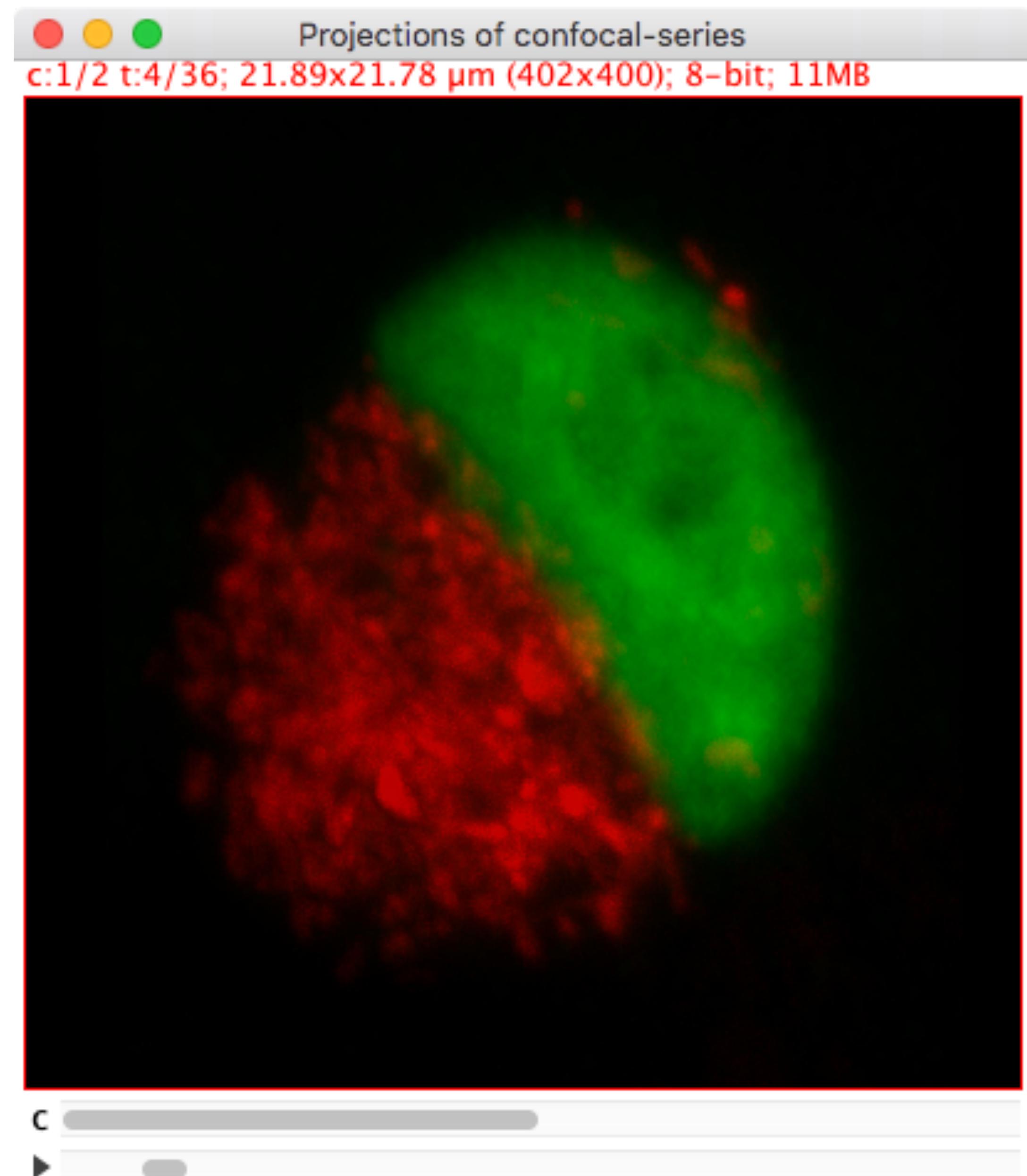
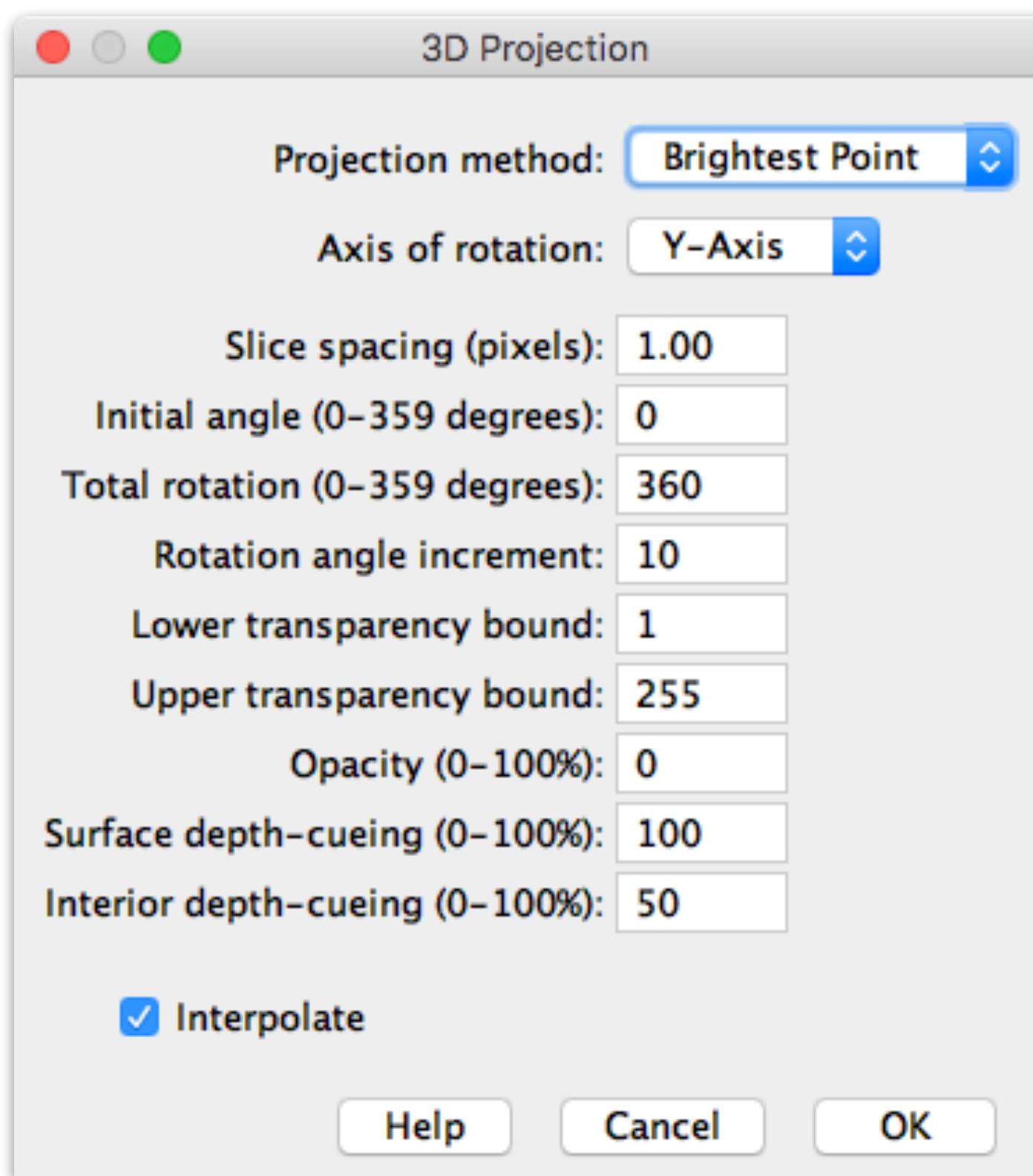


4D STACK - CONFOCAL SERIES

3D PROJECT...

IMAGES:STACKS

- Images to Stack
- Stack to Images
- Make Montage...
- Reslice [/]...
- Orthogonal Views ⌘⌘H
- Z Project...
- 3D Project...**
- Plot XY Profile
- Plot Z-axis Profile
- Label...
- Statistics
- Animation ►
- Tools ►

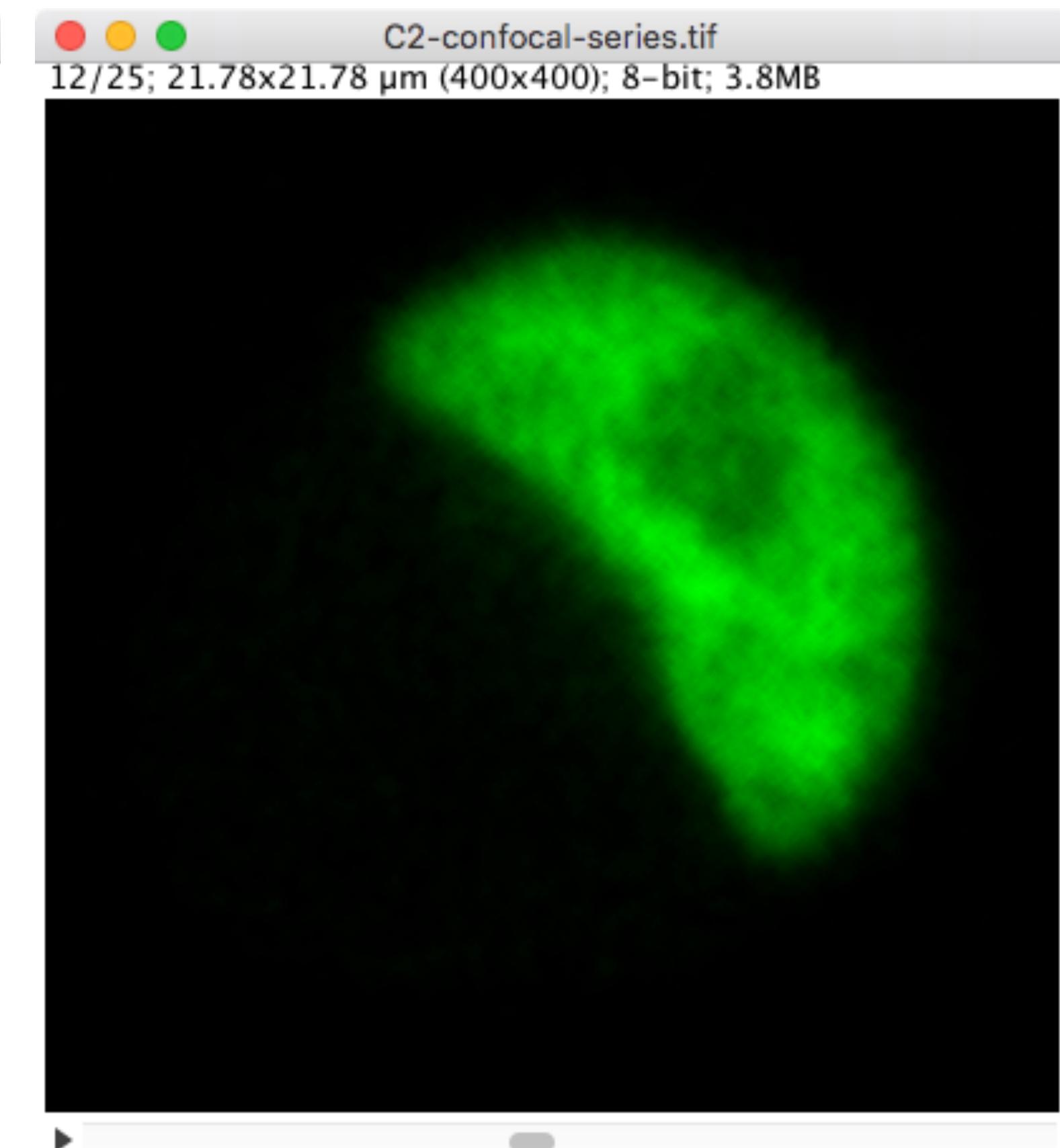
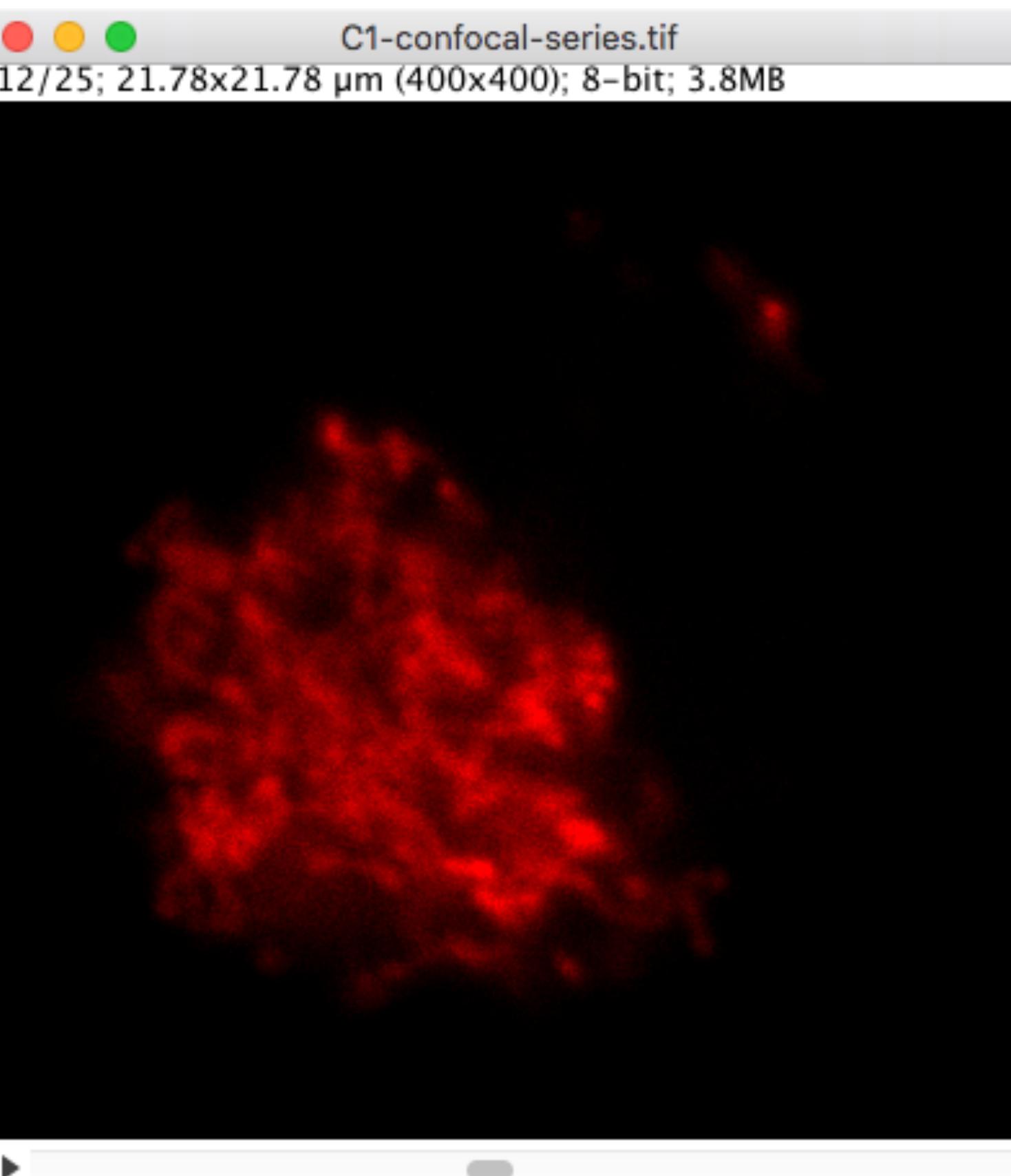


4D STACK - CONFOCAL SERIES

SPLIT CHANNELS

IMAGES:COLOR

- Split Channels
- Merge Channels...
- Arrange Channels...
- Channels Tool... ⇧⌘Z
- Stack to RGB
- Make Composite
- Show LUT
- Display LUTs
- Edit LUT... ⇧⌘K
- Color Picker...
- Colour Deconvolution
- Dichromacy
- Simulate Color Blindness
- Retinex
- Replace Red with Magenta
- Replace Red with Magenta (system clipboard)
- Average Color
- RGB to CIELAB
- RGB to Luminance
- Set Color By Wavelength

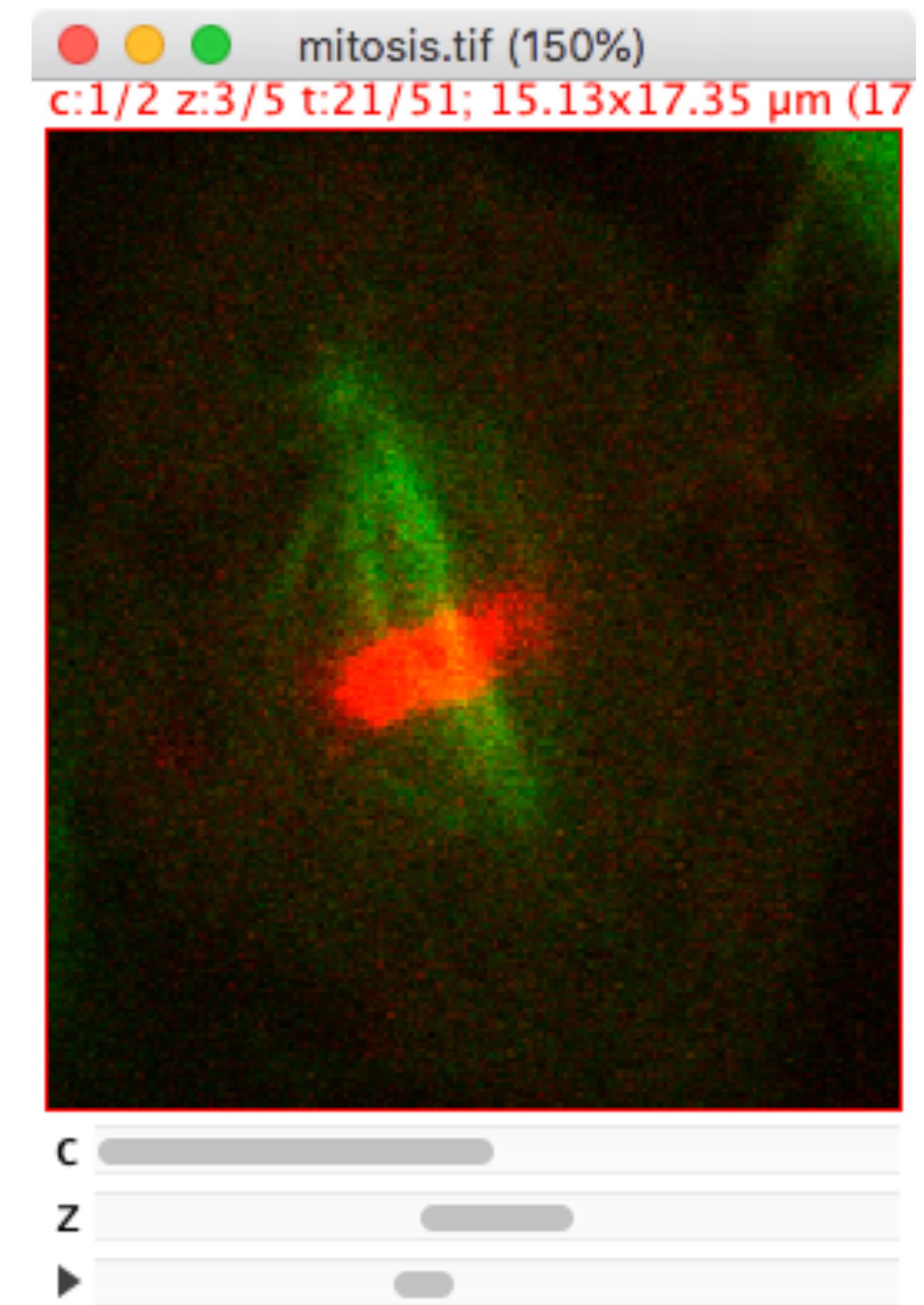


5D

5D STACK

MITOSIS SERIES

- ▶ A 2-Channel, 5 slice, 51 timepoint Series
 - ▶ $2 \times 5 \times 51 = \mathbf{510 \text{ images}}$ in total
- ▶ Notice that there are now 3 scroll bars at the bottom:
 - ▶ **C, Z, and T**
 - ▶ **16-bits** per channel
 - ▶ Frame Interval: **0.14286 s** (~7 frames/sec)
 - ▶ Voxel size = **0.085 x 0.085 x 1 μm^3**
 - ▶ anisotropic voxels (little rectangular prisms - not cubes)
 - ▶ How was this series made?



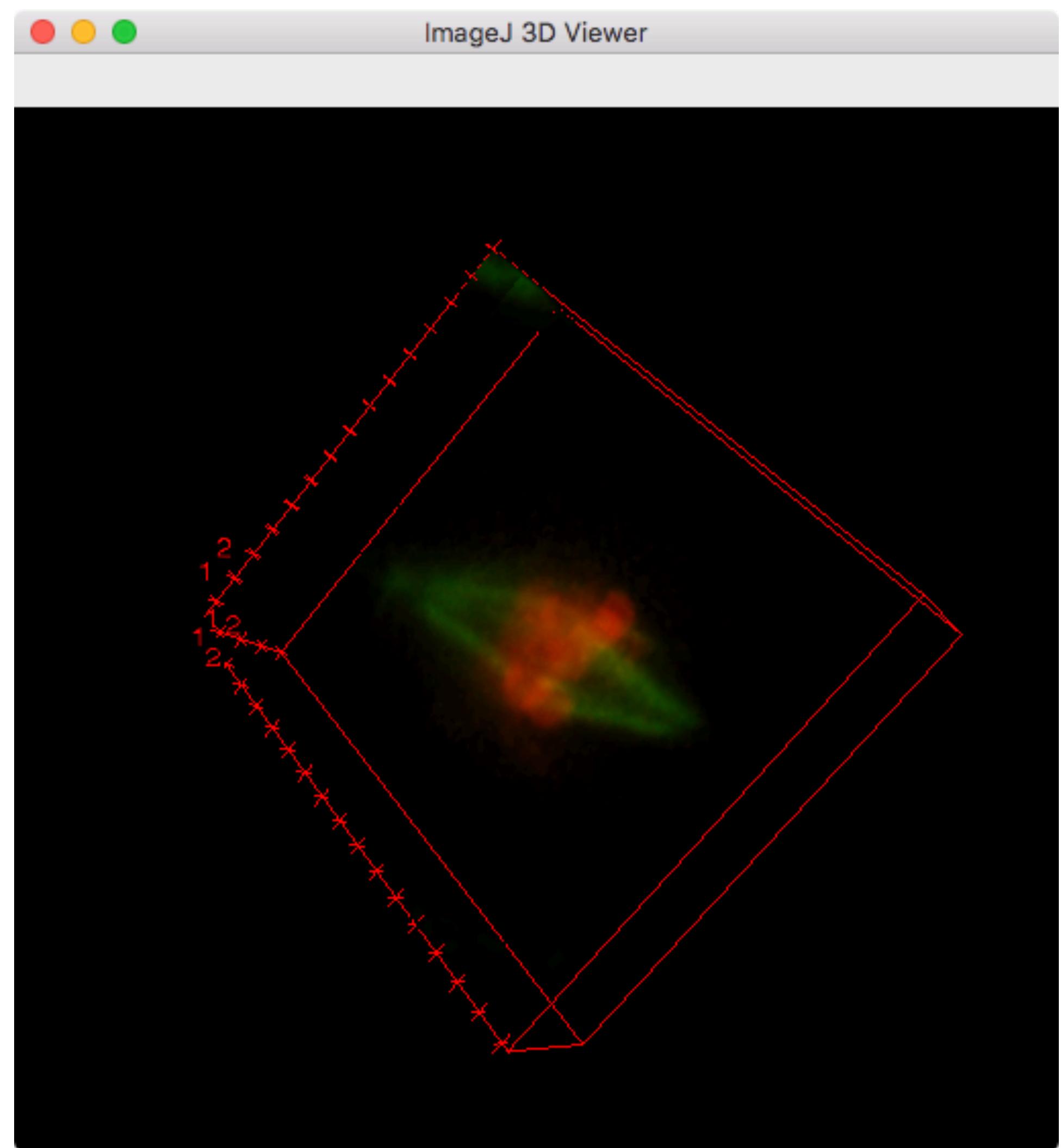
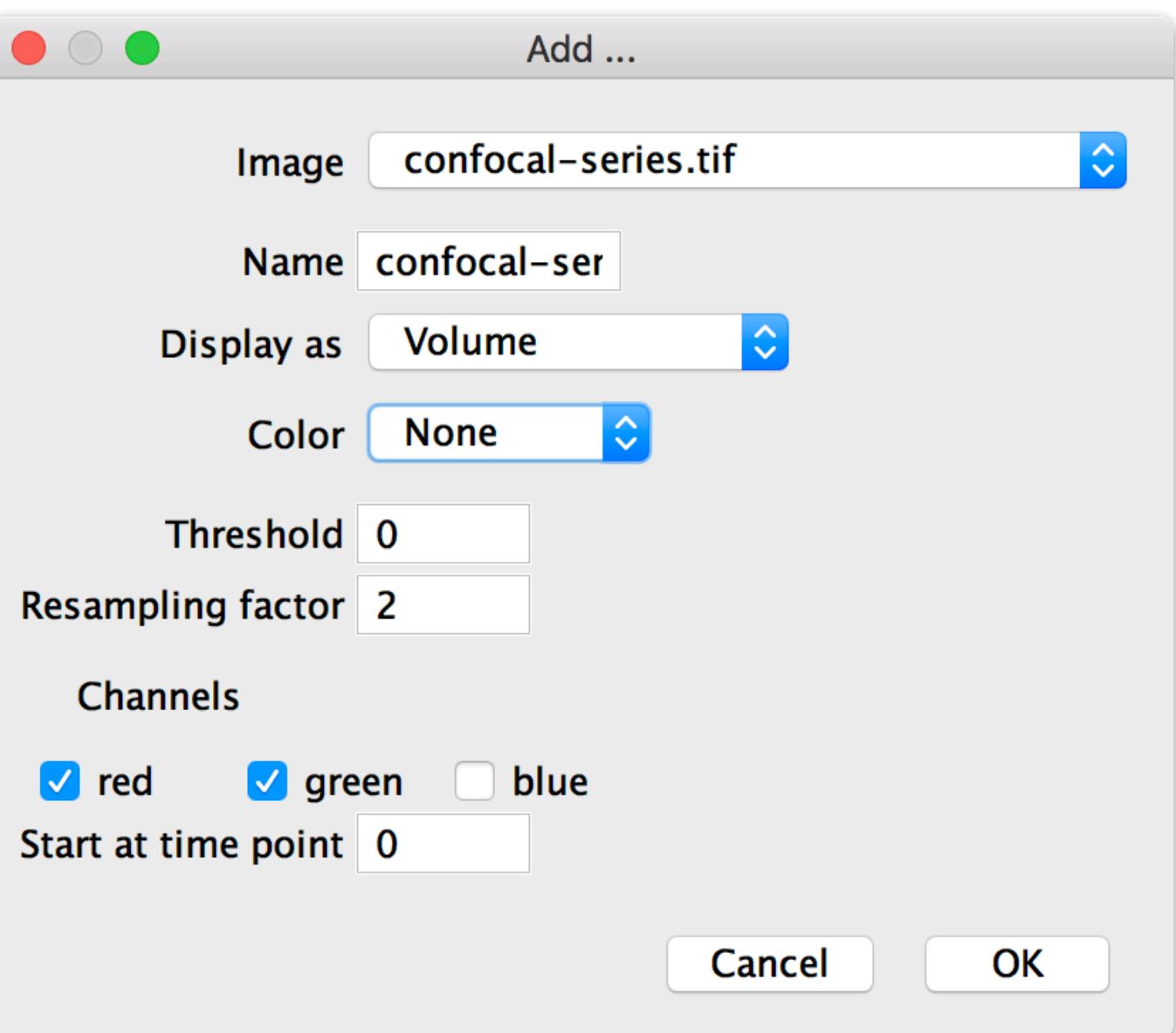
Drosophila S2 cell expressing GFP-Aurora B and mCherry-tubulin fusion protein undergoing mitosis. Courtesy of Eric Griffis.

5D STACK - MITOSIS SERIES

3D VIEW

PLUGINS

- Macros
- Shortcuts
- Utilities
- New
- Compile and Run...
- Install...
- Install Plugin...
- 3D Viewer**
- Analyze
- BigDataViewer
- Bio-Formats
- Cluster
- Color Inspector 3D
- Examples
- Feature Extraction
- HDF5
- Image5D
- Integral Image Filters
- Janelia H265 Reader
- LOCI
- LSM Toolbox
- Landmarks
- Multiview Reconstruction
- Optic Flow
- Process
- Registration
- SPIM Registration
- Segmentation
- Skeleton



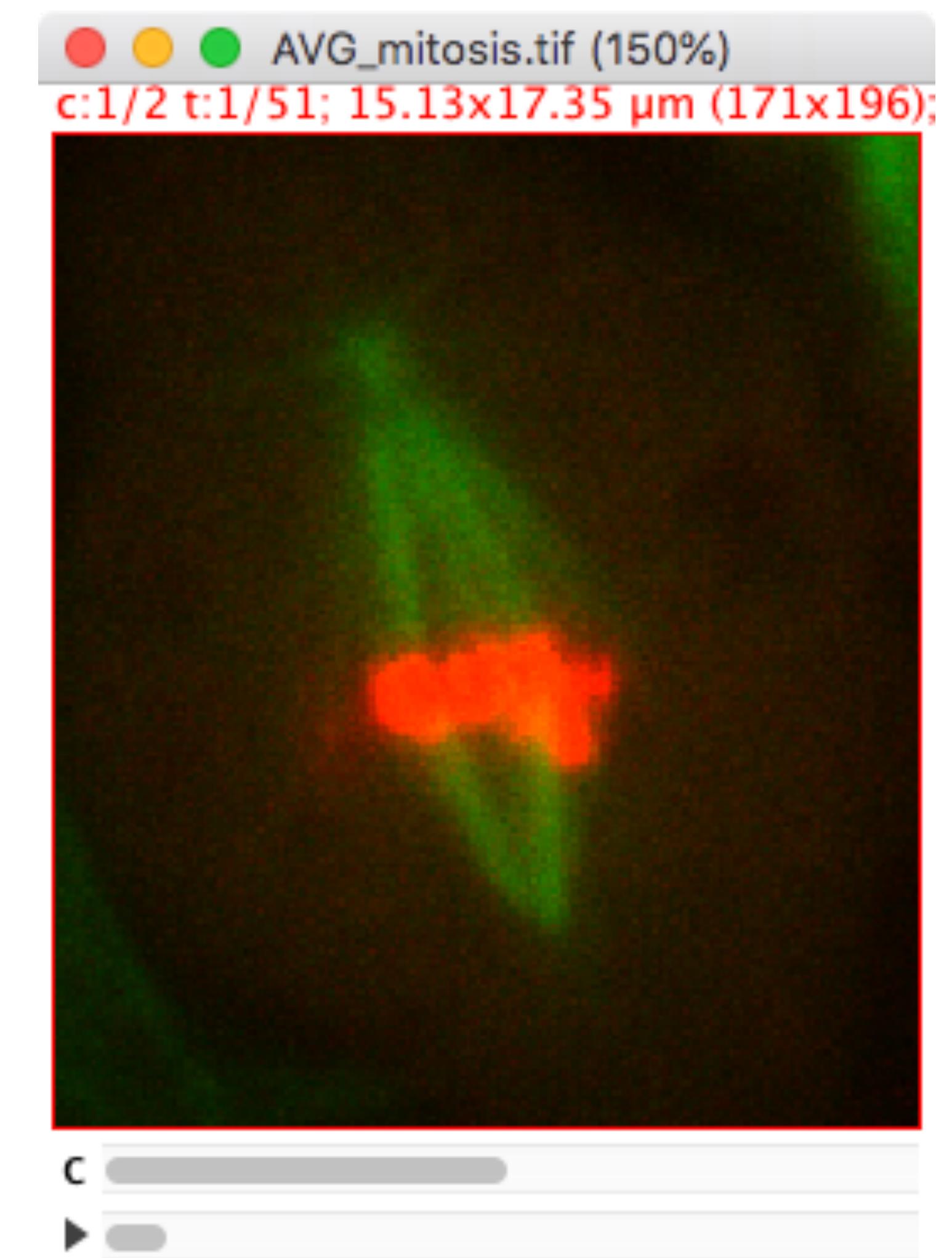
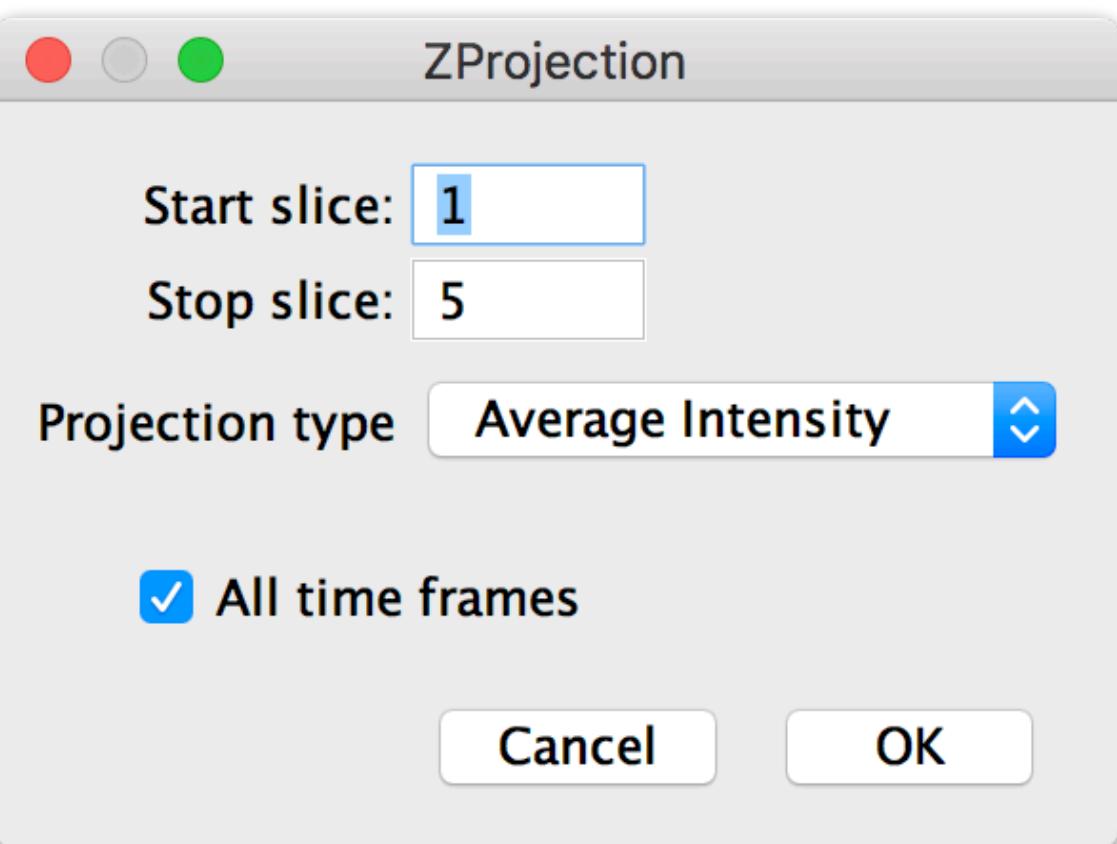
Starting time point can't be adjusted

5D STACK - MITOSIS SERIES

Z PROJECTION

IMAGES:STACKS

- Add Slice
- Delete Slice
- Next Slice [>]
- Previous Slice [<]
- Set Slice...
- Images to Stack
- Stack to Images
- Make Montage...
- Reslice [/]...
- Orthogonal Views ⌘H
- Z Project...**
- 3D Project...
- Plot XY Profile
- Plot Z-axis Profile
- Label...
- Statistics
- Animation ►
- Tools ►
- Radial Reslice
- Dynamic Reslice
- Series Labeler
- Kalman Stack Filter
- Time Stamper
- Reslice Z
- View5D ►



The Projection still has 2 scroll bars, one for color and one for time

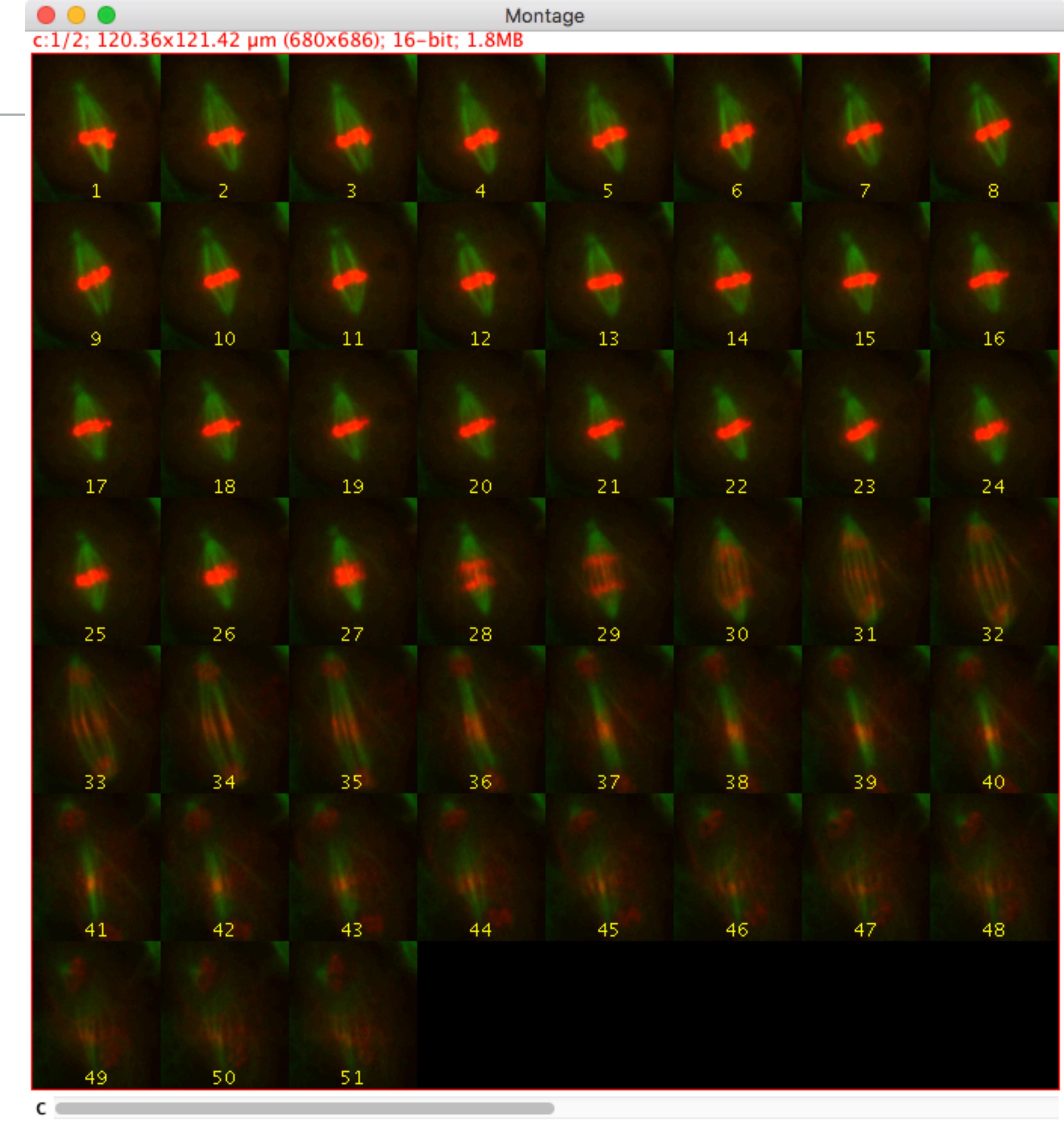
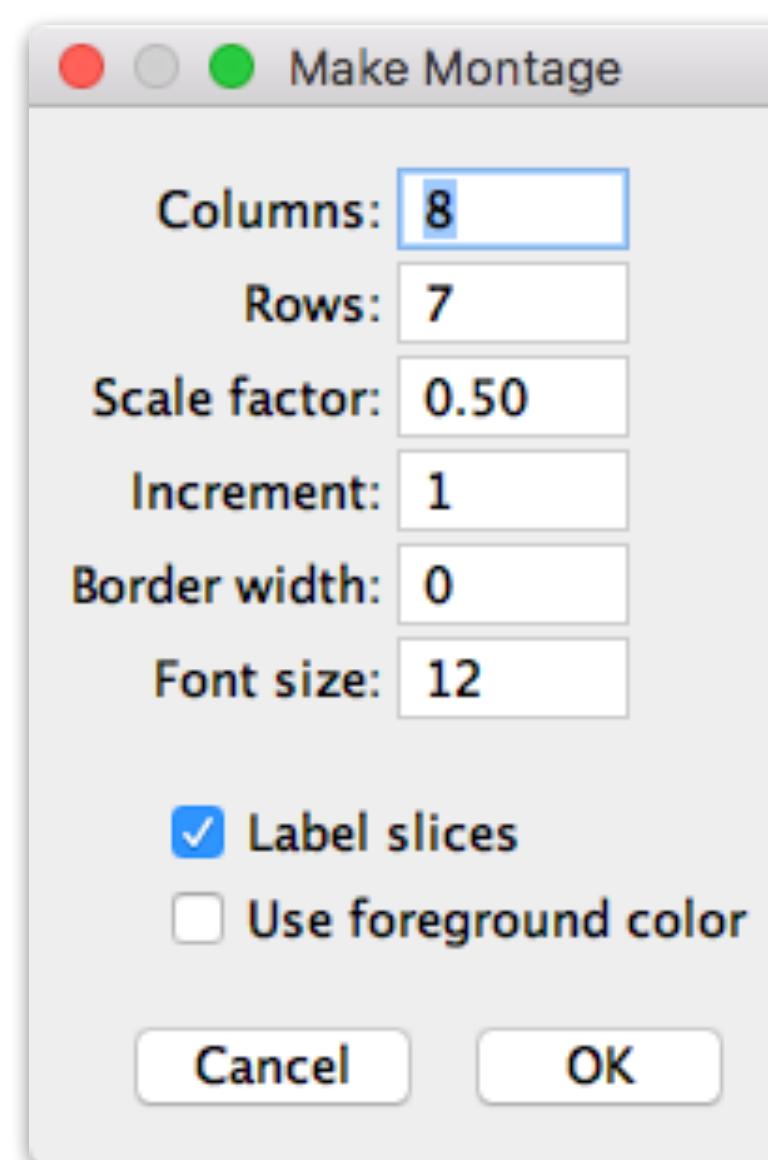
5D STACK - MITOSIS SERIES

MAKE A MONTAGE

Be sure to make a montage of the projection
(AVG_mitosis), not the original stack

IMAGES:STACKS

- Set Slice...
- Images to Stack
- Stack to Images
- Make Montage...**
- Reslice [/]...
- Orthogonal Views ⌘⌘H
- Z Project...
- 3D Project...
- Plot XY Profile
- Plot Z-axis Profile
- Label...
- Statistics
- Animation ►
- Tools ►
- Radial Reslice
- Dynamic Reslice
- Series Labeler
- Kalman Stack Filter
- Time Stamper
- Reslice Z
- View5D ►



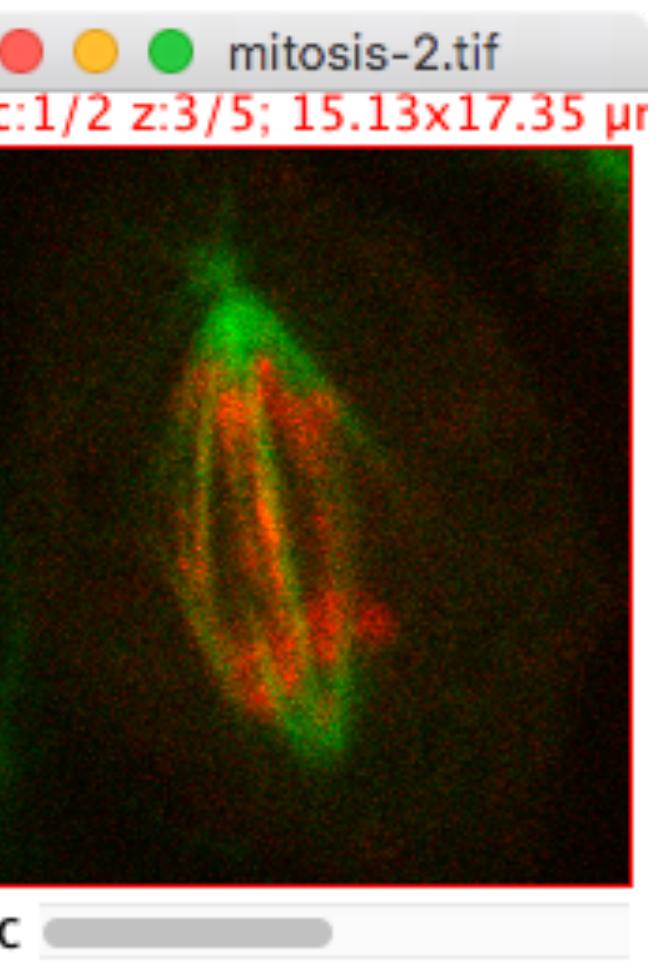
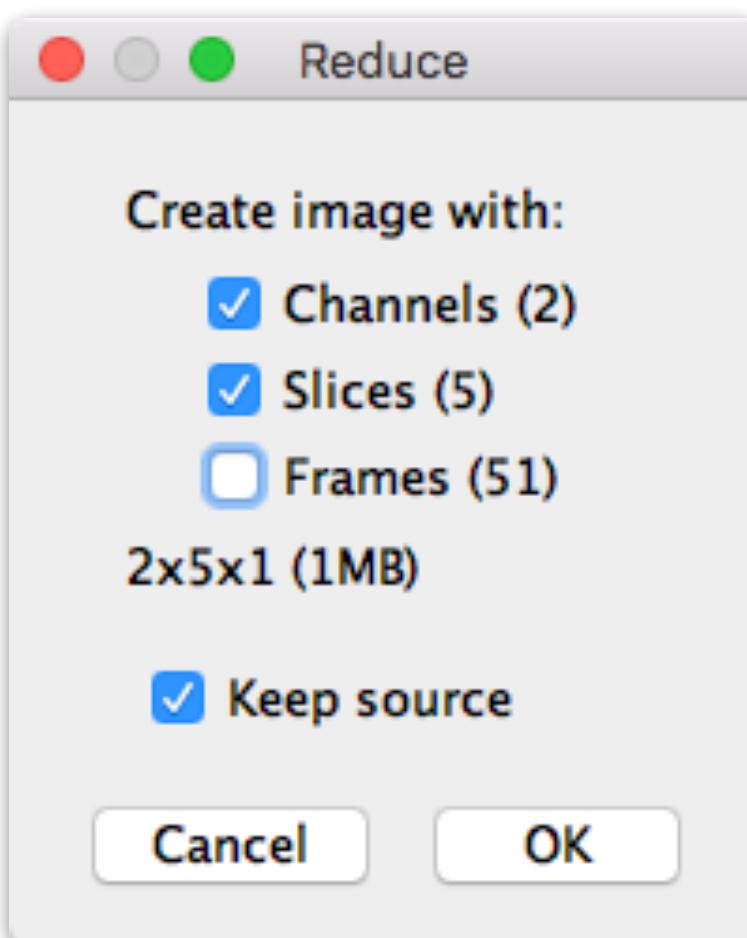
5D STACK - MITOSIS SERIES

REDUCE DIMENSIONS

Grab one time point (the 30th time frame). Create a new stack that only has the colors and volume at that time point

IMAGES:HYPERTACKS

- New Hyperstack...
- Stack to Hyperstack...
- Hyperstack to Stack
- Reduce Dimensionality...**
- Re-order Hyperstack ...
- Temporal-Color Code



3D PROJECT

IMAGES:STACKS

- Images to Stack
- Stack to Images
- Make Montage...
- Reslice [/]...
- Orthogonal Views ⌘H
- Z Project...
- 3D Project...**
- Plot XY Profile
- Plot Z-axis Profile
- Label...
- Statistics
- Animation
- Tools

