Fiji Plugins for 3D and 3D+T Data, aka Big-*ish* Image Data

3/26/2025
QLS Breakfast
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Previous relevant session

Introduction to ImageJ Macros, Justin Savage, 03/06/2024

Outline

1. Virtual stack for viewing large data - macro-capable
2. Drift corrections - macro-capable
3. Big Data Viewer (vs Volume Viewer)
4. TrackMate - macro-capable
5. Mastodon = BDV + TM

Please speak up with your own tips + anecdotes.

Quick tips for large data in FIJI

Allocate FIJI max memory to 75% of computer RAM - best practice -or whatever arbitrary high number you want - so FIJI isn't limiting factor Edit>Options>Memory & Threads

Read data locally or over wired (ethernet) connection

Subset data to only the relevant portion for your analyses (as a duplicate TIF file, etc)

- Convert to 8-bit if you don't need 16-bit res
- Subsample: Image>Scale… reduce to desired pixel dimensions (e.g. 1024 –> 512) also have to reduce Z

When in doubt, clear the memory or close FIJI and reopen

- Sometimes memory not released back to system after images are closed (even virtual)
- More on clearing memory in a minute…

Open images as virtual stacks

Dealing with the memory backlog

Click status bar

or

Plugins>utilities>collect garbage
Plugins>utilities>monitor memory

or

call("java.lang.System.gc");

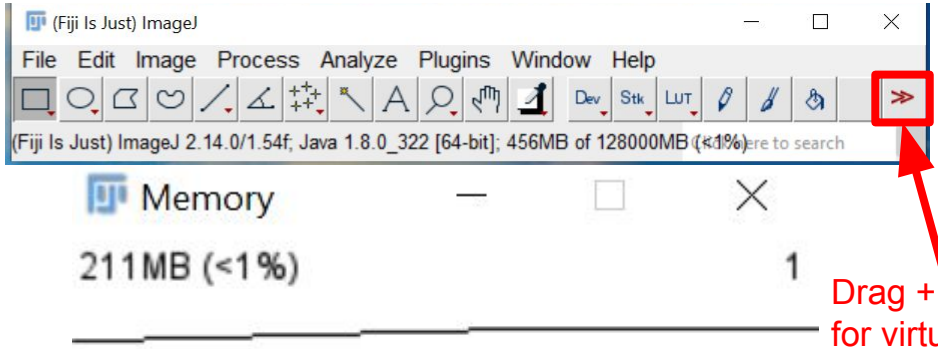
or

run("Collect Garbage");

run("Fresh Start");

or

Restart FIJI



Another user's solution written in Groovy:

```
imp.flush() //flush the image
IJ.wait(5000) //delay to collect the garbage
System.gc() //run the garbage collection
```

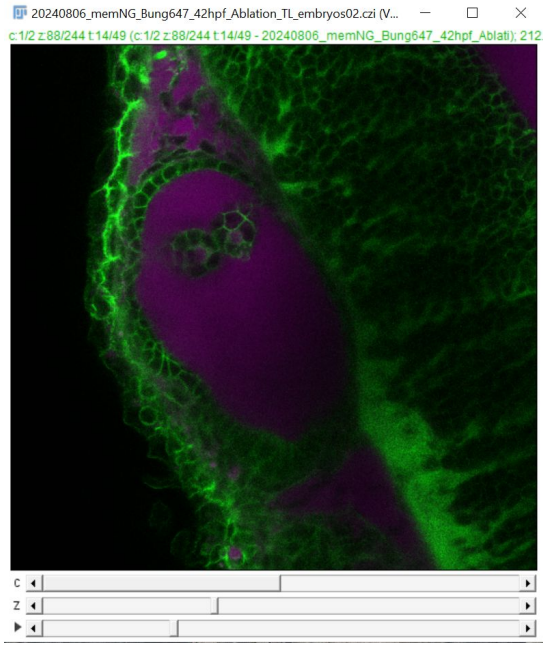
https://forum.image.sc/t/memory-releasing-batch-processing-scripting/60547

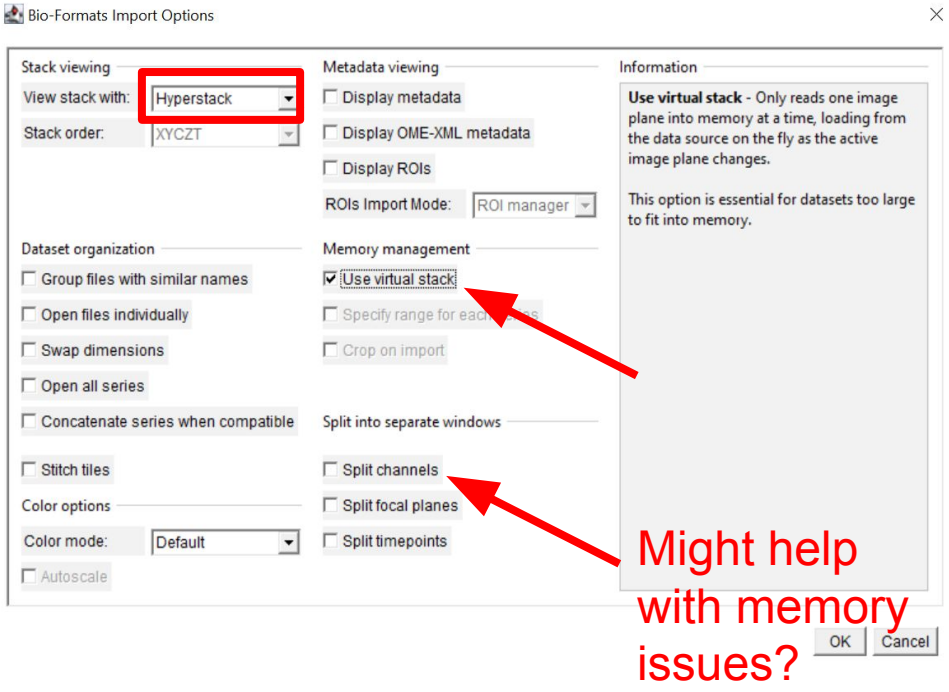
Equivalent in macro language (*I think equivalent*):

```
close("WindowName")
wait(5000) //wait 5000 milliseconds
run("Collect Garbage")
run("Fresh Start")
```

1. Virtual Stack in Bio-Formats Importer

Plugins>Bio-Formats>
Bio-Formats Importer



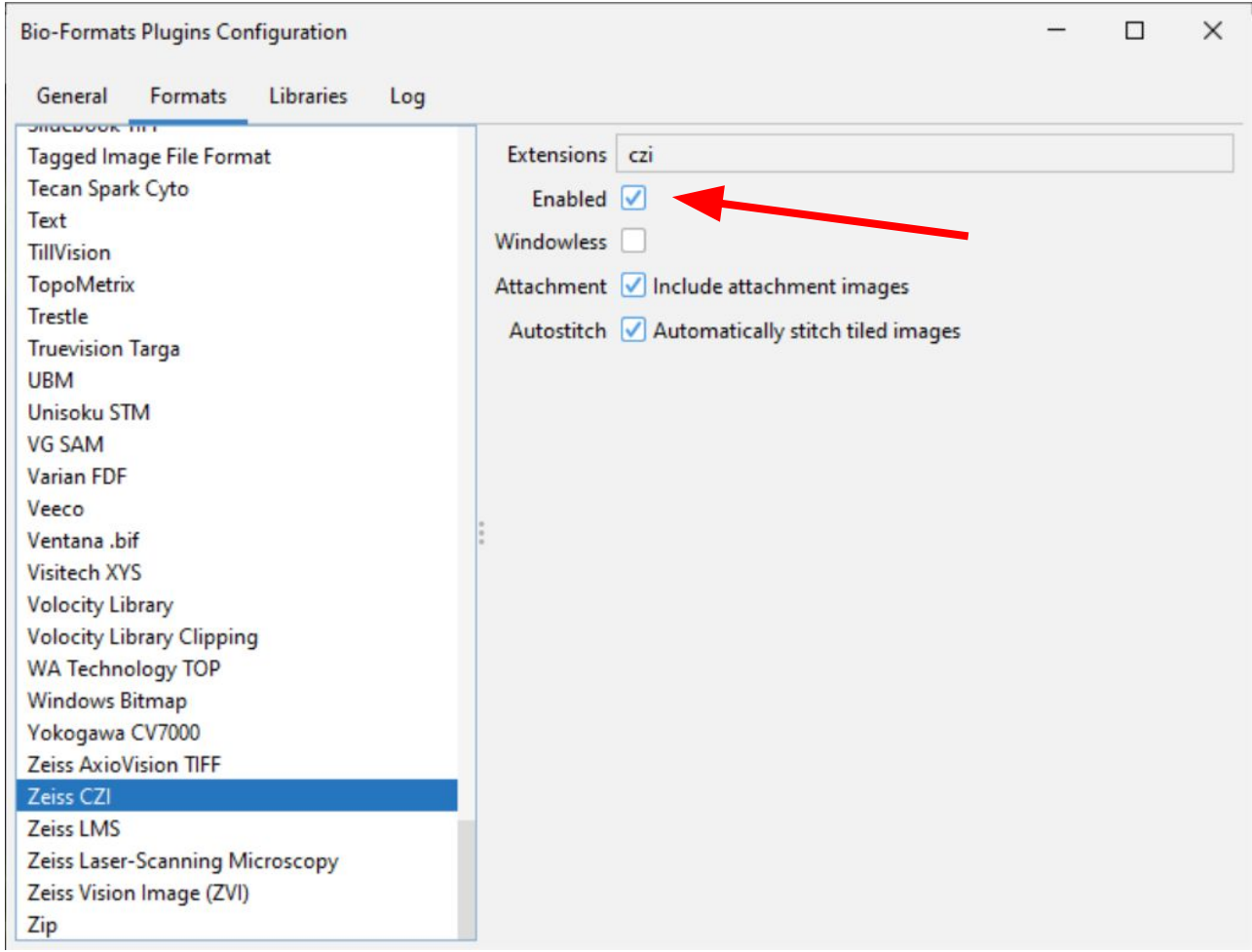


Might help with memory issues?

Plugins > Bio-Formats > Bio-Formats Plugin Configurations

To enable as default (probably already enabled)

Now drag+drop or File>Open will use bio-formats importer for these file types



Get professional help for large datasets and analysis

LMCF staff

IT staff in your Department

Research Computing support at Duke

Duke Center for Data and Visualization Sciences

2. Drift Corrections

Many solutions, but I'll go over…

Plugins>Registration>**Correct 3D Drift**

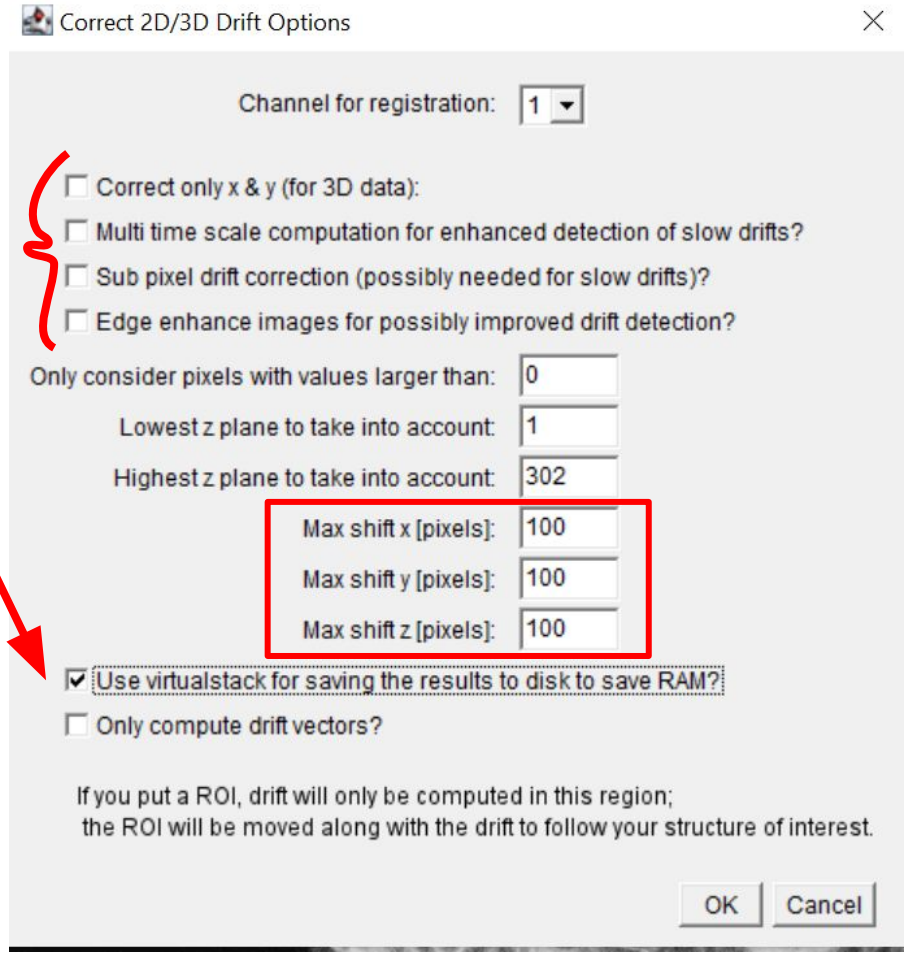
Input: open TIF (as virtual Stack) in FIJI
Start with a fresh instance of FIJI

Output: 3D registered TIF (unsaved) and 2D images saved in folder specified when you run registration

To save yourself the headache, save the output TIF instead of trying to reconstruct it
It will be much larger than original (maybe 2x or more)

Then close FIJI or run garbage collection

Virtual Stack!



How the 3D Drift correction works

Phase Correlation Correction Method

Based on fourier shift theorem

image2(x,y)=image1(x−tx,y−ty) for each pixel of a given two timepoints - but including z

Kuglin, C. D. and Hines, D. C., 1975. The Phase Correlation Image Alignment Method. Proceeding of IEEE International Conference on Cybernetics and Society, pp. 163-165, New York, NY, USA.

Drift corrections of multiple channels

5D (3D, 2 channel, time)

> Limited by computer RAM, even with virtual stack (FIJI not clearing memory properly with 3D drift correct plugin)

I have to drift correct one channel and apply the correction to other channels

***Ask me for python script for applying drift to second channel if interested.

3. Big Data Viewer

For reslicing 3D and 3D+T big image data on any angle, quickly responsive UI

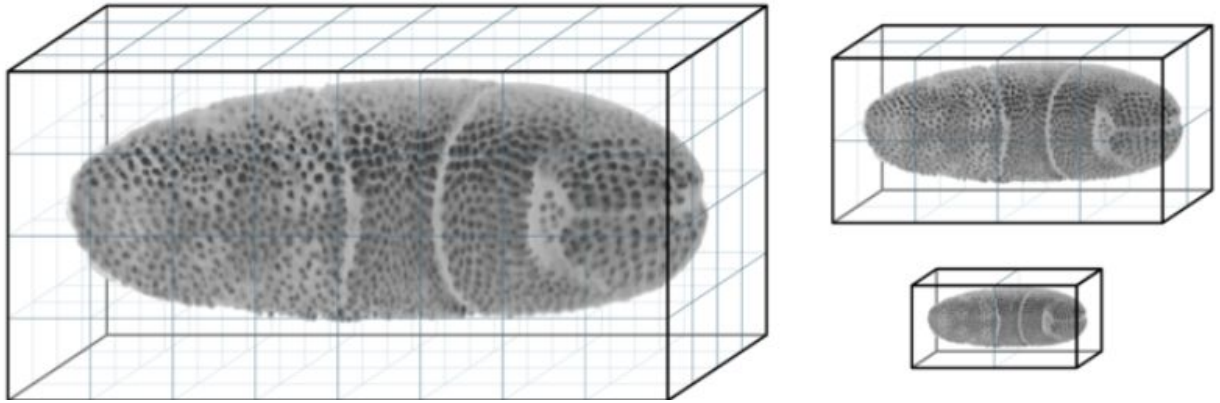
Far more intuitive than orthogonal views (which have their benefits of course)

Check image z dimensions

Image>Properties…. Dimensions

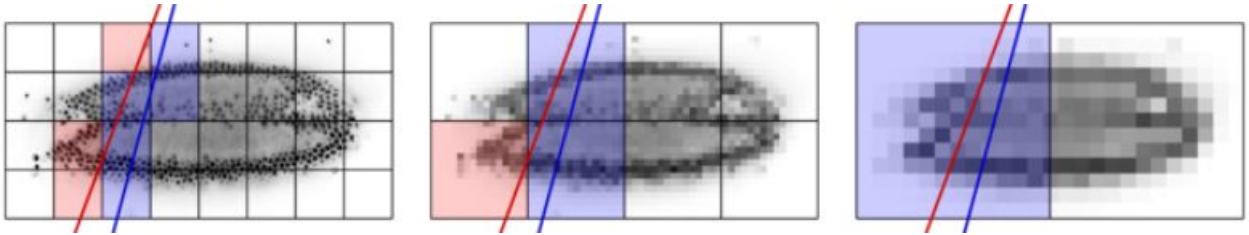
Plugins>BigDataViewer>Open Current Image (I open in FIJI as virtual stack to start)

How BDV works



Starts with low resolution mipmaps and loads higher resolution as those chunks are pulled into cache

Only loads into cache the relevant ‘chunks’ of your data at each resolution (ie blus vs red)

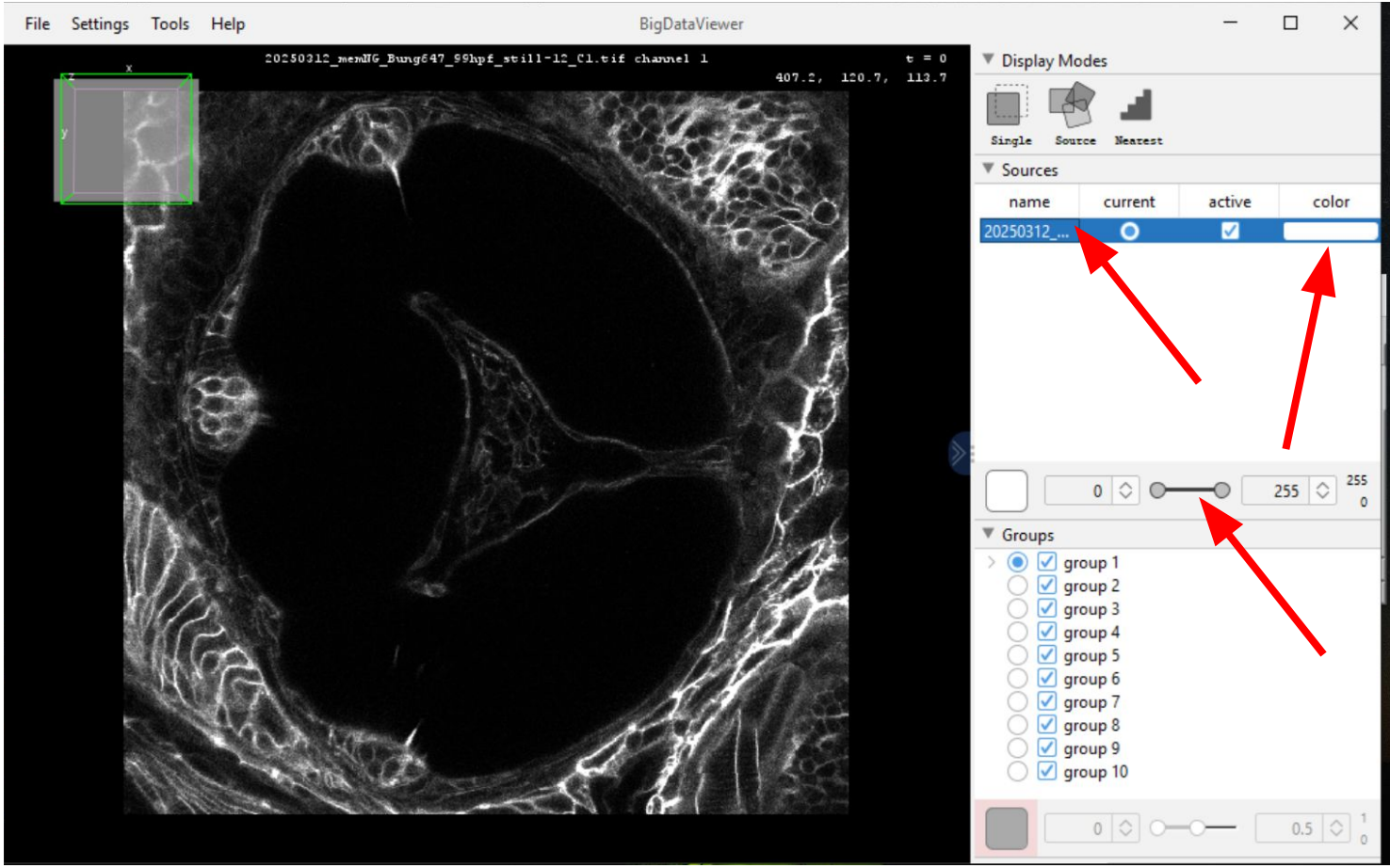


From https://imagej.net/plugins/bdv

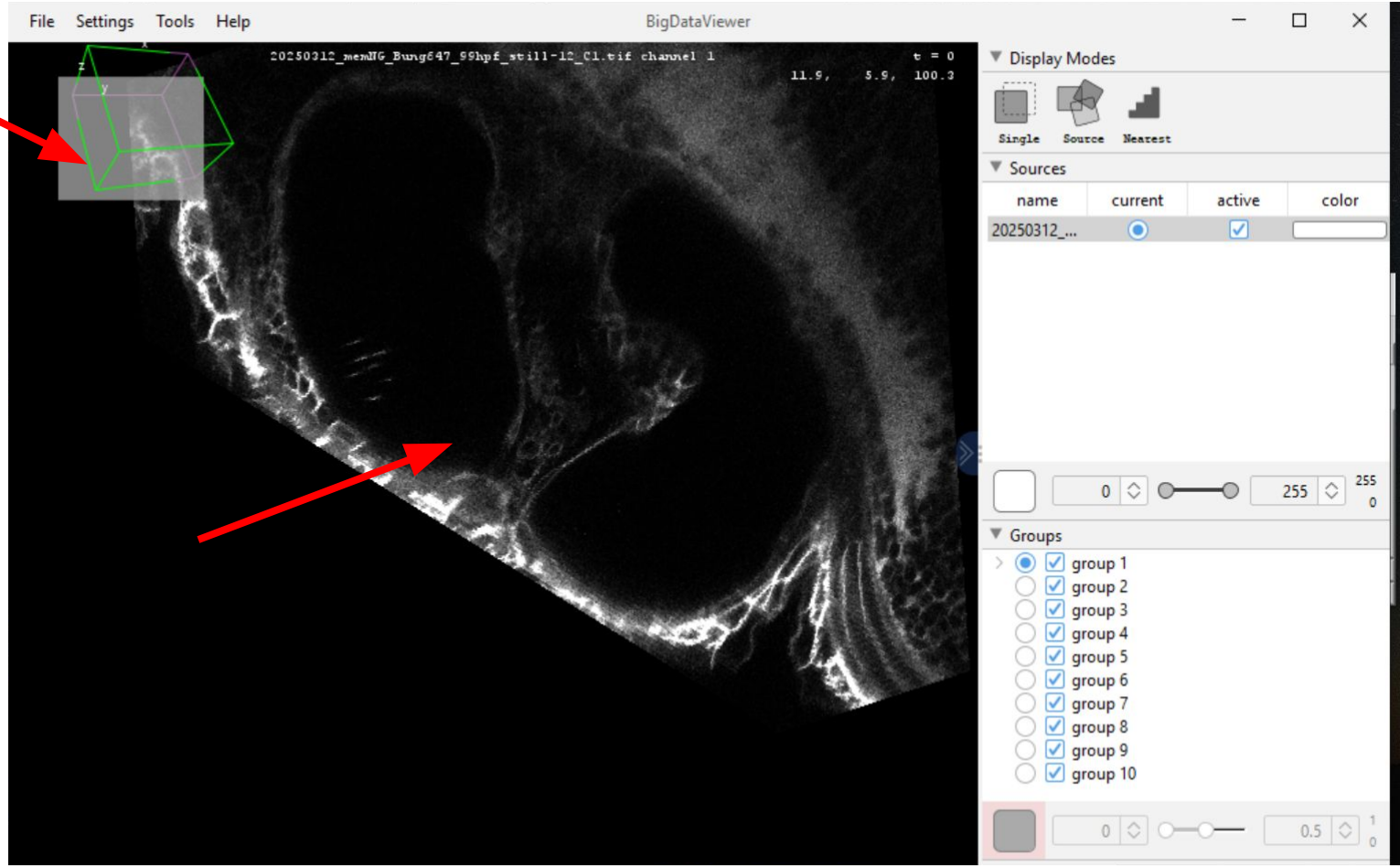
Cursor x,y,z position displayed in microns, as well as timepoint

Hover over righthand side to open display editor

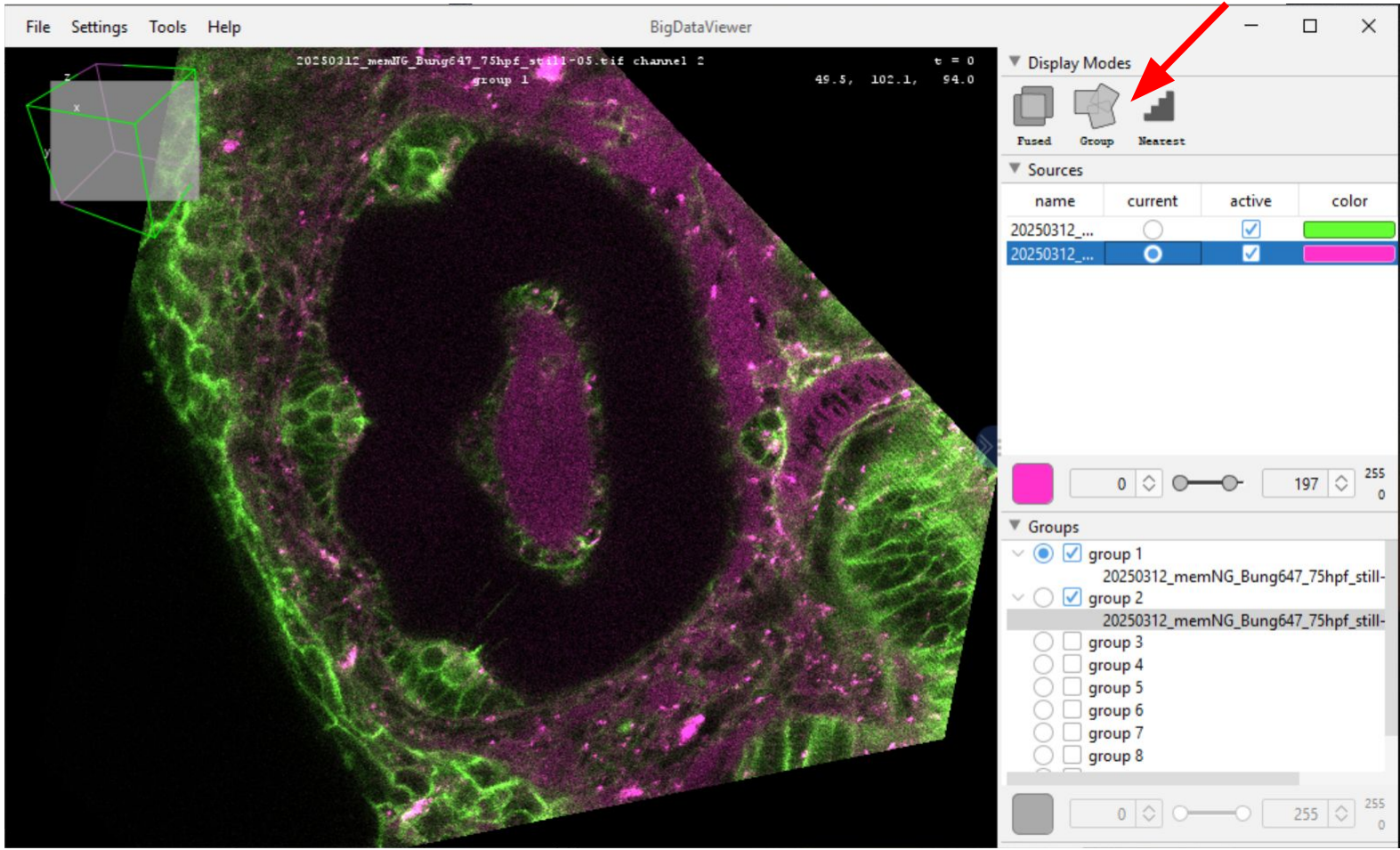
Adjust display for selected source



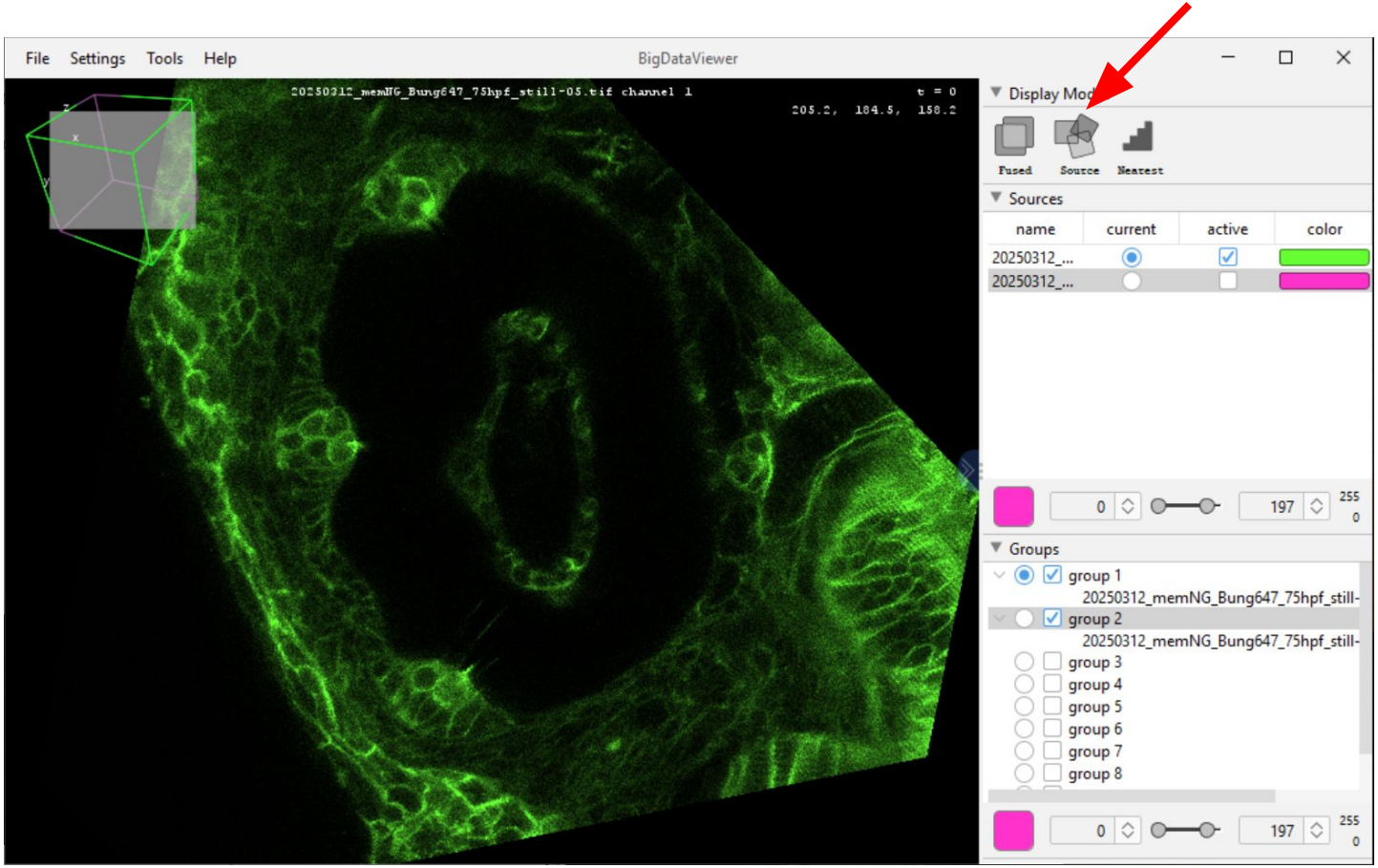
Rotate with image or with cube of axes



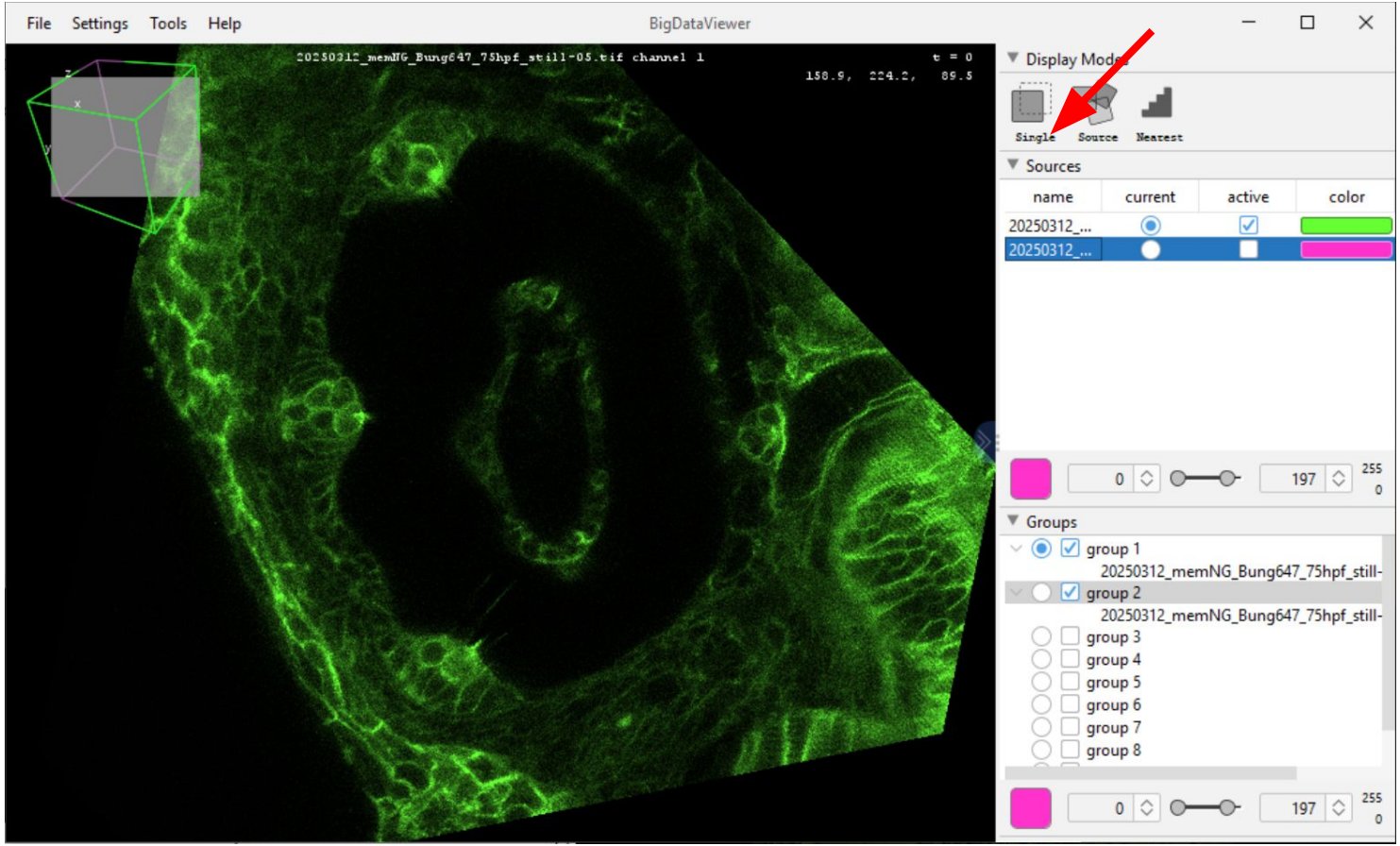
Display
from group



Display from source

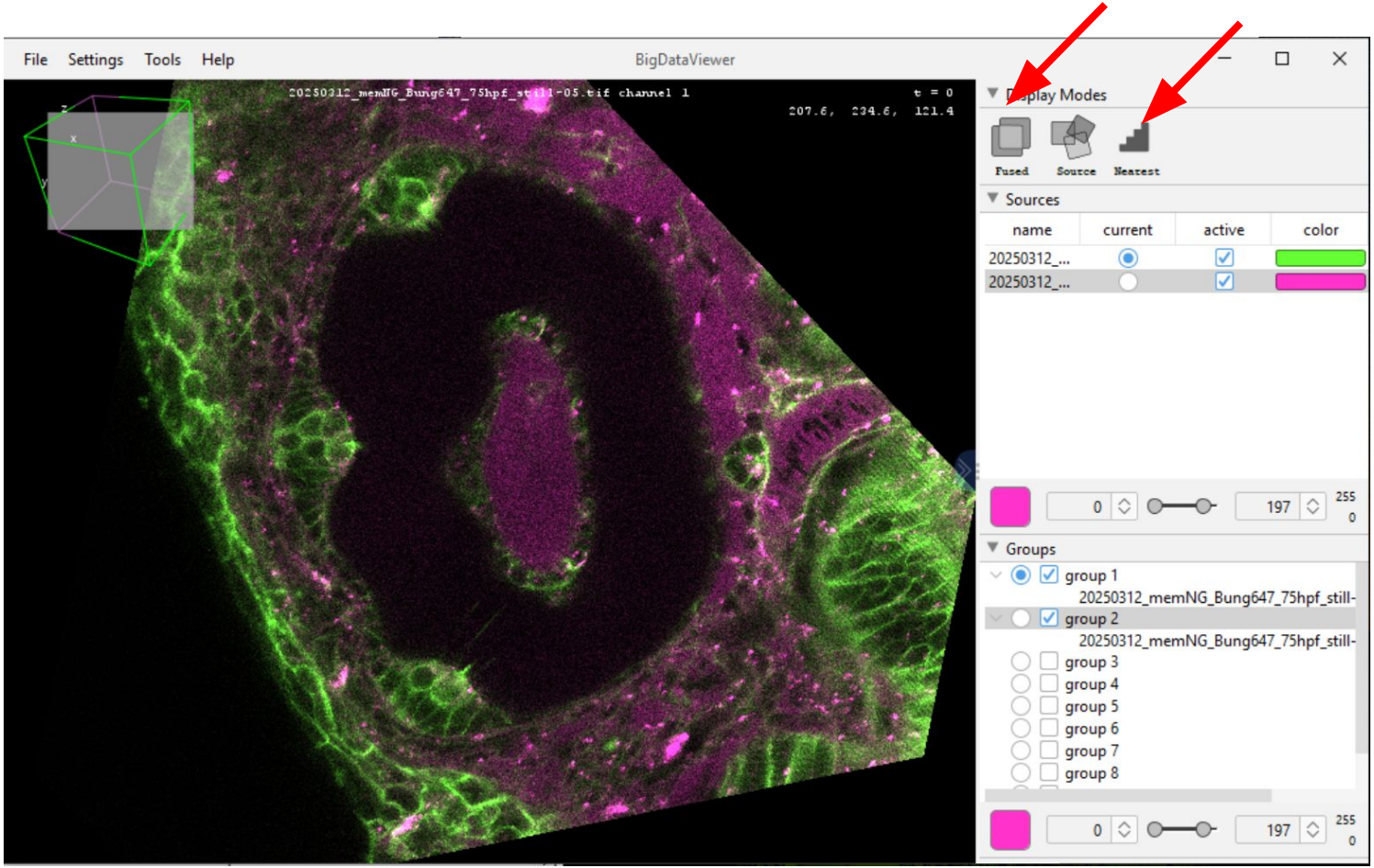


Display from single source

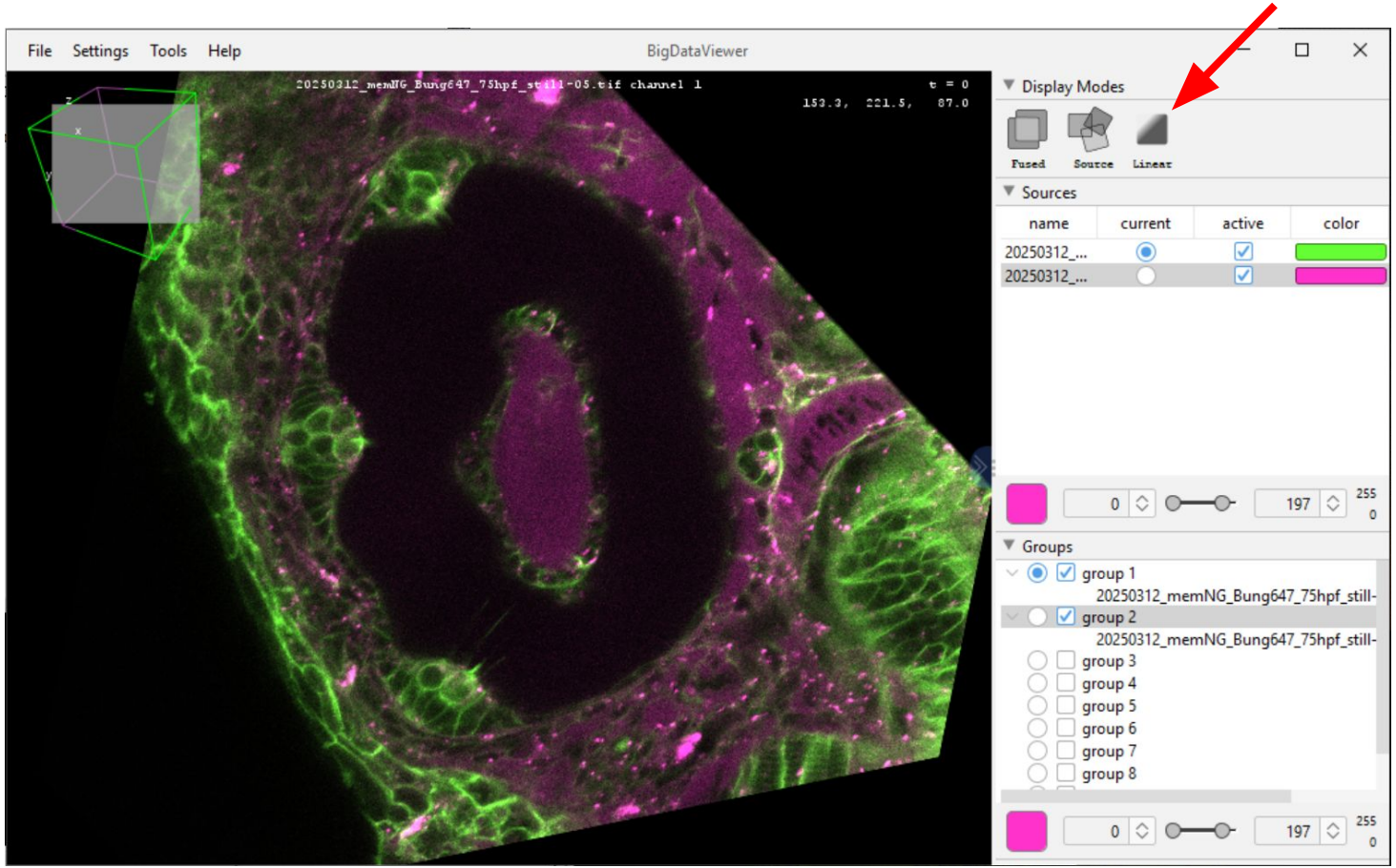


Display fused sources

Display nearest neighbor interpolation



Display tri-linear interpolation



BDV Shortcuts

https://imagej.net/plugins/bdv/

Shift to speed-up

Ctrl to slow-down

these shortcuts

The following table shows the available navigation commands using the mouse:

Left Drag	Rotate (pan and tilt) around the point where the mouse was clicked.
Right Drag or Middle Drag	Translate in the XY-plane.
Mouse Wheel	Move along the z-axis.
⌘ Command + Mouse Wheel or ⇧ Shift + ^ Ctrl + Mouse Wheel	Zoom in and out.

The following table shows the available navigation commands using keyboard shortcuts:

X, Y, Z	Select keyboard rotation axis.
← Left, → Right	Rotate clockwise or counter-clockwise around the choosen rotation axis.
↑ Up, ↓ Down	Zoom in or out.
, , .	Move forward or backward along the Z-axis.
⇧ Shift + X	Rotate to the ZY-plane of the current source. (Look along the X-axis of the current source.)
⇧ Shift + Y or ⇧ Shift + A	Rotate to the XZ-plane of the current source. (Look along the Y-axis of the current source.)
⇧ Shift + Z	Rotate to the XY-plane of the current source. (Look along the Z-axis of the current source.)
[or N	Move to previous timepoint.
] or M	Move to next timepoint.

For all navigation commands you can hold ⇧ Shift to rotate and browse 10x faster, or hold ^ Ctrl to rotate and browse 10x slower. For example, ← Left rotates by 1° clockwise, while ⇧ Shift + ← Left rotates by 10°, and ^ Ctrl + ← Left rotates by 0.1°.

Video tutorial links

Awesome video tutorial on BDV, etc

https://youtu.be/LHI7vXiUUms?si=cbf7zzgbBNHlB_IW

Making videos with BDV

https://www.youtube.com/watch?v=vXu4ZOboEio

Plugins/Software that use BDV

3D Image handling/processing:

BigStitcher - image registration, fusion, and deconvolution

BigWarp - elastic image alignment

BigVolumeViewer - 3D image rendering (vs FluoRender)

BigDataProcessor2 - TB size data processing

Tracking:

MaMuT

Tr2D

Mastodon (more later…)

Segmentation/Pixel Classification:

LabKit

MetaSeg

Other Software:

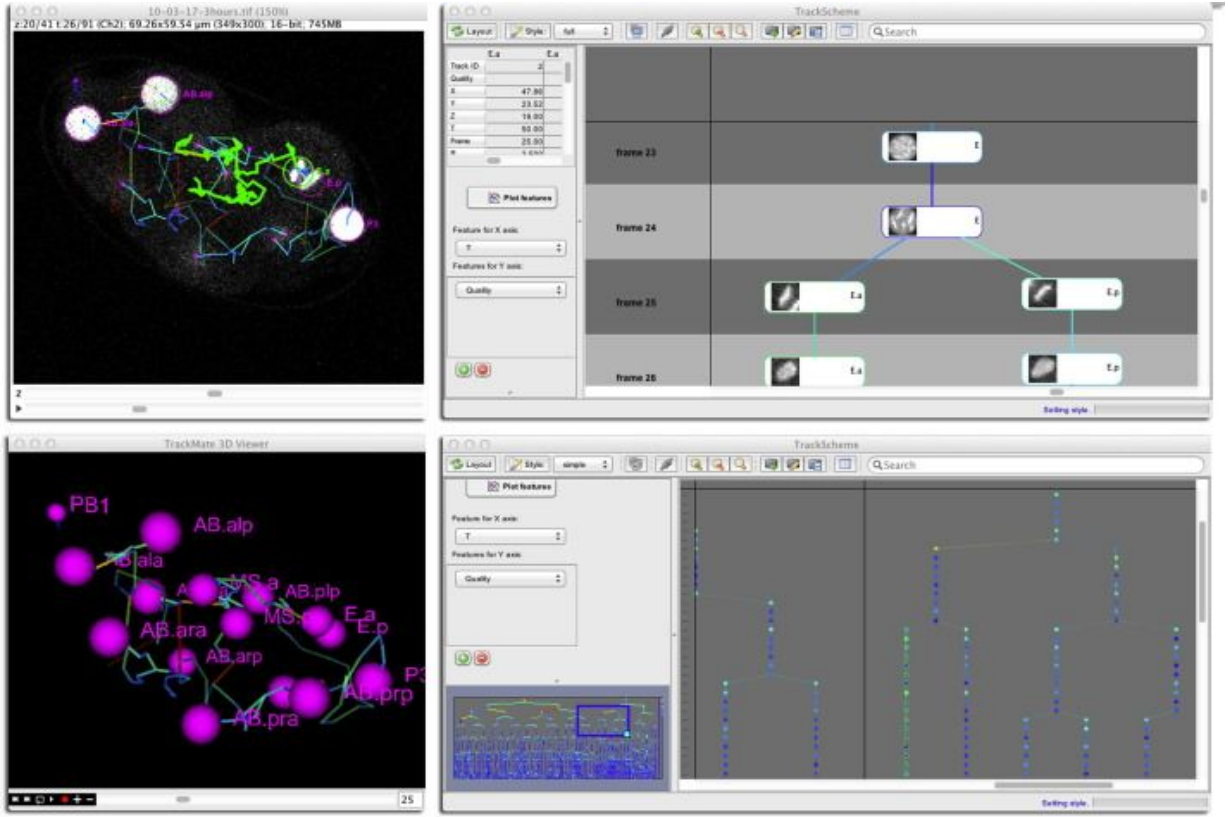
Paintera

4. TrackMate

Automated or manual tracking with nodes to represent what you are tracking and lines of fading color to represent past trajectory

Also makes tree of node-node relationships in time (e.g. cell divisions)

Many different tracking algorithms to try - see documentation if interested!

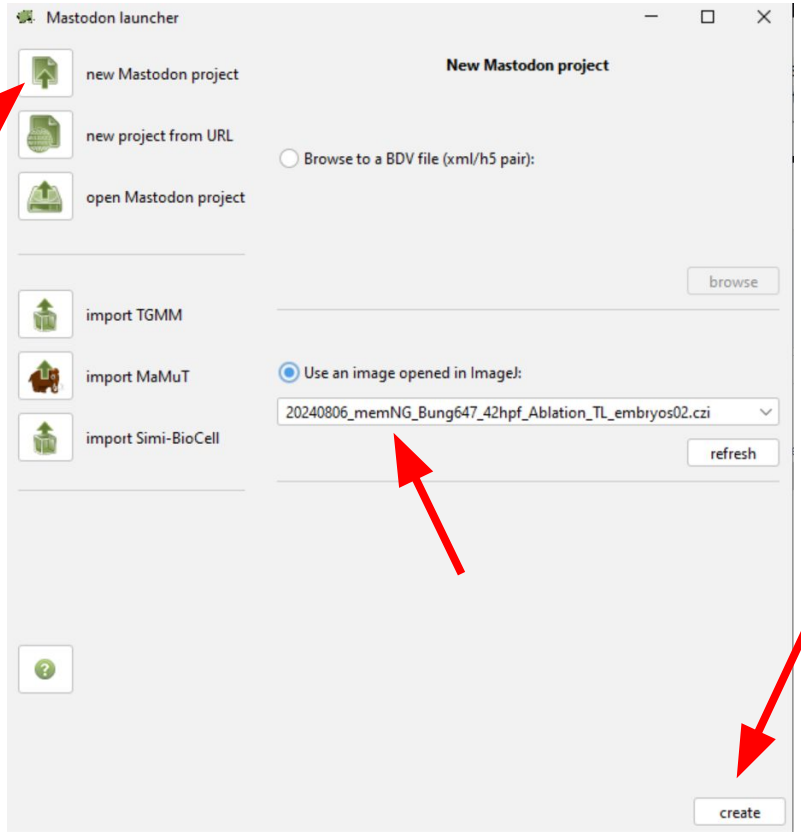


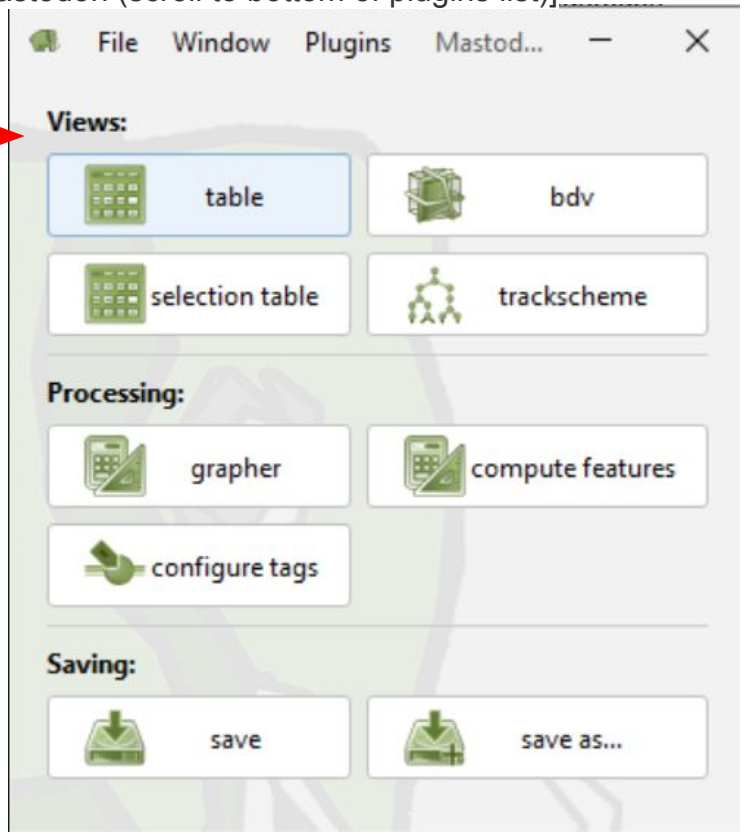
https://imagej.net/plugins/trackmate/tutorials/getting-started

5. Mastodon

Help › Update… wait…. and click Manage update sites. Check the Mastodon box. Close the window. Click Apply changes, then restart Fiji.

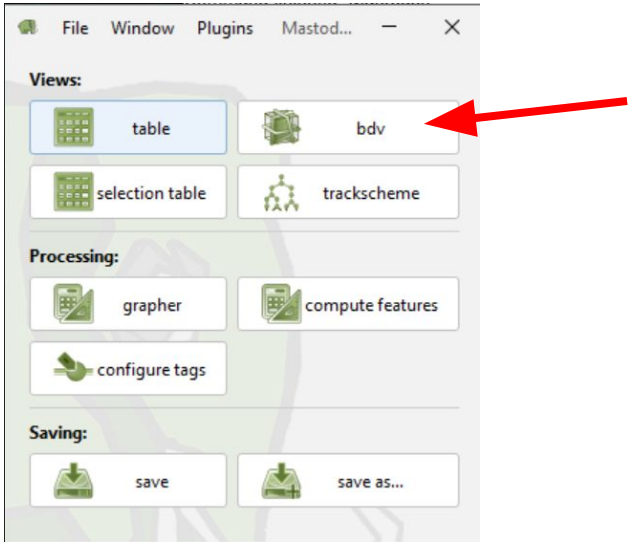
Plugins>Tracking>Mastodon>MastodonLauncher
[or for older version Plugins>Mastodon (scroll to bottom of plugins list)]

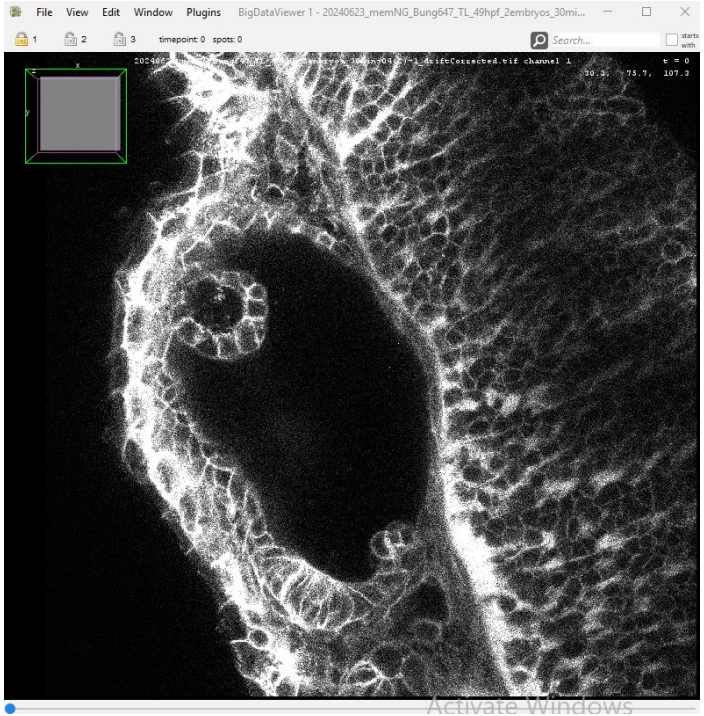




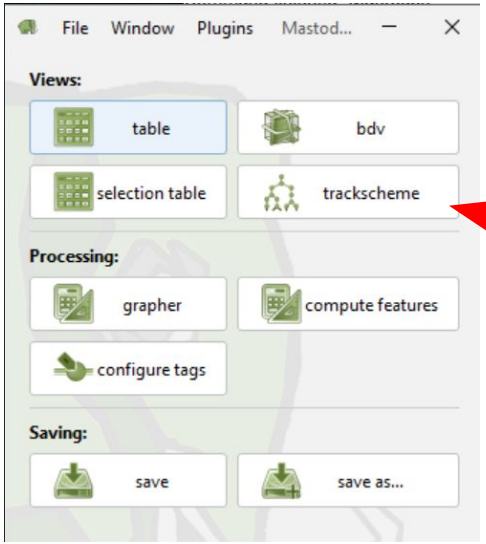
BDV in Mastodon

Can use virtual stack with Mastodon, just like Big Data Viewer!





TrackScheme in Mastodon



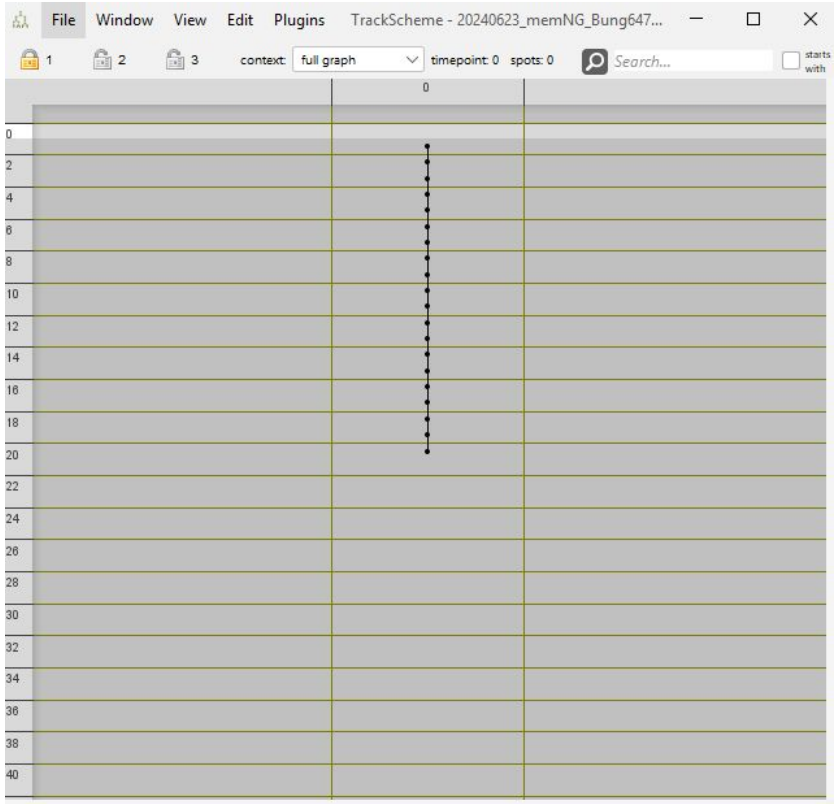
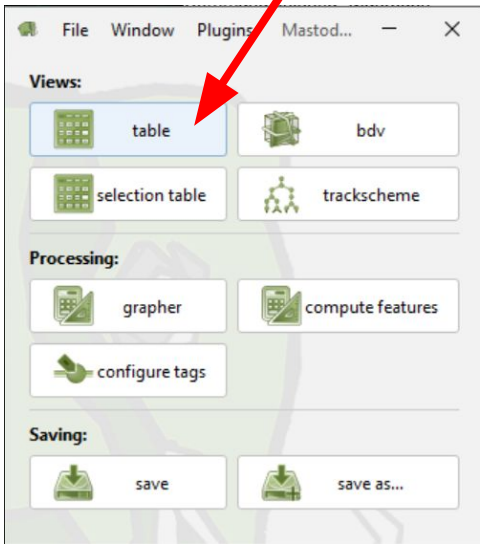


Table in Mastodon (includes x,y,z coordinates)

Can edit label for each node, can export to csv



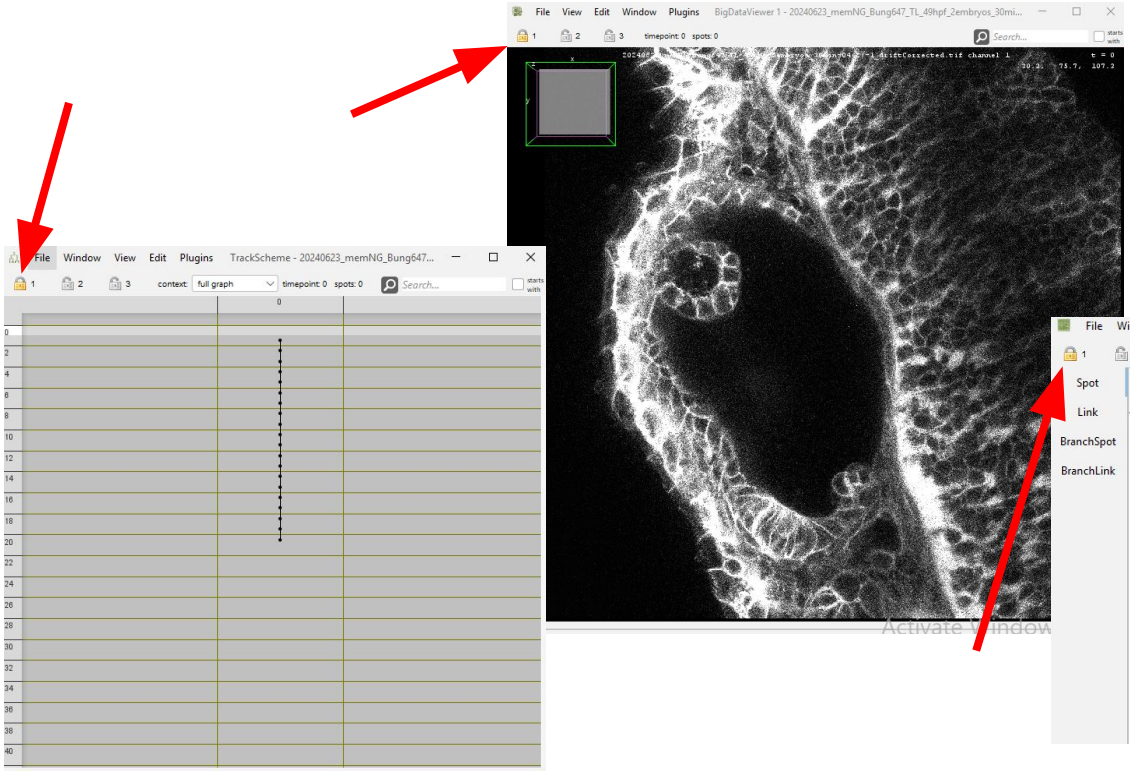
File Window View Edit Settings Plugins Data table - 20240623_memNG_Bung647_TL_49hpf_2embryos_30min-04...

1 2 3 context: full graph Search... starts with

Spot
Link
BranchSpot
BranchLink

Label	ID	Spot N links	Spot N links N incoming...	Spot N links N outgoing ...	Spot frame	Spot position X (micron)	Spot position Y (micron)	Spot position Z (micron)	Spot radius (micron)
0	0	1	0	1	1	149.736	157.267	100.725	1.381
1	1	2	1	1	2	151.339	157.089	100.725	1.381
2	2	2	1	1	3	149.914	157.089	100.725	1.381
3	3	2	1	1	4	150.448	155.842	100.725	1.381
4	4	2	1	1	5	149.558	153.883	100.725	1.381
5	5	2	1	1	6	151.339	153.527	100.725	1.381
6	6	2	1	1	7	154.544	154.24	98.766	1.381
7	7	2	1	1	8	156.325	151.568	96.807	1.381
8	8	2	1	1	9	154.901	150.678	97.342	1.381
9	9	2	1	1	10	151.873	148.363	97.342	1.381
10	10	2	1	1	11	150.327	146.366	89.328	1.381
11	11	2	1	1	12	153.532	146.366	89.328	1.381
12	12	2	1	1	13	154.779	147.613	89.328	1.381
13	13	2	1	1	14	157.628	143.854	95.344	1.381

The three views can be locked together…and you can open as many instances of each as you want!

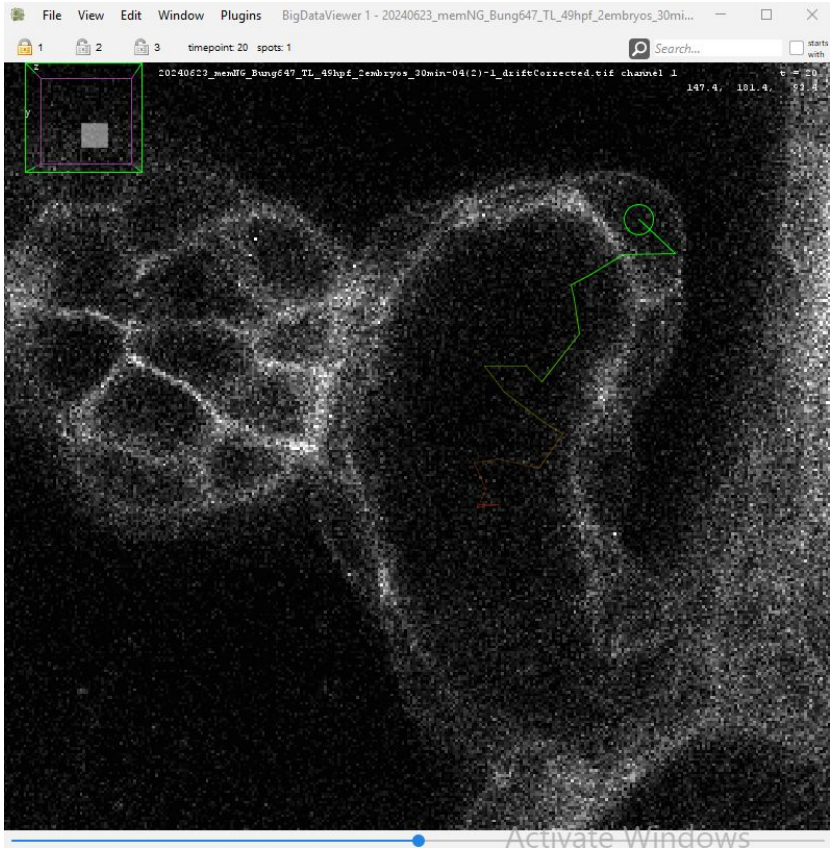


When locked, a node selected in one view will be selected and shown in the other locked views.

Tracking Display in Mastodon BDV

Nodes show as spots

Links over time color from red (oldest) to green (current timepoint in view)

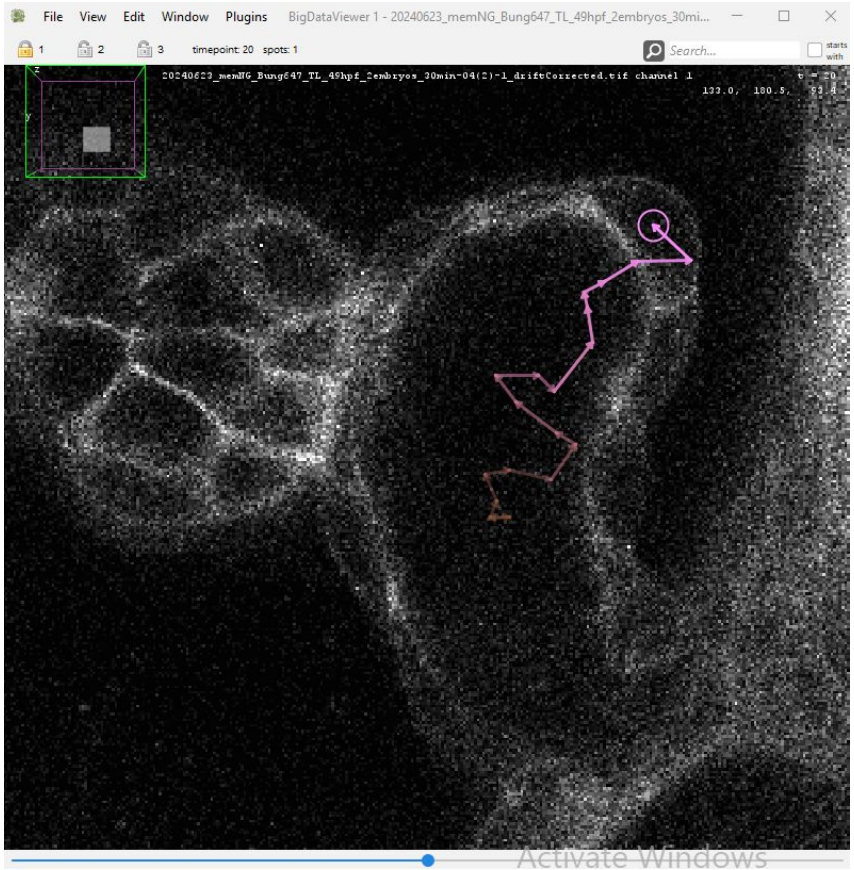


Adjust display of nodes and links

In File>Preferences…

You'll find many settings to change view, including spots and links, adding arrows, coloring, etc…

Selecting a node changes its color, scrolling through z will move things in and out of focus - these settings are all editable



Shortcuts

More in the documentation online

https://mastodon.readthedocs.io/en/latest/docs/partB/table_bdv_navigation_keys.html

Scroll along left-hand side for several tables of shortcuts

Action	Key
Adding, deleting and modifying spots.	
Add a new spot.	Put the mouse at the desired position and press the `A` key.
Move a spot.	Put the mouse inside a spot, press and hold `space`, and move the mouse to the desired position.
Delete a spot.	Put the mouse inside a spot and press `D`.
Change the radius of a spot.	Put the mouse inside a spot and press `Q` (make it smaller) or `E` (bigger). Hold `Shift` to make larger changes or `Control` for finer changes.
Adding and linking spots.	
Link one spot to an existing one in the *next* frame.	Put the mouse **inside** a source spot, and press and hold `L`. The viewer moves to the next time-point and shows a preview of the link. Release the `L` key in the desired target spot. A link is created from the source spot to the target spot.
Link one spot to an existing one in the *previous* frame.	Same procedure, but press `Shift L`.
Add and link to a spot in the *next* frame.	Put the mouse **inside** a source spot, and press and hold `A`. The viewer moves to the next time-point, creates a spot there and links it to the source spot While holding `A`, move the new spot to the desired location, and release `A`.
Add and link to a spot in the *previous* frame.	Same procedure, but press `Shift A`.
Toggle the auto-linking mode.	`Control L`
Removing links.	
Delete a link.	Put the mouse over the link to delete and press `D`.
Selection editing.	
Add a spot / link to the selection.	`Shift click` on a spot or a link to add / remove it to / from the selection.
Clearing the selection.	Click on an empty place of the image.
Remove selection content.	`Shift delete`
Undo / Redo.	
Undo / Redo	`Control Z` / `Control Shift Z`

Mastodon Documentation and Tutorials

https://mastodon.readthedocs.io/

Lean on the imageJ community

https://forum.image.sc/

And the Duke QLS community

Thanks!