MesoSPIM Stitching using BigStitcher

Guide for stitching mesoSPIM data.

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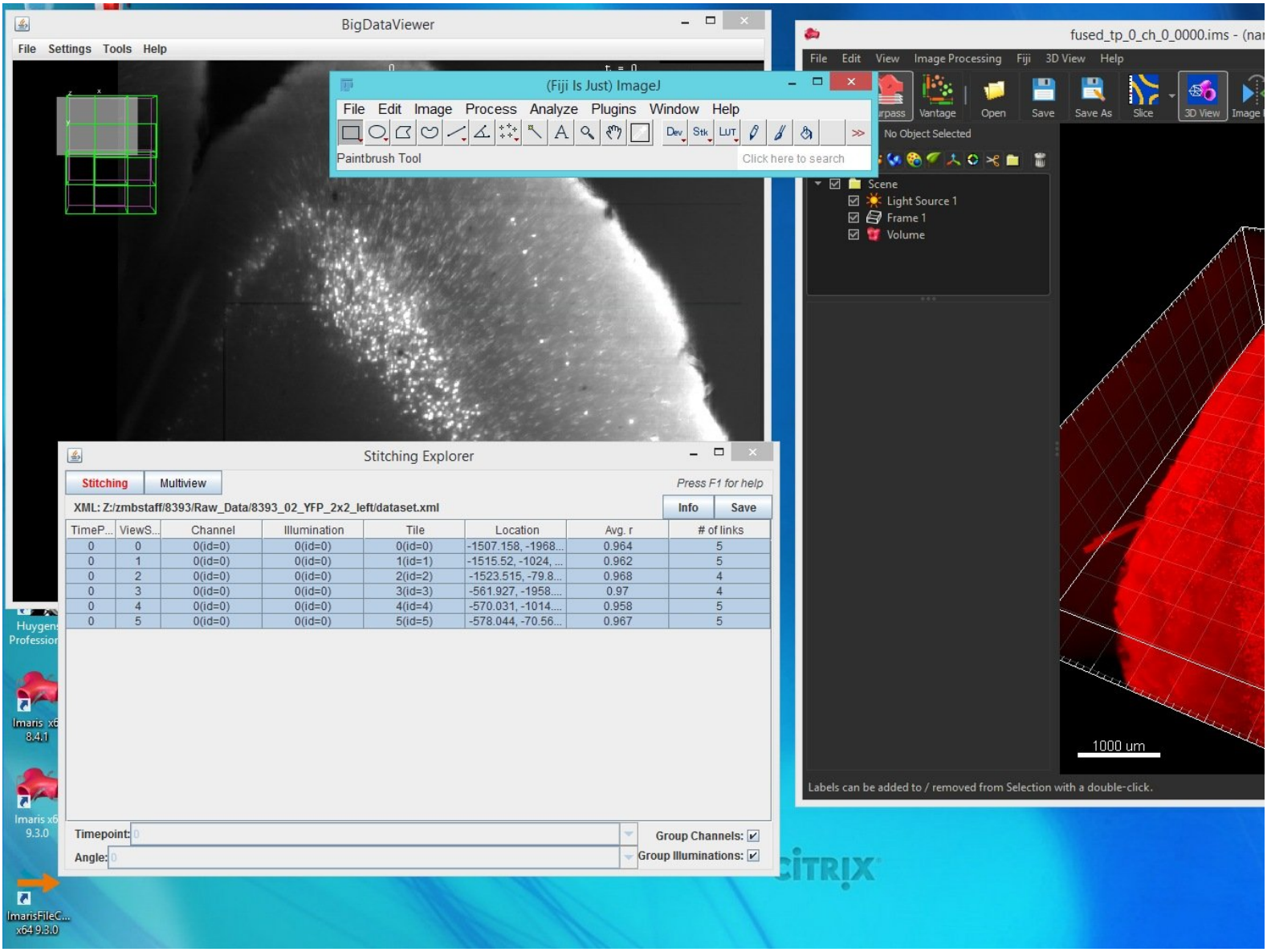

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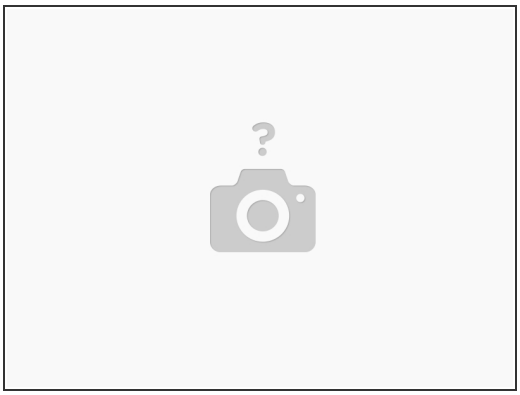
INTRODUCTION

This guide explains how to stitch tiled datasets from the ZMB mesoSPIM using the BigStitcher Fiji plugin. The only thing needed is a lot of computer RAM and depending on the amount of data, 1-2 days of time. This guide only covers stitching and not multiview registration.

Most steps in this guide also apply to other regular volumetric datasets such as from confocal microscopes.

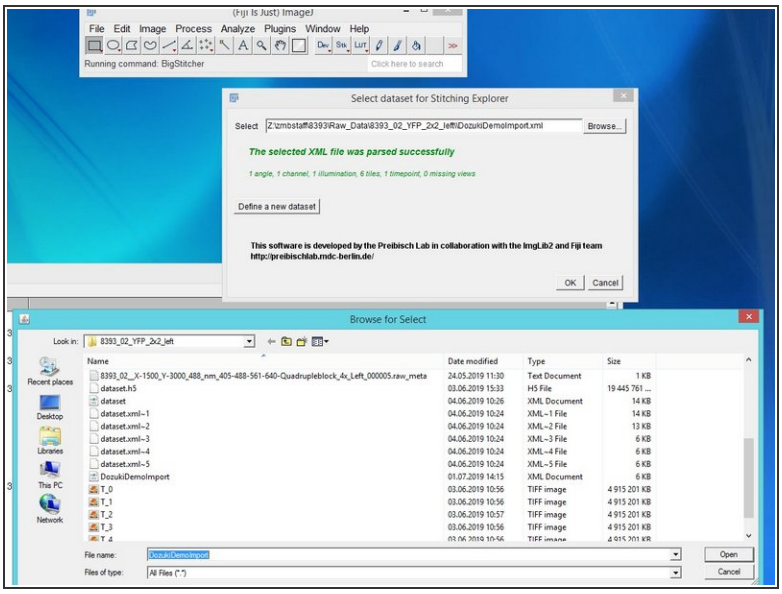
Step 1 — Workflow information

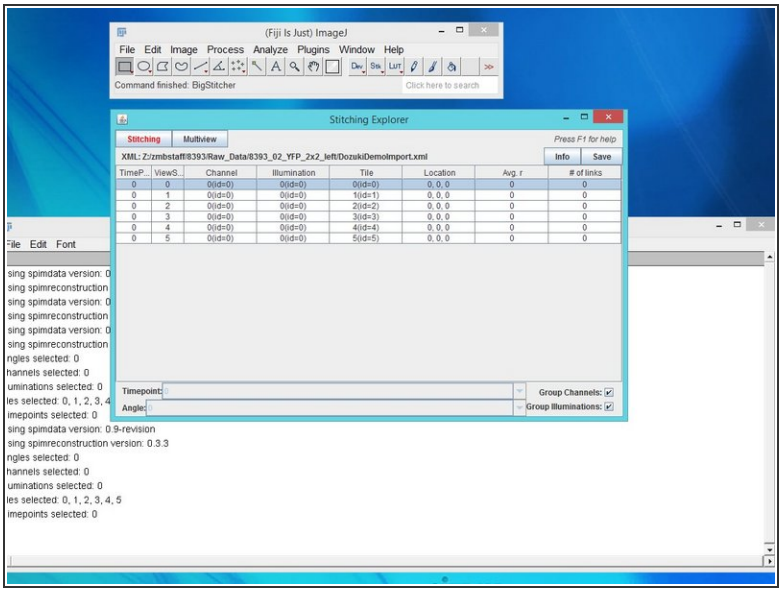


- Useful information concerning this workflow can be found on the BigStitcher webpage!
- This guide covers the multi-tile stitching workflow of BigStitcher without Multiview registration (different angles).

⚠ Be aware that there are two plugins from Preibish "BigStitcher" and "Multiview Reconstruction". Since they also work together, that can be a bit confusing. Here we work with the "BigStitcher" in the "Stitching" mode.

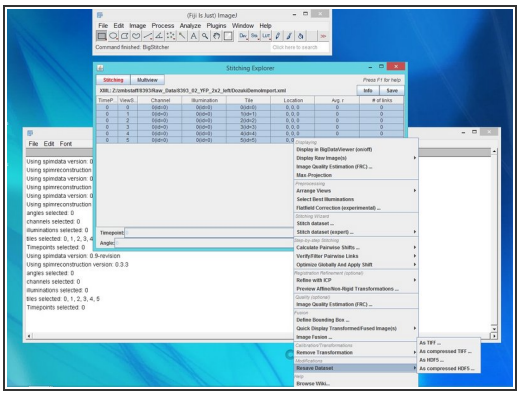
Step 2 — Open .xml and stitching explorer

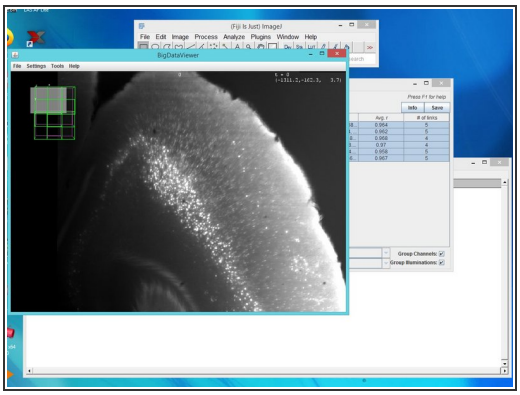


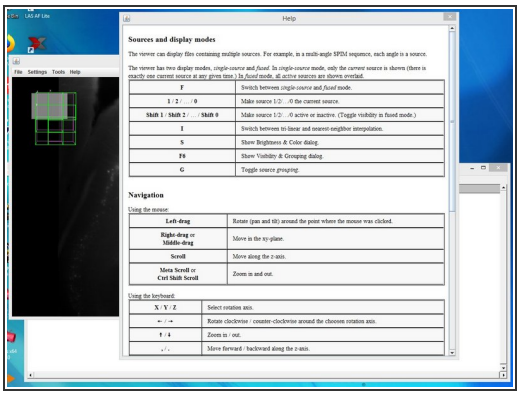


- Open FiJI > Plugins > BigStitcher > BigStitcher
- Drag and drop the .xml file from your MesoSPIM data. A "BigDataViewer" window will appear with your data.

Step 3 — Big data viewer

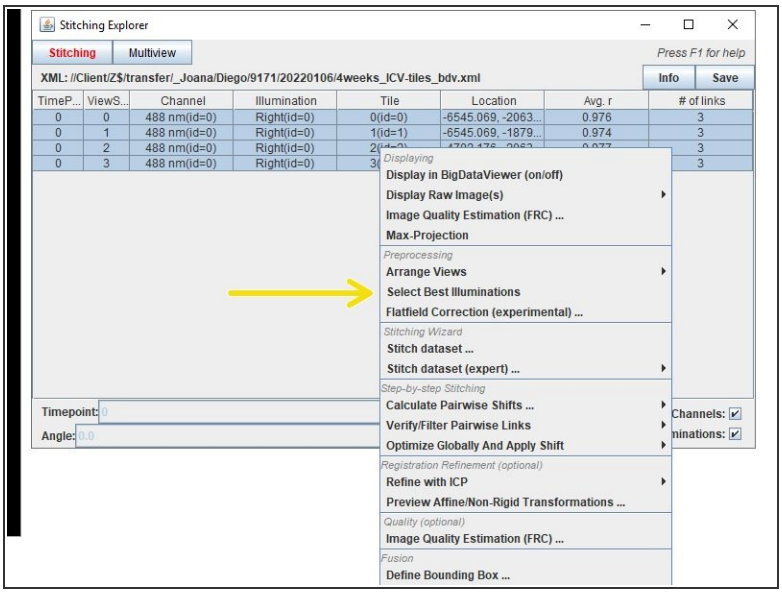


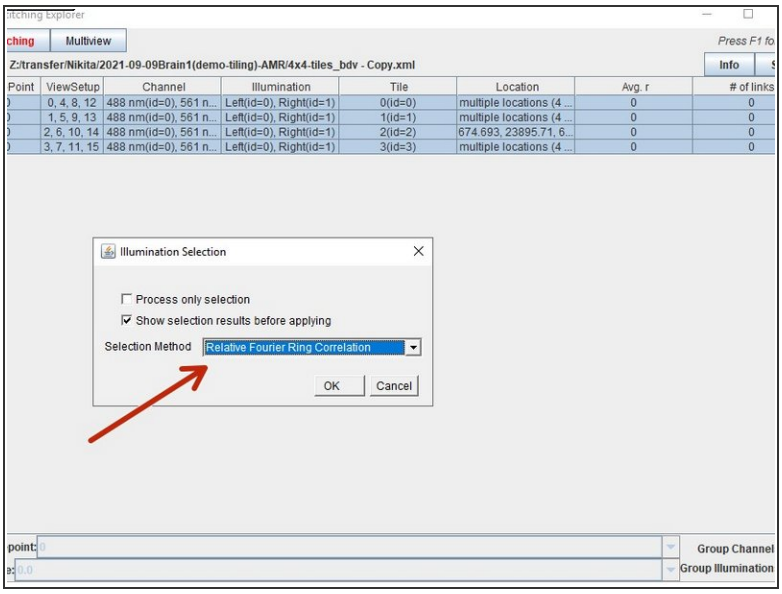




- For visualization, BigStitcher uses the BigData Viewer. Select all views using shift, right click and "Display in BigData Viewer (on/off)" for visualization of your views and later for the stitching progress.
- For learning about how to use the BigData viewer press help in the BigData viewer window.
- If you get lost in the BigData viewer 3D space, just close the window and open it again by the above strategy.

Step 4 — Select best illumination

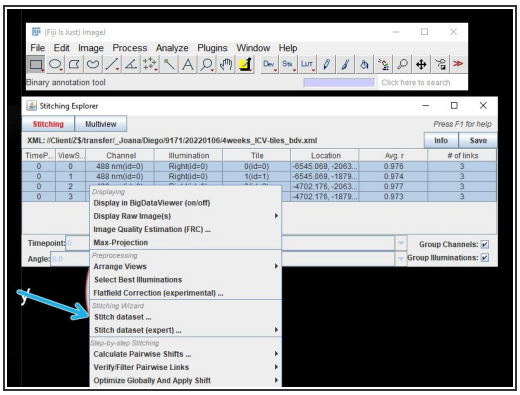


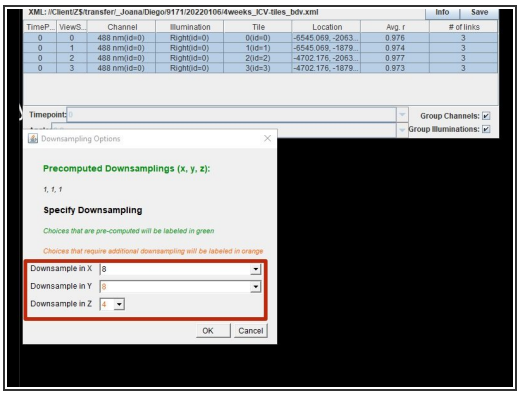


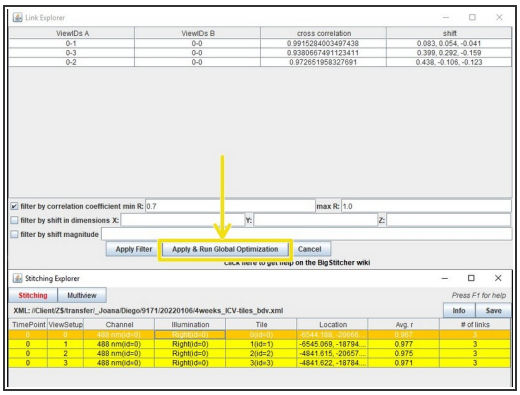
⚠ The BigStitcher workflow does not merge/average/register multiple illuminations from single tiles (e.g. left/right).

- Press right click over your selected data and choose "select best illuminations".
- Then select the "Relative Fourier Ring Correlation method". All three methods provide similar results, with gradient magnitude and FRC providing more differentiation power in close cases.
- When views have more than one illumination, you can automatically select the best for stitching by right click, Preprocessing/Select Best Illuminations.

Step 5 — Stitching

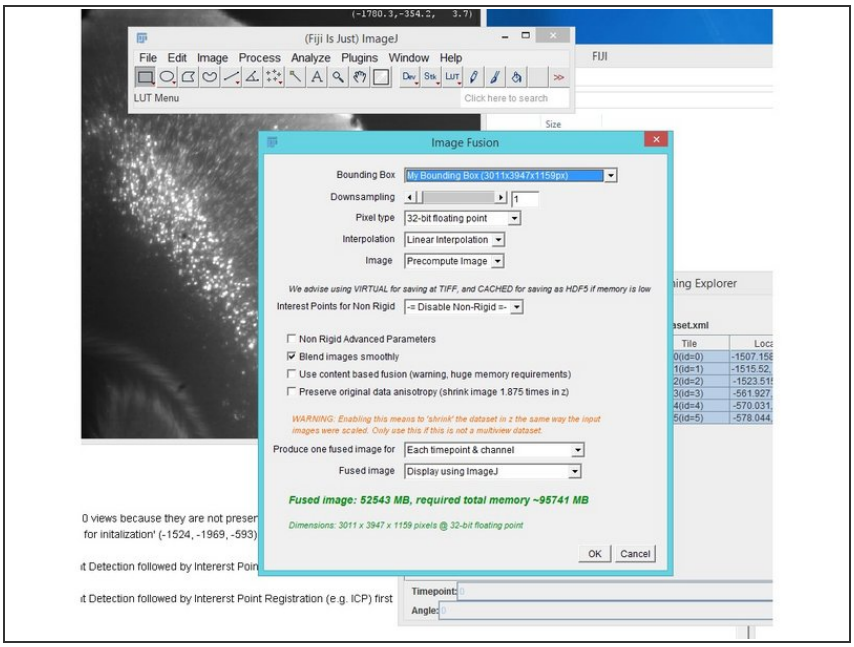






- To stitch your data together, right click on the data you want to stitch and select "stitch dataset".
- Down sample your data, this will be faster and have better quality than running it at full resolution.
- After the the data is done stitching a window will open saying "Pairwise shift calculation done" press yes.
- Another window opens, this time press "Apply & Run global optimization"

Step 6 — Image fusion



- The process of creating single image stacks from multiple transformed/aligned input images is called Image Fusion.
- Select your data, right click Image Fusion.

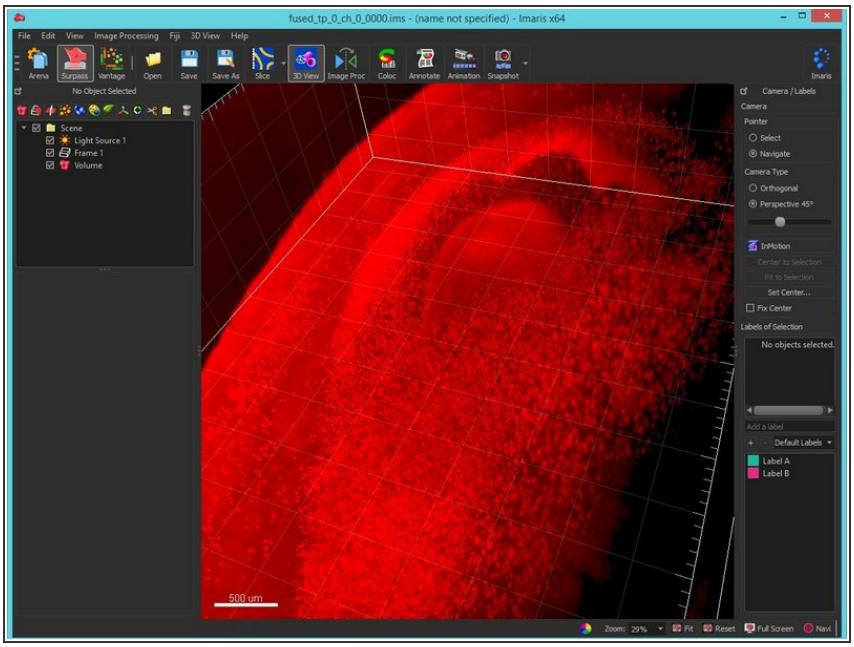
ⓘ Selecting 16-bit output will round the output down to the next whole number (integer) and reduce the files size (and memory requuirenment) by a factor of two.

- In our tests we used "Linear Interpolation", "Precompute Image", "Blend images smoothly"

⚠ Fusion and export also will take a considerable amount of time.

⚠ For fusion roughly twice the memory than the resulting image size is needed. The amount needed can be seen in the "Image Fusion" window.

Step 7 — Saving and cleanup



- In Fiji save your resulting stack as .tif image sequence Save As/Image Sequence.
- Again be sure to save your .xml file.
- Delete all files not necessary (e.g. tiffs).
- By opening the .xml file again all steps of the workflow can be accessed again and rerun.
- Import and visualize the results in a 3D visualization application such as Imaris.