Supplementary information

Whole-mouse clearing and imaging at the cellular level with vDISCO

In the format provided by the authors and unedited



TileConfiguration_{zzz}

e

TileConfiguration_{zzz}.txt.registered

3/27/2021 4:25 PM 3/27/2021 4:25 PM Text Document REGISTERED File

Rename .registered file ending as "{zzz}.new.txt". Use this file, which includes stitching parameters



35_Blaze[00 x 00] C00 xyz-Table Z1973.tif; ; (0.0, 0.0) 35_Blaze[00 x 01] C00 xyz-Table Z1973.tif; ; (1360.4033, 10.487305) 35_Blaze[00 x 02] C00 xyz-Table Z1973.tif; ; (2703.9636, 17.393616) 35_Blaze[01 x 00] C00 xyz-Table Z1973.tif; ; (20.529175, 1308.2139) 35_Blaze[01 x 01] C00 xyz-Table Z1973.tif; ; (1323.0183, 1383.4768) 35_Blaze[01 x 02] C00 xyz-Table Z1973.tif; ; (2651.8105, 1380.3085) 35_Blaze[02 x 00] C00 xyz-Table Z1973.tif; ; (-0.8508301, 2679.6377) 35_Blaze[02 x 01] C00 xyz-Table Z1973.tif; ; (1320.1492, 2656.6377) 35_Blaze[02 x 02] C00 xyz-Table Z1973.tif; ; (2649.397, 2588.2144)

Channel info Section info



This Plugin is maintained by Ignacio Arganda-Carreras http://fiji.sc/Stitching_2D/3D		Run Batch Kill persistent Started Stitching-old-just_txtFile.py at S OK Cancel
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Supplementary Figure 1 2D Fiji stitching

(a) Open FIJI, go to Plugins/Stitching/deprecated/Stitch sequence of Grids of Images. (b) Fill in the prompter as shown and choose an optical section deep in the tissue for "start z". (c) After clicking OK, stitching parameters will be calculated and resulting files will be saved in the input directory. Rename ".registered" file ending as "{zzz}.new.txt". (d) Open this file and change the section number in each line to 0000. Save the changes and copy this file under a new file for the current channel. Create new folders for each channel and place this file into each folder with the changed channel information. (e) Load the custom macro. Click on "Run and open the txt file contacting stitching parameters. Enter the number of sections in the dataset and click "OK" to start running the macro.

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🐓 Import files

Assume same structure for all files?

Analyzing 2997 files can take a long time. You can speed up the process if you know that all files have the same structure.

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Supplementary Figure 2

Arivis conversion and stitching

(a) Rename the FIJI-stitched images (e.g. the ventral side of a whole body) with an orthodox file manager software with a multi-rename tool such as "TotalCommander". The renamed file should include the channel number and the z section number. Use the "Counter" function, set "Start at" as 0 and "Digits" as 4 as shown in red. Put all the renamed files into one

folder and drag this folder into Arivis4D. (b) Click "Assume same structure for all files". This will open another window. Choose "Custom import", "New File" and click on more options. Choose Target Pixel Size to be 16-Bit integer and click "Ok". This will open another window. Click on "Selection" and "Pattern matching". Check that files will be loaded by the correct channel and section numbers. Click "Ok" to start the simultaneous conversion and import session. When the first import is finished, drag the other scan (e.g. the other side of the whole body) that needs to be fused on Arivis and follow the same steps of conversion except for one important change: choose "New Image Set", instead of "New File". (c) When both sides are imported choose double sided view and put the different sides to each window. Using the navigation option find the same structure in both images. Choose "Place object" button, then "Marker" option, and check these landmarks through "Objects table". Rename each landmark by right click "Rename Objects", corresponding to the landmark number. Find at least 3 landmarks.

4 dorsal-naive-86.sis - 2 Viewers - arivis Vision4D 3.3.0







Supplementary Figure 3

Arivis fusion

(a) After three landmarks are set in each side, click on "Data" and then "Volume Fusion". This will open a new window. Choose the base image set and the moving image set and click on New Image Set. Change the "Scale" to 10% to initially test how well the landmarks work. Click on Transformation tab to choose the landmarks for each side. Click on the wheel button at the right bottom of each image and choose "Add All Objects as Landmark". Naming of these will help you to match landmarks from each image. Click on Run to obtain a low resolution version of your fusion. (b) After 10% version is ready, judge the quality of the landmarks and if sufficient start the 100% scale fusion changing the Save as option to "New File". You can view the 3D version of your resulting fusion with the cube shaped button at the bottom left (marked with red box). After the fusion is finished, click on the " Save" button and export the files as a series of images using "Tiff Exporter" under File/Export. The exported files are ready for further processing.