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Stitching Protocol

This document describes the procedure of stitching a set of data acquired by the Tissuecyte.

Operation

1. Be sure to place the data in folders that do not contain any spaces in the name (underscore and dash is ok). If any of the folder names contains spaces, the Fiji code will crash.
2. Navigate to the TV stitch directory and double click Run-stitch batch file. This will start your MATLAB program and begin the process.
    a. If a shortcut of Run-Stitch was made on desktop, double click that instead.
3. The Script will first query if you would like to save the output data (to be processed by Fiji) in a different location. The script will create a modified copy of the dataset that Fiji can stitch, effectively doubling the hard drive space required to contain all the images. Therefore saving the output in a different location is useful when the hard drive is not large enough to contain both the original data and the modified for stitching data.
    a. NOTE: It is highly recommended that the original data is saved until the stitching is verified to be successful.
4. Select the Mosaic file (the main one that contains a variable name) in your main directory
    a. The mosaic file is located in the directory that contains all the section folders, that is the numbered "sample_name-000X" folders.
    b. An example mosaic file: "Mosaic_ff0e63a7-e426-4bdf-8cc8-b13af911c4c4.txt" or "Mosaic_test_sample.txt." The long string is generated by Orchestrator if no sample name is entered into the protocol
5. Provide the required values
    a. Starting section and ending section are the parameters for determining which sections of the data set you would like to prepare for Fiji stitching. The code will create folders with the start/end numbers in their titles, be sure to input the same start and end into Fiji as you did here or it will not be able to access the images.
    b. First/last section for image average generation will determine which part of the data will be averaged to generate field correction. For best possible correction do the entire data set, for faster time do part of the data set.
        i. Typically, 10-15% of the data set is sufficient, but if the data set is very small you may need to use more
    c. Number of channels varies 1 to 3, choose the one you want. As before, be sure to stay consistent in Fiji selections, or the code will crash attempting to access non-existing directories.

   i. Channel selection is Channel 1, channels 1-2 or channels 1-3. If you enter 3 in this field, the code will do all 3 channels, if you enter 2 it will only do channels 1 and 2 etc.

6. The script will now go through all the images and save the new modified images in your main directory, or the directory of your choice if one was selected in step 4, inside folder named "(scan ID number)-Mosaic." Three folders are created per channel.
   a. "Tile_sections" contains all the modified images for Fiji, "Stitched_sections" will contain the stitched images after Fiji is finished.
   b. This will require space on the HDD equivalent to original dataset.
7. Close MATLAB.
8. Open Fiji by clicking the shortcut on the desktop
9. In Fiji, click "Plugins->Tissue vision stitching"
   a. **Alternative:** If only one channel needs to be stitched, click "Plugins->Tissue vision stitching chan." Follow all steps as described below, but note that selecting the channel from the drop window in step 11 will now stitch only that channel.
10. Enter the values as prompted.
   a. Stay consistent with what you originally entered in MATLAB. You can check the folders in /main/(scan ID number)-Mosaic/, the "starting" and "ending" section numbers are at the end of each folder. Example: "/main/(scan ID number)-Mosaic/Ch1_Tile_Sections_1_to_145"
11. Select the image containing folder
   a. You need to find your main directory and then select the "(scan ID number)-Mosaic" folder.
12. Fiji will now execute stitching and contrast adjustment. This will take several hours depending on the size of the dataset.
   a. All actions performed are noted in the Log
13. When stitching is finished, a message box will pop-up indicating so.
   a. Each channel set will generate the message box, to continue to the next channel close the box and the code will proceed. Example: 3 channels of data, Fiji stitches the 1st channel fully, then generates a message box "Stitching finished!" Click ok and Fiji will proceed to contrast adjust those images and stitch the 2nd channel. Repeat after 2nd channel is done.
14. When finished, Fiji will prompt so in the Log: "Operation complete."
15. **Data Cleanup**
   a. At this point you can delete the folders named "Tile_sections," they contain the MATLAB modified data and are equivalent to the original data set in size.
   b. The "stitched sections" folders contain the large, fully stitched images. If you navigate to this folder, you will see that each section has 3 files associated with it. For example, Section 1 of channel 1 will have a

"JPEG_stitched_image_ch1_001.jpg," a "stitched_image_001.tif," and a "stitched image_ch1_001.tif."

c. You should open the JPEG images and stitched image_ch# images to verify that they contain the sections as intended
    i. You can use FIJI to open large sets of these images by clicking "File -> Import -> Image Sequence… , selecting one of the images in the folder containing all of them, and filling out the dialog boxes. You can filter to only open JPEGS or tifs using the "file name contains" box. Note that the tifs are large images, and you will probably not be able to open all of them at once, but will have to import them a set of 5-10 at a time depending on your RAM. You should, however, be able to open all the JPEGS at once.
d. After you have verified that all the JPEGs and Tifs contain your data, you can delete the set of "stitched image_00#.tif" from the folder. Those images should be small blank (all 0) images, and they are a remnant of image conversion done by Fiji.
e. It is highly recommended that you hold on to the original data that was used by MATLAB to begin the process. You can compress it and store it until you are certain that you no longer need it. Occasionally the stitching code will have a bug and create a nonsensical stitched image, which can be restitched and thus fixed using the original data.

Appendix 1: Installation

1. **MATLAB**
    a. Extract TV Stitch zip file to any desired location
    b. Navigate to the extracted files and locate "Run-stitch.bat"
    c. Right-click "Run-stitch.bat", select "send to" and click "Desktop(create shortcut)"
    d. Use the "Shortcut to Run-stitch.bat" link on your desktop to start the MATLAB portion
2. **FIJI (ImageJ)**
    a. Fiji can be downloaded for free from http://fiji.sc/Fiji
    b. Copy the files "Tissue_vision_stitching.ijm", "TV_jpeg_conv.ijm" and "Tissue_vision_stitching_chan.ijm" from the "TV Stitch\ImageJ-Plugins" directory into C:\Program Files (x86)\Fiji.app\plugins folder (or the directory Fiji.app folder was installed in).