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2D EM montage tile stitching

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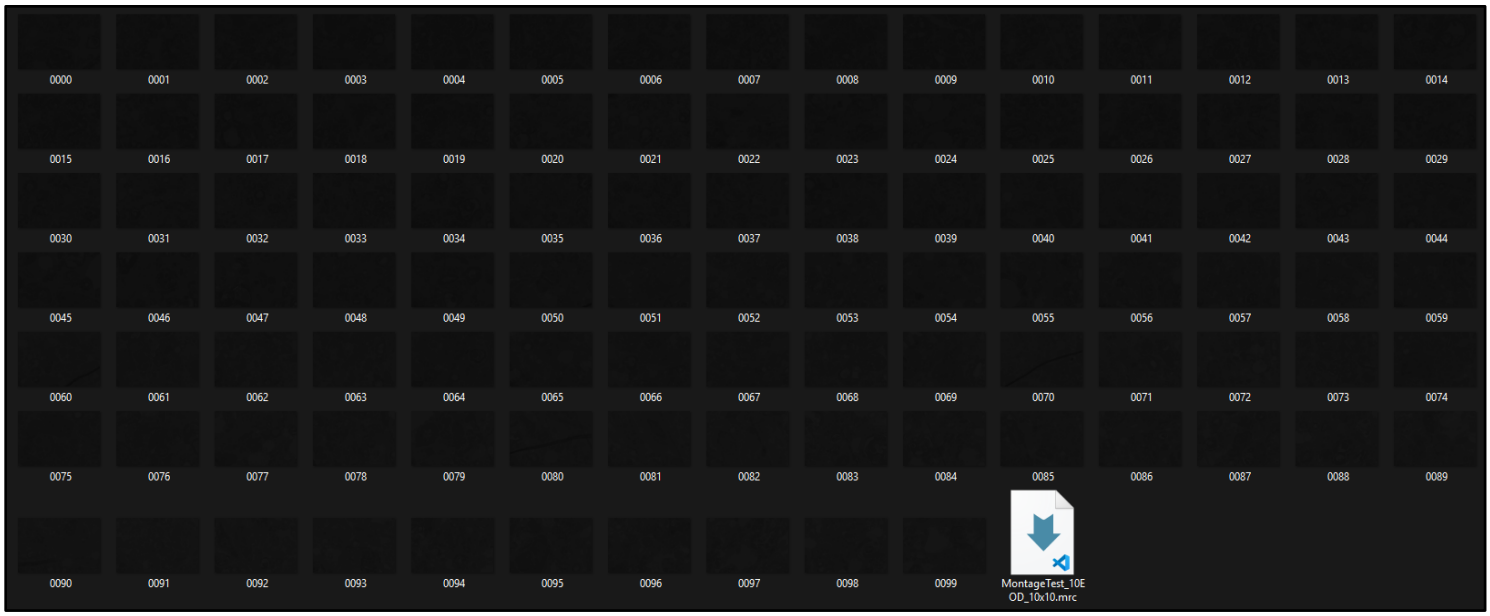
Overview

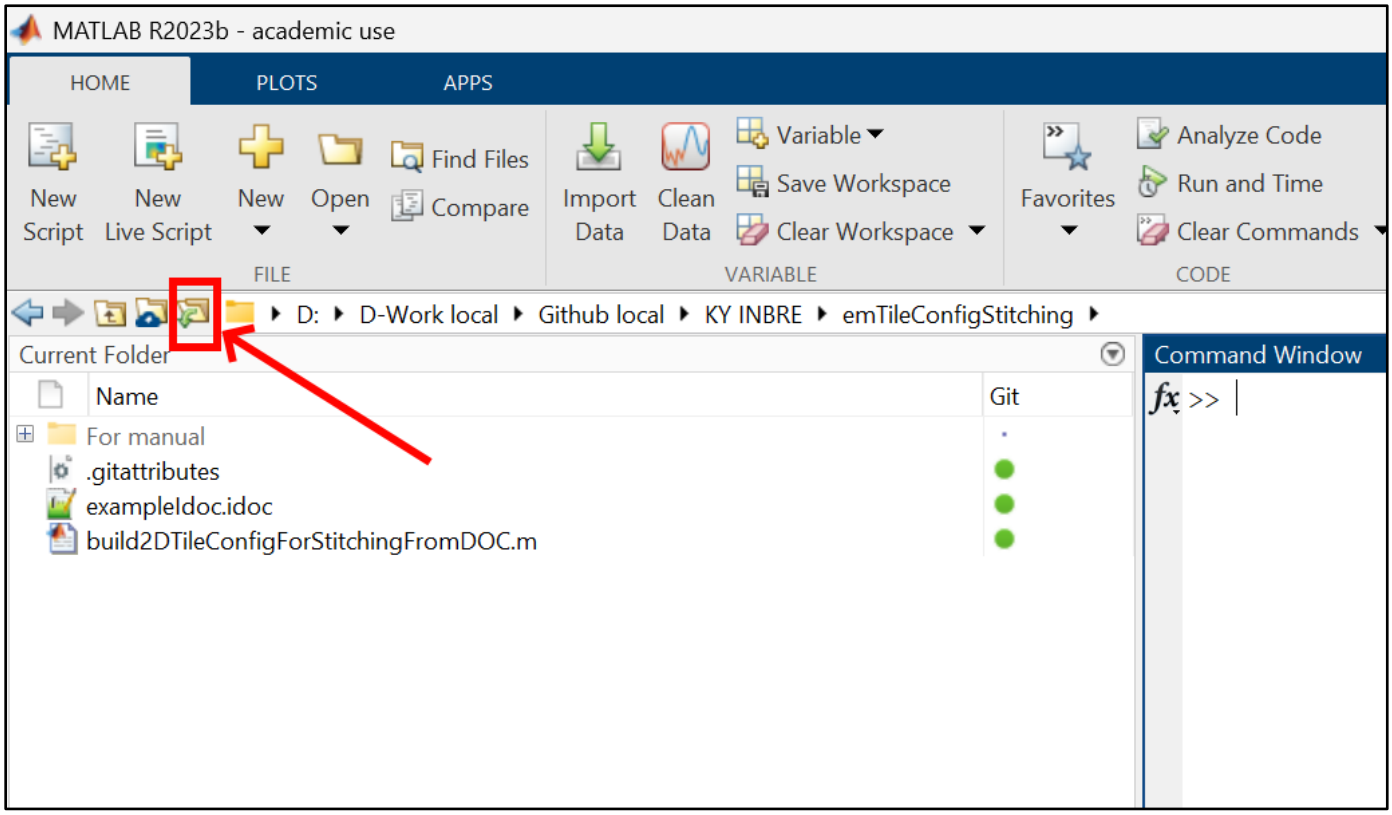
This document plus the code it comes with is designed to stitch 2D EM images taken as individual .tif tiles. The code takes a folder of tifs plus one .idoc/.mdoc file and builds a custom tile configuration text file for use with the FIJI grid stitching plugin (Preibisch, S., Saalfeld, S., & Tomancak, P. (2009). Globally optimal stitching of tiled 3D microscopic image acquisitions. Bioinformatics, 25(11), 1463–1465). The final output is a stitched image that can be opened with FIJI or further analyzed/processed as a single image.

Creating the tile configuration file

1. First you will need to put your tif files and the .mdoc/.idoc file into the same folder:
   a) Make sure the files are named sequentially as shown (same order as what the.mdoc/.idoc has).
   b) You can view the contents of the doc file using the program notepad++.

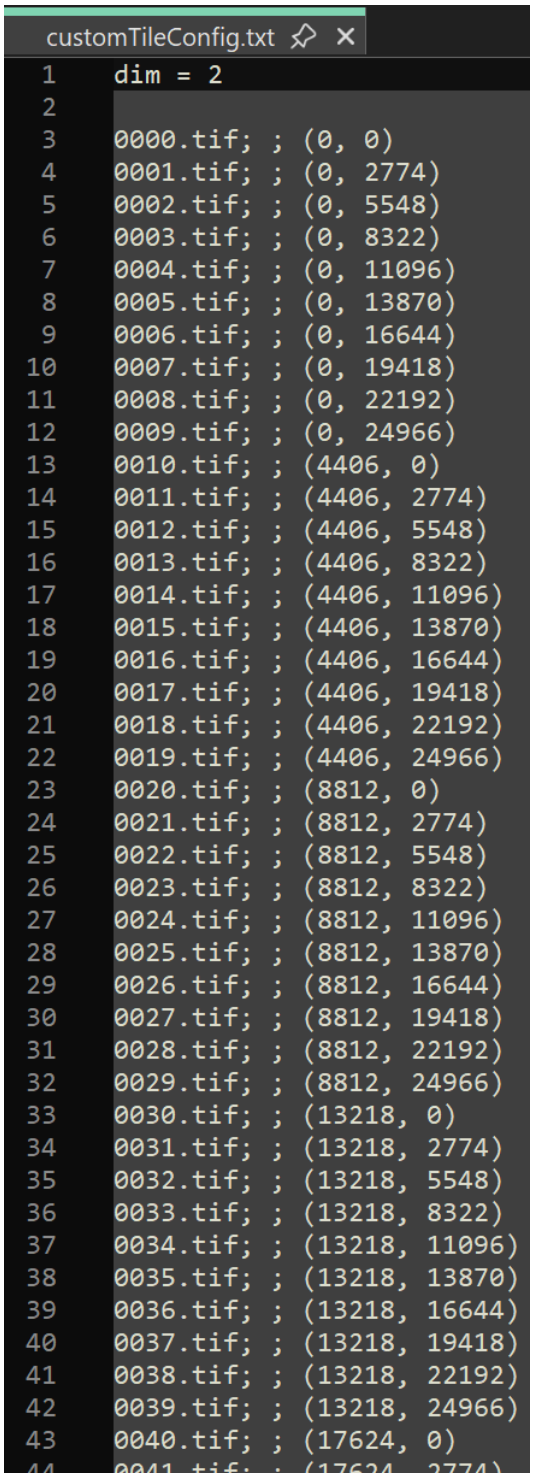


2. Open MATLAB and switch your current folder to the one with the tile configuration script (build2DTileConfigForStitchingFromDOC.m):



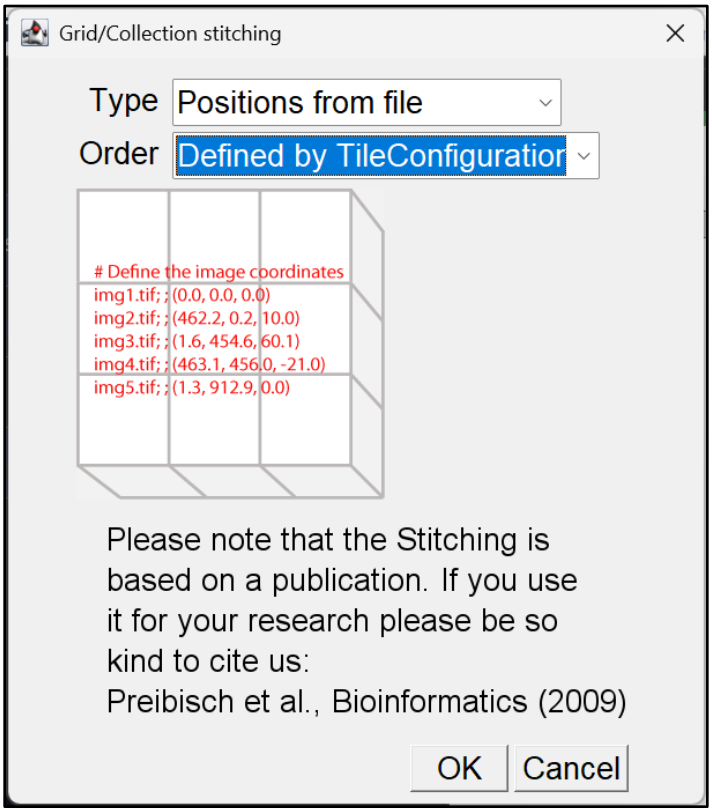
3. Double left click build2DTileConfigForStitchingFromDOC.m to open it, then using the “Editor” tab at the top click the “Run” button. In the window that opens, select the folder you set up in step one.
4. Information about the order/stitching used will be printed in the command window. Double check this looks correct before continuing.

5. If successful a text file called “customTileConfig.txt” will be created in the selected folder, you can open this in notepad++ to confirm it looks correct:

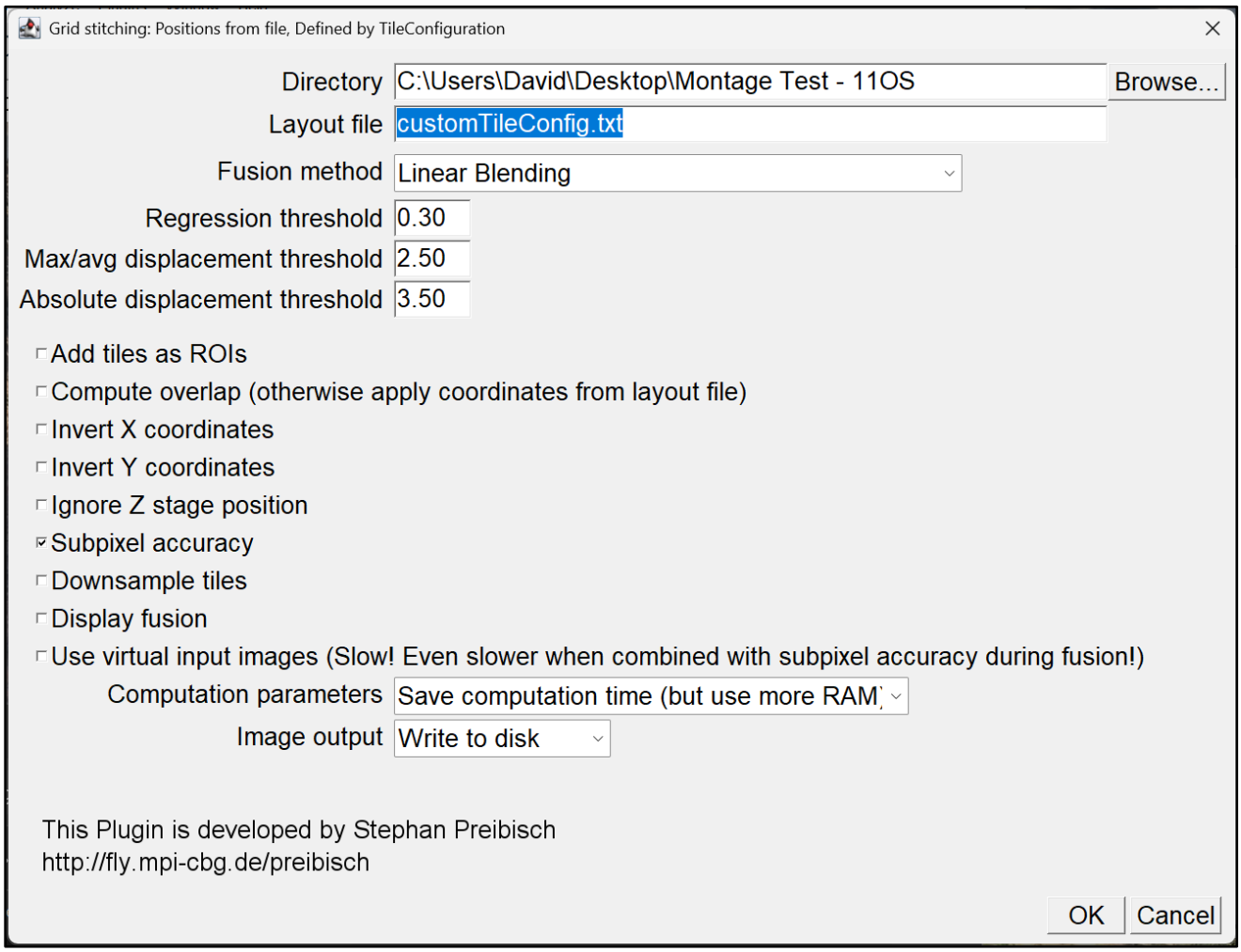


Running the stitching plugin

1. If FIJI is not installed, install it from here (pick the JDK version for your computer): https://imagej.net/software/fiji/downloads
2. Make sure FIJI is using as much RAM as available by going to Edit -> Options -> Memory & Threads.
    a. This is usually set automatically, but you can safely set this as high as 80% of your available RAM.
3. Go to Plugins -> Stitching -> Grid/Collection stitching. In the first window, change the settings to use a custom tile config as shown below:

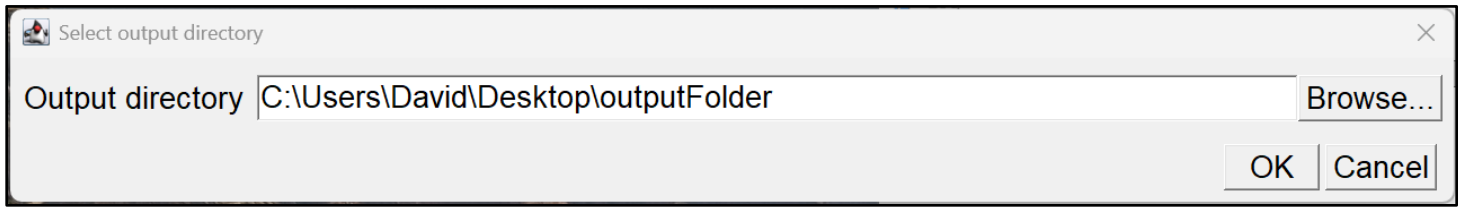


4. In the second window, use the settings shown in this image. Be sure to change the “Directory” to the folder that has your tifs + the customTileConfig.txt:



   a. Note that this process will use a significant amount of RAM. Calculate how large the final stitched image will be in gigabytes, then make sure you have at least twice this amount of RAM.

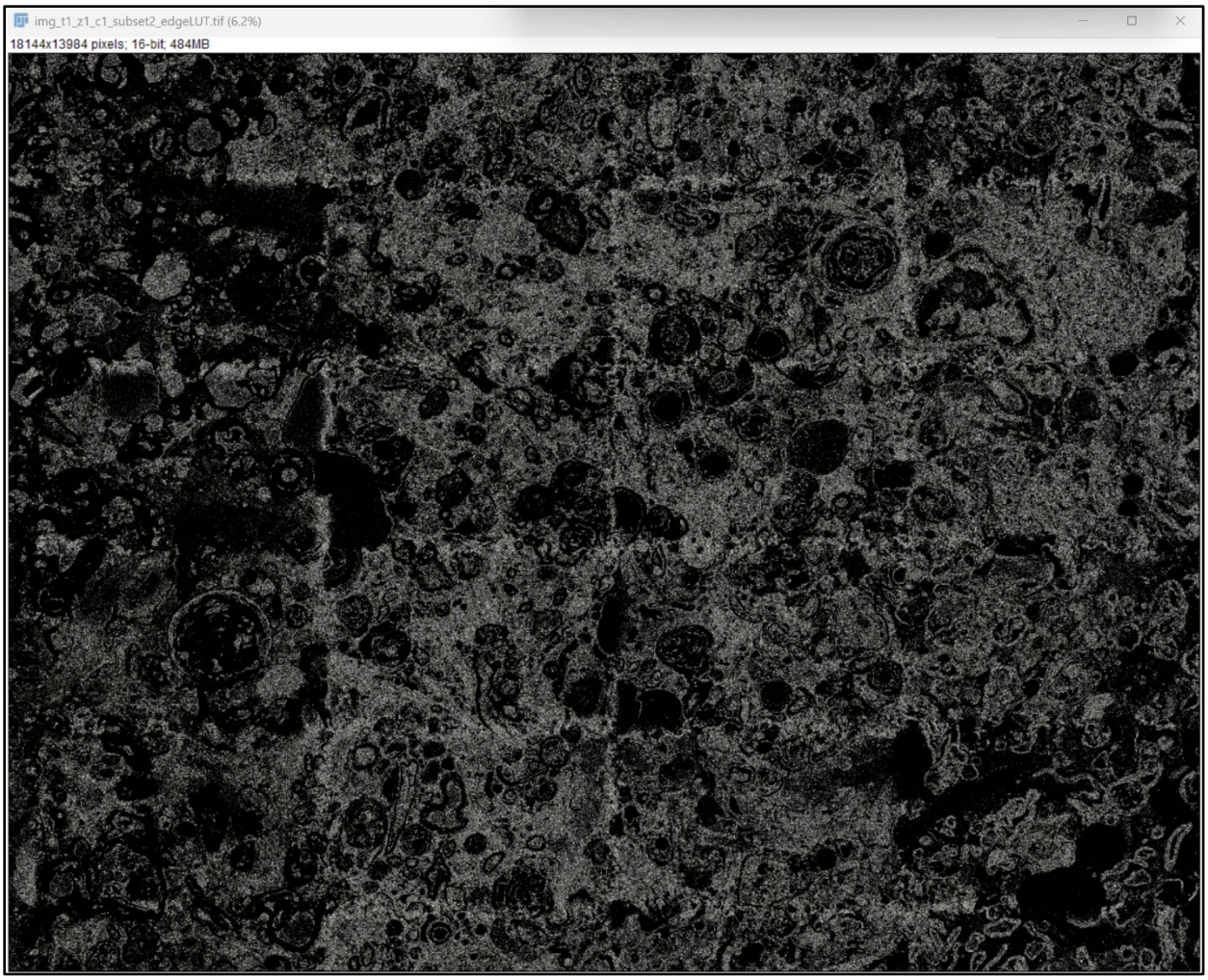
5. In the final window, select the folder where you want to write the output stitched image file:



6. Once you hit OK above, stitching will start. View the “Log” window to see the progress.
    a. It is a good idea to close all other programs (MATLAB etc.) before clicking OK to maximize the amount of available RAM.
7. Once finished, a file called “img_t1_z1_c1” will be created in the selected output directory. This is your stitched file, drag this onto FIJI to open it.

Notes and tips

Overlap artifacts



During EM image collection there is an option to pick percent overlap (~10% for our images by default). Because of this overlap and the way that the tiles are blended (Linear Blending), you will end up with minor grid like artifacts where this overlap occurs. Shown above using the edge LUT in FIJI. The best way to minimize this is to minimize the percentage overlap. You can also try other blending techniques other than the default linear blending.

Bleach correction via histogram matching

Often there will be some brightness drift across your final image. One way to correct this is to use histogram matching to correct all the tiles before running stitching:

- Put only your tif files into a folder, then use FIJI -> File -> Import -> Image sequence. Pick the folder itself.
    - This loads the images as a stack, which is needed for histogram matching in FIJI.

- In FIJI go to Image -> Adjust -> Bleach correction. Use “Histogram Matching” as the correction method.
- Once finished, a new window will open with “DUP_” appended to the window name. Close the original window so that only the “DUP_” window is open.
- In FIJI, go to File -> Save As -> Image Sequence.
    - Change “Dir” to be where you want to save the bleach corrected tiles.
    - Use “TIFF” as the format.
    - Delete everything in the “Name” field so that it is empty.
    - Start at 0, use 4 Digits.
    - Don’t check the box for “Use slice labels…”.
- If you have already created a tile configuration text file, copy that into this folder containing the bleach corrected tifs. Otherwise, go through the steps to create this with the MATLAB script.
- Run through the stitching process as described earlier, using this new folder of bleach corrected tiles as the input.