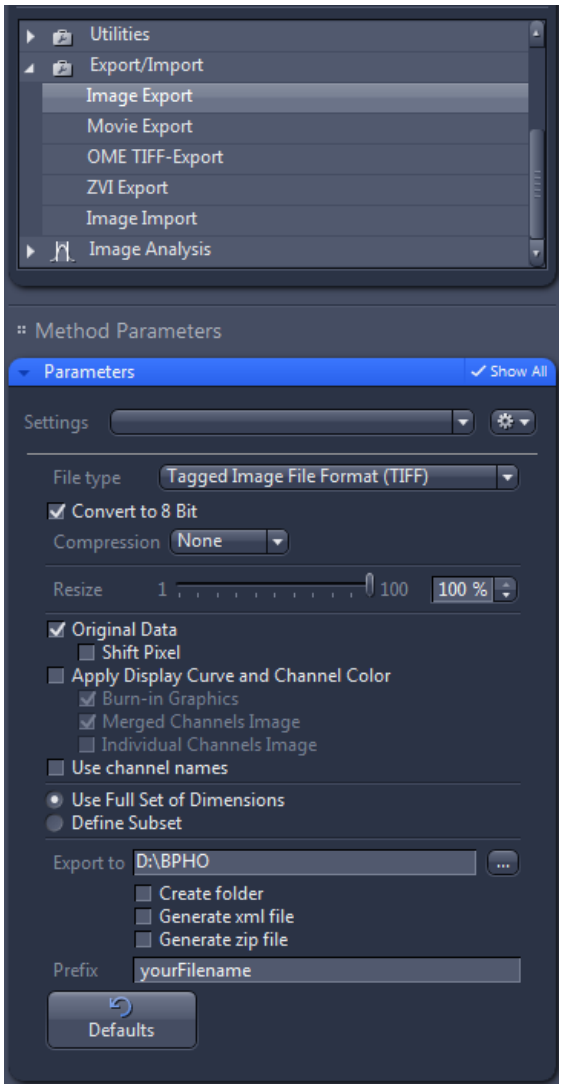
HOW TO STITCH LIGHTSHEET IMAGES

1. Export your file
2. Convert the files into the correct folder hierarchy and names
3. Stitch

This guide is written by Pufai Santisakultarm (puifais@salk.edu) on 3/27/2017. Our Biophotonics Core has Zeiss Lightsheet Z.1. This is how you use TeraStitcher to stitch your data together using open source software.

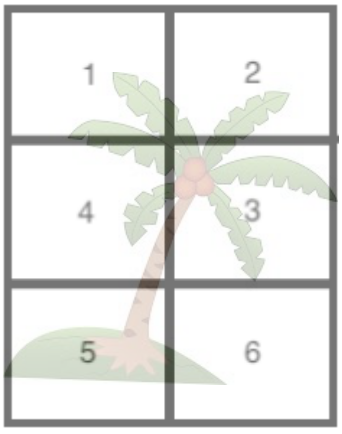
EXPORT YOUR FILE



Open the file in ZEN blue 2.3 (paid or lite versions). Export it to tif using the settings shown here.

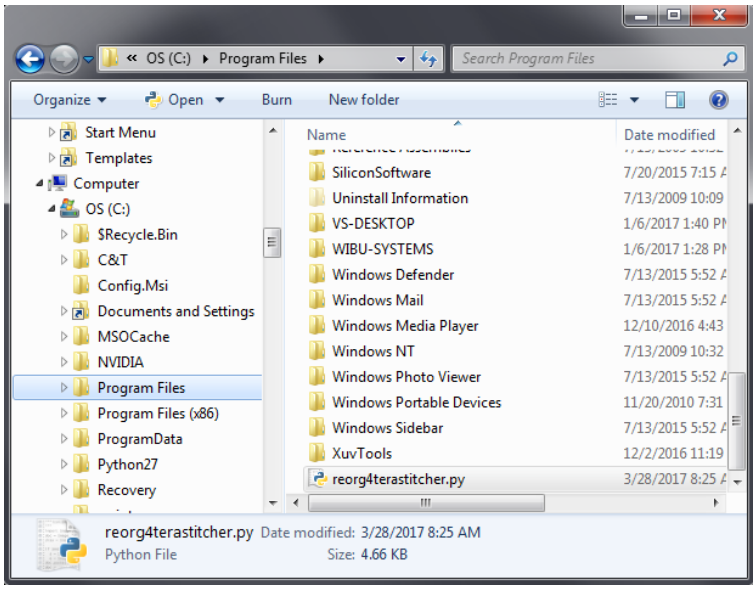
This will create 8-bit gray scale tif images named filename_v01z001_ORG.tif where the numbers after 'v' and 'z' increase up to the max number of views and z-planes.

You should always do 'bidirectional' scan when acquiring lightsheet images since this is the most efficient way. In the next step, we'll assume that you have done bidirectional scanning (i.e. backward 's' pattern of tiles)

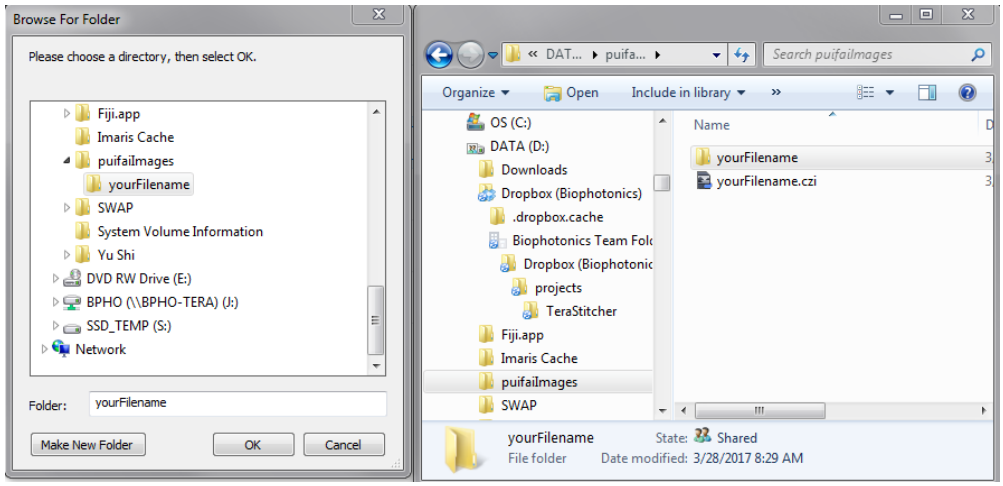


CONVERT THE FILES INTO THE CORRECT FOLDER HIERARCHY AND NAMES

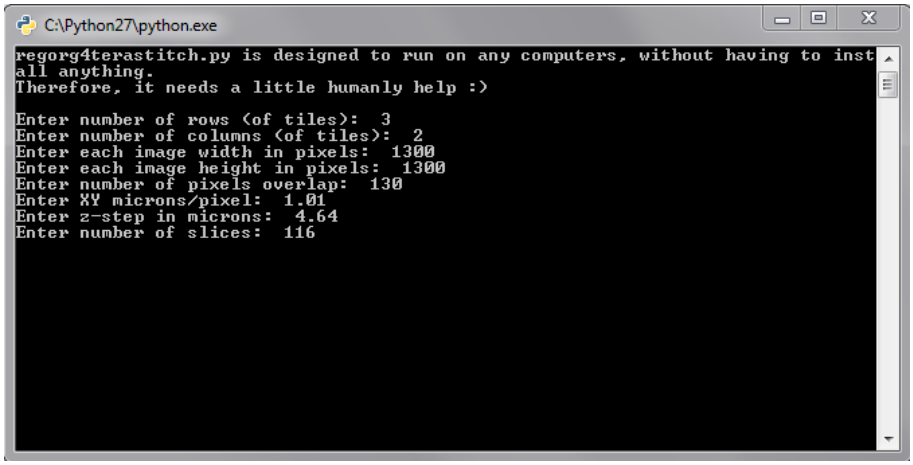
We will use TeraStitcher to stitch your data together. This is an open source software <http://abria.github.io/TeraStitcher/>. It requires a particular folder structure detailed on the website. I wrote a little script < https://github.com/puifais/biomedical_python/blob/master/reorg4terastitcher.py> to organize and rename the files as required by TeraStitcher.



Double click on reorg4terastitcher.py



Select the folder that contains the exported image files

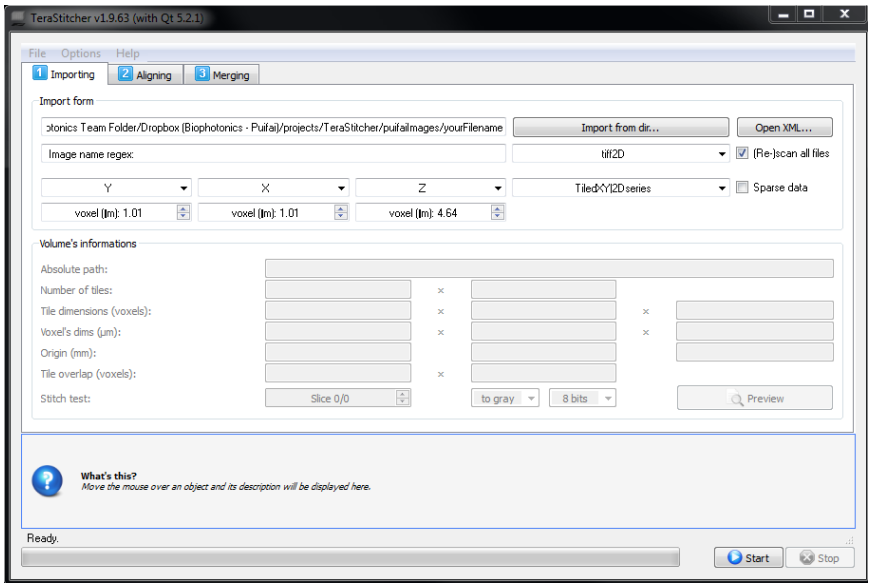


Next, a command line window will pop-up and ask about the images and tiles.

"All done" will appear on terminal and the window will close itself.

You should see a folder called yourFilename_Terastitcher appears.

STITCH



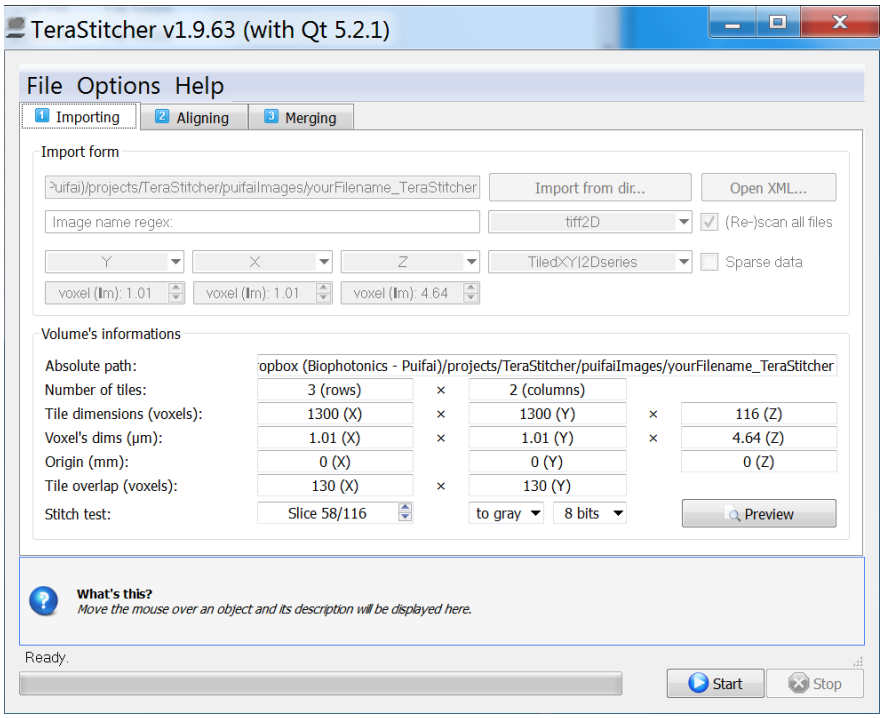
Double click on TeraStitcher icon.

Click Import from dir… and navigate to your yourFilename_TeraStitcher folder

Select tiff2D and TiledXY|2D series

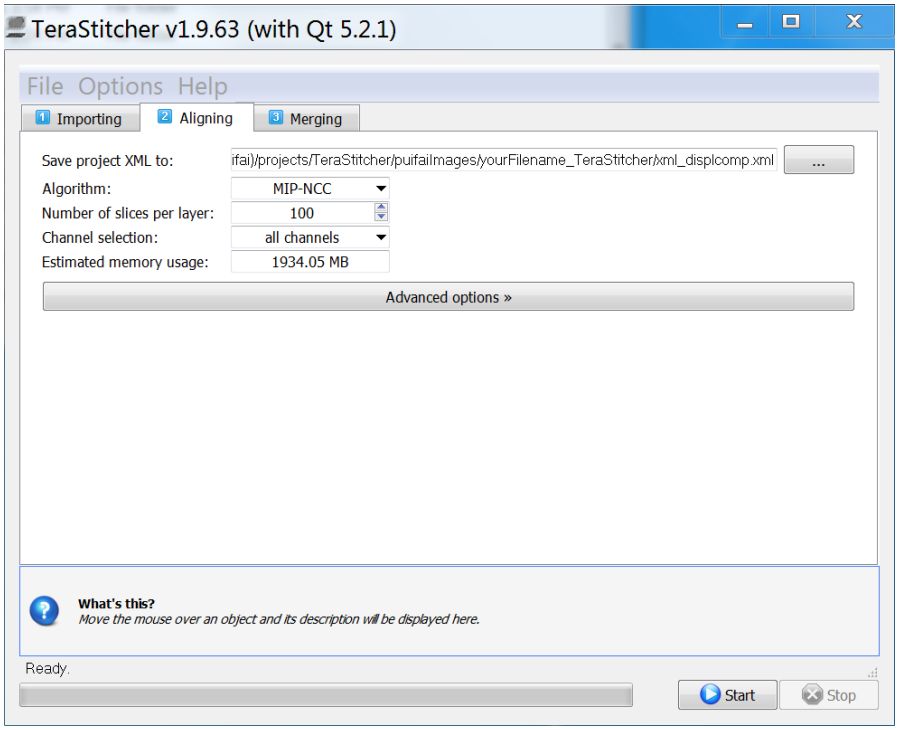
Put in the orientation Y,X,Z, and the correct voxel sizes. In this case, my XY size was 1.01 um and Z step was 4.64 um

Click Start



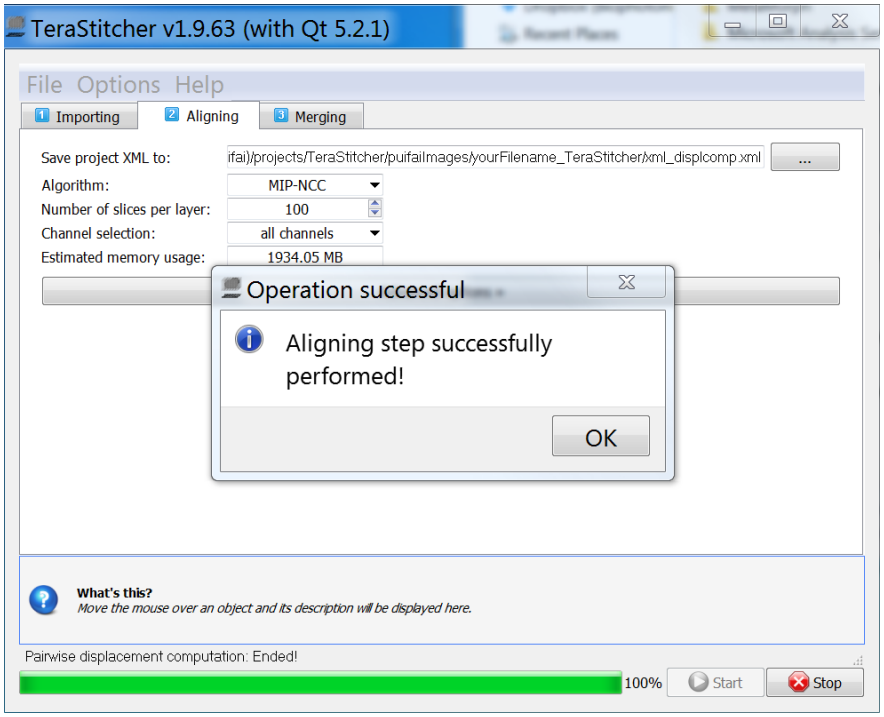
The bottom portion will no longer be grayed out. Check your volume information and make sure it's correct.

If you want, click Preview and 1 slice will be generated in your folder. Open that up with Fiji/ImageJ

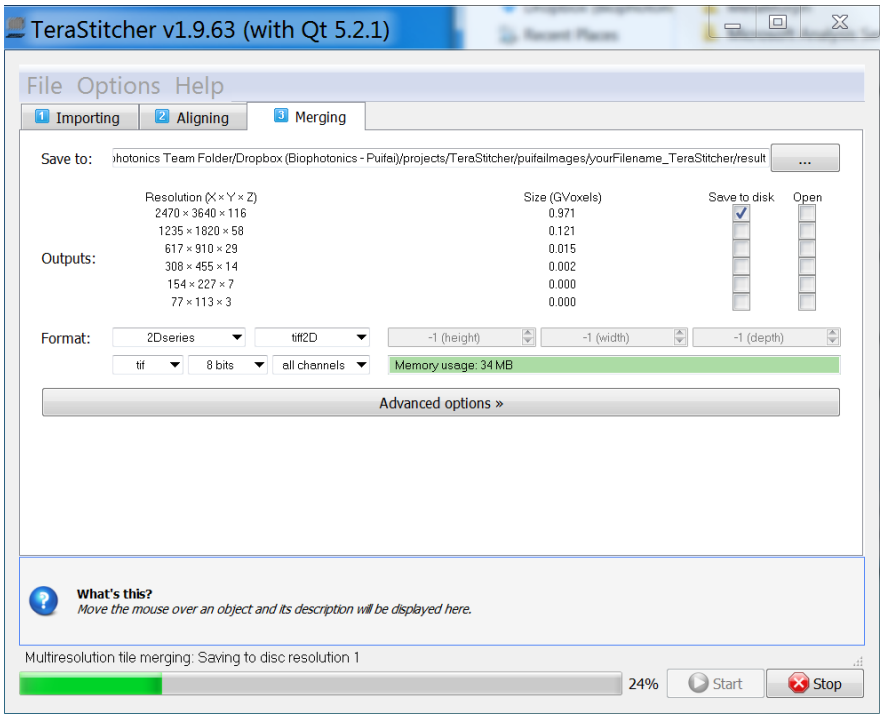


Next, click Aligning tab. Leave all settings alone. Click Start

Updated March 28, 2017



You should see this when aligning is done

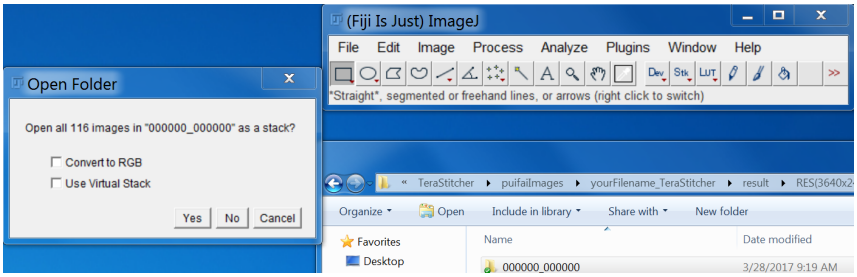


Click Merging tab

Select the directory to save the result to

Select 2Dseries with tiff2D option. Then click Start.

When it's done, go to your folder and there should be stitched tif files there!



If you want to combine these till images into 1 tif file (i.e. stack), drag the folder which contains the file onto Fiji. Click Yes. Then save as tiff

Updated March 28, 2017