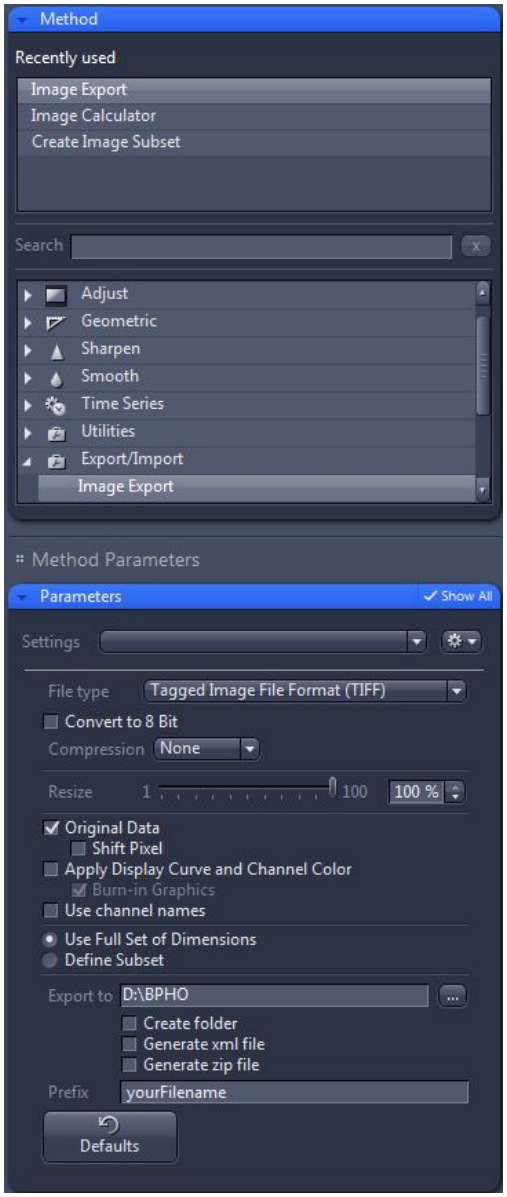
HOW TO STITCH LIGHTSHEET IMAGES

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.

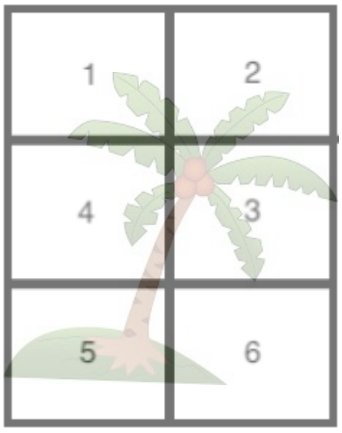
EXPORT YOUR FILE



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

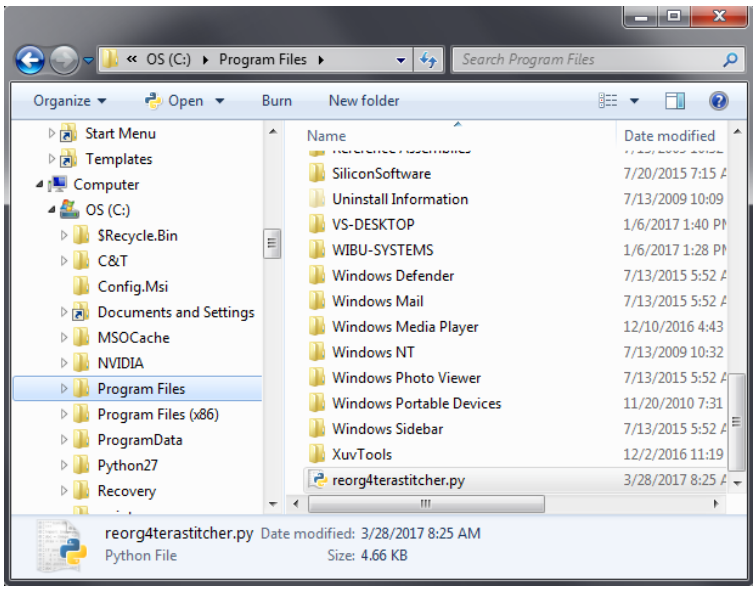
This will create a series of tif images named yourFilename_v01z001_ORG.tif where the numbers after 'v' and 'z' increase up to the max number of views and z-planes.

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)

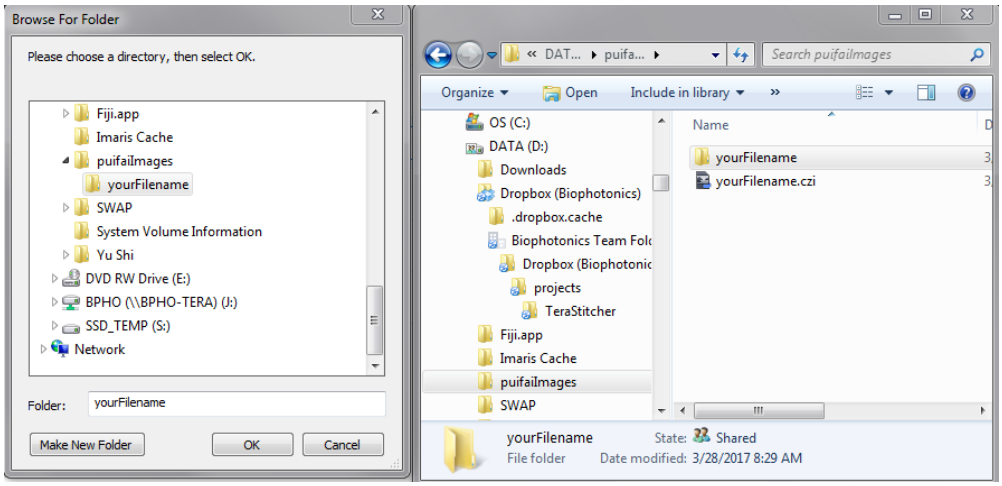


CONVERT THE FILES INTO THE CORRECT FOLDER HIERARCHY AND NAMES

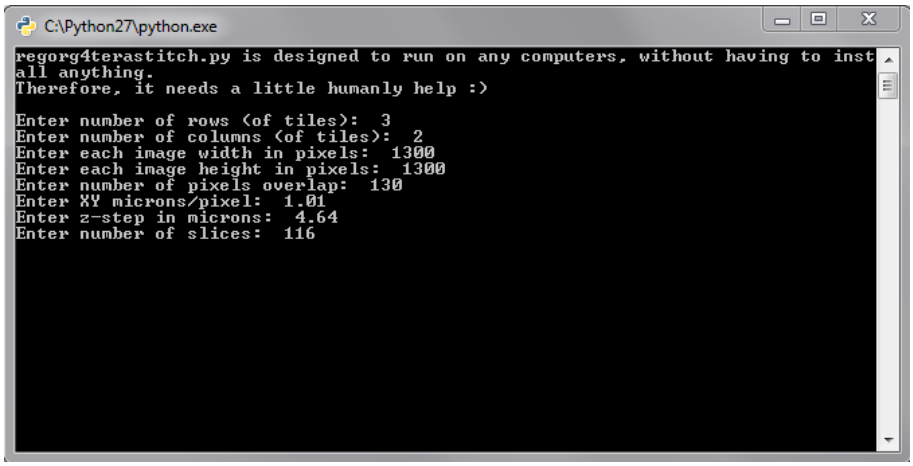
We will use TeraStitcher to stitch your data together. This is an open source software <http://abria.github.io/TeraStitcher/>. It requires a particular folder structure detailed on the website. I wrote a little script < https://github.com/puifais/biomedical_python/blob/master/reorg4terastitcher.py> to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



Double click on reorg4terastitcher.py



Select the folder that contains the exported image files

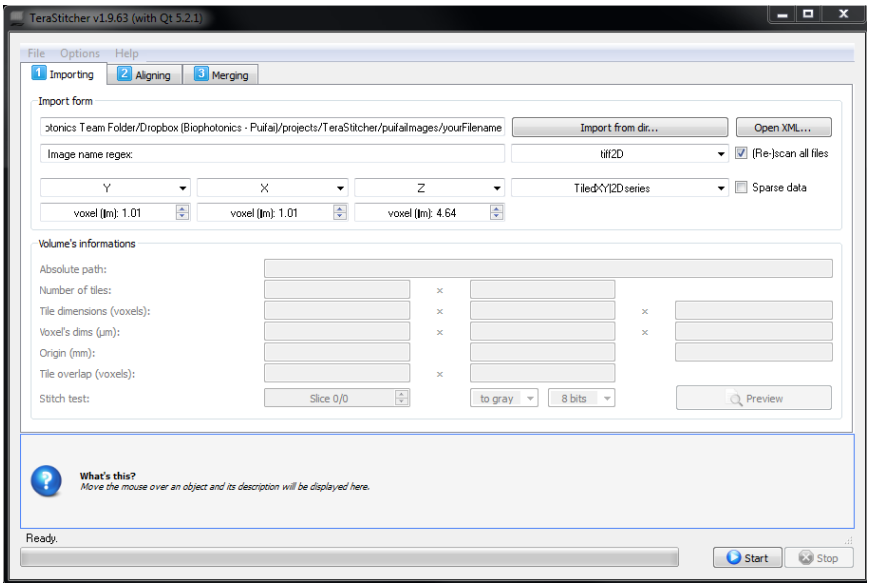


Next, a command line window will pop-up and ask about the images and tiles.

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

You should see a folder called yourFilename_Terastitcher appears.

STITCH



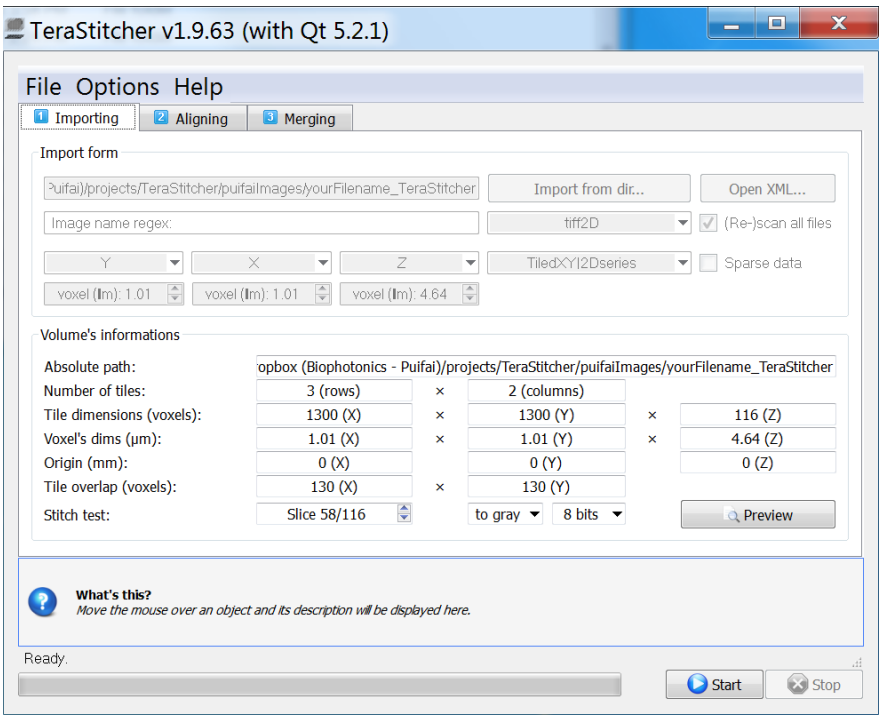
Double click on TeraStitcher icon.

Click Import from dir… and navigate to your yourFilename_TeraStitcher folder

Select tiff2D and TiledXY|2D series

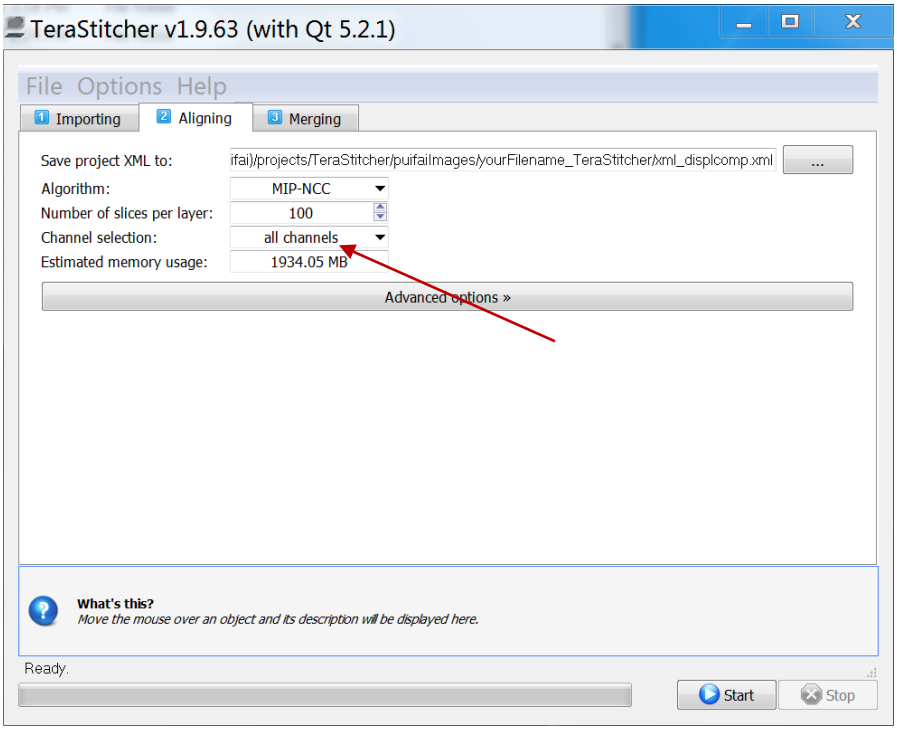
Put in the orientation Y,X,Z, and the correct voxel sizes. In this case, my XY size was 1.01 um and Z step was 4.64 um

Click Start



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

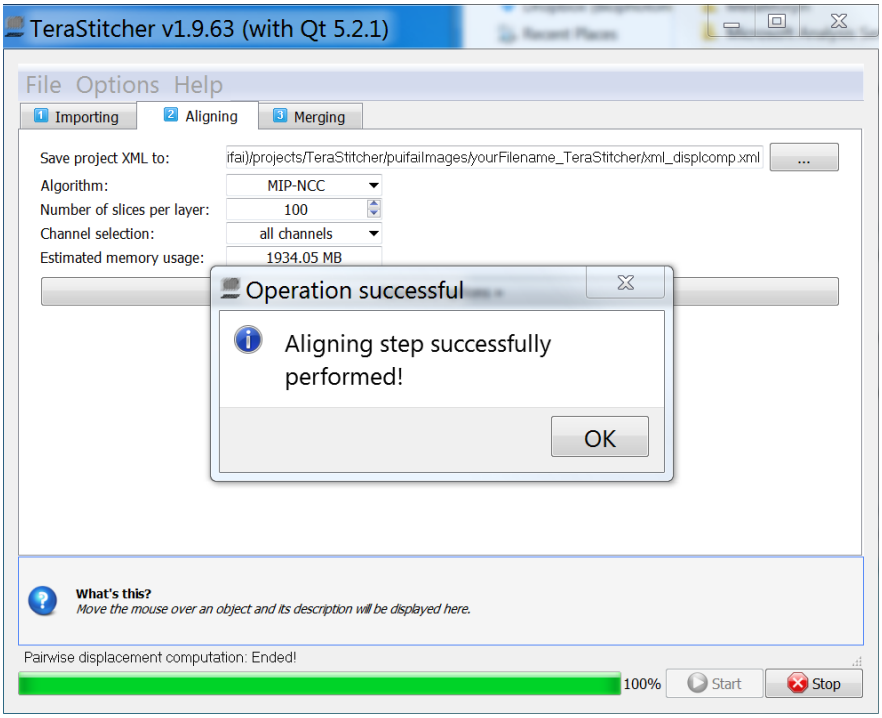
If you want, click Preview and 1 slice will be generated in your folder. Open that up with Fiji/ImageJ



Next, click Aligning tab. Leave all settings alone if the image is 1-channel.

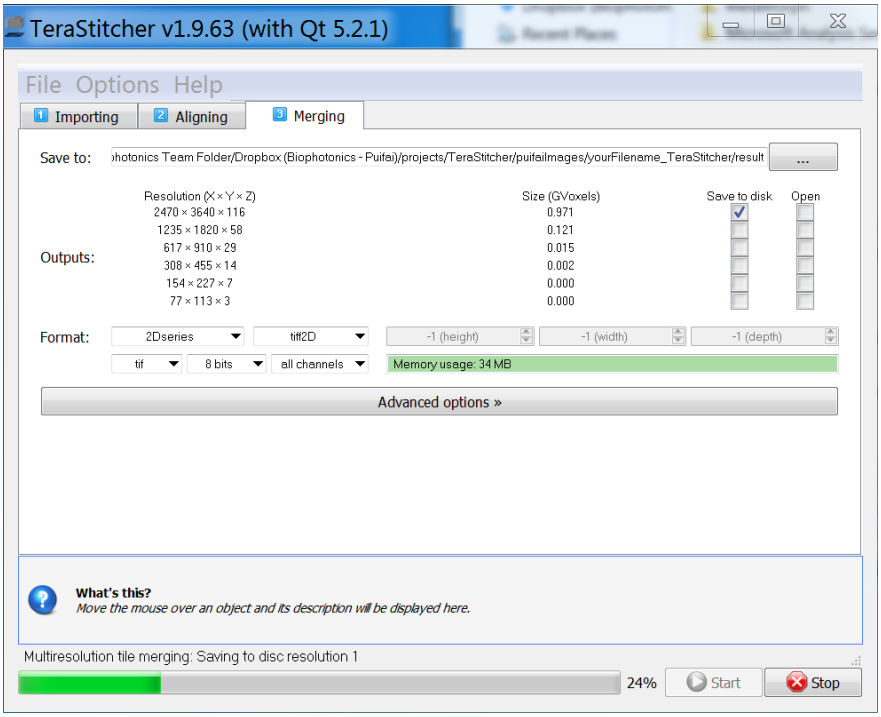
If the image has multiple channels, then select the color (red, green, or blue) with noticeable feature to be used as stitching map.

Click Start



You should see this when aligning is done

FOR 1 CHANNEL IMAGE:



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!

FOR > 1 CHANNEL IMAGE:

Open command line prompt (Windows > cmd)

1. Navigate to the directory where your xml_merging.xml file is located

To change from C drive to D drive, type "D:" and press enter.
To navigate to your image's directory, type "cd D:\Users\yourName\yourFilename_TeraStitcher" and press enter

2. Type this and click enter:

teraconverter --sfmt="TIFF (unstitched, 3D)" -s=xml_merging.xml --dfmt="TIFF (series, 2D)" -d=destination_directory

where "desitnation_directory" can be something like this:
"D:\Users\yourName\yourFilename_Stitched"