# Reshape Protocol + Analysis

Materials:

1. Reshape2 software (Can be found in the Tower or talk to Tuan) – 6.68 Gb. No installation need, just copy the folder and it's ready to use.
2. Fiji or Imagej
3. ZO-1 or phalloidin staining images.

Steps:

1. Start Fiji -> Drag the image to the software. If you have multichannel image, make sure you split channels, and only use the channel that contains cell tight junction staining. Once you have a clean image, convert it to 8- or 16-bit grayscale 2D image. Here I use 8 bit. Save the image and repeat the step with all the images you want to analyze.
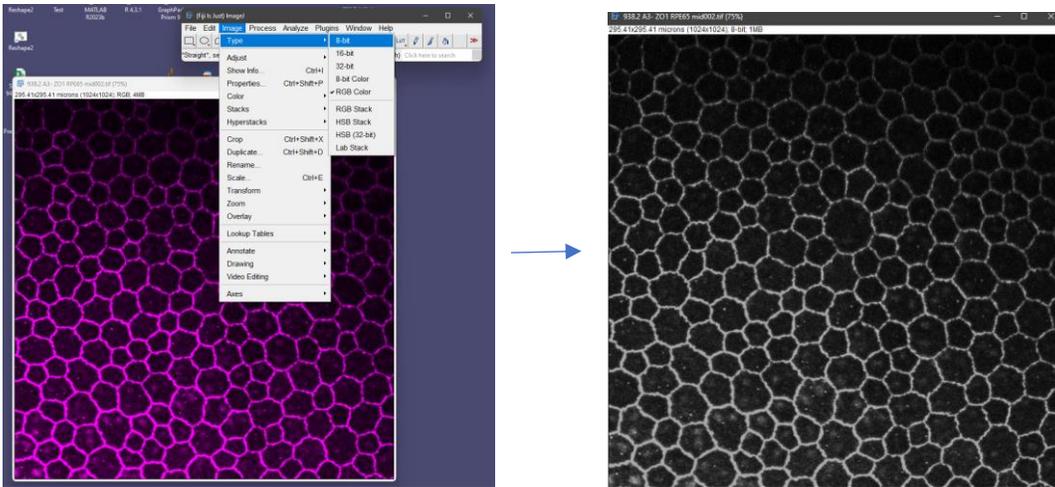

Make sure all images are in the same scale! You also need the scale (microns/pixel) value for later which can be found at Image -> Properties -> Pixel width or height.
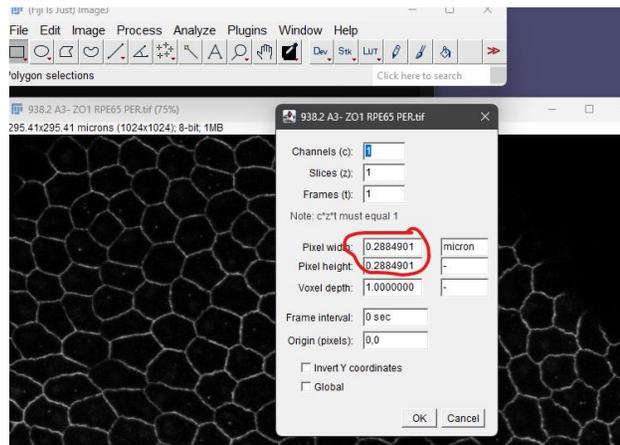

2. Move all the grey scale images to one folder. Start Reshape2 (double click on Reshape2_GUI.bat). See screenshot below for where you need to change.

a. Change input to where your folder is located. Software will automatically create an output folder (named Processed) inside your input folder.
b. 1. This list all the images the software can detect.
   2. The log shows which file is skipped. In this case, one image is skipped because it's not the format Reshape wants.

c. Choose **yes** for convert pixels to microns. **Scale(microns/pixels)**: Put the number you got earlier.

Click start and let the software do the magic.
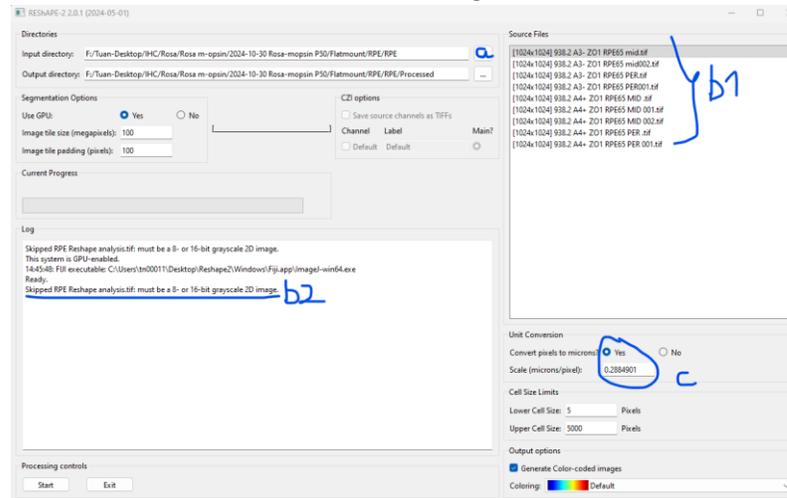
3. The software will give you a lot of analysis, it's totally up to you which piece of information you want to use. However, we can analyze further for 4 parameters (Area, Aspect Ratio, Neighbors and Hexagonality Score). To do that, go to folder Processed -> Analysis. Exclude all the neighbor's files and copy the rest to a new folder.
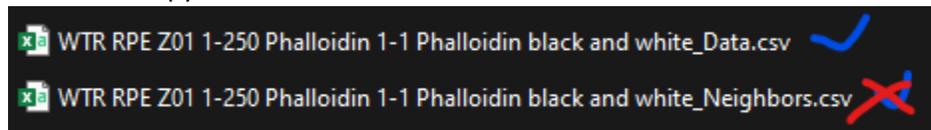
If you open a file, in each file, you want to extract column Area, AR, Neighbors and Hex Ave and combine into one single sheet so you can graph them. You can manually do it 😊 or

a. You can use the code I made (thanks Chatgpt) to one-click it. Open Template(code) for sorting Reshape data.xlsm located in Shared drives\Lab database\Flatmount
b. Click "enable editing". Go to Developer tab -> Macros -> Run. Now you have a sheet with only (Area, Aspect Ratio, Neighbors, Hex Score) data you need to make graphs from.

Examples of the graphs I made in GraphPad (I use column individual values to graph this, as do T test for each group):