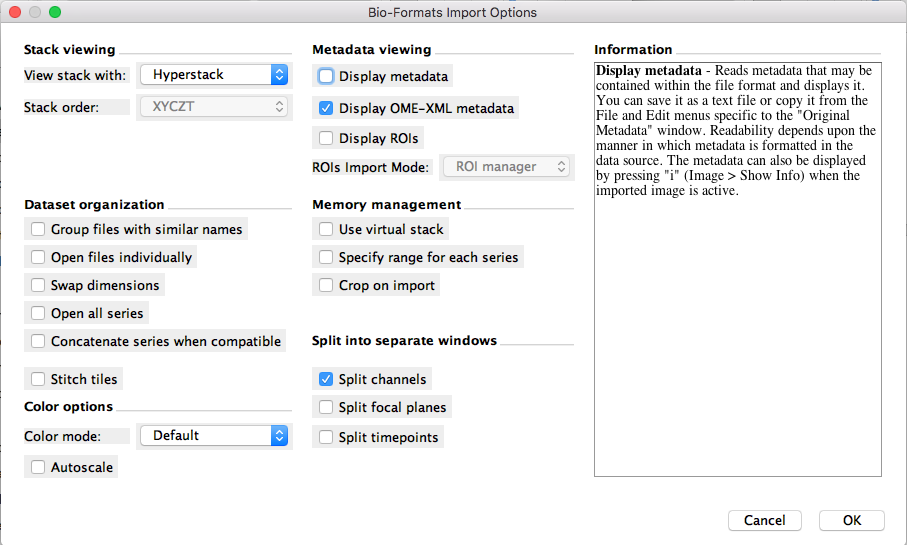
If FIJI is not already installed, install it: <https://imagej.net/software/fiji/downloads>

Open oir file in FIJI with the following settings:

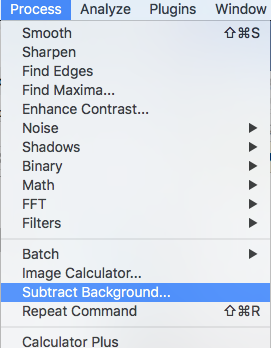
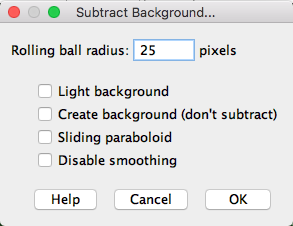


In the metadata window that opens, scroll to find the physical size of the X, Y, and Z axes. Write these down for use with 3D Morph. You only need to get metadata for one image from the data set. The rest should all be the same dimensions.

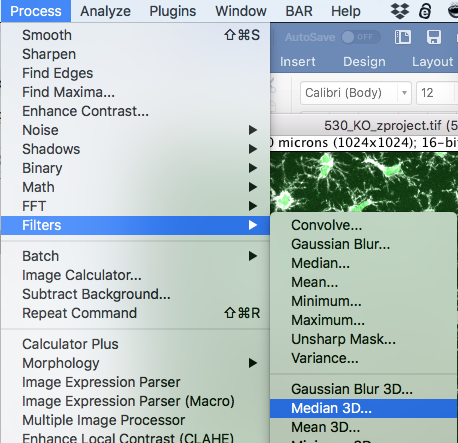
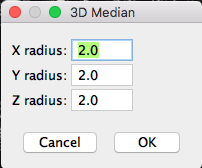
**Manual Image Pre-processing**

If you only want to do a few images for testing, use these steps. If you have an entire folder of images that need pre-processing, use the preprocessing tool, which is described at the end.

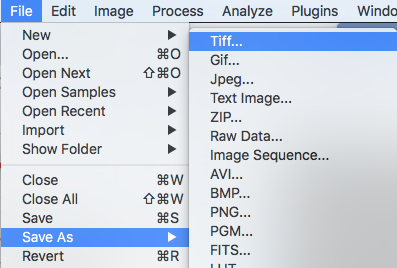
Choose the channel window you want, and do a background subtract with a rolling ball radius of 25:

IF your microglia are GFP and/or you are using the GFP channel, do a 2x2x2 median filter:

Save the file as a tiff:



**Using the pre-processing tool**

Install “preprocess\_3DMorph.jar” as a FIJI plugin: Plugins -> Install, select the jar file.

Restart FIJI

Run the plugin with Plugins -> Preprocess 3DMorph