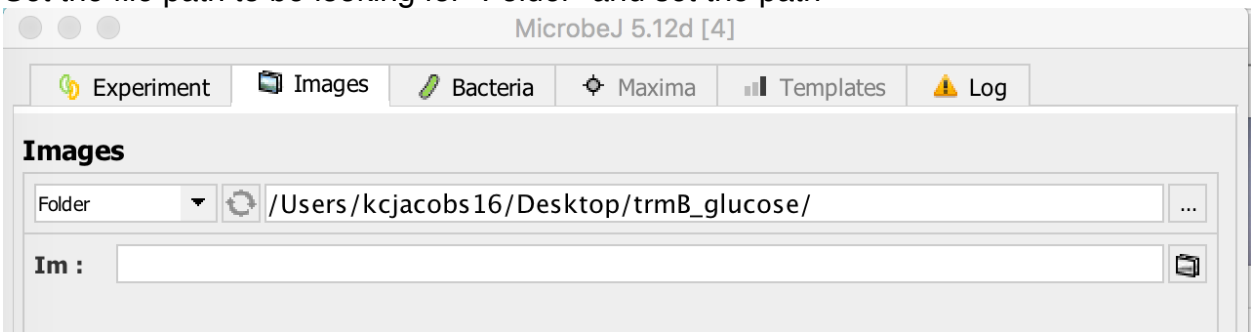
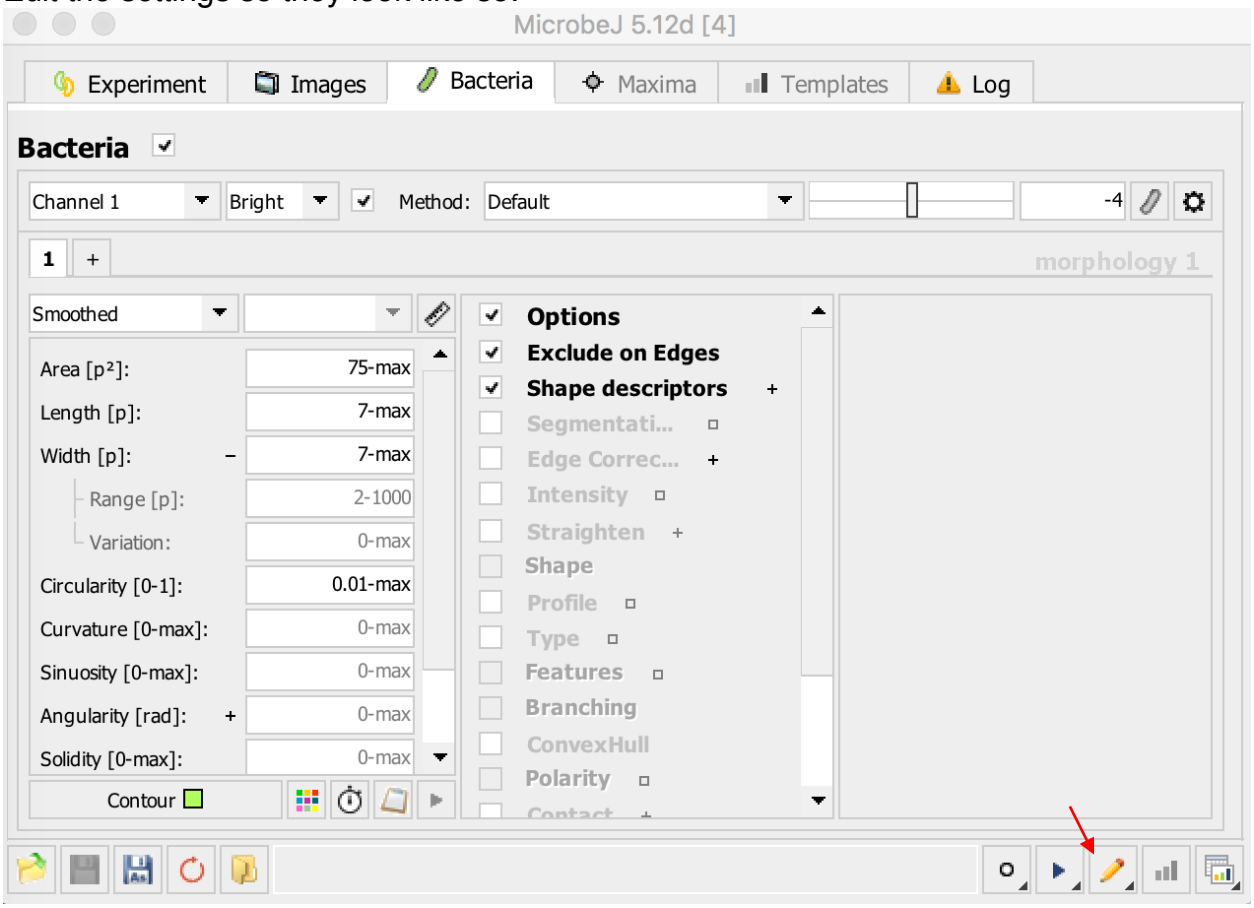
Image Analysis with MicrobeJ

Open Fiji
To open MicrobeJ, go to Plugins > MicrobeJ > MicrobeJ
Go to the “Images” tab
Set the file path to be looking for “Folder” and set the path



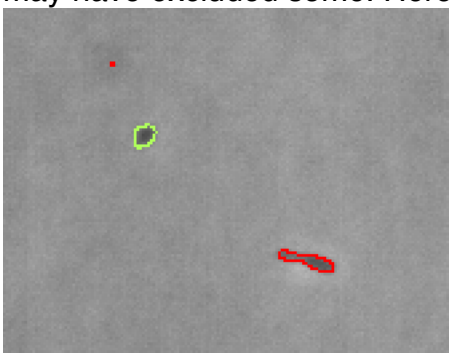
Go to the “Bacteria” tab
Edit the settings so they look like so:



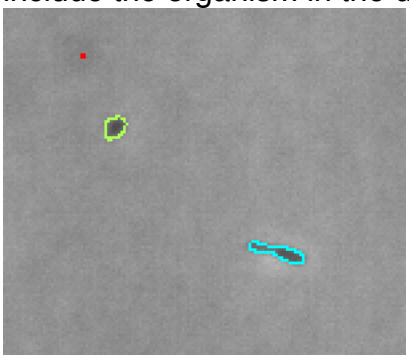
Click the pencil icon (indicated by the red arrow above) ⇒ MicrobeJ will analyze all the images in the folder (this can take several seconds).
The “Experiment Editor” window will open.

Select and right click (or ctrl-click) on an object name (e.g. “b1”) and select “Load Image(s)” – now you can see which organisms were recognized by the software (green outlines).

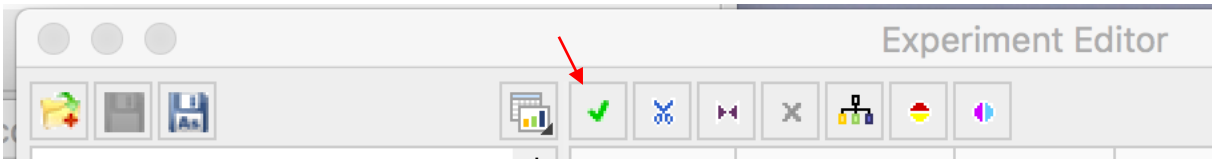
Look through the images to see if all the organisms have been selected – the software may have excluded some. Here’s an example:



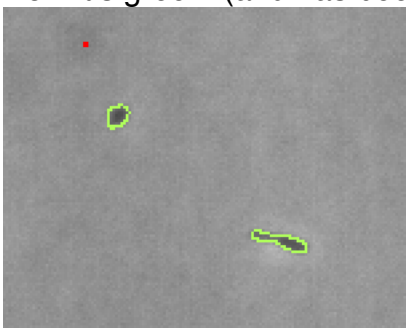
The software has selected the organism in circled green, but not the one in red. To include the organism in the data set, first click on it to select it (it will turn blue).



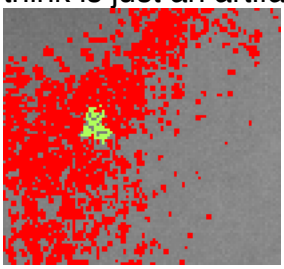
Then, “enable it” by clicking on the “Enable the Particle(s)” button in the “Experiment Editor”.



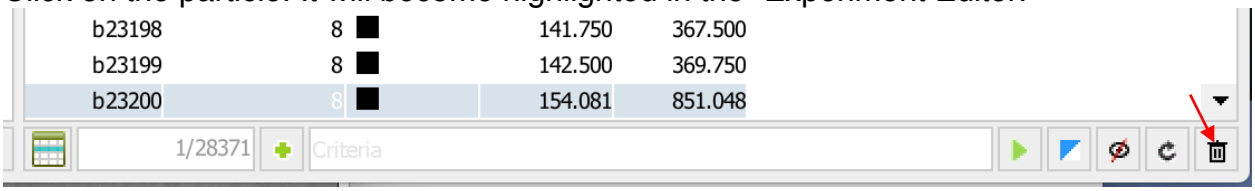
Now it’s green! (and has been added to the data)



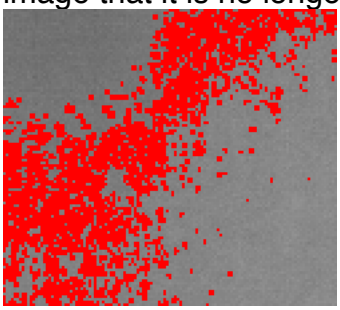
There may also be particles that you want to get rid of. See the example below (which I think is just an artifact from the image, not an organism).



Click on the particle. It will become highlighted in the “Experiment Editor.”

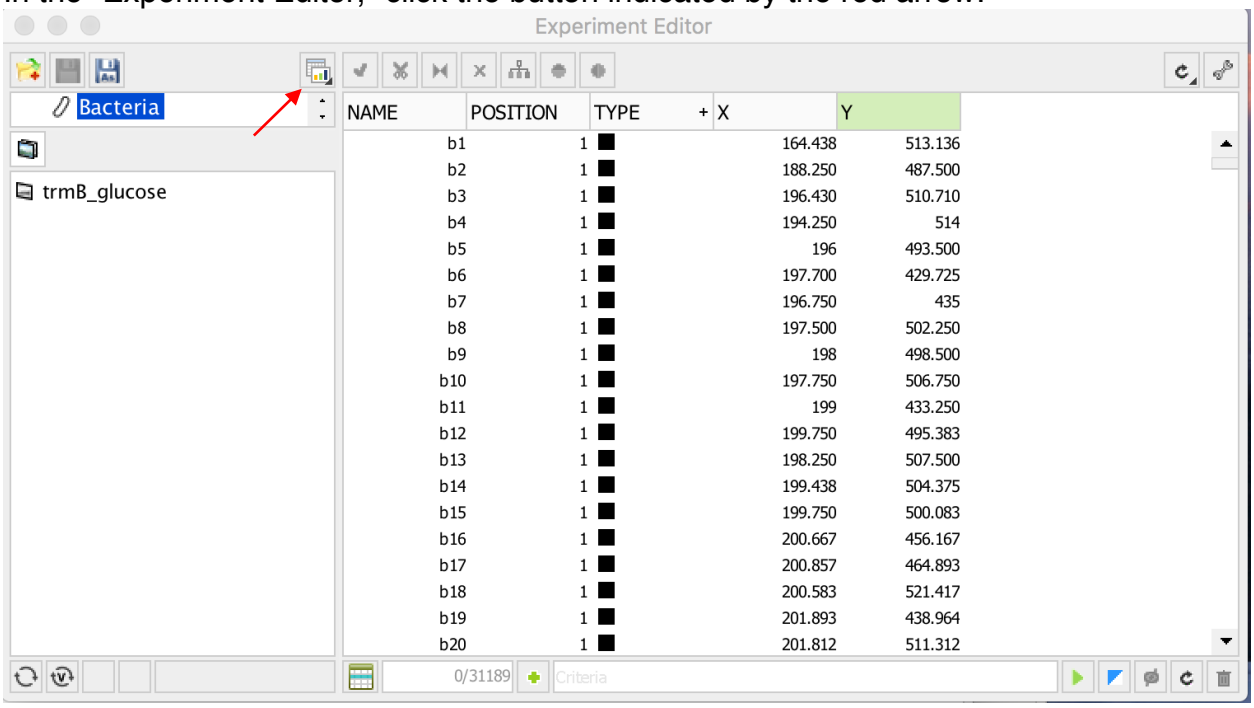


Click the trashcan button to delete the particle from the data. And you can see on the image that it is no longer circled in green!

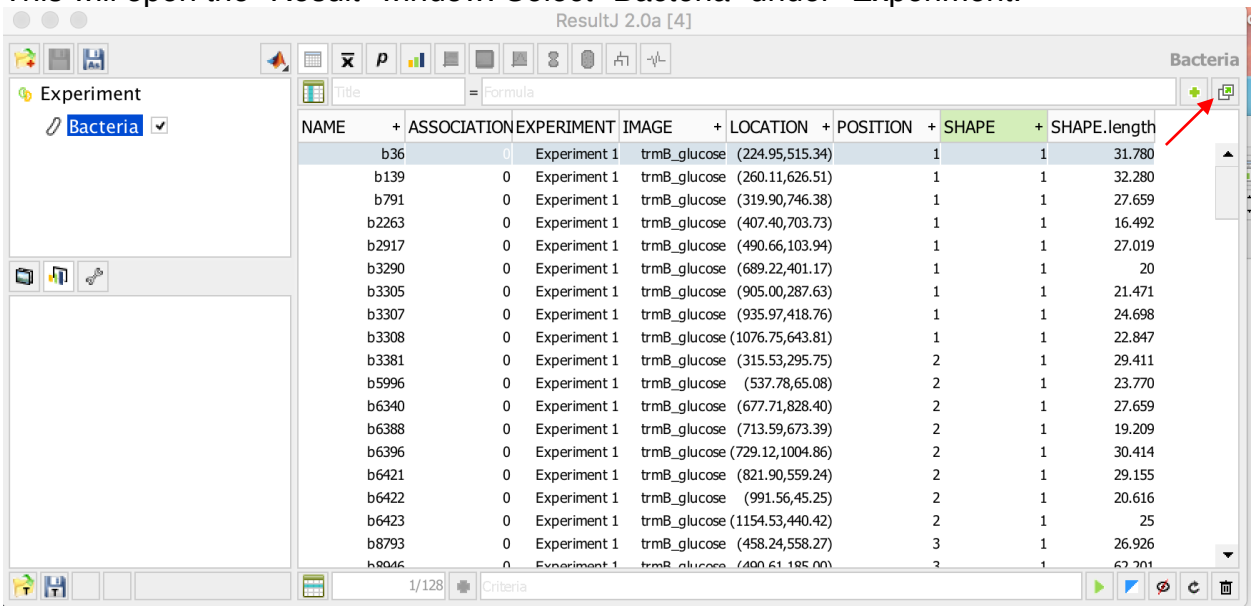


Go through all the images to make sure all the organisms are included and that any particles you recognize as not organisms are excluded.

In the “Experiment Editor,” click the button indicated by the red arrow:



This will open the “Result” window. Select “Bacteria” under “Experiment.”



Right click on the “SHAPE” column and select “length” to get a column displaying the lengths of each object/organism.

Click the button indicated by the red arrow above to export the data. Right click anywhere in the window that opens up, select “Save as…” to save the data in a .csv file. Open your .csv file with Excel. Make an additional column with length in microns (convert from pixels). Save as a .csv file.