

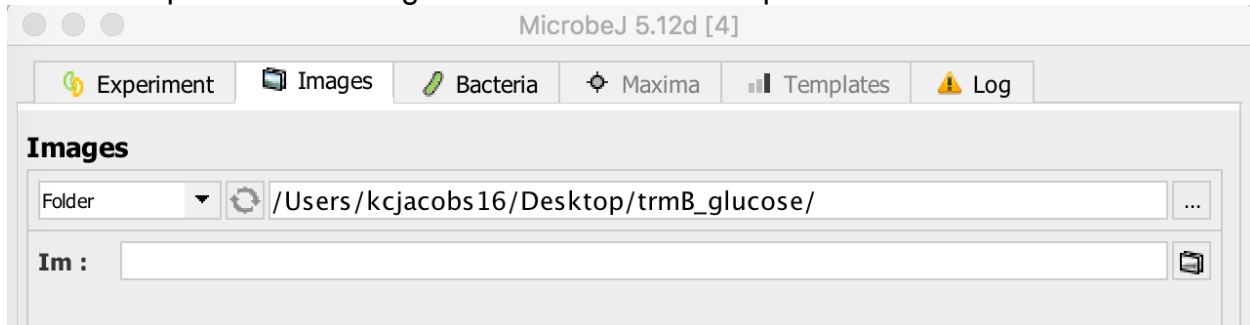
Image Analysis with MicrobeJ

Open Fiji

To open MicrobeJ, go to Plugins > MicrobeJ > MicrobeJ

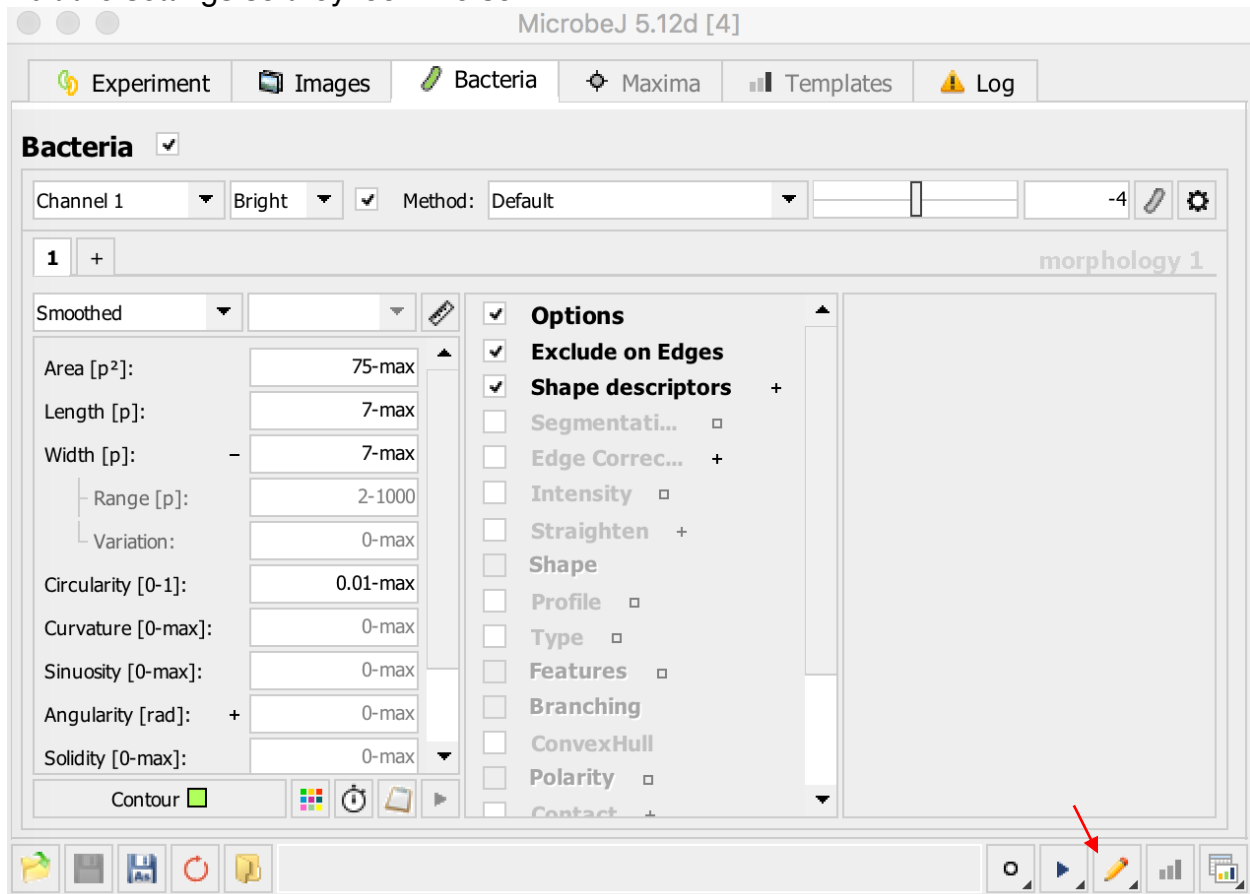
Go to the “Images” tab

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:

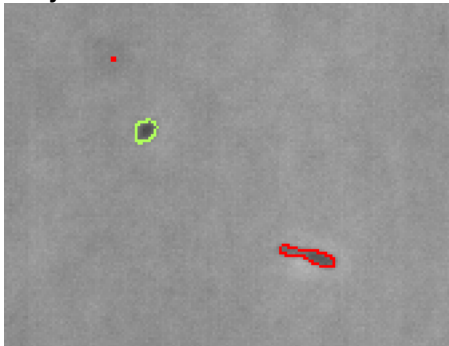


Click the pencil icon (indicated by the red arrow above) ⇒ MicrobeJ will analyze all the images in the folder (this can take several seconds).

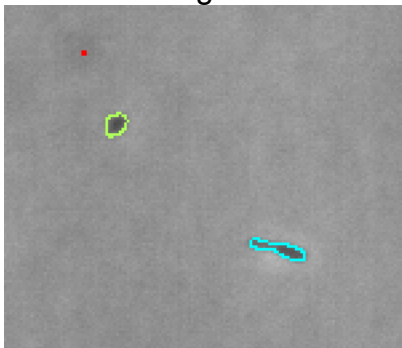
The “Experiment Editor” window will open.

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).

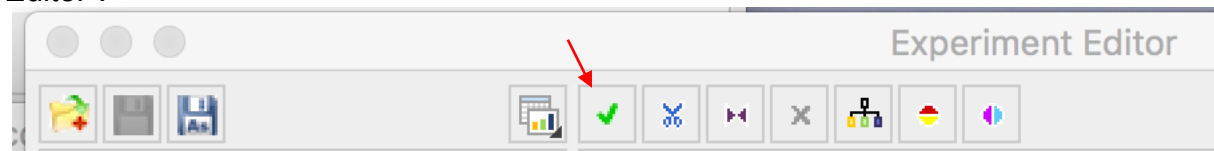
Look through the images to see if all the organisms have been selected – the software may have excluded some. Here’s an example:



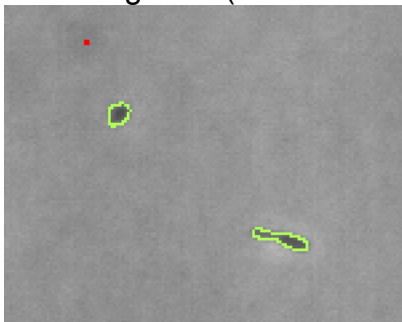
The software has selected the organism in circled green, but not the one in red. To include the organism in the data set, first click on it to select it (it will turn blue).



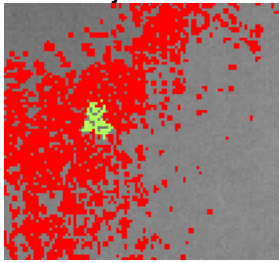
Then, “enable it” by clicking on the “Enable the Particle(s)” button in the “Experiment Editor”.



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).

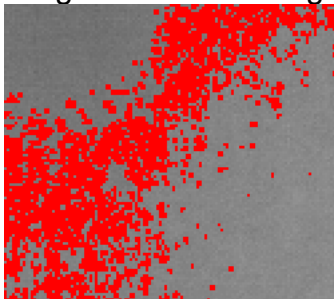


Click on the particle. It will become highlighted in the “Experiment Editor.”

b23198	8	■	141.750	367.500
b23199	8	■	142.500	369.750
b23200	8	■	154.081	851.048

 1/28371  Criteria     

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:

Experiment Editor

NAME POSITION TYPE X Y

b1	1	■	164.438	513.136
b2	1	■	188.250	487.500
b3	1	■	196.430	510.710
b4	1	■	194.250	514
b5	1	■	196	493.500
b6	1	■	197.700	429.725
b7	1	■	196.750	435
b8	1	■	197.500	502.250
b9	1	■	198	498.500
b10	1	■	197.750	506.750
b11	1	■	199	433.250
b12	1	■	199.750	495.383
b13	1	■	198.250	507.500
b14	1	■	199.438	504.375
b15	1	■	199.750	500.083
b16	1	■	200.667	456.167
b17	1	■	200.857	464.893
b18	1	■	200.583	521.417
b19	1	■	201.893	438.964
b20	1	■	201.812	511.312

0/31189 Criteria

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

ResultJ 2.0a [4]

Experiment

Bacteria

NAME ASSOCIATION EXPERIMENT IMAGE LOCATION POSITION SHAPE SHAPE.length

b36	0	Experiment 1	trmB_glucose	(224.95,515.34)	1	1	31.780
b139	0	Experiment 1	trmB_glucose	(260.11,626.51)	1	1	32.280
b791	0	Experiment 1	trmB_glucose	(319.90,746.38)	1	1	27.659
b2263	0	Experiment 1	trmB_glucose	(407.40,703.73)	1	1	16.492
b2917	0	Experiment 1	trmB_glucose	(490.66,103.94)	1	1	27.019
b3290	0	Experiment 1	trmB_glucose	(689.22,401.17)	1	1	20
b3305	0	Experiment 1	trmB_glucose	(905.00,287.63)	1	1	21.471
b3307	0	Experiment 1	trmB_glucose	(935.97,418.76)	1	1	24.698
b3308	0	Experiment 1	trmB_glucose	(1076.75,643.81)	1	1	22.847
b3381	0	Experiment 1	trmB_glucose	(315.53,295.75)	2	1	29.411
b5996	0	Experiment 1	trmB_glucose	(537.78,65.08)	2	1	23.770
b6340	0	Experiment 1	trmB_glucose	(677.71,828.40)	2	1	27.659
b6388	0	Experiment 1	trmB_glucose	(713.59,673.39)	2	1	19.209
b6396	0	Experiment 1	trmB_glucose	(729.12,1004.86)	2	1	30.414
b6421	0	Experiment 1	trmB_glucose	(821.90,559.24)	2	1	29.155
b6422	0	Experiment 1	trmB_glucose	(991.56,45.25)	2	1	20.616
b6423	0	Experiment 1	trmB_glucose	(1154.53,440.42)	2	1	25
b8793	0	Experiment 1	trmB_glucose	(458.24,558.27)	3	1	26.926
b8046	0	Experiment 1	trmB_glucose	(400.61,185.00)	2	1	62.201

1/128 Criteria

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.