

Download image files:
 Halobacterium_salinarum_100X
 WT_starter_100 (Haloarcula hispanica)
 trmB_starter_100 (Haloarcula hispanica)

WT_starter_100 (*Haloarcula hispanica*)trmB starter 100 (*Haloarcula hispanica*)

Open ImageJ, drag and drop files to ImageJ toolbar to open them.

Go to Analyze > Set Measurements and check “Fit ellipse” (uncheck anything else and you can also set “Decimal places” to zero). Click OK.

In the ImageJ toolbar, select the freehand selections tool. Draw an outline around the cell you want to measure (you can zoom using the “+” and “-” keys). Click the “M” key (or go to Analyze > Measure), a “Results” window should appear with the major and minor axes of the fitted ellipse. You can mark the cell you measured on the original image by selecting the text tool from the toolbar, drawing a text box over the cell, typing in an identifier (like a number), and pressing ctrl + D to stamp it on (or go to Edit > Draw) (this can help to keep track of cells you’ve already quantified).

When you're done with an image, select the results from the "Results" window and copy and paste them into an Excel spreadsheet. Save the stamped version of your image.

In Excel, we can calculate the length/width ratio by dividing the major axis by the minor axis for each cell.

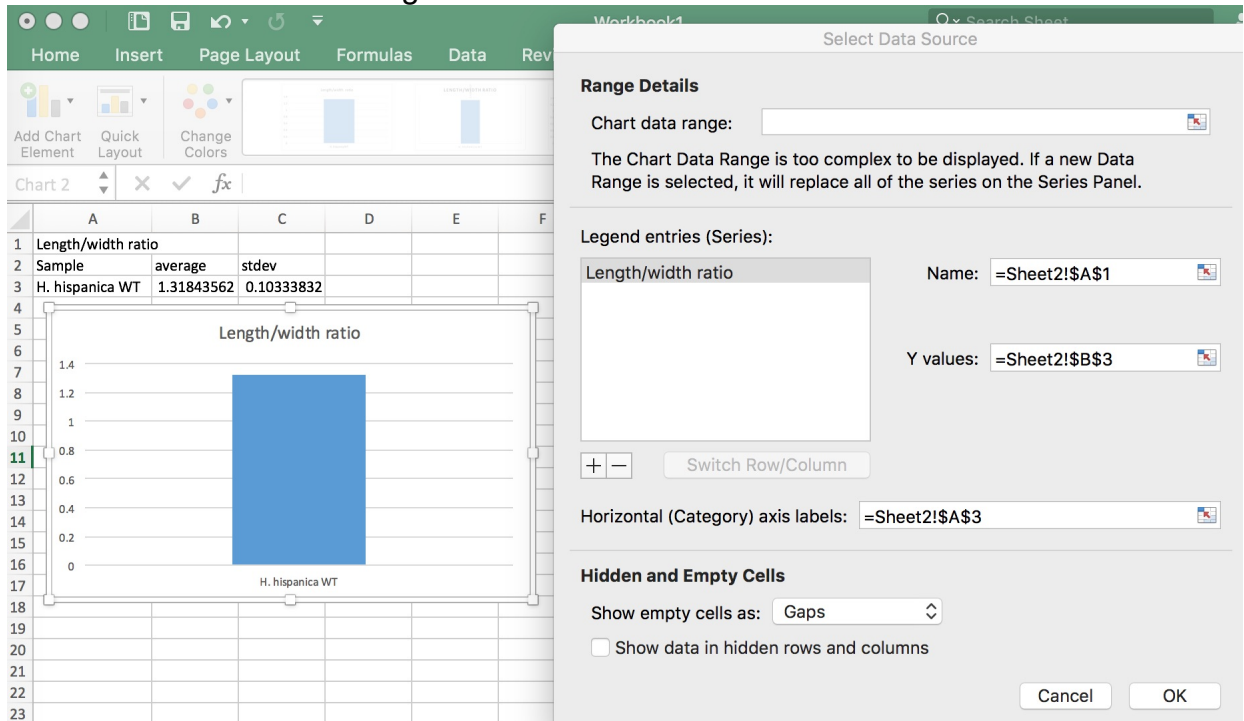
Here's an example:

	A	B	C	D	E	F	G
1	Image	Cell #	Major	Minor	Angle		Length/width
2	WT_starter_100C	1	53	36	7		=C2/D2
3		2	41	32	3		
4		3	45	36	46		
5		4	47	37	155		
6							

You can also use Excel to make graphs of your data. Type “=AVERAGE” into a cell and then select the numbers you want to get the average of. Use the STDEV function to get the standard deviation.

	A	B	C	D	E	F	G
1	Image	Cell #	Major	Minor	Angle		Length/width
2	WT_starter_100C	1	53	36	7		1.472222222
3		2	41	32	3		1.28125
4		3	45	36	46		1.25
5		4	47	37	155		1.27027027
6							
7						average	1.318435623
8						stdev	0.103338319

To make a graph, make a second sheet in your Excel workbook and enter in the data you want to make into a graph. An example using Insert > Chart > Column is shown below. Select the chart and go to Data > Chart Source Data to add the data.



You can add additional data by clicking on the “+” button in the Select Data Source window.

To add error bars, go to Add Chart Element (in the toolbar) > Error Bars > More Error Bars Options. Then select “Custom,” click “Specify Value” and use the standard deviation for the positive and negative error values.