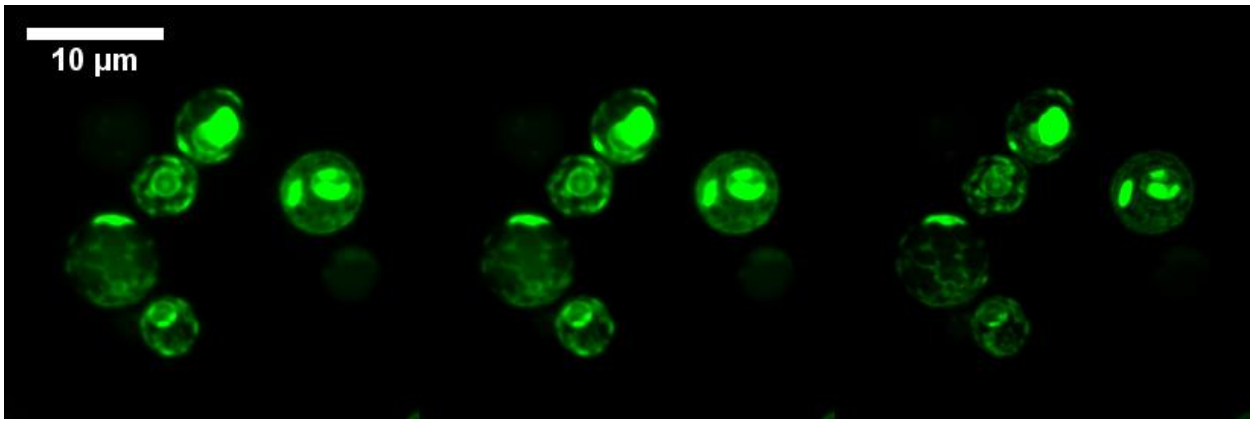
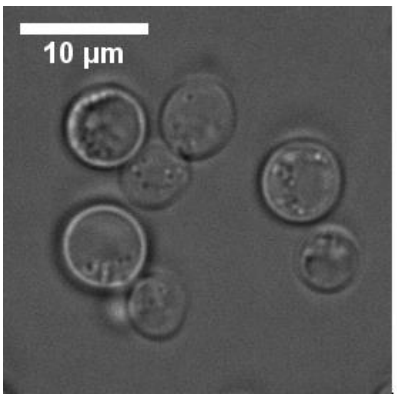
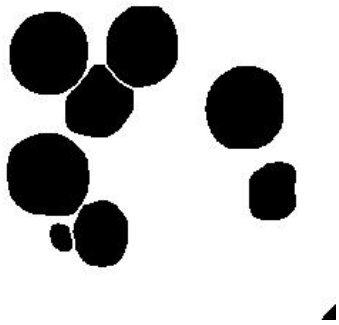
1. Move all .nd2 into a new folder – name it whatever you like!
2. Run batch-nd2-to-maxZ-sumZ-avgZ-plus-bmp.ijm on the folder
    a. Output: splits .nd2 into separate brightfield channel and three Z projections
    b. Also an empty folder called “yeastspotter” that you need to populate by hand with segmented images
    c. You can check the images by opening the Output-RGBbmp folder. Example below. From left to right: Average Z, Sum Z, Max Z of SEN555



3. Go to http://yeastspotter.csb.utoronto.ca/ and upload 10 of the brightfield tifs at a time
    a. I recommend you open a bunch of tabs & upload 10 brightfield images at a time into each new tab
4. Download the zip files when it is finished and extract them into the yeastspotter folder
    a. Output: segmentation of your yeast based on the brightfield image. File names will correspond with the brightfield image name.
    b. The only files in the yeastspotter folder should be the output images. Do not include the zip files or brightfield images in this folder.





5. Next, open and run the quantify_perc_puncta_ver3_yeastspotter_detection.ijm script in imageJ. You will be prompted to select the directory of Z projection you want to analyze

    a. I recommend maxZ for puncta; averageZ for fluorescence quantification if Z stacks are all different sizes (e..g average if you have 10 slices vs 30 slices in different samples), or sumZ if you want total cell fluorescence and all your Z stacks are the same size.
    b. You will need to set the puncta threshold – this is the minimum value of intensity that is counted as a "puncta". I recommend setting at 550 – this is what we used for the manual analysis before – but you might want to spot-check all your mutants and images to test this. Make sure you use the same threshold for ALL the genotypes/experiments you want to plot together! We should also report this value in the methods.
6. Compile results using the same R script as before!