MitoGraph-FISH analysis

1. Run deconvolution for the mitochondrial channel. Remember to choose output files in “.tif” format before launching the job.
2. Change the names of the output files from deconvolution by removing the letters added during deconvolution.
3. Place the mito and FISH images into two folders separately
4. Run GenFramesMaxProjs.ijm This macro is used to generate a z-stack where each slice corresponds to the max projection of a z-stack. The z-stack generated here is used to draw ROIs around the cells you want to analyze individually with MitoGraph.
   1. This macro is in the Desktop folder MitoGraph (also in “MitoGraphTools-1.0” folder)
   2. Open the macro in imagej.
   3. Run the macro and select the folder that contains the original z-stacks.
   4. Save the output file in the same folder with a name of MaxProjs.tif
   5. Run this macro on both FISH and mito images separately.
   6. NOTE: the original macro only recognizes “.tif”, but not “.TIF”. I modified the macro so that one macro version only recognizes “.TIF” and another macro version only recognizes “.tif”
   7. If the order of the stack needs to be changed, open the stack and click: image -> stack -> tools -> stack sorter
5. Run CropCells.ijm
   1. In the MaxProjs.tiff, open ROI manager
   2. Draw ROIs around each cell for analysis. Use DIC as a reference for budding cells.
   3. Save the whole ROI as RoiSet.zip
   4. Copy paste the RoiSet.zip file into both mito and FISH folders
   5. Run the CropCells.ijm
6. I have the MitoGraph program in a Desktop folder called “MitoGraph”
7. Open the terminal, type in

cd ~/Desktop/MitoGraph

./MitoGraph -xy 0.064 -z 0.2 -path ~/Dropbox\ \(EinsteinMed\)/shared\ files/NMD-resistant\ MS2\ Weihan-Anna/092921\_FISH\_ATP2\_Su9-GFP/092921\_W303WT\_ATP2Q570\_Su9-GFP/mt/cells

(NOTE: -xy and -z are the pixel size in xy and z. The folder path should NOT include the upstream folders like: /Users/weihanli/ The folder path should begin at the level of /Desktop or /Dropbox)

1. Perform FISH Quant in matlab. Remember to change the setting so that the XYZ pixel size is correct.

Pixel size xy: 64.5 (always for the left microscope with 100x objective)

Pixel size z: 200

Refractive index: 1.518 (for the oil we use)

Numeric aperature NA: 1.4

Excitation wavelength CY3:548 CY5:750 (647) EGFP: 488

Emission wavelength Cy3: 566, Cy5: 776 (670) EGFP: 509

Microscope: widefield

Pixel-size xy Inml 
Pixel. silo z Inml 
Refracti index 
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1. Copy & paste the mitochondrial and RNA coordinates (.txt files) into two separate files
2. Use the matlab file “change\_FISH\_files\_to\_csv\_files.m” to convert the RNA coordinate files (outputs of FISH Quant) into csv file by removing the headers. The output csv files have file names of “FISH\_\*”. When using “change\_FISH\_files\_to\_csv\_files.m”, remember to change the file address in the script. Sometimes, the first two files in a folder are "." and "..". Thus, start from the 3rd file in the for loop.
3. Only when step 2 was not taken and the names of the mitochondrial files contain the letters added during deconvolution: Use “mito\_file\_renaming.m” to rename the mitochondrial .txt files to remove the letters added during the deconvolution process
4. Double check to make sure that there are same number of mitochondrial coordinate files and FISH RNA coordinate files
5. Run “loop\_RNA\_mito\_distance.m”. Change the folder names in the matlab script. Change the line “mito\_files = dir(append(mito\_folder,'/ATP2\*.txt'));” according to the first letters of the txt files.
6. Copy the matlab script “count\_colocalizing\_RNA\_number.m” to the working folder and run it