**General workflow**

**Helpful links:**

*Coloc live coding*: <https://www.youtube.com/watch?v=GFt2XhUZQy0>

**Overall inputs:** 800 x 1000 pixel images (.tif)

* C1 - granule marker
* C2 - probe
* C3 - construct expression marker

**Overall outputs:**

* Count of granules in each cell in each image for each group that contain probe (granules positive for probe/total number of granules)
* Enrichment value of probe in granules (probe intensity in granule/probe intensity in background)

**Segment cells (in each image in each group)**

Inputs:

* 800 x 1000 pixel images in C2 and C3
* Maybe some manual annotation in a GUI for training data

Workflow:

* Cellpose package in Python
* Segment in C2 to make a mask for cells that have probe in them
* Segment in C3 to make a mask for cells that have the expression marker in them
* Find masks that (mostly) overlap from C2 mask and C3 mask

Output:

* Mask of cells that are expressing both the construct marker and have the probe

**Find granules (in each cell in each image in each group)**

Inputs:

* numpy.array/ scipy: sobel or histogram
* Mask of cells that are expressing the construct marker and have the probe
* 800 x 1000 pixel images in C1 and C2
* Some information about intensities of granules and probe in granules (just look at one image in ImageJ)

Workflow:

* Find all pixels in C1 that are above a certain threshold to make initial granule mask
* Find all pixels in C2 that are above a certain threshold to make initial probe mask
* Clean up masks (filter out things that are too small or too big, smooth edges)

Outputs:

* Mask of granules in cells that we are interested in
* Mask of probe in cells that we are interested in

**Count granules (in each cell in each image in each group)**

Inputs:

* Mask of granules in cells that we are interested in
* Mask of probe in cells that we are interested in

Workflow:

* Find masks that (mostly) overlap between granule mask and probe mask
  + Look to see if granule mask has a probe mask counterpart
* Count number of granule masks
* Count number of granule masks that have a probe mask counterpart

Outputs:

* Number of granules in cells that we are interested in
* Number of granules that are positive for probe in cells that we are interested in

**Find enrichment of probe in granules (in each granule in each cell in each image in each group)**

Inputs:

* Mask of granules in cells that we are interested in
* 800 x 1000 pixel image in C2 (probe)

Workflow:

* Dilate mask (imdilate in MATLAB/ scipy.ndimage.morphology.binart\_dilation) of granules to make a dilated mask
* Subtract original mask from dilated mask to get a ring mask (around the original mask)
* Find average probe intensity in original mask of the granule (max)
* Find average probe intensity in ring mask of the granule (min)

Outputs:

* Average probe intensity in original mask of the granule
* Average probe intensity in ring mask of the granule

**Calculations (in each cell in each group, in each group)**

Inputs:

* Number of granules in cells that we are interested in
* Number of granules that are positive for probe in cells that we are interested in
* Average probe intensity in original mask of the granule (probe in granule)
* Average probe intensity in ring mask of the granule (probe in background)

Workflow:

* Calculate fraction of granules that are positive for probe (number of granules positive for probe / number of granules total)
* Calculate enrichment of probe in granules (average probe intensity in original mask of the granule / average probe intensity in ring mask of the granule)

Output:

* Fraction of granules that are positive for probe in each cell
* Enrichment of probe in granules in each cell

**Plotting and stats**

Inputs:

* Fraction of granules that are positive for probe in each cell
* Enrichment of probe in granules in each cell

Workflow/outputs:

* Violin plot (?) of fraction of granules that are positive for probe in each group with each cell as a data point
  + Would be sweet to plot each cell as a data point of a different color
* Violin plot (?) of enrichment of probe in granules for each group with each granule as a data point
  + Would be sweet to plot each granule as a data point and granules from the same cell are the same color
* Stats to determine if the two groups are different!