Bi1x: Gene Expression: Assignment

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1 Things to turn in:

1. For a phase image of your choice, print out
   (a) the original phase image
   (b) the raw phase mask obtained by binary thresholding (before applying area or other criteria)
   (c) an overlay of the original phase image with your final, filtered phase mask.
2. Using the HG104::NoFluo strain (Pad 2), compute and report the mean autofluorescence pixel value. (In this week's Matlab tutorial, we showed how to compute **integrated** pixel intensities for each cell detected by our phase segmentation. Here, you are asked to compute the **mean** pixel intensity over all pixels in detected cells. This will require you to slightly modify the code from the tutorial.) nnnnnn
3. For each of the other 5 strains, calculate the autofluorescence-corrected integrated fluorescence intensity of each detected cell. If you're not sure how to correct for autofluorescence, see the bottom of page 3 of the Matlab tutorial handout.
4. For each of the four experimental strains, compute the repression as defined by

$$\begin{aligned}\text{Repression} &= \frac{\text{<Gene expression in the absence of repressor>}}{\text{<Gene expression in the presence of repressor>}}. \\ &= \frac{\text{<YFP Fluorescence in the absence of repressor>}}{\text{<YFP Fluorescence in the presence of repressor>}}\end{aligned}$$

   where the brackets are a reminder that we're averaging over all cells from a given pad. For example, let's say that *integrated_intensities_Strain1* is your vector of (autofluorescence corrected!) integrated intensities from Strain1, and *integrated_intensities_HG105* is your vector of (autofluorescence corrected!) integrated intensities from strain HG105 (no repressor).

Then the represssion for Strain1 is

$$\text{Repression} = \frac{\text{mean(integrated\_intensities\_HG105)}}{\text{mean(integrated\_intensities\_Strain1)}}$$

Report the values you obtain in data table format, along with an estimated uncertainty for each value.

5. Now that you have computed the repression for each strain, use the expression discussed in class to compute the number of repressors per cell R for each strain:

$$\text{Repression} = 1 + \frac{R}{N_{NS}} e^{-\Delta\epsilon_{rd}/k_B T}. \tag{1}$$

N_{NS}, the number of possible nonspecific binding binding sites for LacI, is well approximated by the length of the *E. coli* genome, 4.6×10^6 base pairs. $\Delta\epsilon_{rd} = -14.3\, k_B T$ for the O2 LacI binding site used in these experiments. Be sure to report your answer in data table format!

6. You may have noticed while imaging that even genetically identical cells on the same pad exhibited noticeable variability in brightness. Before we finish, let's look briefly at the amount of noise or variability in gene expression for your various strains. A common measure of noise in gene expression is the variance in gene expression normalized by the mean:

$$\text{Noise Strength} = \frac{\text{variance(Gene expression)}}{\text{mean(Gene expression)}} \tag{2}$$

where the mean and variance are computed across the population of cells from a given pad, and where "Gene expression" is represented in this case by integrated fluorescence intensities as in question 4.

Compute the noise strength for each of your 4 experimental strains and for HG105. Make a scatter plot of noise strength vs. mean gene expression for each of your five data points. Does noise strength increase or decrease with increased gene expression?