Bi 1X, Spring 2011

Assignment 6: Rate of Things

Growth curve:

- A write-up on what you did in a lab report format. Follow the guidelines under the "Assignment" section in the Grading Handout on the Bi1x website. These include:
    - Introduction to bacterial growth and antibiotics
    - Procedure that explains each step in the protocol, including ones that the TAs did for you in pre-lab setup
    - Results, your conclusions and discussion, as well as any sources you cited
    - Figures and plots with their own captions such that you could independently understand it without having to refer to the text
    - Remember to use full sentences and walk the reader through each step. Do not write a list. In this case, your lab notebook is a reference for you to look up exactly what you did in the procedure.
- Be sure in your results to include a graph of absorbance vs. time using all the data with different concentrations of the antibiotics from the whole class. Label the lag phase, exponential phase and any other points of interest.
    1. What do you expect to see in such a plot?
    2. Is there anything strange about your plot?
    3. Calculate a doubling time for each concentration of the antibiotics.
- Find the relationship between OD and number of cells for your own concentration of antibiotics. Knowing that you plated 200µL on each plate, the dilution factors, and the number of colonies counted during office hour, calculate the cell density in units of CFUs per mL at each of the three ODs you plated. (Hint: CFU = Colony Forming Unit).
    1. What is the margin of error on each density and how do you determine it?
    2. Assuming each colony grew out of a single cell plated by the student, plot the cell densities for both ODs and fit a line showing the OD-to-cell conversion factor in units of cells per mL. Make sure to show the margin of errors as upper and lower error bars on your plot.
    3. Is it close to the expected value? (OD_{600} 0.1=10^8 cells/mL)
    4. Discuss your error. Where might large errors have come from?
- Be sure to answer the following questions from the handout in your discussion:
    1. Calculate the molar extinction coefficient of E. coli at 600 nm assuming the path length is 1cm.
    2. What conclusions you can draw about challenges to E. coli with antibiotics and why kanamycin might differ from other antibiotics, like penicillin or vancomycin? How do these work?
    3. Can you estimate the MIC (minimum inhibitory concentration) based on the data from the whole class?
    4. What is the clinical significance of the MIC and what can doses of antibiotic below that level lead to in the long run? Can you find an example of this?

Single-cell movies on dividing bacteria:

Please answer the following questions and turn in your answers along with pictures and Matlab codes. You do NOT need to write in report format.

1. Answer the questions in the protocol handout marked in *italics* related to the autofocus and the time resolution.
2. Show the initial and final frames of all the positions you took data from. Did all cells divide? Make sure to include scale bars, time stamps and captions. Time stamps are easy to add in ImageJ using "Image → Stacks → Label". You can add a scale bar by doing "Analyze → Tools → Scale Bar". Consult your TA for the calibration factor.
3. Estimate the cell doubling time by looking at your movies. An easy and fast way to look at the movies is with ImageJ by using the command "File → Import → Image Sequence". Make sure to explain your reasoning carefully by showing a couple of division events of your favorite movie with their respective time stamps. You might want to look into the Matlab command *montage* in order to plot many frames right next to each other. Make sure to also include scale bars and captions. How do you decide that a cell has divided already? Do all cells divide at the same rate? Do you get the same division rate in all positions on the pad?
4. Look at the movies corresponding to one or two positions and manually track the different cell division times. Draw a histogram of the division times you found using Matlab with the command *hist.* Make sure to label your axes and title. Find the mean division time and its standard deviation. Make sure to include units. How does the division time compare to the bulk results? Is there are any difference, why do you think this could be?