Bi 1X, Spring 2013

Assignment 2: Rate of Things

Growth curve:

- Provide 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.
    - 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 it is possible to 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 from the different strains from the whole class.

1. What do you expect to see in such a plot? Specifically, what do these curves tell us about the growth rates of the different strains?
2. Calculate a doubling time for each of the strains. The doubling time was the main objective of these experiments, just to get a sense of how long it takes for a given bacterial cell to reproduce. This helps us understand the time scales of DNA replication and biosynthesis of the various macromolecules of the cell. To arrive at the doubling time, it will be helpful to plot your graph using the logarithm of the optical density. Make sure you explain the logic of the fit you are doing.
3. How does the growth rate of the well studied lab strain compare to the rate of the environmental samples? Is there any relation between the growth rates and the relatedness of the host organisms?
4. What conclusions can you draw about the differences between these strains, and why, if they are members of the same species, do they seem to grow at different rates?
5. Find the relationship between OD and number of cells for the strain you used in the experiment. Knowing that you plated 200μL on each plate, the dilution factors, and the number of colonies counted, calculate the cell density in units of CFUs per mL for each of the two ODs you plated. (Hint: CFU = Colony Forming Unit).
    a. Right now your calculations are in units of CFUs. To convert to number of cells, assume that each colony you counted was grown from a single cell. Plot the cell densities for each of your two ODs.
    b. Calculate the mean and standard deviation of the cells/mL using all three dilution factors for each OD. If you were only able to count one dilution you won't be able to calculate this. If this is the case please discuss the possible sources of error.
    c. Using these mean values (or the single value you were able to count) fit a line between your two ODs in order to obtain the conversion factor between OD and cells/mL. If possible, add error bars to this plot.

    d. Is the conversion factor close to the rule of thumb we quoted in class? (OD_{600} $0.1=10^8$ cells/mL)
6. Calculate the molar extinction coefficient of *E. coli* at 600 nm assuming the path length is 1cm.

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. As you learned in the Matlab session/handout, calculate how fast the cell area increases using the images you took for the growth movie. And from this infer the doubling time of your environmental sample. How do the division times in your movies compare to those found in the bulk assay? If there are differences between the two methods, what hypotheses can you formulate to explain these differences?