**Bi 1x, Spring 2010**

**Week 6**

- **Session 2**
  - Growth Curves

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**Background:**

**Cell Growth and Spectroscopy**

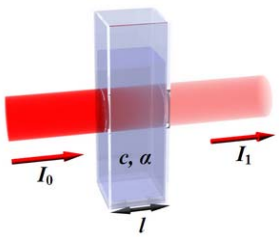
While there are a multitude of important time scales in biology, arguably one of the most important is concerned with the rate of cell division. In the bacteria *E. coli*, rates of cell division are closely linked with evolutionary fitness and hence our basic understanding of natural selection. To better understand the concepts involved in cell division, we will measure the bulk growth rate of *E. coli* in media with different amounts of antibiotic. We measure cell growth using spectroscopy and optical density as outlined in the following section. The included formulas will prove useful in analyzing your spectroscopic data.

**Beer's Law and Optical Density**

It is often extremely useful to know the concentration of cells in a liquid culture, and can be used in various kinetic measurements of fitness and protein production. Light scattering is the primary method by which we measure cell density. In the following section, we will discuss the basic physical concepts that allow determination of concentration as well as the formulas that relate optical properties to concentration.

The basic physics are as follows:

1. Light, with a wavelength comparable to the size of the object being measured (600 nm), enters the sample at an initial intensity ($I_0$)
2. Cells, having a different index of refraction as the surrounding medium, randomly reflect and scatter light out of the incident light path (scattering with no change in momentum). The amount of scattering is proportional to the amount of cells at any given point in the sample. (Caveat: to get an accurate reading, cell density must be low enough that light is only scattered *once* in its journey through the sample. Often, this means that cells must be serially diluted to get an accurate reading.)
3. Upon exiting the sample, the light intensity is reduced by $I_1 = I_0 e^{-\alpha l c}$, where α is the wavelength-dependent molar absorption coefficient of the species in question, l is path length – usually 1 cm in modern spectrophotometers, and c is the concentration, this is known as *Beer's Law*. See the figure below:



4. The transmission efficiency of the sample is the ratio of the final and incident light intensity, $T = I_1 / I_0$, where the measured quantity, called 'absorbance' is $A = \ln(1/T) = \alpha l c$. The general rule of thumb is that accurate absorbance readings lie in the range $0.01 < A < 1$. (Serial dilutions of the sample will be necessary to bring A within this range.)

**Measuring Cell Growth**

For more see:
"Bacterial Growth: Constant Obession with dN/dt" – Neidhardt, *J Bact* **181**: 7405, 1999.
"The Growth of Bacterial Cultures" – Monod, *Ann Rev Micro Biol* **3**: 371, 1949.

This experiment will take ~3 hours and everyone will participate, working in groups of two or three.
Goals:

- Measure baseline growth rate in rich media.
- Measure growth with added antibiotic.
- Determine the correspondence between OD600 and cell density.

*Prior to your involvement:*

1) Groups will be assigned a particular mixture in which to assess growth rates.
   i) Growth 1 – LB + 0 μg /mL Kanamycin
   ii) Growth 2 – LB + 2 μg /mL Kanamycin
   iii) Growth 3 – LB + 4 μg /mL Kanamycin
   iv) Growth 4 – LB + 6 μg /mL Kanamycin
   v) Growth 5 – LB + 8 μg /mL Kanamycin
   vi) Growth 6 – LB + 10 μg /mL Kanamycin
   vii) Growth 7 – LB + 12 μg /mL Kanamycin
   viii) Growth 8 – LB + 14 μg /mL Kanamycin
2) Cultures of wild type (MG1665) *E. coli* were inoculated into 100ml of each of these media, and incubated on a shaker at 37°C for one half-hour prior to the start of the experiment. This ensures that the so-called 'lag phase' has passed, and that (slow) exponential growth has begun.

*Procedure:*

1. Every 10-15 minutes (as time and coordination allows), remove a 1 mL sample of your culture using a sterile pipette. Deposit this into a 1 ml cuvette and immediately bring to the spectrophotometer (the 'spec').
2. Carefully insert the cuvette into the spec in the correct orientation. Make sure the spec is set to read OD600, and measure the absorbance, and record the exact clock time.
   a. You should have been given a 'blank', i.e. a sealed cuvette with sterile media. Absorbance is always measured relative to the blank. Be sure to measure the absorbance of the blank with every culture measurement!
   b. You may want to take readings in more than one of the available specs, to check their consistency.
3. Properly dispose of the sample cuvette, and repeat these steps for the next ~3 hours.
4. When you reach OD600 ~0.1 and at your maximum OD600, remove 100 ul from the culture and put it in a labeled eppendorf.
   a. For the moment, let us assume OD600 0.1 = $10^6$ cells / ml. With that in mind, properly dilute

your sample with LB so that 500ul contains on the order 50 cells (~100 cells / ml). This will require serial dilution – try to be accurate in your pipetting! (Hint: try to bracket your dilution by a factor of 10 on either side.)

b. On LB plates, evenly spread 200 μl with beads.

c. Incubate the plates overnight at 37°C. Later, we can count colonies and determine the exact correspondence between OD600 and cell density.

i. What is the cell density at OD600 0.1?

ii. What is the margin of error on this figure and how do you determine it?

iii. Calculate the molar extinction coefficient of *E. coli* at 600 nm?

5. Afterwards you should have a list of times and absorbances. With the blank's absorbance subtracted from each sample value, make a plot of absorbance vs. time.

a. What do you expect to see in such a plot?

b. Is there anything strange about your plots? Can you calculate a doubling time(s)?

c. Can you estimate the MIC (minimum inhibitory concentration)?

d. What is the clinical significance of the MIC and what can sub-MIC doses of antibiotic lead to in the long run?