Introduction to Counting Cells – How to Use a Hemacytometer

With a lab partner, discuss and answer the following questions:

- Why might an investigator want to determine the total number of cells growing in a Petri dish, in a flask, or in a test tube?
- How could you estimate the number of cells in a Petri dish? What are some limitations of your proposed method?

Today you will learn one method that is used to determine the amount of cells growing in a culture vessel using a counting chamber or slide called a hemacytometer. View the following video before you continue with this activity: http://www.youtube.com/watch?v=wPeNK0pRIpA

The Hemacytometer

Figure 1: Hemacytometer Slide http://en.wikipedia.org/wiki/File:Hemocytometer.jpg#filelinks

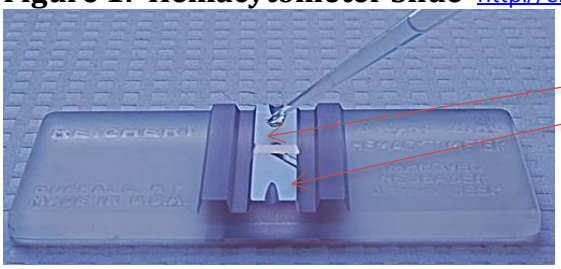
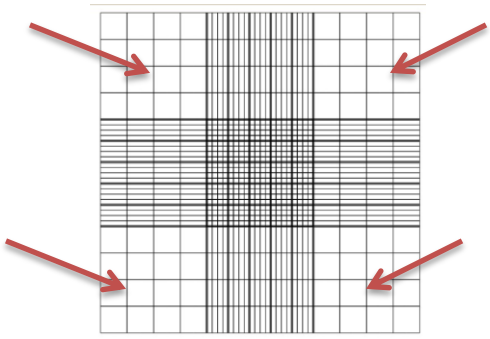


Figure 2: The counting grids are located on the reflective portions* of the hemacytometer and are visible using the microscope.
http://www.microbehunter.com/2010/06/27/the-hemocytometer-counting-chamber/



Note the 4 quadrants in the 4 corners of the counting grid indicated by the arrows. Each is composed of 16 squares.

Preparing Sample

To obtain an accurate estimate of the cell density of your sample the cells need to be evenly suspended in your culture vessel before you remove an aliquot for counting. The method will vary depending on your culture method. For algae, you might be sampling from a flask or test tube. For cell cultures you probably are sampling from a Petri dish. To resuspend cells in a flask or test tube you can simply swirl the flask or invert the test tube and then quickly remove your aliquot of cells. For a cell culture growing in a Petri dish, you need to resuspend the cells growing on the bottom of the dish by gently using a pipette to remove cells and media from a dish and then gently expelling them back into the dish. This aspiration of the contents of the Petri dish should be completed several times, each time expelling the cells and media while moving the pipette across the bottom of the dish to gently discharge cells growing on the entire surface. After

resuspension the aliquot is quickly removed from the vessel before the cells settle to the bottom again.
For cell culture applications cells are often stained with Trypan Blue so that dead cells can be distinguished from live cells. For example, a 0.5 ml suspension of cells would be removed from the Petri dish and mixed with 0.5 ml Trypan Blue solution in an *Eppendorf* or small test tube. Trypan Blue is a stain that selectively stains dead cells. For algal cells, Lugol's solution (KI_3) is commonly used to stain an aliquot of cells in a small test tube. The Lugol's solution will immobilize and kill the algal cells. The amount of stain used to dilute the cell suspension must be measured and recorded so that you can apply it as your dilution factor in the final calculations.

Loading Samples

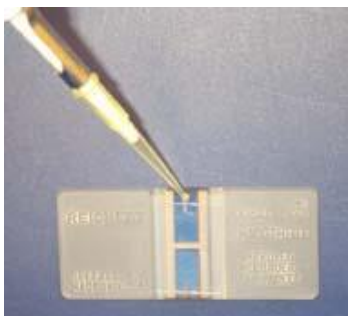
The goal when loading your sample of cells is to obtain an even distribution of cells on the hemacytometer slide. In order to achieve this result the slide has to be very clean and you must load your sample quickly and smoothly. It is important to handle the hemacytometer and the cover slip carefully. Never place your fingers on the reflective surface of the slide.

- Always clean the slide before you load the sample by rinsing the slide and cover slip with 70-95% ethanol. Air dry or gently wipe the slide and cover slip with lens paper or *Kimwipes*. Never use paper towels or soaps.
- Place the clean and dry slide on your work surface and place the coverslip on top to cover the reflective surfaces.

Figure 3: Hemacytometer with cover slip
http://www.vivo.colostate.edu/hbooks/pathphys/reprod/semeneval/hemacytometer.html

- If you have diluted your cells in a test tube, invert the tube several times to resuspend the cells.
- Using a micropipette, quickly and smoothly without interruption, add 10 µl of your cell suspension (or 1 drop from a transfer pipette) to the v-shaped groove on each side of the hemacytometer., If the slide is clean, the suspension should move quickly under the cover slip covering the entire reflective surface of the hemacytometer. The suspension should not flow into the channels or gutters along the slides of the reflective surface. If your sample moves into the gutters you may not have loaded the sample in the correct location or you may have used too large of an aliquot. Practice first and make any adjustments that are necessary. If the sample does not flow quickly across the surface the hemacytometer may not be clean or you may not have expelled the solution quickly enough.

Figure 4: Loading the cells on the hemacytometer using a micropipette
http://www.vivo.colostate.edu/hbooks/pathphys/reprod/semeneval/hemacytometer.html

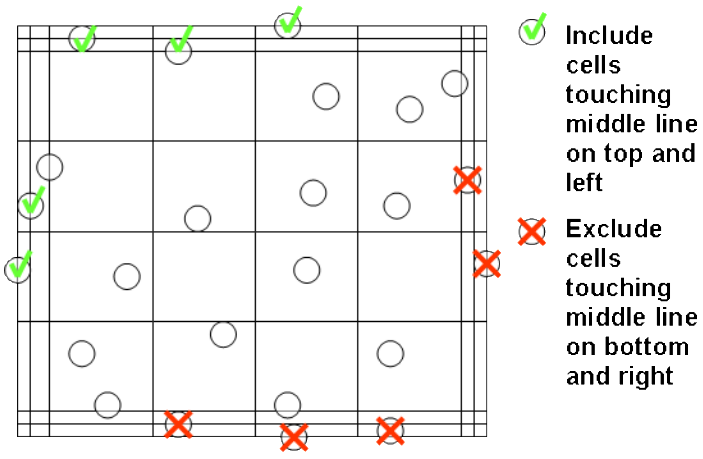


Estimating Cell Density using a Hemacytometer

You should be able to visualize the grid of the hemacytometer when viewing through a compound microscope. You will need to utilize your good microscope skills taking care to focus well and adjust the iris so that you have good contrast to view the grid. The hemacytometer is much thicker than a normal slide so you need to be careful and note the reduced working distance. It is a good idea to examine a hemacytometer without a sample using the microscope before you begin your counts.

Consistency is important in science. In using a hemacytometer it is important use a consistent counting methods. Different laboratories might use different patterns of counting but you will utilize the method figured below today. You will count all of cells within each of the four large quadrants in the four corners of each counting chamber on the hemacytometer (see Figure 2). Count all of the cells within each quadrant except those on the far right edge and lower bottom edge (see Figure 5 below). If the number of cells is high, you should use a tally counter to keep track of your counts. View the hemacytometer at the highest magnification that allows you to see an entire quadrant and then focus to visualize the cells.

Figure 5: Cells in one quadrant of the hemacytometer as viewed through a microscope.
http://www.hpacultures.org.uk/technical/ccp/cellcounting.aspx



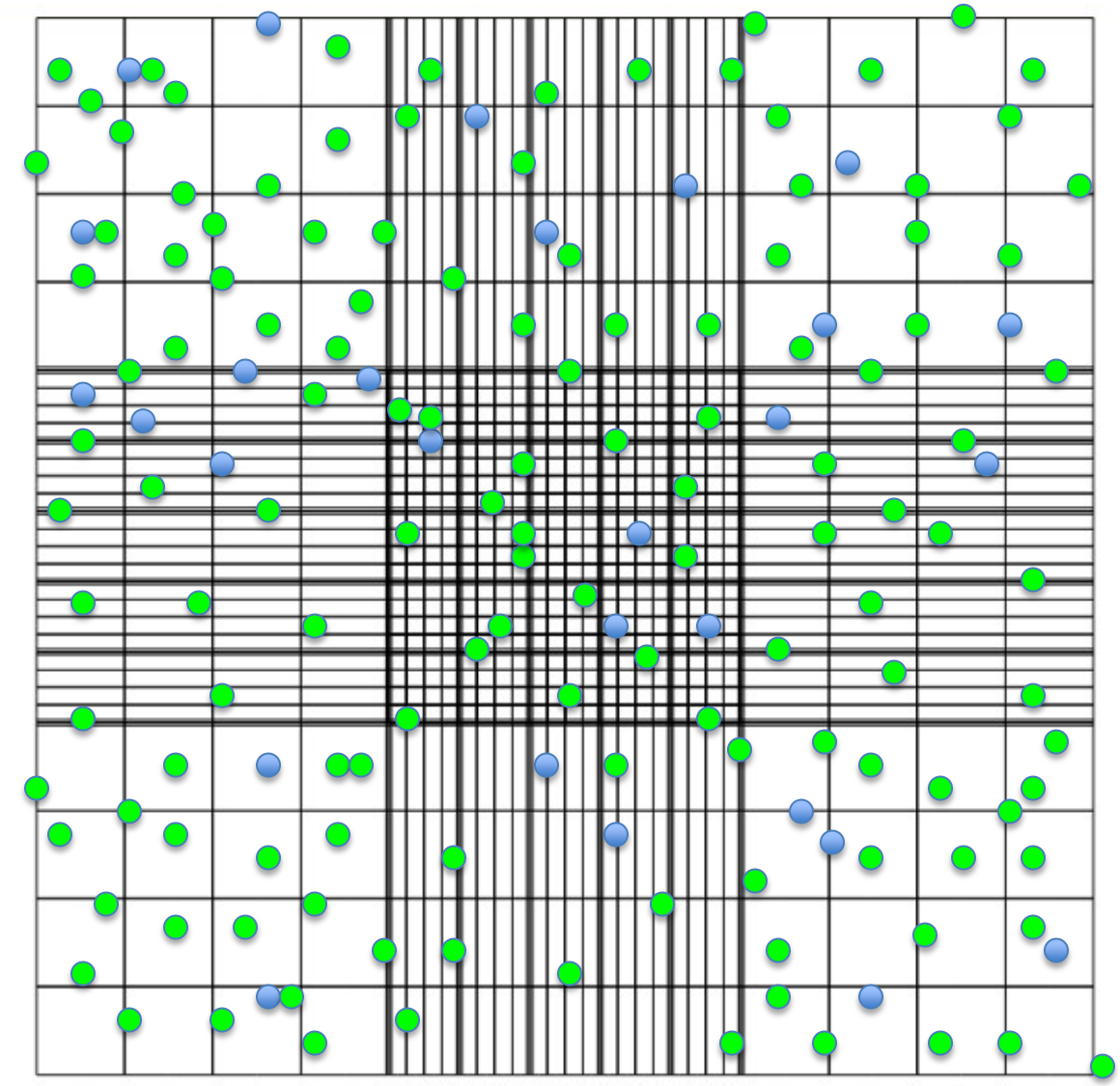
Estimating Cell Density Activity

The figure below represents the view of the hemacytometer through a microscope. The circles represent cells that had previously been cultured in a Petri dish.

A 0.5 ml suspension of cells were removed from the Petri dish and mixed with 0.5 ml Trypan Blue solution. Recall that Trypan Blue is a stain that selectively stains dead cells. The green dots in the figure represent live cells and the blue dots are dead cells that have taken up the Trypan Blue.

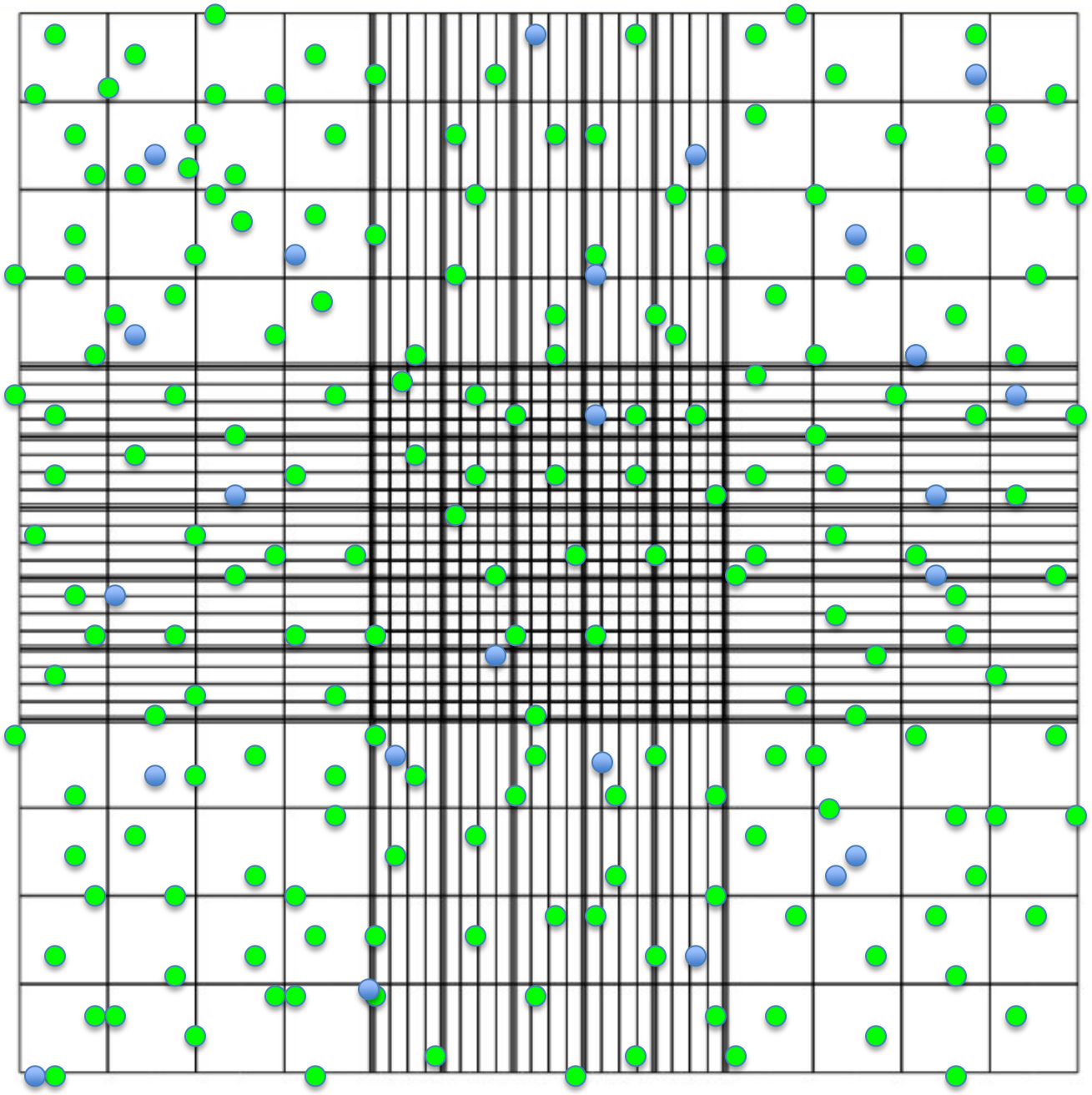
Live cells Dead cells stained with Trypan Blue

Chamber A



Blank grids from: http://www.microbehunter.com/2010/06/27/the-hemocytometer-counting-chamber/

Chamber B



Procedure

1. Count the live and dead cells in each of the four quadrants in each of the counting chambers (A & B) of the hemacytometer. Calculate the averages for each counting chamber.

Chamber A

Quadrant	#Live Cells	#Dead Cells	Total # Cells
1			
2			
3			
4			
Average #cells:			

Chamber B

Quadrant	#Live Cells	#Dead Cells	Total # Cells
1			
2			
3			
4			
Average #cells:			

2. Calculate the average #cells from chambers A & B

Average # Live Cells _____ **Average # Dead Cells_____** **Average # Total Cells_____**

3. Calculate the cell density.

 # Cells/ml = Average # cells X dilution factor x 10^4

The dilution factor for this example is 2 because 0.5 ml of cell suspension was diluted with 0.5 ml Trypan Blue.

 # Cells/ml = _____ X 2 x 10^4

 # Cells/ml = _____

4. Calculate cell viability

Average # live cells / Average # total cells x 100

_____ / _____ x 100

Cell viability = _____%

5. Why did only the dead cells take up the Trypan Blue stain and not the living cells? (Hint: think about the structure and function of a cell)