K Johnson
Fall23-BIOL4140

Protocol

Counting of Monolayer Cells

As we have discussed in lecture, if cells are not regularly subcultured it can lead to many problems – including senescence, apoptosis and transformed phenotypes. As a precaution, cells are often maintained on a regular schedule and when split for experiments cells are quantitated so that every experiment can begin with the same cell density. It is important to become comfortable with using a hemocytometer to quantitate cells.

Materials

Nonsterile

- *Inverted or EVOS microscope*
- *Hemocytometer*
- *10ml Cell Suspension*
- *P20 Pipette and tips*

Procedure (For the trypsin procedure see the Wk3-Protocol Subculture Dilution.)

1. Mix the suspended cells thoroughly by **pipetting the solution into 15mL conical tube.** Cap the tube tightly and mix by inverting the tube several times.
2. In the hood**, transfer 0.2ml of your cell suspension** to a sterile **1.5 mL microtube.**
3. Outside of the hood, **transfer 10µL of your mixed cell suspension to each side of the hemocytometer chamber**. Slowly expel the suspension and let it be drawn up by **capillary action**. Do not overfill and avoid bubbles in the chamber.
4. Under the inverted scope, find and focus on the grid at 4x objective. To count the cells in the center square, move up to the 20x objective.
5. You can either count a **1mm square (red square) or 5 of the smaller squares (yellow-Figure)** in the center. Count the number of cells in **5 of the small squares (indicated in yellow below),** across the diagonal of the larger $1mm^2$ square. **Count both sides of the hemocytometer**, individually, and take the average cell number to feed into the equation below.
    a. Note: for more precise quantitative experiments, **more than 50 cells should be counted.**
    b. If needed, **you can count all 9 of the large squares**. You may need to move to a smaller objective. If you count the **full grid, this will be the number of cells per 900nl, rather than 100nl.**
6. Derive the concentration of your sample using the following formulas:

    When counting 5 yellow squares:

    Cells/ml = {(n x 5)/100nl} x 10^6

    When counting 1 red square:

    Cells/ml = (n/100nl) x 10^6

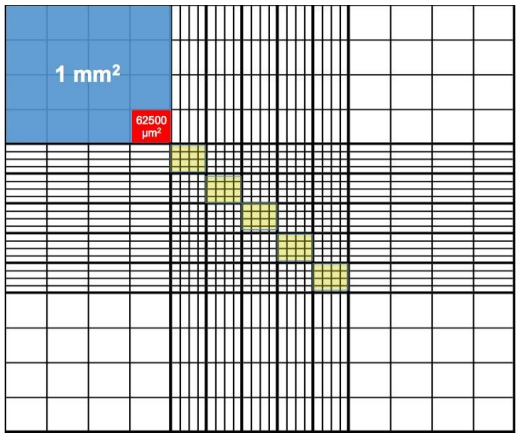
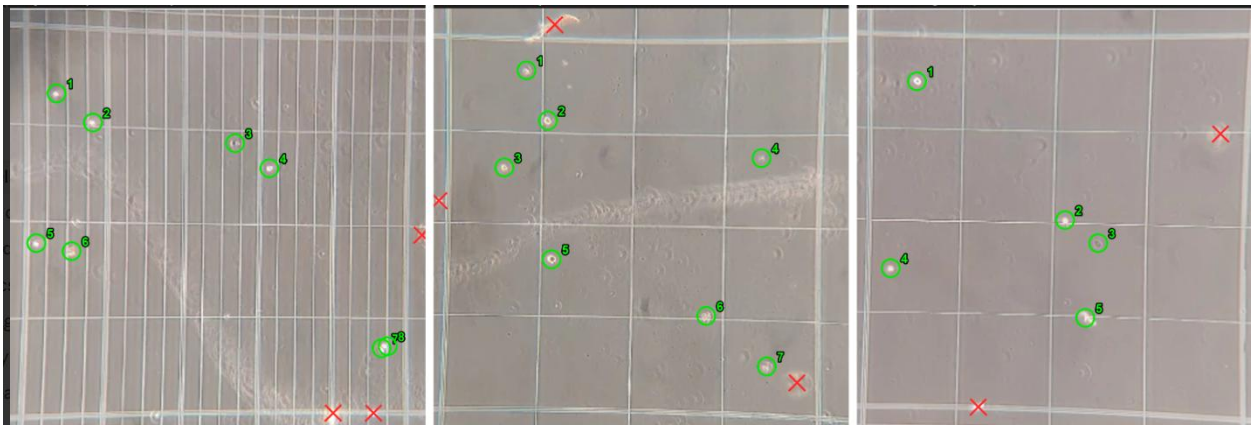


Figure 1. Hemocytometer Grid. The image shows the 9 squares printed on the Hemocytometer chip. The blue square has an area of 1mm^2 and holds a volume of 100nL. The red square has an area of 0.0625mm^2 and hold 6.25nL. The yellow squares have an area of 0.04mm^2 and hold 4nL.



7. **Calculations**
   - **a.** Using the counts you obtain from your cell suspension to calculate the following:
     - i. The number of cells/ml

     - ii. The number of total cells in a 10ml suspension.

   - b. Calculate and describe how you will prepare the following flasks. Note, you have calculated cells/ml, but you are seeding a total number of cells in T25 flasks.
     - i. 1 x 10^6 cells/flask

     - ii. 5 x 10^5 cells/flask