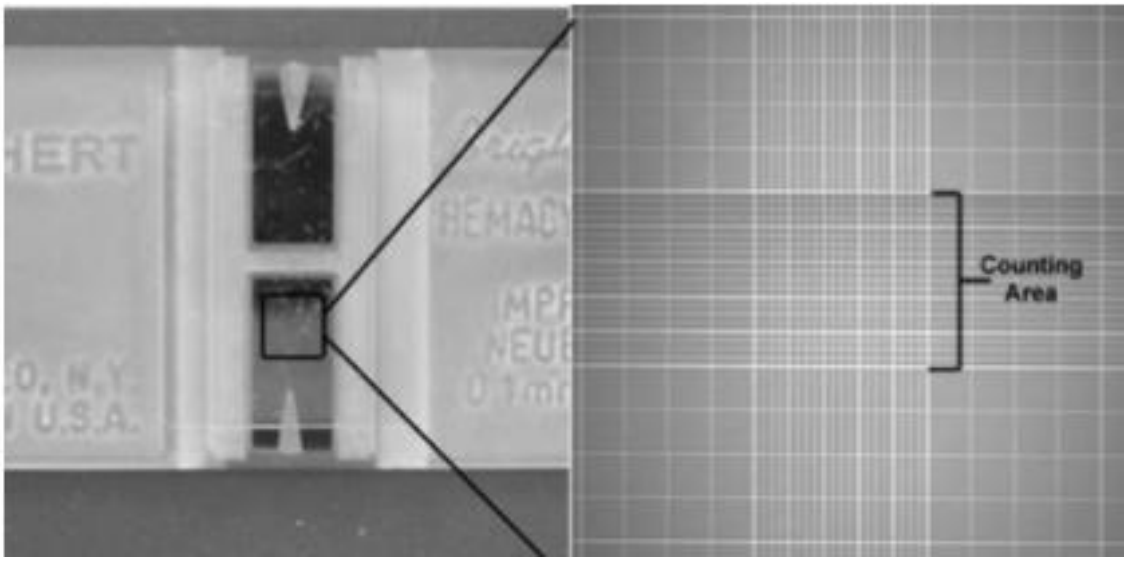
APPENDIX G: Basics of Using a Hemocytometer

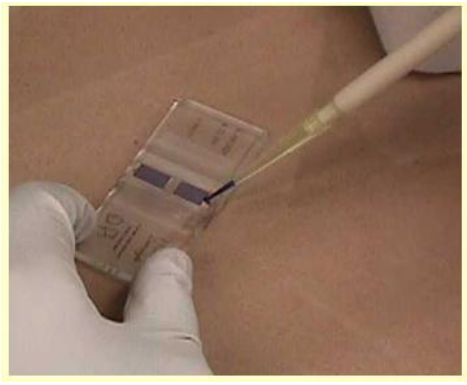
Introduction

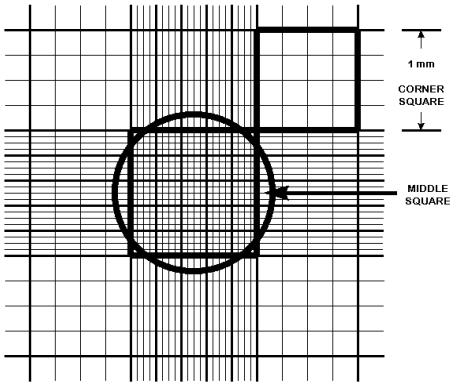
As part of the Signal Transduction exercise you will use a cell counting chamber called a **hemocytometer**. As you prepare for that exercise you should also review the instructions for proper use of a microscope. A hemocytometer is a specialized microscope slide that allows you to easily count the number of cells in the microscope's field of view to determine the number of cells per milliliter in a sample. Familiarity with use of a hemocytometer is essential to accurately collect your data in the signal transduction exercise. The purpose of this document is to familiarize with the use and interpretation of the hemocytometer.

The hemocytometer

A hemocytometer is a specialized microscope slide that contains an etched glass counting chamber for enumerating the number of cells in a suspension. The chamber holds a specific volume of liquid and has grid lines etched into the glass so that cells can easily be counted using a microscope.







At the center of the chamber (there're actually 2 chambers on each hemocytometer) the etched lines form a 25 square (5 squares by 5 squares) grid (Notice that there are 16 smaller squares etched into each of the 25 squares. These are a visual aid for counting cells). This is the area of the chamber that you want to use for counting. When a coverslip is placed on the hemocytometer, the dimensions of ALL 25 squares are:

0.1 cm X 0.1 cm X 0.01 cm or 1 / 10,000th of a milliliter

That makes the volume of the full 25 squares to be 1/10,000 of a milliliter. Therefore, counting the number of cells in one of those 25 squares, then multiple by 25 (since you need to know how many cells are in the full 25 squares) and then multiplying that number by 10,000 would give you the cells/ml of the cell suspension. Counting the cells in only one of the 25 squares would be a statistically unreliable measurement. So you try to count all the cells in the all of the 25 squares or count 5 squares then multiple by 5, instead of 25.

Three DIFFERENT examples of counts made being converted to calculate the concentration (cells/ml) in the starting solution:

Example 1: Added **500 µl of cells to 1000 µl of iodine** then put on a hemocytometer and counted 150 cells in all 25 squares (10X-magnification) on the hemocytometer grid. To calculate the concentration, do the following:

Concentration = 150 cells x 3 (dilution in the iodine) x 10,000 (dilution putting on the hemocytometer) = 4.5×10^6 cells/ml

Example 2: Added **500 µl of cells to 1500 µl of iodine** then put on a hemocytometer and counted 10 cells in ONE square (4x4 square at 40X-magnification) on the hemocytometer grid. To calculate the concentration, do the following:

Concentration = 10 cells x 4 (dilution in the iodine) x 25 x 10,000 (dilution putting on hemocytometer) = 1.0×10^7 cells/ml

Example 3: Added **500 µl of cells to 500 µl of iodine** then put on a hemocytometer and counted 60 cells in FIVE squares (five squares of the 25-square at 10X or five of the 4x4 squares at 40X-magnification) on the hemocytometer grid. To calculate the concentration, do the following:

Concentration = 60 cells x 2 (dilution in the iodine) x 5 x 10,000 (dilution putting on hemocytometer) = 6.0×10^6 cells/ml

Procedure for using a hemocytometer

1 Collect a hemocytometer from your TA *(hemocytometers are expensive; please handle it carefully)*

2 Transfer 10 µl of your cell suspension to the hemocytometer chamber and place cover the chambers with a coverslip.

3 Observe your sample at the lowest magnification and observe and familiarize yourself with the pattern of grid lines ***(Lowering the condenser will increase contrast if you have trouble seeing the grid lines)***. Find the central 25 square counting area.

4 Increase to higher magnifications using the usual focusing procedure *(Be careful though. The thickness of the hemocytometer makes it easy to scratch the higher power objectives.)*

5 Count the number of cells in each of the 25 squares of the counting region and record the values in your lab notebook to get a total number of cells in the 25 sqaures. *(Hint: A good strategy for this is to create a 5 X 5 cell table in your notebook ahead of time to record your data).*

6 Calculate the concentration of your cells in your suspension (cells/ml) as described above. Show your calculations and record your results in your lab notebook.

7 When you are done for the day**, clean your hemocytometer and *return it to your TA*.**