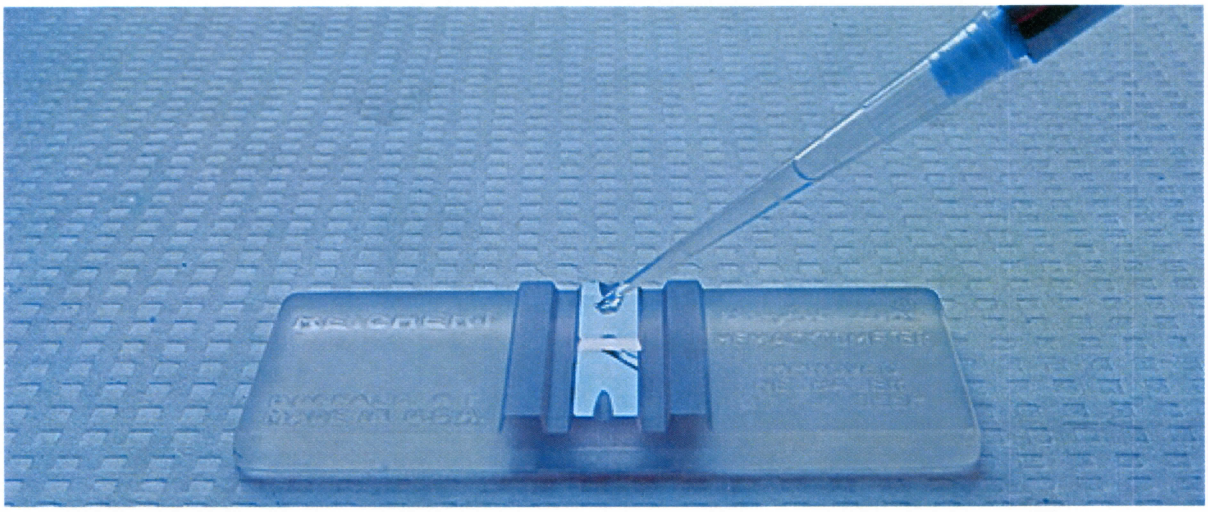
Cell Counting with a Hemocytometer:



Many biological applications such as microbiology, cell culture, blood work and many others that use cells require that we determine cell concentration for our experiment.

Cell counting is rather straightforward and requires a counting chamber called a hemocytometer, a device invented by the 19th century French anatomist Louis-Charles Malassez to perform blood cell counts. A hemocytometer consists of a thick glass microscope slide with a grid of perpendicular lines etched in the middle. The grid has specified dimensions so that the area covered by the lines is known, which makes it possible to count the number of cells in a specific volume of solution.

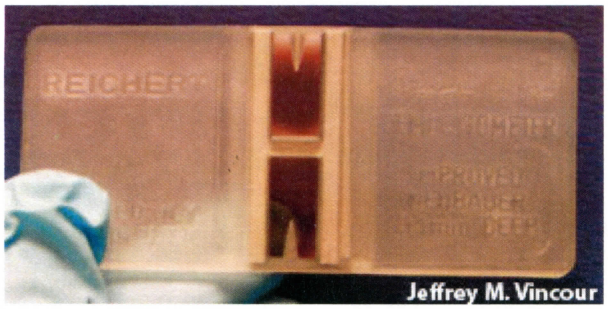


Figure 1. A Classic Hemocytometer

The most common type of hemocytometer has an "H" shape engraved in the middle that encloses two separate mirror-like polished grid surfaces and provides the cover slip mounting area (Figure 1).

Loading the hemocytometer

Before starting ensure that both the hemocytometer and its coverslip are clean by removing any dust particles with lens paper. Coverslips that are used for mounting on hemocytometers are

specially made to be thicker than the conventional microscopy coverslips because they must be able to overcome the surface tension of a drop of liquid.

Make sure to first place the coverslip over the counting surface before loading the cell suspension. Then place the pipette tip with your sample into one of the V-shaped wells, as in Figure 2, and gently expel the sample. The area under the coverslip fills by capillary action. Enough liquid should be introduced so that the mirrored surface is just covered, usually around 10 µl, but do not overfill the surface. You can load two samples on one hemocytometer, one into each of the two grids.

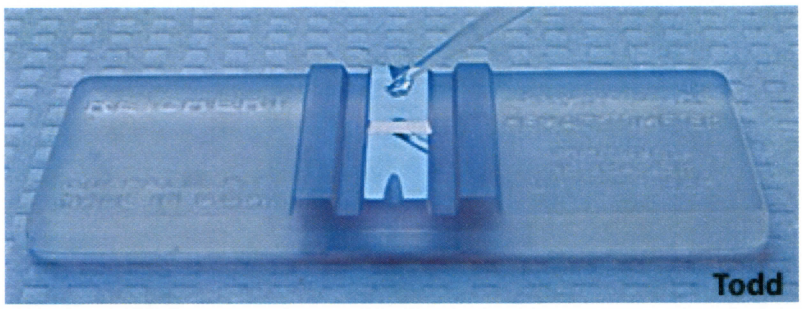


Figure 2. Loading the Hemocytometer

The loaded hemocytometer is then placed on the microscope stage and the counting grid is brought into focus at low power. Allow the sample to settle for a couple of minutes and avoid moving the coverslip as it might introduce air bubbles and make counting difficult.

Counting cells in a hemocytometer

The full grid on a hemocytometer contains nine squares, each of which is 1 mm^2 (Figure 3). The central counting area of the hemocytometer (Figure 3B) contains 25 large squares and each large square has 16 smaller squares. When counting, count only those cells on the lines of two sides of the large square to avoid counting cells twice (Figure 3G). Suspensions should be dilute enough so that the cells or other particles do not overlap each other on the grid, and should be uniformly distributed.

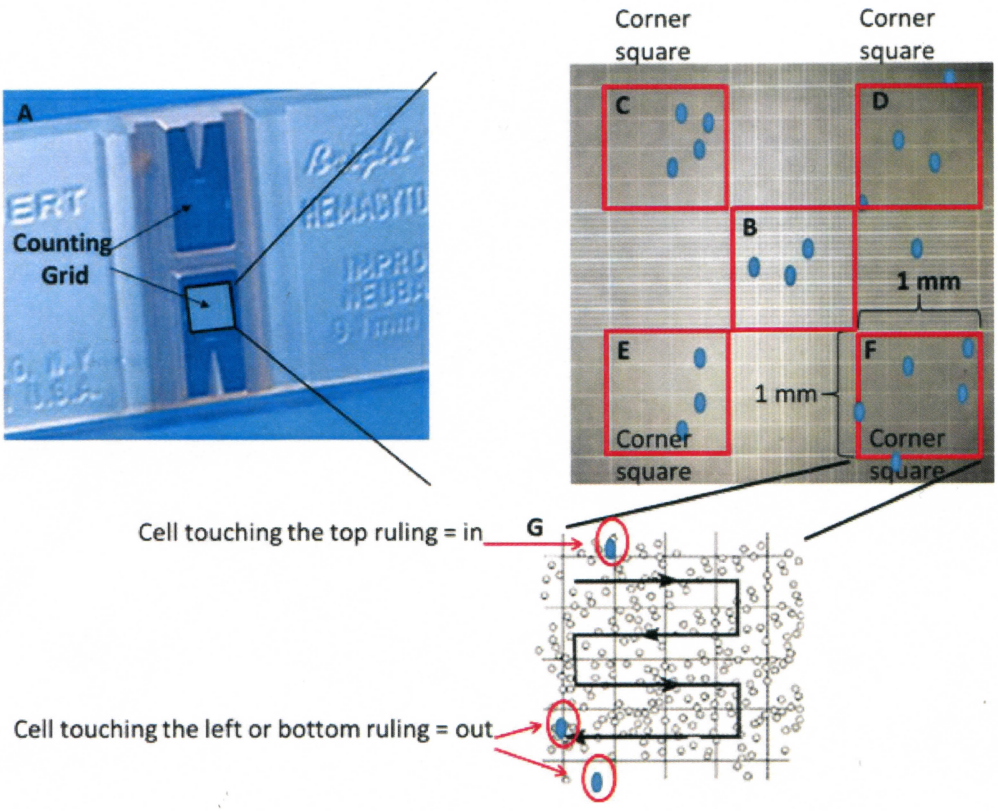


Figure 3. Counting Cells on the Hemocytometer.

To distinguish between dead and viable cells, the sample is often diluted with a particular stain, such as Trypan blue. This staining method, also known as dye exclusion staining, uses a diazo dye that selectively penetrates cell membranes of dead cells, coloring them blue, whereas it is not absorbed by membranes of live cells, thus excluding live cells from staining. When viewed under a microscope, dead cells would appear as dark blue (Figure 4)

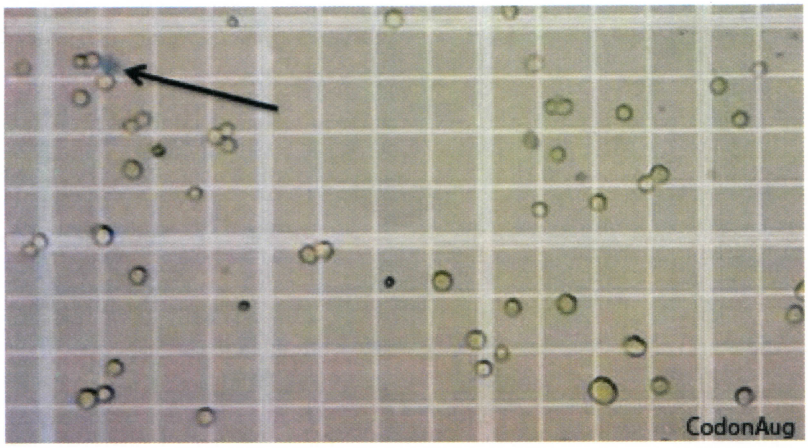


Figure 4. Trypan Blue Exclusion of Live Cells on the Hemocytometer. (Arrow indicates uptake of dye across the membrane of dead cells.)

To perform the count, determine the magnification needed to recognize the desired cell type and systematically count the cells in selected squares so that the total count is approximately 100 cells, a minimum number of cells needed for a statistically significant count.

For large cells, you can simply count the cells inside the four large corner squares (Figure 3C-F) and the middle one (Figure 3B). For a dense suspension of small cells you may wish to count the cells in the four outer and middle squares of the central square (Figure 3B) or make a more dilute suspension.

Remember if a cell overlaps a ruling, count it as "in" if it overlaps the top or right ruling, and "out" if it overlaps the bottom or left ruling (Figure 3G).

The area of the middle (Figure 3B) and each corner square (Figure 3C-F) is 1 mm x 1 mm = 1 mm^2: the depth of each square is 0.1 mm. The final volume of each square at that depth is 100nl.

Once you have obtained the total cell count, cell concentration can be calculated from the following formula:

$$\text{Total cells/ml} = \text{Total cells counted} \times \frac{\text{dilution factor}}{\text{\# of Squares}} \times 10{,}000 \text{ cells/ml}$$

So, for example, if you diluted your sample 1:1 with Trypan blue, and you counted 325 cells in 4 corner squares plus the central big square, total cells per ml =

$$325 \text{ cells} \times \frac{2(\text{dilution factor})}{5 \text{ Squares}} \times 10{,}000 \text{ cell/ml} = 130 \times 10^4 \text{ cells/ml}$$

If you want to know how many cells you have in your original sample, just multiply the cell concentration by total sample volume. For example, if your original sample volume is 5 ml, then your sample has a total =

$$130 \times 10^4 \text{ cells/ml} \times 5\text{ml} = 650 \times 10^4 \text{ cells}$$