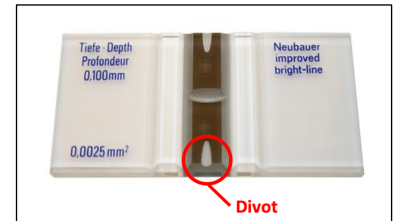


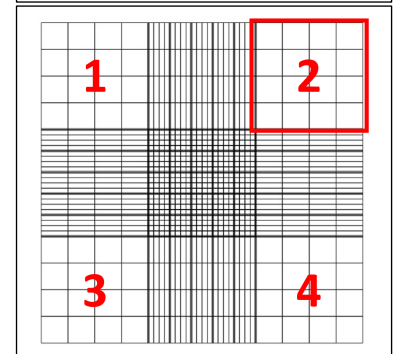
Protocol: Hemocytometer Cell Counting

The hemocytometer is used for counting cells to estimate density. In many cases, it is necessary to use a specific number of cells for an experiment.

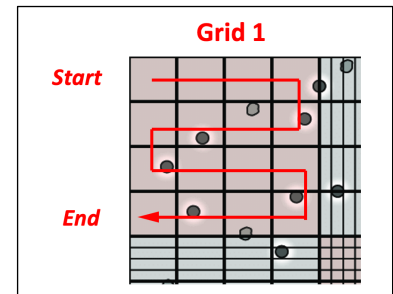
1. Perform a cell trypsinization and harvest in an appropriate volume of media.
2. Ensure cells in suspension are uniformly mixed. Mix by pipetting if the suspension has sat for a prolonged period of time.
3. Remove 20 μ l of the cell suspension to a microcentrifuge tube and bring this out of the hood.
4. Add 20 μ l of Trypan Blue to the cells. Mix by finger vortex.
5. Pipette 10 μ l of the cell-trypan blue mixture onto the divot(s) of the cytometer. Place a coverslip gently on top. This draws the solution into the counting chamber (see right).



6. Place the hemocytometer under the microscope. Using the lowest magnification bring the counting chambers into view. You should see a field of view similar to the one in the box to the right.



7. Turn the magnification to 10X (phase ring Ph1) and navigate to the upper left-hand grid (labeled #1 in the figure above). Count cells using a clicker counter. Start in the upper left-hand corner of grid 1 and move your line of sight following the pattern in the figure to the right, clicking as you come across cells. Count cells that lie on the lines of the grid, just try not to double-count as your eyesight moves.



8. Count each of the grids (1, 2, 3, and 4) and record your numbers for each grid in your lab notebook. For more accurate counting, perform the same procedure for the divot on the other side of the hemocytometer and count that chamber for a total of eight grids. Perform the calculations below.

AVG # of cells (in 4 or 8 squares)	Trypan Blue Correction (multiply by 2)	Hemocytometer Volume Correction (multiply by 10,000; units are cells/ml)

9. When done, add a small squirt of 70% EtOH to a Kimwipe and gently wipe down the coverslip and counting chambers.
10. Perform your dilution using $(M_1)(V_1) = (M_2)(V_2)$.