



# Hemocytometer Counting

*adapted from Mather, J.P., and P.E. Roberts, 1998. Introduction to Cell and Tissue Culture: Theory and Technique. Plenum Press. New York and London.*

The hemocytometer is commonly used to determine the concentration of cells in a cell suspension.

## Materials:

1. Cell suspension
2. Hemocytometer with cover-slip (improved Neubauer)
3. Tally counter
4. Pasteur pipettes
5. Microscope

## Procedure:

1. Place the coverslip over the hemocytometer counting chamber and using a Pasteur pipette, place a drop of the cell suspension at the edge of the "V" shape of the chamber. Allow the suspension to be drawn into the chamber by capillary action. Care should be taken not to overfill or underfill the chamber. Fill the opposite chamber in the same manner.
2. Place the chamber on the microscope stage.
3. The hemocytometer consists of nine 1 mm squares divided into smaller squares. One of the 1 mm squares represents a volume of  $0.1 \text{ mm}^3$  or  $10^{-4} \text{ ml}$ . Using the 10X objective, count the number of cells in a 1 mm square area (see figure 1). If there are fewer than 100 cells in a square mm, 2 or more 1-mm square areas should be counted and the results averaged.
4. Use the same procedure to count the cells on the other side of the hemocytometer.
5. To calculate the concentration of the cells, first calculate the average of all  $1 \text{ mm}^2$  areas counted and apply this formula:

$$c = n/v$$

where:

c = cell concentration in cells/ml

n = avg. number of cells/ $\text{mm}^2$  area

v = volume counted =  $10^{-4}$

Thus:

$$c = n \times 10^{-4}$$