DETERMINING THE RATE OF CELL DIVISION

The rate of cell division can be calculated from the concentrations of cells growing in culture. In part of this week's lab, you will set up a culture of *Tetrahymena* cells and determine the concentration of cells. Next week, after the cells have grown for 7 days, you will determine the concentration of cells in the culture again and, using the two concentrations, calculate the average number of cell divisions occurring per day.

Procedures
1) Collecting the cells.
a. Obtain a sterile, covered Erlenmeyer flask containing growth medium (0.5% proteose peptone, 0.25% soytone, autoclaved). The volume of medium in the flask = 50 mL. Place the flask on an area of the laboratory bench that has been cleaned with 70% ethyl alcohol.
b. This step needs to be done carefully to avoid contaminating the culture with bacteria that are present in the air and on the surfaces of containers (see section on sterile technique below). Using a sterile 1.0 mL pipet, fill the pipet with 0.7 mL of cell suspension from the tube containing stock culture (fill the pipet to the 0.3 mL mark), and then expulse 0.2 mL to the flask containing the medium.
c. Transfer the remaining 0.5 mL of stock suspension to a small, empty test tube.
d. Label the flask with your initials and place it in the cabinet until next week.

2) Determining the starting cell concentration using the hemocytometer.
a. To the test tube of cells (in c above), add an equal volume (0.5 mL) of buffered formalin.
b. Mix the formalin with the cells by covering the tube with Parafilm and inverting the tube 2 or 3 times.
c. Place the tube in a rack or foam rubber holder and let the tube sit for 2 min. Recuperand the cells by inverting the tube 2 or 3 times.
d. Using a non-sterile pipet, draw up some of the uniformly distributed cell suspension.
e. Apply gentle pressure to the bulb, forcing the cell suspension to the tip of the pipet. Carefully fill one chamber of the hemocytometer.
f. Count the cells in the four corner squares and the central square (numbered 1-5 in the figure on the next page) and average them by dividing by 5. Include in your counts cells that lie across the left and top borders of a square, and ignore the cells lying on the right and bottom sides.
g. The average multiplied by 10^4 gives the concentration of cells (in cells/mL) in the tube containing the formalin. To calculate the concentration of cells in the stock culture, multiply by 2 to correct for the dilution with formalin.
h. To calculate the concentration of cells in the new culture flask, first compute the number of cells added to the flask by multiplying the concentration of cells in the stock culture (in cells/mL, determined in g) by the volume of cells added (0.2 mL), and then divide this number by the final volume in the culture flask (50.0 mL). Final units will be cells/mL.

Next week:
3) Determining the final cell concentration.
a. Swirl the flask to mix the cells, and remove 0.5 mL of cell suspension.
b. Place the cell suspension in a small test tube, and add 0.5 mL of buffered formalin.
c. Mix cells with the formalin and let the tube sit for 2 min.
d. Count the cells using the hemocytometer.
e. To calculate the final concentration of cells, determine the average number of cells in the 5 squares, multiply by 10^4 (to convert to cells/mL), and multiply by 2 (to correct for the dilution with formalin).