Counting Cells with Hemocytometer

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Abstract

Counting cells with a hemocytometer is an easy way to determine relatively accurate numbers of viable cells. After determining cell counts, cells can be passaged, frozen away, or used for an experiment at a particular density.

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Guidelines

Keep cells sterile by wearing gloves, ethanol treating your hands and surfaces, using sterile pipette tips, and ***only opening vials with cells in the BSL-2 laminar flow hood***.

Materials

Trypan Blue 100 mL 7050 by Stemcell Technologies

Complete DMEM (DMEM 10% HI FBS 50ug/mL Gentamycin) by Gibco - Thermo Fischer

✓ Hemocytometer (Neubauer) by Contributed by users

✓ Compound Microscope by Contributed by users

Protocol

Step 1.

Collect cell suspension

Cell suspension must be in a known volume. Adherent cells can be detached by methods appropriate for your particular cell line.

Step 2.

Dilute an aliquot of cells in Trypan Blue

Use a 1:10 dilution for many cells, 1:2 dilution if working with fewer cells

Need a total volume of 10ul; Can do 10ul of cell suspension into 90ul Trypan Blue (1:10) to ensure plenty of excess cells and limit pipetting errors associated with pipetting volumes less than 10 ul

Step 3.

Add cells to hemocytometer

Using a P10 micropipette, transfer 10ul of cell suspension + Trypan Blue under the cover slip on a hemocytometer

Step 4.

Count Cells

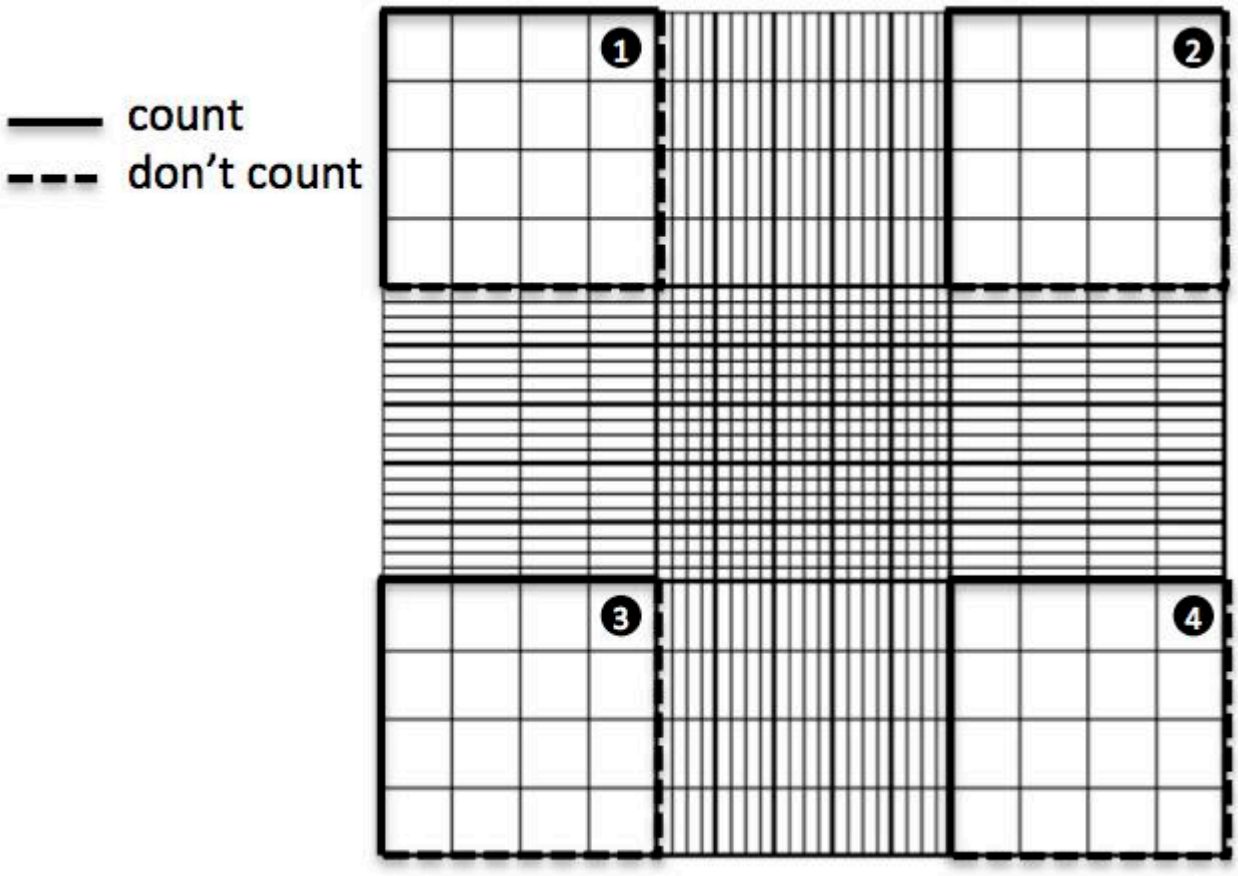
Observe the hemocytometer under the compound microscope

Count the cells within the squares

Count cells on 2 of the 4 perimeter lines

Only count live (non-blue) cells

Valid counts should include at least 50 cells



Step 5.

Calculate cell concentration

Cells/mL = (Cell count)/(number of chambers counted) x dilution x 10^4

EX: If you diluted your cells 1:10 in trypan blue, counted chambers 1 and 4, and counted a total of 150 cells:150/2 = 75 x 10 x 10^4 = 7.5 x 10^6 cells/mL

NOTE: Concentration (cells/mL) x total volume (mL) = total number of cells