Counting Cells with a Hemocytometer

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1 Clean hemocytometer and cover slip

2 Dilute the resuspended cells 1:4 in fresh complete media
Optional: Instead of media stain suspended cells with Trypan Blue (100 uL of cells 400 dye). The digital microscopes in C230 don't usually require the Trypan Blue stain

3 Fill hemocytometer with 10 ul each side cell suspension, on both sides. Filling only one side will cause inaccuracies in the volumes and therefore the calculations.

4 Using a 10x microscope, focus on the grid lines then find the first 16 block (4x4) grid in the upper left quadrant of the hemocytometer

5 Count the number of cells in the 16 block grids found in each corner of the Hemocytometer counting only cells fully within the grid as discussed in the attached file

Hemocytometer Cell Counting example.pdf

6 Record cell numbers and count other quadrants

7 To calculate the number of viable cells/mL:

Take the average cell count from each of the sets of 16 corner squares (1mm2 4x4 grid).

Multiply by 10,000 (10^4).

Multiply by 5 to correct for the 1:5 dilution from the media/Trypan Blue addition.

The final value is the number of viable cells/mL in the original cell suspension.

8 In the biosafety cabinet, clean the hemocytometer and the coverslip with 70% ethanol.