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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 (10⁴).

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.