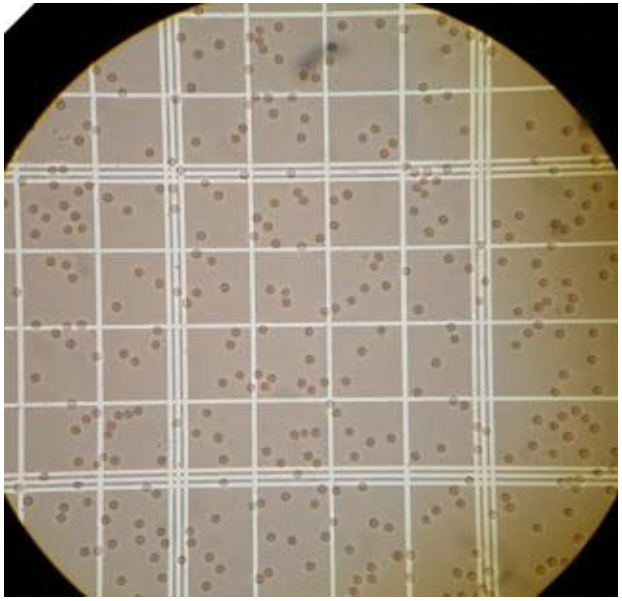


Cell Quantitation

Accuracy and Subculture

1) One flask evenly into several (1:5)

2) Estimate the percent confluence of a flask and determine how much you need for a lower percentage.

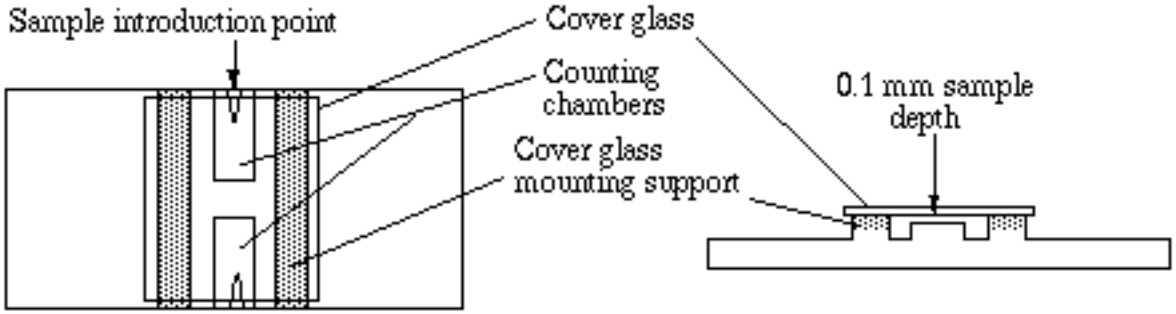
3) Count cell number and seed an exact cell number



QUANTITATION OF CELLS

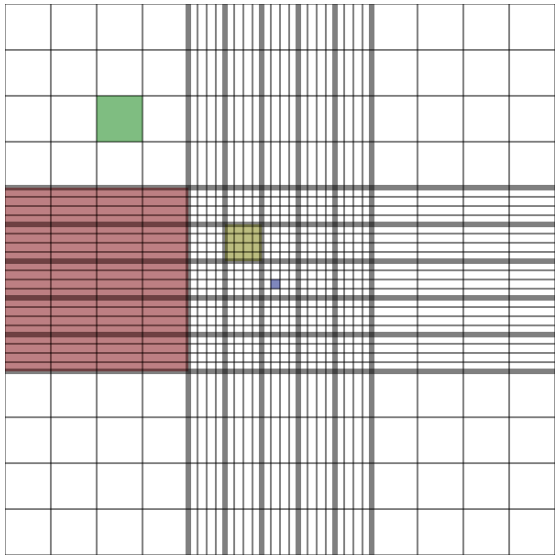
hemocytometry





QUANTITATION OF CELLS

hemocytometry

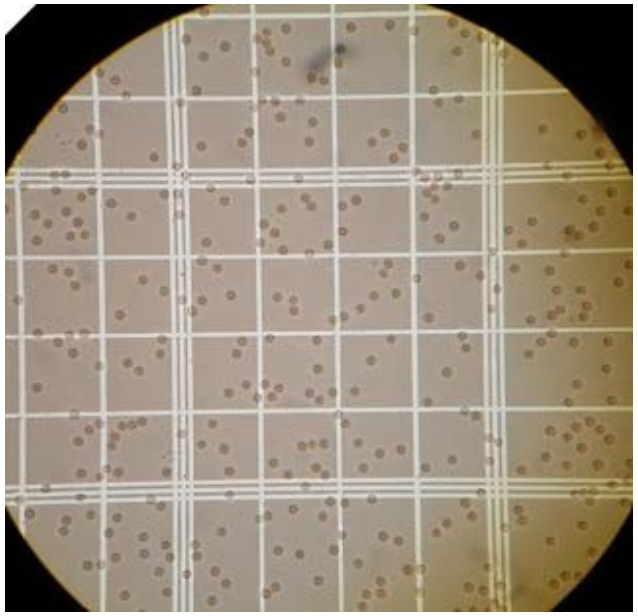


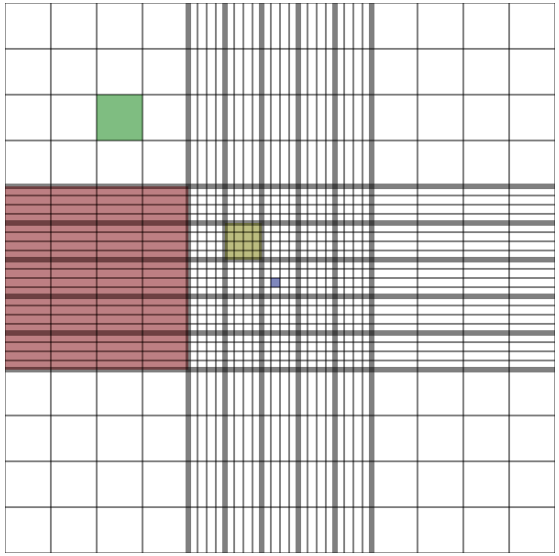
Based on grid counts, you can calculate the number of cells/mL

Dimensions	Area	Volume (0.1mm depth)
1 x 1 mm	$1mm^2$	100nL
0.25 x 0.25 mm	$0.0625mm^2$	6.25nL
0.2 x 0.2 mm	$0.04mm^2$	4nL
0.05 x 0.05 mm	$0.0025mm^2$	0.25nL

QUANTITATION OF CELLS

hemocytometry





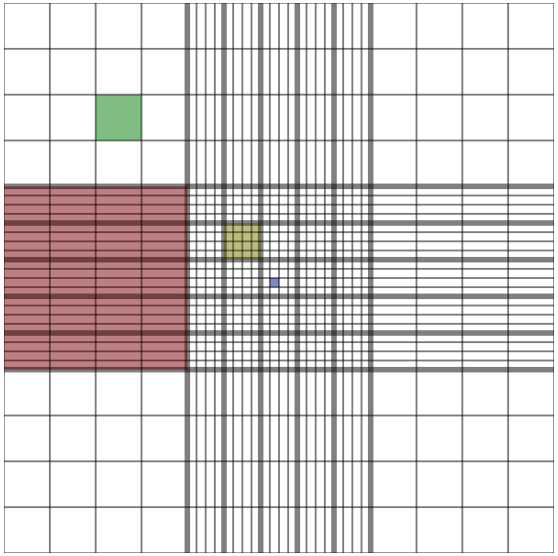
S1 = 56
S2 = 62
S3 = 44
S4 = 65
S5 = 51

When counting 1 red square:

Cells/ml = (n/100nl) x 10^6

When counting 5 yellow squares:

Cells/ml = {(n x 5)/100nl} x 10^6



When counting 1 red square:

Cells/ml = (n/100nl) x 10^6

When counting 5 yellow squares:

Cells/ml = {(n x 5)/100nl} x 10^6

1390 cells/100nL = 13.90 cells/nl

nl is 1,000,000th of a ml

Cells/ml= 13.9 x 10^6 cells/ml = 13,900,000 cells/ml

13.9 cells/ml then in 3ml 41.7 x 10^6 cells

Cells / flask?

a. 1×10^6 cells/flask

1×10^6 cells/5ml = 2×10^5 cells/ml

C1 V1 = C2 V2

~~13.9×10^6 cells/ml (V1) = (2×10^5 cells/ml) (5ml)~~

13.9×10^6 cells/ml (V1) = 1×10^6 cells

V1 = 1×10^6 cells/ 13.9×10^6 cells/ml

V1 = 0.0719 ml or 72 ul

Determine Seeding Density

You know how to count and how to get a certain percentage of confluence when splitting into the same size flask.

For experiments, you will often need to split your cells from a flask to a multiwell plate.

Calculate the difference in surface area to determine what to seed.



Plate	Surface Area (mm^2)	Seeding Density	Growth Medium (ml)
24-well	200	0.05×10^6	0.5-1.0
12-well	401	0.1×10^6	1-2
6-well	962	0.3×10^6	3-5
60mm	2827	0.8×10^6	5
100mm	7854	2.2×10^6	10