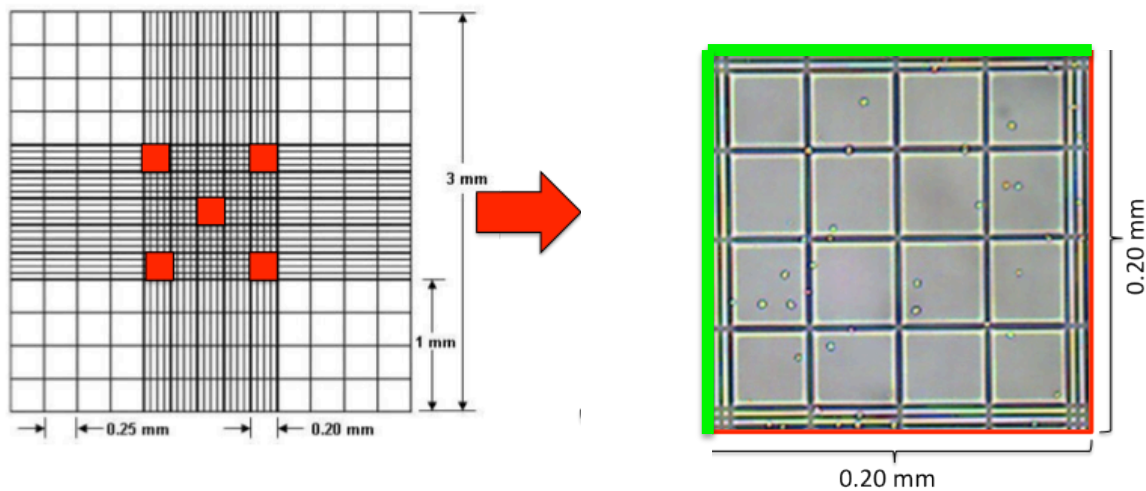


## Algae to Energy - Using and Re-using a Hemocytometer to Count Algae Cells

- 1) Prepare your sample by shaking your photobioreactor for at least 30 seconds and use a transfer pipet to remove 1 ml of algae. Add 10 ul of algae sample to slot A.
- 2) Count the cells. Using a compound microscope, count the number of cells in a square. Which size square you choose will depend on the density of your sample. If the sample contains a high density of cells, you will want to choose one of the smaller squares. If the sample contains a low density of cells, you will want to choose one of the larger squares. For the Algae to Energy lab, you will probably want to look at the small squares within the center square, highlighted in red, below. Choose 5 squares and count the number of cells in each square. If cells are on the border of the square, count only the cells touching the top and left sides of the square, as shown in green, below. Do not count the cells on the right and bottom sides of the square (shown in red)



- 3) Find the average cells/square. Add the number of cells from each square together and divide by 5 to get the average number of cells per square.
- 4) Calculate cell density.

$$\frac{\text{Average \# of cells per square} \times \text{Dilution Factor (if any)}}{\text{Volume of the square}} = \text{Cell Density}$$

In this example, the average cells per square is 27. *If your sample was diluted, you will need to multiply this number by your dilution factor.*

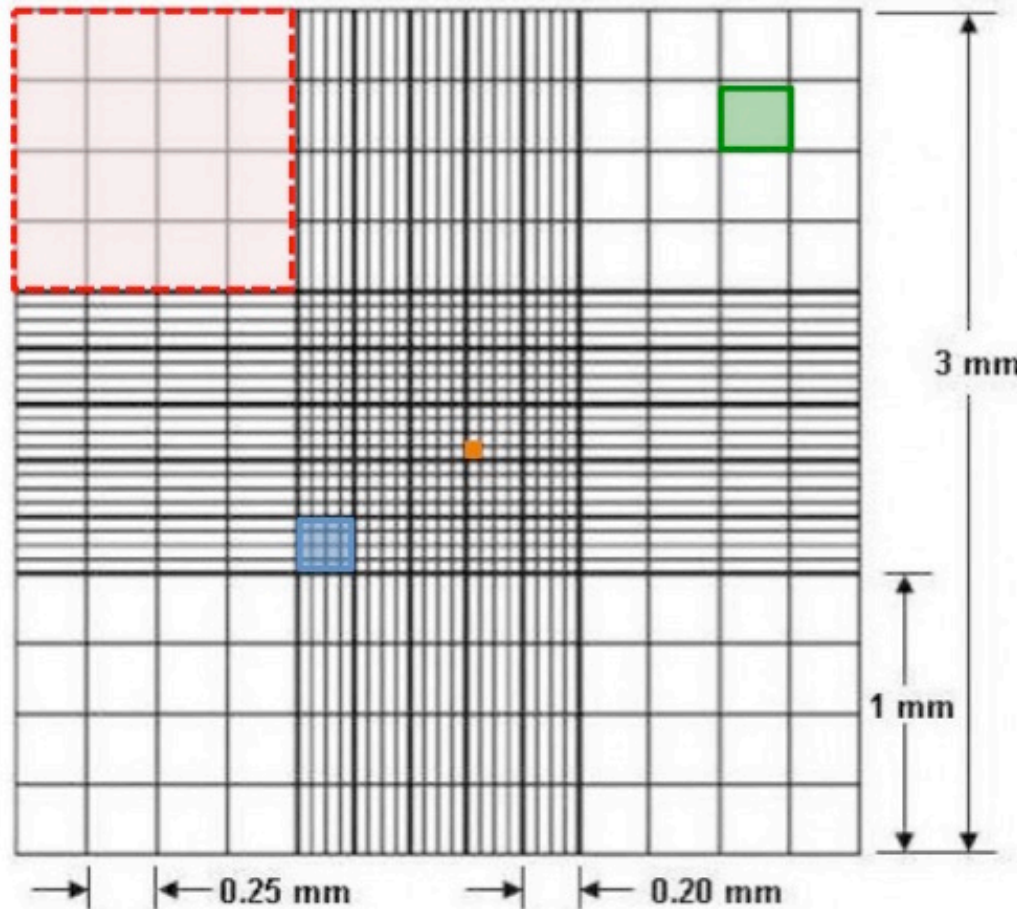
If you are counting the small center squares, shown above in red, the volume of the square is 0.000004 ml.

$$\text{Example: } \frac{27 \text{ cells}}{0.000004 \text{ ml}} = 6,750,000 \text{ cells/ml}$$

Refer to the table on the next page for the volume of other squares.

This table, courtesy of [hemocytometer.org](http://www.hemocytometer.org), will help you calculate the volume per square that you are counting. Colors refer to the diagram, below.  
(<http://www.hemocytometer.org/2013/04/11/hemocytometer-square-size/>)

Unit	Width	Area	Volume (mm <sup>3</sup> )	Volume (mL)	#	
chamber	3 mm	9 mm <sup>2</sup>	0.9 mm <sup>3</sup>	0.0009 mL	2	per hemocytometer
Square (red)	1 mm	1 mm <sup>2</sup>	0.1 mm <sup>3</sup>	0.0001 mL	9	per chamber
Small square (green)	0.25 mm	0.0625 mm <sup>2</sup>	0.00625 mm <sup>3</sup>	0.00000625 mL	16	per corner square
Smaller square (blue)	0.2 mm	0.04 mm <sup>2</sup>	0.004 mm <sup>3</sup>	0.000004 mL	25	per central square
Smallest square (orange)	0.05 mm	0.0025 mm <sup>2</sup>	0.00025 mm <sup>3</sup>	0.00000025 mL	16	per smaller square



- 5) Clean out the hemocytometer for reuse. Though labeled as disposable, these hemocytometers can be cleaned and reused multiple times. Once the sample has been counted, use compressed air to expel the sample from the chamber by making a quick burst of air into the overflow space. Add distilled water to the A slot and expel it with the compressed air. Repeat this process three more times. Check your hemocytometer under the microscope to confirm that it is clean before reusing.
- a. The cleaning process can be seen here:  
<https://www.youtube.com/watch?v=69T755-J33A>  
(cleaning begins at the 4:15 mark)

