"Counting Stars Cells"

S4 - S6 Senior Phase

Aim - To investigate the effect of fertiliser concentration on the growth of algae.

N5 Biology, Unit 3, KA5: Food production.
N5 Environmental Science, Unit 3, KA2: Food.
Higher Biology, Unit 3, KA1: Food Supply.

Step 1: Growing Chorella in the presence and absence of fertiliser.

Algae are grown in distilled water and in three different concentrations of fertiliser solution (0.1, 0.2 and 0.3%). Students can compare the size of algal populations after a defined period of time by performing a cell count using a haemocytometer.

Materials

- 50 ml measuring cylinder
- 4 x 250 ml conical flask
- Cotton wool
- 1 x 5ml syringe
- distilled water
- suitable liquid plant fertiliser, e.g. BabyBio.

- 5 x 1ml plastic pipette for setting up cultures
- Access to a discard jar containing 1% Virkon
- Stock algae culture, e.g. Chlorella.
- Marker pen

1 Label each flask with the fertiliser concentration and add 250ml distilled water to each flask. Add the correct volume of fertiliser to each flask and 5ml of stock algal culture. Place used syringes and pipettes in discard jar.

Final concentrations of fertiliser are 0, 0.1, 0.2 and 0.3%.

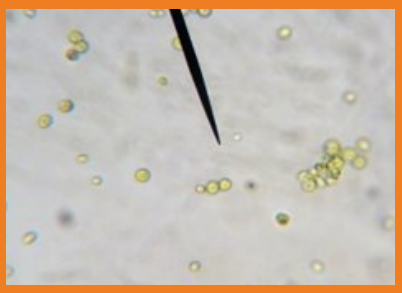
2 Swirl to mix the contents, plug flask necks with cotton wool and then leave cultures for 10-14 days.

Step 2: Performing a cell count using a haemocytometer.

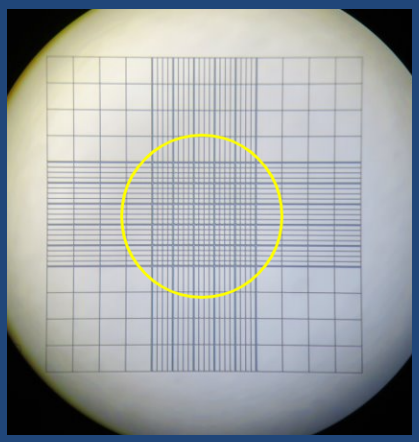
Following growth of algae in varying concentrations of fertiliser, the number of algal cells in the culture can be estimated using a haemocytometer.

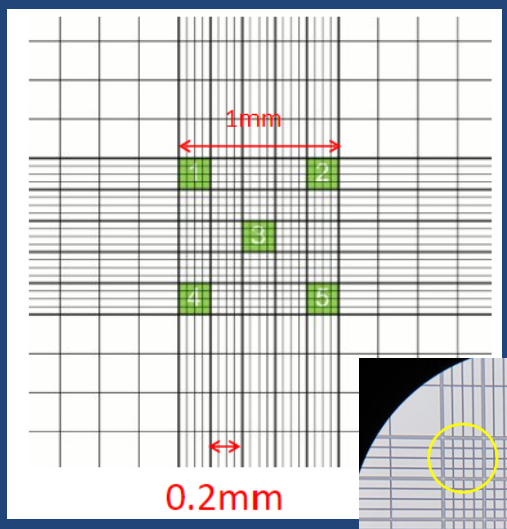
Materials

- Light microscope
- C-Chip/glass haemocytometer
- Capillary tube
- Chlorella grown in 0, 0.1, 0.2 and 0.3% plant fertiliser.

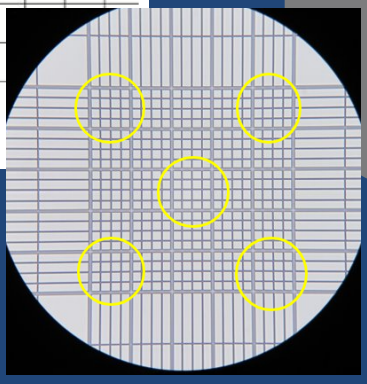


1 Mount the haemocytometer on the microscope stage and use the x4 objective lens to locate the counting grid within the chamber.



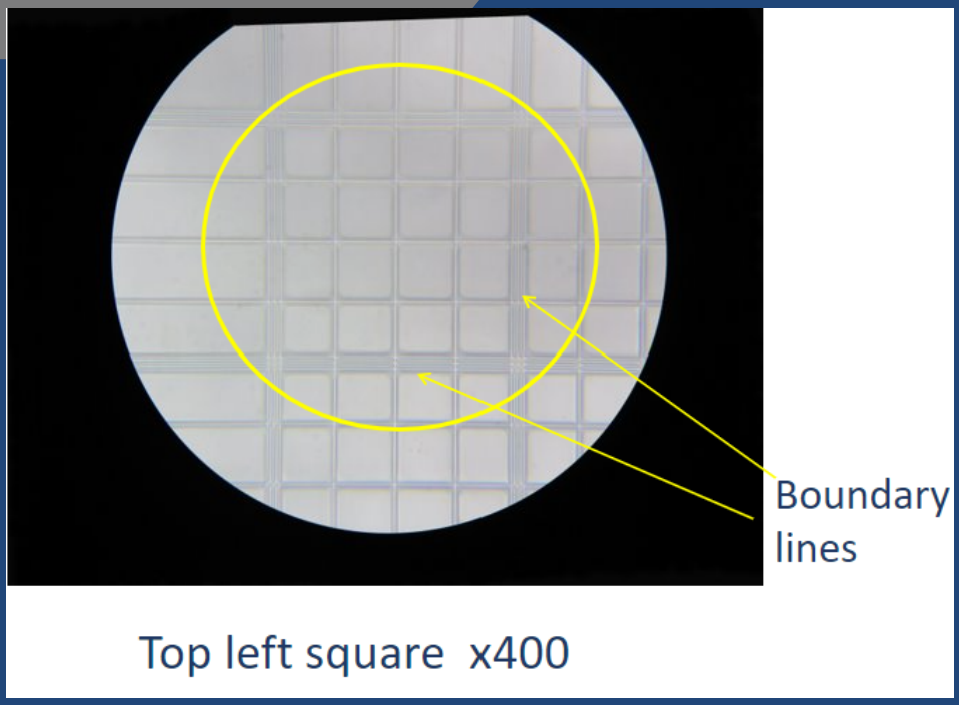


2 View the counting chamber at x100 magnification to identify the "sample" squares. For more concentrated samples, you will count the number of cells found within the green-coloured "sample squares" highlighted opposite.



3

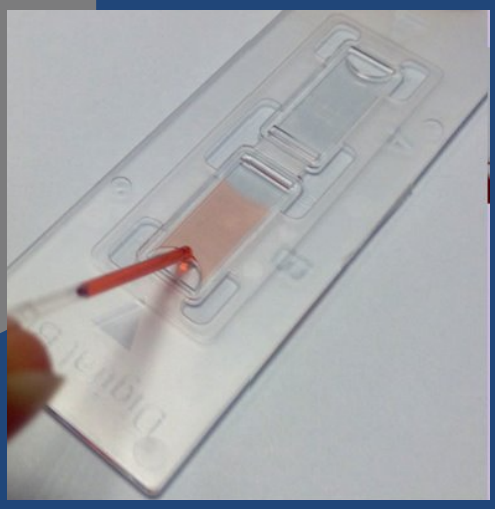
Adjust the objective lens to achieve x400 total magnification of the counting chamber. Find the "sample squares", as highlighted in green in Step 2. Practise moving around to locate all "sample squares" and take note of the boundary lines: each of the sample squares is composed of 16 boxes, shown by the yellow circle below.



Top left square x400

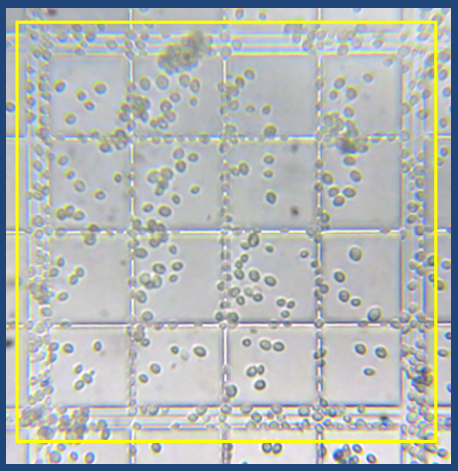
4

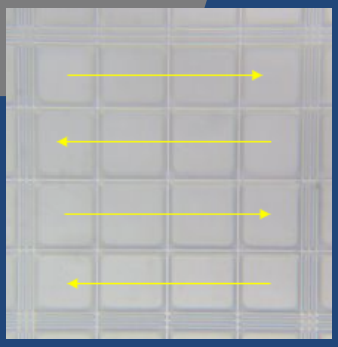
Using a piece of capillary tubing, load one of the sample chambers of the haemocytometer with the algae suspension that has been grown in water lacking Baby Bio (negative control). Observe at x40, x100 and x400 magnification. Check that you can locate the sample squares at x400. The image opposite shows a practise attempt using a red dye.



5

Count the number of cells in the 16 boxes of the top left sample square systematically, as highlighted in the image below. There are various apps available to help you record your counting if required.

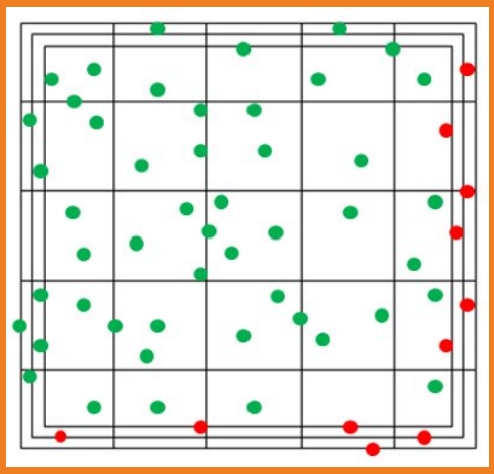




What should be counted?

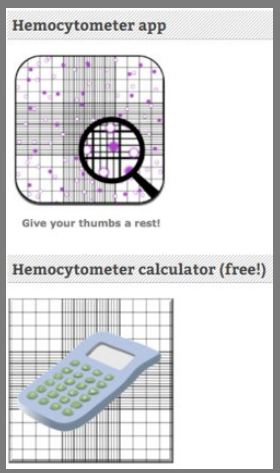
Count all the cells within the sample square and those touching the top and left boundary lines (as highlighted in green).

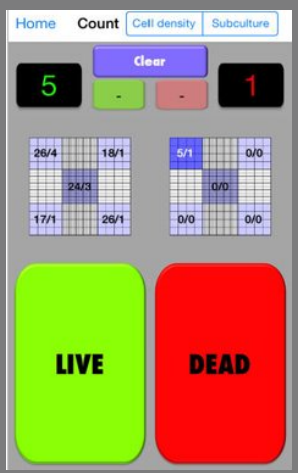
Do not count those touching the bottom and right boundary lines (example cells shown in red).



Apps for counting cells

- Hemocytometer App
- Hemocytometer Calculator (free)
- iPhone HemocyTap





6 Count the cells in all 5 sample squares and record these values.

Number of sample square	Number of cells counted
1	
2	
3	
4	
5	

7 Now you know the number of cells in 5 squares, it is time to estimate the number of cells in the whole grid. See orange box below.

<u>Volume in sample area (5 squares)</u>

Length of side of grid = 1 mm

Area of grid = 1 mm^2

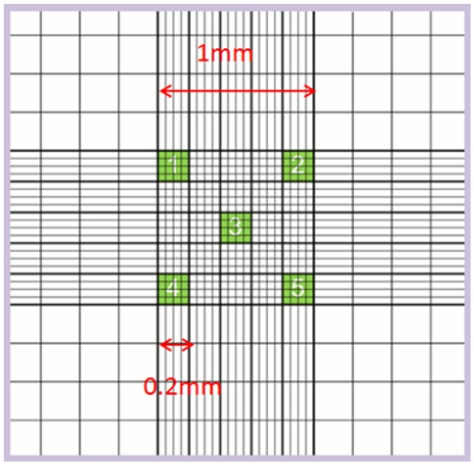
Depth between coverslip & slide = 0.1 mm

Volume under grid (25 squares) = 1 mm^2 x 0.1 mm

= <u>0.1 mm^3</u>

Volume under 5 squares = 0.1 / 5 mm^3

= <u>0.02 mm^3</u>



<u>Estimating cell concentration (5 squares)</u>

Number of cells in sample = n

Number of cells in 1 mm^3 = n x 50 (0.02 mm^3 is 1/50 of 1mm^3).

Number of cells in 1 cm^3 = <u>(n x 50) x 1000</u>.

If the concentration of cells is very low, all the cells in the 25 square grid should be counted. Remember the principles of a representative sample.