Counting cells using a haemocyto

Figure 1 - A conventional glass haemocytometer.

Within the *Cells and Proteins* unit of Advanced Higher Biology [1], "Aseptic technique and cell culture" is a mandatory course key area. Learners are required to be able to use a haemocytometer to make an estimate of cell count in a microbial culture and to use staining methods to estimate viable cell counts.

A haemocytometer is a specially designed microscope slide with a counting chamber of known depth which has a grid etched on to its lower surface. The grid enables direct counts of cells in a standard volume of culture to be carried out. There has for some time been a set of guidelines for the use of a conventional glass haemocytometer (Figure 1) on the SSERC website [2].

However, in recent workshops with teachers of biology we have been using disposable plastic *C-Chip™* haemocytometers (Figures 2 and 3).

Figure 2

Figure 3

There are several advantages to using these haemocytometers in school:
• They are much cheaper than the conventional glass version: approximately £50 for a pack of 50, as opposed to £50 for 1 [3].
• They do not require coverslips
• They are virtually non-breakable

Here we wish to outline a suggested approach to using the *C-Chip™* disposable haemocytometer in the classroom to make an estimate count of yeast cells in a liquid culture. Supporting resources are available from the SSERC website [4].

The challenges for students learning to use a haemocytometer successfully are often related to poor microscope skills. For those new to haemocytometers it is therefore important that they know their way around the microscope they are using and that they can use it appropriately to observe cell samples at various magnifications. We suggest that learners are supplied with a pre-prepared microscope slide and that they are given instruction and time to practise focusing, adjusting brightness, adjusting magnification and moving the slide around on the stage. Prior to introducing the haemocytometer, it might also be valuable for learners to examine a drop of yeast cell culture on an ordinary microscope slide so that they are familiar with the appearance of the cells at various magnifications before trying to count them. This will also provide further microscopy practice. Once learners are confident with the microscope, they can begin to become familiar with the haemocytometer. The *C-Chip™* haemocytometer has two 'detection areas' (Figure 4) and therefore cells in two samples of culture can be counted.
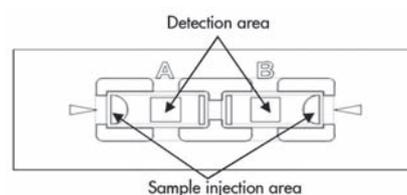

Figure 4 - Image : https://www.labtech.com/disposable-haemocytometers.

It is just possible to see the grids etched on the detection areas with the naked eye (Figure 5).

Once learners have seen the location of the grids they should be able to position the haemocytometer on the stage of the microscope so that light passes through one of the grids. It should now be possible to view the grid, initially using the x 4 objective lens (Figure 6).
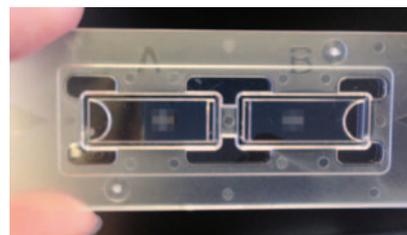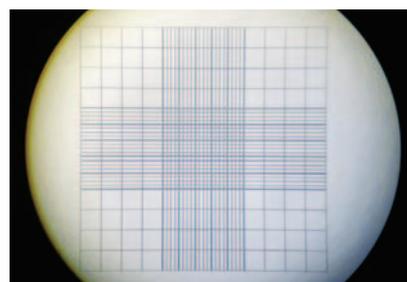

Figure 5 - The grids.

Figure 6 - Grid viewed at x 40 (Magnification = x 4 objective lens multiplied by x 10 eyepiece lens).
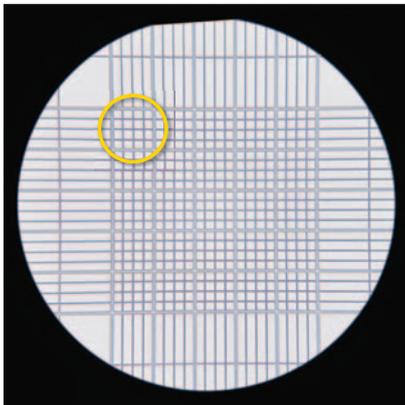
meter (it beats counting sheep!)

Figure 7 - *Central area of grid at x 100 magnification.*

Figure 8 - *Smaller sample squares.*

Figure 9 - *One sample square; notice the 3 boundary lines around the edges.*

Counting cells is done using the smaller squares which make up the central area. One of these is circled in Figure 7. At x 100 magnification learners should be able to see these 25 smaller squares and identify the 5 which will be used to sample the number of cells in the central area. A typical set of 5 sample squares is shown in Figure 8.

At x 400 magnification the sample squares (which themselves are comprised of 16 smaller squares) can be viewed individually - this is the magnification which will be used for counting cells (Figure 9).

Of course at this magnification the whole of the central area is not visible within the field of view
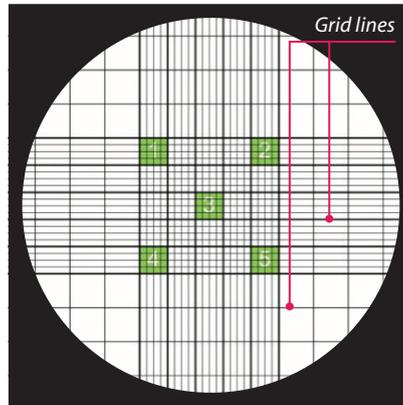
and, therefore, it is tricky to find the sample squares without a bit of practice. The grid lines around the edges (Figure 8) help with negotiating the central area, but learners will need time to practise finding their way to each of the 5 sample squares.

As stated earlier, the *C-Chip™* haemocytometer does not require a coverslip. Liquid samples are drawn into the detection area, from a micro-syringe, or capillary tube, by capillary action via the sample injection area (Figure 4). In our experience it is difficult to see colourless liquid samples being drawn in and the 'drawing in' happens quickly. We have therefore allowed learners to practise loading
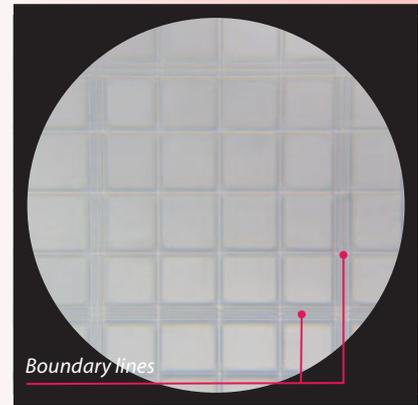
the haemocytometer using a coloured liquid before attempting to load a sample of yeast cell culture (Figure 10).

Once the yeast cell culture sample has been loaded, learners should be able to see cells at the different available magnifications, for example, x 40, x 100, x 400, and begin to think about counting cells at x 400 in the 5 sample squares (Figure 11).

The final instruction for learners relates to HOW to count the cells. Different laboratories will have different protocols for this, but the key point is to count systematically within the square (Figure 12) and to decide 'what's in and what's out'. ▶

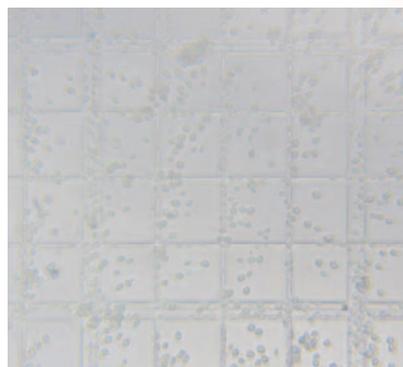**Figure 10** - *Use a coloured liquid to practise loading the haemocytometer.*

Figure 11 - *Yeast cells in one sample square of the haemocytometer at x 400 magnification.*
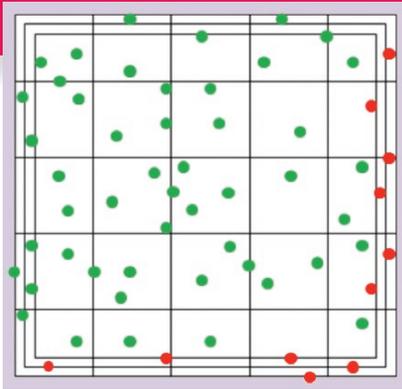

Figure 12 - *Count systematically.*

Figure 13 - *Count all the cells within the sample square and those touching the top and left boundary lines (●). Don't count those touching the bottom and right boundary lines (●).*
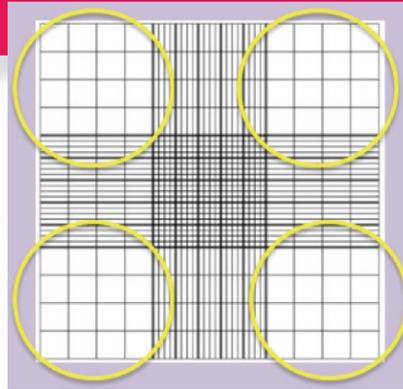

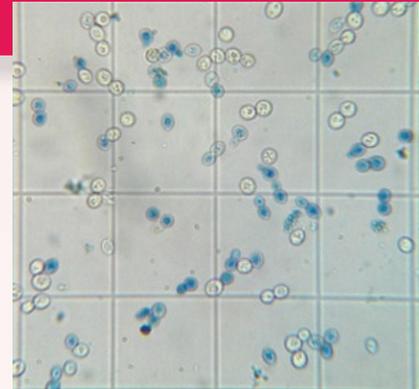*Figure 14* - *The 'large' squares of the haemocytometer grid.*

Figure 15 - *Non-viable cells are blue (image: http://braukaiser.com/blog/).*

Figure 13 illustrates a system that might be used to ensure accuracy and consistency of cells counted.

Once the cells in the sample squares have been counted, an estimate of the total number of cells in the central grid of the haemocytometer can be made.

If the concentration of cells is very low, all the cells in the 25 square grid could be counted.

For counting large cells, some protocols advocate using the 'large' squares of the haemocytometer grid (Figure 14).

If cell viability is to be taken into account, methylene blue can be added to the sample. Since live cells actively pump the dye out, viable cells are colourless and non-viable cells become blue (Figure 15).

For enthusiastic cell counters, or perhaps students carrying out an investigation involving enumerating cells over a period of time, useful 'apps' are available. We have used iPhone's 'HemocyTapp' (Figure 16).

This will helpfully record counts of live and dead cells and will calculate cell densities and ratios in cultures. Data can be stored and e-mailed to the user. ◄

Volume in sample area (5 squares)		
Length of side of central area	= 1 mm	
Area of central area	= 1 mm^2	
Depth under central area	= 0.1 mm	
Volume under central area (25 squares)	= 1 mm^2 x 0.1 mm = 0.1 mm^3	
Volume under 5 squares	= 0.1 / 5 mm^3 = 0.02 mm^3	

Estimating cell concentration	
Number of cells in sample	= n
Number of cells in 1 mm^3	= n x 50 (because 0.02 mm^3 is 1/50 of 1 mm^3)
Number of cells in 1 cm^3	= (n x 50) x 1000

References
[1] Adv Higher Support notes: http://www.sqa.org.uk/files_ccc/AHCUSNBiology.pdf (accessed January, 2016).
[2] http://www.sserc.org.uk/index.php/biology-2/biology-resources/microbiological-techniques265/enumerating-micro-organisms141/982-counting-cells-using-a-haemocytometer.
[3] Sourced from labtech.com: https://www.labtech.com/disposable-haemocytometers#slide-0-field_image-604.
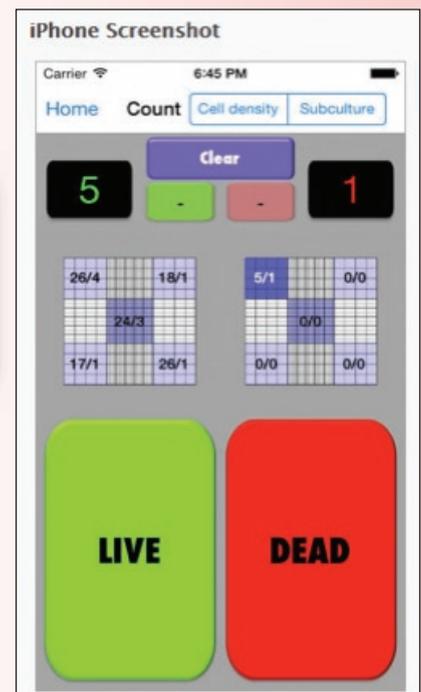[4] http://www.sserc.org.uk/index.php/biology-2/biology-resources/microbiological-techniques265/enumerating-micro-organisms141/3964-counting-cells-using-a-disposable-plastic-haemocytometer.

Figure 16 - *16 iPhone HemocyTapp (https://itunes.apple.com/us/app/hemocytap/id617767138?mt=8&ign-mpt=uo%3D4).*