THIRUTHANGAL NADAR COLLEGE

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Selavayal, Chennai-51.

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NAME OF THE DEPARTMENT: PLANT BIOLOGY & PLANT BIOTECHNOLOGY

SUBJECT : BIOINSTRUMENTATION & BIOSTATISTICS

TOPIC : HEMOCYTOMETER

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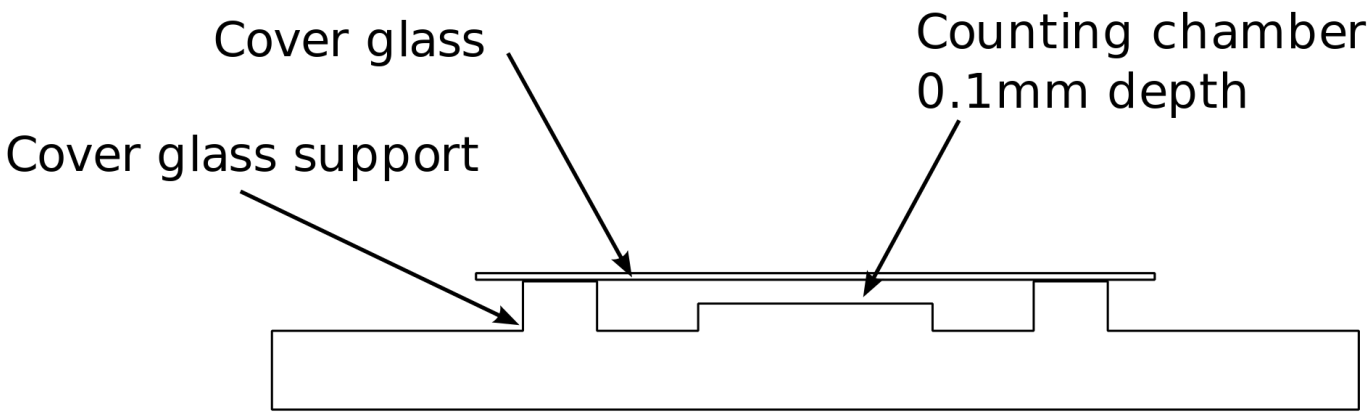
Significance of hemocytometer

- Many fields such as microbiology, cell culture, blood bank etc. are involved in cell counting and determine cell concentration in a given medium.
- Cell counting requires a counting chamber called a hemocytometer, a device invented by the 19th century French anatomist Louis-Charles Malassez to perform blood cell counts.

Fig. 1. Classic hemocytometer

Components of hemocytometer



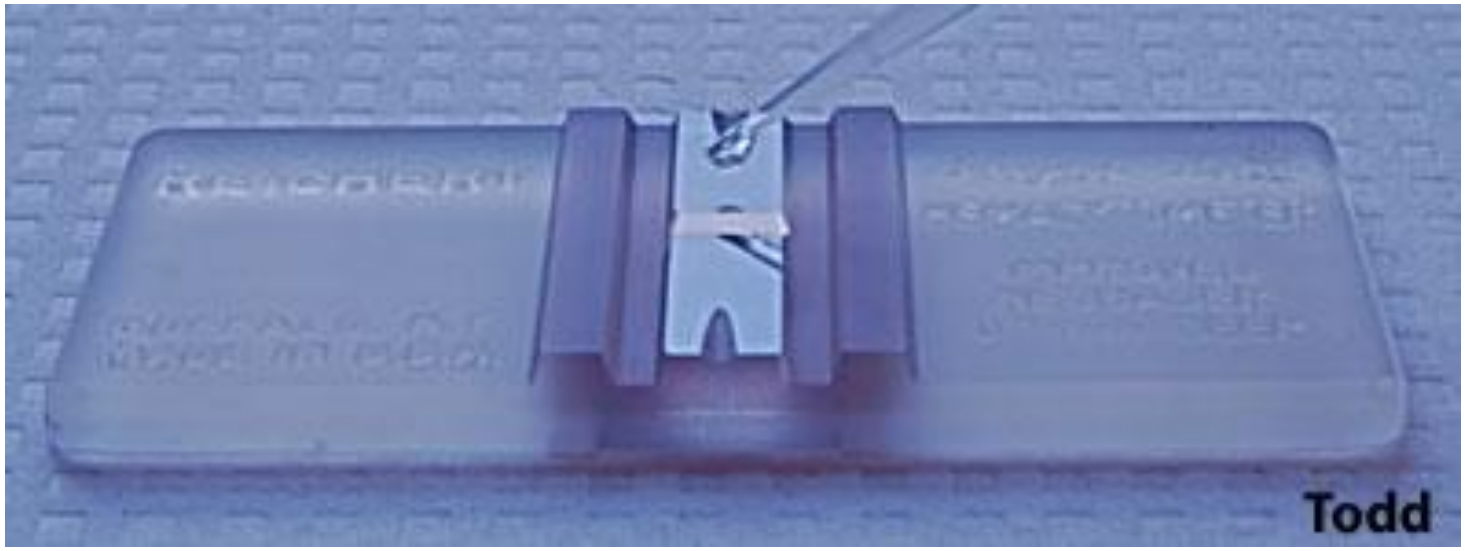
Components of hemocytometer

- A hemocytometer consists of a thick glass microscope slide with a grid of perpendicular lines etched in the middle.
- The grid has specified dimensions so that the area covered by the lines is known, which makes it possible to count the number of cells in a specific volume of solution.

Components of hemocytometer

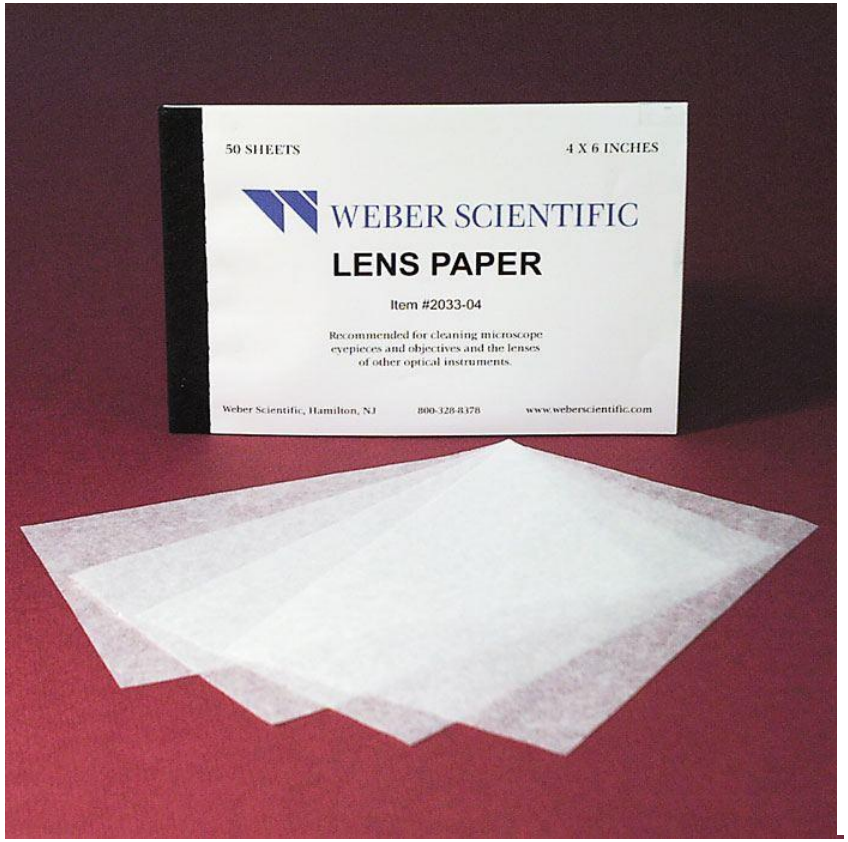
- The most common type of hemocytometer has an “H” shape engraved in the middle that encloses two separate mirror-like polished grid surfaces and provides the cover slip mounting area (Fig. 1.).

Fig. 2. Loading the hemocytometer



Loading the hemocytometer

- Before starting ensure that both the hemocytometer and its cover slip are clean by removing any dust particles with lens paper.
- Cover slips that are used for mounting on hemocytometers are specially made to be thicker than the conventional microscopy cover slips because they must be able to overcome the surface tension of a drop of liquid.


50 SHEETS
4 X 6 INCHES
WEBER SCIENTIFIC
LENS PAPER
Item #2033-04
Recommended for cleaning microscope eyepieces and objectives and the lenses of other optical instruments.
Weber Scientific, Hamilton, NJ
800-328-8378
www.weberscientific.com

Loading the hemocytometer

- First place the cover slip over the counting surface before loading the cell suspension.
- Then place the pipette tip with sample into one of the V-shaped wells and gently expel the sample.
- The area under the cover slip fills by capillary action.
- Enough liquid should be introduced so that the mirrored surface is just covered, usually around 10µl, but do not overfill the surface.

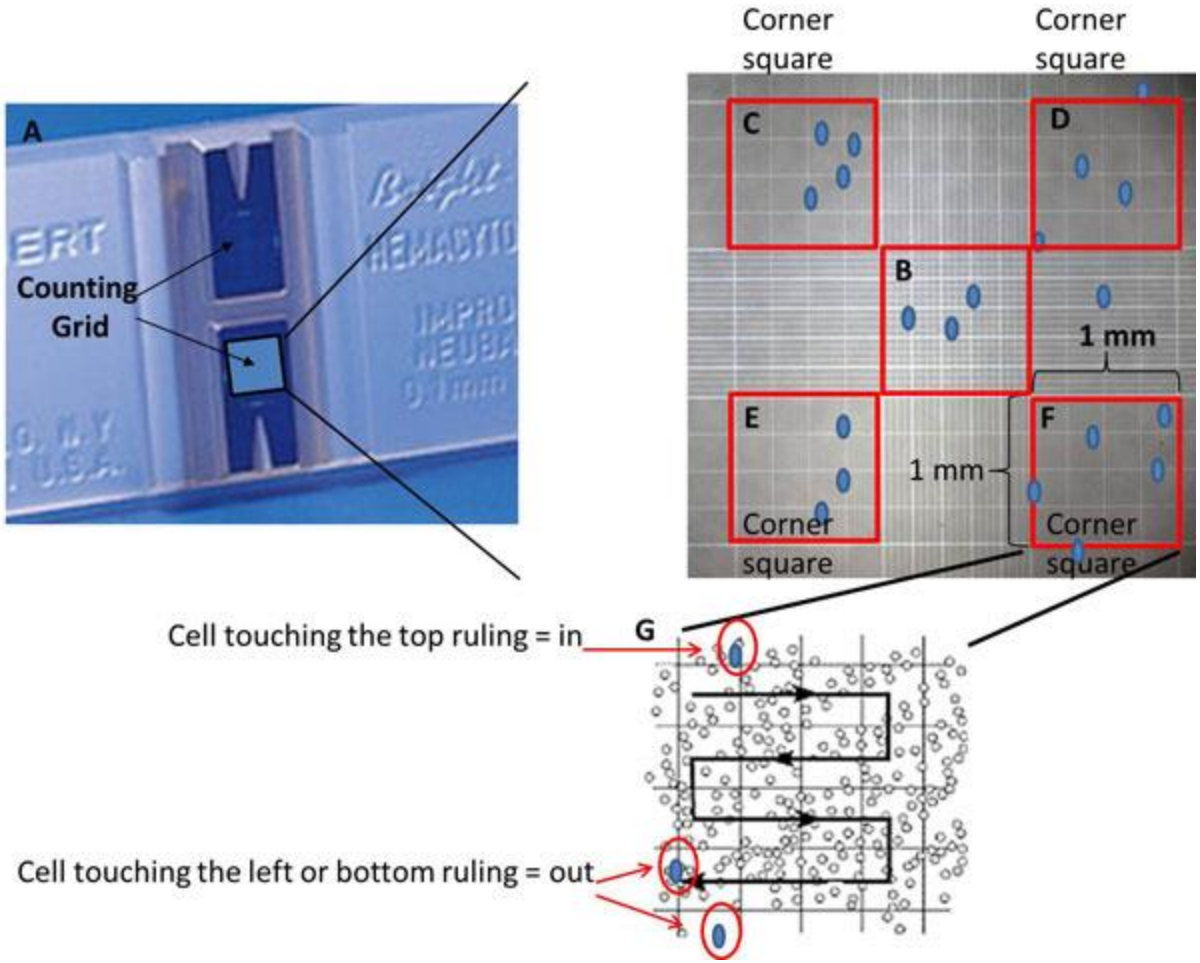
Loading the hemocytometer

- We can load two samples on one hemocytometer, one into each of the two grids.
- The loaded hemocytometer is then placed on the microscope stage and the counting grid is brought into focus at low power.
- Allow the sample to settle for few minutes and avoid moving the cover slip as it might introduce air bubbles and make counting difficult.

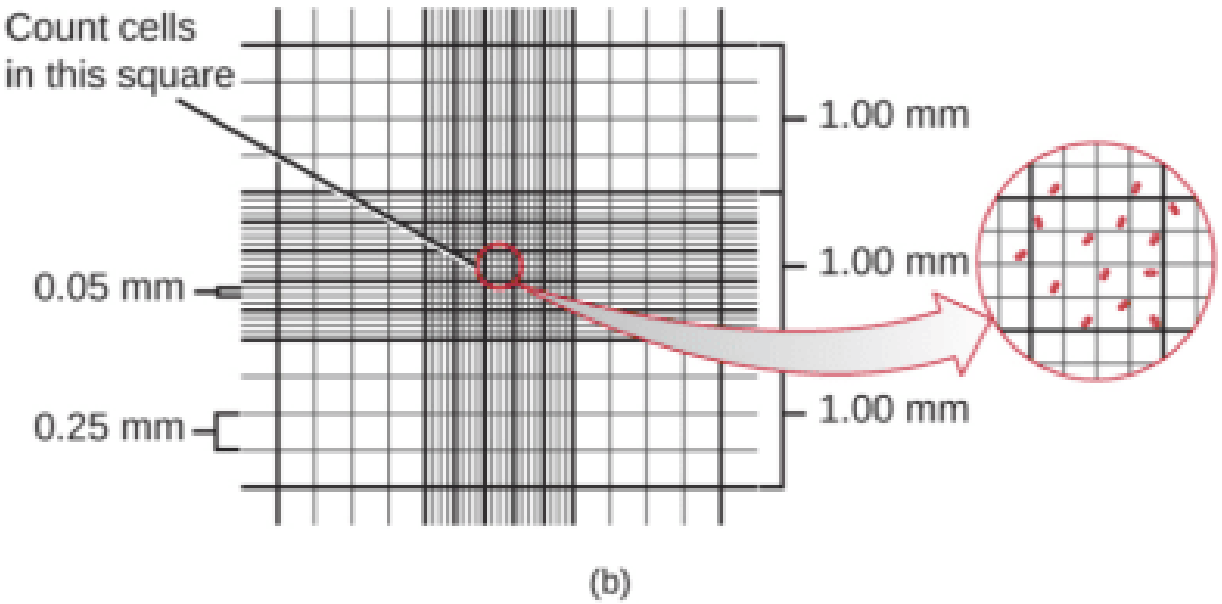
Counting cells in hemocytometer

- The full grid on a hemocytometer contains nine squares, each of which is $1mm^2$ (Figure 3).
- The central counting area of the hemocytometer (Fig 3B) contains 25 large squares and each large square has 16 smaller squares,
- When counting, count only those cells on the lines of two sides of the large square to avoid counting cells twice (Fig 3G).
- Suspensions should be dilute enough so that the cells or other particles do not overlap each other on the grid, and should be uniformly distributed.

Fig. 3. Hemocytometer



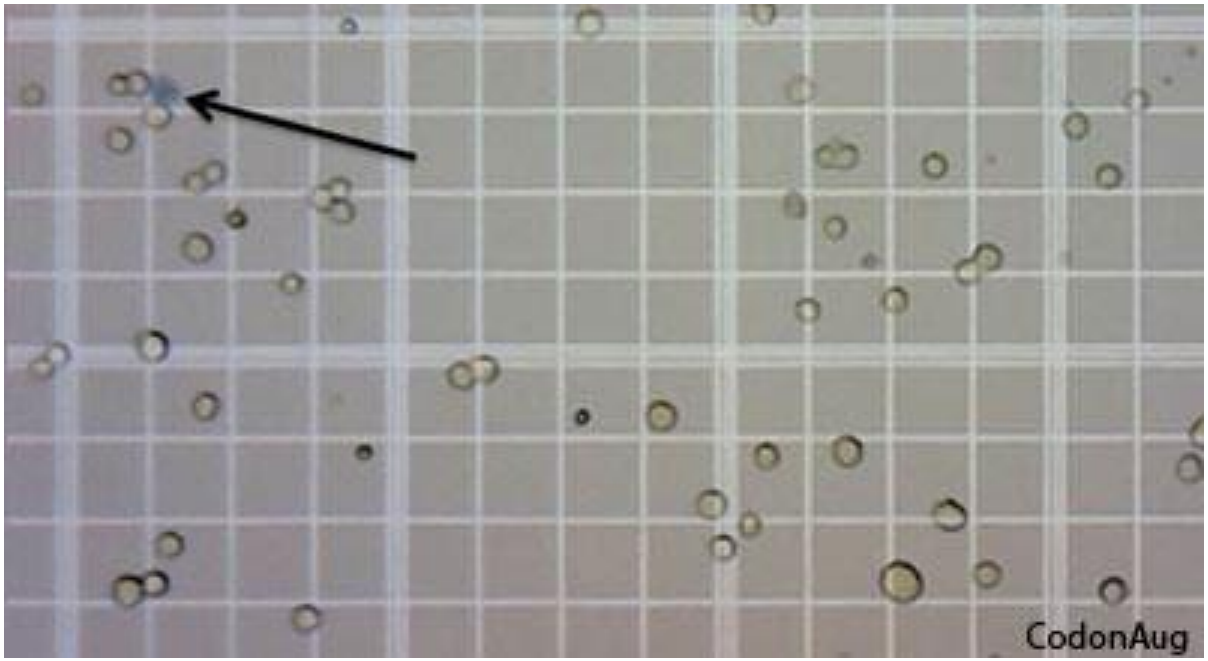
Hemocytometer



Counting cells in hemocytometer

- To distinguish between dead and viable cells, the sample is often diluted with a particular stain, such as Trypan blue.
- This staining method, also known as dye exclusion staining, uses a diaze dye that selectively penetrates cell membranes of dead cells, colouring them blue, whereas it is not absorbed by membranes of live cells, thus excluding live cells from staining.
- When viewed under a microscope, dead cells would appear as dark blue (Fig 4).

Fig. 4. Counting cells in hemocytometer



Counting cells in hemocytometer

- To perform the count, determine the magnification needed to recognize the desired cell type and systematically count the cells in selected squares so that the total count is approximately 100 cells, a minimum number of cells needed for a statistically significant count.
- For large cells, we can simply count the cells inside the four large corner squares (Fig 3c-f) and the middle one (Fig 3B).
- For a dense suspension of small cells, count the cells in the four outer and middle squares of the central square (Fig 3B) or make a more dilute suspension.

Counting cells in hemocytometer

- If a cell overlaps a ruling, count it as “in” if it overlaps the top or right ruling, and “out” if it overlaps the bottom or left ruling (Fig 3G).
- The area of the middle (Fig 3B) and each corner square (Fig. 3c – f) is 1 mm x 1mm = 1 mm^2, the depth of each square is 0.1mm.
- The final volume of each square at that depth is 100nl.

Counting cells in hemocytometer

- After obtaining the total cell count, cell concentration can be calculated from the following formula:
- **Total cells/ml = Total cells counted x diluted factor x 10,000 cells/ml**

  No. of squares
- Eg. Dilution sample 1:1 with trypan blue and count 325 cells in 4 corner squares plus the central big square, total cells per ml =
- 325 cells x 2 (diluted factor) x 10,000 cell/ml = 130×10^4 cells/ml

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  5 squares

Counting cells in hemocytometer

- To count the number of cells in original sample, multiply the cell concentration by total sample volume. For example, if original sample volume is 5ml, then sample has a total = 130×10^4 cells/ml x 5ml = 650×10^4 cells