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# MEDICAL BIOLOGY PRACTICALS. CYTOLOGY.

## Practical 5. The estimation of the number of cells in suspension or culture using hemocytometer

*By Boris M. Sharga, Diana B. Pylypiv, Volodymir P. Feketa*

**Theoretical background.** In practice, the number of cells in a culture or suspension can be determined by the several methods:

- "on the eye" by the turbidity of suspensions in the test tube: if the frame of the window is still visible, the concentration of the suspension is about $10^7$-$10^8$ cells /ml, if it is already not visible, then the cell number is $10^9$ cells/ml and above. This method is inaccurate, does not distinguish between living and dead cells.

- use of an optical turbidity standards, in which latex suspension filled and sealed in tubes is compared with suspension in experiment. The micro-particles of latex, when agitated, simulate a certain number of cells in suspension. The liquid in a standard is agitated by shaking and compared in cloudiness with cell suspensions in front of the font and standard figures printed on paper. This is more accurate, but also does not distinguish between living and dead cells.

- determination of cell suspension concentration in an electrical cell counter, in which a known volume of liquid is passed as drops through a hole with electrodes connected to it, allowing to determine the current (resistance) in the drops with and without a cell. The disadvantage of this device is that it does not distinguish between living and dead cells, rubbish and cell aggregates. This affects the accuracy of the count. The counter provides more precious cell number estimation for large cells (human, animal and plant cells, yeasts, conidia, etc.).

- "plate count" method of counting the number of cells as colony-forming units in Petri dishes is most accurate, since each colony is formed from single cells sown from cell suspension dilution to Petri dishes with a fresh solid nutrient medium. But this method takes time to incubate sowings for the development of colonies.

- measurement of the optical density (OD) on photoelectrocolorimeter or absorbance and light transmission (T%) on spectrophotometer, corresponding to the cells concentration in suspensions and which is determined on the previously constructed graph of dependence of OD, absorbance or T% from the concentration of cell cultures. This is the most accurate method [6]. However, this method requires the preliminary determination of correlation “optical density *versus* plate count” and the construction of calibration graph.

The use of hemocytometer and light microscope is a simplest way of accurate determination of the cells quantities in a sample [1, 6, 7]. It was invented by Louis-Charles Malassez (1842-1909), the French anatomist and histologist.

Hemocytometer is a special thick slide that has 2 depressions with square mesh grid creating counting chambers of known volume when spaces between depressions and cover slips are filled with liquid culture or cell suspension. The counting chambers are divided into nine 1 $mm^2$ squares, etched on silvered surfaces separated by 'H' shaped furrow. A coverslip placed on raised sides of the furrow enclosing both chambers. Most modern hemocytometers have 'V' shaped notch at either side where the cell suspension or culture is loaded into the chambers using pipette. The engraved grid ensures the counting of cells in a defined volume.

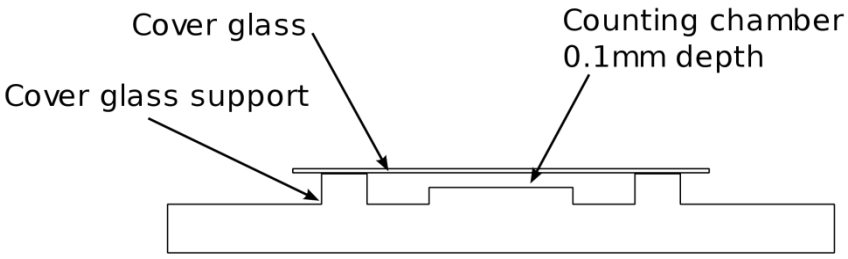


**Fig.1. Hemocytometer side view [3].**

This counting chamber is used to enumerate blood cells, sperm, eggs, pollen, fungal spores, large bacteria, etc. or non-living particles visible in light microscope.

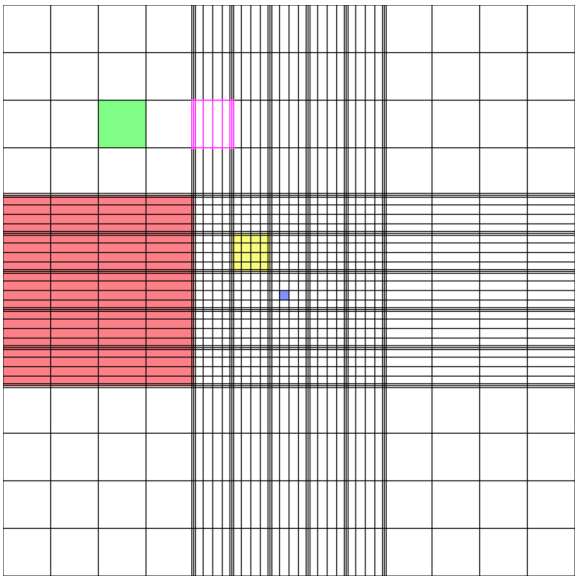

Table 1. Chamber metrics

| Dimensions, mm | Area, $mm^2$ | Volume at 0.1 mm depth, nL |
|---|---|---|
| 1 × 1 | 1 | 100 |
| 0.25 × 0.25 | 0.0625 | 6.25 |
| 0.25 × 0.20 | 0.05 | 5 |
| 0.20 × 0.20 | 0.04 | 4 |
| 0.05 × 0.05 | 0.0025 | 0.25 |

**Fig. 2. Hemocytometer grid of one of two chambers (see Table 1):**
It has its nine 1 mm² large squares separated by the triple lines. Within each of the larger 1 mm² corner squares are 16 small squares, to avoid counting a given cell more than once. The central 1 mm² area consists of 25 small squares of 0.04 mm² each, and each of these is divided into a further 16 tiny (0.0025 $mm^2$) squares. Modified, from R.Wheeler (Zephyris) 2007 [4].

## Experiment. Estimation of the cells number in suspension or in liquid culture

**Materials.** A hemocytometer with cover slip, light microscope, pipette (10-100 µl) sterile tubes, sterile tap water, paper towel, laboratory gloves, commercial bakery yeasts or Sabouraud dextrose broth culture of *Saccharomyces cerevisiae.*

**Method**

1. Remove the small portion of the yeast mass by scalpel and suspend it in 3-4 ml of tap water by thorough mixing. Dilute suspension, if necessary, to allow see the window frame through it. Alternatively 3 day Sabouraud dextrose broth culture of *Saccharomyces cerevisiae* can be used.
2. Fill clean hemocytometer with yeasts suspension as it shown on Fig. 3. Do not overfill or insufficiently fill the camera.

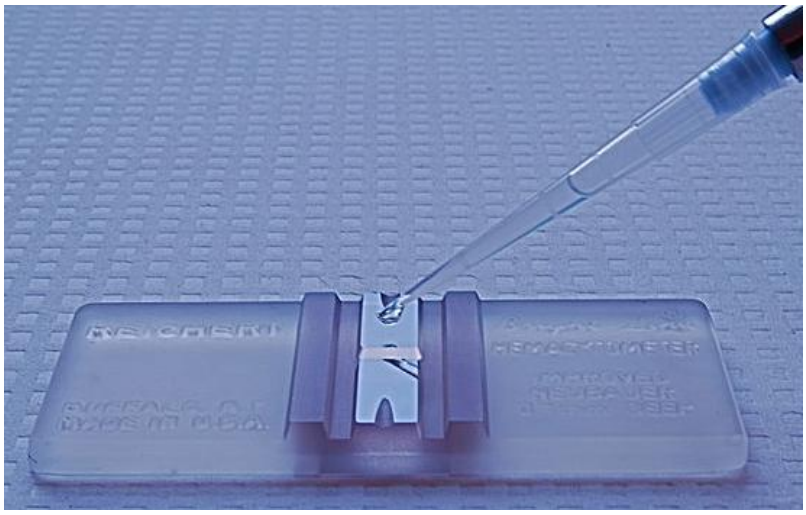

**Fig. 3. Loading a chamber.** By Jacopo Werther 2008 [2].

3. Fix the hemocytometer onto microscope stage.
4. Use the objective with smallest magnification directing it to observe the Neubauer grid (Fig. 4 ) and cells in one-half (i. e., in one chamber) of a hemocytometer.

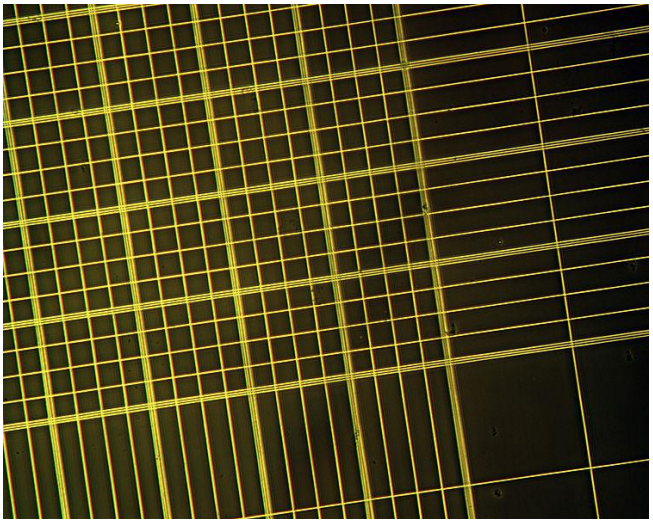

**Fig. 4. Hemocytometer grid. Magnification 100 ×. By J.M. Vinocur [5].**

5. Count all yeasts cells within 1 $mm^2$ of the central square and in four 1 $mm^2$ corner squares. To avoid the confusion about the count of cells on the border gridlines take into account the cells touching left and upper square sides, but not bottom and right sides. Particularly, in one of the corner squares you may see the picture, as in Fig. 5.
6. Repeat the procedure for the camera 2.

*Note*. If more than 10% of the cells are aggregated, repeat the procedure by separating the cells in clusters with intensive movements of suspension through the pipette several times. If more than 500 cells and less than 200 cells are counted in 10 squares (less, than 20 and more, than 50 cells per square), repeat the procedure by establishing the required dilution.

7. Procedure to repeat for another sample.
8. Calculations:

*Number of cells/mL= average per square (count in 10 squares)* $\times$ *dilution* $\times 10^4$.

*Total number of cells in whole volume of suspension = cells/mL* $\times$ *dilution of the suspension from which the sample was taken.*

Each square of a hemocytometer with an overlaying suspension over it forms a volume of 0.1 $mm^3$ or $10^{-4}$ $cm^3$. Since 1 $cm^3$ is equivalent to about 1 ml, the concentration of cells per ml will be determined as in following example. With the mean of 40 cells per square and dilution factor 5, the number of cells per ml in stock suspension will be as follows:

$$40 \times 5 \times 10^4 = 2 \times 10^6 \text{ cells/ml.}$$

Total cells number in the initial volume can be calculated as a product:

$$2 \times 10^6 \text{ cells/ml} \times 10 \text{ ml (stock suspension volume)} = 2 \times 10^7 \text{ cells.}$$

Statistical error of the method usually does not exceed 10-14%, if uniform suspensions, proper filling of hemocytometer chambers and cell counting method are applied.

## Literature:


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