Counting cells

A hemocytometer is a specialized microscope slide with a precision-etched grid to determine the number of cells in a known volume. The most common variant for mammalian cells is the Neubauer or "Improved Neubauer" chamber. By staining with a viability dye such as Trypan blue, the proportion of live and dead cells can be determined simultaneously.

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-1 or BSL-2)
▷ Wear gloves, safety glasses, lab coat
□ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE

Reviewed: Mar 14, 2023

Procedures

›› *Cell counting with a Neubauer cell chamber*

□ Neubauer cell chamber
□ Hand tally
□ 0.4% Trypan blue solution, 10 µL ⟨*R*⟩

(1.) *Optional:* Gently mix the cell suspension with an equal volume of 0.4% Trypan blue.

This is why: Trypan blue is a diazo dye excluded from live cells, allowing simultaneous viability assessment.

Hint: Automated cell counters use the same Trypan blue exclusion principle and accept 10 µL samples in disposable slides. They reduce user-to-user variability and are preferred for high-throughput work. Follow the manufacturer's instructions for sample preparation and calibration.

(2.) Clean the hemocytometer and coverslip with a lint-free tissue to remove dust and debris.

(3.) Moisten the raised glass rails of the hemocytometer. Place the coverslip over the counting surface.

Note: The coverslip is thicker than standard types to withstand surface tension and ensure correct chamber depth.

(4.) Carefully load about 10 µL of the sample into the chamber. The void will fill by capillary action.

(5.) Place the hemocytometer on the microscope stage and focus. Cells should be evenly distributed and not overlapping.

- Using the hand tally, count the cells in the four corner grids of the chamber.
- Exclude cells on the left or bottom border, count cells on the right and top border (or vice versa).
- Tally unstained (live) and stained (dead) cells separately.

Quality assurance: Repeat with a more concentrated sample if the overall tally is 200 cells or less.

Hint: Most hemocytometers have nine large 100 µm^2 grids that are arranged in a 3 × 3 pattern. The four corner grids with 16 small squares are used to count white blood cells and mammalian cells; the center grid is used for smaller particles such as red blood cells, platelets, or cell nuclei.

(6.) Average the number of cells across the counted grids and multiply by $1{\times}10^4$ mL^{-1} to determine the cell concentration. Be sure to account for any dilution factor introduced by Trypan blue.

Hint: For example, if the average count across four corner grids is 150 cells and the sample was diluted 1:2 with Trypan blue, the concentration is $3.0{\times}10^6$ /mL cells.

This is why: The Neubauer and "Improved Neubauer" chambers have a depth of 100 µm between the counting grid and coverslip. Other chamber designs may differ.

Troubleshooting

Cell counting with a Neubauer cell chamber

In Step 5:

– Cells are unevenly distributed under the coverslip

- Ensure chamber and coverslip are clean and properly assembled.
- Mix cell suspension gently but thoroughly before loading.

Recipes

Trypan blue solution, 0.4%

Amount	Ingredient		Stock	Final
0.4 g	Trypan Blue	[72-57-1]	960.8 g/mol	0.4%
10 mL	Phosphate-buffered saline (PBS), pH 7.4	R0037	10 ×	
To 100 mL	Water, reagent-grade			

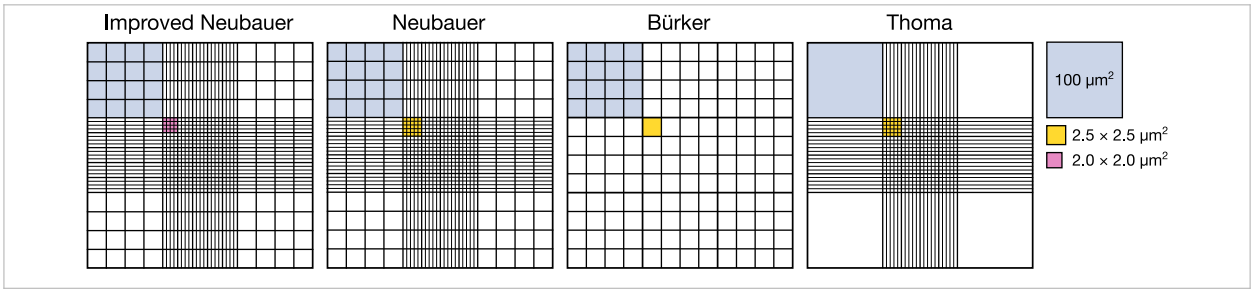
Dissolve the dye in 80% of the final volume; bring to a slow boil, cool down and make up with water.

0.4% Trypan blue solution

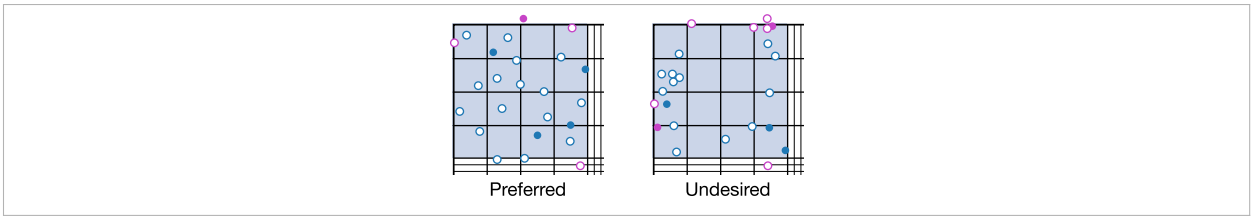
Date: Sign: R0133

Resources

Cell counting with a Neubauer cell chamber



In Step 5: The design of the nine large 100 µm² grids varies between hemocytometer models.



In Step 5: Particles touching the top or right borders of a grid are counted; those touching the bottom or left are excluded. Uniform cell distribution is essential.

Change log

2000-01-01	Unknown	Initial protocol; Jue Chen lab.
2015-08-07	Martin Wilson	Haemocytometer types; online resource (Agar Scientific Ltd.).
2020-05-27	Michael Haugbro	Initial protocol; Tom Muir lab.
2023-03-14	Benjamin C. Buchmuller	Adaptation as SOP.