

COUNTING CELLS

Two methods will be described: a) using a microscope and a chamber of known dimensions (the hemocytometer) and b) using an electronic cell counter. Most types of cells may be counted by either method, but the electronic cell counter is more suitable when many determinations are to be made on cells of the same kind under comparable conditions.

COUNTING THE CELLS WITH THE HEMOCYTOMETER

1. The Hemocytometer (Neubauer ruling)

A hemocytometer is a microscope slide modified to have two ruled areas. These areas are isolated and so located that when a cover slide is placed over them the space between a ruled area and the cover slide forms a "chamber." The distance between the ruled area and the cover slide is 0.1 mm, and a ruled area is 3 mm. on a side or 9 mm. Therefore, one chamber has a volume of 0.9 mm. The square millimeter (W) occupying each corner is ruled into 16 squares. The central square millimeter is ruled into 25 squares (five of which are marked R in the figure) each separated by triple lines, the middle line being the boundary. Each of these 25 squares contains 16 of the smallest squares. Volumes of some of these subdivisions are given in Table 1.

Table 1

Unit	Length of One Side mm	Area mm ²	Depth mm	Volume mm ³
One chamber	3	9	0.1	0.9
W	1	1	0.1	0.1
Z	0.25	0.0625	0.1	0.1
R	0.20	0.04	0.1	0.004

Expel at least 6 drops of fluid from the diluting pipette. A chamber is filled by lacing the tip of a diluting pipette at the edge of the cover slide overlying the ruled area and allowing the chamber to fill by capillarity. For filling the chamber, the pipette is best held at an angle of 35° from the horizontal. The suspension of cells must NOT overflow the chamber, or the count will be inaccurate.

Counting chambers must be cleaned immediately after use to prevent drying of protein. Wash with warm tap water and leave in alcohol bath- Avoid touching the chambers since grease from the hands prevents uniform spreading of fluid. Coverglasses should be dried when cleaned.

Dilutions of cells should be made such that each "W" square contains ~200 cells.

For trypan blue vital stain

1) mix 4 parts, filtered 0.2% trypan blue in distilled H₂O with 1 part 5x saline

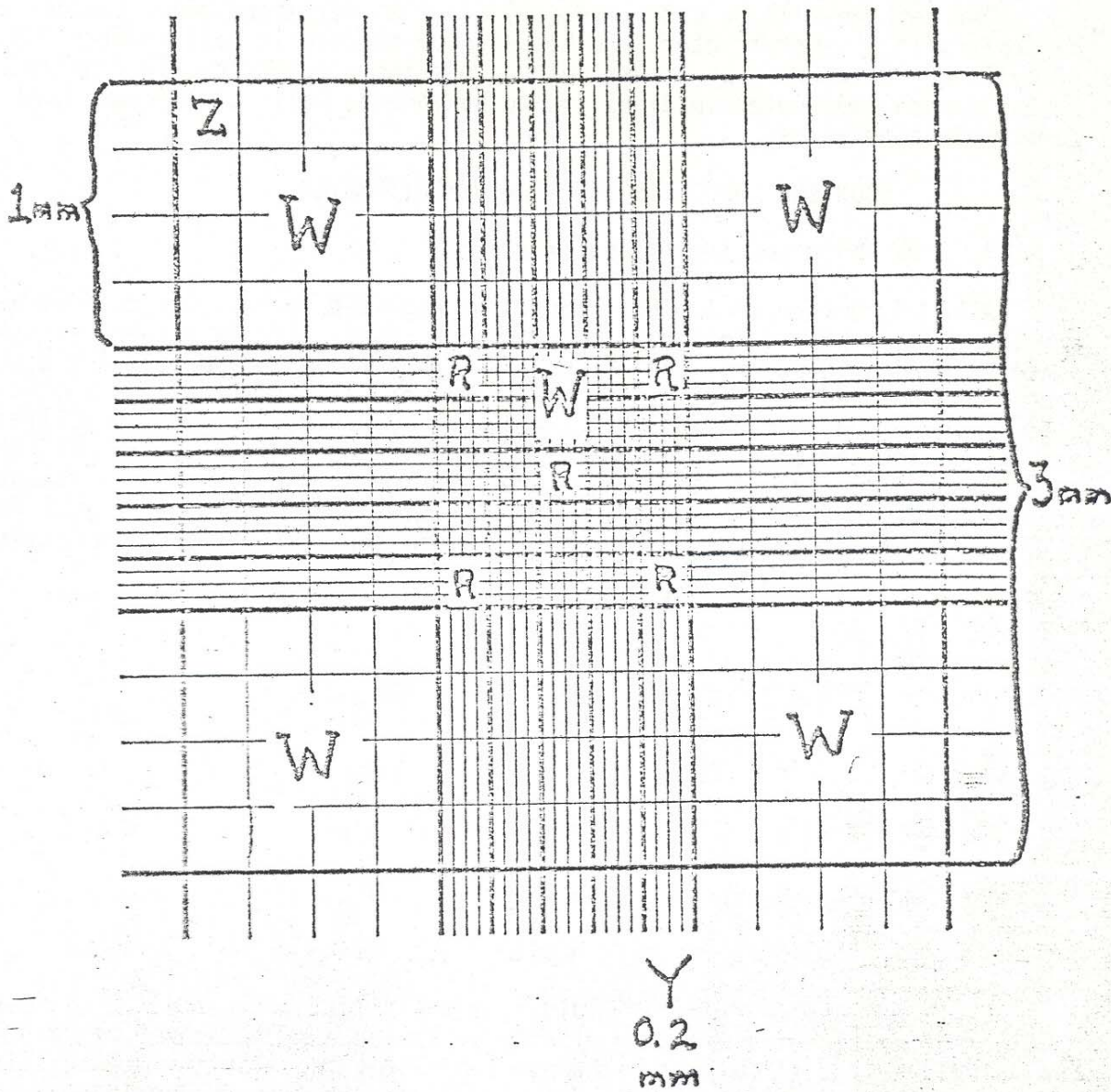
2) mix equal parts (100 λ each) of the above solution with your cell suspension (1:2 dilution of cells)

3) after thorough mixing, place drop from pasteur pipet in chamber.

Live cells are clear or opalescent

Dead cells are blue and swollen.

Counting Cells 2



IMPROVED NEUBAUER RULING FOR A COUNTING CHAMBER

$$\# \text{ cells in a "W" sq} \times \text{dil.} \times 10^4 = \text{cells/ml}$$