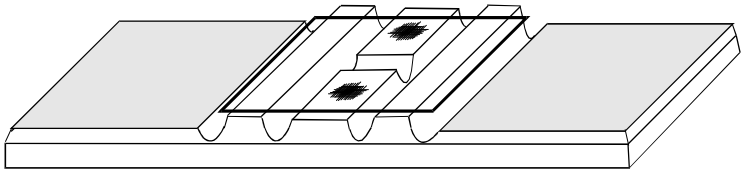
Counting Chamber (Hemacytometer)

1) What is a counting chamber and what is it used for?

A counting chamber is a precision measuring instrument made of special optical glass. It is used to count cells or other particles in suspensions under a microscope. Counting chambers are mainly used for counting blood cells (e.g. leucocytes, erythrocytes, thrombocytes, …) and cells in fluid of the brain and spinal cord. Furthermore, counting chambers are also used to count bacteria, sperm and fungus spores.

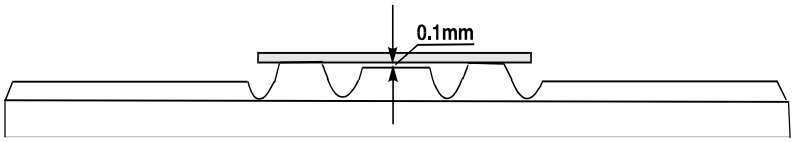
2) Design principle:

All counting chambers have the same basic design principle.



There are four longitudinal grooves in the central third of a rectangular base plate made of special optical glass.

The grooves run parallel to the short edges of the base plate and the central third has the same size as the coverglass used with the counting chamber.
The two larger external surfaces are unfinished and are used for marking purposes.

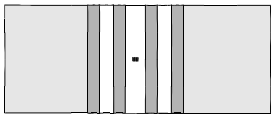


The central support and the two external supports are ground flat, parallel and polished. The surface of the central support is slightly deeper than the ones of the two external supports. The counting grids are engraved into the central support (chamber base).

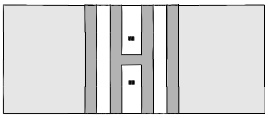
When a cover glass is placed on to these external supports this forms a capillary gap between the rear side of this cover glass and the central support of the counting chamber.

3) Design and identification of a counting chamber:

Design:



single net ruling:
middle support without division
(one counting grid)



double net ruling: middle support with one division
(two counting grids)

Furthermore there are two different designs of grids:

- with **dark lines**: Standard counting chambers have counting grids which are **engraved** into the chamber bottom.
- with **bright lines**: the chamber base is initially coated with metal and then the counting grid is **etched into the metal coating**.

Identification:
The following details are printed on both unworked surfaces of the counting chamber:

- counting grid's system
- name and trademark of Marienfeld
- chamber depth in mm
- area of the smallest square in mm^2

4) Production and quality descriptions:

Counting chambers are precision instruments and in-vitro diagnostic devices. They are predominantly used in medical laboratories. For application and distribution in the European Union counting chambers have to be approved and CE-marked.

All counting chambers sold by Marienfeld are manufactured in compliance with the relevant Calibration Ordinance and DIN standard.

Production:
The production of counting chambers is described herewith briefly. It includes several individual processes, each of which is followed by stringent quality checks.

The central support (chamber base) as well as the two external supports are ground and polished. The flatness and the accuracy of the depths are the most important requirements. They are described in the standard DIN 12874. The central support (chamber base) is lowered according to the specific system (for example the depth of the standard Neubauer system has to be 0.1 mm). Besides the standard depths there are special depths available (e.g. 0.2; 0.5; 0.01 and 0.02 mm).
After these processes the counting grid is engraved into the chamber base which is followed by the lettering on the outer surfaces and by the annealing. The production of the counting chamber is finished by a final check which makes sure that the counting chamber is in accordance with the DIN standards and the regulations of the German Board of Weights and Measures.

Requirements on quality controls:
The maximum deviations allowed according DIN 12847 are as follows:

- for the chamber depth in the area of a counting net ± 2% of the nominal value
- for distances of less than 0.4 mm between any net lines ± 2µm
- for distances of 0.4 mm or more between any net lines ± 0.5% of the desired value
- for the angle of the net division ± 1°
- the width of the division marks must not be greater than 5 µm.

The flatness tolerance acc. to DIN 7184 Part 1 is as follows:

- for the chamber base near the counting net 2 µm
- for the support areas 2 µm
- for the cover glasses 3 µm (according to DIN 58 884)

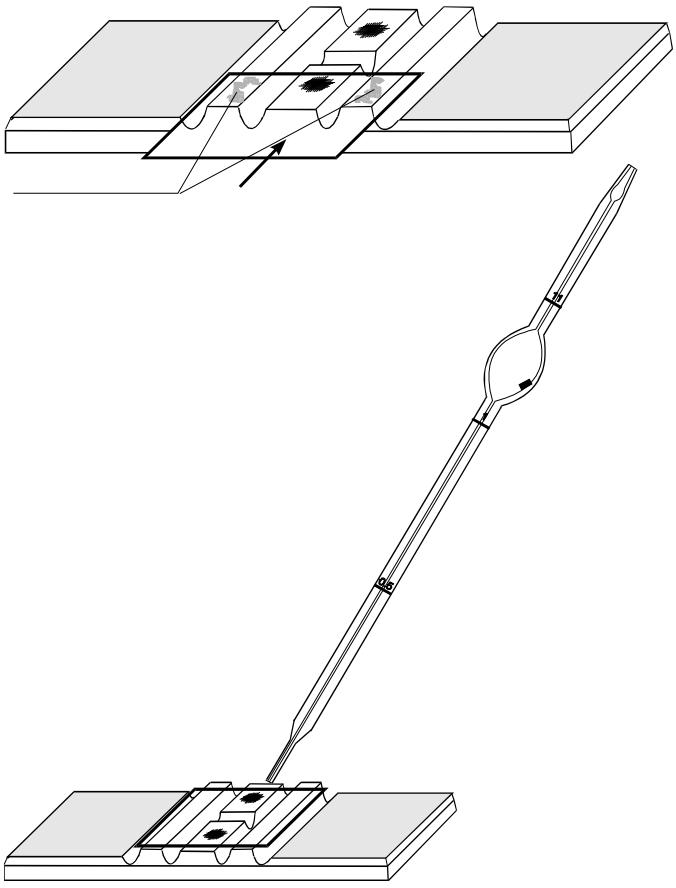
5) How to fill the counting chamber:

Sliding on the cover glass:

The external supports are to be moistened with distilled water and the cover glass then is gently pushed onto the counting chamber from the front.

Important: The cover glass is fragile!

The formation of interference lines (Newton rings) between the external support and the cover glass shows that the cover glass is correctly positioned.



Feeding:

Take a well mixed pipette from the shaker and dispose off the first few drops.

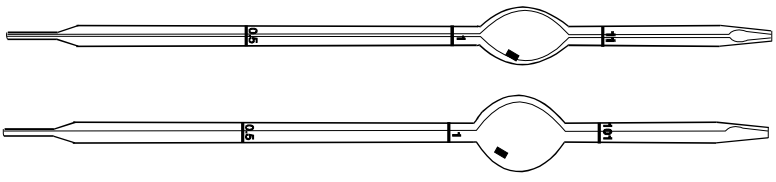
Wipe the pipette dry on the outside and then hold it at an angle until a small drop has arisen at the tip of the pipette.

This drop is then to be placed between the cover glass and the counting chamber.

As a result of the capillary effect the gap between the cover glass and the chamber base fills up. Before the thinned blood solution can overflow at the edges of the chamber section, the tip of the pipette must be removed. If any air bubbles are visible or if the liquid has overflown over the edges into the grooves, the chamber should be cleaned and feeding be started again.

Blood mixing pipettes to be used:

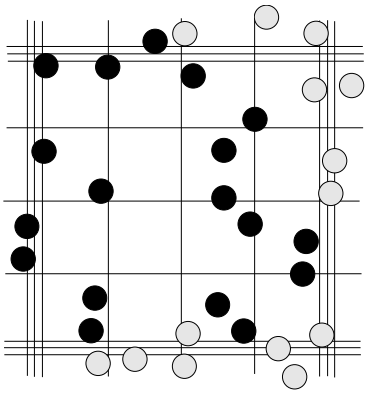
- Leucocyte pipette (white bulb)
- Erythrocyte pipette (red bulb)



6) Counting the particles:

Counting technique:

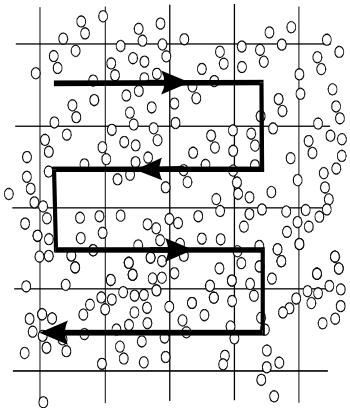
Counting assumes precise knowledge of the grid lines of the counting chamber used. The picture shows the threefold lines of the Neubauer improved system.



To ensure that cells which are on or along the limit lines are not counted twice or are not missed during the count, certain rules have to be observed (eg. see illustration to the right).

The cells which are on or along the two (one) sides are counted, e.g. on the left and top lines (shown in black). This also applies for the type of the actual counting process which is supposed to be completed in a meandering pattern.

The count should be started at the top left-hand corner and follow the direction shown by the arrow.



Notes on counting:

a) The trim of the capacitor on the microscope must be almost closed for all chamber counts.

b) The difference of the counted cells in the large squares and the group squares must not exceed 10 cells.

c) Double checks must be performed for all cell counts. After counting the cells o one grid the other one is to be counted in the same way as a double check. When doing this it is important to be sure that the chamber has not yet dried out. This can be prevented by filling the second grid only shortly before the count and counting it after the sedimentation time.

d) The difference between the totals of the counts for the two counts must not exceed 10 cells. The average value of the counts is then used in the calculation formula or multiplied by the corresponding factor.

7) Calculation

Formula:

$$\frac{\text{Number of cells}}{\text{Counted area } (mm^2) \text{ x chamber depth (mm) x dilution}} = \text{Cells per 1 µl of blood}$$

Example: Chamber: Neubauer improved

a) Leucocytes:

1. Counted cells 161 leucocytes
2. Counted area: four squares (= 4 x 1 mm^2) = 4 mm^2
3. Chamber depth 0.1 mm
4. Dilution 1:20

$$\frac{161}{4\ mm^2 \text{x } 0.1\ mm \text{ x } 1/20} = \frac{161 \text{ x } 20}{4 \text{ x } 0.1 \text{ x } 1\ \mu l} = \text{8050 leucocytes per 1 µl of blood}$$

b) Erythrocytes:

1. Counted cells 507 erythrocytes
2. Counted area: five squares (= 5 x 0.04 mm^2) = 0.2 mm^2
3. Chamber depth 0.1 mm
4. Dilution 1:200

$$\frac{507}{0.2\ mm^2 \text{ x } 0.1\ mm \text{ x } 1/200} = \frac{507 \text{ x } 200}{0.2 \text{ x } 0.1\ \mu l} = 5.07 \text{ x } 10^6/l\ \mu l$$

In other words 5.07 million erythrocytes per 1 µl of blood.

8) How to clean the counting chamber:

Immediately after completing the count the cover glass is to be removed and the counting chamber has to be cleaned with water or (if necessary) with a mild cleaning solution. Afterwards, the chamber is to be dried with a soft cloth or rinsed with acetone.

9) Short description of the mostly used counting chamber:

The various systems used for counting chambers differ in the design of the counting net and the chamber depth. The counting net is made up of a square net division which is not visible until it is placed under a microscope (approx. 100 times magnification). Here is a short description of the most common system:

Neubauer improved :
Largest square size: 1 mm^2
Group square: 0.04 mm^2
Smallest square size: 0.0025 mm^2

Depth of chamber is 0.100 mm. The net division of these chambers has 3 times 3 large squares, each with an area of 1 mm^2.
The four corner squares are used for leucocyte counts.
The large square in the middle is also divided into five times five group squares with an edge length of 0.2 mm each and an area of 0.04 mm^2 each. The group squares in turn are divided into sixteen very small squares each with an area of 0.0025 mm^2
Five of these group squares are used for erythrocyte counts.

Special attention should be given to the fact that the chamber has triple lines on all sides, of which the central line is to be regarded as the actual dimension line. This is important for deciding whether cells in the border area are to be counted or not.

10) Why are counting chambers still used in laboratories although there are electronical counters?

- For smaller laboratories this equipment is too expensive
- For special requirements e.g. research and non-routine examinations (counting of liquor or effusion, worm eggs, bacteria and fungus spores) counting chambers are required
- Counts are less accurate in case of small number of cells (e.g. liquor or few thrombocytes)

Possible sources of error:
- counting chamber is not clean
- cover glass is not placed correctly onto the chamber
- chamber is not filled without bubbles
- chamber is overfilled
- not enough time for sedimentation of the cells
- the wrong cover slip is used. Standard cover slips for microscopy are too thin and bend because of the capillary forces.

Electronical counters:
- are used in large laboratories (enormous investments)

- **disadvantage:** Cells are identified only by size. Therefore, dust or other particles may cause counting errors.
- **advantage**: The counting of many cells reduces **statistic errors the formula** of which is.

  Statistic error = 1 : n

  n = 100 counted cells 1 : 100 = 0.1 = 10 %

  n = 10'000 counted cells 1 : 10.000 = 0.01 = 1 %

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