

IVD

CE en

# Hemacytometer

## General instructions for the use of Hemacytometer - Counting chambers

### Counting Chambers

Counting chambers are precision instruments for the visual microscopic counting of living or dead cells of various types and/or for the quantitative selection between living and dead cells.

In addition to visual counting, there are also counting chambers that are used especially for automatic counting as well as disposable counting chambers that are not covered in this manual.

In principle, reference is made here only to the general use of a counting chamber. For special use (counting, calculation) the analysis instructions (e.g. instructions for use of the reagent manufacturer) are always decisive.

#### General information

The following information should appear on each counting chamber:

= Counting chamber depth  
 mm<sup>2</sup> = Smallest division

#### Human in-vitro diagnostics

Counting chambers for in-vitro diagnostics (IVD) must in accordance with the Act of Medical Devices and the IVD Directive 98/79/EC be marked with the following symbols, for the basis of which a declaration of conformity by the manufacturer is required [1]:

= Complies with IVD Directive 98/79/EC.  
 = Approved for human in-vitro diagnostics.  
 = Manufacturer.

Counting chambers which do not or only partly show the above symbols, must NOT be used for human in-vitro diagnostics [1].

= Information symbol for the availability of a user manual

#### Veterinary medicine

All counting chambers that are also approved for human in-vitro diagnostics can be used for this purpose. The above symbols do not have to be placed on the counting chamber.

#### Life Science & Microbiology

In the field of life science and non-human diagnostic microbiology, the same applies as described for veterinary medicine.

#### Industry

Other regulations may apply to the industry (e.g. foodstuffs, beverages).

### Counting Chamber Types

#### Line execution

Two basic distinguishing features apply to almost all counting chamber types:

- counting chambers with dark mesh lines (standard) and
- so-called bright-line counting chambers, in which the glass surface was provided with a metal coating (rhodium) prior to scratching the counting lines. This darkens the image and the engraved lines appear bright.

As far as bright-line counting chambers are not prescribed for special counting methods, we generally do NOT recommend the use of them!

Disadvantages of bright-line counting chambers:

- Lower light transmission with the consequence that the microscope lamp is turned up brighter. This leads to faster heating (drying, cell movement) of the preparation.
- Higher sensitivity of the metallized surface.
- Higher acquisition costs.

#### Counting methods and counting nets

Depending on the cell type to be counted and the expected number of cells per volume, different counting methods or counting chambers are available. The counting methods differ mainly in the dilution and composition of the dilution solutions.<sup>[1]</sup> The counting chambers differ in the counting nets and partly in the depths of the chambers.

In the following, the specifications of some counting chamber types (different counting nets) are listed.

#### Neubauer improved<sup>[2]</sup>

Standard counting chamber for blood tests, e.g. for erythrocyte, leukocyte and platelet counting.

Note/recommendation: For repeat orders insist on "Neubauer improved" (improved model).

CC-NEUI Counting net = 9 mm<sup>2</sup> total area. Chamber depth 0,100 mm. Divided into 9 square groups of 1 mm<sup>2</sup> area each.

In the middle square 5 x 5 = 25 squares with 16 least squares each of 0.05 mm side length (= 0.0025 mm<sup>2</sup>). Incl. 2 cover glasses.

CC-NEUI-B As above, but with bright lines (brightline, metallized surface).

#### Neubauer (conventional)<sup>[2]</sup>

Conventional counting chamber for blood tests, e.g. for erythrocyte, leukocyte and platelet counting.

Note/recommendation: For repeat orders insist on "Neubauer improved" (improved model).

CC-NEUI Counting net = 9 mm<sup>2</sup> total area. Chamber depth 0,100 mm. Divided into 9 square groups of 1 mm<sup>2</sup> area each.

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#### Thoma new

Counting chamber for blood tests, e.g. for erythrocyte and platelet counting.

Note/recommendation: The counting field corresponds to the inner area of the Neubauer improved counting chamber. You should therefore buy these because you can also count leukocytes.

Attention! Contrary to some literature and textbooks, in principle no leukocytes may be counted in the Thoma counting chamber!

#### Thoma (conventional)<sup>[2]</sup>

Counting chamber for blood tests, e.g. for erythrocyte and platelet counting.

Note/recommendation: The counting field corresponds to the inner area of the Neubauer counting chamber. But even this one is outdated. Insist on a Neubauer improved when buying a new one.

Attention! Contrary to some literature and textbooks, in principle no leukocytes may be counted in the Thoma counting chamber!

#### Fuchs-Rosenthal<sup>[2]</sup>

Counting chamber for small cell numbers, e.g. for counting cells in the cerebrospinal fluid.

CC-FURO Fuchs Rosenthal - Counting chamber  
Counting net = 16 mm<sup>2</sup> total area. Chamber depth 0.2 mm. Divided into 16



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square groups of 1 mm<sup>2</sup> area each.  
Per mm<sup>2</sup> 16 least squares of 0.25 mm side length each (= 0.0625 mm<sup>2</sup>).  
Incl. 2 cover glasses.

### Nageotte [2]

- Use e.g. for counting the residual cells in blood and plasma cans.
- CC-NAGE Nageotte counting chamber with horizontal lines (usually in the western world).  
Counting net = 100 mm<sup>2</sup> total area. Chamber depth 0.5 mm. Volume = 50 µL.  
Divided into 40 strip fields (rectangles) each with side length of 10 x 0.25 mm (= 2.5 mm<sup>2</sup> area). The centerline is marked by an additional double line. Incl. 2 cover glasses.
- CC-NAGV Nageotte counting chamber with vertical lines, otherwise as above.

### Cover glasses for counting chambers

- Simple cover glasses cannot be used. Only use cover glasses approved for the counting chamber.
- CC-CS.20x26 Counting chamber cover glasses (spare) in precision design with optically flat surface grinding on both sides. Pack of 10.  
Thickness = 0.4 mm; size = 20 x 26 mm (standard size, others on request).

### Safety

Observe the necessary precautions when using laboratory reagents and body fluids; as well as microbiological samples. Applications should be performed by expert personnel only. Follow the national and laboratory internal guidelines for work safety and infection control. Wear suitable protective clothing and disposable gloves while handling.

It is important to ensure effective protection against infection according to laboratory guidelines.  
Use a capillary holder for capillaries.

## Structure of the Counting Chamber

Counting chambers consist of a glass block of special optical glass. The base plate is divided into two wide fields (outside) for lettering and three narrow webs (inside). The three narrow webs are divided up as follows:

- A centre web (bottom of the chamber) on which a network of lines (counting net) is engraved, which differs according to the type of chamber. Normally, two counting nets are engraved, separated from each other by a groove.
- The cover glass is pushed onto the supporting webs located on the left and right of the centre web. These carrier webs have a precisely defined height in relation to the plane of the counting nets and thus specify the distance between the cover glass and the counting net (= chamber depth; e.g. 0.100 mm).

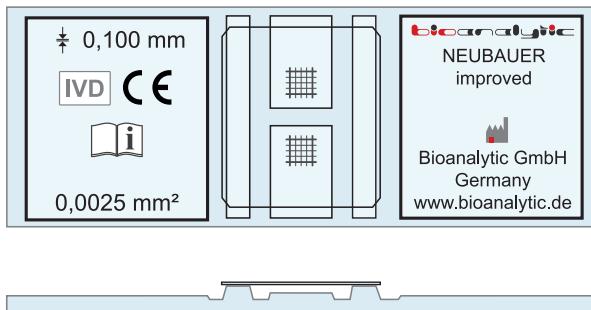


Figure 1:  
Structure of the counting chamber: top view (top) and side view (bottom).

The cover glasses for counting chambers differ from cover glasses for normal light microscopy in that their surfaces are flat-ground and polished. In addition, they are slightly thicker than normal cover glasses to avoid being bent through by capillary forces.

Cover glasses MUST match the counting chamber! These are rectangular and not square. The 4 corners are bevelled and not 90°-angled. Damaged counting chambers must be replaced. Damaged cover glasses must not be used any longer.

## Preparation

Before using the counting chamber, it must be prepared for use. For this purpose, the ground cover glass must be "slid" onto the carrier webs. A special technique is required to ensure that there is no distance between the flat-ground support beams and the cover glass. Proceed as follows:

- Check the counting chamber and cover glass for cleanliness! No lint, no fingerprints in the counting net area or on the cover glass!
- Place the counting chamber on a clean, smooth and firm table surface.
- Use a slightly damp cotton swab and lightly moisten the carrier webs. There must not be any water left on it that could run down when inclined. For better wetting of the glass surface, one drop of glycerine per 1 mL can be added to the water and mixed.
- The cover glass is placed parallel to the counting chamber on the carrier webs, not in the middle but 3 mm deeper.

• Immediately afterwards, the cover glass is pushed upwards by a slight pressure on the carrier webs by about 3 mm, so that the cover glass lies in the middle. Due to the adhesion of the water, the cover glass adheres to the support webs.

The cover glass is properly positioned when:

- the cover glass can no longer be moved.,
- Newtonian rings (interference lines) are formed by oblique incidence of light (rainbow colored edges)
- the cover glass covers both counting fields correctly, i. e. sits as centred as possible on the counting chamber.

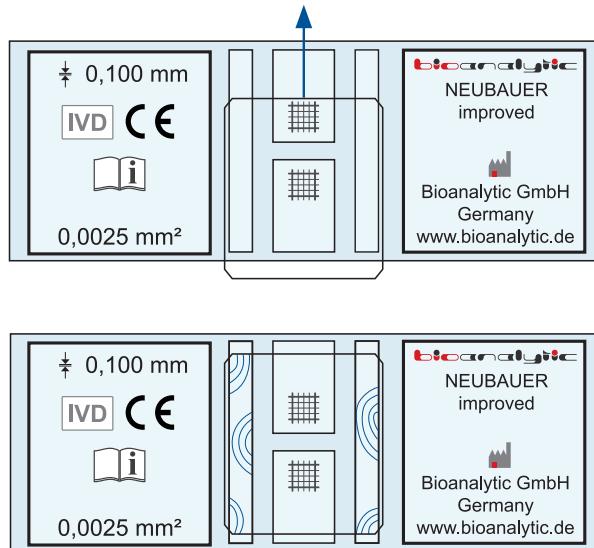


Figure 2:  
Preparation of the counting chamber. Sliding of the cover glass onto the carrier webs (top) and Newtonian rings (bottom).

## Filling the Counting Chamber

See also the instructions for your test method, especially with regard to special features such as the cell sedimentation prior to counting in a wet chamber. The most important thing is the sufficient homogenization of the sample (suspension) DIRECTLY BEFORE filling the counting chamber in order to transfer a representative number of cells. Otherwise, results may be both too high and too low.

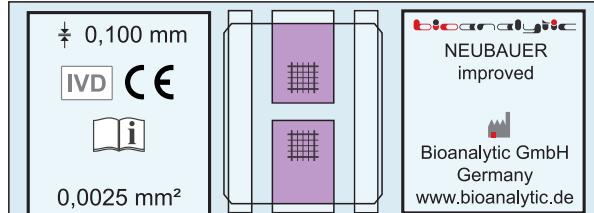
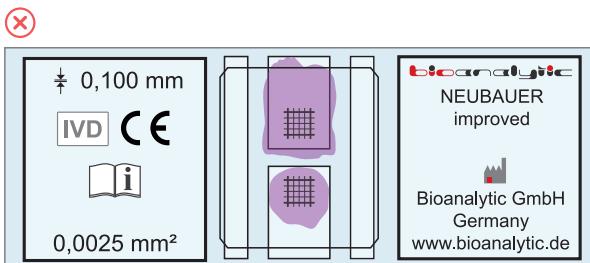
Attention!

The counting chamber must not be underfilled or overfilled. The counting area must be filled exactly without sample overflowing into the bevelled area or liquid accumulating there. Excess liquid must not be removed with a swab. In this case, the chamber must be cleaned and refilled.

The counting chamber is correctly filled if only the complete area of the centre web (counting net) is filled underneath the cover glass.

In the case of coloured dilutions, only a very narrow colour fringing is produced at the edges.

The correct filling of a counting chamber requires some practice. Only count the cells if the filling has been carried out correctly.



**Figure 3:**  
Filling the counting chamber. Overfilled and underfilled counting chamber (top) and correctly filled counting chamber (bottom).

Various instruments and procedures are available for the correct filling of the counting chamber:

#### Chamber filling capillaries

Chamber filling capillaries are the standard in ready-to-use TIC systems supplied by Bioanalytic GmbH.

The chamber filling capillary is filled with freshly resuspended sample dilution by capillary action about half to 2/3. The upper end is closed with one finger. Wipe off any outside droplets at the bottom end. Then bring the open lower end diagonally to the cover glass gap / cover glass edge. By slightly opening the upper end, the sample is allowed to run between the counting net and the cover glass until it is correctly filled.

#### Piston-stroke pipette

A counting chamber can also be filled with a piston-stroke pipette. The exact volume depends on the counting chamber and the area below the cover glass. Therefore, an exact volume cannot be given. Either the counting chamber is filled visually according to filling level or the filling volume is determined empirically. As a rule, this lies between 15 and 50 µl.

#### Blood-mixing pipette

Blood-mixing pipettes have already been declared obsolete (inaccurate, dangerous) by the WHO (World Health Organization) in 1988. Therefore, the chamber filling is not discussed here further.

## Microscope

A good laboratory microscope with a cross-table is expected for cell counting. It must usually have the magnifications 100× and 400×.

The magnification is obtained by multiplying the lens and the ocular. 40x objective × 10x ocular = 400× magnification.

Please always follow the instructions of the microscope manufacturer, as only general information can be given here.

The microscope should be clean and well-kept and should be serviced / inspected regularly. Please contact your microscope manufacturer or supplier.

#### Approach

If slightly stained or unstained preparations are used, the aperture on the condenser of the microscope must be set to narrow (dimmed out) for cell counting. Alternatively, or if there is no condenser aperture, the condenser must be placed at the very bottom.

The counting chamber is mounted on the cross-table and the lens is swivelled 10 times into the optical path. The coarse/fine focus adjustment regulates the distance between the cross-table and the lens in such a way that the counting net is displayed.

This presetting can also be prepared with an empty counting chamber. After that, a higher magnification (larger lens) can be adjusted if required. Then only a slight correction in the sharpening plane is necessary.

## Counting Method

Depending on the cell type of the cells to be counted, different groups of squares are counted and an average value is calculated. The counting of cells requires precise knowledge of the counting method and how to deal with the boundary lines. The rules are explained below.

#### Meander-shaped counting

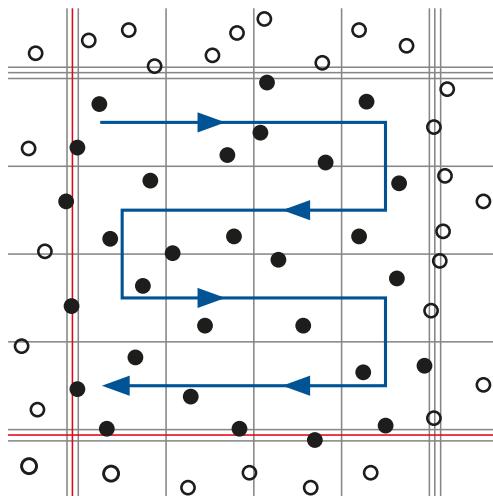
To avoid erroneous counting, the counting of squares is done in a meandering pattern.

#### Boundary line rule (L-rule)

When counting, the boundary lines must be carefully observed. For counting chambers with triple boundary lines, such as the Neubauer improved counting chamber, the middle line represents the actual boundary line.

When counting, it is important to note that two boundary lines of a counting field that are not opposite each other are discriminated against. This means that all cells within the field are counted, as well as the cells lying on the so-called "L". The cells opposite the "L" are not counted. This avoids that cells lying on the lines of the counting net are not counted twice and only in this way the counted area is exactly maintained.

Therefore, it is important to consider which boundary lines should be excluded from the count before counting. These can be the upper and right boundary lines, for example, or the combination of lower and left boundary lines (see figure). This counting technique must not be deviated from within the counting process.



**Figure 4:**  
Schematic representation of the cells of a field according to the "L-rule" (red lines) and meandering counting (blue).

#### Double determination

In principle, double determinations are prescribed for in-vitro diagnostics (IVD). For other applications, please observe the applicable regulations.

For double determination, both chamber fields (upper and lower counting net) are filled with the same sample and both chamber fields are counted. The result of both counts may deviate by a maximum of 10 %, then the mean value from both chamber fields is used as the result. In case of larger deviations, repeat the counting with freshly prepared dilution solution if necessary.

## Microscopic Counting of Cells

Cells are counted and calculated in accordance with the usual instruction for use or the manufacturer's instructions (dilution solution, test kit).

Use the magnification, counting area and calculation specified there.

## Calculation

The calculation of the number of cells depends on the dilution and the chamber volume.

The information on this can usually be found in the instructions for use of your counting method, in the information provided by the dilution solution manufacturer or in the instructions contained in the test kit.

#### Calculations

$$\text{counted chamber volume } [\mu\text{l}] = \text{counted chamber area } [\text{mm}^2] \times \text{chamber depth } [\text{mm}]$$

$$\text{Number of cells / } \mu\text{l} = \text{counted cells} \times (1 / \text{counted chamber volume}) \times \text{dilution factor}$$

## Cleaning and Disinfection

Please refer to the literature [3].

Only use undamaged and clean counting chambers and cover glasses!

## Error Possibilities

If the following errors occur, the counting chamber must be cleaned, re-prepared and refilled.

### Too high cell counts

- Dilution was not resuspended immediately before chamber filling.  
⇒ Attention! Prepare new dilution. The existing dilution was falsified by the extraction and can no longer be used for further counting.
- Cover glass not fixed or floating, can be moved.  
No Newtonian rings visible.
- Counting chamber filling incorrect.
- Wrong fields were counted.
- L-rule not observed.
- Boundary lines not correctly observed.

### Too low cell counts

- Dilution was not resuspended immediately before chamber filling.  
⇒ Attention! Prepare new dilution. The existing dilution was falsified by the extraction and can no longer be used for further counting.
- Counting chamber filling incorrect.
- Wrong fields were counted.
- L-rule not observed or only cells within counted.
- Boundary lines not correctly observed.

### Implausible results

- Wrong calculation  
⇒ Verify and correct.
- Wrong dilution.  
⇒ Attention! Prepare new dilution!
- Incorrect counting chamber, wrong counting chamber depth (special depths exist).

## Notes

### Classifications

EU: IVD, CE  
AU: Class I, IVD  
CA: HC: Class I; exempt; for in-vitro diagnostic use.  
US: FDA: GHO; Class I; exempt; for in-vitro diagnostic use.

## Waste Management

For disposal, please observe the regulations of your country. The counting chambers are made of glass.

## Literature & Footnotes

Observe the instructions of the reagent or kit manufacturer. In particular, in-vitro diagnostics (IVD) must not deviate from this standard.

- [1] Act of Medical Devices; Directive 98/79/EC on in-vitro diagnostics.
  - [2] Product information Bioanalytic GmbH, counting chamber descriptions with coloured illustrations and calculation examples.  
Available via download at the respective counting chamber under [www.bioanalytic.de](http://www.bioanalytic.de).
  - [3] Product Information Bioanalytic GmbH, Counting chambers - Cleaning and disinfection of haemacytometers.  
Available via download at the respective counting chamber under [www.bioanalytic.de](http://www.bioanalytic.de).
- (1) Dilution solutions and test kits are available for different examination methods: [www.bioanalytic.de](http://www.bioanalytic.de) or information on request:  
[support@bioanalytic.de](mailto:support@bioanalytic.de).