# Cell Counting with Neubauer Chamber

## Basic Hemocytometer Usage


## Introduction

Despite the fact of the recent technical development of scientific laboratories, the Neubauer chamber remains the most common method used for cell counting around the world.

This article has been written in order to help newbies and experimented researchers to perform a proper cell counting using a Neubauer chamber or Hemocytometer.

The principles described in this article apply to any cell counting chamber, although the dimensions and volumes of each chamber may differ.

First, the parts and basic principle of the Neubauer chamber are described

Second, the article describes how to perform a cell count step by step, in order to achieve reliable and reproducible results. The article describes best practices and recommendations when performing a cell count.

## Materials

The necessary elements to perform a cell count with Neubauer chamber are as follows:

a) cellular dilution to measure
b) hemocytometer, or Neubabuer chamber
c) optical microscope
d) cover glass
e) pippette / micropippete with disposable tips.
f) Dilution buffer / PBS (if needed)

### THE NEUBABUER CHAMBER, OR HEMOCYTOMETER

The Neubauer chamber is a thick crystal slide with the size of a glass slide. (30 x 70 mm and 4 mm thickness)

In a simple counting chamber, the central area is where cell counts are performed. The chamber has three parts.

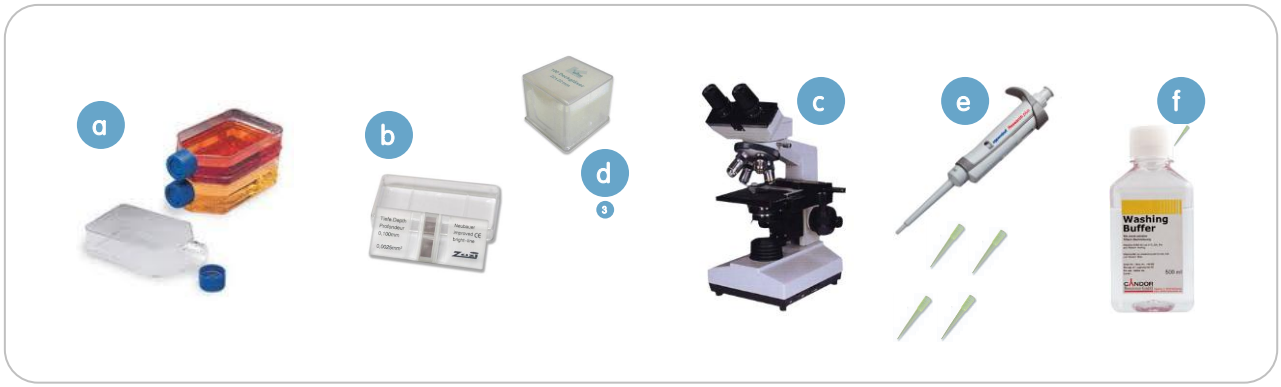


Fig 1. Necessary equipment to perform a cell count with hemocytometer.

The central part, where the counting grid has been set on the glass.

Double chambers are most common than simple chamber. In this case, the chamber has two counting areas than can be loaded independently.

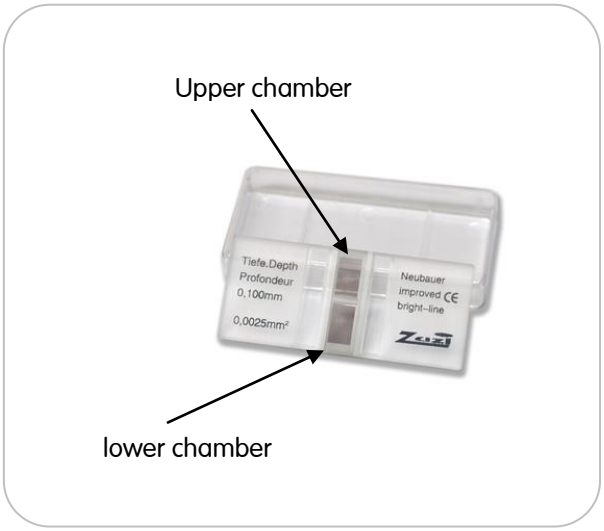


Fig 2. Neubauer comercial chamber

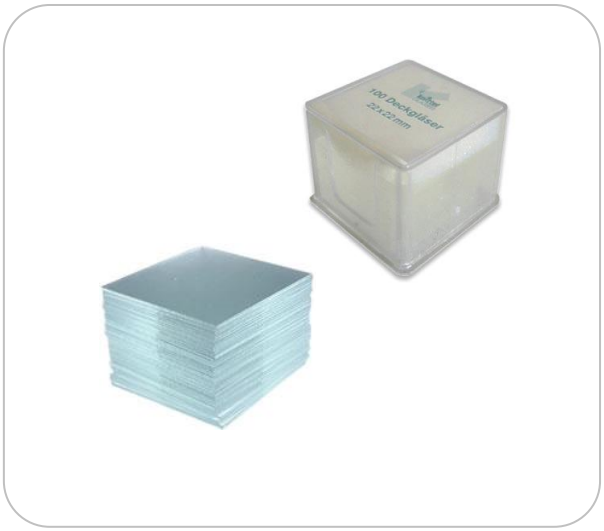

Fig 3. Pile of glass covers, and box.

Neubauer chamber's counting grid is 3 mm x 3 mm in size. The grid has 9 square subdivisions of width 1mm. (See Fig. 4-1)

In case of blood cell counting, the squares placed at the corners are used for white cell counting. Since their concentration is lower than red blood cells a larger area is required to perform the cell count.

The central square is used for platelets and red cells. This square is split in 25 squares of width 0,2 mm ( 200µm). See Fig. 4-2. Each one of the 25 central squares is subdivided in 16 small squares. Fig 4-3.

Therefore, the central square is made of 400 small squares.

## GLASS COVER.

The glass cover is a squared glass of width 22 mm. The glass cover is placed on the top of the Neubabuer chamber, covering the central area. The glass cover leaves room for the cell concentration between the bottom of the chamber and the cover itself. The chamber is designed so that the distance between the bottom of the chamber and the cover is 0,1 mm.

It is not uncommon that the glass cover remains slightly lifted when we introduce more liquid than necessary in the chamber. To avoid this, some counting chambers have two special clamps to avoid the cover glass to avoid edge-lift.
If the glass cover is lifted, the distance between the chamber and the cover will be higher than 0,1 mm, and the cell counts will not be accurate.

## PIPETTE

The pipette allows for the introduction of a precise amount of liquid into the Neubauer chamber. Historically, they have been manufactured in glass. Nowadays, glass pipettes have been replaced by micropipettes, than can be calibrated with a maximum capacity of 20, 200 and 2000 µl.

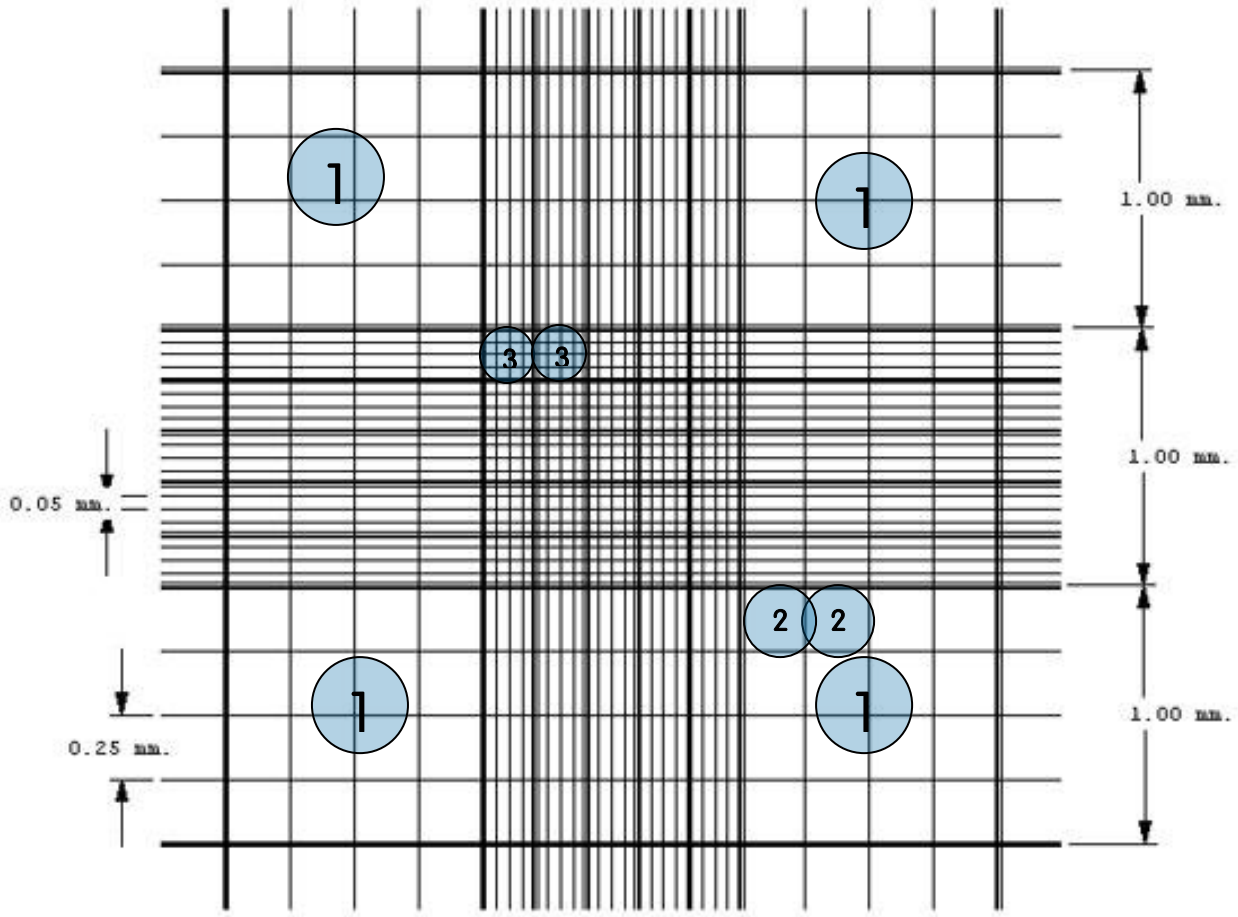


Fig 4. Neubauer-improved chamber counting grid detail.

## Cell count, step by step

**STEP 1. Sample preparation.**

Depending on the type of sample, a preparation of a dilution with a suitable concentration should be prepared for cell counting.

Typically, the concentration range for a cell count with Neubauer chamber is between 250.000 cells / ml and 2,5 million cells / ml.

It is recommended for the dillution concentration to be around $10^6$ cells / ml (1 millón cells / ml) applying the required dilutions.

With concentrations below 250.000 cells per ml, ($2,5 * 10^5$ cells / ml) the amount of cells counted will not be enough to obtain a fair estimation of the original concentration. [1]

Above 2,5 million cells / ml ($2,5 * 10^6$), the probability of counting errors increases as well as the time and effort required to achieve a reliable cell count.

Above 2,5 million, it is preferable diluting the sample to obtain a final concentration closer to the optimum 1 million per ml. It is important to write down the dilution performed to the original sample.

[1] See: http://www.celeromics.com/conteo-celular-con-concentraciones-bajas.htm, for an statistical explanation

**STEP 2. Introducing the sample into the Neubauer chamber**

Take 10 µl of dilution prepare in STEP 1 with the micropipette.

1) Put the glass cover on the Neubauer chamber central area. Use a flat surface to place the chamber, like a table or a workbench.
2) Put a disposable tip at the end of the micropipette.
3) Adjust the micropipette to suck 10 µl. You can adjust it by turning the upper plunger roulette to select the required pipetting volume.
4) Introduce the micropipette tip on the dilution previously prepared (STEP 1)
5) Push the pipette plunger slowly until you feel it has arrived to the end of its travel.
6) Remove the pipette tip from the dilution, and bring it to the Neubauer chamber. When the pipette is loaded, it must always be held in vertical position.
7) Place pipette tip close to the glass cover edge, right at the centre of the Neubauer chamber.
8) Release the plunger slowly watching how the liquid enters the chamber uniformly, being absorbed by capillarity. See Fig. 5
9) In case of the appearance of bubbles, or that the glass cover has moved, repeat the operation.

Congratulations!. The Neubabuer chamber has been loaded, and it is ready to perform the cell count.

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Fig.5. Sample filling a Neubauer chamber.

**STEP 3. Microscope set up and focus.**

1. Place the Neubauer chamber on the microscope stage. If the microscope has a fixing clamp, fix the Neubauer chamber.
2. Turn on the microscope light.
3. Focus the microscope until you can see a sharp image of the cells looking through the eyepiece and adjusting the stage.
4. Look for the first counting grid square where the cell count will start. In this example, 5 big squares from a Neubauer-Improved chamber will be counted. See Fig. 6

See http://www.celeromics.com/easy-formula-for-manual-cell-counting.htm, for the formulas to be applied for the most common counting chamber (Thoma, Fuchs-Rosenthal, Nageotte, etc)

5. Start counting the cells in the first square.

   Different laboratories have different counting protocols, but there is a popular unwritten rule that states:

   "Cells touching the upper and left limits should be counted, unlike cells touching the lower and right limits which should not be taken into account"

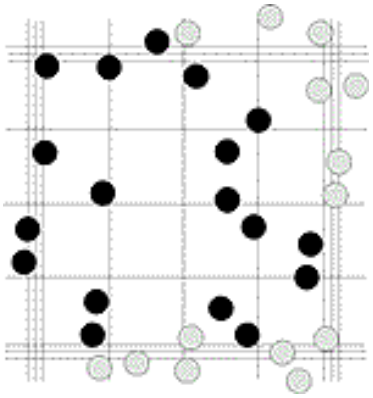

Fig. 6. Count in a Neubauer chamber big square.

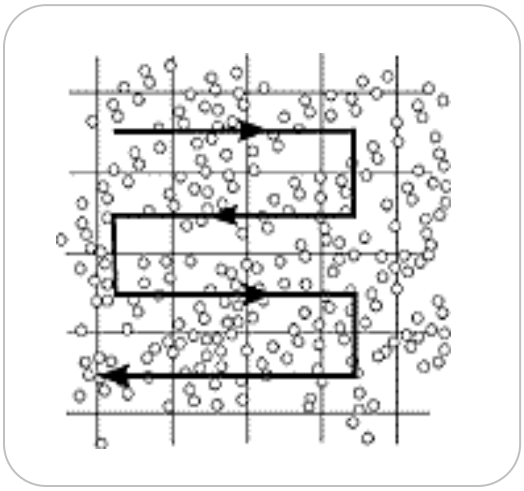

Fig. 7. High cell concentration cell count.

In case of high cell concentration, it will become very easy to get lost when counting cells. In this case, a counting technique in zig-zag is used.

6. Write down the amount of cells counted in the first square.

7. Repeat the process for the remaining squares, writing down the counting results from all of them. The higher the number of cells counted, the higher the accuracy of the measurement.

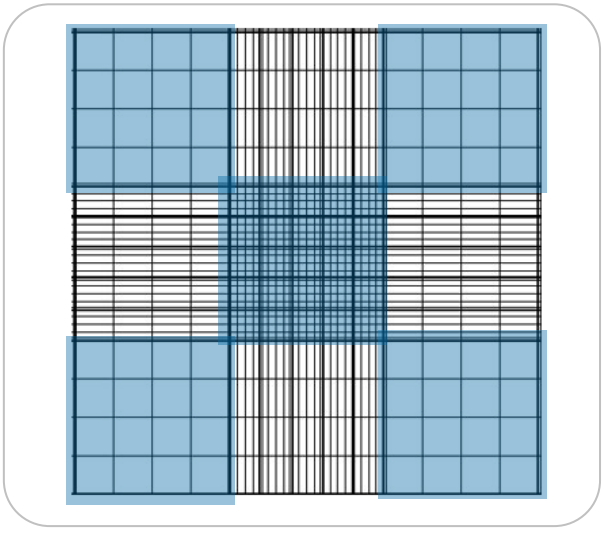

Fig. 6. Example of cell count in one of the 9 big squares of a Neubauer chamber.

**STEP 4: Concentration calculation**

We apply the formula for the calculation of the concentration

$$\text{Concentration (cel / ml)} = \frac{\text{Number of cells}}{\text{Volume (in ml)}}$$

The number of cells will be the sum of all the counted cells in all squares counted.

The volume will be the total volume of all the squares counted.

Since the volume of 1 big square is:

0,1 cm x 0,1 cm = 0,01 $cm^2$ of area counted.
Since the depth of the chamber is 0,1mm
0,1 mm = 0,01 cm
0,01 $cm^2$*0,01 cm = 0,0001 $cm^2$ = 0,0001ml = 0,1 µl

So, for the Neubauer chamber, the formula used when counting in the big squares.

$$\text{Concentration} = \frac{\text{Number of cells} \times 10.000}{\text{Number of squares}}$$

In case a dilution was applied, the concentration obtained should be converted to the original concentration before the dilution.

In this case, the concentration should be divided by the dilution applied.

The formula will be:

$$\text{Concentration} = \frac{\text{Number of Cells x } 10.000}{\text{Number of square x dilution}}$$

Example:
For a 1 : 10 dilution. Dilution = 0,1
For a 1 : 100. Dilution = 0,01

**Error**

Errors in the range of 20%-30% are common in this method due to pipetting errors, statistical errors, chamber volume errors, and errors from volume of simple introduced into the chamber.

Even though, the Neubauer chamber remains the most widely used cell counting method in the world.

To obtain a detailed analysis of the error introduced in a cell count, go to:

http://www.celeromics.com/cell-count-error.htm