General Information: CellVision counting chambers

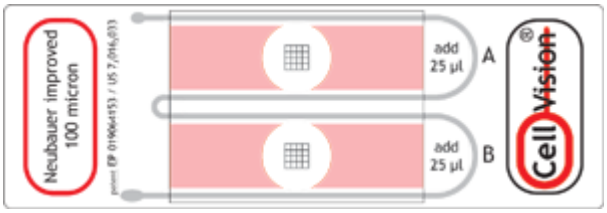
INTRODUCTION:

CellVision glass disposable counting chambers (hemocytometers) with fixed coverslips, built-in grids and precise chamber-depths are the most innovative development of the last decade in the field of manual and automated cell counting and assessment of motility and viability in sperm. CellVision hemocytometers minimize the risk of infectious handling; changing manual cell-counting into a normal and safe laboratory practise.

CellVision hemocytometers are especially designed to eliminate non-hygienic, time-consuming and non-productive handling associated with the use of re-usable hemocytometers, providing its users maximum cell-visibility.

In comparison to plastic counting chambers, and old re-usable hemocytometers; the accuracy and reproducibility of manual cell counting results when using CellVision hemocytometers is unsurpassed due to the absence of filling errors associated with conventional loading combined with a stable and reproducible chamber depth.

Absolutely no changes in counting-protocols were a prerequisite for the development of CellVision; lab-technicians can easily adapt to this new counting device (a wide variety of types with international known grids and chamber-depths are available).



Neubauer improved counting chamber

TECHNICAL DESIGN:

The CellVision hemocytometer consists of a microscopic-slide with a fixed attached coverslip. The printed ink-pattern on the back-side of the microscopic-slide is showing type name, height, loading and counting areas. A glue-pattern between the microscope-slide and coverslip divides the area into 2, 4, 6 and even 8 chambers (depending on type), creating loading-zones and providing an exact chamber-depth over the whole counting area.

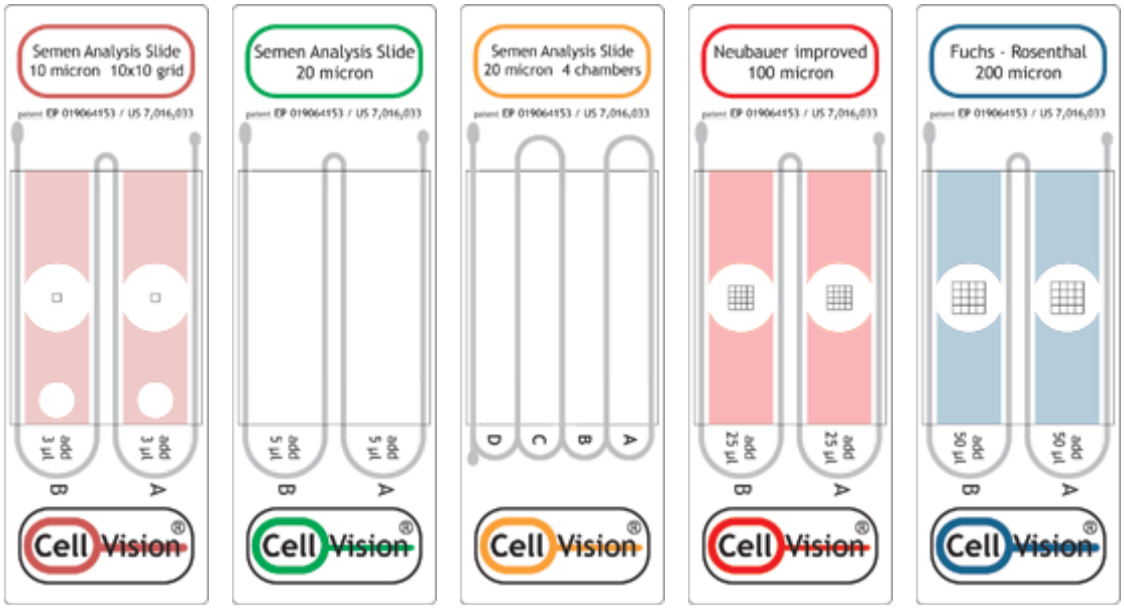
CellVision counting chamber types with a built-in grid on the bottom of the microscope-slide surface (only available in a 2 chamber design) carry a circular thin foil layer inside the counting area which holds the grid-pattern. The counting grid consists of lines with the same dimensions as in re-usable devices allowing cells – located on the lines - to be noticed; in contrast with the much wider and overexposed lines in plastic hemocytometers.

All CellVision counting chambers are specially cleaned, washed, rinsed and coated, to prevent sticking of cells to the glass and to minimise the adverse effects of glass-ions to living cells.

CELLVISION TYPES AVAILABLE:

Article number	Chamber height	Grid type	2/4/6/8 chamber	Special Sperm Coating
CV 1010	10 µm	N.A.	2	Yes
CV 1010-4ch	10 µm	N.A.	4	Yes
CV 1010-102	10 µm	Makler / 10^2	2	Yes
CV 1012	12 µm	N.A.	2	Yes
CV 1012-4ch	12 µm	N.A.	4	Yes
CV 1016	16 µm	N.A.	2	Yes
CV 1016-4ch	16 µm	N.A.	4	Yes
CV 1020	20 µm	N.A.	2	Yes
CV 1020-4ch	20 µm	N.A.	4	Yes
CV 1020-4cv	20 µm	N.A.	4	Yes
CV 1020-6ch	20 µm	N.A.	6	Yes
CV 1020-8ch	20 µm	N.A.	8	Yes
CV 1020-102	20 µm	Makler / 10^2	2	Yes
CV 1020-PV	20 µm	Post Vasectomy	2	Yes
CV 1030	30 µm	N.A.	2	Yes
CV 1030-4ch	30 µm	N.A.	4	Yes
CV 1100	100 µm	N.A.	2	Yes
CV 1100-4ch	100 µm	N.A.	4	Yes
CV 1100-NI	100 µm	Neubauer improved	2	No
CV 1100-BT	100 µm	Bürker-Türk	2	No
CV 1100-T	100 µm	Thoma	2	No
CV 1100-B	100 µm	Bürker	2	No
CV 1200-FR	200 µm	Fuchs Rosenthal	2	No

Appendix is showing some Grid images and dimensions



CellVision hemocytometers are disposable: no disassembling, washing, cleaning, drying and re-assembling steps are needed to perform manual cell-counting. The risk of contact with potential infectious material decreases dramatically using CellVision hemocytometers compared to re-usable devices. Nevertheless, CellVision users should take care at all times when working with material from biological origin.

GENERAL PREPARATION FOR CELL COUNTING:

Microscope setting:
CellVision hemocytometers are used for counting cells and particles and can be used with standard light- , phase-contrast or dark-field microscopes. For optimum view and concentration results; the microscope settings must be in accordance to the microscope manufacturers manual. The best contrast is gained with the capacitor of the microscope almost shut (light-microscope) or 10X phase-contrast.

Counting technique:
To obtain a correct concentration, counting should be performed using international accepted protocols.

Example of a counting protocol:

- Determine the total surface (the number and dimensions of boxes) of the grid that has to be counted

- Always start in the upper-left corner and proceed in the direction that is shown on figure 1.
- Count all cells which are completely within each box and all cells that touch the left and upper lines of the box (figure 2.) In this example (type: Neubauer improved) the outer line of the box to be counted is the middle line. The cells "painted black" (18 pc.) are correct

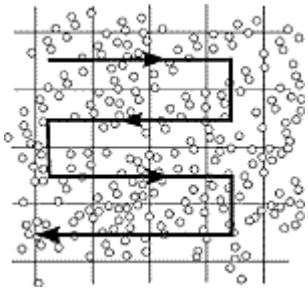


Figure 1.

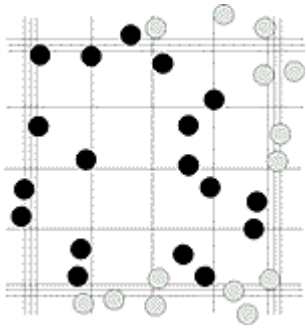


Figure 2.

Normally a simple factor is used to directly obtain the concentration of cells after the counting is performed (see Counting Examples). The standard method of calculation is described here. A precise chamber depth is essential for concentration purposes. All CellVision hemocytometers consist of a fixed coverslip ensuring accurate chamber-depth and reproducible concentration determinations.

Standard formula for concentration of cells:

$$\text{Total Cells in 1µl sample} = \frac{\text{Number of counted cells}}{\text{Total surface counted } (mm^2) * \text{chamber-depth } (mm) * \text{dilution}}$$

As valid for all concentration determinations: the more cells counted - the higher the accuracy and predictive value of the test.
In general: the best concentration results with a hemocytometer are yielded when approx. 200 cells or more per test are counted.

APPLICATIONS:

Main fields of application for CellVision hemocytometers are situated in haematology, human and animal fertility, tissue-culture, immuno-fluorescence and microbiology. Also exotic applications are known; e.g. fat-bulbs in milk, resin-particles in paper-pulp, plankton in sea-water, yeast cells in beer-concentrate etc..

FERTILITY - SEMEN APPLICATIONS:

Two different ways of analysis can be distinct in semen applications; manual and automated (CASA) analysis.

Manual application:

During manual analysis of semen, the technician uses a microscope and a CellVision hemacytomer with a built-in grid to be able to determine both concentration and motility of spermcells.

Most suited for manual semen analysis is the CellVision hemocytometer consisting of a 2-chamber slide design with the 10^2 built-in grid. For human semen-analysis a chamber depth of 20µm is most advisable. It allows the technician to have a constant sharp view of moving spermcells with a standard magnification of 100x or 200x. More over, human spermcells are not hindered in their motion (as seen with hemocytometers with a lower chamber-depth), thus showing more actual motility-figures. For laboratories having experience with the re-usable Makler® counting chamber, the CellVision CV 1010-102 is the perfect replacement because both chamber depth and grid dimensions are exactly the same.

Preferable slides to use in manual applications:

CV 1010-102:	10 µm depth	10^2 grid	(Makler replacement)
CV 1020-102:	20 µm depth	10^2 grid	

Automated application:

Automated semen-analysis is performed with the use of a CASA-system (Computer Aided Sperm Analyser). A CASA-system consists of a microscope-camera-software combination, able to detect and follow spermcells in the counting chamber. The software calculates concentration and a wide-variety of motion-parameters; some systems also offer morphology determination of spermcells.

The CellVision 10, 12, 16 and 20µm depth chamber types without a grid are mostly suited for human and animal CASA applications. The 30 and 100 µm depth counting chambers are used for toxicology tests in, for example, mice or rat sperm.

Preferable slide to use in automated CASA applications:

CV 1100:	100 µm depth	no grid	
CV 1100-4ch	100 µm depth	no grid	
CV 1030:	30 µm depth	no grid	
CV 1030-4ch:	30 µm depth	no grid	
CV 1020:	20 µm depth	no grid	
CV 1020-4ch:	20 µm depth	no grid	
CV 1020-4c**v**:	20 µm depth	no grid	***
CV 1020-6ch:	20 µm depth	no grid	
CV 1020-8ch:	20 µm depth	no grid	
CV 1016:	16 µm depth	no grid	
CV 1016-4ch	16 µm depth	no grid	
CV 1012:	12 µm depth	no grid	
CV 1012-4ch:	12 µm depth	no grid	
CV 1010:	10 µm depth	no grid	
CV 1010-4ch:	10 µm depth	no grid	

*** The CellVision CV 1020-4c**v** is the type of choice for all Hamilton Thorne IVOS® CASA systems (IVOS, TOX IVOS and UltiMate), because the direction of the chambers on this slide is **vertical**. These mentioned CASA systems are not able to measure outside the middle-line of a microscope slide thus need vertical chamber direction.

HAEMATOLOGY APPLICATIONS:

Hemocytometers are widely used in haematology laboratories. Leucocytes, erythrocytes and platelets are counted manually in bodyfluids where automated-instruments aren't deployable or unavailable; e.g.

- concentration of the cells is lower than the detection-threshold of the instrument (i.e. spinal-fluid; disturbed bloodcell-growth; blood from patients during cancer treatments).
- body-fluid to be counted is highly viscous and / or is containing cell-cloths or other disturbing particles blocking the counting flow of instruments (i.e. ascitis, peritoneal or pleural-fluid; or fluids from inflammatory reactions).
- small number of samples.

The 100µm depth counting chambers are commonly used for counting a wide variety of bloodcells and other cells / particles. Based on region and historical grounds, some counting grids are widely used, others are rare. Although grid-types are slightly different, every type provides the same counting result when the right protocol is followed. The Neubauer improved grid is most commonly used worldwide and therefore expressed in counting examples in this manual. Specifications of the Neubauer improved and other available counting grids are shown in the Appendix.

Preferable slides to use: All 100 µm and 200 µm depth counting chambers

CV 1100-NI	100 µm depth	grid - Neubauer improved
CV 1100-B	100 µm depth	grid - Bürker
CV 1100-BT	100 µm depth	grid - Bürker-Türk
CV 1100-T	100 µm depth	grid - Thoma
CV 1200-FR	200 µm depth	grid – Fuchs Rosenthal

TISSUE-CULTURE APPLICATIONS:
In the field of Tissue-Culture it can be very important to exactly determine cell-concentration and or their viability in the culture-flasks. All kinds of cells can be counted and mainly the Neubauer improved grid type is used.

Preferable slide to use: see Haematology Applications

MICROBIOLOGY APPLICATIONS:
Determination of the concentration of bacteria in a growth-testing system is mostly done using photometry / turbimetry. But when a precise concentration is requested the hemocytometer is the product of choice.

Preferable slide to use: see Haematology Applications

COUNTING EXAMPLES:

Sometimes it is advisable to determine the concentration of an undiluted sample (when very low cell-counts are to be expected). In all other cases samples are diluted with a reagent/solution appropriate to the needs of the test.

Leukocyte count:
Normally in whole-blood; erythrocytes will disturb the counting of leucocytes because of the number (approx. 1000 x erythrocytes more than leucocytes). To improve the visibility of leucocytes; Türk Reagent which dyes the leucocytes and induces erythrocyte-lyses is added.

Platelet count:
For counting platelets the samples are diluted and cells are stained with i.e. Brecher's Reagent or Platelet Count.

Erythrocyte count:
Samples are diluted and cells are stained with i.e. Hayem's Reagent.

Also differentiation between living cells and dead cells (viability) can be performed with a staining method; i.e. Tryptan Blue, Erythrocin B or Nigrosin. The nuclei of a damaged cell will take up these reagents thus showing a stained nucleus in contrast to unstained nuclei of living cells.

Of course there are many more methods, reagents and solutions for specific applications, but all with the same target; to enhance the visibility of cells, making it easier to detect the cells to be counted and thus improving the accuracy of the counting result.

Example of Leukocyte, Erythrocyte and Sperm cell count

Leucocyte Count:

10µl of anticoagulated (K3-EDTA) whole-blood is added to a small test tube containing 90µl Türk-reagent. Mix carefully and allow the solution to stay for 2 minutes.
Using a microliterpipet, transfer 20µl of the blood-solution onto the loading-zone of 1 of the 2 chambers of the CellVision hemocytometer (Type: CellVision Neubauer-improved). See Figure 3. The chamber will fill itself by capillary action. After the chamber is completely filled; wipe away any possible excess from the loading zone. Allow the hemocytometer to stand for approx. 1-2 minutes so cells can settle by gravity onto the counting grid. Then count all leucocytes in 4 big boxes.

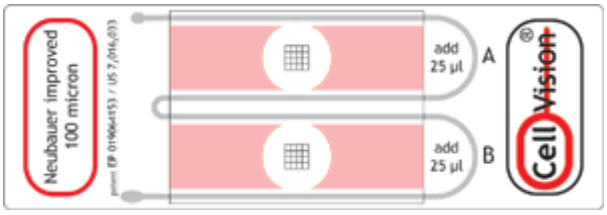
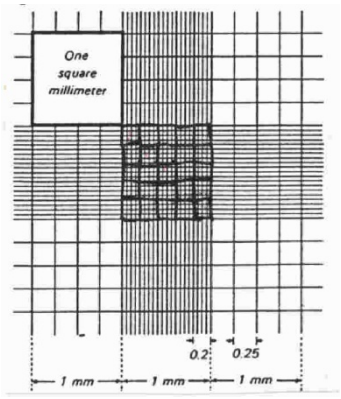


Figure 3.

Hemocytometer type:	CellVision Neubauer-improved
Total cells counted:	263
Surface:	4 big boxes 1mm² = 4mm²
Chamber-depth:	100µm (0.1mm)
Dilution:	1 : 10 with Türk reagent

$$\frac{263}{4 * 0.1 * 1/10} = \frac{263 * 10}{4 * 0.1 * 1} = 6575 \text{ cells per µl} = \underline{6{,}6 \times 10^{9}/L}$$



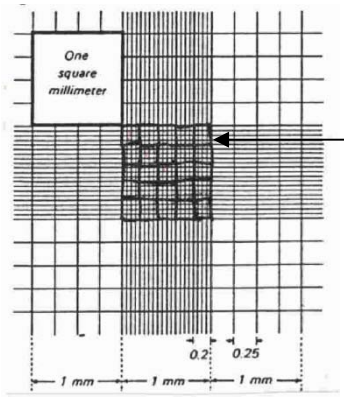
Neubauer-improved grid

Erythrocyte Count:

5µl of anticoagulated (K3-EDTA) whole-blood is added to a small test tube containing 495µl Hayem-reagent. Mix carefully and allow the solution to stay for 2 minutes. Using a microliterpipet, transfer 20µl of the blood-solution onto the loading-zone of 1 of the 2 chambers of the CellVision hemocytometer (Type: CellVision Neubauer-improved). The chamber will fill itself by capillary action. After the chamber is completely filled; wipe away any possible excess from the loading zone.
Allow the hemocytometer to stand for approx. 1-2 minutes so cells can settle by gravity onto the counting grid. Then count all leucocytes in 4 big boxes.

Hemocytometer type:	CellVision Neubauer-improved
Total cells counted:	312
Surface:	1 middle boxes 0.04mm²
Chamber-depth:	100µm (0.1mm)
Dilution:	1 : 100 with Hayem reagent

$$\frac{168}{0.04 * 0.1 * 1/100} = \frac{168 * 100}{0.04 * 0.1 * 1} = 4{,}2 \times 10^{6}/\mu l = \underline{4{,}2 \times 10^{12}/L}$$



Middle box = 0.2 x 0.2 mm = 0.04mm²
1 Middle box contains 16 small boxes

Neubauer-improved grid

Sperm cell count:

Mainly all sperm cell counts are preferably performed in a 20µm depth chamber. Depending on the use of a CASA-system (some can't handle 20µm depth) or species type (i.e. rat-sperm is much bigger and needs a chamber depth of 100µm), other chamber depths can be chosen.

A. Sperm cell count with CASA-system:

Setup the CASA system to be used with the CellVision chamber of choice (20µm depth is advised). Allow the semen to liquefy completely (normally after 30 minutes liquification is complete) and transfer the appropriate amount of semen with a microliterpipette (depends on CellVision type used; as shown in table 1.) onto the loading-zone of a chamber. The chamber will fill itself by capillary action. Directly after the chamber is filled the analysis can be performed. If an excess of sample material is added to the loading zone, this excess should be wiped away after filing , before analysis. Continue according to the CASA user-manual

B. Sperm cell count manually:

Allow the semen to liquefy completely (normally after 30 minutes liquification is complete) and transfer the appropriate amount of semen with a microliterpipette onto the loading-zone of a chamber (depends on CellVision type used; as shown in table 1.). The chamber will fill itself by capillary action. Directly after the chamber is filled the analysis can be performed. If an excess of sample material is added to the loading zone, this excess should be wiped away after filing , before analysis.
Count all cells in 5 boxes (for the 20 µm type with grid).

Hemocytometer type:	CellVision 20µm with 10^2 grid (CV 1020-102)
Total cells counted:	204
Surface:	5 boxes 0.01mm² = 0.05mm²
Chamber-depth:	20µm (0.02mm)
Dilution:	non

$$\frac{204}{0.05 * 0.02 * 1} = \frac{204 * 1000}{1} = 204 \times 10^3/\mu l = 204 \text{ mil. } / \text{ml}$$

To differentiate spermcells (in WHO- ABCD-class), classify the movements of 100 sperm cells in the CellVision hemocytometer in the special motility-window provided in the chambers nearby the entrance. The total number of cells in each class represents the percentage.
To obtain absolute cell numbers; multiply the counting result of each class by the original concentration and subsequently divide this result by 100.

Chamber volume different CellVision designs:

Type CellVision counting chamber	Sample volume to be added to 1 chamber:
10 µm 2 chambers	3 µl
10 µm 4 chambers	2 µl
10 µm with 10² grid	3 µl
12 µm 2 chambers	3 µl
12 µm 4 chambers	2 µl
16 µm 2 chambers	4 µl
16 µm 4 chambers	3 µl
20 µm 2 chambers	5 µl
20 µm 4 chambers horizontal direction	4 µl
20 µm 4 chambers vertical direction	3 µl
20 µm 6 chambers	3 µl available up on request
20 µm 8 chambers	3 µl available up on request
20 µm with 10² grid	5 µl
20 µm with Post Vasectomy grid	5 µl
30 µm 2 chambers	8 µl
30 µm 4 chambers	5 µl
100 µm 2 chambers	25 µl
100 µm 4 chambers	15 µl
100 µm with all grid types	25 µl
200 µm with Fuchs Rosenthal grid	50 µl

All CellVision, glass disposable counting chambers are manufactured in a low-dust environment under strict GMP regulations all having the CE and IVD mark for In-Vitro Diagnostic Medical Device.
The CellVision / Attain Technologies B.V. production plant in Heerhugowaard, The Netherlands is ISO 9001:2008 accredited

CellVision / Attain Technologies B.V. also manufactures counting chambers for fluorescence applications in automated detection systems from a variety of manufacturers. If you have any request in any direction, please do not hesitate to contact us.

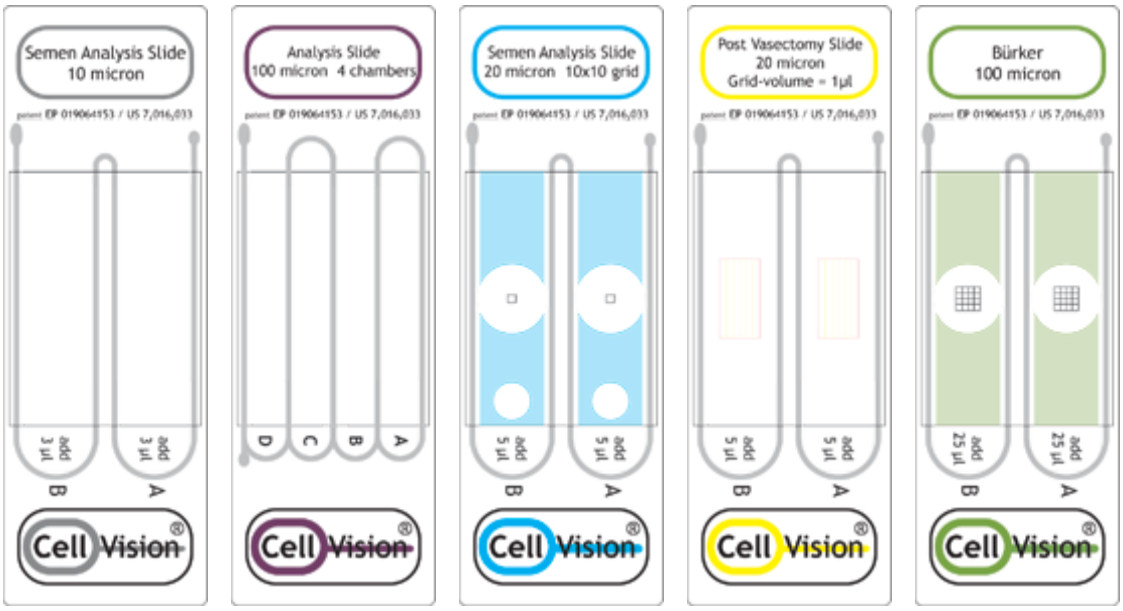
APPENDIX:

Ordering specifications

Please refer to your local CellVision, glass disposable counting chamber distributor for available types, articlenumbers, description, pricing and terms.

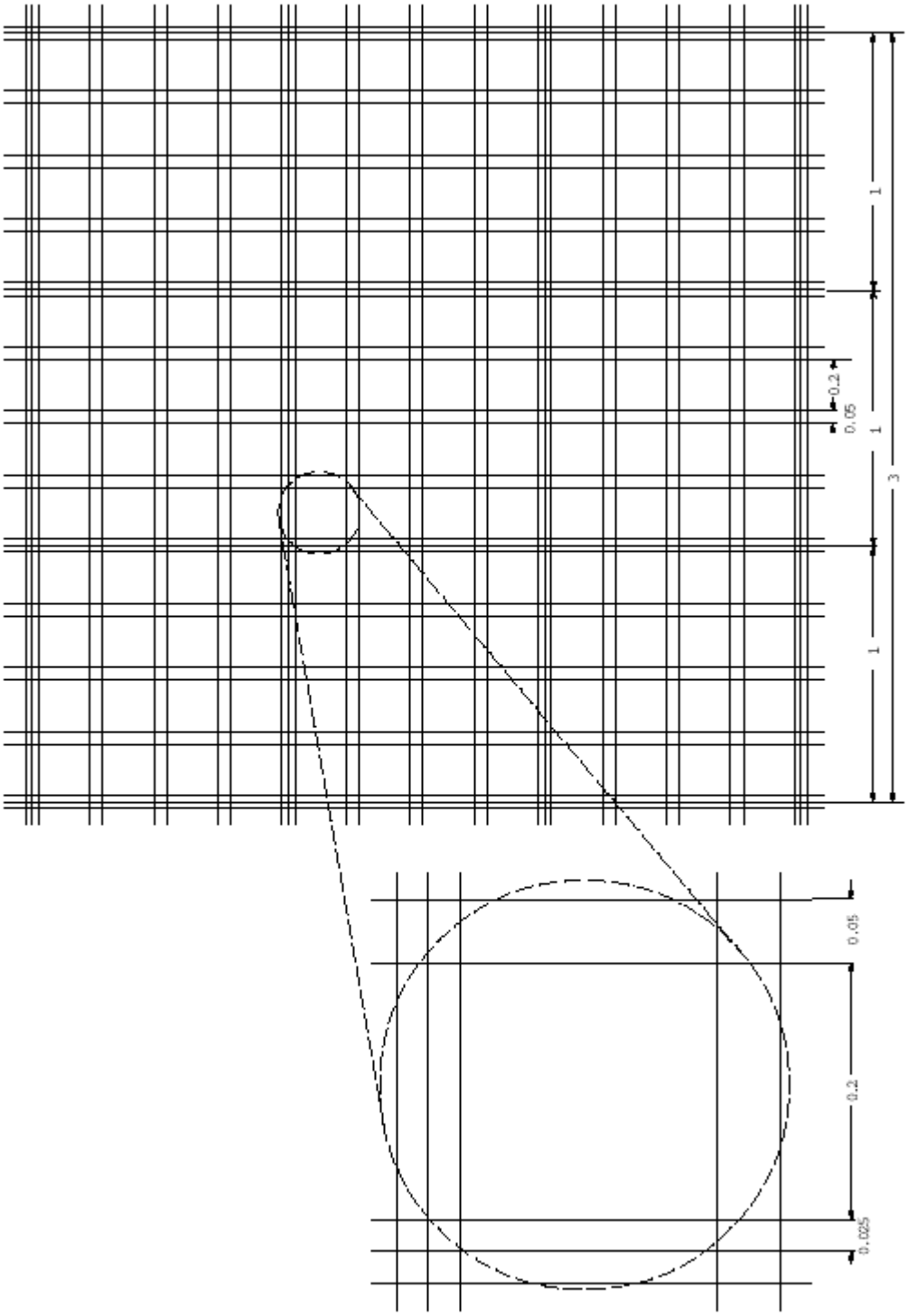
Local Distributor: Sticker or Stamp

CellVision Design types:

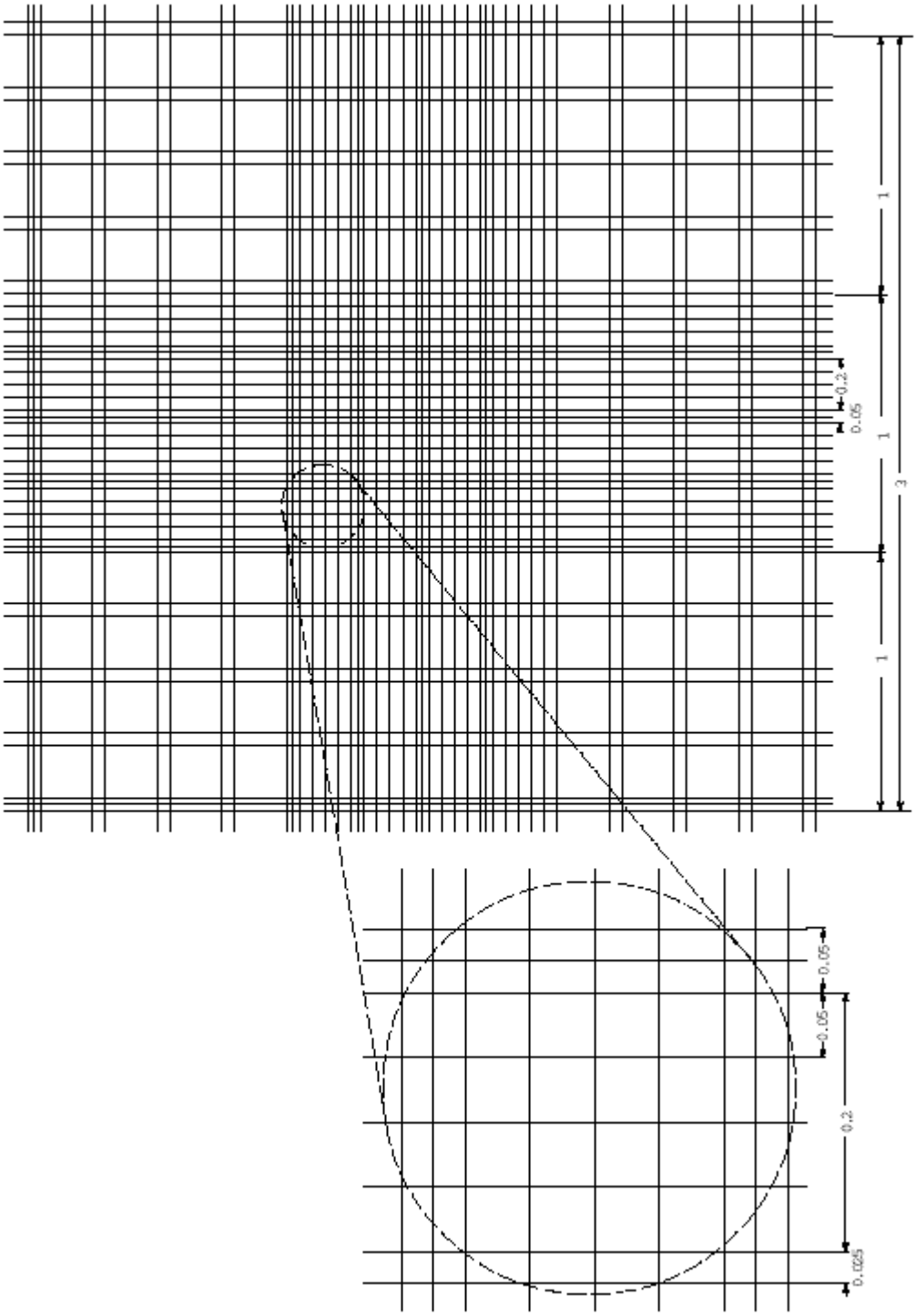


Type 1. Type 2. Type 3. Type 4. Type 5.

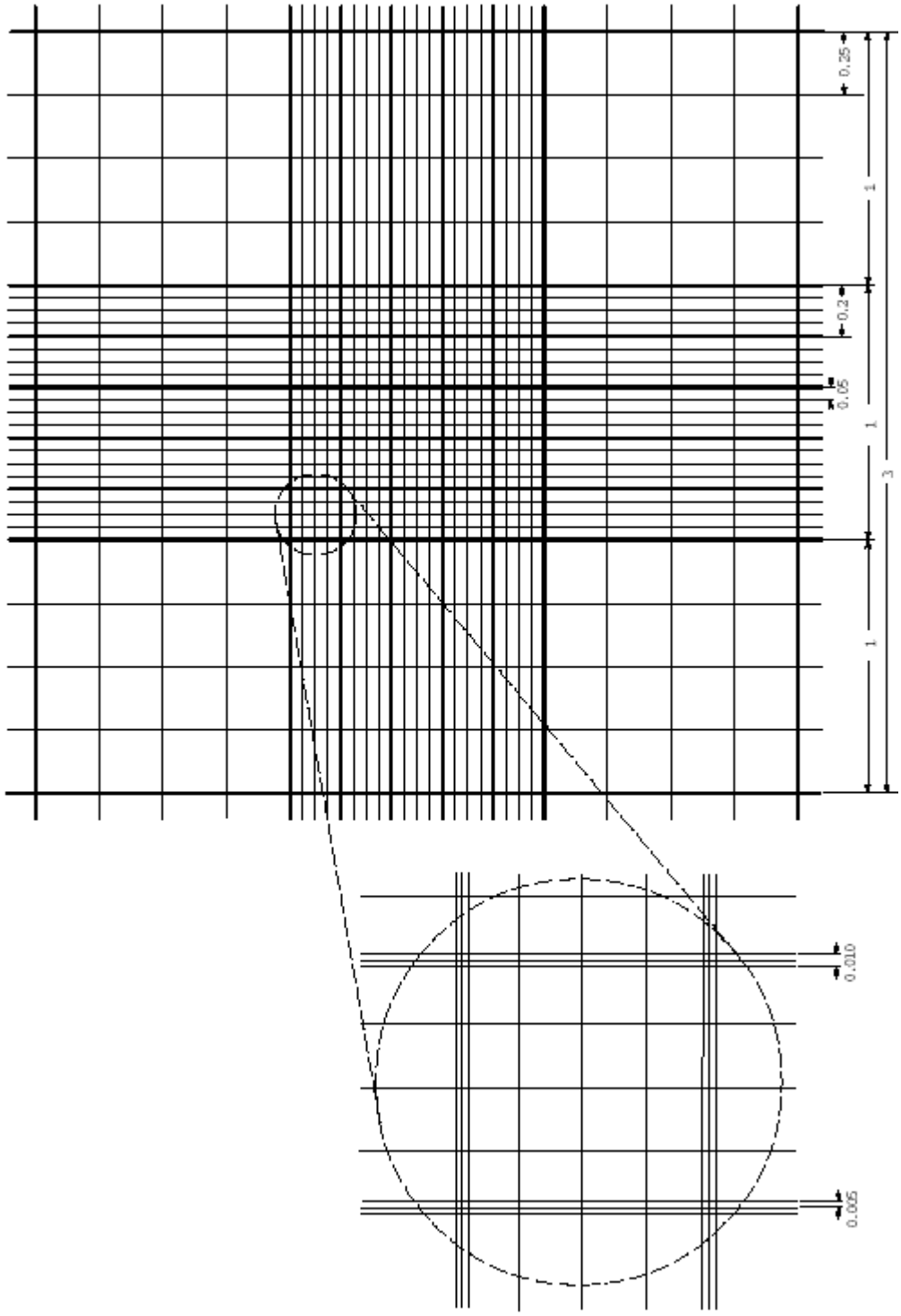
BÜRKER grid Figure and dimensions:



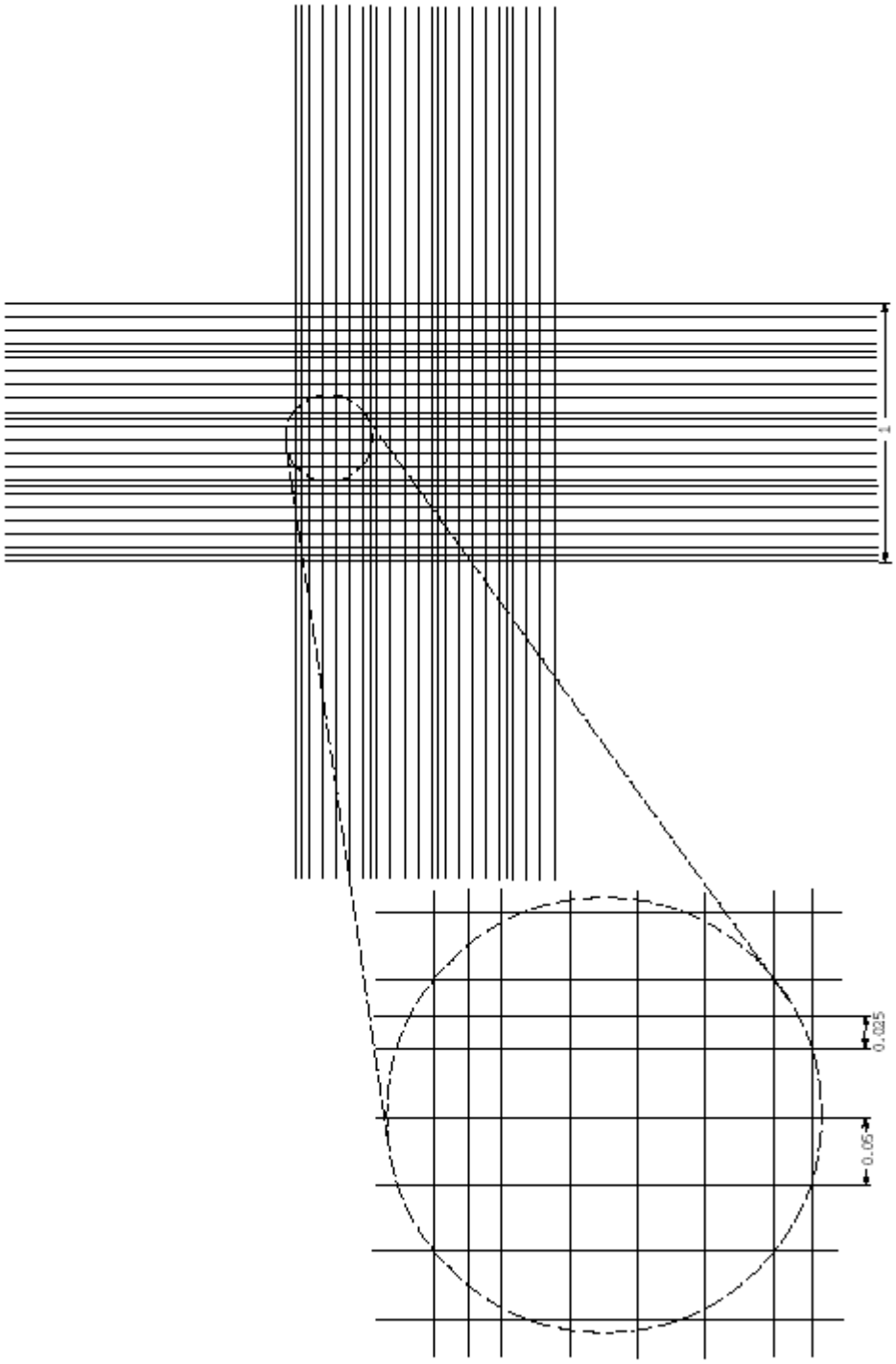
BÜRKER-TÜRK grid Figure and dimensions:



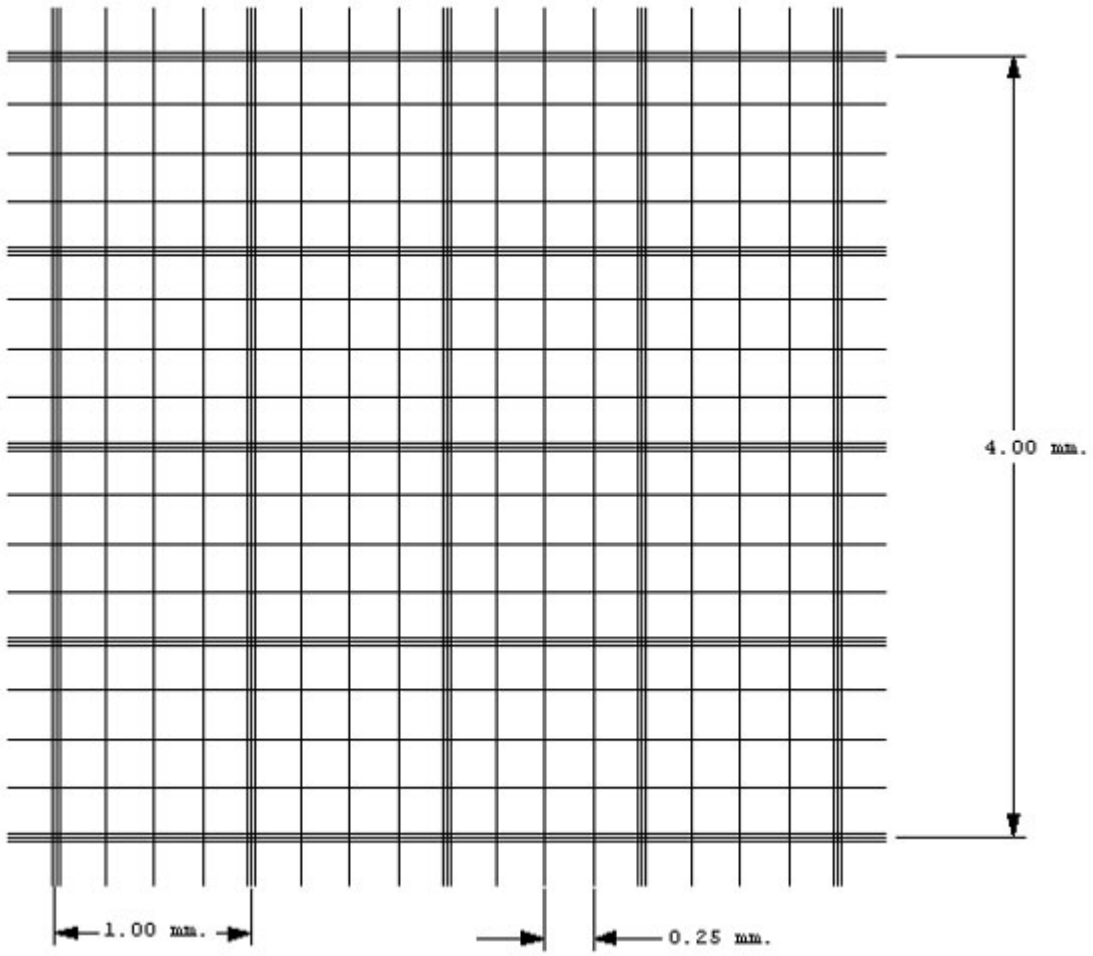
NEUBAUER improved grid Figure and dimensions:



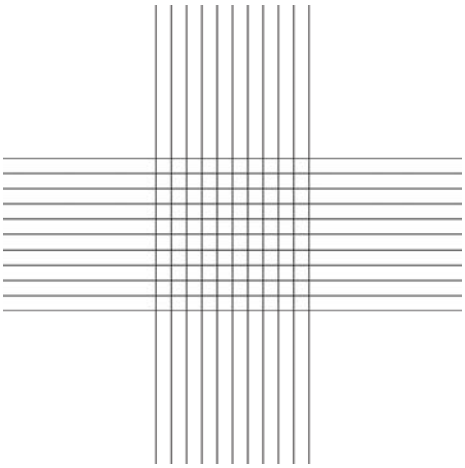
THOMA grid Figure and dimensions:



FUCHS ROSENTHAL grid Figure and dimensions:



MAKLER / 10² grid Figure and dimensions:



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