LECTURE PLAN

COURSE NAME: Experimental Embryology (Zoo 424) **LECTURE No.** 2 **TERM & YEAR**: 1/1438-1439

LECTURE TITLE: **Sperm cells counting using Hemocytometer**

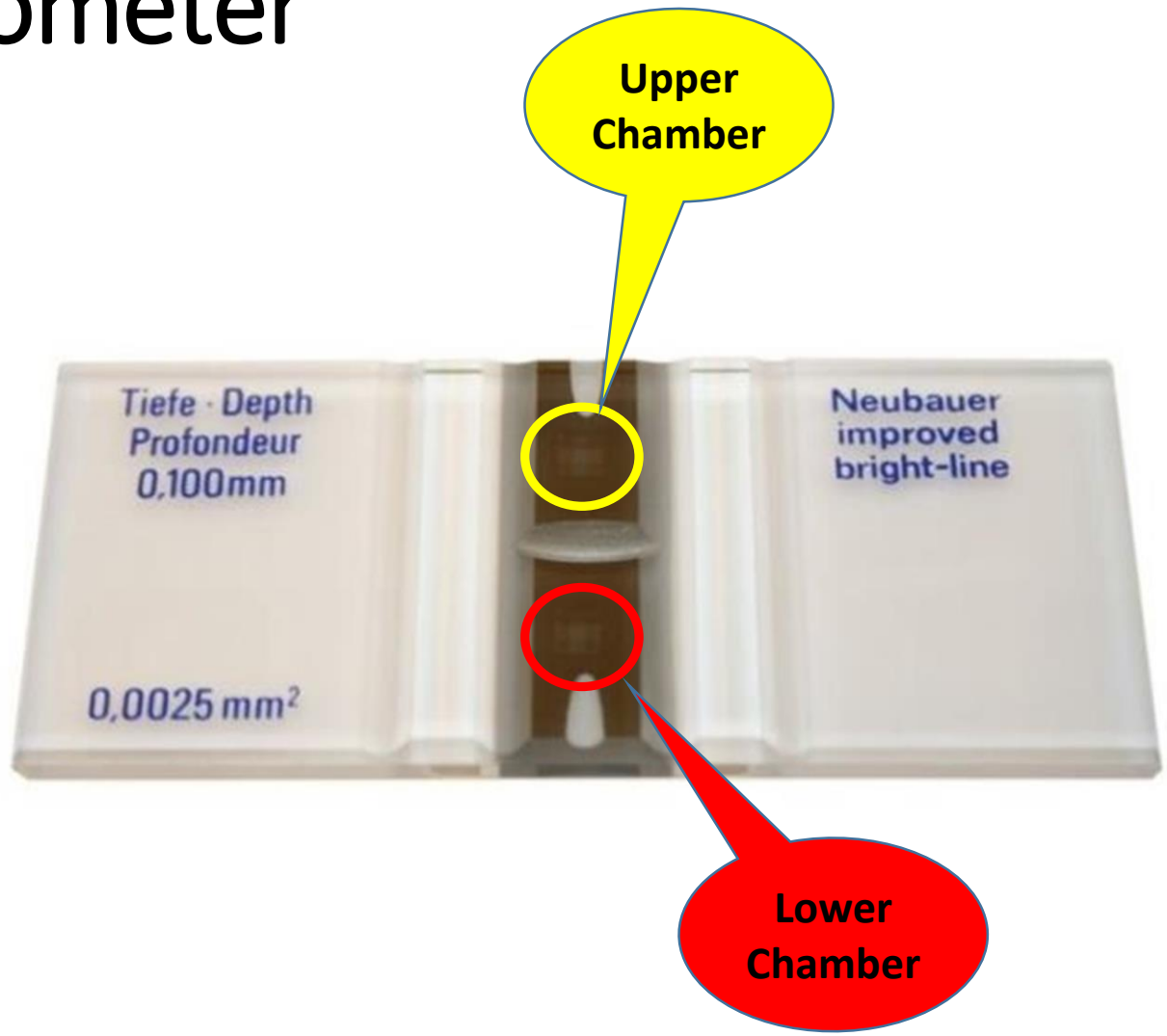
INSTRUCTOR: Dr. Muath Alghadi, Ph.D. **DEPARTMENT**: Zoology Dep. / College of Science

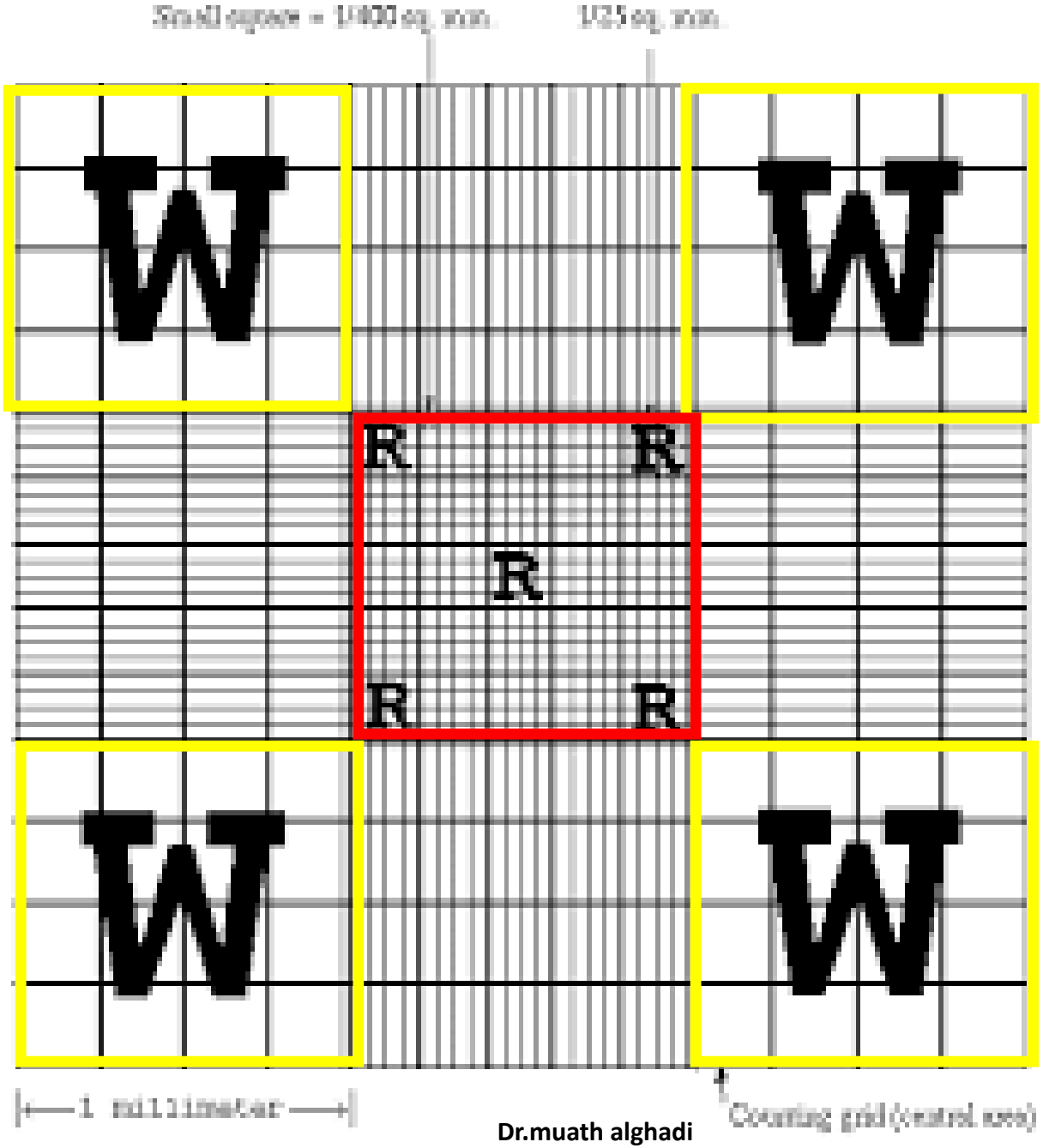
LECTURE GOALS: After this lecture you should be able to answer any questions related to the following topics:

1- Hemocytometer protocol
2- Hemocytometer calculation
3- Calculating dilutions
4- Counting sperm cells

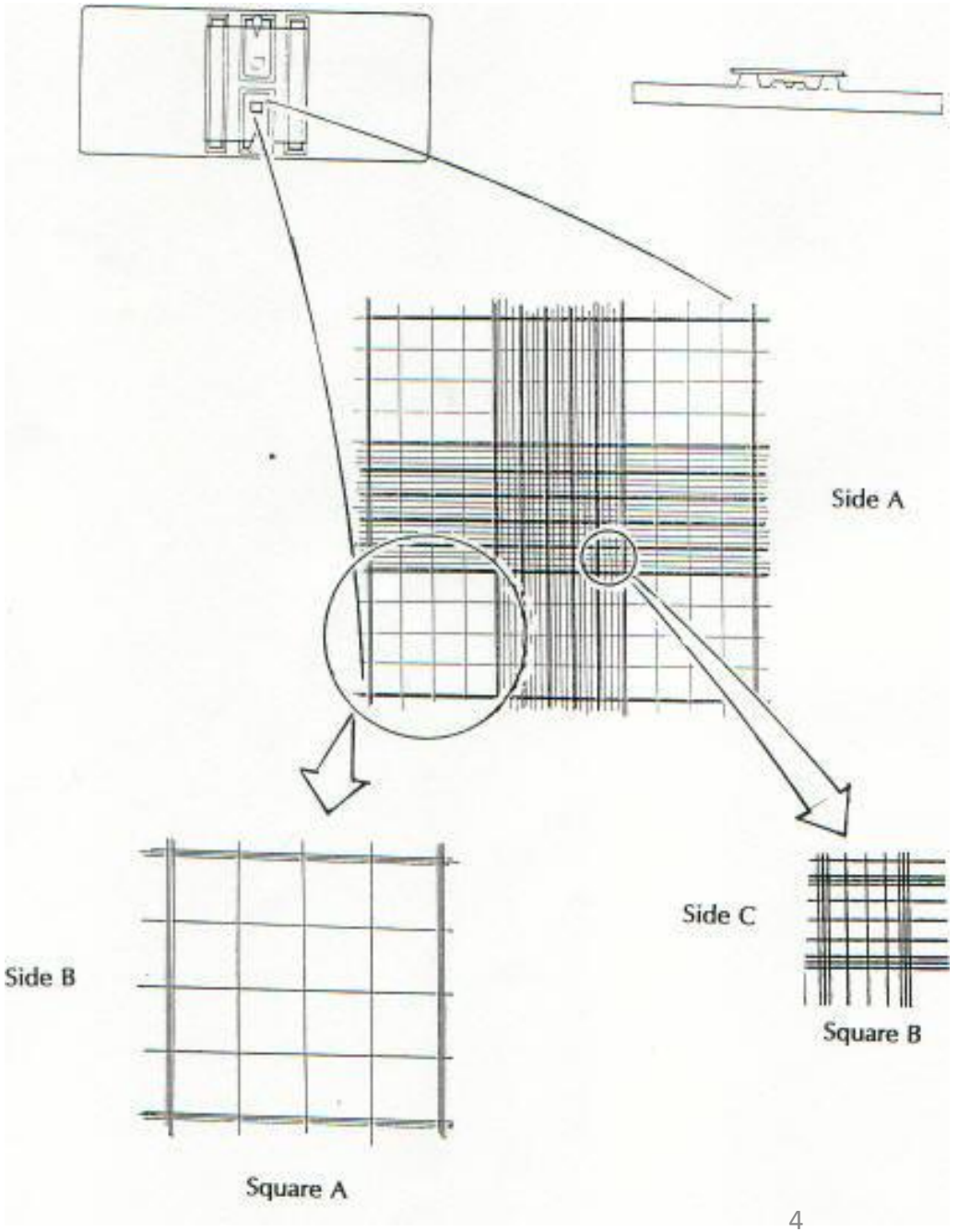
Hemocytometer

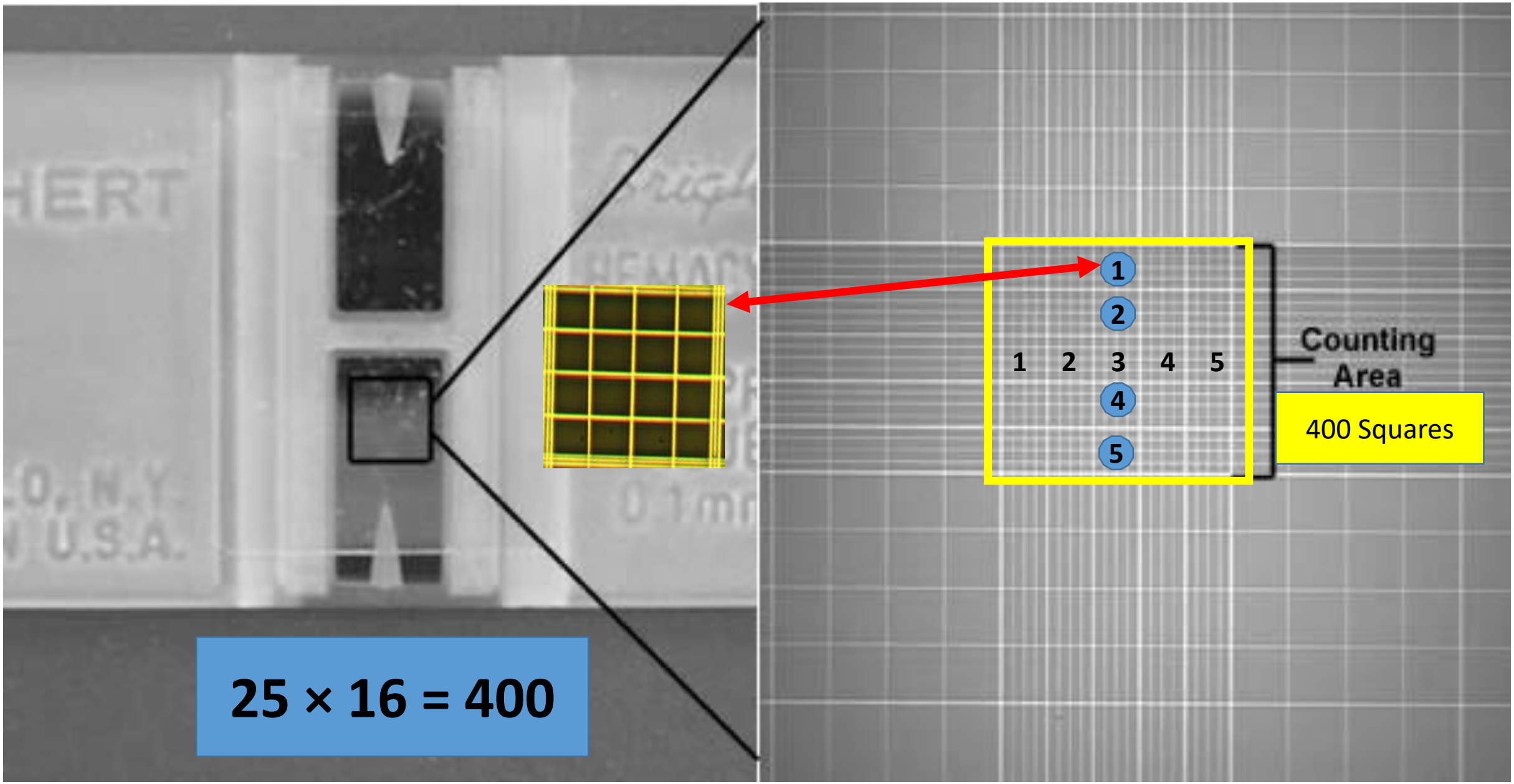
- Originally used in counting RBCs
- Consists of a thick glass microscope slide with a rectangular indentation that creates a counting chamber
- Counting chamber - engraved with a laser-etched grid of perpendicular lines
- Raised edges hold cover slip above these marked grids

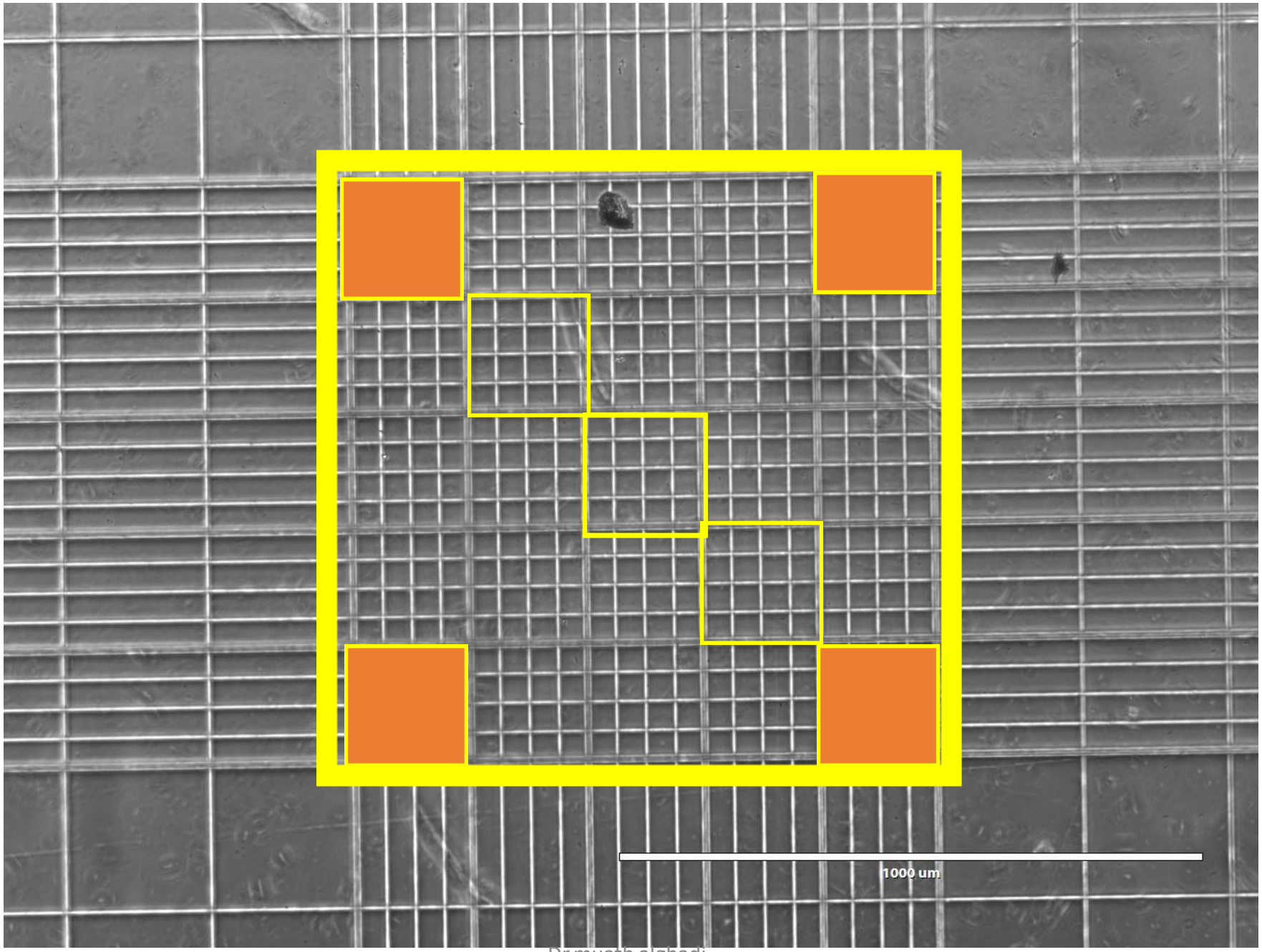



Small square = 1/400 sq. mm.
1/25 sq. mm.
W
W
W
W
R
R
R
R
R
1 millimeter
Counting grid (central area)

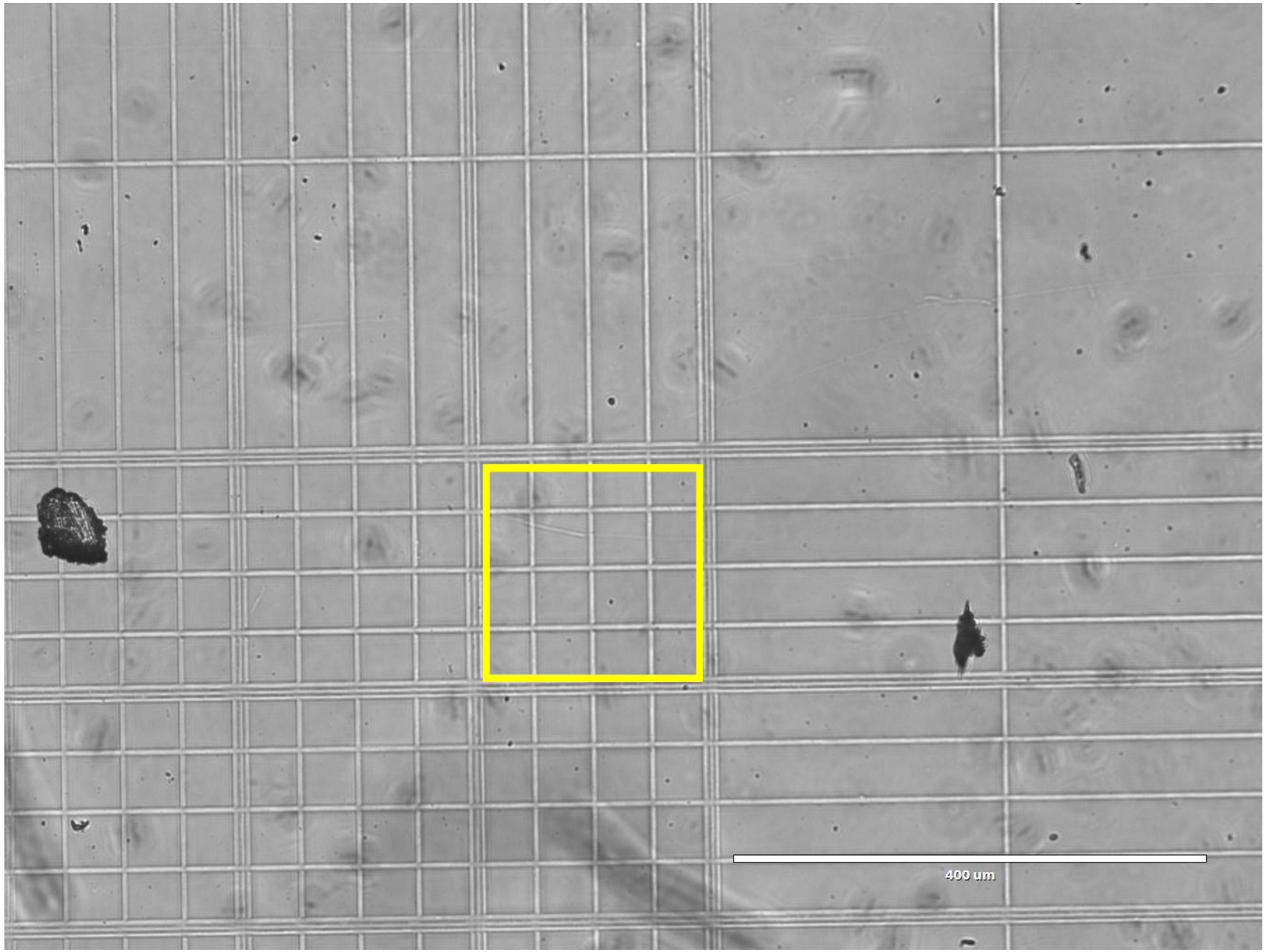
- Counting chamber is a 3 x 3 mm square and divided into nine large squares
- Each resulting square measures 1 mm^2, which are used for white blood cell counting (Square A)
- The center square is divided into 25 smaller squares. (1/25 mm^2)
- Each smaller square is subdivided into 16 even smaller squares, which are used for counting red blood cells. (Square B = 1/400 mm^2)

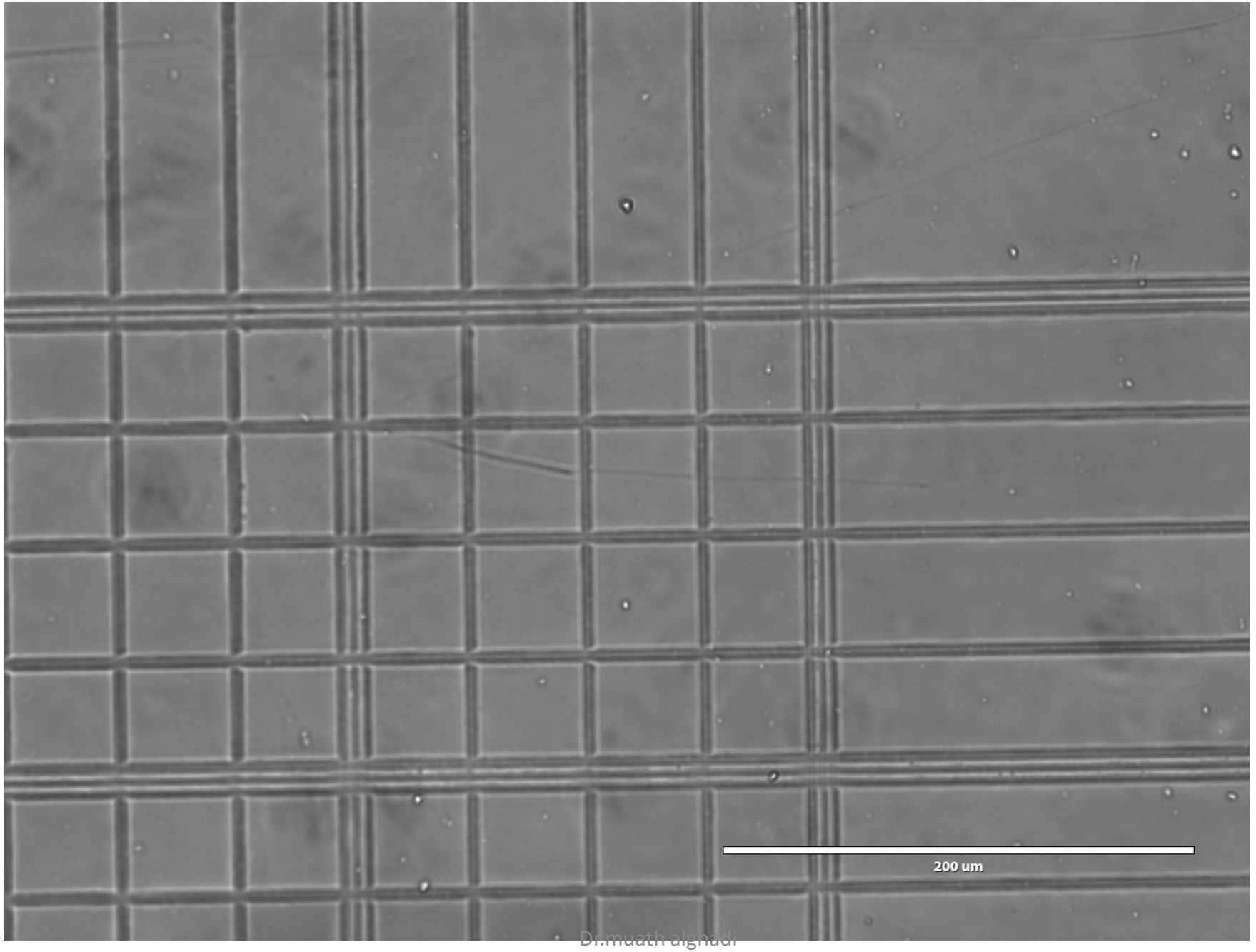


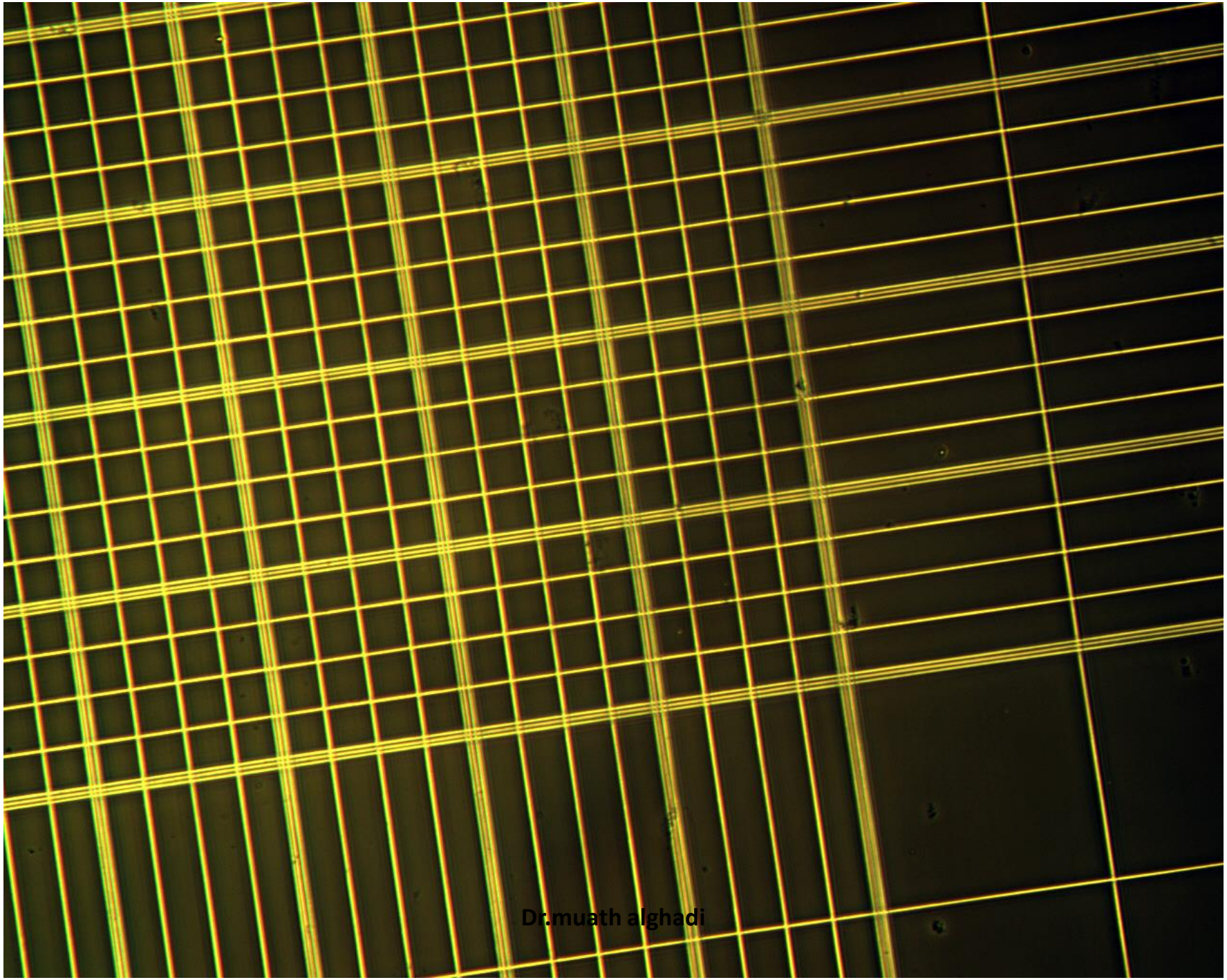

1
2
1 2 3 4 5
4
5
Counting Area
400 Squares
25 × 16 = 400

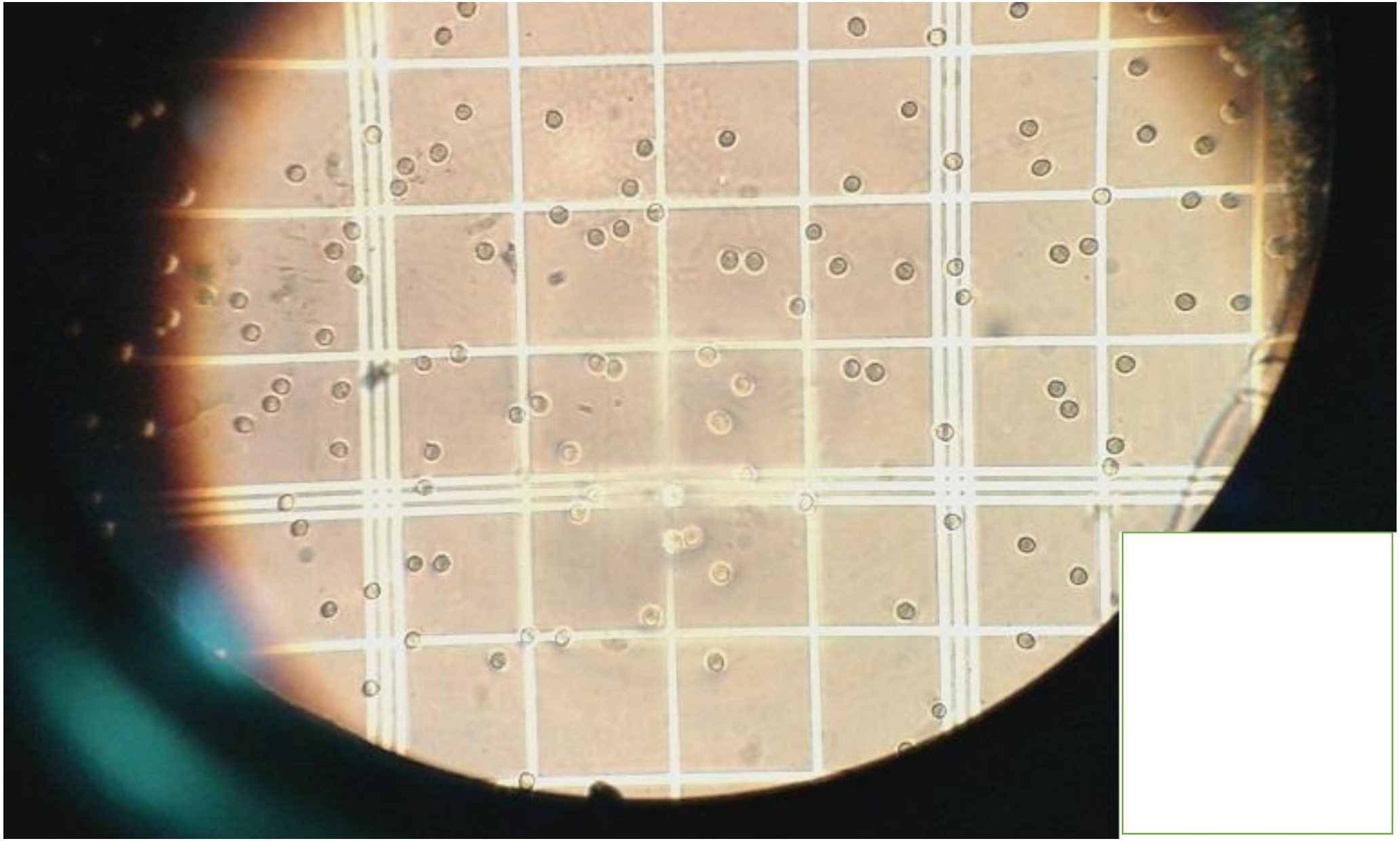

1000 um


400 um

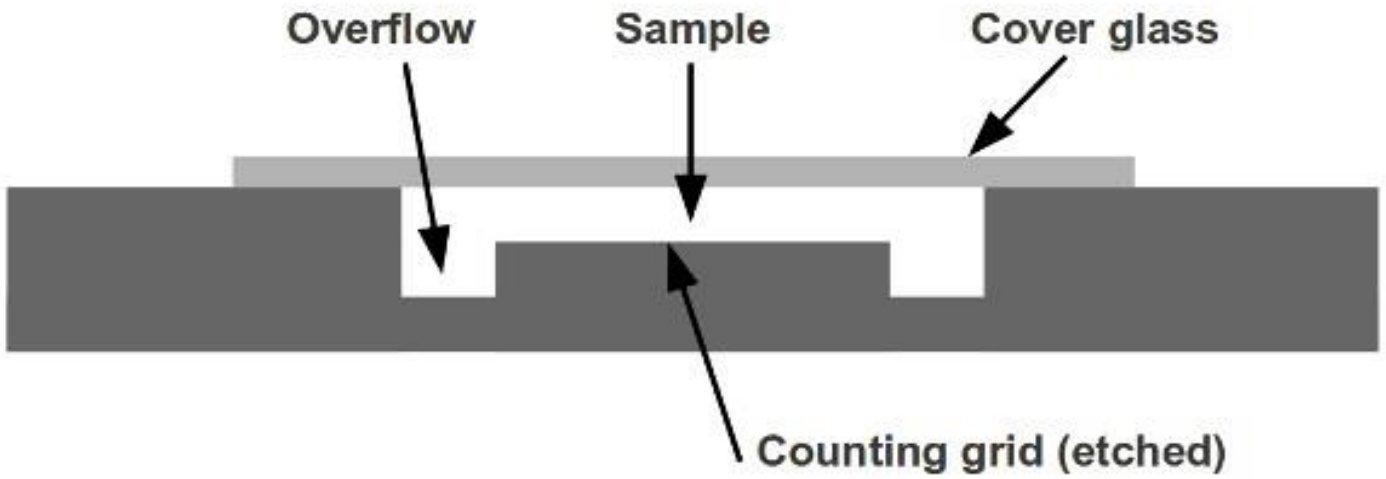

400 um

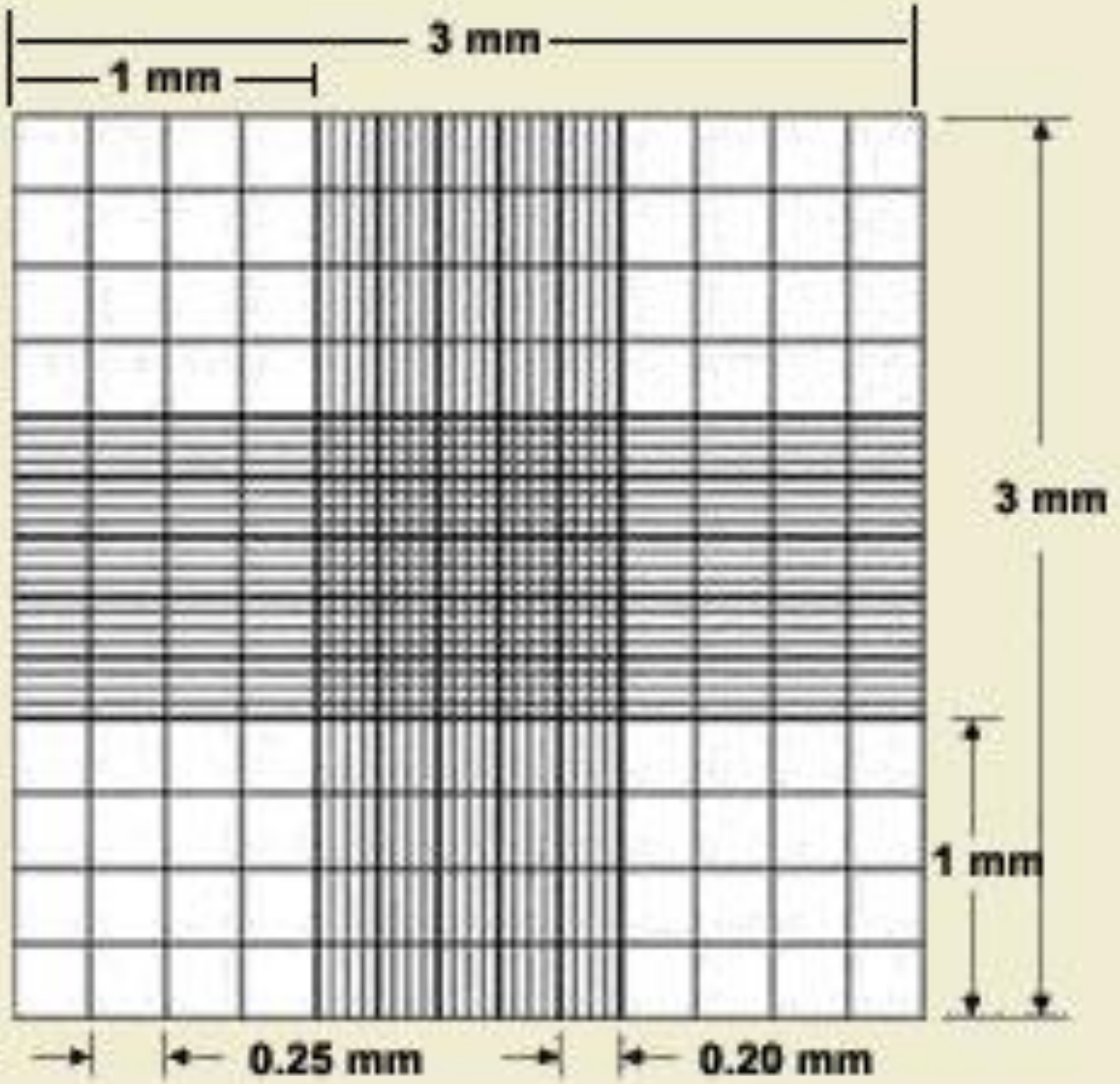

200 um

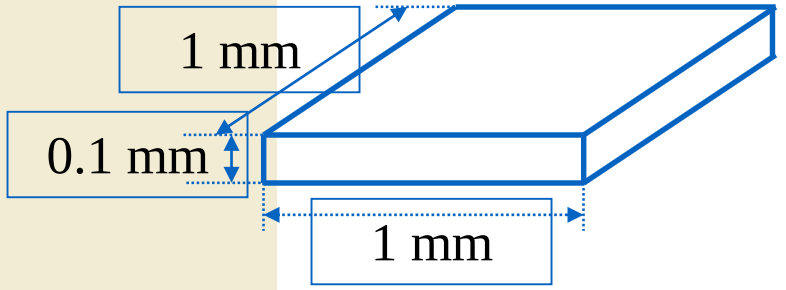

Dr.muath alghadi



Hemocytometer: side view

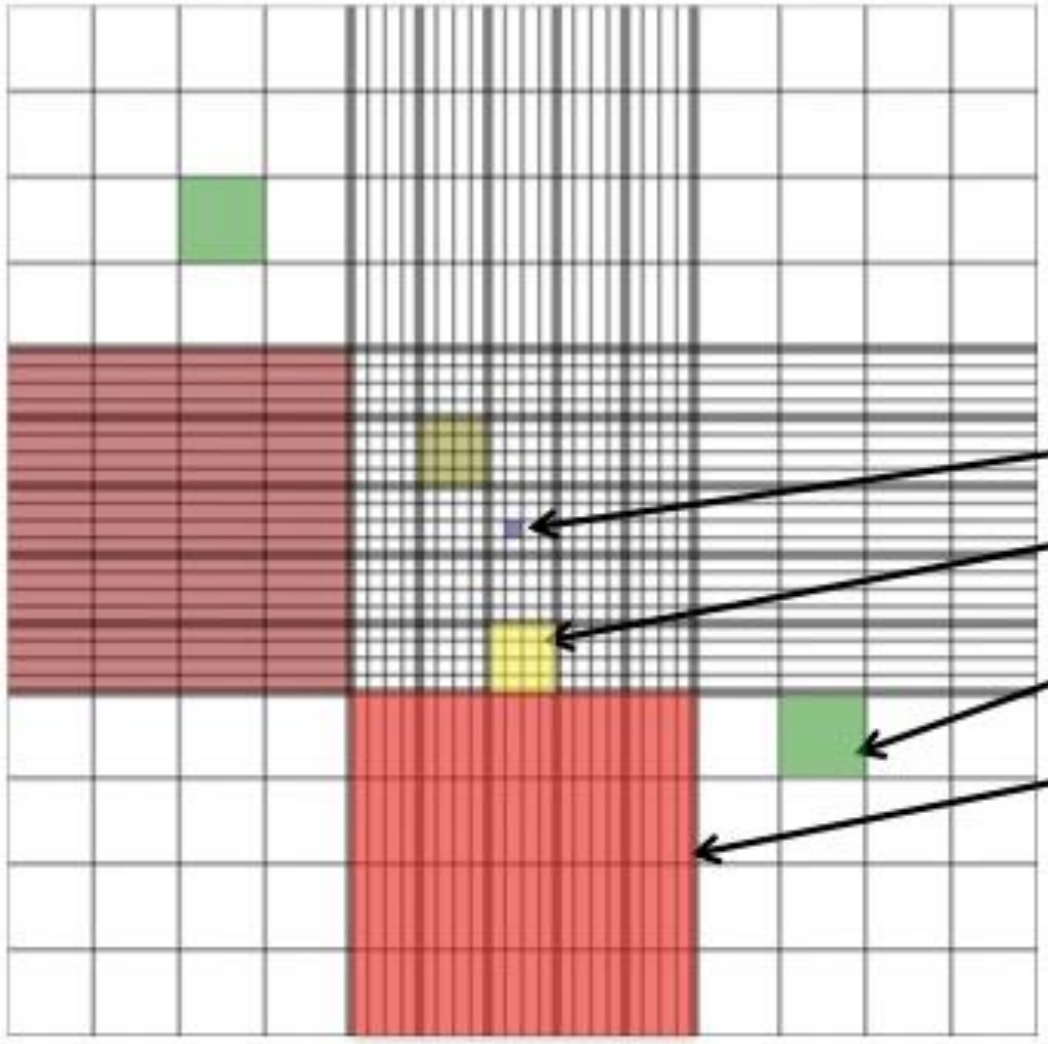






Volume : $0.1 mm^3$

$1 ml = 1 cm^3 = 1000 mm^3$

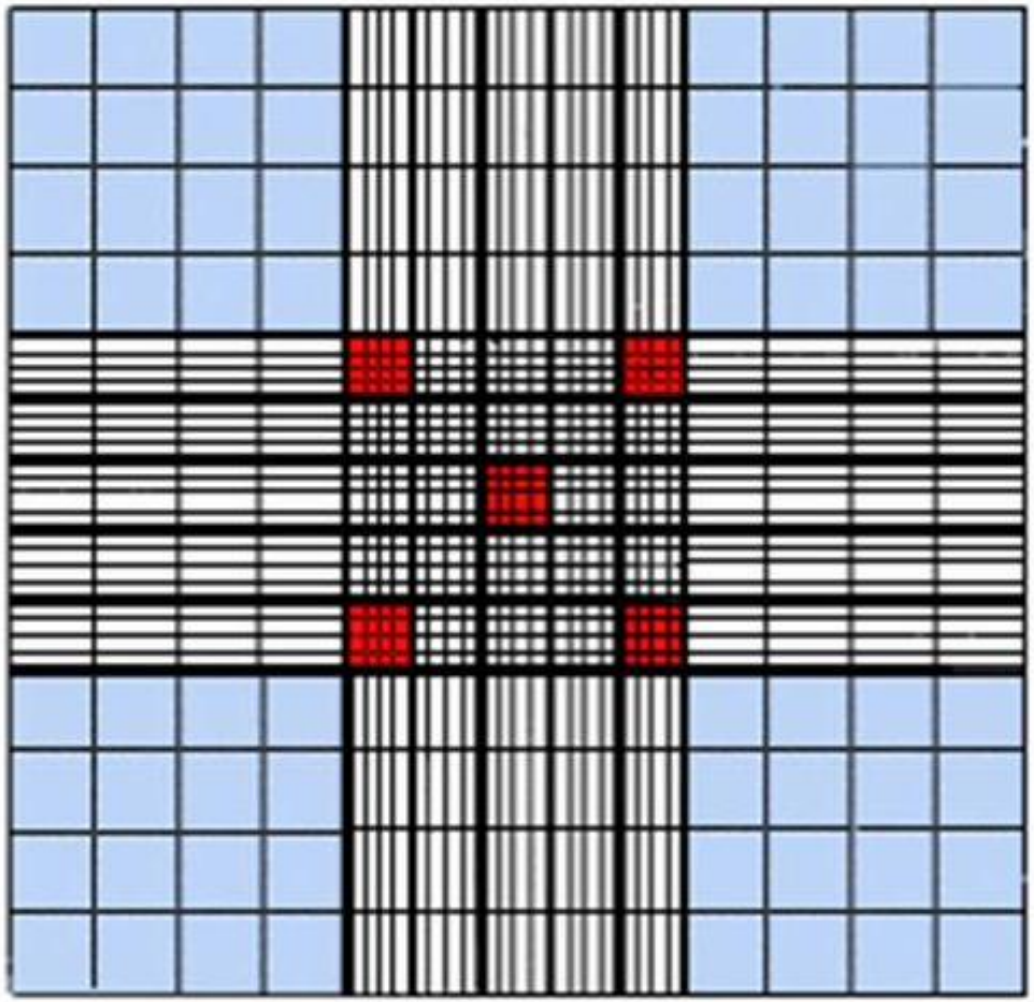


$cm^3 = ml / mm^3 = \mu l$

$L = 1000\ ml / ml = 1000\ \mu l / \mu l = 1000\ nl$

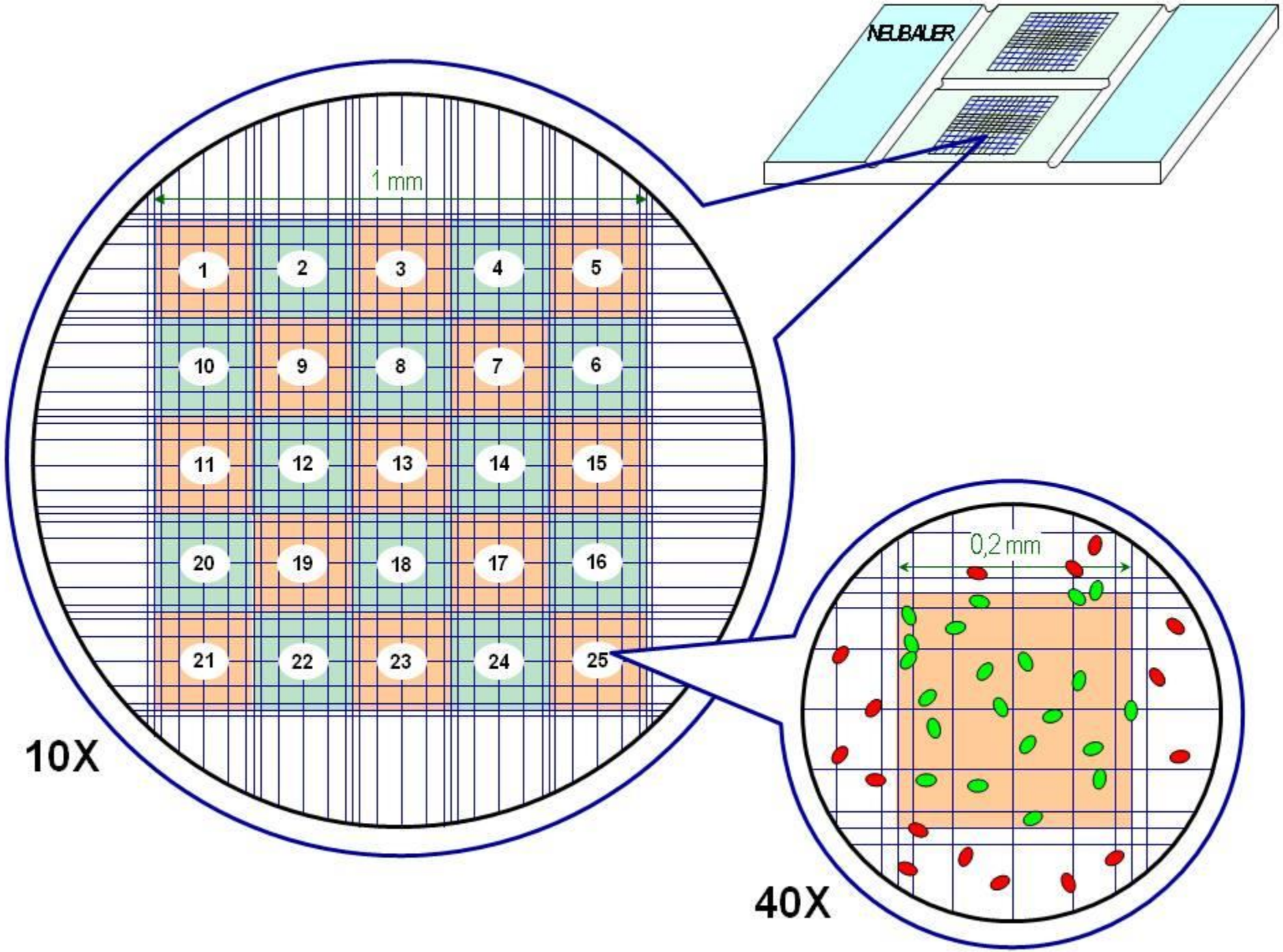
	Size	Volume
Blue	0.0025 mm^2	0.25 nl
Yellow	0.04 mm^2	4 nl
Green	0.0625 mm^2	6.25 nl
Red	1 mm^2	100 nl

$$\text{concentration of cells in original mixture} = \left(\frac{\text{number of cells counted}}{(\text{proportion of chamber counted})(\text{volume of chamber})}\right)\left(\frac{\text{volume of sample dilution}}{\text{volume of original mixture in sample}}\right)$$



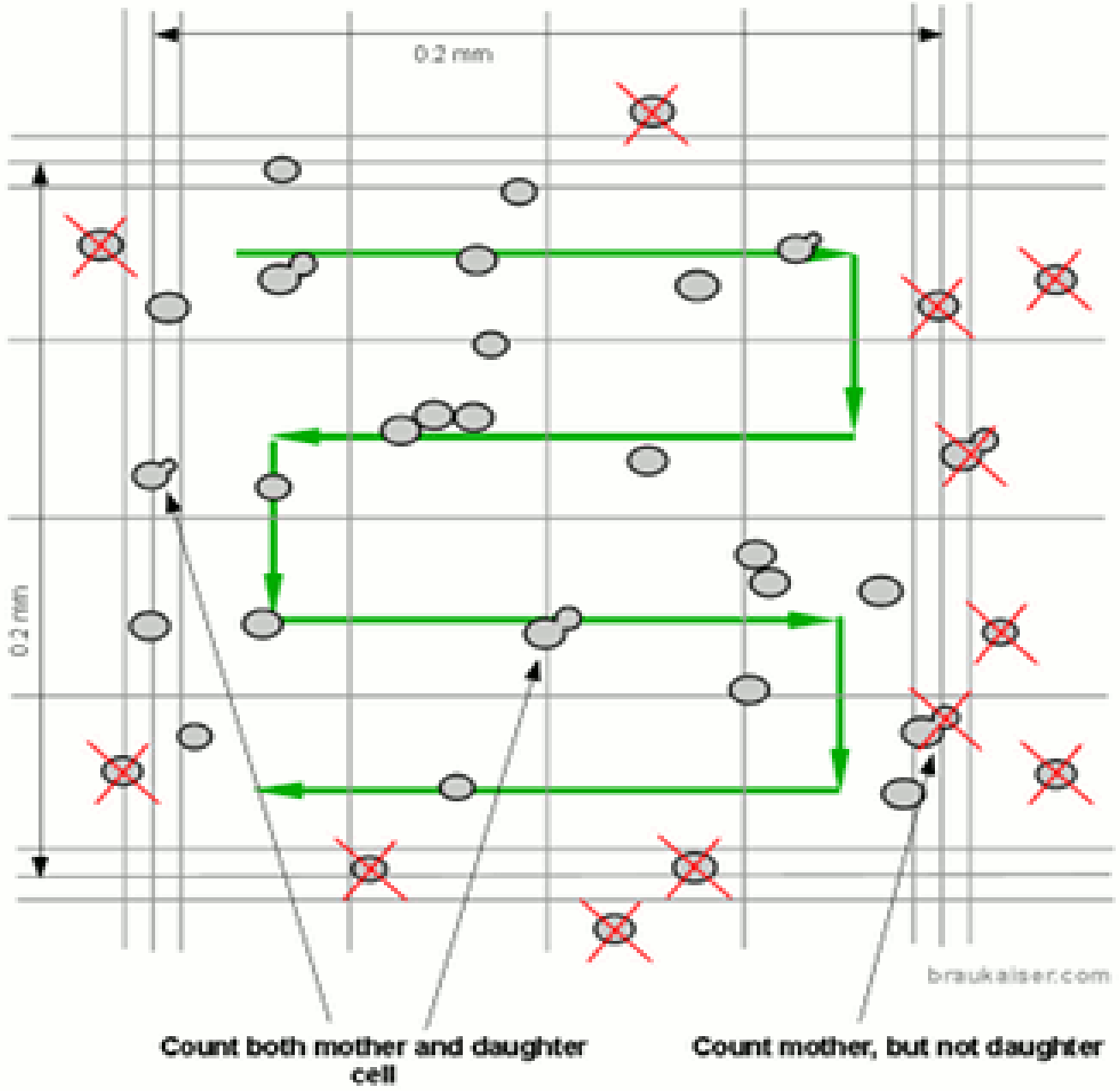
- # of cells in upper left = 30
- # of cells in upper right = 35
- # of cells in lower right = 26
- # of cells in lower left= 29
- # of cells in middle= 33
- Total = 153 cells found in 5 squares
- 153 cells × 5 = 765 cells / 25 squares
- 765 cells / 0.1 µl
- 765cells × 10^4 = 7650000 cells / ml

The end result is (7650000 × DF) cells / ml

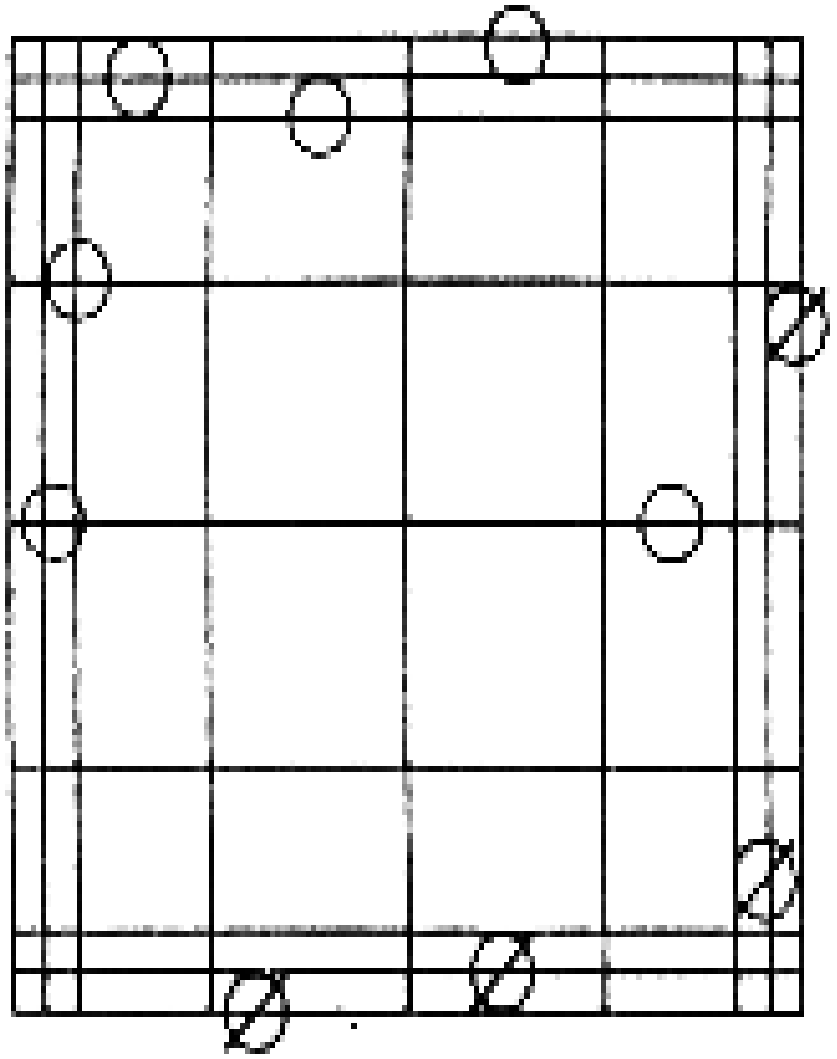

NEUBAUER
1 mm
1
2
3
4
5
10
9
8
7
6
11
12
13
14
15
20
19
18
17
16
21
22
23
24
25
10X
0,2 mm
40X

Counting Rule

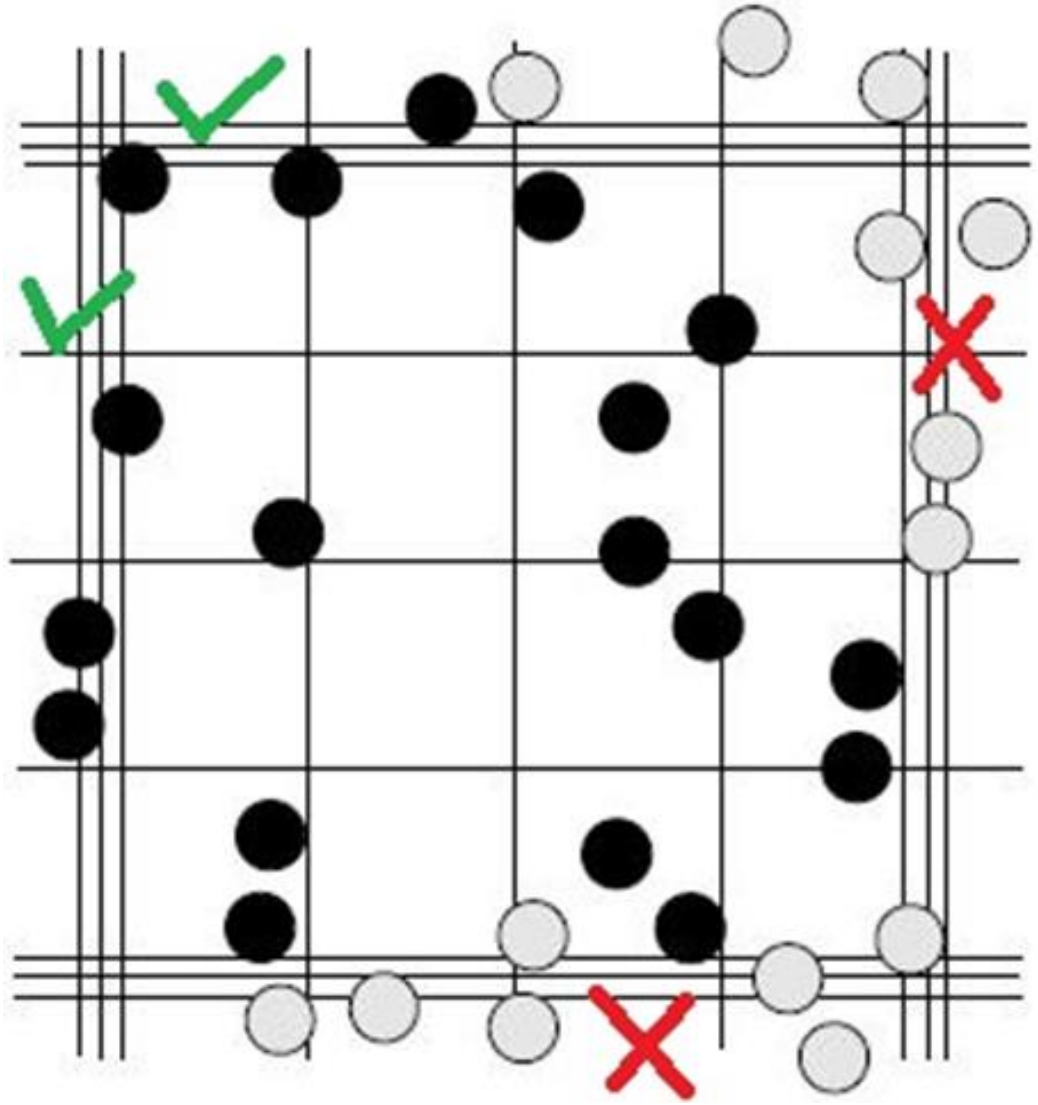
- Do not count cells touching
  - Bottom line
  - Right line

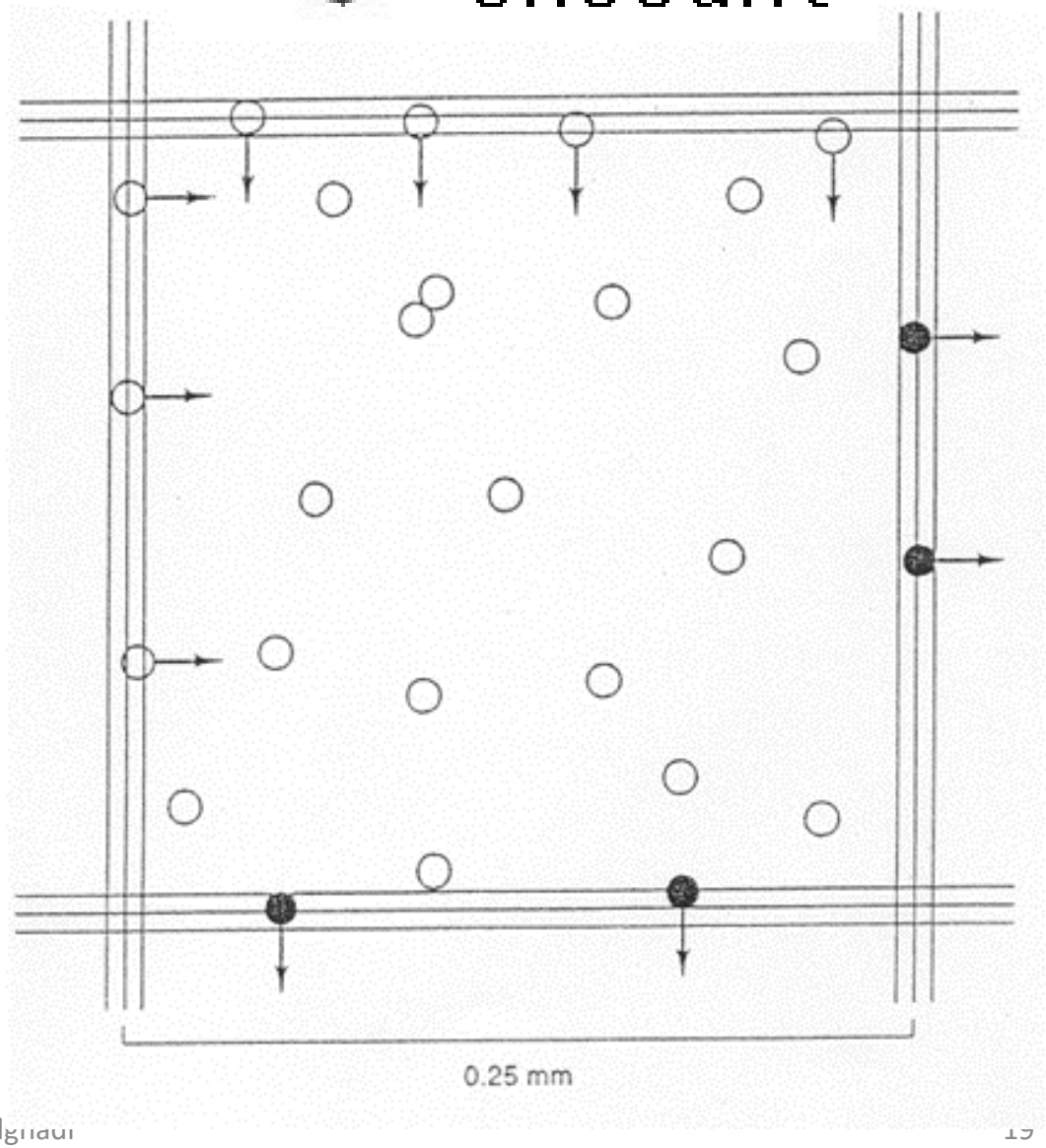


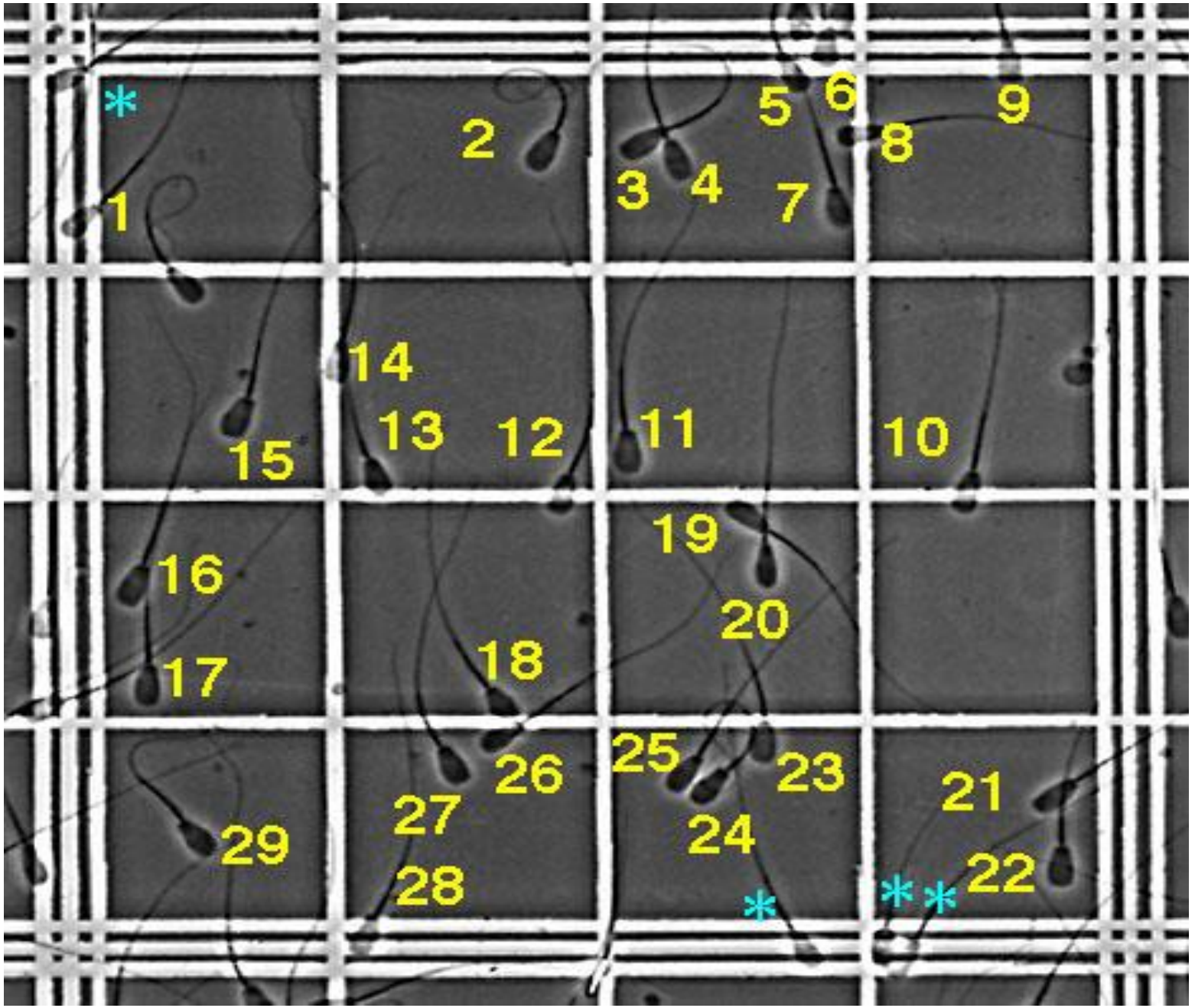
CORNER SQUARE (ENLARGEMENT)



Count cells on top and left touching middle line (○).
Do not count cells touching middle line at bottom and right (Ø).




Count
Uncount
0.25 mm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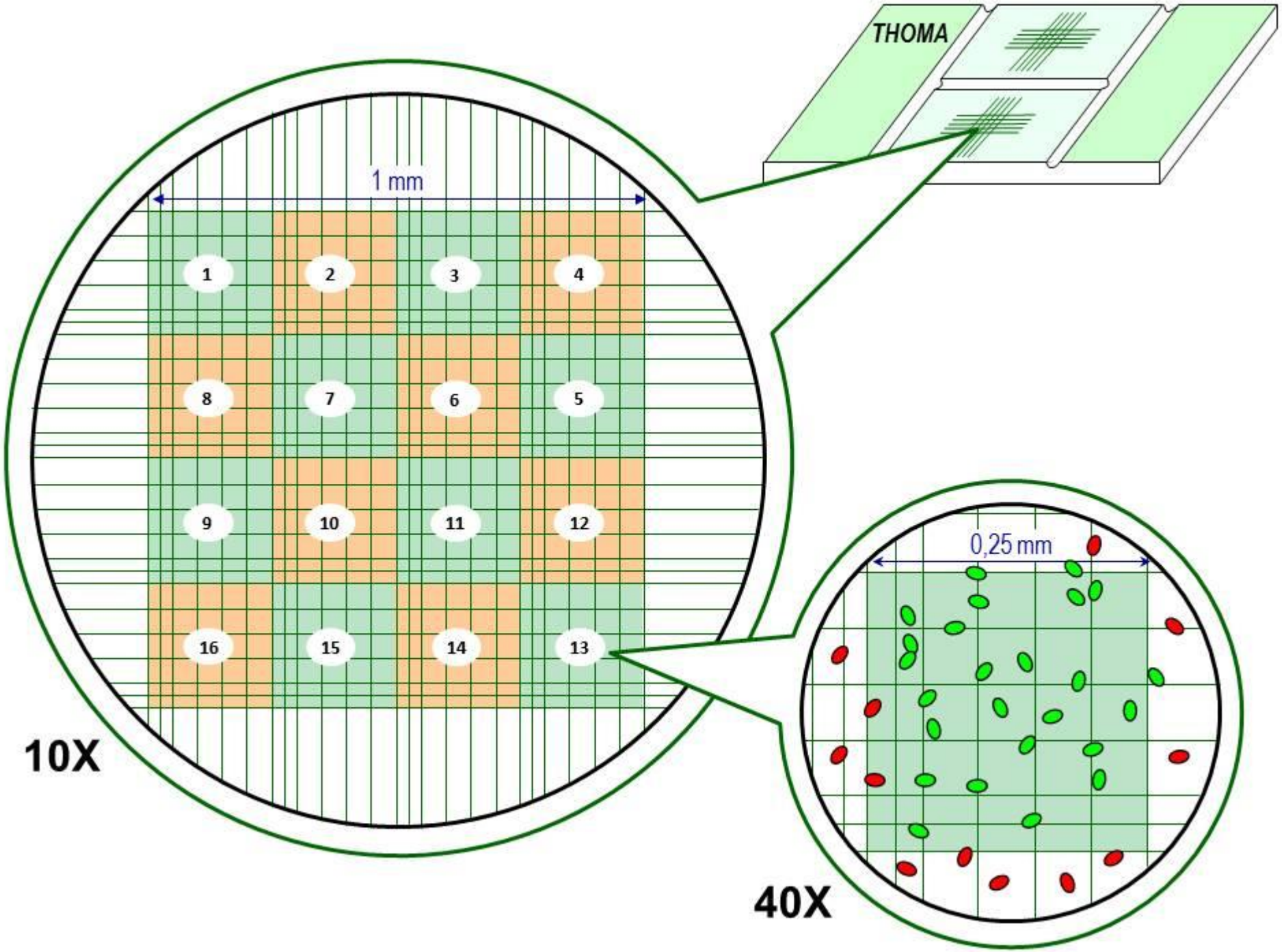
28
29

Counting chambers

- Thoma counting chambers
- Neubauer counting chambers
- Improved Neubauer counting chambers
- Buerker counting chambers
- Tuerk counting chambers
- Buerker-Tuerk counting chambers
- Nageotte counting chambers
- Thoma new counting chambers
- Fuchs-Rosenthal counting chambers
- Jessen counting chambers
- Schilling-Kreuznetz counting chambers
- Malassez counting chambers
- McMaster counting chambers


THOMA
1 mm
1
2
3
4
8
7
6
5
9
10
11
12
16
15
14
13
10X
0,25 mm
40X