Al-Rasheed University College
Pharmacy Department
2nd Stage / 2nd Course
(2019 – 2020)

TOTAL WBC COUNTING

PHYSIOLOGY LAB.

2ND STAGE / 2ND COURSE

LAB. (4)

INTRODUCTION:

- Leukocytes are originated in bone marrow from stem cells (hemocytoblasts)
- Once they are fully mature, they leave bone marrow and enter the blood stream
- Normal value: 4000 – 11000 WBCs / $1mm^3$ of blood
- When talking about WBCs, there are important terms that must be known:
  - Leukocytosis
  - Leukopenia
  - Leukemia

INTRODUCTION (CONTINUE):

- **Leukocytosis:**
  - It means an increase in the number of WBCs above upper normal limit
  - Mostly occurs in
    1. acute infections (severe bacterial infection, severe malarial infection)
    2. After hemorrhage
    3. During pregnancy
    4. Postoperative (after surgery)
    5. And some types of anemia
  - Total WBCs count up to 20000 WBCs / 1mm^3 of blood is considered leukocytosis

INTRODUCTION (CONTINUE):

- **Leukopenia:**
  - A decrease in the number WBCs below the lower normal limit
  - Mostly occurs in
    1. Viral infection
    2. Typhoid fever
    3. After radiation therapy and chemotherapy
    4. Chronic use of steroid medication
    5. Pernicious anemia
  - Total WBCs count below 4000 WBCs / 1mm^3 of blood is considered leukopenia

INTRODUCTION (CONTINUE):

- **Leukemia:**
  - Is characterized by uncontrolled increase and proliferation of one type or more of the different hematopoietic cells, and associated with many changes in the circulating cells of the blood
  - The most 2 common types of leukemia are:
    - Lymphocytic leukemia
    - Myelocytic leukemia
  - Leukemia can be further subdivided into:
    - Acute: disease progressive rapidly and there is marked morphological changes in cells
    - Chronic: the disease is slow and there is no obvious changes
  - Total WBCs count exceeding 20000 WBCs / $1 mm^3$ of blood could be considered leukemia

IMPORTANT NOTES:

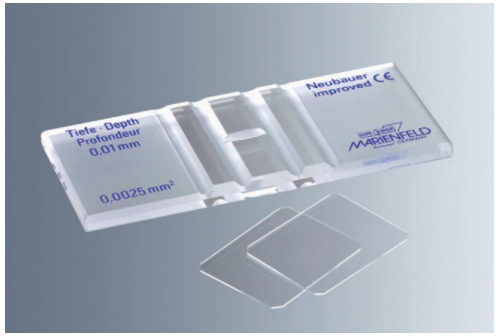
- Total WBCs counting can be done manually using (special slide, stain and microscope)
- and can be done by automated method (hematology analyzer)
- Manual method in total WBCs counting is still superior in certain circumstances like (counting WBCs in cerebrospinal fluid and synovial fluid)

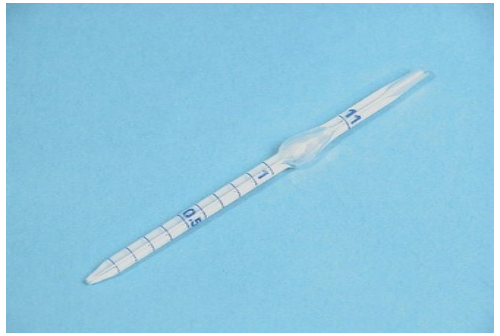
OBJECTIVE (AIM) OF THE EXPERIMENT:

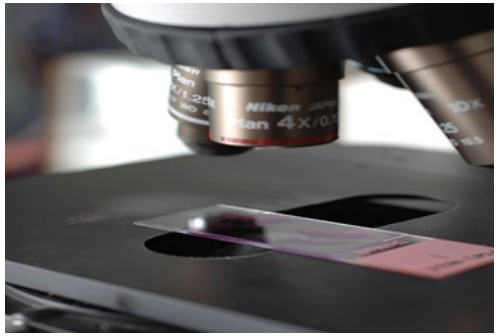
1. The presence of infection and follow the progress of certain diseases
2. Presence of Leukemia
3. Monitor the body response to various treatments
4. Conditions or diseases that may weaken the immune system, like HIV infection, chemotherapy, radiation therapy causing a drop in WBC count.
5. Monitor bone marrow function

BASIC REQUIREMENTS:

- 70% alcohol and cotton
- Sterile blood lancet
- Special WBC pipette
- Special slide (hemocytometer)
- Cover slip
- Special dilution colored fluid (Turk's solution)
- Microscope







PROCEDURE: 1- WBC PIPETTE

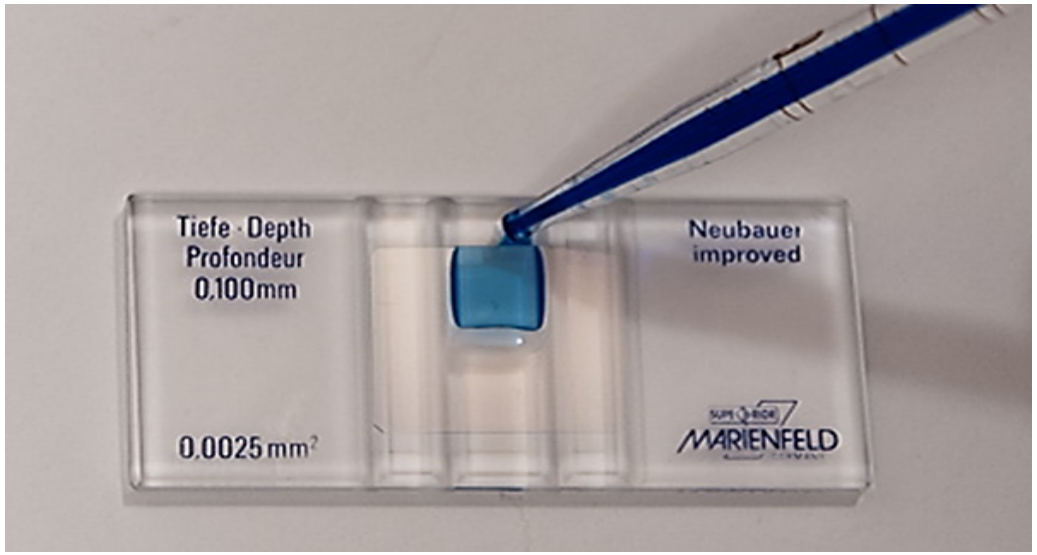
- Small and graduated (0.5 IU – 11 IU)
- Used to withdraw blood sample, and then dilute it with **Turk's solution**

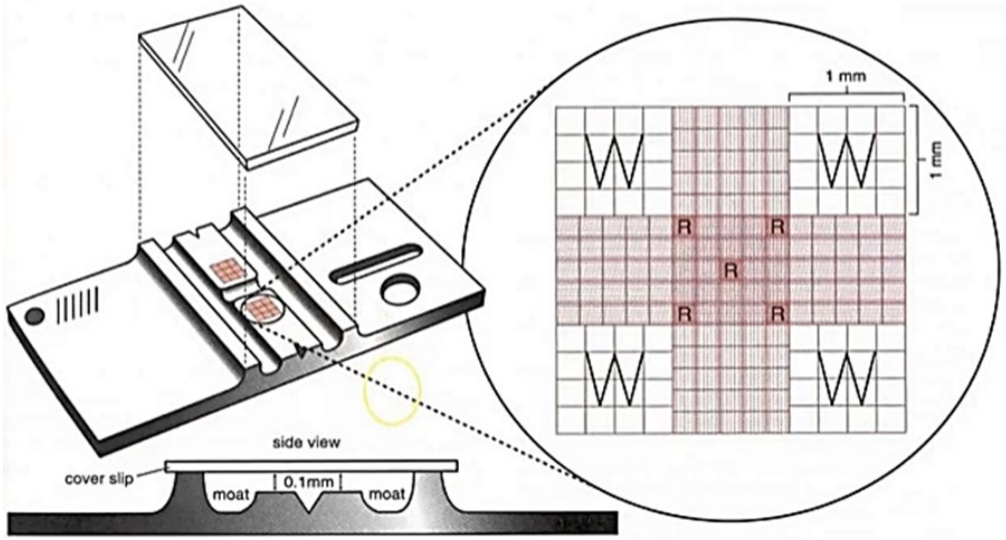
PROCEDURE: 2-TURK'S SOLUTION

- **A special dilution colored fluid**
- **Composed of:**
  - Glacial acetic acid (for destruction of RBCs)
  - Gentian violet or methylene blue (for staining WBCs)
  - Distilled water (for dilution of sample)

PROCEDURE:
3-HEMOCYTOMETER (COUNTING CHAMBER)

- Hemocytometer is a special slide containing a counting chamber
- It is used for manual measurement of different types of cells in blood and other body fluids
- Used to determine the total number of:
  - WBCs in a blood sample, cerebrospinal fluid sample, and synovial sample
  - RBCs in a blood sample
  - Platelets in a blood sample
  - Sperms in a seminal fluid sample





HOW TO CALCULATE THE TOTAL NUMBER OF WBC?

- **Part one: derived from the (WBC pipette)**

0.5 unit of blood is diluted to 11 units with Turk's solution

- 0.5 unit (B) → 11 unit (B+T)

Mix by rotating the pipette for 2 – 5 min then **discards 3 drops which are equivalent to 1 (IU) unit.**

- 0.5 unit (B) → 11 unit (B+T) – 1 unit (B+T)
- 0.5 unit (B) → 10 unit (B+T)
- [0.5 unit (B) ≡ 10 unit (B+T)] x 2
- 1 unit (B) ≡ 20 unit (B+T)

Since [1 mm^3 (B) ≡ 20 mm^3 (B+T)] / 20

So 1/20 mm^3 (B) ≡ 1 mm^3 (B+T)

1 mm^3 (B+T) ≡ 1/20 mm^3 (B)

HOW TO CALCULATE THE TOTAL NUMBER OF WBC?

- **Part Two: derived from (hemocytometer)**

Calculate the volume of (1) corner square

V = L(length) * W(width) * D(depth)

= 1mm * 1mm * 0.1mm

= 0.1 mm³ (B+T)

Volume	Cells
0.1 mm³ (B+T)	W
1 mm³ (B+T)	?

Then 1 mm³ (B+T) = 10W

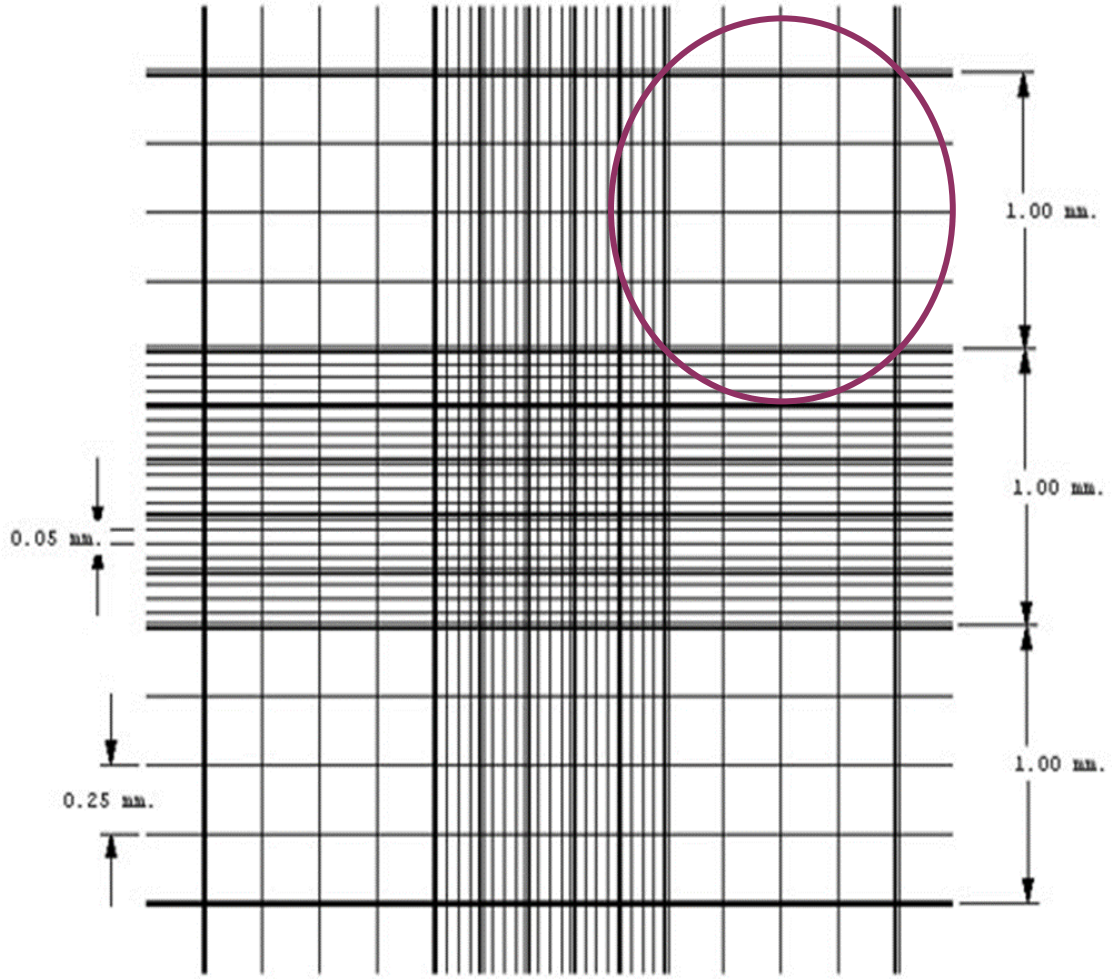
Since 1mm³ (B+T) ≡ 1/20 mm3 (B) ---------- (from the pipette equation)

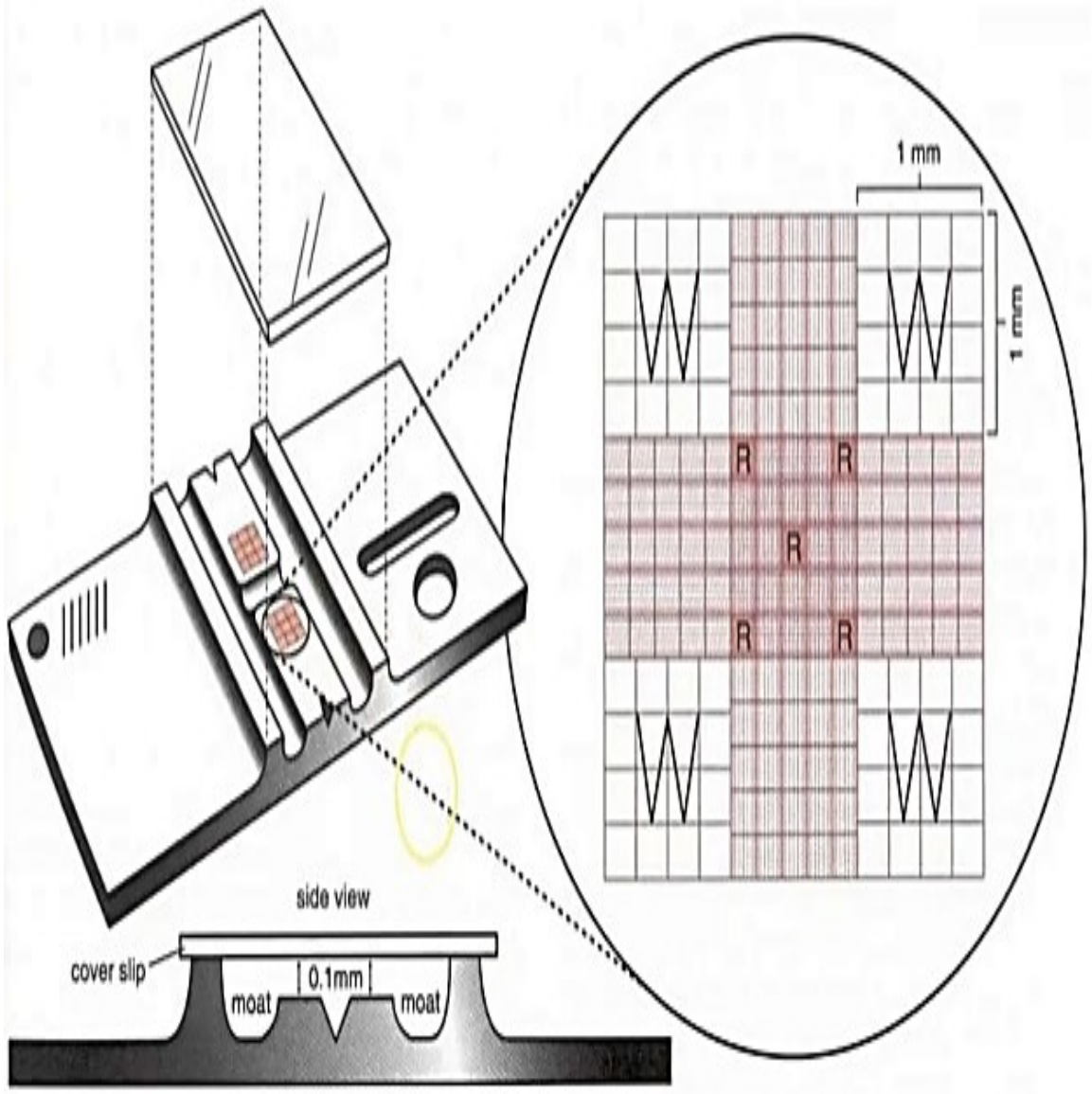
Then:

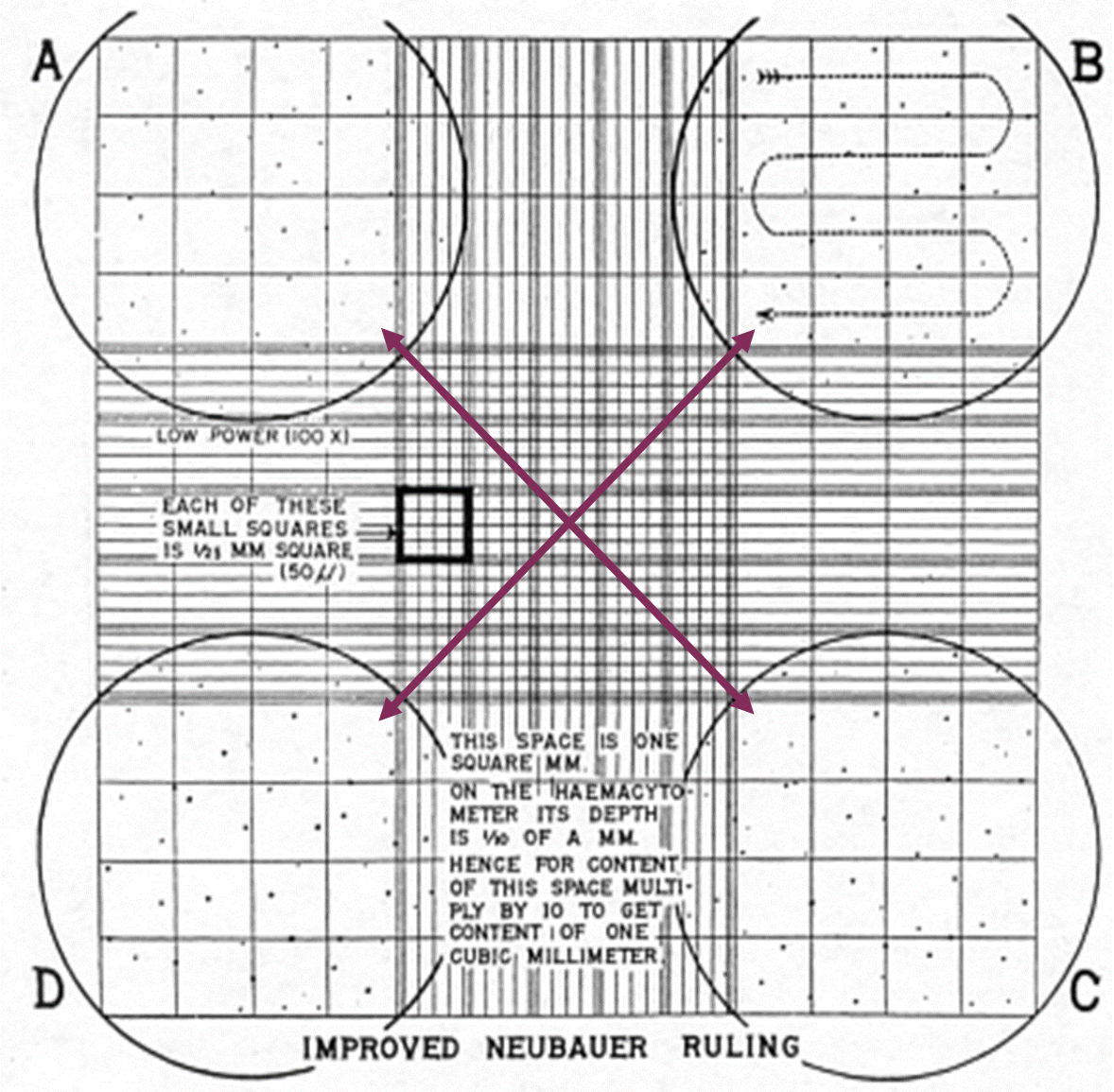
1/20 mm³ (B)	10W
1 mm³ (B)	?

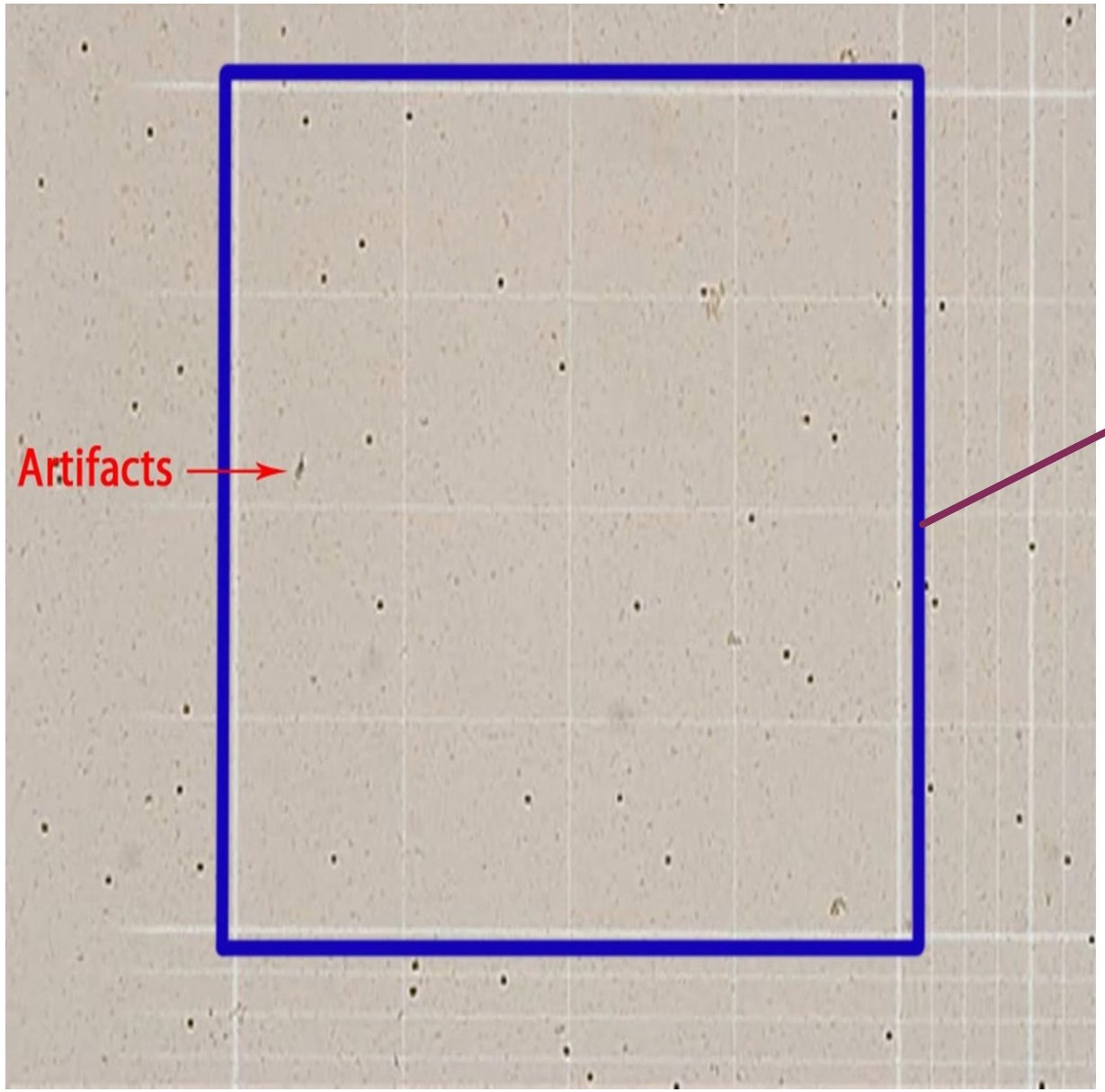
W1+W2+W3+W4 / 4 =
Result (Average W)

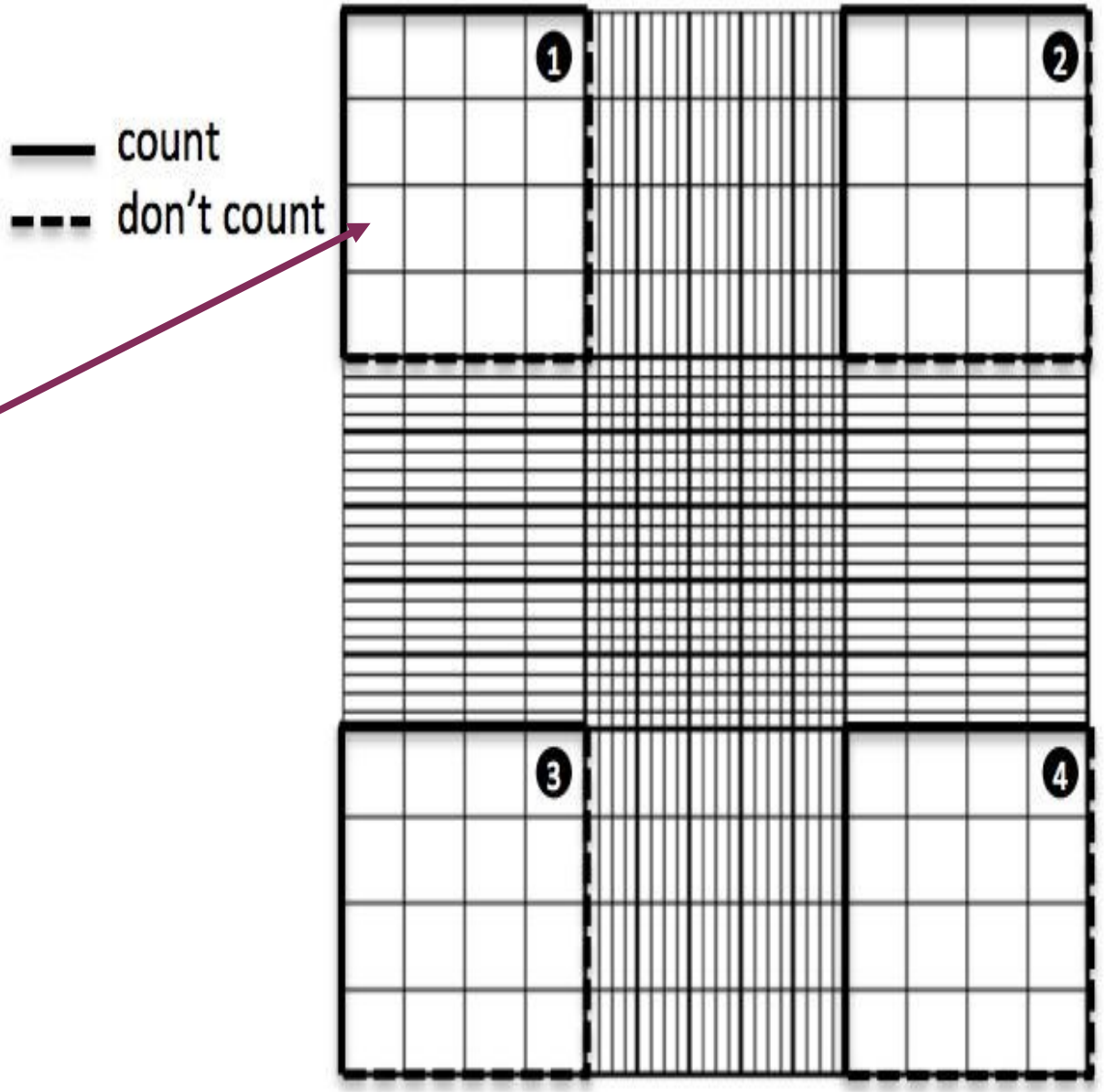
1 mm³ (B) = 200W




1 mm
1 mm
R
R
R
R
R
side view
cover slip
moat
0.1mm
moat


A
B
C
D
LOW POWER (100 X)
EACH OF THESE SMALL SQUARES IS 1/25 MM. SQUARE (50 μ)
THIS SPACE IS ONE SQUARE MM.
ON THE HAEMACYTOMETER ITS DEPTH IS 1/10 OF A MM.
HENCE FOR CONTENT OF THIS SPACE MULTIPLY BY 10 TO GET CONTENT OF ONE CUBIC MILLIMETER.
IMPROVED NEUBAUER RULING


Artifacts


count
don't count
1
2
3
4

EXAMPLE:

- Calculate the total WBC count for a blood sample when the total count for square A= 80, square B= 100, square C= 120 and square D= 88 cells. State if the result is normal, leukocytosis, leukopenia or leukemia?

Answer:

A + B + C + D = ?

80 + 100 + 120 + 88 = 388

388 / 4 = 97 (average)

Since 1mm³ (B) = 200 W

Then 200 * 97 = 19400 WBC / 1mm³ of blood *(Leukocytosis)* because the normal value is

4000 – 11000 WBC / 1mm³ of blood

EXAMPLE:

- calculate the total WBC if **the total WBCs of two chambers (A and C) is 48** and state if the result is normal, leukocytosis, leukopenia or leukemia?

Answer: we have **2 squares**

A + C = 48

48 / 2 = 24 (average)

Since 1mm³ (B) = 200 W

Then 200 * 24 = 4800 WBC / 1mm³ of blood

the result is ***normal*** because the normal value is

4000 – 11000 WBC / 1mm³ of blood

EXAMPLE:

- calculate the total WBC count for a blood sample when the total count of square A= 19, square B= 20, and square C= 10 cells. State if the result is normal, leukocytosis, leukopenia or leukemia?

Answer 1: we have **3** squares
We will choose (A and C) squares only
A + C = ?
19 + 10 = 29
29 / 2 = 14.5 (average)

Since 1mm³ (B) = 200 W
Then 200 * 14.5 = 2900 WBC / 1mm³ of blood

Result: *Leukopenia* because normal value is
4000 – 11000 WBC/1mm³ of blood

Answer 2: we have **3** squares **(the best answer)**
We will take the summation of all (3) squares
A + B + C = ?
19 + 20 + 10 = 49
49 / 3 = 16.3 (average)

Since 1mm³ (B) = 200 W
Then 200 * 16.3 = 3260 WBC / 1mm³ of blood

Result: *leukopenia* because normal value is
4000 – 11000 WBC/1mm³ of blood


STAY SAFE!
Keep Your Distance!
Thank you for your patience
2 metres
2 metres