

Platelet Count Using Haemocytometer

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- Platelets or thrombocytes are elements of blood. Platelets are small, colourless and non-nucleated cells.
- Normally, platelets are of several shapes, for example spherical, rod-shaped and may attain oval or disk-shaped in inactive condition.

- Normal platelet count is 2,50,000/cu mm of blood. It ranges between 2,00,000 and 4,00,000/cu mm of blood.
- Average lifespan of platelets is 10 days. It varies between 8 and 11 days.
- Platelets are destroyed by tissue macrophage system in spleen. So, **splenomegaly** (enlargement of spleen) decreases platelet count and **splenectomy** (removal of spleen) increases platelet count .
- During activation, the platelets change their shape with elongation of long filamentous pseudopodia which are called processes or filopodia.

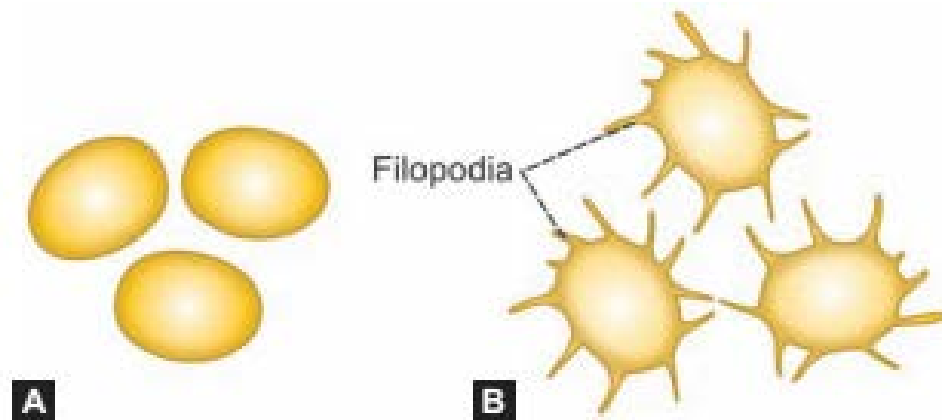


FIGURE 18.2: A. Inactive platelets. B. Activated platelets.

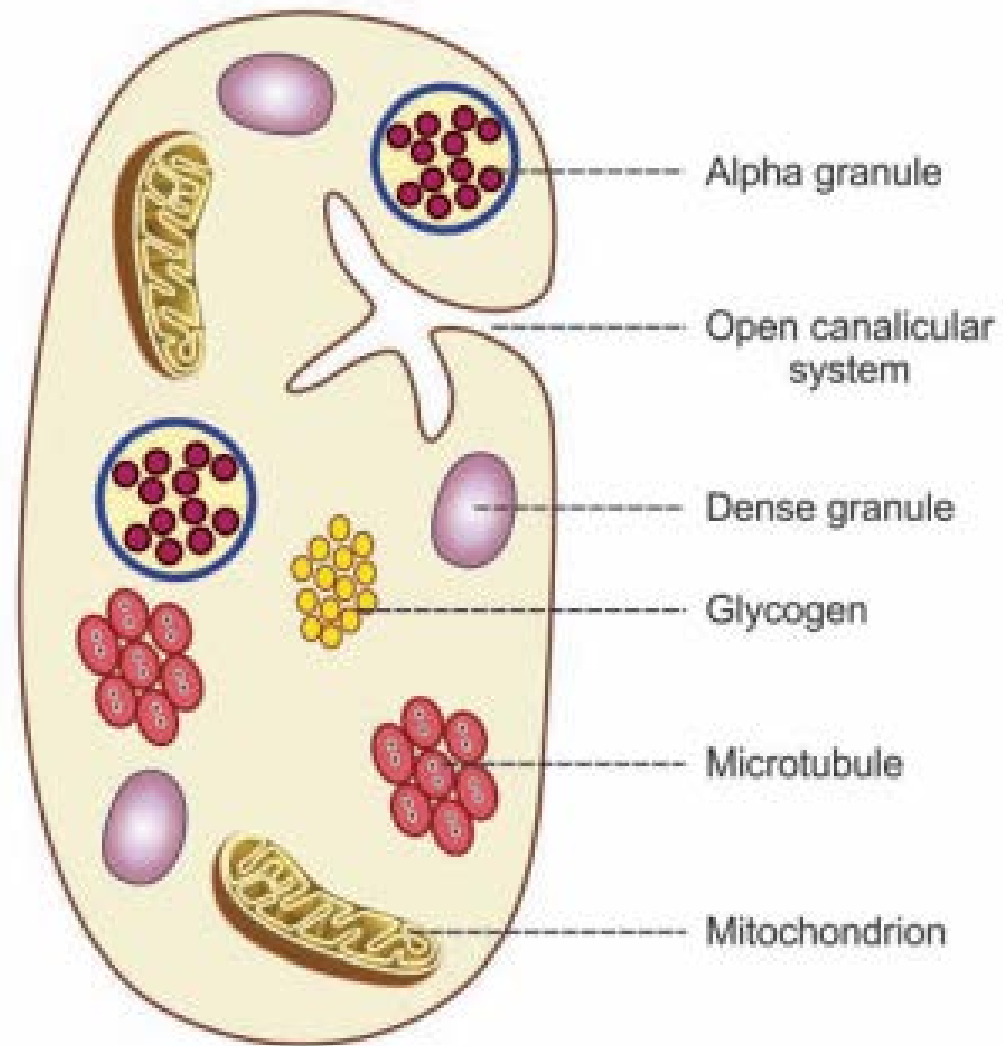


FIGURE 18.1: Platelet under electron microscope

Functions of platelets

- **Role in blood clotting**
- **Role in clot retraction**- this is the process by which the volume of a formed clot is reduced through contraction of the intracellular actin-myosin cytoskeleton of activated platelets incorporated in the clot.
- **Role in prevention of blood loss (hemostasis)**
- **Role in defence mechanism**
By the property of agglutination, platelets encircle the foreign bodies and destroy them.

- **PHYSIOLOGICAL VARIATIONS**

1. *Age*: Platelets are less in infants (1,50,000 to 2,00,000/cu mm) and reaches normal level at 3rd month after birth.
2. *Sex*: There is no difference in the platelet count between males and females. In females, it is reduced during menstruation.

- Platelet disorders occur because of pathological variation in platelet count and dysfunction of platelets.

Platelet disorders are:

1. Thrombocytopenia
2. Thrombocytosis
3. Thrombocythemia
4. Glanzmann's thrombasthenia.

- **Decrease in platelet count is called thrombocytopenia.**

Thrombocytopenia occurs in the following conditions:

- i. Acute infections
- ii. Acute leukemia
- iii. Aplastic and pernicious anemia

Aplastic anemia Blood cells are made from stem cells in your bone marrow. In this case, stem cells in bone marrow are damaged and can't make enough new blood cells.

Pernicious anemia is one of the vitamin B-12 deficiency anemias. It's caused by an inability to absorb the vitamin B-12 needed for body to make enough healthy red blood cells.

- iv. Chickenpox
- v. Smallpox
- vi. Splenomegaly
- viii. Typhoid
- ix. Tuberculosis
- x. Purpura

Purpura occurs when small blood vessels burst, causing blood to pool under the skin. This can create purple spots on the skin that range in size from small dots to large patches.

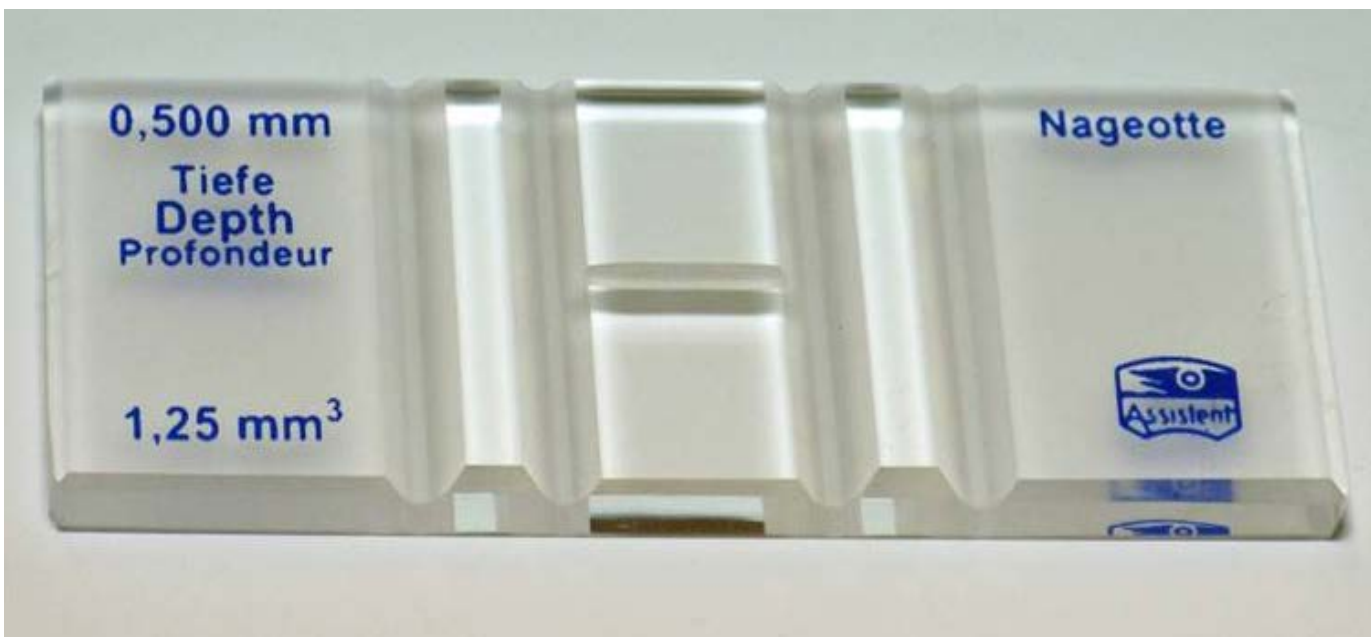
- Increase in platelet count is called thrombocytosis. Thrombocytosis occurs in the following conditions:
 - i. Allergic conditions
 - ii. Asphyxia - a condition arising when the body is deprived of oxygen, causing unconsciousness or death; suffocation.
 - iii. Hemorrhage-. Hemorrhaging is excessive bleeding.
 - iv. Bone fractures
 - v. Surgical operations
 - vi. Splenectomy
 - vii. Rheumatic fever
 - viii. Trauma (wound or injury or damage caused by external force).

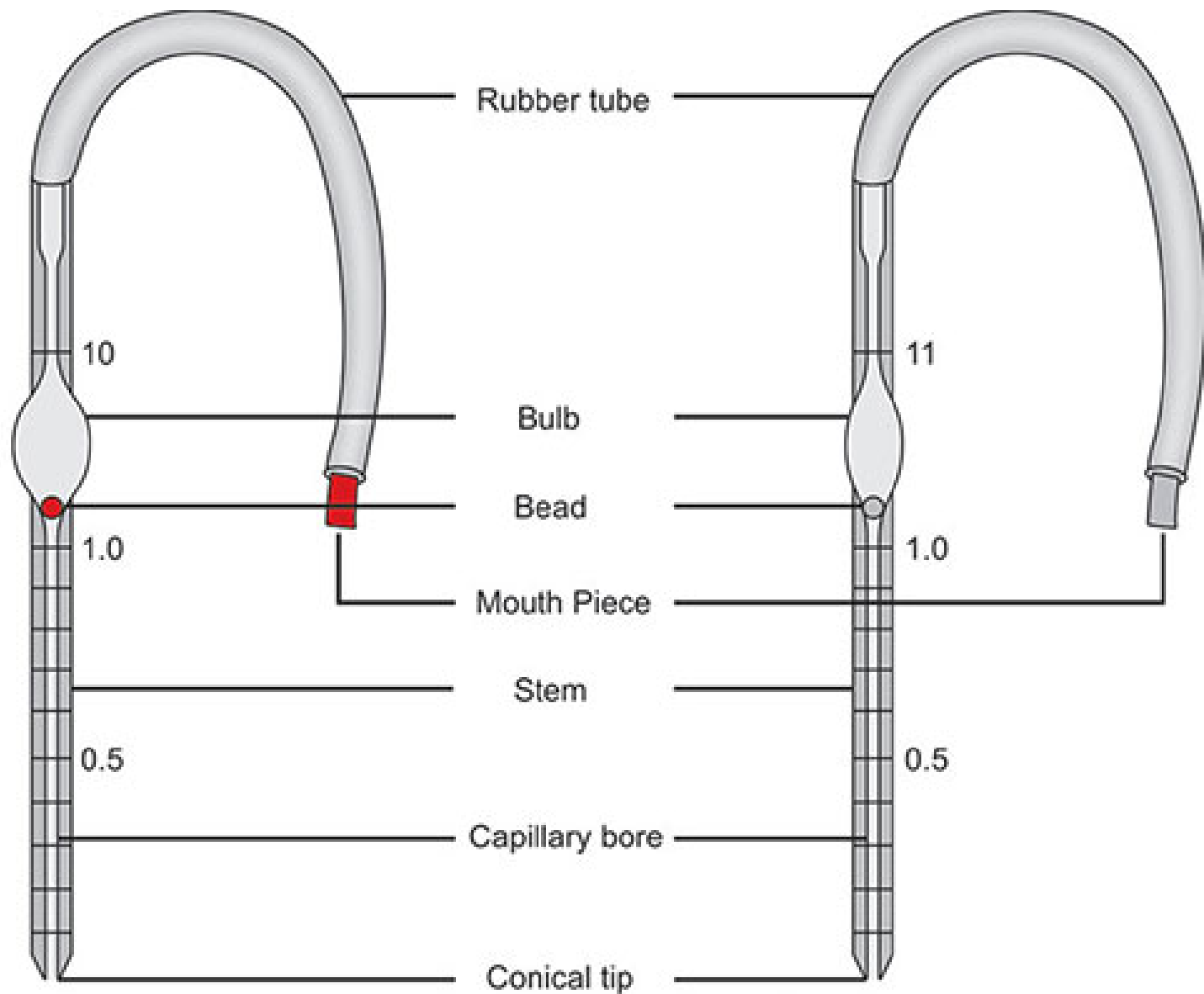
- Thrombocythemia is the abnormal increase in plate occurs in the following condition:
 - i. Carcinoma
 - ii. Chronic leukemia
 - iii. Hodgkin's disease.

- ***Glanzmann's Thrombasthenia***

Glanzmann's thrombasthenia is an inherited hemorrhagic disorder, caused by structural or functional abnormality of platelets. It leads to **thrombasthenic purpura**.

Methodology of Platelet count by haemocytometer



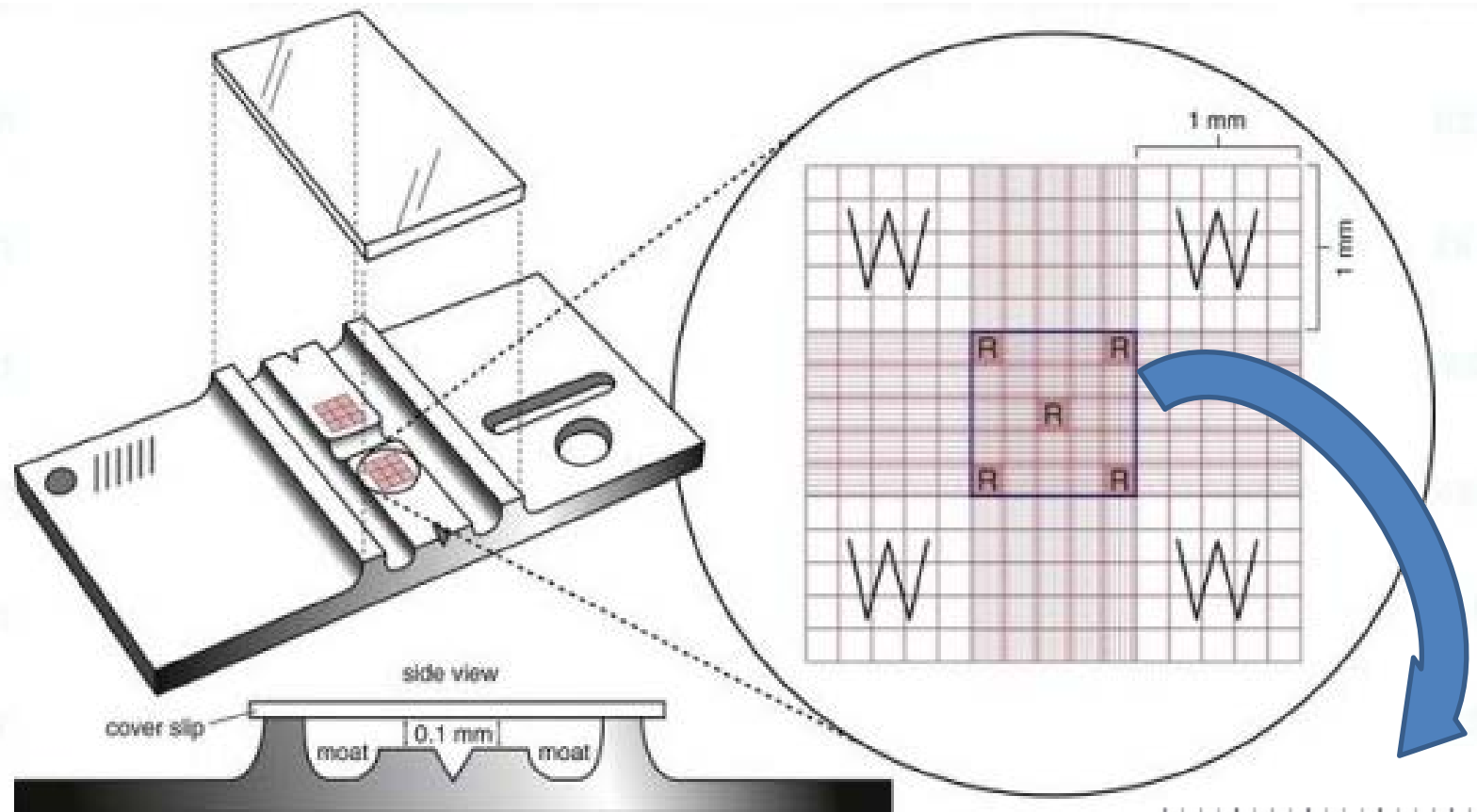


RBC pipette

WBC pipette

DIFFERENCES BETWEEN RBC AND WBC PIPETTE

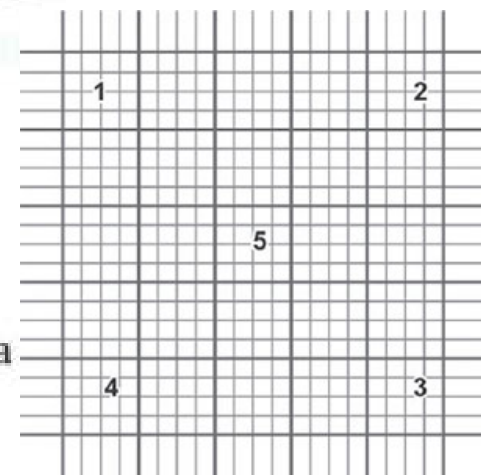
	RBC pipette	WBC pipette
1)	It has a red bead	It has a white bead
2)	It has graduations upto mark 101	It has graduations upto mark 11
3)	Size of bulb is larger	Size of bulb is smaller
4)	Size of lumen is smaller	Size of lumen is larger



Platelet calculation details

$$\text{Platelet count (per litre)} = \frac{\text{Cells counted} \times 20^* \times 10^6}{0.2^\dagger \times 0.1^\ddagger}$$

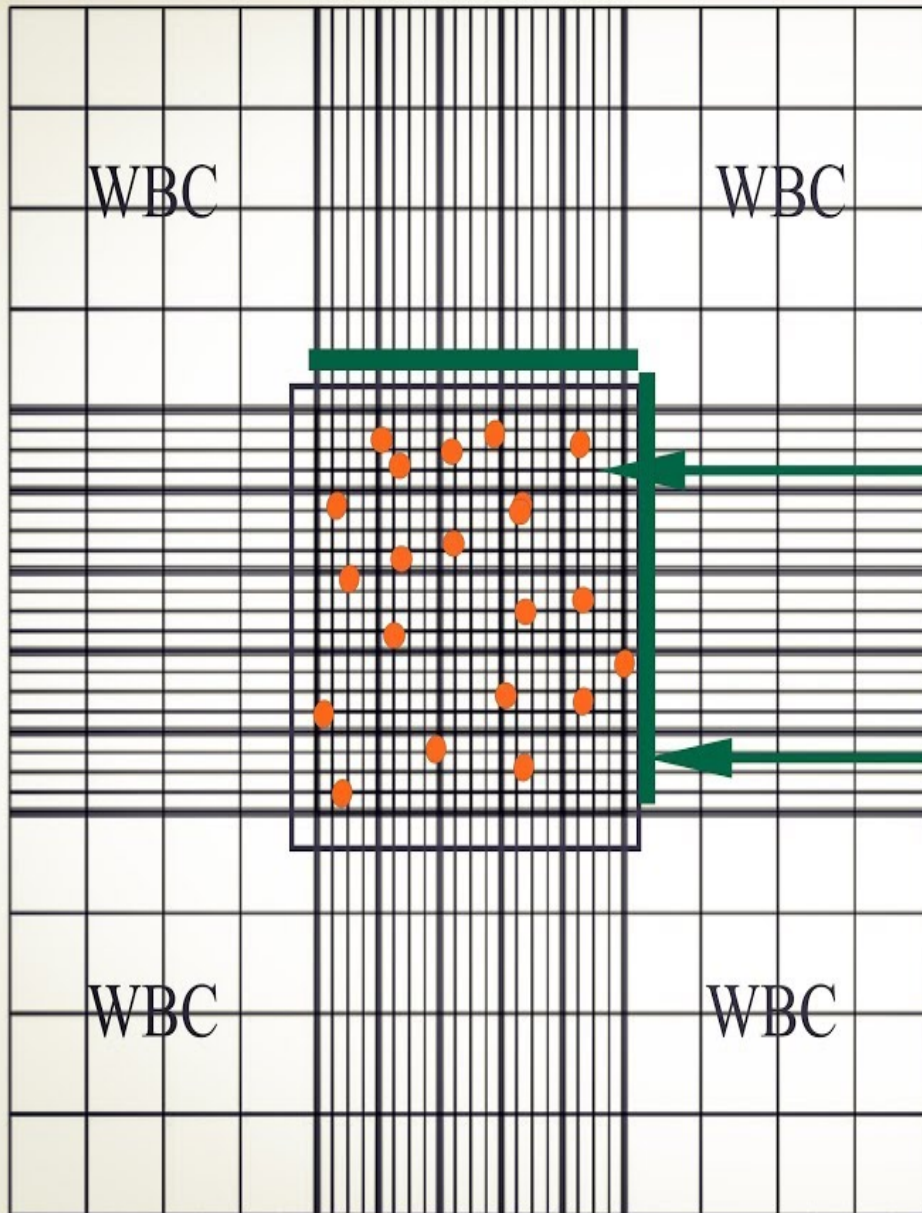
where $*$ = 1 in 20 dilution of blood, † = 0.2 mm² area counted ‡ = 0.1 mm depth of chamber



RBC Chamber

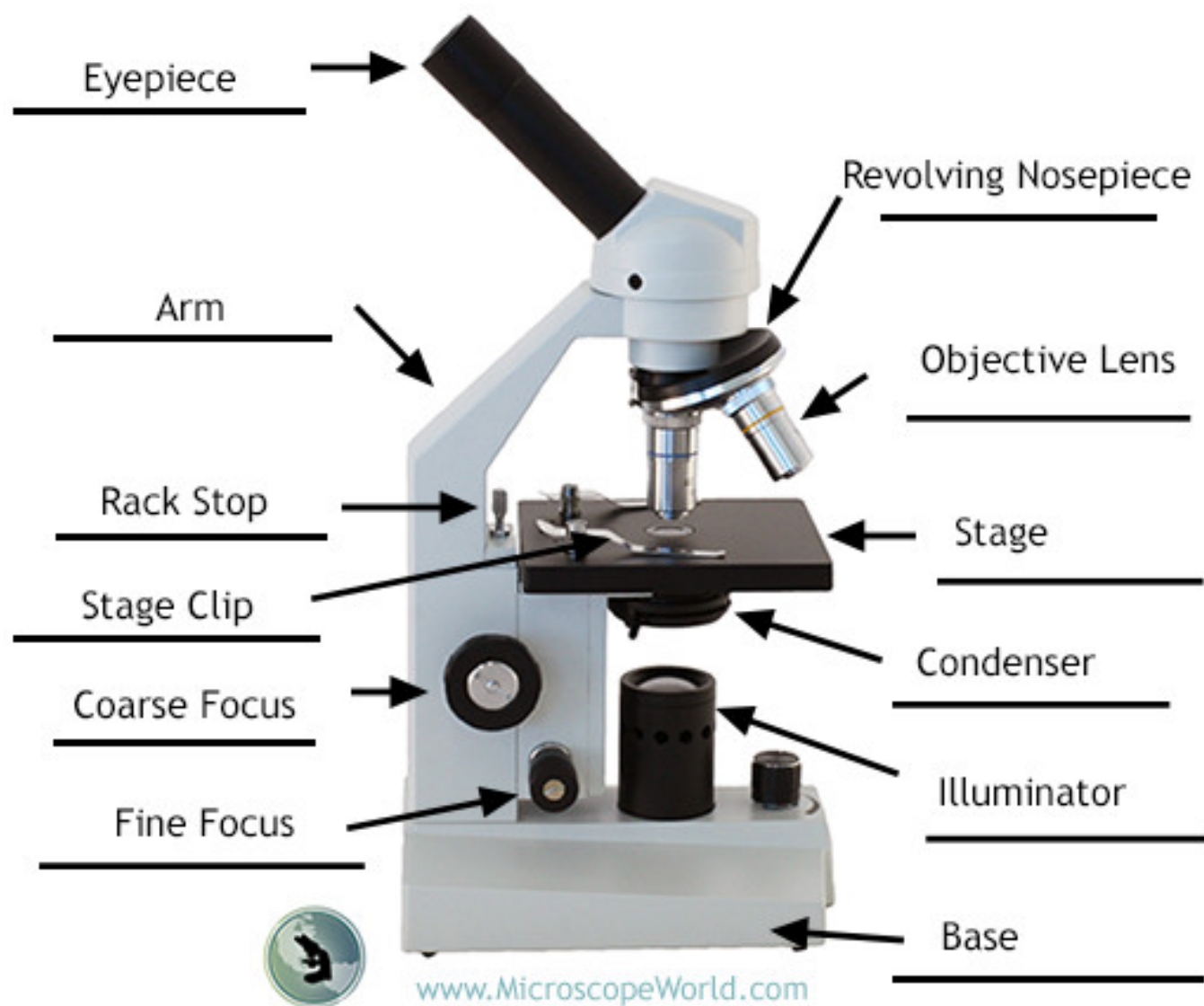
Platelets count

MANUAL METHOD



Count the central square

Don't count the platelets along the Green lines(left and upper line)



- Platelet Count: Blood is diluted 1 in 20 by taking 0.1 ml of blood into 1.9 ml of diluent.
- The diluted blood is well mixed.
- The diluted sample was loaded in Neubauer chamber after blowing out at least a third of the contents of the bulb.
- The Chamber is then stood in a petri dish, containing a piece of moist filter paper, for 20 min to allow the cells to settle.
- The moist filter paper prevents evaporation during the long period of standing.
- The cells are counted as in **chamber number 1,5,13, 21 and 25 of RBC chamber** (that is in 80 small squares).
- Counting of platelets was done in compound light microscope under 40 X objective.

Principle –:

1% Ammonium oxalate is isotonic to platelets and lyses the RBC. WBC remains but they are less in count so they do not interfere in counting the platelets.

$$\text{Platelets count (per ml)} = \frac{\text{No. of cells counted}}{\text{Volume of the chamber } (\mu\text{l})} \times \text{dilution} \times 1,000$$

$$= \frac{N}{0.02} \times 20 \times 10^3$$

$$= N \times 10^6$$