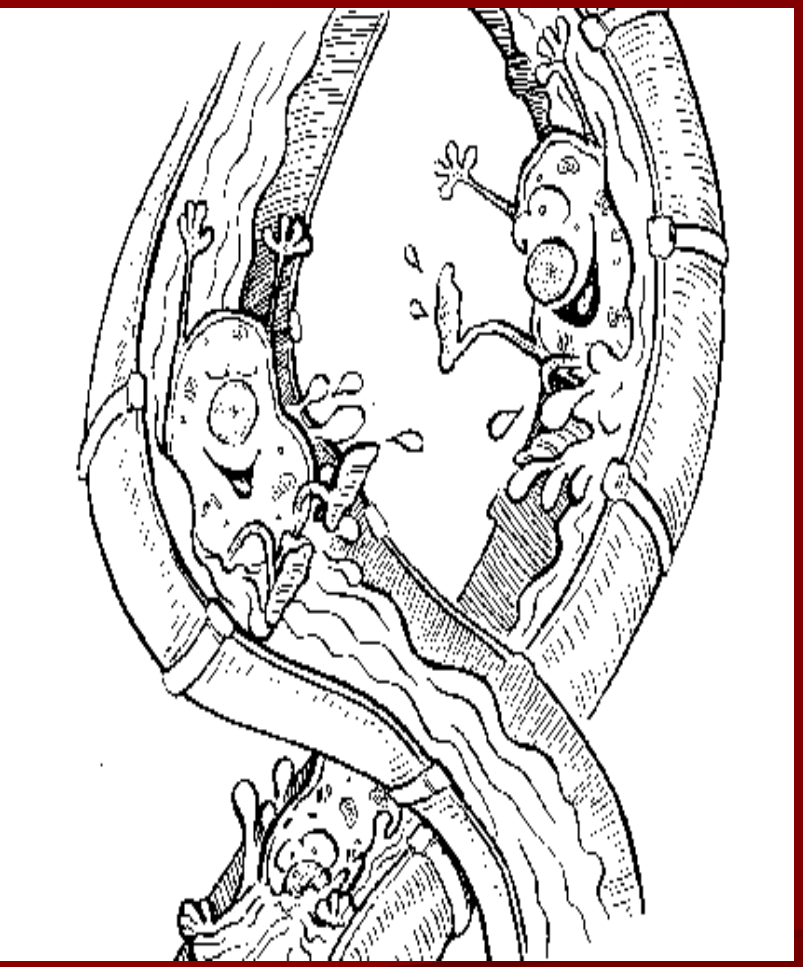
Attention

- Don´t manipulate the light microscopes, please.
- They are prepared for your work following presentation.
- You´ll receive an instruction how to use the LM and how to study blood smears.

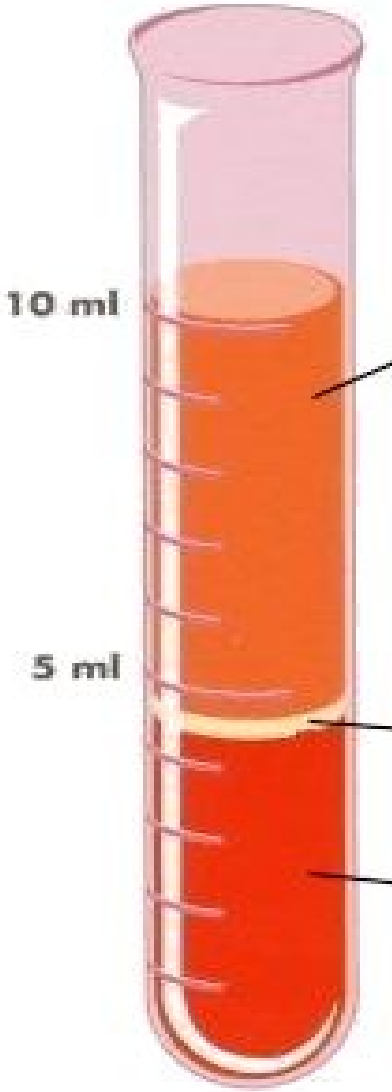


Blood

Plasma [redacted]lood cells

Hematocrit:

the volume of blood cells per unit volume of blood



PLASMA - 55% of Total Blood Volume
91% Water
7% Blood Proteins (fibrinogen, albumin, globulin)
2% Nutrients (amino acids, sugars, lipids)
Hormones (erythropoietin, insulin, etc.)
Electrolytes (sodium, potassium, calcium, etc.)

CELLULAR COMPONENTS - 45% of Total Blood Volume

Buffy Coat
White Blood Cells (7000-9000 per mm^3 of blood
Platelets (250,000 per mm^3 of blood

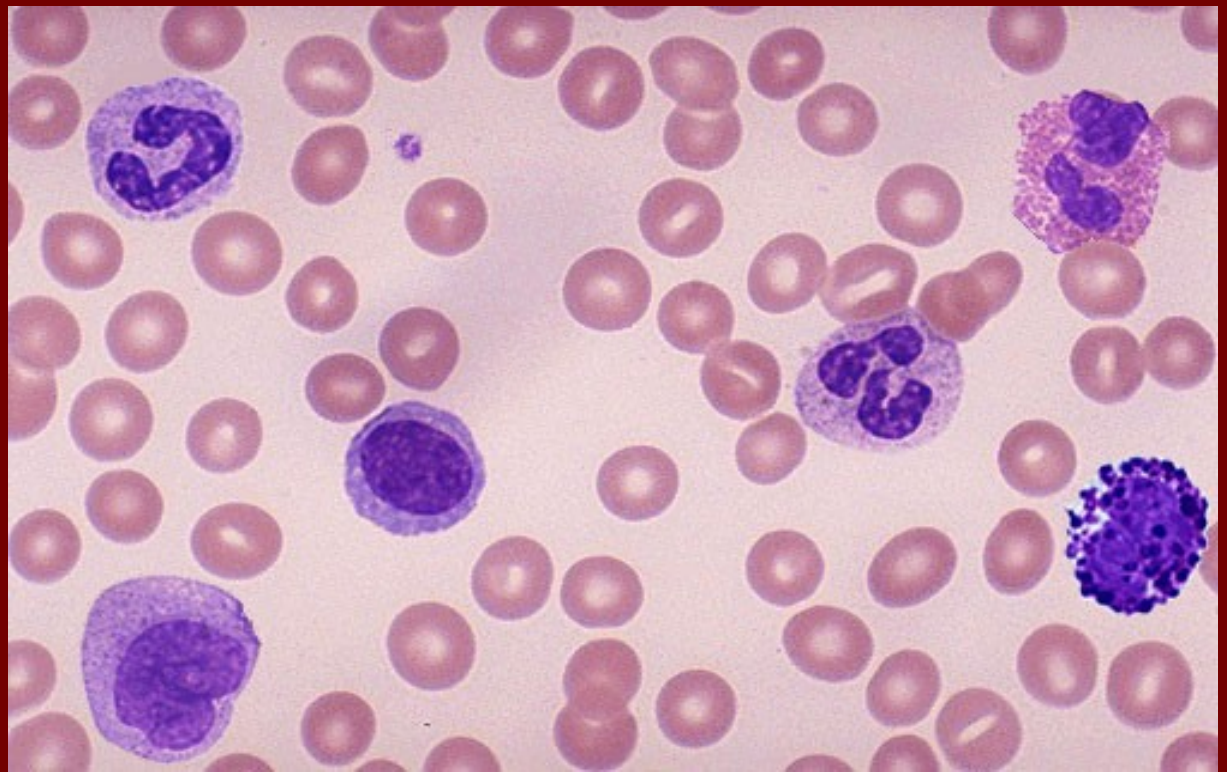
Red Blood Cells (RBCs)
About 5,000,000 per mm^3 of blood

Blood cells (formed elements)

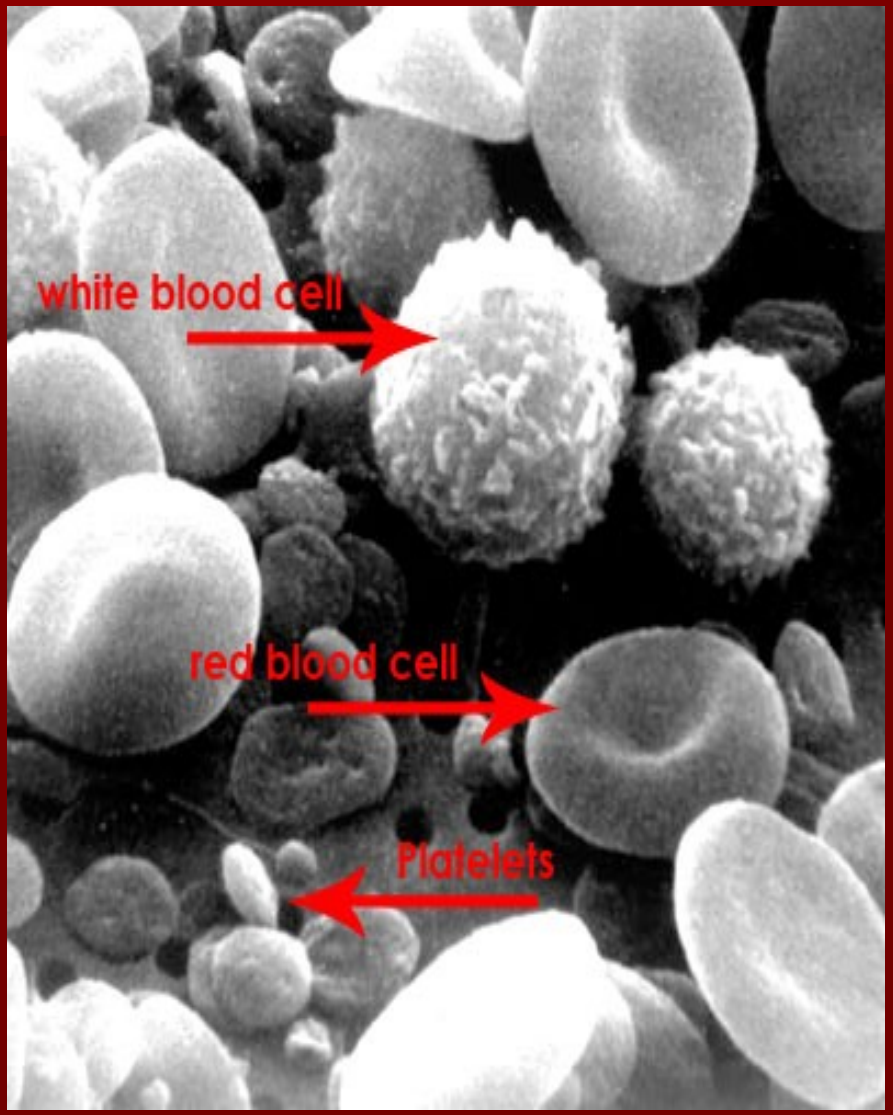
- Red blood cells – **erythrocytes**
- White blood cells – **leukocytes**
- Platelets – **thrombocytes**

Granulocytes: Neutro-, Eosino-, Baso-

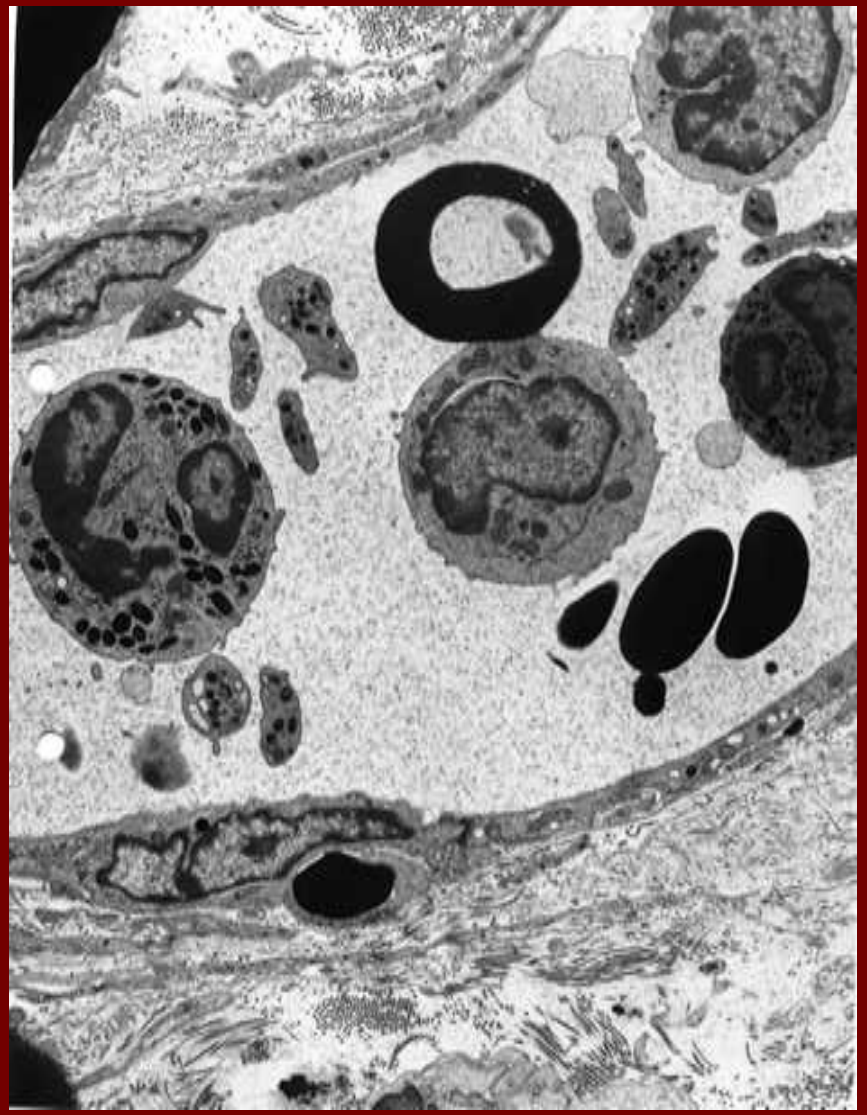
Agranulocytes: Lymfo-, Mono-



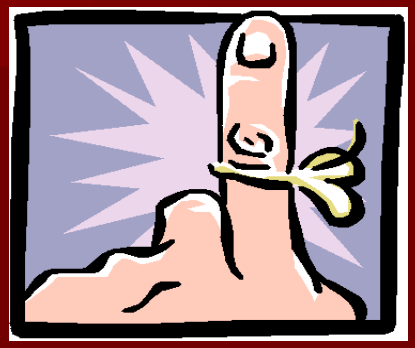
REM



TEM



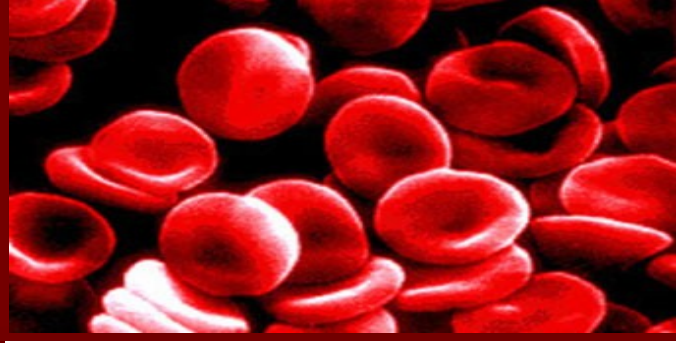
REMEMBER!



- Erythrocytes: 4 – 6 millions/ 1 □ **of blood**
- Leukocytes: 5,000 – 9,000 / 1 □
- Thrombocytes: 150,000 – 250,000/ 1 □

ERYTHROCYTES

- 4 – 6 million/µl
- Shape: biconcave disc,
  dumble-shaped *(cross section)*
- Size: 7.4 µm in diameter
  (= normocyte)
- Structure: plasmalemma,
  cytoplasm + hemoglobin 33 %
  absence of the nucleus and cell organelles
- Lifespan: 120 days



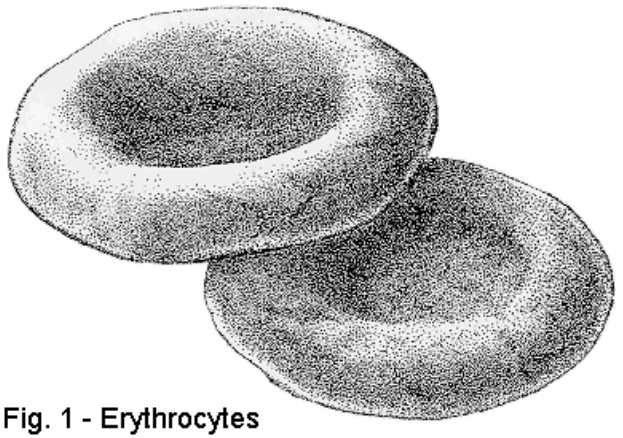
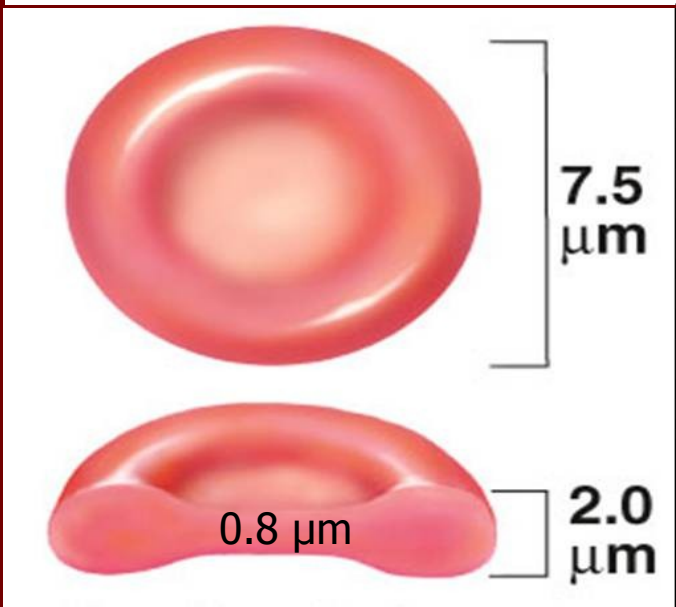
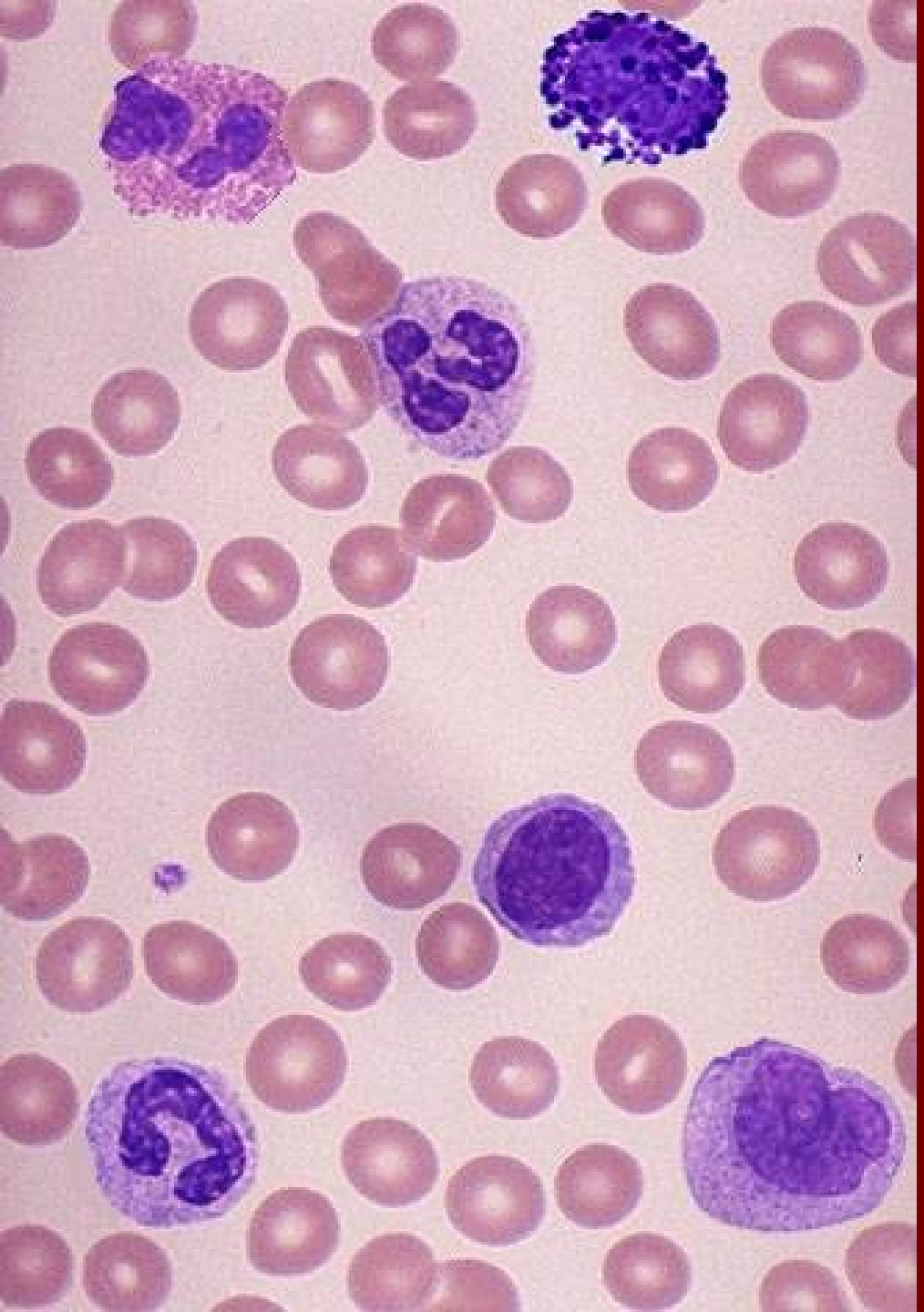
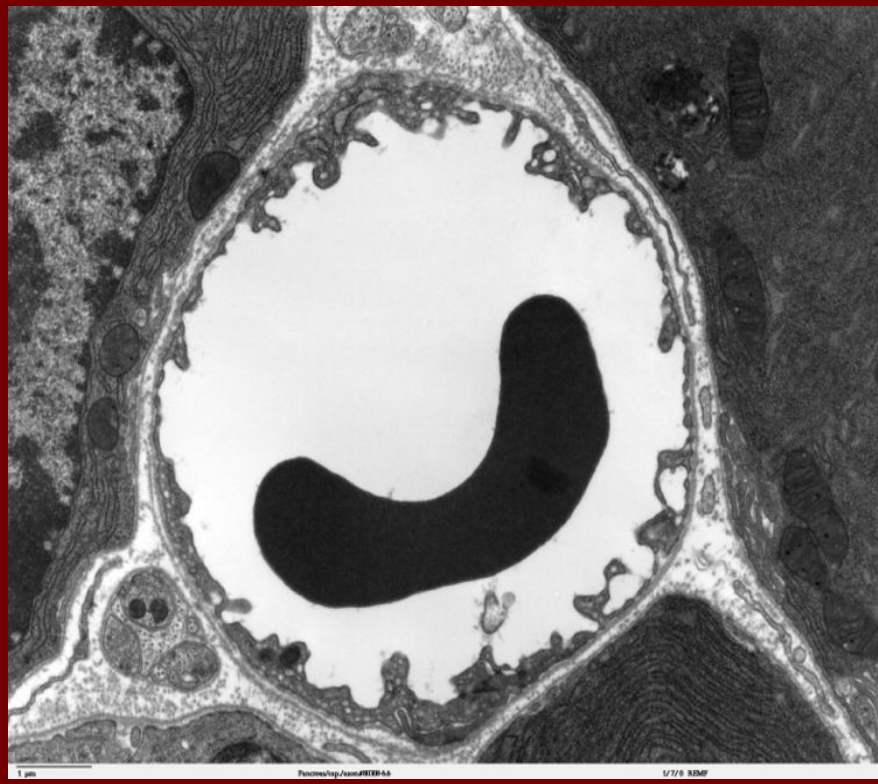


Fig. 1 - Erythrocytes





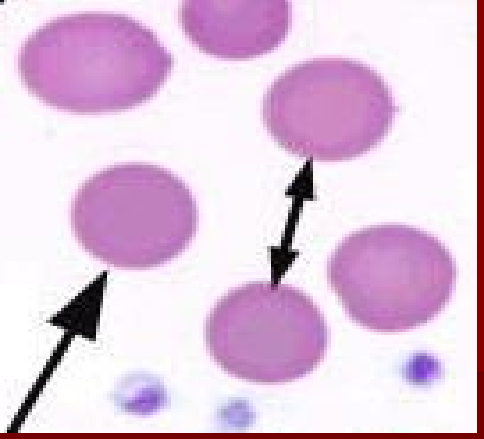


<Important terms>

- **Polyglobulia** – *an increased number of ery*
- **Anemia** – *a decreased number of ery*

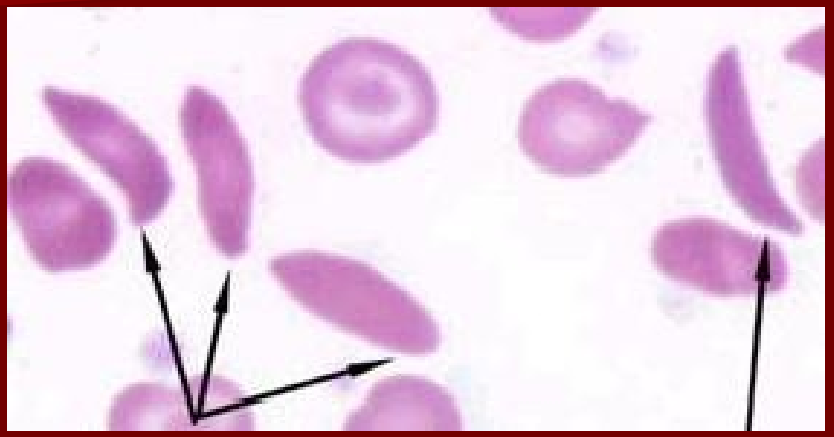
- **Poikilocytosis** – *an occurrence of variously shaped ery (spherocytes, elliptocytes, drepanocytes = sickle cells, etc.)*
- **Anisocytosis** – *an occurrence of variously sized ery (microcytes, macrocytes)*

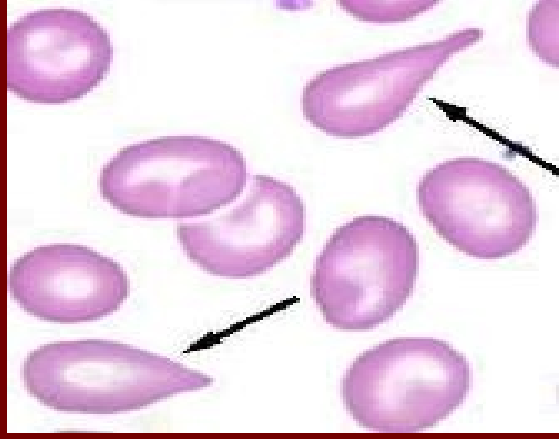
poikilocytosis



spherocytes

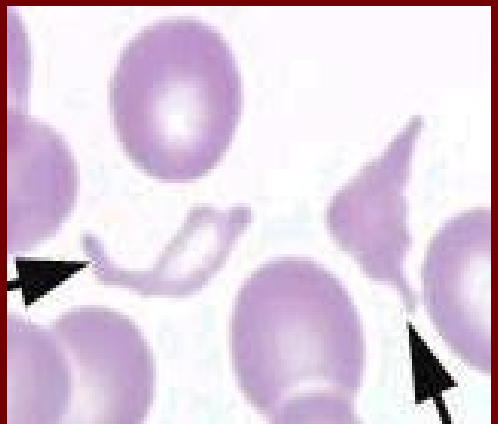
drepanocytes



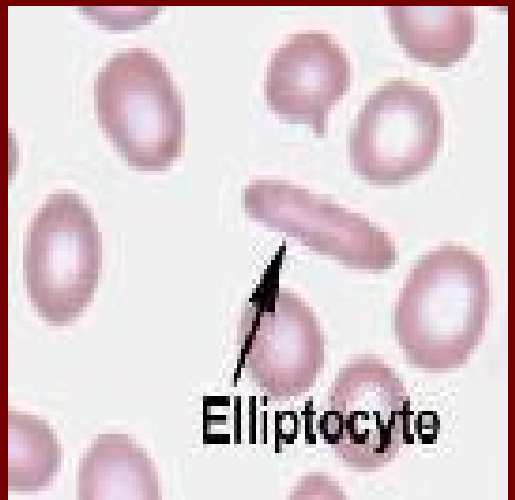


dacryosytes
(teardrop)

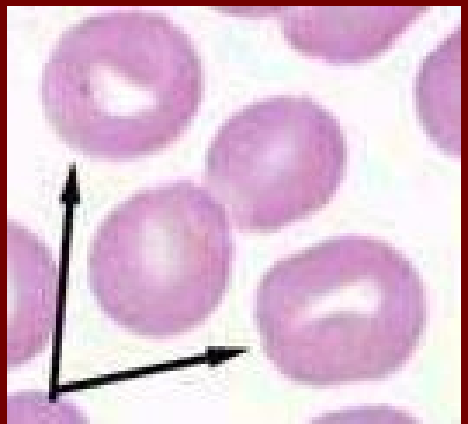
schystocytes
(keratocytes)



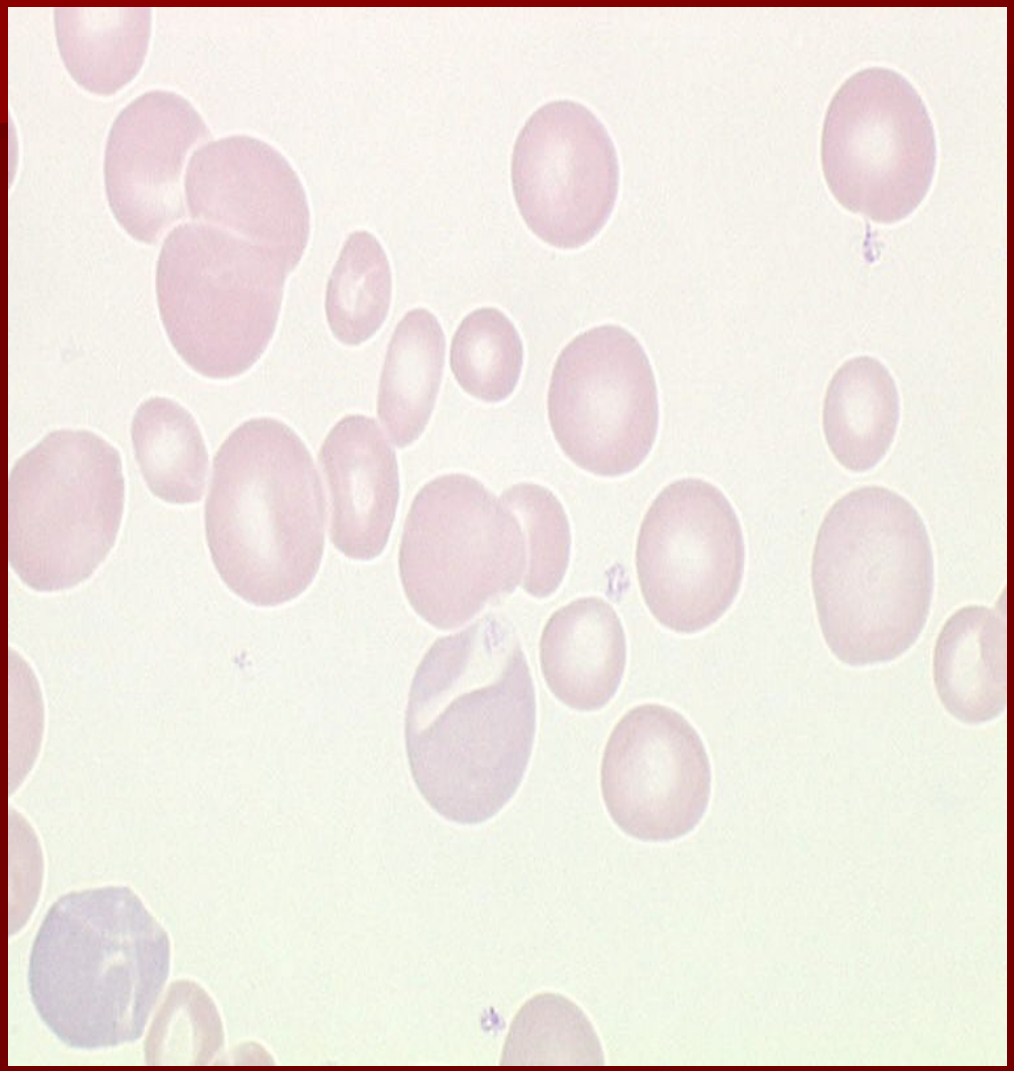
elliptocytes



stomatocytes



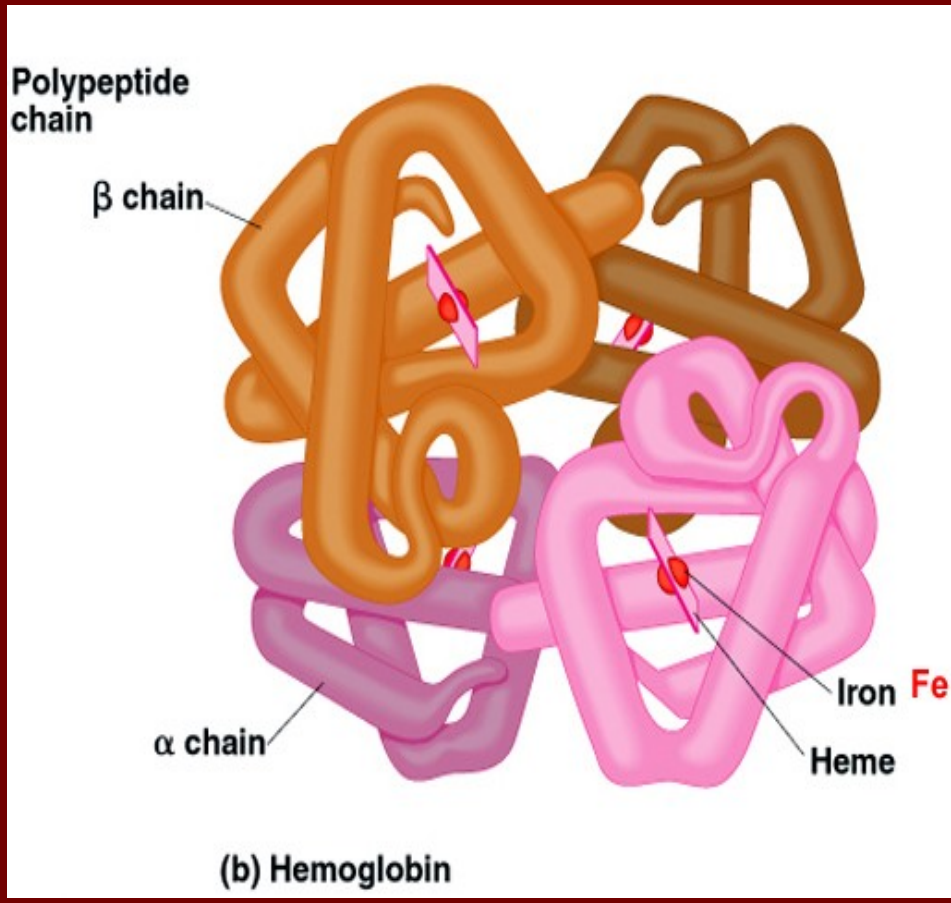
Anisocytosis



- Microcytes
  - < 6.5 µm
- Normocytes
  - 7.4 µm
- Macrocytes
  - > 8 µm

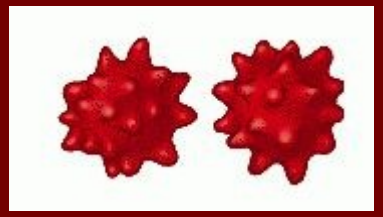
Hemoglobin

- a conjugated protein:
  4 polypeptide chains + heme groups = protoporphyrin ring with ferrous iron (Fe^{2+})
- Hb F (fetal)
- Hb A (adult)
- normochromatic ery:
  32±2 picogramms
  (*hyper-, hypo-*)



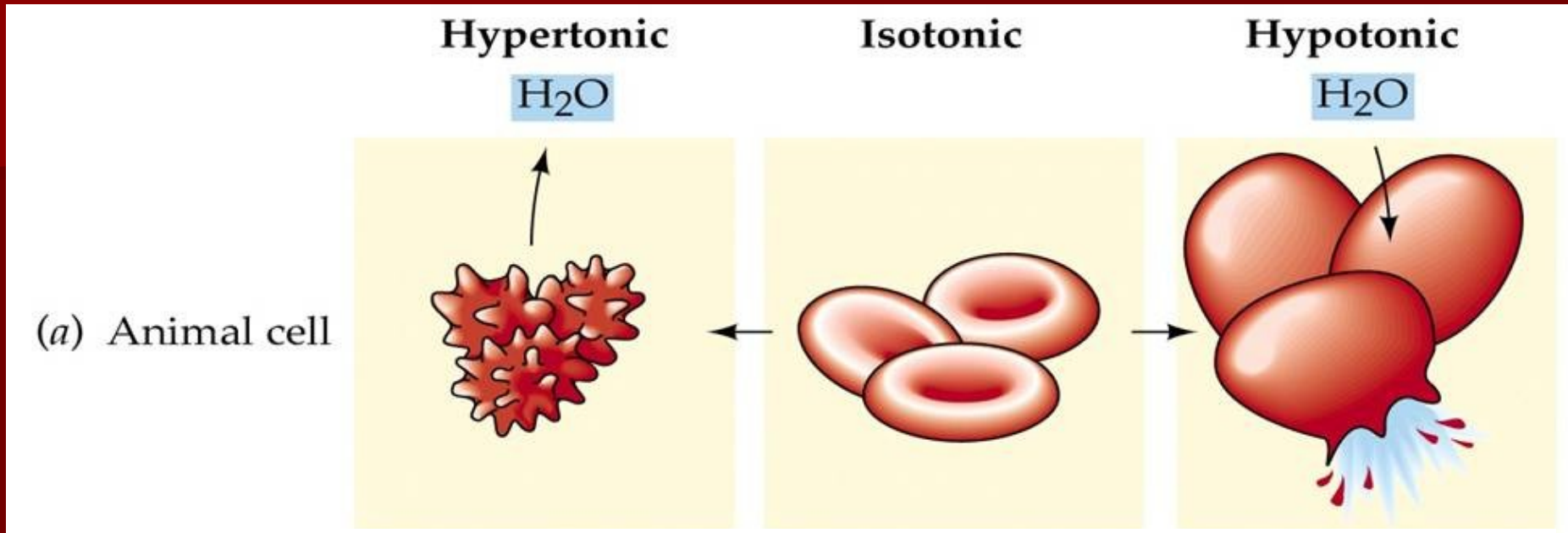
<Important terms>

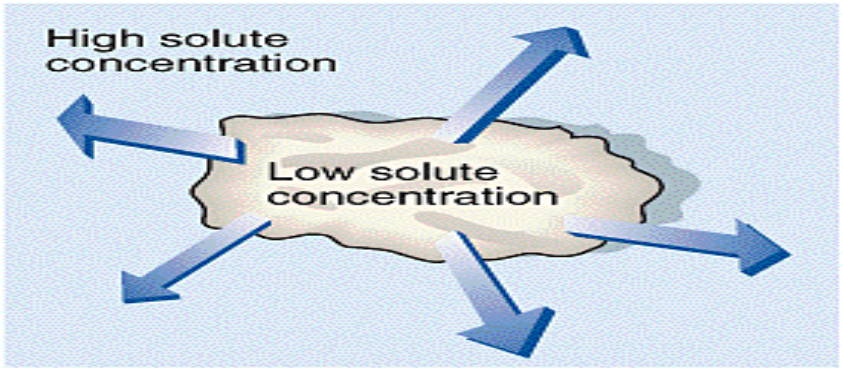
- Osmotic resistance and hemolysis
  (osmotic pressure has a great effect on living cells, because their walls are semipermeable membrane)
- Isotonic conditions
- in hypertonic solution – ery shrink irregularly and become crenated



- in hypotonic solution – ery swell, their plasmalemma come to the rupture, Hb is released – **hemolysis** – (the rest of ery = ghost)

(a) *crenation* is caused by **water** movement **out of** a cell in a *hypertonic* solution.
(b) *hemolysis* is caused by **water** movement **into** a cell in a *hypotonic* solution.

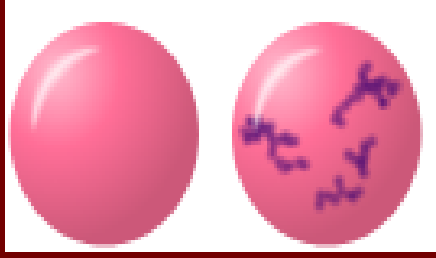




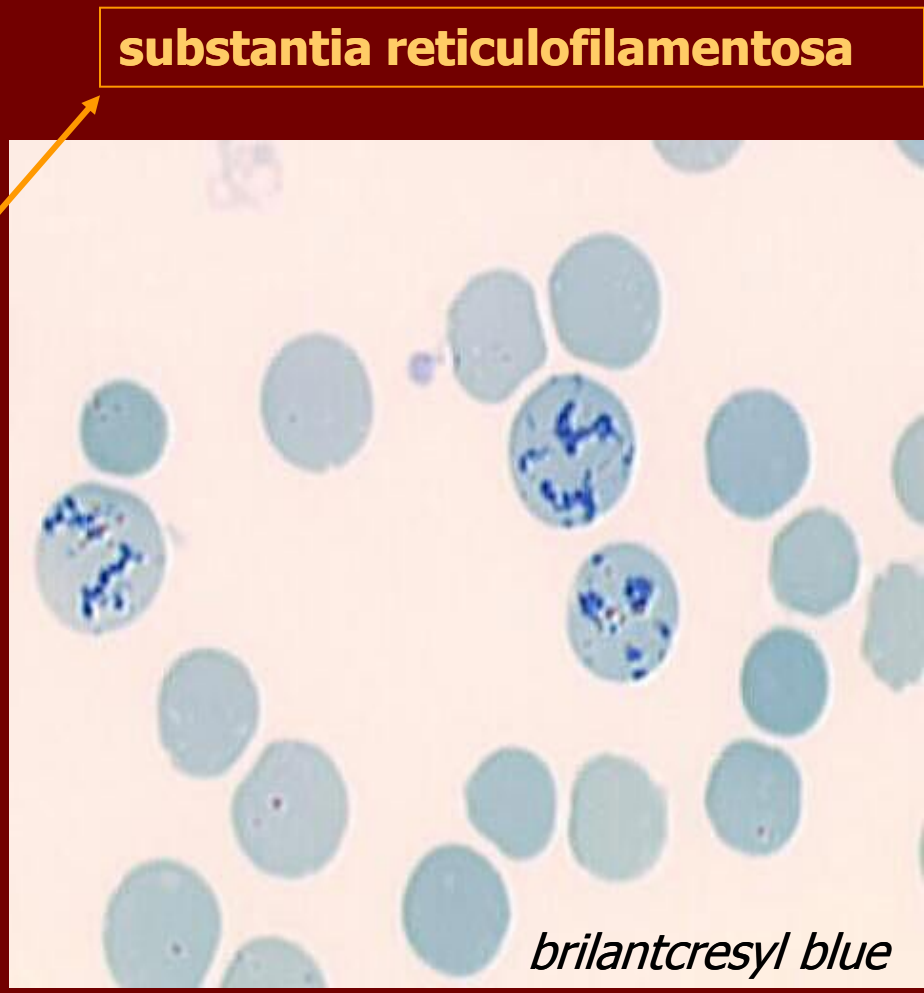
(a)

(b)

Reticulocytes



- Immature ery are released from the bone marrow into the peripheral blood (0.5 – 1.5 %)
- the rests of organelles – ribosomes, mitochondria
- maturation into ery during 24 hours



Functions of ery

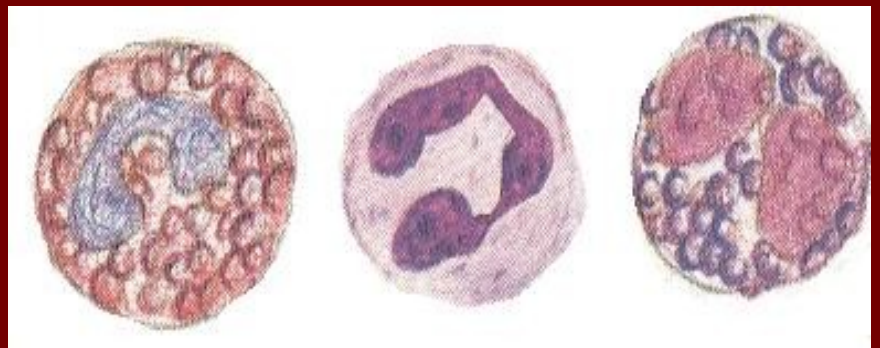
- transport of oxygen from the lungs
- transport of carbon dioxide from the tissues

LEUKOCYTES

- Granulocytes:
  - neutrophils
  - eosinophils
  - basophils

General characteristic:

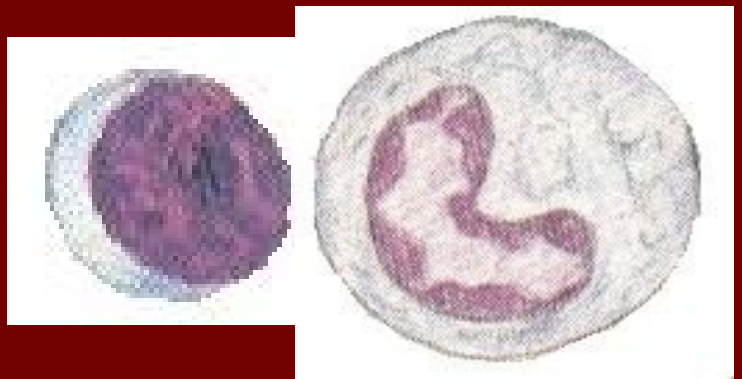
Polymorphonuclears with acidophilic cytoplasm and Specific + azurophilic granules

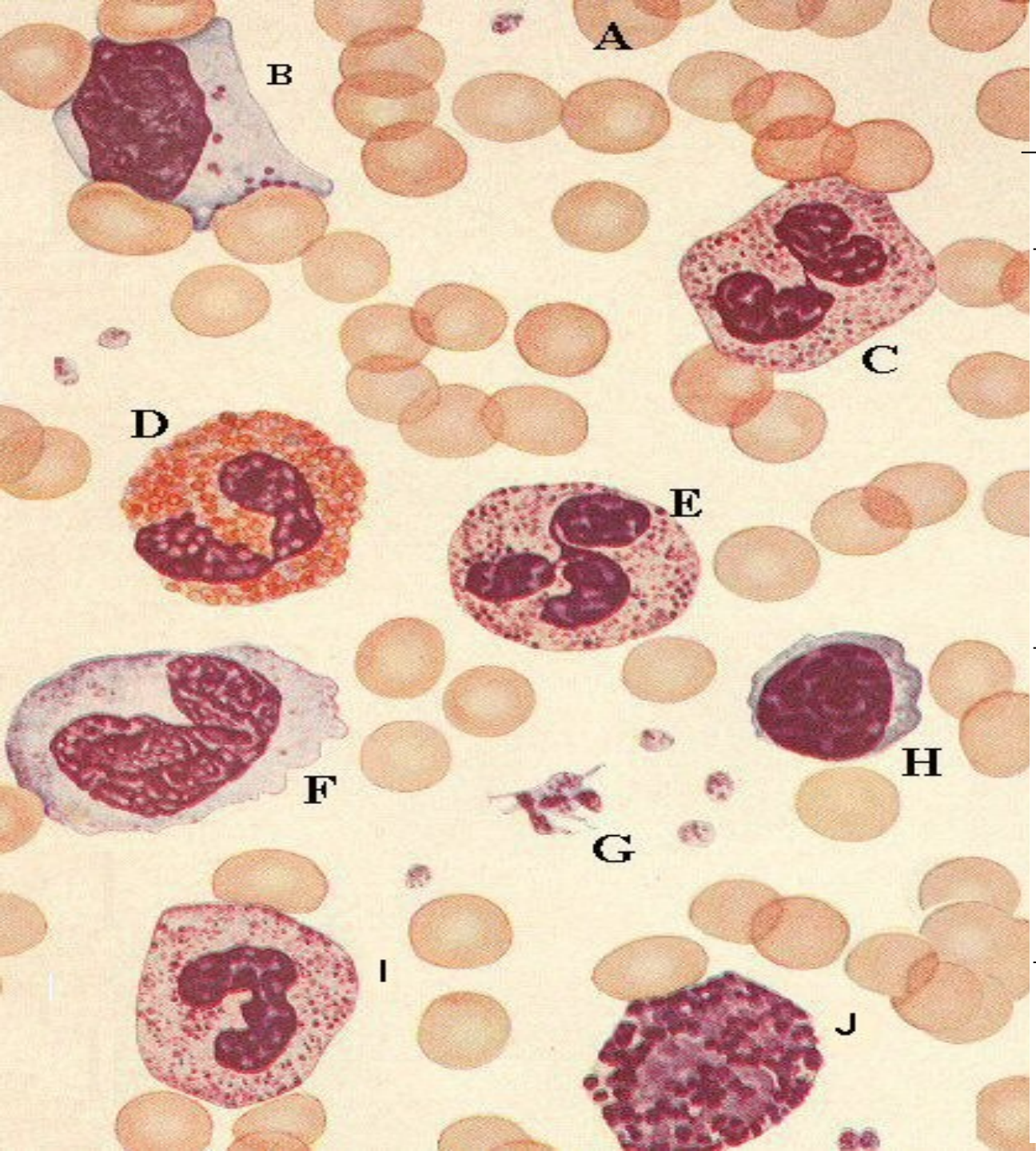


- Agranulocytes
  - lymphocytes
  - monocytes

General characteristic:

Mononuclears with basophilic cytoplasm and azurophilic granules





Erythrocytes (A)

Thrombocytes (G)

Granulocytes:
(pink cytoplasm,
specific granules)

- neutrophil segment (E,C)
- neutrophil band (I)
- eosinophil (D)
- basophil (J)

Agranulocytes:
(blue cytoplasm,
azurophilic granules)

- lymphocyte (H, B)
- monocyte (F)

Granulocytes

- General charcteristic:

- polymorphonuclears – different shape of nuclei

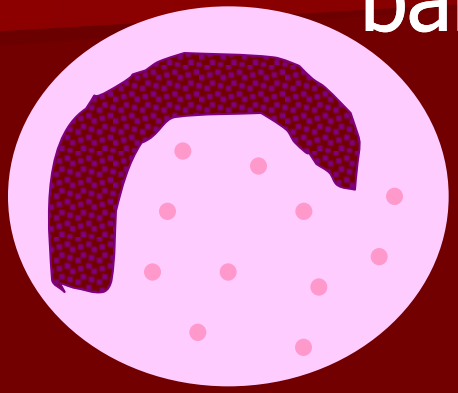
band segmented with chromatin bridges

- **acidophilic cytoplasm** – bright-pink
- **specific granules** – with special enzymes
- **azurophilic granules** – with lysosomal enzymes
- all granulocytes are able to migrate from the vessels and by diapedesis invade a site of inflamation

Granulocytes

neutrophils – eosinophils - basophils

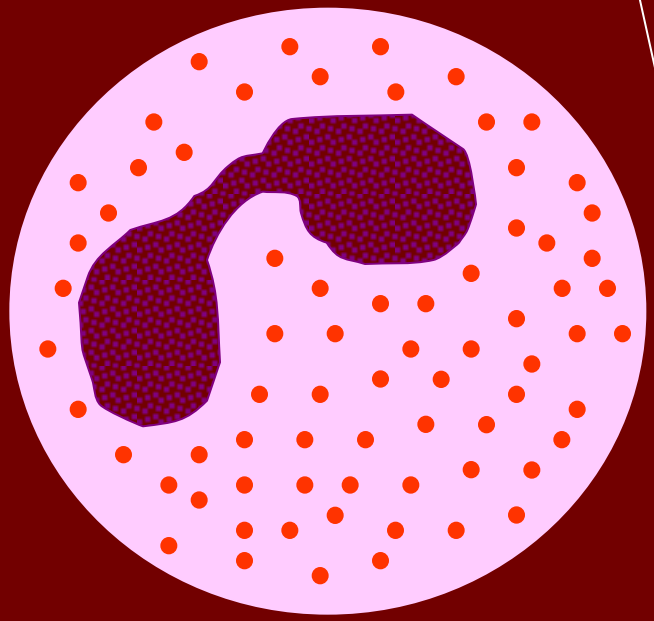
band

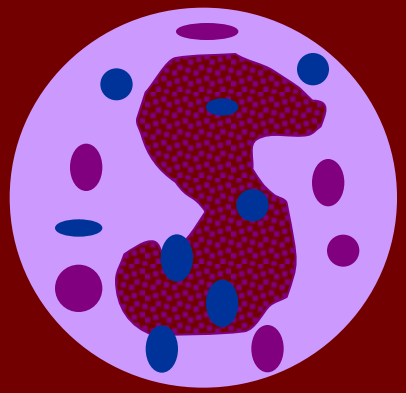


10-12 µm

segment

12-14 µm





10 µm

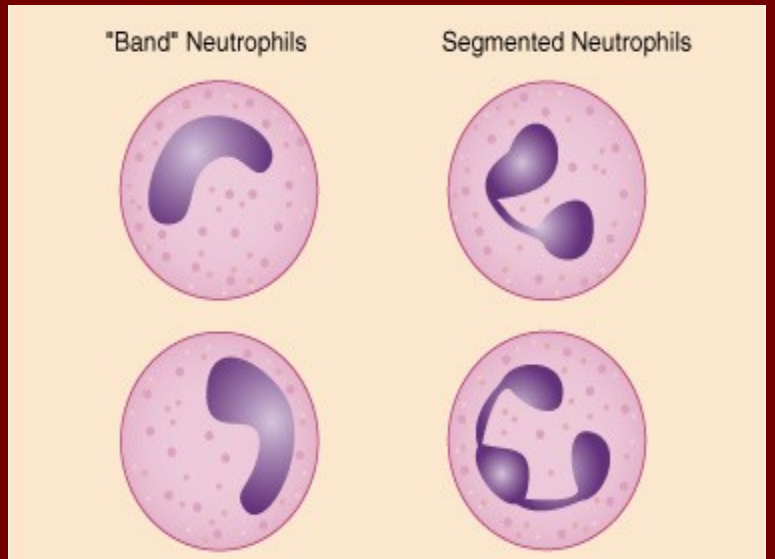
Differential white cell count (DWCC)

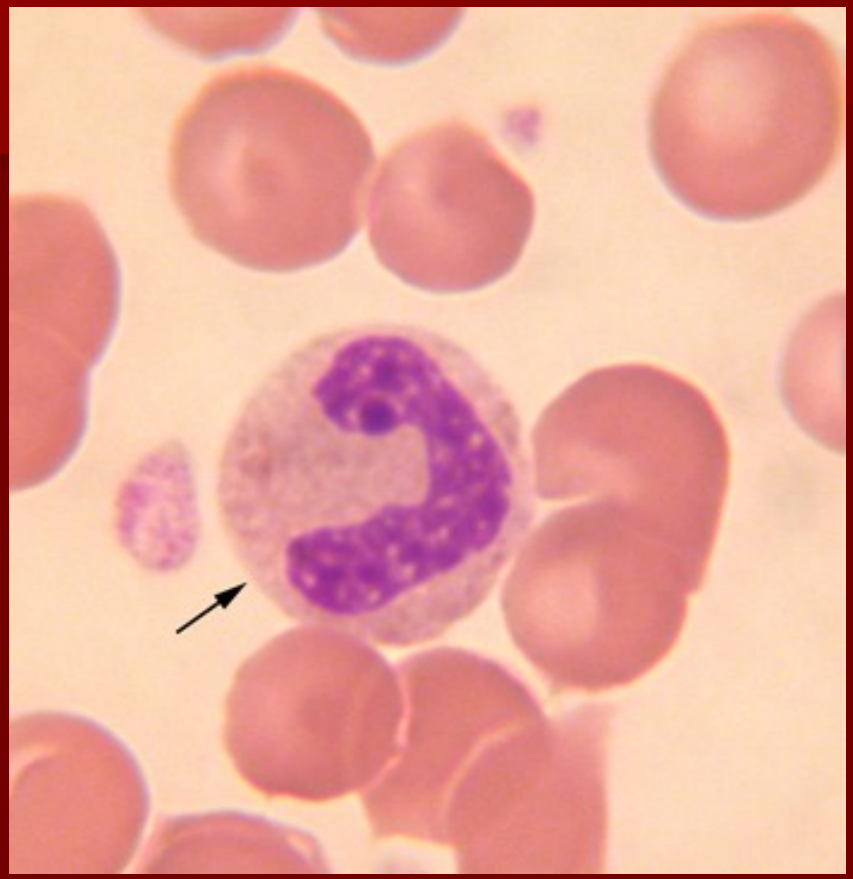
- Total number of leukocytes: normal values

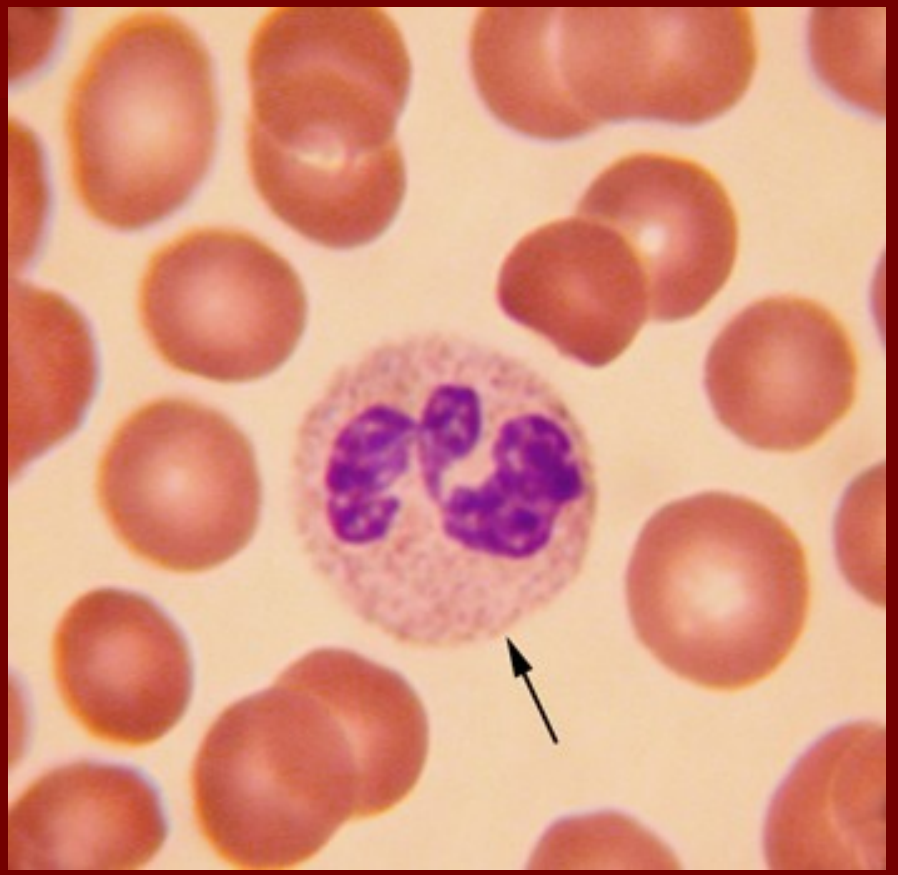
Neutrophils - bands	4 %
- segments	68 %
Eosinophils	3 %
Basophils	1 %
Lymphocytes	20 %
Monocytes	4 %
	Σ = 100 %

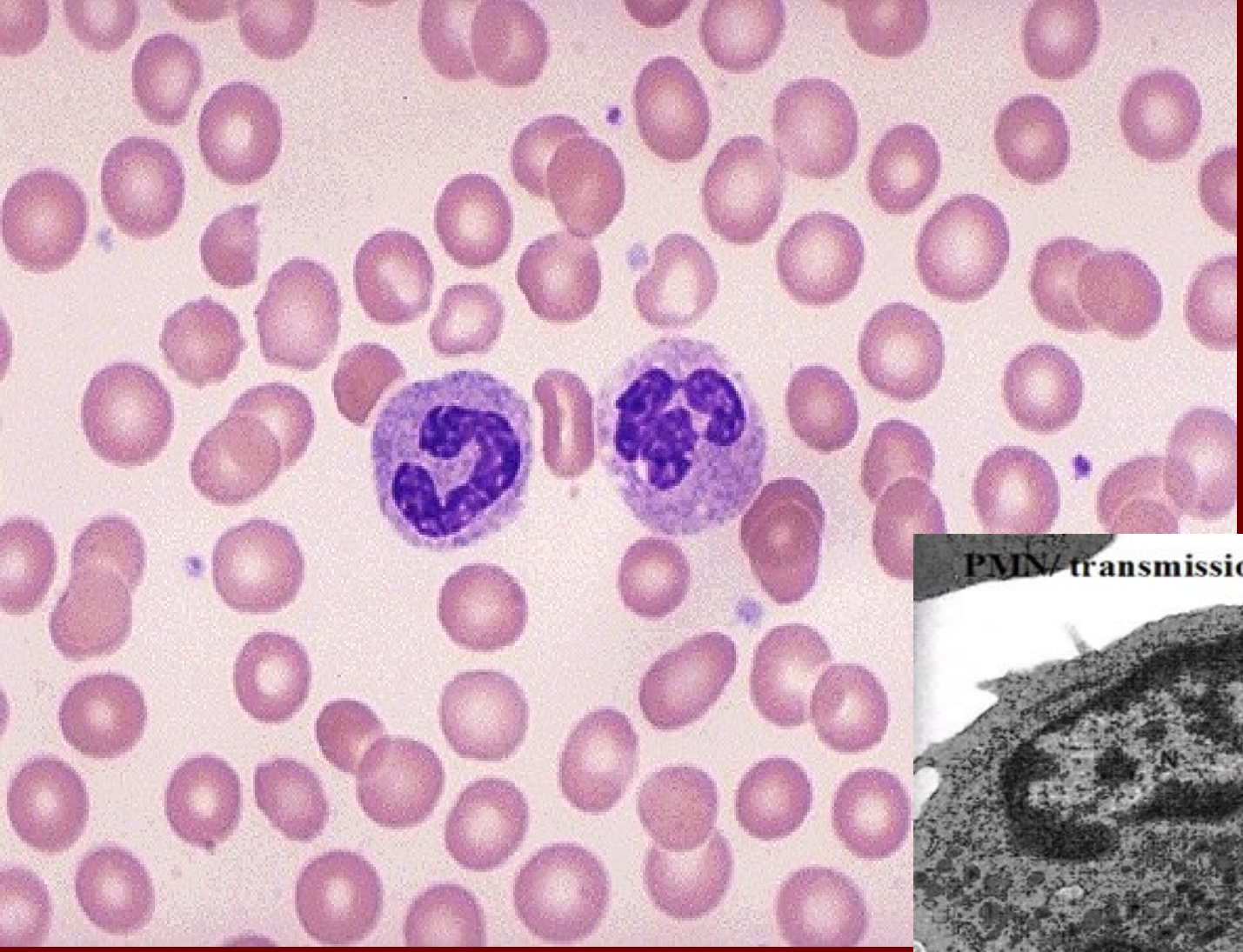
Neutrophil granulocytes (neutrophils)

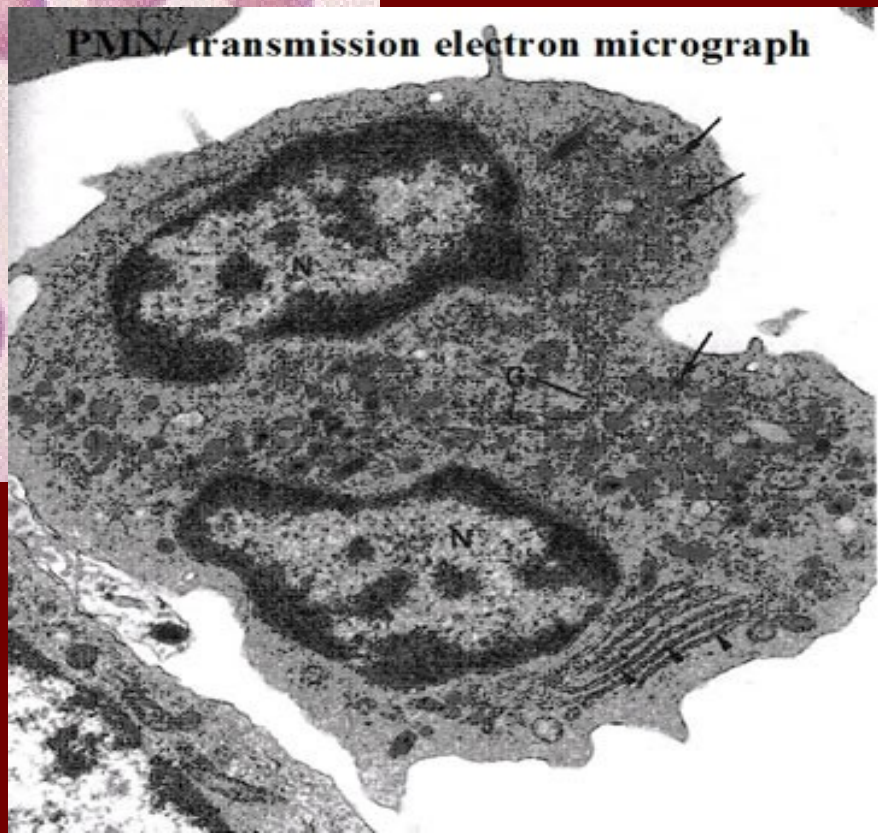
- 71 % of all white blood cells (DWCC)
- 10 – 12 µm
- Cytoplasm: bright pink (eosinophilic = acidophilic)
- Specific granules: neutrophilic (0.3 µm)
  (enzymes:alcaline phosphatase, kolagenase, lysozyme, …)
- Nucleus:
  band-shaped (4 %)
  or segmented (67 %)
  (*2-5 segments*)










PMN/ transmission electron micrograph
N
G
N

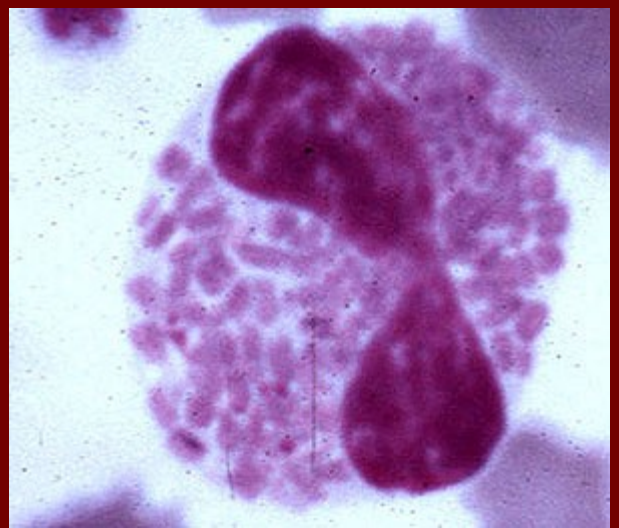
Functions of Neu

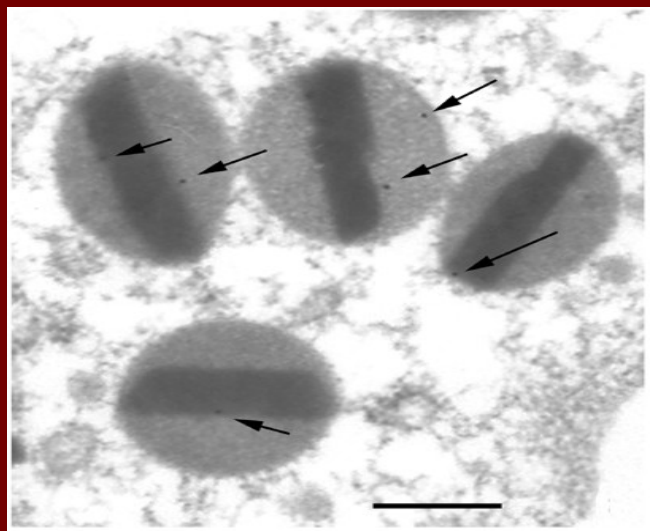
- a central role in inflammatory processes – Neu invade, by diapedesis from the vessels into sites of infection and release some factors (e.g. cytokines)
- cell membrane receptors allow Neu to recognise foreign bodies (bacteria, tissue debris), which they begin to phagocytose and destroy.

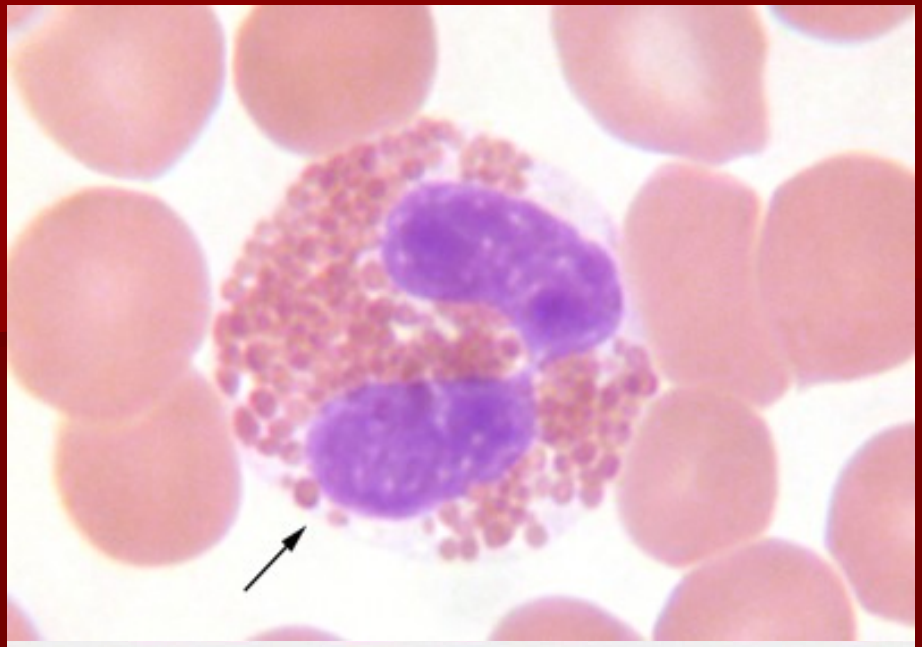
The Neu die once their supply of granules has been exhausted. Their lifespan is only about one week. Dead neutrophils and tissue debris are the major components of pus.

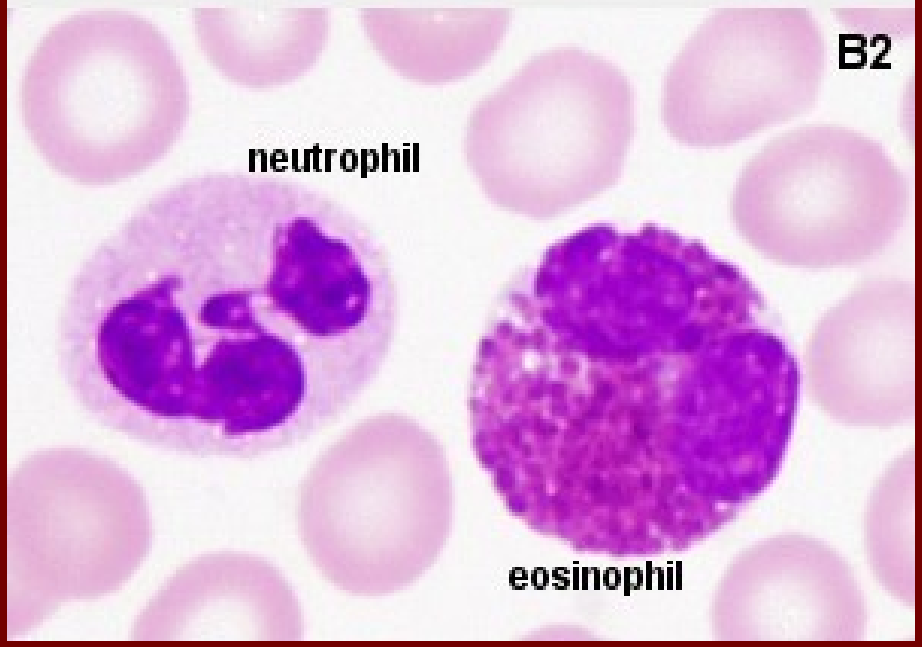
Eosinophil granulocytes (eosinophils)

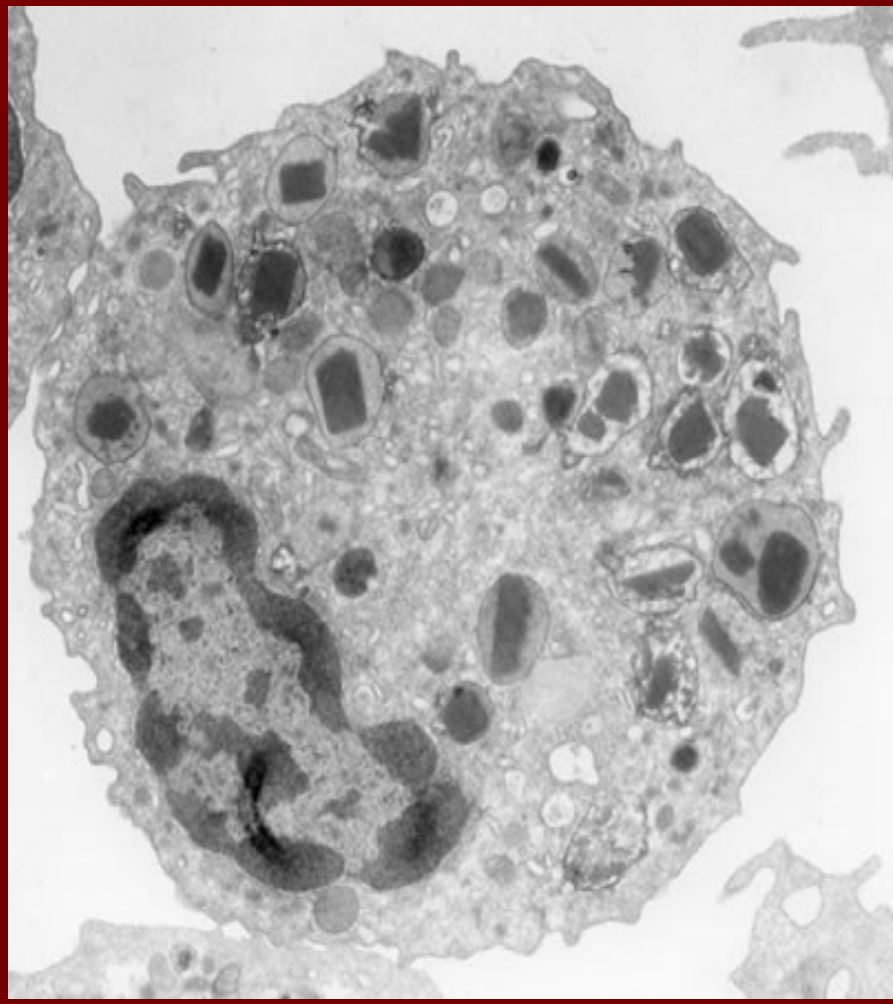
- 1– 4 % of all white blood cells (DWCC)
- 12 – 14 μm
- Cytoplasm: bright pink (eosinophilic = acidophilic)
- Specific granules: eosinophilic (0.5 – 1 μm)
  (enzymes: acid phosphatase, peroxidase,histaminase, arylsufatase …)
- Nucleus: dumb-belt, (2 segments)
  chromatin bridge








B2
neutrophil
eosinophil

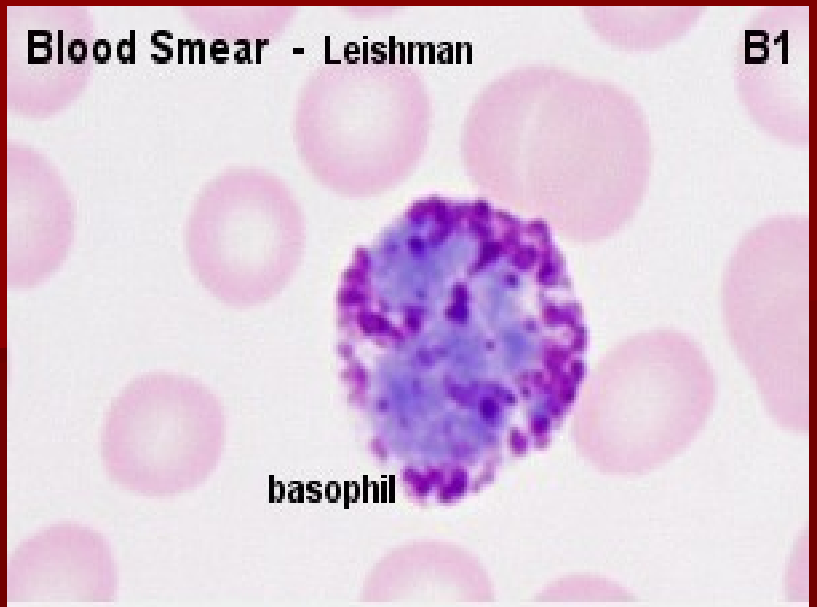


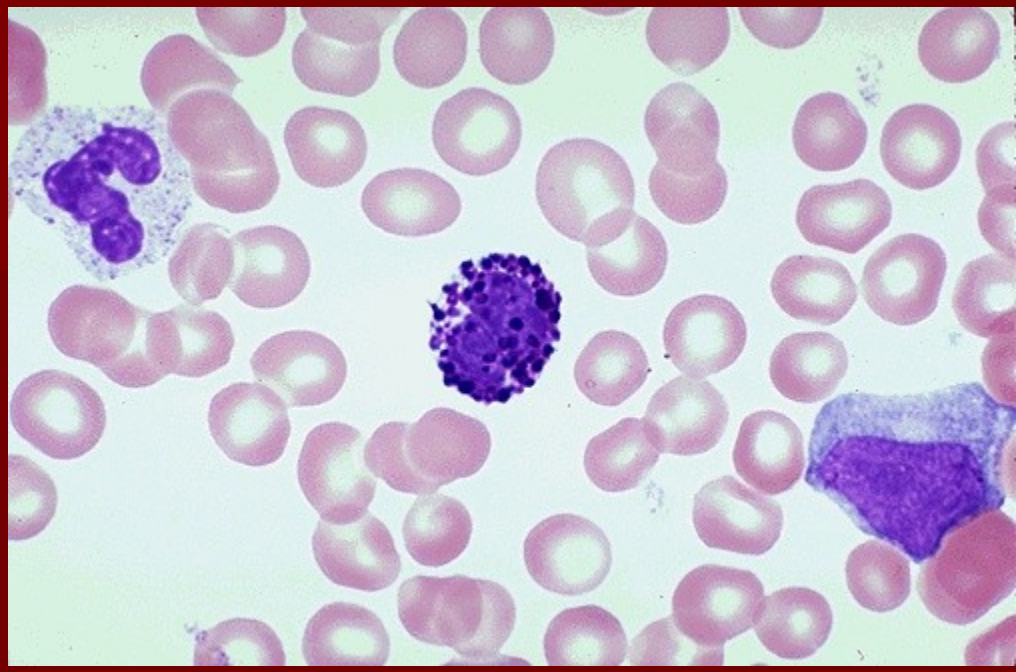
Functions of Eos

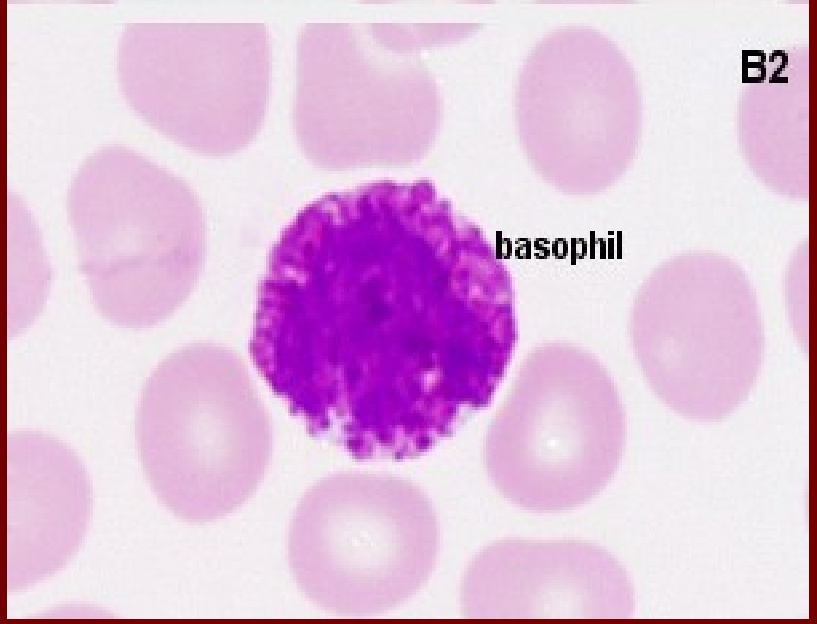
- phagocytosis of antibody-antigen complexes and prevention of the immune system from "overreacting„
- eos are involved in the response of the body against parasitic infections, which are accompanied by an increase in the number of eosinophils.

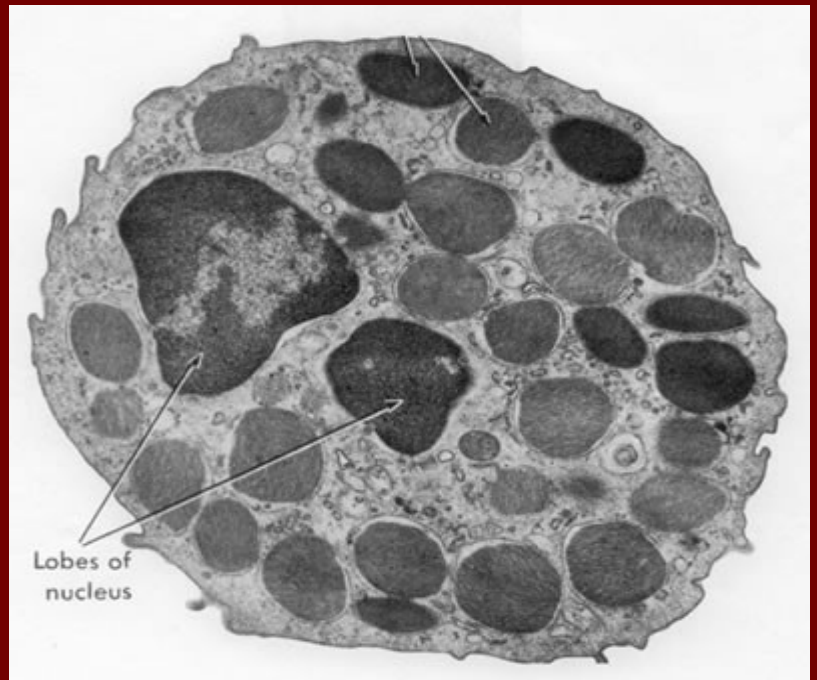
Basophil granulocytes (basophils)

- up to 1 % of all white blood cells (DWCC)
- [redacted]p to 10 [redacted]m
- Cytoplasm: bright violet-pink (lightly basophilic)
- Specific granules: ([redacted]m)
  (heparin, histamin, …)
- Nucleus:
  „shape of dick S"


Blood Smear - Leishman
B1
basophil




B2
basophil


Lobes of
nucleus

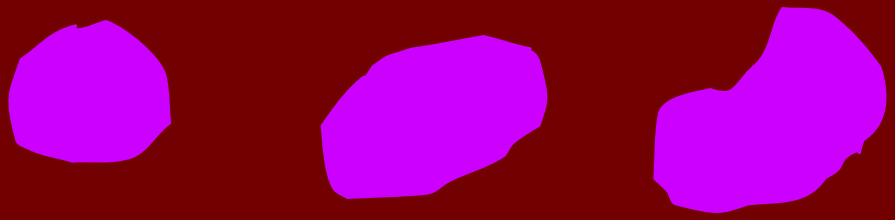
Functions of Baso

- heparin and histamine are vasoactive substances. They dilate the blood vessels, make vessel walls more permeable and prevent blood coagulation. They facilitate the access of heparinocyte in a site of infection.
- antibodies produced by plasma cells (activated B-lymphocytes) bind to the receptors on the plasma membrane of basophils. If these antibodies come into contact with antigens, they induce the release of the contents of the basophil granules.

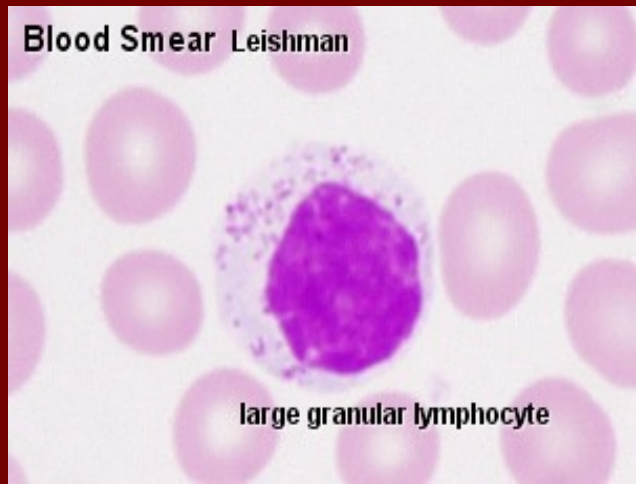
Agranulocytes

- General charcteristic:

- mononuclears – shape of nuclei is spherical (*in Ly*), oval or bean-shaped (*in Mono*)



- basophilic cytoplasm – blue
- NO specific granules
- azurophilic granules – with lysosomal enzymes



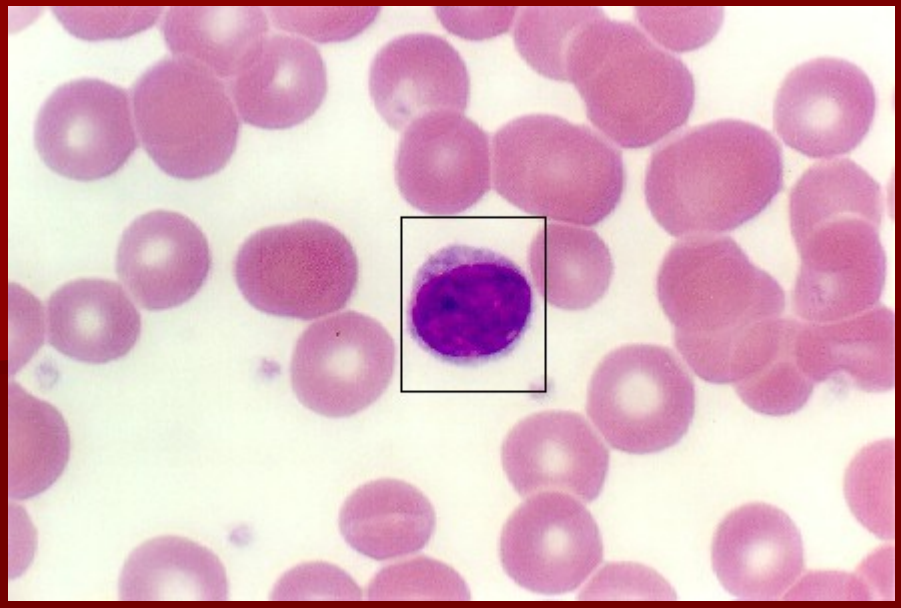
LYMPHOCYTES

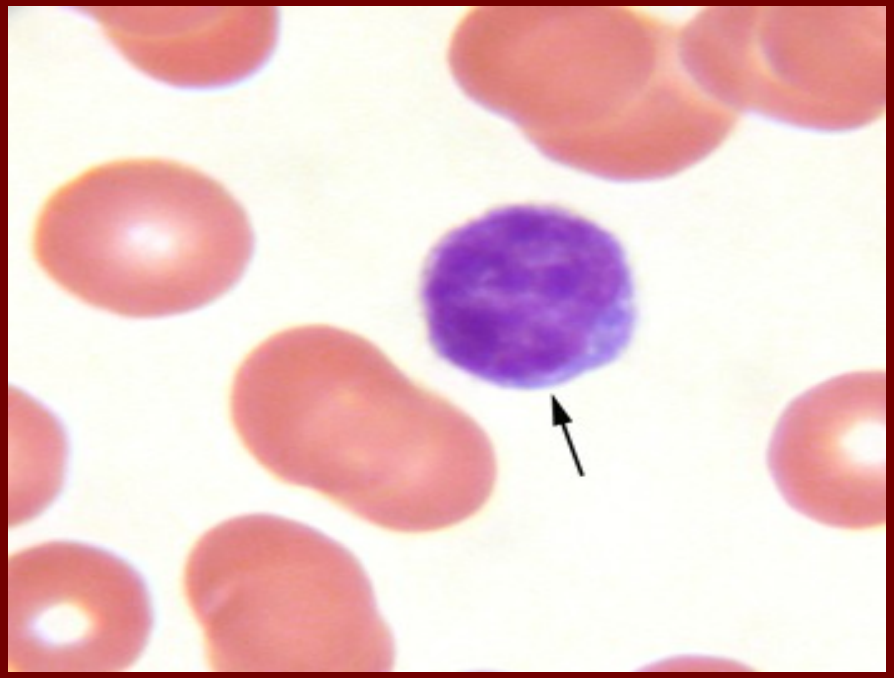
- Classification:
  - according to origin – T-Ly (*thymus*), B-Ly (bone marrow – bursa of Fabricius in birds)
  - according to the size – small (∅ 8 μm), medium (∅ 10-12 μm), large (∅ 16-18 μm),
  - according to the function – natural killer cells, helper cells, memmory cells, supressor cells,
  - according to life-span (long, short)

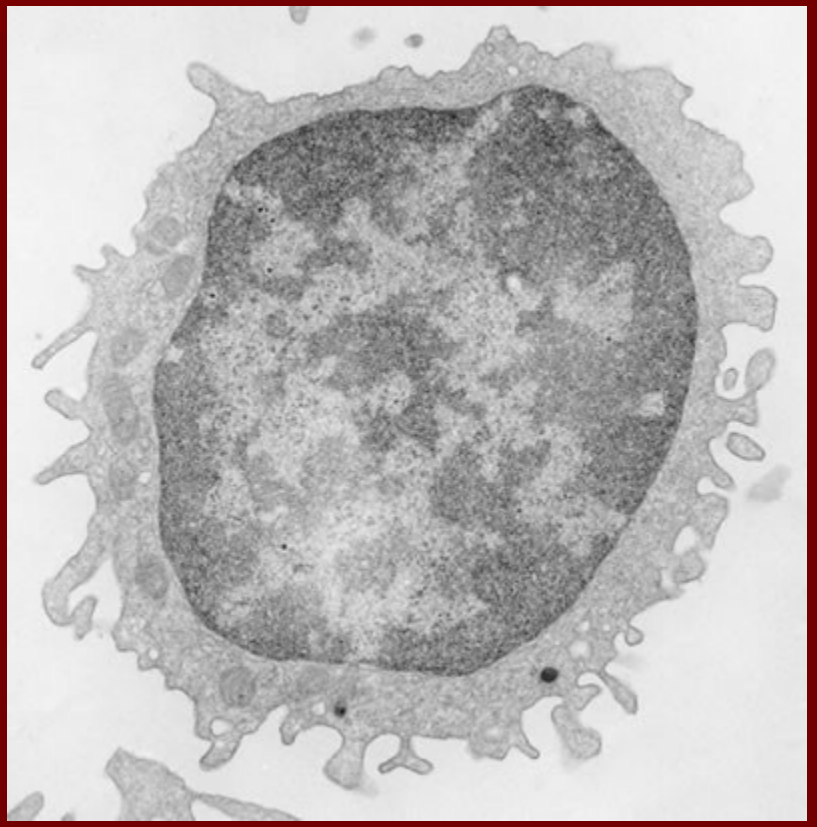
Lymphocytes - structure

- 20 % of all white blood cells (DWCC)

- small, medium-sized, large Ly
- cytoplasm – dark blue, contains non-specific azurophilic granules with lysosomal enzymes (hydrolases) and numerous ribosomes
- nucleus – round, hyperchromatic – coarse grains of heterochromatin (*dark violet colour*)





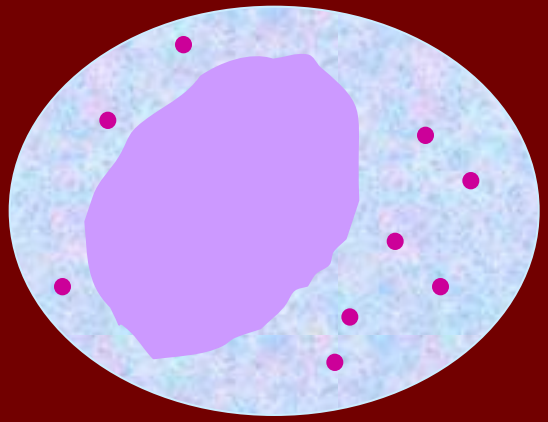


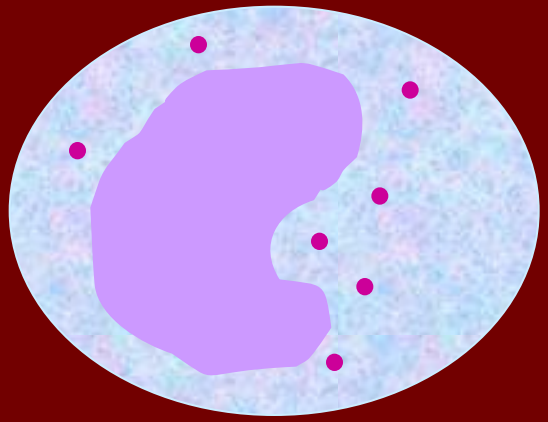
Functions of Ly

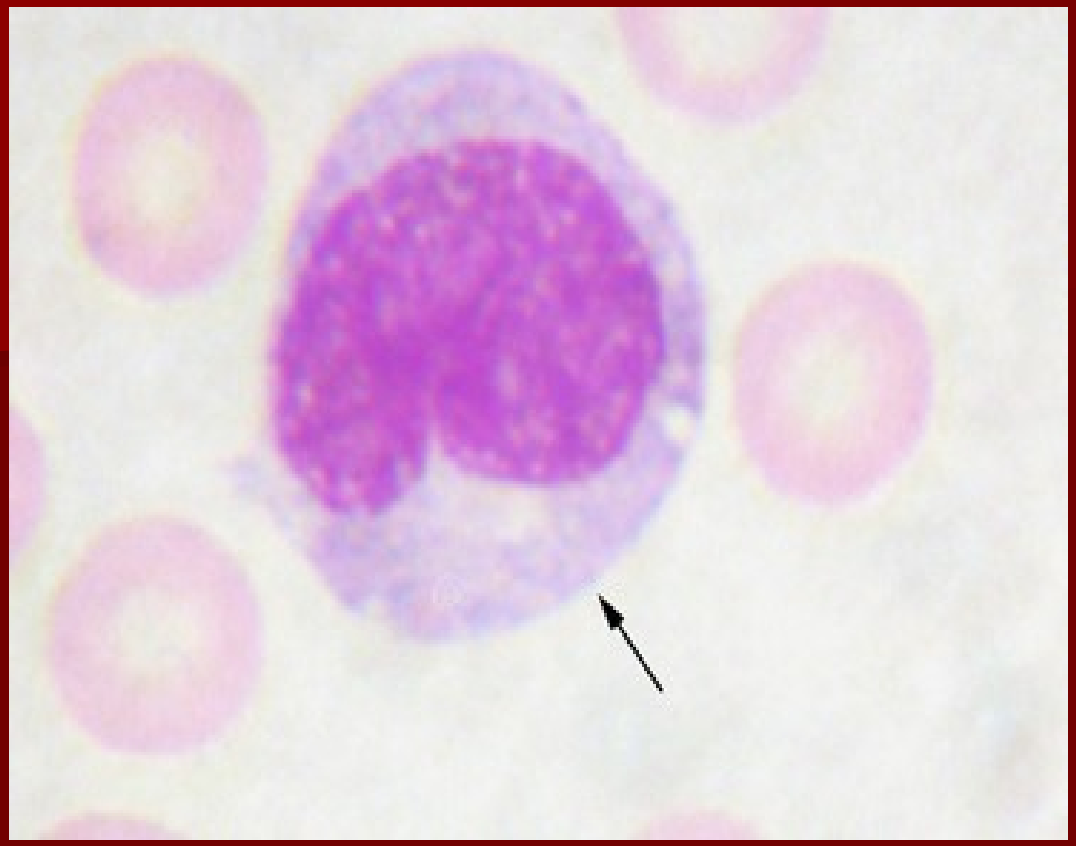
- B-lymphocytes differentiate into antibody producing plasma cells and so they represent "humoral immunity"
- T-lymphocytes represent the "cellular immunity" and may attack foreign cells, cancer cells and cells infected by e.g. a virus

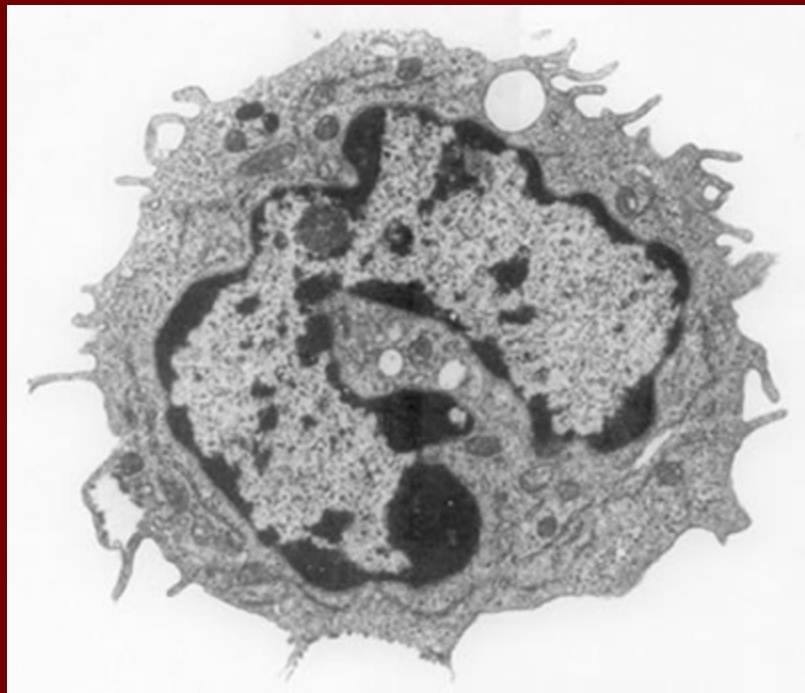
MONOCYTES

- 5 % (DWC), [redacted]5 – 20 [redacted]m
- cytoplasm – voluminous, bright blue, contains non-specific azurophilic granules with lysosomal enzymes (hydrolases) and numerous ribosomes
- nucleus – oval to bean-shaped, finely dispersed chromatin









Functions of Mono

- *monocytes enter the connective tissue they differentiate into macrophages*. At sites of infection macrophages are the dominant cell type after the death of the invading neutrophils.
- macrophages phagocyte microorganisms, tissue debris and the dead neutrophils.

- *mono also give rise to osteoclasts, which are able to distroy bone. They are of importance in bone remodelling.*

THROMBOCYTES (blood platelets)

- 150,000 – 300,000 / 1 of blood
- thrombocytosis X thrombocytopenia
- - are not cells, but cytoplasmic fragments of large cell (megakaryocyte) in bone marrow
- shape: spindle-shaped discoid plate
- size: 2 – 4
- cytoplasm – basophilic (bright violet-blue), contains microtubules and α,δ and λ granules:

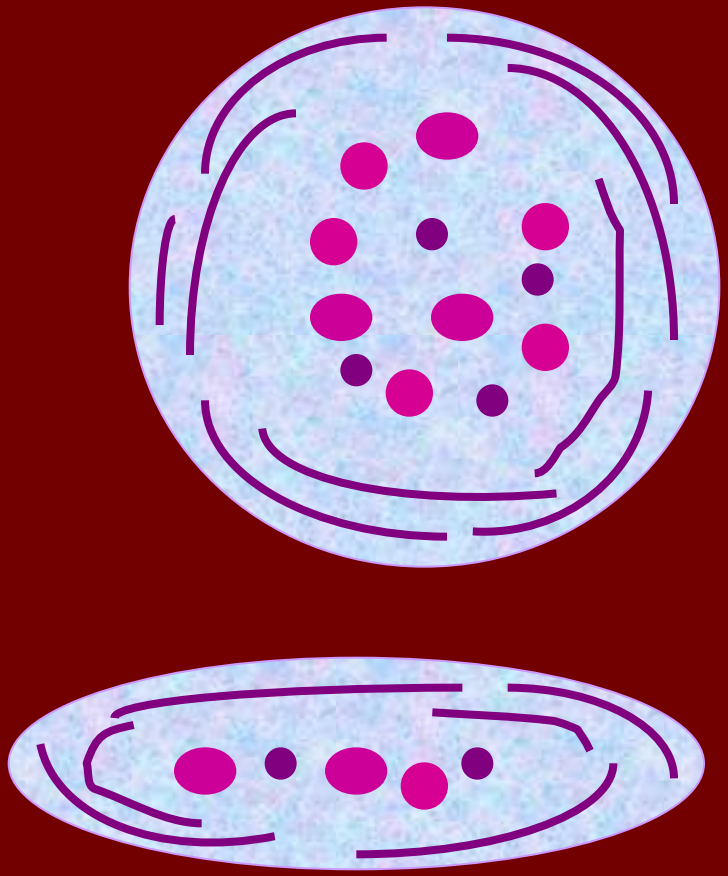
  alpha granules – fibrinogen, …

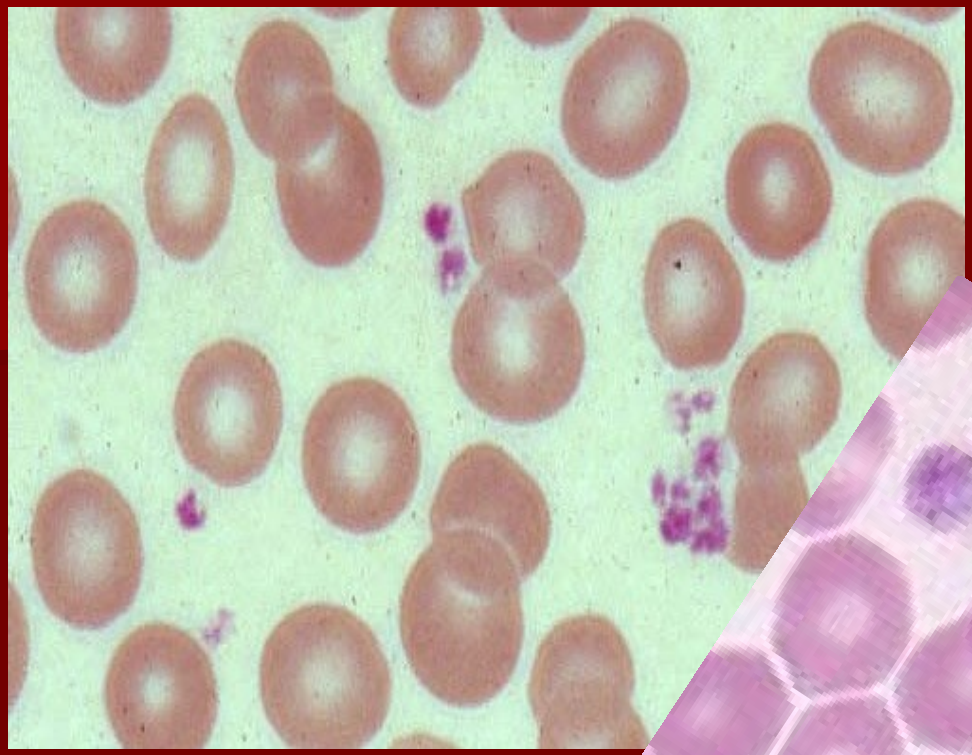
  delta granules – serotonin, Ca ions, ATP and ADP,…

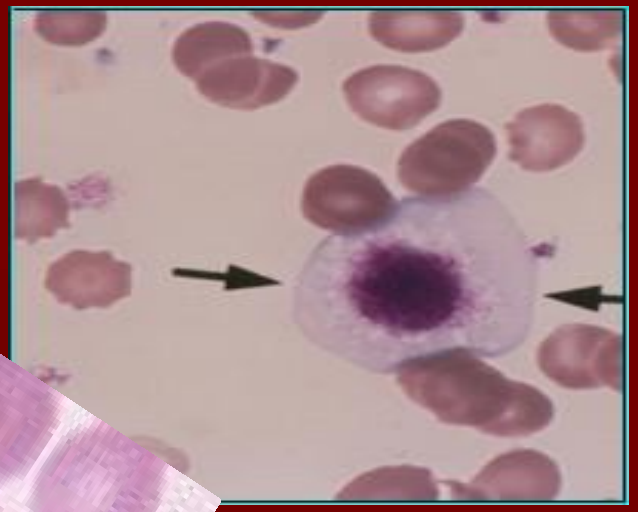
  lambda granules – are small lysosomes

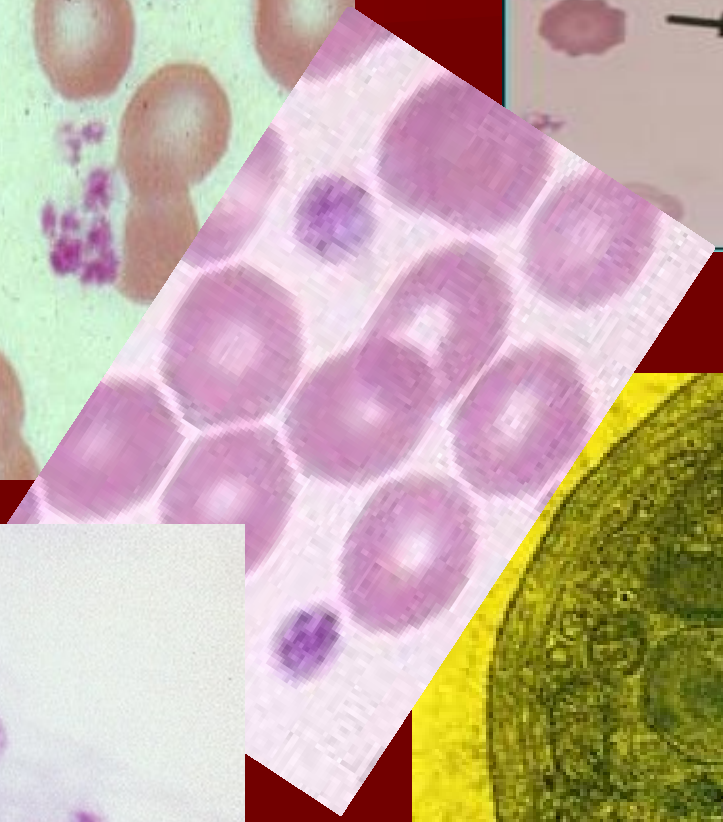
Platelet structure

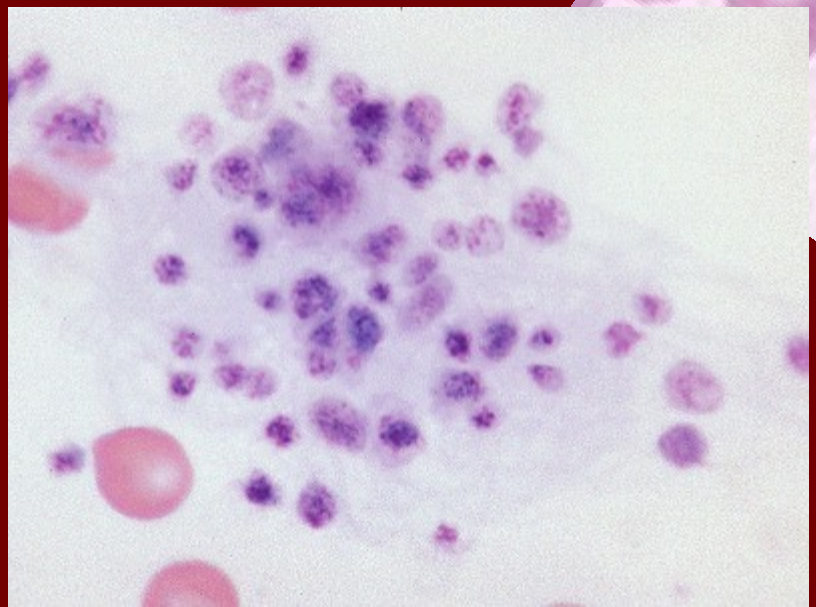
- Hyaloplasm contains microtubules (on the periphery of platelet)
- Granuloplasm contains granules

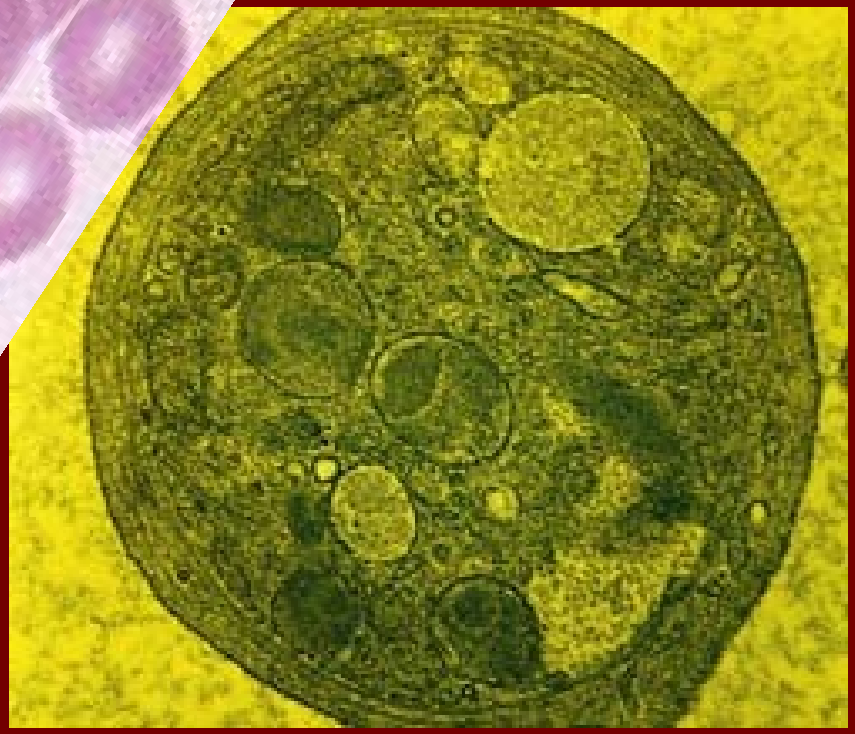










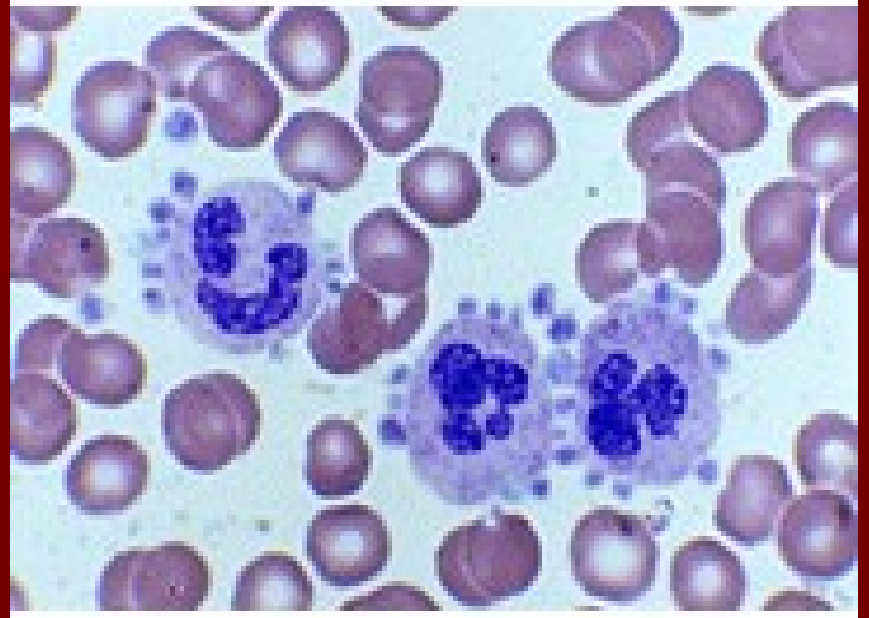


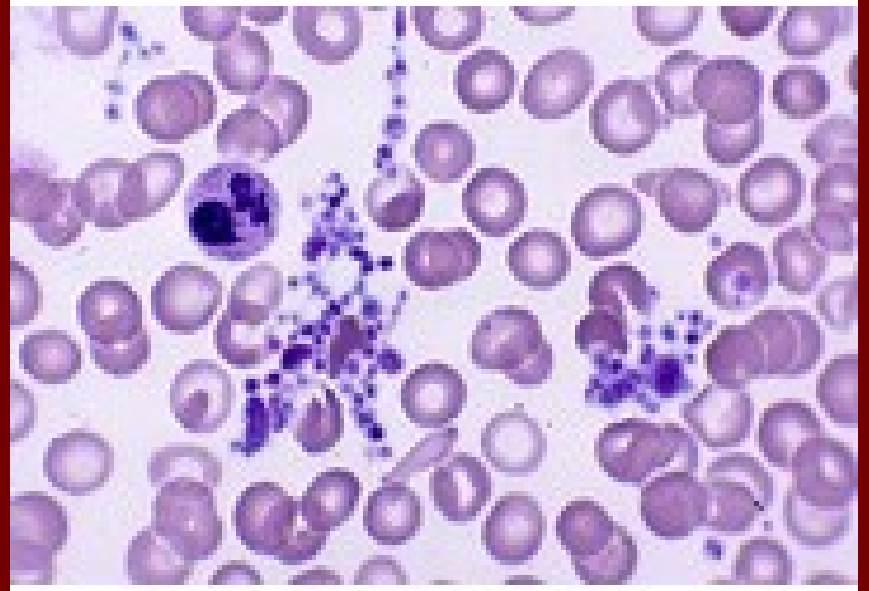
Blood Smear - Leishman B1

blood platelet

B2

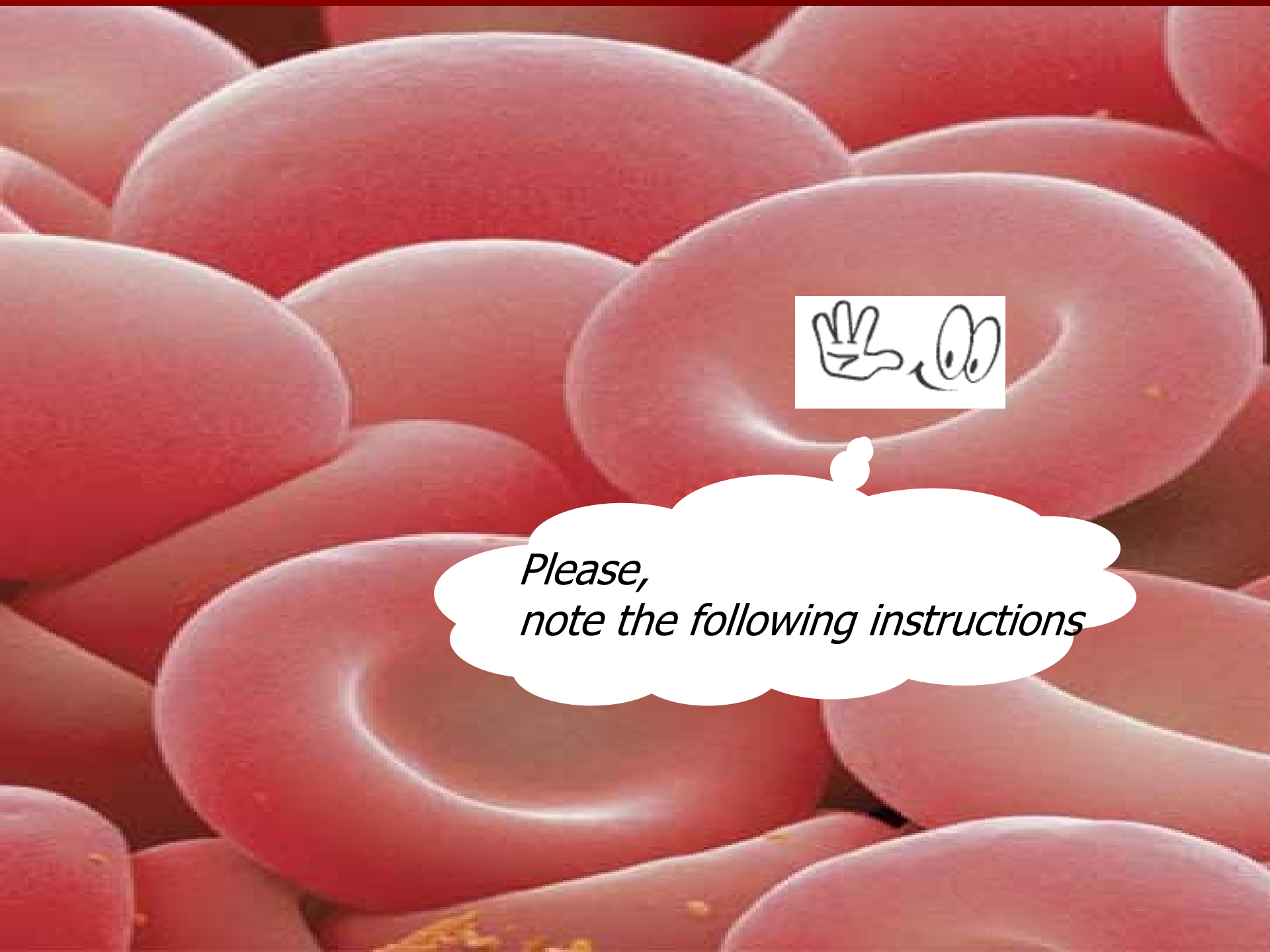
blood platelet



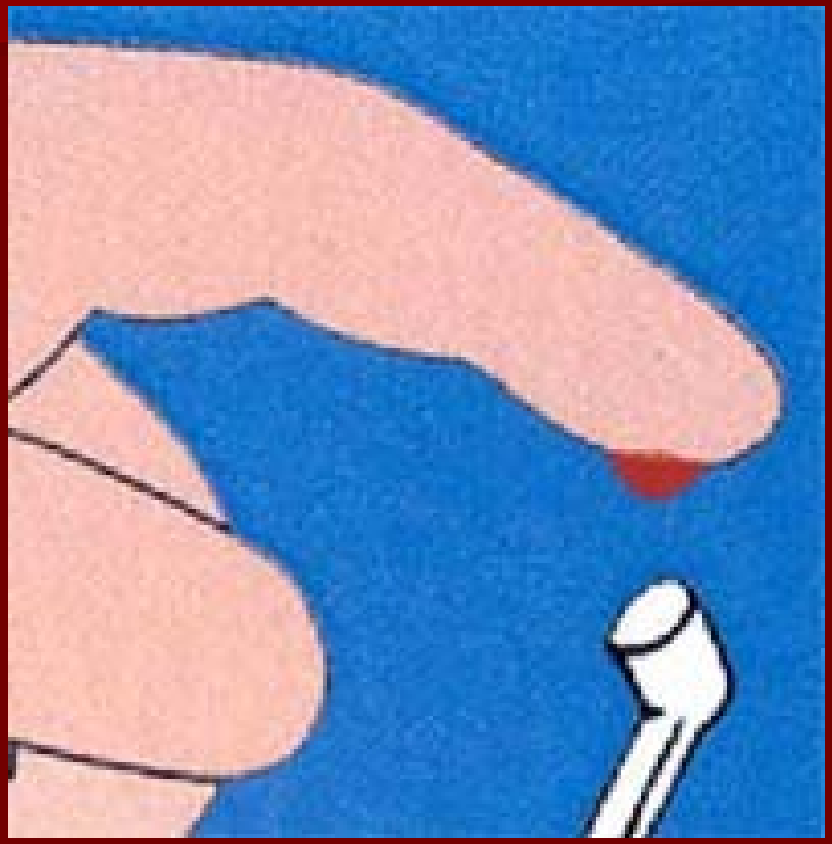


Functions of thrombocytes

- Platelets assist in ***haemostasis***, *the arrest of bleeding*.
- Serotonin is a vasoconstrictor. Its release from thrombocytes, adhering to the walls of a damaged vessels, is sufficient to close even small arteries. Platelets, which come into contact with collagenous fibers in the walls of the vessel, swell, become "sticky" and activate other platelets to undergo the same transformation. This cascade of events results in the formation of a *platelet plug (or platelet thrombus)*. Finally, activating substances are released from the damaged vessel walls and from the platelets. These substances mediate the conversion of the plasma protein *prothrombin* into *thrombin*. *Thrombin catalyzes the conversion of fibrinogen into fibrin*, which polymerizes into fibrils and forms a fibrous net in the arising blood clot. Platelets captured in the fibrin net contract leading to *clot retraction*, which further assists in haemostasis.

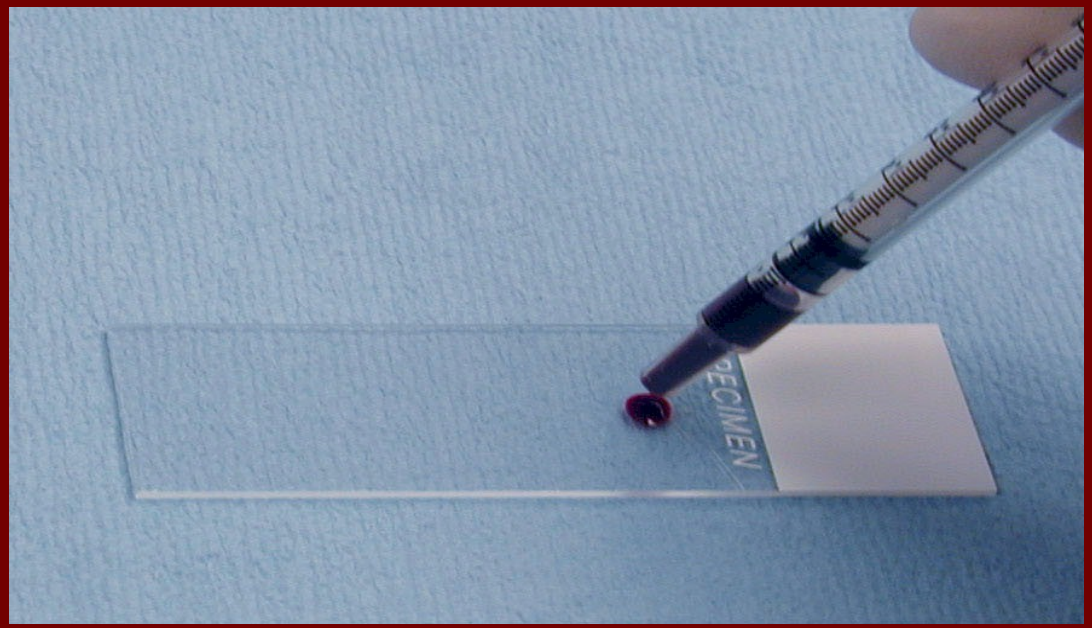

Please,
note the following instructions

How to prepare blood smear?



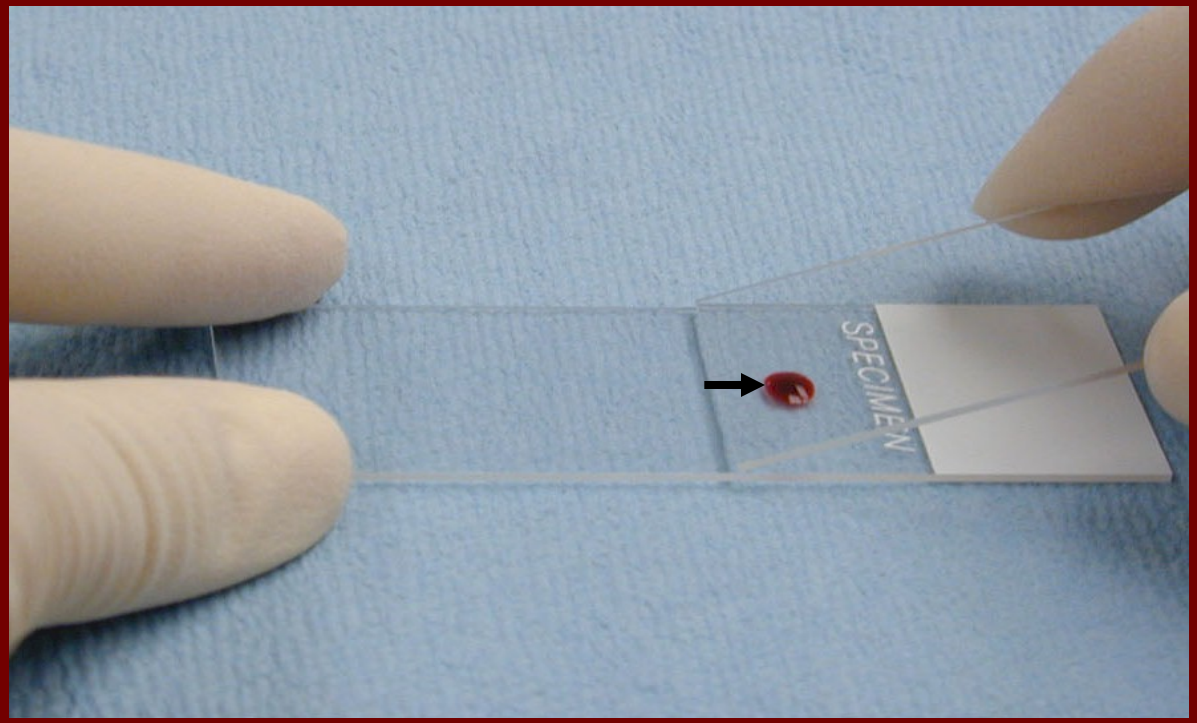
How to prepare blood smear - I

- Smears of peripheral blood must be made immediately.
- Step 1: Place drop of blood about 1cm from the frosted end of a clean slide.



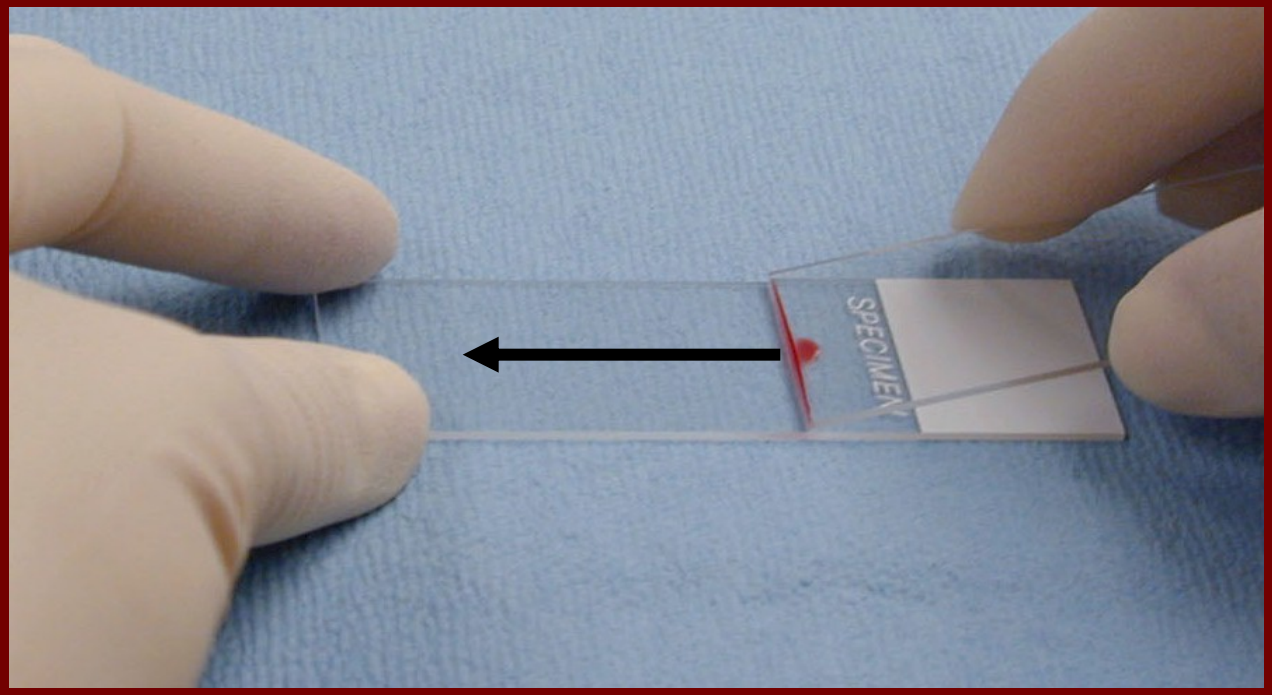
How to prepare blood smear - II

- Step 2: hold the end of a second slide ("spreader") against the surface of the first slide at an angle of 30-45 degrees.



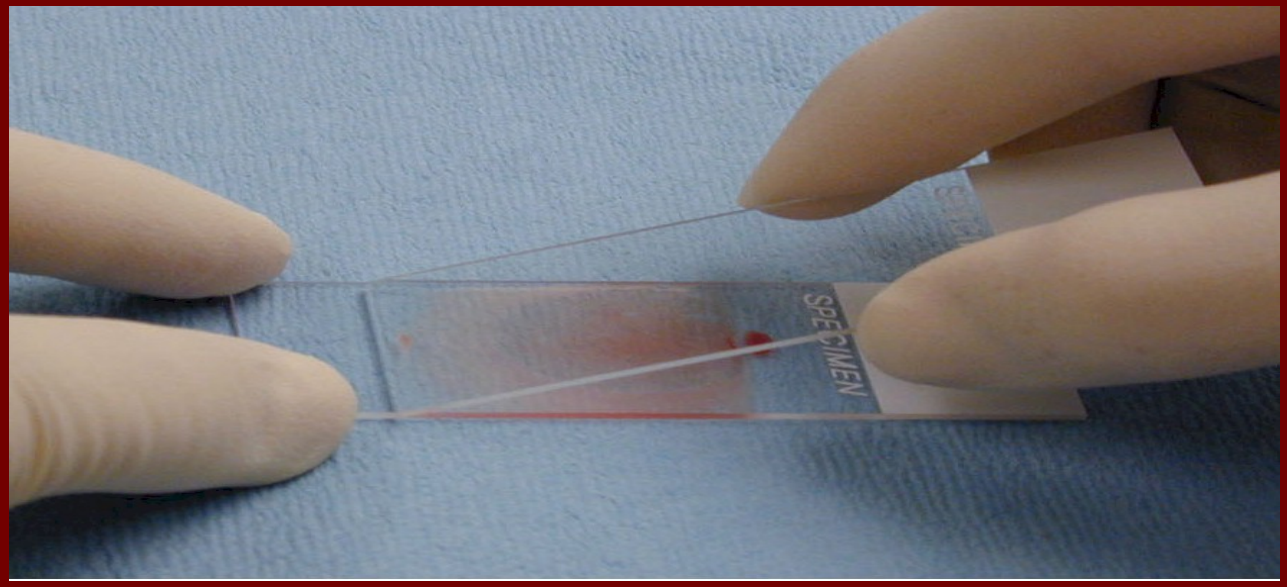
How to prepare blood smear - III

- Step 3: draw it back to contact the drop of blood. Allow the blood to spread and fill the angle between the two slides.



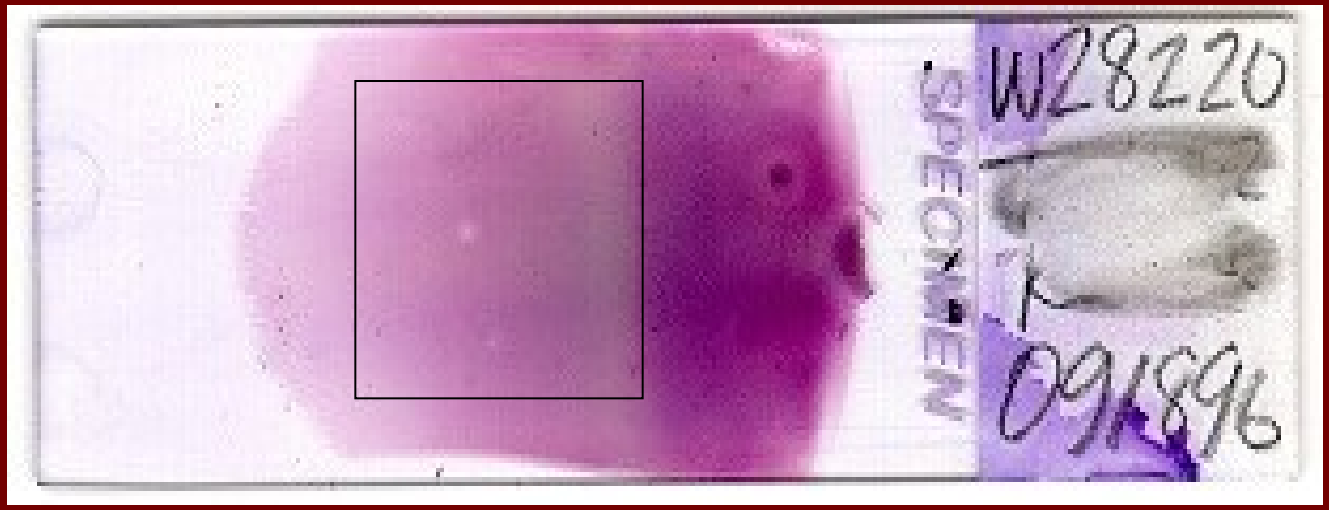
How to prepare blood smear - IV

- Step 4: Push the "spreader" slide at a moderate speed forward until all of the blood has been spread into a moderately thin film.



How to prepare blood smear - V

- Smear is prepared for fixation (methyl alcohol, 3-5 minutes) and staining (special panoptic method according to Pappenheim can be used)

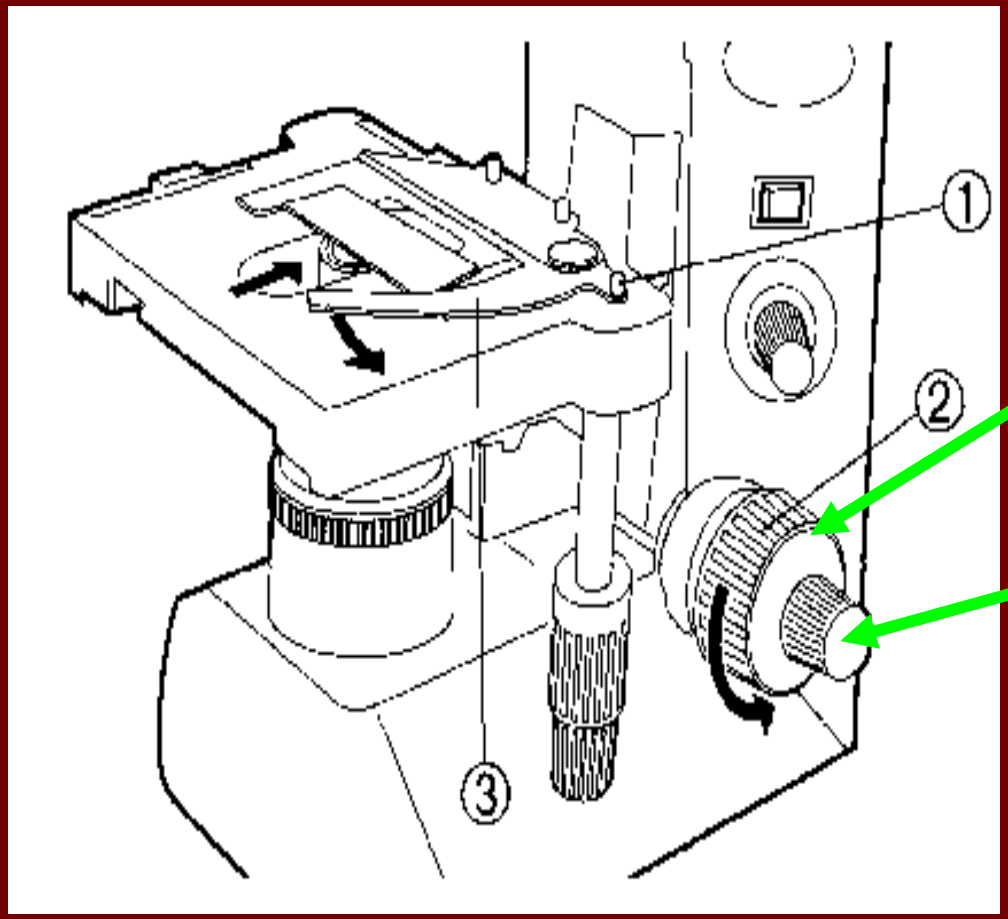


How to study blood smear in the light microscope (LM)?

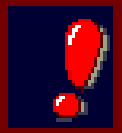
- the lense of immersion objective (magnif. 100x) is immersed into a drop of oil and blood smear is prepared for study in the LM;
- switch on your LM and see into the eypice: blood cells should be visible in the light field
- if not, try to focuse picture – you may use **ONLY** the fine adjustment knob!
- if you are not succesfull, ask for help your teacher, do NOT use the coarse adjustment knob!

Light microscope manipulation

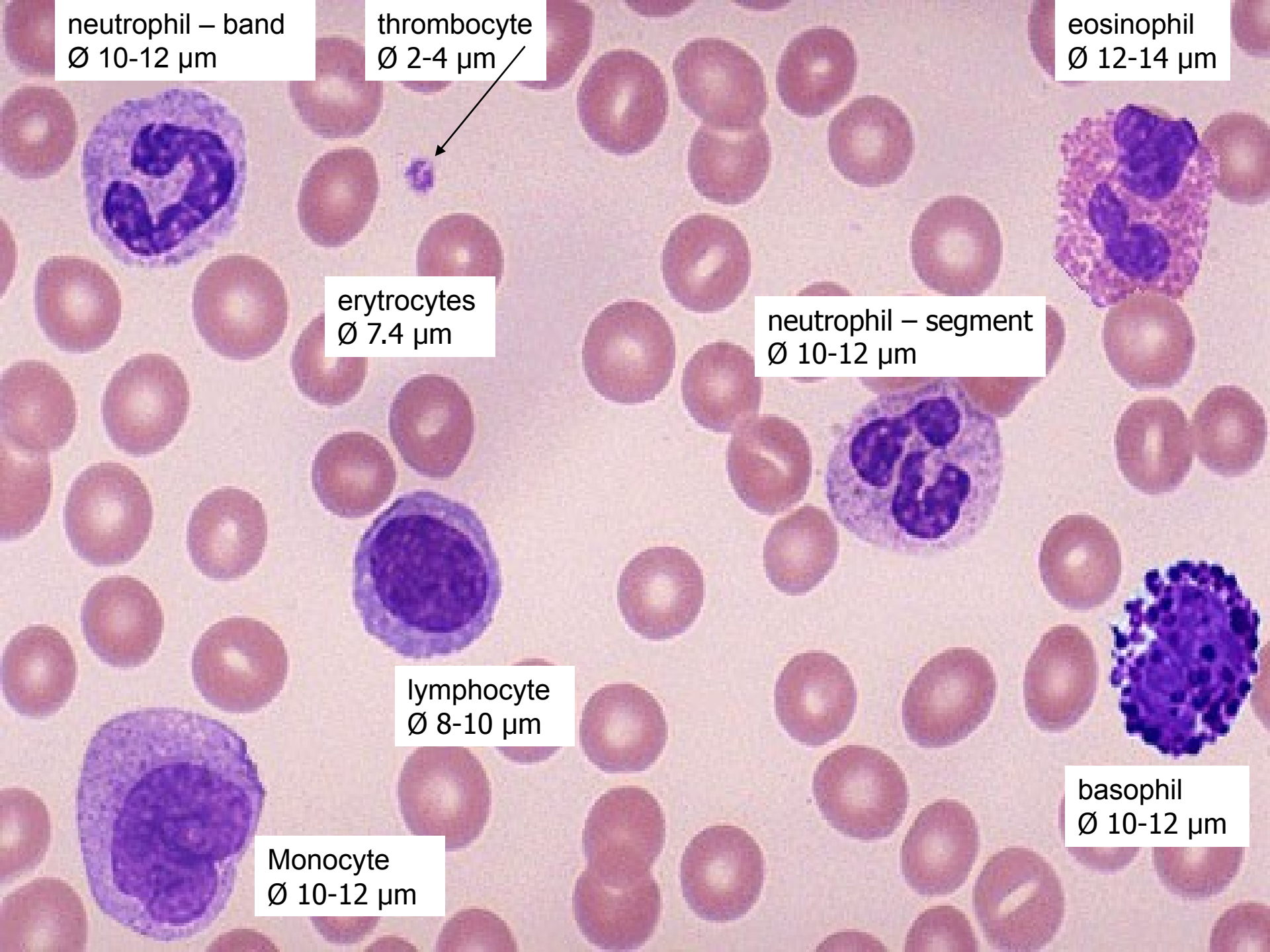
Stage with slide holder (3)
Lever of holder (1)
Focusing knobes (2).



Course adjustment knob
don't use today



Fine adjustment knob
only that can be used
to focuse the image

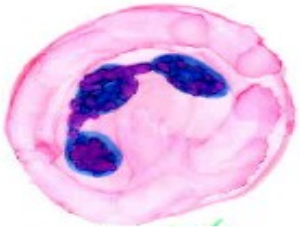

neutrophil – band
Ø 10-12 µm
thrombocyte
Ø 2-4 µm
eosinophil
Ø 12-14 µm
erytrocytes
Ø 7.4 µm
neutrophil – segment
Ø 10-12 µm
lymphocyte
Ø 8-10 µm
basophil
Ø 10-12 µm
Monocyte
Ø 10-12 µm

P R O T O C O L

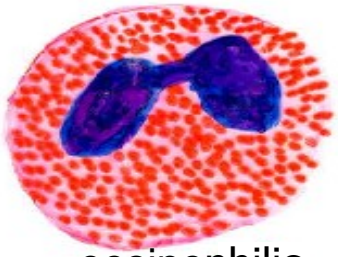
Erythrocytes (Ø 7,4 µr

Thrombocytes (2-4 µm)

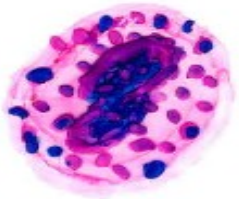
Granulocytes:



neutrophilic
10-12 µm

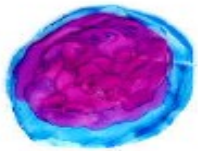


eosinophilic
12-14 µm

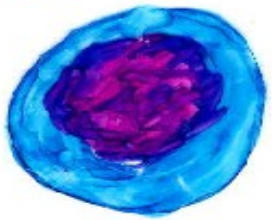


basophilic
10 µm

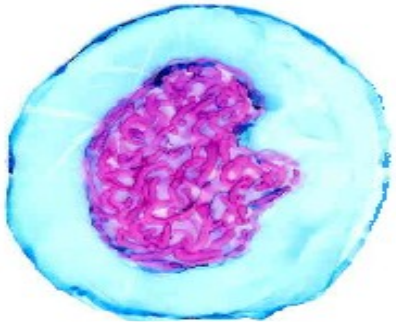
Agranulocytes:



Lymfocytes (8-10 µm)



Monocyte (15-20 µm)

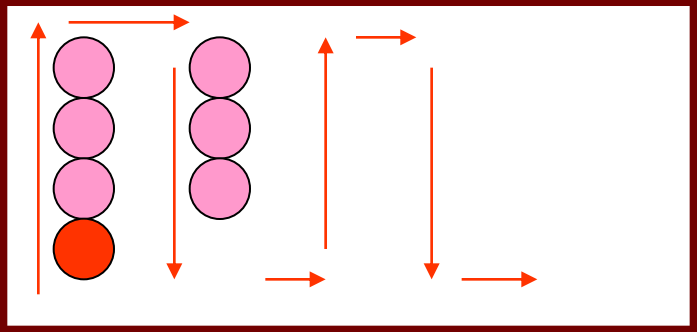


How to count leukocytes in blood smear?

- differential white cell count (DWCC) is an important hematologic screening which helps to diagnose
- leukocytes percentage is the result of this investigation
- 100 white cells must be count and registered in the table prepared for all types of leukocytes (Neu-bands, Neu-segments, Eos, Baso, Ly, Mono)
- arithmetic sum of each type of leukocytes represents their percentage (%)

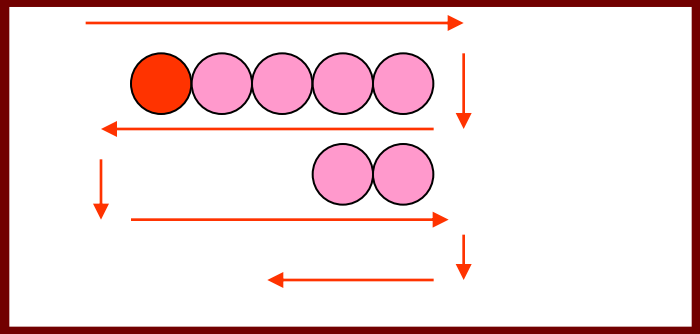
How to count leukocytes in blood smear?

- blood smear have to be systematicaly viewed (it avoids repeatedly count the same cells)



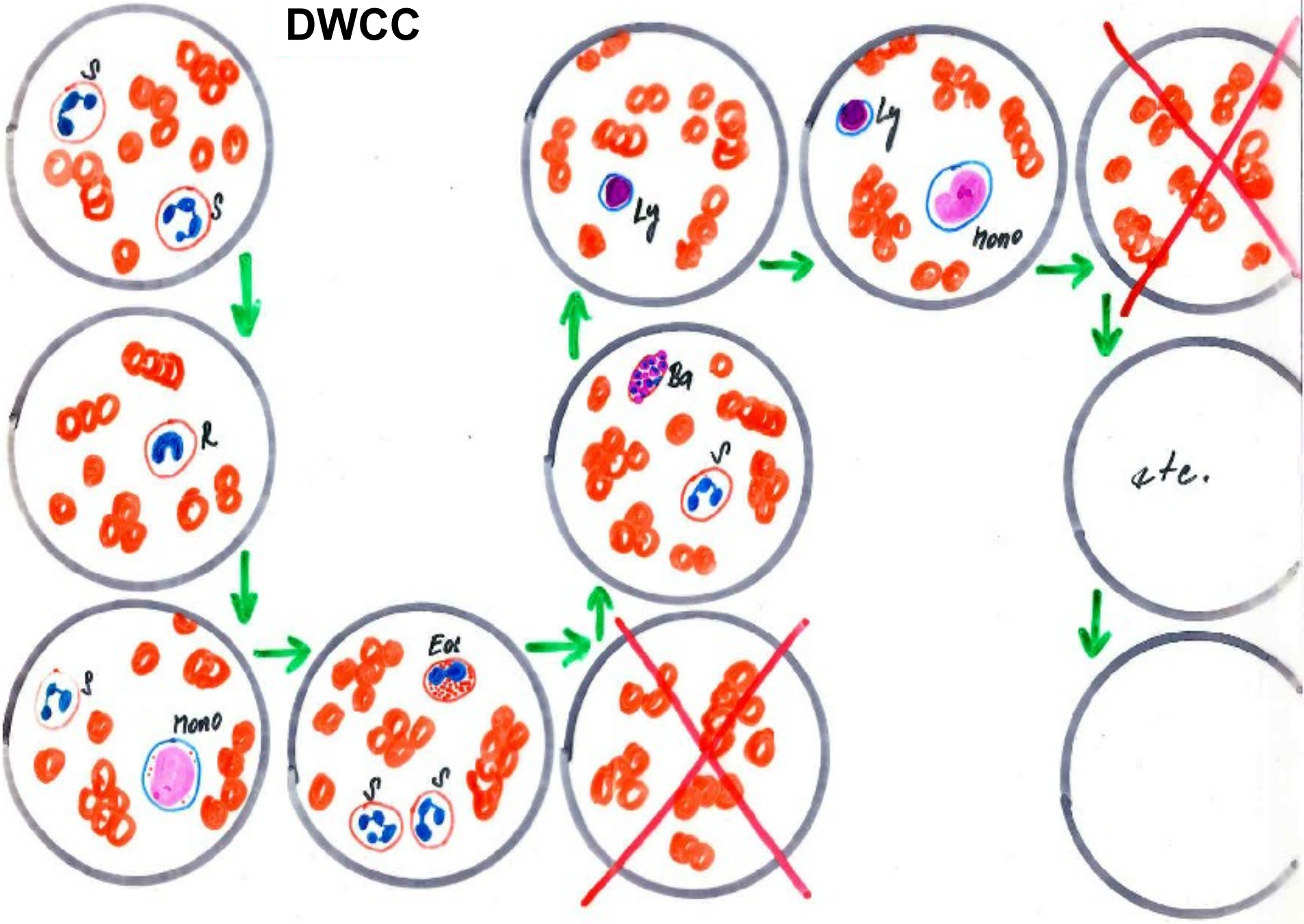
vertical browsing

or



horizontal browsing

DWCC



Table

	1	2
Neu bands	/	
Neu segments	//// //	///
Eos		/
Baso		
Ly	//	////
Mono		//
	10 cells	10 cells

→

9	10	results	norm
//			4 %
//// /	///		68 %
/	//		3 %
	/		1 %
/	////		20 %
			4 %
10 cells	10 cells	100%	100 %

Differential white cell count (DWCC)

- Total number of leukocytes: normal values

Neutrophils - bands	4 %
- segments	68 %
Eosinophils	3 %
Basophils	1 %
Lymphocytes	20 %
Monocytes	4 %
	Σ = 100 %

Anomalies of DWCC

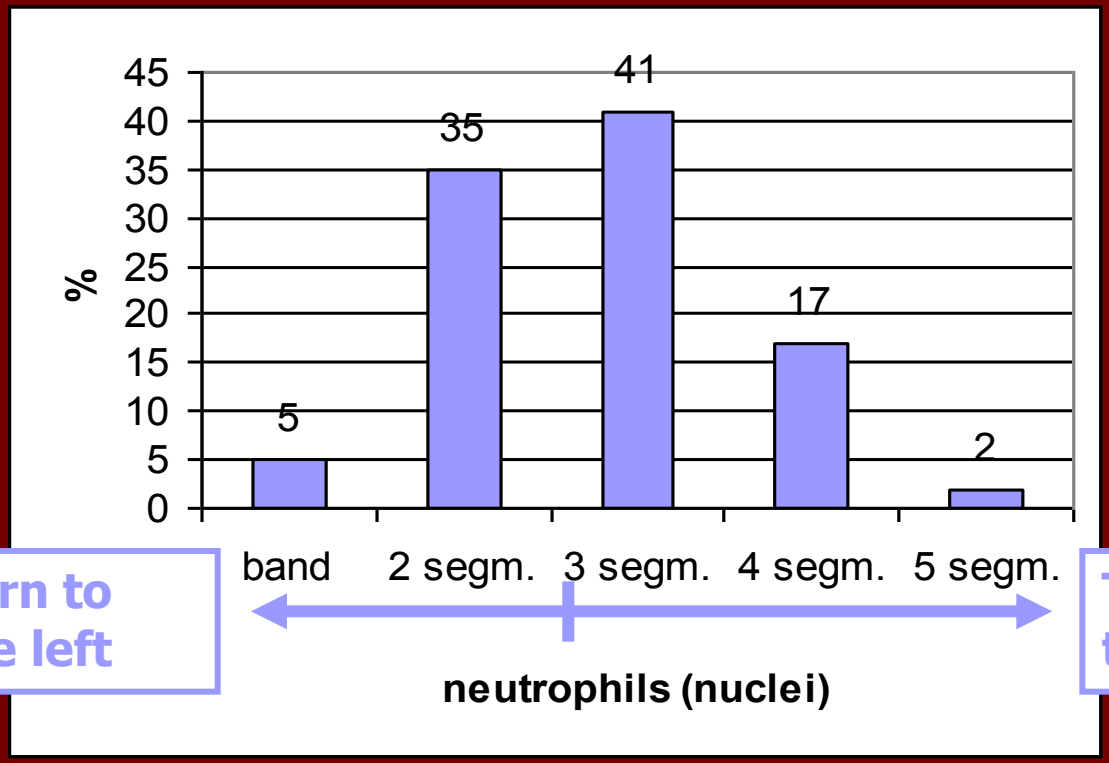
	↑ Increased number	↓ Decreased number
Neutrophils*	neutrophilic granulocytosis	neutrophilic granulocytopenia
Eosinophils	eosinophilic granulocytosis	eosinophilic granulocytopenia
Basophils	basoophilic granulocytosis	basoophilic granulocytopenia
Lymphocytes	lymphocytosis	lymphocytopenia
Monocytes	monocytosis	monocytopenia

* sum total of bands and segments has to be compared with norm; normal value is 71 % (4 % bans + 68 % segments)

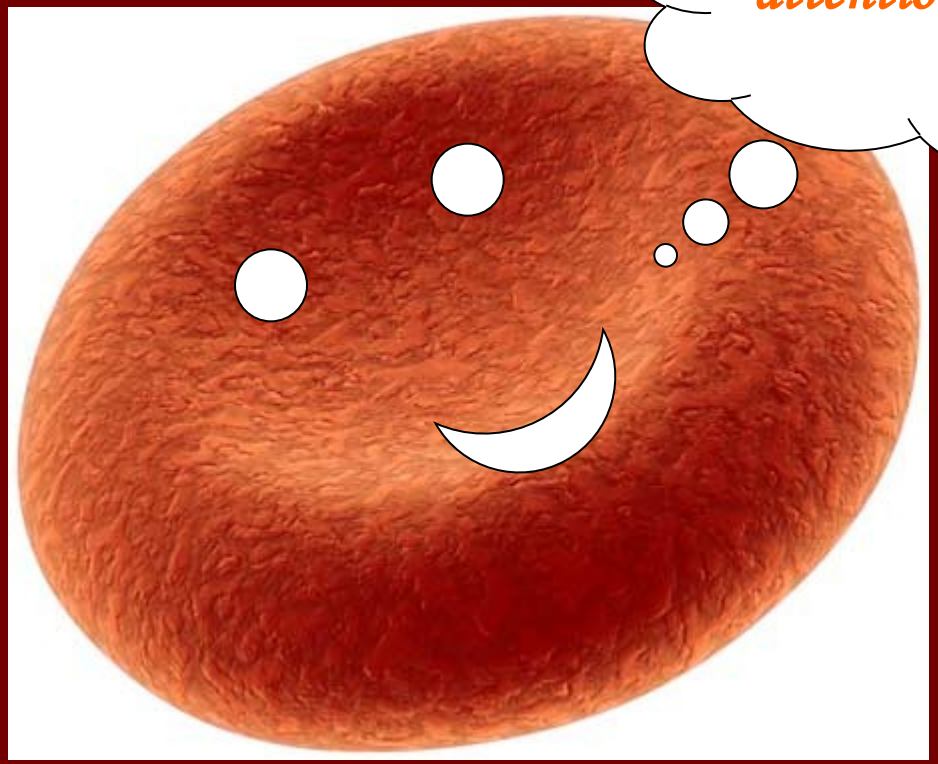
Normal ratio of neutrophil bands and segments

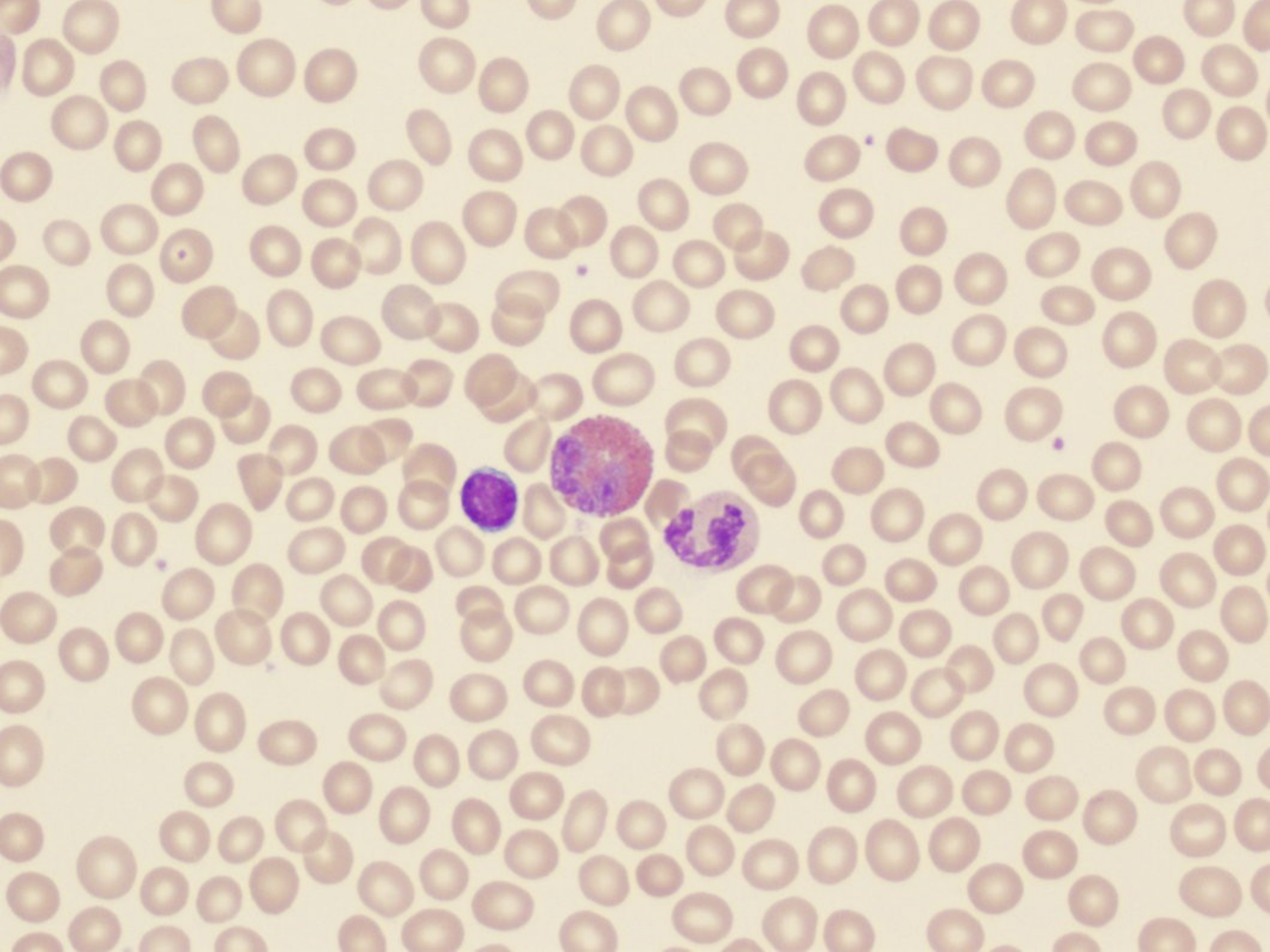
Bands : Segments ratio is 4 % : 68 % = 1 : 17

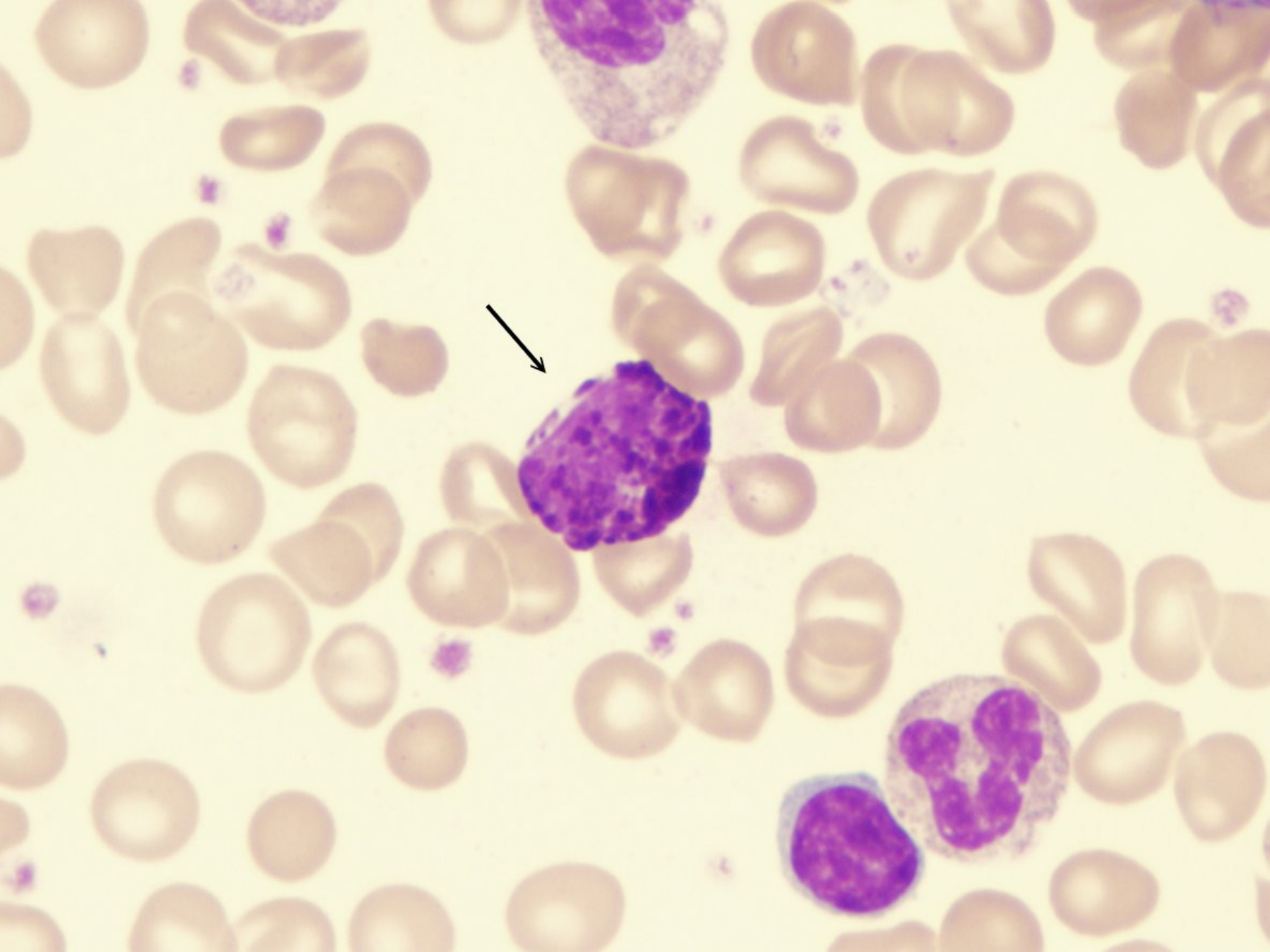
- **Turn to the left** – bands are increased
- **Turn to the right** – segments are increased in peripheral blood

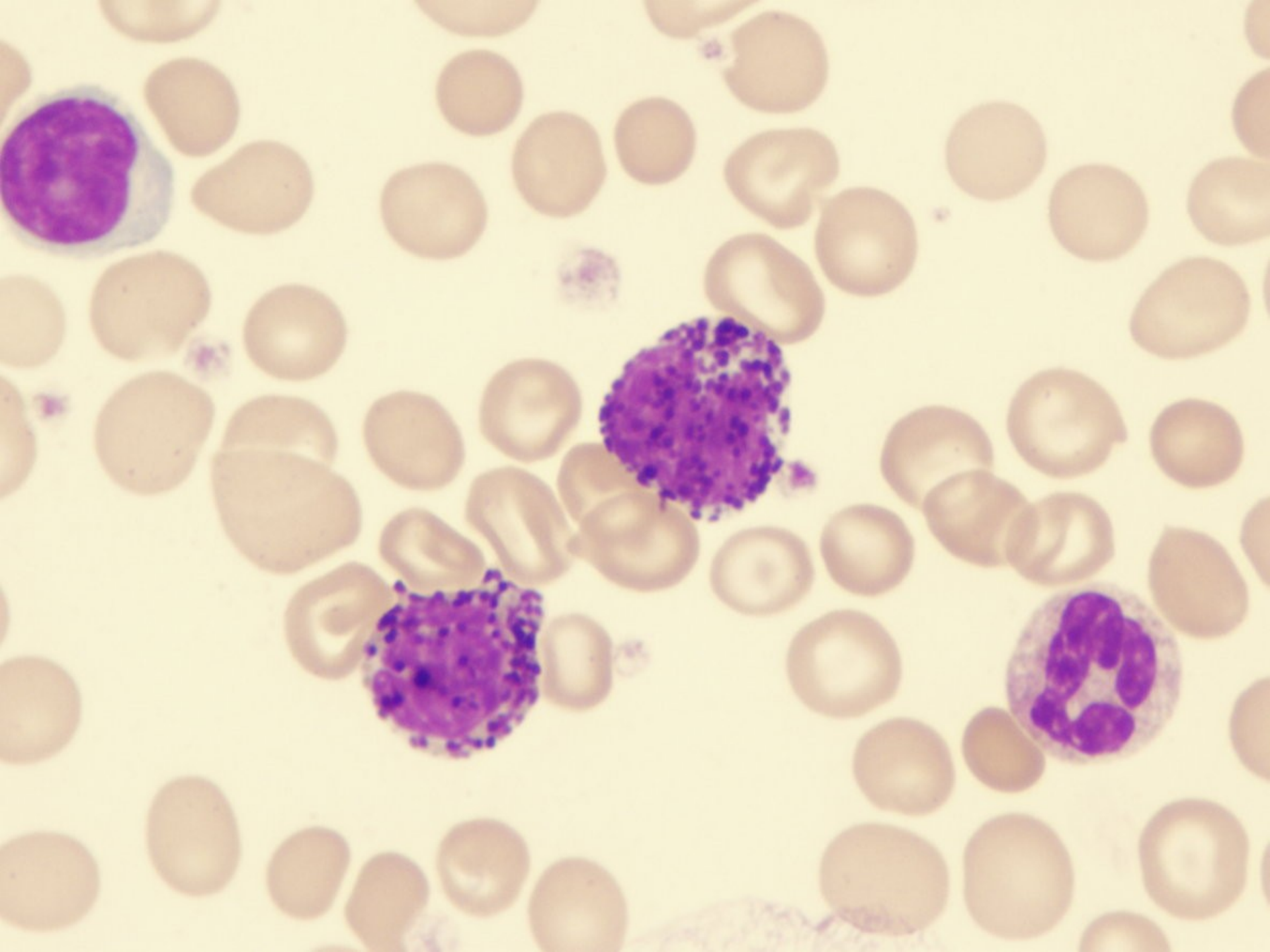


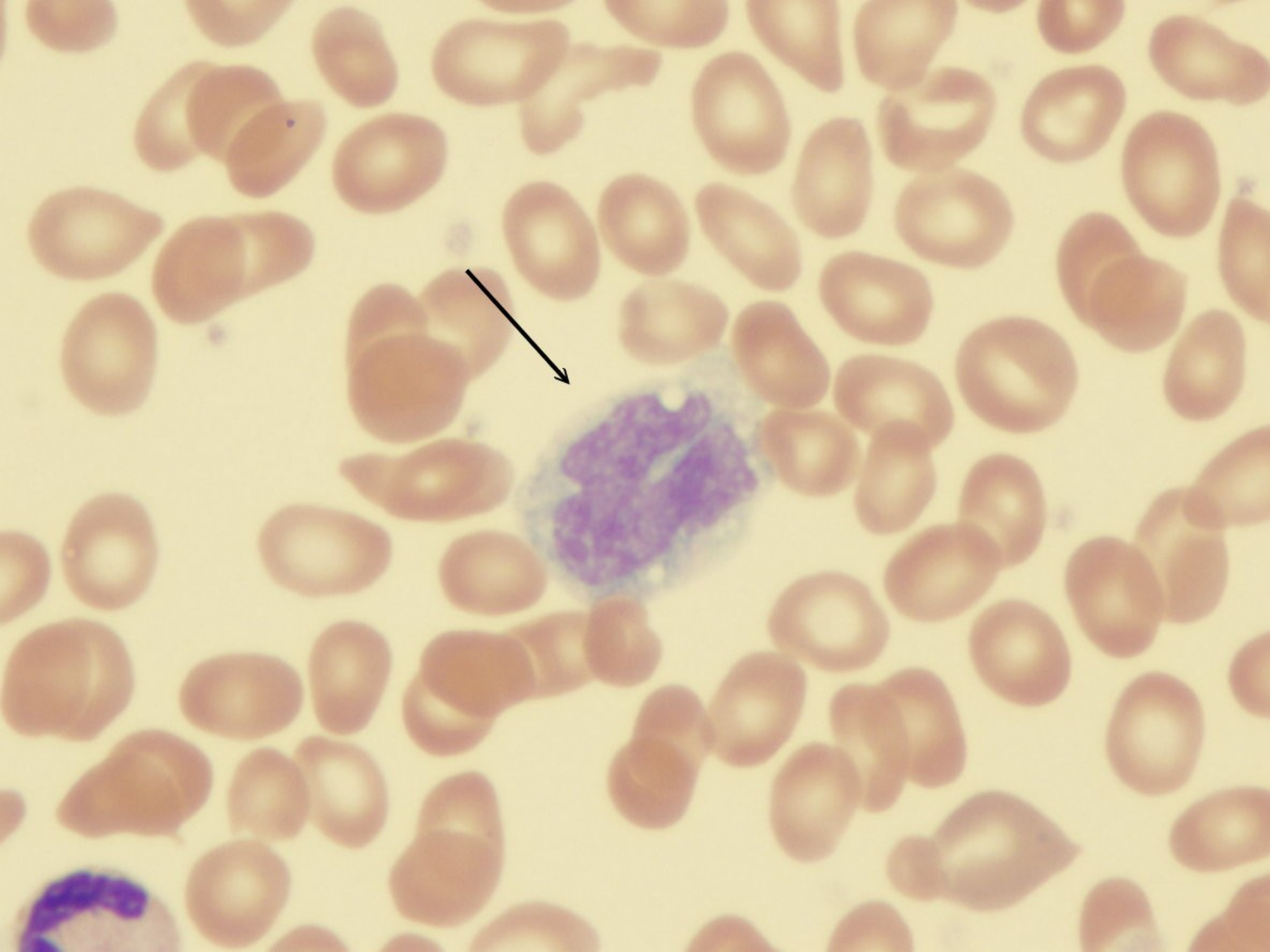
... thank you for attention ...

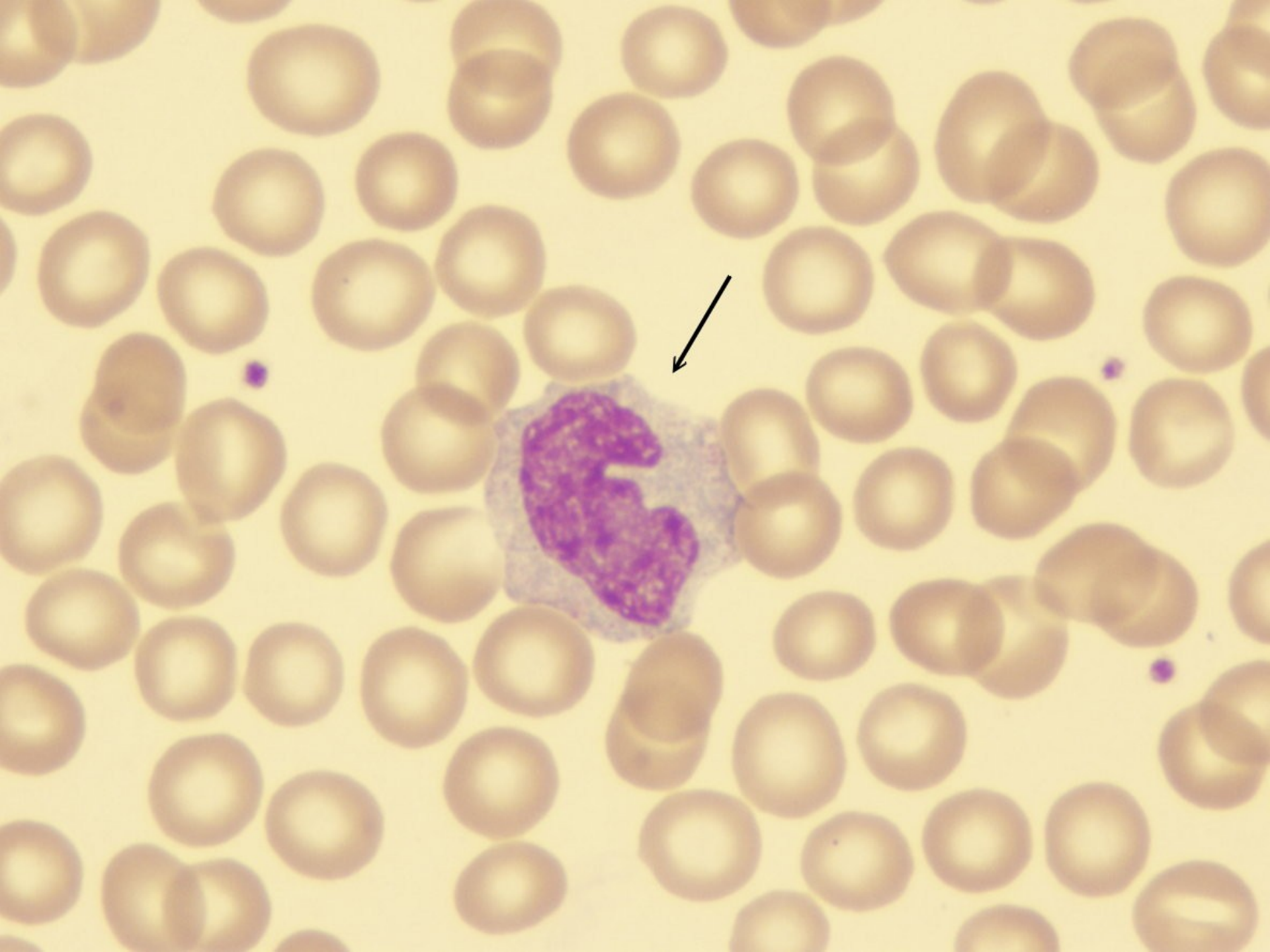


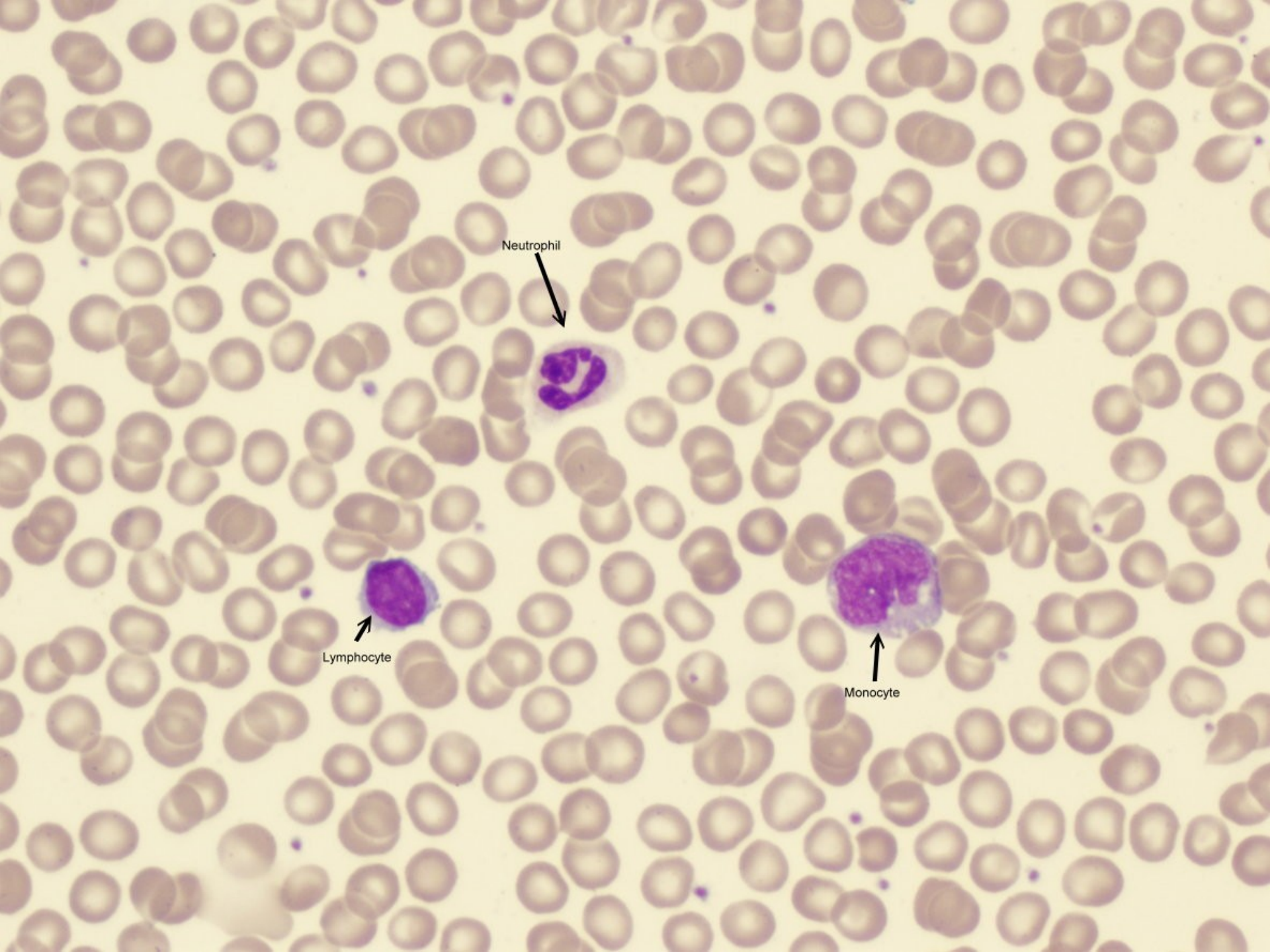


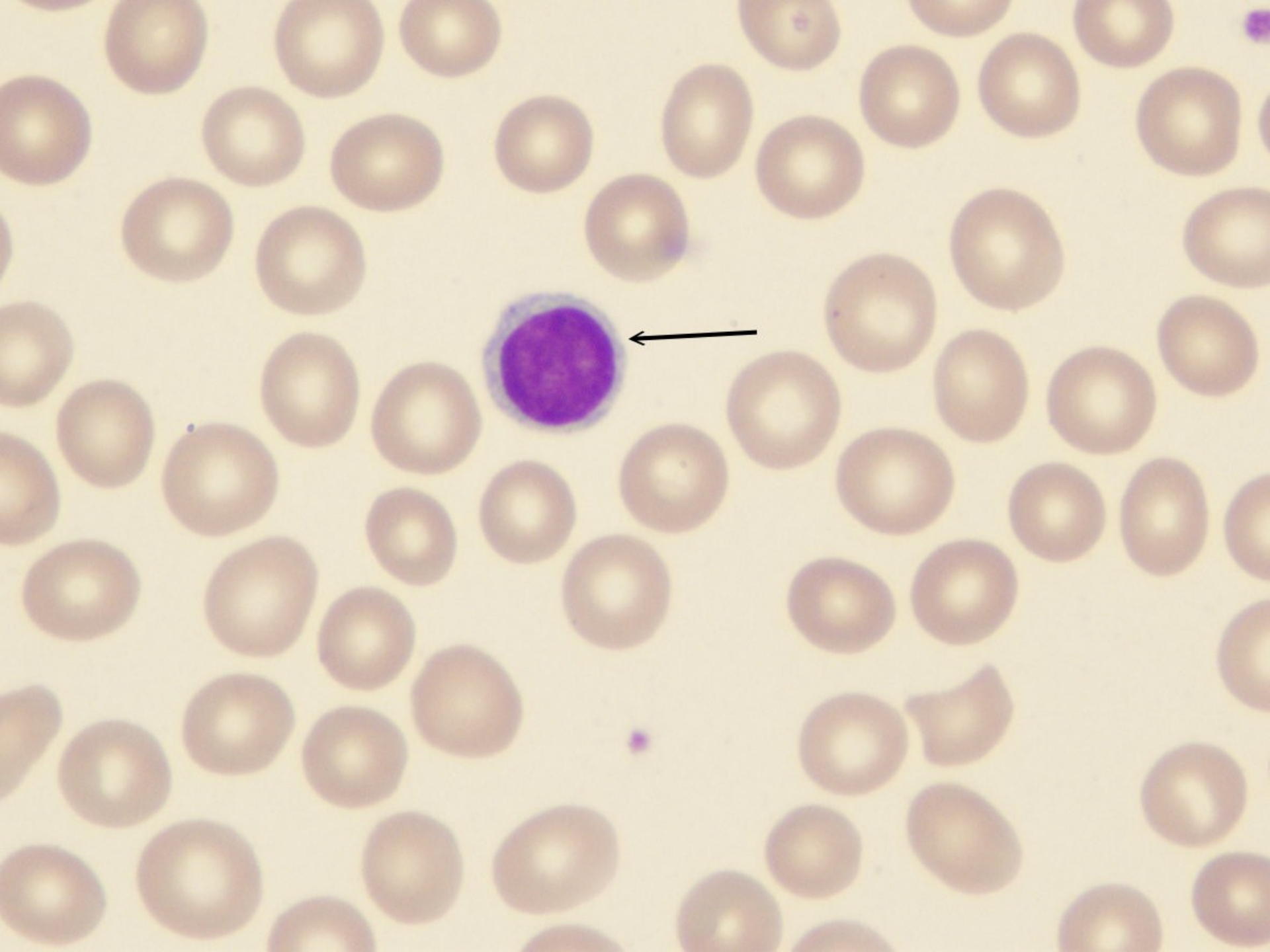


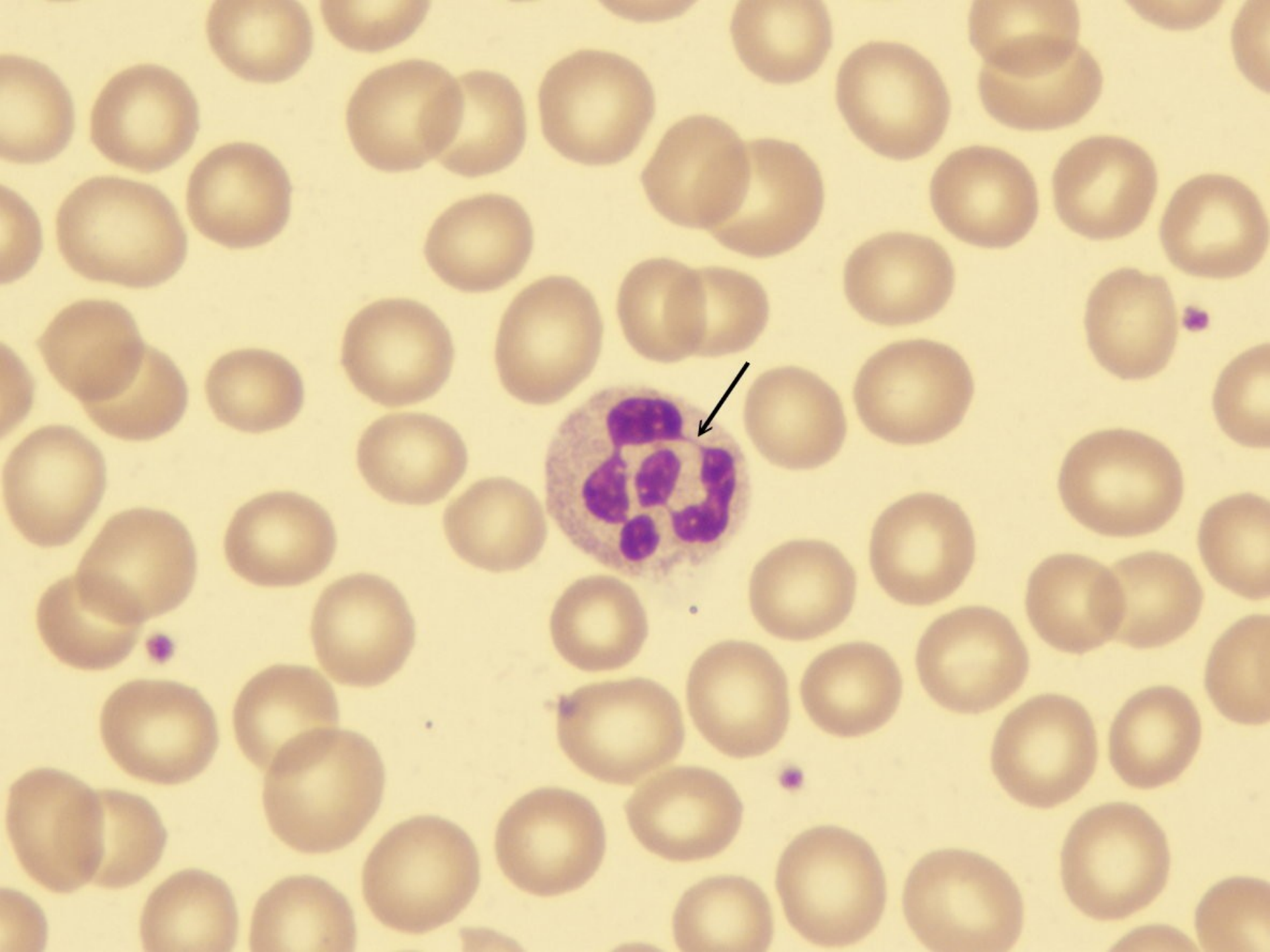


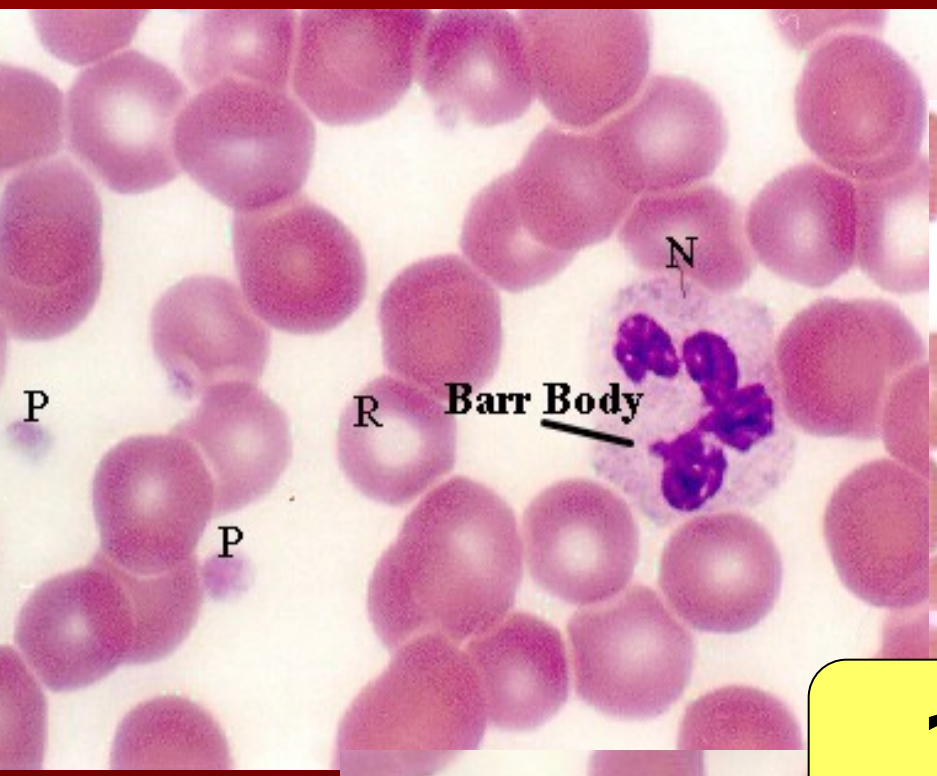


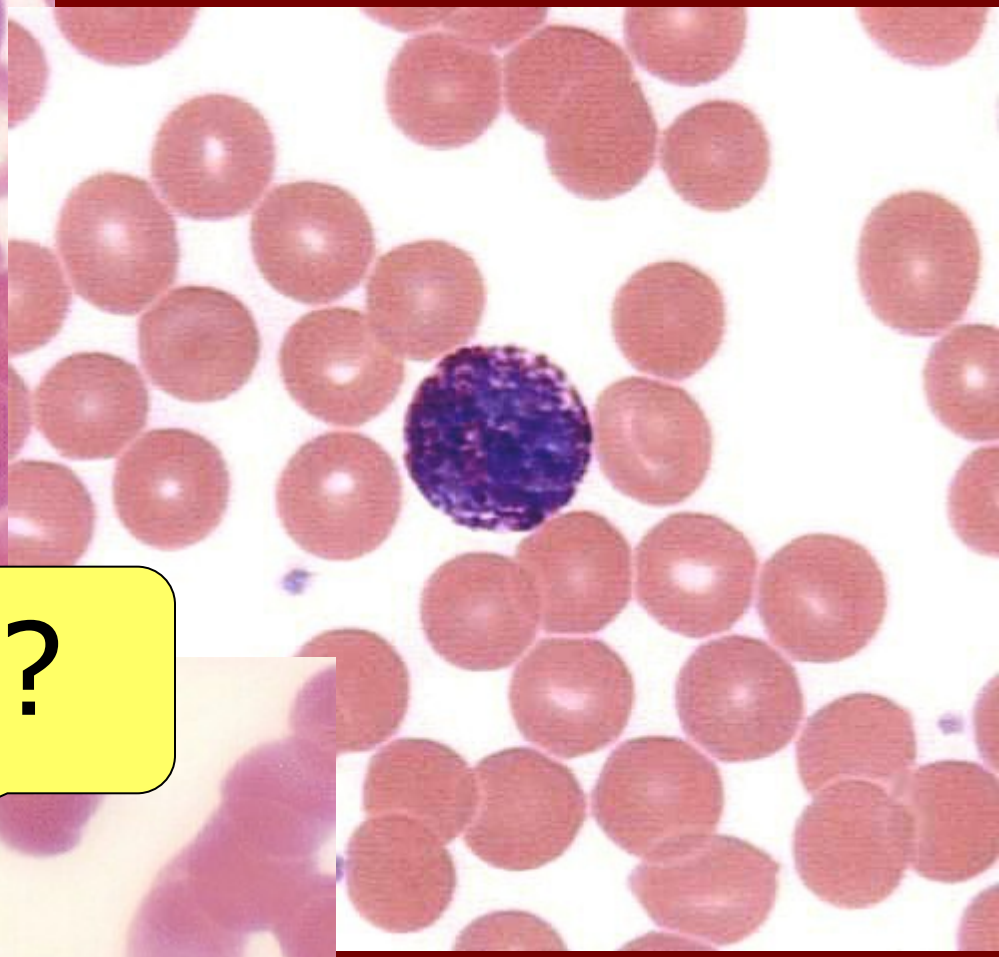



Neutrophil
Lymphocyte
Monocyte

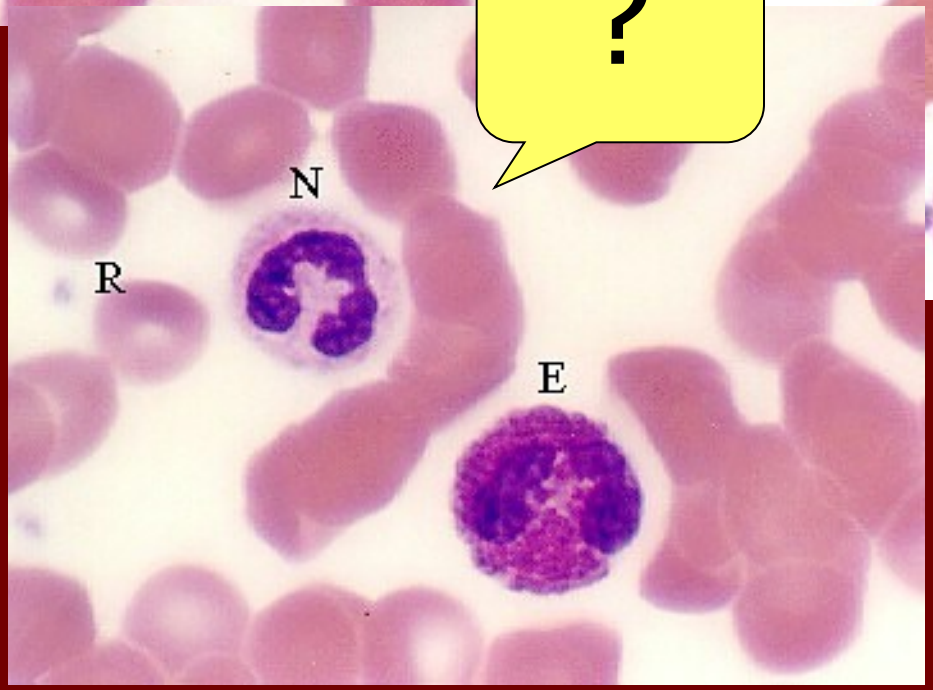


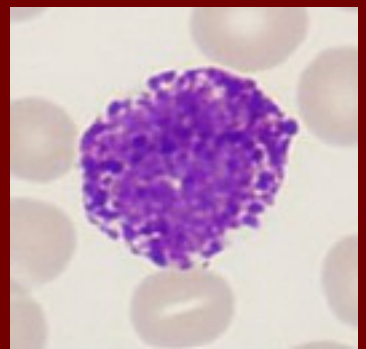


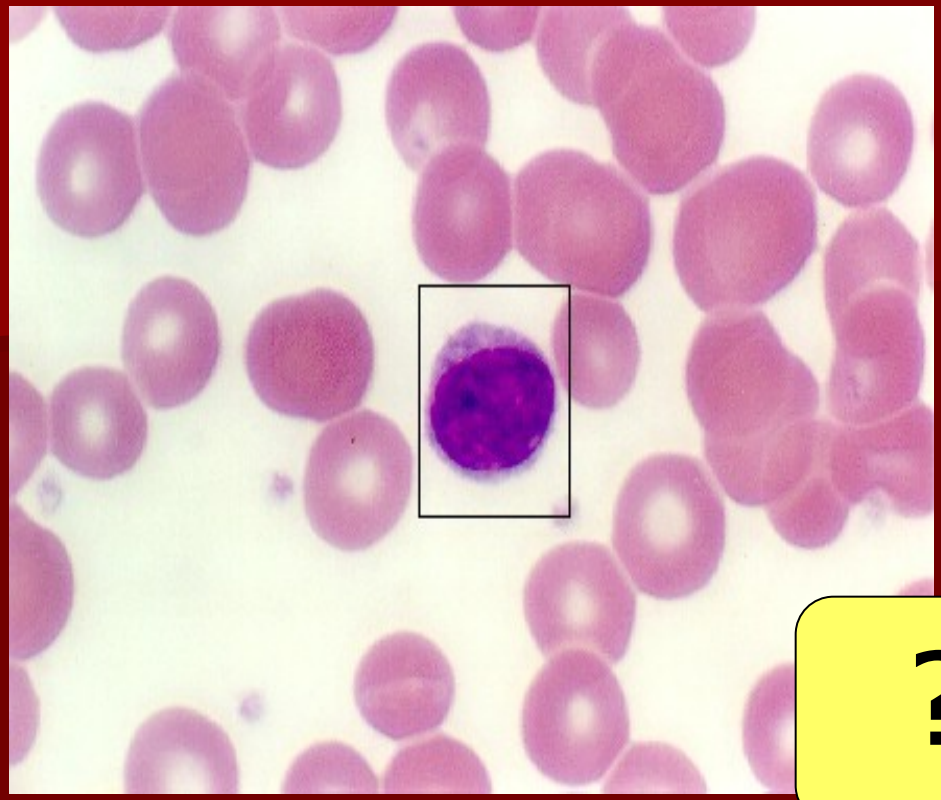

N
P
R
Barr Body
P



?


N
R
E





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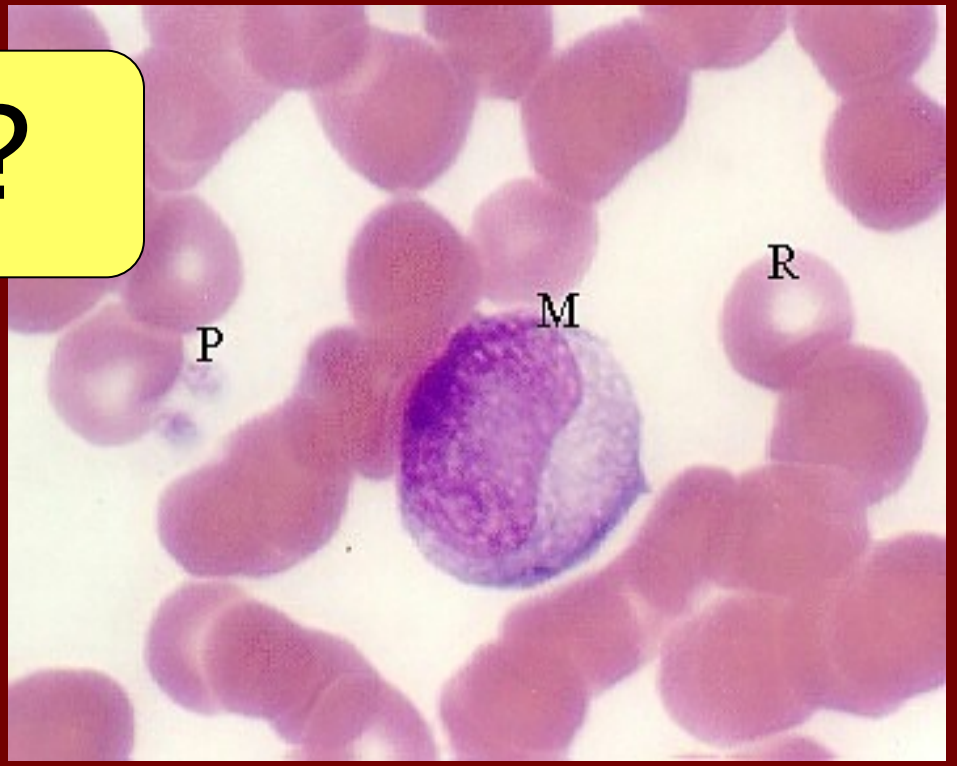
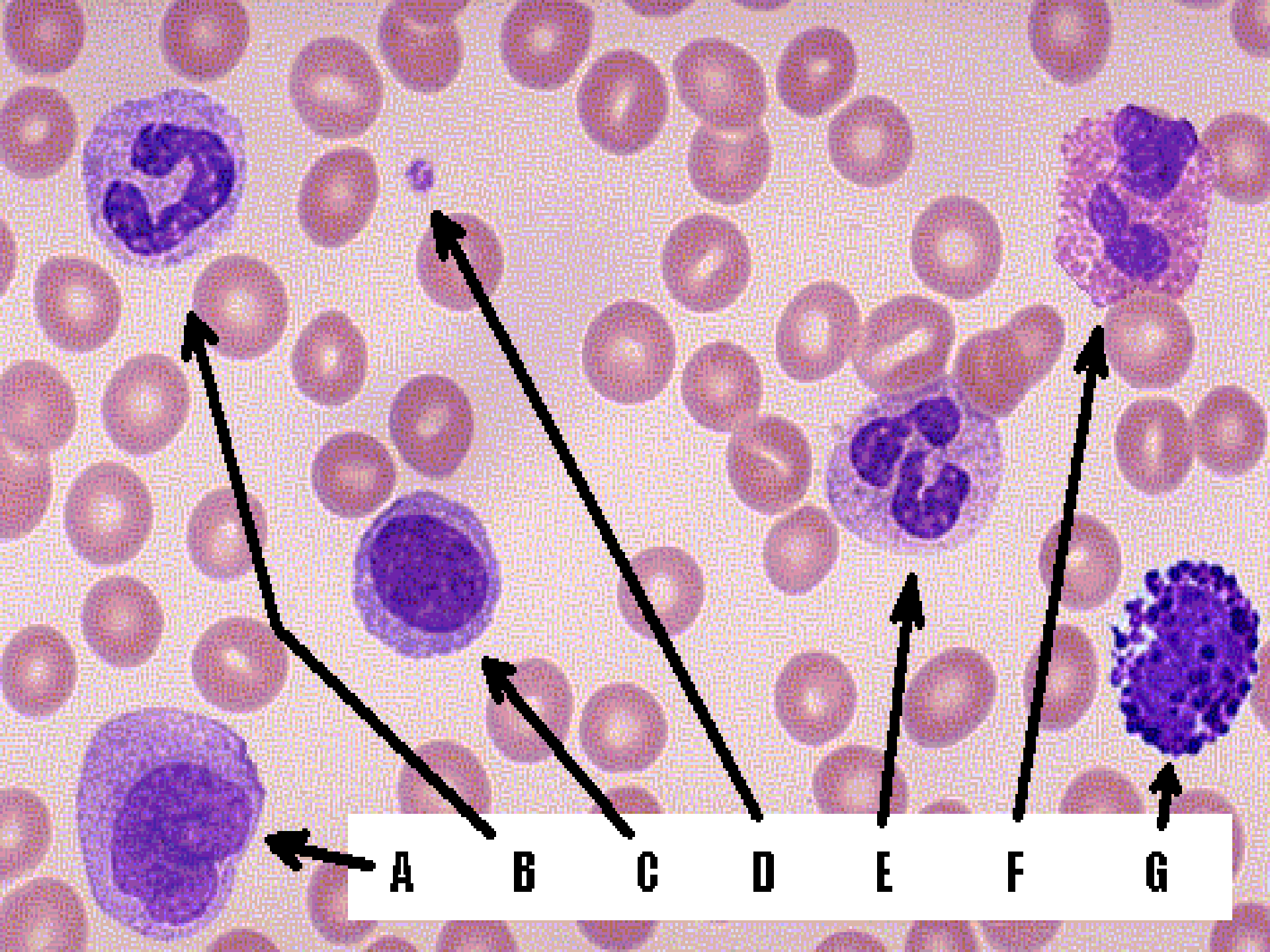
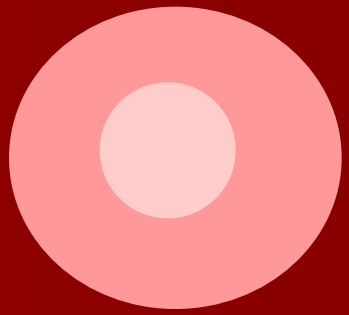
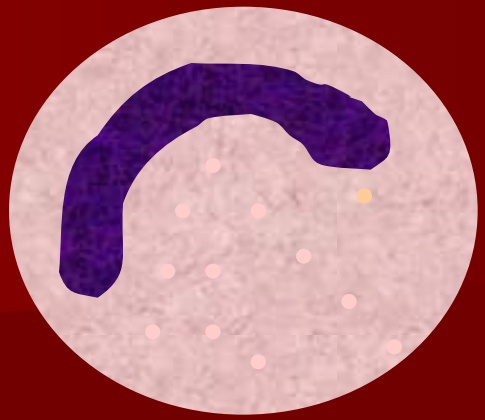

P
M
R


A
B
C
D
E
F
G

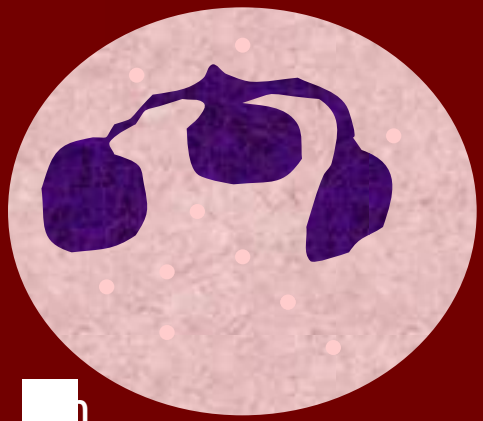


Ery 7.4 µm

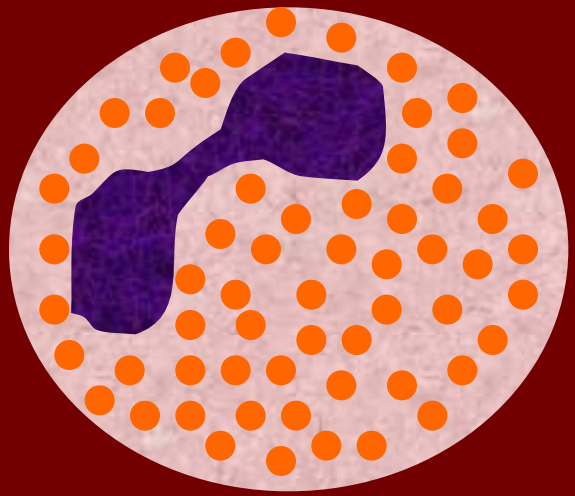


Neu – band

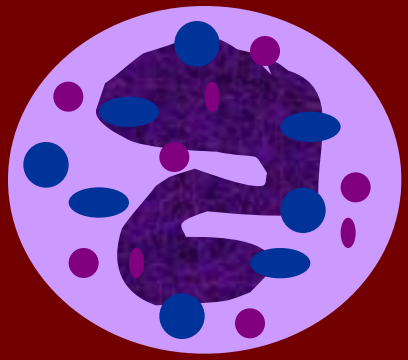
10 – 12 µm



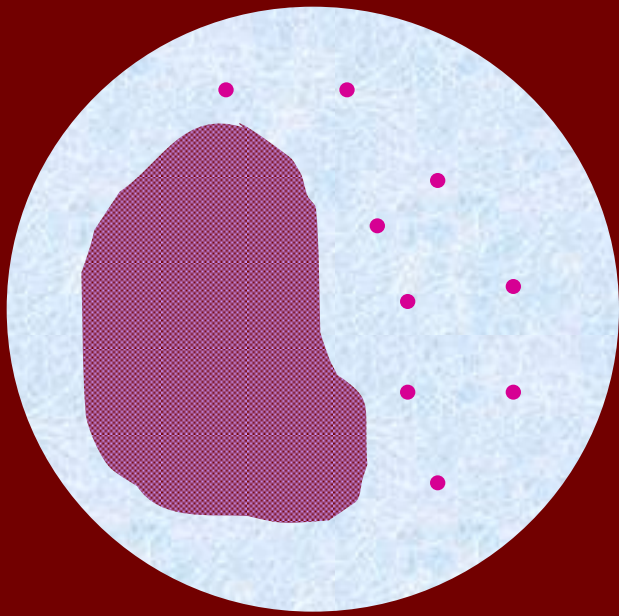
Neu – segment



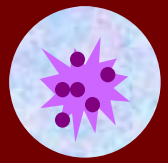
Eos 12-14 µm



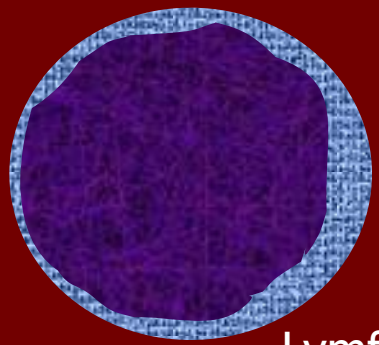
Baso 10 µm



Mono 15 – 20 µm

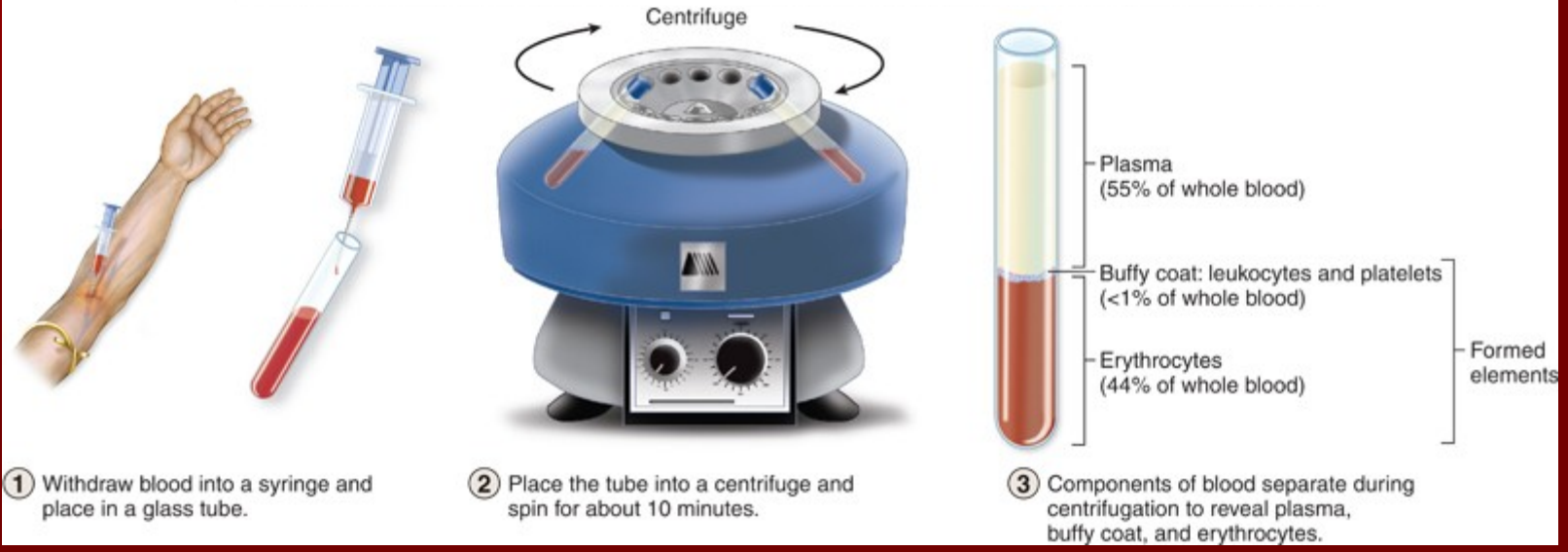


Platelet 2 – 4 µm

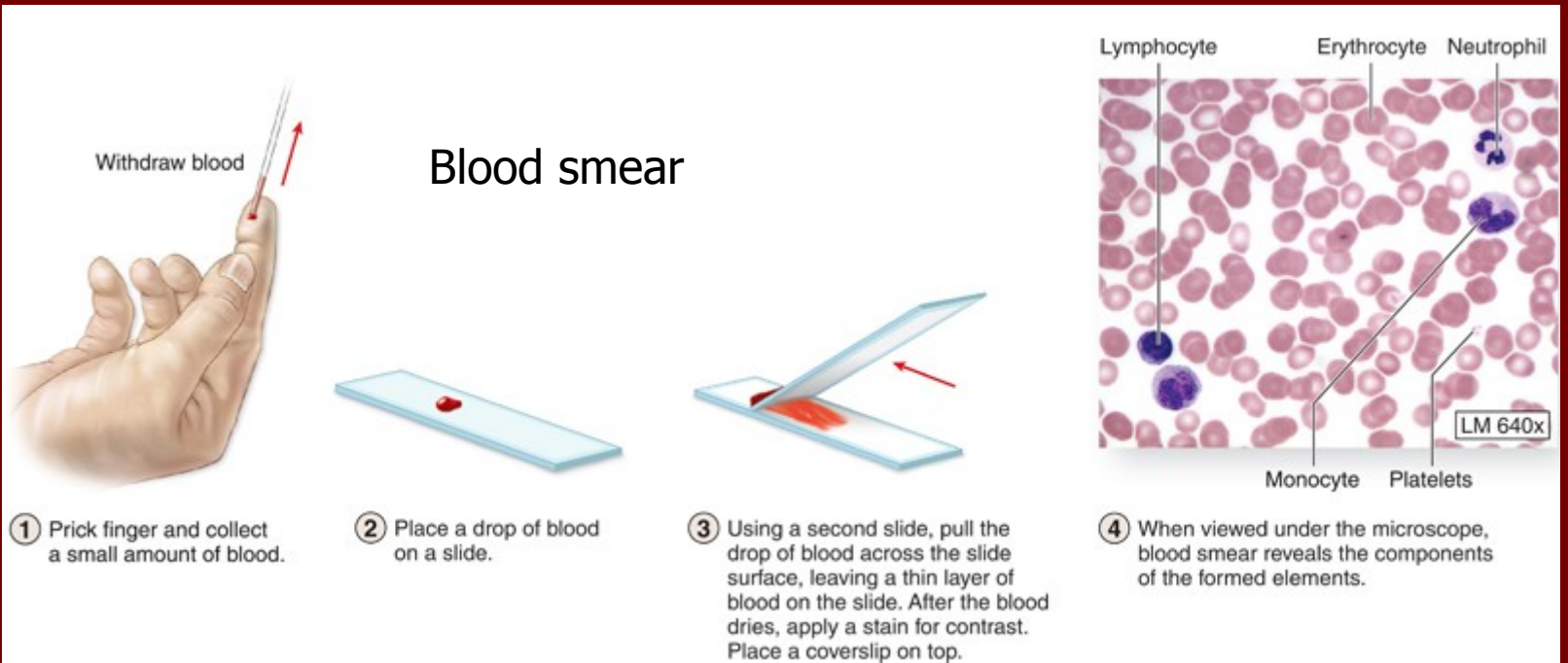


Lymfo 8 µm

Blood investigation

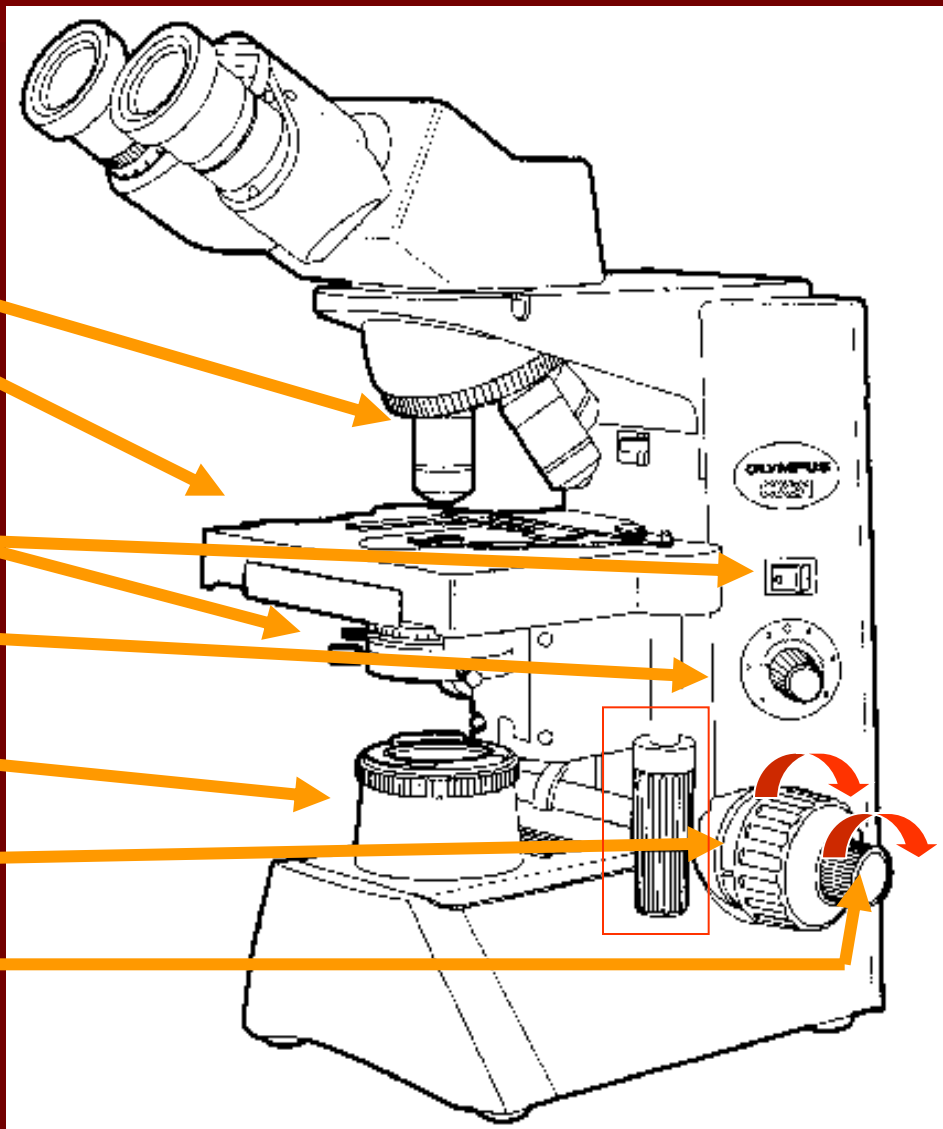


Blood smear



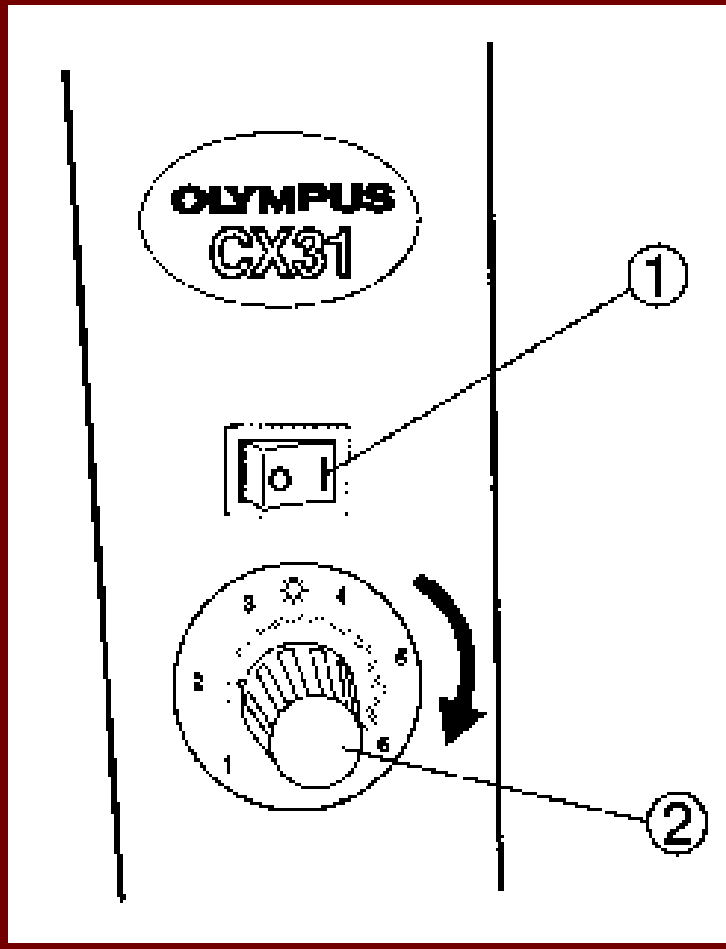
Light microscope

- Eyepieces
- Objective lense
- Stage with slide holder
- Condenser
- On/off switch
- Light intensity control
- Source of light beam
- Course adjustment knob
- Fine adjustment knob



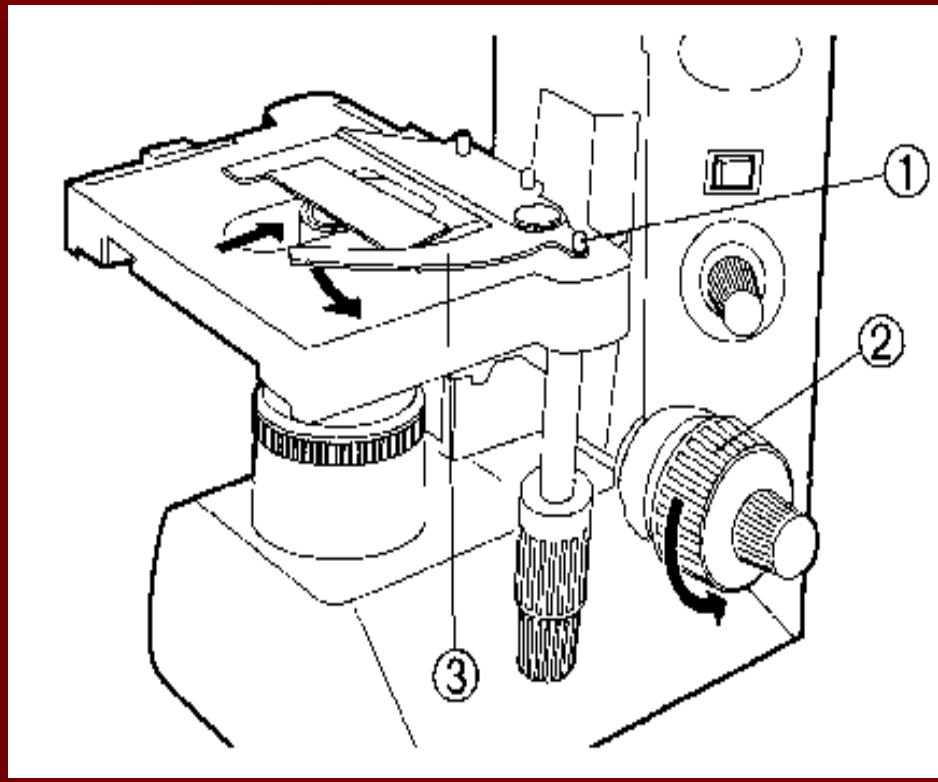
Light microscope manipulation

- on/off switch knob (1) (rocker or wheel SW)
- pivoted potenciometer (2) regulate intensity of emited light



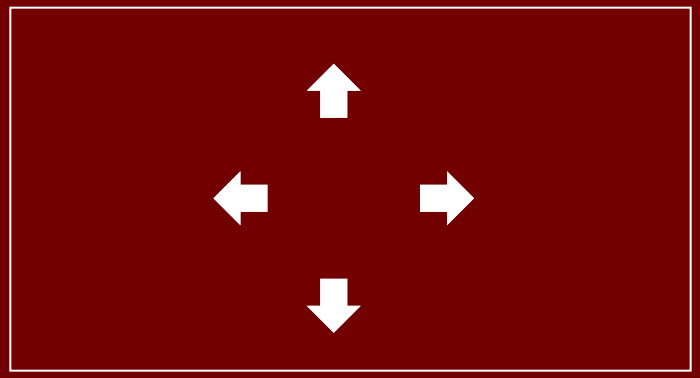
Light microscope manipulation

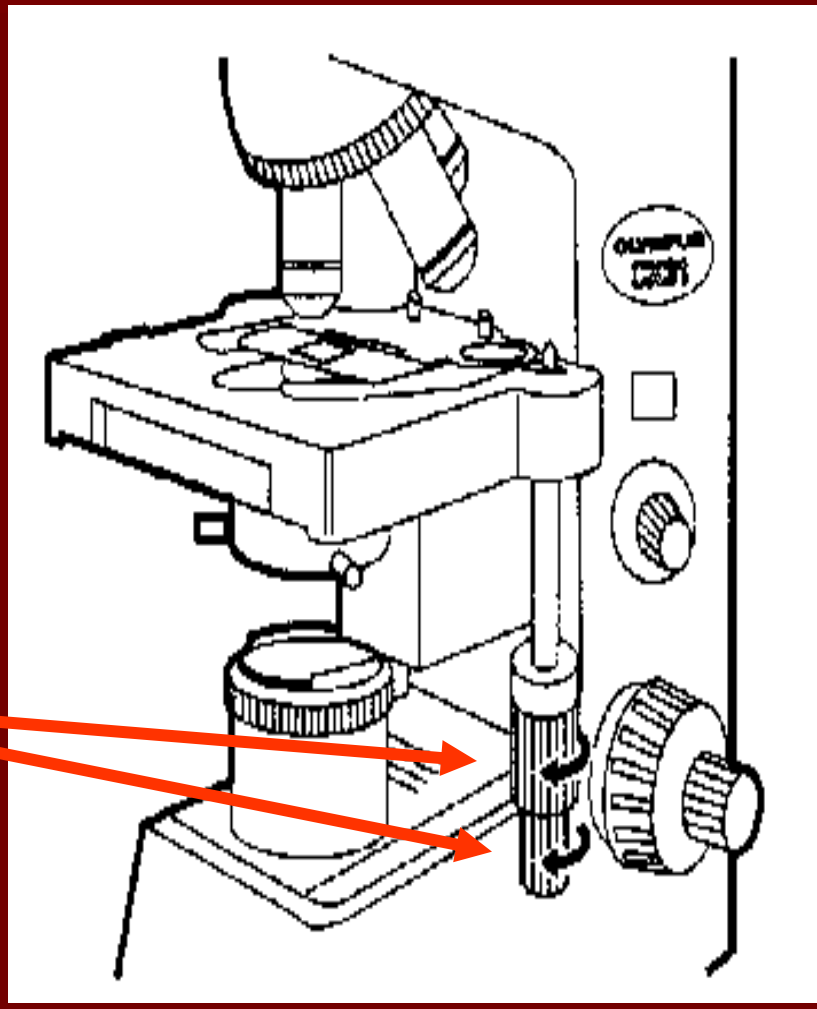
- Stage with slide holder (3)
- Lever of holder (1)
- Focusing knobes (2).



Light microscope manipulation

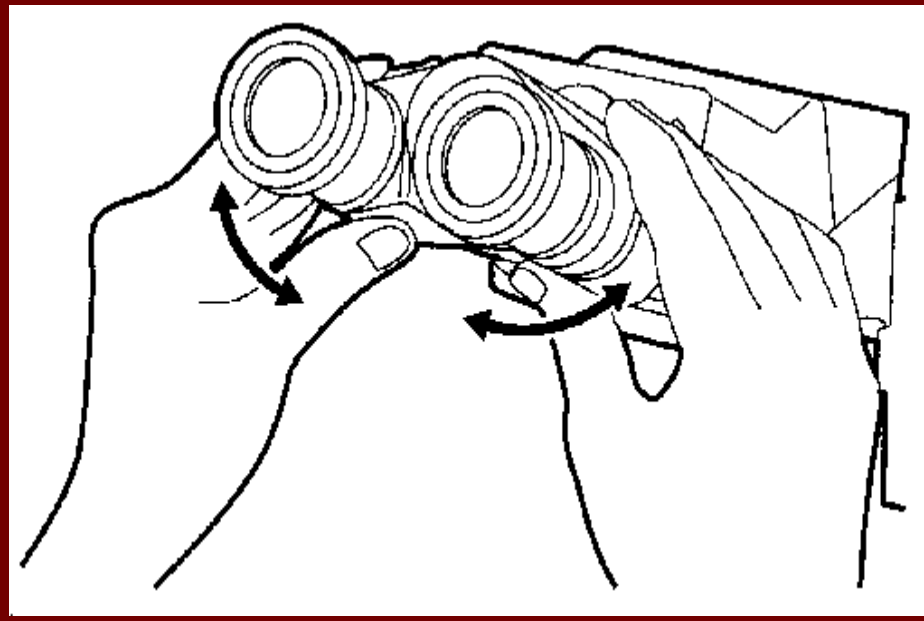
- Use mechanism of cross shift for shift of the slide on the stage

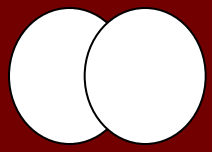




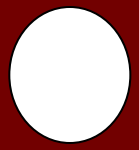
Light microscope manipulation

- focuse a picture in LM and look at it with both eyes
- regulate a distance between the eyepices so, you can see one focused circular field





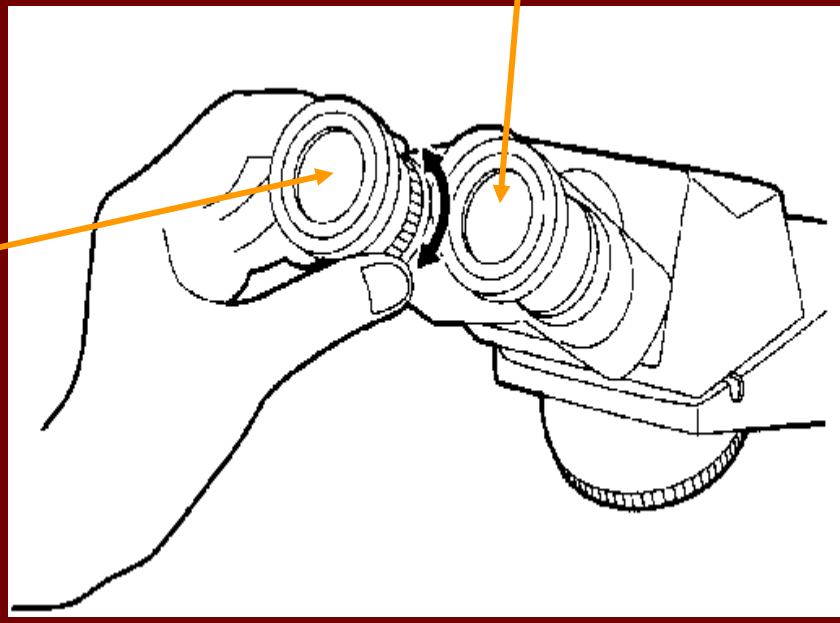
bad



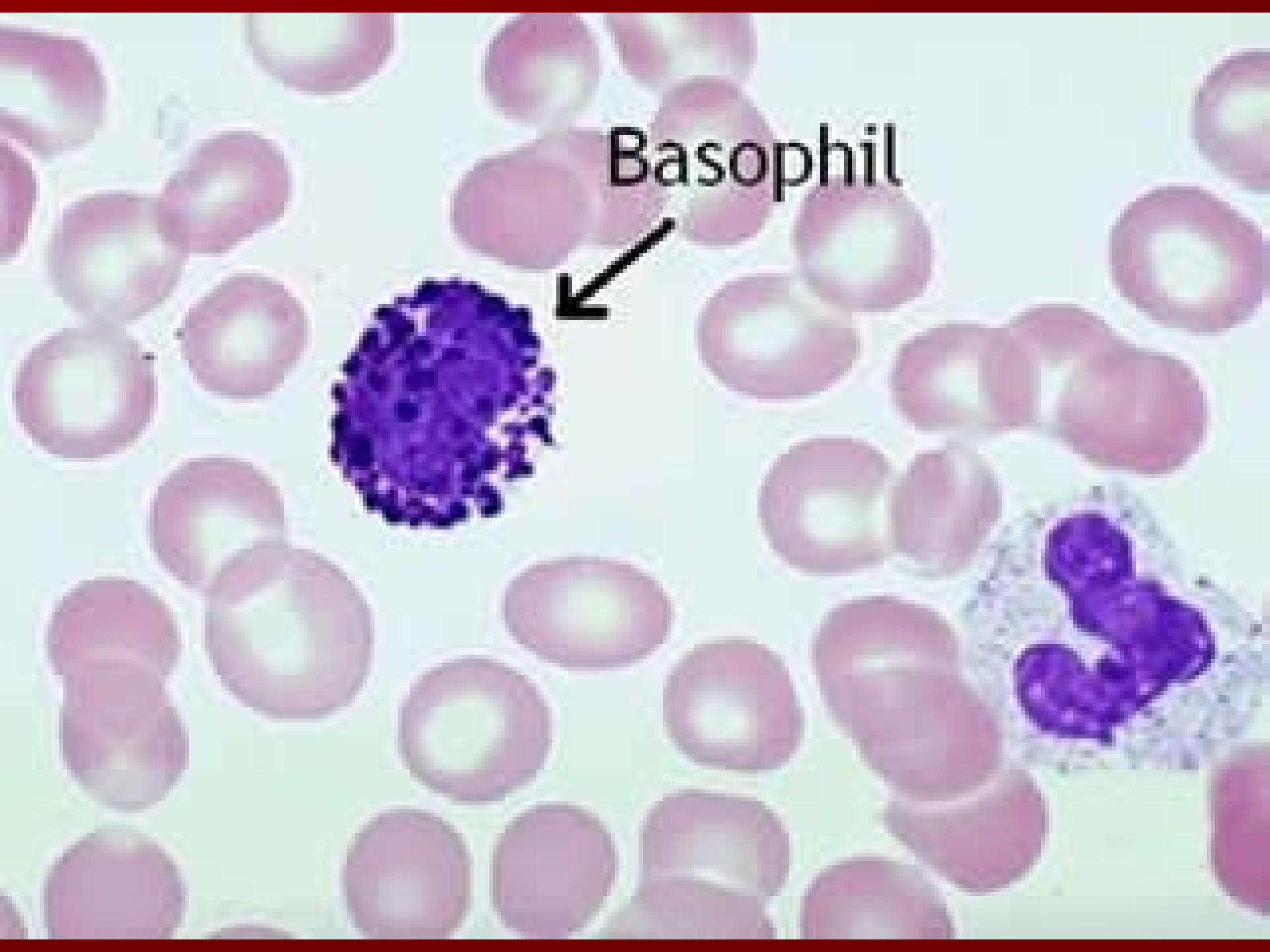
correct

Light microscope manipulation

- Look at the slide only through the right eyepiece and focuse some point in the picture.
- Without refocusing, look at the left eyepiece.
- In doing so, screw the ring below the left eyepiece to focuse the same point.
- So, the dioptric correction is set up.



Now, you can start to study blood smear in your LM ☺


Basophil