Professional Practice in Clinical Chemistry

Basic Hematology

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Presented by AACC and NACB

LEARNING OBJECTIVES

1. Define “CBC”
2. Define and explain red cell indices including *derived values* such as MCV, MCHC and MCH
3. Define and explain erythrocyte sedimentation rate
4. Describe the main cell types observed in peripheral blood.
5. Describe what is meant by the term “differential count”.
6. Describe the distribution & morphology of cells in certain common “Blood Pictures”

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Clinical Diagnostic Laboratories offer:

1. "**CBC**"
2. "**CBC plus Diff.**" [CBC + Differential count]
3. Above ± "**Reticulocyte count**"

What do these terms mean, and how are they used?

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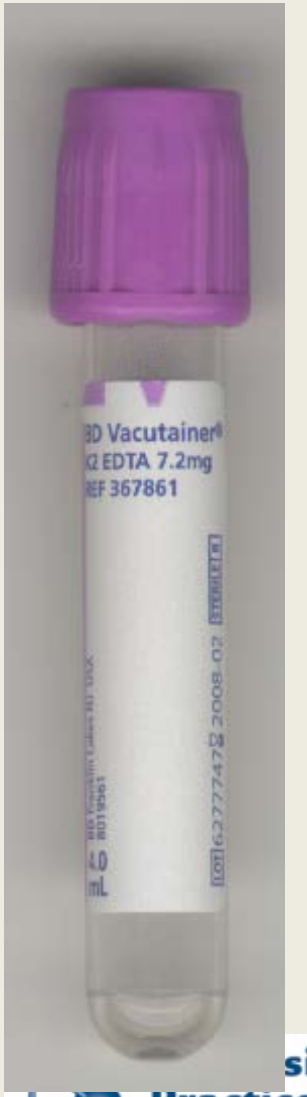
CBC

1. White Cell count (leukocyte count)
2. Platelet count
3. Red cell count, hemoglobin concentration and red cell indices

What Specimen?

- Whole blood, uncentrifuged, anticoagulated with EDTA (Purple or lavender cap)



Cell Counts

- White cells, red cells (erythrocytes) and platelets are counted **per unit volume** of whole blood.
- Unit volume: per **cubic millimeter** (mm^3) which is the same as **µL**

- **WBC** 4.0-10.0 x 10^3/cu mm
- **Platelets** 150-450 x 10^3/cu mm
- **RBC** 4.5-5.9 x 10^6/cu mm

Cell Counts

- Unit volume: per **liter (SI units)**.

- **WBC** $4.0\text{-}10.0 \times 10^9/L$
- **Platelets** $150\text{-}450 \times 10^9/L$
- **RBC** $4.5\text{-}5.9 \times 10^{12}/L$

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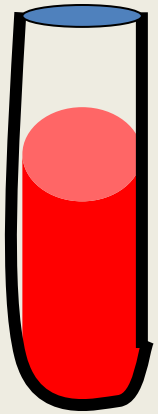
Hemoglobin & Red Cell Indices

1. Hemoglobin
2. Hematocrit
3. Red cell count
4. Mean cell (corpuscular) *volume* or MCV
5. Mean cell *hemoglobin concentration* or MCHC
6. Mean cell *hemoglobin content* or MCH
7. Red cell distribution width or RDW

Red Blood Cell Indices

1) **Hemoglobin concentration**
in grams/dL (grams per 100 mL)



Normal: 12 -16 g/dL or
120 – 160 g/L

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Hemoglobin concentration

- **Hemoglobin is converted to CYANMETHEMOGLOBIN which absorbs maximally at 540 nm**

- Hb plus KCN plus potassium ferricyanide + $NaHCO_3$
- Heme iron (Fe^{2+}) $\rightarrow$ Fe^{3+}
- Methemoglobin binds with **CN**

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Hemoglobin concentration

- **Most current hematology analyzers have switched to a new, alternative method: *sodium lauryl sulfate* (SLS, SDS) binds to hemoglobin.**
- **Sulfated derivative absorbs at 535 nm**

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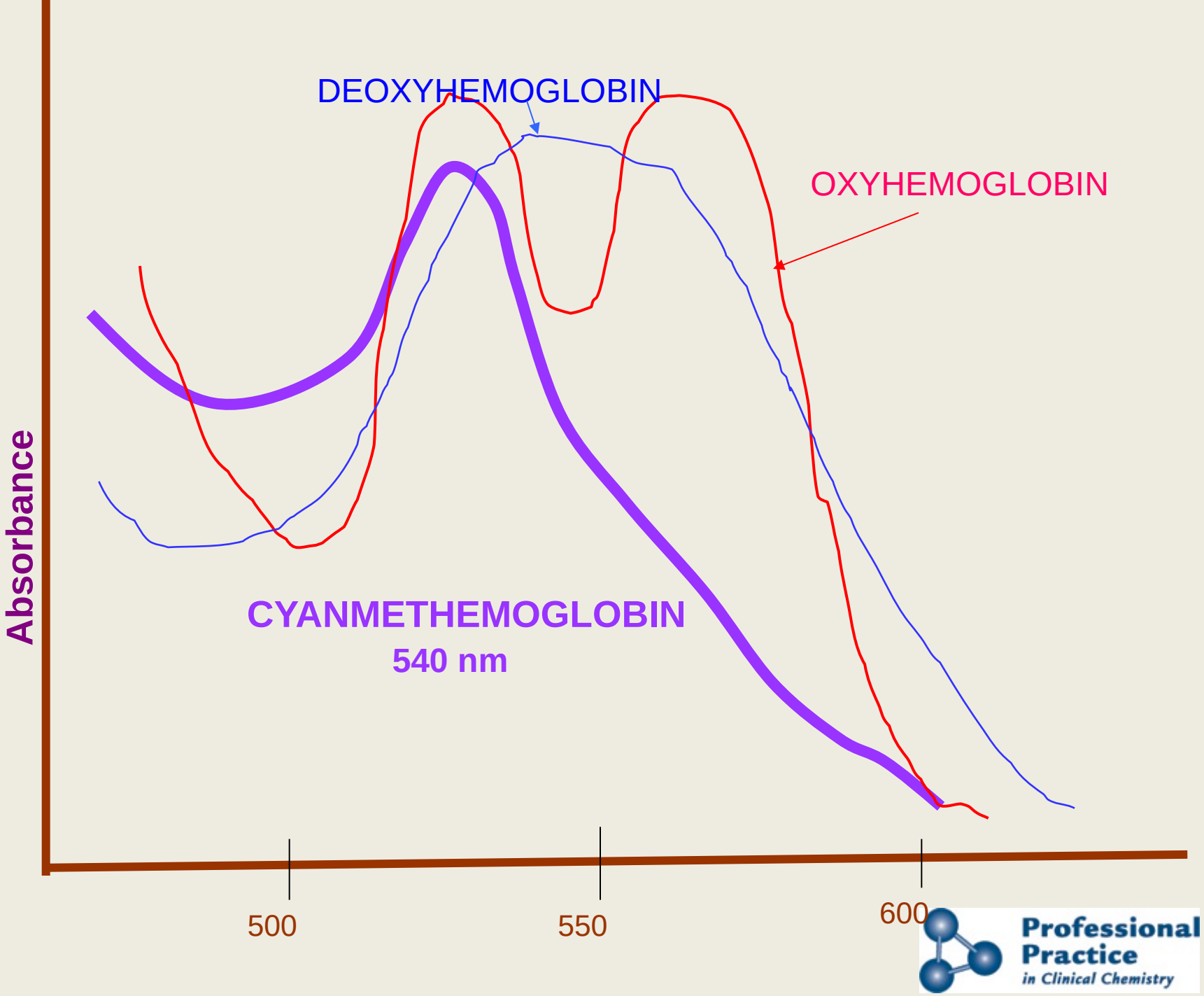

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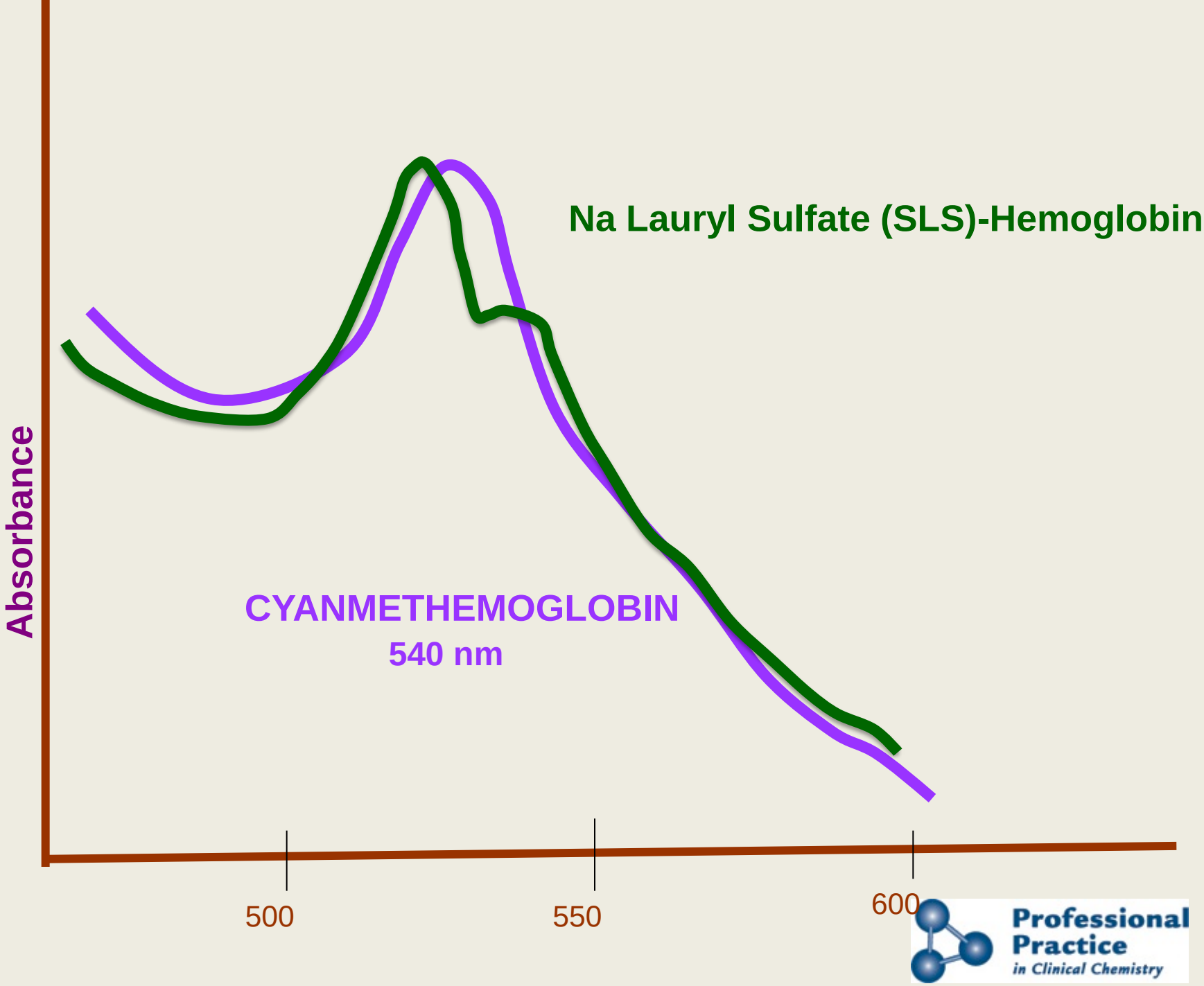

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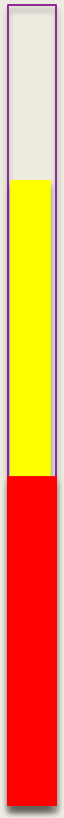
2) **Hematocrit** (the percentage of blood that is represented by the packed red cells)

Hematocrits determined by blood centrifugation are termed "*spun hematocrits*"

60%



40%

Normal: 35 - 45%

vol/vol

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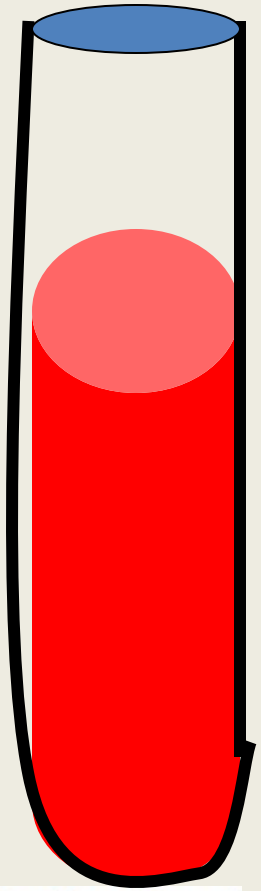
3) **Red cell count**. *Number* of red blood cells per microliter of blood (or per liter)

Normal: 4 - 5.5 x 10^6/uL



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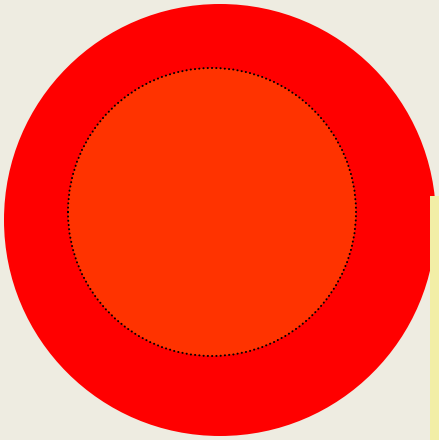
4) **Mean Cell Volume** (MCV) = red cell *volume* in *femtoliters* or 10^{-15} liter



Low MCV = Microcytic

High MCV = Macrocytic

Normal: 78 -100 *femtoliters*

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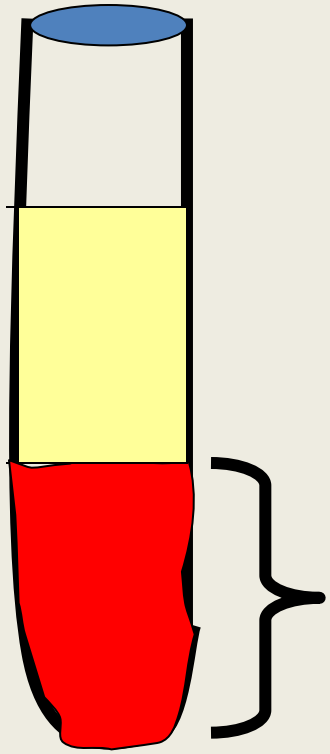
5) **MCHC** (Mean cell hemoglobin concentration:

Hemoglobin concentration of the packed red cells (minus plasma)



Low MCHC: *Hypo*chromic

High MCHC *Hyper*chromic

Normal: 31-37 g/dL (*of erythrocytes*)

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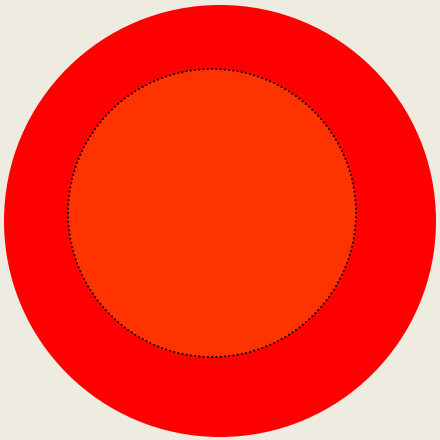
6) **Mean Cell Hemoglobin** (**MCH**) = red cell hemoglobin **content** in picograms or 10^{-12} grams



Normal: 26 - 32 pg per red cell

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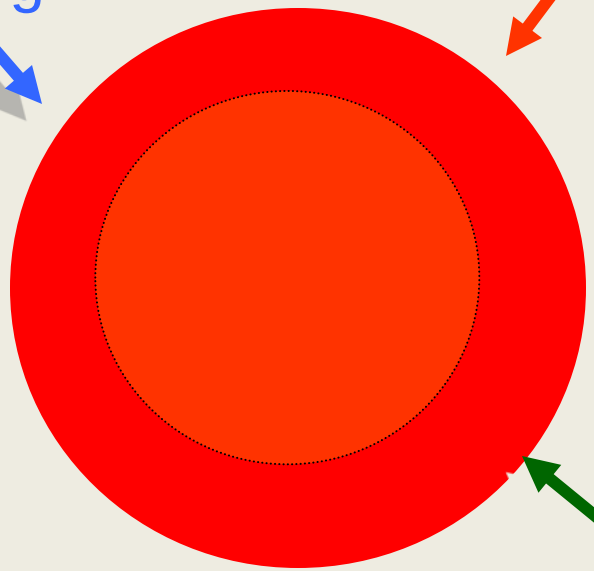
MCV: reflects the Cell *Volume* in femtoliters

Small vs Big

MCHC: reflects the *concentration* of Hb in the red cell (g/dL)

"Pale" vs "Deep Red"
Hypo- vs Hyperchromic



Both

MCH: reflects the Hb *CONTENT* (in picograms) of each red cell

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MEAN CELL VOLUME (MCV) **femtoliters** $= \frac{\text{Hematocrit x 10}}{\text{RBC}}$

MEAN CELL HEMOGLOBIN (MCH) **pg per RBC** $= \frac{\text{Hemoglobin x 10}}{\text{RBC}}$

MEAN CELL HEMOGLOBIN CONC (MCHC) $= \frac{\text{Hemoglobin x 100}}{\text{HCT}}$

g/dL of erythrocytes

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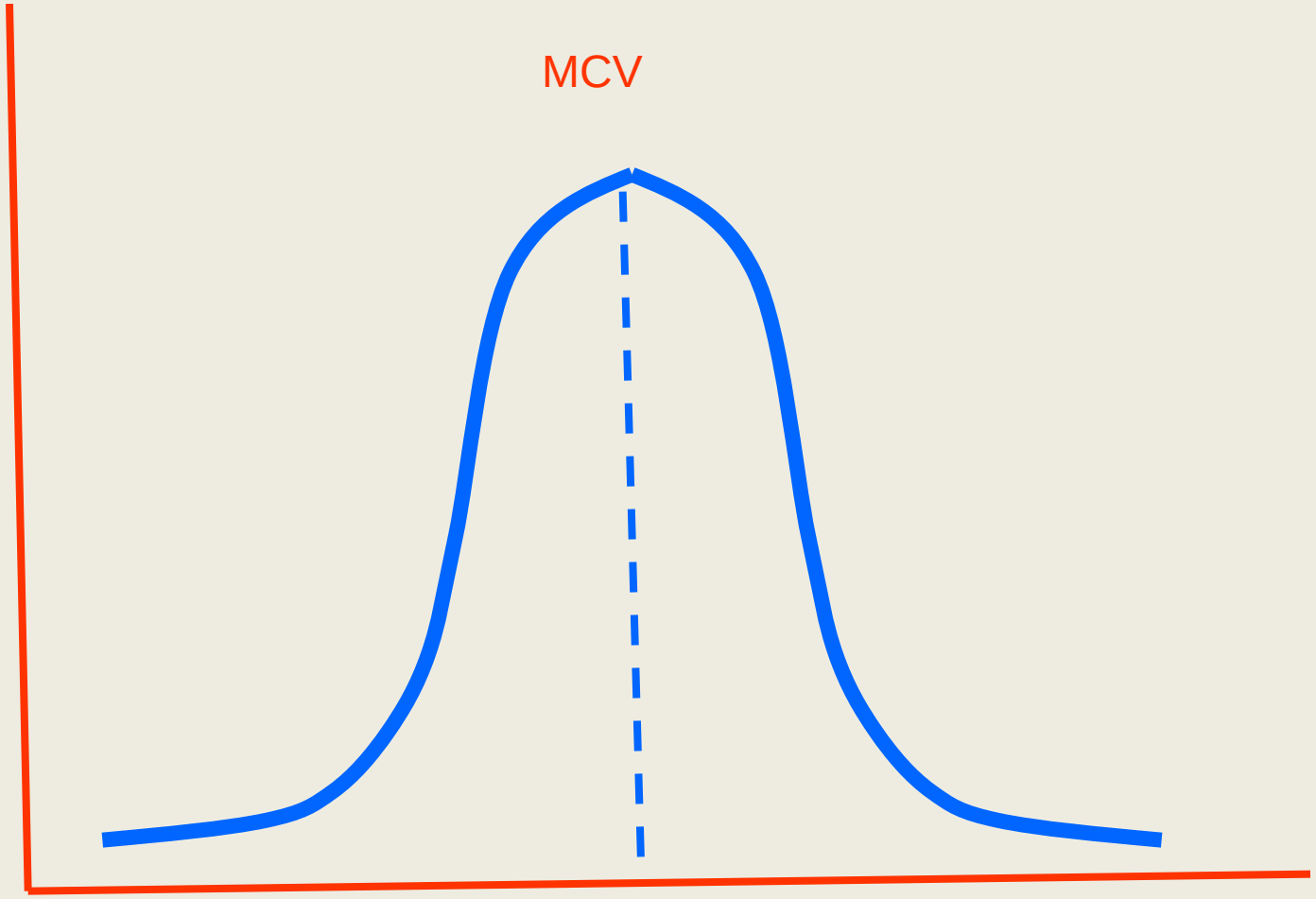
All automated cell counters can generate a red cell volume - distribution histogram



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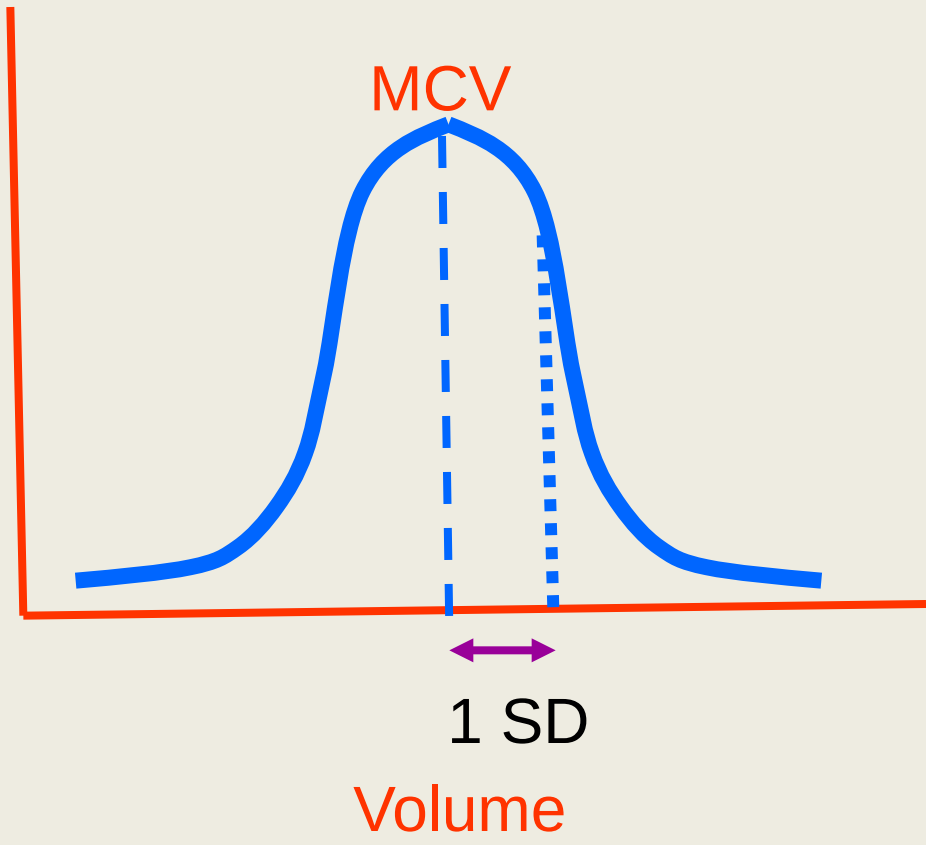
7) Red cell Distribution Width (RDW)

- The *coefficient of variation* of the red cell volume - distribution histogram



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MEAN CELL VOLUME (MCV)	=	$\frac{\text{Hematocrit} \times 10}{\text{RBC}}$
MEAN CELL HEMOGLOBIN (MCH)	=	$\frac{\text{Hemoglobin} \times 10}{\text{RBC}}$
MEAN CELL HEMOGLOBIN CONC (MCHC)	=	$\frac{\text{Hemoglobin} \times 100}{\text{HCT}}$

$$\text{Hematocrit} = \frac{\text{MCV} \times \text{RBC}}{10}$$

Directly measured

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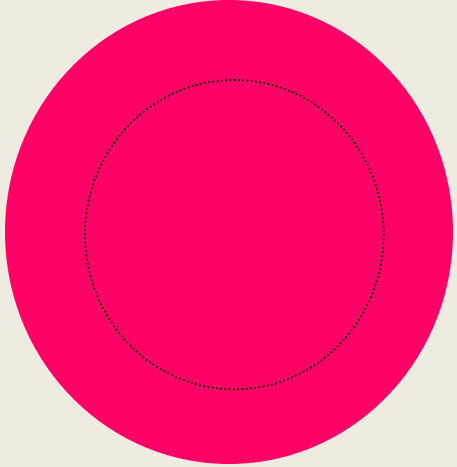
Reticulocytes

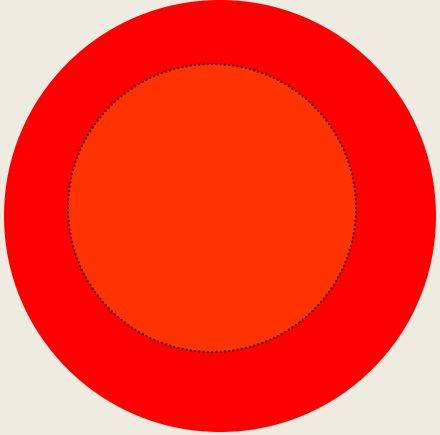
1. Immature red cells containing residual RNA
2. Indicate *rate of new RBC production*

The % reticulocytes assess the bone marrow response to anemia.

- In a hemolytic anemia → ↑ reticulocytes
- In bone marrow disease (impaired erythrocyte production) → ↓ reticulocytes

Normal stain

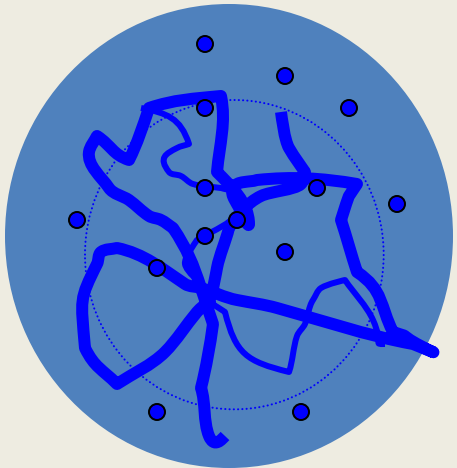


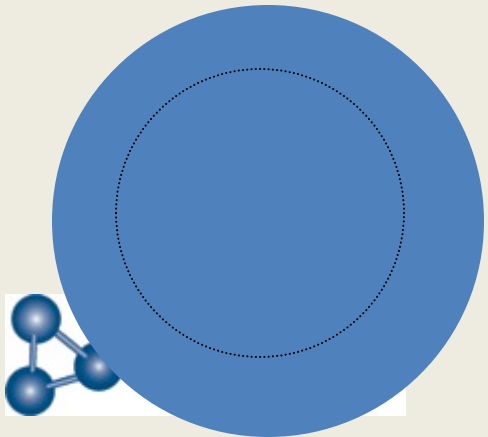


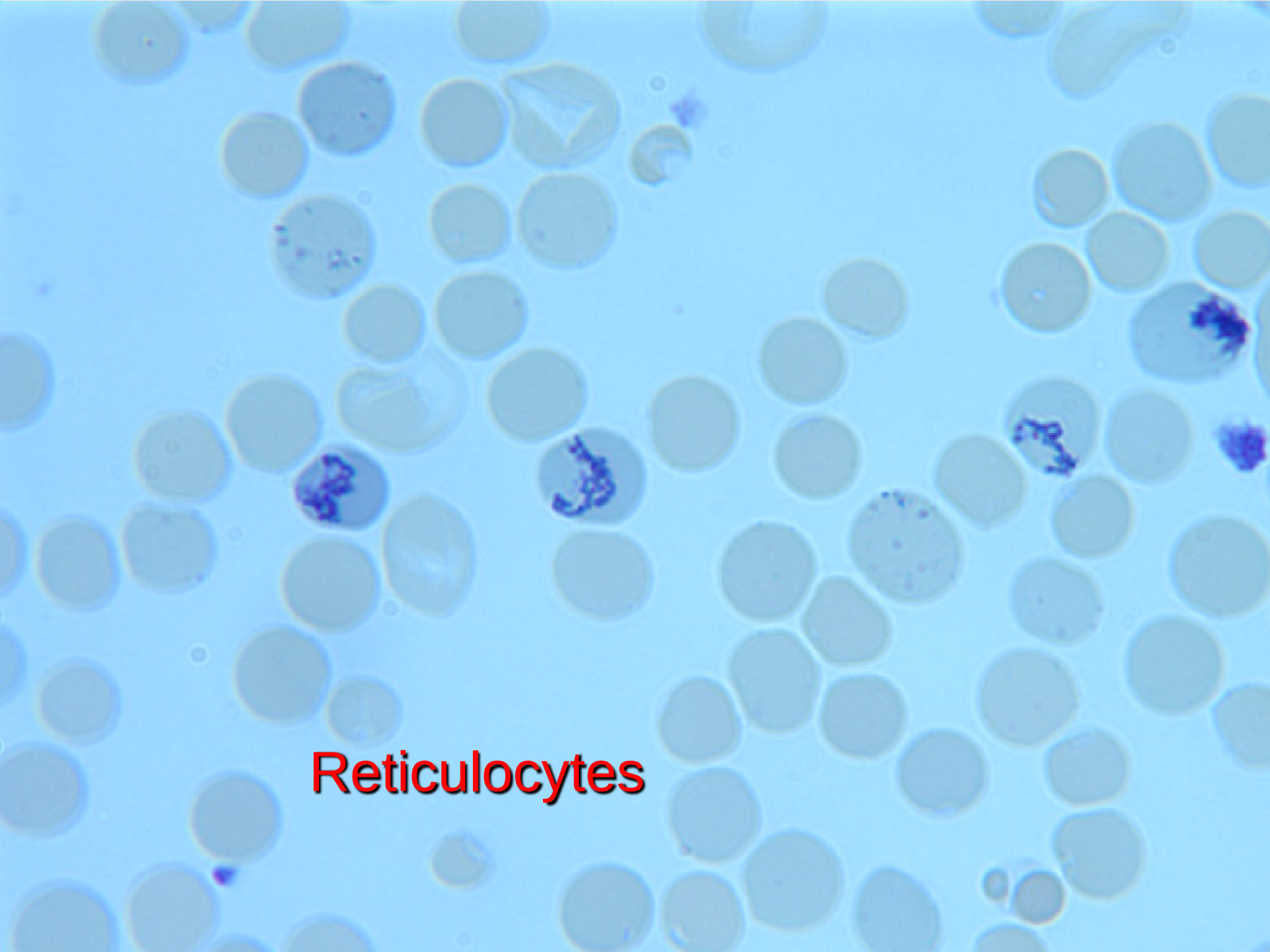
Reticulocyte

Normal

Using special reticulocyte (supravital) stain






Reticulocytes

RETICULOCYTES

- The retic count multiplied by the patient's hct divided by the expected hct (45%) is the standard **reticulocyte correction formula**.

$$\frac{\text{Patient HCT}}{\text{Standard HCT (45\%)}} \times 100$$

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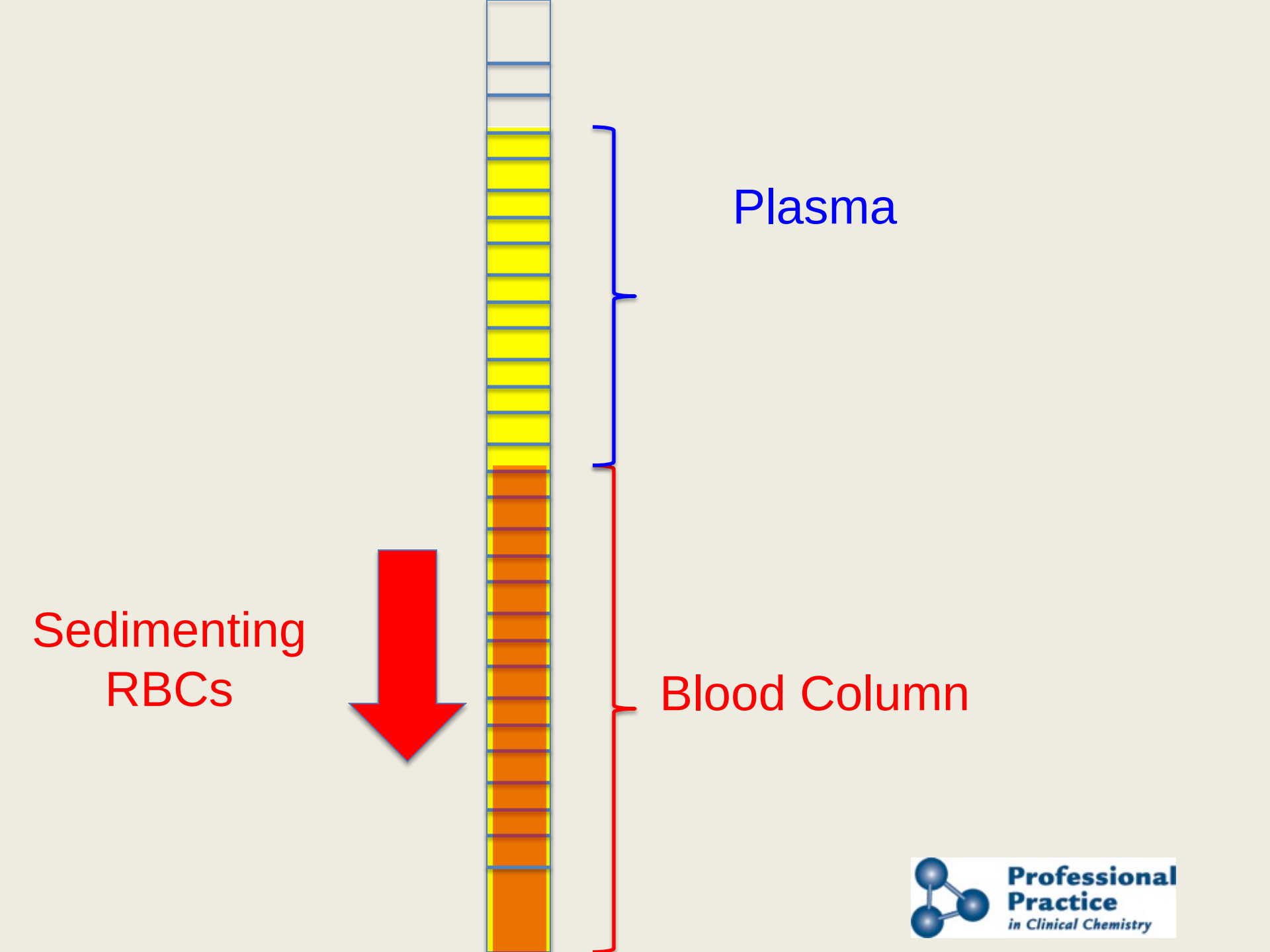
Erythrocyte sedimentation rate or ESR

- Anticoagulated whole blood (4 parts whole blood: 1 part Na Citrate) is placed in a narrow-bore glass tube tube. Erythocytes are allowed to sediment at room temperature.
- ESR measures the distance, in millimeters, that erythrocytes fall in 1 hour.


Plasma
Sedimenting
RBCs
Blood Column

Erythrocyte sedimentation rate or ESR

- Rate of sedimentation is determined by plasma proteins. ESR *increases* with acute phase response
- This is an *indirect* determination of inflammation
- Used to follow rheumatoid arthritis, SLE, vasculitis and many inflammatory conditions
- VERY LOW SPECIFICITY

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Erythrocyte sedimentation rate or ESR

- Westergren Method: 200 mm tube
- Wintrobe Method: 100 mm tube
- Modern Methods: Semi-automated systems that measure sedimentation by infra-red light. Measured over a shorter period than 1 hour & extrapolated to give Westergren ESR.
- Can interface with LIS

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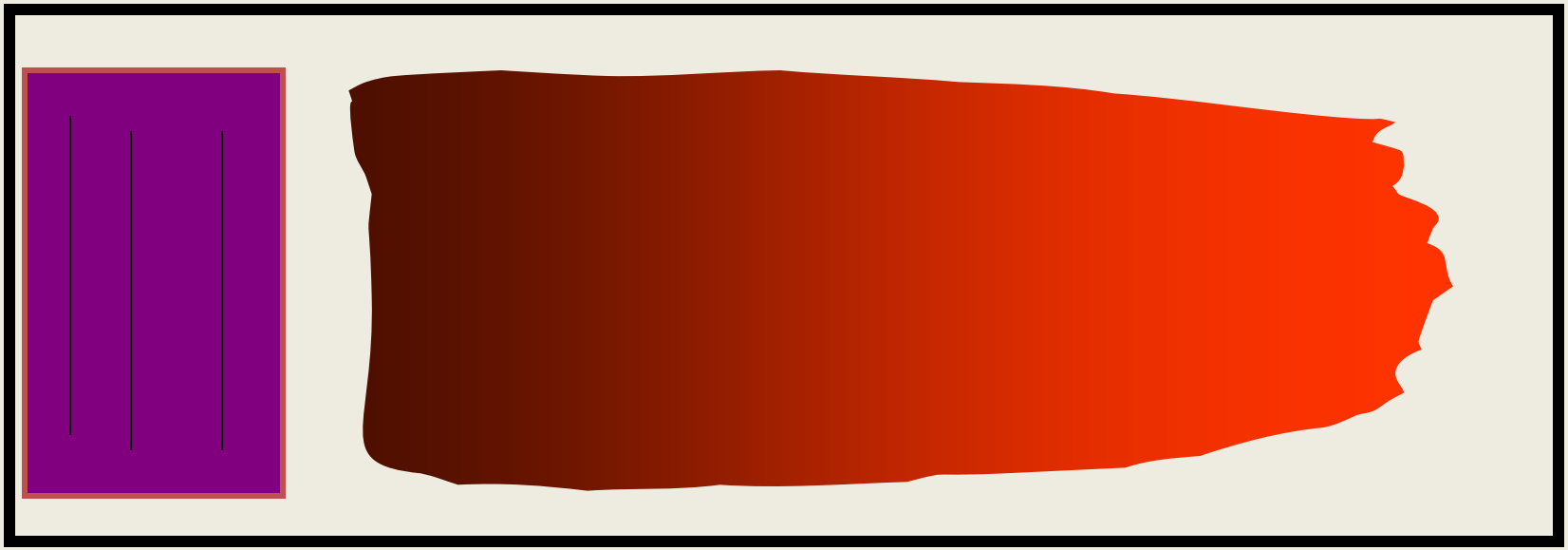
BLOOD SMEAR

Presented by AACC and NACB



The Blood Smear

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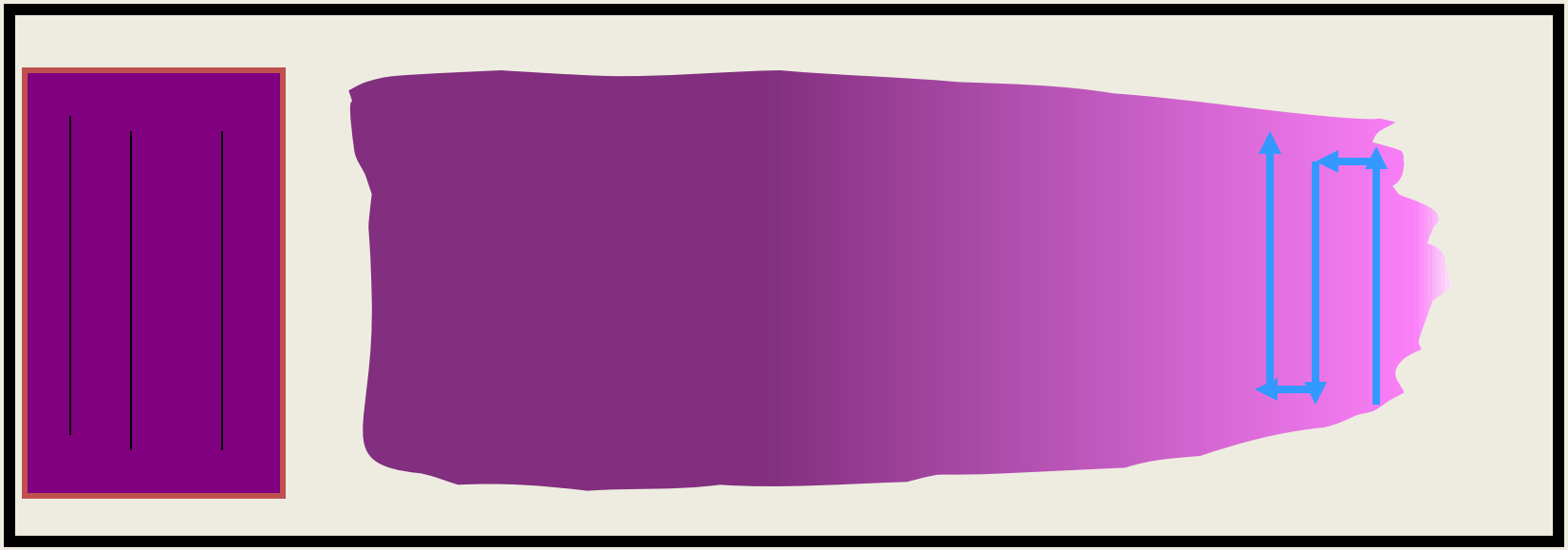
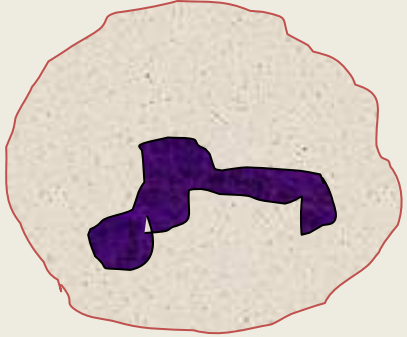
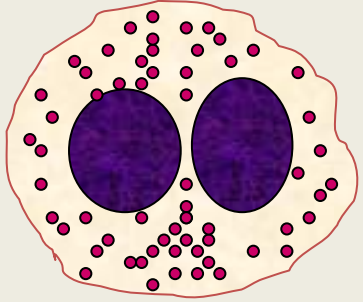


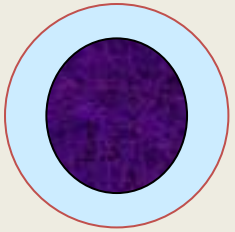
Stained
Blood
Smear



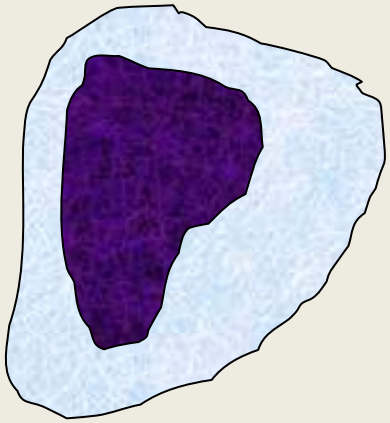
Neutrophil



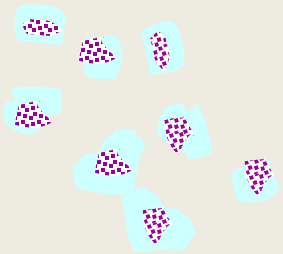
Eosinophil



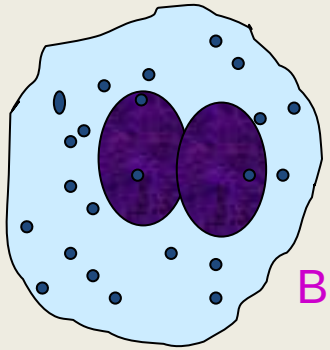
Lymphocyte



Monocyte



Platelets



Basophil

MYELOID

LYMPHOID

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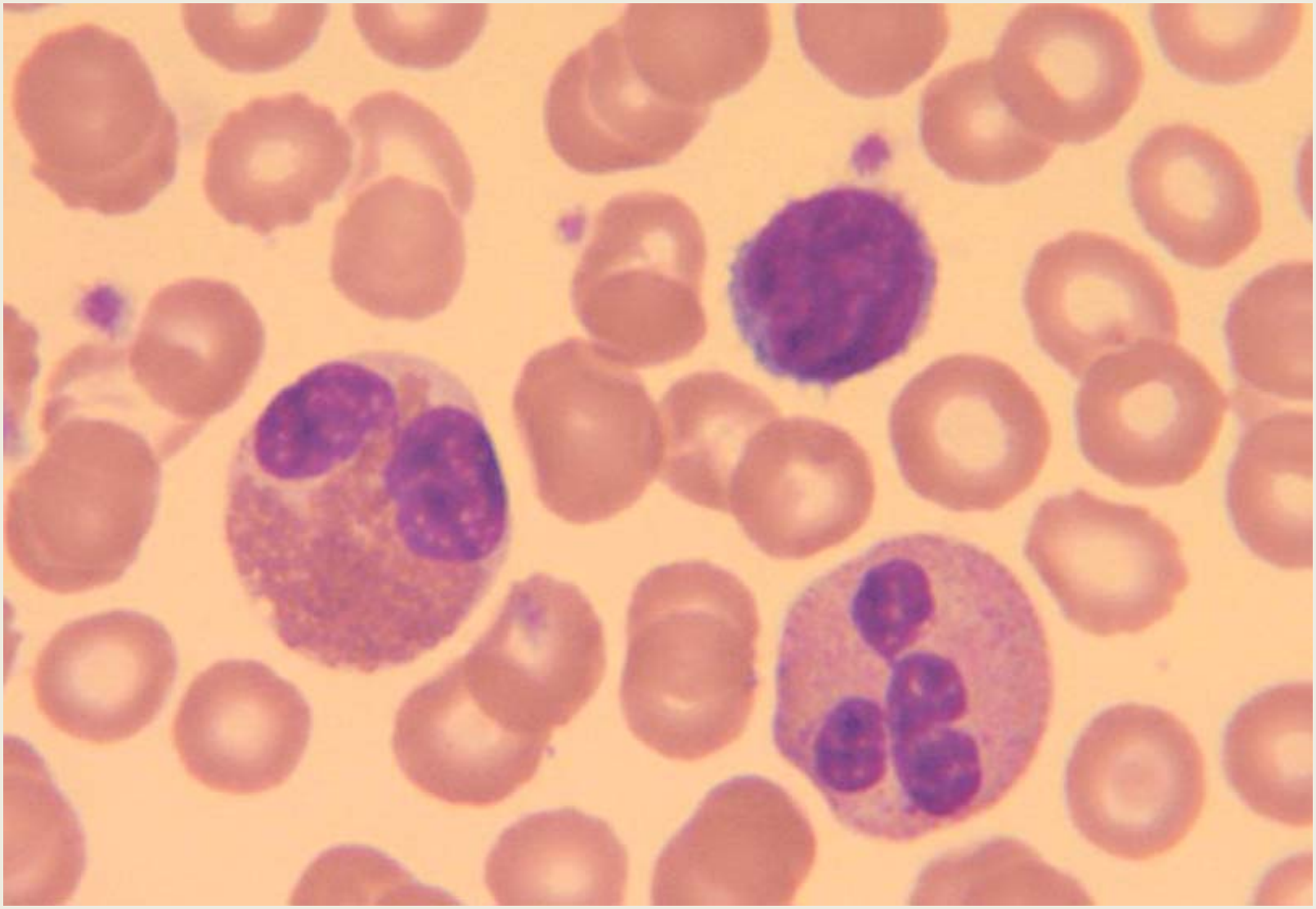
A "Differential Count"

1. Stained peripheral blood smear is examined carefully using 40 x to 100 x objective – usually oil immersion lens
2. 100 white blood cells are counted
3. Cells are classified by morphology→

Neutrophils
Bands
Lymphocytes
Monocytes
Eosinophils
Basophils
Metamyelocytes
Myelocytes
Promyelocytes
Blasts



Eosinophil, Lymphocyte & Neutrophil

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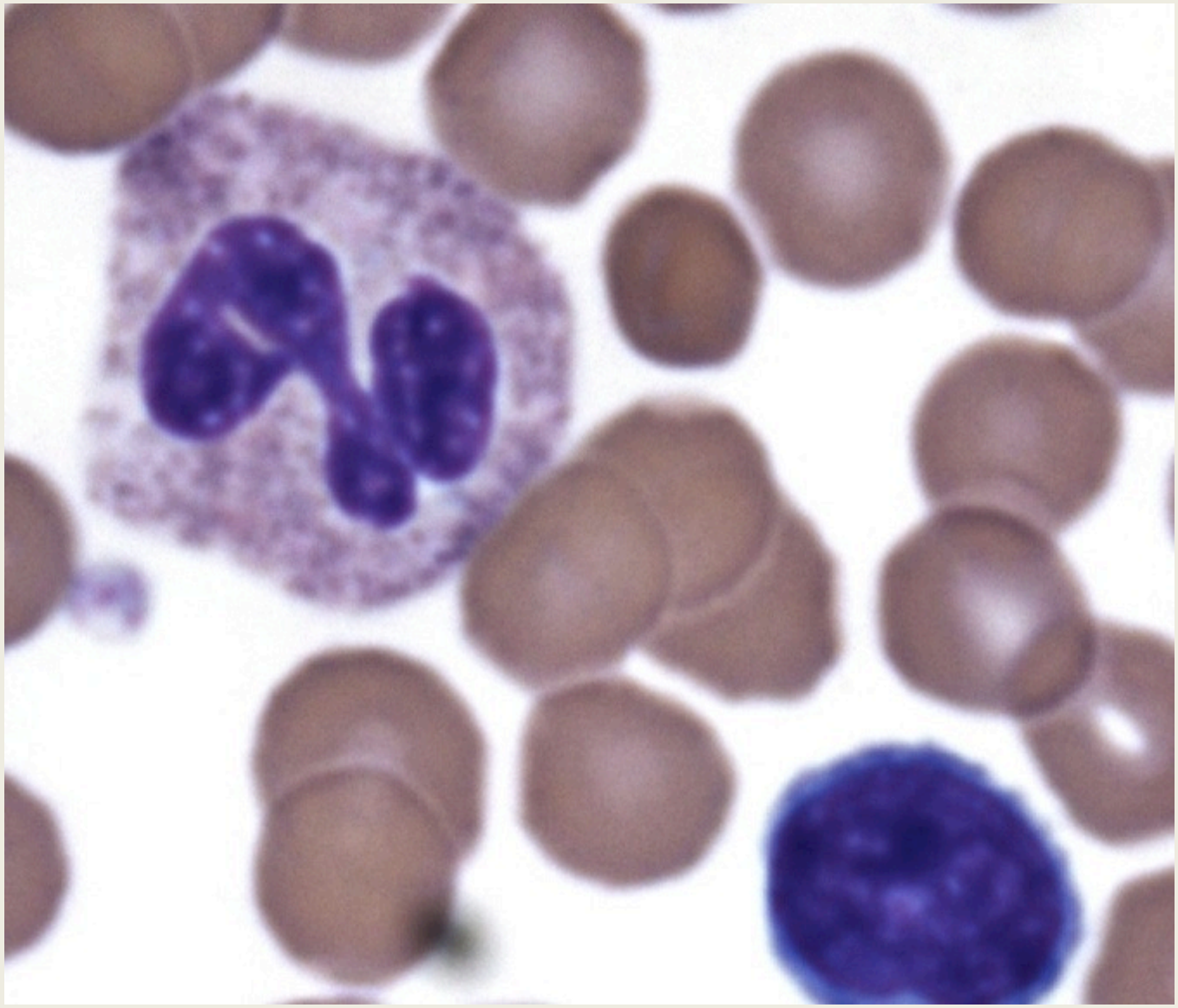

Neutrophil and Lymphocyte

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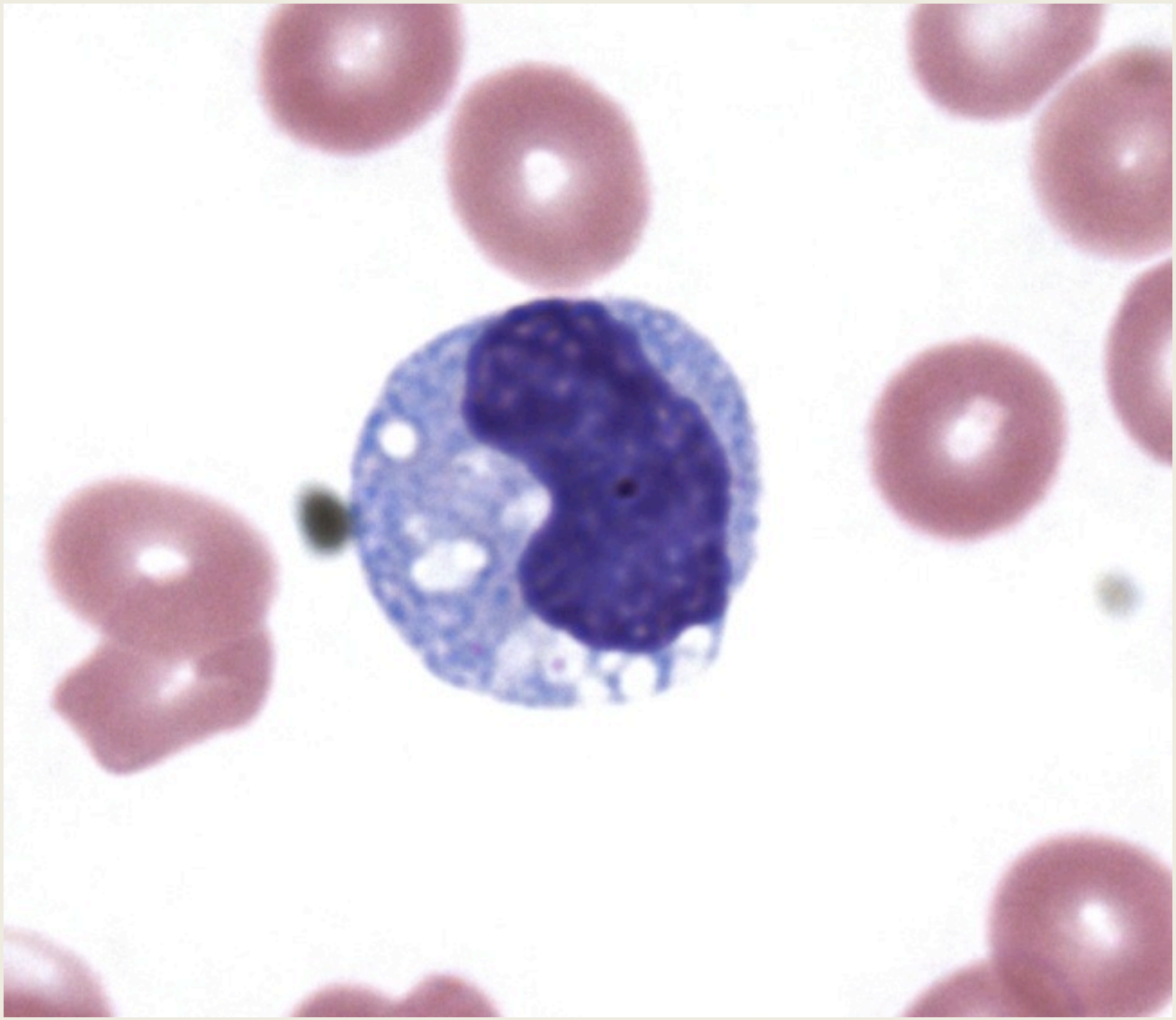
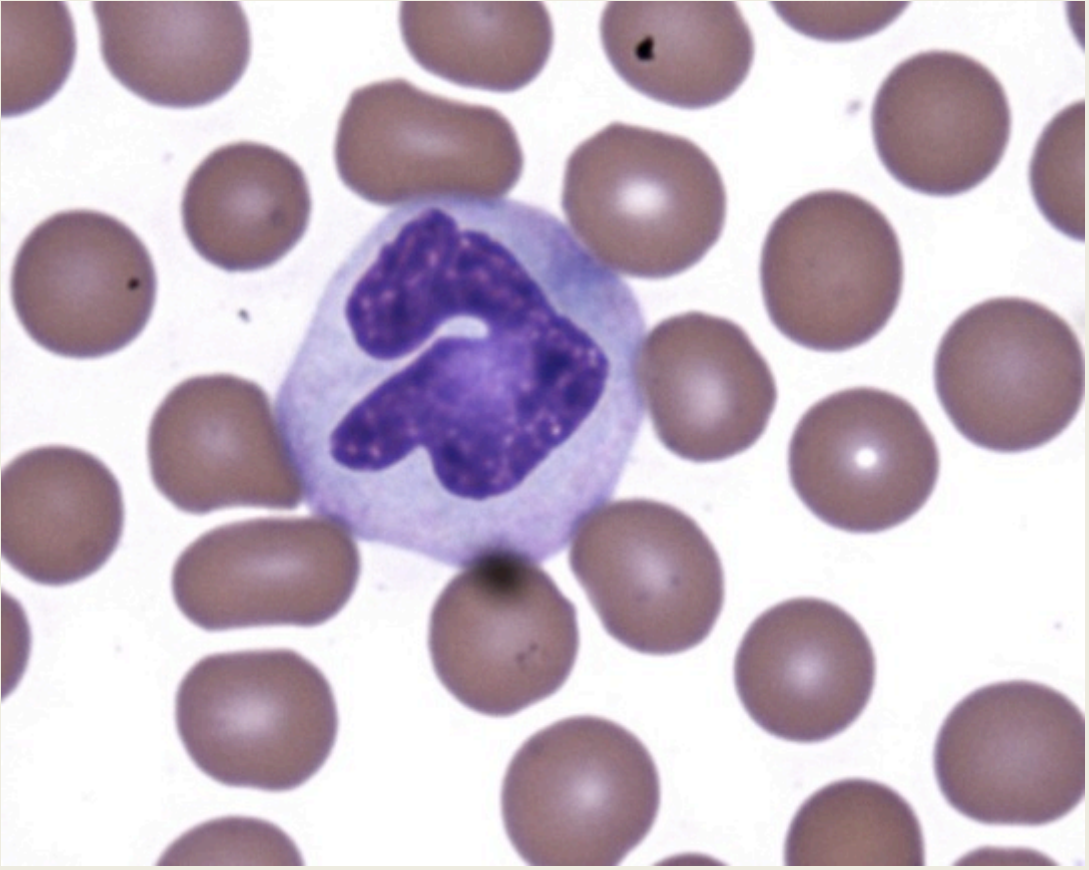
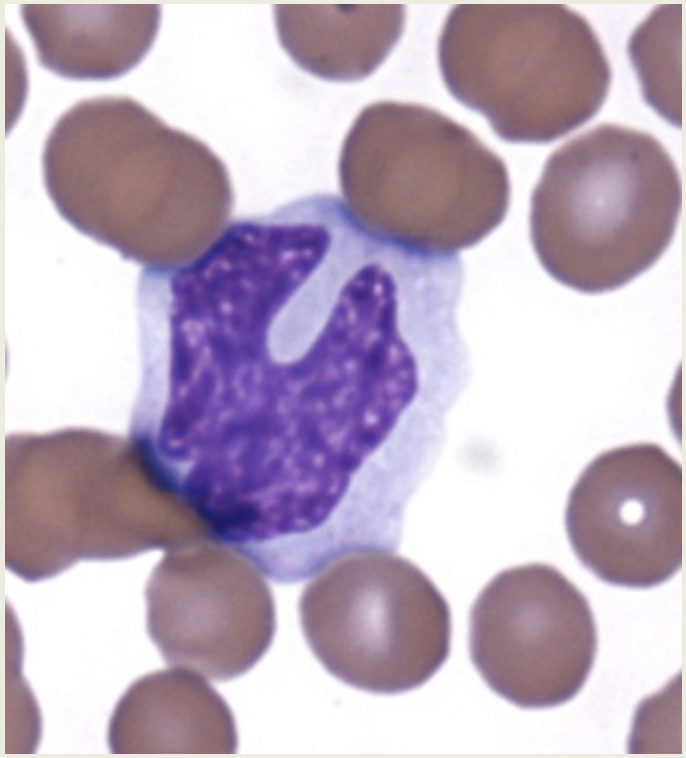
Neutrophils - The main phagocytic cells of peripheral blood.



Monocyte





Monocytes

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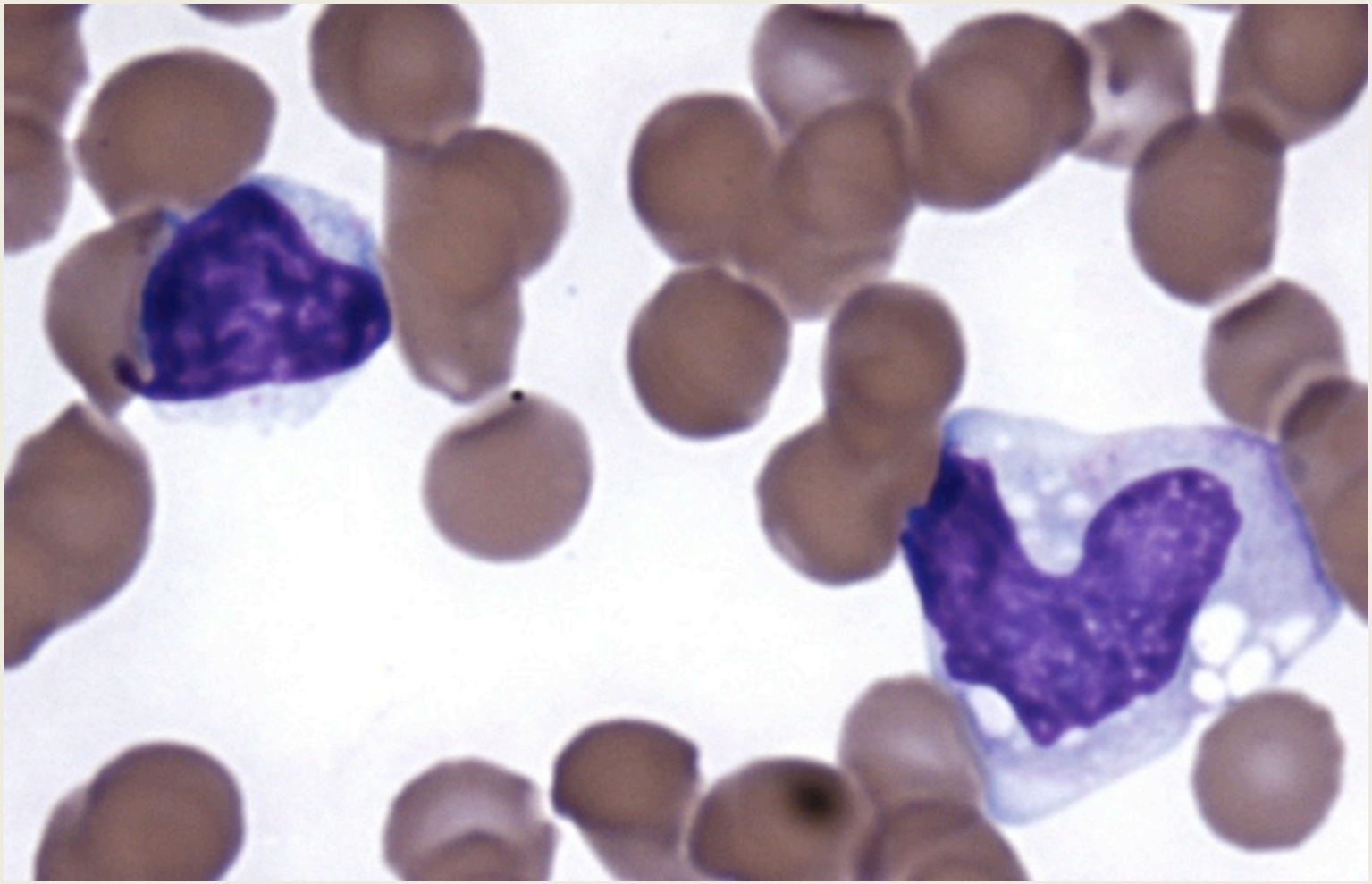

Monocyte and Lymphocyte

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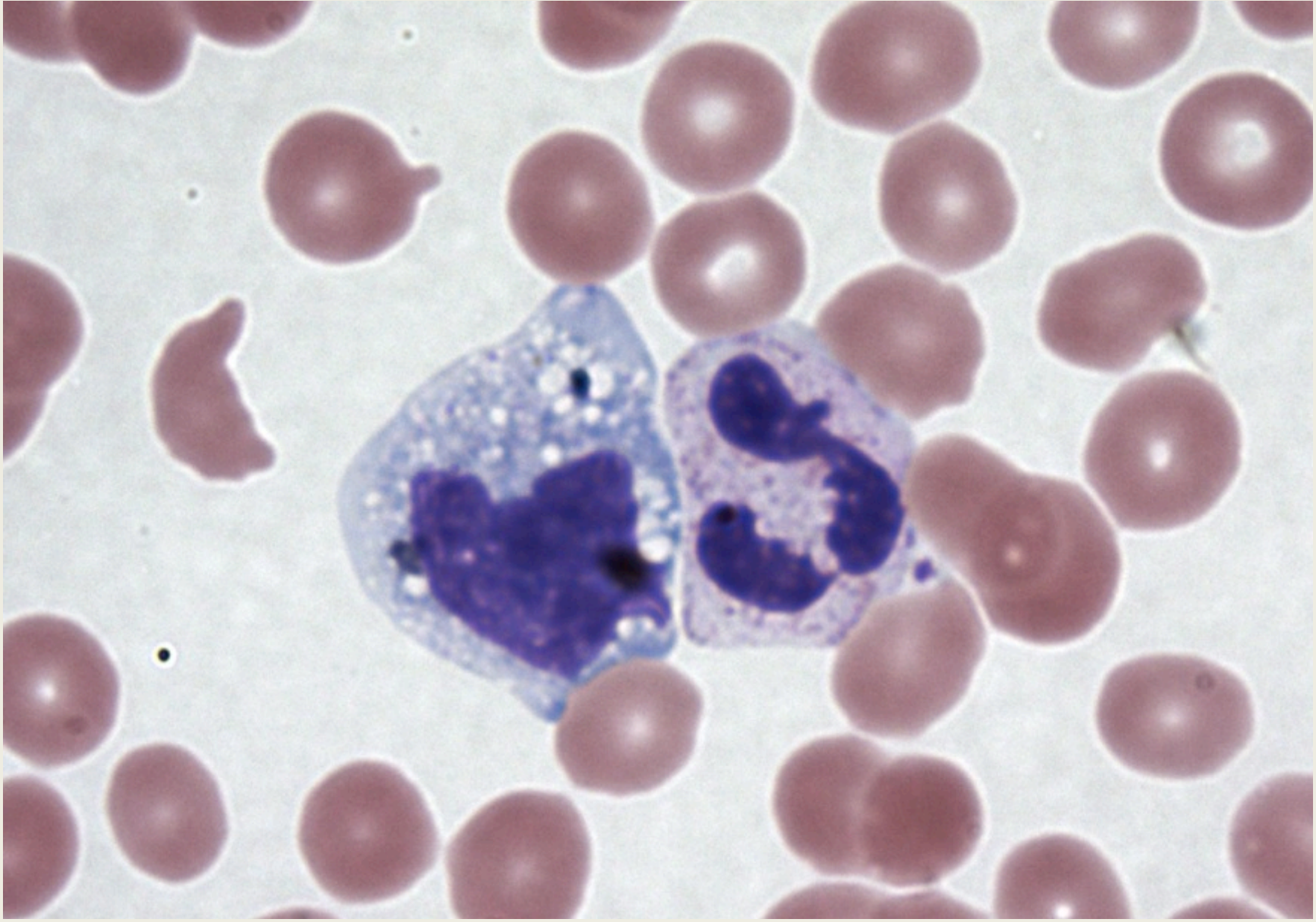

Monocyte and Neutrophil

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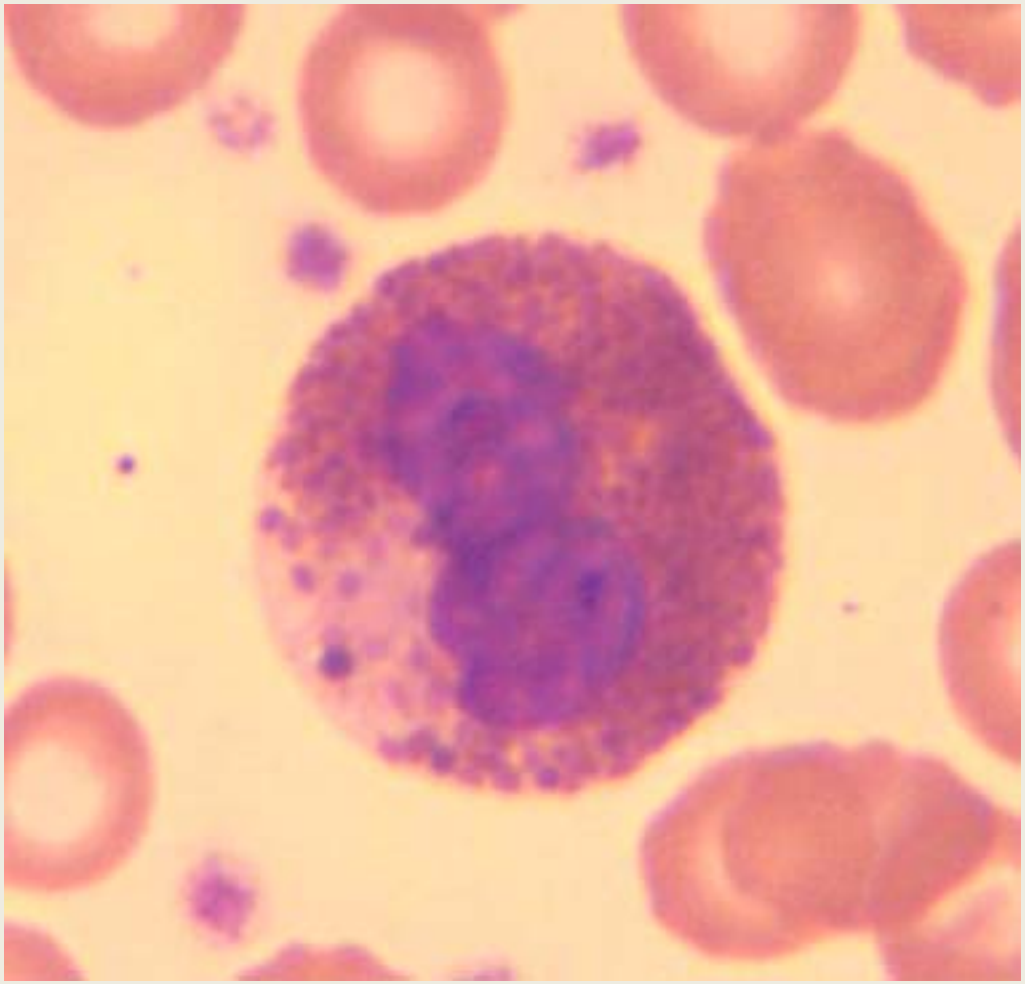
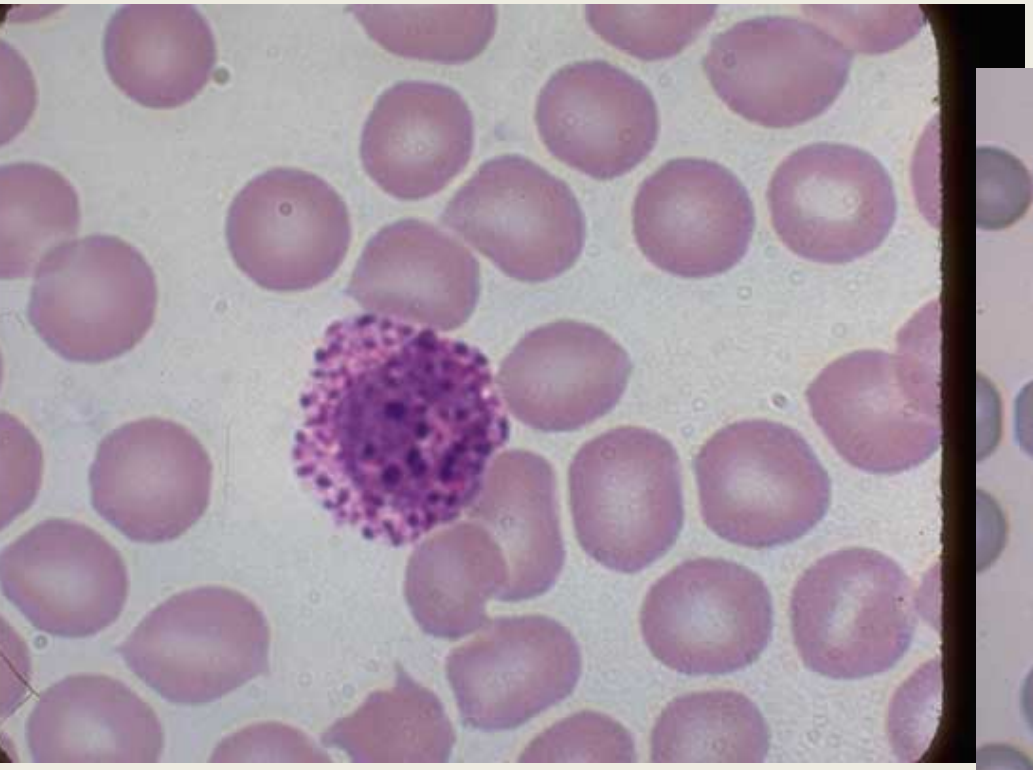
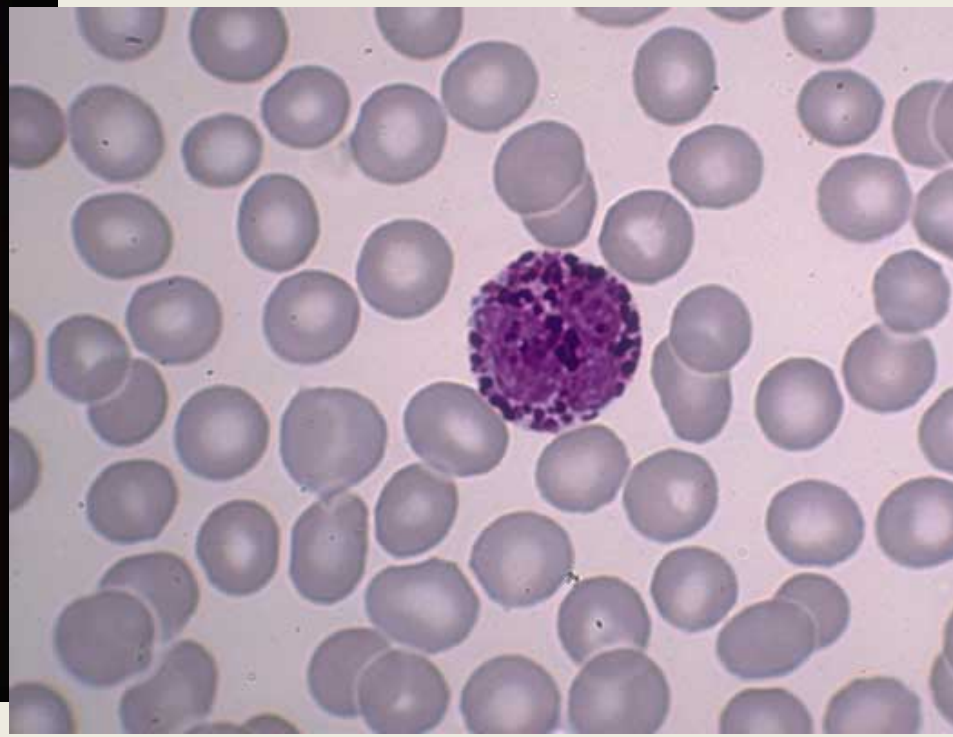


Eosinophil





Basophils - involved in IgE-mediated hypersensitivity response

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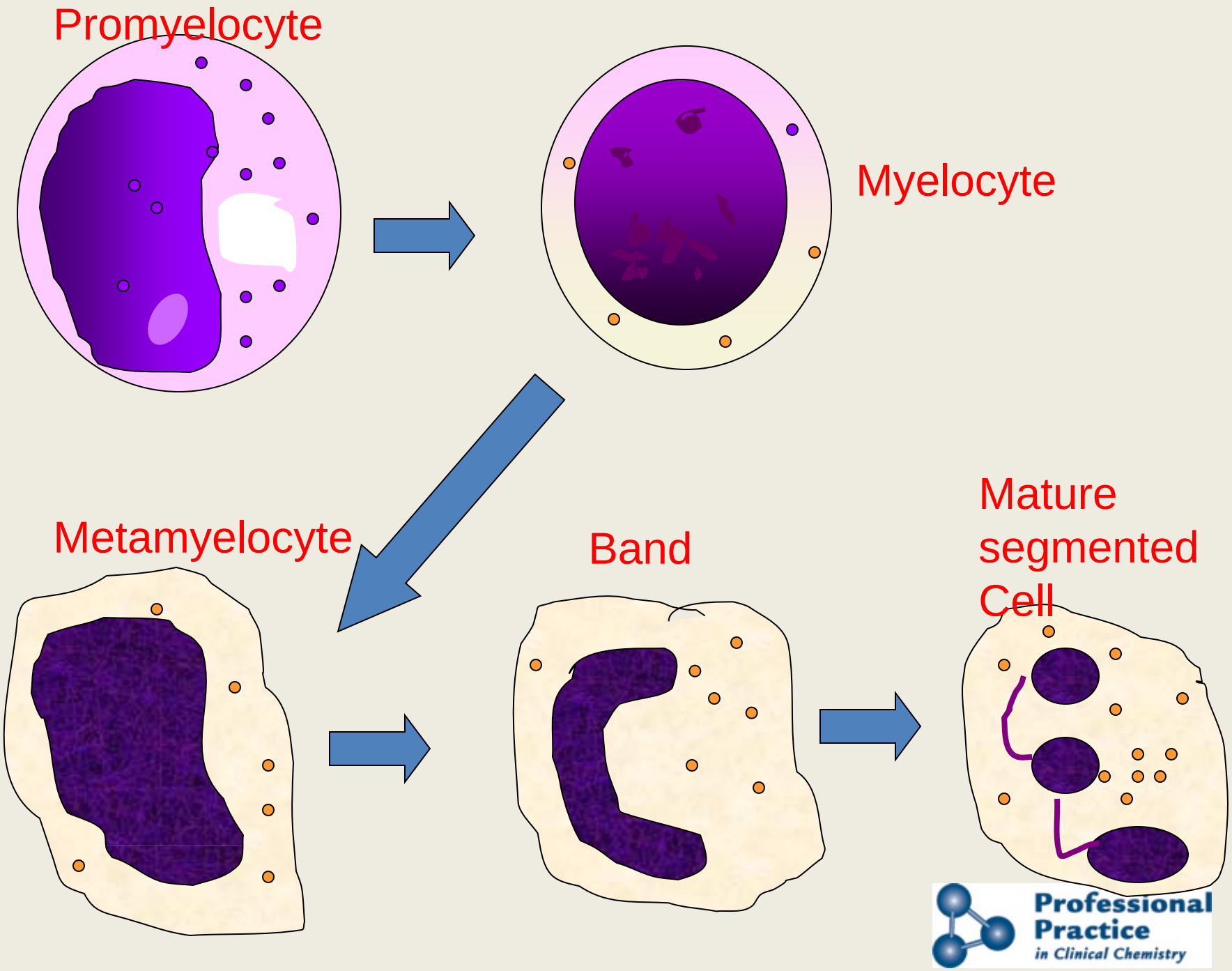
Neutrophil maturation

Presented by AACC and NACB


Promyelocyte
Myelocyte
Metamyelocyte
Band
Mature segmented Cell
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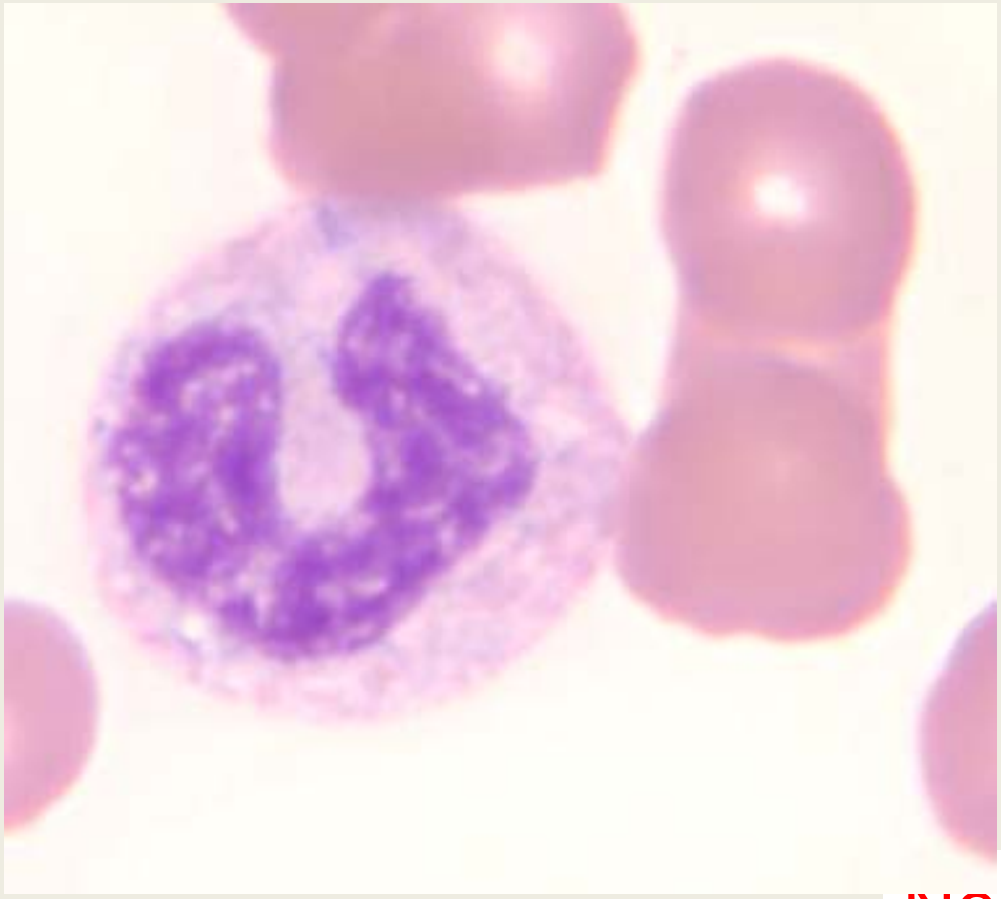
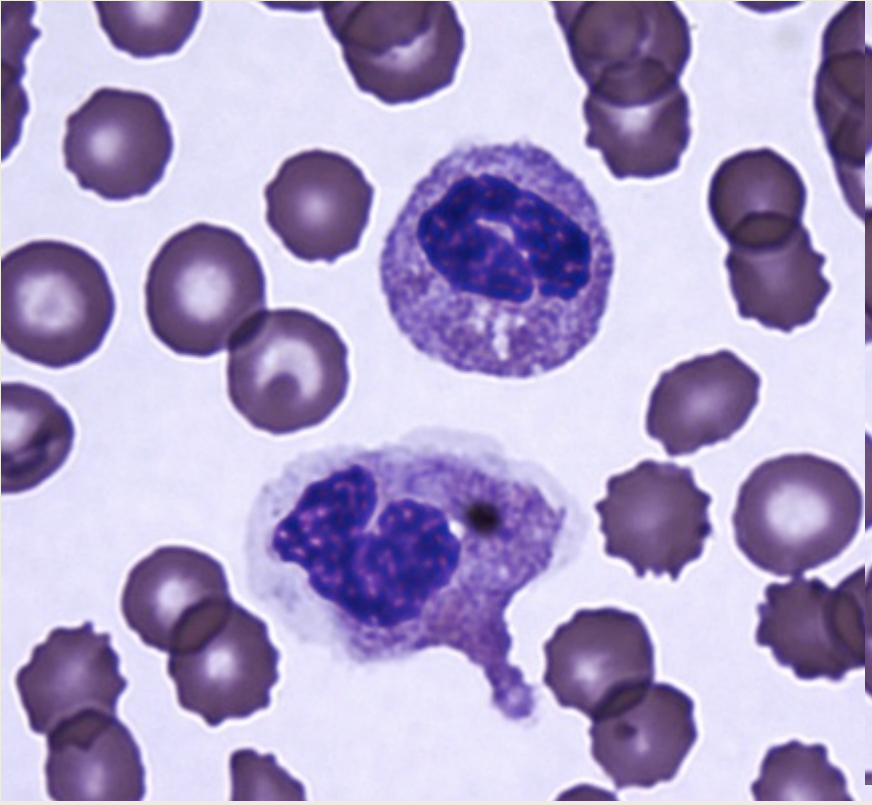
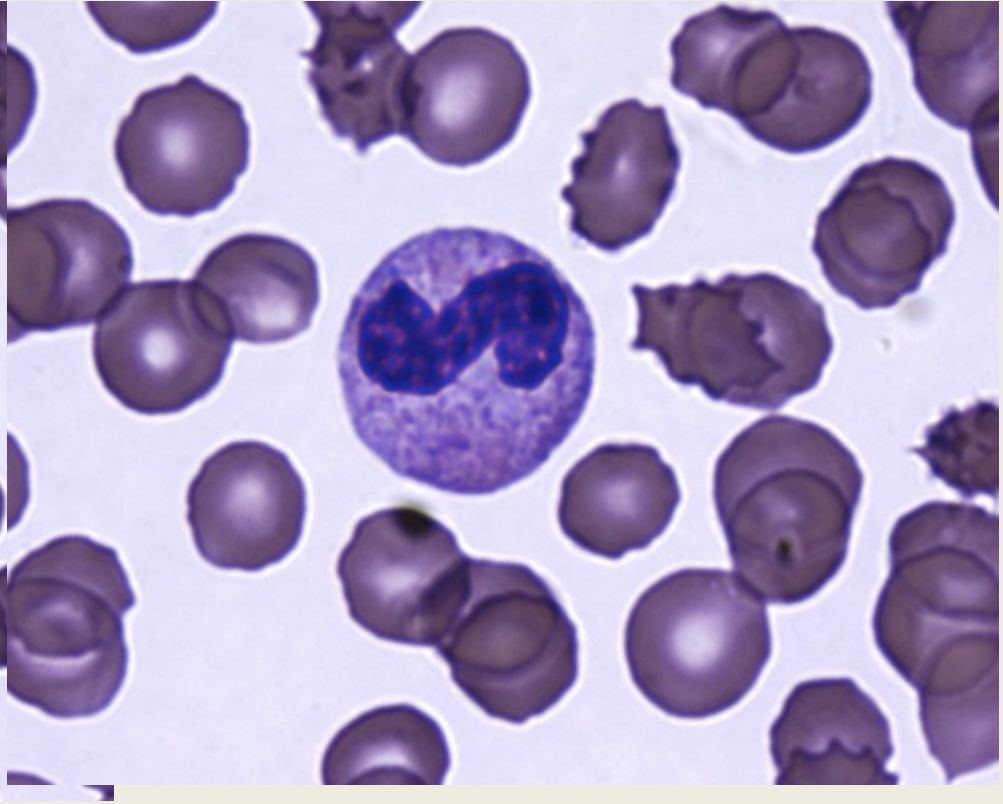
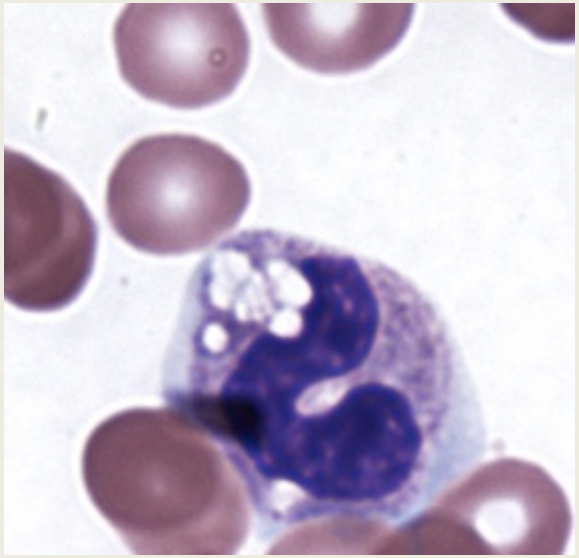


Neutrophil “Bands” - immature cells, classically with a “horseshoe-shaped” nucleus.







BANDS

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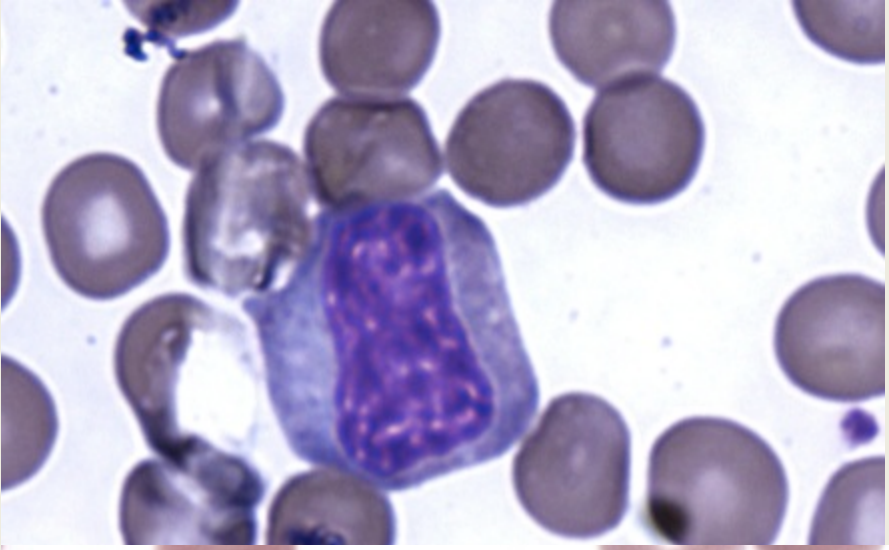

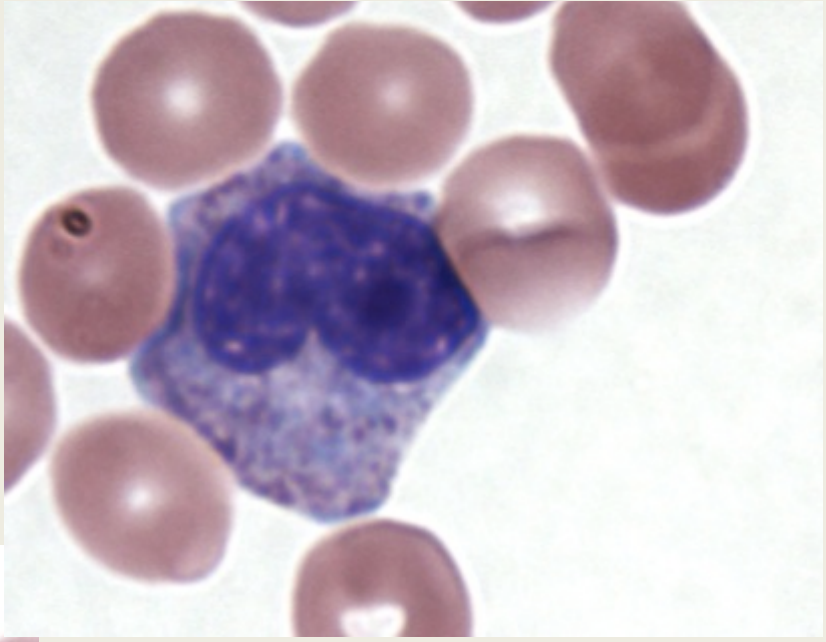

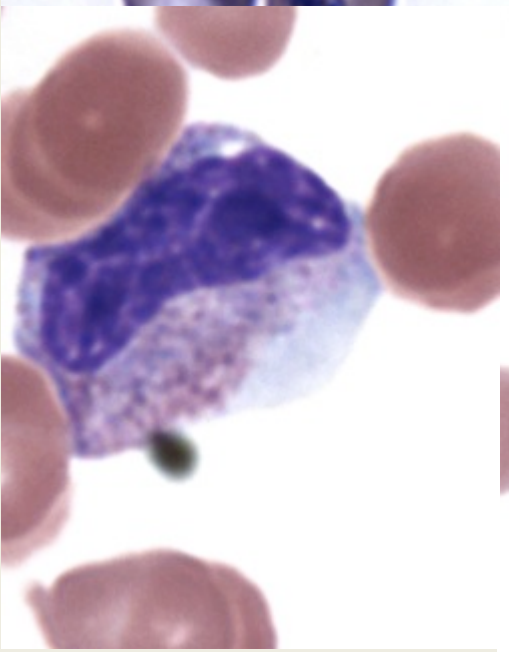

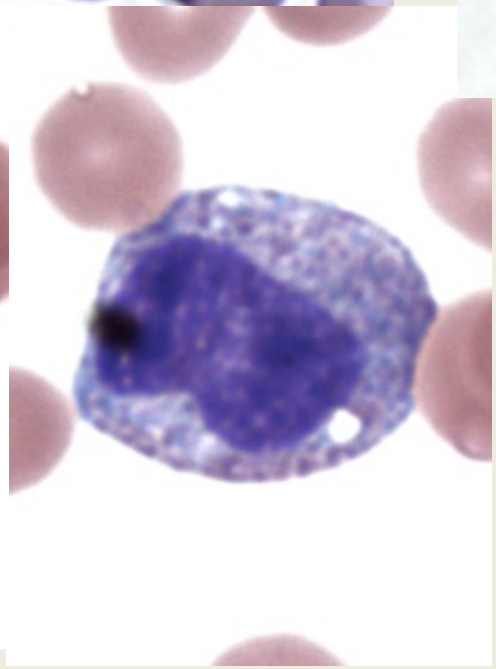

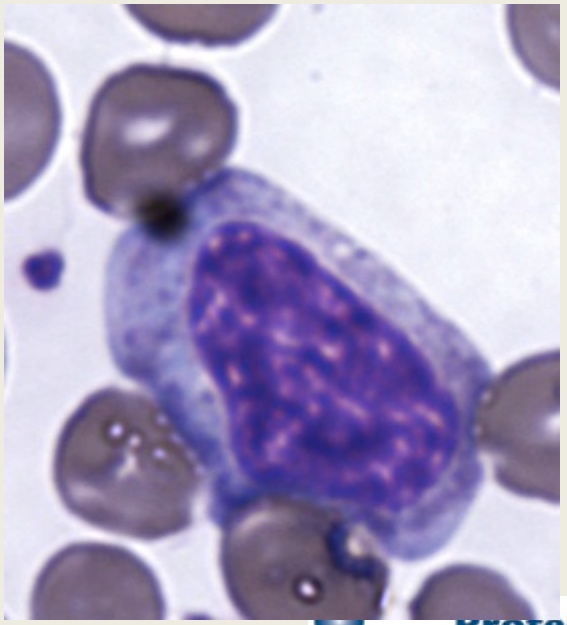

METAMYELOCYTES

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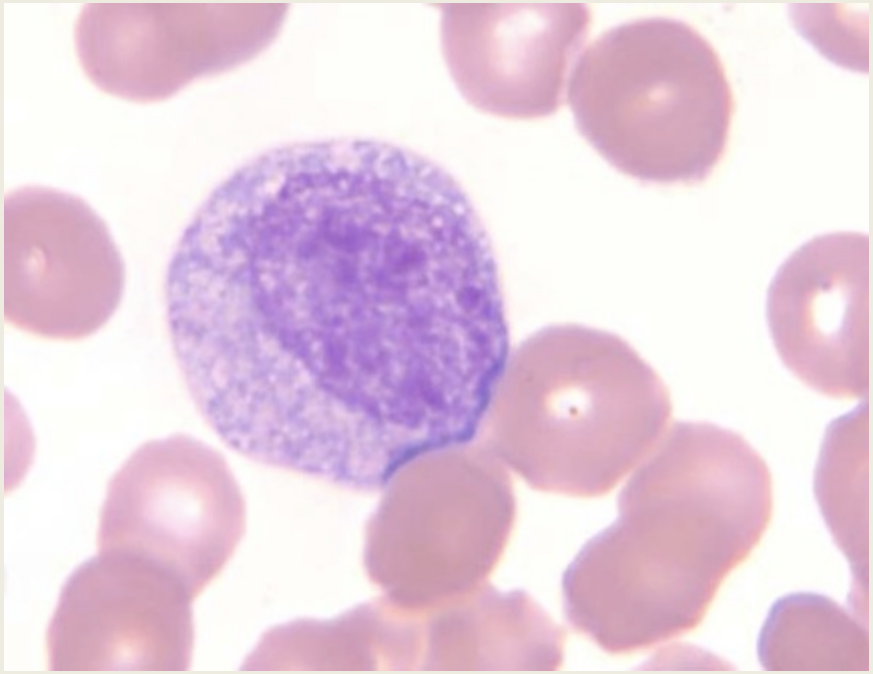
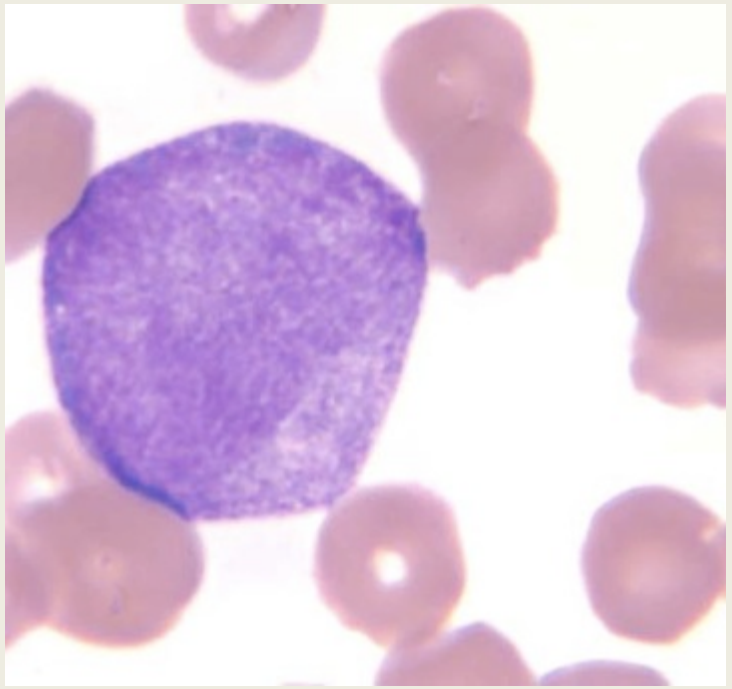
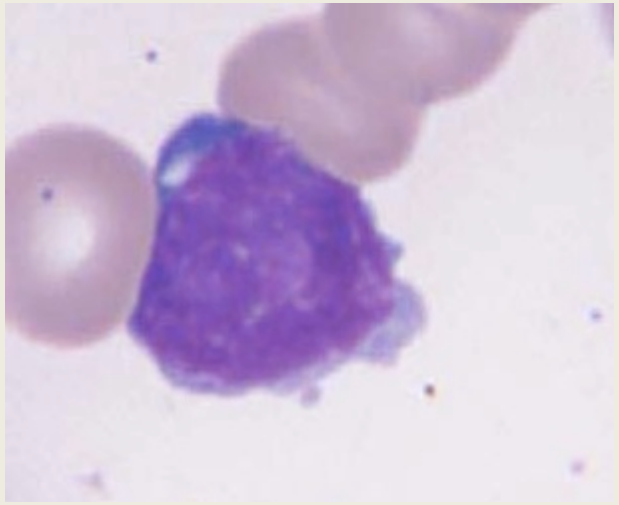
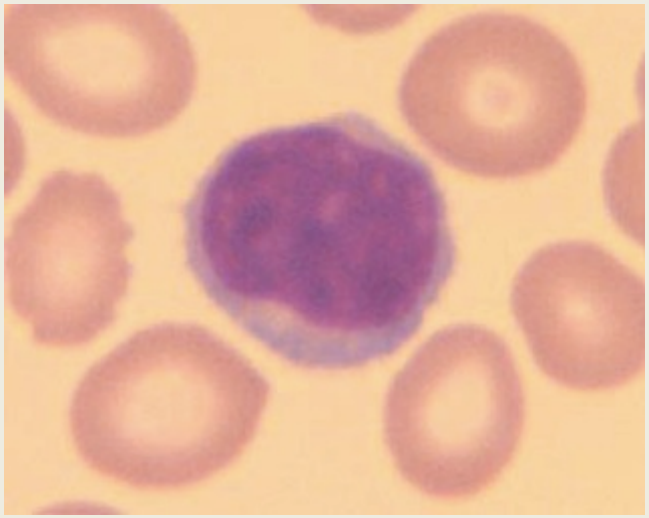
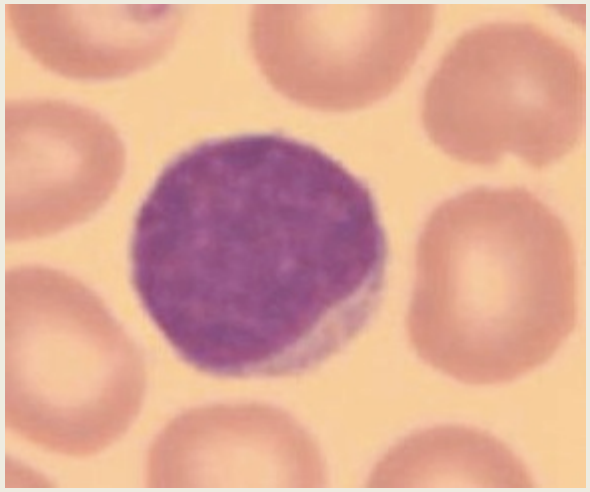




Myelocytes - very immature neutrophils with a round or near-round nucleus







Lymphocytes -classic small variety;

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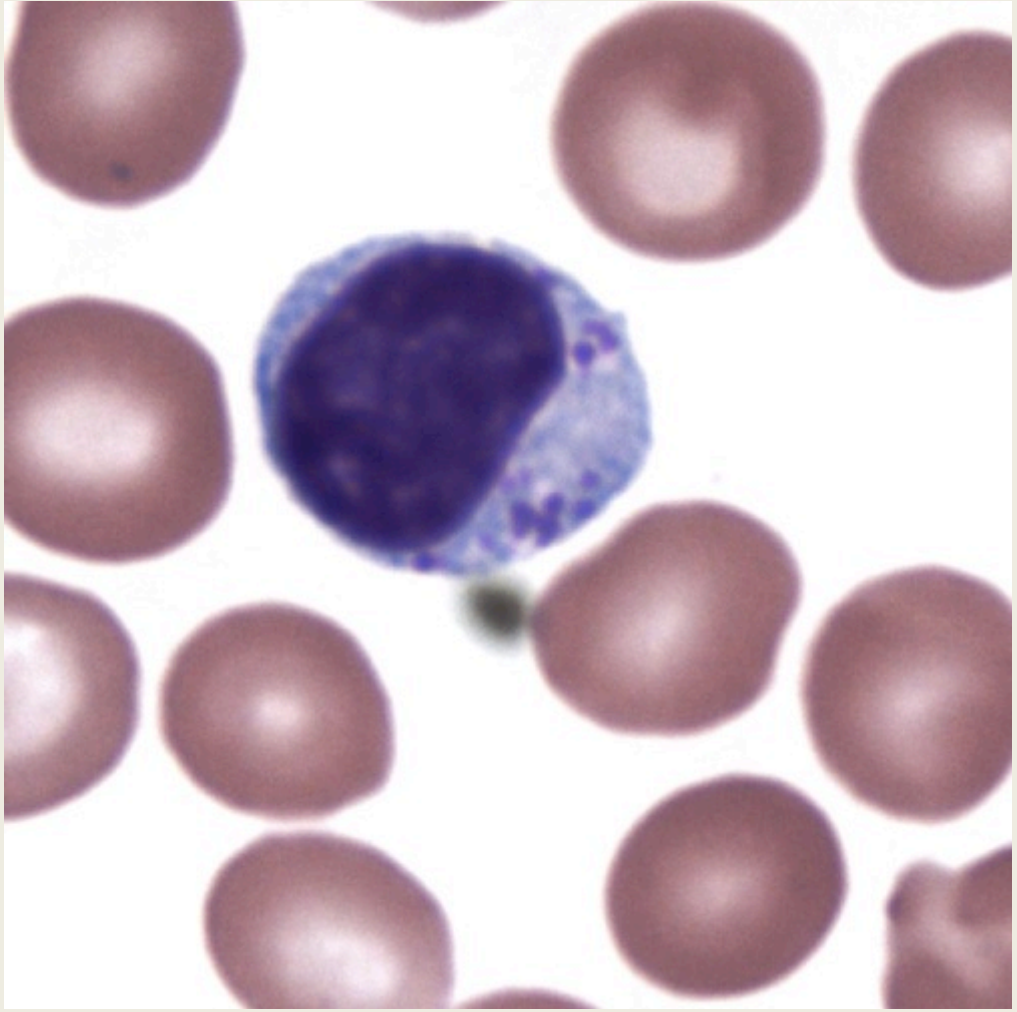
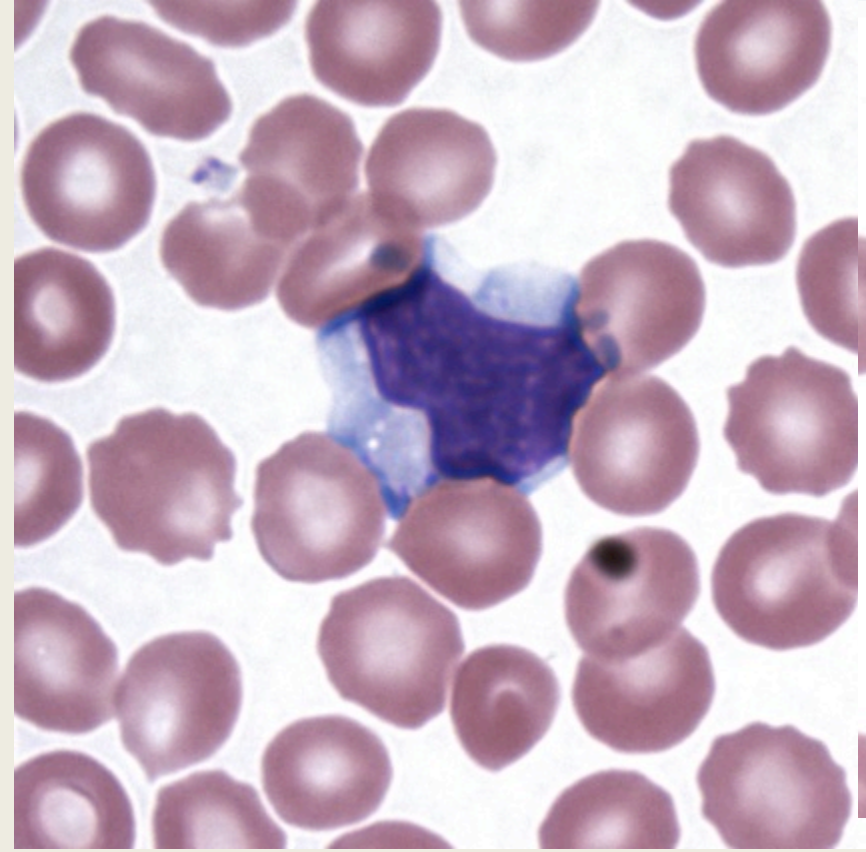
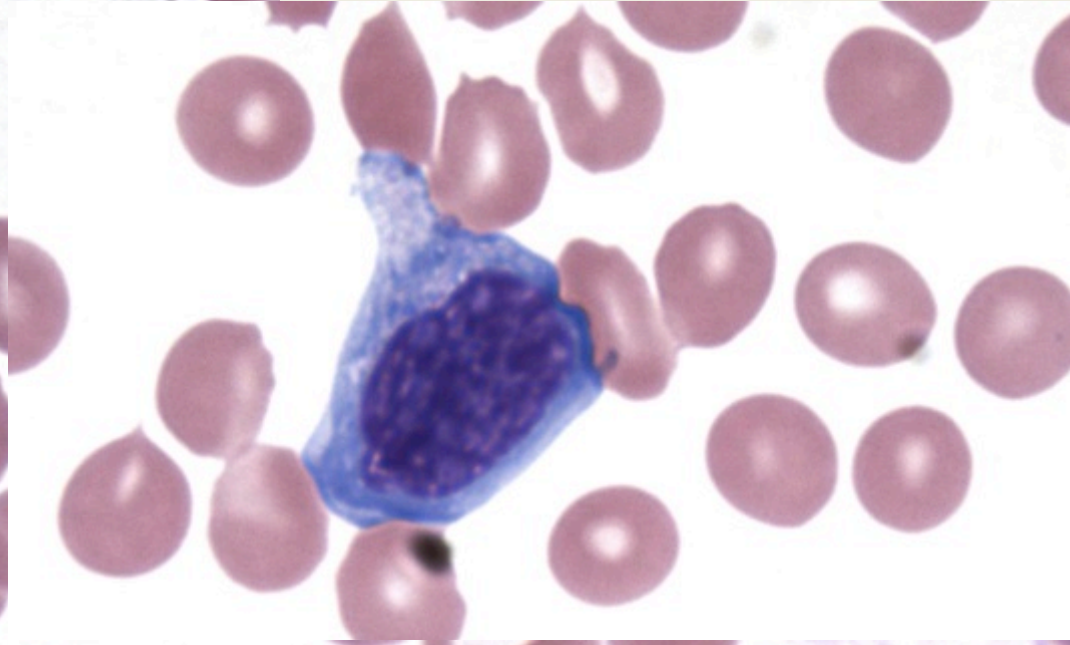
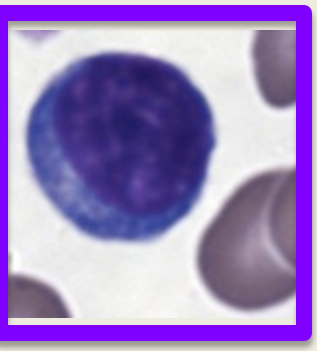


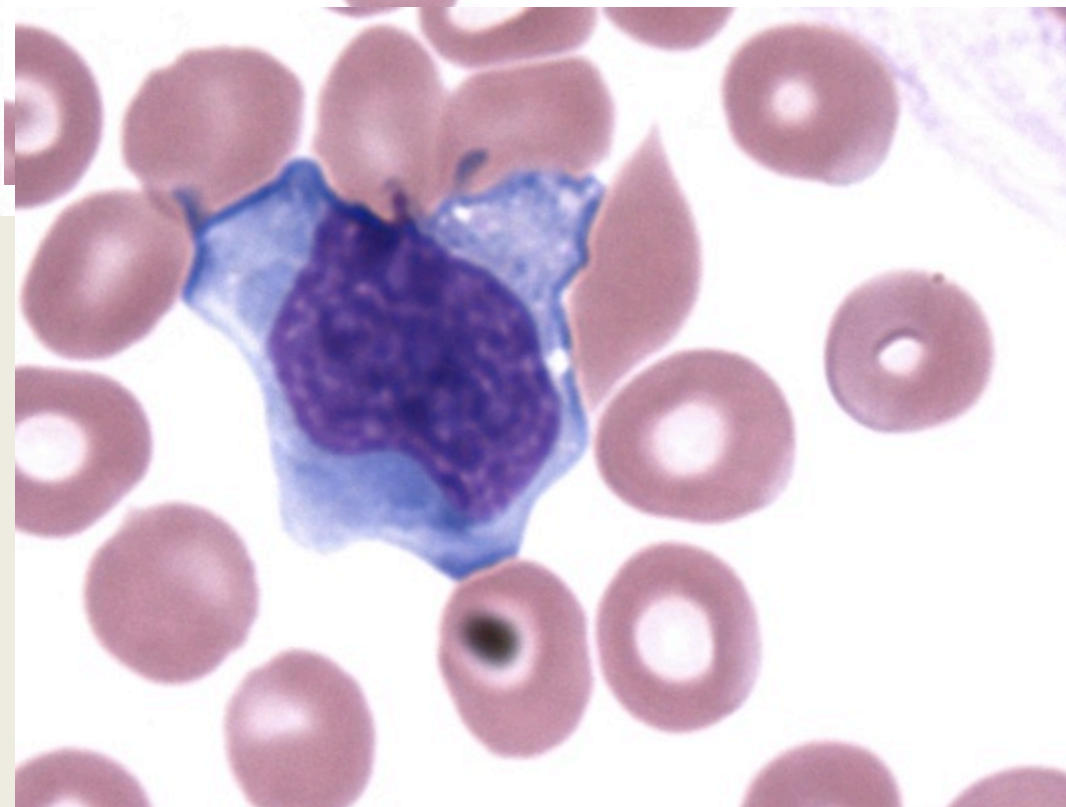
Lymphocytes –Large granular (“LGL”)



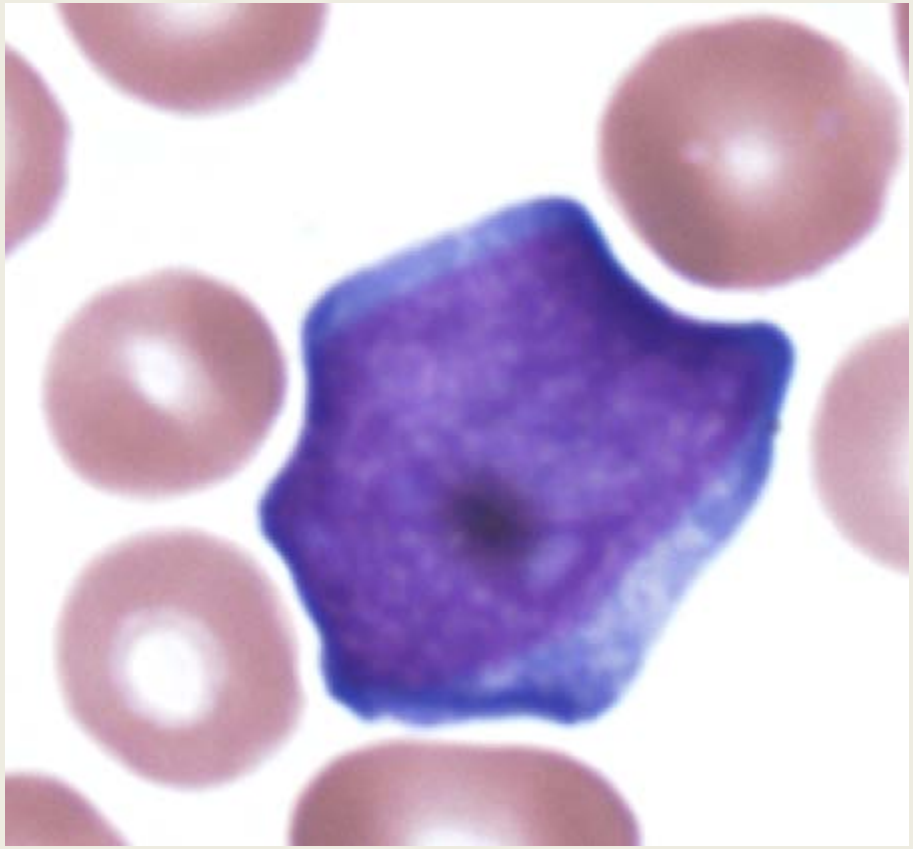




NORMAL



Atypical Lymphocytes



Blast

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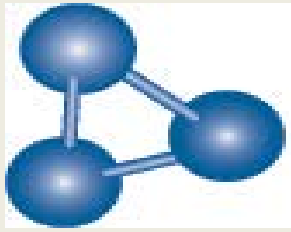

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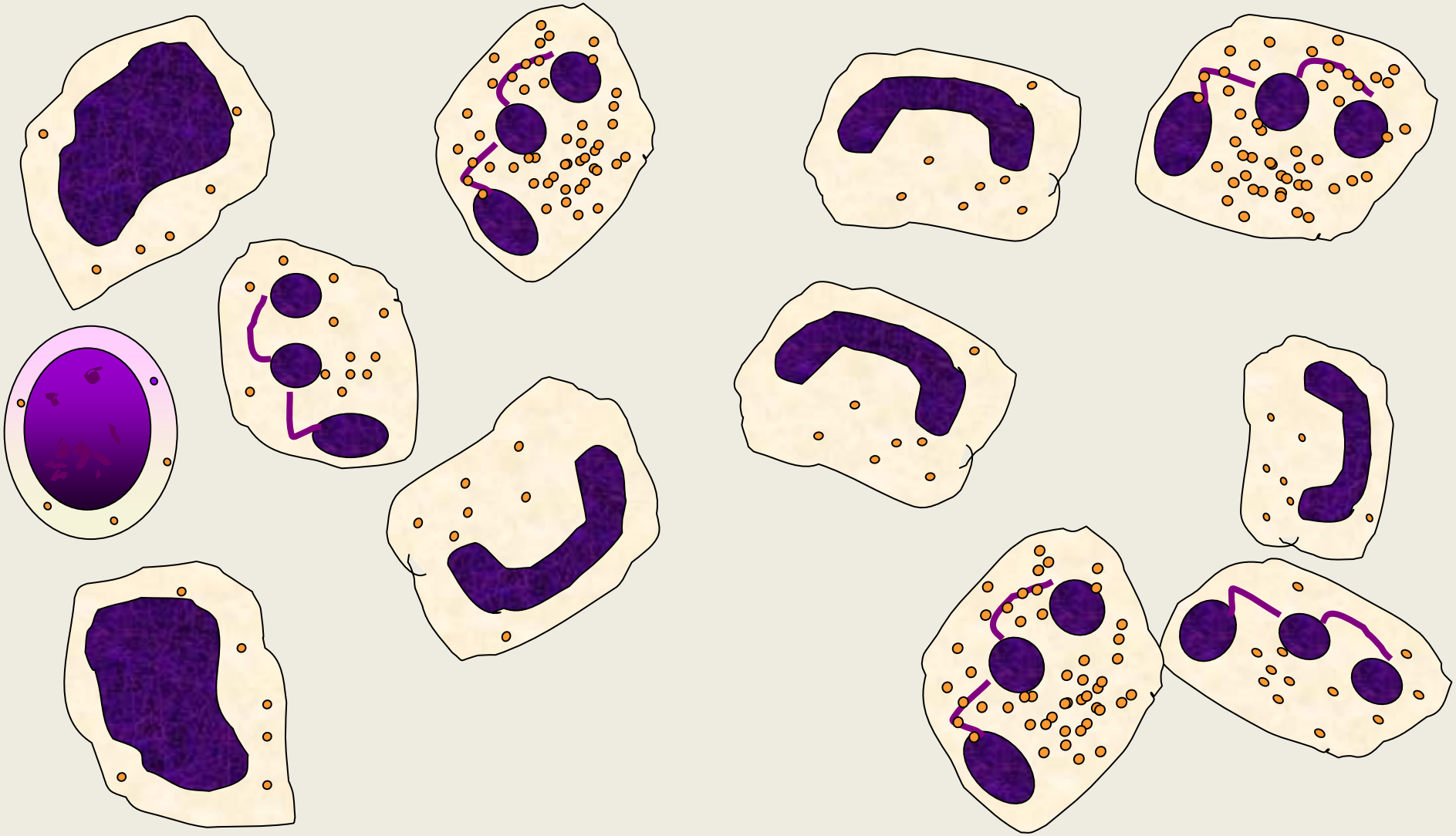
Common Blood "Pictures"

Presented by AACC and NACB



Reactive neutrophilia with “left shift”

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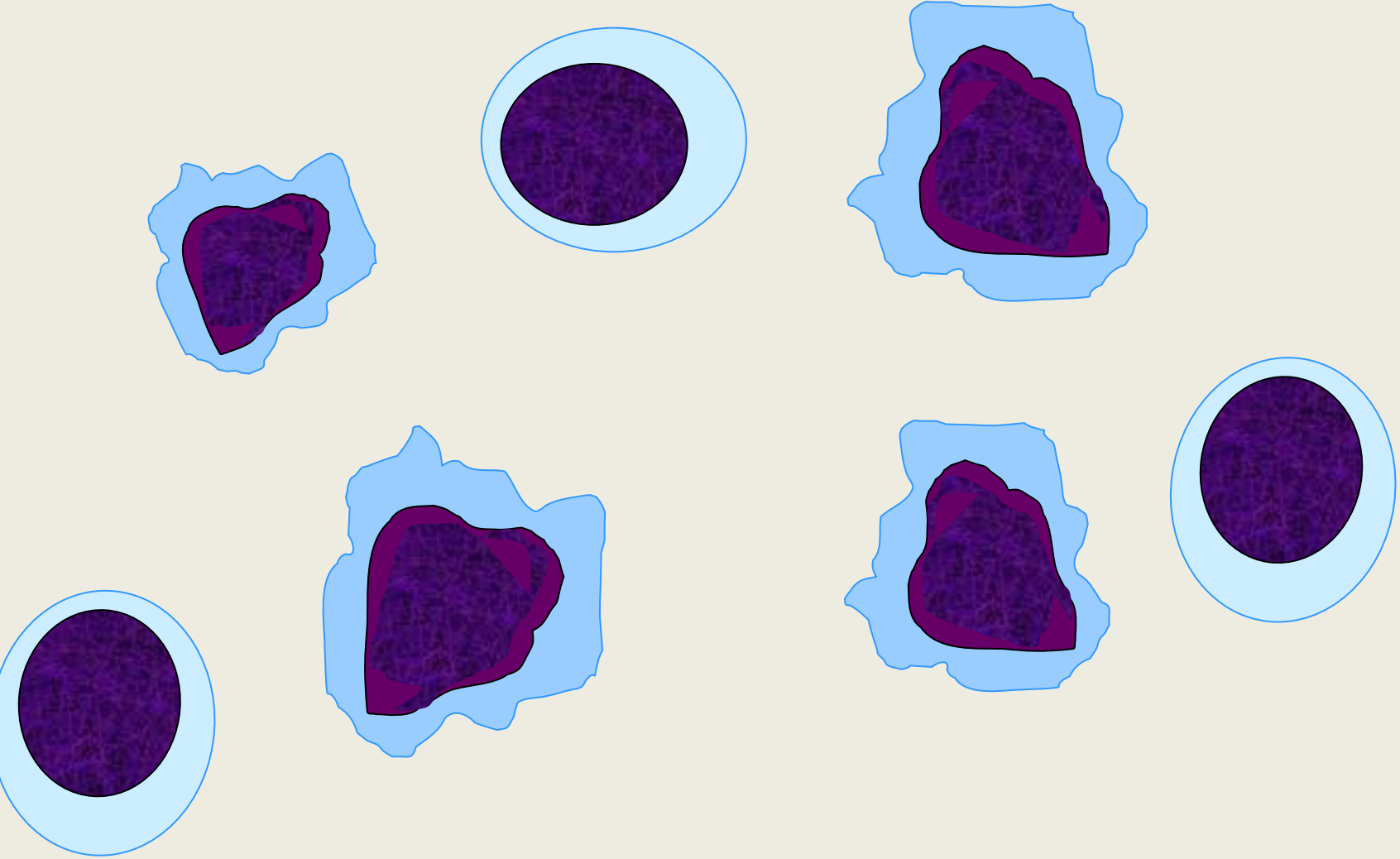

Atypical Lymphocytes (reactive)

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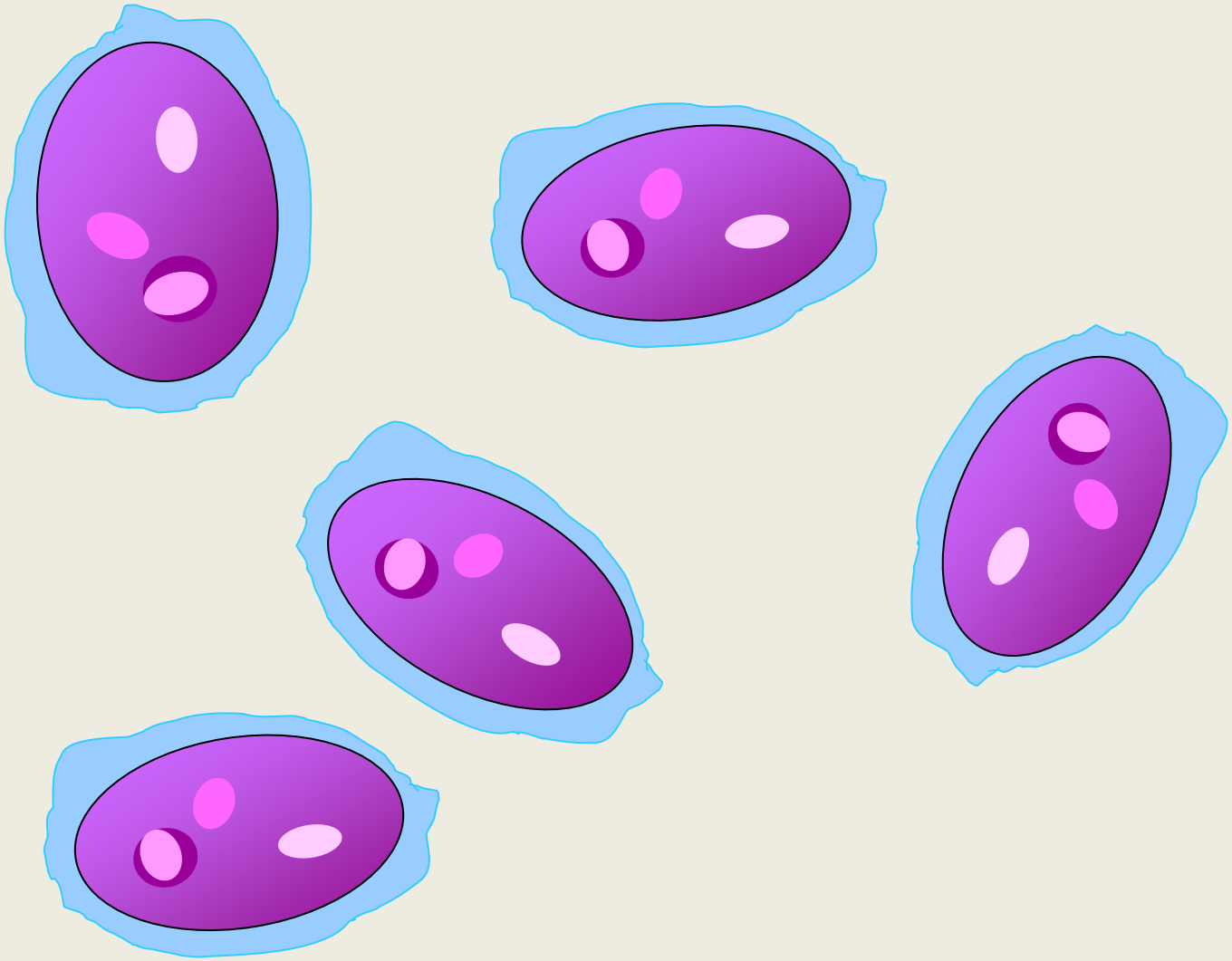
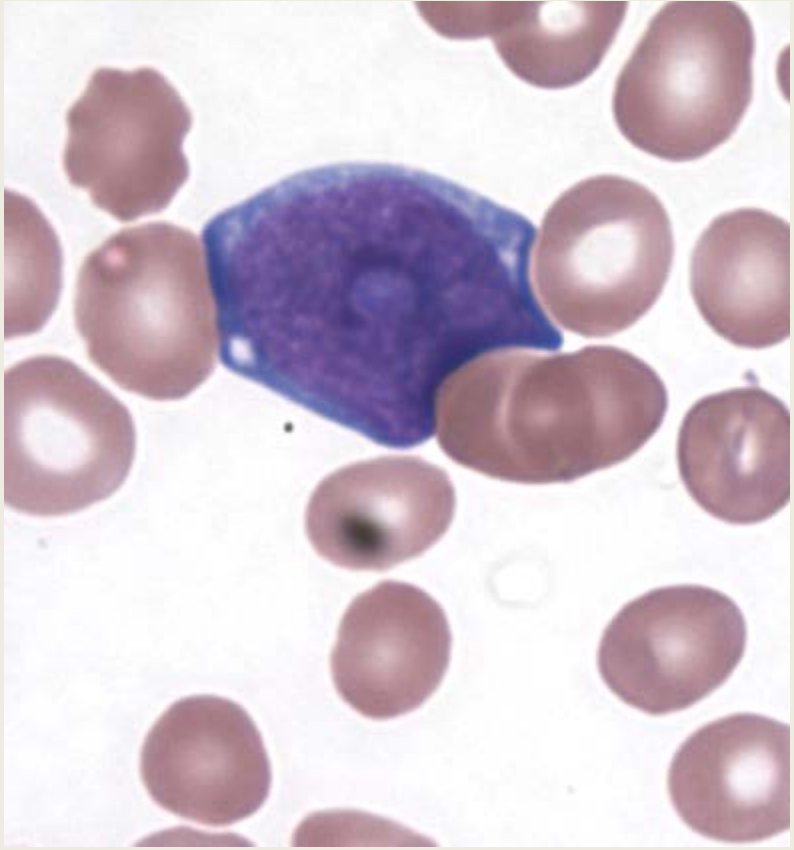
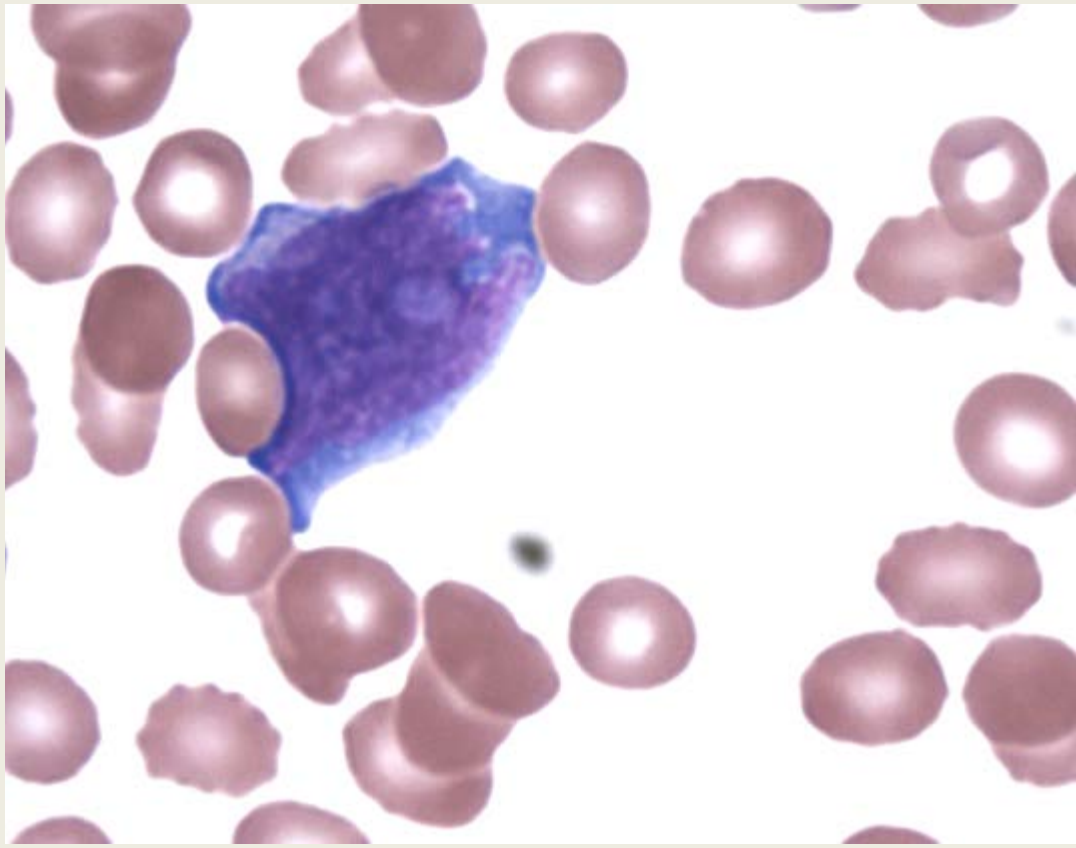


Acute Leukemia with larger blasts





Blast

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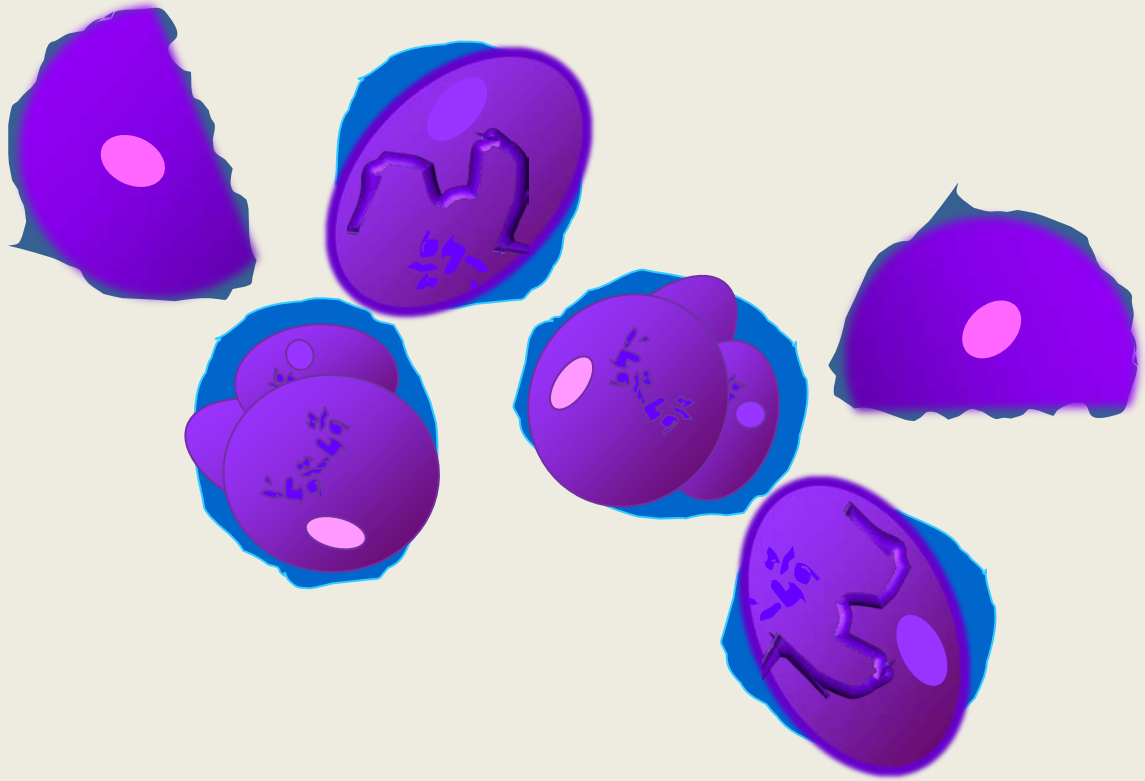
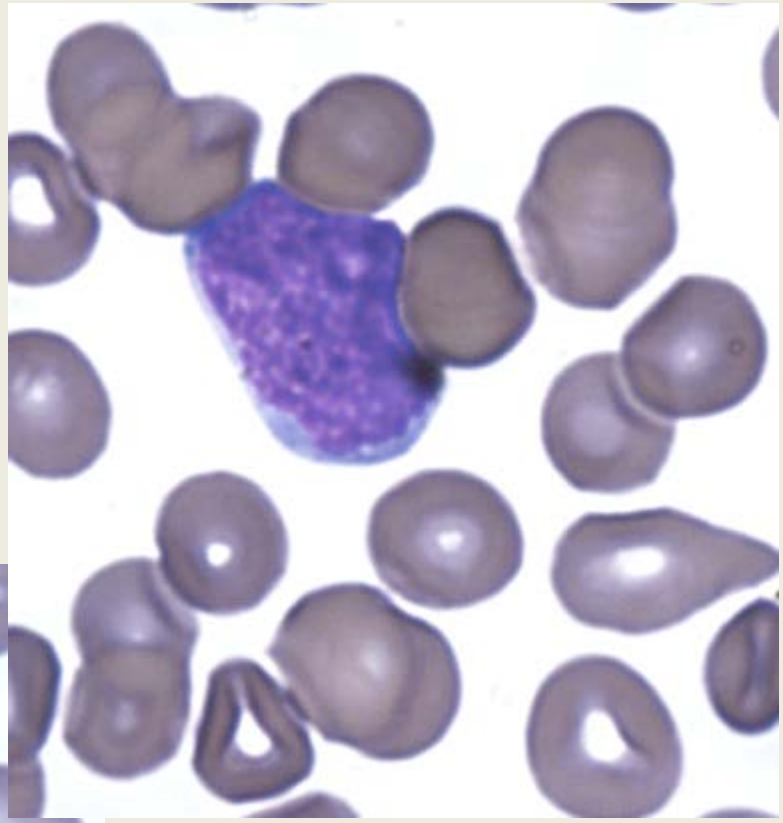
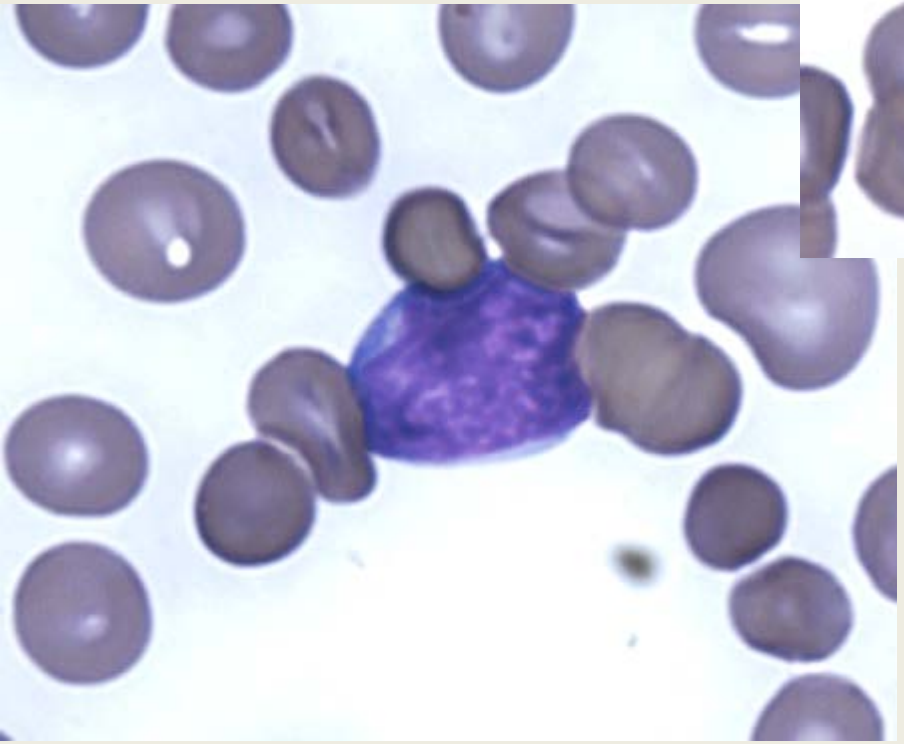
Acute Leukemia with smaller blasts







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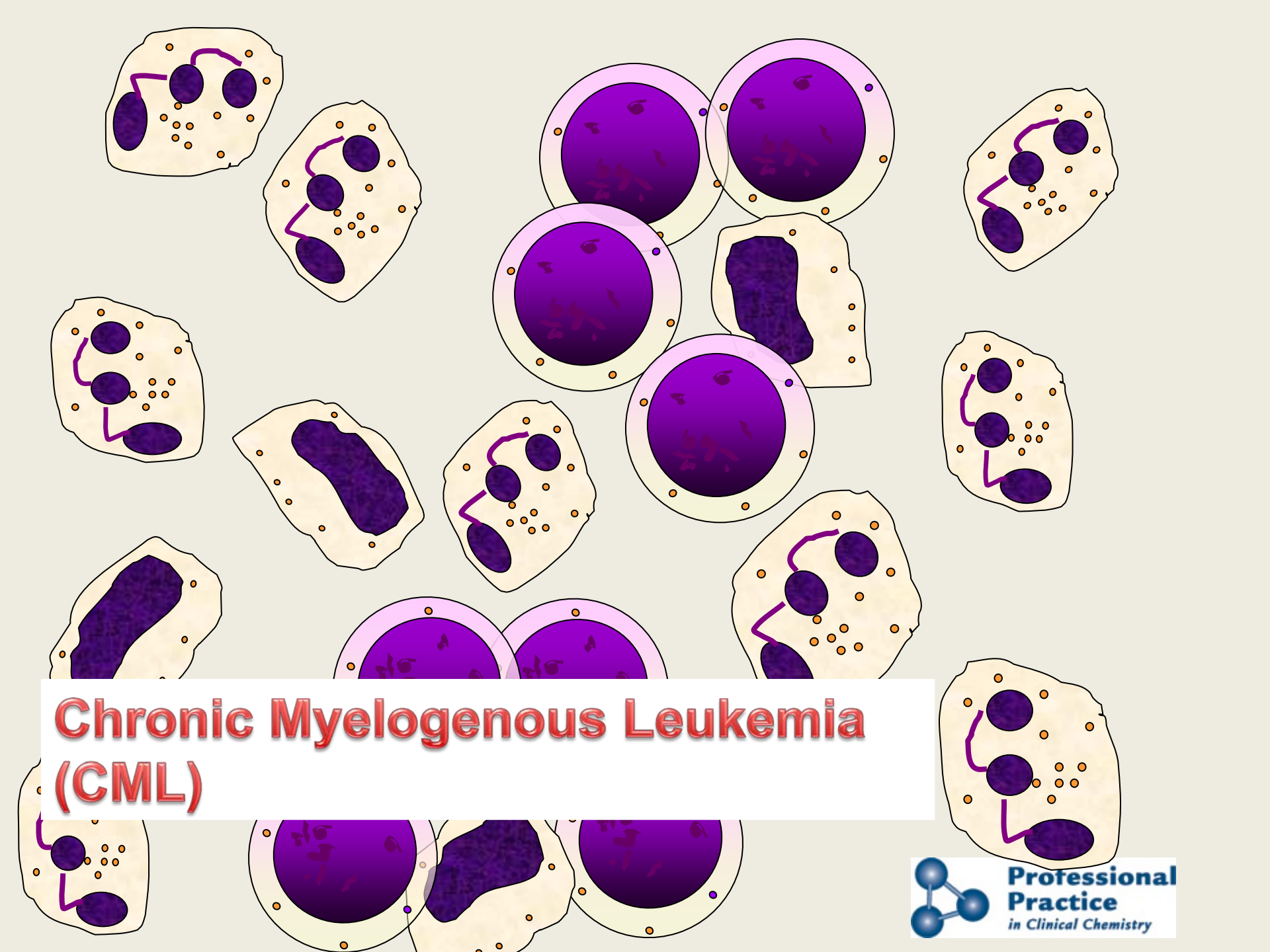

Chronic Myelogenous Leukemia (CML)

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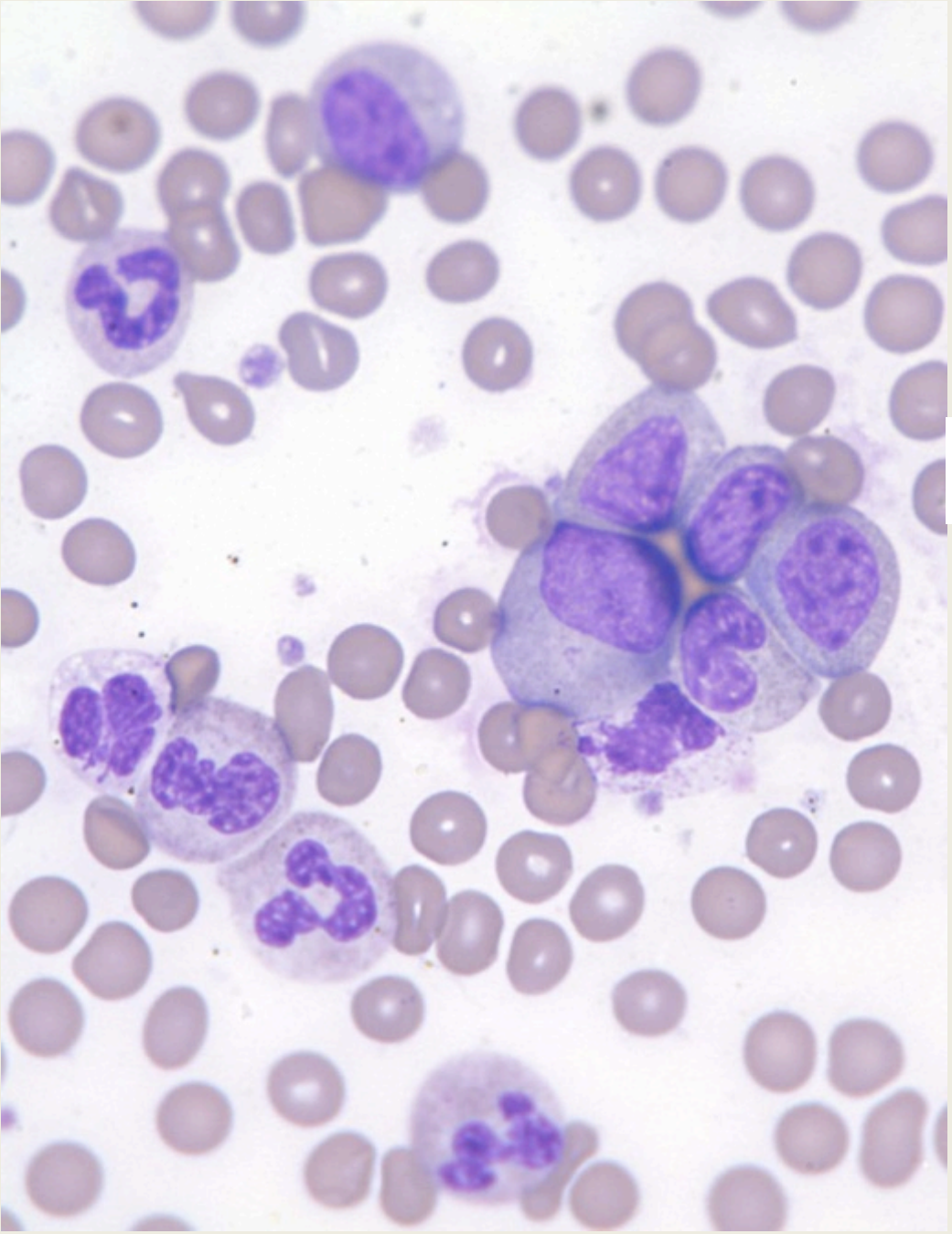

CML

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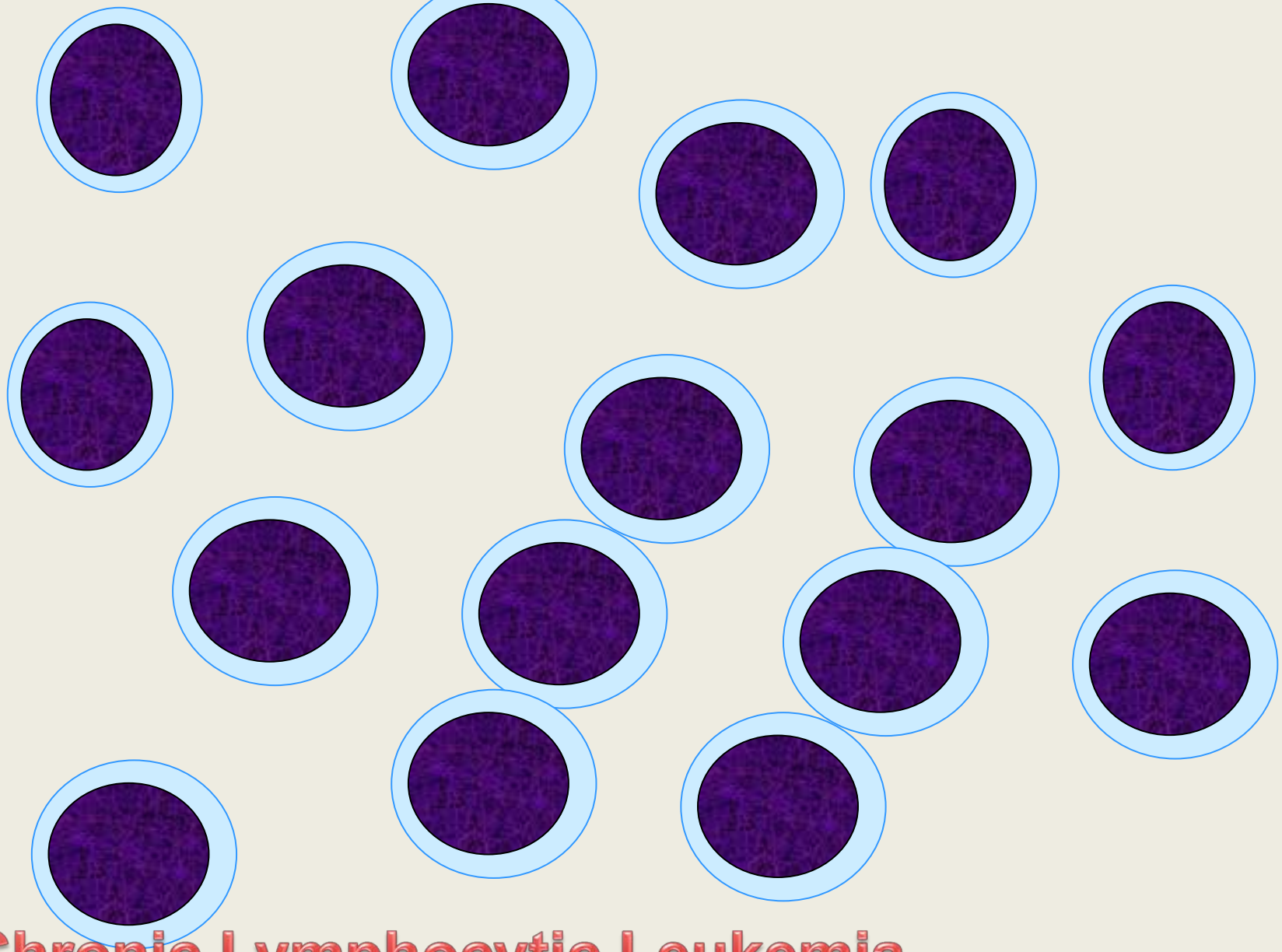

Chronic Lymphocytic Leukemia (CLL)

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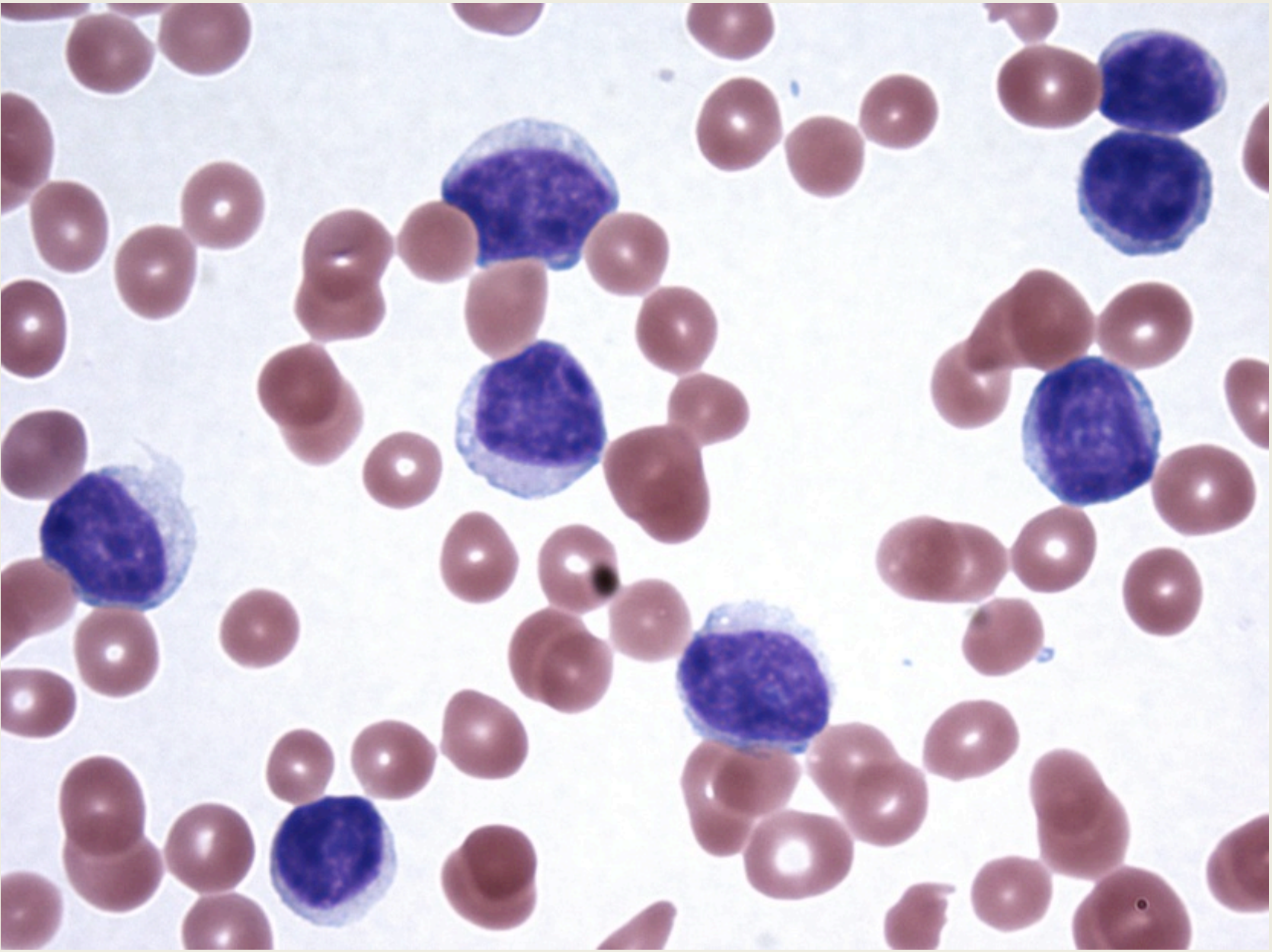

CLL

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END

Presented by AACC and NACB

Self-Assessment Question #1

Regarding the *mean cell volume* or MCV:

a) **This is now determined directly by electrical or flow cytometric methods**.

b) it is always calculated from the spun hematocrit.

c) it is determined by light microscopy.

d) it is determined by electron microscopy.

e) it is derived from the white cell count.

Explanation: The red cell MCV is measured directly on cell counters; this is true whatever method is employed to enumerate cells. The counter is able to plot a red cell volume histogram, and the mean is determined. MCV can be calculated from the spun hematocrit as in option b. This was the original method for determining MCV but it is not commonly used anymore.

Self-Assessment Question #2

The Erythrocyte Sedimentation Rate (ESR) is:

a) the rate of sedimentation of fibrinogen in a 5 ml glass tube.

b) the rate of sedimentation of white cells in a standard narrow-bore glass tube.

c) the rate of clotting in the presence of erythrocytes

d) a test of platelet function

e) **the rate of sedimentation of erythrocytes in a standard narrow-bore glass tube**

Explanation: The ESR is the rate of sedimentation of red cells in a whole blood specimen enclosed in a narrow-bore glass tube. ESR is increased in acute inflammation.

Self-Assessment Question #3

Acute leukemia is characterized by presence of ____ in the stained peripheral blood smear:

a) **blasts**

b) neutrophils

c) lymphocytes

d) platelet clumps

e) basophils

Explanation: The hallmark of acute leukemia is the increased presence of blasts in the bone marrow and in peripheral blood. Blasts are immature progenitor cells with characteristic morphology and cell surface markers.

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APPENDIX

Presented by AACC and NACB

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Derivation of RBC Indices

Presented by AACC and NACB

MCV = HCT/RBC

- 0.45 L/L divided by 5.0×10^{12}/L
- $= 0.09 \times 10^{-12}$ L
- $= 90 \times 10^{-15}$ L or 100 fL (femtoliter)
- A femtoliter (fL) is = "*cubic micron* (μM^3)"

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MCHC = Hb/HCT

- 150 g/L divided by 0.45 L/L
- = 333 g/L
- = 33.3 g/dL

MCH = Hb/RBC

- 150 g/L divided by 5.0×10^{12}/L
- $= 30 \times 10^{-12}$g
- = 30 pg

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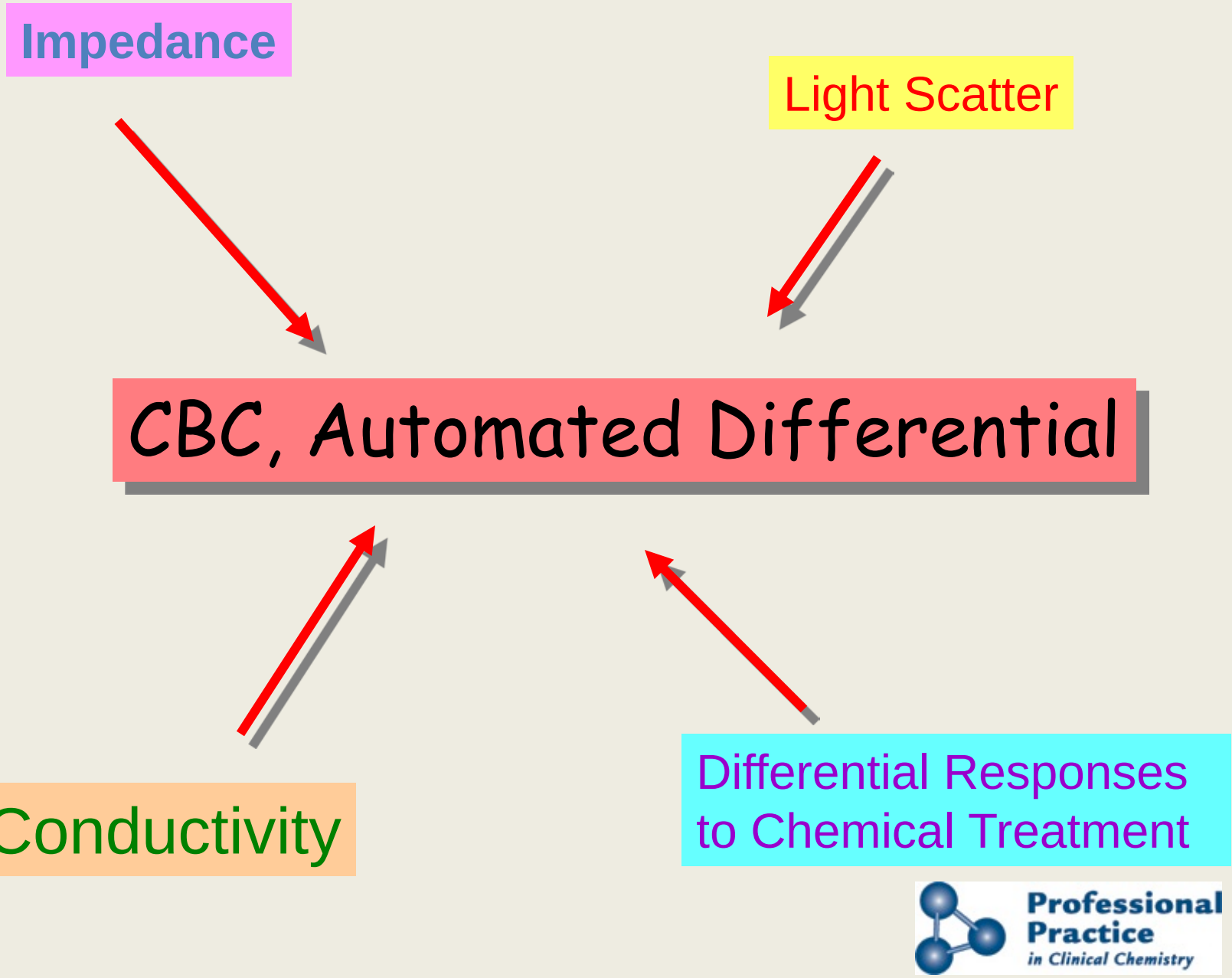
AUTOMATED CELL Analysis

Presented by AACC and NACB


Impedance
Light Scatter
CBC, Automated Differential
Conductivity
Differential Responses
to Chemical Treatment
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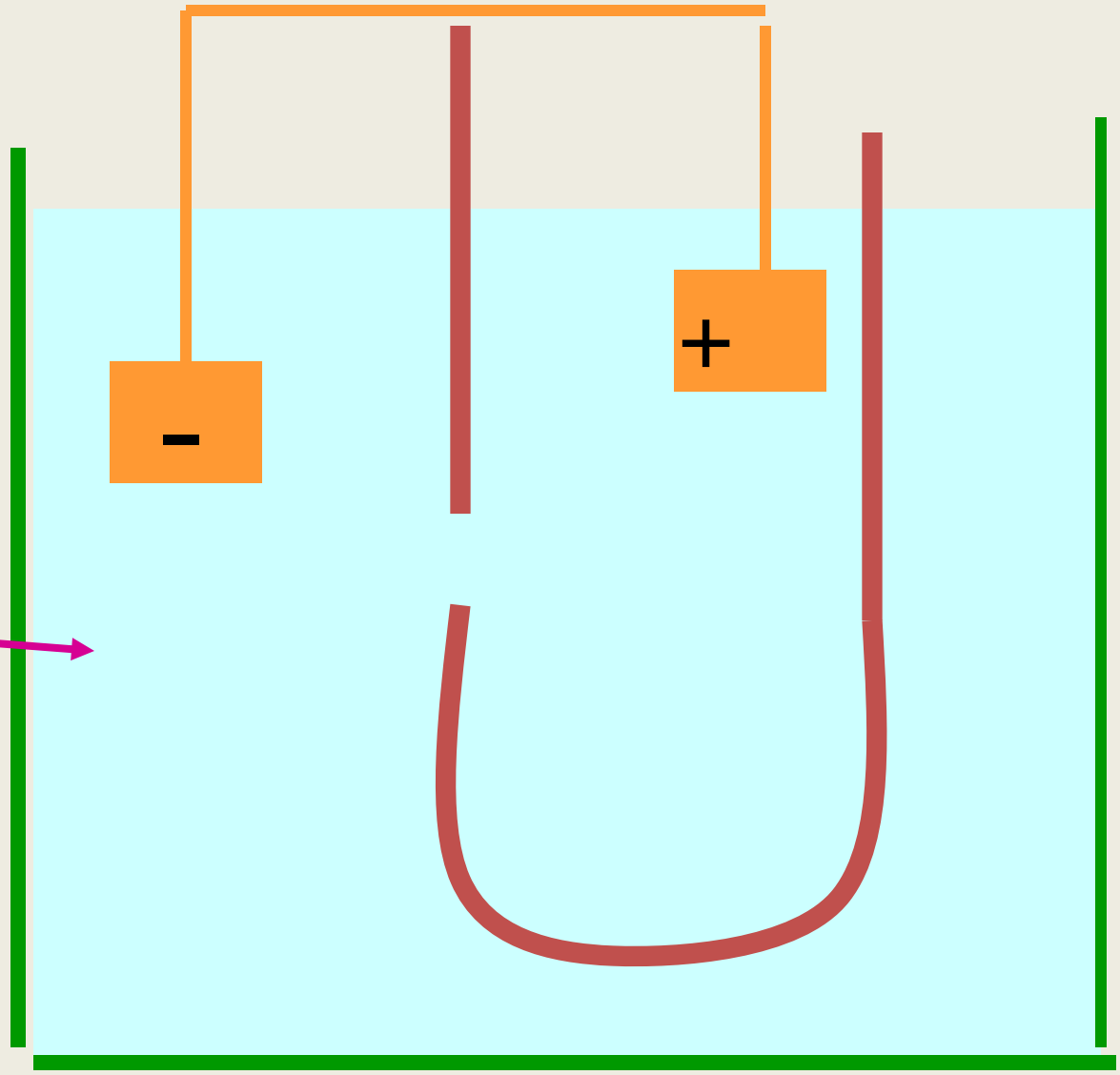
1) IMPEDANCE

Presented by AACC and NACB



Electrically conductive diluent

A low-voltage direct current is established

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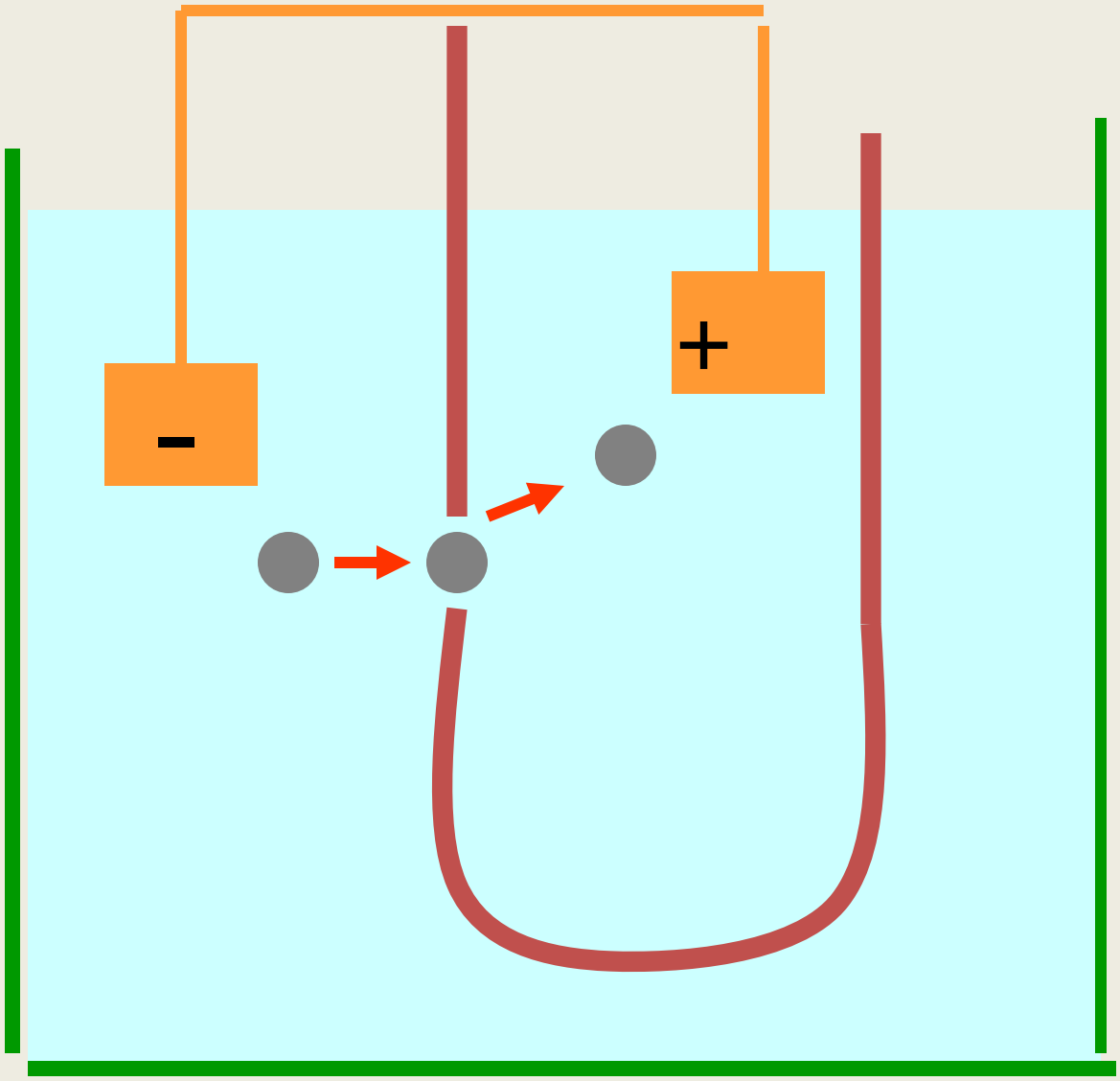
A cell pulled through the aperture interrupts the current



Impedance

- Current interruption = “Impedance”
- Impedance can be recorded as *voltage pulses*
- The number of pulses = Cell Number
- Pulse height = *Cell Volume* [femtoliters]

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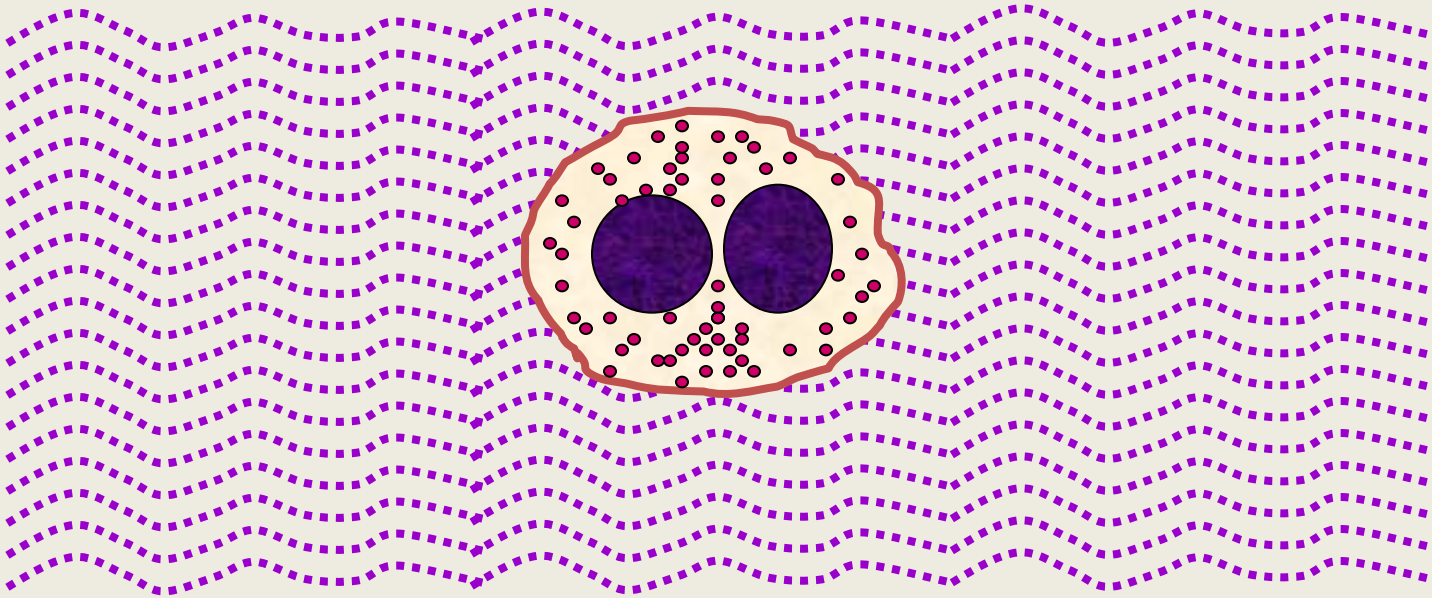
2) CONDUCTIVITY

Presented by AACC and NACB

Conductivity is measured by a high voltage radio-frequency current



Conductivity is determined by *cell granularity, nuclear density, nuclear/cytoplasm ratio*

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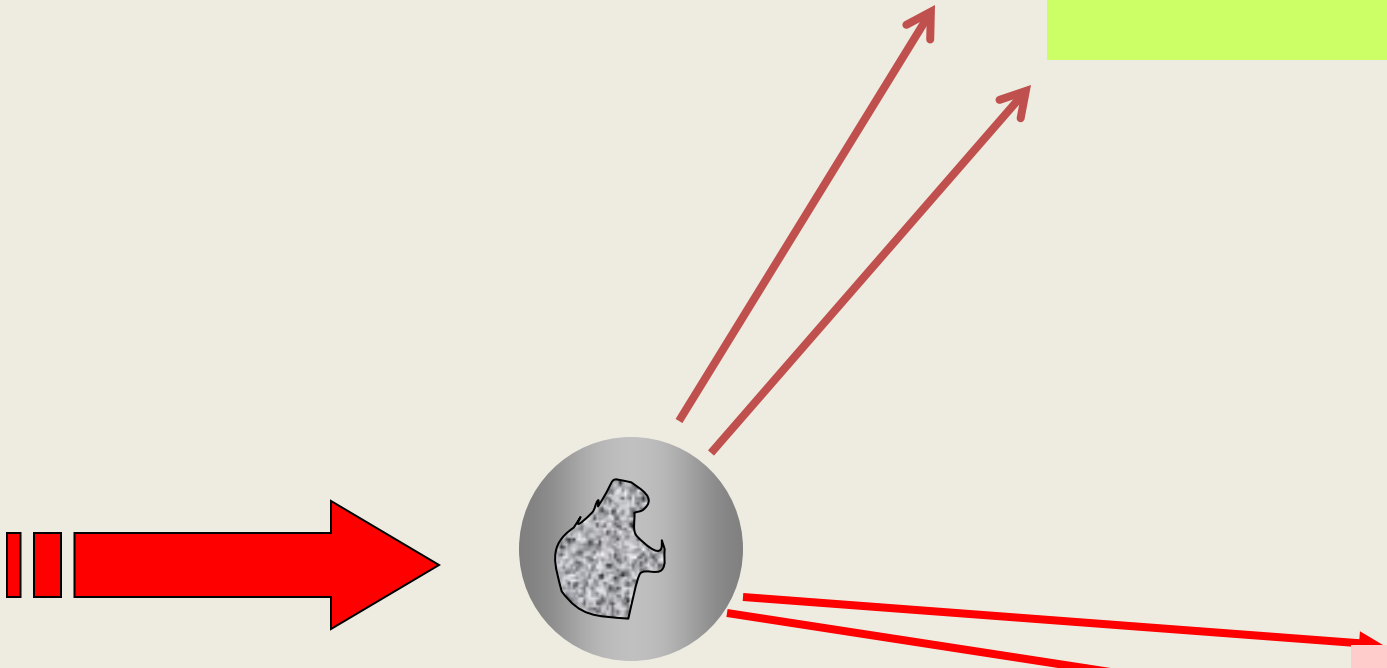
3) LIGHT SCATTER (Flow Cytometry)

Presented by AACC and NACB

SIDE SCATTER: Internal complexity



LASER

CELL

FORWARD SCATTER: Cell Volume

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4) Differential Responses to Chemical treatment

Presented by AACC and NACB

Differential Responses to Chemical treatment

- Lysis of RBCs $\rightarrow$ Allows counting of white cells
- Selective *shrinking* and/or *lysis* of certain white cell populations
- Selective *stripping* of cytoplasm $\rightarrow$ Allows analysis of cell nuclei
- Selective staining of cells e.g., *myeloperoxidase*

New Generation Cell Counters

- High sensitivity & specificity for blasts
- Ability to distinguish lymphoblasts from normal lymphocytes
- Automated enumeration of nucleated RBCs with correction of WBC count
- Reticulocyte counting, unaffected by red cell shape and capable of providing *immature reticulocyte fraction* and/or CHr
- High sensitivity & specificity for *immature granulocytes*
- Some will enumerate immature granulocytes
- Automated slidemaker/stainer
- Can be connected to automated front-end processing