Automated cell counter / Hematology analyzer

The sample is loaded into an automated cell counter and it is forced through a small tube while the automated cell counter uses optical or electrical impedance (a measure of the opposition to electrical flow) sensors to count how many cells go through the tube.

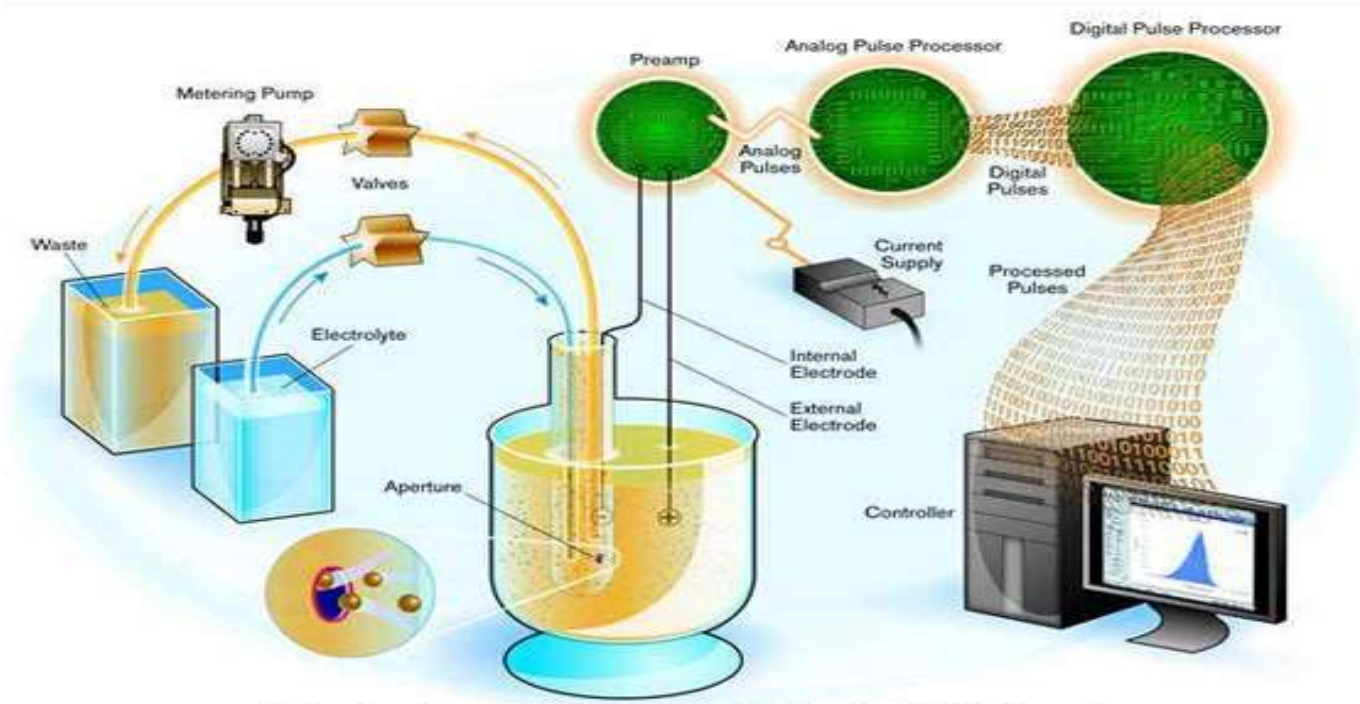


Figure 1. Schematic of a COULTER COUNTER

TYPES OF CELL COUNTING

MANUAL

SEMIAUTOMATED

AUTOMATED

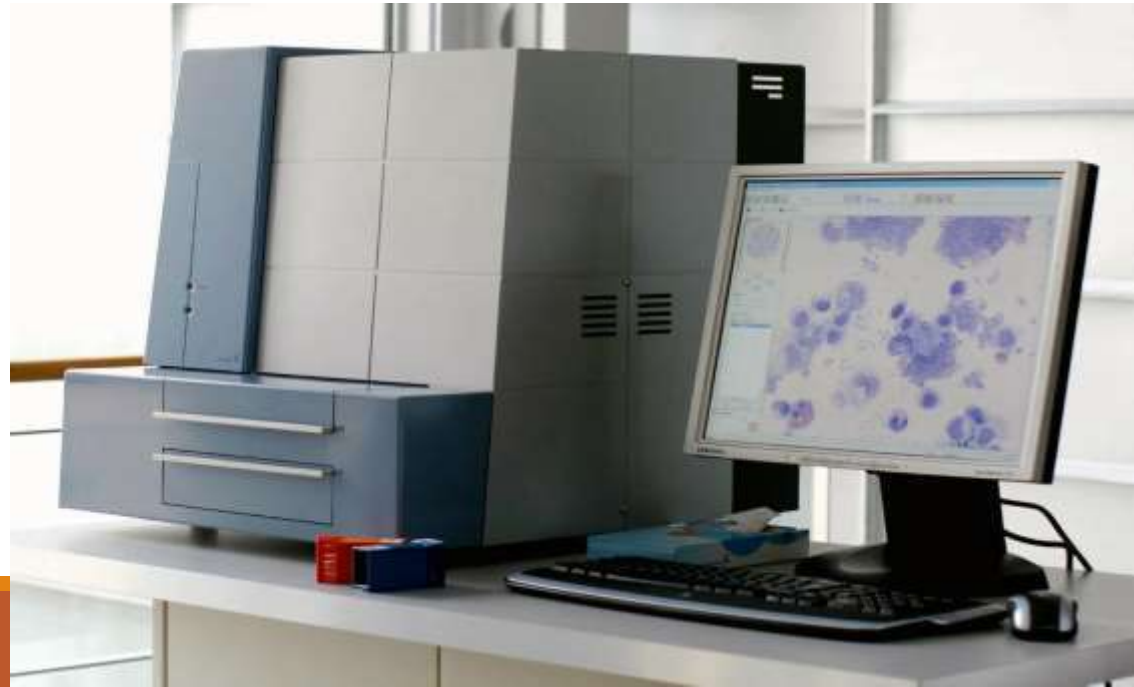
Types Of Automated

- **Fully Automated**

  - CoulterSTKS
  - SysmexSE series,XE2100
  - Bayer H SeriesAdiva
  - Abbot

Morphology Based

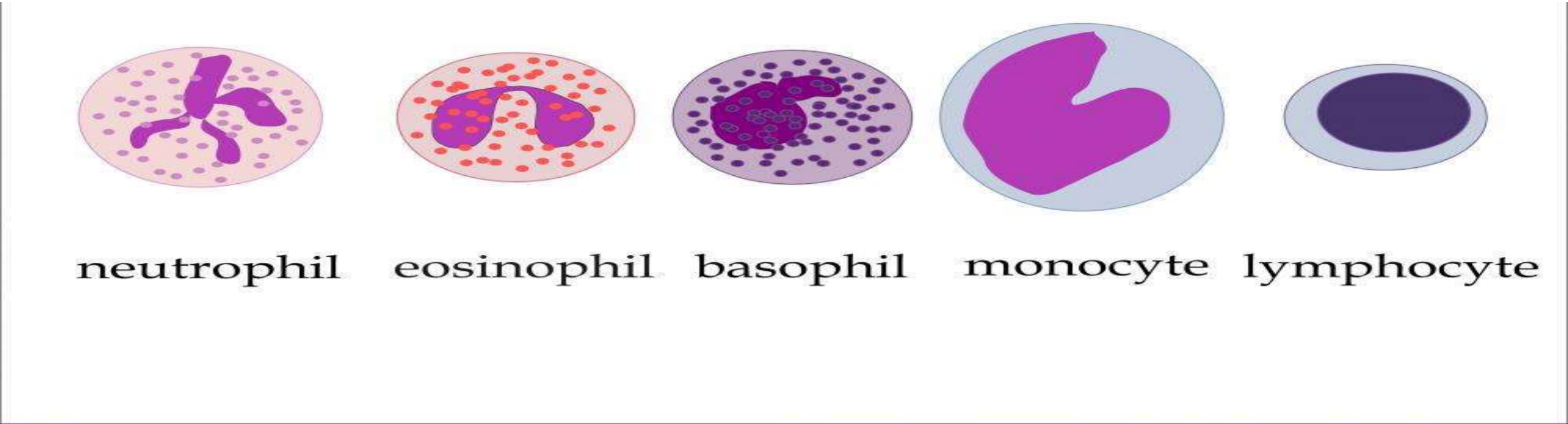
- Cella Vision DM96
- DiffMaster
- SE-223 70



Disadvantages Of Manual Cell Counting:

- Cell identification errors in manual counting:

  Mostly associated with distinguishing lymphocytes from monocytes, bands from segmented forms and abnormal cells (variant lymphocytes from blasts)



Disadvantages Of Manual Cell Counting

- Slide cell distributionerror:

  increased cell concentration along edges also bigger cells found there i.e. monocytes, eosinophils, and neutrophils
- Statistical sampling error.

Slow: Counting and recording large numbers of cells is a laborious,

Time-consuming process through which relatively small volumes of cells are counted.

Hemocytometers must be thoroughly cleaned between samples.

Volumetric errors: Pipetting errors and poor siting of the cover slip can lead to measurement inaccuracies.

Challenging differentiation between cells and debris

No standardized protocol

Advantages Of Automated Cell Counting:

- Objective (no inter-observer variability)
- No slide distribution error
- Eliminate statistical variations associated with manual count based on high number of cells counted
- Many parameters not available from a manual count, e.g. MCV, RDW…
- More efficient and cost effective than manual method.
- Less time consuming
- More accurate results

History

The world of cells was colorless until **Paul Ehrlich** stained blood cells. 1870s allowed, for the first time, differentiation between different white cell types.

Methods based on optical measurements In 1896 a new method for blood cell counting was proposed by George Oliver

Wallace Coulter :First automated analyzer for counting and sizing cells and presented it in 1956

In the early 1970s, Julius and colleagues demonstrated fluorescence-based cell sorting

Definition:

Hematology analyzers are computerized, highly specialized and automated machines that count the number of different kinds of cells eg WBCs (White Blood Cells) RBCs (Red Blood Cells) and platelets in a blood sample.

History:

The original hematology analyzers first appeared in the **1950s,** but the truly functional and automated versions of these machines did not become available for **two more decades**. Before this time, cell counts were performed manually.

AUTOMATION IN HEMATOLOGY

- Cell counts(Automated hematology analyzers)
- Diagnosis of hemoglobinopathies(HPLC)
- Immunophenotyping(Flow cytometry)
- Coagulation(Coagulometers)

Automated Hematology Analyzers

As a routine investigation

Including anaemia, polycythemia, infection, inflammation, allergy, drug toxicity, malignancy, bleeding tendency etc

Aim-to study RBC, WBC series and platelets, Hb and Absolute Values (MCV, MCH, MCHC)

The results they provide are collectively known as complete blood counts (CBCs) .

complete blood with differentiation of cells (CBCs with diff).

Some cell counters can process 120-150 samples per hour .

Advantages

- Speed with efficient handling of large number of samples
- Accuracy and precision in quantitative blood tests
- Ability to perform multiple test on a single platform
- Significant reduction of labor requirements

Disadvantages

- Flagging of a laboratory test result demands labour intensive manual examination of a blood smear.
- Red cell morphology cannot be generated.
- Platelet Clumps are counted as single, so low count.
- Increased or decreased results due to interfering factors.
- Expensive with high running costs

Types Of Counters

- **Semi automated** :
  Some steps carried out manually like dilution of blood
  Measures only a few parameters

- **Fully automated:**
  Require only anticoagulated blood samples. Measures multiple parameters

Components Of A Cell Counter

3 Basic components

- **Hydraulics:**
  Includes aspirating unit, dispencers, diluters,mixing chambers, aperture baths & hemoglobinometer
- **Pneumatics:**

Vacuums &pressure for operating valves

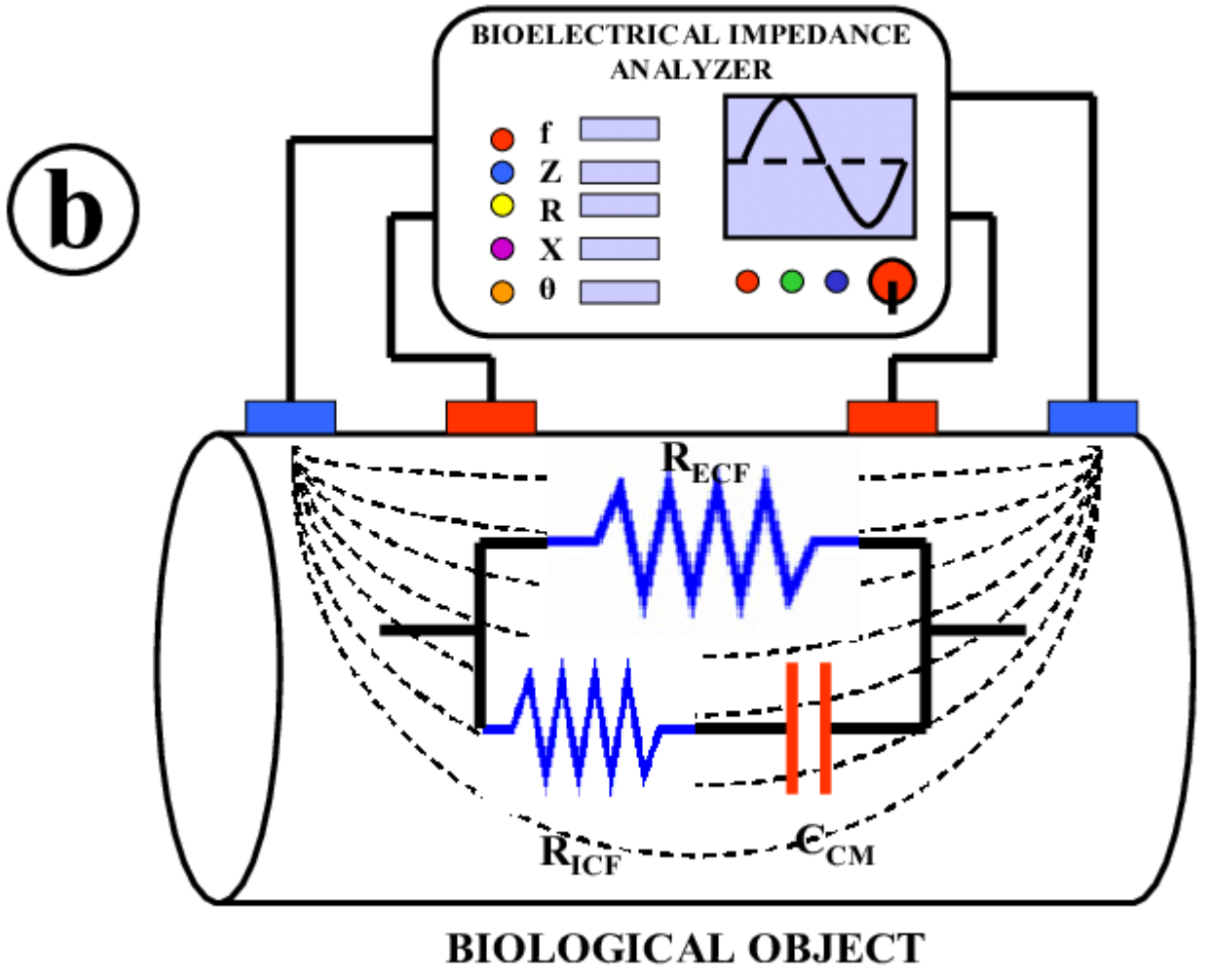
- **Electronics :**
  Analyzer &computing circuit

Principles of Working

- Electrical Impedance
- Optical Light Scatter
- Fluorescence
- Light absorption
- Electrical conductivity

Electrical Impedance

- First introduced by Wallace Coulter
- Blood cells are poor conductor of electricity
- 2 chambers filled with a conductive buffered electrolyte solution
- Separated by a small aperture
- DC current between two electrodes



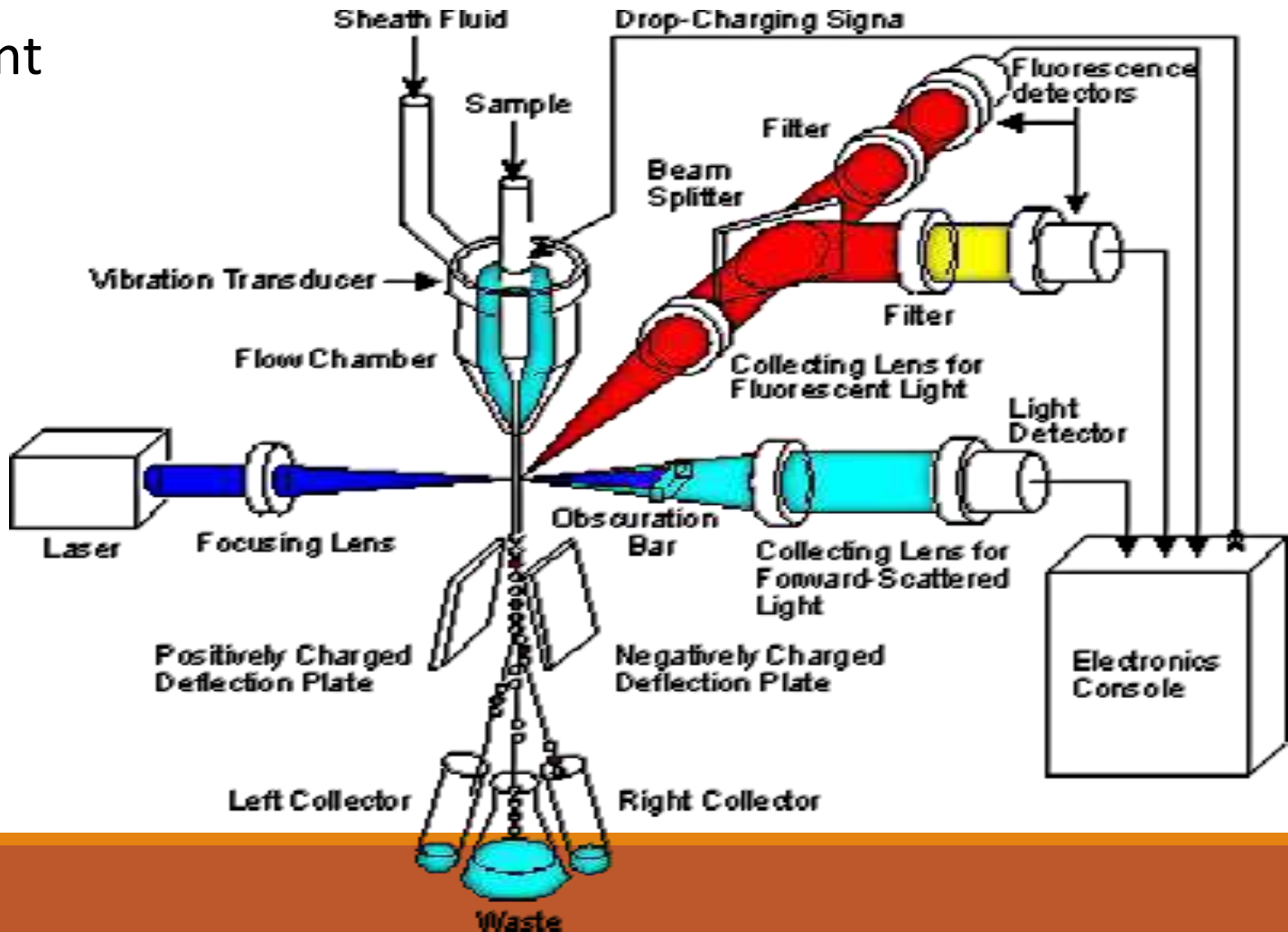
- Cell counting and sizing is based on the detection and measurement of changes in electrical impedance (resistance) produced by a particle as it passes through a small aperture

- Particles such as blood cells are nonconductive but are suspended in an electrically conductive diluent

- As a dilute suspension of cells is drawn through the aperture, the passage of each individual cell momentarily increases the impedance (resistance) of the electrical path between two electrodes that are located on each side of the aperture

Optical Light Scatter

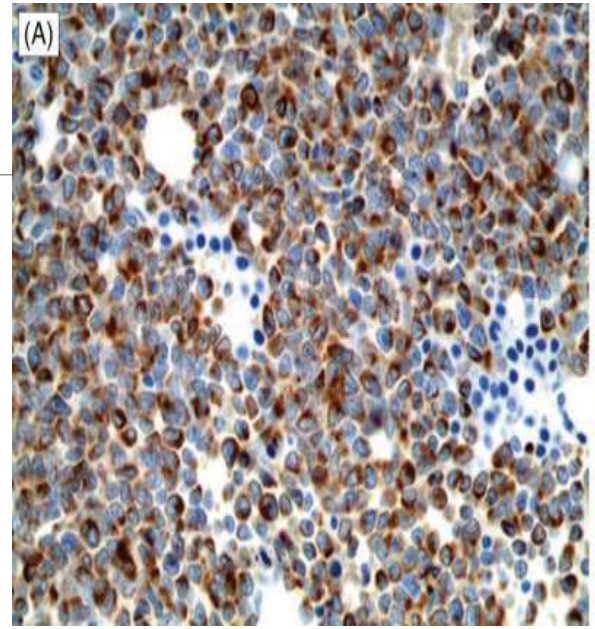
- Each cell flows in a single line through a flow cell
- A laser device focussed
- On striking on cells scattering in different directions
- Sensor capture & multiplies
- Forward angle light scatter (FALS)-Cell Size
- Side scatter(SS)-Granularity

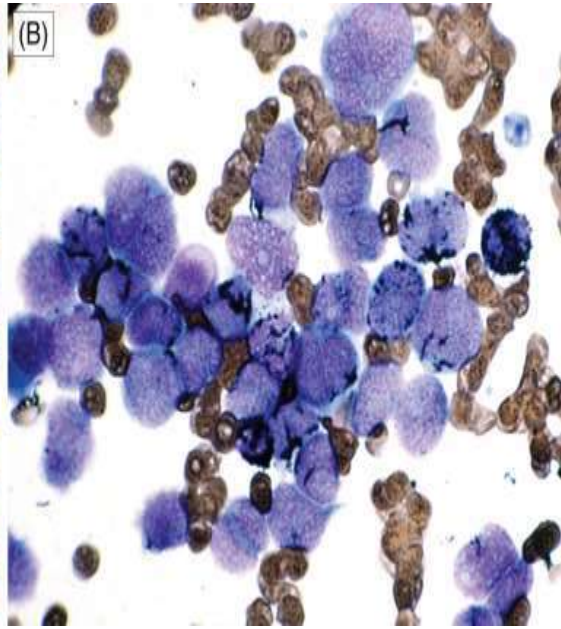


Other Methods

- **Peroxide based counters:**

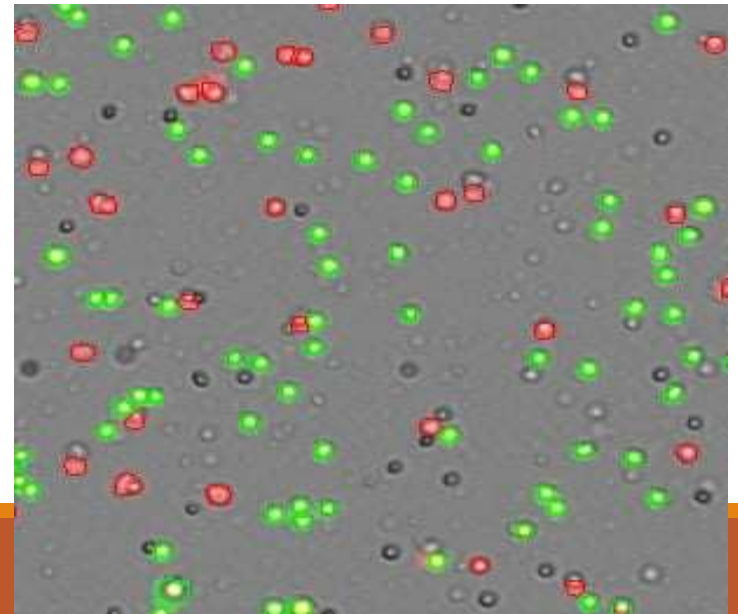
Myeloperoxidase (MPO), Staining recognizes the primary granules in the cytoplasm of granulocytes, eosinophils, and their precursors MPO is used to count neutrophils.Lymphocytes not stained





- **Fluroscence based:**

For platelet count.Immature pltlets detected best



- **Immunological based:**

Accurate platelet count using CD41/CD61 antibodies

IP Messages

- Interpretive messages
- Assist the laboratory in screening for abnormal samples that may need verification
- Seen at the bottom end of hemogram

Indicators that may appear after the data

@ : Data is outside the linearitylimit

* : Data is doubtful

+ or – :Data is outside the reference limits.

---- : Data doesn’t appear due to analysis error or abnormal sample++++ : Data exceeds displaylimit.

Histograms

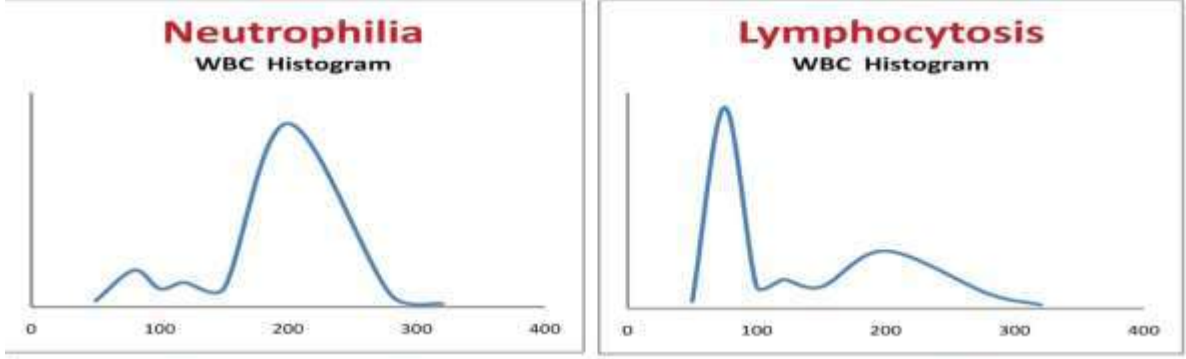
❖These are the graphical presentation of numerical datas of different cell populations in a cell counter.

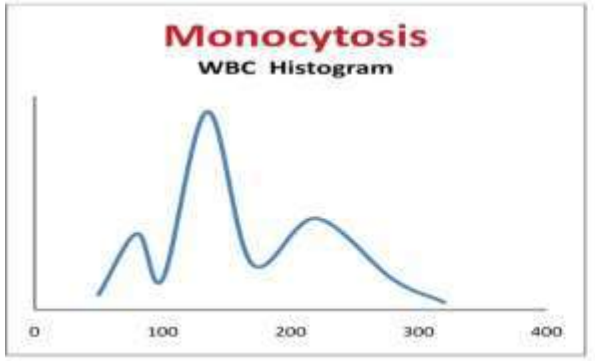
❖On the X-axis is the cell size and on the Y-axis is the number of cells.

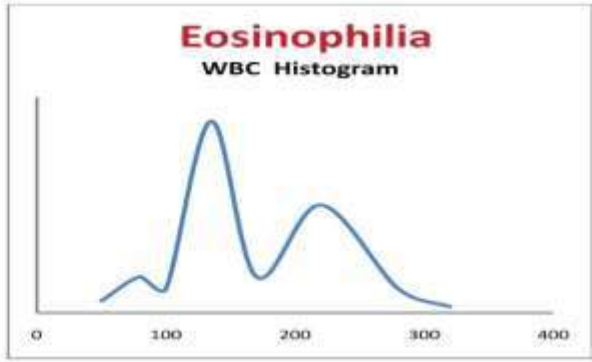
❖Used to determine

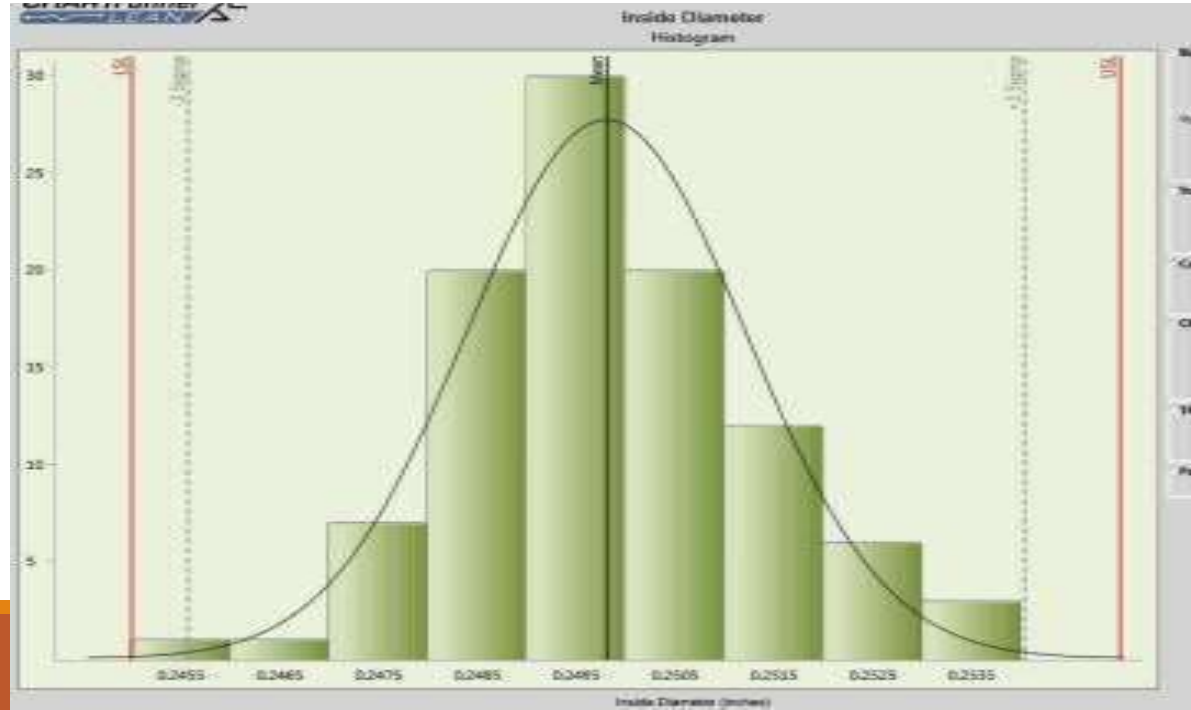
- The average size
- Distribution of size
- Detect subpopulation

(Homogenous or Heterogeneous)







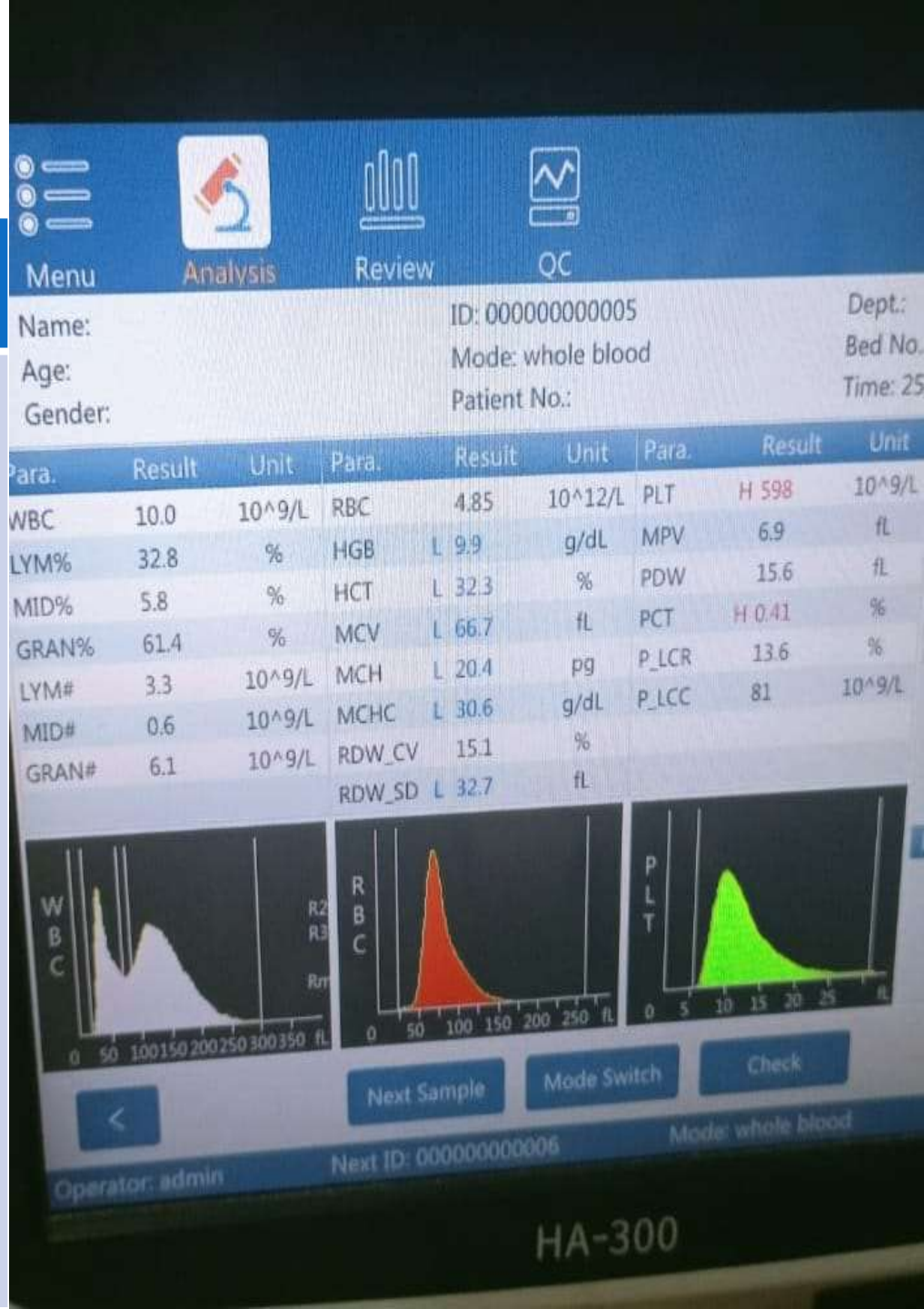


Flagging System

- Whenever any significant abnormalities of any cell present , signaled by certain ‘asteriks’ on the report.
- Every instrument has its own flagging system.

Parameters Measured

Directly Measured	Derived From Histograms	Calculated
1.RBC Count 2.WBC Count 3.Platlet count 4.Hemoglobin 5.Reticulocyte Count	1.MCV 2.RDW 3.DLC 4.PDW	1.Hematorit (MCV/RBC Count) 2.MCH (Hb/RBC Count) 3.MCHC (Hb/Hct)



Terminologies

- **Quality Assessment:**
  Adequate control of the pre & post analytical from sample collection to report dispatch.
- **Quality Control:**
  Measures that must be included during each assay run to verify the test working properly.
- **Proficiency Testing:**
  Determines the quality of results generated by lab.
- **Internal Quality control:** Continuous evaluation of the reliability of the daily works of the labwith validation of tests.
- **External Quality Control:** Evaluation by an outside agency of between-laboratory & between-method comparability.