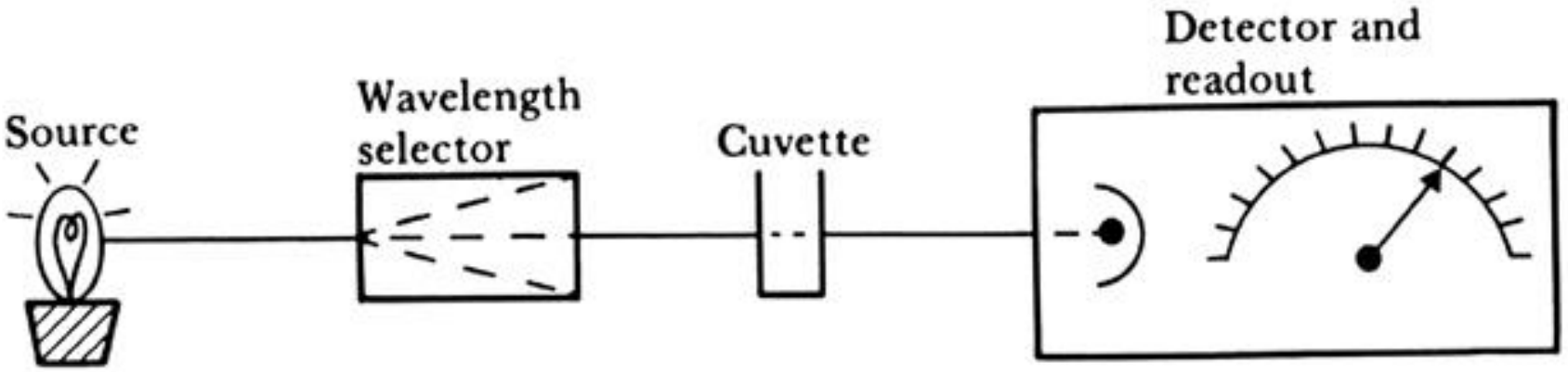
Clinical Laboratory Instrumentation

❑ Spectrophotometry

- The source supplies the radiant energy used to analyze the sample.
- The wavelength selector allows energy in a limited wavelength band to pass through.
- The cuvette holds the sample to be analyzed in the path of the energy.
- The detector produces an electric output that is proportional to the amount of energy it receives



Block diagram of a spectrophotometer (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry*, 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

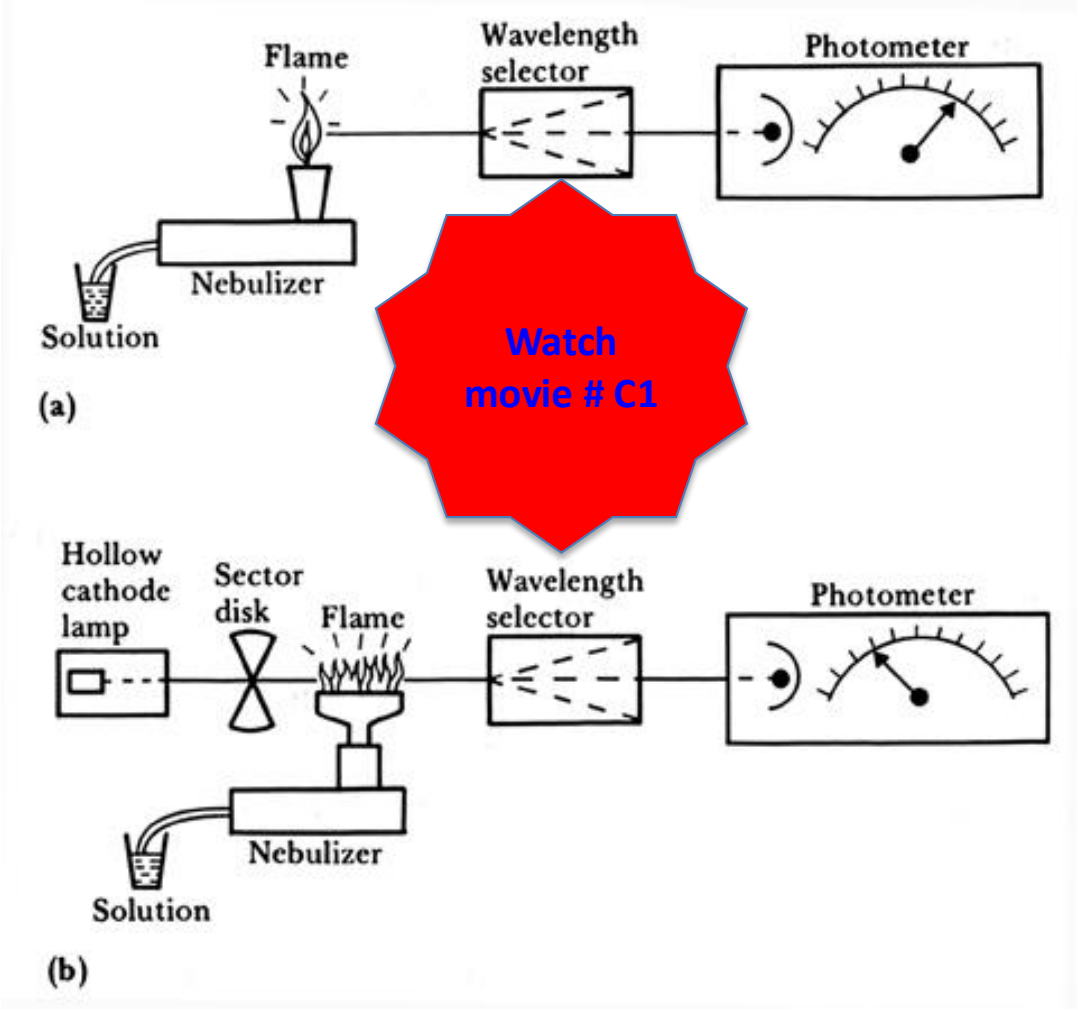
The basic principle of a spectrophotometer:

Using the energy-absorption properties of a substance of interest to measure the concentration of that substance

❑ Flame photometers

Flame photometers differ in three important ways from spectrophotometer:

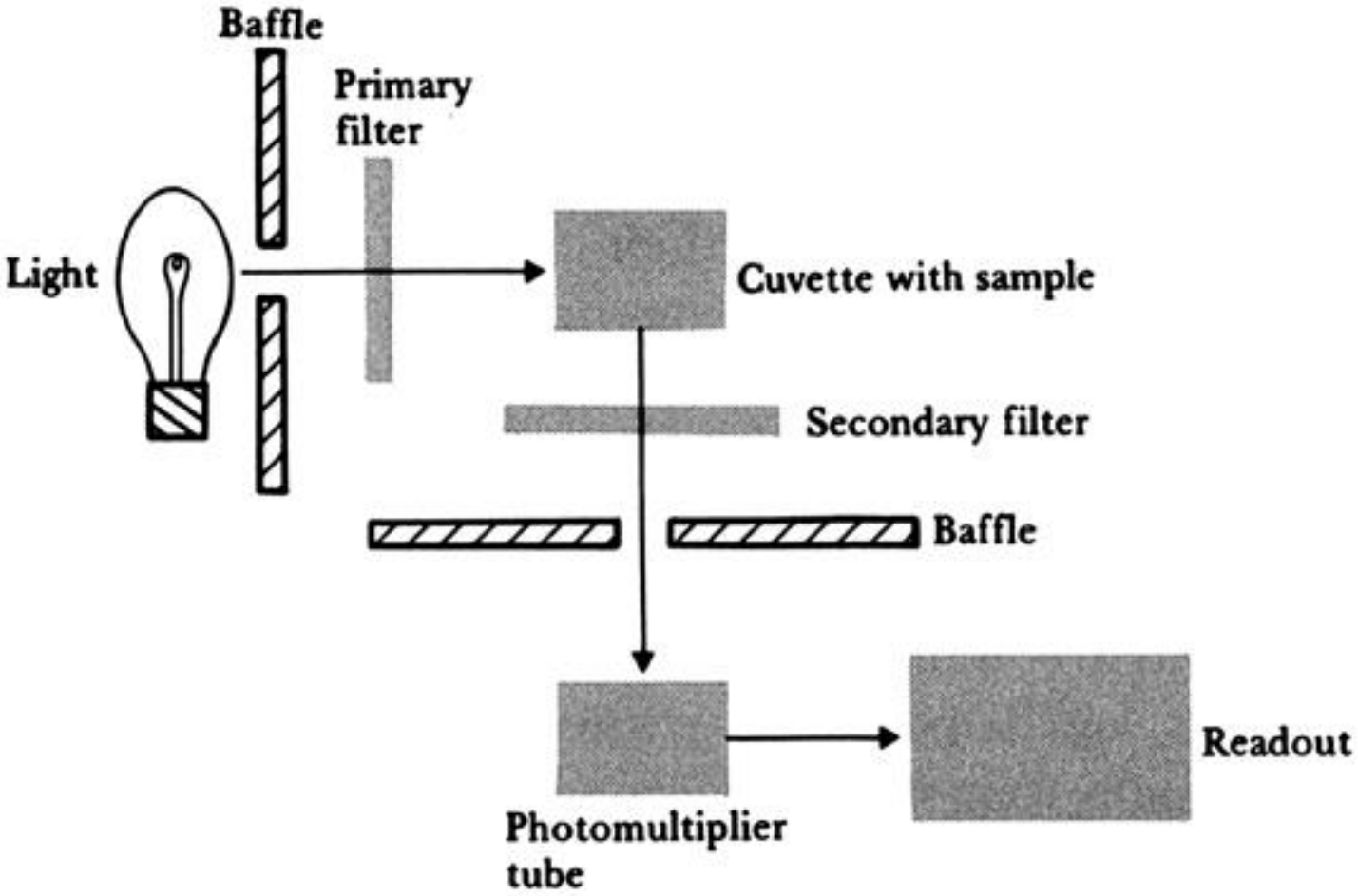
- the power source and the sample-holder function are combined in the flame
- the objective is measurement of the sample's emission of light rather than its absorption of light
- flame photometers can determine only the concentrations of pure metals.



Block diagram of instruments for (a) flame emission and (b) flame absorption. (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry*, 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

❑ Fluorometry

- Fluorometry is based on the fact that a number of molecules emit light in a characteristic spectrum—the emission spectrum—immediately after absorbing radiant energy and being raised to an excited state.

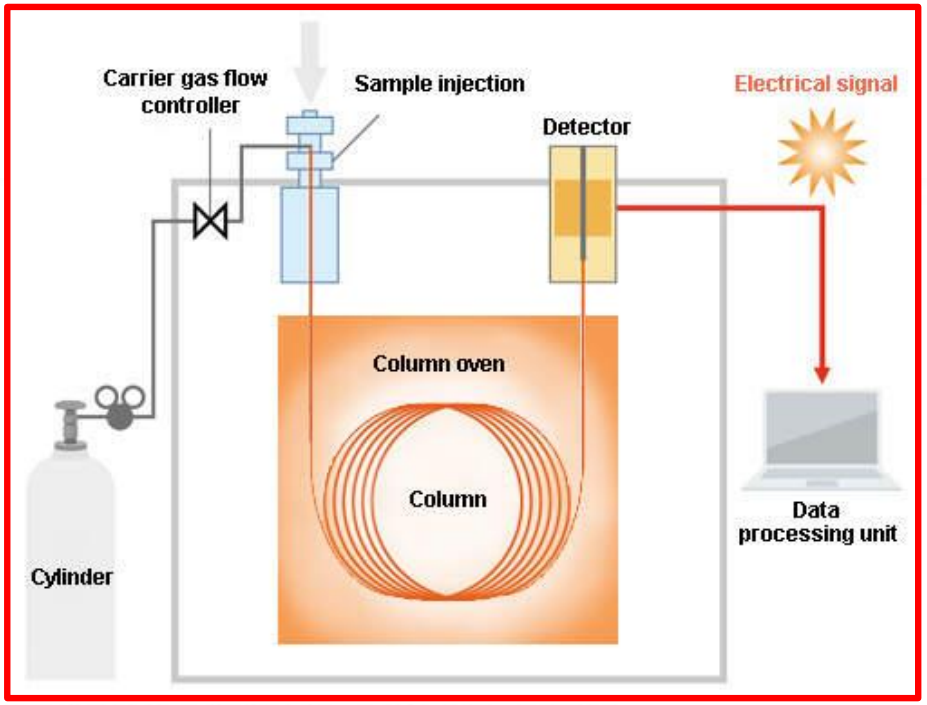


Block diagram of a fluorometer (Based on R. Hicks, J. R. Schenken, and M. A. Steinrauf, *Laboratory Instrumentation*. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

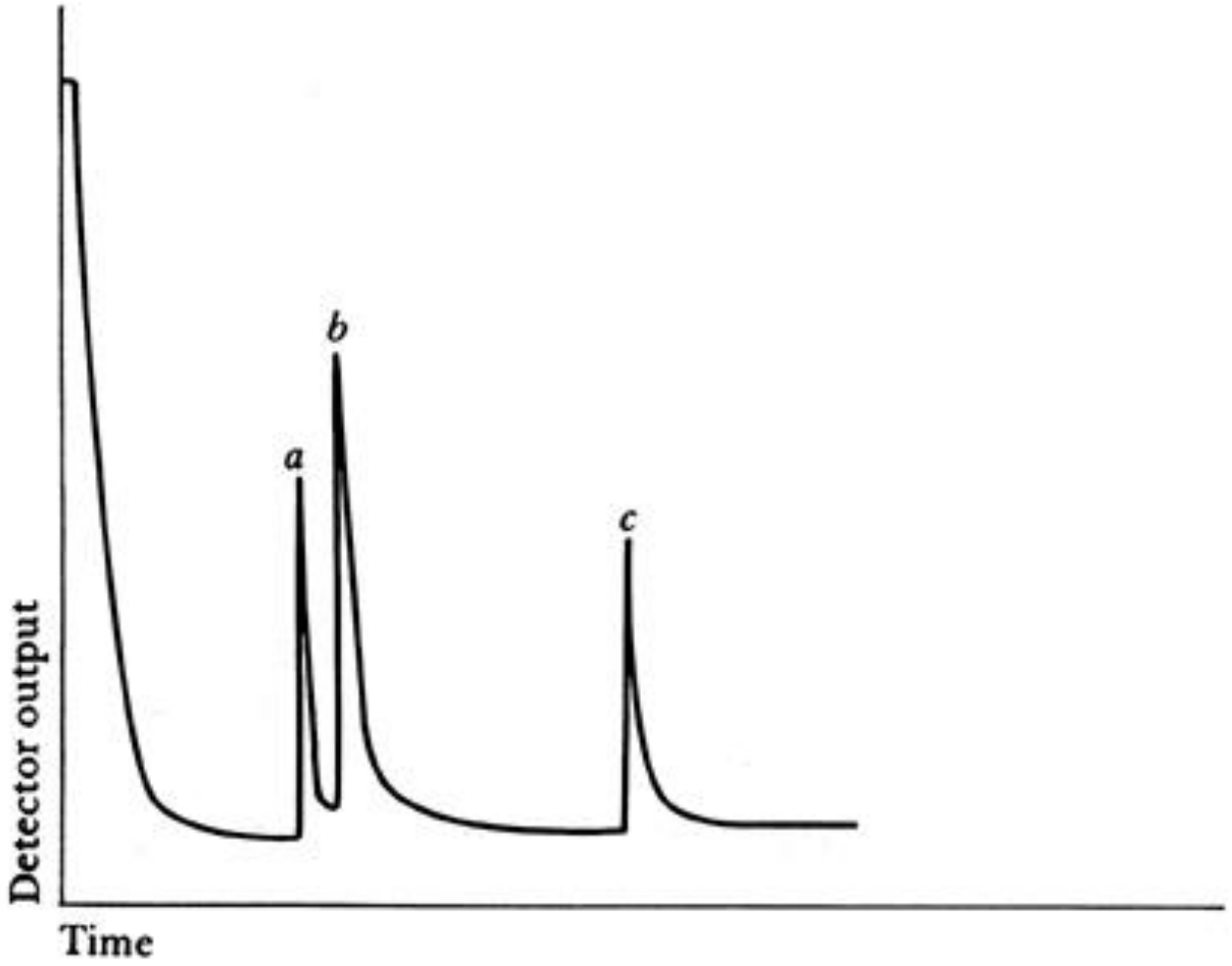
❑ Chromatology

- Chromatology is basically a group of methods for separating a mixture of substances into component parts.

Gas–liquid Chromatographs



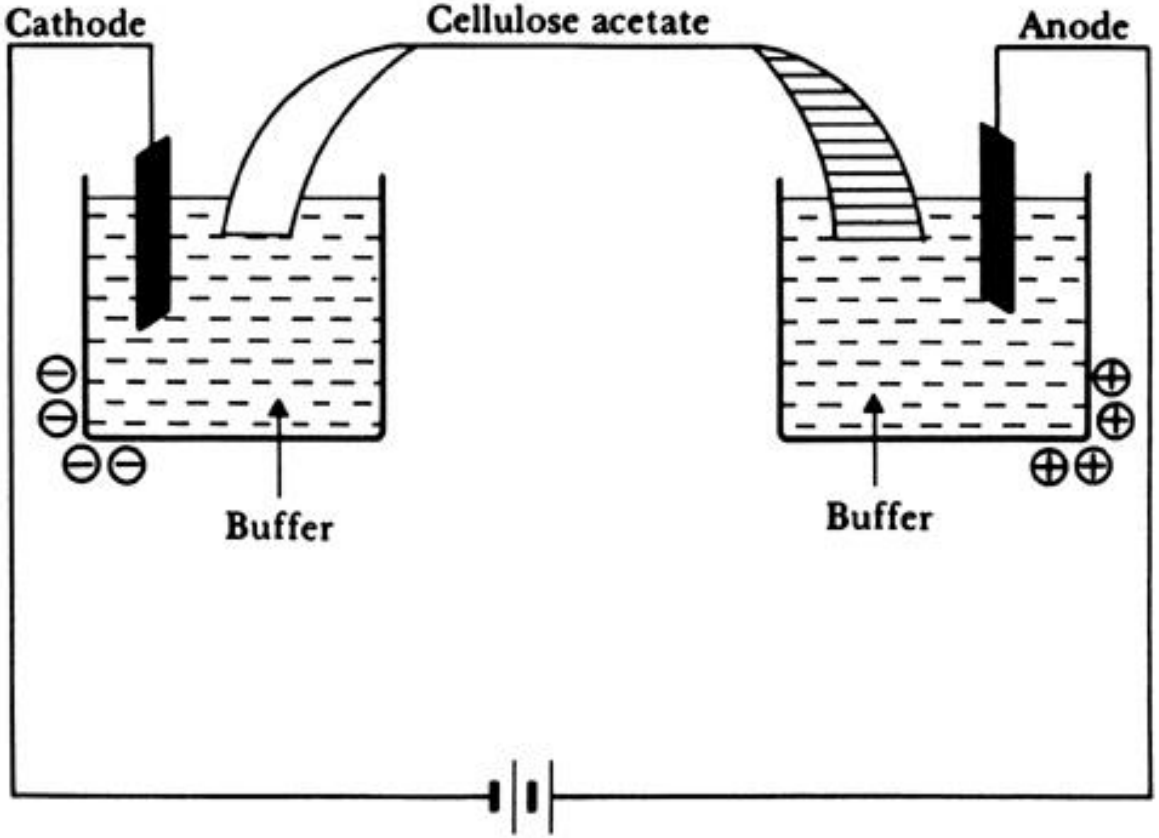
Block diagram of a gas-liquid chromatograph (GLC)



Example of a GLC recording for the analysis of blood levels of phenobarbital (peak *a*) and phenytoin (peak *c*). Peak *b* corresponds to the level of heptabarbital (the internal standard).

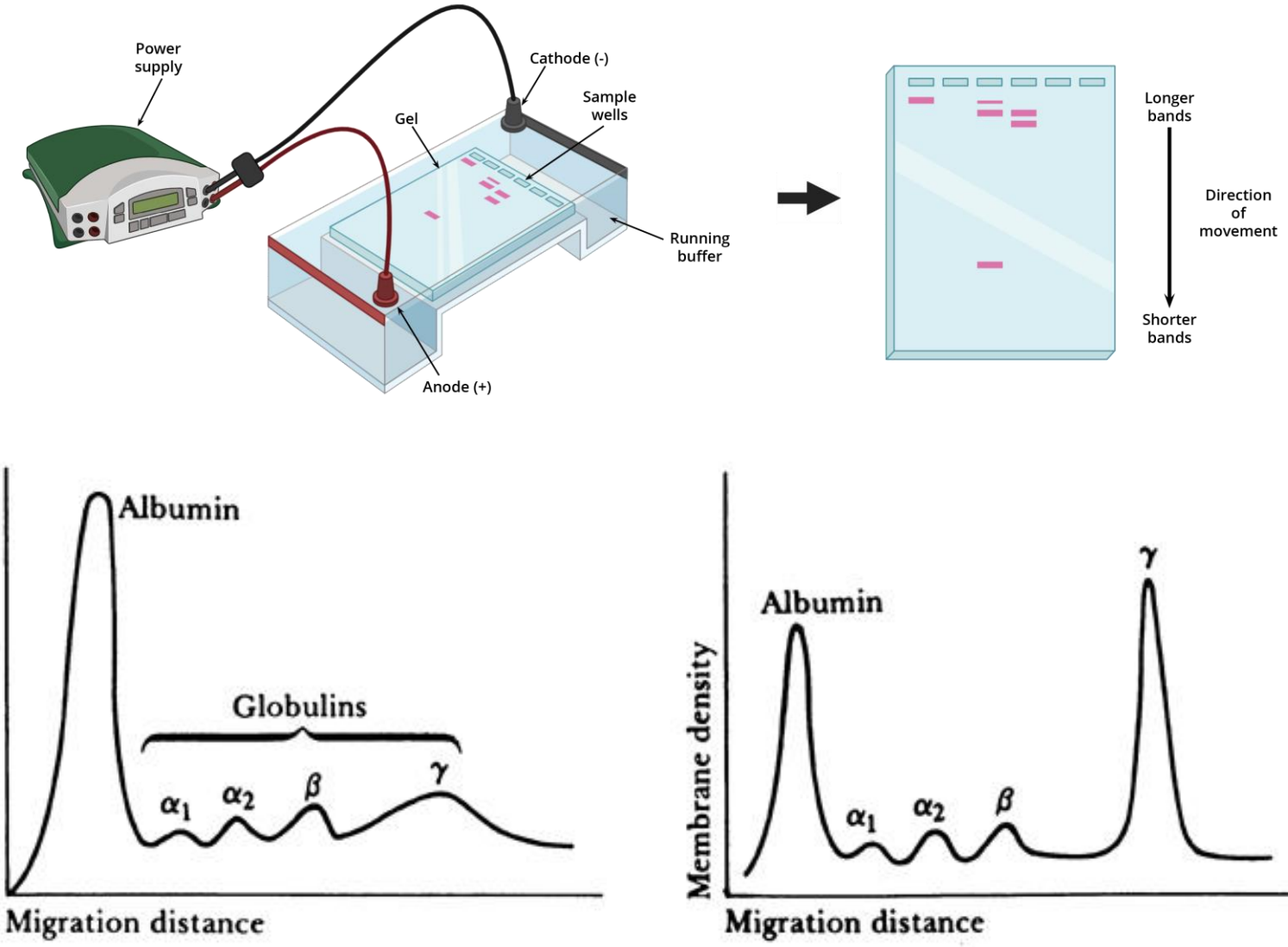
❑ Electrophoresis

- Devices based on electrophoretic principles are used in the clinical laboratory to measure quantities of the various types of proteins in plasma, urine, and CSF; to separate enzymes into their component isoenzymes; to identify antibodies; and to serve in a variety of other applications.



Electrophoresis may in general be defined as the movement of a solid phase with respect to a liquid (the buffer solution).

Cellulose acetate electrophoresis (Based on R. Hicks, J. R. Schenken, and M. A. Steinrauf, *Laboratory Instrumentation*. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)



Examples of patterns of serum protein electrophoresis The left-hand pattern is normal; the right-hand pattern is seen when there is an over production of a single type of gamma globulin.

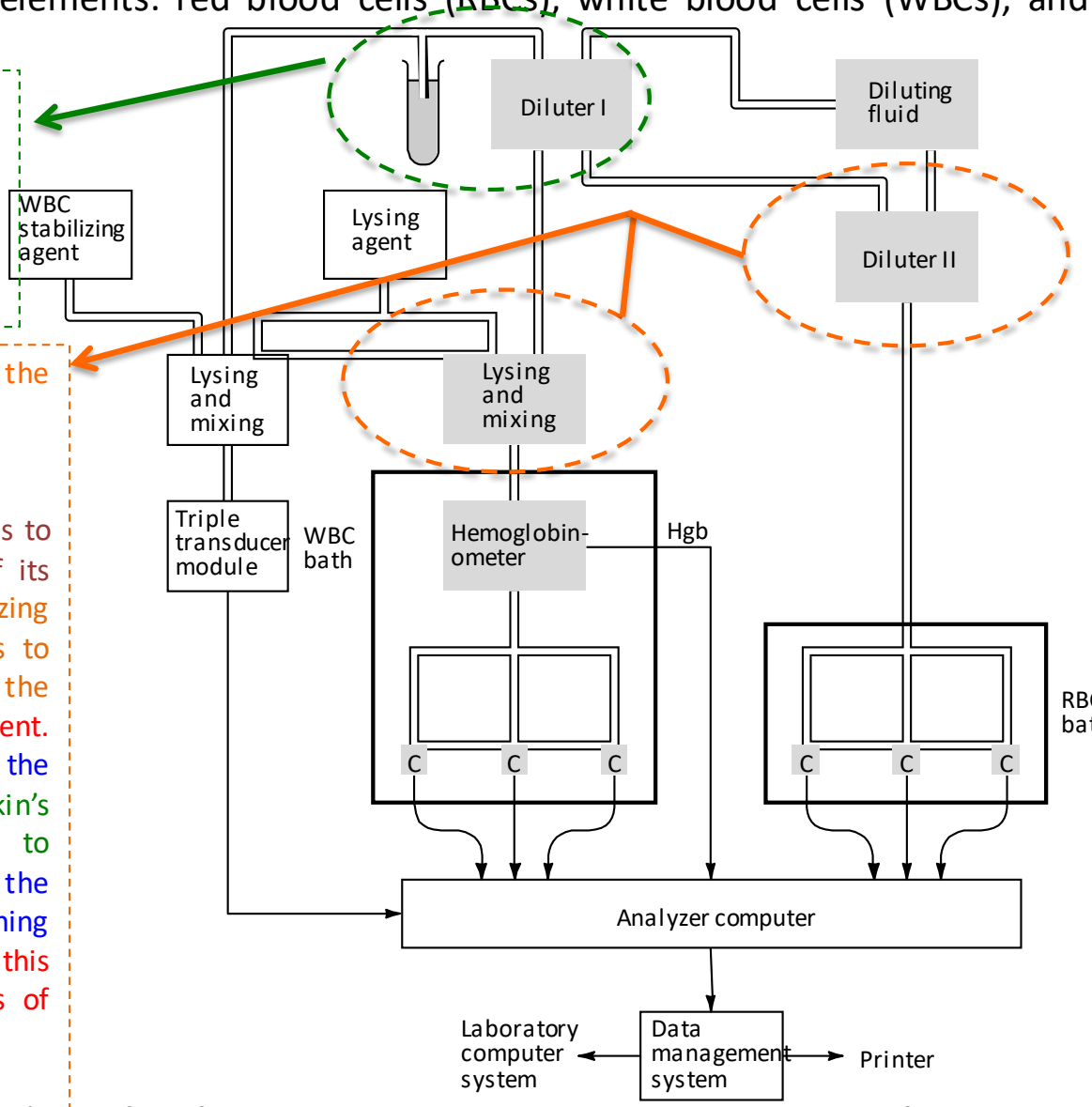
Hematology

- The blood consists of formed elements, substances in solution, and water.
- Characteristics of the blood formed elements: red blood cells (RBCs), white blood cells (WBCs), and platelets.

- The initial step in the analysis procedure is the automatic aspiration of a carefully measured portion of the specimen.
- Next the specimen is diluted to 1:224 with a solution of approximately the same osmolality as the plasma in Diluter I

The diluted specimen is then split, part going to the mixing and lyzing chamber and part to Diluter II.

The function of the diluting and lyzing chamber is to prepare the specimen for the measurement of its hemoglobin content and WBC count. The lyzing agent causes the cell membranes of the RBCs to rupture and release their hemoglobin into the solution. The WBCs are not lyzed by this agent. Adding the volume of lyzing agent increases the dilution to 1:250. A second substance, Drabkin's solution, is present; it converts hemoglobin to cyanmethemoglobin. This is done to conform to the accepted standard method for determining hemoglobin concentration. The advantage of this method is that it includes essentially all forms of hemoglobin found in the blood.

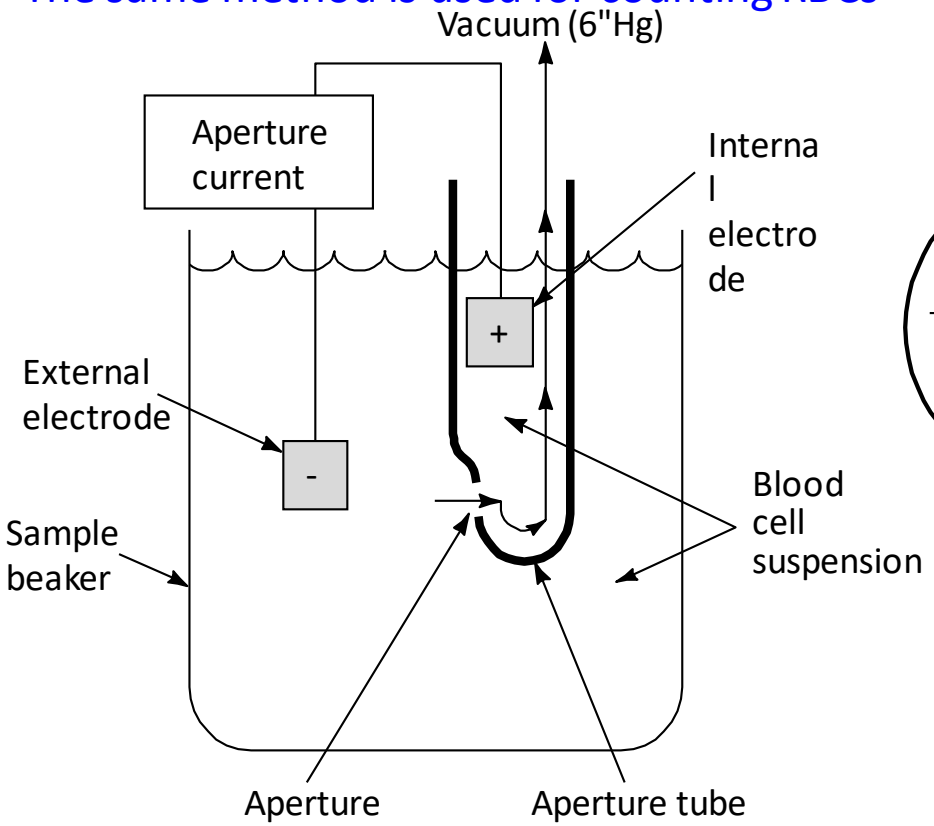


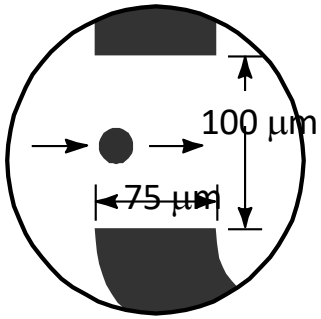
A block diagram of a Coulter Model STKS. (Modified from J. Davidsohn and J. B. Henry, Todd Sanford Clinical Diagnosis by Laboratory Methods, 15 ed. Philadelphia: W. B. Saunders Co.)

- The specimen is next passed through the WBC bath, which functions as a cuvette for the spectrophotometric determination of the hemoglobin content.
- The final step in this process is measurement of the WBC count.

A vacuum pump draws a carefully controlled volume of fluid from the WBC-counting bath through the aperture. A constant current passes from the electrode in the WBC- counting bath through the aperture to the second electrode in the aperture tube. As each WBC passes through the aperture, it displaces a volume of the solution equal to its own volume. The resistance of the WBC is much greater than that of the fluid, so a voltage pulse is created in the circuit connecting the two electrodes. The magnitude of that voltage pulse is related to the volume of the WBC.

- The same method is used for counting RBCs





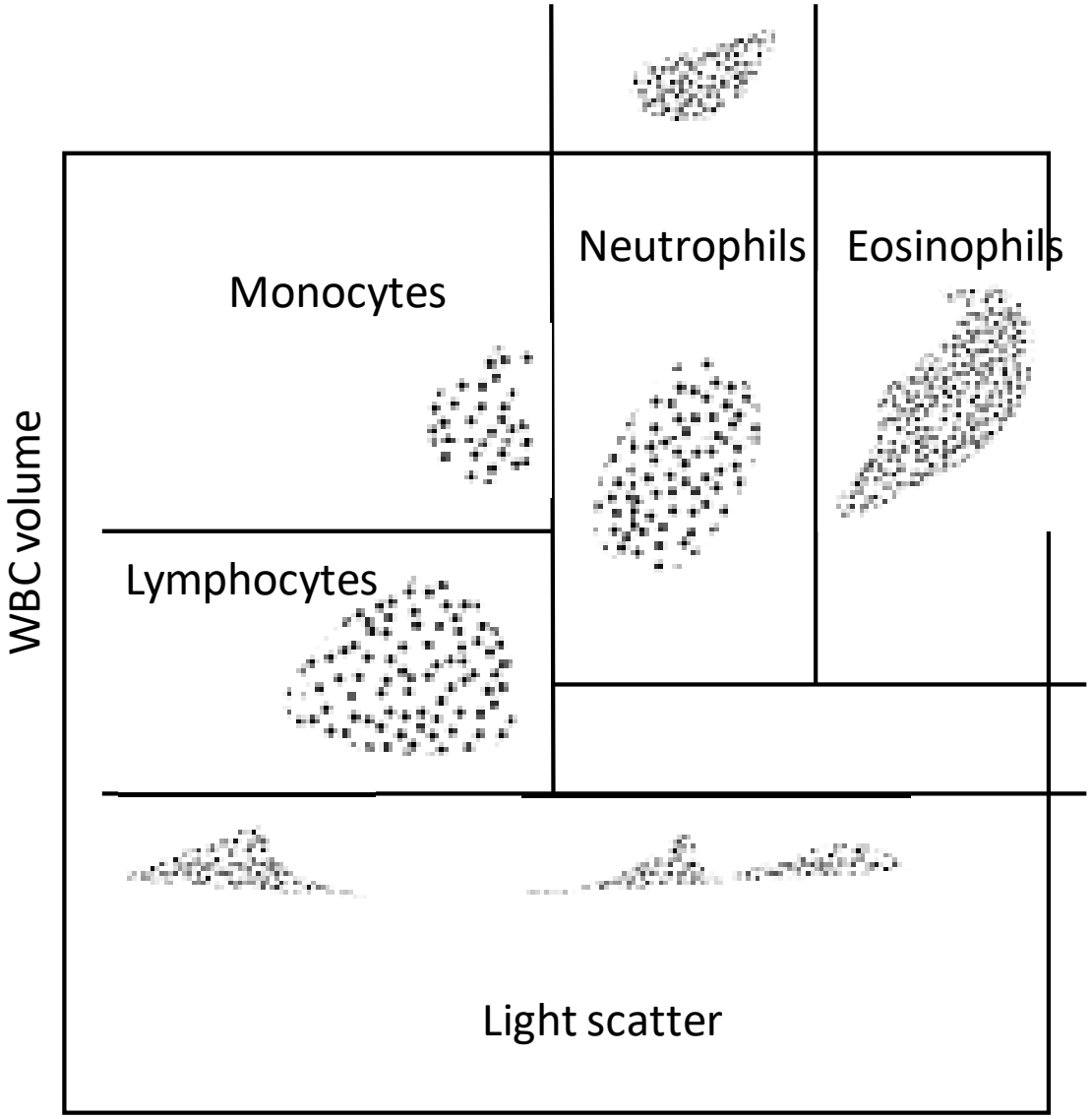
Detail of aperture (WBC)

Cell volumes are important for the determination of cell type wheter it is WBC,RBC, platelet.

Cells with volumes greater than 35.9 fl are classified as RBCs.

Cells whose volumes are in the 2 to 20 fl range are classified as platelets.

Watch movie # C5



Two-dimensional scatterplot.