Chapter 11 Clinical Laboratory Instrumentation

- Analysis of patient specimens: to aid in the diagnosis and evaluate the effectiveness of therapy
- Clinical phathology or clinical laboratory department
  - Chemistry: blood, urine, cerebrospinal fluid (CSF), and other fluids
  - Hematology: RBC, WBC, platelets, function of physiological systems in blood (clotting)
  - Microbiology: pathological microorganisms in body tissues and fluids
  - Blood bank: ABO grouping, blood bank
- Accuracy and precision are extremely important
- Fast response is required
- Automation using computer technology

11.1 Spectrophotometry

- A general term for a class of instruments including photometers and colorimeters
- Enough accuracy and precision, suitable for automation ⇒ widely used
- Substances of clinical interests selectively absorb or emit electromagnetic energy at different wavelengths: ultraviolet (200 ~ 400 nm), visible (400 ~ 700 nm), and near infrared (700 ~ 800 nm)
- Beer's law: $P = P_0 10^{-aLC}$ where
  $P_0 =$ radiant power arriving at the cuvette
  $P =$ radiant power leaving the cuvette
  $a =$ absorptivity of the sample
  $L =$ length of the path through the sample
  $C =$ concentration of the absorbing substance

  Percent transmittance, $\%T = \frac{100P}{P_0} = 100 \times 10^{-aLC}$

  Absorbance, $A = \log\left(\frac{P_0}{P}\right) = \log\left(\frac{100}{\%T}\right) = 2 - \log(\%T) = aLC$
- Keep a and L constant and calibrate to get the absorbance, A_s of the same substance with a known concentration, C_s . Then, the unknown sample concentration,

$$C_u = C_s\left(\frac{A_u}{A_s}\right)$$

- Block diagram in Fig. 11.1
  - Power sources:
  - Wavelength selectors
  - Cuvette
  - Sample
  - Photometric system

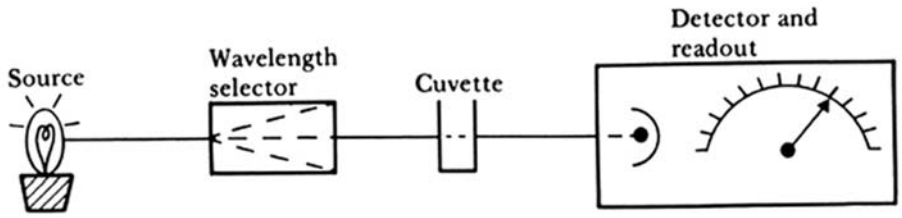

Figure 11.1 Block diagram of a spectrophotometer (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry*, 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

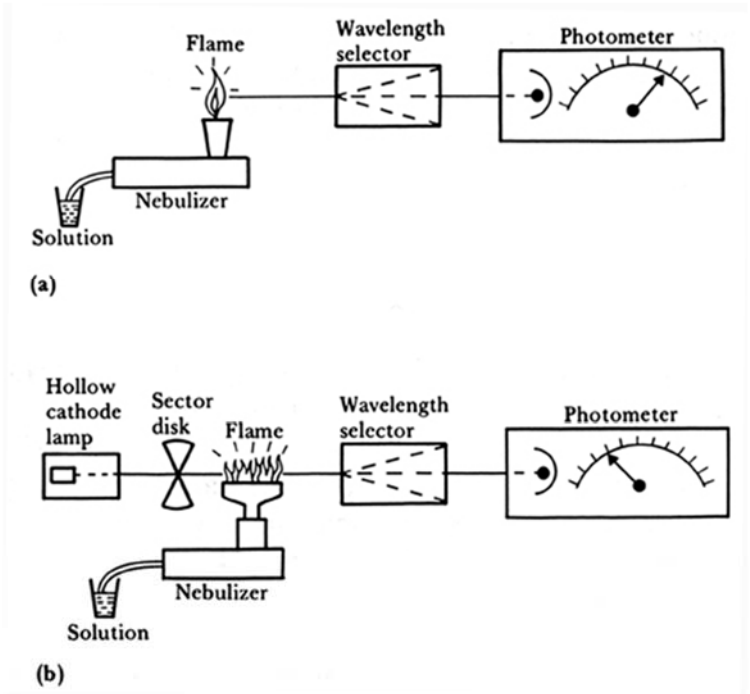

Figure 11.2 Block diagram of instruments for (a) flame emission and (b) flame absorption. (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry*, 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

Flame Photometers

- Power source and sample-holder function are combined in the flame as in Fig. 11.2
- In most cases, measure the sample's emission of light
- Only for determining the concentrations of pure metals

Atomic Emission Flame Photometry

- Limited use for only Na^+, K^+, and Li^+ (with complicated optical system, Ca^{2+})
  - Only 1% of the atoms are raised to an excited level
  - Only a few elements produce enough power at a single wavelength as they move back to lower-energy orbits
- Fig. 11.2(a): sample combined with a solvent ⇒ nebulizer ⇒ flame
  - Fuel: propane or natural gas mixed with compressed air
  - Solvent evaporates and particles disintegrate to yield atoms
  - Atoms emit light as they move back to lower-energy orbits
- Parallel determinations of Na^+, K^+, and Li^+ ⇒ Li^+ is used as the internal standard to correct the errors due to variations in the rate of solution uptake, aerosol production, and flame characteristics
  - Good for small variations
  - Cannot be used for patients receiving Li^+ to treat a psychotic disorder

Atomic Absorption Flame Photometry

- Very accurate concentration determination for calcium, lead, copper, zinc, iron, magnesium
- Majority of atoms in a flame absorb energy at a characteristic wavelength
- Block diagram (Fig. 11.2(b))
  - Power source: placed in an atmosphere of an inert gas, hollow cathode lamp constructed from the metal to be determined (or coating)
  - Heat the cathode ⇒ atoms leaves the cathode ⇒ cathode cavity is filled with atomic vapor ⇒ atoms are excited due to collisions with electrons and ions ⇒ emit light when returning to the ground state ⇒ this light is directed to the flame ⇒ the amount of the light absorption is proportional to the concentration
  - Monochromator
  - Detector: PM tube
  - Rotating-sector disk between the source and the flame ⇒ pulse output from power source ⇒ phase-sensitive demodulator to differentiate the light emitted

by the atoms

Fluorometry

- Molecules: absorption of radiant energy ⇒ being raised to an excited state ⇒ emit light in a characteristic spectrum
- Block diagram (Fig. 11.3)
  - Power source: mercury arc lamp (365, 405, 436, and 546 nm)
  - Wavelength selector
  - Detector: PM tube, at the right angle to the power source to avoid direct light transmission
- Higher sensitivity (10^4 higher than spectrophotometry) and great specificity ⇒ picogram can be detected
- Only a small number of substances have fluorescence property
- Sensitive to pH and temperature

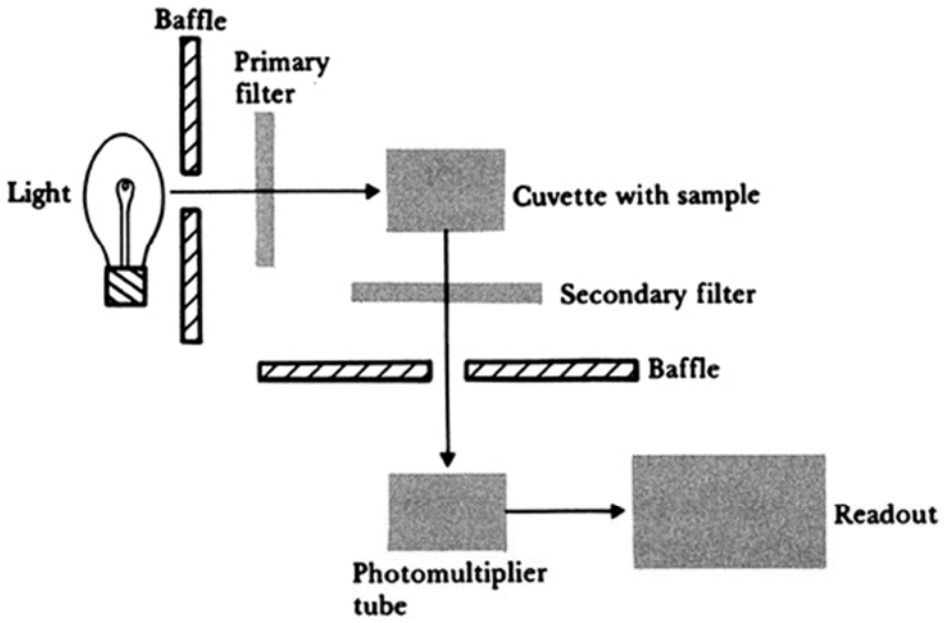

Figure 11.3 Block diagram of a fluorometer (Based on R. Hicks, J. R. Schenken, and M. A. Steinrauf, *Laboratory Instrumentation*. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

11.2 Automatic Chemical Analyzers

❍ Spectrophotometric methods

- Specimen aspiration, dilution, combination of sample with reagents, movement of samples, computation, recording
- Enhanced productivity and reduced response time

Synchron CX4

- High-capacity specimen processing chemistry analyzer
- Microcomputer-controlled discrete random-access clinical analyzer
- Automated specimen handling
- Performance of a variety of analytical test techniques
- Extensive use of microcomputers
- Bar code identification technique
- End-point and rate assays at 30 and 37 °C

Automatic Clinical Analyzer (ACA)

- Flexibility rather than high-capacity
- Serial determination of any of 40 tests for each sample

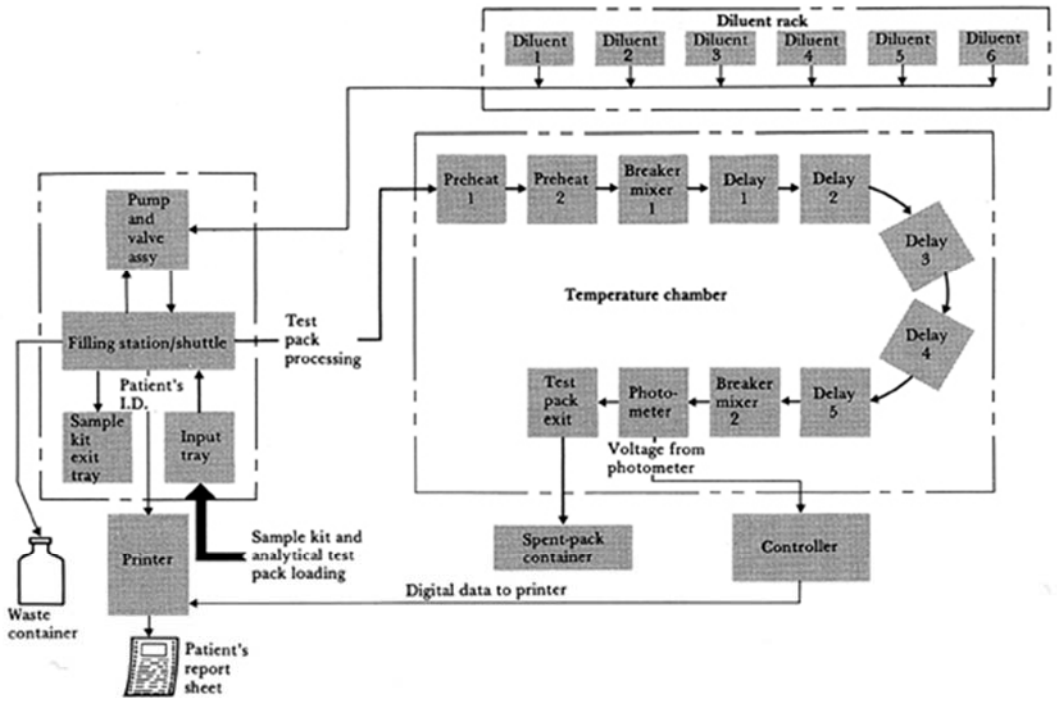

Figure 11.5 Block Diagram of ACA (From *ACA Instrument Instruction Manual*, Dupont Company, Automatic Clinical Analysis Division, Wilmington, DE 19898.)

11.3 Chromatology

- A group of methods for separating a mixture of substances into component parts
- Differences in the rate of movement of components of the mixture in the mobile phase (gas or liquid) due to the interaction of these components with the stationary phase (liquid or solid) ⇒ four possible combinations
  - Liquid stationary phase: partition
  - Solid stationary phase: adsorption
- Detection of complex substances such as drugs or hormones: gas-liquid chromatographs (GLC) and thin-layer chromatographs (TLC)

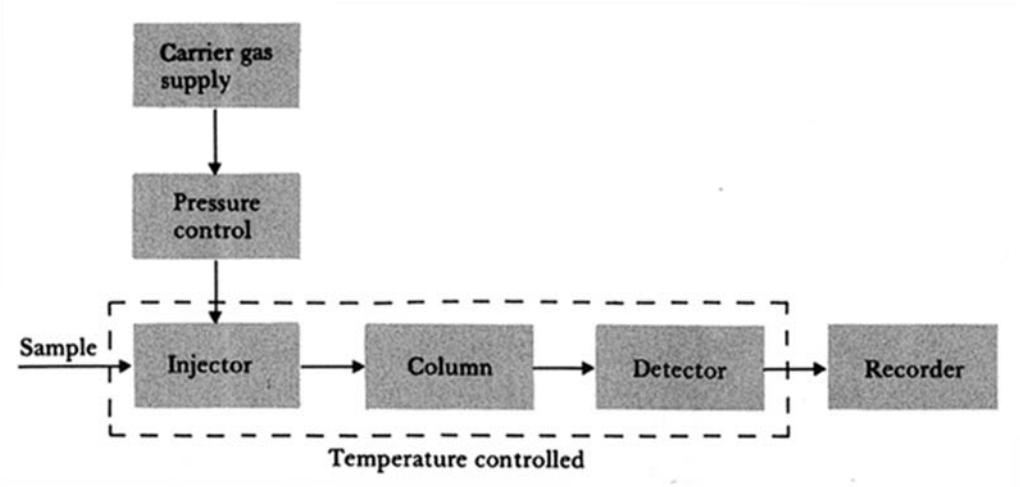

Figure 11.7 Block diagram of a gas-liquid chromatograph (GLC)

Gas-Liquid Chromatographs (GLC)

- Block diagram (Fig. 11.7): fast response (15 min or 1 h), great sensitivity (1 ng), small amount of sample (a few mL)
- Injector
  - 5 mL of sample in the solvent
  - Temperature is set to flash-evaporate he sample and solvent
- Carrier gas: mobile phase
  - N_2 or He (inert gas)
  - Sweeps the evaporated sample and solvent gas down the column
- Column: stationary phase
  - 1 m long, less than 7 mm diameter

- Packed with solid support material (such as diatomaceous earth)
- Solid support is coated with the liquid phase,
- Enclosed in a temperature-controlled oven: temperature controller gradually increases the temperature of the column for the best separation

- Detector
  - At the end of the column
  - Output electrical signal proportional to the quantity of the compound in the effluent gas (Fig. 11.8)
  - Ionization detector, thermal conductivity detector, electron capture detector
- Recorder
  - X-axis (time) distinguishes the components
  - Y-axis (detector output) determines the quantity of the components

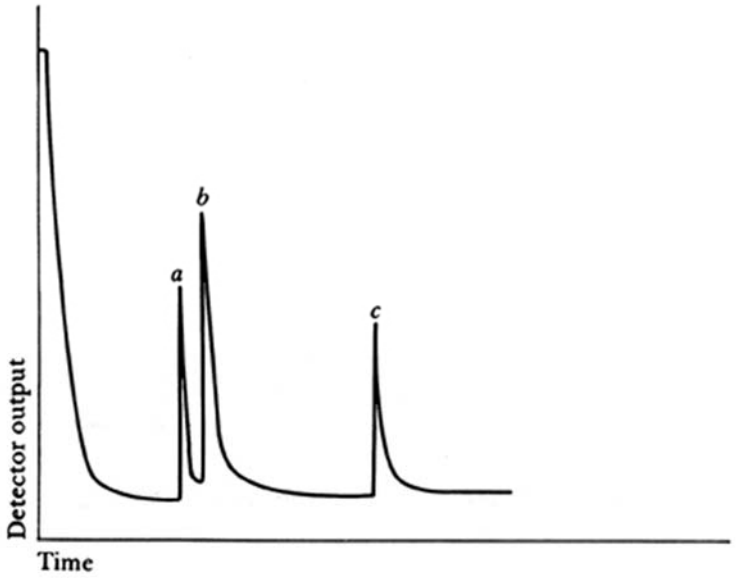

Figure 11.8 Example of a GLC recording for the analysis of blood levels of phenobarbital (peak *a*) and phenytoin (peak *c*). Peak *b* corresponds to the level of heptabarbital (the internal standard).

11.4 Electrophoresis

- Measure proteins in plasma, urine, and CSF
- Separate enzymes into their component isoenzymes
- Identify antibodies

Basic Principles

- Movement of a solid phase with respect to a liquid (buffer solution)
- Buffer solution
  - Carry current
  - Keep the pH of the solution constant during migration
  - Supported by a solid substance (medium)

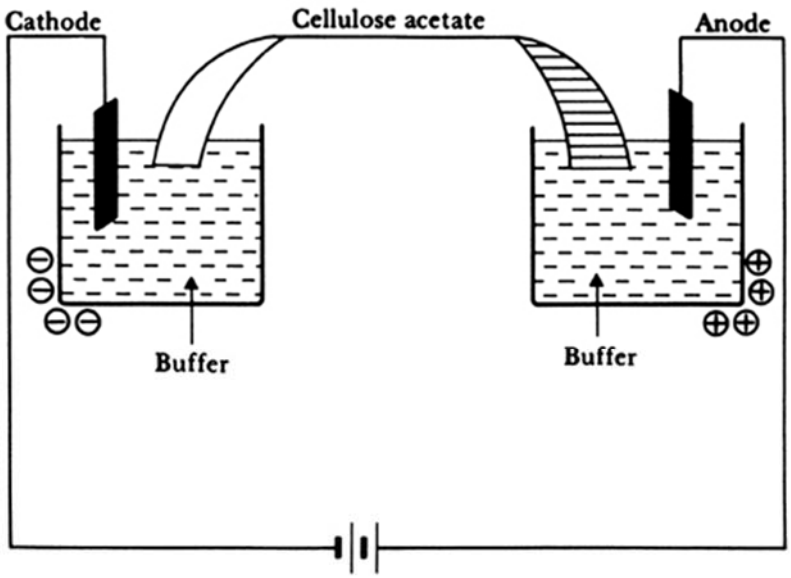

Figure 11.9 Cellulose acetate electrophoresis (Based on R. Hicks, J. R. Schenken, and M. A. Steinrauf, *Laboratory Instrumentation*. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

Zone Electrophoresis

- Sample is applied to the medium under electric field ⇒ particles with similar size, charge, and shape migrate at similar rate ⇒ separation of particles into zones
- Magnitude of charge: mobility (distance a particle moves in unit time per unit field strength, $cm^2/V \cdot s$) of a particle is directly related with its net charge
- Ionic strength of buffer: higher buffer concentration ⇒ slower migration
  - Buffer ions themselves carry current
  - Interaction between buffer ions and particles
- Temperature: mobility is directly related with temperature
  - Current flow ⇒ heat production ⇒ temperature of medium increases ⇒ resistance decreases ⇒ increased migration rate
  - Heat ⇒ water evaporation ⇒ increased particle concentration ⇒ increased

migration rate
    - For gel type medium, constant-current source is used to minimize the heat production
- Time: distance of migration is directly related to the time
- Types of support media: paper, cellulose acetate, starch gel, agar gel, acrylamide gel, sucrose
    - Cellulose acetate (Fig. 11.19) is widely used
    - Constant voltage of 250 V for 15 ~ 20 min, initial current of 4 ~ 6 mA
    - Use fixative to fix the migrated protein bands to the buffer
    - Use dye to stain the bands
    - Dried for densitometry
- Densitometer (Fig. 11.10): light source, filter, holder, detector (photodiode), recorder
    - X-axis: migration distance
    - Y-axis: membrane density (the amount of the component)
    - Integrator
- Other factors: electroendosmosis, chromatography, particle shape, "barrier" effect, "wick flow", streaming potential

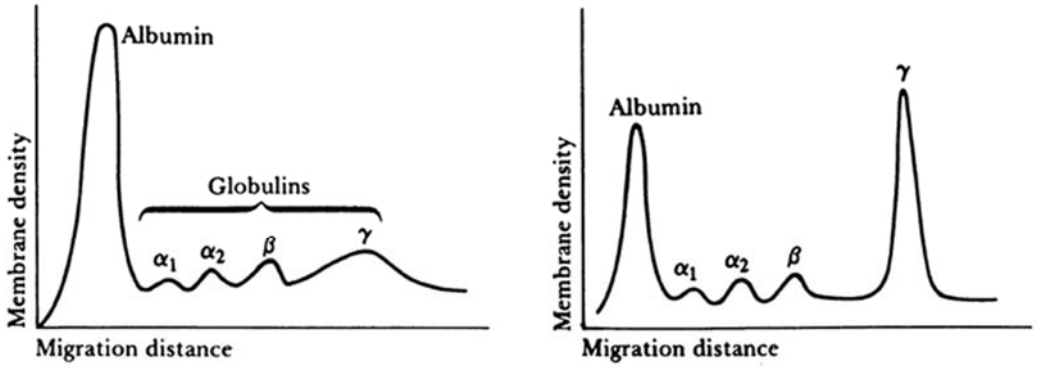

Figure 11.10 Examples of patterns of serum protein electrophoresis The left-hand pattern is normal; the right-hand pattern is seen when there is an over production of a single type of gamma globulin.

11.5 Hematology

Basic Concepts

- Blood: formed elements (RBC, WBC, and platelet), substances in solution, and water
- Red blood cell (RBC)

  - Carry oxygen and carbon dioxide
  - RBC count: 4.6 ~ 6.2×10^6/μL (normal adult male), 4.2 ~ 5.4×10^6/μL (normal adult female),
- White blood cell (WBC)
  - Defend the body against infection
  - Five types (decreasing order): neutrophils, lymphocytes, monocytes, eosinophils, and basophils
  - WBC count: 4 500 ~ 11 000/μL (normal adult male and female)
- Platelet
  - Plug small breaks in the walls of the blood vessels
  - Participate in the clotting mechanism
  - Platelet count: 150 000 ~ 400 000/μL (normal adult male and female)
- Hematocrit (HCT):
  - % of the volume of all formed elements to the total volume of blood sample
  - 40 ~ 54 % (normal adult men), 35 ~ 47 % (normal adult female)
- Hemoglobin (Hb)
  - Conjugated protein in RBC
  - Transports most of O_2 and a portion of CO_2
  - 13.5 ~ 18 g/dL (normal adult men), 12 ~ 16 g/dL (normal adult female)
- RBC indices: characterization of RBC volume and Hb concentration
  - MCV (mean corpuscular volume): 82 ~ 98 μm^3

$$\mathrm{MCV} = \frac{10\,\mathrm{HCT}}{\mathrm{RBC\,count}}$$

  - MCH (mean corpuscular hemoglobin): 27 ~ 31 pg

$$\mathrm{MCH} = \frac{10\,\mathrm{Hb}}{\mathrm{RBC\,count}}$$

  - MCHC (mean corpuscular hemoglobin concentration): 32 ~ 35 %

$$\mathrm{MCHC} = \frac{100\,\mathrm{Hb}}{\mathrm{HCT}}$$

  - RDW (volume distribution width): measure of the spread of the RBC volume distribution

Electronic Devices for Measuring Blood Characteristics

- Detection of changes in electric resistance of a solution when a formed blood element is passed through an aperture: Coulter, Clay Adams, Lors & Lundberg, Baker
- Detection of deflection of light beam caused by the passage of formed blood elements:

Technicon

- Coulter STKS (Fig. 11.11)
  - Blood sample is anticoagulated with EDTA
  - Accurate automatic aspiration
  - Dilution (1:224) with a solution similar to plasma (Diluter I)
  - Path 1 for Hb and WBC
    - Mixing and lysing chamber ⇒ Drabkin's solution converts hemoglobin to cyanmethemoglobin and lysing agent ruptures RBC ⇒ Hb and WBC count
    - Aperture bath (Fig. 11.12): constant-current source ⇒ WBC or RBC ⇒ voltage pulse
    - Counts from three channels are within a range (common WBC-counting-bath electrode and individual aperture tube electrodes) ⇒ average them to produce the result ⇒ correction for coincidence using statistical signal processing
    - Calibration using a specimen with a known WBC count ⇒ threshold for pulse detection
  - Path 2 for RBC
    - Diluter II: further dilution of 1:224 due to greater RBC concentration
    - RBC counter is identical to WBC counter (Fig. 11.12)
    - Cells with volumes greater than 35.9 fL are classified as RBCs
    - 256 channel RBC size histogram ⇒ MCV and RDW
    - Cells with volumes in the 2 ~ 20 fL range are classified as platelets
    - 64 channel platelet size histogram ⇒ MPV, PDW ⇒ used for quality control
  - Path 3 for WBC differential count
    - WBC differential mixing and lysing chamber: RBC removal and WBC stabilization
    - Flow cytometry: low frequency impedance, high frequency conductivity, and light scatter (laser illumination)
    - Three-dimensional scatter plot (two-dimensional view in Fig. 11.13)
  - Reticulocytes count
    - RBCs are dyed with New Methylene Blue
    - Separate run of the instrument
  - RBC, Hb, MCV ⇒ computer ⇒ HCT, MCH, MCHC

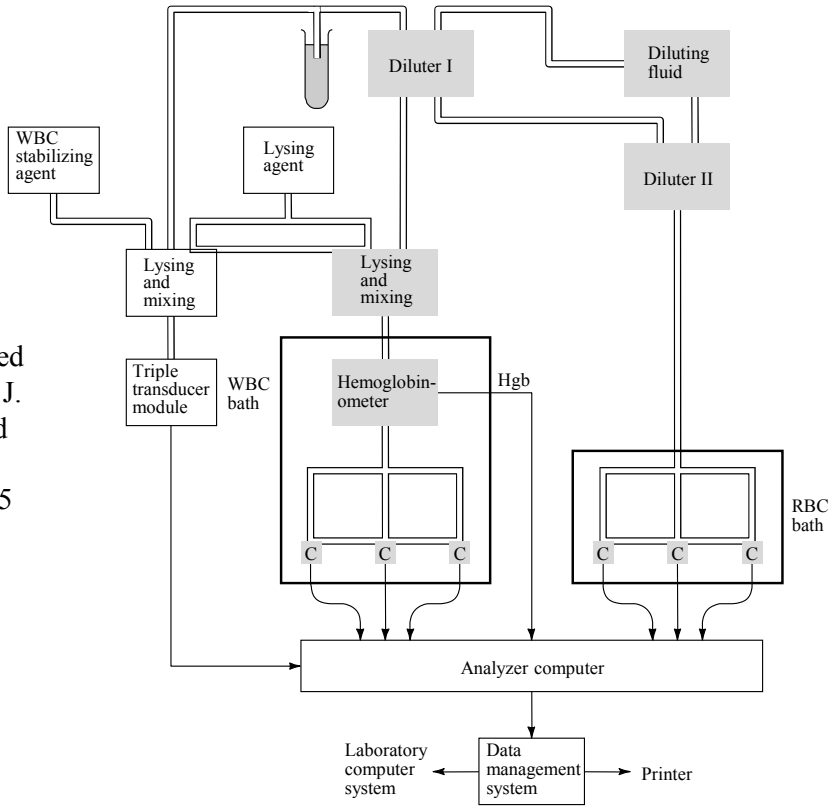

Figure 11.11 A block diagram of a Coulter Model STKS. (Modified from J. Davidsohn and J. B. Henry, Todd Sanford Clinical Diagnosis by Laboratory Methods, 15 ed. Philadelphia: W. B. Saunders Co.)

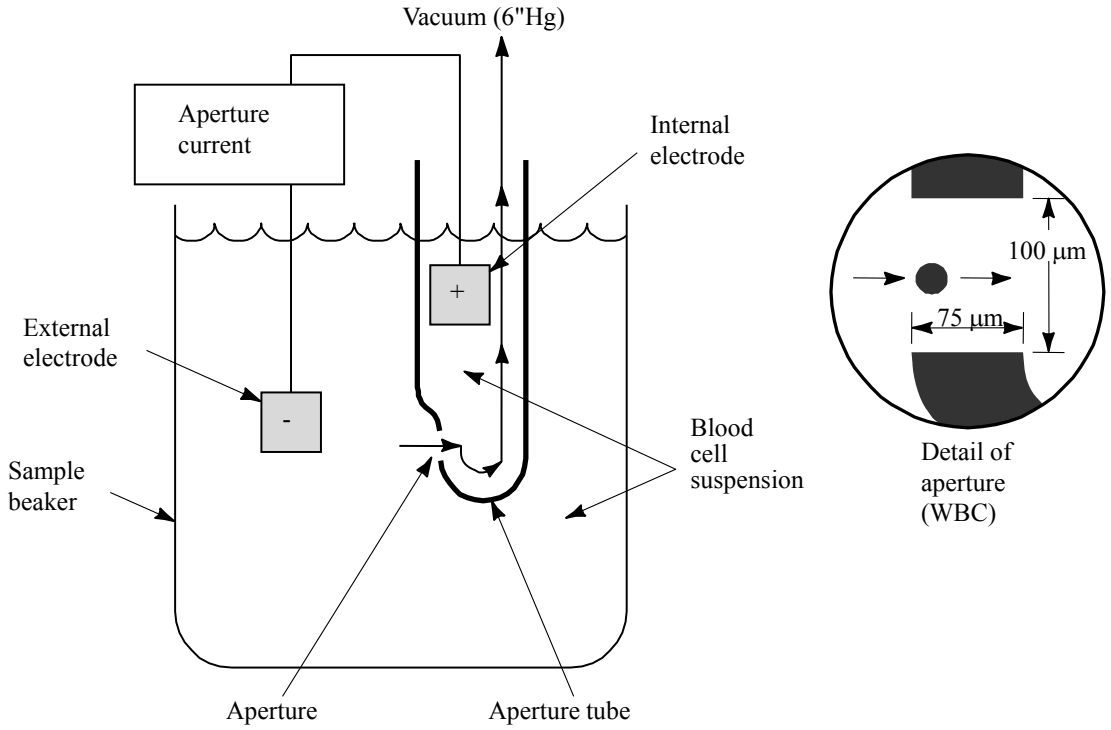

Figure 11.12 Coulter STKS aperture bath

Automatic Differential Counts

- Pattern recognition technique
- Hematrak (Geometric Data Co.): Fig. 11.14
- Used in cytology lab for automatic screening of pap smears for abnormal cells detection of early cervical carcinoma
- Color video scanner with microscope optics ⇒ image memory ⇒ morphological analyzer

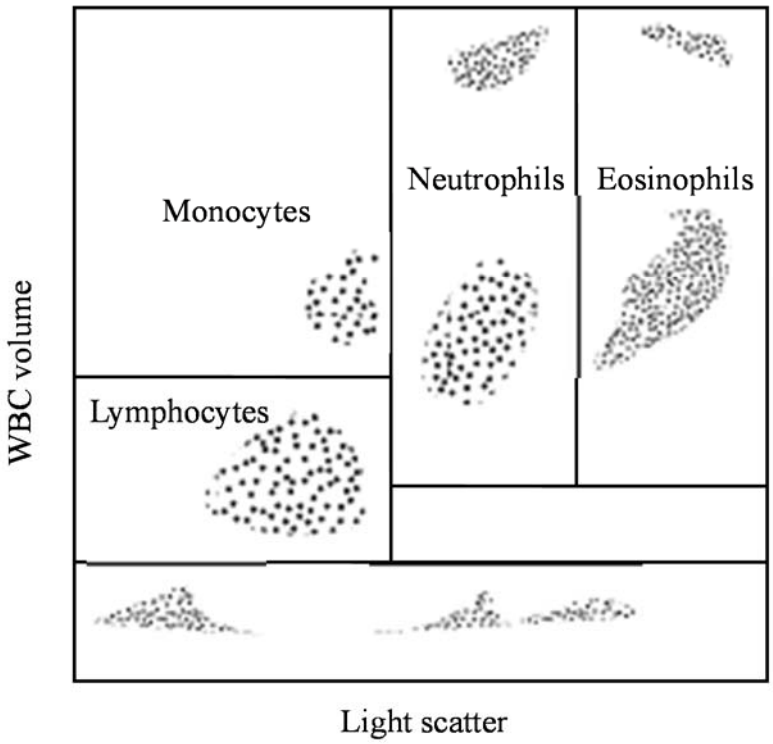

Figure 11.13 Two-dimensional scatterplot.

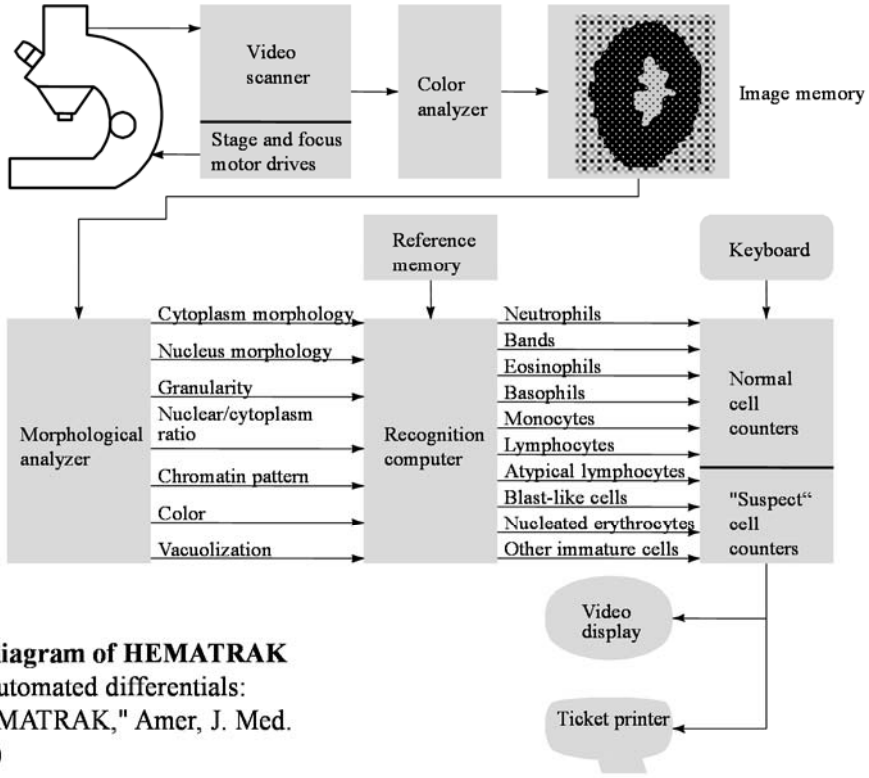

Figure 11.14 Block diagram of HEMATRAK
(From M. Levine, "Automated differentials: Geometric Data's HEMATRAK," Amer, J. Med. Tech., 40: 464, 1974.)