CLINICAL ANALYTICAL INSTRUMENTATION

EE 471 – F2016

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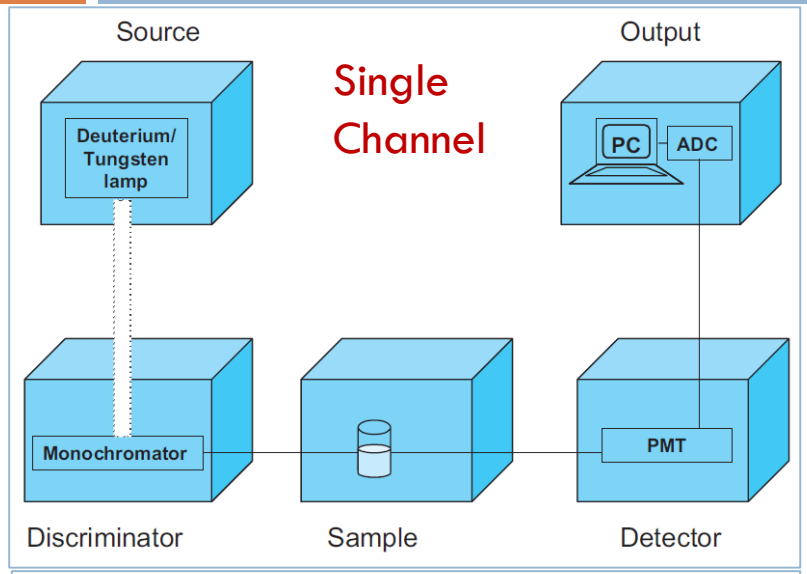
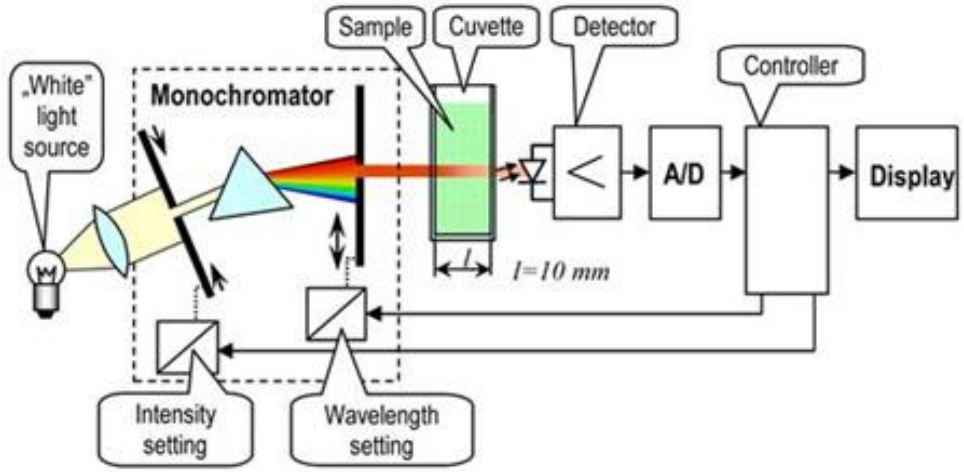
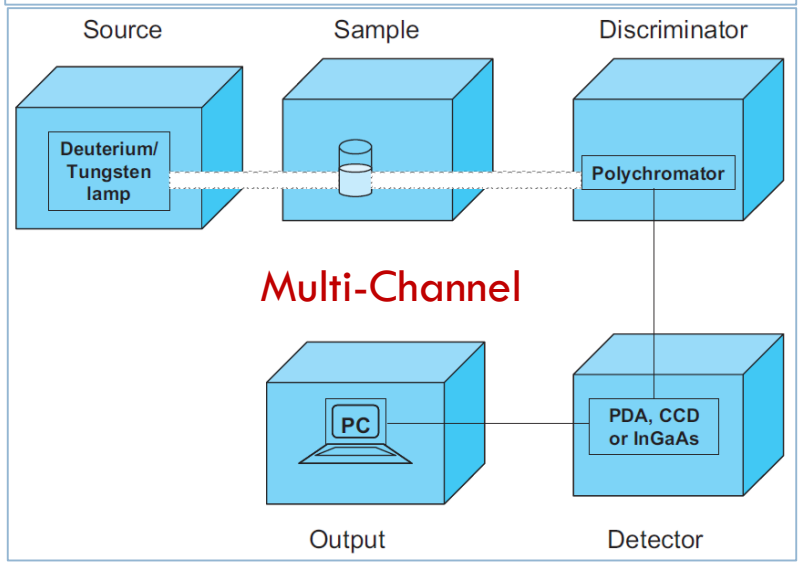
Spectrometric Instruments

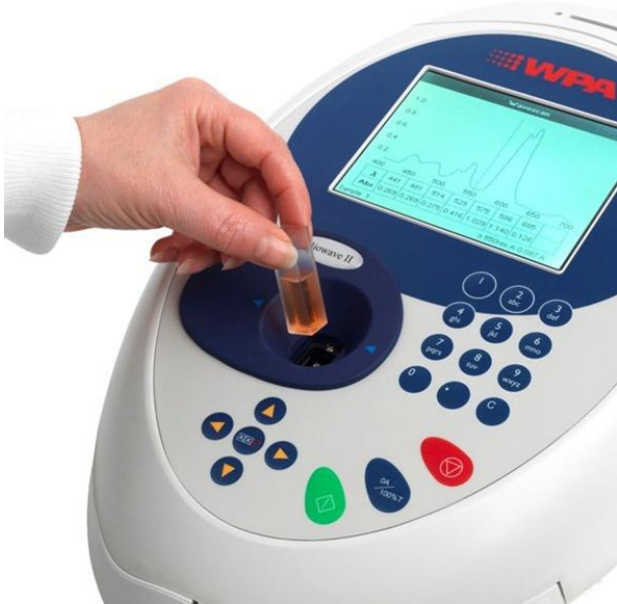
- At room temperature, most compounds are in their lowest energy or ground state
- Upon interaction with appropriate type of electromagnetic radiation, characteristic transitions can occur: Excited State
  - Excited states usually decay back to ground state very quickly, by emitting energy absorbed with same or lower frequency or by 'radiationless' relaxation through heat loss
  - Infrared radiation causes the vibrations in molecules to increase in amplitude
  - Absorption of visible and ultraviolet radiation cause electrons to move to higher electronic orbitals
  - X-rays actually break bonds and ionize molecules
  - Molecular spectra obtained by measuring radiation absorbed or emitted by gases, liquids or solids yield much analytical information about a molecule
- These phenomena are exploited by spectrometric instruments

Spectrophotometer







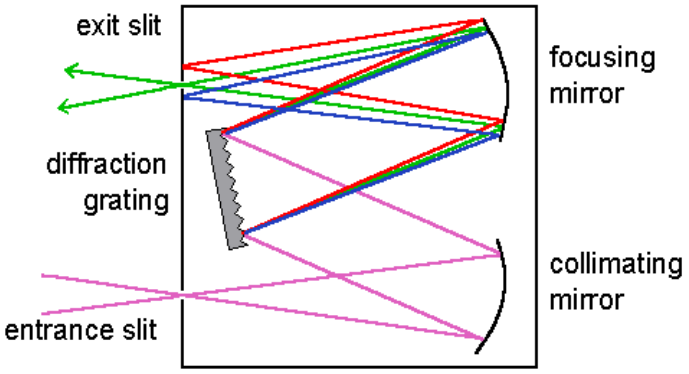


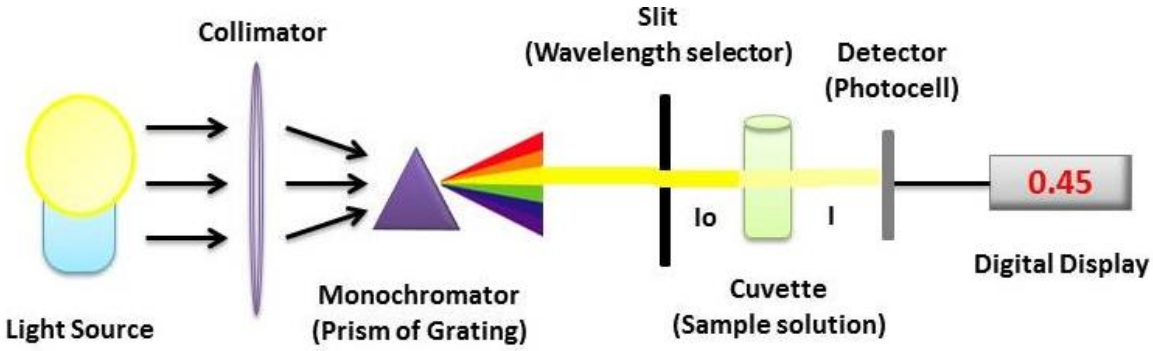
Source

- Deuterium lamps are commonly used as UV radiation source in range 200–400 nm
- Tungsten incandescent lamps as sources for visible and NIR regions covering range 400–2500 nm
  - For NIR, source operated at 2500–3000 K: more intense radiation

Discriminator

- Monochromator is usually used as wavelength selector
- Components: dispersing medium to separate wavelengths of polychromatic radiation from source, slits to select narrow band of wavelengths of interest and lenses or mirrors to focus chosen radiation
  - Dispersing medium can be diffraction grating(+), prism or optical filter
  - Interferometers more common in Fourier Transform (FT) instruments: more effective at longer wavelengths (IR and NIR) and also used for UV–Vis



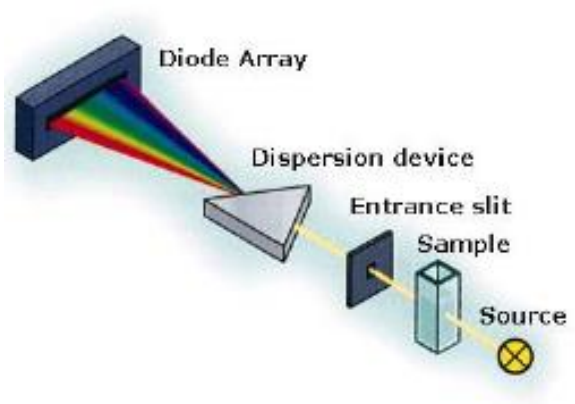


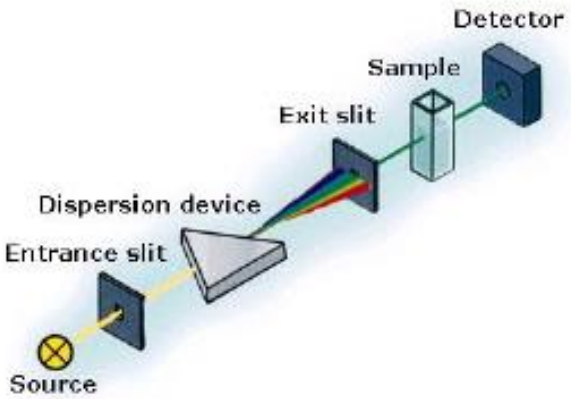
Sample Holder

- Sample holder must be transparent in wavelength region being measured
  - Quartz cuvettes are normally used for UV–Vis and NIR measurements
  - Flow-through, cylindrical, micro and thermal cells can also be used
- For UV–Vis absorbance, cuvettes are usually 1 cm in path length in laboratory based instruments, but shorter path lengths can be employed
- For NIR, longer path lengths of 5–10 cm used in short wavelength NIR (750–1100 nm) and shorter path lengths of 0.1–2 cm used for the long wavelength NIR (1100–2500 nm)
- Cuvettes and cells should be handled carefully to avoid leaving fingerprints
- Sample compartment must prevent stray light and dust from entering
  - Adversely affect the absorbance readings if allowed
- Sample should also not be too concentrated
  - Beer–Lambert Law starts to deviate at high absorbance levels

Detectors

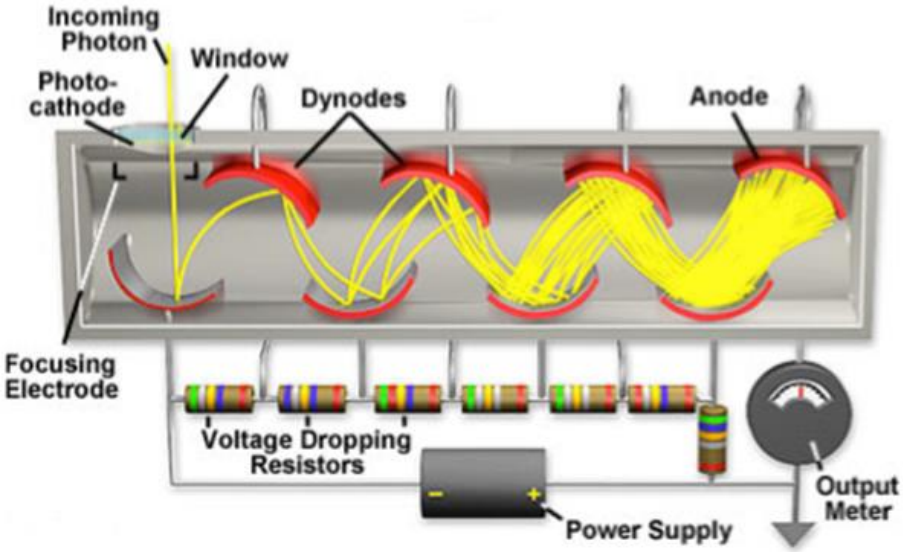
- Typically photomultiplier tube (PMT), photodiode array (PDA) or charge-coupled device (CCD)
- Mono-channel systems use one detector (one wavelength at a time)
  - Measures intensity of one at a time as monochromator slowly scan through spectrum
- Multi-channel systems use array detector (many wavelengths measured simultaneously)
  - Two advantages: Multichannel advantage (SNR↑), and throughput advantage (single deuterium source for whole UV–Vis range - no monochromator)





Photomultiplier Tube (PMT)

- Popular monochannel detector
- Consist of photosensitive surface and series of electrodes (dynodes), each at increased potential compared to one before
- When photon strikes photosensitive surface, primary electron is emitted and accelerates towards first dynode
- Electron impacts dynode and causes release of a number of secondary electrons, which hit next electrode and so on, until the signal is amplified many times over (typical gain: 10^6)
- Can detect extremely small signals

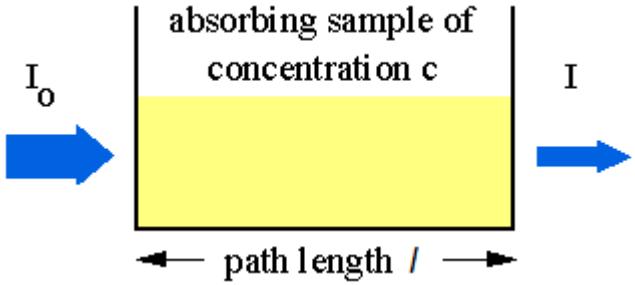


Beer–Lambert Law

- Concentration is related to absorbance by

$$A_\lambda = \log\left(\frac{I_o}{I}\right) = \varepsilon_\lambda c\, l \quad \text{or} \quad I = I_o \exp(-\varepsilon_\lambda c\, l)$$

  - A_λ : absorbance at a particular wavelength (λ),
  - ε_λ : extinction coefficient at a particular wavelength (λ)
  - c : concentration
  - *l* : path length.
- During most experiments, ε and l remain constant, so absorbance is proportional to concentration
  - Exploited for quantitative analysis

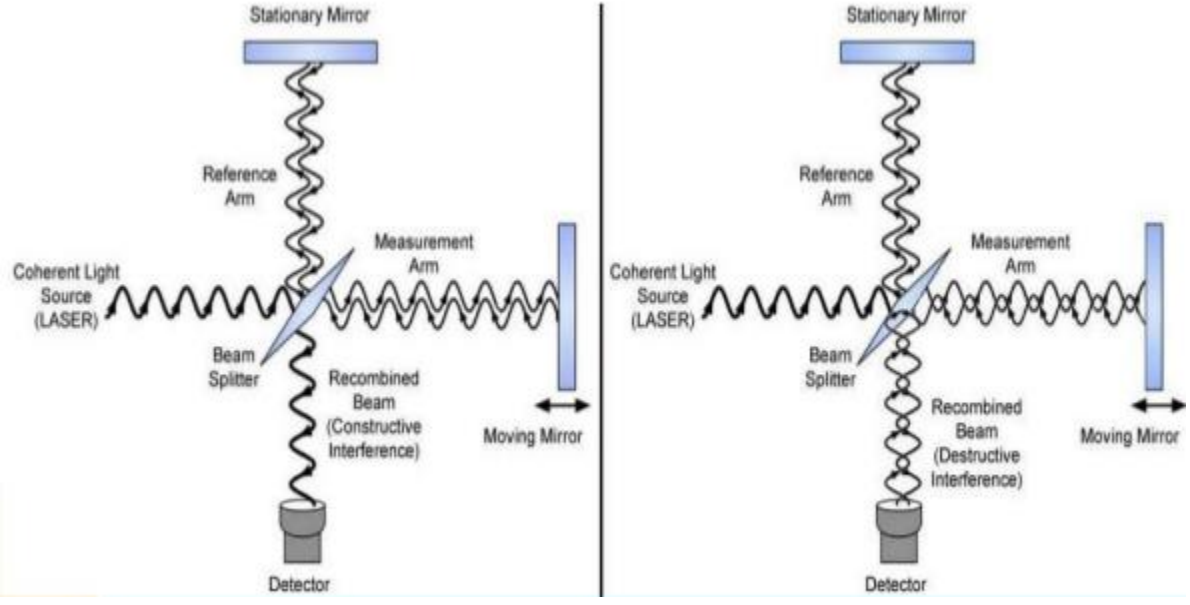


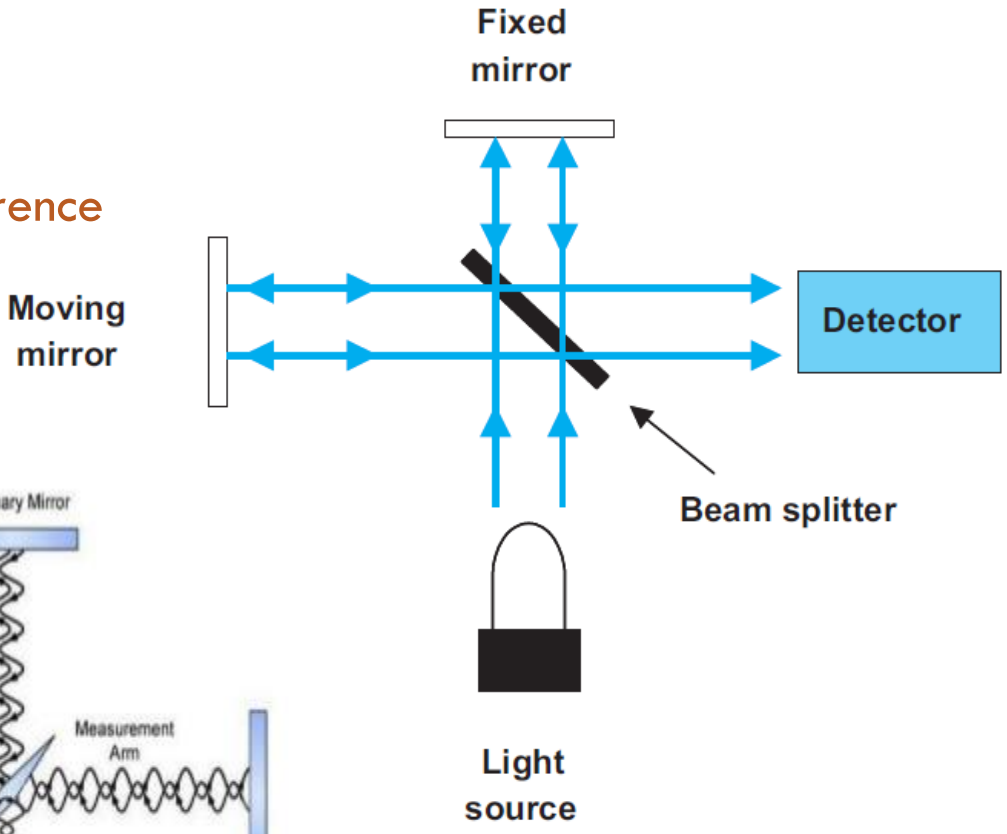
Output

- PC collects the data, converts it from transmission to absorbance and displays spectrum
- PC can often carry out baseline subtraction and smoothing and filtering tasks as well as qualitative and quantitative analysis
- PC may also compare spectrum to those in spectral library and to carry out peak purity checks

Michelson Interferometer

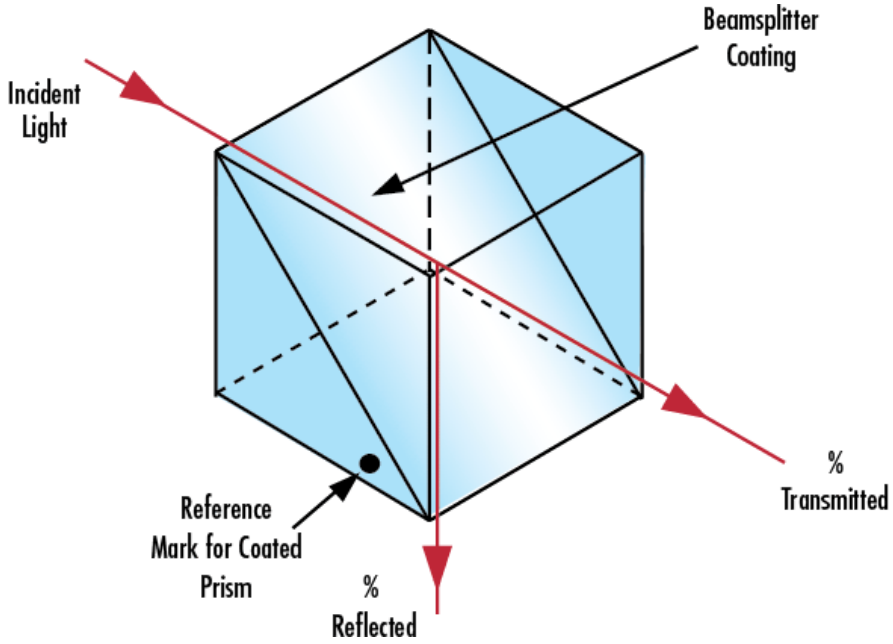
- Selection of wavelength
  - Moving mirror position
  - Based on constructive interference
  - Scanning all wavelengths

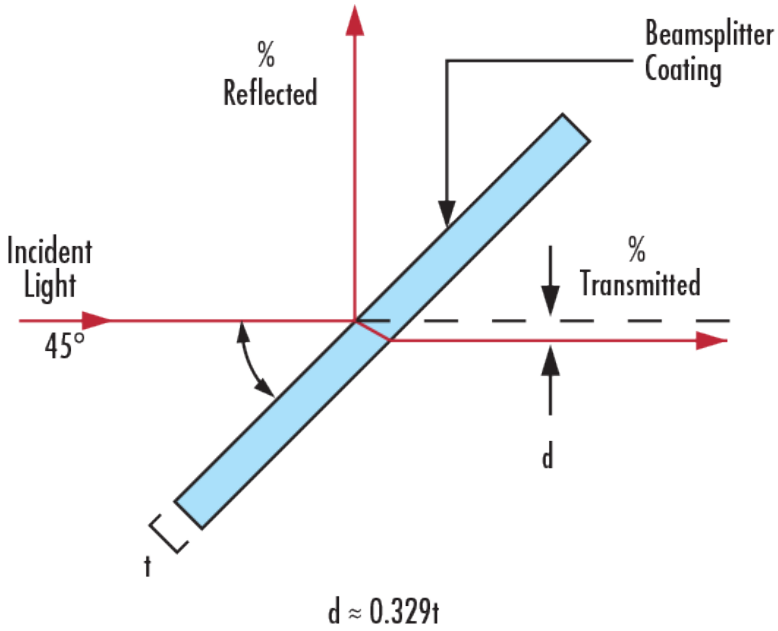




Beam Splitters

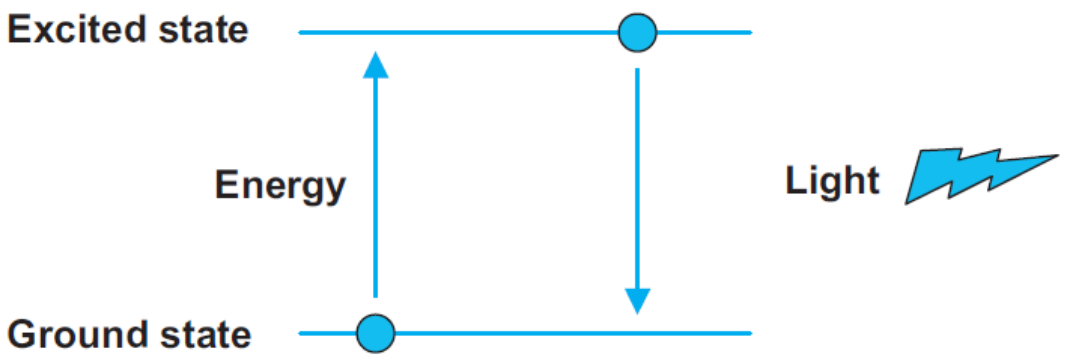
- Optical components used to split incident light at designated ratio into two separate beams
- Can also be used in reverse to combine two different beams into a single one
- Classified according to their construction into cube or plate

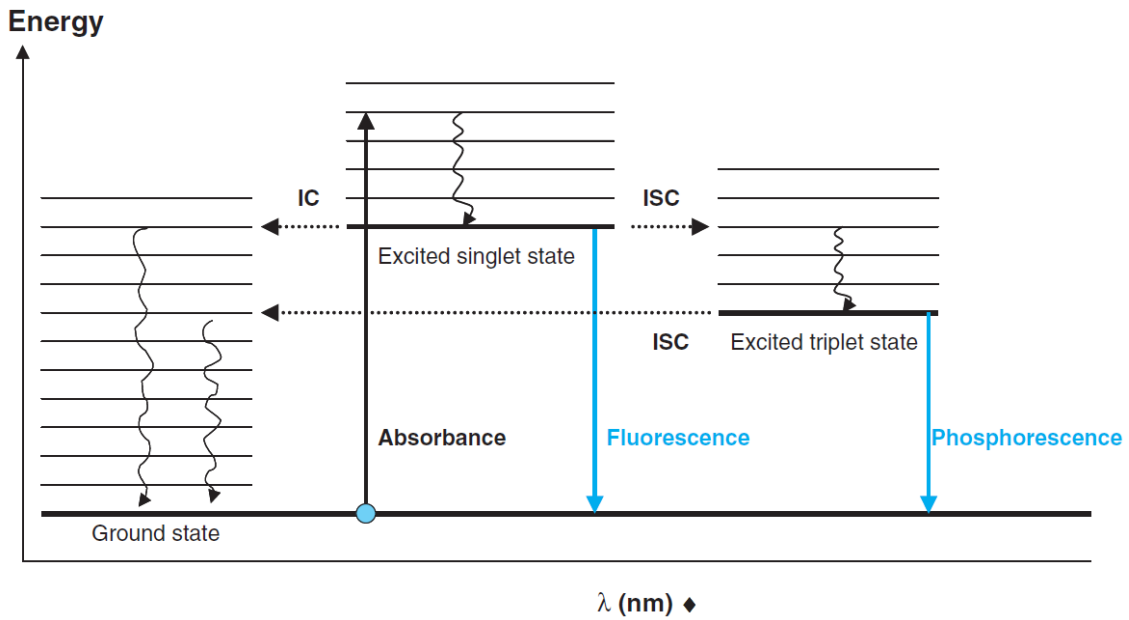




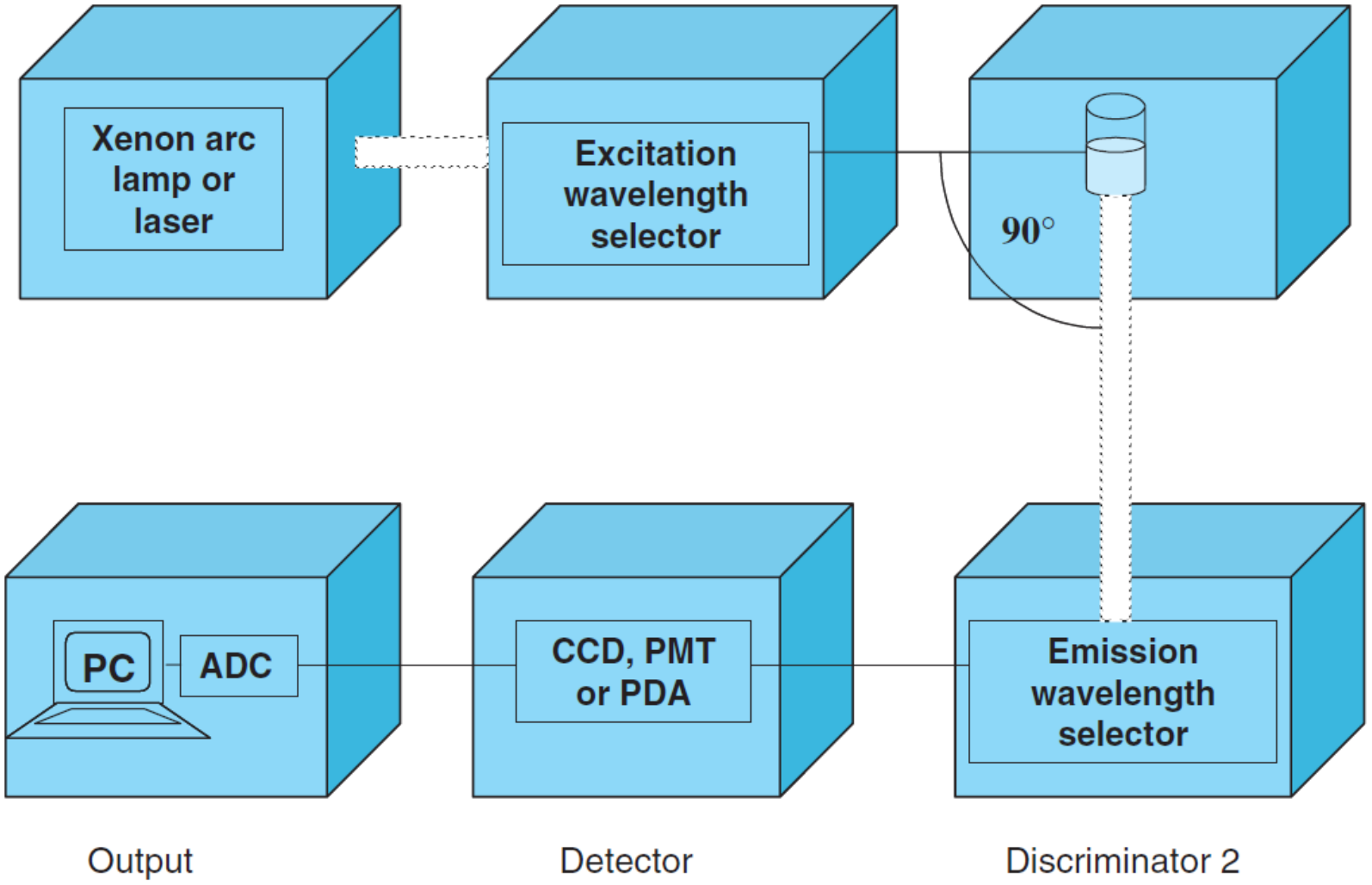
Luminescence Phenomena

- Photoluminescence
  - Fluorescence
  - Phosphorescence
- Radioluminescence
- Bioluminescence
- Chemiluminescence

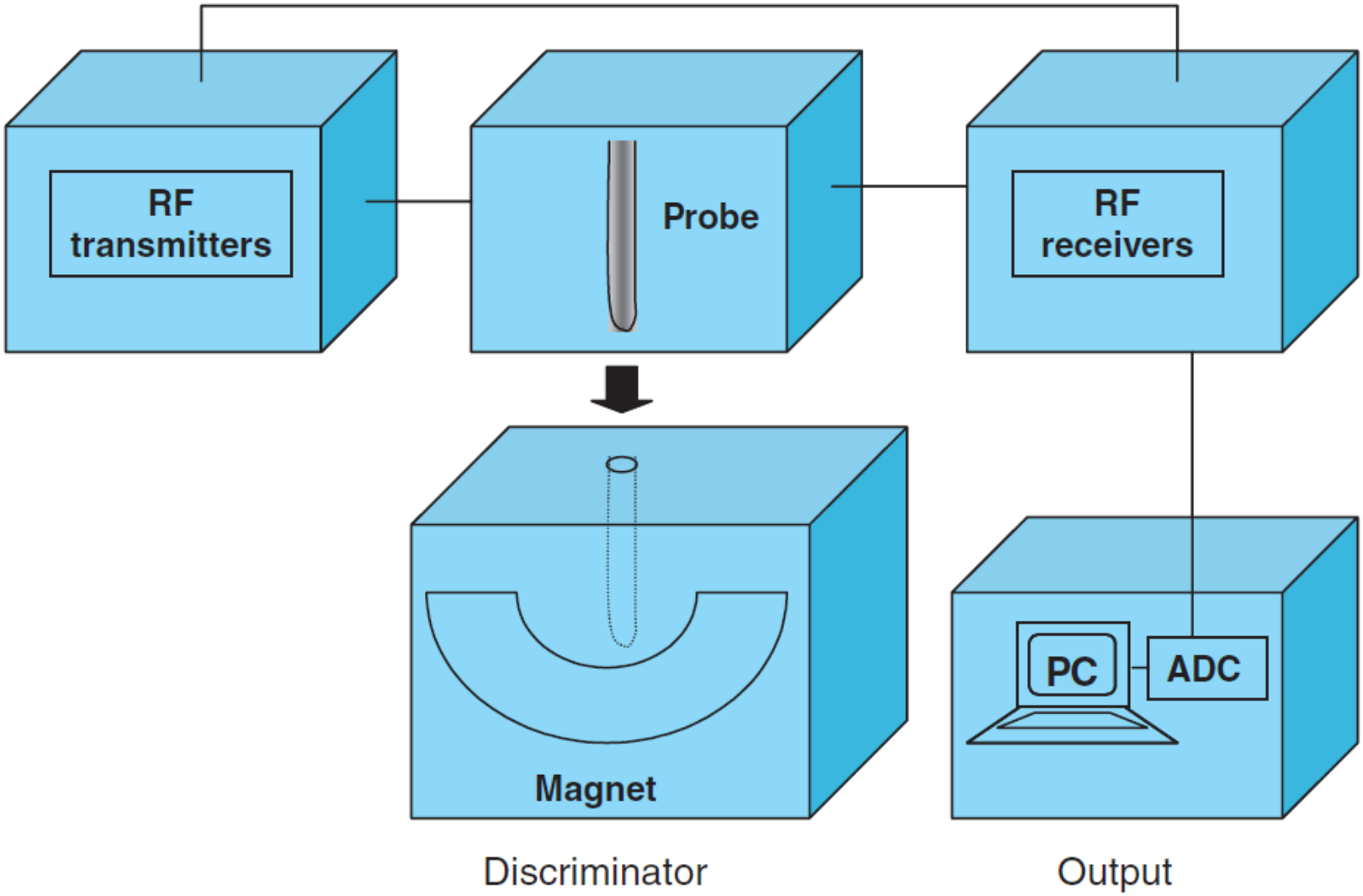




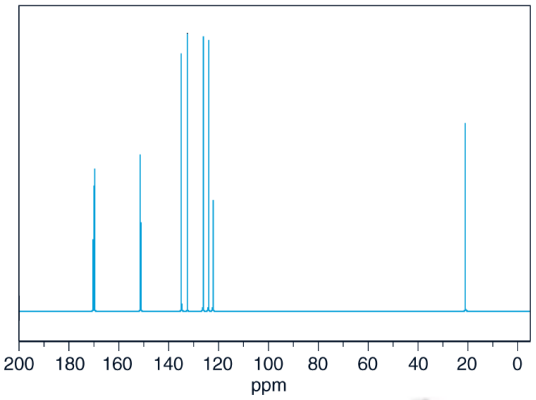
Spectrofluorometer Instrumentation

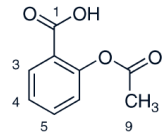


NMR Spectrometer



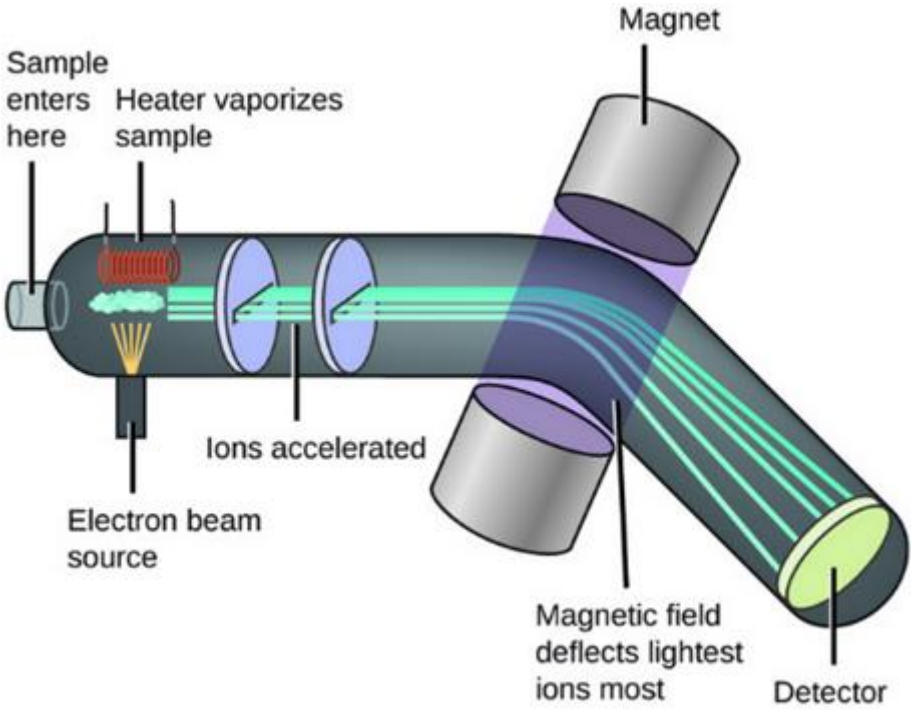
NMR Instrumentation





Mass Spectrometer

- Mass spectrometry is based on generating ions in gaseous state, separating them according to their mass-to-charge ratio (m/z) and detecting them
  - In fact, MS provides more information about composition and structure of compound from less sample than any other analytical technique

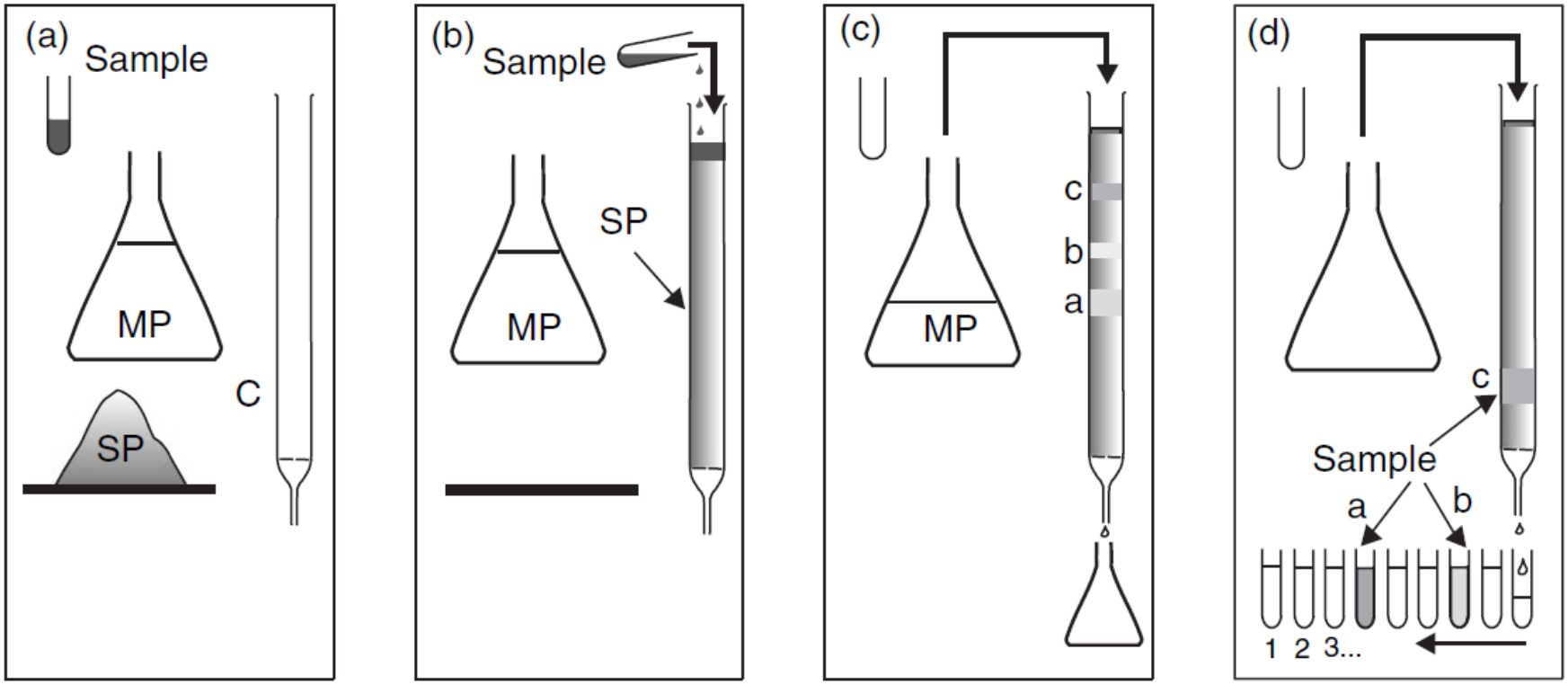


Separation Instruments

- Separation instrumentation is based mainly on chromatography, which is a procedure for separating the analyte(s) of interest from interferences (matrix) and other compounds in sample mixture
- Chromatographic techniques depend on differing distributions of individual compounds between two immiscible phases – mobile and stationary
  - Stationary phase is fixed in a column or on a solid surface
  - In practice, sample mixture is added to one end of stationary phase and mobile phase then passes through or over it carrying the sample
  - Mixture of compounds is eluted, compound appearing first at the end of stationary phase being that which has the smallest distribution into stationary phase
  - As separated compounds appear at the end of stationary phase they are detected
  - Detector used may be general purpose detector or specific for analyte of interest
  - Actual identification and quantitation of separated compounds is made by detector
- Many types of chromatography including , e.g. thin layer, gas and liquid

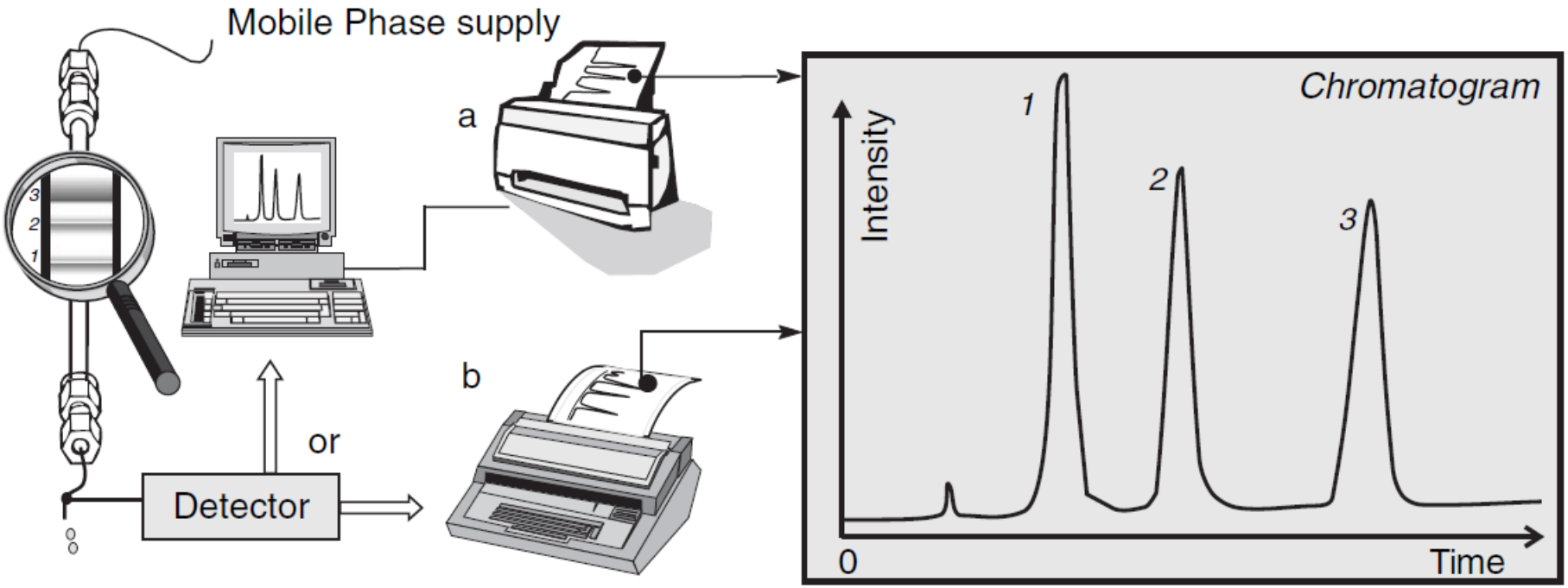
Basic Experiment in Chromatography

- (a) Ingredients – C: column, SP: stationary phase, MP: mobile phase, and S: sample
- (b) Introduction of sample
- (c) Start of elution
- (d) Recovery of products following separation



Analysis by Chromatography

- Essential recording obtained for each separation is called chromatogram
  - It corresponds to diagram traced on chart paper or screen that reveals variations of composition of eluting mobile phase as it exits column
  - order of appearance of compounds corresponds to relative position of each constituent on column



Reading Assignment

- Read Chapter 2 and 3 of *Analytical Instrumentation*