HPLC detectors

HPLC detectors

- Corresponding sensitivity
- Stability and reproducibility
- Wide dynamic linear range
- Fast response
- Minimal volume of detection cell

- detectors can be universal or selective
  - A universal detector depends on the characteristic of a system as a complex (refractive index)
  - A selective detector depends on the certain characteristic of the sample (fluorescent)
- detectors can be destructive (AAS, AES) or non-destructive (UV/Vis)
- detectors can with combined benefits (multiple detection)
- detectors can work in several domains of one characteristic (diode array detector)
- some detectors need a special interface to the LC system

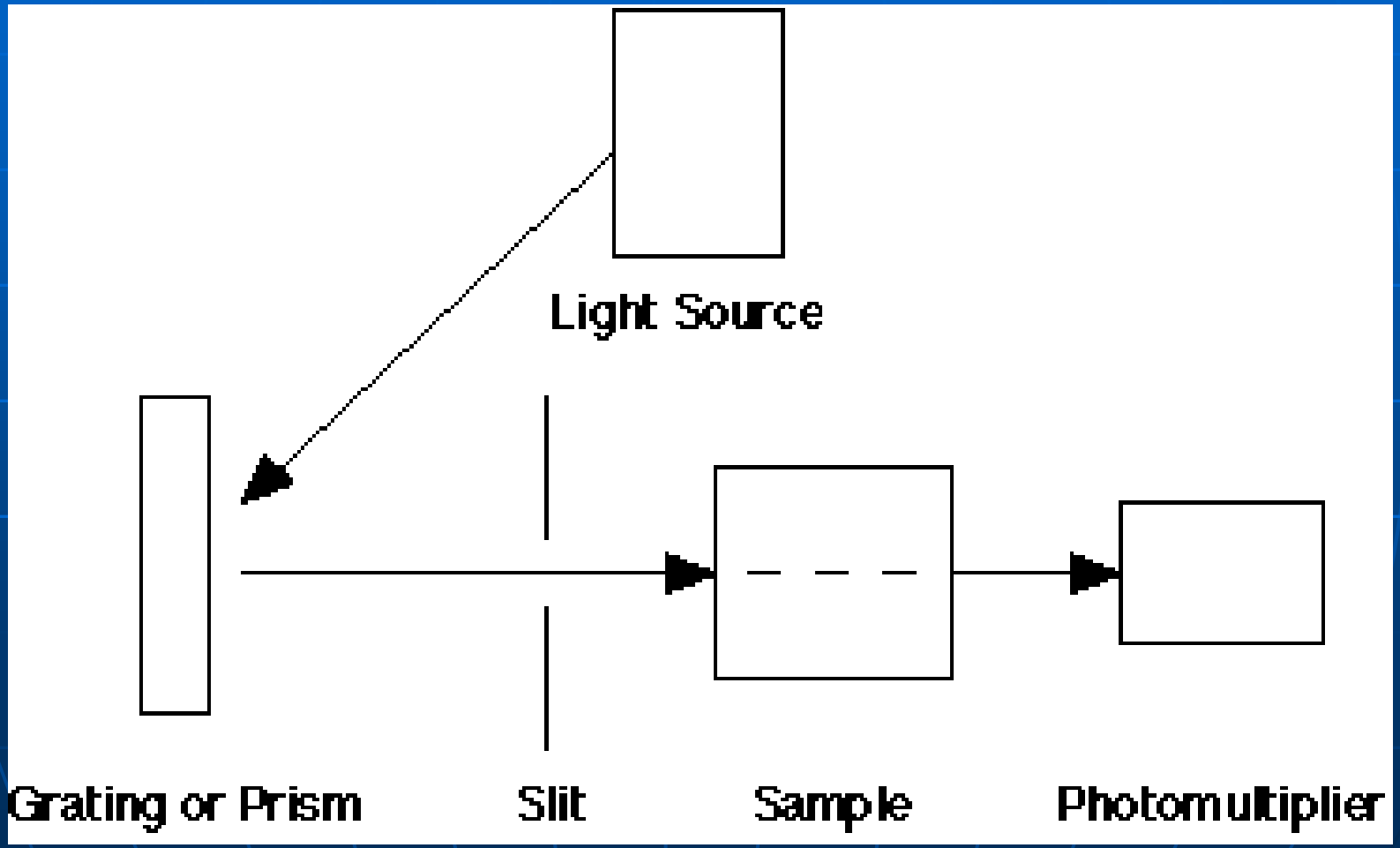
- UV/Vis detection
- Fluorescent detectors
- Radiochemical detectors
- Electrochemical detectors
- Nuclear Magnetic Resonance (NMR) detectors
- Light-Scattering (LS) detectors
- Refractometric detectors
- Polarometric detectors
- IR detectors

HPLC detectors

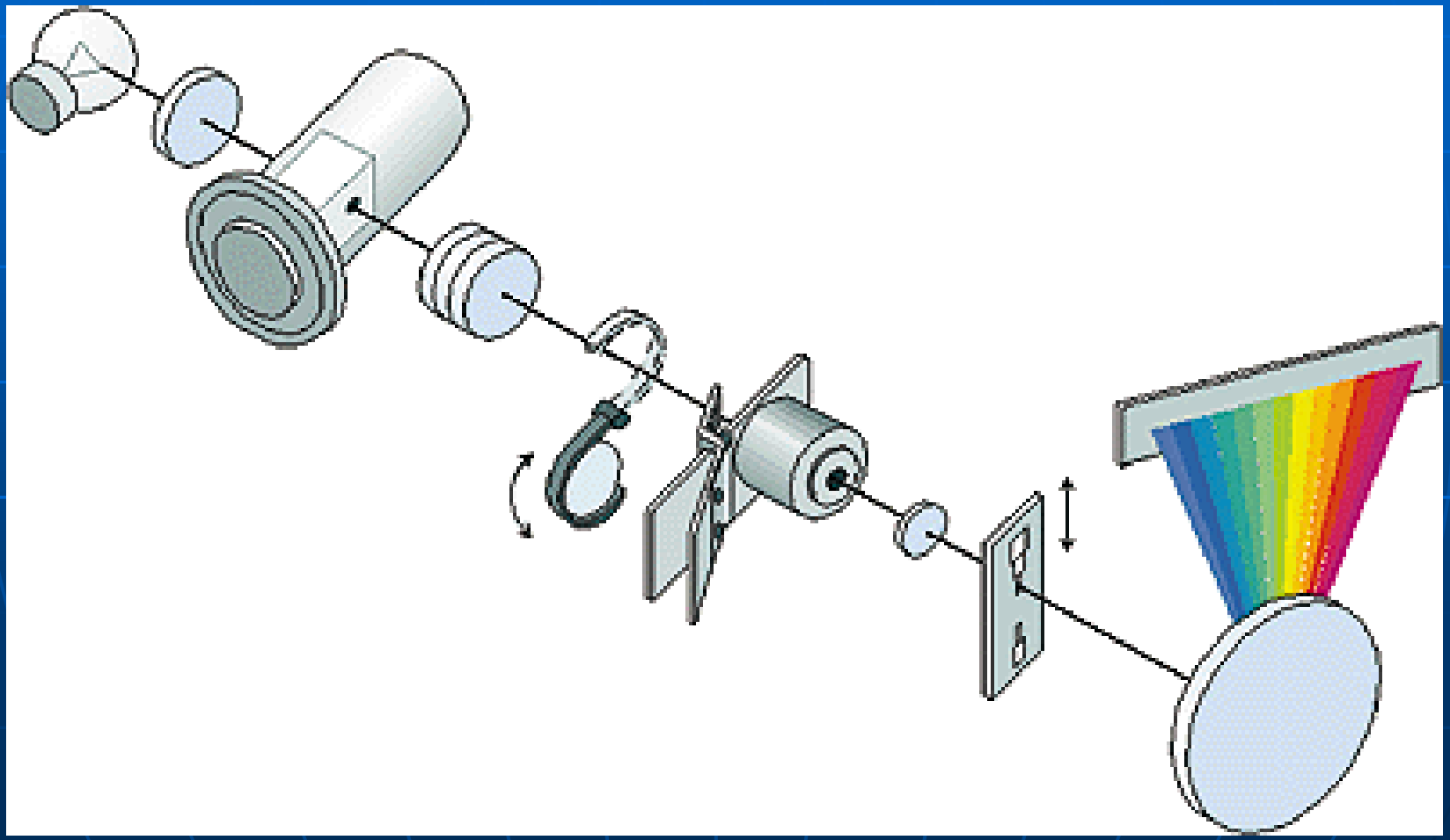
- UV/Vis detection
  - Fixed wavelength
  - Variable wavelength
  - DAD
- standard monochromatic spectrophotometer, deuterium lamp, wavelength is changed by the turning of a prism, at any moment one wavelength only

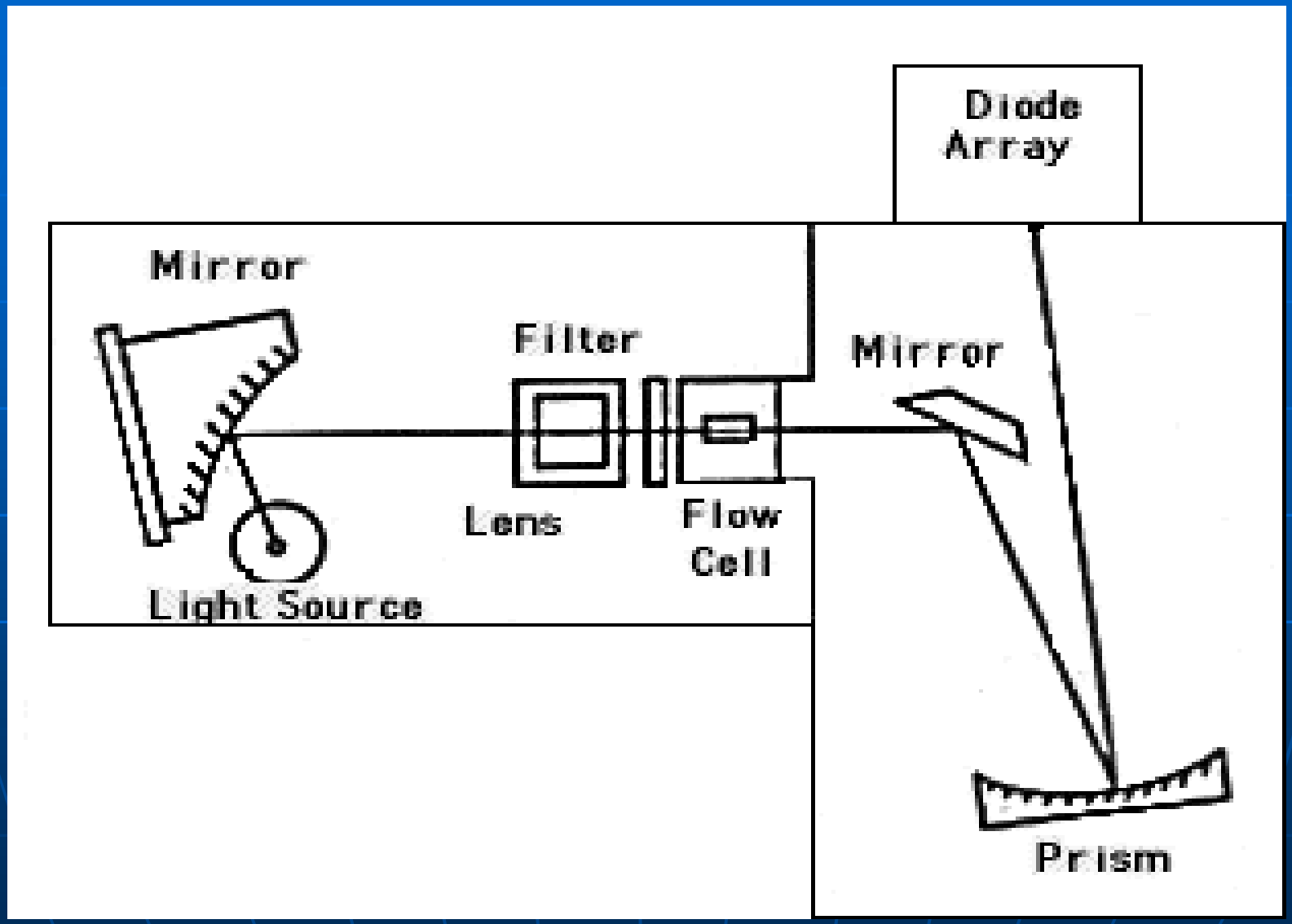
- continuous monitoring of the complete UV/Vis spectrum:
- a) several standard spectrophotometers on-line in line – several UV/Vis regions
- b) DAD - Diode Array Detector – simultaneous measurement of the whole area, data available immediately, possibility of creation of 3D chromatogram

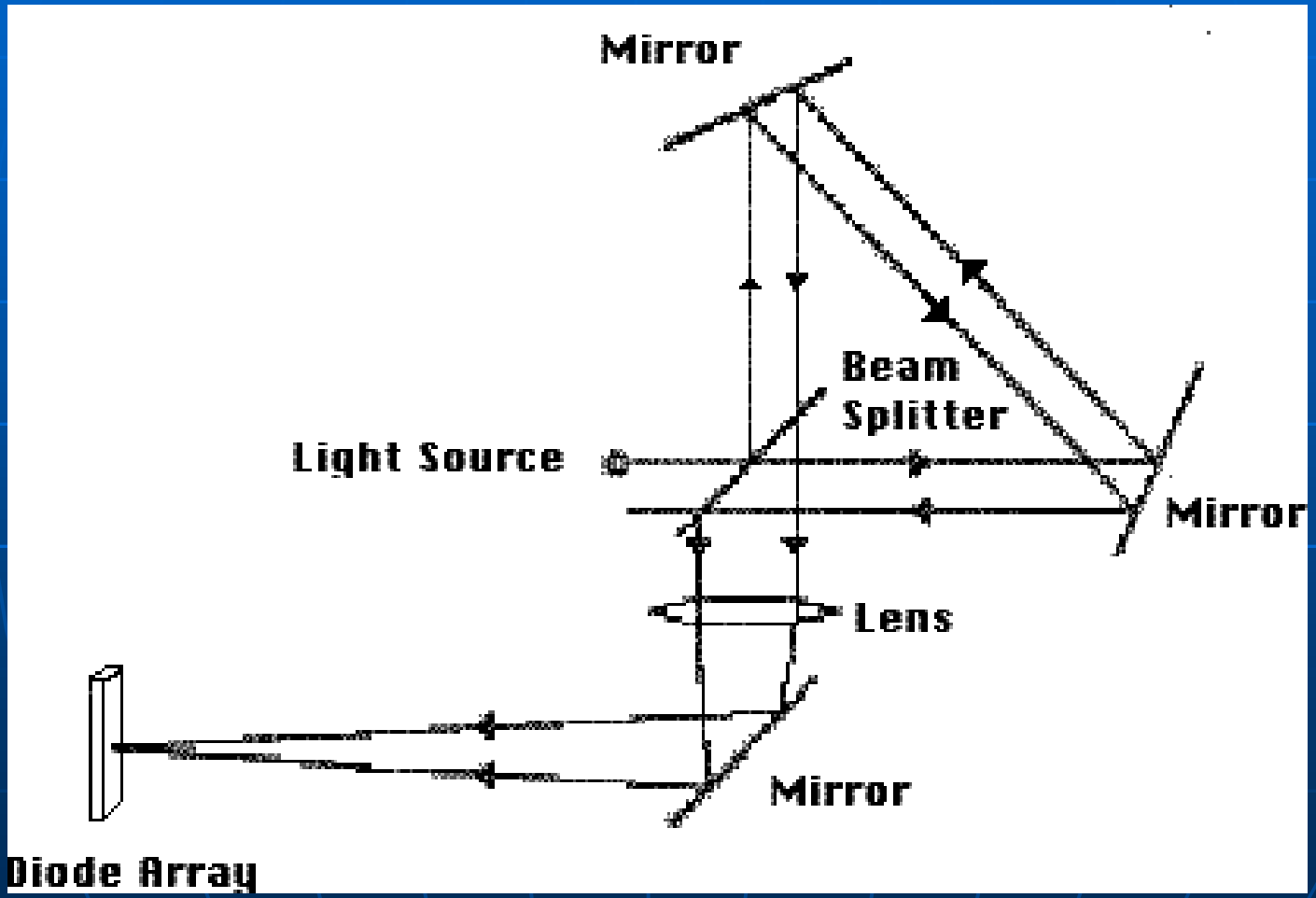
Standard monochromatic spectrophotometer

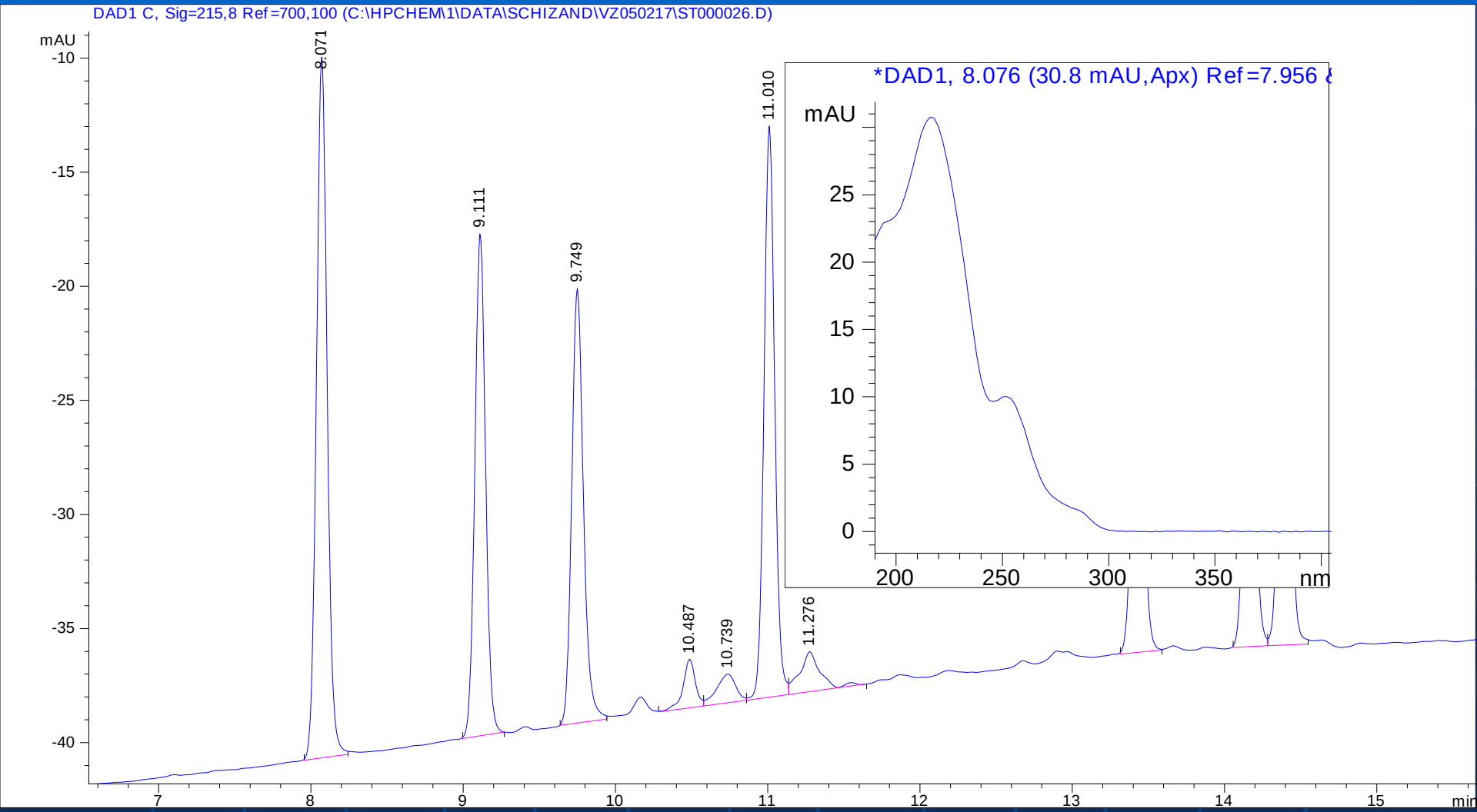


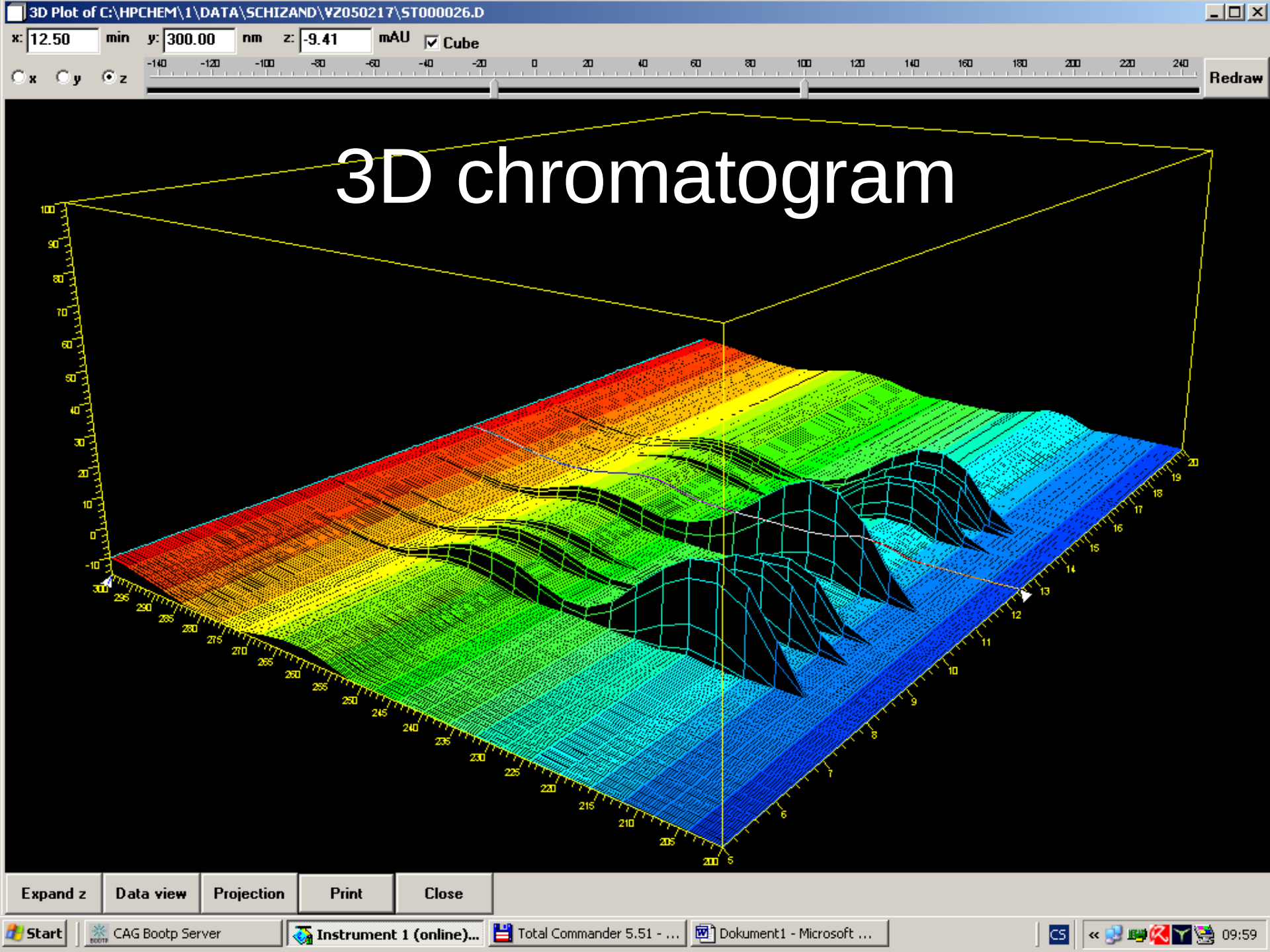
Scheme of DAD




Diode
Array
Mirror
Filter
Mirror
Lens
Flow
Cell
Light Source
Prism

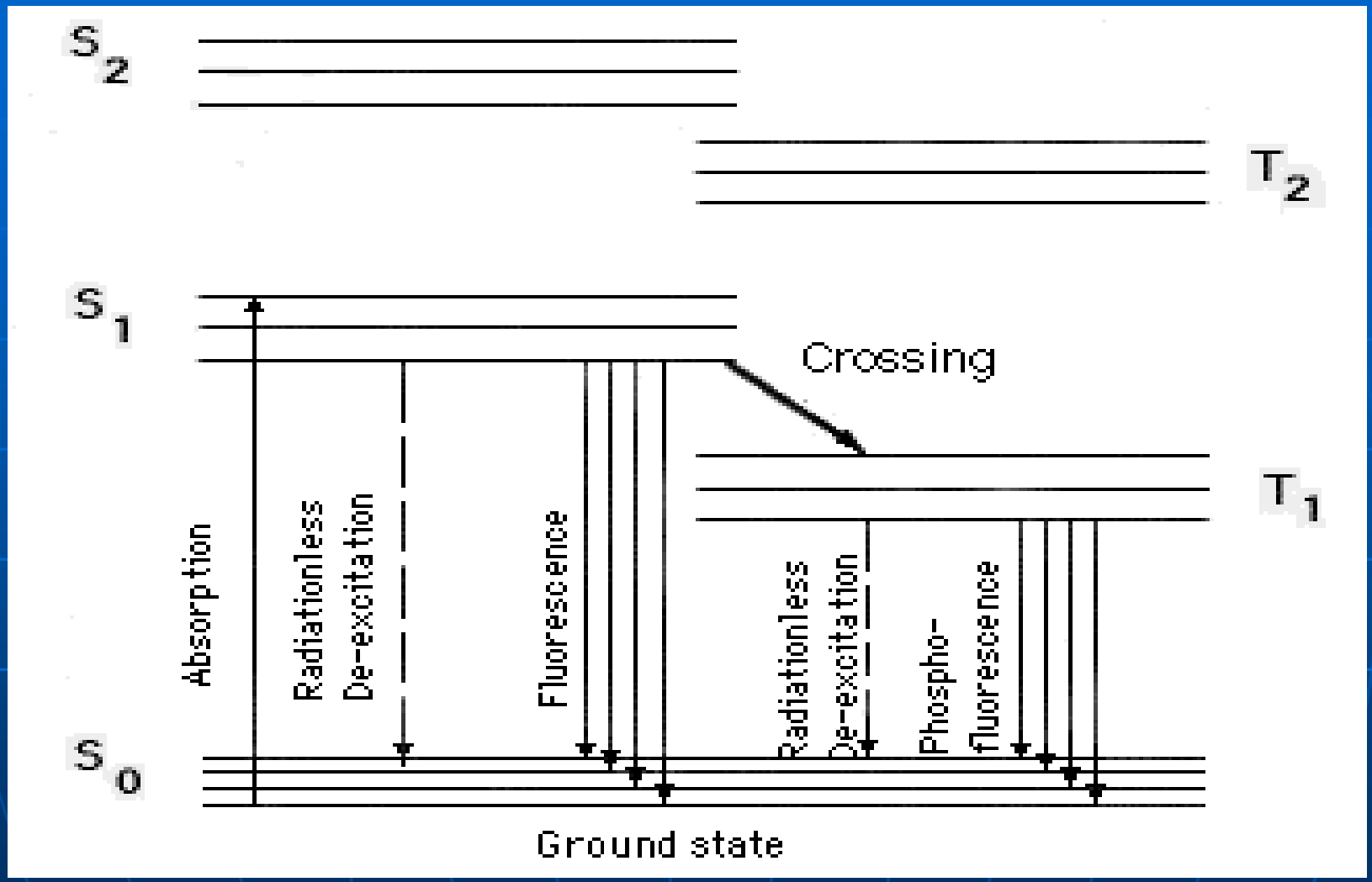

Mirror
Beam
Splitter
Light Source
Mirror
Lens
Mirror
Diode Array


DAD1 C, Sig=215,8 Ref=700,100 (C:\HPCHEM\1\DATA\SCHIZAND\VZ050217\ST000026.D)
mAU
-10
-15
-20
-25
-30
-35
-40
8.071
9.111
9.749
10.487
10.739
11.010
11.276
7
8
9
10
11
12
13
14
15
min
*DAD1, 8.076 (30.8 mAU,Apx) Ref=7.956
mAU
25
20
15
10
5
0
200
250
300
350
nm


3D Plot of C:\HPCHEM\1\DATA\SCHIZAND\VZ050217\ST000026.D
x: 12.50 min
y: 300.00 nm
z: -9.41 mAU
Cube
x
y
z
Redraw
3D chromatogram
Expand z
Data view
Projection
Print
Close
Start
CAG Bootp Server
Instrument 1 (online)...
Total Commander 5.51 - ...
Dokument1 - Microsoft ...
09:59

Fluorescent detectors

- Measurement of the ability to absorb and emit a quantum of light at a certain wavelength
- Compounds can characteristically fluoresce. Source of excitation goes through the flow cell into the photo detector, the intensity of emitted light at a certain wavelength is measured
- Sensitivity 10^{-9} to 10^{-11} g/ml.


S2
T2
S1
Crossing
T1
Absorption
Radiationless De-excitation
Fluorescence
Radiationless De-excitation
Phospho-fluorescence
S0
Ground state

Usage of fluorescent detection

- 1) DNA/RNA
- 2) Enzymatic methods
- 3) Protein-Ligand Interactions
- 4) Fluorescent compounds detections

Refractive index detectors – refractometers

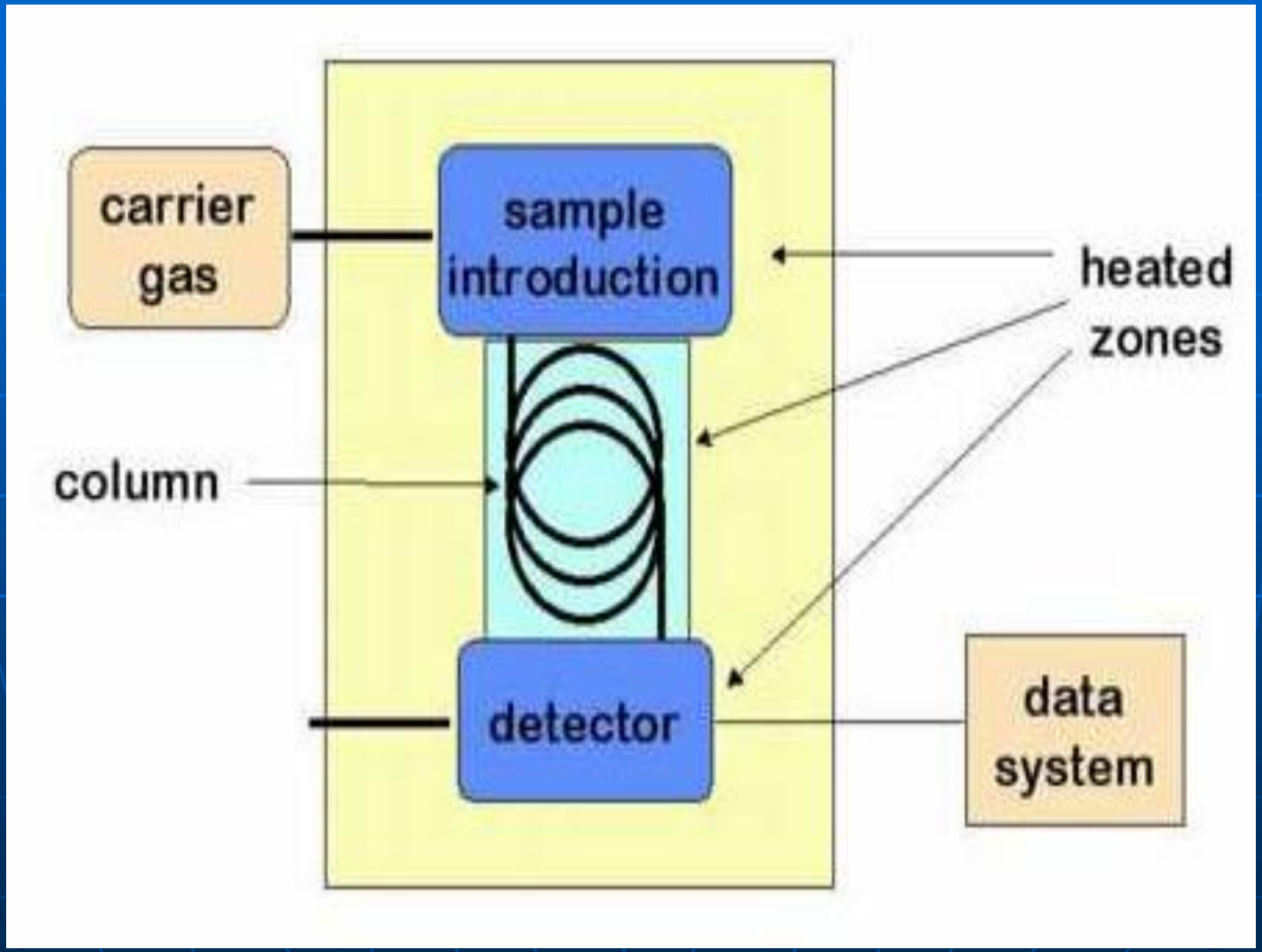
- Reliable
- Affected by the flow rate of the mobile phase
- Usable for all types of solvents
- Low sensitivity to impurities and air bubbles
- Sensitive to temperature
- Expensive

GC - gas chromatography

- Analytical separation technique useful for separation of volatile organic substances
- Separation based on different distribution and selective retardation in column
- mobile phase - inert gas, only transportation of the analyte through the column, no interaction between the analyte and the mobile phase

Equipment is composed of:

- Reservoir of the mobile phase - inert gas (He, Ar)
- Injector – maintained at stable temperature
- Separation column containing the stable stationary phase – tempered to controlled stable temperature
- Detector


carrier gas
sample introduction
heated zones
column
detector
data system

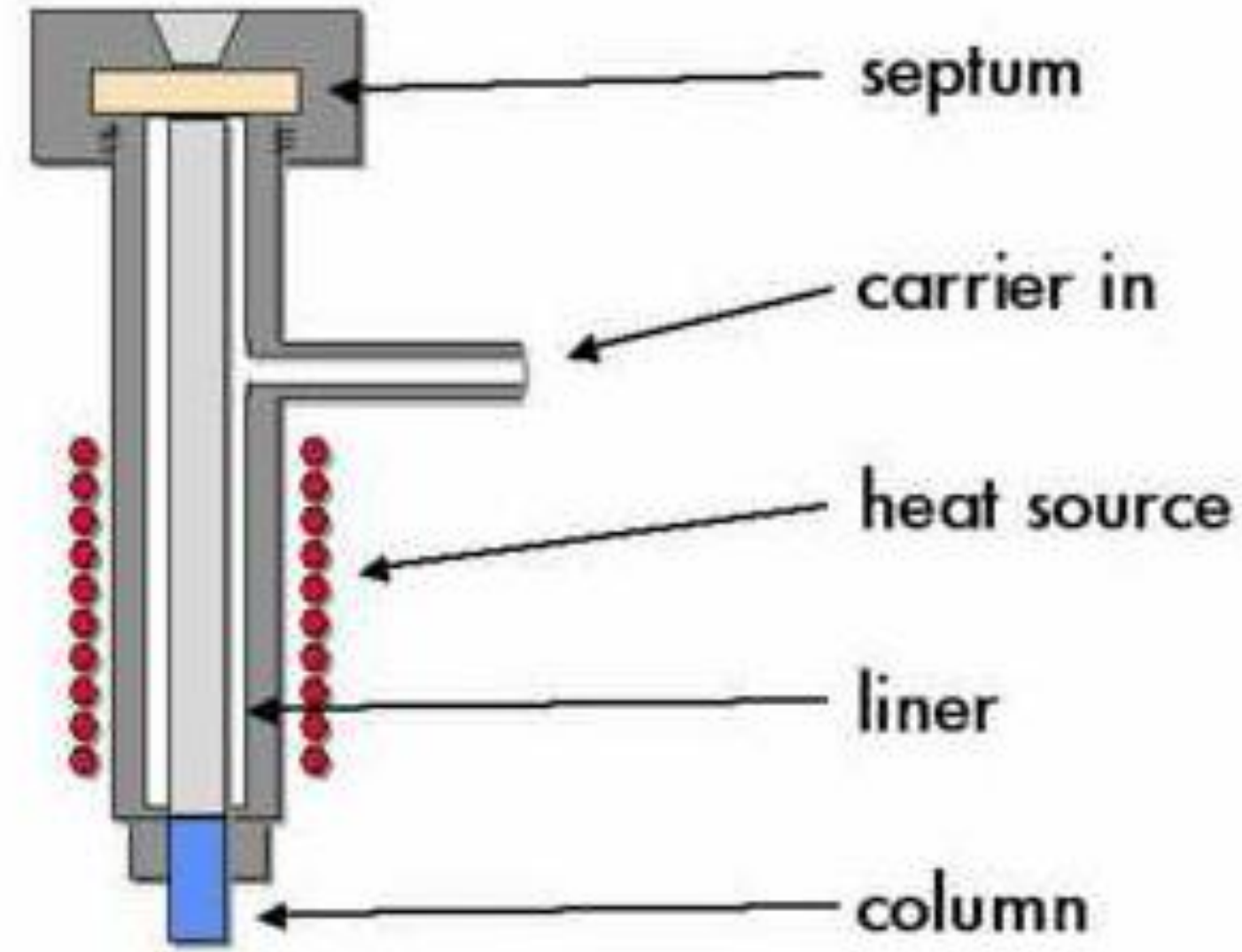

GCQ
GCQ
Ceres
soft

Mobile phase

- Carrier gas
- The task is the transport of the analyte
- Inert both to analyte and stationary phase
- N_2, H_2, He in the case of ionization detectors with radioactive Ar

Injector

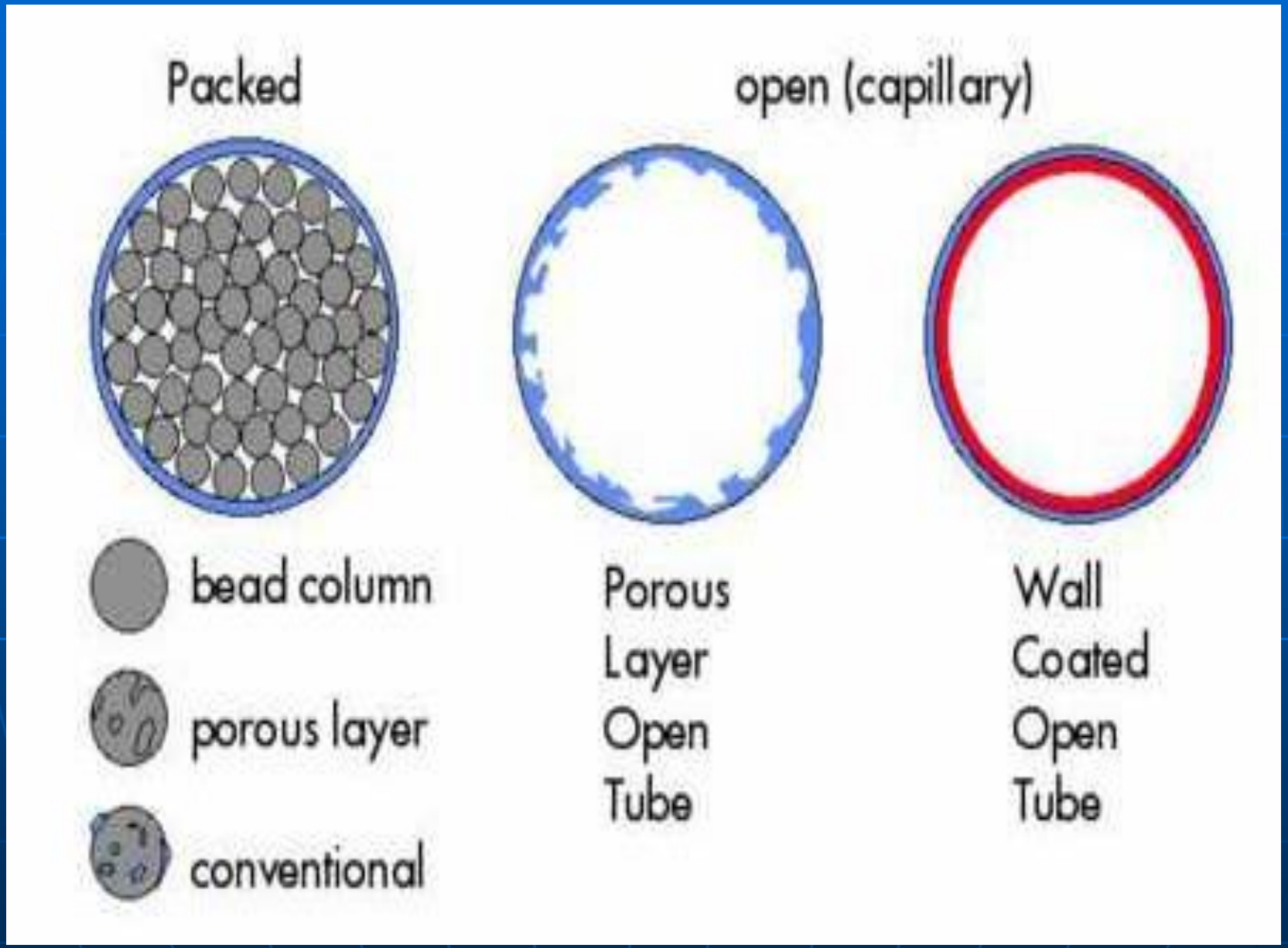
- At the entrance into the column
- Small space for the gasification of sample
- Volume of sample as low as possible (0.1 - 1 µL)
- Temperature of chamber higher than boiling point of the least volatile component in the sample

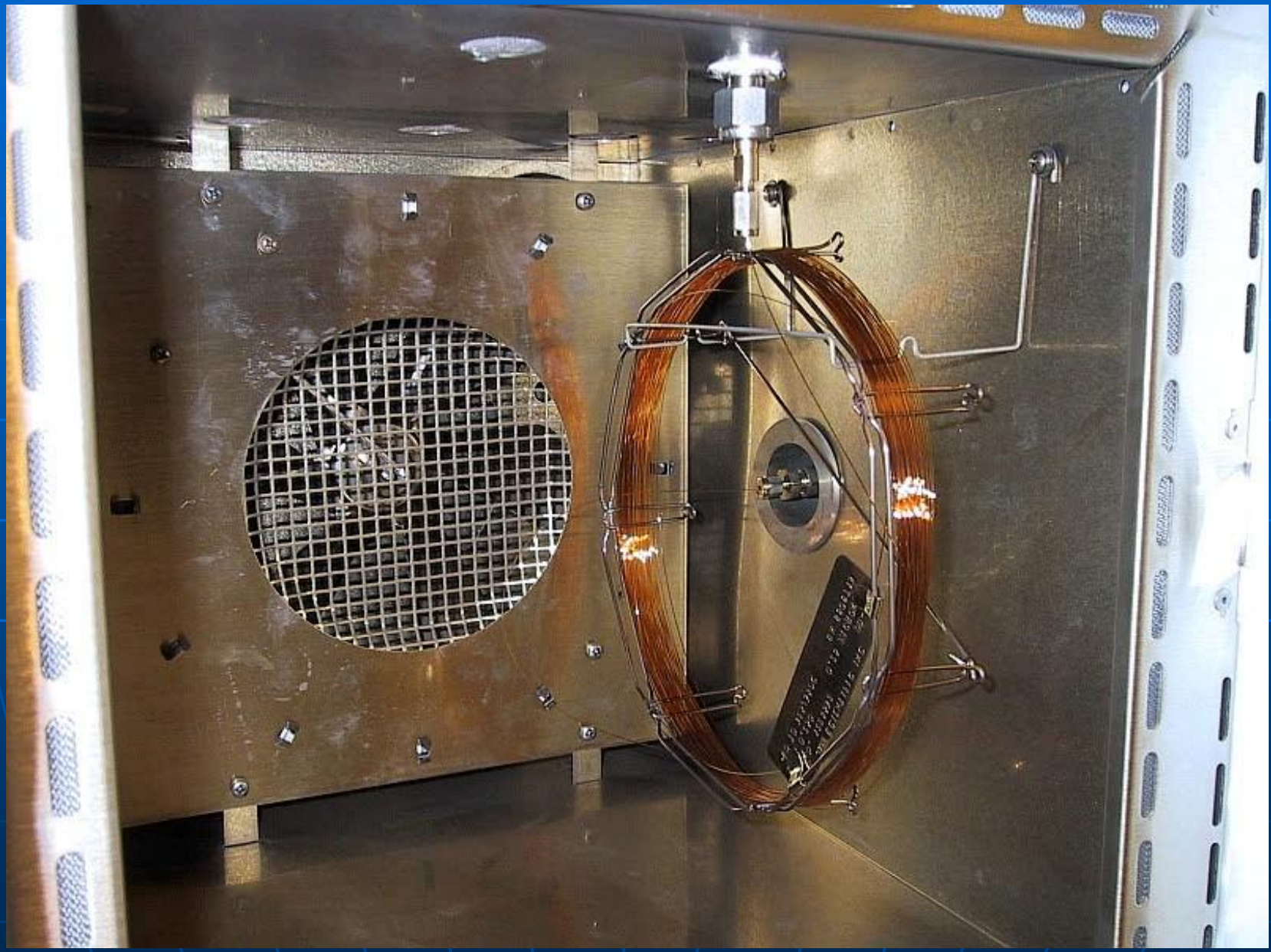

septum
carrier in
heat source
liner
column


Slow
Fast

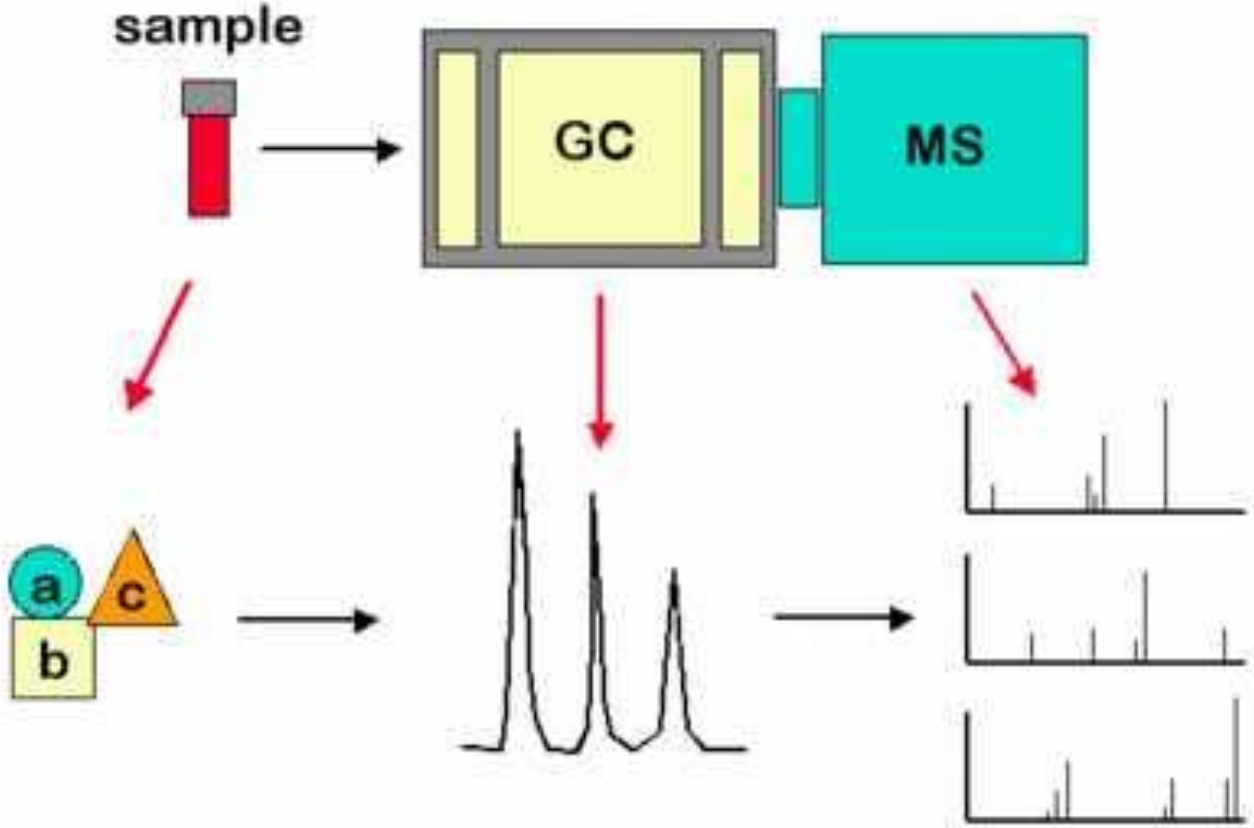
Columns

- Packed x capillary
- Different materials
- Shape according to the type of chromatograph
  - direct
  - U-shaped
  - helix
- Necessary to maintain the stable set temperature – velocity of movement compounds dependent on temperature


Packed
open (capillary)
bead column
porous layer
conventional
Porous
Layer
Open
Tube
Wall
Coated
Open
Tube



GC/MS process



Mass detectors
Mass spectroscopy MS

- combination of ionization, fragmentation and separation process
- formation of ions by different methods
- fragmentation of compounds
- after trapping – mass spectrum
- interface between GC/MS
  - high vacuum
  - molecular separators

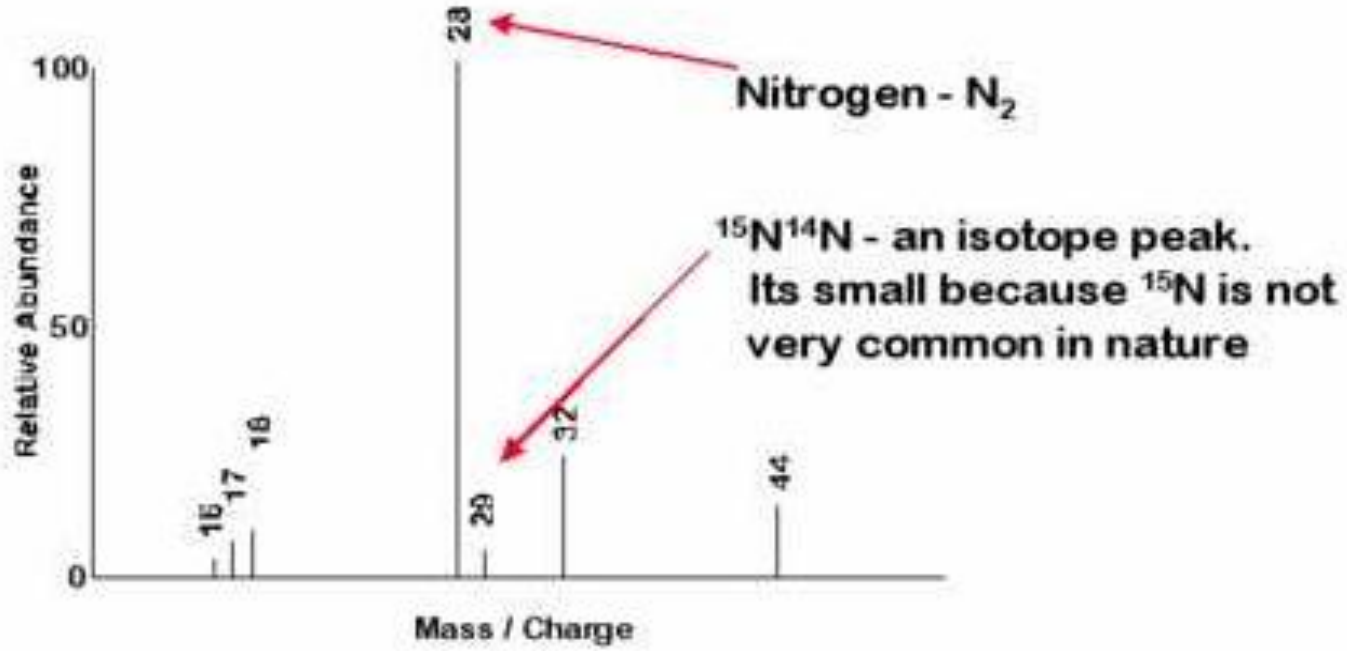
Mass Spectroscopy (MS) – mass detectors

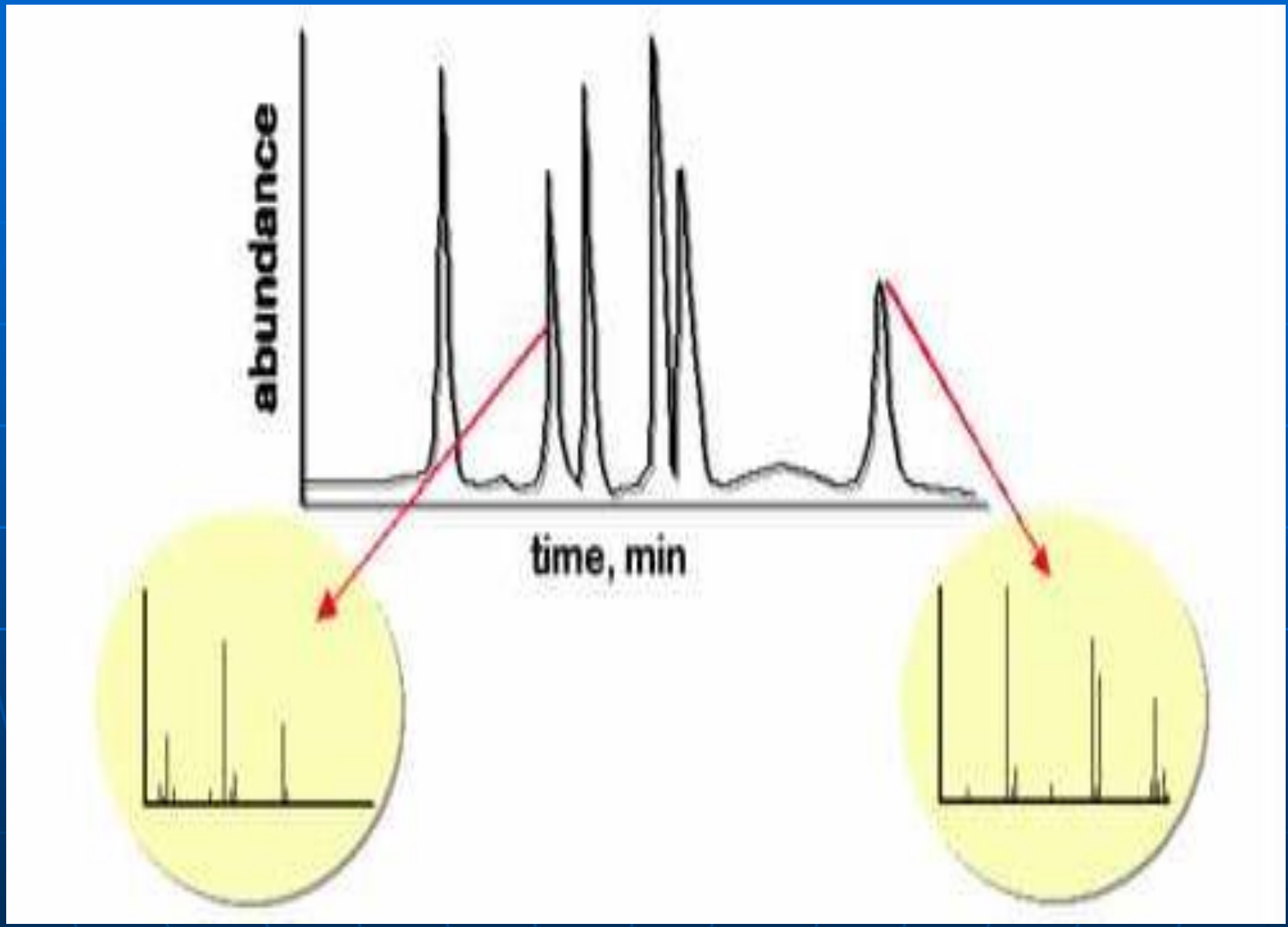
- a) Electron Impact (EI)- ionization by impact of electron beam or strong electric field
- b) Chemical Ionization- ionization via ionized gas
- c) Fast Atom Bombardment (FAB) – bombarding by Xenon atoms
- sensitivity 10^{-8} to 10^{-10} g/l.

A simple mass spectrum

The masses in this example should look familiar.

This is a spectrum for air - a mixture.




abundance
time, min