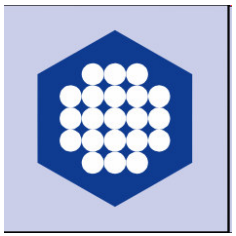
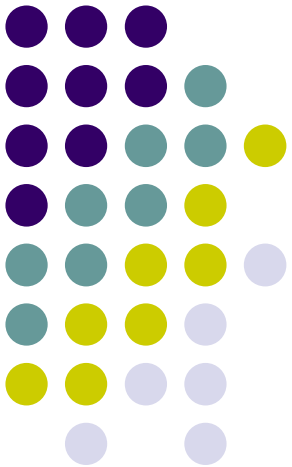
MS instrumentation

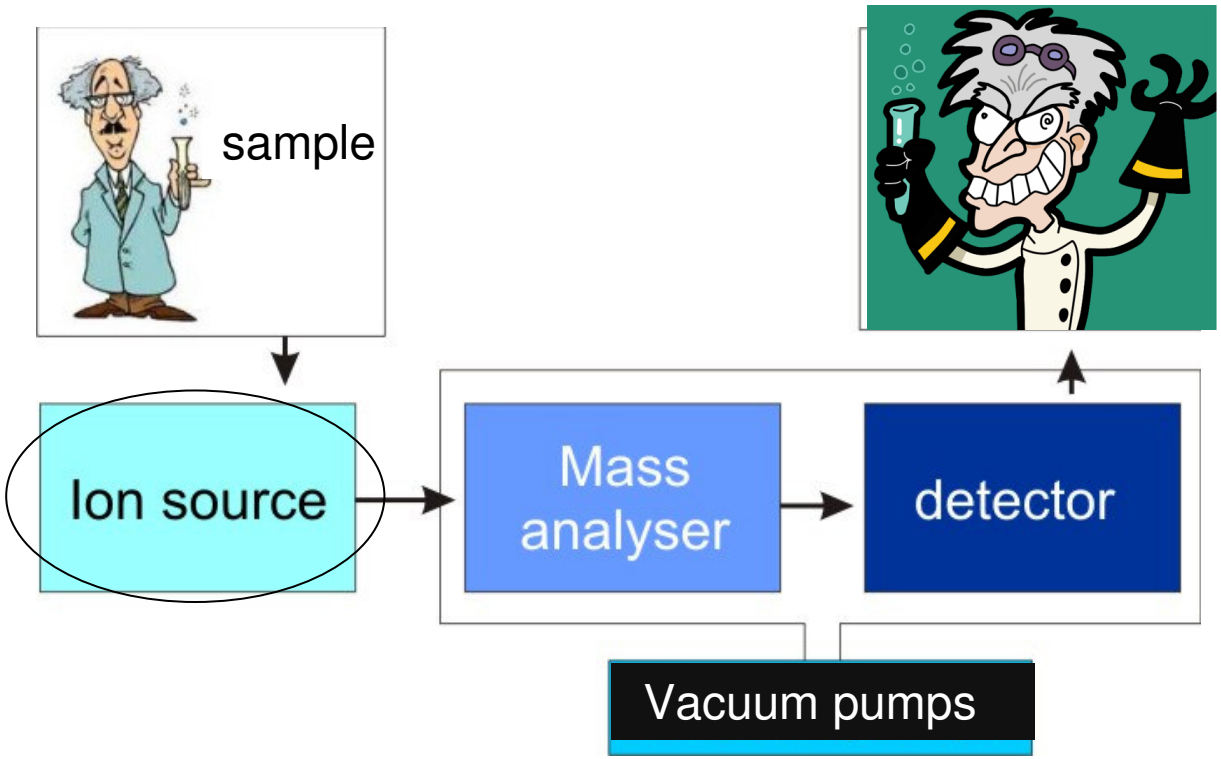


Vladimír Vrkoslav
MS Group

Institute of Organic Chemistry and Biochemistry AS CR, v.v.i.



Mass spectrometer



- Ion source - device which produces positive or negative electrically charged molecules in gas phase
- Mass analyser - separates the ions according to their mass-to-charge ratio (m/z)
- Detector - records the charge induced or the current produced, when an ion passes by or hits a surface

Ion source

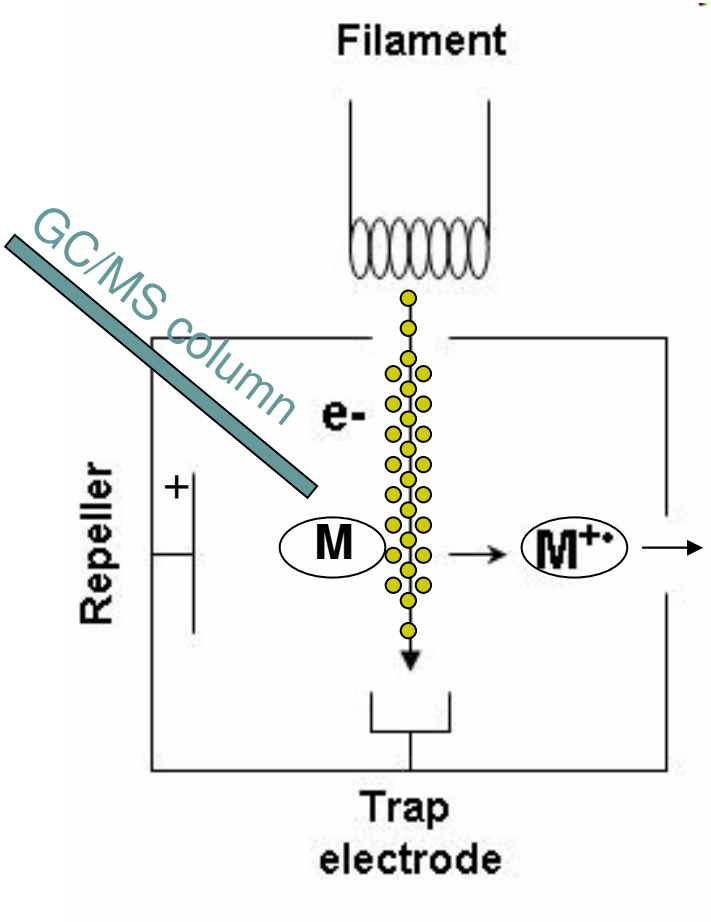
Produce **positive** or negative electrically charged molecules in **gas phase**

- Choice depends on compound
  - Universal ionization technique does not exist
- Differentiation
  - By energy
    - Hard (EI)
      - Cation radical with high energy - fragmentation i ion source – many fragments in the spectra
    - Soft (CI, ESI, APCI, ESI,...)
      - molecular adduct with low energy – no or a few fragments in the spectra
  - By pressure
    - Vacuum (EI, CI, MALDI)
    - Atmospheric pressure (ESI, APCI, AP MALDI, ...)
- Ions
  - $M + e^- \rightarrow M^{+\bullet} + 2\ e^-$ Cation radicals
  - $M + HA \rightarrow [M+H]^+ + A^-$ Molecular adducts
  - $M + B^- \rightarrow [M-H]^- + HB$ Deprotonated molecules

Different ionization techniques

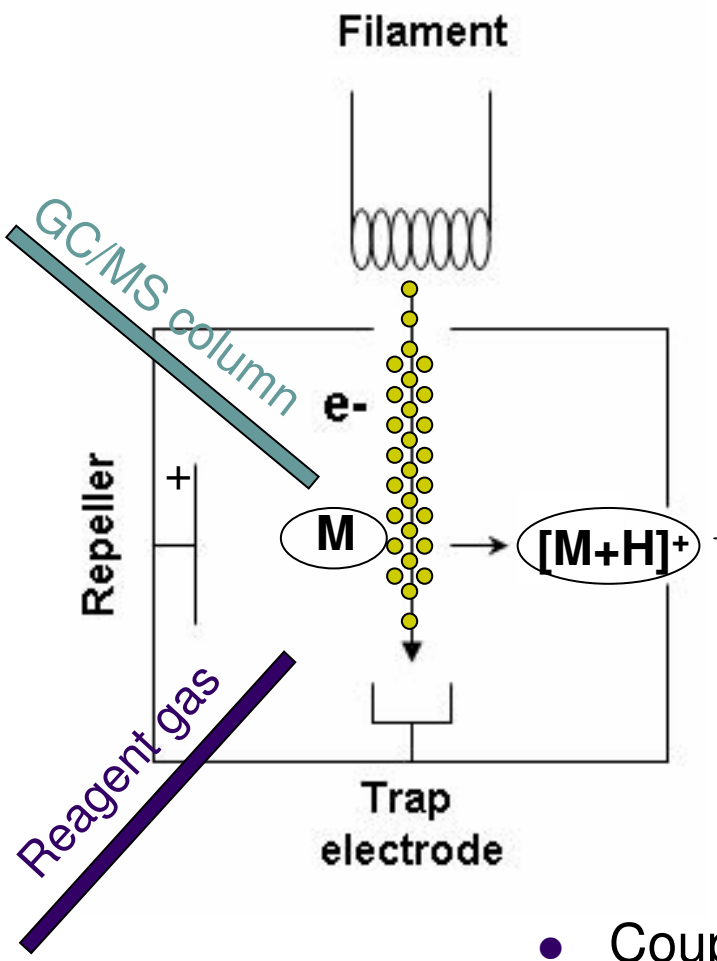
- **Molecular Analysis**
  - **Electron Ionization** (EI)
  - **Chemical Ionization** (CI)
  - **Electrospray** (ESI)
  - **Nanospray** (nanoESI)
  - **Atmospheric Pressure Chemical Ionization** (APCI)
  - Atmospheric Pressure Photoionization (APPI)
  - **Matrix-Assisted Laser Desorption/Ionization** (MALDI)
  - Laser Desorption Ionization (LDI)
  - Secondary Ion Mass Spectrometry (SIMS)
  - Fast Atom Bombardment (FAB)
  - Desorption Electrospray Ionization (DESI)
  - Desorption Atmospheric Pressure Chemical ionization (DAPCI)
  - Direct Analysis in Real Time (DART)
  - Termospray (TSI)
  - .....
  - .....

Electron ionization (EI)



- An ionization method in which energetic electrons interact with gas phase molecules to produce ions.
  - Electron emission by heating a tungsten wire filament
  - Good reproducibility – spectral library – easy interpretation
    - (energy of the electrons 70eV)
- $M + e^- \rightarrow M^{+\bullet} + 2\ e^-$
  - M is the analyte molecule being ionized
  - e^- is the electron and
  - $M^{+\bullet}$ is the resulting ion
- Often coupled with GC/MS
- Widely used for volatile organic molecules

Chemical Ionization (CI)



- Analyzed ions are produced through the collision of the analyte with ions of a reagent gas that are present in the ion source
  - Methane, ammonia, isobutane, acetonitrile,.....
- Soft ionization technique
  - $[M+H]^+$, $[M+\text{reagent gas}]^+$, fragments (depend on condition)
- Example
  - CH_4 as a reagent gas

Primary ion formation0

$$CH_4 + e^- \rightarrow CH_4^+ + 2e^-$$

Secondary reagent ions

$$CH_4 + CH_4^+ \rightarrow CH_5^+ + CH_3$$

$$CH_4 + CH_3^+ \rightarrow C_2H_5^+ + H_2$$

Product ion formation

$$M + CH_5^+ \rightarrow CH_4 + [M+H]^+$$

$$AH + CH_3^+ \rightarrow CH_4 + A^+$$

$$M + CH_5^+ \rightarrow [M+CH_5]^+$$

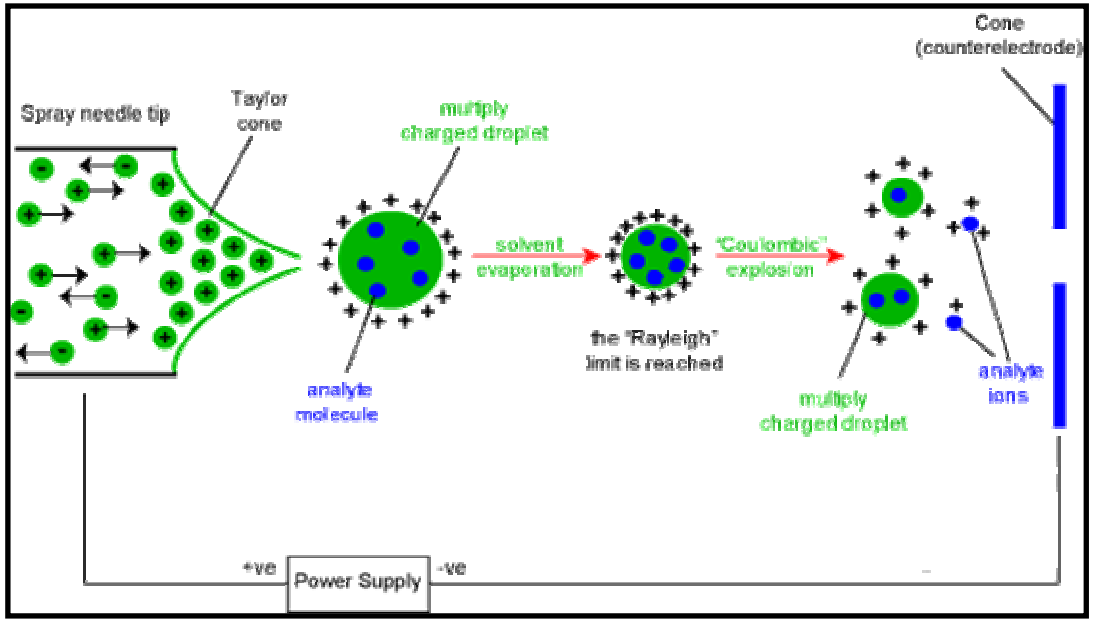
$$A + CH_4^+ \rightarrow CH_4 + A^+$$

- Coupled with GC/MS
- Used for gases and volatile organic molecules

Electrospray (ESI)

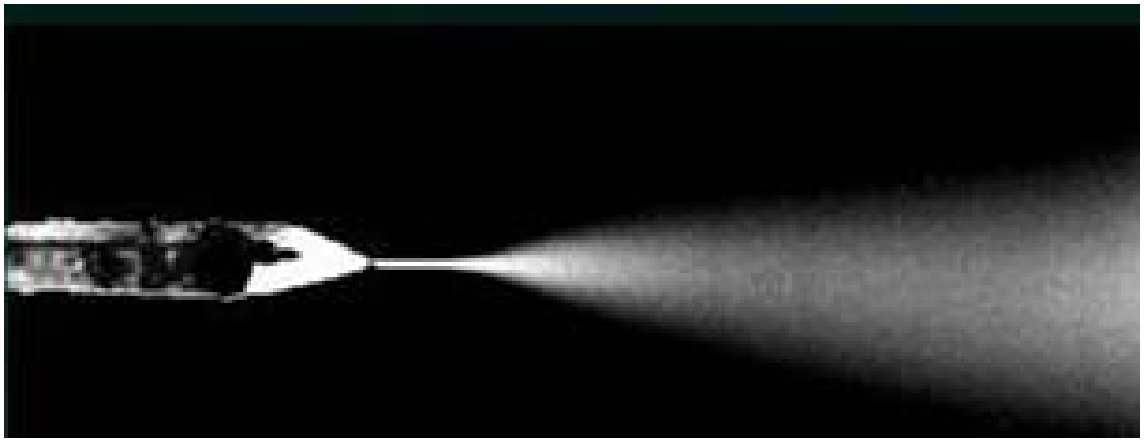
- The liquid containing the analyte(s) is dispersed by electrospray into an aerosol
  - Charged droplets
  - Solvent evaporation
  - Coulombic explosion
- Soft ionization technique
  - **[M+H]** $^{+}$, $[M+ Na]^{+}$, $[M+ K]^{+}$, ... molecular adducts

John B. Fenn
Nobel prize in Chemistry
2002



http://www.chm.bris.ac.uk/ms/theory/esi-ionisation.html

Nanoelectrospray (nanoESI)



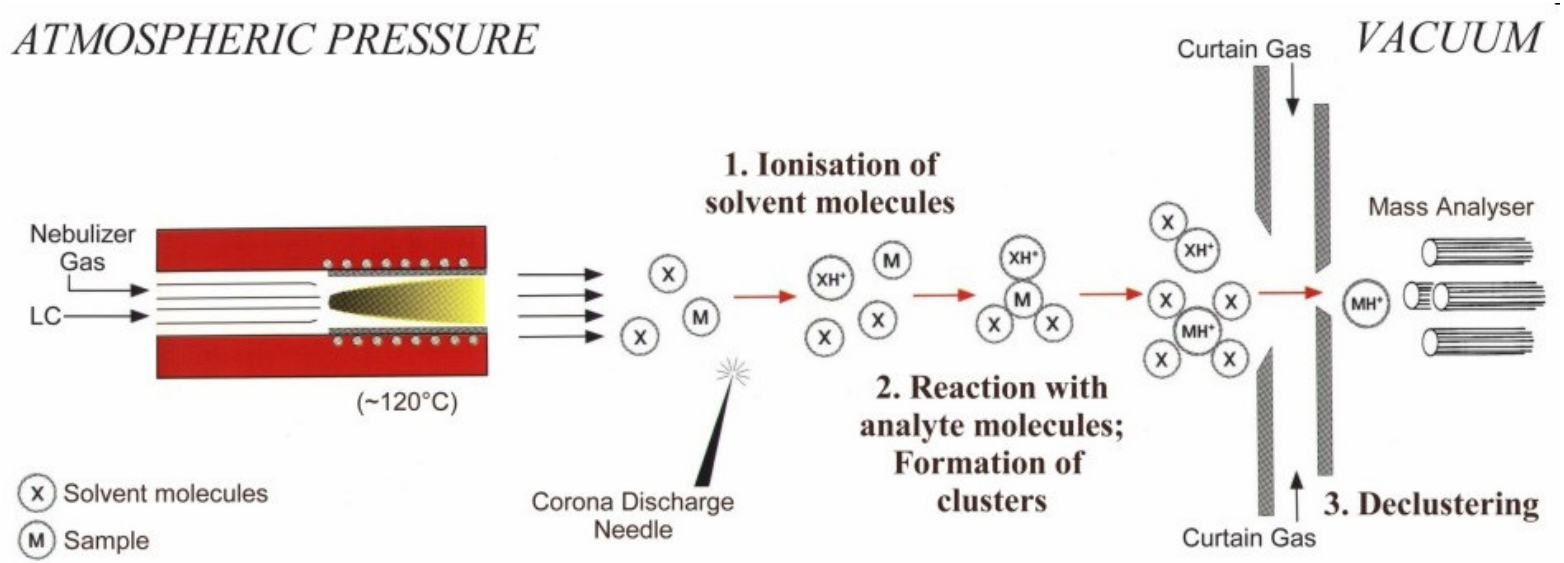
- Flow of mobile phase usually hundreds nl/min
  - $[M+H]^+$

Electrospray technique

- Polar analytes in broad mass range
- Obtaining more charged ions
  - Possibility to analyzed molecules with Mr behind the range of analyser
- Coupled with HPLC or UHPLC
  - Polar solvent (mobile phase) as a donor of H^+

Atmospheric Pressure Chemical Ionization (APCI)

- The mobile phase containing eluting analyte is heated to high temperature (above 400℃), sprayed with high flow rates of nitrogen
- Molecules of solvent and gas are ionized by corona discharge
- Analyte are ionized by ionized solvent and gas molecules



Atmospheric Pressure Chemical Ionization (APCI)

- APCI can be performed in a modified ESI source
  - Device is similar to ESI source
  - However, mechanism of ionization is similar to CI
- The ionization occurs in the gas phase

- APCI is a less "soft" ionization technique than ESI
  - Generates more fragment ions

- Coupled with HPLC or UHPLC
  - Advantage of APCI - it is possible to use a nonpolar solvent (mobile phase)

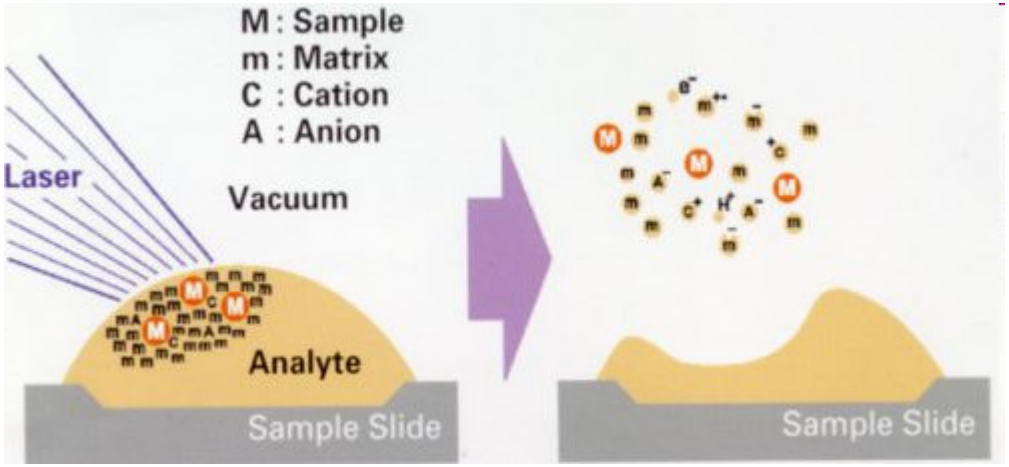
Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Laser-based soft ionization method
  - Matrix and analyt are mixed on the target plate
  - The laser (**UV**, IR) shoots the mixture
  - The energy is transferred to the matrix, which is vaporized, carrying analyte into the vapour phase and charging it

- The mechanism of MALDI is still debated

Koichi Tanaka

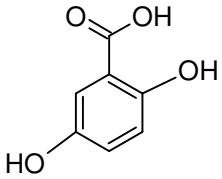
Nobel prize in Chemistry 2002

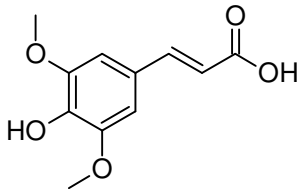


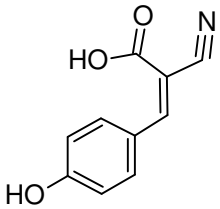
http://www.fasmatech.net/content-61-2.html

MALDI Matrices

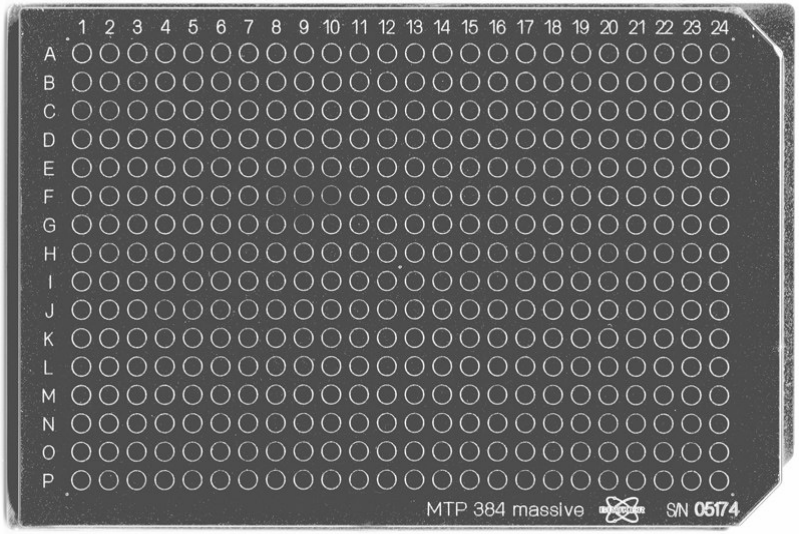
- Small molecules, usually small organic acids
  - 2,5-dihydroxybenzoic acid, sinapic acid, α-cyano-4-hydroxycinnamic acid,.....





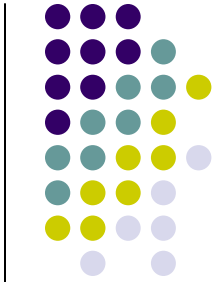


  - MALDI plate



www.ms-textbook.com

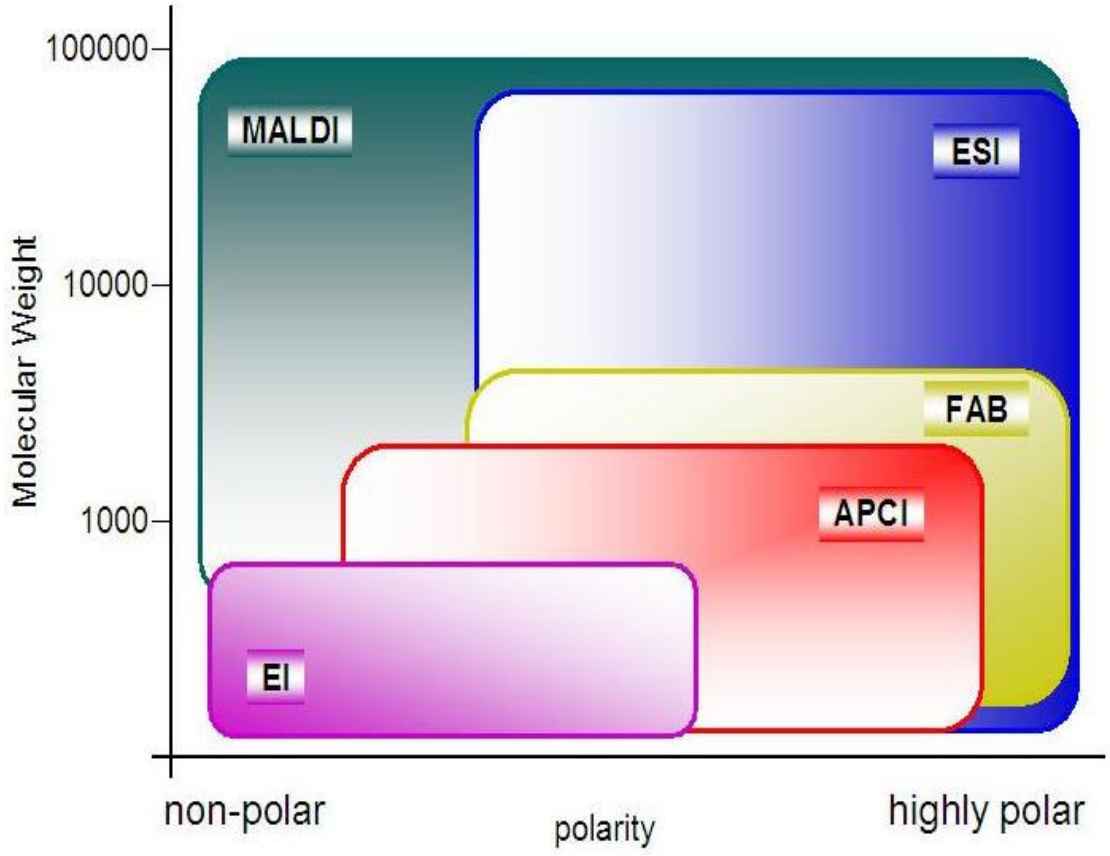
MALDI



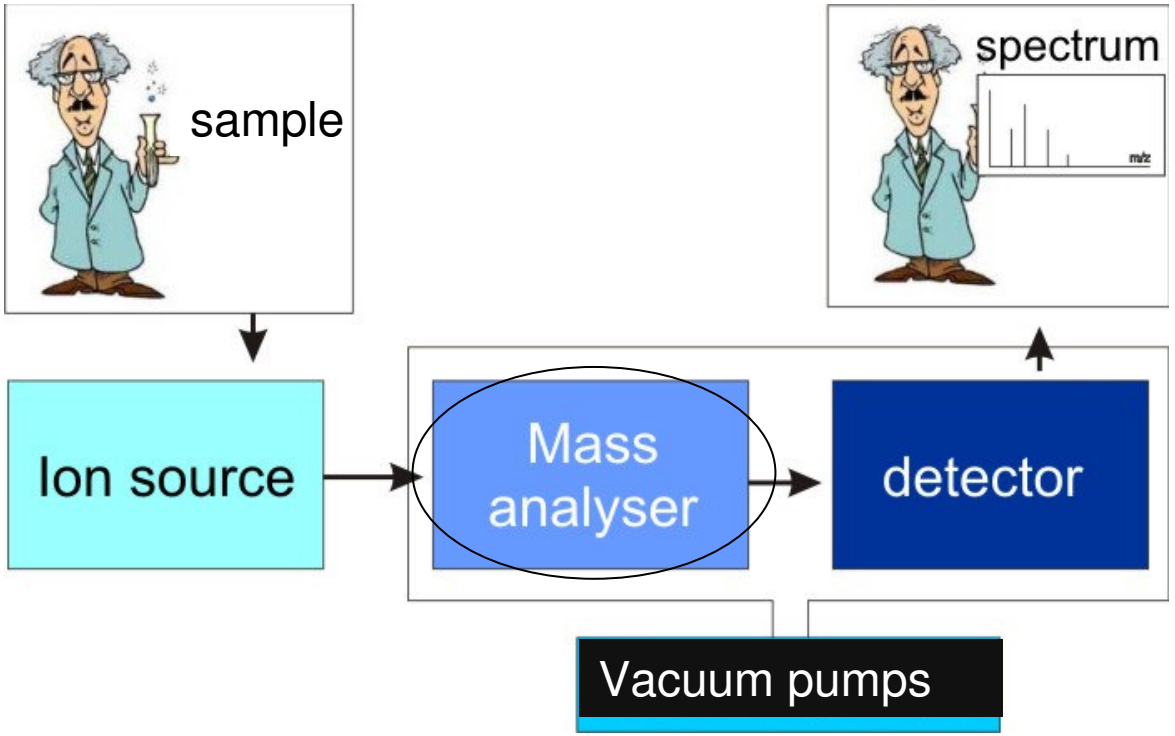
- Analysis of
  - Biomolecules (DNA, proteins, peptides and sugars)
  - Large organic molecules (polymers, dendrimeres,...)
    - Which tend to fragment, when are ionized by more conventional ionization methods.
  - Singly charged molecular adducts
    - Molecular adducts (**$[M+H]^+$**, $[2M+H]^+$, $[M+2H]^{2+}$) or loss of proton $[M-H]^-$
    - Other molecular adducts $[M+metal]^+$ with salts in sample (**Na**, K, ...,)

Choice of ionization technique

- Almost all compounds can by ionized by more then one technique
  - Depends on **molecular mass, polarity, ionization energy,** solubility, ...



Mass analyser



- Mass analysers - separate the ions according to their mass-to-charge ratio

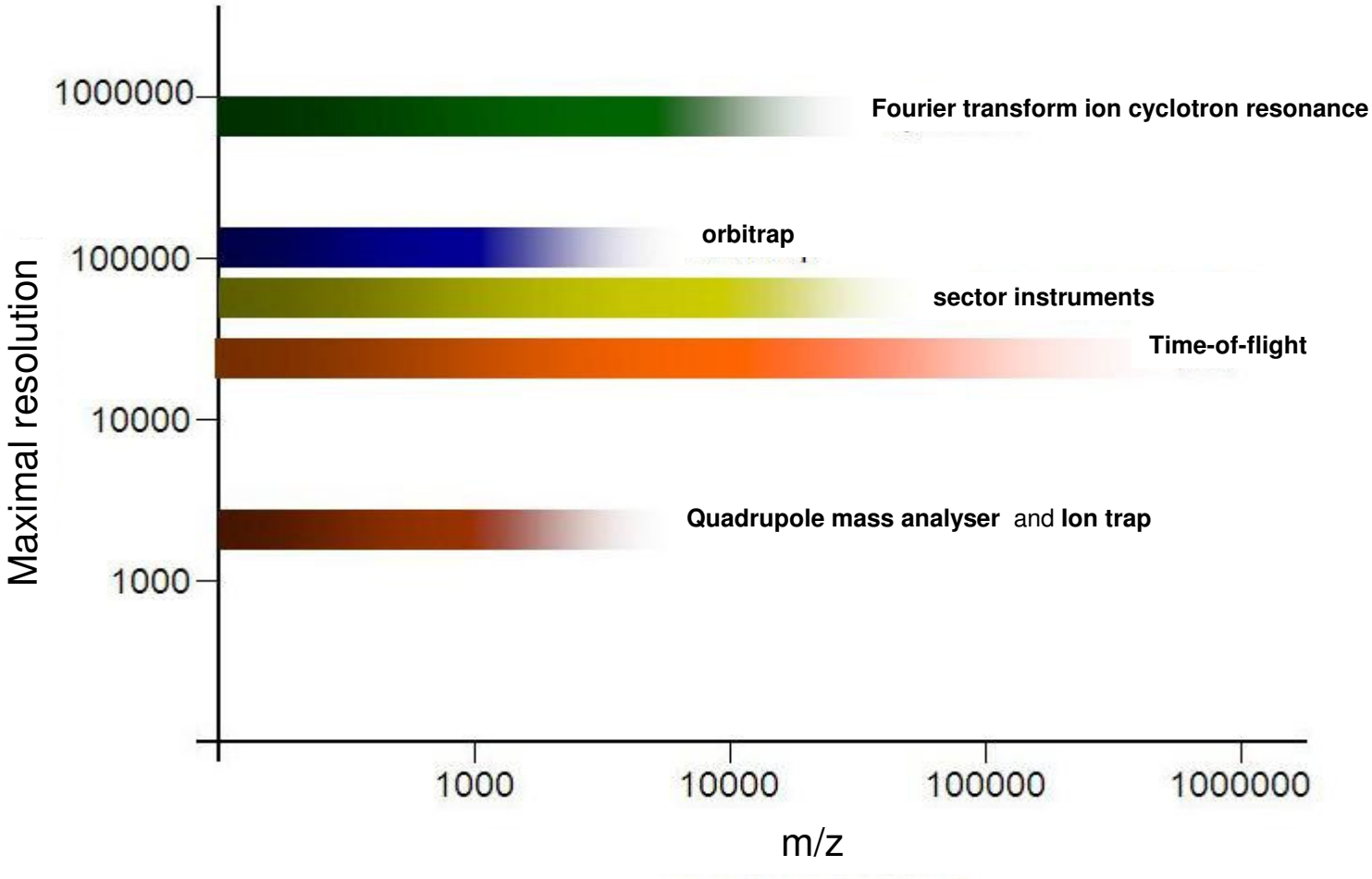
Mass analyser

Separate the ions according to their mass-to-charge ratio in space or time

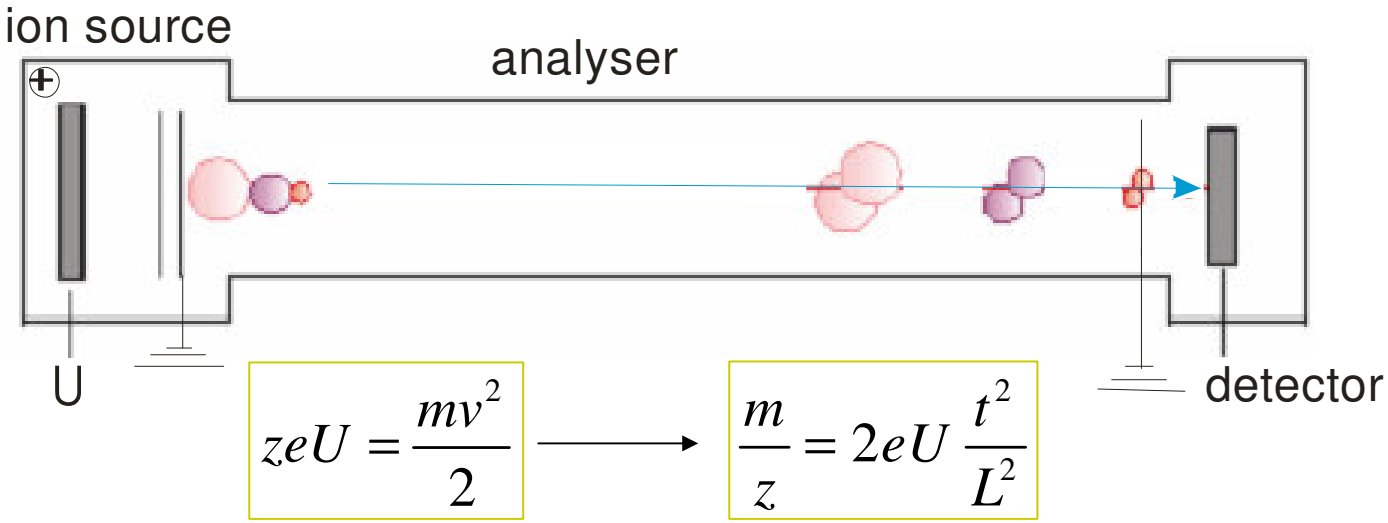
- Magnetic Sector (MAG)
- Electrostatic Sector (ESA)
- **Time-of-flight** (TOF)
- **Quadrupole mass analyser** (Q)
- **Ion trap (IT)**
  - **Three-dimensional quadrupole ion trap (3D)** (QIT)
  - **Linear ion trap (2D)** (LIT)
- **Fourier transform ion cyclotron resonance**
  - **Fourier transform ion cyclotron resonance** (FT-ICR-MS)
  - **Orbitrap (FT-Orbi)**

- Tandem mass spectrometry (MS/MS or MS^n)
  - fragmentation of analyte

Mass analysers

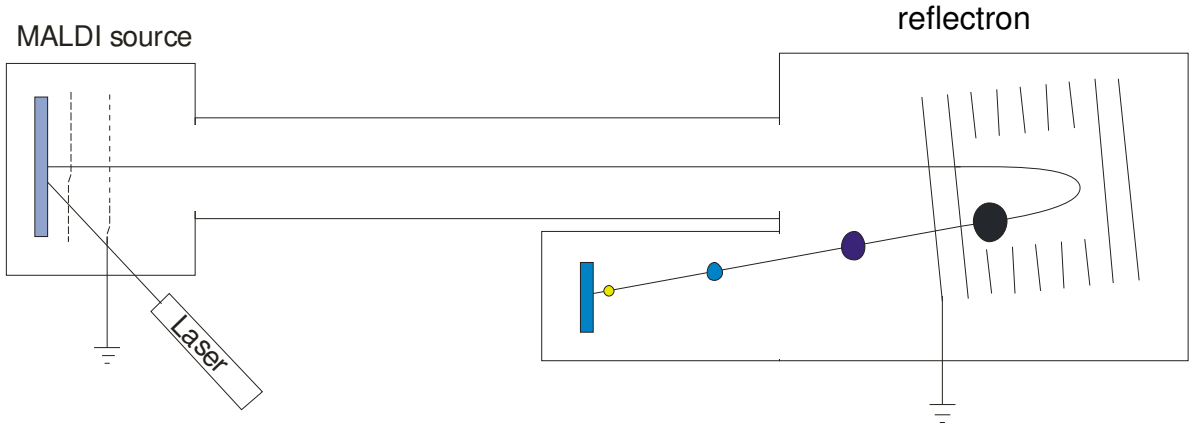


Time-of-flight (TOF)

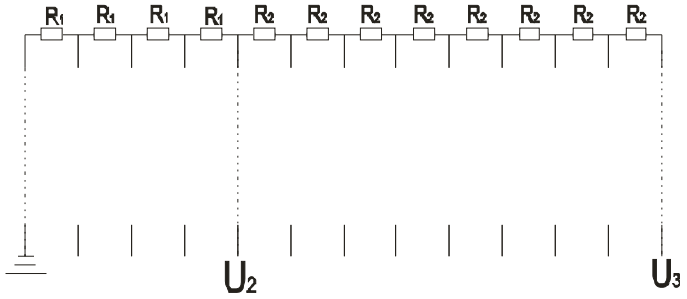


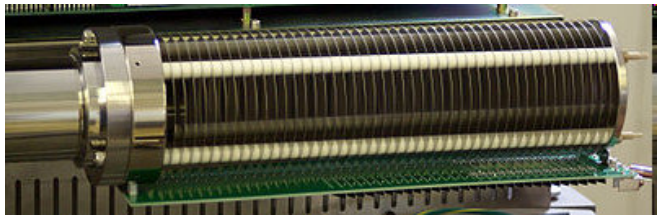
- Ions are accelerated by an electrostatic field - travel over a drift path to the detector
  - Measuring the flight time for each ion allows the determination of its mass
- Resolution depends on the length of the path
- Major advantages are
  - The extremely high transmission
  - The detection of all masses (all spectrum for each pulse)
  - The theoretically unlimited mass range
- Suitable for MALDI (MALDI-TOF instruments)
- Can be use for accurate mass spectra

TOF with reflectron

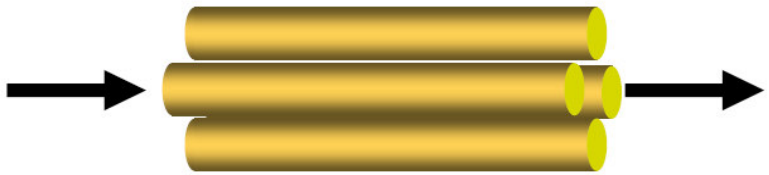


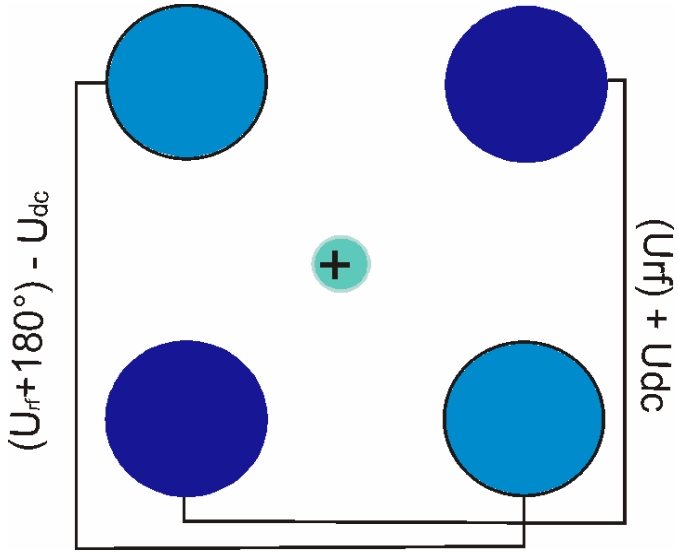
- The reflectron uses an electrostatic field to reflect the ion beam toward the detector.
  - Ring electrodes
- Advantage – better resolution
  - Longer path of ions
  - Focusing of ions in reflectron
- Disadvantage
  - Not suitable for protein – too long pass for large molecules





Quadrupole mass analyser (Q)

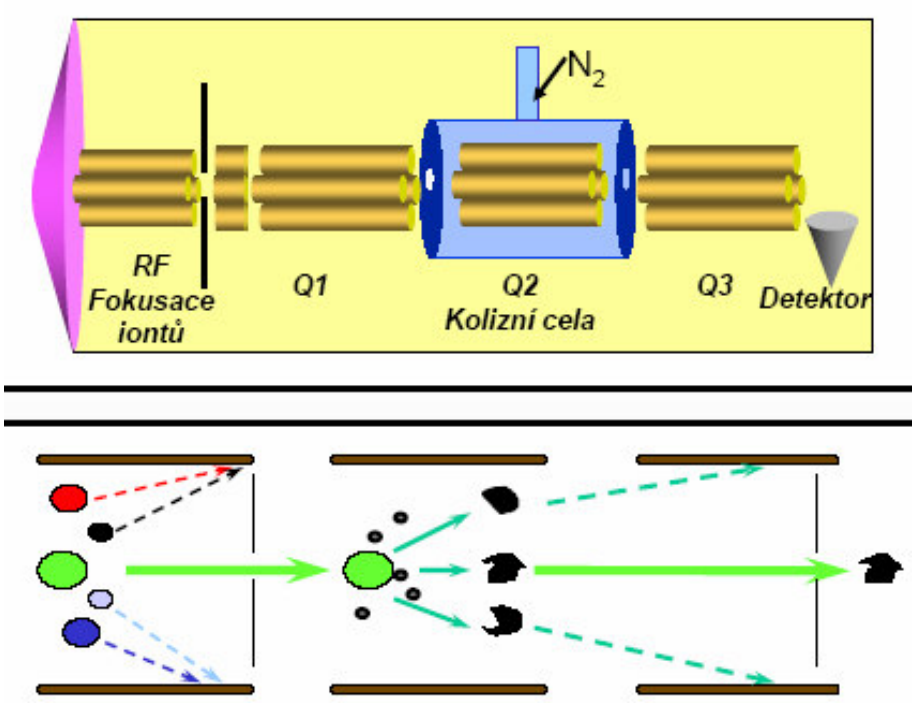




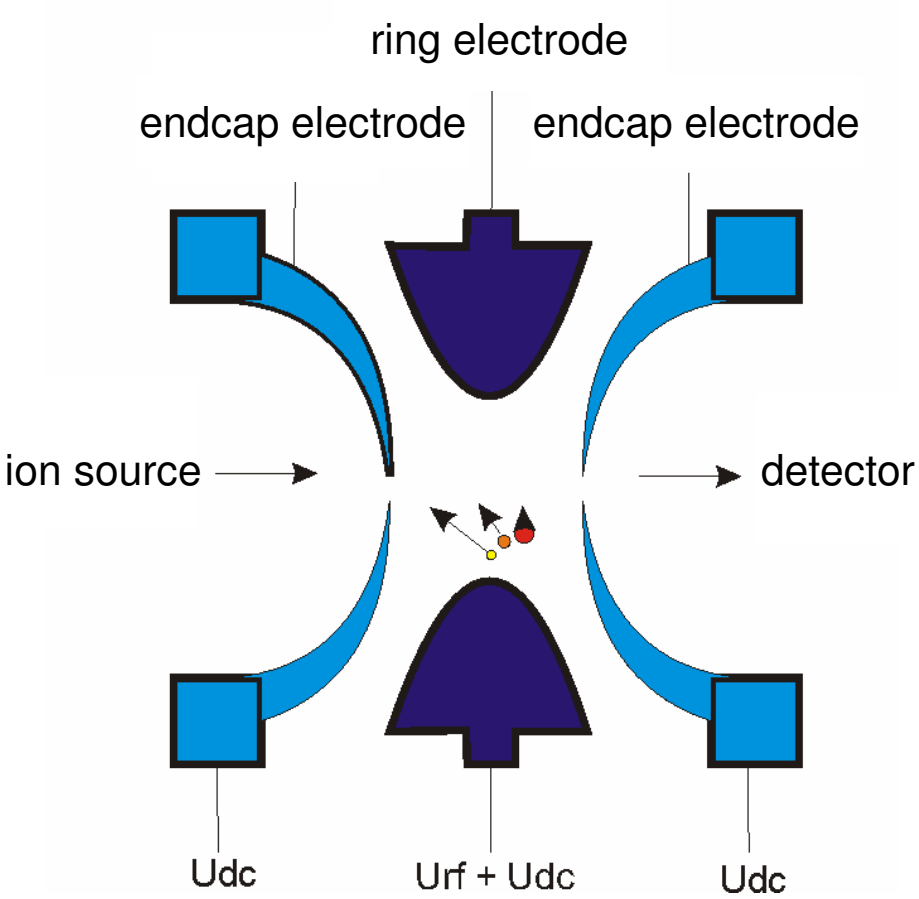
- Use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (U_{RF}) quadrupole field created between 4 parallel rods
  - Only the ions in a certain range of m/z are passed through the system at any time
- Limits m/z 2000 – 4000
- Low resolution spectra (not for accurate mass measurement)
- One Q can not be use for MS/MS

Collision-induced dissociation (CID) in collision cell

- QqQ
  - Q1 mass analyser can isolate one m/z (precursor ion)
  - **Q2 as a collision cell** - they collide with a gas - they are fragmented.
  - Q3
    - Scan all fragment – identification of compound
    - Scan one or a few ions – **quantitative analysis**



Three-dimensional quadrupole ion trap (QIT)

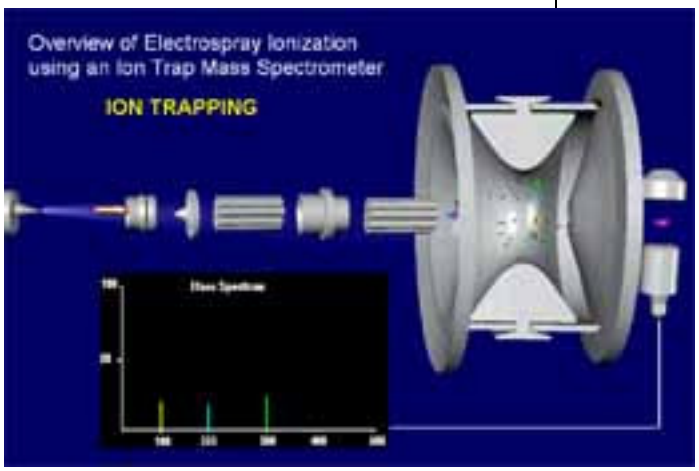


- The ions enter into the trap through the inlet and they are trapped through action of the three hyperbolic electrodes.
- Tthe ions are in a stable oscillating trajectory
- The ions are ejected in order of increasing *m/z* by a gradual change in the potentials

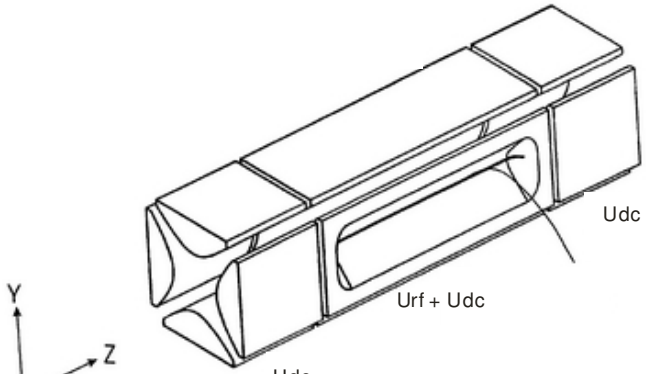
Ion trap (IT)

- Possibility MS/MS (CID) (to MS^{10}, in real life MS^3)
  - Rule 30:70 – ions at low 30% of m/z range are not stabile in ion trap – lose information
- Limits m/z 2000 – 4000
- Low resolution spectra (not for accurate mass measurement)
- Three-dimensional x lineat ion trap
  - **Linear ion trap (2D)** (LIT) better sensitivity, resolution, capacity and scanning faster

3D

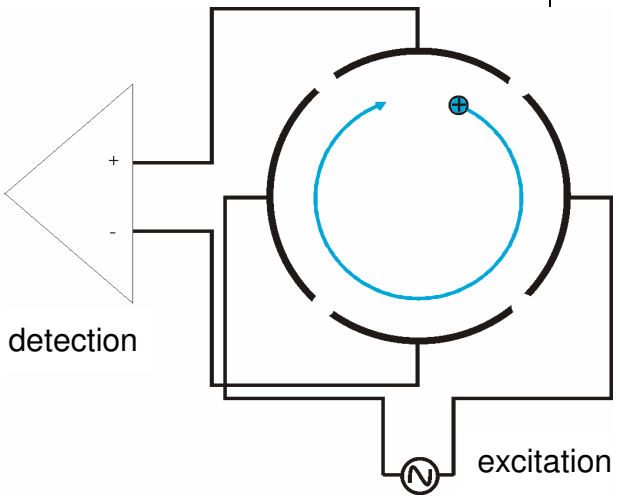


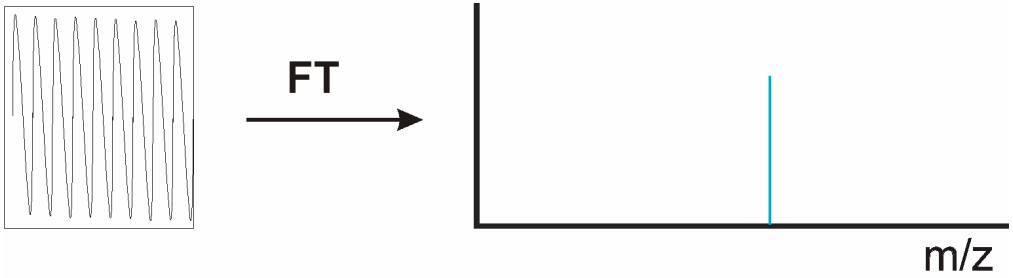
2D

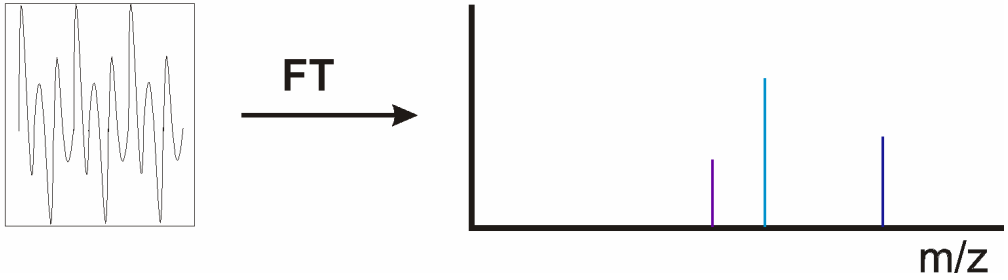


Fourier transform ion cyclotron resonance (FT-ICR -MS)

- Based on the circular movement of charged particles in a strong magnetic field (cyclotron movement)
  - The cyclotron frequency depends directly on the mass-to-charge ratio of the ions







$$\omega = \frac{v}{r} = \frac{Be}{m/z}$$

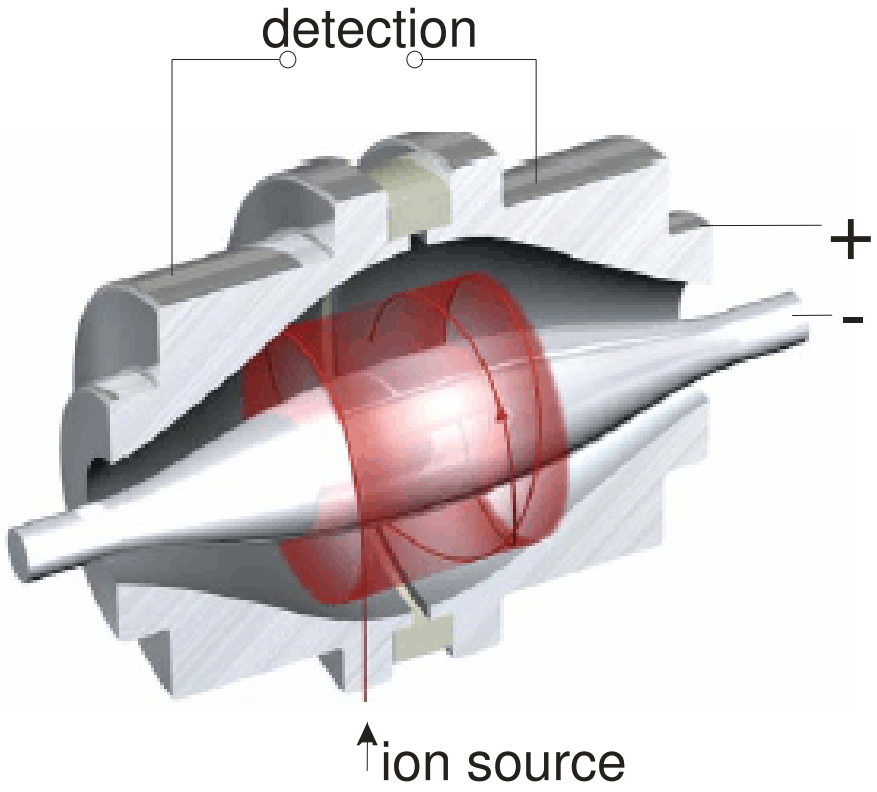
- Detector electrodes measure the electrical signal of ions which pass near them, producing a periodic signal

Fourier transform ion cyclotron resonance (FT-ICR-MS)

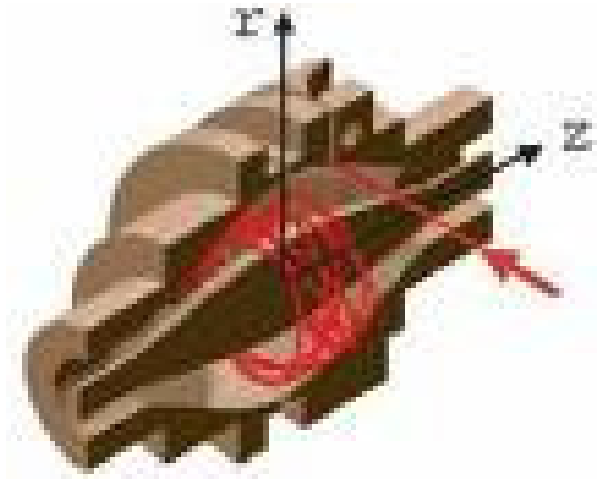
- Advantage
  - High accuracy (about 1 ppm)
  - High resolution (900 000)
  - Possible measured of MS^n
    - CID
      - Proteomic - primarily *b*- and *y*- type of fragment
    - Electron capture dissociation (ECD)- by capturing the thermal electron
      - Proteomic - primarily *c*- and *z*- type of fragments
    - Infrared multiphoton dissociation (IRMPD) - by IR laser

Orbitrap

- Similar principle to FT-ICR-MS
- The Orbitrap is an ion trap – but there are not RF or magnetic fields!
- Ions in Orbitrap
  - Moving around a central electrode
  - **Moving in *z* axis**
  - Detector electrodes measure the electrical signal of ions

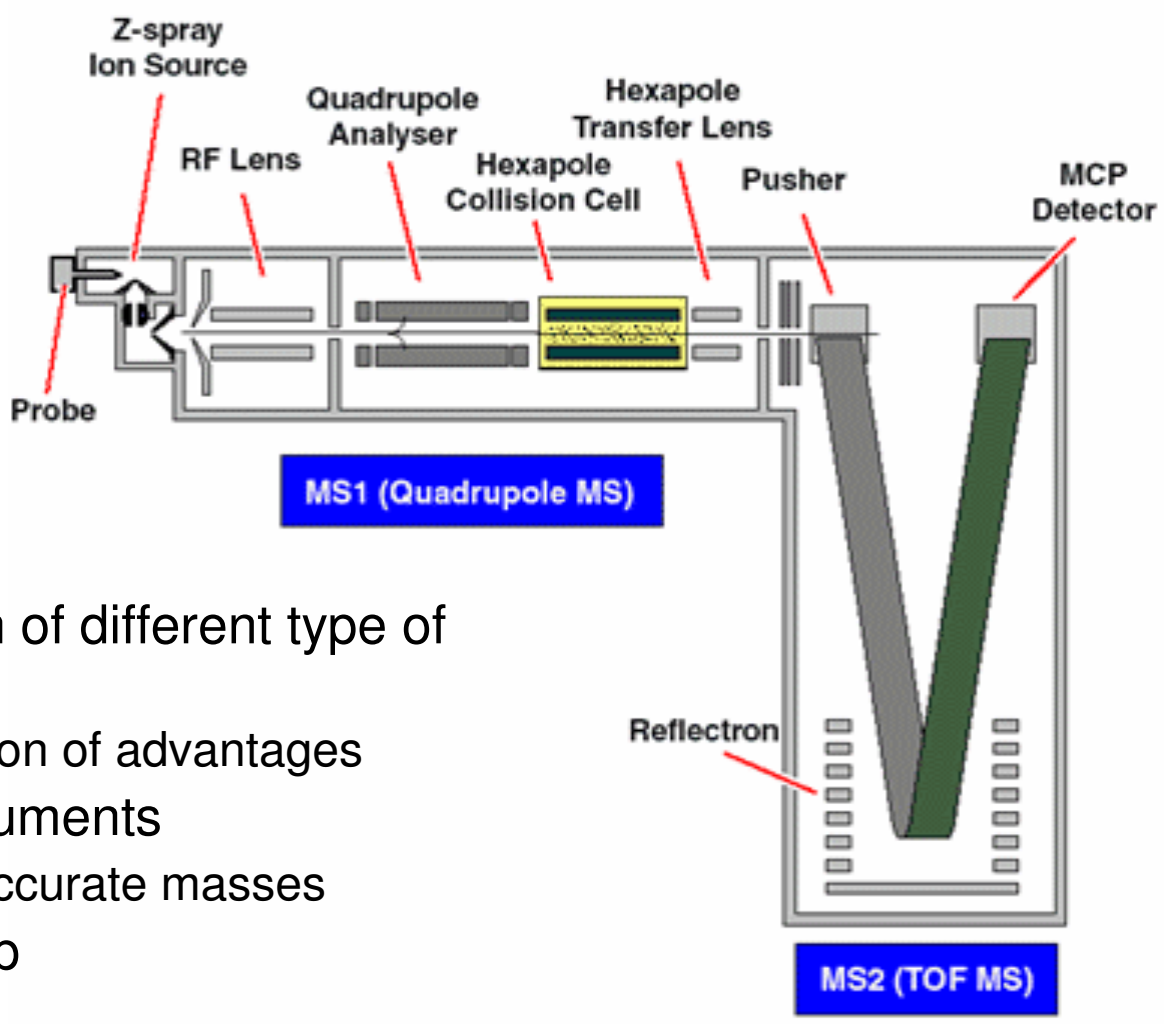


Orbitrap

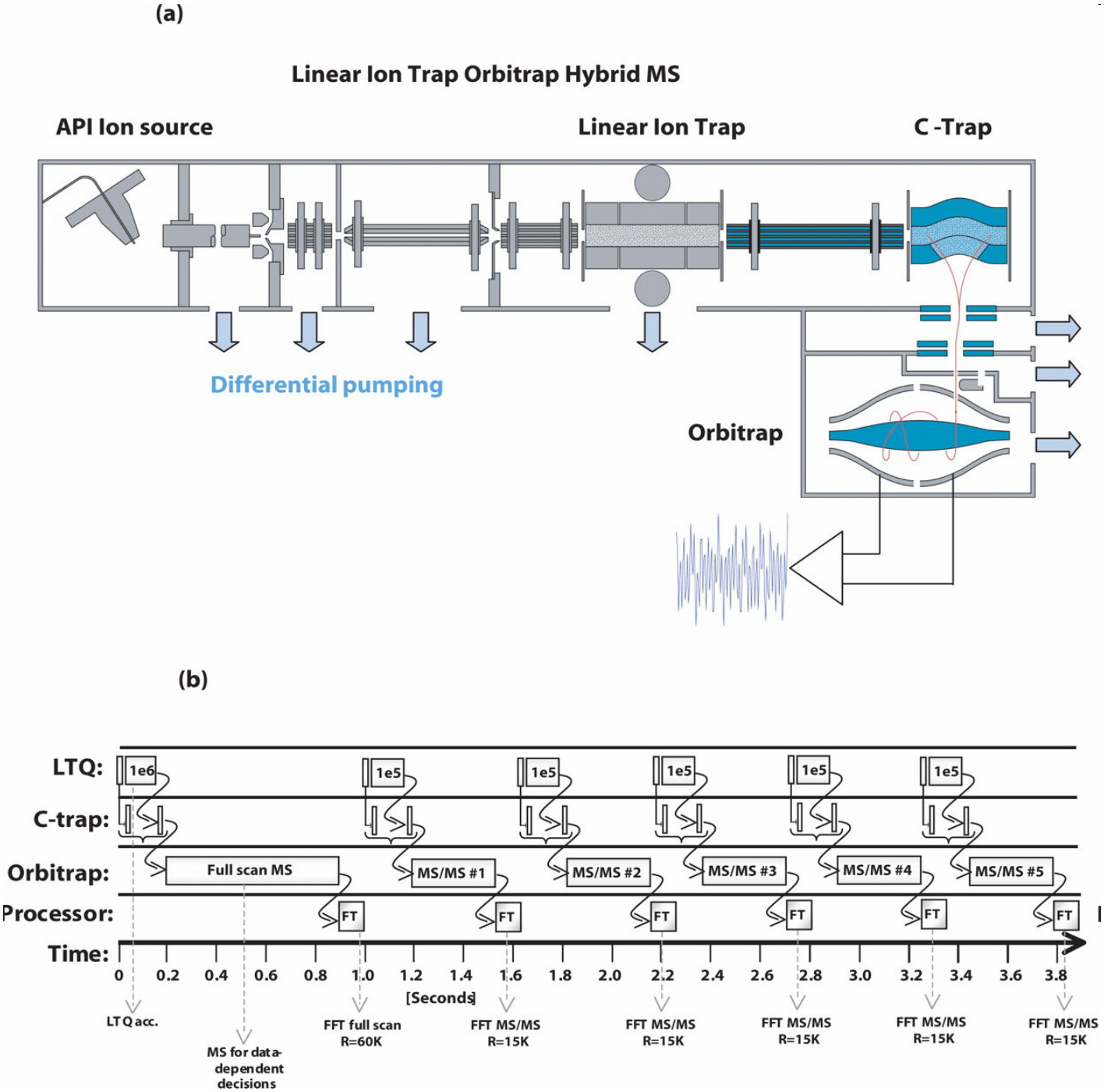


- Advantage
  - High accuracy (about 1 ppm)
  - High resolution (100 000)
    - New generation of instrument 250 000
  - Does not need magnet – the most expensive part of instrument
- Electron-transfer dissociation
    - Proteomic - c- and z-type of fragments (similar to ECD)
      - ETD does not use free electrons but employs radical anions (e.g. anthracene, azobenzene,.....)

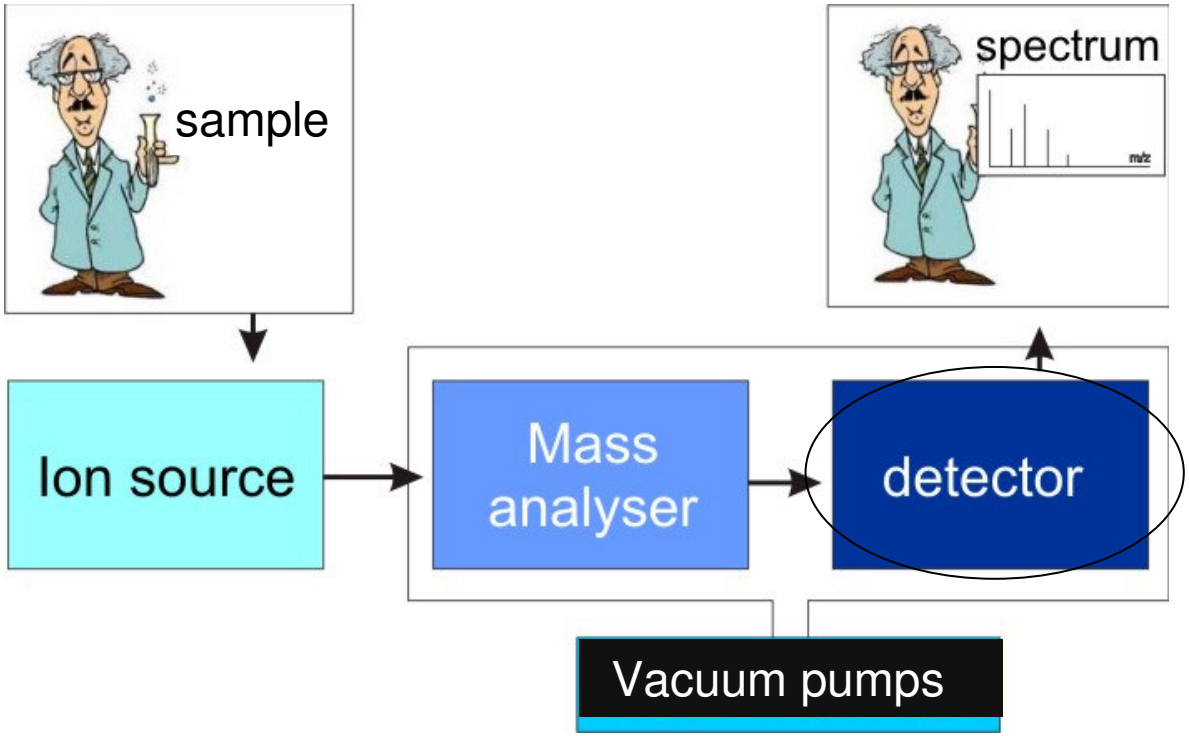
Hybrid mass spectrometers



- Combination of different type of analysers
  - Combination of advantages
- Q-TOF instruments
  - MS/MS, accurate masses
- LTQ-Orbitrap
- ……..


(a)
Linear Ion Trap Orbitrap Hybrid MS
API Ion source
Linear Ion Trap
C -Trap
Differential pumping
Orbitrap
(b)
LTQ:
C-trap:
Orbitrap:
Processor:
Time:
1e6
1e5
Full scan MS
MS/MS #1
MS/MS #2
MS/MS #3
MS/MS #4
MS/MS #5
FT
0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8
[Seconds]
LTQ acc.
MS for data-dependent decisions
FFT full scan R=60K
FFT MS/MS R=15K

Detector



- Detectors - record either the charge induced or the current produced, when an ion passes by or hits a surface

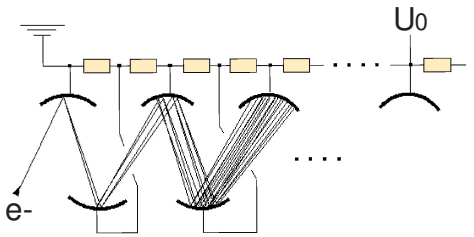
Detectors

- Records either the charge induced or the current produced, when an ion passes by or hits a surface.
- Detectors
  - In commercial instrument detectors with conversion dynode
    - Ions strike a conversion dynode to produce electrons – electron multiplied by
      - Electron multiplier
      - Ion-to-photon detector

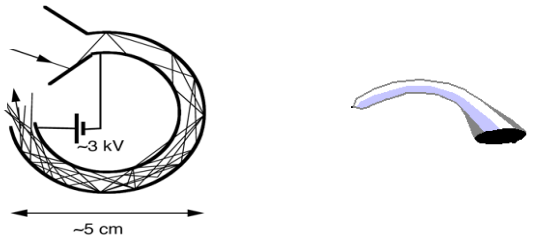
- In FT-ICR-MS and Orbitraps
  - The detector is part of analyser
  - Ions only pass near the electrodes

Detectors

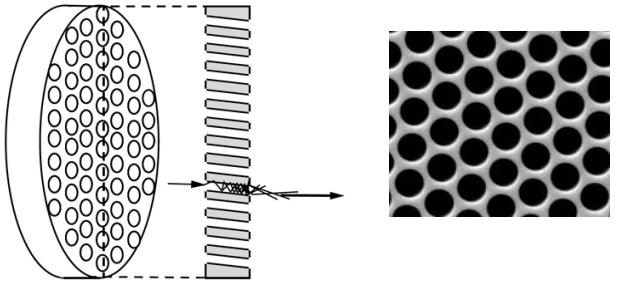
- Elektromultiplier with discrete dynodes
  - Amplification 10^6



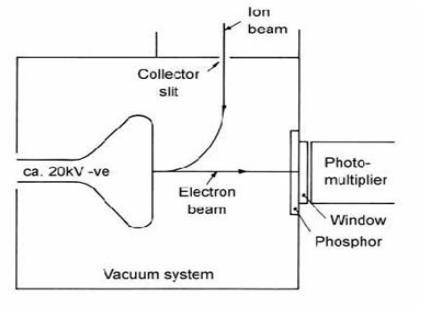
- Chaneltron
  - PbO - sensitive surface
  - Amplification 10^6



- Microchannel Plate Detectors (MCP)
  - PbO - sensitive service
  - Amplification 10^3
    - Two detectors – 10^6
  - For TOF analyser

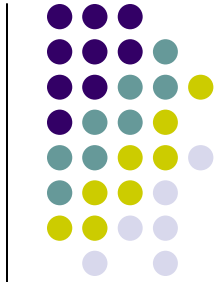


- Ion-to-photon detector
  - Electron strike a phosphor and the resulting photons are detected by a photomultiplier



Vacuum pumps

- Usually two steps
  - Rough vacuum (roughing pump - membrane pump, oil-sealed roughing pump)
    - 100 – 0.1 Pa
    - all type of instruments
  - High vacuum (turbomolecular pump, diffusion pump)
    - 0.1-10^{-6} Pa,
    - TOF, Q, IT
  - Ultra-high vacuum (turbomolecular pump)
    - (10^{-10}-10^{-12} Pa)
    - Orbitrap, ICR



Thank you for your attention