Mass spectrometry – part II

SEPARATION and DETECTION of IONS

Ion separation	*Ion detection*
Spectrum quality - **stability, range** - **resolution - accurate mass** **true mass** **Speed of measurement** **LOD / LOQ**	**LOD / LOQ** **Stability** **Lifetime / Durability**

Principles of ion separation

*** separation is realized by mass analyzer (mass filter)**

*** mass analyzer = part of mass spectrometer,**
which distinguishes ions
according to their value of *m/z*

**** basic parameters of mass analyzers:***

a) range of m/z

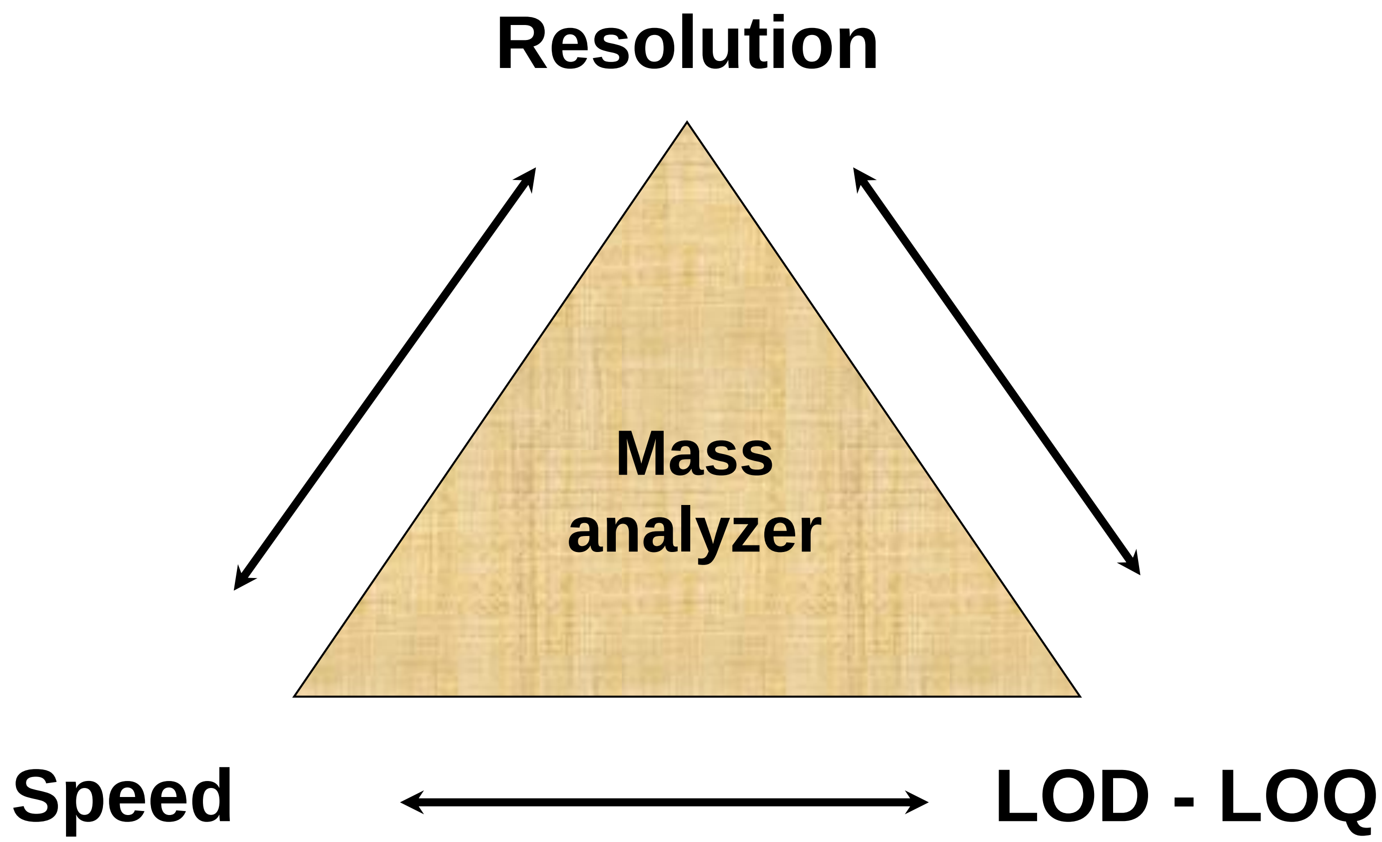
b) scan speed, switching of scan mode

c) resolution power X resolution

c) accurate mass X true mass

e) multistage mass analysis (MS^2 etc.)

Contradictory of parameters of mass analyzer



Parameters of mass analyzers (1)

* range of m/z:

a) lower limit: from technical "0" or from higher values

b) upper limit: up to a certain maximum value

(singly charged X multiply charged ions)

* scan speed and possibility of switching of scan modes:

a) speed = number of scans per second:

< 1	-	slow
1 to 10	-	medium
10 to ≈ 500	-	fast

b) mode: full scan, segment scan, SIM, SRM, MRM

Parameters of mass analyzers (2)

* resolving power - RP ($10^3 – 10^6$); RP = 1/R

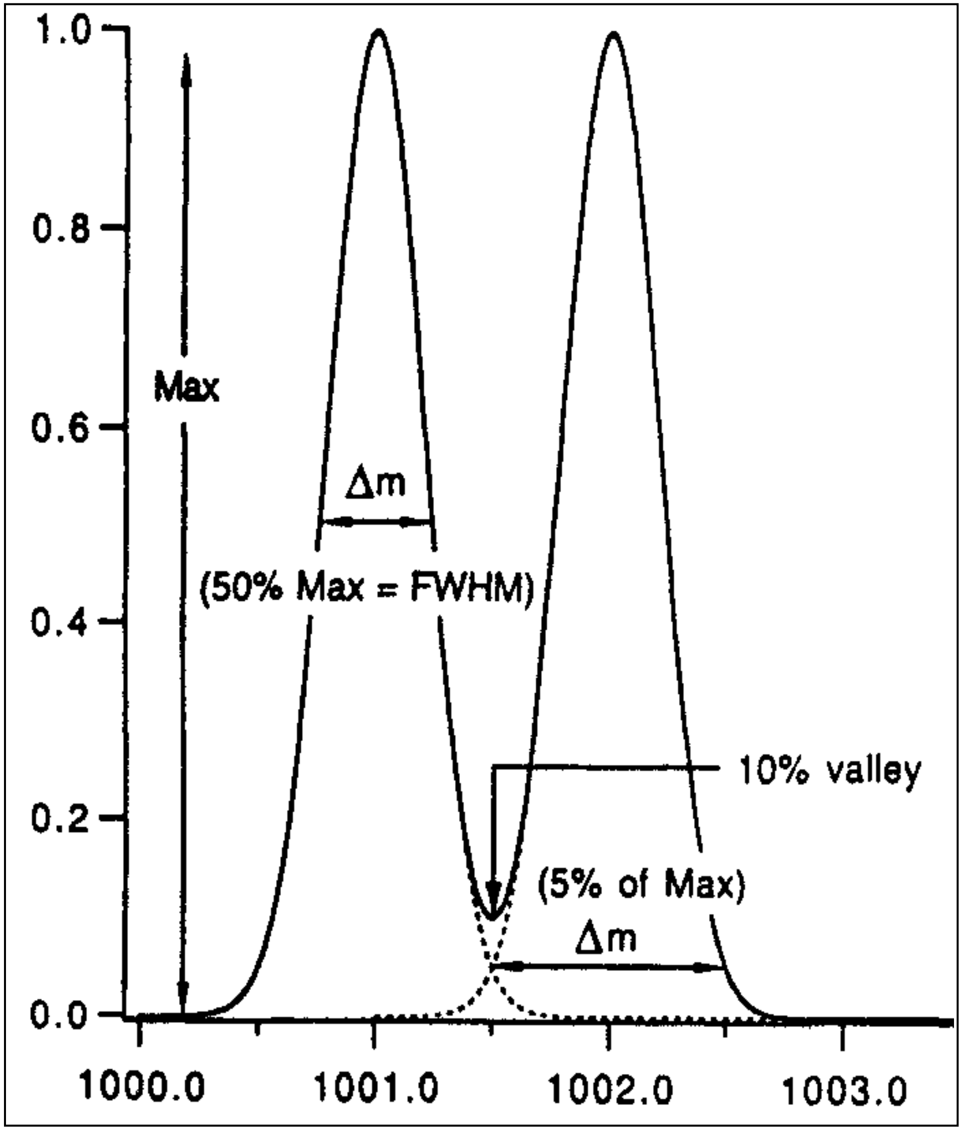
* a measure of the ability of mass analyzer to distinguish between close m/z values, i.e. what the smallest difference can be record

Relative measure - depends on m/z value

a) definition for two close ions: RP = m_1 / (m_1 - m_2); z_1, z_2 = 1
peaks m_1 and m_2 with 10 % overlaying at the same height

b) definition for one ion: m / Δm (or t / 2 Δ t for TOF)
based on concept of FWHM (full width at half maximum)

Parameters of mass analyzers (3)



Parameters of mass analyzers (4)

* **resolution - R (10^{-1} – 10^{2} ppm); R = 1/RP**

* **a measure of the ability of mass analyzer to distinguish between close *m/z* values, i.e. what the smallest difference can be record**

Relative measure - depends on *m/z* value

Expressed in ppm; $R = m_1 – m_2 / m_1$

Parameters of mass analyzers (5)

* <u>accurate mass</u>

* a measure of match between measured and calculated (exact) *m/z*

* a measure of the ability of mass analyzer to determine the correct *m/z*

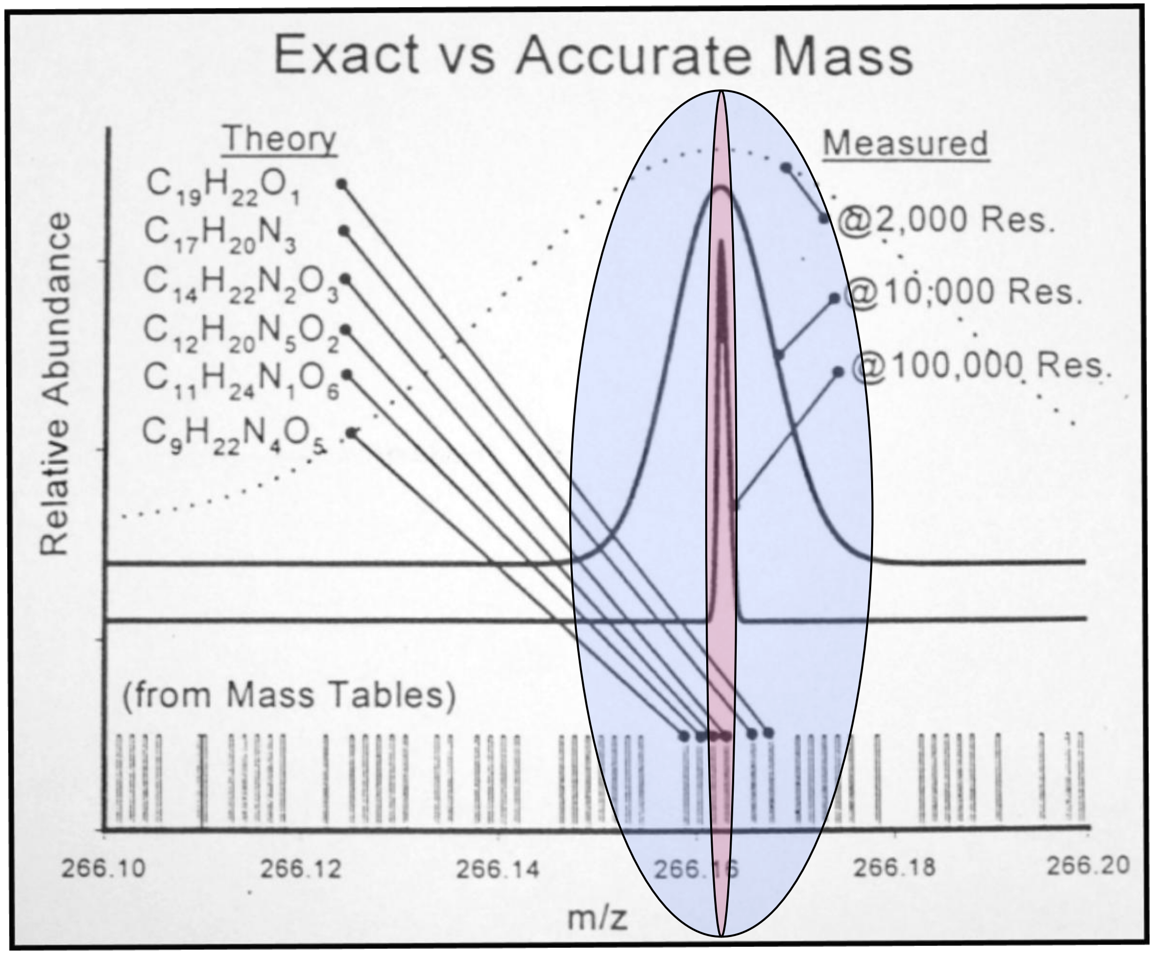
Relative measure - depends on *m/z* value

Expressed in ppm: ppm = $10^6 . \Delta m / m$

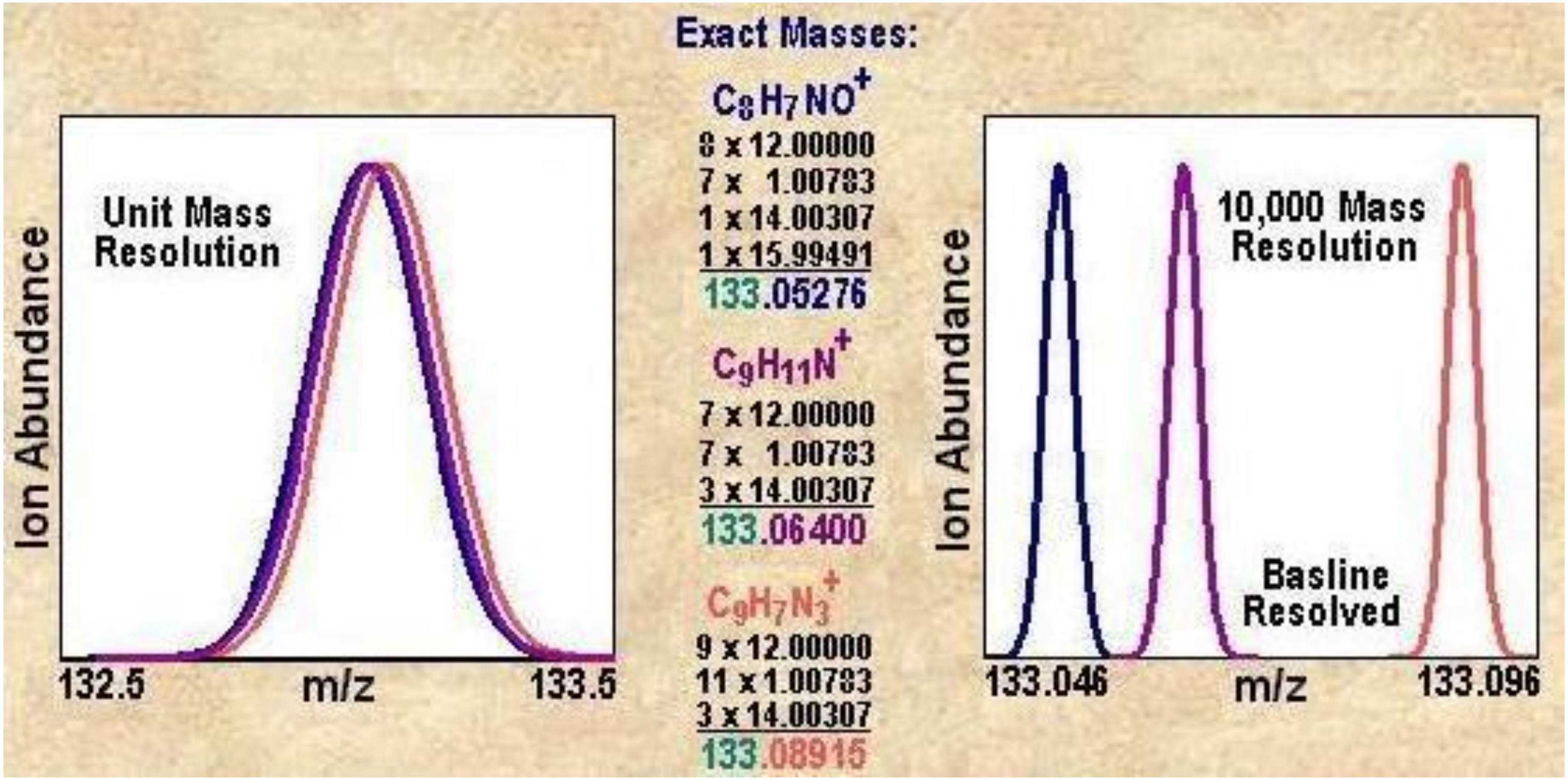
10^6. (exact value – measured value / exact value)

e.g.: ppm = 10^6. 212,14124 – 212,14072 / 212,14124 = 2,45 ppm

Parameters of mass analyzers (6)



Parameters of mass analyzers (7)



Parameters of mass analyzers (8)

Examples

Resolving power (RP) = 20000 (usually given as FWHM resolution)

- for *m/z* = 1000 ion distinguishing with difference ±0.05 (1000/20000 = 0.05)

- for *m/z* = 100 ion distinguishing with difference ±0.005 (100/20000 = 0.005)

Resolution (R) for RP above is 50 ppm (1/20000 = 50.10^{-6})

Accurate mass

a) high resolution with accuracy 3 ppm

100 ± 0.0003 or 1000 ± 0.003 etc.

b) Low resolution with accuracy 100ppm

100 ± 0.01 or 1000 ± 0.1 etc.

Parameters of mass analyzers (9)

*** multistage or multiple mass analysis**
→ MS/MS = MS^2, MS/MS/MS = MS^3 etc.

a) in space – combination of the same or various mass analyzers in series: *Q1 → Q2 → Q3* vs. *Q1 → T-wave → Q2*

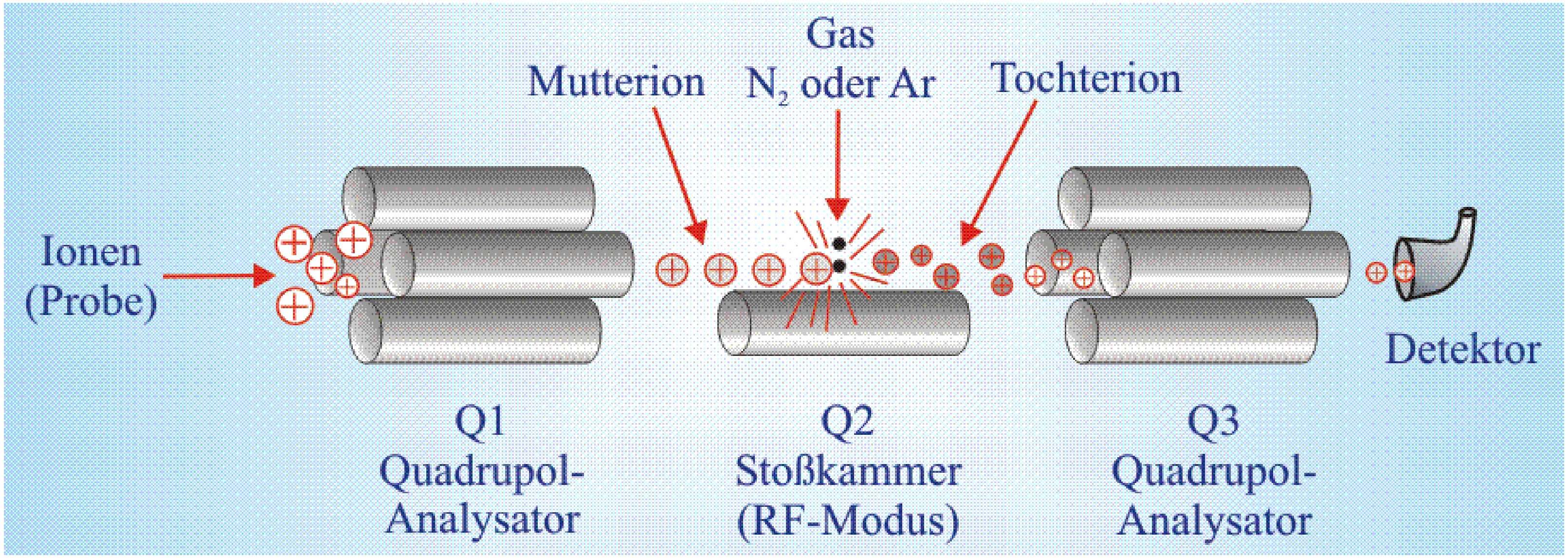
analyzer → collision cell → analyzer

b) *in time* – realized in the same space (analyzer) with changes of parameters of the analyzer - ion trap

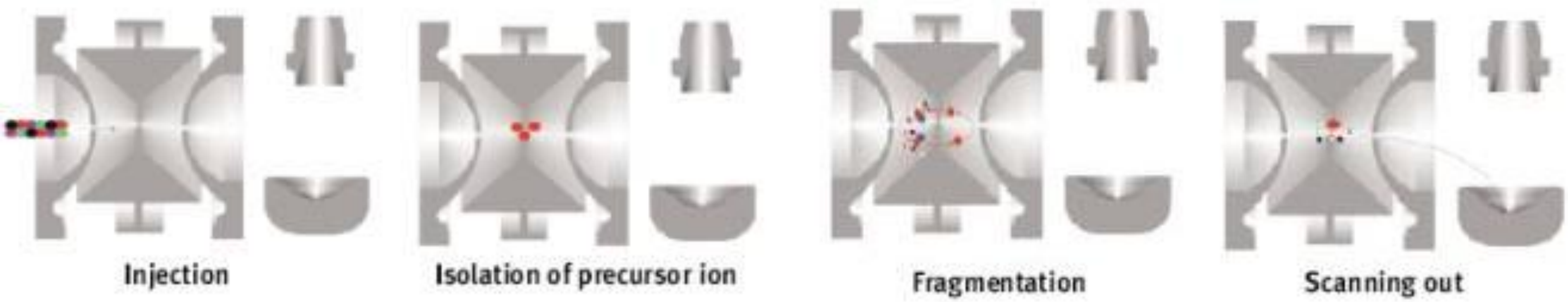
primary analysis → collision → secondary analysis

Parameters of mass analyzers (10)

In space

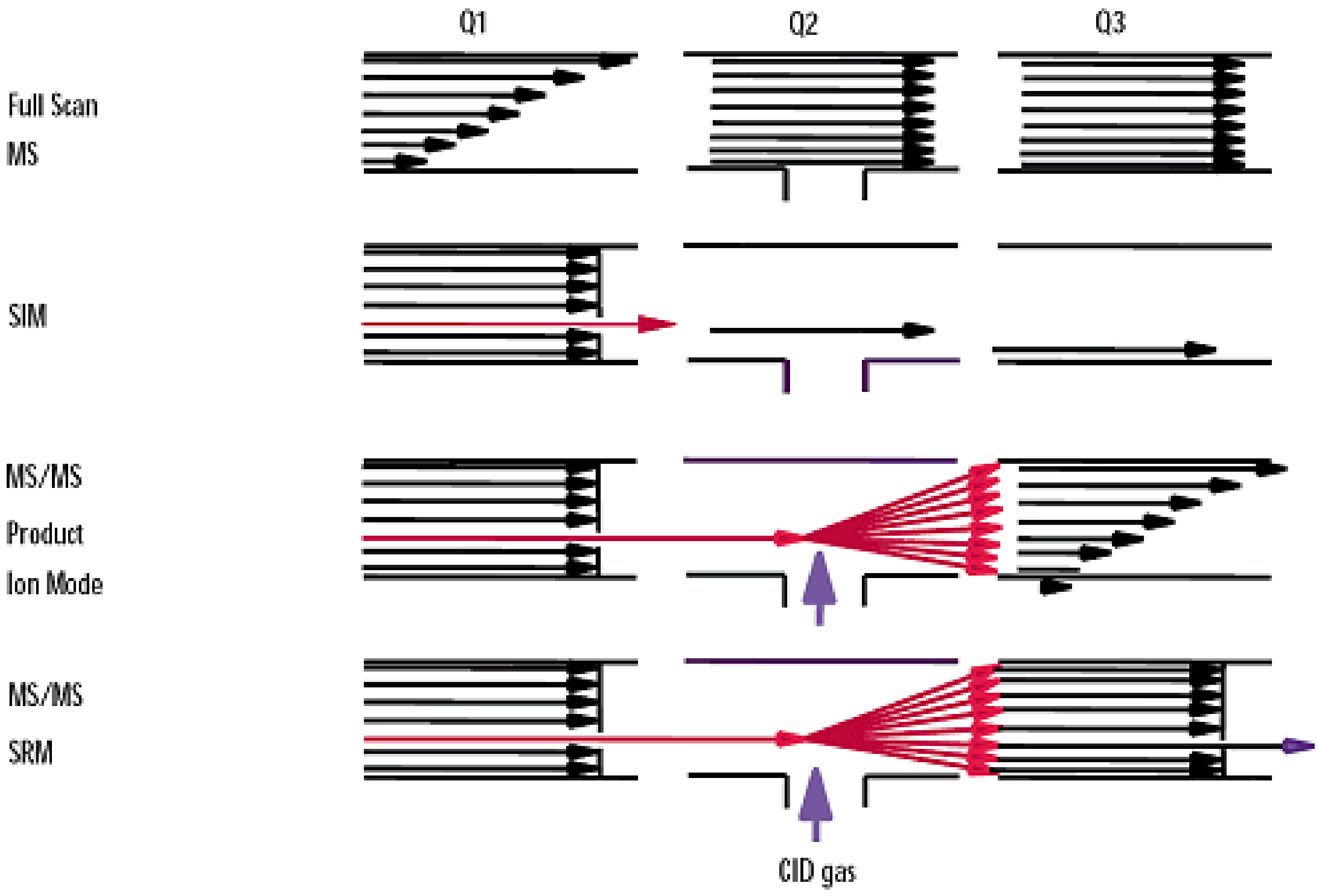


In time



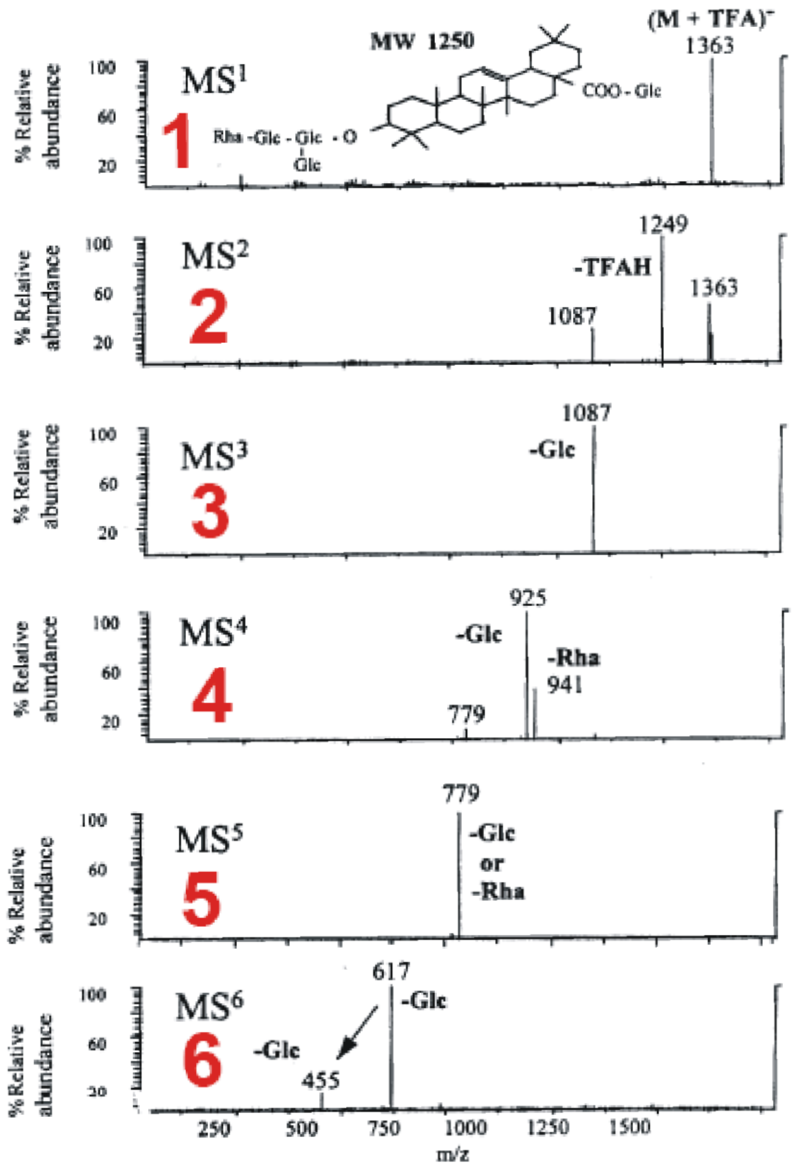
Parameters of mass analyzers (11)

Scan functions of triple quadrupole

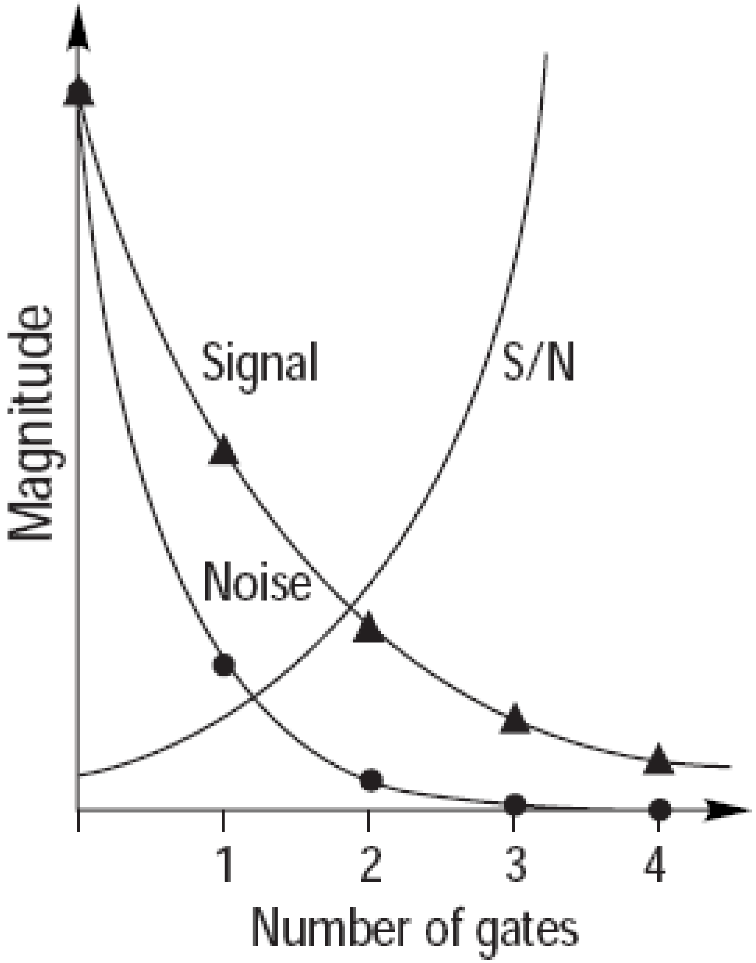


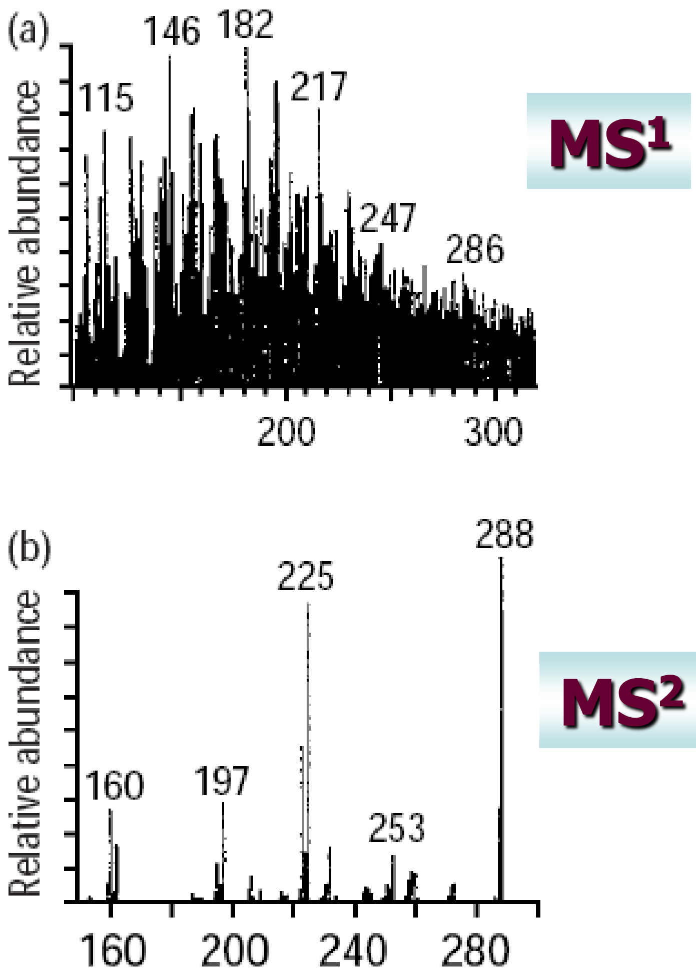
Parameters of mass analyzers (12)

Sequential fragmentation the ion trap



Parameters of mass analyzers (13)





Technical principles of mass analyzers (1)

* <u>different principles allowing ion separation</u>

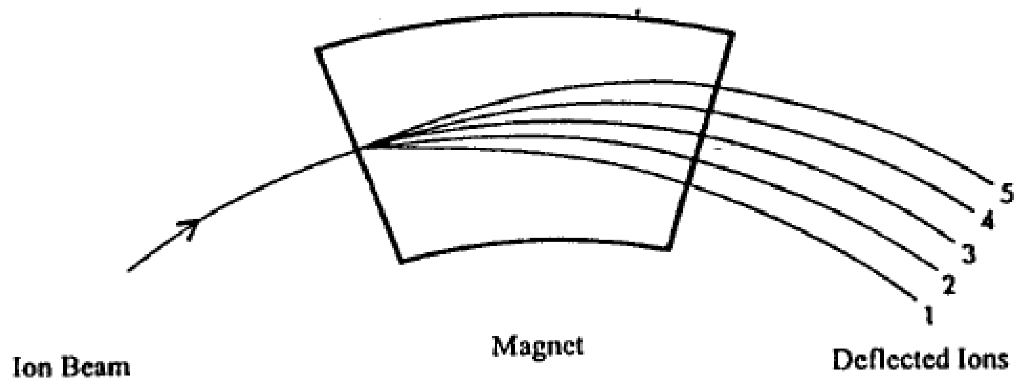
a) magnetic analyzer with single focusation
b) magnetic sector analyzer with dual focusation
b) quadrupole analyzer: four rods X segment
   ion trap (3D, 2D - linear)
e) time of flight: straight X reflex (includes reflectron)
f) ion mobility - transport waves (travelling wave) X drifting tube
g) ion cyclotron resonance X orbitrap

* <u>mass analyzers used in ion optics applied for ion focusation:</u>

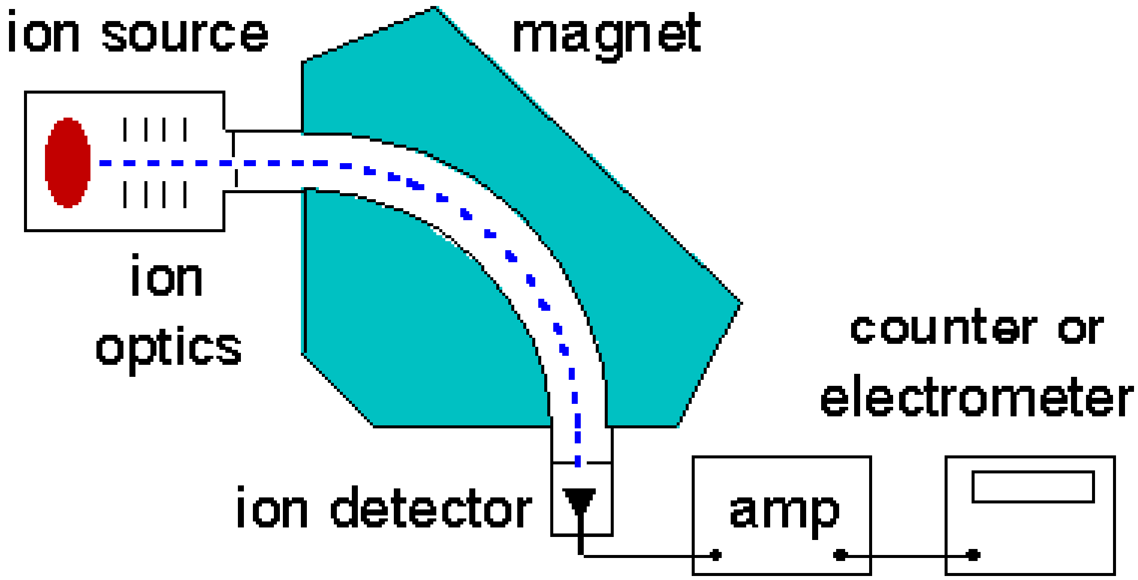
a) multipole analyzers: hexa-, octa-pole
b) ion optics - plates, rings

Technical principles of mass analyzers (2)

Magnetic sector



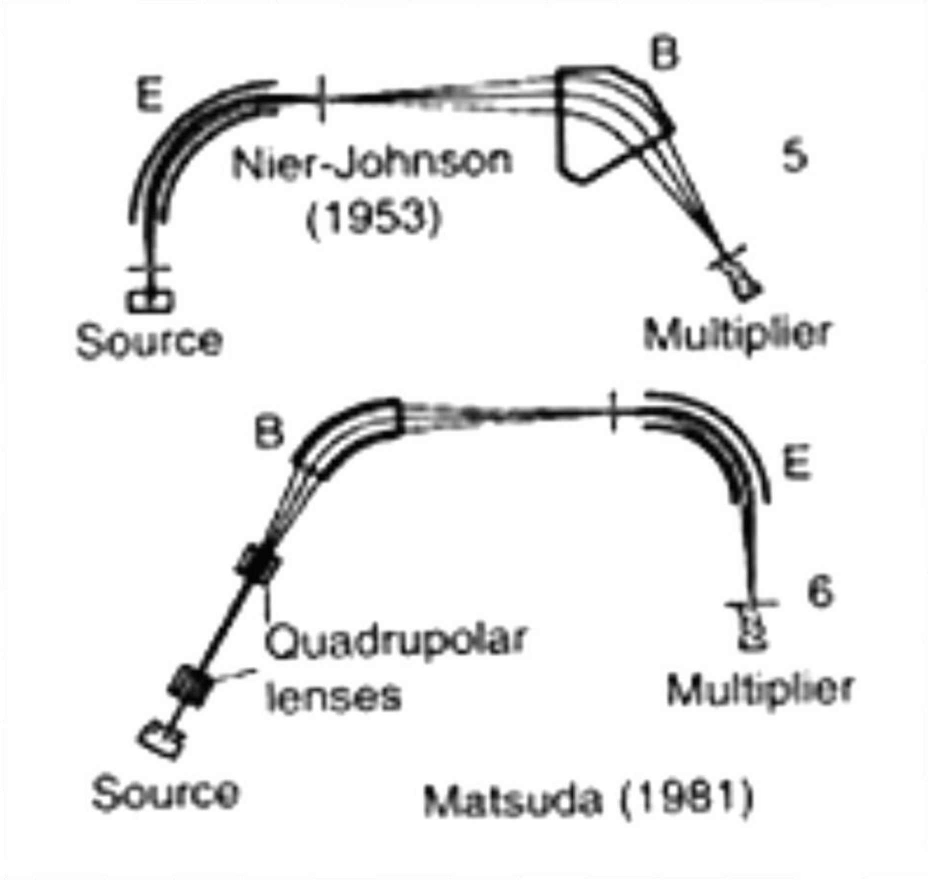
$(m/z)_1 < (m/z)_2 < \ldots < (m/z)_5$



Technical principles of mass analyzers (3)

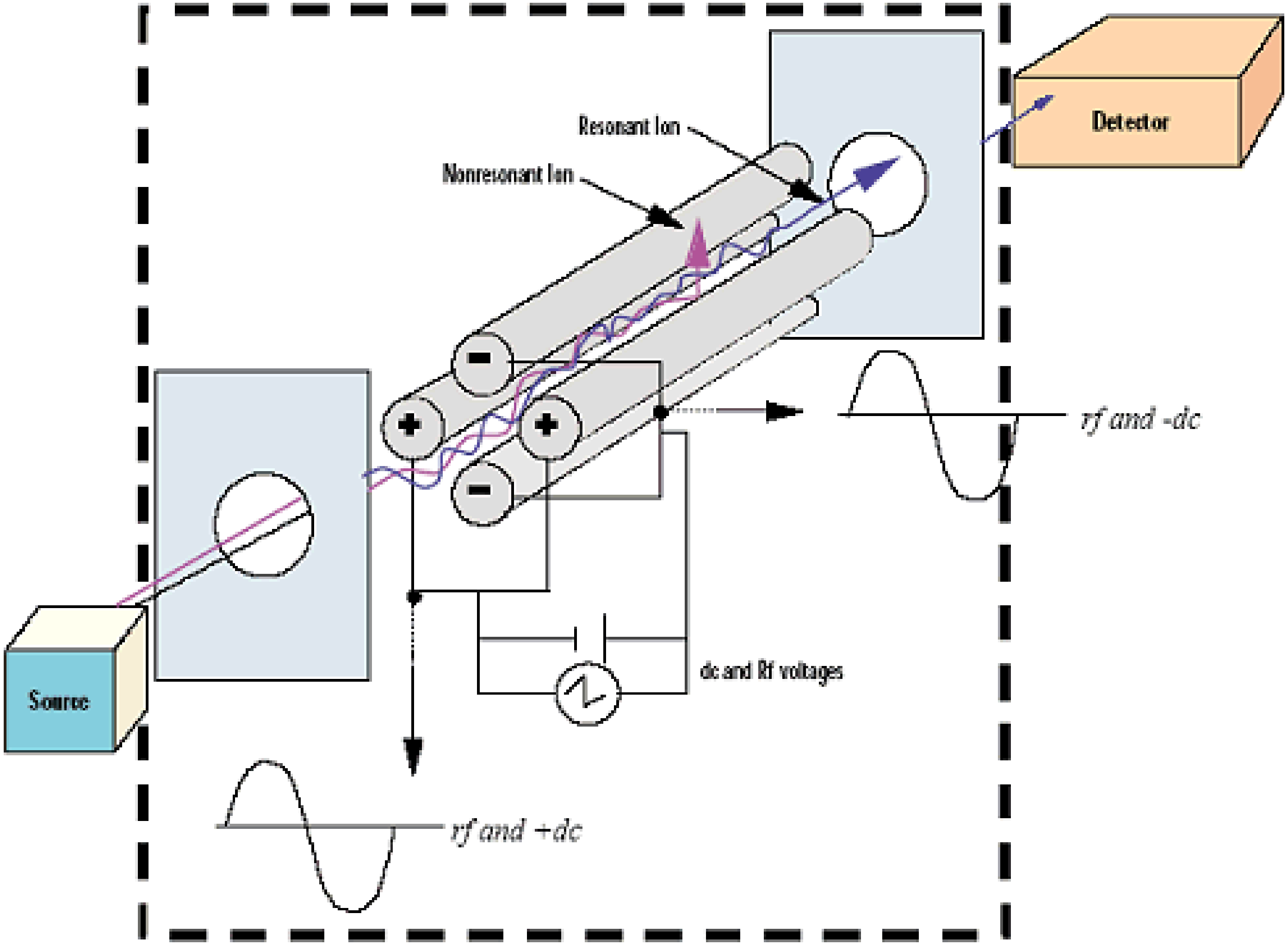
Magnetic sector - dual focusation

→ electrostatic / magnetic



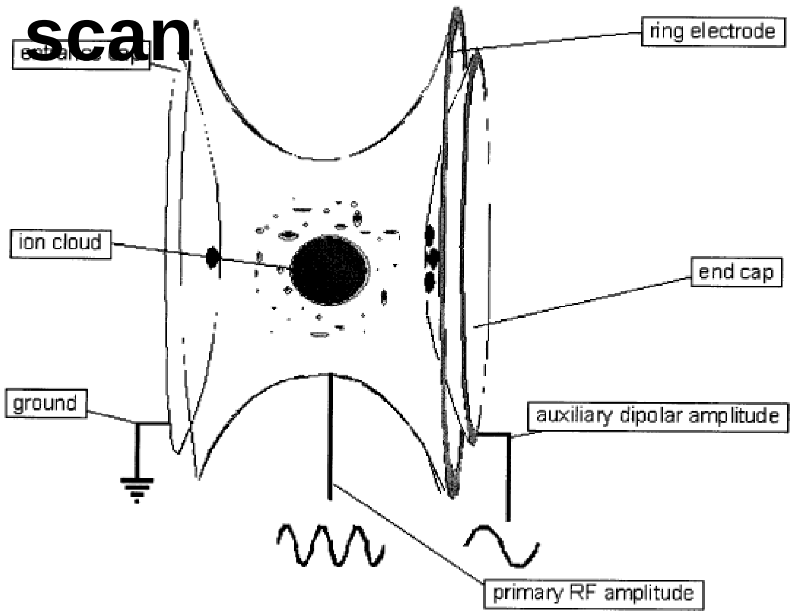
Technical principles of mass analyzers (4)

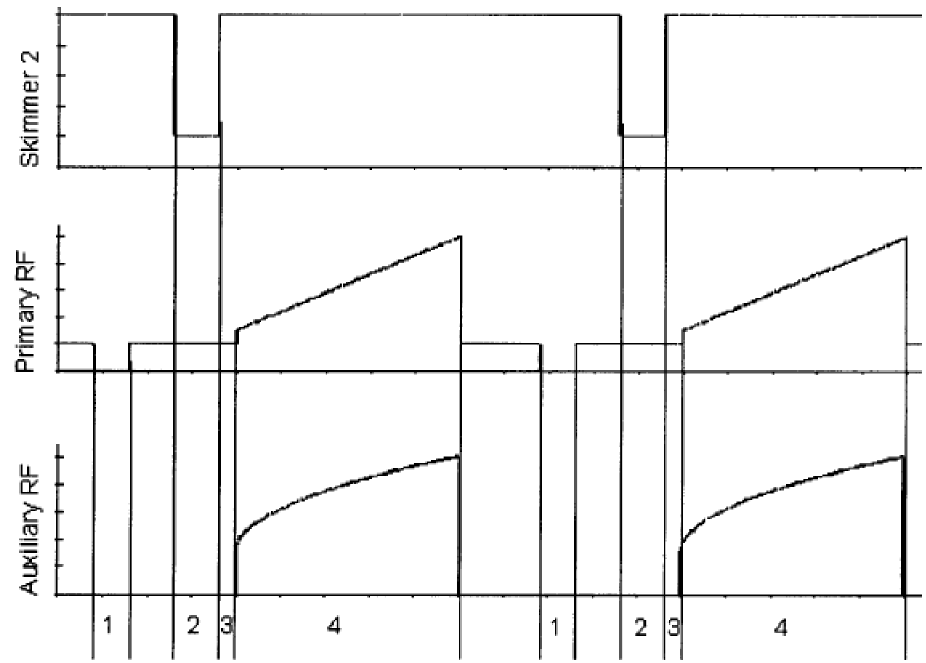
Quadrupole: straight - 4 rods

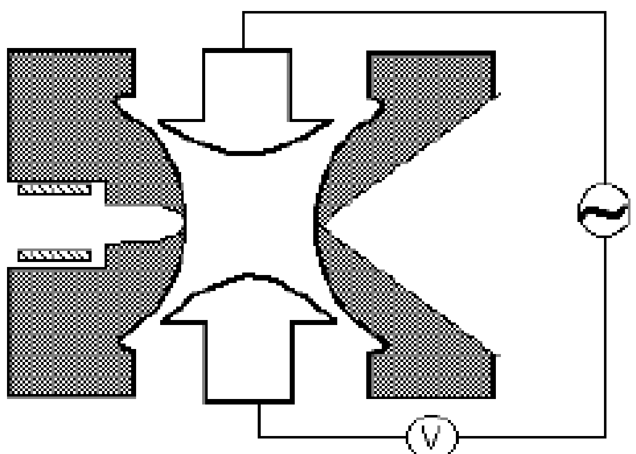


Technical principles of mass analyzers (5)

Quadrupole: ion trap (3D) - MS scan







Ion trap - MS scan

1. Trap emptying

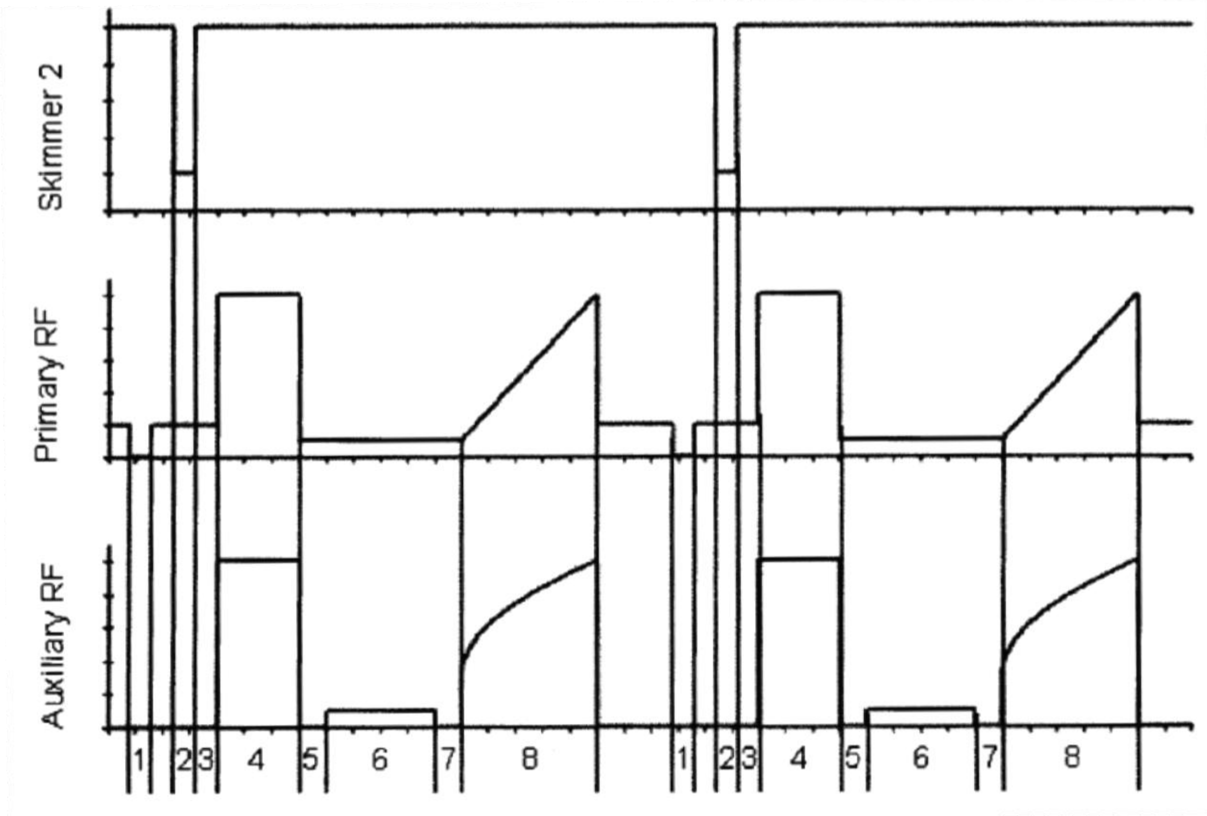
2. Accumulation of ions

3. Scan dwell

4. Mass analysis

Technical principles of mass analyzers (6)

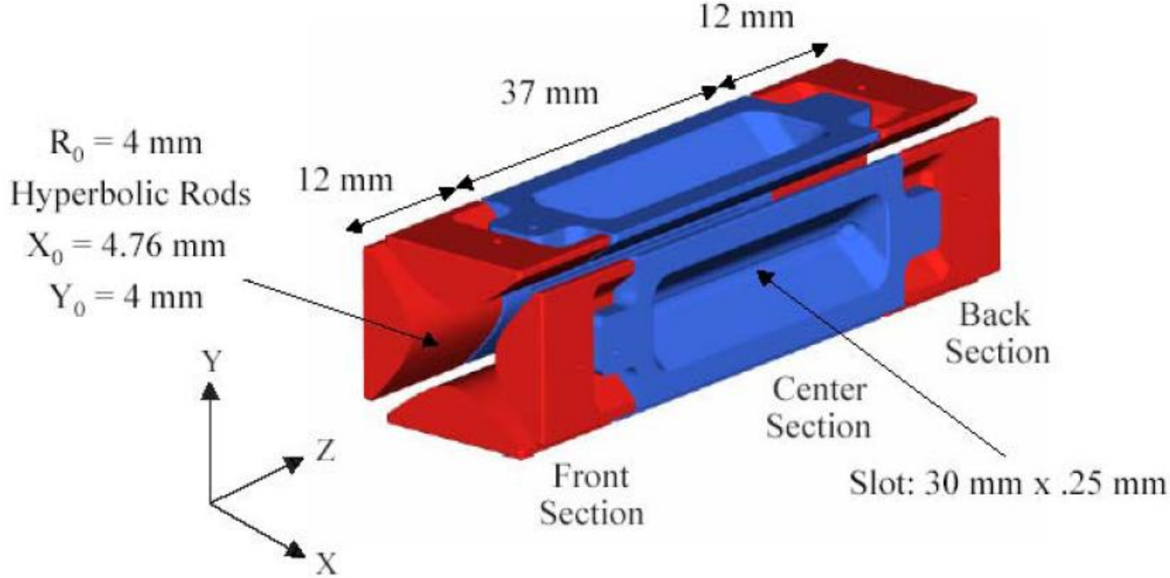
Quadrupole: ion trap - MS/MS scan

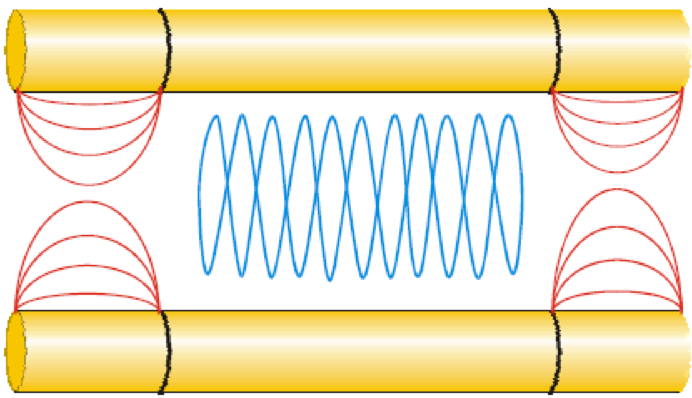


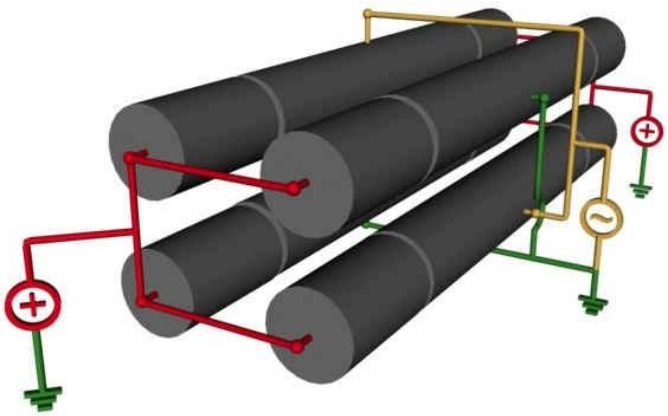
1. Trap emptying	5. Fragmentation dwell
2. Accumulation of ions	6. Fragmentation
3. Isolation dwell	7. Scan dwell
4. Isolation of parent ions (precursors)	8. Mass analysis

Technical principles of mass analyzers (7)

Quadrupole: linear ion trap (segmented quadrupole)

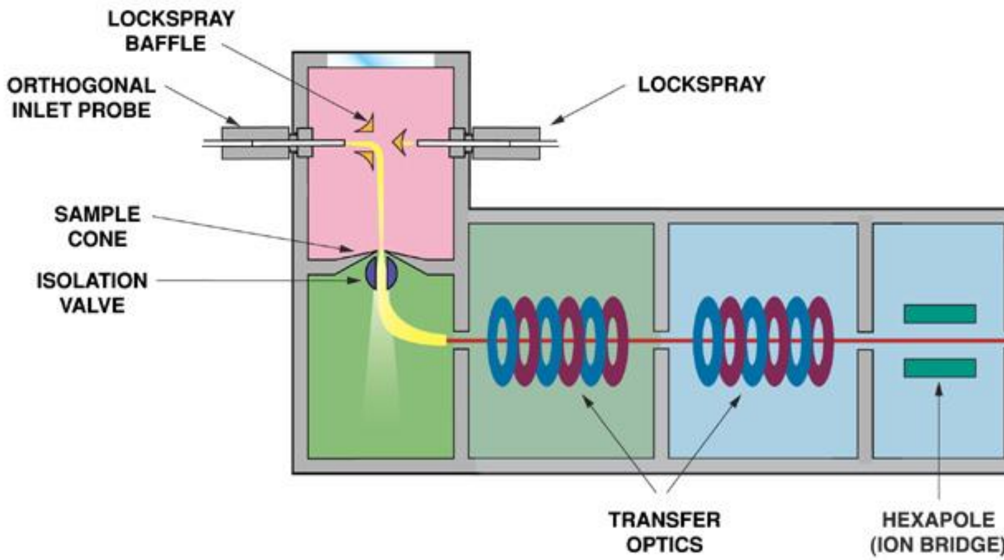


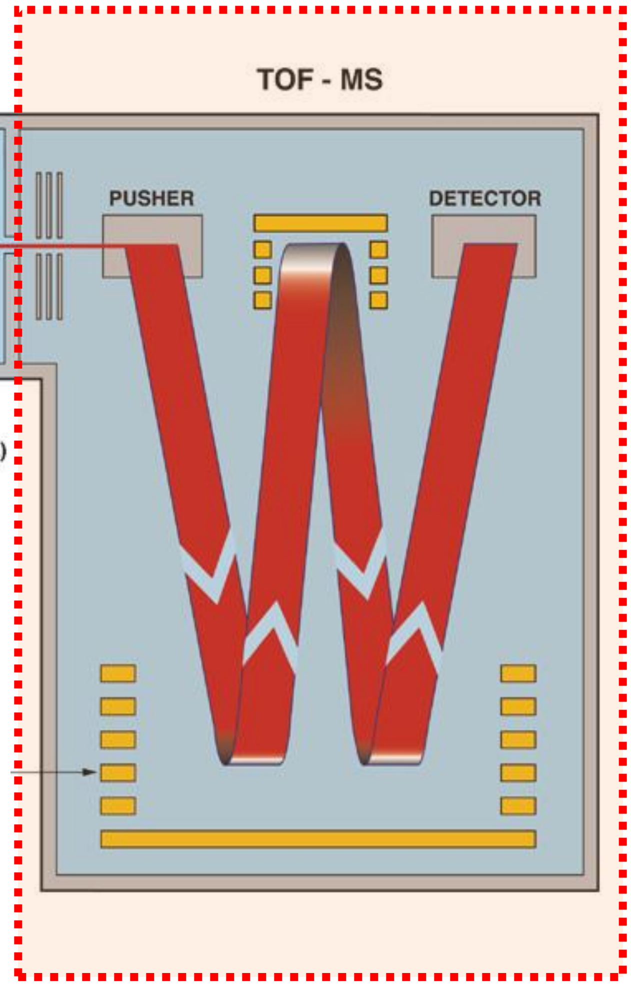


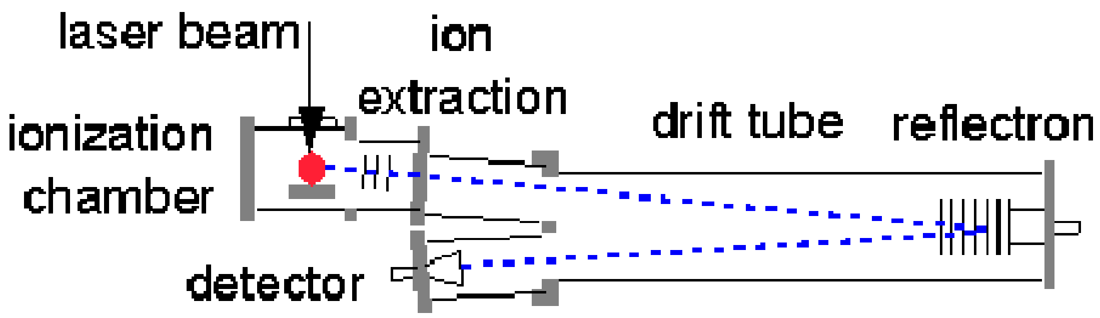


Technical principles of mass analyzers (8)

Time Of Flight (TOF)



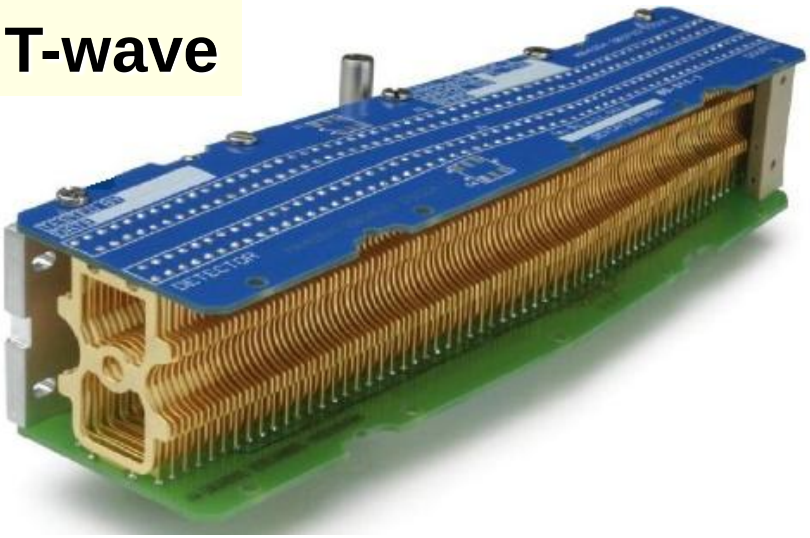




Technical principles of mass analyzers (9)

Travelling wave

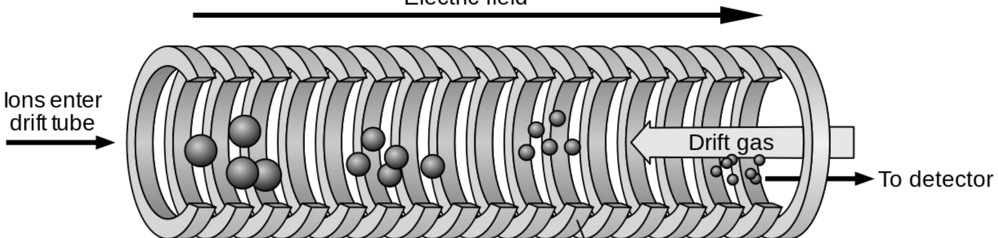
T-wave



Reflectron - TOF

Drift tube

Ion mobility



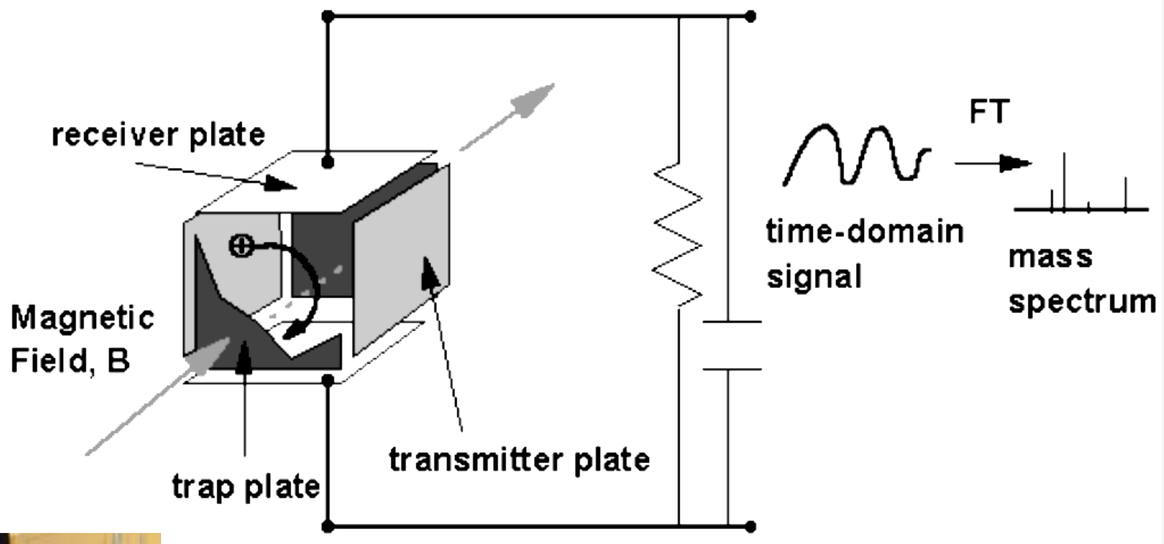
Technical principles of mass analyzers (8&9)

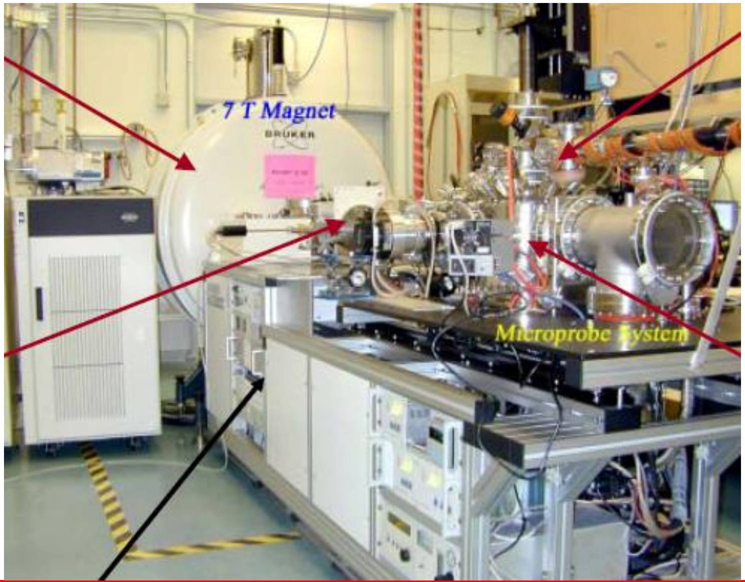
Ion Mobility (Travelling wave) - TOF MS (with reflectron)

VIDEO

Technical principles of mass analyzers (10)

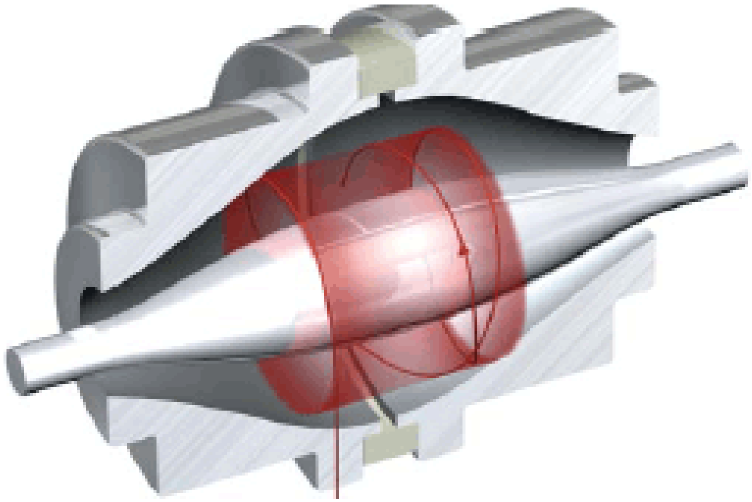
Fourier transform ion cyclotron resonance





Technical principles of mass analyzers (11)

ORBITRAP



Comparison of mass analyzers

Mass analyzer	RP (FWHM)	RANGE *m/z*	SCAN SPEED	PRICE [mil. CZK]
Magnet-sector	10^5	10^4	low	10 – 15
Quadrupole – straight	10^3	10^3	medium	2 – 4
Quadrupole – 3D trap	10^3	10^4	high	3 – 6
TOF	10^4	10^3 (10^6)	very high	6 - 8
ICR	10^6	10^8	high	30
Orbitrap	10^5	10^4	high	10

Ion detectors and signal multipliers (1)

Direct detection:

a) photographic plate (past)

b) Faraday cup - accurate number of ions

Detection using signal multipliers:

a) multiplier with conversion - photomultiplier

b) electron multiplier producing measurable current

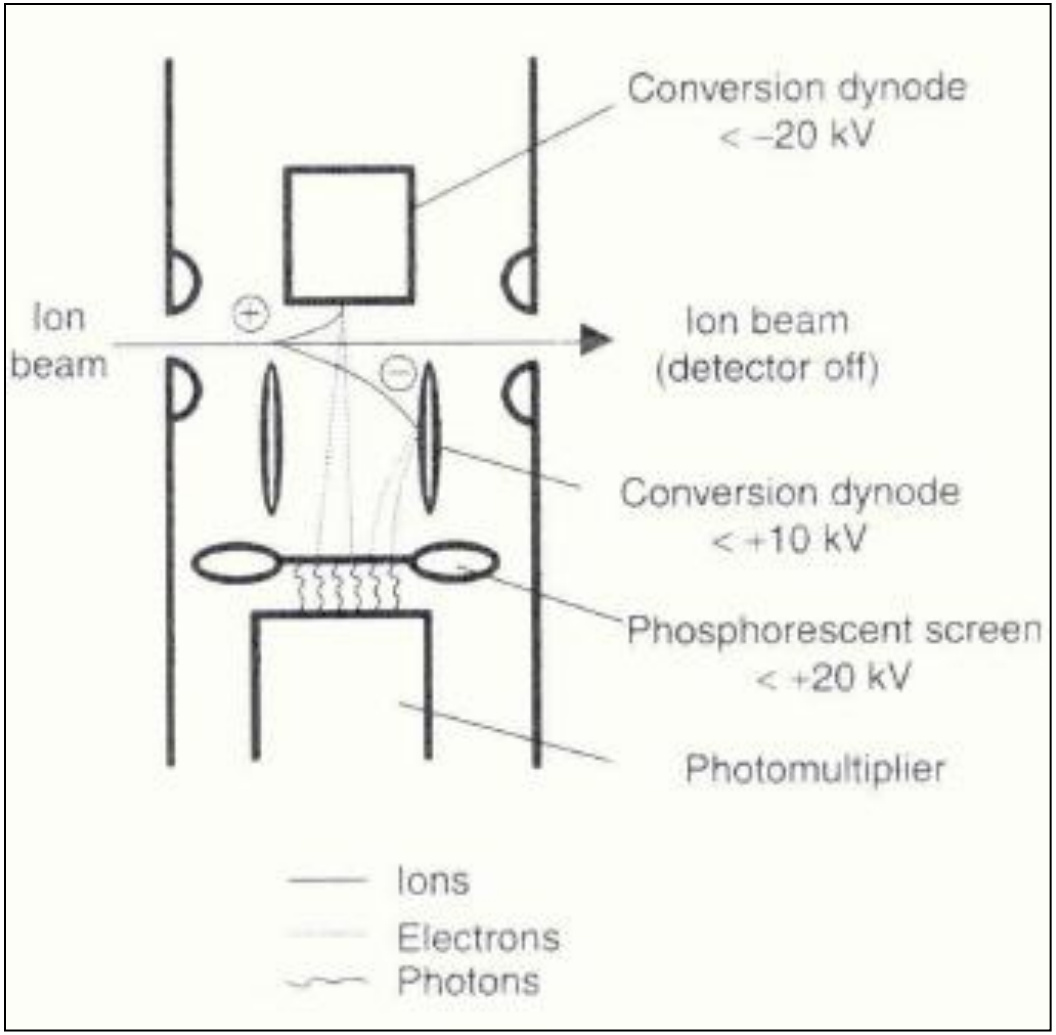
- dynode plates
- dynode continuous (tube - straight, curved)
- microchannel plate

Parameters:

amplification, durability, undesirable detection (neutral fragments)

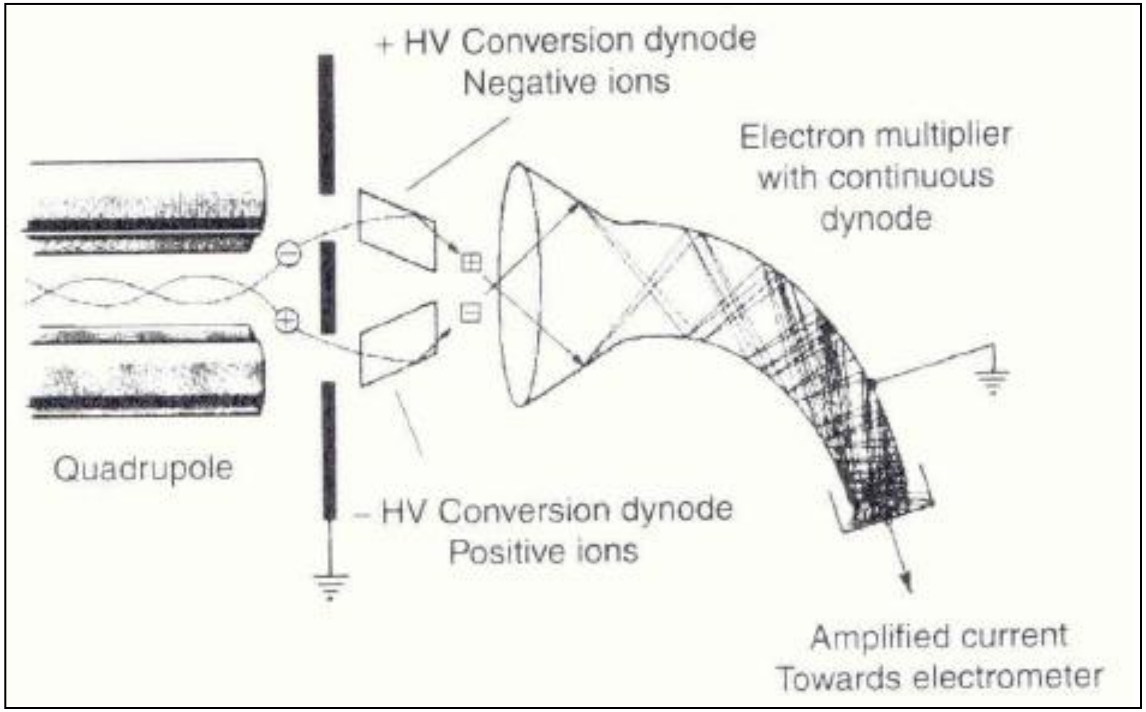
Ion detectors and signal multipliers (2)

Photomultiplier



Ion detectors and signal multipliers (3)

Electron multiplier



Ion detectors and signal multipliers (4)