Mass Accuracy and Absolute Mass

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Masses of elements and their isotopes

- Mass is defined using the mass of carbon-12 being 12.0000 (exactly)
- On this scale,
  - ^{1}H is 1.007825 and ^{2}H is 2.014102
  - ^{14}N is 14.003074 and ^{15}N is 15.000108
  - ^{16}O is 15.994915, ^{17}O is 16.999132 and ^{18}O is 17.999161
  - ^{31}P is 30.973761
  - ^{32}S is 31.972071 and ^{34}S is 33.967867

How is mass defined?

Assigning numerical value to the intrinsic property of “mass” is based on using carbon-12, ^{12}C, as a reference point.

One unit of mass is defined as a Dalton (Da).

One Dalton is defined as 1/12 the mass of a single carbon-12 atom.

Thus, one ^{12}C atom has a mass of 12.0000 Da.

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

+Why do we care?

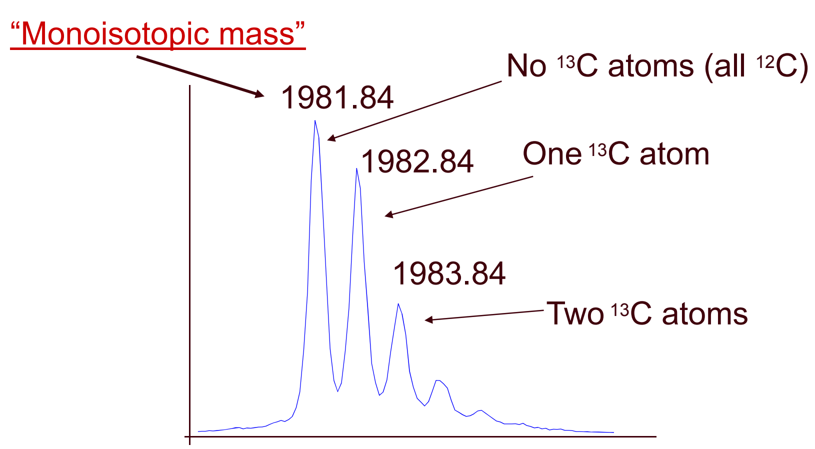
Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

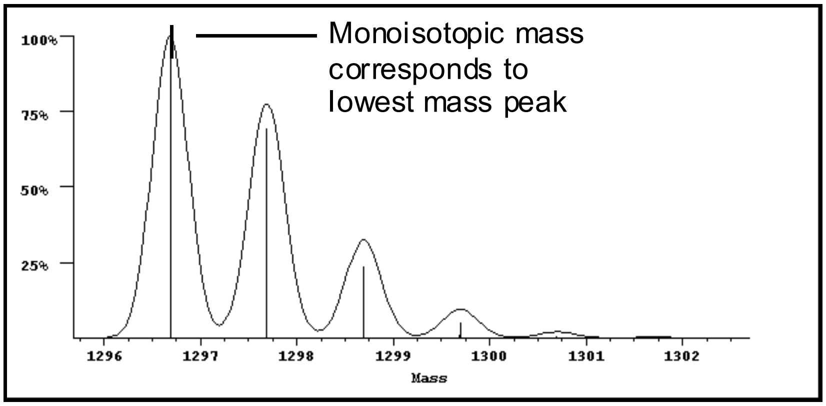
Stable isotopes of most abundant elements of peptides

Element	Mass	Abundance
H	1.0078 2.0141	99.985% 0.015
C	12.0000 13.0034	98.89 1.11
N	14.0031 15.0001	99.64 0.36
O	15.9949 16.9991 17.9992	99.76 0.04 0.20

Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)

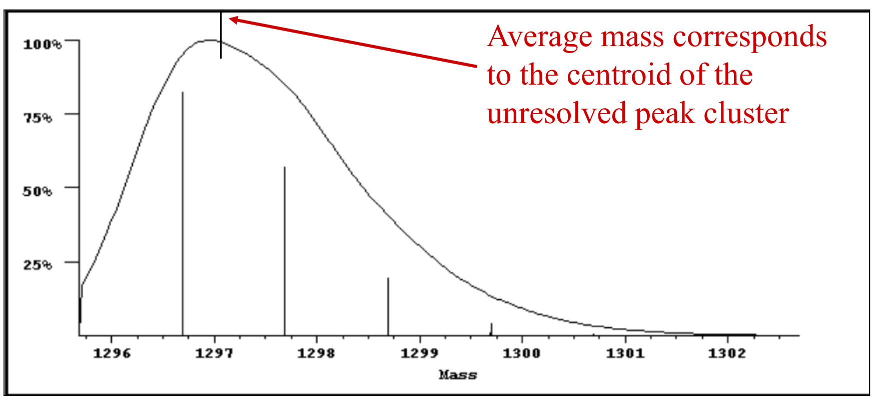


Monoisotopic mass



When the isotopes are clearly resolved the **monoisotopic mass** is used as it is the most accurate measurement.

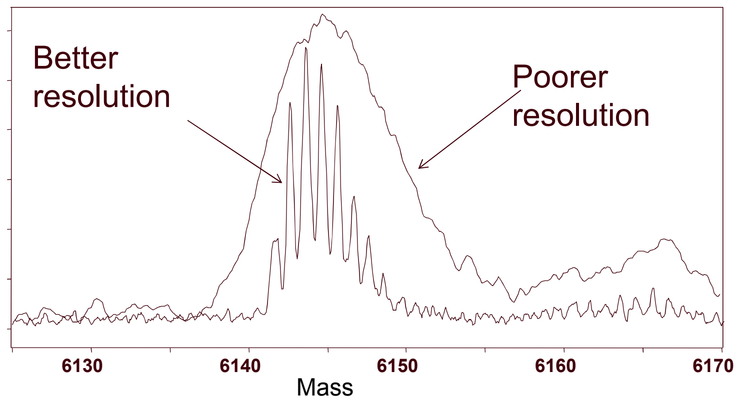
Average mass



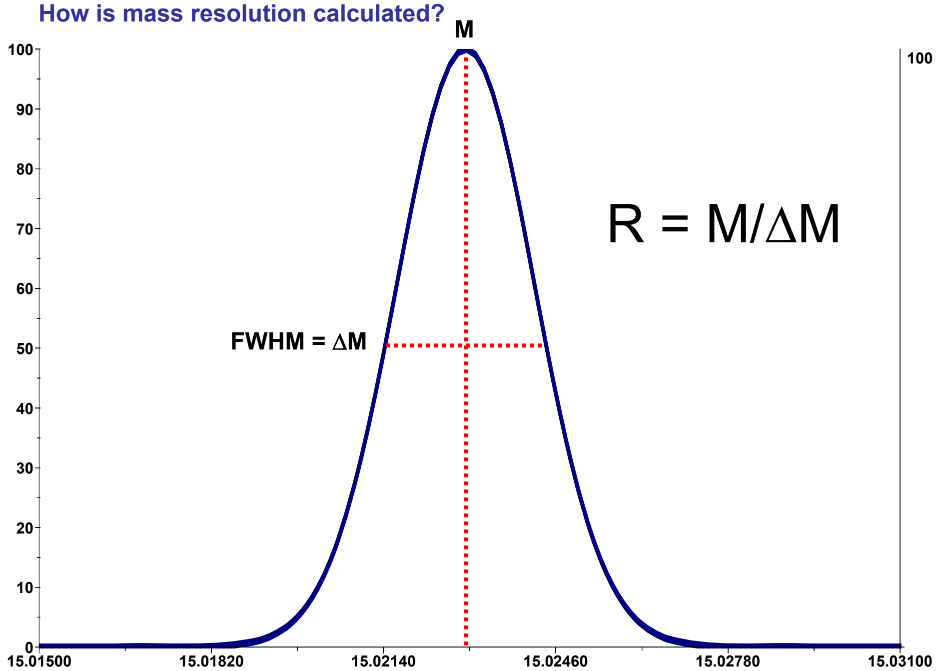
When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the the isotope peaks in the cluster, which is the same as the average or chemical mass.

What if the resolution is not so good?

At lower resolution, the mass measured is the average mass.

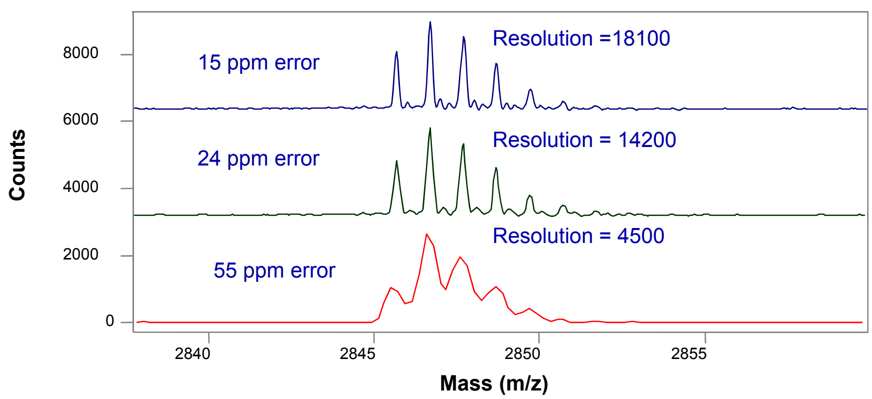


How is mass resolution calculated?



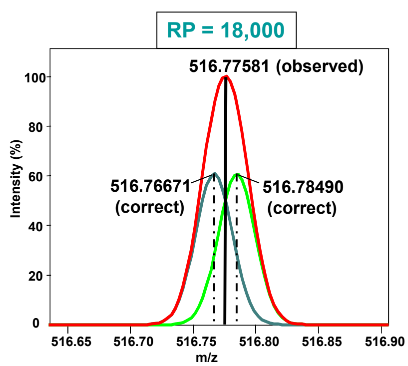
Mass measurement accuracy depends on resolution

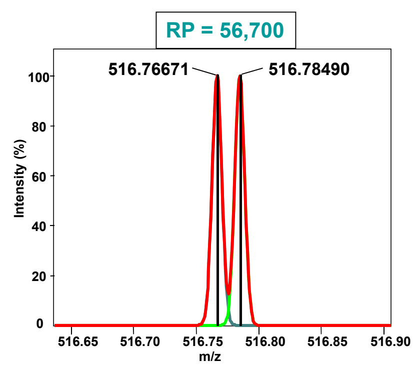
High resolution means better mass accuracy



Two peptides - same nominal mass - simulation

Peptide mixture:	**[Val^5]-Angiotensin II**	**Lys-des-Arg^9-Bradykinin**
Sequence:	**DRVYVHPF**	**KRPPGFSPF**
Formula:	**$C_{49}H_{69}N_{13}O_{12}$**	**$C_{50}H_{73}N_{13}O_{11}$**
Exact mass:	**$[M+2H]^{2+}$ = 516.76671**	**$[M+2H]^{2+}$ = 516.78490**
Δm (mmu):	**18.2 mmu**	



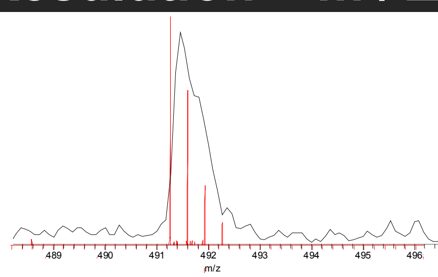


Is Mass Accuracy Important ?

Results for error limit up to 5 ppm

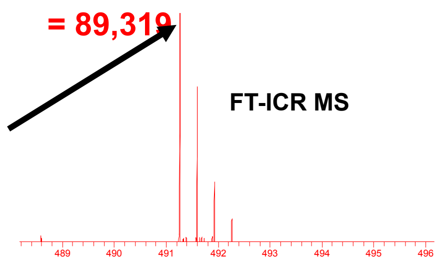
	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	516.76671	0.0	0.0	21.0	C_{49} H_{71} O_{12} N_{13}
	516.76647	0.5	0.2	15.0	C_{49} H_{79} O_{11} N_{9} S_{2}
	516.76638	0.6	0.3	12.0	C_{41} H_{75} O_{14} N_{15} S_{1}
	516.76705	-0.7	-0.3	11.5	C_{43} H_{77} O_{15} N_{12} S_{1}
2 ppm (10)	516.76604	1.3	0.7	16.0	C_{48} H_{75} O_{16} N_{9}
	516.76738	-1.3	-0.7	20.5	C_{51} H_{73} O_{13} N_{10}
	516.76604	1.3	0.7	21.5	C_{47} H_{69} O_{11} N_{16}
	516.76580	1.8	0.9	15.5	C_{47} H_{77} O_{10} N_{12} S_{2}
	516.76772	-2.0	-1.0	16.5	C_{44} H_{73} O_{11} N_{16} S_{1}
	516.76773	-2.0	-1.0	11.0	C_{45} H_{79} O_{16} N_{9} S_{1}
5 ppm (23)	516.76805	-2.6	-1.3	25.5	C_{52} H_{69} O_{9} N_{14}
	516.76537	2.6	1.3	16.5	C_{46} H_{73} O_{15} N_{12}
	516.76807	-2.6	-1.4	7.0	C_{38} H_{79} O_{14} N_{15} S_{2}
	516.76513	3.0	1.6	10.5	C_{46} H_{81} O_{14} N_{8} S_{2}
	516.76513	3.1	1.6	16.0	C_{45} H_{75} O_{9} N_{15} S_{2}
	516.76839	-3.3	-1.7	16.0	C_{46} H_{75} O_{12} N_{13} S_{1}
	516.76479	3.7	1.9	20.0	C_{52} H_{75} O_{11} N_{9} S_{1}
	516.76872	-3.9	-2.0	25.0	C_{54} H_{71} O_{10} N_{11}
	516.76470	3.9	2.0	17.0	C_{44} H_{71} O_{14} N_{15}
	516.76874	-3.9	-2.0	6.5	C_{40} H_{81} O_{15} N_{12} S_{2}
	516.76446	4.3	2.2	11.0	C_{44} H_{79} O_{13} N_{11} S_{2}
	516.76897	-4.4	-2.3	12.5	C_{40} H_{73} O_{16} N_{16}
	516.76907	-4.6	-2.4	15.5	C_{48} H_{77} O_{13} N_{10} S_{1}

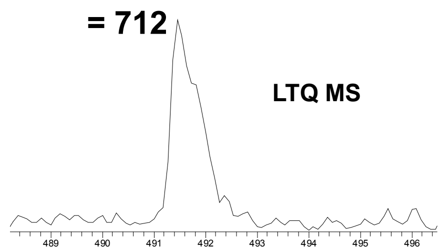
Mass Resulution = m / Δm $_{50\%}$



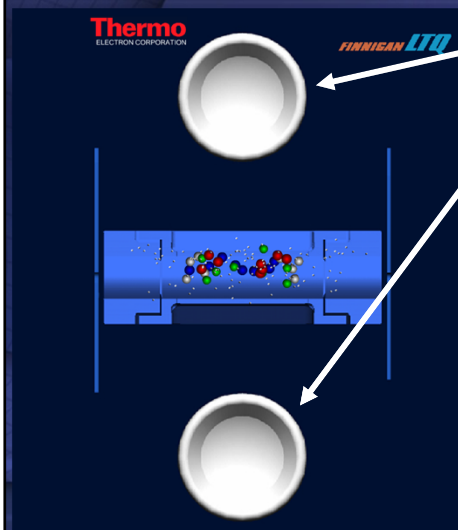
RP = 491.2594 / 0.0055 amu
= 89,319

RP = 491.45 / 0.69 amu
= 712



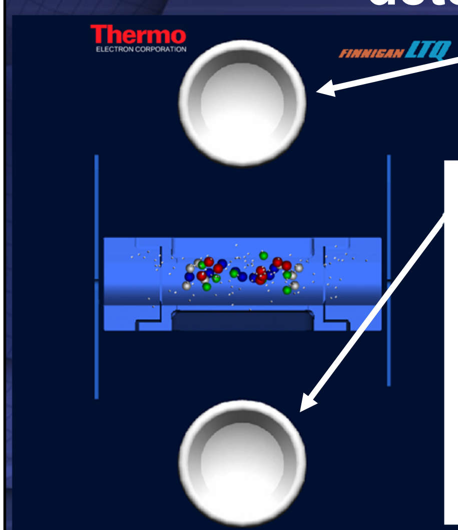


2D ion trap detection

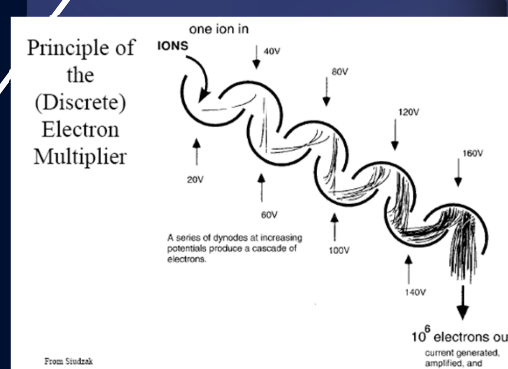


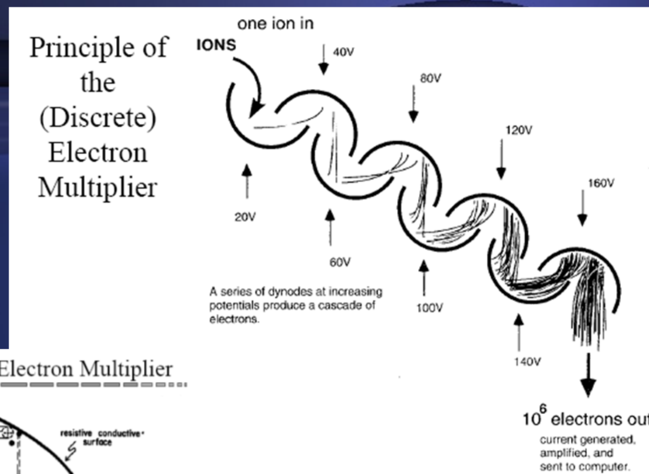
Conversion dynodes

2D ion trap detection



Conversion dynodes (electron multipliers)





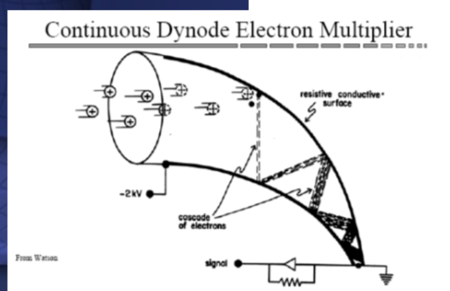
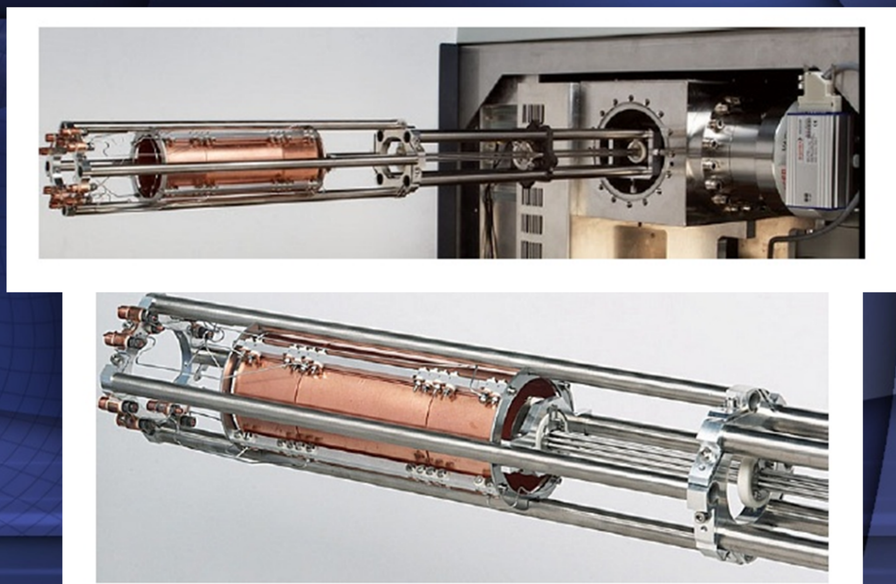


FIG. 13.3. Conceptual diagram of a nonmagnetic electron multiplier; the field gradient along the resistive conductive internal surface of the cornucopia attracts the cascading electrons toward the preamplifier.

Put the trap in a high magnetic field
Ion cyclotron resonance

Penning Trap (ICR cell)



Penning Trap (ICR cell)

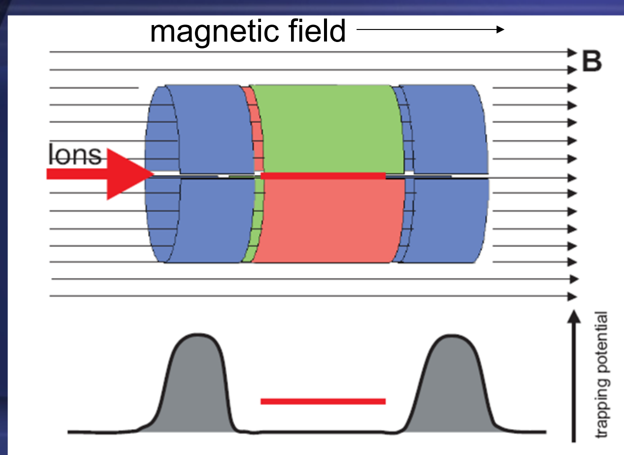
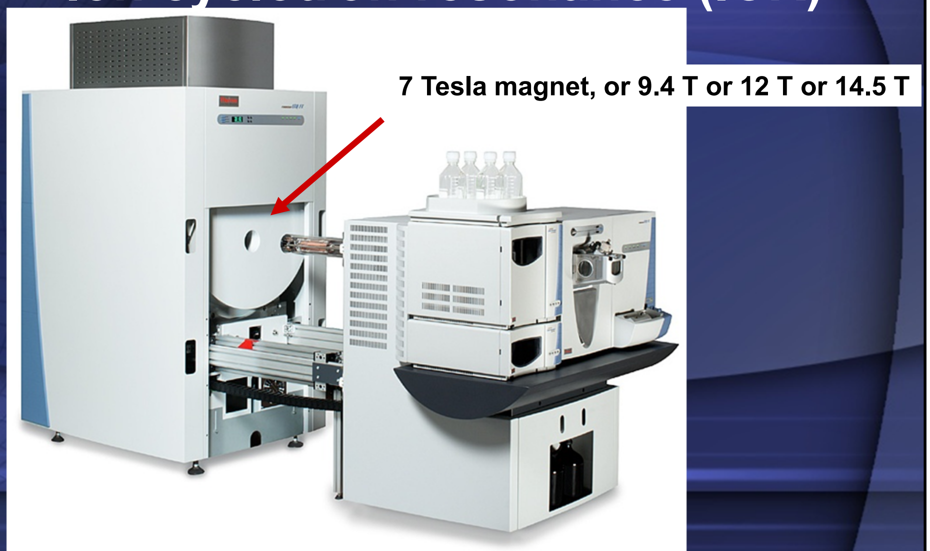
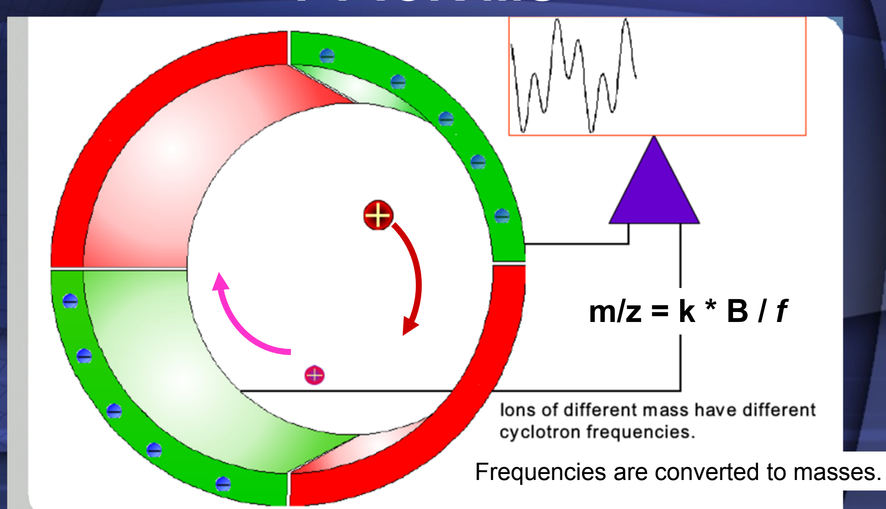


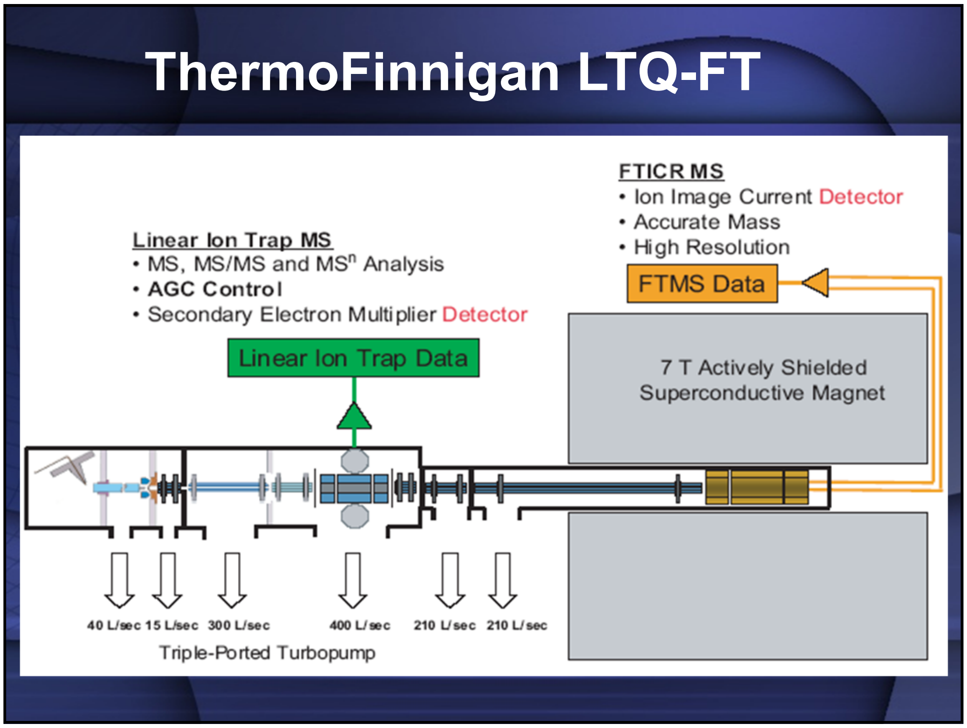
Figure 1-14. Operating principle of the Penning trap

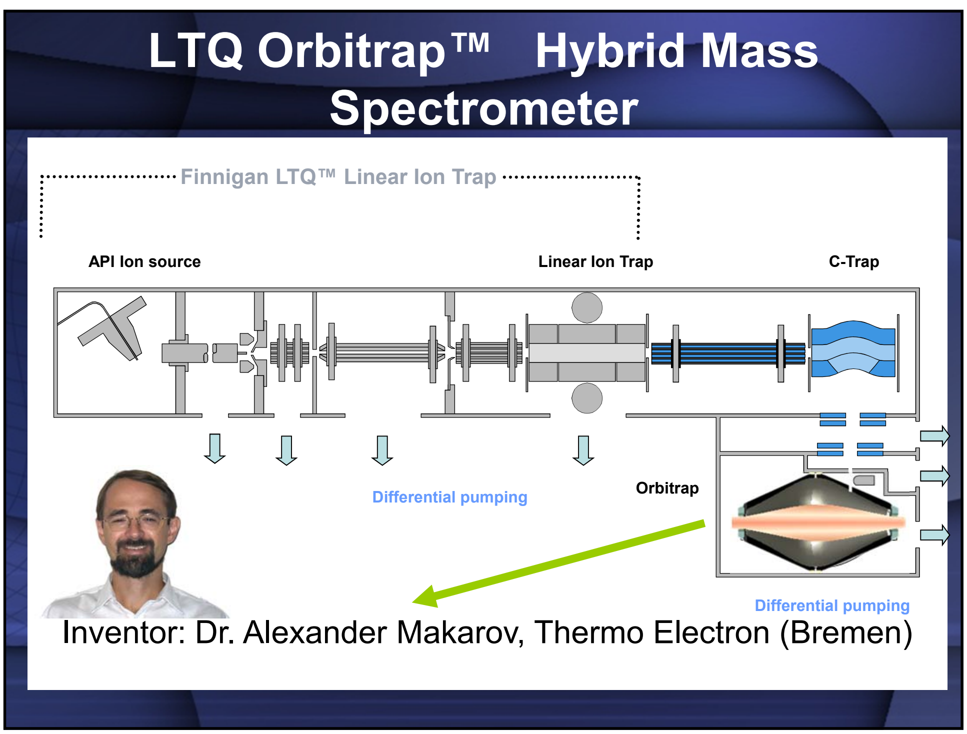
Detecting in the ion trap
Ion cyclotron resonance (ICR)



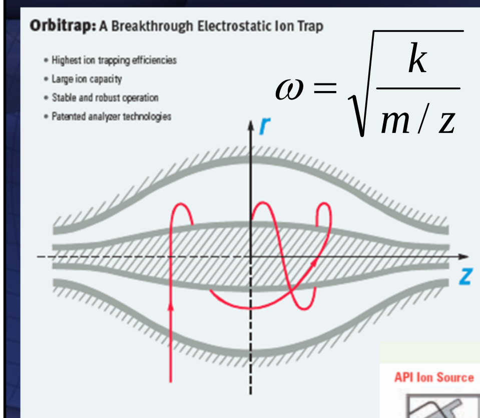
Fourier transform-
Ion cyclotron resonance
FT-ICR MS

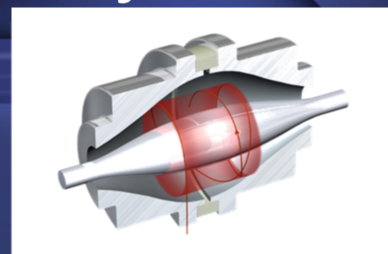


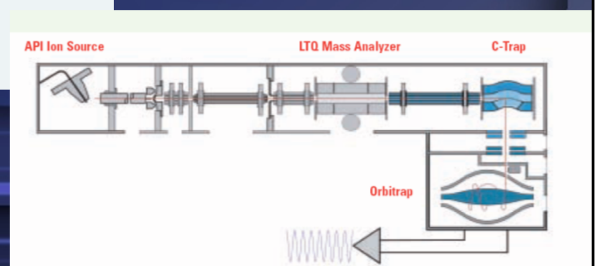

ThermoFinnigan LTQ-FT
FTICR MS
• Ion Image Current Detector
• Accurate Mass
• High Resolution
Linear Ion Trap MS
• MS, MS/MS and MSⁿ Analysis
• AGC Control
• Secondary Electron Multiplier Detector
FTMS Data
Linear Ion Trap Data
7 T Actively Shielded Superconductive Magnet
40 L/sec 15 L/sec 300 L/sec 400 L/sec 210 L/sec 210 L/sec
Triple-Ported Turbopump


LTQ Orbitrap™ Hybrid Mass Spectrometer
Finnigan LTQ™ Linear Ion Trap
API Ion source
Linear Ion Trap
C-Trap
Orbitrap
Differential pumping
Differential pumping
Inventor: Dr. Alexander Makarov, Thermo Electron (Bremen)

Orbitrap Mass Analyzer







https://www.youtube.com/watch?v=fqfyyravJkA

https://www.youtube.com/watch?v=zJagpUbnv-Y