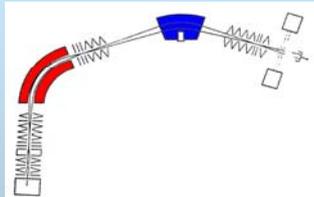
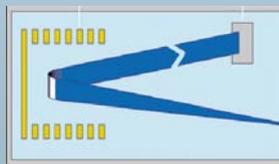
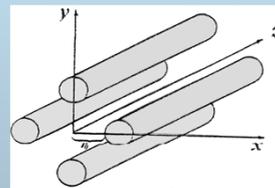


Separazione degli ioni nello spazio



Settori: elettrostatico e magnetico

Quadrupolo



Tempo di volo

$E_{pot} = zeV$
 $E_{cin} = \frac{1}{2} m v^2$

$zeV = \frac{1}{2} m v^2$

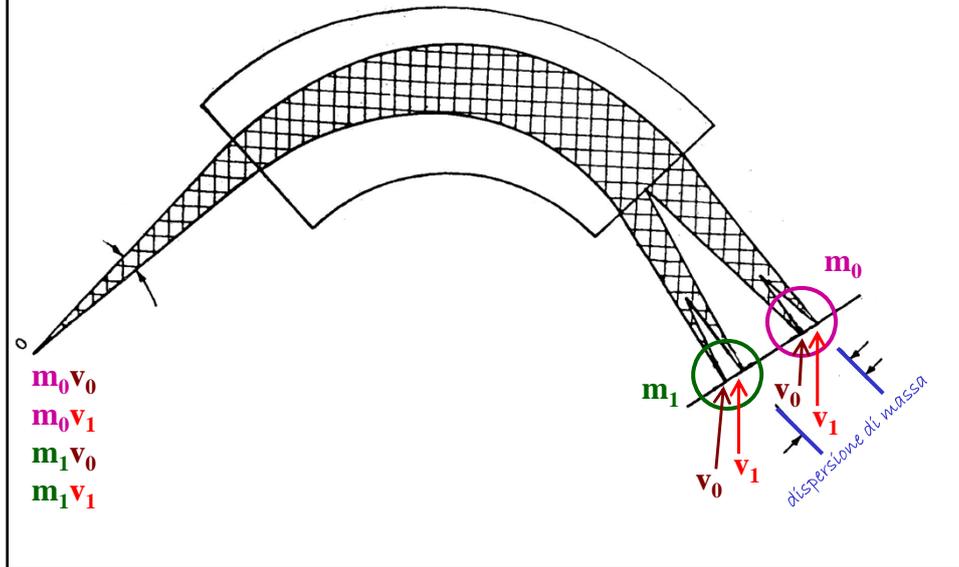
$F. \text{ centripeta} = Bzev$
 $F. \text{ centrifuga} = \frac{m v^2}{r}$

$\frac{m}{ze} = \frac{B^2 r}{2V}$

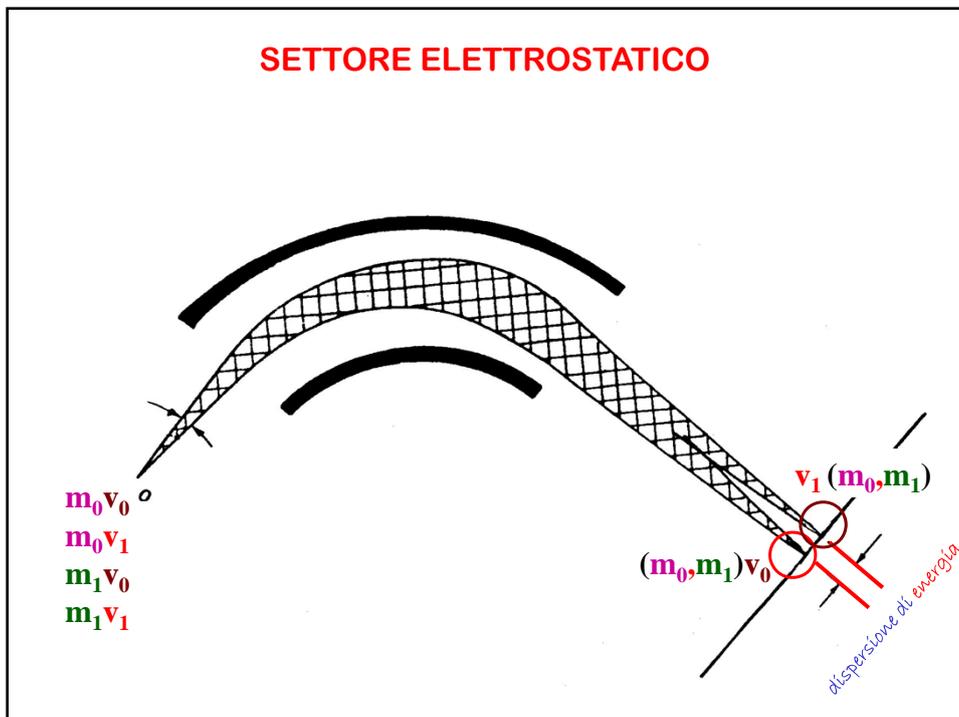
$r^2 \propto \frac{m}{z}$
 (B, V costante)

Campo
 Cornea
 Direzione della deflessione

PRINCIPIO DI FUNZIONAMENTO DI UN SETTORE MAGNETICO



SETTORE ELETTROSTATICO



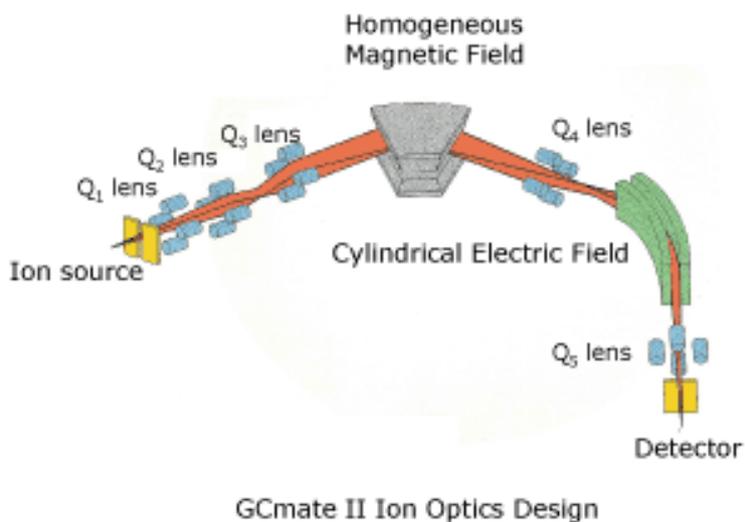
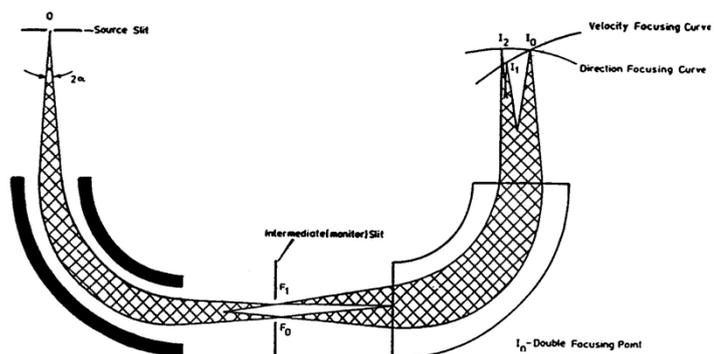
DOPPIO FUOCO

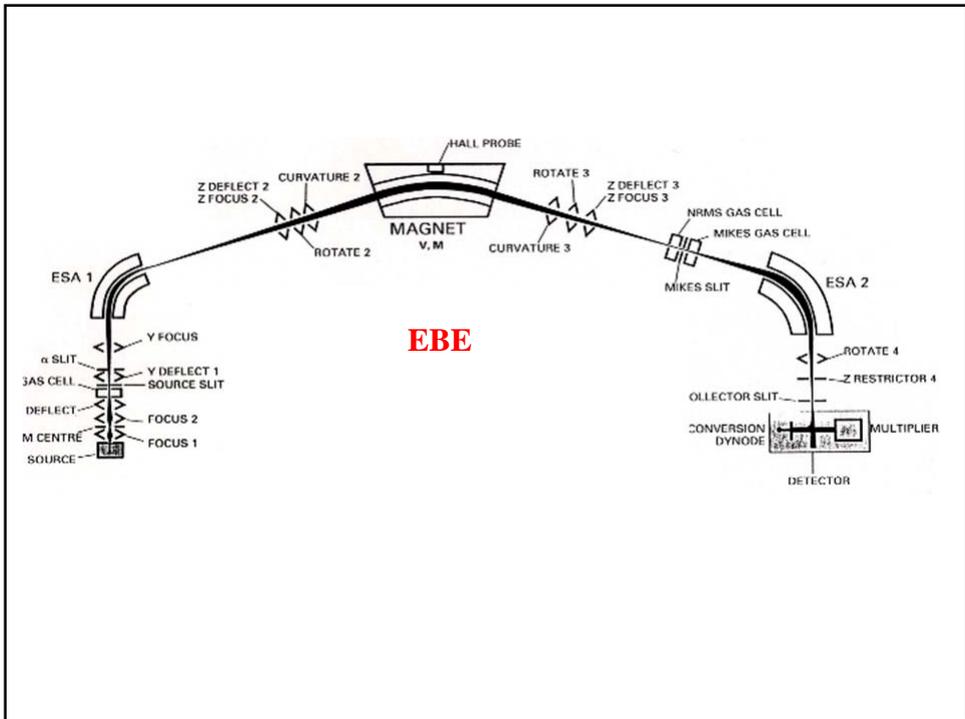
Focalizzazione di un fascio ionico

- **divergente**

e

- **non omogeneo in energia**



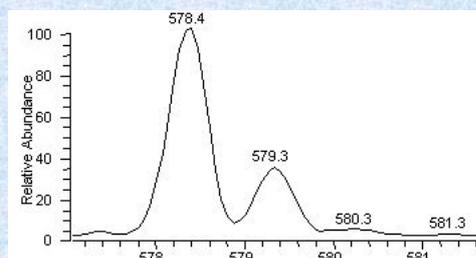




EBEBE

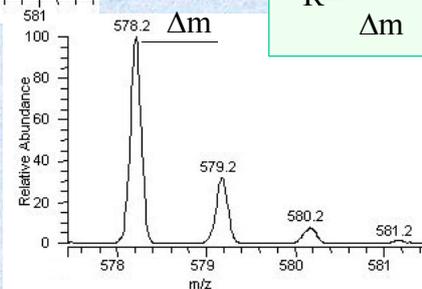
1985-1993

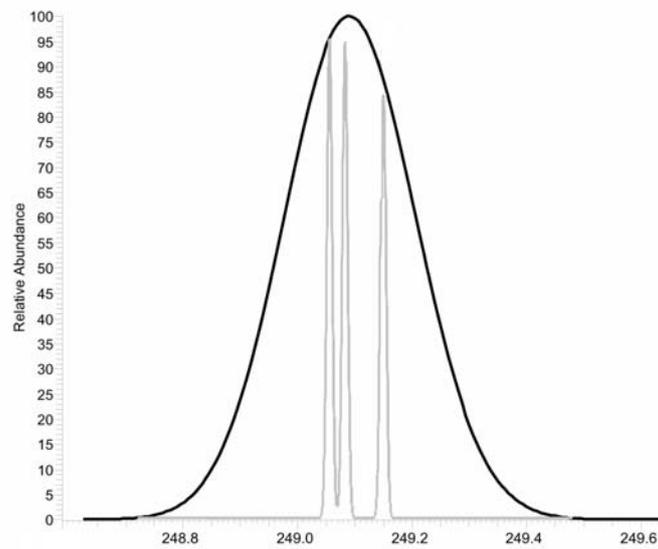
Proprietà degli analizzatori:



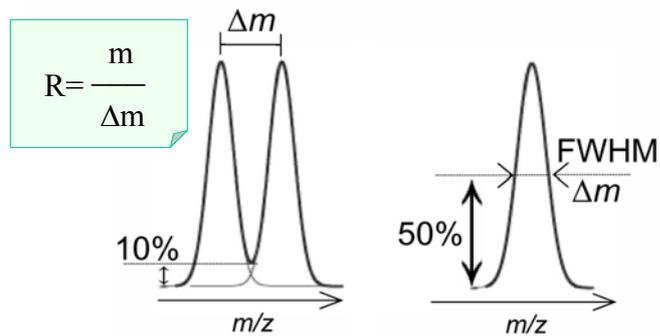
RISOLUZIONE

$$R = \frac{m}{\Delta m}$$





Mass spectra recorded at different resolutions: mass spectrum of a mixture of three isobaric species $[C_{19}H_7N]^+$, $[C_{20}H_9]^+$, $[C_{13}H_{19}N_3O_2]^+$ obtained at low resolution (black line) and at resolving power 50,000 (grey line). It is noteworthy that at low resolution the three peaks are completely unresolved.



Resolution at 10% valley (left) and at full width at half maximum (FWHM, right).

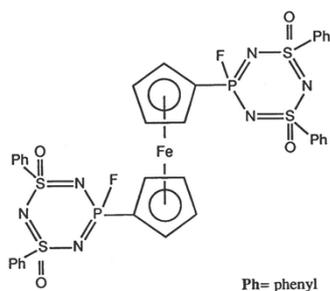
Exact mass

The calculated mass of an ion or molecule containing a single isotope of each atom

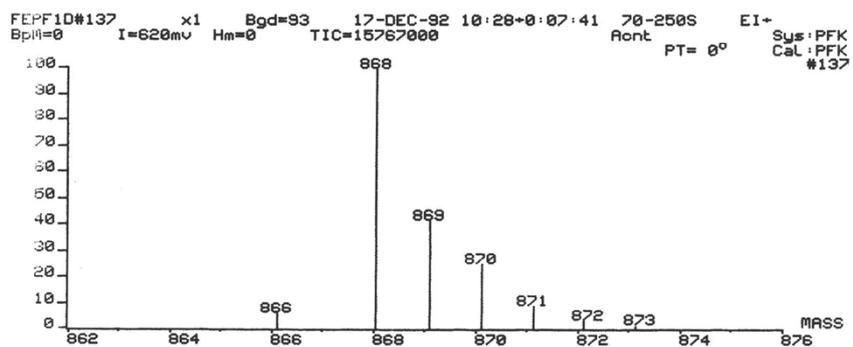
Accurate mass

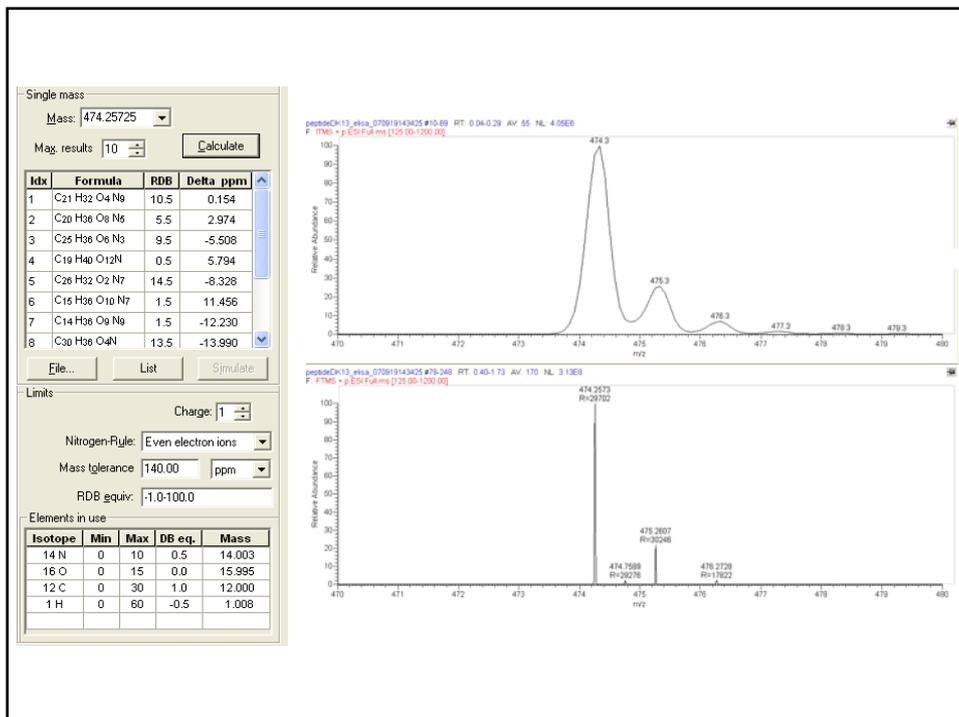
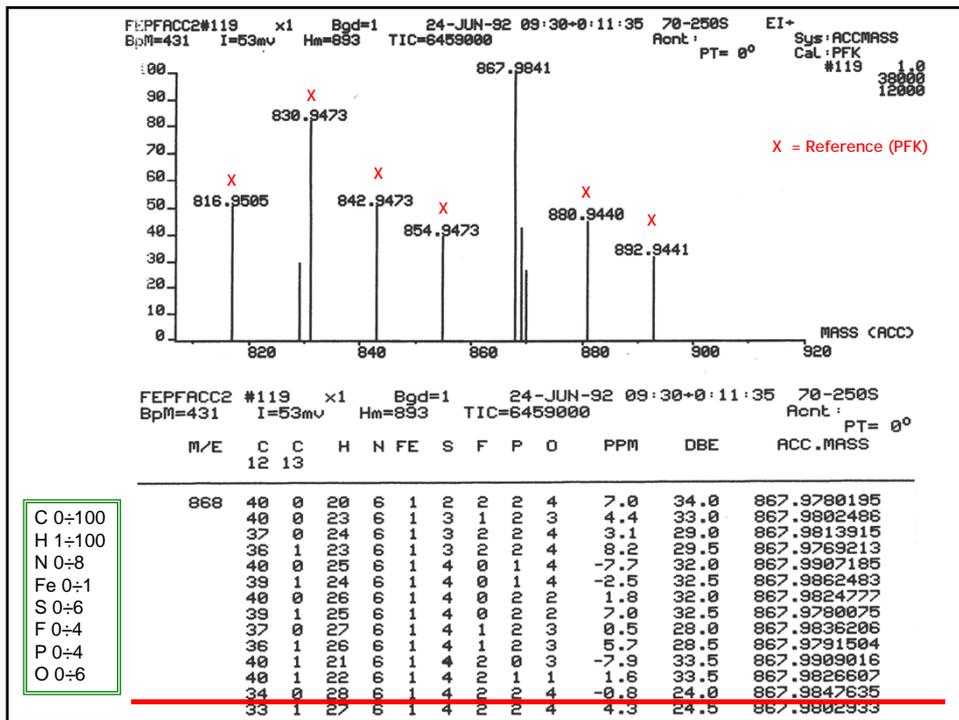
An experimentally determined mass of an ion that is used to determine an elemental formula

Mass Spec Terms Project: http://mass-spec.lsu.edu/msterms/index.php/Category:Mass_spectrometry_terms



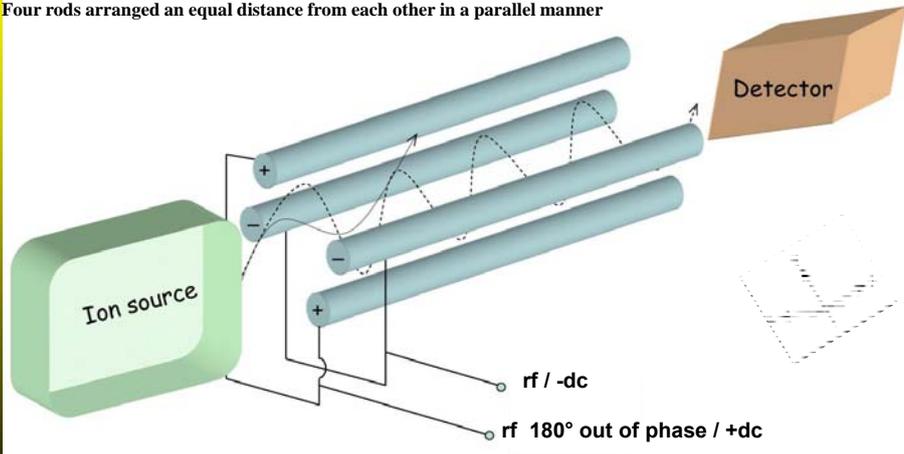
VG 70-250S
m/z 900-200
10 sec/dec
Res=8.000 (10% valley)



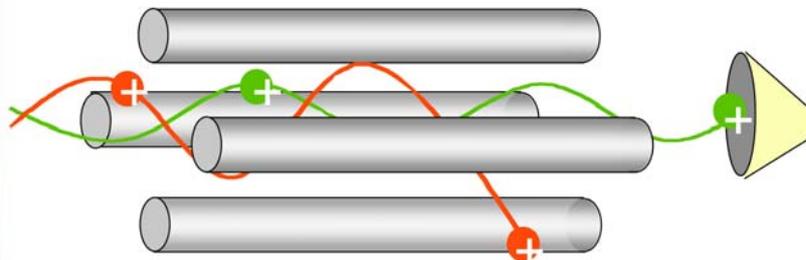


Analizzatori: il quadrupolo

Four rods arranged an equal distance from each other in a parallel manner

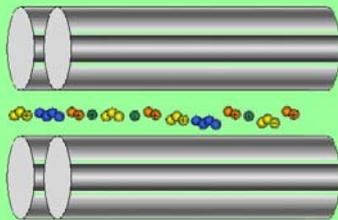


Quadrupole Mass Analyser

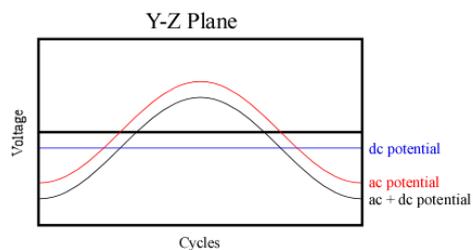
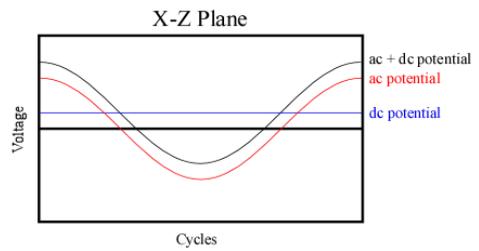
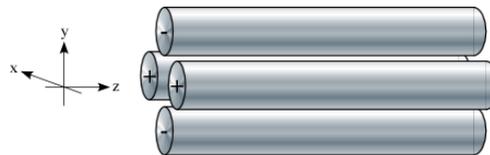


The ● ion is transmitted along the quadrupole in a stable trajectory Rf field. The ● ion does not have a stable trajectory and is ejected from the quadrupole.

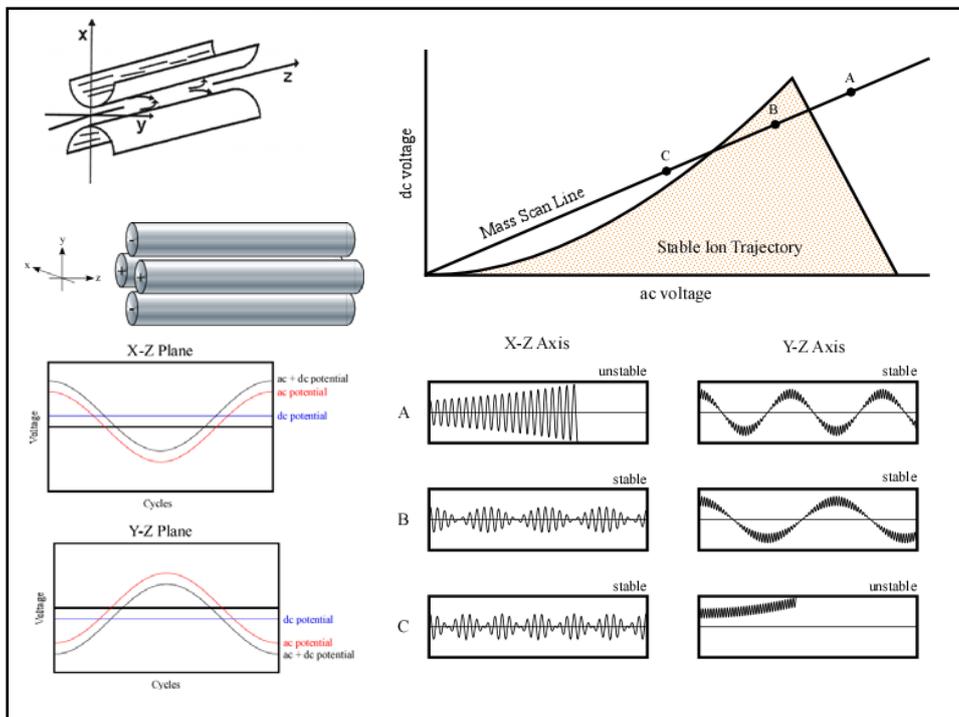
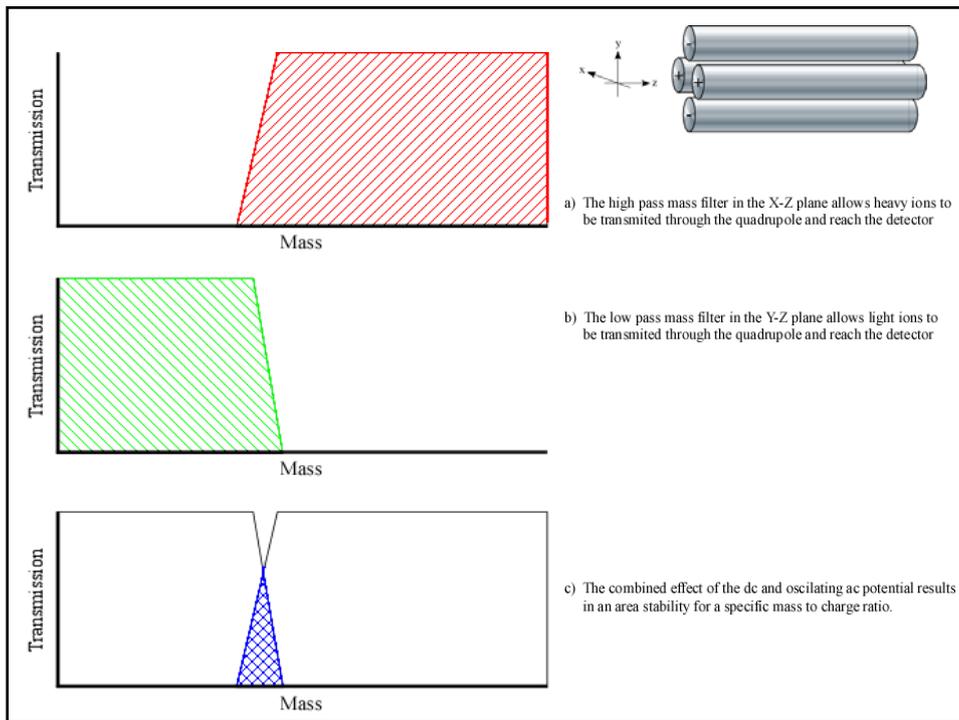
Il movimento degli ioni all'interno del quadrupolo dipende da:



1. Il loro rapporto m/z
2. Le dimensioni del quadrupolo
3. Intensità potenziale dc (U)
4. Intensità potenziale rf (V)
5. La frequenza del potenziale rf

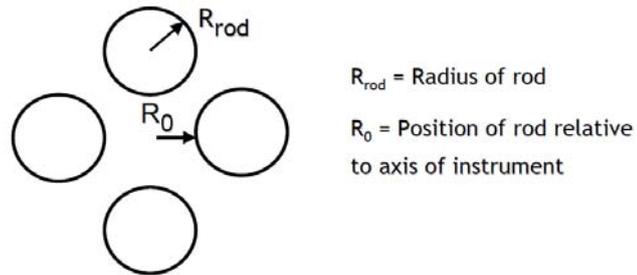


http://people.whitman.edu/~dunnivm/C_MS_Ebook/CH5/5_5_2.html

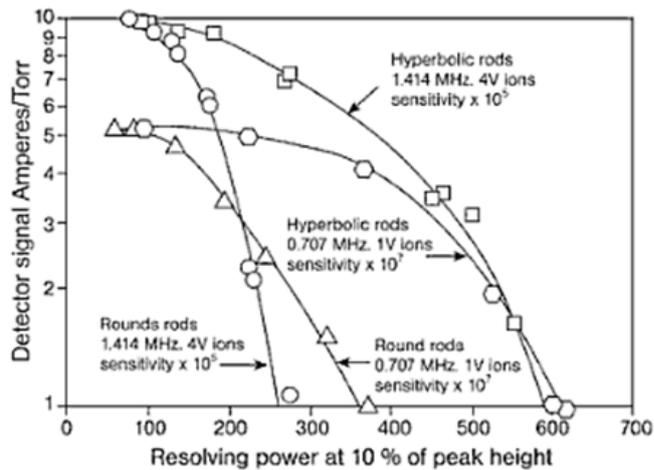


Hyperbolic rods

Round rods can only approximate the field generated by hyperbolic rods - most ideal when $R_{\text{rod}}/R_0 = 1.16$.



Manufacturing such a kind of rods is non-trivial

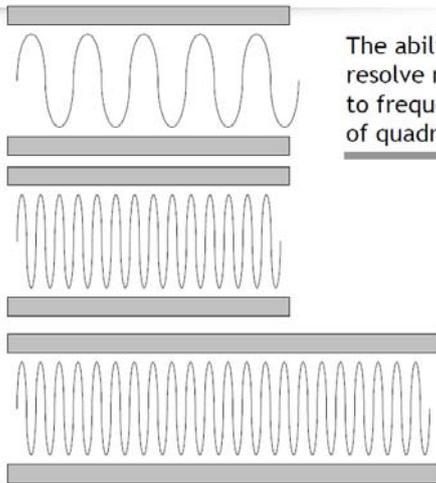


Ion transmission of round and hyperbolic quadrupole systems (resolving power ag. ion transmission), first documented investigation by Brubaker 1968 (two curves left: round rods; two curves right: hyperbolic rods).

Hyperbolic Rods Quadrupoles



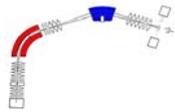
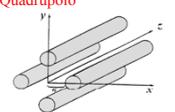
Resolving Power of a quadrupole



The ability of a quadrupole to resolve masses is proportional to frequency of AC and length of quadrupole

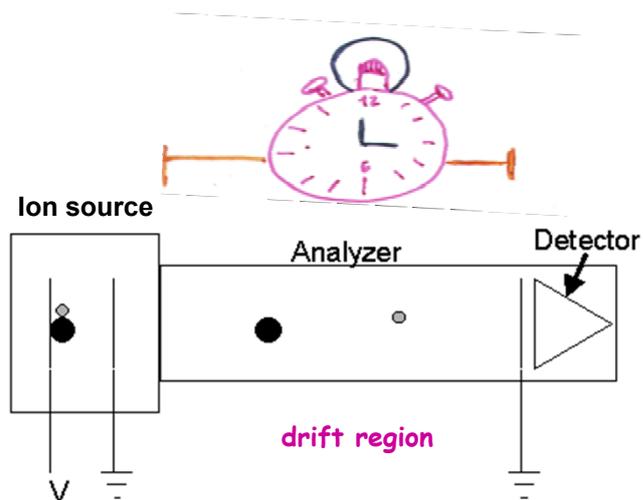
Separazione degli ioni nello spazio



Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
Doppio fuoco (EB, BE) 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000	10.000	< 1ppm
Quadrupolo 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000	Unitaria (0.2 u FWHM)	No (>20ppm)

1946

William Stephens at the Univ. of Pennsylvania proposed the concept of **time of flight**



In an ideal case, all ions leave the source with the same kinetic energy:

$$eV = \frac{1}{2}mv^2$$

where V = accelerating voltage, v = ion velocity.

As

$$v = \frac{D}{t}$$

where D = drift length and t = the flight time

$$t = \left(\frac{m}{2eV} \right)^{1/2} D$$

MW = 1000 Dalton; z=+1; D=1.5m; V=15 kV

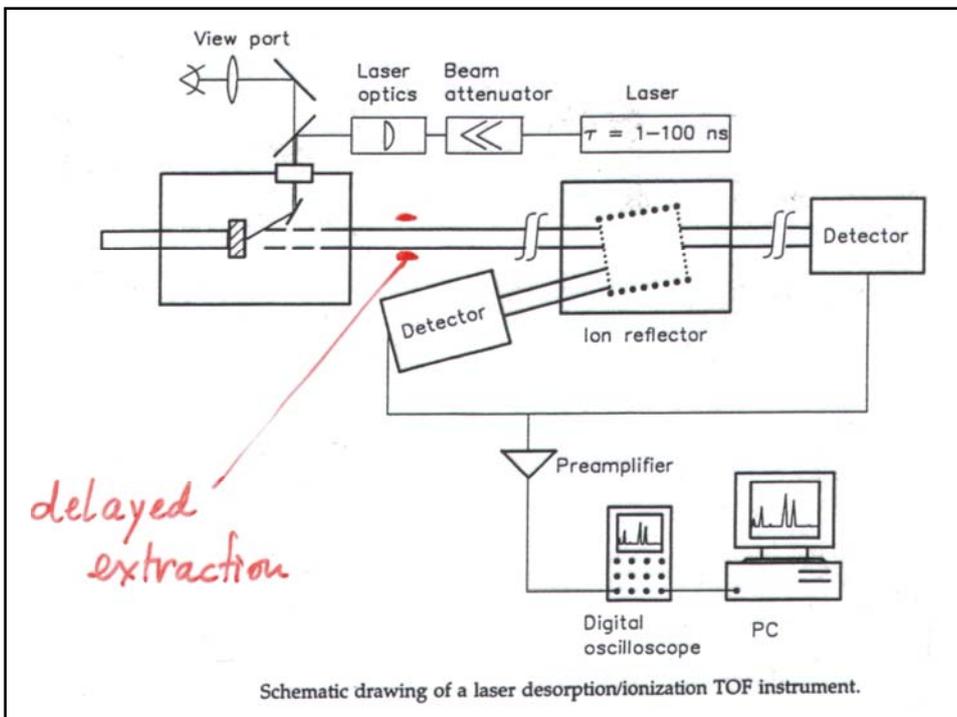
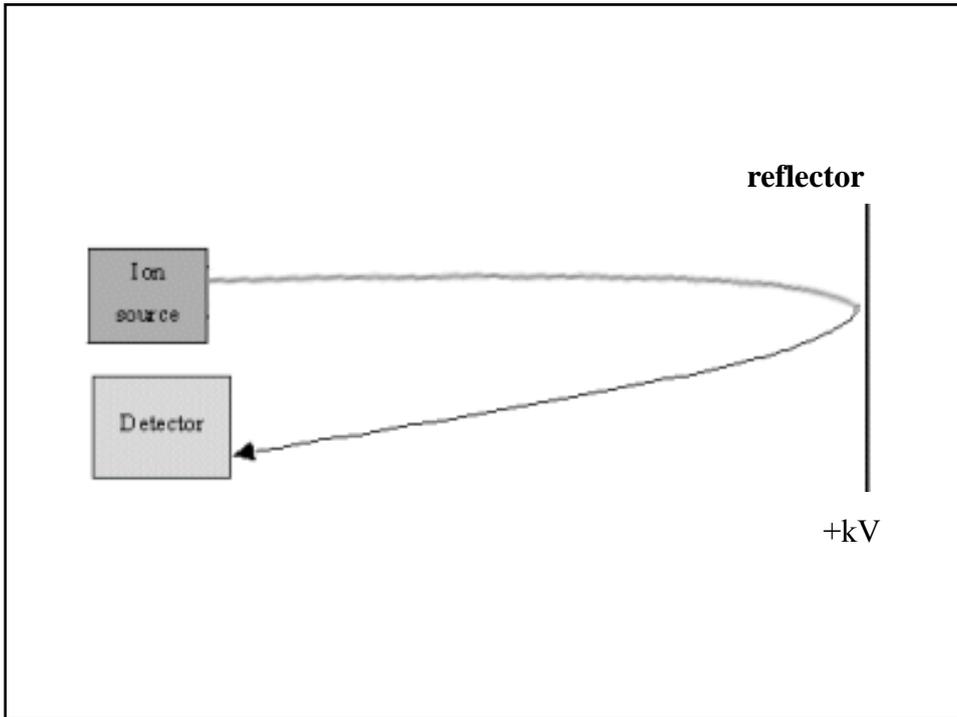
$$t = \left(\frac{m}{2eV} \right)^{1/2} D$$

$$t = \sqrt{\frac{1000\text{Da} \times 1.672621 \times 10^{-27} \text{ kg Da}^{-1}}{2 \times 15000 \text{ V} \times 1.602 \times 10^{-19} \text{ C}}} \times 1.5 \text{ m} = 2.792 \times 10^{-5} \text{ s} \approx 28 \mu\text{s}$$

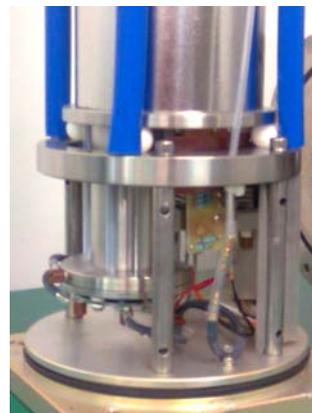
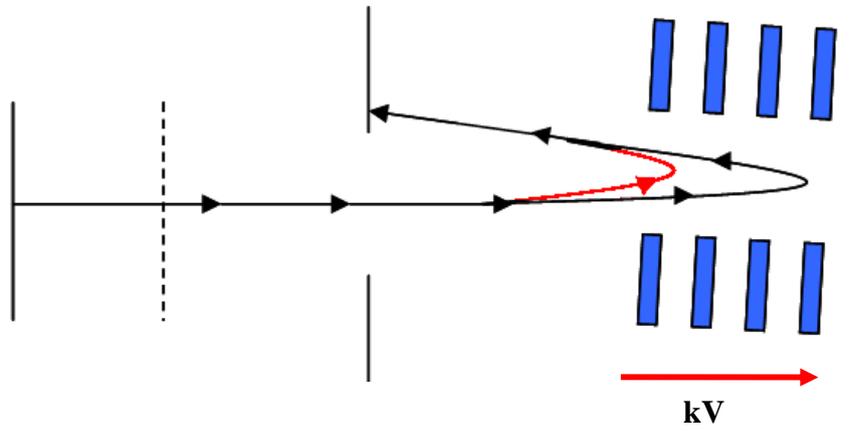
MW = 10000 Dalton V=15 kV t ≈ 88 μs

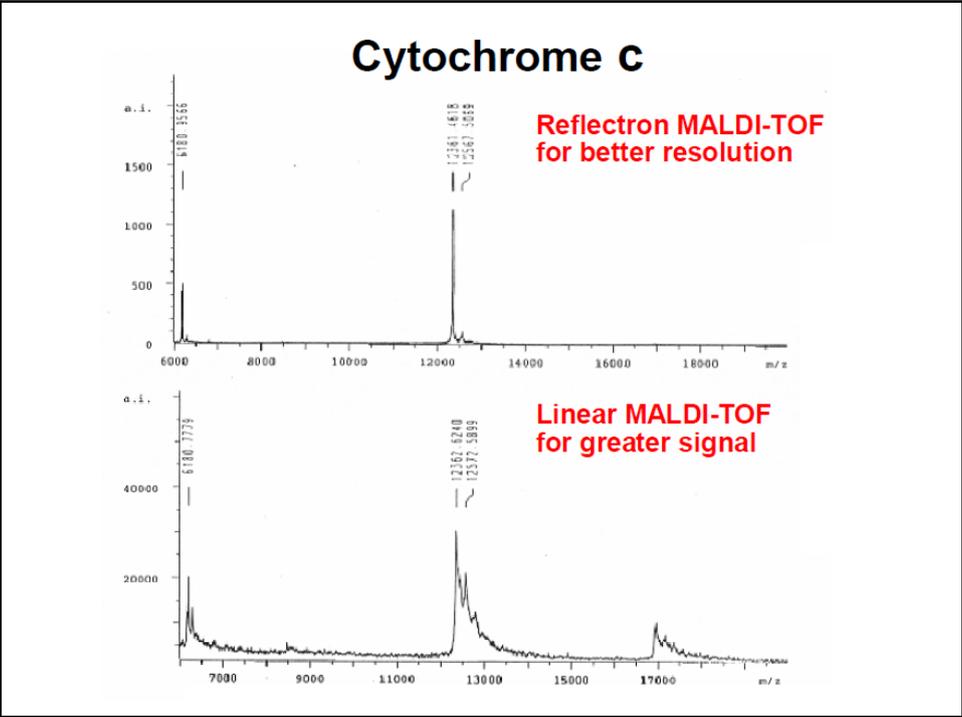
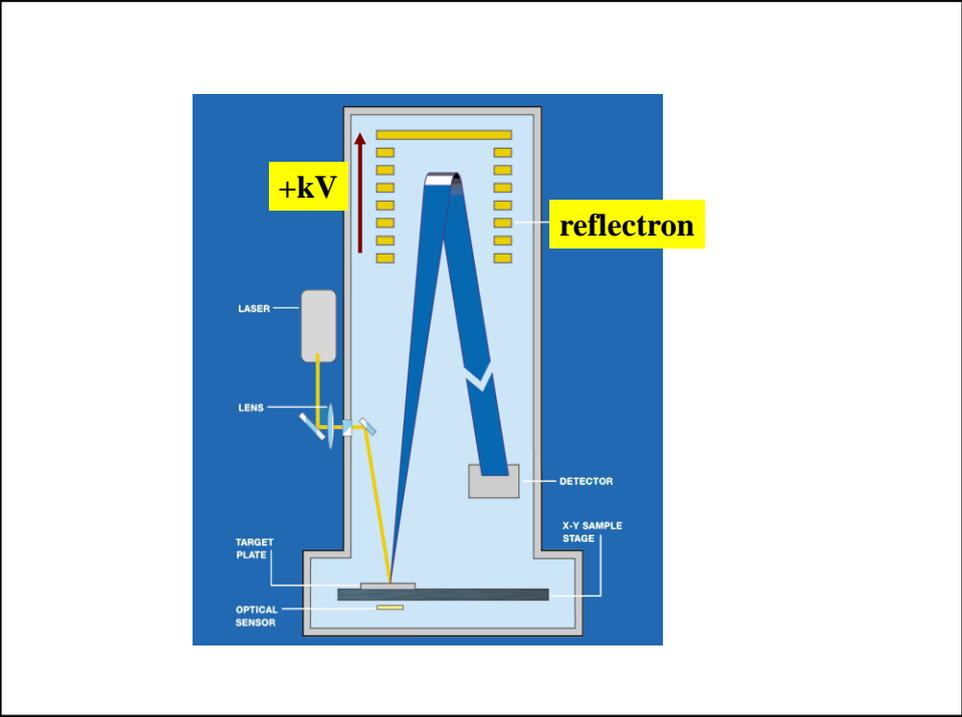
MW = 10000 Dalton V=30 kV t ≈ 63 μs

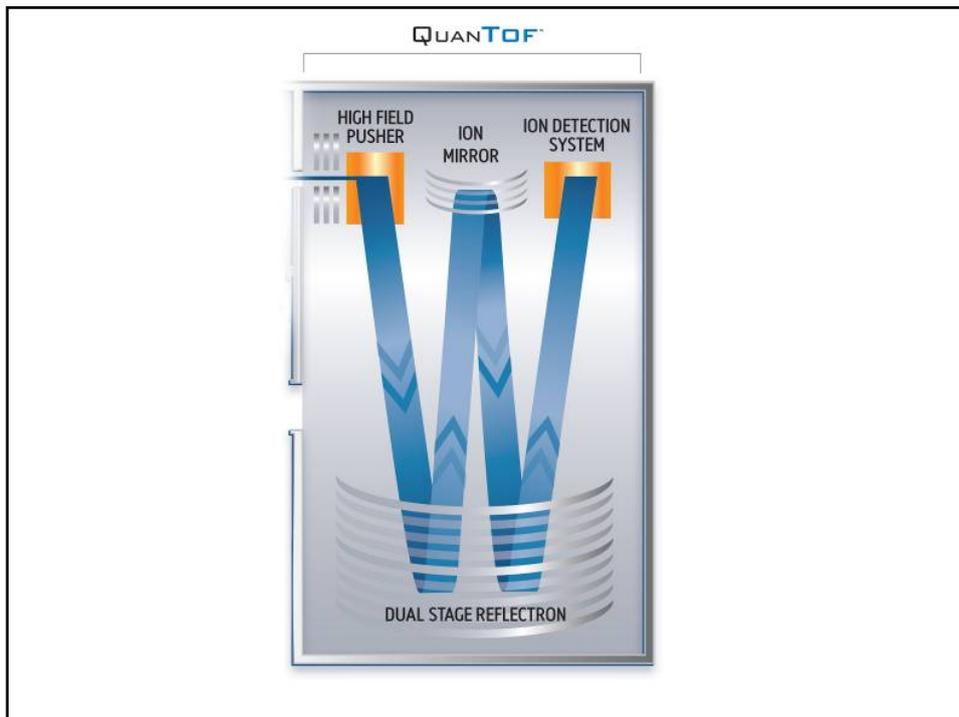
MW = 100000 Dalton V=30 kV t ≈ 20 μs



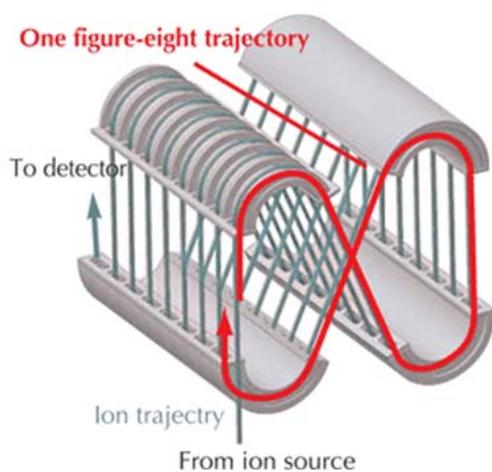
Reflectron







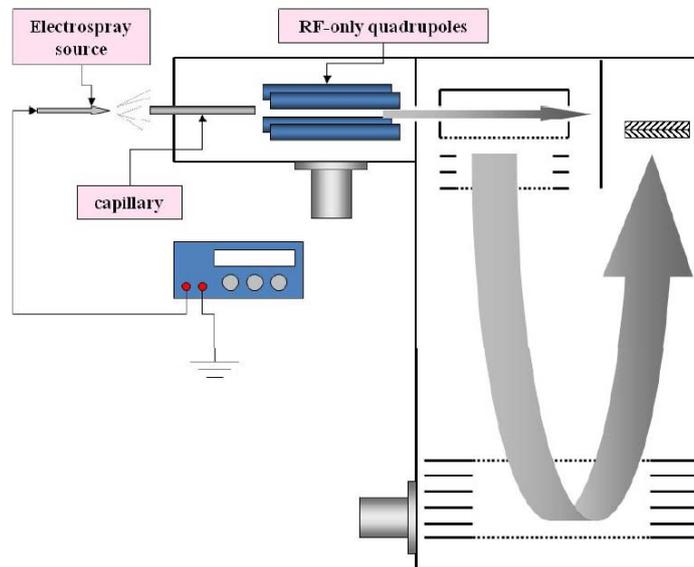
Spiral TOF



The spiral path is composed of 4 layered toroidal electrodes each incorporating 9 Matsuda plates inside its cylindrical electrode. Ions are accelerated to 20 kV in the ion source, and then pass through each layer of the 4 layered toroidal electrodes, reaching the detector.

Ion path = 17m

oa-TOF with an ion guide and reflectron



Separazione degli ioni nello spazio



Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
 Doppio fuoco (FB RF) 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000	10.000	< 1ppm
Quadrupolo 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000	Unitaria (0.2 u FWHM)	No (>20ppm)
Time of flight 		Velocità	>100.000	>10.000	2-5 ppm

Separazione degli ioni

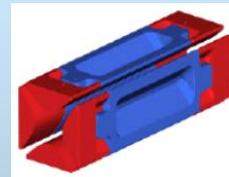
..... nel tempo



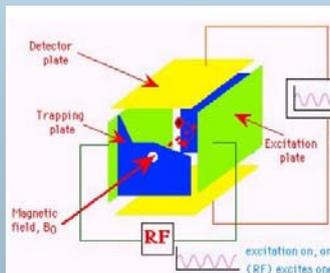
Separazione degli ioni nel tempo



Trappola ionica 3D

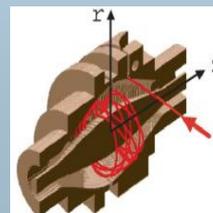


2D



Cella a risonanza ciclotronica

Orbitrap



The Nobel Prize in Physics 1989

The Royal Swedish Academy of Sciences has awarded this year's Nobel Prize in Physics for contributions of importance for the development of atomic precision spectroscopy



Hans Dehmelt
University of Washington
Seattle, USA

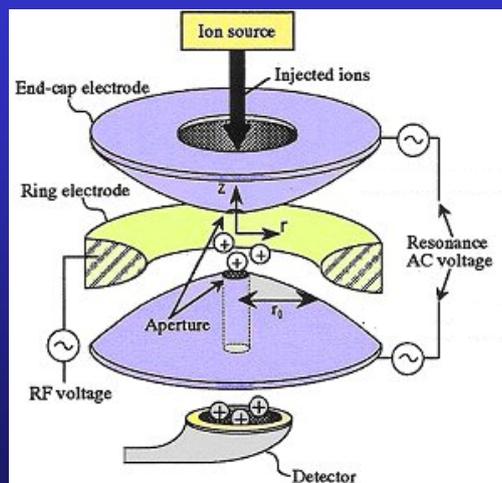
Wolfgang Paul
Universität Bonn
Federal Republic of Germany

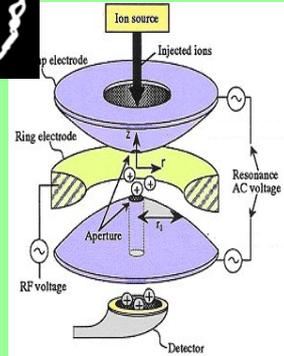
Norman F. Ramsey
Harvard University
Cambridge, USA

for the development of the ion trap technique

for the invention of the separated oscillatory fields method and its use in the hydrogen maser and other atomic clocks

La trappola ionica





Il movimento degli ioni all'interno della trappola dipende da:

1. Il loro rapporto m/z
2. Le dimensioni della trappola (r)
3. Il potenziale dc (U) (elett. anello)
4. Il potenziale rf (V) (elett. anello)
5. La frequenza Ω del potenziale (elett. anello)

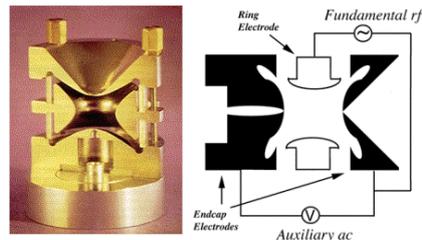
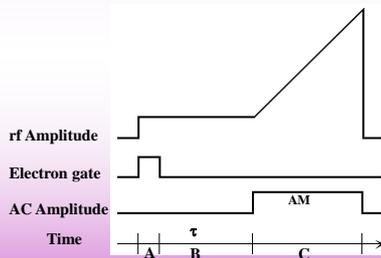
$$a_u = \frac{8eU}{mr_0^2 \Omega^2}$$

$$q_u = \frac{4eV}{mr_0^2 \Omega^2}$$

Equazione di Mathieu

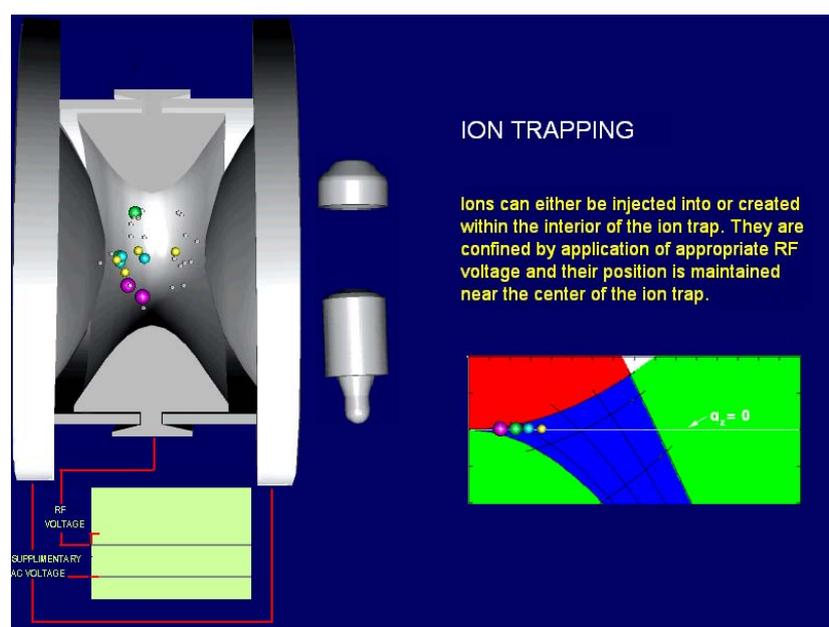
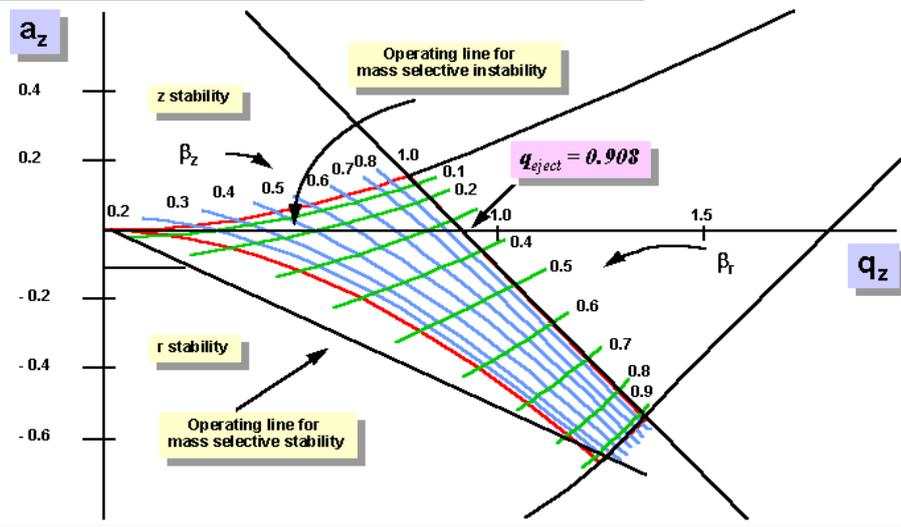
Analizzatori:

La trappola ionica



Separazione degli ioni nel tempo

Mathieu Stability Diagram



L'elio, presente all'interno della trappola ionica, aiuta a confinare gli ioni al centro

RF VOLTAGE

SUPPLEMENTARY AC VOLTAGE

$a_z = 0$

The diagram shows a cross-section of an ion trap with a central ion cloud and surrounding helium gas. A stability diagram on the right shows the ion's position in the $a_z = 0$ plane. Below the trap, a control panel shows the RF voltage and supplementary AC voltage levels.

SEQUENTIAL MASS SCANNING

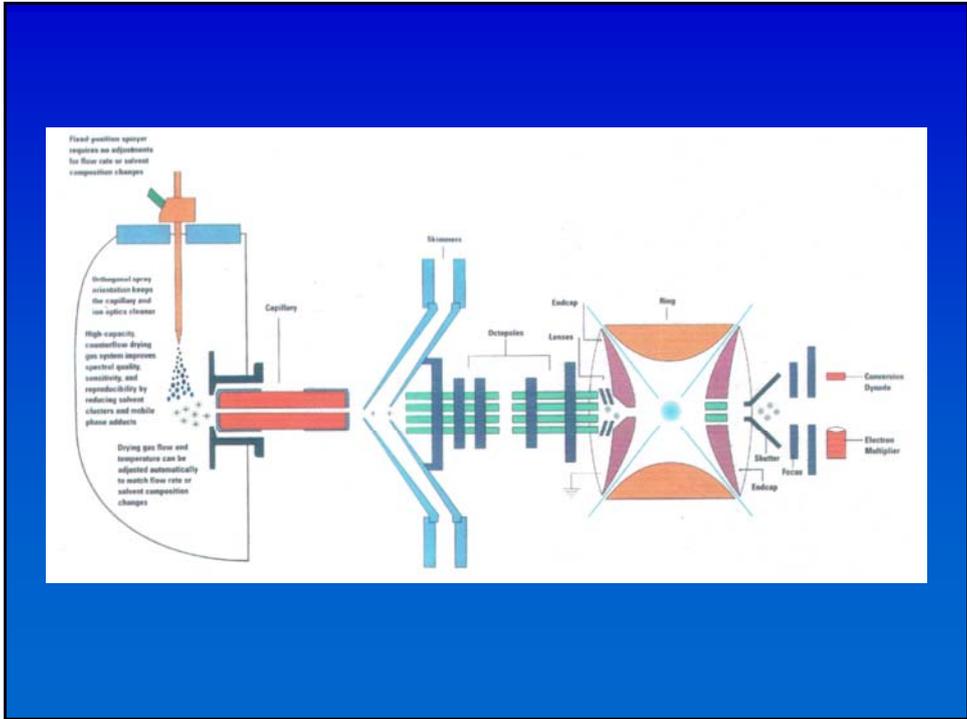
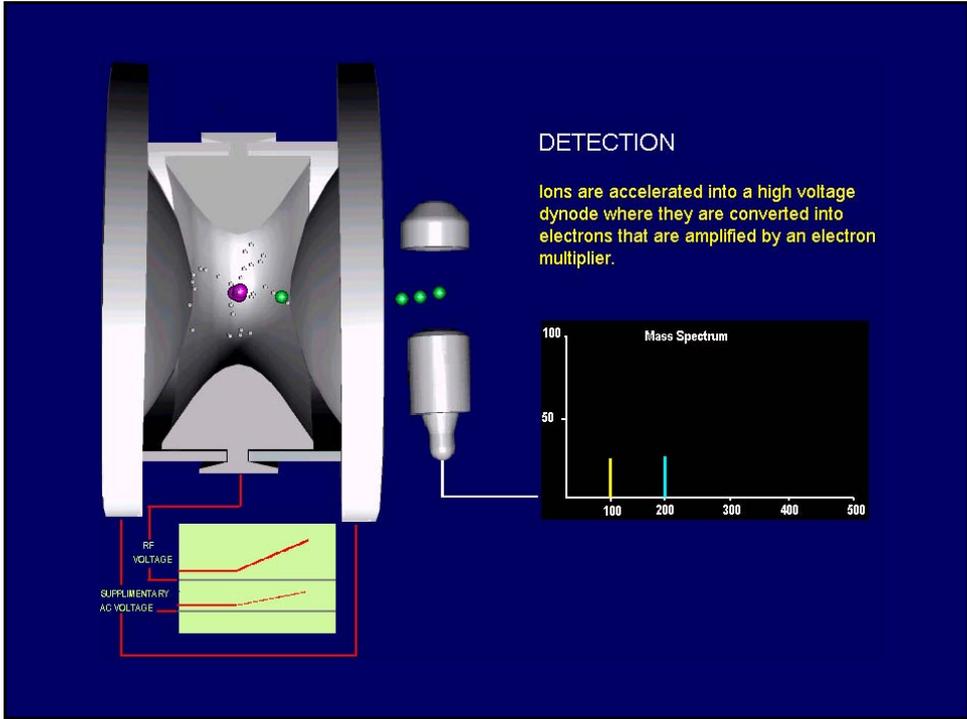
Scanning is accomplished by changing the RF voltage applied in the ring electrode of the ion trap. This change moves the ions in the stability diagram to a position where they become unstable in the z-direction and leave the trapping field for detection.

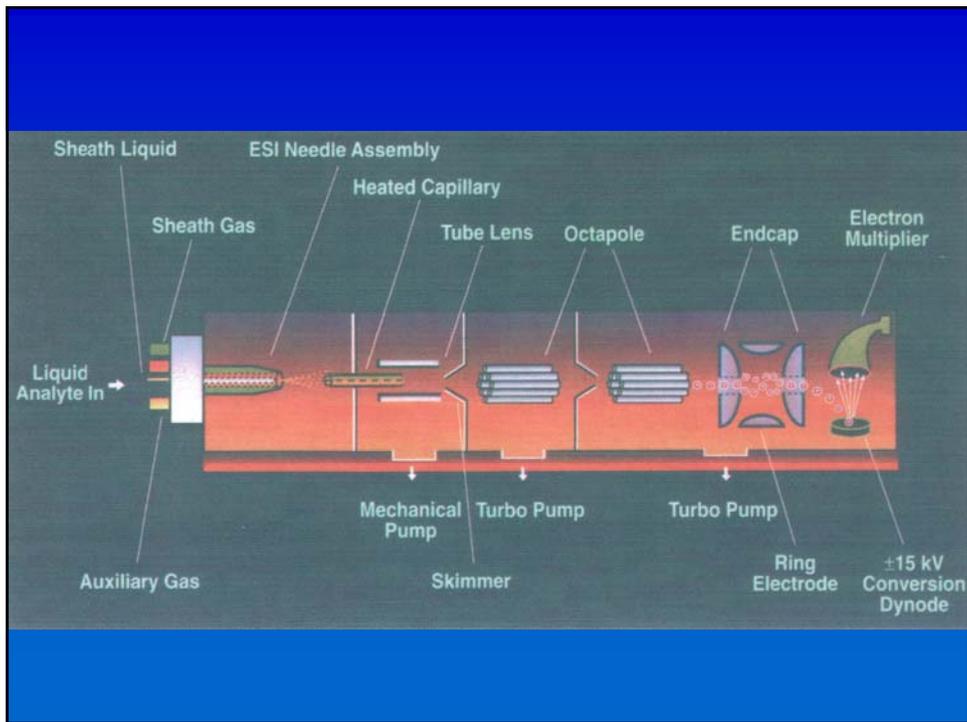
RF VOLTAGE

SUPPLEMENTARY AC VOLTAGE

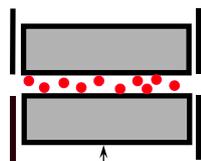
$a_z = 0$

The diagram illustrates the process of sequential mass scanning. The ion cloud is shown moving towards the ring electrode as the RF voltage is changed. The stability diagram shows the ion's position moving towards the edge of the stable region, where it becomes unstable and is ejected for detection. The control panel shows the RF voltage and supplementary AC voltage levels.

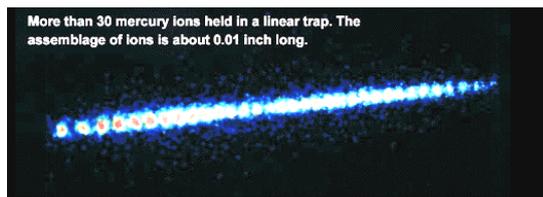
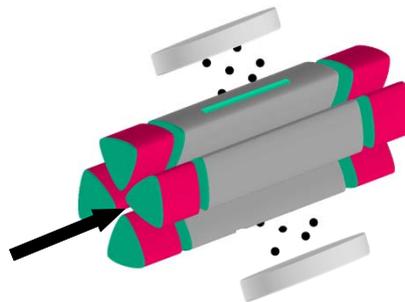




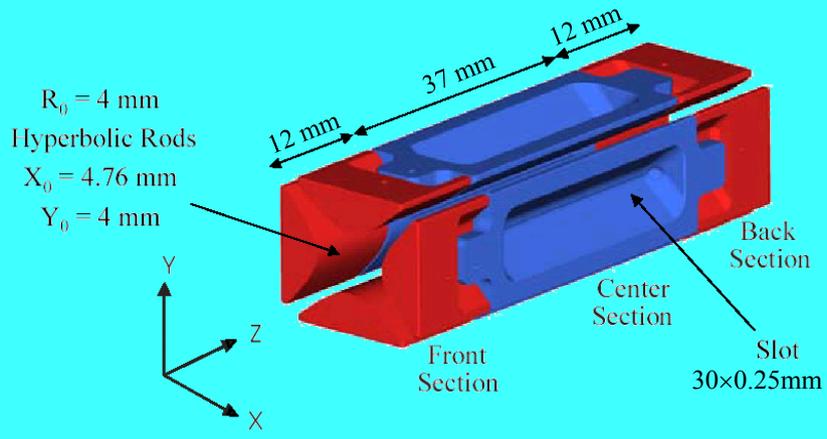
Trappola ionica lineare (2D)



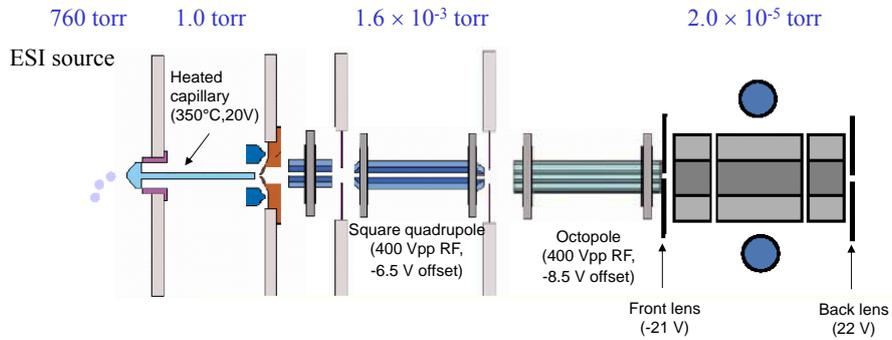
Drive RF &
auxiliary AC



Basic Linear Trap Structure

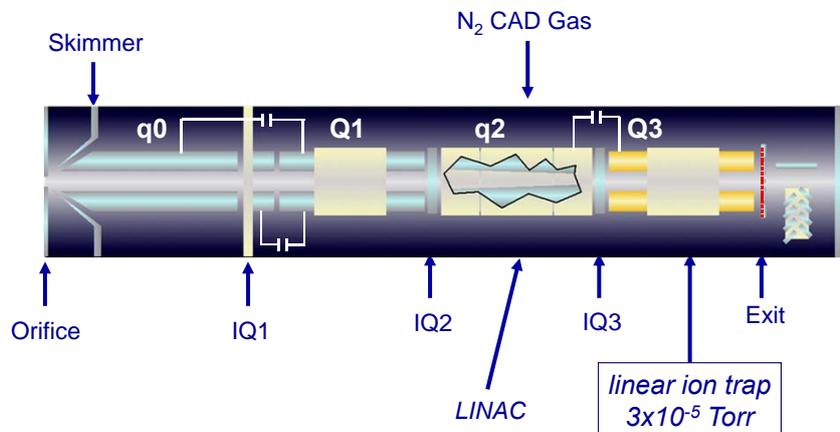


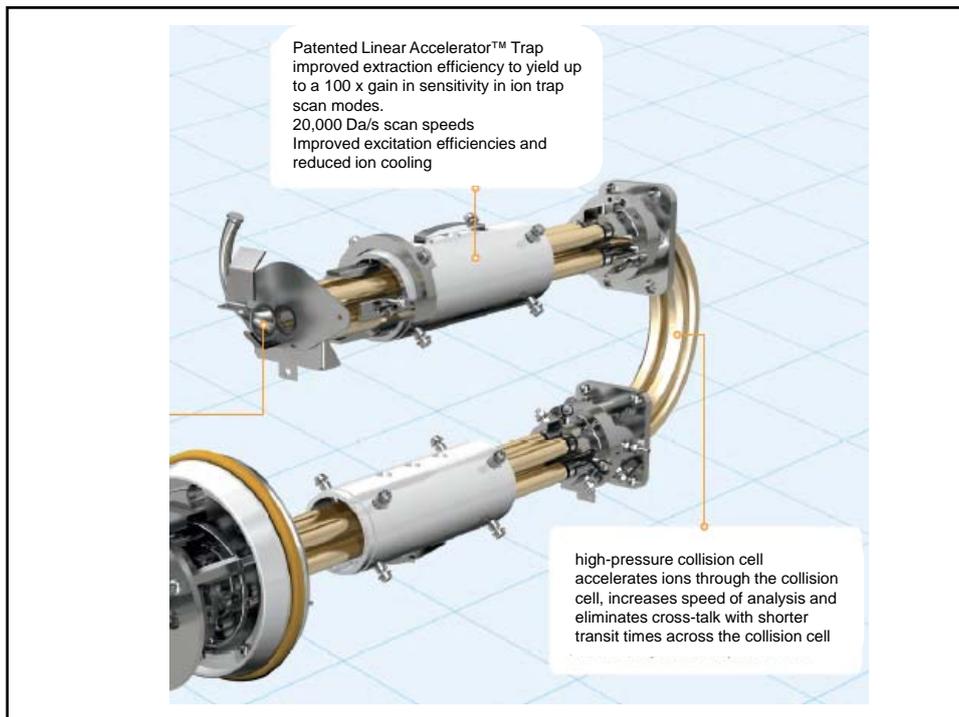
Linear Ion Trap



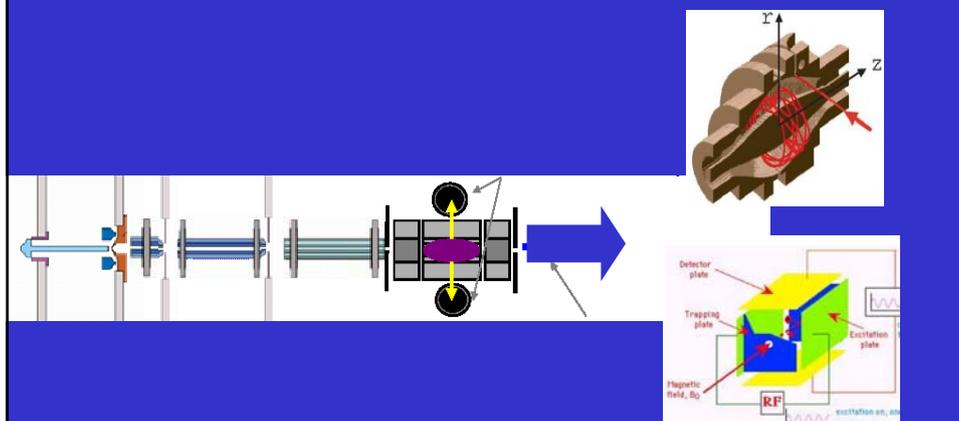
Analizzatori ibridi QqIT

Hybrid Instrument with Q3 Linear Ion Trap



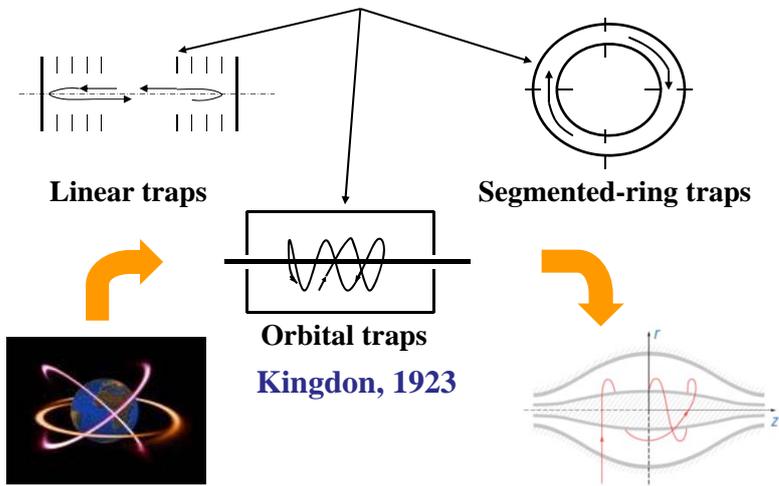


L'espulsione ASSIALE degli ioni permette di accoppiare la trappola ionica lineare ad altri analizzatori



Electrostatic Traps

"...Static charges can not be stable in electrostatic fields..."



The Orbitrap: a new mass spectrometer

Qizhi Hu,^a Robert J. Noll,^a Hongyan Li,^a Alexander Makarov,^b Mark Hardman^c and R. Graham Cooks^{a}*

^a Purdue University, Chemistry Department, West Lafayette, IN 47907, USA

^b Thermo Electron (Bremen), Hanna-Kunath-Str. 11, Bremen 28199, Germany

^c Thermo Electron (San Jose) 355 River Oaks Parkway, San Jose, CA 95134 USA

JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2005; **40**: 430–443



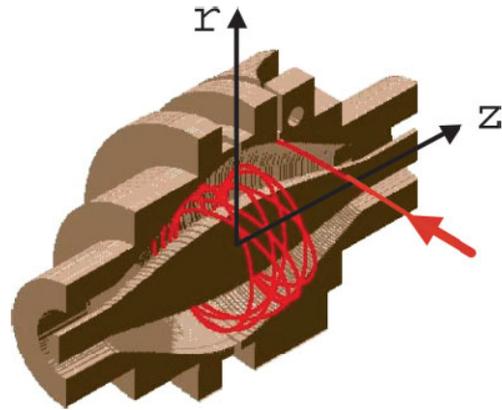
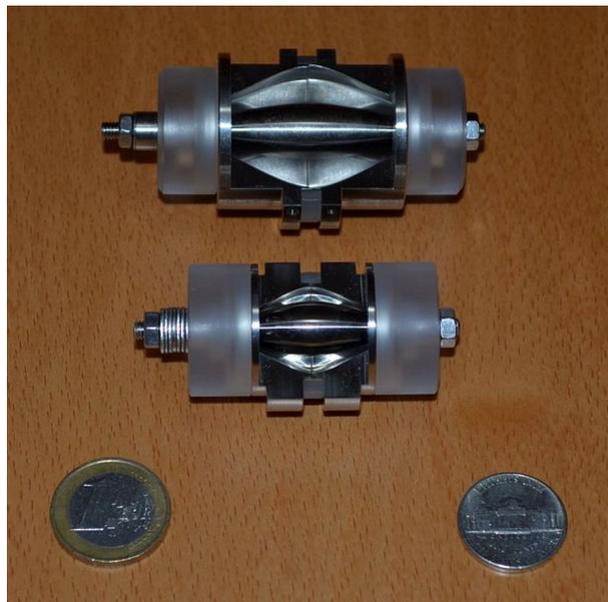


Figure 1. Cutaway view of the Orbitrap mass analyzer. Ions are injected into the Orbitrap at the point indicated by the red arrow. The ions are injected with a velocity perpendicular to the long axis of the Orbitrap (the z-axis). Injection at a point displaced from $z = 0$ gives the ions potential energy in the z-direction. Ion injection at this point on the z-potential is analogous to pulling back a pendulum bob and then releasing it to oscillate.



Standard

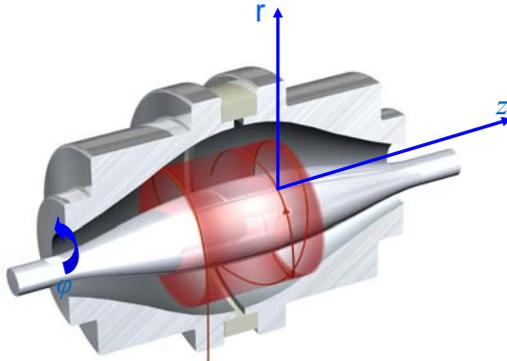
High-field

Orbitrap mass
analyzers

Trajectories in the orbitrap

- Characteristic frequencies:

- Frequency of rotation ω_ϕ
- Frequency of radial oscillations ω_r
- Frequency of axial oscillations ω_z



$$U(r, z) = \frac{k}{2} \cdot \{z^2 - r^2 / 2 + R_m^2 \cdot \ln(r / R_m)\}$$

$$\omega_\phi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1}$$

$$\omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

$$\omega_z = \sqrt{\frac{k}{m/z}}$$

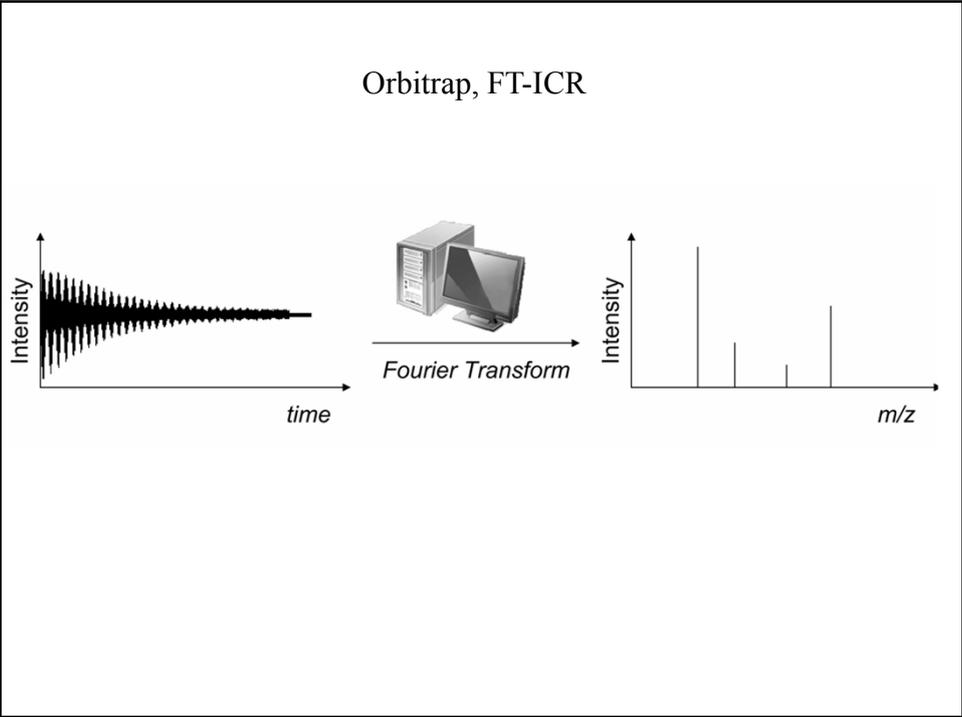
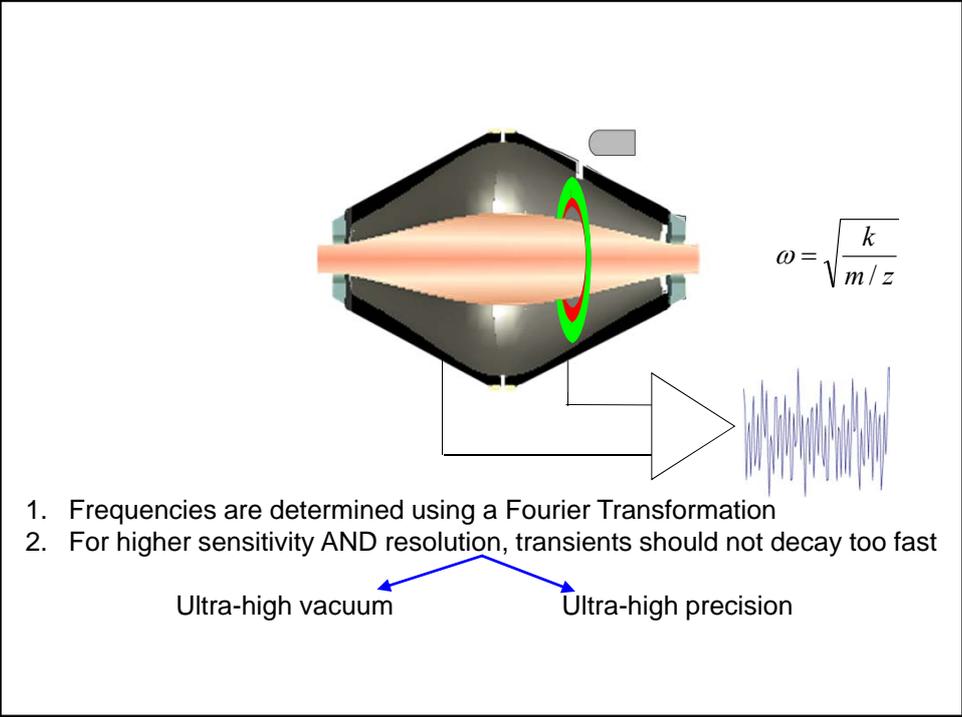
Ion Motion Along z Axis in the Orbitrap

$$\omega = \sqrt{z / m \times k}$$

k = field curvature

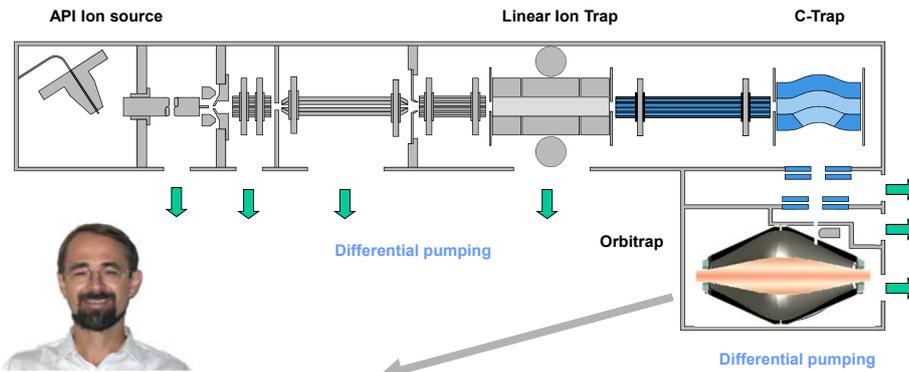
ω is to first order independent of ion energy and spatial spread

A Makarov. *Anal. Chem.* 2000, 72, 1156.



LTQ Orbitrap™ Hybrid Mass Spectrometer

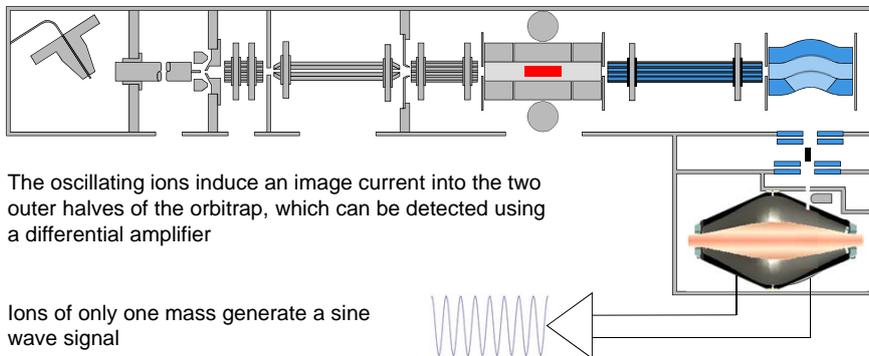
2005 - 2006



Inventor: Dr. Alexander Makarov, Thermo Electron (Bremen)

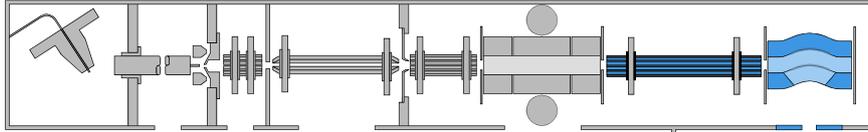
LTQ Orbitrap Operation Principle

1. Ions are stored in the Linear Trap
2. are axially ejected
3. and trapped in the C-trap
4. they are squeezed into a small cloud and injected into the Orbitrap
5. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation

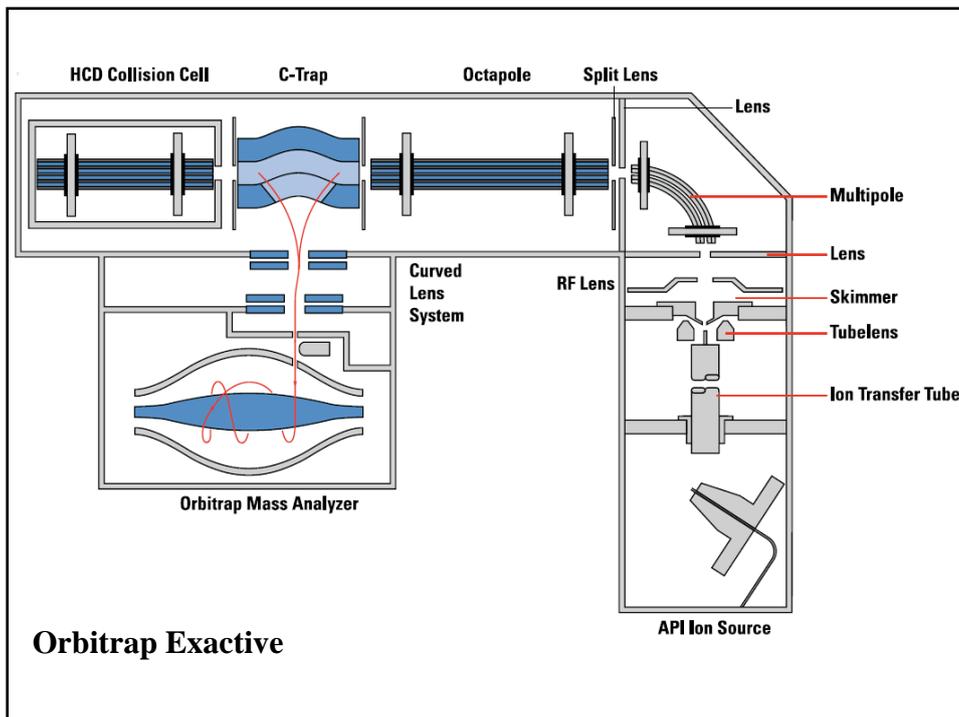
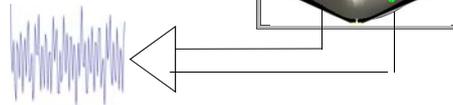


Frequencies and Masses

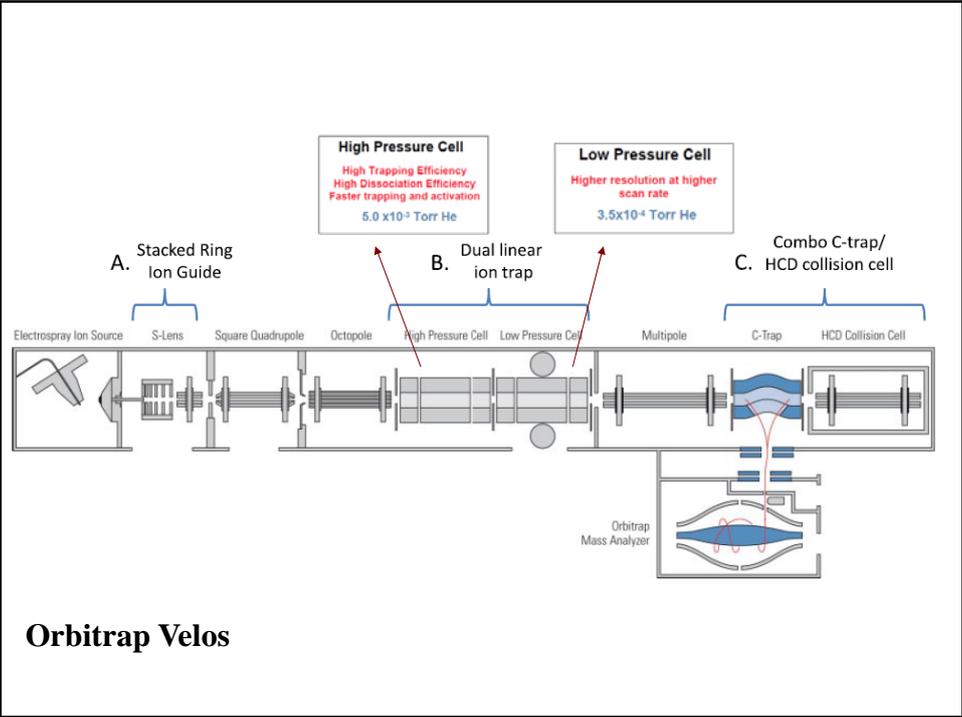
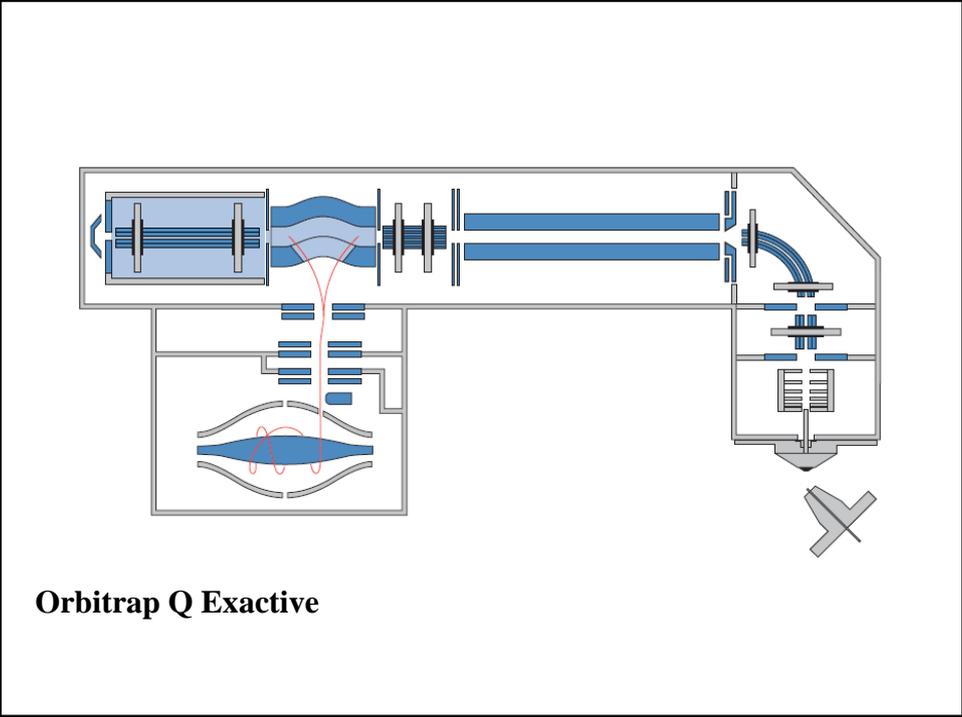
The axial oscillation frequency follows the formula $\omega = \sqrt{\frac{k}{m/z}}$
 Where ω = oscillation frequency
 k = instrumental constant
 m/z = well, we have seen this before

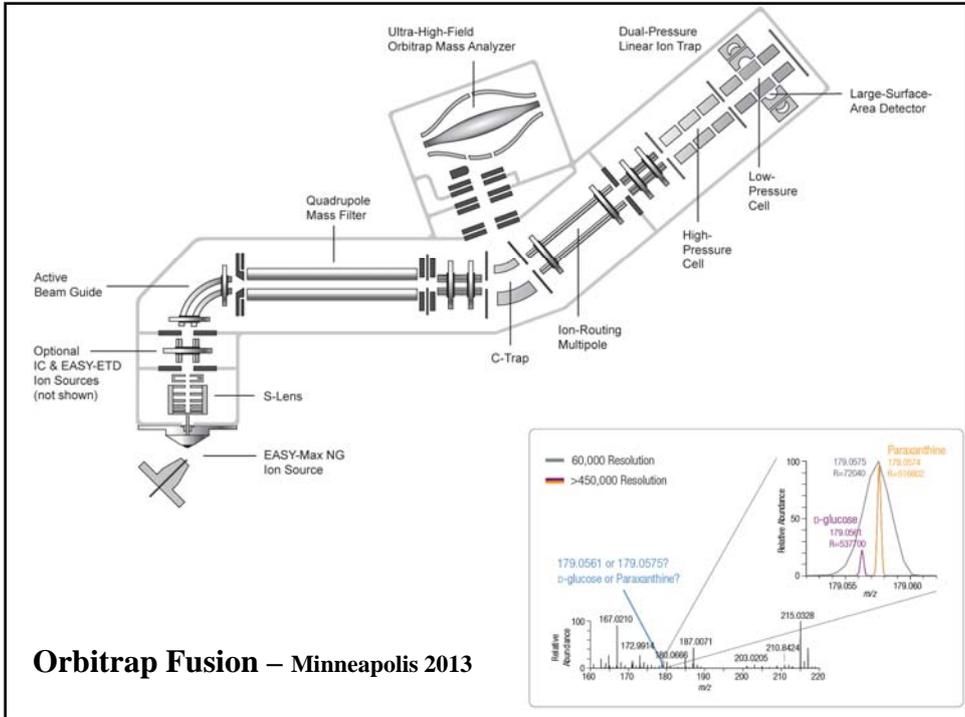
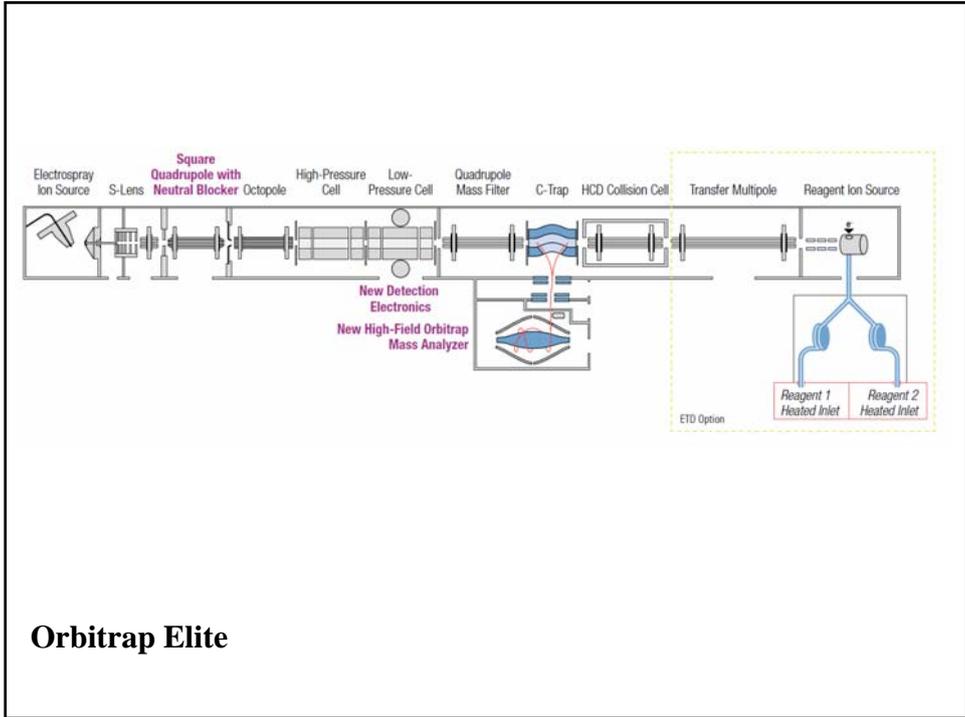


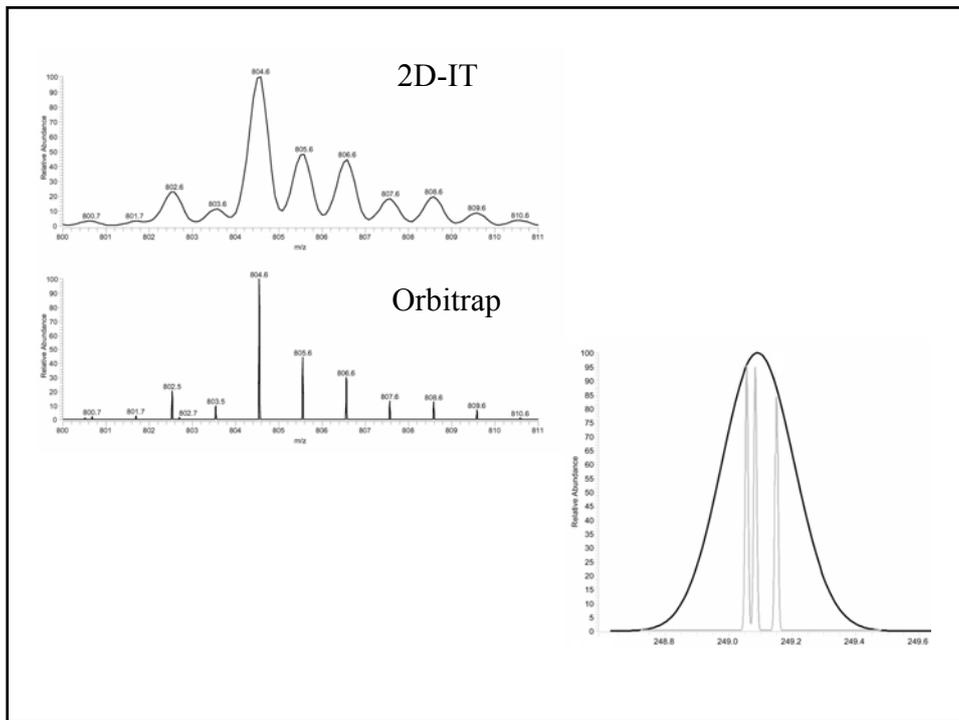
Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation



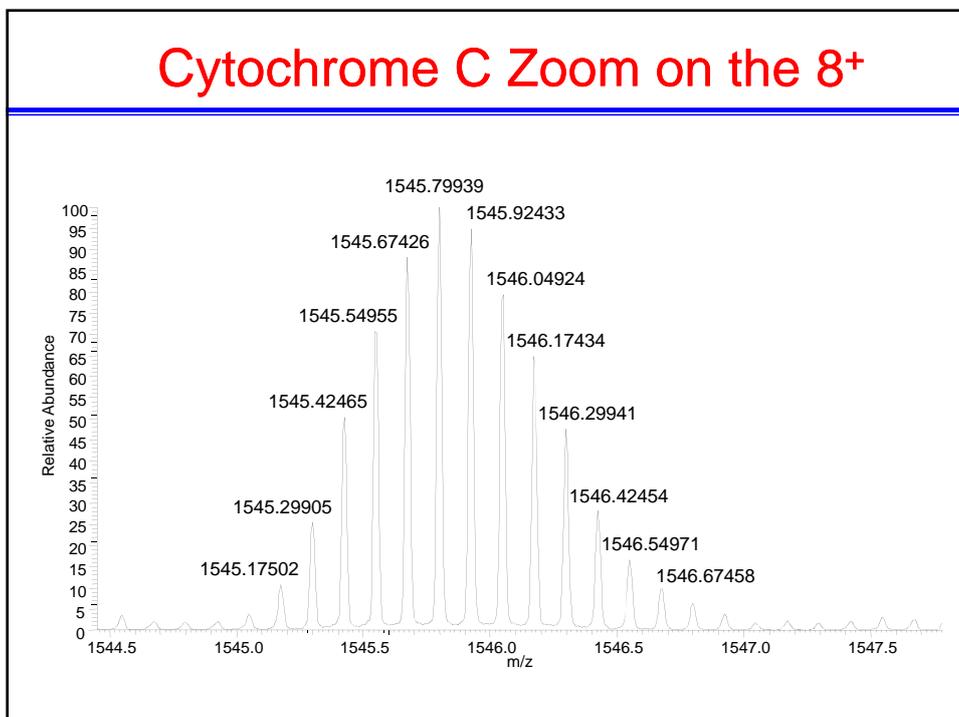
Orbitrap Exactive







Cytochrome C Zoom on the 8⁺



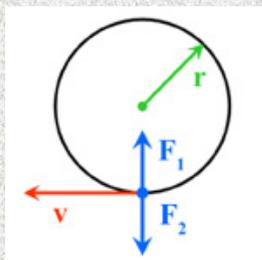
FT-ICR

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

LA CELLA A RISONANZA CICLOTRONICA

<http://www.youtube.com/watch?v=7EHngA4S3Ws>

Movimento Ciclotronico



Magnetic field directed upward

F_1 = Magnetic Force

F_2 = Centrifuge Force

r = radius of gyration

v = velocity

Per mantenere lo ione in un'orbita circolare stabile occorre che:

$$F_1 = F_2$$

$$q v B = m v^2/r$$

$$m v^2/r = q v B$$

Ne consegue che la frequenza angolare ω di uno ione con massa m e carica q è data da:

$$v/r = q B/m$$

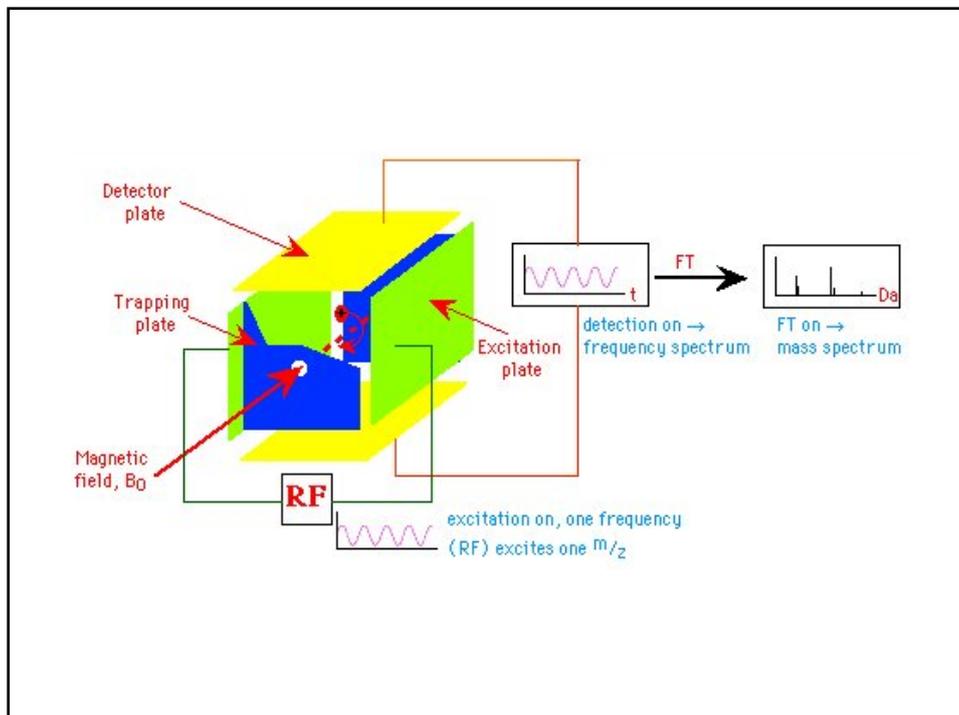
Frequenza ciclotronica ω

$$\omega = q B/m \quad (\text{radianti/sec})$$

$$\omega = (q B/m)/2\pi \quad (\text{Hz})$$

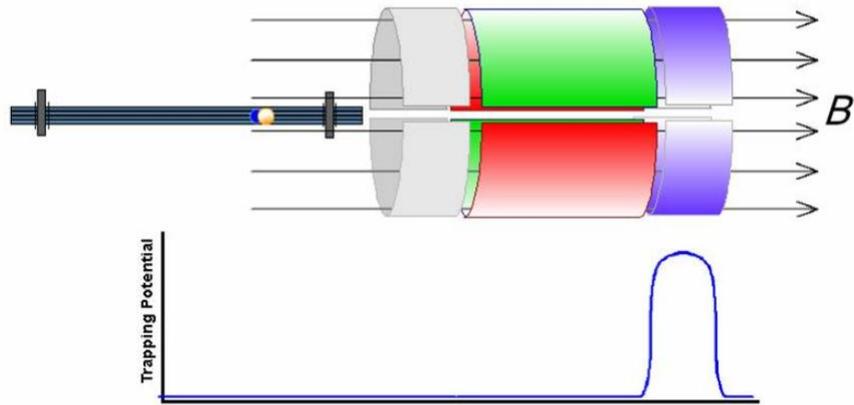
Da notare che **la frequenza ciclotronica NON dipende dalla velocità dello ione.**

Perciò ioni di una stessa massa avranno tutti la stessa frequenza ciclotronica indipendentemente dal tempo in cui entrano nella cella o dalla velocità con cui vi entrano.



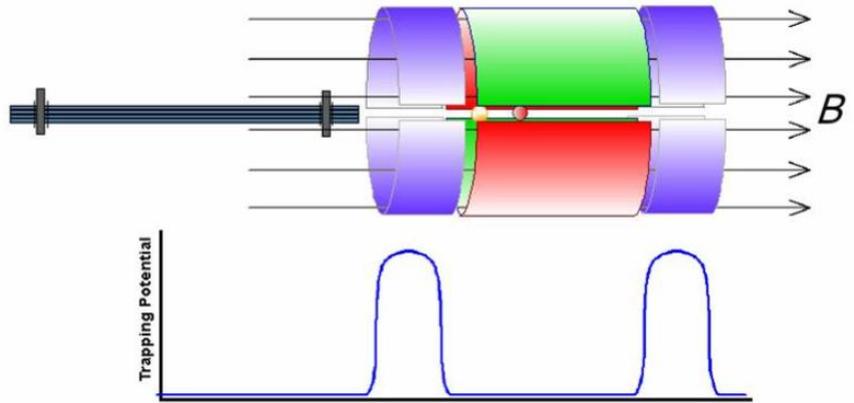
Packets of ions are injected into the ICR cell from the linear ion trap.

They are trapped in the ICR cell by the coaxial magnetic field and voltages applied to the trapping electrodes.



Packets of ions are injected into the ICR cell from the linear ion trap.

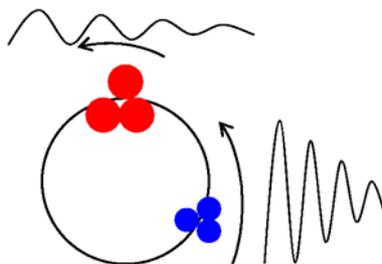
They are trapped in the ICR cell by the coaxial magnetic field and voltages applied to the trapping electrodes.

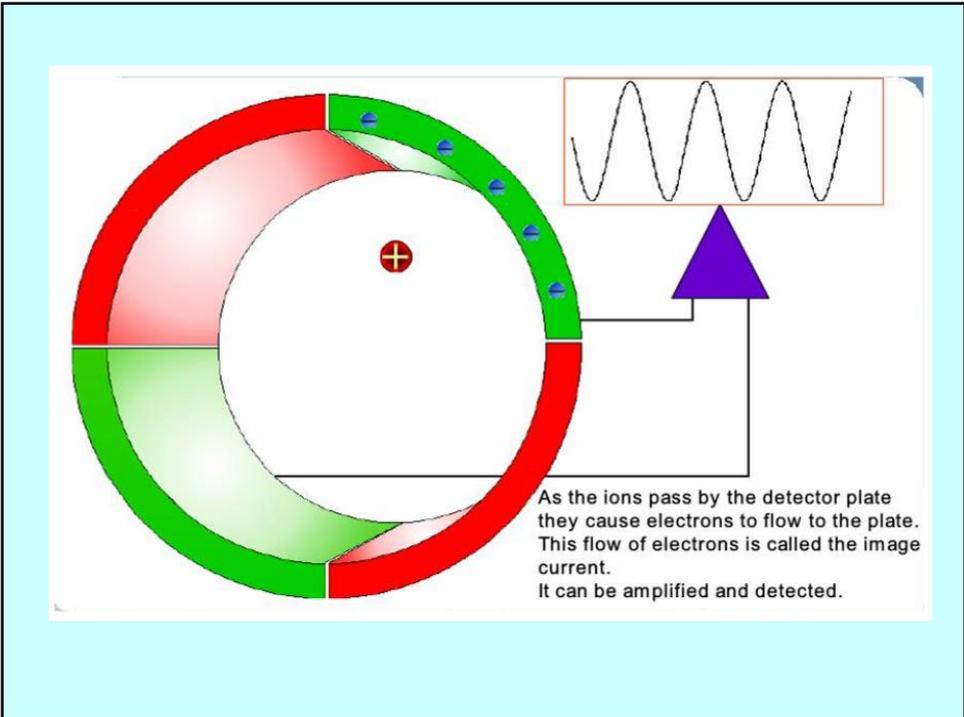
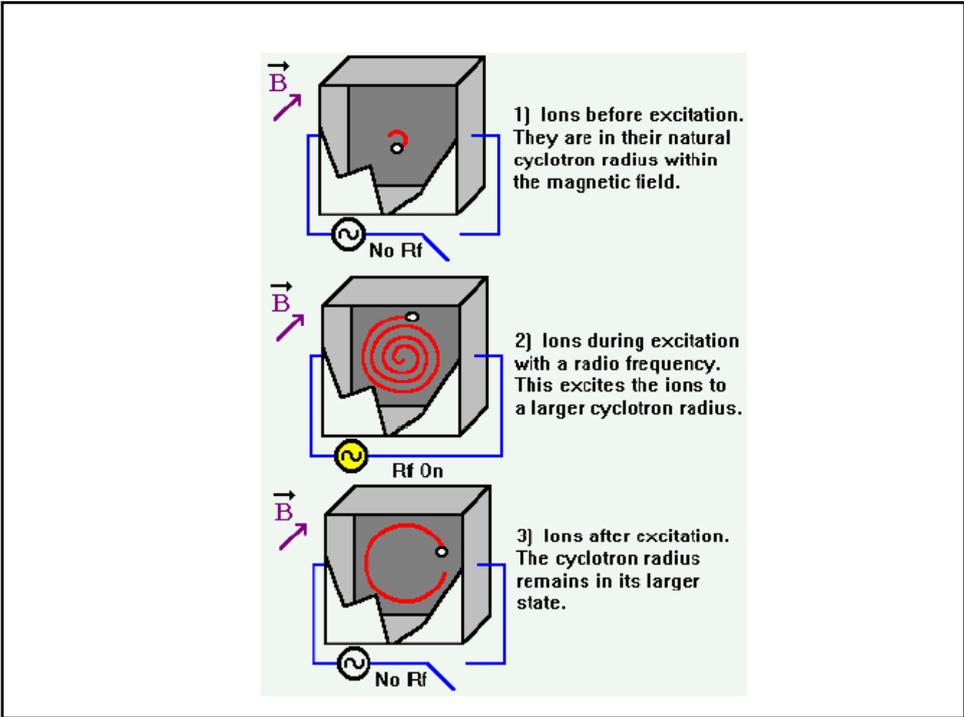


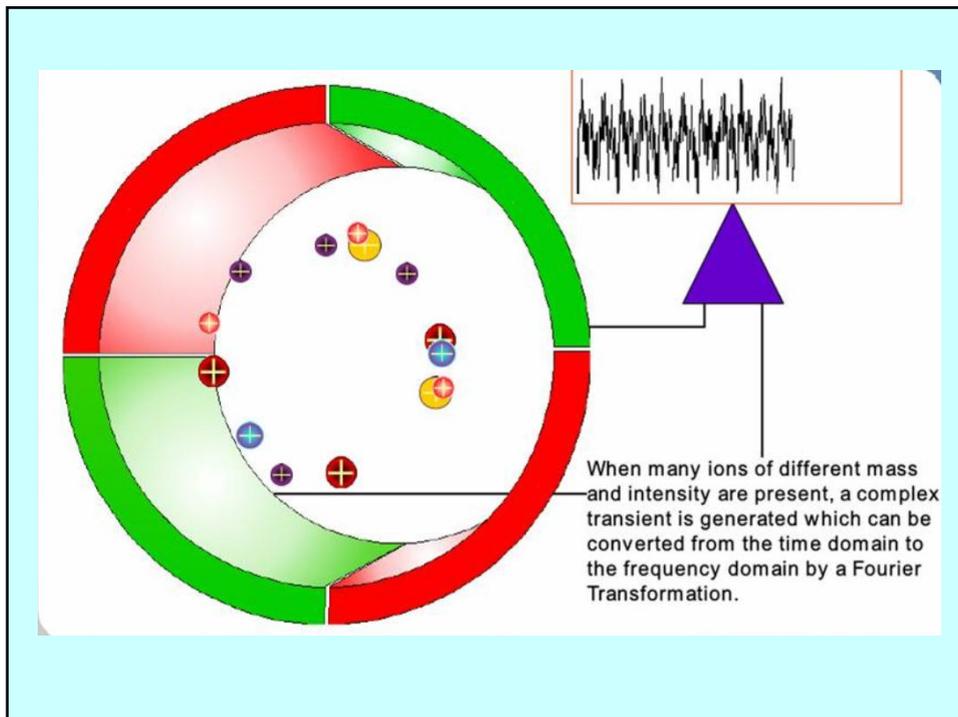
Ad esempio, la frequenza ciclotronica di uno ione monocaricato, con m/z 1000 e un campo magnetico di 7.0 Tesla, è di 107.45 kHz, ovvero compie circa 100.000 orbite al secondo.

Se il suo stato di carica è +4 il numero di orbite al secondo diventa circa 400.000.

La cella è situata all'interno di una regione di campo omogeneo di un grande magnete superconduttore. Gli ioni si muovono in orbite circolari alle loro frequenze ciclotroniche. Se all'interno della cella vi sono ioni con diverso valore di m/z , essi gireranno su orbite con un raggio molto simile ma avranno frequenze diverse. Normalmente il raggio di un'orbita di uno ione è di circa 0.1 mm. Se un impulso RF, sufficientemente largo (8MHz÷100kHz) da coprire l'intervallo delle diverse frequenze ciclotroniche degli ioni che si vogliono osservare, viene inviato all'interno della cella, gli ioni acquisteranno energia dal campo RF e si muoveranno su orbite più larghe.

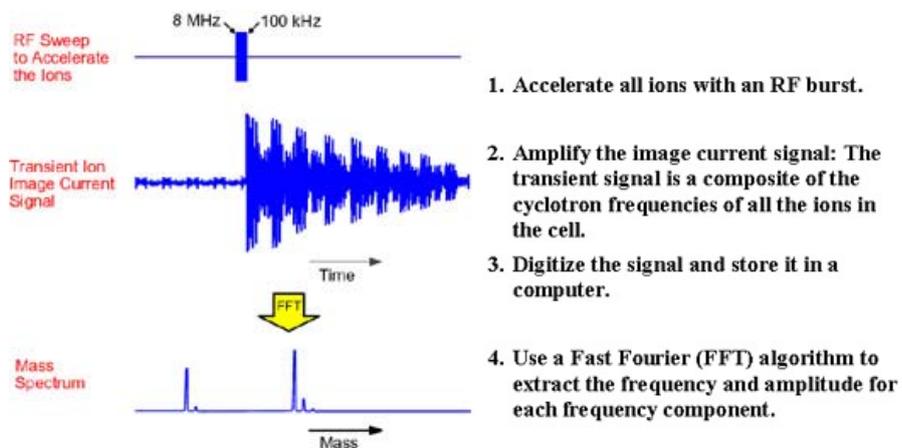






Così il segnale complessivo che esce dalla cella è un **segnale composito** costituito dalla sovrapposizione delle frequenze ciclotroniche di tutti gli ioni presenti all'interno. Inoltre questo segnale diminuisce lentamente mentre gli ioni rilassano e ritornano alle loro orbite circolari stabili al centro della cella.

Usando la trasformata di Fourier è possibile deconvolvere il segnale complessivo e identificare le singole frequenze.



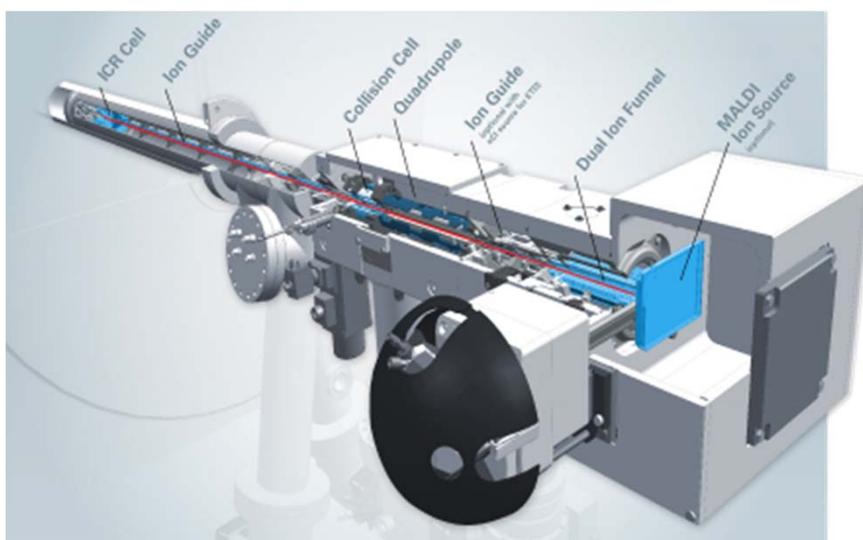
A new ultra-high resolution FT-ICR cell with dynamic harmonization

extreme mass resolution greater than 10 million



Nikolaev EN, Boldin IA, Jertz R, Baykut G., *J Am Soc Mass Spectrom.* 2011, **22**:1125-33

Qq-FTICR



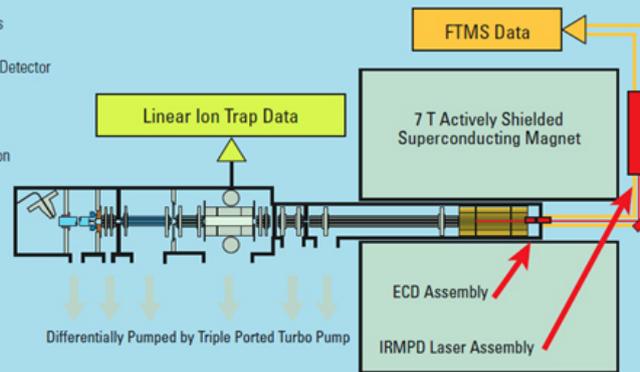
IT-FTICR

Linear Ion Trap MS

- MS, MS/MS and MSⁿ Analysis
- AGC Control
- Secondary Electron Multiplier Detector

FTICR MS

- Ion Image Current Detector
- Accurate Mass, High Resolution
- ECD, IRMPD



FT-ICR

ESI (+) of Ubiquitin

1,040,000 Resolution at m/z 779

