

TANDEM MS: PRESENTE E FUTURO

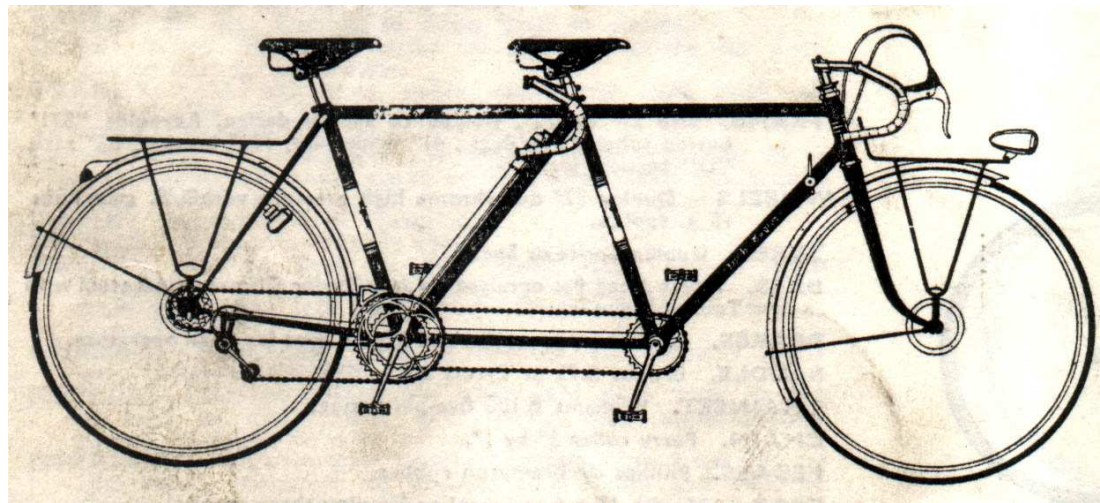
PIETRO TRALDI

CNR ISTM PADOVA



Tandem: *sm*, bicicletta a due sedili e due coppie di pedali, che serve per due pedalatori uno dietro l'altro

Latino: Tandem=infine, finalmente







Tandem, *av.*, uno dietro l'altro, in
fila indiana

Da Mario Hazon, Dizionario inglese-italiano,
Garzanti (1981)

Come aumentare la specificità di una misura di spettrometria di massa:

- Aspetti fisici: aumentare la risoluzione e l'accuratezza di massa relativa allo ione di interesse;
- Aspetti chimici: studiare la reattività dello ione di interesse.

Come aumentare la sensibilità di una misura MS/MS:

- Elevata resa della reazione di decomposizione;
- Elevata trasmissione ionica nella zona di collisione e di analisi di massa.

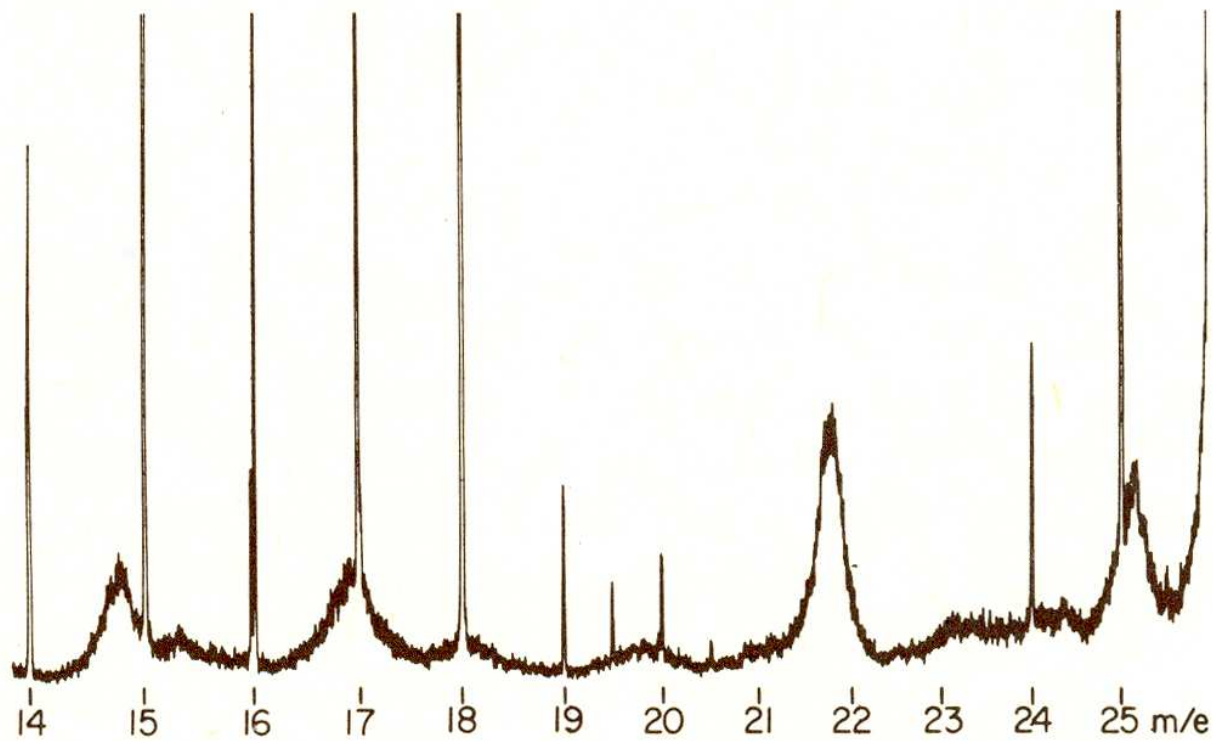
La caratterizzazione strutturale di uno ione di interesse si ottiene studiando la sua decomposizione indotta da collisione con un gas non reattivo.

L'esperimento MS/MS (tandem mass spectrometry) si ottiene quindi da:

- Selezione dello ione di interesse;
- Decomposizione dello ione mediante collisioni;
- Analisi di massa dei frammenti ottenuti dalla collisione

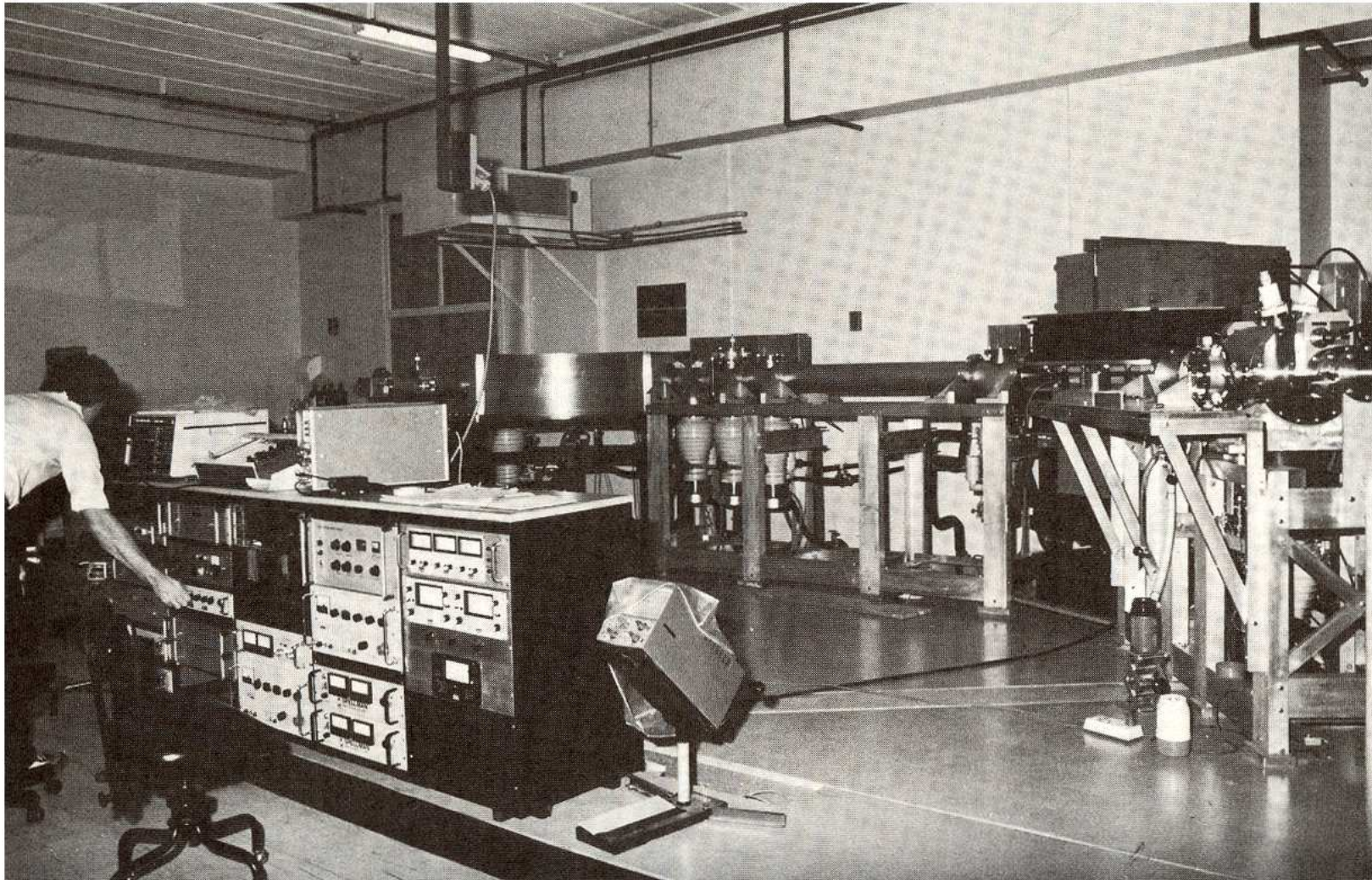


Politecnico di Milano - 1975

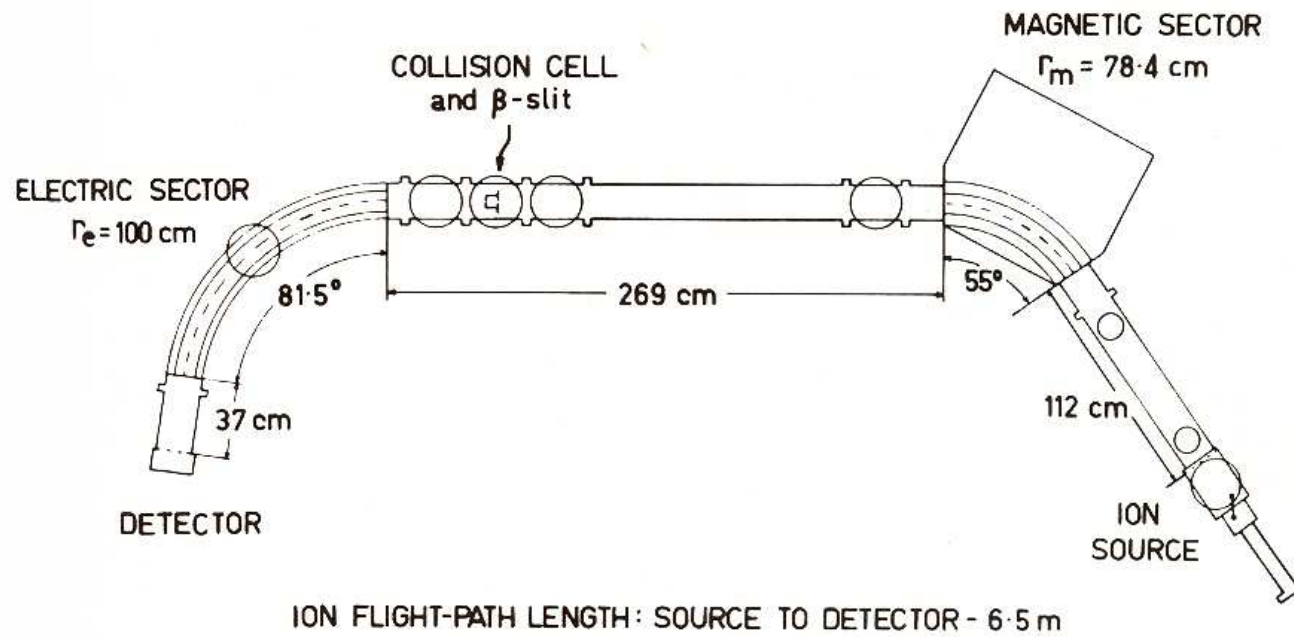


$$m^* = \frac{m_2^2}{m_1}$$

Part of a mass spectrum between mass-to-charge ratios 14 and 26 showing a variety of diffuse (metastable) peaks.



The grade-scale double-focusing mass spectrometer at the University of New South Wales.



Schematic drawing of the instrument of Figure 10.1.

ANALIZZATORI MAGNETICI A 5 SETTORI



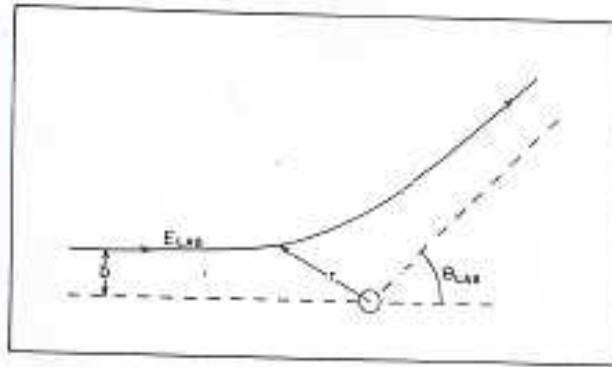


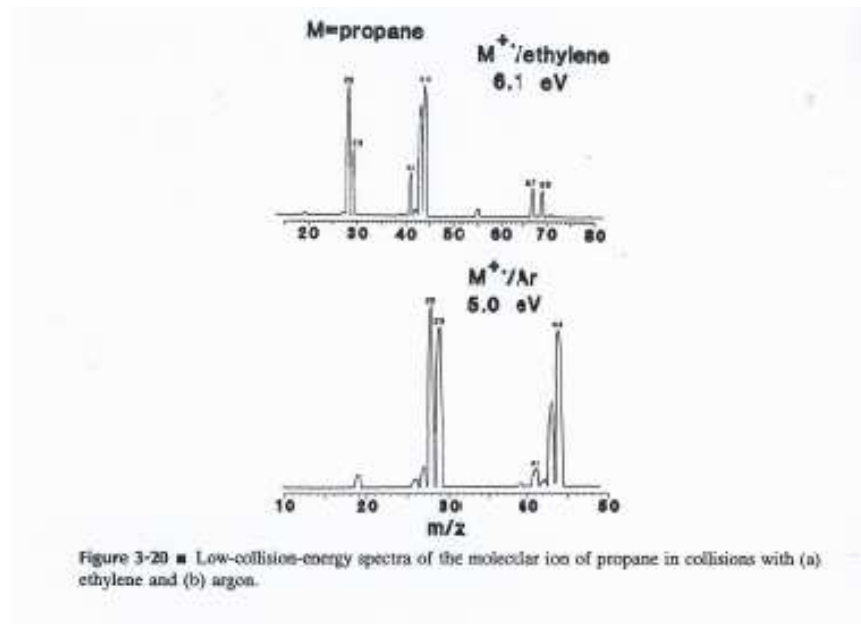
Figure 3-12 ■ Generalized depiction of a collision between a high-velocity projectile and a stationary target in which the interaction potential, $V(r)$, is repulsive over the entire range of r . A $V(r)$ that is strongly attractive at some values of r could result in a more complicated trajectory depending upon the impact parameter and the collision energy.

Domanda: Collisioni in fase gas tra ioni e atomi (o molecole) bersaglio: elastiche o anelastiche?

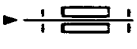
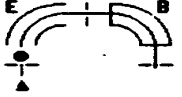





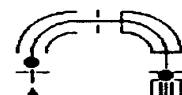
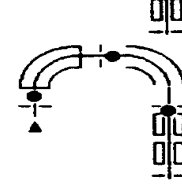

Risposta: essenzialmente anelastiche, con deposizione di energia nella specie ionica.

L'efficienza di una collisione dipende da:

- Massa dell'atomo (o molecola) bersaglio;
- Sezione d'urto dell'atomo (o molecola) bersaglio;
- Energia cinetica dello ione selezionato (?)

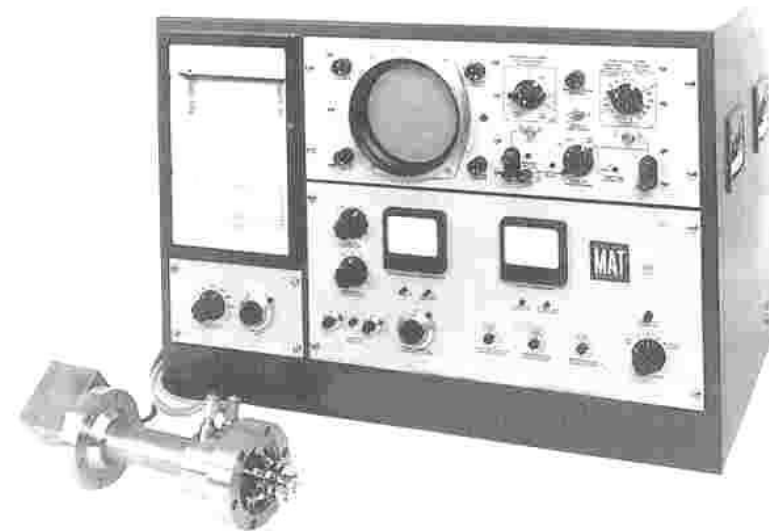
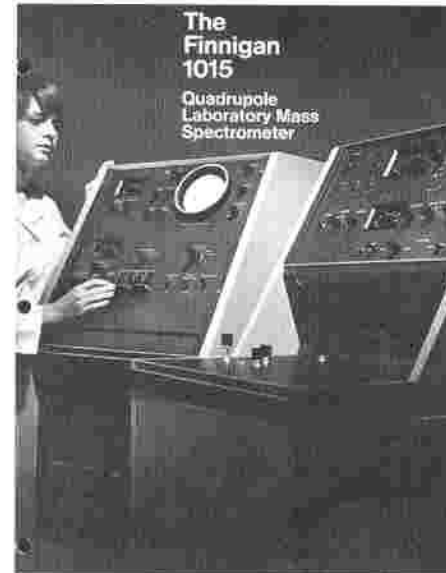


Configurazioni degli strumenti a settore e a quadrupolo per lo studio delle relazioni tra ioni precursori / prodotto e perdite neutre

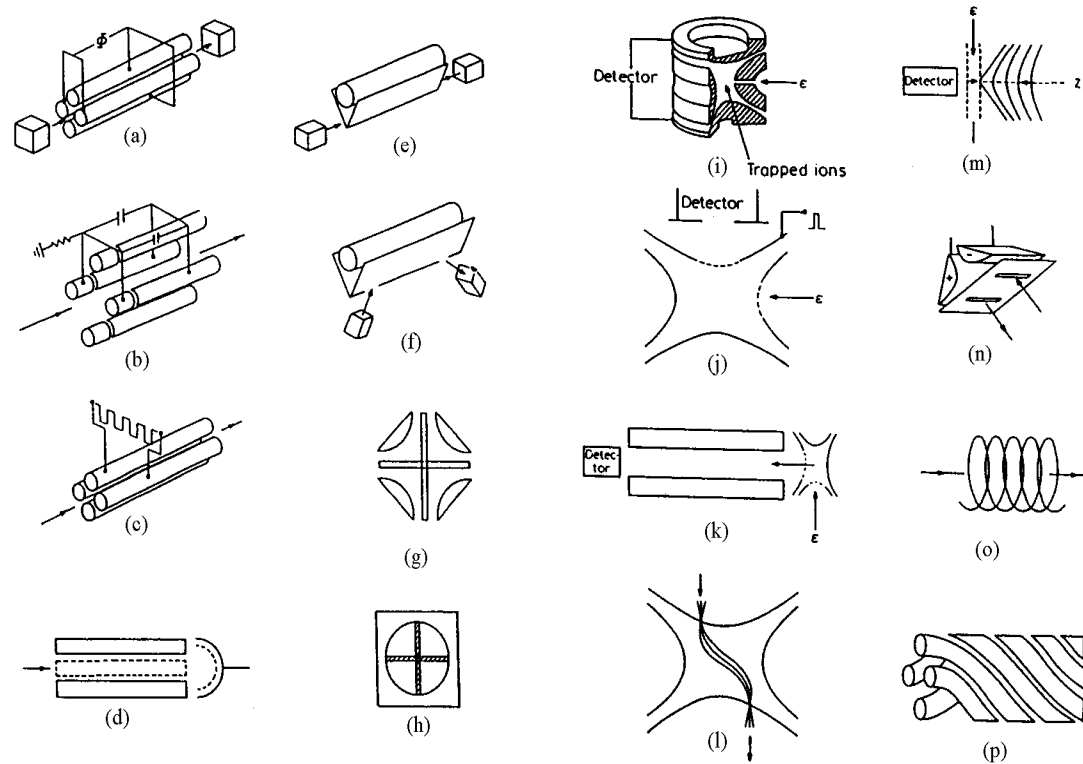
		Modi operativi in MS/MS			
Configurazione		regione di frammentazione	risoluzione parent ion	risoluzione daughter ion	energia di collisione
	Q	Q			
	EB	EB	200	2000	KeV
	BE	B ↓ E	2000	200	KeV
	BEB	BE ↓ B	<100.000	2000	KeV
	EBE	EB ↓ E	<100.000	200	KeV
	BEBE	BE ↓ B ↓ E	<100.000	<10.000	eV-KeV
	BEEB	BE ↓ E ↓ B	<100.000	<10.000	eV-KeV
	EBQQ	EBQQ	<20.000	1	eV
	BEQQ	BEQQ BE ↓ QQ	<20.000 <20.000	1 1	eV KeV
	QQQ	QQQ	1	1	eV



Wolfgang Paul
Università di Bonn
Premio Nobel 1989

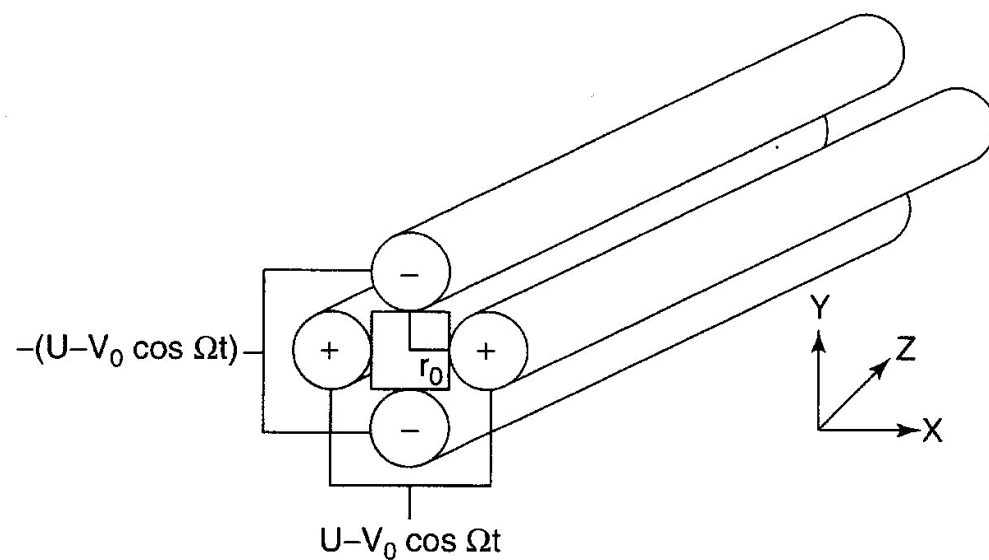


Analizzatori quadrupolari



Alcuni dispositivi basati su campi quadrupolari studiati dal gruppo di Paul (Univ. Di Bonn) negli anni sessanta.

Filtro di massa quadrupolare



In funzione di U , V_0 , ω , r , m e z si ottiene il **diagramma di stabilità** che permette di individuare le condizioni che portano a traiettorie stabili all'interno del campo quadrupolare

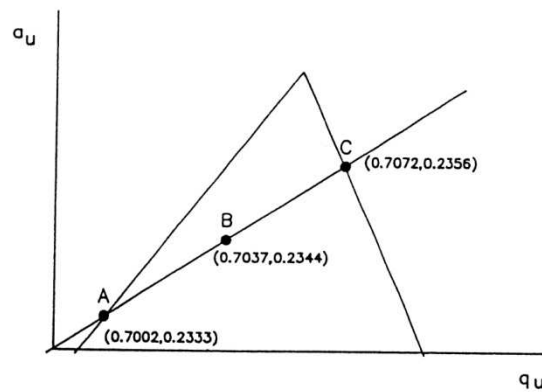


Fig. 9. The apex of the a , q stability region with a mass scan line corresponding to a resolution of 100.

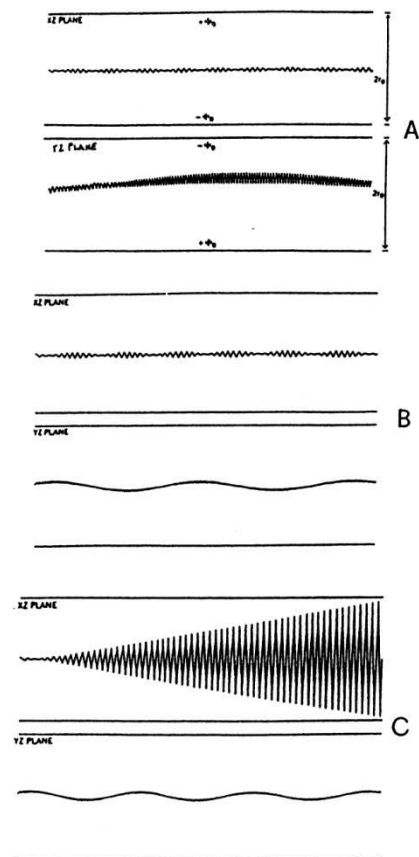


Fig. 10. The trajectories of the m/z 100 ion calculated at points A, B and C in fig. 9.

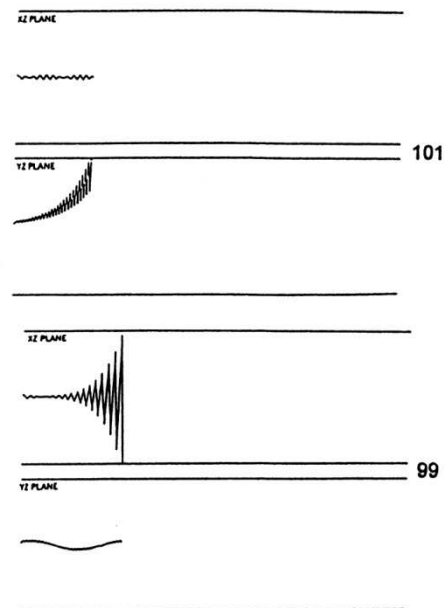
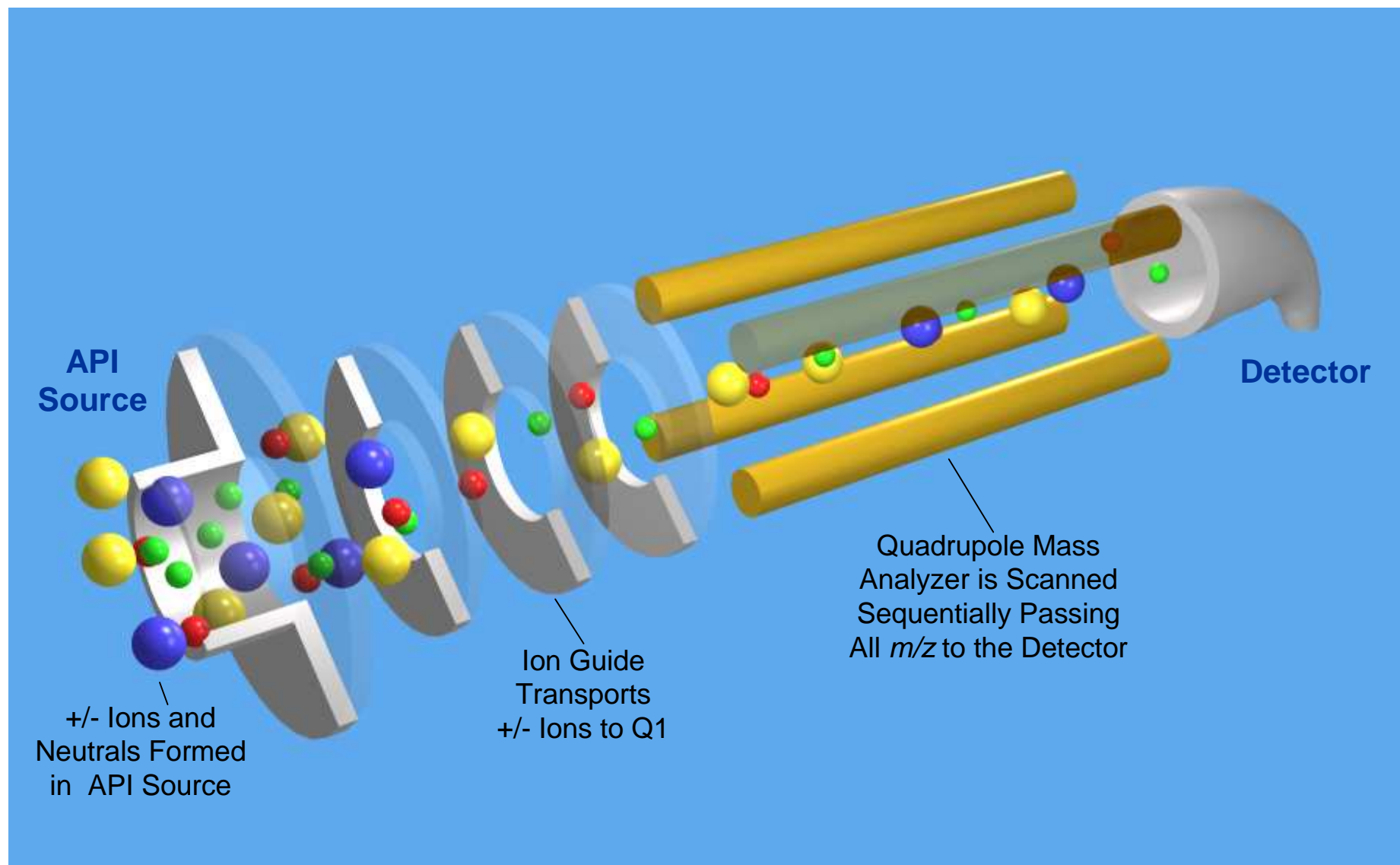


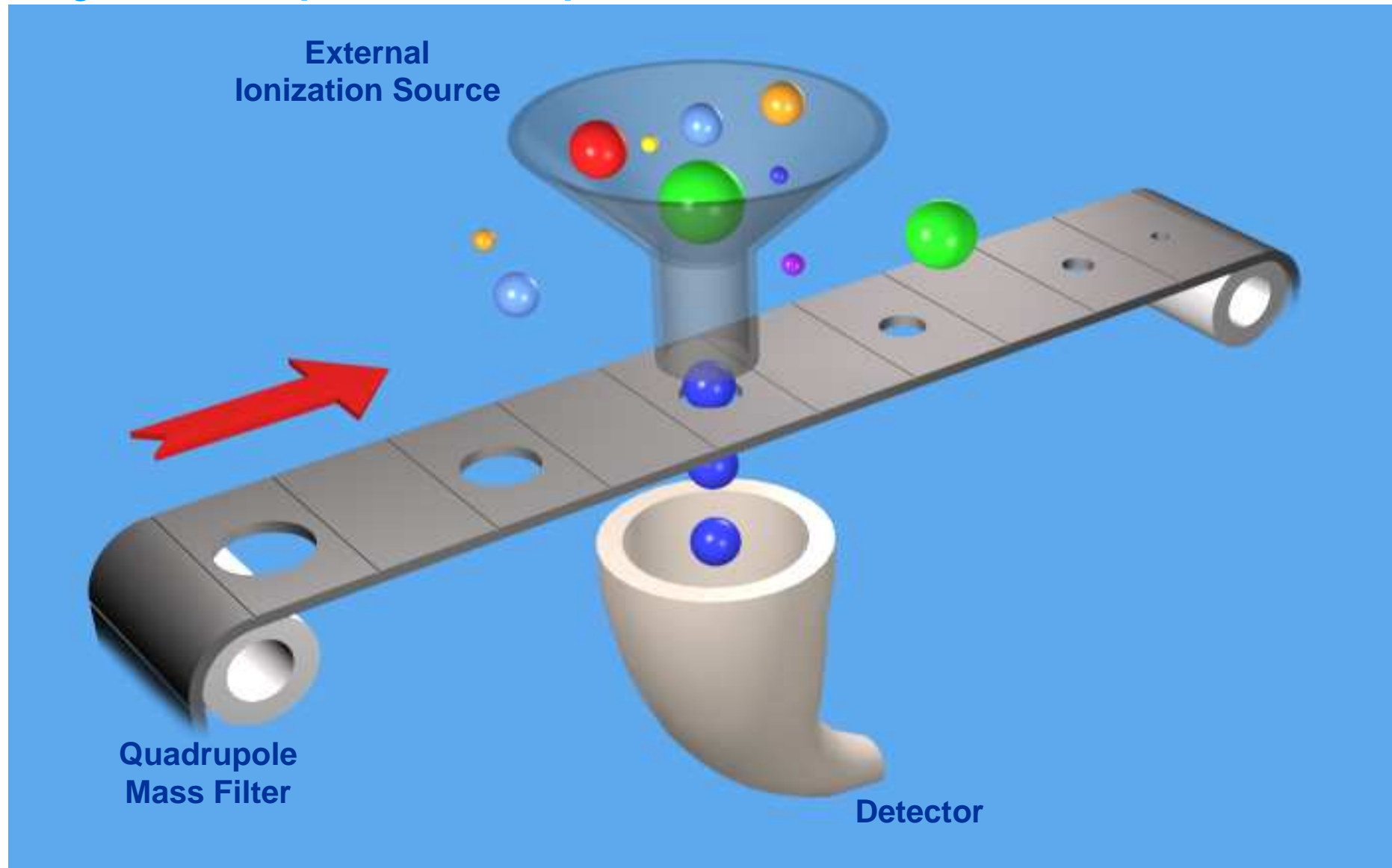
Fig. 11. The trajectory of the m/z 101 and m/z 99 ions calculated at operating values set to transmit only m/z 100.

Effettuando una scansione contemporanea di U e di V , mantenendo il rapporto U/V costante, si trasmettono selettivamente attraverso il campo quadrupolare tutti gli ioni generati in sorgente

Single Quadrupole: Full Scan MS

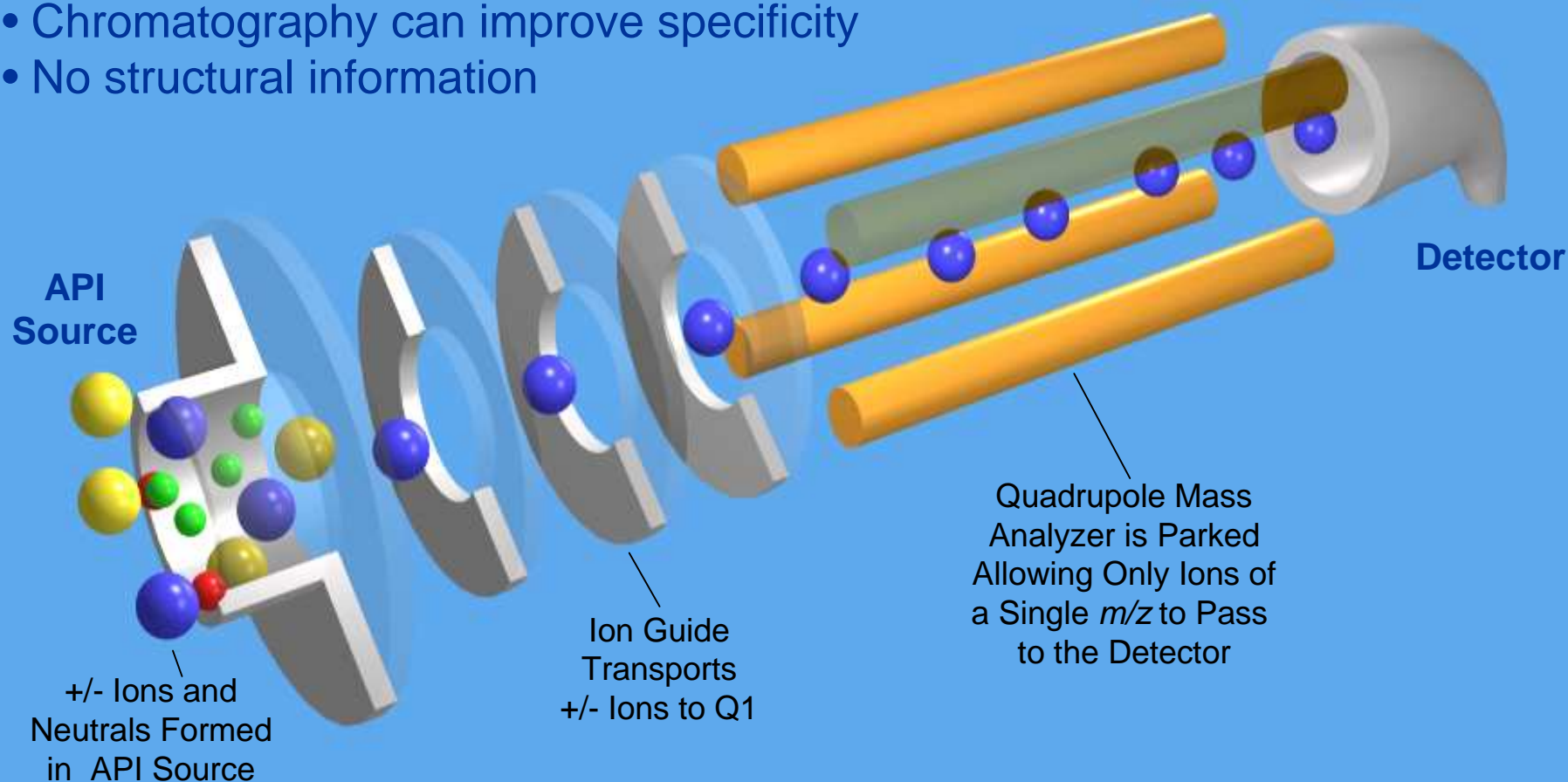


Conceptual Model of a Single Quadrupole Mass Spectrometer

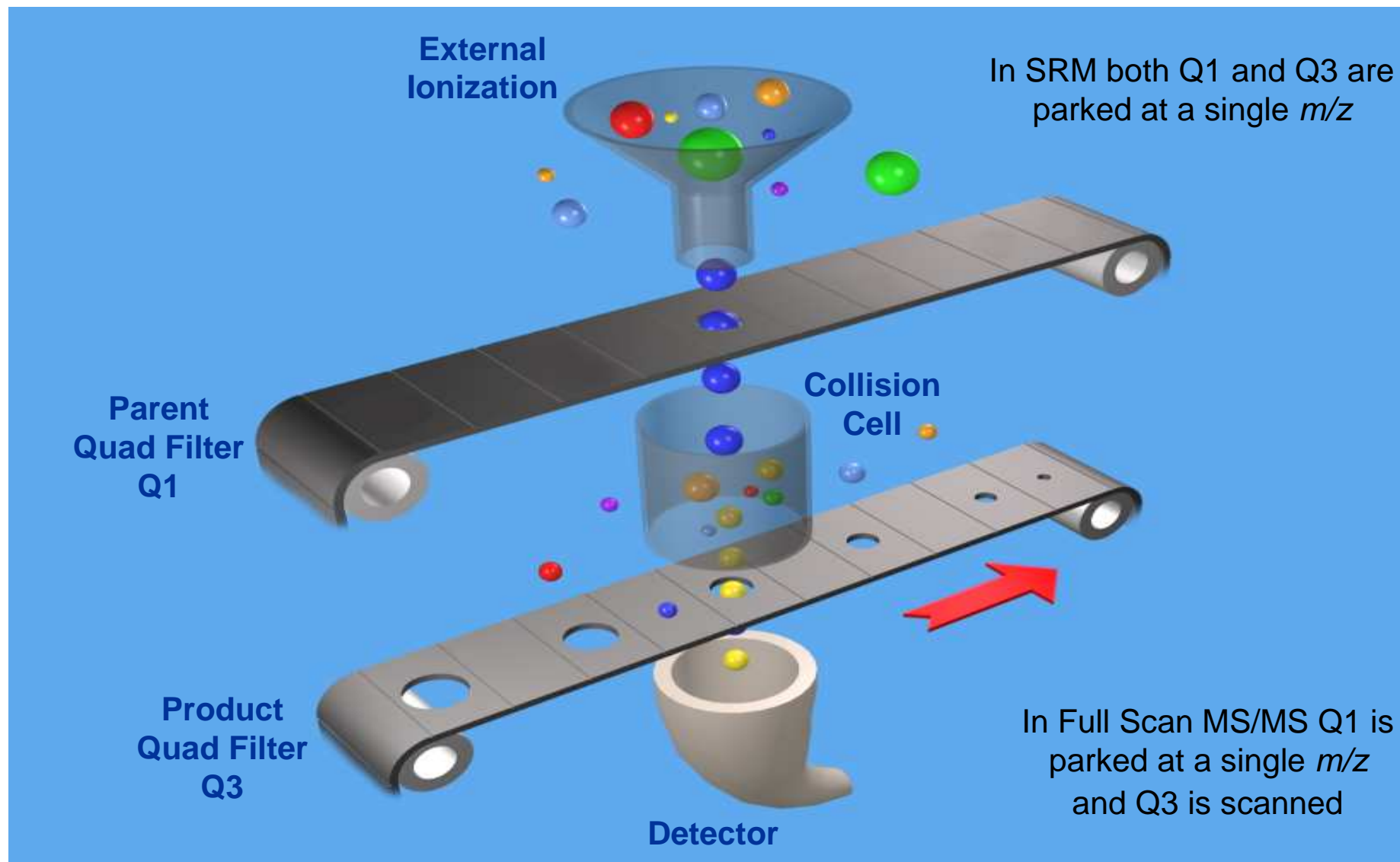


Single Quadrupole: SIM

- Best sensitivity for quantitation
- Provides increased selectivity
- Chromatography can improve specificity
- No structural information

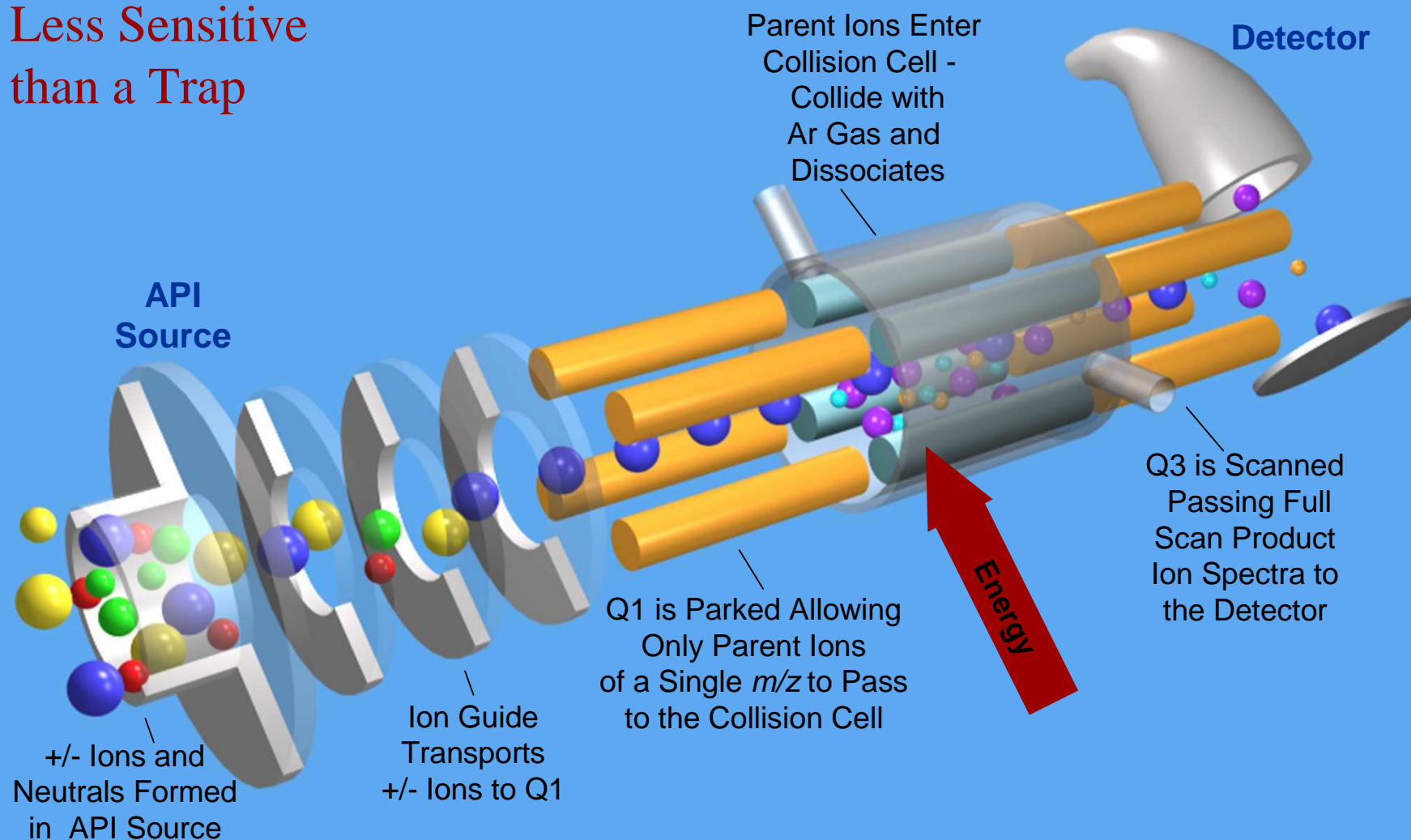


Conceptual Model of a Triple Quadrupole Mass Spectrometer



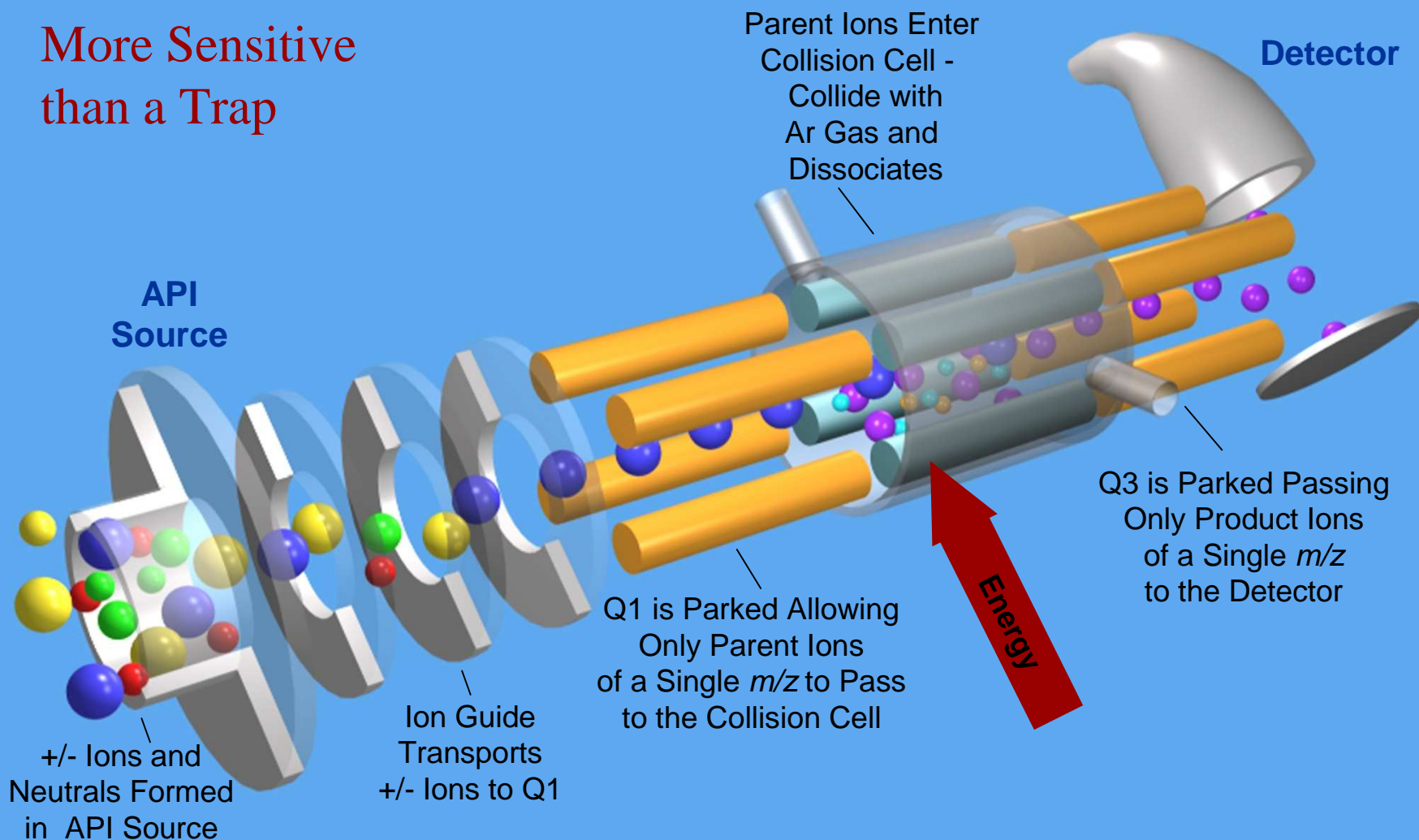
Triple Quadrupole: Full Scan MS/MS

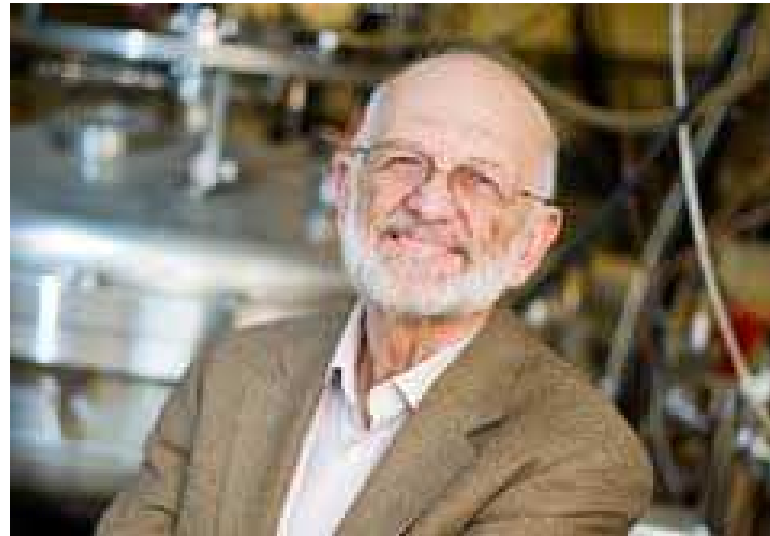
Less Sensitive
than a Trap



Triple Quadrupole: SRM

More Sensitive
than a Trap





Prof. Robert Graham Cooks
Purdue University

QqQ

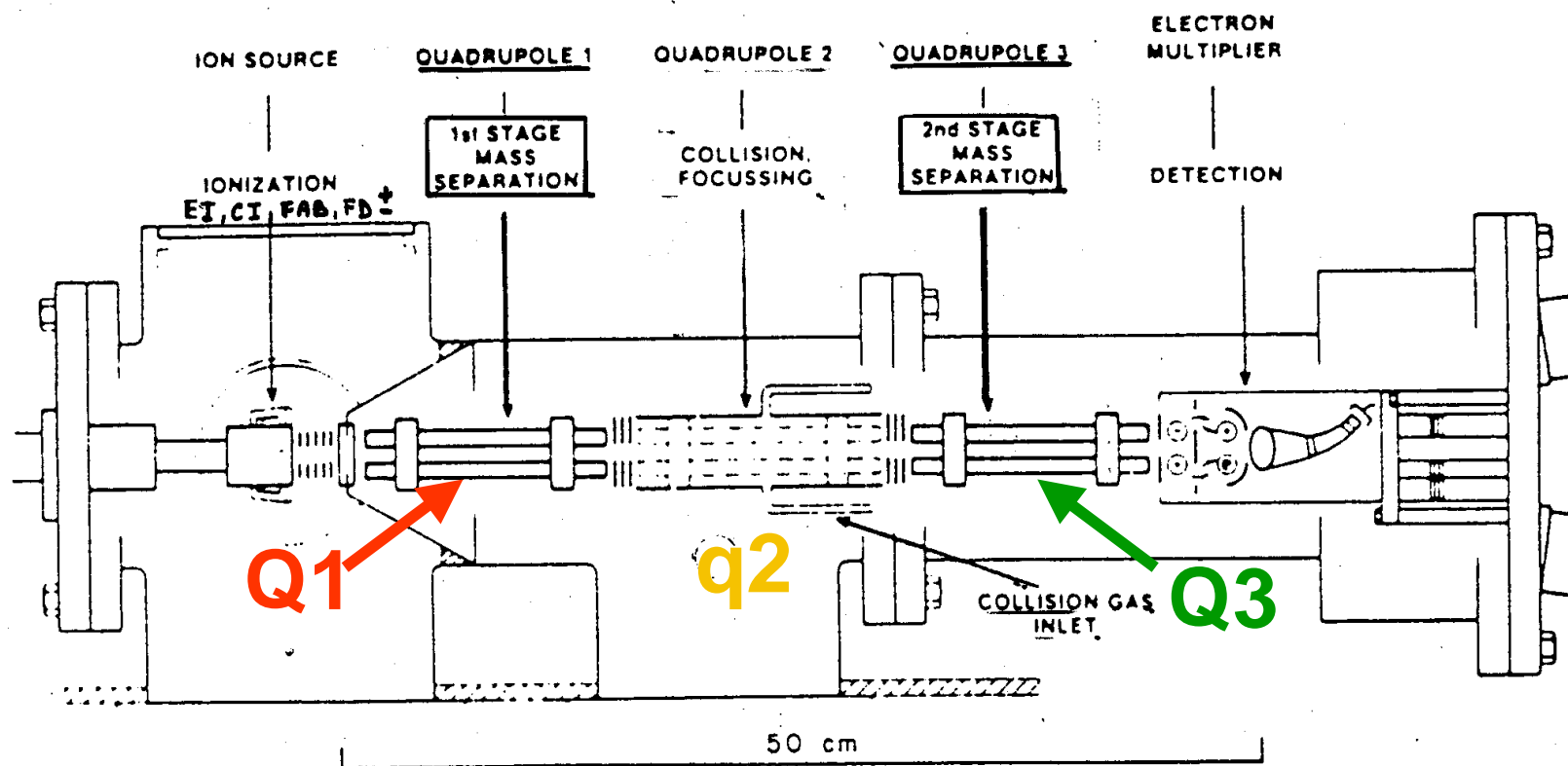
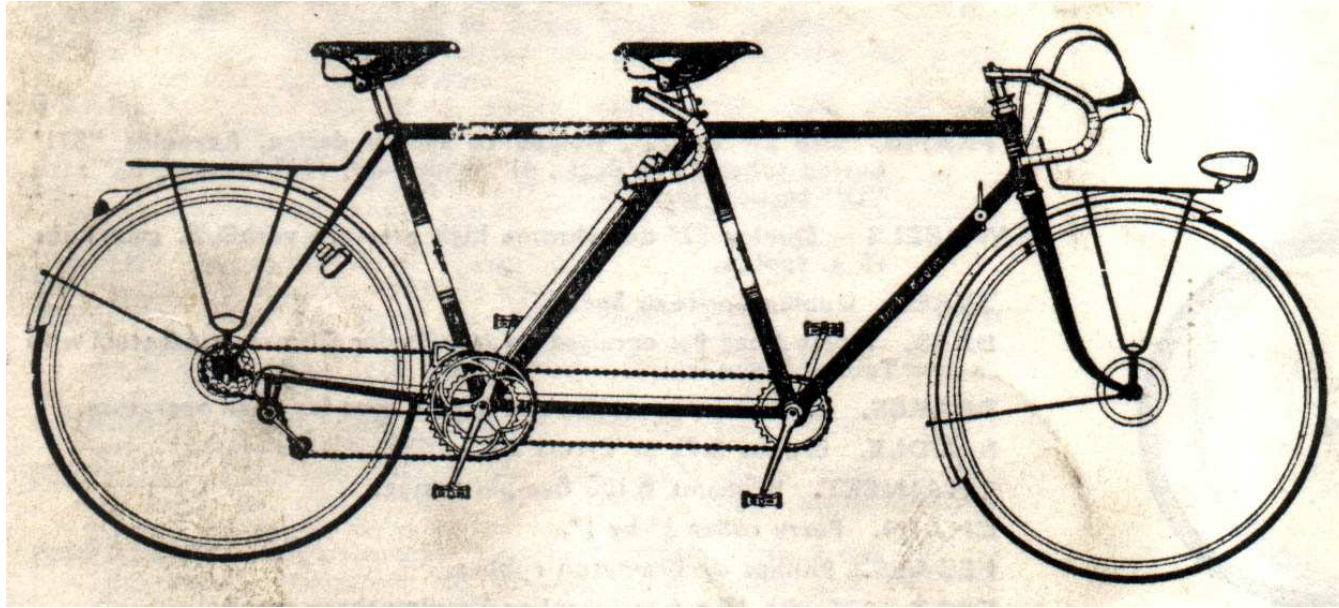


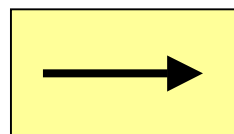
Figure 5: Scale drawing (side view) of the Finnigan MAT TSO triple-stage quadrupole MS/MS instrument (15).

1980

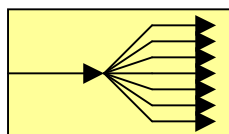


Main processes in tandem mass spectrometry

MS/MS



selected m/z

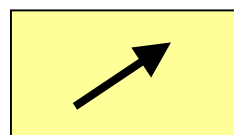
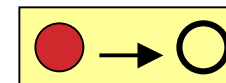


CID

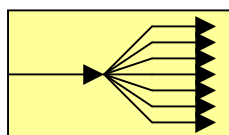


scanned

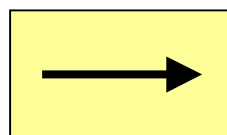
Product ion scans



scanned

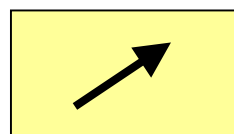
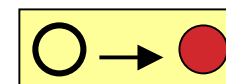


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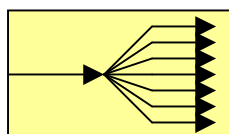


selected m/z

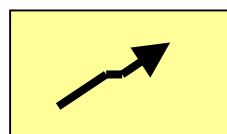
Precursor ion scan



scanned
m/z = x

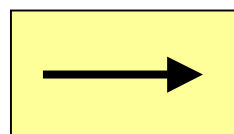
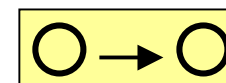


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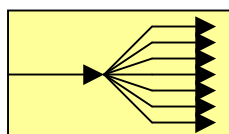


scanned
m/z = x-a

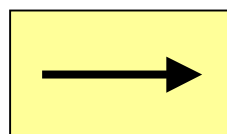
Neutral loss scan



selected
precursor
m/z = a

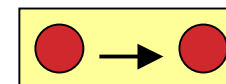


CID



selected
fragment
m/z = b

Selected reaction
monitoring

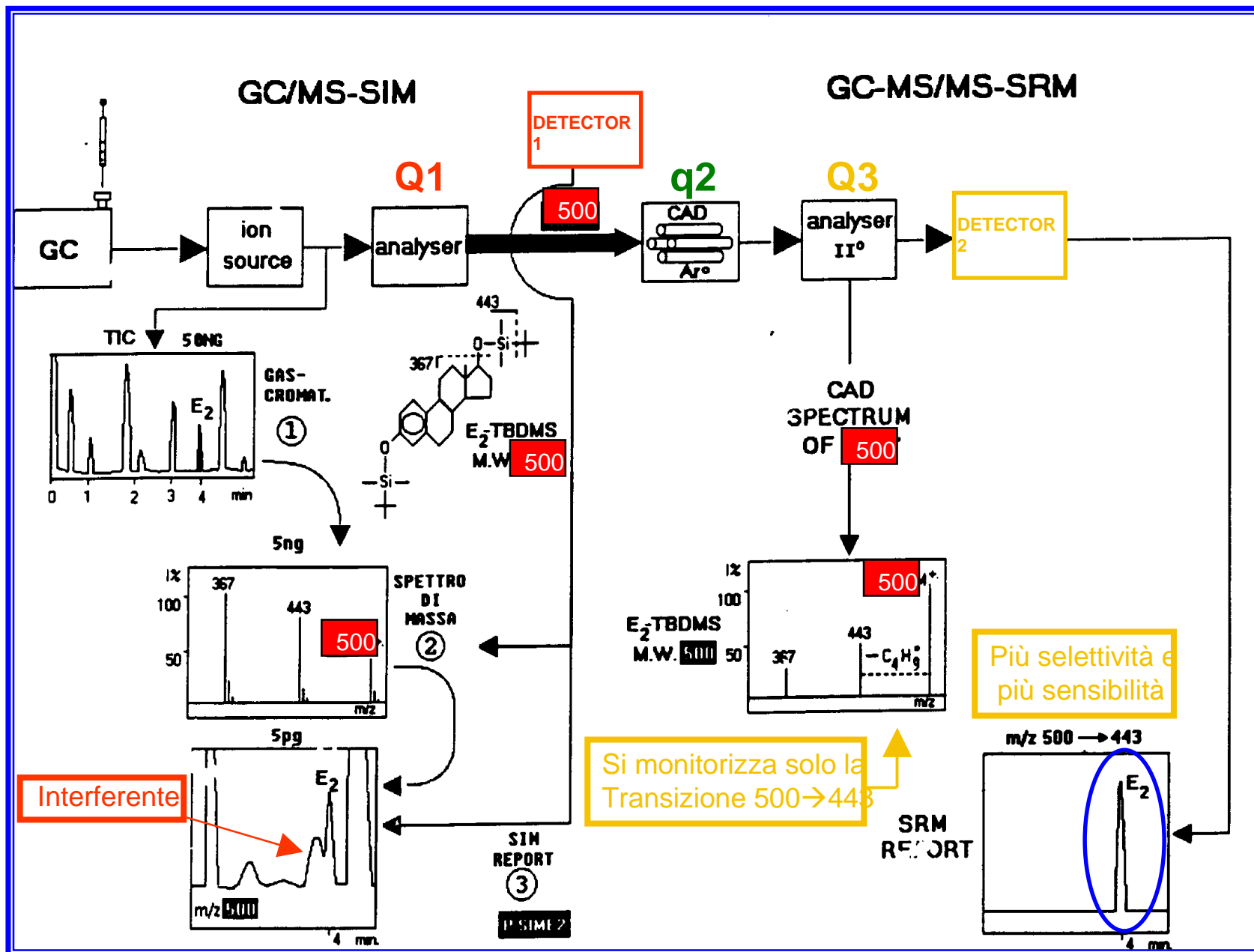


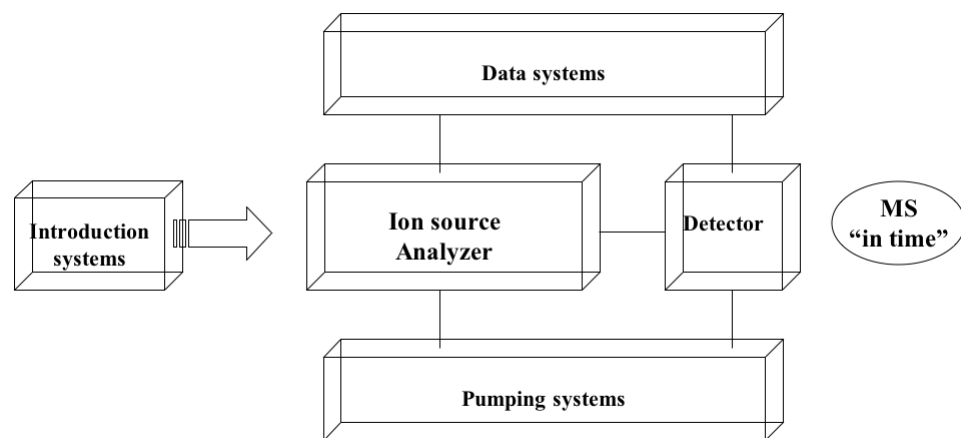
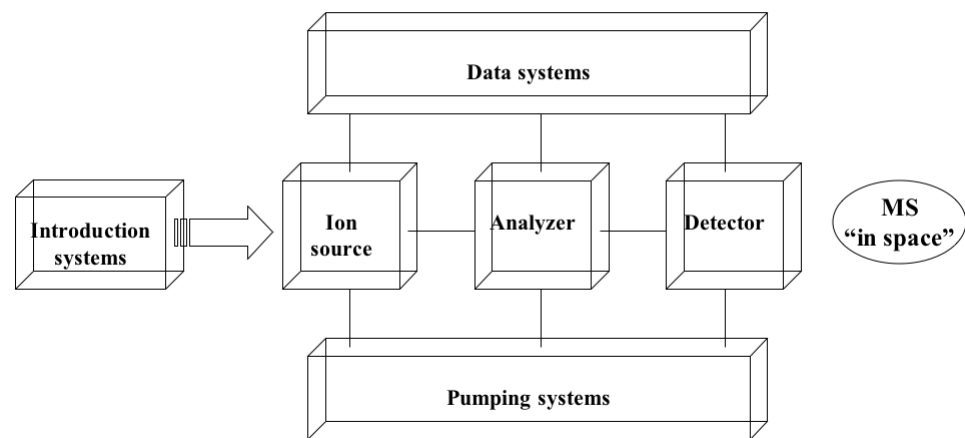
fixed ms



scanning ms

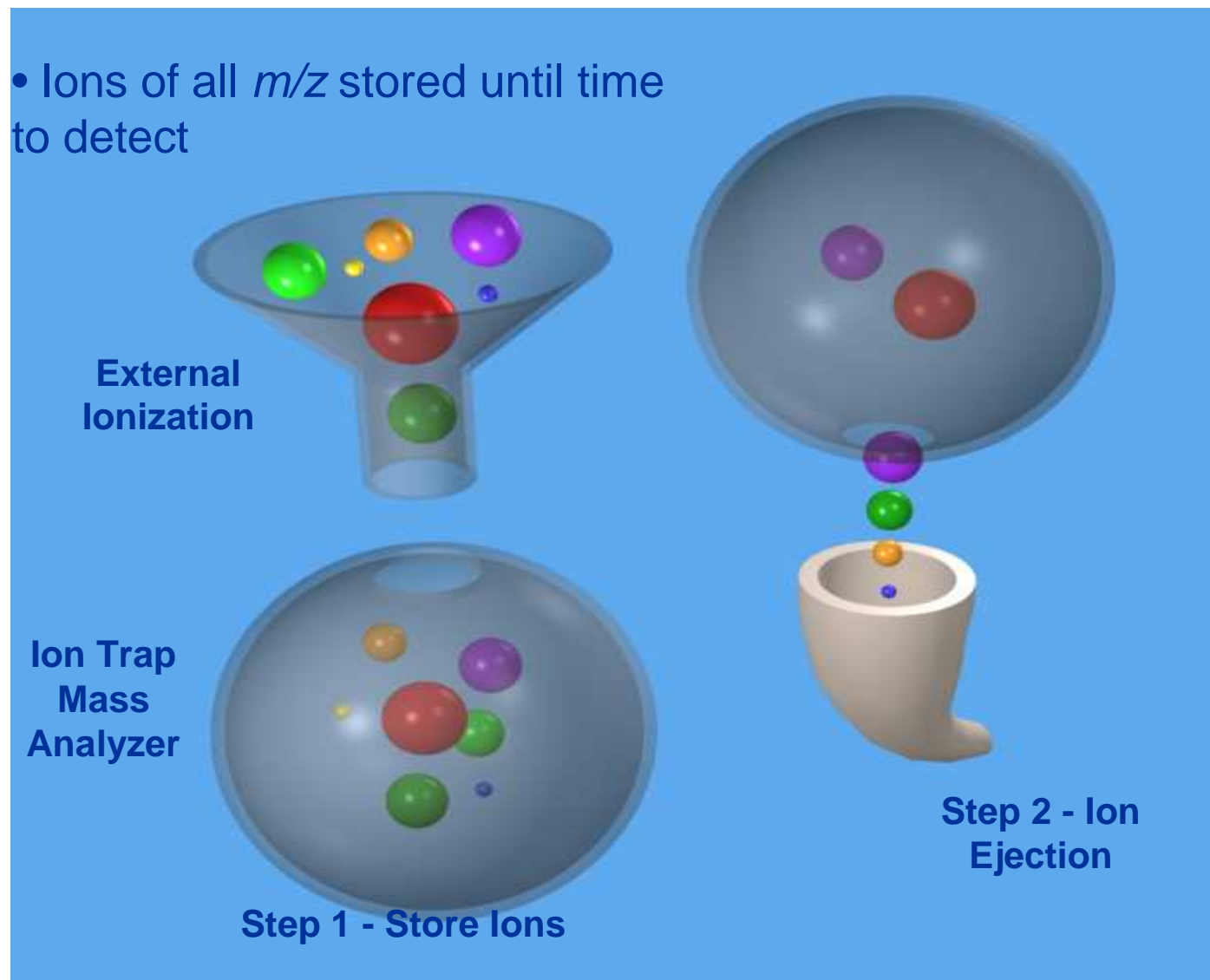




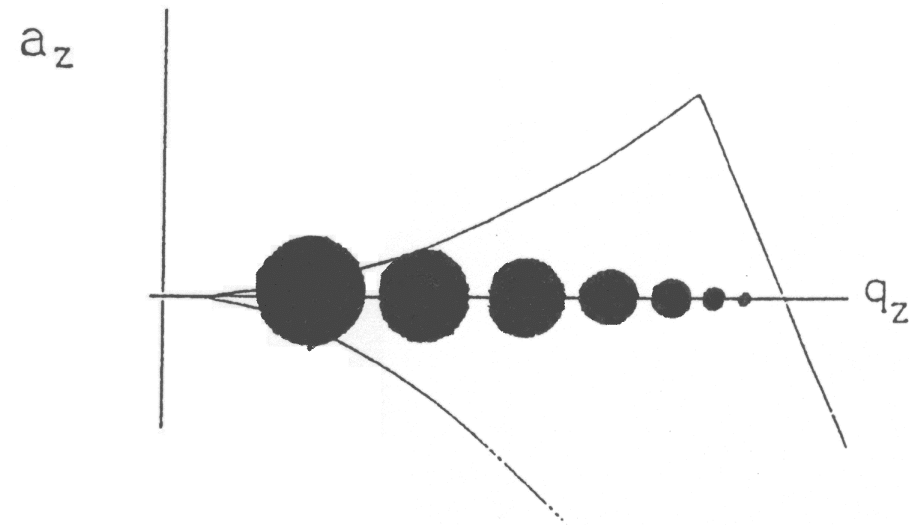


Conceptual Model of an External Ion Trap Mass Spectrometer

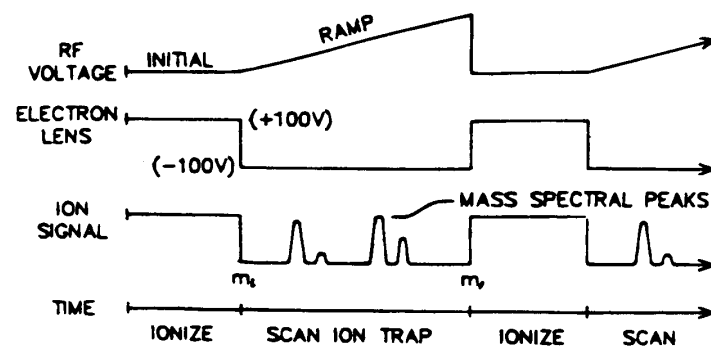
- Ions of all m/z stored until time to detect



$$q = -4zV/mr^2\omega^2$$

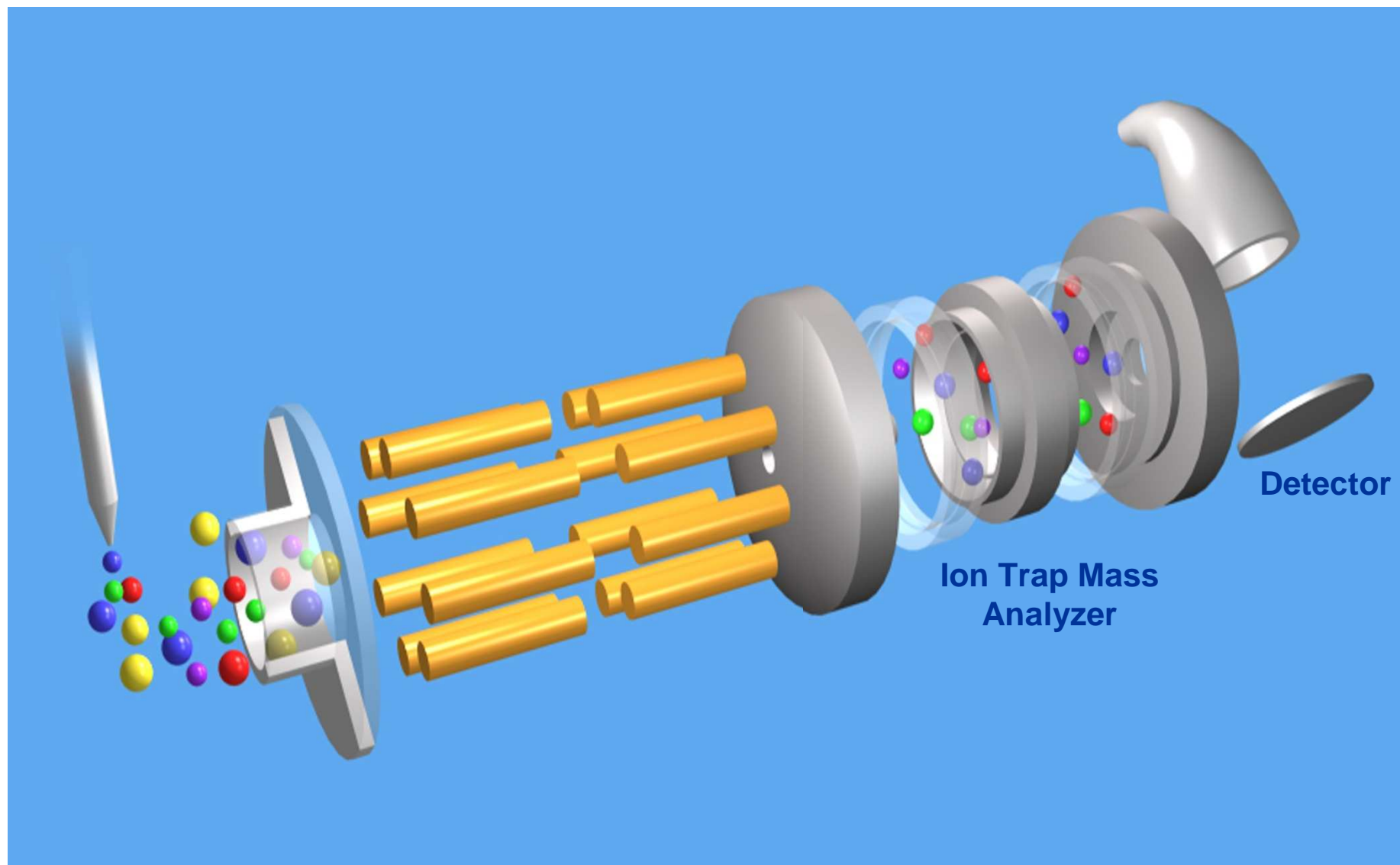


PRACTICAL ASPECTS OF ION TRAP MASS SPECTROMETRY VOL. III

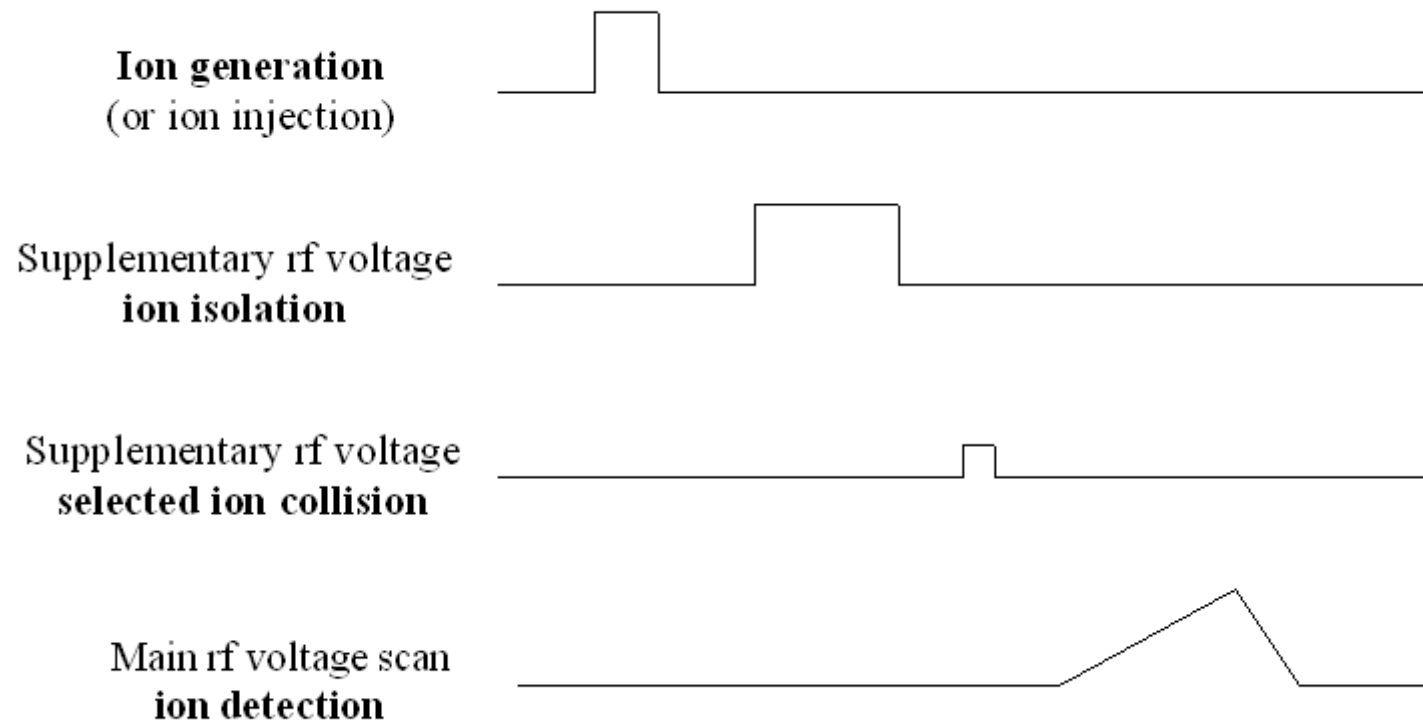


Simple timing sequence (the *scan function*) for the ion trap mass spectrometer. Reproduced with permission from Ref. 3. Copyright 1984 Finnigan Corporation. All rights reserved.

Ion Trap MS/MS: Full Scan MS

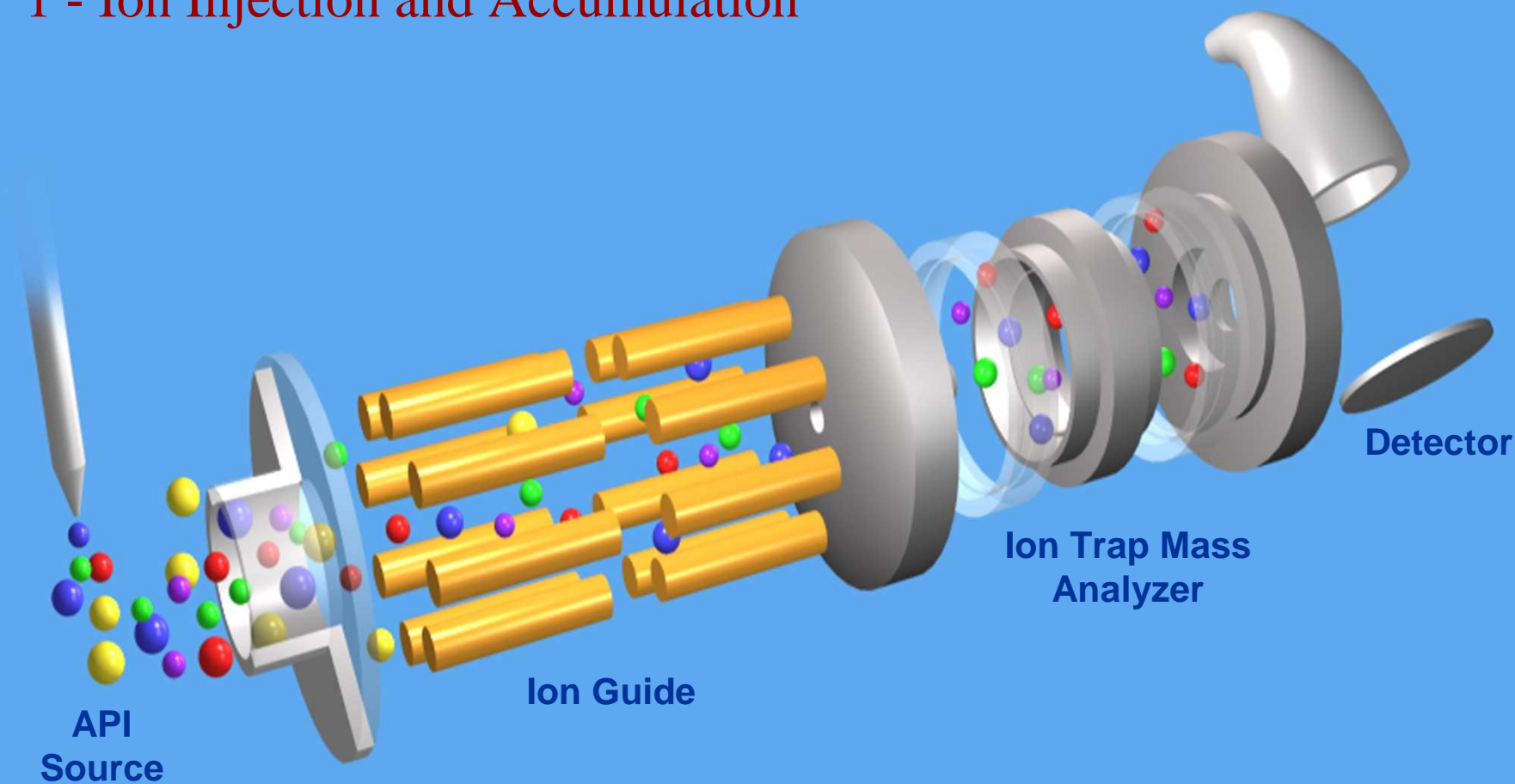


MS/MS in time: ion trap



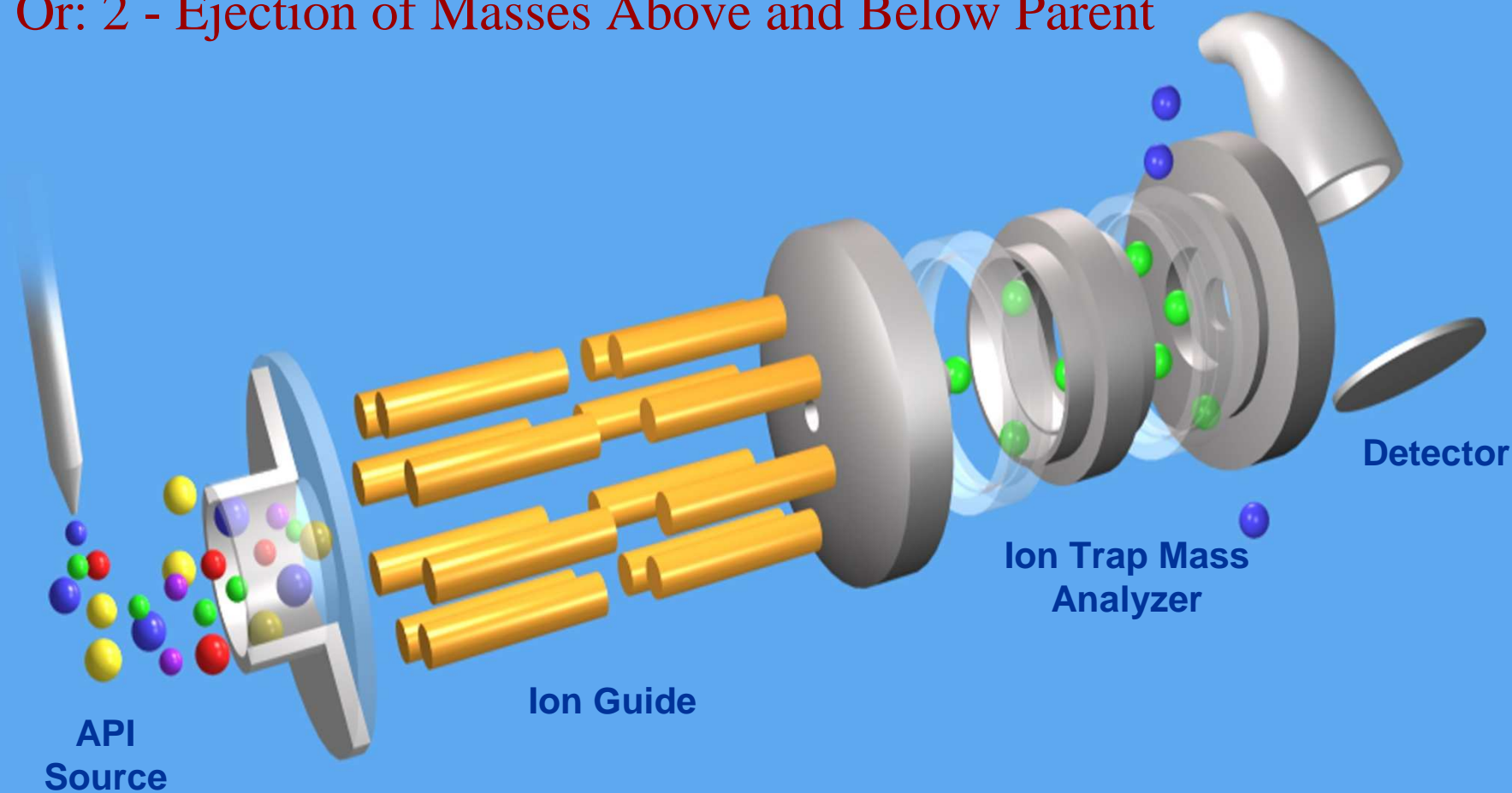
Ion Trap MS/MS

1 - Ion Injection and Accumulation

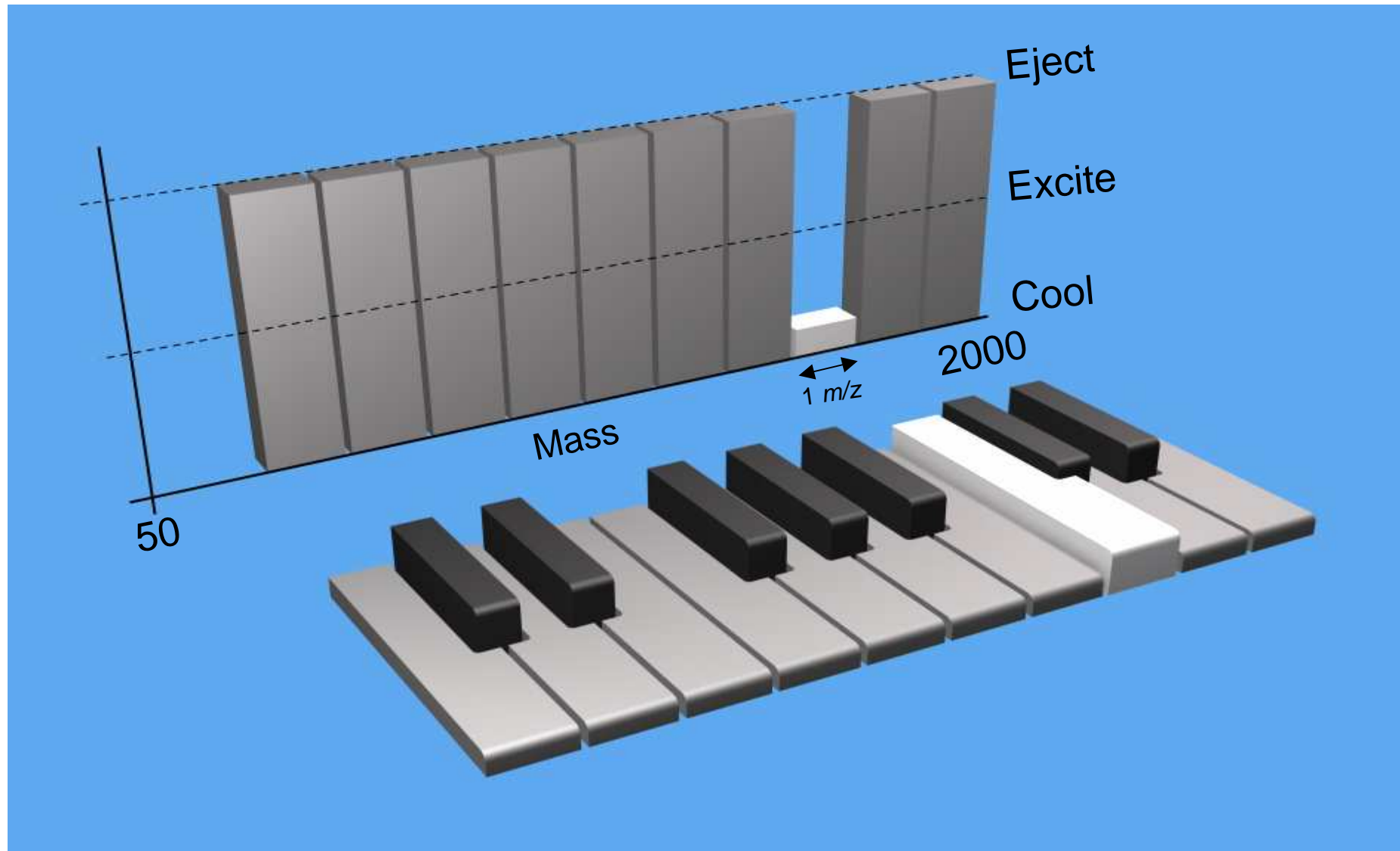


Ion Trap MS/MS: Isolation

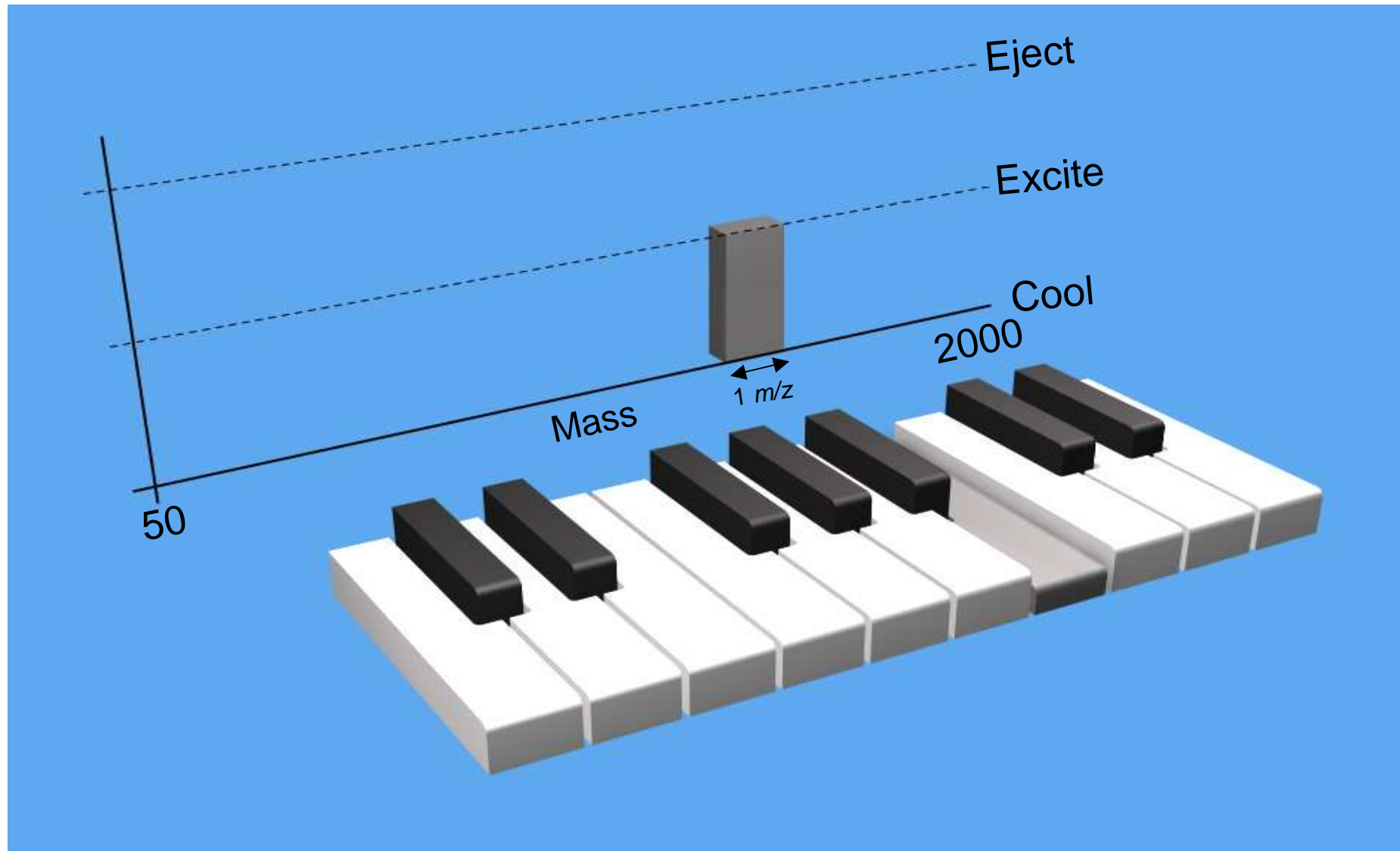
Or: 2 - Ejection of Masses Above and Below Parent



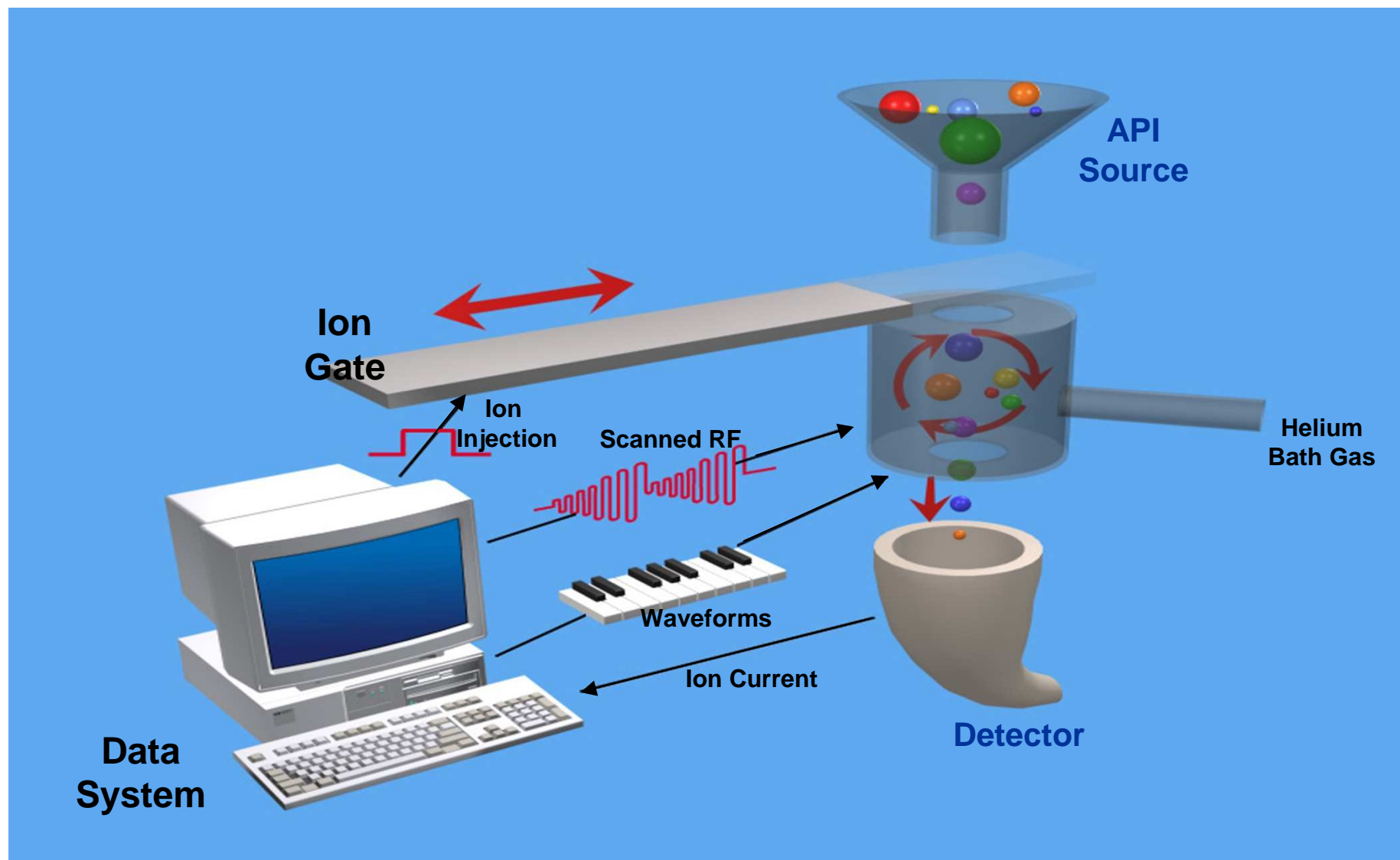
Apply Waveform to Isolate Parent Ion



Apply Waveform: Excite and Fragment



Summary: Conceptual Ion Trap Operation



Mediante la trappola ionica si possono
effettuare esperimenti MS/MS sequenziali

MSⁿ

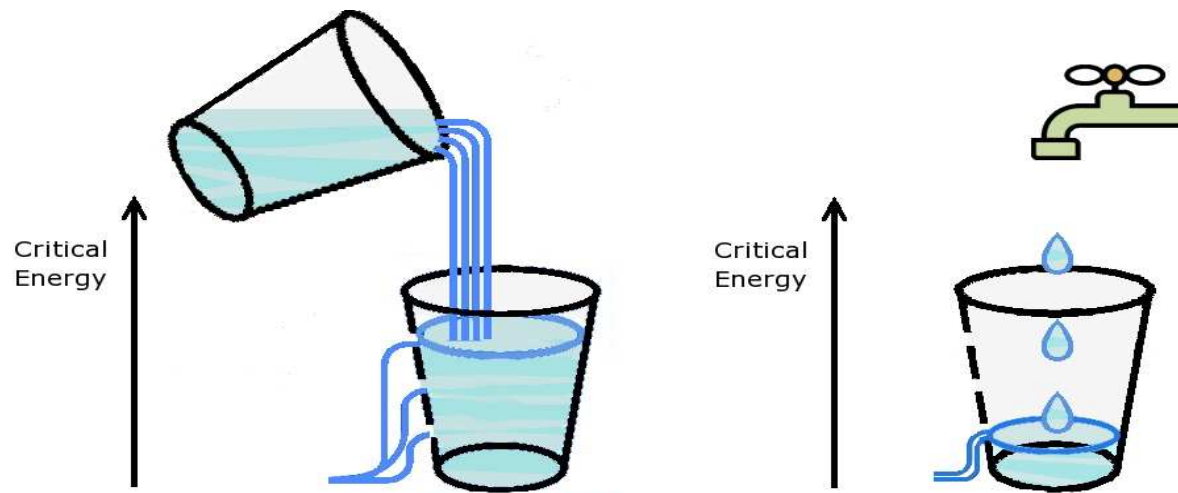


I fenomeni collisionali che avvengono nei sistemi QQQ e Ion Trap sono profondamente differenti:

- Diversi gas di collisione (Ar nel caso di QQQ, He nel caso di IT);

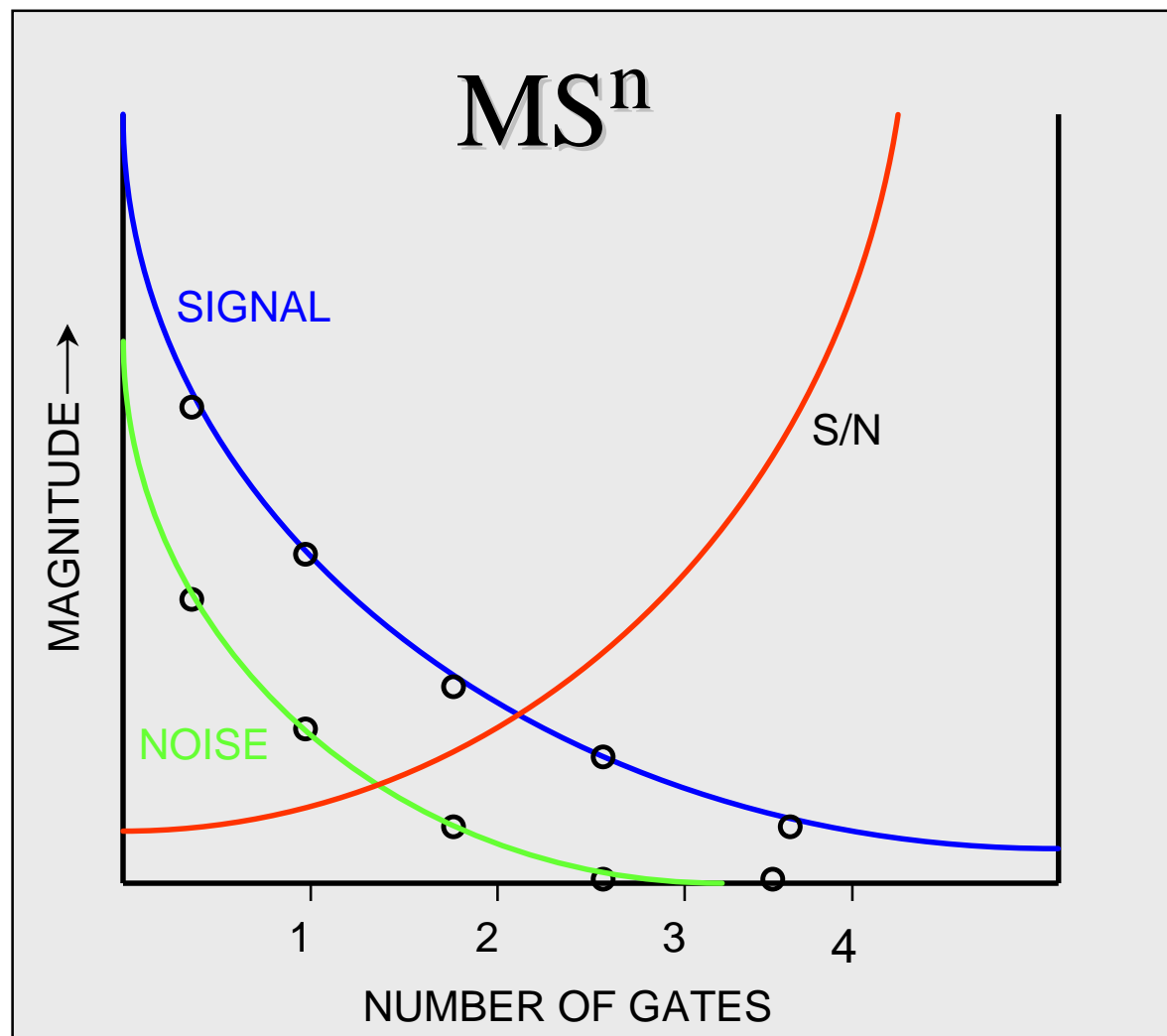
- Acquisizione di energia interna da collisione attraverso un processo step-by-step nel caso di IT.

Conseguentemente i processi a più bassi valori di energia di attivazione (critical energy) sono favoriti nel caso di IT.



A didactical view of the difference existing between MS/MS experiments performed by QQQ (left side) or by resonant excitation by ion trap (right side).

MS^n AUMENTA LA SENSIBILITA'



From: Busch K.L., Cooks G. in "Tandem Mass Spectrometry"
Ed. Mc Lafferty Wiley, N.Y. 1983, 11-39

I limiti della trappola ionica sono dovuti a fenomeni di densità di carica spaziale, che limitano il numero di ioni che possono essere confinati al suo interno.

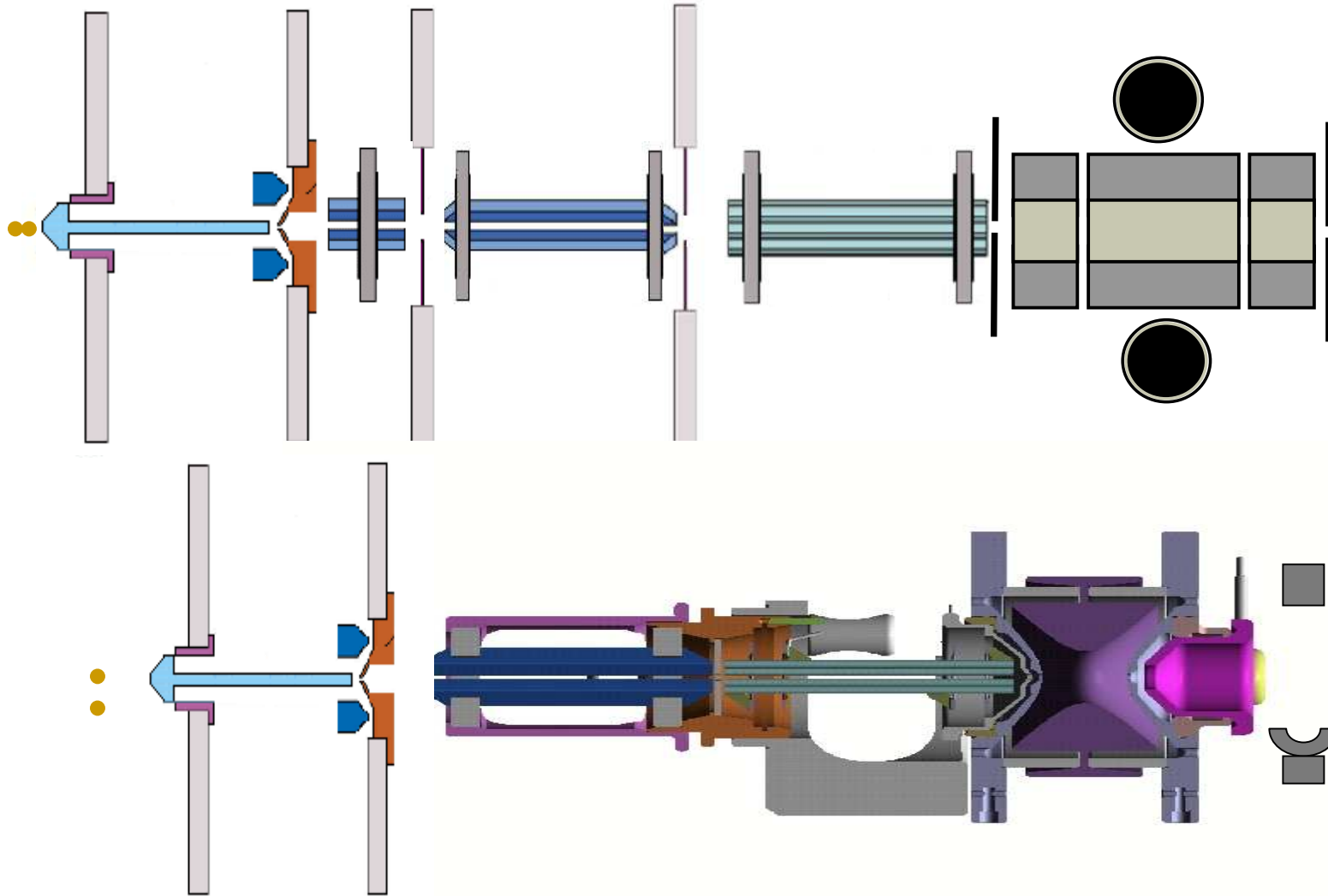
Soluzione:

Aumentare il volume di intrappolamento o aumentare l'intensità del campo elettrico.

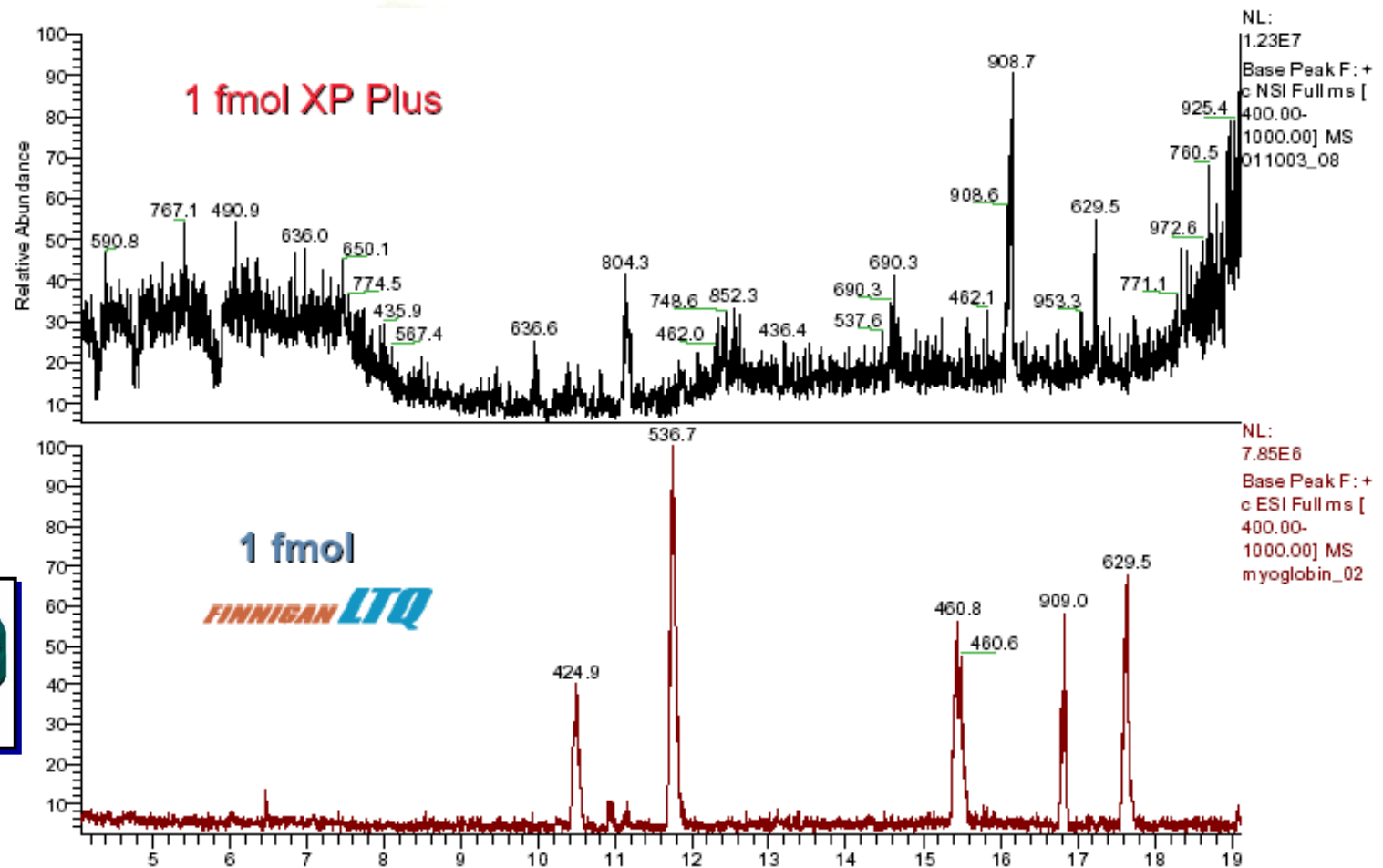
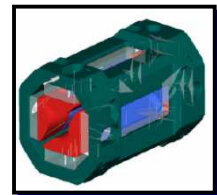
Nel primo caso, sviluppo di

Linear Ion Trap

Ion Ejection – 2D vs 3D



Myoglobin Tryptic Digest – 1fmol LCMS/MS

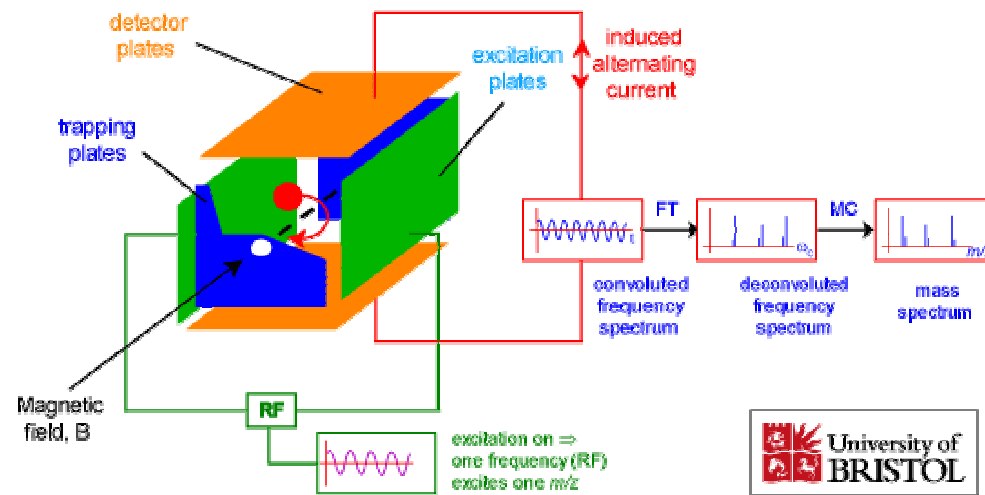


Come aumentare la specificità di un esperimento MS/MS?

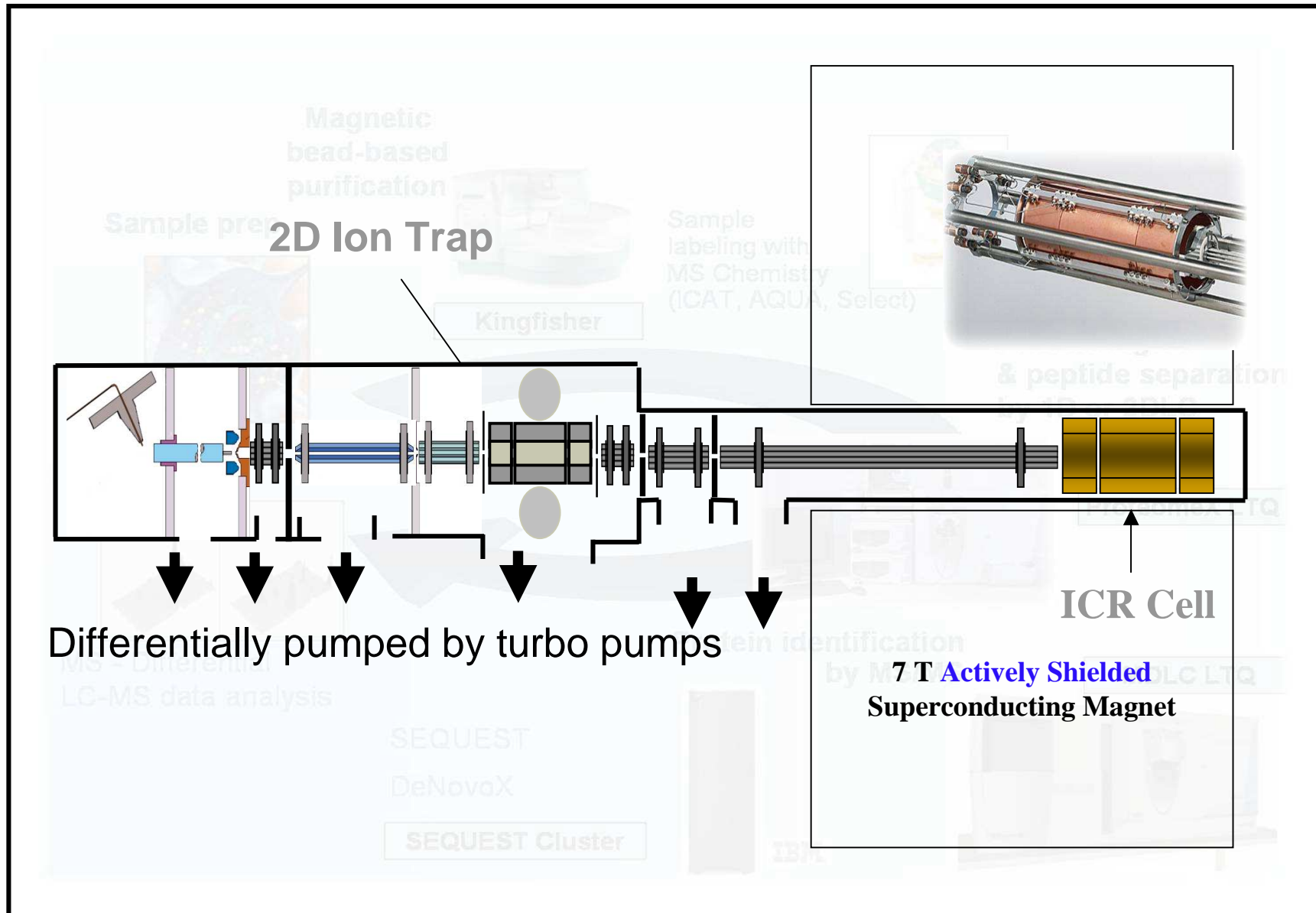
Aumentando la risoluzione del secondo analizzatore, così da determinare la massa accurata dei frammenti ottenuti da collisione.

Soluzioni:

- Q (o LIT)/FTICR (R=10⁶)**
- Q (o LIT)/TOF (R=10⁴)**
- Q (o LIT)/Orbitrap (R=10⁵)**



LINEAR ION TRAP – FTICR HYBRID



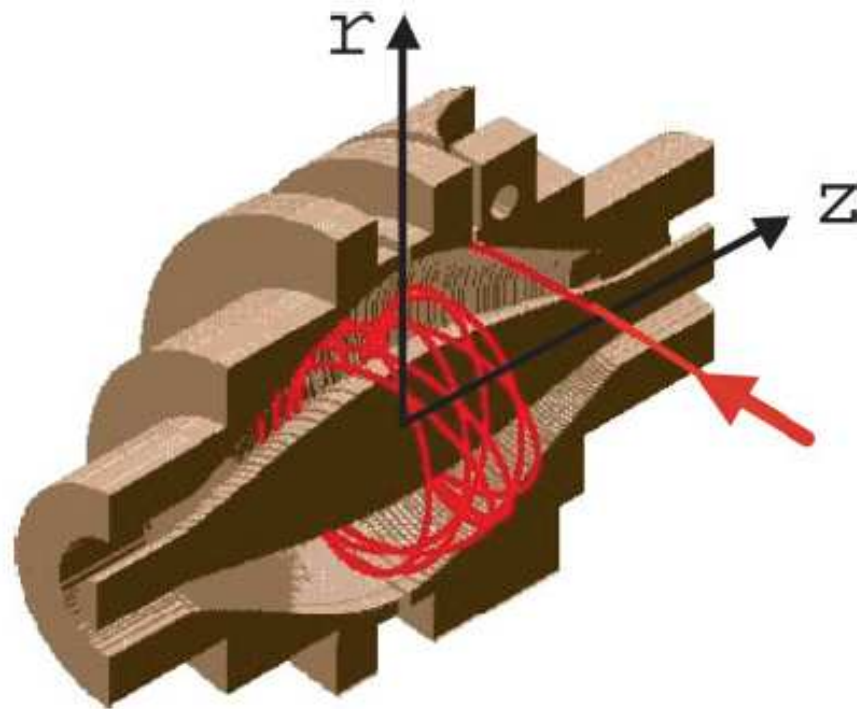
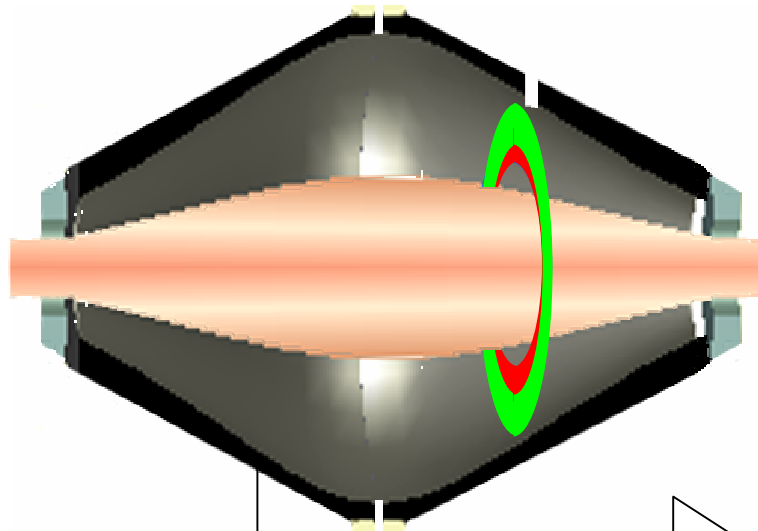
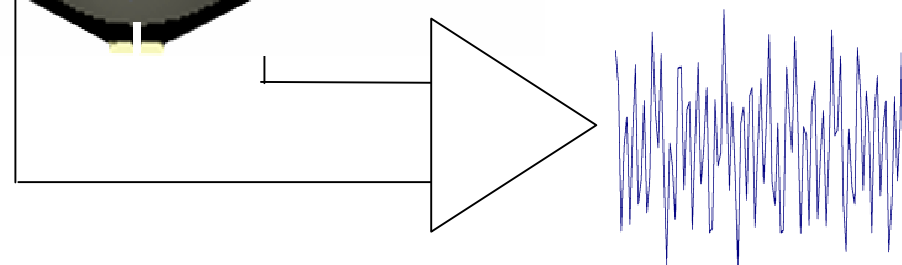


Figure 1. Cutaway view of the Orbitrap mass analyzer. Ions are injected into the Orbitrap at the point indicated by the red arrow. The ions are injected with a velocity perpendicular to the long axis of the Orbitrap (the z -axis). Injection at a point displaced from $z = 0$ gives the ions potential energy 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.



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

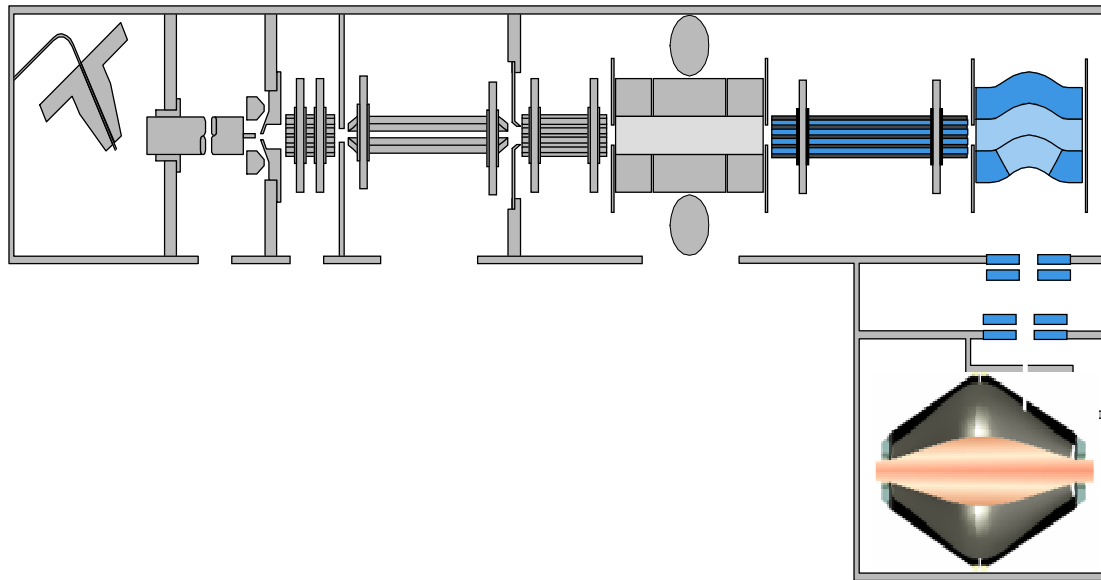


1. Frequencies are determined using a Fourier Transformation
2. For higher sensitivity AND resolution, transients should not decay too fast

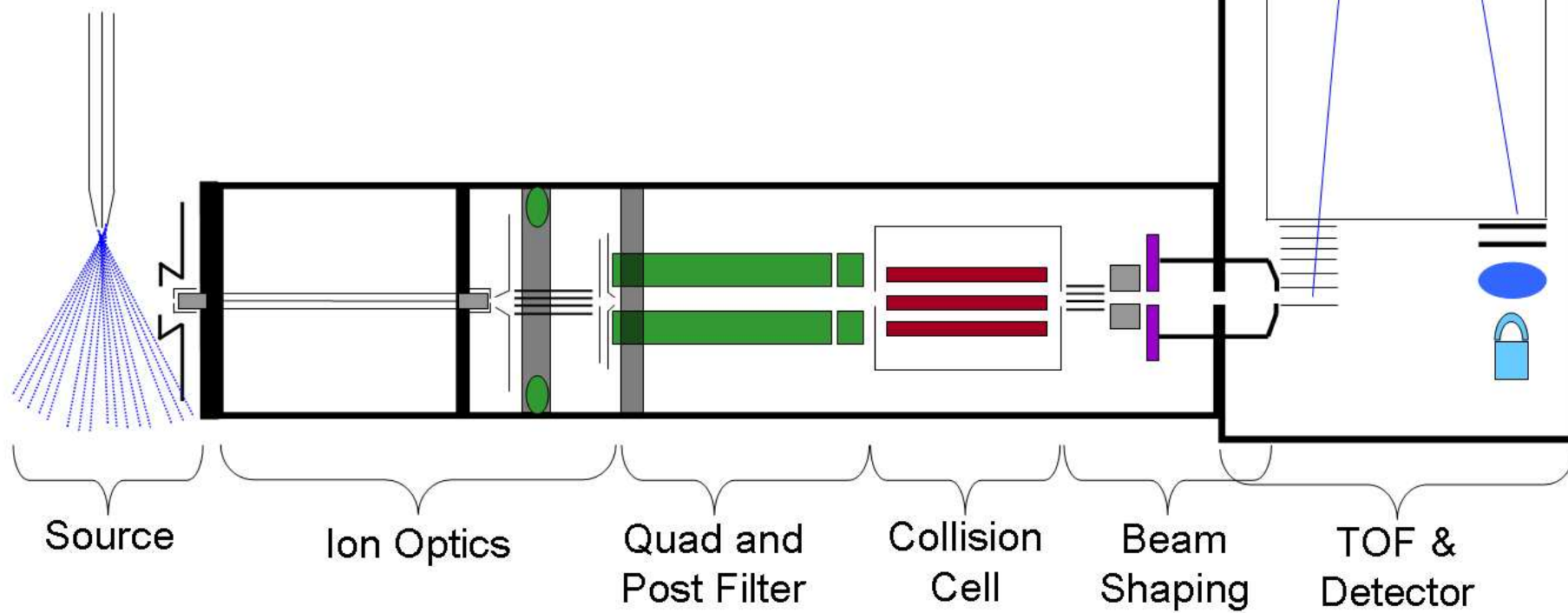
Ultra-high vacuum

Ultra-high precision

Linear ion trap - Orbitrap



Q-TOF

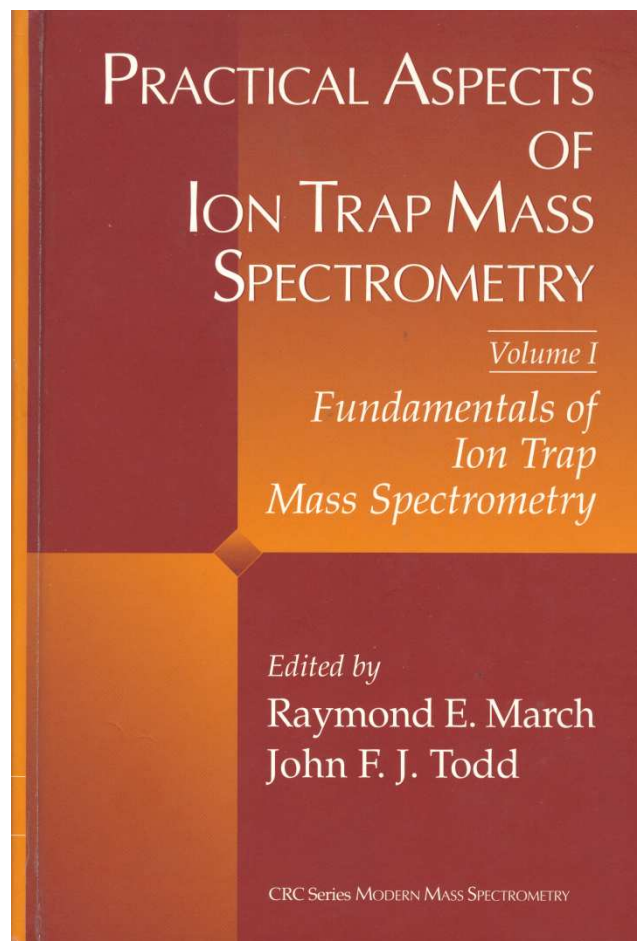


Agilent Technologies

Il futuro

*In una concezione lineare del tempo, il **futuro** è la parte di tempo che ancora non ha avuto luogo; nella concezione relativistica il settore dello spaziotempo nel quale si trovano tutti gli eventi che ancora non sono accaduti dato uno specifico sistema di riferimento.*





DEDICATION

To ion trappers, young and old, everywhere.