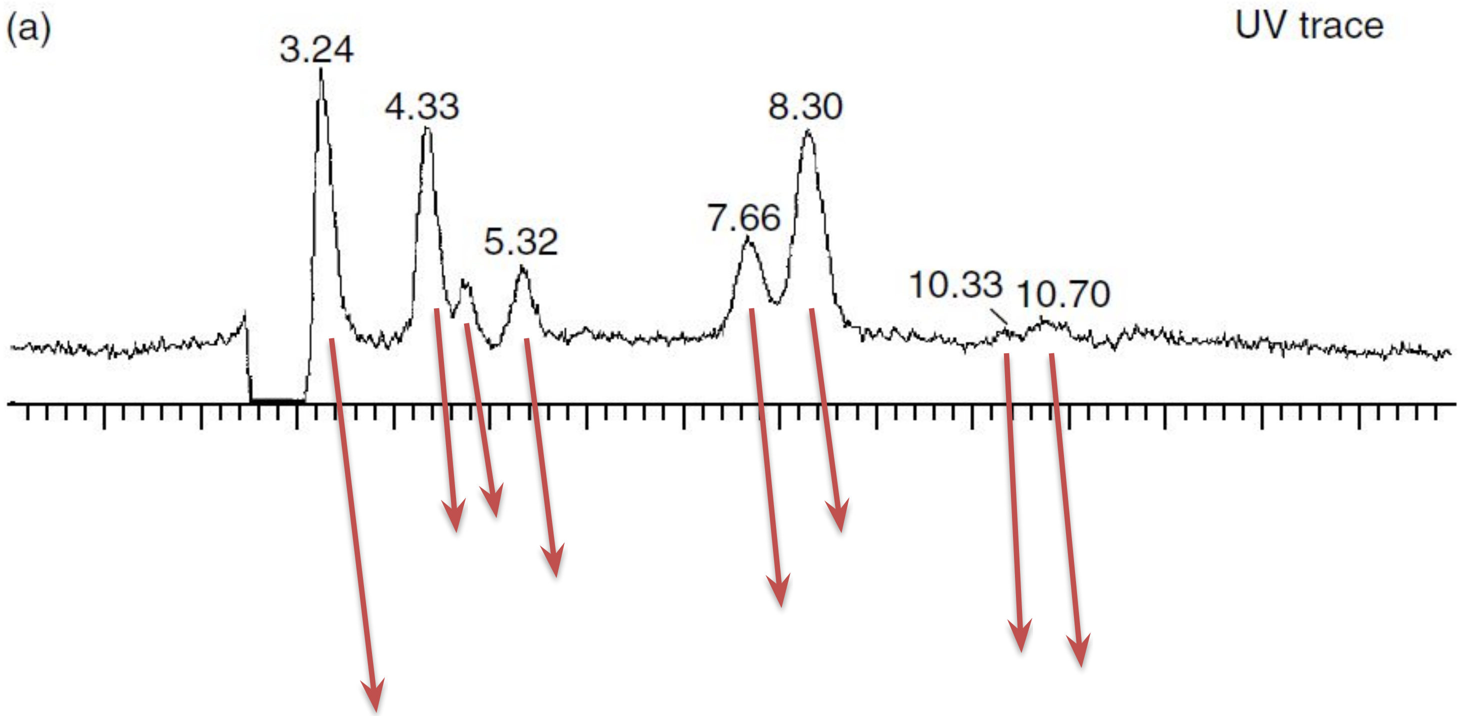
Espectrometria de Massas:

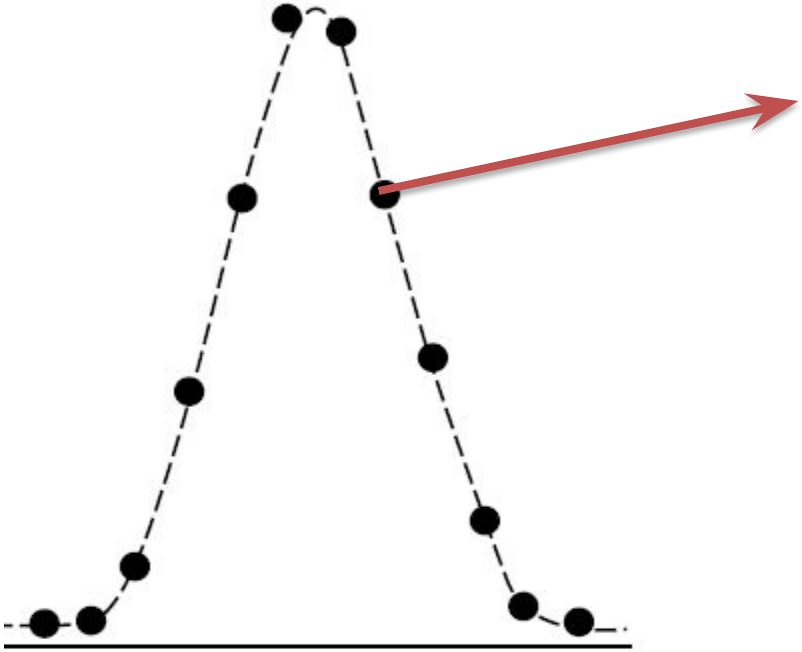
- Analisadores de massa
  - Resolução e Exatidão de Massa
- Detectores

Álvaro José dos Santos Neto

Comparação entre cromatograma de LC-UV e LC-MS (modo TIC)



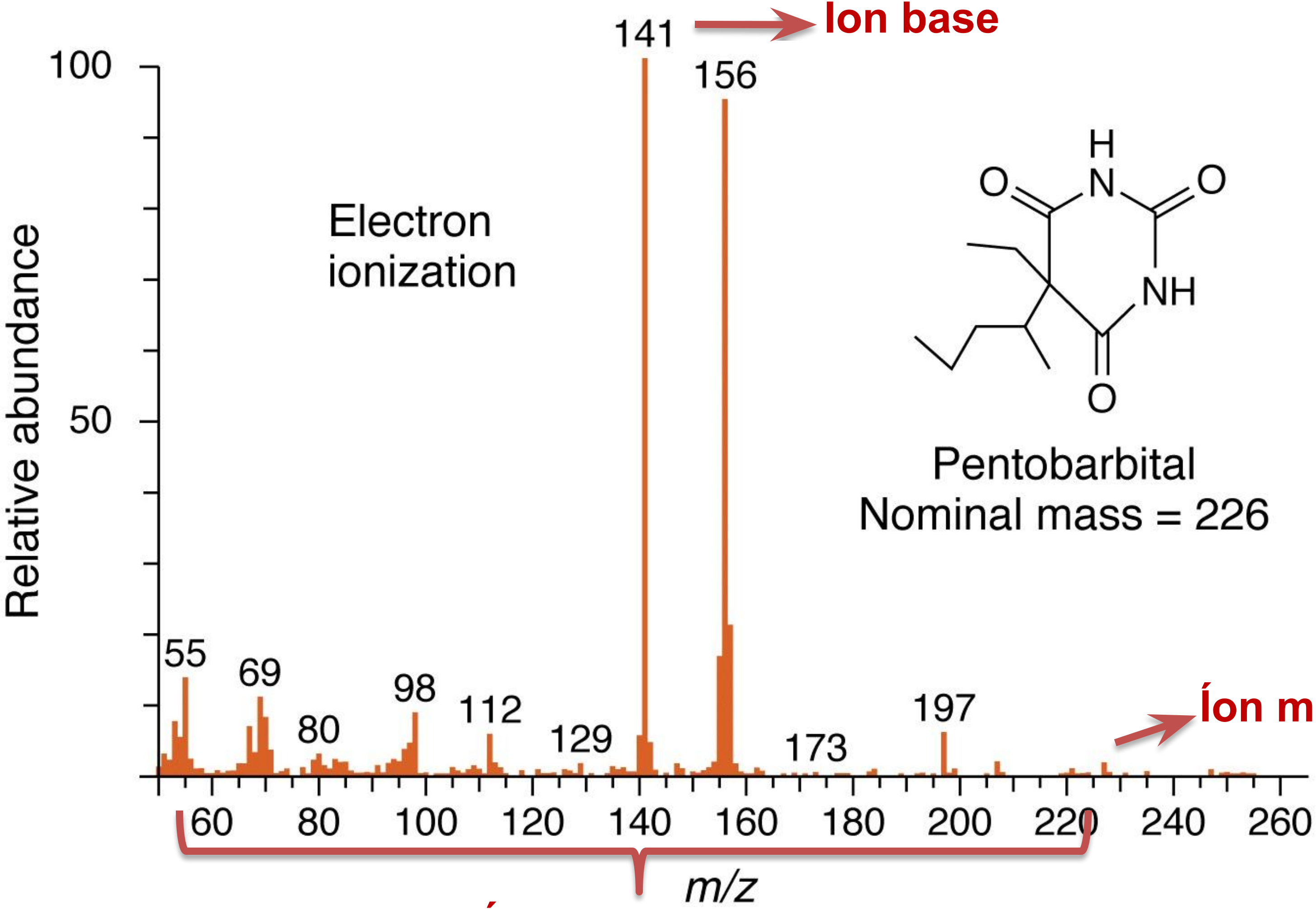
O pico cromatográfico em MS



Contém toda a informação de MS:
- Espectro completo (TIC)
- Íons selecionados (SIM)

Ou toda a informação de MS/MS:
- Reações específicas (MRM)
- Perda neutra (*neutral loss*)
- Íons precursores
- Íons produtos

O Espectro de Massas

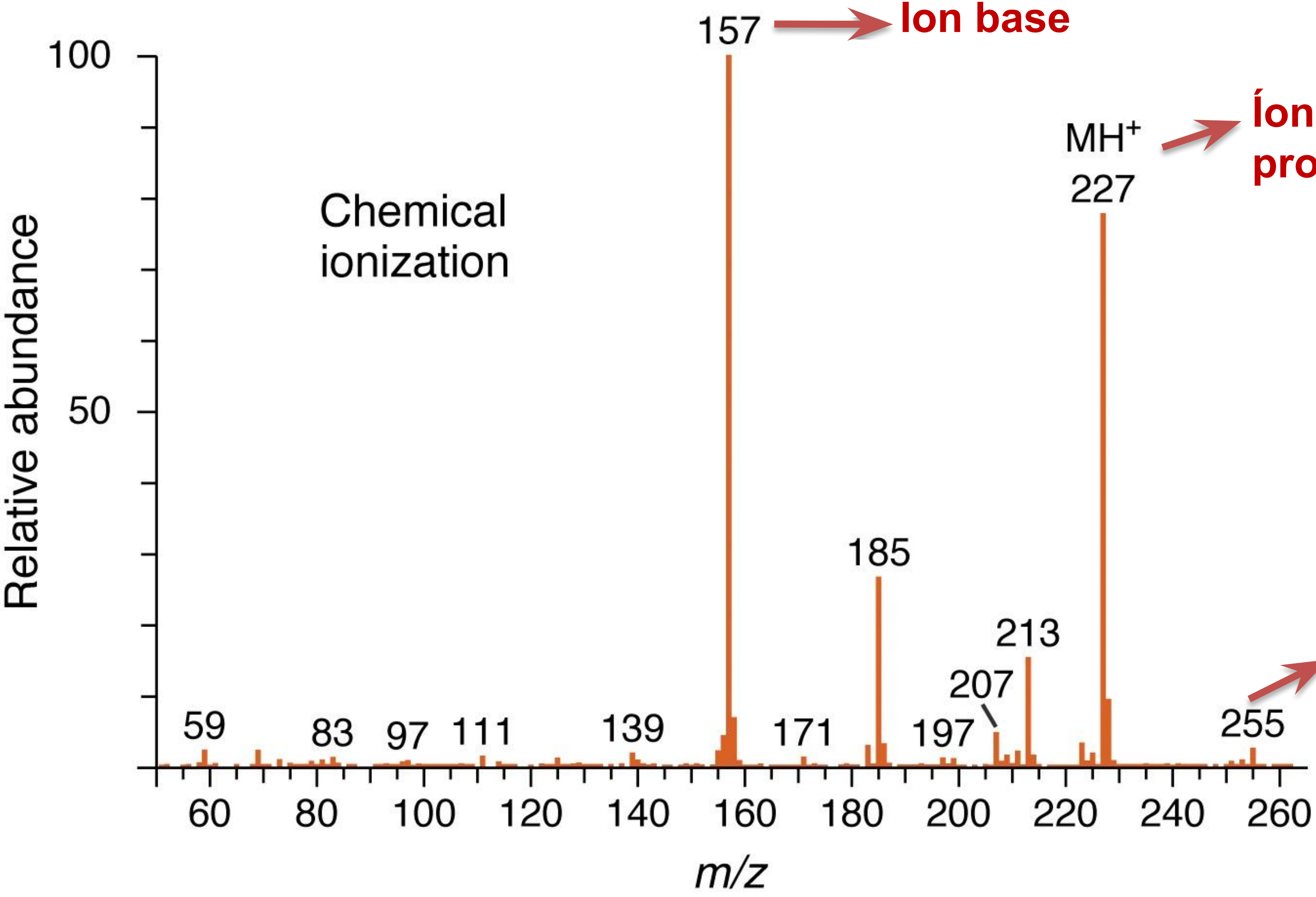


Íon base

Íon molecular

Íons fragmentos

O Espectro de Massas



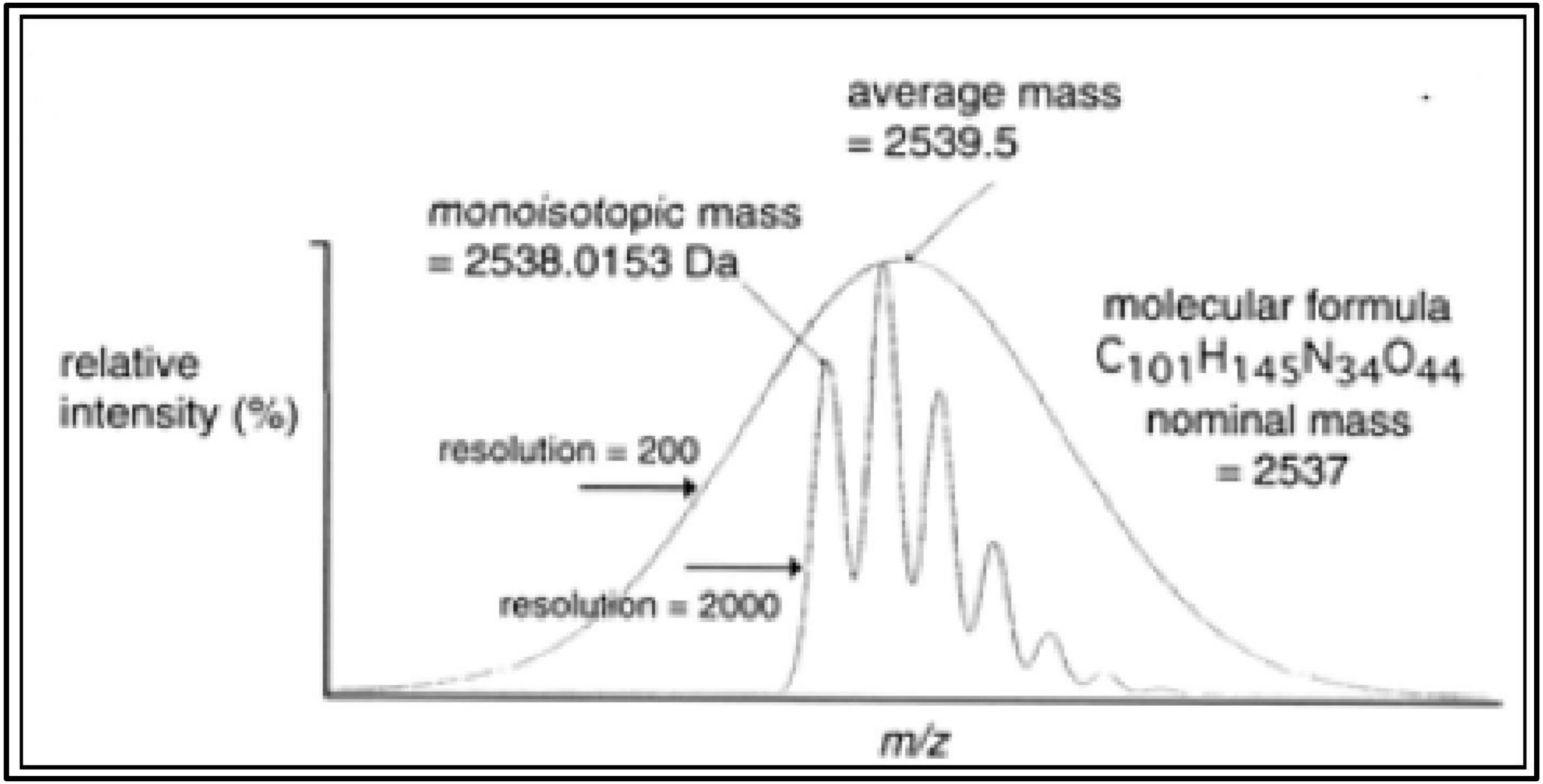
Íon base

Íon molecular protonado

Adutos ?

Diferentes termos em MS:

- **Massa nominal:** a massa calculada para um íon levando em conta a massa inteira do isótopo mais abundante de cada elemento.
- **Massa monoisotópica:** a massa calculada a partir da massa exata do isótopo mais abundante de cada elemento.
- **Massa do íon mais abundante:** a massa correspondente ao pico mais intenso dentro do conjunto de picos isotópicos de um mesmo composto.
- **Massa média:** é a média ponderada de todos os picos isotópicos de um determinado composto.


average mass
= 2539.5
monoisotopic mass
= 2538.0153 Da
molecular formula
$C_{101}H_{145}N_{34}O_{44}$
nominal mass
= 2537
relative
intensity (%)
resolution = 200
resolution = 2000
m/z

Etapas na análise por MS

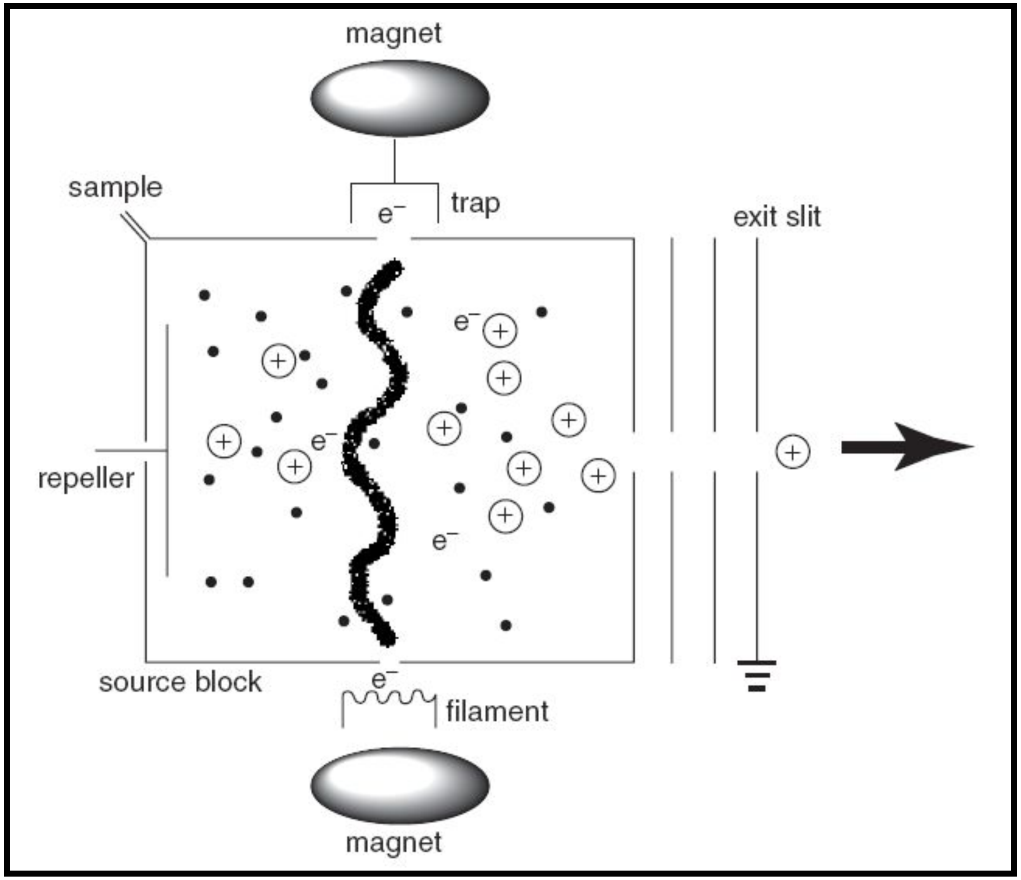
1. Ionização dos analitos

2. Seleção/Separação das m/z dos íon*

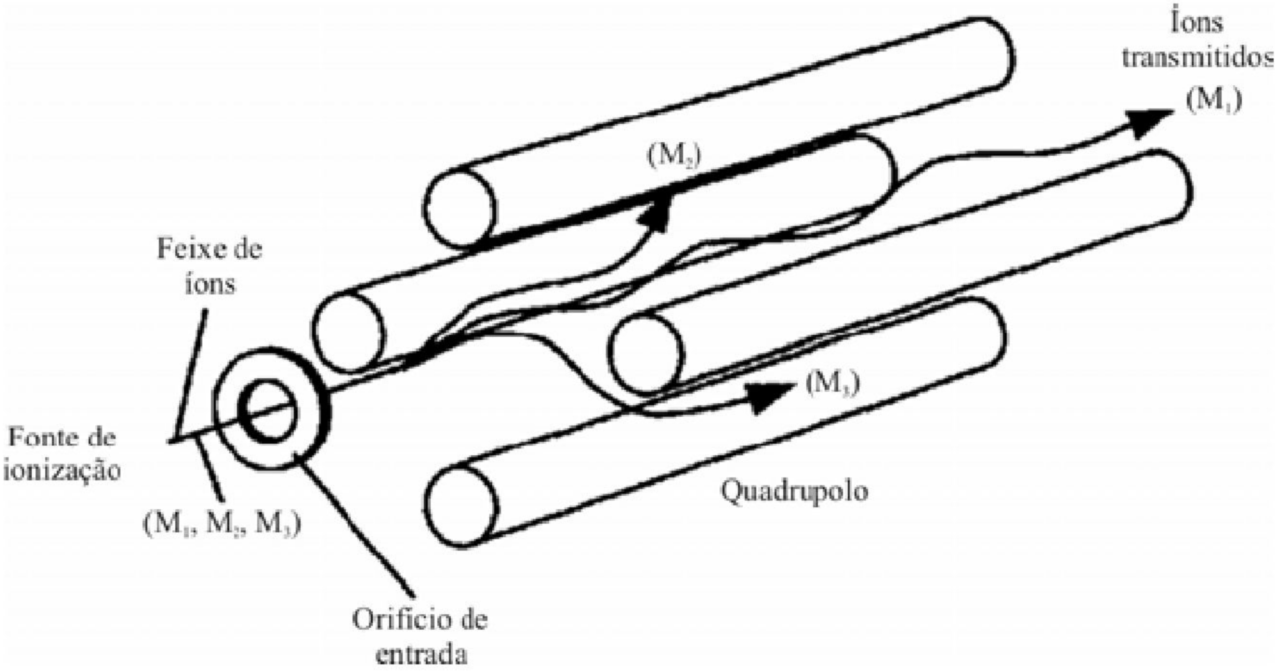
3. Detecção

1. Ionização

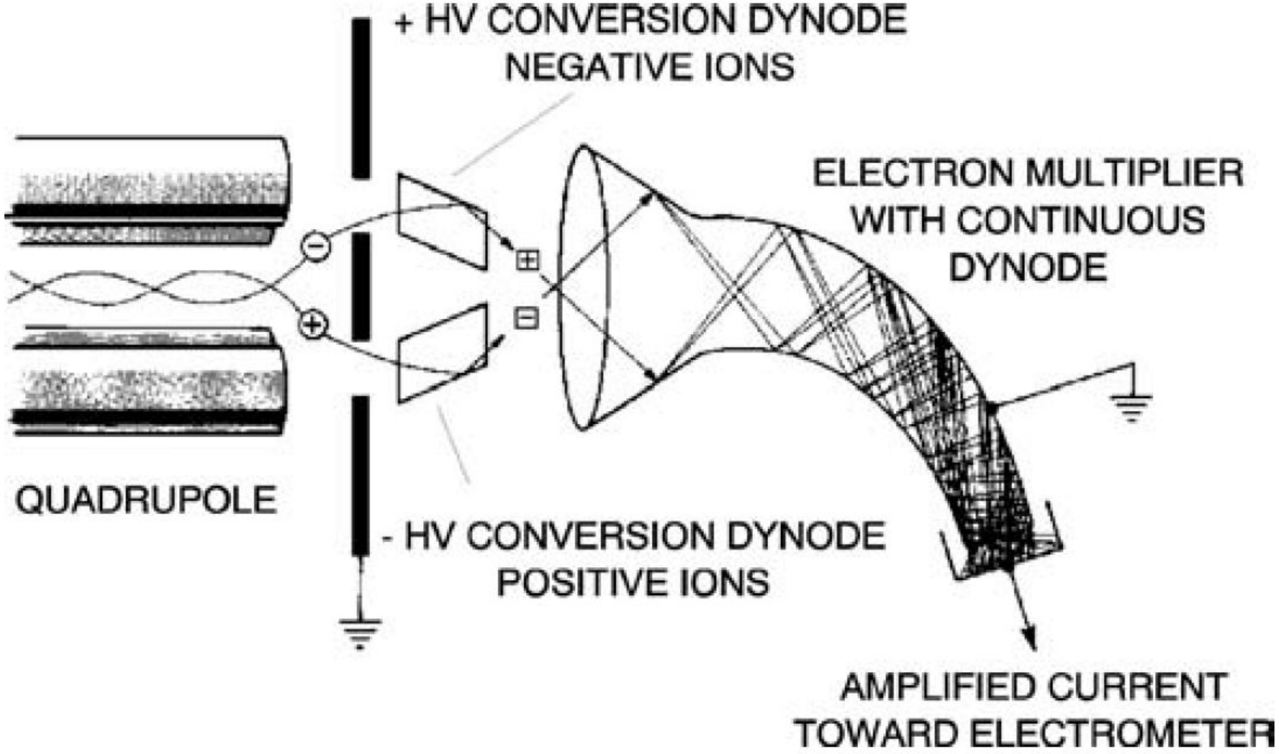
$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$



2. Análise/separação



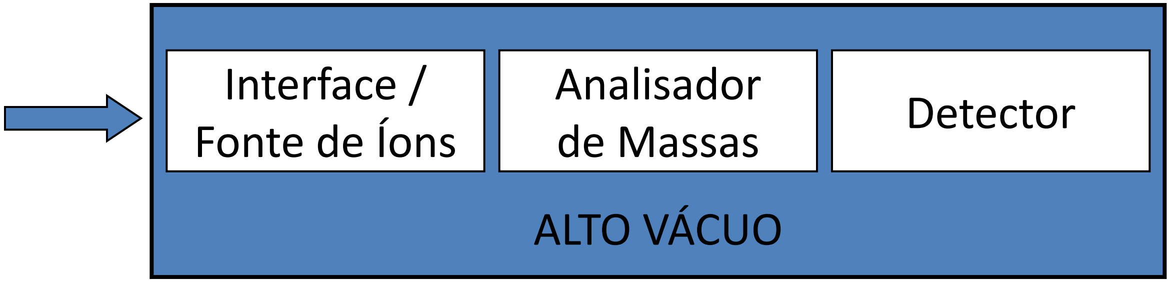
3. Detecção


+ HV CONVERSION DYNODE
NEGATIVE IONS
ELECTRON MULTIPLIER
WITH CONTINUOUS
DYNODE
QUADRUPOLE
- HV CONVERSION DYNODE
POSITIVE IONS
AMPLIFIED CURRENT
TOWARD ELECTROMETER

Diagramas de blocos de um MS

Modo de Ionização no Vácuo

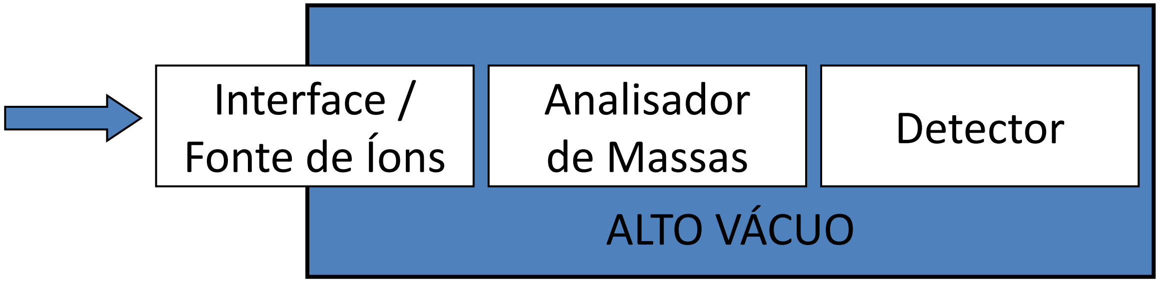
Introdução dos analitos (sistema de cromatografia)



Exemplos: EI, CI, PI, MALDI, TSI, FAB, SIMS, FI/FD

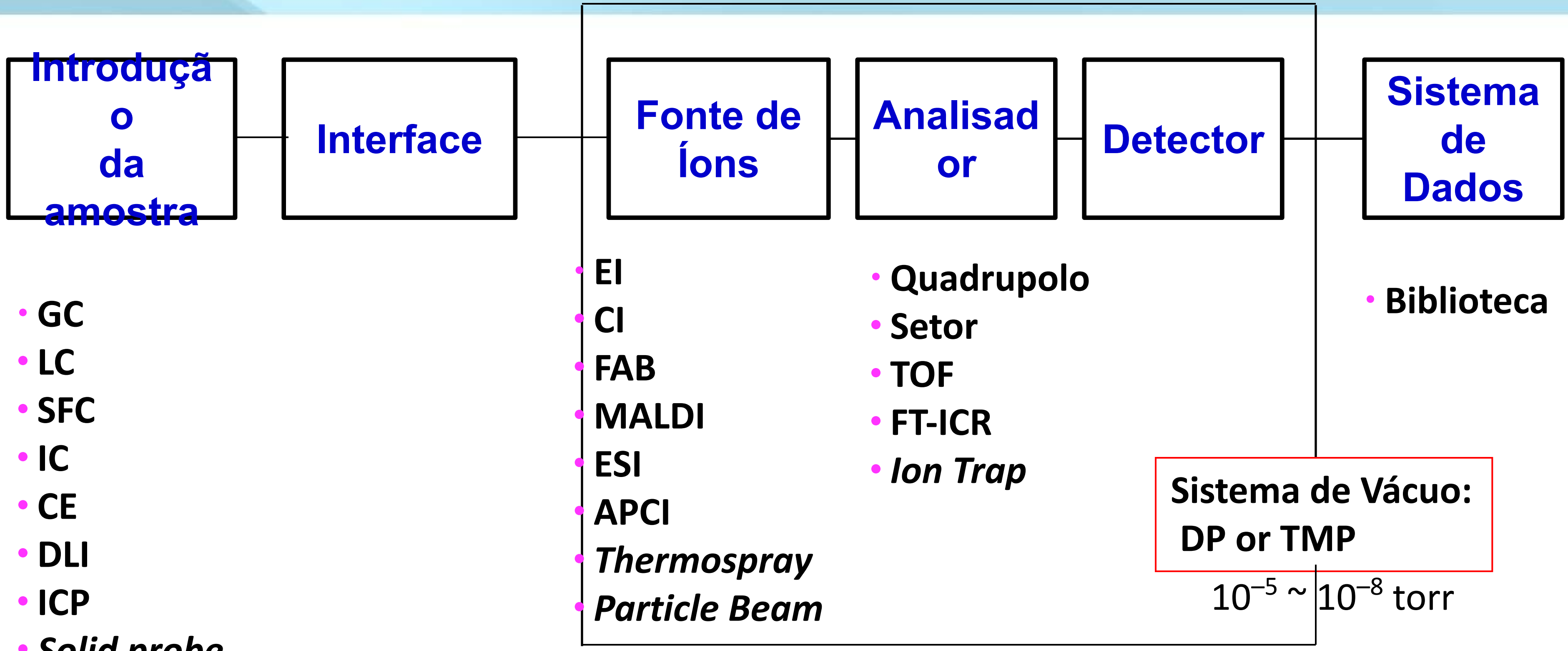
Modo de Ionização à Pressão Atmosférica (API)

Introdução dos analitos (sistema de cromatografia)



Exemplos: ESI, APCI, APPI, AP-MALDI, DESI, DART, EASI

Tipos de componentes para um espectrômetro de massas



Introdução da amostra

Interface

Fonte de Íons

Analisador

Detector

Sistema de Dados

- GC
- LC
- SFC
- IC
- CE
- DLI
- ICP
- *Solid probe*

- EI
- CI
- FAB
- MALDI
- ESI
- APCI
- *Thermospray*
- *Particle Beam*

- Quadrupolo
- Setor
- TOF
- FT-ICR
- *Ion Trap*

- Biblioteca

Sistema de Vácuo:
DP or TMP

$10^{-5} \sim 10^{-8}$ torr

- MS/MS – Q/TOF, TOF/TOF
- HRMS
- LRMS

Sistemas de vácuo – Bomba rotativa (*roughing* ou *rotary*)

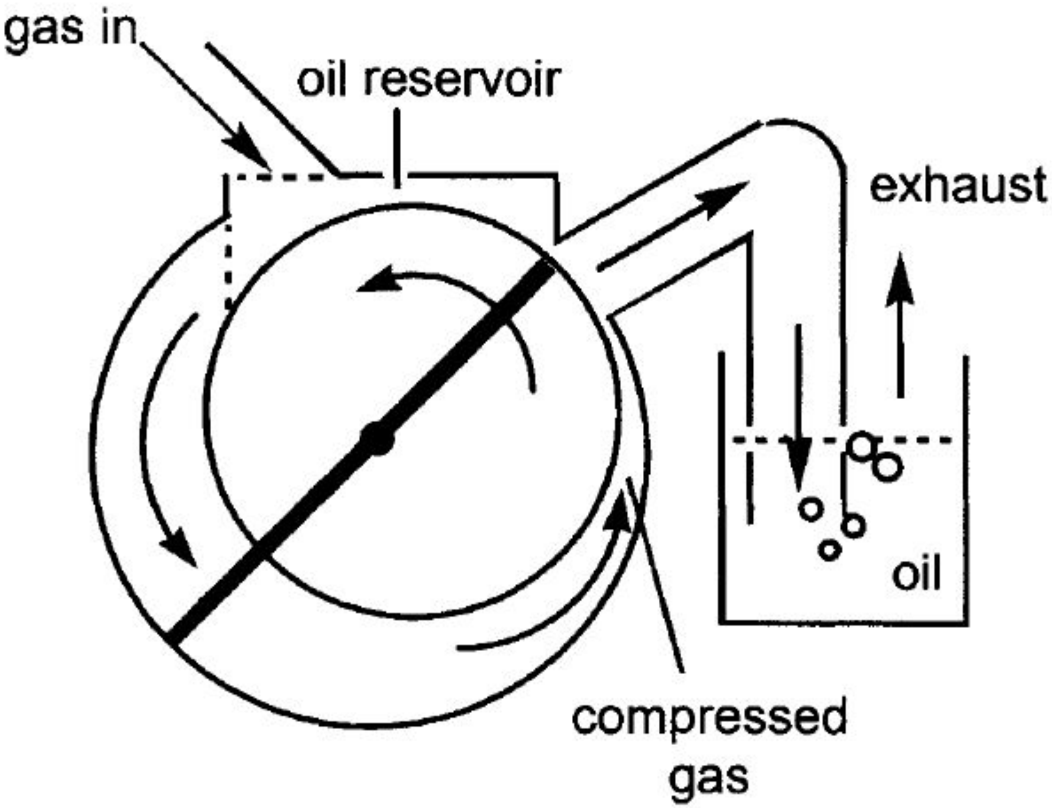


Figure 3.28 *Cross-section of a rotary vacuum pump*

Sistemas de vácuo – bomba turbomolecular

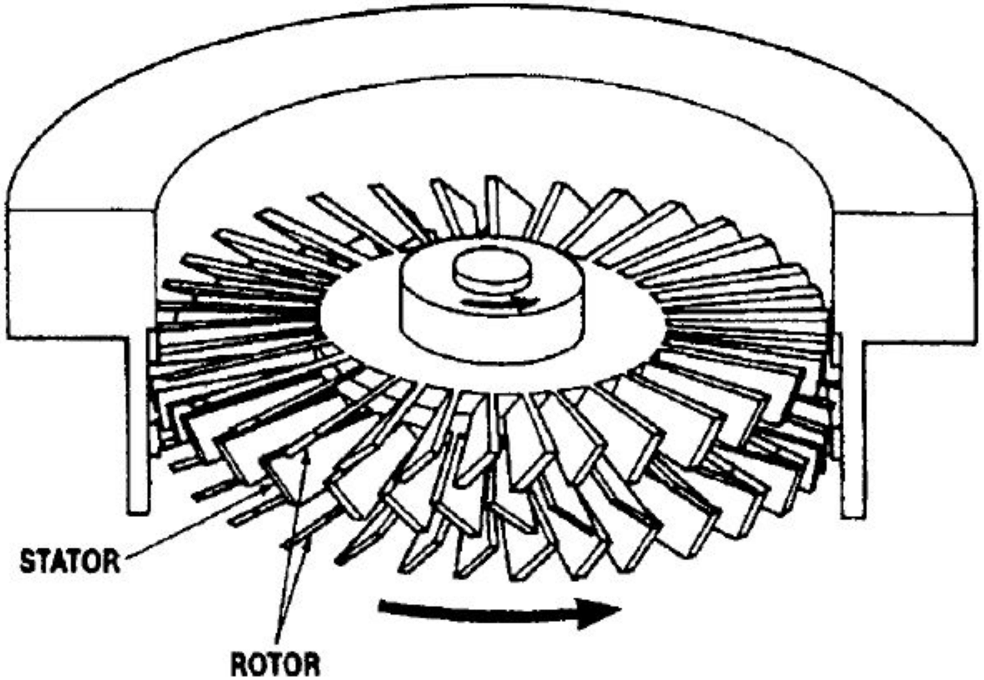


Figure 3.30 *View of the rotor of a turbomolecular pump*
(Source: J.T. Watson, *Introduction to Mass Spectrometry*, 3rd edition, Lippincott-Raven, Philadelphia, 1997, Figure 19.7, p. 431)

Sistemas de vácuo – Bomba criogênica

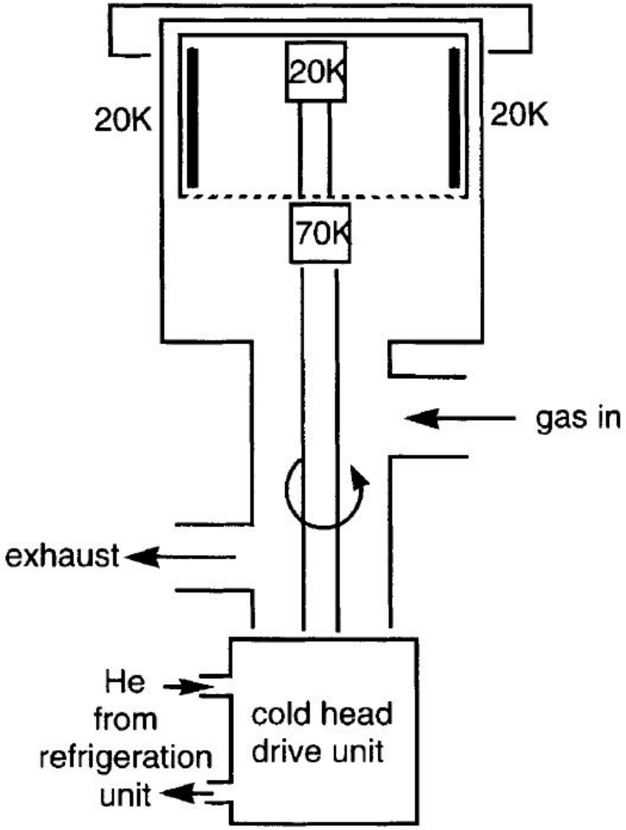
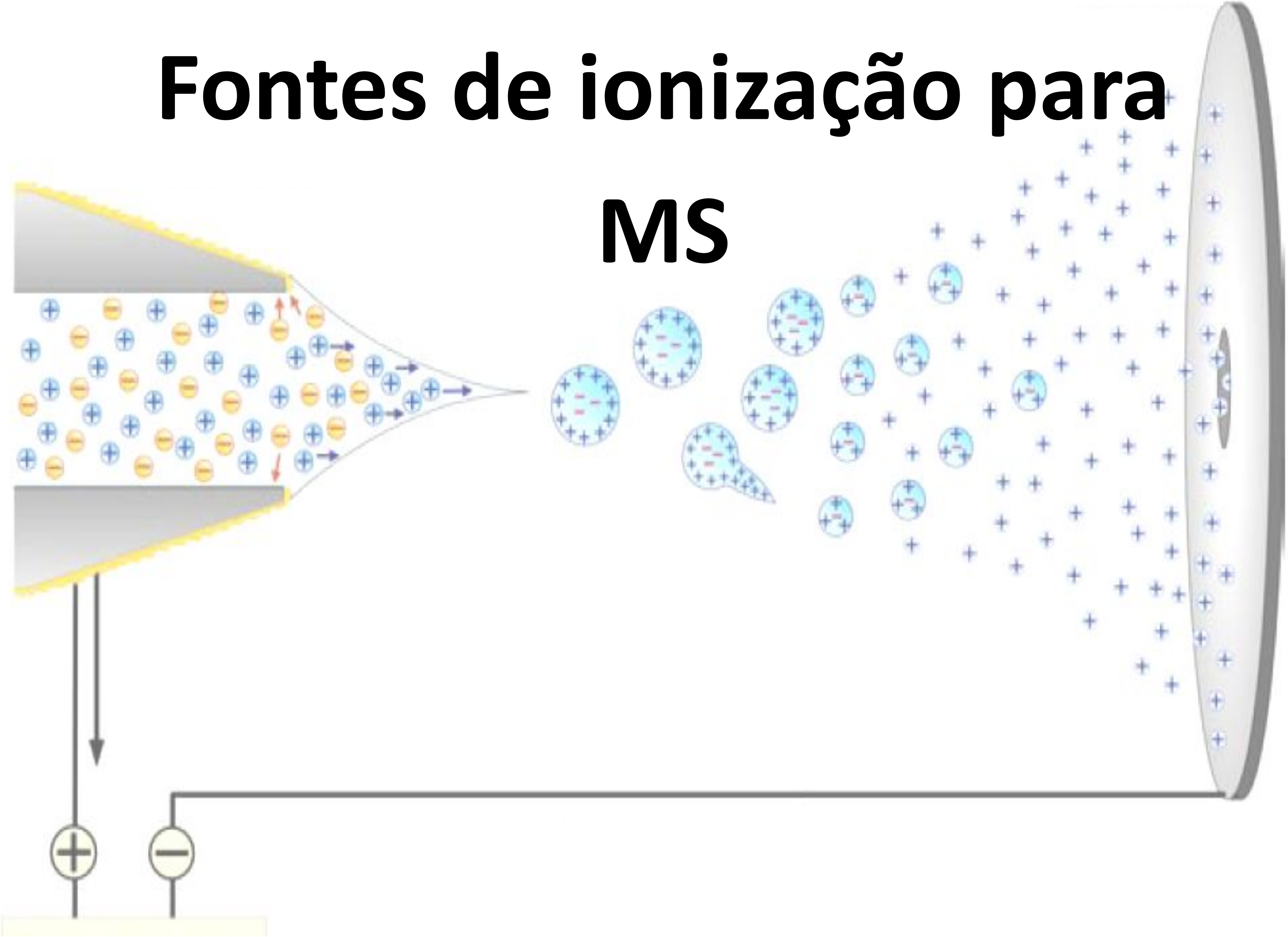


Figure 3.31 *Cross-section of a cryopump*

Fontes de ionização para MS

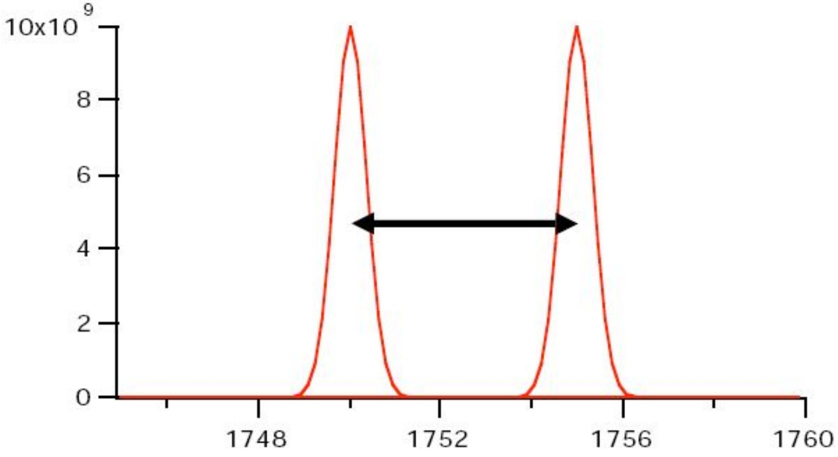


Definições de resolução em MS

- Resolução de Massa (*Mass Resolution*)

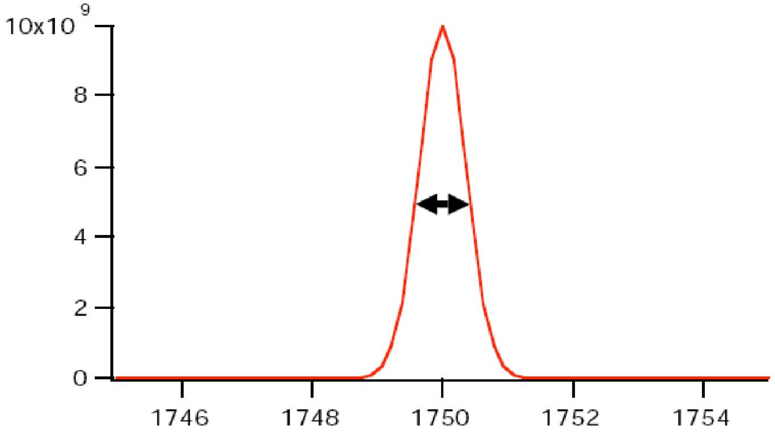
vs.

- Poder de Resolução de Massa (*Mass Resolving Power – RP)*



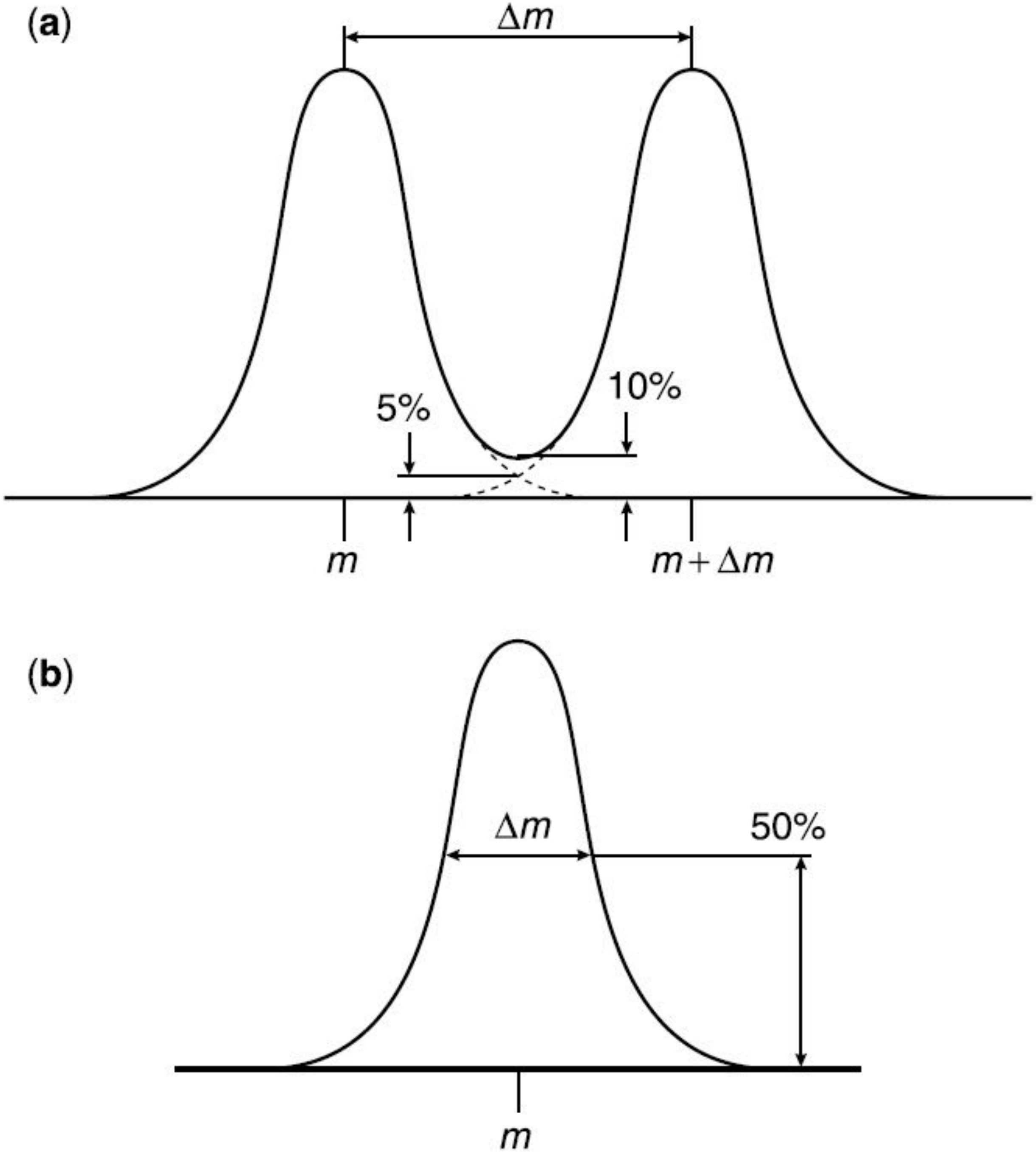
Ex.: 10% do vale =

5% do máximo



FWHM = *Full Width at Half Maximum*

(Largura total da metade do pico)


(a)
Δm
5%
10%
m
m + Δm
(b)
Δm
50%
m

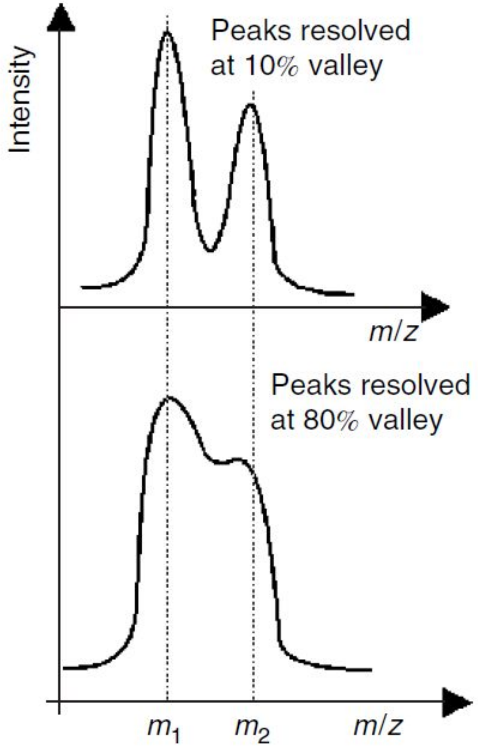


Figure 2.1
Diagram showing the concepts of peak resolution and valley.

Definições de exatidão em MS

- Massa exata (*exact mass*) = massa isotópica calculada

vs.

- Massa acurada (*accurate mass*) = massa medida com a exatidão do equipamento

Erro da medida (Δm) = $m_{medida} - m_{exata}$ (u)

Grau de Exatidão (*Degree of accuracy*) = $10^6 \times \Delta m/m_{exata}$ (ppm)

Exemplo

N_2^+

m/z = 28,006148

CO^+

m/z = 27,994915

Δm = 0,011233

Requer grau de exatidão de pelo menos 200 ppm

RP (10% vale) ≈ 2.500

RP (FWHM) ≈ 4.250

Analisadores para MS

- Setor Magnético e Setor Eletrostático
- Quadrupolo
- *Ion Trap*
- Tempo de Voo
- FT-ICR
- (FT) Orbitrap

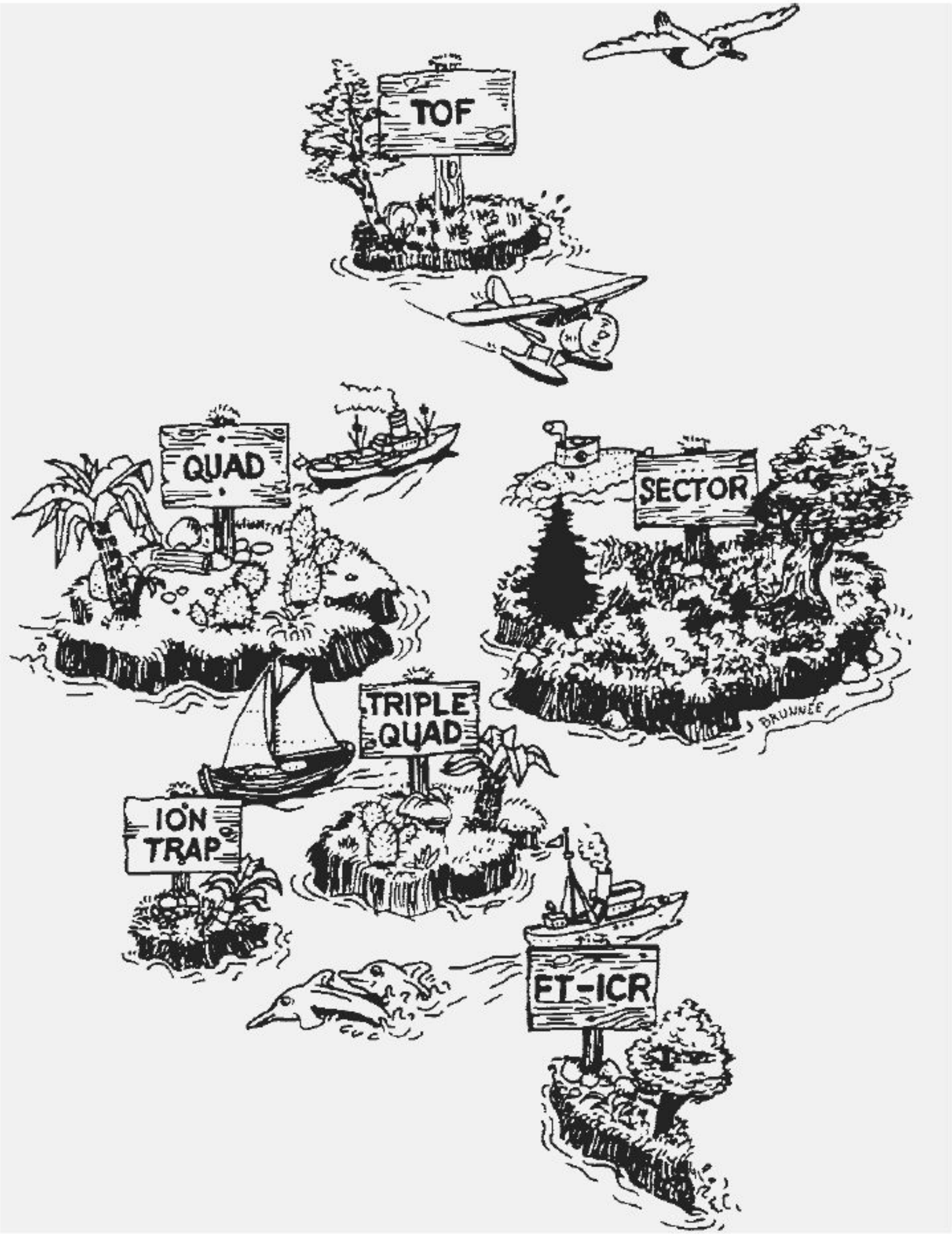
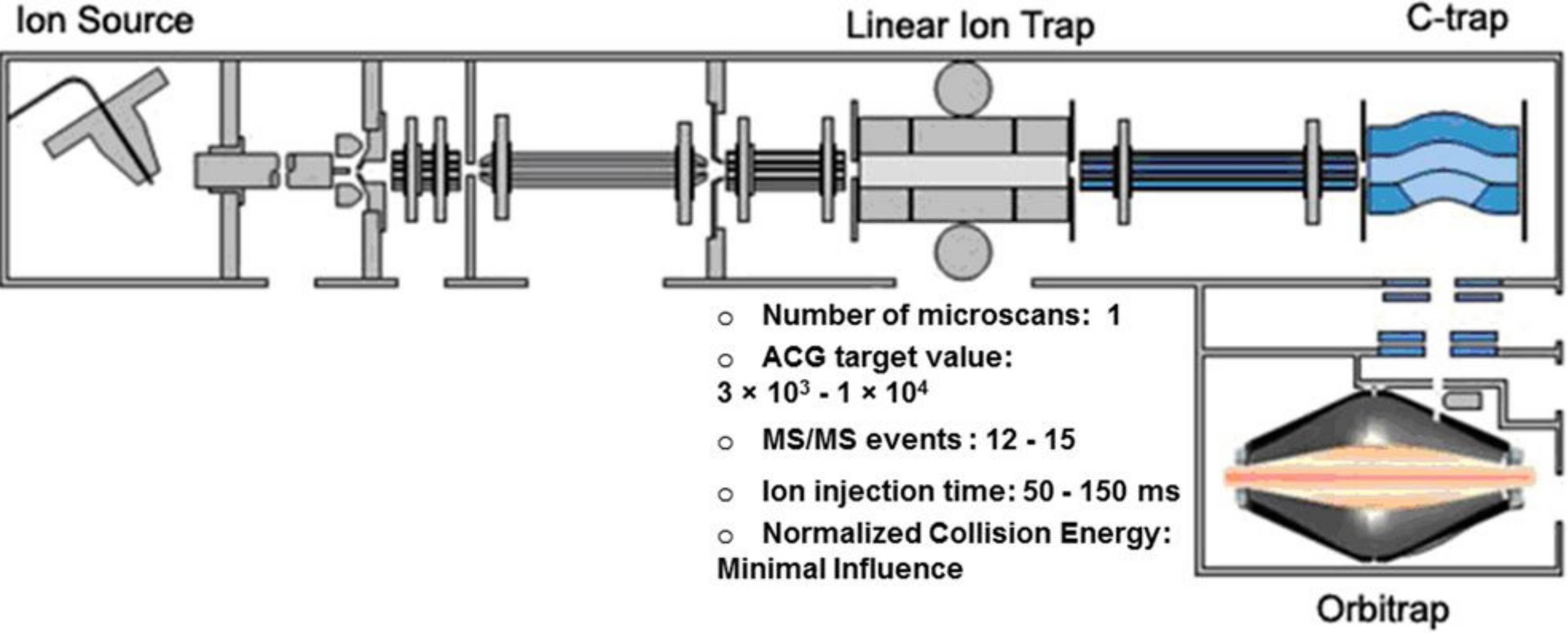


Fig. 4.1. Mass spectrometer islands. A cartoon by C. Brunnée. Reproduced from Ref. [2] with permission. © Elsevier Science, 1987.

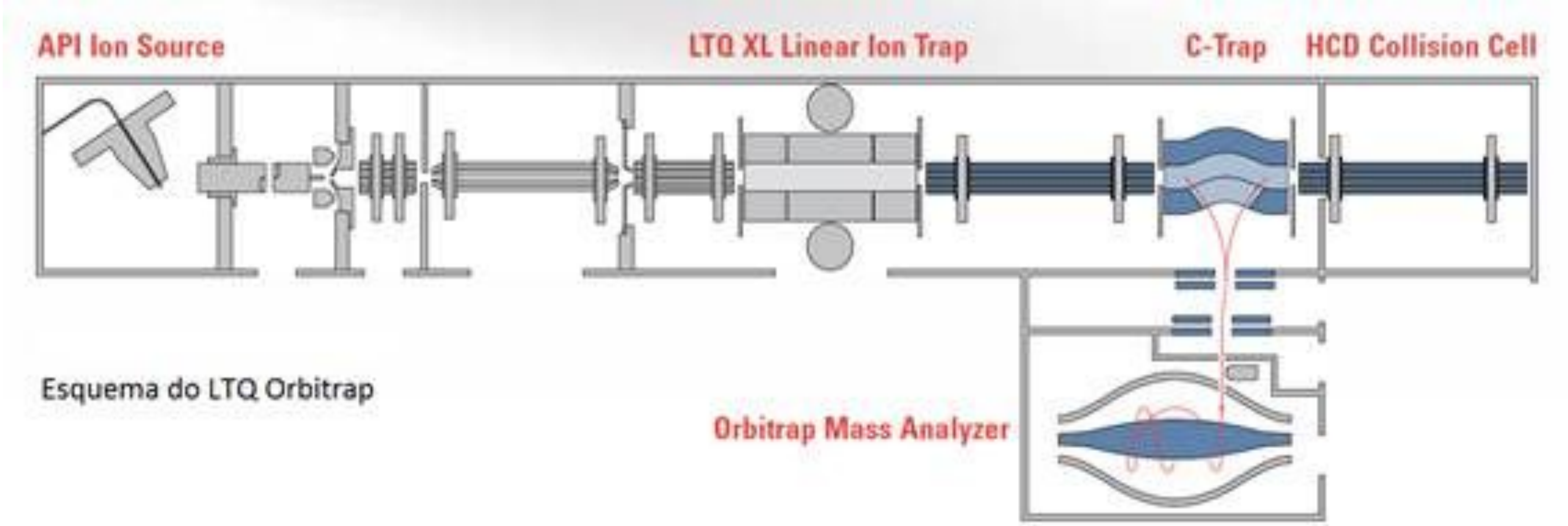
LTQ-Orbitrap



- Number of microscans: 1
- ACG target value:
$3 \times 10^3 - 1 \times 10^4$
- MS/MS events : 12 - 15
- Ion injection time: 50 - 150 ms
- Normalized Collision Energy:
Minimal Influence

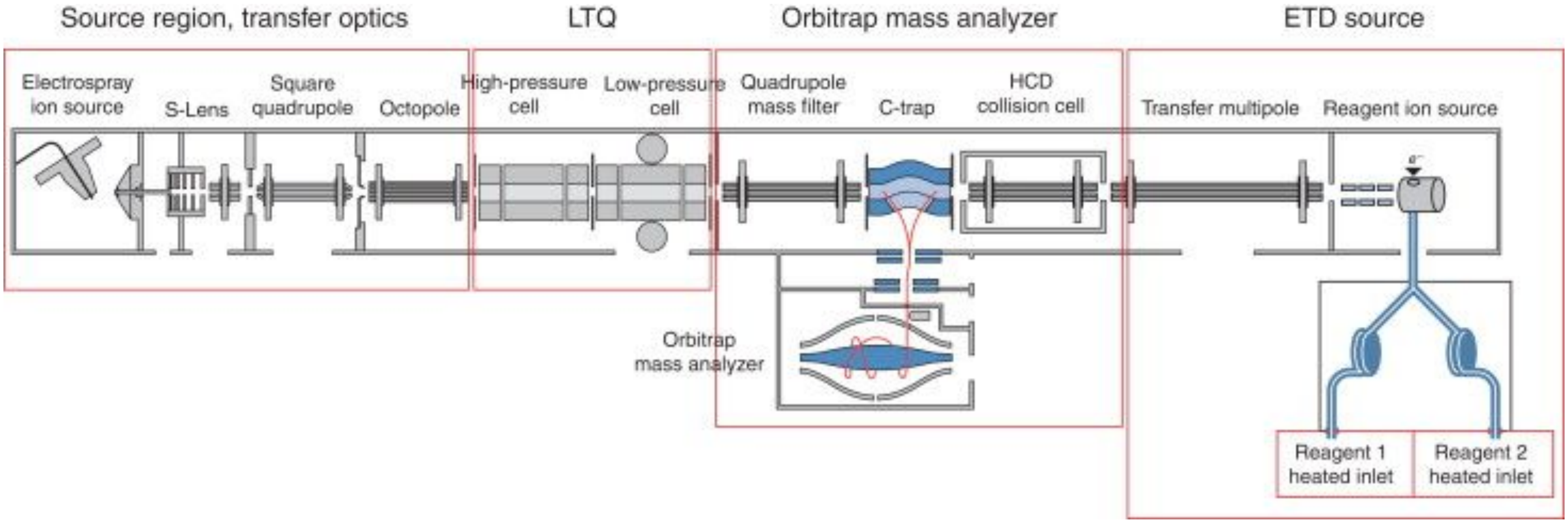
- Mass resolving power:
30 000 or 60 000
- Monoisotopic precursor selection:
Enabled
- ACG target value: $5 \times 10^5 - 1 \times 10^6$
- Preview Mode for FTMS Scan:
Minimal influence
- Dynamic exclusion duration:
Minimal influence

LTQ Orbitrap XL

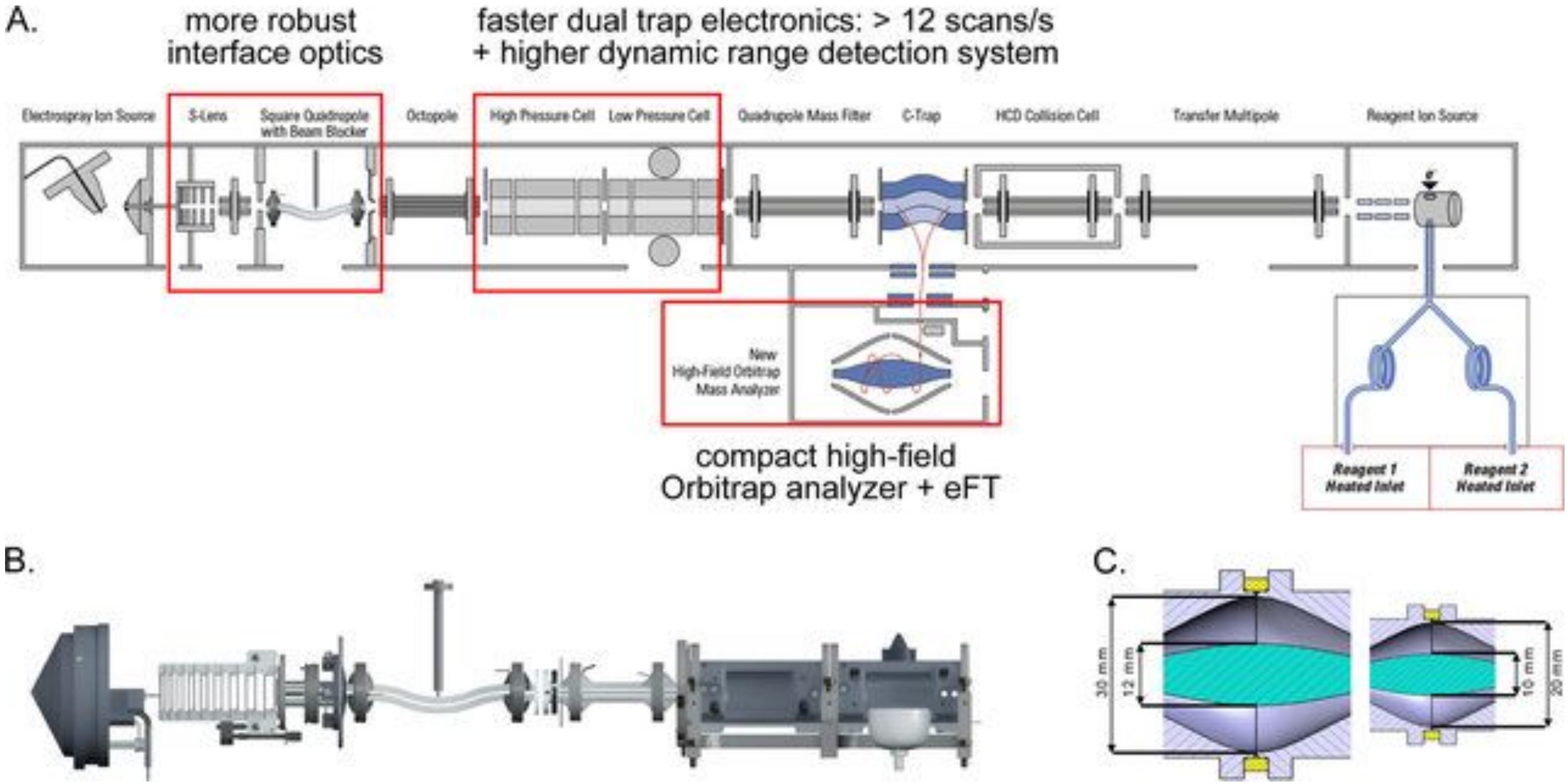


Esquema do LTQ Orbitrap

LTQ-Orbitrap Velos c/ ETD



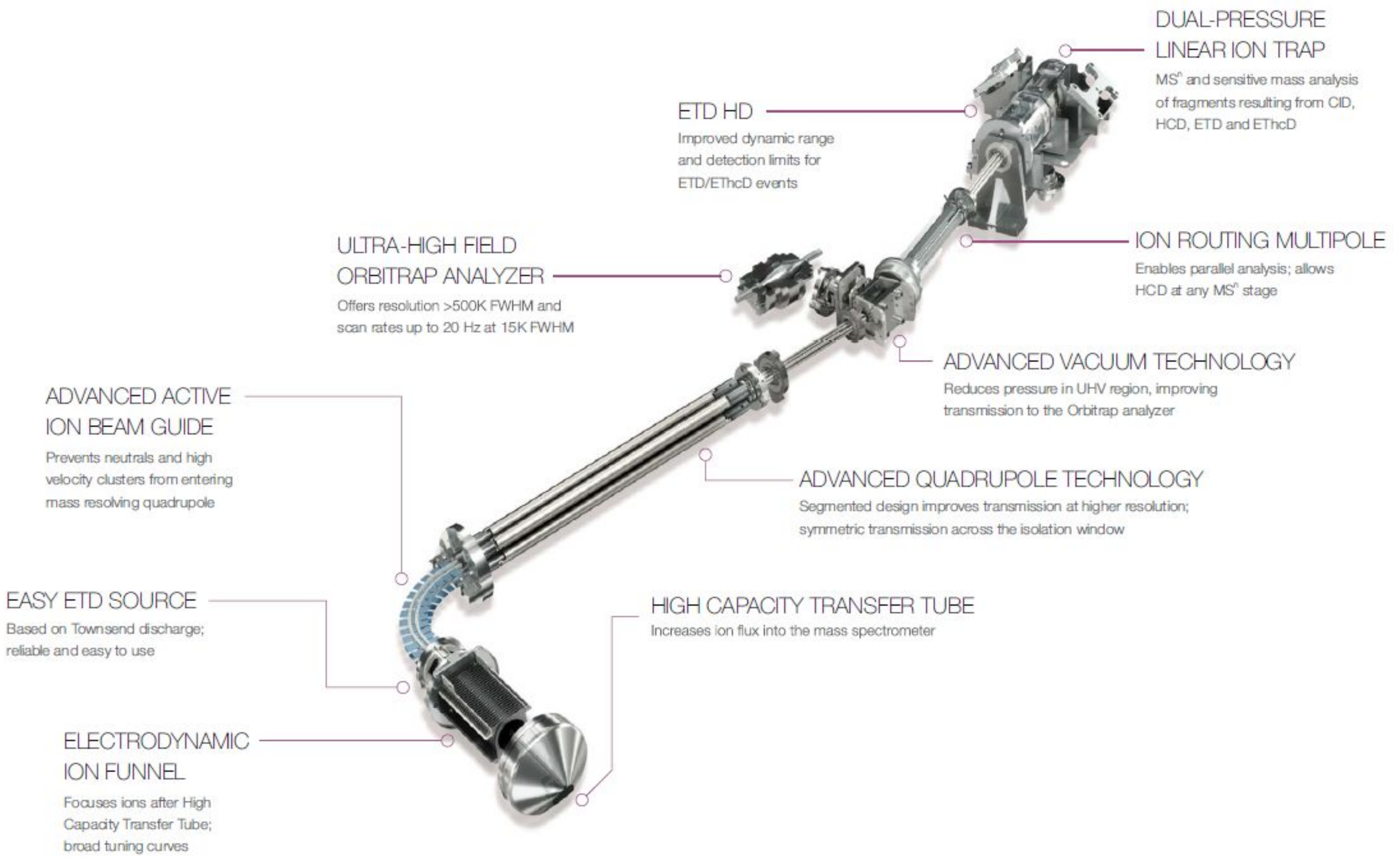
(LTQ) Orbitrap Elite c/ ETD


more robust interface optics

faster dual trap electronics: > 12 scans/s + higher dynamic range detection system

compact high-field Orbitrap analyzer + eFT

Tribrid technology



DUAL-PRESSURE LINEAR ION TRAP
MS^n and sensitive mass analysis of fragments resulting from CID, HCD, ETD and EThcD

ETD HD
Improved dynamic range and detection limits for ETD/EThcD events

ULTRA-HIGH FIELD ORBITRAP ANALYZER
Offers resolution >500K FWHM and scan rates up to 20 Hz at 15K FWHM

ION ROUTING MULTIPOLE
Enables parallel analysis; allows HCD at any MS^n stage

ADVANCED VACUUM TECHNOLOGY
Reduces pressure in UHV region, improving transmission to the Orbitrap analyzer

ADVANCED ACTIVE ION BEAM GUIDE
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

ADVANCED QUADRUPOLE TECHNOLOGY
Segmented design improves transmission at higher resolution; symmetric transmission across the isolation window

EASY ETD SOURCE
Based on Townsend discharge; reliable and easy to use

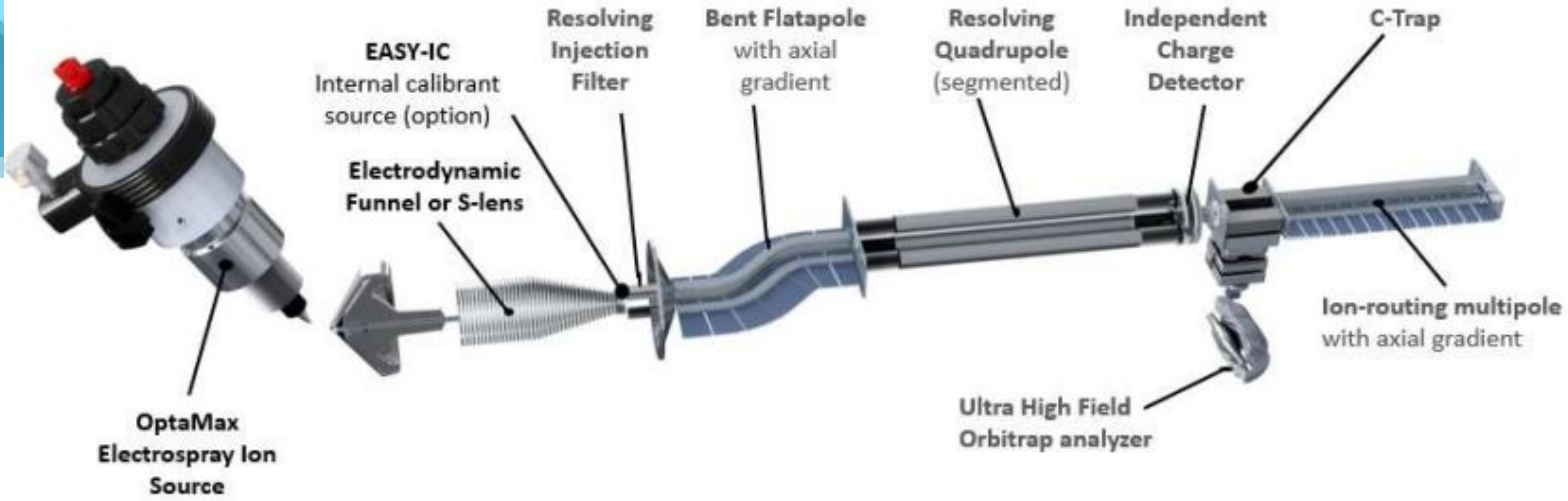
HIGH CAPACITY TRANSFER TUBE
Increases ion flux into the mass spectrometer

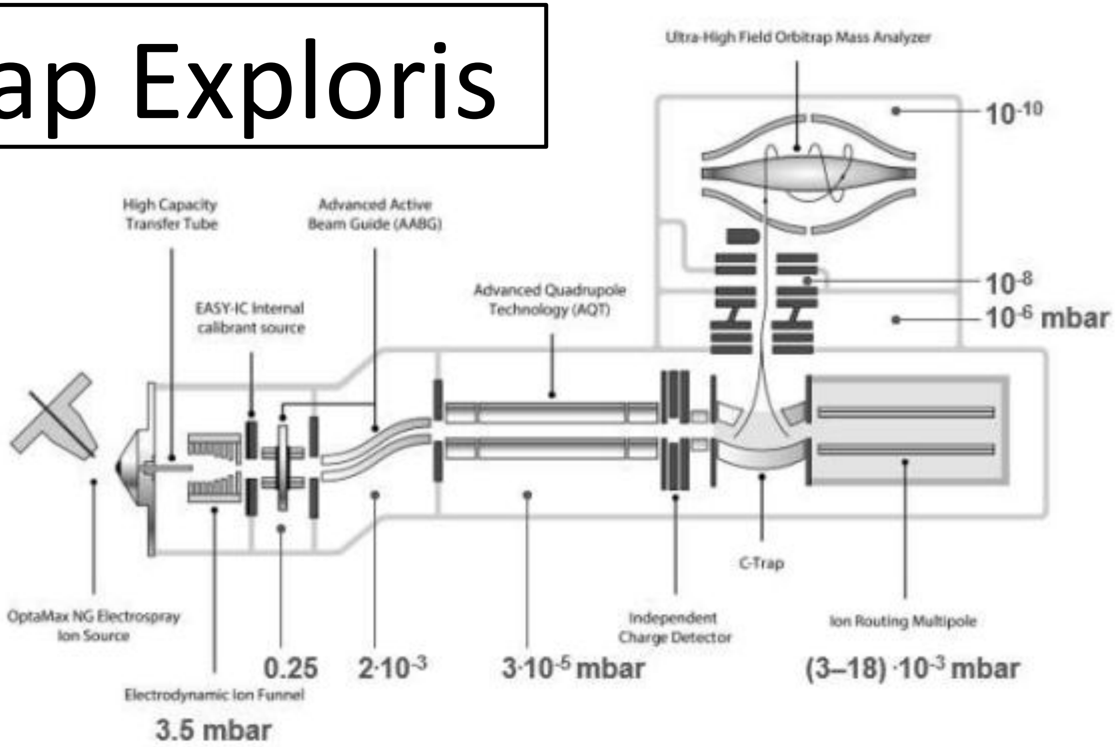
ELECTRODYNAMIC ION FUNNEL
Focuses ions after High Capacity Transfer Tube; broad tuning curves

Figure 1. Hardware benefits.

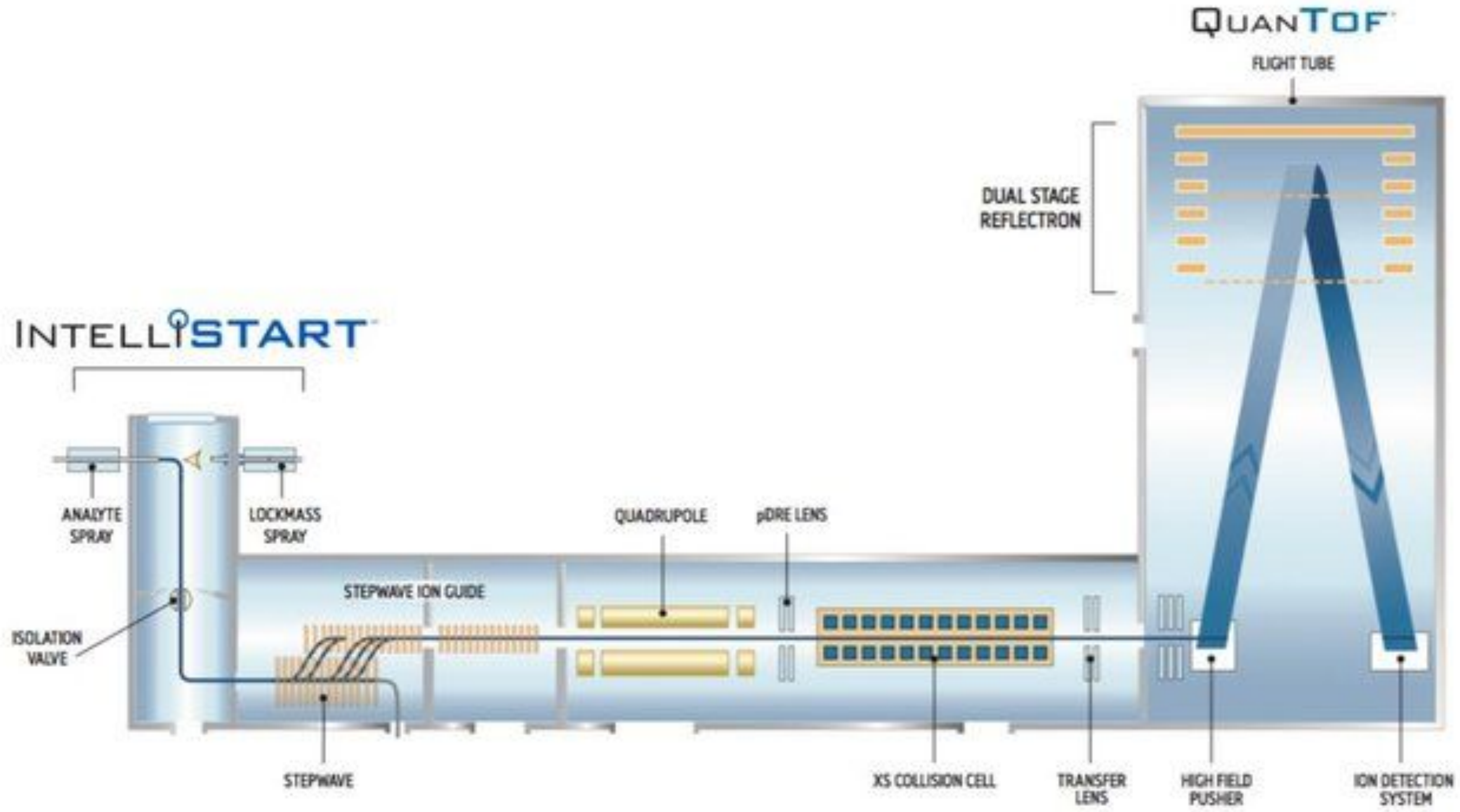
Q Exactive Orbitrap

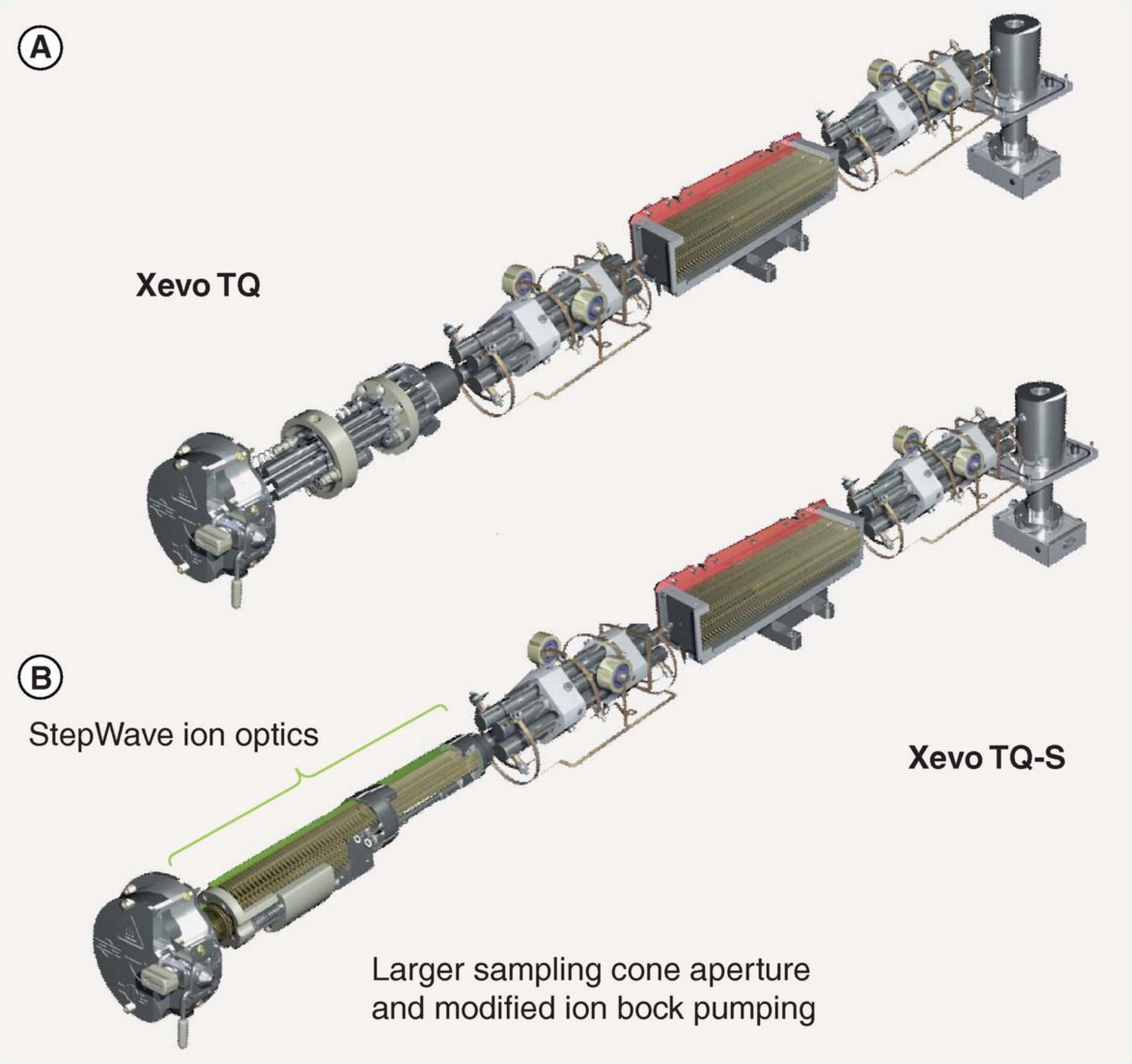
Orbitrap Exploris





Xevo G2-XS


QUANTOF
FLIGHT TUBE
DUAL STAGE REFLECTRON
INTELLISTART
ANALYTE SPRAY
LOCKMASS SPRAY
QUADRUPOLE
pDRE LENS
STEPWAVE ION GUIDE
ISOLATION VALVE
STEPWAVE
XS COLLISION CELL
TRANSFER LENS
HIGH FIELD PUSHER
ION DETECTION SYSTEM


A
Xevo TQ
B
StepWave ion optics
Xevo TQ-S
Larger sampling cone aperture
and modified ion bock pumping

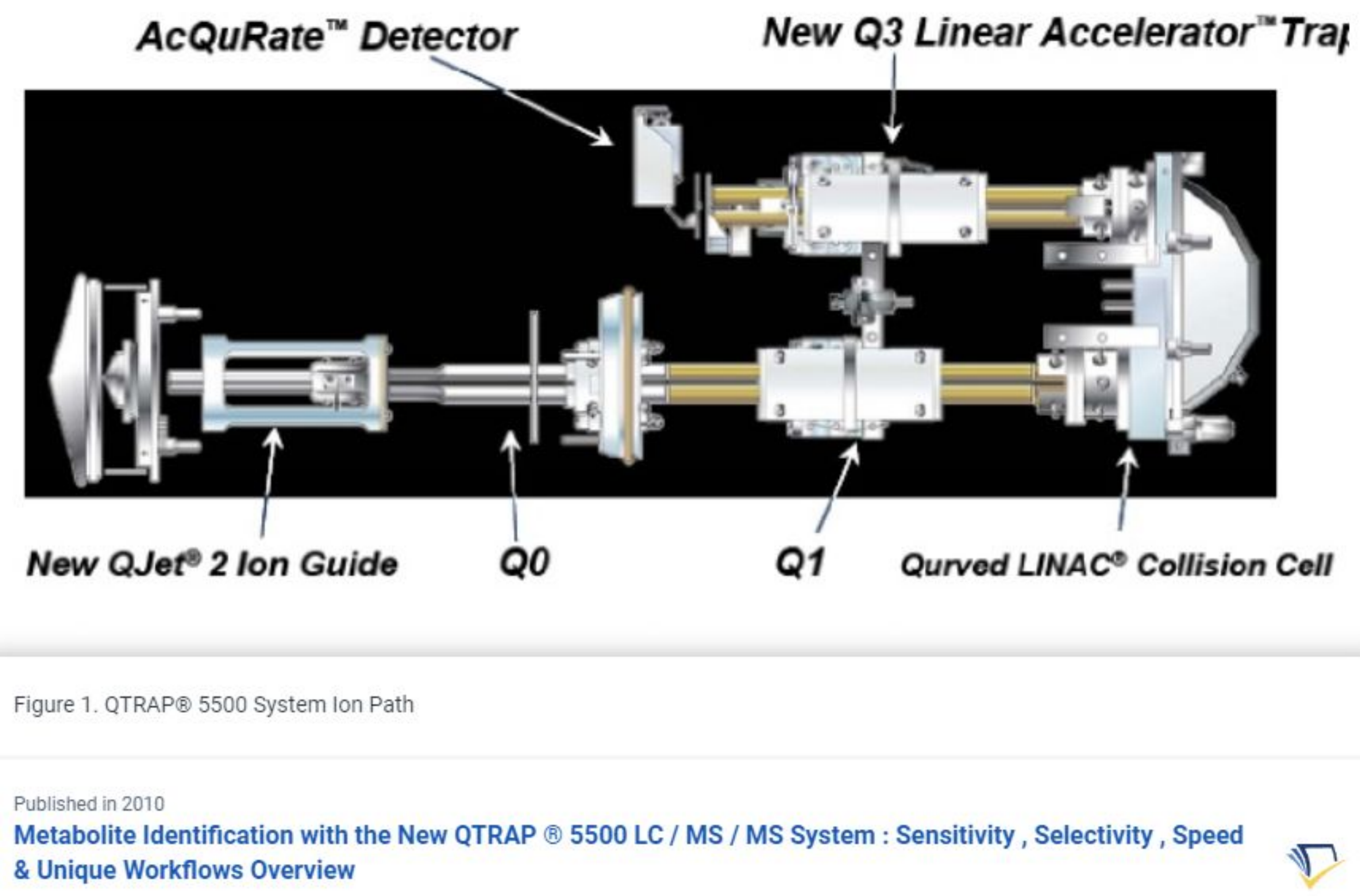


Figure 1. QTRAP® 5500 System Ion Path

Published in 2010

Metabolite Identification with the New QTRAP ® 5500 LC / MS / MS System : Sensitivity , Selectivity , Speed & Unique Workflows Overview

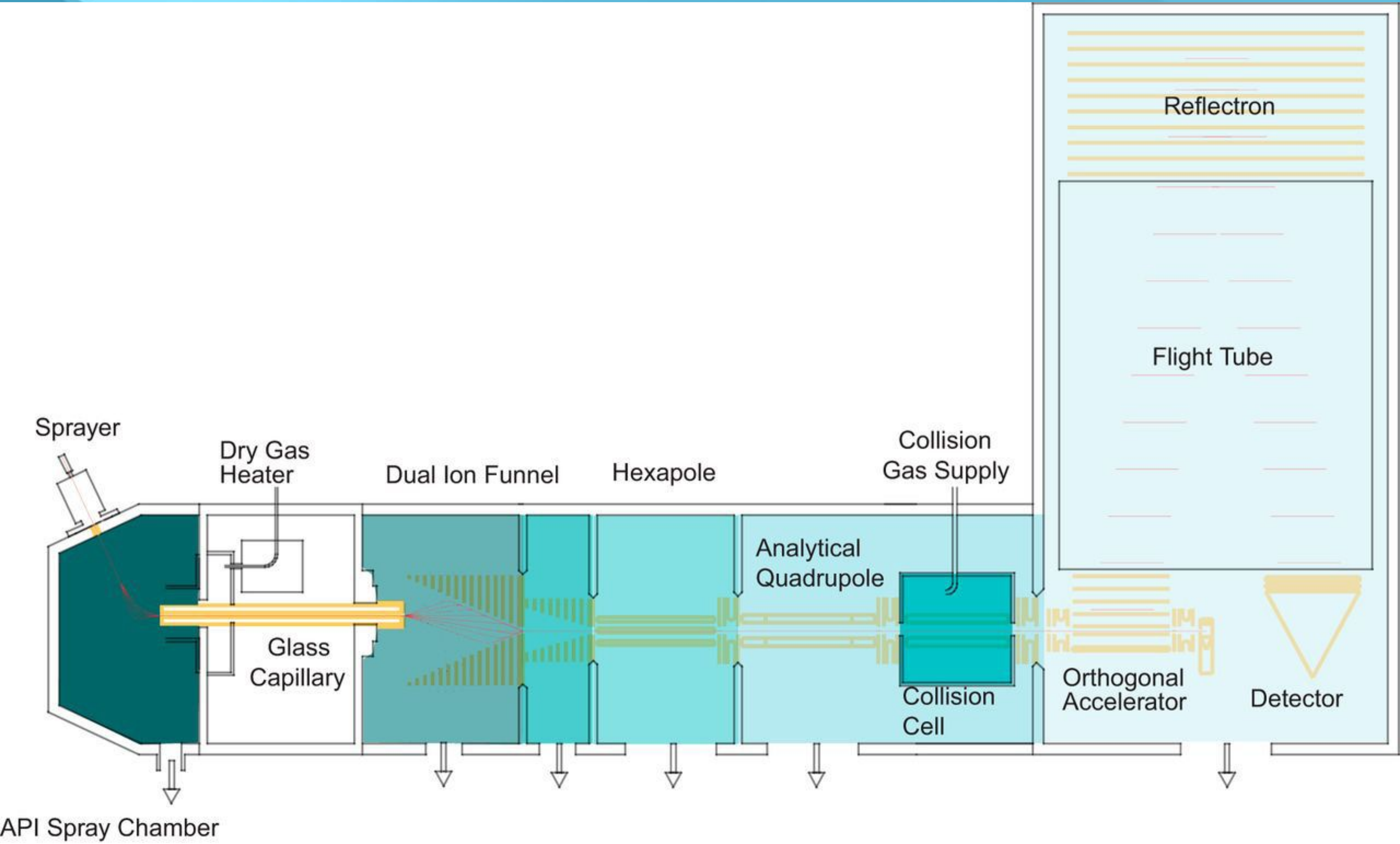
Flight Tube with in-flight focussing and dual stage reflector for Full Sensitivity Resolution (FSR)

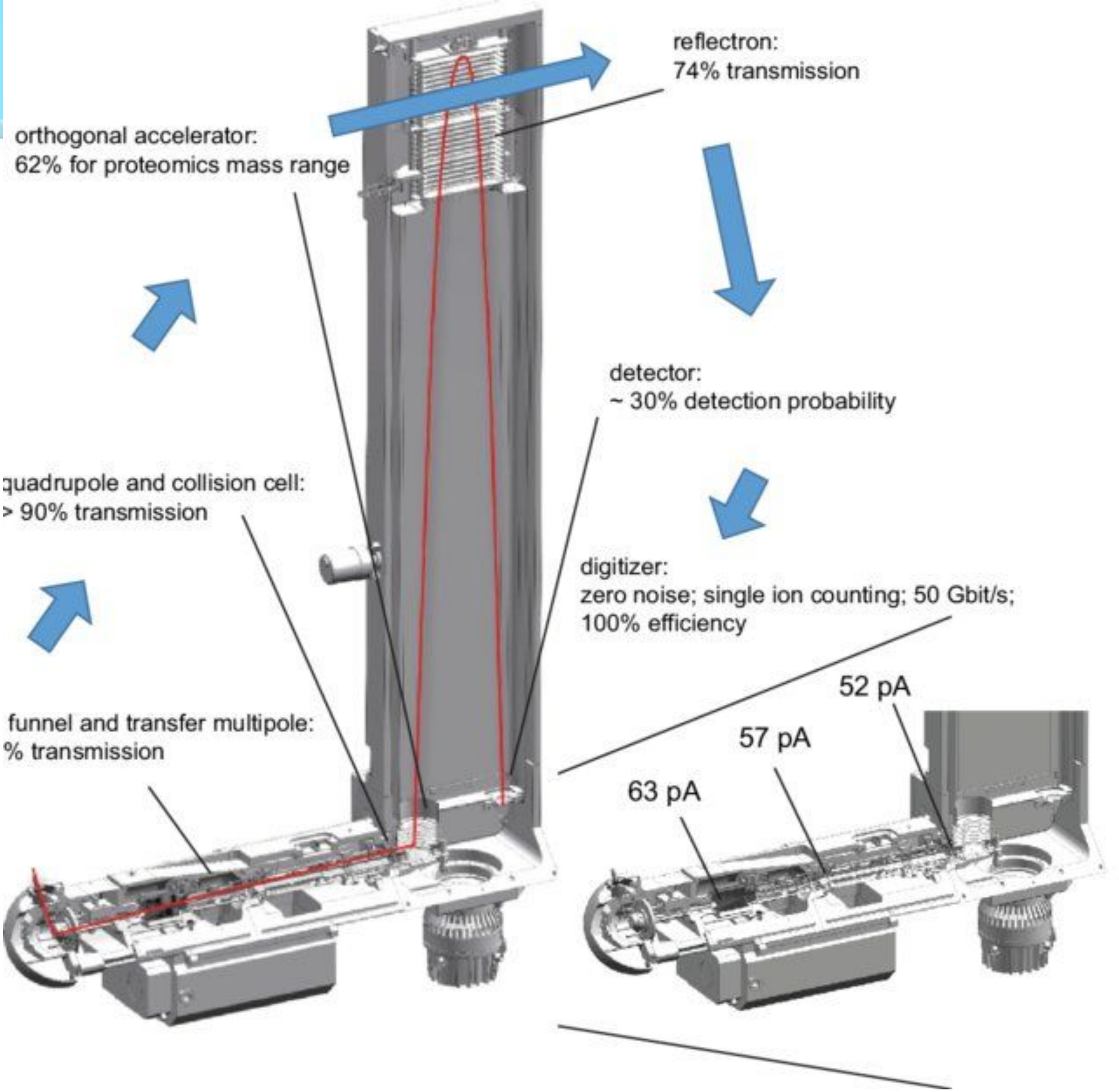
Novel high-definition 10 bit detection system

Broad mass transfer Quadrupole CID cell

Patented dual Ion-Funnel: Triple-quad like sensitivity

Choice of rugged and sensitive ion sources


Reflectron
Flight Tube
Sprayer
Dry Gas
Heater
Dual Ion Funnel
Hexapole
Collision
Gas Supply
Analytical
Quadrupole
Glass
Capillary
Collision
Cell
Orthogonal
Accelerator
Detector
API Spray Chamber



funnel and transfer multipole:
% transmission

quadrupole and collision cell:
> 90% transmission

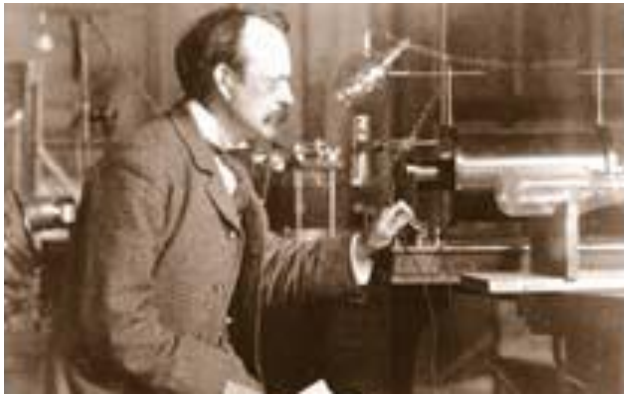
orthogonal accelerator:
62% for proteomics mass range

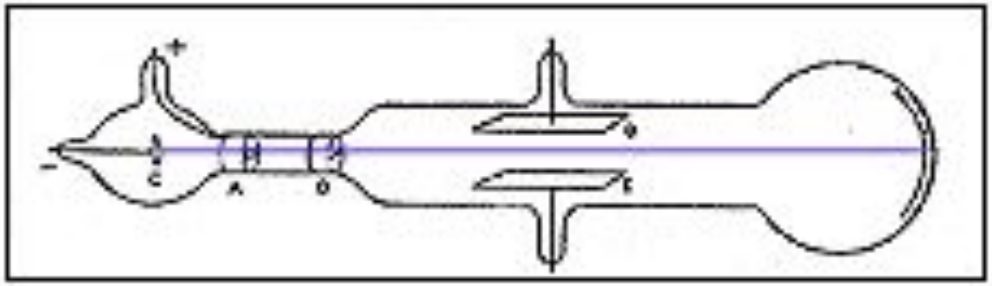
reflectron:
74% transmission

detector:
~ 30% detection probability

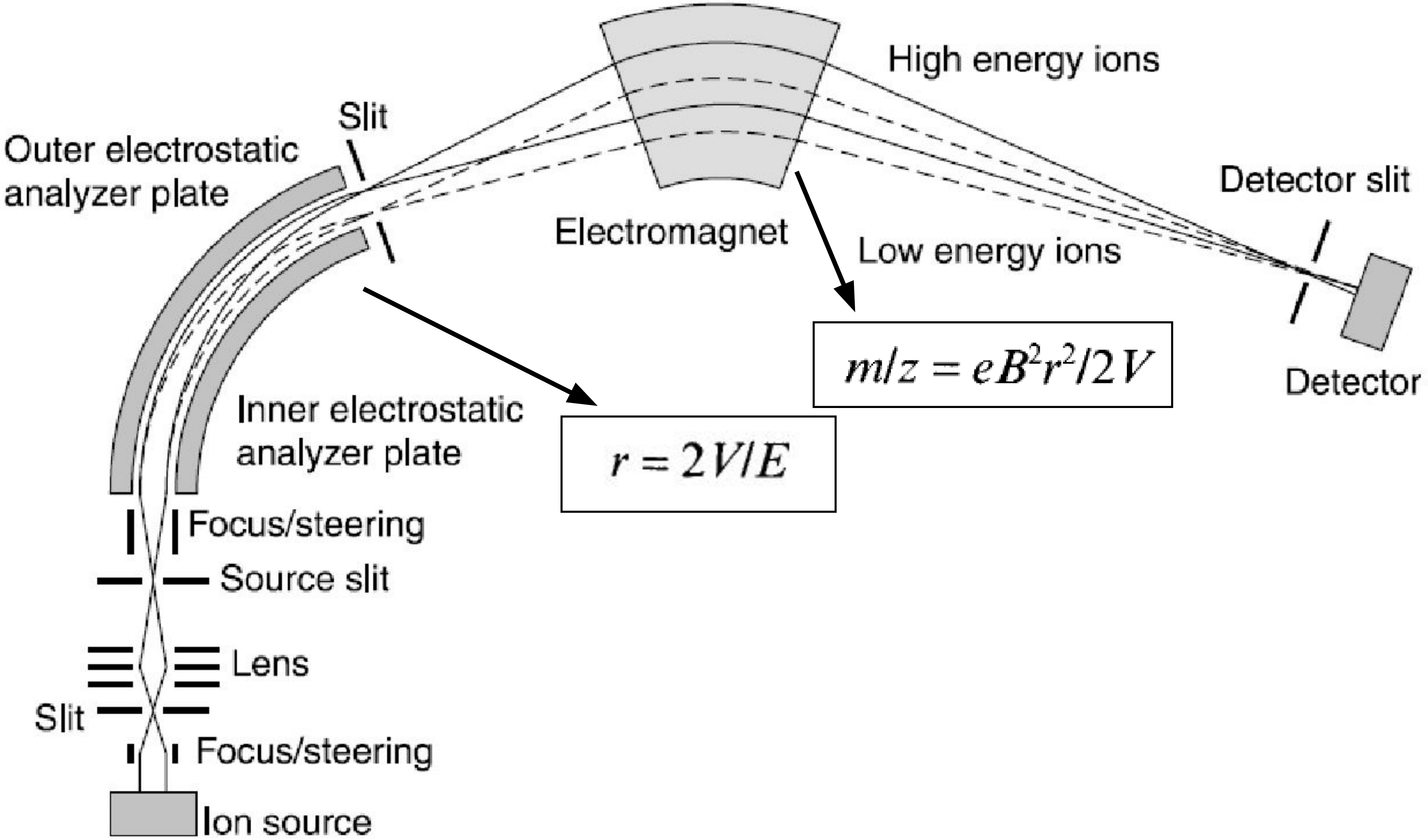
digitizer:
zero noise; single ion counting; 50 Gbit/s;
100% efficiency

J.J. Thomson e o tubo de raios catódicos





Setor Magnético e Setor Eletrostático (Focalização Dupla)



$$m/z = eB^2r^2/2V$$

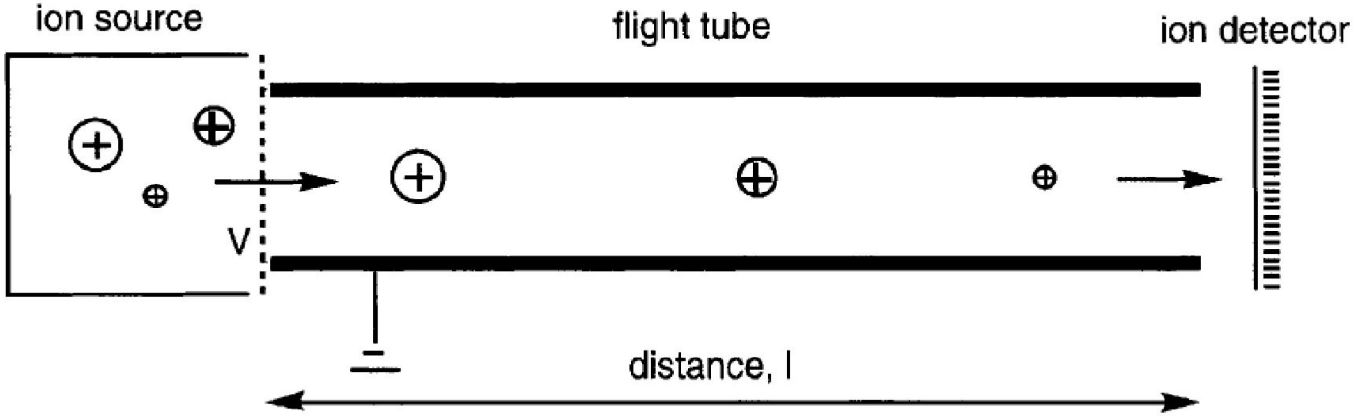
$$r = 2V/E$$

Focalização Dupla

- Vantagens
  - Alta resolução e exatidão
  - Excelente estabilidade = resultados quatitativos

- Desvantagens
  - Caro -- Difícil de usar
  - Velocidade de varredura limitada (histerese)
  - Detectabilidade é dependente da velocidade

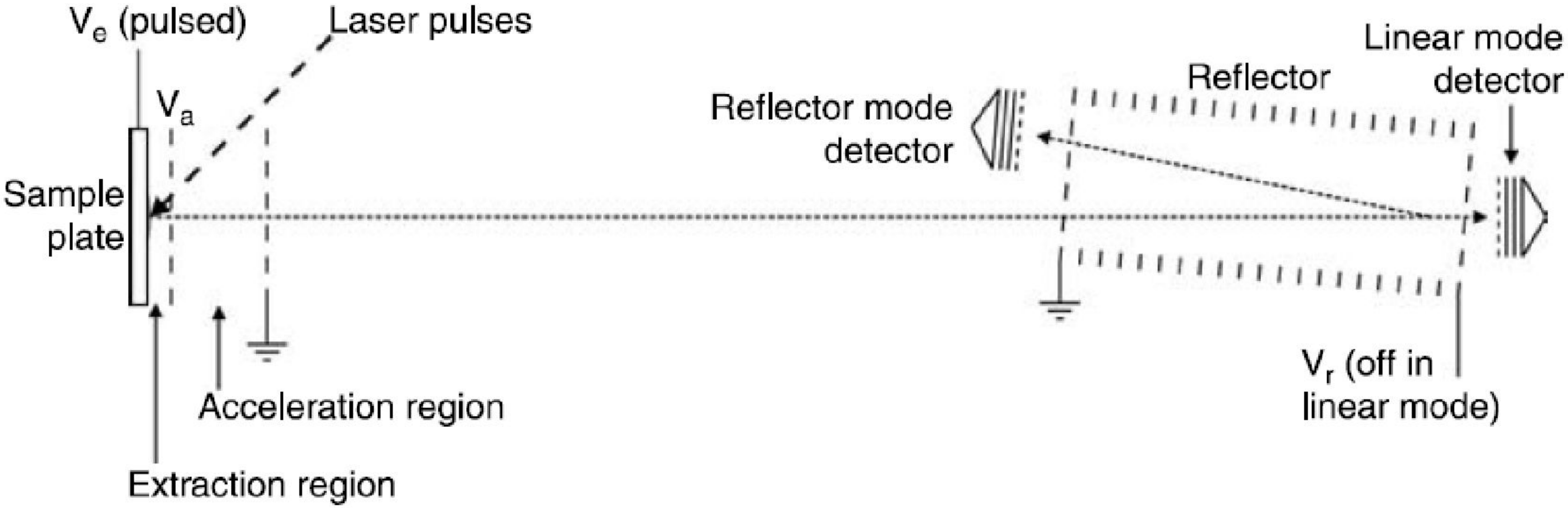
Tempo de Voo - TOF



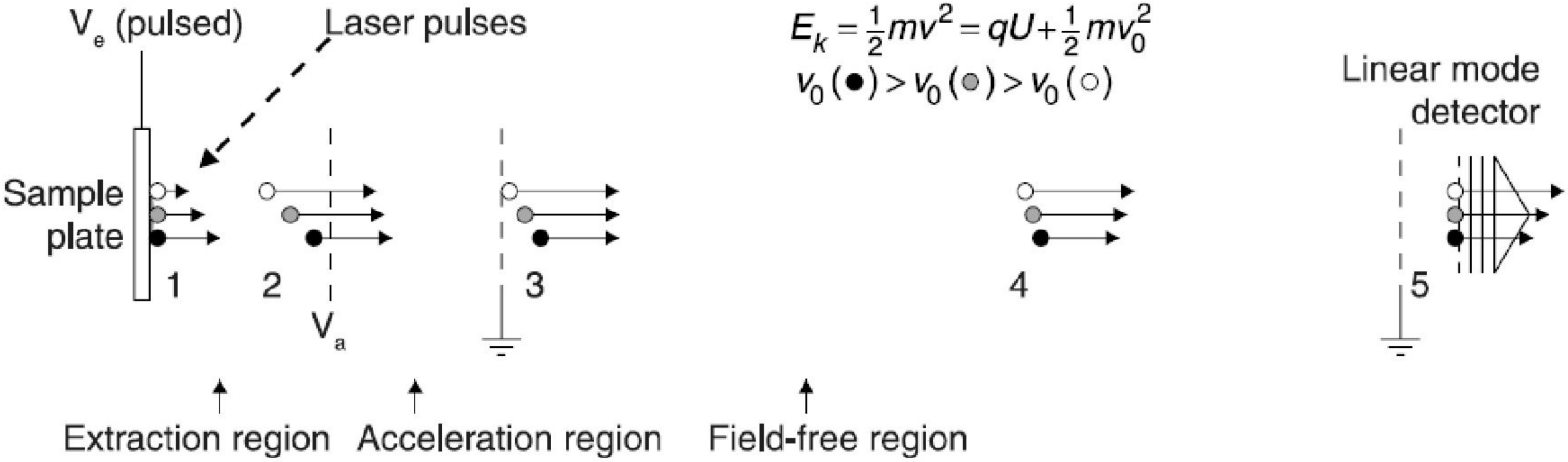
$$t = \frac{L}{v} = L\left(\frac{m}{2zeV}\right)^{1/2}$$

$$m / z = \frac{2eVt^2}{L^2}$$

TOF de alta resolução

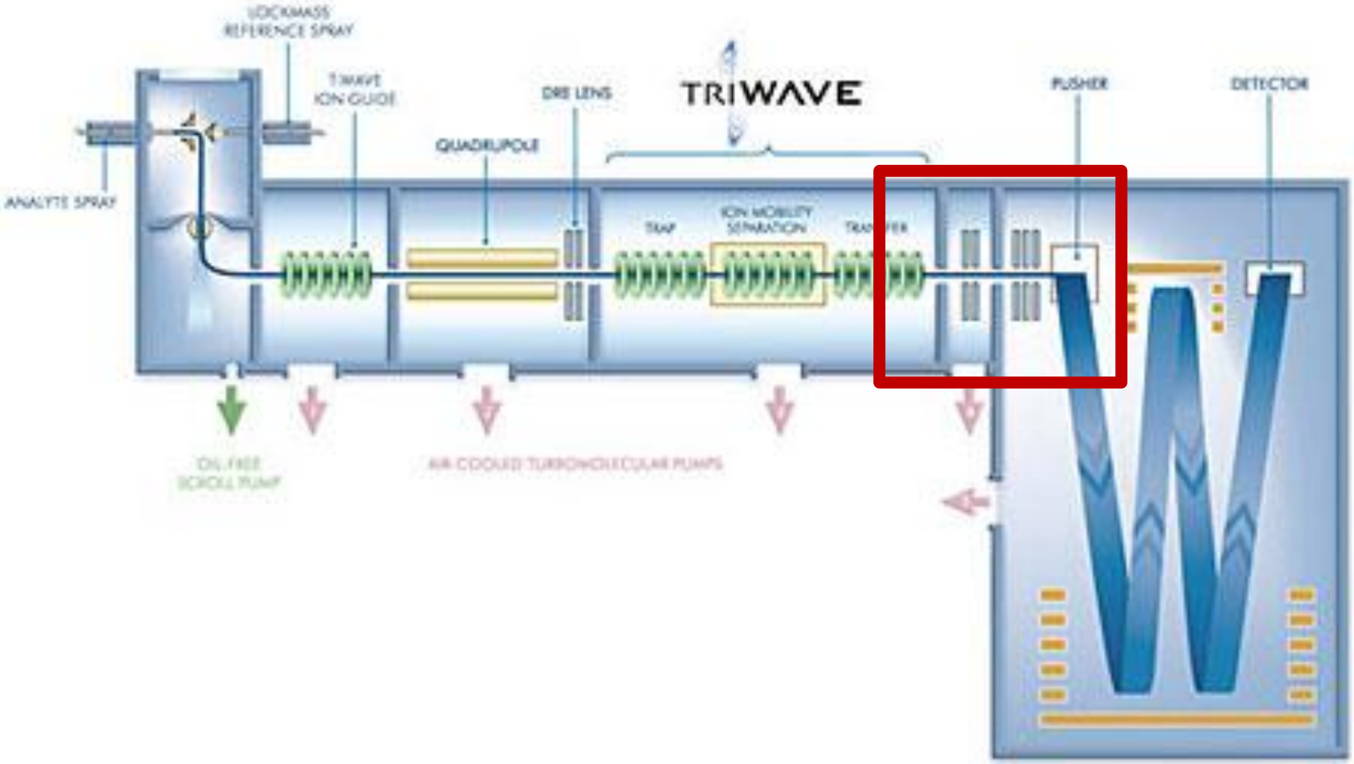


TOF de alta resolução – *Delayed Extraction (DE)*



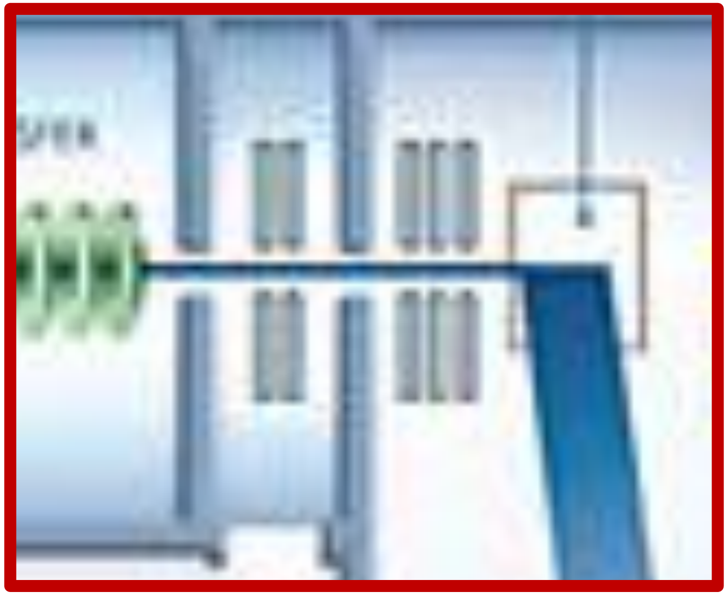
TOF de alta resolução – oa-ToF

Orthogonal Acceleration



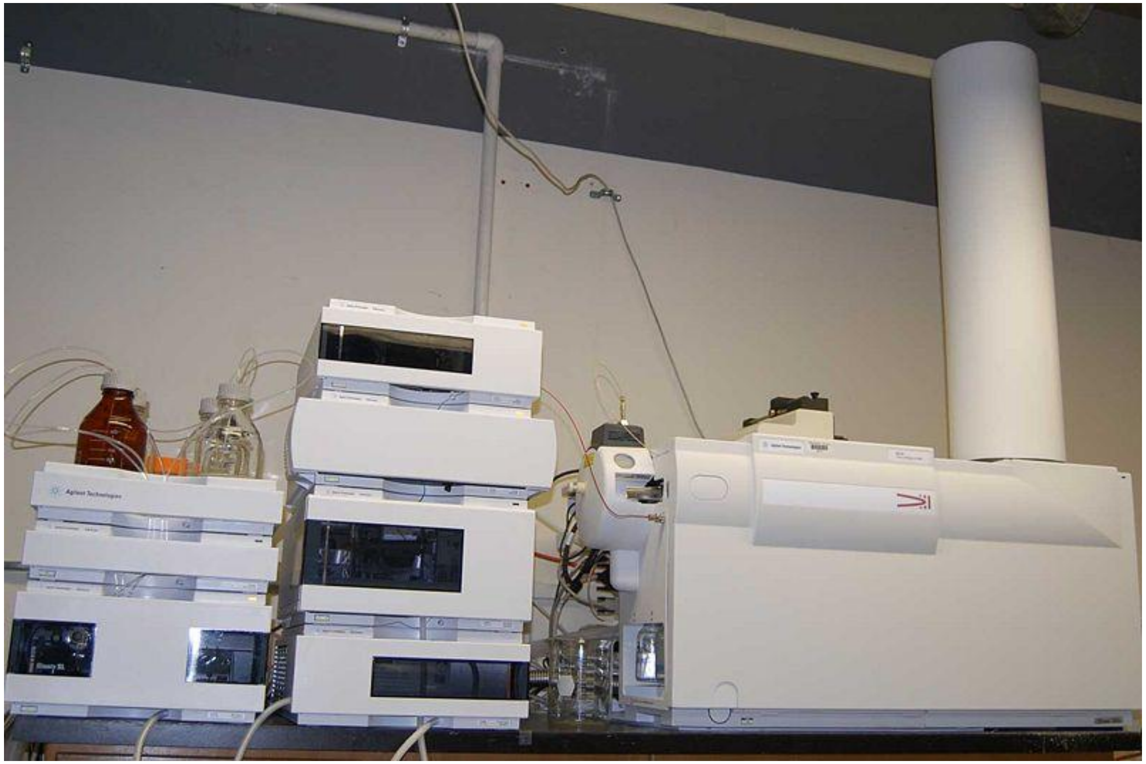
TOF de alta resolução – oa-ToF

Orthogonal Acceleration

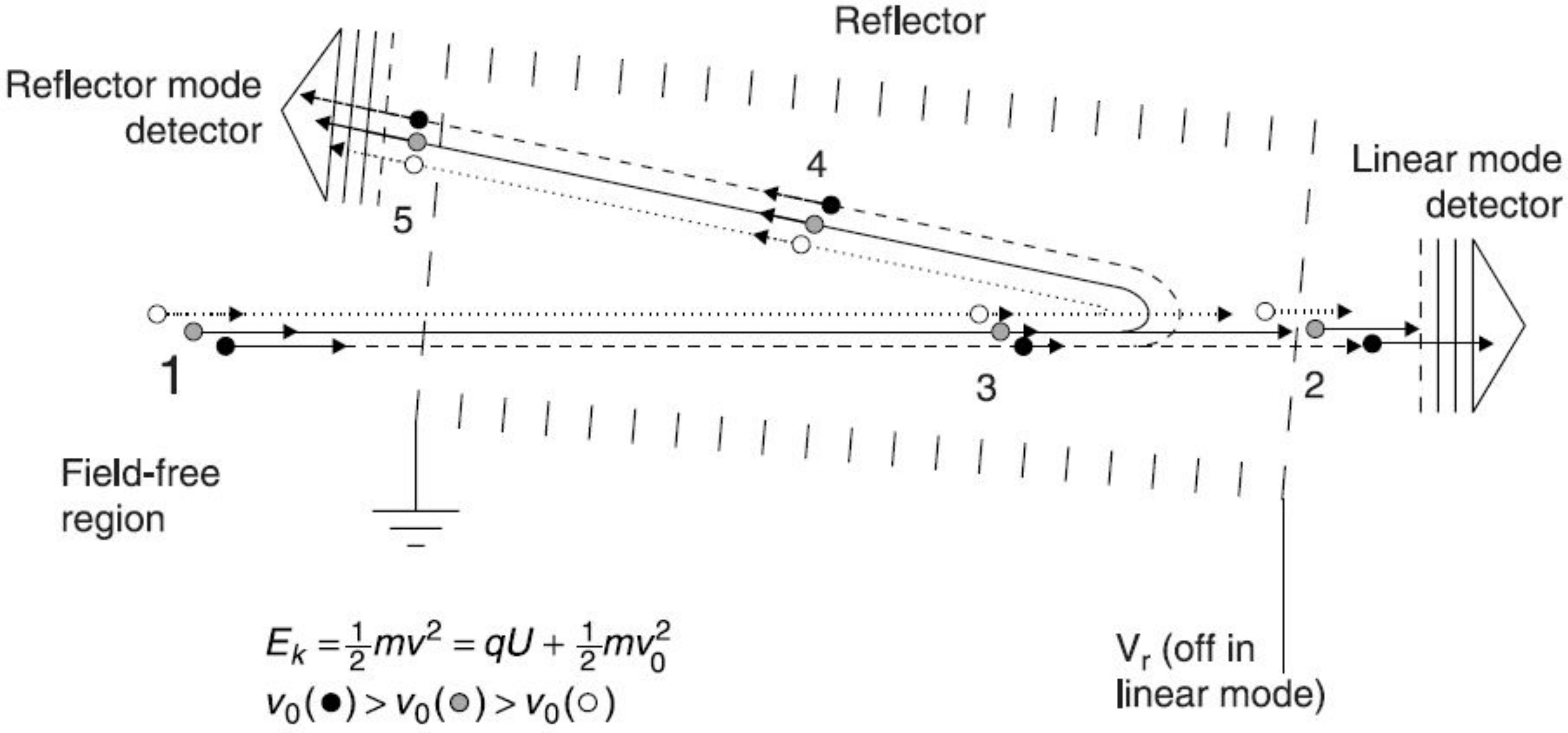


TOF de alta resolução – oa-ToF

Orthogonal Acceleration



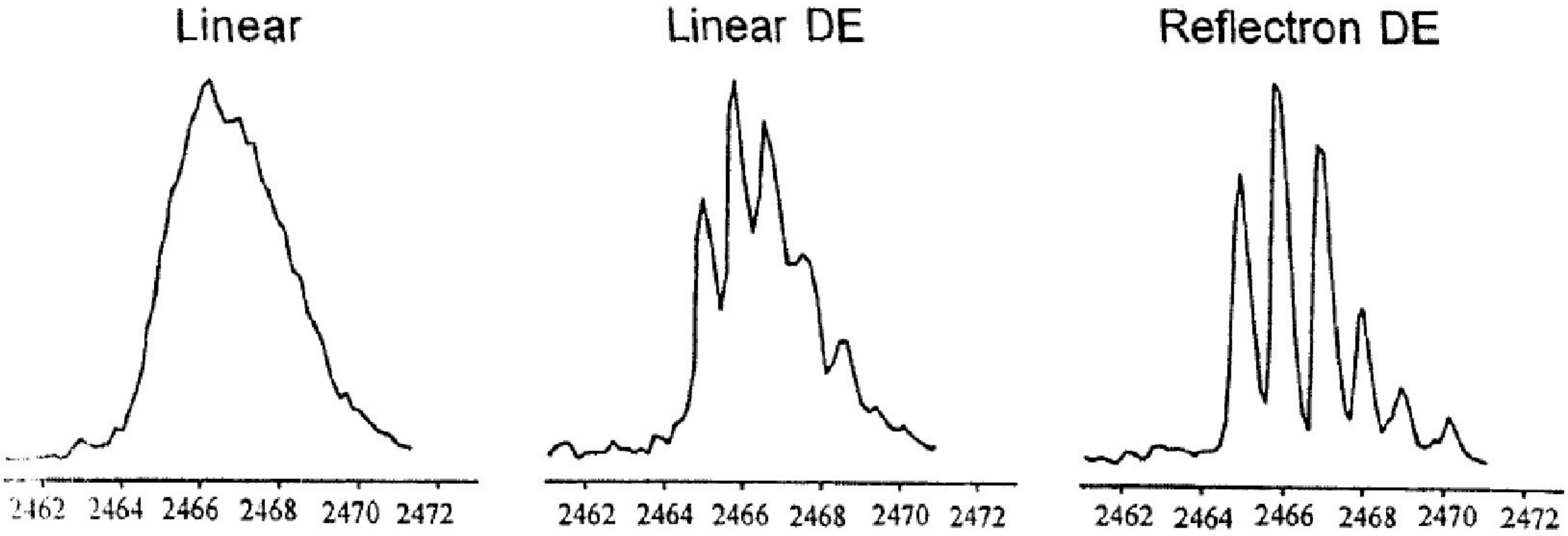
TOF de alta resolução – *Reflectron*



$$E_k = \frac{1}{2}mv^2 = qU + \frac{1}{2}mv_0^2$$

$$v_0(\bullet) > v_0(◉) > v_0(\circ)$$

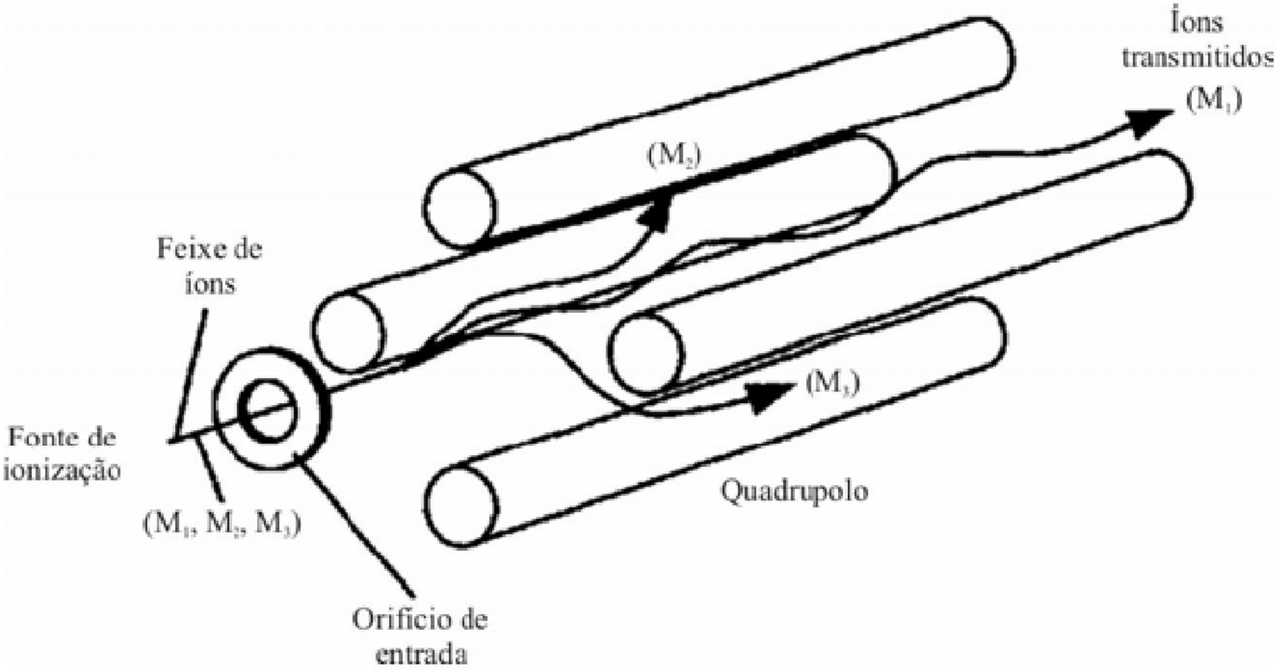
TOF de alta resolução – *DE* + *Reflectron*

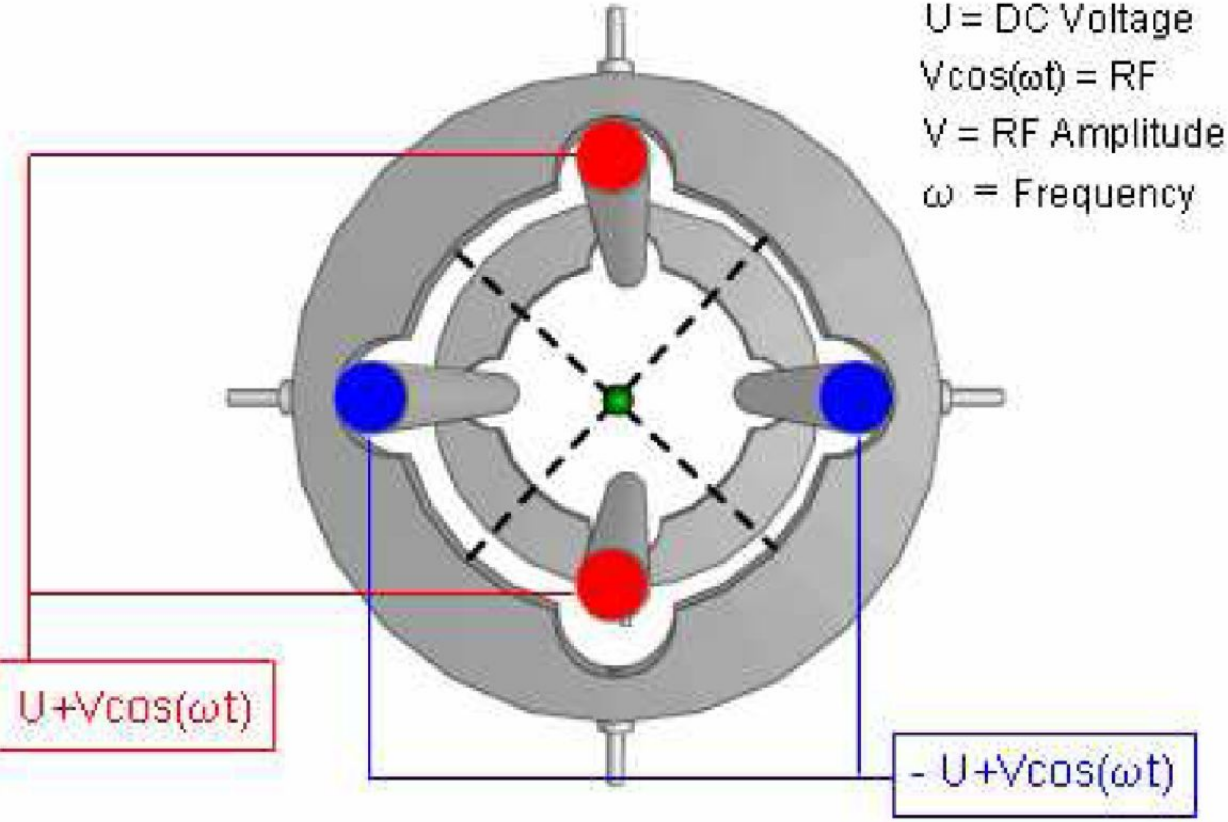


Características do TOF

- Aquisição veloz do espectro com sensibilidade
- Virtualmente sem limite de massas (modo sem reflexão)
- Bom poder de resolução : 10.000 a 50.000 (FWHM)
  - para melhor RP: ortogonal, DE (*delayed extraction*) e refletor
  - geralmente < 20.000 (FWHM)
- Grau de Exatidão: 20 a 100 ppm
- Exige calibração interna para melhorar exatidão

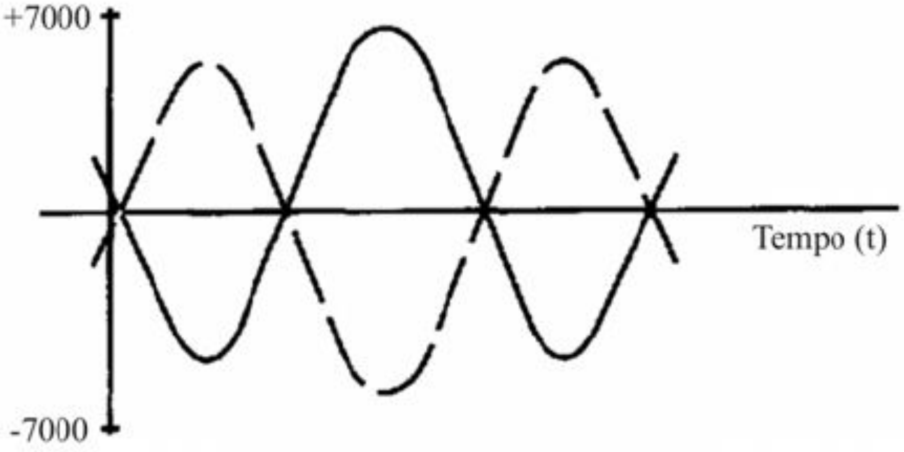
Quadrupolo


Íons transmitidos
(M_1)
(M_2)
Feixe de íons
Fonte de ionização
(M_1, M_2, M_3)
(M_3)
Quadrupolo
Orifício de entrada

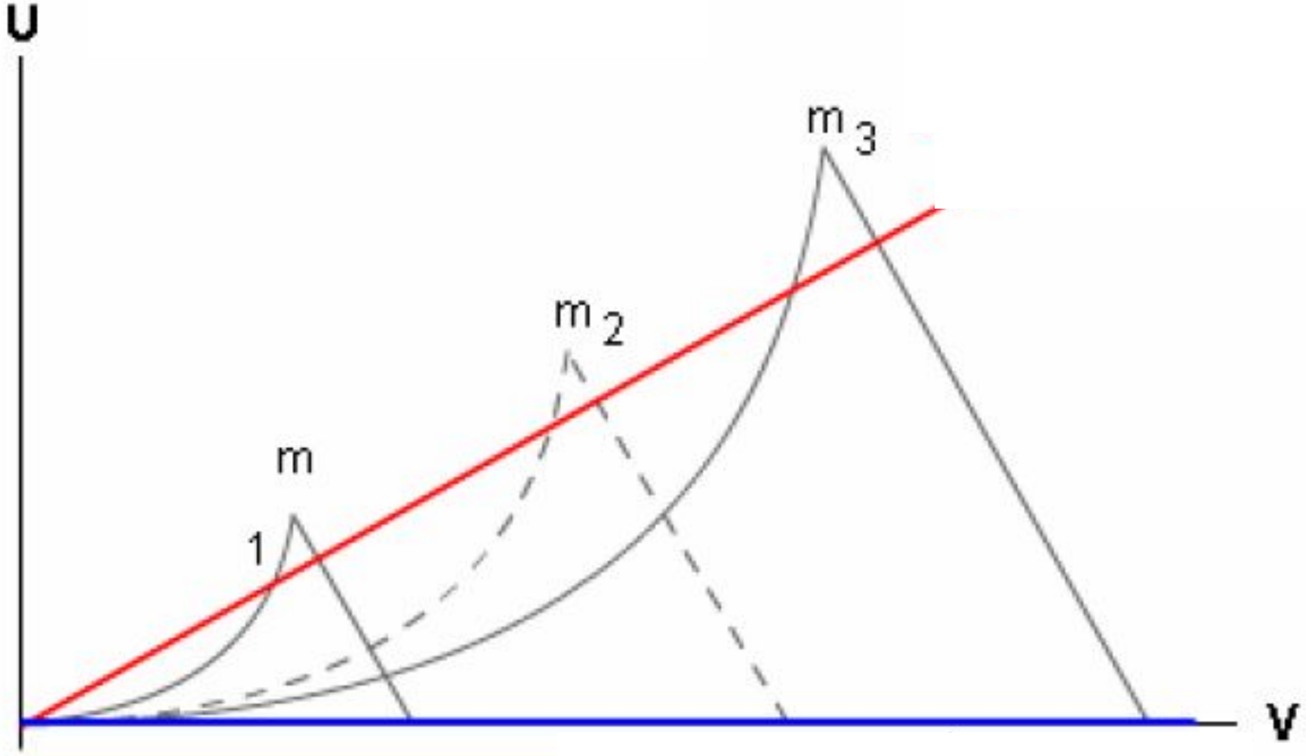


Quadrupole rods

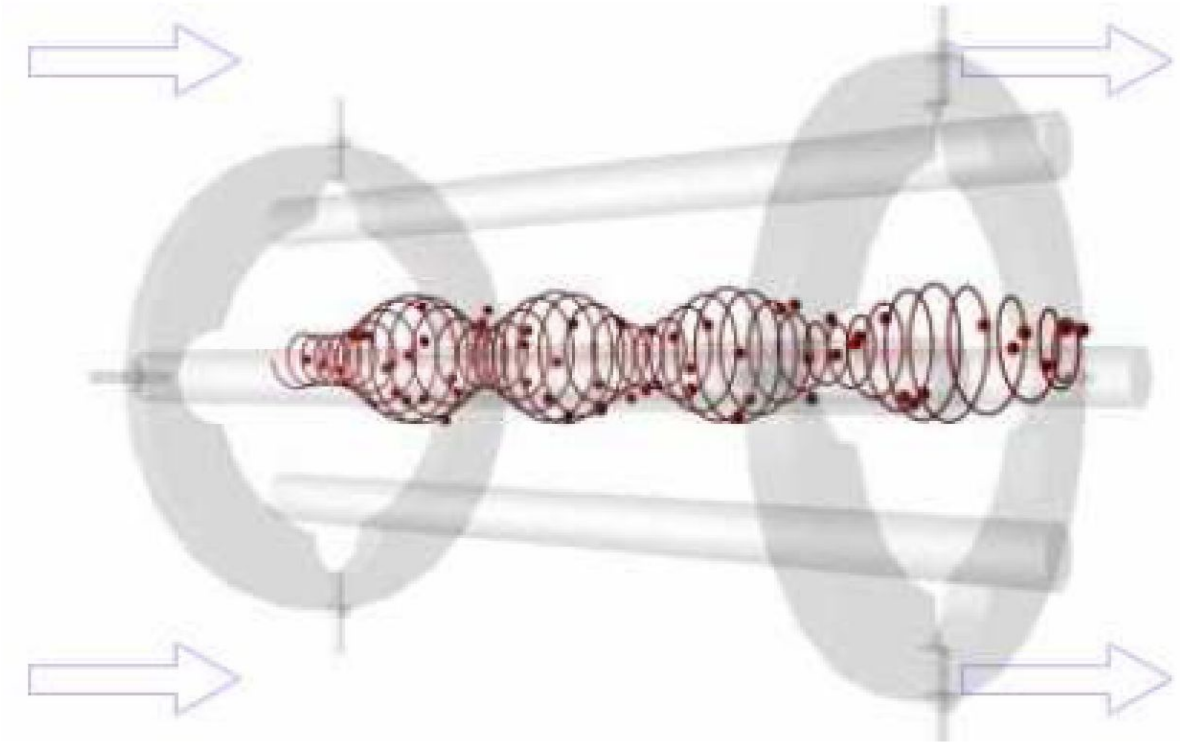
Sobreposição de AC e DC ►



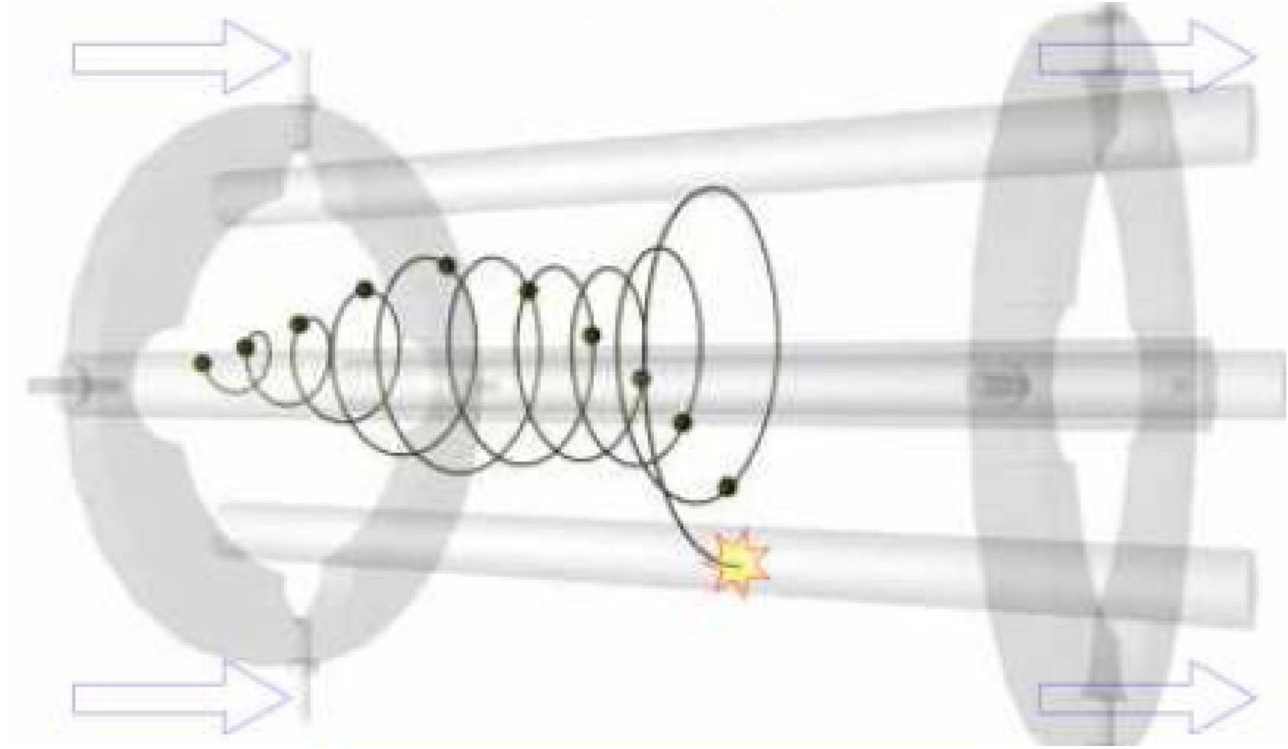
$$a = \frac{8zU}{mr_o^2\omega^2} \qquad q = \frac{4zV}{mr_o^2\omega^2}$$



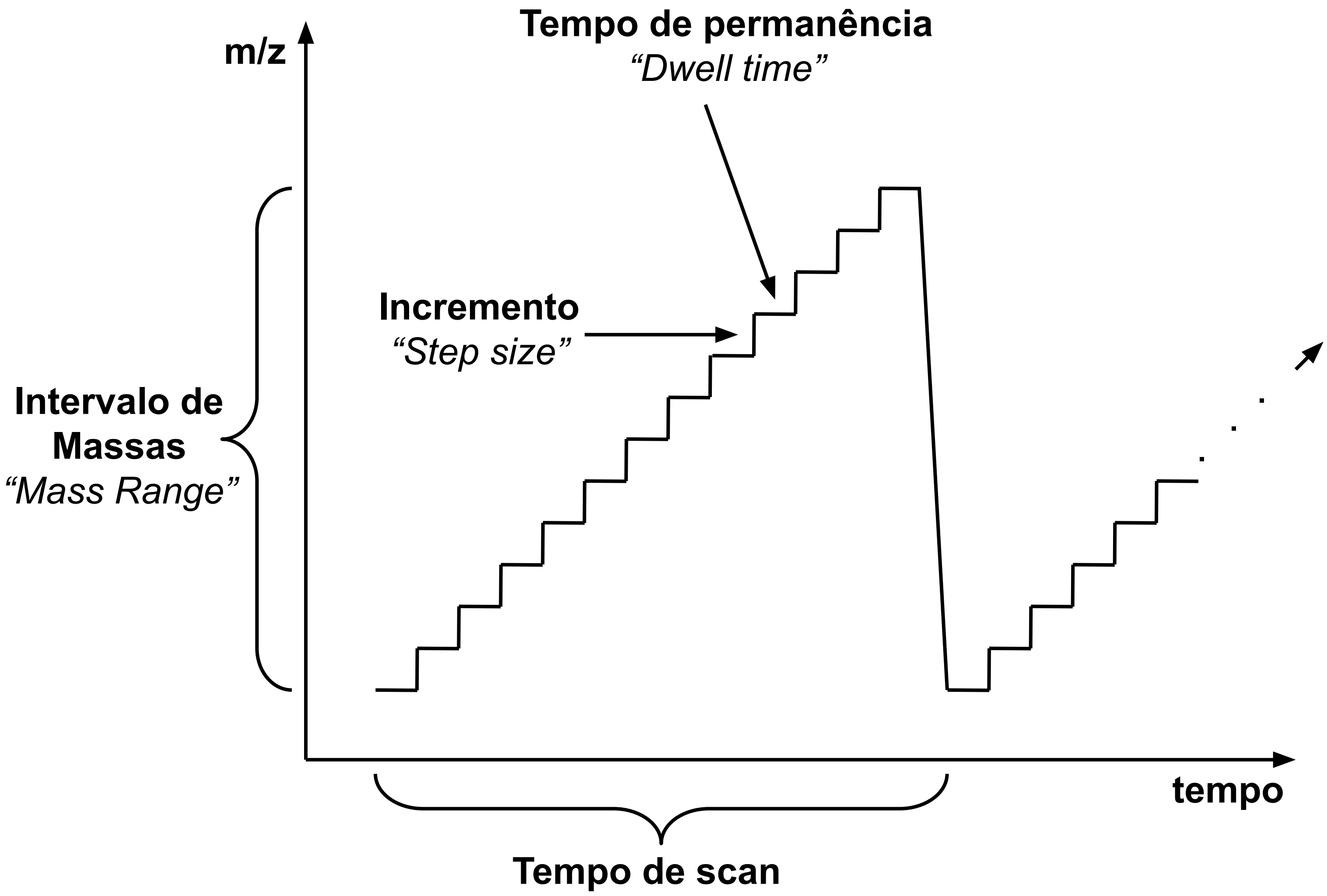
Trajetória de íon em movimento harmônico



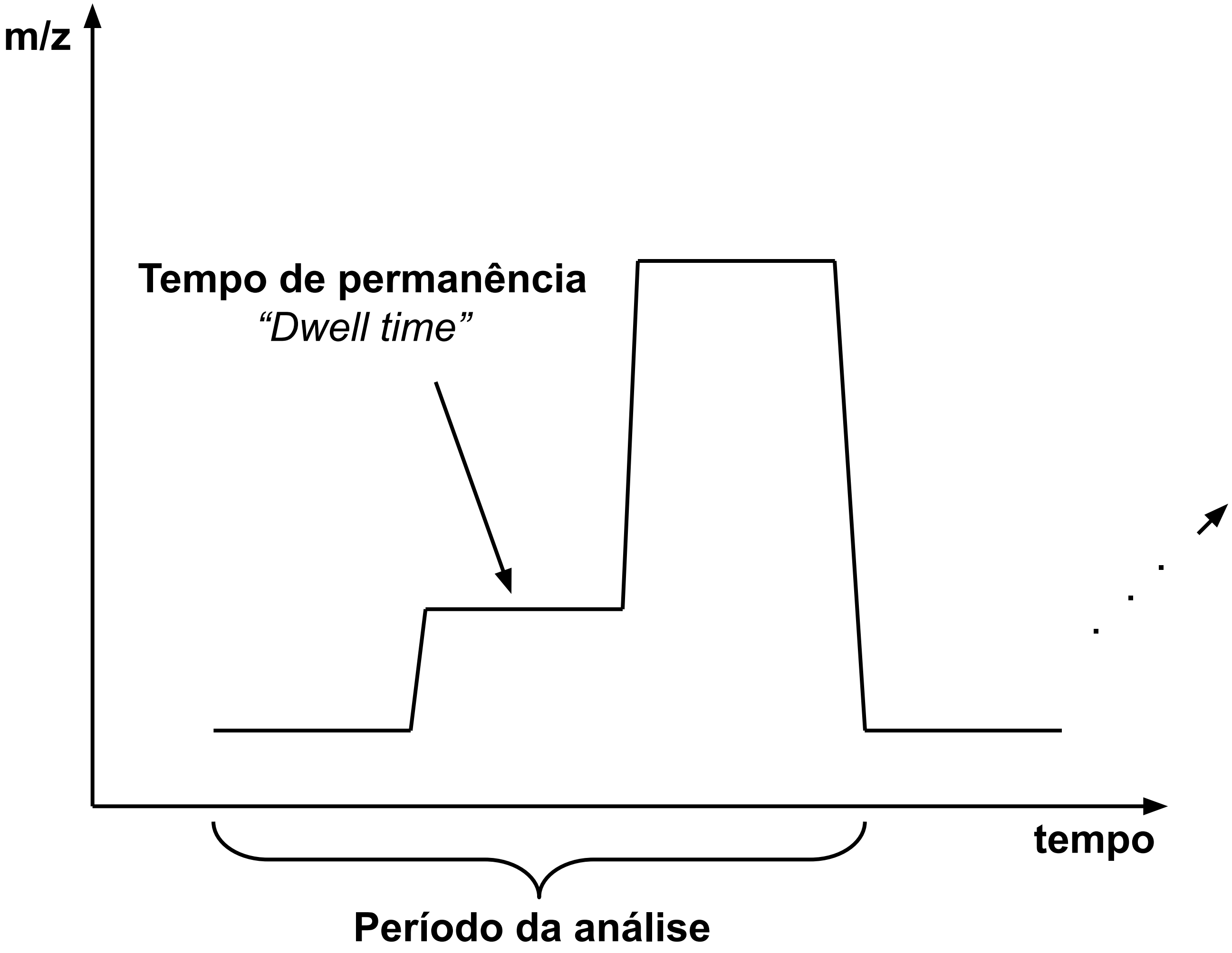
Trajetória de íon em movimento não harmônico



Modo Scan


m/z
Tempo de permanência
"Dwell time"
Incremento
"Step size"
Intervalo de Massas
"Mass Range"
tempo
Tempo de scan

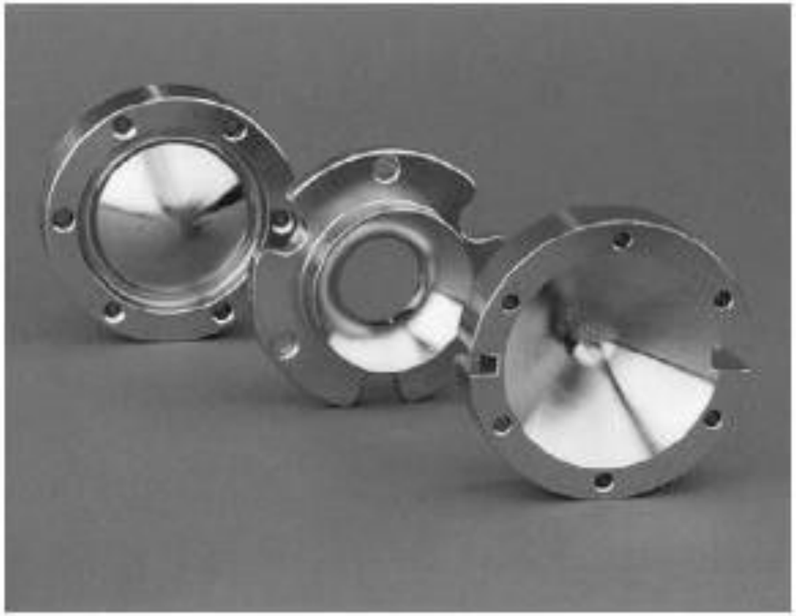
Modo SIM


m/z
Tempo de permanência
"Dwell time"
tempo
Período da análise

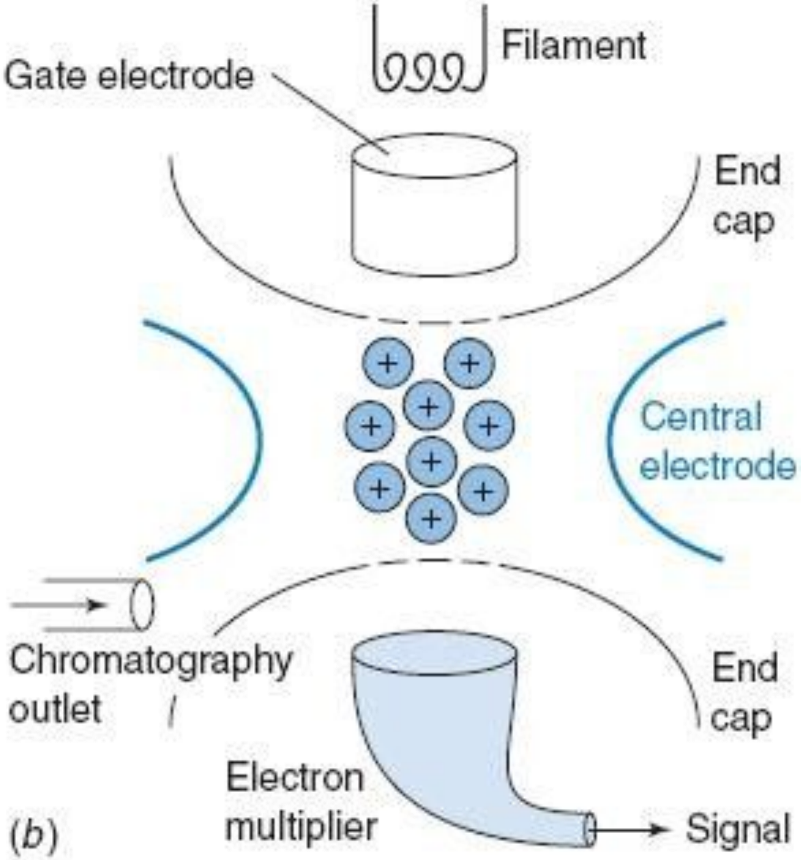
Características de um Quadrupolo

- m/z < 4000 Da/e
- Baixo RP: 1000-4000 (FWHM)
  - geralmente 0,7 a 1,0 Da
- Grau de Exatidão: 100 a 200 ppm
- Baixo custo
- Velocidade de varredura razoável
- Velocidade de varredura influencia na detectabilidade

Quadrupolo Aprisionador de Íons - *Ion trap*



(*a*)



(*b*)

Ion trap

DC on the Ring

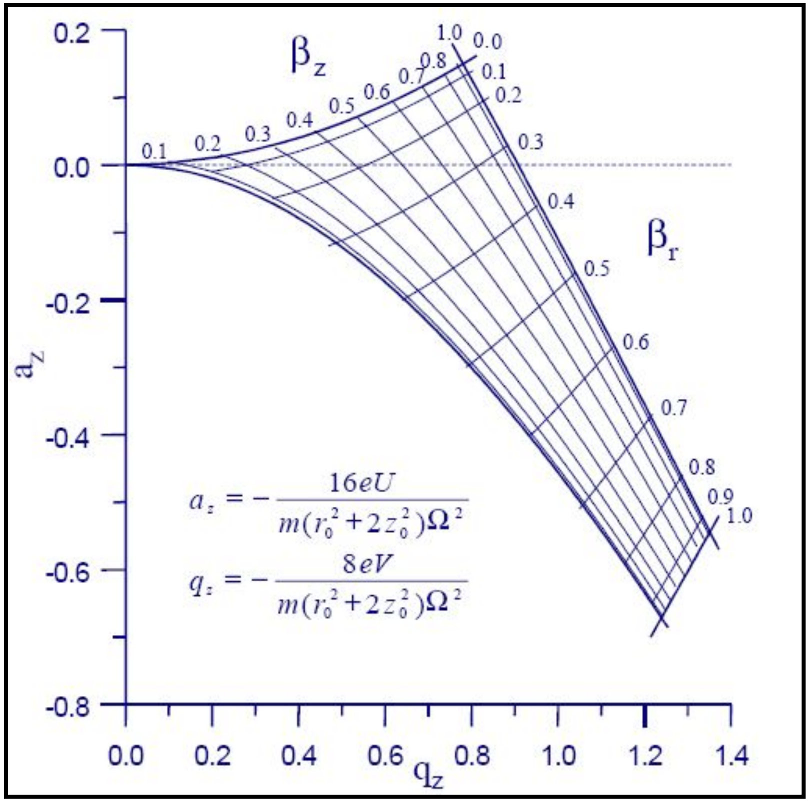
$$a_z = -2a_r = -\frac{16eU}{m(r_0^2 + 2z_0^2)\Omega^2}$$

RF on the Ring

$$q_z = -2q_r = -\frac{8eV}{m(\underbrace{r_0^2 + 2z_0^2)\Omega^2}_{\text{Ring Parameters}}}$$

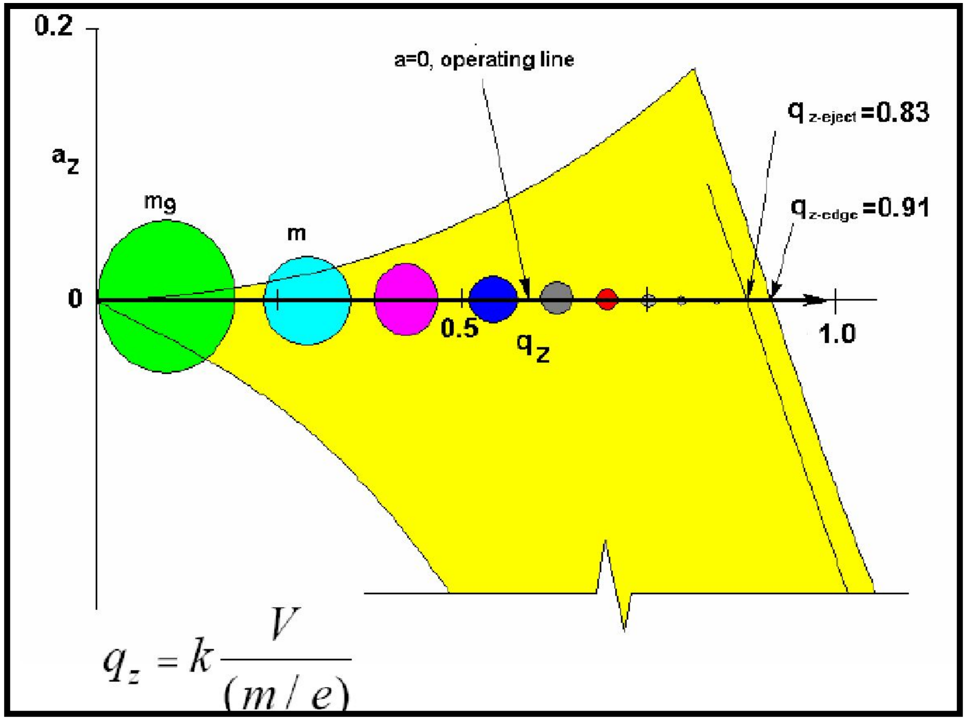
Mass

Ion trap



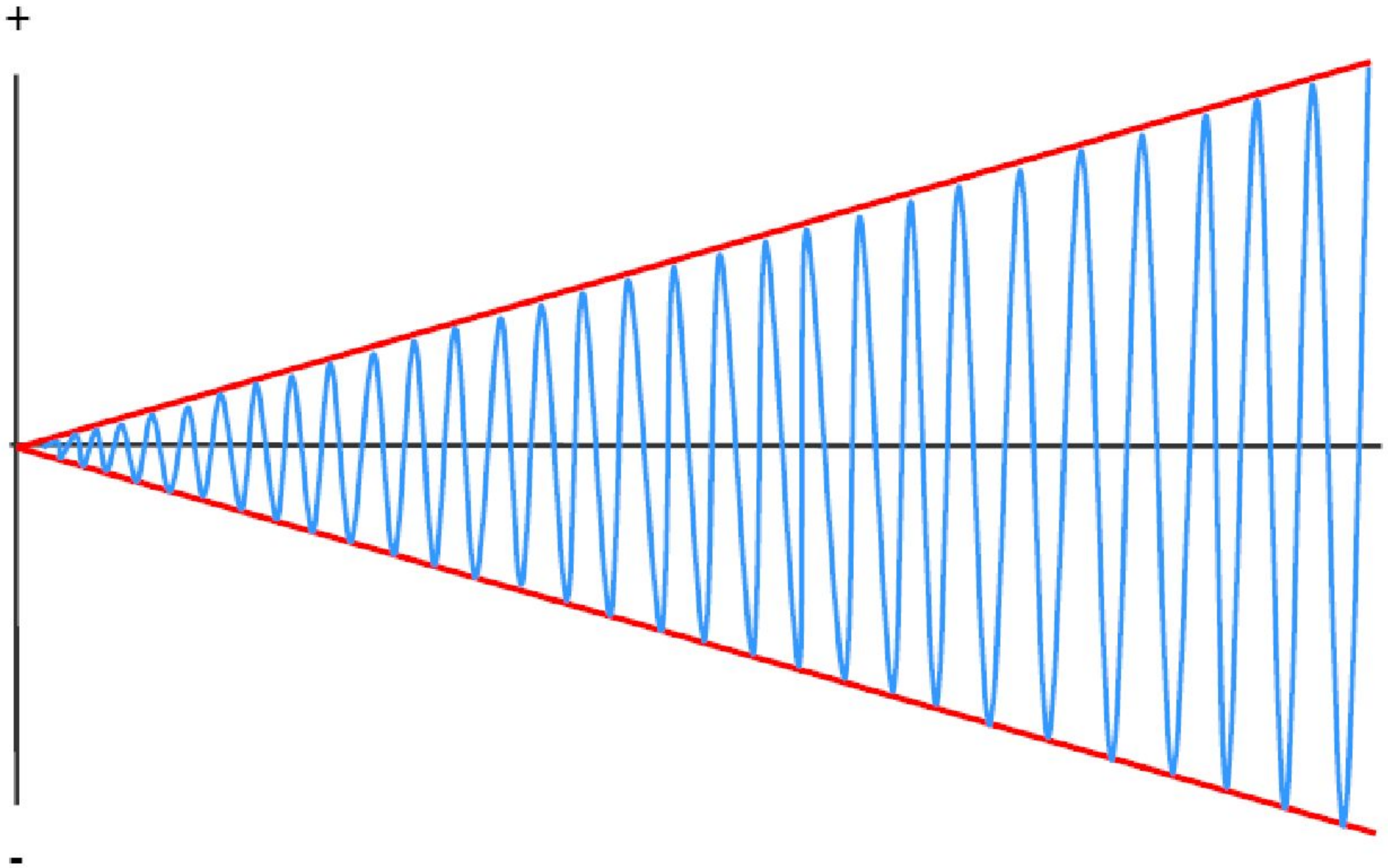
$$a_z = -\frac{16eU}{m(r_0^2+2z_0^2)\Omega^2}$$

$$q_z = -\frac{8eV}{m(r_0^2+2z_0^2)\Omega^2}$$

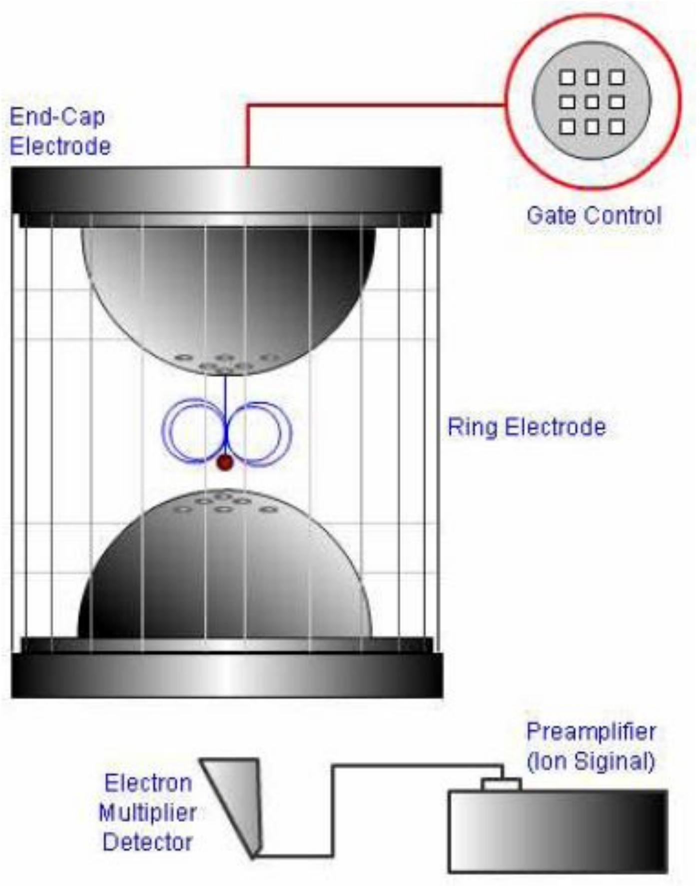


$$q_z = k\frac{V}{(m/e)}$$

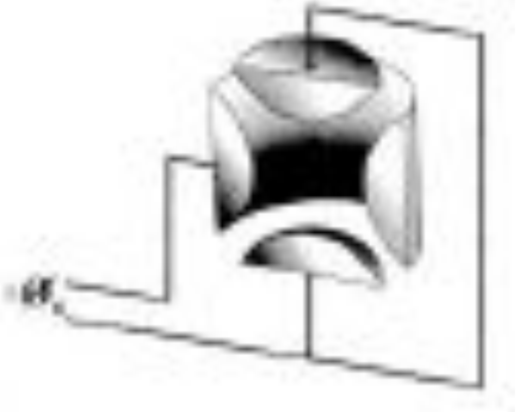
Varredura do potencial V



Varredura do potencial V

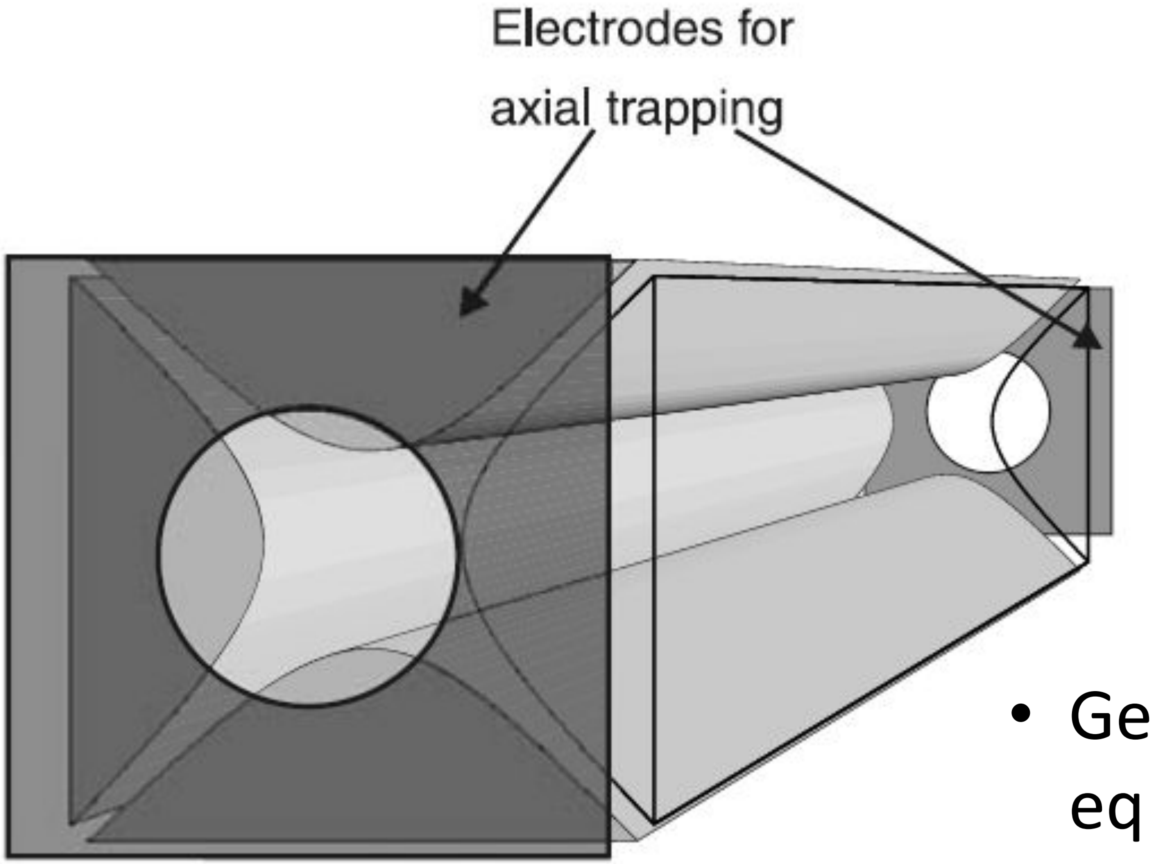


Características de um IT



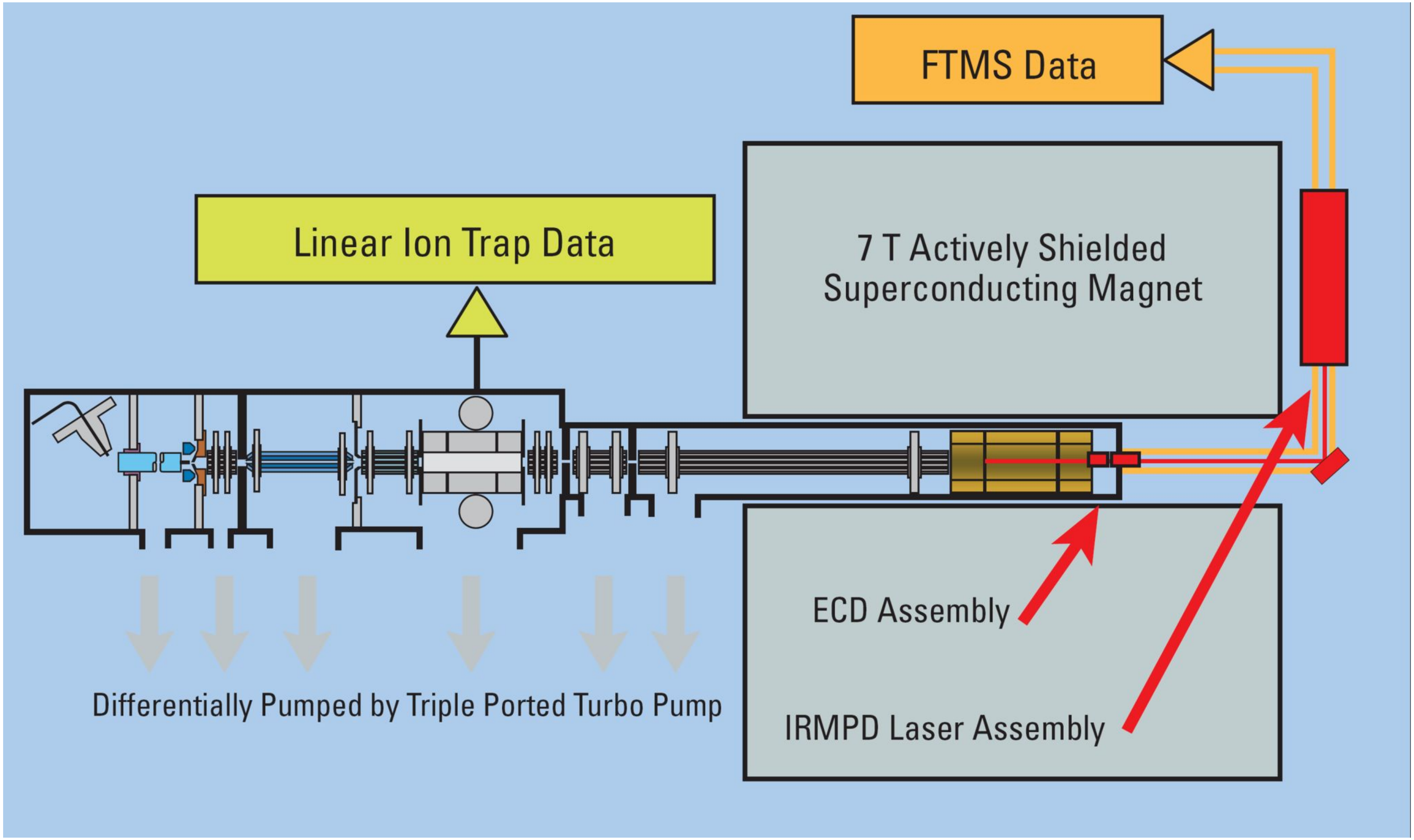
- m/z < 6.000 Da/e
- RP: 1000 a 5000 (FWHM)
  - Geralmente 0,7 a 1,0 Da
- Grau de Exatidão: 200 a 300 ppm
- Baixo custo
- MS^n (n < 4 ou 5)
- *Charge-space effects*

Ion trap linear - LIT

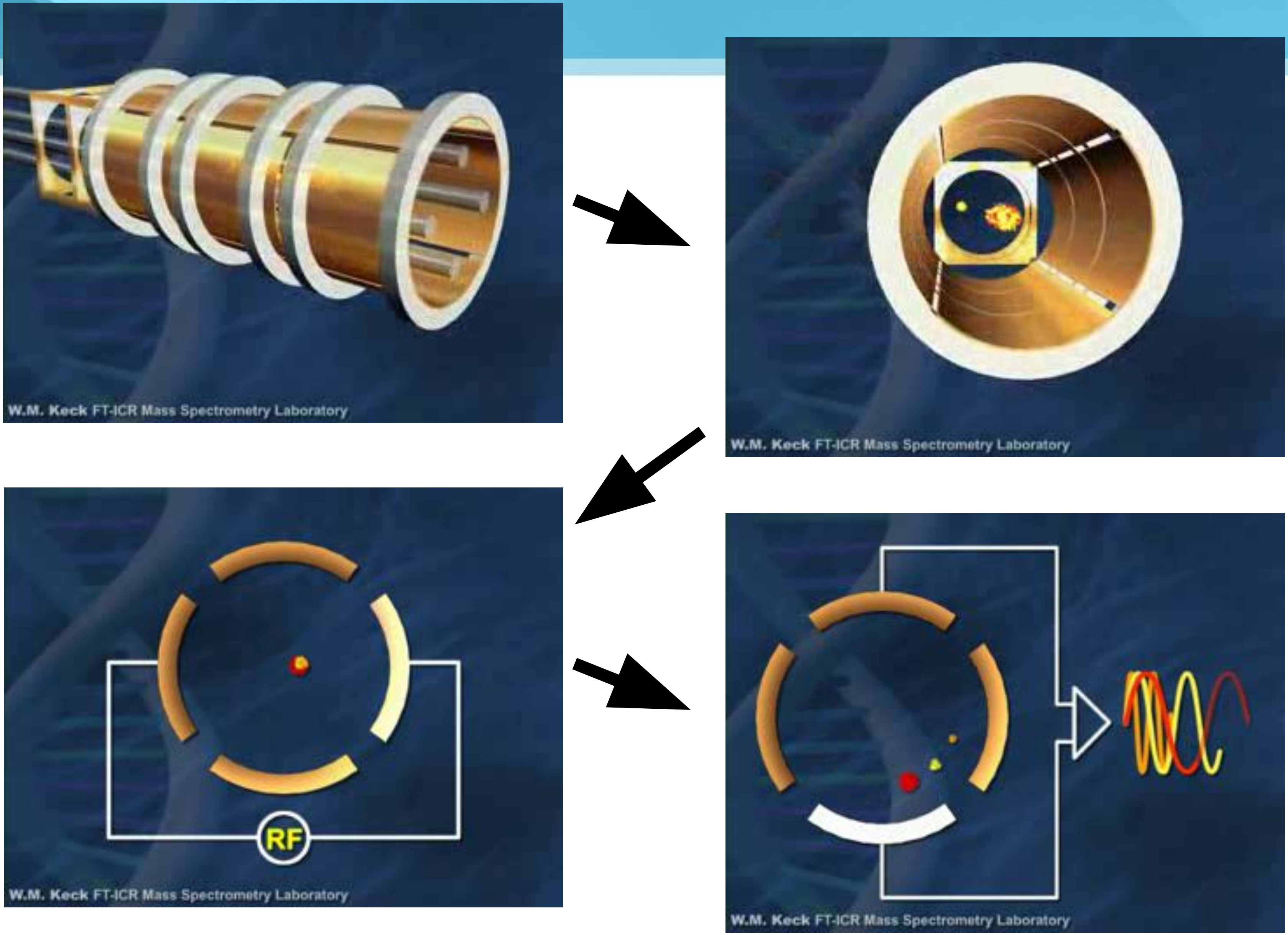


- Geralmente usado em equipamentos híbridos

Ressonância Ciclotrônica de Íons com Transformada de Fourier (FT-ICR)



FT-ICR



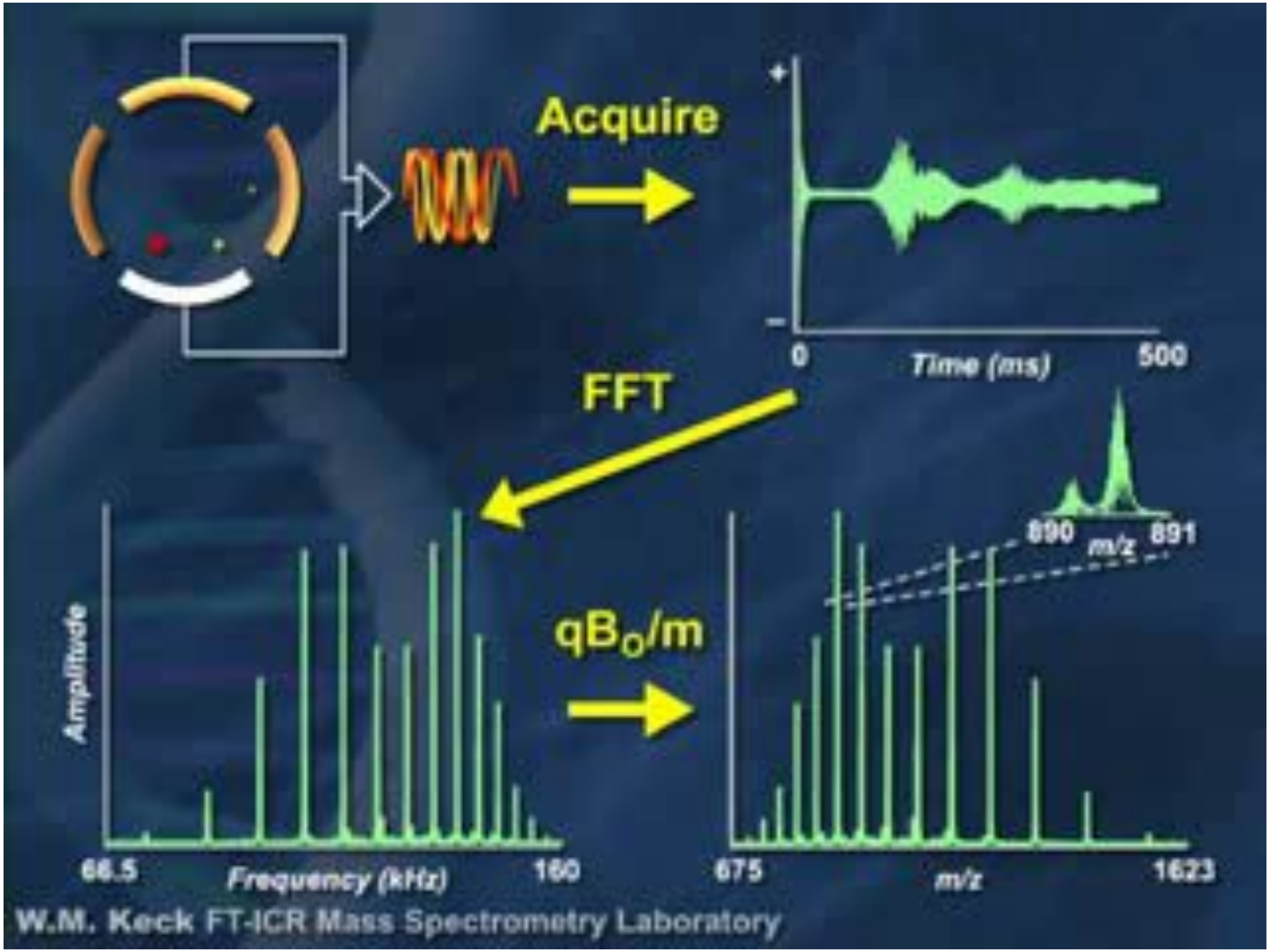
W.M. Keck FT-ICR Mass Spectrometry Laboratory

W.M. Keck FT-ICR Mass Spectrometry Laboratory

W.M. Keck FT-ICR Mass Spectrometry Laboratory

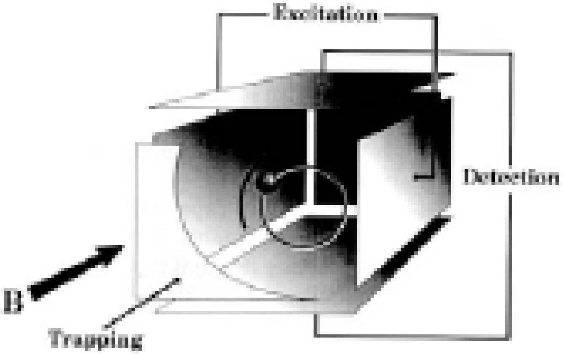
W.M. Keck FT-ICR Mass Spectrometry Laboratory

FT-ICR


Acquire
+
−
0
Time (ms)
500
FFT
890
m/z
891
Amplitude
qB$_0$/m
66.5
Frequency (kHz)
160
675
m/z
1623
W.M. Keck FT-ICR Mass Spectrometry Laboratory

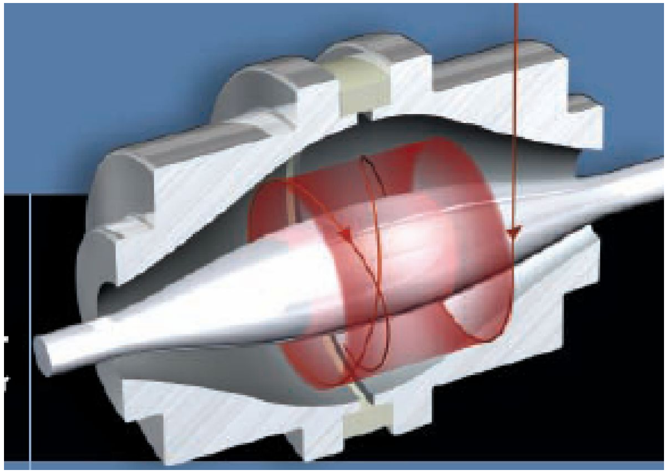
Características de um FT-ICR

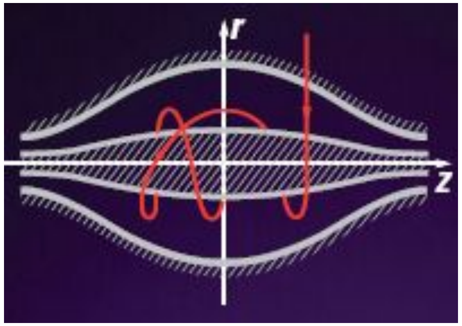
- – m/z: < 4000 híbrido ou até 70.000 Da/e
- – RP: 100.000 a 1.000.000 (FWHM)
- – Exatidão: < 1 ppm (int) ou <1,2 ppm (ext)
- – Custo alto
- – Dificuldade de operação
- – Manutenção
    - 150 L He líquido (troca cada 100 dias)
    - 100 L N_2 líquido (troca cada 7 dias)

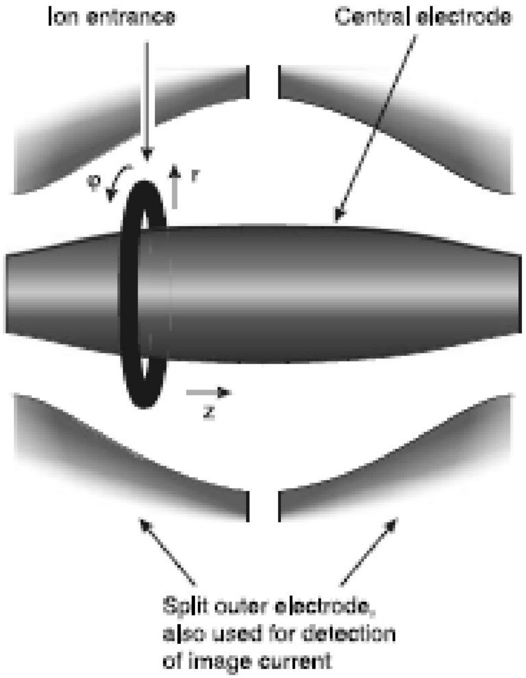


Orbitrap

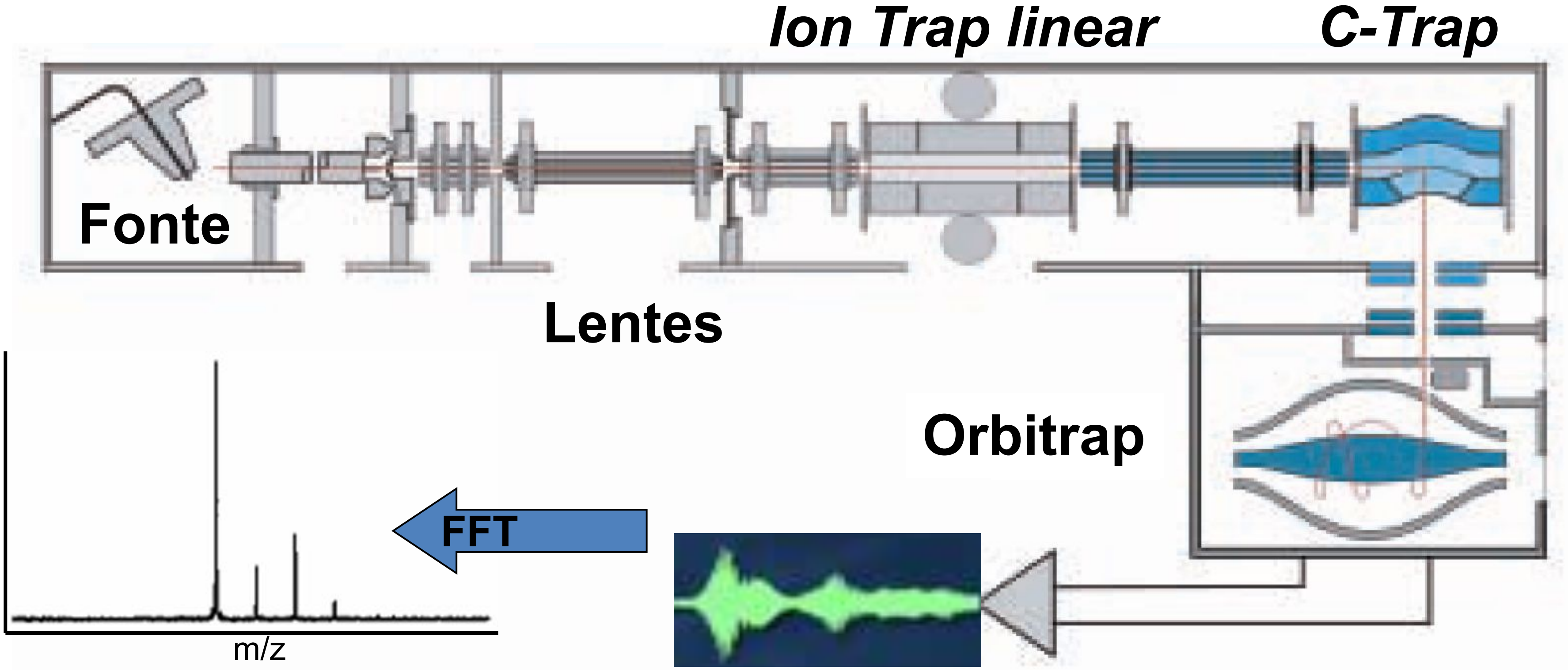
- Aprisionador eletrostático orbital axialmente harmônico
- Também utiliza FFT
- Mais nova tecnologia de análise de massas (2002)





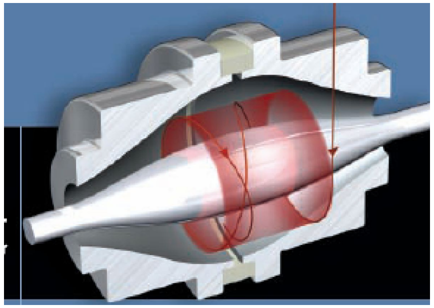


LTQ Orbitrap®



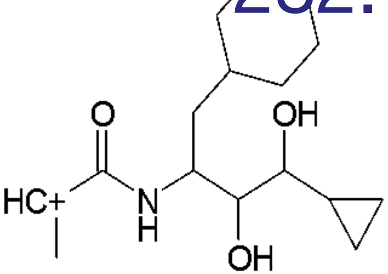
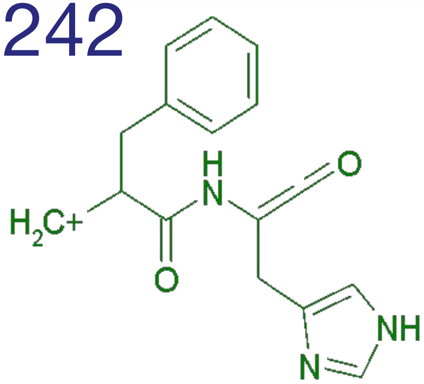
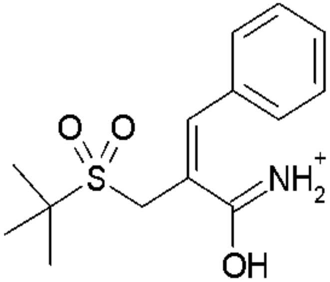
Características de um Orbitrap

- m/z < 50000 Da/e
- Resolução até 200.000 (FWHM)
  - Geralmente 60.000 ou 100.000
- Exatidão: 1 a 2 ppm (com PI)
- Custo relativamente alto

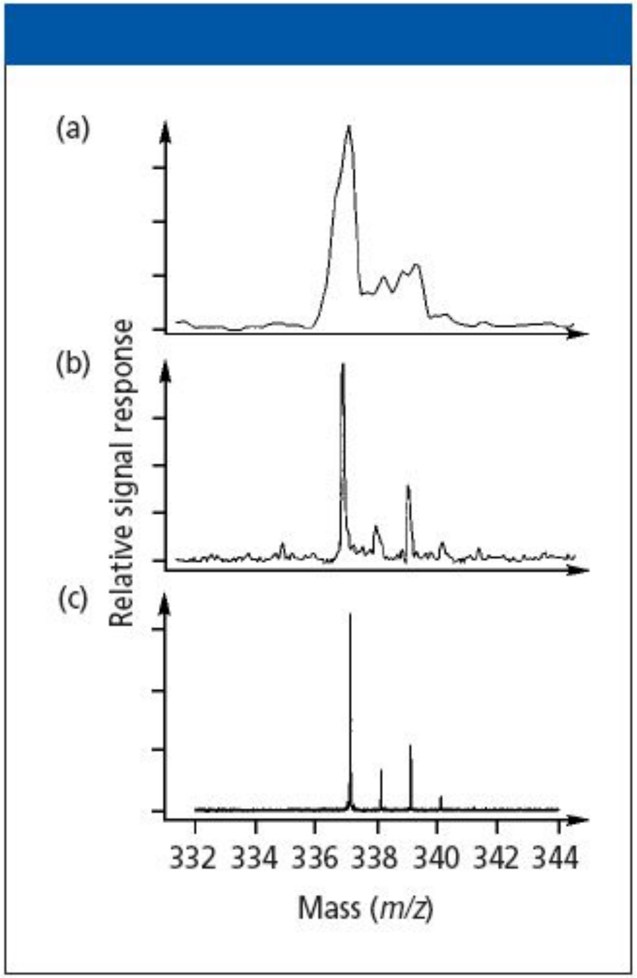


Qual a importância da Exatidão de Massa?

$m/z_{\text{medida}} = 282.1242$

		
(A)	(B)	(C)
$C_{16}H_{28}NO_3$ 282.2069 82.7 mmu 293 ppm	$C_{16}H_{16}N_3O_2$ 282.1242 0.0 0.1 ppm	$C_{14}H_{20}NO_3S$ 282.1164 7.8 mmu 28 ppm

Qual o efeito da resolução do analisador?



Comparação de espectros de massas obtidos por diferentes analisadores:

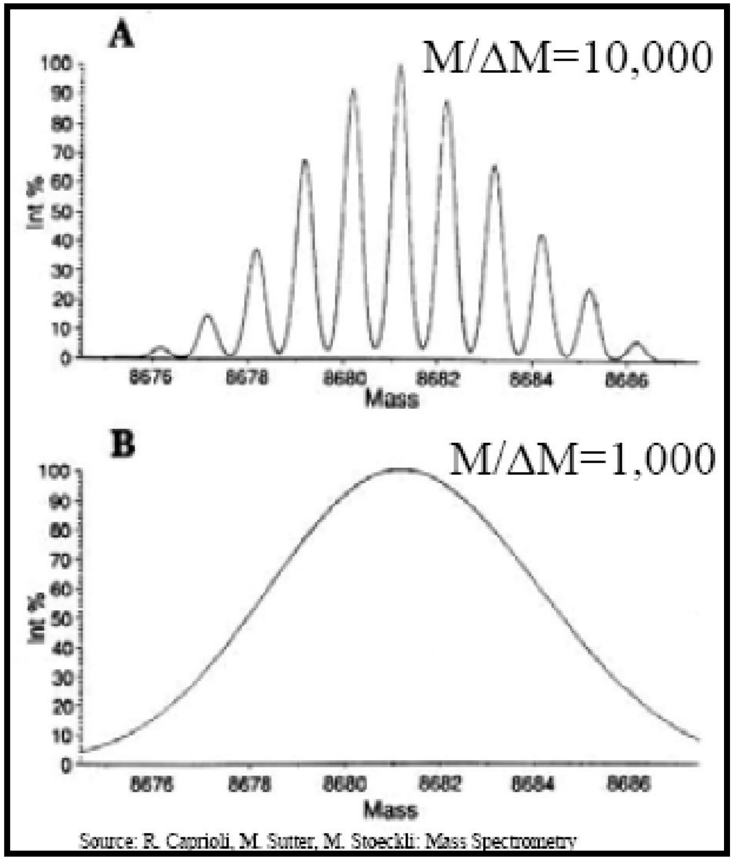
a)Quadrupolo

b)ToF

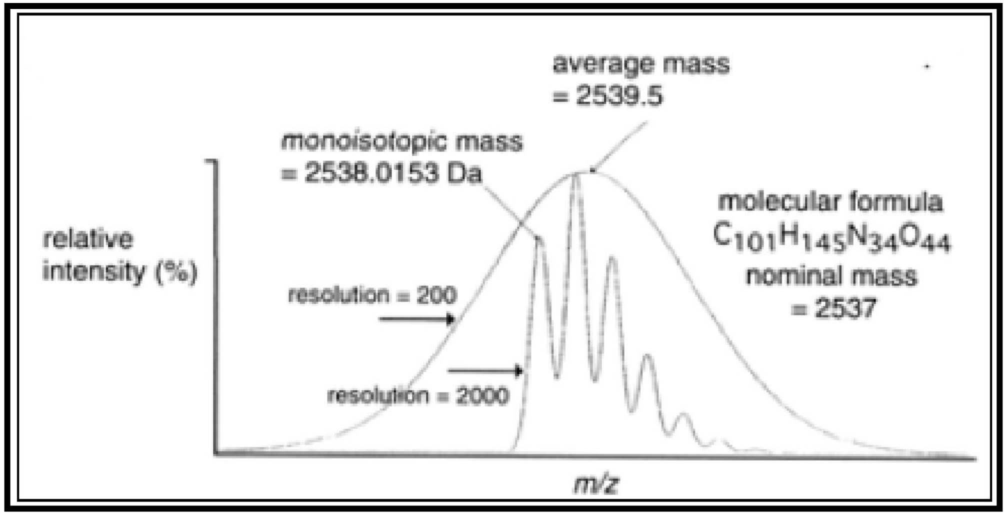
c)Transformada de Fourier

***LC-GC.**, 2001, 19, 514-523.*

Para que a resolução é importante?



Source: R. Caprioli, M. Sutter, M. Stoeckli: Mass Spectrometry



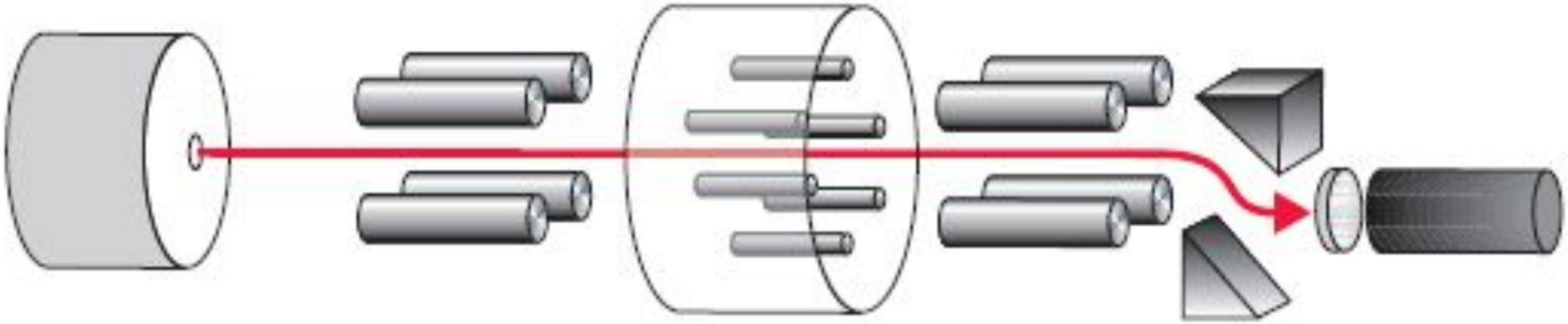
“Sem resolução boa não há exatidão adequada!”

Analisadores MS/MS

- Instrumentos para *Tandem* MS
  - “Triplo” Quadrupolo (QqQ)
  - Ion Trap (IT)
    - Convencional
    - Linear
  - Q-ToF
  - Q-Ion Trap
  - Ion Trap-Orbitrap
  - Ion Trap-FTICR

Híbridos

“TANDEM-IN-SPACE” – ex. QqQ



Fonte

MS1

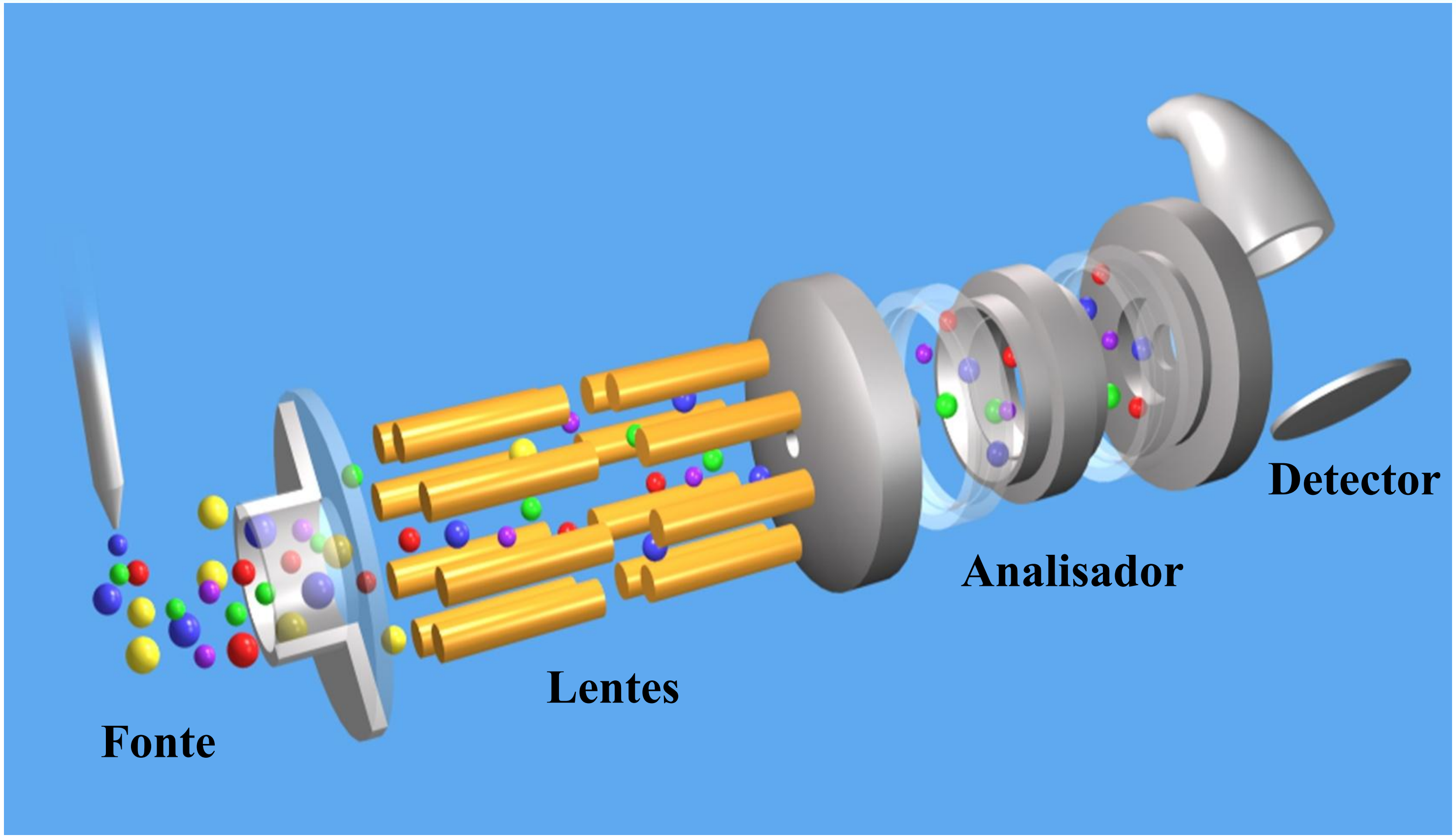
Colisão

MS2

Detector

Esquema de um instrumento do tipo “triplo” quadrupolo (QqQ)

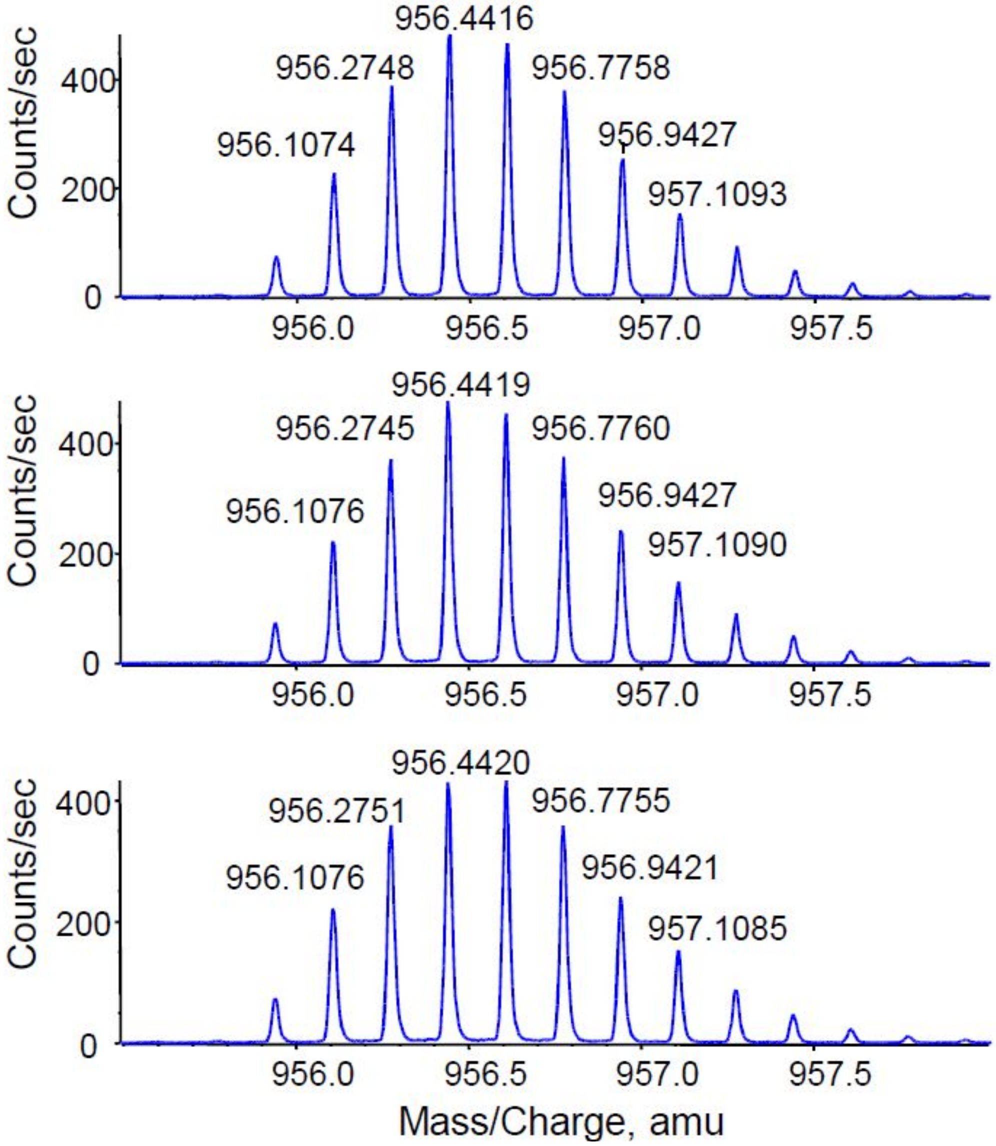
“TANDEM-IN-TIME” – ex. Ion Trap



Esquema de um instrumento do tipo Ion Trap MS/MS

High Resolution at the Highest Speed

SmartSpeed™ Acquisition - Bovine Insulin, 6+ Charge State



1 s

R=43,750

100 ms

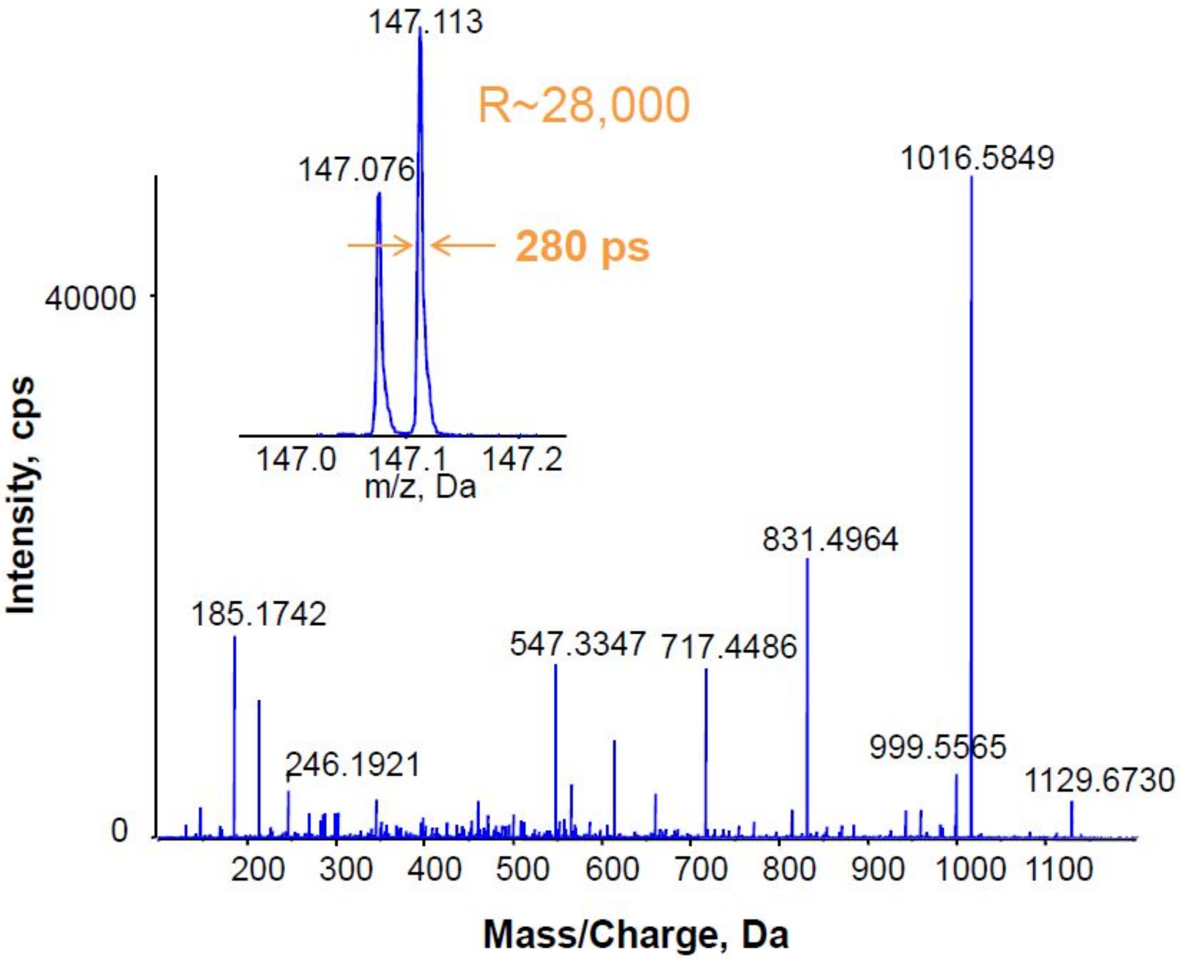
R=44,020

10 ms

R=44,000

High Resolution at Low m/z

10x More Data Points Across a Peak than 4 GHz ADC



- Ultra fast 40 GHz Time to Digital Converter (TDC) with 25 ps per bin
- Resolution across the mass range provides more information content
- Y1 ions of Lys and Gln can be resolved!

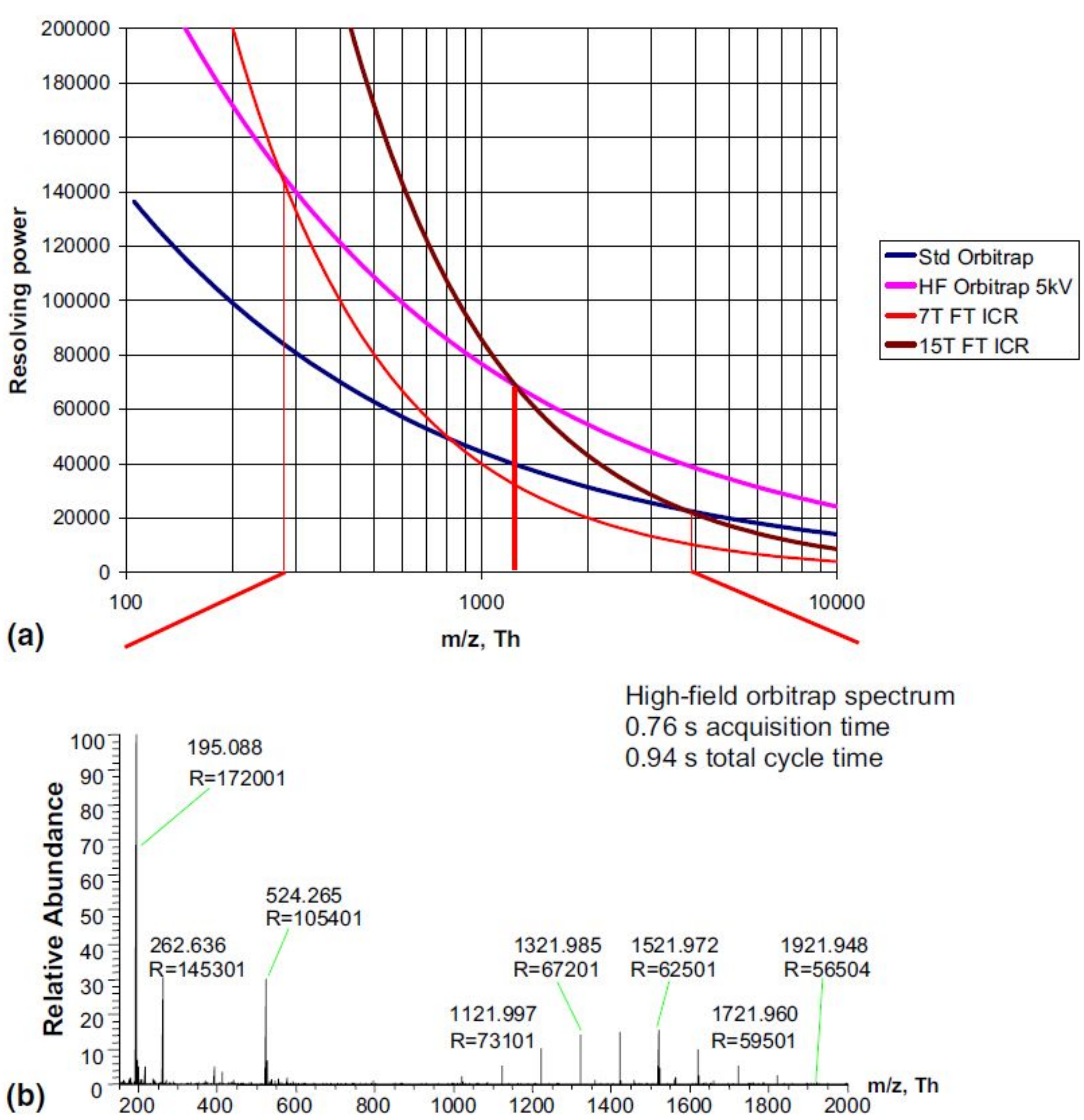


Figure 2. (a) Resolving power as a function of *m/z* for different FTMS instruments: Standard Orbitrap analyzer [4]; high-field Orbitrap analyzer; FT ICR with 7 Tesla superconducting magnet; FT ICR with 15 Tesla superconducting magnet. Acquisition parameters are the same in all cases (0.76 s detection time, single zero-filling and Kaiser-Bessel apodization). (b) An example of a spectrum with the mass range *m/z* 150–2000 acquired using HF Orbitrap.

MAKAROV ET AL. J Am Soc Mass Spectrom 2009, 20, 1391–1396

1396 MAKAROV ET AL. J Am Soc Mass Spectrom 2009, 20, 1391–1396

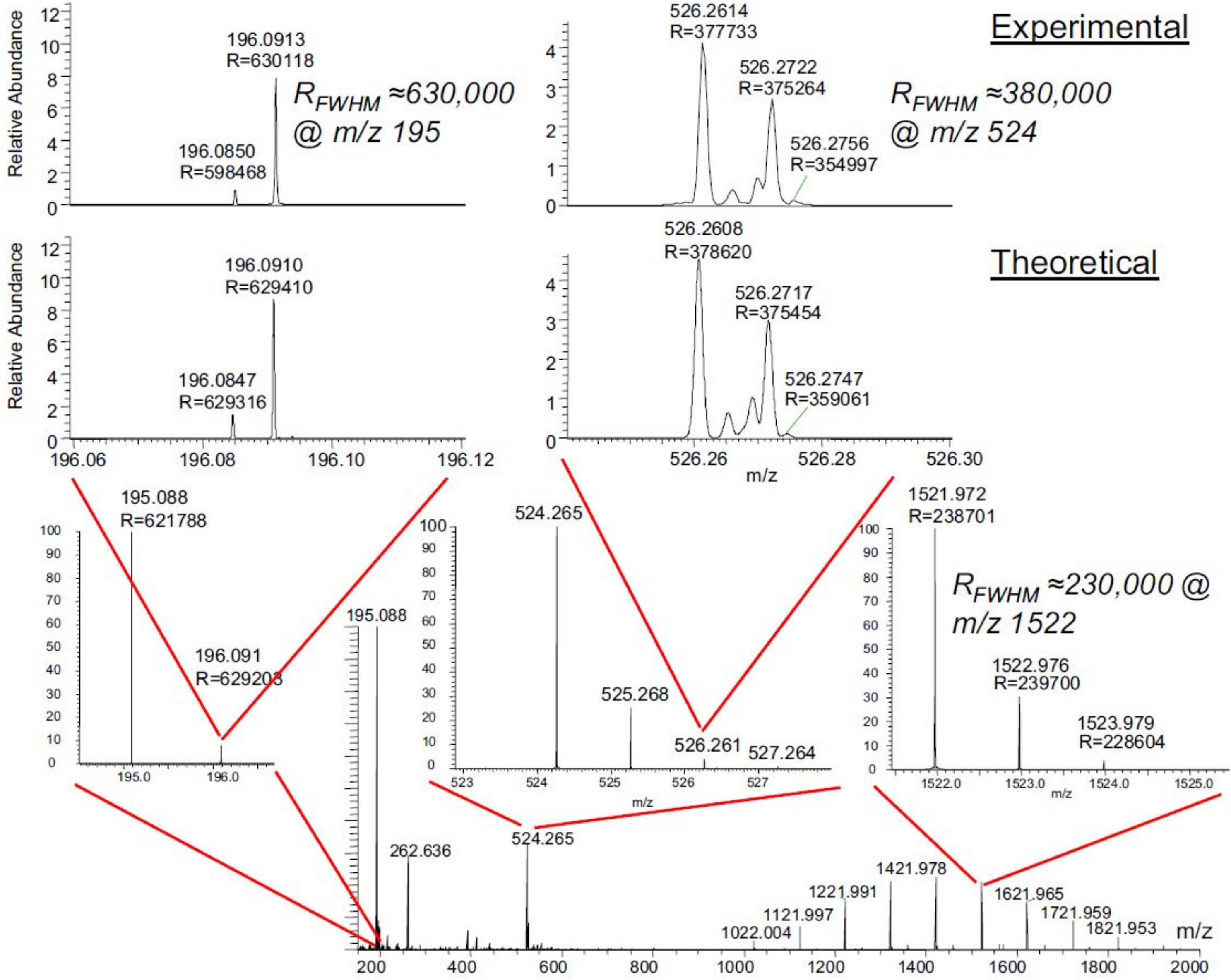
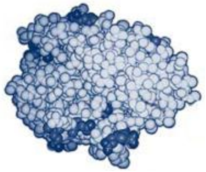
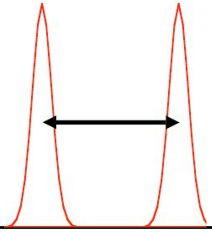
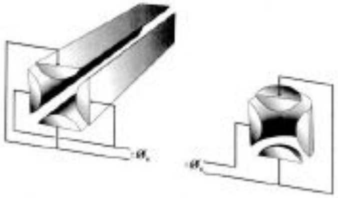
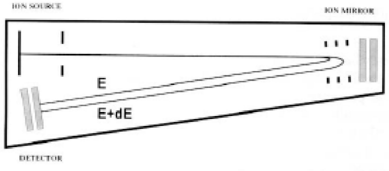
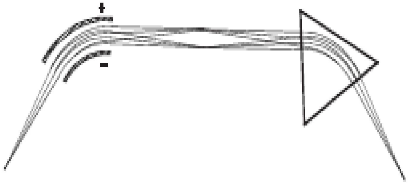
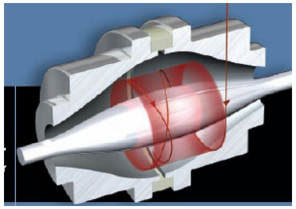
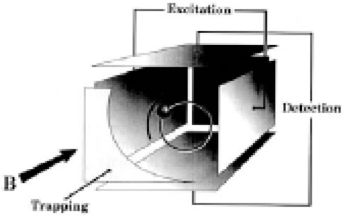
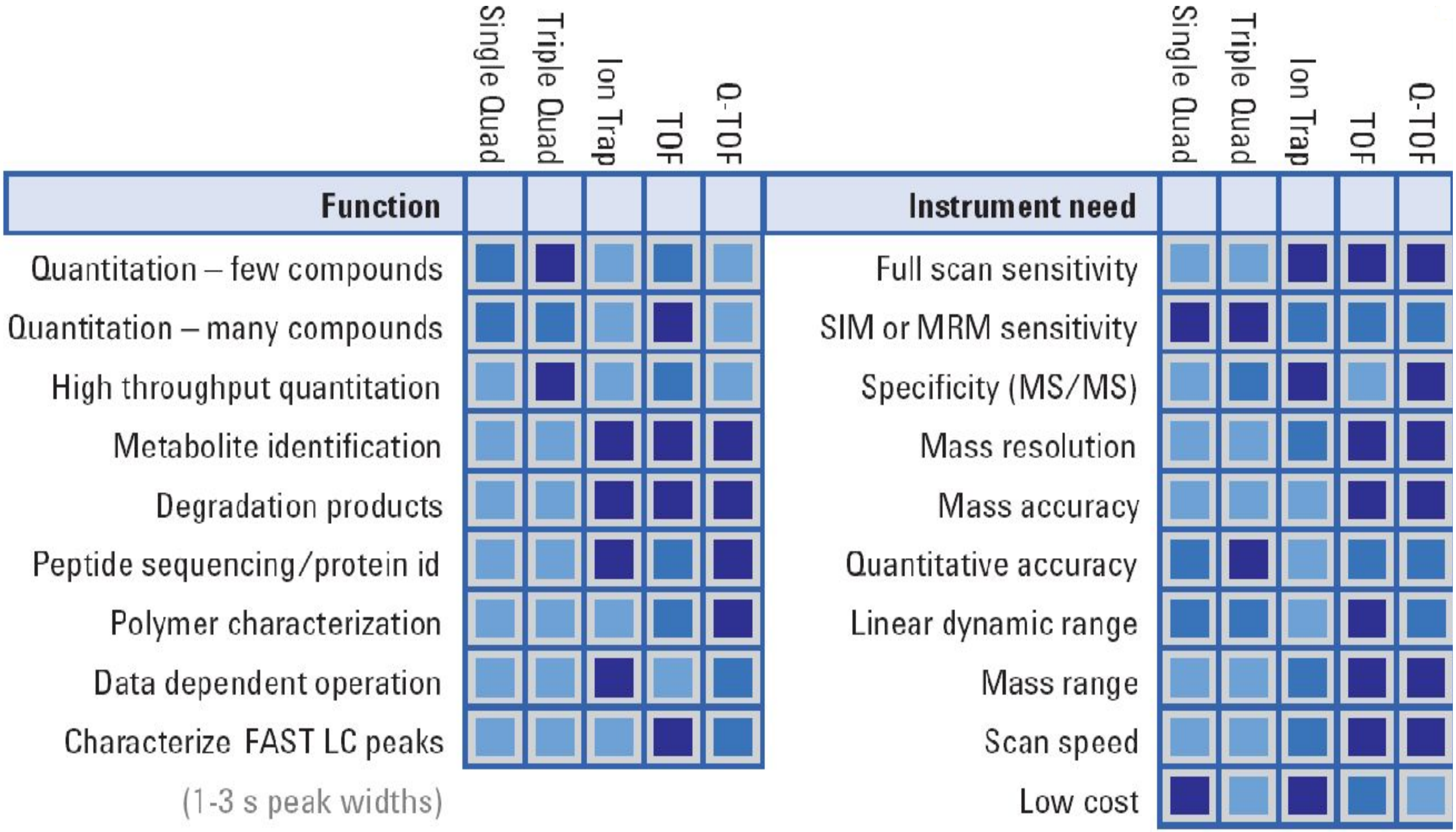


Figure 6. Ultimate resolving power obtained using 3 s detection time (four times longer comparing to detection time in Figure 2) and EXTERNAL calibration. Experimental values are presented on panels for corresponding *m/z*. Isotope clusters with fine structure are presented in insets along with corresponding theoretical patterns normalized to monoisotopic peak.

			Δm/m (ppm)		Amostragem
					
					
					
					
					

Escolhendo o analisador em LS-MS



Function	Single Quad	Triple Quad	Ion Trap	TOF	Q-TOF
Quantitation – few compounds	▣	■	▨	▣	▨
Quantitation – many compounds	▣	▣	▨	■	▨
High throughput quantitation	▨	■	▨	▣	▨
Metabolite identification	▨	▨	■	■	■
Degradation products	▨	▨	■	■	■
Peptide sequencing/protein id	▨	▨	■	▣	■
Polymer characterization	▨	▨	▨	▣	■
Data dependent operation	▨	▨	■	▨	▣
Characterize FAST LC peaks (1-3 s peak widths)	▨	▨	▨	■	▣

Instrument need	Single Quad	Triple Quad	Ion Trap	TOF	Q-TOF
Full scan sensitivity	▨	▨	■	■	■
SIM or MRM sensitivity	■	■	▣	▣	▣
Specificity (MS/MS)	▨	▣	■	▨	■
Mass resolution	▨	▨	▣	■	■
Mass accuracy	▨	▨	▨	■	■
Quantitative accuracy	▣	■	▨	▣	▣
Linear dynamic range	▣	▣	▨	■	▣
Mass range	▨	▨	▣	■	■
Scan speed	▨	▨	▣	■	■
Low cost	■	▨	■	▣	▨

■ Best chance of success
▣ Good chance of success
▨ Chance of success
□ Not recommended

Fonte: Agilent Technologies
Publication #: 5989-5848EN

Detectores para MS

Detectores – Copo de Faraday

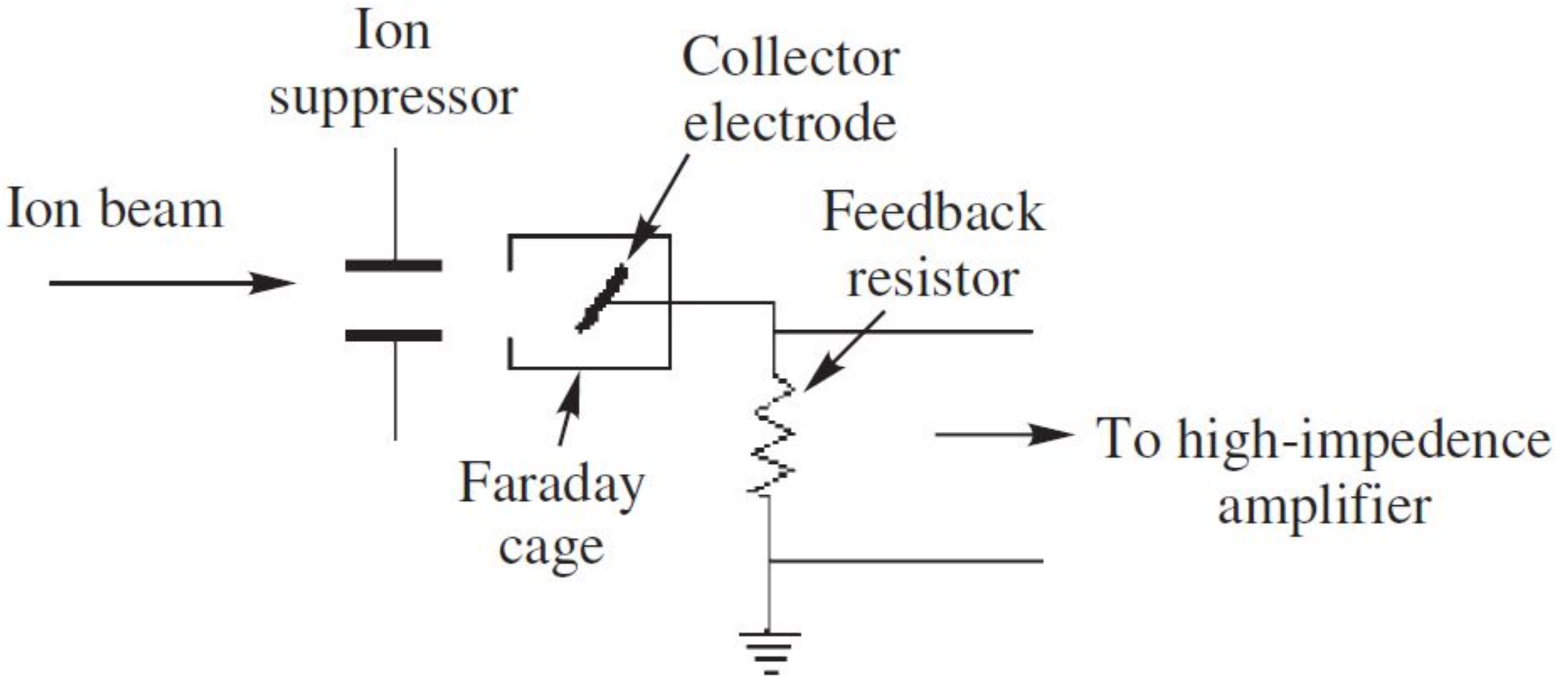


Figure 3.27. Faraday cup detector.

Detectores – Multiplicadora de elétrons com dinodo discreto

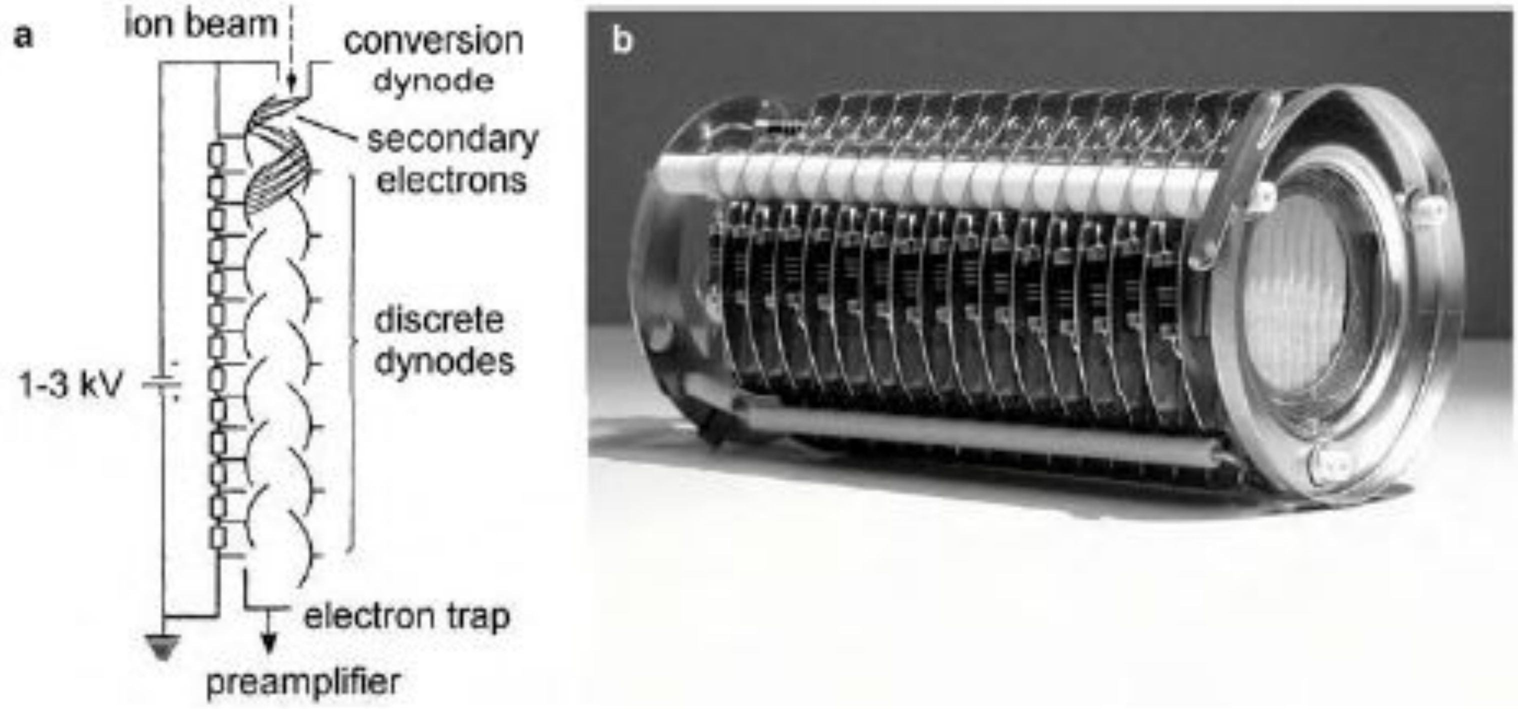


Fig. 4.57. Discrete dynode electron multipliers. (**a**) Schematic of a 14-stage SEM. (**b**) Photograph of an old-fashioned 16-stage Venetian blind-type SEM clearly showing the resistors and ceramics insulators between the stacking dynodes at its side. (**a**) Adapted from Ref. [238] by permission. © Springer-Verlag Heidelberg, 1991.

Detectores – Multiplicadora de elétrons contínua - *channeltron*

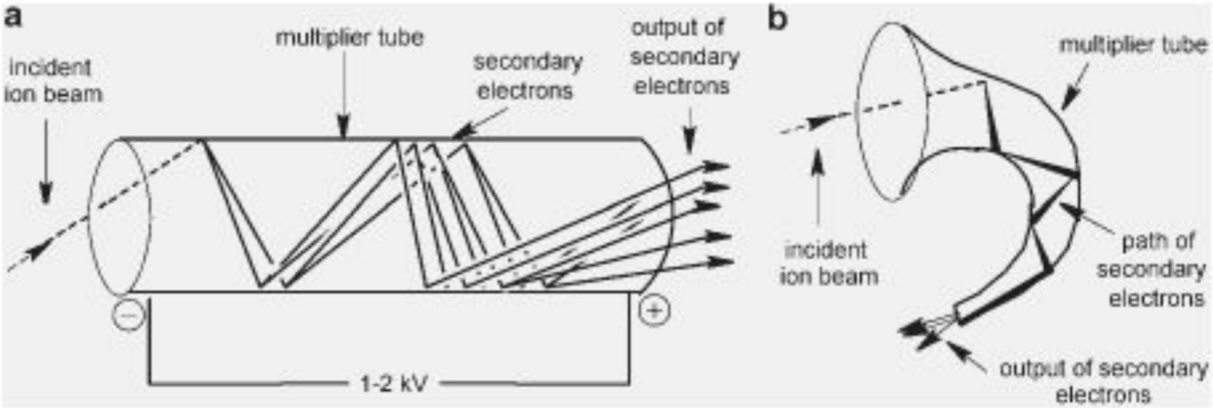


Fig. 4.58. Schematic of linear channel electron multiplier (**a**) and curved channel electron multiplier (**b**). By courtesy of Waters Corporation, MS Technologies, Manchester, UK..

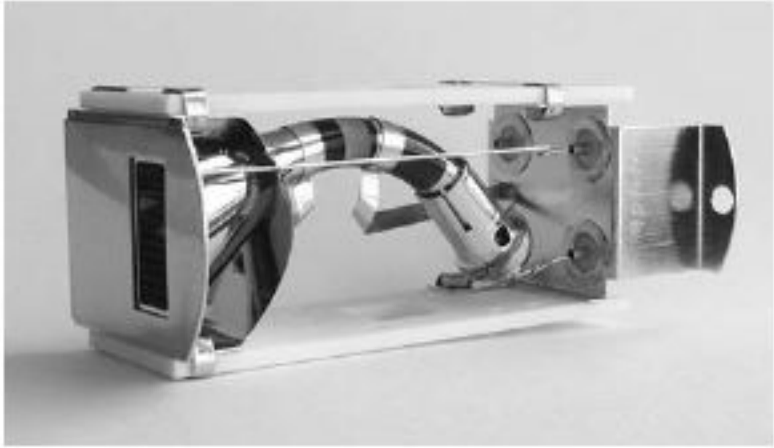


Fig. 4.59. Photograph of a channeltron multiplier.

Detectores – Placas com microcanais – *microchannel plates*

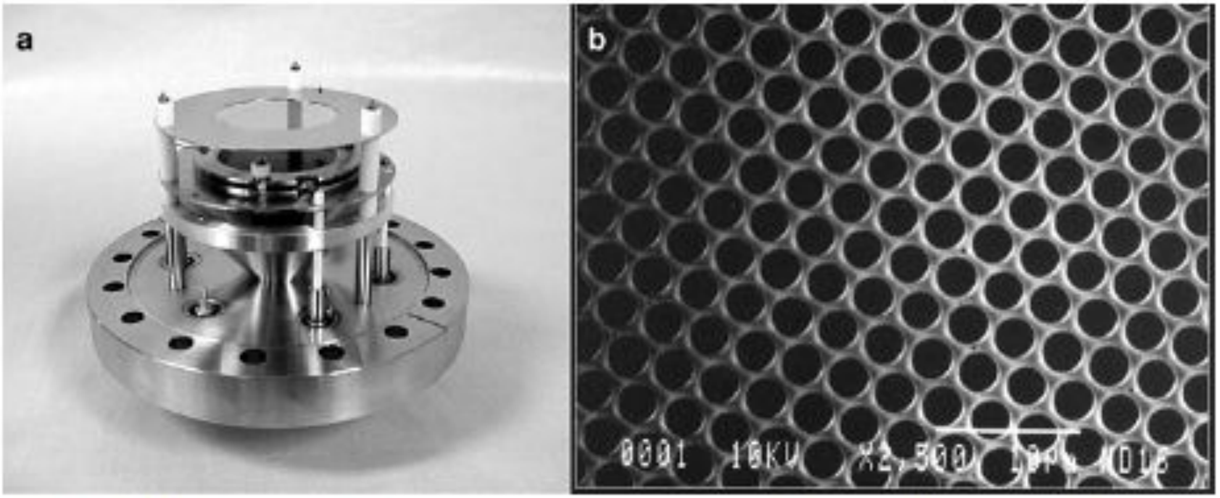


Fig. 4.60. MCP detector (shimmering surface) mounted on top of a flange (**a**) and SEM micrograph of a high resolving MCP showing channels of 2 μm diameter (**b**). By courtesy of (**a**) R.M. Jordan Company, Grass Valley, CA and of (**b**) Burle Industries, Baesweiler.

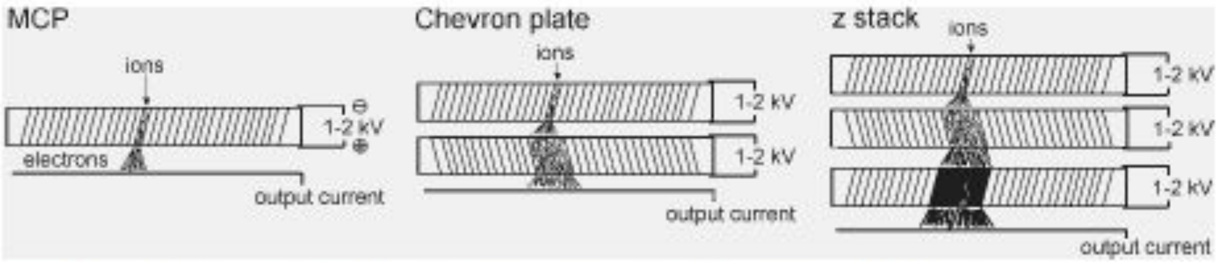


Fig. 4.61. Stacking of MCPs to increase gain. From left: single MCP, Chevron plate, and z-stack configuration. Note the loss of spacial resolution upon stacking.

ADC ou TDC

- Analog-to-Digital Converter Systems – ADC

- Time-to-Digital Converter Systems - TDC

ADC

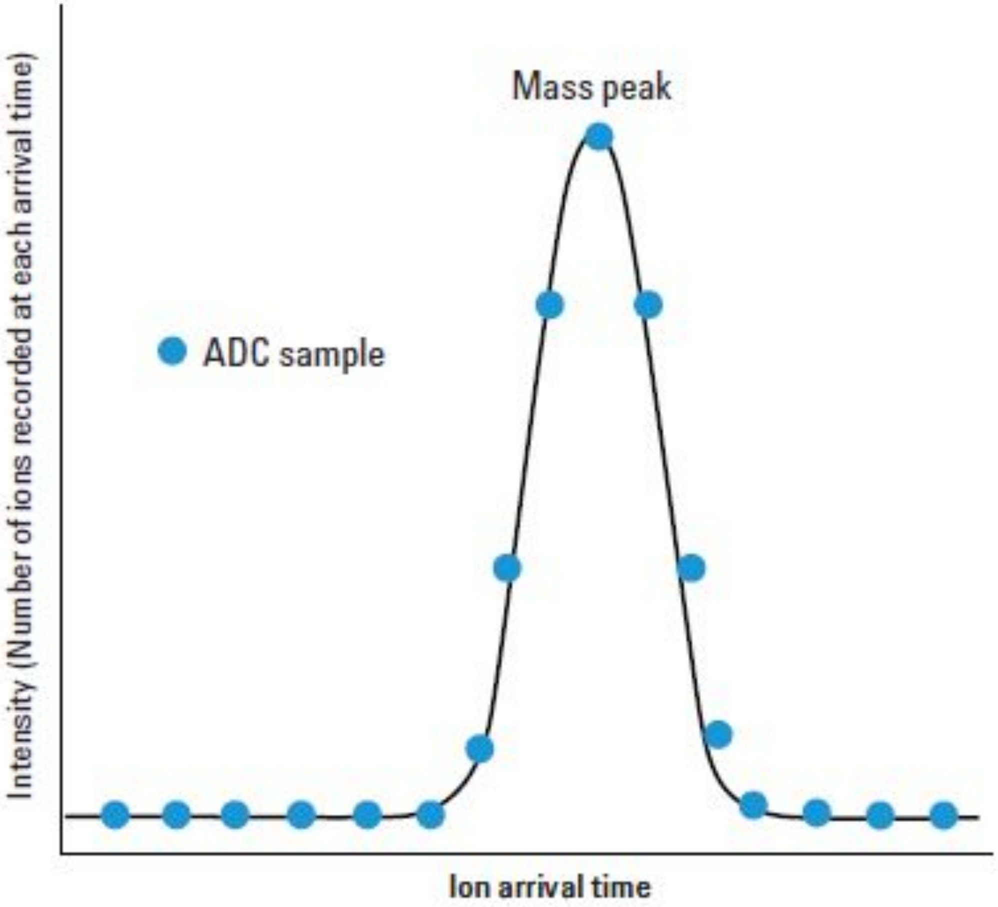


Figure 4. An ADC can record multiple ions per transient, so it accurately tracks ion signal intensity.

TDC

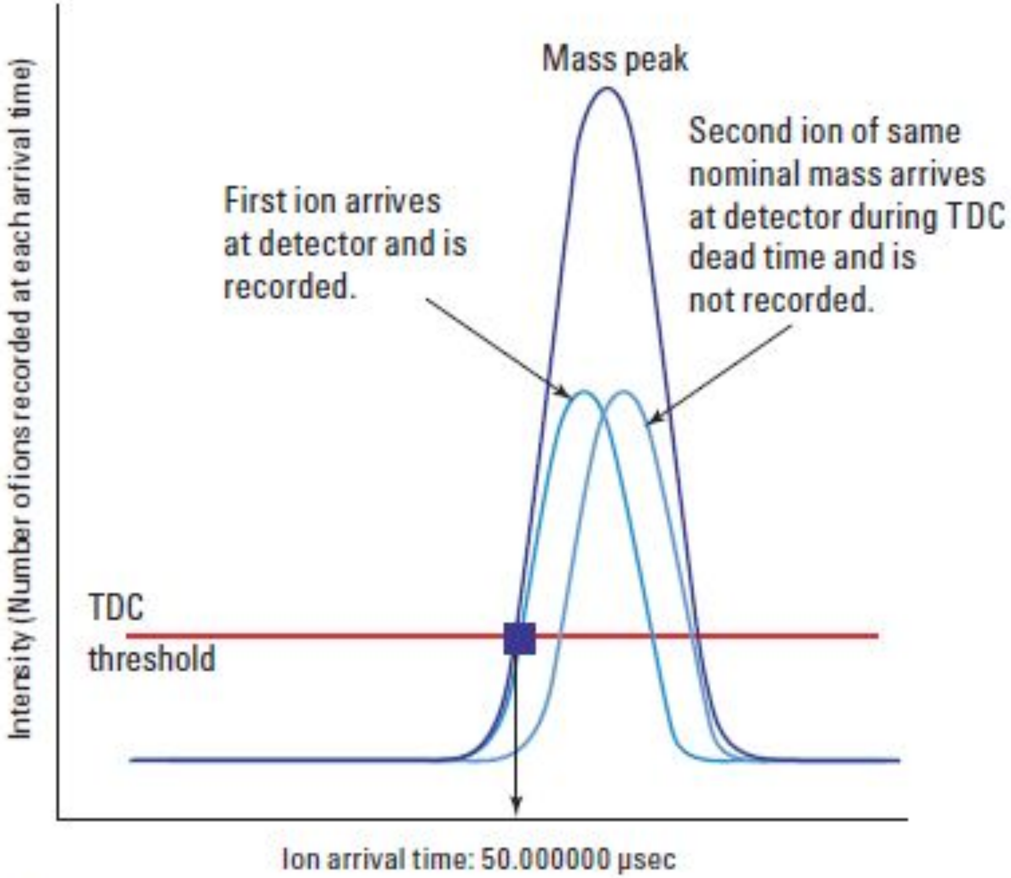


Figure 6. TOF detector with potentials shown for positive ion operation.

ADC ou TDC

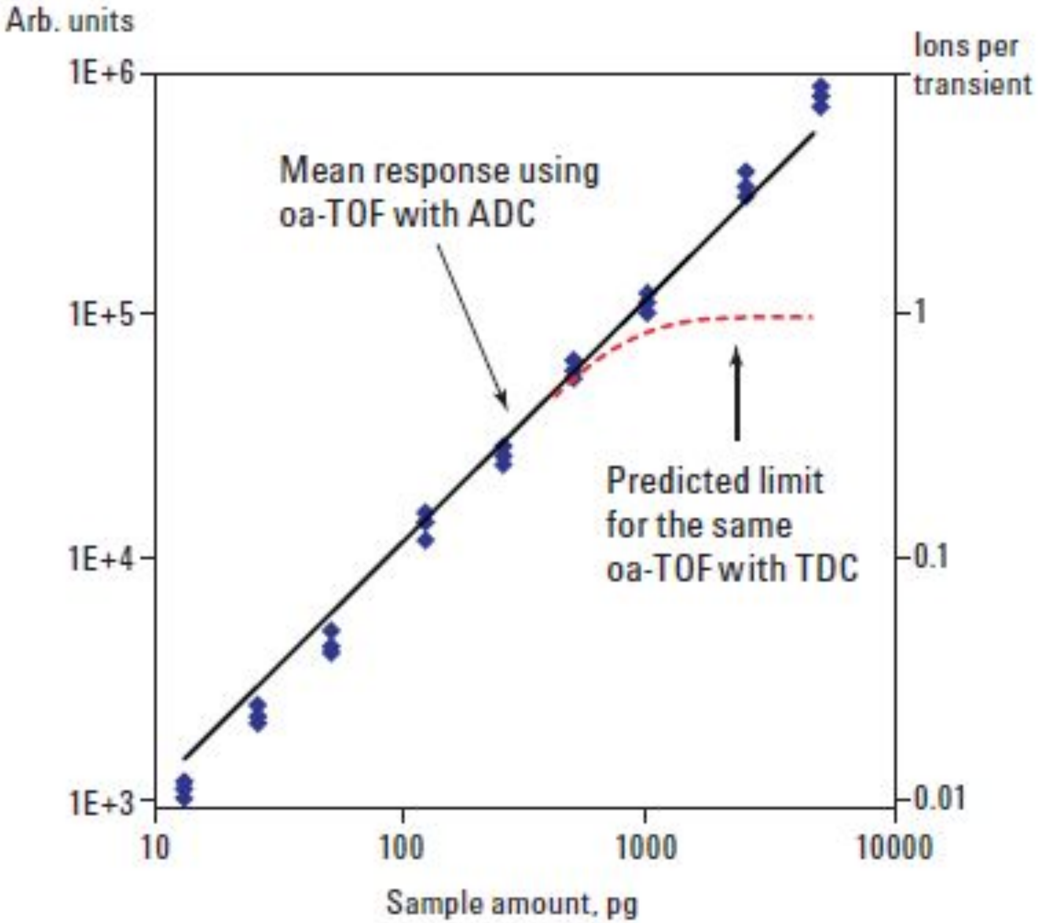
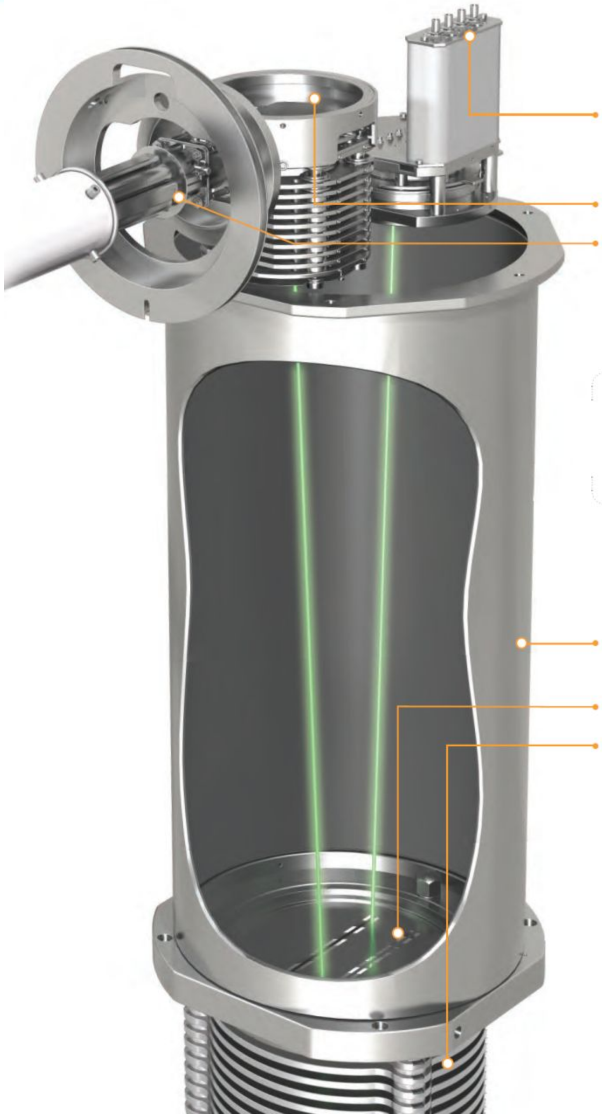


Figure 5. Ions per transient as a function of sample amount, showing TDC limitations.

Summary - TOF operation



40 GHz four channel TDC and detector provide highest sampling speeds and maintains high resolution – even at low mass

30kHz accelerator for highest acquisition rate

New entrance optics improves ion beam focusing post LINAC

Accelerator TOF™ Analyzer
improves resolution and sensitivity

15kV acceleration voltage for higher sensitivity and resolution

High transparency grids throughout for minimal ion loss

Two-stage ion reflector compensates for energy dispersion to maximize resolution