Introduction to Orbitrap™

HRAM (High-Resolution, Accurate-Mass) Technology

Sensitive and Stable High Resolution Mass Spectrometry

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LCMS Product Specialist

Sci Spec
Your Scientific Specialist

Thermo SCIENTIFIC

HPLC – Block Diagram

General ideas for analyzing small molecules from mixtures.

(U)HPLC System

- **Pump** : - Mix two or more solvents
  - Control the flow rate of mobile phase and analytes
- **Degasser** : Remove air bubble in solvents
- **Autosampler** : Inject the sample into a running system
- **Column** : Separate each components
- **Column Compartment** : Control a column temperature
- **Detector** : Detect signal from analytes after separation

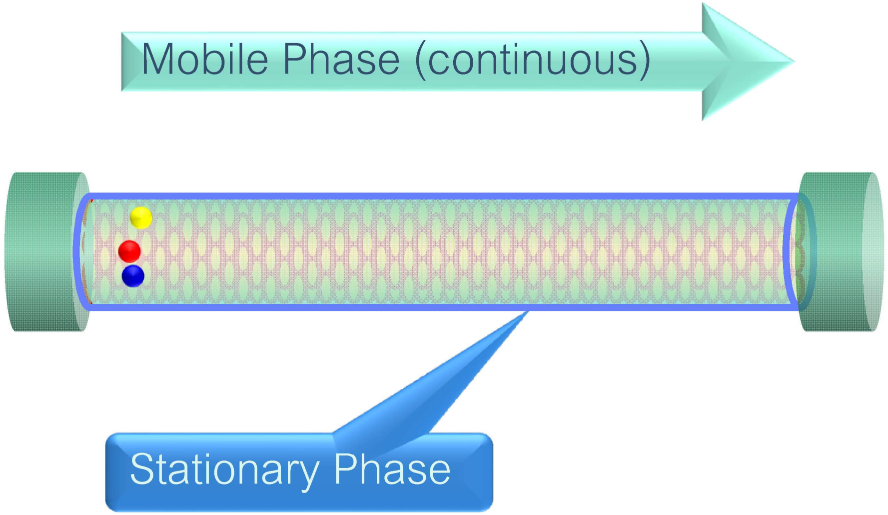
DAD (UV, VIS)

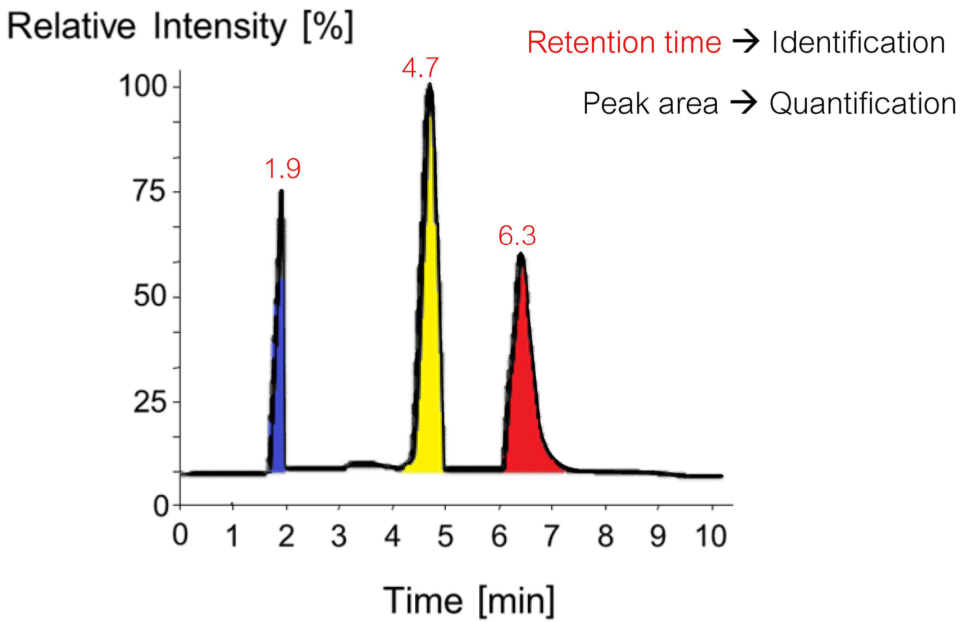
Fluorescence

Reflective Index

Mass Spectrometer

Liquid Chromatography (LC)





- **Liquid Chromatography (LC)** : Separation technique which liquid is used as mobile phase.
- **Separation** : Between two phases (Stationary phase and Mobile phase)
- Compounds are separated from each other based on their difference in affinity for the stationary or mobile phase.

(U)HPLC System

- **HPLC:** High Performance Liquid Chromatography
- **UHPLC:** Ultra High Performance Liquid Chromatography

	Typical Particle Size	Typical Back Pressure	Typical Column Diameter
Preparative HPLC	100 – 10 µm	10 - 100 bar	21 mm
Conventional HPLC	5 – 3 µm	100 – 300 bar	4.6 mm
UHPLC	≤ 3 µm	≥ 600 bar	2.1 mm

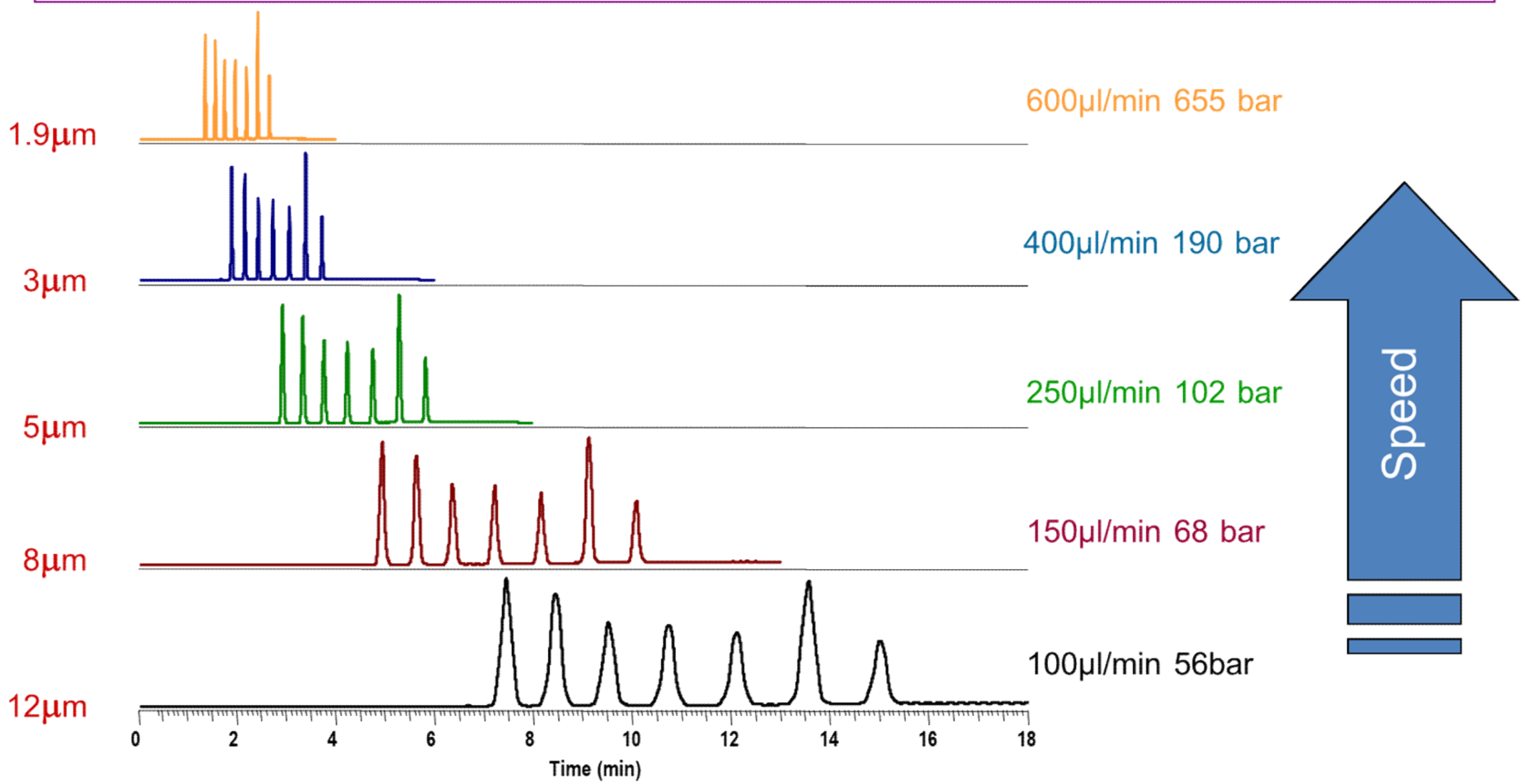
The smaller the particles, the better the separation performance

But!! Smaller particles generate higher back pressure

Advantage of Small Particle & High Flow

Increase Speed, Maintain Resolution *200x2.1mm*

Speeding up analysis with 1.9 µm Hypersil GOLD



(U)HPLC Portfolio

HPLC Systems — Routine Analysis

UHPLC Systems — Low-Flow | Flexibility, More Performance and Method Development | Research & HT

Vanquish Duo System Workflows

Thermo Scientific™ UltiMate™ 3000 Basic Automated System
- Highly economic & reliable

Thermo Scientific™ UltiMate™ 3000 SD System
- Workhorse for standard HPLC applications

620 bar

Thermo Scientific™ UltiMate™ 3000 RSLCnano System

Thermo Scientific™ EASY-nLC™ 1200 System
- UHPLC systems for Nano/Cap/Micro range
- Ideal front-end for proteomics applications

800-1200 bar

Thermo Scientific™ UltiMate™ 3000 RS/BioRS System
- Specialty workflow support
- Binary and Quaternary UHPLC systems

Thermo Scientific™ Vanquish™ Flex System
- High pressure binary and low pressure quaternary solvent mixing options
- Two thermostatting modes
- Biocompatible
- Integration of multiple detection technologies

Up to 1000 bar

Thermo Scientific™ Vanquish™ Horizon System
- High pressure binary solvent mixing
- Industry-leading precision and accuracy
- Two thermostatting modes
- Unmatched detection sensitivity
- Biocompatible
- Integration of multiple detection technologies

1500 bar

Perfect (U)HPLC System

Performance

To improve separations

- Binary and Quaternary systems
- Widest flow-pressure footprint

Sensitivity

To find the complete picture

- Diverse detector portfolio to provide highly sensitive and selective detection
- Unique near universal charged aerosol detection

Productivity

To get more done

- Charger Module extends capacity
- Chromeleon (CDS)

Accuracy

To control experiments

- Unsurpassed sample dosage
- Active and passive pre-heating
- Multiple thermostatting options

Confidence

To ensure highest data quality

- Unmatched retention time precision with SmartInject
- Automation of workflows with barcode reading

Flexibility

To grow with your research

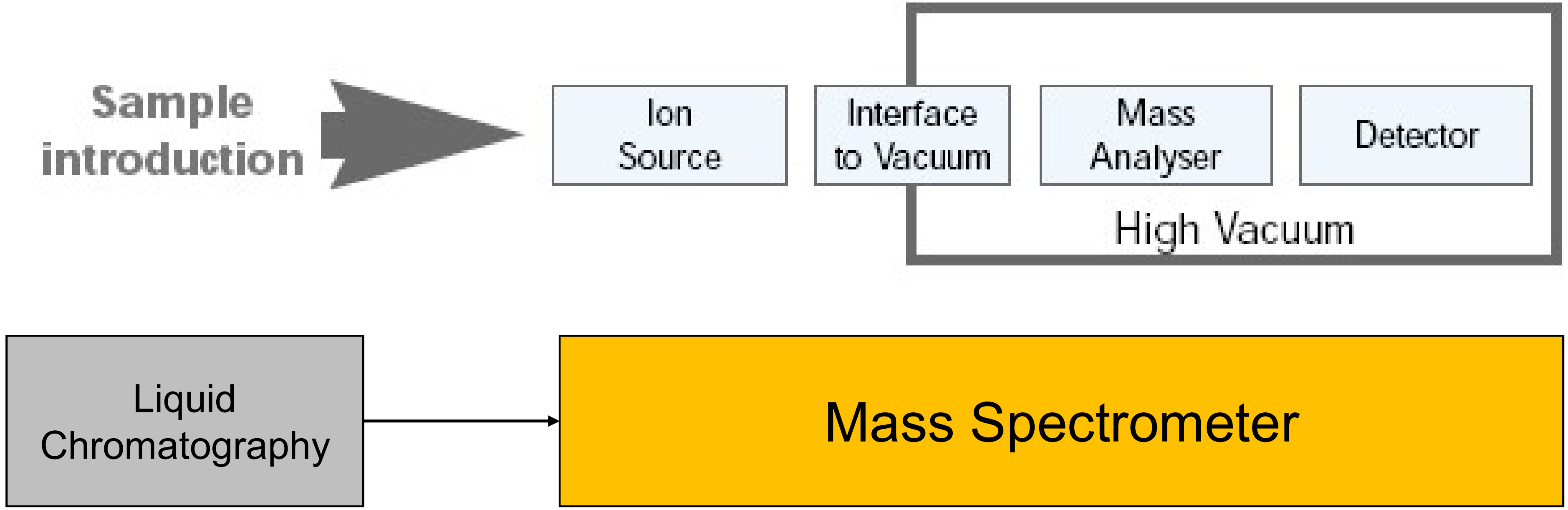
- Biocompatible and tool-free set up by default with optimized fluidics
- Integrated modularity – swap modules

https://www.thermofisher.com/th/en/home/products-and-services/promotions/industrial/explore-vanquish-hplc-uhplc-systems.html

HPLC: Block Diagram

General ideas for analyzing small molecules from mixtures.

LCMS: Block Diagram



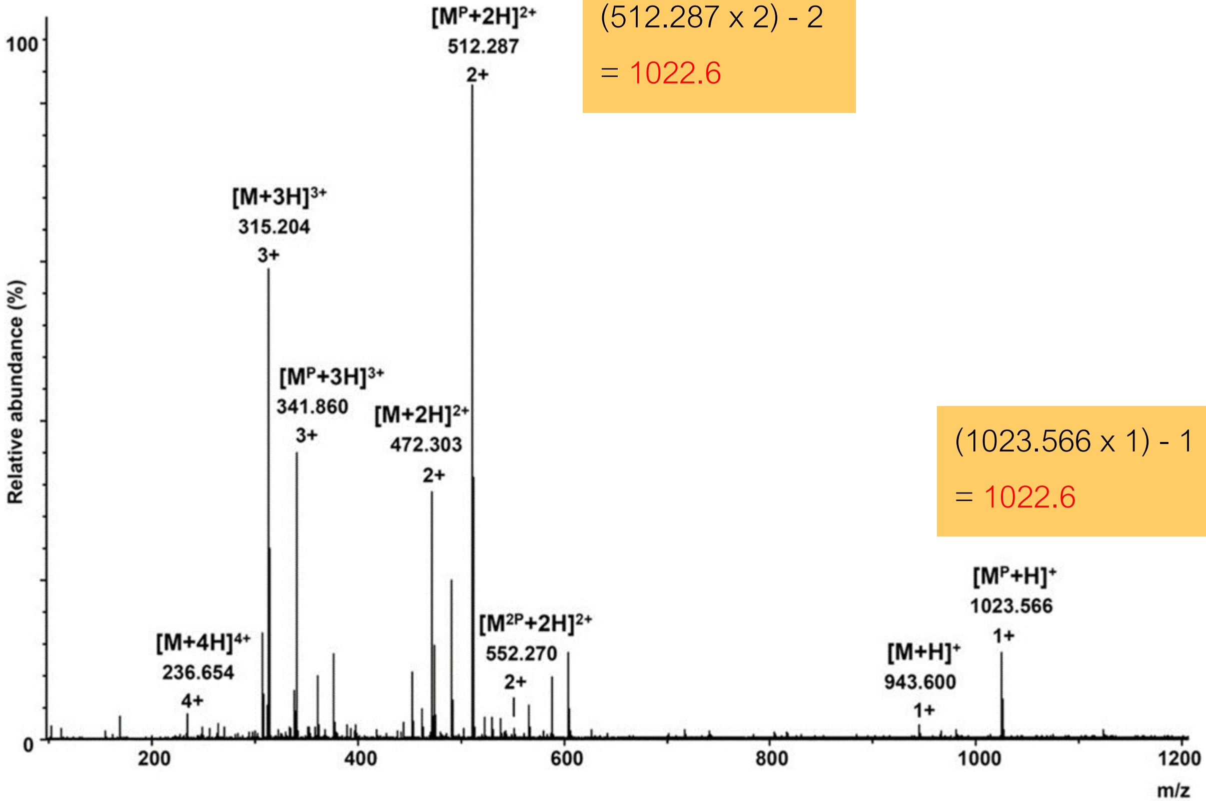
Mass Spectrometry (MS)

Niessen *et al.*, *LC-MS: Principles and Applications*, 1992, Marcel Dekker, Inc., New York, p. 29.

"The basis in mass spectrometry (MS) is the production of ions, that are subsequently separated or filtered according to their **mass-to-charge (m/z) ratio**, and detected."

- Measure gas-phase ions
- Operate at very low pressure (10^{-5} to 10^{-7} torr)
- Mass spectrometer works with **IONS**
- Determine the mass are separated according to their mass-to-charge (m/z) ratio

Information Rich Data & Mass Spectrum



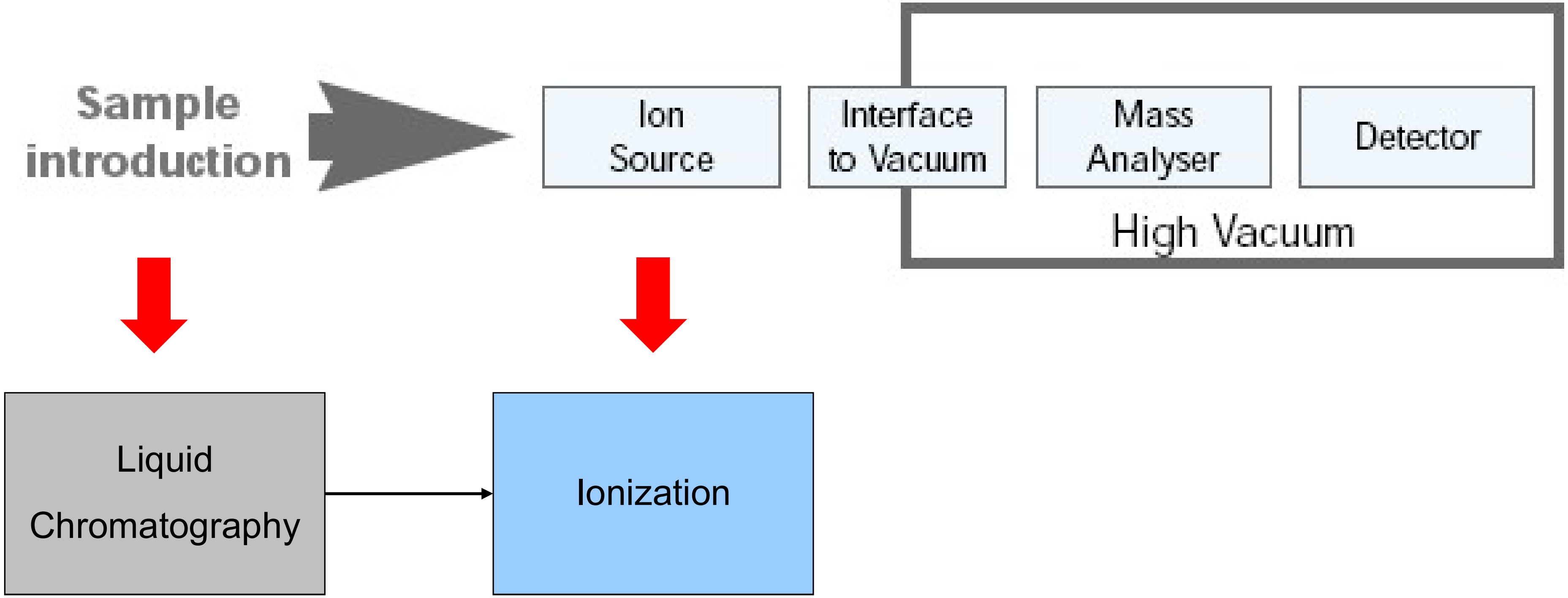
(512.287 x 2) - 2
= 1022.6

(1023.566 x 1) - 1
= 1022.6

- Molecular weight
- Elemental composition
- Empirical formula
- Molecular structure
- Unambiguous Identification
- Mass specific response

Mass to charge (m/z) = (molecular weight + charge) / charge

LCMS: Block Diagram



- Columns
- Solvent systems

- ESI
- APCI

Ionization

Ion Source

- **Ion source** : Converts sample molecules (neutral) into charged molecules or molecular ions.
- Type of ionization techniques
  - Matrix Assisted Laser Desorption Ionization (MALDI)
  - Atmospheric Pressure Ionization (API)

    - Electrospray Ionization (ESI)

    - Atmospheric Pressure Chemical Ionization (APCI)

$$M: \xrightarrow{-e^-} M^{+\bullet}$$

$$M: \xrightarrow{+H^+} [M+H]^+$$

$$M: \xrightarrow{-H^+} [M-H]^-$$

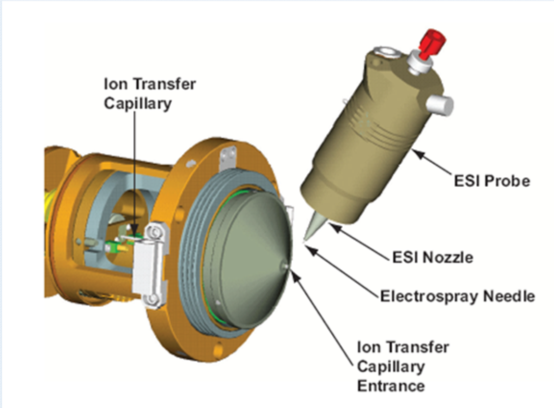
neutral analyte: cannot be detected by MS

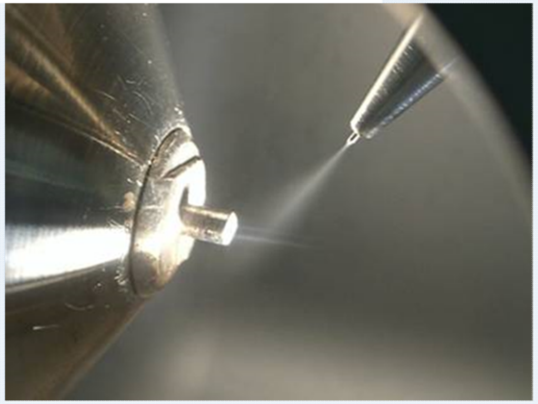
No one ionization technique is applicable to all classes of chemical species !

API (Atmospheric Pressure Ionization)

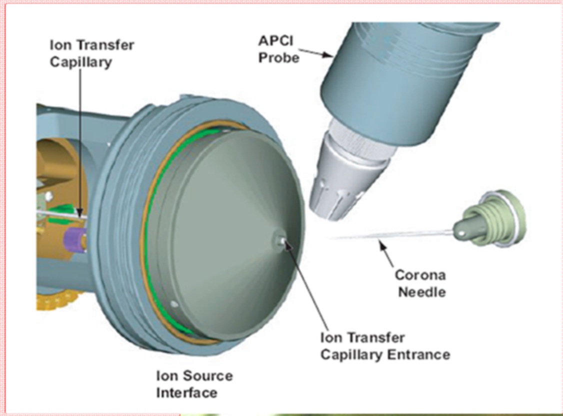
Ion Source

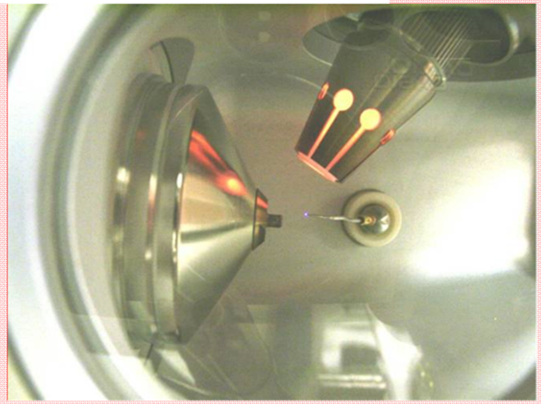
ESI



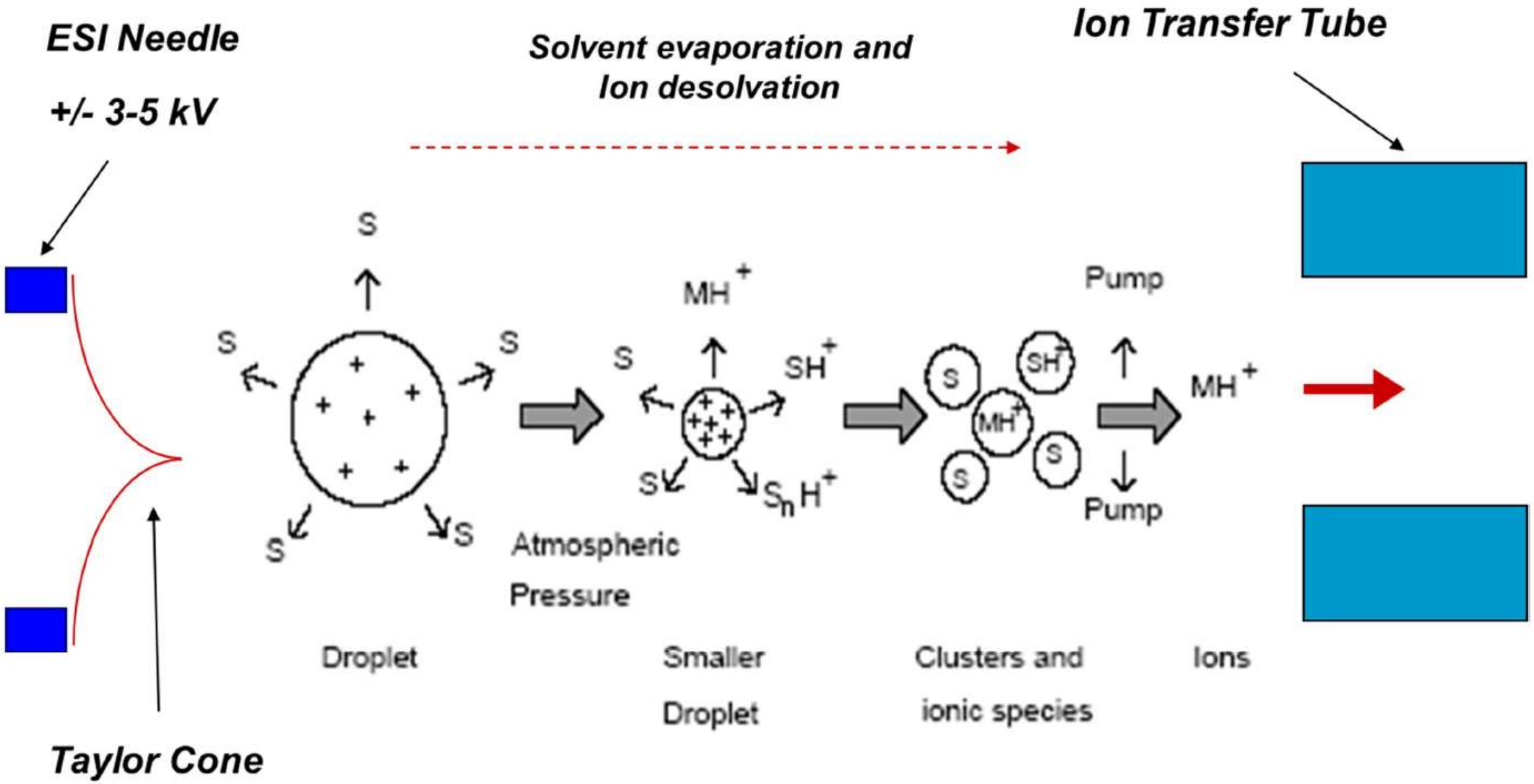


APCI

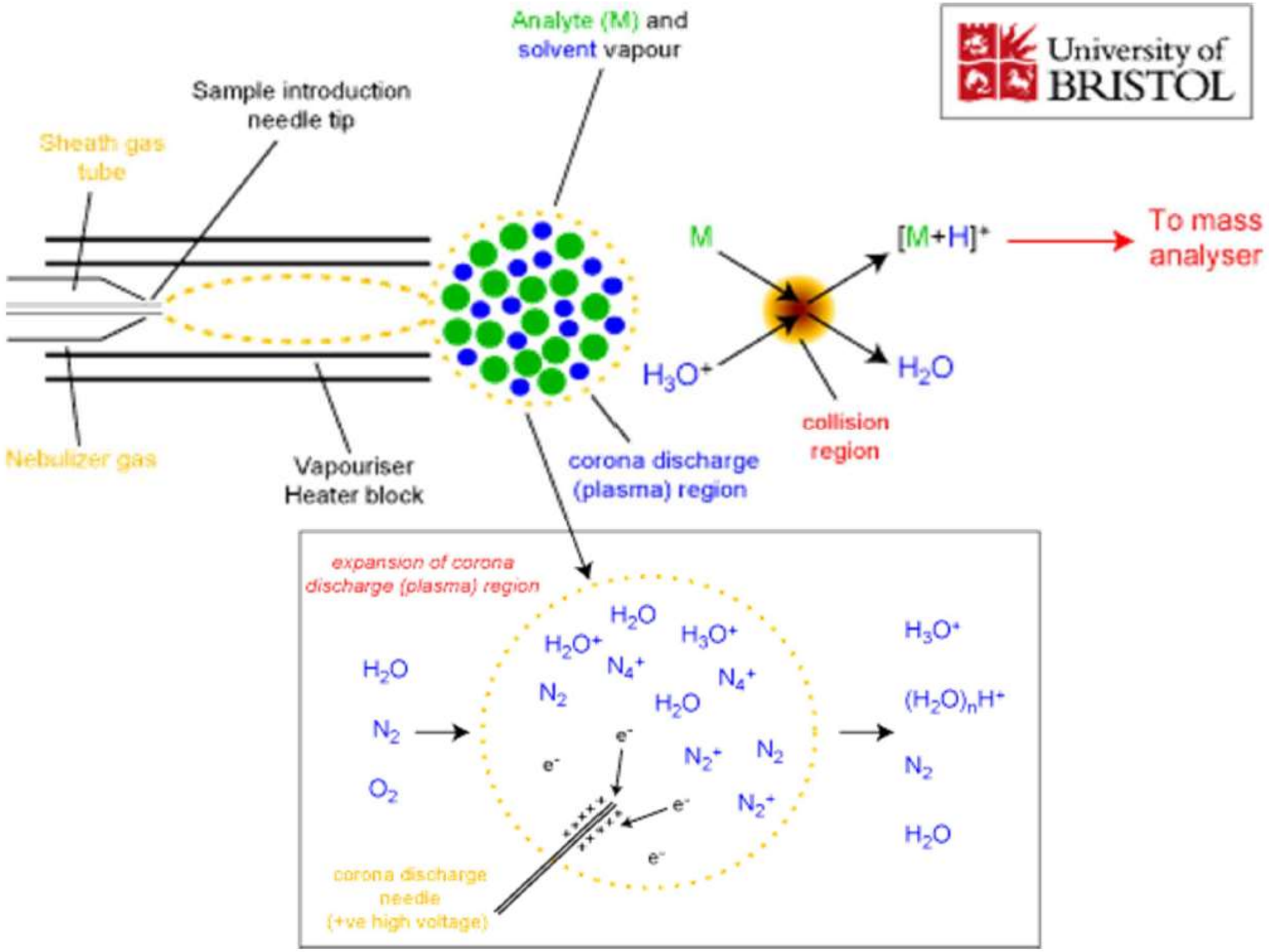




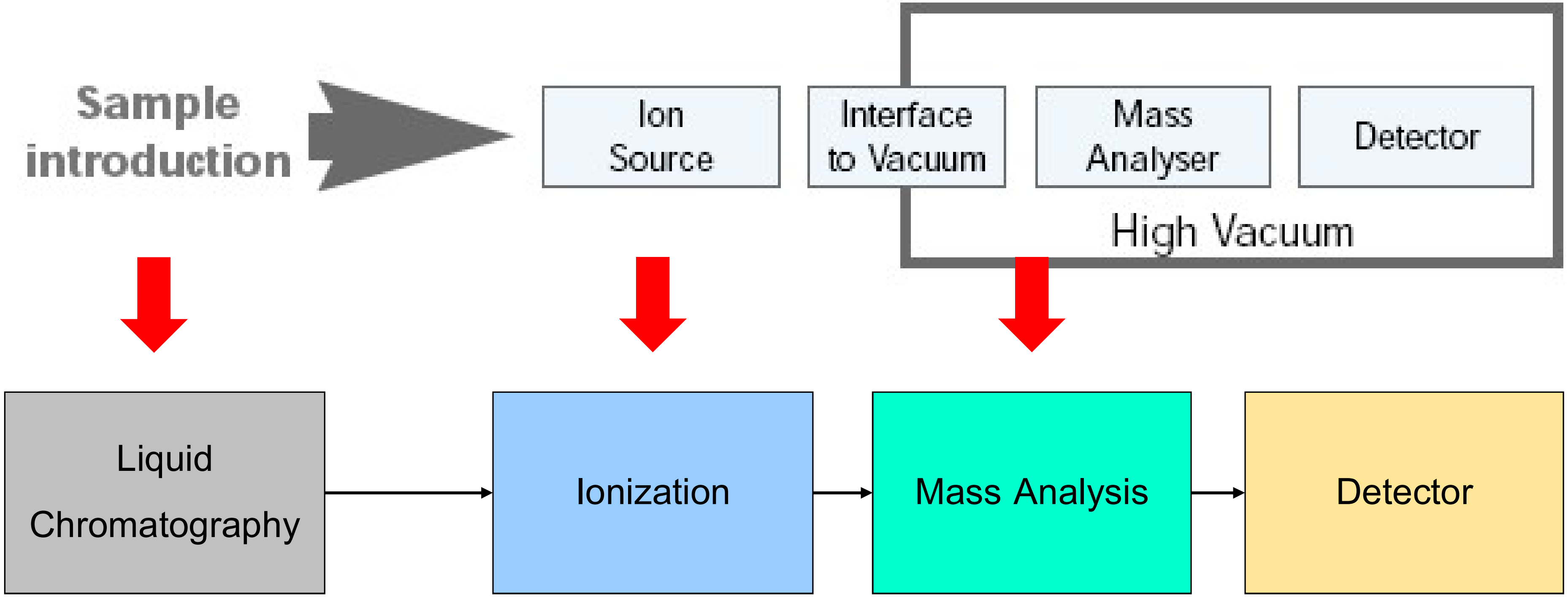
Electrospray Ionization (ESI)



Atmospheric Pressure Chemical Ionization (APCI)



LCMS: Block Diagram



- Columns
- Solvent systems

- ESI
- APCI

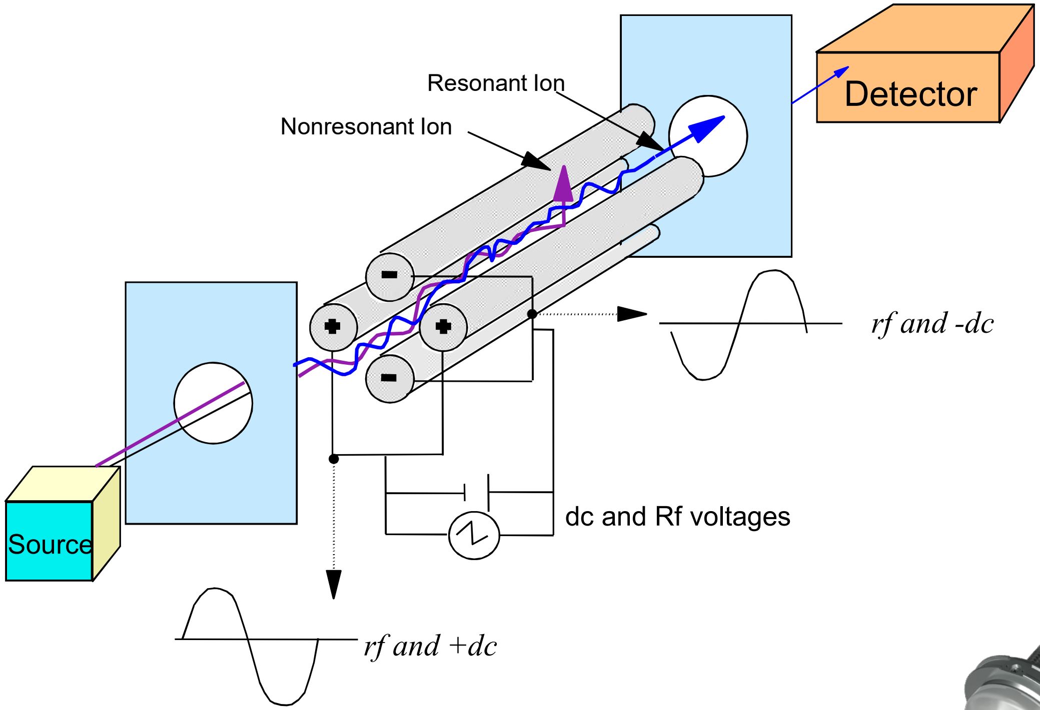
- Ion Trap
- Triple Quadrapole
- ToF
- Orbitrap
- Etc.

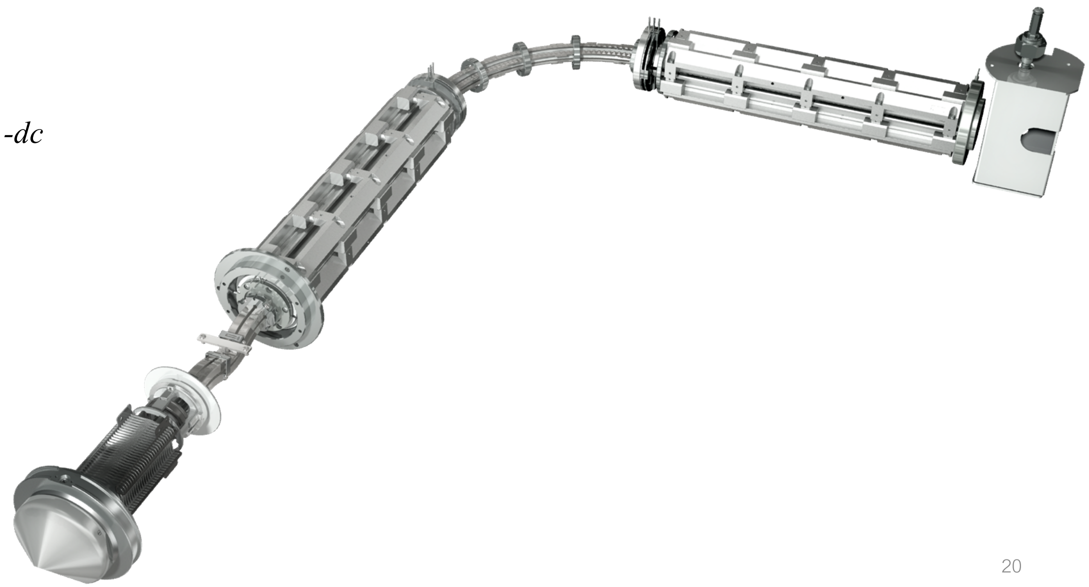
Type of Mass Analyzers

- Scanning or Filtering (Separation in Space)
  - Quadrupole
  - Magnetic Sector
- Pulsed or Batch (Separation in Time)
  - Ion Trap
  - FT-ICR
  - Time-of-Flight
  - Orbitrap

Scan or Filter (Separation in space)

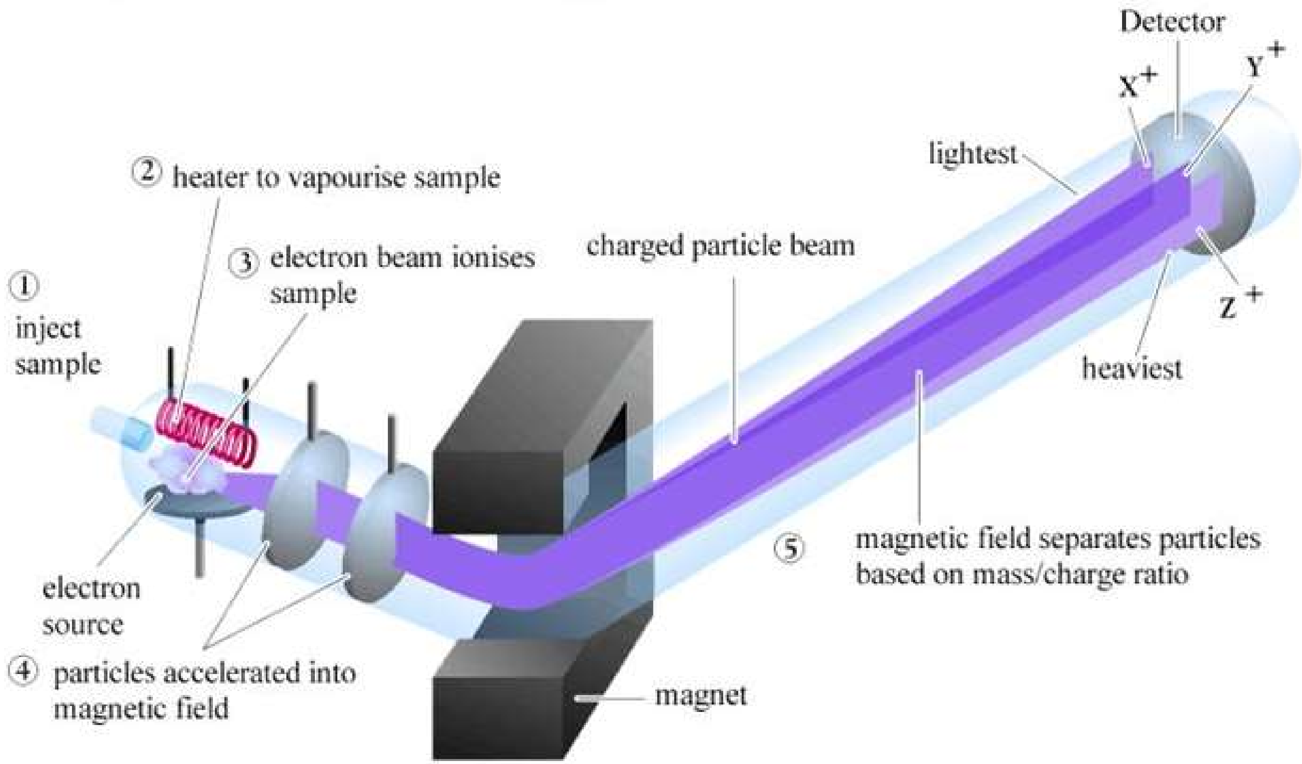
Quadrupole or Triple Quadrupole





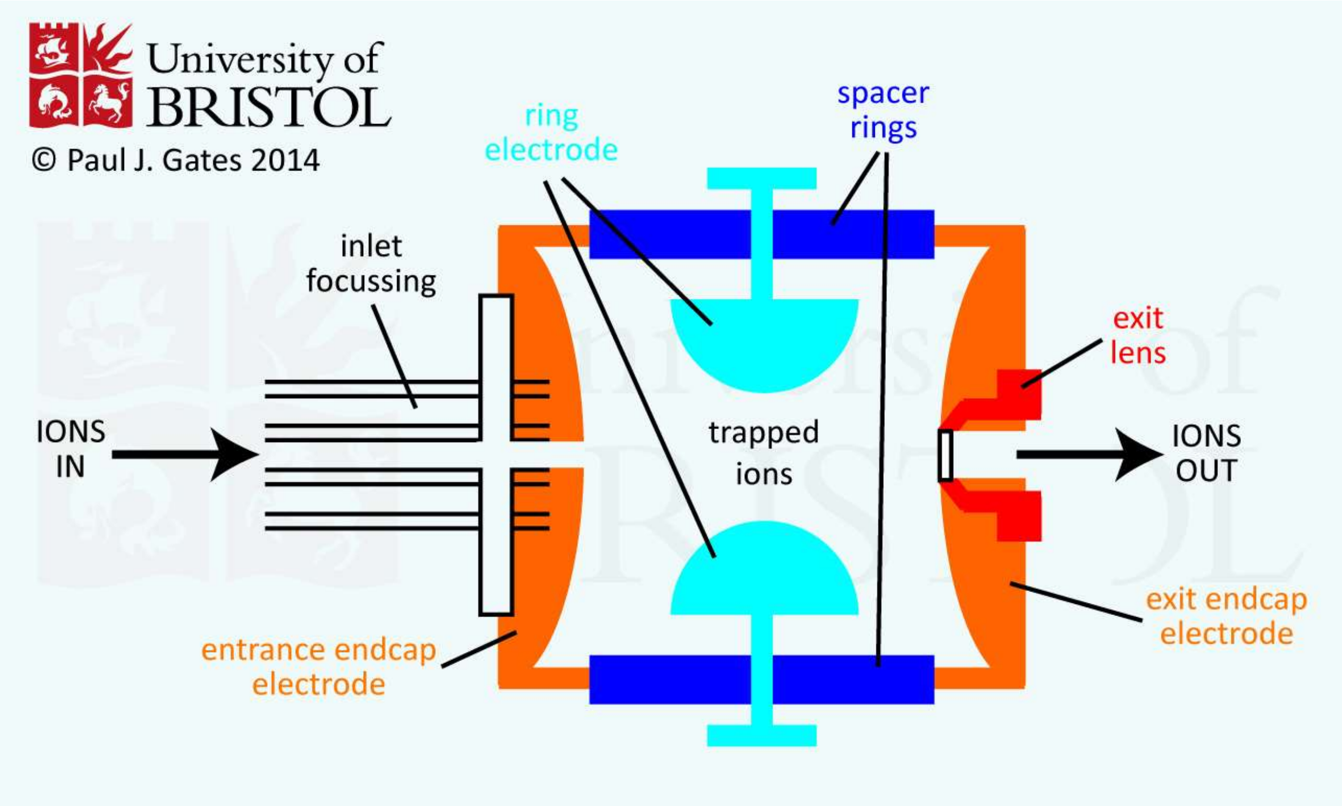
Scan or Filter (Separation in space)

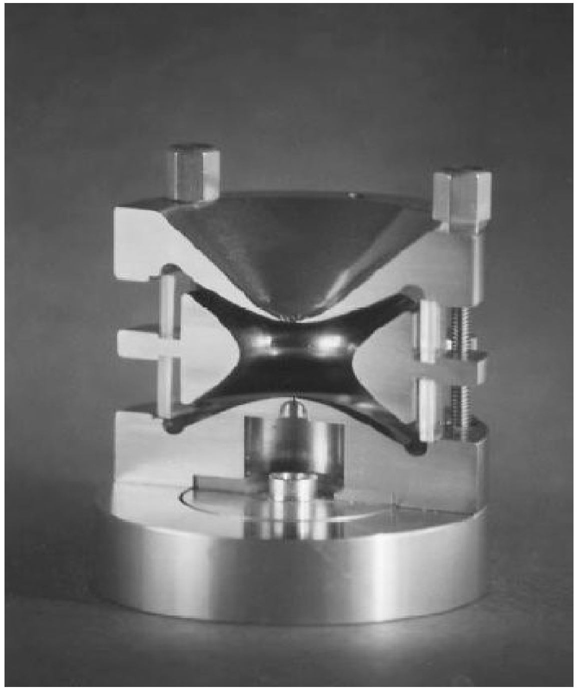
Magnetic Sector



Pulsed or Batch (Separation in time)

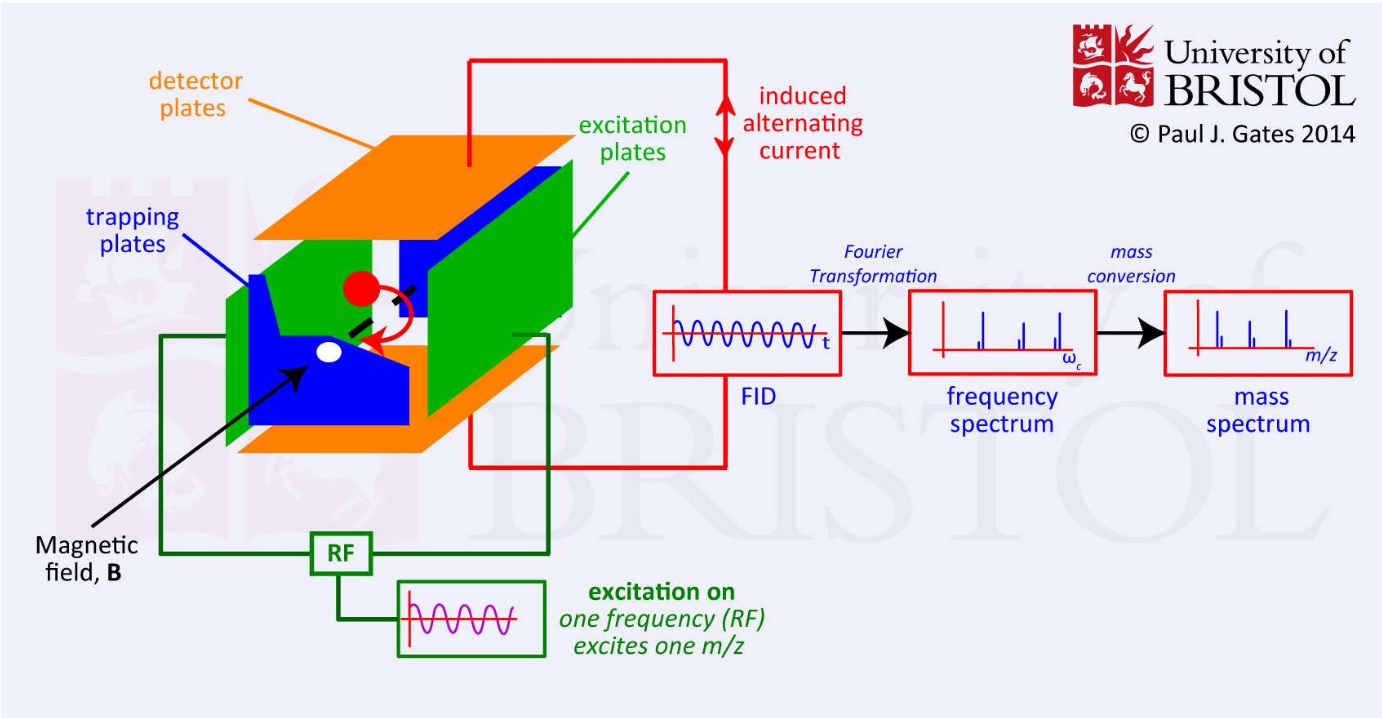
Ion Trap





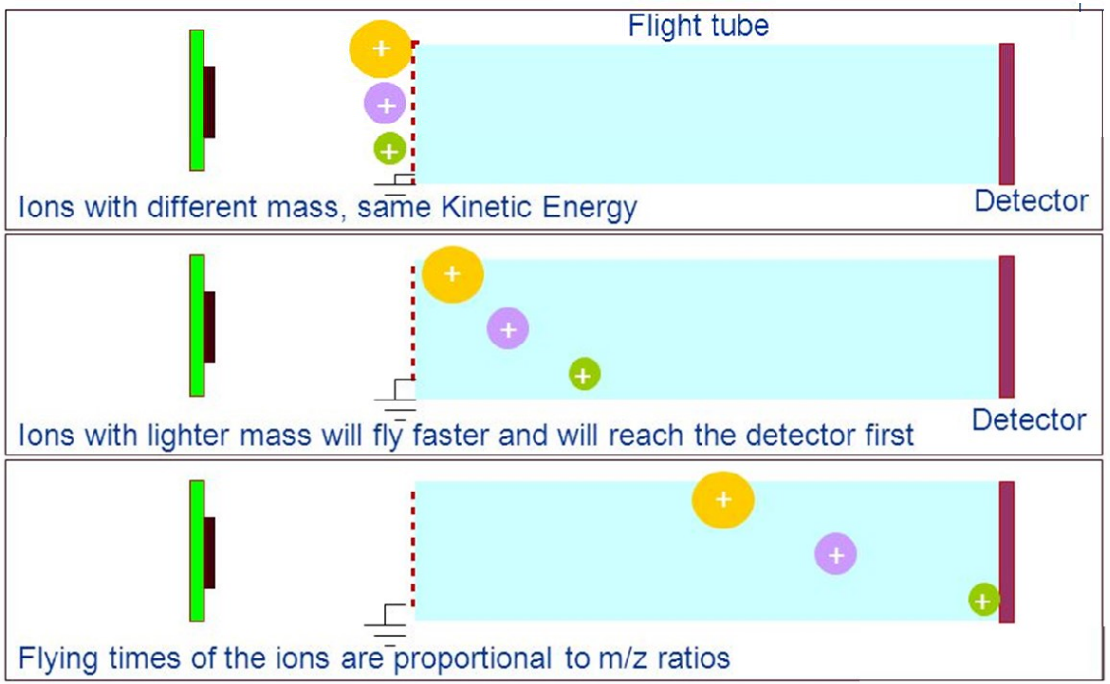
Pulsed or Batch (Separation in time)

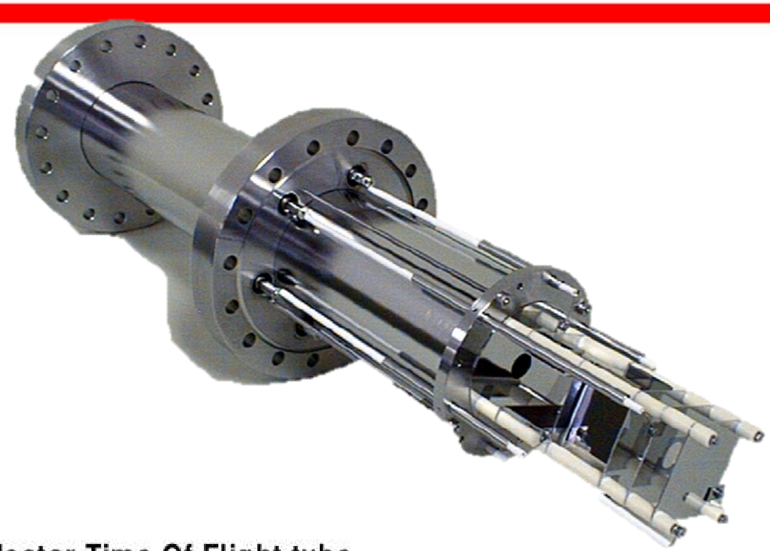
FT-ICR (Fourier-Transform Ion Cyclotron Resonance)



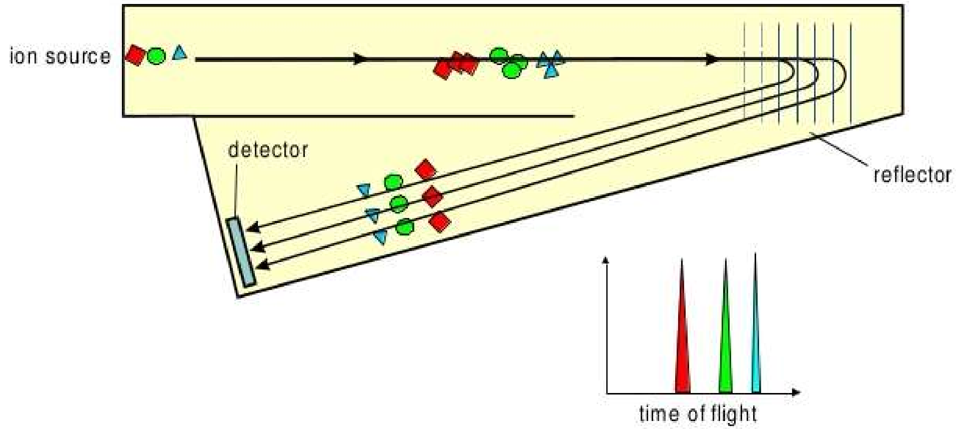
Pulsed or Batch (Separation in time)

ToF (Time of Flight)





Reflector Time Of Flight tube



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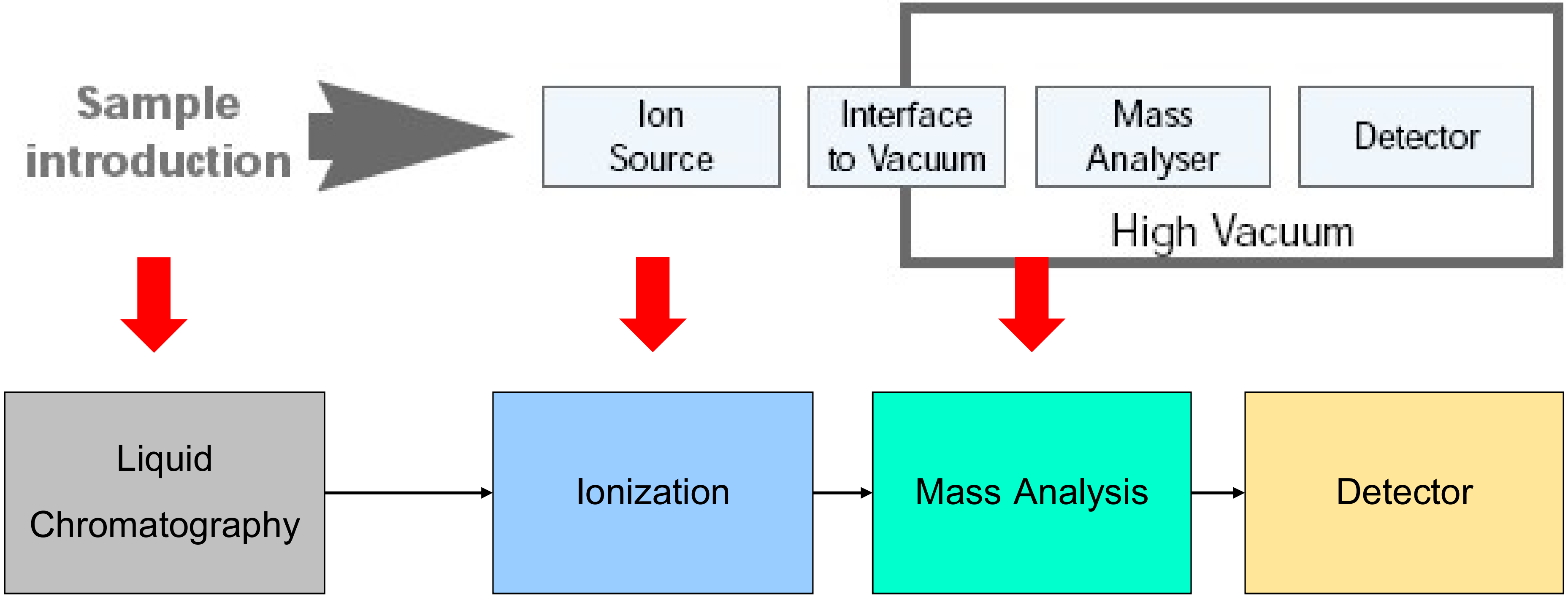
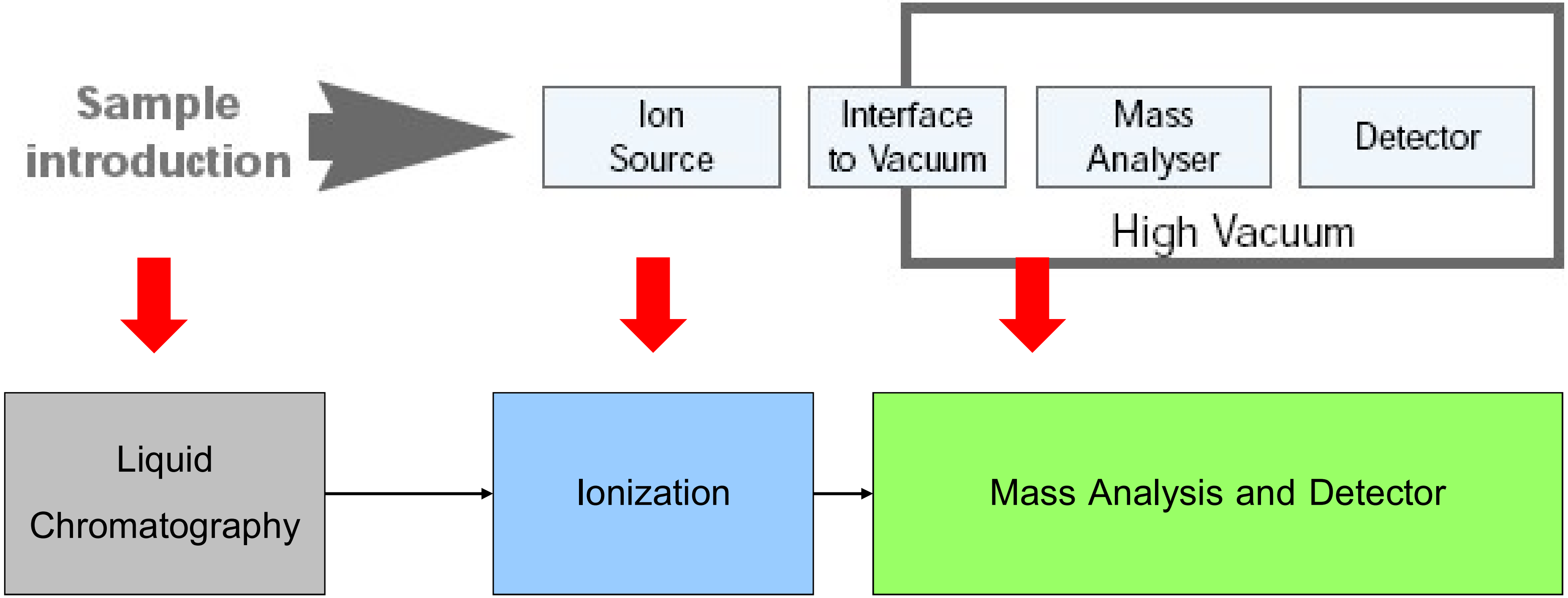


- Ion Trap
- Triple Quadrapole
- ToF
- **Orbitrap**
- Etc.

LCMS: Block Diagram



- **Orbitrap**

Orbitrap

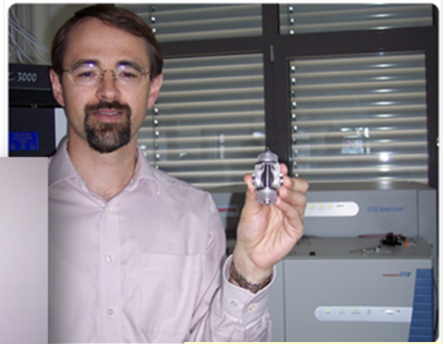
Anal. Chem. **2000,** *72,* 1156−1162

Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

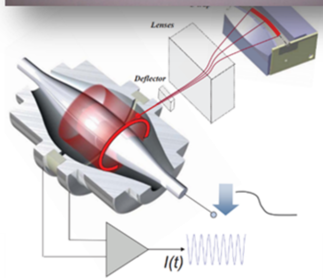
Alexander Makarov*

HD Technologies Ltd., Atlas House, Simonsway, Manchester, M22 5PP, U.K.

This work describes a new type of mass analyzer which employs trapping in an electrostatic field. The potential distribution of the field can be represented as a combination of quadrupole and logarithmic potentials. In the absence of any magnetic or rf fields, ion stability is achieved only due to ions orbiting around an axial electrode. Orbiting ions also perform harmonic oscillations along the electrode with frequency proportional to $(m/z)^{-1/2}$. These oscillations are detected using image current detection and are transformed into mass spectra using fast FT, similarly to FT ICR. Practical aspects of the trap design are presented. High-mass resolution up to 150 000 for ions produced by laser ablation has been demonstrated, along with high-energy acceptance and wide mass range.



Alexander Makarov



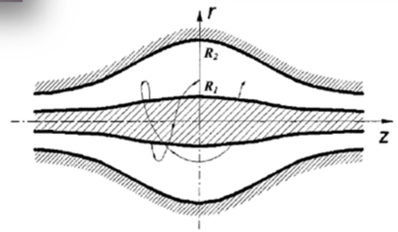
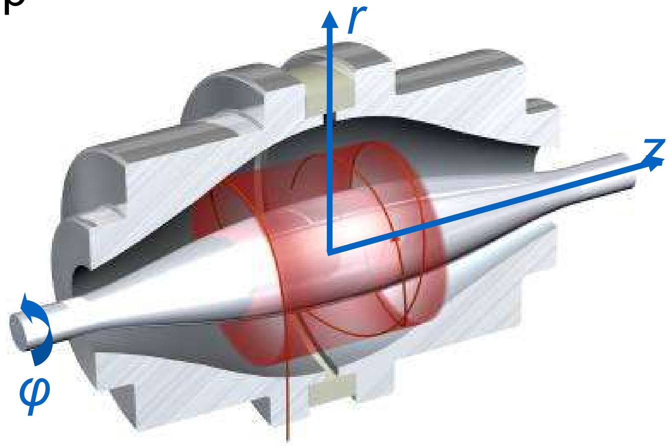


Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

Orbitrap Analyzer – An Electrostatic Trap

- Ions trapped in an electrostatic field
- Central electrode kept on high voltage
- Outer electrode is split and able to pick up an image current induced by ion packets moving inside the trap

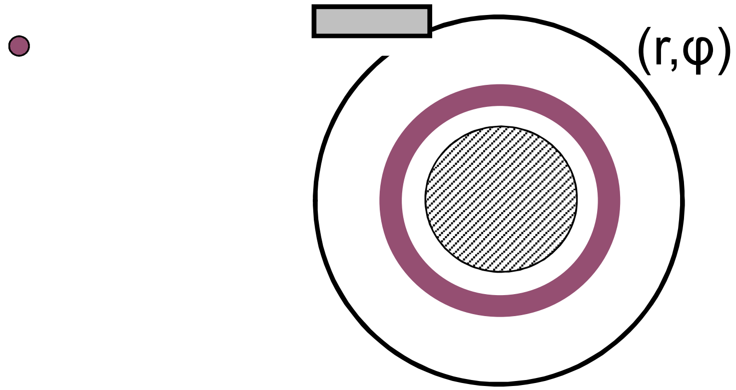


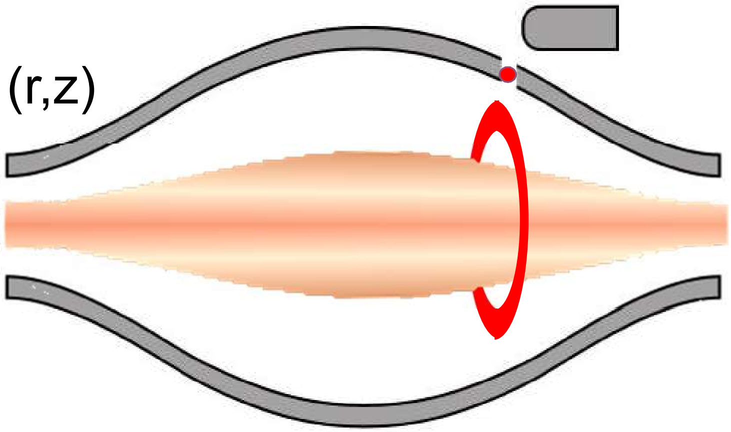
$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

A. Makarov, Anal. Chem 2000, 1156-1162

Ion Injection and Formation of Ion Rings

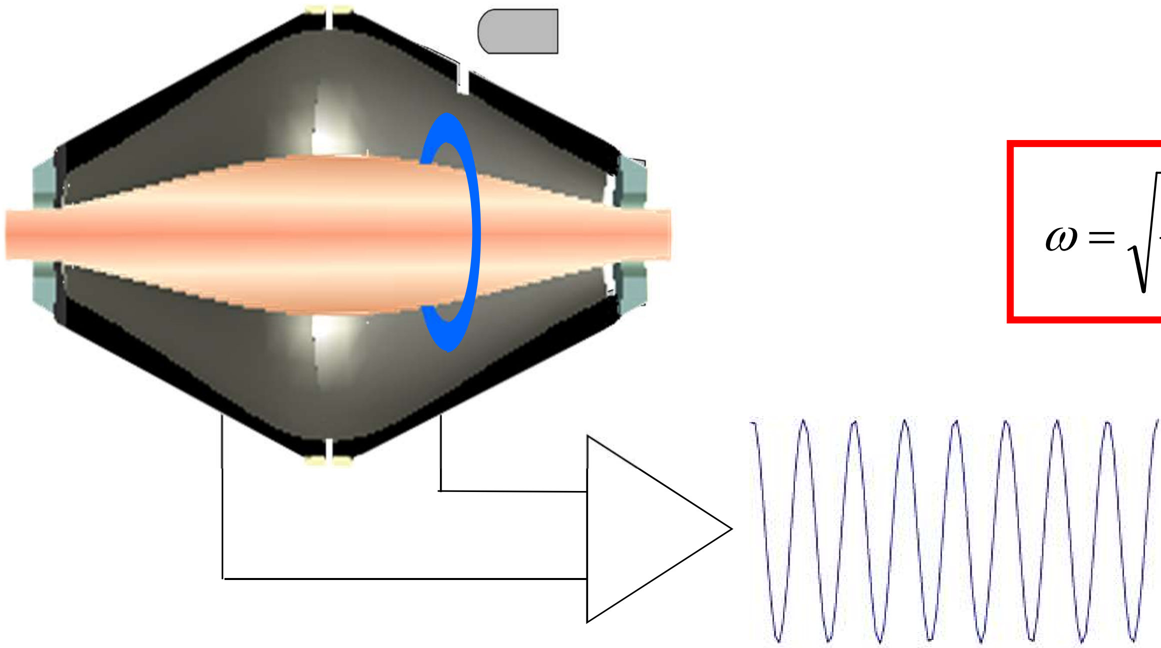
- An ion packet of a selected *m/z* enters the field
- Increasing voltage squeezes ions
- Voltage stabilises and ion trajectories are also stabilized
- Angular spreading forms a ROTATING RING





Detection of Ions

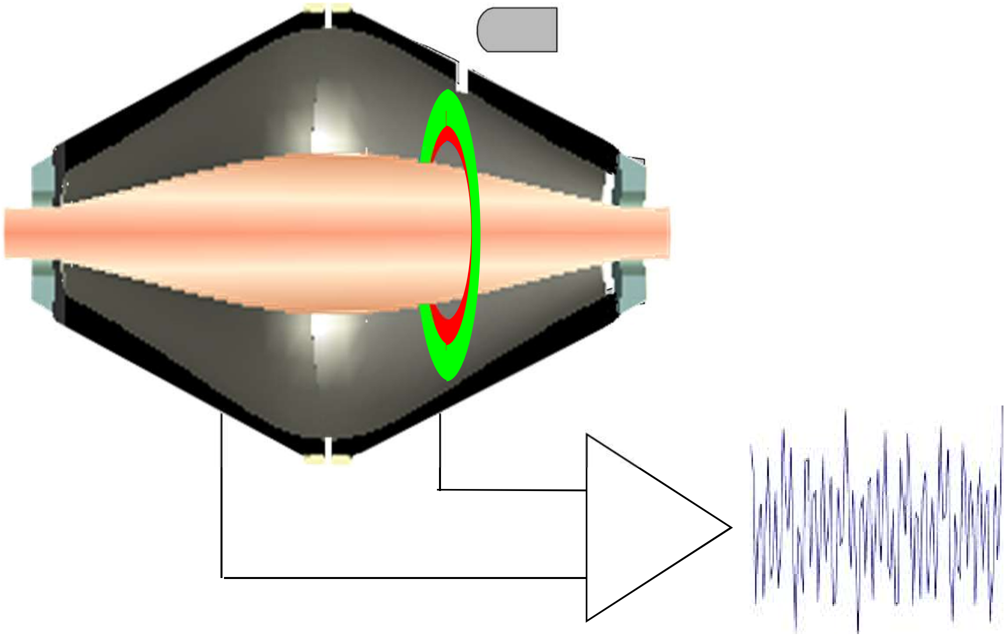
- Ion packets enter the analyzer slightly off axis
- The field inside the trap effects an oscillation of the ion packets/rings
- The moving ion rings induce an image current on outer electrodes
- The frequency of harmonic oscillations is proportional to ions' *m/z*



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

Fourier Transform

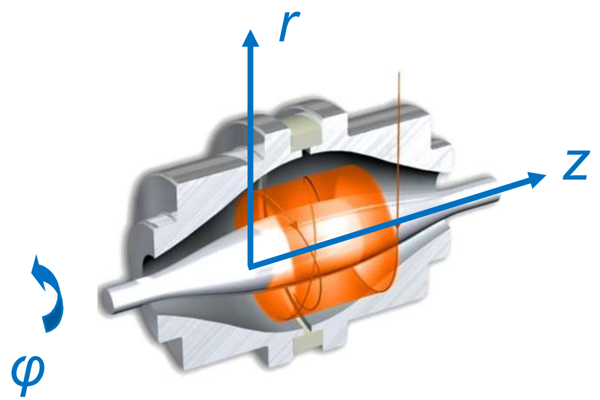
- Mathematical operation transforms frequency signal into a time domain spectrum
- Orbitrap is a Fourier transform-based mass analyzer



Baron Joseph Fourier

Scigelova et al. Mol. Cellular Proteomics 2011, 10: M111.0009431

Orbitrap



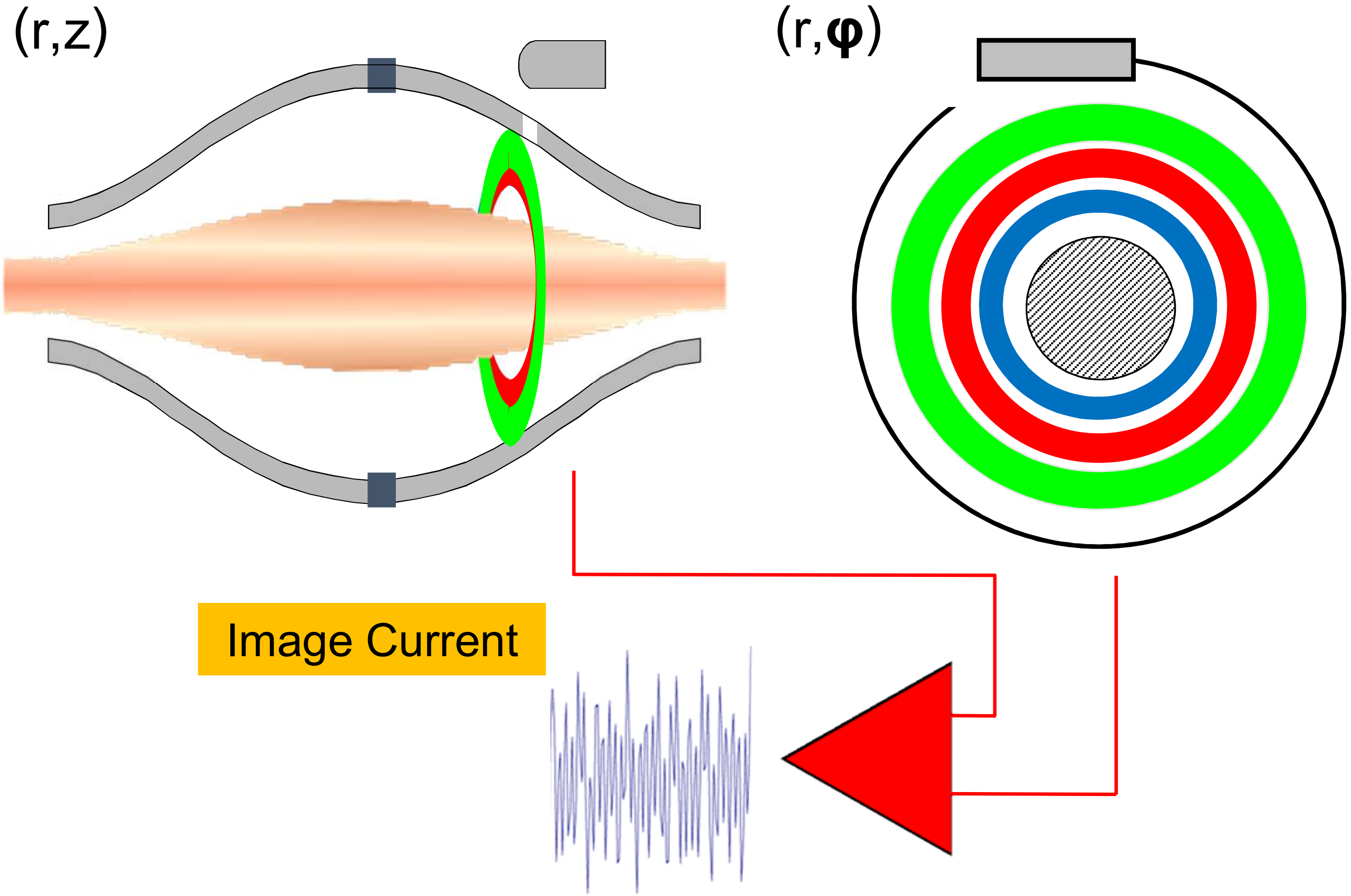
Hyper-logarithmic potential distribution:
"ideal Kingdon trap"

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

Characteristic frequencies:

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

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



Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

Orbitrap

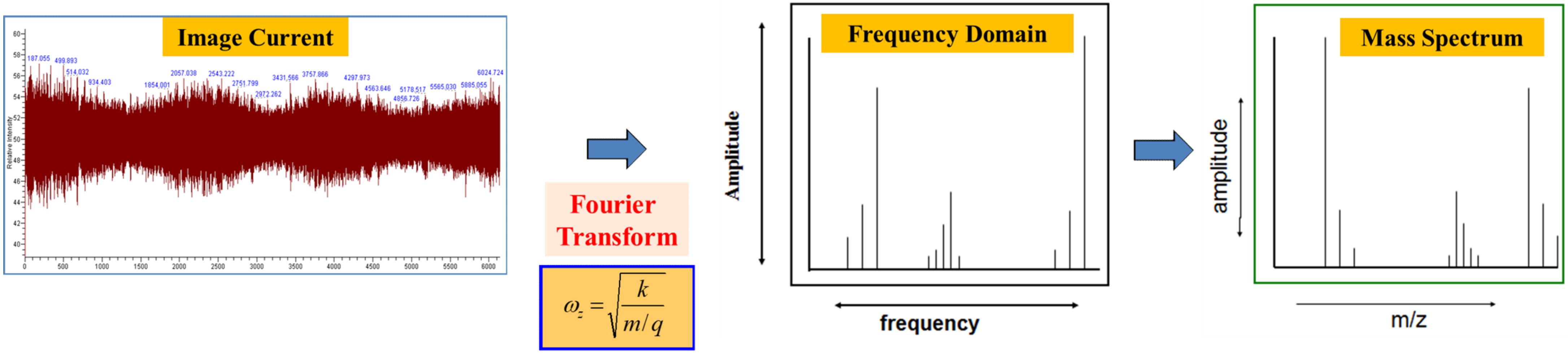


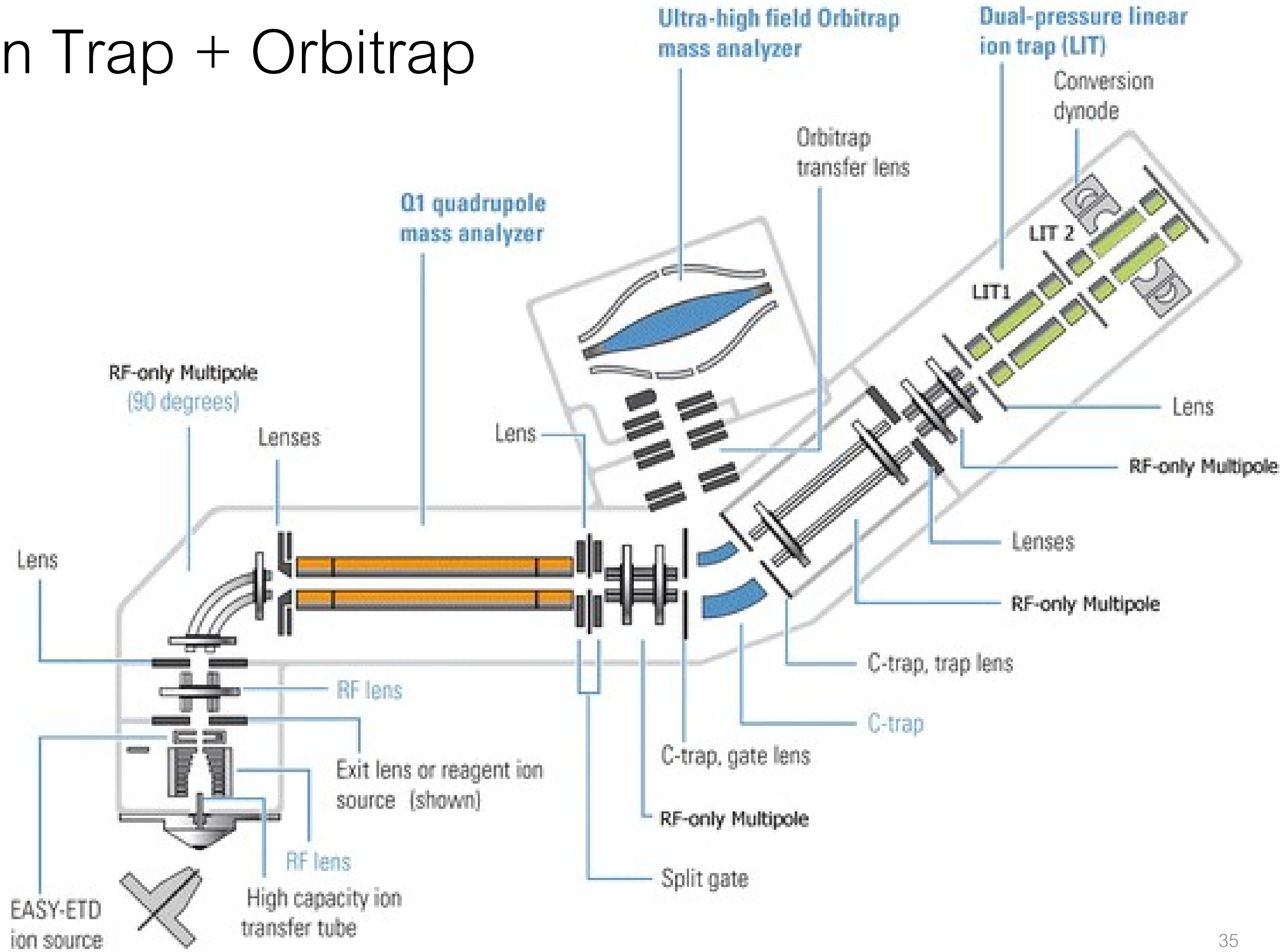
Image Current
Fourier Transform
$\omega_z = \sqrt{\frac{k}{m/q}}$
Frequency Domain
Amplitude
frequency
Mass Spectrum
amplitude
m/z

Hybrid Mass Analyzers

- QTrap = Quadrupole + Ion Trap
- IT Tof = Ion Trap + Time of Flight
- QTof = Quadrupole + Time of Flight
- LTQ Orbitrap = (Linear) Ion Trap + Orbitrap
- Q Exactive = Quadrupole + Orbitrap

Tribrid Mass Analyzers

- Quadrupole + Linear Ion Trap + Orbitrap



Orbitrap Families

- Hybrid quadrupole with orbitrap mass analyzer families
  - Exactive and Q-Exactive
    - Faster scan speed than Orbitrap Elite
    - Fitted for small molecule and big molecule application (wide mass range and high dynamic range)
    - QE HF is done same resolution with OT Elite
    - Can be operated like Elite and much more*
- Hybrid iontrap with orbitrap mass analyzer families
  - Orbitrap Elite
    - Dedicated for proteomic and intact protein application (max resolution >240K)
    - Multiple fragmentation support (CID,HCD and ETD)
- Tribrid quadrupole with orbitrap with iontrap families
  - Orbitrap Fusion/Lumos
    - Highest performance among all OT HRMS model
    - Fastest scan speed
    - Highest mass resolution
    - Multiple fragmentation support (CID, HCD, UVPD and ETD)
    - Can be operated like Elite or QE and much more

Orbitrap MS Portfolio

- ✓ *Ultra high resolution, fast scan speed and good sensitivity*
- ✓ *Excellent mass measurement accuracy and precision*
- ✓ *High quality MS/MS spectrum (MS^n capability)*

Q Exactive Focus

70,000 @m/z 200
12 Hz @17,500

Q Exactive Plus

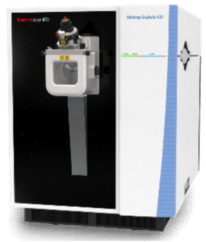
140,000 @m/z 200
12 Hz @17,500

Q Exactive HF

240,000 @m/z 200
18 Hz @15,000

Q Exactive HF-X

240,000 @m/z 200
40 Hz @7,500



Exploris 480

480,000 @m/z 200
40 Hz @7,500

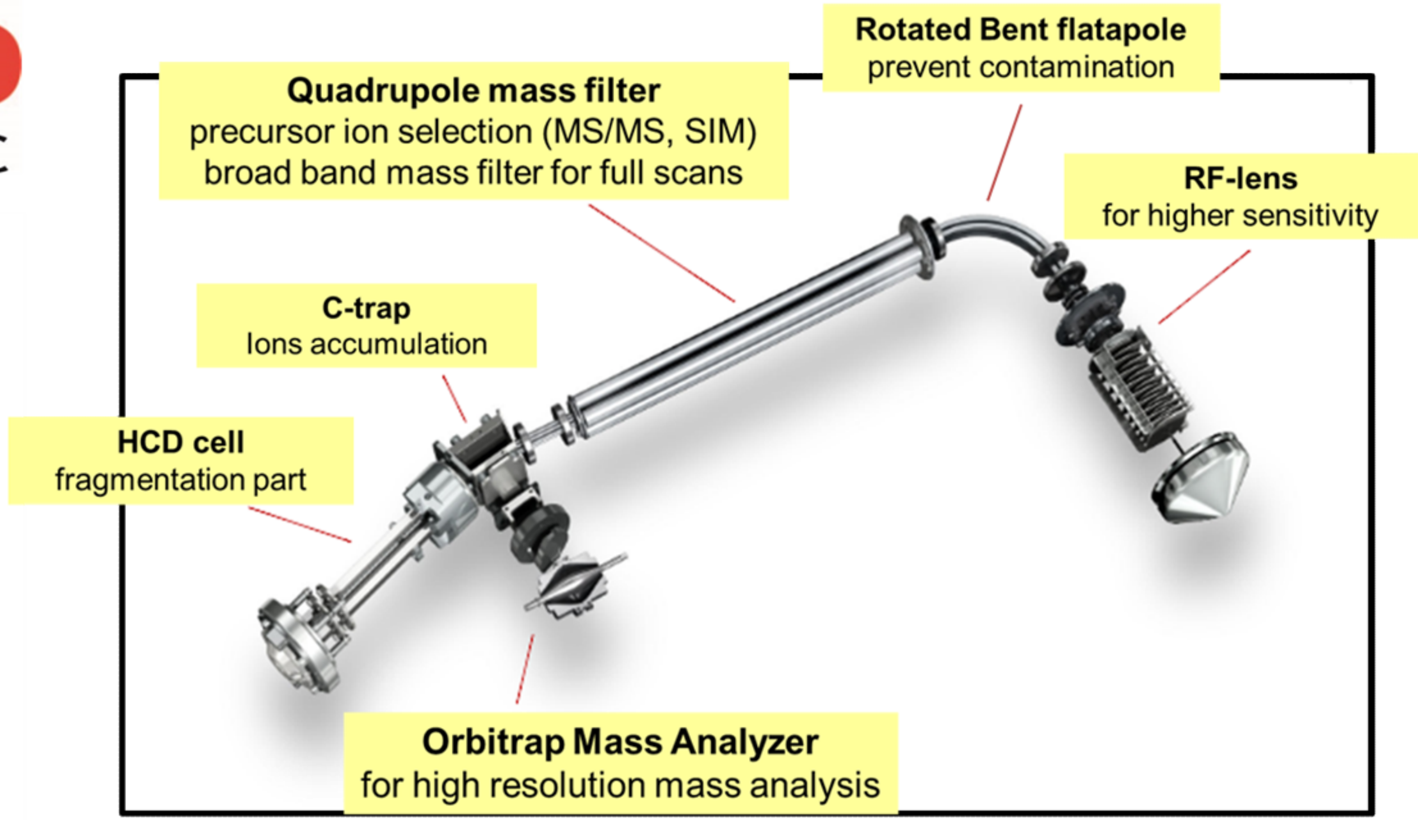
Orbitrap Eclipse

1,000,000 @m/z 200
40 Hz @7,500

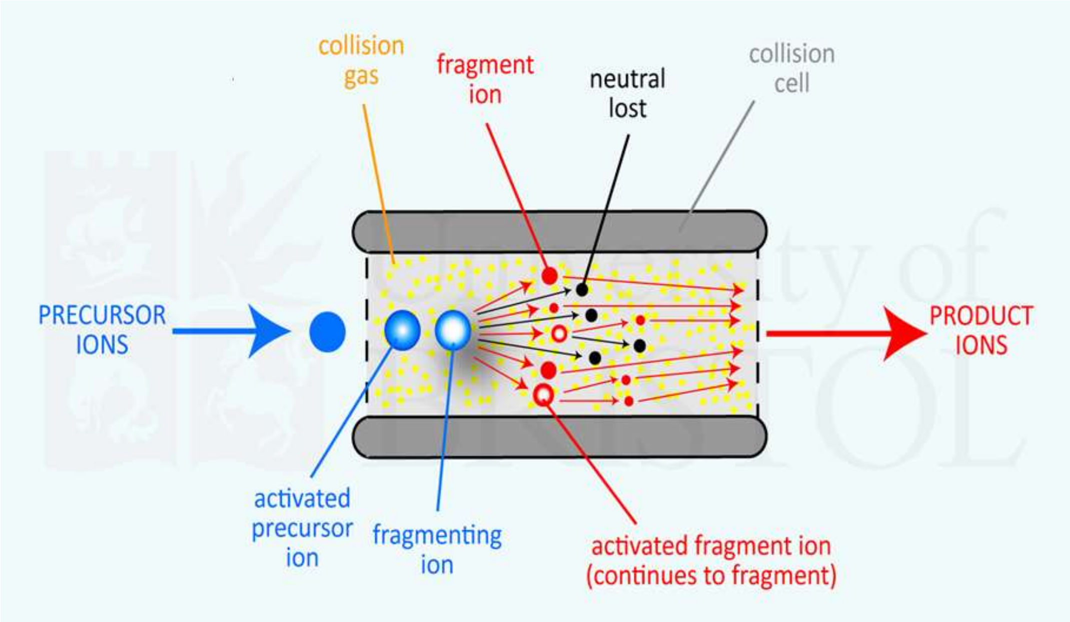
Thermo SCIENTIFIC

QExactive

Thermo
SCIENTIFIC



QExactive (HCD)



- Common fragmentation method
- Increase ion energy to move faster and collide with neutral gas molecules
- High pressure

QExactive

Thermo
SCIENTIFIC

Q Exactive Plus Mass Spectrometer

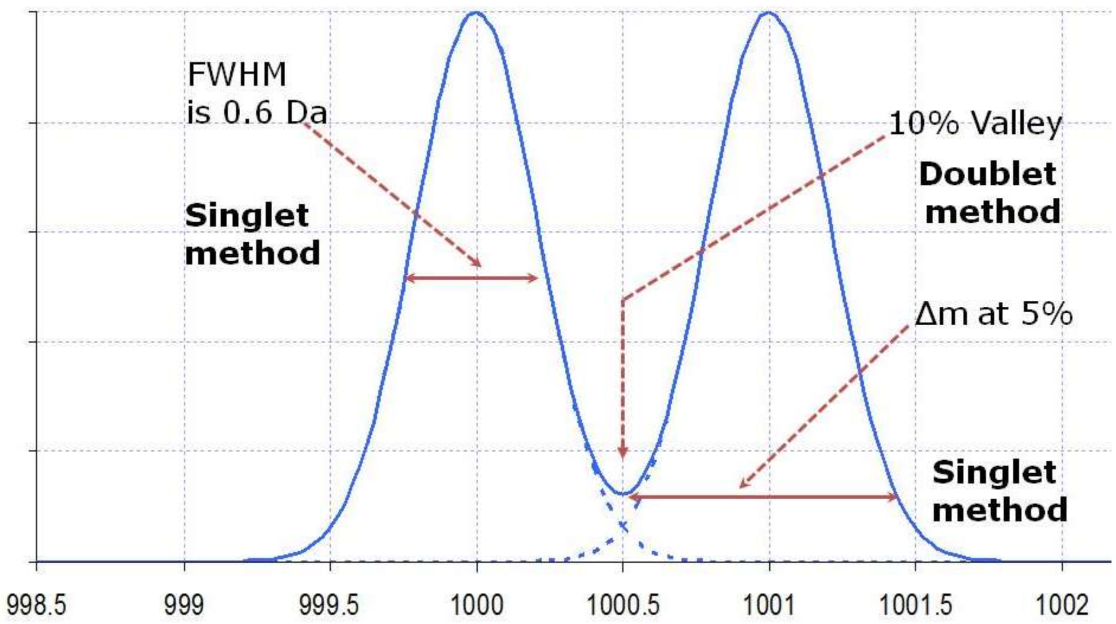
www.planetorbitrap.com/q-exactive-plus#.WmoCMeRG31X

Orbitrap Technology HRAM

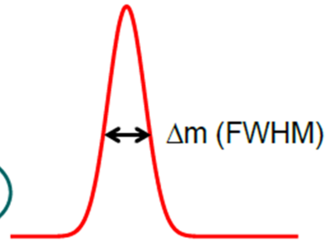
- **HRAM** = High Resolution Accurate Mass spectrometry
- **Mass Resolution** = ability of a mass spectrometer to distinguish between ions of nearly qeual m/z ratios (isobars).
- **Mass Accuracy** = the precision of witch the mass is measured by the mass spectrometer.

Mass Resolution

- Ability of a mass spectrometer to distinguish between ions of nearly equal m/z ratios (isobars).



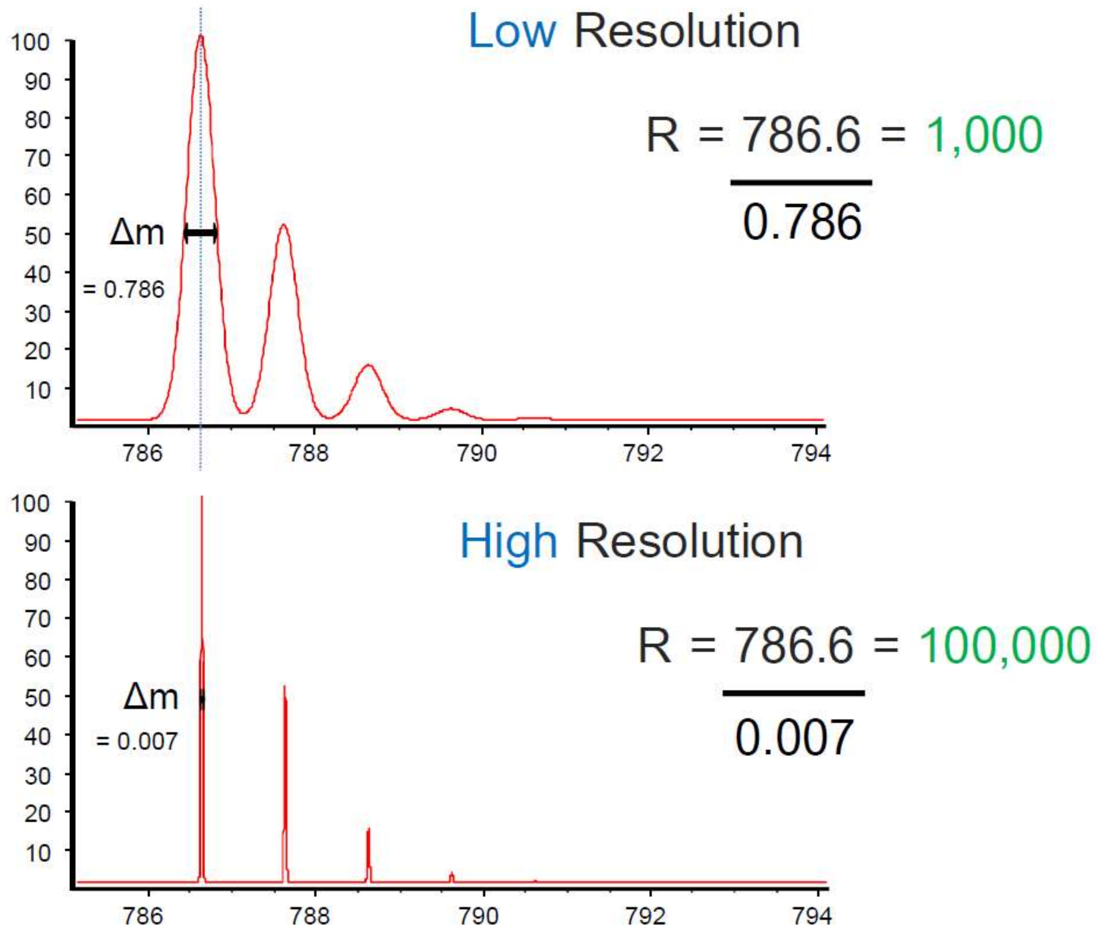
$$R = \frac{m}{\Delta m}$$



- m - measured mass
- Δm - peak width measured at 50% peak intensity (Full Width Half Maximum)

Mass Resolution: What is it?

- Typical values of resolution for <u>Low resolution mass analyzers</u> (e.g. quadrupoles and ion traps) are below **5000**.
- <u>High resolution instruments</u> have a resolution exceeding **15000**.



Why High Mass Resolution

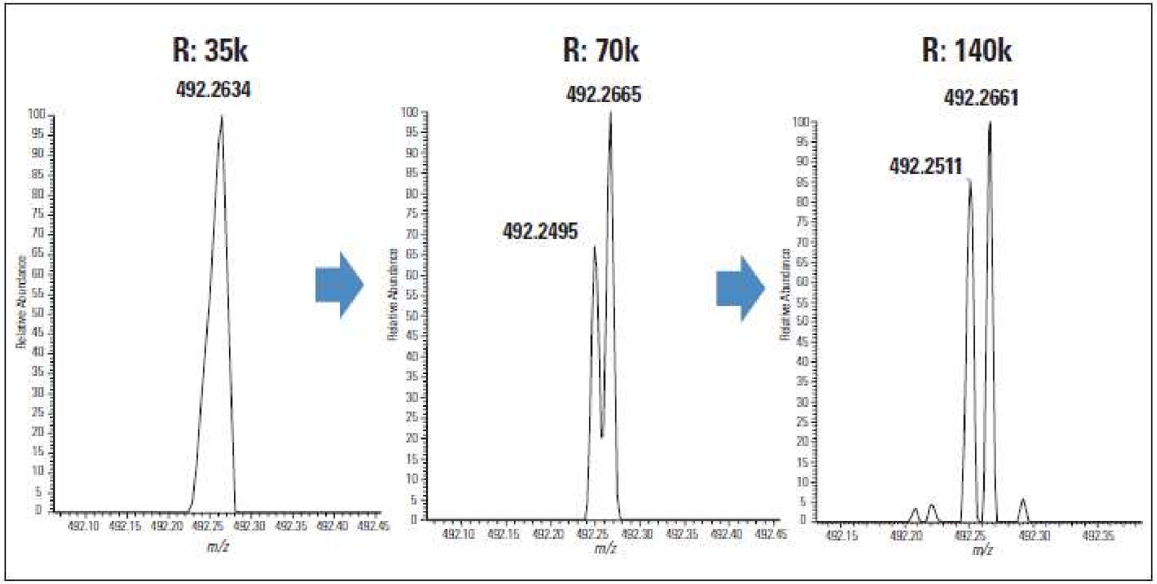
C = 12.0000
H = 1.0078
N = 14.0031
O = 15.9949
S = 31.9721

CO	=	27.9949
N_2	=	28.0061
C_2H_4	=	28.0313

- It is possible to have combinations of atoms which have the same nominal mass but different accurate mass
- Nominal mass measurements cannot distinguish these compounds
- These elemental combinations have the same nominal mass but different accurate mass
- If such compounds can be mass measured with sufficient accuracy it is possible to determine elemental composition

Why High Mass Resolution

- Enables accurate mass
- Increases confidence of identification
- Improves quantitative accuracy
- Gives access to qualitatively different information



More on the topic: N. Cortes-Francisco *et al.,* Accurate mass measurements and ultrahigh-resolution: evaluation of different mass spectrometers for daily routine analysis of small molecules in negative electrospray ionization mode. ***Anal. Bioanal.Chem*. 2011, 400: 3595-3606.**

Mass Accuracy

- **Mass Accuracy** is the precision of which the mass is measured by the mass spectrometer.
- Exact Mass is the mass of an ion with a given empirical formula calculated using the exact mass of the most abundant isotope of each element
- Mass error reporting in **ppm** (relative mass error)

$$\text{Mass error} = \left(\frac{\text{Theoretical}}{\text{Measured} - \text{Theoretical}} \right) \times 10^6 = \text{ppm}$$

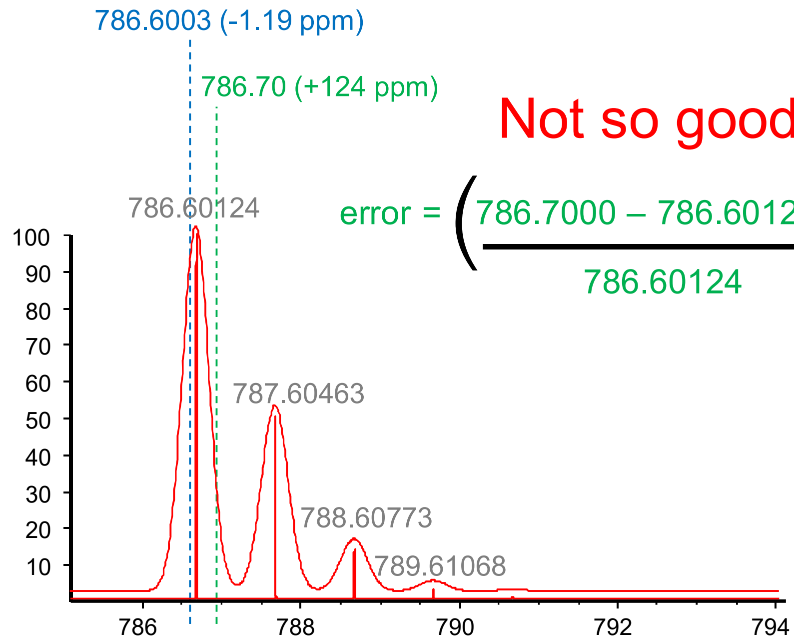
C = 12.0000	H = 1.0078	O = 15.9949
	N = 14.0031	S = 31.9721

Good

$$\text{error} = \left(\frac{786.6003 - 786.60124}{786.60124} \right) \times 10^6$$

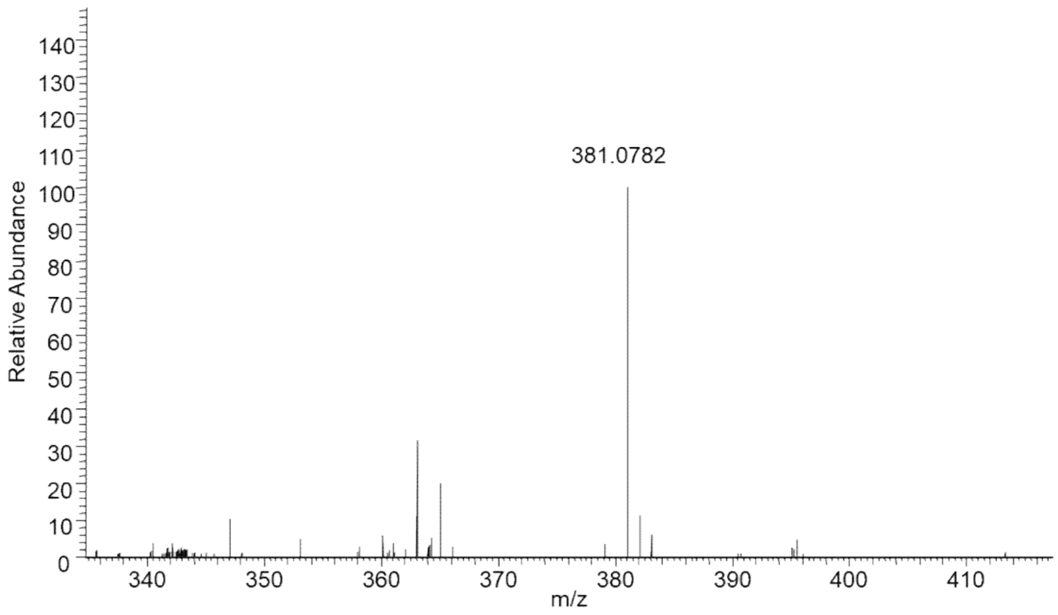
Not so good

$$\text{error} = \left(\frac{786.7000 - 786.60124}{786.60124} \right) \times 10^6$$



Mass Accuracy

- Increases confidence in identification



$[M+H]^+$ 381.0782

Mass Accuracy	Number of hits*
± 200 ppm	265
± 100 ppm	133
± 30 ppm	39
± 10 ppm	14
± 5 ppm	5
± 3 ppm	4
± 1 ppm	1

± 200 ppm to ± 3 ppm: $C_xN_xO_xH_x$

± 1 ppm → $C_{12}H_{22}O_{11}$

* Compounds containing CNOH

Mass Resolution & Accuracy

Measured Mass	Mass Error (Da)	Possible Formula	Exact Mass
32.0	± 0.2	O_2	31.9898
		CH_3OH	32.0261
		N_2H_4	32.0374
		S	31.9721
32.02	± 0.02	CH_3OH	32.0261
		N_2H_4	32.0374
32.0257	± 0.002	CH_3OH	32.0261

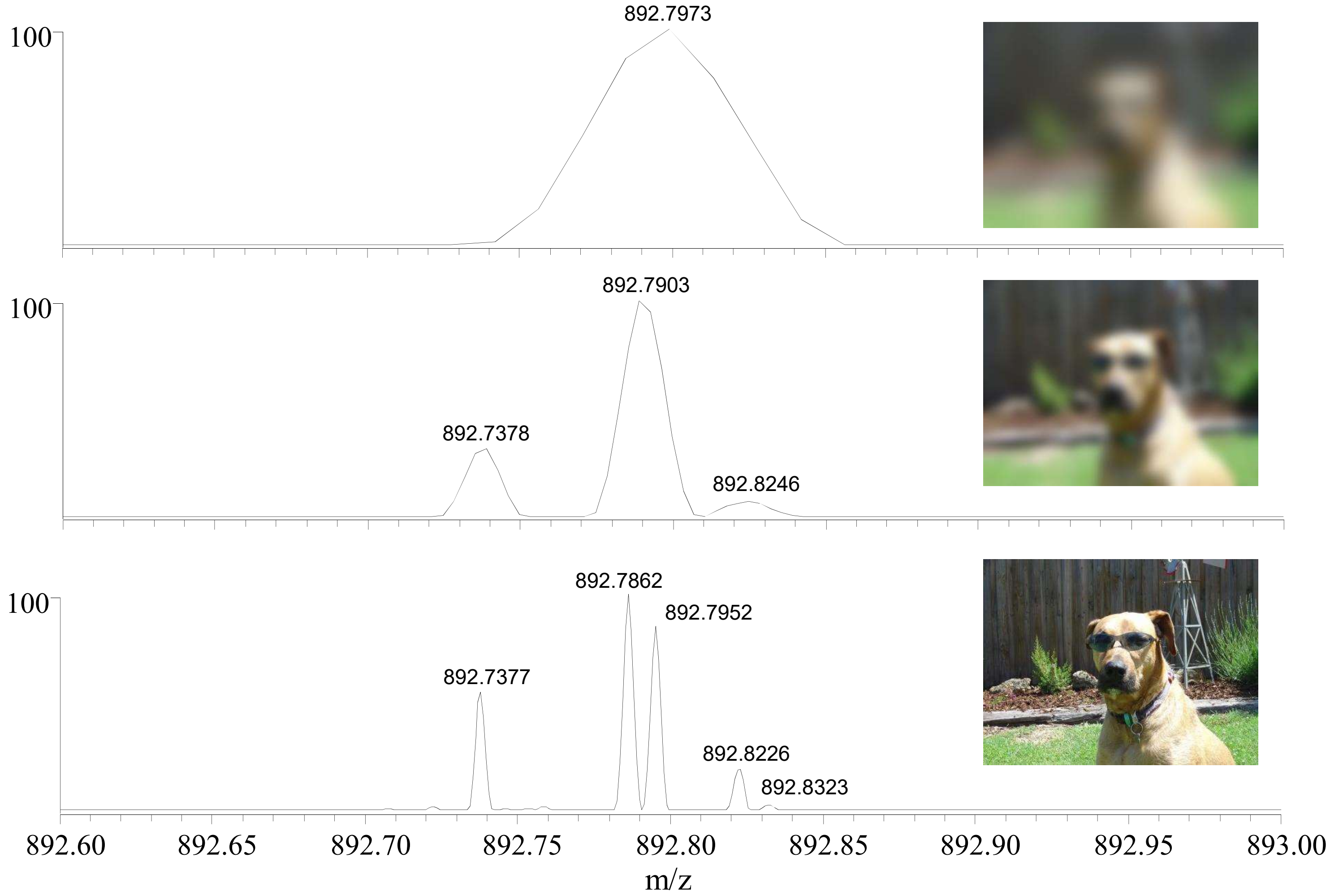
C = 12.0000

O = 15.9949
S = 31.9721

H = 1.0078
N = 14.0031

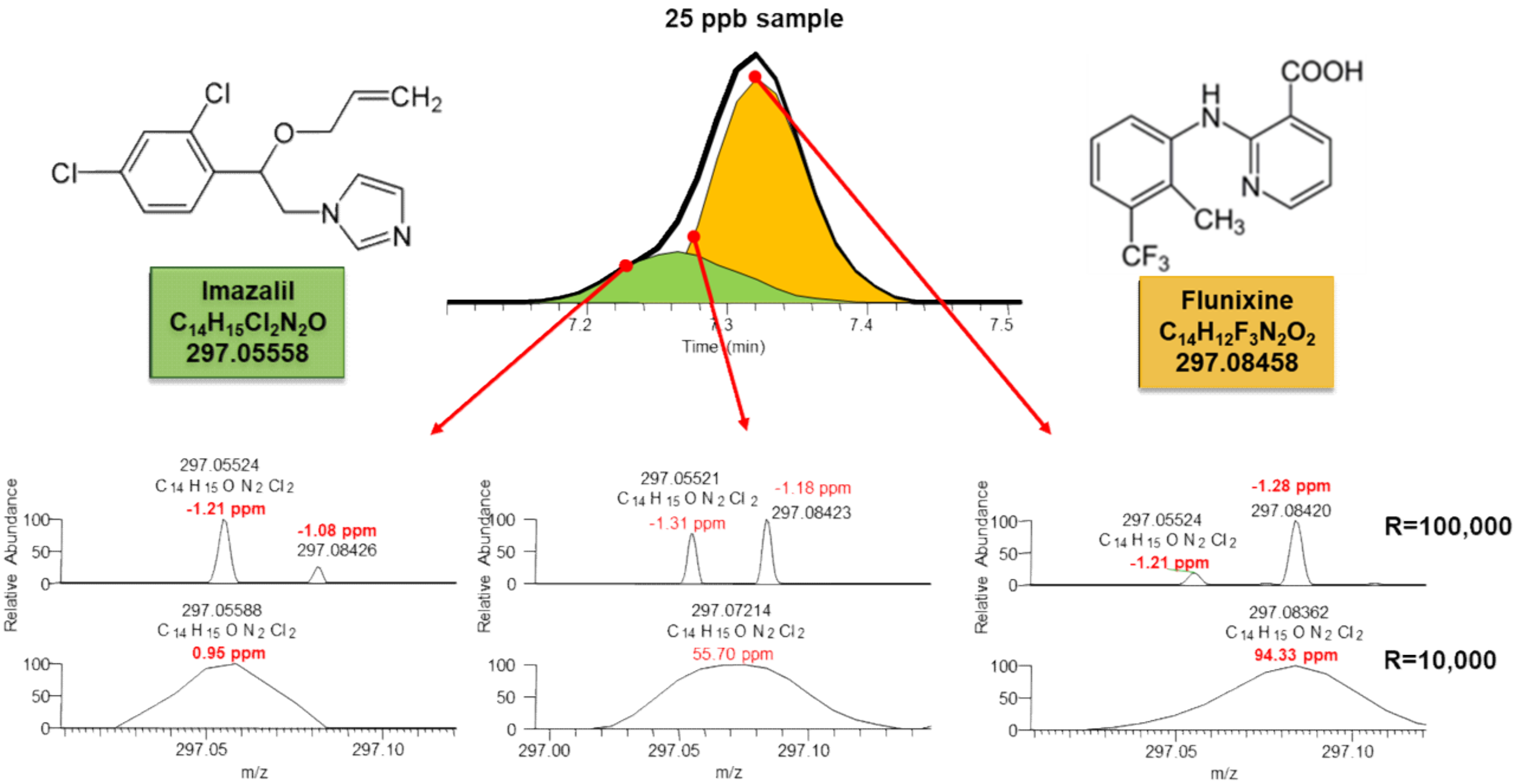
- **Main advantage:** the possibility to determine the elemental composition of individual molecular or fragment ions, a powerful tool for the structural elucidation or confirmation.

Mass Resolution & Accuracy



Mass Resolution & Accuracy

- Isobaric compounds separation



Mass Resolution & Accuracy

- Removing interferences

High resolution is very important for samples with complex matrix (e.g. biological, food), since they will contain a significant number of background ions

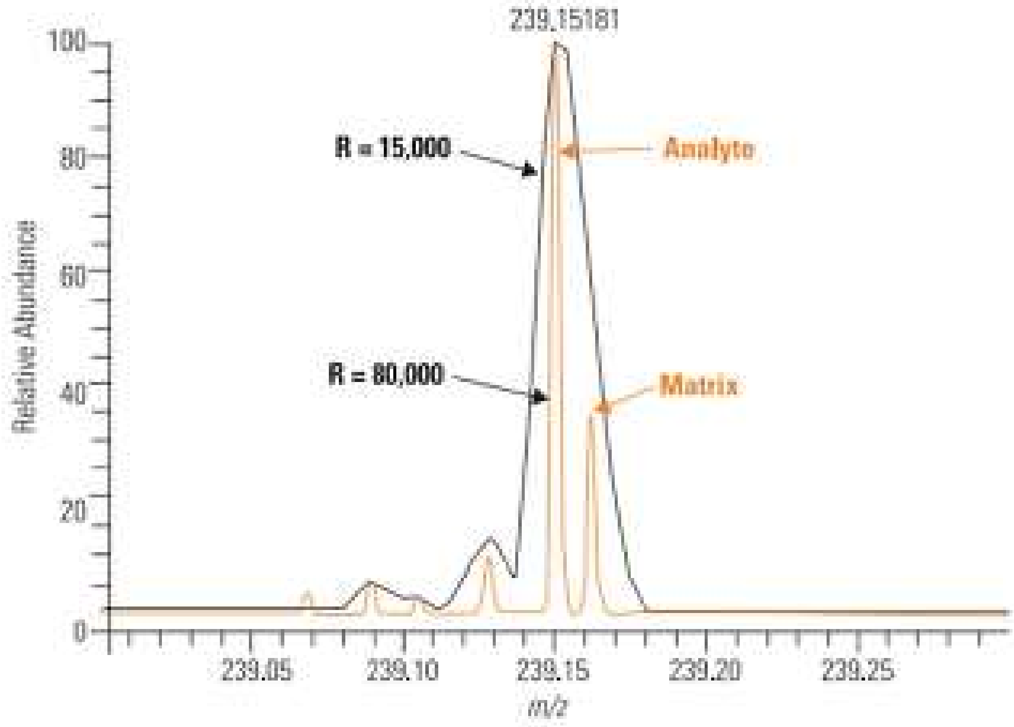
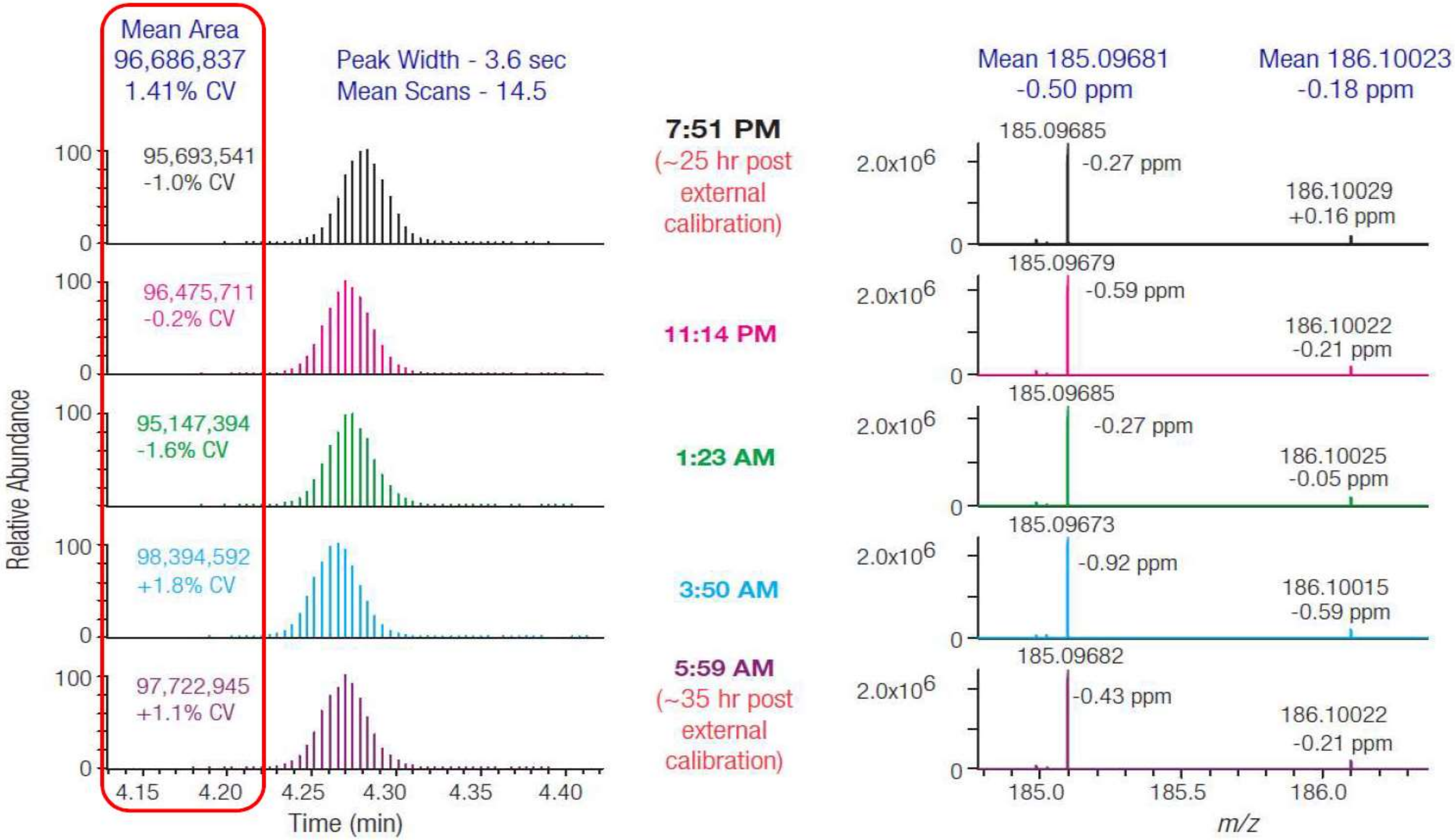


Figure 1: Analysis of the MH⁺ peak of Pirimicarb at 15,000 and 80,000 resolution.

Stability: Robust and Reproducible HRAM



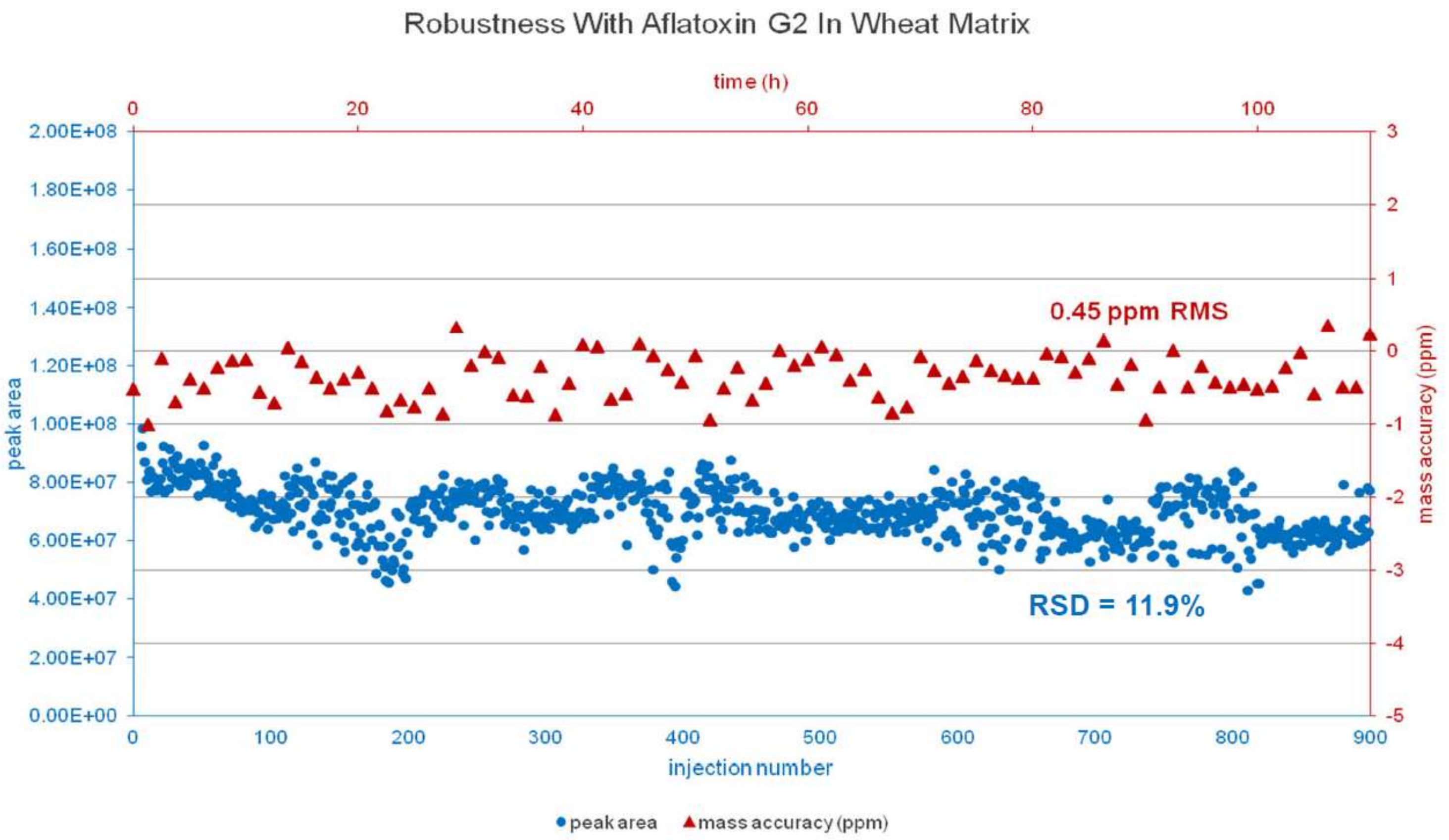
Instrument: UHPLC UltiMate3000 coupled with Q Exactive

Mode: Full scan MS Resolution 70,000

D_5-Hippuric acid, theoretical m/z 185.0969

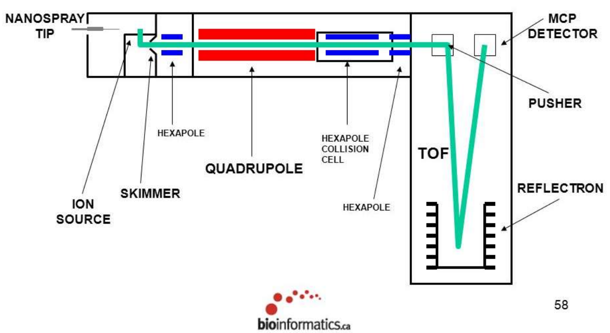
Stability: Robust and Reproducible HRAM

- Peak intensity (area) variation during 900 injections (blue) with according mass accuracy (peak apex scan, red)



Stability: Robust and Reproducible HRAM

Accuracy and stability of mass measurement are crucial for metabolite identification

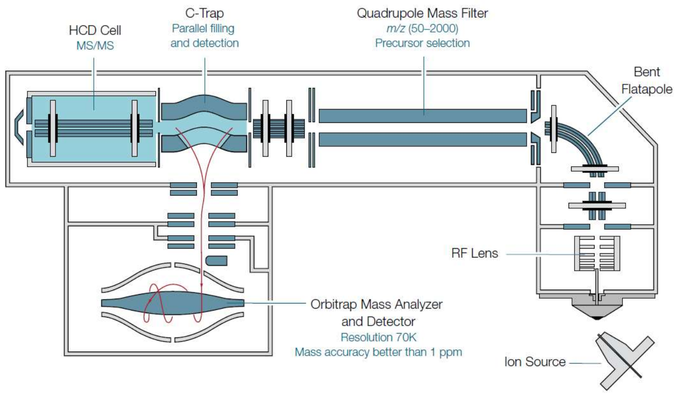


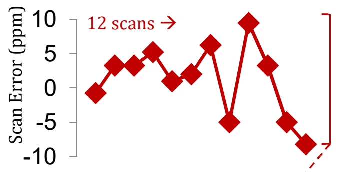
QToF

Scan	Error
1	-0.75
2	3.23
3	3.23
4	5.22
5	0.99
6	1.99
7	6.22
8	-4.97
9	9.45
10	3.23
11	-4.97
12	-8.20

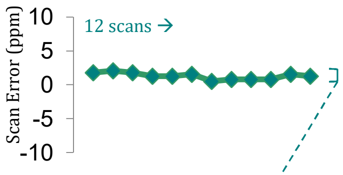
QExactive

Scan	Error
1	1.81
2	2.07
3	1.81
4	1.29
5	1.29
6	1.55
7	0.52
8	0.78
9	0.78
10	0.78
11	1.55
12	1.29





Q ToF
Error Range = 17.65 ppm !

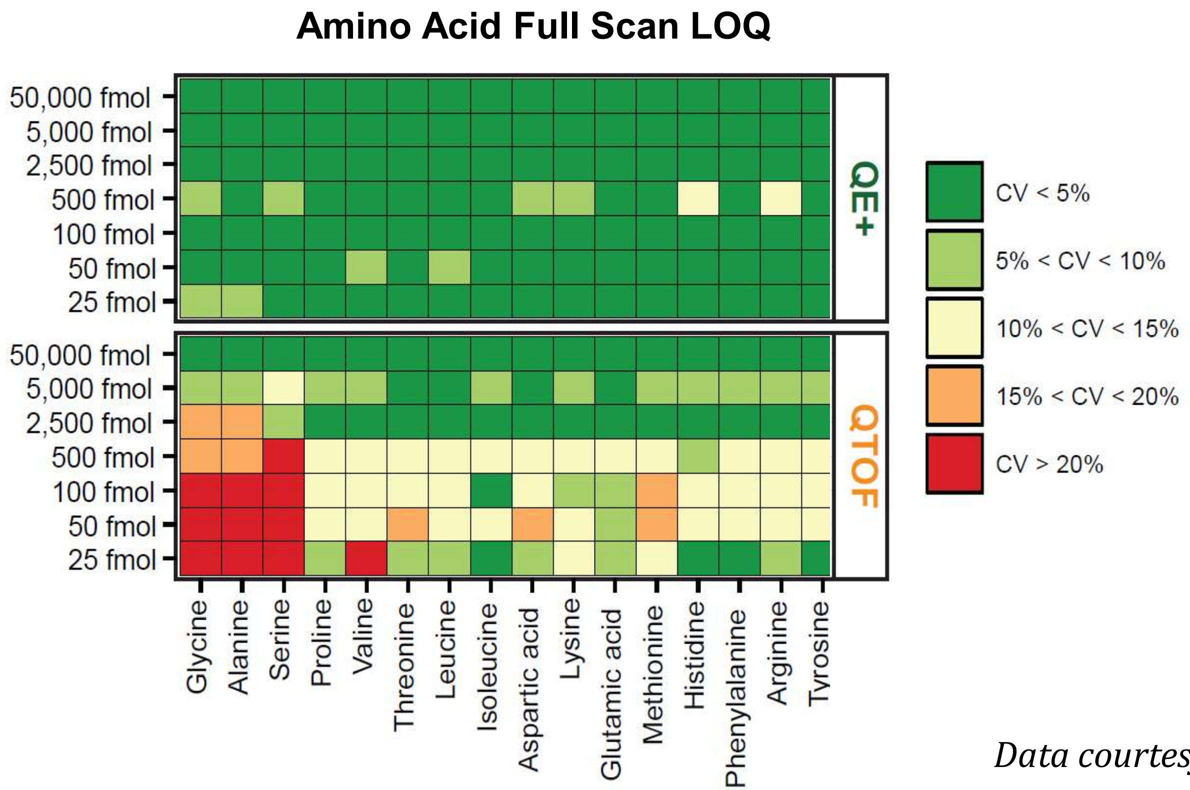


Q Exactive
Error Range = 1.55 ppm ✓

Data From Bristol-Myers Squibb Company

Stability: Robust and Reproducible HRAM

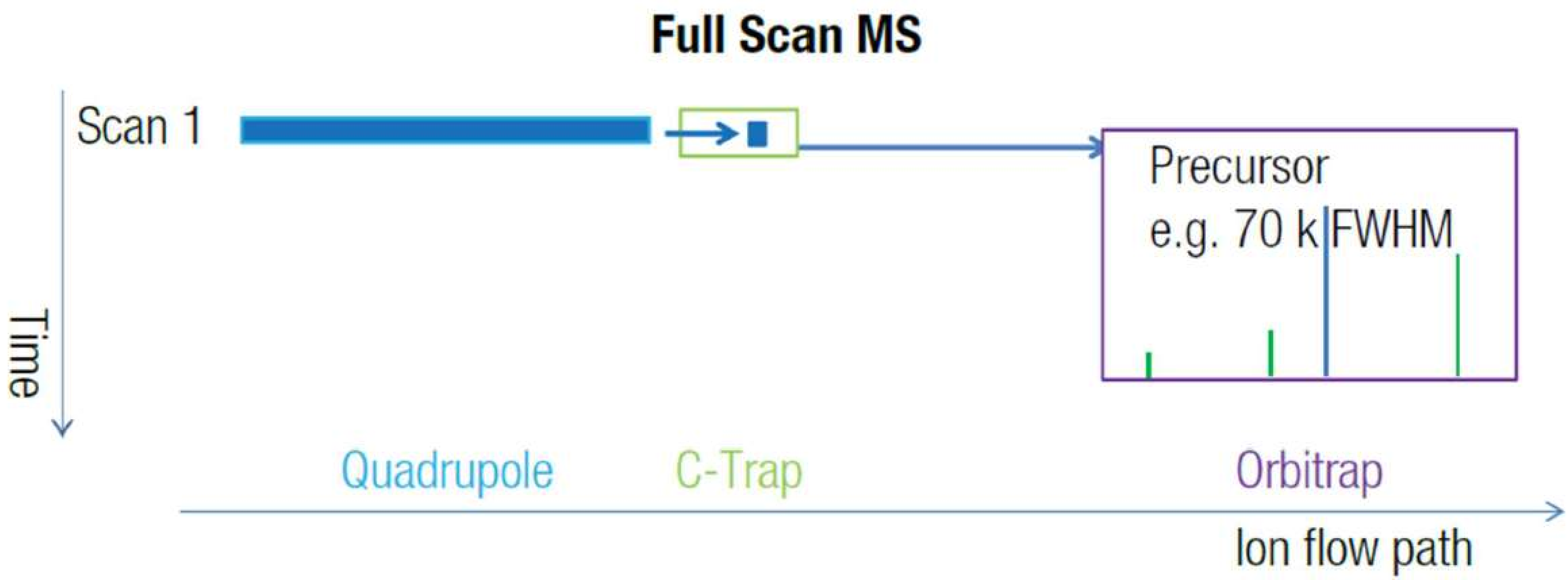
Good sensitivity is fundamental need for metabolite analysis



Data courtesy Stanford University

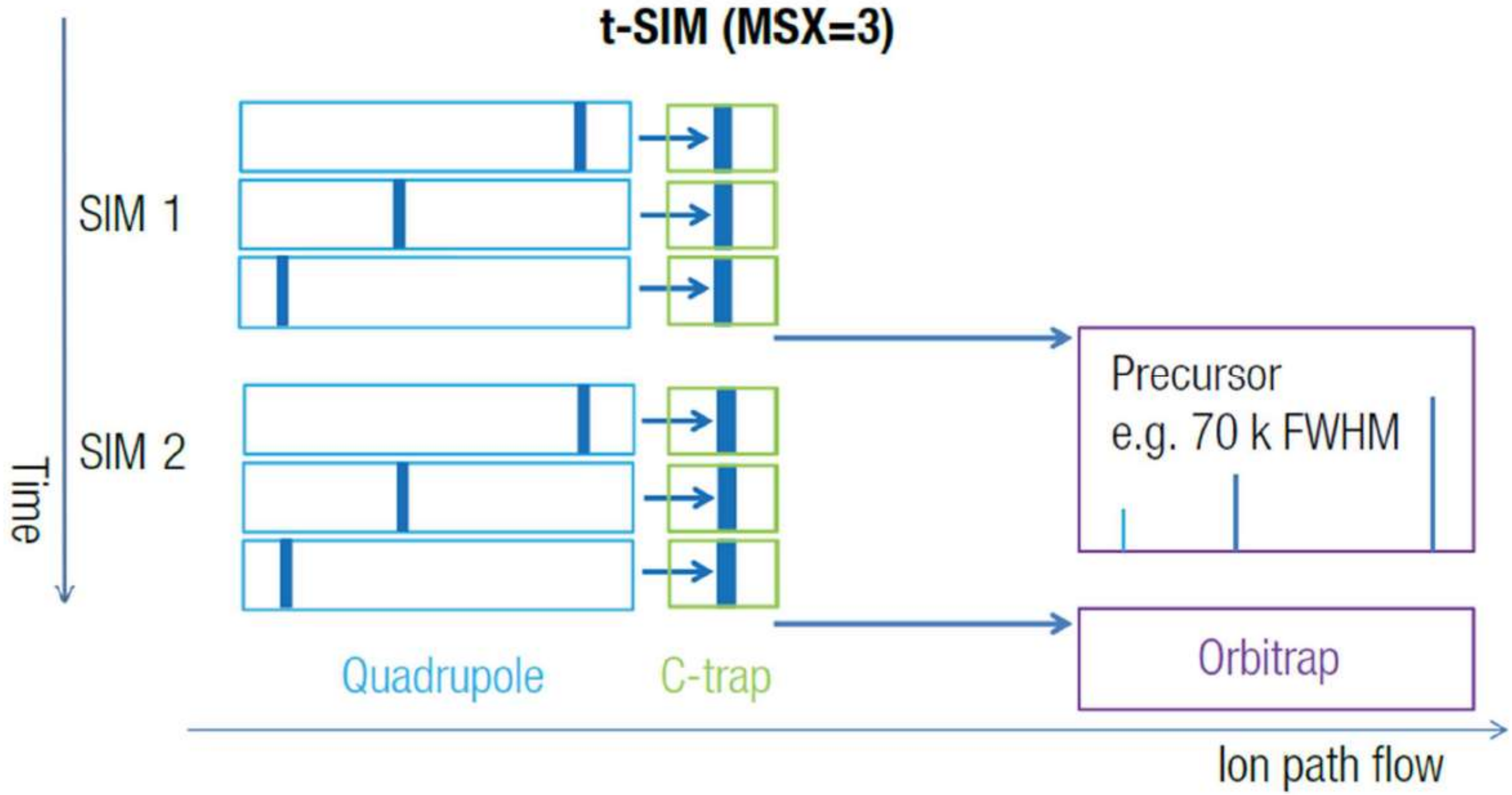
Mode of Acquisition

- Full Scan



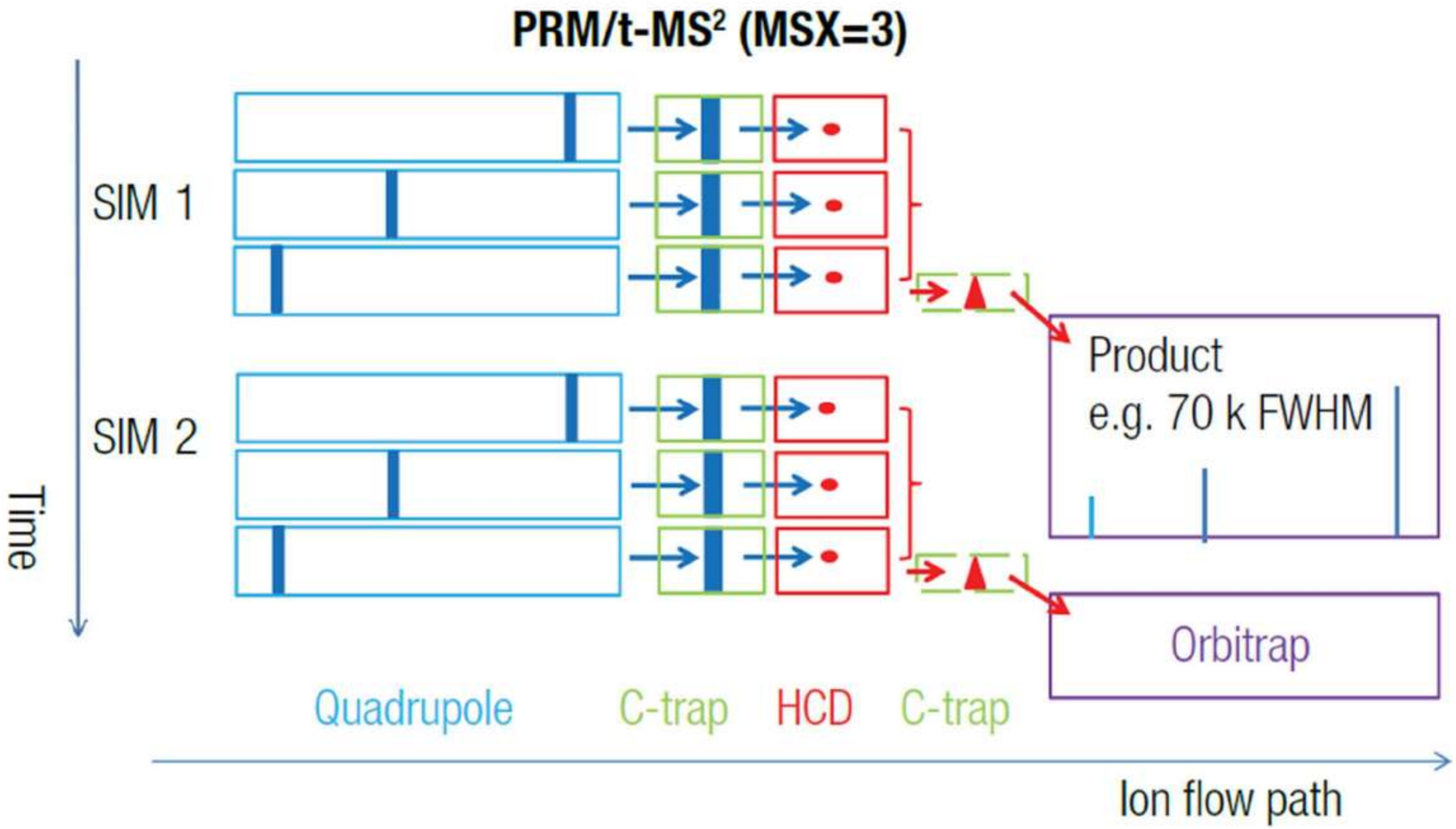
Mode of Acquisition

- SIM: **S**elected **I**on **M**onitoring



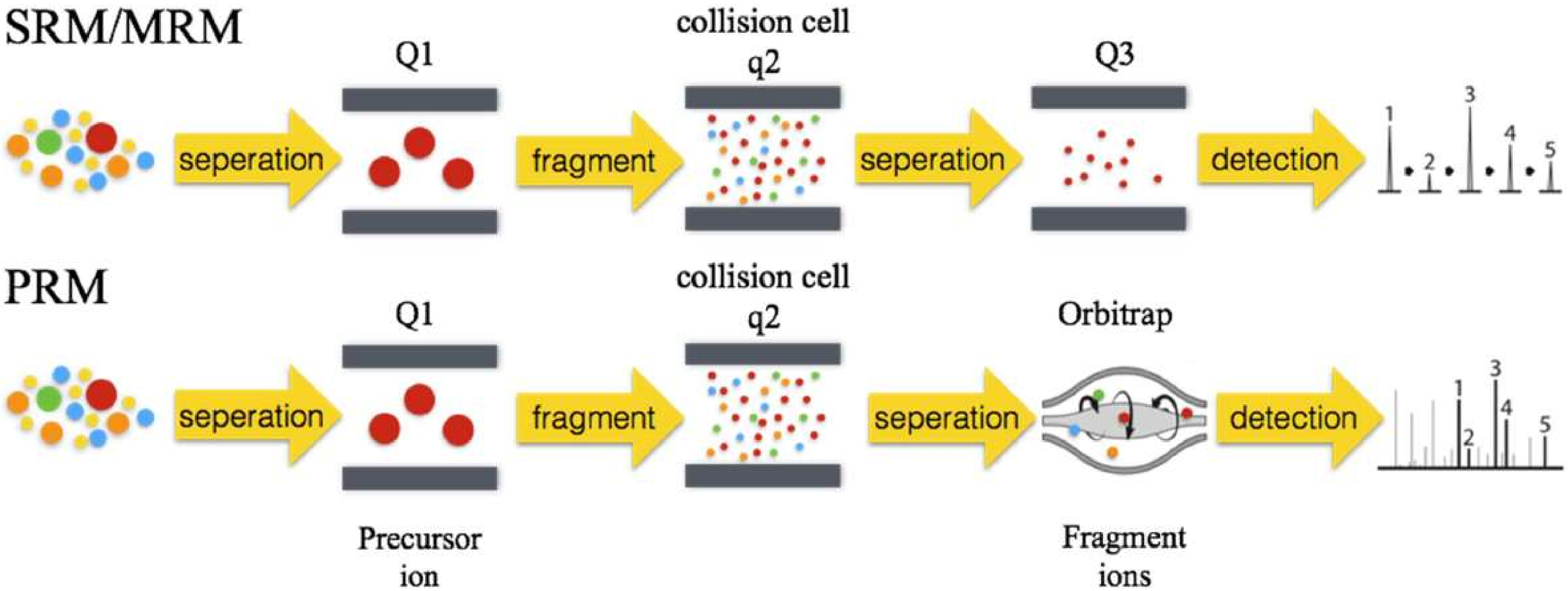
Mode of Acquisition

- PRM: **P**arallel **R**eaction **M**onitoring



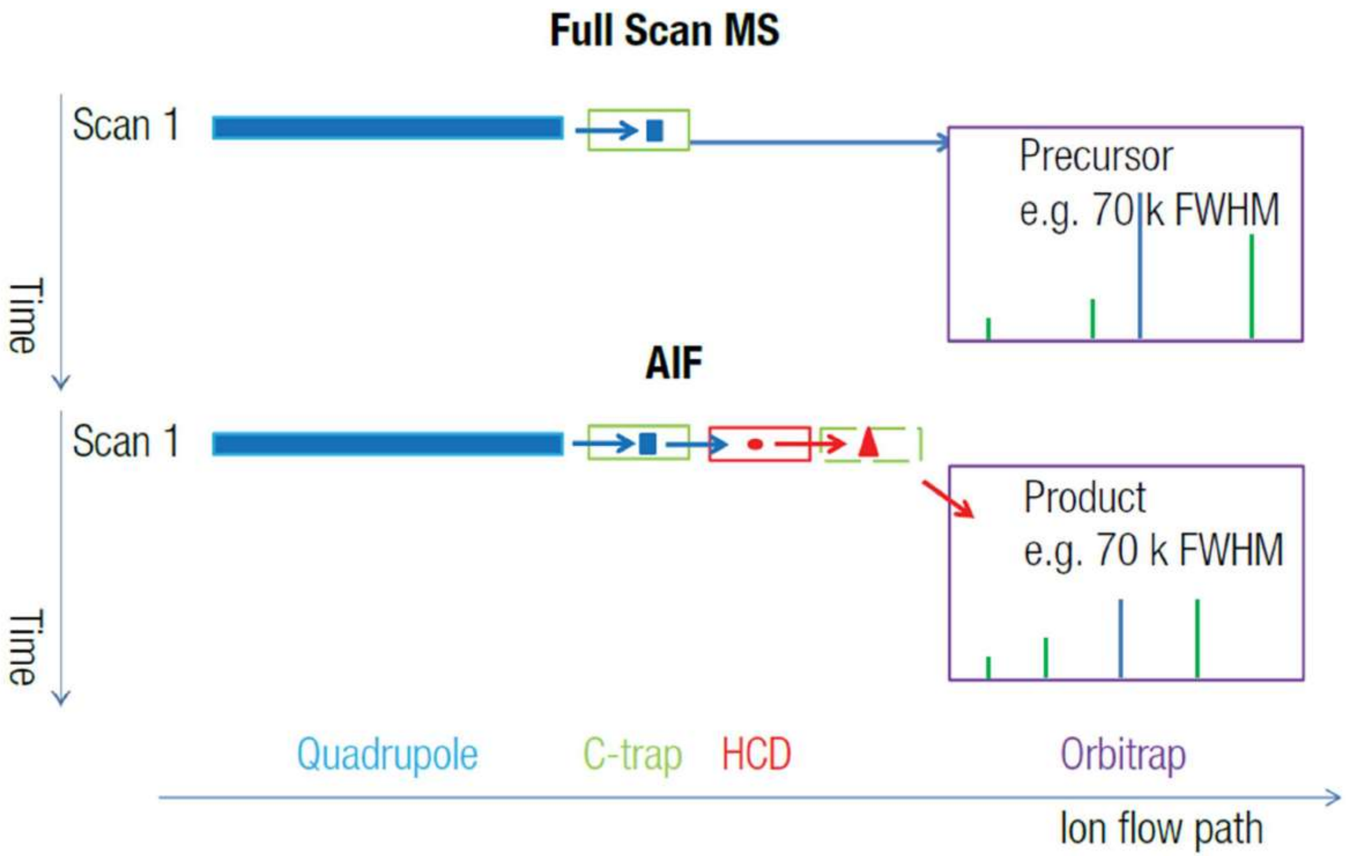
Mode of Acquisition

- PRM: **P**arallel **R**eaction **M**onitoring



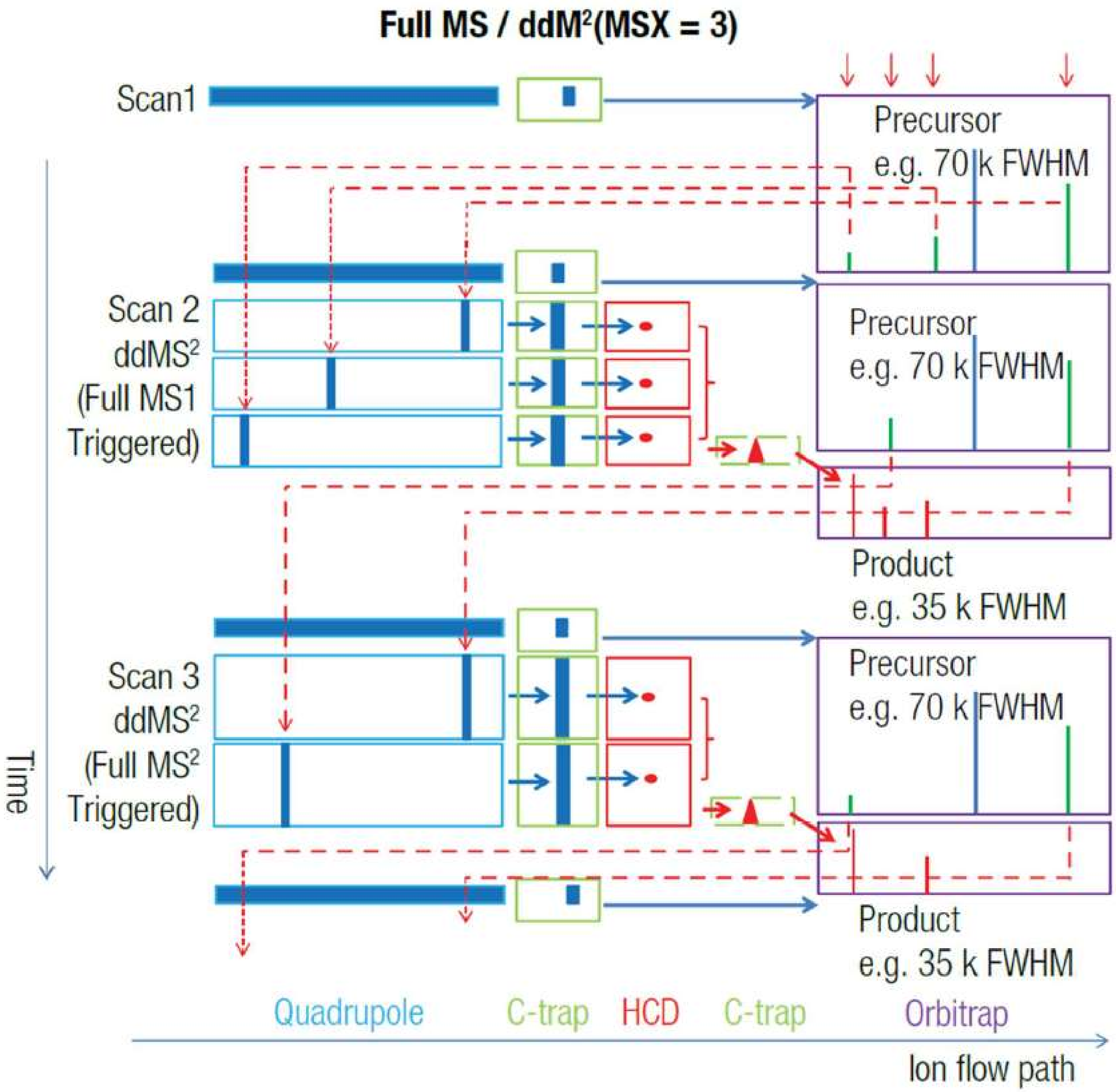
Mode of Acquisition

- AIF: **A**ll **I**on **F**ragmentation



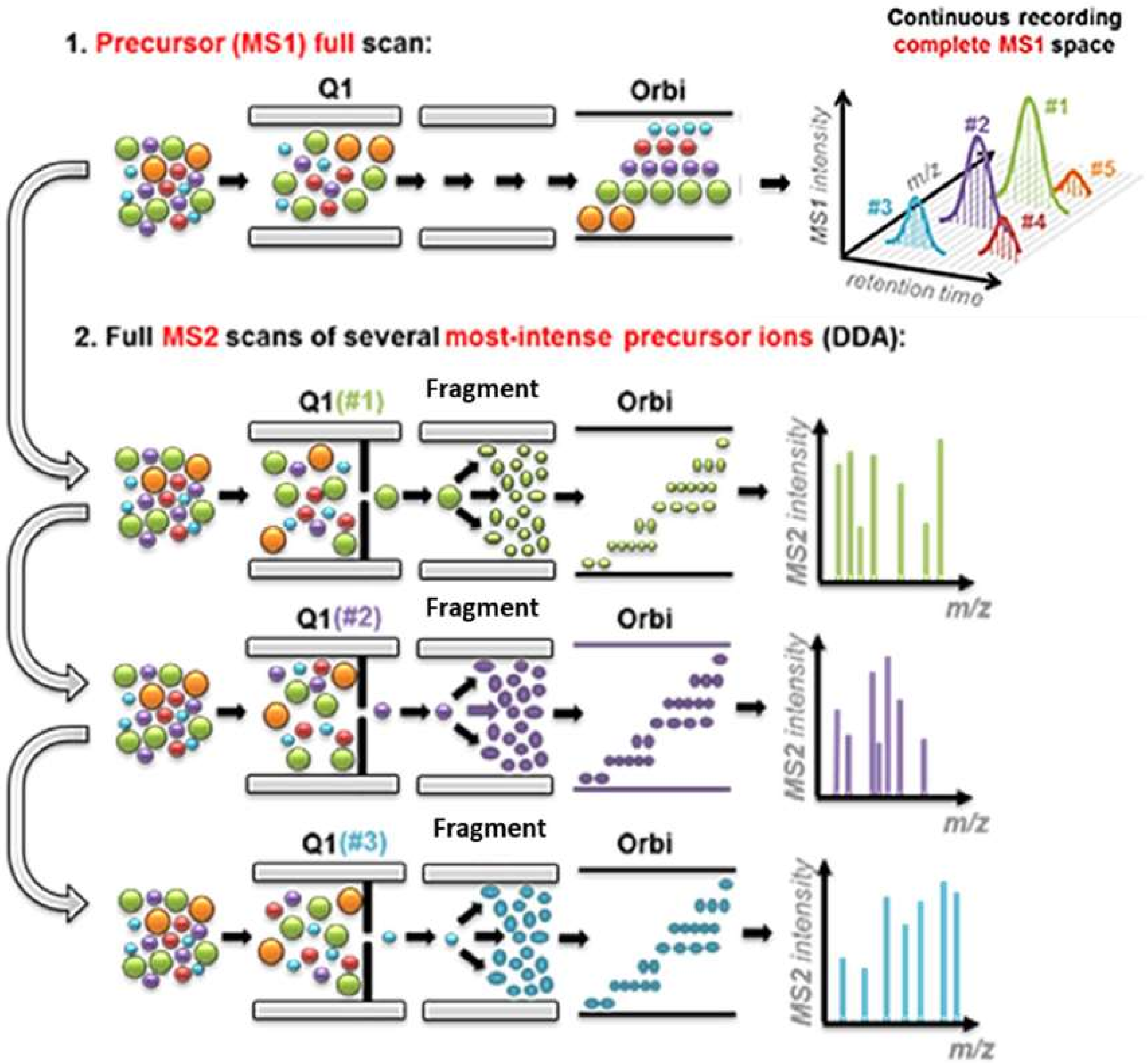
Mode of Acquisition

- DDA: **D**ata **D**ependent **A**cquisition (or **ddMS²**)



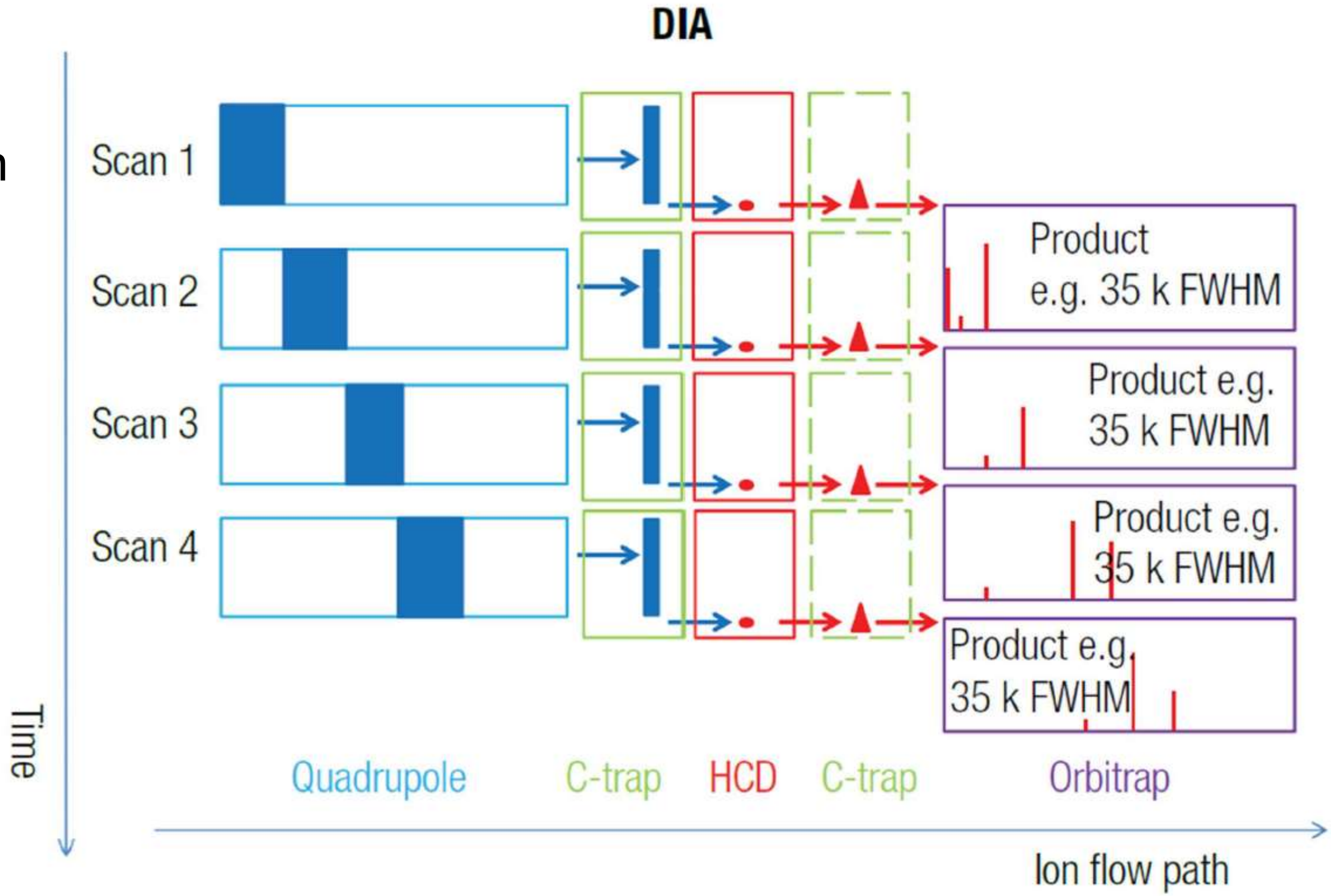
Mode of Acquisition

- DDA: **D**ata **D**ependent **A**cquisition (or **ddMS^2**)



Mode of Acquisition

- vDIA: (**v**ariable) **D**ata **I**ndependent **A**cquisition



Mode of Acquisition

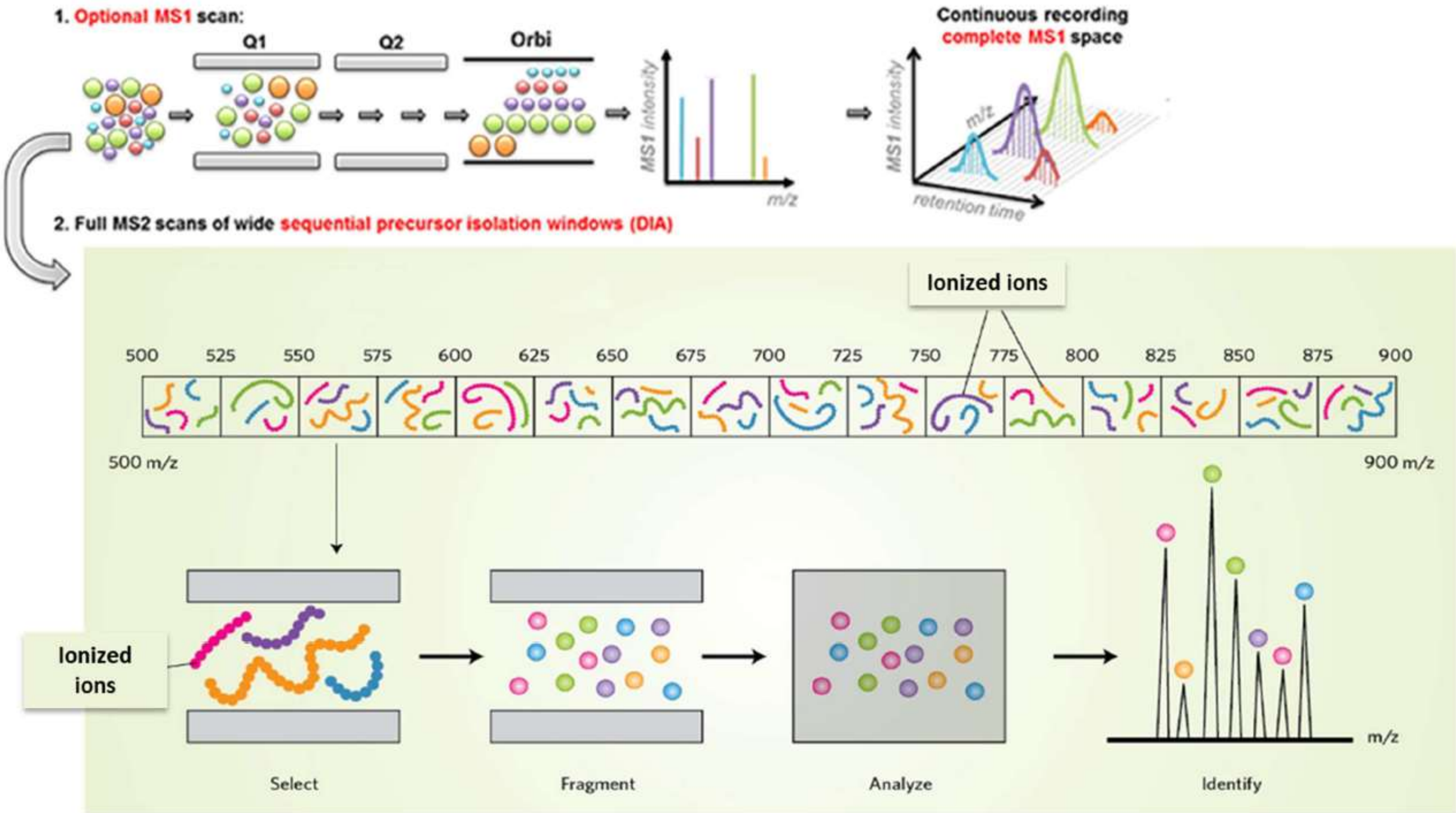
- vDIA:

  (**v**ariable)

  Data

  Independent

  Acquisition



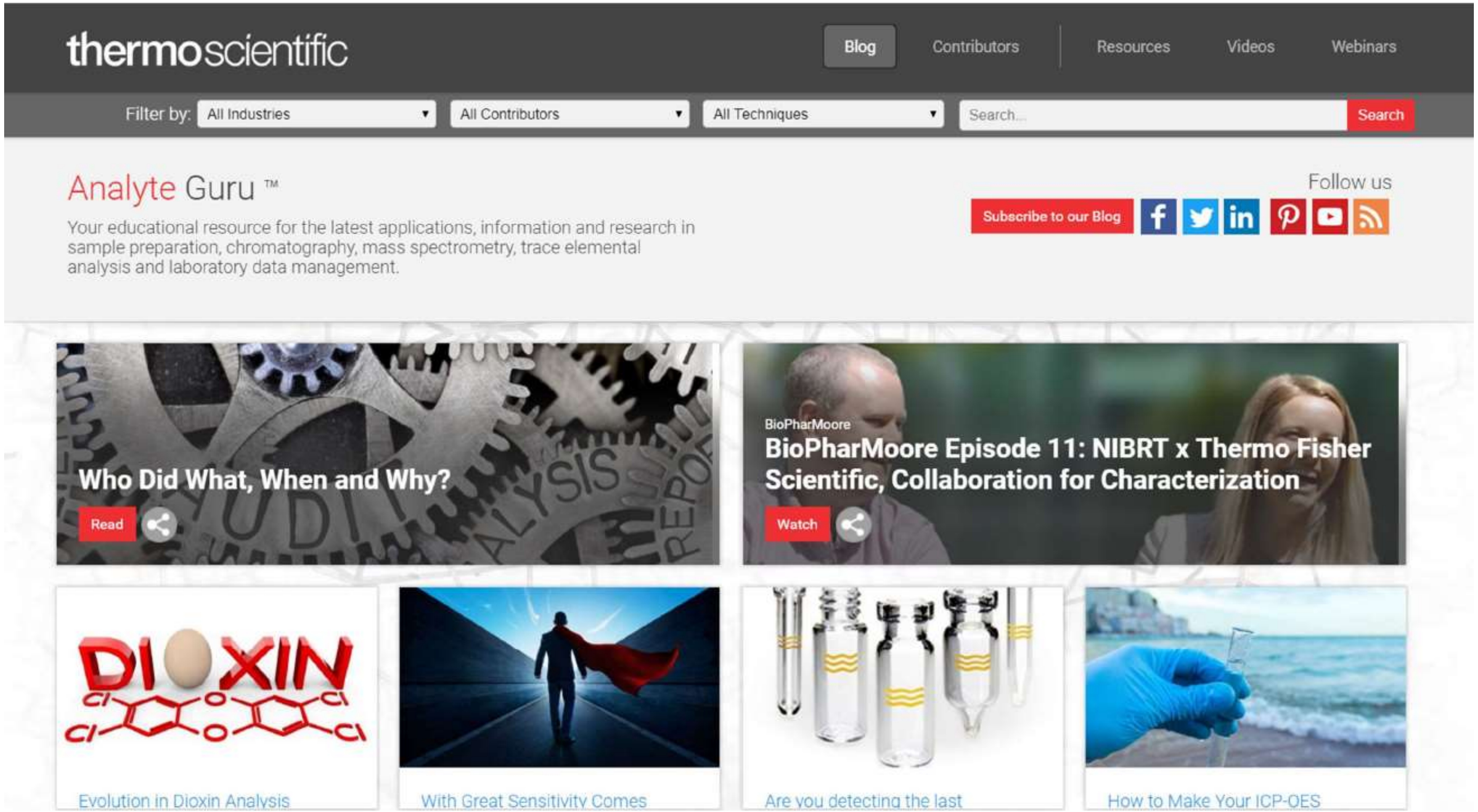
Orbitrap Application Universe

Additional Information

http://planetorbitrap.com/

Additional Information

http://analyteguru.com/



Thank you for you attention

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