DEPARTMENTS OF CHEMISTRY AND BIOCHEMISTRY

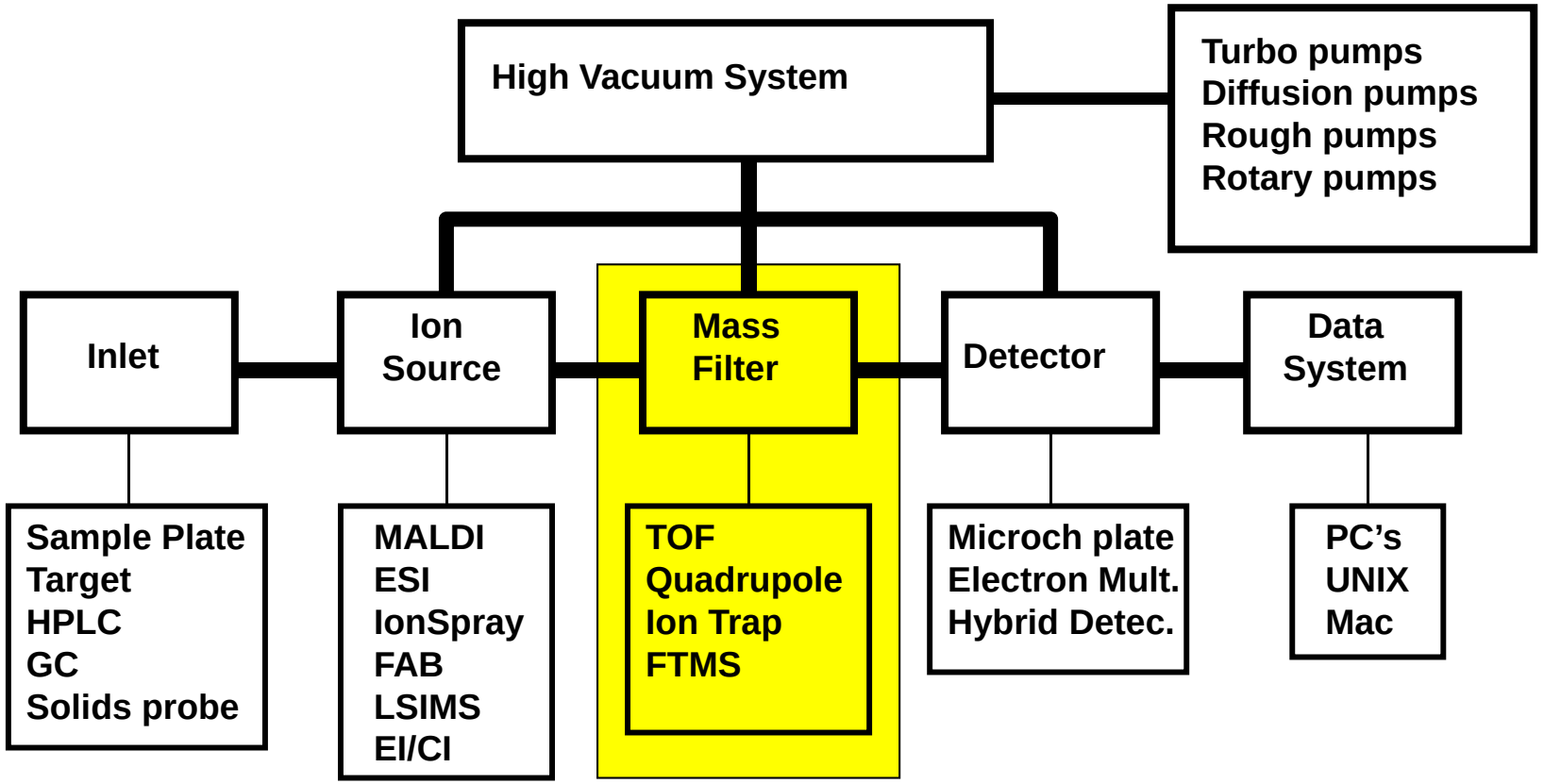
UNIVERSITY OF OXFORD

GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 2

Mass Analysers

Shabaz Mohammed

October 20, 2015

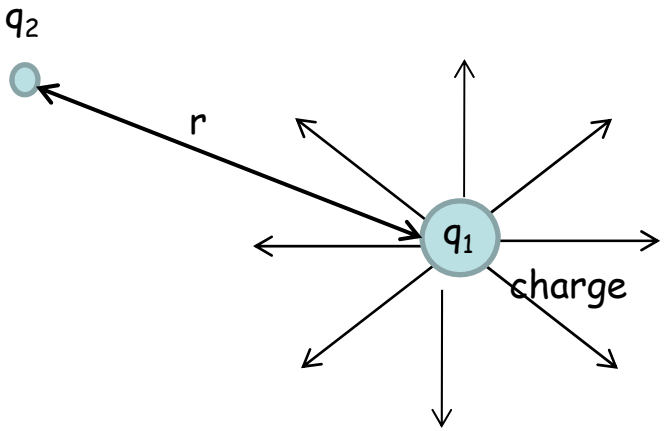

High Vacuum System
Turbo pumps
Diffusion pumps
Rough pumps
Rotary pumps
Inlet
Ion Source
Mass Filter
Detector
Data System
Sample Plate
Target
HPLC
GC
Solids probe
MALDI
ESI
IonSpray
FAB
LSIMS
EI/CI
TOF
Quadrupole
Ion Trap
FTMS
Microch plate
Electron Mult.
Hybrid Detec.
PC's
UNIX
Mac

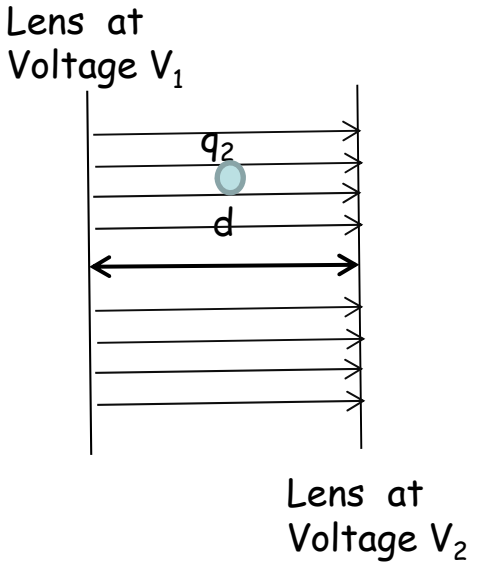
Mass Analyzers

- Quadrupole Analyzer (Q)
- Ion Trap Mass Analyzer (QIT)
- Time-of-Flight Analyzer (TOF)
- Orbitraps(Orbi)

Electromagnetism

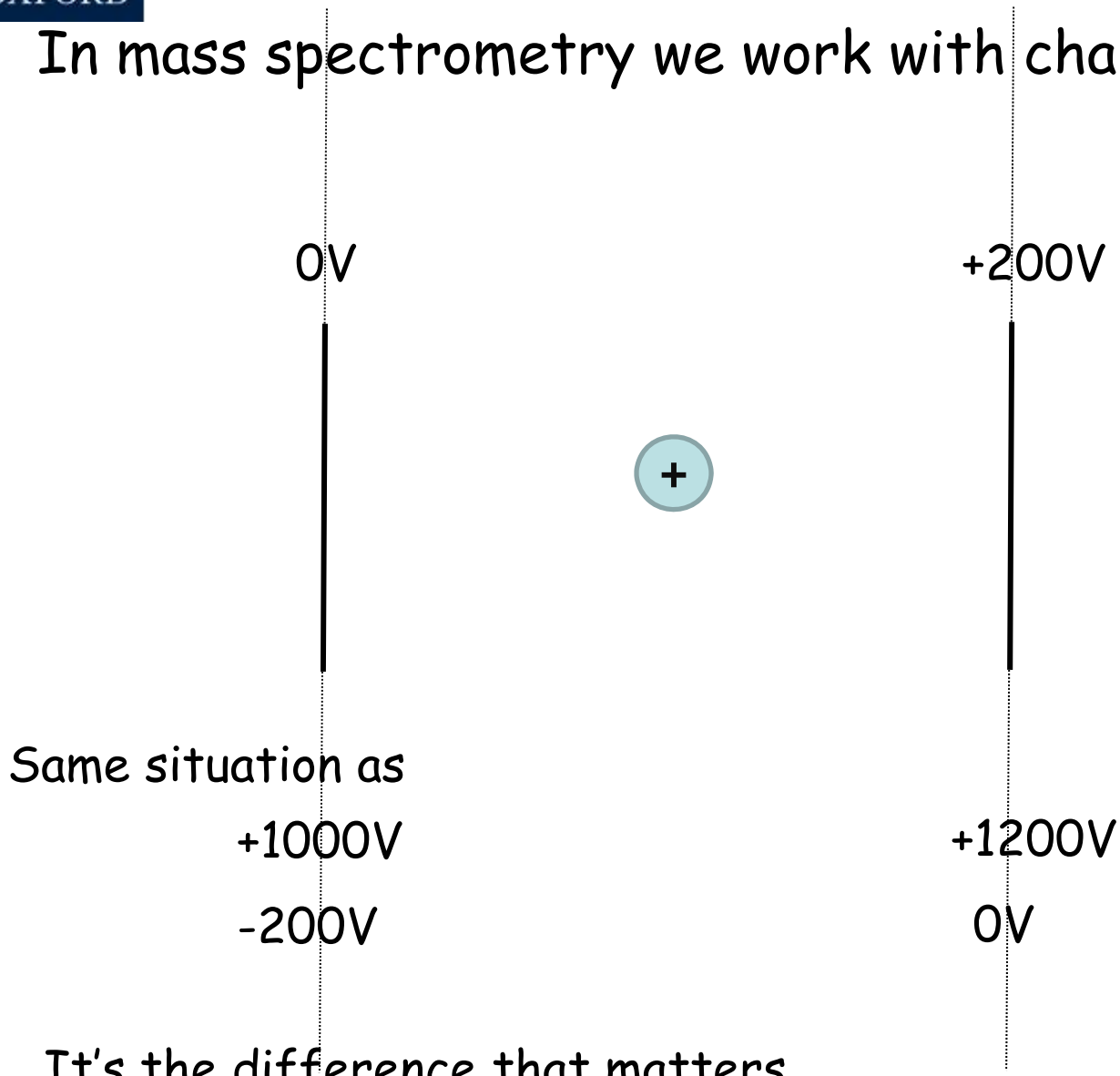
Force or F = $q_2 \times \frac{q_1}{4\pi\varepsilon r^2}$ or $q_2 \times \frac{V_1 - V_2}{d}$





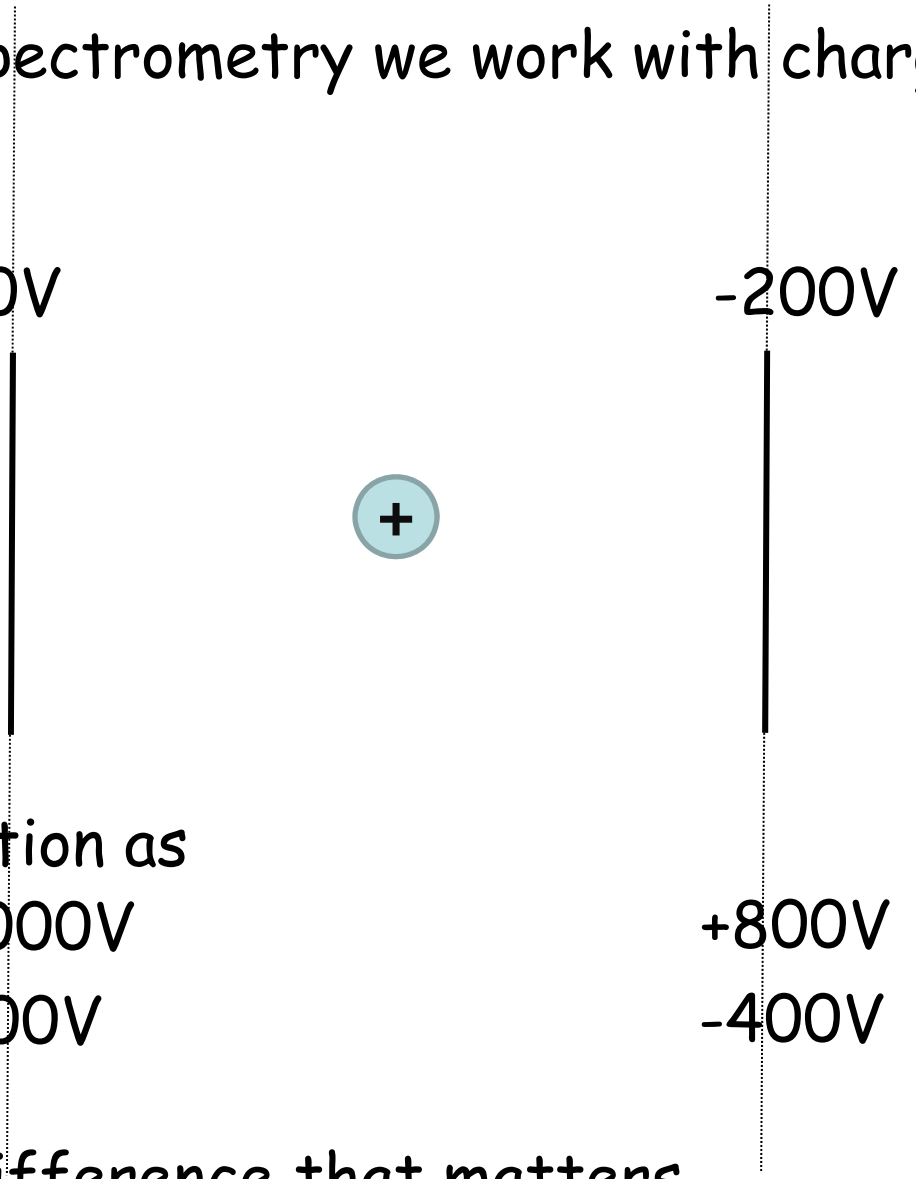
Electromagnetism

In mass spectrometry we work with charges and lenses



Electromagnetism

In mass spectrometry we work with charges and lenses

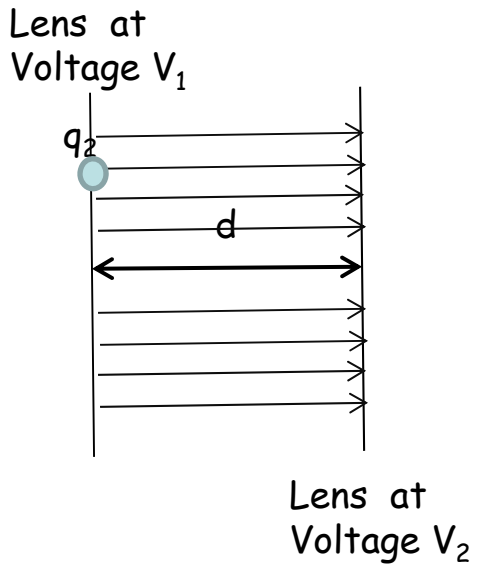


Same situation as

It's the difference that matters

Electromagnetism

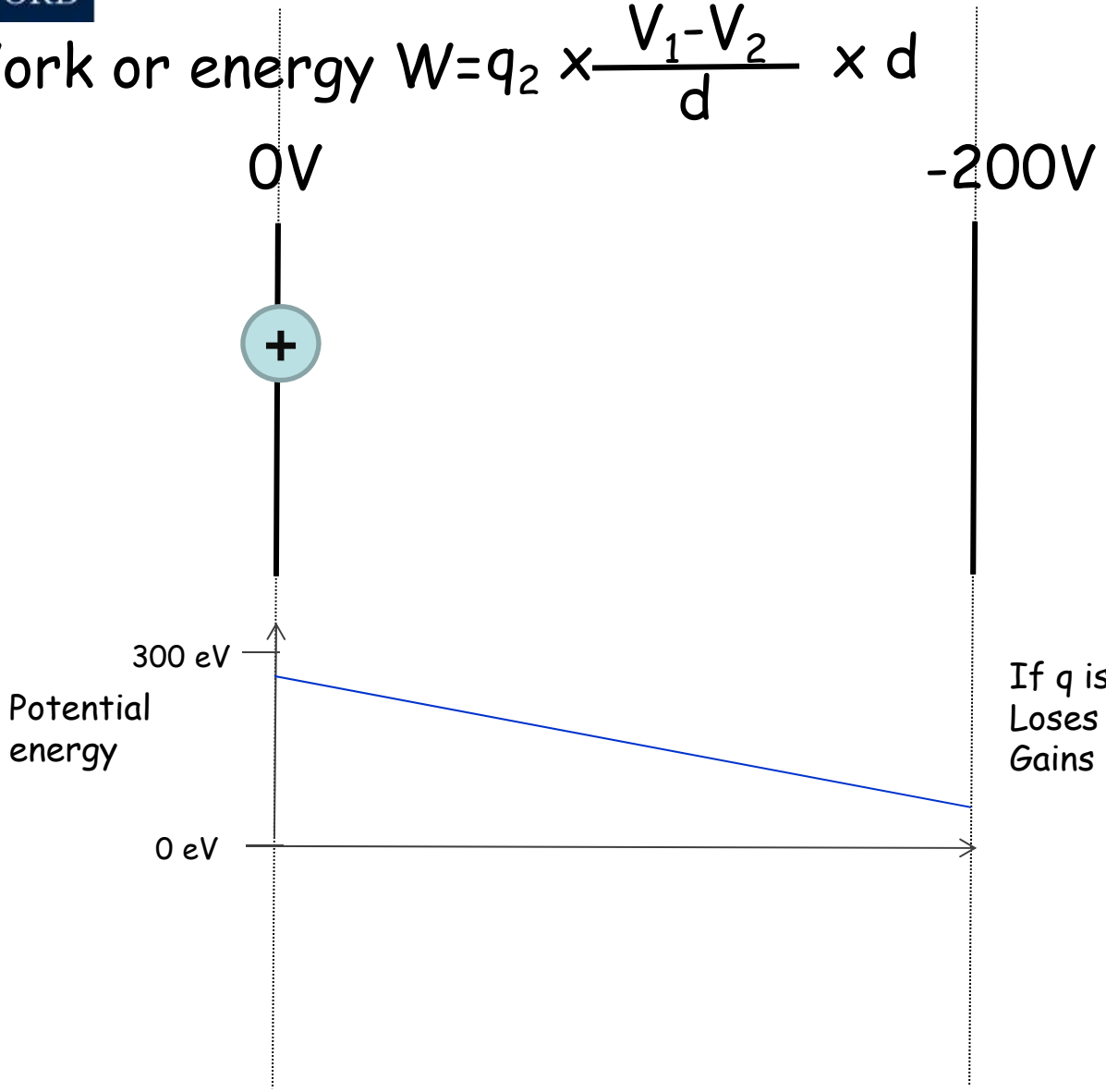
Work or energy $W = q_2 \times \frac{V_1 - V_2}{d} \times d$



q is often expressed in multiples of e (charge on an electron)
i.e energy is expressed in eV (electron volts)

Electromagnetism

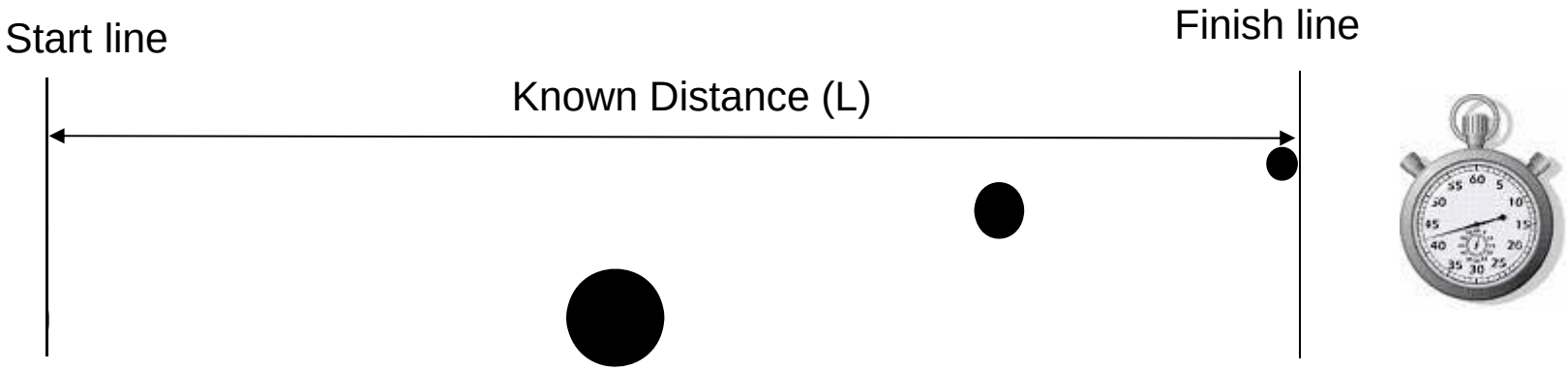
Work or energy $W = q_2 \times \frac{V_1 - V_2}{d} \times d$



If q is 1 charge then:
Loses 200 eV of potential energy
Gains 200 eV of kinetic energy

Time-of-flight Mass Analyzer

Analyser performs the equivalent of the 100m sprint for ions



•Ions are given the same amount of energy through a pulse
(Energy is proportional to charge and the applied potential. E=zeV, z is number of charges, e is the amount of charge on an electron, V is volts)

•Ions then move at the speed determined by their mass (E=0.5mv^2, *v* for velocity (L/t))
i.e. velocity goes down as mass goes up

•Distance to finish line is established and stopwatch (t) is accurate to 1 nanosecond or better…

•So….

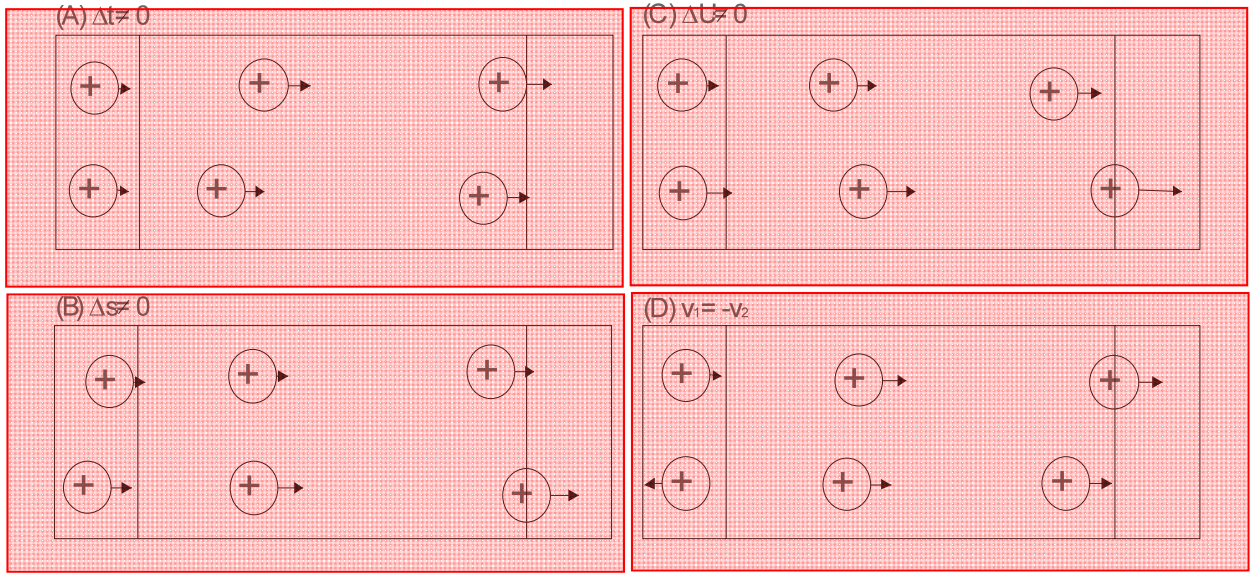
$$zeV = \frac{mv^2}{2}$$

which can be turned into

$$\frac{m}{z} = 2eV\left[\frac{t}{L}\right]^2$$

ToF: Problems

Starting position is not always the same!



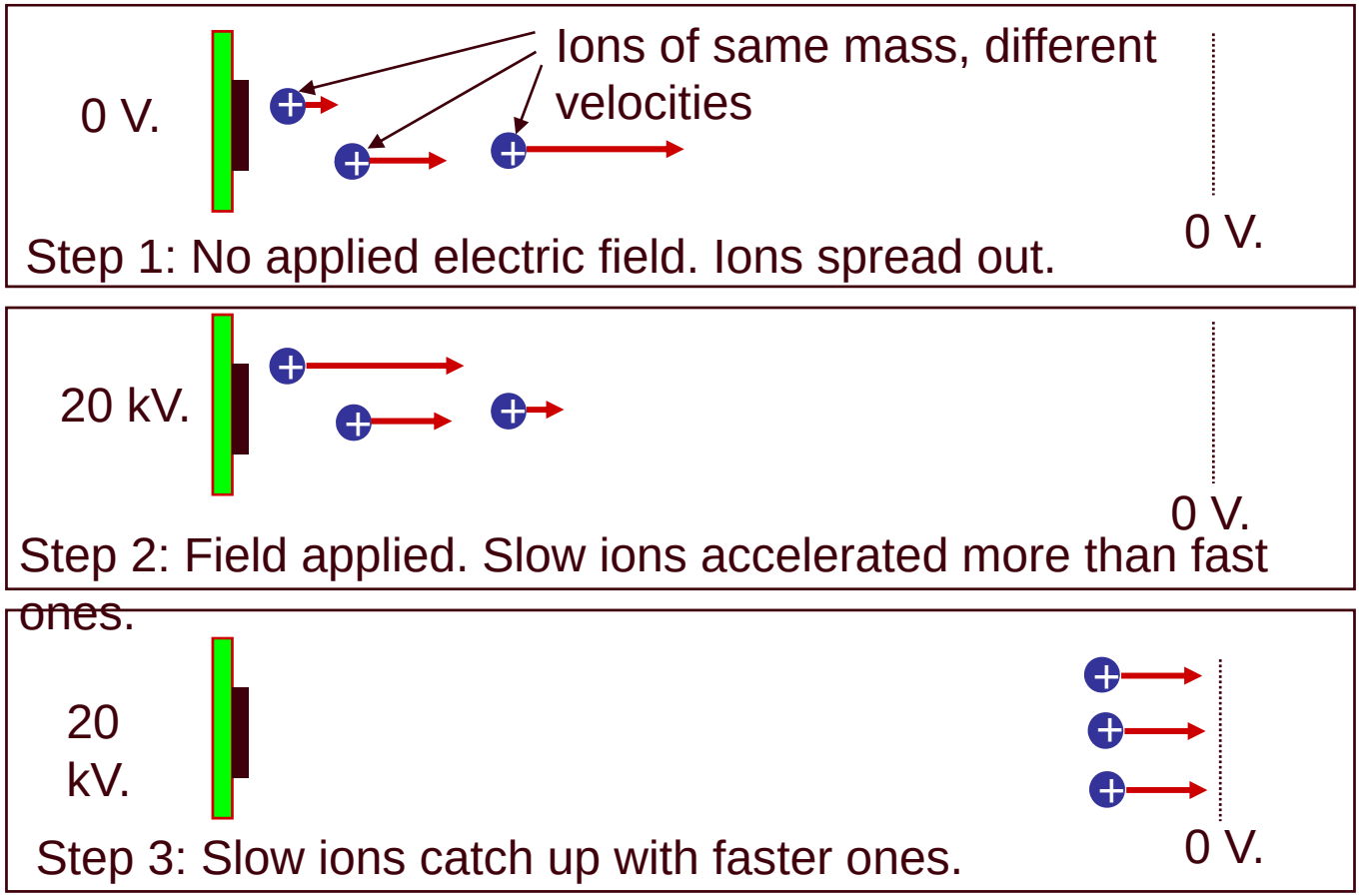
A) Temporal: Ions of the same mass can form at different times but have the same KE.

B) Spatial: Ions of the same mass form having the same KE but in different parts of the source;

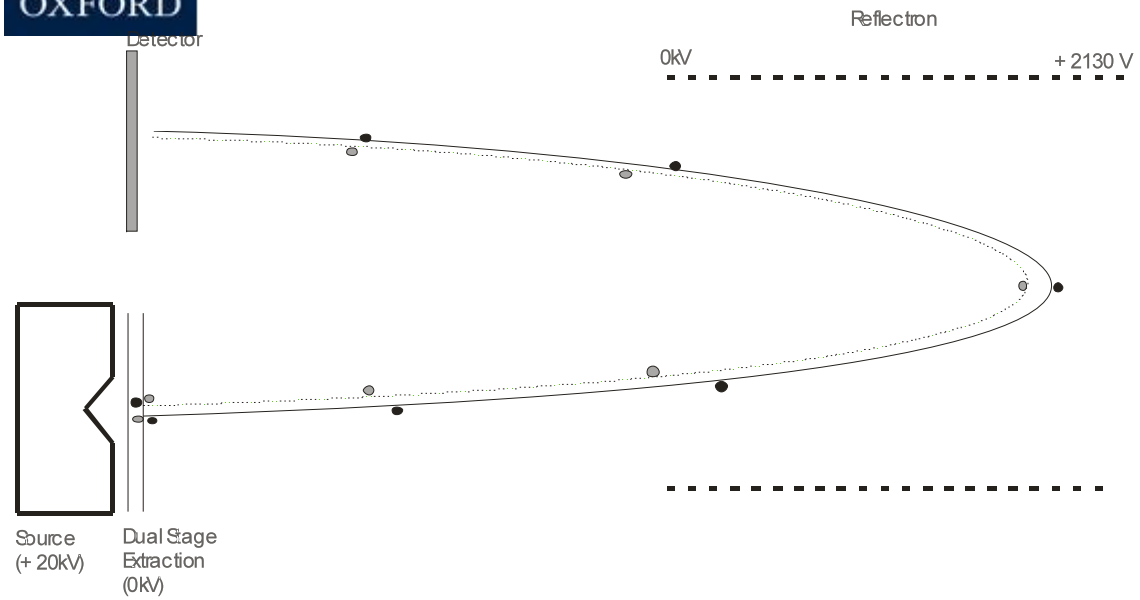
C) Kinetic Energy: Ions of the same mass can form with different KEs.

D) Direction: There is also the problem that when an ion forms, it may do so with an initial velocity in the opposite direction.

Ion formation problem solution: Time Lag Focussing/Delayed extraction



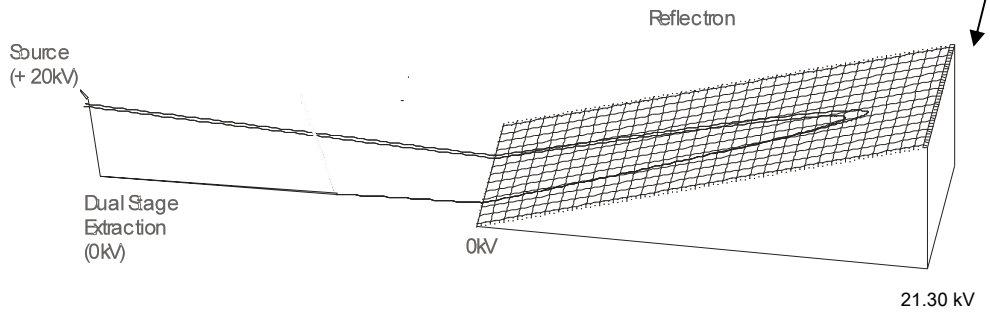
Improving resolution: The reflectron



Reflectron is an electrostatic hill for the ions to try and overcome

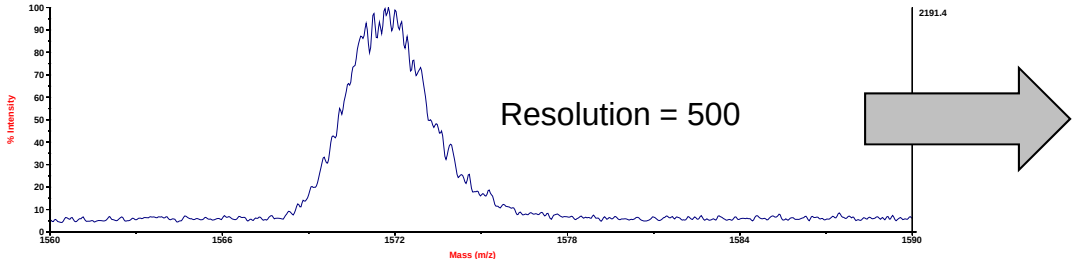
In reality they fail and are turned around

Ions that have a higher velocity will have more kinetic energy and will climb the hill further before being turned around.



SIMION

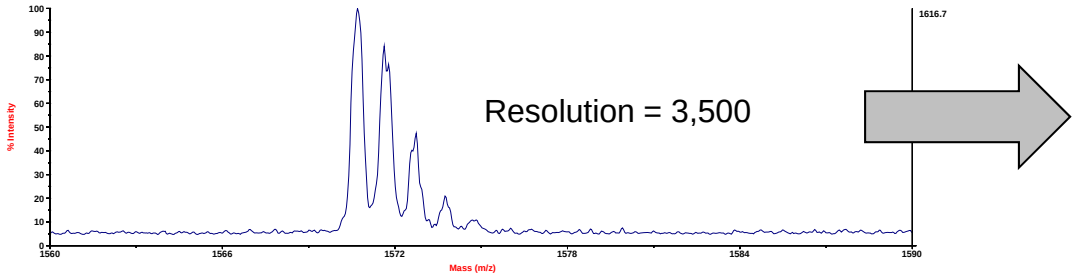
Improvement of resolution.



Continuous extraction

÷ Delayed extraction

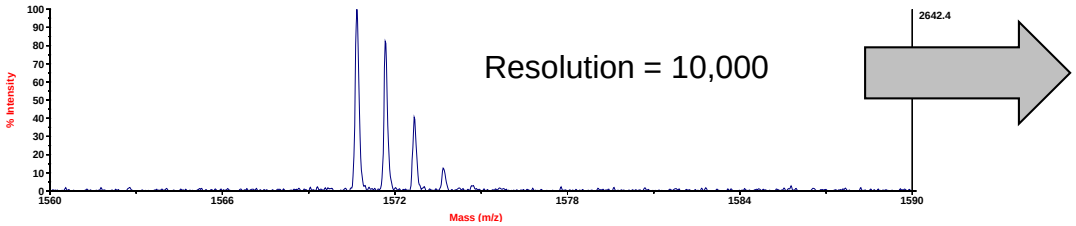
÷ Reflector



Linear mode

+ Delayed extraction

÷ Reflector

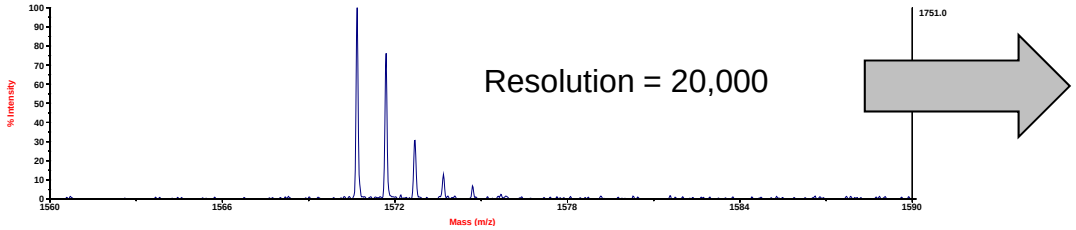


m/z = 1570.68

Reflector mode

÷ Delayed extraction

+ Reflector

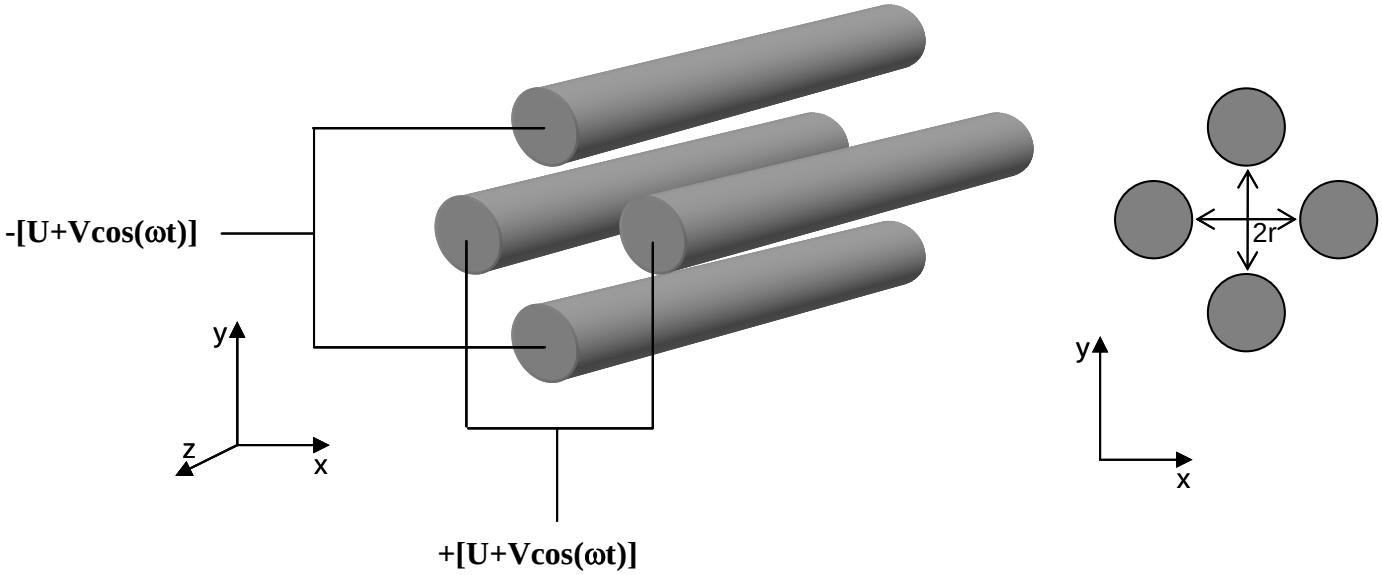


Reflector mode

+ Delayed extraction

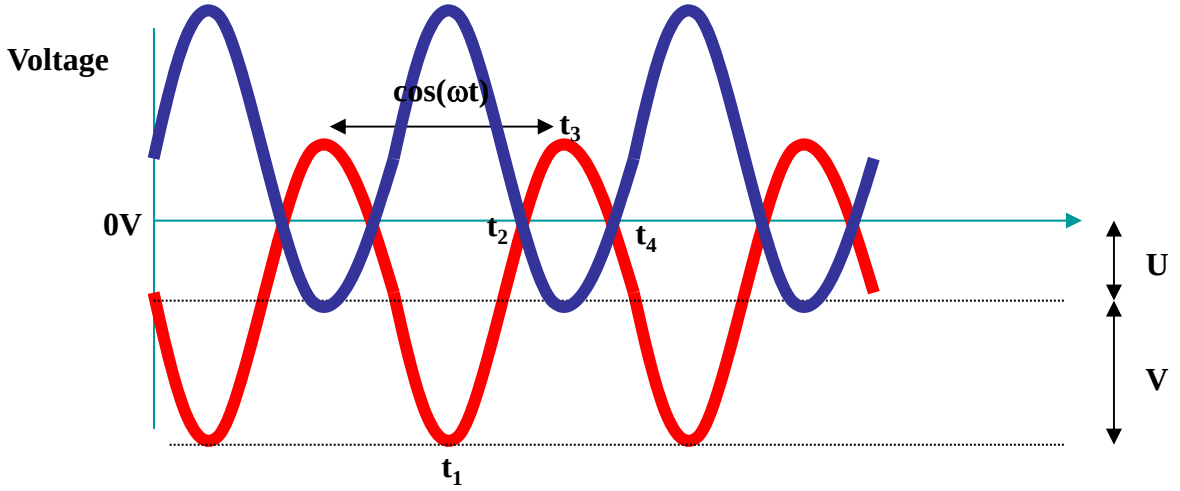
+ Reflector

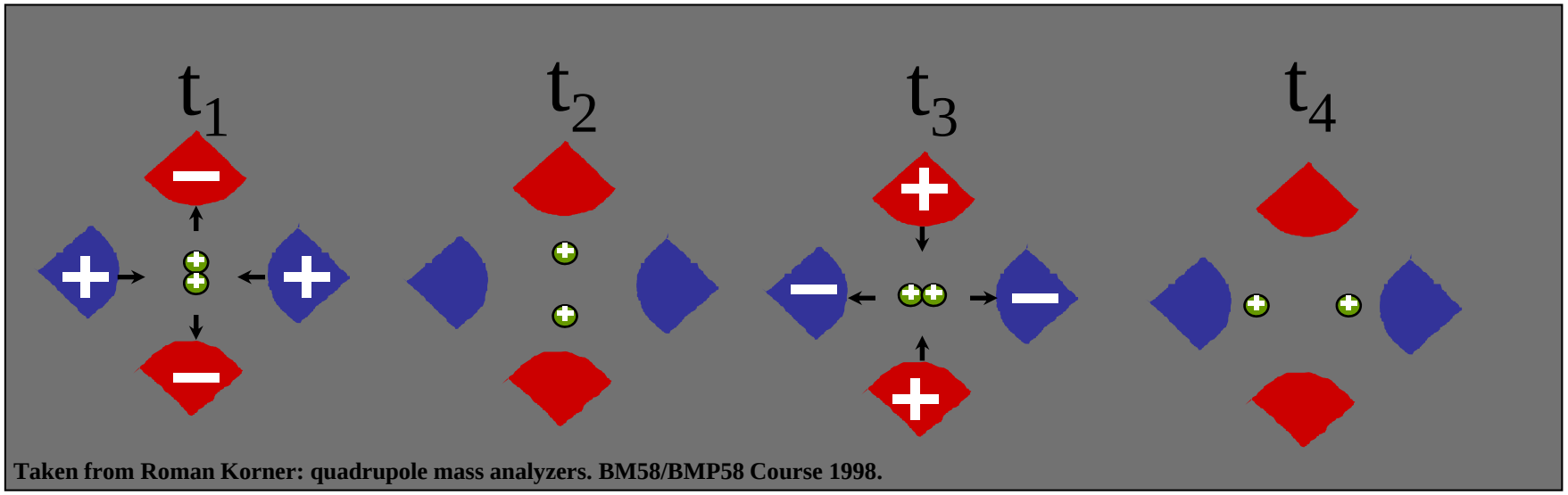
Quadrupole Mass Analyser: Description



- **Consists of Four Metal Rods**
- **Opposite Rods have <u>same</u> voltage and adjacent rods have <u>opposite</u> voltage**
- **The voltage is a combination of a constant potential (U) and an alternating potential (V)**

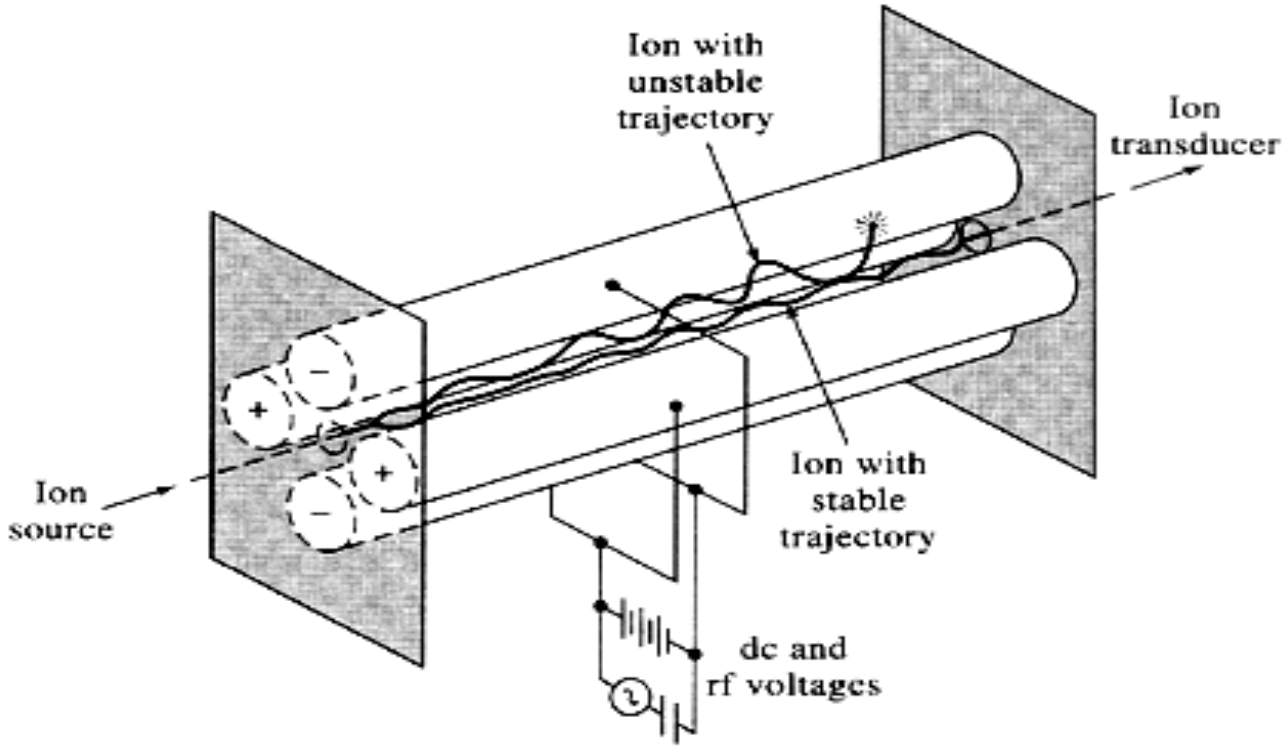
Quadrupole Mass Analyser: Description





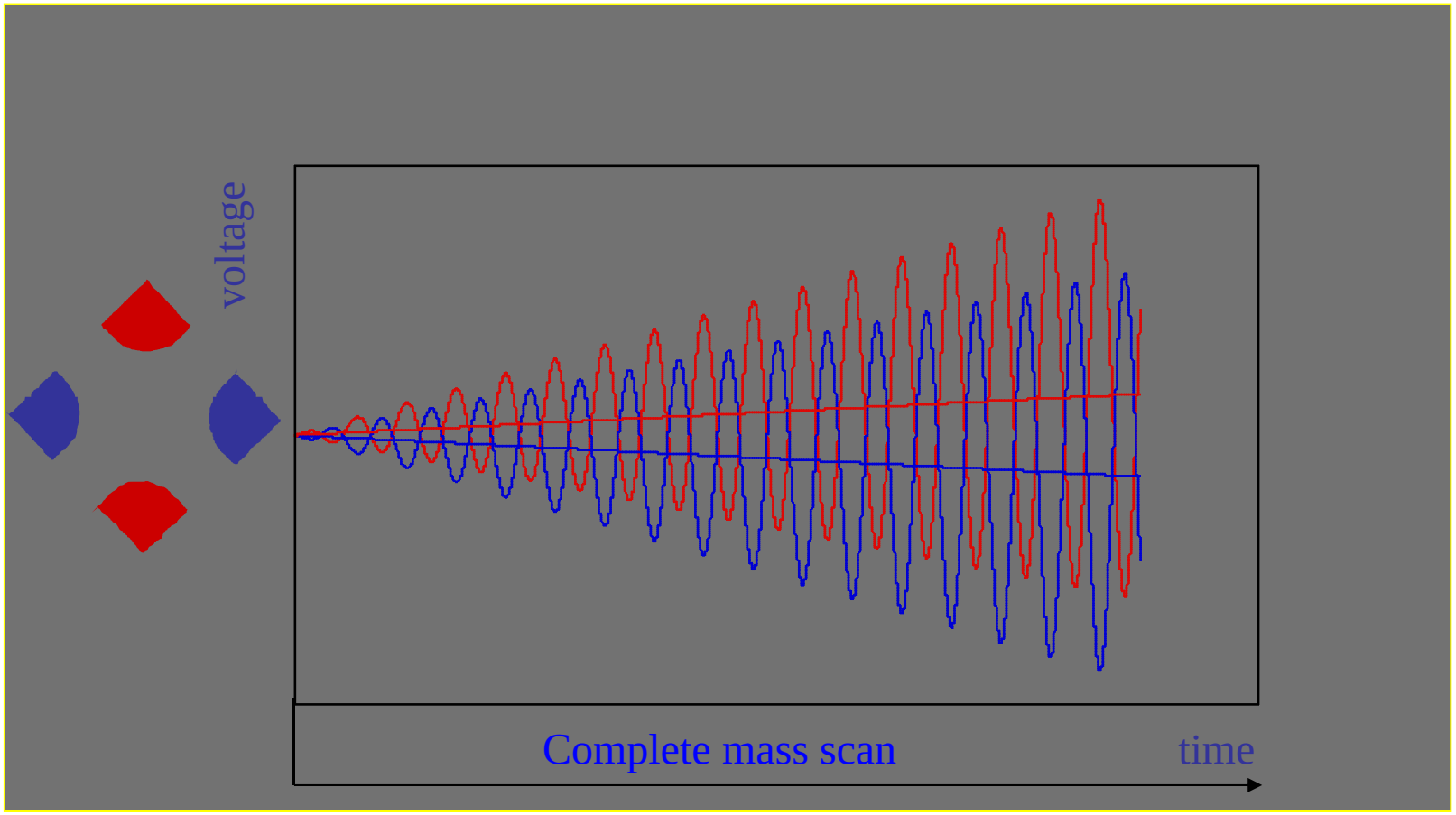
Taken from Roman Korner: quadrupole mass analyzers. BM58/BMP58 Course 1998.

Quadrupole Mass Analyser: Description

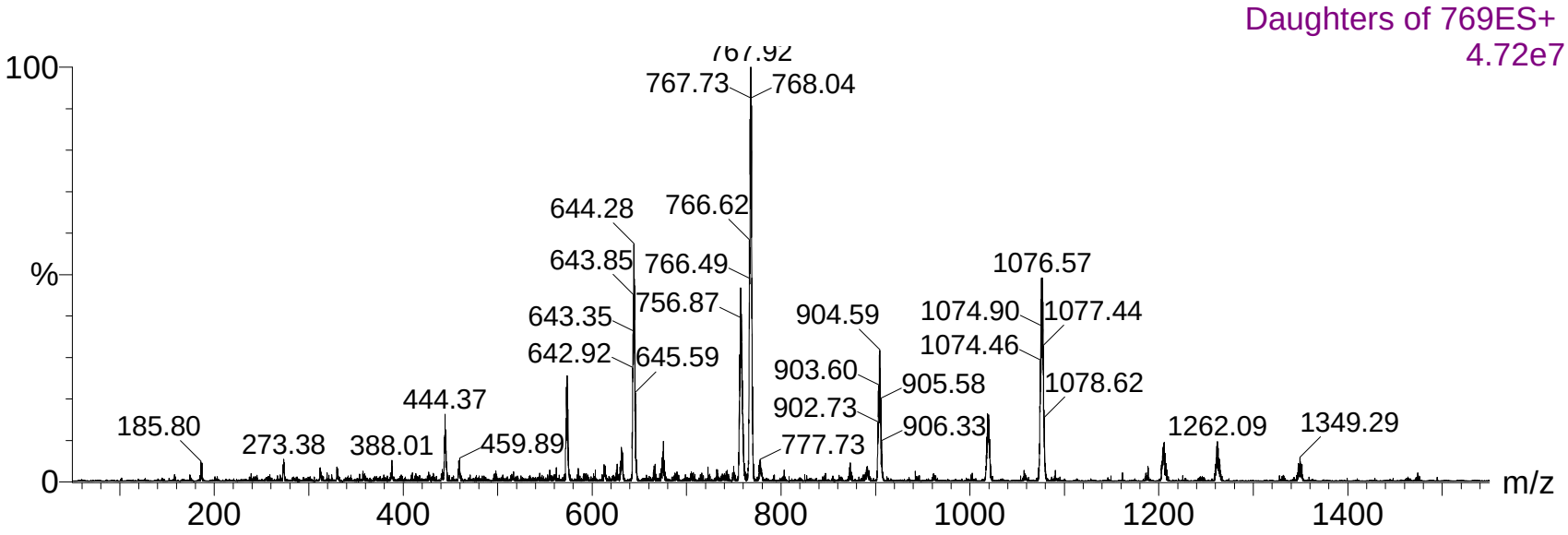


Ions are given a small amount of kinetic energy (1 - 2 eV) to allow them to traverse the quadrupole to the detector (assuming they have stable trajectories)

Quadrupole Mass Analyser: Scanning

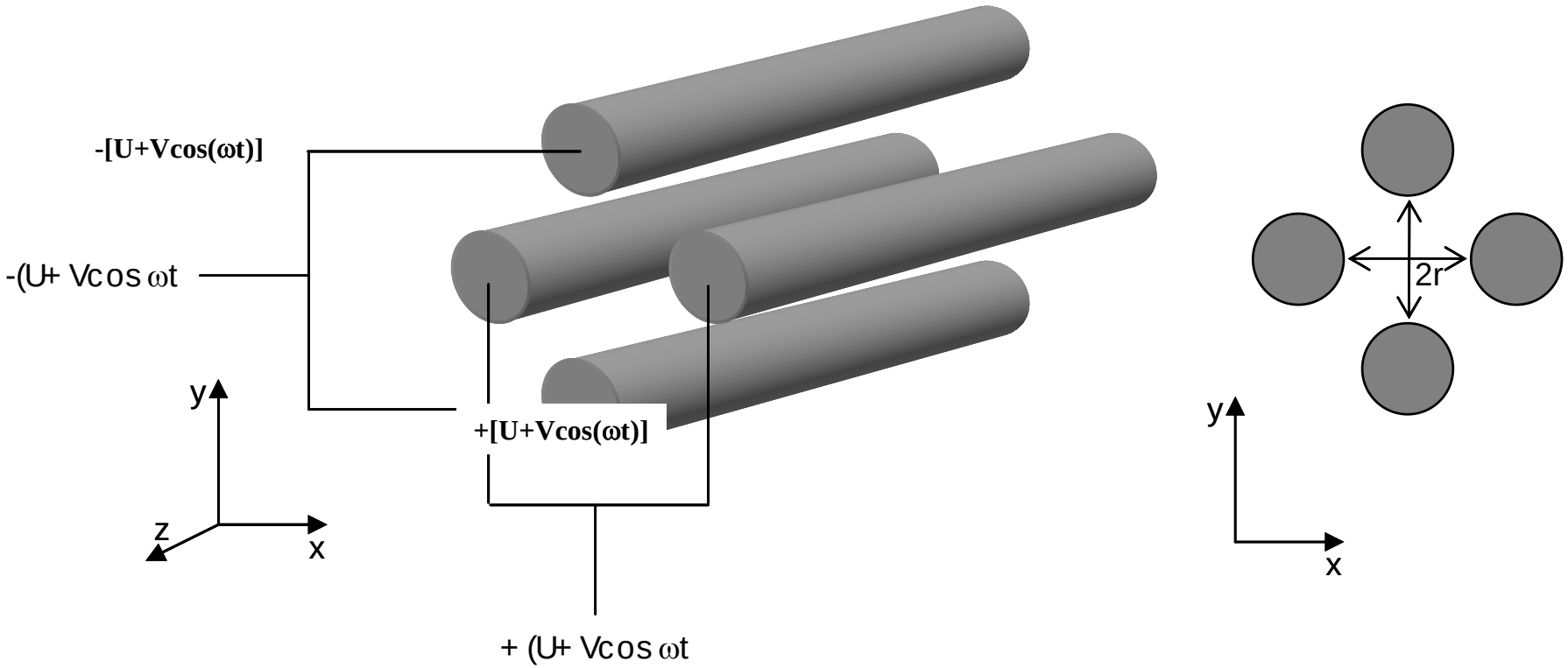


Quadrupole Mass Analyser: Scanning

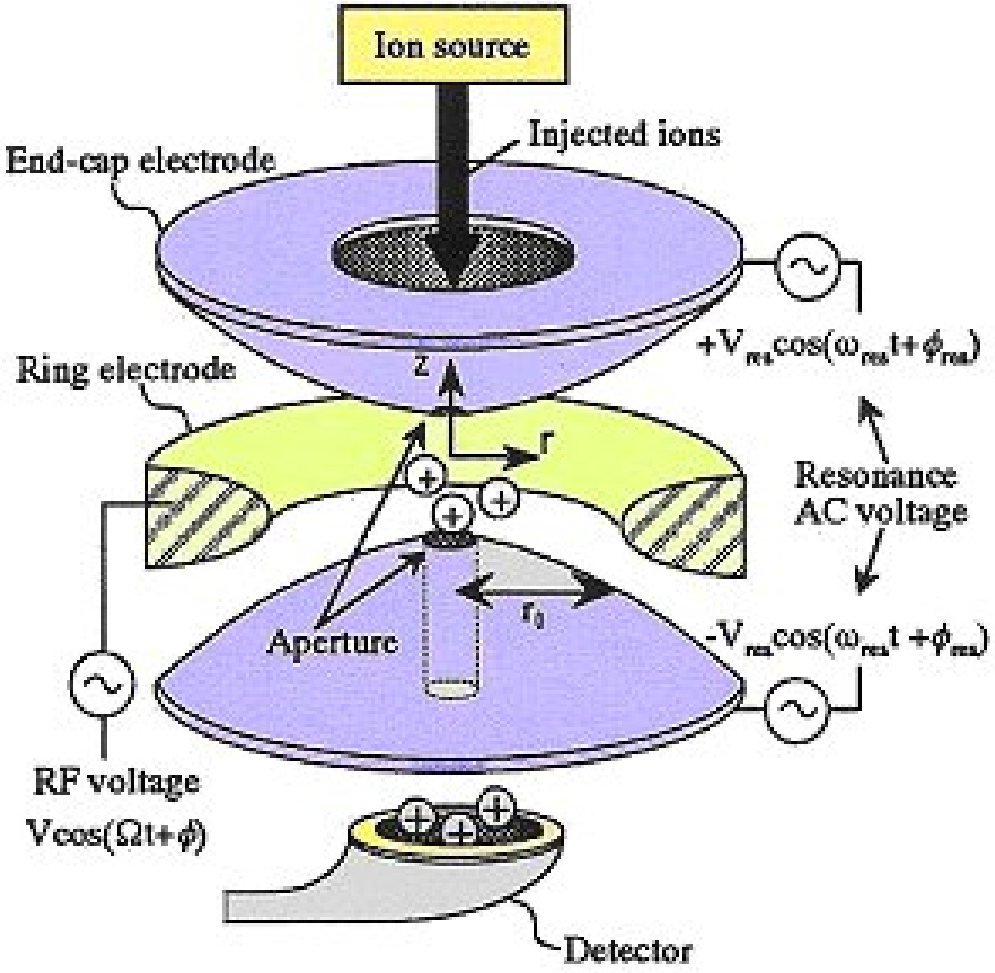


Quadrupole Mass Analyser

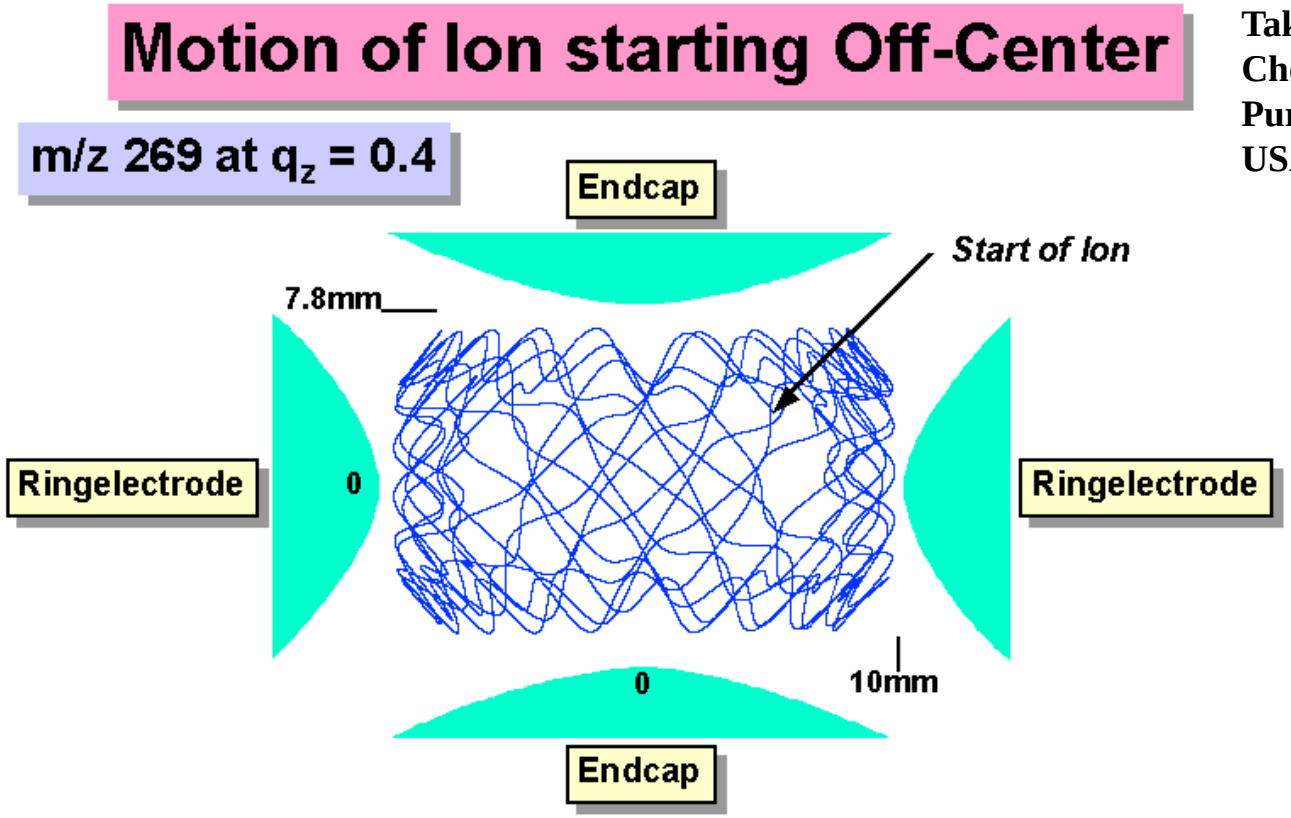
- **Used to be one of the cheapest mass spectrometers on the market**
- **Very good for precursor ion scanning and selective reaction monitoring**


-[U+Vcos(ωt)]
-(U+ Vcos ωt
+[U+Vcos(ωt)]
+ (U+ Vcos ωt
y
z
x
2r
y
x

Quadrupole Ion Traps: Description



Quadrupole Ion Traps: Description



Taken from: Web Site of Chemistry Department Purdue University,Indiana , USA

RF voltage is applied to ring electrode to produce a three-dimensional quadrupole electric field for trapping ions.

Quadrupole Ion Traps

Figure 2. The three electrodes of the quadrupole ion trap shown in open array.

Taken from: Web Site of Chemistry Department. Purdue University, Indiana , USA

2D Ion Trap Mass Analyzer – 'LTQ'

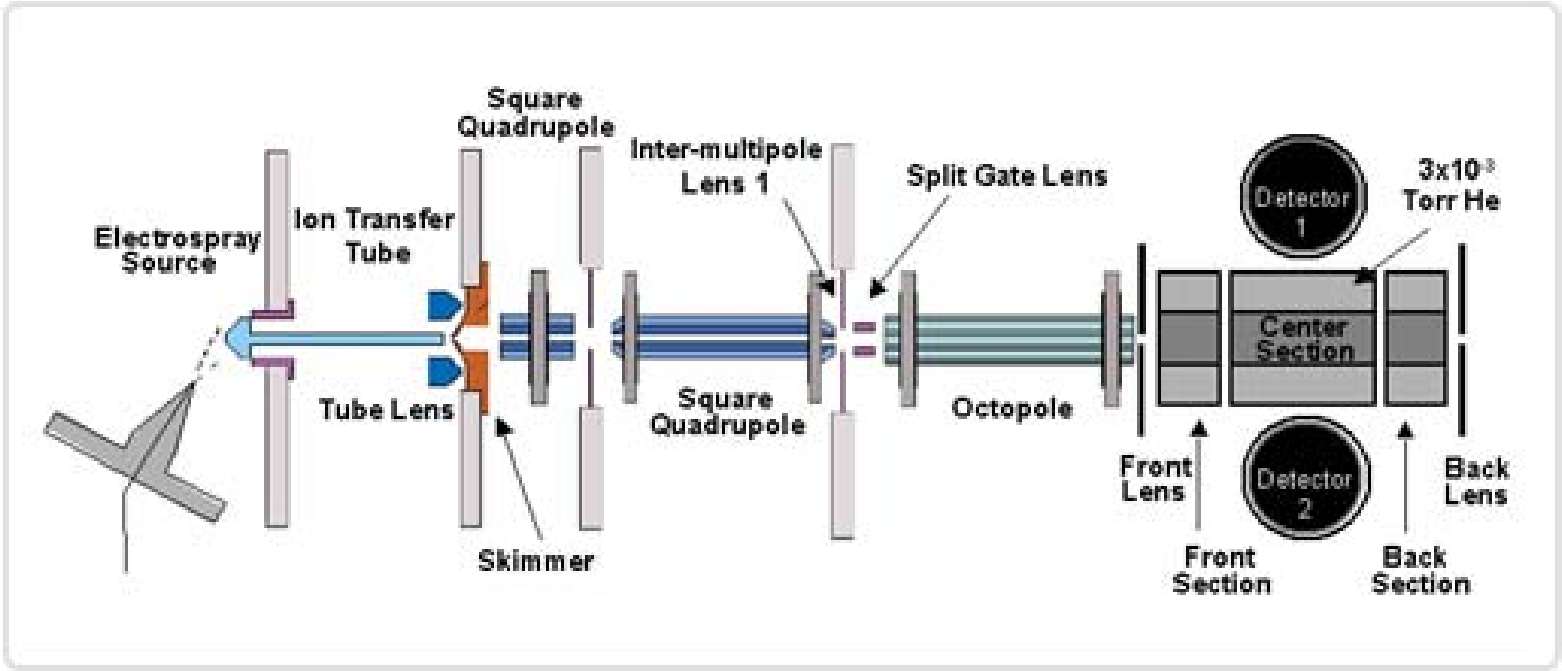
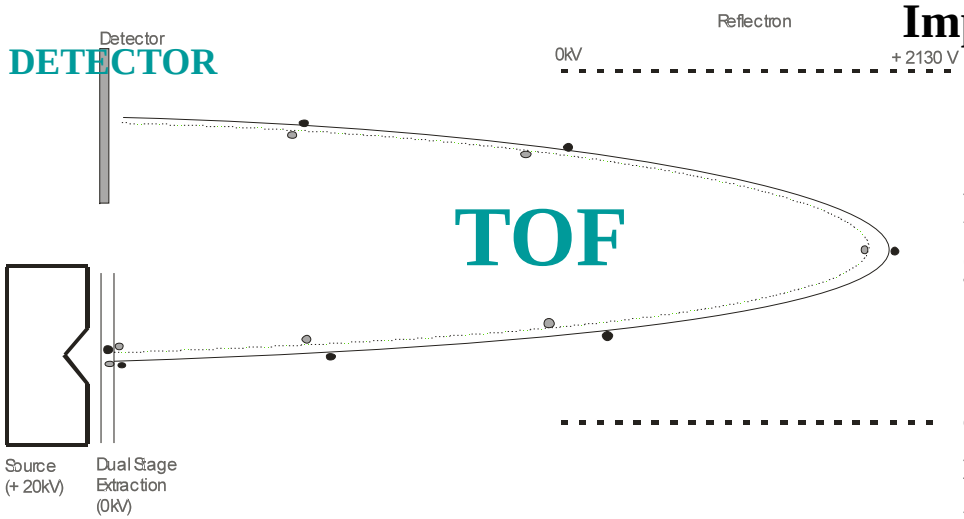
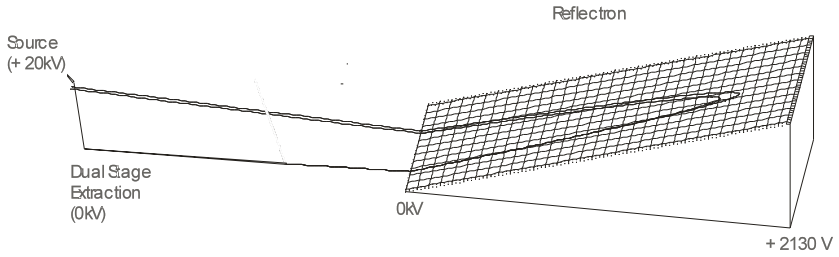


그림 4 . Two-Dimensional Linear Quadrupole Ion Trap Instrument

A Little Bit About Orthogonal ToFs



SOURCE

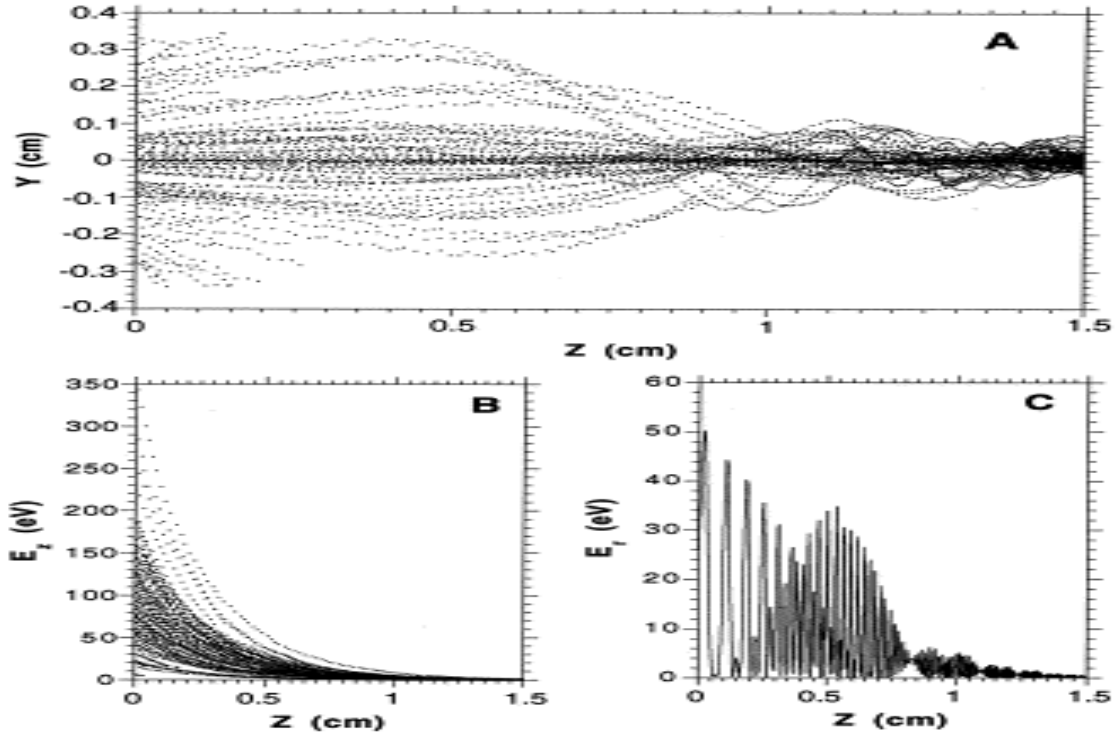


SIMION

Important Factors in on-axis MALDI-ToF:

- **Resolution and mass accuracy depend on knowing exact distance, time and energies given to the ions**
- **Ions form in the same direction as ToF analyser and so the source conditions are important factors in mass accuracy and resolution e.g.**
- **Starting position of ions**
- **Starting kinetic energy and direction of ions**

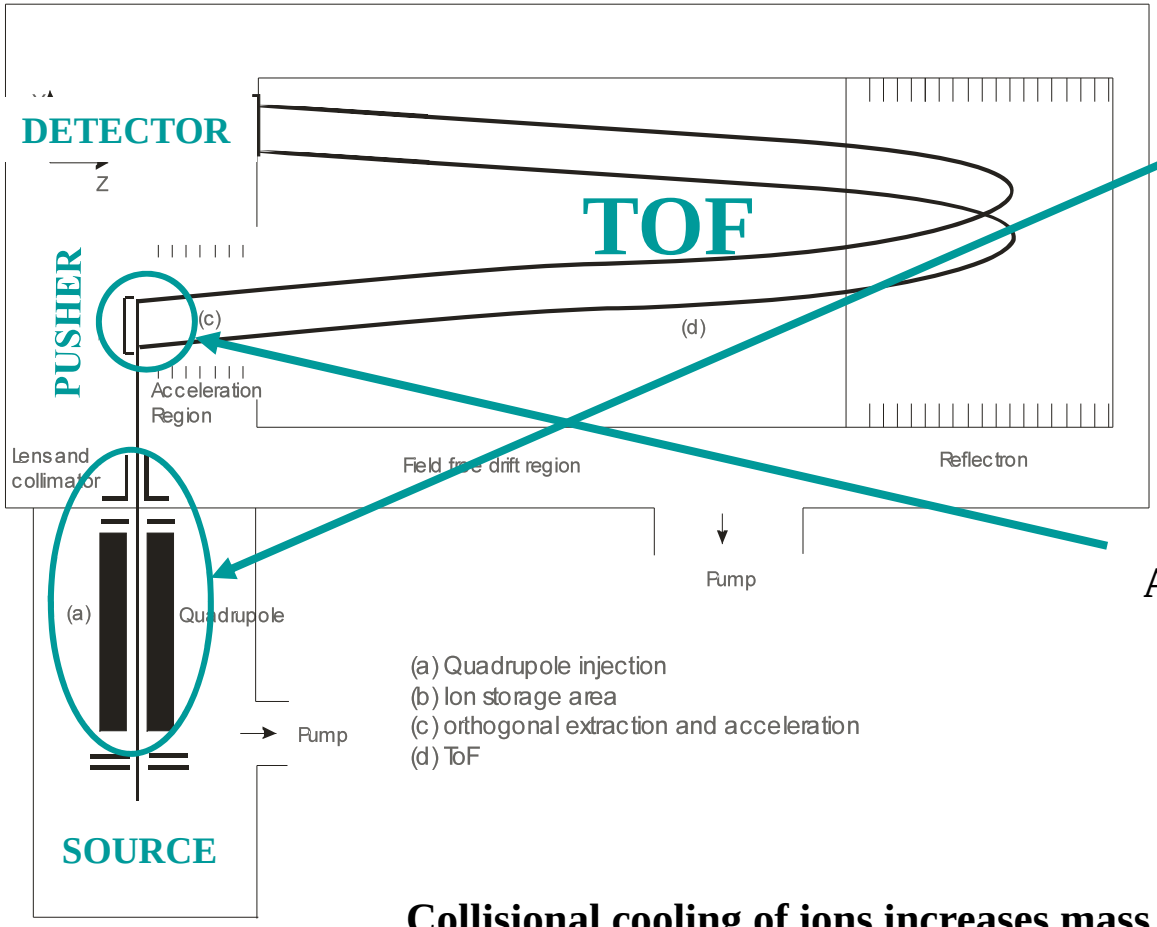
A Little Bit About Orthogonal ToFs



Collisional Cooling of ions (usually in a quadrupole) before they enter the ToF region helps further improves ToF sensitivity and Resolution.

Cooling allows better positioning of ion packet into pusher region of the ToF

A Little Bit About Orthogonal ToFs



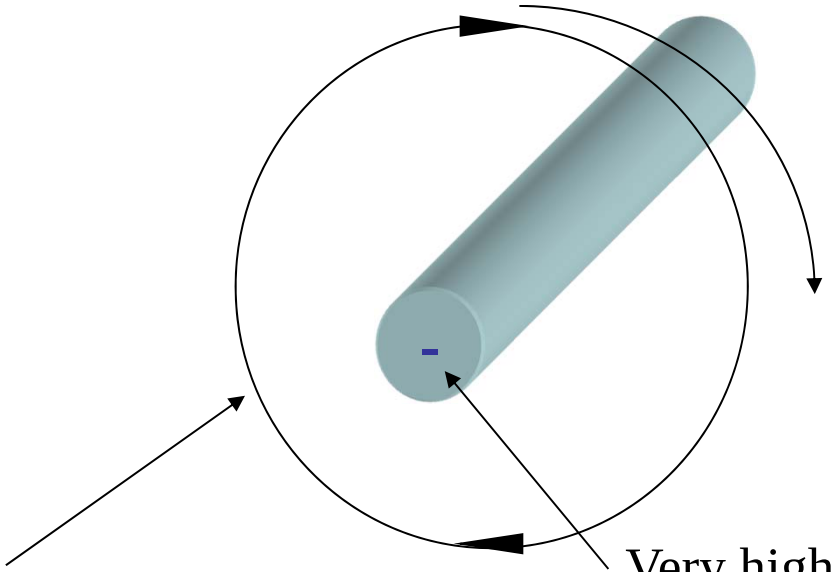
Reducing the ions velocity here

Allows better focusing of ions here

Collisional cooling of ions increases mass accuracy and resolution

The Orbitrap

Positive ion moving very fast



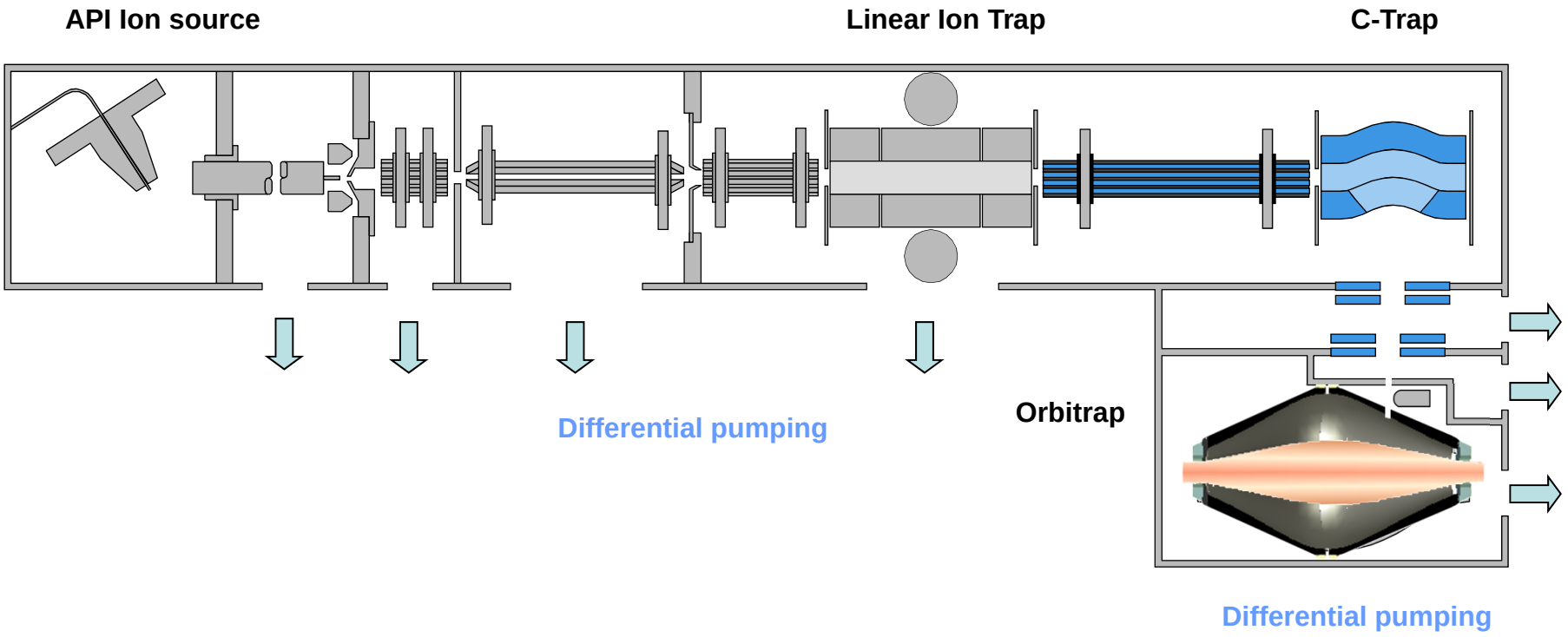
Very high negative potential

Attraction of rod is high,
Velocity of ion is high..
Ion makes an orbit around rod

Similar to a satellite around the earth

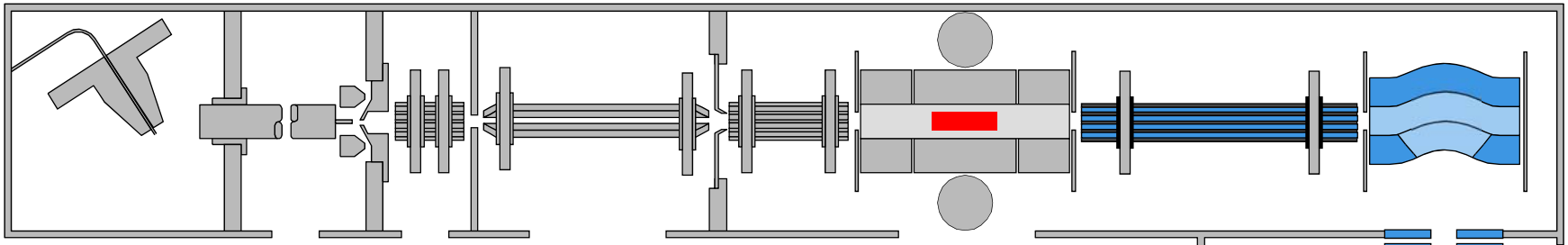
LTQ Orbitrap Hybrid Mass Spectrometer

Finnigan LTQ™ Linear Ion Trap

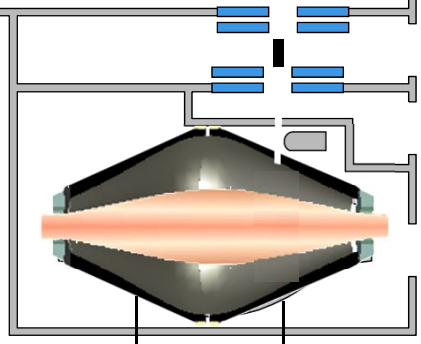


LTQ Orbitrap Operation Principle

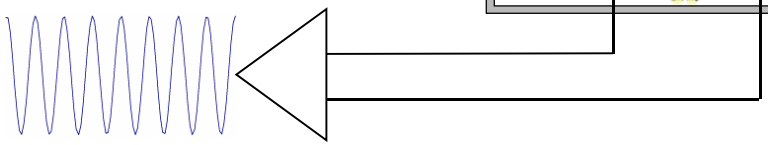
1. Ions are stored in the Linear Trap
2. …. are axially ejected
3. …. and trapped in the C-trap
4. …. they are squeezed into a small cloud and injected into the Orbitrap
5. …. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation



The oscillating ions induce an image current into the two outer halves of the orbitrap, which can be detected using a differential amplifier



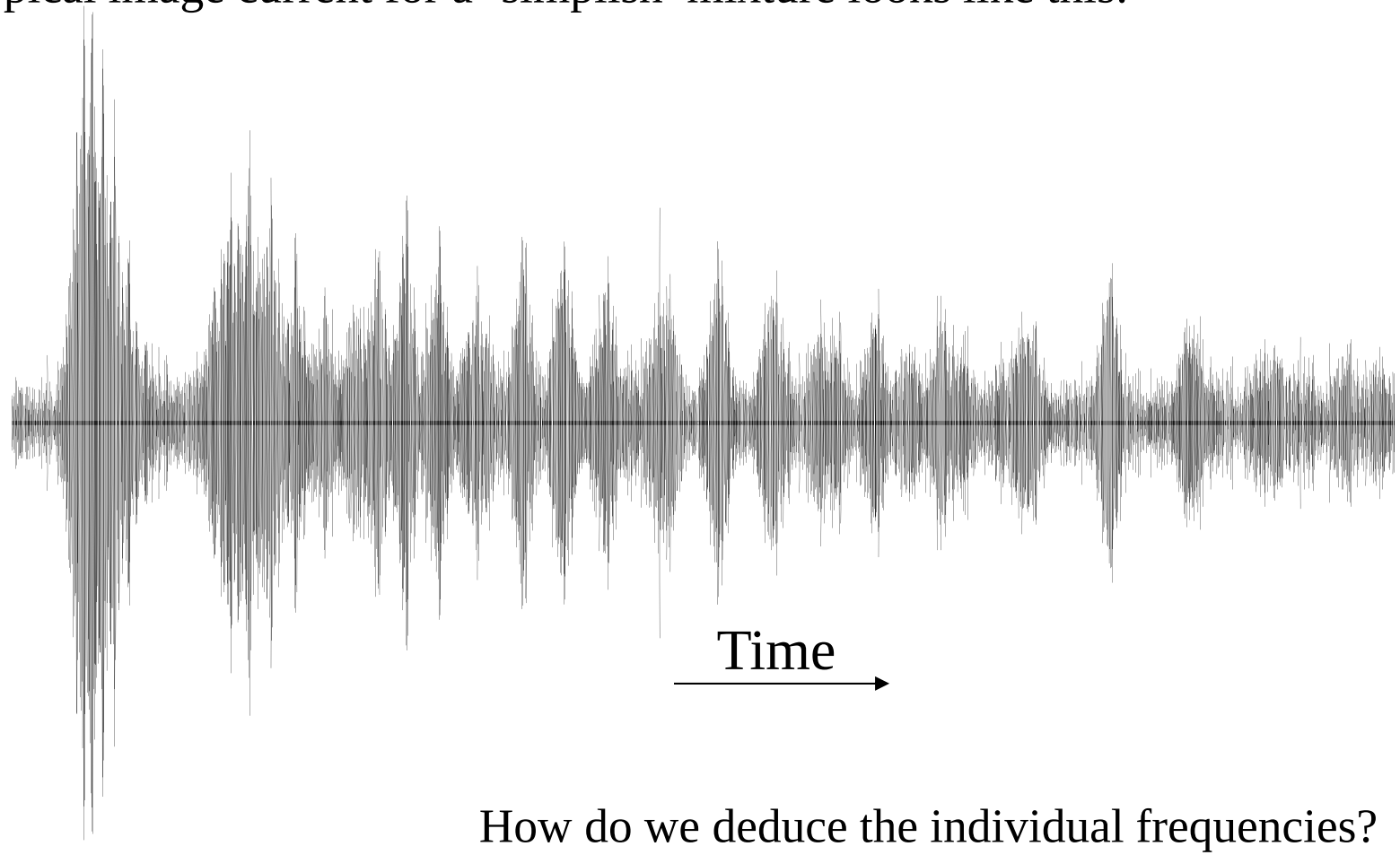
Ions of only one mass generate a sine wave signal



Detection:Fourier Transform

Samples are typically not just 1 ion but several and on top there will be ions with several different m/z values.

Typical image current for a ‘simplish’ mixture looks like this:

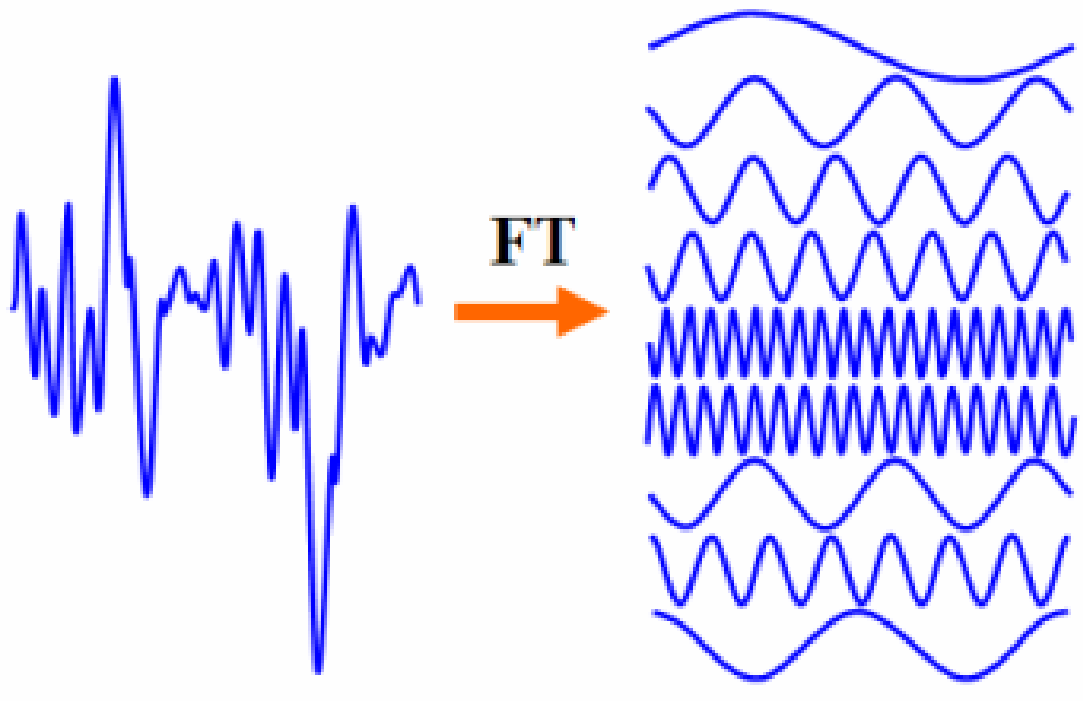


How do we deduce the individual frequencies?

Detection:Fourier Transform

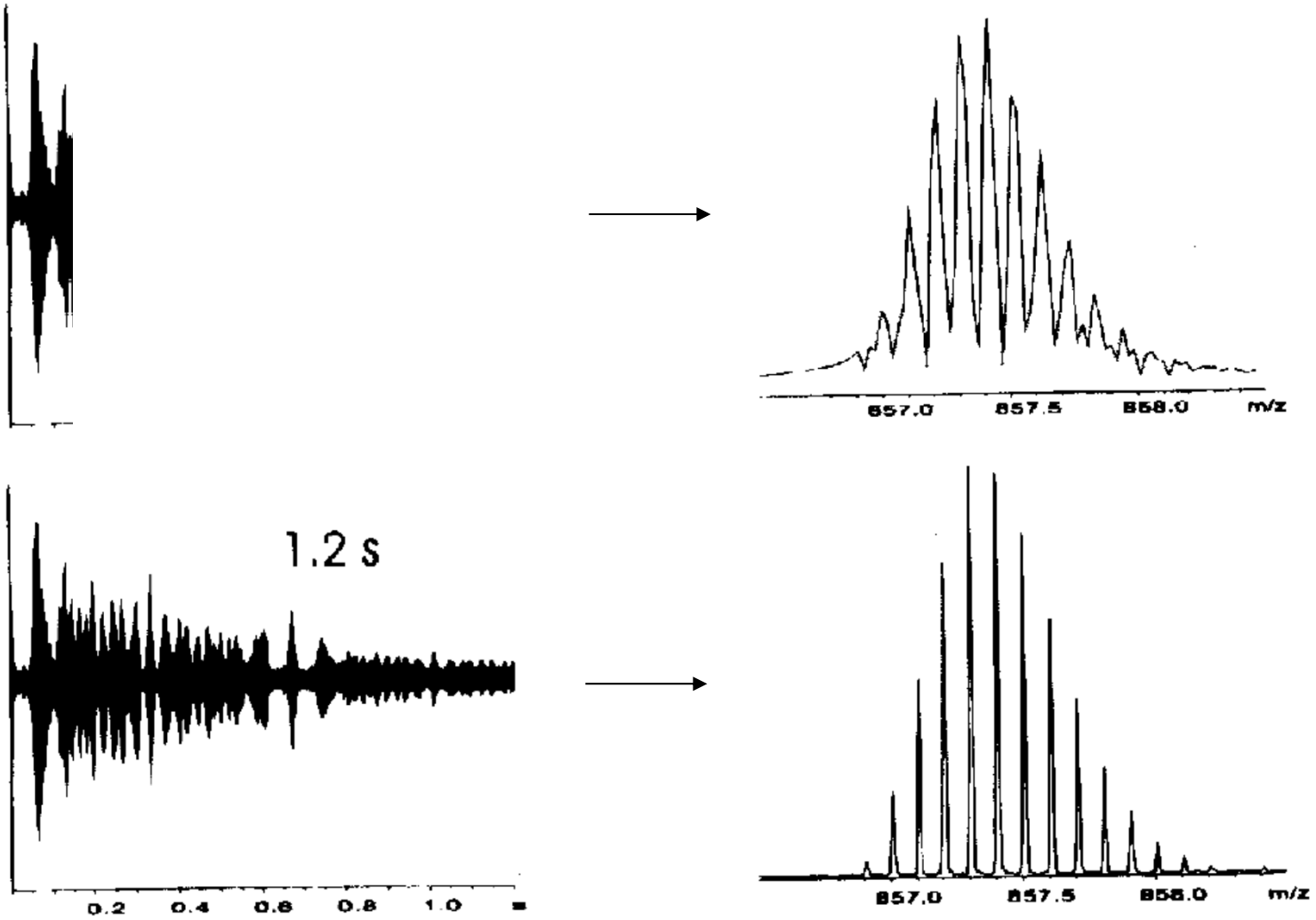
Luckily waves do not affect each other and so within the ‘messy’ image current, the waves are present intact.

Fourier transform is a mathmatical technique which can deduce the frquencies present.



Resolution

The longer you measure, the better the spectrum….more datapoints for final deduction



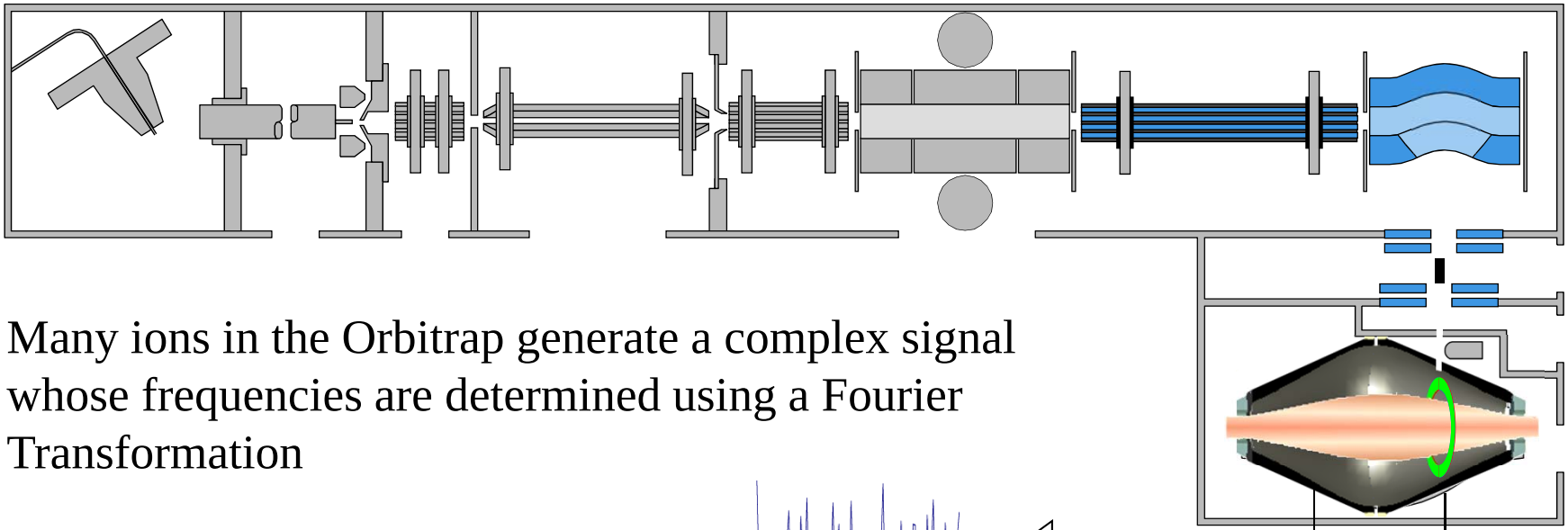
Frequencies and Masses

The axial oscillation frequency follows the formula $\omega = \sqrt{\frac{k}{m/z}}$

Where w = oscillation frequency

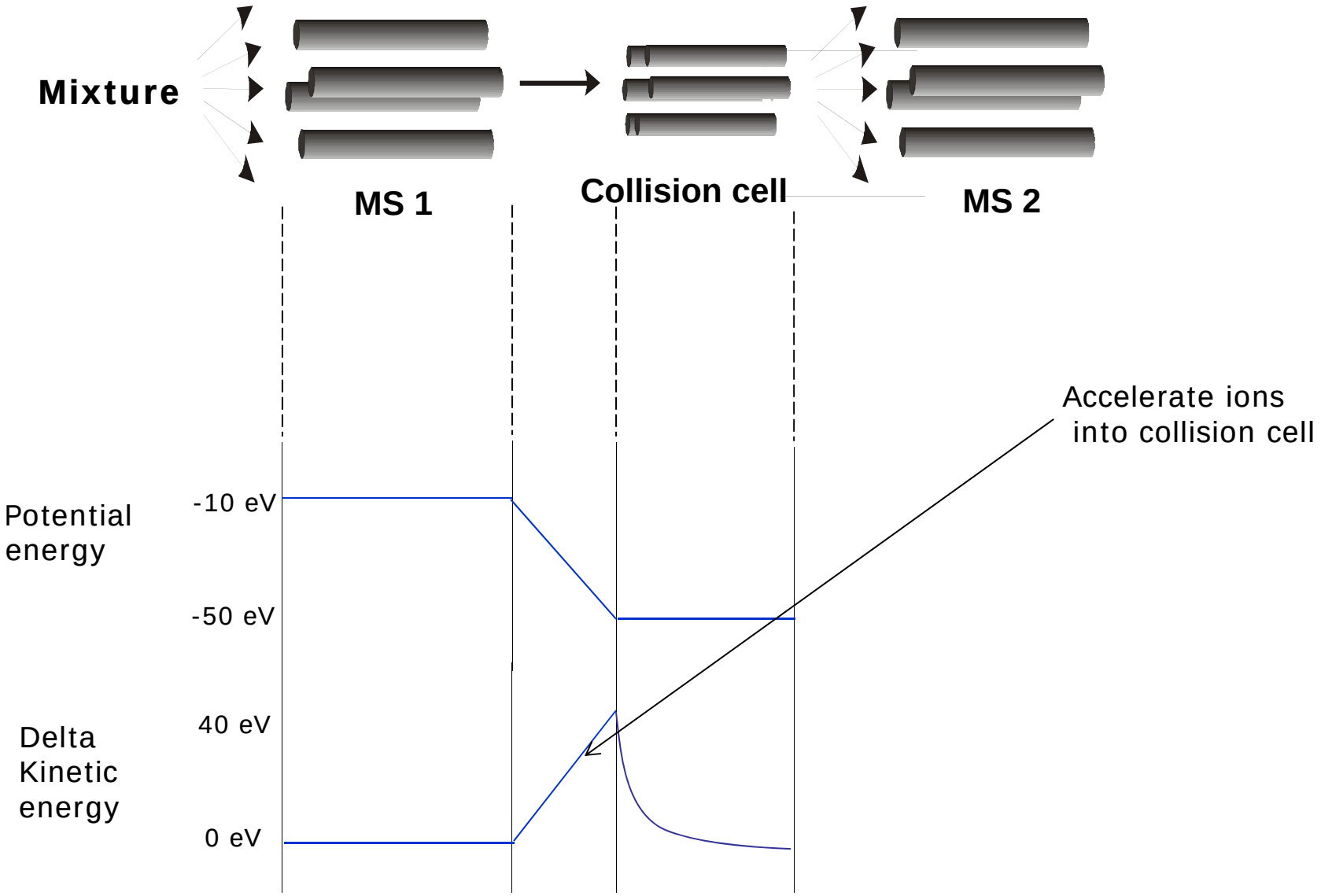
k = instrumental constant

m/z = …. well, we have seen this before

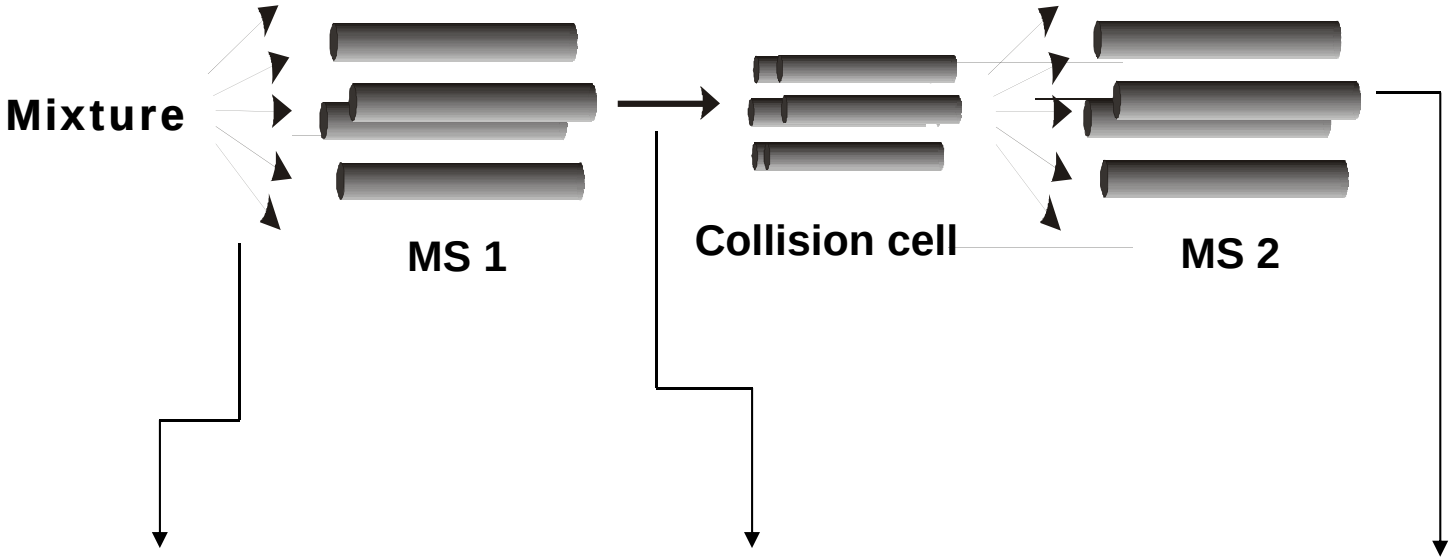


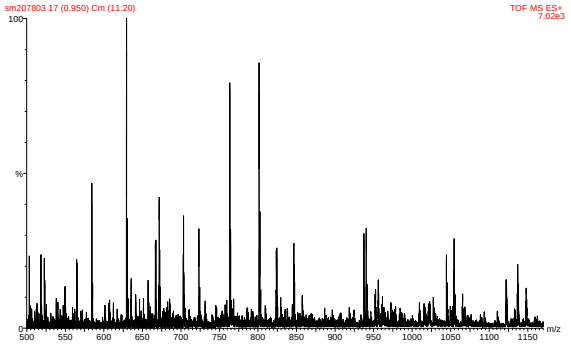
Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

Collision induced dissociation

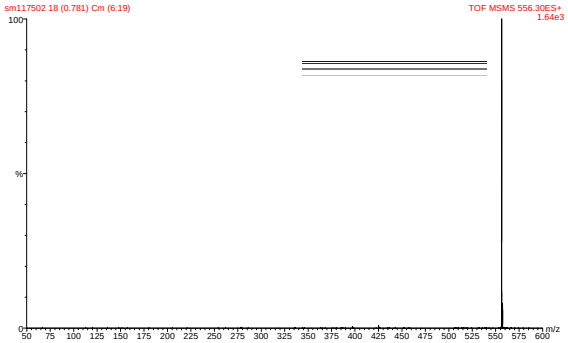


Collision induced dissociation

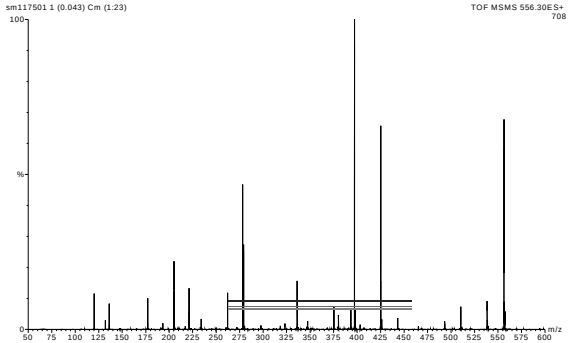




A mass spectrum of a mixture



Isolation of an ion
In our case a protonated peptide

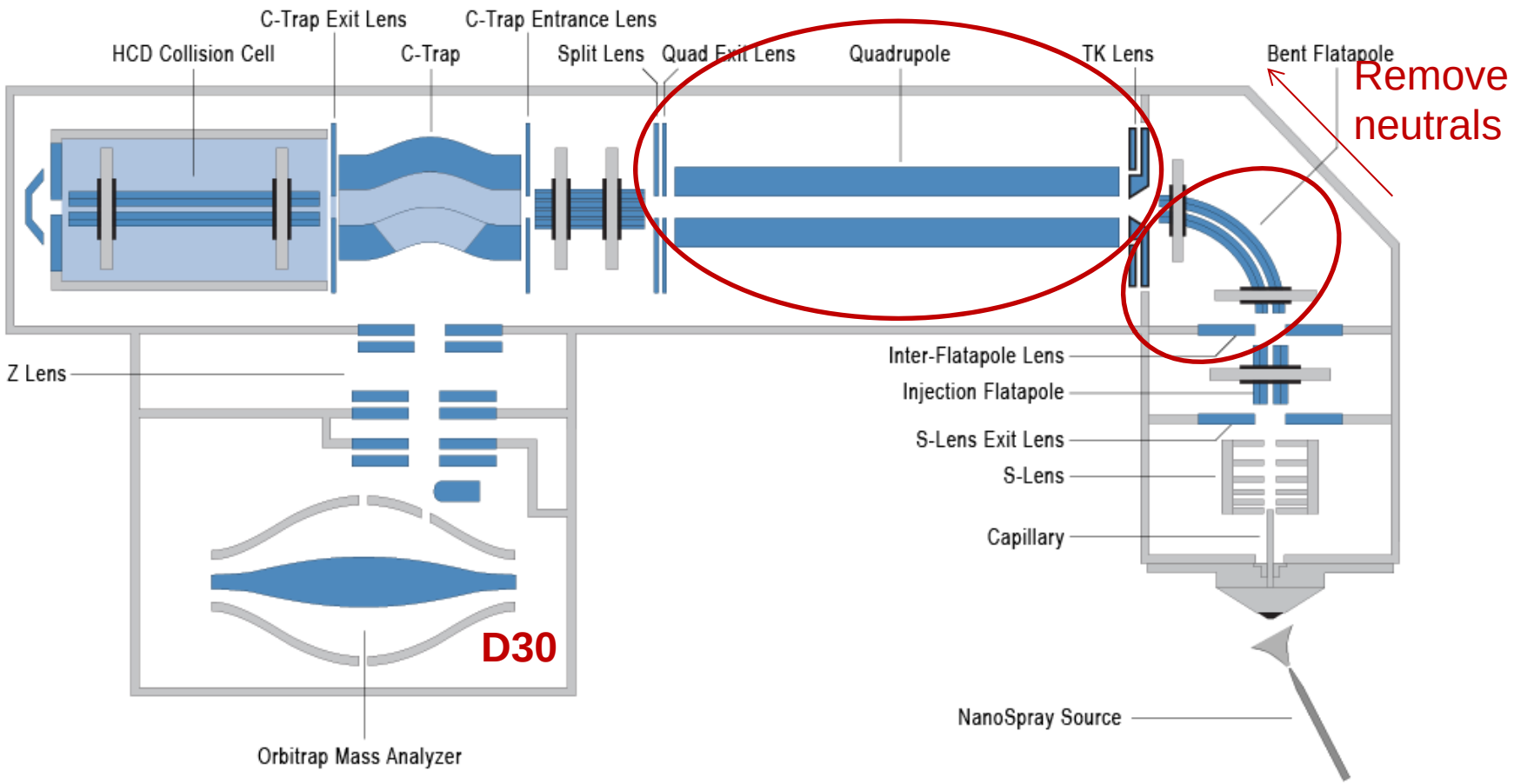


A mass spectrum of the fragments
Produced by the ion

Q Exactive

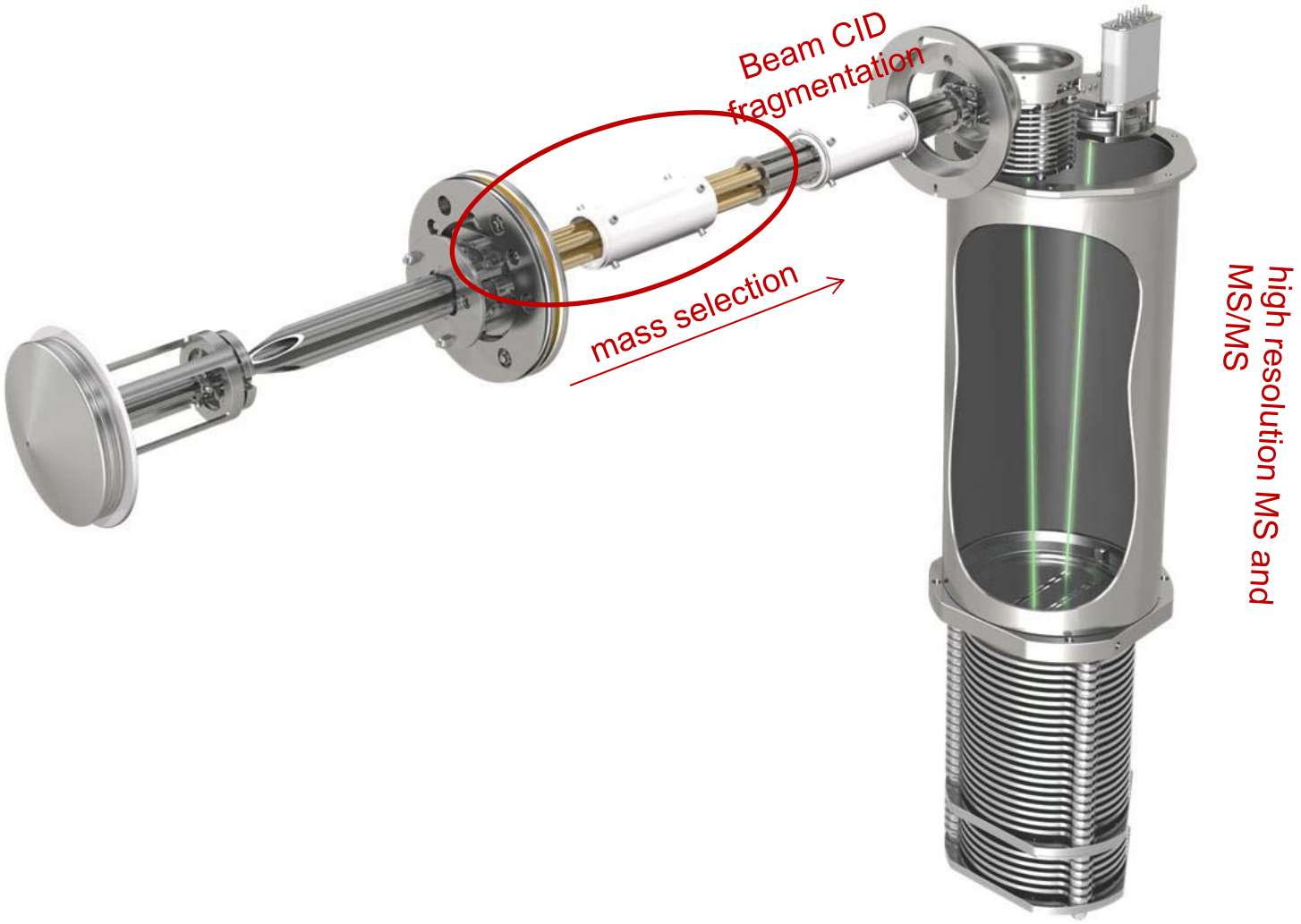
Beam CID fragmentation

mass selection

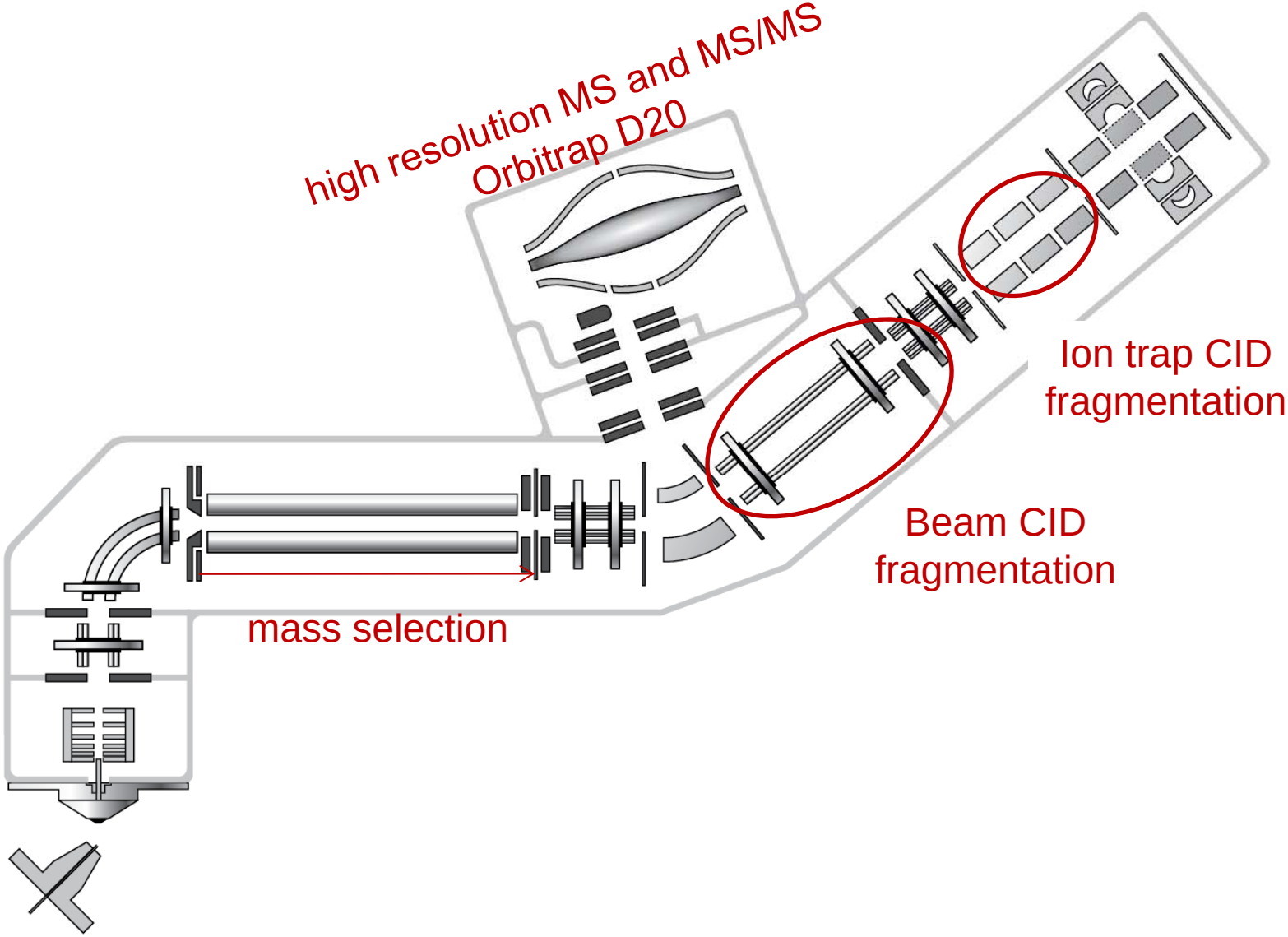


high resolution MS and MS/MS

Q- ToFs


Beam CID
fragmentation
mass selection
high resolution MS and
MS/MS

Orbitrap Fusion CID



Redesigned instrument capable of: 15 MSMS HCD (orbitrap) per second
20 MSMS CID (ion trap) per second

Mass Analyzers

- Quadrupole Analyzer (Q)
  - Low (1 amu) resolution, fast, (relatively) cheap
- Ion Trap Mass Analyzer (QIT)
  - Fair resolution, all-in-one mass analyzer
- Time-of-Flight Analyzer (TOF)
  - Good resolution, exact mass, fast, no upper m/z limit, costly
- Orbitraps(Orbi)
  - High resolution, exact mass, costly