Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS)

The first steps along the way to FT-ICR-MS date back to the 1930s, when Ernest Lawrence, at the University of California, Berkeley, developed the theory of cyclotron resonance, i.e. the acceleration of charged particles inside a cyclotron, that had been invented by himself in 1924.

Other milestones in the FT-ICR-MS history are:

✓ 1950, Sommer et al.: incorporation of ICR principle into a mass spectrometer, the Omegatron

✓ 1978, Comisarow and Marshall: adaptation of Fourier Transform methods to ICR spectrometry and development of the first FT-MS instrument
ICR cell

The heart of a FT-ICR-MS instrumentation is the ICR cell, an ion trap located within a spatially uniform, static magnetic field of intensity $B$. Two geometries have been commonly explored for ICR cells, cubic and cylindrical open-ended:

The arrow indicates the direction and verse of the magnetic field. Ions can enter the ICR cells either through a hole in a cube face or through one of the open surfaces in the cylindrical cell.
The cubic ICR cell actually derives from the spatial arrangement of six metal plates, that can be divided into three groups:

- **trapping plates**: perpendicular to the magnetic field; one of them has a hole to allow ion injection into the cell;

- **excitation plates**: parallel to the B vector plane and normal to the trapping plates; a RF Voltage is applied to these plate during ICR operation;

- **detector plates**: normal to the other plate couples; they collect the time dependent signal from which a mass spectrum is subsequently obtained.
In the **cylindrical ICR cell**:

- the **trapping plates** correspond to the two metal cylinders at the ends;

- the **excitation and detector plates** are four identical cylindrical sectors, arranged in the center section in an alternate configuration:

The shape and dimension of the **cylindrical cell** make it more suitable to fit into the bore of a **superconducting magnet**, while the **cubic cell** is better matched with the narrow gap between the pole caps of an **electromagnet**.
Cyclotronic ion motion

When entering the ICR cell, if no electric field is applied a ions is subject only to the Lorentz force due to the magnetic field:

\[ \mathbf{F} = q(\mathbf{v} \times \mathbf{B}) \]

The force is a vector normal both to the magnetic field and to the ion velocity vector, thus the ion moves along a circular trajectory.

Actually, the same phenomenon occurs on any charged particle, including electrons, as shown in the picture on the right, referred to the motion of electrons in a gas-filled bulb put in a magnetic field. The electron trajectory is visible due to excitation of the gas:
If no external perturbation occurs, the cyclotronic motion is periodic and characterised by a **cyclotron frequency**, depending on B and on the m/z ratio:

\[ f_c = \frac{zB}{2\pi m} \]

For m/z=1000 and B = 7 Tesla, \( f_c = 104.7 \text{ kHz} \). The cyclotron frequencies are usually in the kHz-MHz range.

The m/z ratio of an ion can then be measured if the frequency of its cyclotronic motion is known.

A peculiarity of the above relationship is that **the m/z ratio of an ion is not influenced by its initial velocity**, a unique feature of ICR-MS.

**Ion kinetic energy spreading cannot influence mass resolution in this case**; this explains why ICR-MS can reach ultra-high resolving powers (up to R = 1000000).
For a given m/z ratio, the radius of the cyclotron orbit scales directly with the ion velocity or with the square root of kinetic energy. If typical kinetic energies are considered, cyclotron radii in the order of 100 μm can be calculated.

The angular frequency of cyclotronic motion can be easily calculated by \( f_c \) and then related to the velocity, \( v \), and radius, \( r \):

\[
\omega_c = 2\pi f_c = \frac{zB}{m} \quad \omega_c = \frac{v}{r}
\]

The relationships between radius, m/z ratio and velocity or kinetic energy \( E \) can be then easily obtained:

\[
r = \frac{mv}{zB} \quad r = \sqrt{\frac{E}{2m}}/zB
\]

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Trapping motion

Trapping motion is triggered by the application of a small symmetrical positive (negative) voltage to the trapping plates (that are perpendicular to the magnetic field), leading to the storage of positive (negative) ions within the ICR cell:

Ions undergo simple harmonic oscillation between the trapping plates along the magnetic field axis.
Magnetron motion

The combination of the magnetic and electric fields creates a three-dimensional ion trap, allowing ions to be stored in the ICR cell for seconds, minutes or even hours, i.e. times many orders of magnitude longer than typical residence times of most other mass spectrometers.

Although it would seem that the magnetic and electric fields operate independently, their combination introduces a third fundamental ion motion in the ICR cell, the magnetron motion.

In order to understand magnetron motion, the actual electric potential in the cell has to be considered. Indeed, while the trapping plates are subject to the trapping potential, $V_t$, the detection and excitation plates are grounded:

The potential at the center of the cell is then given by the equation:

$$\frac{(2V_t + 4 \times 0)}{6} = \frac{V_t}{3}$$
If a section of the ICR cell, normal to the z axis, is considered, the plot of the electric potential in the corresponding x-y plane is:

The potential surface is radially repulsive, thus the field acts to drive ions away from the center of the analysed cell, although it keeps them close to the central x-y section.
While being pushed outwards in the central x-y plane, the ion is subject to the Lorentz force, due to the magnetic field.

Indeed, any radial ion motion (red continuous vector) can be divided into two components, along x and y directions (dotted vectors), that are normal (not parallel) to the magnetic field.

The resulting effect is the magnetron motion: a precession of the cyclotron motion of an ion around the center of the cell.

The reported simulation refers to the following parameters:

4.4 cm cubic cell, m/z = 500,

\[ V_t = 3 \text{ V}, \; B = 1 \text{ Tesla} \]
The magnetron radius (red) of an ion (in the figure it is purposely emphasized with respect to the cyclotronic motion radius, the blue one) depends on its initial displacement from the z axis when the ion itself is injected or created in the ICR cell.

The magnetron motion frequency is a function of the trapping potential, V, the distance, a, between the trapping plates and the geometry of the ICR cell (\( \alpha = 1.39 \) for a cubic cell):

\[ f_m = \frac{\alpha V}{\pi a^2 B} \]

The magnetron frequency is usually in the order of 1-100 Hz, thus much lower than the cyclotron one. Moreover, it is independent on the m/z ratio.
The three motions present in a ICR cell can be schematically represented by the following picture:
Effect of collisions on magnetron motion

When ions are detected the pressure inside a ICR cell is about $10^{-9}$ Torr or lower, so that collisions do not happen frequently.

However, during some FT-MS experiments the pressure needs to be raised, sometimes by several orders of magnitude.

Ion-neutral collisions cause a decrease in ion kinetic energy/velocity.

The cyclotron radius ($r_c = \frac{mv}{zB}$) is then rapidly reduced; contemporarily, ions drift towards the cell edge and are finally neutralised by hitting the plates (collisionally mediated radial diffusion):

$P = 0.01$ Torr
Events sequence in a FT-ICR-MS measurement

Differently from most other mass spectrometers, in a FT-ICR-MS instrument ionization, mass analysis and ion detection occur in the same space (the ICR cell) but spread out in time.

A typical experimental sequence consists in four main events:

**Quench**: the cell is emptied by any ions deriving from previous experiments by applying antisymmetric voltages to the trapping plates. In a few ms the ions are ejected axially.

**Ionize**: ionization can occur inside the ICR cell (i.e. by electron ionization). Ions generated outside the cell can be guided to the cell through electrostatic or RF ion gates.
Ion excitation / detection

Excitation and detection of ions in a ICR cell are strictly related, the former being mandatory for the latter to be performed.

Ions in the trap are excited by applying a sinusoidal voltage to the excitation plates: when their cyclotron frequency $f_c$ is in resonance with the exciting field frequency they absorb energy and spiral outwards into a larger cyclotron orbit:

Ions having the same m/z ratio will be excited coherently, i.e. grouped as tightly as they were initially.
When a packet of isobaric positive (negative) ions pass near a detection plate of the ICR cell, due to cyclotronic motion, electrons are attracted from (given to) the plate.

An alternate current, known as image current, is generated into the external circuit connecting the detection plates and then amplified, digitised and stored for subsequent computer processing.

Its frequency is given by $f_c - f_m$, thus nearly equal to the cyclotronic frequency (since the magnetronic frequency is much lower).

The measurement of the image current frequency can be performed with extreme precision, thus giving FT-ICR-MS the highest accuracy currently achievable in m/z measurements.

Moreover, ion detection is completely not destructive, an almost unique feature between mass analysers (although some experiments show that image currents could be registered also in quadrupolar traps).
Ions with different m/z ratios can be detected simultaneously by FT-ICR-MS if a broad-band excitation is performed.

In this case a frequency synthesizer is programmed to sweep over frequencies from 10 kHz to 1 MHz in a 1 ms period:

each ion population is subject to resonance at a characteristic cyclotron frequency and the image current becomes a composite of sinusoids of different frequencies and amplitudes.

The Fourier transform of the time transient provides frequency components and then the distribution of m/z ratios (by applying a calibration formula derived from the cyclotron frequency equation):
The image current transient and the multi-charge mass spectrum obtained by FT-ICR-MS for ubiquitin (8500 Da) are shown in the following picture:
Resolution in FT-ICR-MS

The maximum resolving power achievable by FT-ICR-MS is expressed by the equation:

\[ RP = \frac{f_c T}{2} = \frac{zBT}{\pi m} \]

where \( T \) is the duration of the time transient.

Note that the RP is proportional to the magnetic field \( B \).

The length of \( T \) can be limited by collisions between ions and neutrals, with loss of coherence.

At \( 10^{-10} \) Torr pressures transients of 60 s can be achieved and \( R > 10^6 \) can be reached.
An astonishing example of the extreme resolving power achievable by FT-ICR-MS is shown in the following spectrum:

It is a detailed view of the region related to nominal mass 35 u, where a positive and a negative $^{35}$Cl ion were detected by switching the ion mode during the scan between the two peaks.

The m/z difference between the two peaks is equivalent to 2 electrons, i.e. 0.00109 u.
MS/MS measurements with FT-ICR-MS

A **typical MS/MS experimental sequence** with FT-ICR-MS can be described as follows:

In this case, after ionization, a **mass selection stage** is required to isolate a specific precursor ion: a suitable **excitation pulse with appropriate frequencies and amplitudes** is applied to the cell to eject unwanted ions.
A very efficient approach to mass selection is the so-called Stored Waveform Inverse Fourier Transform (SWIFT), developed by Marshall et al. in the mid 1980s.

The stages of SWIFT are:

- elaboration of the desired excitation spectrum
- calculation of the excitation waveform by Inverse Fourier Transform (IFT)
- application of the waveform to the ICR cell

In the reported example, also the precursor ion is excited, but with half energy, so that its isolation and fragmentation occur contemporarily.
Quadrupolar excitation and axialization in FT-ICR-MS

In 1991 it was demonstrated that cyclotron and magnetron motions can periodically interconvert if an ion is excited at its cyclotron frequency by a quadrupolar symmetry:

As cyclotron motion is much faster than magnetron one, exposing a precursor ion to quadrupolar excitation in the presence of a collision gas (up to $10^{-5}$ Torr) will result in a more significant damping of the cyclotron motion.

The final effect is ion relaxation to the center of its orbit, a process usually known as axialization.
When axialization is accomplished on a specific ion, the other ones will be subject to typical radial diffusion and will be finally ejected.

Precursor ions are then selected and several ions can be isolated in the same experiment if a composite or a SWIFT waveform is applied for the quadrupolar excitation.

Further applications of axialization are:

- **thermalization of excited state ions** by quadrupolar excitation when they are placed near the cell center;

- **signal-to-noise enhancing by repeated detection** of the same set of ions: in this case quadrupolar excitation is used to return ions to the center after a dipolar excitation/detection stage.

Repeating the procedure several times and averaging the image current transients thus obtained leads to a significant increase of sensitivity. **Attomoles** ($10^{-18}$ moles) sensitivities have been reported for MALDI-FT-ICR-MS adopting this approach.
Translational excitation

After selection, **translational excitation** of the precursor ion is accomplished by enlarging its cyclotron radius and thus **increasing its kinetic energy** $E$, since $r = (E^2 / m)^{1/2} / zB$.

As an example, a mono-charged ion with $m/z$ 1000, excited to a 2 cm radius orbit, at $B = 7$ Tesla, will acquire a kinetic energy of **950 eV**.

Collisional activated dissociation (CAD)

After precursor ion selection and excitation an inert gas is admitted into the ICR cell through a pulsed valve. Ion-atoms (or molecules) collisions occur and product ions are generated by **collisional activated dissociation** (CAD).

The main drawback of this procedure is that **product ions are generated far from the center cell**, which limits the efficiency and resolution for the MS/MS experiment.
Linear Ion Trap – FT-ICR-MS hybrid system

The MS/MS, MS^n capabilities of linear ion trap have been coupled to the extreme mass resolution of a ICR cell to obtain a powerful mass spectrometer (LTQ-FT Ultra, from Thermo Electron):

**ECD**: Electron Capture Dissociation  
**IRMPD**: InfraRed Multi Photon Dissociation
The main **figures of merit** of this LIT-ICR instrument are:

- **mass range**: 50-4000 Da
- **resolution**: 100000 at m/z 400 at 1 s/scan; > 750000 at m/z 400 at slower scan repetition rates (ICR operated in broadband mode)
- **mass accuracy**: < 1.2 ppm RMS error, with external calibration; < 1 ppm RMS error with internal calibration
- **sensitivity**: sub femtomoles (10^{-15} moles) - attomoles (-10^{-18}) for peptides
- **dynamic range**: > 4000
Another very important feature is the possibility of double detection, that can be performed even in a parallel approach:

In the first part of the experiment, a pre-scan is performed with the LT, then ions are partly injected in the ICR trap for high accuracy/resolution MS scan. The remaining ions are subject to parallel MS/MS acquisitions in the LT.
An example of LT-ICR power: discrimination between isobaric compounds (two mutant peptides).

- Angiotensin mutant DRVYVHPF [M+H]$^+$ 1032.5261
- Bradykinin mutant KRPPGFSPF [M+H]$^+$ 1032.5625

$\Delta m = 0.0364$ u