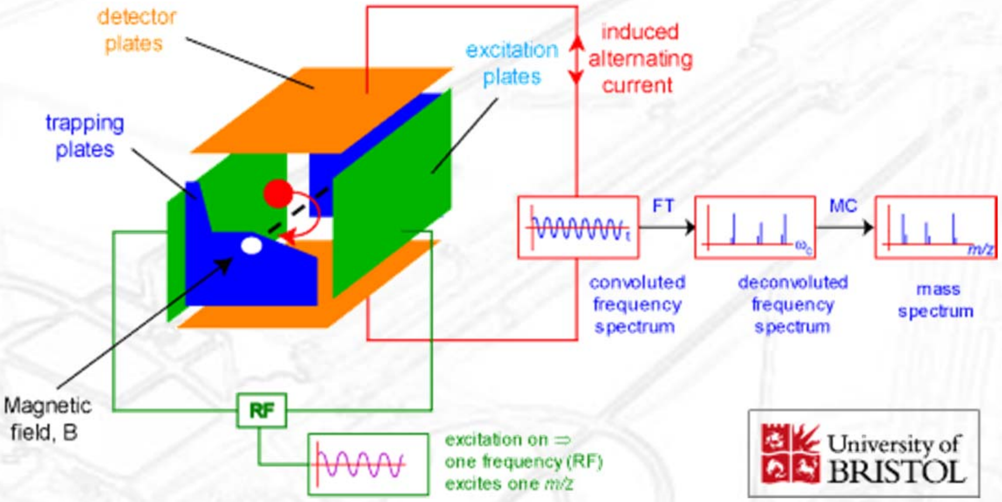
Week 5: Fourier Tranform-based Mass Analyzers: FT-ICR and Orbitrap



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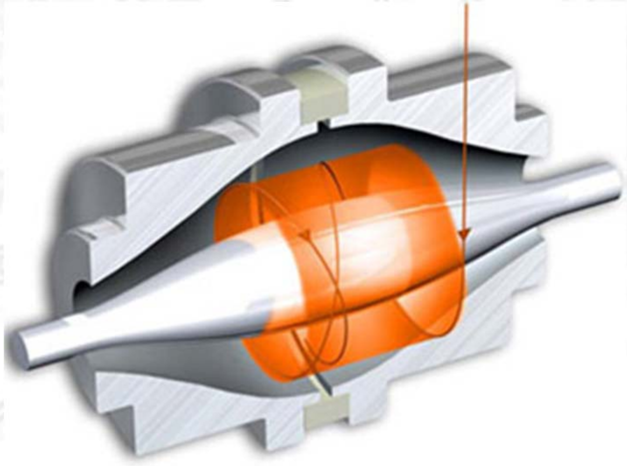
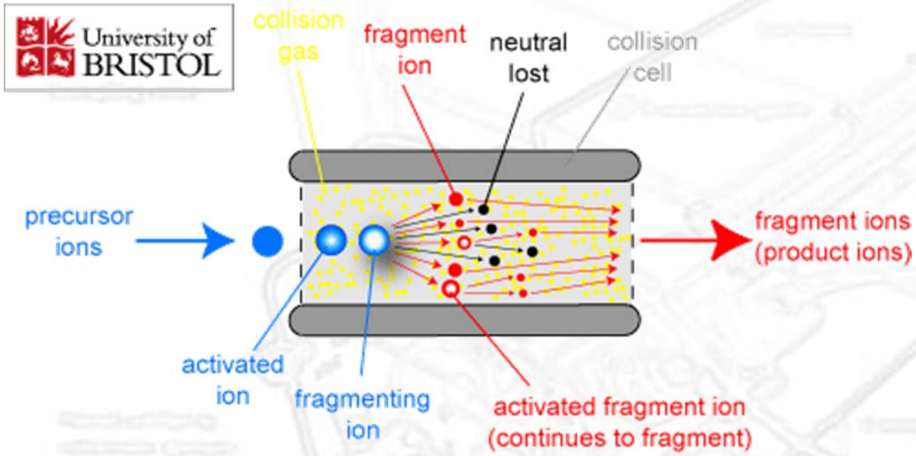
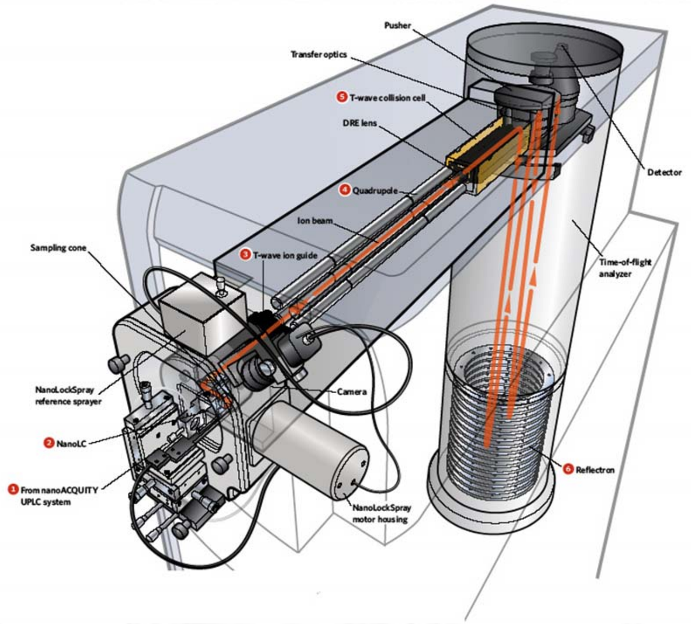


Last Time…

- Mass Analyzers; CAD and TOF mass analyzers:



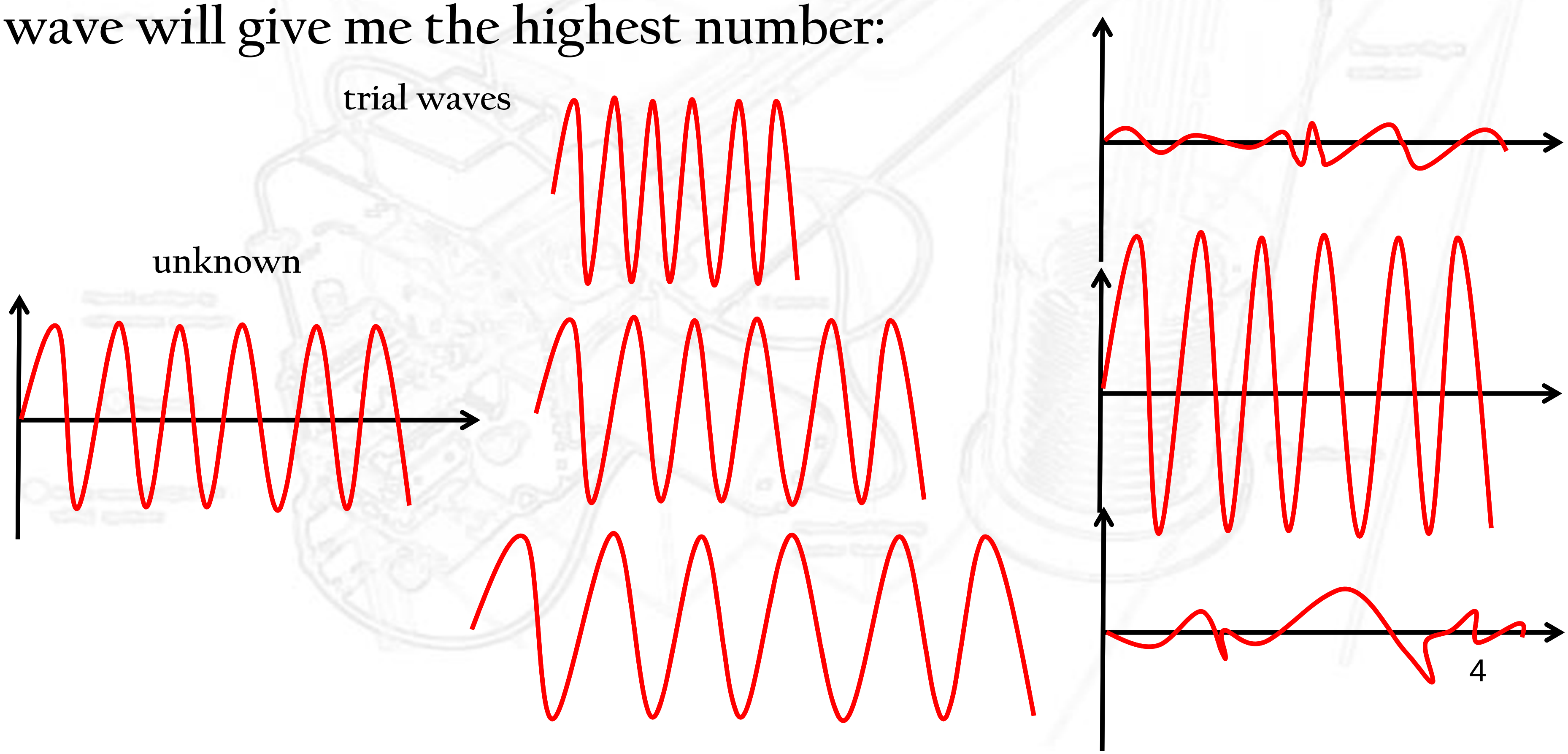


Fourier Transforms

- A ‘transform’ is when you change your analytical ‘space’ without changing the demensionality or scaling.
- For 2D datasets, this means changing the domain of the analysis (almost always the thing on the x-axis).
- We’ve seen this before when we switched from ‘time’ to ‘number of oscillations’ in the Mathieu equations.
- In a fourier transform we go from the ‘time’ domain to the ‘frequency’ domain.

How Does a Fourier Transform Work?

- Fundamentally, an FT is based more or less on constructive and destructive interferance.
- Lets say I have a wave of unknown frequency. If I add a set of ‘trial waves’ to it, the trial that exactly matches the unknown wave will give me the highest number:



Fourier Transform Lineshape

- If FT worked perfectly, we would get a single ‘Dirac peak’ (zero width) at the frequency where the trial wave exactly matches the actual wave.

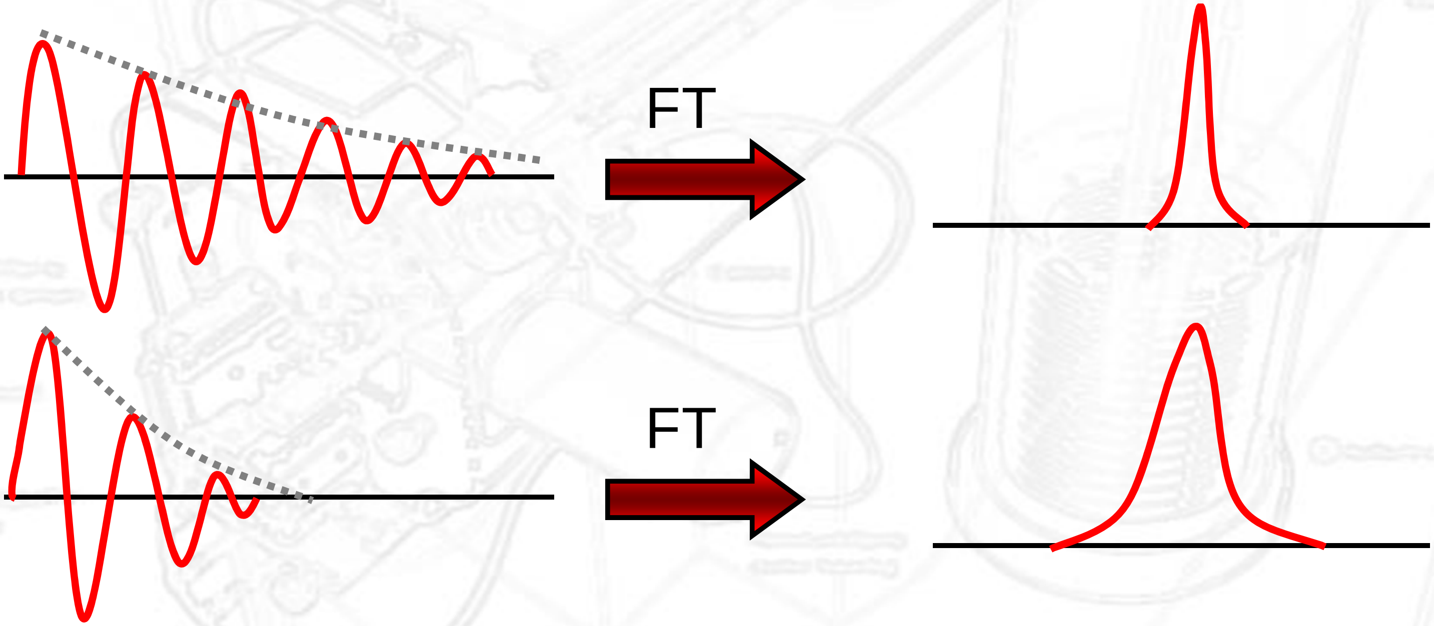
- However, you may have guessed that as our trial waves get closer to the actual wave, the sum gets bigger. This results in a gaussian lineshape centered on the correct frequency.

- The width of the distribution depends on how much bigger the ‘perfect fit’ sum is compared to an ‘almost perfect fit’ sum, which depends on the number of oscillations over which you are summing.

Fourier Transform Linewidth

- In an ideal world, we’d be summing over an infinite amount of time, so the perfect fit would be infinitely bigger than the next perfect fit, thus the zero width ‘Dirac’ peak.

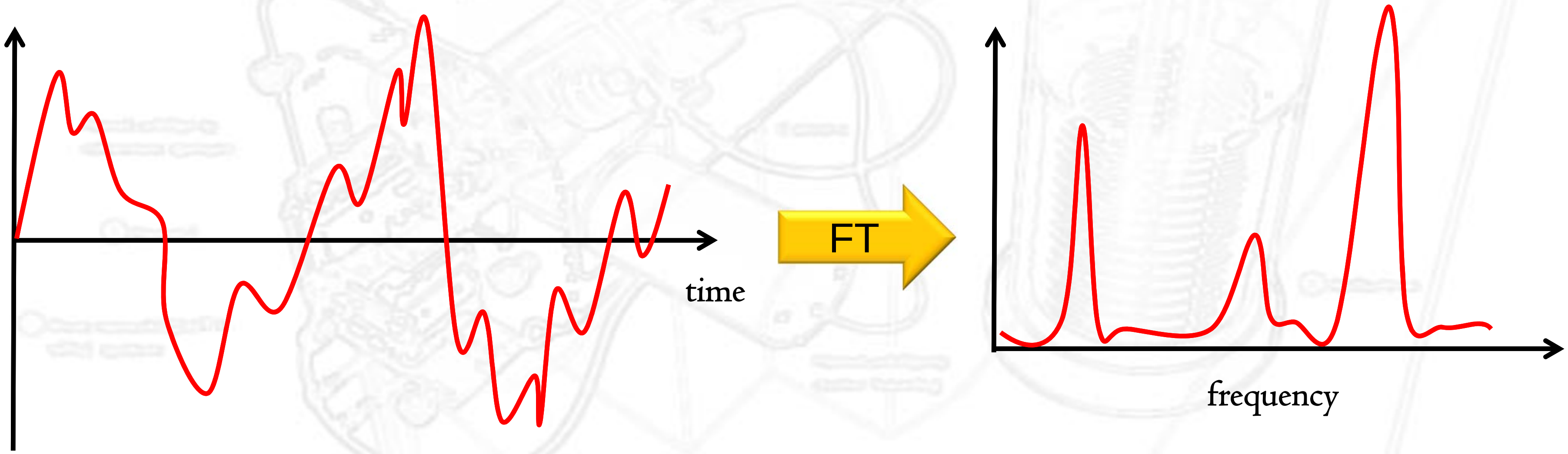
- Unfortunately, orbits tend to decay over time, so:



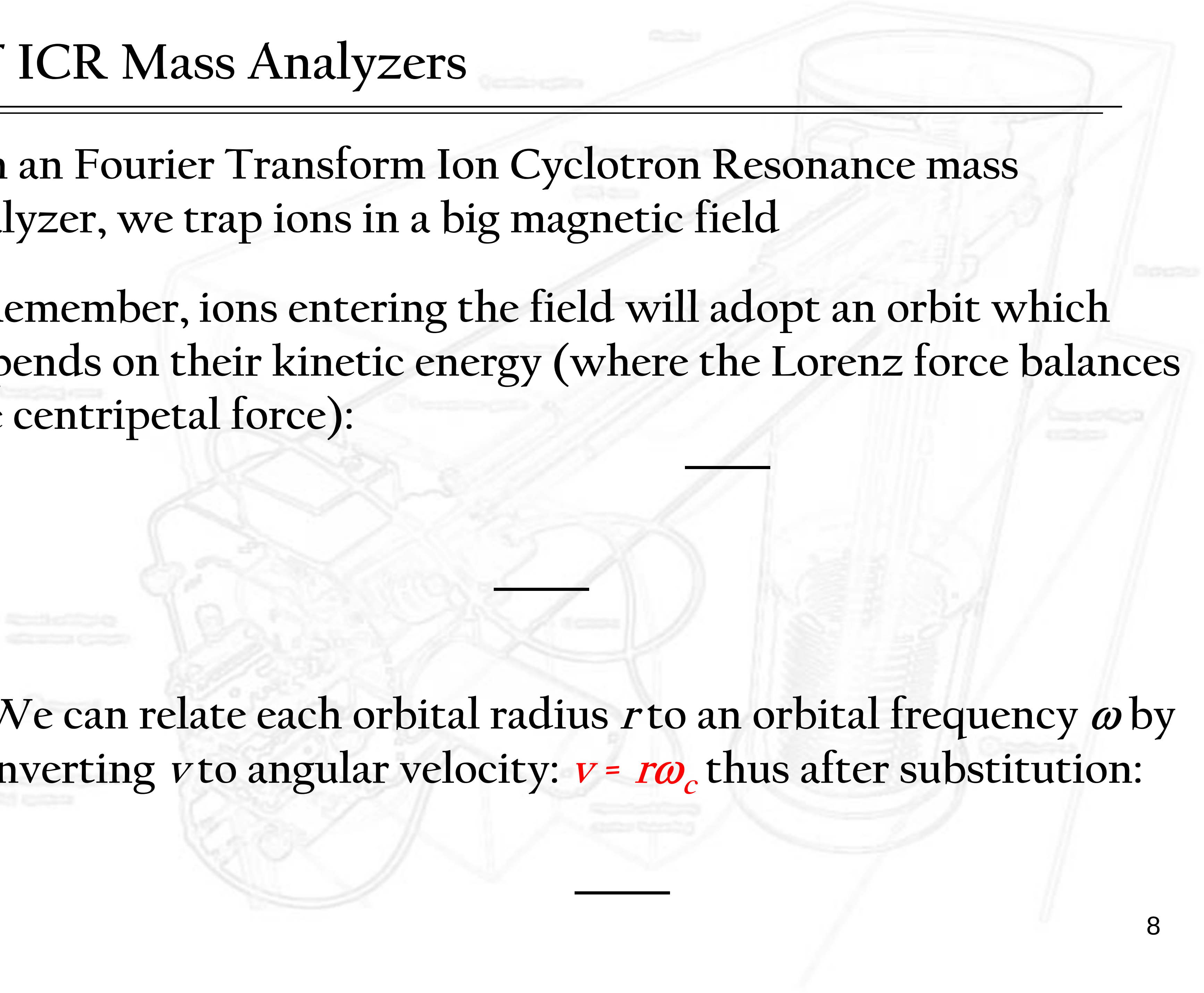
Fourier Transforms of Complex Waveforms

- What if we have multiple individual frequencies contributing to the measured waveform?

- The great thing about FTs is that (ideally) it doesn't matter how many different frequencies you have contributing to the time domain. You'll just get a series of peaks where different trial waves match individual components:



FT ICR Mass Analyzers



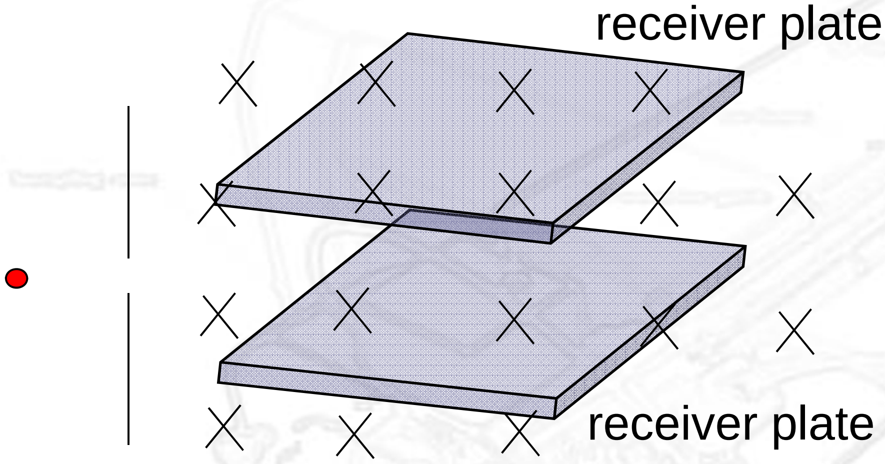
- In an Fourier Transform Ion Cyclotron Resonance mass analyzer, we trap ions in a big magnetic field

- Remember, ions entering the field will adopt an orbit which depends on their kinetic energy (where the Lorenz force balances the centripetal force):

- We can relate each orbital radius r to an orbital frequency ω by converting v to angular velocity: $v = r\omega_c$ thus after substitution:

Measuring Orbital Frequency in an ICR Cell

- Now we've got these ions orbiting, but how do we measure their ω_c?

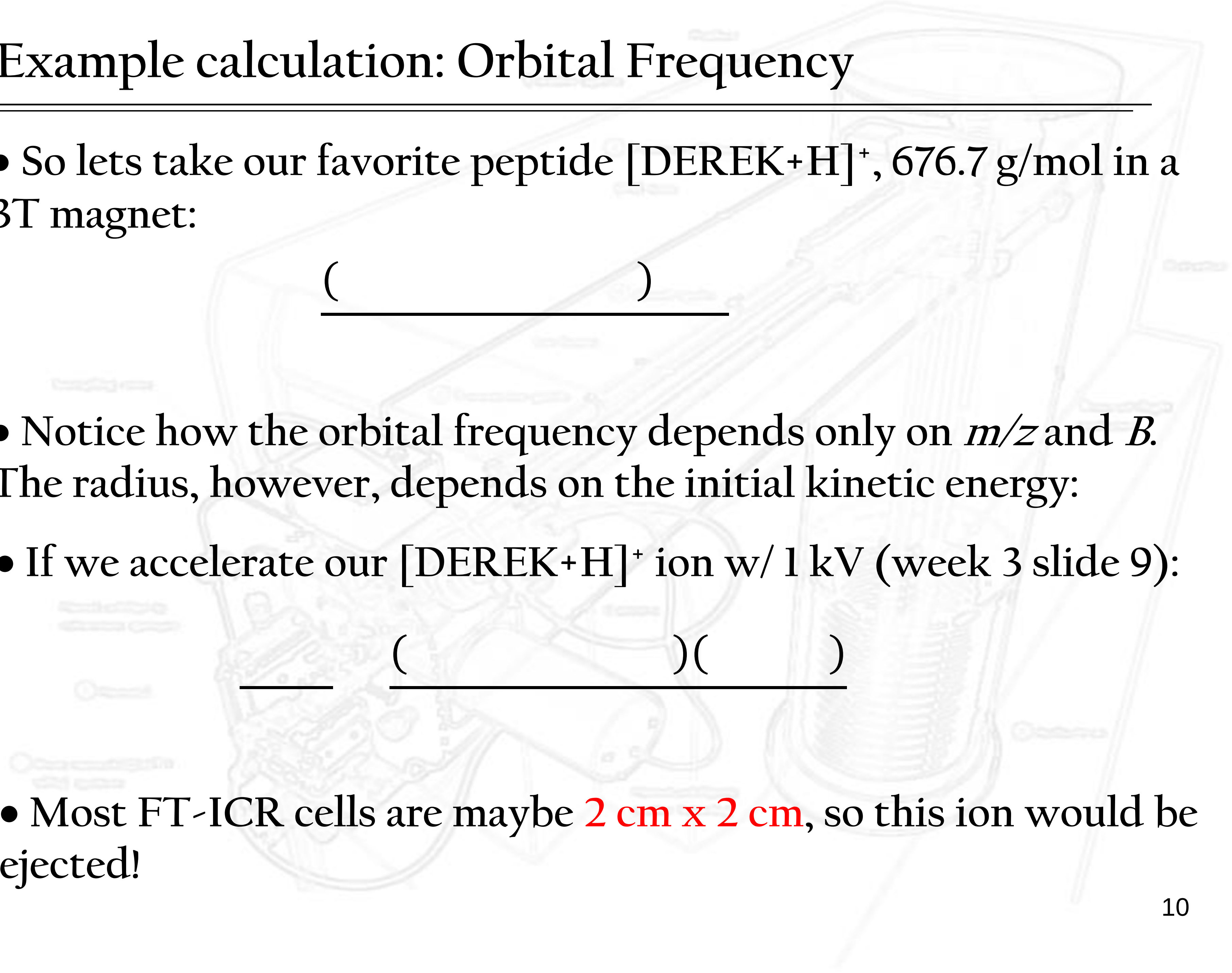


Our signal…

- Ions passing close to receiver plates induce a current

- BUT, only if their orbit is excited!

Example calculation: Orbital Frequency



- So lets take our favorite peptide [DEREK+H]$^+$, 676.7 g/mol in a 3T magnet:

$$\underline{(\qquad\qquad\qquad\qquad)\qquad\quad}$$

- Notice how the orbital frequency depends only on *m/z* and *B*. The radius, however, depends on the initial kinetic energy:

- If we accelerate our [DEREK+H]$^+$ ion w/ 1 kV (week 3 slide 9):

$$\underline{\qquad\quad} \quad \underline{(\qquad\qquad\qquad\qquad)(\qquad\quad)}$$

- Most FT-ICR cells are maybe 2 cm x 2 cm, so this ion would be ejected!

Coherence Signal Decay in FT-ICR

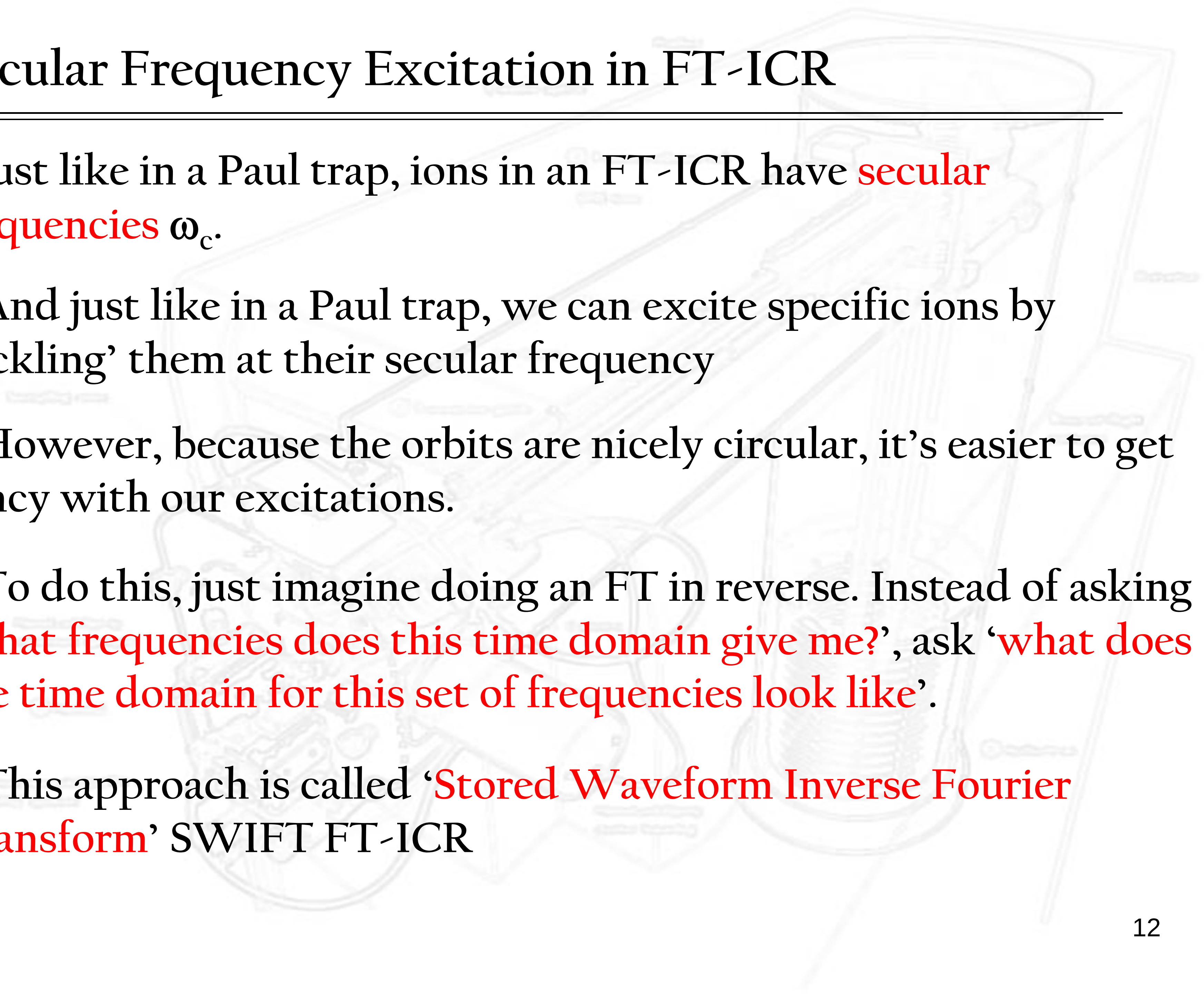
- FT-ICR detection requires not only that all ions with a particular *m/z* have a particular orbit, but also that they all pass by the detector plates at the same time, i.e. in little 'packets'. This is called Coherence.

- Coherence is created initially using a train of, broadband excitation RF pulses.

- However, over time, repulsive forces between ions results in expansion of the ion 'packets' and loss of coherence.

- As with a Paul trap, a partial solution is to use low energy collisions with neutral gas to slow cooling. Unlike a Paul trap, you can also reorganize the packets by letting them cool down and then 'reordering' with an RF pulse train.

Secular Frequency Excitation in FT-ICR



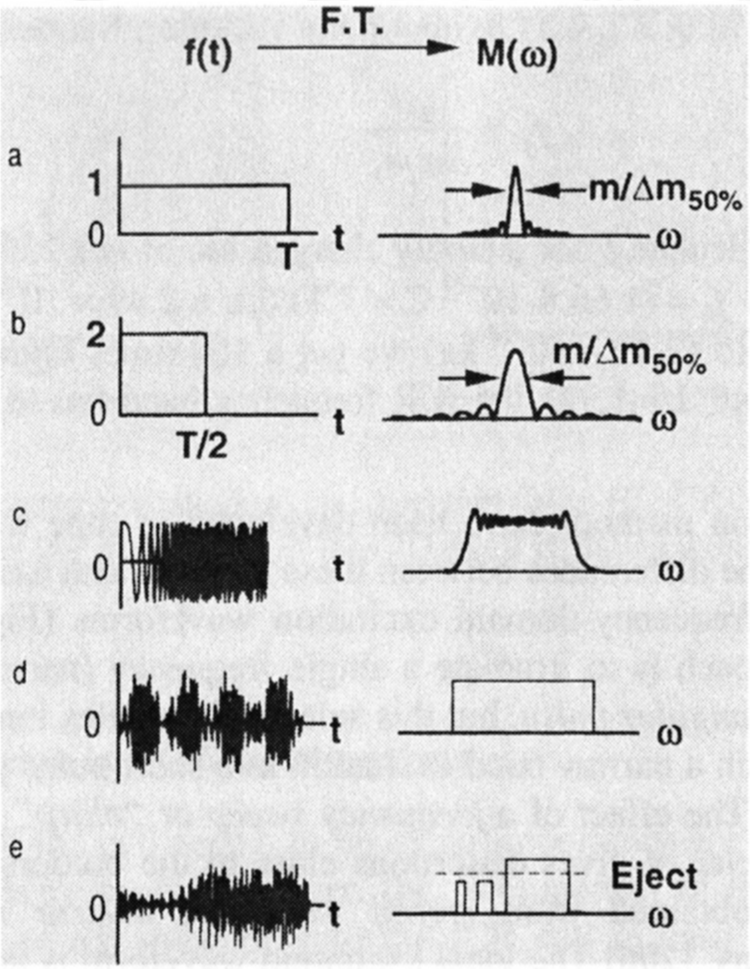
- Just like in a Paul trap, ions in an FT-ICR have secular frequencies ω_c.
- And just like in a Paul trap, we can excite specific ions by 'tickling' them at their secular frequency
- However, because the orbits are nicely circular, it's easier to get fancy with our excitations.
- To do this, just imagine doing an FT in reverse. Instead of asking 'what frequencies does this time domain give me?', ask 'what does the time domain for this set of frequencies look like'.
- This approach is called 'Stored Waveform Inverse Fourier Transform' SWIFT FT-ICR

Inverse FTs

- SWIFT is a powerful technique that allows us to select not only specifically which ions are excited, but also how much they are excited.

- It is implemented by adding a complex waveform RF voltage to the ICR cell plates.

- This is rather like NMR



CAD in FT-ICR

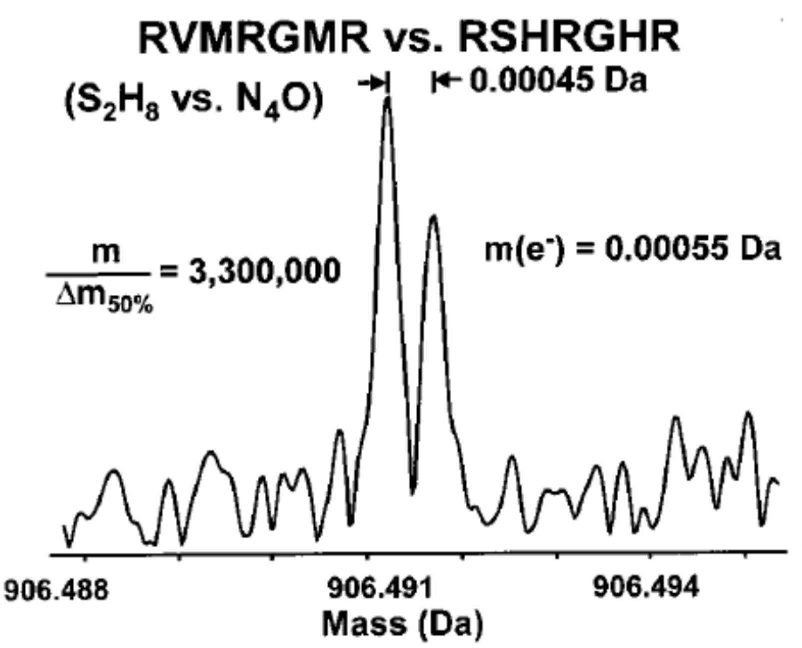
- MS^n can be performed in FT ICR instruments in pretty much the same was are resonant excitation in a Paul trap.
- Step 1: Select an ion (SWIFT)
- Step 2: Tickle it (secular frequency excitation)
- Step 3: Collide with background gas (also used for cooling)
- Step 4: Analyze fragments, repeat
- Example: Our $[DEREK+H]^+$ ion was going 16885 $m \bullet s^{-1}$ for a 4 cm radius. That corresponds to a kinetic energy of:

$$(\qquad\qquad)(\qquad)$$

… which is more than enough energy for fragmentation

Properties of FT-ICR Spectra

- FT-ICR is the highest resolution mass analyzer. As a reminder:

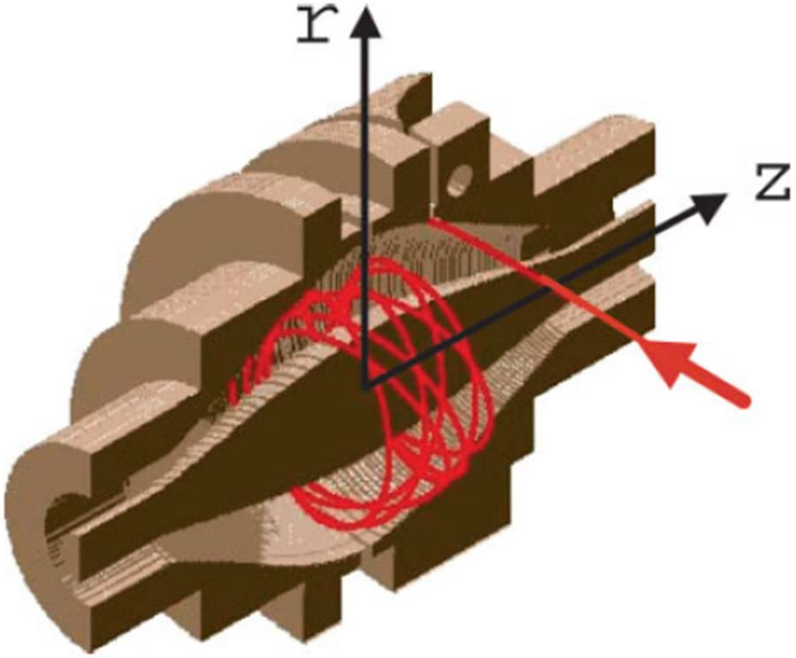


- On high *B* field instruments, resolutions of 2,000,000+ are easily achievable.

Orbitrap Intruments

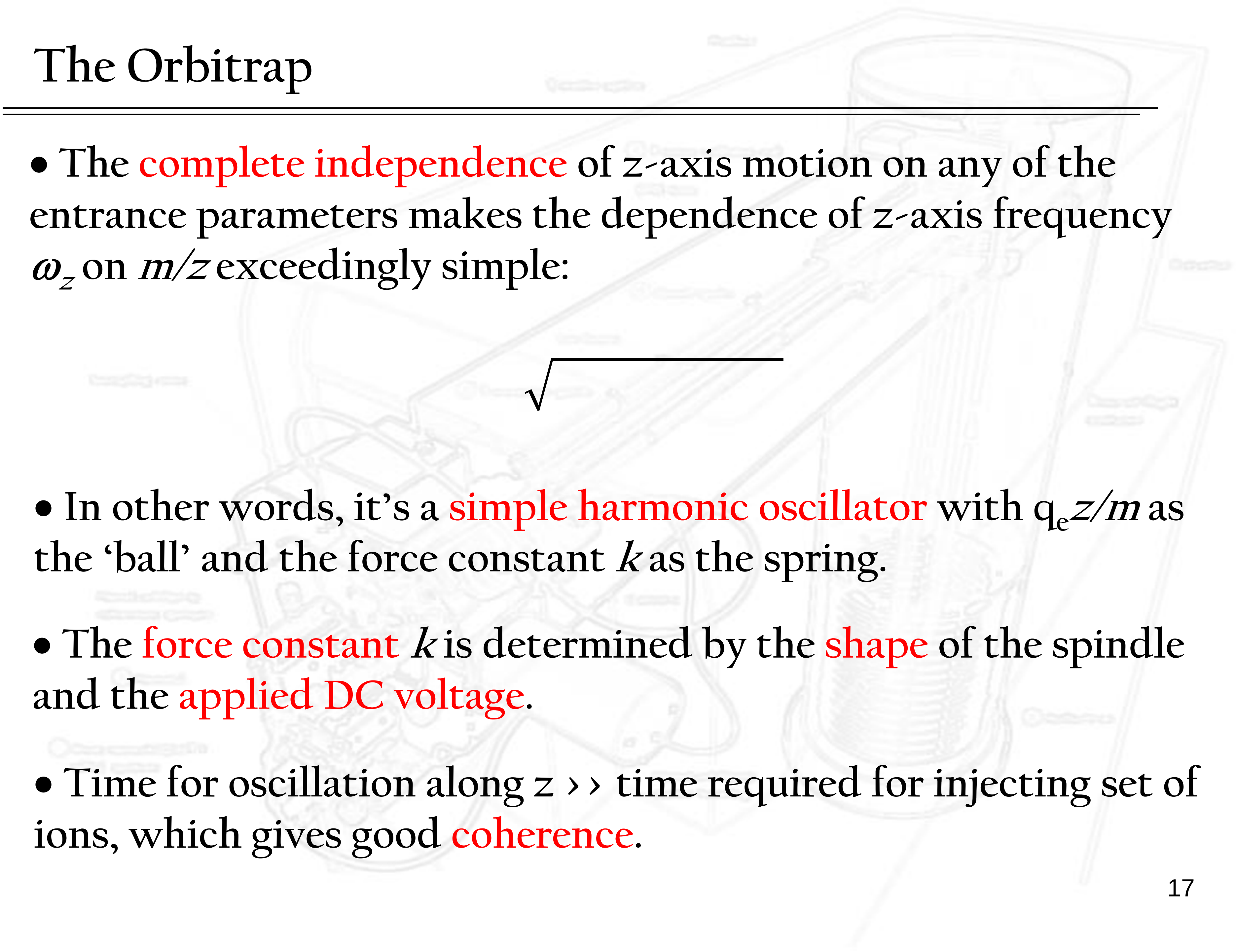
- The Orbitrap is an entirely new type of mass analyzer invented by Marakov in 1999.

- Ions are injected ‘off center’ into a radial ‘spindle-like’ DC-only trap. Because of the shape of the spindle and the off center injection, the ions obtain a velocity component in the z direction.



- Because of the shape of the spindle, the z direction velocity component is oscillating, and so the ions have an oscillating trajectory in z which is independent of the entrance kinetic energy.

The Orbitrap



- The complete independence of *z*-axis motion on any of the entrance parameters makes the dependence of *z*-axis frequency ω_z on m/z exceedingly simple:

$$\sqrt{\quad}$$

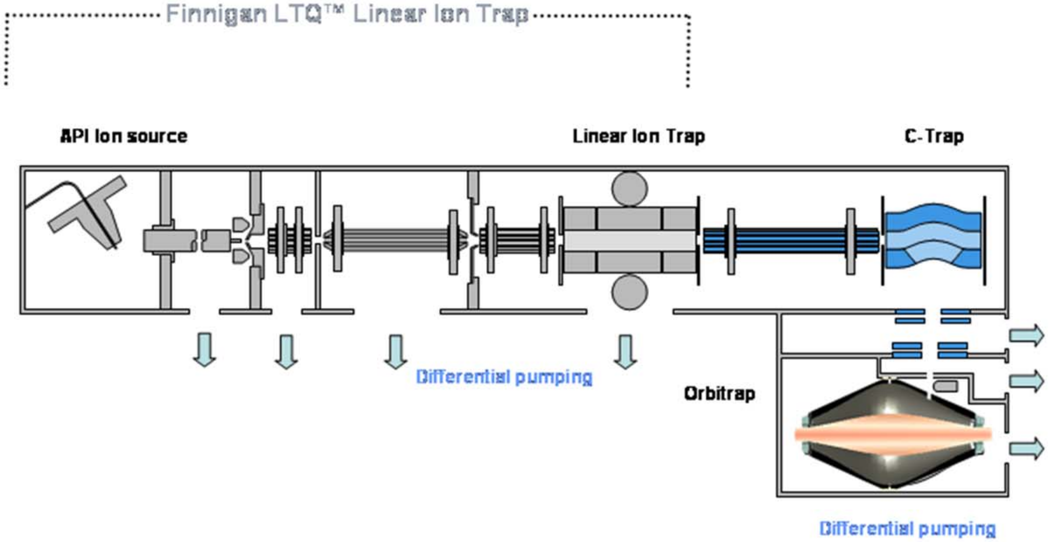
- In other words, it's a simple harmonic oscillator with $q_e z/m$ as the 'ball' and the force constant k as the spring.

- The force constant k is determined by the shape of the spindle and the applied DC voltage.

- Time for oscillation along z ›› time required for injecting set of ions, which gives good coherence.

Orbitrap Instruments

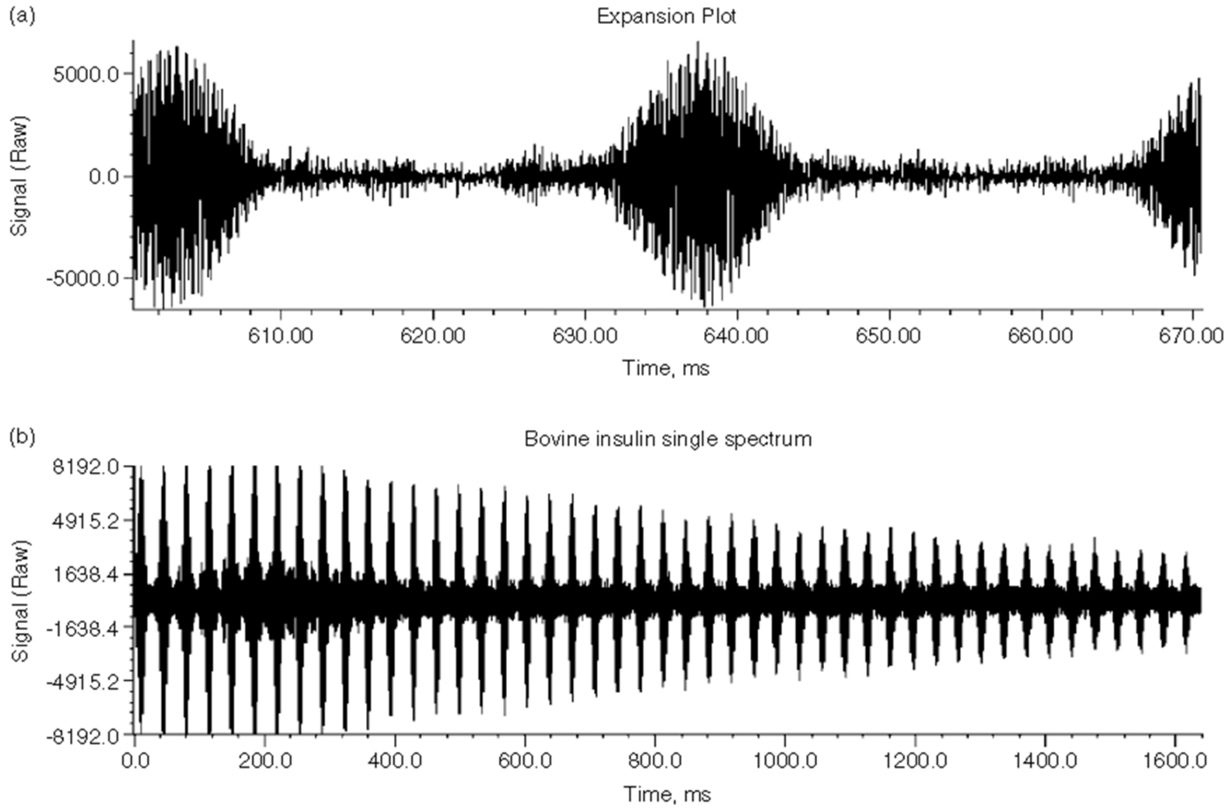
- At the moment, orbitrap technology is owned entirely by Thermo/Fisher. Their instrument includes an oddly-shaped quadrupole ion trap, called a ‘C-trap’ for injecting bundles of ions into the orbitrap for mass analysis:



- This has the unfortunate side effect of limiting the mass range on orbitrap instruments, to roughly that of a quadrupole.

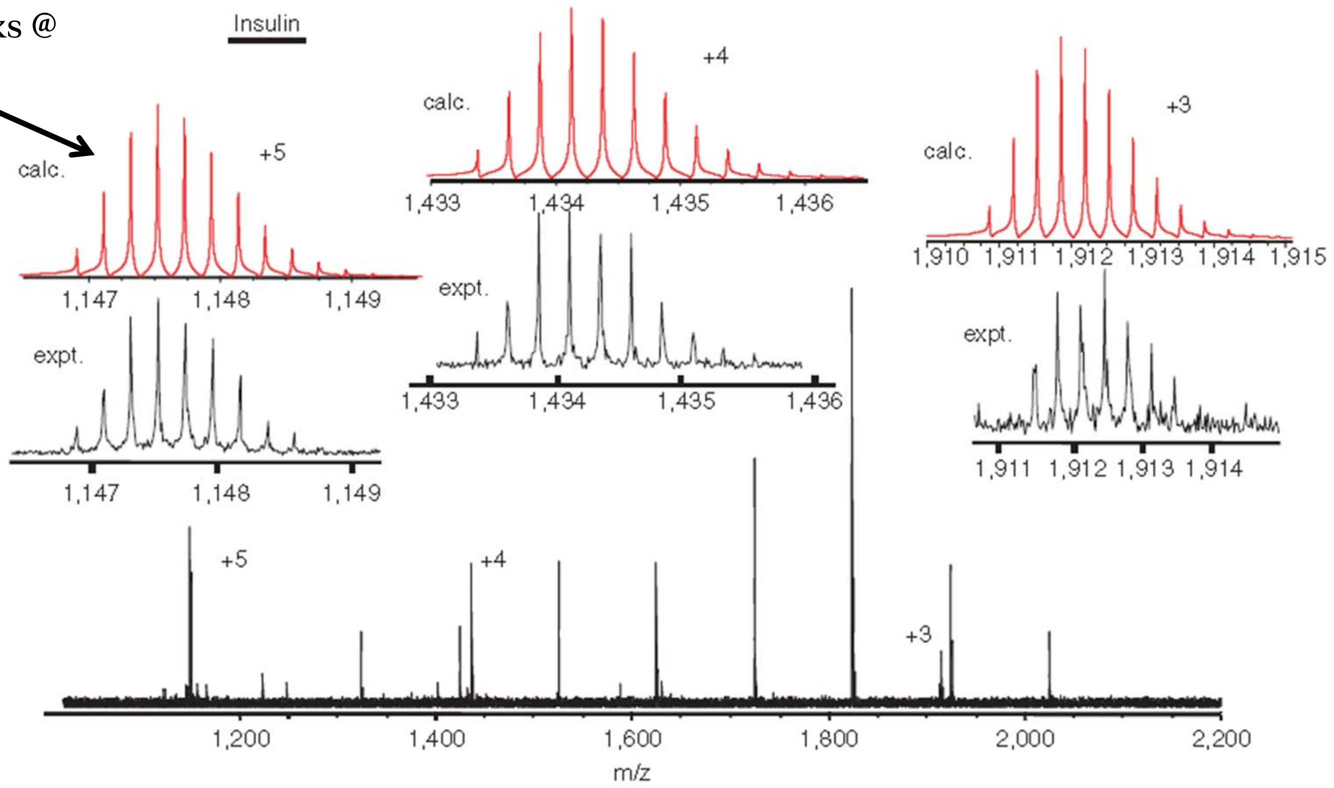
Properties of Orbitrap Instruments

- Compared to FT-ICR, the oscillations we measure in an orbitrap are slow (by around a factor of 10). As a result, scan times tend to be a bit slower in order to achieve high resolution.



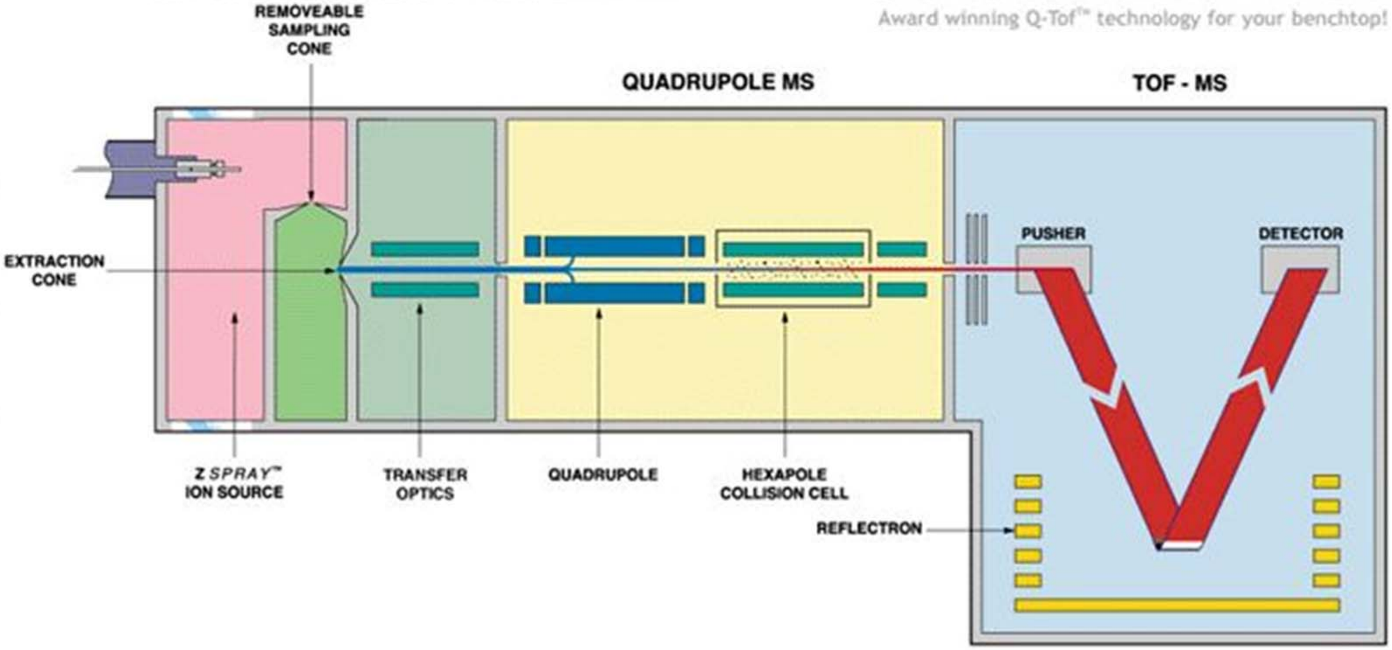
More properties of Orbitraps

- Orbitraps can achieve a resolution of 150,000+ FWHM at around 400 m/z
- They also have super-high mass accuracy – regularly achieve 2 ppm with internal calibration.


Theoretical peaks @ 100,000 FWHM

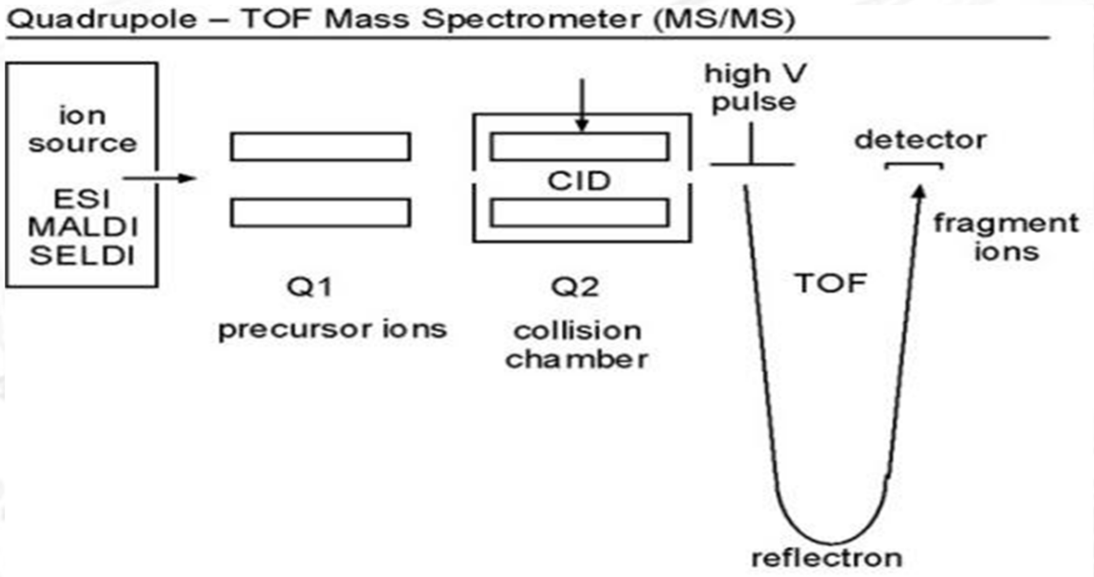
Hybrid instruments

- So far, we've focused on instruments with one (or at least one type) of mass analyzer.
- Of course, it is sometimes helpful to 'mix and match' mass analyzers to take advantage of the qualities of both.
- The most common type of hybrid is the Q-TOF:


Award winning Q-Tof™ technology for your benchtop!

Q-TOF Instruments

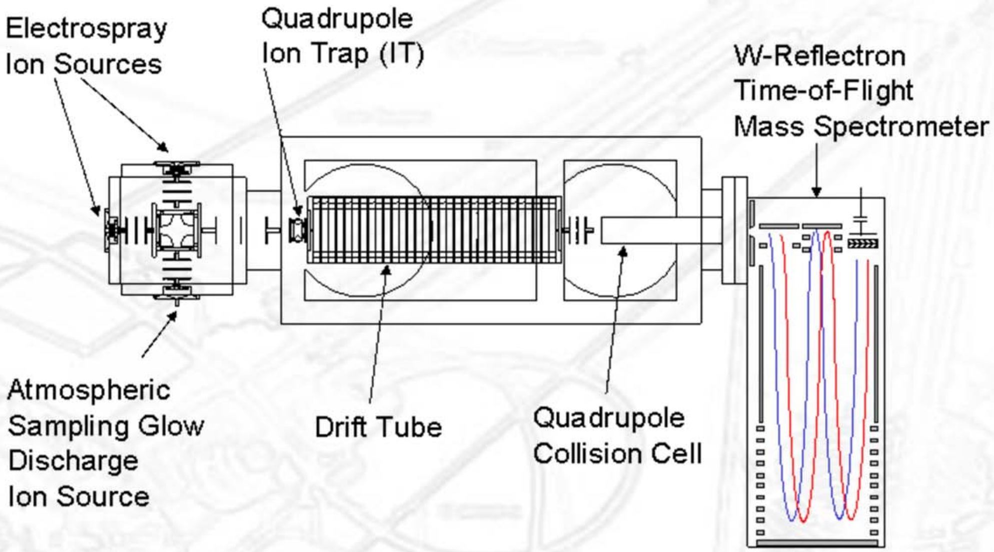
- These instrument were designed to allow MS/MS on a TOF:



- In principle, because the TOF measures entire mass spectra in a second or less, you can do any type of scan on these instruments as you can on a triple quad, but with a TOF mass analyzer.

Linear Paul Traps and TOF

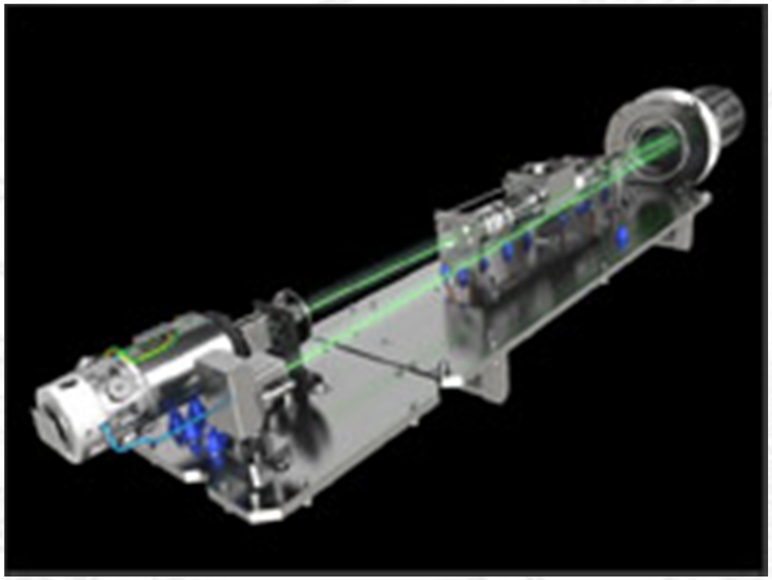
- Fairly recently, a number of companies have started to incorporate linear ion traps into their Q-TOFs



- Traps allow us to: Do some gas phase chemistry, fragmentation and thermalize the ions before they go into the TOF
- Also increases duty cycle by sending ions in packets.

TOF/TOF

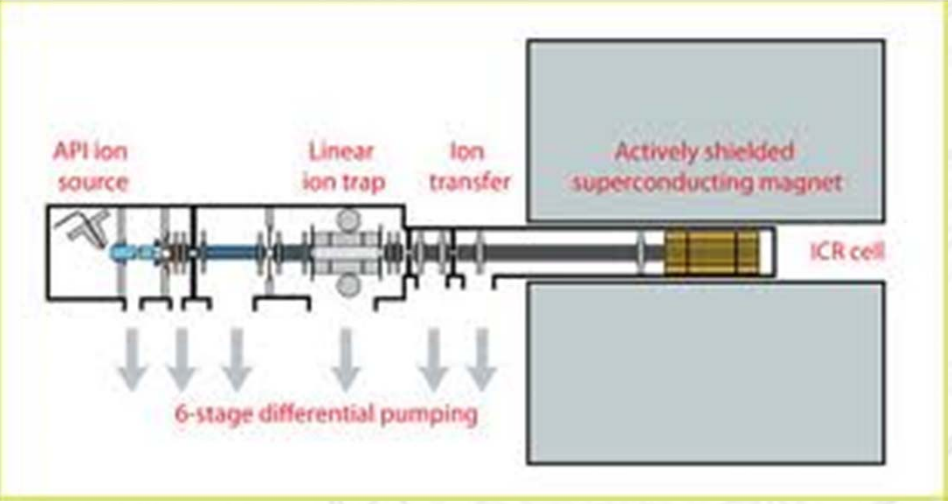
- TOF/TOF instruments are only kindof hybrids – they are basically a way of doing MS/MS inside the TOF flight tube…

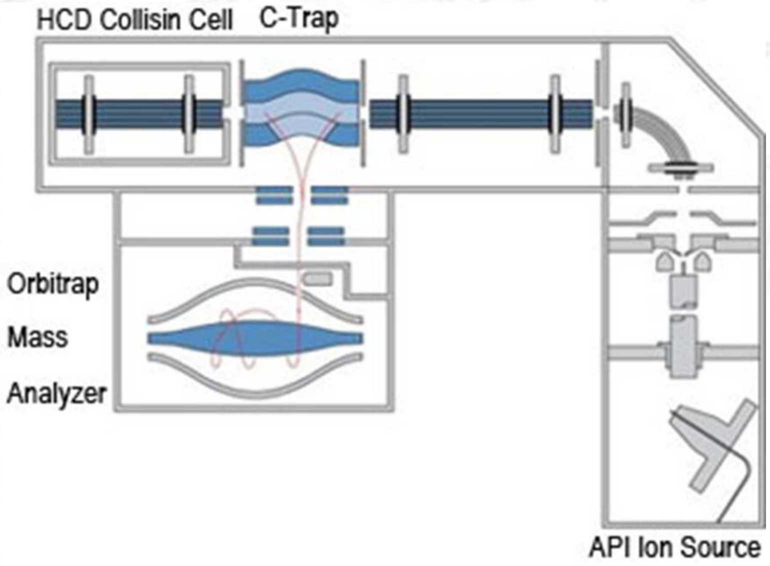


- These instruments use a 'timed ion selector' to select ions based on their 'first' flight time. Then fragment, then fragments fly.

FT-ICR and Orbitrap

- All (current) FT-ICR and orbitrap instruments are hybrids





- This is so that you can do efficient fragmentation and then analyze the mass… in FT-ICR is also helps with thermalization.

- Ions come into the mass analyzer as packets.

Other Types of Dissociation: BIRD

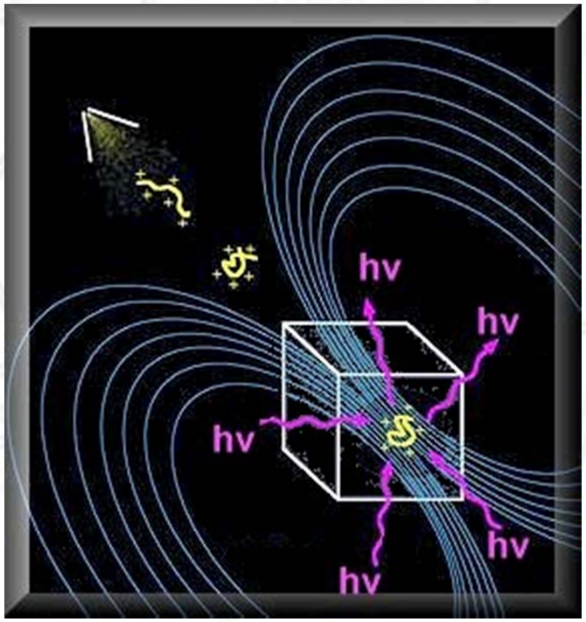
- So far, we've only talked about CAD as a method of doing MS/MS. In traps and ICRs, you can do much more.

- Some people wrap warm blankets around their FTICR instruments: Black Body Radiation Dissociation.

- When they warm the cell up, they release IR photons (black body radiation)

- This eventually provides enough energy to break bonds

- Used for measuring fundamental dissociation energies.



Other Types of Dissociation: IRMPD

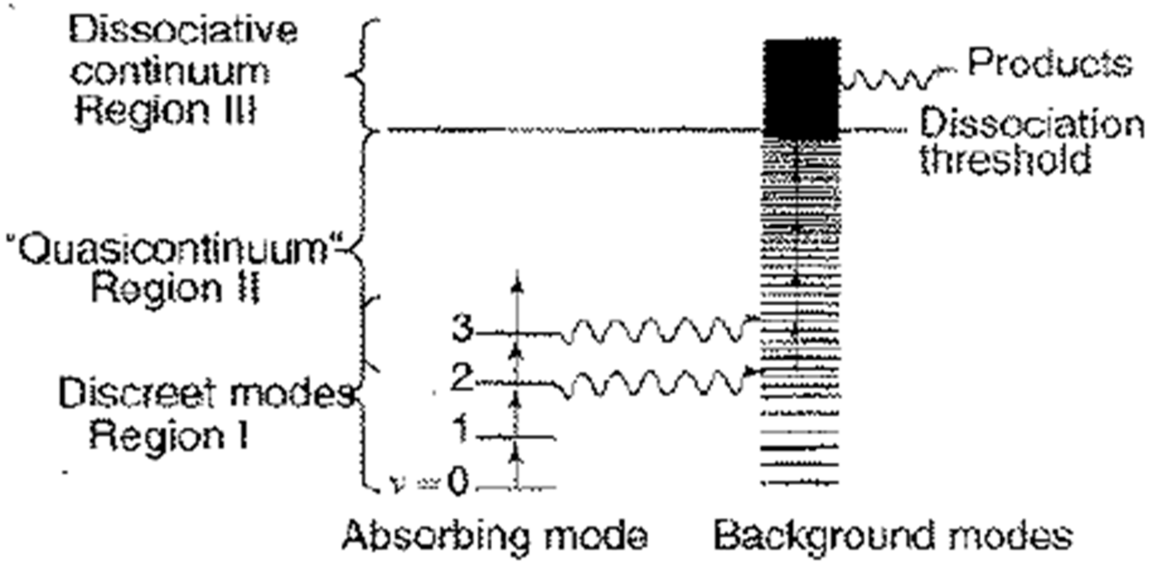
- In BIRD, the flux of photons is pretty low, so it has to be done in an FTICR where the trapping times are long.

- A more direct approach is to shoot an IR laser at the orbiting ions. This is called Infer-red Multiphoton Dissociation (IRMPD).

- Why 'multiphoton'? Because an IR photon has ~ 1 eV of energy = 96 kJ/mol. Not enough to break a bond on it's own.

- A typical IRMPD experiment will use a CO2 laser (1060 nm) at 30 – 45 W.

- Not great in Paul traps (cooling too quick).

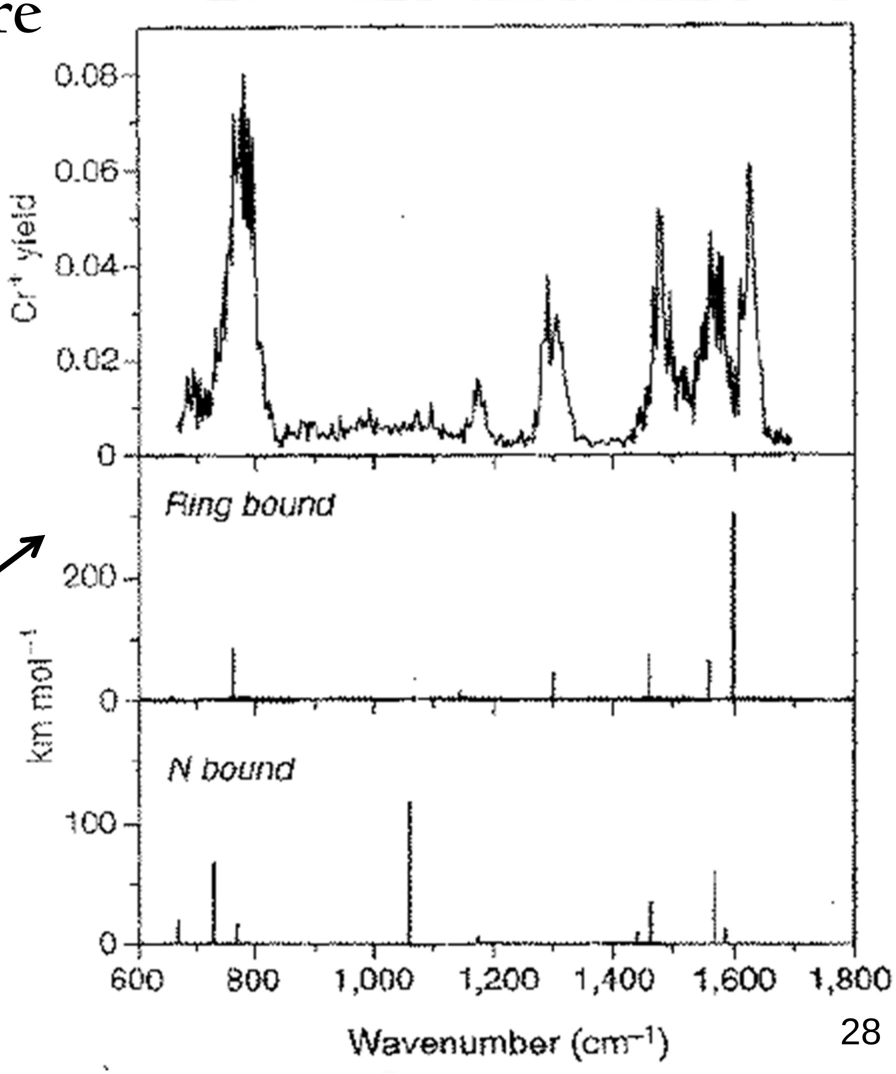


Action Spectroscopy

- IRMPD allows for MS^n, but with a tunable laser, it can also be used to probe molecular structure

- This is called ‘action spectroscopy’ or ‘consequence spectroscopy’ where you measure dissociation as a function of irradiation wavelength.

- Chromium bound to an aniline ring… Based on action spectrum (top) vs. theoretical IR spectra (bottom 2), which way is Cr+ bound?



Electron Capture Dissociation

- This method is basically like EI on trapped ions, though the electrons are at substantially lower energy (~0.1 eV).

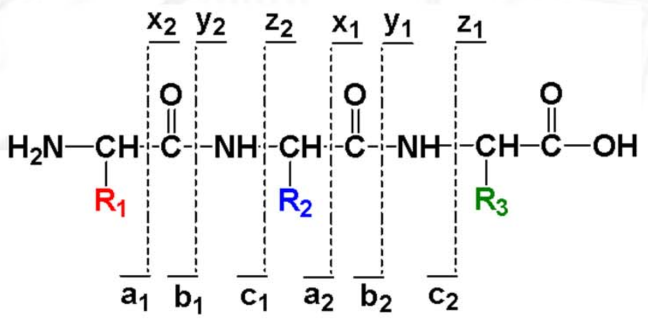
- This results in the 'capture' of an electron. The energy from partial neutralization is converted very rapidly into internal energy of about 7 – 9 eV, which is enough to break a bond.

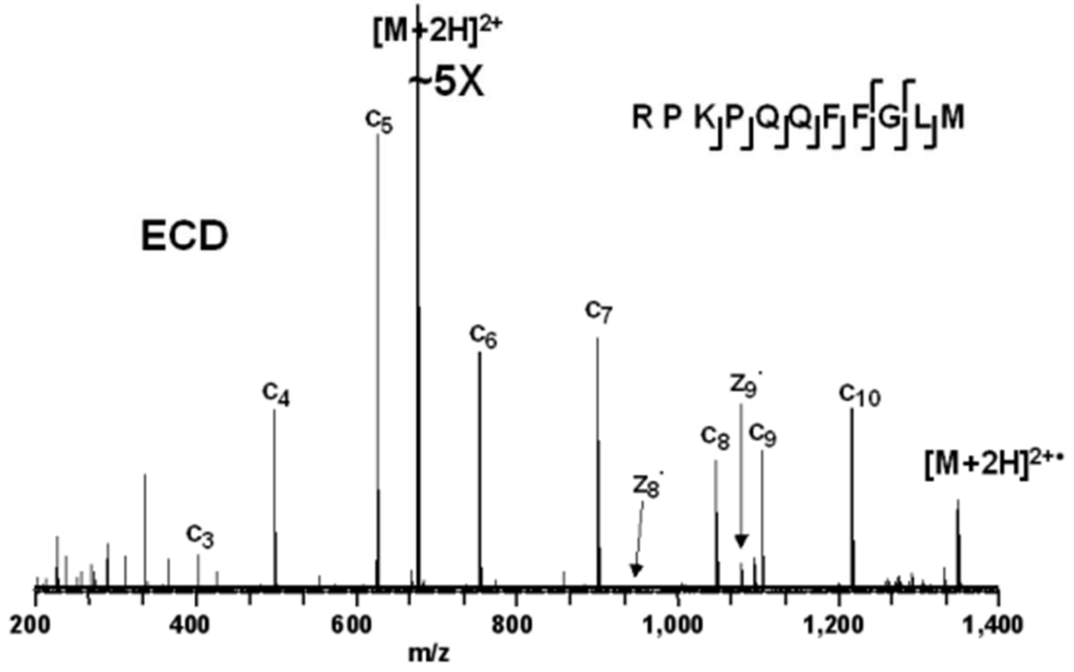
- Because dissociation is so fast (shorter than a bond vibration), there is no redistribution of internal energy during heating (like in CAD and IRMPD). This is called 'non-ergotic'.

ECD Cont.

- Bond breaking in ECD occurs near positive charges where the electron is captured, rather than where bonds are weakest. This means fragmentation is often at different sites compared to CAD and IRMPD.

- For example, with peptides:

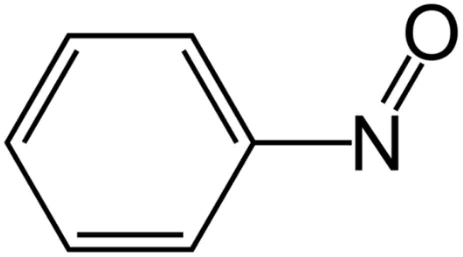




Electron Transfer Dissociation (ETD)

- Electron transfer dissociation is basically like CI of trapped ions.

- Radical anions (e.g. nitrosobenzene) are generated by plasma or glow discharge. These chemically transfer electrons to positively charged trapped ions.

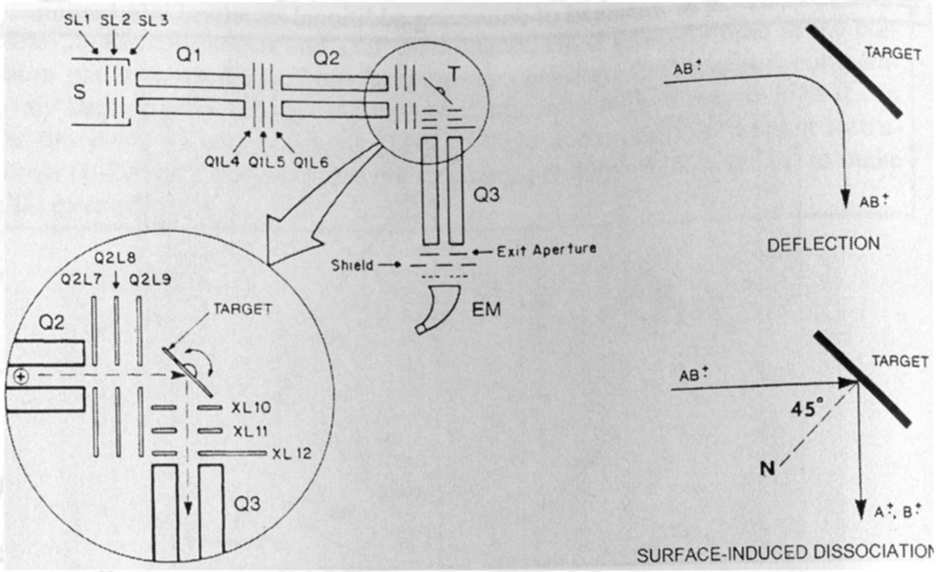


- Was originally designed to allow ECD-like experiments in a Paul trap.

- Fragmentation is identical to ECD… both of these are often used for retaining weakly-bound covalent modifications on proteins during fragmentation (such as phosphotyrosine).

Surface Induced Dissociation

- Finally, another way to dissociate ions is to ‘bang’ them into a surface, known as Surface Induced Dissociation

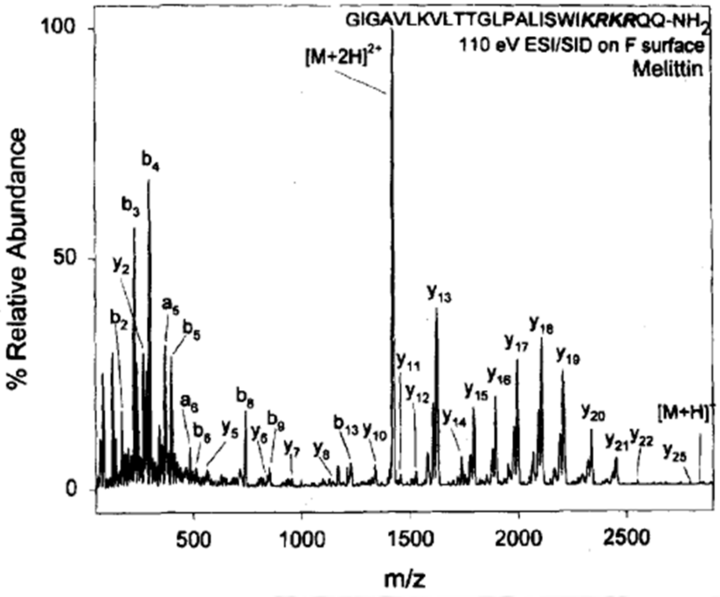


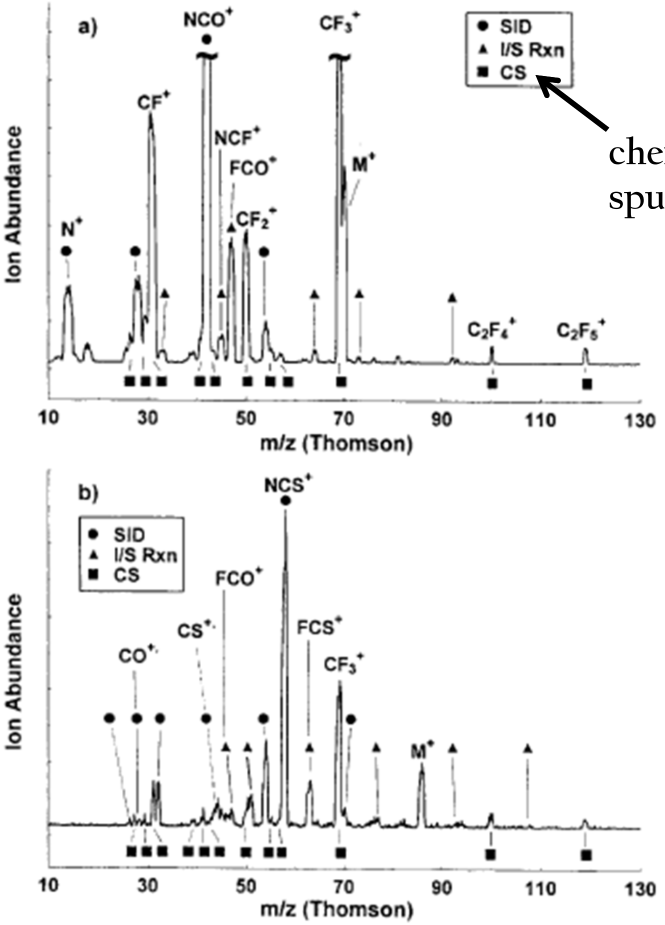
- The surface is usually a metal, held at ~45° to the incident angle of the ion beam.

- SID can be used for chemistry as well… at lower collision energies (<100 eV) incoming ions can react with the surface material.

SID-based Fragmentation

- SID fragmentation is similar to CAD, except energy is more easily controlled, and can be higher. Can also be done at low pressure.





chemical sputtering

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