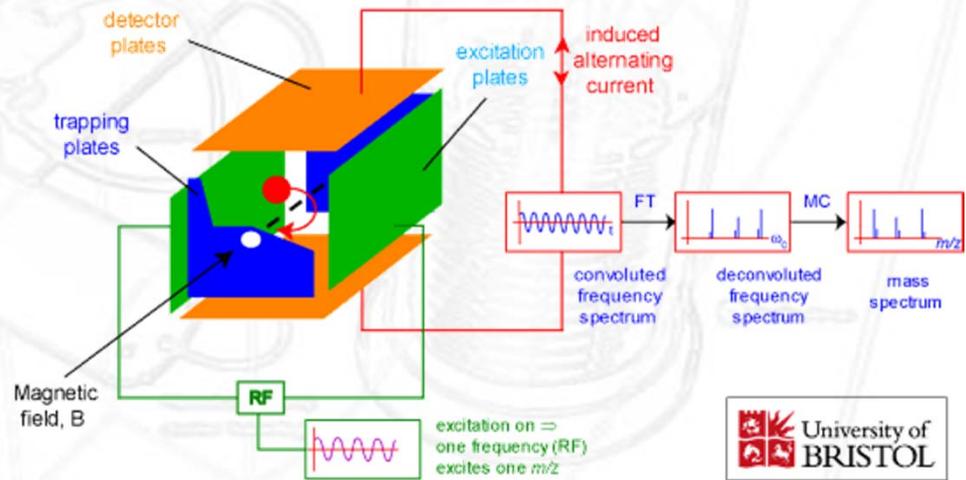
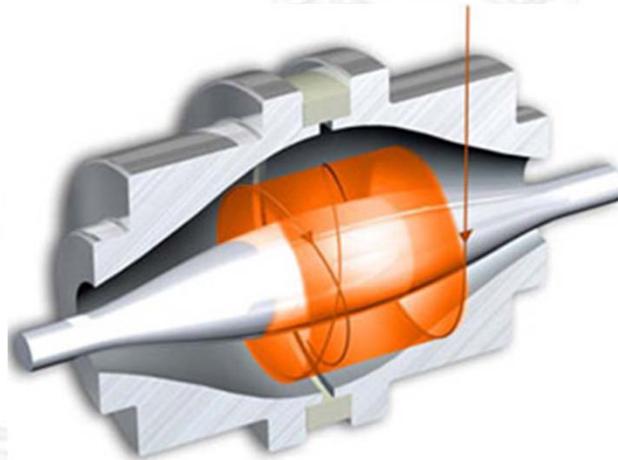
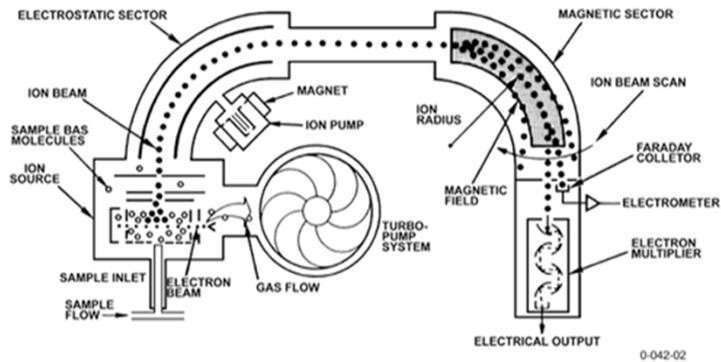
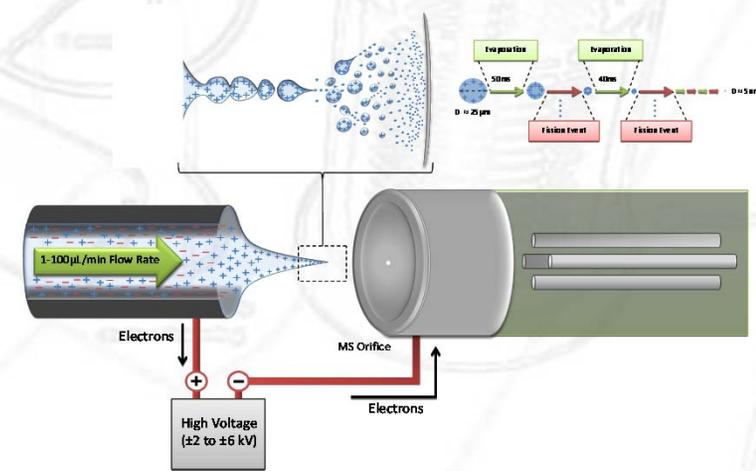
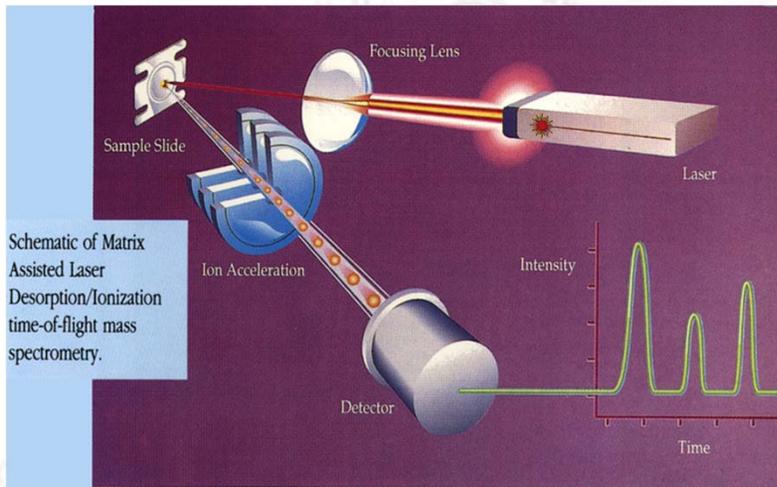
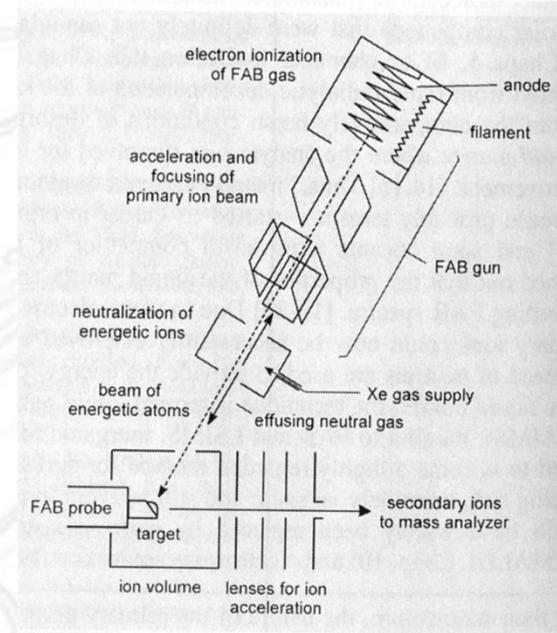
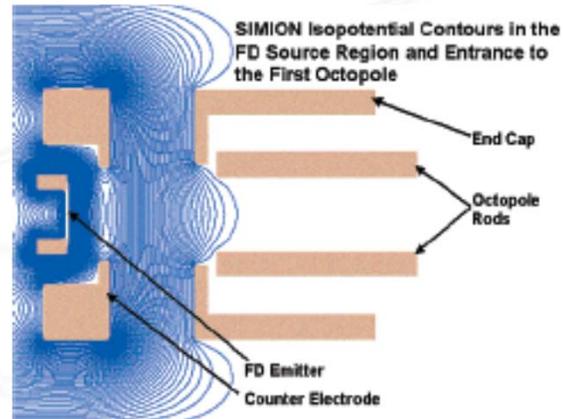


Week 3: Mass Analyzers



Last Time...

- **Soft Ionization:**

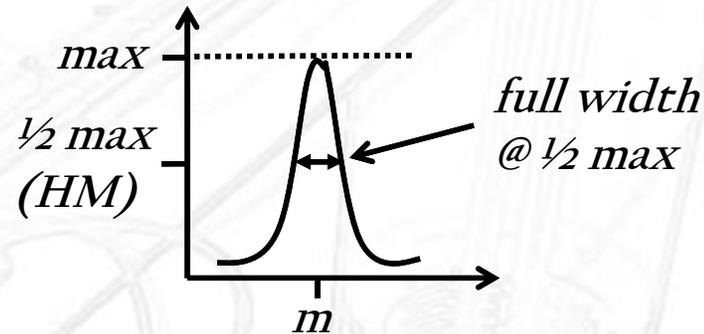


Mass Analyzer = the ♥ of the Mass Spectrometer

- The mass analyzer is what makes a mass spectrometer a mass spectrometer.
- There are three classes of analyzer (that I made up):
 - **Separating**: Ions are **separated in space** according to their m/z and detected at different times or locations.
e.g. Thomson, some sector, TOF
 - **Filtering**: All ions except for a specific m/z are removed. Ions with particular m/z are detected **one at a time**.
e.g. Aston, some sector, linear quadrupole, ion trap (sortof)
 - **Resonant**: Mass to charge is determined by measuring the **resonant oscillations** of ions in electric/magnetic fields.
e.g. Ion trap (sortof), FT-ICR, orbitrap

Properties of Mass Analyzers

- The mass analyzer used will determine a number of properties of the mass spectrum:
 - **Resolution**: Probably the most important feature related to the mass analyzer.

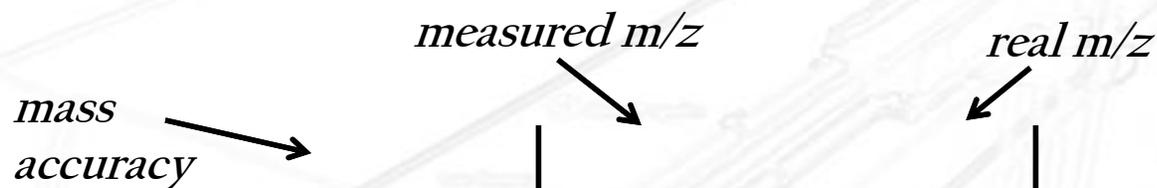


- e.g. I have a peak at 500 m/z, intensity 100 cps. The width of the peak at 50 cps is .2 m/z:

- pretty crappy

Other Properties of Mass Analyzers

- The next most important feature (arguably) is **mass accuracy**:



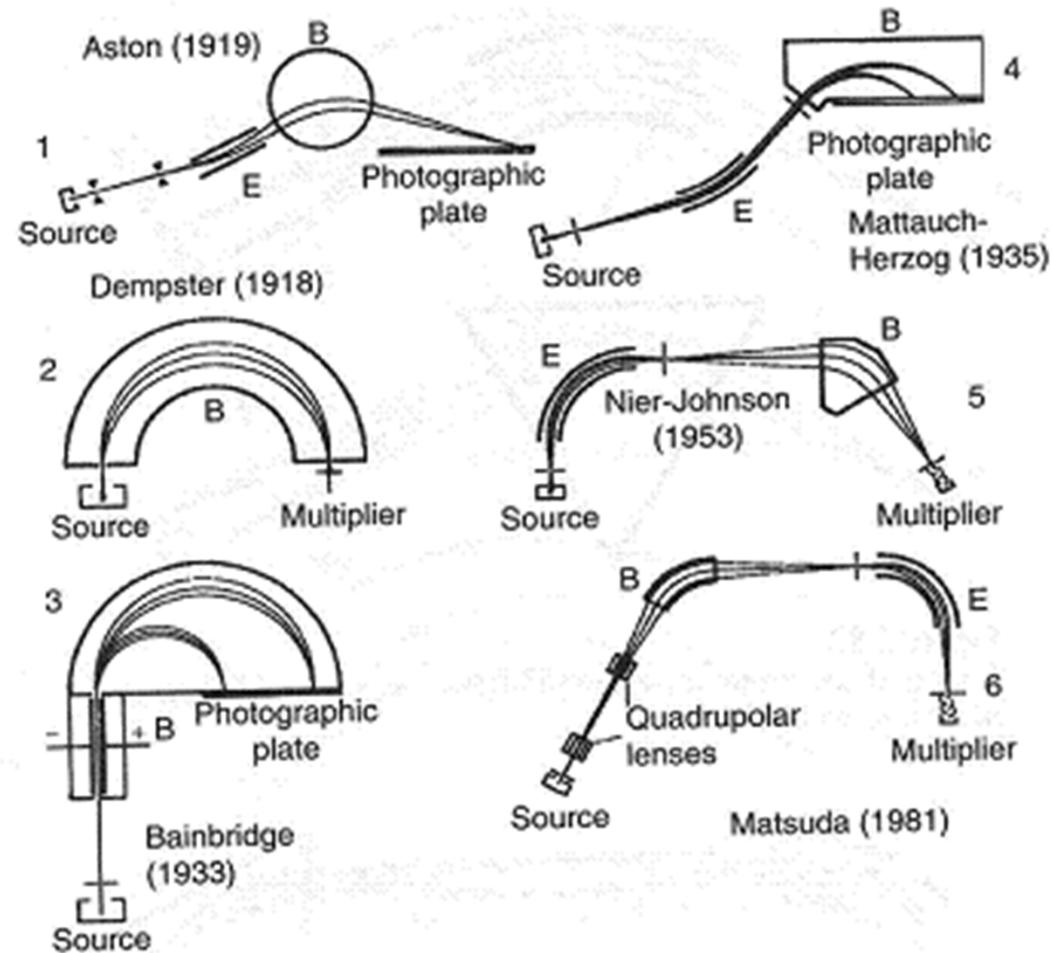
- Modern instruments can usually achieve **5 ppm** mass accuracy or less, which means:
 - If I measure a m/z of 1000, I can expect to get it right to within $1000 \times (5 \times 10^{-6}) = .005$ m/z
 - Or put another way, at 5 ppm mass accuracy, my m/z measurement of 1000 is actually 1000.000 ± 0.005

Still More Properties of Mass Analyzers

- Mass analyzers also determine or influence:
 - **What types of experiment you can do.** MS/MS? What Type? MSⁿ? Ion Mobility?
 - **Sensitivity.** Not as important as ionization or detector.
 - **Mass Range.** Quadrupoles limited to about 3,000 m/z. TOFs not limited (in theory – in practice around 20,000 m/z)
 - **Linear Dynamic Range.** Not as important as detector; may contribute to ‘m/z bias’ in spectrum.

Sectors: The First Mass Analyzers

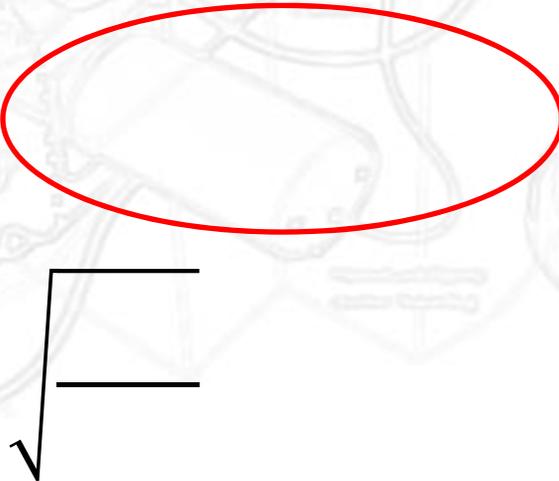
- Aston's mass spectrometers incorporated 'sector' mass analyzers, so called because they are divided into electric and magnetic sectors.



Sector Instrument, Step 1: Acceleration

- In a sector instrument, the first step is to accelerate our ions using an electric field.
- The energy supplied by the electric field E_e is equal to the product of the basic electric charge ($q_e = 1.6022 \times 10^{-19}$), the number of charges z and the applied voltage U .
- If we assume that all of the energy is converted into kinetic:

rearrange for v .


$$\sqrt{\quad}$$

Velocity Due to Electric Field: Example

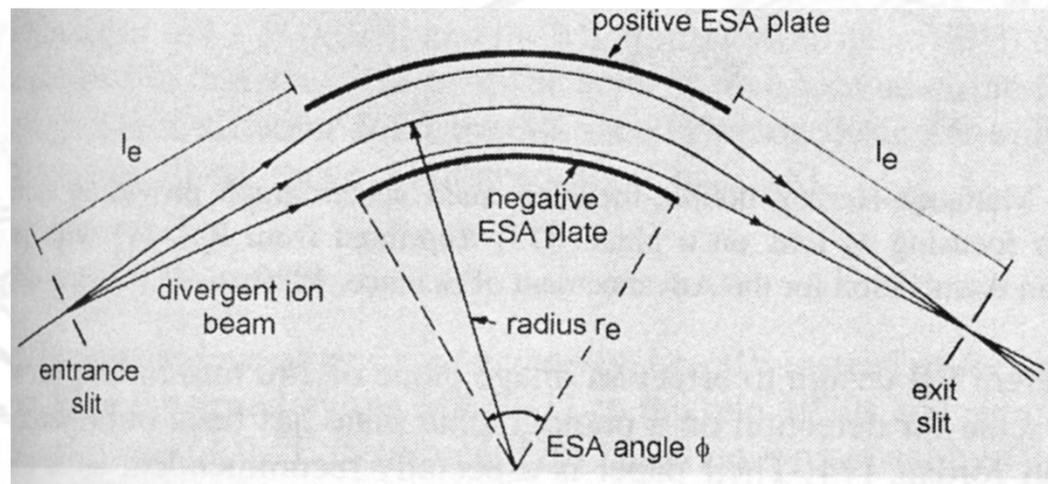
- Let's suppose we're accelerating the awesome peptide $[\text{DEREK}+\text{H}]^+$ which would have a molar mass of 676.7 g/mol, with 10kV.
- We need to know the mass of the molecule in *kg*, so:

- Then we calculate the velocity:

√ _____

Sector Instrument Step 2: Electric Sector

- In modern instruments, the electric sector produces a radial electric field between two opposite charged plates.

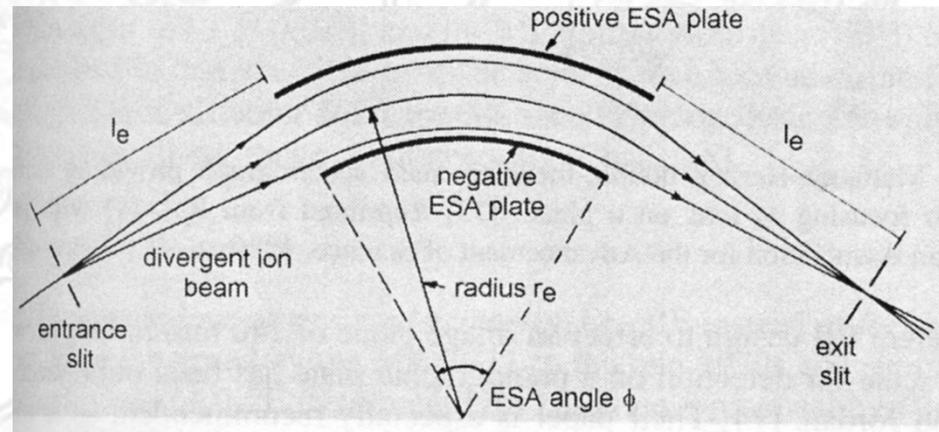


- Particles entering the electric field feel a force F_e due to the electric field:

rearrange for r_e
sub in U (see
slide 8) :

Electric sector, con't...

- What this tells us is intuitively obvious: That the 'orbit' of a **particular m/z** is bigger for faster ions (high accelerating voltage U) and smaller when the electric field between the plates is large (high E).
- Another property is that ions with divergent paths, but the same kinetic energy will have a set of orbits that focus at a particular length l_e .



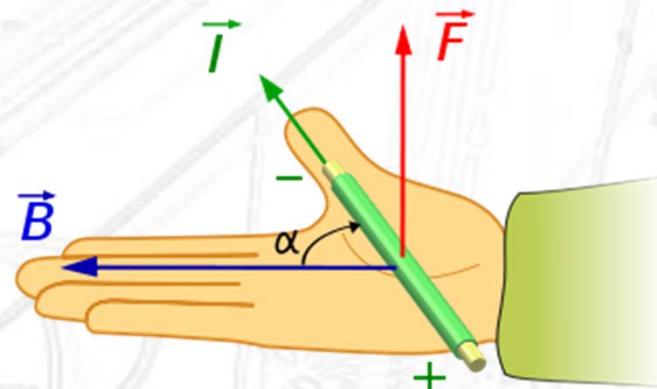
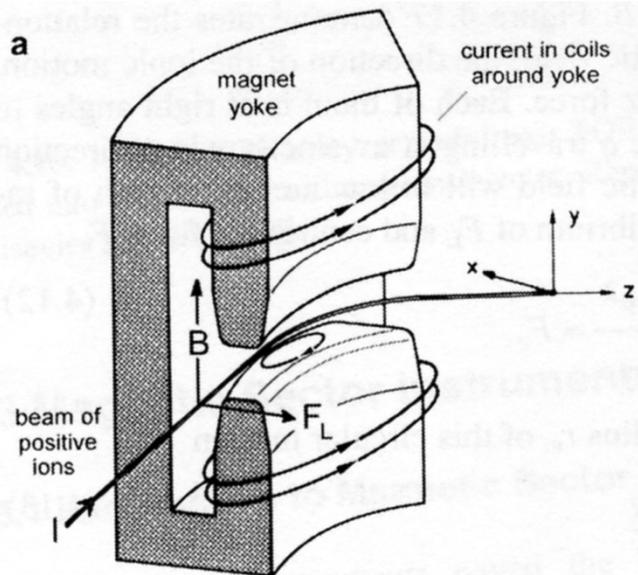
Sector Instruments Step 3: The Magnetic Sector

- The electric sector can focus ions with different trajectories **slightly** different kinetic energies onto a single point. Substantial differences in kinetic energy (*e.g.* due to different m/z and same U) will push the ion well out of focus (or ‘crash’).
- Magnetic sectors focus ions based on their momentum, based on the Lorentz force:
- Ions with a **particular E_k** will adopt a **circular orbit with radius r_m** when the Lorentz force is balanced by the centripetal force:

_____ or _____

(One of) The Right Hand Rule(s)

- The direction of the Lorentz force can be shown using the right hand rule

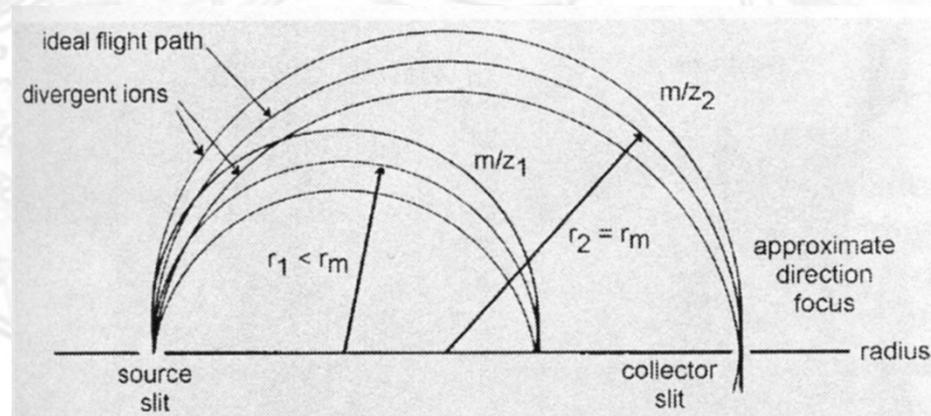


The Magnetic Sector, Con't...

- We know the accelerating voltage U , and the relation to kinetic energy (see slide 8), so...

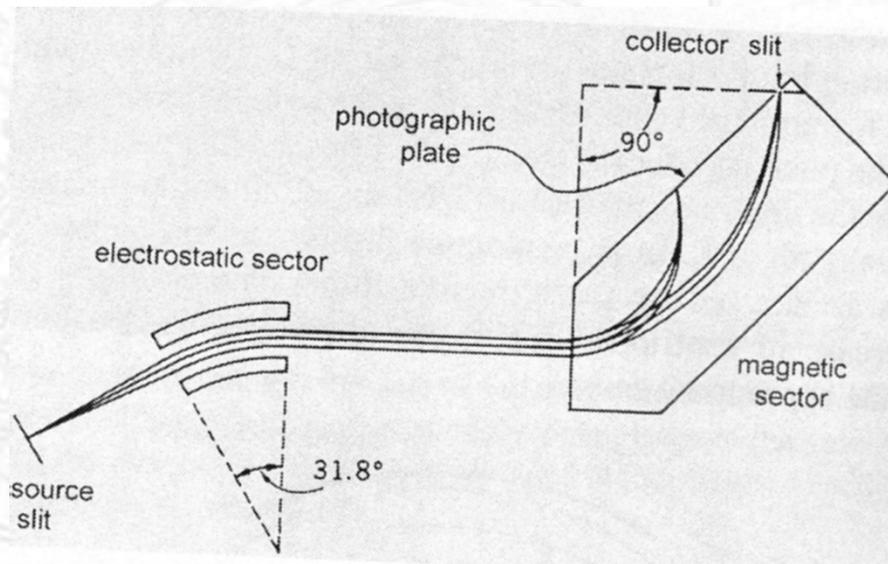
$$\sqrt{\quad} \quad \sqrt{\quad}$$

- ‘Spatial focusing’ of two ions is shown below. Divergent ions with same kinetic energy are focused at the **radius of the ideal path** (in which the ion enters at 90° to the field)



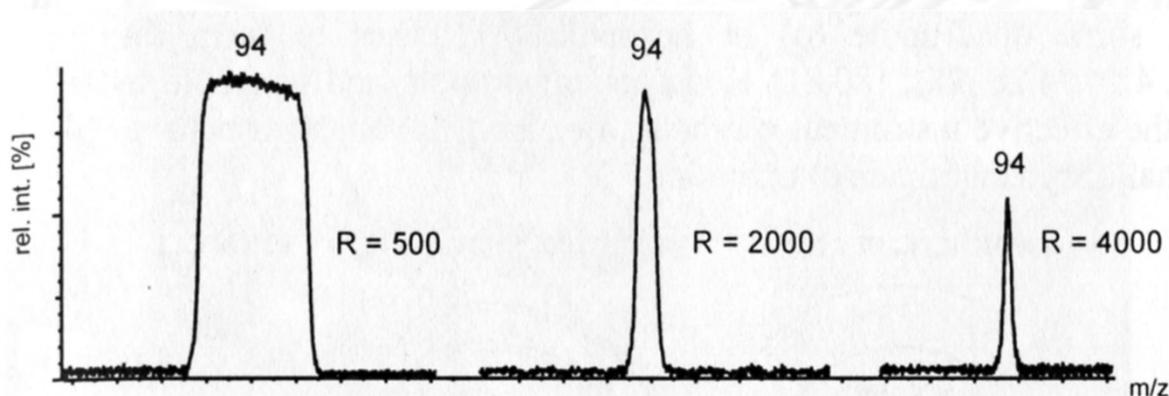
Sector Instruments: Magnet Scans

- Sectors can come in all shapes and sizes. One of the main differences is the order of the sectors, i.e. *EB* vs. *BE*.
- The most common type of instrument is probably *EB* and the most common type of scan uses *E* for energy dispersion with spatial focussing, followed by *B* for mass dispersion. *B* is scanned to bring successive m/z through the detector slit.



Sector Instruments: Properties

- Ultimately, the resolution of sector instruments depends on the amount of focusing, but mostly on the slit widths:



- Note that decreasing slit width also reduces signal intensity, so there's a tradeoff
- With slow scanning and μm slits, modern double focusing sector instruments can achieve resolutions of 60,000 or more with <5 ppm mass accuracy.

Sector Instruments: Other Properties

- Sector instruments are capable of (and actually singularly good at) MS/MS studies with Collisional Dissociation.
- This is because sector instruments are set up to accelerate ions to an extremely high velocities through the instrument. This makes **single collision bond breaking possible**.
- Modern sector instruments are sometimes equipped with linear quadrupoles or hexapoles, which can transmit a wider range of m/z for separation in the magnetic sector.

Linear Quadrupole Mass Analyzers

- Quadrupoles are resonance mass filters. They consist of four parallel rods in a square configuration

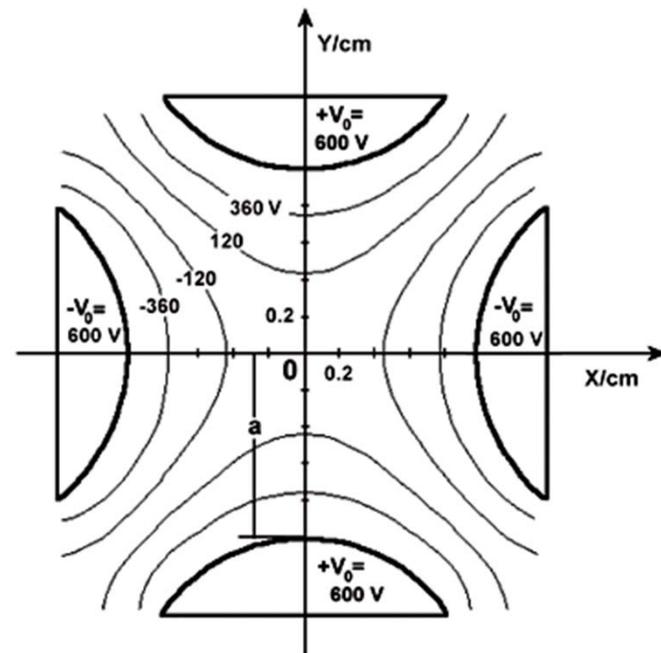
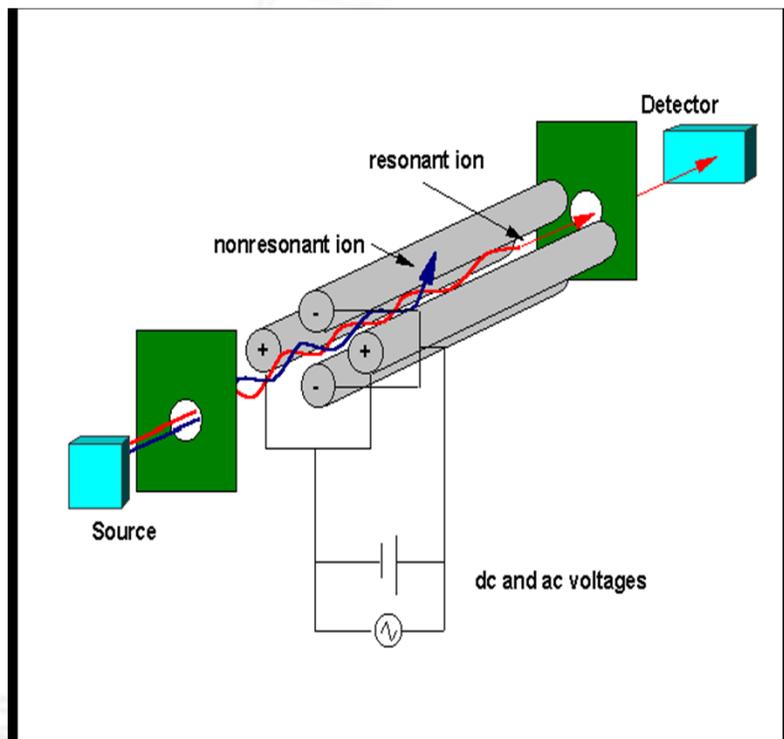
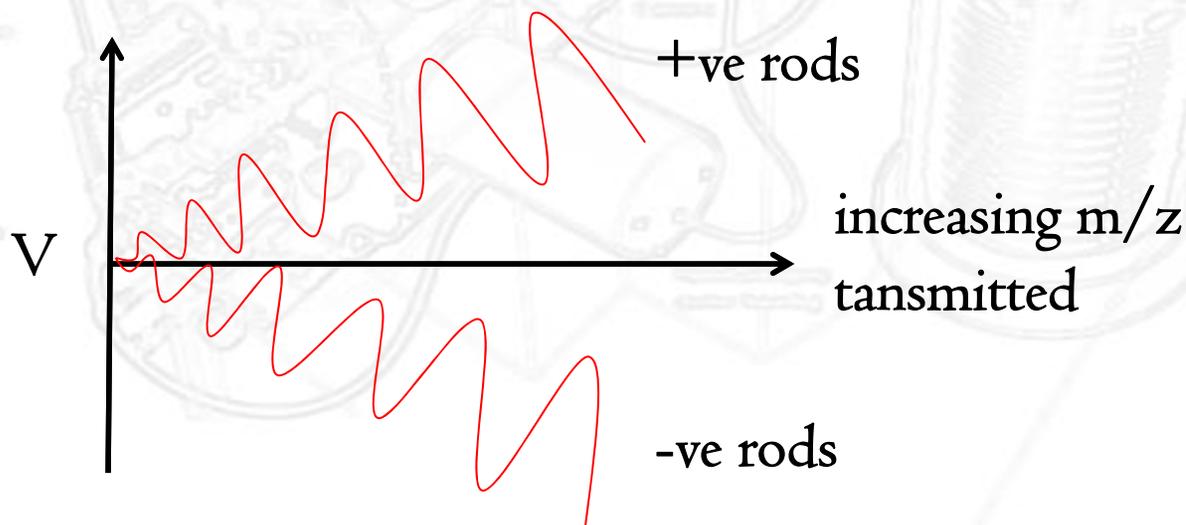


Figure 1. Electrodes and equipotential lines in an electrostatic quadrupole.

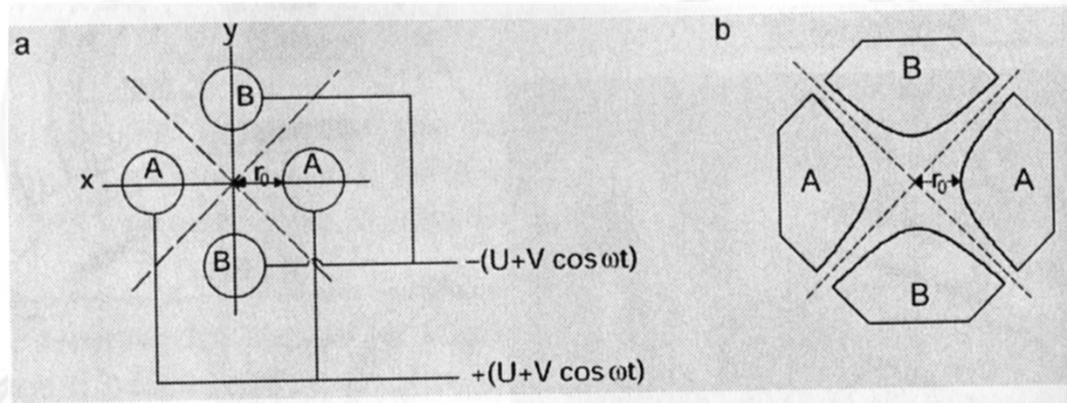
The Quadrupole Field

- The quadrupole field used in mass analyzers is a combination of a direct current (DC) and an alternating current (RF).
- Each m/z will have a range of DC and RF values in which it's trajectory through the quadrupole is stable.
- In a typical quadrupole scan, the DC and RF voltages are ramped together at a particular ratio, which has the effect of letting one m/z through at a time:



What Ions Feel in a Quadrupole

- Ions in a linear quadrupole mass analyzer are exposed to the following fields:



- As a result, they will move in the following way (these ‘equations of motion’ are called the **Paul** equations):

$$\frac{d^2x}{dt^2} + \left(\frac{2qU}{m r_0^2} - \frac{qV \cos \omega t}{m r_0^2} \right) x = 0$$

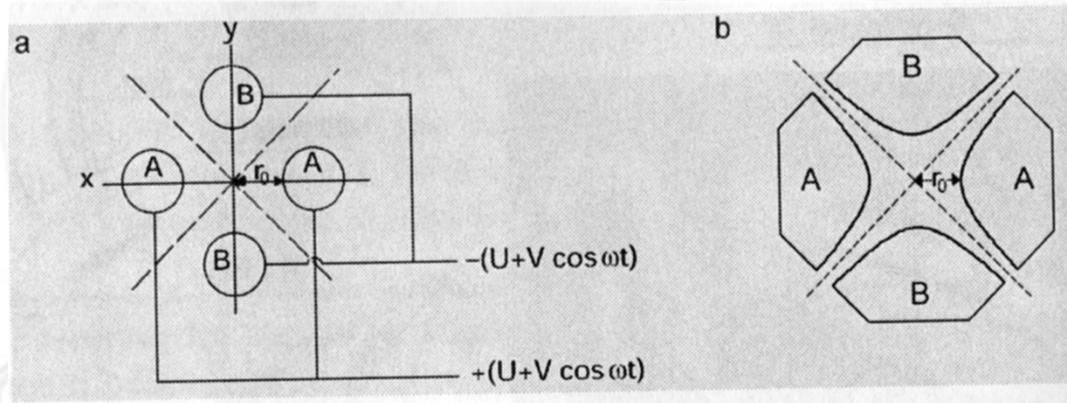
$$\frac{d^2y}{dt^2} + \left(\frac{2qU}{m r_0^2} + \frac{qV \cos \omega t}{m r_0^2} \right) y = 0$$

Frequency of RF



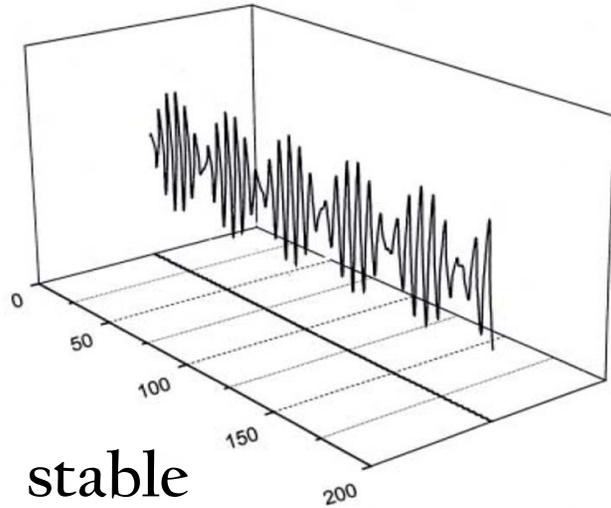
Stability in the Quadrupole

- For an ion to have a stable trajectory, its x or y position must never exceed r_0 (distance from the center to edge of rod).

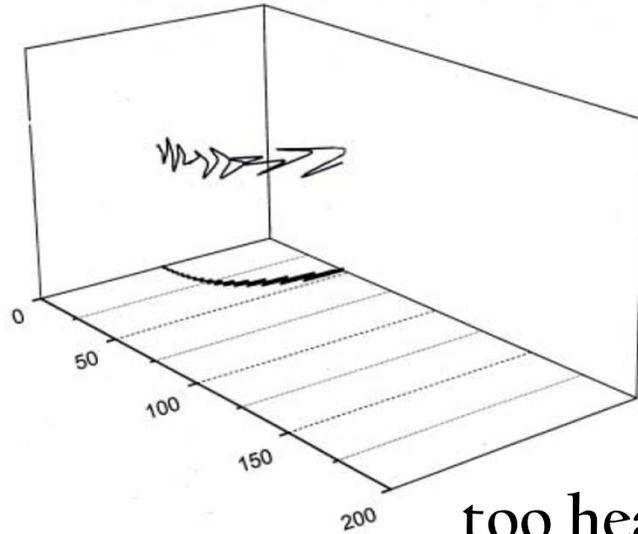


- To determine if an ion is stable, we could integrate the Paul equations over the amount of time t that it takes to traverse the quadrupole ($t = l / v_z$), however, direct integration of the Paul equations is difficult.
- Instead, we need to change the ‘space’ of our analysis so that we’re not dealing with time, but rather a **number of oscillations**.

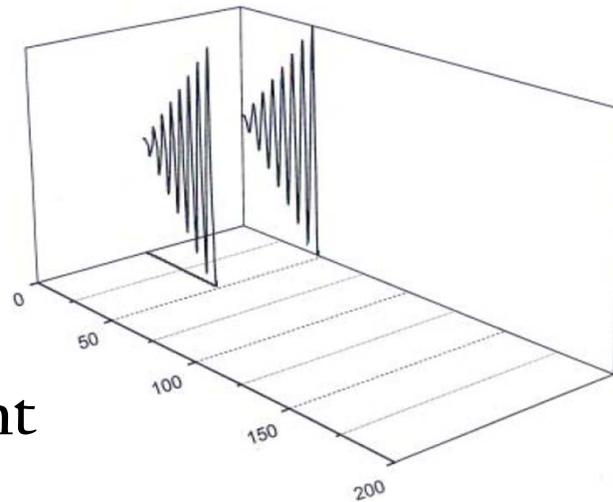
Stable vs. Unstable trajectories



stable



too heavy



too light

The Mathieu Equations

- This was worked out in 1866 by **Walter Mathieu**, who was working on **propagating waves in drums**.
- To change space (transform), we need to describe the time spent in the quadrupole in terms of number of oscillations by introducing a new term ξ :

— and —

- Incorporating ξ and rewriting the equations of motion:

$$— \left(\begin{array}{c} () \\ () \end{array} \right) \quad — \left(\begin{array}{c} () \\ () \end{array} \right)$$

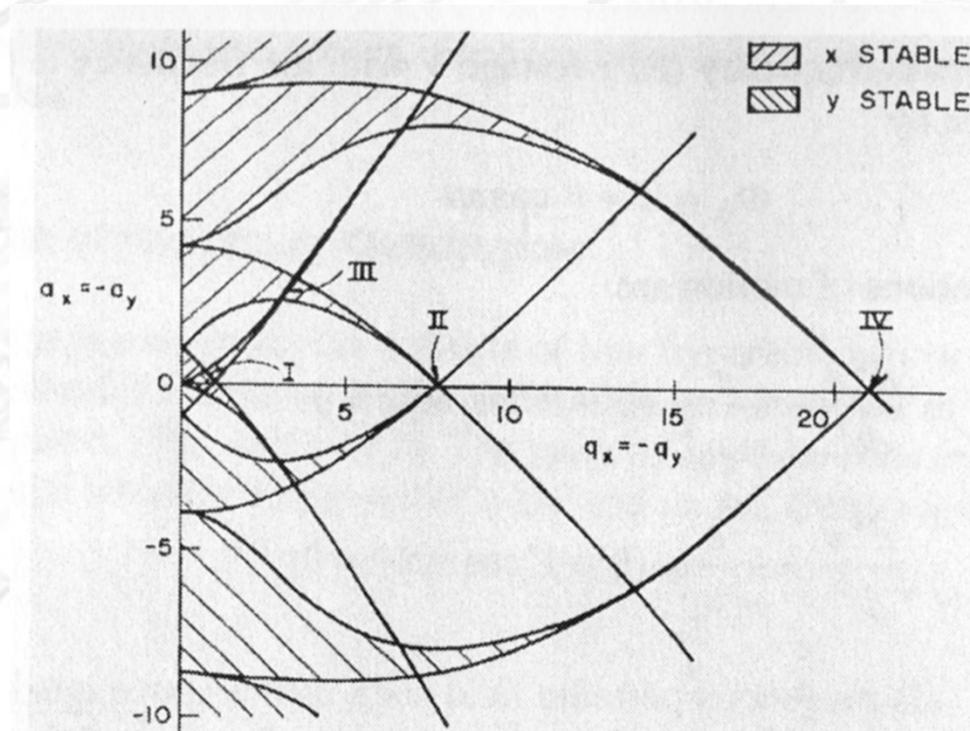
The a terms and q terms

- You may noticed that the a terms have something to do with the DC voltage U and the q terms have something to do with the RF voltage V .

- Notice that the direct current term is multiplied by 8 whereas the RF term is only multiplied by 4. This implies that the DC voltage has a greater influence on the ion flight path than the RF on a V/V basis.

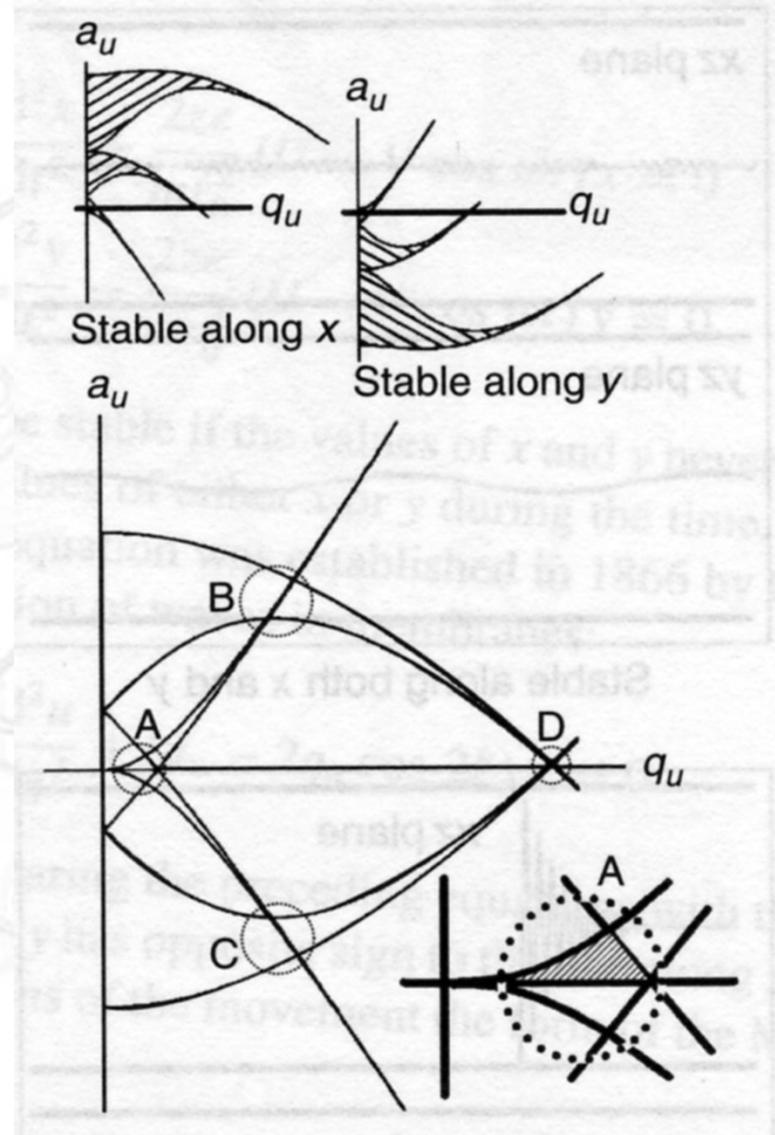
Stability Regions

- By integrating the Mathieu equation (see slide 22), we can determine which values of a and q lead to stable trajectories (i.e. both x and $y < r_0$ for the length of the quadrupole)
- If make a plot of a vs. q , and shade in all of the regions that correspond to a stable trajectory, we get:



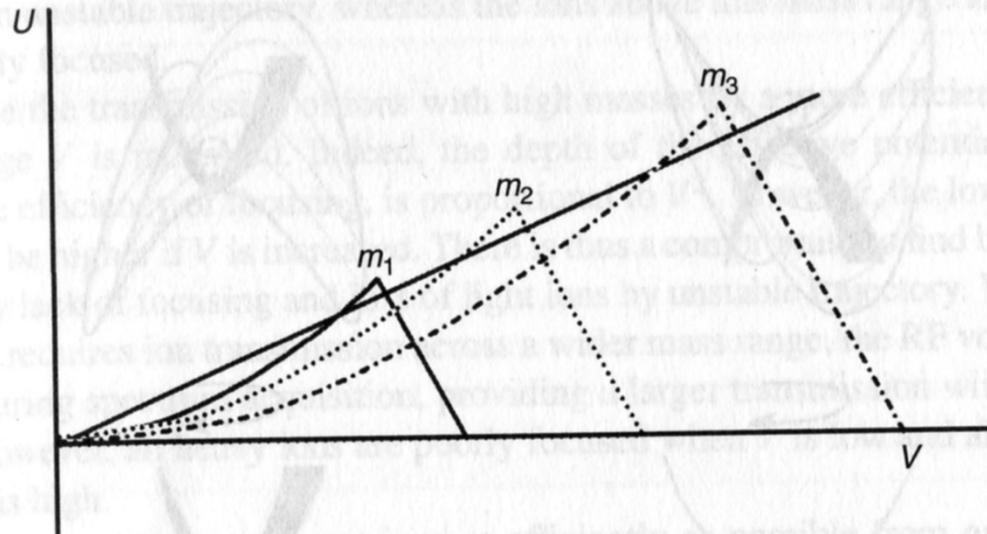
Stability Regions Con't

- Note that the ion must be stable in the xz plane and the yz plane. Therefore, we are looking for regions where $a_x/-a_y$ and $q_x/-q_y$ intersect.
- This gives 4 possible 'stable regions':



The Best Stability Region

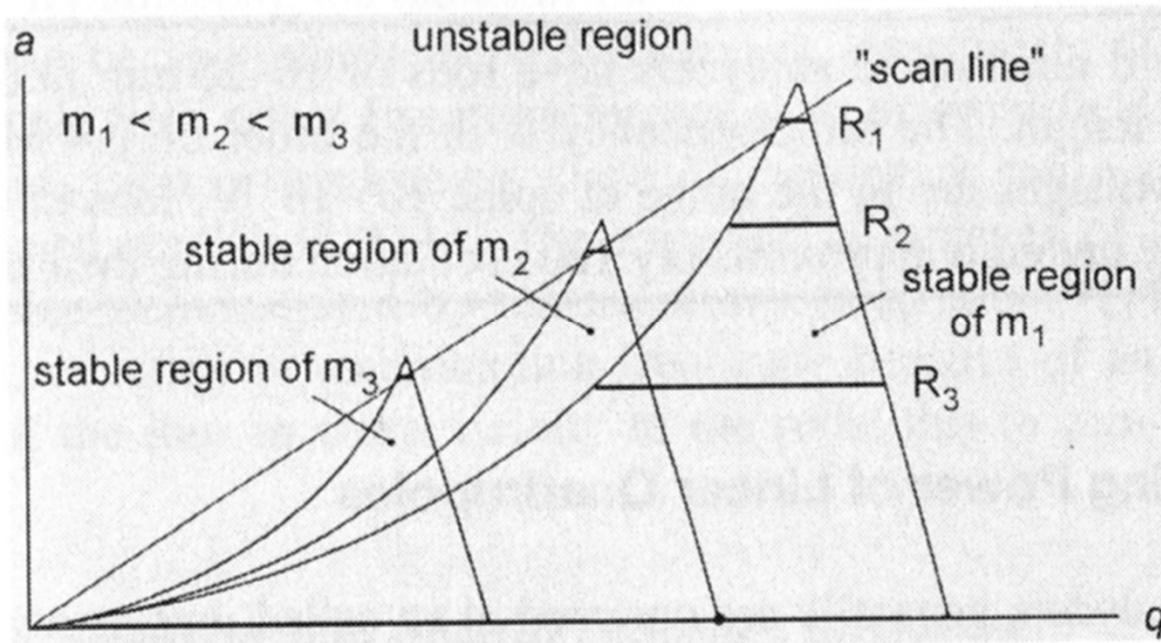
- So that we don't have to use huge voltages, we want the stability region closest to 0,0.
- This region looks like a triangle that varies in size depending on the m/z in question:



- So to conduct a scan in which I allow progressively larger masses through, I have to **ramp U and V together** in a certain ratio which gives the slope on the above plot.

Resolution in Quadrupoles

- Resolution in a quadrupole is determined by how close we can get our line to the 'tip' of the triangular stability region

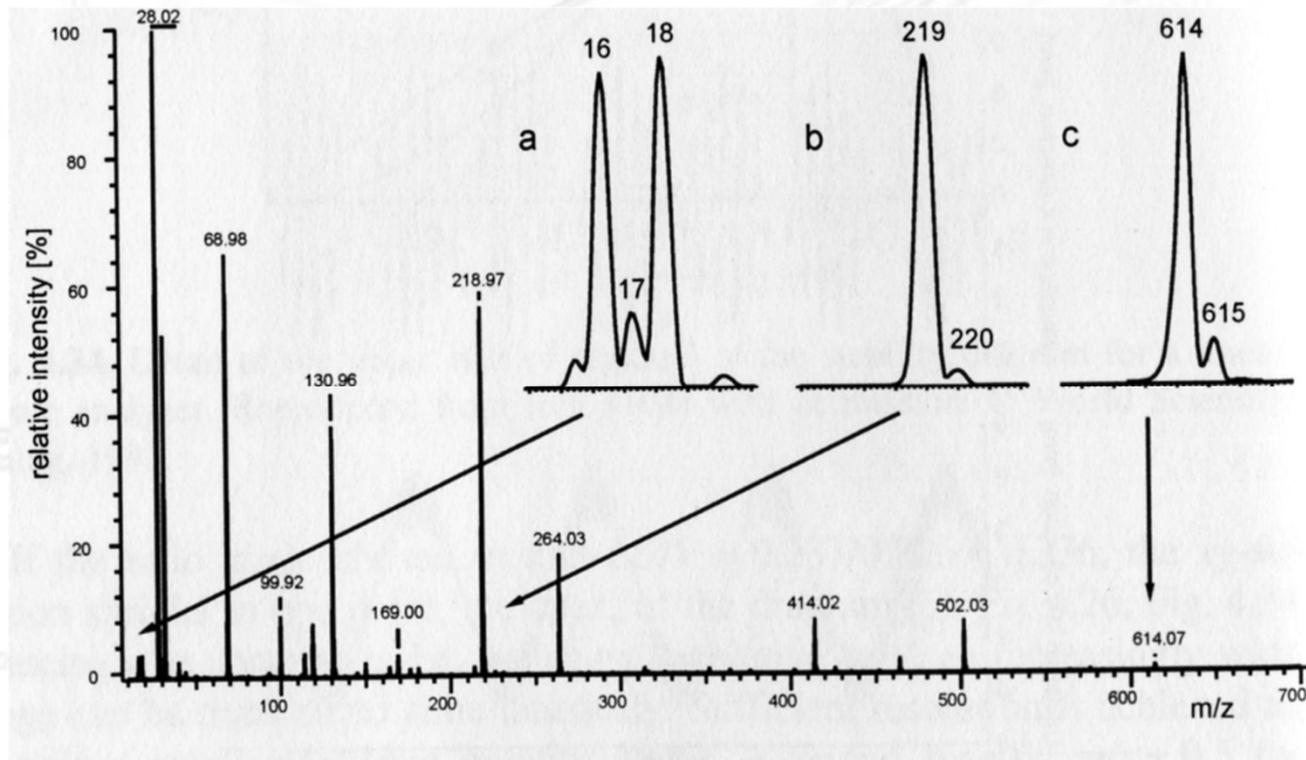


This graph is wrong!!

- Of course, ions with slightly different kinetic energies or initial paths might be lost if we're too choosy, so high resolution comes at a cost to sensitivity.

Resolution in Quadrupoles Cont.

- In practice, what this means is that quadrupoles tend to have 'unit' resolution across their entire mass range:

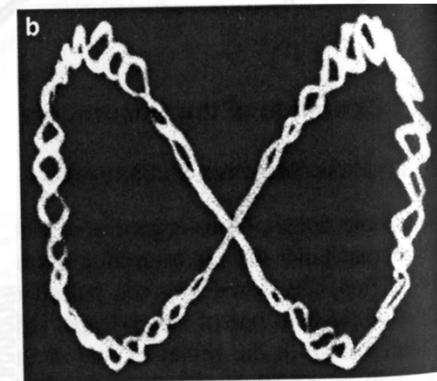
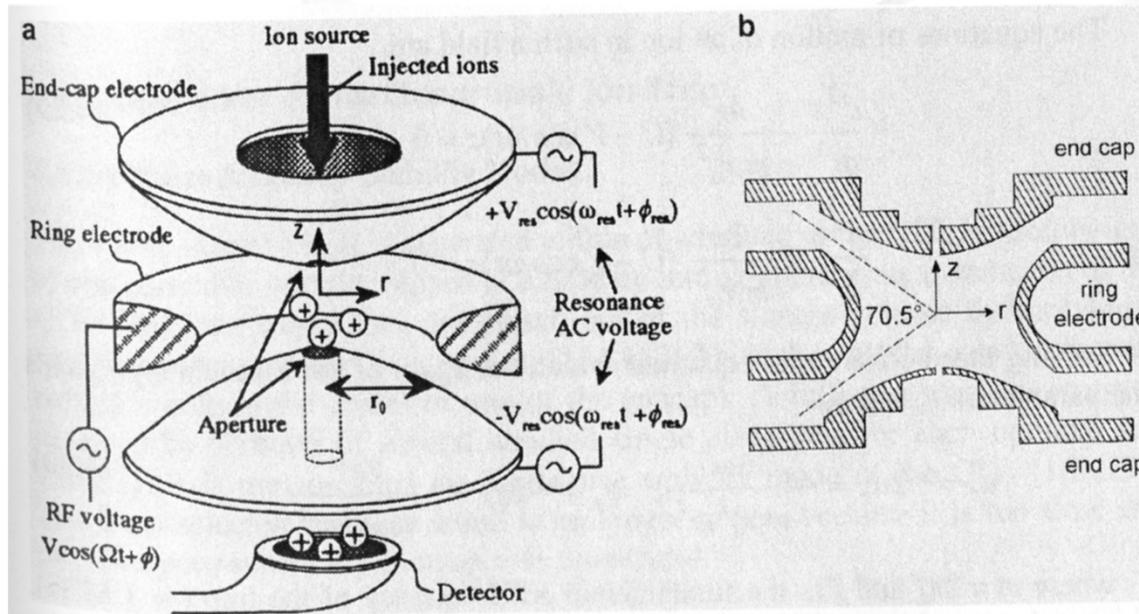


RF-only Quadrupole Ion guides

- Note that if you set the DC voltage to 0, a quadrupole will simply transmit any ion that passes through, providing some spatial focusing.
- In theory, any ion can be transmitted, but in practice, the limitation is around 30,000 m/z at normal RF frequencies. Larger ions can be transmitted (and even mass analyzed) using low, frequency, high power RF (see Carol Robinson).
- Ion guides can be used for **collisional cooling**, in which an ion with an initially high amplitude oscillation is damped by **low energy collisions** with neutral gas.

Quadrupole Ion Traps

- Quadrupole (Paul) ion traps consist of a ring electrode with end caps on the top and bottom.



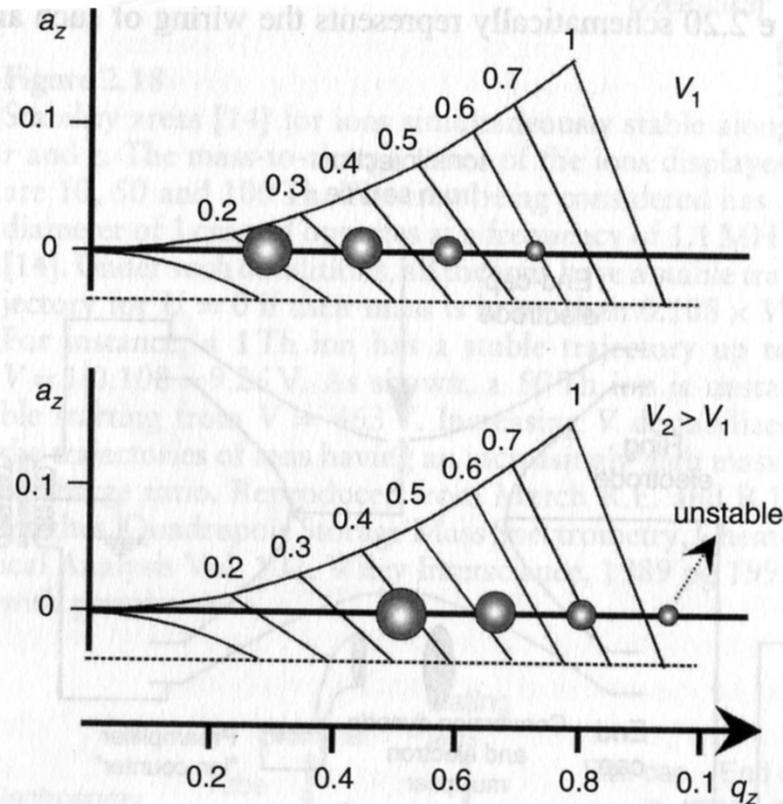
- The result is a '3D quadrupolar field' with a null in the dead center. In one plane, ions in the trap have a 'figure 8'-like orbit.

Stability in a 3D Ion Trap

- Stability of an orbit in the ion trap can be determined more or less in the same way that it is for linear quadrupoles (we won't go through it again)
- However, this time our goal will be to overexcite ions with specific masses, ejecting them from the trap.
- To do this, we make the DC portion of the field 0, going 'RF only'.
- We also need to reverse how we've been looking at our plot, noting that q is also a function of m/z . Higher m/z = lower q at the same voltage. Higher voltage = higher q for all ions!

Ion Traps as Mass Analyzers

- OK, so imagine a set of ions in the trap at a particular RF voltage



- When we increase V , q increases for all ions, and the lighter ones fall outside the stability region.

Ion Traps as Mass Analyzers, Cont.

- It turns out that for Paul ion traps, an ion is stable at or below $q_z = .908$.
- This means we can easily calculate the voltage required to observe a particular mass, or, as below, the maximum observable singly charged mass on an instrument that can put 8000V RF on the end caps:

()

()()

Calculating q_z : Example

- Let's say we have a trap mass spectrometer with trap parameters $r_0=7$ mm and $z_0=7.8$ mm. We want to figure out q_z for our favorite peptide ion $[\text{DEREK}+\text{H}]^+ = 676.7$ g/mol. Our RF frequency is .76 MHz.

$$\frac{(\quad)}{(\quad)}$$

- First we have to establish the trap geometry parameters **in m**:

$$\left(\left(\quad \right) \left(\quad \right) \right) \quad \text{m}^2$$

- Also need to convert RF frequency **in Hz** into angular frequency:

$$\left(\quad \right) \quad \text{rad}\cdot\text{s}^{-1}$$

Calculating q_z : Example Cont.

- We also need the mass of the ion **in kg** = (see slide 9)

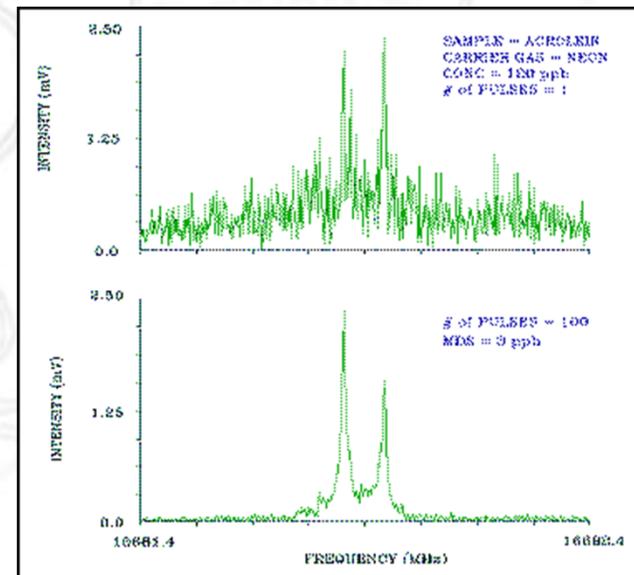
- And now we're ready:

$$\frac{\left(\frac{\text{C}}{\text{mol}} \right) \left(\frac{\text{mol}}{\text{kg}} \right)}{\left(\frac{\text{C}}{\text{mol}} \right) \left(\frac{\text{mol}}{\text{kg}} \right)}$$

- In other words, the ion is **trapped!**

Resolution in an Ion Trap

- In an ion trap, resolution is determined primarily by your **scan speed**, *i.e.* how quickly you decrease the RF.
- Another important consideration is **space-charge effects**: The ‘cloud’ of ions at the center of the trap will repel itself and spread out over time. This hurts resolution since it introduces non m/z -related variation in kinetic energy within the trap.
- The solution to the above problem is twofold: *i*) Don't have too many ions in the trap. *ii*) Take away the extra kinetic energy by collisions with neutral bath gas (usually He).



Mass Analyzer = the ♥ of the Mass Spectrometer

- The mass analyzer is what makes a mass spectrometer a mass spectrometer.

