Lecture 2

Mass Analyzers for Protein Analysis

Nancy Allbritton, M.D., Ph.D.
Department of Physiology & Biophysics
824-9137 (office)
nlallbri@uci.edu

Office- Rm D349
Medical Science D Bldg.

Mass Analyzers- Measure m/z

Properties of Analyzers:

1. Upper Mass Limit (Mass Range)- highest measurable m/z

2. Ion Transmission (Sensitivity)- ratio of ions reaching detector to that produced at the source

3. Mass Accuracy- difference between theoretical & measured mass
   mass accuracy = $(m_{real} – m_{measured})/m_{real}$
   express as ppm

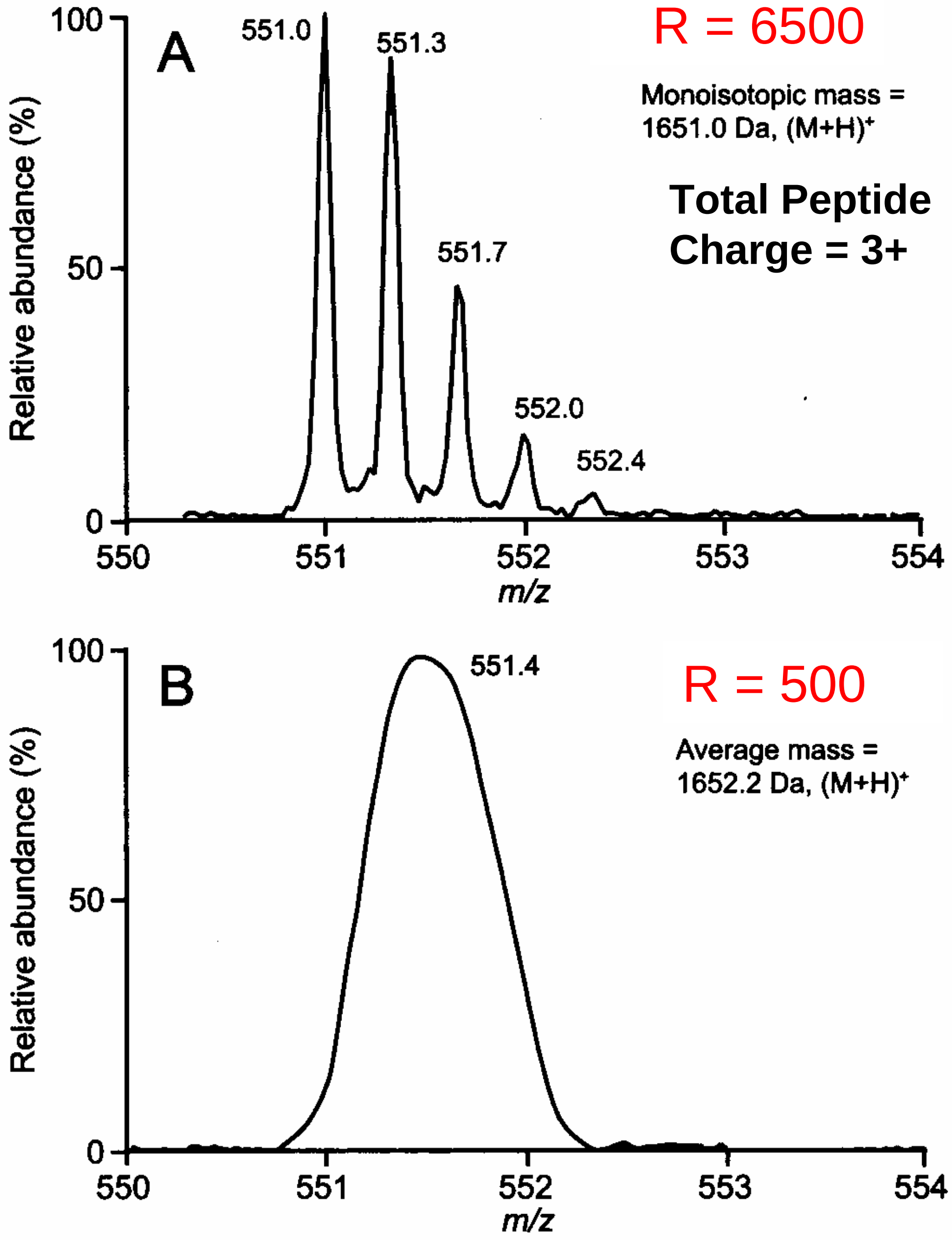
Ex: measured mass = 1000.2 real mass = 1000
$\Delta m = 0.2$
mass accuracy = 0.2/1000
= 200/1,000,000
= 200 ppm

Mass Analyzers

Properties of Analyzers:

4. Mass Resolution-
ability to resolve 2 ions
with a small mass difference

Resolution = R = m/Δm
Δm = mass difference of
 2 nearby masses,
 m & (m+Δm),
 with a signal
 overlap of 10%



R = 6500

Total Peptide Charge = 3+

R = 500

Mass Analyzers for Proteins/Peptides

Types of Analyzers:

1. Quadrupole Analyzer- R ~ 3000; m/z < ~2000;
   mass accuracy ~400 ppm, poor sensitivity

2. Ion Trap- R ~ 5000; m/z < ~2000;
   mass accuracy ~200 ppm; excellent sensitivity

3. Time of Flight (TOF)- R ~ 10,000; m/z < 500,000;
   mass accuracy ~10 ppm; very good sensitivity

4. Fourier-Transform Ion Cyclotron Resonance (FTICR)
   R ~ 1,000,000; mass accuracy ~1 ppm;
   Very expensive (limited availability);
   Ions are confined in a high B field;
   B field is created by a superconducting magnet;
   Circling frequency of ions ~ z/m

Time of Flight (TOF) Analyzer

Principle:

For a constant kinetic energy, different masses move at different v.

1. Accelerate a bolus of ions with a potential V_s-

$$\text{Energy}_{\text{kinetic}} = mv^2/2 = qV_s$$

for m = mass; v = velocity

q = charge = z e

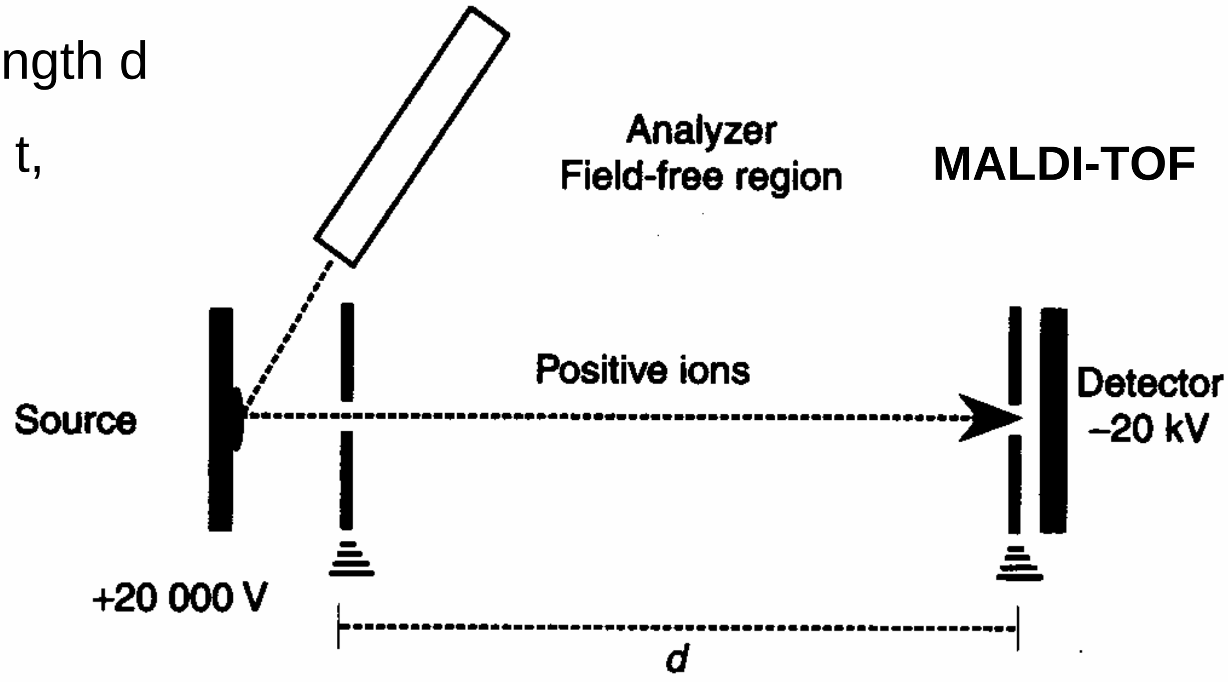
2. Ions then enter a field-free region of length d

3. Measure the time, t, for the ions to cover the distance, d

$$v = d/t$$

Solve for t-

$$t^2 = (m/z)(d^2/2V_se)$$



MALDI-TOF

Time of Flight Analyzer

Example:

V_s = 20,000 V;   Drift Tube- d = 1 meter
m = 500 D with a single charge
Then
$v = 9 \times 10^4$ m/s
$t = 1 \times 10^{-5}$ s = 10 μs

Advantages:

1. No theoretical mass limit (in practice $\leq$500 kD)

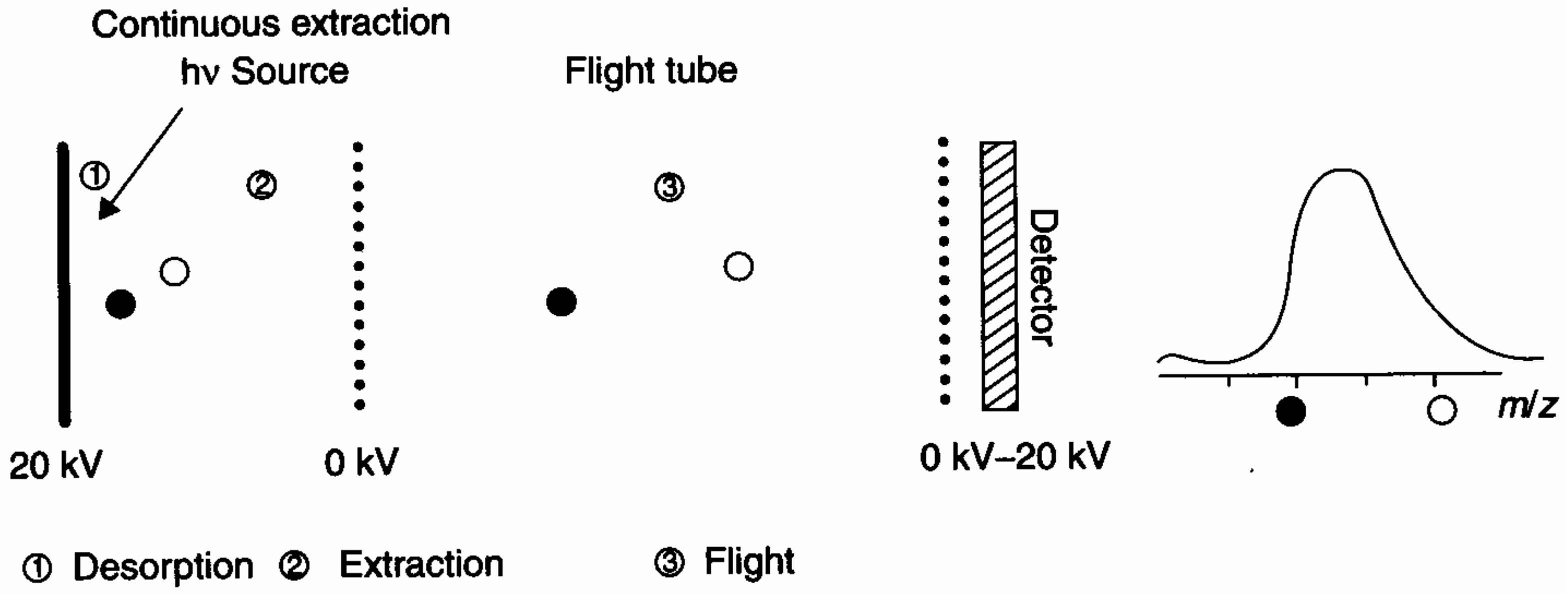
2. Very high transmission efficiency *i.e.* sensitivity
   (attomole detection limits)

3. Typically combined with MALDI (ns-length ion pulses)

Improving TOF Resolution

Can Have Poor Mass Resolution Due To:

1. Temporal distribution of ions *i.e.* varying times of ion formation
2. Spatial distribution of ions *i.e.* ions form at different locations
3. Kinetic energy distribution *i.e.* variation in ion energy

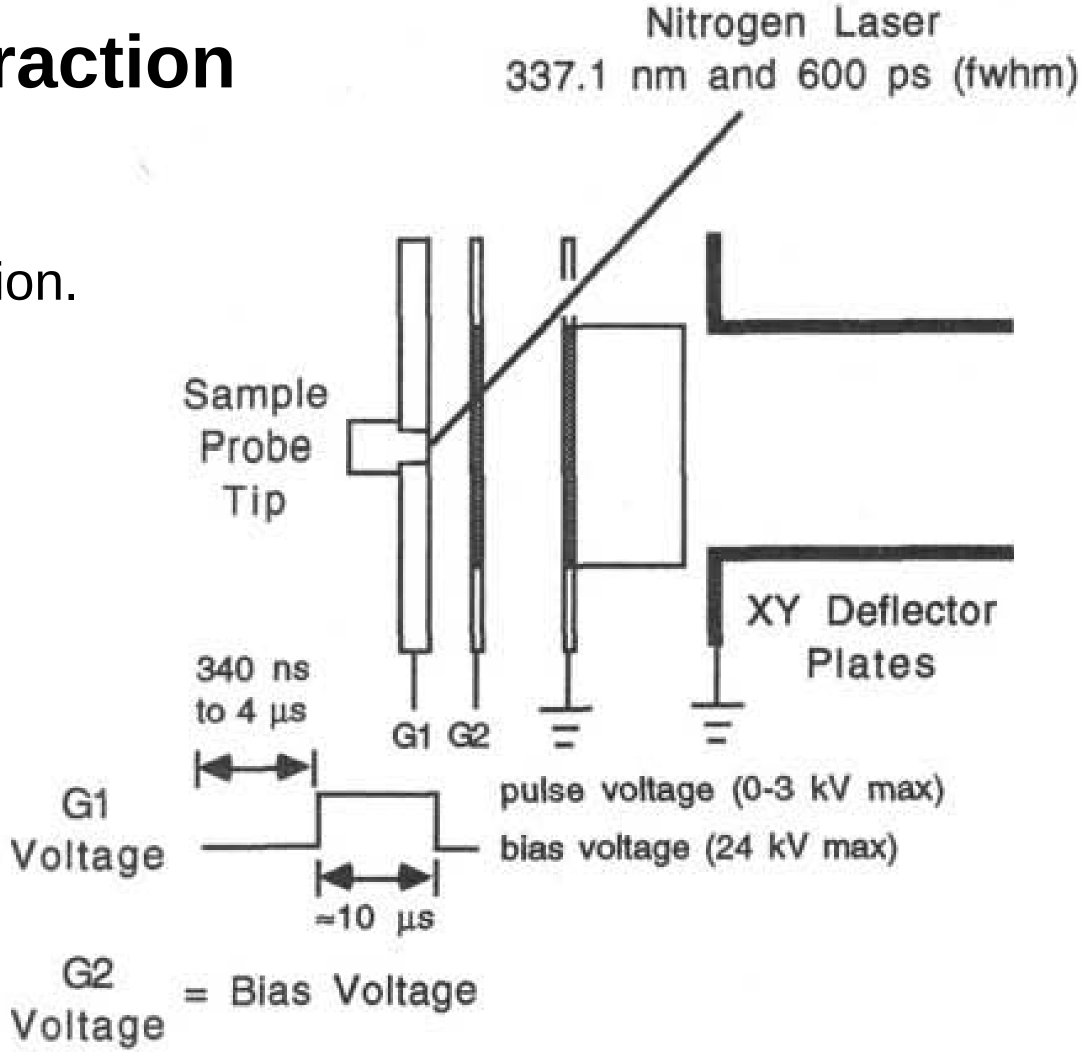


Solutions: 1. Delayed Pulse Extraction

2. Reflectron

Delayed Pulse Extraction

1. Allow ions to form and expand in a field-free region.
2. After a time delay (ns-μs), apply a voltage.
3. Apply a potential gradient across the source.



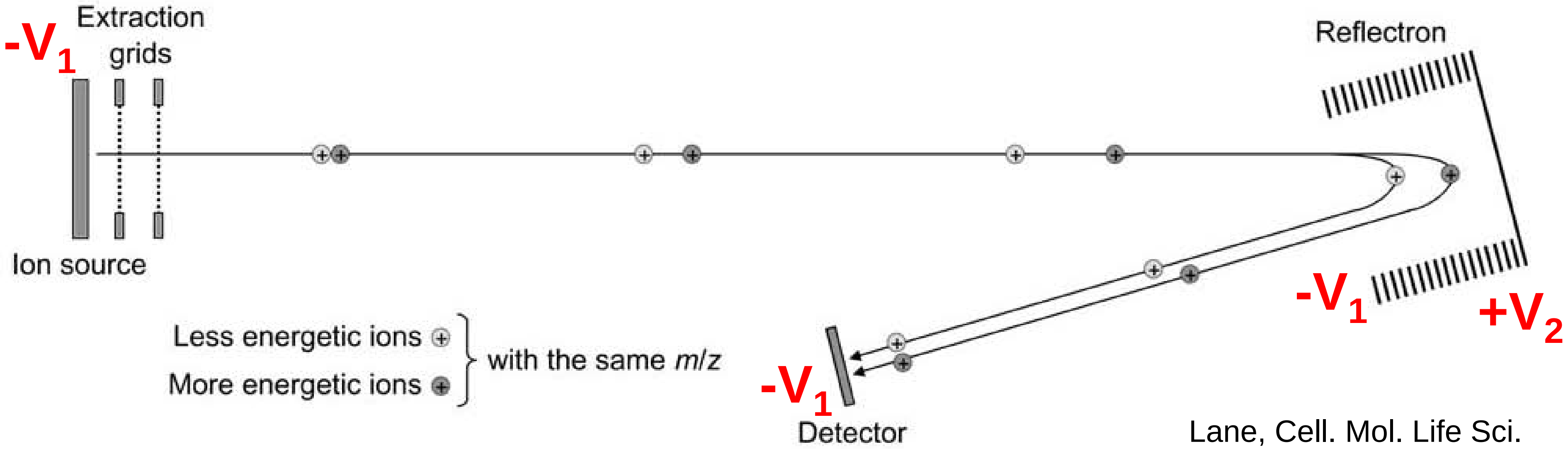
Effects

1. Delay- All ions have formed at time of voltage pulse.
2. Faster ions are farther into the instrument- will acquire less energy
3. Slower ions acquire more energy

Brown & Lennon, Anal Chem. 1995, 67:1998

Reflectron

Electrostatic Reflector- Retarding field reflects ions & returns them.
Corrects energy dispersion for ions with same m/z.



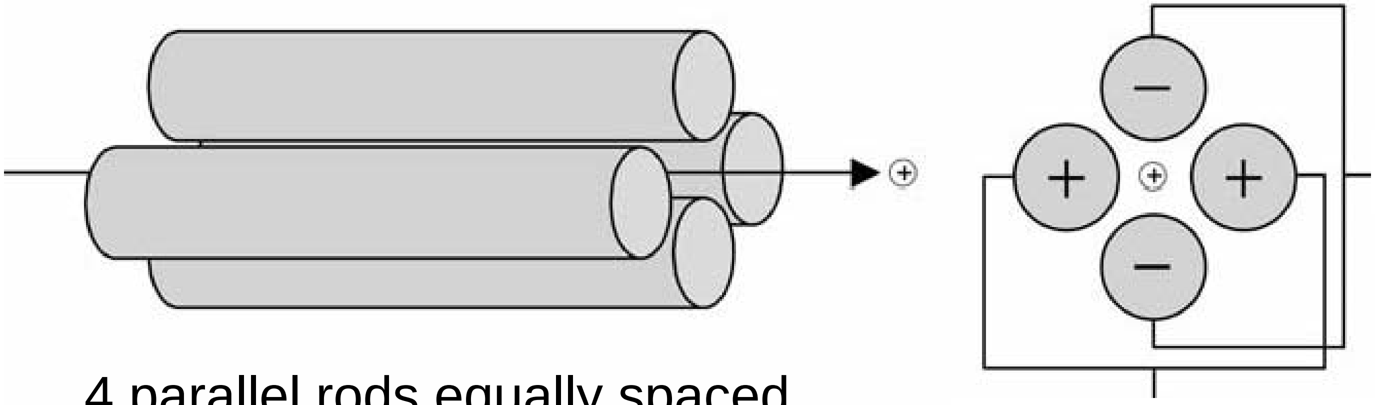
Lane, Cell. Mol. Life Sci. 2005, 62:848

Ions with hi'er kinetic energy penetrate deeper & stay in reflectron longer.

Increases Resolution but Decreases Sensitivity

Quadrupole Mass Analyzer

Separates ions by their stability in an oscillating E field.



$V(t) = -V_{dc} + V_{rf}\cos\omega t$

4 parallel rods equally spaced

$V(t) = V_{dc} + V_{rf}\cos\omega t$

V_{dc} = constant potential applied to the rods
V_{ac} = peak-to-peak amplitude of the AC or rf voltage
$\omega = 2\pi\gamma$ = angular frequency (rad/s)
γ = frequency of rf field
$2r_o$ = distance between opposing rods

Typically: $500V \leq V_{dc} \leq 2000V$ & $0 \leq V_{rf} \leq 3000V$
γ = 1-2 MHz

Equations for Ion Trajectory In A Quadrupole

$$F_x = m\,\frac{d^2x}{dt^2} = -q\,\frac{\delta V(x,y,t)}{\delta x} \qquad\qquad F_y = m\,\frac{d^2y}{dt^2} = -q\,\frac{\delta V(x,y,t)}{\delta y}$$

But- $V(x,y,t) = V(t)\dfrac{(x^2-y^2)}{r_o{}^2} = \dfrac{(x^2-y^2)}{r_o{}^2}\,(V_{dc} + V_{rf}\cos\omega t)$

$$\text{Integrate To Find X, Y Trajectories}\ \left\{\begin{array}{l} \dfrac{d^2x}{dt^2} + \dfrac{2q}{mr_o{}^2}\,(V_{dc} - V_{rf}\cos\omega t)\,x = 0 \\[2ex] \dfrac{d^2y}{dt^2} - \dfrac{2q}{mr_o{}^2}\,(V_{dc} - V_{rf}\cos\omega t)\,y = 0 \end{array}\right.$$

Ion Has A Stable Trajectory If:

$-r_o < x < r_o$ AND $-r_o < y < r_o$

Note: $q = ez$

If x, y $\geq r_o$ or x, y $\leq -r_o$, the ion hits a rod and is lost.

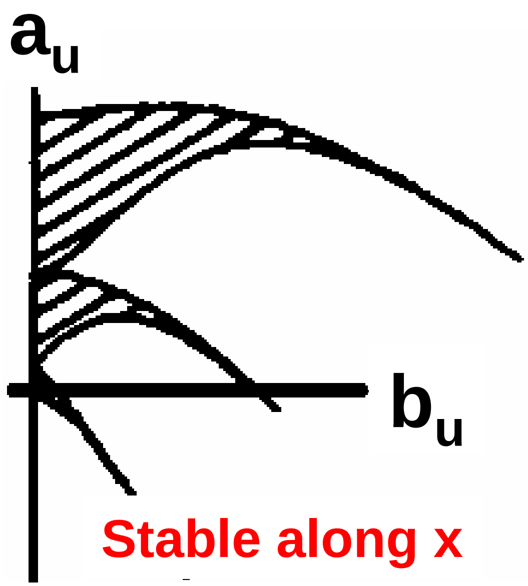
Stable Ion Motion In A Quadrupole

Perform a change of variables: $\xi = \omega t/2$
Rewrite x & y equations in the form (Mathieu Eqn):

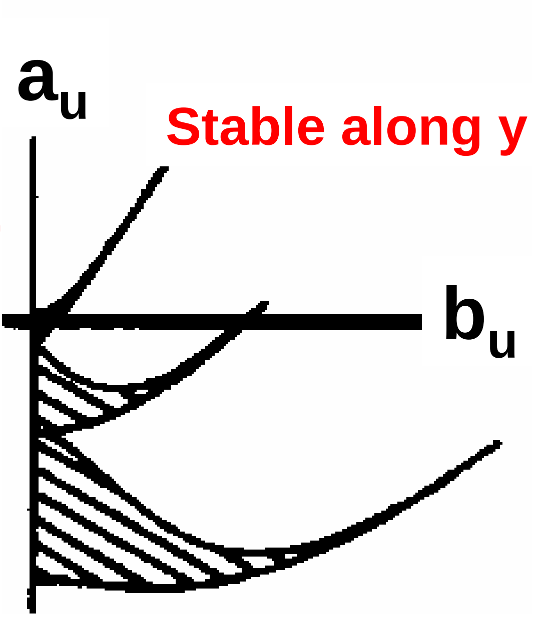
$$\frac{d^2u}{d\xi^2} + (a_u - 2b_u \cos 2\xi)\, u = 0 \qquad \text{for u being x or y}$$

Mathieu Parameters: $a_u = a_x = -a_y = \dfrac{8zeV_{dc}}{m\omega^2 r_o^2}$ & $b_u = b_x = -b_y = \dfrac{4zeV_{rf}}{m\omega^2 r_o^2}$

a & b for
$|x| < r_o$



a & b for
$|y| < r_o$

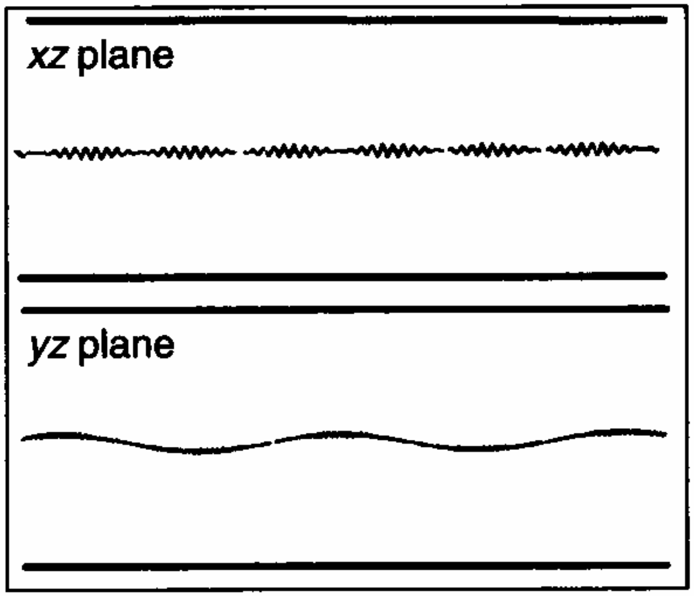


Quadrupoles Act As An m/z Band-Pass Filter

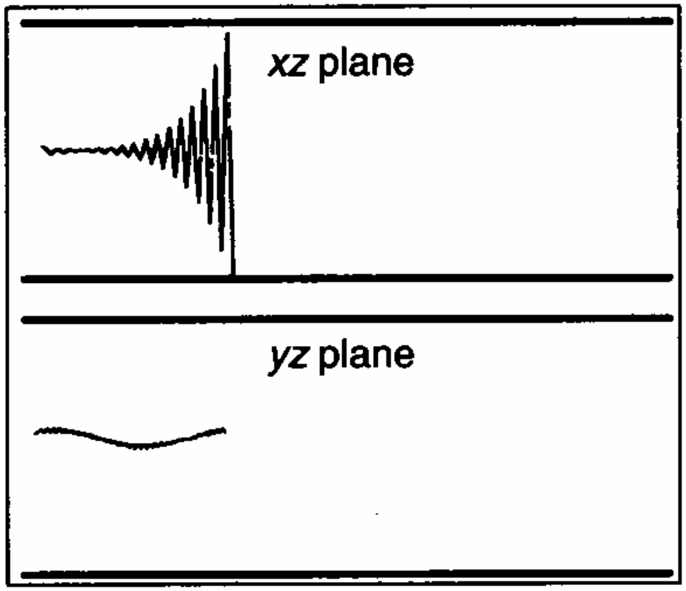
For a + ion:

1. + rods act as a high-pass m/z filter
2. - rods act as a low-pass m/z filter
3. Together + & - rods act as a band-pass filter

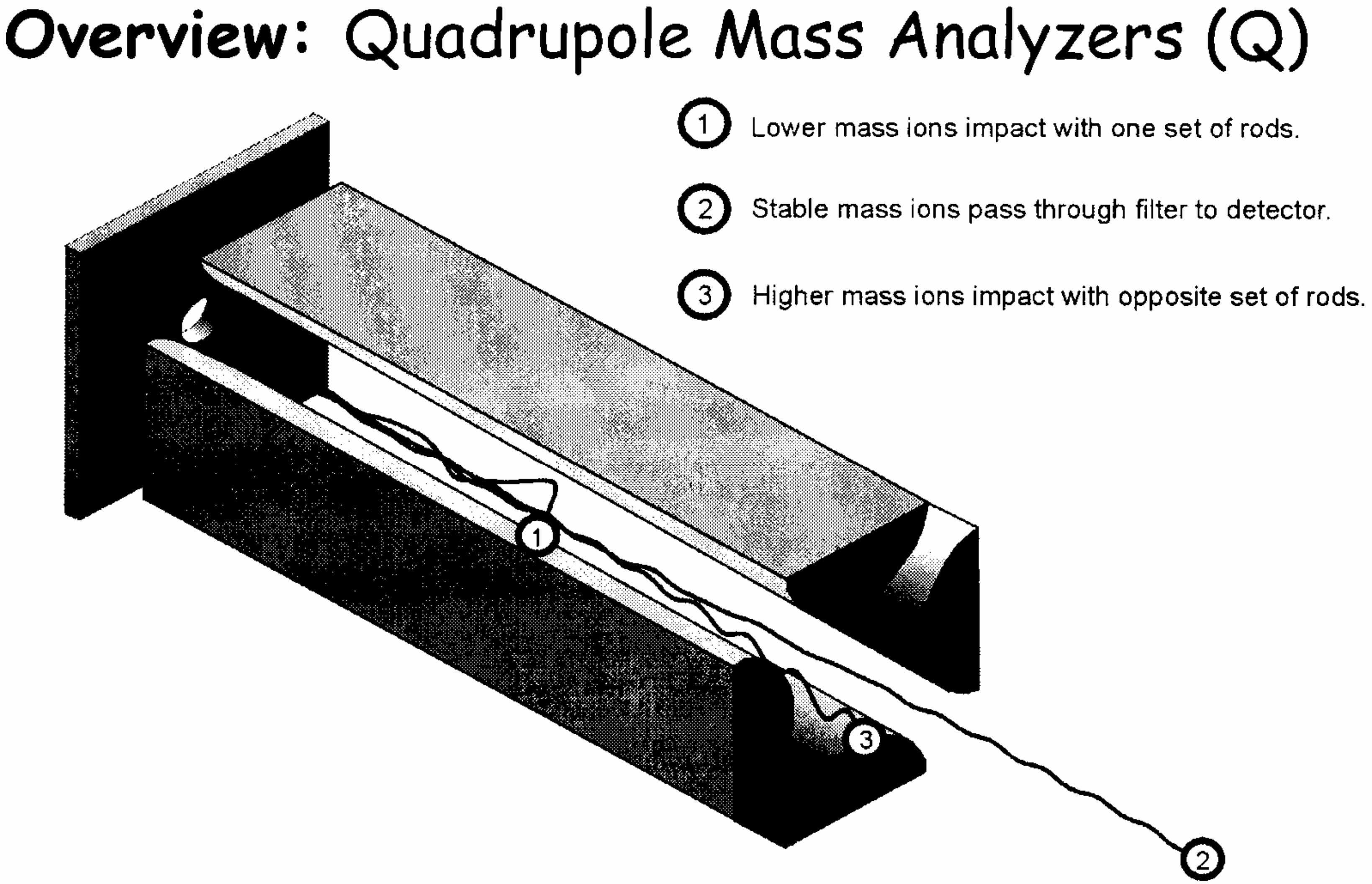
For a given V_{dc} & V_{rf}, only a small range of m/z's can pass through the rods.



Stable along both *x* and *y*



Stable along *y*, unstable along *x*



Overview: Quadrupole Mass Analyzers (Q)

① Lower mass ions impact with one set of rods.

② Stable mass ions pass through filter to detector.

③ Higher mass ions impact with opposite set of rods.

Figure 2.2- Quadrupole Mass Analyzer

Acquiring A Mass Spectrum

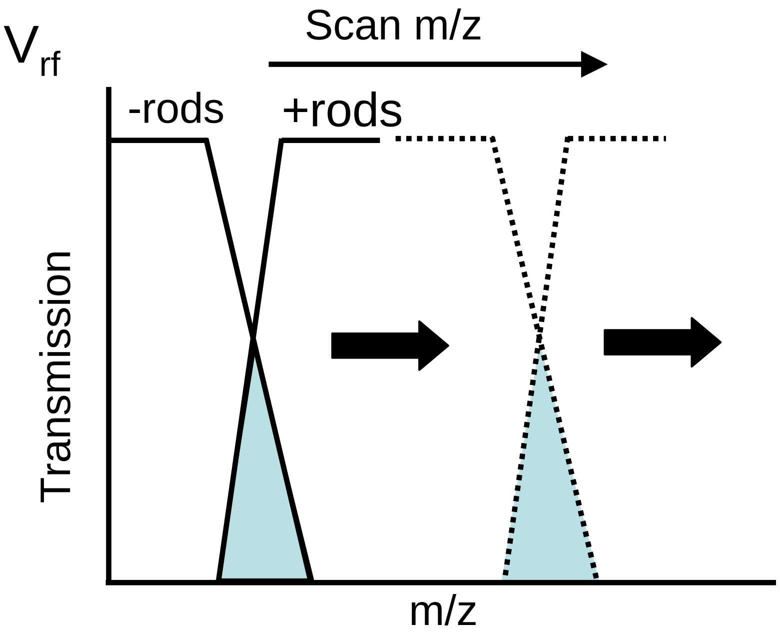
1. Higher V_{dc} & V_{rf}- Permits Higher m/z's to Pass.

   A. To Acquire Mass Spectrum- Scan V_{dc} & V_{rf} so different m/z reach the detector.

   B. Scan Rates- 1000 Th/s

      i. Scan from 200-2000 Th requires 1.8 s
         Good match for chromatography

      ii. 0.001 s at each m/z
         0.1% ions at each m/z detected
         Poor sensitivity



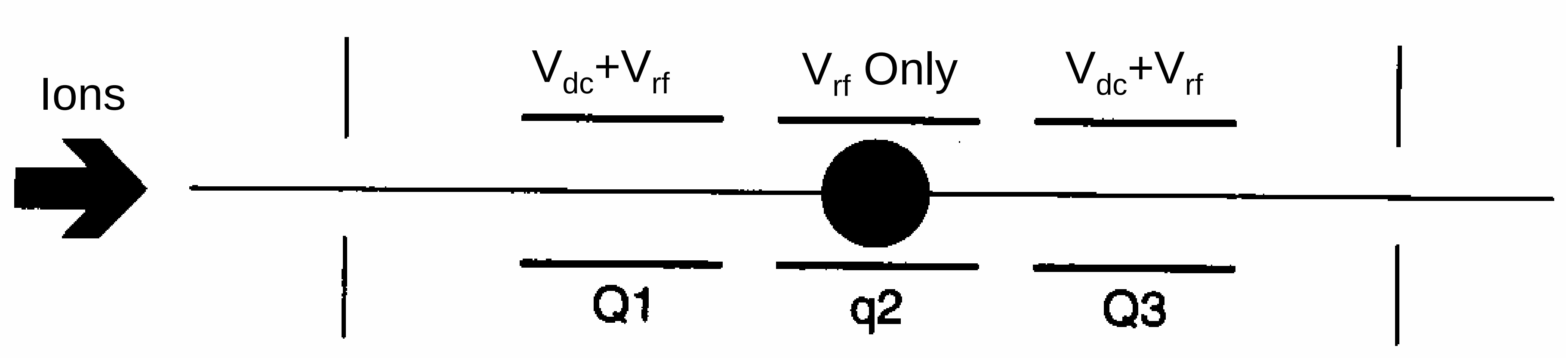
2. Increasing V_{rf}/V_{dc}- Increases Band-Pass or Decreases Resolution

   A. Typically operate at "unit resolution"
      Resolve m/z differing by 1 Th

   B. Compromise between resolution & sensitivity

RF-Only Quadrupole

1. If V_{dc} = 0, then Resolution = 0
2. V_{rf} imposes a minimum stable m/z
3. The alternating E field focuses stable ions to the center of the 4 rods
4. RF-only quadrupoles- Collision Cells

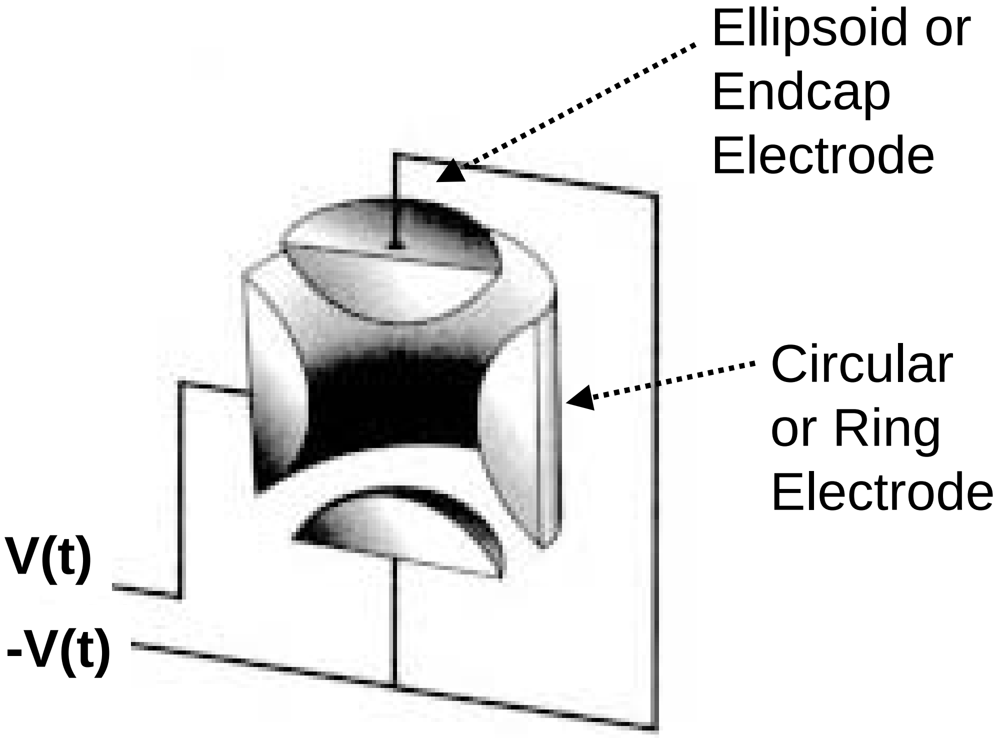


"Daughter Scan"- 1. Q1 selects ions of a given m/z
2. q2 collides ions with inert gas to fragment
3. Q3 scan m/z to analyze reaction products

Ion Traps

Quadrupole with 3-D Ion Trajectories
Circular electrode + Ellipsoid electrodes above and below

1. Apply $V(t) = V_{dc} + V_{rf}\cos\omega t$ to the Ring Electrode
2. Apply -V(t) to Endcaps
3. Inject ions through endcap.
4. For a given set of V, all ions are stable.
5. Ions oscillate in the cavity, frequency dep. on m/z



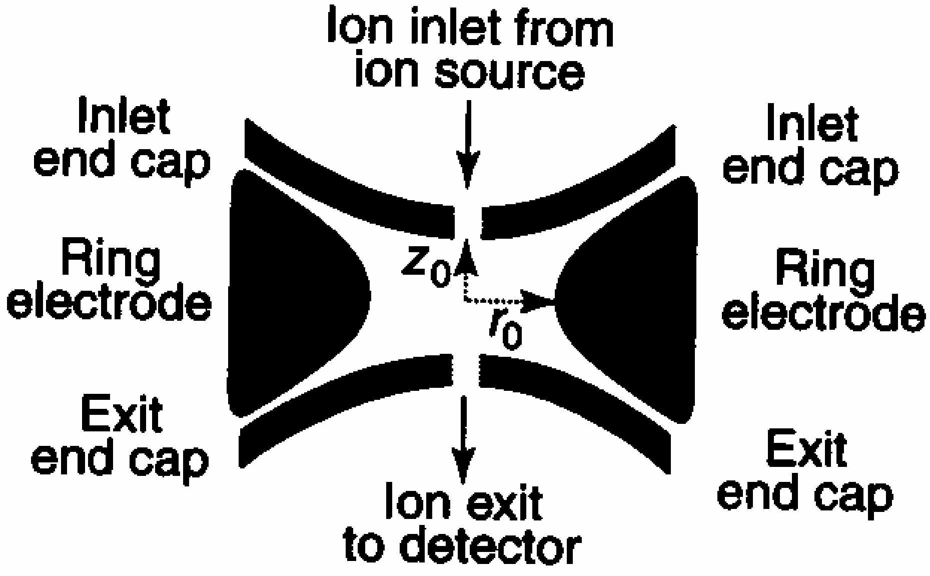
http://staging.mc.vanderbilt.edu/msrc/tutorials/ms

Stable Ion Motion In An Ion Trap

Equation of Motion:
(Cylindrical Coord.)

$$\frac{d^2r}{dt^2} + \frac{2q\,(V_{dc} - V_{rf}\cos\omega t)\,r}{m(r_o^2 + 2z_o^2)} = 0$$

$$\frac{d^2z}{dt^2} - \frac{4q\,(V_{dc} - V_{rf}\cos\omega t)\,z}{m(r_o^2 + 2z_o^2)} = 0$$



Ion Has A Stable Trajectory If:

$-r_o < x < r_o$ AND $-z_o < z < z_o$

Stable Ion Motion In An Ion Trap

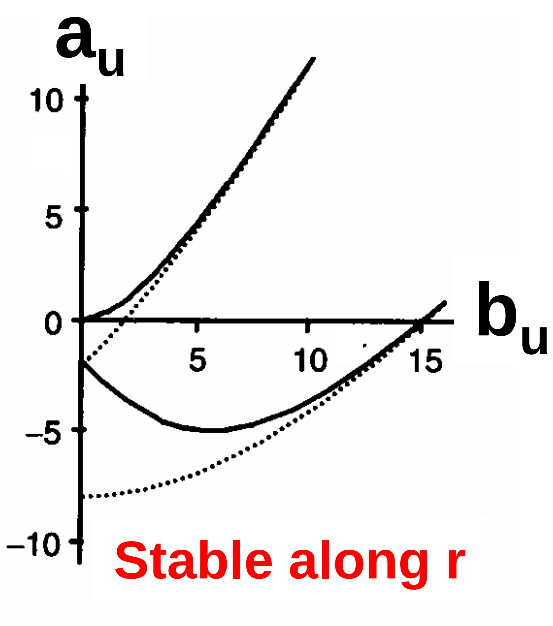
Perform a change of variables: $\xi = \omega t/2$

Rewrite r & z equations in the form of the Mathieu Eqn:

$$\frac{d^2u}{d\xi^2} + (a_u - 2b_u\cos 2\xi)\, u = 0$$ for u being r or z

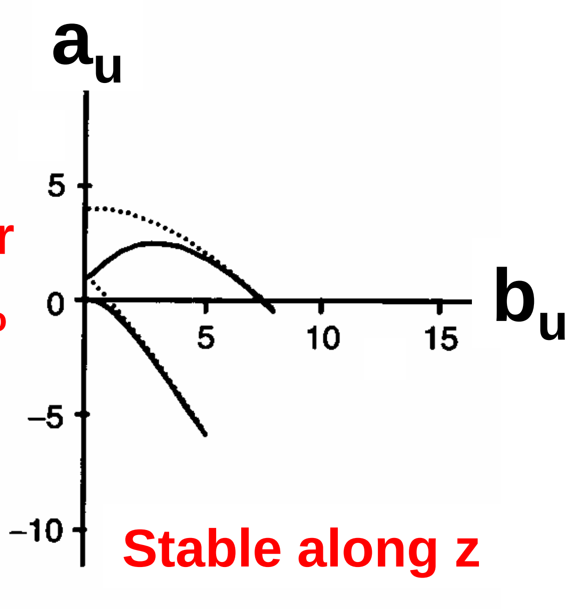
Mathieu Parameters: $a_u = a_z = -2a_r = \frac{-16qV_{dc}}{m\omega^2(r_o^2 + z_o^2)}$ & $b_u = b_z = -2b_r = \frac{8qV_{rf}}{m\omega^2(r_o^2 + z_o^2)}$

Plot for $|r| < r_o$



Stable along r

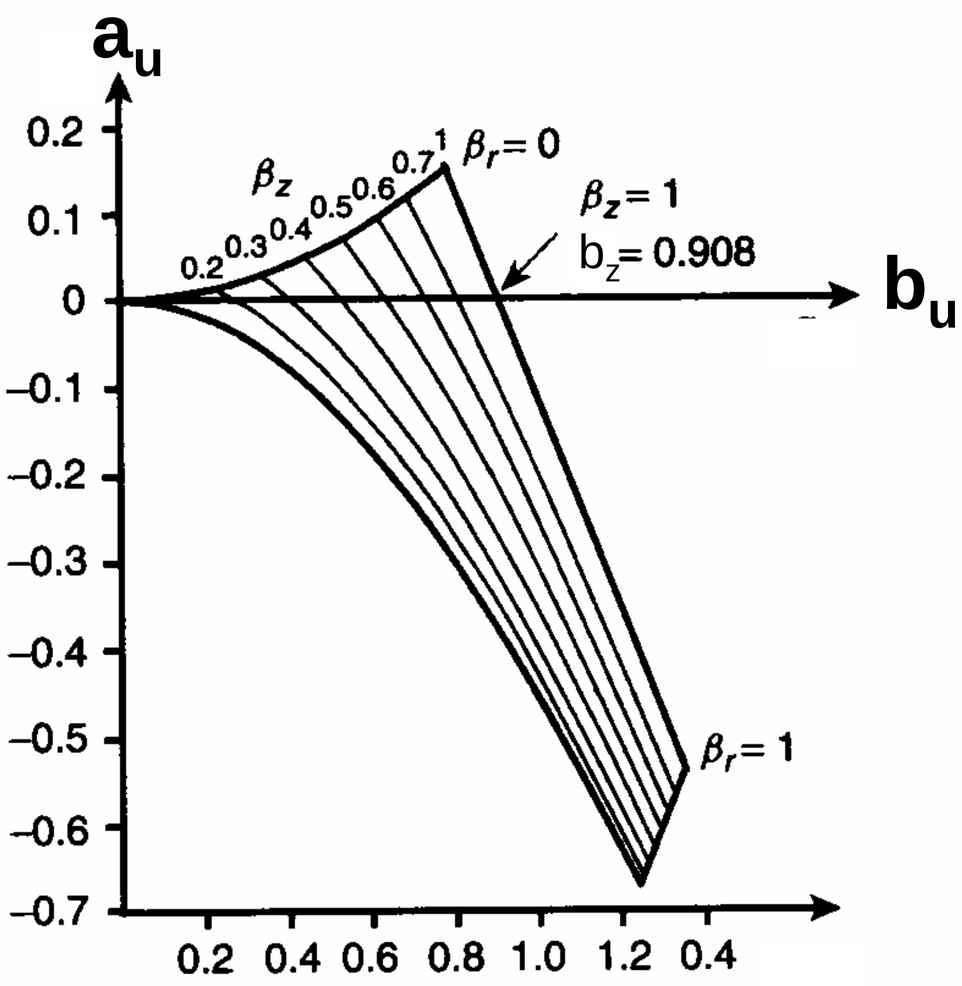
Plot for $|z| < z_o$



Stable along z

Stability Region Along z For An Ion Trap

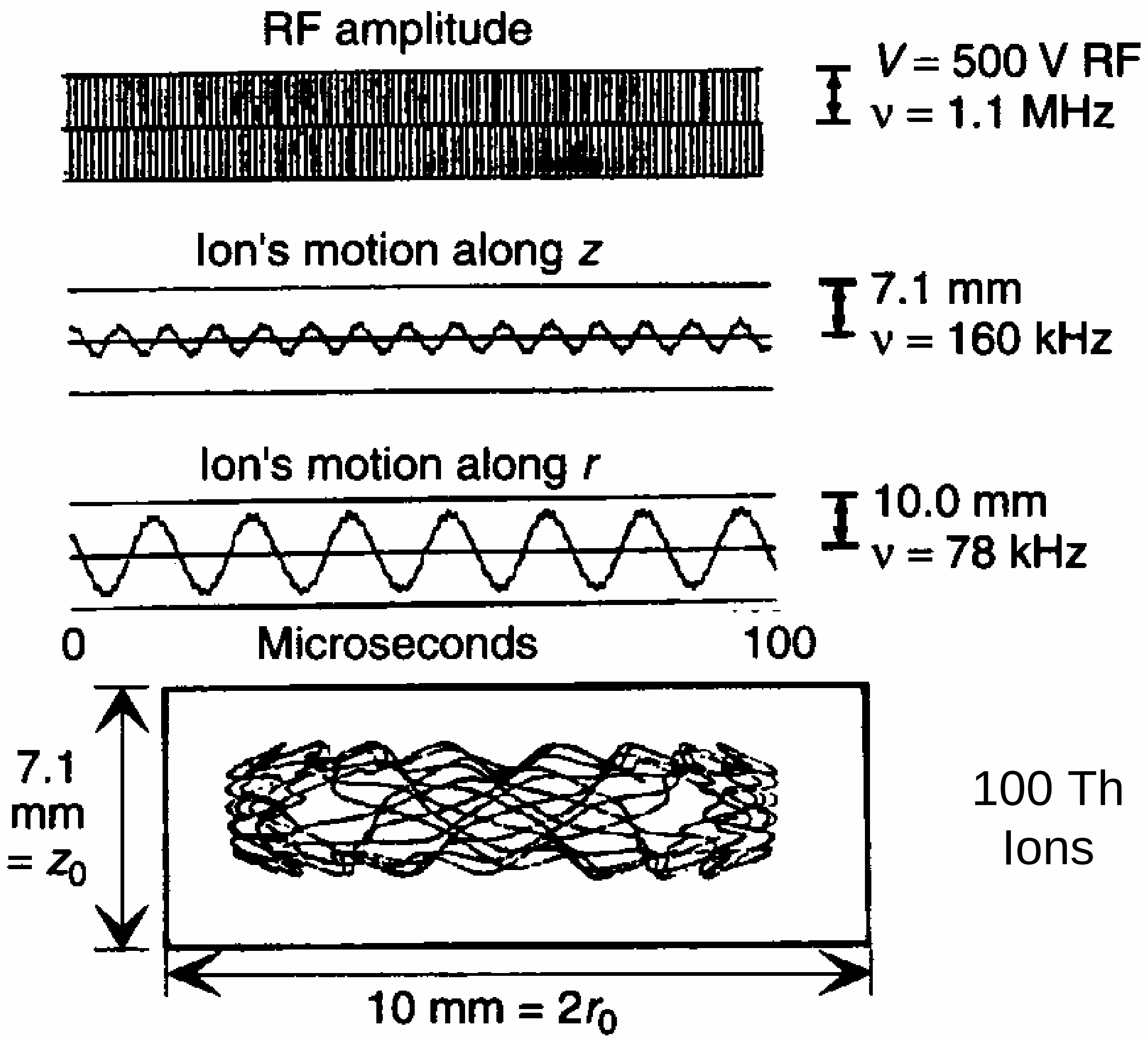
1. RF voltage with $\omega = 2\pi\nu$
2. Ions oscillate along the z axis with a frequency of $f_z = \beta_z \nu/2$
3. β_z depends on z/m
4. Maximum $\beta_z = 1$ for a stable ion in z



For $a_u = 0$ ($V_{dc} = 0$), $b_{ueject} = 0.908$

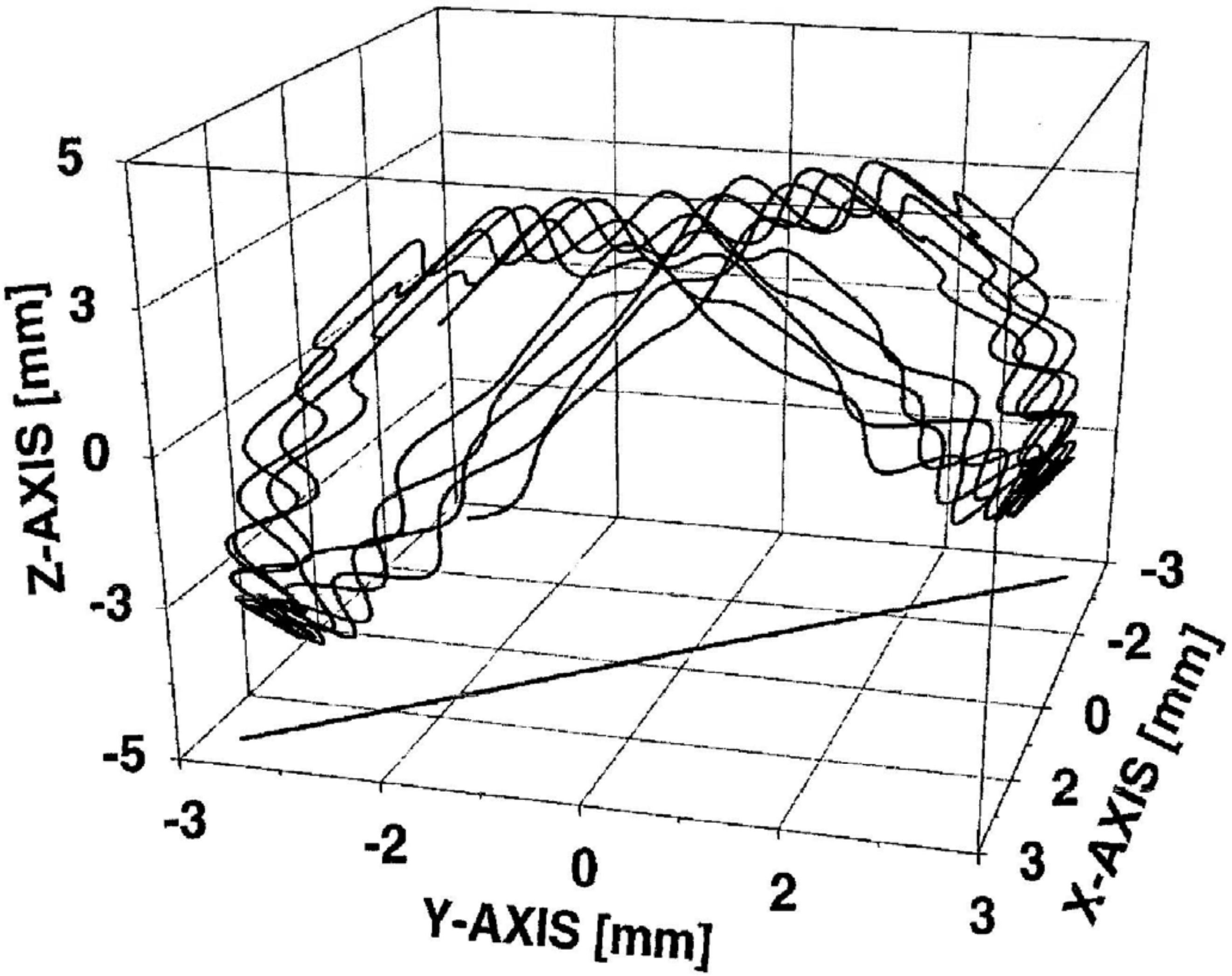
Scan V_{rf} to scan across b_u & eject along z axis

Example Of An Ion In An Ion Trap



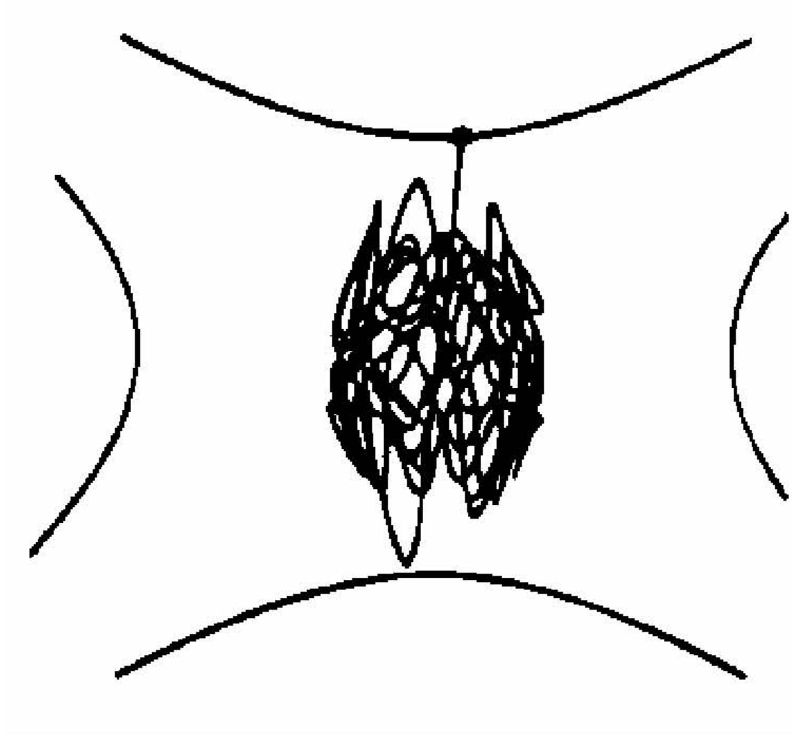
100 Th
Ions

Example Of An Ion In An Ion Trap



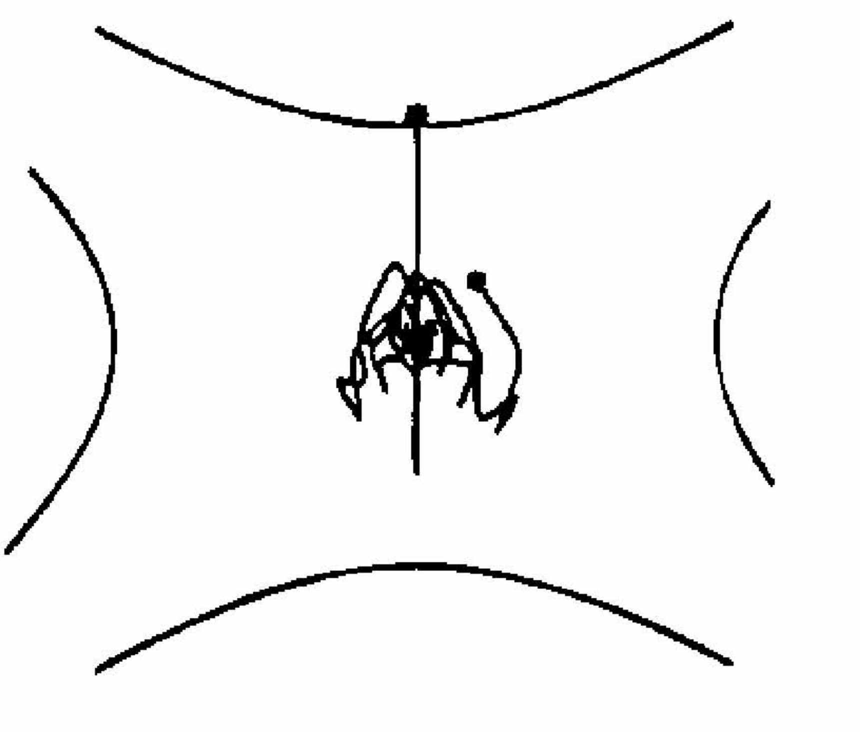
Over Time, Ions Repel Each Other

Expanding Trajectories



Without He

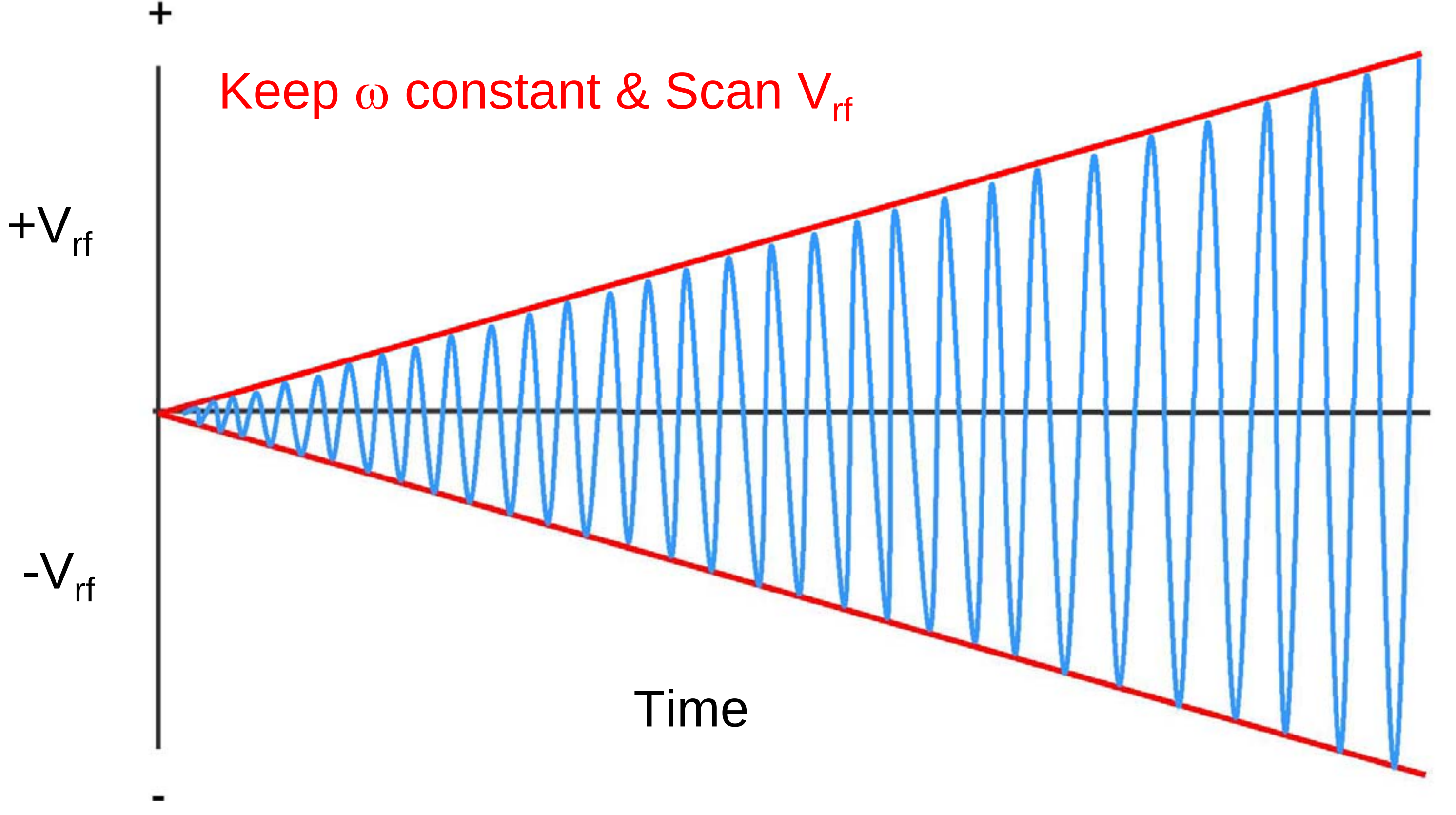
Use Collisions with He
to Remove Excess Energy



With He

Ion Ejection From An Ion Trap- Mass Selective Instability

Set $V_{dc} = 0$ $\quad$ $a_z = \dfrac{-16qV_{dc}}{m\omega^2(r_o^2 + z_o^2)} = 0$ $\quad$ & $\quad$ $b_z = \dfrac{-8qV_{rf}}{m\omega^2(r_o^2 + z_o^2)}$

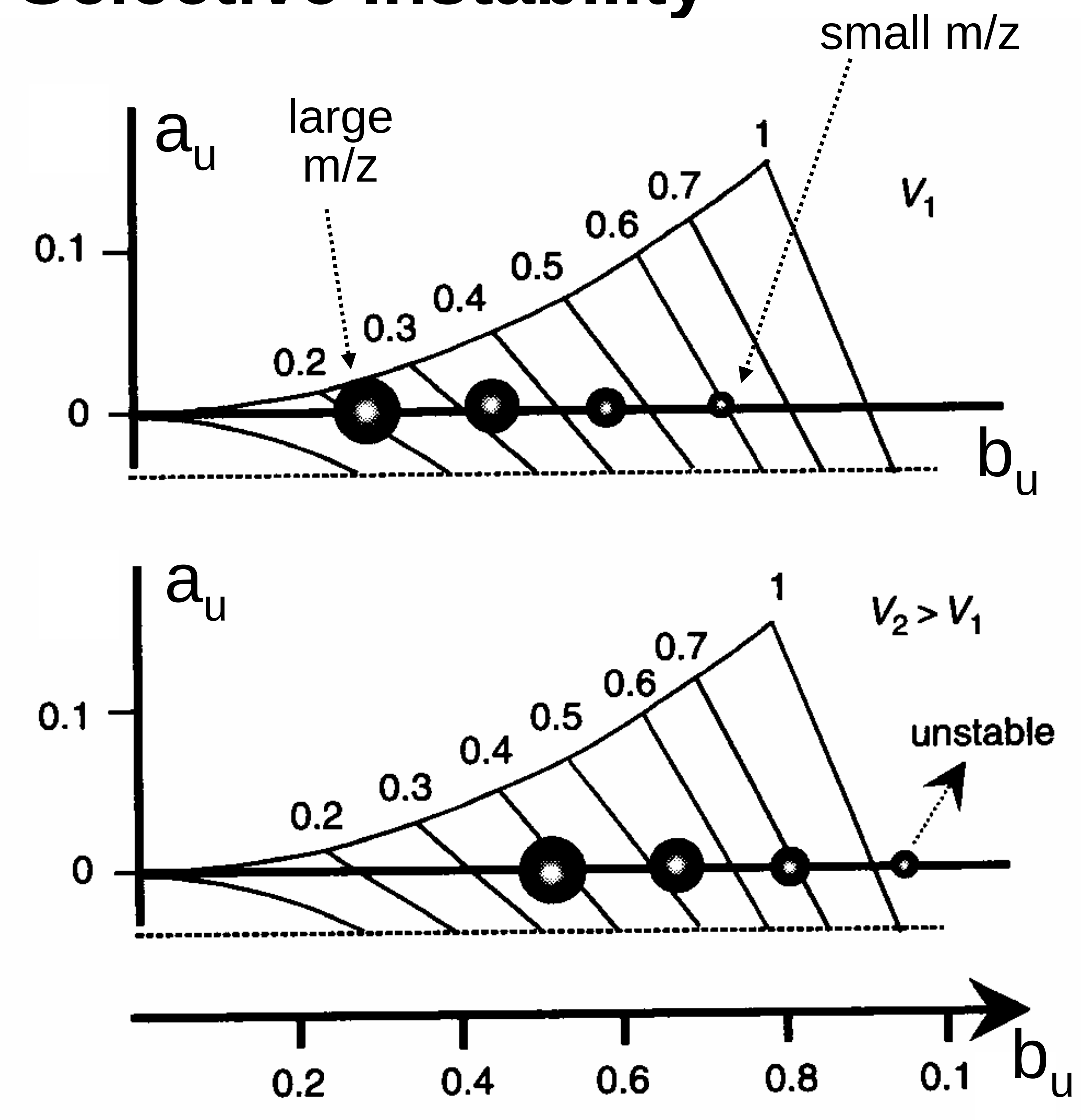


Ion Ejection From An Ion Trap- Mass Selective Instability

$$b_z = \frac{-8qV_{rf}}{m\omega^2(r_o^2 + z_o^2)}$$

An ion with $b_z \geq 0.908$, is ejected!

Low m/z ions
are ejected 1st.



ω & V_{rf} Determine The Maximum Mass That Can Be Ejected.

$$\frac{m_{max}}{q} = \frac{-8V_{rfmax}}{0.908\omega^2(r_o^2 + z_o^2)}$$

for $b_z = 0.908$

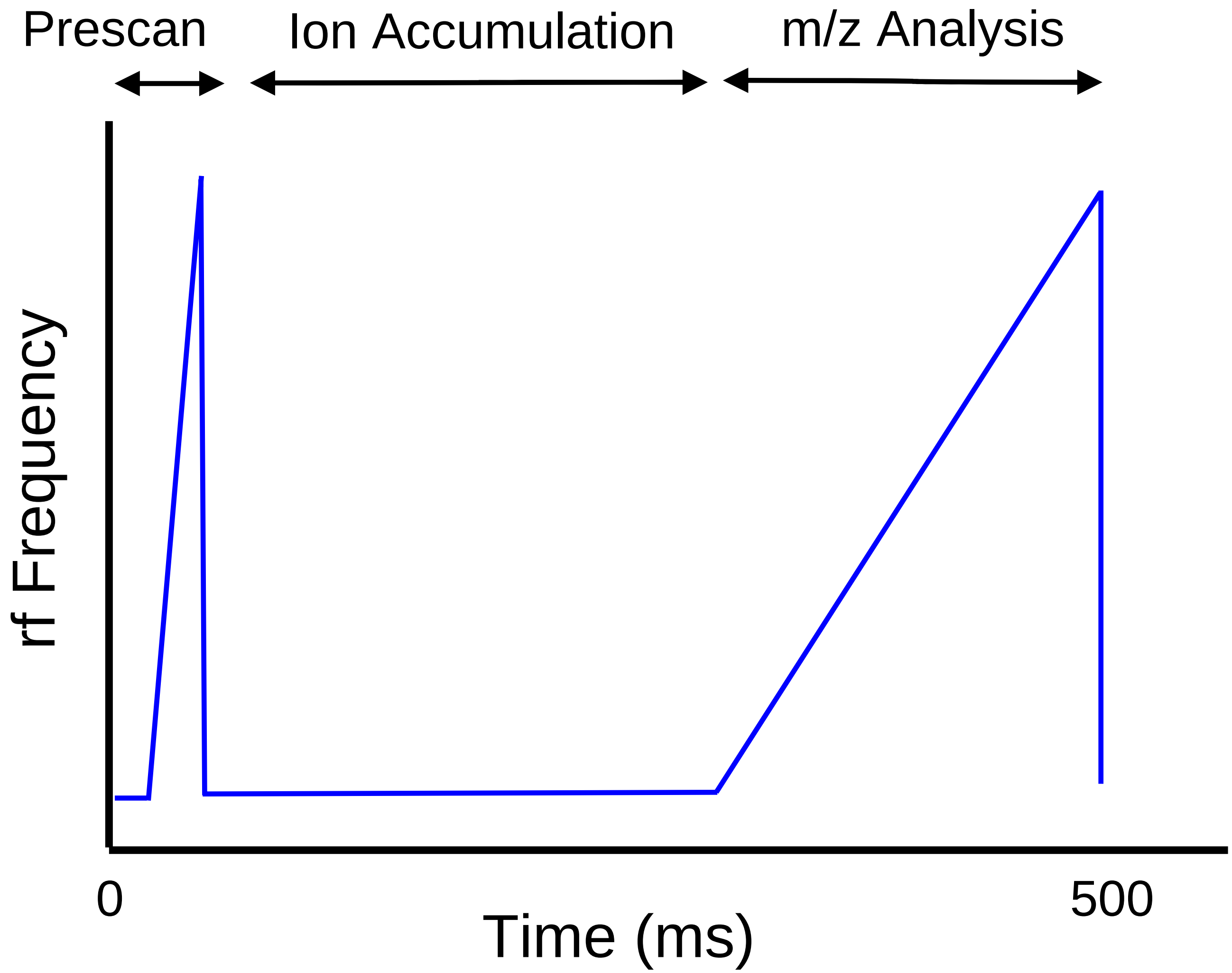
For:

$\nu = 0.6$ MHz
$r_o = 7$ mm
$z_o = 7$ mm
$V_{rfmax} = 8000$ V
charge = 1

Note:
50% ions go
go out each
endcap.

$$\frac{m_{max}}{q} = 3260 \text{ Th}$$

Operating An Ion Trap



TOFs, Quads, & Ion Traps

Types of Analyzers:

1. Time of Flight (TOF)- R ~ 10,000; m/z < 500,000;
   mass accuracy ~10 ppm; very good sensitivity
   MALDI-TOF: easy to use, low costs

2. Quadrupole Analyzer- R ~ 3000; m/z < ~2000;
   mass accuracy ~400 ppm, poor sensitivity
   very easy to interface with LC & GC

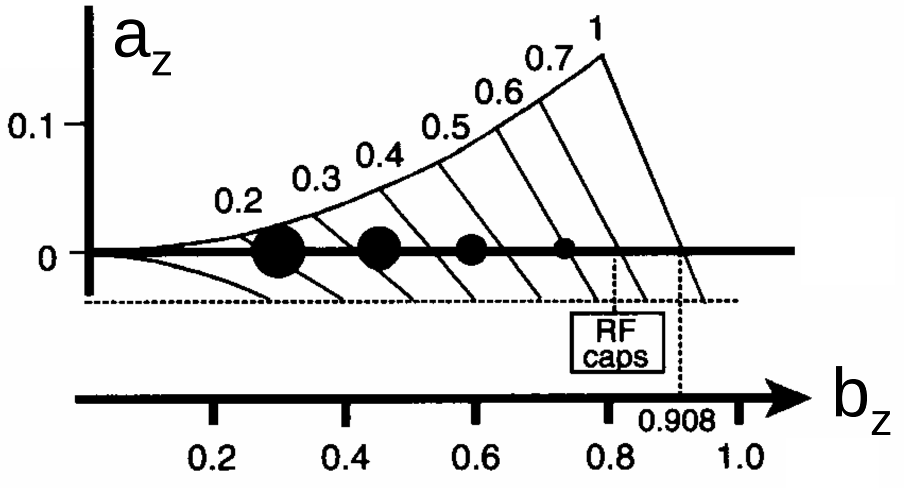
3. Ion Trap- R ~ 5000; m/z < ~2000;
   mass accuracy ~200 ppm; excellent sensitivity
   robust, sensitive, inexpensive
   can act as a collision cell & mass analyzer

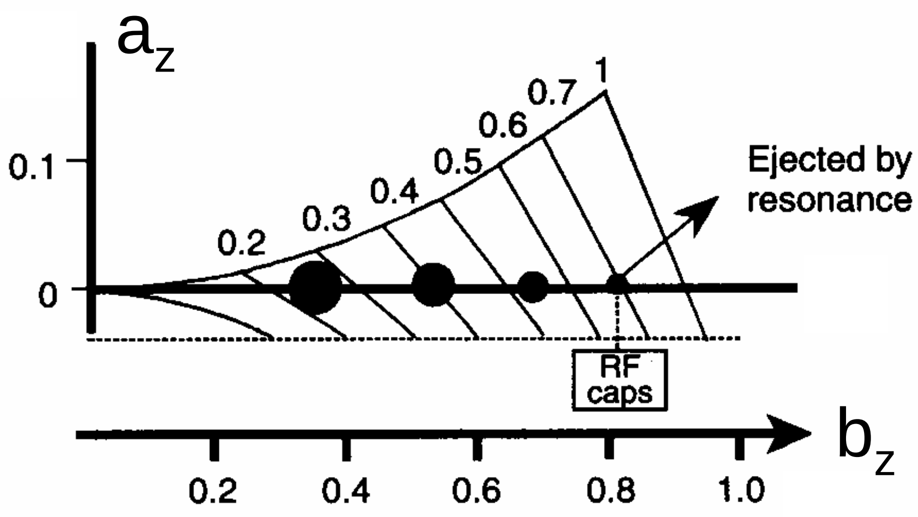
References

1. Caprioli, R, Sutter, M, Stoeckli, M. Online Mass Spectrometry Tutorial http://staging.mc.vanderbilt.edu/msrc/tutorials/ms/ms.htm
2. Lane CS. 2005. Mass spectrometry-based proteomics in the life sciences. Cellular and Molecular Life Sciences. 62:848-869.
3. Thermofinigan web site.
4. Steen H, Mann M. 2004. The ABC’s (and XYZ’s) of peptide sequencing. Nature Reviews: Molecular Cell Biology. 5:699-711.
5. Herbert CG, Johnstone RAW. Mass Spectrometry Basics. 2003. CRC Press, Boca Raton.
6. de Hoffman, E. Stroobant, V. Mass Spectrometry Principles and Application. 2001. 2nd Ed., John Wiley & Sons, New York.
7. Kinter, M., Sherman, N.E. Protein Sequencing and Identification Using Tandem Mass Spectrometry. Wiley, New York, 2000.
8. Cotter, R.J. The new time-of-flight mass spectrometry. Analytical Chemistry News & Features. (1999) pp. 445A-451A.
9. March, R.E. Quadrupole ion trap mass spectrometry: a view at the turn of the century. International Journal of Mass Spectrometry (2000) 200:285–312.
10. Hager, J.W. A new linear ion trap mass spectrometer. Rapid Communications in Mass Spectrometry. (2002) 16:512-526.
11. Brown, R.S., Lennon, J.J. Mass resolution improvement by incorporation of pulsed ion extraction in a matrix-assisted laser desorption/ionization linear time-of-flight mass spectrometer. Analytical Chemistry. (1995) 67:1998-2003.
12. Jonscher1, K.R., Yates, J.R. The quadrupole ion trap mass spectrometer—a small solution to a big challenge. Analytical Biochemistry (1997) 244:1–15.

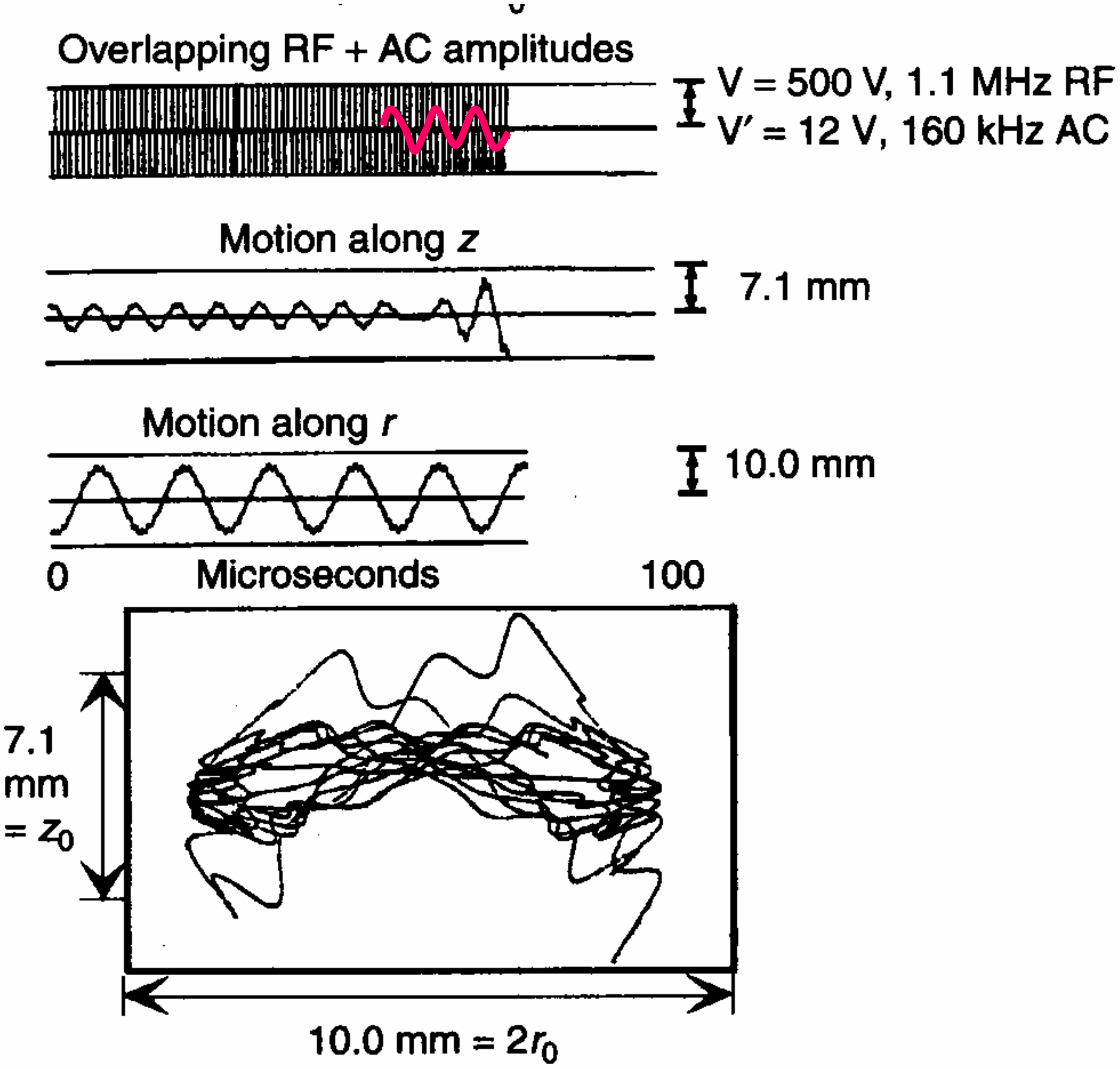
Ion Ejection From An Ion Trap- Resonant Ejection

1. Same as ejection by mass selective instability
2. But also apply a supplemental V_{rf} to end caps
3. Effect is to decrease b_{zeject}





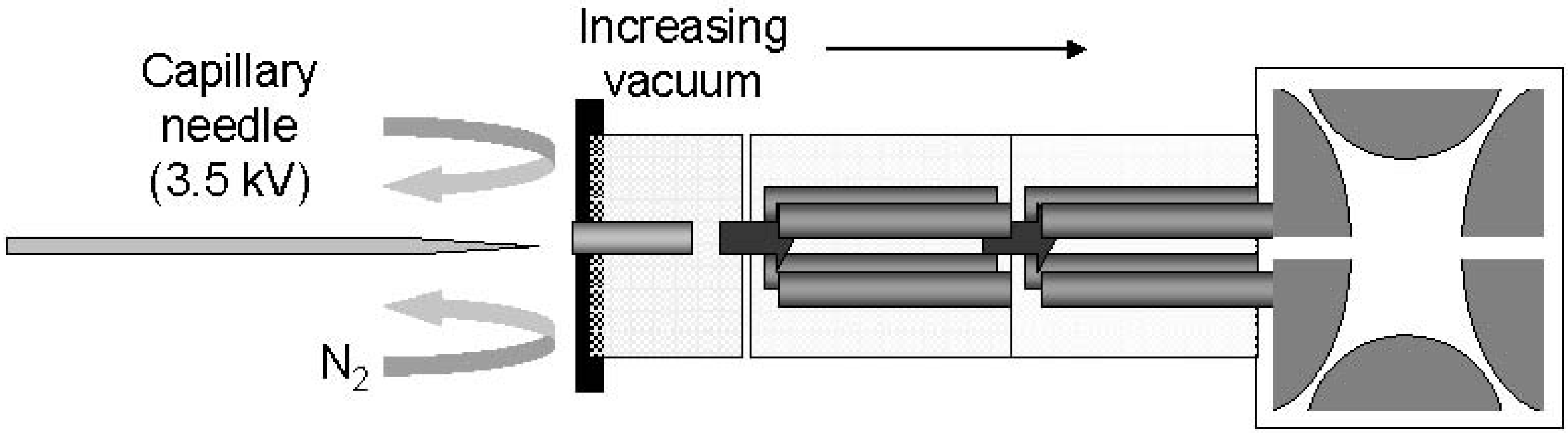
Resonant Ejection



100 Th
Ion

Extends the
mass range
of the ion trap.

ESI-Ion Trap



Electrospray source at atmospheric pressure

RF-only quadrupole or octapole filters that collimate ions from high pressure ESI source.

Ion trap mass spectrometer