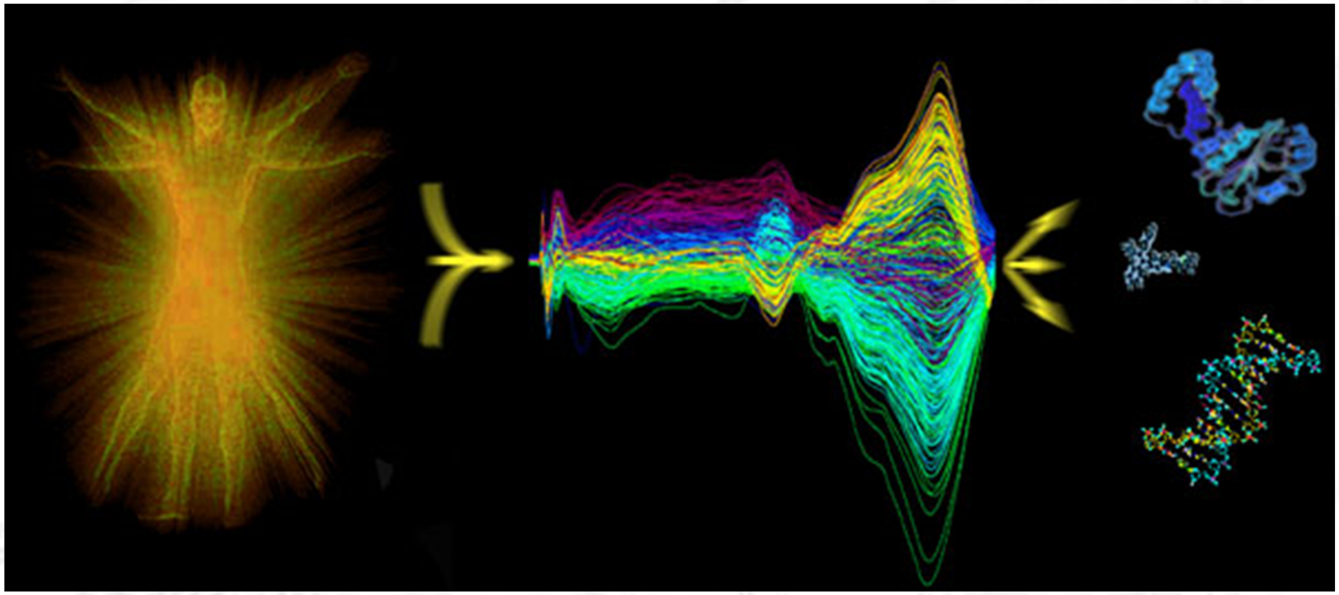
Welcome to 4081: Principles and Applications of Mass Spectrometry



Course Director: **Derek J. Wilson**

Email: **dkwilson@yorku.ca**

Office Hours: **After Lecture, CB318**

http://www.google.ca/imgres?imgurl=http://masspec.scripps.edu/images/main_ms_image.jpg&imgrefurl=http://masspec.scripps.edu/redirect.html&usg=__hRzC
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mass%2Bspectrometry %2Bimages%26start%3D40%26um%3D1%26hl%3Den%26sa%3DN%26ndsp%3D20%26tbs%3Disch:1

Course Outline

*This course will develop **Mass Spectrometry** as an **analytical tool** in **chemistry and biochemistry**, at an advanced level. The course will be delivered in three sections: **Instrumentation**, **Theory** and **Applications**. Topics will include ionization, mass analyzers, ion dissociation, ion mobility, qualitative and quantitative analysis with various applications.*

Week 1 (Sept 14th, 16th): History of Mass Spectrometry. Ionization.

Week 2 (Sept 21st, 23rd): Soft Ionization

Week 3 (Sept 28th, 30th): Mass Analyzers, Ion mobility

Week 4 (Oct 5th, 7th): Vacuum Systems, Detectors, Quadrupole Theory

Reading Week (Oct 11th – 15th)

Week 5 (Oct 19th, 21st): Ion traps, FT-ICR and Orbitrap Theory

Week 6 (Oct 26th, 28th): TOF theory, Review

Week 7 (Nov 2nd, 4th): Mid-term! (2nd), Tandem MS / Ion Dissociation

Week 8 (Nov 9th, 11th): Applications: Small Molecule

Week 9 (Nov 16th, 18th): Applications: Proteomics

Week 10 (Nov 23th, 25th): Applications: Protein / Protein Assembly Structure

Week 11 (Nov 30th, Dec 2nd): Applications: Protein Dynamics and Function

Week 12 (Dec 7th, Dec 9th): Project Presentations

Assessment

Midterm exam I *- 25% November 2nd*

Project Assignment *- 35% Due: Dec 9th, Presentations Dec 7th and 9th*

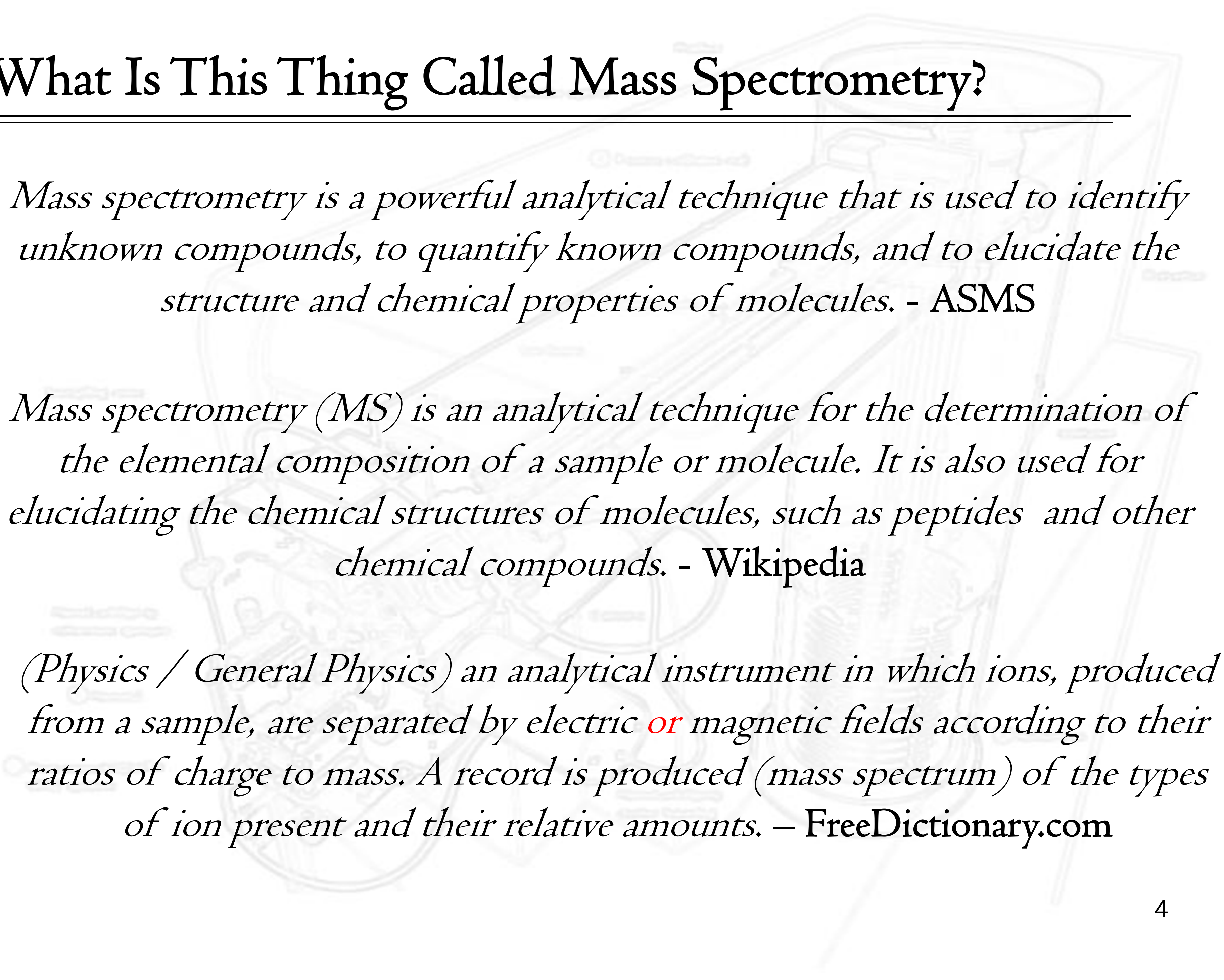
Final exam *- 40%*

Academic Honesty:

York students are required to maintain high standards of academic integrity and are subject to the Senate Policy on Academic Honesty: (http://www.yorku.ca/secretariat/policies/document.php?document=69)

Students should also review materials on the Academic Integrity website: (http://www.yorku.ca/academicintegrity)

What Is This Thing Called Mass Spectrometry?



Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds, and to elucidate the structure and chemical properties of molecules. - ASMS

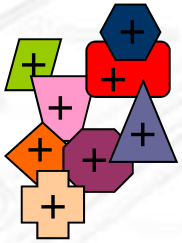
Mass spectrometry (MS) is an analytical technique for the determination of the elemental composition of a sample or molecule. It is also used for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. - Wikipedia

(Physics / General Physics) an analytical instrument in which ions, produced from a sample, are separated by electric or magnetic fields according to their ratios of charge to mass. A record is produced (mass spectrum) of the types of ion present and their relative amounts. – FreeDictionary.com

Mass Spectrometry in a Very Small Nutshell.

Stuff

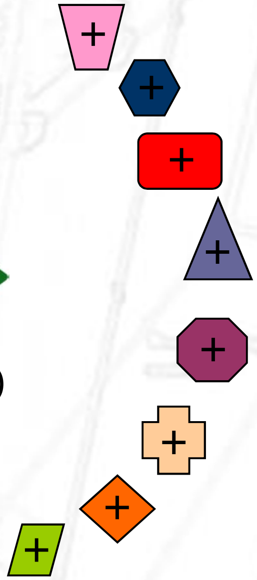
Ionize



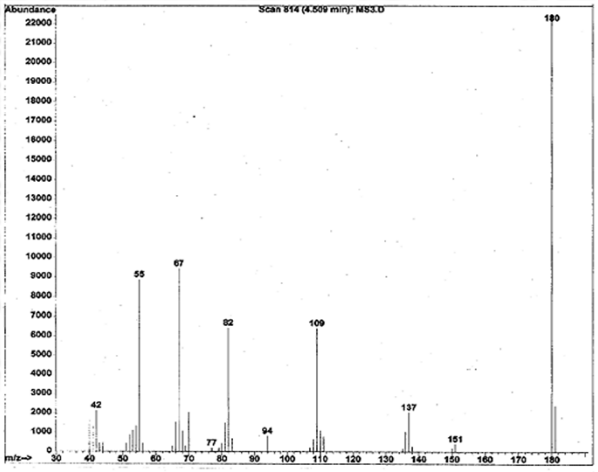
(negative ions also possible)

Manipulate according to ***mass/charge***

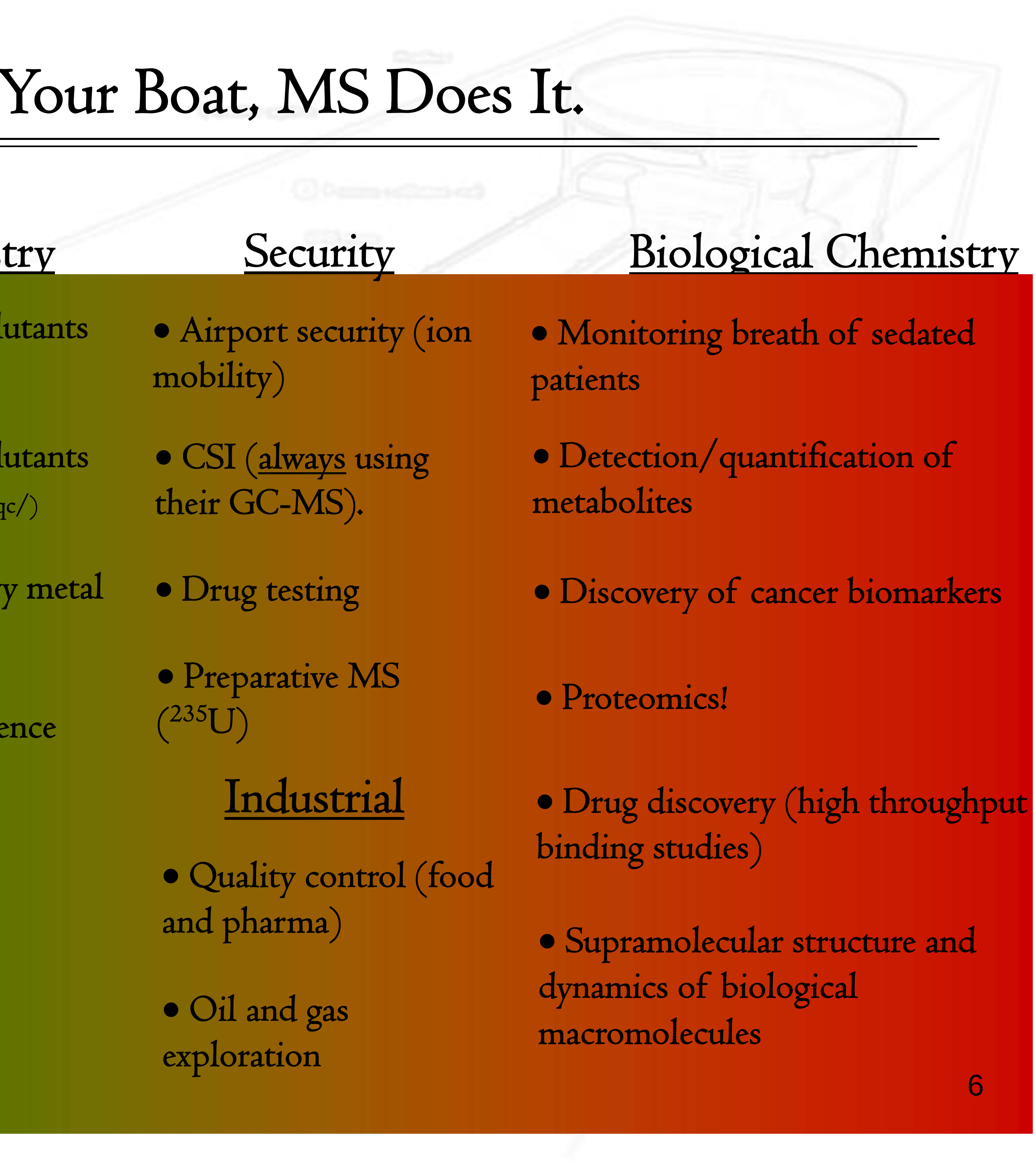
(or **size** to charge)



Detect



Whatever Floats Your Boat, MS Does It.



Environmental Chemistry

- Detecting/quantifying pollutants in atmosphere
- Detecting/quantifying pollutants in water… (http://www.trentu.ca/wqc/)
- Analysis of pollutant/heavy metal uptake in plants/fish
- Atmospheric and space science

Security

- Airport security (ion mobility)
- CSI (always using their GC-MS).
- Drug testing
- Preparative MS (^{235}U)

Industrial

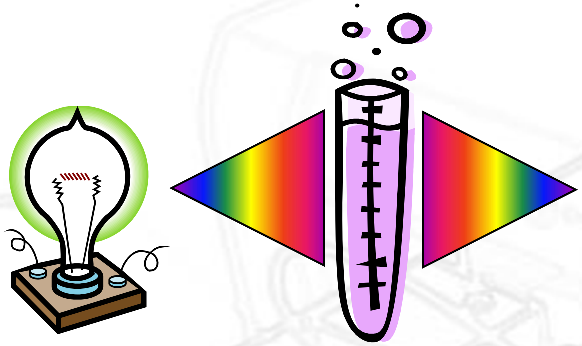
- Quality control (food and pharma)
- Oil and gas exploration

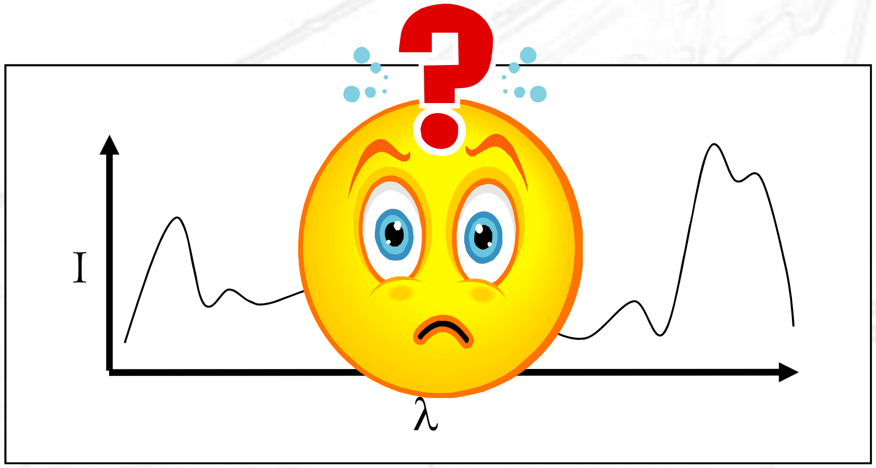
Biological Chemistry

- Monitoring breath of sedated patients
- Detection/quantification of metabolites
- Discovery of cancer biomarkers
- Proteomics!
- Drug discovery (high throughput binding studies)
- Supramolecular structure and dynamics of biological macromolecules

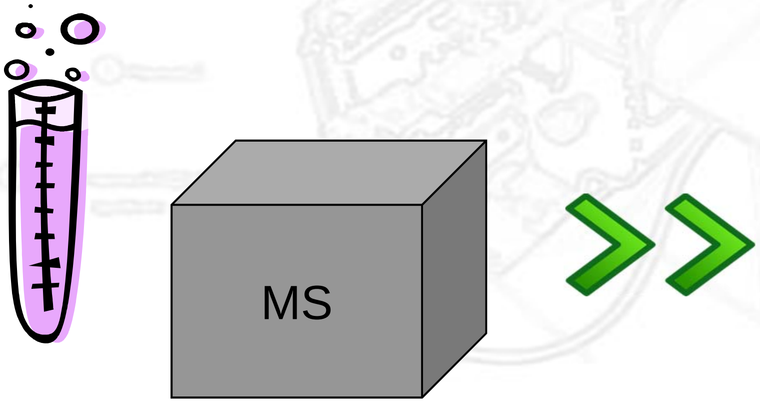
The Coolest Thing About MS or Why MS is Awesome

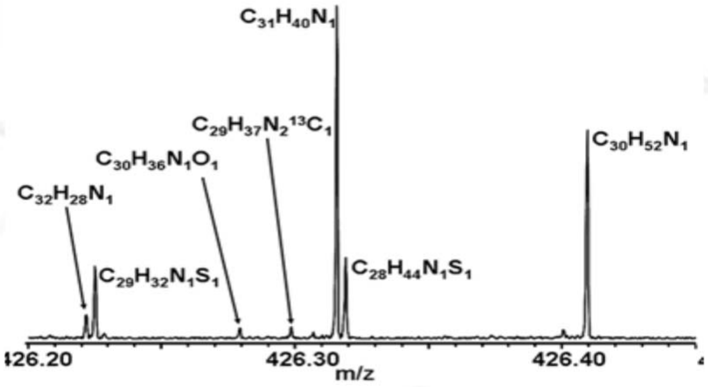
- The most powerful feature of MS is it’s selectivity. Watch:





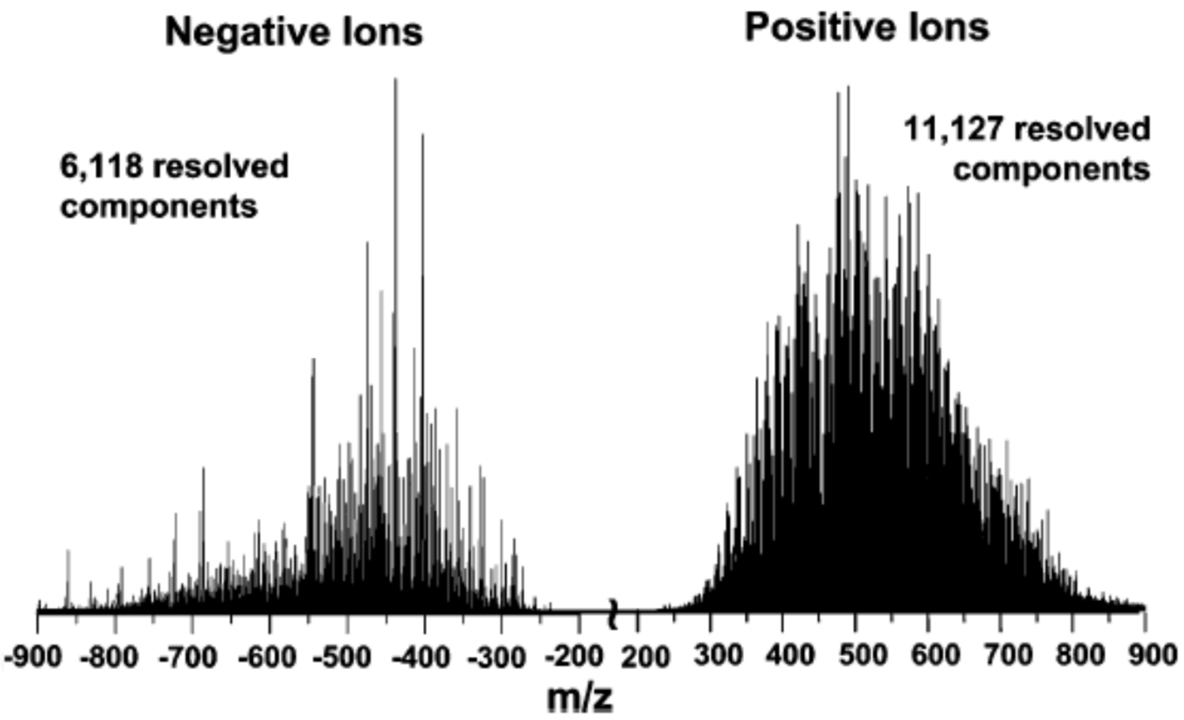
- UV/visible Spectrophotometry of complex mixture: What am I looking at??





Selectivity…

- To what extent do different things in sample give me a different signal?
- World record for selectivity:

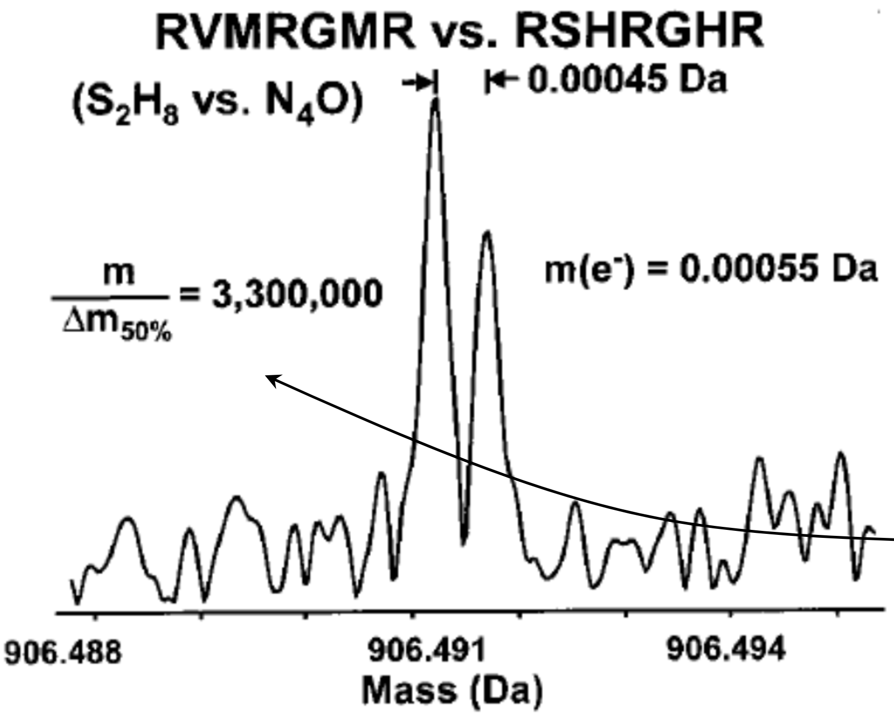


Hughey *et al*. Anal. Chem. **2002**, 74, 4145-4149

- This is what happens when you put crude oil into an FT-ICR mass spectrometer

Possibly the Coolest Thing Ever

- Another way of looking at specificity is in terms of mass resolving power. For this we ask: What is the minimum separation of two masses that I can distinguish?



- The answer (so far) is: .00045 Da

- Which is *less than the mass of an electron*

- Sneaky inclusion of the most common formula for measuring resolution (FWHM)

The Next Coolest Thing About MS

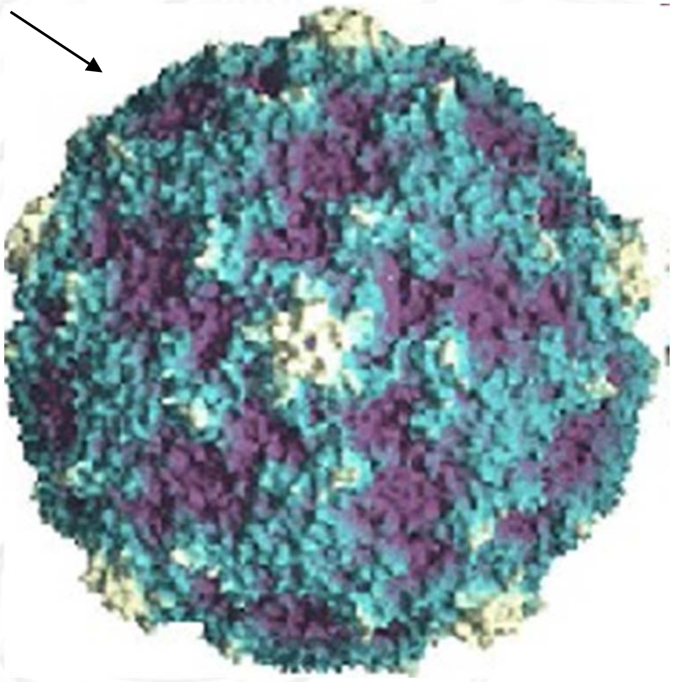
- Probably the next coolest thing about MS is that almost anything can be analyzed

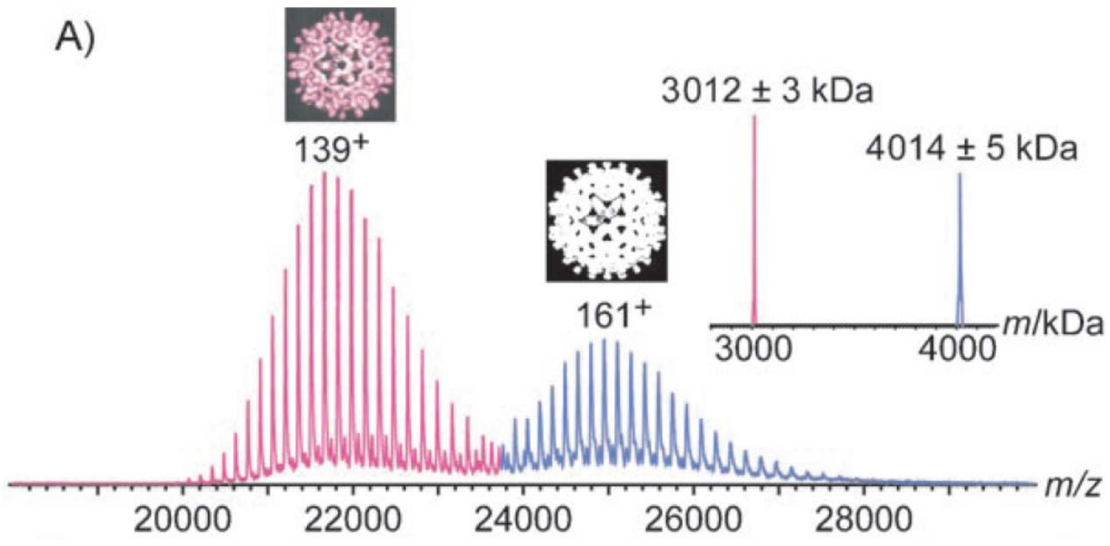
from this

Very small molecule (2 Da)

Viral Capsid (~10,000,000 Da)

to this





Uetrecht *et al.*, *Angew. Chem. Int. Ed*. 2008, 47, 6247 –6251

Another Totally Awesome Thing About MS

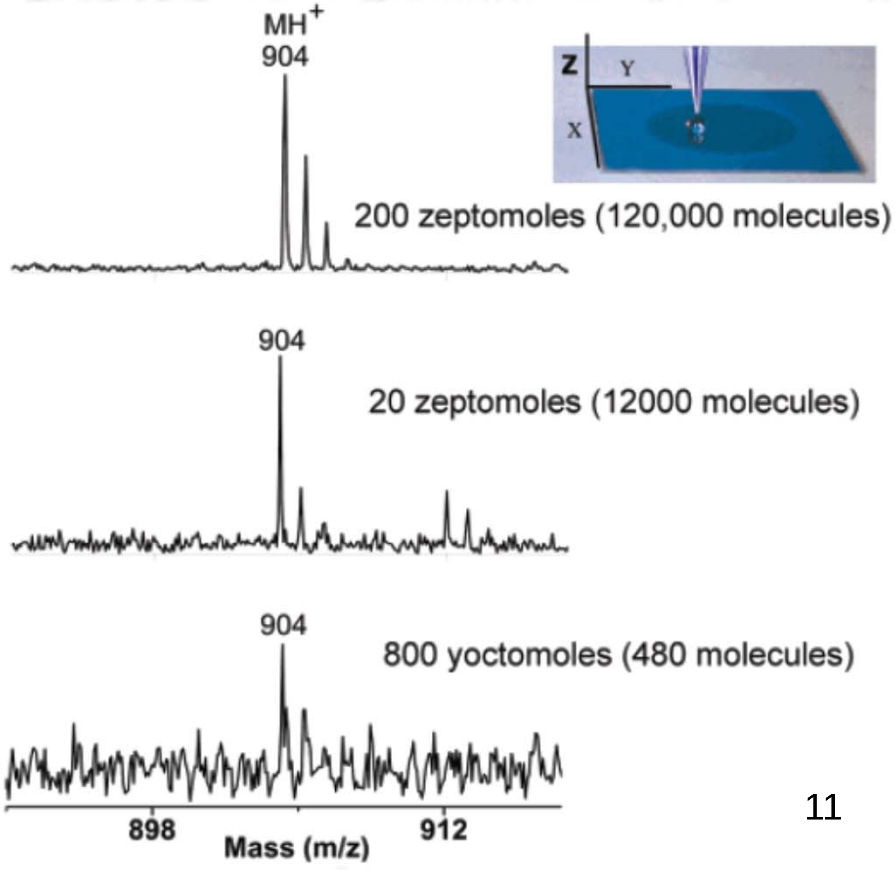
- Mass Spectrometry is very sensitive.

- Only fluorescence spectroscopy is more sensitive (the fluorescence guys have been down to 1 molecule for a while now).

- Sensitivity has no specific definition, but the Limit of Detection is: The lowest number of molecules that I can detect

- FYI: zepto- = 10^{-21}

  yocto- = 10^{-24}



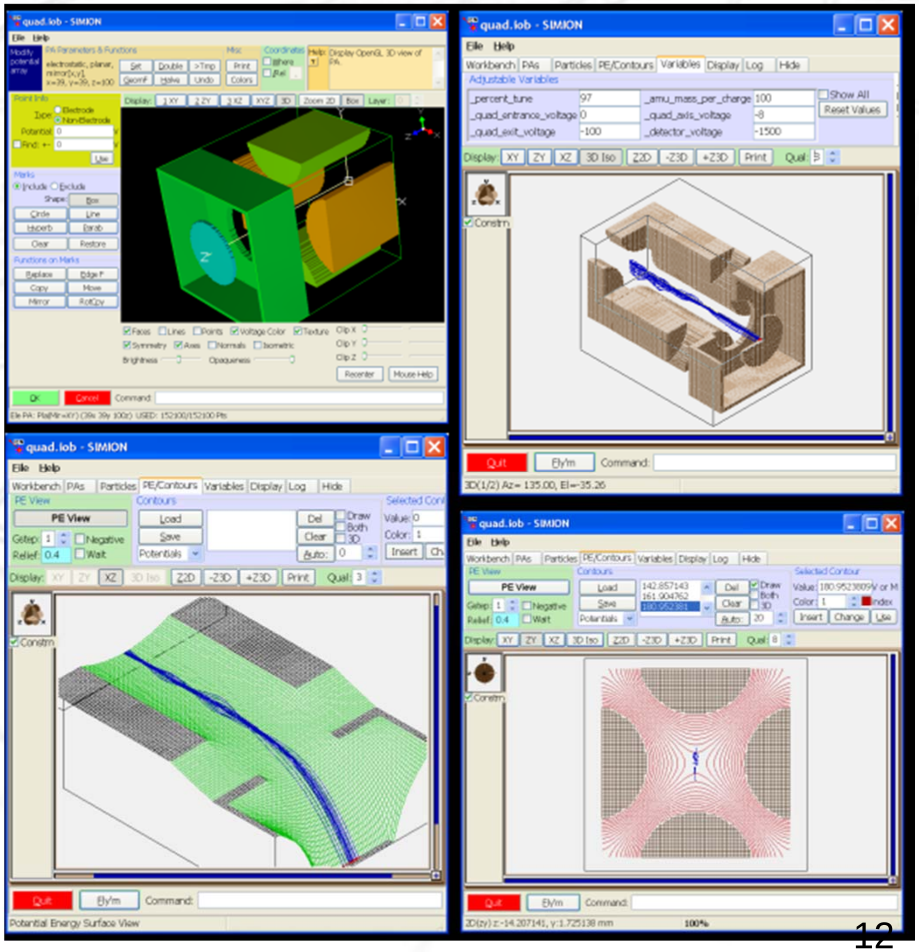
Trauger *et al*., Anal. Chem. 2004, 76, 4484-4489

The Basic Principles of MS are Simple!

- Unlike some other powerful analytical techniques (cough, cough NMR cough cough) the basic principles of MS are relatively easy to understand.

- In MS, the signal comes from an ionized particle (usually) crashing into a surface to create a cascade of electrons

- In NMR, the signal comes from net magnetization of the sample in a plane perpendicular to the big magnetic field axis and spinning at a multitude of frequencies governed by the microenvironments of the nuclei being analyzed



http://simion.com/

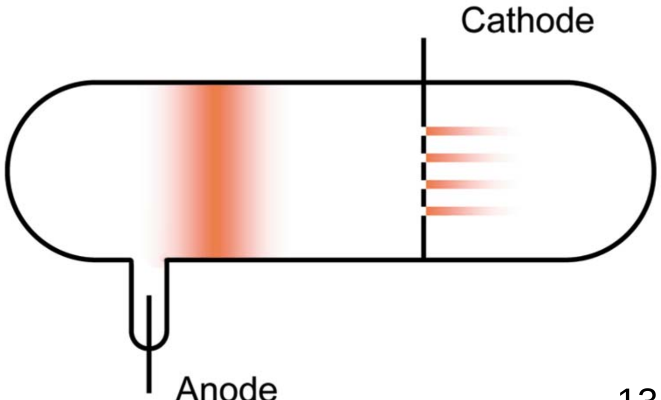
The History of Mass Spectrometry

- In the 1870's, many an amateur (and professional) scientist was fooling around with electricity flowing through vacuum tubes.

- What they were generating were streams of electrons traveling through space from the cathode (-ve) to the anode (+ve), which Eugen Goldstein dubbed "cathode rays".

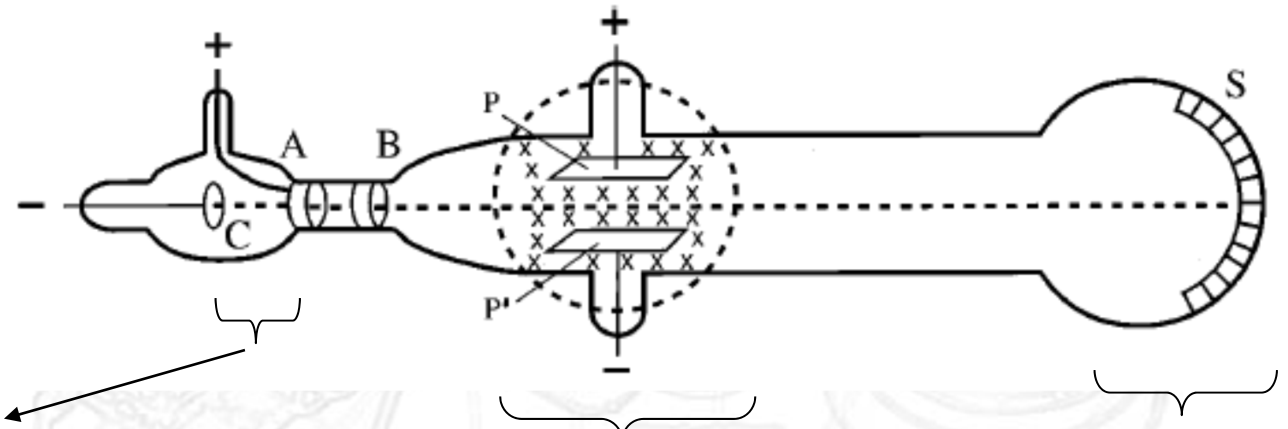
- Goldstein also noticed that when he cut slits in the cathode, rays traveling from the anode to (actually past) the cathode were visible.

- The color of these rays depended on the background gas: air yellow, hydrogen rose, neon red.



Playing With 'Corpuscles'…

- So now we have charged particles to play around with, +ve and –ve.
- What next? How about trying to deflect the rays using electric and magnetic fields!
- For cathode rays, this turned out to be pretty easy. Enter J. J. Thomson and the desire to measure m/z of an electron:

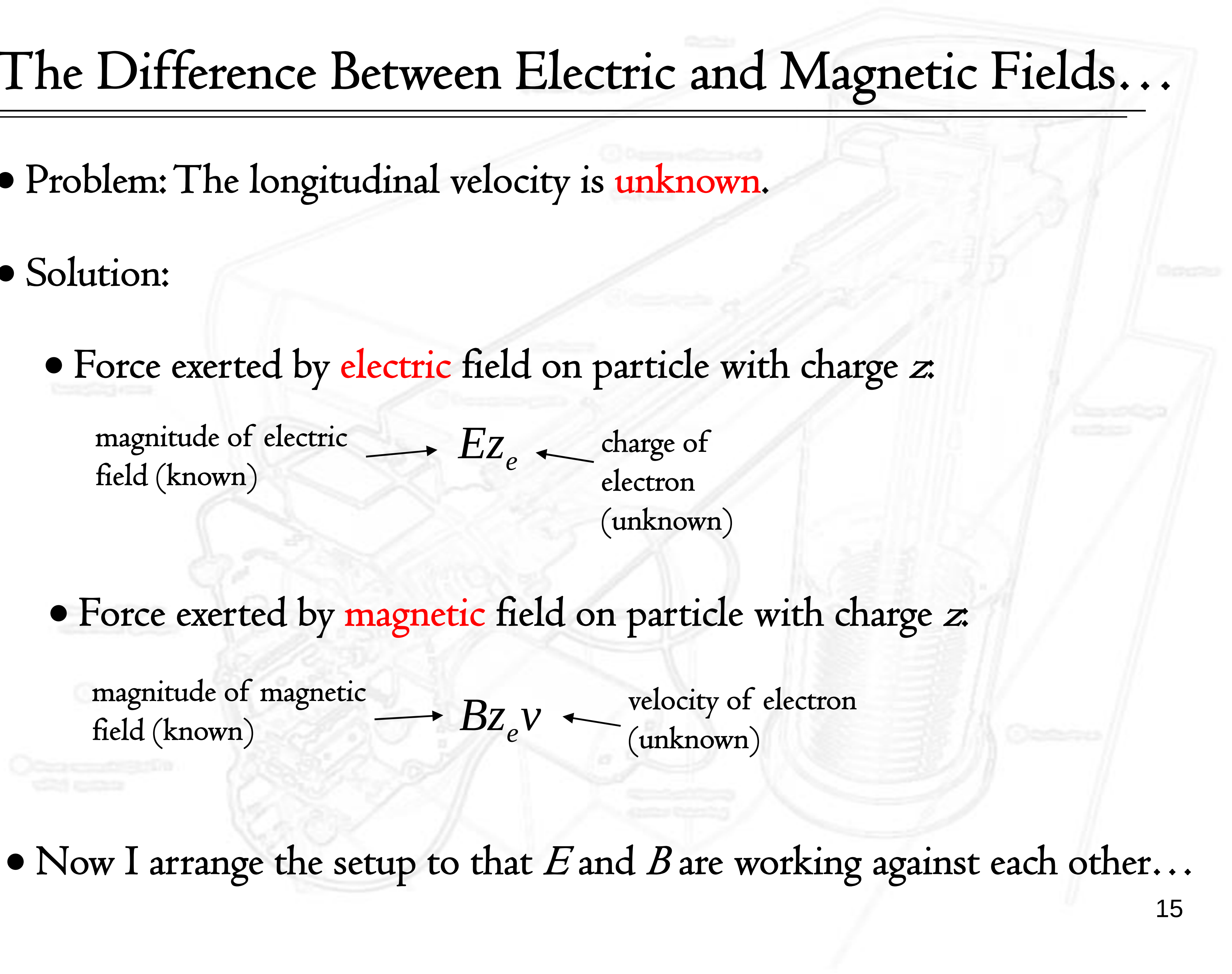


electrons accelerated to a particular longitudinal velocity (v_x)

electron beam deflected by electric and magnetic field

extent of deflection measured

The Difference Between Electric and Magnetic Fields…



- Problem: The longitudinal velocity is unknown.
- Solution:
  - Force exerted by electric field on particle with charge *z*:

magnitude of electric field (known) → Ez_e ← charge of electron (unknown)

  - Force exerted by magnetic field on particle with charge *z*:

magnitude of magnetic field (known) → $Bz_e v$ ← velocity of electron (unknown)

- Now I arrange the setup to that *E* and *B* are working against each other…

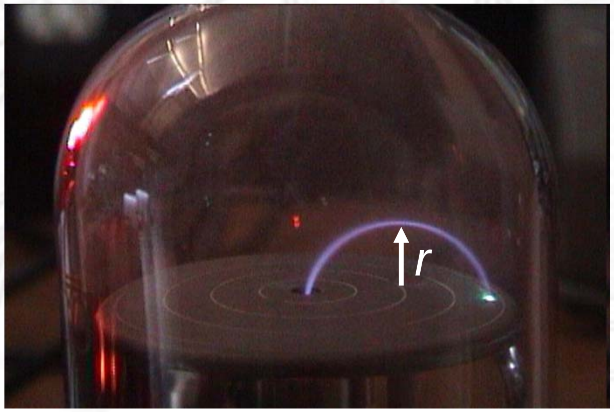
Bend it (Better Than) Beckham…

- If I adjust the magnetic field strength so that there is no net deflection, then: $Ez_e = Bz_e v$ or (more usefully after the z_e's cancel)… $v = \dfrac{E}{B}$

- Once v is known it's actually very easy to measure m/z by taking advantage of the fact that the magnetic force is always 90° to the velocity, giving charged particles with a particular m/z a characteristic orbit:

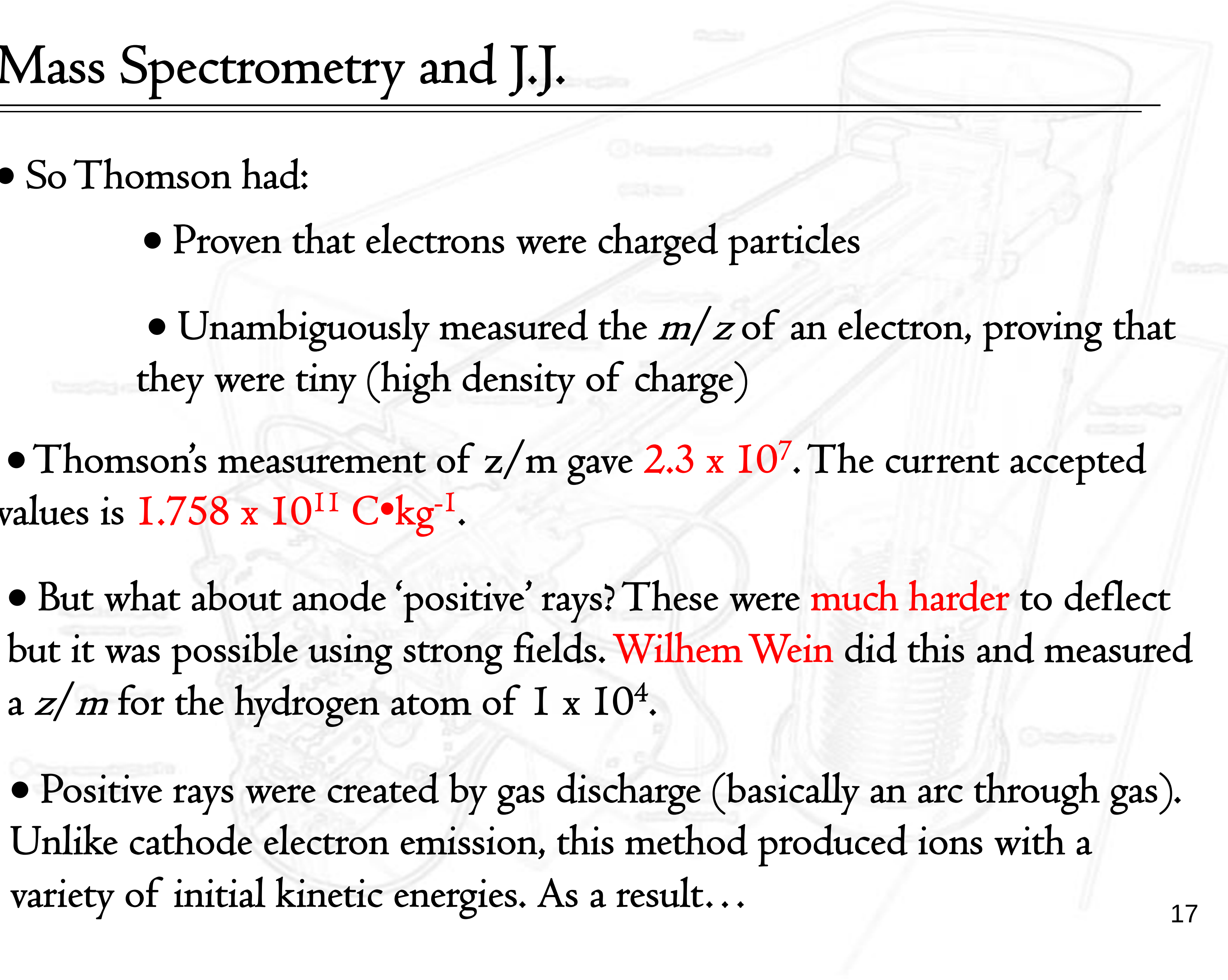
radius of orbit

$$r = \frac{m_e v}{B z_e}$$



- Thomson didn't do it this way… (he used electric field only)

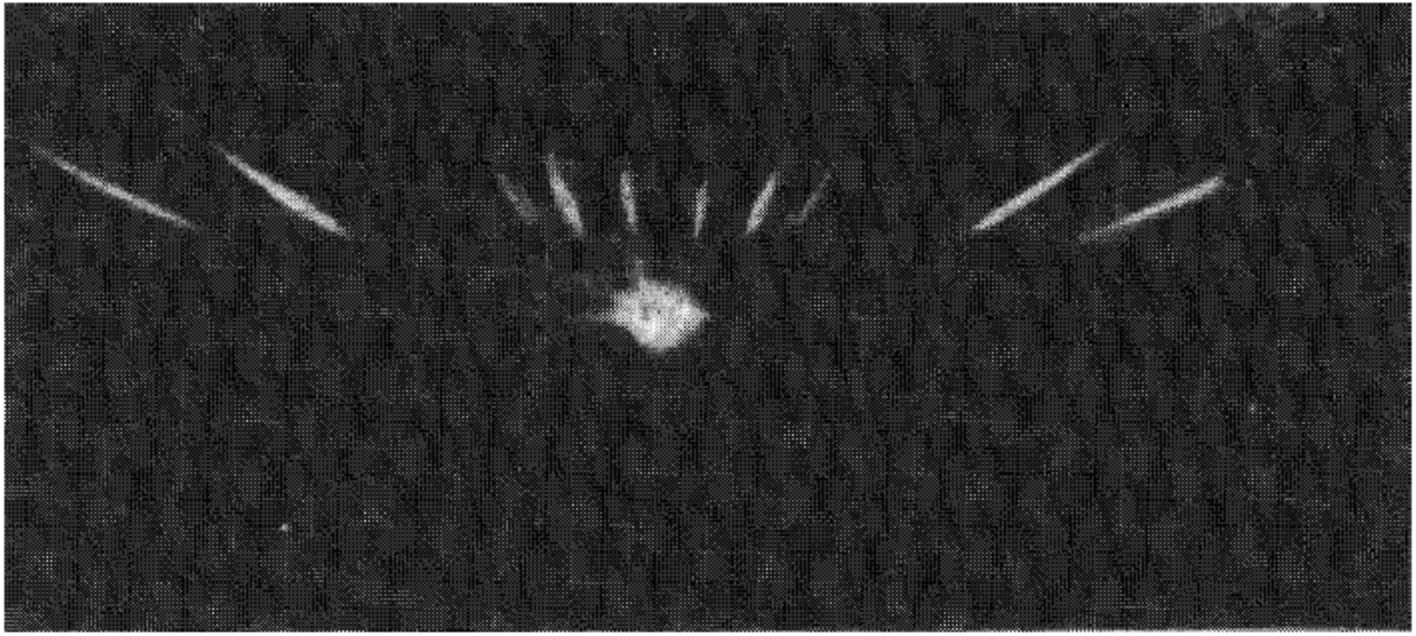
Mass Spectrometry and J.J.



- So Thomson had:
    - Proven that electrons were charged particles
    - Unambiguously measured the m/z of an electron, proving that they were tiny (high density of charge)
- Thomson's measurement of z/m gave 2.3×10^7. The current accepted values is 1.758×10^{11} C•kg^{-1}.
- But what about anode 'positive' rays? These were much harder to deflect but it was possible using strong fields. Wilhem Wein did this and measured a z/m for the hydrogen atom of 1×10^4.
- Positive rays were created by gas discharge (basically an arc through gas). Unlike cathode electron emission, this method produced ions with a variety of initial kinetic energies. As a result…

The First ‘Mass Spectrum’

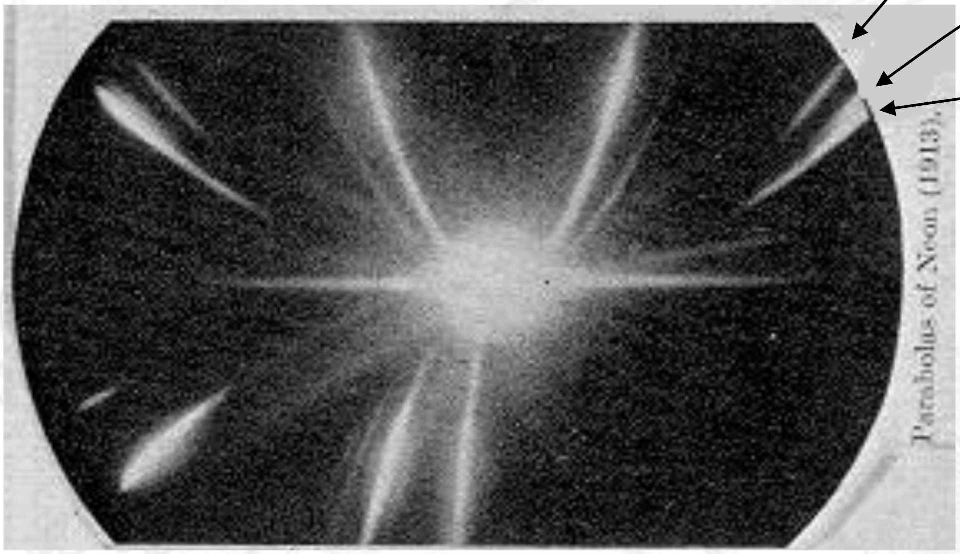
- Positive rays made parabolas instead of single points on the photographic plate detectors
- Each positively charged species with a different mass gave a different parabola…



Iwan W. Griffiths, RAPID COMM MASS SPECTROM, Vol. **11**, 2–16 (1997)

The mystery of Neon

- The mass spectrum of Neon showed something that could not be explained by available theory:

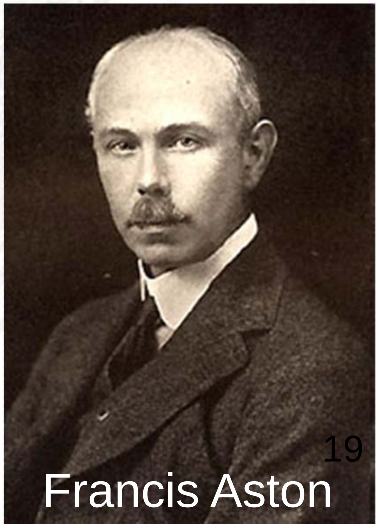


CO^+ (m/z 34)

??? (m/z 22?)

Ne (m/z 20)

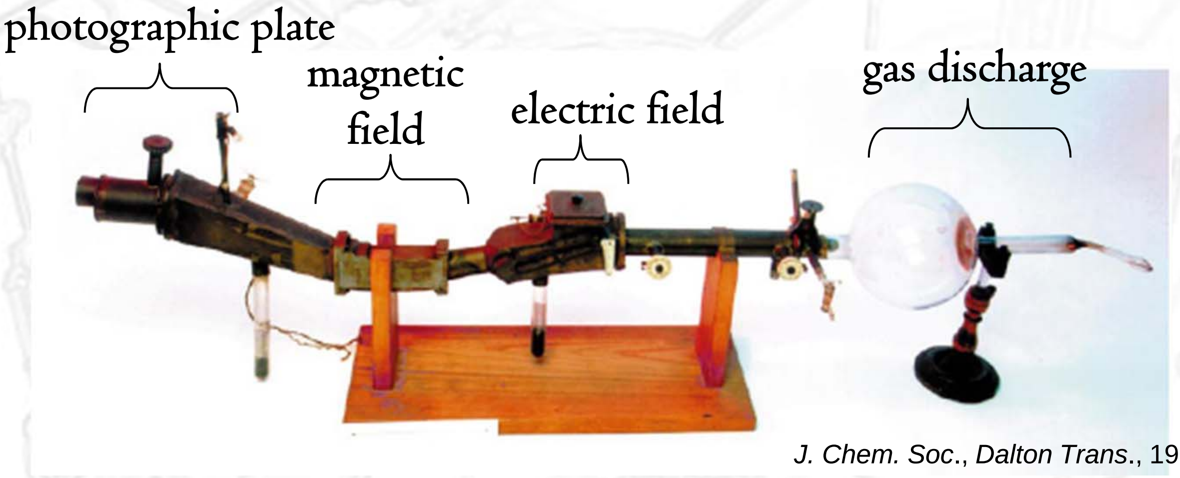
- Thomson thought he had discovered a new element, and so did his student Francis Aston, who worked very very hard to separate the 'new element' from Neon by conventional methods… to no avail…


Francis Aston

Aston's First Mass Spectrometer

- After WWI, Aston realized that the odd signal in Neon might be explained using the new theory of radioactive 'isotopes' (pioneered by Frederich Shoddy)… except that Neon wasn't radioactive…

- To prove that there were isotopes of stable elements, Aston built himself a fancy new mass spectrometer in which the electric and magnetic fields were applied in tandem rather than simultaneously:

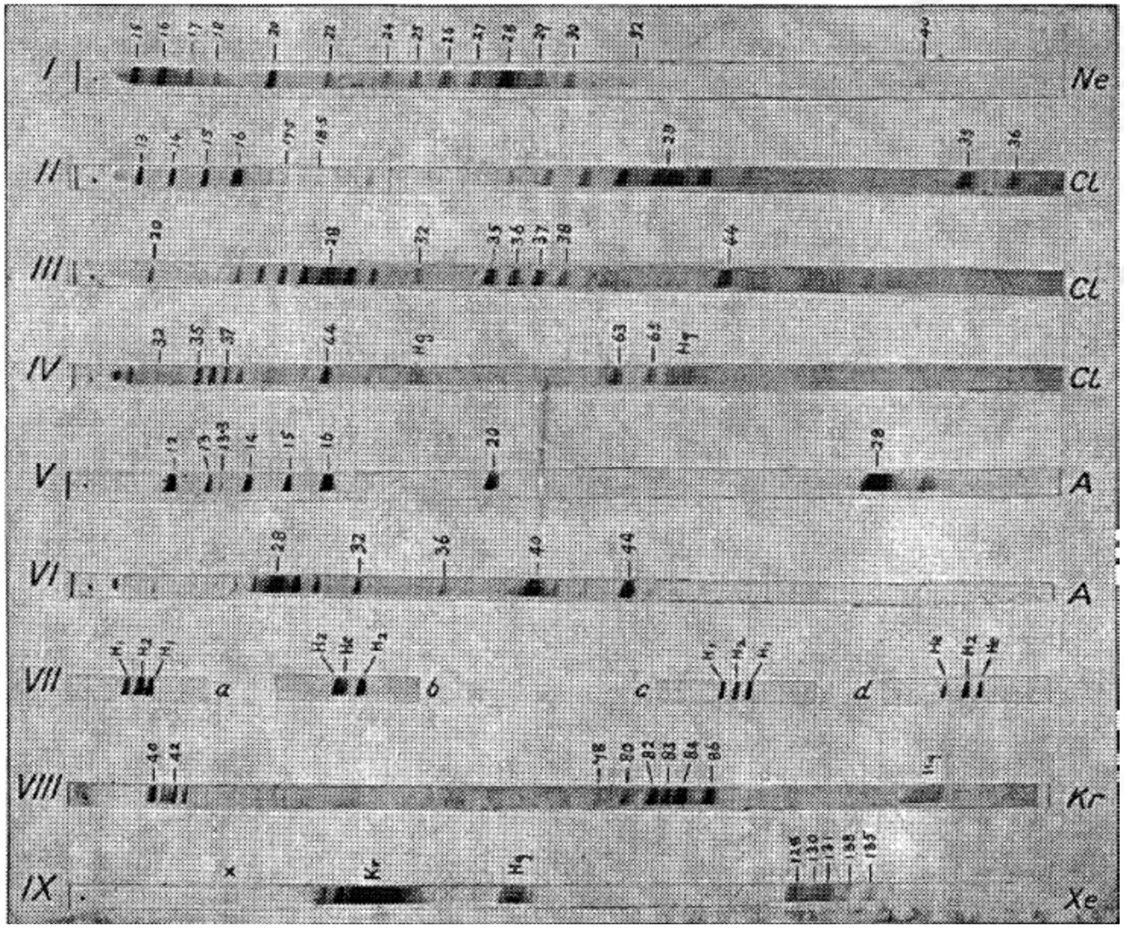


J. Chem. Soc., *Dalton Trans.*, 1998, 3893–3899

- The result is that ions exiting the electric 'sector' with different velocities (which would make the parabola) are focused onto the plate at a single point

The Mass Spectrograph

- This allowed Aston to get real 'mass spectrographs' in which he could detect many stable isotopes:

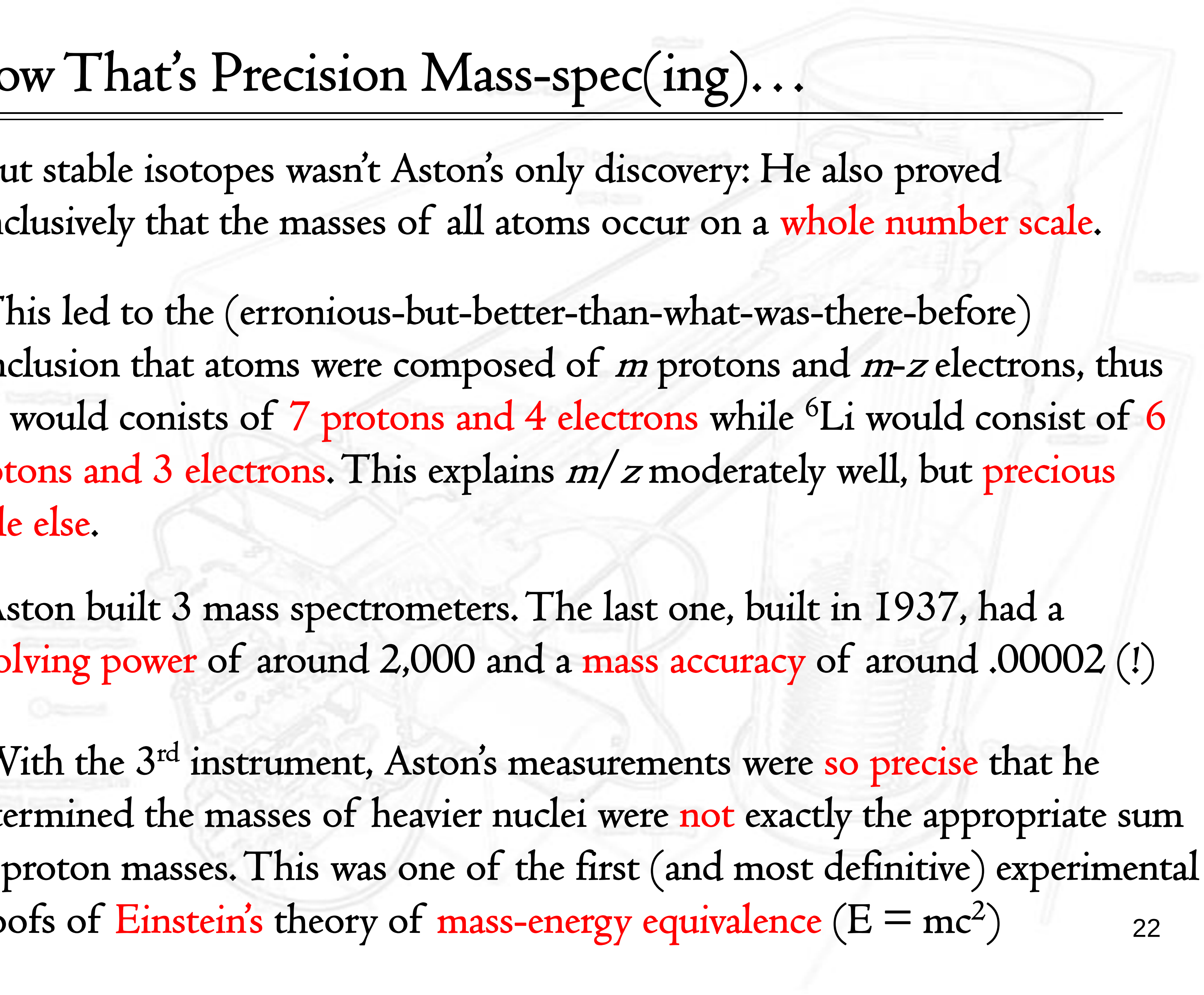


Neon isotopes at 20 and 22

Cl isotopes at 35 and 37

- Other peaks are impurities and/or compounds (often hydrides like $H^{35}Cl$ and $H^{37}Cl$)

Now That's Precision Mass-spec(ing)…



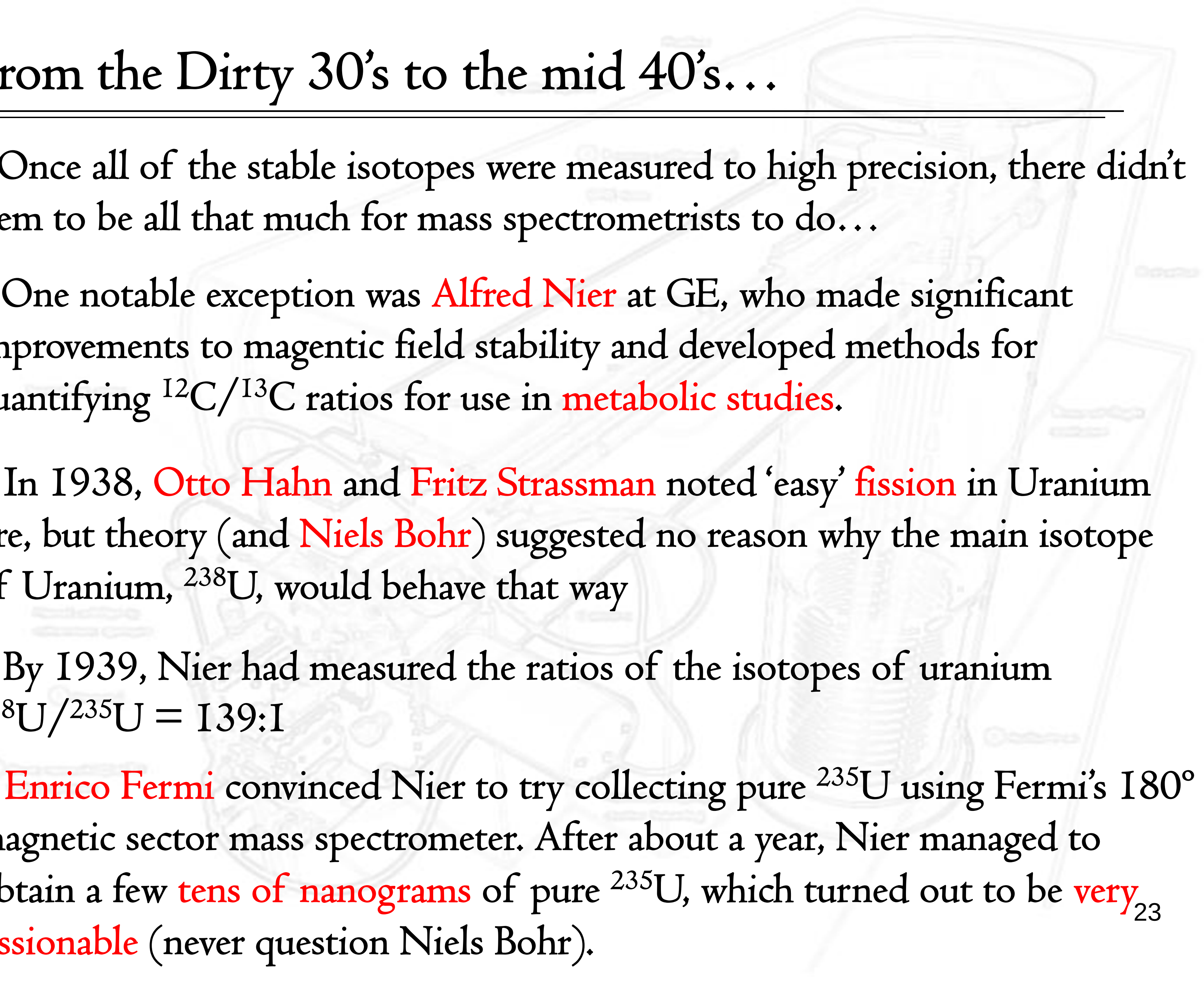
- But stable isotopes wasn't Aston's only discovery: He also proved conclusively that the masses of all atoms occur on a whole number scale.

- This led to the (erronious-but-better-than-what-was-there-before) conclusion that atoms were composed of m protons and m-z electrons, thus ^{7}Li would conists of 7 protons and 4 electrons while ^{6}Li would consist of 6 protons and 3 electrons. This explains m/z moderately well, but precious little else.

- Aston built 3 mass spectrometers. The last one, built in 1937, had a resolving power of around 2,000 and a mass accuracy of around .00002 (!)

- With the 3rd instrument, Aston's measurements were so precise that he determined the masses of heavier nuclei were not exactly the appropriate sum of proton masses. This was one of the first (and most definitive) experimental proofs of Einstein's theory of mass-energy equivalence ($E = mc^2$)

From the Dirty 30's to the mid 40's…



- Once all of the stable isotopes were measured to high precision, there didn't seem to be all that much for mass spectrometrists to do…

- One notable exception was Alfred Nier at GE, who made significant improvements to magentic field stability and developed methods for quantifying $^{12}C/^{13}C$ ratios for use in metabolic studies.

- In 1938, Otto Hahn and Fritz Strassman noted 'easy' fission in Uranium ore, but theory (and Niels Bohr) suggested no reason why the main isotope of Uranium, ^{238}U, would behave that way

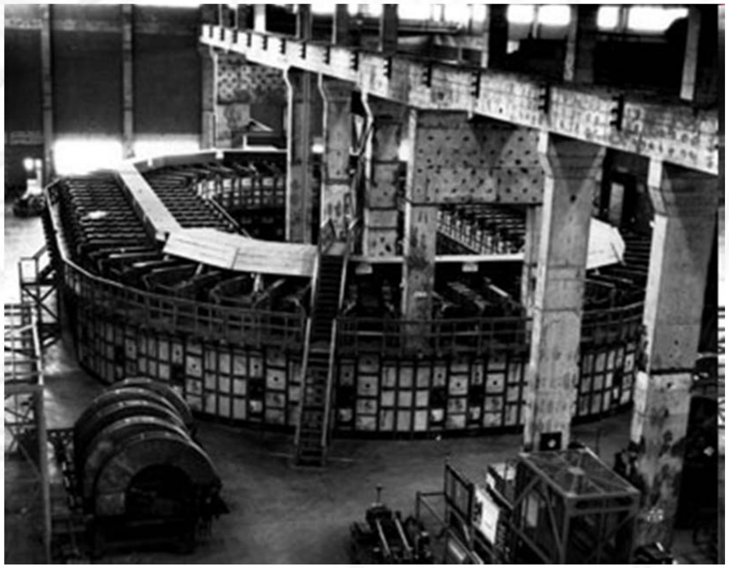
- By 1939, Nier had measured the ratios of the isotopes of uranium $^{238}U/^{235}U = 139{:}1$

- Enrico Fermi convinced Nier to try collecting pure ^{235}U using Fermi's 180° magnetic sector mass spectrometer. After about a year, Nier managed to obtain a few tens of nanograms of pure ^{235}U, which turned out to be very fissionable (never question Niels Bohr).

Makin the Bomb Ain't Easy…

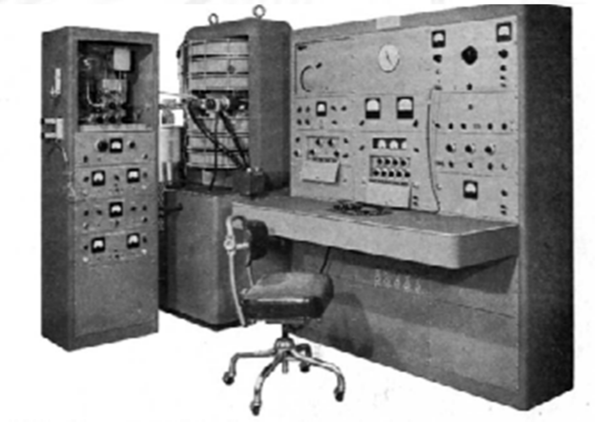
- Making a few nanograms of ^{235}U is all well and good, but to make a bomb (everybody wanted to make a bomb in the early 40's) would require hundreds of grams (10,000,000,000 x more)

- To do this, Earnest Lawrence developed the first (and kindof the last) preparative mass spectrometer, the Calutron. To make the bomb, 96 calutron units were used in series: The α-racetrack



After WWII to the late 70's…

- After the war, most advances in mass spectrometry were dominated mainly by incremental advances:
  - The first gas phase ion dissociation reactions were observed (called 'metastable peaks')
  - Industry, most especially oil and gas started to make use of MS… the first commercial instruments appear
  - Time-of-flight (TOF) mass analysis was introduced, but not widely used

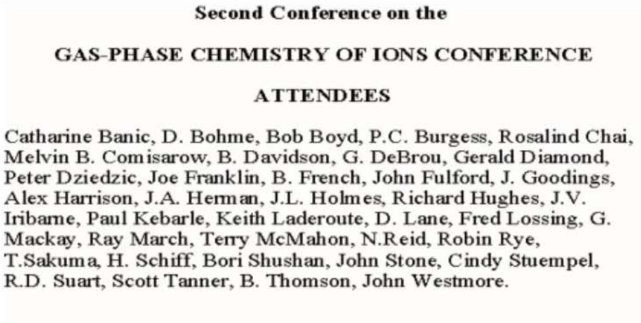


CEC 21-103C (sector) was one of the first commercial MS instruments

- Two notable exceptions to 'incremental':
  - 1957 – Quadrupole mass analyzer described. Not commercially available until 1968.
  - 1967 – McLafferty and Jennings introduce collisional dissociation

The late 70's to the present…

- Things started to heat up in the late 70's and early 80's, mostly thanks to new mass analyzers and ionization methods
- Two major advances helped things along:
  - The increased availability of Quadrupole mass analyzers, which were much more versatile (and cheaper) than sectors. Diethard K. Bohme built the first quadrupole MS in Canada!
  - The development of atmospheric pressure ionization techniques



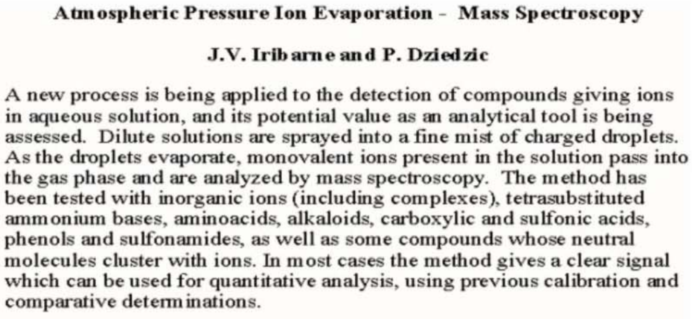
Second Conference on the

GAS-PHASE CHEMISTRY OF IONS CONFERENCE

ATTENDEES

Catharine Banic, D. Bohme, Bob Boyd, P.C. Burgess, Rosalind Chai, Melvin B. Comisarow, B. Davidson, G. DeBrou, Gerald Diamond, Peter Dziedzic, Joe Franklin, B. French, John Fulford, J. Goodings, Alex Harrison, J.A. Herman, J.L. Holmes, Richard Hughes, J.V. Iribarne, Paul Kebarle, Keith Laderoute, D. Lane, Fred Lossing, G. Mackay, Ray March, Terry McMahon, N.Reid, Robin Rye, T.Sakuma, H. Schiff, Bori Shushan, John Stone, Cindy Stuempel, R.D. Suart, Scott Tanner, B. Thomson, John Westmore.

Figure 3. Attendees at the 2nd Gas-Phase Chemistry of Ions Conference



Atmospheric Pressure Ion Evaporation - Mass Spectroscopy

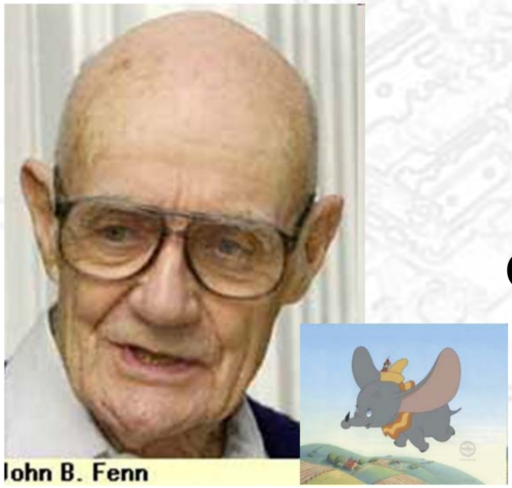
J.V. Iribarne and P. Dziedzic

A new process is being applied to the detection of compounds giving ions in aqueous solution, and its potential value as an analytical tool is being assessed. Dilute solutions are sprayed into a fine mist of charged droplets. As the droplets evaporate, monovalent ions present in the solution pass into the gas phase and are analyzed by mass spectroscopy. The method has been tested with inorganic ions (including complexes), tetrasubstituted ammonium bases, aminoacids, alkaloids, carboxylic and sulfonic acids, phenols and sulfonamides, as well as some compounds whose neutral molecules cluster with ions. In most cases the method gives a clear signal which can be used for quantitative analysis, using previous calibration and comparative determinations.

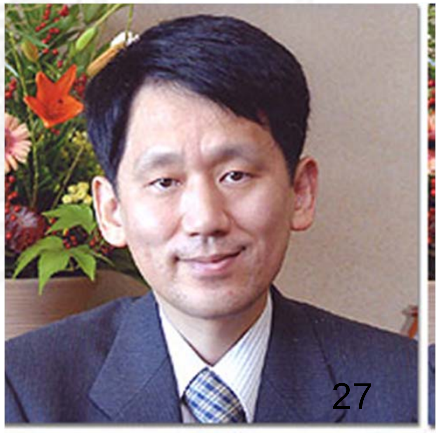
Figure 4. The abstract from J.V. Iribarne and P. Dziedzic for the Second Gas-Phase Chemistry of Ions Conference, 1980.

Late 1980's: Soft Ionization

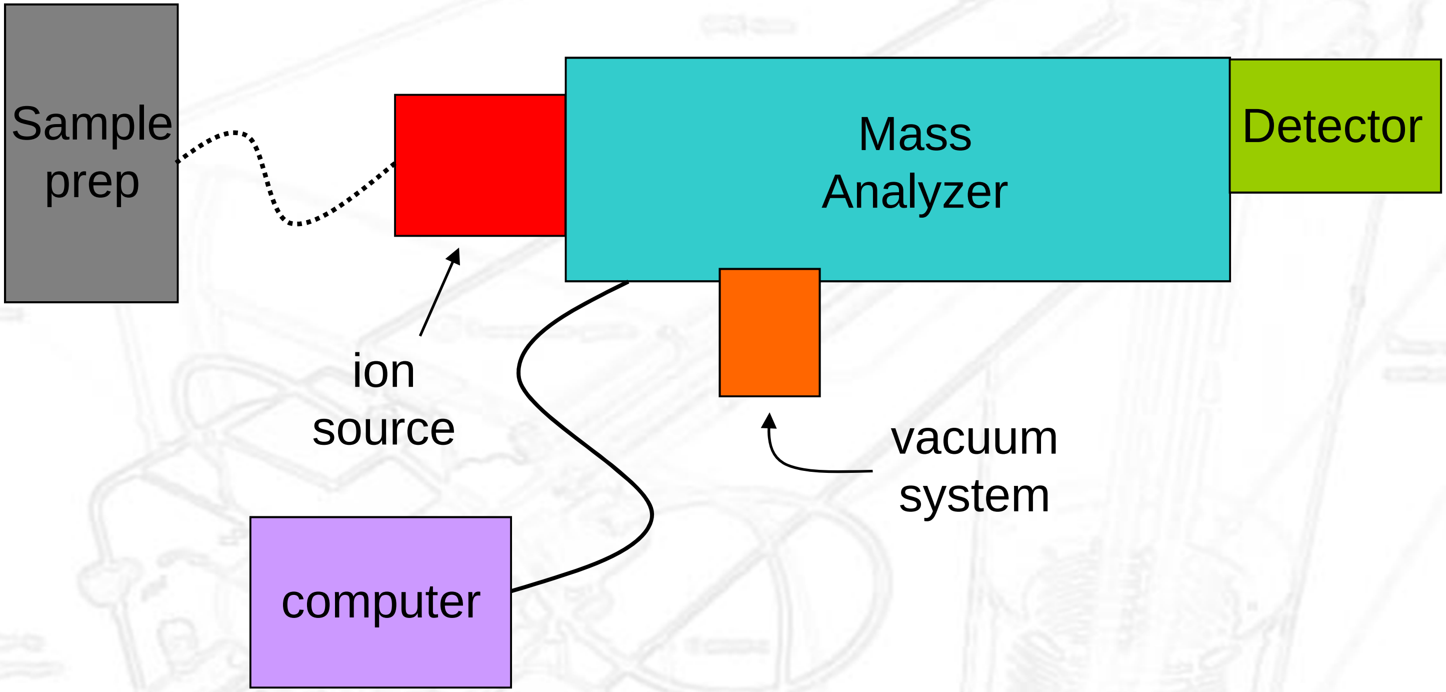
- In the late 1980's two ionization techniques appeared that would spark a huge increase in interest in Mass Spectrometry. These were:
  - Matrix Assisted Laser Desorption Ionization (MALDI)
  - Electrospray Ionization
- These ionization techniques were revolutionary because they were soft, meaning that they transferred whole molecules intact into the gas phase… *even proteins and non-covalent protein complexes*!



John Fenn and Koichi Tanaka
won the 2002 Nobel prize in
Chemistry for Electrospray and
MALDI, respectively.



The Modern Mass Spectrometer



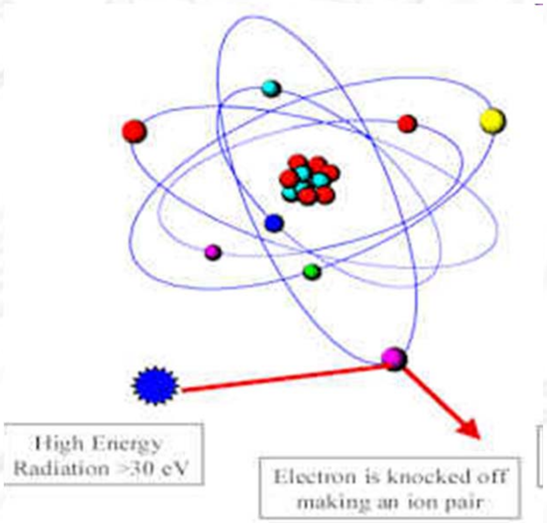
- Sample prep isn't part of the mass spectrometer, strictly speaking, but it is so integral that common sample prep instruments (*i.e.* LC, GC) are often directly coupled to the ion source and sold with the instrument.

- The first half of this course is arranged around these components and the theory associated with their use. The second half deals with applications. . .

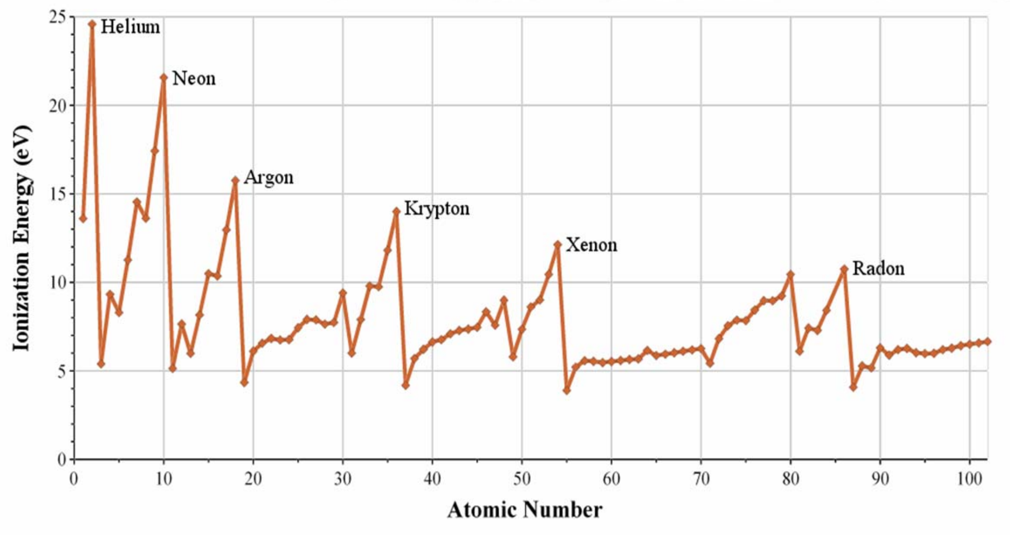
Ionization

What is ionization?

- In the simplest case, ionization is the removal of an electron to give an atom with an overall +ve charge.



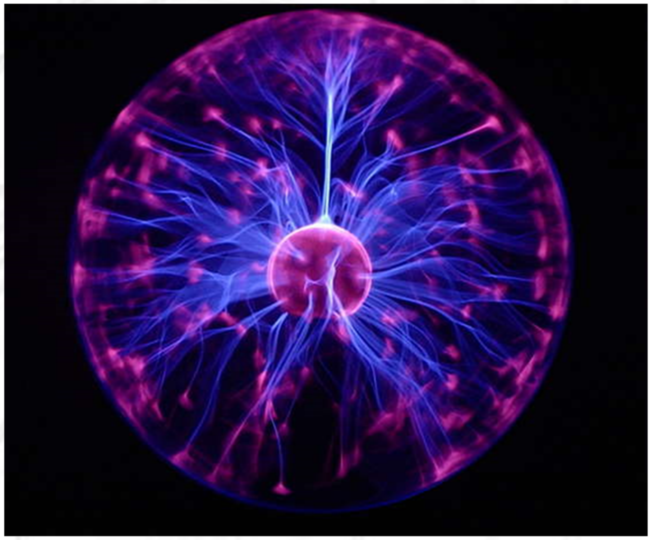
- The amount of energy required to do this (ionization energy) depends on how tightly the nucleus binds it's highest energy electrons:



- The earliest mass spectrometrists used gas discharge, supplying hundreds of eV. More than enough for a single ionization and often multiple ionizations.

Gas Discharge Ionization…

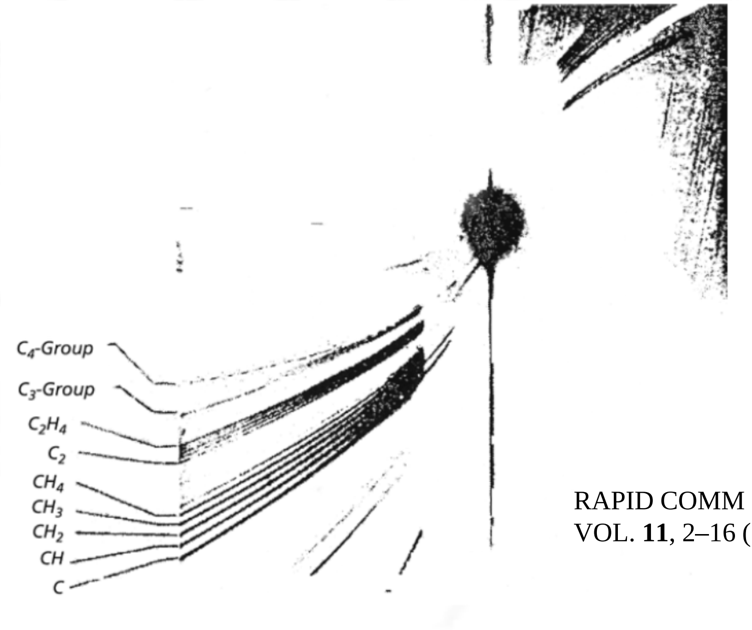
- Gas discharge makes pretty colours, depending on the gas being ionized. Street lamps are often low pressure sodium discharge lamps.



- It was not uncommon for anode ray parabola people to detect things like O^{2+}

- Because of the amount fo energy, gas discharge is also quite destructive.

- Here’s a gas discharge spectrum for methane:

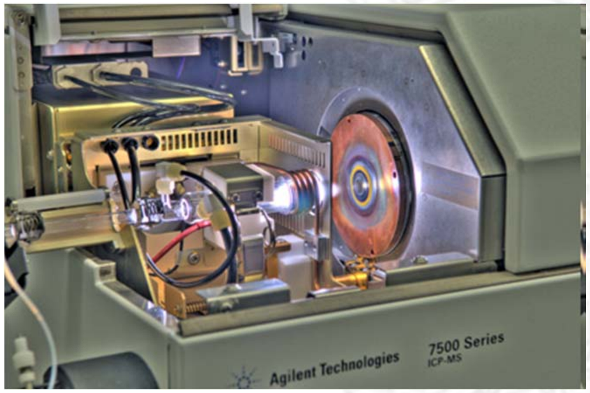


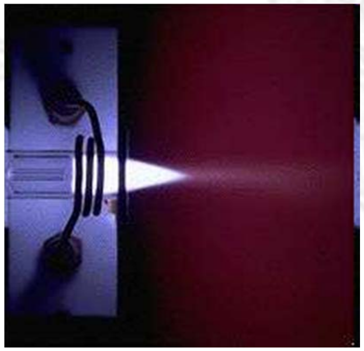
RAPID COMM MASS SPECTROM, VOL. **11**, 2–16 (1997)

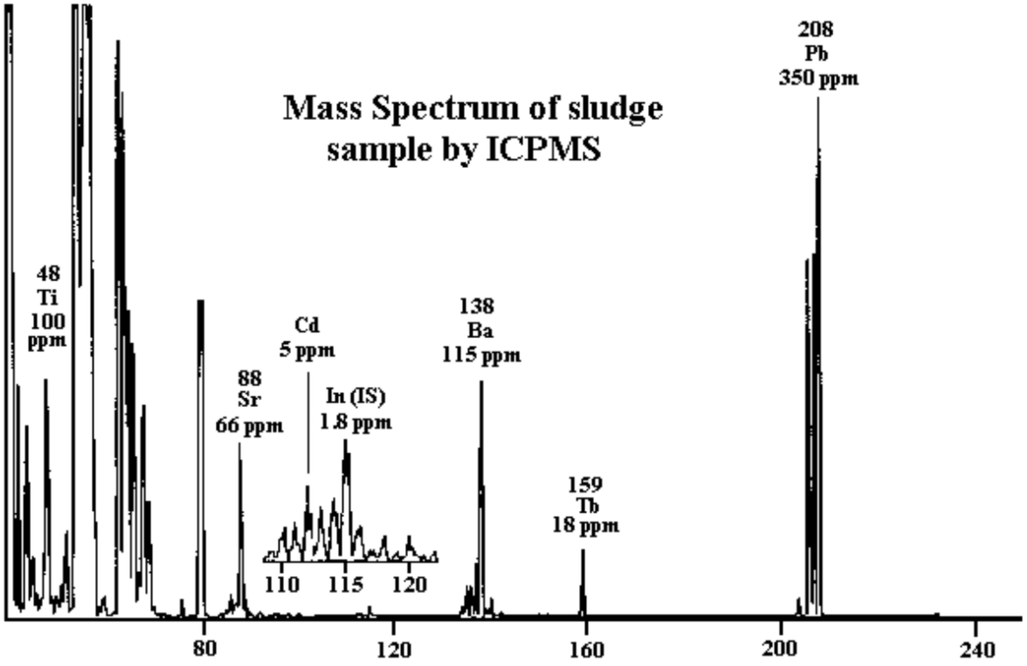
Inductively Coupled Plasma Ionization

- Probably the most direct modern descendent of gas discharge is Inductively Coupled Plasma (ICP) ionization mass spectrometry

- In fact, the actual process of ionization (sample interacting with plasma) is almost identical, except for the fact that the ‘plasma torch’ used in ICP is stable, providing a consistent amount of energy for ionization.






Mass Spectrum of sludge sample by ICPMS

ICP-MS Con't…

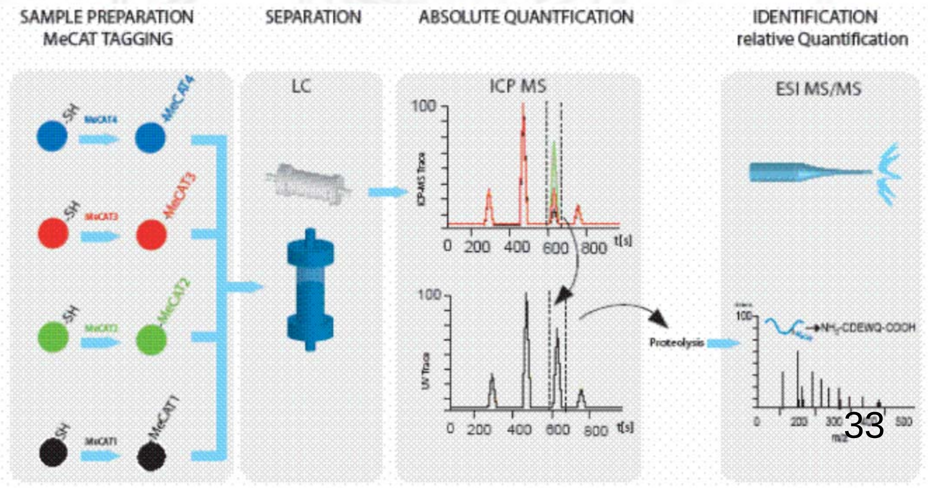
- ICP ionization is used mainly because it can efficiently ionize absolutely anything. This makes it highly sensitive and more quantitative.

- Unfortunately, in so doing, it also destroys pretty much everything.

- It is therefore relegated mostly to the analysis of metal containing compounds or complex mixtures containing metals (like the sludge you saw on the last page).

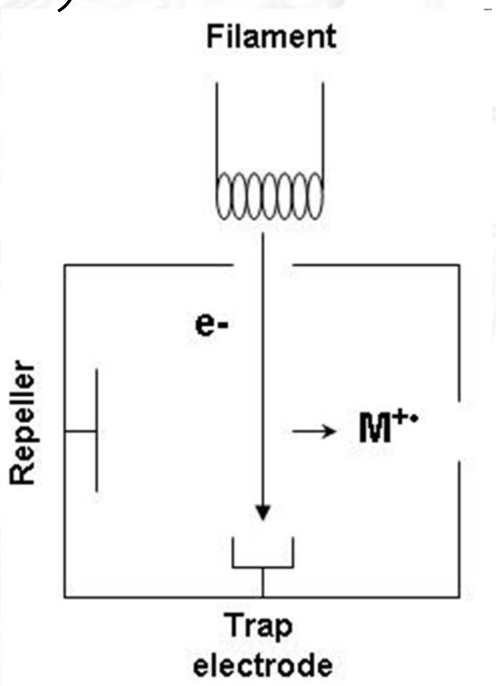
- ICP-MS can also be used for trace metal measurements in biological samples.

- A recent biological application is quantitation of metal-tagged proteins (MeCAT).



Electron Ionization…

- Electron Ionization (formerly electron impact ionization) is sortof a lower energy version of gas discharge.



- Electrons are emitted from a hot filament (usually tungsten, *i.e.* a lightbulb)

- These electrons are accelerated towards an electrode so that their energy > 60 eV. As a result:

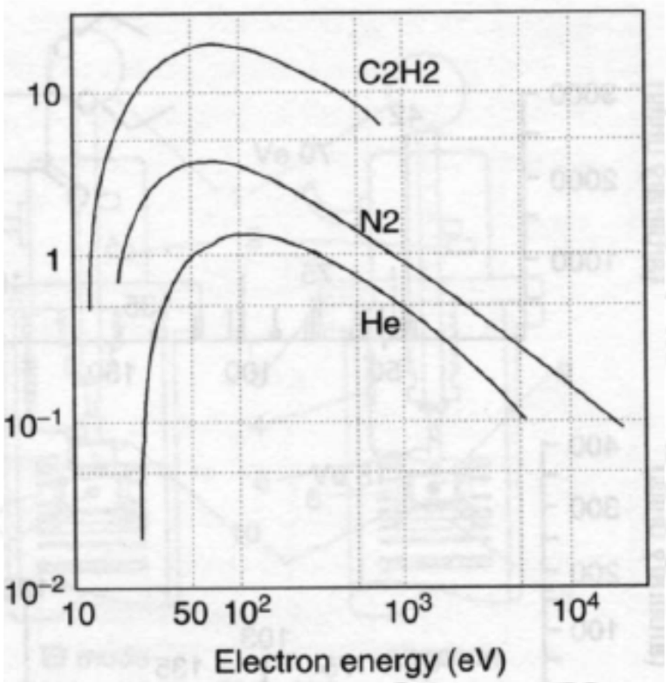
$$M + e \rightarrow M^{\bullet +} + 2e$$

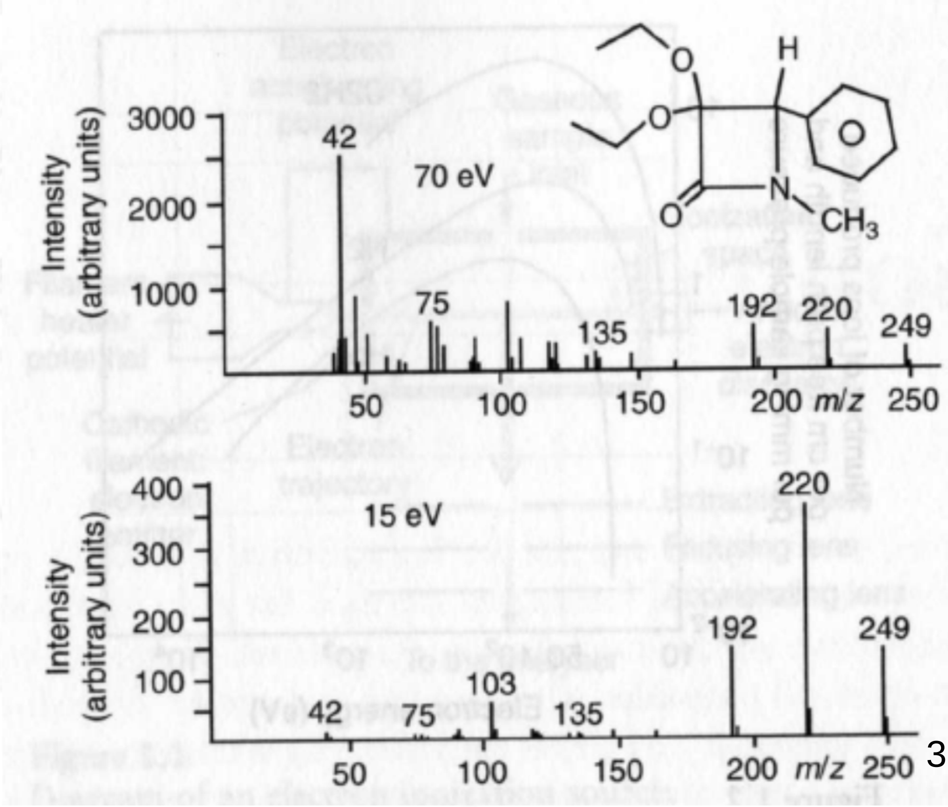
- ~ 70 eV electrons are quite resonant with electrons in C-C and C-H bonds, so that energy tends to give the best ionization of organic molecules. However…

Electron Ionization Cont'…

- Ionization events typically transfer more energy than is required for ionization (around 10 eV). This results in fragmentation.

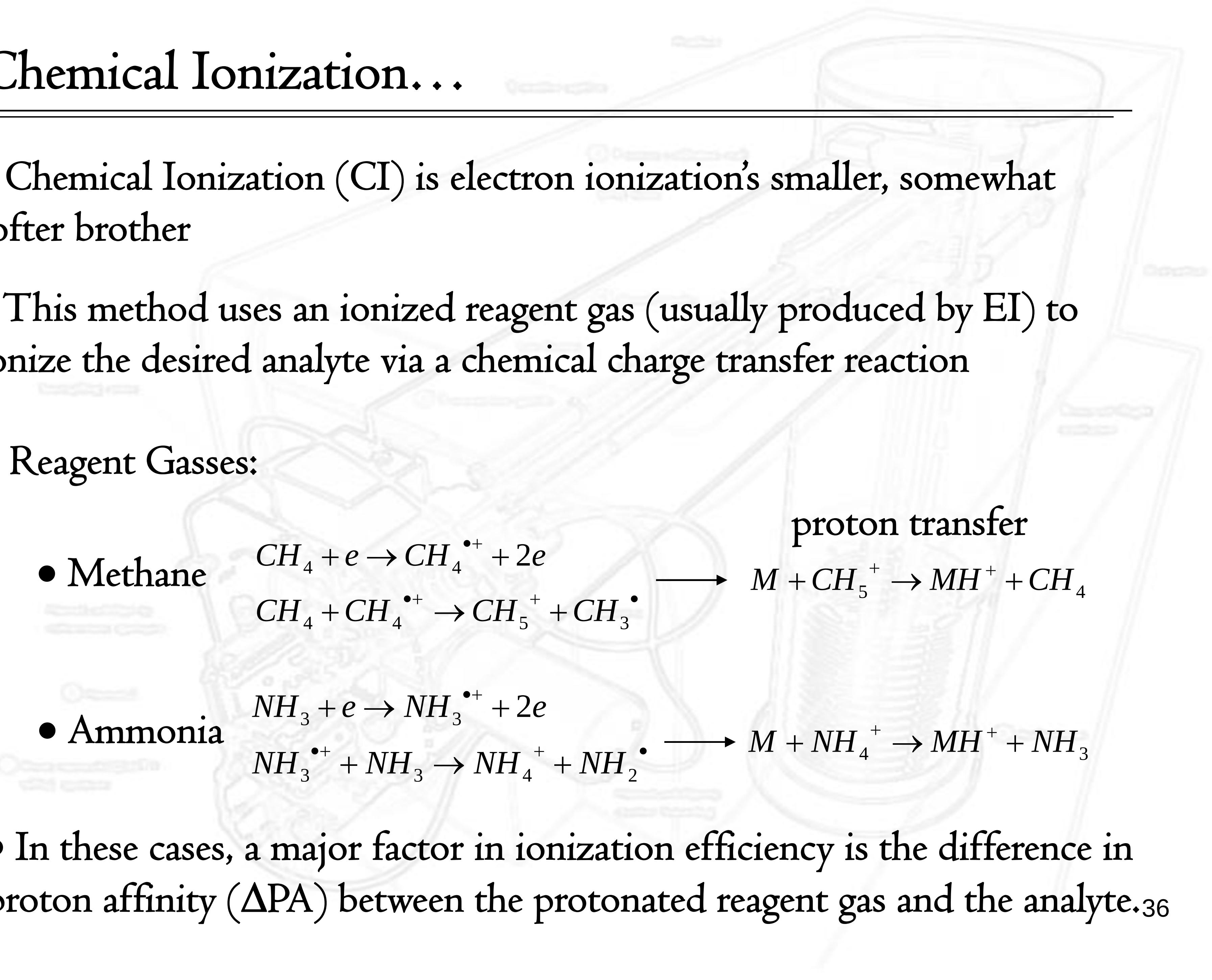
- As a result, there is a tension between ionization efficiency and fragmentation in EI mass spectrometry.





Hoffmann and Stroobant (2007) 'Mass Spectrometry: Principles and Applications', John Wiley and Sons

Chemical Ionization…



- Chemical Ionization (CI) is electron ionization's smaller, somewhat softer brother

- This method uses an ionized reagent gas (usually produced by EI) to ionize the desired analyte via a chemical charge transfer reaction

- Reagent Gasses:
  - Methane

$$CH_4 + e \rightarrow CH_4^{\bullet +} + 2e$$
$$CH_4 + CH_4^{\bullet +} \rightarrow CH_5^{+} + CH_3^{\bullet}$$
$$\xrightarrow{\text{proton transfer}} \quad M + CH_5^{+} \rightarrow MH^{+} + CH_4$$

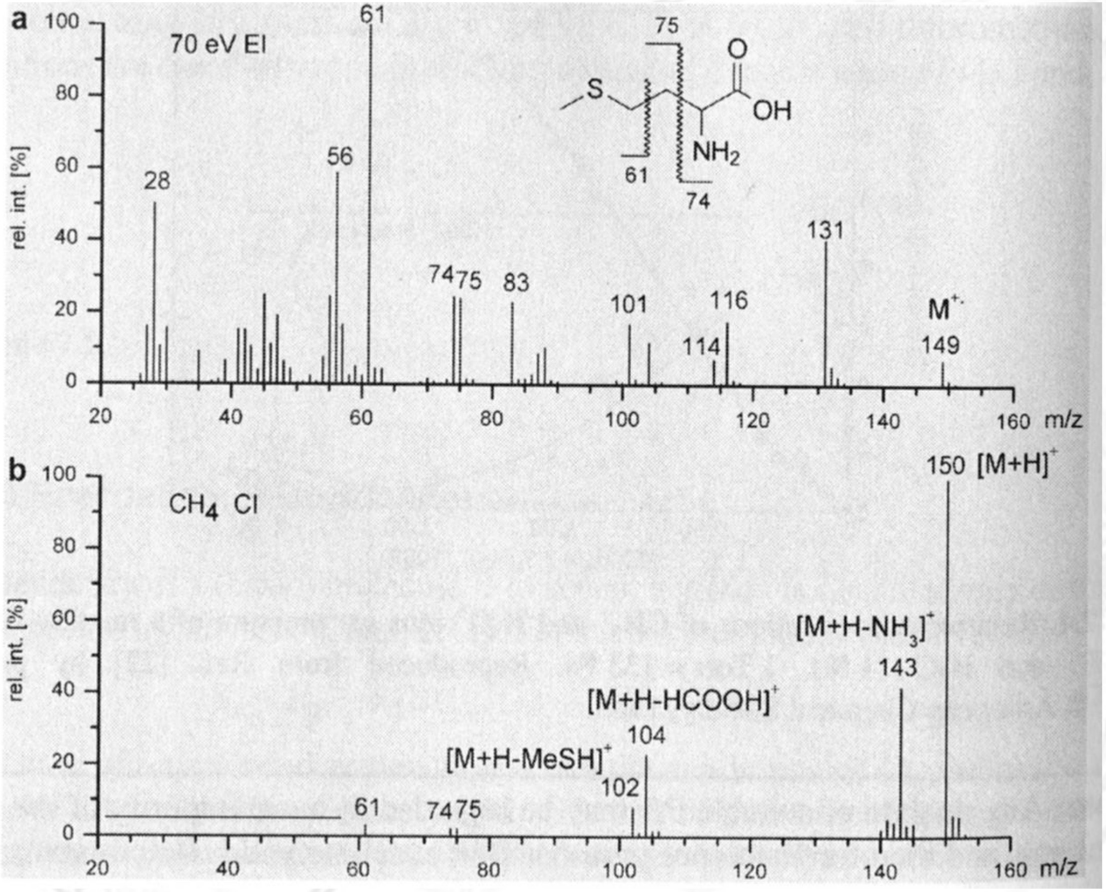
  - Ammonia

$$NH_3 + e \rightarrow NH_3^{\bullet +} + 2e$$
$$NH_3^{\bullet +} + NH_3 \rightarrow NH_4^{+} + NH_2^{\bullet}$$
$$\longrightarrow \quad M + NH_4^{+} \rightarrow MH^{+} + NH_3$$

- In these cases, a major factor in ionization efficiency is the difference in proton affinity (ΔPA) between the protonated reagent gas and the analyte.

Chemical Ionization Cont'd…

- A comparison of ionization: EI (top) and CI (bottom, with CH_4Cl as the bath gas)



Mass Spectrometry: A Textbook, Jurgen H. Gross, Springer (c)2004