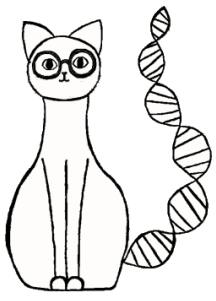
Basic Mass Spectrometry: Instrumentation

Professor Jana Roithová



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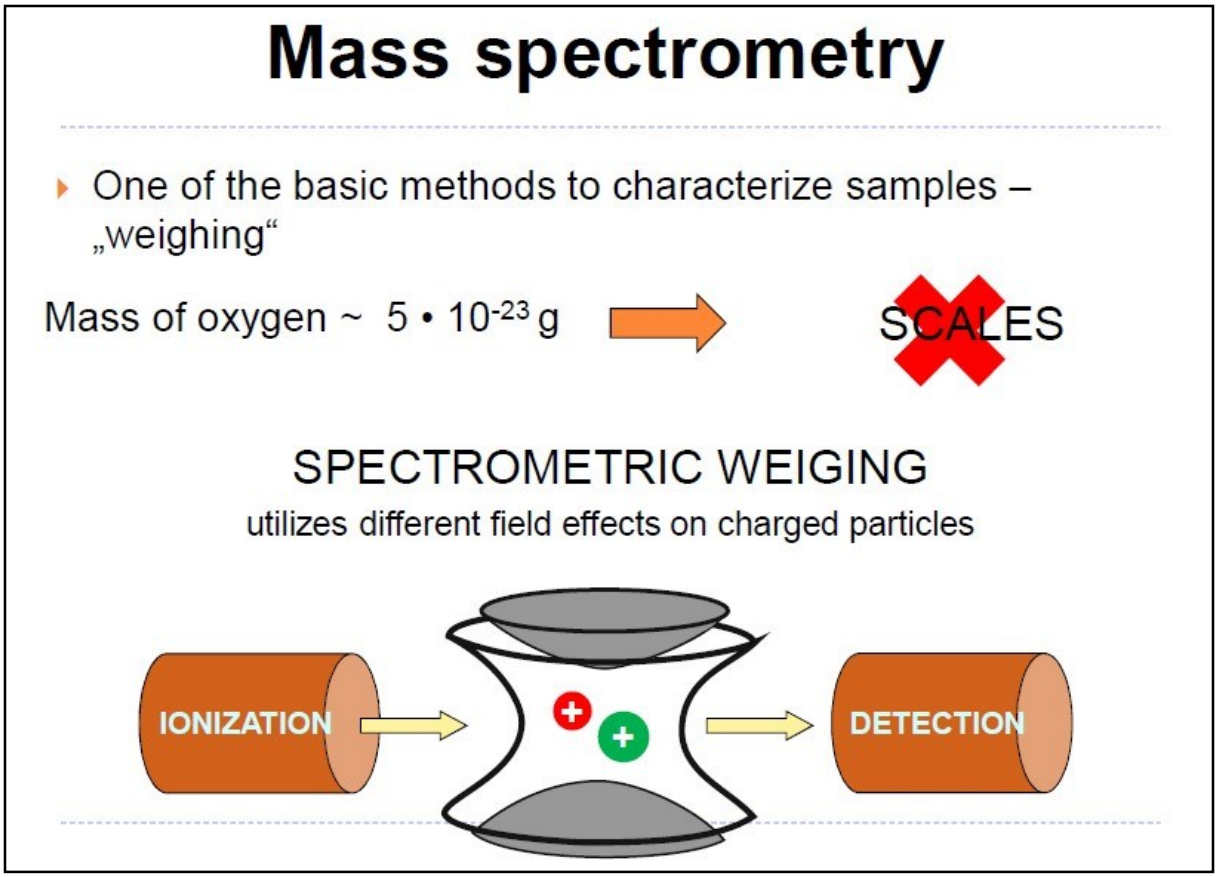
Lecture 1. Introduction

Welcome to the course on Mass Spectrometry. During this course, you will learn the basics of mass spectrometry instrumentations and the basics of interpretation of mass spectra.

What is mass spectrometry?

Mass spectrometry is a method that serves for "weighing" molecules. You probably know that we cannot weigh molecules on high precision balances but we have to do it differently and for that we have tricks.



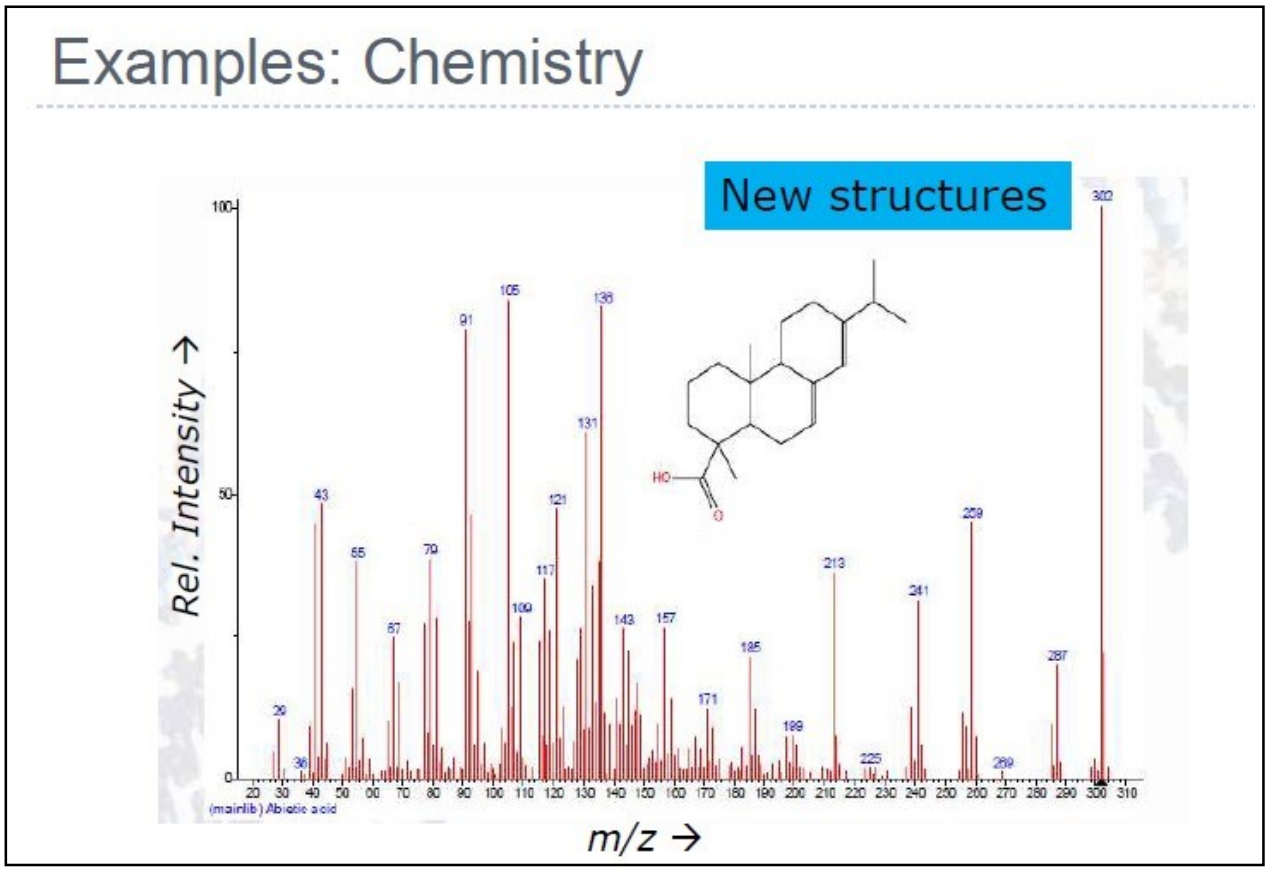
The first trick involves the ionization of a given molecule and its transfer to the gas phase. The second trick is that we evaluate the response of these ionized molecules to different fields; for example, magnetic field or electrostatic field. From the response of the ions to the field forces, we can deduce their masses.

Why is mass spectrometry important? it is important in various fields. I will give only a few examples.

In chemistry, mass spectrometry serves for the identification of the compounds.

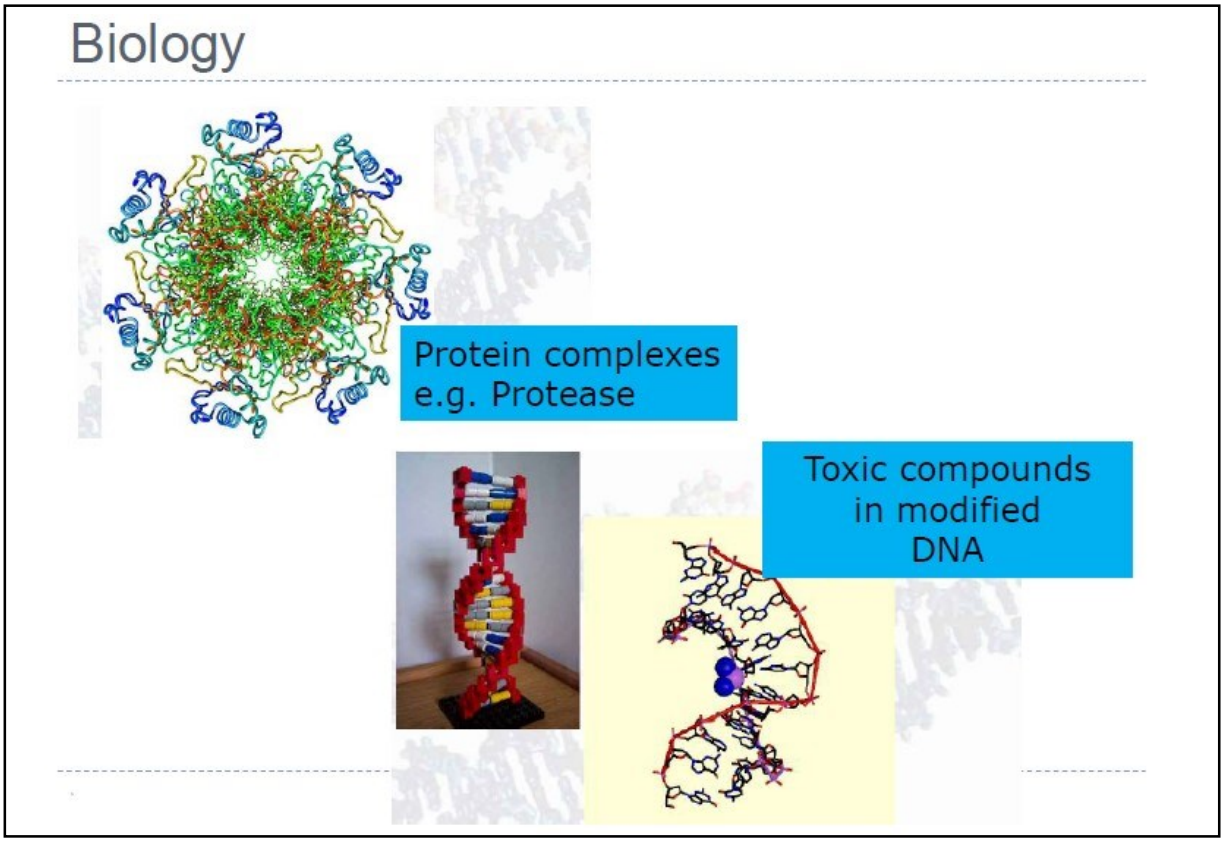
It's one of the analytical methods that give you information about the identity of a compound (that you perhaps synthesized).

It can also serve in the exploration of new structures. For example, in the natural-product analysis field, it's an important method, because there you usually extract only a tiny amount of a natural product from e.g., a plant. And then using mass spectrometry, which is a very sensitive method that needs only a tiny amount of a sample, you can determine what kind of new molecules you received.



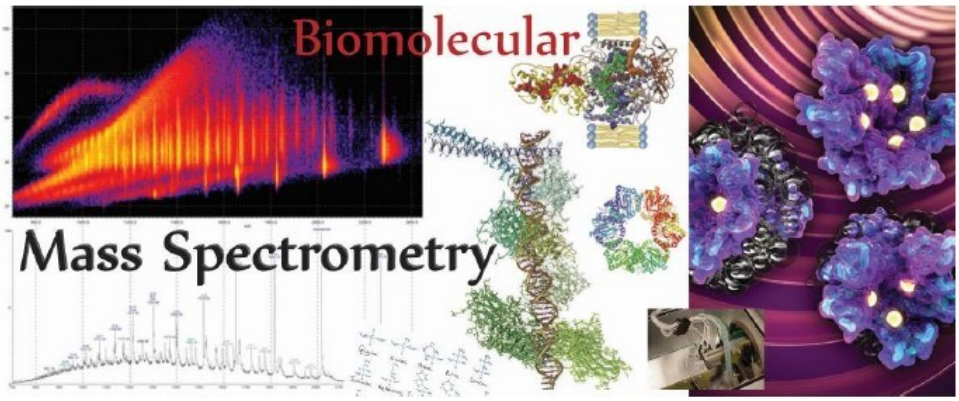
Another field that profits from mass spectrometry is molecular biology.

Another field that profits from mass spectrometry is biology or better say molecular biology. And these pictures show that nowadays we can analyze huge biomolecules using mass spectrometry:



We can look into the structure of proteins or we can look into the structure of DNA. We can even determine complexes of DNA with drugs. We can determine where the drugs bind, for example.

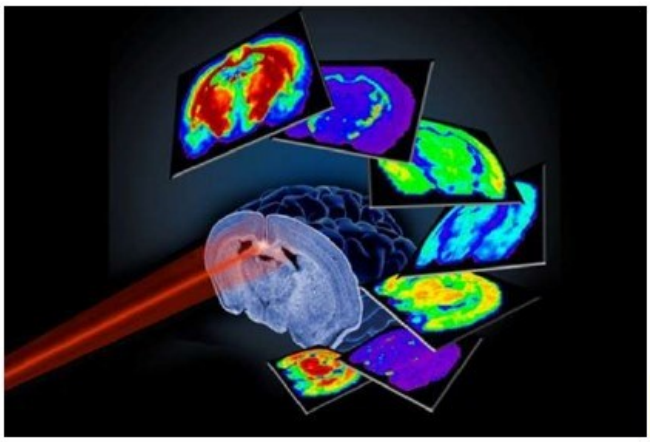
Another example is shown here:



Credit: Prof Frank Sobott: http://www.astbury.leeds.ac.uk

This is an application of mass spectrometry to the investigation of huge biomolecule systems. This picture shows you that we can analyze the masses of different fragments, for example. The second dimension of the map in this picture corresponds to ion mobility of these fragments. Using ion mobility in combination with mass spectrometry we can determine the shapes of these analyzed ions.

Another example is shown here and it stresses yet another feature of mass spectrometry:

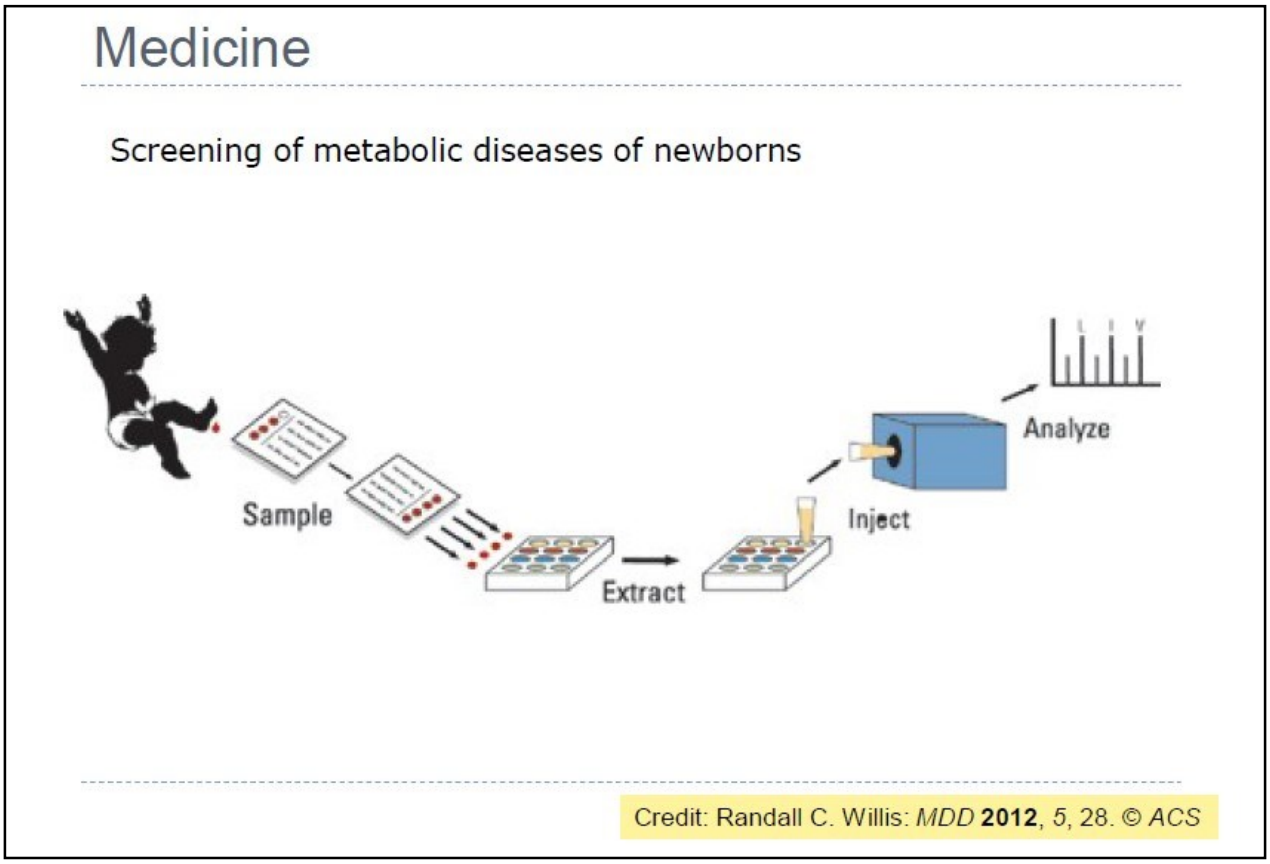


Credit: https://medschool.vanderbilt.edu/ims/

Namely, the use of mass spectrometry for imaging. You probably know that we can image tissues by histology. However, you can look at these slices of tissues also by mass spectrometry. You can measure mass spectra at each point of the 2D slice. For the determined ions (with a given mass), you obtain one picture showing you a 2D distribution of these ions. Using this approach, you can study distribution of a drug, for example, in some tissue.

Medicine is another field, where mass spectrometry is an important method.

Medicine is another field where mass spectrometry is an important method. Mass spectrometry is great for the detection of metabolites in blood or urine. And this example shows that, for example, from a drop of blood from a newborn a series of possible metabolic diseases that can be detected. This method is used routinely for screening metabolic diseases in newborns.



Analysis in environmental sciences also often relies on mass spectrometry.

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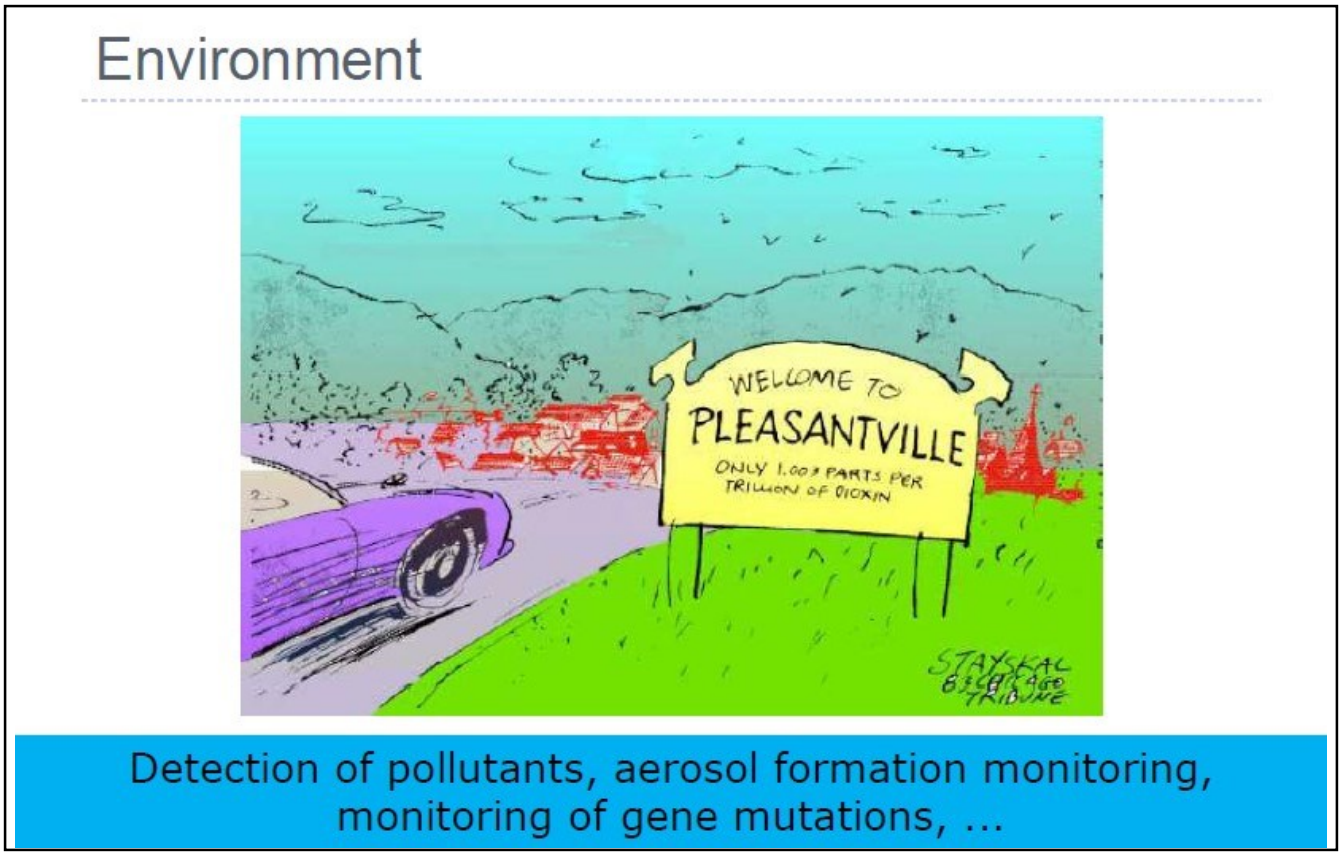
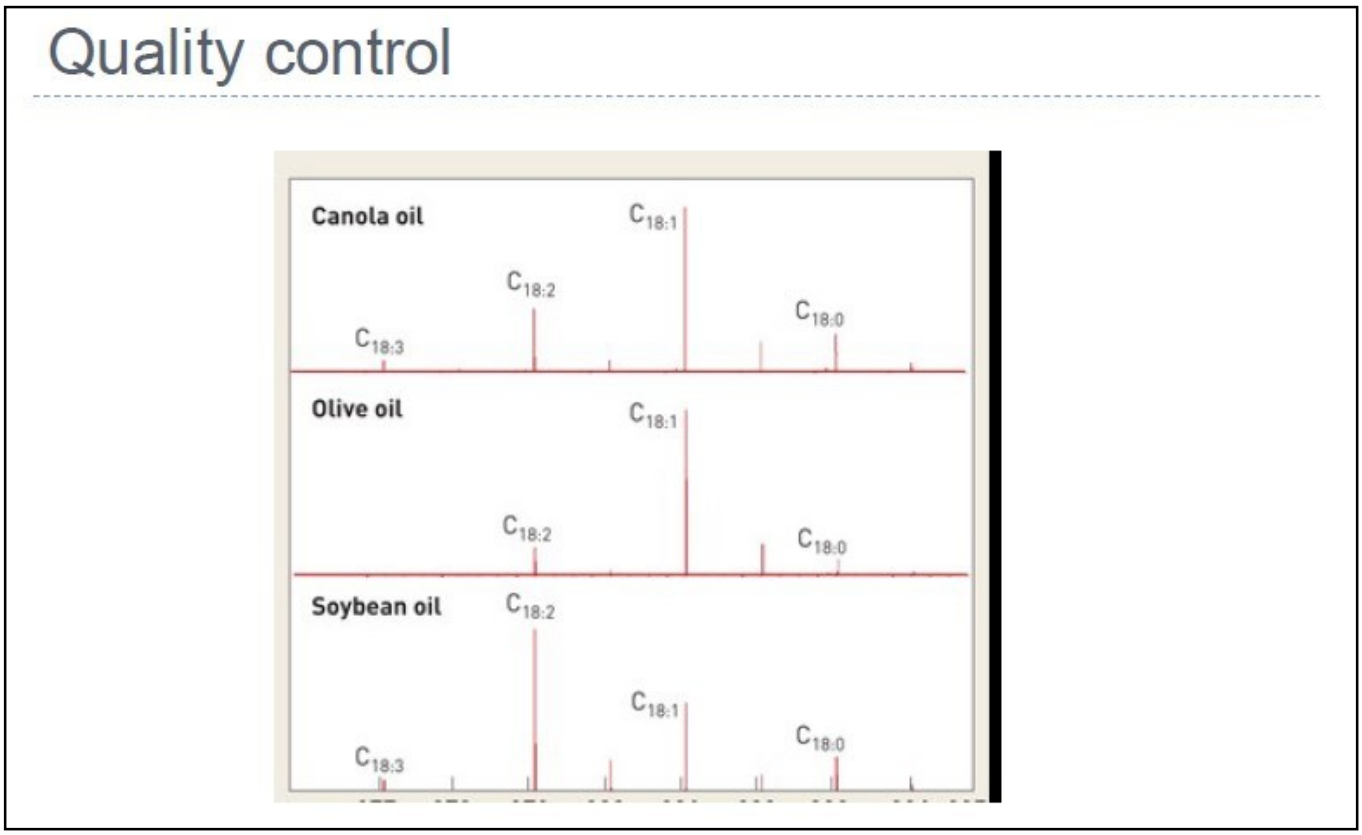


Mass spectrometry can be used for the investigation of the quality of air or the quality of water. You can study pollution in plants by some compounds, you can even monitor gene mutations. This all can be done using mass spectrometry.

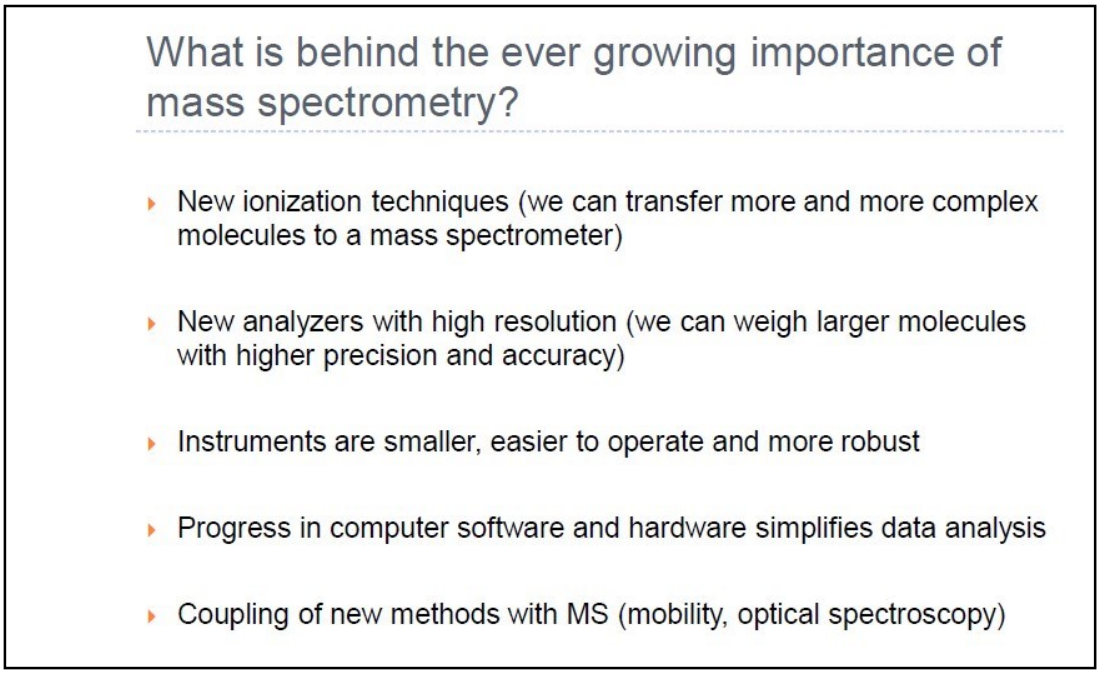
This is a simple example of how you can investigate different oils.



You can measure the mass spectrum of oil. You will see a composition of fatty acids that are in the oil. In addition, you will also determine various admixtures in the oil. This information can tell you, what kind of oil it is and it can also tell where from geographically the oil comes.

What is behind the ever growing importance of mass spectrometry.

Mass spectrometry is an important analytical method and its importance is all the time growing. And why is it growing?



Mass spectrometry is developing towards the implementation of new techniques. The innovations are on different levels. One of them is the implementation of new ionization techniques. As a result, we can nowadays transfer and ionize more

and more complex molecules to a mass spectrometer. This is what made the breakthrough in molecular biology. There is also a big advancement in the field of analyzers. New analyzers have advantages such as a higher resolution. This contributes to our ability to analyze larger molecules with higher precision and accuracy.

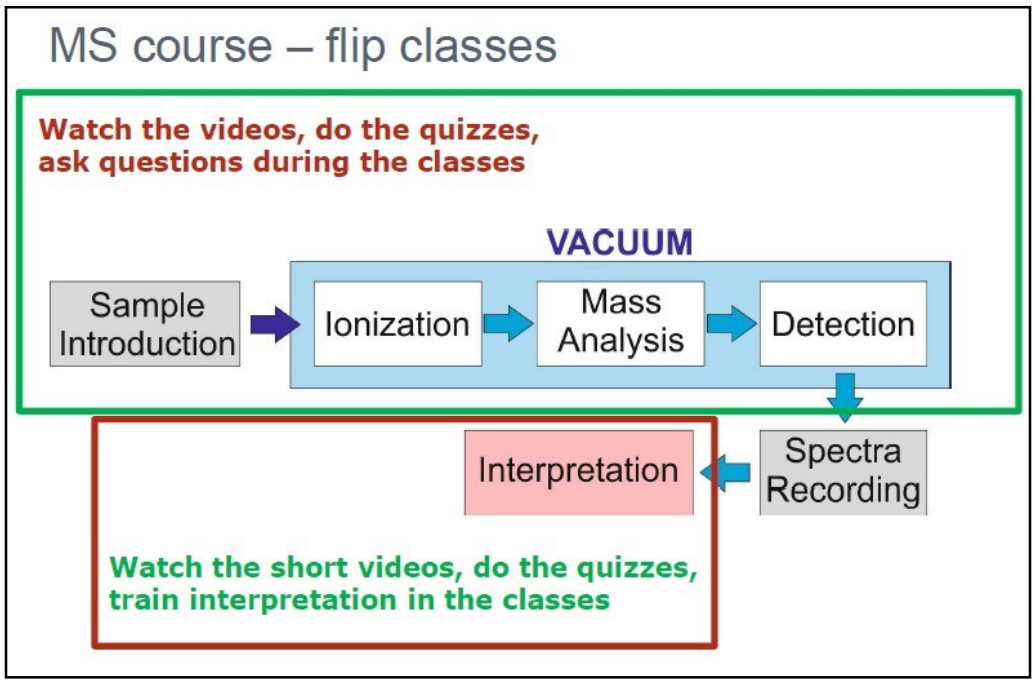
Another direction of development is towards making the instruments smaller, easier to operate, and more robust. This is especially important if you want to make use of mass spectrometry as a routine method in, for example, hospitals or at police stations, or environmental-control stations. So, this development is significant for the growth of mass spectrometry.

The emergence of mass spectrometry as an important method in molecular biology led to the production of enormous amounts of data. Hence, the progress in electronics and computer science is also very important for the applicability of mass spectrometry for studying of complex problems.

And last but not least, mass spectrometry develops in the direction of couplings with other methods. The most important coupling nowadays is the one with ion mobility. This coupling allows us not only to measure the mass of the ions but also their shape. So, it gives you additional information and it's extremely important for the analysis of large molecules.

Another coupling is the one with optical spectroscopic methods. This is mostly at a research-level now, but you should know about it because Radboud University is an important player in this field. We have a FELIX facility where we can investigate infrared spectra of mass-selected ions.

The structure of this course.



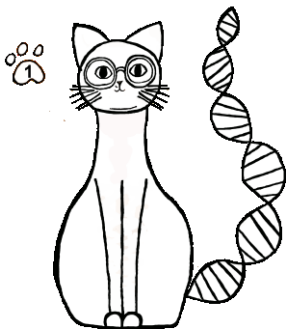
You will have to learn about instrumentation because unlike in other methods in mass spectrometry it's very important what kind of instrumentation and the method you use for your analysis. The way, how you ionize the sample and the way how you mass analyze it, determines the information that you will obtain.

The second part of the course is to learn to understand mass spectra. You will have to learn the basics of mass spectra interpretation.

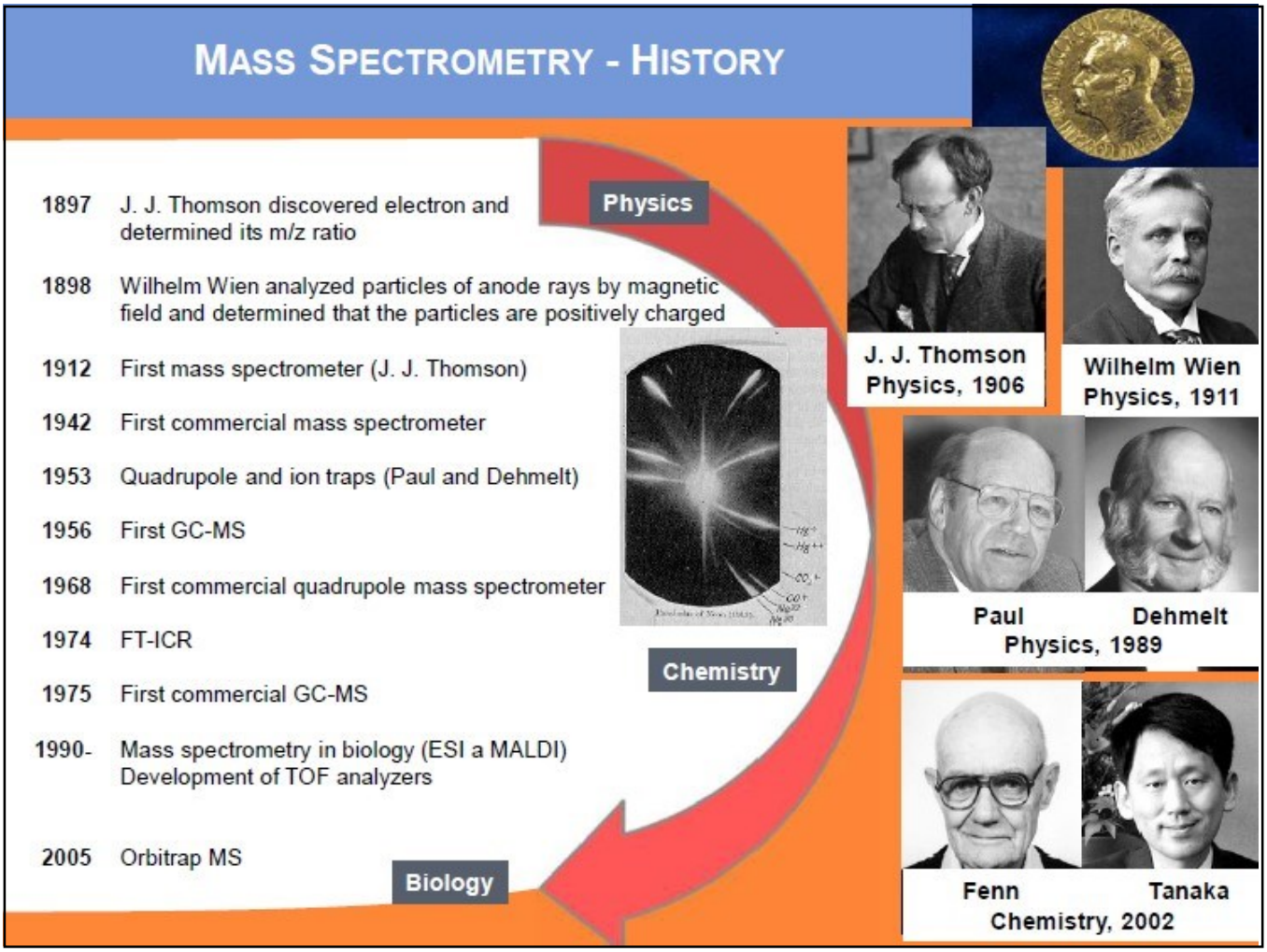
Depending on the year and conditions, I will use a different strategy for the course. It may be a frontal, classic approach or it may be the flip class approach. In any case, you will have available videos and quizzes that can prepare you at home. For the flipped classroom approach, you must prepare yourself before coming to the classroom, because we practice the knowledge, I will not explain the content again.

The interpretation of mass spectra will be always taught in person.

I hope that you will enjoy the course.

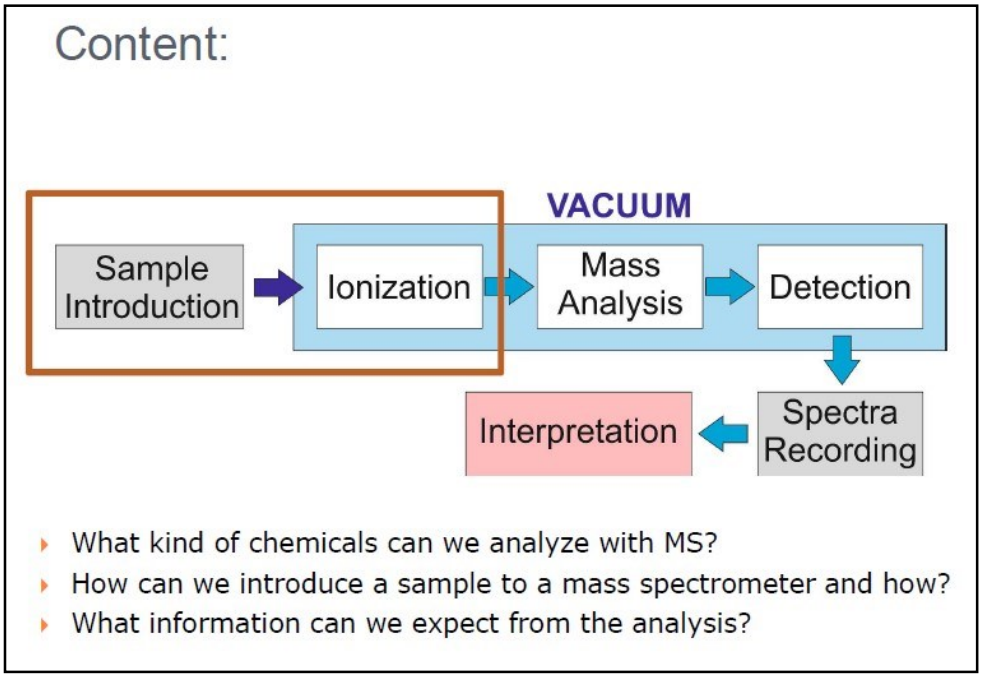


Additional materials:



Lecture 2. Ionization methods

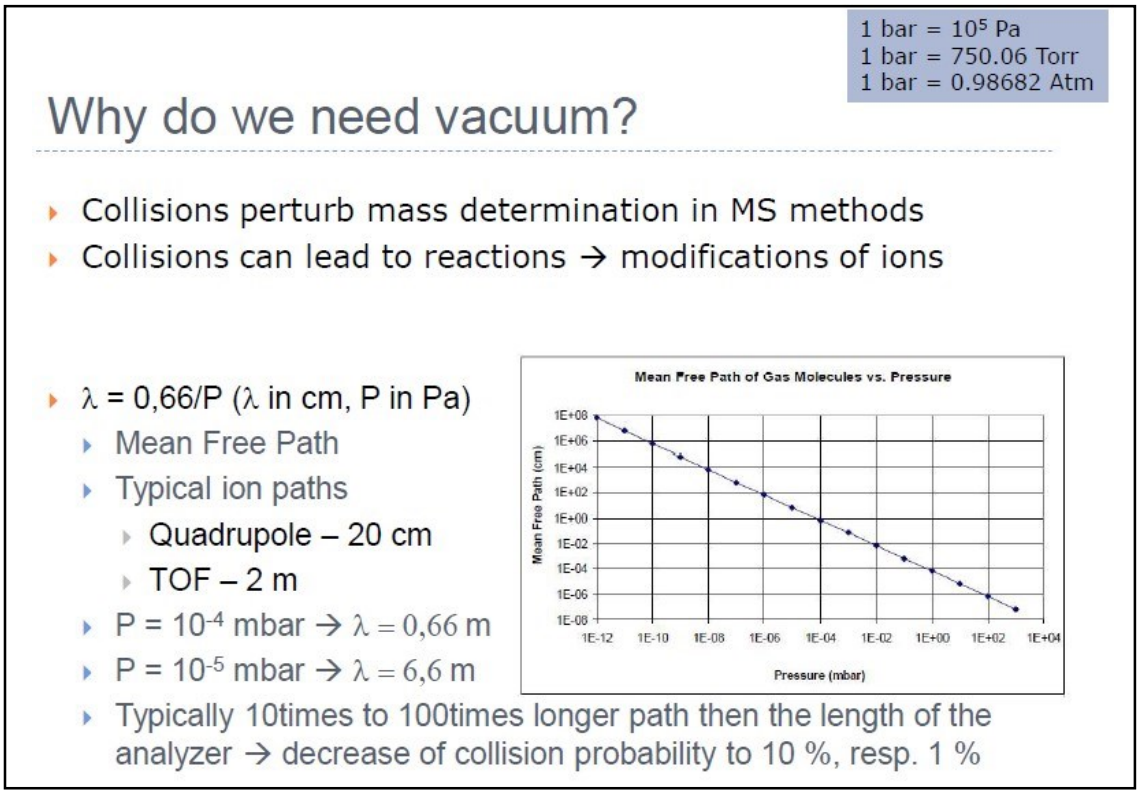
The second lecture of the mass spectrometry course is devoted to the methods for generating ions.



First, we have to introduce a sample to the mass spectrometer and during this process or afterward we ionize the molecules of the sample and genAtate ions. In the end of this lecture, you should be able to answer these questions:

- What kind of chemicals can we analyze with mass spectrometry?
- How can we introduce a sample to a mass spectrometer?
- And what information can we expect from the analysis?

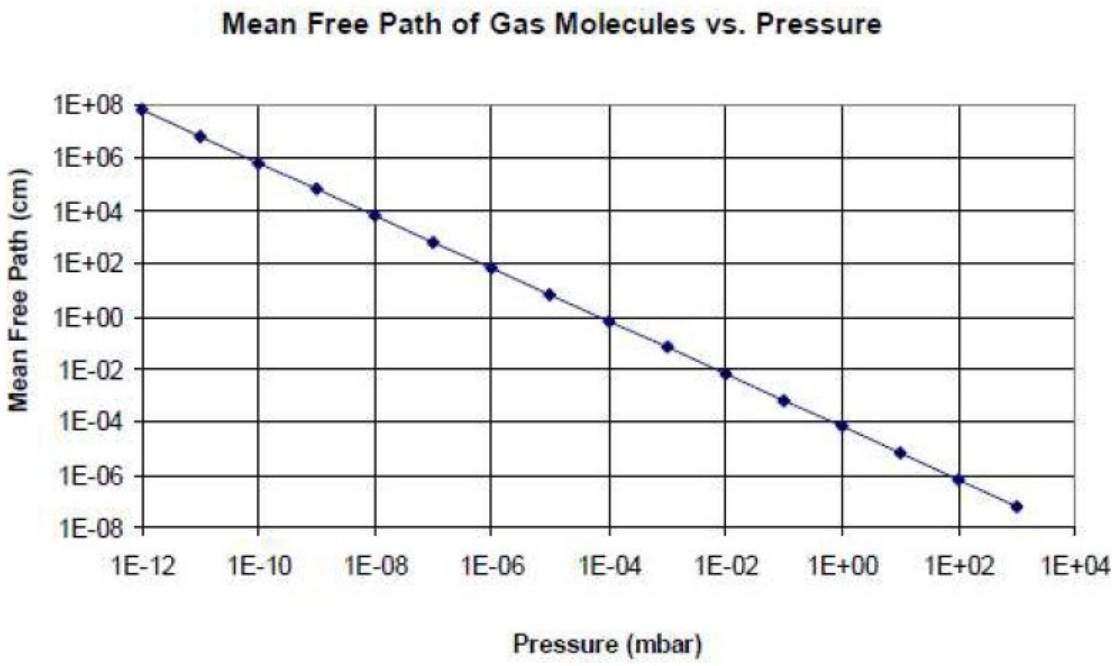
Why do we need vacuum?



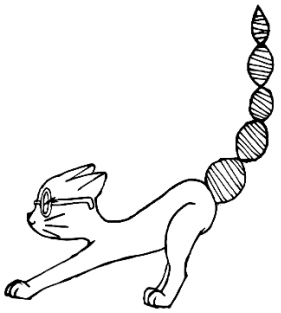
All mass spectrometers work at low pressures. Hence, we create an environment with pressures close to "a vacuum". Why do we need it? As I explained to you in the previous lesson, we measure the mass of an ion by looking at the path of the given ion in a field; e.g., in a magnetic field or electrostatic field. Hence, if the ion would collide with molecules from the air, it would perturb its pathway and therefore it would perturb the determination of its mass.

Secondly, collisions of ions with other molecules can lead to reactions. We need to avoid this because such reactive collisions could modify the structures of the ions and/or it could modify the mass of the ions. Hence, these encounters are undesired, therefore we have to work at very low pressures.

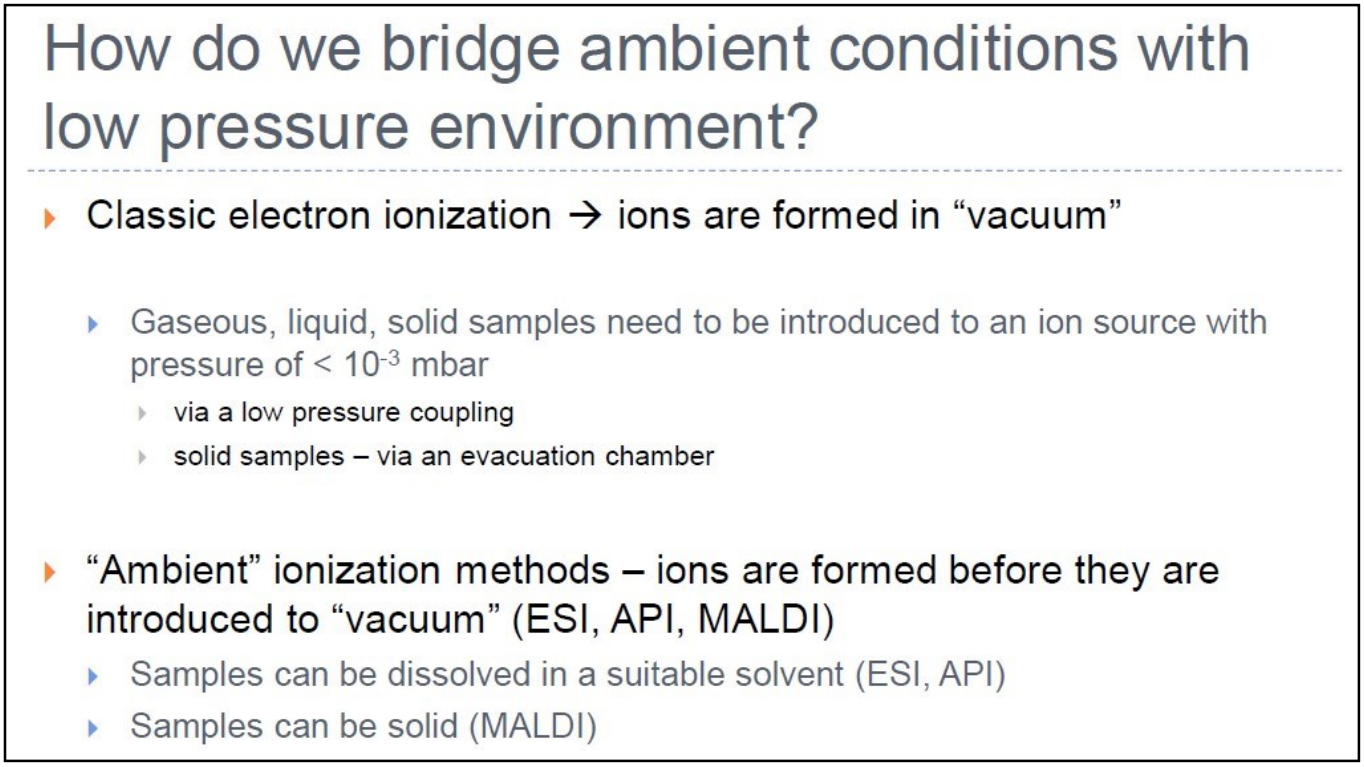
How low pressure do we need? To understand it, we will have a look at the Mean Free Path. Mean Free Path is the path of an ion achieved at a given pressure without any collision. What are the mean-free-paths that are typically required in mass spectrometry? To understand it, we must think about the dimensions of mass spectrometers that we are going to work with. For example, using quadrupole-based mass spectrometer, the ions must travel about 20 cm without any collision. Another typical example is a mass spectrometer with the time of flight analyzer. It is much longer analyzer than a quadrupole. The ions must achieve about 2 m paths without any collision, but this number can differ for different analyzers.



If we look at the correlation between the pressure and Mean Free Paths of molecules or ions at the given pressure, we see that for the pressure of 10^{-4} mbar (millibars) the ions achieve around 66 cm without colliding. When we decrease the pressure 10 times, we increase the Mean Free Path 10 times. If we want to have an unperturbed determination of the mass, we should reduce the probability of the collision during the required path to about 10% or 1%. This means that we will work at even lower pressures than determined here. We will typically work at 10^{-6} mbar and lower pressures.



How do we bridge ambient conditions with low-pressure environment?



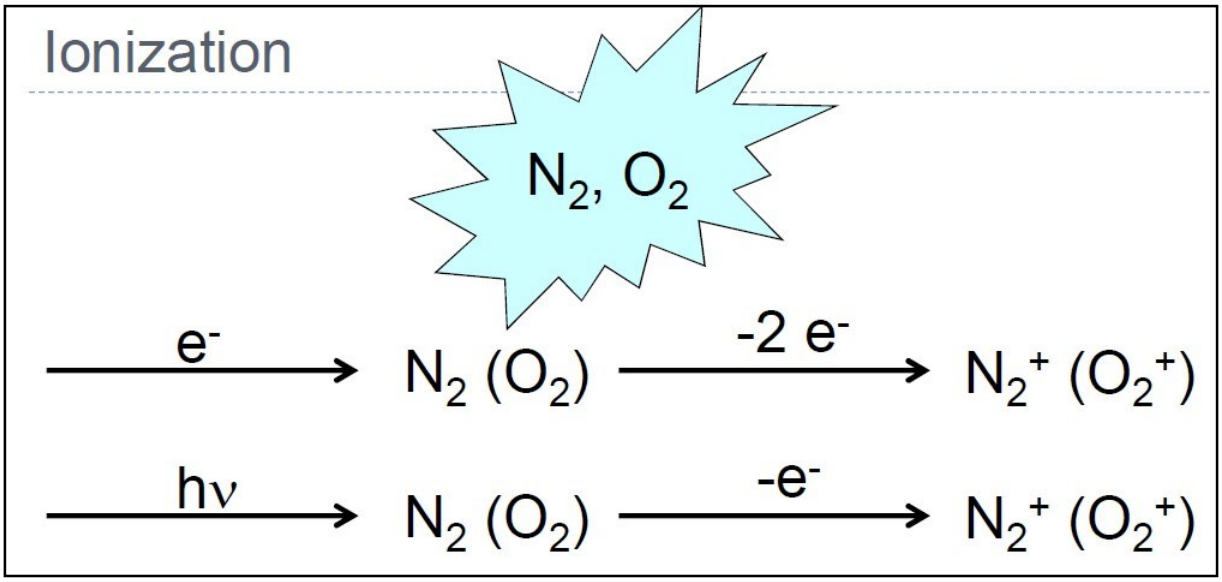
The requirement of working under variable pressure means that we somehow have to introduce the sample from the laboratory conditions (one atmosphere) to the mass spectrometer (very low pressure). How do we do it? It depends on the ionization method that we choose to work with.

The first type of ionization is classical electron ionization. During this method, the ions are formed in a vacuum. It means that you have to introduce the analyte to the vacuum. The analyte can be gaseous, liquid, or solid. It can be introduced through a septum to the low-pressure range. This is available with gaseous or liquid samples. For solid samples, we need to use a probe and an evacuation chamber. In any case, you must be always very careful because during the sample/analyte introduction, you must bridge the 1 atm and "vacuum" environments and during such action, you can damage the instrument if you don't work sufficiently carefully.

Another type of ionization methods are ambient ionization methods. These methods were developed much later than the classical ionization methods and they allow us to transform the molecules of our interest into the ions at the atmospheric pressure and guide them from the range of atmospheric pressure to the mass spectrometer by an electric field while decreasing the pressure. For this type of analysis, the samples can be dissolved in a suitable solvent, then we use so called electrospray ionization or atmospheric pressure ionization. The samples can be also solid, then we would use matrix assisted laser desorption ionization.

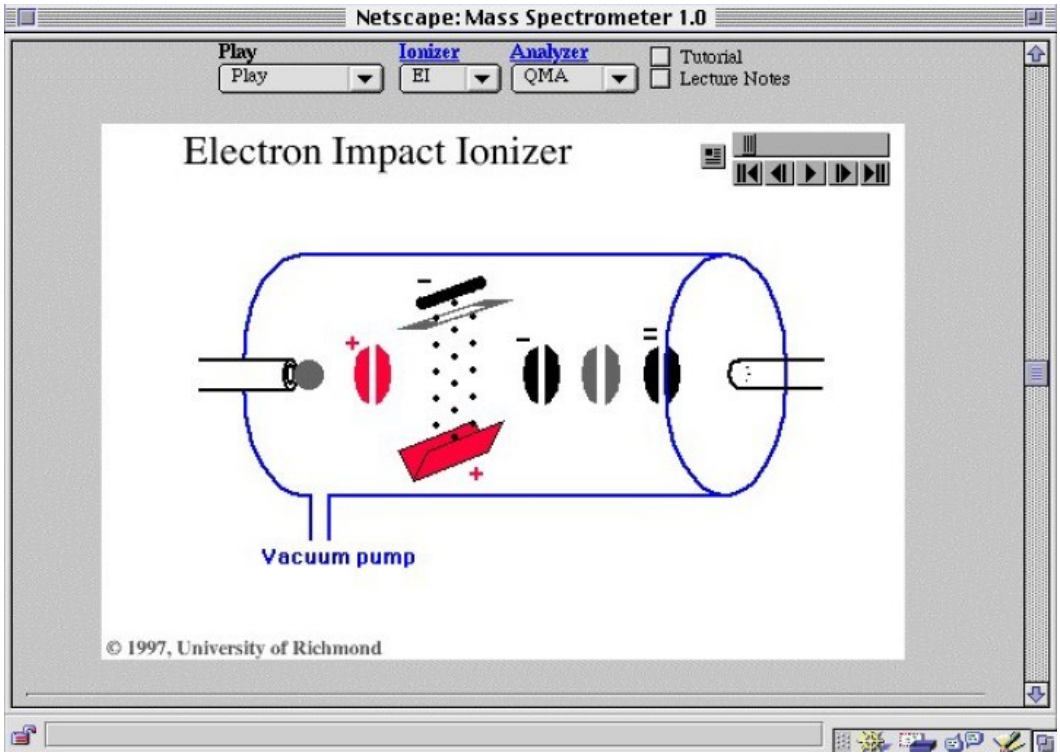
Electron ionization

I will explain the principles of electron ionization using the example of the analysis of air. The electron ionization source contains a metal (W) filament that leads an electric current. As a result, the filament gets hot and emits electrons. The electrons are accelerated through the source by the attractive field of a trapping electrode. The fast electrons collide with the molecules of the analyte and the collision leads to an ejection of another electron from the neutral molecule.

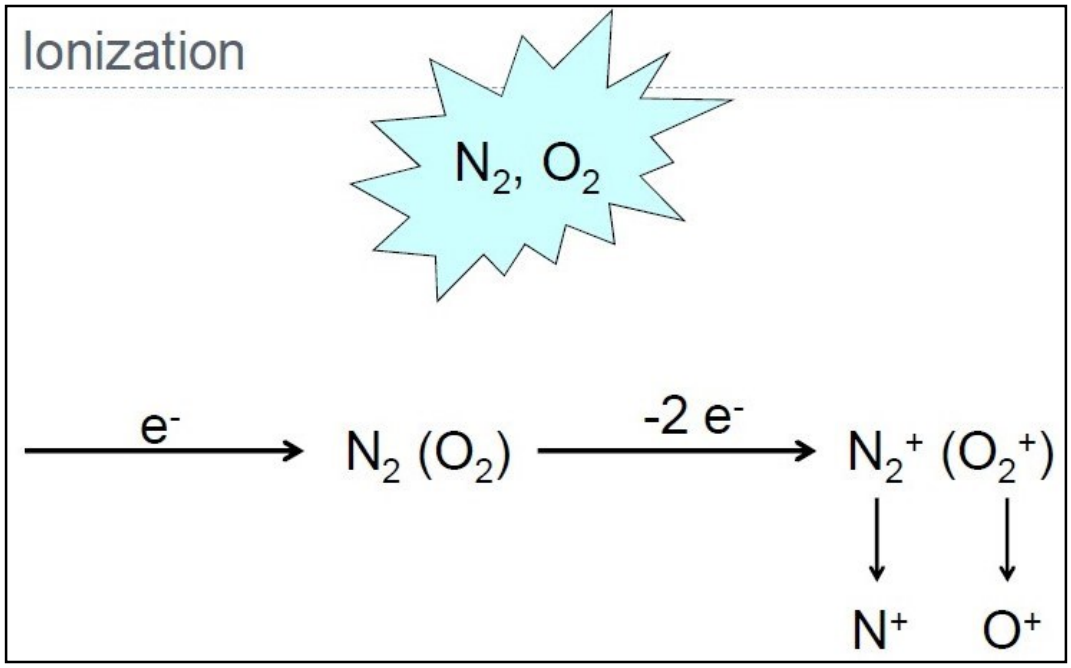


In this example, the neutral molecules will be nitrogen or oxygen molecules. Hence, the high collision-energy collisions generate positively charged ions. We can achieve the same effect by using high-energy photons instead of electrons. This would be photoionization.

This is a schematic of an electron ionization source.

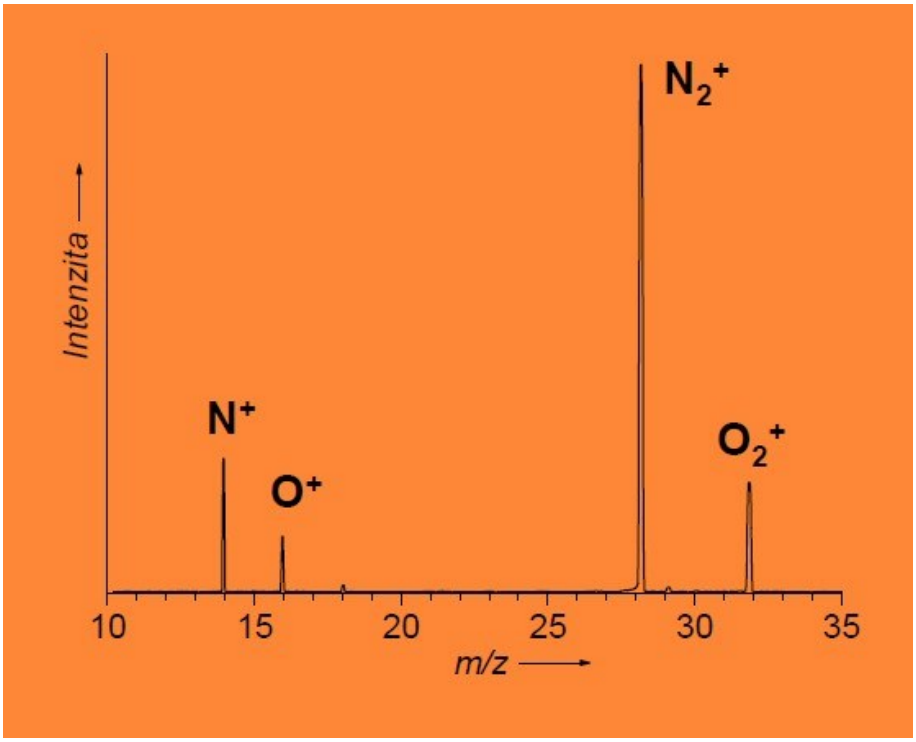


The electron ionization source operates at a low pressure. The schema of an ion source shows you a capillary at the left side with which you introduce a gaseous sample. At the top, you can see a filament that ejects electrons that are then accelerated towards the anode in the bottom. Electron are usually accelerated to reach 70 eV of kinetic energy. These fast electrons collide with our analyte and eject another electron. As a result, we create positively charged ions. These ions are guided by lenses towards the rest of mass spectrometer on the right.



As explained, the electrons have large energy. Therefore, they will not only ionize the neutral molecules, but they will also transfer a large internal energy to the ionized molecules. This means that electron ionization spectra show not only the "molecular" ions of your analyte, but also many fragment ions.

This is a typical spectrum of air where you see the ionized molecules of nitrogen and oxygen, but you can also see their fragments:



The energy required for the ionization of organic compounds is usually between 6 to 15 eV. However, the electrons carry as much as 70 eV of translational energy.

Ionization energy

Ionization energy of common chemicals up to ~ **15 eV**
Kinetic energy of electrons: **70 eV**

From Lambert

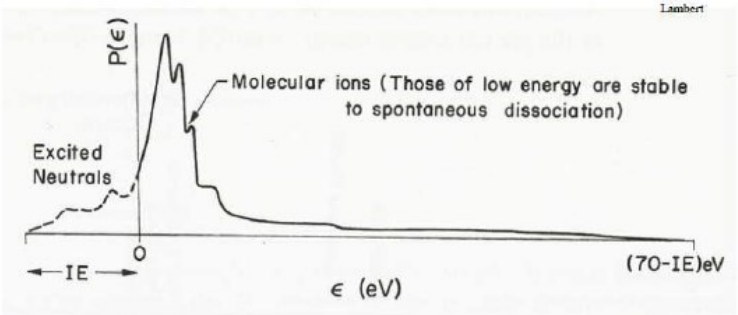


Figure 14-28 Stylized internal energy distribution $P(\varepsilon)$ resulting from impact by electrons of 70 eV kinetic energy on an organic molecule. The maximum internal energy that an ion can acquire is 70 eV, minus the ionization energy (IE). Most ions acquire small internal energies.

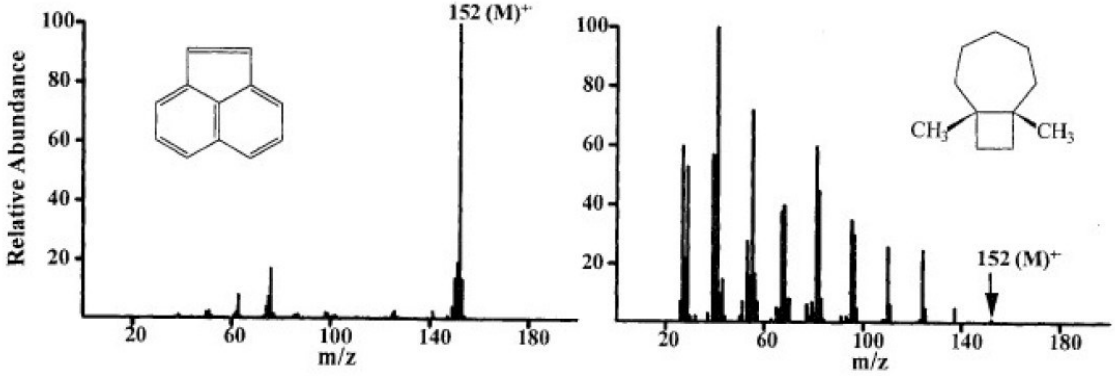
EI generates ions with large distribution of internal energy

~0 – 15 eV

→ many fragments in the EI mass spectra

This slide shows the internal energy distribution in ions after the collision with 70 eV electrons. A part of the collision energy stays in the form of the kinetic energy. However, some (variable) part of the energy is transformed into the internal energy of the colliding molecule. A part of this energy is consumed for the ionization (ionization energy), but the remaining part stays as an internal energy of the generated ion. This can be a substantial amount of energy. Therefore, this ion can fragment. How much the ions will fragment depends on their structure.

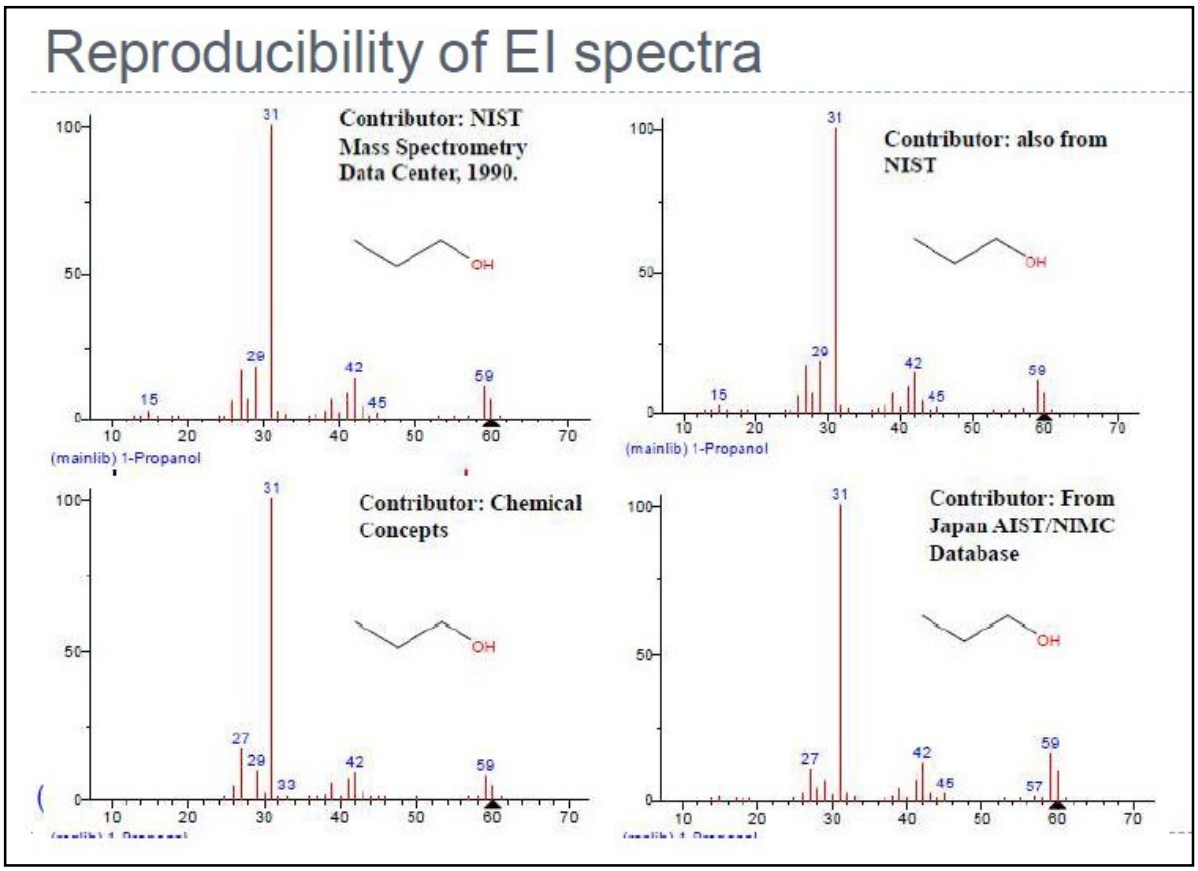
There are two examples here:



These are both hydrocarbons. Both have a molecular mass of 152. The left molecule is an aromatic hydrocarbon that is stable after the ionization. The molecular ions can withstand a lot of internal energy before they fragment because the energy required for the fragmentation of this stable skeleton is large. As you can see, most of the ions survive as intact molecule ions after the 70 eV electron ionization.

The hydrocarbon on the right is a labile bicyclic compound. The ionization energy was also 70 eV, but almost no molecular ions survived. This amount of internal energy deposited in the ions after the ionization is sufficient to overcome the energy barriers for fragmentations.

The advantage of electron ionization mass spectra is that the spectra are typically measured under the same conditions. Ionization sources are very similar, the ionization conditions are similar, and electron energy is typically 70 eV. Therefore, the spectra are very similar regardless of which instrument they were measured with. This allows us to make libraries of spectra. The big advantage is that we can assign structures based on the comparisons with the standards in the libraries.



Advantages and disadvantages of EI

Advantages	Consequences
Reproducibility	Libraries of EI spectra → identification
Large fragmentation	We can deduce molecular structure
Large ionization efficiency	Sensitive method (1 molecule out of 1000 is ionized)
Disadvantages	**Consequences**
Only positive ions	Not suitable for all kinds of compounds
Radical cations	Rearrangements
Sample most be volatile	Works only for compounds with low molecular weight (~ 600 Da)
Ionization is not selective	All molecules present in the ion source contribute to the mass spectrum
Hard ionization method	Pronounced fragmentation sometimes precludes determination of molecular mass

Summary: The advantage of the EI-MS method is that it is highly reproducible because it's made under standard conditions. Therefore, we have EI spectra libraries. Another advantage is that next to our molecular ions we can see a large fragmentation pattern and from that we can deduce the molecular structure of the ions (this is what we will learn in the interpretation part of the course). Another very important advantage is that we have a large ionization efficiency. This

method can ionize 1 molecule out of 1000. As mass spectrometry is an extremely sensitive method being able to almost detect singular ions, a tiny amount of a sample is usually sufficient for the mass-analysis of an analyte.

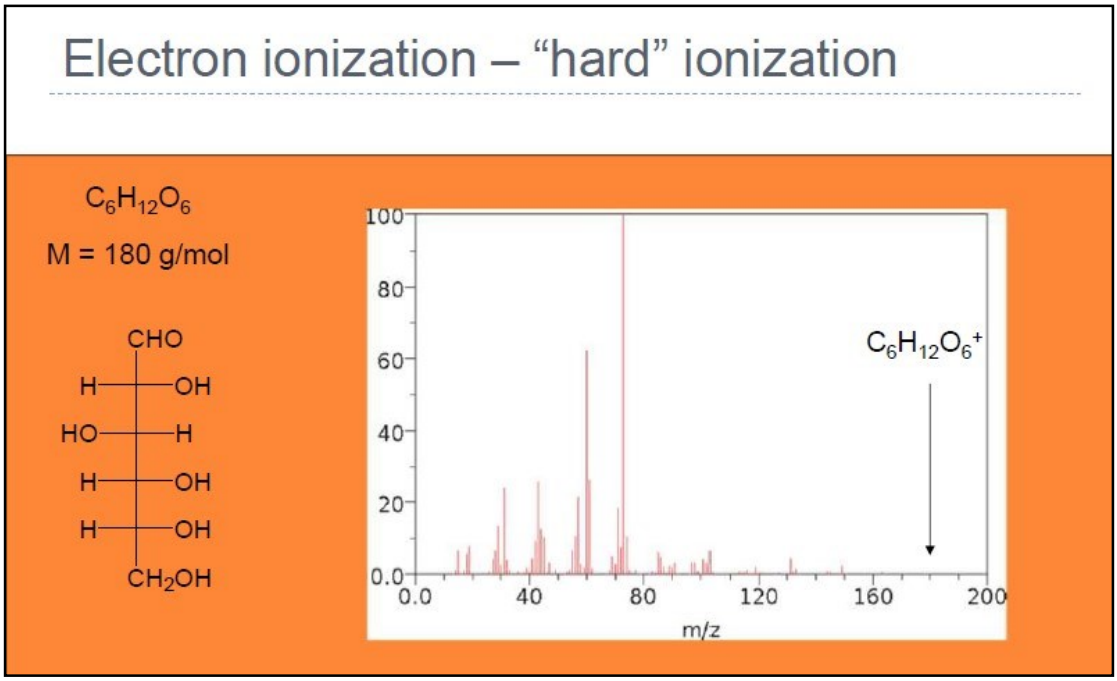
There are also some disadvantages. One disadvantage is that we can generate only positive ions and not all molecules can be studied as positive ions. For example, think about carboxylic acids. The generated ions are radical cations because in electron ionization an electron is ejected leading to a radical cation. As you know from organic chemistry, working with radicals is not easy because they are very reactive, and they can make a lot of rearrangements. This can of course complicate the interpretation of our spectra.

Another disadvantage is that the sample must be volatile. We can also use liquid or solid samples, but they must be volatile at low pressures. This means that we can work with compounds up to roughly 600 Da. The heavier compounds will not be volatile under the conditions of the experiments.

Another disadvantage is that ionization is not selective. This means that if your sample is not pure or you have impurities in the ionization chamber, you will ionize also the impurities, and these will contribute to the measured spectrum which will complicate the analysis.

The last disadvantage might be that it's a hard ionization method. While it is an advantage for the molecular structure interpretation, it can be a disadvantage, if the molecular ions are fragile and the pronounced fragmentation precludes the determination of the molecular mass.

An example is here:



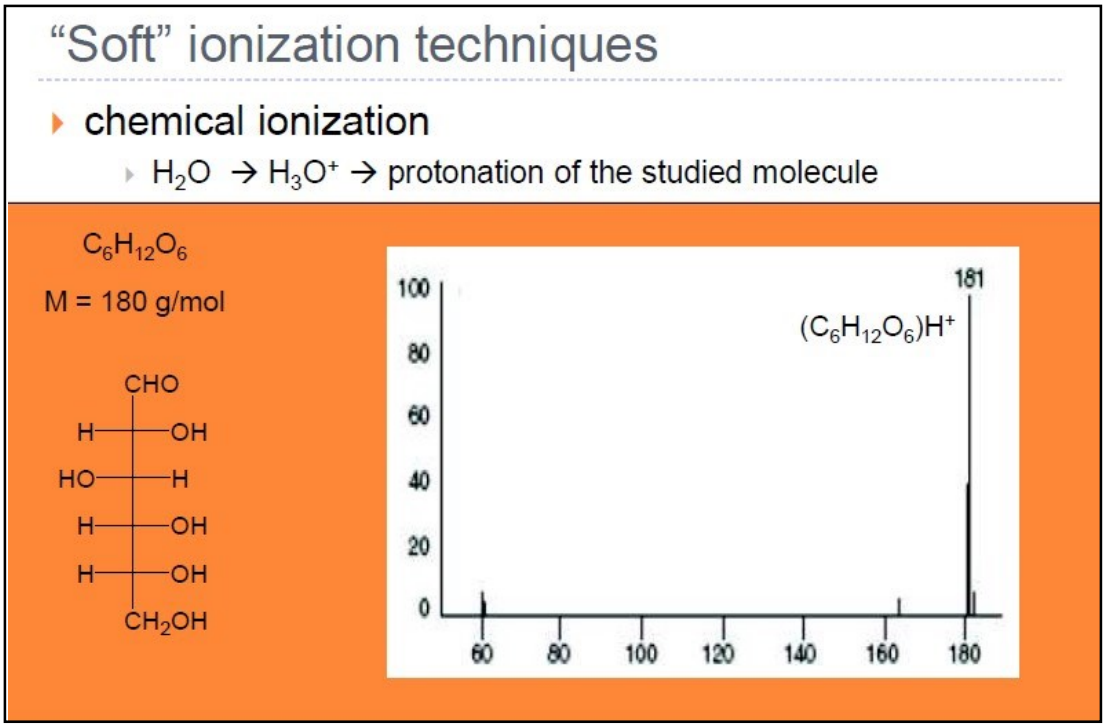
This is electron ionization spectrum of glucose. And you can see that we do not see the molecular mass of glucose at all in the spectrum. You can assign the spectrum by comparison with standards in the spectra libraries. Hence, you would probably still be able to assign this molecule. However, you don’t see the mass of molecule ion.

“Soft” ionization techniques

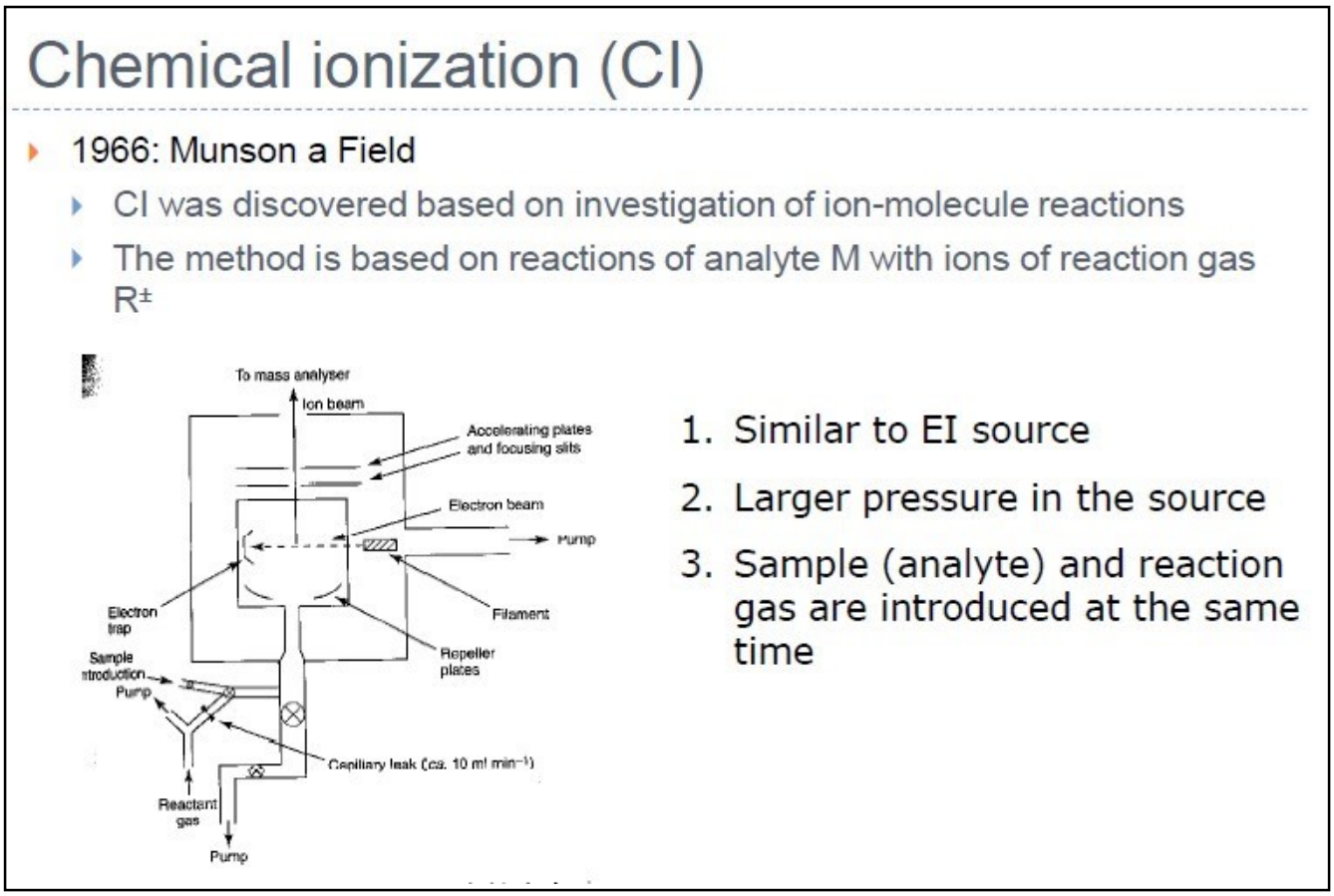
Because of that, researchers developed so-called “soft” ionization techniques.

Chemical ionization

First of the soft ionization techniques is chemical ionization. The key difference is that the ions are not formed by ejection of electrons but by a gas-phase ion-molecule reaction.



The most frequent reaction used for the soft ionization of molecules is proton transfer. For example, we can form the H_3O^+ ions that can then protonate the glucose molecules. Instead of the pronounced fragmentation pattern that you saw in the EI mass spectrum, you can almost exclusively protonated molecule ions in the chemical ionization (CI) mass spectrum.



Details of chemical ionization. The ion source looks almost exactly the same like the ion source for electron ionization. The ionization chamber is more closed because we will want to achieve a larger pressure within the ionization volume. It has

a filament, and it has an electron trap/anode. We introduce the sample in the same way as we did it for electron ionization, but together with the sample, we also introduce a large excess of a so-called reaction gas. The reaction gas could be water, methane, isobutane, N_2O molecules, many gases can serve as reactant gases.

The principle of chemical ionization is that the electrons emitted from the filament ionize by electron ionization the molecules of a reactant gas that is in a large excess. The formed radical cations of the reactant gas react with other molecules of the reactant gas until they form long lived, usually closed-shell cations. These cations then reacts by proton transfer with the molecules of the analyte.

General steps during CI

- Reaction gas R is ionized by electron ionization

  For example: $CH_4 \rightarrow CH_4^{+\bullet}$

  Large excess of R over M (<100 : 1) ensures preferential ionization of R

I have here example of methane. Electron ionization leads to methane radical cation.

This radical cation reacts with other molecules in the source.

- The initially formed ions $R^{\pm}$ react with other molecules of R to form longer lived ions $R'^{\pm}$

  For example: $CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet$

  The reactions require collisions → we need a larger pressure in the source

We have a large excess of methane over our analyte. Therefore, methane radical cation reacts preferentially with methane and forms the more stable CH_5^+ cation and the methyl radical. The cation CH_5^+ is already sufficiently stable with sufficient lifetime to react also with a molecule of the analyte.

In the example of the analysis of glucose, CH_5^+ reacts with a molecule of glucose. It transfers a proton, and we detect protonated glucose.

- In collisions of $R'^{\pm}$ with analyte M, the analyte is ionized to $M_1^{\pm}$

  $R^{\pm} + M \rightarrow M_1^{\pm} + N_1$

  For example: $CH_5^+ + C_6H_{12}O_6 \rightarrow (C_6H_{12}O_6)H^+ + CH_4$

The result of chemical ionization and the appearance of the CI spectra depend on the thermodynamics of the proton transfer processes in the ion source.

- Analyte ions can fragment

  $M_1^{\pm} \rightarrow M_2^{\pm} + N_2$ or $M_3^{\pm} + N_3$ or $M_4^{\pm} + N_4$

If the proton transfer reaction proceeds with a large exothermicity, the protonated molecule can be formed with a huge internal energy and can subsequently fragment. Hence, even chemical ionization spectra can show fragmentations and not only molecular ions.

Next to proton transfer reactions, we can have also other processes during chemical ionization. Alternative processes are electron transfer, electron capture and adduct formation. The last option means that in chemical ionization spectra, ions can be formed that are heavier than the molecular ions!

Chemical ionization

- Common reactions:

Proton transfer:	$RH^+ + M \rightarrow MH^+ + R$
Electron transfer:	$R^{+\bullet} + M \rightarrow M^{+\bullet} + R$
Electron capture:	$R + e \rightarrow R^-$
Adduct formation:	$RH^+ + M \rightarrow (M\text{-}RH)^+$
	$MH^+ + M \rightarrow (M\text{-}MH)^+$

- Fragmentation upon CI depends on exothermicity of the given reaction:
  Proton transfer: $\Delta H = PA(R) - PA(M)$

  Note: Proton transfer occurs if PA(M) > PA(R)

- MS spectra are affected by selection of reaction gases → possible to obtain different information

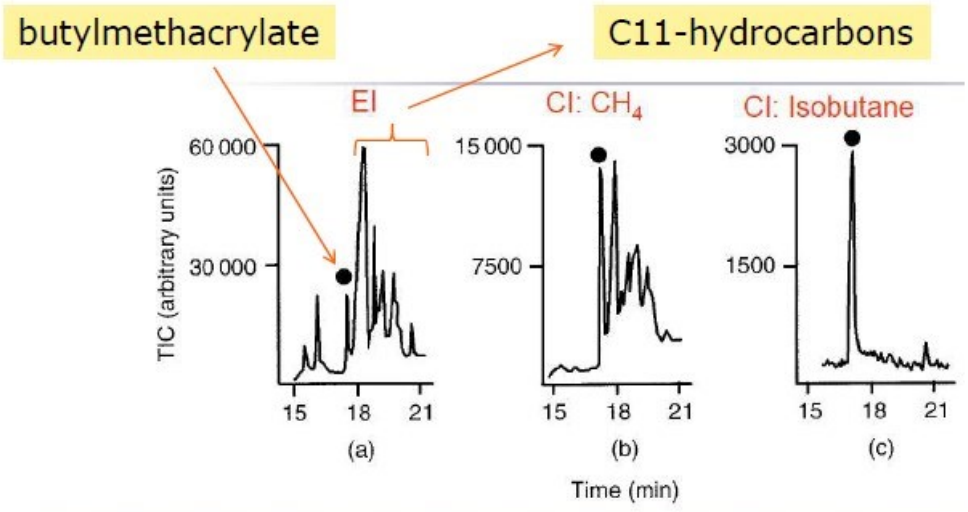
Fragmentation upon chemical ionization depends on the exothermicity of the given reaction. If we consider proton transfer ionization, then the lower the proton affinity of the reaction gas is, the more exothermic protonation reaction of a sample will be. For example, if you compare protonations by CH_5^+ and H_3O^+, CH_5^+ will protonate with a larger exothermicity. You can use this effect to adjust what the CI spectra will display.

This is an example of a selective detection, where we use the trick with different reactant gases.

Selective detection

GC-MS

Problem: We analyse a mixture of C11-hydrocarbons with a small admixture of butylmethacrylate. We want to selectively detect butylmethacrylate, therefore we will take an advantage of its larger proton affinity compared to the pure hydrocarbons. The spectra show that using a reactant gas with a larger proton affinity (isobutane) will more selectively detect butylmethacrylate than using a reactant gas with a very low proton affinity (methane). Note, that electron ionization is a non-selective method.



Proton affinity of reaction gas determines what molecules can be protonated

This is a recording of the chemicals coming out of a gas chromatograph. We have a mixture of gases and we can choose how we will detect it. One option is a detection by mass spectrometry (GC-MS). The first example is detection by electron ionization. EI is a non-selective method, therefore it will detect everything that is coming out of the gas chromatograph.

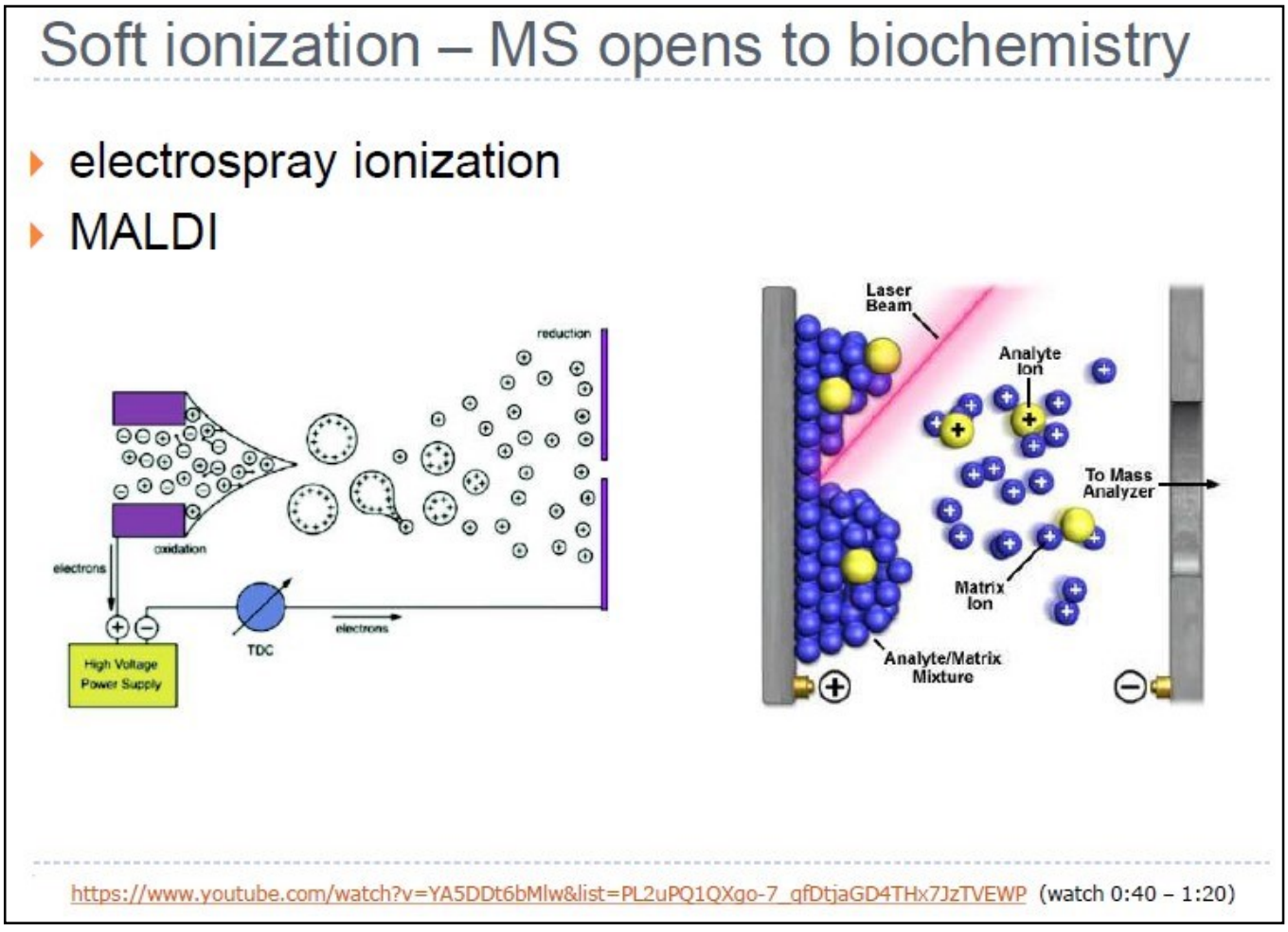
However, imagine that you are interested in a selective detection of butyl methacrylate. The peak corresponding to butyl methacrylate is very small using EI. Hence, if the concentration of this gas further decreases, the sensitivity of the detection could drop and we might not be able to detect it at all. Therefore, we can develop procedures that allow us to detect it more selectively. The alternative approach can use chemical ionization and I will explain it on a selective detection of butylmethacrylate.

(b) and (c) shows the results with CI using two different reactant gases, methane and isobutane. Methane has a lower proton affinity than isobutene. Therefore, CH_5^+ will transfer a proton with a larger exothermicity than $C_4H_{11}^+$. From the same reason, CH_5^+ can protonate a broader scope of organic compounds than $C_4H_{11}^+$. The protonation of butylmethacrylate is exothermic for both reactant gases. However, the protonation by $C_4H_{11}^+$ of compounds with larger proton affinities (such as butylmethacrylate) is more selective that that by CH_5^+. Therefore, we achieve much more selective detection.

As a conclusion: the proton affinity of the reaction gas determines which molecules can be protonated.

Ambient ionization methods. Electrospray ionization

Ambient ionization methods are all “soft” ionization methods. This means that the ions are formed by protonation or other gas-phase reactions. Two most often used techniques are electrospray ionization and MALDI.



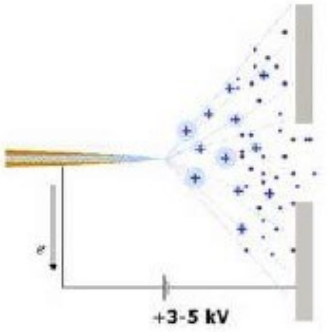
You can watch the illustration of the electrospray ionization (ESI) works is a presentation from Thermo. In short, the solution of the analyte comes to the ESI source with a capillary. The capillary can be coupled to a liquid chromatograph or to a syringe for so called direct injection. The solution sprays from the end of the capillary. The spray from the capillary is

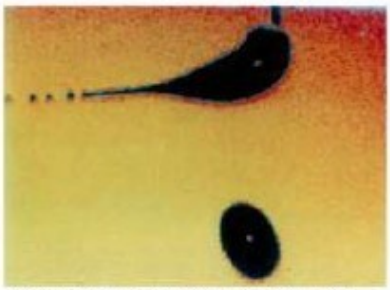
being surrounded by a flowing nitrogen that helps formation of the spray and the desolvation process. Between the capillary and the entrance to the instrument, we have a big potential difference that helps the ionization process and guiding of the ions towards the mass spectrometer. The capillary connects the one-atmosphere end with the ion source range, where we reduce the pressure.

Different companies have different solutions of the coupling with the mass spectrometer that must reduce the pressure further to the pressures required for the mass analysis. Thermo uses a long metal capillary through which they transfer the ions. The capillary reduces the transfer of other gases.

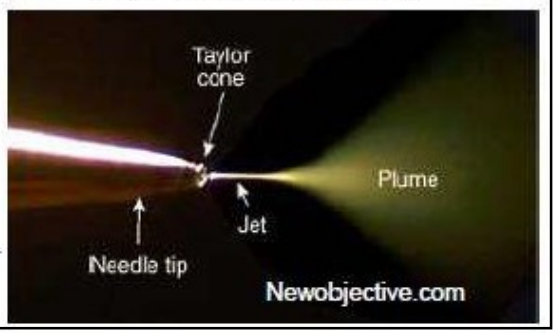
Electrospray ionization

- Atmospheric pressure ionization
- Ionization of large nonvolatile molecules (proteins) without fragmentation
- Connection to HPLC
- Mechanism
  - Solution infused by a capillary to the interface with a potential difference of 3 – 5 kV
  - Liquid stream → Taylor cone → strand → droplets
  - Evaporation of solvent from droplets increases charge density
  - Raylegh limit – repulsion force between cations is equal to the surface tension of the droplet → explosion (charge residue model or ion evaporaton model)
  - Coulombic explosions lead to generation of isolated ions (positive or negative)



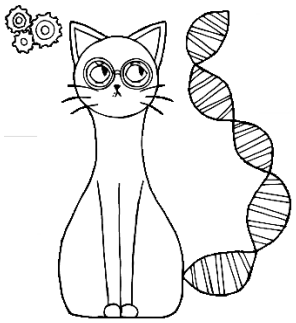


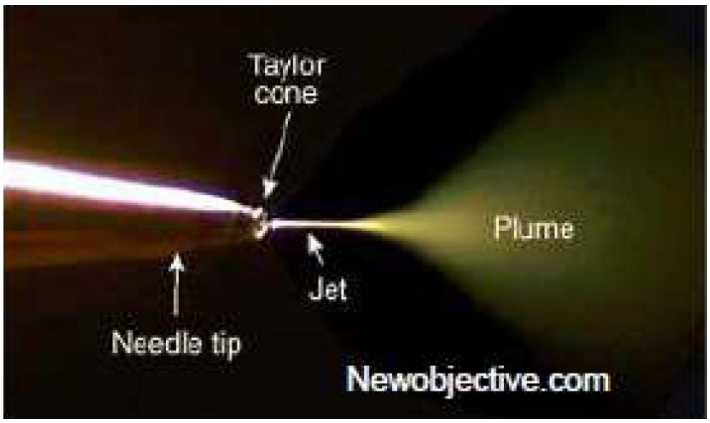
Gomez & Tang, *Phys Fluids*, 1994, 6:404–414



In summary: Electrospray ionization is an atmospheric pressure ionization. It can be used to ionize large non-volatile molecules such as proteins without fragmentation. Solutions of analytes are directly introduced allowing a direct connections with LC/HPLC.

The detail of the ESI process:





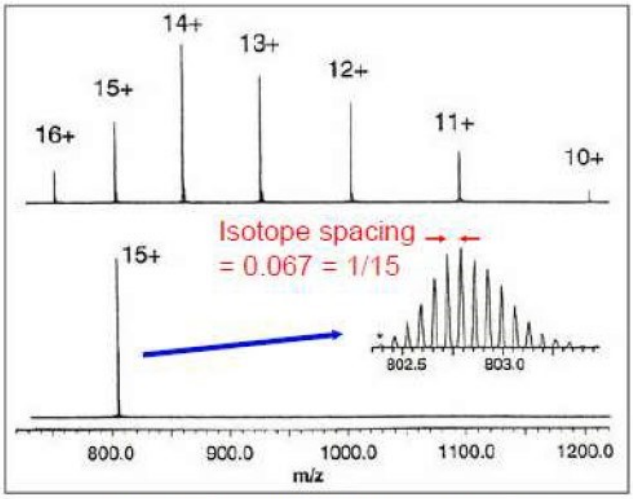
This picture shows you that the liquid goes out of the capillary in a so-called Taylor cone which transforms to a jet and the jet transforms to droplets.

The liquid contains charged particles (because of the large potential difference) therefore is can be oriented by the field. Hence, you can orient the flow of the jet and the droplets perpendicular with respect to the original direction of the capillary flow. This geometry protects the mass spectrometer from undesired neutral molecules that would increase the pressure inside of the mass spectrometer. During the spray process, the solvent molecules evaporate from the droplets leaving the isolated individual ions in the end. There are several models that explains how the ESI ionization happens, but the details are out of the scope of this course.

How does a characteristic electrospray ionization spectrum look like?

Characteristic ESI-MS spectrum

- Multiply charged ions
- Multiple charge → small *m/z* → advantageous for most of the mass analyzers
- *z* can be determined from the spacing of the peaks



ESI-MS of Cytochrome C, ~12,360 Da

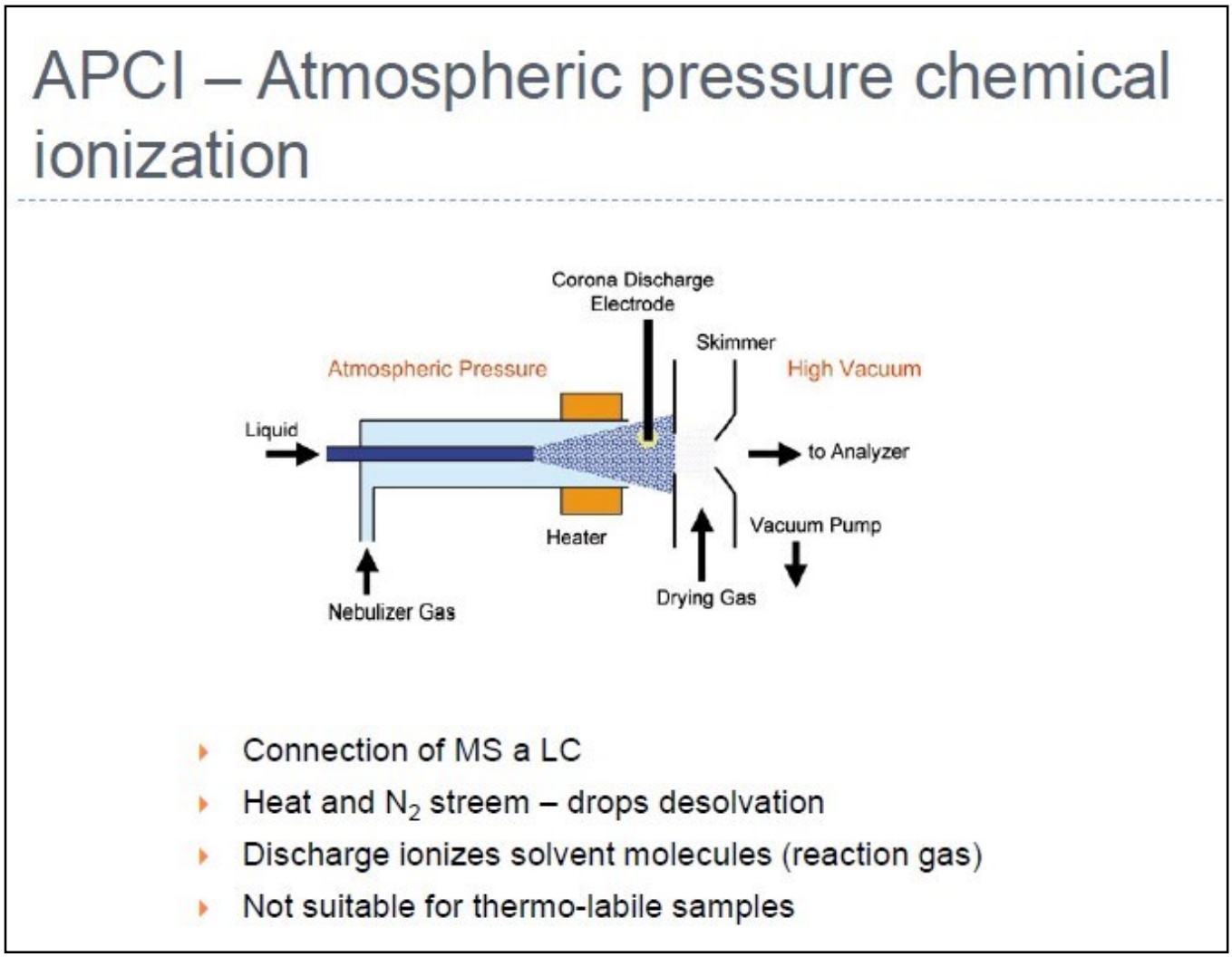
From Fig 13-18 Lambert

Typically, you will have multiply charged ions. This slide shows an example of a protein detected as 14 times protonated molecular ions. Proteins have many basic amino acids, therefore they gat easily protonated. They can be detected from solutions in methanol or water or from other mixtures of solvents.

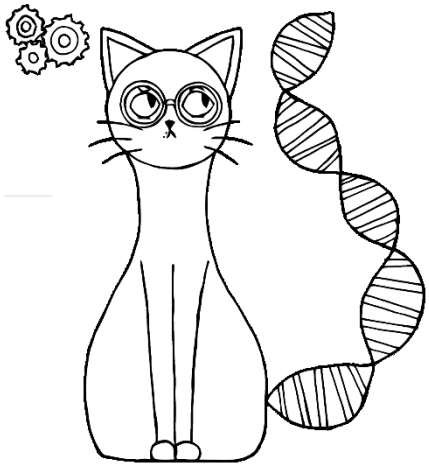
This figure should also remind you that we are always measuring the ***m/z*** ratio. Hence, not only mass but mass divided by the number of charges. So, typically, in electrospray ionization spectra where you have multiple charged ions, the peaks will be separated by a much smaller distance than 1 mass. So, for ion with 15 charges, the isotopic peaks will be separated by 1/15. This means you need a large m/z resolution to resolve the isotopic patterns in order to identify what the molecular ions are.

Ambient ionization. APCI - Atmospheric pressure chemical ionization

Atmospheric pressure chemical ionization is another variant of ambient ionization. The setup is similar to electrospray ionization.



The sample is introduced as a solution by a capillary. The ionization is secured by ion-molecule reactions. The primary ions are formed in a discharge. The discharge is initiated by a needle at a large potential. The corona discharge formed around the needle leads to the ionization of the molecules of the solvent. The ions formed by the ionization of solvent transfer (typically) a proton to your analyte. The ionization process bears similarities with the chemical ionization explained above. Therefore, is also has a similar name.



What do we know about ionization of neutral molecules?

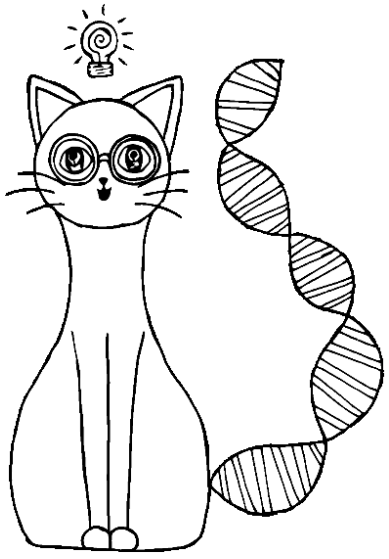
- Where and how are neutral molecules ionized?
  - In solution, in droplets, in the gas phase
  - By formation of adducts with Na^+, Li^+, NH_4^+ from salts, with H^+ from acids, with Cl^- from salts/solvent
  - Electrochemically (H^+ from water, radical cations)
  - Proton transfer reactions in the gas phase
- (Dis)similarity of chemistries in the gas phase and in solutions
  - Proton transfer in solution → pK_a
  - Proton transfer in the gas phase → PA (proton affinity)
  - They are not necessarily analogous

Details of the ionization process:

How are neutral molecules ionized? They can be ionized in various stages. Firstly, the ions can be already present in solution. Secondly, the neutral molecules can get ionized in the state of droplets. Thirdly, the molecules can be also ionized in the gas phase.

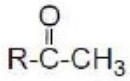
The molecules can be charged in different ways. They can make an adduct with sodium cations, lithium cations, ammonium cations. This process is important to consider, if you prepare your compound by synthesis. If it's not completely clean or purified, then you usually see such adducts.

The most frequent type of the ionization is by proton transfer. If you look at negative ions, you can have adducts with anions. The anions usually originate from salts or from solvents. Hence, often adducts with chloride anions are observed.



ESI in praxis

- ESI chemistry has many control parameters and unknown effects
- Understanding requires a good knowledge of organic chemistry and ion chemistry
- ESI is one of the fastest and most powerful ionization methods in mass spectrometry

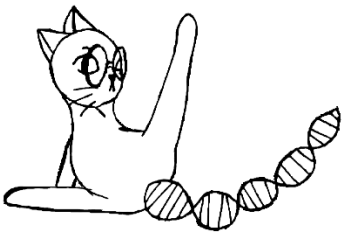
Analyte	ESI polarity	ESI reagent	ΔPA (vs. H_2O, R=CH_3)
carboxylic acid 	- (or +)	methanol (or ACN + trace H_2O)	22.3 kcal/mol
ketones 	+	methanol (or ACN + trace H_2O)	29.0 kcal/mol
aldehydes 	+	methanol (or ACN + H_2O) + HCOOH, LiCl, or NaCl	18.7 kcal/mol

cf. Lithium ion attachment MS (Selvin, Iwase and Fujii, Anal.Chem.74, (2002) 2053

Mostly, you will be interested in electrospray ionization as an analytical methods. You should be aware that it has many control parameters. The mechanism can be complex and several ionization pathways can combine during the ESI. Understanding all effects that can affect the formations of the ions requires a good knowledge of organic chemistry and ion chemistry. However, for using the electrospray ionization as a fast and powerful method to ionize analytes, you will only need to know a selection of solvents that you can use.

This table shows you a simple comparison of three types of analytes: carboxylic acids, ketones, and aldehydes. You can choose to analyze them in the positive mode, which will work for all of them. Or in the negative mode and this will work only for the carboxylic acids. For all of them you can use methanol as a preferred solvent. But you can use also other combinations. A popular combination is acetonitrile with a trace of water.

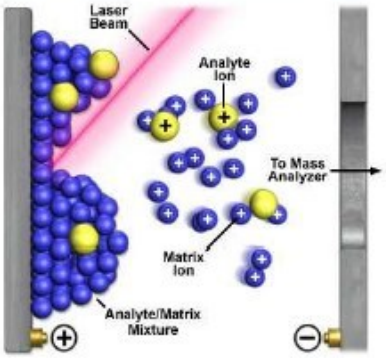
What will be the outcome? Your sample gets easier protonated if there is a big difference between the proton affinity of your sample and the solvent. Ketones have the largest proton affinity, therefore they are the easiest compounds to be identified as positive ions. Aldehydes have a lower proton affinity, therefore simple protonation of H_3O^+ will not work. In order to see the protonated ions, we have to increase the acidity of the solvent by addition of an acid (often we add a trace of HCOOH). We can also make a use of detecting the molecules as adducts with alkali cations (e.g., Li^+ or Na^+).



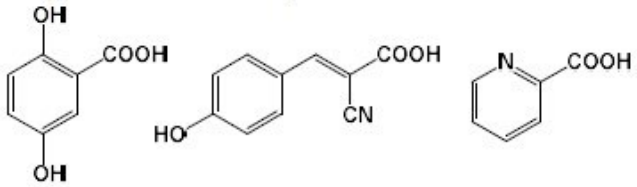
MALDI - Matrix assisted laser desorption ionization

The principles of MALDI can be illustrated by the graphics from Bruker. The sample is solid co-crystallized in beads a matrix. The ionization is achieved by short laser pulses. The laser irradiates the sample together with the matrix, the matrix absorbs the radiation which leads to a heat-up of the sample. The matrix as well as the analyte are evaporated to the gas phase. The matrix transfers a proton to an analyte molecule. Once the molecules are ionized, they are responsive to the potential field of the mass spectrometer and they get accelerated toward the entrance of the mass spectrometer. In principle, we use aromatic carboxylic acids as the matrix. This is because they are aromatic, therefore they absorb UV irradiation. They are acidic and therefore they easily protonate the analyte molecules.

Matrix assisted laser desorption ionization



- Analyte mixed with a crystalline matrix



- Dry mixture irradiated by a short, intense laser pulse at a wavelengths absorbed by the matrix (usually UV)
- Fast matrix heat-up → sublimation and expansion to the gas phase
- Ionization – proton transfer
- Usually singly charged ions, suitable for large molecules

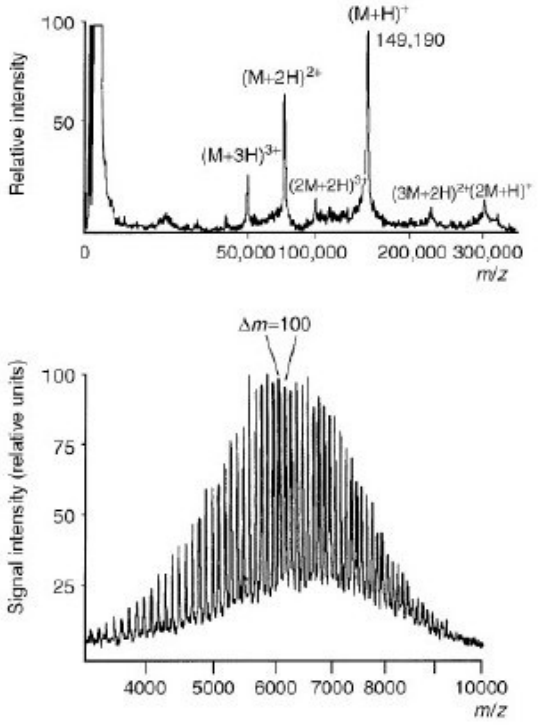
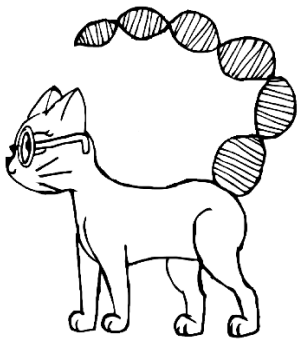


Figure 1.15
The MALDI spectra of a monoclonal antibody (*top*) and poly(methyl methoacrylate) of average mass 7100 Da (*bottom*) (Reproduced (modified) from Ref. 24 and from Finnigan MAT documentation, with permission)

The important information is that we usually detect singly charged ions. You can also detect doubly or triply charged ions but you will not detect 15-times protonated proteins, for example, as we had it with electrospray ionization. MALDI produces typically singly charged ions. This is an example for of the spectra for monoclonal antibodies.



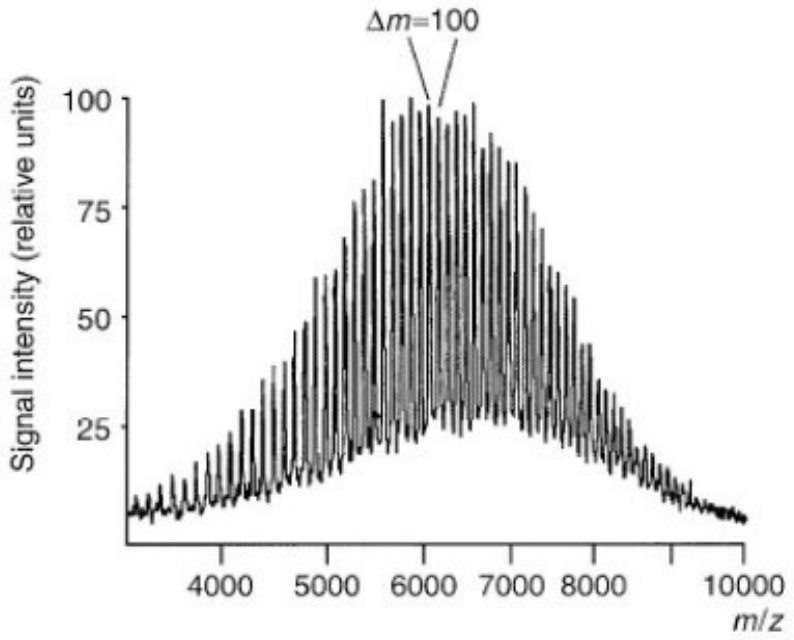


Figure 1.15
The MALDI spectra of a monoclonal antibody (*top*) and poly(methyl methoacrylate) of average mass 7100 Da (*bottom*) (Reproduced (modified) from Ref. 24 and from Finnigan MAT documentation, with permission)

These are all singly charged ions. The envelop shows the distribution of proteins in the sample.

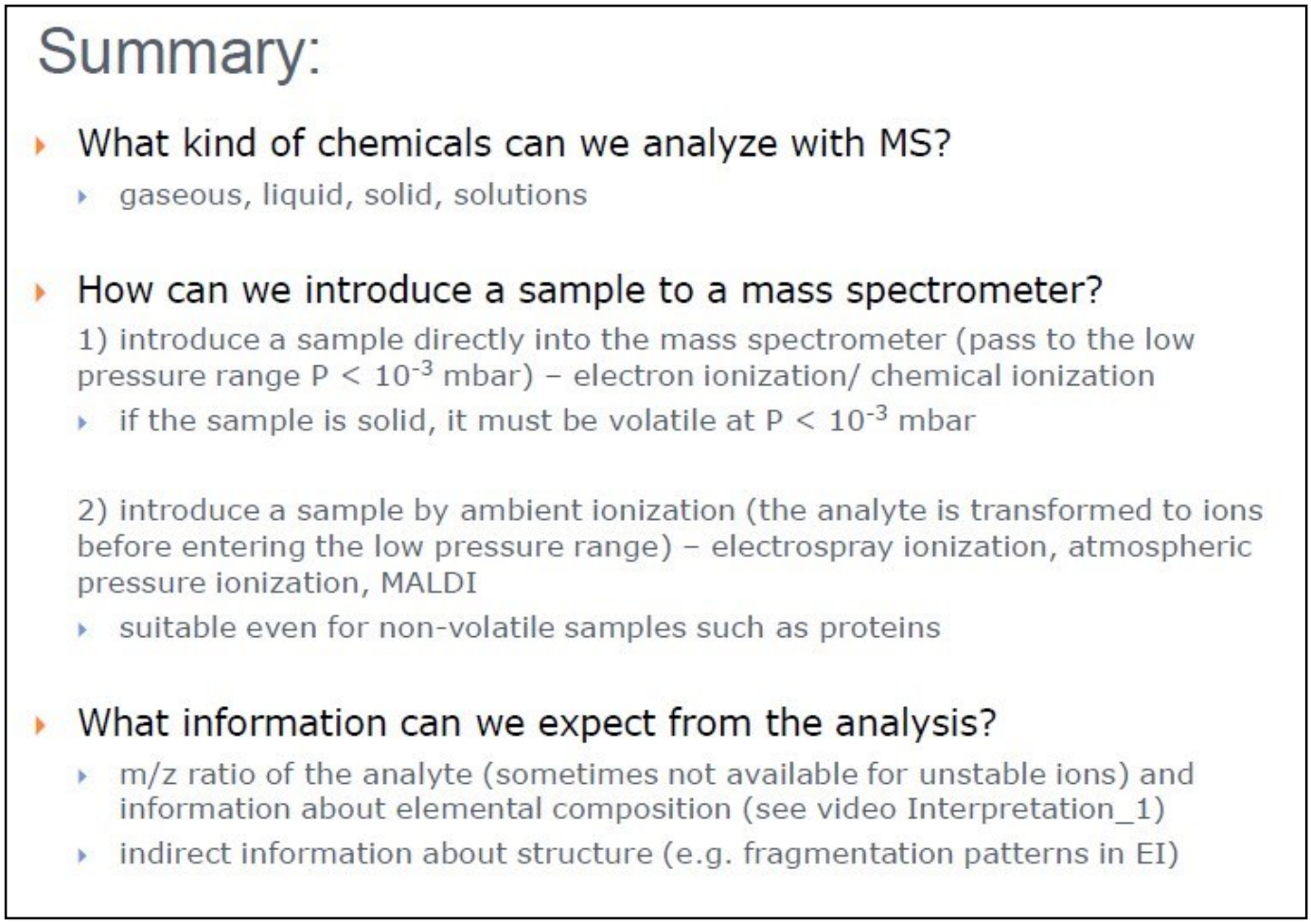
Matrices

Matrix	Solvents	Wavelength (nm)	Analytes
2,5-dihydroxybenzoic acid	ACN, H_2O, CH_3OH, acetone, $CHCl_3$	337, 355, 266	Peptides, (oligo)nucleotides, oligosacharides
3,5-dimethoxy-4-hydroxycinnamic acid	ACN, H_2O, acetone, $CHCl_3$	337, 355, 266	Peptides, proteins, lipids
α-cyano-4-hydroxycinnamic acid (CHCA)	ACN, H_2O, EtOH, aceton	337, 355	Peptides, lipids, nucleotides
Picolinic acid (PA)	EtOH	266	oligonucleotides

Matrices - you will use them together with your sample. You will dissolve the matrix and the analyte in a suitable solvent and put the solution on a bead. Then you must wait until the solvent is evaporated. Once the mixture is dry it can be irradiated by a laser at the wavelength at which the matrix molecules absorb photons which will lead to the ionization.

For different types of molecules, different matrices were optimized. I advise you to always look up what kind of matrices work best for the given analyte. As you can see from this table, MALDI was optimized for biomolecules. But in recent years it gets also applications in material sciences, so you can use MALDI for analysis of different polymeric material compounds.

Summary



In the beginning, I have outlined that you should be able to answer three questions.

The first question: what kind of chemicals can we analyze with MS?

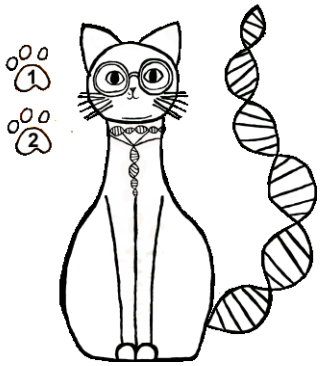
The answer: We can analyze chemicals in various states: gases, liquid, solids. We can also analyze dissolved molecules in a suitable solvent. A huge variety of solvents exist; usually, they have to be at least a little bit polar but even THF is working.

Q: How can we introduce a sample to a mass spectrometer?

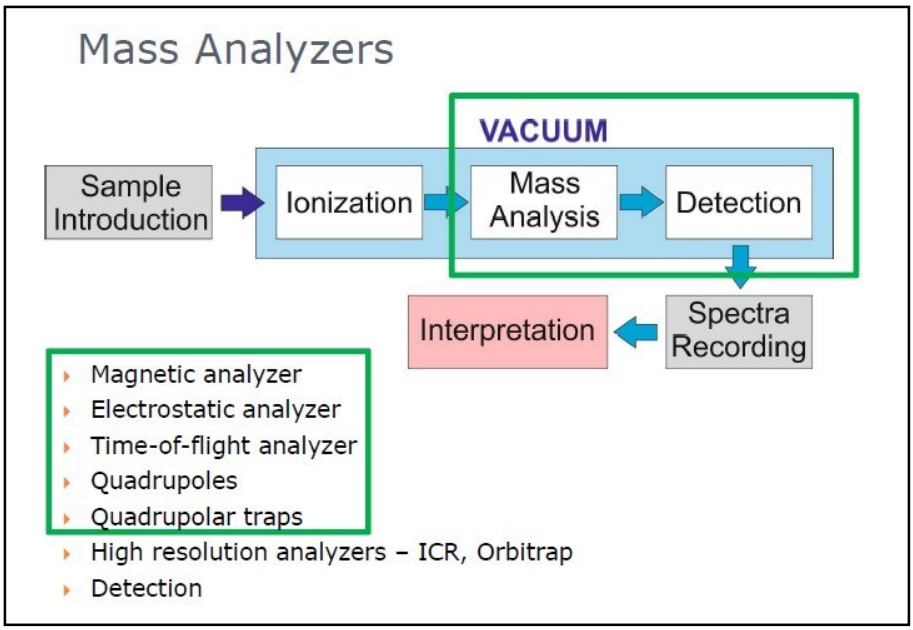
A: You can put a sample directly to the mass spectrometer – in that you must know that it is necessary to bridge to the low pressure range. An example would be the methods of classical electron ionization or chemical ionization methods. If the sample is solid and you want to use EI or CI, then the sample has to be volatile at reduced pressures. It means that we are limited by about 600 Da. Another option is ionization at ambient conditions. This means that the analyte is transformed to ions before entering the low pressure range. You should know some details about electrospray ionization and MALDI. The ambient methods are suitable even for non-volatile samples such as proteins.

Q: What kind of information can we expect from the MS analysis.

A: We will obtain the ***m/z*** ratio of the analyte. Sometimes, the mass of the molecular ions is not available as explained for the electron ionization method. You should know alternative methods how to obtain the molecular ions. As you will learn in the interpretation part of the course, the mass spectra also give you information on the structure of the elemental composition and the structure of the molecular ions.



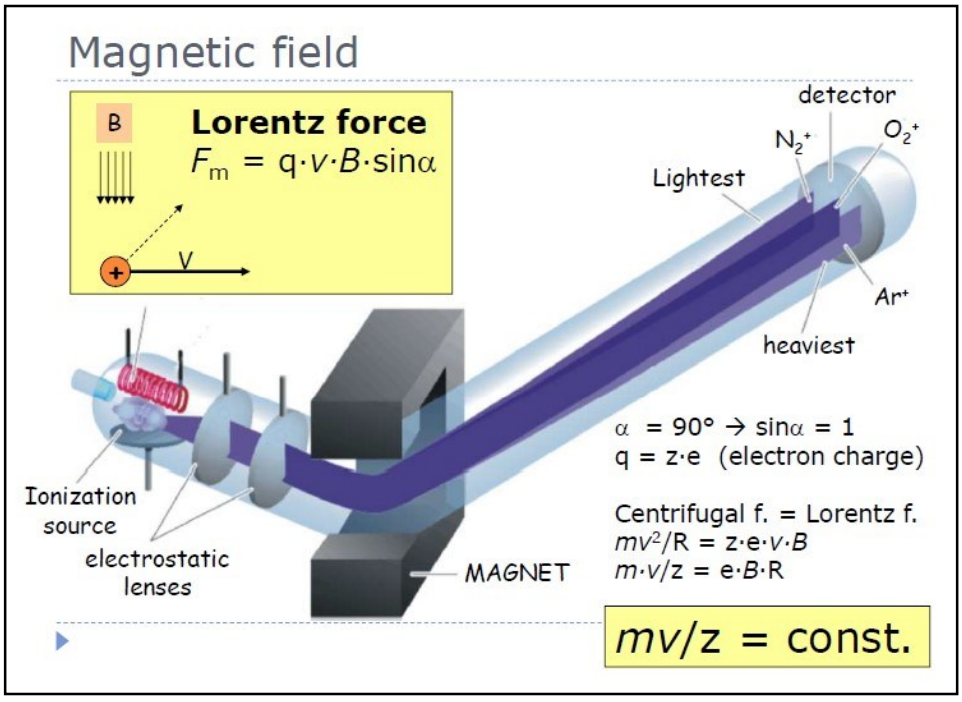
Lecture 3_ Mass Analyzers - Part 1



In the following two lectures we will discuss mass analyzers and the ion detection system. We will talk about magnetic analyzer, electrostatic analyzer, time-of-flight analyzer, quadrupoles, quadrupole traps, high resolution analyzers and about the ion detection systems. After this lecture you should be aware of principles, advantages and disadvantages of the first five types of mass analyzers (green box).

Magnetic analyzer

Magnetic analyzer is one of the first- or the first mass analyzer that was used.



This scheme shows a spectrometer with an electron ionization source. After the formation, the ions are accelerated towards the mass analyzer. They pass through several electrostatic lenses for focusing. The acceleration potential extracting the ions from the source defines the velocity of the ions. Hence, focused with a given velocity the ions enter the magnetic field. The magnetic field acts at moving charged particles by the so-called Lorentz force. For the magnetic

field direction from top to down and the ions moving from left to right, the ions' paths will be deflected to the back of the screen/paper.

In this hypothetical case we analyze the molecules/atoms present in the air. Air is mostly composed of nitrogen, oxygen, but we can also detect argon. The lightest radical cations will be deflected most, whereas the heaviest argon radical cations will be deflected least.

Why is that? It's because, when we look at the deflection, we have to always consider equilibrium between the centrifugal force and the Lorentz force. I have simplified the formula for the Lorentz force by assuming that the field is perpendicular to the path of the ion which it always is.

$\alpha = 90° \rightarrow \sin\alpha = 1$
$q = z \cdot e$ (electron charge)

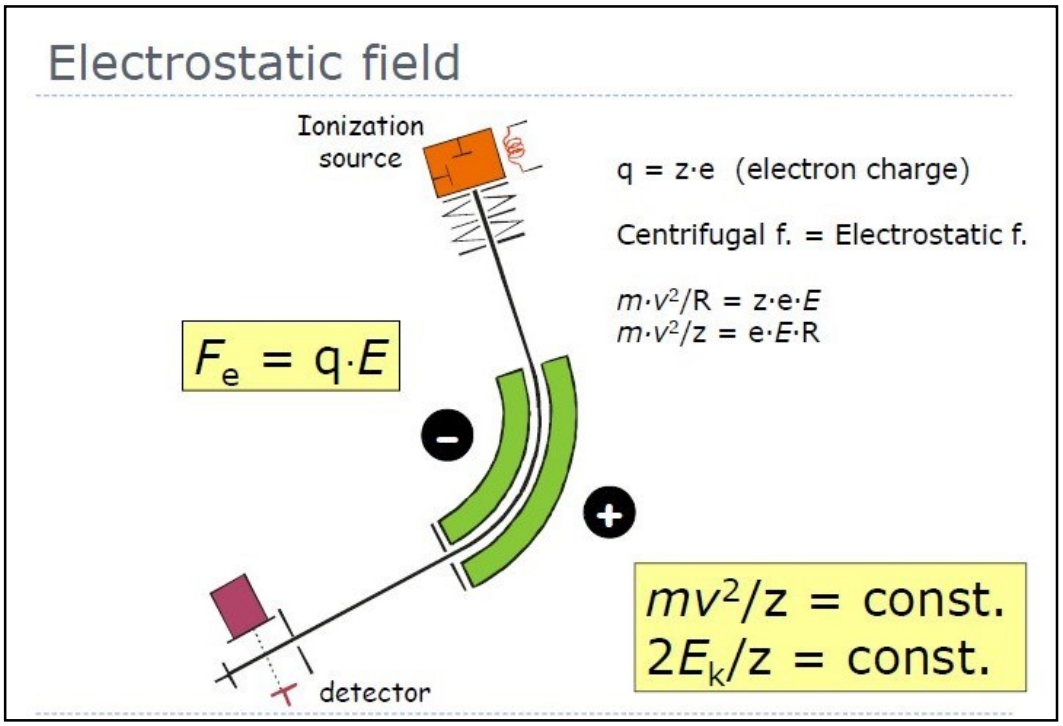
Centrifugal f. = Lorentz f.
$mv^2/R = z \cdot e \cdot v \cdot B$
$m \cdot v/z = e \cdot B \cdot R$

$$mv/z = \text{const.}$$

And I have also set the charge as a product of the number of the charges of the ion and the charge of the electron, which is a constant. The centrifugal force is a mass times velocity to the square divided by ***R***. ***R*** is always constant for the given mass spectrometer because there is a constant geometry of the instrument with the constant position of the analyzer. And this will be equal to Lorentz force.

After simplification, we can find out that ***m*** (mass) times velocity divided by the number of the charges is equal to an expression that is constant for the given mass spectrometer. Hence, the conclusion is that the magnetic field analyzes the ions according to their momentum ($m.v$). It means that for a good mass resolution (Δm), you need a very narrow distribution of the ion velocities (Δv).

Electrostatic field



The electrostatic analyzer looks somewhat similar to the magnetic analyzer. We again generate ions in an ion source and accelerate them towards the analyzer. The velocity of the ions is defined by the acceleration potential applied to the extraction lenses. The ions are accelerated and focused towards the electrostatic analyzer. The electrostatic analyzer works along the path. It has negatively and positively charged electrodes. Positive ions will be attracted to the negative electrode and repelled from the positive electrode which will lead to the deflection of their paths.

The electrostatic force acts according to the intensity of the electrostatic field and the number of charges.

$$F_e = q \cdot E$$

When we look at how the electrostatic field acts on the ions, we have to assume the equilibrium between the centrifugal force and the electrostatic force.

$q = z \cdot e$ (electron charge)

Centrifugal f. = Electrostatic f.

$$m \cdot v^2/R = z \cdot e \cdot E$$
$$m \cdot v^2/z = e \cdot E \cdot R$$

We know already the formulas. When we rearrange the equations, then we find out that in the electrostatic field the $\boldsymbol{m.v^2/z}$ expression is constant for a given mass spectrometer (with a given geometry parameter R). Hence, the electrostatic field separates the ions according to their kinetic energy ($E_k = ½\ m.v^2$).

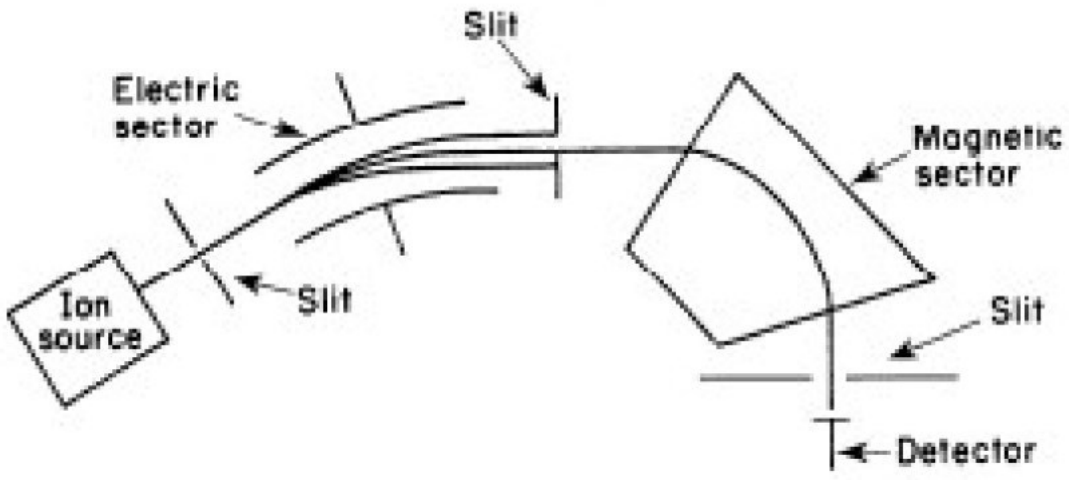
$$mv^2/z = \text{const.}$$
$$2E_k/z = \text{const.}$$

The resolution of this analyzer again depends on the initial resolution of the ion velocities. However, the resolution of the electrostatic analyzer will be smaller than that of the magnetic sector because it depends on the square of the velocity.

Double-focusing analyzers

Separately, magnetic and electrostatic fields do not have big resolutions as mass spectrometers. That is why we always using them in a combination as so-called double-focusing analyzers.

And here is a schematic of one of these combinations.



Such mass spectrometer will have a source and some lenses to extract, accelerate and focus the ions. Then, the ions will enter the electrostatic sector where they will be filtered according to their kinetic energy (see the formula above).

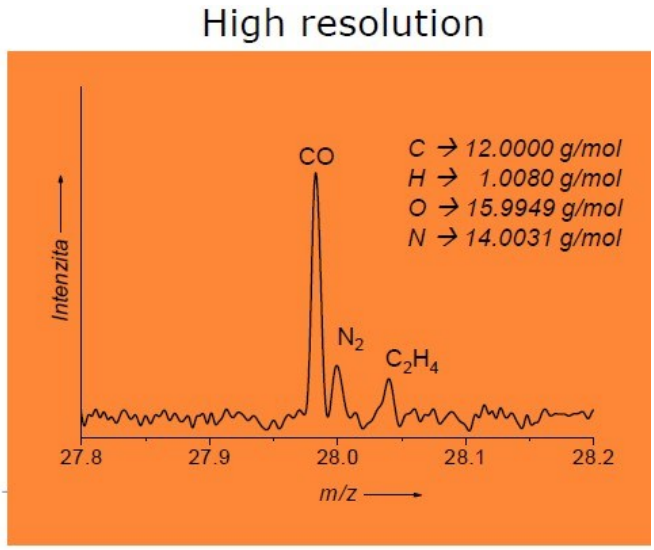
Electrostatic analyzer reduces E_k distribution

→ $2E_k/z$ = const.

Magnetic analyzer filters *m/z*

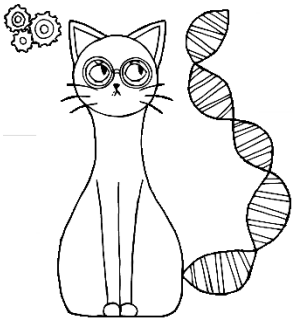
$$R = \frac{mv}{qB} = \frac{\sqrt{2mE_k}}{qB}$$

Afterwards, the ions enter the magnetic field, where they will be analyzed according to their momentum. The initial separation according to the kinetic energy reduced the spread of the velocities of the given ions. Hence, the following mass-analysis according to the ion momentums reaches so-called high resolution. We refer to the combinations of electrostatic and magnetic field analyzers as sector analyzer. These analyzers can achieve a high resolution as shown below.



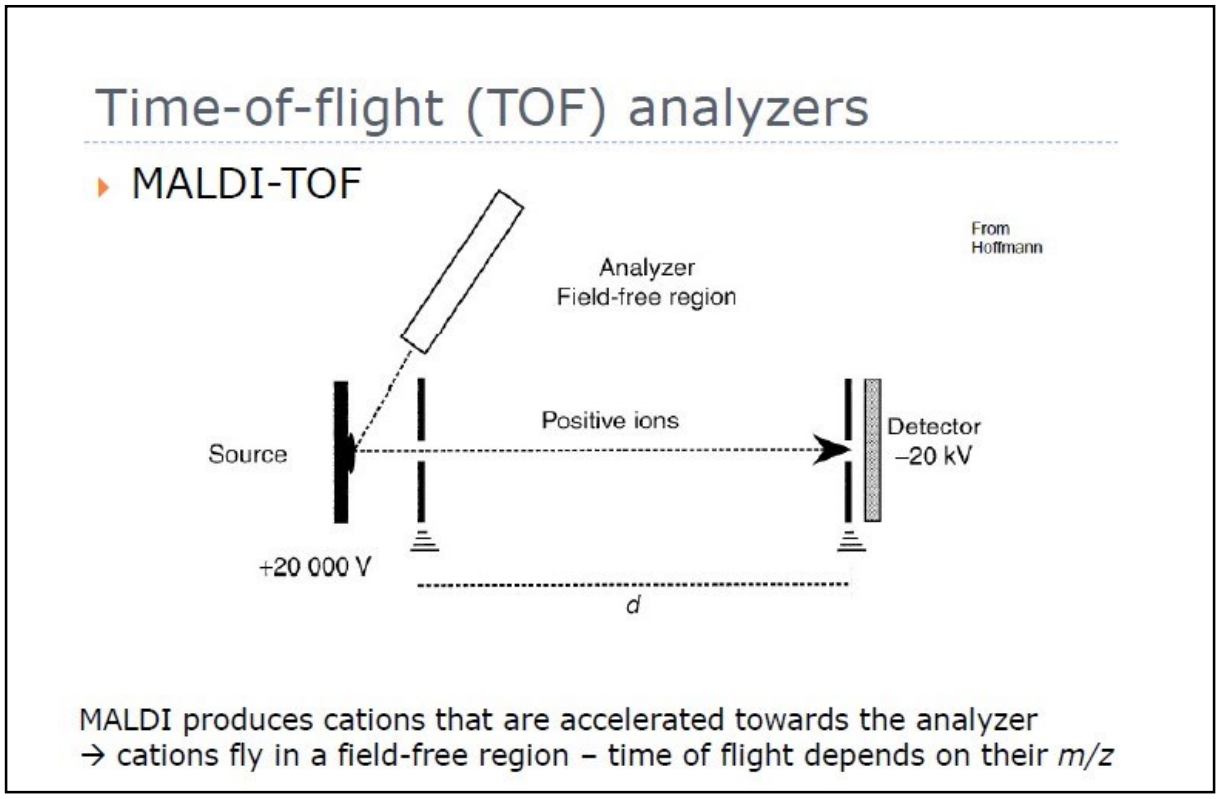
This examples shows separation of a mixture of molecules that all have nominally mass 28. Because of the high resolving power, we can separate them, although the masses differ only at small digits behind the comma.

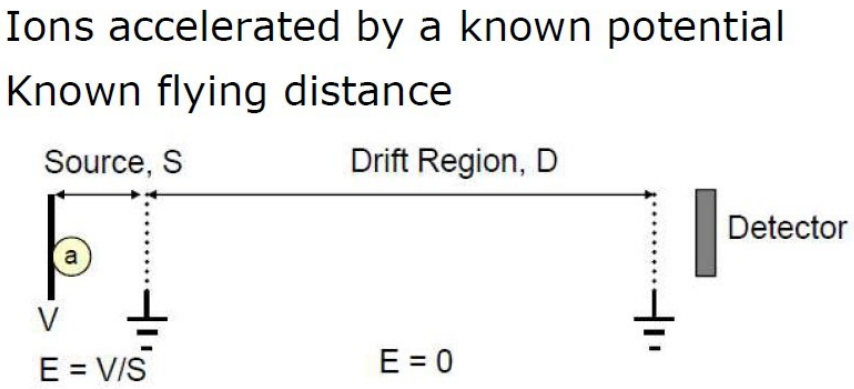
This particular mass analyzer has the source in front, the ions enter first the magnetic sector and only afterwards they are analyzed in the electrostatic sector. In general, it does not matter what the order of these mass analyzers is.



Time-of-flight (TOF) analyzers

Another type of a mass analyzer is a time-of-flight analyzer. In this type of analysis we will measure the time that the ions need to fly a certain distance.





The sequence of the TOF analysis:

1) The ions are generated in an ion source.
2) The ions are put onto the start with a certain velocity. We do it by accelerating them with a given potential towards the start line. The given field (V) accelerates the ions to a given kinetic energy (i.e., the lighter ions achieve larger velocities than the heavier ones).
3) The ions fly in a field-free region a certain, known distance. As the velocity of the ions with given m/z is defined by the initial acceleration field, the time required for the flight is dependent on m/z of the ions. See the formulas explaining, why m/z depends on the square of the time-of-flight.

$$E_k = \frac{1}{2}mv^2 = qV$$

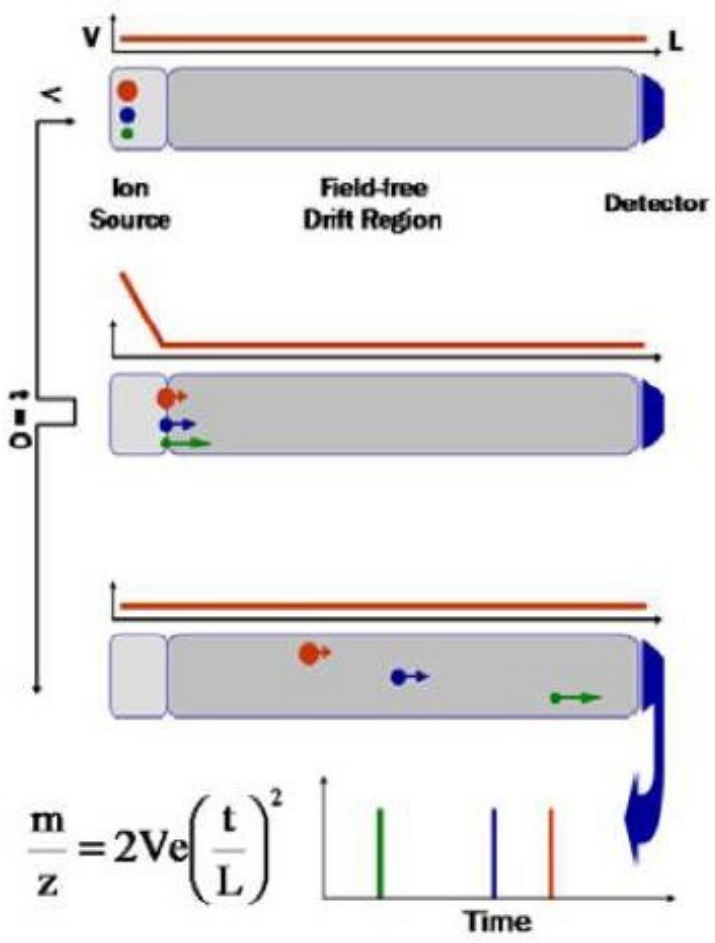
$$zeV = \frac{1}{2}m\frac{x^2}{t^2}$$

$$\frac{m}{z} = 2Ve\left(\frac{t}{x}\right)^2 = Kt^2$$

Important factors:

- All ions measured simultaneously → faster spectra scanning
- Time resolution
- "Infinite" range of masses
- cheap

Mass analysis by the time-of-flight is fast because all ions are measured simultaneously. This means,

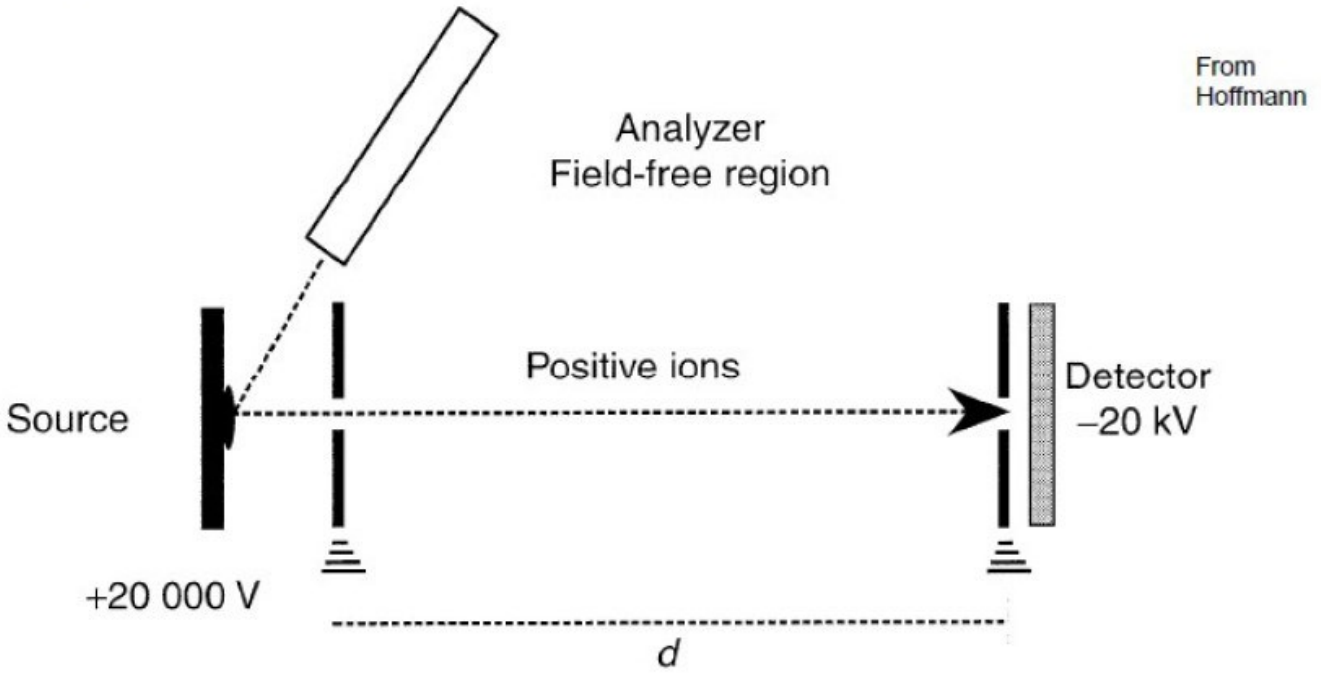


that we generate them at once in an ion source. Then we accelerate them to the start line. And then they all fly at once. The only sequential event is their detection at the finish line.

The time that the ions need to pass the flight distance is usually on the order of nanoseconds. It means that we must be able to measure the arrival times very precisely. Also, it gives us a straightforward option how to increase the resolution of such mass analyzer - just increasing the flight distance. This is why you will see in laboratories many time-of-flight analyzers that have long tubes pointing usually to the ceiling.

Another advantage of the TOF analyzers is that we basically do not have the upper mass range. TOF has an infinite range of masses that we can detect or determine. In principle, we just need a really good vacuum conditions in order to have sufficient long flight distances for the resolution of heavy ions.

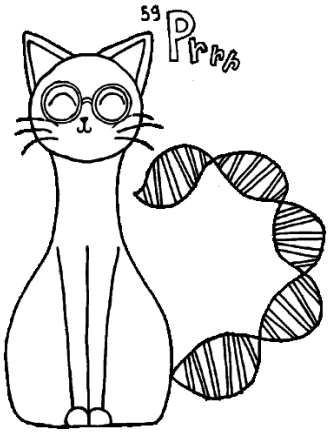
TOF renaissance



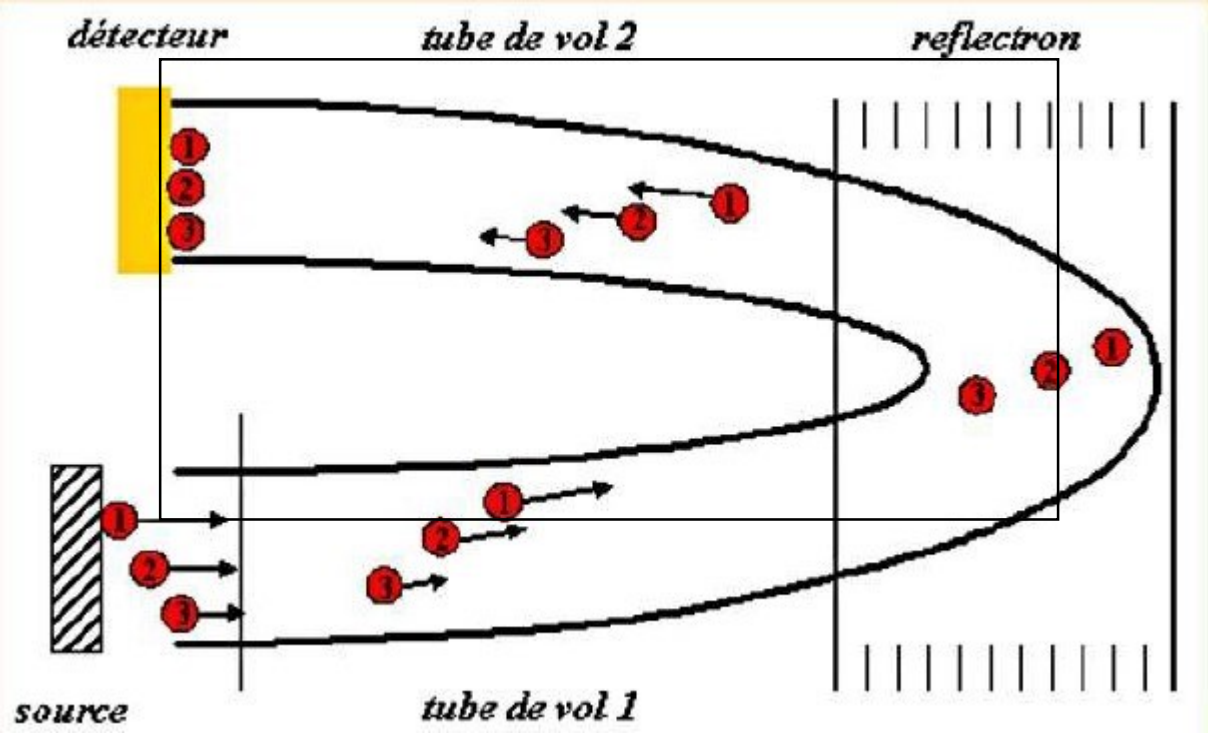
This picture shows you a combination of the time-of-flight mass spectrometer with a MALDI ionization. This is a typical combination in mass spectrometry because MALDI generates ions at once at a very well time-defined event, therefore the ions are ideally prepared to be accelerated to the start of the mass analysis and for the measurements of their time of flight. See also the video of the Bruker company.

Problems of the TOF analysis:

The problem for the TOF analysis (and also for others) is that the ionization sources are not the ideal ionization sources. The ions are not generated in an indefinite small volume and they are not accelerated by ideal homogeneous field to the same velocity. For the TOF analysis is means that the ions are not at the start of the analysis with the uniform velocities which compromises the resolution. We have two inventions to correct for this problem: reflectrons and delayed ion extraction.



Reflectron



Reflectrons

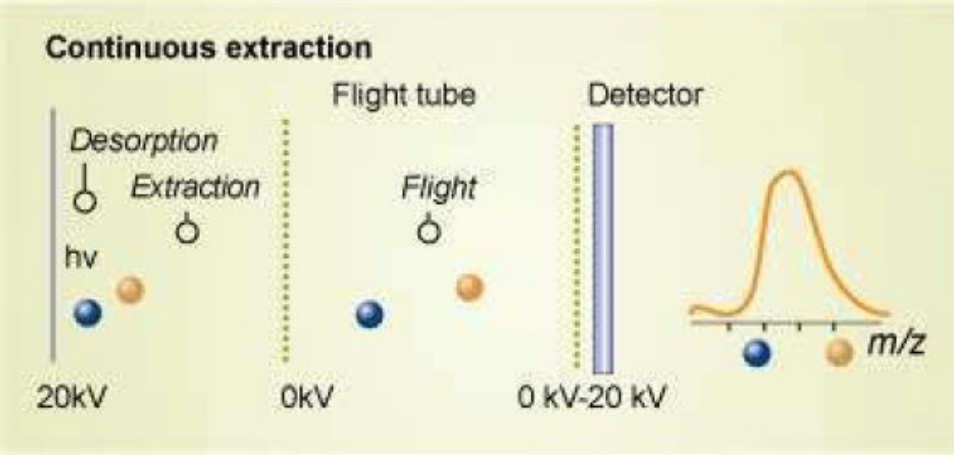
- Series of electrodes creating a linear field with an opposite sign to the initial accelerating field
- Ions are decelerated and turned to the opposite direction
- Constructed so that ions are focused to the plane of the detector

→ Ions with different kinetic energy, but the same m/z, fly a different distance → In the end, they have the same time of flight

Reflectron is composed from a series of electrodes that make a linear field with the opposite sign to the source acceleration field. The ions fly into the reflectron field and get decelerated and turned around by the field. On the way back they get again accelerated to the precisely the same speed with which they entered the field and then they travel towards the detector. The reflectrons are constructed so, that the ions focus on the detector plate. The reflectron correction means that the ions with the same mass, but a different kinetic energy, arrive at the same time to the detector. Invention of this trick significantly increased the resolution of TOF and made it one of the most popular analyzer for biomolecules.

Example: Suppose that you have two ions with the same mass, but a different velocity. The ion with a larger kinetic energy penetrates the reflectron field more and therefore makes a longer path towards the detector than the ion that had a smaller kinetic energy. The difference in the path length compensates the different kinetic energies of these two ions and therefore they arrive to the detector at the same time.

Delayed ion extraction



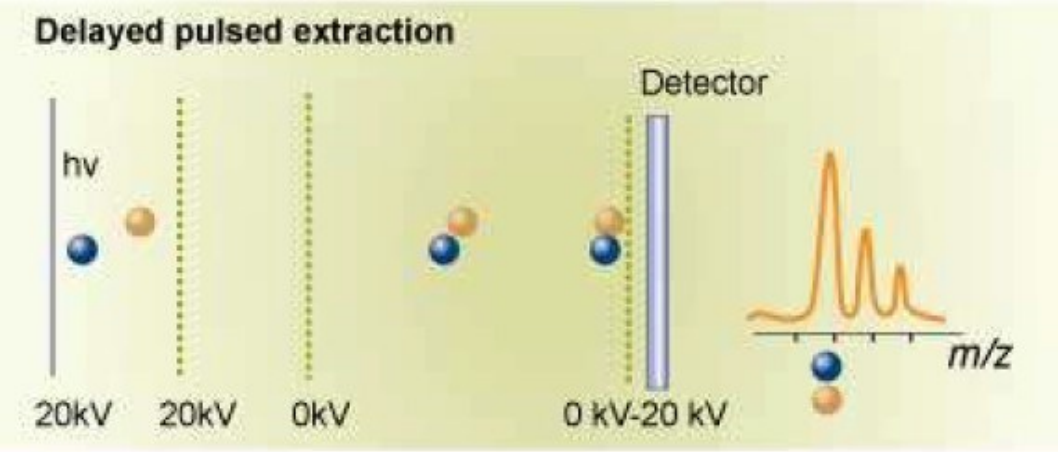
The second problem of the TOF analysis stems from generating the ions at different positions of the ionization volume and they have different initial velocities in different directions. Once the ions are generated, they feel an extraction field that accelerates them towards the start-line (see the continuous extraction figure). Because of the different initial situation of the ions, the acceleration field act differently and they achieve different velocities in the end.

We can correct this problem with a delayed pulsed extraction.

Delayed pulsed extraction

- Extraction of the ions is delayed by 200 – 500 ns
- During the delay, faster ions move closer to the extraction electrode than the slower ones
  → extraction pulse accelerates the faster ions shorter time → final velocities are similar

→ Initial distribution of velocities is corrected → the same time of flight

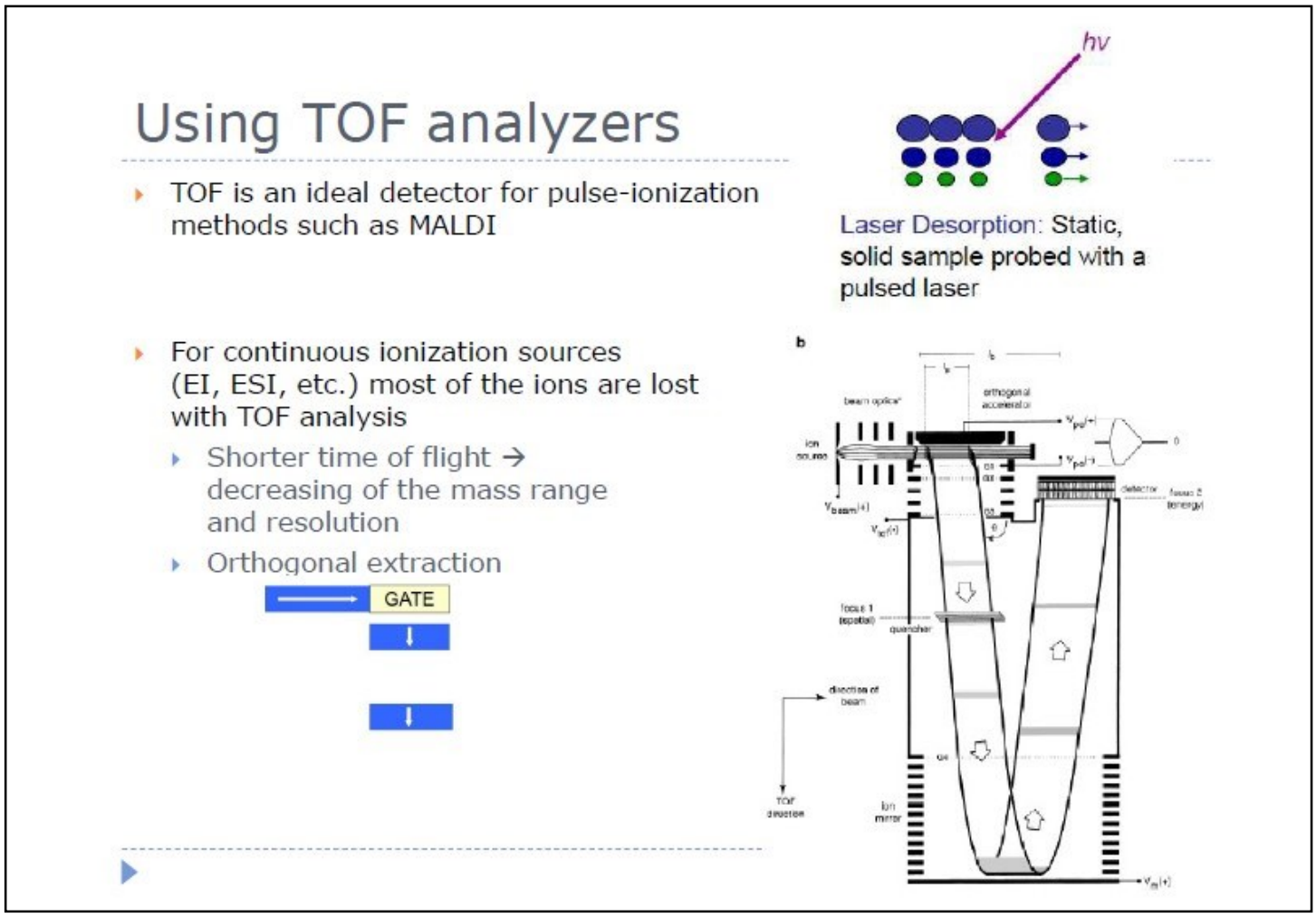


The trick of the delayed extraction is very simple. First, we generate the ions and let them move in a field that there is no potential difference (see the figure above). This means that the faster ions will fly closer to this first green line than the

slower ions. After this delay we will extract them. During the extraction pulse, the faster ions will be already closer to the extraction plate than the slower ions. This means that the faster ions experience a weaker electric field that the slower ones. Accordingly, the faster ions will be accelerated less than the slower ones which will lead to their focusing at the start line. Hence, using delayed pulsed extraction, we can correct the initial velocity distribution during the ion formation.

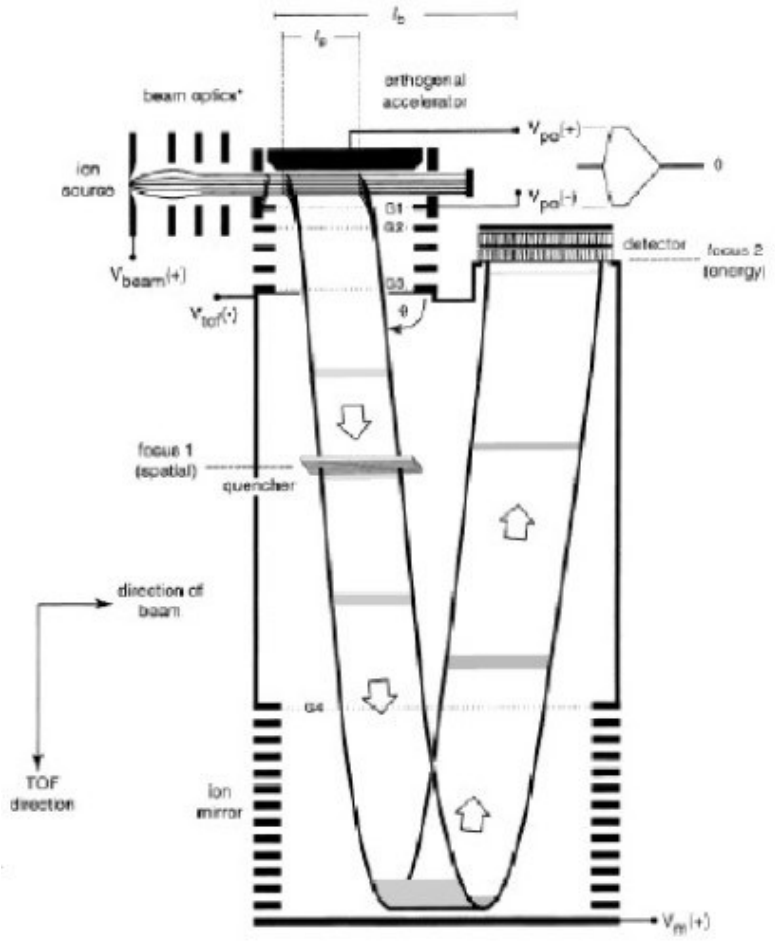
These two inventions - reflectron and delayed pulsed extraction led to the TOF renaissance. Without these two tricks the TOF has a very small resolution, but with these two tricks the TOF became one of the high resolution mass spectrometer instruments.

The final slide about TOF is about their practical use.



The combination of TOF and MALDI is an ideal one, because it combines a pulsed ionization method with a pulsed mass analysis. But sometimes we would like to use TOF also with continuous ion sources. Continuous ion ionization sources are sources that are producing ions all the time, like electron ionization, chemical ionization, or electrospray ionizations.

Such situation is not suitable for a pulse analysis. However, we have various tricks how to overcome this problem.



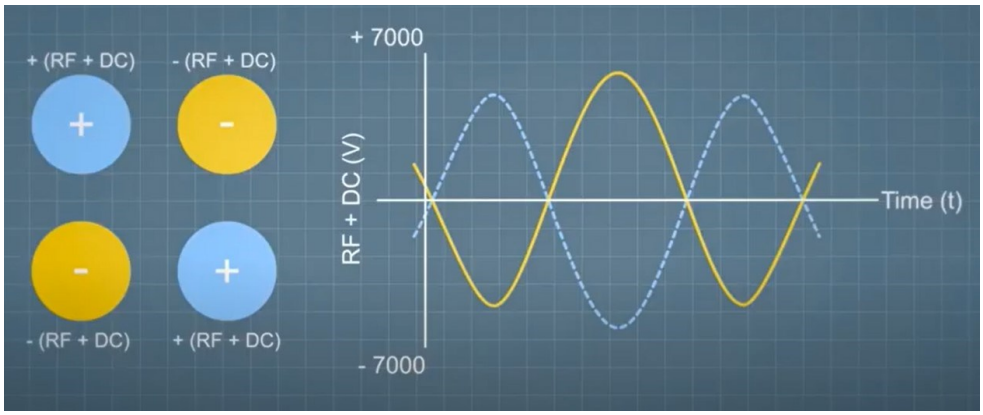
The figure shows that we can combine continuous ionization source (e.g., ESI) with a TOF analysis by perpendicular geometry. The ions are produced continuously, but only in pulsed they are pushed orthogonally towards the TOF analyzer. The problem is that we lose most of the ions because only a part of them is extracted in pulses for the mass analysis. To overcome this drawback, we can work with shorter times-of-flights. This, however, decreases the resolution and limits the mass range that you can study. This type of analysis is called orthogonal extraction of the ions from continuous sources.

Quadrupole

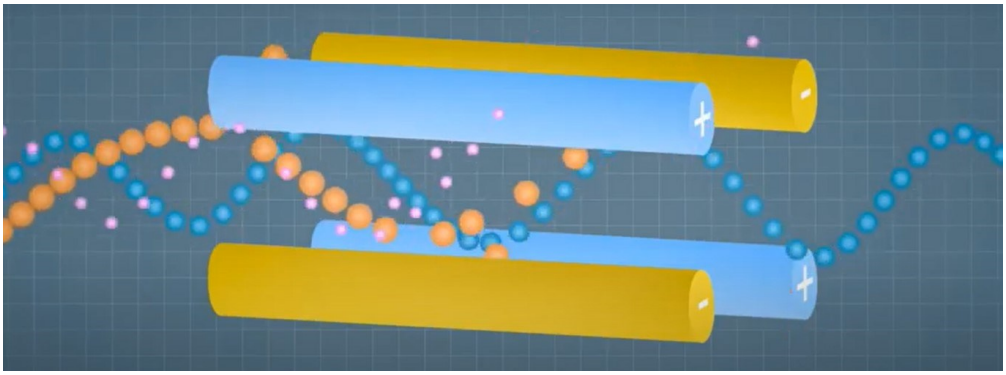
The next mass analyzer is a quadrupole. Please, watch a video from the Waters company that explains the analysis. Here, you can read a script of the video:

"*Hello my name is Dave. I work at Waters corporation as a development scientist. I'm going to give a brief overview of what a quadrupole is and how it works. So, a quadrupole is a mass analyzer and it consists of four parallel rods arranged around the central axis, as shown. Static potentials or DC voltages and alternating potentials or RF voltages are applied to the quadrupole rods with one pair of rods having the opposite polarity to the other pair.*

These voltages create a fluctuating electric field between the rods. The field oscillates as shown. Typically, at frequencies of approximately 1 MHz (one Megahertz).

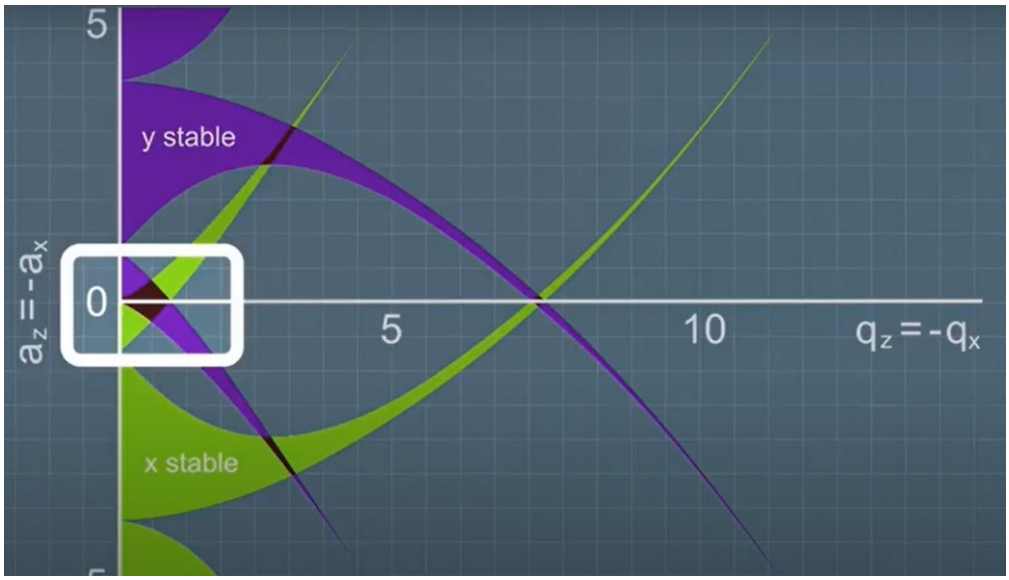


The stability of an ion when passing through this field is dependent on the master charge ratio of the ion. Since the voltages define the field and we control the voltages they can be set such that only a single master charge ratio is stable and can pass down the length of the quadrupole. Ions whose master charge ratio is too large or too small have unstable motion they strike the quadrupole rods and therefore aren't detected.



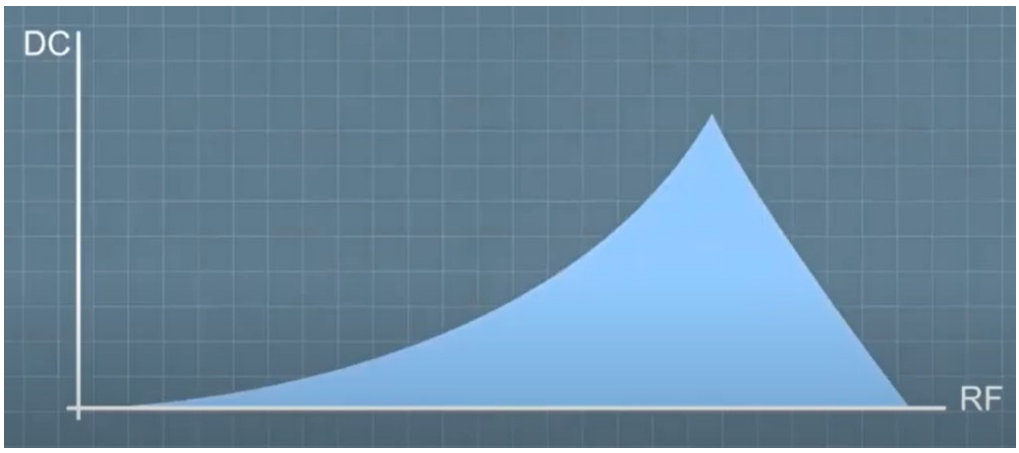
The ion motion within a quadrupole is relatively complex so we won't go into details here.

*It's possible to plot ratios of the RF and DC voltages for which the ion motion does not extend beyond the inscribed radius of the rods in the **X** and the **Y** axis. In other words, we can derive ratios where the ion motion is stable. So, plotting this gives the following.*

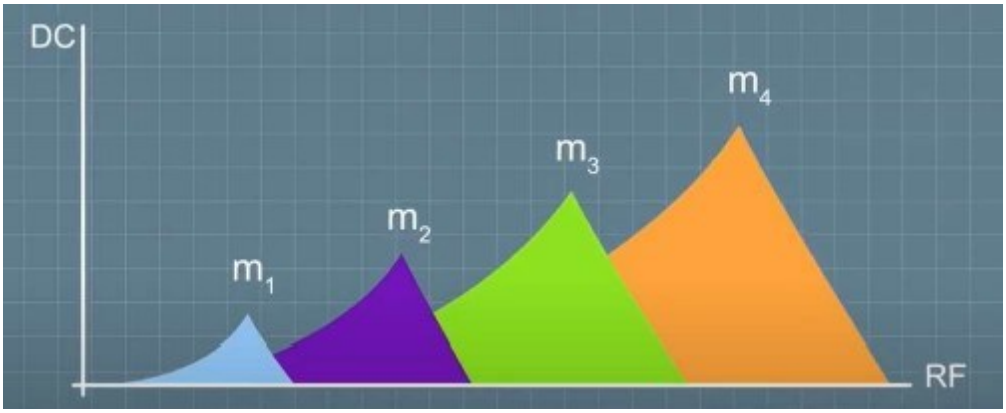


*Obviously for an ion to make it through the quadrupole it needs to be stable in both the **X** and **Y** axis. And from this plot we can see that there are several regions where the stability zones overlap. But all commercial quadrupoles use what's called the first stability region. Mainly because this is where the voltage requirements are the lowest.*

If we look closer at this region, we can see it has the following shape.

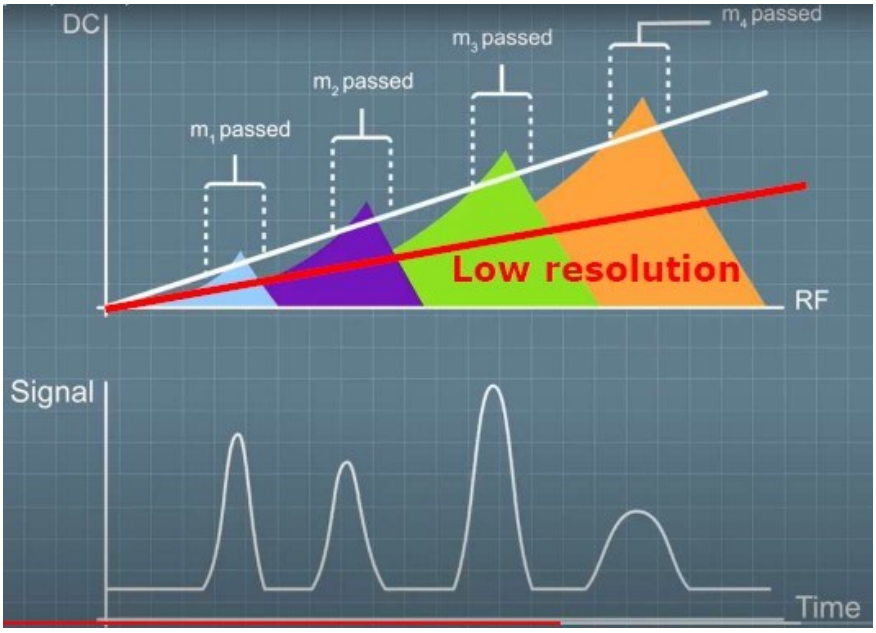


The stability region is dependent on the mass to charge ratio of the ion. So, if we plot the stability regions of different masses on a single axis, we get the following.



Typically, when scanning with a quadrupole, the RF and DC are ramped linearly with time. As the voltages are increased, the scan line cuts through the stability regions of different masses, and the ions of that particular mass are transmitted through the quadrupole to the detector.

A plot can then be created as signal versus time, which is translated into a plot of signal versus mass or in other words, a mass spectrum.



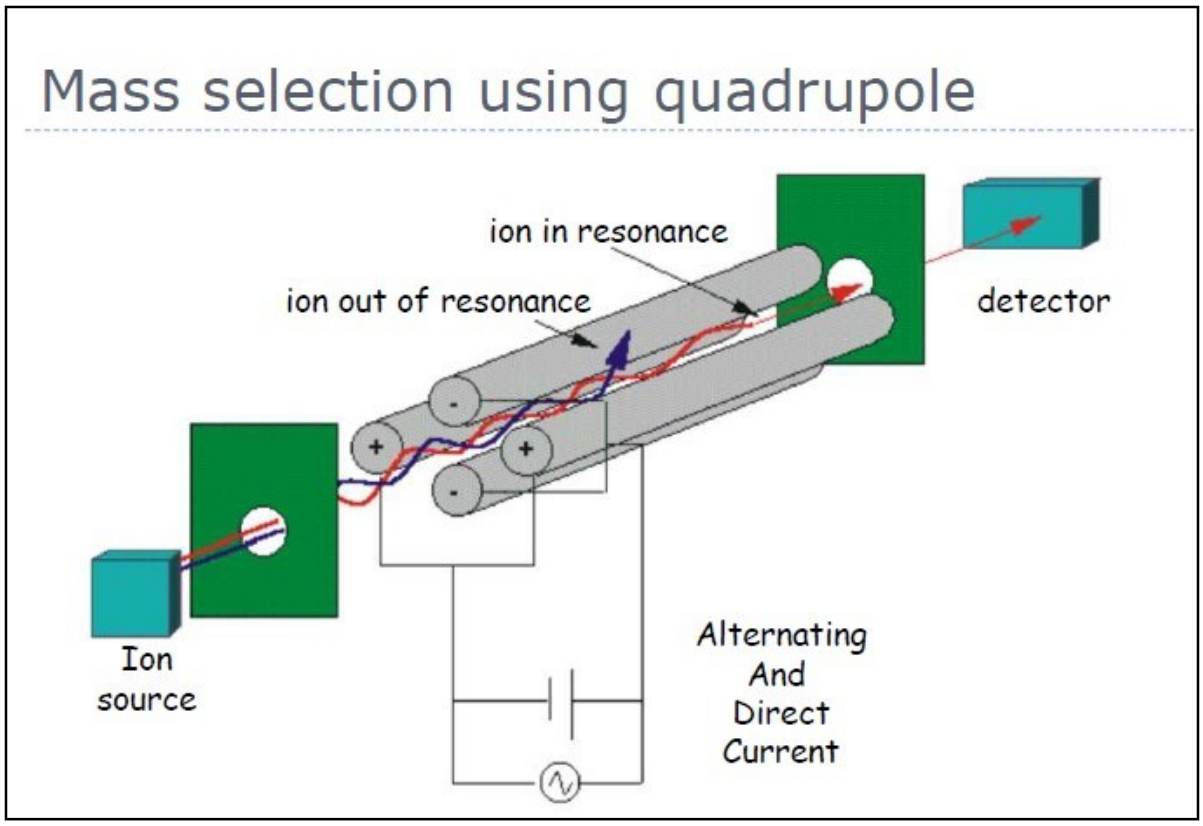
We're able to resolve the masses from each other because the scan line doesn't cross two stable regions at the same time. We can also increase the DC to RF ratio to the point where the scan line crosses the very tips of the stability regions. As you can see as we do that, the peak widths are reduced. Therefore, the resolution is increased.

As we attempt to operate a quadrupole at higher and higher resolution the transmission reduces because the entry conditions become more and more stringent. In other words, the ions must enter closer and closer to the central axis to make it through.

The theoretical limit of the resolution of the quadrupole relates to the number of cycles of RF the ion is exposed to. So, increasing the frequency of the RF, making the quadrupole longer or slowing the ions down will increase the theoretical maximum.

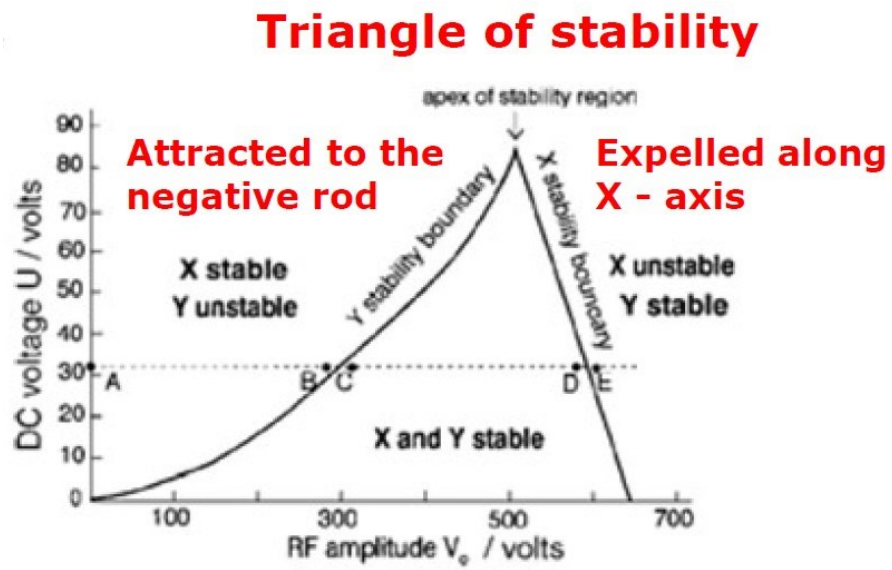
However, there are practical limitations to the resolution performance of quadrupoles, which are dominated by the mechanical accuracy of the quadrupole itself. Because of this, quadrupoles are generally operated with a resolution set to what is known as the unit resolution. This is the point at which ions with a mass difference of 1 amu can be resolved from each other."

Let me repeat several key points from the video.



Quadrupole is mass analyzer that is composed of four metal rods. On these rods we put a combination of alternating and direct currents. Two of the rods are positively charged and two others are negatively charged by the DC current and on top of the DC we put an alternating current (see the figure).

Whether or not the ions pass the quadrupole filter depends on the DC-AC combination and is determined by a so-called triangle of stability.



To understand the triangle of stability, let's make a thought experiment. Imagine that you have a positive ion that enters somewhere at the axis the quadrupole. Imagine that we have just DC potential, so the rods are charged, but we do not apply RF potential.

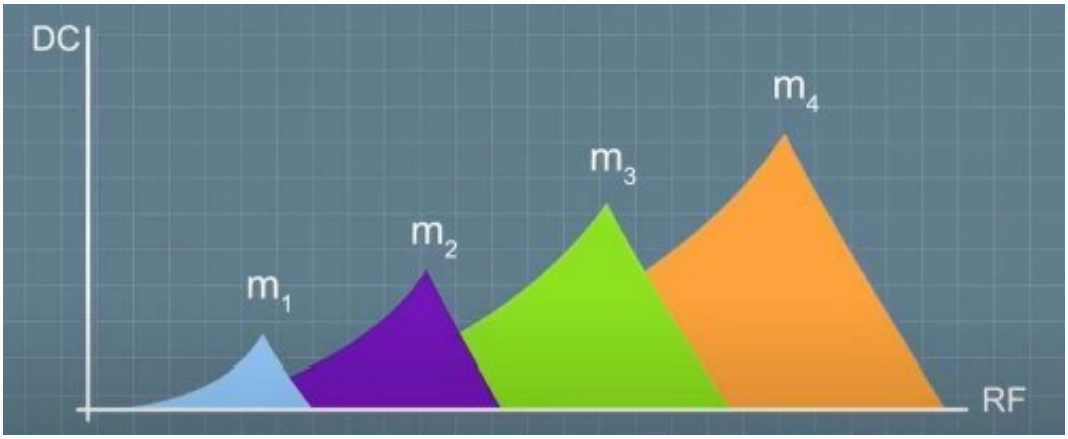
What will happen? The positively charged ion will immediately be attracted to one of the negative rods and it will not go through the quadrupole. In order to prevent this falling onto one of the negative rods we have to apply sufficiently large the RF potential. The falling on the negative rod (being unstable along the y-axis) happens in regime with a small RF field (left part from the triangle of stability).

Then, there is a certain range (the triangle of stability), where the DC and RF amplitudes are suitable for the ion to pass the quadrupole.

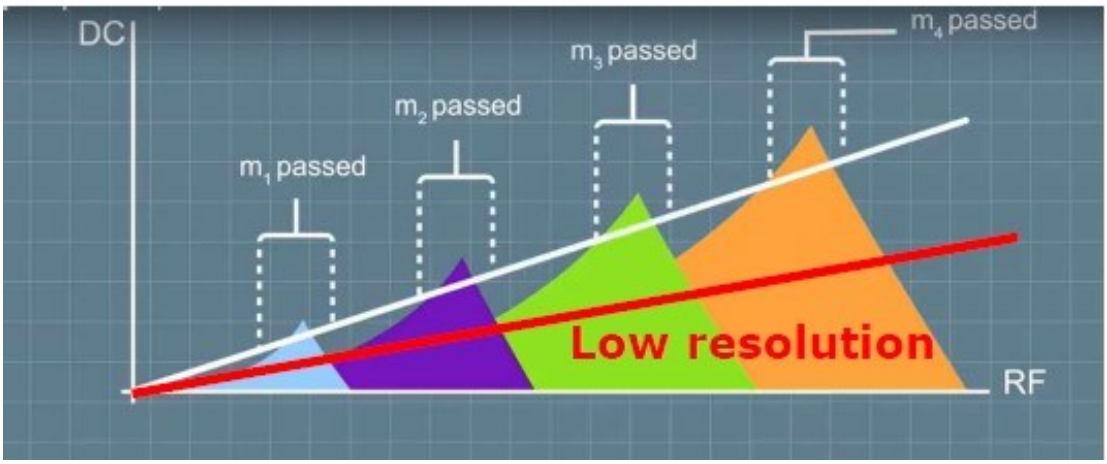
Finally, when the RF amplitude is too large, then the positively charged ions get huge amplitudes along the ***X*** axis. Meaning that they start to oscillate to the vicinity of the positively charged rods and eventually get expelled out of the quadrupole.

Quadrupole as mass analyzer

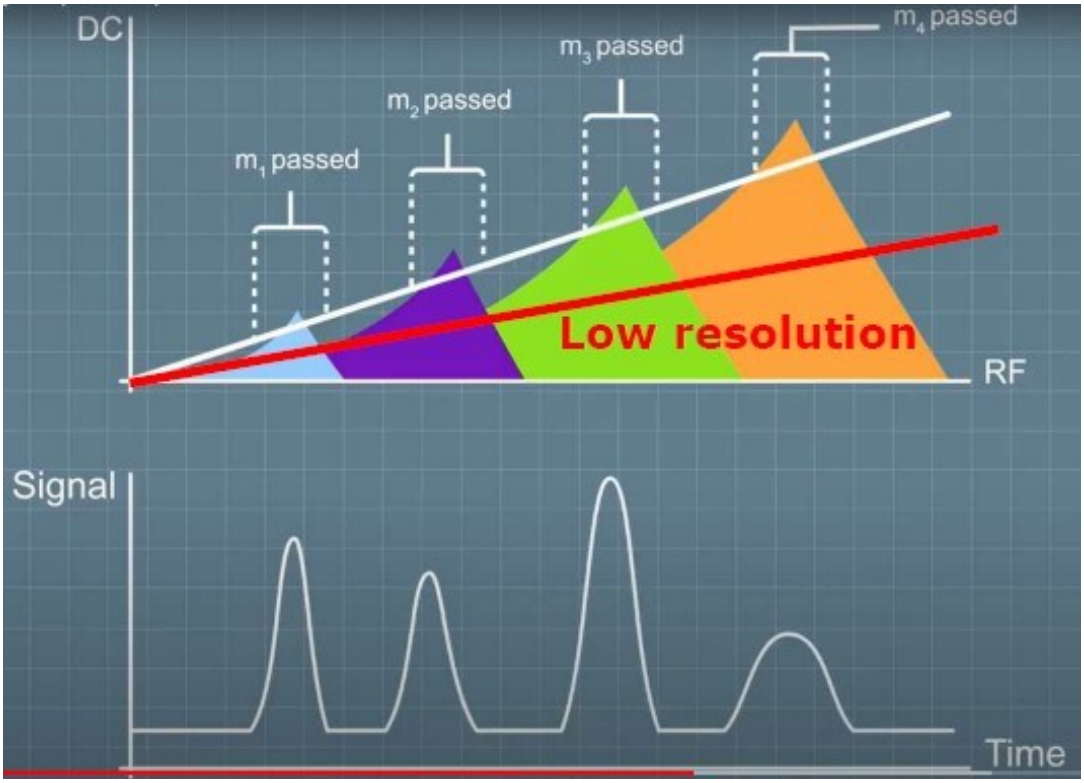
For each mass we have a separate triangle of stability that determines the combination of DC and RF potential suitable for the given ions with the given masses. Usually, we scan the masses by scanning the DC and RF potentials with the constant DC/RF ratio.



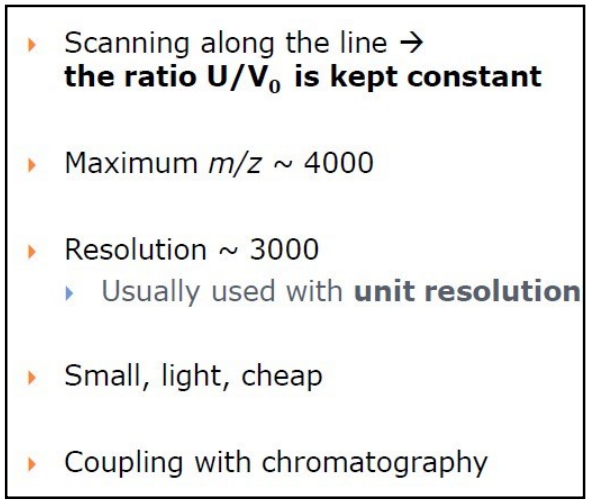
Scanning DC and RF potentials with two different DC/RF ratios (along the red and while lines):



The resolution of the quadrupole analysis is tuned by the DC/RF ratio. The red line indicates a scan with a low DC/RF ratio. You can see that at certain points, ions with different masses have stable trajectories and therefore they can pass the quadrupole. It means that we will not be able to resolve them, because they can simultaneously pass the quadrupole filter. In order to resolve all ions, we must scan with a larger DC/RF ratio (see the white line). Scanning along the white line means that at the given combinations of DC and RF (at the white line), only one particular kind of ions (with a given m/z ratio) can pass the filter at a time.



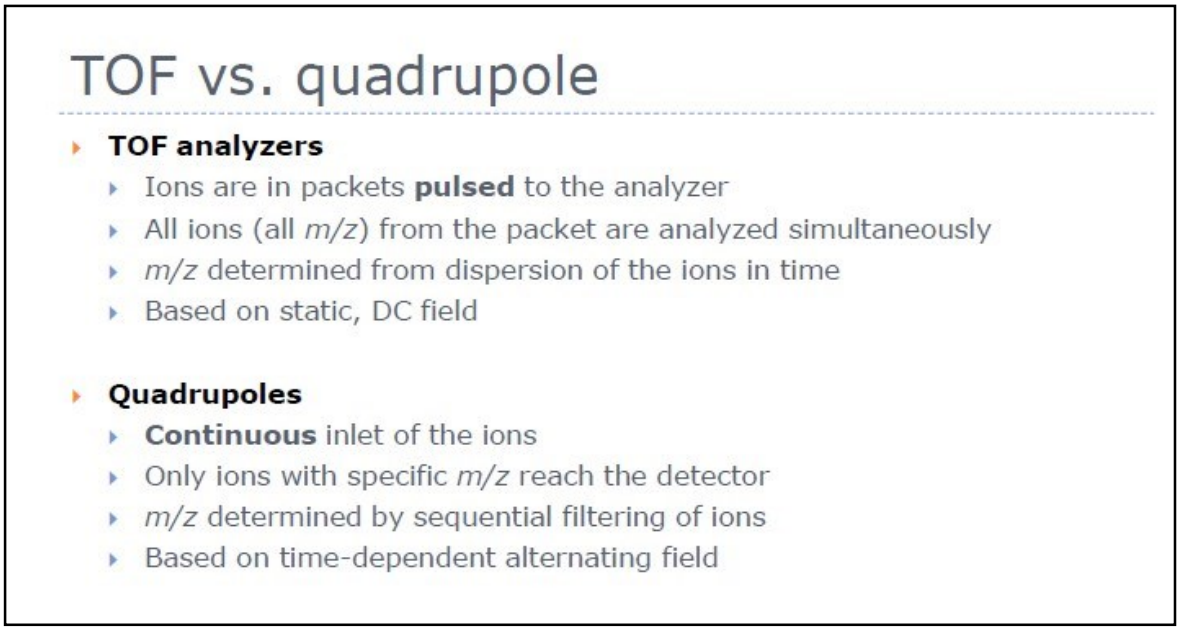
Usually, we work with a unit resolution so that you can determine the nominal mass. But you increase the resolution by scanning at the tips of the triangles. However, the intensity of the signal will be much smaller.



Maximum range of masses that you can analyze with classical quadrupole analyzers is up to 4000. It's not suitable for biomolecules. Usually, resolution is up to 3000 and we are usually work with unit resolution. Quadrupole is a typical mass analyzer with a low resolution. The advantage is that it's small, light and cheap. It's among the cheapest mass spectrometers that you can buy.

TOF vs. quadrupole

Finally let me compare a time-of-flight analyzer with a quadruple analyzer. What are the differences between these two analyzers?



TOF analyzer analyzes ions in packets that are pulsed towards the analyzer, so at once all ions are pulsed to the analyzer. TOF are ideal for coupling with pulsed sources such as MALDI.

Quadrupoles analyze continuously. They are usually coupled with continuous sources and they analyze by filtering through the masses of the ions.

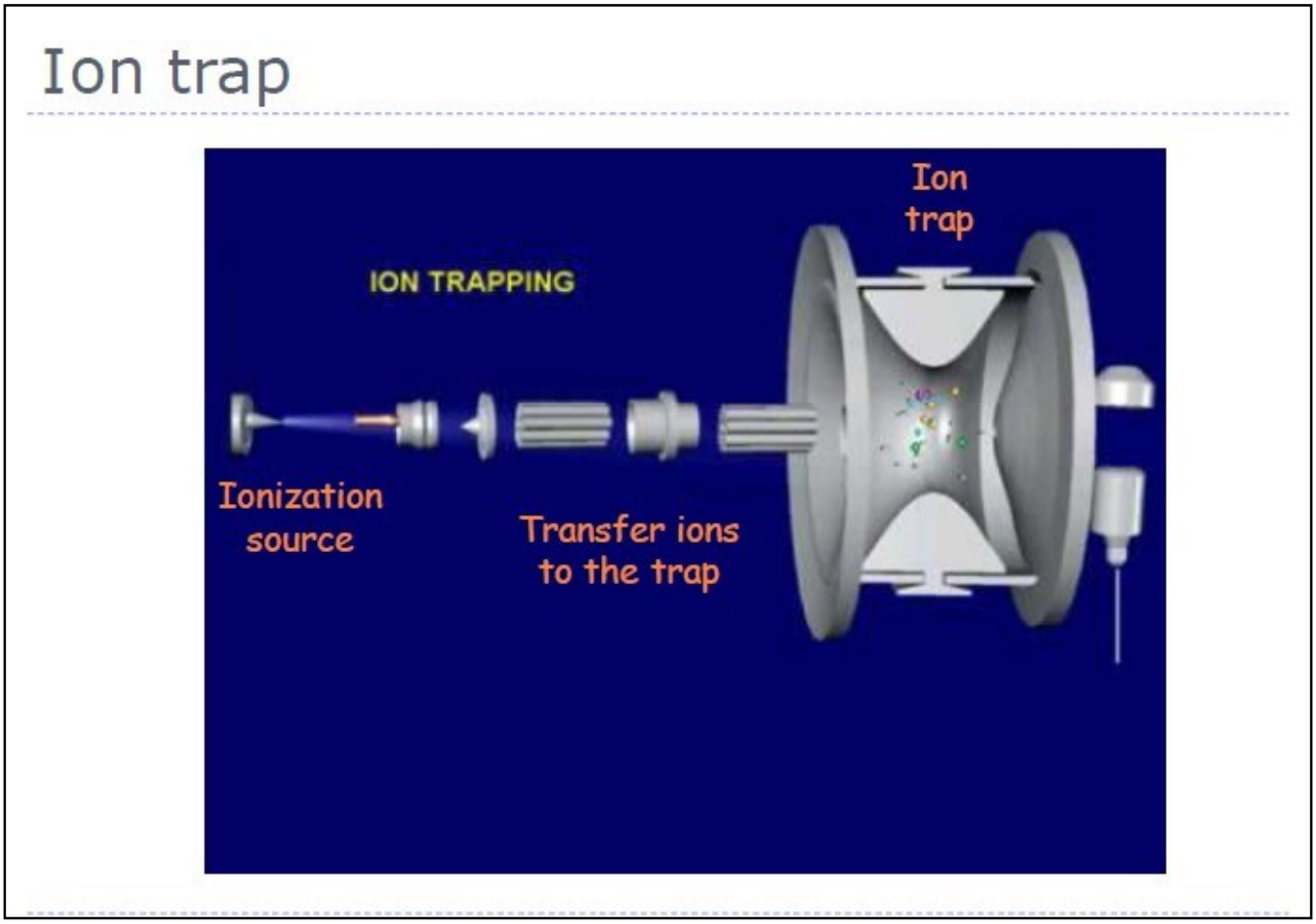
In TOF analyzers, all ions go towards the detector simultaneously, whereas in quadruple analyzers only ions with a specific mass-to-charge ratio have a stable trajectory in the quadrupole and reach the detector.

In TOF analyzers, the mass-to-charge ratio is determined from the dispersion of the ions in time (from the arrival times). In quadrupole mass analyzers, the mass to charge ratio is determined by a sequential filtering of the ions. You scan the DC and RF potentials with a fixed DC/RF ratio.

TOF analyzers are based on a static DC field. The ions are accelerated by a static DC field and then we measure their time-of-flight in a field-free region. In quadrupoles, the analysis is based on time-dependent alternating fields.

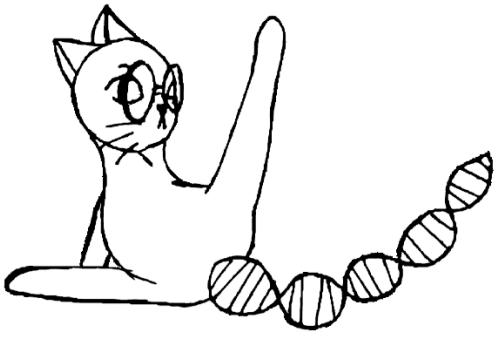
Ion trap

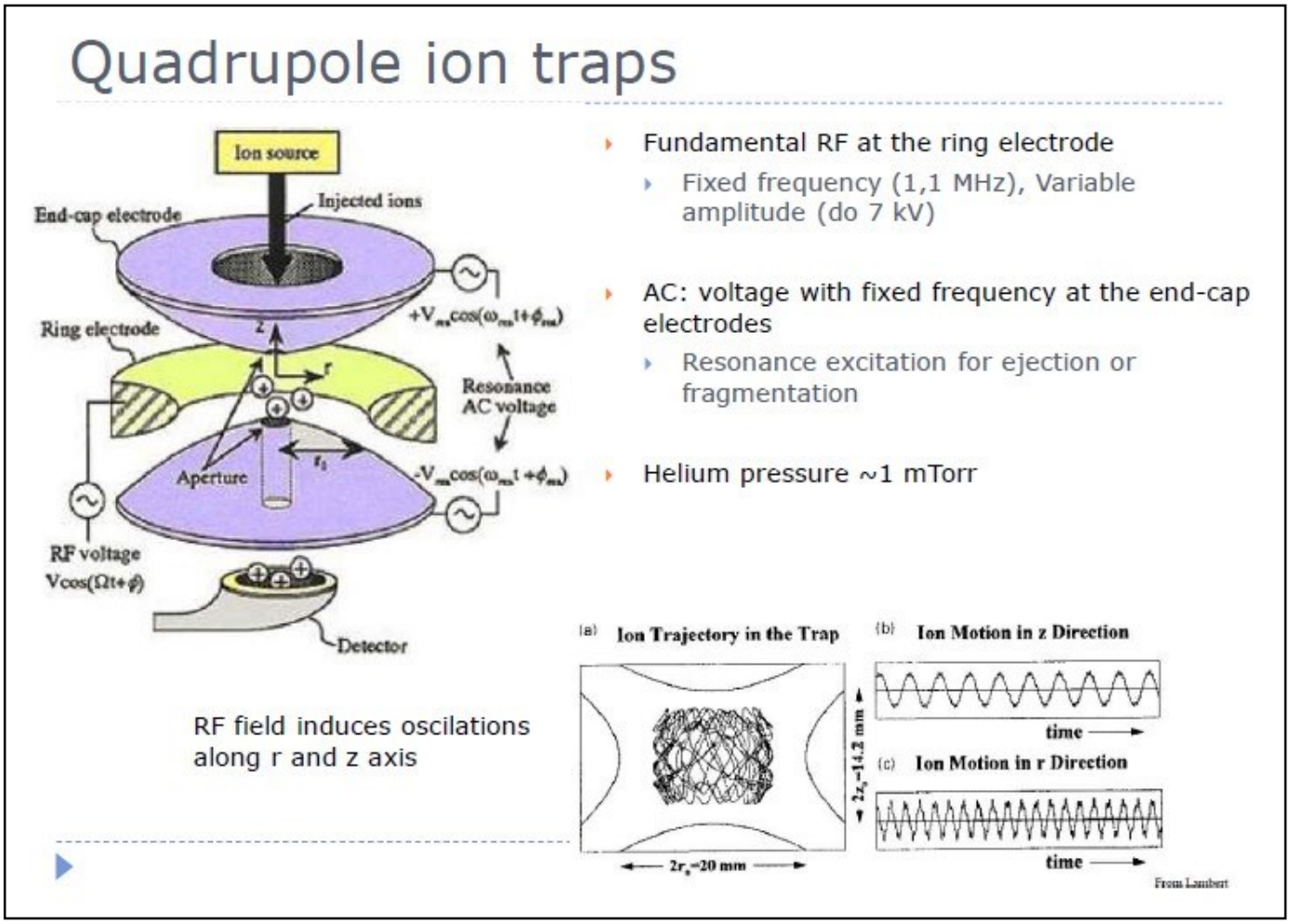
The last mass analyzer of this lecture is an Ion trap. I will explain the function of ion traps on a so-called Paul-type mass analyzer.



The typical instrument has an electrospray ionization source. We generate ions and transfer them with some ion optics towards the quadrupole ion trap. The trap is filled with helium (~10^{-5} mbar). The helium (so called buffer gas) is necessary for stabilizing the trajectories of the ions in the trap. The ions get into the trap and they are decelerated and thermalized by collisions with helium atoms. This stabilizes their trajectories and allows their trapping.

For the mass analysis, we use the RF field. The increasing RF field with increase the oscillations of the ions in the trap and eventually eject them out of the trap. The response of the ions to the RF field depends on their m/z ratio. Hence, one can use the increasing RF field for sequential ejection of the ions from the trap from the lightest ions to the heaviest and thereby measure the mass spectra.

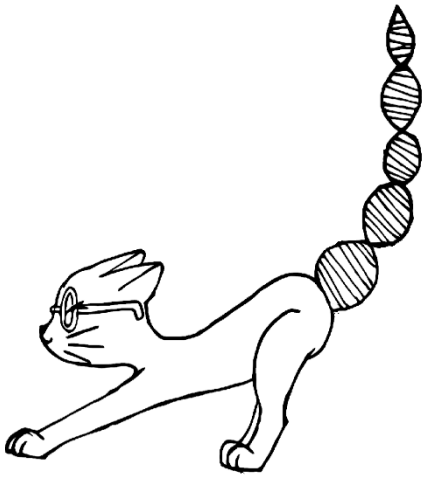




A Paul trap is composed from a ring electrode and the end-caps electrodes. We put so-called fundamental RF to the ring electrode. This creates a radio-frequency field with a fixed frequency in the trap. We can vary the amplitude of this fundamental RF field. The ions oscillate inside the trap and they oscillate along the ***R*** axis but also along the ***Z*** axis.

Then we have the end-cap electrodes. We can also put an alternating current voltage to the end-cup electrodes and thereby manipulate the trajectories of the ions.

The important feature of ion traps is that they are working with helium inside the trap. All mass spectrometers work with very low pressures. The ion traps are a sort of an exception because they work at higher pressures. It is still a reduced pressure, of course, with respect to the atmospheric pressure. The helium gas is necessary inside the trap because it helps trapping the ions inside the trap and stabilizing their trajectories.



Stability diagram

- Stability of ion trajectories affected by combination of AC and DC → mostly DC is set to zero
- For zero DC, stability given by q_Z:

$$q_Z = \frac{8ezV}{m(r_0^2 + 2z_0^2)(2\pi\upsilon)^2}$$

- Stable trajectories up to q_Z = 0.908

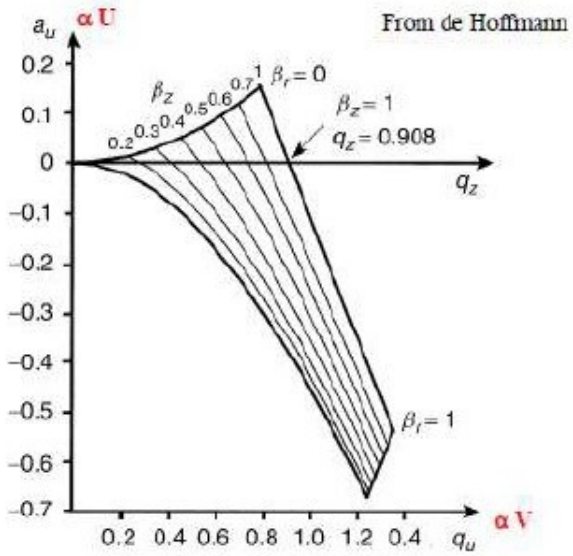


Figure 2.16
Typical stability diagram for a quadrupole ion trap. The value at $\beta_z = 1$ along the q_z axis is $q_z = 0.908$. At the upper apex, $a_z = 0.149998$ and $q_z = 0.780909$. (Data from Ref.12)

The stability of the trajectories of the ions in traps are described by a similar diagram as we saw for quadrupoles. With the ion traps, we will work with the DC potential set to zero. Hence, we will be interested just in the line along the x-axis.

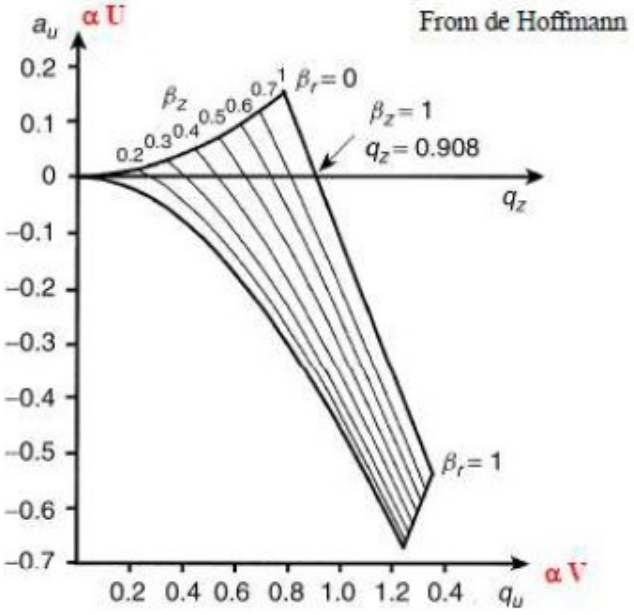


Figure 2.16
Typical stability diagram for a quadrupole ion trap. The value at $\beta_z = 1$ along the q_z axis is $q_z = 0.908$. At the upper apex, $a_z = 0.149998$ and $q_z = 0.780909$. (Data from Ref.12)

In principle, you should know that the ions are stable up to a certain amplitude of the RF field above which they are expelled out of the trap. This amplitude is characteristic for each mass and is given by so-called $\mathbf{q_z}$ value.

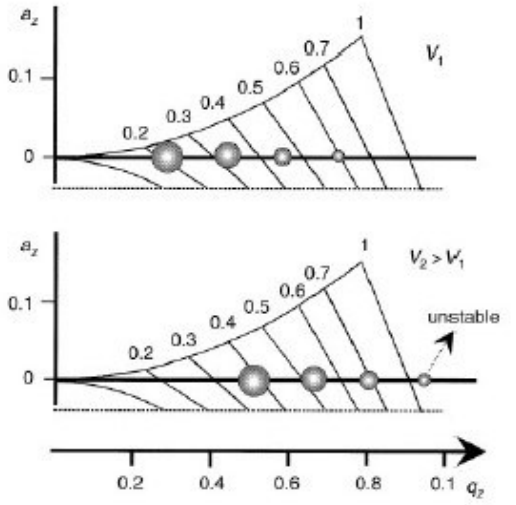
$$q_Z = \frac{8ezV}{m(r_0^2 + 2z_0^2)(2\pi\upsilon)^2}$$

In this formula, $(r_0^2 + 2z_0^2)$ are the dimensions of the trap, so they are fixed, υ is the frequency of the RF field at the ring electrode. It is fixed and will be given for each instrument. The only thing that we will vary is the amplitude of the RF field V.

The trajectories are stable up to $\mathbf{q_z}$ value equals 0.908. If we increase the amplitude above this value, we expel the given ions out of the trap.

By scanning V towards higher values, we sequentially expel the ions with increasing m/z from the trap.

Ejection of ions



$$q_Z = \frac{8ezV}{m(r_0^2 + 2z_0^2)(2\pi\upsilon)^2}$$

- With increasing V → larger and larger *m/z* beyond q_Z = 0.908
- Pressure determines the highest V (discharges) – usually ~2000 D

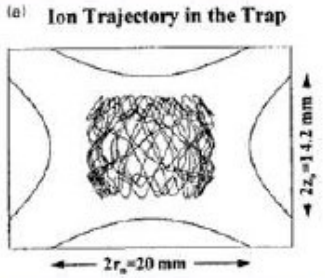
Figure 2.20
At a fixed value of the RF potential V applied to the ring electrode, heavier ions will have lower β_z values and thus lower secular frequencies. If V is increased, β_z values increase for all the ions, as do the secular frequencies. In the example given, the lightest ion now has a β_z value larger than unity and is thus expelled from the trap. The highest mass that can be analyzed depends on the limit V value that can be applied: around 7000–8000 V from zero to peak. For a trap having $r_0 = 1$ cm and operating at a υ frequency of 1.1 MHz, the highest detectable mass-to-charge ratio is about 650 Th

In principle, we could increase the amplitude up to very high values and thus expel heavy ions out of the trap for the analysis. However, in practice we are limited by discharges. So, above certain amplitudes we would start discharges in the trap and therefore, we cannot work with very heavy ions in the traps. Usually, we are limited to up to 2000 D.

Secular frequency

- Ions oscillate at secular frequency f that is smaller than ν
- Along z axis, f_z is proportional to q_z
- If RF with frequency f_z is applied to the end-cap electrodes, ions with secular frequency f_z will be in resonance and amplitude of their oscillation along the z axis will increase

→ for sufficiently large amplitudes are ions expelled
→ collisions with helium (use for fragmentation of the excited ions)

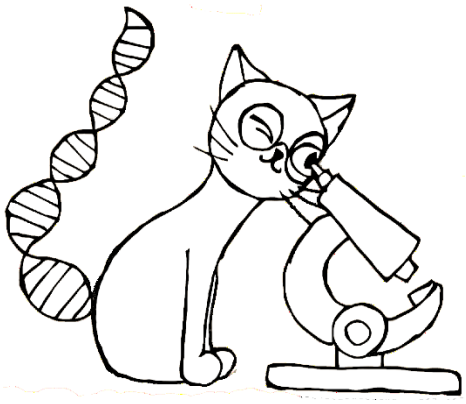


$$q_z = \frac{8ezV}{m(r_0^2 + 2z_0^2)(2\pi\upsilon)^2}$$

Secular frequency is another important feature of the trap instruments. The RF field in the trap makes the ions to oscillate along the r-axis and along the z-axis. Ions a given mass-to-charge ratio have a typical frequency of this oscillation in the trap which is called secular frequency. This frequency is smaller than the frequency of the RF field. The typical RF fields are usually around 1 MHz (Megahertz). Secular frequencies (the frequencies with which the ions oscillate) are about one order of magnitude smaller.

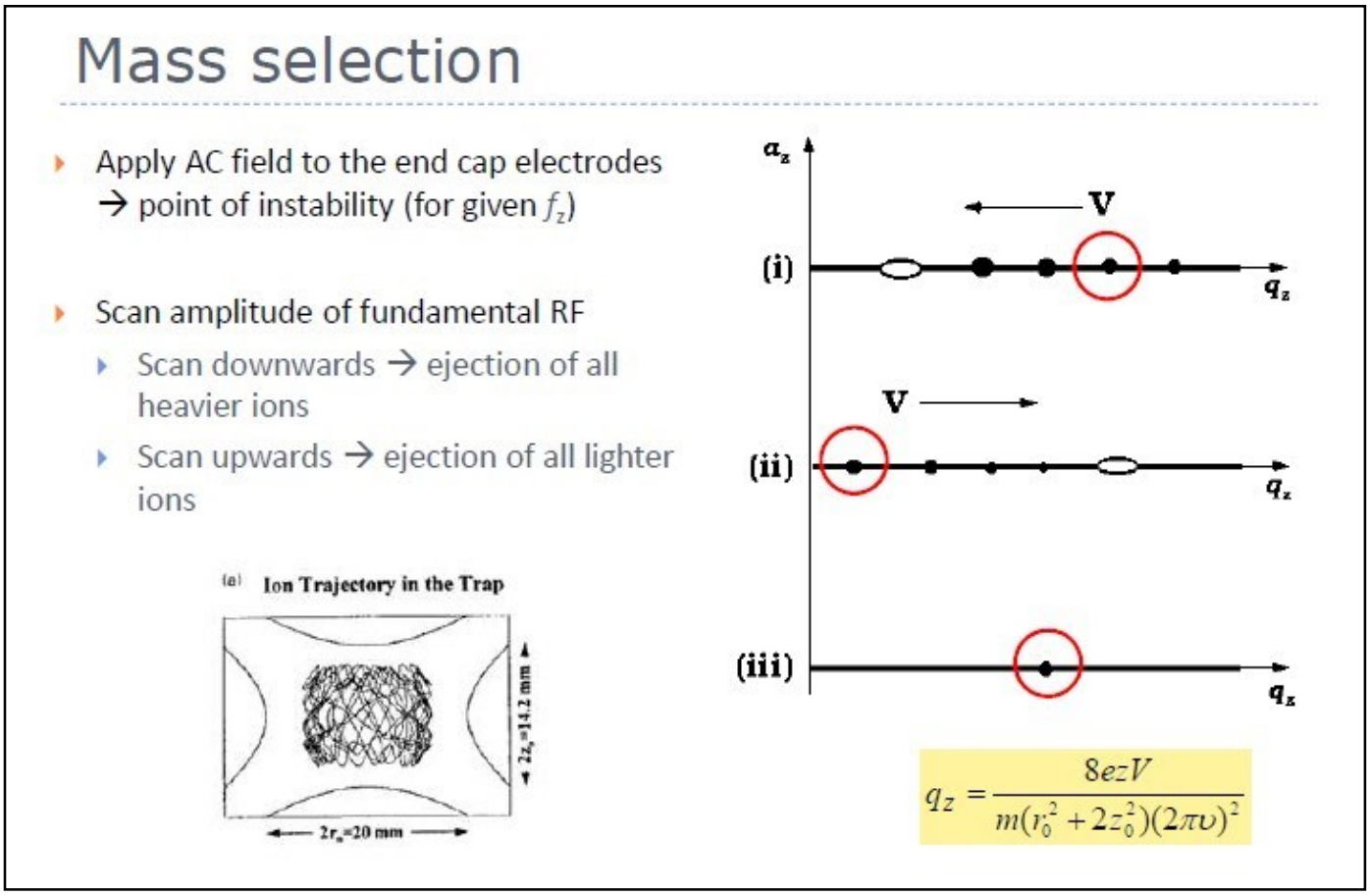
The secular frequency for each ion along the z-axis (in between the end-cap electrodes) is proportional to the $\mathbf{q_z}$ value of this given ion and thus to its m/z. We can use it for addressing particular ions in the trap. If we apply an AC current to the end cap electrodes with a frequency identical to the secular frequency of ions with particular m/z ratio, we will selectively excite these ions. These ions would be in resonance with the AC field and start to absorb the energy and thus oscillate with larger and larger amplitudes.

This effect can be used for two purposes. One purpose could be that that you can selectively eject these particular ions. A second purpose could be that you just excite the ions a little bit, therefore you enlarge amplitudes of their trajectories and accelerate them. It will cause collisions between these accelerated ions and helium present in the trap. The collisions with internally excite the ions and therefore, the ions will fragment. This is the principle of so called collision induced dissociation experiments in the traps.



Mass selection

The manipulation of the ion trajectories using secular frequency can be used for so called MS/MS experiments. First, I will explain mass-selection.



Imagine that you are interested only in particular ions. For example, you electrospray solution with a mixture of peptides. However, you are interested only in one particular peptide and you want to determine the sequence of this given peptide. Let's imagine that this peptide is the ion in the red circle (i.e., an ion with a given m/z ratio).

Firstly, we don't want to have the other ions in the trap. We will do it by sequential expelling the heavier and lighter ions out of the trap. We will use the circular frequency trick. We will start with the heavier ions. Therefore, we will apply an AC field on the end-cap electrodes corresponding to the secular frequency of heavy ions with a given $\boldsymbol{q_z}$ (imagine the heaviest ions present in the trap). You can imagine it as a secular frequency "hole". Every ion that starts to oscillate with this particular frequency gets ejected from the trap. In order to push the ions to the "hole", we will scan the amplitude of the RF field. This will sequentially change the $\boldsymbol{q_z}$ values of the ions and they will sequentially fall into the "hole". We will stop the scan before the ions of our interest (in the red circle) would fall into the "hole" too.

Once we have ejected all the heavier ions, we will put this excitation AC frequency at the end cap electrodes at a higher frequency typical for lighter ions. And we will again scan the amplitude of the RF field in order to push all the lighter ions into this "secular-frequency hole". In the end, the trap will contain only the ions that we are interested in. This is called mass selection.

As outline in the begging of the example, we are not just interested in the isolation of the given peptide ions, but we would like to know its sequence. How can we do it?

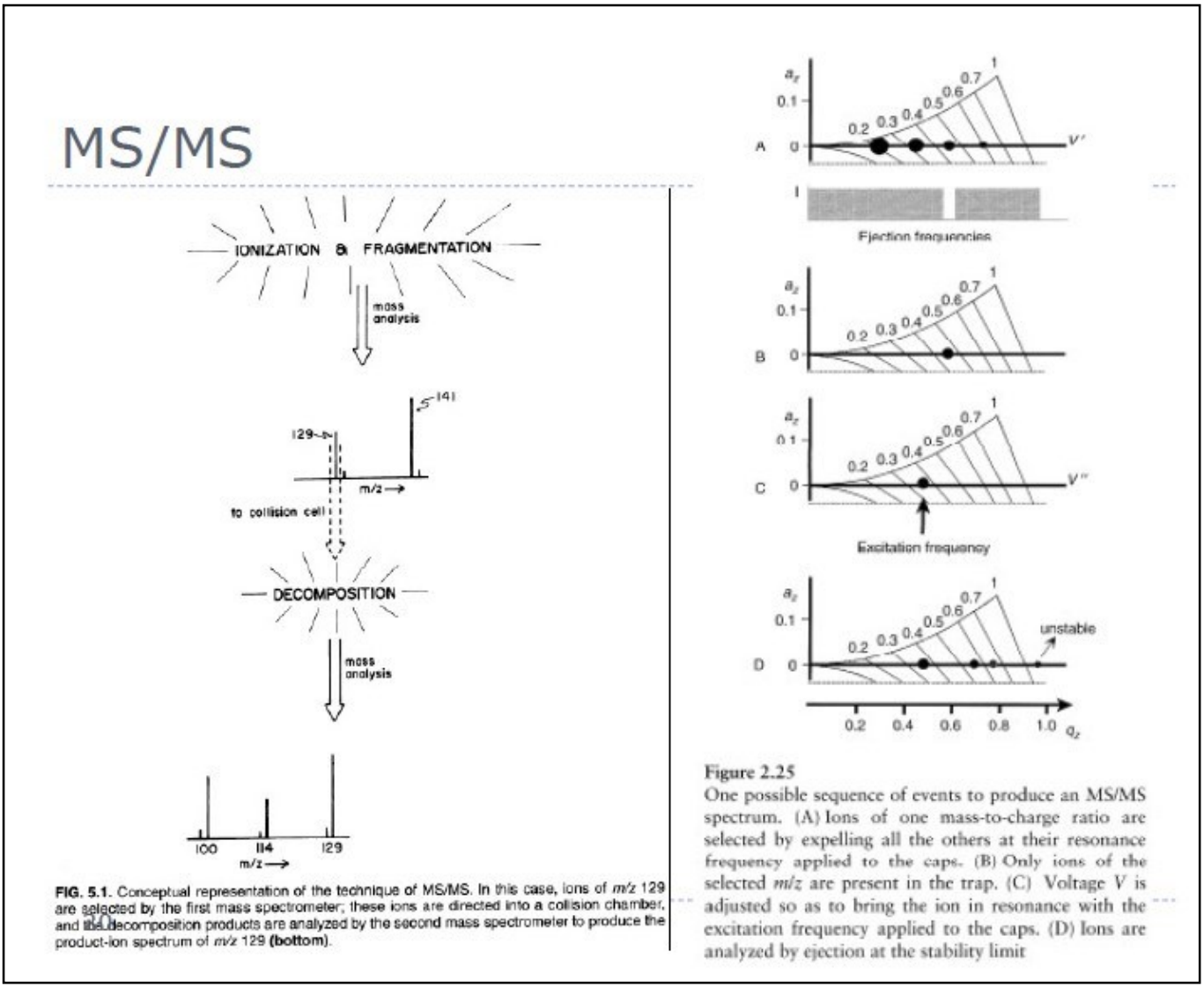


FIG. 5.1. Conceptual representation of the technique of MS/MS. In this case, ions of *m/z* 129 are selected by the first mass spectrometer; these ions are directed into a collision chamber, and the decomposition products are analyzed by the second mass spectrometer to produce the product-ion spectrum of *m/z* 129 **(bottom)**.

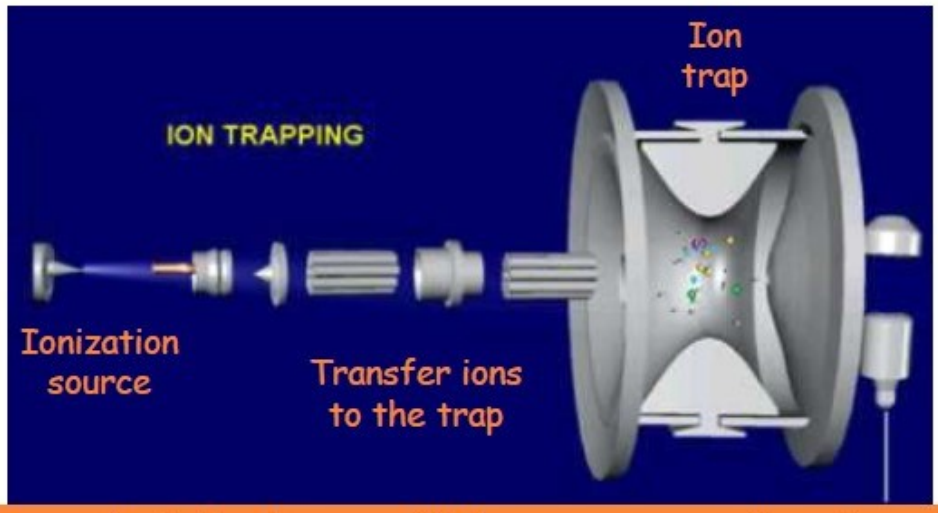
Figure 2.25
One possible sequence of events to produce an MS/MS spectrum. (A) Ions of one mass-to-charge ratio are selected by expelling all the others at their resonance frequency applied to the caps. (B) Only ions of the selected *m/z* are present in the trap. (C) Voltage *V* is adjusted so as to bring the ion in resonance with the excitation frequency applied to the caps. (D) Ions are analyzed by ejection at the stability limit

Hence, we have mass-selected ions with particular m/z ratio. Now, we will apply the excitation frequency at the secular frequency of these ions, but we will not eject them from the trap. We will just excite them it so that they start to oscillate between the end-cap electrodes and collide with helium atom present in the trap. This will lead to the fragmenting of these ions.

After this excitation we will have in the trap our "parent" ions that survived the collisions and their fragment ions. We can use usual mass ejection for recording a mass spectrum of the ions present in the trap. We call such spectrum collision-induced dissociation (CID) spectrum. The CID spectrum shows the parent ion and its fragments. For our example of a peptide ion, the CID spectrum would show the mass of the parent peptide ion and the masses of various fragments showing how individual amino acids cleaved of from the parent ion. Thereby, you could reconstruct the structure of the peptide that you were interested in.

The advantage of the ions traps is that we can do this sequence up to 12 times. Therefore, you can imagine, that you can get a very detailed information on complex ions.

Ion trap



Ions have complicated pathways with frequency proportional to *m/z*

Ions can be analyzed according to *m/z*

Ions can be mass-selected → up to MS^{12}

Mass-selected ions can be collided with buffer gas and their fragmentations can be studied

Summary: Ions in the ion traps have complicated pathways with frequencies that are proportional to their ***m/z*** ratios, Ions can be analyzed according to these ***m/z*** ratios by sequentially expelling them out of the trap. The ions can be mass selected and this we can do up to the 12 times. The mass selected ions can be collided with the helium buffer gas present in the trap. Thereby, we can study the fragmentation of the mass-selected ions.

Finally, you should be aware that there are many types of these traps. Paul trap is just one type.

Many other ions traps with similar properties

- Linear quadrupole traps
- Higher multipole traps

Another popular type is a linear quadrupole ion trap or also higher multiple traps. They all work with a similar principle.

Summary of the lecture today is, that we learned about several types of mass spectrometers.

Summary:

- Magnetic analyzer (*B*)
  - continuous analysis of ions according to their momentum
- Electrostatic analyzer (*E*)
  - continuous analysis of ions according to their kinetic energy
- *B* and *E* combinations
  - double focusing – high resolution
- Time-of-flight analyzer (*TOF*)
  - pulsed analysis of kinetic energy of the ions by measuring the time required for passing a fixed distance
  - Increase of resolution – reflectrons, delayed pulsed extraction → high resolution
- Quadrupoles (*Q*)
  - continuous analysis of ions in an alternating field (combination of DC and RF)
  - usually unit resolution
- Quadrupolar traps
  - pulsed analysis of trapped ions by their sequential ejection
  - mass selection/fragmentation up to 12 times

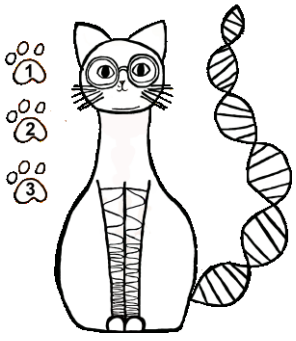
See you in the classroom!

Magnetic analyzer separates ions according to their momentum. Electrostatic analyzer separates ions according to the kinetic energy. Standalone magnetic and electrostatic analyzers do not achieve high resolution, but in a combination they do, because they are double focusing and with that we can achieve a very high resolution.

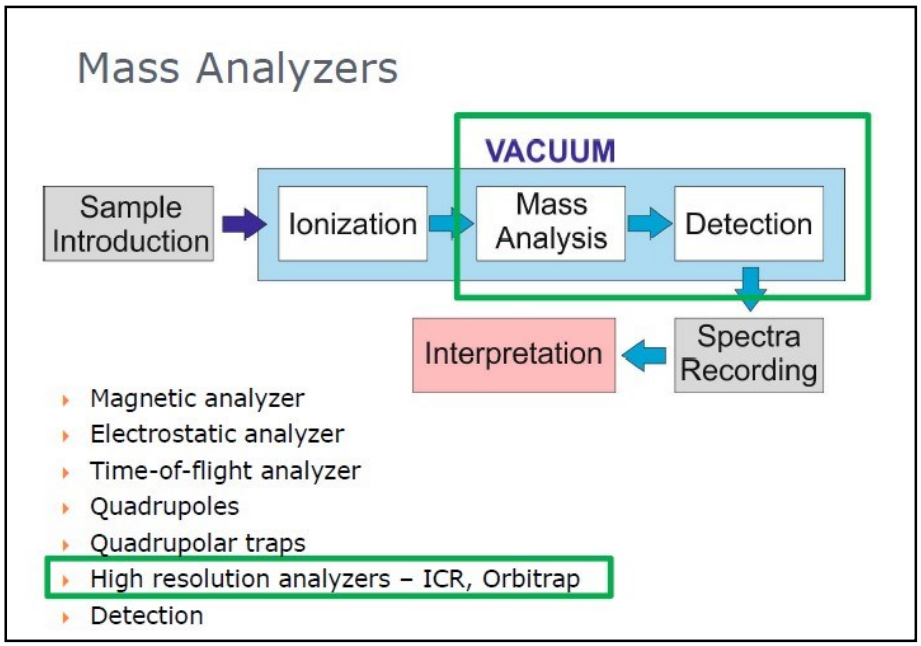
Time-of-flight analyzers use a pulsed analysis of kinetic energy of the ions that they achieve by the acceleration from the source. We measure the time required for the ions to pass a certain distance defined by the instrument. As such the resolution of TOF is low, but using reflectron and delayed pulsed extraction we can achieve very high resolutions. TOF is one of the high-resolution type mass analyzer.

Quadrupoles work continuously and separate ions in an alternating electric field. We use a combination of DC and RF potentials on the rods of the quadrupoles and by that we sequentially transmit all ions through the quadrupole and mass-analyze them. We usually work with a unit resolution. So, quadrupoles are low resolution mass spectrometers.

Quadrupole traps use pulsed analysis. We trap the ions in the ion trap using the RF field and helium buffer gas. A mass spectrum is measured by sequentially ejecting the ions out of the trap. The advantage of the quadrupole traps is that we can work in cycles with a mass selection and fragmentation of the mass-selected ions. This approach can give us information about the structure of the ions.



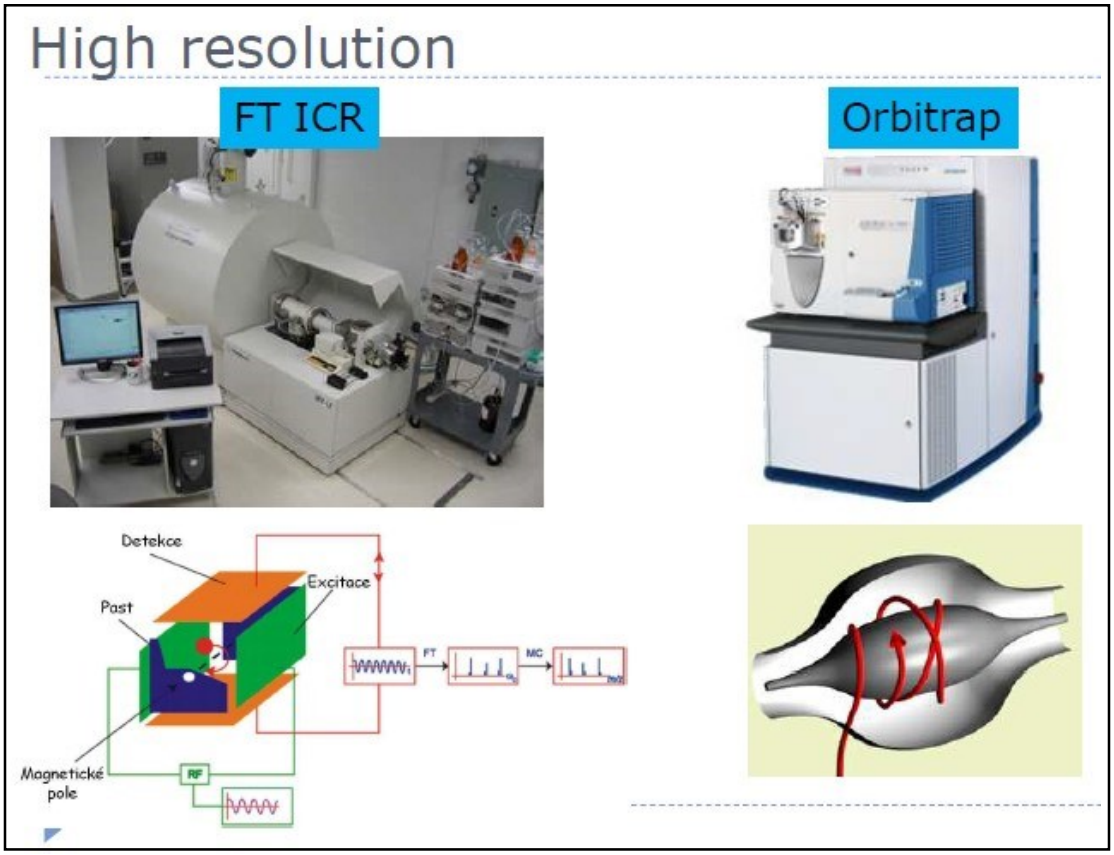
Lecture 4. Mass Analyzers. Part 2



This lecture is devoted to high resolution analyzers: ion cyclotron resonance (ICR) mass spectrometers and Orbitrap mass spectrometers. In the previous lecture I told you that time-of-flight analyzers with reflectrons are also considered as a high resolution analyzer. However, ICR and Orbitrap analyzers achieve still much higher resolution.

High resolution

Both ICR and orbitrap analyzers are working on the trap principles.



ICR is a trap that uses a high magnetic field. Orbitrap uses an electrostatic field. The fields must be strong so that the ions make circular movements in the magnetic- or electrostatic field. These circular movements are dependent on the mass-to-charge ratio of the given ions.

The circulating ions also create an electromagnetic field. Hence, we can use this field to detect the ions and we can use the frequency of the field to determine their mass-to-charge ratio. We do is using receiver plates as shown on the schematics for the ICR analyzer. The current detected by the receiver plates can be transformed by Fourier transformation directly to a mass spectrum. This works for both of these traps.

Ion cyclotron resonance (ICR)

Ion cyclotron resonance (ICR)

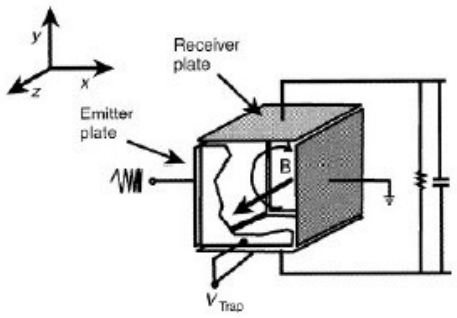
Penning trap

- electric trapping in a magnetic field

→ pulsed analysis

1. Ions are trapped
2. Ions are excited (emitter)
3. Ions circulate in the magnetic field at their cyclotron resonance (dependent on m/z)
4. Oscillating ions generate a current (receiver)

ICR can be also used for mass selection and CID experiments similarly to the quadrupole ion traps

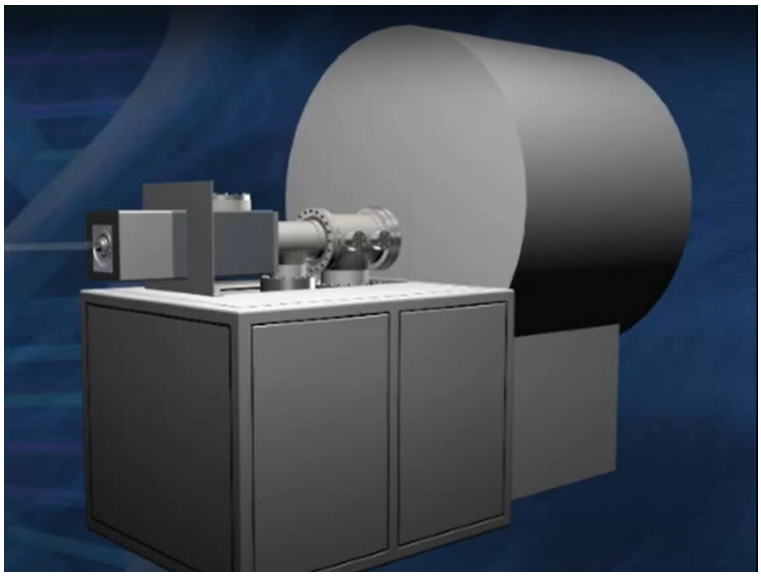


From Hoffmann

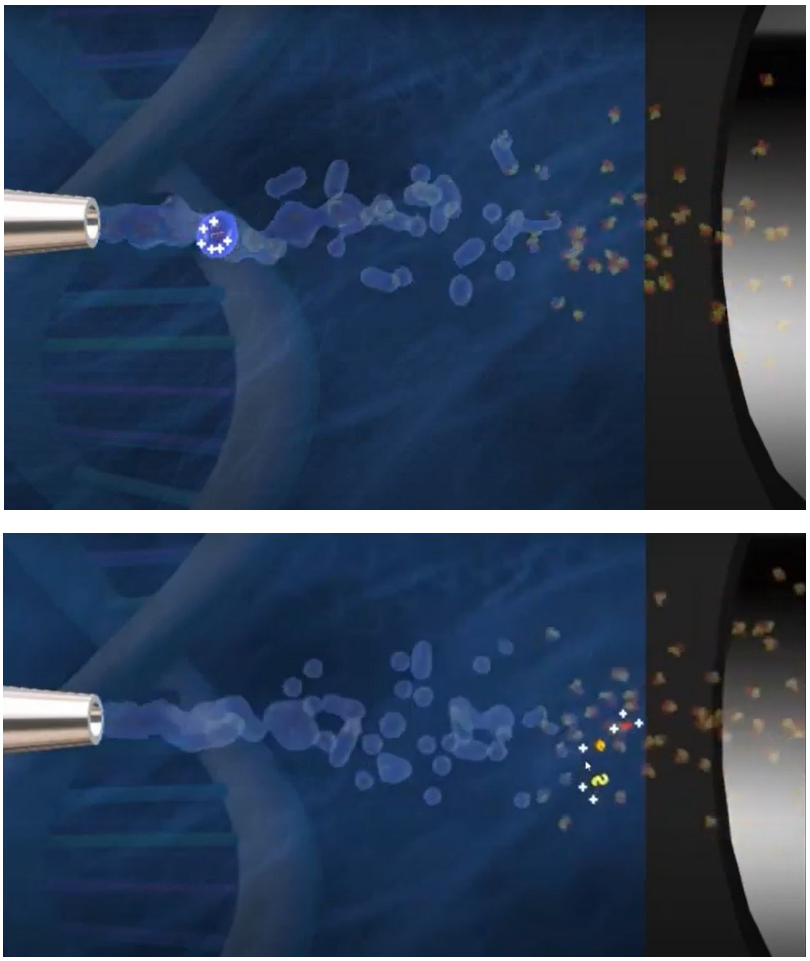
Figure 2.50
Diagram of an ion cyclotron resonance instrument. The magnetic field is oriented along the *z*-axis. Ions are injected in the trap along the *z*-axis. They are trapped along this axis by a trapping voltage, typically 1 V, applied to the front and back plates. In the *x*,*y* plane, they rotate around the *z*-axis due to the cyclotronic motion and then go back along the *z*-axis between the electrostatic trapping plates. The sense of rotation indicated is for positive ions. Negative ions will orbit in the opposite direction

http://www.youtube.com/watch?v=a5aLlm9q-Xc&feature=related

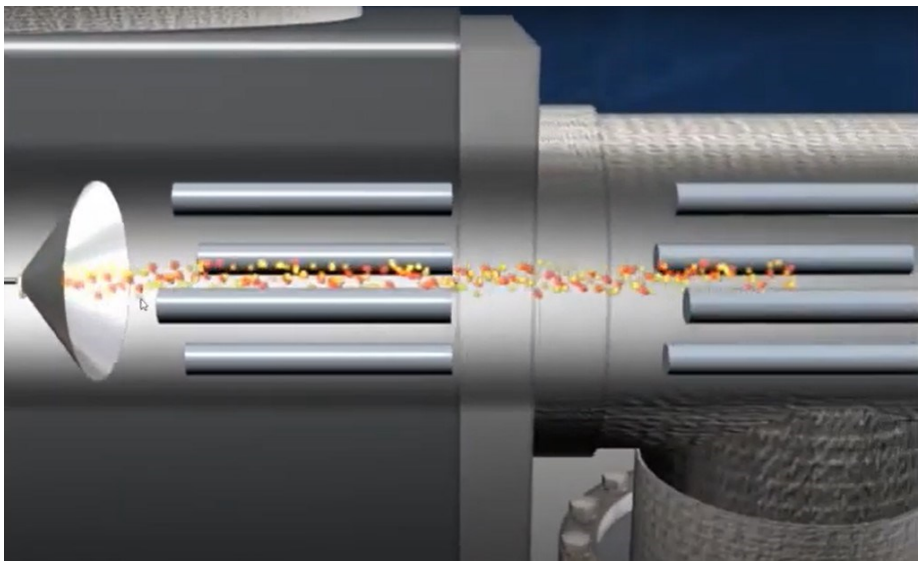
The trap in the ICR spectrometry works with a strong magnetic field. We trap the ions inside of the trap by an electric field. See the YouTube video for details.



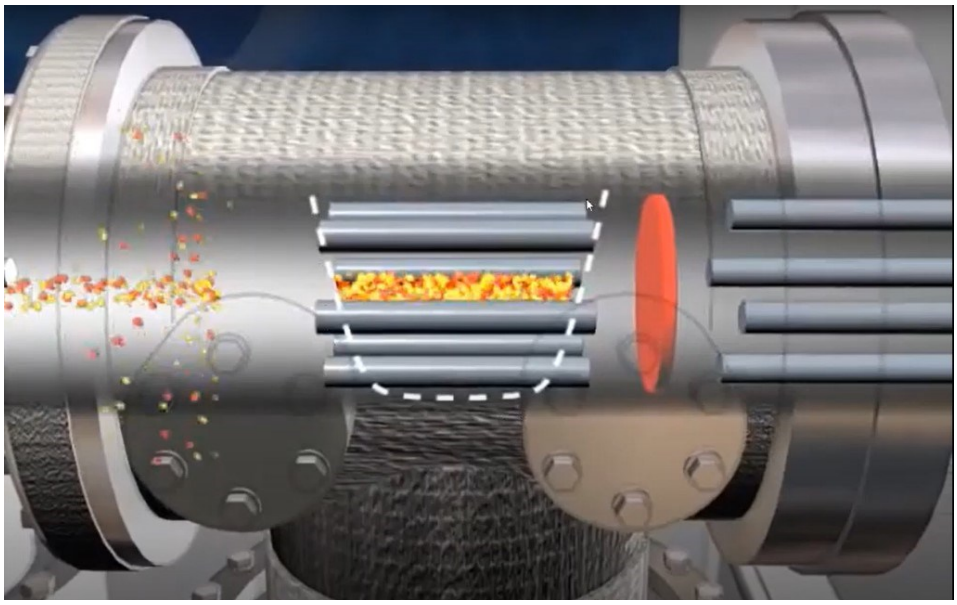
I will shortly comment on the YouTube vides: The video shows a typical ICR mass spectrometer. It is a relatively large instrument with a large magnet. The ions are generated by electrospray ionization. You have learned about the technique, so you know that we generate protonated or multiply protonated ions.



The ions are then transferred to the mass spectrometer using a capillary serves to reduce the pressure. Then they pass so called quadrupole guides. The quadrupole do not mass-select, they just serve for guiding ions inside this large instrument. We can achieve this function by working with RF field only (no DC potential on the rods).



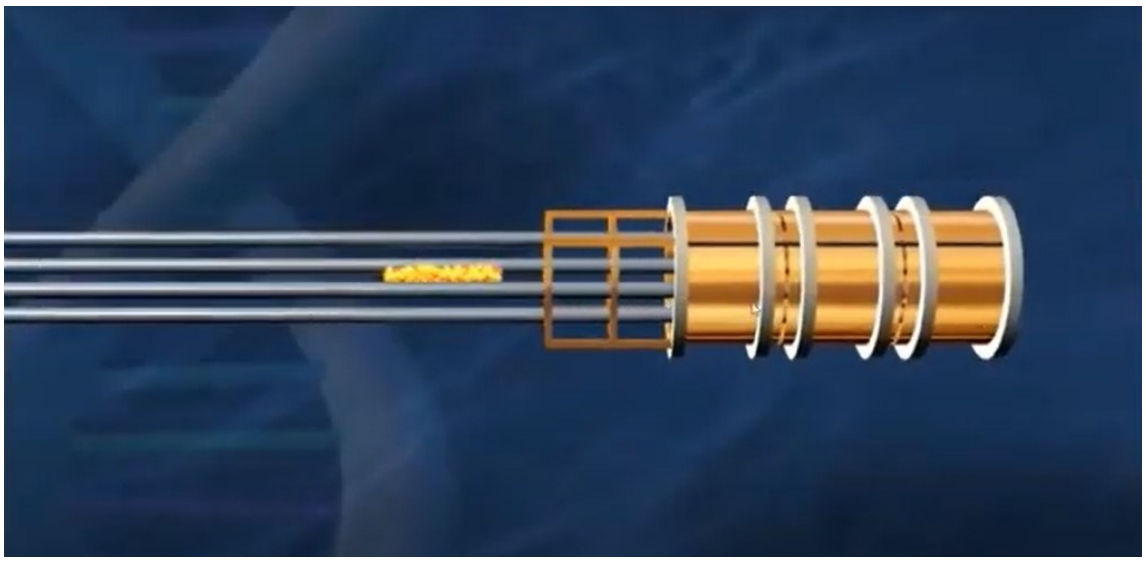
It is a function of quadrupoles that we often use for transferring ions from one side of a mass spectrometer to another. And these don't need to be just quadrupoles, they can be also octopoles or hexapoles.



Next, we transfer the ions to a linear ion trap. You can see that it's a higher multiple. The principles of all RF traps (quadrupole, hexapole, octopole, …) are similar as those that I explained for the Paul trap. The ions are trapped. The advantages of these linear traps is that at certain point we can reduce the electrostatic potential that keeps the ions inside and eject all ions at ones out of the trap.

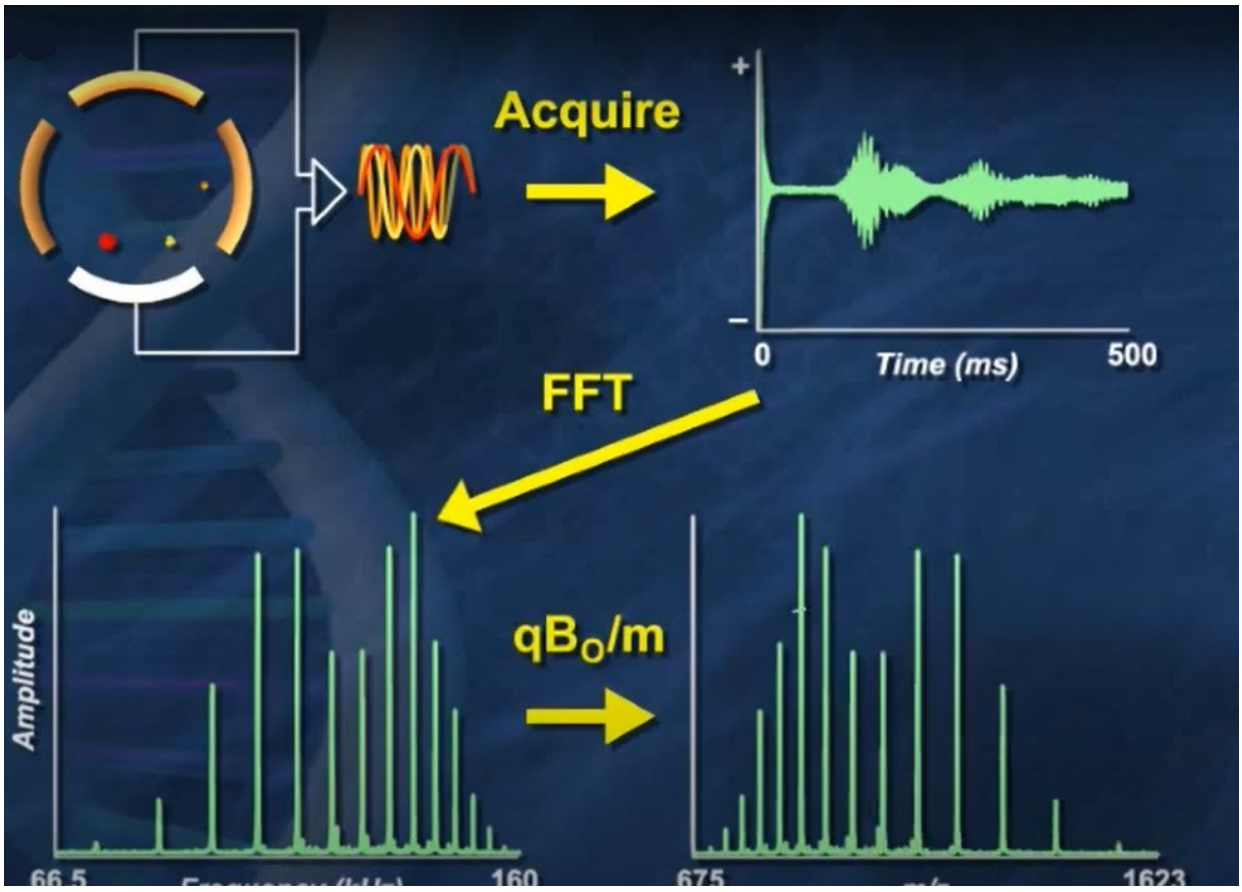
And why do we do it? As I explained you, the ICR is a trap, so we need to do a pulsed analysis. The trapping of the ions before the ICR analysis bundles the ions into bunches. Hence, although we produce the ions with electrospray ionization in a continuous way, we transform them into a pulsed bunches of ions.

As we eject the ions from the trap to the ICR, we start to mass-analyze the ejected bunch and simultaneously we also already prepare a new bunch by the trapping. This approach increases duty-cycle of the analysis

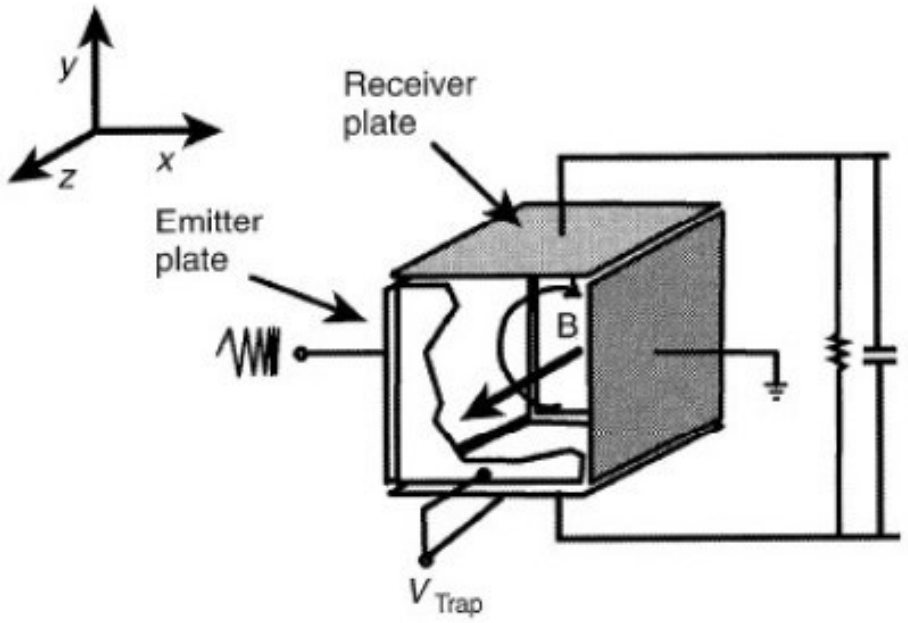


As the ions get into the ICR cell, we first trap them by the electrostatic potential and then we start to excite them by an emitter so that they start to cycle at a larger diameter orbit. As the ions circulate in the magnetic field, the frequency of the circulation depends on their mass to charge ratio.

Then we switch off the emitting field and let the ions freely circulate in the magnetic field. We “listen” to the ions by recording the RF current that they generate at the receiver plates. The ions circulate at their typical cyclotron resonance frequencies that are dependent on their mass to charge ratio.



The recording is in time domain, it is a complicated record that contains signals of all ions with all masses. We can transform it to the frequency domain by Fourier transformation. Finally, as the frequencies are dependent on the mass of the ions, we can transform the frequency spectrum to the final mass spectrum.



From Hoffmann

Figure 2.50
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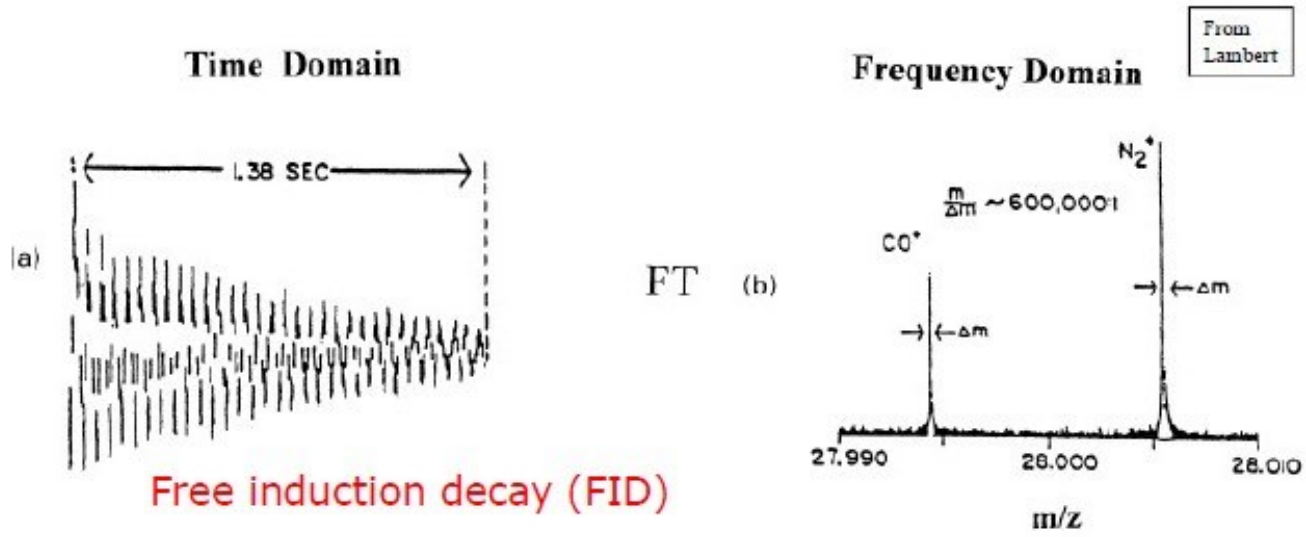
Summary: ion cyclotron resonance mass analyzer works with a strong magnetic field. Ions are trapped in the electric field. It's a pulsed analysis, so first we trap the ions. Then we excite the trajectories of the ions, so that they start to oscillate at a higher diameter of the trajectory. Then we switch off this exciting field and let the ions oscillate just in the magnetic field. As the ions oscillate at their cyclotron frequency they generate an RF field. We are listening to this RF field by measuring this RF field. And from that we can by Fourier transformation get information about the number of the ions in the trap and about their masses.

ICR cells can be used in a similar manner as quadrupole ion traps. So, you can also mass select the ions and you can do CID experiments with the ions.

This is an example of a spectrum recorded for a mixture of carbon monoxide radical cation and nitrogen radical cation.

FT-MS



Free induction decay (FID)

Figure 13-25 (a) Time domain signal (transient) recorded for a mixture of $CO^{+\cdot}$ and $N_2^{+\cdot}$ ions of nominal *m/z* 28 and (b) the corresponding frequency (mass) domain signal. (Courtesy of A.G. Marshall.)

You can see that we can distinguish ions that have nominally the same mass and the resolution here is 600 000. So, this should just demonstrate that the ICR cells are mass analyzers with very high resolution.

I will finish with this picture,

Instruments with highest resolution

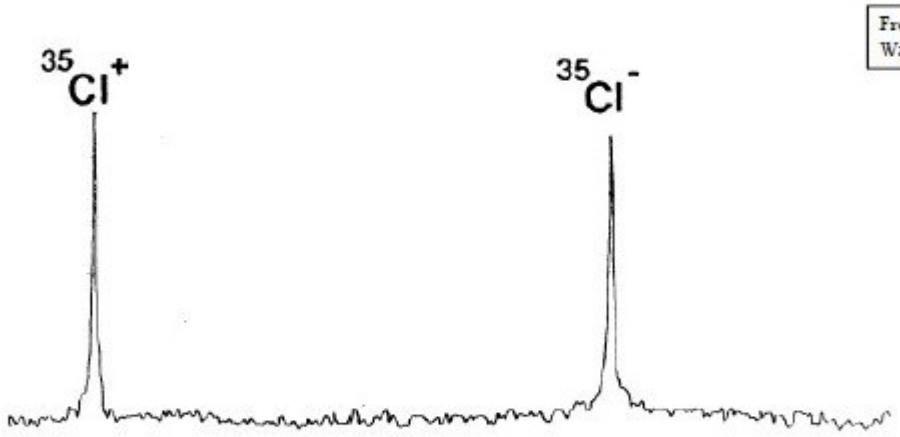
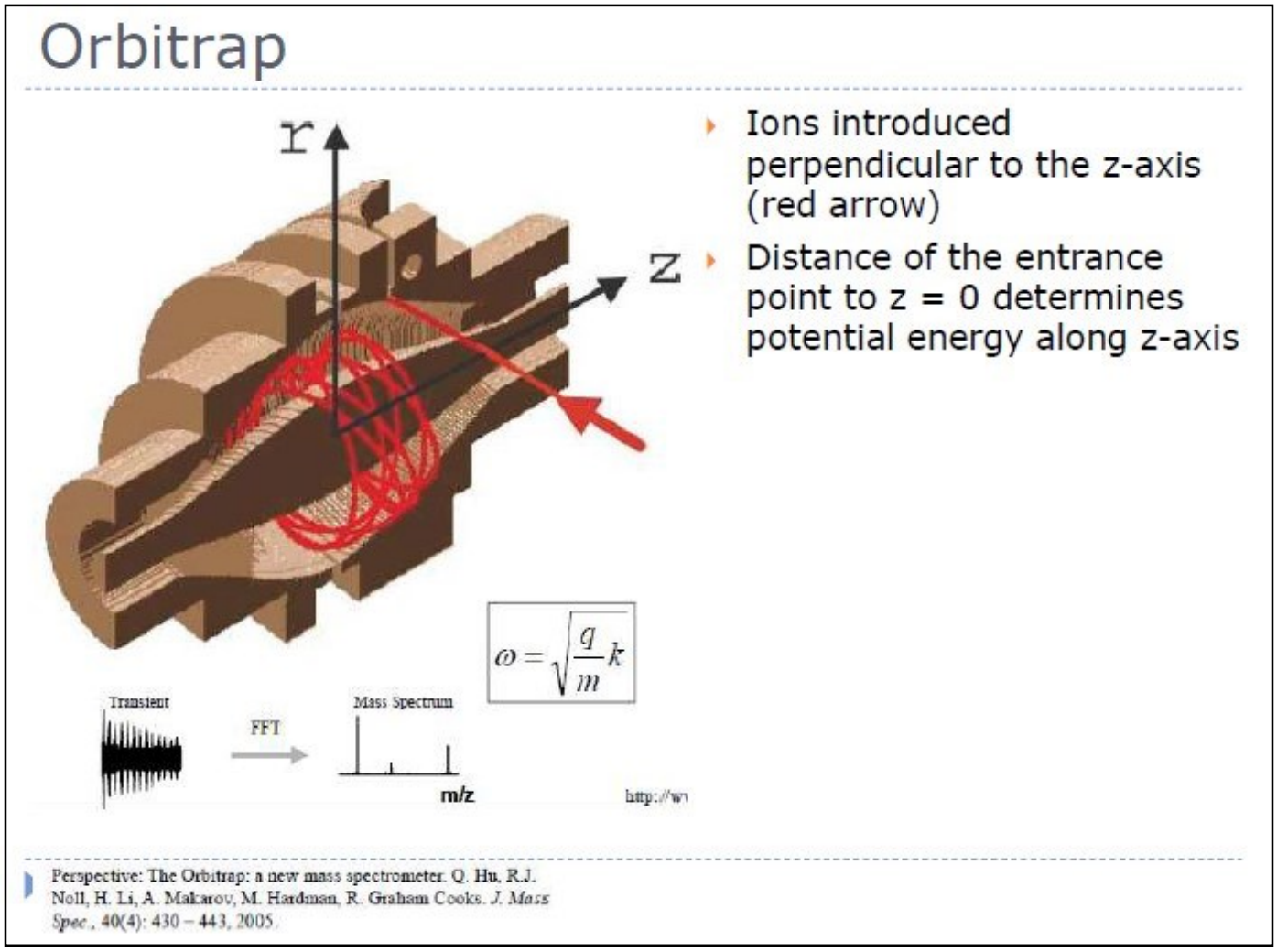


FIG. 4.22. Segment of mass spectrum in region of nominal mass 35 showing a resolution greater than 1,000,000 (FWHM definition) when using FT-MS. The peaks represent the positive and negative ions of ^{35}Cl that have a difference in mass equivalent to the mass of two electrons. The spectrum was obtained using a FT-ICR mass spectrometer with a superconducting magnet (4.7 tesla); the instrument was switched from the positive-ion-detection mode to the negative-ion-detection mode during the scan between the two peaks (Courtesy of Spectrospin AG.)

that shows analysis of a mixture of positively and negatively charged chlorine ions. It shows that we can separate ions that differ in mass of only two electrons. You should know that ICR mass spectrometers are now the mass spectrometers that achieve the highest resolution and that's why they are used for analysis of biomolecules.

Orbitrap



Orbitrap is a trap that works with the electrostatic potential. The electrodes have unusual shapes reminding a spindle. Thanks to this shape, the electrodes create a harmonic potential in which the ions behave like pendulums (see the figure). The ions are injected at one side and then here oscillate back and forth along the spindle. The frequency of the oscillation in the harmonic potential is dependent on the mass-to-charge ratio of the ions.

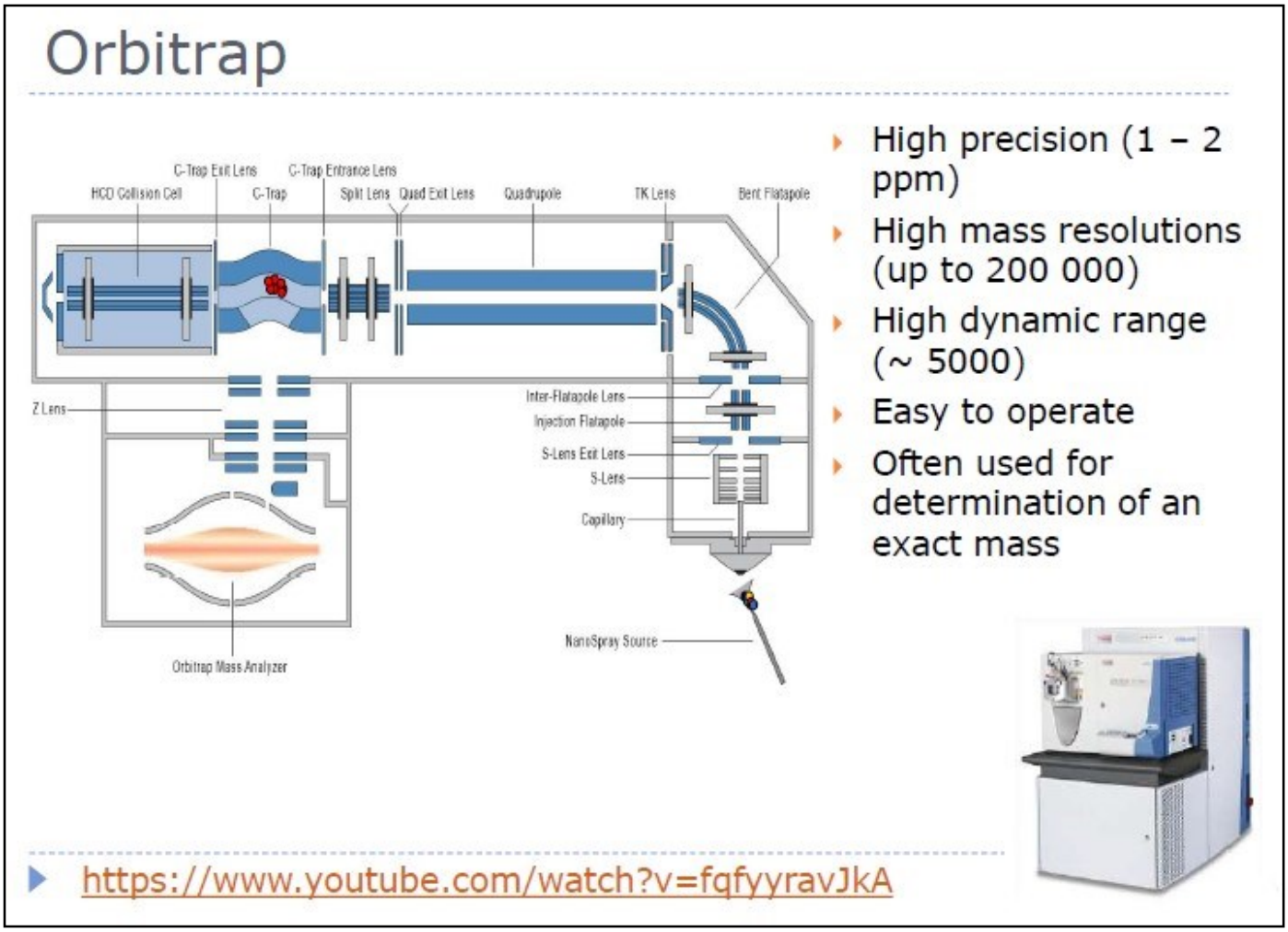
Similarly, as for the ICR signal, we record the RF current that the oscillating ions generate. The recording of the current in the time domain can be transformed by the Fourier transformation to the frequency domain and from there transformed to the final mass spectrum.

The YouTube video from Thermo shows the operation of one of the mass spectrometers with the orbitrap mass analyzer (see the video).

Short comments on the video: You can see that what we call “orbitrap” is usually an instrument combining various mass-analyzing elements and ion traps (similarly as you saw it earlier for an ICR instrument). The ions from the ion source focused by ion optics and guided by quadrupole guides (working in the RF regime only as explained above) towards the orbitrap analyzer. The ions can be also mass-selected by the quadrupoles (setting an appropriate combination of DC and RF potential to the quadrupole rods). You can also see some ion traps in the video. One ion trap (so called C-trap) serves to accumulate the ions before injecting them to the orbitrap analyzer. Another ion trap serves for collision induced dissociation of the mass-selected ions.

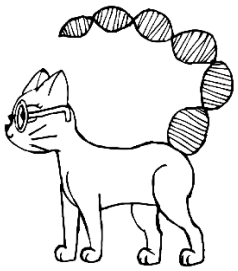
In the video, you see how the ions circulate there in this electrostatic field of the orbitrap analyzer and how the frequency of their side-to-side oscillation is dependent on their mass-to-charge ratio.

The video also shows analysis of mass-selected ions and their CID fragments. You can also see how the instrument performs several activities at the same time in order to keep a high duty cycle.



Orbitrap mass analyzer is a high resolution mass analyzer, it achieves resolution in the range of ~10^5. Using a sequential analysis, it achieve a high dynamic range. The dynamic range in mass spectrometry means that you can detect ions that have low abundance next to the ions that have higher abundance.

Another advantage of Orbitraps is that they are easy to operate. Therefore, they became a standard equipment of analytical laboratories or analytical mass spectrometry laboratories and they are often used for determination of the exact mass. Hence nowadays, Orbitraps are common high resolution mass spectrometers. They are also used in biochemistry but one should know that the resolution is so far still lower than that of the ICR mass analyzers.



Comparison of mass analyzers

Comparison of mass analyzers

Mass analyzer	Mass range Resolution	Advantages	Disadvantages
Quadrupole	Range m/z ~ 3000 R ~ 2000	Well suited for ESI Small, cheap	Small mass range, poor adaptability to MALDI
Ion traps	Range m/z ~ 2000 R ~ 1500	Small, cheap CID: up to MS^{12}	Small mass range
Magnetic sector	Range m/z ~20 000 R ~ 10 000	High resolution High dynamic range Reliable	Not tolerant to high pressures Expensive Slow scanning
TOF	Range m/z ∞ R ~ 10^3 - 10^4	Good resolution Very fast scanning Cheap	Suboptimal adaptability to ESI
Orbitrap	Range m/z 6 000 R ~ 10^5	High resolution Easy to operate (compared to ICR)	Requires high vacuum Expensive
ICR	Range m/z 10 000 R ~ 10^6	High resolution CID: up to MS^4	Superconducting magnet → expensive, massive Requires high vacuum

Summary

Low resolution quadrupole and ion traps: Quadrupoles, they have a resolution of about 2000; we work with a unit resolution and they can reach a mass range up to the mass-to-charge ratio 3000 – 4000. The advantage is that they are well suited for electrospray ionization, they are small and cheap. The disadvantage is that they have a relatively small mass range and they are poorly adaptable to MALDI.

Ion traps – a similar mass range as quadrupoles. They have slightly lower resolution than quadrupoles. They are also small and cheap and the big advantage is that you can make up to MS^{12} experiments. So, you can do mass selections and collision induced dissociations with mass selected ions. The disadvantage is a relatively small mass range.

Sector instruments: Magnetic sectors can operate to a very large mass-to-charge ratio range. In the combination with the electrostatic sector, the instruments achieve a high resolution. They have the highest dynamic range from all mass spectrometers that we were talking about. Hence, they are the best for the detection of tiny signals next to large ion currents. They are also very reliable. The disadvantage of the sector instruments is the requirement of a high vacuum (very low pressures). They are very expensive and they are relatively slow in scanning. Usually, they are used only for special applications.

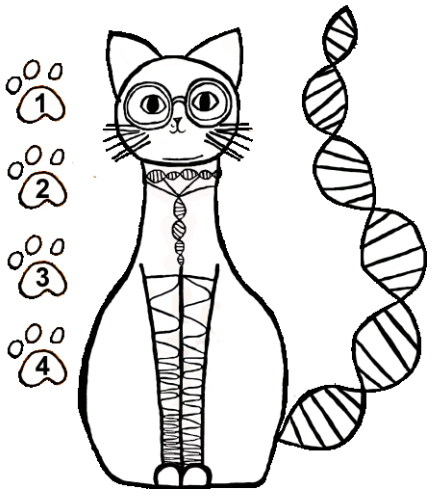
Time-of-flight mass analyzers: The big advantage is that they have basically an indefinite mass range that they can study. They achieve a relatively large resolution with the reflectron. They are very fast for scanning and they are cheap. The disadvantage is that they are pulsed. However, they can be nowadays connected to the continuous sources (such as ESI) using either the orthogonal extraction or the trapping techniques that increase the duty cycle of the instrument.

High resolution mass spectrometers: The orbitrap analyzer works up to the mass-to-charge ratio of 6000 or 8000. It achieves resolution up to 10^5 or higher nowadays. The instruments are easy to operate, therefore they became the most frequent high-resolution analyzers nowadays. The disadvantage is that they are quite expensive and they require a high vacuum for the orbitrap analysis. The ICR instruments are even more expensive than the orbitrap instruments. They work up to the mass range of ~10.000. They achieve the highest resolutions up to 10^6. The ICR cell is also suitable for MS/MS experiments, so you can mass-select ions within the ICR cell and you can make a collision-induced dissociation for studying of the fragmentation patterns. The disadvantage is that the ICR instruments are very expensive, because they need superconductive magnets and they require the best vacuum out of all the mass spectrometers on the market.

Both types of the instruments (orbitrap-based and ICR-based) usually combine several mass-analyzers from the list above. The instruments frequently contain a quadrupole for mass-selection, and ion trap for CID experiments and an ion trap for the ion ejection to the high-resolution analyzer (orbitrap or ICR cell).

Summary

- High resolution mass analyzers
  - Time of flight (reflectron)
  - Penning trap (ion cyclotron resonance)
    - high magnetic field, ions circulate in the magnetic field at their cyclotron frequency → AC current (frequency and amplitude → m/z and intensity)
    - proteomics
  - Orbitrap
    - ions oscilate in a harmonic electrostatic field → AC current (frequency and amplitude → m/z and intensity)
    - biochemical applications
    - chemistry – exact mass analysis

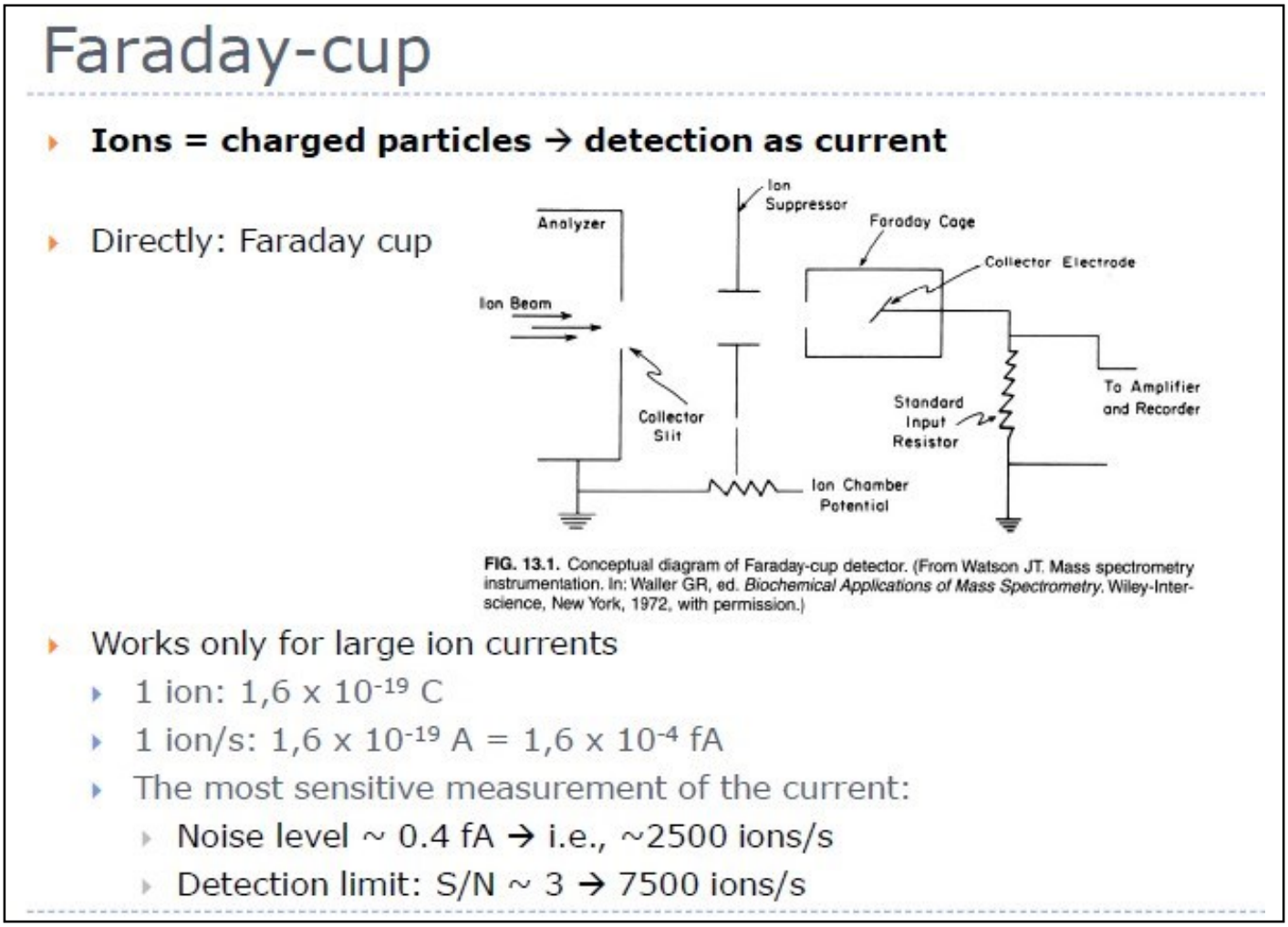


Lecture 5. Detectors

The last video is devoted to detectors. I have explained you a series of mass analyzers that allow us to mass analyze the ions. After the mass-analysis, the ions have to be detected. How do we do it?

Faraday-cup

Ions are charged particles. Hence, in principle we can detect them as a current. We do it by using so called Faraday cup.



The principle of the detection with a Faraday cup is that an ion beam is accelerated towards a collector electrode where the ions get neutralized. The neutralization proceeds with electrons from the metal of the electrode which generates a current that we can measure.

However, this detector works only for relatively large ion currents. One ion with a charge of 1 electron corresponds to $1.6*10^{-19}$ C which means that it generates a current 1 ion/second. This value converted to the current is 10^{-4} fA. Our best measurements of the current have a noise level of about 0.4 fA. This noise corresponds to a current of about 2 500 ions/second. In order to have a meaningful detection, you need to have at least three times larger signal than the noise level. This means that we can use a Faraday cup for the currents that are larger than 7500 ions/second.

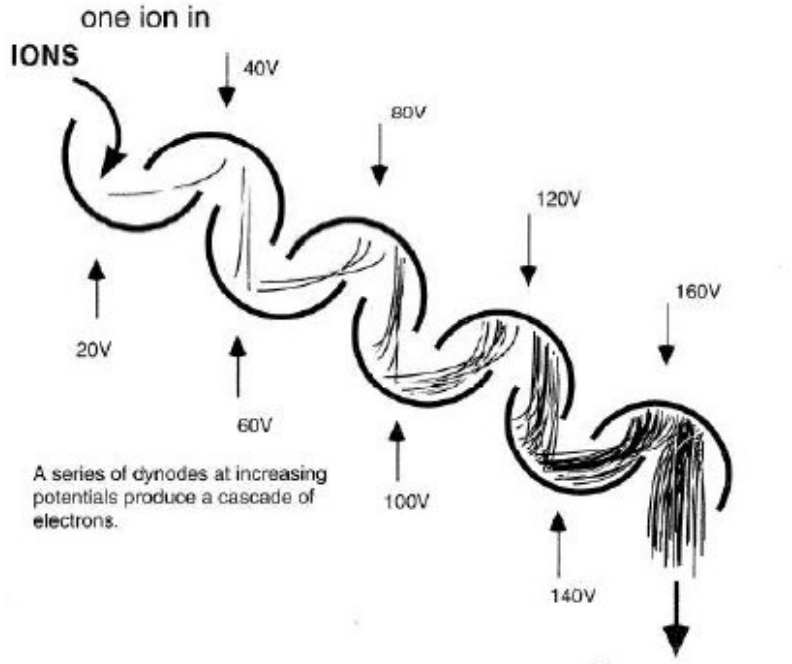
Electron multiplier

In mass spectrometry we have often small currents. Therefore, the Faraday-cup is usually not used and we have to use more sensitive methods of the detection. They are based on so-called electron multipliers.

Electron multiplier

- Ion collides with the first dynode → emission of electrons
- Electrons are attracted by the second dynode → each electron knocks out further electrons
- And so on

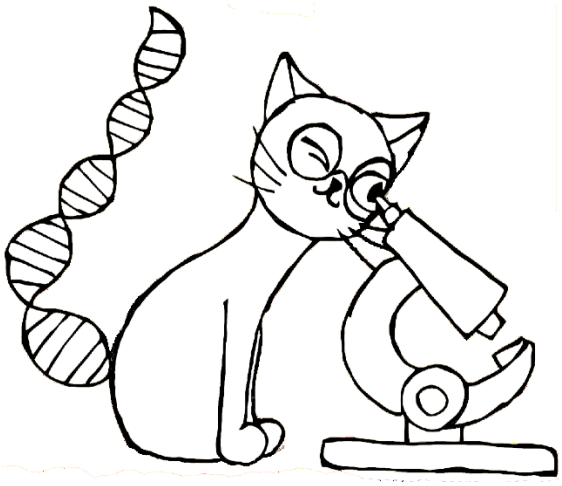
- Multiplication by ~ 10^6
- High sensitivity
- Low noise level
- Life-span 1-2 years



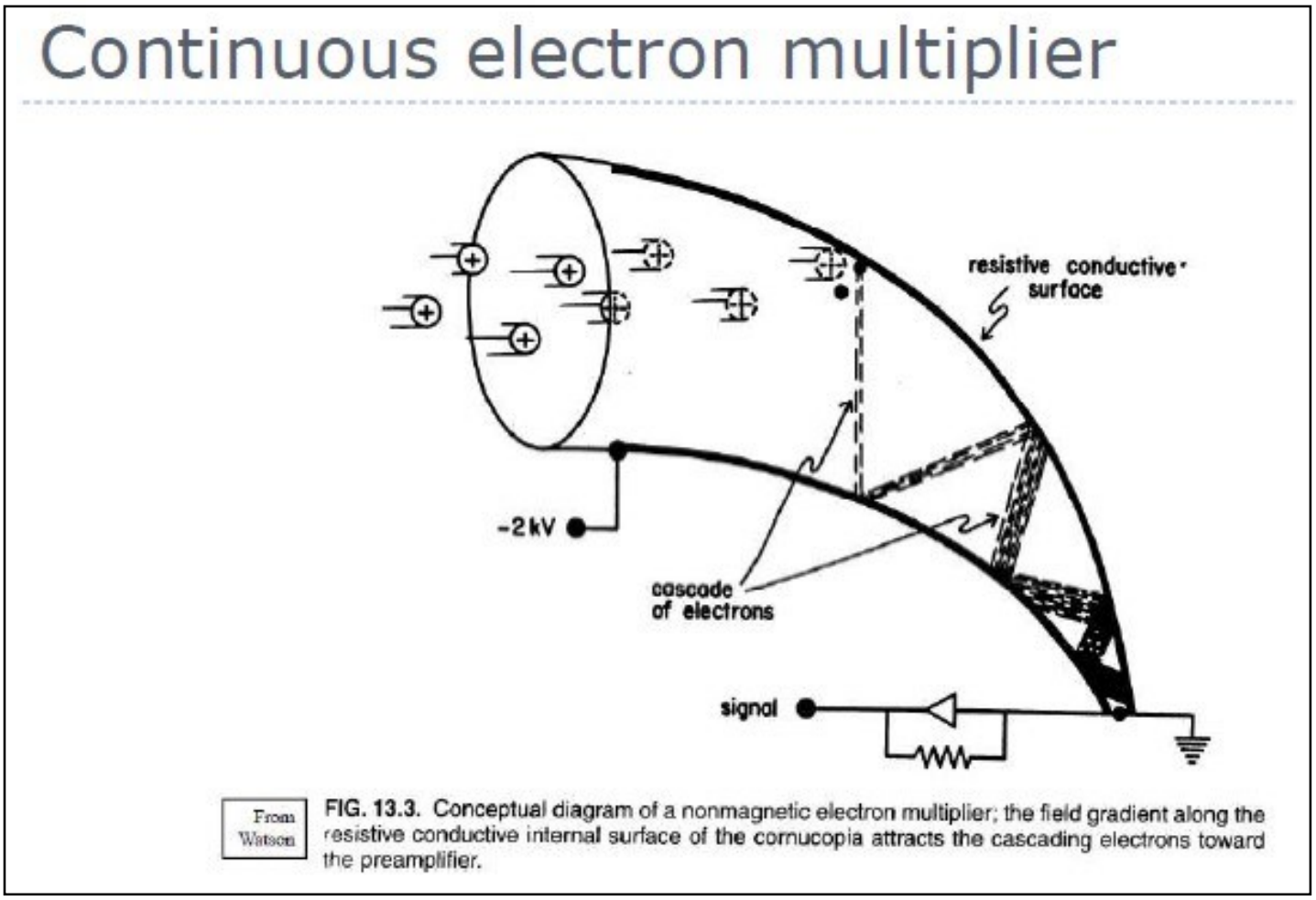
A series of dynodes at increasing potentials produce a cascade of electrons.

http://www.vias.org/simulations/simusoft_emultiplier.html

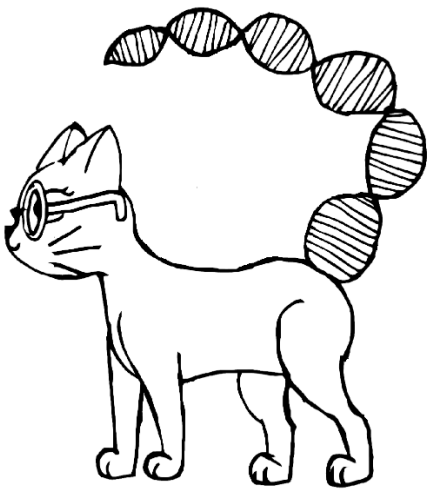
Electron multiplier is a set of dynodes. The ions are accelerated towards the first dynode, where they collide with the dynode and knock out an electron. Hence, the ions get converted to the electrons at the first dynode. The electrons are accelerated to the second dynode where they collide and knock out more electrons. This goes as a cascade in which each colliding electron knocks out more of the electrons at each dynode. Hence, we have an exponential growth of the number of electrons and in the end one ion is multiplied to about 10^6 of the electrons. This results in a tremendous increase of the sensitivity of the ion detection. In addition, electron multipliers have a low noise level. The disadvantage is that it has a life-span of only two years or so.



Continuous electron multiplier



Alternatively, we can have a continuous geometry of the multiplier. Hence, instead of a set of the discrete dynodes, we can have one continuous dynode with a cornucopia shape. The ions are coming into this dynode, ejecting electrons and electrons are multiplied up to the 10^6 value of electrons in the analogous manner as it was described above.



Conversion dynode

Another improvement of electron multipliers is a combination with a conversion dynode.

Conversion dynode

From Sindzak

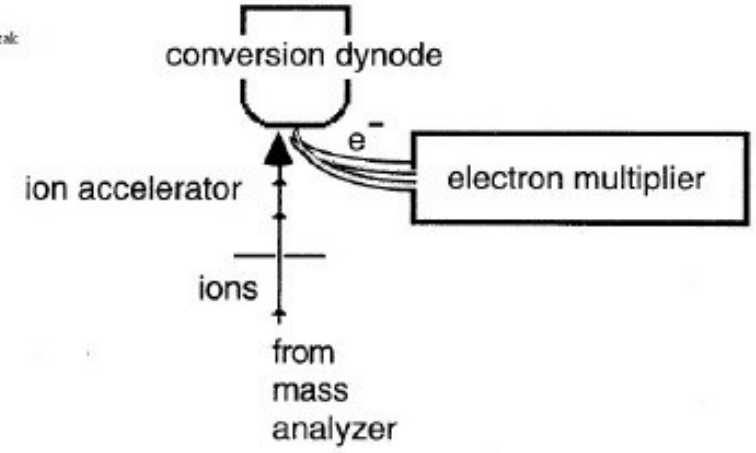


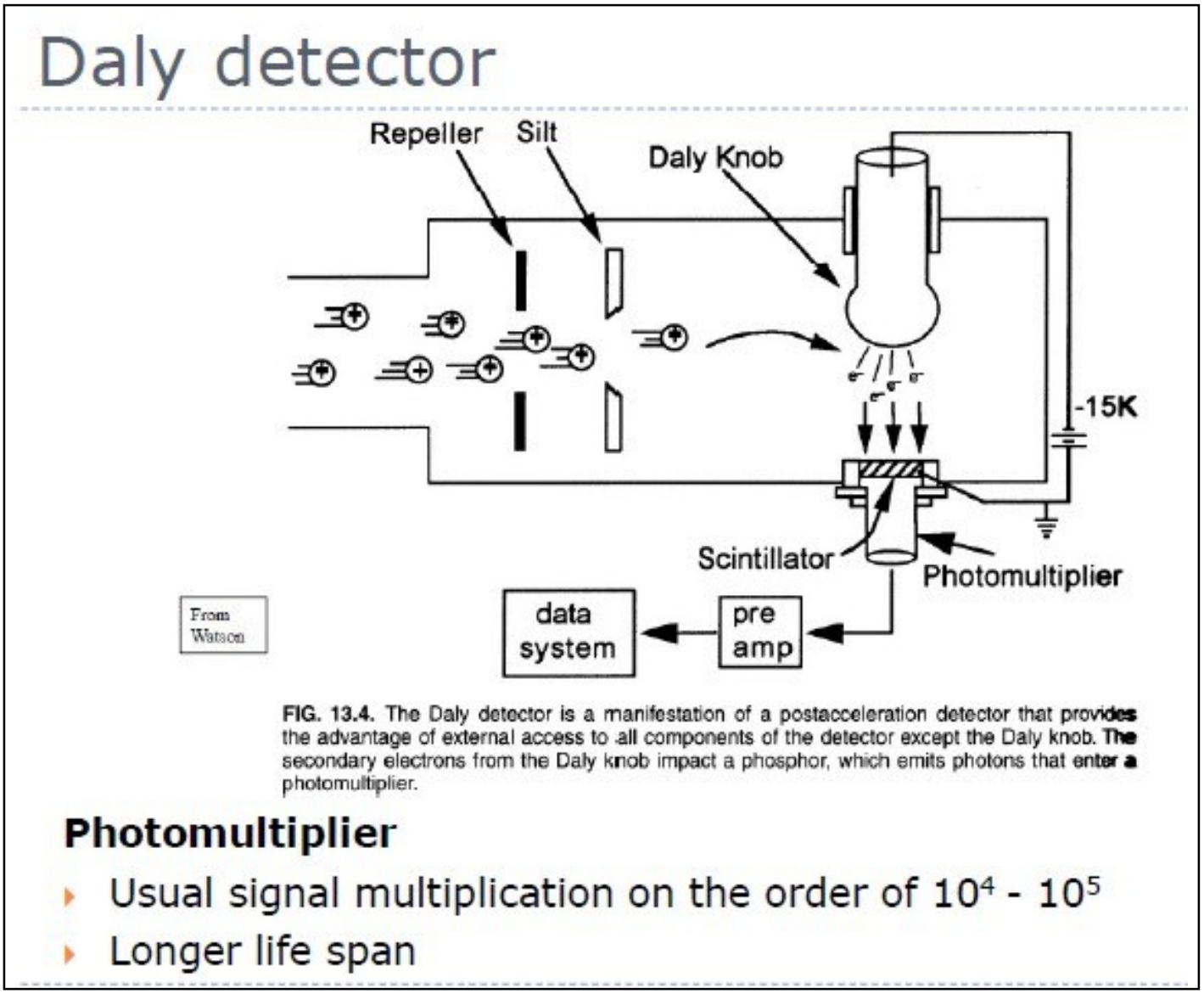
Figure 2.21 High-energy dynode detector.

- Ions are first converted to electrons and only the electrons enter the electron multiplier
- Suitable for larger masses, because with higher m/z decreases the response of the classical detectors

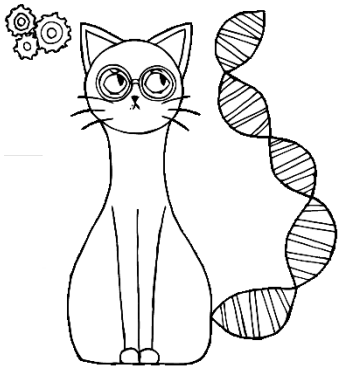
The ions from a mass analyzer are first accelerated towards a knob, which we call a conversion dynode. At this knob, the ions are converted to electrons. The electrons are then accelerated to a classical electron multiplier. Hence, the difference is that the ions do not go directly to the multiplier but first to the conversion dynode and only electrons go to multiplier. The advantage of this arrangement is that it's suitable also for negative ions and for ions with larger masses. Therefore, this is the most often used arrangement nowadays in mass spectrometers.

Daly detector

The last detector I will mention is a Daly detector.



The Daly detector contains a conversion dynode, called a Daly Knob, where we convert the ions to electrons. The electrons are accelerated towards a photomultiplier. First, they go to a scintillator where they are converted to photons. Photon are then multiplied in a photomultiplier and detected. The photomultiplier works for photons in an analogous manner as electromultiplier for electrons. The advantage of photomultipliers is that they have much longer life-span. The multiplication is slightly lower than for electromultipliers, but it is still on the order of 10^4 to 10^5.



Summary

- Ions are detected as a current → **Faraday cup**
- Usually, the number of ions is insufficient for direct detection → multipliers
  - **Electron multiplier** – discrete dynodes
  - Continuous dynode
- **Conversion dynode** – ions are converted to electrons before entering a multiplier
- **Daly detector** – ions are converted to electrons, electrons are converted to photons, photons are detected in a **photomultiplier**

Summary: Ions are detected as a current. We can do it directly by a Faraday cup, but for that we need a large amount of the ions to be detected. In order to increase the sensitivity and to measure currents of low abundant ions, we use an indirect detection of the ions by their conversion to electrons and the subsequent electron multiplication. We achieve the electron multiplication in electron multipliers that can be either composed of discrete dynodes or it can be one continuous dynode. This allows us to increase the currents by six orders of magnitude.

For larger masses and for detection of anions, we need a conversion dynode that is separate from an electron multiplier. Using a conversion dynode, we first convert ions to electrons and only afterwards electrons enter the electron multiplier.

Finally, we can also use photomultipliers. We have to first convert ions to electrons, the electrons are then converted to photons and only afterwards are the photons multiplied and detected in a photon multiplier.