What is mass spectrometry?
1) The mass to charge ratio (m/z) is used to describe ions observed in mass spectrometry.
2) m is the numerical value for the mass of the ion and z is the numerical value for the charge of the ion. The unified atomic mass (u) and the elementary charge units (e) are used for these values.
3) The unified atomic mass is defined as 1/12 the mass of an atom of $^{12}$C. Older terms still in use but not accepted as SI units include the atomic mass unit (amu) and the dalton (Da). The amu is no longer acceptable due to conflicting definitions. The dalton is frequently used for polymers, peptides and other large molecules.
4) Because the unified atomic mass and the charge number are pure numbers the mass-to-charge ratio is a number and does not have any units.

Definition of Dalton:
Measure of molecular weight or mass. One hydrogen atom has mass of 1 Da. The dalton is one twelfth of the mass of the nuclide $^{12}$C.

$1 \text{u} = \frac{1}{N_A} \text{g}$ (where $N_A$ is Avogadro's number)

$1 \text{u} \approx 1.66053886 \times 10^{-27} \text{kg}$

What is mass spectrometer?
Vacuum environment is required for ionization and mass separation. Hence, sample introduction is to convert a sample into gas-phase molecules without loss of vacuum.

Batch inlet (reservoir) for sample introduction
1) Gas phase analyte is introduced directly into the source region of the mass spectrometer through a needle valve.
2) Pump out lines are usually included to remove air from the sample.
3) This inlet works well for gases, liquids, or solids with a high vapor pressure.
4) Samples with low vapor pressure are heated to increase the vapor pressure.
5) Since this inlet is limited to volatile compounds and modest temperatures, it only works for some samples.

Characteristics of reservoir:
- Used for volatile liquids or solids
- Advantage: Constant signal for a while
- Disadvantage: require large amount of sample
Mass spectrometer-ionization method

Ionization method:
Mass spectrometers measure the mass-to-charge (m/z) ratios of gas phase ions. Creating gas phase ions is the role of the ionization method.

- Electron Impact (EI)
- Chemical Ionization (CI)
- Spray ionization (SI) such as Electrospray
- Desorption ionization (DI)
  -- Fast Atom Bombardment (FAB)
  -- Matrix Assisted Laser Desorption (MALDI)
  -- secondary ion mass spectrometry (SIMS)

Ionization accompanied by different degree of excitation of the molecular ion:
- Soft ionization: transfer little excess energy to the ionized molecular, which is observed intact.
- Hard ionization: give rise to the fragment ions frequently seen in the mass spectra

Mass spectrometer- Electron ionization (EI)

The method of electron ejection for positive ion formation proceeds:
- The sample is thermally vaporized.
- Electrons ejected from a heated filament are accelerated through an electric field at 70 V to form a continuous electron beam.
- The sample molecule is passed through the electron beam.
- The electrons, containing 70 V of kinetic energy, transfer some of their kinetic energy to the molecule. This transfer results in ionization (electron ejection) with the ion internally retaining no more than 6 eV excess energy.

\[ M + e^- (70 \text{ eV}) \rightarrow M^+ (~5 \text{ eV}) + 2e^- (~65 \text{ eV}) \]

- Excess internal energy (6 eV) in the molecule leads to fragmentation:

\[ M^+ \rightarrow \text{molecular ions + fragment ions + neutral fragments} \]

Mass spectrometer-Chemical ionization (CI)

Chemical Ionization (CI) is applied to samples similar to those analyzed by EI and is primarily used to enhance the abundance of the molecular ion. Chemical ionization uses gas phase ion-molecule reactions within the vacuum of the mass spectrometer to produce ions from the sample.

A possible mechanism for ionization in CI occurs:

\[ \text{Reagent (R)} + e^- \rightarrow R^+ + 2e^- \]
\[ R^+ + RH \rightarrow RH^+ + R \]
\[ RH^+ + \text{Analyte (A)} \rightarrow AH^+ + R \]

For example (Ar+, CH5+ as ionization agents):

Mass spectrometer-Chemical ionization (CI)

Example of chemical ionization: Methane as Reagent Species

The reagent ions are produced by introducing a large excess of methane into an electron impact (EI) ion source. Electron collisions produce CH4+ and CH3+ which further react with methane to form CH5+ and C2H5+:

\[ \text{CH}_2^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{H}_2 \]
\[ \text{CH}_3^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2 \]

- good for most organic compounds
- usually produces [M+H]+, [M+CH3]+ adducts
- M + CH4+ \rightarrow CH5+ + M + H+ (protonation)
- M + CH3+ \rightarrow [M + CH3]+ (adduct formation)
- adducts are not always abundant
- extensive fragmentation

Ionization effect on spectra

Effects of ionization method and the reagent

Butyl methacrylate

\[ \text{Cl, R= Methane (PA=5.7 eV)} \]
\[ \text{Cl, R= Isobutane (PA=8.5 eV)} \]

The EI (top), methane CI (middle) and isobutane CI (bottom) mass spectra of butyl methacrylate. The ionization techniques (EI vs. CI) and the reagent gases (methane vs. isobutane) influence the amount of fragmentation and the prominence of the protonated molecular ions detected at 143 Th.
MALDI-MS was first introduced in 1988 by Tanaka, Karas, and Hillenkamp. MALDI is a widespread analytical tool for peptides, proteins, and most other biomolecules (oligonucleotides, carbohydrates, natural products, and lipids). The efficient and directed energy transfer during a matrix-assisted laser-induced desorption event provides high ion yields of the intact analyte, and allows for the measurement of compounds with sub-picomole sensitivity.

MALDI is designed to enhance mass spectra by solving two main problems:
– Thermal instability and low volatility
– Large and heavy biomolecules

Characteristics of MALDI:
• Analyte molecules are embedded in a crystalline matrix composed of a low molecular weight organic species.
• Dried mixture is struck with a short, intense laser pulse that is strongly absorbed by the matrix (often UV or IR).
• Rapid heating of matrix causes sublimation and expansion into gas phase. Intact analyte molecules carried with little internal energy.
• Most widely accepted ionization mechanism is gas phase proton transfer.

Role of MALDi matrix:
1) Isolation of analyte molecule to prevent aggregation
2) Absorption of energy from the laser
3) Energy transfer to allow des-integration without destruction of the molecule (soft desorption/soft ionization)
4) Stimulation of analyte ionization

Commonly used MALDI matrices:
- aspartic acid (SA)
- cysteic acid (CA)
- sinapic acid (SA)
- 2,5-dihydroxybenzoic acid (DHB)
- pentafluoropropionic acid (PFP)
- o-cyano-4-hydroxy-cinnamic acid (CICA)

Spectra contain mostly single-charged ions.
• Fragmentation due to excess energy imparted on analyte during ionization process is possible.

UV MALDI mass spectrum of bovine serum albumin (MW=66,429 Da)

<table>
<thead>
<tr>
<th>Ionization method</th>
<th>Typical Analytes</th>
<th>Sample Introduction</th>
<th>Mass Range</th>
<th>Method Highlights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Impact (EI)</td>
<td>Relatively small volatile</td>
<td>GC or liquid/solid probe</td>
<td>&lt;600 Daltons</td>
<td>Hard method versatile provides structure info</td>
</tr>
<tr>
<td>Chemical Ionization (CI)</td>
<td>Relatively small volatile</td>
<td>GC or liquid/solid probe</td>
<td>&lt;800 Daltons</td>
<td>Soft method molecular ion peak (M+H)+</td>
</tr>
<tr>
<td>Electrospray (ESI)</td>
<td>Peptides Proteins non-volatile</td>
<td>Liquid Chromatography or syringe</td>
<td>&lt;200,000 Daltons</td>
<td>Soft method ions often multiple charged</td>
</tr>
<tr>
<td>Fast Atom Bombardment (FAB)</td>
<td>Carbohydrates Organometallics Peptides nonvolatile</td>
<td>Sample mixed in vacuo matrix</td>
<td>&lt;6,000 Daltons</td>
<td>Soft method but harder than ESI or MALDI</td>
</tr>
<tr>
<td>Matrix assisted Laser Desorption (MALDI)</td>
<td>Peptides Proteins Nucleotides</td>
<td>Sample mixed in solid matrix</td>
<td>&lt;500,000 Daltons</td>
<td>Soft method very high mass</td>
</tr>
</tbody>
</table>
Mass Analyzer

The mass analyzer separates these ions according to their m/z value.

The selection of a mass analyzer depends upon the mass resolution, mass range, scan rate, and detection limits required for an application.

Mass Analyzer - Magnetic Sector

- The earliest mass analyzers separated ions with a magnetic field.
- In magnetic analysis, the ions are accelerated into a magnetic field using an electric field.
- A charged particle traveling through a magnetic field will travel in a circular motion with a radius that depends on the speed of the ion, the magnetic field strength, and the ion’s m/z.
- A mass spectrum is obtained by scanning the magnetic field and monitoring ions as they strike a fixed point detector.

Mass Analyzer - Time-of-flight (ToF) analyzer

The ions are then electrostatically accelerated into a field-free drift region with a nominal kinetic energy of:

\[ E_k = eV_0 = \frac{1}{2}mv^2 \]

Where \( V_0 \) is the accelerating voltage, \( m \) the mass of ion, \( v \) the flight velocity of ion, \( e \) its charge. It is obviously seen from the above formula that the ion with lower mass has higher flight velocity than one with higher mass. Thus they will reach the ion detector earlier. As a result, the mass separation is obtained in the flight time \( t \) from the sample to the detector. The flight time \( t \) is expressed by:

\[ t = \frac{L}{2V_0\sqrt{2/m}} \]

Where \( L \) is the effective length of the mass spectrometer. A variety of mass ions are recorded by the detector with the time sequence to give the mass spectrum.

Mass spectrometer - mass analysis

- Magnetic Sector or Electrostatic Sector
- Time-of-Flight (TOF)
- Quadrupole
- Ion trap
- Ion Cyclotron Resonance (ICR)

TOF Mass analyzer

Advantages of reflection TOF mass analyzer:

- High Resolution (> 20,000 in some models)
- High Accuracy (< 5 ppm)
- Highest practical mass range of all MS analyzers (>10,000 Mass Range)
- Fastest MS analyzer
- Suitable for pulsed ionization methods such as MALDI or some models of SIMS

Limitations:

- Requires pulsed ionization method or ion beam switching
Mass analyzer- Quadrupole mass filter
• Quadrupole consists of four parallel rods; Precise dimensions and spacing;
  Rods connected diagonally in pairs.
• Quadrupole mass analyzers are connected in parallel to a radio frequency
  (RF) generator and a DC potential.
• At a specific RF field, only ions of a specific m/z can pass through the
  quadrupoles as shown in Figure, where only the ion of m/z 100 is detected.
• In all three cases in Figure the DC and RF fields are the same. Therefore by
  scanning the RF field can be achieved in approximately one second (m/z
  20~800).

Mass analyzer- Quadrupole ion trap
RF fields induce oscillations in r and z directions.
A “trapped” ion is stable along both axes.

Mass analyzer- Ion Cyclotron Resonance
Ion Cyclotron Resonance: Fourier Transform MS
• Ions with motion normal to a magnetic field have circular
  trajectories with a characteristic angular frequency, ω.
• For a strong magnetic field, this is a radio frequency. e.g., B = 4.7 Tesla,
  72 MHz for H+.
• At room temperature the orbits are about 1mm in diameter.
• Ions absorb energy from a resonant RF electric field and move in phase
  with it to higher radii.
Ions moves in circular orbits with a cyclotron angular frequency, which is
expressed by:
ω = \frac{Bz}{m} = 2\pi f
m / z = B / 2\pi f

Definition of Mass Resolution
Mass resolution represents the ability to separate two adjacent masses. It
measures the “sharpness” of the MS peak.

<table>
<thead>
<tr>
<th>Quadrupole</th>
<th>Ion Trap</th>
<th>Time-of-Flight</th>
<th>Time-of-Flight</th>
<th>Magnetic Sector</th>
<th>Ions Cyclotron FTMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>100 ppm</td>
<td>100 ppm</td>
<td>5 ppm</td>
<td>5 ppm</td>
<td>5 ppm</td>
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<tr>
<td>Resolution</td>
<td>4,000</td>
<td>4,000</td>
<td>1,000</td>
<td>10,000</td>
<td>100,000</td>
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<tr>
<td>m/z Range</td>
<td>4,000</td>
<td>-500,000</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
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<tr>
<td>Scan Speed</td>
<td>-a second</td>
<td>-a second</td>
<td>a second</td>
<td>a second</td>
<td>a second</td>
</tr>
<tr>
<td>General Comments</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Good accuracy</td>
<td>Instrument is massive</td>
</tr>
<tr>
<td></td>
<td>Ease of</td>
<td>Ease of</td>
<td>High</td>
<td>Good resolution</td>
<td>High resolution</td>
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<td></td>
<td>switching</td>
<td>switching</td>
<td>through-put</td>
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<td>MS high</td>
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<td></td>
<td>ion tagging</td>
<td>ion tagging</td>
<td>ion tagging</td>
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<td>vacuum,</td>
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<td></td>
<td>Small size</td>
<td>Small size</td>
<td>Small size</td>
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<td>super</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>expensive</td>
</tr>
</tbody>
</table>

Summary of mass analyzer
A general comparison of mass analyzers typically used for electrospray.
Interpretation of mass spectra

General information which will aid mass spectra interpretation:

Molecular ion (M+): If the molecular ion appears, it will be the highest (or second highest) mass in a spectrum. This peak will represent the molecular weight of the compound. Its appearance depends on the stability of the compound. Double bonds, cyclic structures and aromatic rings stabilize the molecular ion and increase the probability of its appearance.

Reference Spectra: Mass spectral patterns are reproducible. The mass spectra of many compounds have been published. Instrument computers generally contain spectral libraries which can be searched for matches.

Isotopes: Isotopes occur in compounds analyzed by mass spectrometry in the same abundances that they occur in nature. This approach is very useful for identifying metal associated ion cluster.

Fragmentation: General rules of fragmentation exist and are helpful to predict or interpret the fragmentation pattern produced by a compound. Functional groups and overall structure determine how some portions of molecules will resist fragmenting, while other portions will fragment easily. A detailed discussion of those rules is beyond the scope of this introduction, and further information may be found in your organic textbook or in mass spectrometry reference books.

Interpretation of MS- molecular ion

Molecular ion (M-). If the molecular ion appears, typically it will be the highest mass in a spectrum if no additional chemical reagent or matrix is involved into the ionization. This peak will represent the molecular weight of the compound.

A mass spectrum of methanol (CH₃OH) is shown below. CH₃OH⁻ (the molecular ion) and the fragment ions appear in this spectrum.

Interpretation of MS- ion fragmentation

The output of the mass spectrometer shows a plot of relative intensity vs the mass-to-charge ratio (m/z). The most intense peak in the spectrum is termed the base peak and all others are reported relative to its intensity. The peaks themselves are typically very sharp, and are often simply represented as vertical lines.

The process of fragmentation follows simple and predictable chemical pathways and the ions which are formed will reflect the most stable cations and radical cations which that molecule can form.

Interpretation of MS- ion fragmentation

Interpretation of MS- ion fragmentation

What is SIMS?

1) SIMS is one type of mass spectrometer
2) SIMS needs UHV
3) Only solid samples are used in SIMS
4) Primary beam is ion beam
5) SIMS (SSIMS) is surface-sensitive technique, sampling depth is 1-2 monolayer
6) SIMS is capable of high-resolution chemical imaging

The bombarding primary ion beam produces particles of sample material and resputtered primary ions, along with electrons and photons. The secondary particles carry negative, positive, and neutral charges and they have kinetic energies that range from zero to several hundred eV.

- Primary beam species include Au⁺, Ga⁺, Ca⁺, O²⁺, and Ar⁺ at energies between 1 and 30 keV. Primary ions are implanted and mix with sample atoms to depths of 1 to 10 nm.
- Sputter rates typically vary between 0.1 and 5 nm/s. Sputter rates depend on primary beam intensity, sample material, and crystal orientation.
- The sputter yield is the ratio of the number of atoms sputtered to the number of impinging primary ions. Typically, sputter yields fall in a range from 5 to 15.

The collision cascade model quantitatively explains how the primary beam interacts with the sample atoms.

- In this model, a fast primary ion transfers energy to target atoms in a series of binary collisions.
- Energetic target atoms (called recoil atoms) collide with more target atoms.
- Target atoms that recoil back through the sample surface constitute sputtered material. Atoms from the sample's outer monolayer can be driven in about several nanometers generating surface mixing.

### Primary Ion Sources
- Ion sources with electron impact ionization: Duoplasmatron Ar⁺, O²⁺, O⁻
- Ion sources with surface ionization: Cs⁺ ion source.
- Ion sources with field emission: Ga⁺ Liquid metal ion source.

### Mass Analyzers for secondary ion mass separation
- Magnetic sector analyzer
- Quadrupole mass analyzer
- Time-of-flight analyzer

### Secondary ion detectors
- Faraday cup
- Dynode electron multiplier
- Ion imaging detector

This mass filter eliminates impurity species in the beam. For example, Cr, Fe, and Ni ions sputter from stainless steel surfaces within a duoplasmatron.

The electrostatic lenses and the apertures control the intensity and width of the primary ion beam.

Electrostatic deflectors steer the primary beam in a raster pattern onto the sample.
**SIMS- mass analyzer**  
Time-of-flight mass analyzer

**SIMS- ion detector**  
**Secondary Ion Detectors**

- Ion counting electron multiplier. The ion counting electron multipliers are the most sensitive detectors. They must be protected from intense ion beams.
- Faraday cup. The Faraday cup detector moves on a solenoid to cover the electron multiplier when the incoming ion signal is too high.
- Ion image detectors.

- High energy neutral species form by charge exchange when an ion beam strikes a surface. These neutrals contribute noise to the ion signal.
- If an electrostatic sector precedes the electron multiplier, the neutrals can be eliminated from the ion signal.
- Quadrupole mass analyzers also use electrostatic sectors or deflectors to minimize the contributions of high energy neutral species to the ion signal.

**SIMS- ion detector**  
**Electron Multipliers**

An electron multiplier consists of a series of electrodes called dynodes, each connected along a resistor string. The signal output end of the resistor string attaches to positive high voltage. The other end of the string goes to the electron multiplier case and ground.

- The dynode potentials differ in equal steps along the chain. When a particle strikes the first dynode, it produces secondary electrons.
- The secondary electrons are accelerated into the next dynode where each electron produces more secondary electrons. A cascade of secondary electrons ensues.
- The dynode acceleration potential controls the electron gain.

**SIMS- ion detector**  
**Faraday Cups**

1) A Faraday cup is just an electrode from which electrical current is measured while a charge particle beam (electrons or ions) impinges on it.
2) The shape helps minimize loss of secondary electrons that would alter the current measurement. A deep cup with an electron repeller plate minimizes secondary electron loss.

**Positive Charge Build-up in positive mode**

- Total Ion Yields are typically $10^{-2}$ - $10^{-3}$. Therefore, the secondary ion induced negative charge is very small.
- The positive charge originates from implantation of positive primary beam.

**Positive Charge Build-up in Negative SIMS Analysis**

Secondary electron yields are significant, making negative SIMS more difficult than positive SIMS.
Effect of surface charge on mass spectra

- Loss of mass resolution — peak broaden
- Loss of spatial resolution — imaging fuzzy
- Loss of signal — peak intensity reduce

Charge compensation by electron flood gun

Primary ion beam
Ga+

20 eV

Electron gun
e-

Sample

- electron beam must be well focused
- electron beam must be aimed at the spectrometer acceptance area

SIMS analytical modes- static and dynamic SIMS

Static SIMS (SSIMS):
- Typically, the low primary ion dose in the SSIMS mode is \( <10^{12} \) ions/cm\(^2\) for mass analysis,
- Outmost surface (1-2 monolayers) affected
- Pulse mode of primary beam (short pulses of \(<1\) ns).
- Complete analysis before loss of surface integrity
- Elemental analysis
- Molecular information

Dynamic SIMS (DSIMS):
- Large primary ion dose
- Continuous mode of primary beam
- Material removal during analysis
- Elemental analysis
- No molecular information available

Material removal during SIMS analysis

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Image width (μm)</th>
<th>Atoms per nonreact</th>
<th>Masses removed in 100s under:</th>
<th>Dynamic ion current (μA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>3.6 x 10^4</td>
<td>0.0001</td>
<td>0.0105</td>
<td>0.05</td>
</tr>
<tr>
<td>2000</td>
<td>3.6 x 10^5</td>
<td>0.0002</td>
<td>0.0013</td>
<td>0.02</td>
</tr>
<tr>
<td>5000</td>
<td>3.6 x 10^6</td>
<td>0.0003</td>
<td>0.0015</td>
<td>0.03</td>
</tr>
<tr>
<td>10000</td>
<td>3.6 x 10^7</td>
<td>0.0005</td>
<td>0.0015</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note: For a 256 x 256 pixel image with a beam spot size equal to 100 μm, the diameters range is 0 to 400 μm. With increasing magnification, the beam damage becomes more intense within the beam footprint and the surface integrity is reduced. It is recommended to perform a low beam power at high magnification to avoid damage.

Capability of SIMS

1) Identifying the elemental composition and the chemical status near the surface (1-2 monolayers) with high sensitivity (\(<10\) ppm) and high mass resolution (\(>9000\)).
2) Distinguishing the different isotopes of the same element.
3) Imaging the topography of surface using the secondary electrons.
4) Line-scanning of chemical species.
5) Mapping chemical species on the submicron scale.
6) Ultra-thin depth profiling.

SIMS can be used for surface analysis of inorganic, organic materials, and biological cells, applied to conductors, insulators, and semiconductors.

SIMS Spectra

High-sensitivity SIMS spectra, 40 ppm Si in the GaN film

SIMS is capable of detecting trace elements
SIMS Spectra

- High resolution SIMS spectrum
- SIMS spectrum of as-received TiO_2 particles
- SIMS spectrum of silane-coated TiO_2 particles
- SIMS Spectra - examination of surfactant on nanoparticles

Depth profiling of SiO_2 film on the Si substrate, showing the sharp SiO_2/Si interface

- SIMS Depth profile of thin film on the substrate
- SIMS image of organic film pattern (fatty acid/thiol/Au sample), left: total ion image, middle: OH^- map showing the fatty acid film; right: S^- map showing the thiol film; scale: 1mm, sample provided by A. Pannier

Secondary ion mass spectrometry (SIMS) images

- SIMS image of surface-modified AFM cantilever, scale: 100um, sample provided by M. Su