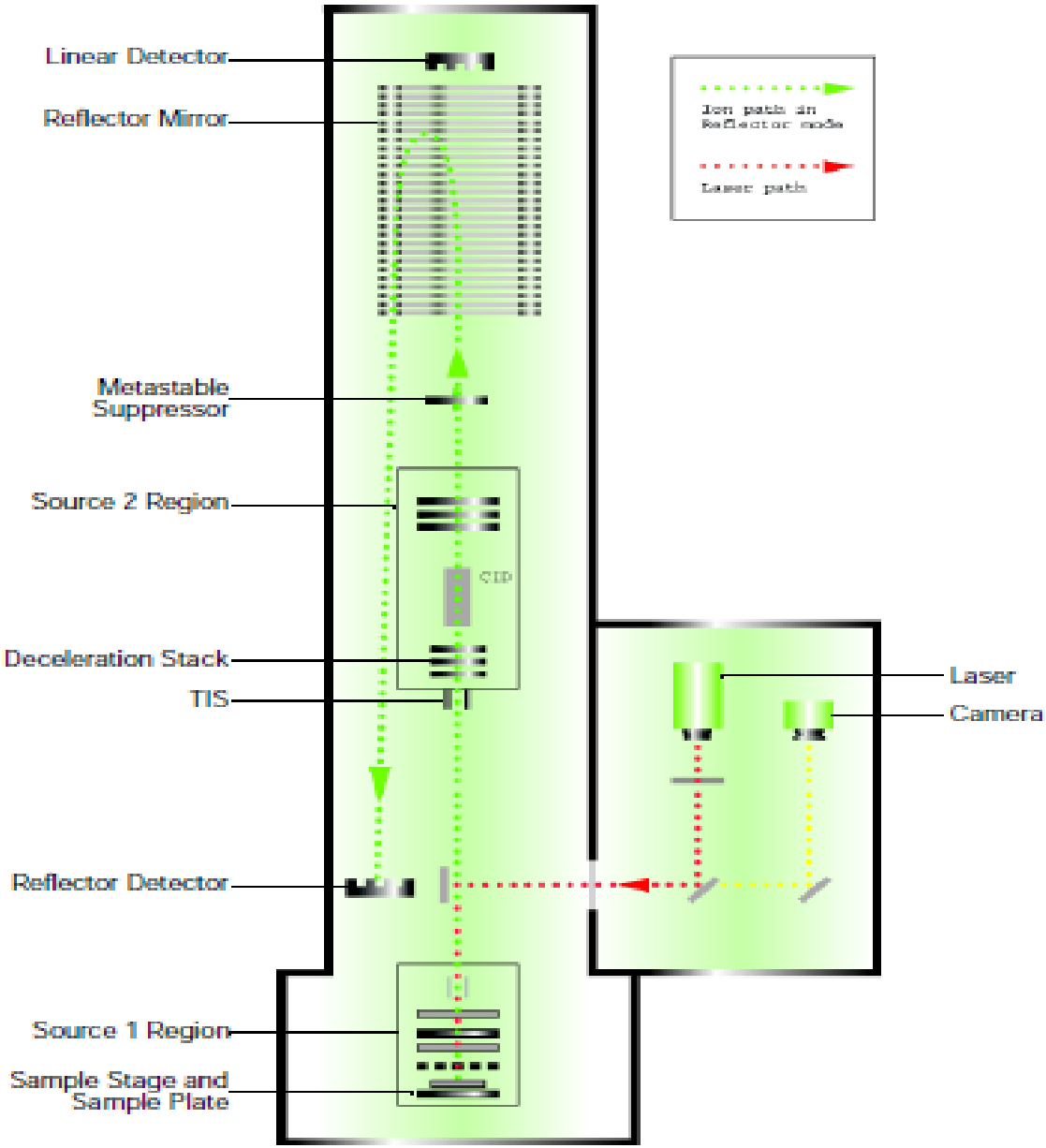
4800 System Overview: Concepts and Principles

4800 MALDI TOF/TOF™ Analyzer mass spectrometer



Applied Biosystems

ABI 4800 Proteomics Analyzer

- **MALDI TOF/TOF system with Linear (optional), Reflector, and MS/MS Acquisition modes**
- **High speed Data Acquisition**
  - 200 Hz Laser
  - ＞400 samples per hours
- **Sample plate**
  - Uses 3×5” sample plates
  - Automated single-plate sample loading system
- **Ultra-Wide Mass Range TOF Analyzer**
- **TOF/TOF with High Energy CID**
  - More Fragmentation - Immonium ions, side chain cleavages, internal fragments (Leu/Ile differentiation)
  - Consistent fragmentation patterns for rapid and accurate Protein Post Translational Modification ID

ABI 4800 Overview

1. MS overview
2. MALDI Ionization
3. TOF Mass Analyzer
4. Delayed Extraction
5. MS/MS
6. Laser
7. Reflector

Basic Components of a Mass Spectrometer

Vacuum Envelope

Ionization

Ion Source

Forms ions
(charged molecules)

Mass Sorting (filtering)

Mass Analyzer

Sort Ions by Mass (m/z)

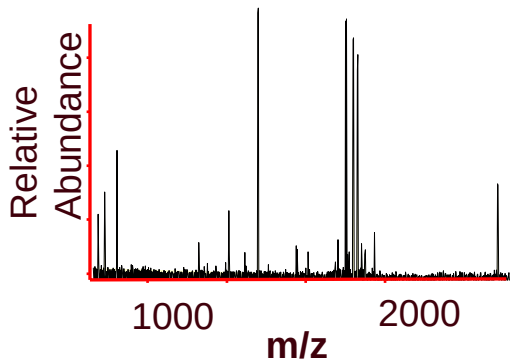
Detection

Ion Detector

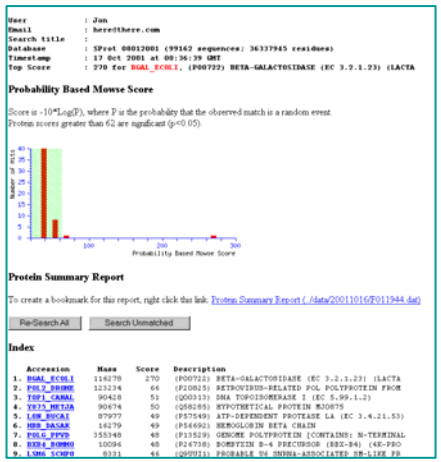
Detect ions

Data System

Mass Spectrum



Data Processing



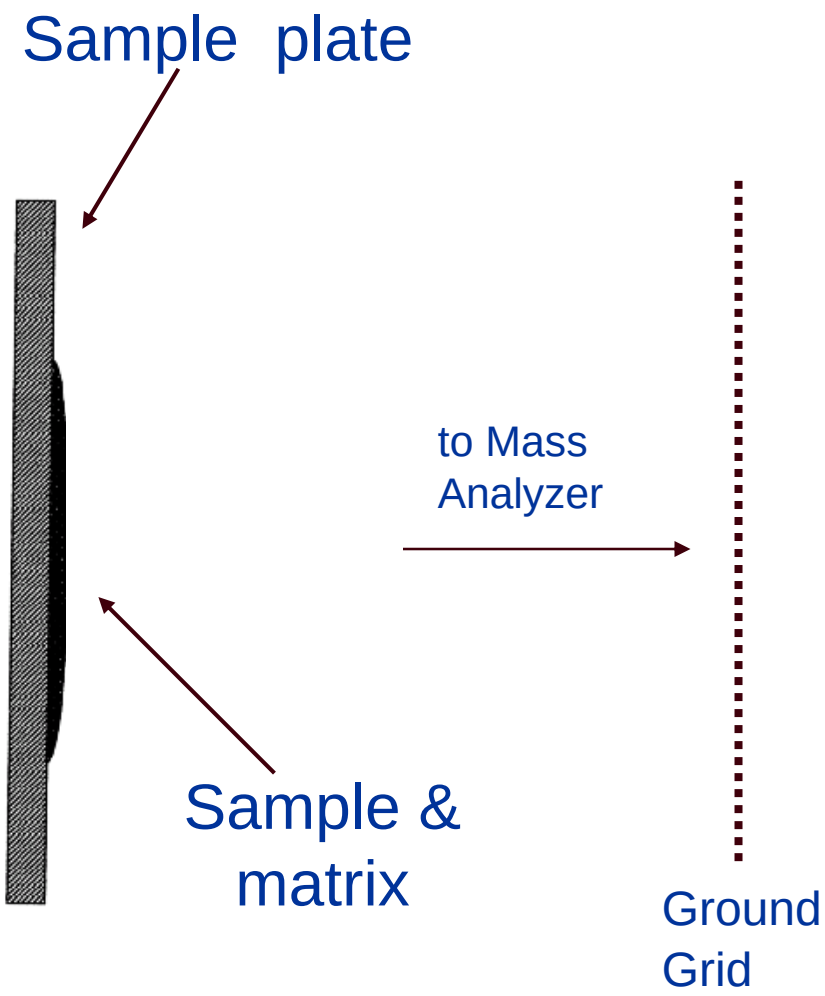
Sample Inlet

Sample/Matrix dried
on MALDI Plate
(Autoloader Cassette)

Ion Sources Make Ions From Sample Molecules

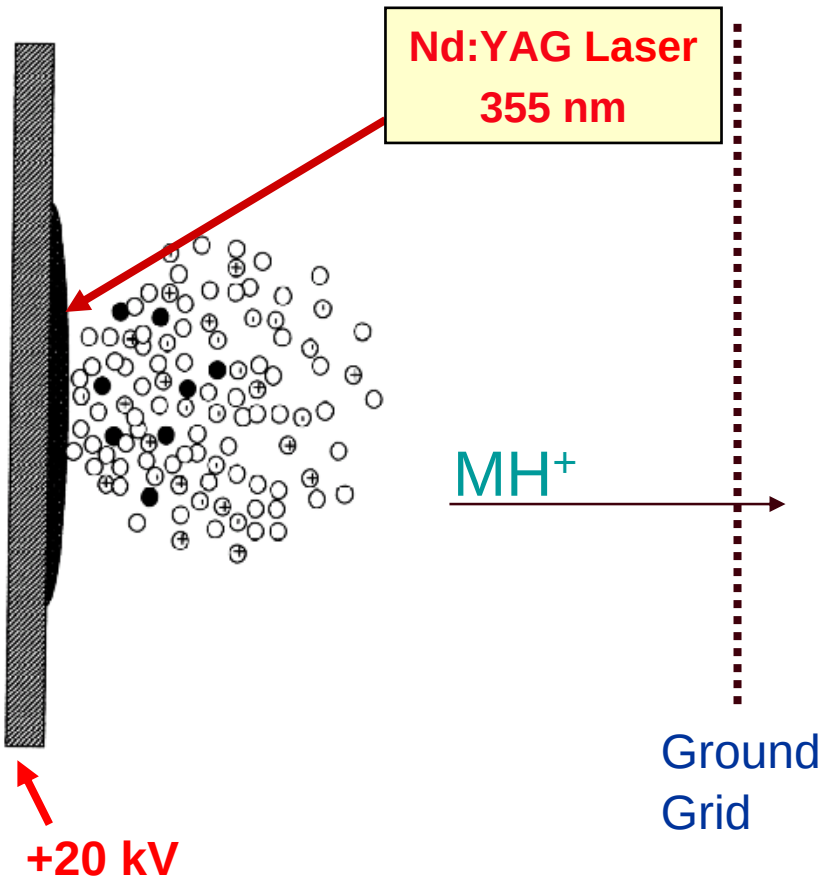
- For biological applications, ion sources convert neutral molecules to ions by adding or taking away one or more protons.
- Ions may be singly or multiply charged.
- Ions are easier to control in the mass spectrometer than neutral molecules. Ion beams can be focused, aligned or reflected with electrical fields.
- Ions are easier to detect than neutral molecules.

MALDI:
Matrix Assisted Laser Desorption Ionization



1. Sample (M) is mixed with excess matrix (X) and dried on a MALDI plate.

MALDI:
Matrix Assisted Laser Desorption Ionization



2. Laser flash produces matrix neutrals (X), matrix ions $(XH)^+$, $(X\text{-}H)^-$, and sample neutrals (M).

3. Sample molecules are ionized by proton transfer from matrix ions:

$$XH^+ + M \rightarrow X + MH^+.$$

4. High voltage is applied to the sample plate, accelerating ions out of the Ion Source into the Flight Tube

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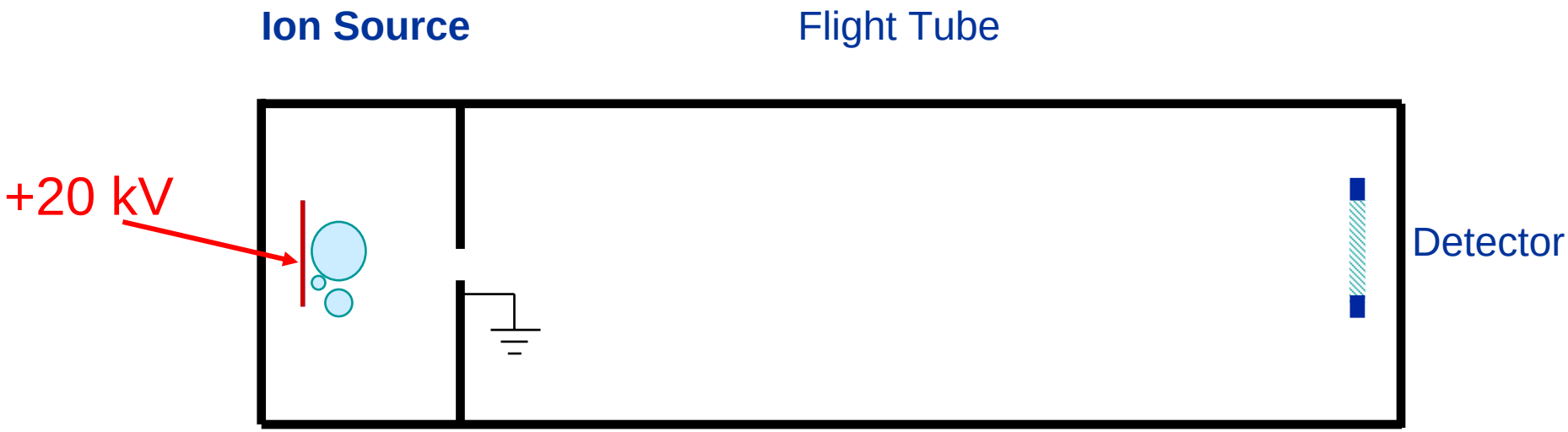
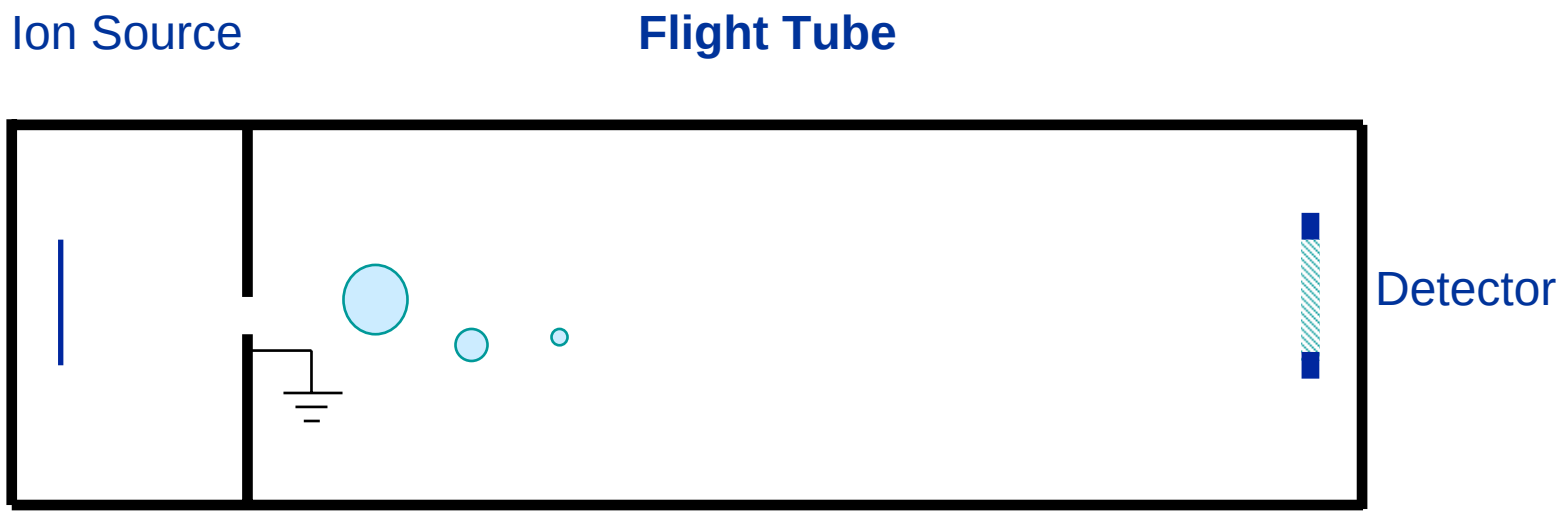
Time-of-Flight Mass Analyzer



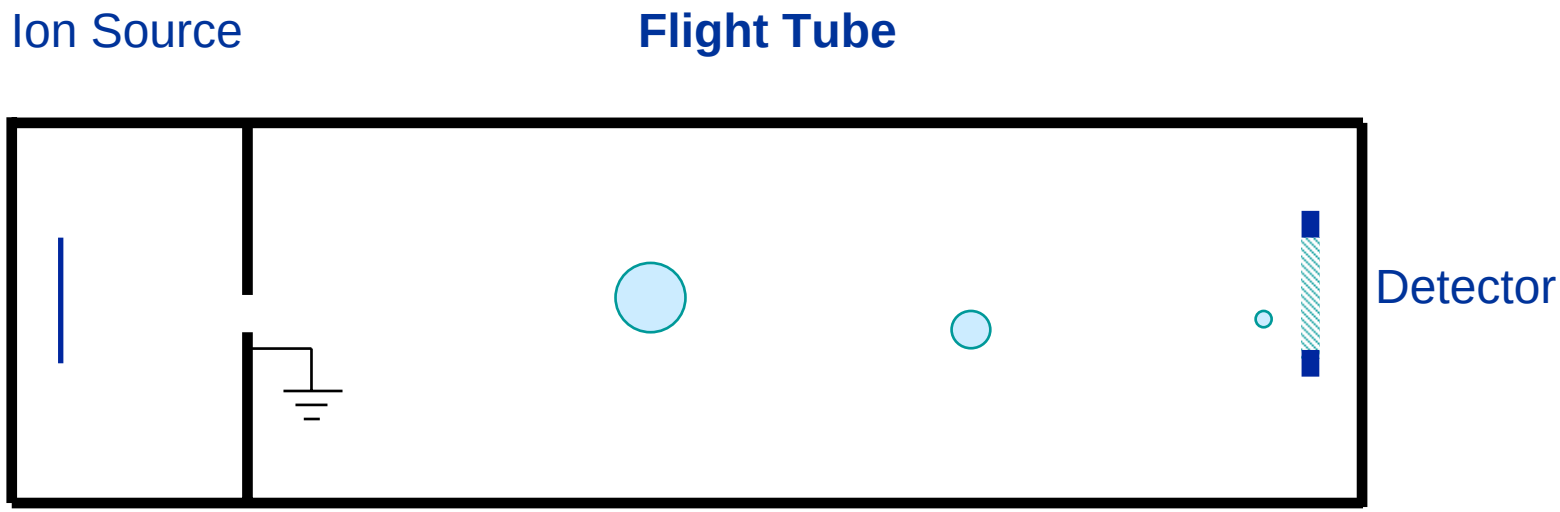
Principle: If ions are accelerated with the same potential at a fixed point and a fixed initial time and are allowed to drift, the ions will separate according to their mass-to-charge ratios.

Time-of-Flight Mass Analyzer



The ions enter the flight tube with the lighter ions travelling faster than the heavier ions

Time-of-Flight Mass Analyzer



The lighter ions strike the detector before the heavier ions.
This “time of flight” (TOF) can be converted to mass

Delayed Extraction

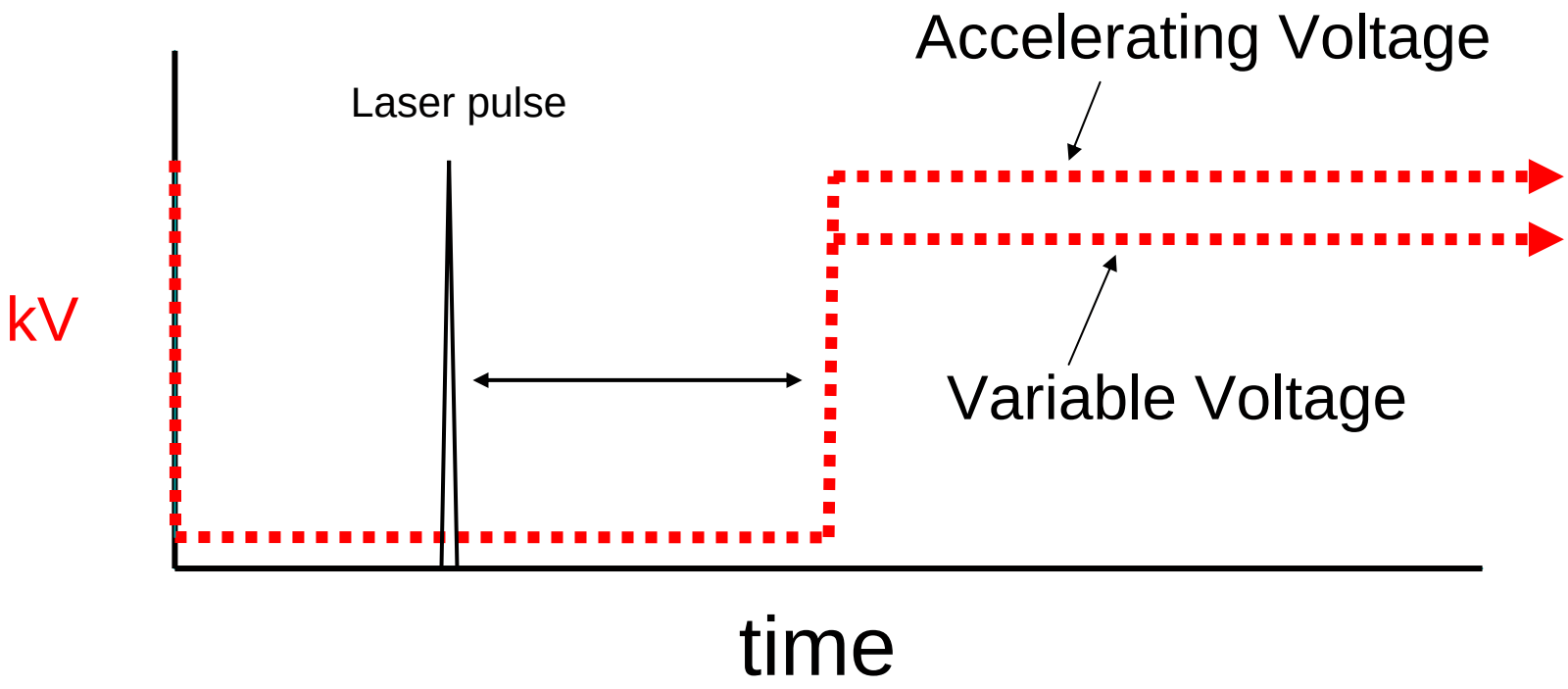
When ions are formed in MALDI they have a range of translational kinetic energies due to the ionization process. This leads to peak broadening. By forming ions in a weak electric field, then applying a high voltage extracting field only after a **time delay**, the effect of this energy spread can be minimized when used in conjunction with an appropriate potential **gradient**.

Field gradients are formed and controlled in the ionization region by the voltages applied to the sample plate and the variable voltage plate.

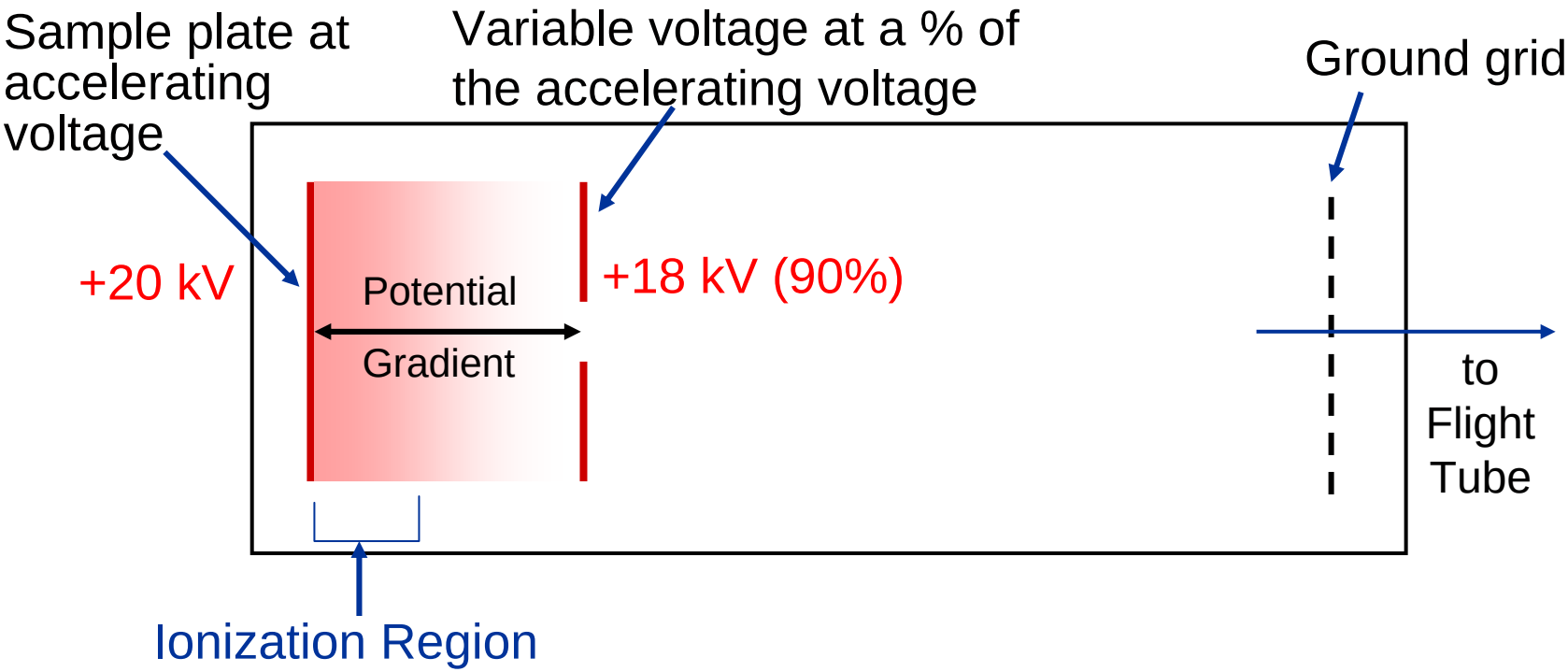
Ref: W.C. Wiley and I.H. McLaren, *Rev. Sci. Instrum.* (1953) **26**, 1150-1157.

Pulse Delay Time

with Delayed Extraction Technology

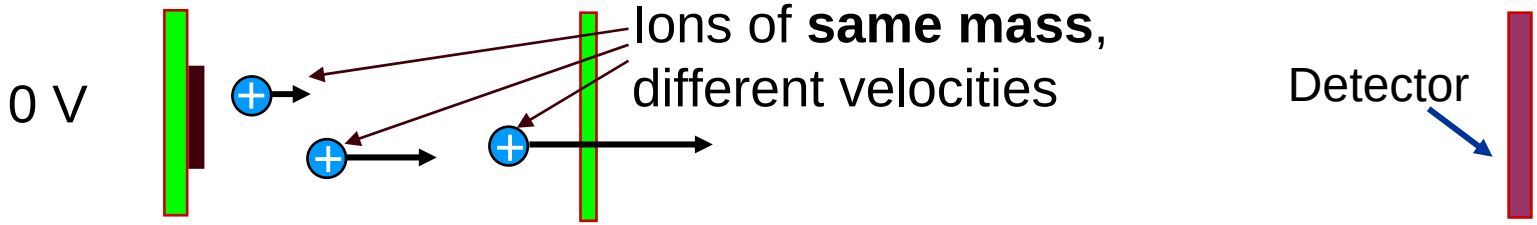


Extraction Voltages



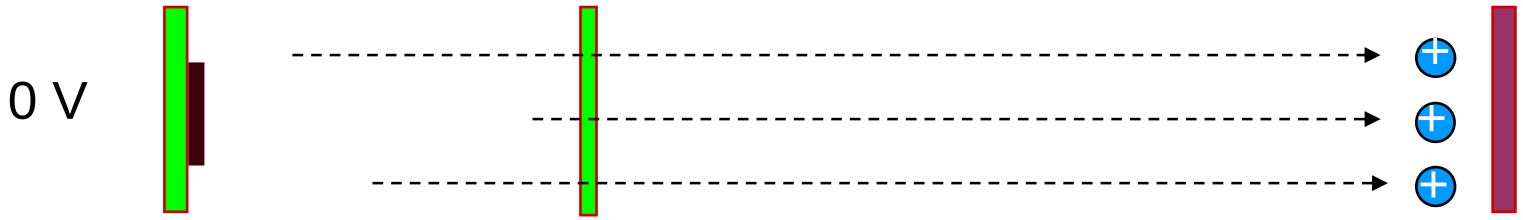
The variable voltage works together with the accelerating voltage to define the potential **gradient** in the ionization region near the target. It and the **delay time** must be adjusted to obtain optimum resolution for a given mass range.

Velocity Focusing with DE



1: No electric field. Ions spread out during **delay time**.

2: Field applied. **Gradient** accelerates slow ions more than fast ones.

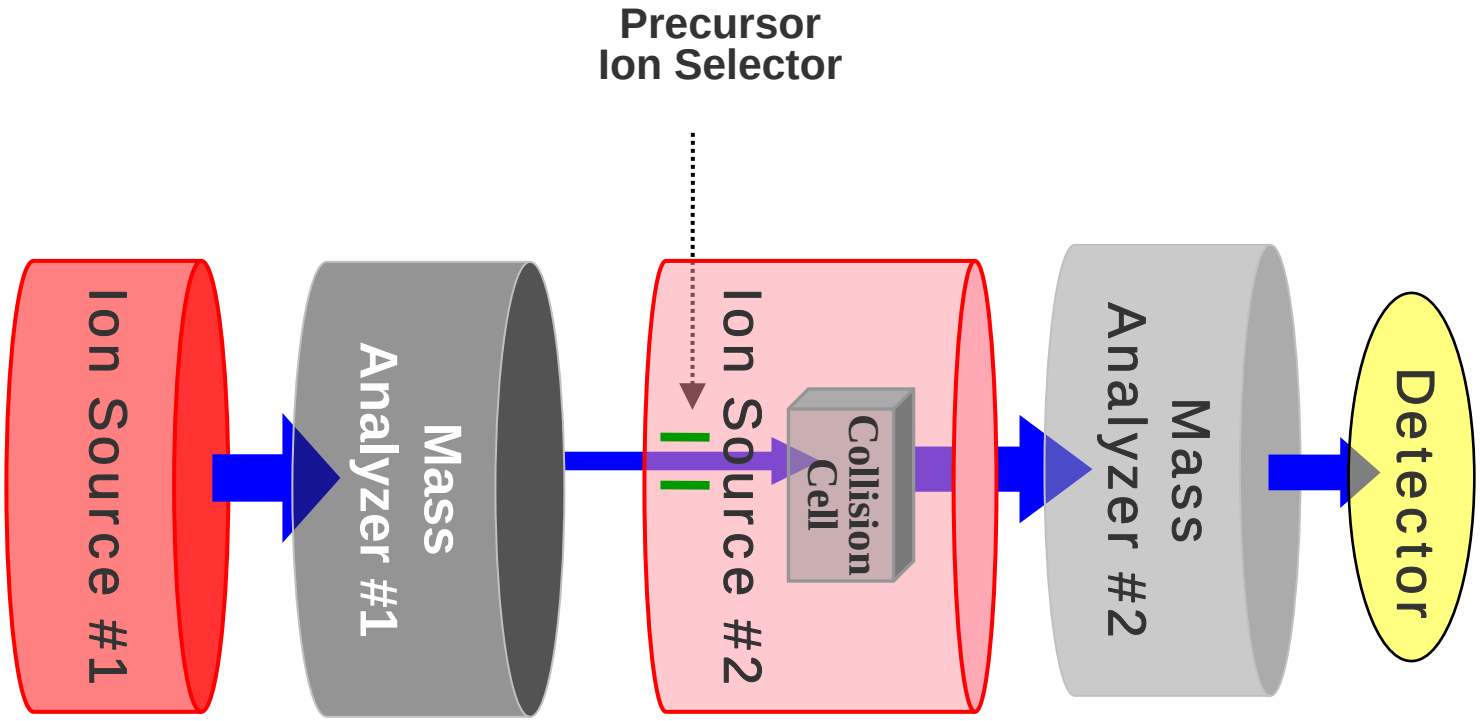


3: Slow ions catch up with faster ones **at the detector**.

Sample Plate

Variable Voltage

Tandem MS/MS Spectrometer



Collision Induced Dissociation

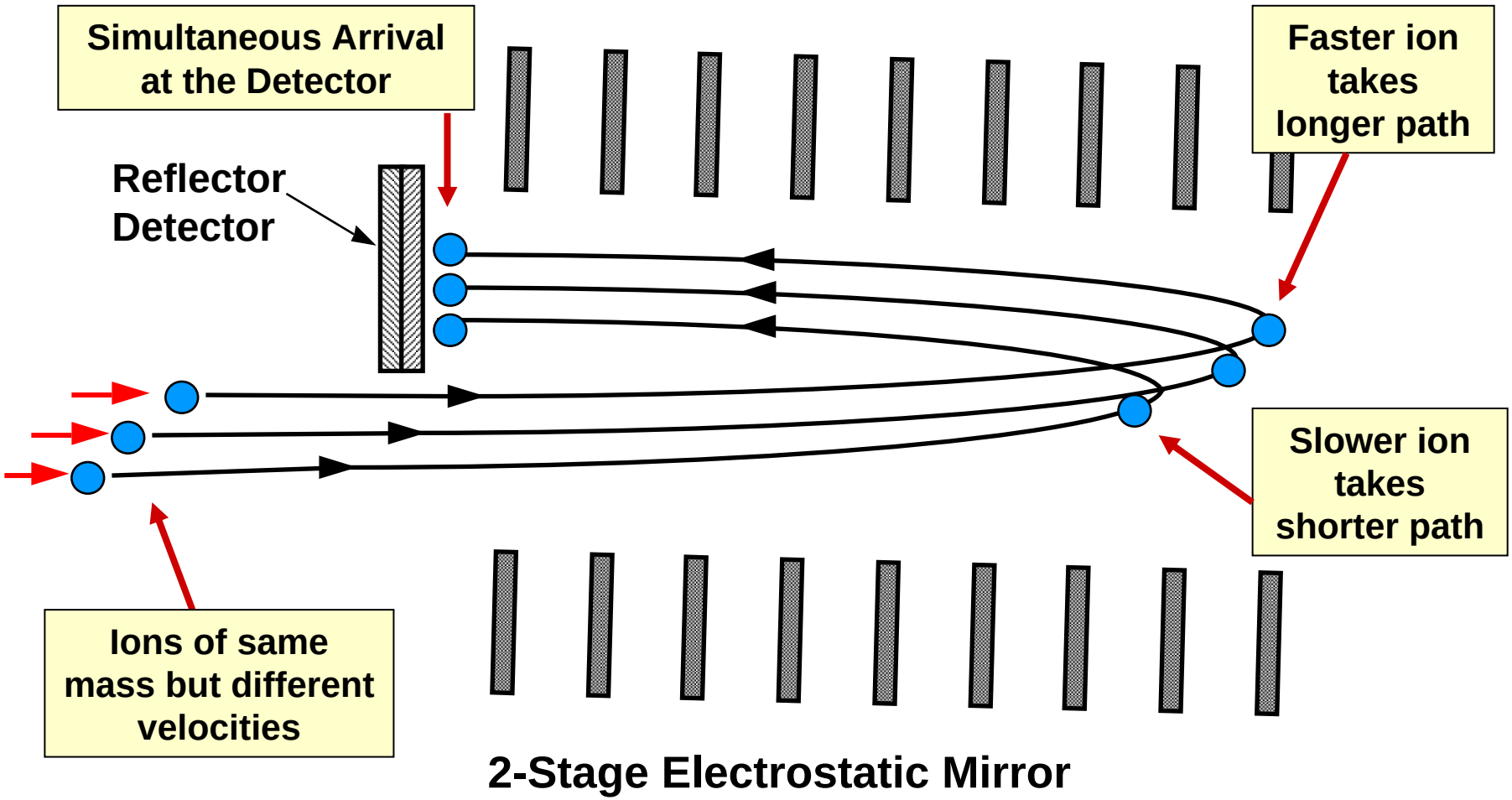
1. An inert gas (commonly used are Ar, He, N_2, Air) is introduced into the collision cell at certain pressure.
2. The precursor ion of interest is selected and transmitted into the collision cell.
3. Collisions occur between the gas molecules and the precursor ions.
4. Energy transfer occurs during the collisions, which induces fragmentation of the precursor ions producing charged and neutral fragments.

Laser

- Diode-pumped Nd:YAG at 355 nm
- Pulse rate up to 200 Hz with <500 psec duration/pulse
- Different samples (analytes) may need different laser intensity to ionize
- Different acquisition modes (linear, reflector, MS/MS) may be optimized at different laser intensity.
- Laser intensity affects both resolution and S/N.

Velocity focusing in Reflector Mode

Ions with higher energy (velocity) follow a longer path such that their **arrival times at the detector** are the same as ions of the same mass with lower energy.



4800 Target Applications

- **Biomarker Discovery**
  - Protein ID and Quant
- **Protein ID**
  - Includes both MS and MS/MS techniques
  - ID of proteins in gel slices (routine ID from 1D, 2D gels)
  - MDLC fractions and complex samples with LC-MALDI
- **Protein Expression Analysis**
  - ID and quantification of differentially expressed proteins (iTRAQ and ICAT)
- **Protein Characterization**
  - Characterization of phosphorylation and glycosylation peptides by MS and MSMS techniques
  - Intact protein analysis
- **Carbohydrates, lipids, oligosaccharides, etc**

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