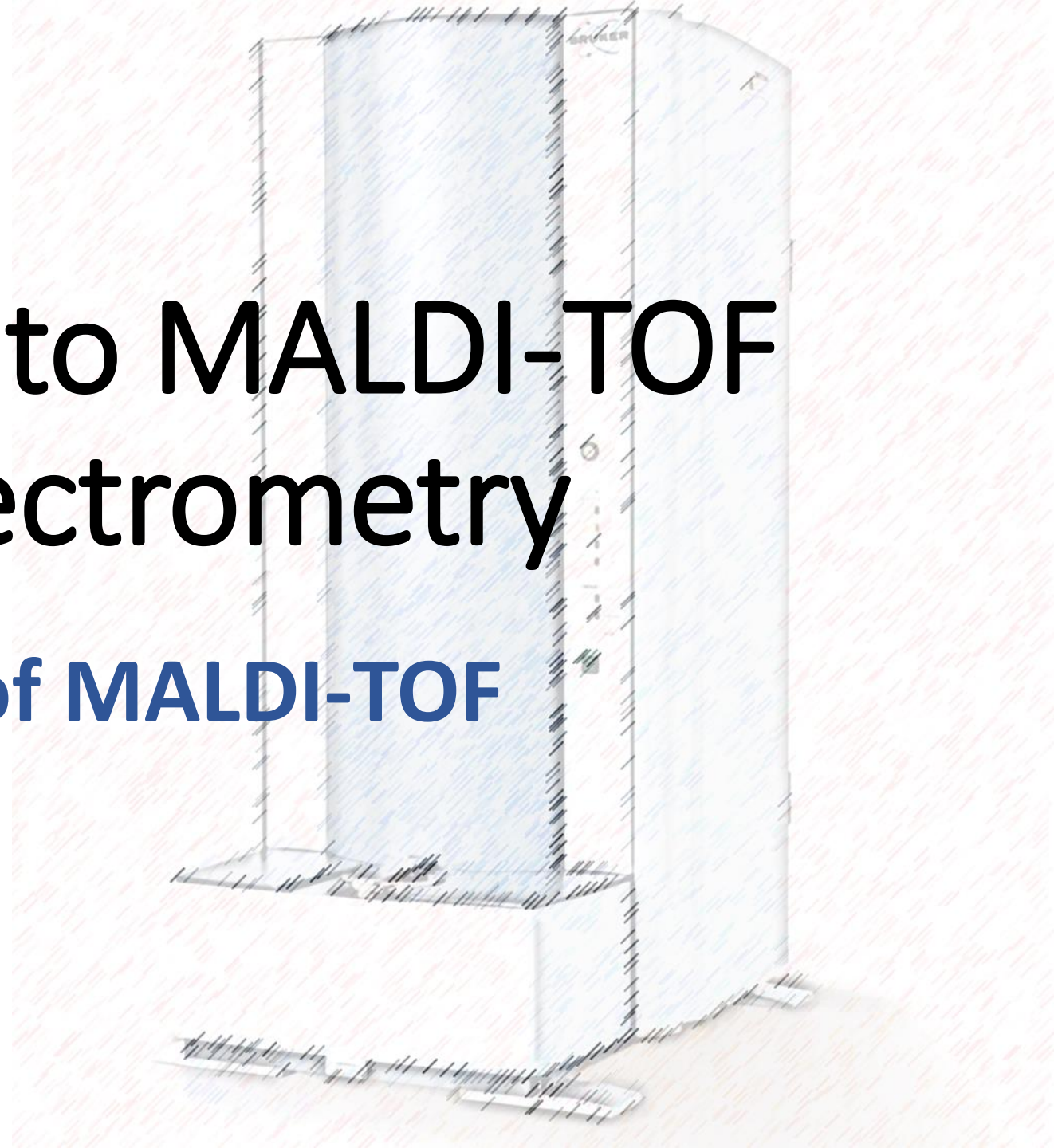


Introduction to MALDI-TOF Mass Spectrometry

Principles of MALDI-TOF



Overview

- Matrix Assisted Laser Desorption/Ionisation – Time of Flight (MALDI-TOF) mass spectrometry used to detect and characterize mixtures of organic molecules.
- In micro used as a rapid, accurate and cost-effective method for ID of microbes
- 2 commercially available:
 - Vitek MS (bioMerieux)
 - MALDI Biotyper CA system (Bruker Daltonics)

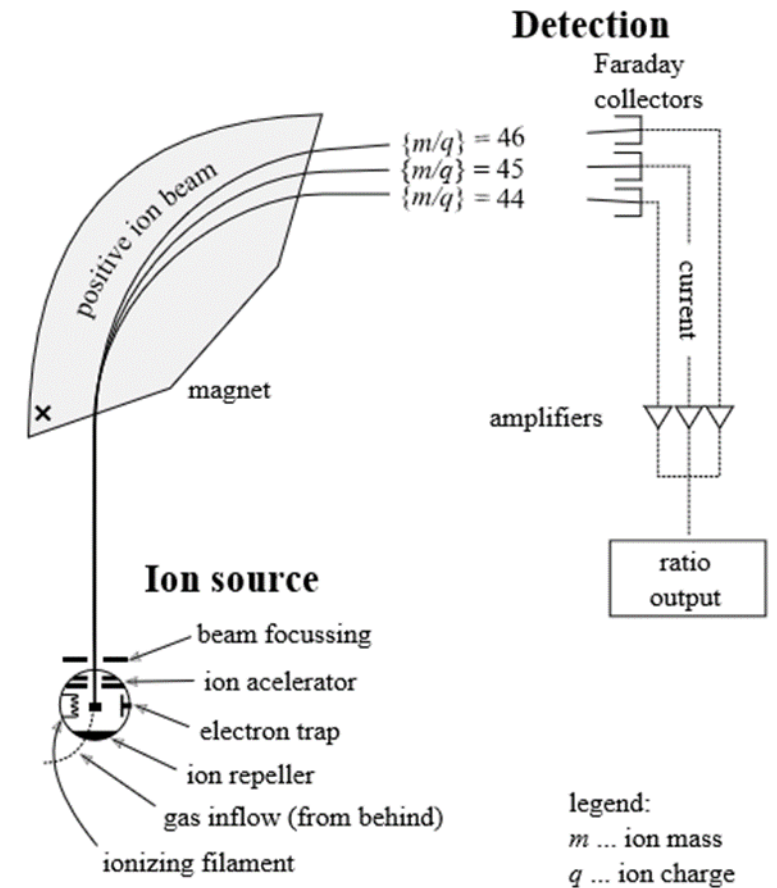


Mass Spectrometry

- Mass spectrometry (MS) measures mass-to-charge ratio (m/Q) of ions.
- Results presented as a mass spectrum - a plot of the ion signal as a function of the mass-to-charge ratio.
- Spectra used to determine
 - elemental or isotopic signature
 - the masses of particles
 - the chemical ID or structure

Three components of MS

- An ion source
 - Sample (solid, liquid, or gaseous) is ionized - for solids via electrons or MALDI
- A mass analyzer
 - Ions then separated based on mass-to-charge ratio
 - Done by acceleration and subjecting to an electric or magnetic field
 - Ions with a lower mass will reach the detector first.
- A detector
 - records either charge or current produced when ion passes by or hits a surface
 - produces a mass spectrum (mass-to-charge ratio)
 - Usually an electron multiplier



MALDI – TOF MS

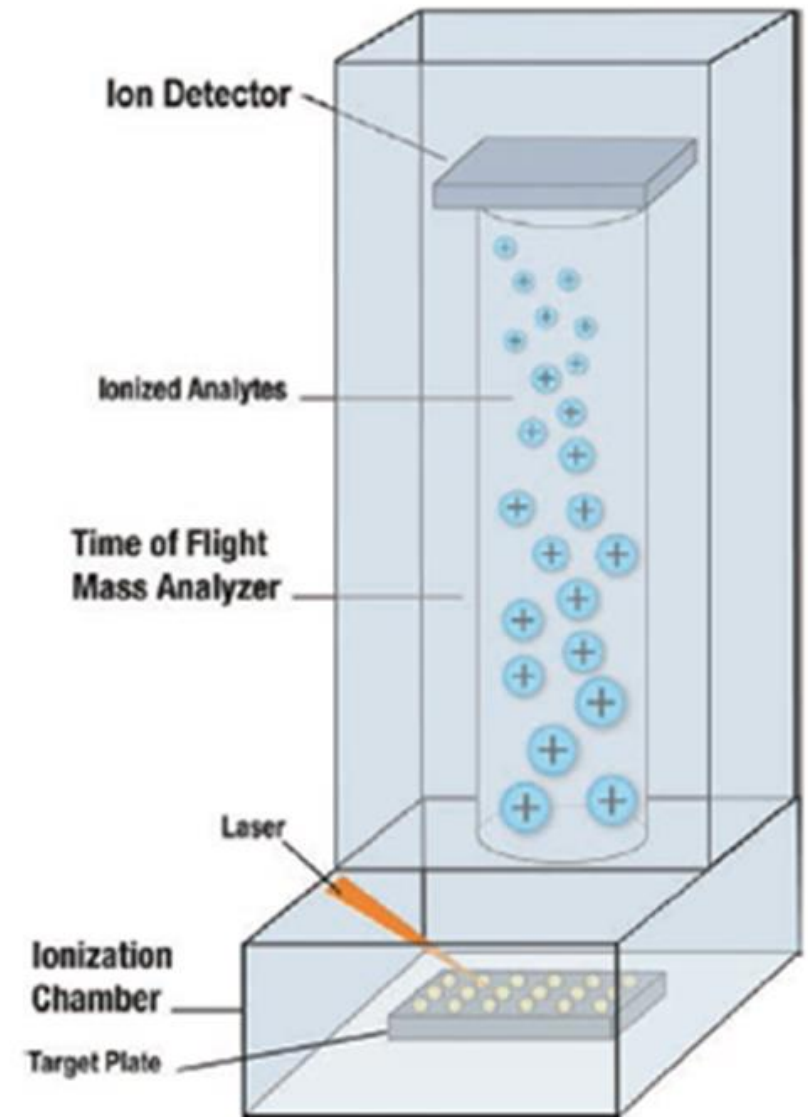
1. An ion source

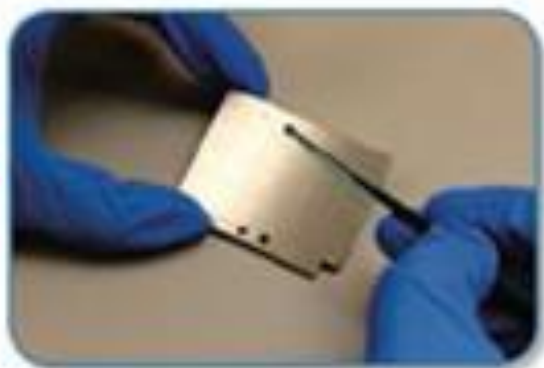
- Laser and ionization chamber to ionize sample and transfer into a gas phase
- Uses laser energy-absorbing matrix to create ions from large molecules

2. A mass analyser

- separates ionized analytes according to their mass (all same charge)
- TOF uses electric field to accelerate ions & measure time to reach detector.

3. A detection device to monitor separated ions





Add Formic Acid
and Dry; Add Matrix
and Dry

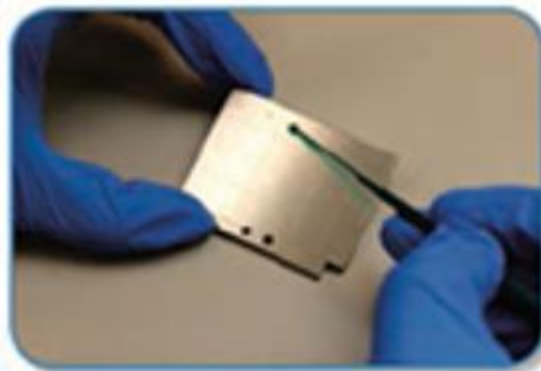


| | 1 | 2 | 3 | 4 | 5 | 6 | |
|---|---|---|---|---|---|---|-------------------|
| A | ● | ● | ● | ■ | ○ | ○ | Not assigned |
| B | ○ | ○ | ○ | ○ | ○ | ○ | ● Blood |
| C | ○ | ○ | ○ | ○ | ○ | ○ | ● Urine |
| D | ○ | ○ | ○ | ○ | ○ | ○ | ● Tissue specimen |

| ID | Protein | Abundance | Score |
|-----|------------------|-----------|-------|
| 101 | Actin | 1000 | 1.25 |
| 102 | Albumin | 2000 | 1.50 |
| 103 | Myoglobin | 500 | 0.80 |
| 104 | Insulin | 100 | 0.30 |
| 105 | Glucagon | 100 | 0.30 |
| 106 | Epinephrine | 100 | 0.30 |
| 107 | Norepinephrine | 100 | 0.30 |
| 108 | Dopamine | 100 | 0.30 |
| 109 | Serotonin | 100 | 0.30 |
| 110 | Histamine | 100 | 0.30 |
| 111 | Adrenaline | 100 | 0.30 |
| 112 | Noradrenaline | 100 | 0.30 |
| 113 | Acetylcholine | 100 | 0.30 |
| 114 | GABA | 100 | 0.30 |
| 115 | Glutamate | 100 | 0.30 |
| 116 | Aspartate | 100 | 0.30 |
| 117 | Glutamine | 100 | 0.30 |
| 118 | Proline | 100 | 0.30 |
| 119 | Serine | 100 | 0.30 |
| 120 | Alanine | 100 | 0.30 |
| 121 | Valine | 100 | 0.30 |
| 122 | Isoleucine | 100 | 0.30 |
| 123 | Leucine | 100 | 0.30 |
| 124 | Phenylalanine | 100 | 0.30 |
| 125 | Tyrosine | 100 | 0.30 |
| 126 | Threonine | 100 | 0.30 |
| 127 | Methionine | 100 | 0.30 |
| 128 | Cysteine | 100 | 0.30 |
| 129 | Proteinase K | 100 | 0.30 |
| 130 | Trypsin | 100 | 0.30 |
| 131 | Chymotrypsin | 100 | 0.30 |
| 132 | Subtilisin | 100 | 0.30 |
| 133 | Carboxypeptidase | 100 | 0.30 |
| 134 | Endonuclease | 100 | 0.30 |
| 135 | Exonuclease | 100 | 0.30 |
| 136 | Phosphatase | 100 | 0.30 |
| 137 | Kinase | 100 | 0.30 |
| 138 | Hydrolase | 100 | 0.30 |
| 139 | Transferase | 100 | 0.30 |
| 140 | Lyase | 100 | 0.30 |
| 141 | Isomerase | 100 | 0.30 |
| 142 | Ligase | 100 | 0.30 |
| 143 | Translocase | 100 | 0.30 |
| 144 | Channel | 100 | 0.30 |
| 145 | Carrier | 100 | 0.30 |
| 146 | Receptor | 100 | 0.30 |
| 147 | Enzyme | 100 | 0.30 |
| 148 | Protein | 100 | 0.30 |
| 149 | Peptide | 100 | 0.30 |
| 150 | Small molecule | 100 | 0.30 |

Procedure

- Combine isolated colony (analyte) , formic acid & matrix on MALDI plate
- Solvents vaporize, leaving only the recrystallized matrix with analyte embedded



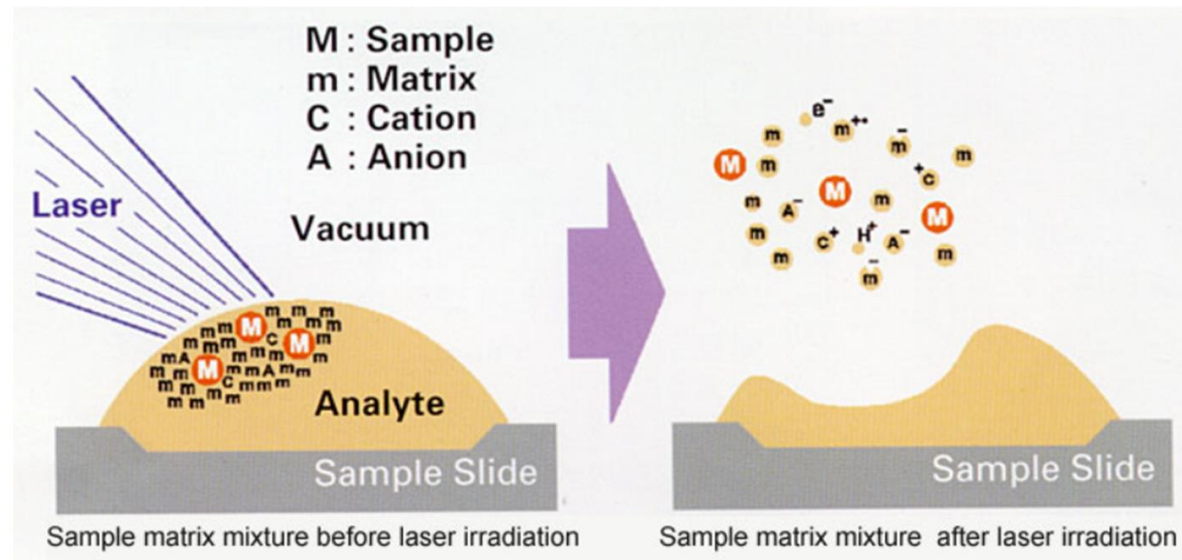
**Add Formic Acid
and Dry; Add Matrix
and Dry**

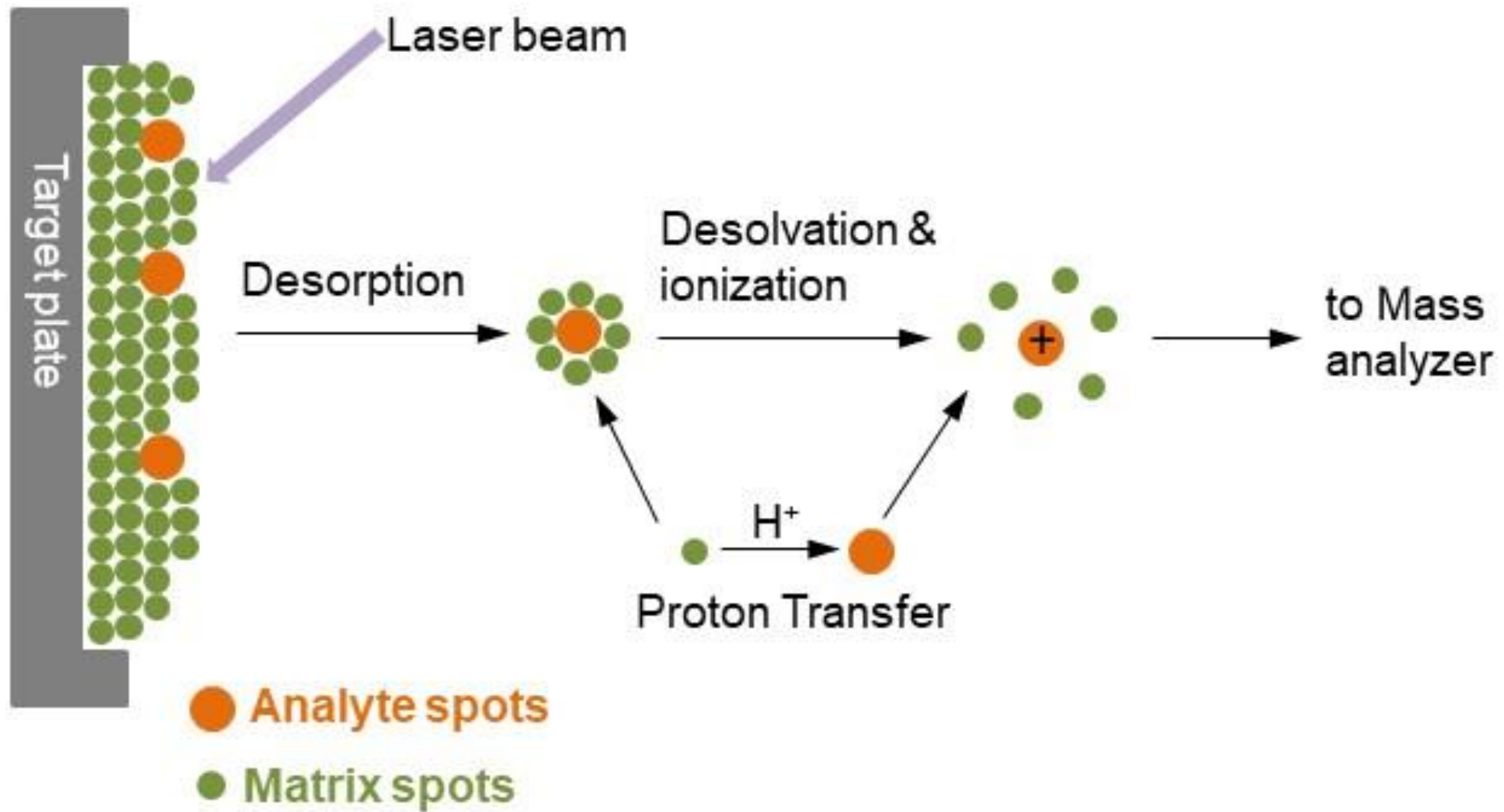
Matrix

- Matrix isolates molecules from each other, protecting them from fragmentation and enabling desorption by laser energy
- Consists of:
 - Small crystallised acid molecules – usually sinapinic acid, alpha-cyano (alpha matrix) or DHB acid.
 - Purified water
 - Organic solvent (alcohol or acetonitrile)
 - Trifluoroacetic acid 2.5%

Ion Source - Laser

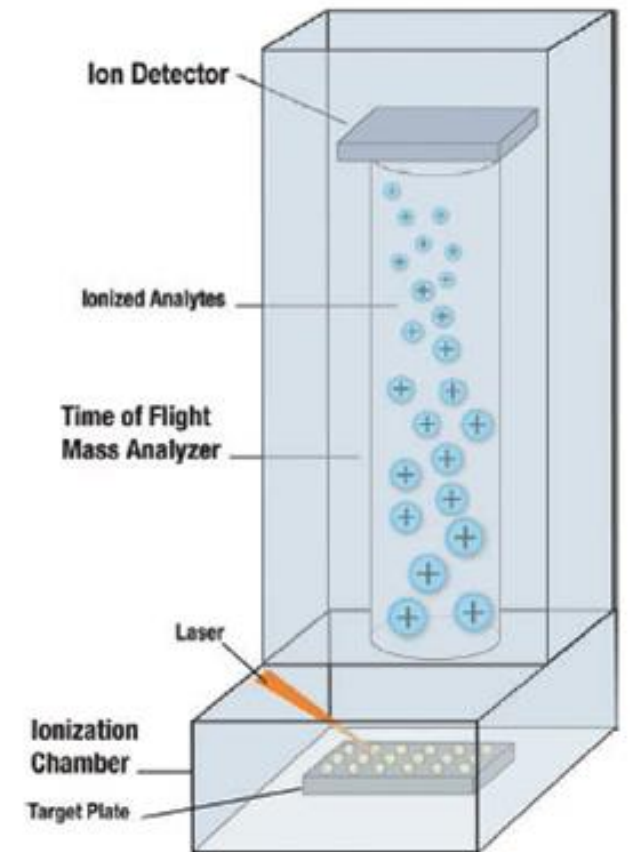
- Uses UV lasers (nitrogen laser light, wavelength 337nm)
- Laser pulses fired at the matrix crystals in the dried-droplet spot.
- Matrix absorbs the laser energy converting it to an ionised state
- Charge is transferred to analyte (random collision in the gas phase)
- Ionised analyte and matrix molecules are desorbed from the plate

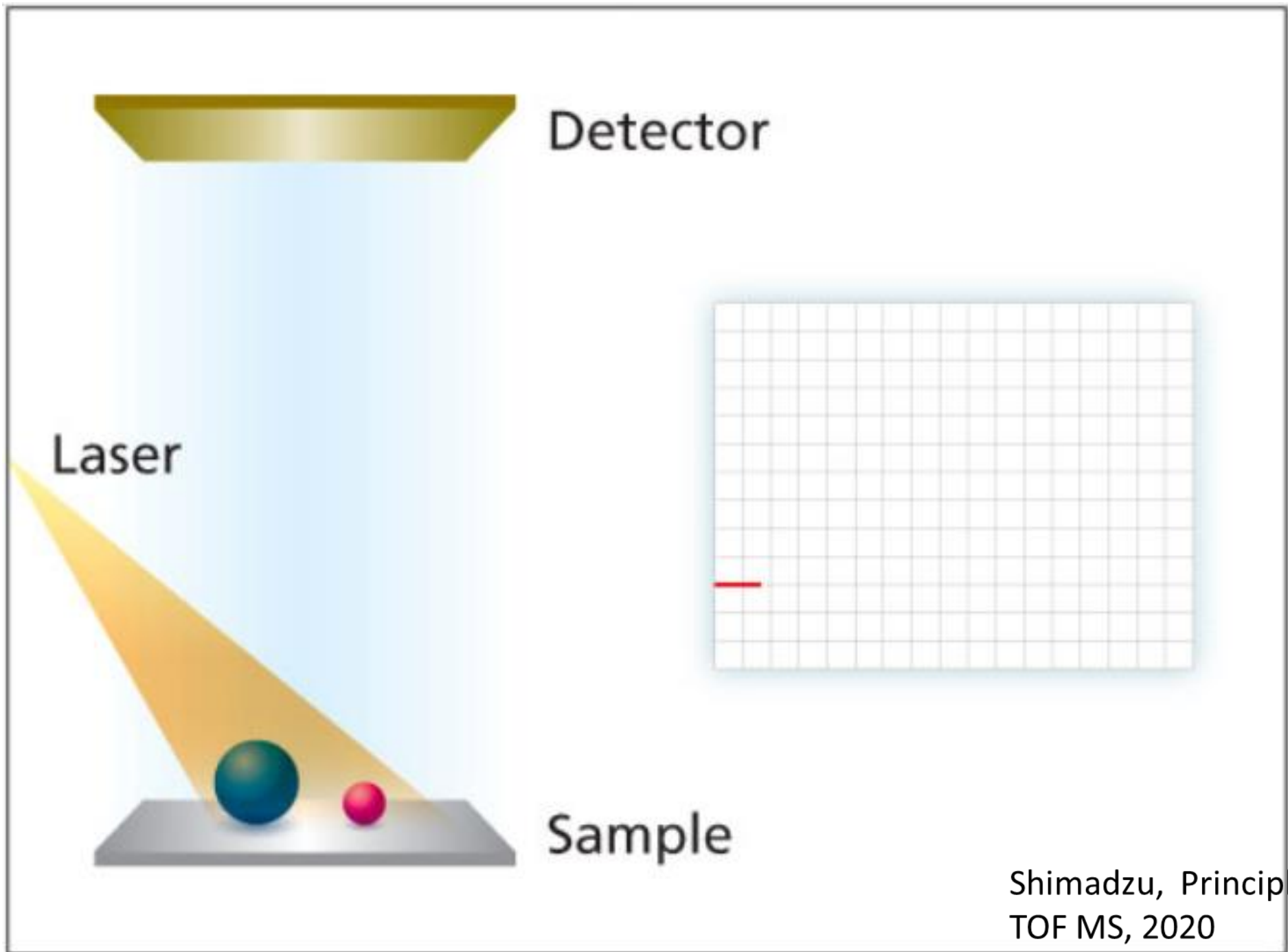


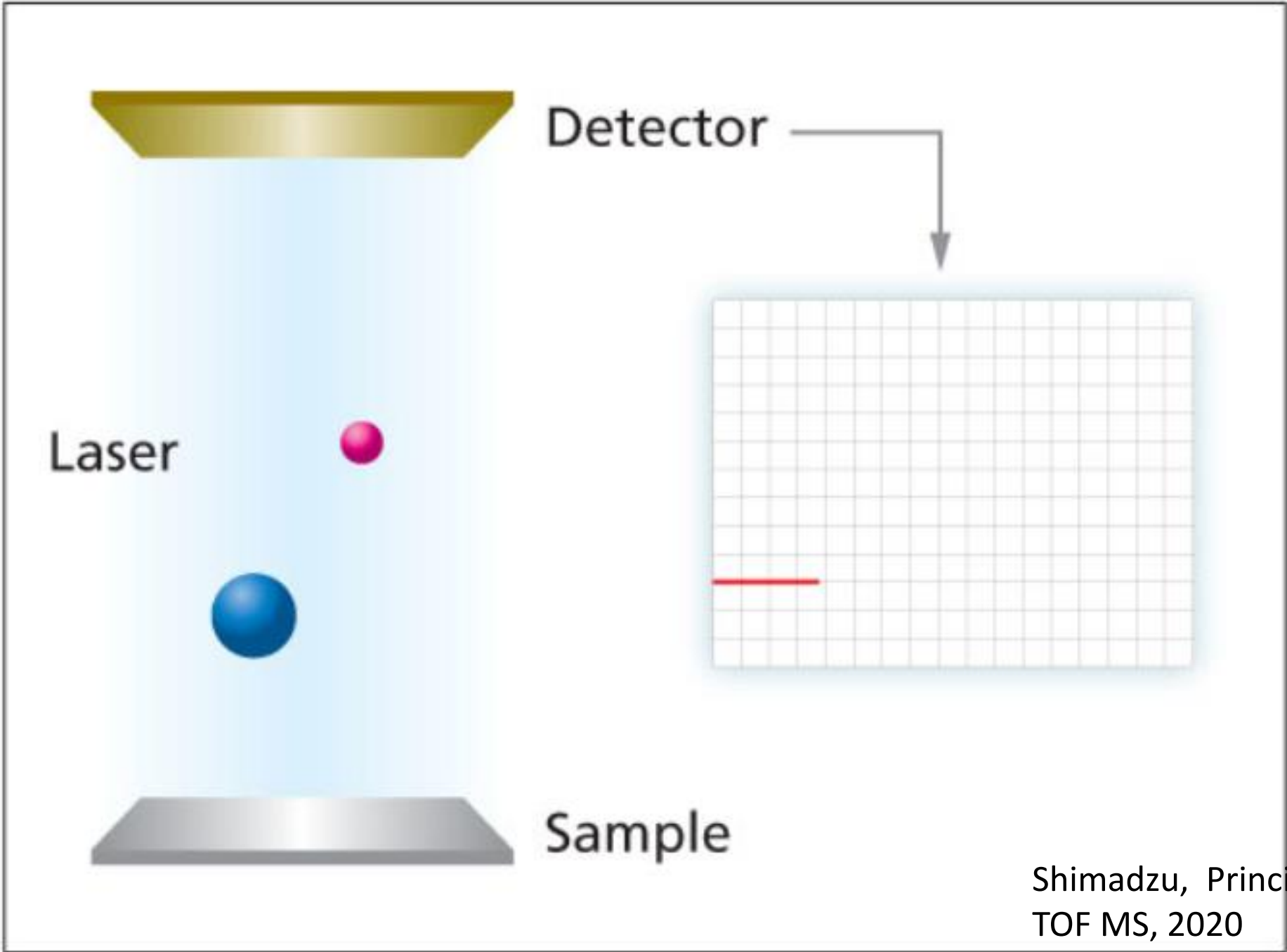


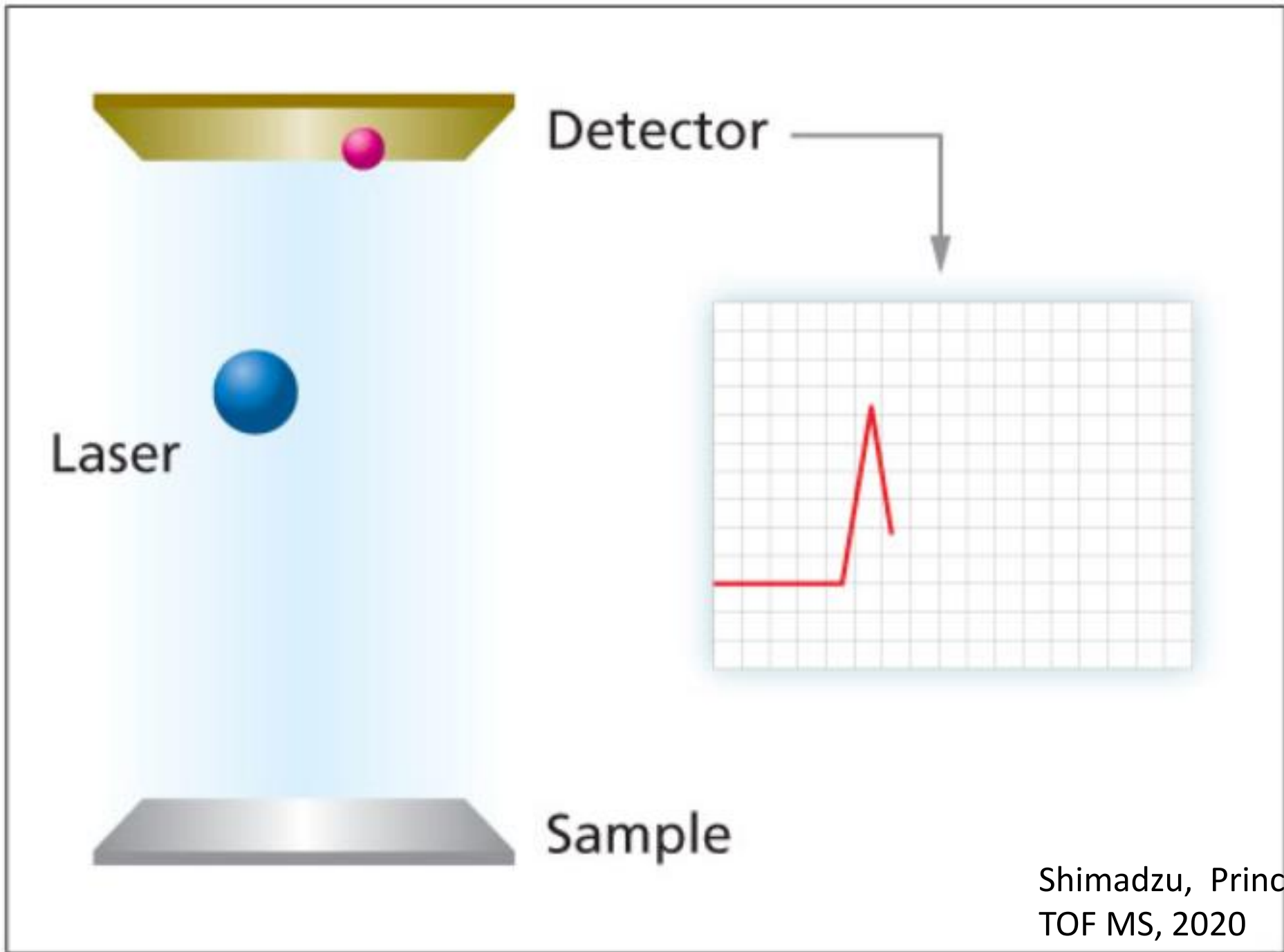
Mass Analyser – Time of Flight

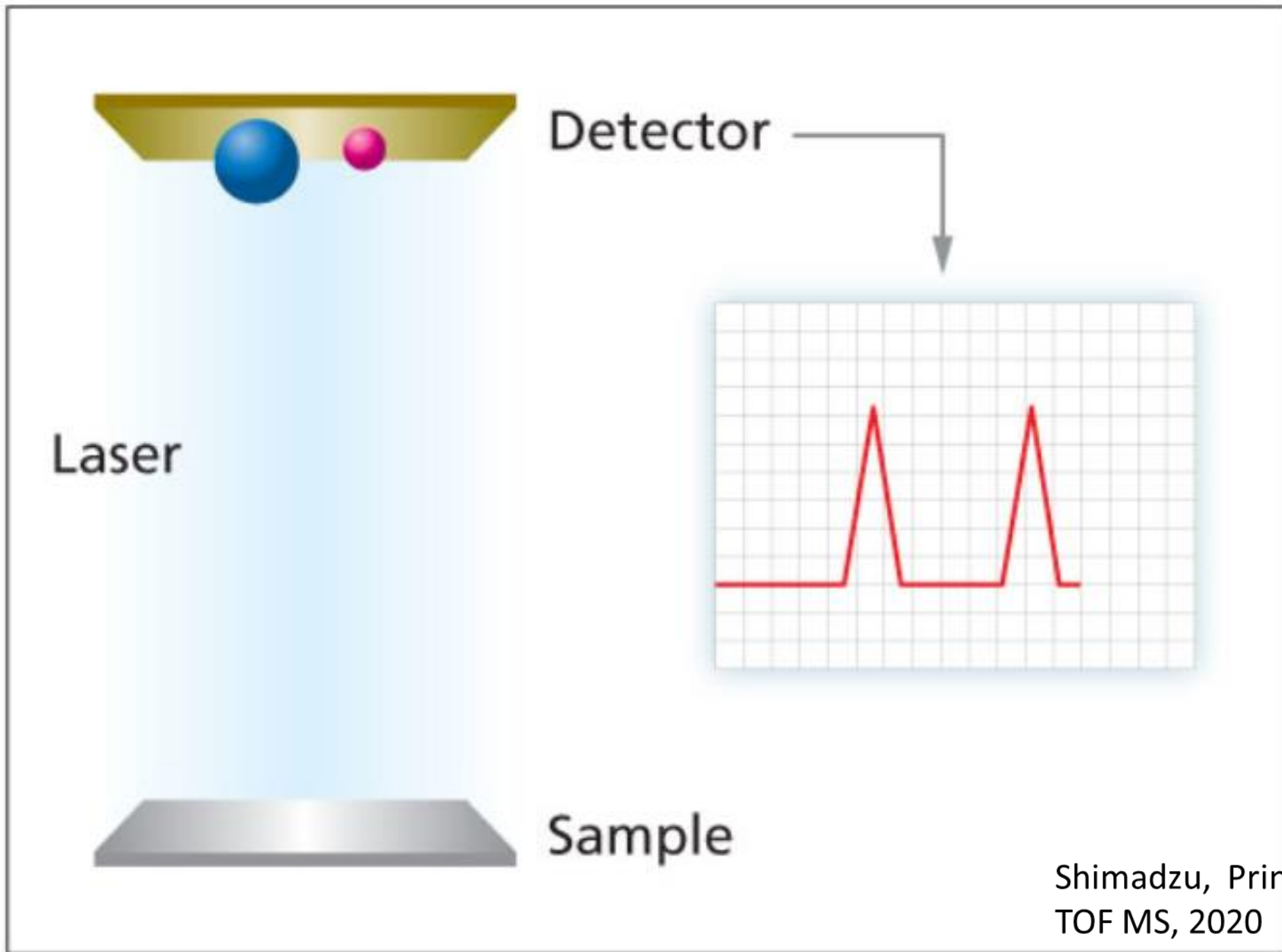
- Ionized microbial molecules accelerated through a positively charged electrostatic field into time of flight (TOF) tube
- Inside vacuum tube ions travel toward an ion detector
- Small analytes travel the fastest (generating mass spectrum)
- Ions emerge from the mass analyser and hit the ion detector → generate a mass spectrum representing the number of ions of a given mass impacting the detector over time

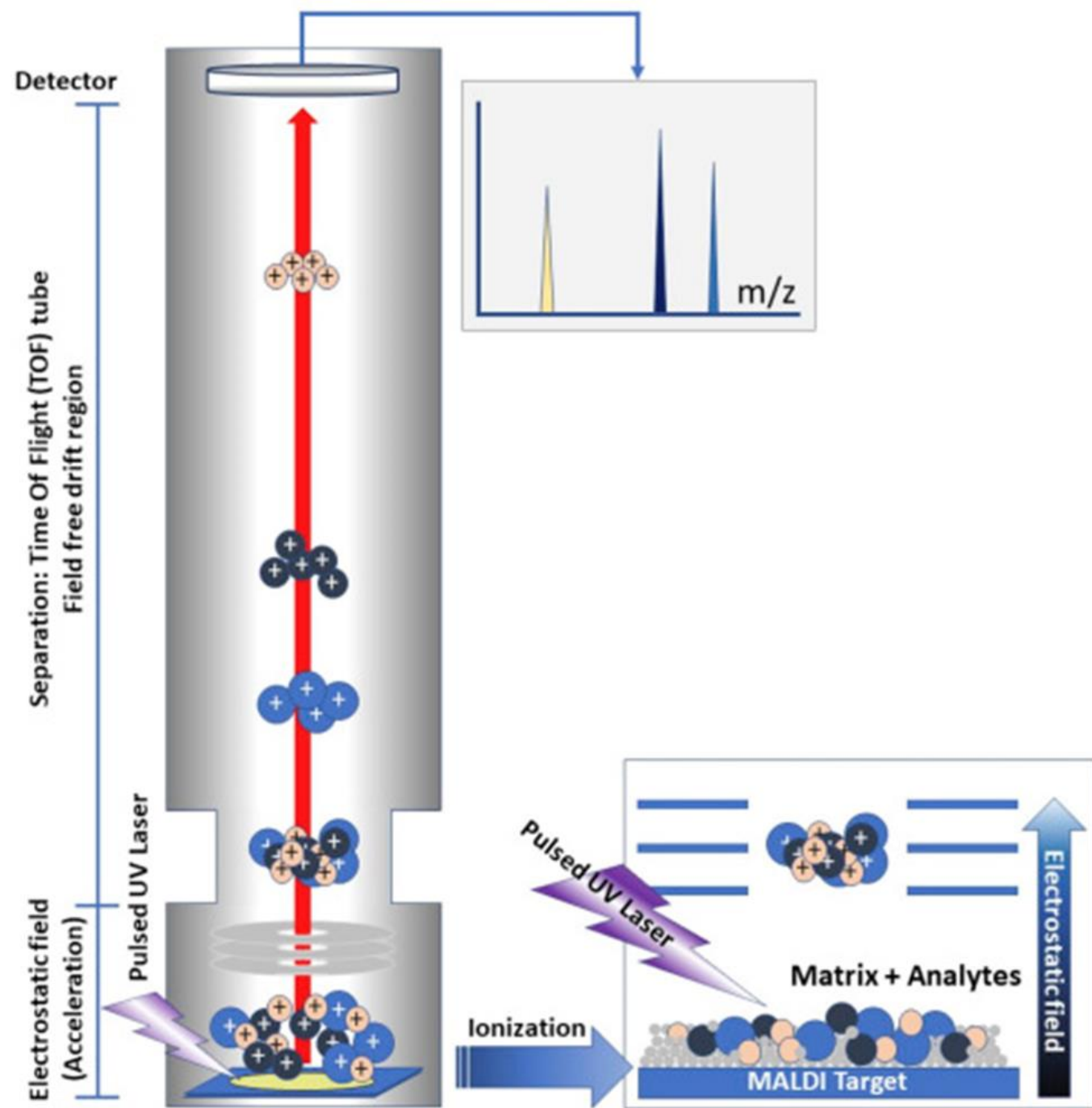


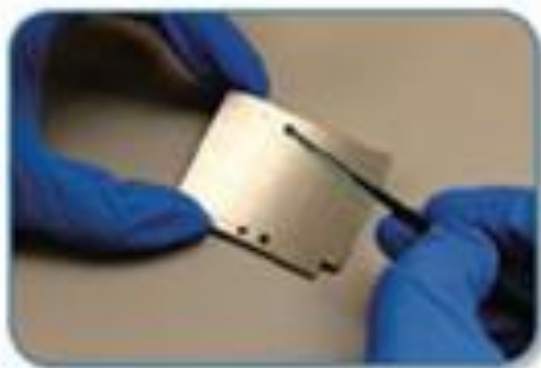










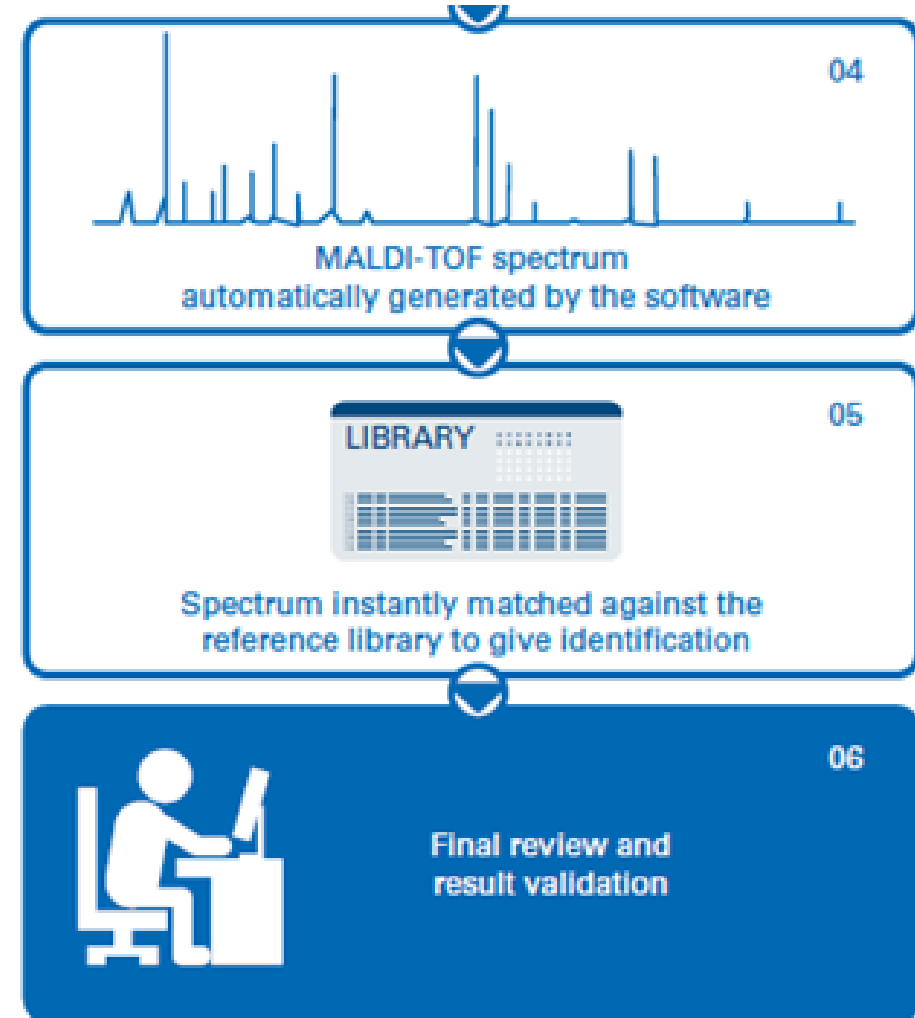


Add Formic Acid
and Dry; Add Matrix
and Dry

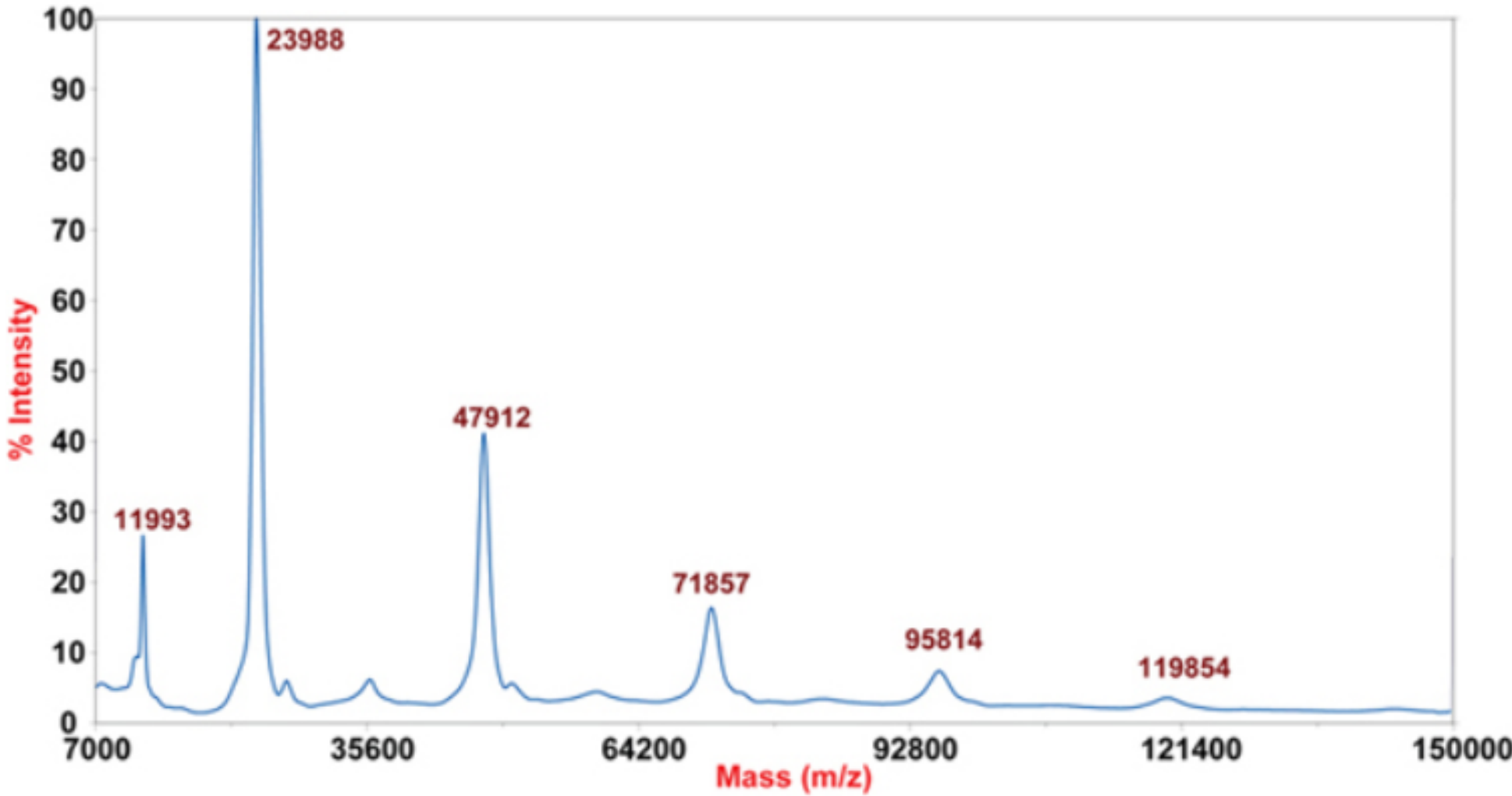


Results

- Mass spectrum provides profile unique to individual types of microbes, with peaks specific to genera and species
- Once acquired compared to a database of reference spectra
- A value - percentage or score is produced

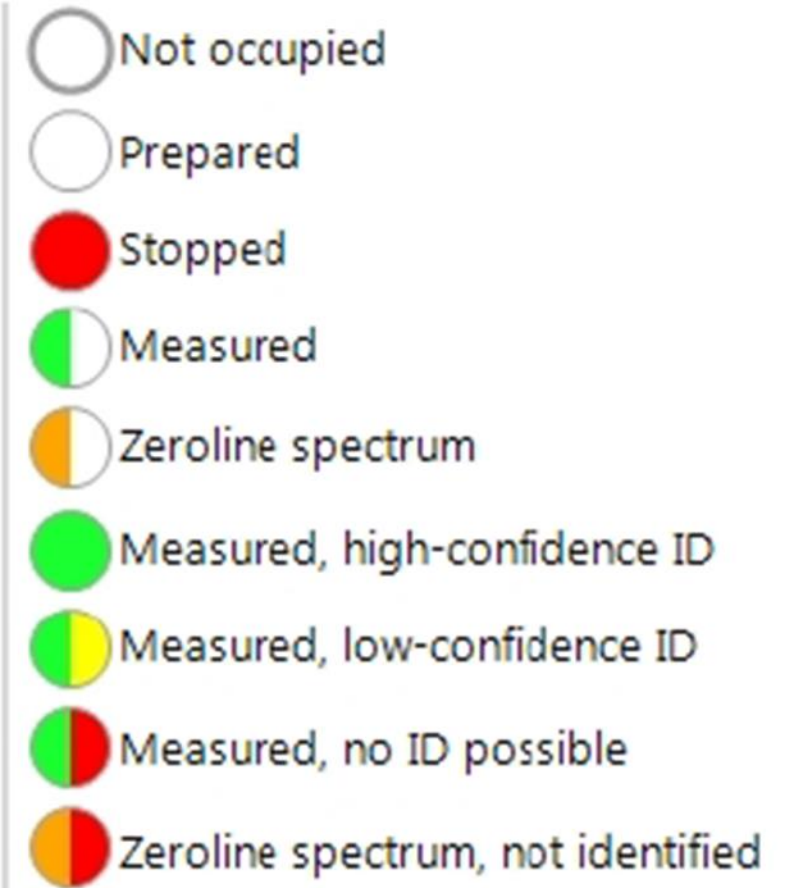


Results



Results

- ID is started immediately after mass spectrum available.
- During the run the appearance of sample and QC positions in the MALDI plate display reflects the success of the measurement and ID at each position
 - If spectrum measurement successful - left half of the sample is green.
 - If measurement fails - left half of the sample is orange
 - Colouring of right half of sample position indicates the score value of ID



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Hide Identified | Group by spec. | Advanced





















































| ID | Position | Detected Species | Score | Comment | Description | Confidence | Export State | Species to Export |
|-------------|----------|------------------|-------|--|-------------------|---|----------------|-------------------|
| ▶ ID of A1 | A1 | Escherichia coli | 2.68 | closely related to Shigella and not d... | Description of A1 | high <input checked="" type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A1 | A2 | | | | Description of A2 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A3 | A3 | | | | Description of A3 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A4 | A4 | | | | Description of A4 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A5 | A5 | | | | Description of A5 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A6 | A6 | | | | Description of A6 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A7 | A7 | | | | Description of A7 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of A8 | A8 | | | | Description of A8 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of B1 | B1 | | | | Description of B1 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of B2 | B2 | | | | Description of B2 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of B3 | B3 | | | | Description of B3 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of B4 | B4 | | | | Description of B4 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |
| ▶ ID of B5 | B5 | | | | Description of B5 | high <input type="radio"/> low <input type="radio"/> unknown <input type="radio"/> | < Best Match > | ▼ |

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Report
run



Hide Identified | Group by spec. | Advanced

| ID | Position | Detected Species | Score | Comment | Description | Confidence | Export State | Species to Export |
|---|----------|------------------|-------------------------------------|---------|---------------------------------------|-------------------|--|--|
| ▶  | ID of A1 | A1 | Escherichia coli | 2.68 | closely related to Shigella and no... | Description of A1 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A1 | A2 | Escherichia coli | 2.75 | closely related to Shigella and no... | Description of A2 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A3 | A3 | Cupriavidus necator | 2.61 | | Description of A3 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A4 | A4 | Staphylococcus aureus | 2.29 | | Description of A4 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A5 | A5 | Escherichia coli | 2.69 | closely related to Shigella and no... | Description of A5 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A6 | A6 | No Organism Identification Possible | 1.41 | | Description of A6 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A7 | A7 | Proteus mirabilis | 2.67 | | Description of A7 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of A8 | A8 | No Organism Identification Possible | 1.10 | is a member of Pseudomonas put... | Description of A8 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of B1 | B1 | Escherichia coli | 2.48 | closely related to Shigella and no... | Description of B1 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of B2 | B2 | Gardnerella vaginalis | 2.51 | | Description of B2 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of B3 | B3 | No Organism Identification Possible | 1.57 | | Description of B3 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of B4 | B4 | Staphylococcus aureus | 2.28 | | Description of B4 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |
| ▶  | ID of B5 | B5 | Enterococcus faecalis | 2.29 | | Description of B5 | high  low  unknown  | <input type="checkbox"/> export < Best Match > ▼ |

MALDI Scores




- Higher the log (score), higher the similarity between mass spectrum of isolate & the database entry in the reference library











| Consistency | Description |
|-------------|--|
| High (A) | Best match is a high-confidence ID Second-best match is: <ul style="list-style-type: none"> high- confidence ID identical sp ID to best match low- confidence ID identical to genus in best match non-identification |
| Low (B) | Requirements for high consistency not met. Best match is a high- or low- confidence ID Second-best match is: <ul style="list-style-type: none"> high- or low-confidence ID identical to genus in best match non-identification |
| None (C) | Requirements for high or low consistency not met |

| Range | Interpretation | Symbols | Color |
|-------------|-------------------------------------|---------|--------|
| 2.00 - 3.00 | High Confidence Identification | (+++) | green |
| 1.70 - 1.99 | Low Confidence Identification | (+) | yellow |
| 0.00 - 1.69 | No Organism Identification Possible | (-) | red |

| Sample Name | Sample ID | Organism (best match) | Score Value | Organism (second best match) | Score Value |
|-----------------------|------------------------|-------------------------------------|-------------|-------------------------------------|-------------|
| <u>A1</u> (+++)(A) | ID of A1 (standard) | <u>Escherichia coli</u> | <u>2.68</u> | <u>Escherichia coli</u> | <u>2.30</u> |
| <u>A2</u> (+++)(A) | ID of A1 (standard) | <u>Escherichia coli</u> | <u>2.75</u> | <u>Escherichia coli</u> | <u>2.35</u> |
| <u>A3</u> (+++)(A) | ID of A3 (standard) | Cupriavidus necator | <u>2.61</u> | Cupriavidus necator | <u>2.15</u> |
| <u>A4</u> (+++)(A) | ID of A4 (standard) | Staphylococcus aureus | <u>2.29</u> | Staphylococcus aureus | <u>2.27</u> |
| <u>A5</u> (+++)(A) | ID of A5 (standard) | <u>Escherichia coli</u> | <u>2.69</u> | <u>Escherichia coli</u> | <u>2.30</u> |
| <u>A6</u> (-)(C) | ID of A6 (standard) | No Organism Identification Possible | <u>1.41</u> | No Organism Identification Possible | <u>1.38</u> |
| <u>A7</u> (+++)(A) | ID of A7 (standard) | Proteus mirabilis | <u>2.67</u> | Proteus mirabilis | <u>2.66</u> |
| <u>A8</u> (-)(C) | ID of A8 (standard) | No Organism Identification Possible | <u>1.10</u> | No Organism Identification Possible | <u>1.10</u> |

Result overview table--continued on next page

| | ID | Position | Detected Species | Score | Comment |
|---|--|----------|-------------------------------------|-------|---------------------------------------|
| ▶ |  ID of A5 | A5 | Escherichia coli | 2.69 | closely related to Shigella and no... |
| ▶ |  ID of A6 | A6 | No Organism Identification Possible | 1.41 | |
| ◀ |  ID of A7 | A7 | Proteus mirabilis | 2.67 | |

| | Score ▾ | Detected Species | Comment | Link |
|---|---------|---|---------|---------------------|
|  | 2.67 | Proteus mirabilis DSM 18254 DSM | | 584 |
|  | 2.66 | Proteus mirabilis 9482_2 CHB | | 584 |
|  | 2.54 | Proteus mirabilis DSM 30115 DSM | | 584 |
|  | 2.53 | Proteus mirabilis DSM 46227 DSM | | 584 |
|  | 2.52 | Proteus mirabilis DSM 788 DSM | | 584 |
|  | 2.49 | Proteus mirabilis DSM 50903 DSM | | 584 |
|  | 2.46 | Proteus mirabilis RV412_A1_2010_06b LBK | | 584 |
|  | 2.39 | Proteus mirabilis 13210_1 CHB | | 584 |
|  | 2.19 | Proteus mirabilis (PX) 22086112 MLD | | 584 |
|  | 1.72 | Proteus vulgaris DSM 13625 DSM | | 585 |

| | | | | | |
|---|--|----|-------------------------------------|------|----------------------------------|
| ▶ |  ID of A8 | A8 | No Organism Identification Possible | 1.10 | is a member of Pseudomonas pu... |
|---|--|----|-------------------------------------|------|----------------------------------|

Advantages

- Rapid (≤ 3 minutes per isolate)
- Inexpensive - low reagent cost
- Small amounts of organism are required
- Direct sample ID possible
- Reduced labour
- Accurate ID

| Microorganism group | Number of processed samples | Number of correct identifications | Number of incorrect identifications |
|---------------------------------------|-----------------------------|-----------------------------------|-------------------------------------|
| Non-fermenting Gram-negative bacteria | 229 | 215 (93.89%) | 14 (6.11%) |
| Enterobacteriaceae | 265 | 263 (99.25%) | 2 (0.75%) |
| Other Gram-negative bacteria | 204 | 195 (95.59%) | 9 (4.41%) |
| Gram-positive bacteria | 230 | 224 (97.39%) | 6 (2.61%) |
| Yeasts | 225 | 219 (97.33%) | 6 (2.67%) |
| Total number | 1153 | 1116 (96.79%) | 37 (3.21%)* |

* Of the 1153 samples, 0.61% (7 samples) provided low-confidence identifications; 1.91% (22 samples) could not be identified; and 0.69% (8 samples) gave a false result.

Limitations

- Databases are proprietary unlike publicly available sequence databases
- Difficulties in ID with some organisms
- Difficulty analysing mixed cultures
- Identifying organisms from liquid cultures
- Low identification scores - repeat testing for 10% of isolates
- Growth on some media may be associated with low scores
- Small/mucoid colonies may fail ID
- ID of biosafety level 3/4 organisms
- Requires room temperature (20-25 °C)
- Human error

Difficulties in Identification

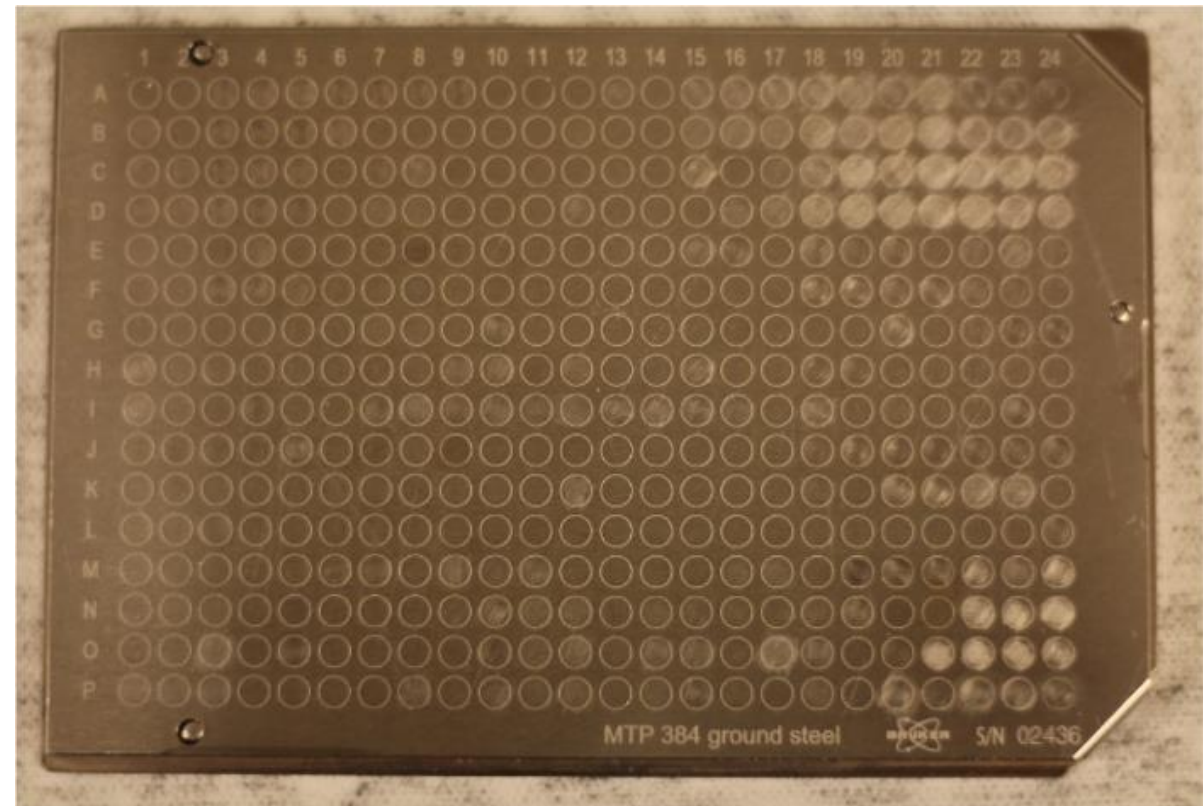
- Misidentification rare
- Can occur with closely related organisms
 - E. coli and Shigella
 - Discrimination between species from same complex eg. E cloacae complex
- Salmonella can only be ID to genus level - No typing
- Difficulties with some species - alpha haemolytic strep
- Mycobacteria & filamentous fungi

Considerations for other organisms

- Mycobacteria:
 - Requires processing to kill tested bacteria, break down cell envelopes, disrupt clumped cells
 - Can ID most clinically relevant species
 - MTB complex ID to complex level only
 - Some related mycobacterium species not well differentiated (*M. chimaera* and *M. intracellulare*)
- Enhanced databases ID *Nocardia* – often specific extraction processes needed
- Fungi:
 - Can identify yeast well
 - Filamentous fungi limited - variable phenotypes & protein spectra vary with growth conditions
 - Available for *Aspergillus*, *Fusarium* & *Mucorales*

Common Sources of Error

- Colony inoculation in erroneous target plate locations
- Testing impure colonies
- Smearing between spots
- Failure to clean target plates
- Entry of wrong results



Conclusion

- MALDI-TOF MS utilizes:
 - Laser & matrix as an ion source
 - TOF (electric field) as a mass analyser
 - Ion detector
- Provides a rapid, accurate and cost-effective method for ID of many bacteria & yeast
- Several limitations which operators need to be aware of when reporting & troubleshooting