# Mass spectrometry Technologies in Lipid chemistry

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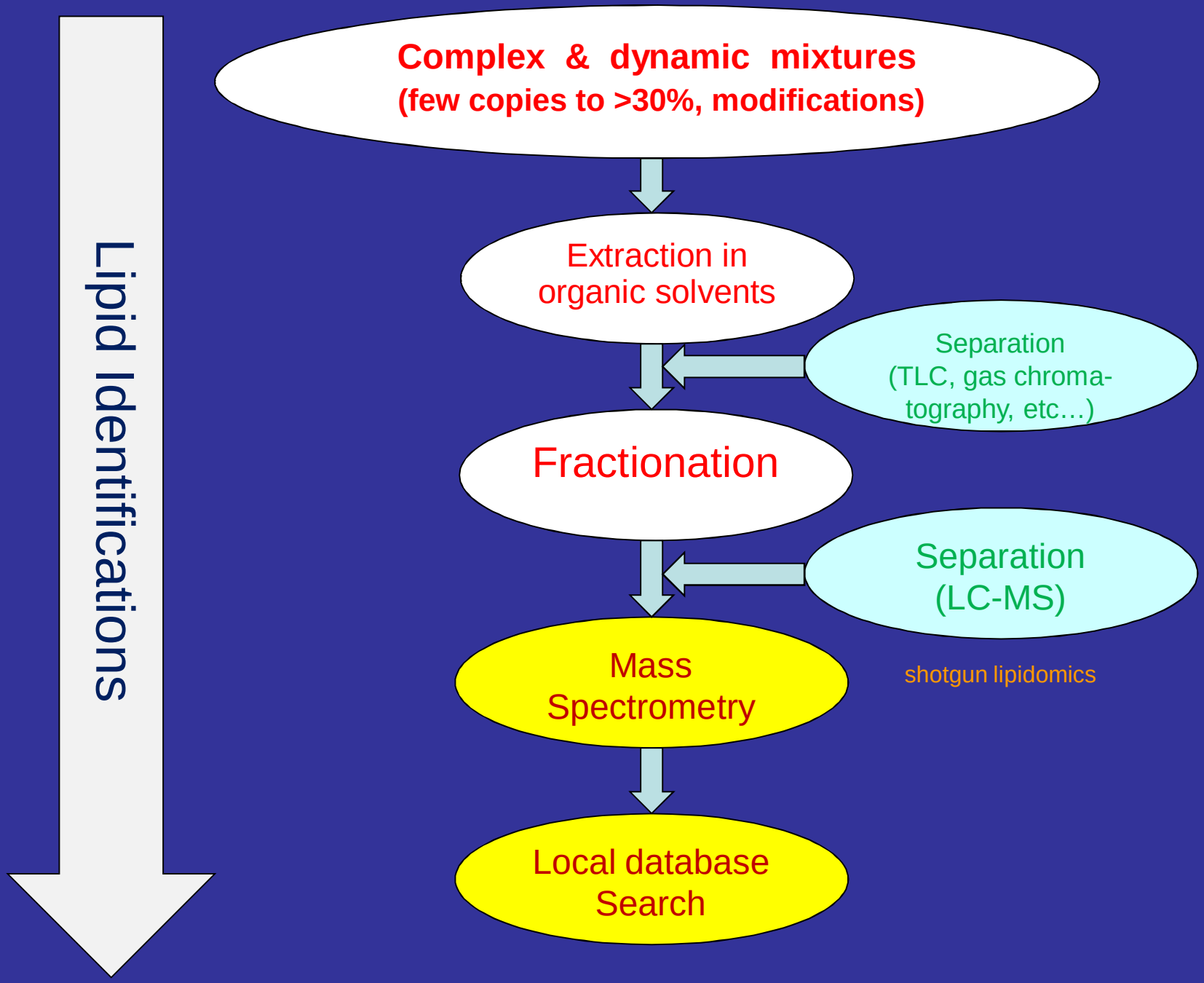

Lipid Identifications
Complex & dynamic mixtures
(few copies to >30%, modifications)
Extraction in organic solvents
Separation (TLC, gas chroma-tography, etc…)
Fractionation
Separation (LC-MS)
shotgun lipidomics
Mass Spectrometry
Local database Search

# Lipids

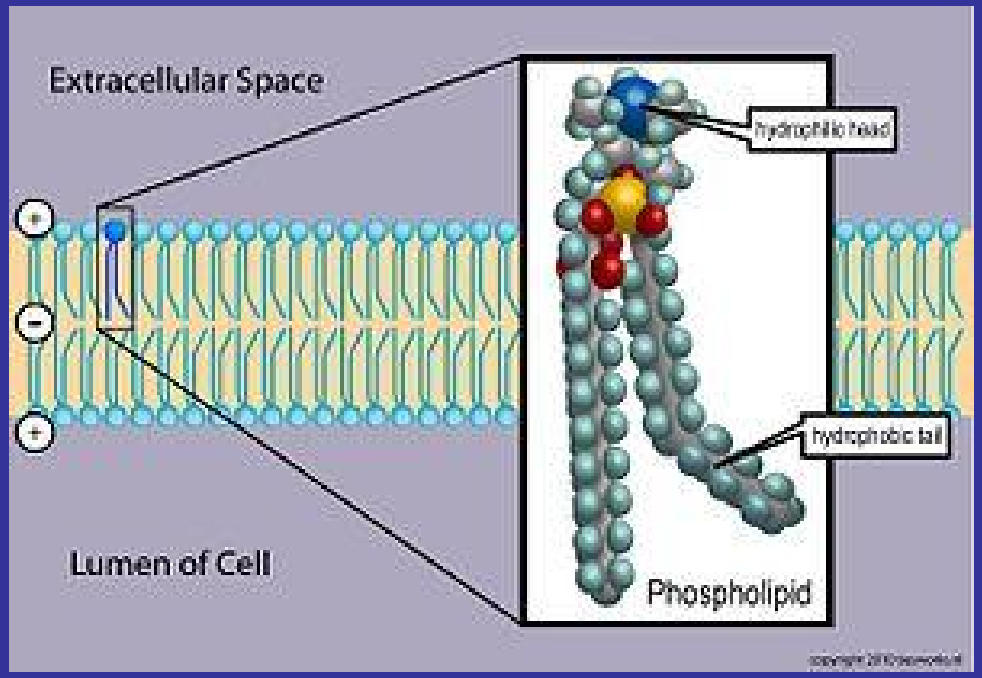
- All Lipids are hydrophobic at one end and hydrophilic at the other end.
- That's the one property they have in common

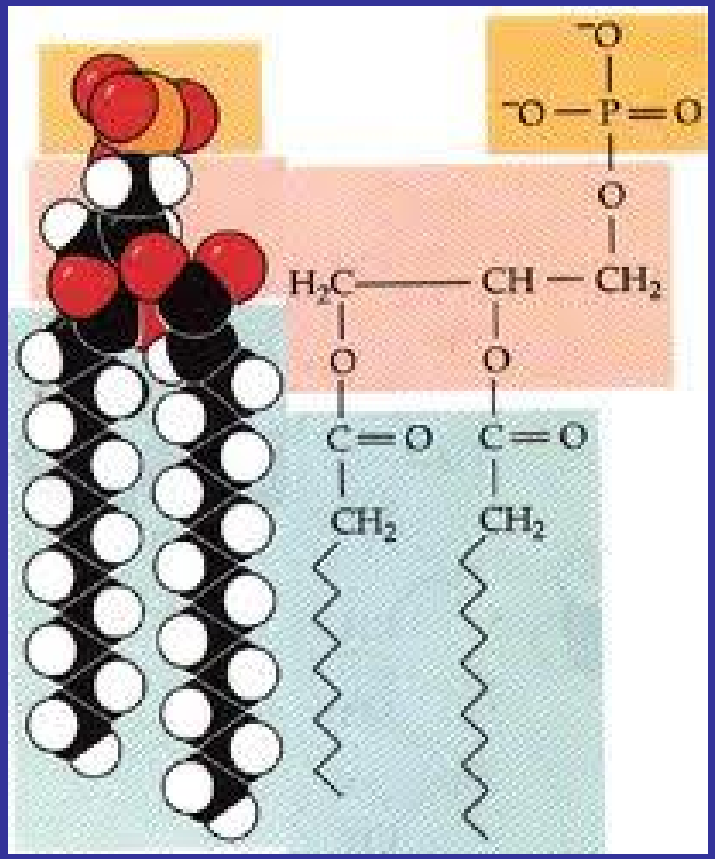
- Lipids includes fats and oils, waxes, **phospholipids**, steroids (like cholesterol), and some other related compounds

# Phospholipids

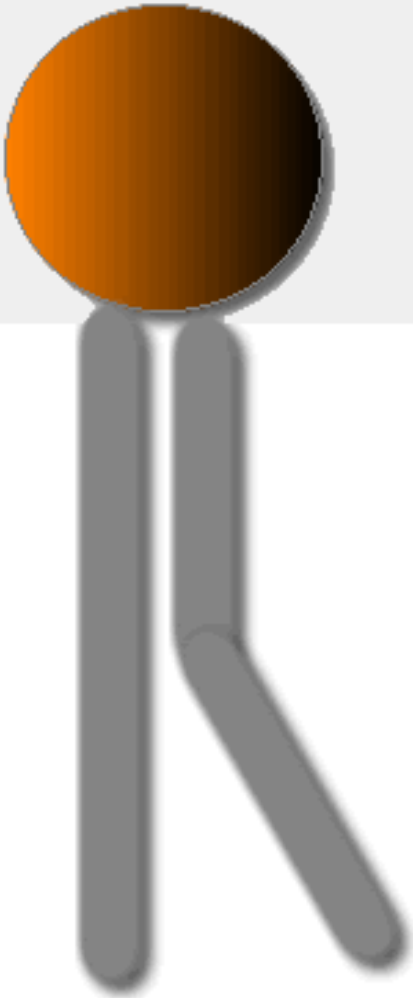
- phospho-, glycero-, or sphingolipids
- major component of all cell membranes as they can form lipid bilayers
- contain a diglyceride, a phosphate group, and an organic molecule such as choline
- *except* sphingomyelin (sphingosine instead of glycerol)

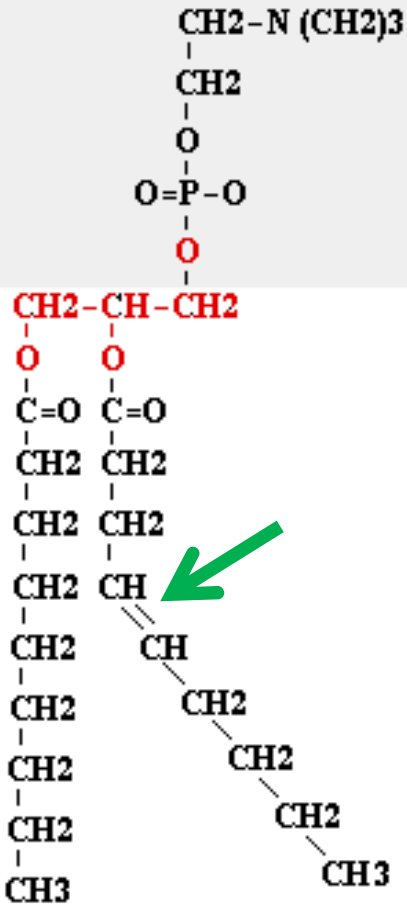
# Phospholipids

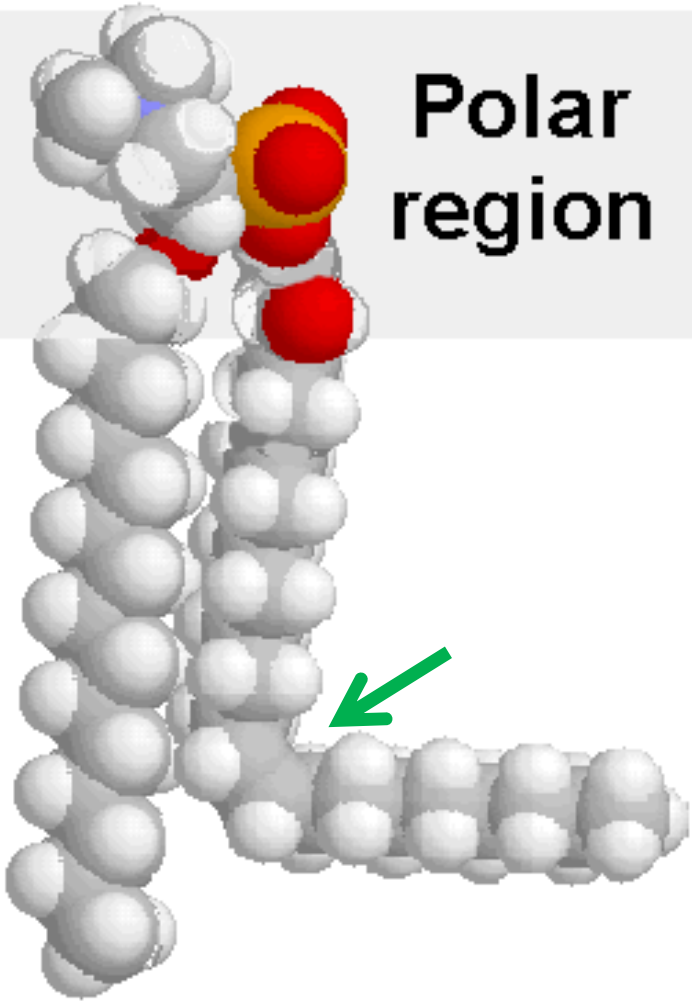


# Phospholipids





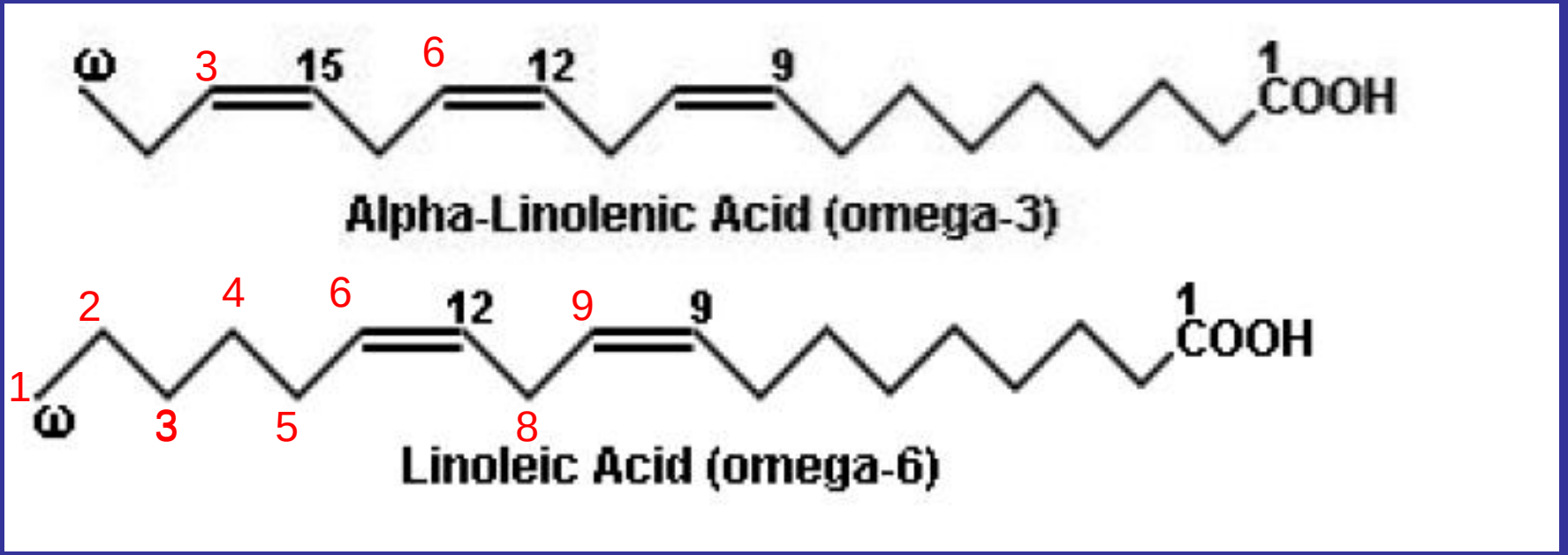



# Fatty acids

Table 1-B-1. Cellular fatty acids.

| Chemical Formula | Name |
|---|---|
| **Saturated fatty acid** | |
| $CH_3(CH_2)_{10}COOH$ | Lauric |
| $CH_3(CH_2)_{12}COOH$ | Myristic |
| $CH_3(CH_2)_{14}COOH$ | Palmitic |
| $CH_3(CH_2)_{16}COOH$ | Stearic |
| $CH_3(CH_2)_{18}COOH$ | Arachidic |
| $CH_3(CH_2)_{22}COOH$ | Lignoceric |
| **Unsaturated fatty acid** | |
| $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$ | Palmitoleic |
| $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ | Oleic |
| $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$ | Linoleic |
| $CH_3(CH_2)_4(CH=CHCH_2)_3CH=CH(CH_2)_3COOH$ | Arachidonic |
| $CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$ | Linolenic |

# Essential fatty acids

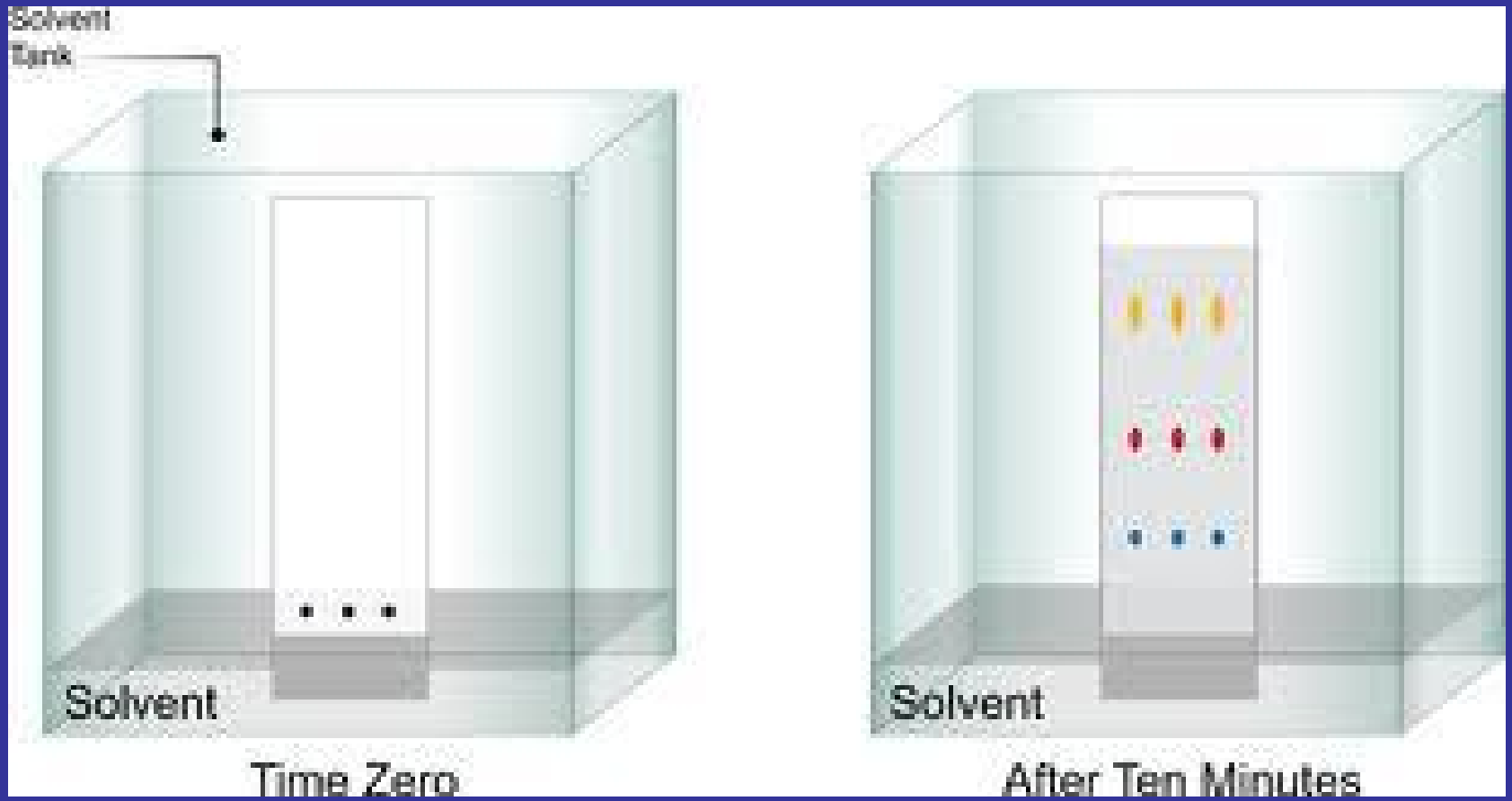


# Lipid separation / detection

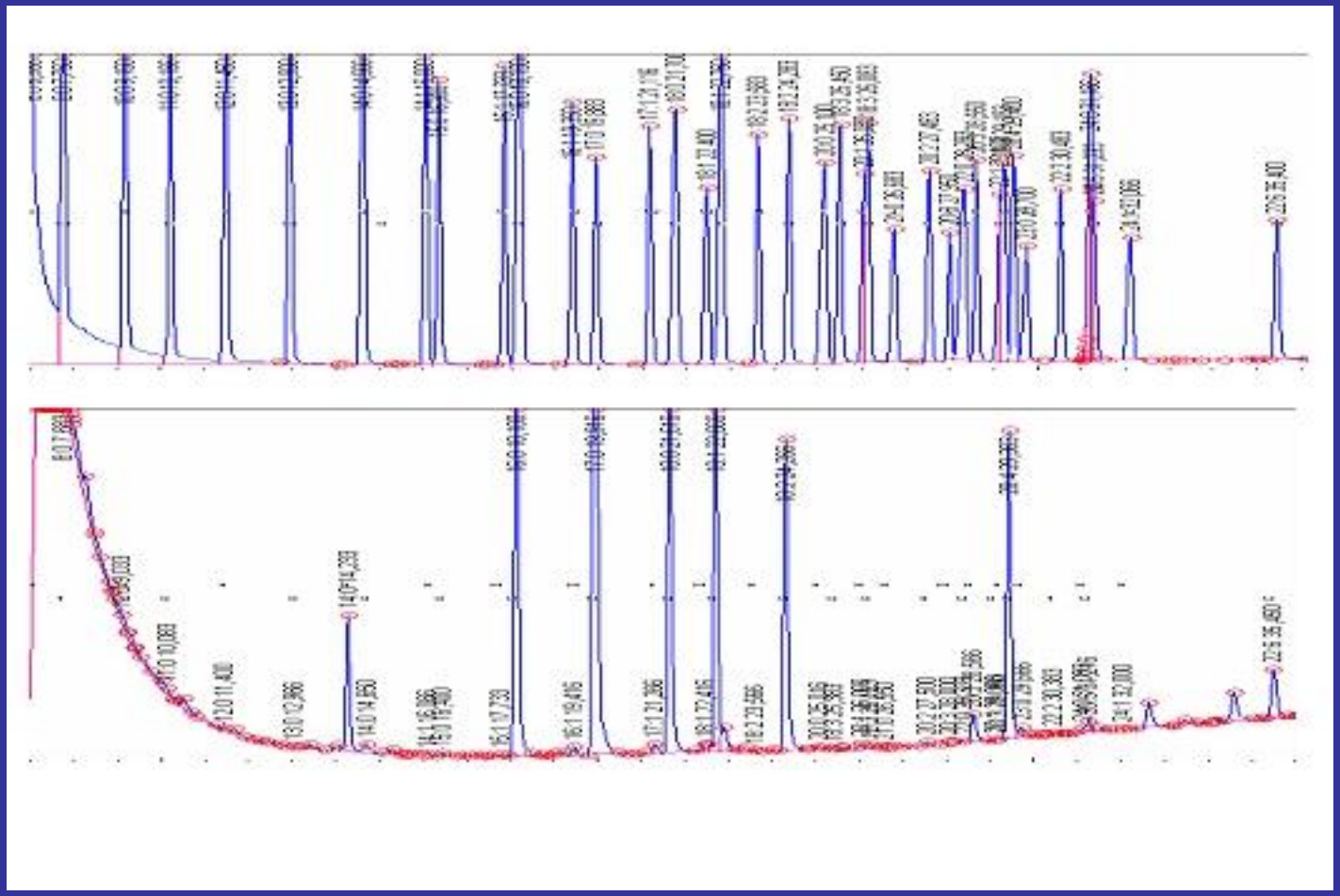
- Liquid chromatography (HPLC, UPLC, UHPLC)
- Gas chromatography
- Thin layer chromatography

- Mass spectrometry (detection)
- Colorimetric detection

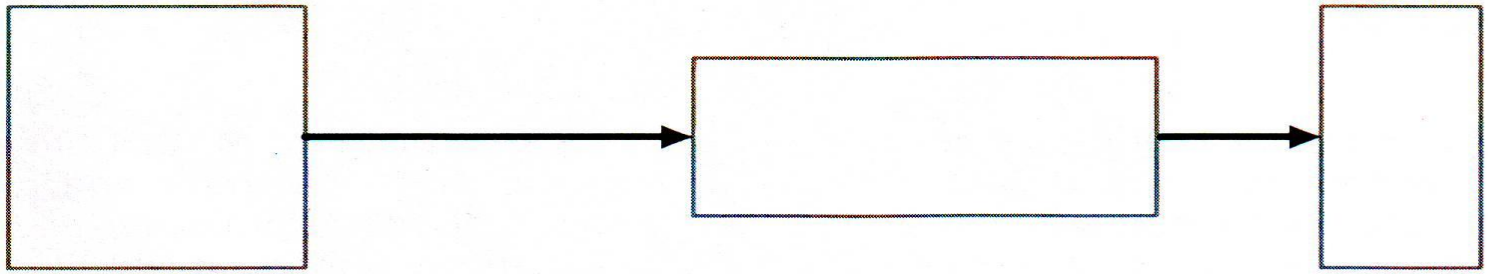
# TLC, a separation technique on the basis of components differences in polarity



# Gas chromatogram with 37 resolved fatty acids of a fatty acid standard



# Mass spectrometry as a tool to analyze lipids




Ion formation → Ion separation → Ion detection

| Ion formation | Ion separation | Ion detection |
|---|---|---|
| Electron Impact (EI) | Quadrupol | Faraday Cup |
| Chemical Ionization (CI) | Magnetic Sector | Secondary Electron Multiplier (SEM) |
| Fast Atom Bombardment (FAB) | Electric Sector | Scintillator |
| Electrospray Ionization (ESI) | Ion Trap | Micro Channel Plate (MCP) |
| Matrix Assisted Laser Desorption/Ionization (MALDI) | Ion Cyclotron | |
| | Time-Of-Flight | |
| | Ion Mobility | |

**Figure 1 Components of a mass spectrometer**

# MALDI vs ESI

- **Matrix-Assisted Laser Desorption Ionization (MALDI):**
- **ElectroSpray Ionization (ESI):**
- both ESI and MALDI belongs to the "soft" ionization techniques.

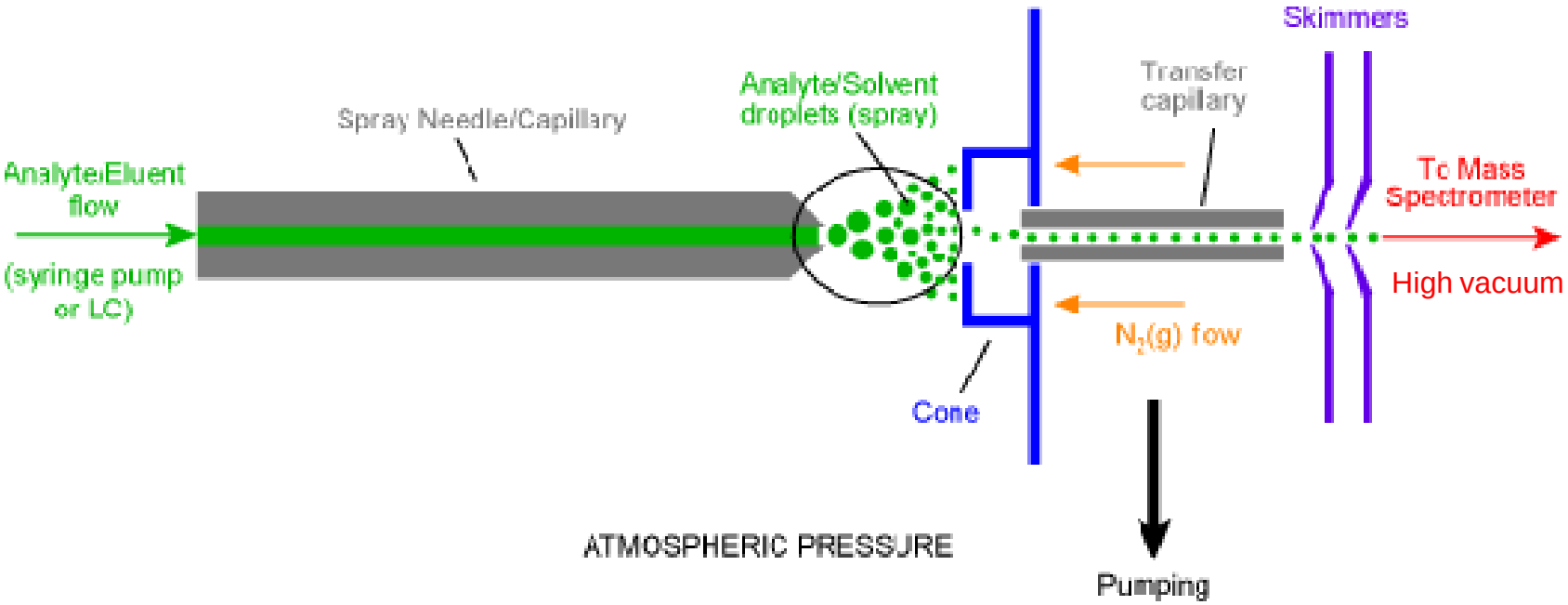
# Biomolecule sample requirements for MS

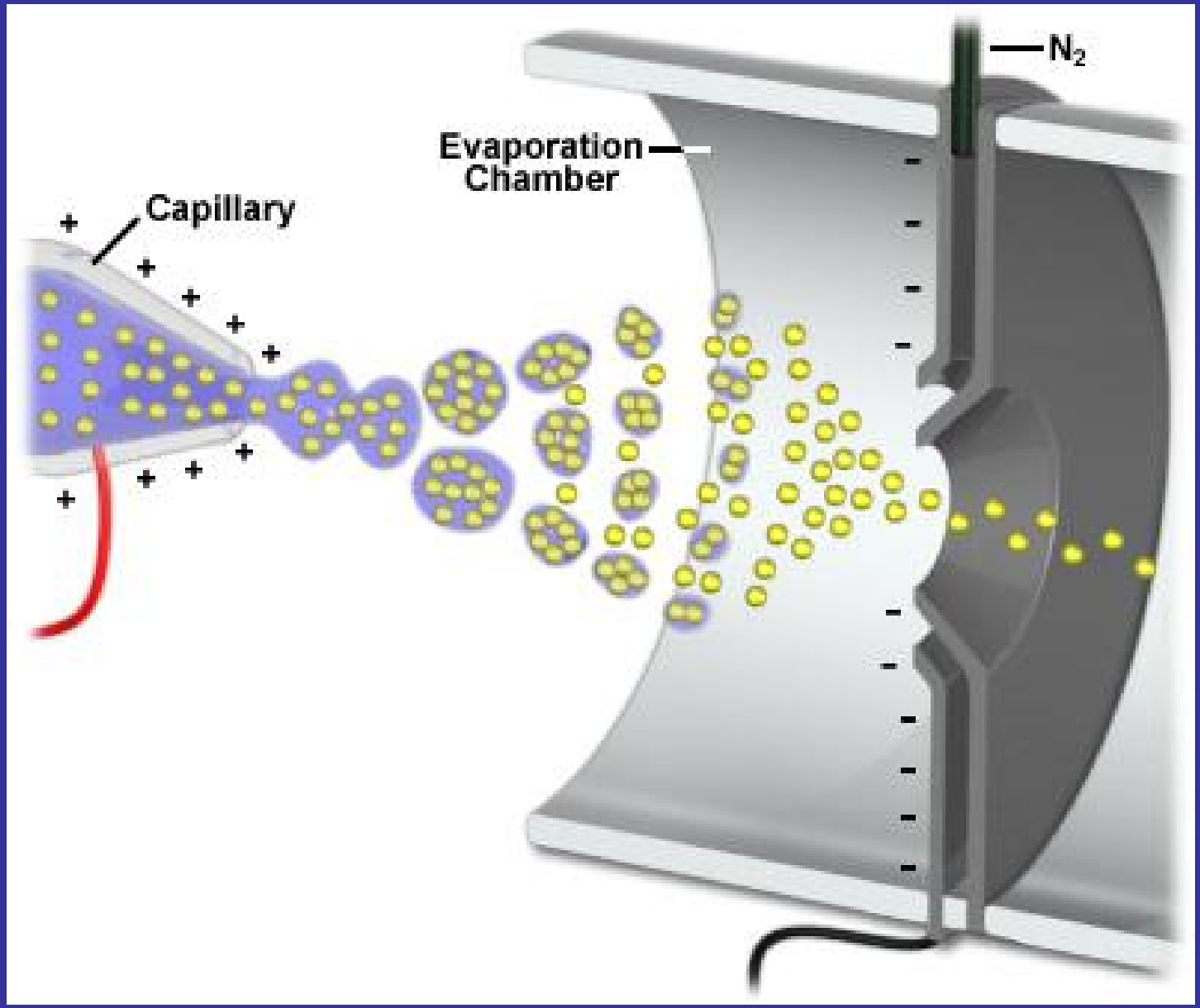
For better ionization, sample should preferably be in a volatile solvent

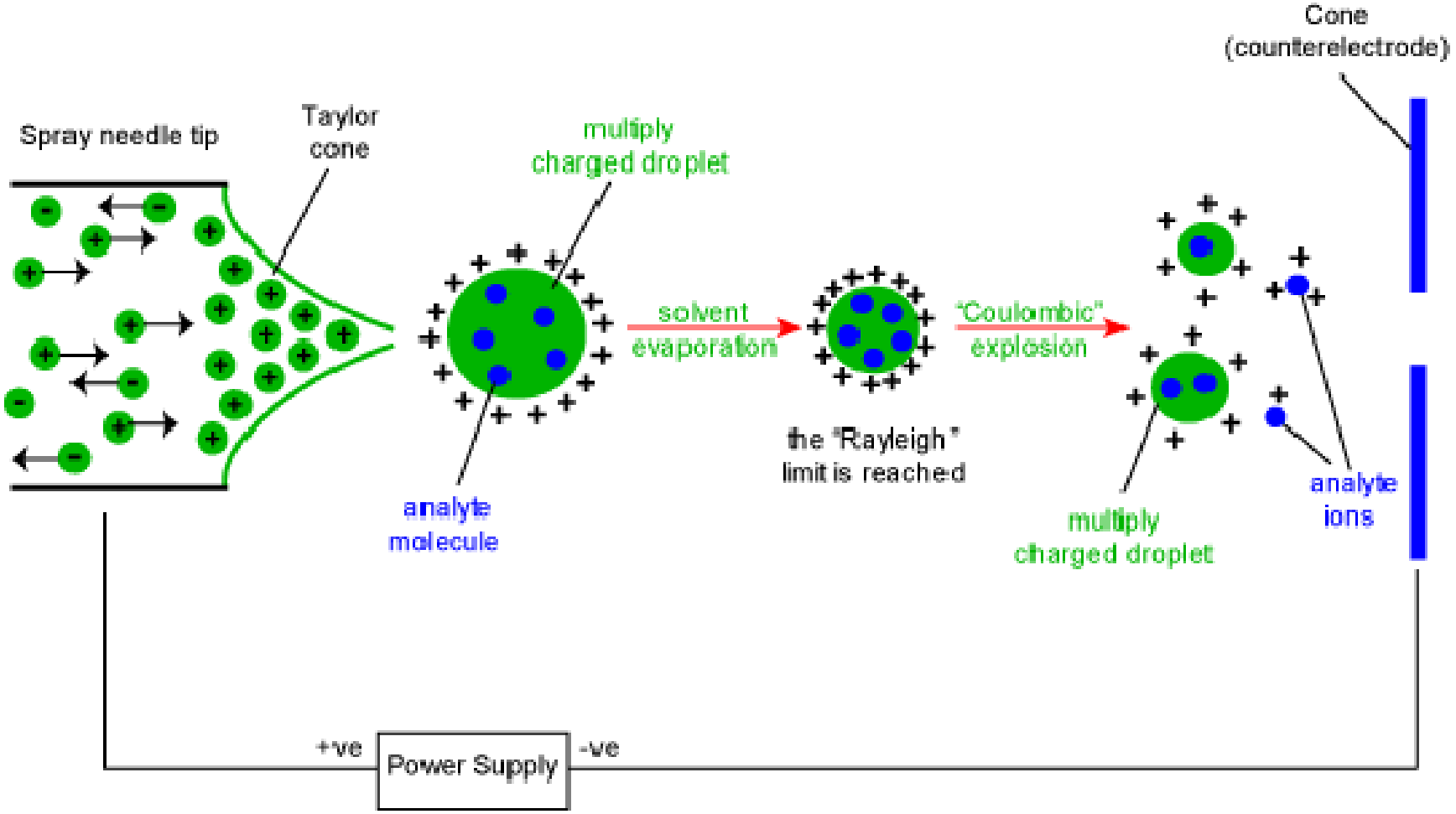
- e.g. ACN (acetonitrile e.g. 50%), methanol, propanol, etc…

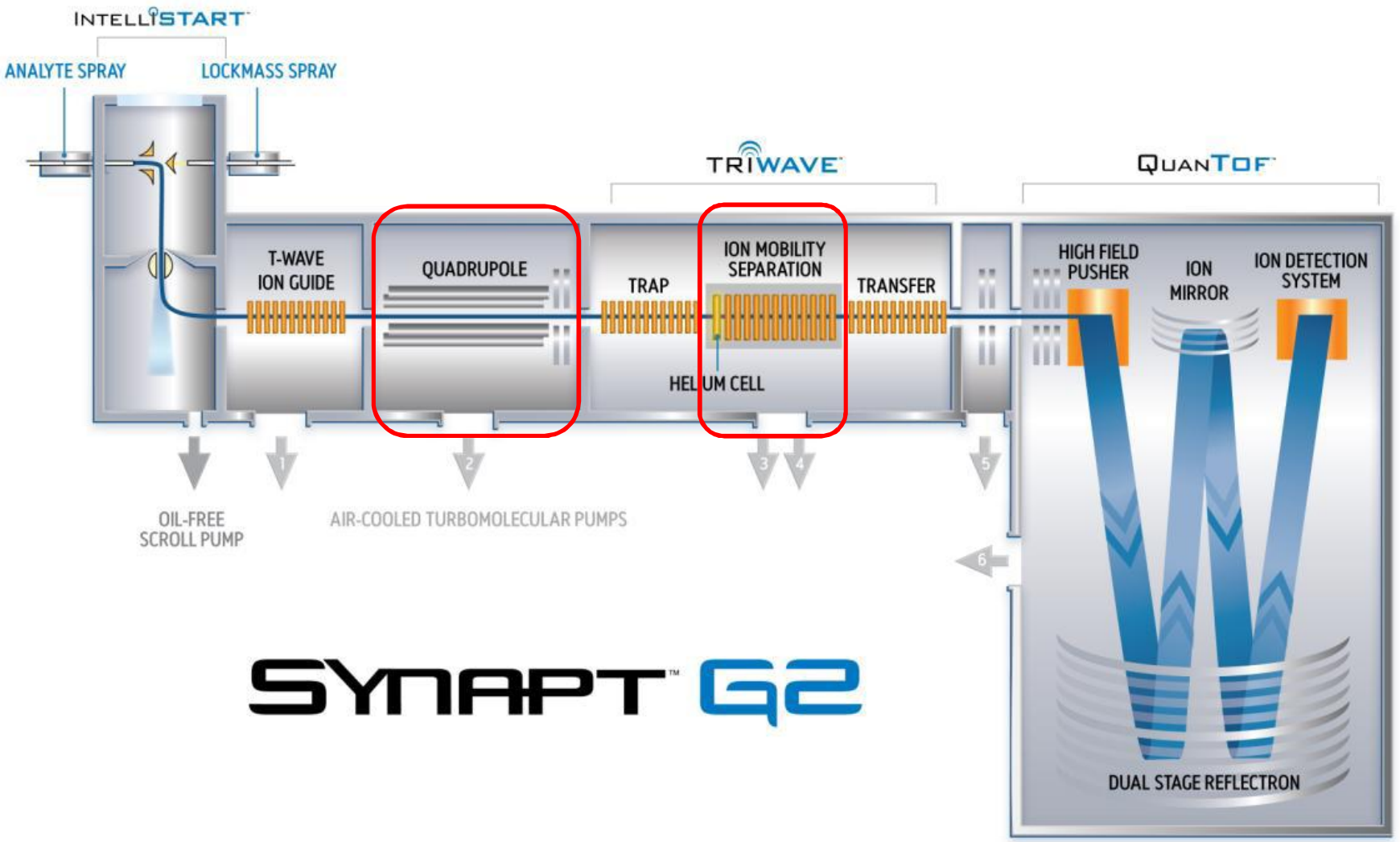
- Samples from liquid chromatograph

# Electrospary Ionization

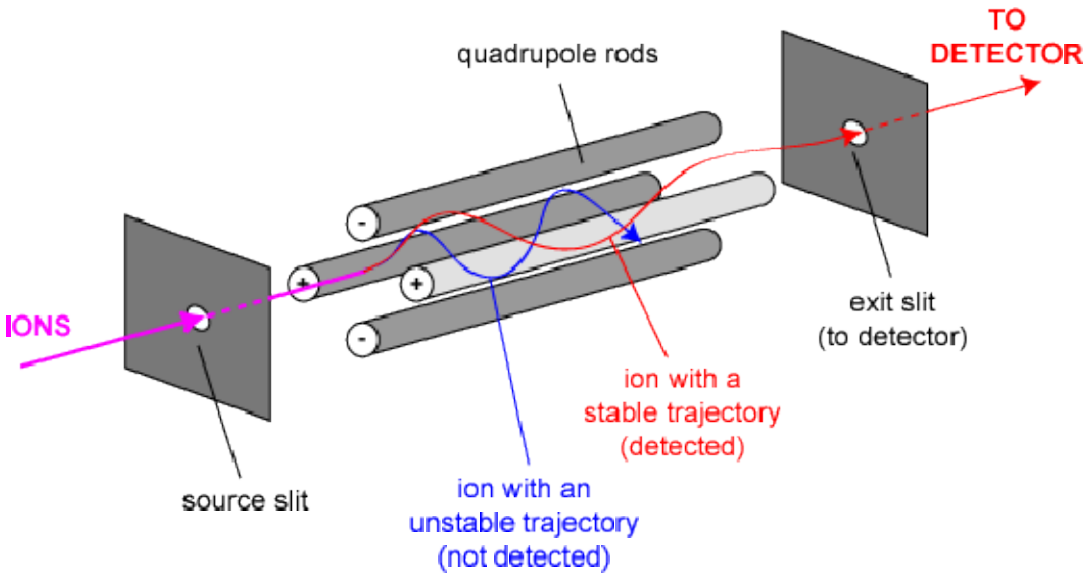


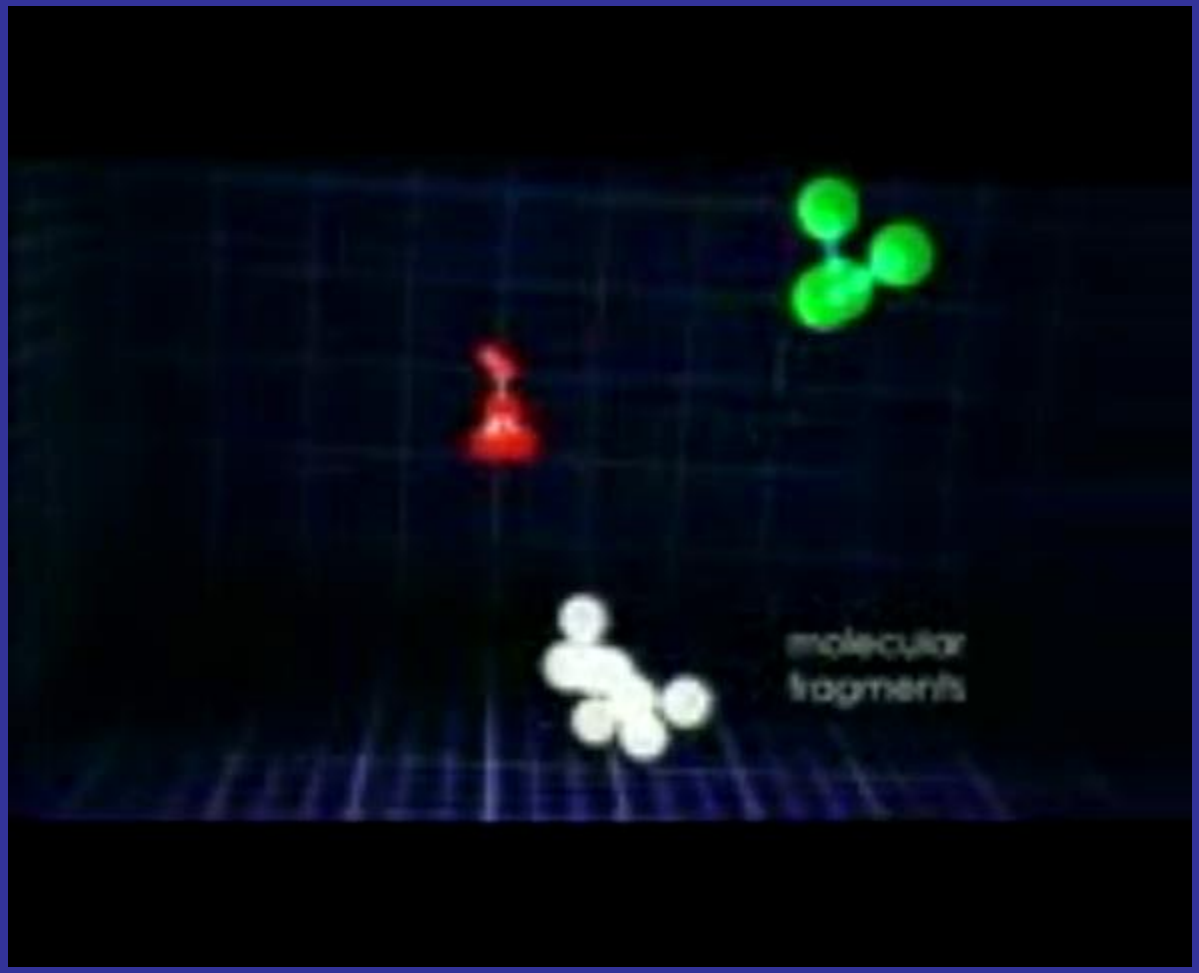

$N_2$
Evaporation Chamber
Capillary


Cone
(counterelectrode)
Spray needle tip
Taylor
cone
multiply
charged droplet
solvent
evaporation
"Coulombic"
explosion
the "Rayleigh"
limit is reached
analyte
molecule
multiply
charged droplet
analyte
ions
+ve
Power Supply
-ve

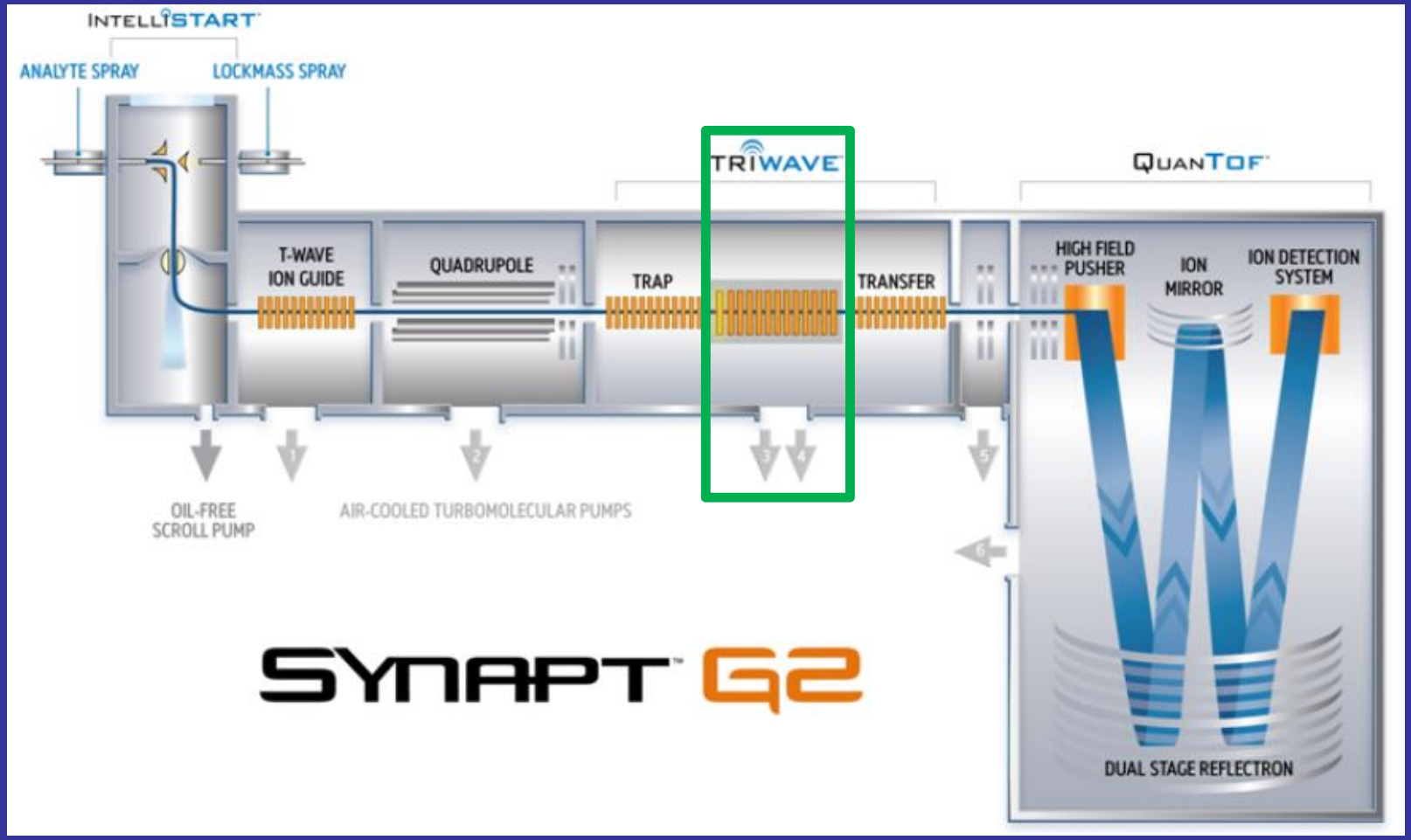

INTELLISTART
ANALYTE SPRAY
LOCKMASS SPRAY
TRIWAVE
QUANTOF
T-WAVE
ION GUIDE
QUADRUPOLE
TRAP
ION MOBILITY
SEPARATION
TRANSFER
HELIUM CELL
HIGH FIELD
PUSHER
ION
MIRROR
ION DETECTION
SYSTEM
1
2
3
4
5
6
OIL-FREE
SCROLL PUMP
AIR-COOLED TURBOMOLECULAR PUMPS
DUAL STAGE REFLECTRON
SYNAPT G2

Ions are separated in a quadrupole based on the stability of their trajectories in the oscillating electric fields that are applied to the rods

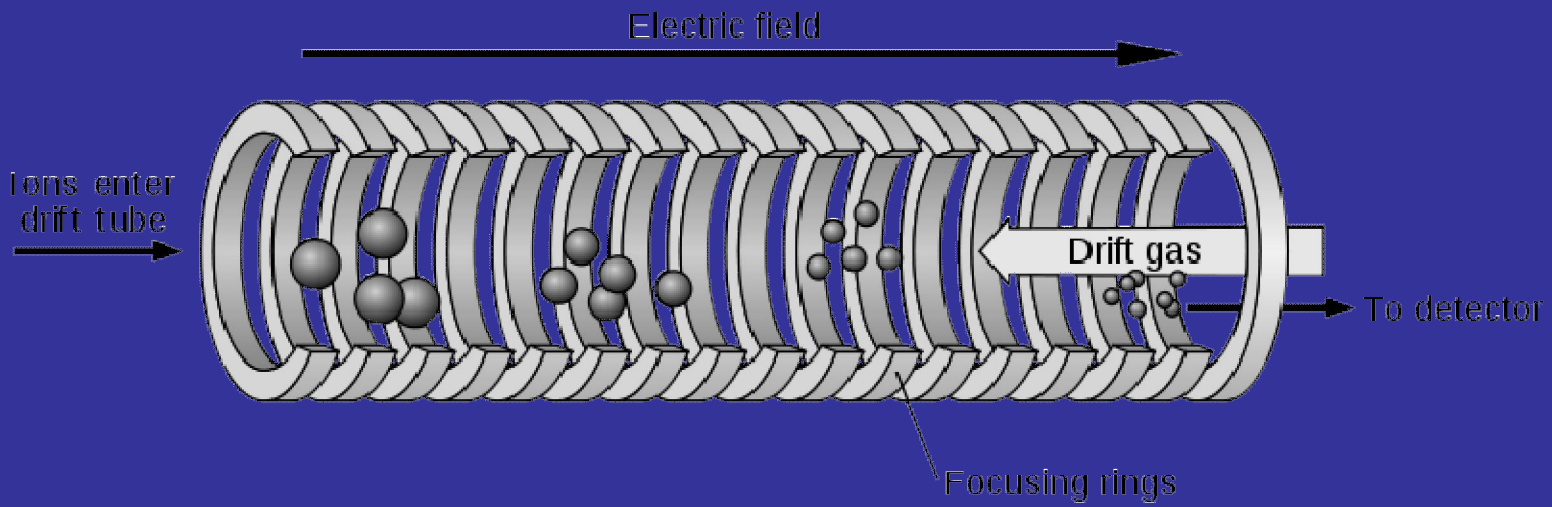



molecular
fragments

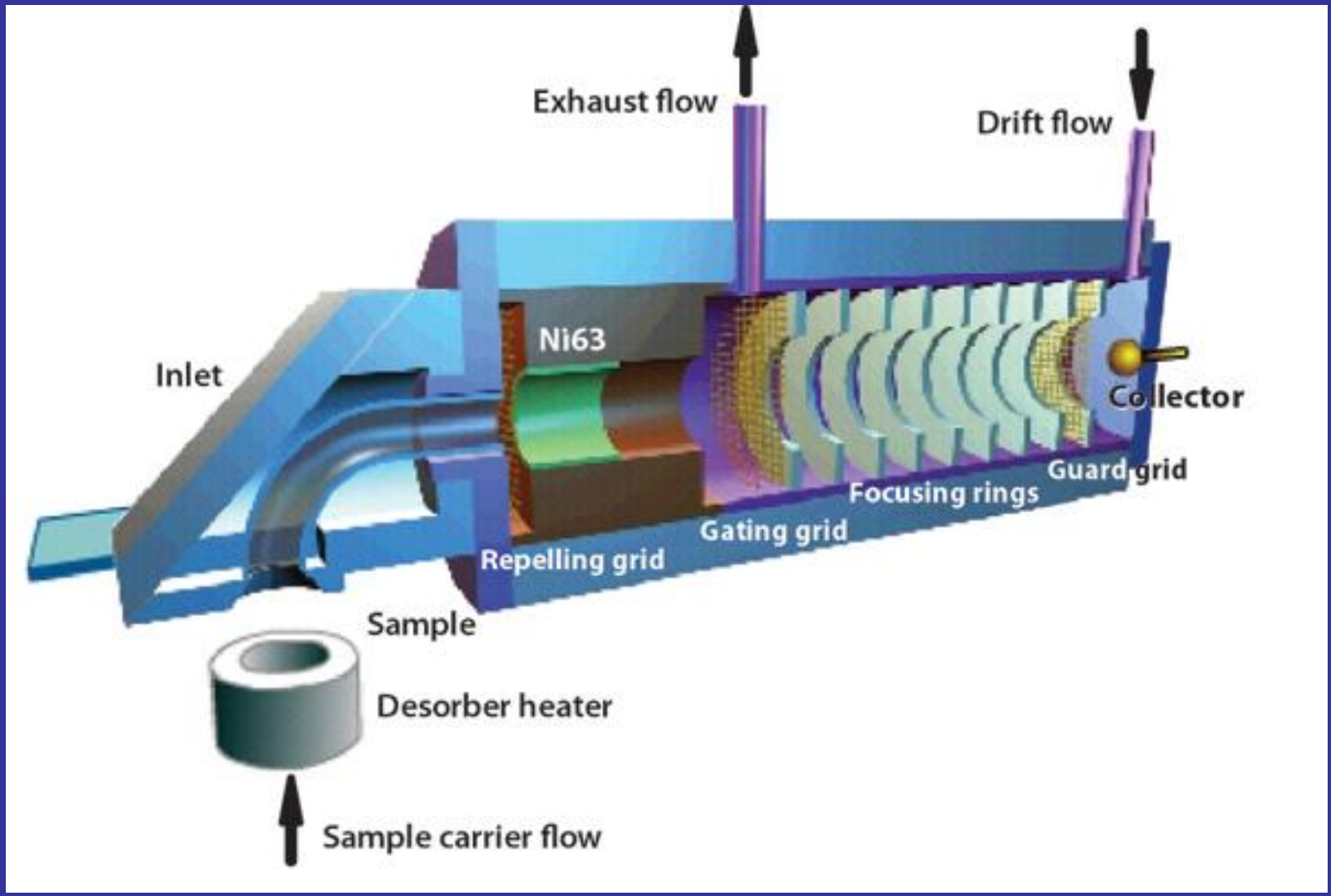
# Ion Mobility Mass Spectrometry



# Ion Mobility Mass Spectrometry




Ion funnel / ring electrodes in Synapt G2



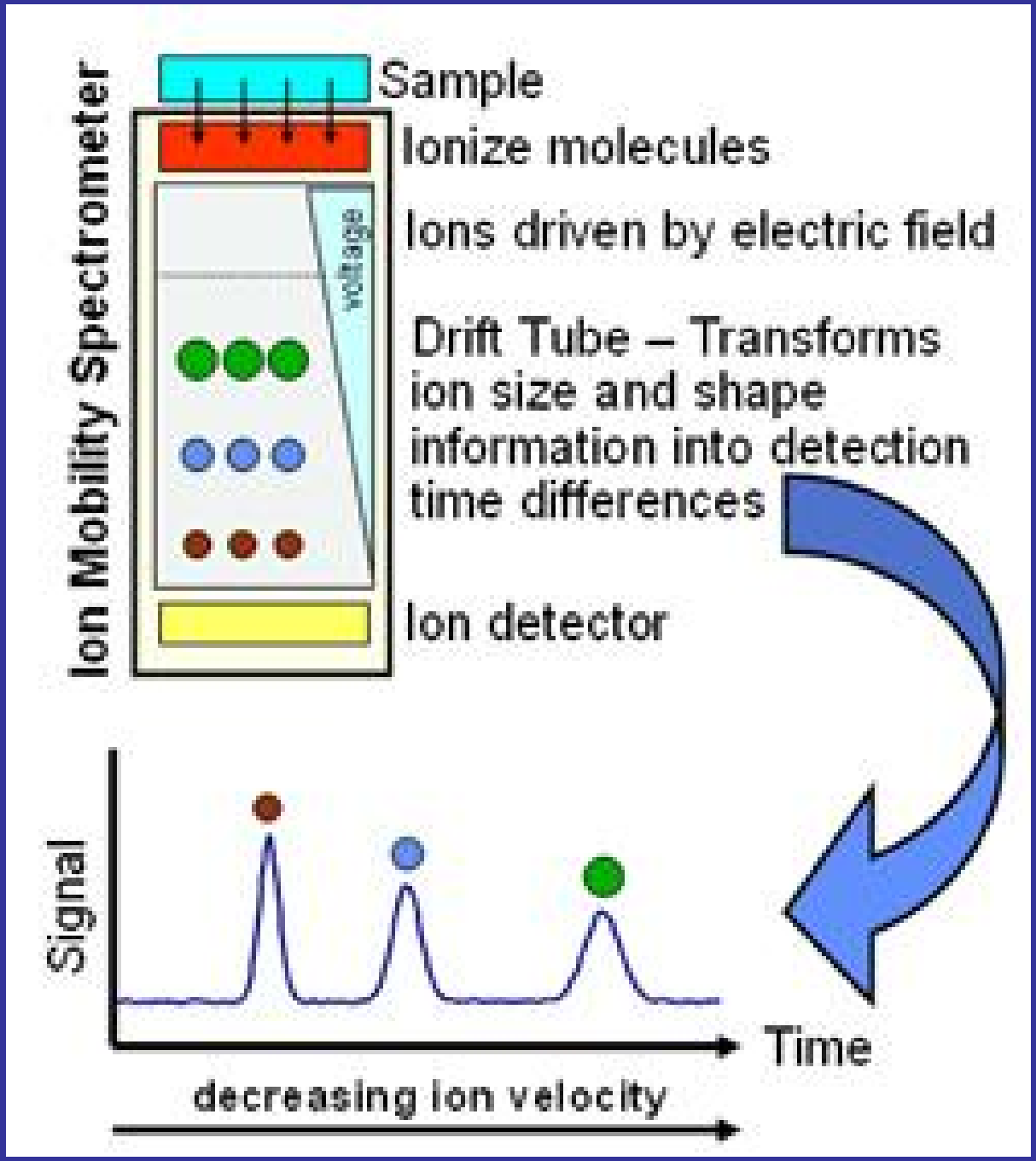

Cross-sectional view of the ion mobility spectrometer sample inlet, ionization chamber, drift tube, and detector.

# IMS

- IMS is a type of separation technique, similar to time-of-flight mass spectrometry, Where ions in gases are separated by their relative mobility within an electric field.
- IMS can separate ions of isomers, isobars, and conformers.
- IMS may measure ion size also

# IMS types

1. Drift-times IMS => provides the highest IMS resolving power
2. Aspiration IMS (AIMS)
3. Differential (also called field-asymetric waveform ion mobility spectrometry, FAIMS) MS
4. Travelling-wave IMS

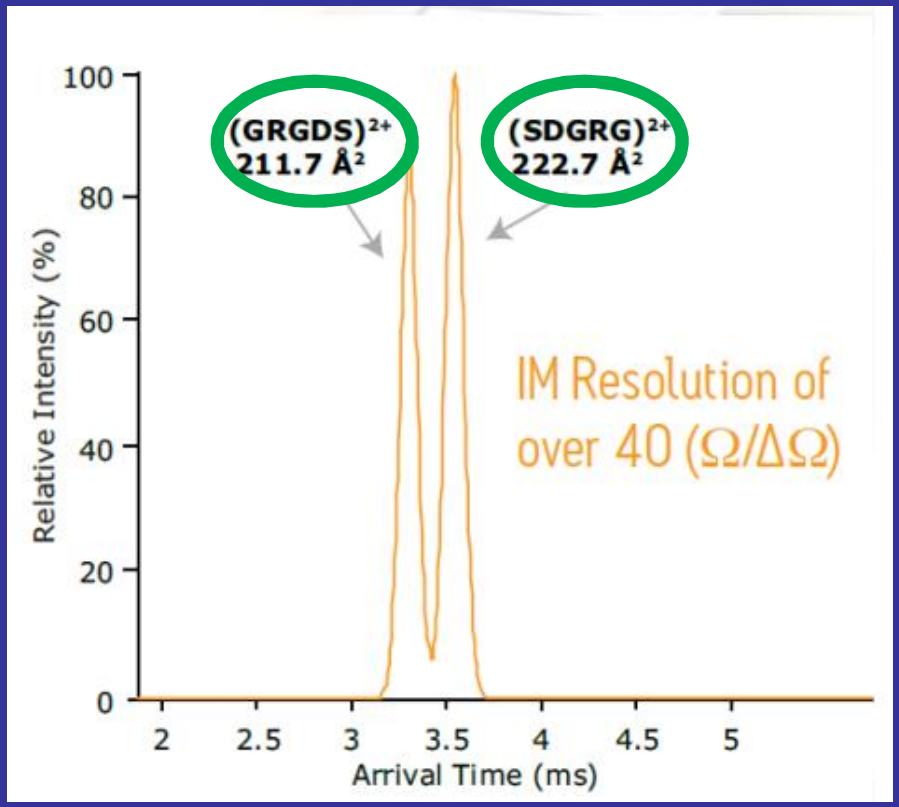

Ion Mobility Spectrometer
Sample
Ionize molecules
Ions driven by electric field
voltage
Drift Tube – Transforms ion size and shape information into detection time differences
Ion detector
Signal
Time
decreasing ion velocity

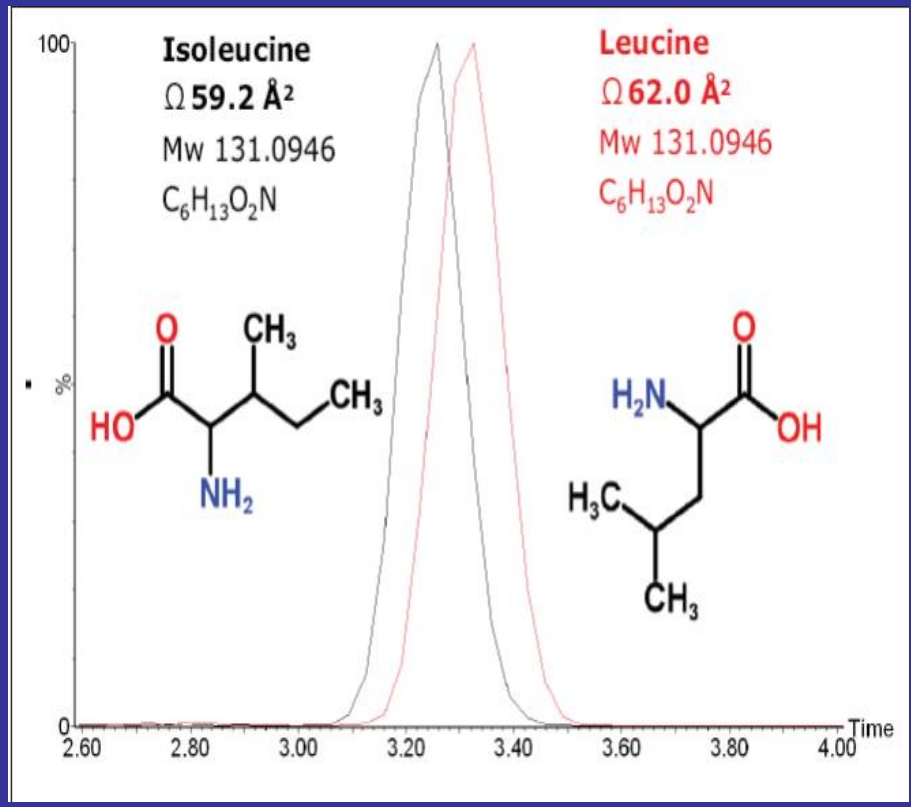
- Eg. Peptide

  KLFTSY MH+ **757.42 Da** and
- Bradykinin peptide

  RPPGFSP MH+ **757.40** Da
- These 2 peptides are of the same size, but the structures are different.

- ION MOBILITY can separate them from each other


100
80
60
40
20
0
Relative Intensity (%)
(GRGDS)2+
211.7 Å2
(SDGRG)2+
222.7 Å2
IM Resolution of
over 40 (Ω/ΔΩ)
2
2.5
3
3.5
4
4.5
5
Arrival Time (ms)


100
%
0
Isoleucine
Ω 59.2 Å2
Mw 131.0946
C6H13O2N
Leucine
Ω 62.0 Å2
Mw 131.0946
C6H13O2N
O
CH3
HO
CH3
NH2
O
H2N
OH
H3C
CH3
2.60
2.80
3.00
3.20
3.40
3.60
3.80
4.00
Time

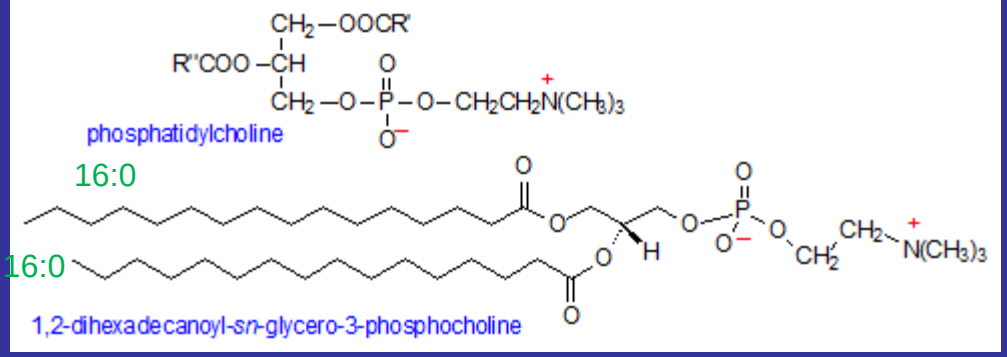



**Table 1. Positional distribution of fatty acids in the phosphatidylcholine of some animal tissues.**

| Position | Fatty acid | | | | | | |
|---|---|---|---|---|---|---|---|
| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 20:4 | 22:6 |
| **Rat liver [1]** | | | | | | | |
| *sn*-1 | 23 | 1 | 65 | 7 | 1 | trace | |
| *sn*-2 | 6 | 1 | 4 | 13 | 23 | 39 | 7 |
| **Rat heart [2]** | | | | | | | |
| *sn*-1 | 30 | 2 | 47 | 9 | 11 | - | - |
| *sn*-2 | 10 | 1 | 3 | 17 | 20 | 33 | 9 |
| **Rat lung [3]** | | | | | | | |
| *sn*-1 | 72 | 4 | 15 | 7 | 3 | - | - |
| *sn*-2 | 54 | 7 | 2 | 12 | 11 | 10 | 1 |
| **Human plasma [4]** | | | | | | | |
| *sn*-1 | 59 | 2 | 24 | 7 | 4 | trace | - |
| *sn*-2 | 3 | 1 | 1 | 26 | 32 | 18 | 5 |
| **Human erythrocytes [4]** | | | | | | | |
| *sn*-1 | 66 | 1 | 22 | 7 | 2 | - | - |
| *sn*-2 | 5 | 1 | 1 | 35 | 30 | 16 | 4 |
| **Bovine brain (gray matter) [5]** | | | | | | | |
| *sn*-1 | 38 | 5 | 32 | 21 | 1 | - | - |
| *sn*-2 | 33 | 4 | trace | 48 | 1 | 9 | 4 |
| **Chicken egg [6]** | | | | | | | |
| *sn*-1 | 61 | 1 | 27 | 9 | 1 | - | - |
| *sn*-2 | 2 | 1 | trace | 52 | 33 | 7 | 4 |

**Table 1.** Presently Investigated Lipids and $E_C$ Values for their Ions (Ammoniated when Marked by an Asterisk and Protonated Otherwise) at DV = 4 kV in 1:1 He/$N_2$. Lipids are Represented as X:a/Y:b, where "X" and "Y" are the Numbers of Carbons in the FA Chain(s) and "a" and "b" are the Number(s) of Double Bonds Therein. The Error Margin of $E_C$ Values is ~0.5 V/cm

| Subclass | Identity | Mass,[a] Da | $E_C$/peak width[b] |
|---|---|---|---|
| Phosphatidylcholines (PC) | 14:0/14:0 | 677.50 | 62.9/1.6 |
| | 15:0/15:0 | 705.53 | 60.4/2.1 |
| | 14:0/16:0 | 705.53 | 60.3/2.1 |
| | 16:0/16:0 | 733.56 | 58.3/2.0 |
| | 14:0/18:0 | 733.56 | 58.7/1.9 |
| | 17:0/17:0 | 761.59 | 55.8/2.1 |
| | 18:0/18:0 | 789.62 | 53.3/1.8 |
| | 23:0/23:0 | 929.78 | 45.0/1.8 |
| | 16:1/16:1 (9) | 729.53 | 54.7/2.1 |
| | 18:3/18:3 (9, 12, 15Z) | 777.53 | 49.9/2.4 |
| | 18:1/18:1 (9) | 785.59 | 52.6/2.0 |
| | 20:4/20:4 (5, 8, 11, 14Z) | 829.56 | 46.5/2.1 |
| | 20:1/20:1 (11Z) | 841.66 | 48.4/1.9 |
| Phosphatidylethanolamines (PE) | 16:0/16:0 | 691.52 | 60.5/1.6 |
| | 18:0/18:0 | 747.58 | 56.3/1.6 |
| Phosphatidylserines (PS) | 17:0/17:0 | 763.54 | 56.9/2.3 |
| | 18:0/18:0 | 791.57 | 55.7/2.2 |
| Lysophosphatidylcholines (LPC) | 14:0 | 467.30 | 70.3/1.7 |
| | 15:0 | 481.32 | 67.5/1.9 |
| | 16:0 | 495.33 | 64.0/1.7 |
| | 17:0 | 509.35 | 61.6/1.9 |
| | 18:0 | 523.36 | 58.3/1.9 |
| | 18:1 (9Z) | 521.35 | 62.1/1.8 |
| Lysophosphatidylethanolamines (LPE) | 14:0 | 425.25 | 74.4 |
| | 16:0 | 453.29 | 69.9 |
| | 18:0 | 481.32 | 65.4 |
| Lysophosphatidylglycerols (LPG) | 14:0 | 456.25 | 68.2/1.7 |
| | 16:0 | 484.28 | 65.5/1.6 |
| | 18:0 | 512.31 | 61.4/1.7 |
| Monoacylglycerols (MG)[c] | 17:0 | 344.29 | 70.5/1.4 |
| | 19:0 | 372.32 | 66.3/1.6 |
| | 11:1 (10Z) | 258.18 | 77.2/1.2 |
| | 24:1 (15Z) | 440.39 | 56.5/1.5 |
| Diacylglycerols (DG)* | 12:0/12:0 (sn1/sn2) | 456.38 | 75.4/1.5 |
| | 14:0/14:0 (sn1/sn2) | 512.44 | 70.7/1.7 |
| | 16:0 (sn1)/12:0 (sn2) | 512.44 | 67.3/1.9 |
| | 16:0 (sn1)/12:0 (sn3) | 512.44 | 70.0/1.9 |
| | 16:0/16:0 (sn1/sn2) | 568.51 | 63.0/2.1 |
| | 19:0/19:0[d] | 652.60 | 57.2/1.9 |
| | 18:1/18:1 (sn1/sn2, 9Z) | 620.54 | 50.0/2.1 |
| | 20:3/20:3 (sn1/sn3, 11, 14, 17Z) | 668.54 | 49.0/1.8 |
| | 24:1/24:1 (sn1/sn3, 15Z) | 788.73 | 41.6/2.3 |
| Triacylglycerols (TG)* | 19:0/19:0/19:0 | 932.88 | 42.0/2.7 |
| | 11:1/11:1/11:1 (10Z) | 590.45 | 62.3/1.4 |
| | 14:1/14:1/14:1 (9Z) | 716.60 | 50.8/1.4 |
| | 18:1/18:1/14:0 (9Z) | 830.74 | 44.2/2.3 |
| | 18:1/18:1/16:0 (9Z) | 858.77 | 43.0/ |
| | 21:1/21:1/21:1 (12Z) | 1010.92 | 37.2/2.2 |
| | 24:1/24:1/24:1 (15Z) | 1137.06 | 34.1/2.2 |
| Monogalactosyl diacylglycerols (MGDG)* | 16:0/18:0 | 758.59 | 56.4/2.0 |
| | 18:0/18:0 | 786.62 | 54.6/1.9 |
| Digalactosyl diacylglycerols (DGDG)* | 16:0/18:0 | 920.64 | 46.4/2.0 |
| | 18:0/18:0 | 948.67 | 45.4/2.0 |
| Ceramides | d18:1/18:0 | 565.54 | 62.4/1.7 |
| | d18:1/24:0 | 649.64 | 55.5/1.9 |
| Sphingomyelins | d18:1/12:0 | 646.50 | 60.6/1.8 |
| | d18:1/14:0[e] | 674.54 | 59.1/1.8 |
| | d18:1/16:0[e] | 702.57 | 56.6/2.0 |
| | d18:1/18:0[e] | 730.60 | 53.8/1.8 |
| | d18:1/20:0[e] | 758.63 | 51.5/1.8 |
| | d18:1/22:0[e] | 786.66 | 49.5/2.2 |
| | d18:1/24:1[e] | 812.68 | 47.7/2.3 |

[a] Mass of the lightest isotopomer
[b] For low-intensity peaks of poor shape, no widths are stated and $E_C$ values are less accurate
[c] Mostly sn1 isomers, sn2 impurity possible (private communication from the vendor)
[d] Mixture of sn1/sn2 and sn1/sn3 in unknown ratio
[e] Composition assigned based on measured *m/z*

Shvartsburg AA et al.,
J Am Soc Mass Spectrom. 2011;22(7):1146-55

# MALDI Imaging (MALDI-MSI)

- TLC MALDI
- MALDI Imaging (MALDI-MSI)
  - Application: Tissue imaging

# MALDI Principles

- Mix analytes with excess matrix compound to crystallize (1:1000 or more)

- Matrix absorbs at the same laser wave length

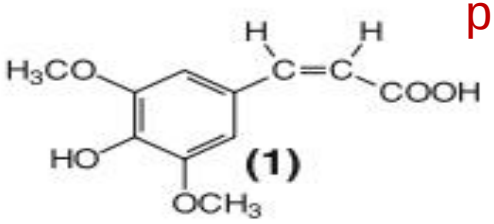
- (e.g. $N_2$-laser@337nm
- Nd:YAG (**neodymium-doped yttrium aluminium garnet**; **Nd:$Y_3Al_5O_{12}$**) laser @355nm,
- infrared Er-YAG@2.94um...)

- Short waved laser pulses

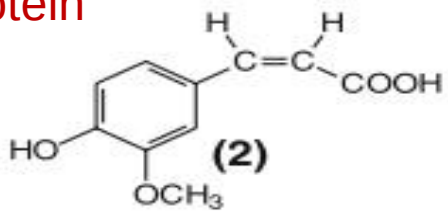
# Example of matrices that are used in MALDI-TOF MS

protein

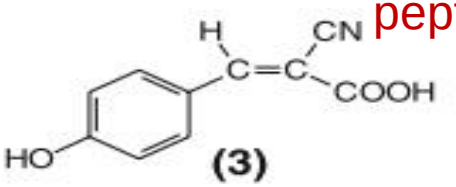
peptide



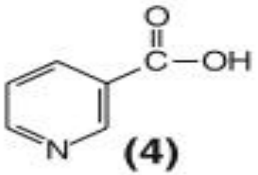

SA



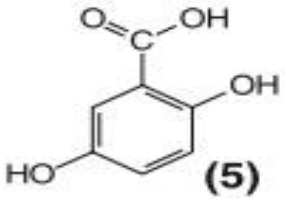

FA




CHCA

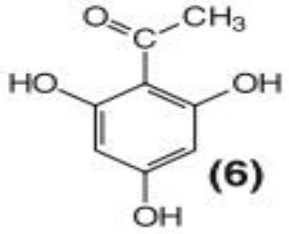



NA

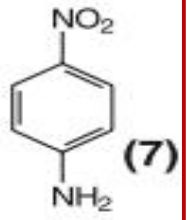



DHB

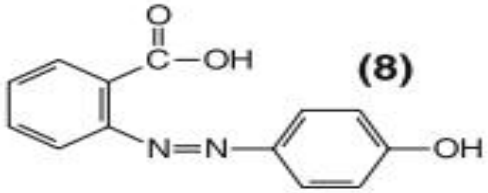
Neg mode




THA




PNA



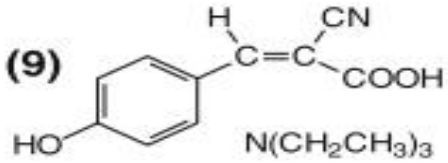
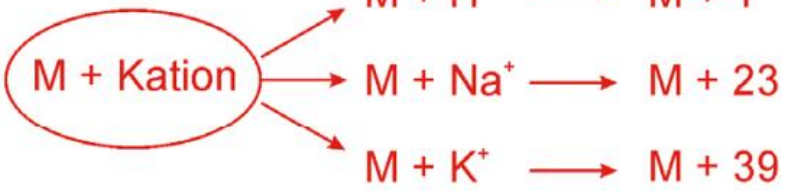
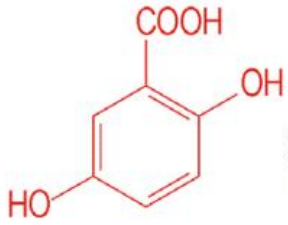




**Fig. 2.** Chemical structures of important MALDI UV matrix compounds. (**1**) Sinapinic acid (SA); (**2**) ferulic acid (FA); (**3**) α-cyano-4-hydroxycinnamic acid (CHA); (**4**) nicotinic acid (NA); (**5**) 2,5-dihydroxybenzoic acid (DHB); (**6**) 2,4,6-trihydroxyacetophenone (THA); (**7**) *p*-nitroaniline (PNA); (**8**) 2-(4-hydroxyphenylazo)benzoic acid (HABA); (**9**) triethylamine/α-cyano-4-hydroxycinnamic acid. Please note that THA and PNA are particularly useful for recording negative ion spectra because they do not possess acidic functions

Fuchs et al.; Chromatographia Volume 69, Supplement 1 (2009), 95-105

## a) Positive Ion Spectra




2,5-Dihydroxy-benzoic acid (*DHB*)

**For neutral PL (PC etc.)**

## b) Negative Ion Spectra



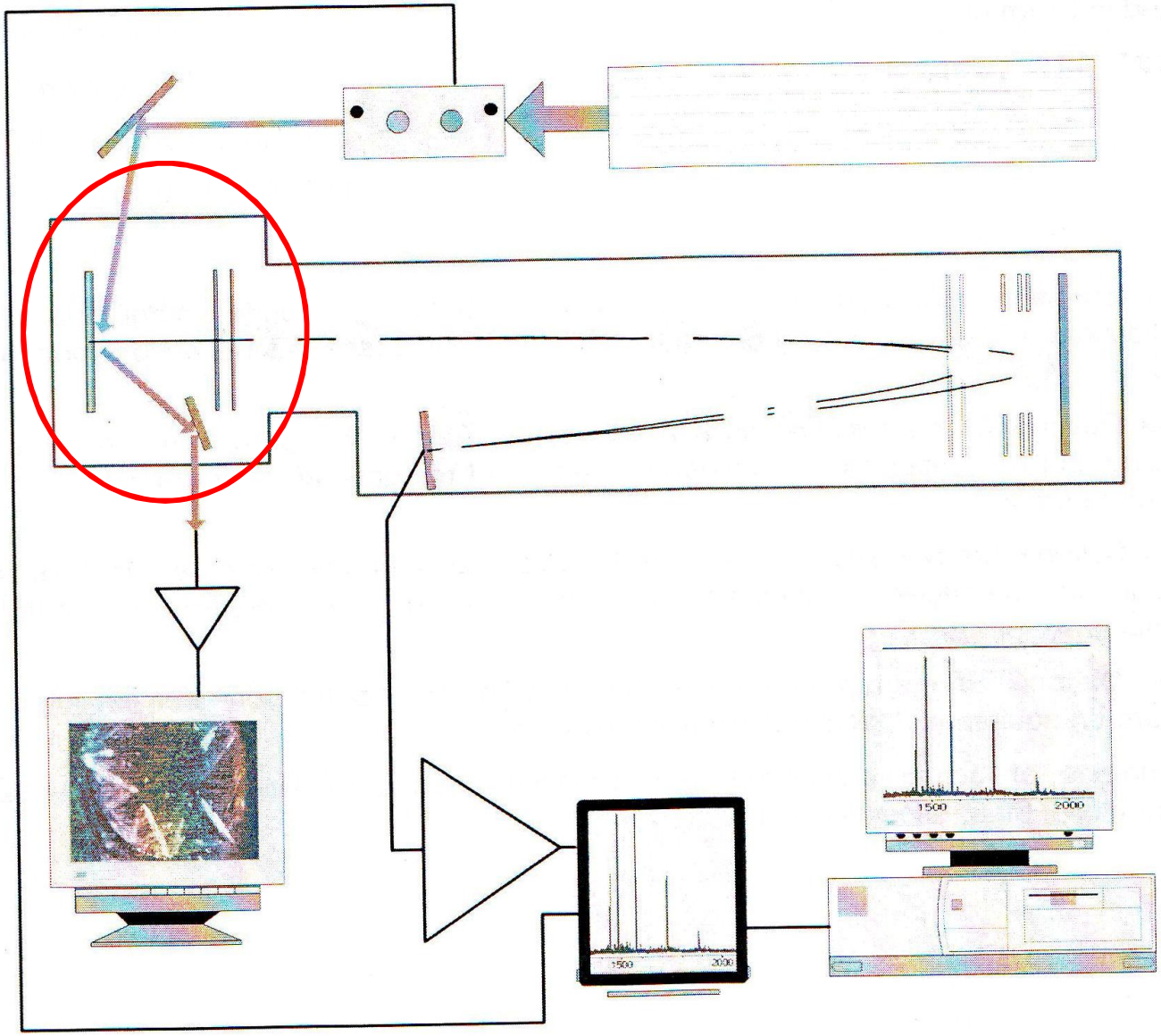
*para*-Nitroaniline (*PNA*)

**Alkaline!**

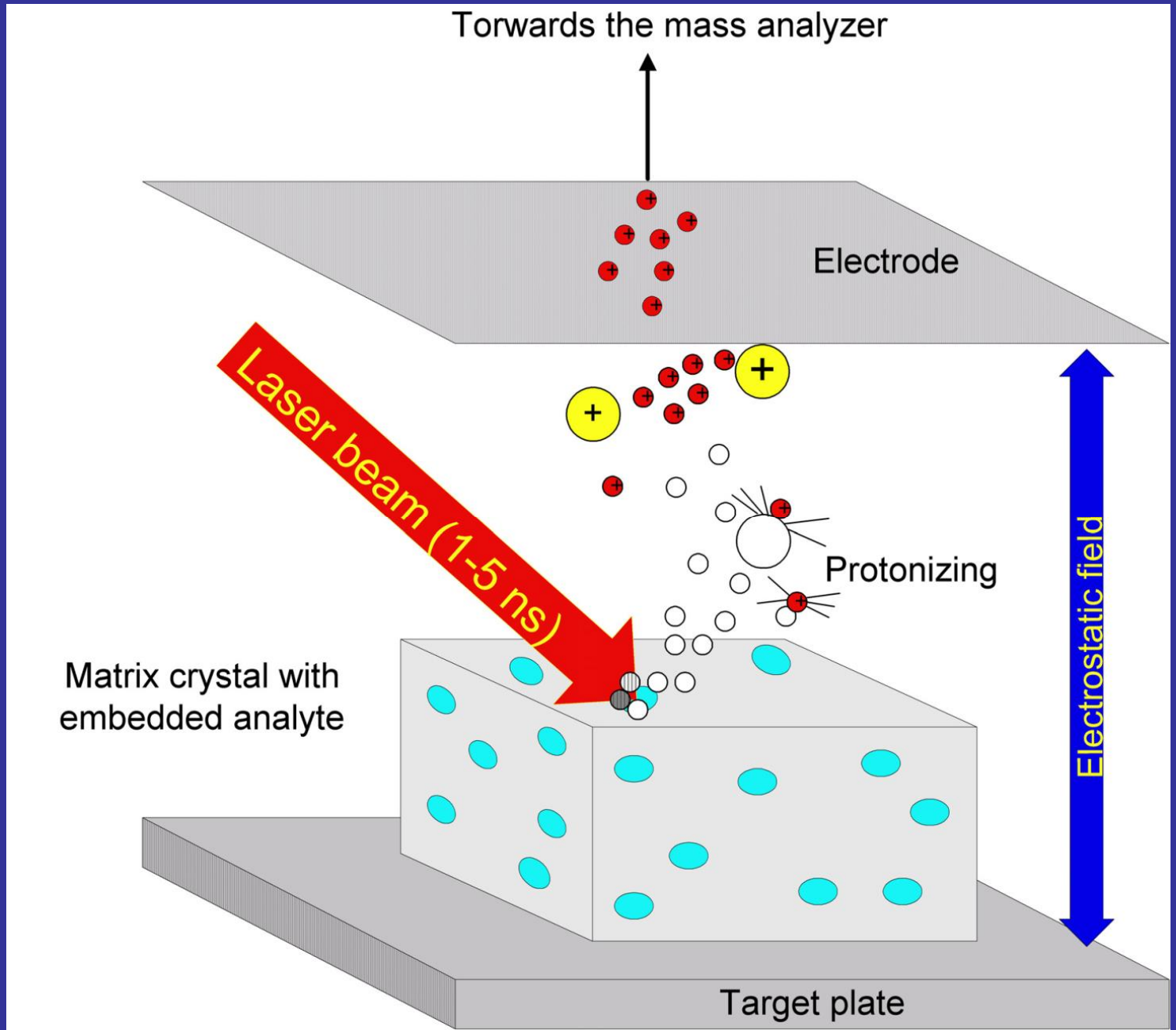
**Ideal for acidic PL (PS, PI etc.) and for PE in mixtures!**

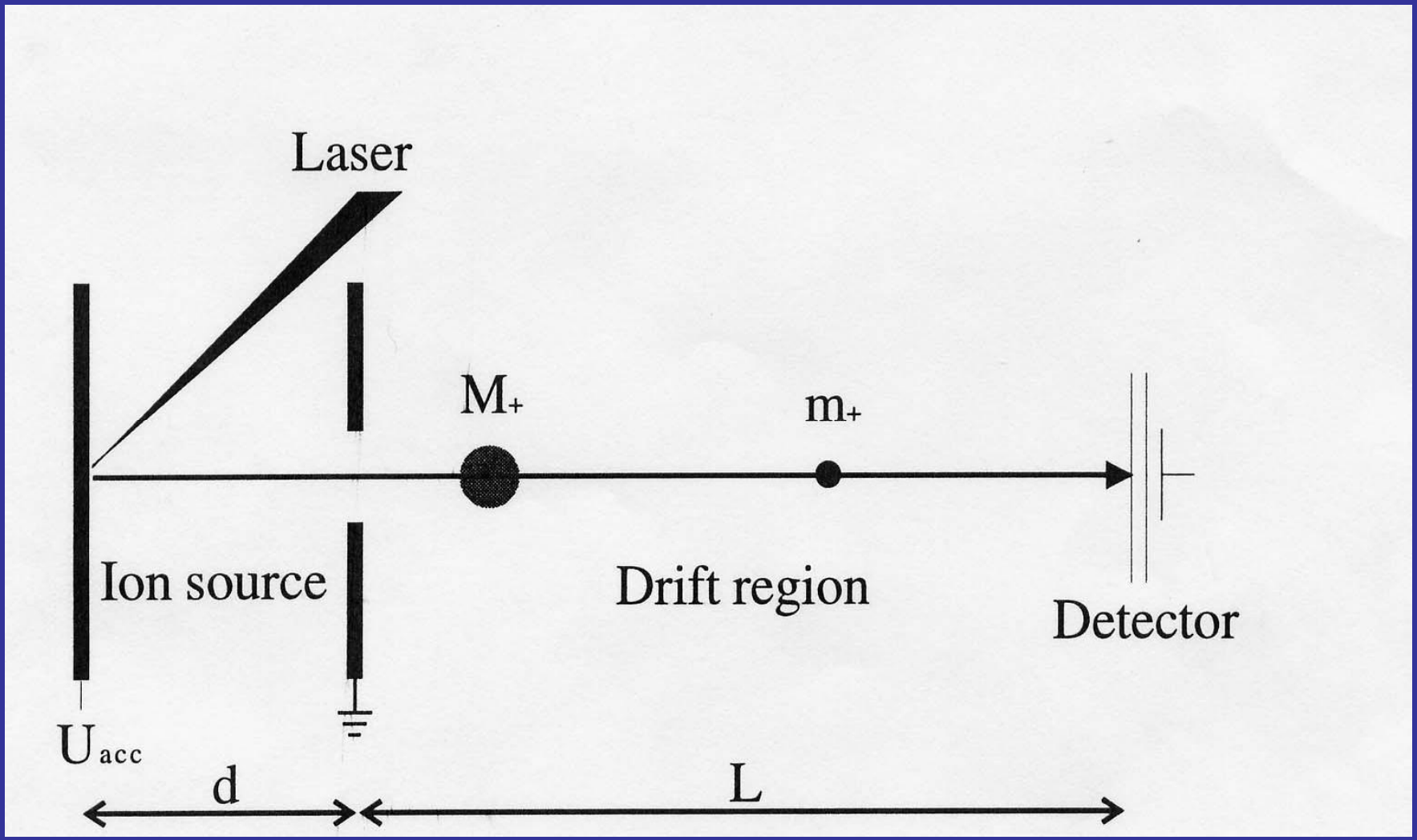
**The pK Values of Matrix and Analyte determine the Sensitivity of Detection!**

# MALDI Mass Spectrometry

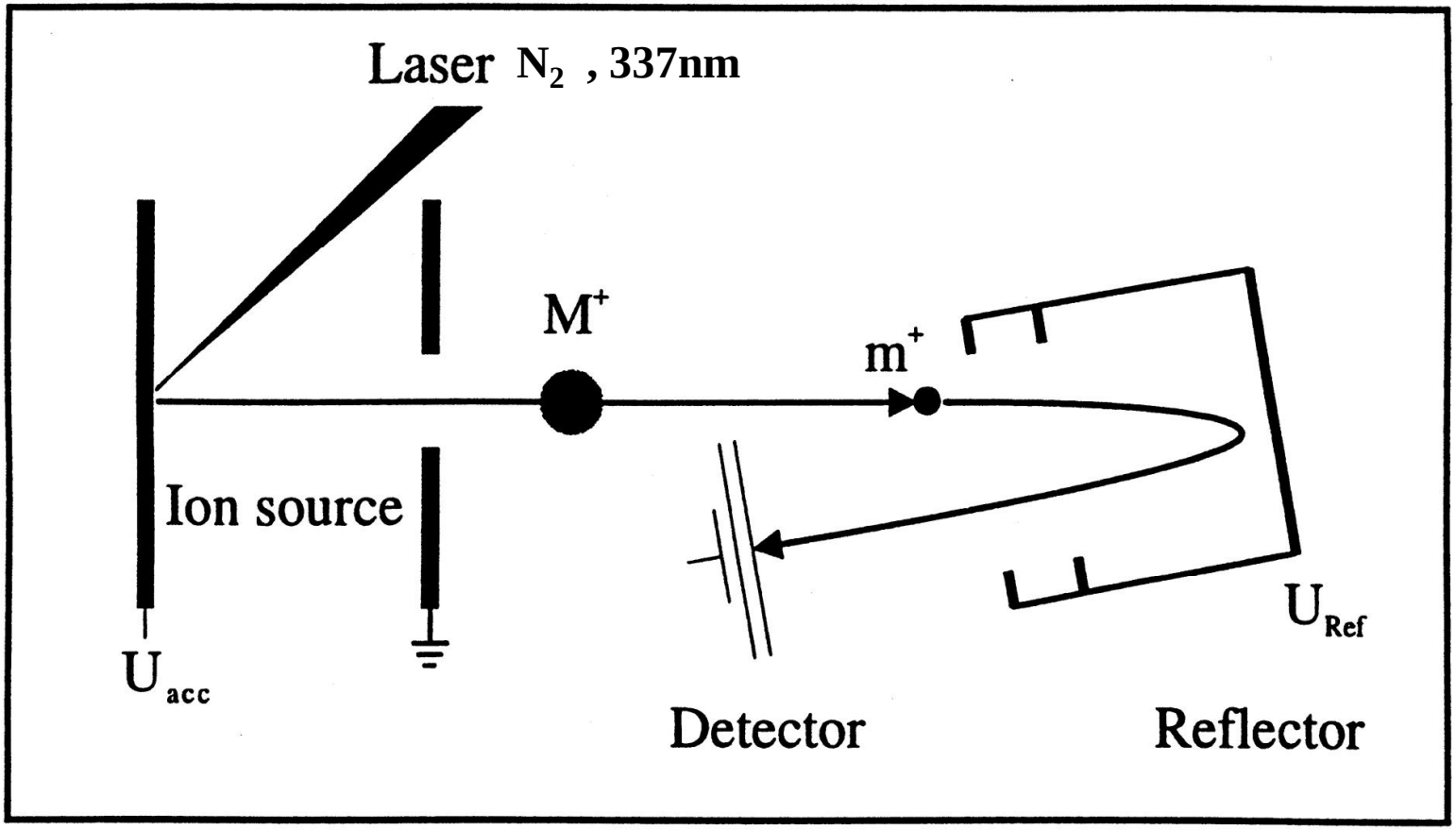


# Principles of MALDI process





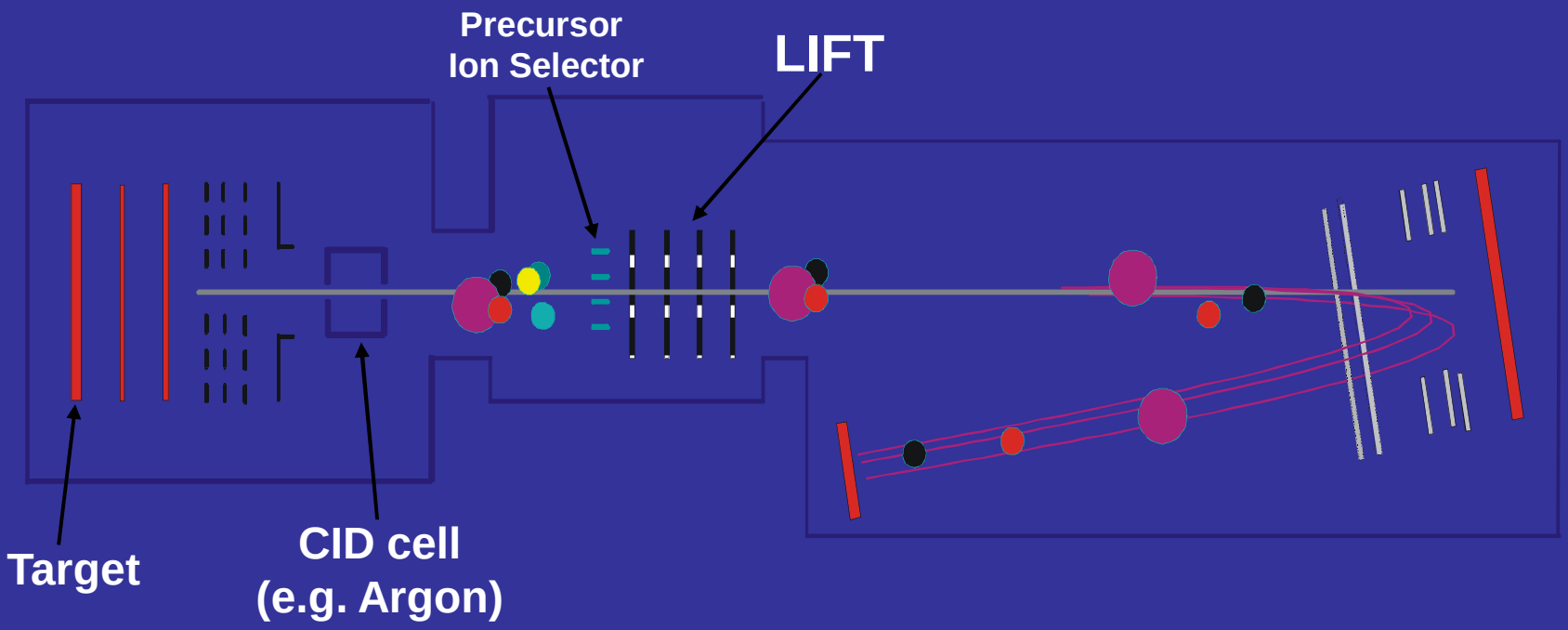

**Schematic diagram of a linear MALDI-TOF mass spectrometer. Delayed extraction (pulsed ion extraction) occurs at the ion source**



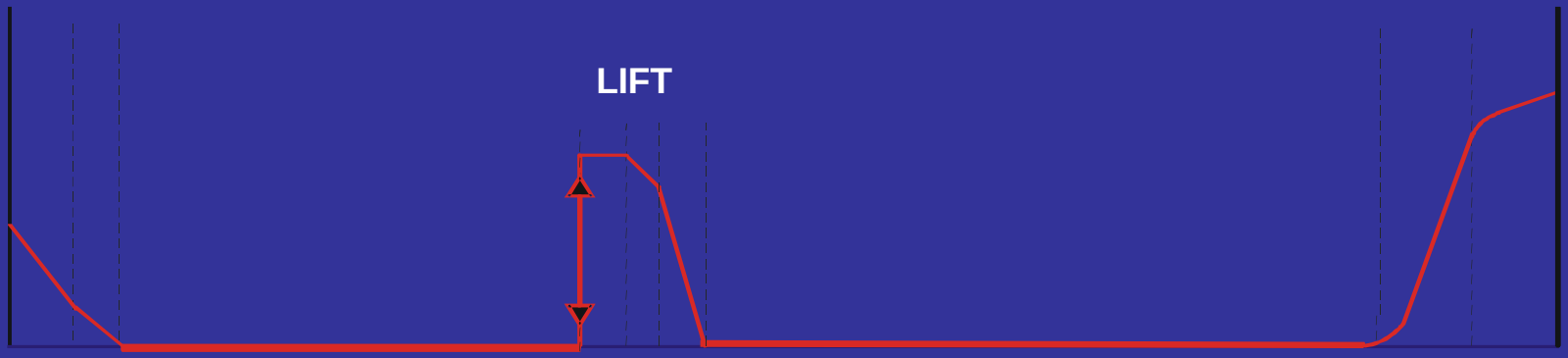

**Schematic diagram of a Reflector MALDI-TOF mass spectrometer.**

**MALDI-TOF = Matrix Assisted Laser Desorption/Ionization - Time Of Flight**

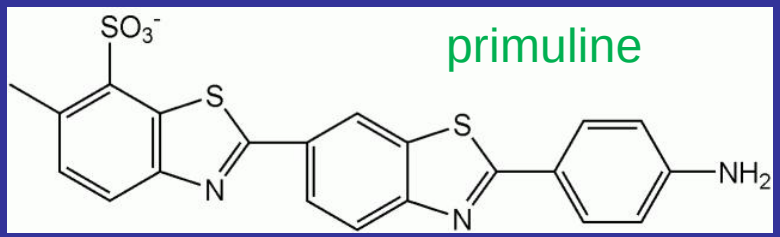
# MALDI-TOF/TOF for LID-LIFT and high energy CID-LIFT




***Potential Profile during LIFT***



1. TLC separation (nmol level)
2. Staining with primuline
3. Densitometry
4. Spot Marking
5. Matrix solution (eg. DHB)
6. Mount and acquire spectra



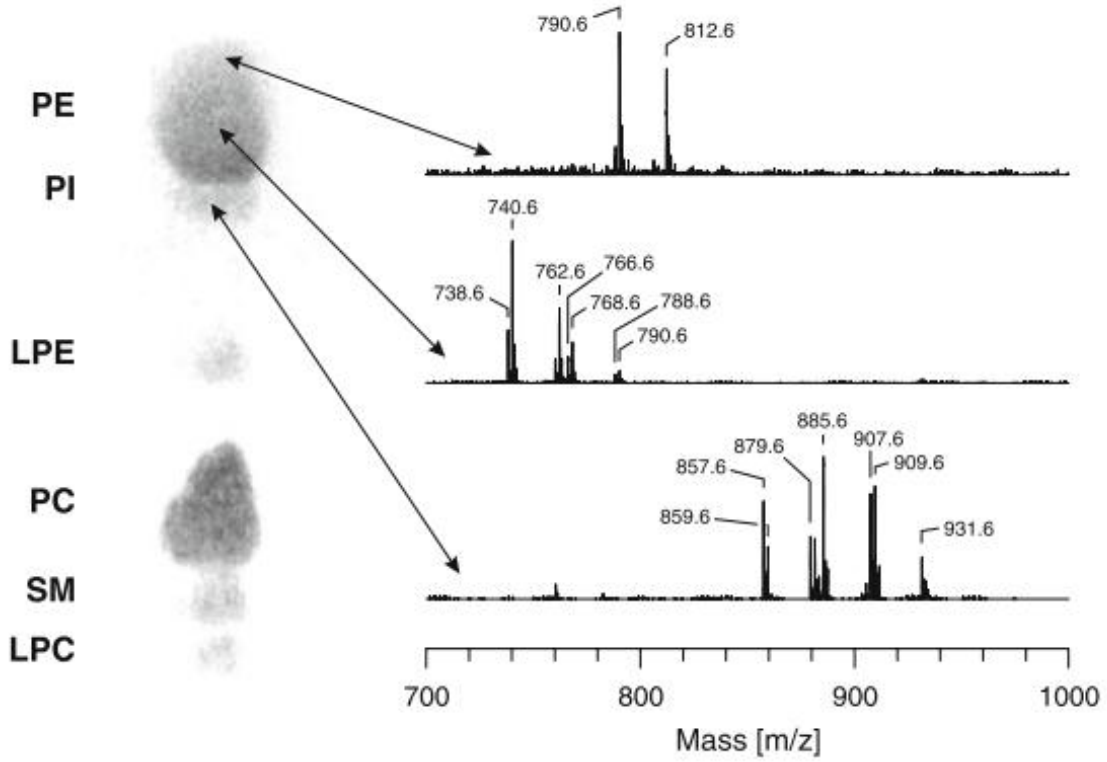


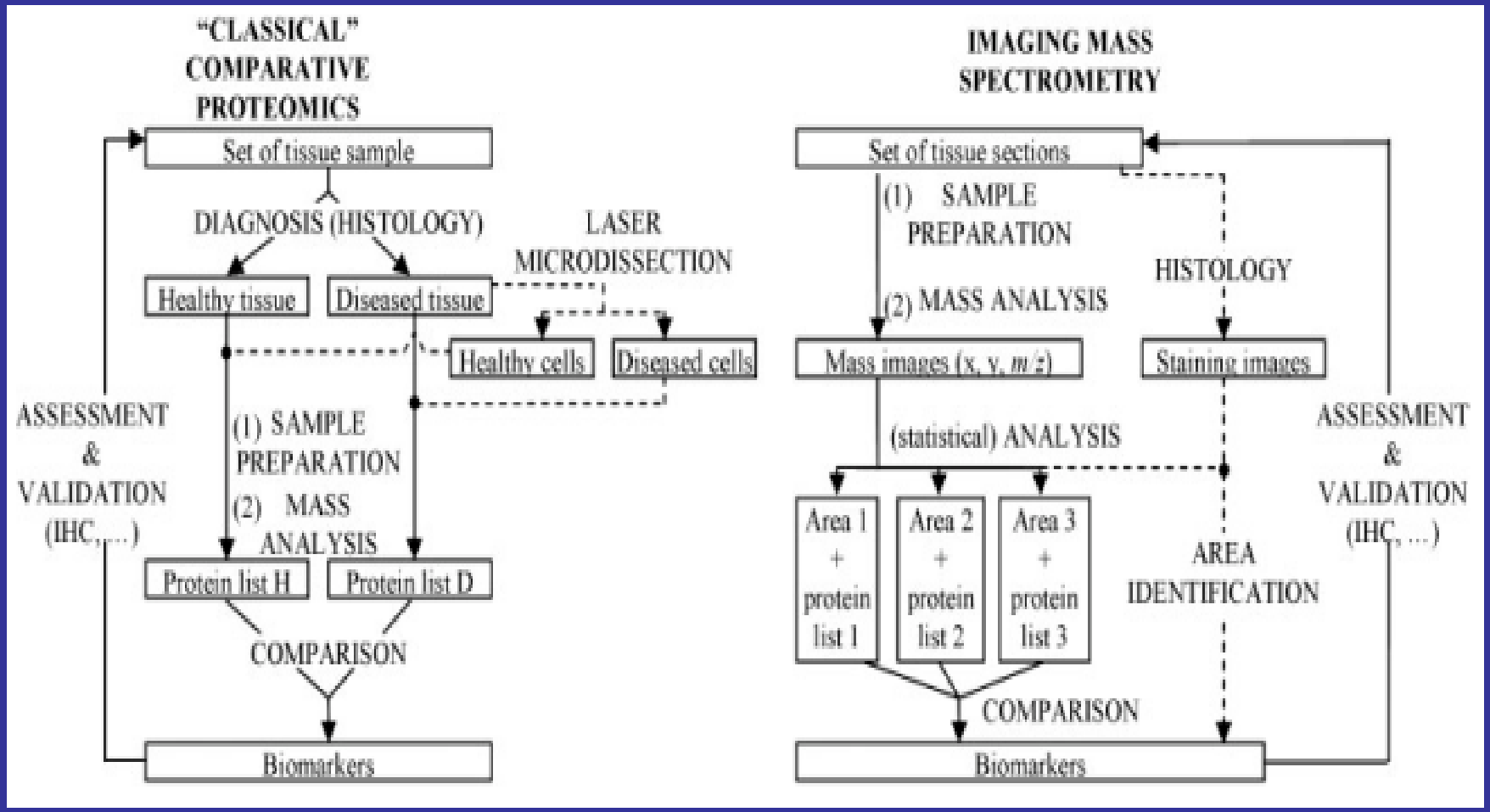

**Fig. 5.** Video image (left) of a typical TLC plate of hen egg yolk extract subsequent to Primuline staining. About 35 µg of PL in total were used in order to obtain reasonable MALDI signals. Selected positive ion MALDI-TOF mass spectra were directly recorded on the TLC sample plate subsequent to soaking the plate with DHB matrix solutions. Please note the high quality of spectra allowing the clear identification of even very minor PLs as well as the differentiation of PE species in dependence on their saturation degree

Fuchs et al.; Chromatographia Volume 69, Supplement 1 (2009), 95-105

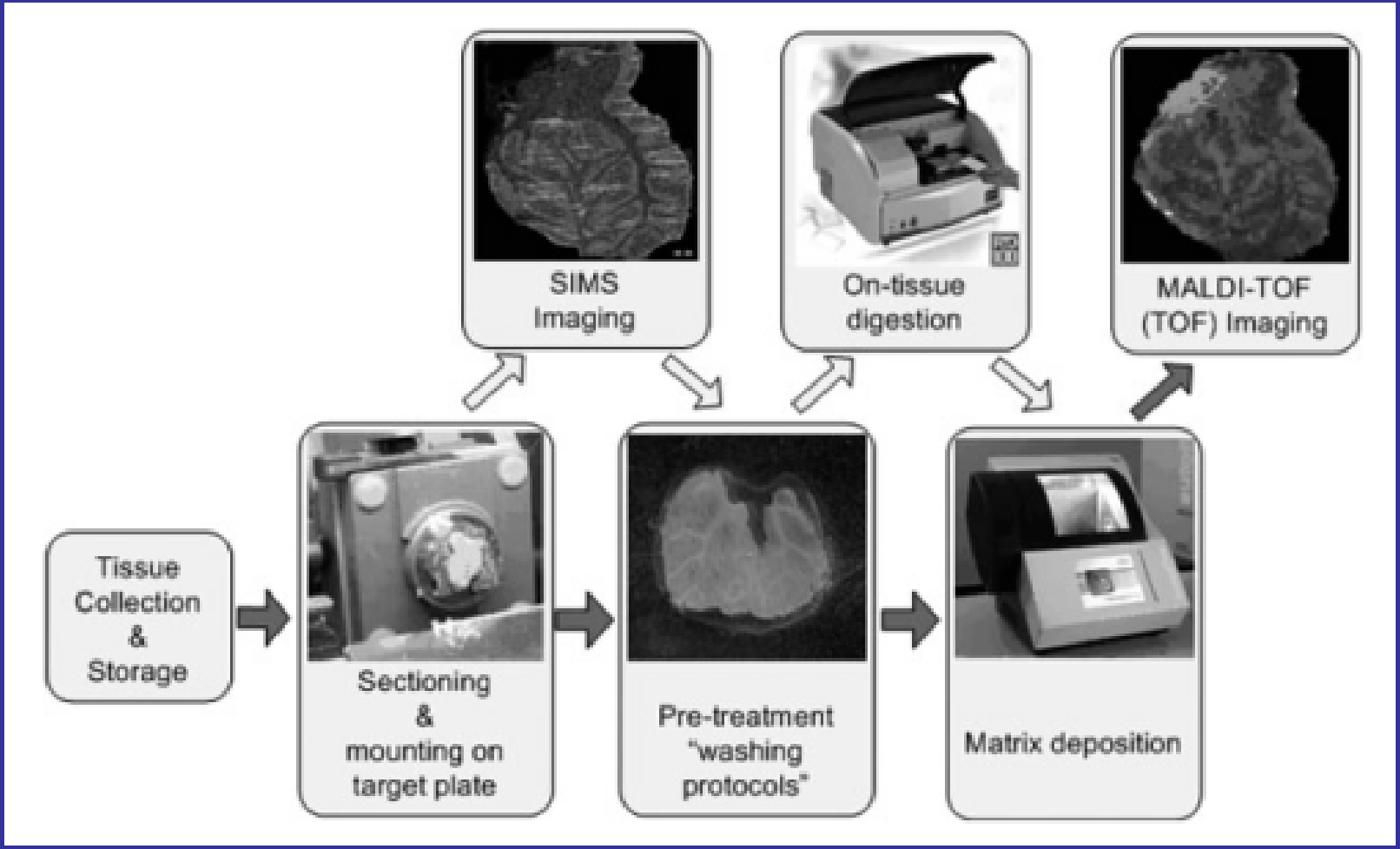
# Definitions:
## MALDI Imaging (MALDI-IMS)

- allows spatial localization of multiple different compounds that are recorded in parallel without the need of a label

# Reviewing proteomics workflows

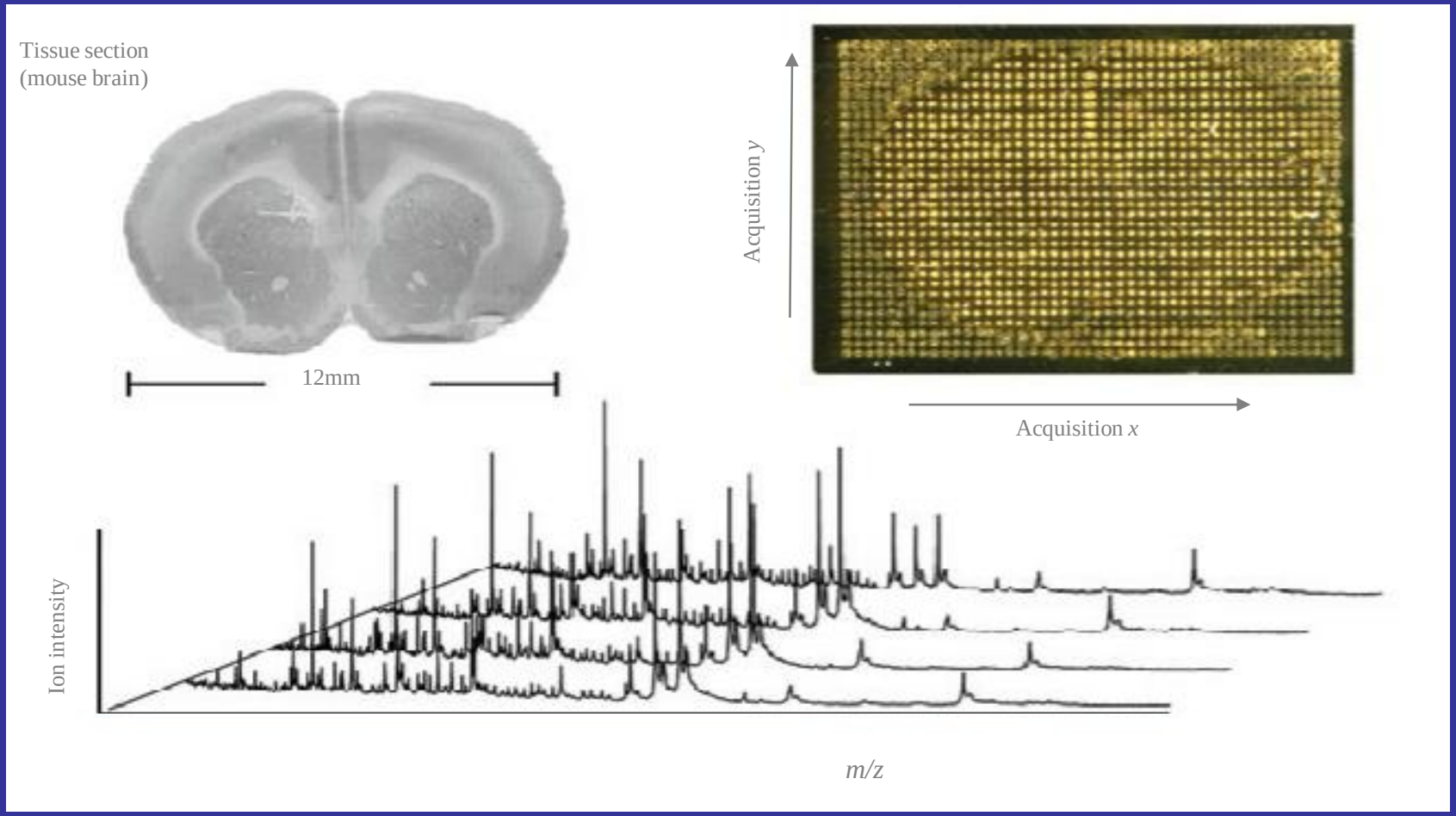


# Key steps in tissue imaging




Kaletas et al.; Proteomics 2009, 9, 2622–2633

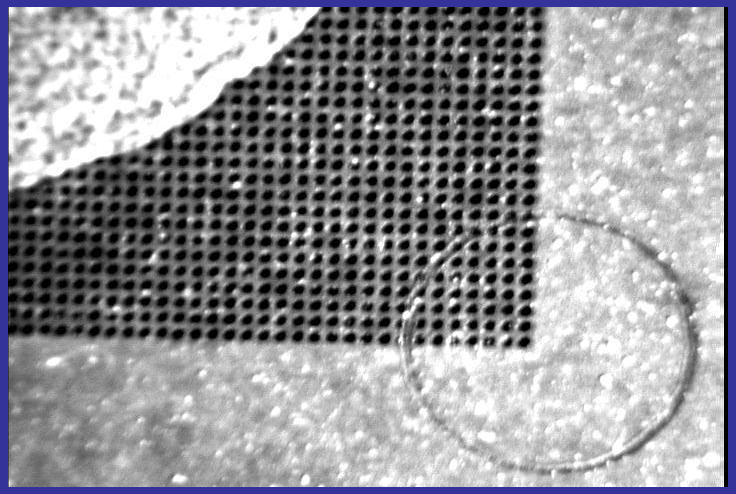
# Principles

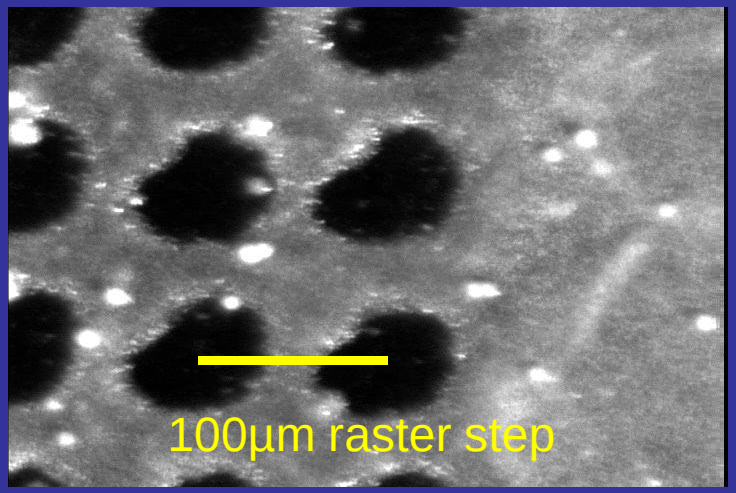



- A laser is rastered over a defined area while acquiring a complete mass spectrum from each position, resulting in molecular images for multiple analytes Cornett, et al., Nature Methods 2007

# Matrix Application

- Matrix application is vital for quality image resolution
- Must contact sample as fine, liquid mist
- Current procedure involves manual application with airbrush

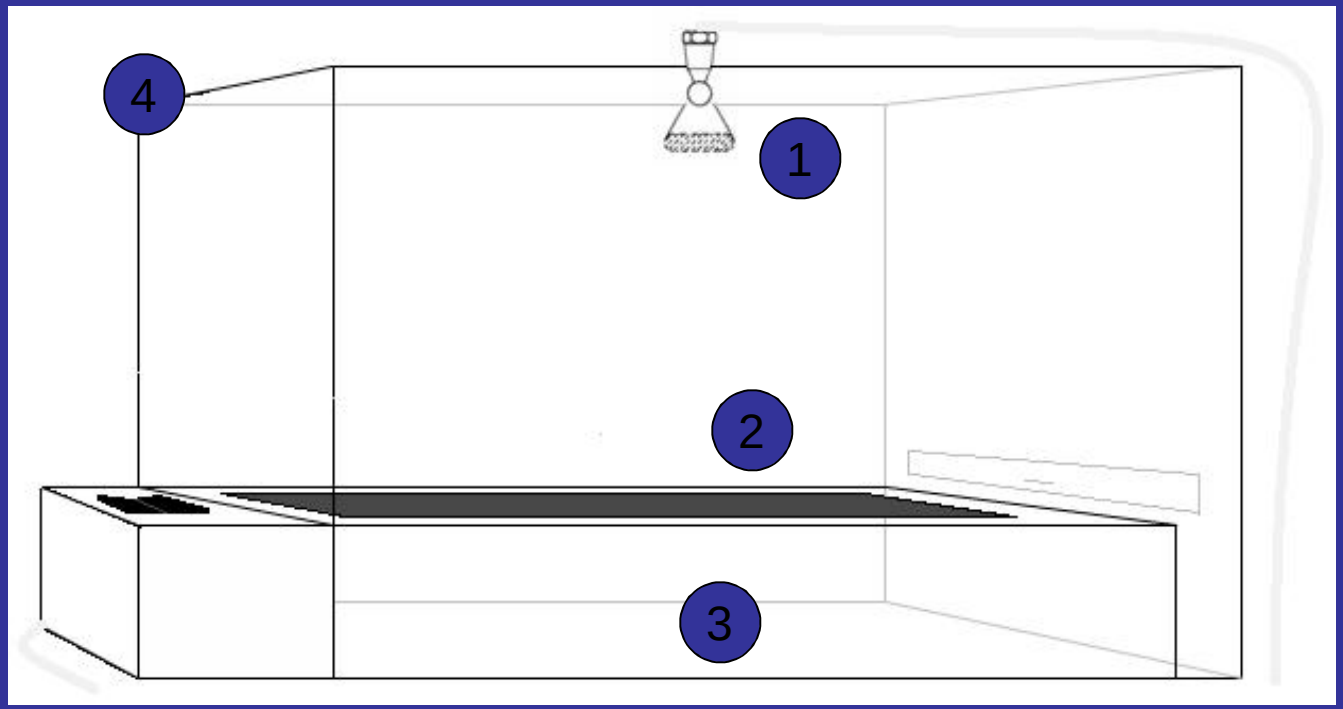





Barrett-Wilt, G., USA

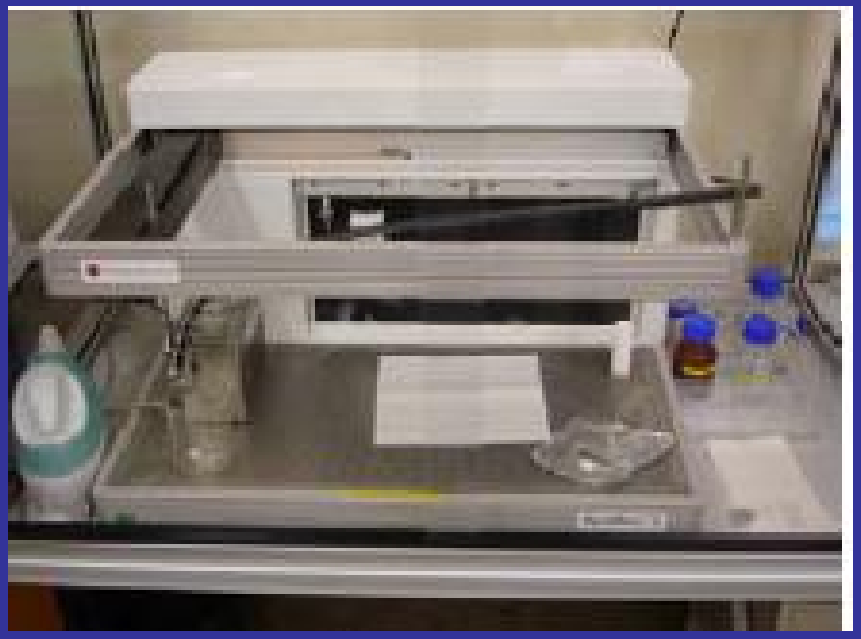
# Home made spray device

1. Automatic Spray Gun
2. Conveyor
3. DC Timing Motor
4. Integrated Polyethylene Box

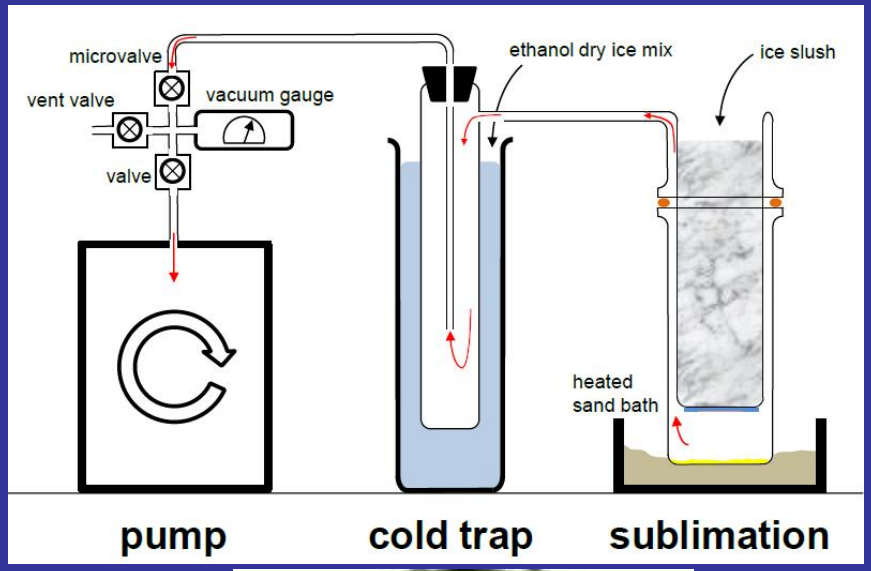


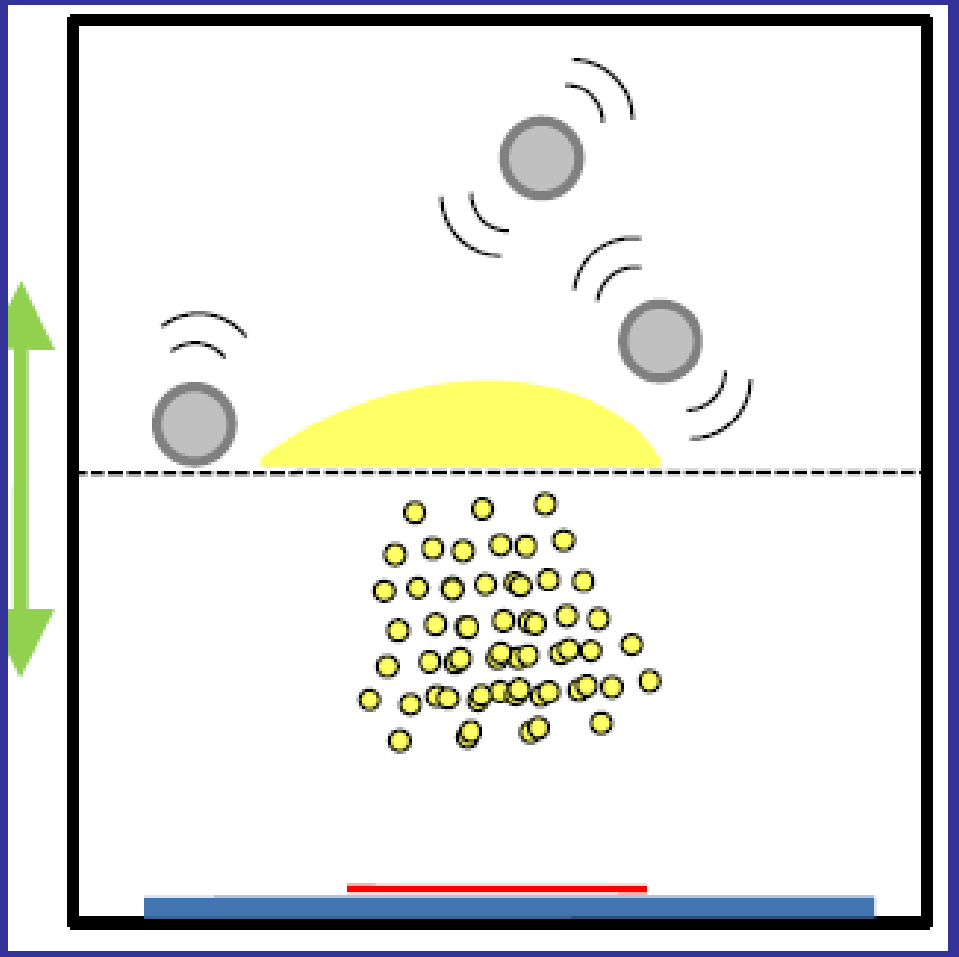

# Bruker IMAGE-PREP With SPR

Nano spotter

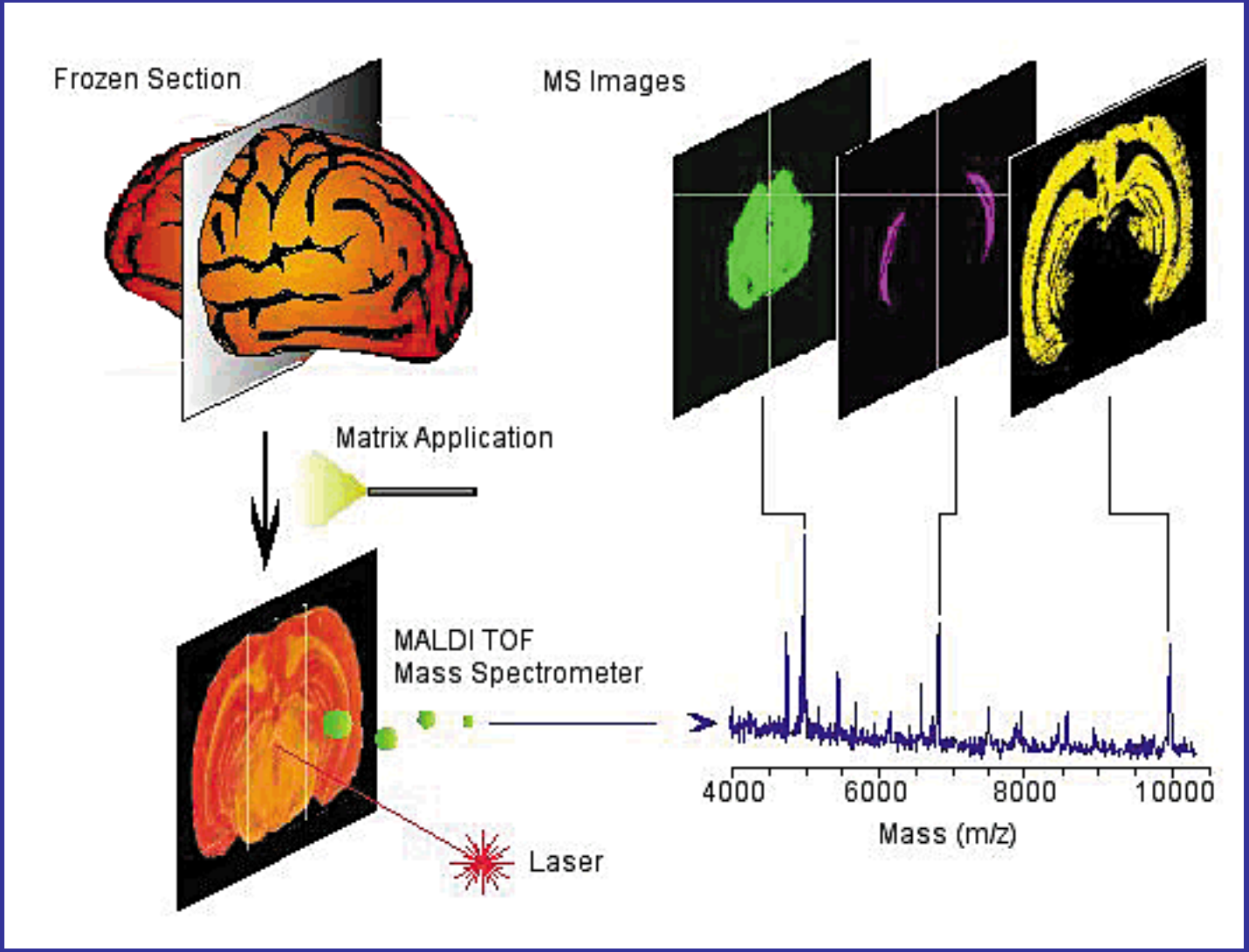


# Sublimation vs dry coating



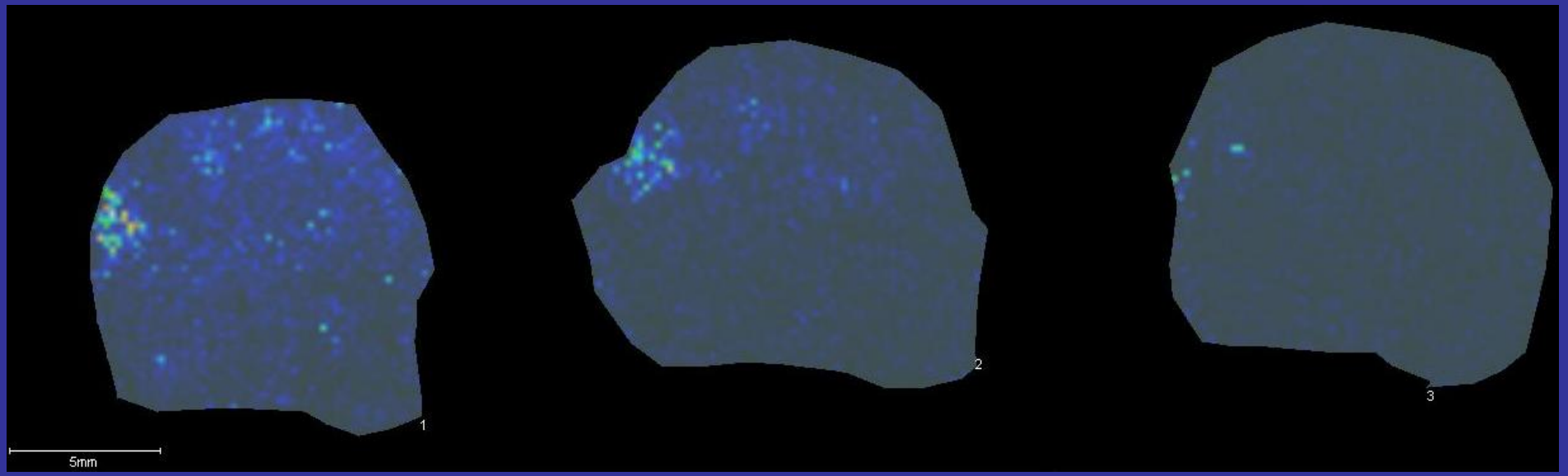



# MSI workflow

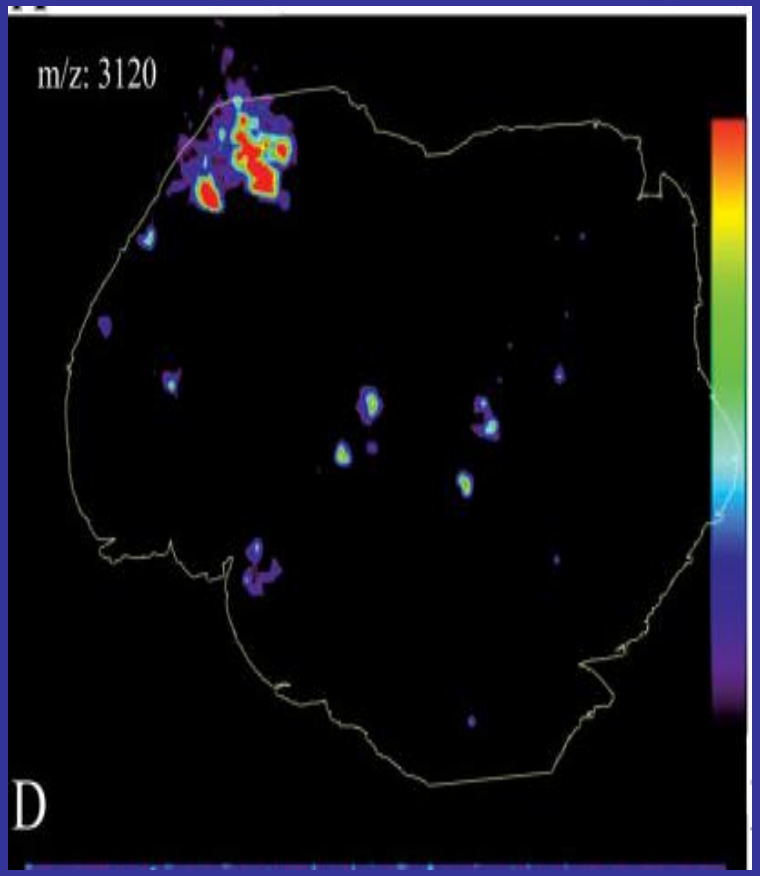


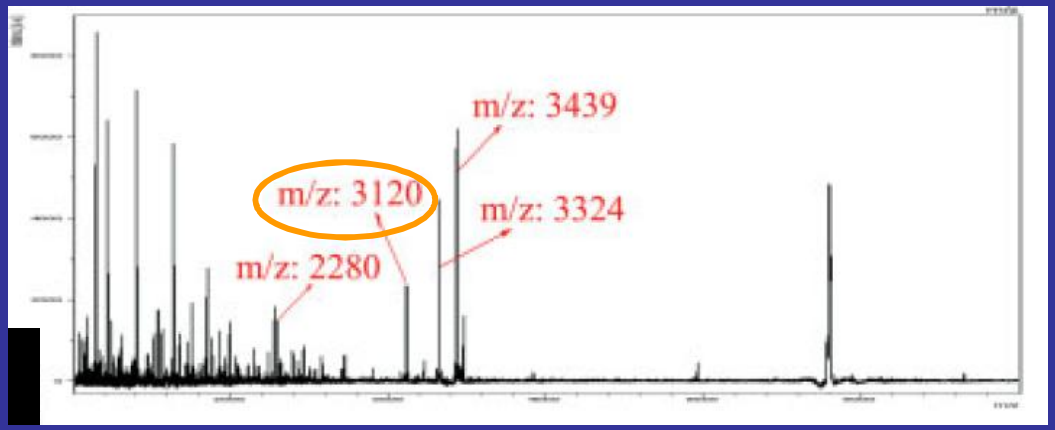

Stoeckli, M, et al., Nature Medicine 2001; vol 7, 4, p493-496

# Consecutive tissue sections



# Peptide IMS of pancreatic tissue of normal mouse



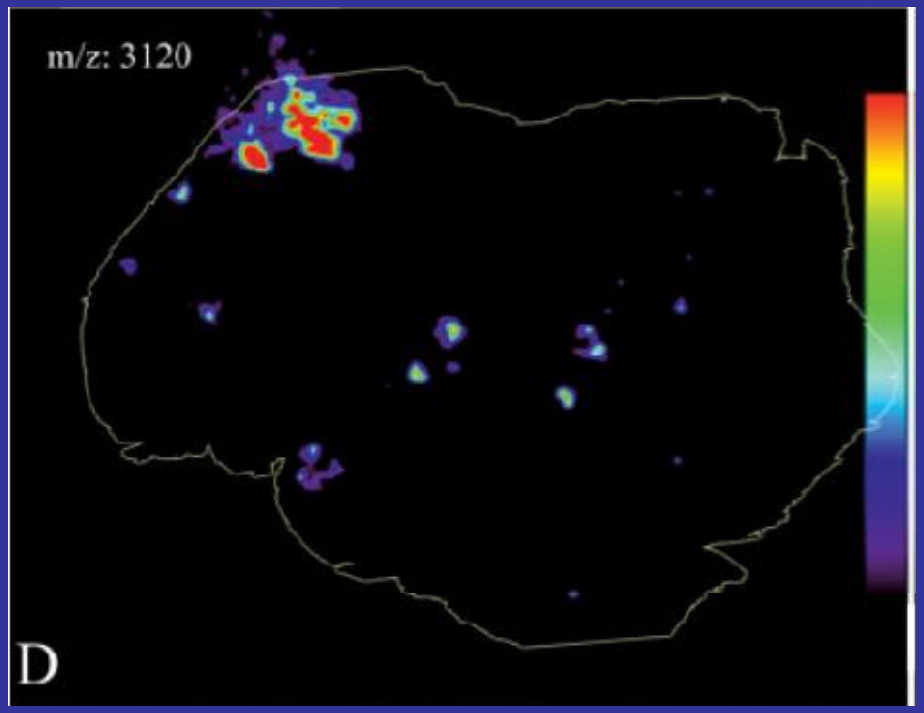




Islet-specific spectrum

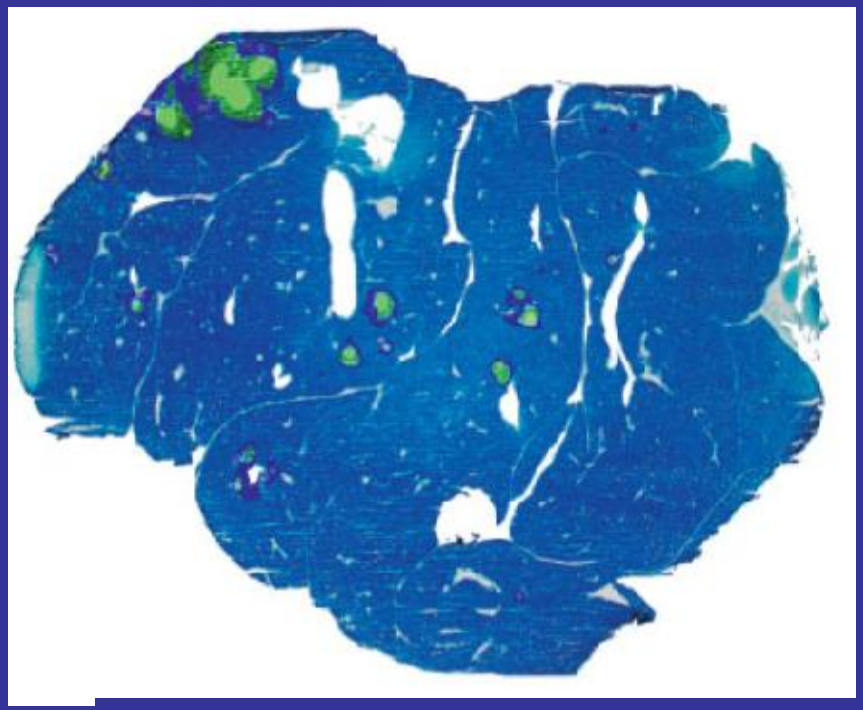
IMS image for ion ***m/z* 3120** (insulin C peptide)

*Minerva, et al., Proteomics 2008*

# IMS vs. histochemical stain




IMS image for ion *m*/*z* 3120 (insulin C peptide)



Overlay image of ion *m*/*z* 3120 and Methylene blue stained of the pancreas

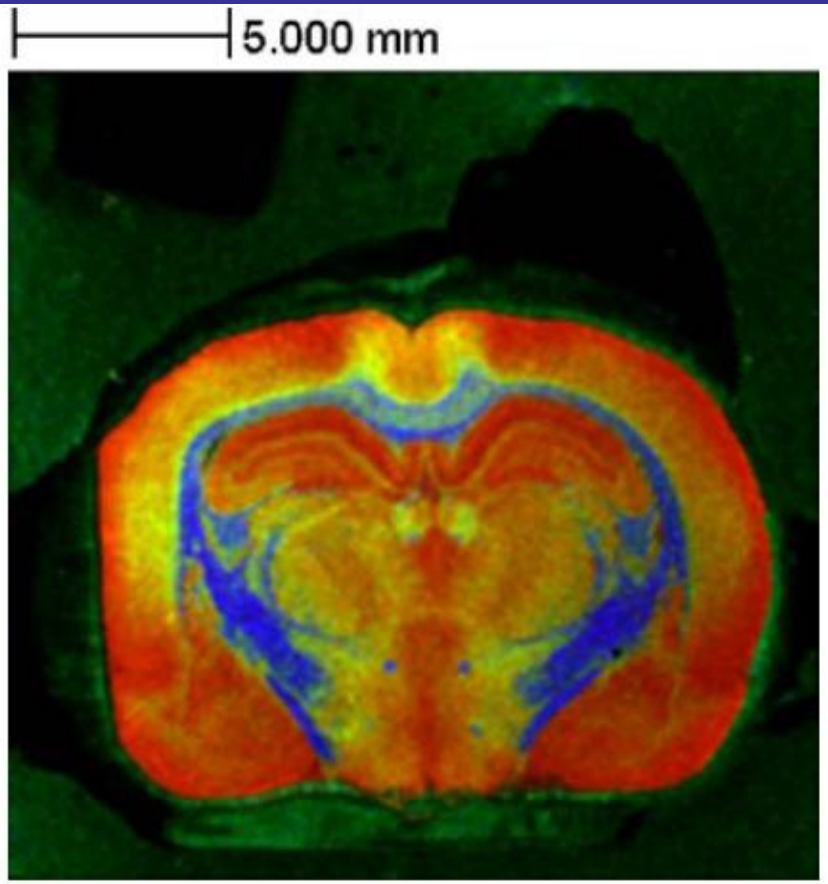
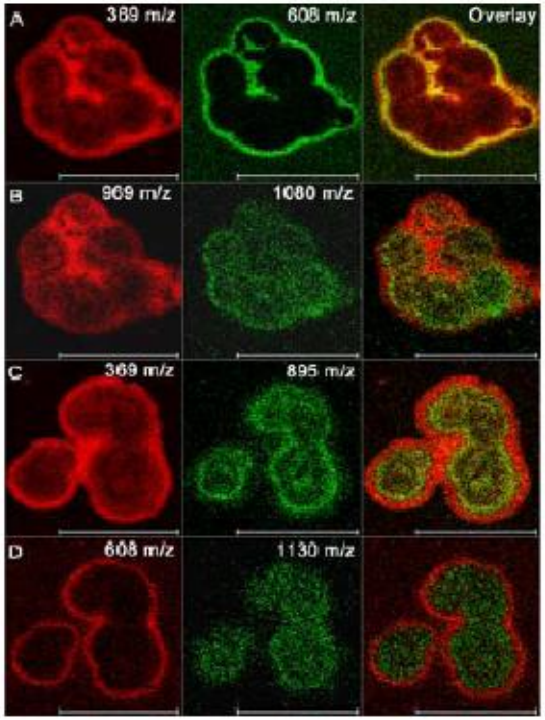


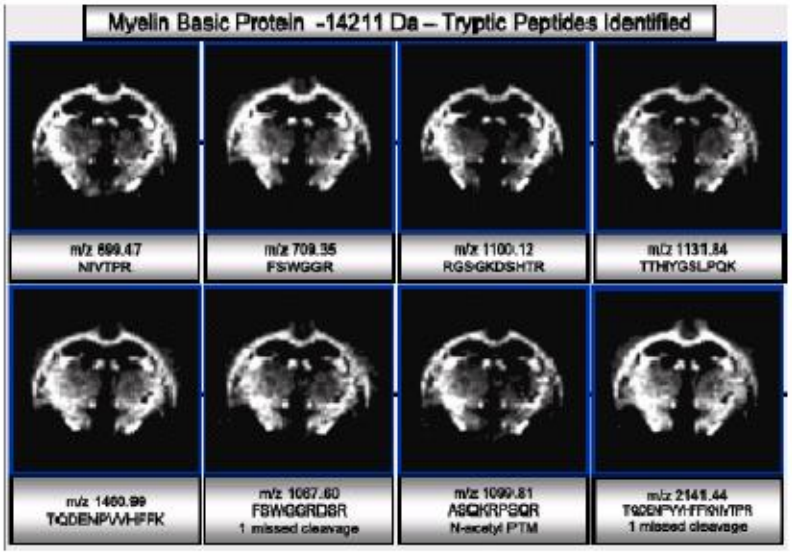

**Fig. 1** Color overlay showing the different localizations of the following negative ions: sum of C16:0 and C18:0 fatty acid carboxylate ions, *m/z* (255+283) (*red*); sulfatides, mean *m/z* 892 (*green*); and cholesterol dimers, *m/z* 771 (*blue*). Obtained from secondary ion images recorded from a rat brain section under the irradiation of $Bi_3^+$ primary ions. Field of view 18×18 $mm^2$, 256×256 pixels, pixel size 70×70 $\mu m^2$. Reprinted by permission of Elsevier from ref. [7], Copyright 2008, the American Society for Mass Spectrometry

Brunelle et al., 2009; Anal Bioanal Chem (2009) 393:31–35

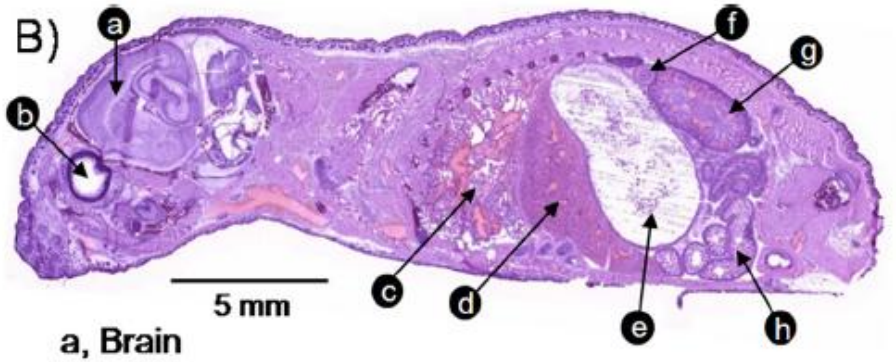
# Imaging Lipidomics




# Imaging Proteomics

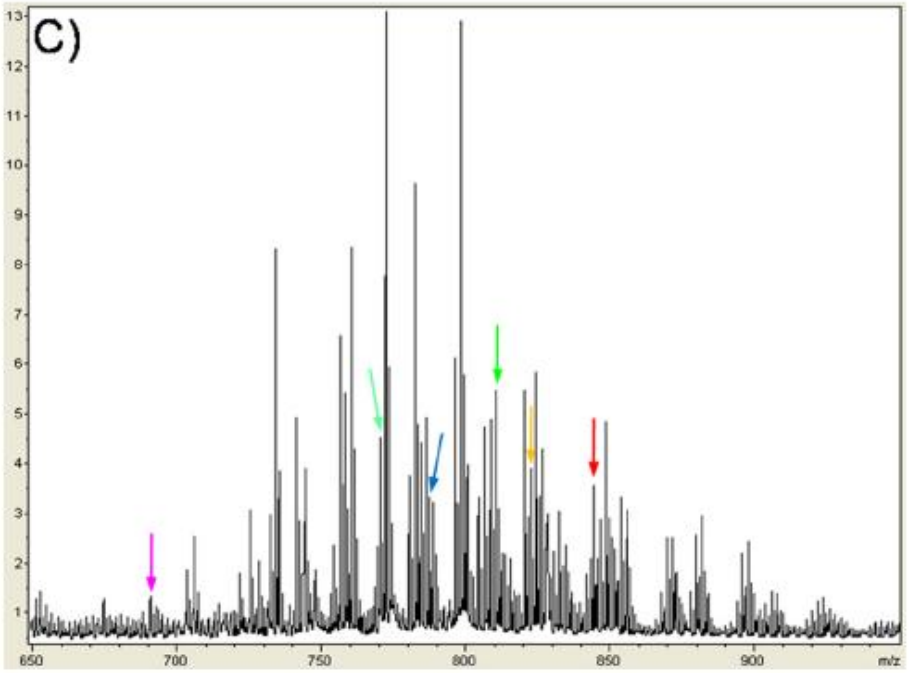



M. Reid Groseclose, Malin Andersson, Richard M. Caprioli ASMS 2006, poster presentation


a, Brain
b, Eye
c, Lung
d, Liver
e, Stomach
f, Adrenal gland
g, Kidney
h, Intestines

*m/z* 691.04
*m/z* 770.53
*m/z* 787.63
*m/z* 810.60
*m/z* 822.51
*m/z* 844.51



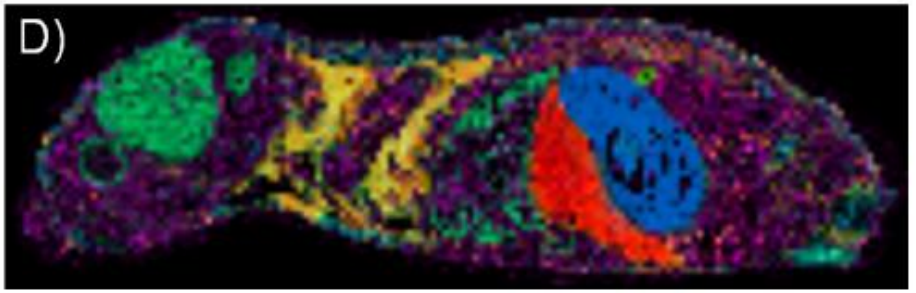
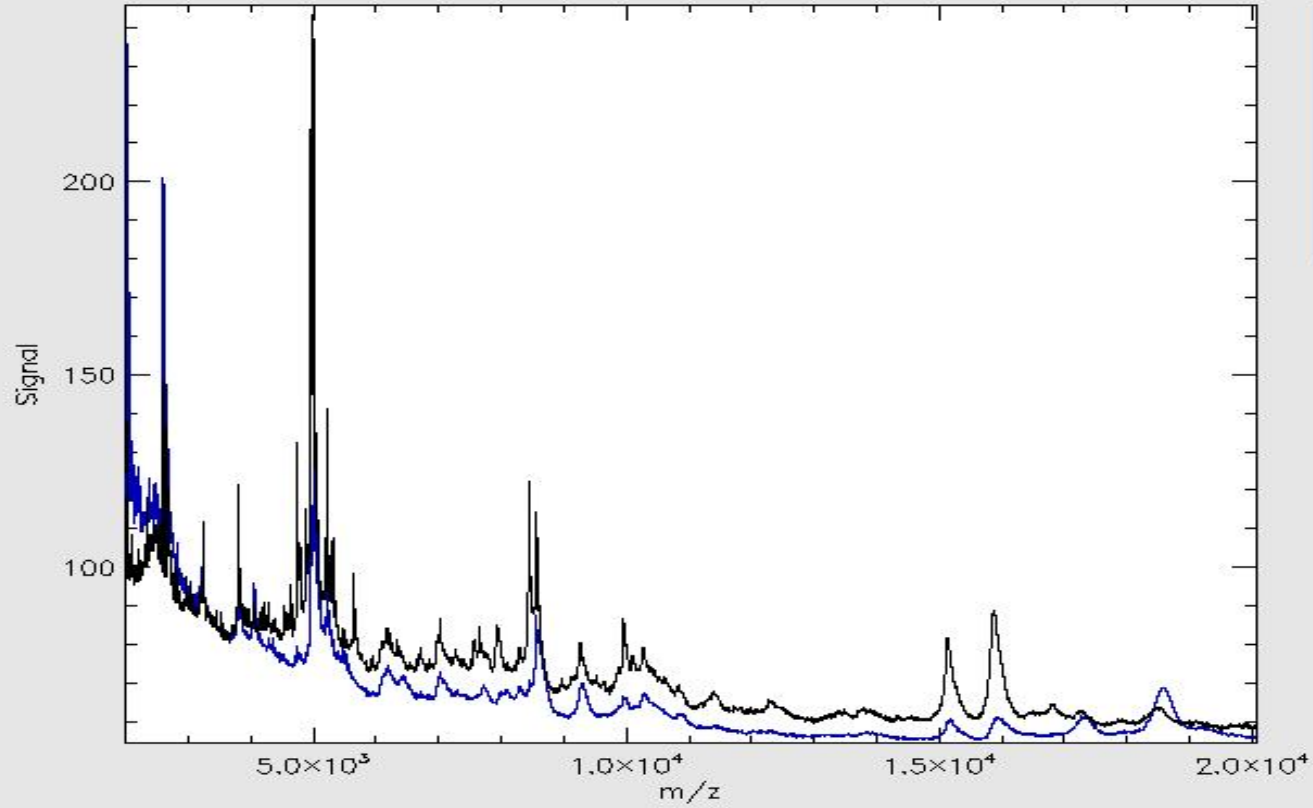



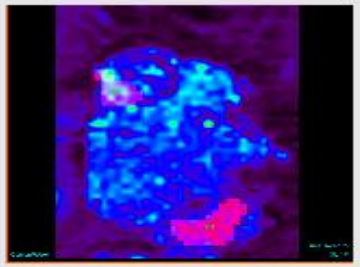

FIG. 1. **Imaging MS of phospholipids in positive ionization mode from a whole-body 1-day-old mouse pup section.** *A*, photomicrograph of the section after 2,5-DHB matrix deposition by dry coating. *B*, photomicrograph of a H&E-stained serial section. Numerous organs constituent of the animal are observed. *C*, sum spectrum after imaging MS data acquisition with a lateral resolution of 200 $\mu$m. *D*, composite ion image made from the overlay of six different lipid ion distributions with observed abundances in various tissues or organs.

Chaurand et al., 2011; Molecular & Cellular Proteomics 10.2: p 1-11

# Comparing Regions of interest

Control vs. patient



R1
R2

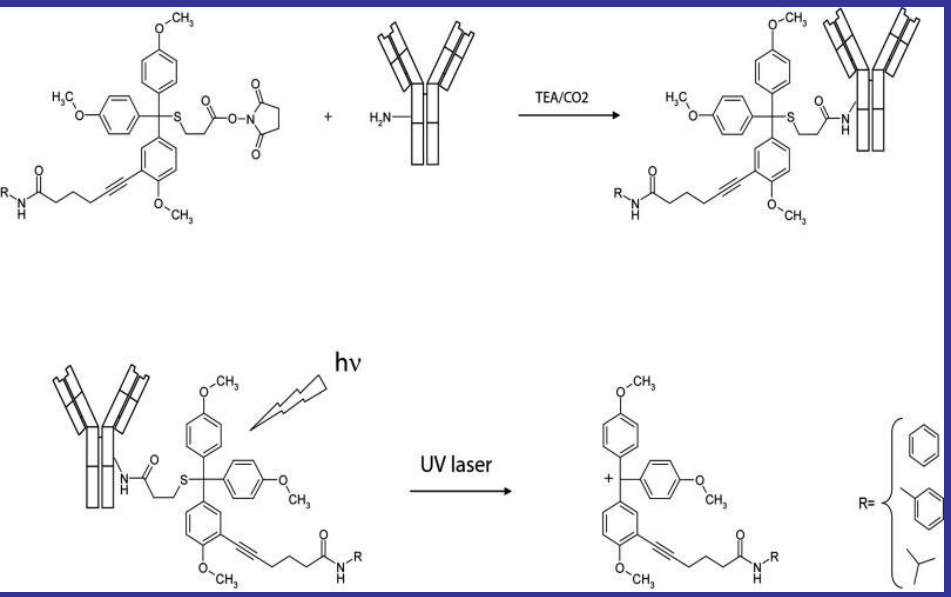
# Application

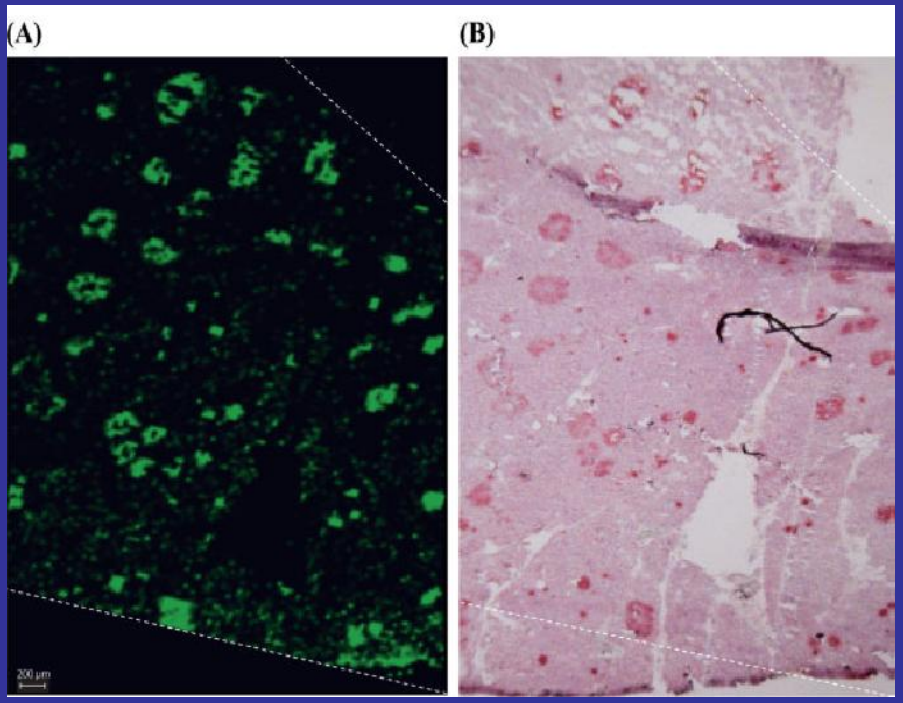

Fig. 1 . (A) DESI image of distribution of cocaine on a LFP blotted on glass. (B) Computer-generated fingerprint from DESI image. (C) Ink fingerprint blotted on paper and optically scanned. (D) Computer-generated fingerprint from optical image. Some of the automatically detected points of interest (minutiae) are represented by dots in (B) and (D).

Ifa et al.; Science. 321(5890):805,

# Special applications

- Immuno-MS-IMS
- **Microchips on IMS**




Imaging of regions immunoreactive with anti-synaptophysin Ab in healthy human pancreas.

(A) Localization of synaptophysin positive cells by TAMSIM. The monoclonal rabbit anti-synaptophysin is conjugated with the **tag El 307 (498 *m/z*)**. The false color green points in the section show the presence of the tag El 307 and thus synaptophysin positive cells.

(B) Classical IHC image with the anti-insulin Ab. The dark pink spots correspond to Langerhans islets and so the synaptophysin-positive cells. The distribution of synaptophysin positive cells in (A) is very similar to that in (B).

*Thiery, G., et al., Proteomics 2008*

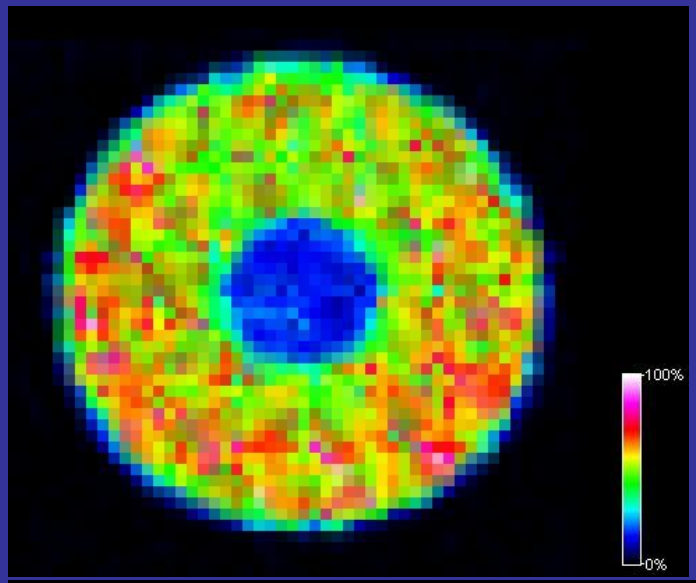
# Surface interaction with peptides

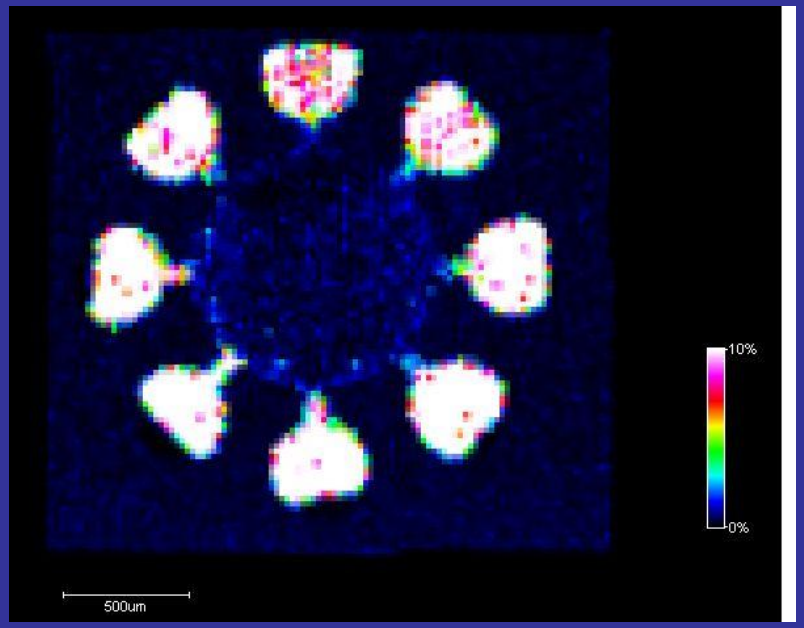
Angiotensin 2, 1 µl, 1 pmol

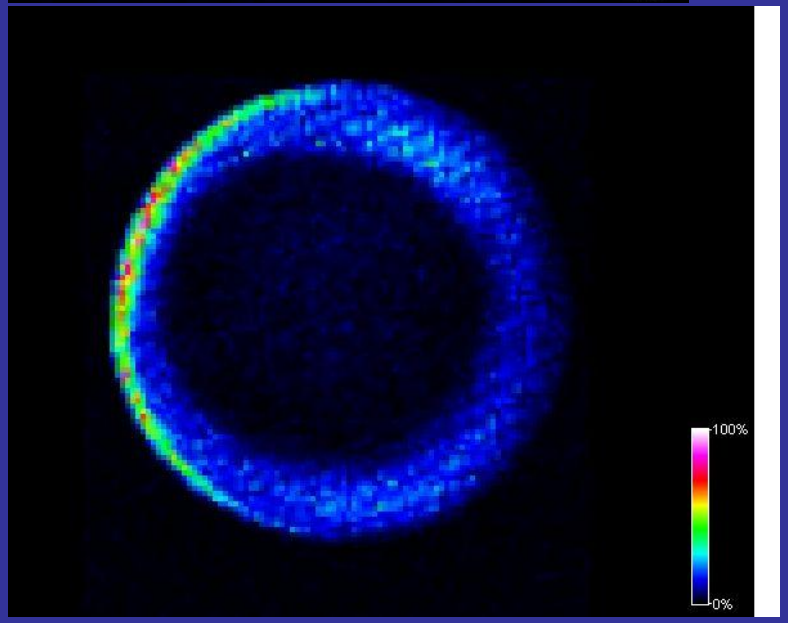
1 mm spot $[A+H]^+$

2 mm spot $[A+H]^+$

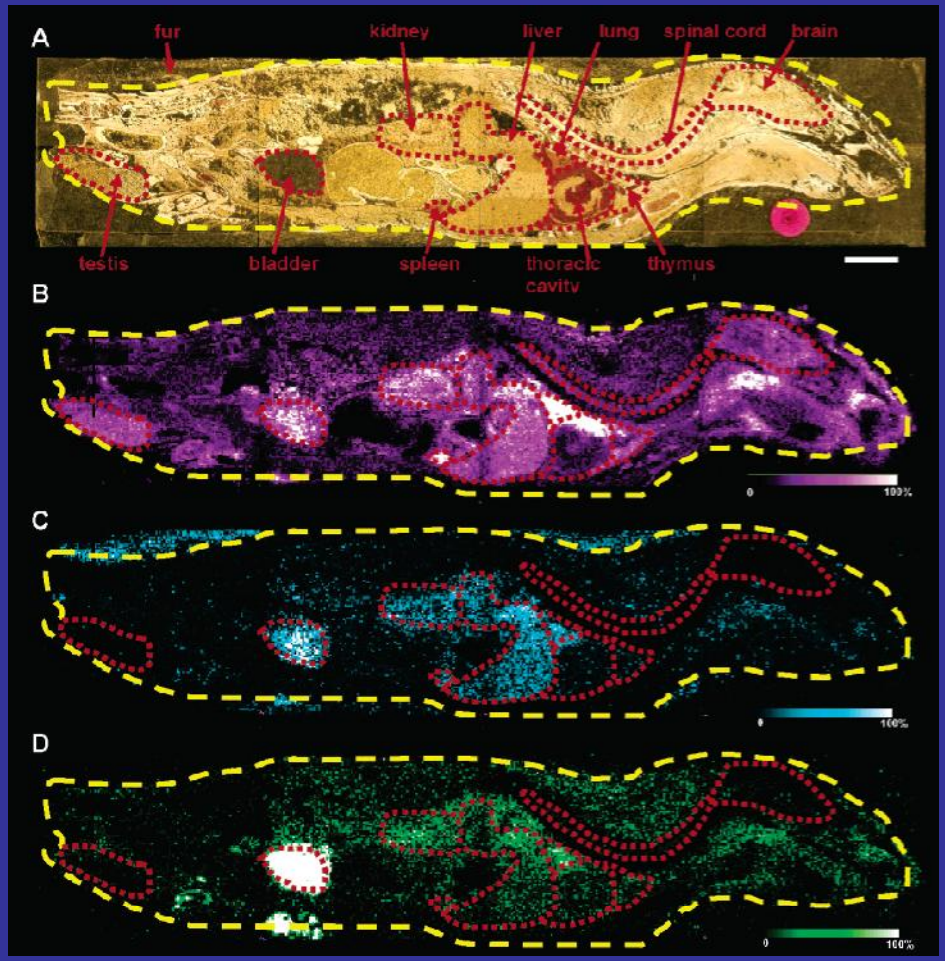
Divider/concentrator spot



# Whole organism



# Advantages of MALDI-IMS

- Analysis of entire tissue/organ/whole organism section at once
- Prior detailed knowledge of tissue molecular composition is not necessary
- Comparison of disease versus healthy may be possible
- Biomolecules are in native state (no modification / no label applied)
- Imaging of biomolecular modifications PTM’s, Metabolites is possible
- Detailed information on molecular identity
- Large scope of different elements and molecules

# Limitations, so far …

- Scans are for <30 kDa ranges

- Sample preparations challenges
  - Tissue cryosections (mostly)
  - Autolysis possible
  - Tissue fixation
  - Suppression effect
  - Reproducibility is a concern

- Interpretation of complex spectra is not straight forward
  - Protein identifications challenges
  - Possible displacement or loss of small molecules after enzyme digestion for MSMS

- Minor proteins enrichment is a handicap
- Requires other staining methods for comparison

“It is always wise to look ahead, but difficult to look further than you can see.” (Winston Churchill)

- Thank you for your attention

- Questions / comments