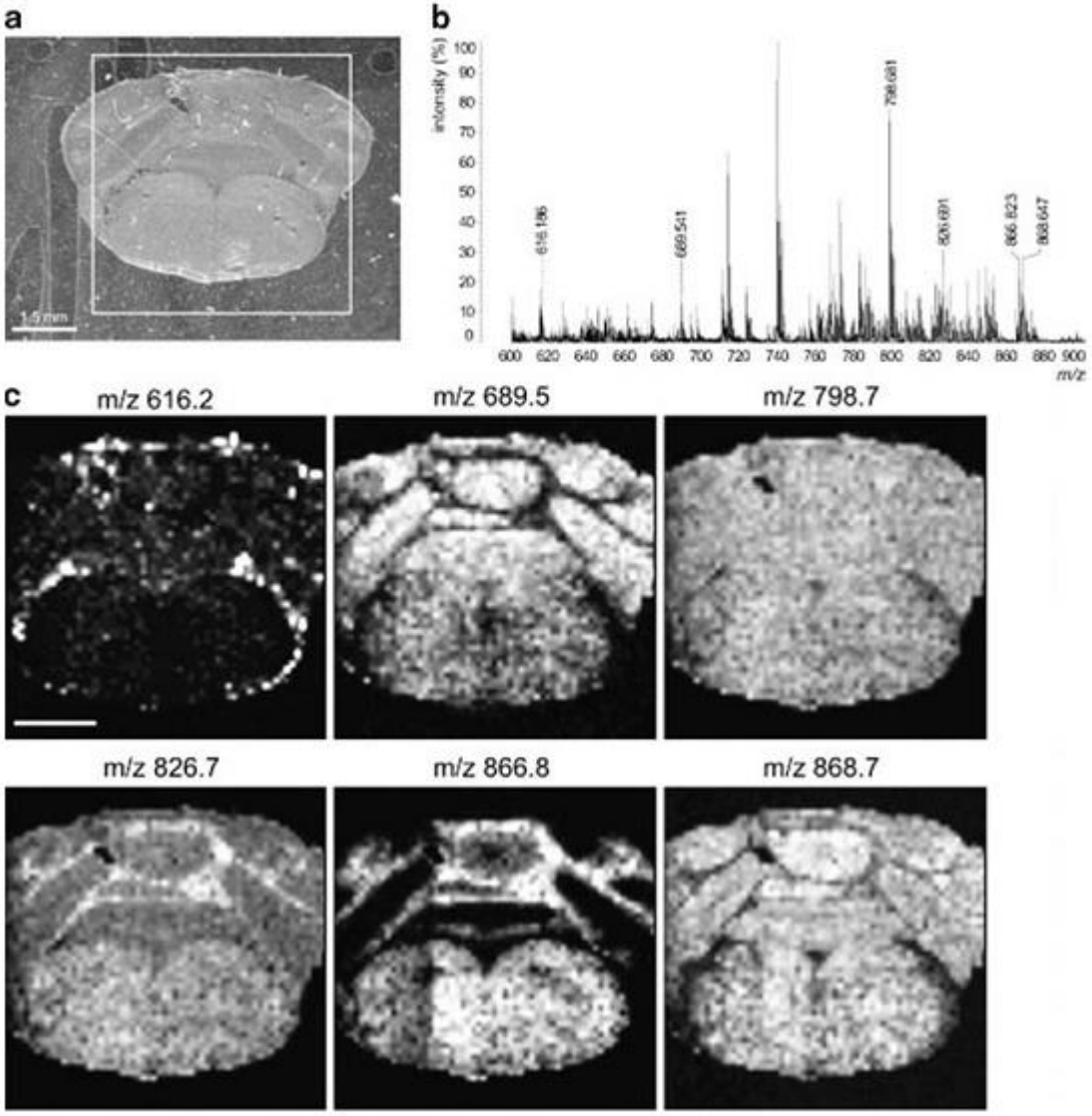
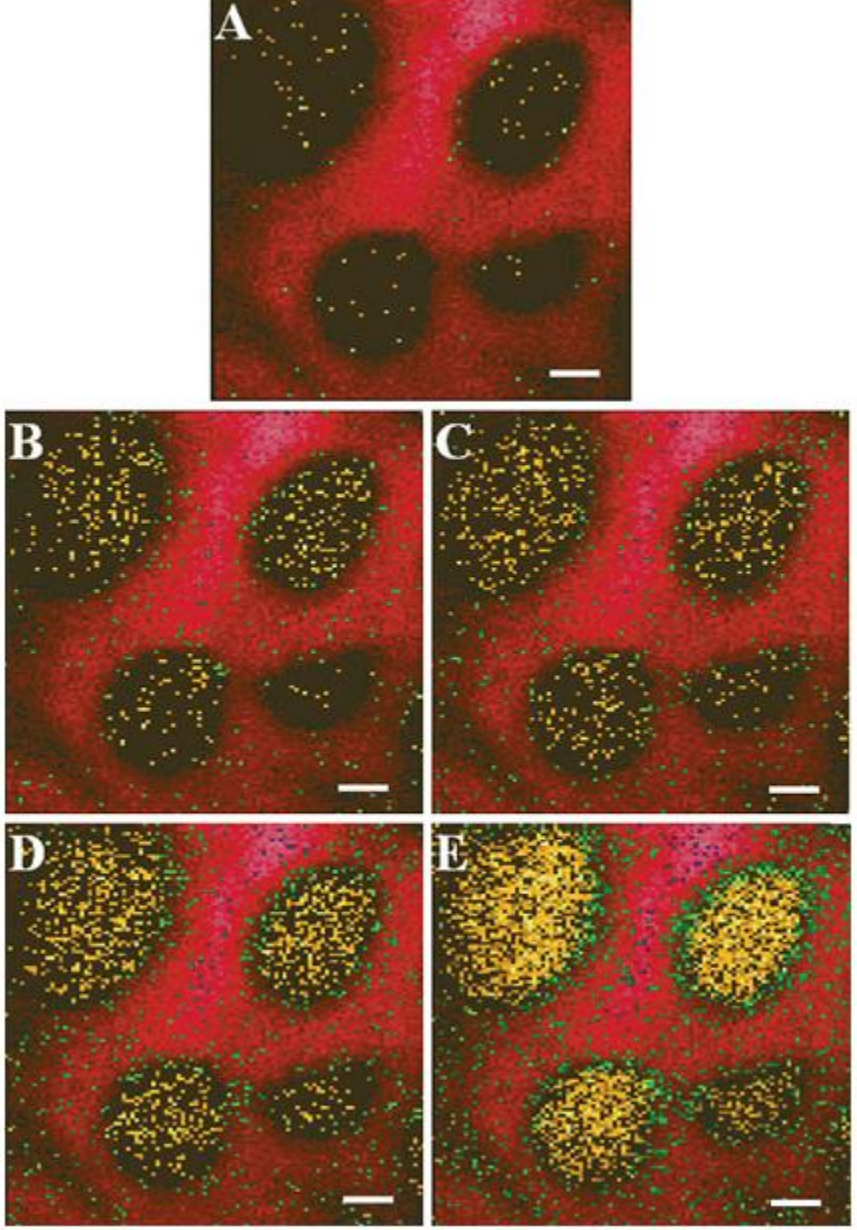
# *IMAGING MASS SPECTROMETRY*

With the advantage of no labeling, IMS has opened a new frontier in medical and clinical applications. Other molecular imaging techniques such as green fluorescent protein (GFP) labeling or immunohistochemistry **require labeling**.

Lipids and low molecular weight compounds in tissue sections cannot be observed with those conventional microscopic and electron microscopic techniques; therefore, no distribution map of these molecules in tissue structure has to date been described in the scientific literature or medical textbooks.




Characteristic lipid distribution in a rodent brain.



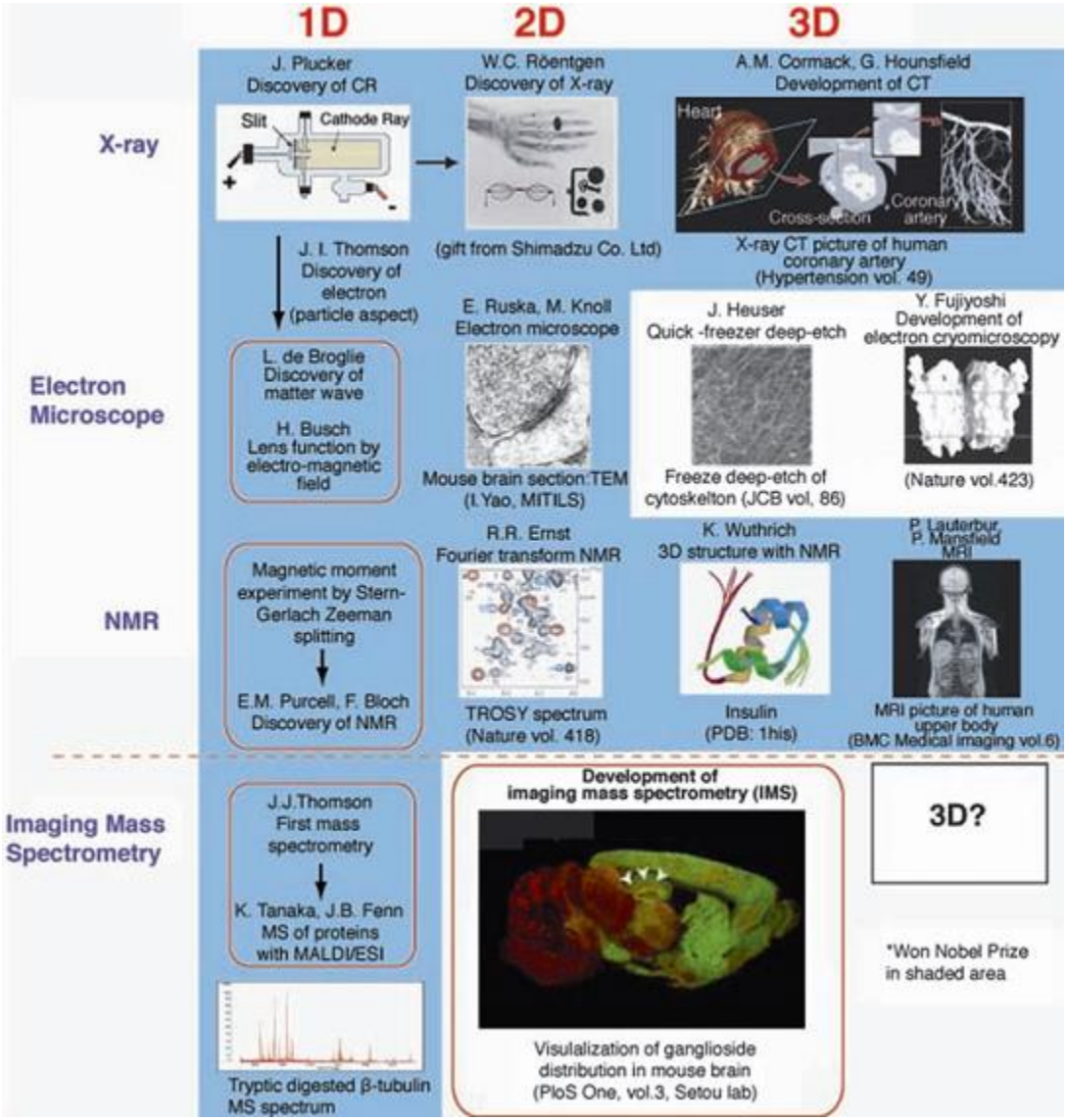

**SIMS** imaging of macrophage cells. SIMS overlay images of the cholesterol fragment ions (yellow) and Na (m/z 23, red) are presented. Green pixels denote the presence of both ions on the macrophages. (a) Cholesterol [m–18] pseudomolecular ion at m/z 369, (b) cholesterol fragmentation ion at m/z 161, (c) cholesterol fragmentation ion at m/z 147, (d) cholesterol fragmentation ion at m/z 109, (e) cholesterol fragmentation ion at m/z 95

The mass microscope is now standing alongside computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) in our hospital at Hamamatsu University School of Medicine

| | MRI | NIRF | PET | Biolu. | WBAL | Opt. | IMS |
|---|---|---|---|---|---|---|---|
| In vivo | ✓ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ |
| Sensitivity | μM | nM | pM | nM | nM | nM | μM |
| Resolution | 50 μm | 5 mm | 5 mm | 1 mm | 10 μm | 1 μm | 50 μm |
| Time required | min | min | min | s | day | min | min |
| Labeling | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Dimension | 3D | 2(3)D | 3D | 2(3)D | 2D | 2D | 2D |
| Cost | ¥¥¥ | ¥ | ¥¥¥¥ | ¥ | ¥¥ | ¥ | ¥¥ |

*MRI* magnetic resonance imaging, *NIRF* near-infrared fluorescence imaging, *PET* positron emission tomography, *Biolu.* bioluminescence, *WBAL* whole-body autoradioluminography, *Opt.* optical, *IMS* imaging mass spectrometry

Historical view of the development of analytical instruments. The progress of imaging technology has been categorized by its technology ( longitudinal axis ) and its analytical dimensions ( horizontal axis ). NMR nuclear magnetic resonance. Work shown with blue area has received a Nobel Prize. CR cathode ray, NMR nuclear magnetic resonance, CT computed tomography, TEM transmission electron microscopy.



**What kinds of molecules** are dominantly ionized via the MALDI imaging?

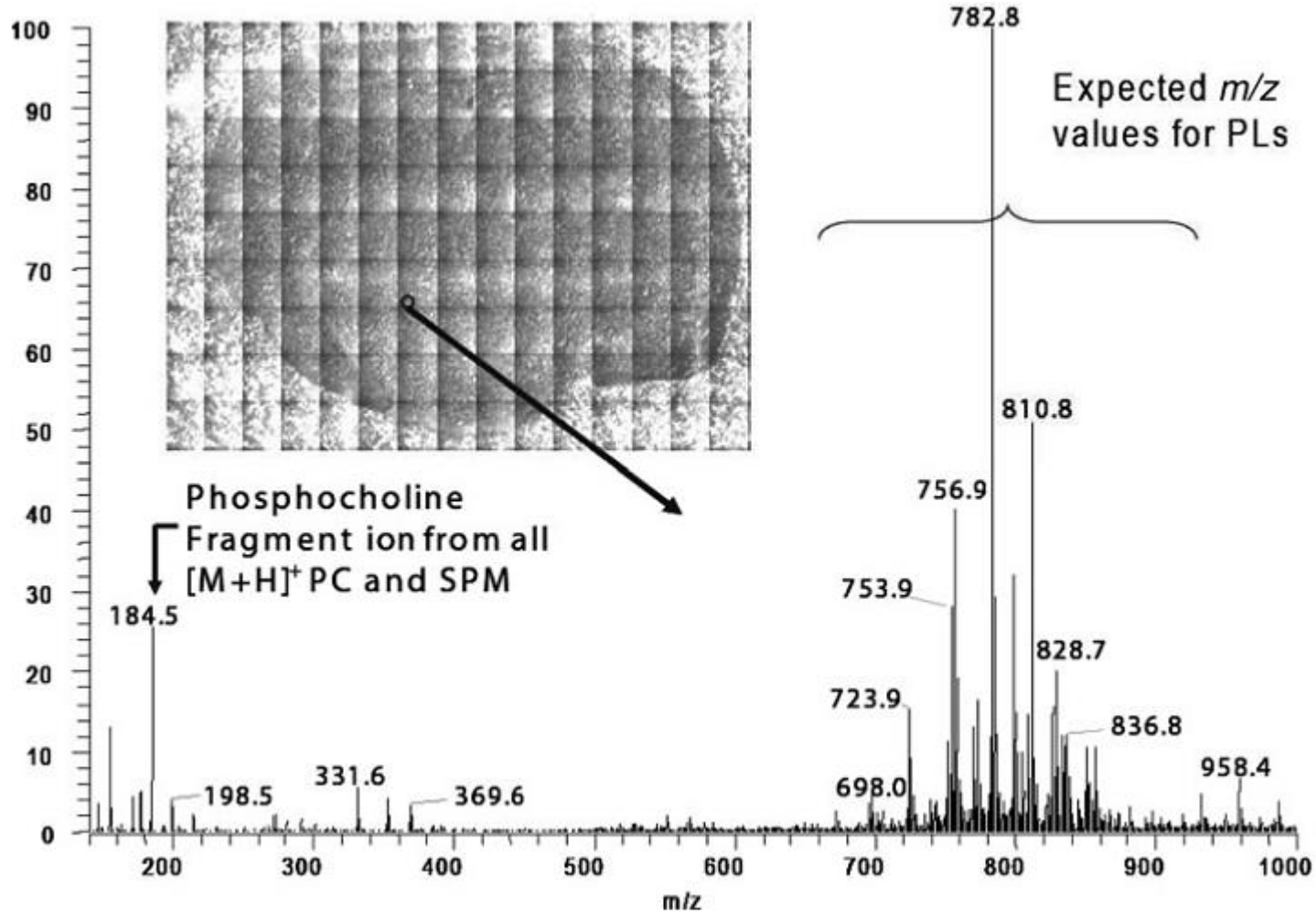
1. Large amount
2. Contains a chemical structure that can be easily charged (e.g., basic groups such as amine- and nitrogen-containing heterocyclic compounds)
3. Has a chemical structure that absorbs UV light (e.g., cyclic organic compounds) (when a UV laser is used for ionization)
4. Easily vaporized
5. Co-crystallizes easily with the matrix
6.

When an infrared (IR) laser is used, the compounds that absorb IR are easily ionized.

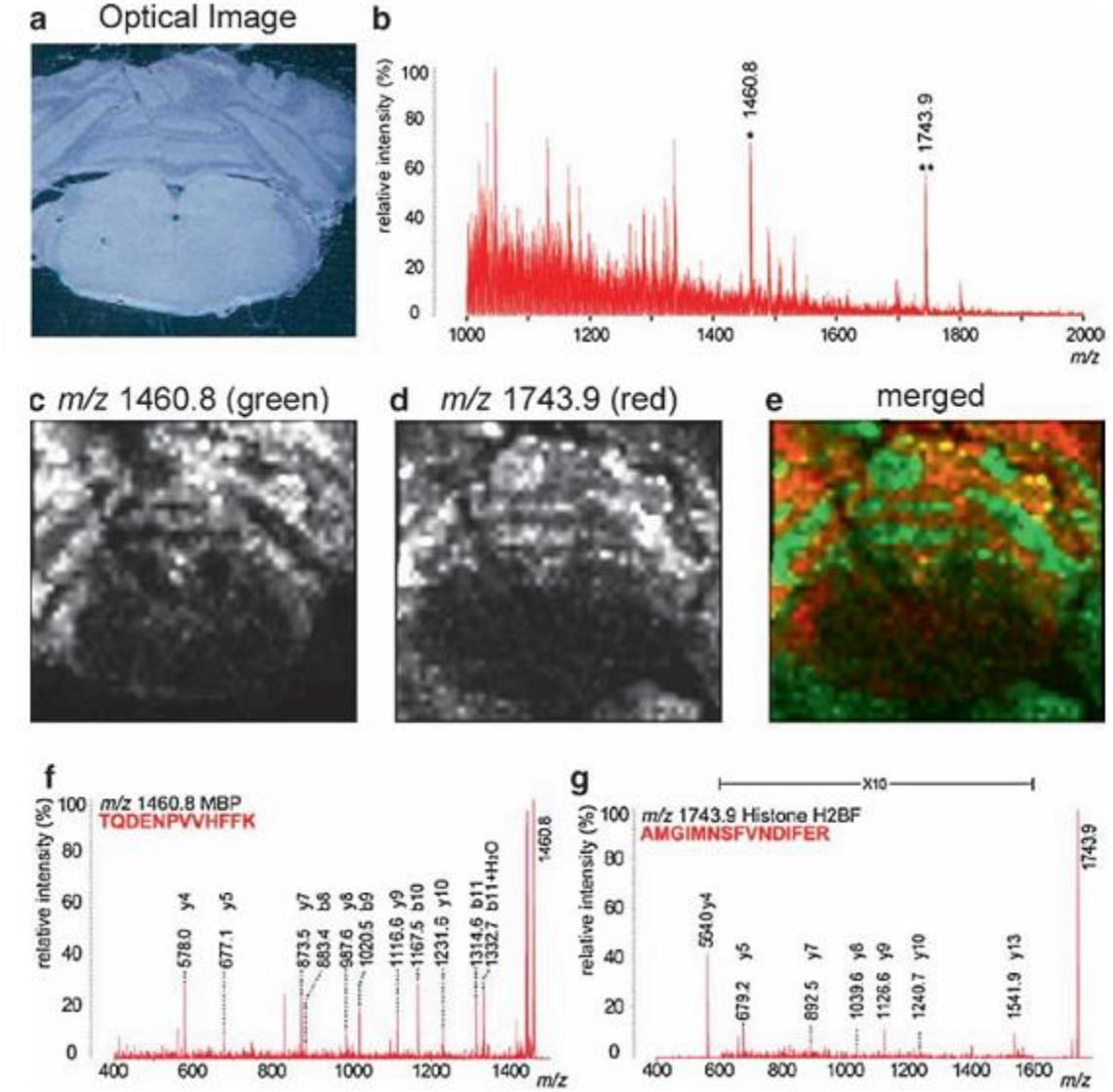
In brain tissues, phospholipids satisfy most of these criteria;

•First, a large amount more than 60% in dry weight – of a mouse brain consists of lipids.

•Second, phospholipids have easily ionized structures; polar lipids, particularly phosphatidylcholine, contain a phosphate and trimetylamine group both of which facilitate addition of charge to the molecule.

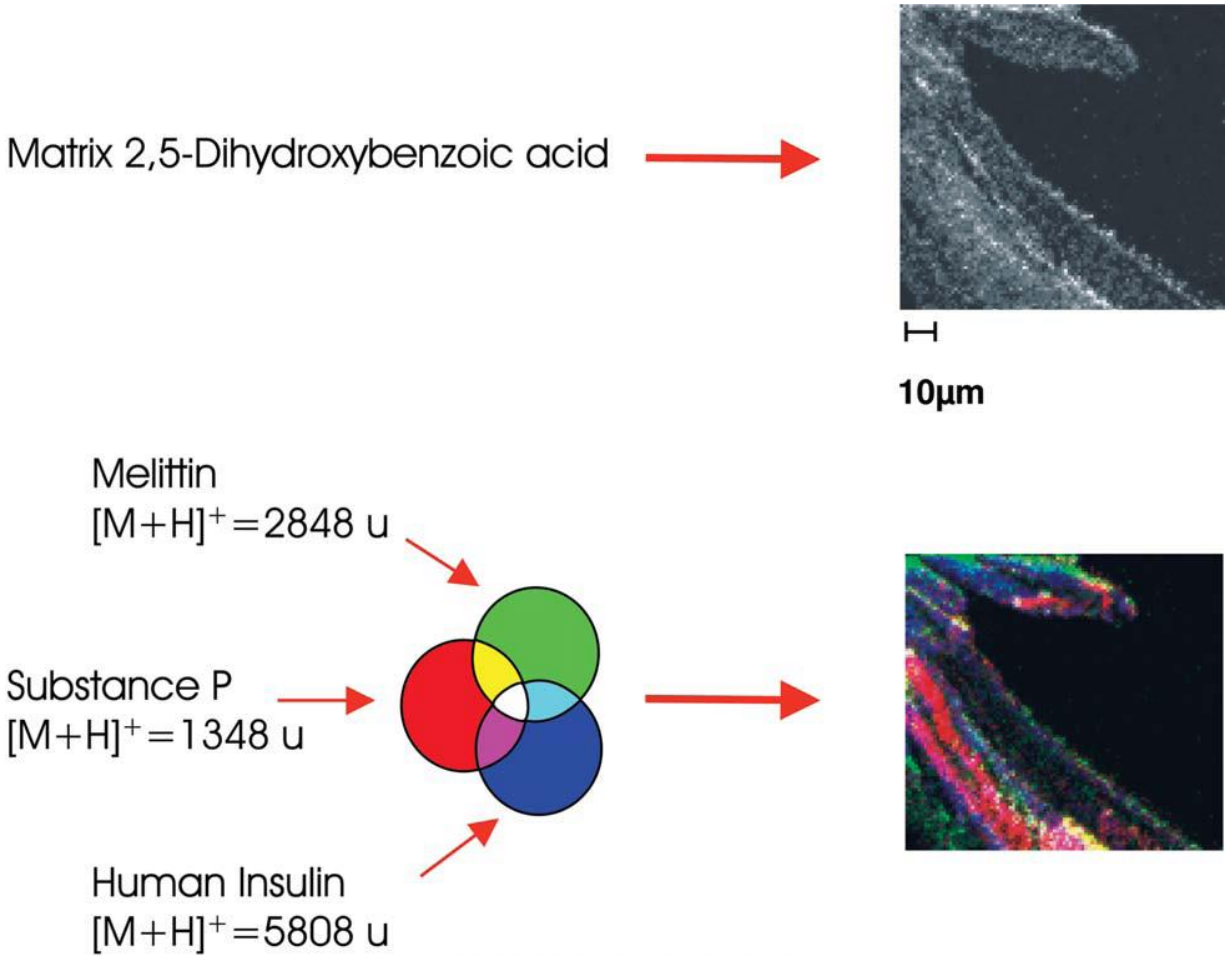


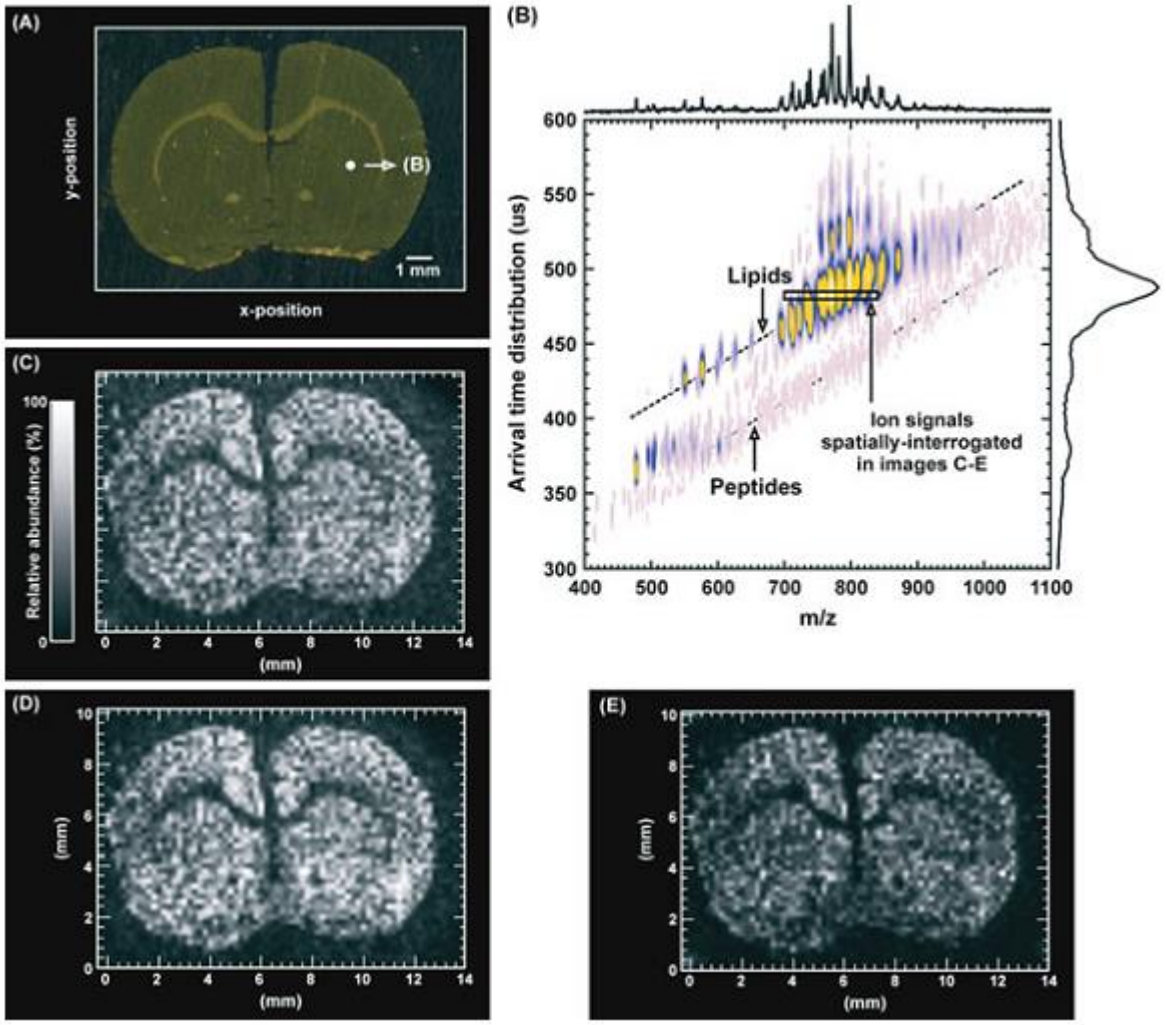

The inset is an optical image (9×15 mm) generated from inside the mass spectrometer of a rat brain section coated with DHB matrix. It was acquired with 1×1 mm square pictures that are stitched together; this creates the lines in the picture. The mass spectrum shows the signal from the area on the tissue indicated by the circle and arrow. The open circle is approximately equal to the laser spot size (120 μm).The spectrum was acquired with 10 laser shots. A total of 11,156 spectra were collected across the tissue section.




Tryptic-digested protein imaging and precursor ion mass spectra with positive ion detection mode. (a) Optical image of imaging region and (b) accumulated mass spectrum from imaging region. (c, d) Imaging results of m/z 1,460.8 and 1,743.9, which are labeled by asterisks in (b). Merged image ( red , m/z 1,460.8; green, m/z 1,743.9) is shown in (e). These peaks were identified by direct multistage tandem mass spectrometry (MSn) MSn as the fragment ions of myelin basic protein (MBP) (f) and histone H2B (g).

High-resolution MALDI image of a dried droplet preparation of a homogeneous solution of three peptides, substance P, melittin and insulin using 2,5-dihydroxybenzoic acid as a matrix. The distribution image indicates that analyte ions segregate during solvent evaporation in different areas of the matrix crystals.






(a) Optical image of a thin coronal rat brain tissue section adjacent the section analyzed by IMS. (b) Lipid imaging of the analytes indicated at an ion mobility arrival time of 480–484 μs and m/z 700–840 is illustrated in (c)–(e). Extracted ion density maps for two phospholipids at m/z 771–776 and 819–823, respectively.

# IONIZATION MECHANISMS

Most imaging mass spectrometry experiments now use either MALDI or SIMS

MALDI can record the spatial distribution of high mass molecules using the chemically specific molecular ions, however, typical spatial resolutions are approximately 25 mm or more (though 10 mm sources are now available).

SIMS imaging is able to provide high spatial resolution images, sub-micron is routine and 50 nm is commercially available, however, the molecular ion mass range is much lower than that of MALDI, most imaging experiments use ions of $m/z<500$.

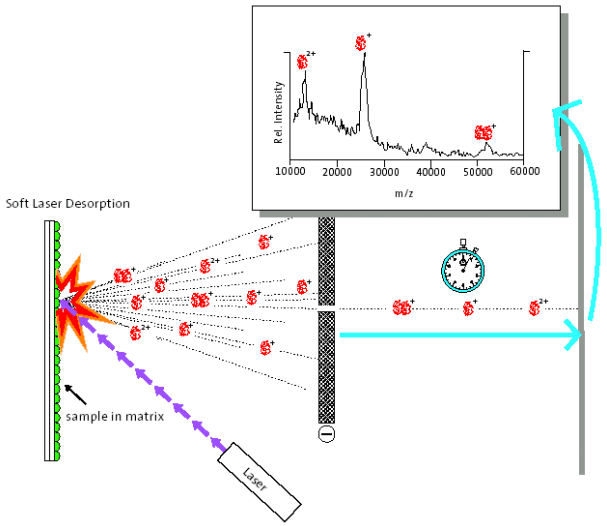
M. Setou, Imaging Mass Spectrometry, Springer, 2010.

# MS Imaging: desorption techniques

## MALDI

**(Matrix Assisted Laser Desorption Ionization)**

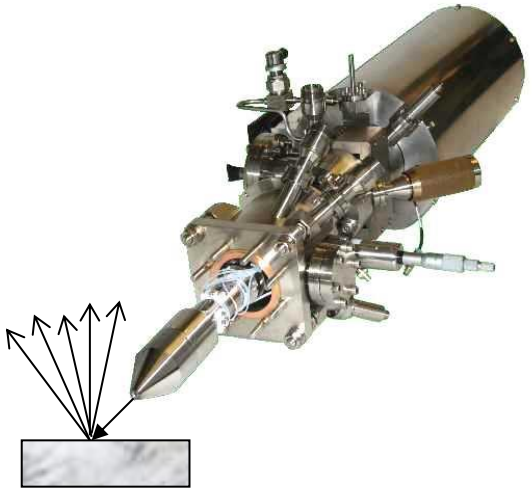
- High mass range
- IR- / UV-Laser
- Suitable for peptides / Proteins
- Limited spatial resolution

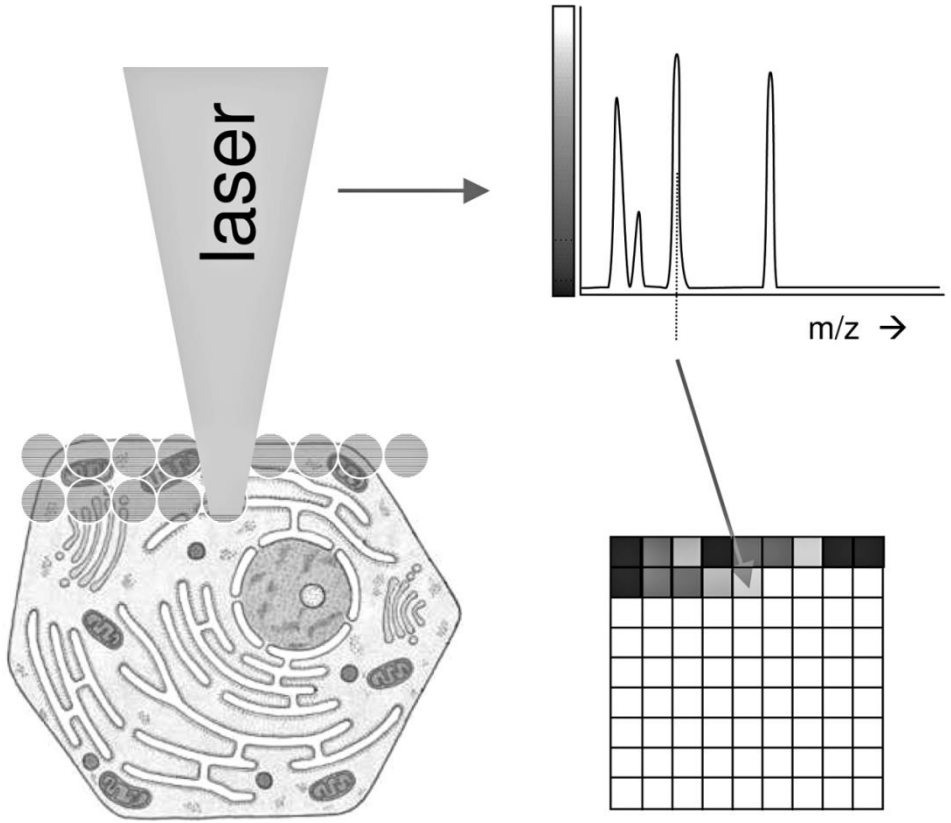



## SIMS

**(Secondary Ion Mass Spectrometry)**

- High spatial resolution (nm range)
- Cluster SIMS ($Au_n$, $Bi_n$, $C_{60}$)
- Highly suitable for lipids
- Low mass range (up to 1 kDa)





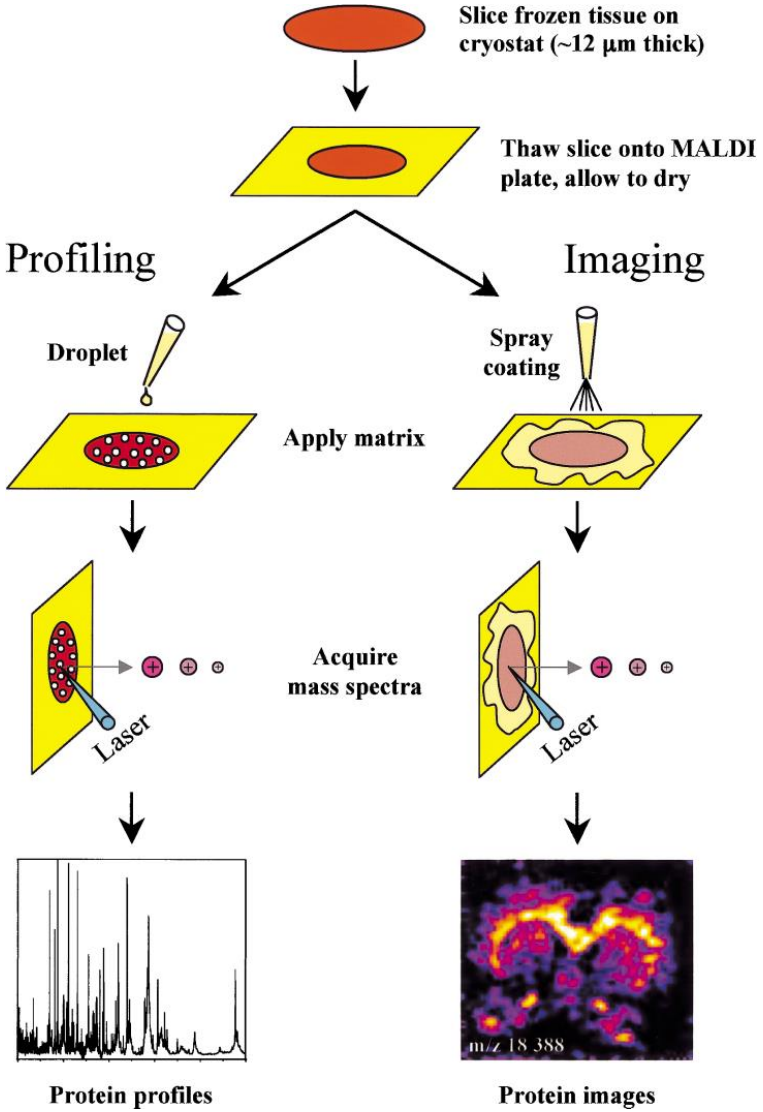

A pulsed laser scans the surface (e.g., a biological sample), producing a mass spectrum for each spot. The intensity of a selected mass signal (a molecular species or “biomarker”) is transformed into a grayscale value and drawn to the according pixel map.

Matrix is first uniformly deposited over the surface of the section, utilizing procedures optimized to minimize protein migration.

Proteins are then desorbed from discrete spots or pixels upon irradiation of the sample in an ordered array or raster of the surface. Each pixel thus is keyed to a full mass spectrum consisting of signals from protonated species of molecules desorbed from that tissue region.

A plot of the intensity of any one signal produces a map of the relative amount of that compound over the entire imaged surface.

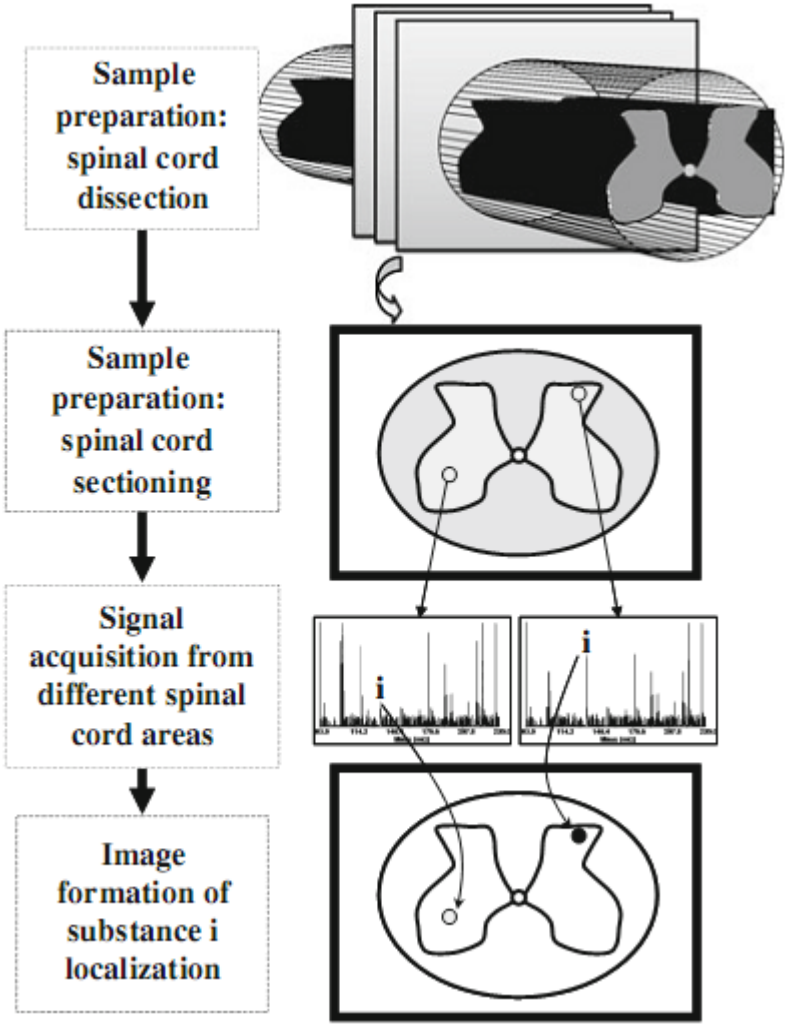
Scheme outlining the different steps involved for profiling and imaging mass spectrometry of mammalian tissue samples.



# Two approach in Imaging MS microprobe and microscope

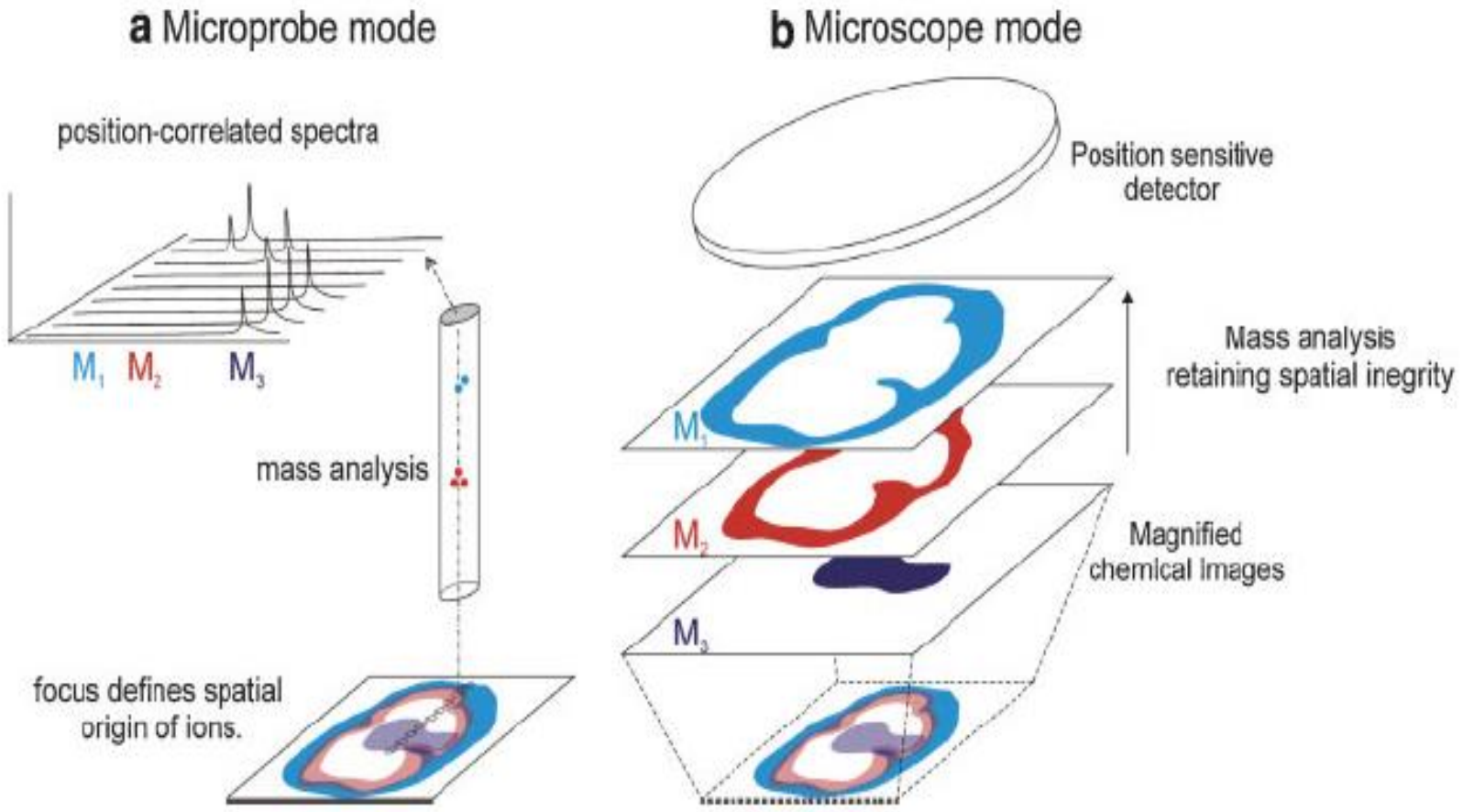
Microprobe : A focused ionization beam is used to analyze a small, localized region of the sample. The resulting mass spectrum is stored along with the spatial coordinates of the spot, as defined by the focus of the ionization beam. This process is repeated until the entire sample area has been examined and mass spectra associated with each location have been obtained. The molecular images are then reconstructed from the individual mass spectra after completion of the experiment.

Microscope: Microscope mode imaging uses ion-optical microscope elements to project the spatial origin of the ions generated at the sample surface onto a position-sensitive detector. With this technique, spatial information is obtained from within the ionization spot and is thus independent of the spot size of the ionizing beam.

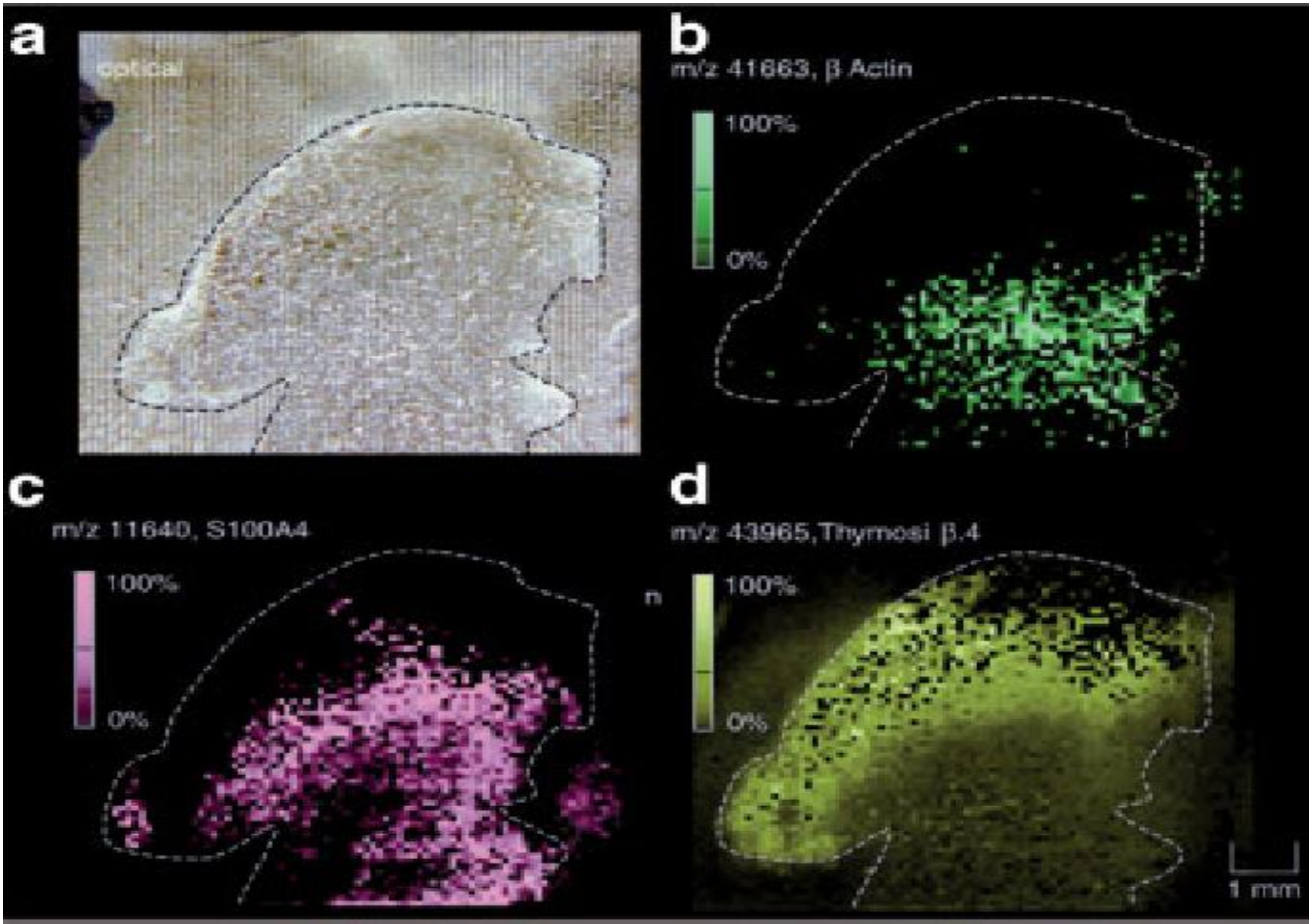


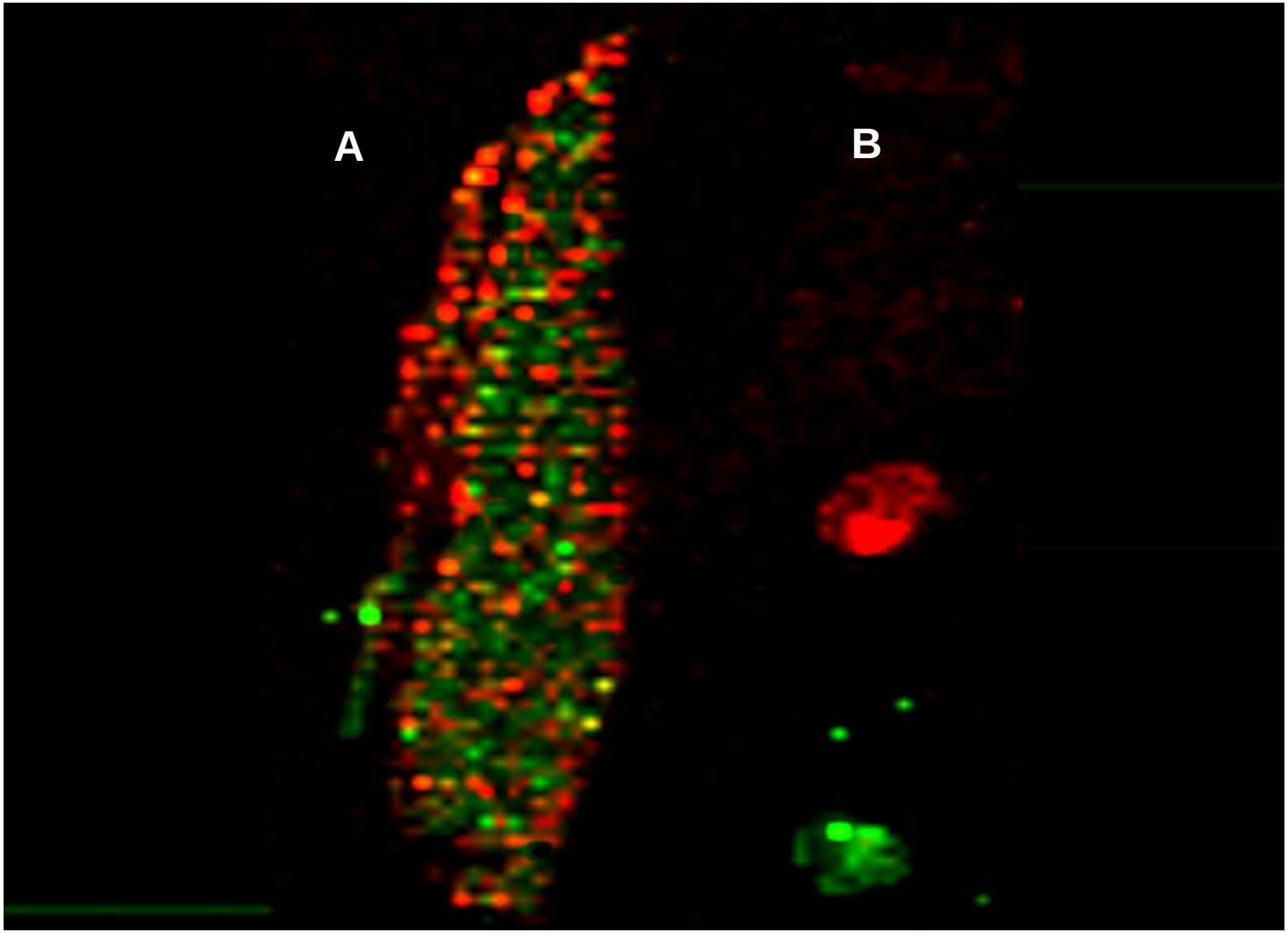

Schematic of the generalized mass spectrometry imaging process utilizing the microprobe mode of operation. The major steps involved include sample preparation and interrogation with a probe, signal acquisition, processing, and final image formation. Distribution of a single or multiple analytes is determined in an array of spots covering the sample surface. Only two mass spectra containing signals from different analytes are shown in the signal acquisition step. Detected signal intensity is often encoded by color or grayscale. Here, the more intense signal is presented in the color black, which provides the most contrast from the background color.

Schematics illustrating the two approaches in molecular imaging mass spectrometry. (a) Microprobe mode imaging records mass spectra from an array of designated positions to construct a molecular image. Using an ion-optical microscope, (b) magnified images of mass resolved ion distributions are recorded with a 2D detector.



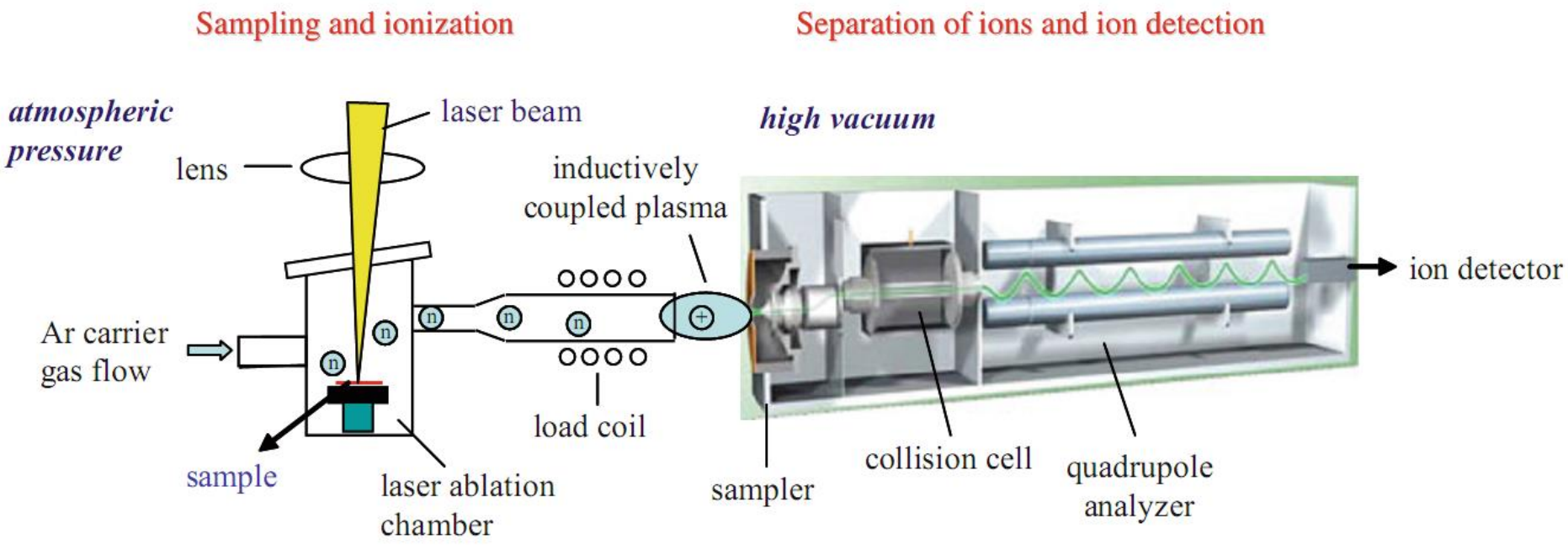
Protein images from a glioblastoma section. (a) Picture of the tissue section mounted on a metal plate, coated with matrix. Images showing the distribution of (b) b Actin, (c) S100A4, and (d) Thymosin b4 within the tissue section.



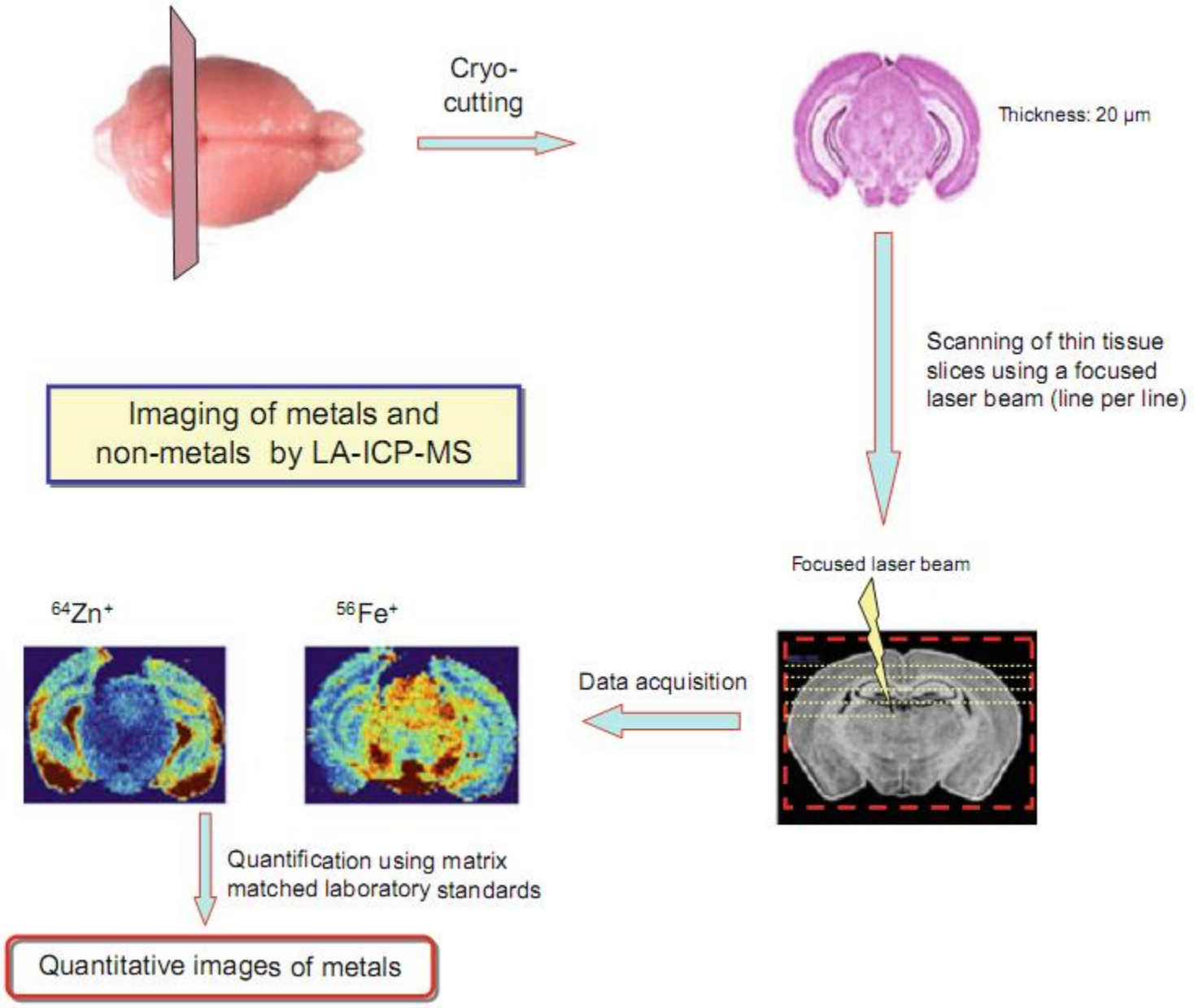



**Images of the Distribution of An Anti-Cancer Pro-Drug and Its Active Metabolite in a Tumour (A = real sample) (B=Control)**

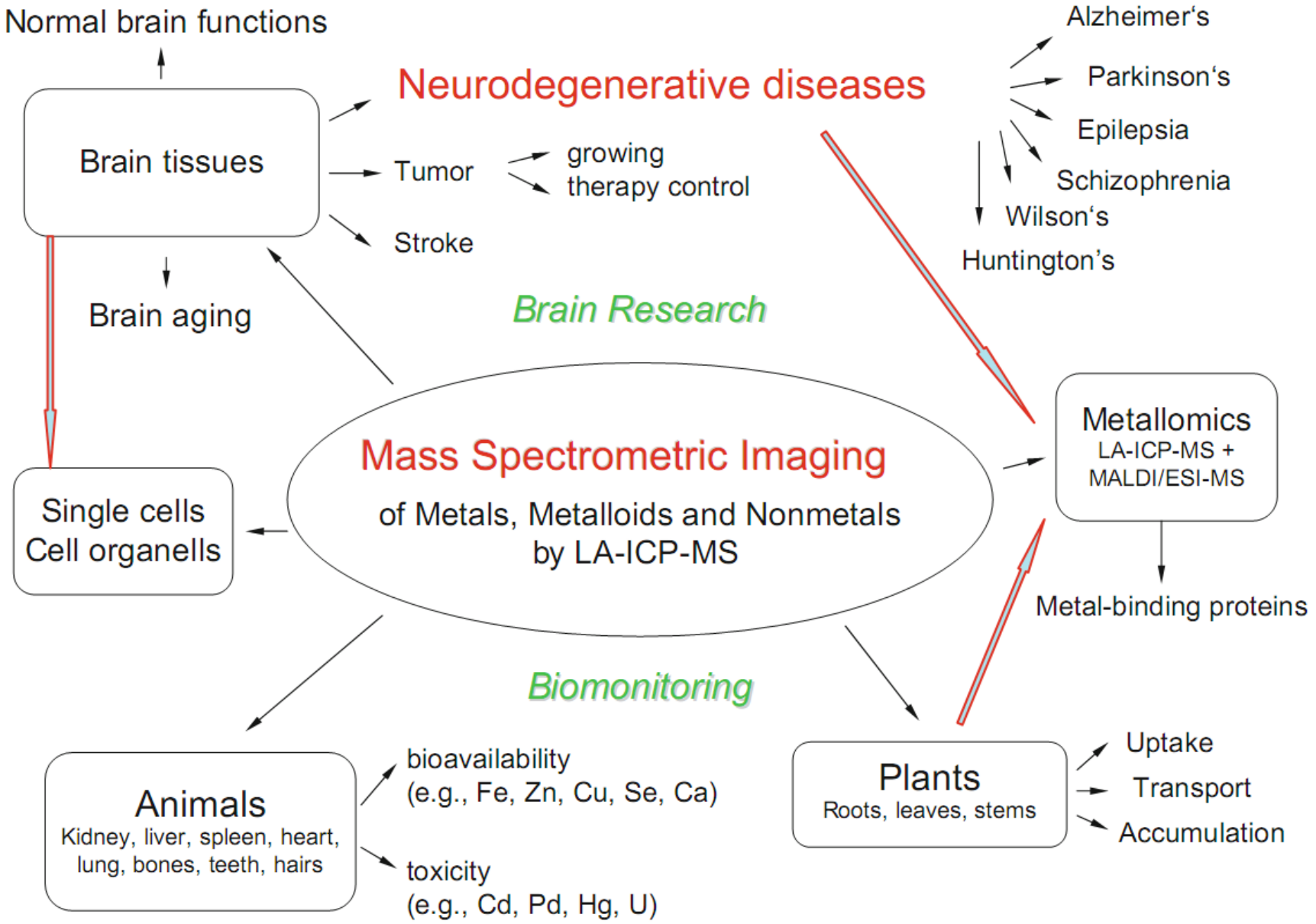
Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used for the quantitative analysis of trace elements in hard biological tissue samples such wood, teeth, and shells but is rarely applied to soft tissues and does not provide the molecular information of MALDI and increasingly SIMS.



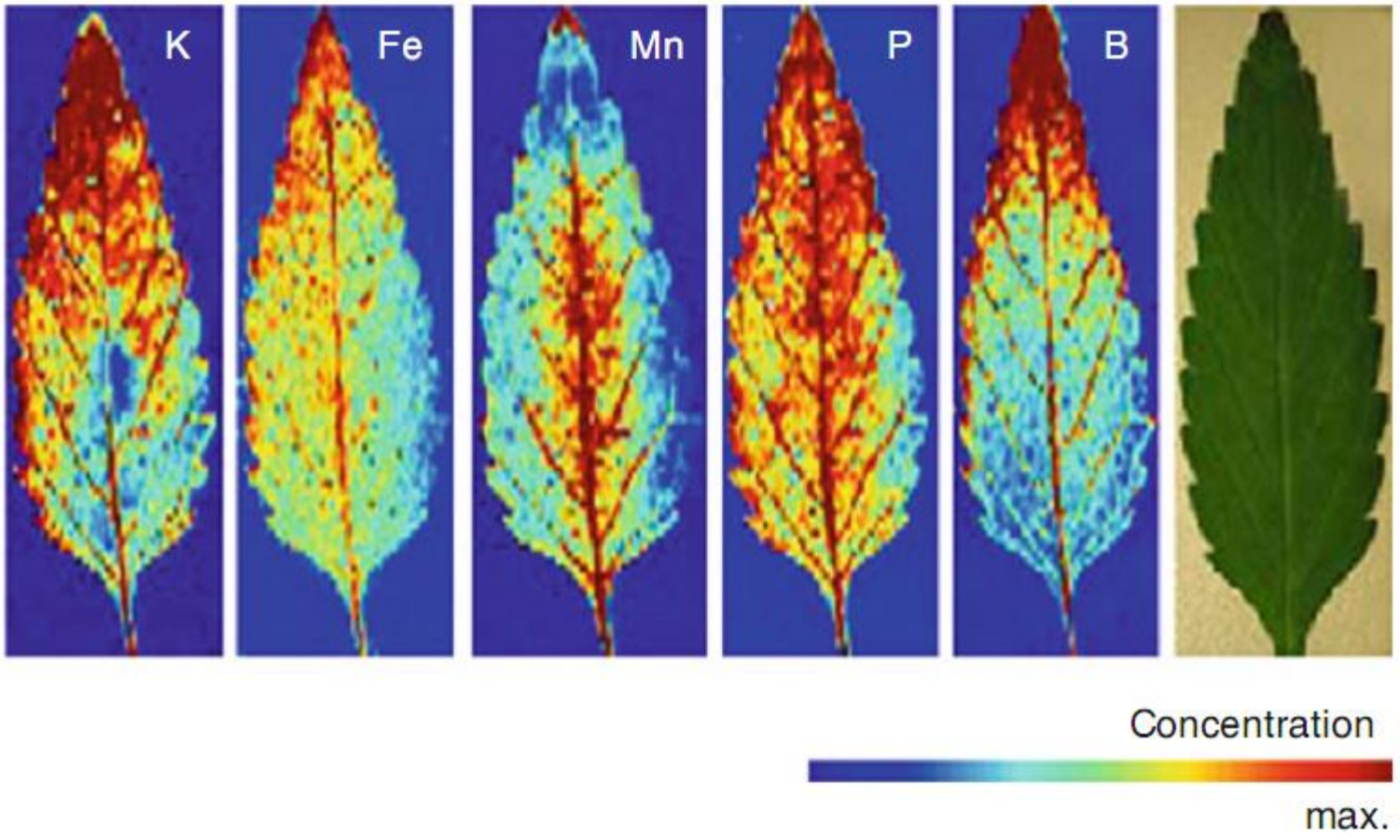

Instrumental outline of LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometer), the laser ablation system is coupled to an ICP quadrupole mass spectrometer with hexapole collision cell.




Schematic of mass spectrometric imaging procedure by LA-ICP-MS on thin section of mouse brain tissue.




Application fields of mass spectrometric imaging by LA-ICP-MS in brain research and for biomonitoring of essential and toxic metals.

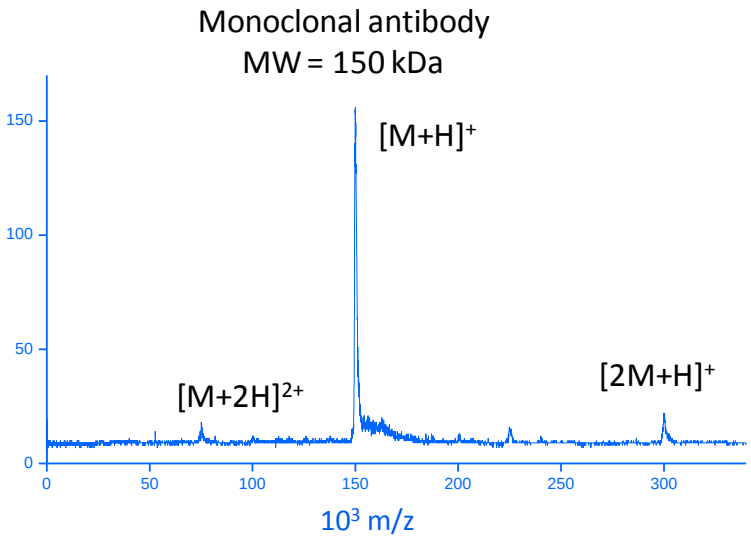



Quantitative images of nutrient elements K, Fe, Mn, P and B measured by LA-ICP-MS in the leaves of E. splendens after Cu treatment.

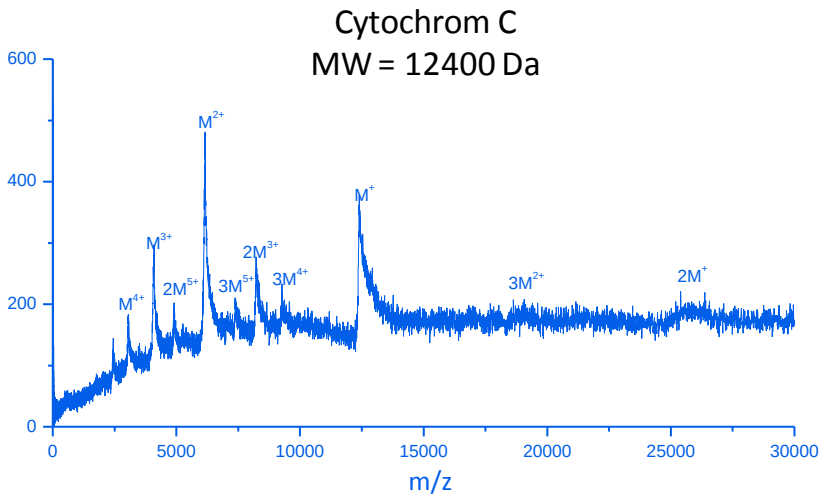
# **Infrared** Imaging Mass Spectrometry

## Advantages of IR-MALDI

### High mass range



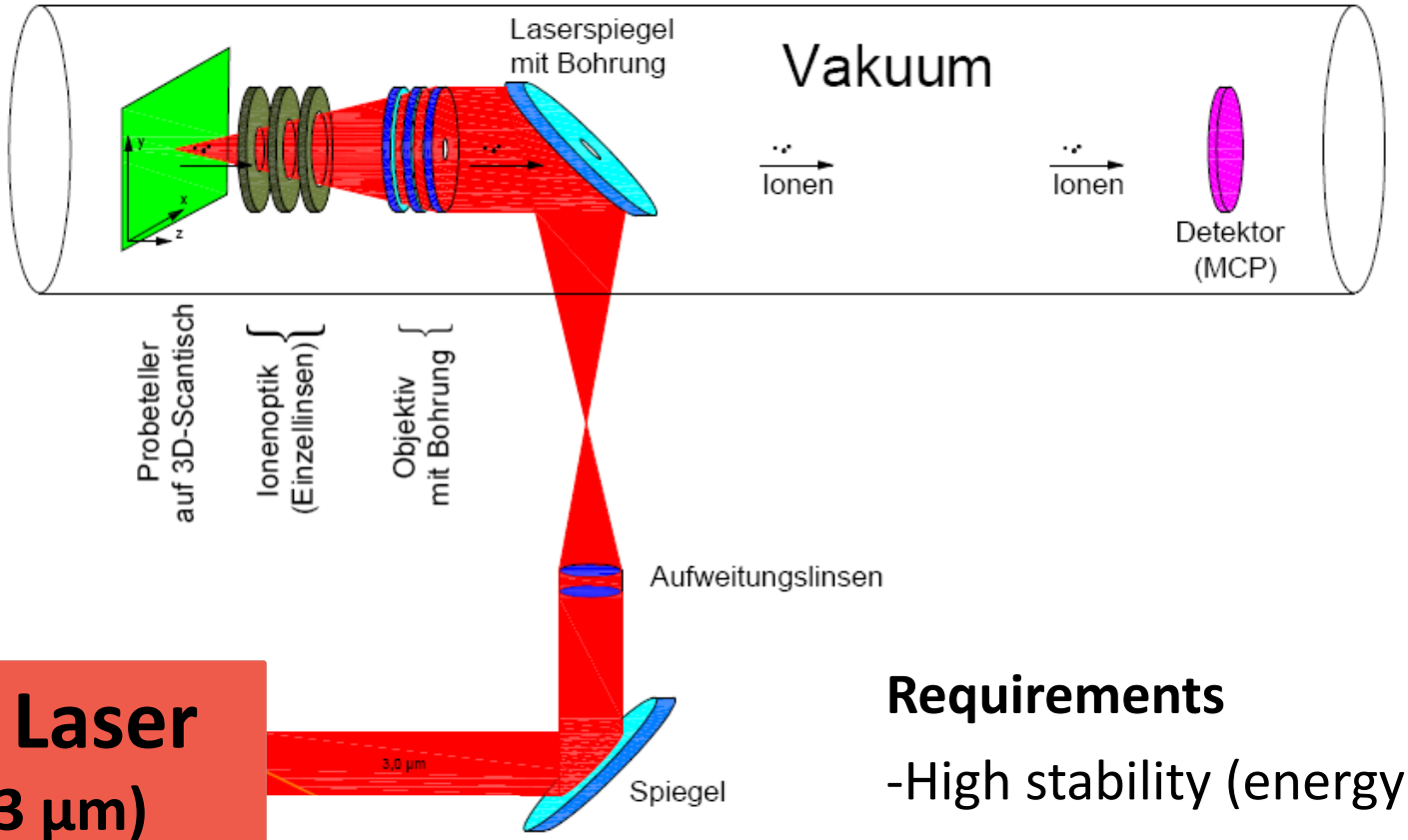

### **Water acts as matrix**




[Leisner, 2004]

# **Infrared** Imaging Mass Spectrometry

Instrumental setup




**IR Laser**
**(3 µm)**

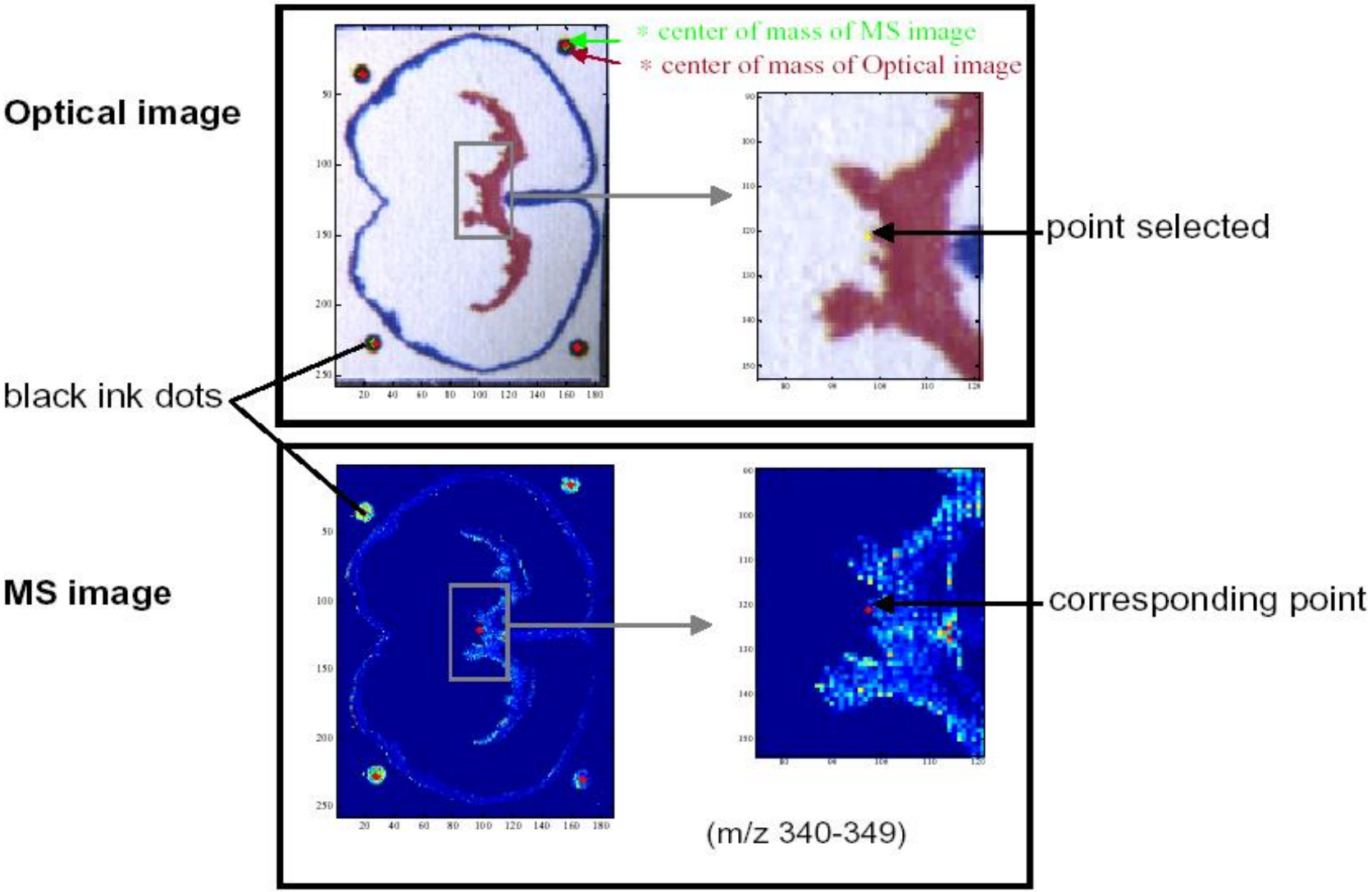
**Requirements**

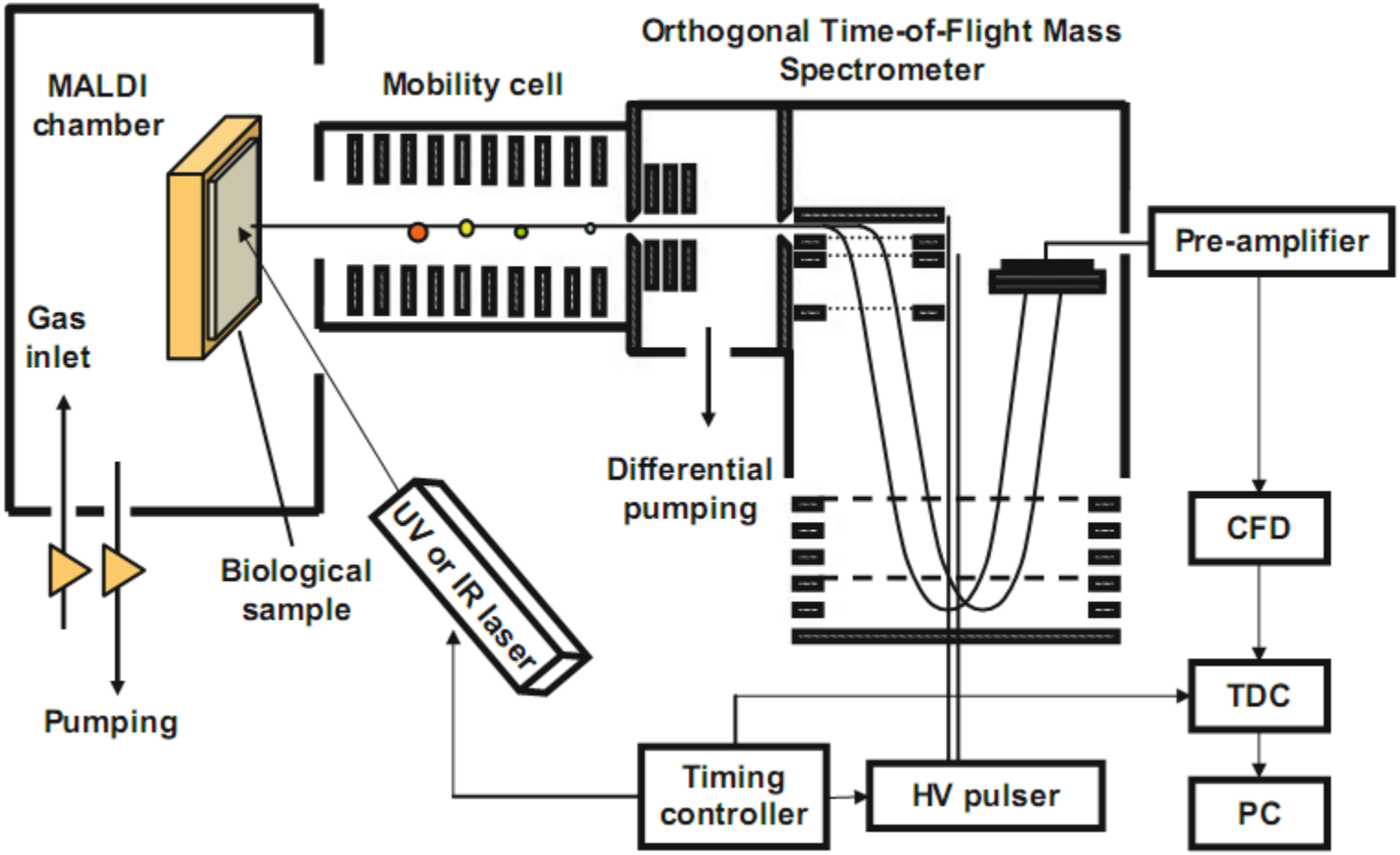
-High stability (energy)

-Fast / high ferquency

-Short pulses

# Registration of optical image to corresponding MS image.






Schematic diagram of MALDI-IM-TOFMS instrument.

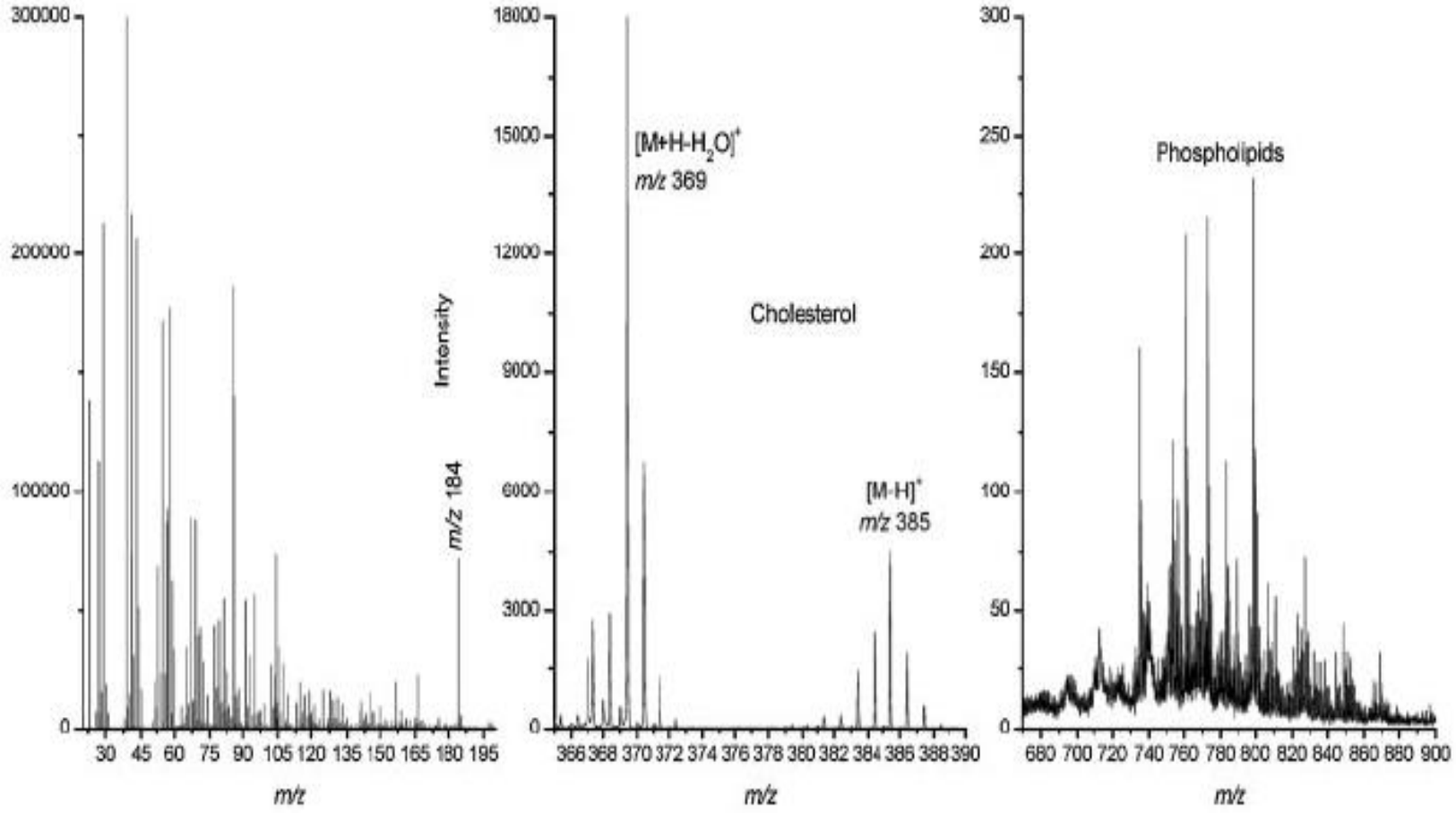
SIMS has traditionally been limited to low mass molecular fragments and atomic ions and high spatial resolution imaging experiments have utilized diagnostic fragments.

In SIMS, the sample surface is bombarded with a high-energy primary ion beam, either single- or polyatomic, between 5 and 25 kiloelectronvolts (keV).

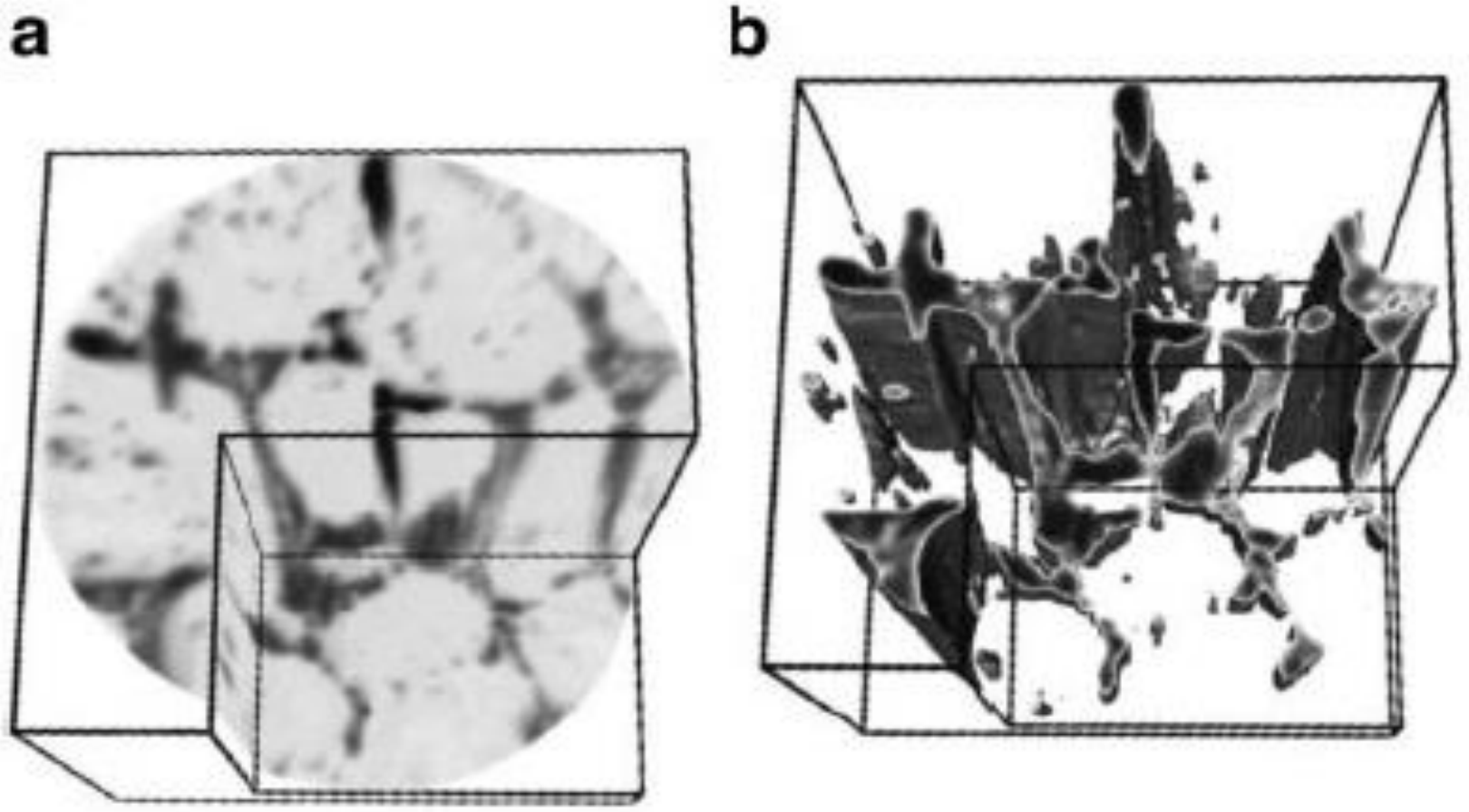
Typical primary ions used in SIMS include $Ga^+$, $Cs^+$, $In^+$, $C60^+$ and $Au^n$ with $Ga^+$ being able to provide the smallest probe size (less than 10 nm).

The rastering of the primary ion beam over the sample surface results in desorption (or sputtering) of both neutral and charged species, consisting of atoms, clusters of atoms and molecular fragments. The charged species (secondary ions) are extracted into the mass spectrometer for mass analysis.

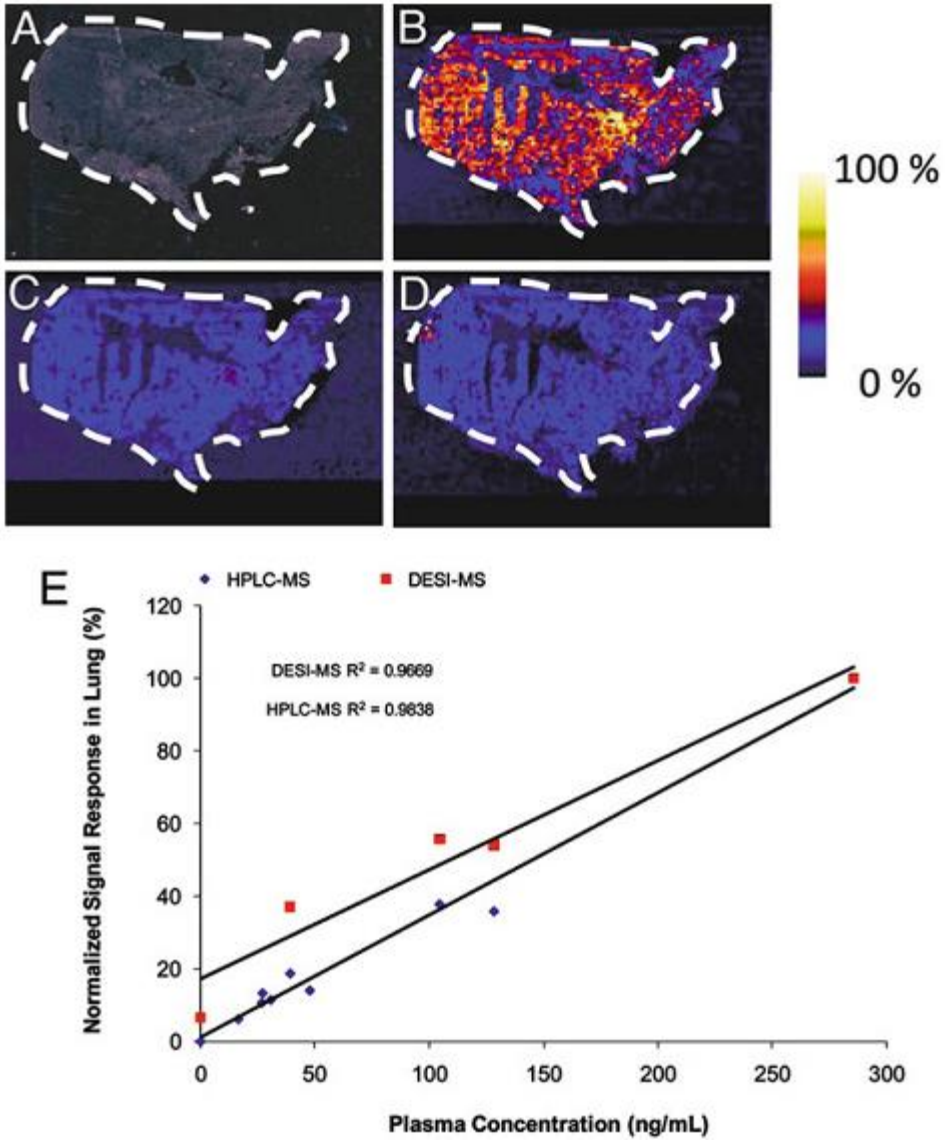
Au3 SIMS mass spectrum in positive ion mode of a mouse brain tissue section. The secondary-ion yields decrease rapidly (and non-linearly) with increasing m/z. It is clear that the number of counts decreases rapidly with increasing mass.



3D SIMS analysis of the distribution of vanadium in highspeed steel.
Dimensions are 15015010 mm. Left is block view and right an iso-surface view.



# DESI Imaging




DESI imaging of a drug in lung tissue. (a) Optical image. (b) Image of clozapine at m/z 327.1. (c) Image of desmethylclozapine at m/z 313.1. (d) Image of sodiated PC 16:0/16:0 at m/z 756.4. (e) DESI-MS imaging and LC/MS/MS results. The signal response in each method was normalized to the maximum response in each experiment and plotted against the clozapine plasma concentration as determined by LC/MS.