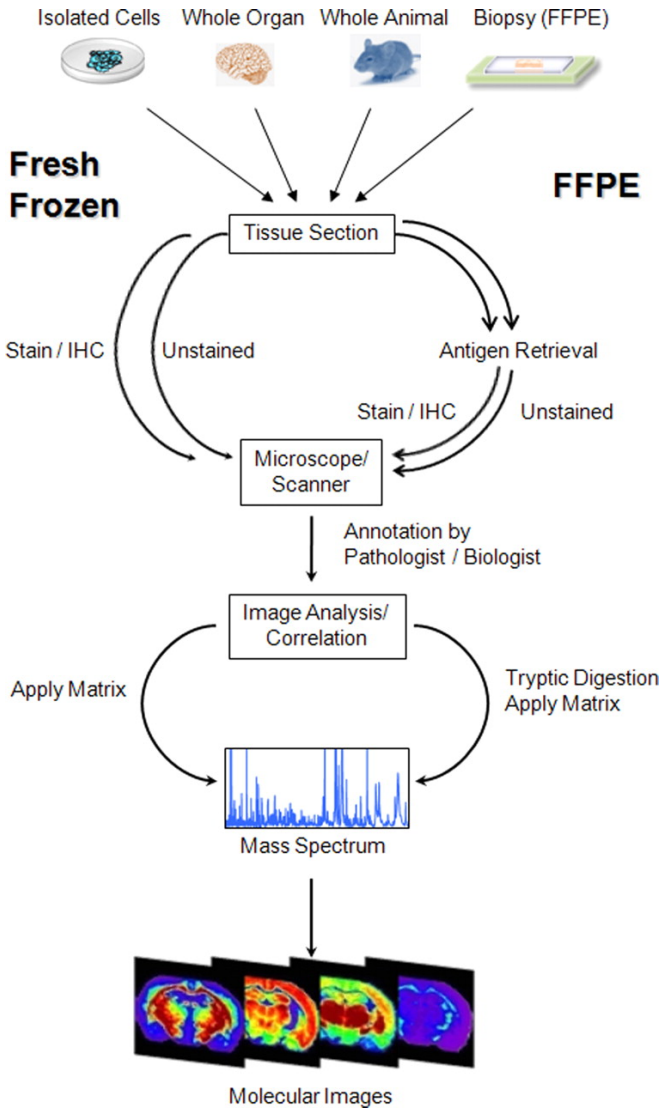
MALDI-IMAGING

- New tool for analysing biological and clinical samples
- Analysis of proteins, peptides, lipds, metabolites and small molecules
- The spatial relationships between the molecules are maintained in the sample, because the tissue is analyzed intact, without homogenization.
- It allows to visualize the distribution of the analytes without the use of antibody or other systems of marking.

Types of mass Spectrometers

- Spettrometro (TOF, TOF-TOF, orbitrap, synapt, FT-ICR, Q-TOF...)
- Ionizzazione
  - MALDI → Proteine, peptidi, lipidi, farmaci
  - SIMS → Piccole molecole, elementi
  - DESI → Piccole molecole, lipidi
- Risoluzione Spaziale
  Ø area colpita
  - MALDI → 10 μm -200 μm
  - SIMS → 100 nm – 10 μm
  - DESI → 300 μm - 500 μm
- Range di Massa
  - MALDI → 0-30 K *m/z*– fino 150 K *m/z*
  - SIMS → < 1000 *m/z*
  - DESI → < 3000 *m/z*
- Velocità dell'acquisizione Frequenza del laser 200Hz – 2kHz

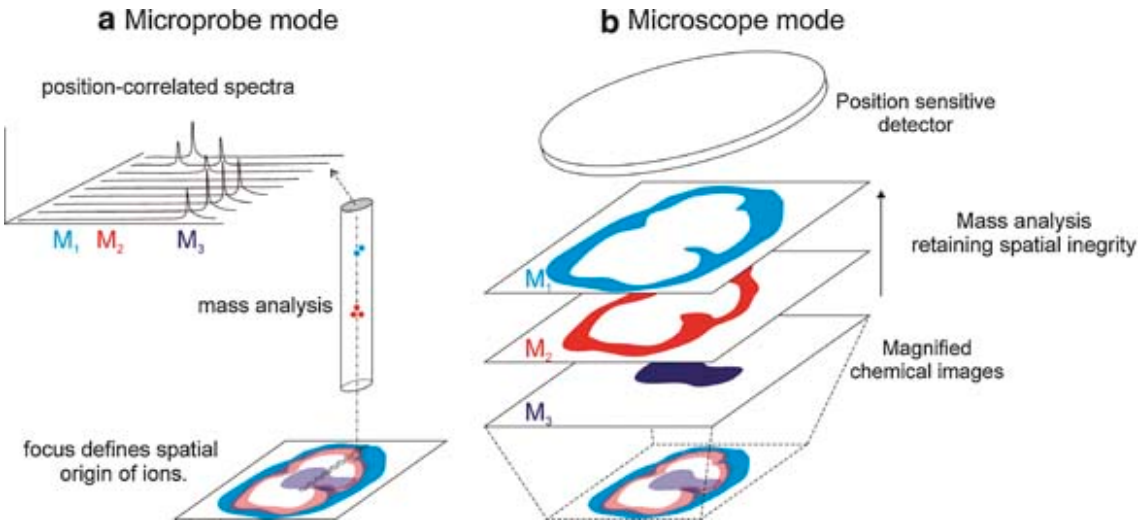
Schematic outline of a typical workflow for tissue samples.



Seeley E H et al. J. Biol. Chem. 2011;286:25459-25466

Analytica background:

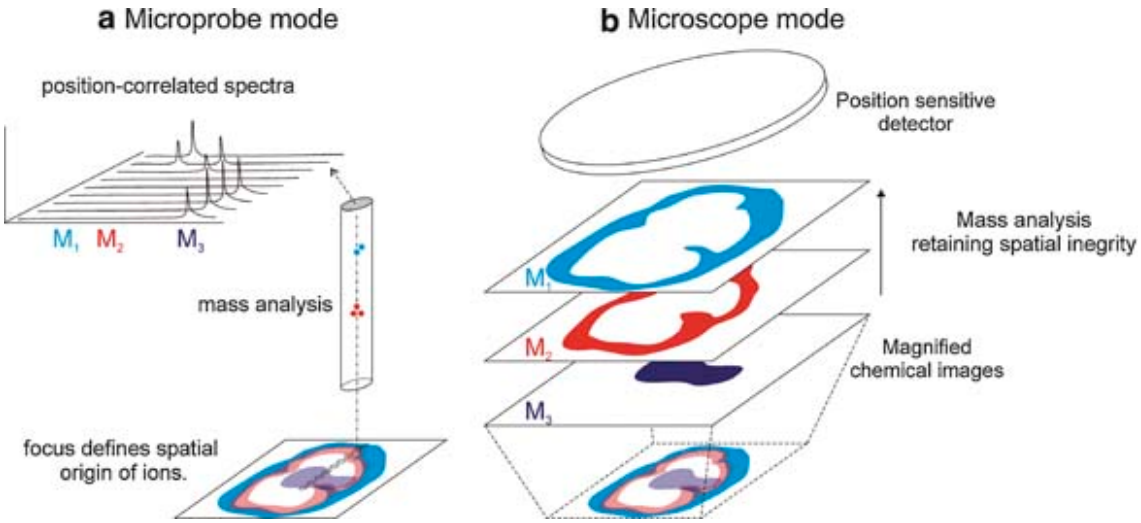
- Mass spectrometry is a technique that analyzes ions of a given mass according to their intensity.
- The mass spectrum of a sample is usually a plot I vs m / z. There is no fixed mass spectrum for a substance as it depends on the type of instrument used.
- In **Maldi Imaging** spatial information is obtained mainly by two methods: the **microprobe** and the **microscope**.



Mass Spectrometry Reviews
Volume 26, Issue 4, pages 606-643, 30 APR 2007 DOI: 10.1002/mas.20124
http://onlinelibrary.wiley.com/doi/10.1002/mas.20124/full#fig3

The microprobe uses an ionizing beam that focuses a given small region of the sample to analyze. The spectrum is preserved together with the radius location coordinates. Then proceed to the next area. The images are then reconstructed using the spectra obtained in the different positions of the laser beam.

In the microscope model, elements of the optical microscope are used alongside the ionization to project the spatial origin of the ions obtained on the surface of the sample onto a position-sensitive detector.



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INSTRUMENT:
1. IONIZATION
2. AANALYZER
3. DETECTOR AND RECORD
4. VACUUM

The most common ionization system for biological systems is MALDI-Matrix assisted laser deasorption

In this technique the matrix has a fundamental role.

The application of the matrix on the sample allows obtaining matrix crystals doped by analyte. When the UV laser beam reaches the partially vaporized matrix, it carries the analyte in the vapor phase. Sinapinic acid is the most used matrix for tissue analysis. Ac is used for small molecules. - 4-hydroxy cinnamic. ac. α-cyano- 4-hydroxy cinnamic is used for small molecules.

•tipo di matrice:

✓**Matrice:**

One of the major requirements of successful MALDI-PMS and MALDI- IMS is the proper incorporation of tissue analytes into a thin matrix layer deposited directly on the tissue and the choice of suitable matrices for different molecular classes.

1. Sinapinic acid (3, 5-dimethoxy-4-hydroxycinnamic acid, SA) at ~10-30 mg/ml, has been reported as a matrix of choice for protein analysis both in the linear MALDI-TOF MS and higher resolution MALDI-IMS. It has a high gas-phase basicity (206 kcal/mol) that is particularly suitable for protein MALDI ionization, given its low tendency in analyte fragmentation].

2. CHCA, α-cyano-4-hydroxycinnamic acid, on the other hand is more suitable for the analysis of smaller molecules, especially peptides (below 4 kDa).

3. DHB, 2,5-dihydroxybenzoic acid, ordinarily known to be suitable for negatively charged less than 4 kDa molecules, such as carbohydrates, is less commonly used as the crystals it forms are larger and mainly suited for certain profiling experiments requiring lower resolution images.

4. Ferulic acid [(E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid] has been recently reported for the detection of high molecular weight proteins on thin tissue sections.

Analyzer

TOF time-of-flight analyzer - It is a dynamic analyzer. Ions enter a long linear area where there is a ddp. Here they travel along a straight path until they reach the detector.

$1/2\ mv^2=zV\ \ s/ti= vi= (2zV/mi)^{1/2}$

Ions with different masses travel the space s at different times, reaching the detector in ≠ time.

Detectors-the most common is the oscillograph

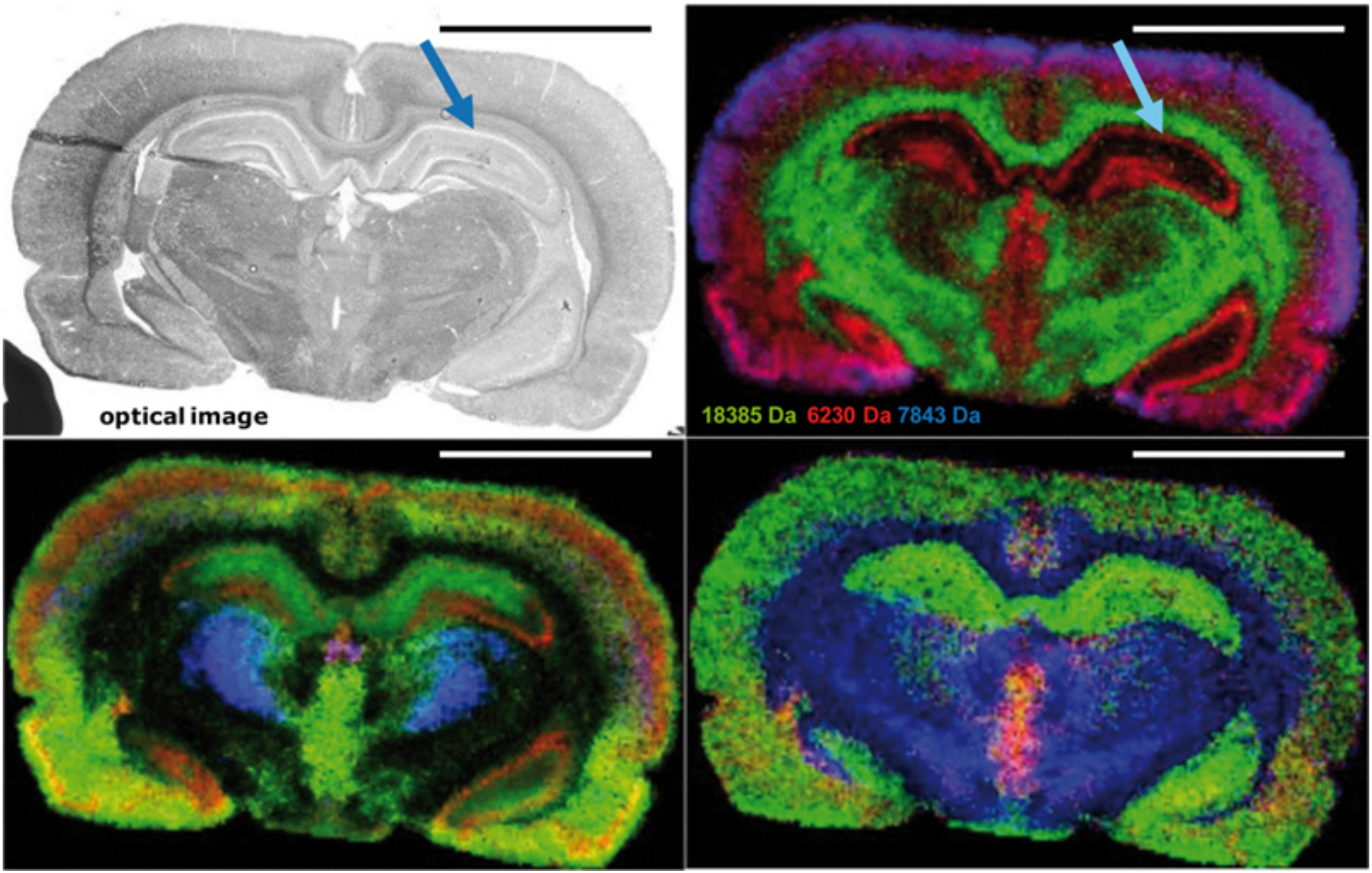
RESOLUTION

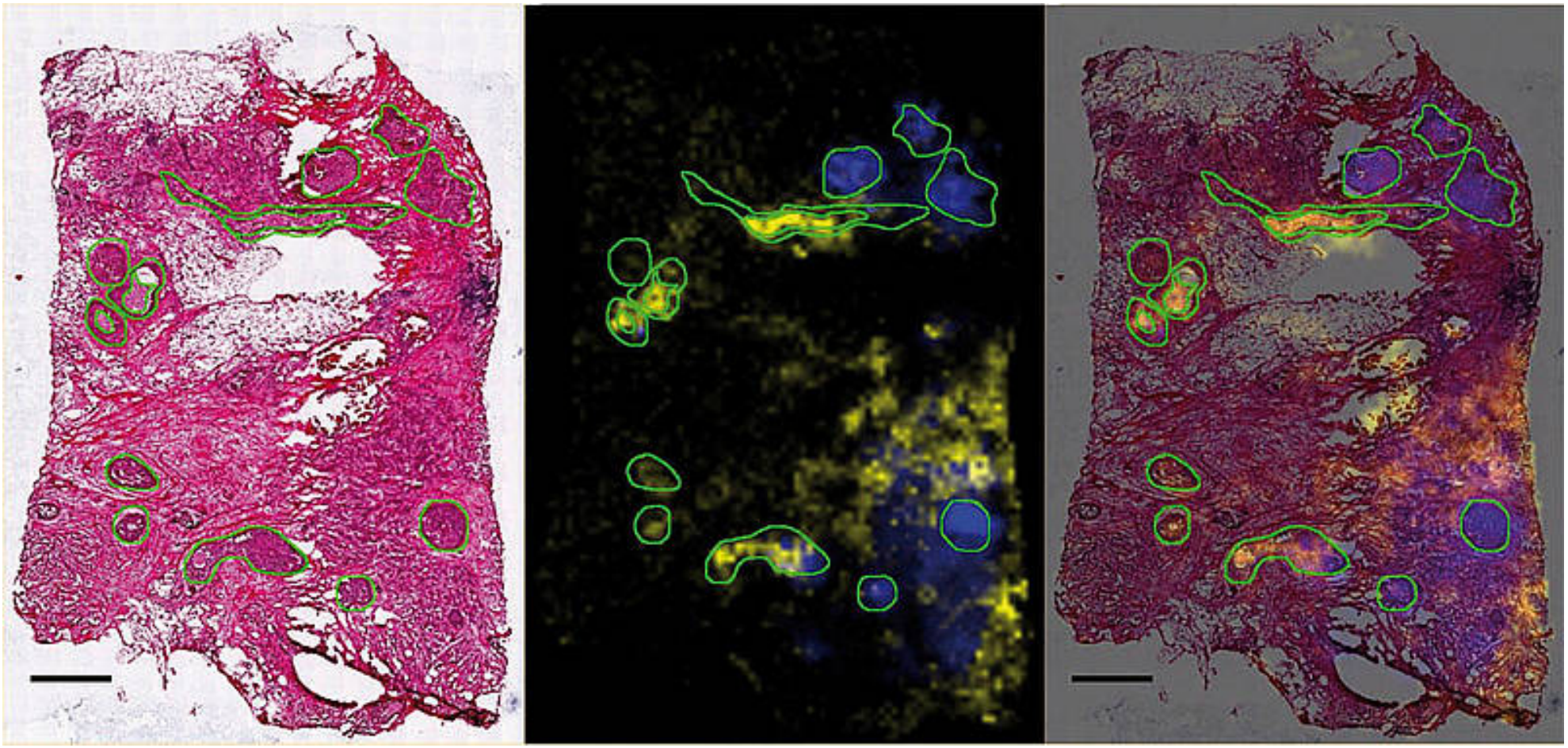
There are 3 types of resolution:

Mass-gives the chemical specificity of the analyte

Spatial- or lateral resolution is the degree of detail visible in the image, or the minimum measurable or solvable distance (pixel dim). It depends not only on the instrument, but also on how the sample is prepared.

Depth-More advanced depth-tools allow reconstructing a 3D image due to the fact that the samples are not monolyers.


optical image
18385 Da 6230 Da 7843 Da



FFPE: these samples are ananlized using an antigen retrieval step followed by a partial in situ tryptic digestion before the matrix is deposited. This allows a partial and controlled digestion of the protein to peptides in the areas of interest. The peptides that result from distinct cellular regions are recorded by intact mass and the peptides of interest are subjected to MS / MS for identification.

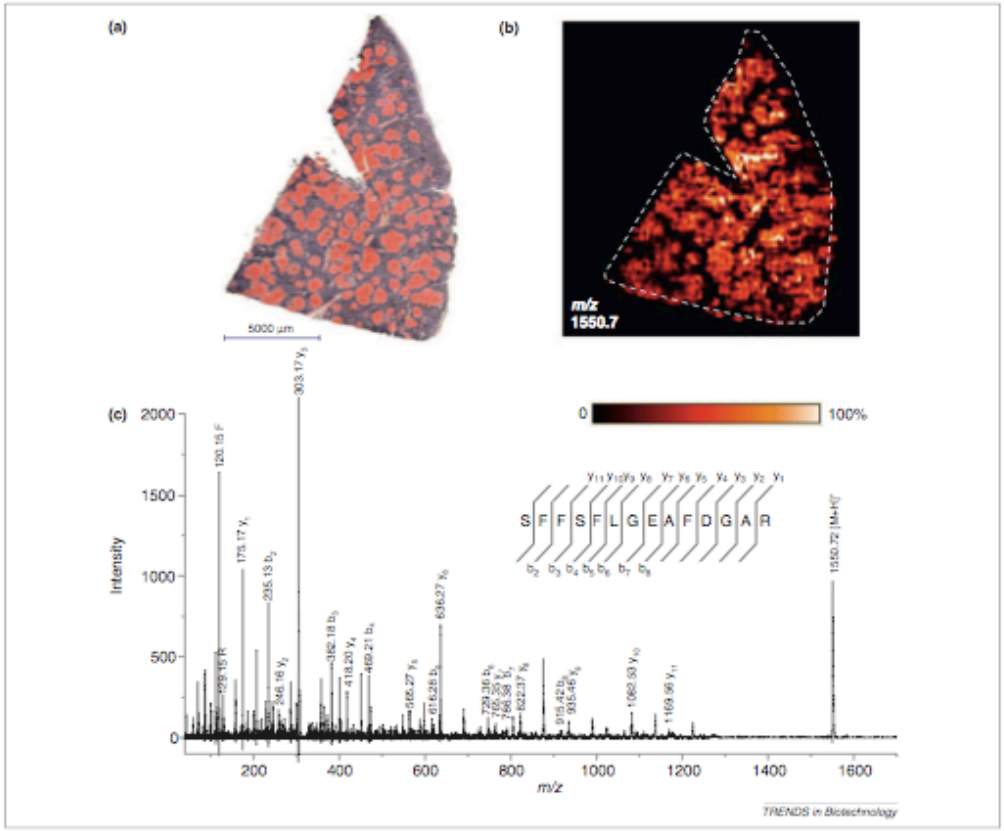


Figure 3. IMS analysis of a 109-year-old FFPE sample. **(a)** Contrast-enhanced Congo Red staining of a human spleen biopsy shows extensive amyloid deposits (in red) throughout the section. Scale bar, 5 mm. **(b)** MS image of a peptide at *m/z* 1550.7 from serum amyloid A localized to the areas of amyloid deposition. **(c)** MS/MS of this peptide directly from the tissue section resulted in nearly complete sequence coverage and identification of the peptide SFFSLGEAFDGAR.

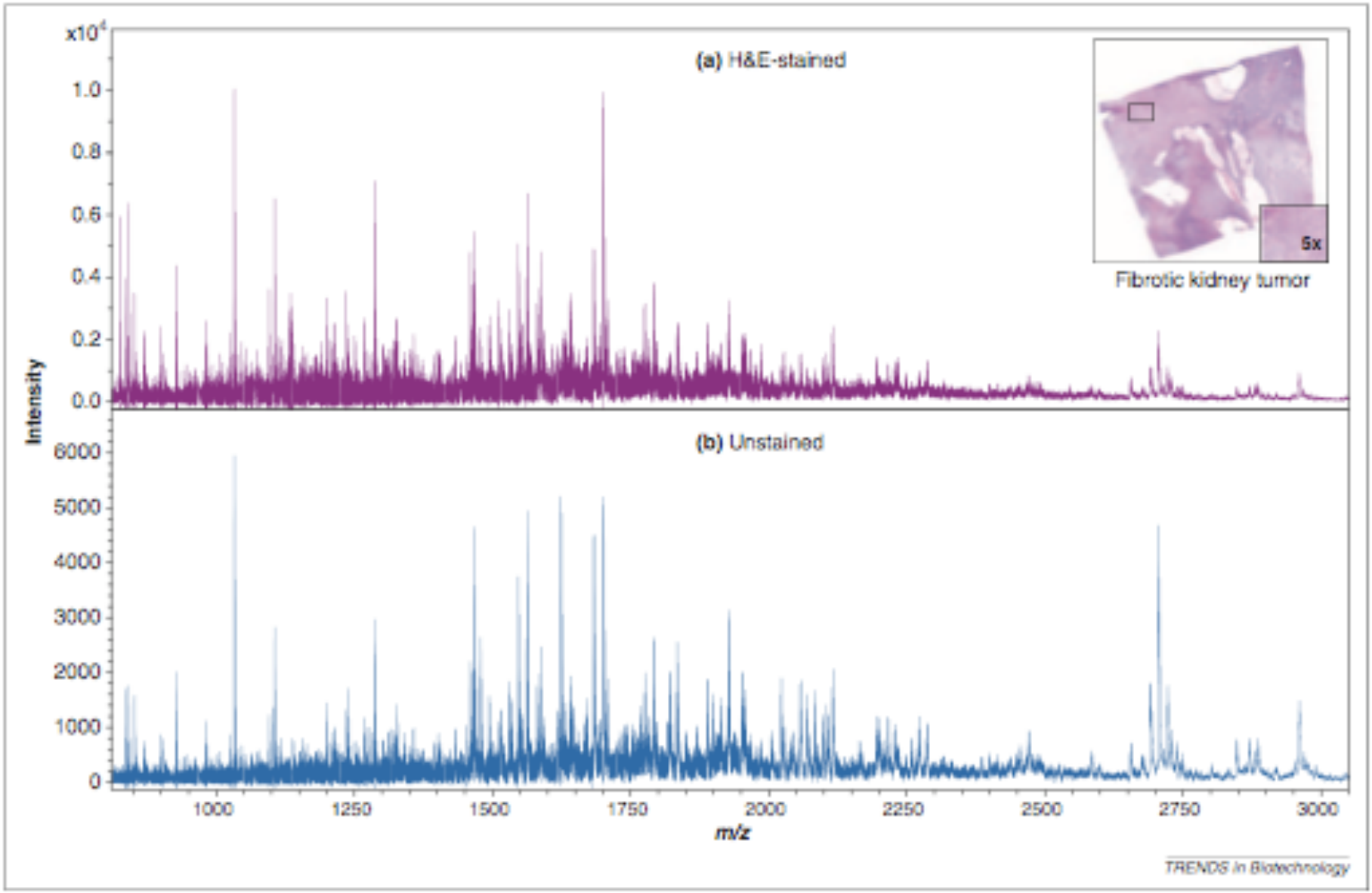
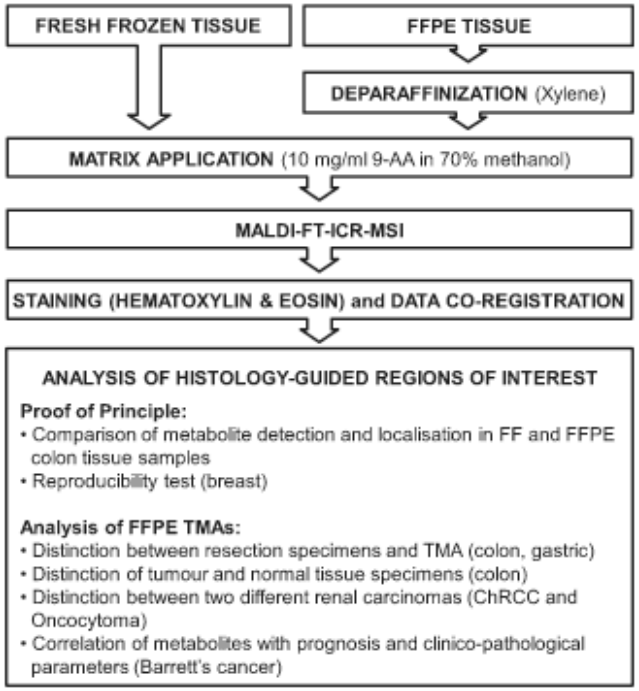


Figure 4. H&E staining coupled with MS of an FFPE human kidney tumor. **(a)** A mass spectrum obtained from an H&E-stained section of a human kidney tumor. The inset shows the tissue that was analyzed, with 5× magnification of the area from which the spectrum was acquired. **(b)** The mass spectrum from an unstained serial section.

High-resolution MALDI-FT-ICR MS imaging for the analysis of metabolites from formalin-fixed, paraffin-embedded clinical tissue samples



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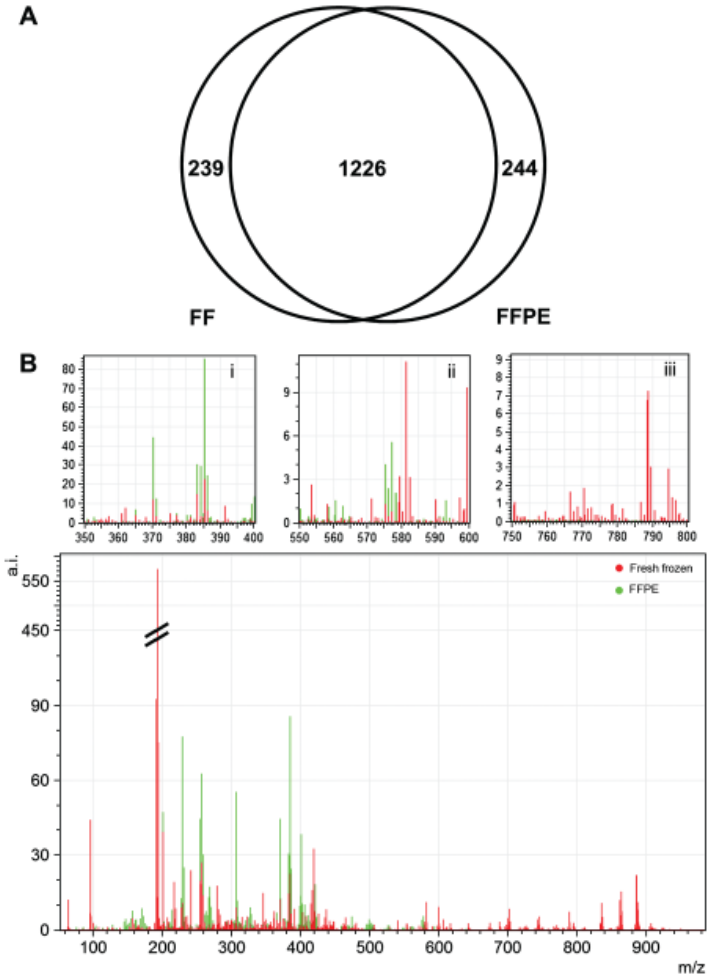

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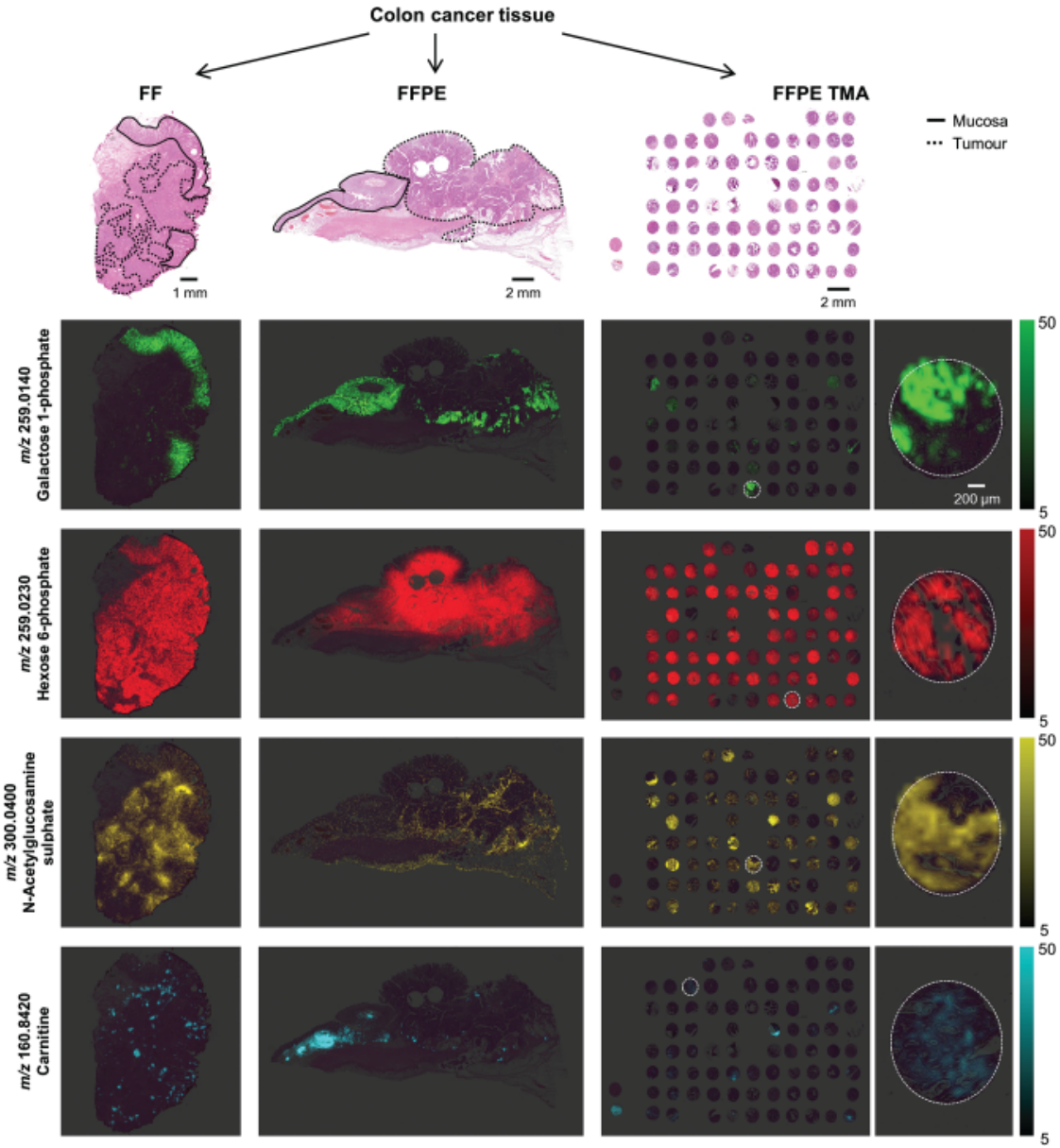
High-resolution MALDI-FT-ICR MS imaging for the analysis of metabolites from formalin-fixed, paraffin-embedded clinical tissue samples

tumour (black dotted line) and mucosa (black solid line

Comparison of *m/z* localization in colon cancer samples from whole resected FF, FFPE, and FFPE TMA. Top panels are H&E-stained FF, FFPE, and TMA colon cancer tissue sections showing regions corresponding to tumour (black dotted line) and mucosa (black solid line). *m/z* 259.0140 (galactose-1-phosphate; green) localizes to mucus. *m/z* 259.0230 (H6P; red) is most intense in the tumour regions in FF and FFPE samples. *m/z* 300.0400 (*N*-acetylglucosamine sulphate; yellow) corresponds to tumour stroma – tissue which supports tumour growth. *m/z* 160.8420 (carnitine; blue) is found in the vicinity of blood.



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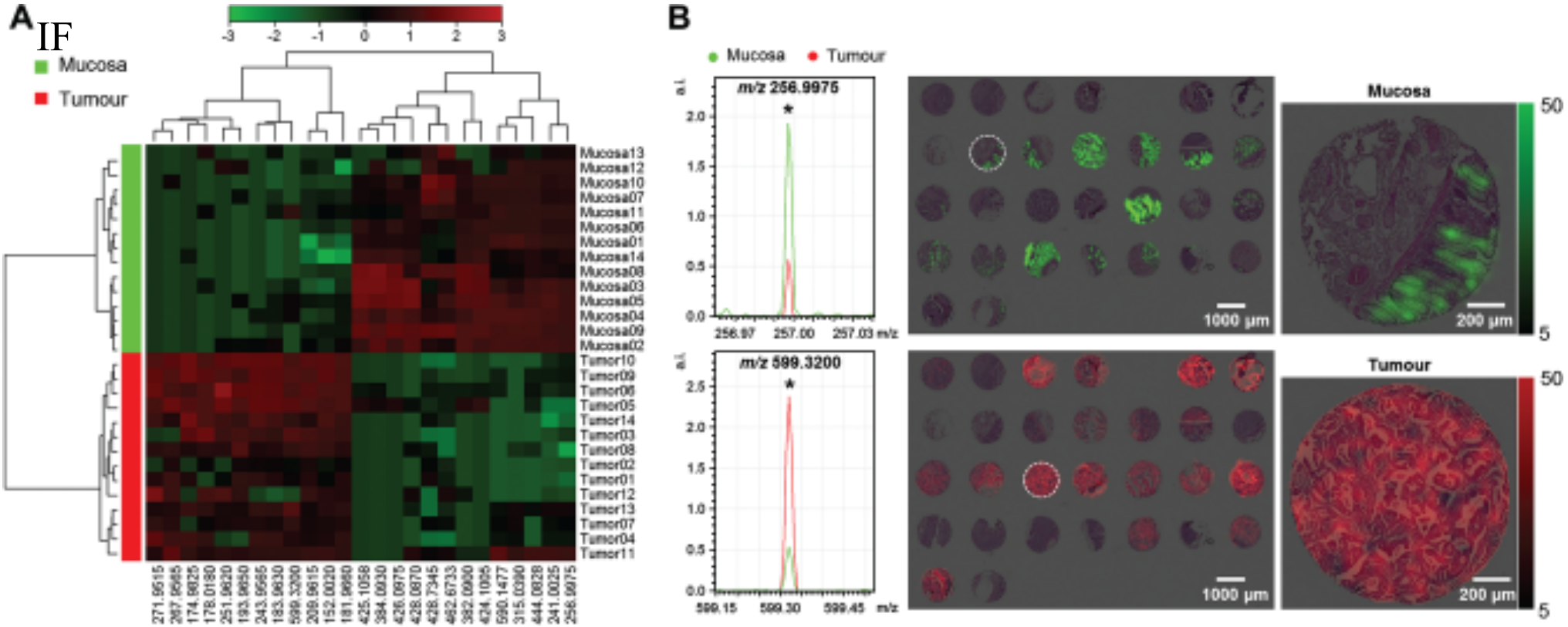
High-resolution MALDI-FT-ICR MS imaging for the analysis of metabolites from formalin-fixed, paraffin-embedded clinical tissue samples

Discrimination of tumour tissue from normal colonic mucosa in FFPE TMA. (A) Heatmap of the top 25 significant *m/z* values demonstrates different *m/z* expression patterns in mucosa versus tumour. (B) Average spectra of *m/z* 256.9975 and *m/z* 599.3200 and localization overlaid on corresponding H&E-stained samples. *m/z* 256.9975 was more intense in mucosa (green) than in tumour (red); a close-up shows it to be specific for mucus-producing epithelium. *m/z* 599.3200 is more intense in tumour regions.



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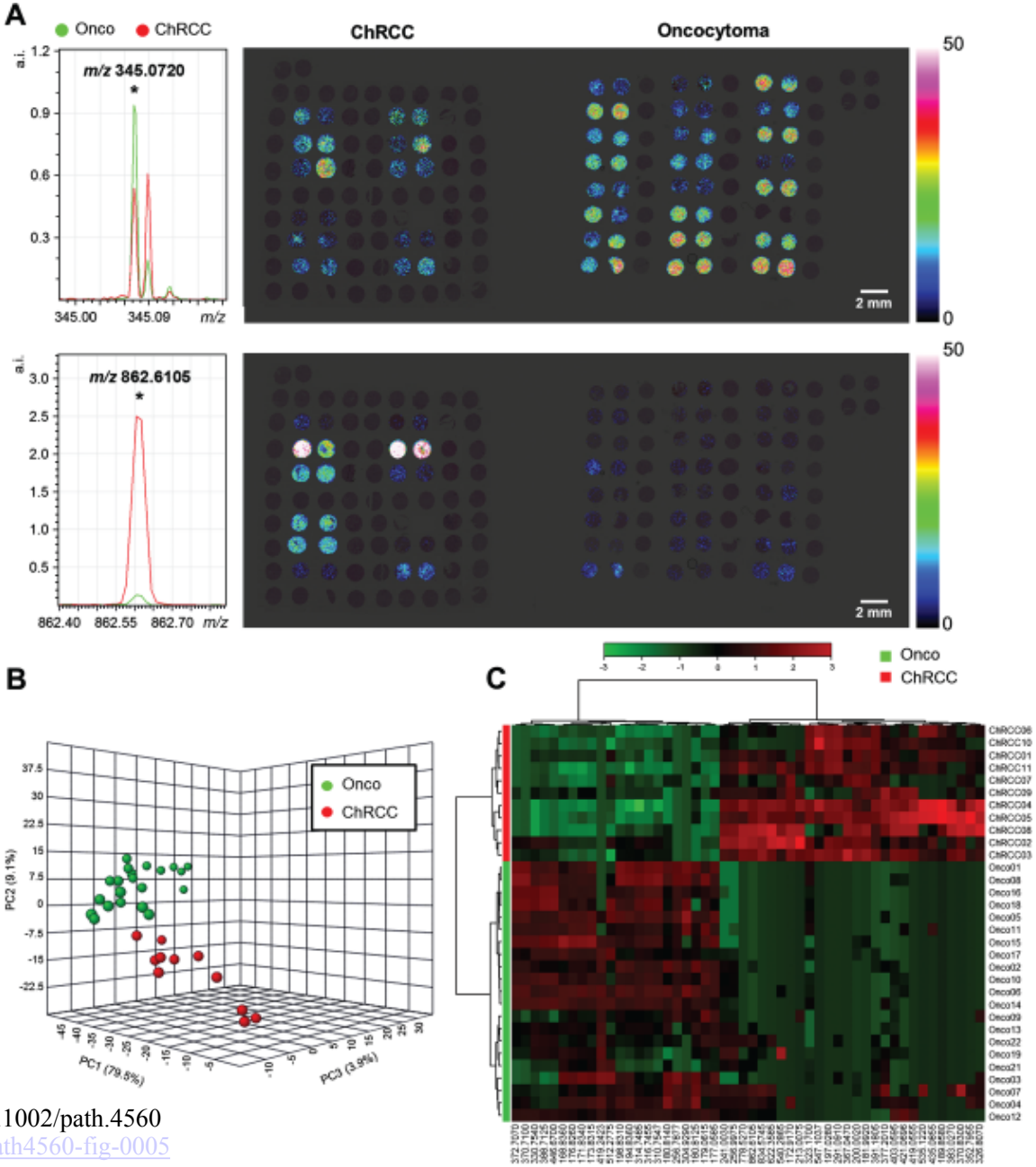
High-resolution MALDI-FT-ICR MS imaging for the analysis of metabolites from formalin-fixed, paraffin-embedded clinical tissue samples

Metabolite MALDI-FT-ICR MSI of renal oncocytoma versus ChRCC. (A) Spectra and ion maps for *m/z* 862.6105 (phosphoethanolamine) and 345.0720 (2-amino-AMP) show differences in distribution and intensities in oncocytoma and ChRCC. (B) PCA accurately distinguishes ChRCC (red) from oncocytoma (green). (C) Heatmap of the top 50 differentially intense *m/z* values.



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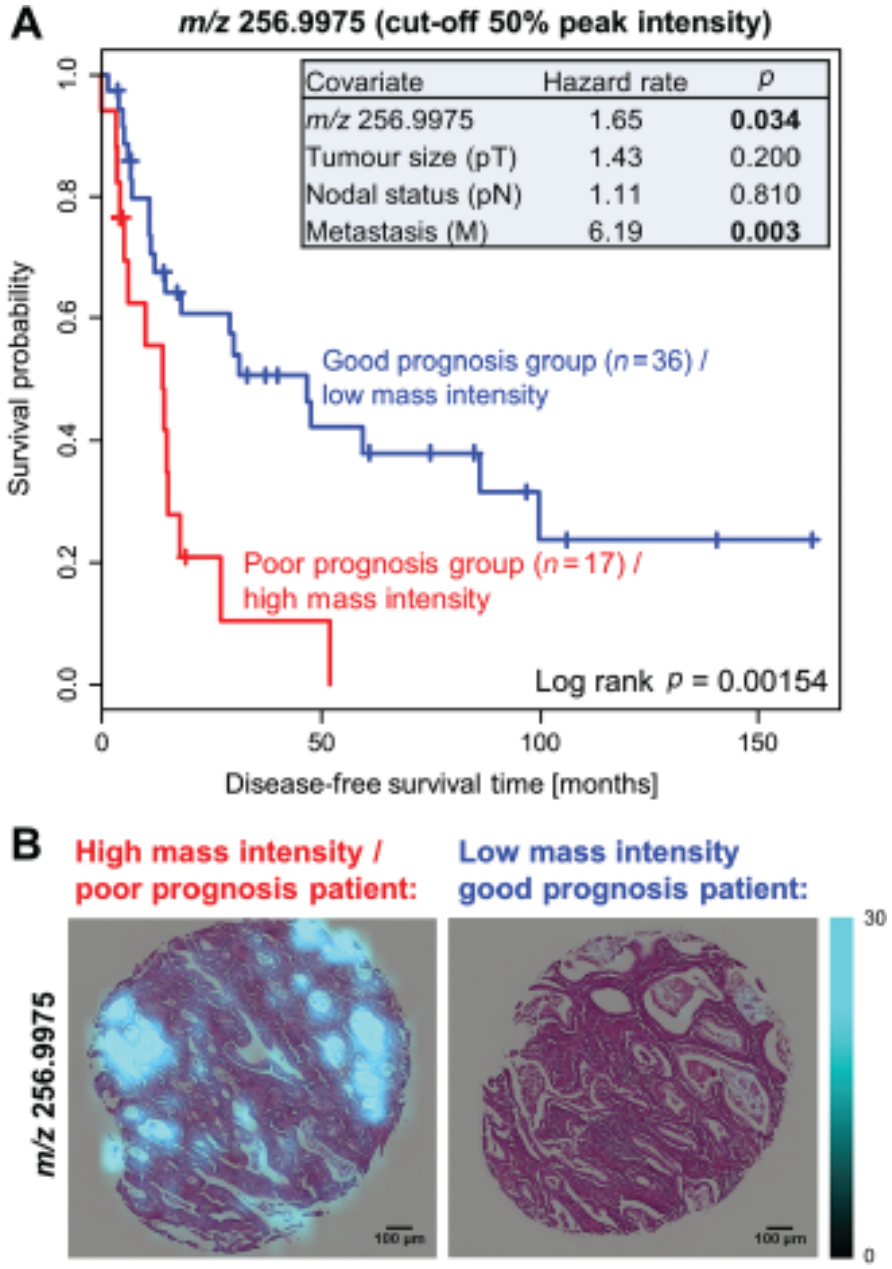
High-resolution MALDI-FT-ICR MS imaging for the analysis of metabolites from formalin-fixed, paraffin-embedded clinical tissue samples

Metabolite imaging for addressing patient survival outcome. (A) Tumour-specific metabolomic data from a TMA containing 53 oesophageal adenocarcinomas were submitted to univariate and multivariate statistical analysis. *m/z* 256.9975 was found to be a significant prognostic factor for disease-free survival of the patients ($p = 0.00154$), independently of other survival determinants given by the clinical TNM classification (A, inset; $p = 0.034$). (B) Ion distribution maps showing localization of deoxy sugar acid with ester sulphate (*m/z* 256.9975) in regions of mucus (visualization in blue).



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