Unveiling Boundless Discoveries: The Dynamic Landscape of the New Mexico Mass Spectrometry Laboratory

Jim Feng, PhD
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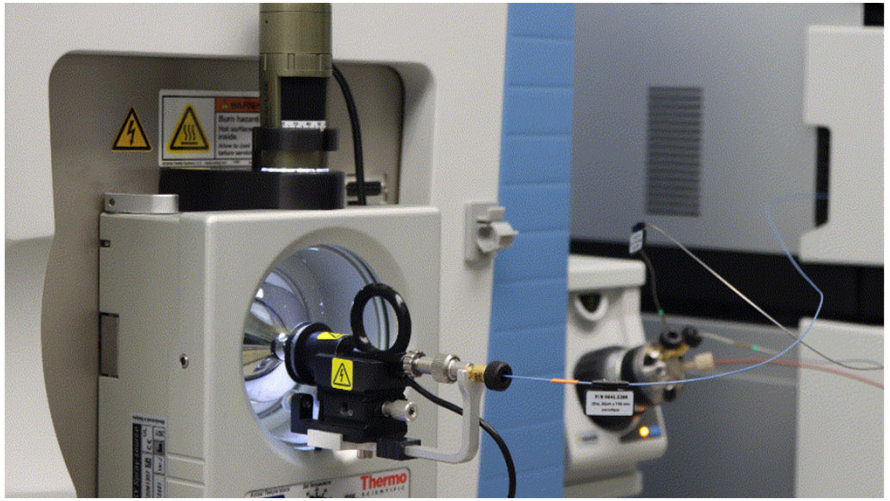


UNM COLLEGE OF PHARMACY

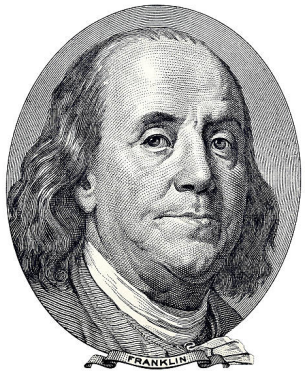
Outline

- Overview of the UNM Integrative Molecular Analysis Core (IMAC) & BioAnalytical Chemistry Core (BACC)
- Current BioAnalytical Capacities
- Emerging Approaches – Mass Spectrometry Imaging (Molecular Microscope)
- Touring the Core labs (B60 and B54)

IMAC & BACC – *Research Excellence*

Mission. Our mission is to undertake leading research and to enhance and expand the collaborative capabilities of research as a shared resource.

Vision. The Core provides a top-notch research infrastructure that enables scientific excellence and novel methodology development for researchers on campus and the scientific community.



"Tell me and I will forget,

Teach me and I may remember,

Involve me and I will learn."

- Benjamin Franklin

UNM Bioanalytical Sciences and Mass Spectrometry Laboratory (**MSL**)

Integrative Molecular Analysis Core (**IMAC**)

Advancement of research on metals interactions in biology and environmental health

BioAnalytical Chemistry Core (**BACC**)

Focus on method development (including non-metals)

Our Team

Matthew Campen, Ph.D.
CMBM P20 Center Director
Regents' Professor
UNM College of Pharmacy

Sarah Blossom, Ph.D.
NM-INSPIRES P30 Director
Professor
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Huayu Zheng, Ph.D.
LC-MS Specialist
Research Scientist
UNM College of Pharmacy

If you're the smartest person in the room, you're in the wrong room.

We hire smart people so they can tell us what to do. – Steve Jobs

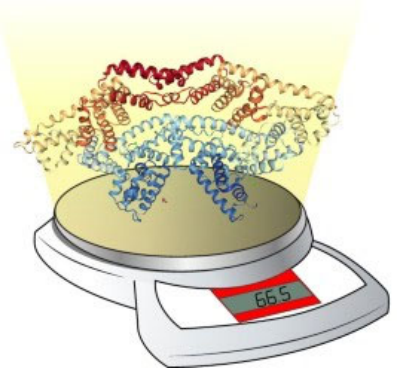
Embracing the Journey - Milestones

In 2023: 28 principal investigators, 11 staff scientists, 19 graduate students, 6 post-doctoral scholars, across 13 departments at UNM HSC and UNM, and 3 other Universities (New Mexico State University, Oklahoma State University, and University of Arizona).

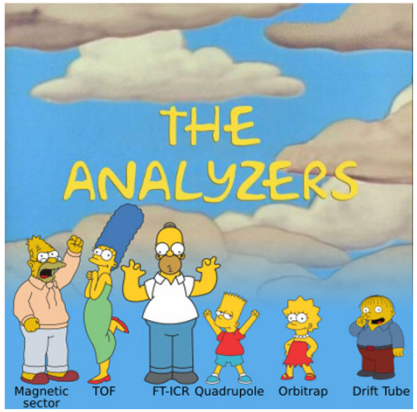
What is Mass Spectrometry

Mass spectrometry (MS) is the smallest scale in the world, not because of the mass spectrometer's size, but because of the size of what it weighs - molecules. With MS, we are looking at the mass of a molecule, or of different fragments of that molecule.

Mass spectrometry is a technique that enables the identification of unknown compounds within a sample, the quantification of known materials, the determination of the structure, and chemical properties of different molecules.



High-End MS Instruments in the Core



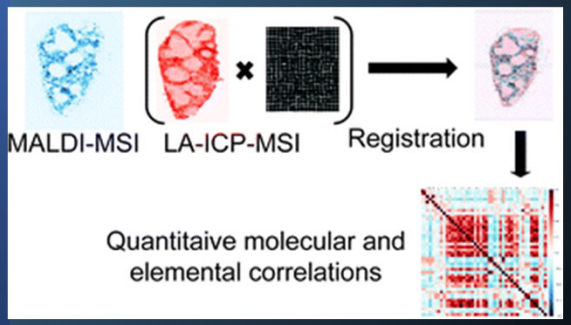
LC-MS
Huayu Zheng Ph.D.
Small molecule & metabolomics

ICP-MS
Rui Liu, Ph.D.
Trace Metal Analysis & Imaging

LC-MS/MS
Ting Jiang Ph.D.
Protein/peptide & proteomics

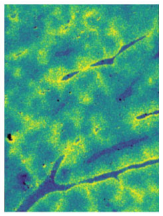
GC-MS
Rui Liu, Ph.D.
Volatile compounds/ microplastics

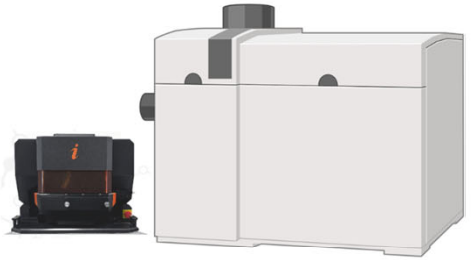
Imaging Mass Spectrometry – New Features at the Core



timsTOF fleX MALDI-2

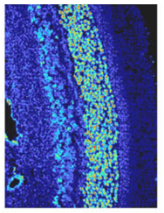
Spatial profiling of molecular constituents of tissue section





LA-ICP-MS

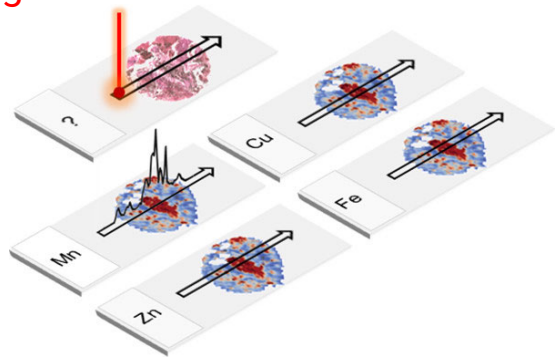
Metal elemental distribution information

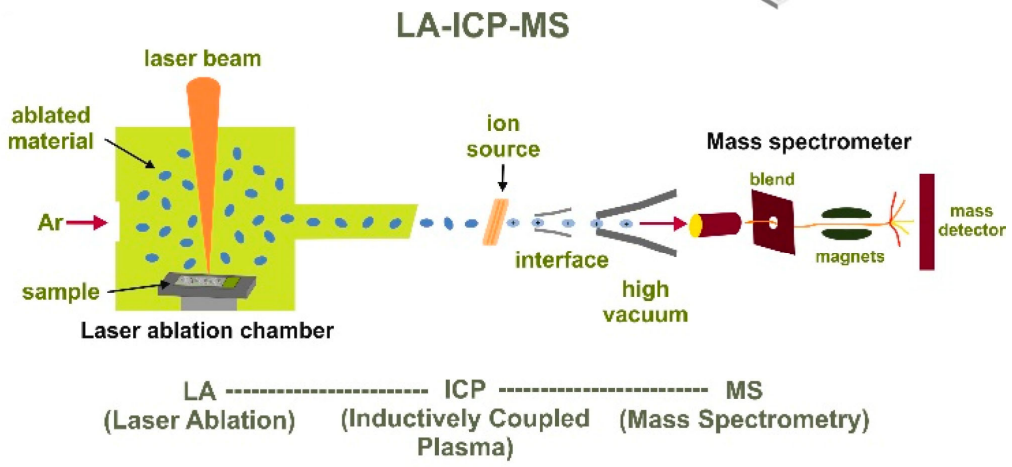


Previously Introduced LA-ICP-MS Imaging Technique

Q: What does LA-ICP-MS image?

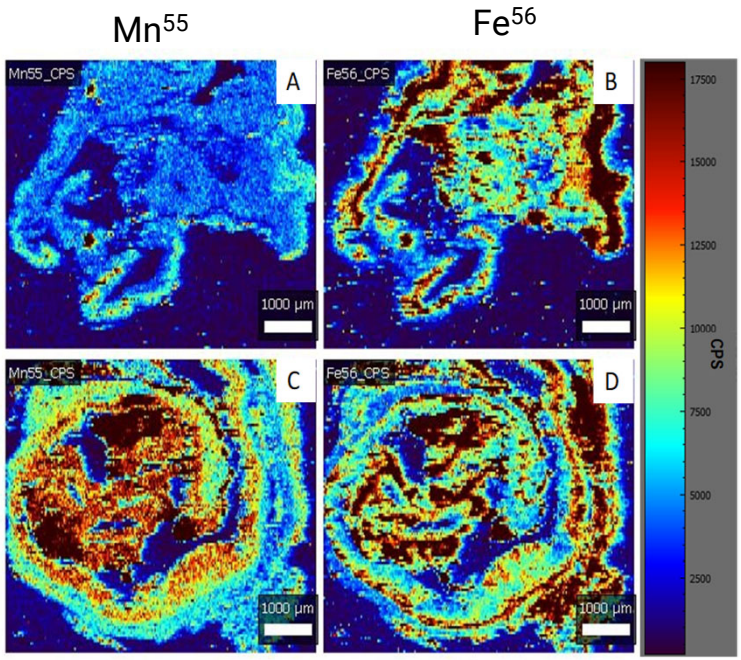
A: Elements!





Low Mn-diet sample

Control sample



Weiskirchen, S.; Kim, P.; Weiskirchen, R. Laser Ablation Inductively Coupled Plasma Spectrometry: Metal Imaging in Experimental and Clinical Wilson Disease. Inorganics 2019, 7, 54

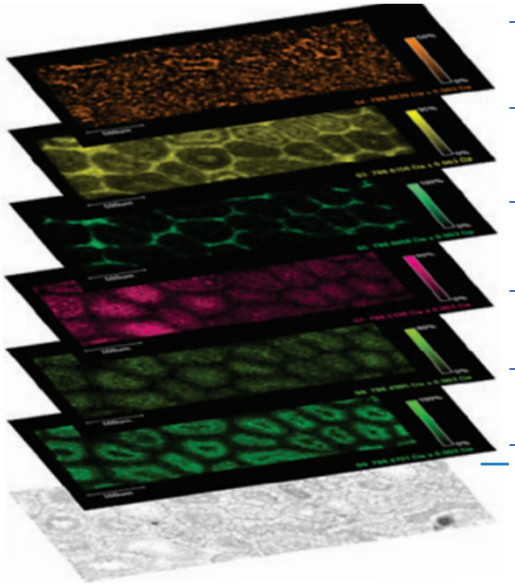
Courtesy of Drs. Xiang Xue & Rui Liu

New MALDI-MS Imaging Technique

Bruker timsTOF Flex MALDI-2

Q: What does MALDI-MS image?

A: Molecules!



Metabolites

Lipids

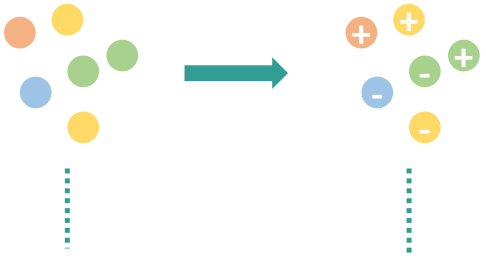
Small molecules

Glycans

Peptides

Targeted proteins

Introduction to Mass Spectrometry



Mass Analyzer

Detector

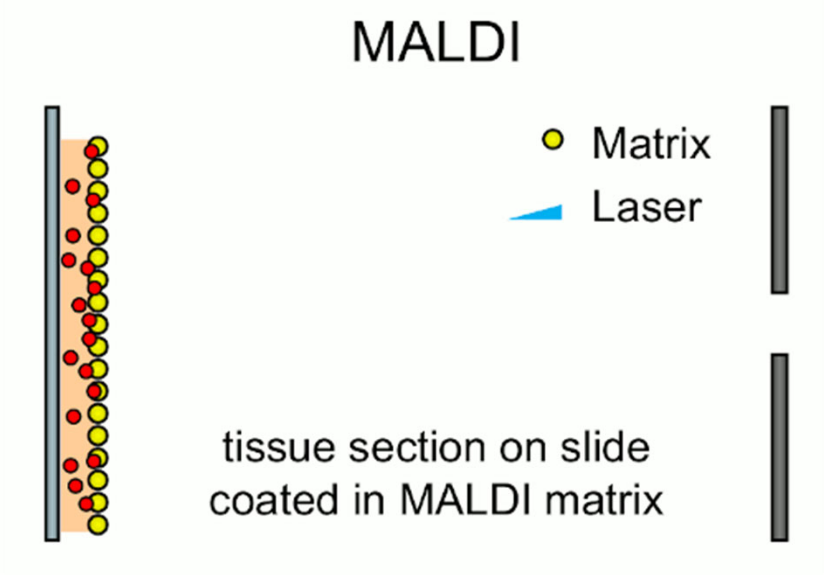
Sample Inlet:
(Related to front-end)
Autosampler,
LC or GC interface
…

Ionization source:
ICP-MS: plasma torch – M^+ elemental
GC-MS: electron impact – M^+, $[M+H]^+$, $[fragment]^+$, $[fragment+H]^+$
LC-MS: electron spray ionization – $[M+nH]^{n+}$, $[M-nH]^{n-}$
MALDI-MS: Matrix-Assisted Laser Desorption/Ionization

e.g.: quadrupole
triple quadrupole
Orbitrap
time of flight

Typically, electron multiplier

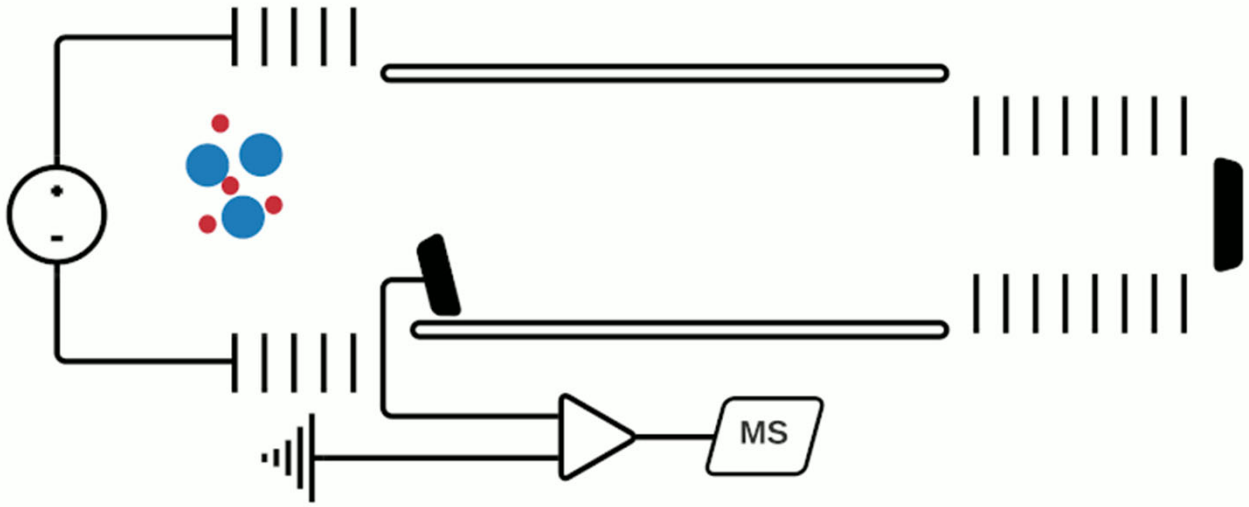
Illustration of the MALDI ionization technique



In MALDI biological molecules are mixed with a matrix – typically an organic aromatic acid (sometimes base) – which absorbs the laser energy and aids molecular ionization.

MSI hardware part 1: ionization techniques | Aspect Analytics | Bring your spatial multi-omics data together (aspect-analytics.com)

Illustration of the time-of-flight (TOF) mass analyzer



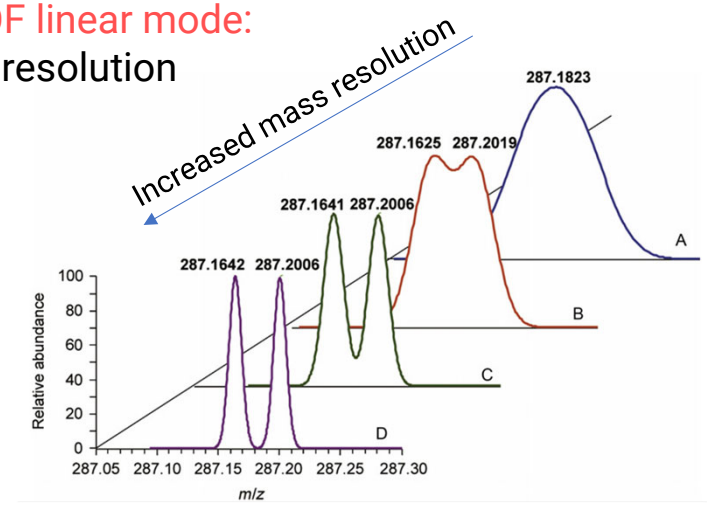
$$zeqV = \frac{1}{2}mv^2 \longrightarrow 2eqV = \frac{m}{z}v^2$$

TOF mass analyzers separate and detect analyte ions based on how long it takes them to "*fly*" along a specified distance.

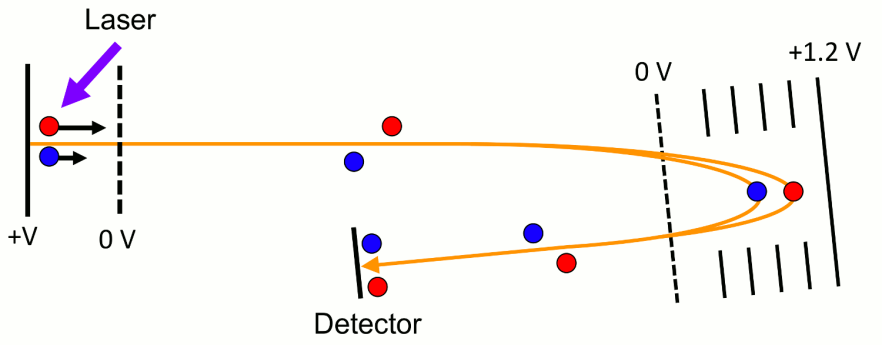
MSI hardware part 1: ionization techniques | Aspect Analytics | Bring your spatial multi-omics data together (aspect-analytics.com)

Variants of TOFs with High Mass Resolution

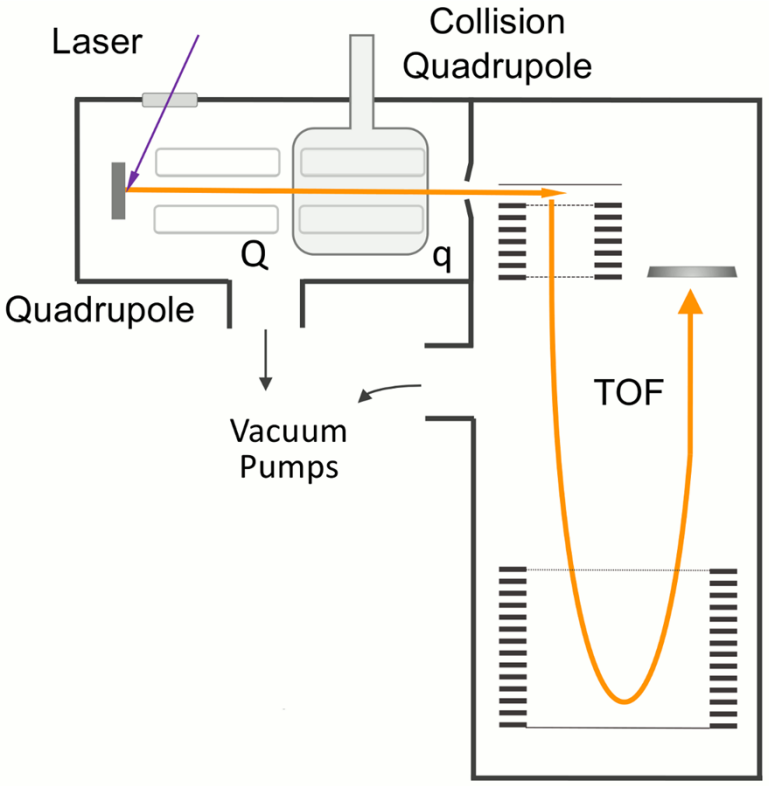
Problem of axial TOF linear mode:
relatively low mass resolution



Axial TOF reflectron mode

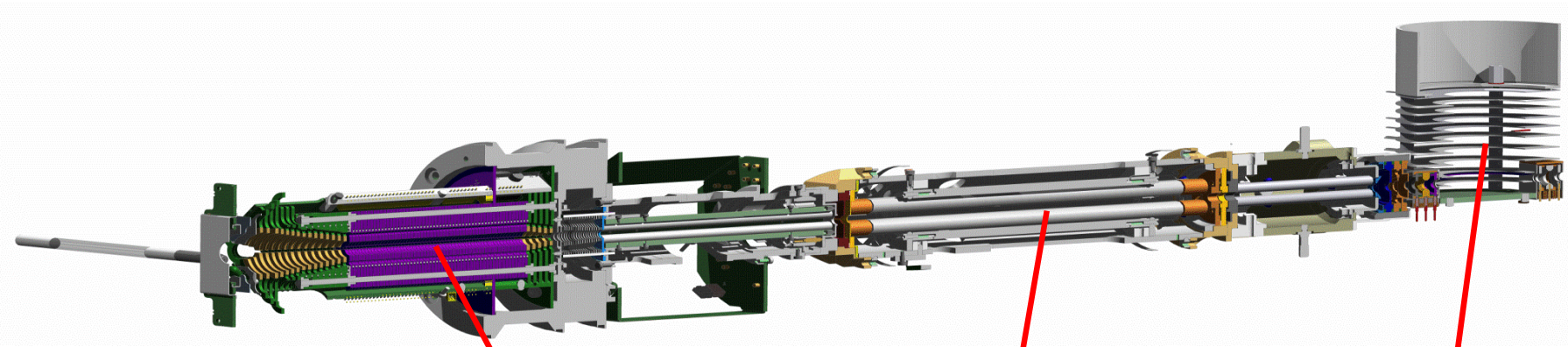


Orthogonal TOF (e.g., QTOF)



Feature #1 of Our New timsTOF – Orthogonal TOF

Orthogonal time-of-flight MS analyzer ---> high resolution and scan rate

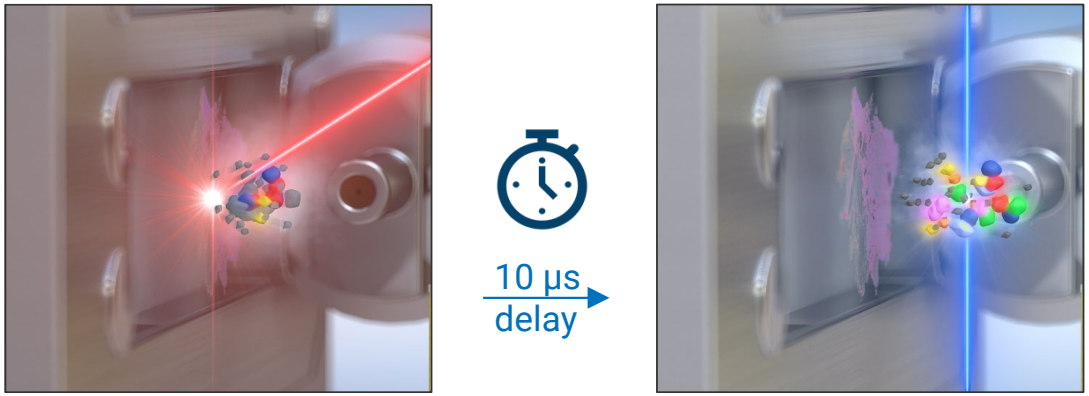


TIMS	Quadrupole Filter	Time of Flight MS
Ion Mobility Separation	Mass Selection	Mass scan
20 - 100 ms	2.5 – 10 ms	110 µs
10 - 50 Hz	100 – 350 precursors/s	9.1 kHz

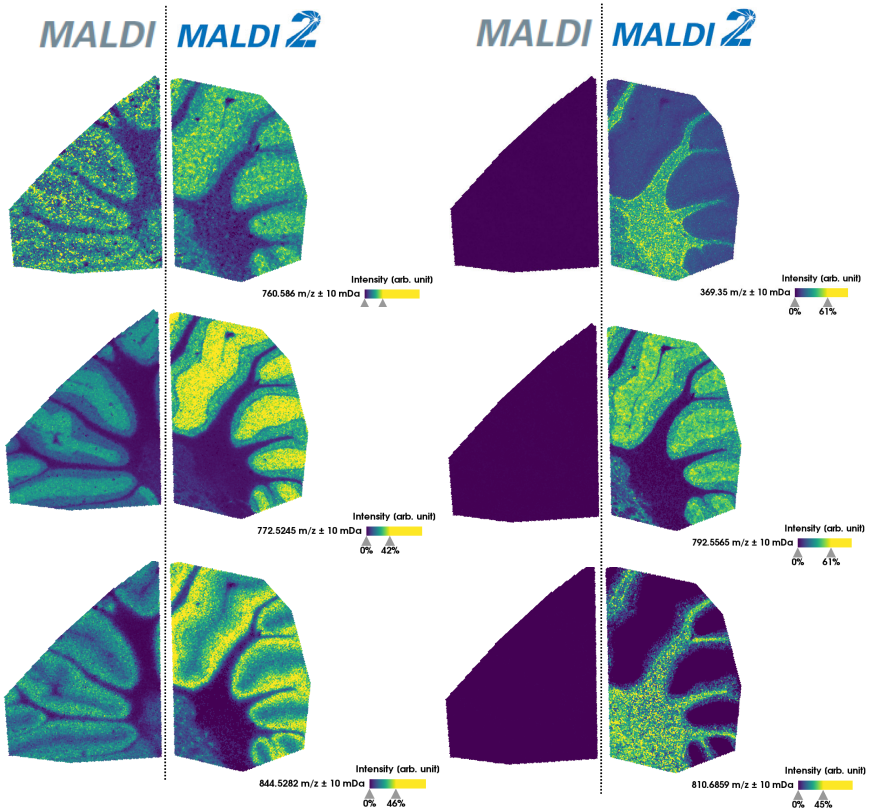
Feature #2 of Our New timsTOF– MALDI-2 Source

MALDI-2 Ionization Source

MALDI-2: 2nd laser enhance ionization thus signal for some molecules



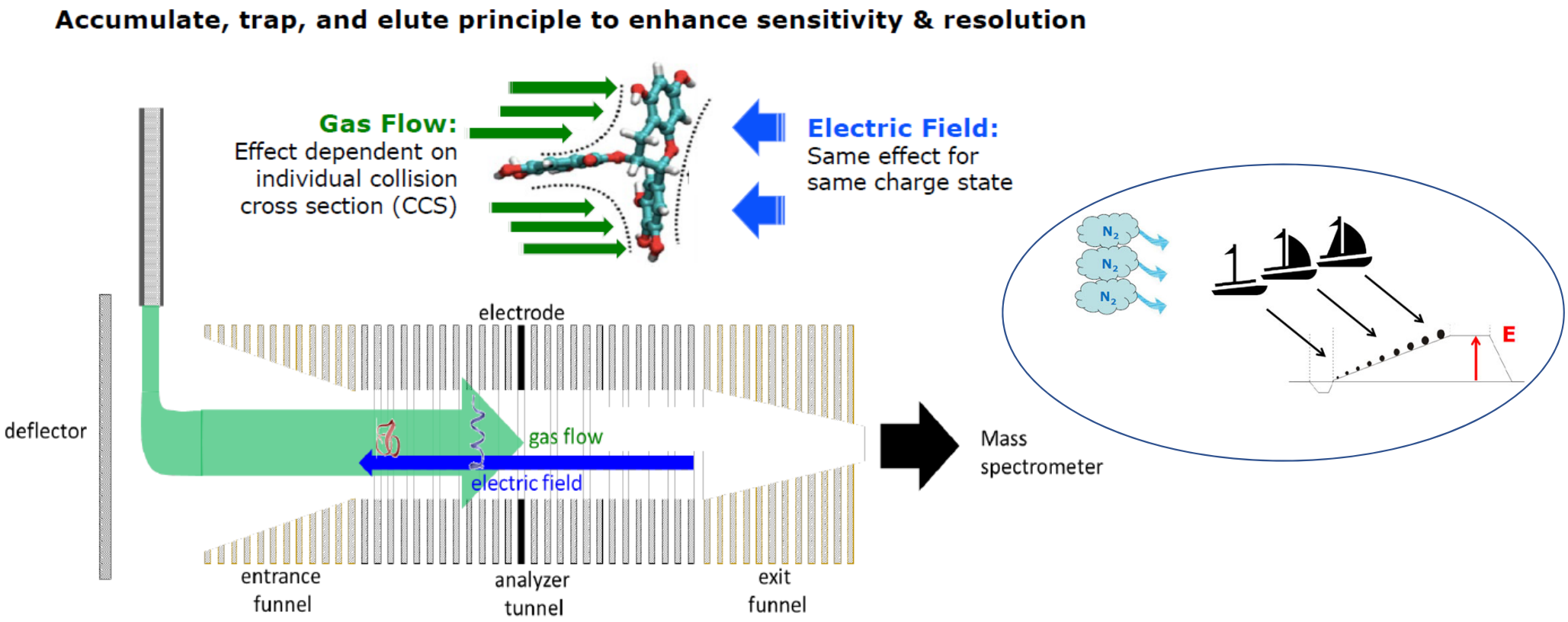
1. Desorption/Ionization **2. Postionization**



- Improve sensitivity by orders of magnitude depending on sample, matrix and analyte
- No ions are lost compared to traditional MALDI (but some signals may decrease due to in-source decay)

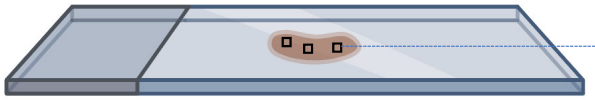
Feature #3 of Our New timsTOF – TIM Separation

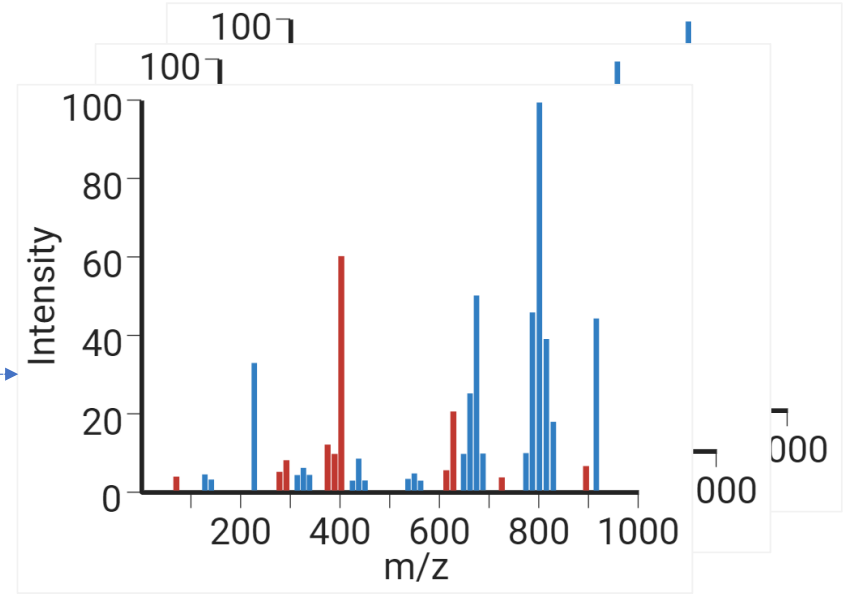
Trapped Ion Mobility (**TIM**) analyzer help separate analytes with the same m/z by their shape/size in *gas phase*

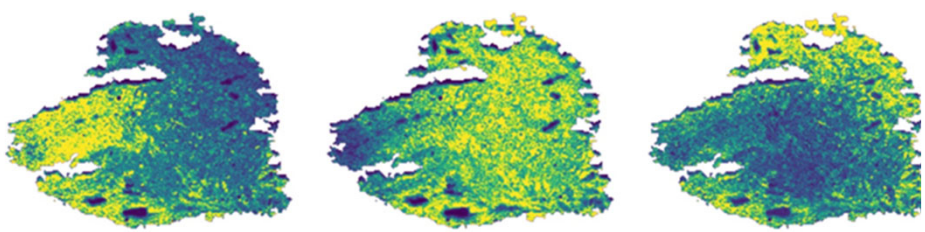


MALDI Imaging

- High mass resolution
- High spatial resolution
- High sensitivity
- Relatively fast acquisition
- Hundreds-thousands of images in one run







MALDI Imaging Workflow

Fresh Frozen or FFPE

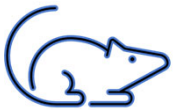
Bruker Intellislides
or normal ITO slides

Sample/biorepository → Section/mount → Apply matrix → Acquire data → View/Analyze Data

FFPE samples can be used for protein imaging, but generally are not good for small molecules including metabolites and lipids as they tend to be lost during processing.

MALDI Imaging Sample Preparation Basics

- Fresh frozen tissue sectioned (< 10 µm thick)
- Mount tissue sections on Bruker's Intellislides or ITO coated slides
- Some embedding materials (e.g., OTC) brings interfering MS signals
- We will provide guidance for the users to prepare the sections on the slide

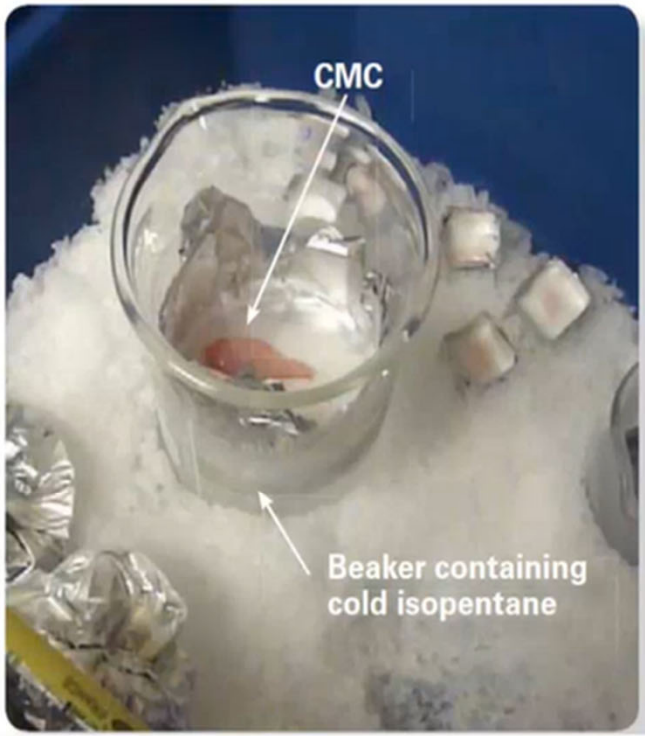


Collection and Storage of samples

- Preparation from fresh frozen tissue is ideal for MALDI Imaging.
- A progressive freeze in liquid nitrogen is most commonly used technique:
  - Loosely wrap organ in aluminum foil, and let the tissue float
  - Or, place tissue in boat on top of nitrogen
- If the tissue is damaged using this technique, you can also use isopentane pre-cooled at -80° C and placed on dry ice.
- Store long-term at -80 ° C

Embedding Tissue

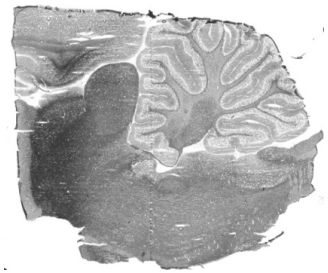
- Most tissues can be sectioned and mounted without embedding, and this is ideal for MALDI Imaging.
- If necessary, 1-2% carboxymethyl cellulose (CMC) can be used for embedding.
- Avoid OCT!
- Store long-term at -80 °C

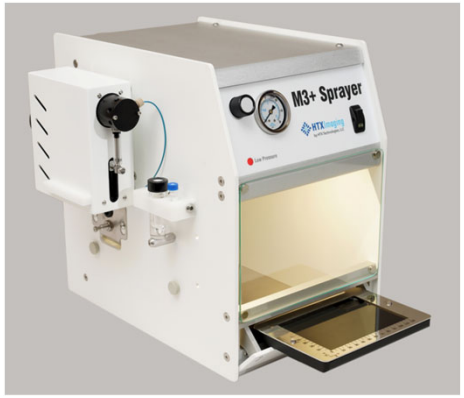


Transporting Tissue for Sectioning

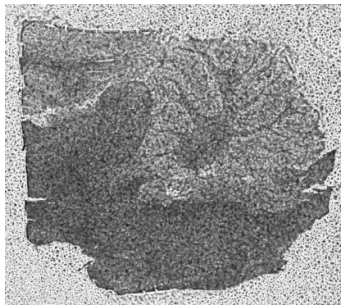
- We recommend transporting the tissue on dry ice between the -80 °C freezer and the -20 °C cryostat.
- The tissue must be stored in the cryostat for 15 minutes for temperature equilibration
- Deliver the tissue section on slide to us on dry ice

Applying Matrix



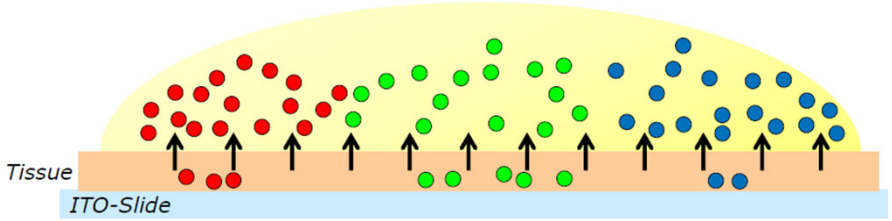


MALDI matrix preparation

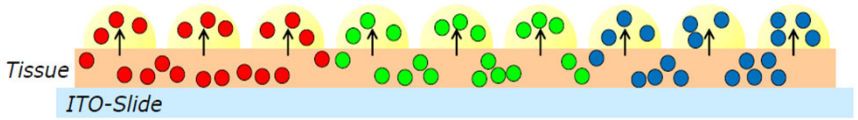


Different matrices for different molecules
e.g., NEDC and 9AA for metabolites
DHB, CHCA, DHAP, DAN for lipids
CHCA, DHB for peptides

Imaging spatial resolution affected by:
Matrix crystal size,
Dry/Wet extraction methods,
etc.



Large droplets: good extraction, low spatial resolution



Small droplets: reduced extraction, high resolution

Applying Matrix

Data Acquisition

The equipment may allow for imaging with spatial resolution as high as 10 μm.
However, a very important question to ask first is

What resolution is good enough to answer my research questions?

Estimated scan time

	20 μm	50 μm
1 cm^2	25 min	4 min
10 cm^2	4.17 h	40 min

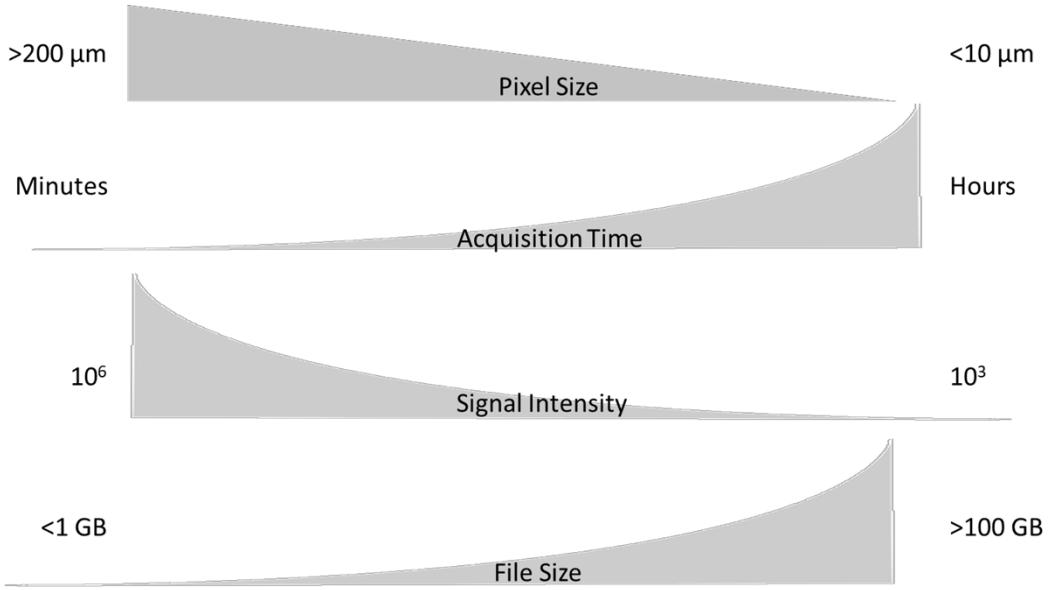
Downside of high resolution:

- Lower signal as less materials are ablated per pixel
  ---> lower sensitivity
- Need better sample prep, more instrument time and more data storage space
  ---> longer time (trial-and-error & acquisition) and (much) higher costs

An alternative is to first profile/scan/screen the whole section at typical resolution, identify the smaller area of interest, and then image that region in an adjacent section sample at higher resolution.

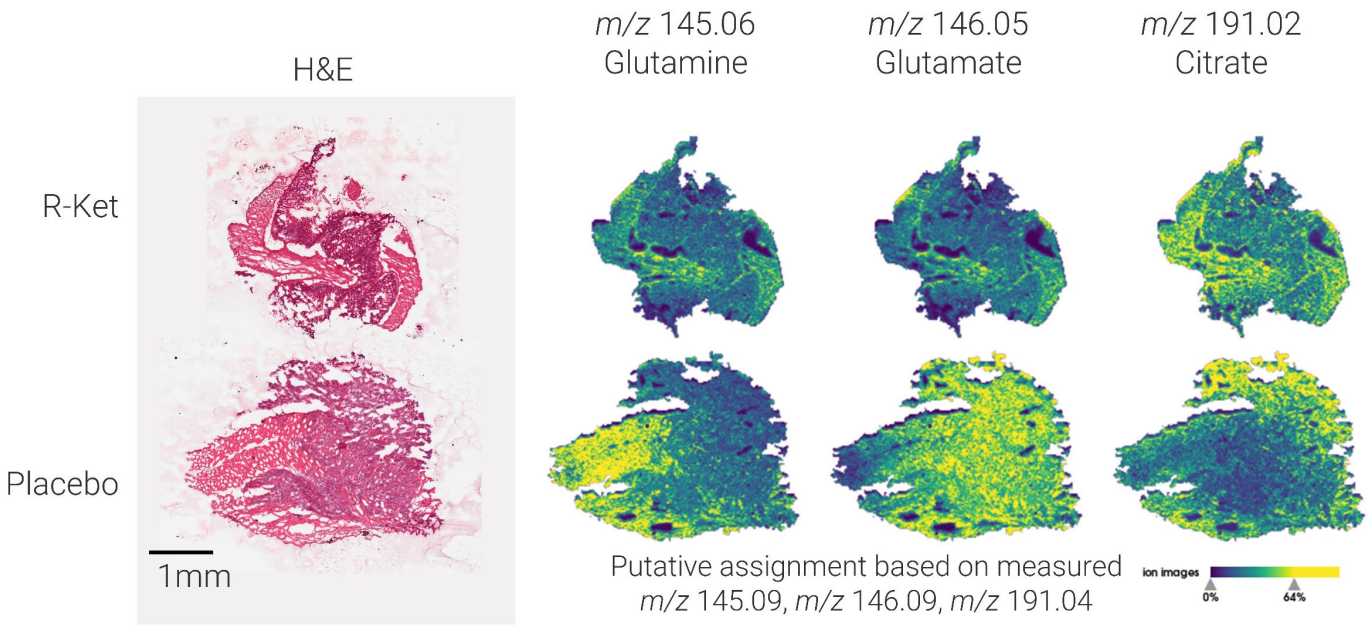
Imaging Tradeoff

- Trade-off between spatial resolution and acquisition time, sensitivity & file size.
- When designing experiment, the spatial resolution should be limited to what is necessary to answer the biological question being asked.



Demo Data from Bruker for the S10 Application

Demo data for samples provided by Hudson Lab:



Tissue: ovarian tumor xenograft on mice

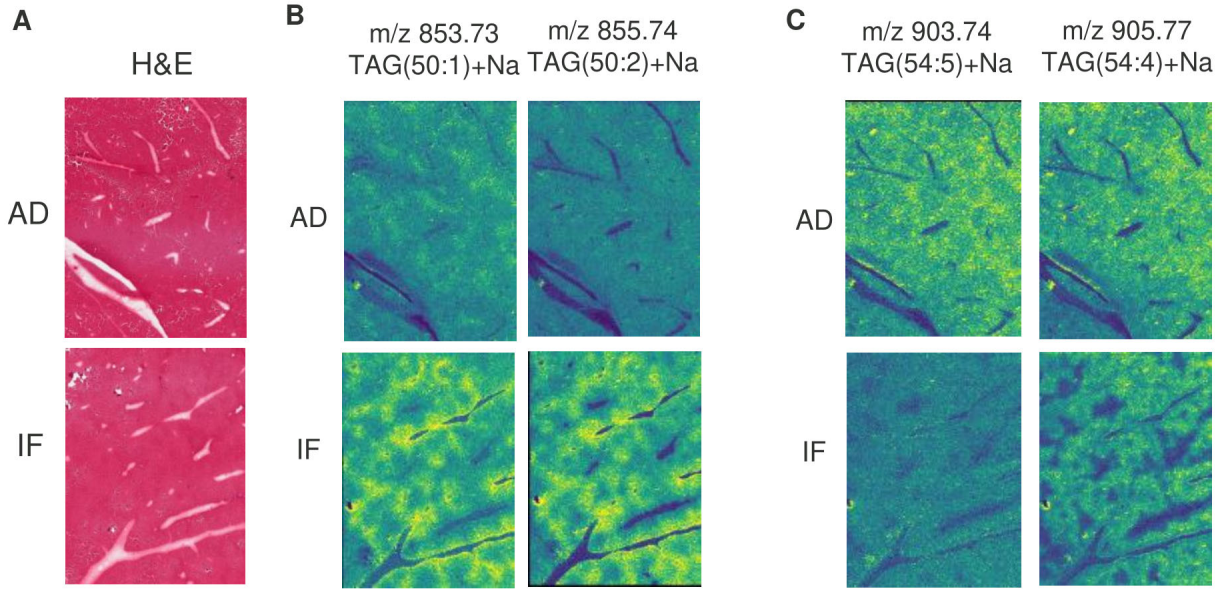
MALDI matrix: NEDC

Spatial resolution: 20 µm

- R-Ket drug has been shown to inhibit ovarian tumor cell engraftment and growth in vivo.
- Several metabolites of interest (e.g., glutamine, glutamate, and citrate) were putatively identified with evident decreases within tumor regions of R-ketorolac treatment.
- Other studies have revealed higher levels of these metabolites lead to poor prognosis in cancer patients

Demo Data from Bruker for the S10 Application

Demo data for samples provided by Liu Lab (Dr. Meilian Liu, School of Medicine):



Tissue: mouse liver, no embedding

MALDI matrix: DHB

Spatial resolution: 20 µm

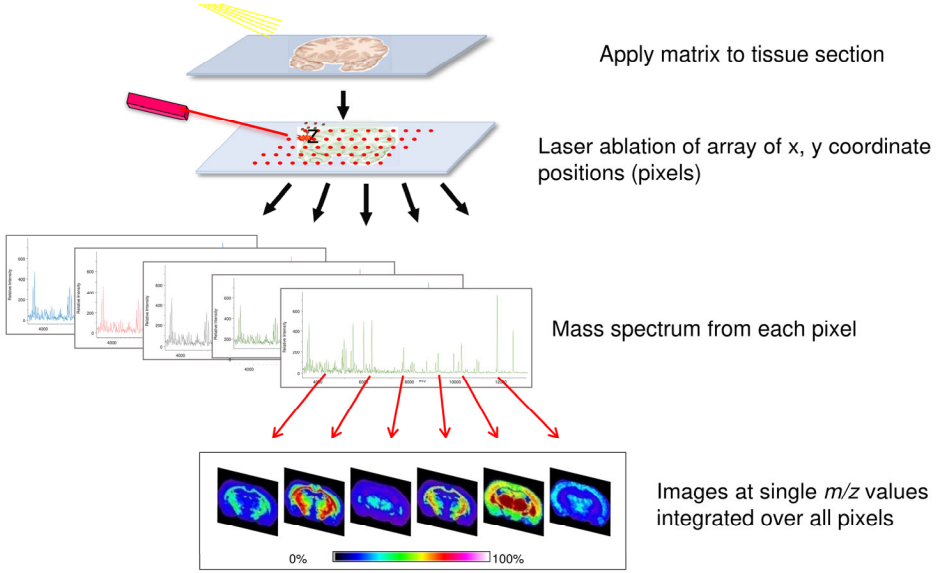
IF: alternate-day fasting
AD: ad libitum feeding

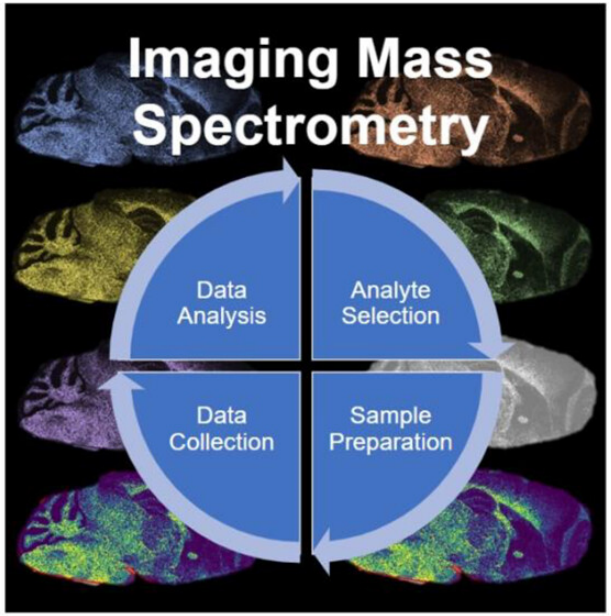
- The distributions of TAG generally line up with the lipid droplets in H&E images.
- IF increased the levels of TAGs with shorter FA tails and fewer C=C chains like TAG (50:1) and (50:2), but decreased TAGs with longer FA tails and more C=C chains like TAG (54:4) and (54:5).
- TAG (50:1) and (50:2) were mobilized into vasculature regions and redistributed by IF.

Summary

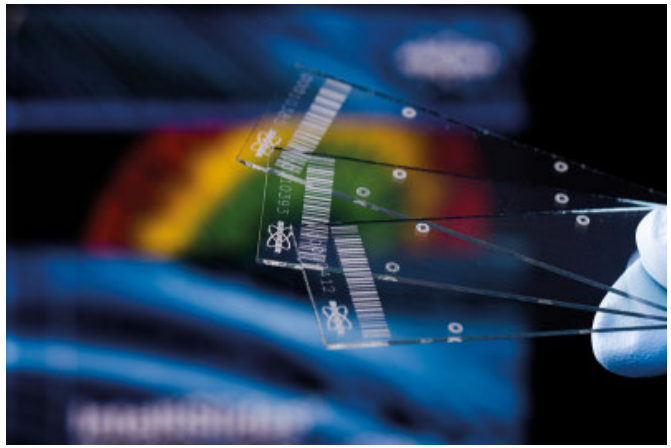
Mass spectrometric imaging research and service

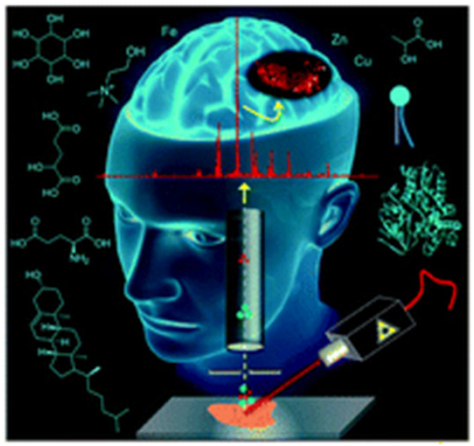
- Timeline and next steps
- The tests will be charged by hours





Questions





Touring the MSL

- Location: B60 (Mass Spec Imaging), B54 (LC-MS, ICP-MS, LA-ICP-MS)
- Time: Till ~ 1:30 PM
- Housekeeping Rules:
  - No food or drinks within the facility. Contamination of the equipment can have significant consequences on experimental results.
  - Do not touch equipment without permission.
  - Ask the staff for clarification.