Matrix-Assisted Laser Desorption/Ionization (MALDI):
Matrix molecules absorb laser light, enter an excited state, and collide with sample molecules, facilitating charge transfer to create ions.

Mass Spectrometric Imaging for biomedical tissue analysis
Kamila Chughtai and Ron M.A. Heeren
Cryosectioning onto Indium Tin Oxide (ITO) coated glass slides and scanning digital image of slide for “teaching” FlexControl software on MALDI-TOF.

Vacuum sublimation is used to apply an even microscopically thin uniform layer of matrix compound onto tissue section without the need for solvents.

Sublimation: *the transition of a substance from solid to gas phase without an intermediate liquid phase.*

MALDI matrices for lipid imaging:

- **DHB:** 2,5-dihydrobenzoic acid (+ve mode)
- **1,5-diaminonapthalene** (-ve mode)
How do we apply matrix for MALDI Imaging?
We built a vacuum sublimation apparatus.

Vacuum at 0.05 Torr pressure is required in sublimation chamber and is monitored by electronic Pirani vacuum gauge.

750 Torr (atmospheric pressure)  0.05 Torr
Matrix deposition by vacuum sublimation.

Deposition of the matrix compound is at the molecular level because gaseous molecules recrystallize at the relatively cold surface of the tissue section attached to the cold condenser.

The uniformity of matrix deposition onto the slide attached to the cold condenser surface reflects the random Brownian motion of the released gaseous matrix molecules.

Slides with matrix applied by vacuum sublimation.
Adapted MALDI plate holds slides for MALDI-imaging Mass Spectrometry.

Conventional MALDI plate  
MALDI plate for cryosections

Setting up a MALDI-IMS run after matrix sublimation.

(1) The slide is placed into a Bruker slide adaptor and into the MALDI-TOF instrument

(2) Regions to image are selected from the scanned photo of the slide
How are we applying MALDI-IMS to our research?

- Lipid based mechanisms of immune suppression and anti-inflammatory action by HDL in Lupus.
- Acute kidney injury (UAB/UCSD O’Brien Center).
- Lipids as mediators of age-related changes in eye lens.

Zebrafish - an emerging model in biomedical research

- $0.39/tank/day (max fish per tank)
- 100-200 embryos weekly per sexually mature female
- Functional vision by 5 days of age
- Transparent embryos permeable to small molecules
- Various mutants model human ocular disorders

- $0.75/cage/day (max 4-5 mice per cage)
- 6-8 pups monthly per sexually mature female
- Functional vision by 2 weeks of age

Stephen Watts, Ph.D., Department of Biology, Director UAB Aquatic Animal Research Core for NORC.
Age associated changes in the lens lipidome of Mouse and Zebrafish.

Specific aims of the study:

1) To characterize and compare the lens lipidomes of Mice and Zebrafish.
2) To analyze the changes that occur in the lens lipidome with aging.
3) To determine where lipid changes are occurring in the lens.

Analyzed using ESI-MS/MS on Triple-TOF Mass Spectrometer.

Normalized weight.

Stephens Barnes, Ph.D., Department of Pharmacology & Toxicology, Director UAB TMPL.
ESI-MS/MS on Triple-TOF Mass Spectrometer.

Complete and Comprehensive Data Collection

TOF MS Scanning Storing all Product Ions

Lipidomic Profiling Map of Rat Brain Extracts Acquired in Negative Mode

Mouse lens positive mode (Total lipids).
Sphingomyelins are increased with ageing in mouse (Black) and Zebrafish (white) lens.
Mouse lens Phosphatidylcholines (white) and Lysophosphatidylcholines (black).

- Log10p

Zebrafish lens Phosphatidylcholines (white) and Lysophosphatidylcholines (black).

- Log10p
ESI-MS/MS fragments from 782.6 m/z 36:4 PC (+mode).

phosphocholine head group

ESI-MS/MS fragments from 840.7 m/z 36:4 PC acetate adduct (-mode).
So where in the lens are these changes in 16:0/20:4 PC occurring?

Tissue processing for matrix application and eye lens MALDI-IMS is challenging

Formalin fixed paraffin sections of lens (B) cannot be used for MALDI-IMS (although certain modifications of fixation protocols might allow for subsequent lipid MALDI-IMS). Cryosectioning (after tissue embedding in 10% gelatin) must therefore be optimized for the tissue being studied. In the case of lens, this is very challenging as the dense lens material has a propensity to crack (A).

Positive ion mode MALDI average mass spectra of (A) 8 week old and (B) 12 month old Zebrafish eyes, showing the location of the peaks for which images were subsequently taken.

Peaks correspond to expected protonated, sodiated, and potassiated adducts of PC(36:4).
MALDI-IMS on protonated, sodiated, and potassiated adducts of PC(36:4) in zebrafish eye.

MS/MS on protonated adduct of PC(36:4) in Zebrafish eye.

MS/MS on m/z 782.6 shows fragmentation indicative of PC(16:0/20:4)
MALDI-IMS on 16:0/20:4 PC(36:4) in mouse eye.

1 year old MOUSE LENS #2 (9-5-2014)
Restricted diacyl phospholipids in lens core.

Mouse eye, 9-5-14
Quantitative and Spatial Analysis of Lipids Involved in Acute Kidney Injury.

Specific aims of the study:

1) To characterize the kidney lipidome of mice following acute injury (quantitatively).

8-10 weeks old C57Bl6/J

Normalized kidney weight

Analyzed using ESI-MS/MS on 5600 Triple-TOF Mass Spectrometer
Specific aims of the study:

2) To analyze WHERE the changes in lipids occur in the kidney (spatially) and to characterize those lipids that remain unchanged quantitatively but might be spatially altered.

8-10 weeks old C57Bl6/J

MALDI-Imaging MS on a Bruker-TOF Mass Spectrometer
**In vivo mouse model of AKI:**

1. **Sham or IR left kidney**
2. nephrectomy
3. 30 mins renal ligation
4. 6 hours reperfusion
5. Euthanization...
6. PBS/BHT perfusion
7. 30 mins renal ligation
8. Kidney homogenization in 1ml PBS/BHT
9. MALDI-IMS
10. Kidney homogenization in 1ml PBS/BHT
11. SWATH 5600 TOF
12. Resuspend lipids in MetOH
13. Dried lipids under Argon
14. Extract lipids under Argon
15. -80°C
16. Samples
17. Irradiation (IR) plasma creatinine 1-2mg/dL

**Mouse kidney lipids changed following IR (0.5/6hrs)**

(5600 Triple-TOF SWATH positive mode).

- **PE O-40:5**
  - 780.6_639.6
- **PC O-38:1**
  - 802.7_184.1
- **PE O-40:4**
  - 782.6_641.6
- **GD2 32:2;2**
  - (LCB 18:2;2-H2O, LCB 18:1;3-3H2O)
  - 900.8_284.3
- **GT2 26:0:2**
  - (LCB 18:0;2-H2O)
  - 900.8_284.3
- **PE O-42:3**
  - 812.7_198.1
- **SM 39:3:2**
  - 769.7_184.1
- **CE 27:3 +NH4 (chol)**
  - 790.7_369.4
- **LPC 22:6**
  - 568.4_184.1

**8-10 weeks old C57Bl6/J (n=6)**
Mouse kidney lipids changed following IR (0.5/6hrs) (5600 Triple-TOF SWATH negative mode).

- Log10p

Log2FC

8-10 weeks old C57Bl6/J (n=6)

PC 16:0/18:2

LPI O-28:0

PE 14:0/20:4

PE O-40:1

PE 46:2

PE 14:0/20:4

PE 46:2

PE O-40:4 intensity

r=0.771

p=0.00228

PE O-40:5 intensity

r=0.724

p=0.0062

1-alkyl, 2-acyl PE

1-acyl, 2-acyl PE

Plasma creatinine (IR 0.5/6hrs)

Plasma creatinine (IR 0.5/6hrs)
1-alkyl, 2-acyl PC

\[
\begin{align*}
&\text{PC O-38:1 intensity} \\
&\quad r=0.730 \\
&\quad p=0.0062 \\
&\text{Plasma creatinine (IR 0.5/6hrs)}
\end{align*}
\]

1-acyl, 2-acyl PC

\[
\begin{align*}
&\text{SM O-39:3;2 intensity} \\
&\quad r=-0.695 \\
&\quad p=0.0121 \\
&\text{Plasma creatinine (IR 0.5/6hrs)}
\end{align*}
\]

1-acyl, 2-acyl PC

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\begin{align*}
&\text{CE 27:3 +NH4 (chol) intensity} \\
&\quad r=0.886 \\
&\quad p=0.0333 \\
&\text{Plasma creatinine (IR 0.5/6hrs)}
\end{align*}
\]

GD2 32:2;2 intensity

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\begin{align*}
&\text{GD2 32:2;2 intensity} \\
&\quad r=-0.582 \\
&\quad p=0.0446 \\
&\text{Plasma creatinine (IR 0.5/6hrs)}
\end{align*}
\]
MALDI-IMS on PE O-40:4 (782.6_641.6)
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