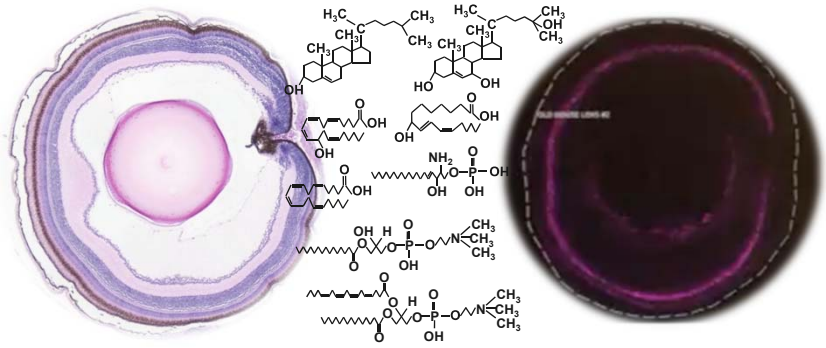
Lipid Imaging Mass Spectrometry.

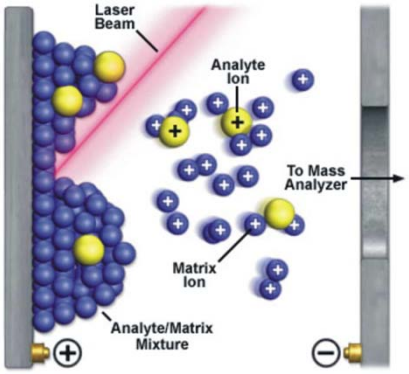


Advanced Graduate Course in Metabolomics
3-11-2015
Janusz Kabarowski, Dept. of Microbiology, UAB.

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.

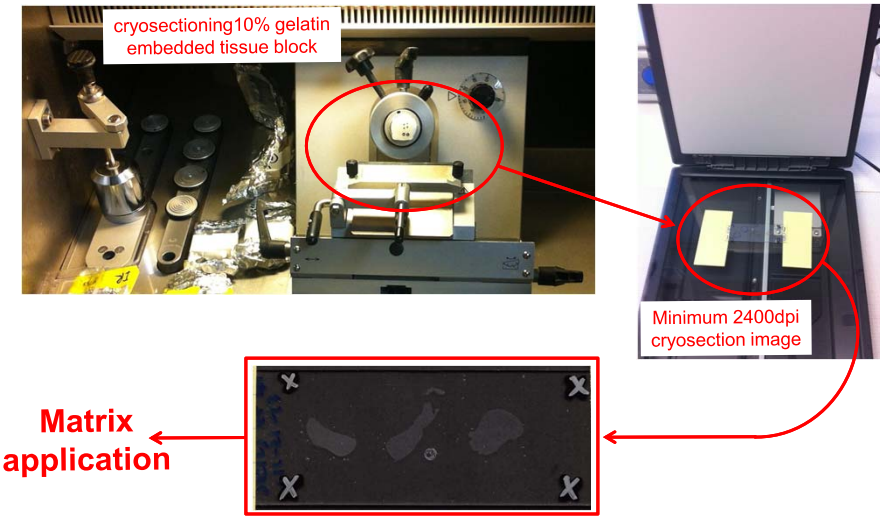

Conventional MALDI plate

MALDI-TOF instrument



Mass Spectrometric Imaging for biomedical tissue analysis
Kamila Chughtai and Ron M.A. Heeren
Chem Rev. Vol.110(5): pp3237–3277, 2010.

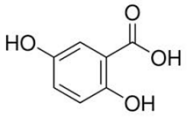
Cryosectioning onto Indium Tin Oxide (ITO) coated glass slides and scanning digital image of slide for "teaching" FlexControl software on MALDI-TOF.

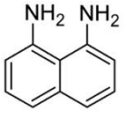


Vacuum sublimation is used to apply an even microscopically thin uniform layer of matrix compound onto tissue section without the need for solvents.

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

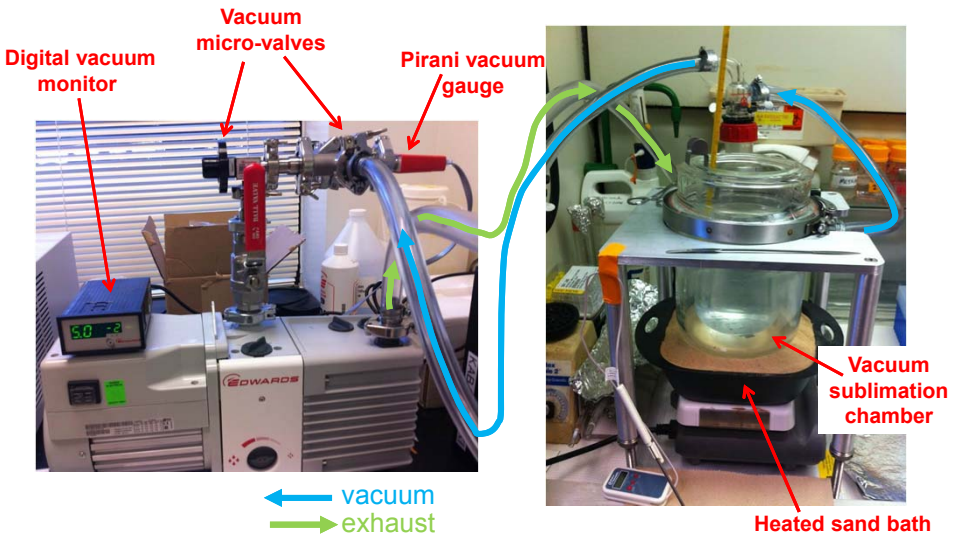
MALDI matrices for lipid imaging:

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.

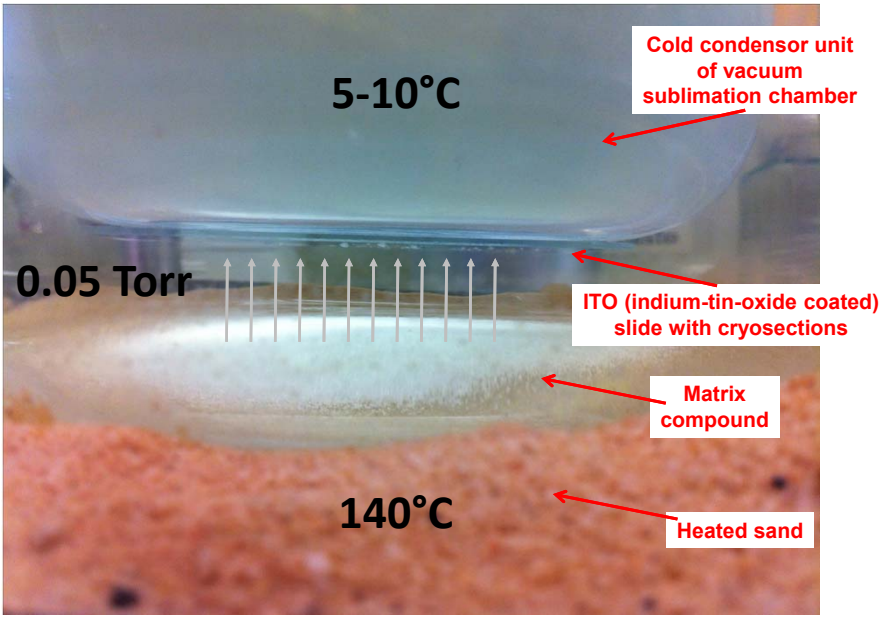


Vacuum at 0.05 Torr pressure is required in sublimation chamber and is monitored by electronic Pirani vacuum gauge.

750 Torr (atmospheric pressure)

0.05 Torr

Matrix deposition by vacuum sublimation.



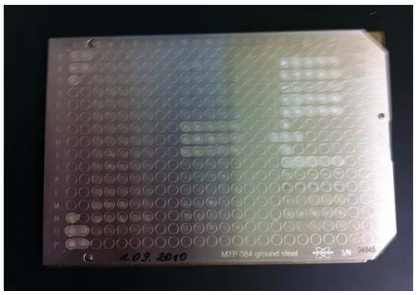
Slides with matrix applied by vacuum sublimation.

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.

Adaptated MALDI plate holds slides for MALDI-imaging Mass Spectrometry.

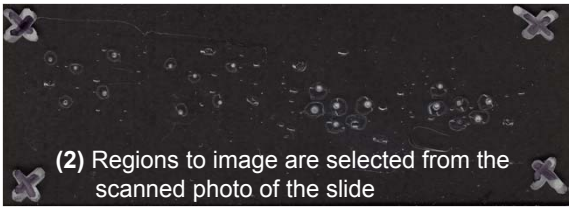
Conventional MALDI plate



MALDI plate for cryosections

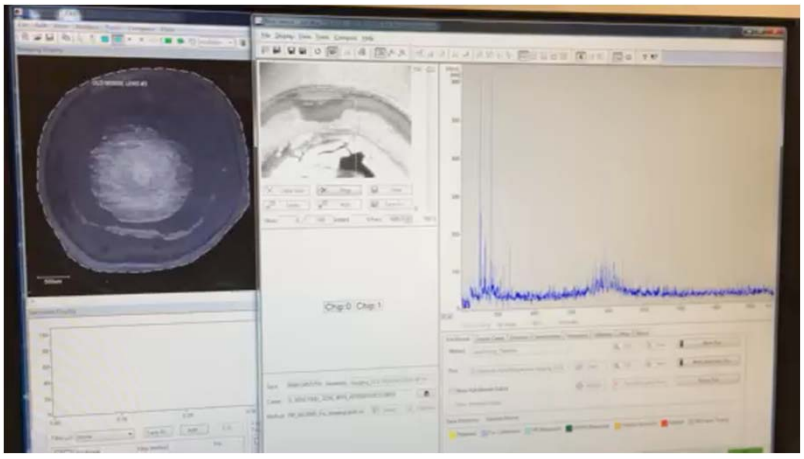
Setting up a MALDI-IMS run after matrix sublimation.

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

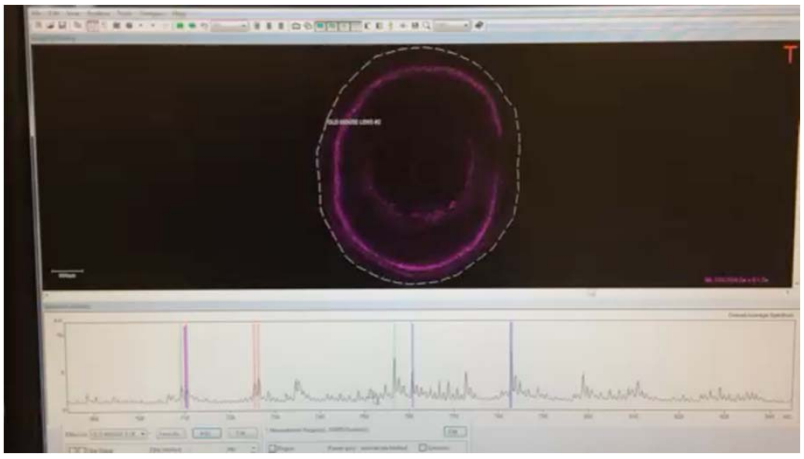


(2) Regions to image are selected from the scanned photo of the slide

MALDI-IMS in action.



MALDI-IMS in action.

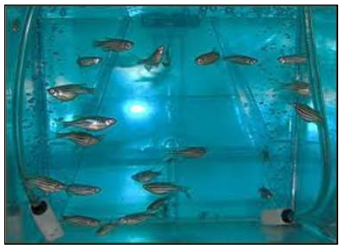


How are we applying MALDI-IMS to our research?

- 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

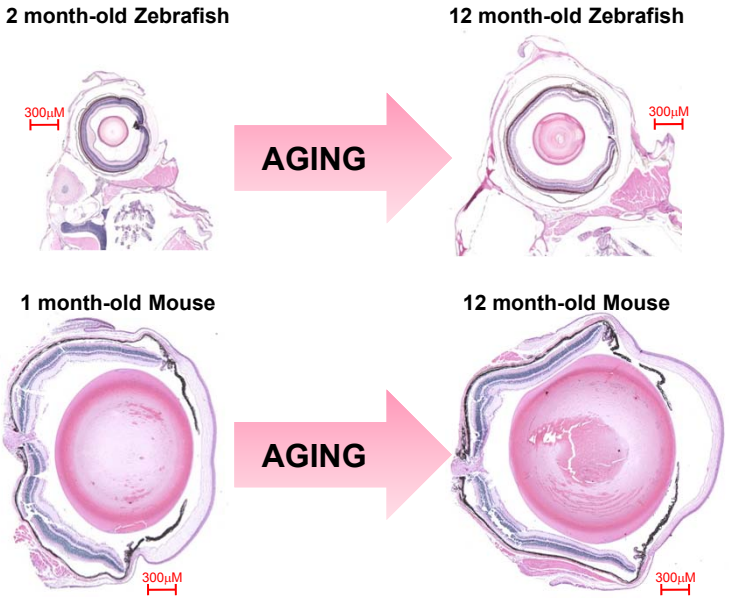
- $0.39/tank/day (max fish per tank)
- 100-200 embryos weekly per sexually mature female
- Functional vision by 5 days of age
- Transparent embryos permeable to small molecules
- Various mutants model human ocular disorders



- $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

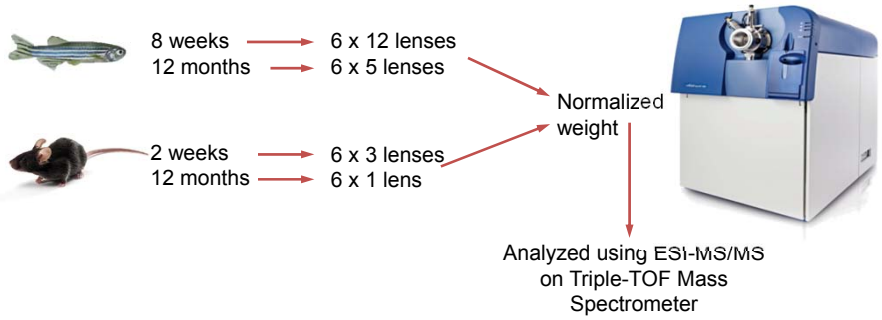
Stephen Watts, Ph.D., Department of Biology, Director UAB Aquatic Animal Research Core for NORC.

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 lipdome with aging.
3) To determine where lipid changes are occurring in the lens.

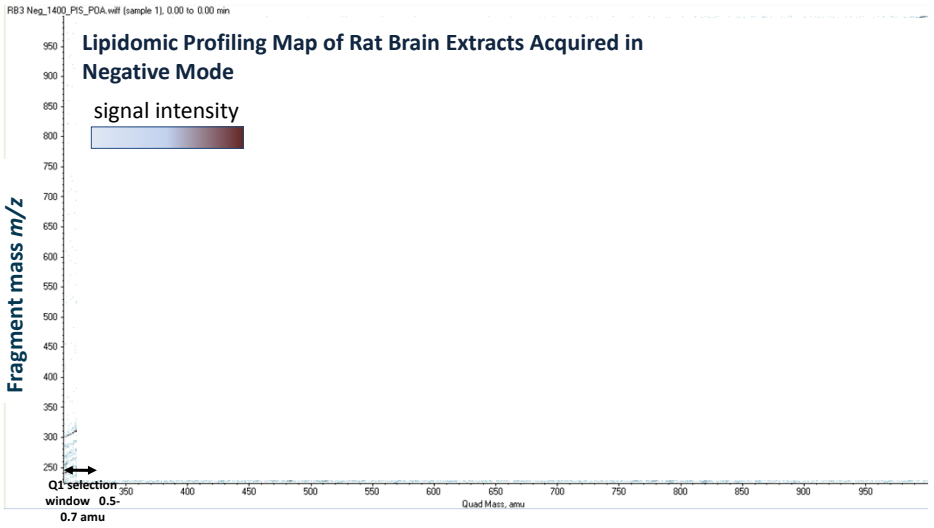


Stephen Barnes, Ph.D., Department of Pharmacology & Toxicology, Director UAB TMPL.

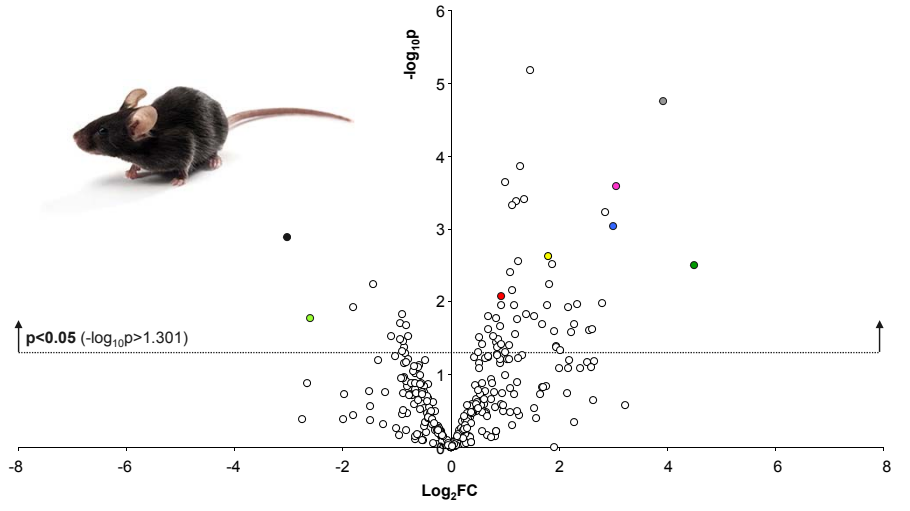
ESI-MS/MS on Triple-TOF Mass Spectrometer.

Complete and Comprehensive Data Collection

TOF MS Scanning Storing all Product Ions

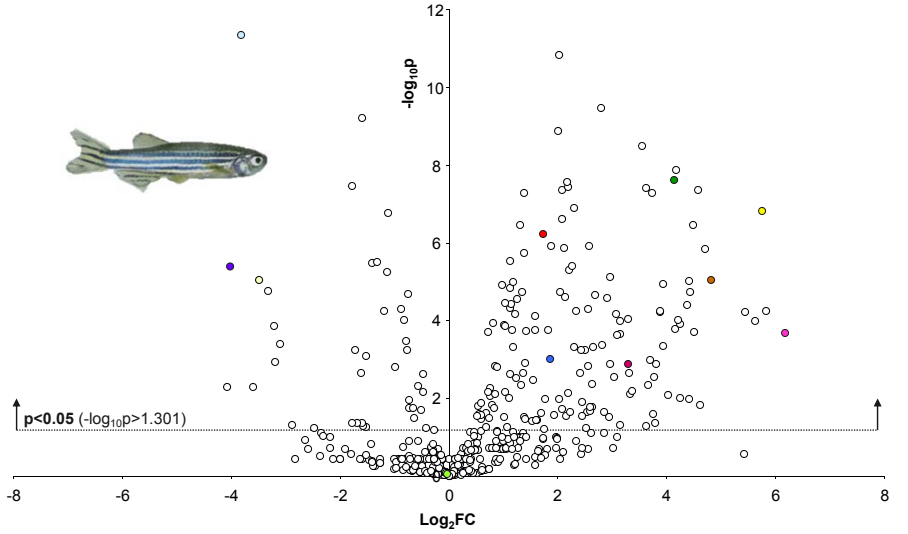


Mouse lens positive mode (Total lipids).



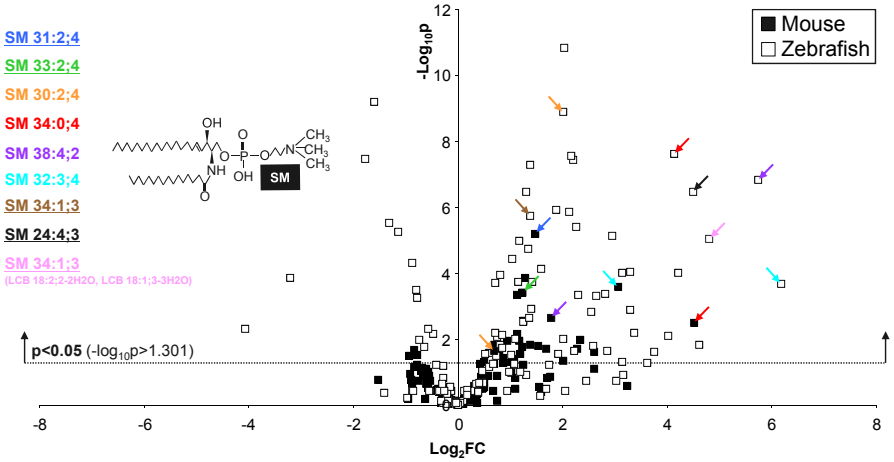
PG O-44:2 (-PG) | SM 38:4;2 (SM) | LPE 12:3 (LPE_p) | LPC 16:0 | PC 36:4 (16:0/20:4) | PC O-32:1 (PC) [alkyl link]
PE 36:0 (-PE) | SM 24:1;2 (SM) | SM 32:3;4 (SM) | SM 34:0;4 (SM) | SM 34:1;3 (LCB 18:2, 2-2H2O, LCB 18:1;3-3H2O)
LMMPE 18:0 (-MMPE) | MM(monomethyl) PE 36:0 (-MMPE)

Zebrafish lens positive mode (Total lipids).

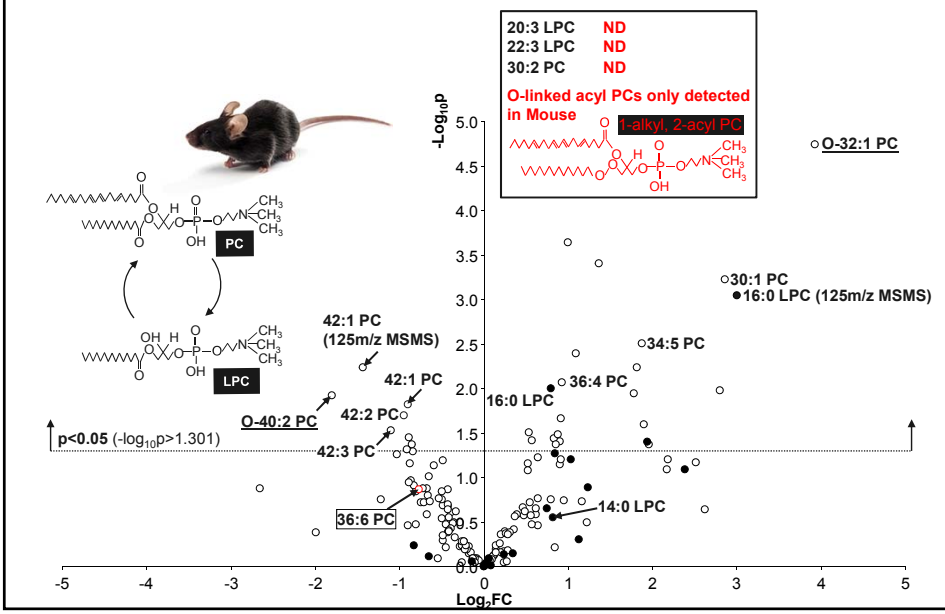


PG O-44:2 (-PG) | SM 38:4;2 (SM) | LPE 12:3 (LPE$_p$) | LPC 16:0 | PC 36:4 (16:0/20:4) | PC O-32:1 (PC) [alkyl link]
PE 36:0 (-PE) | SM 24:1;2 (SM) | SM 32:3;4 (SM) | SM 34:0;4 (SM) | SM 34:1;3 (LCB 18:2, 2-2H2O, LCB 18:1;3-3H2O)
LMMPE 18:0 (-MMPE) | MM(monomethyl) PE 36:0 (-MMPE)

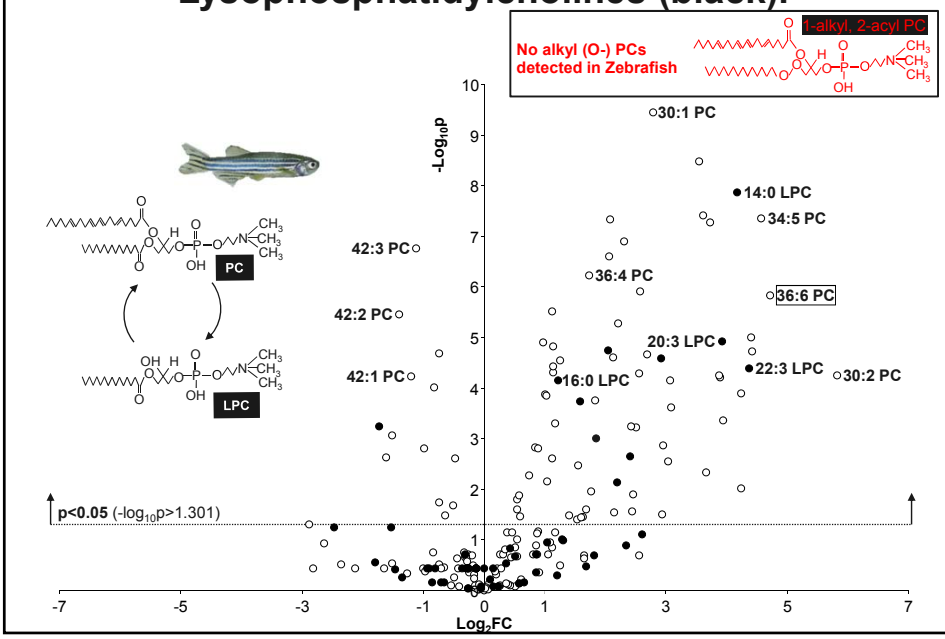
Sphingomyelins are increased with ageing in mouse (Black) and Zebrafish (white) lens.



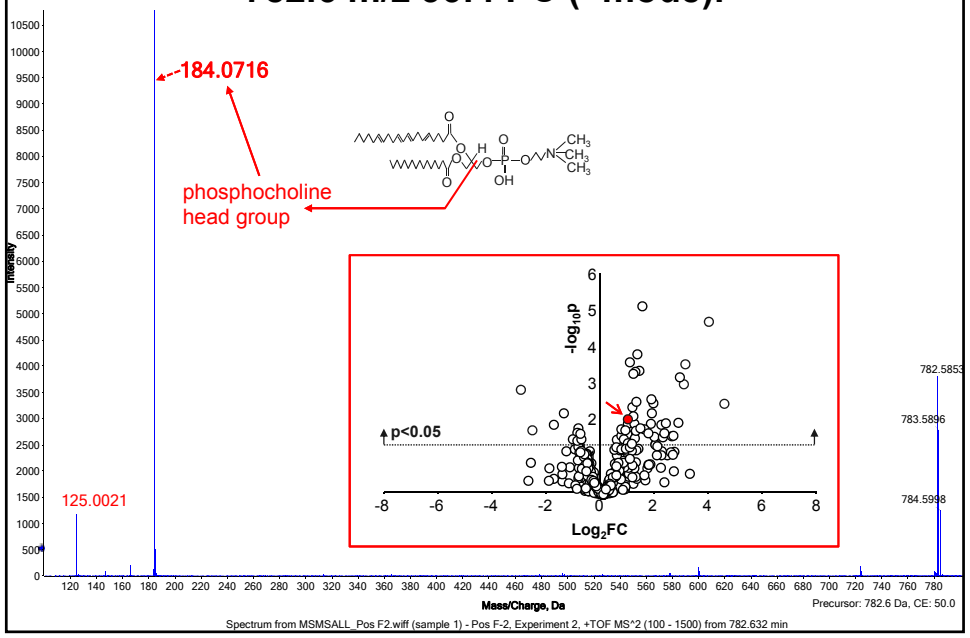
Mouse lens Phosphatidylcholines (white) and Lysophosphatidylcholines (black).



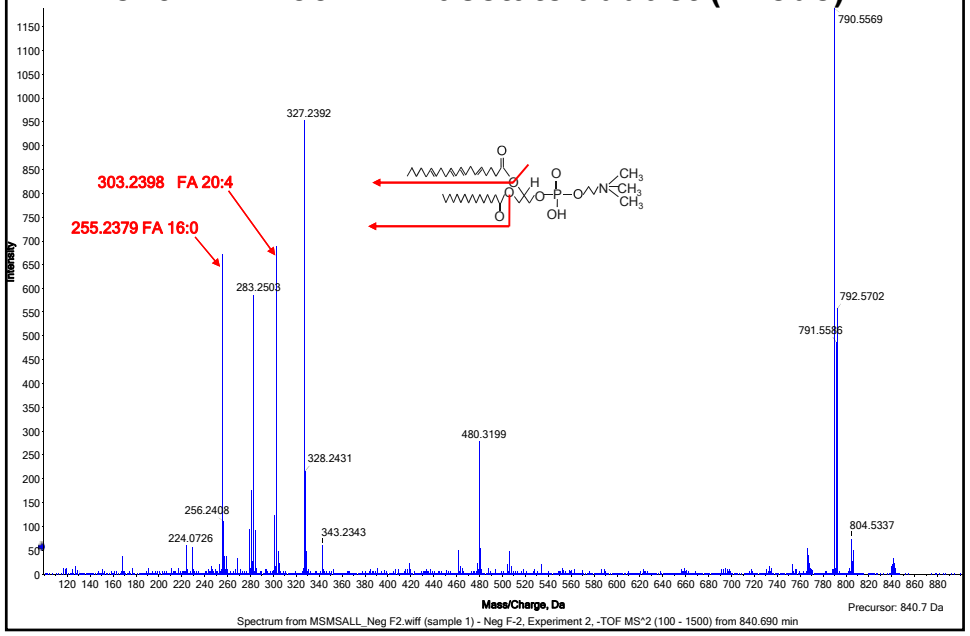
Zebrafish lens Phosphatidylcholines (white) and Lysophosphatidylcholines (black).



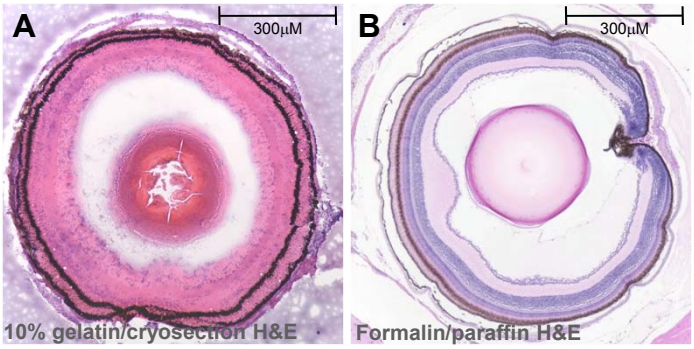
ESI-MS/MS fragments from 782.6 m/z 36:4 PC (+mode).



ESI-MS/MS fragments from 840.7 m/z 36:4 PC acetate adduct (-mode).



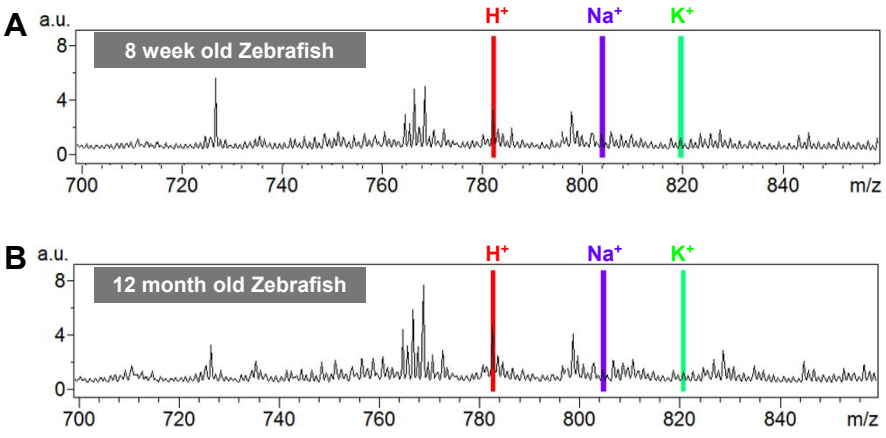
So where in the lens are these changes in 16:0/20:4 PC occuring?



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

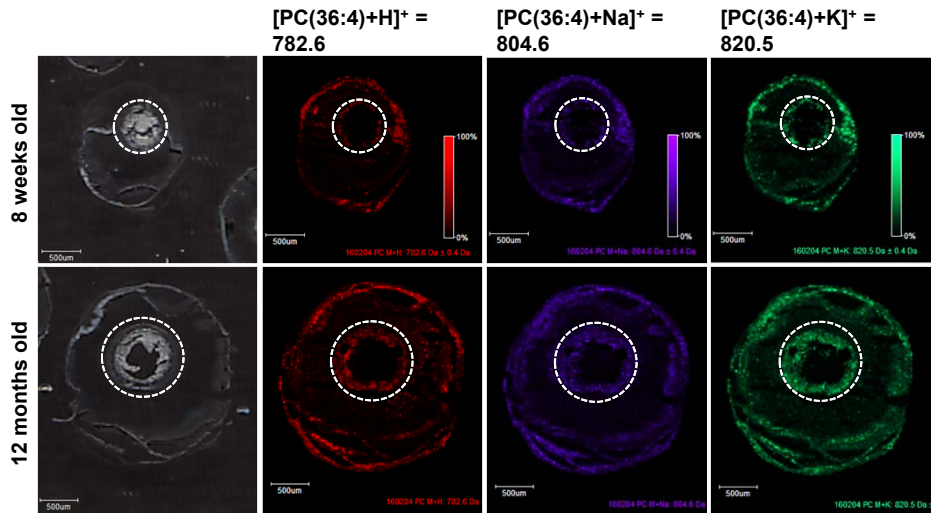
Formalin fixed paraffin sections of lens **(B)** cannot be used for MALDI-IMS (although certain modifications of fixation protocols might allow for subsequent lipid MALDI-IMS). Cryosectioning (after tissue embedding in 10% gelatin) must therefore be optimized for the tissue being studied. In the case 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.

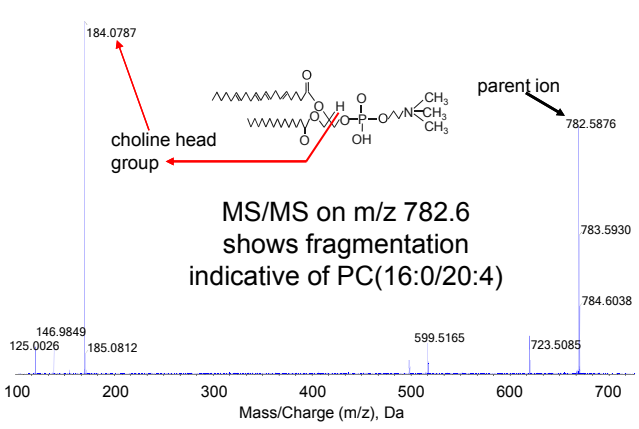


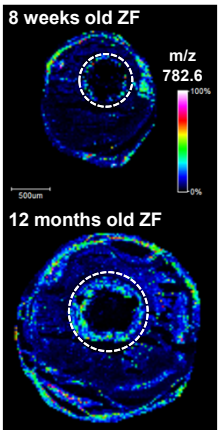
Peaks correspond to expected protonated, sodiated, and potassiated adducts of PC(36:4).

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

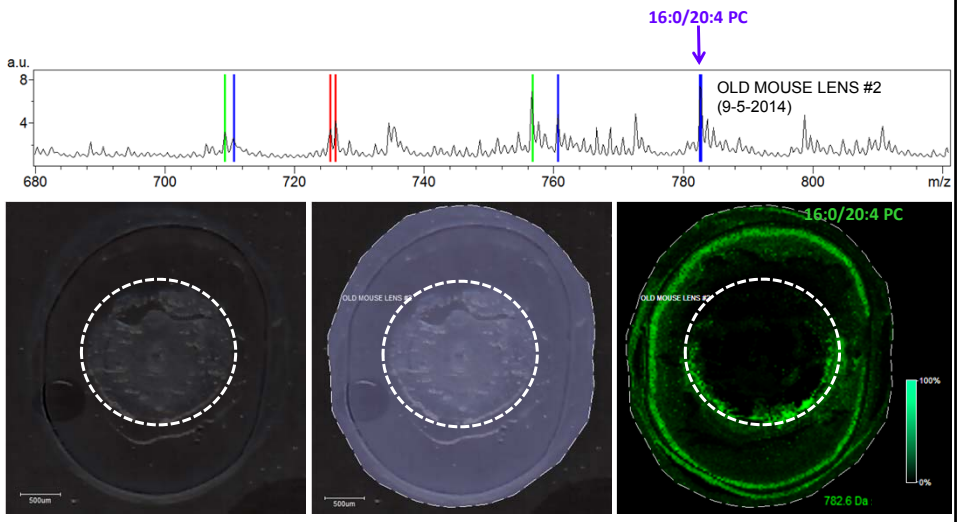


MS/MS on protonated adduct of PC(36:4) in Zebrafish eye.

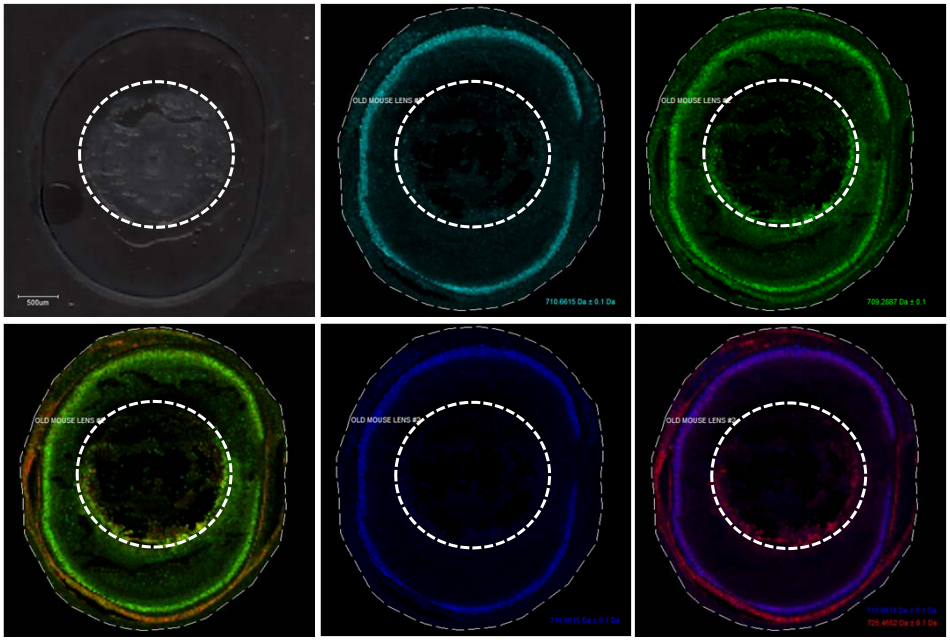


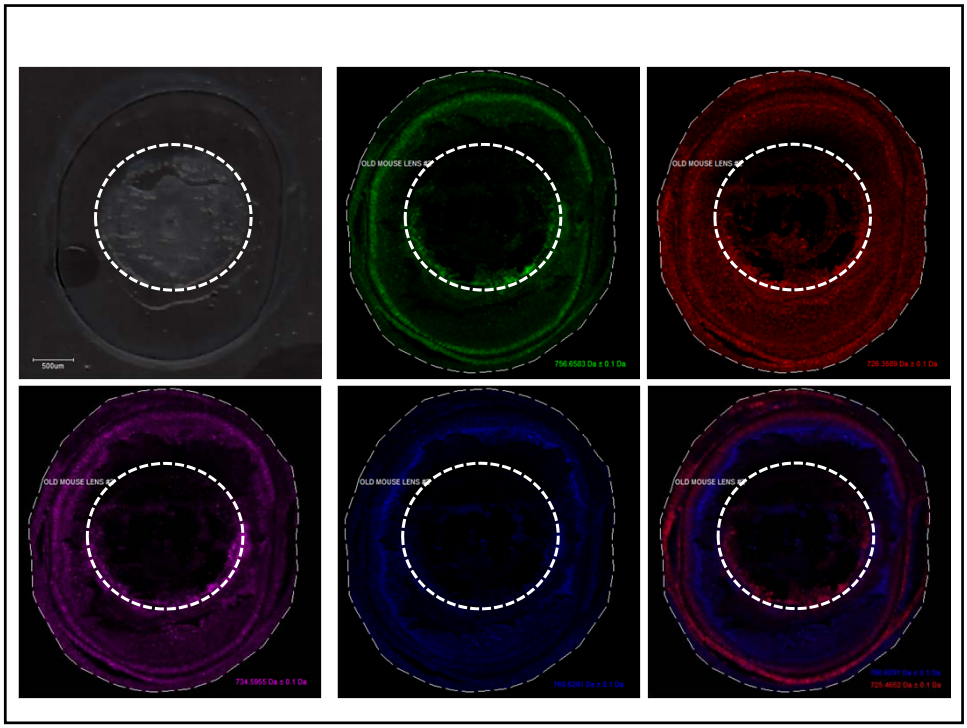


MALDI-IMS on 16:0/20:4 PC(36:4) in mouse eye.

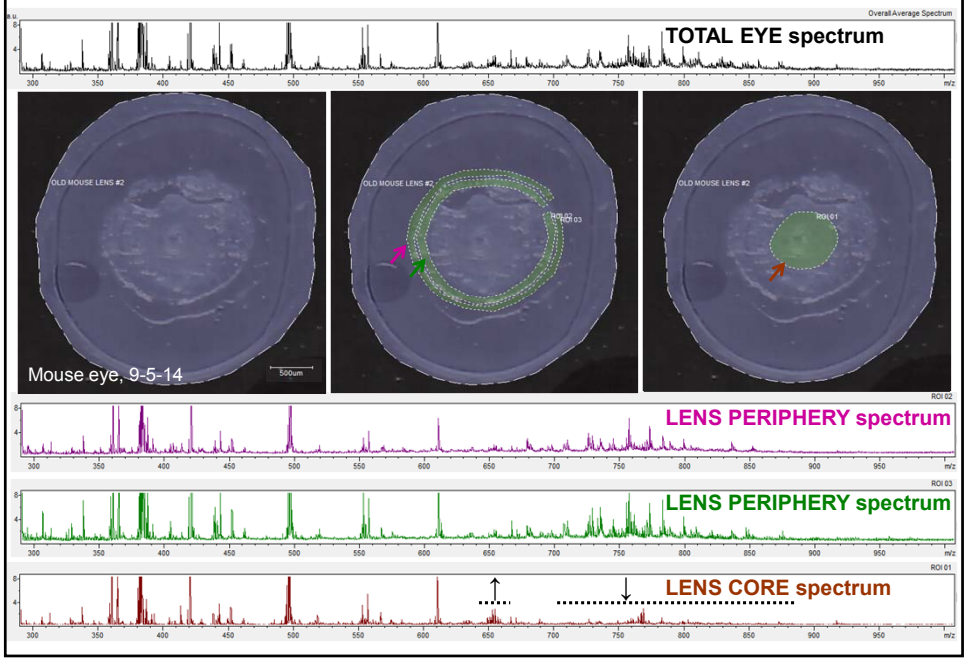


1 year old MOUSE LENS #2 (9-5-2014)

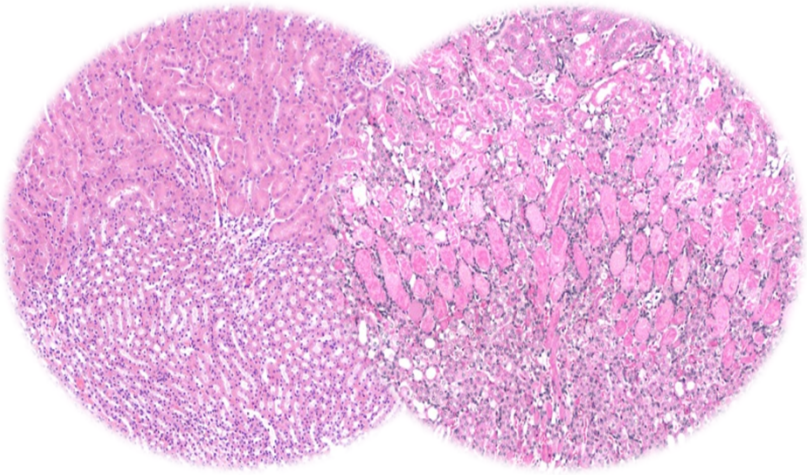




Restricted diacyl phospholipids in lens core.

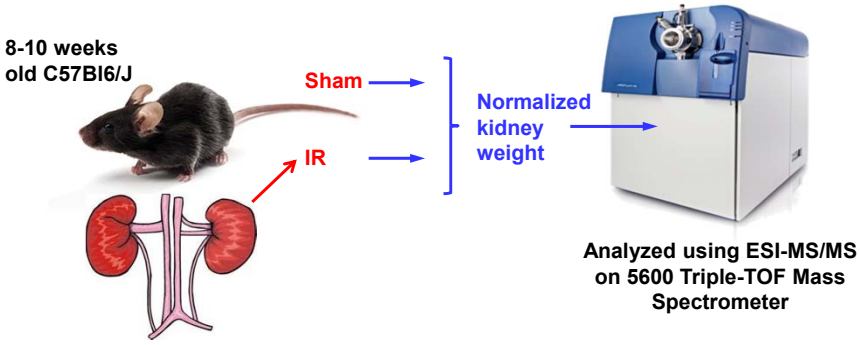


Quantitative and Spatial Analysis of Lipids Involved in Acute Kidney Injury.



Specific aims of the study:

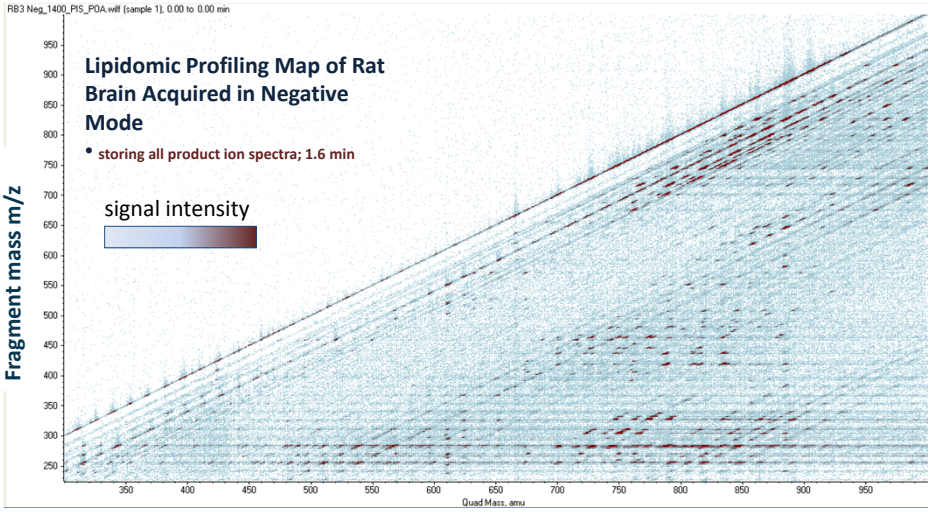
1) To characterize the kidney lipidome of mice following acute injury (quantitatively).



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

MS/MS of Every Precursor

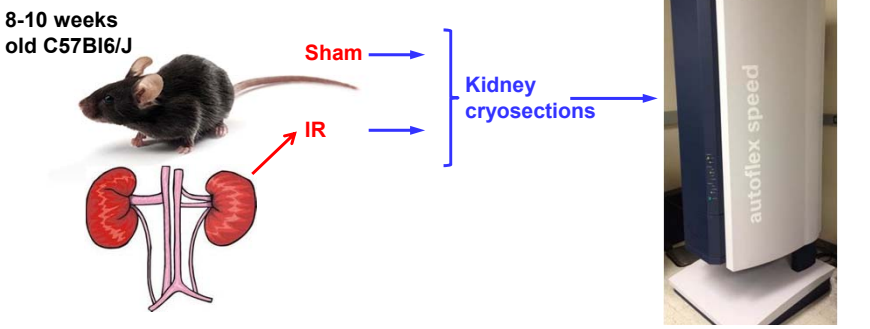
Product Ion Scan of Every Lipid



Precursor mass m/z

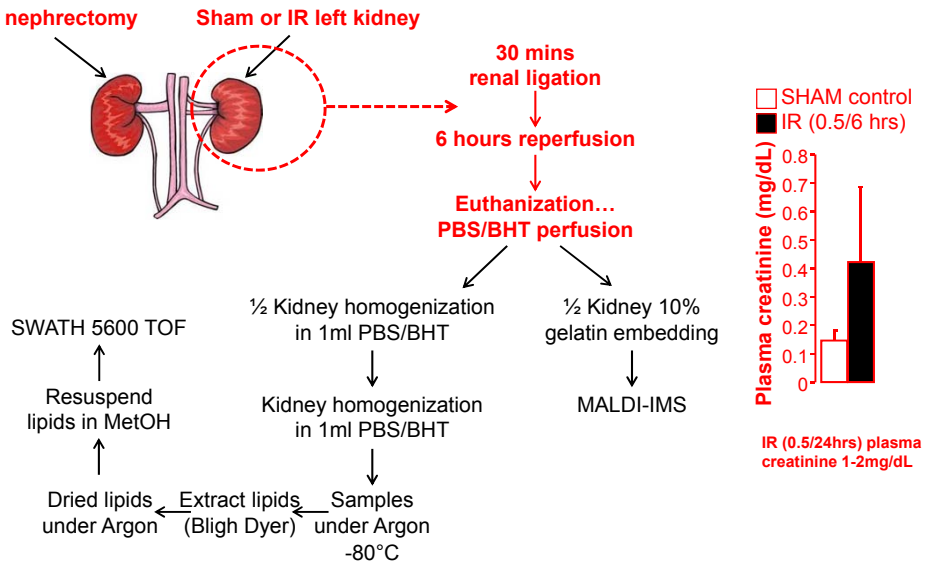
Specific aims of the study:

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.

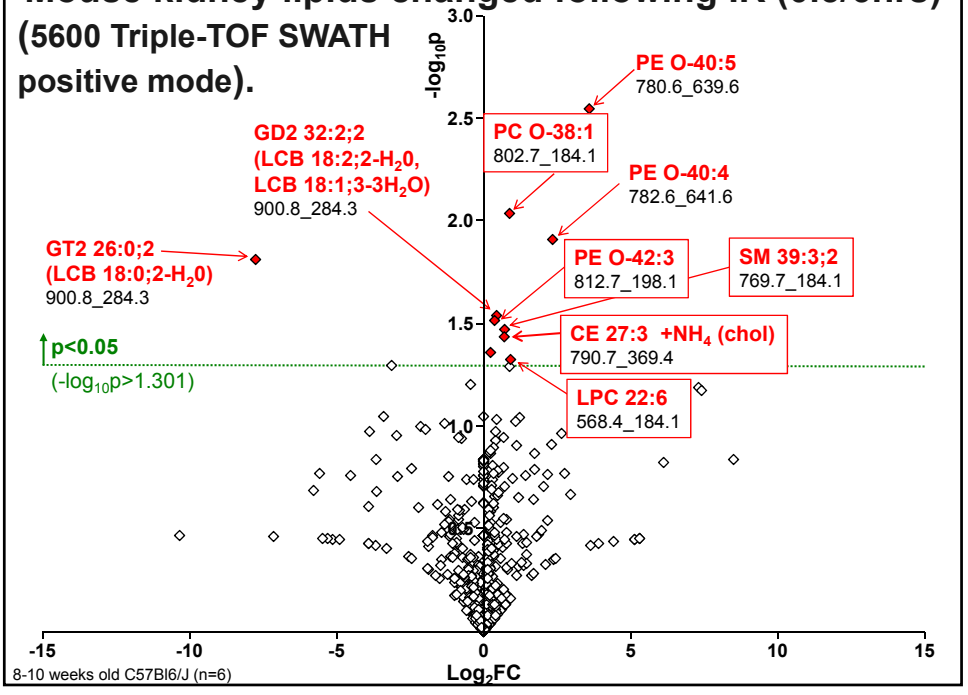


MALDI-Imaging MS on a Bruker-TOF Mass Spectrometer

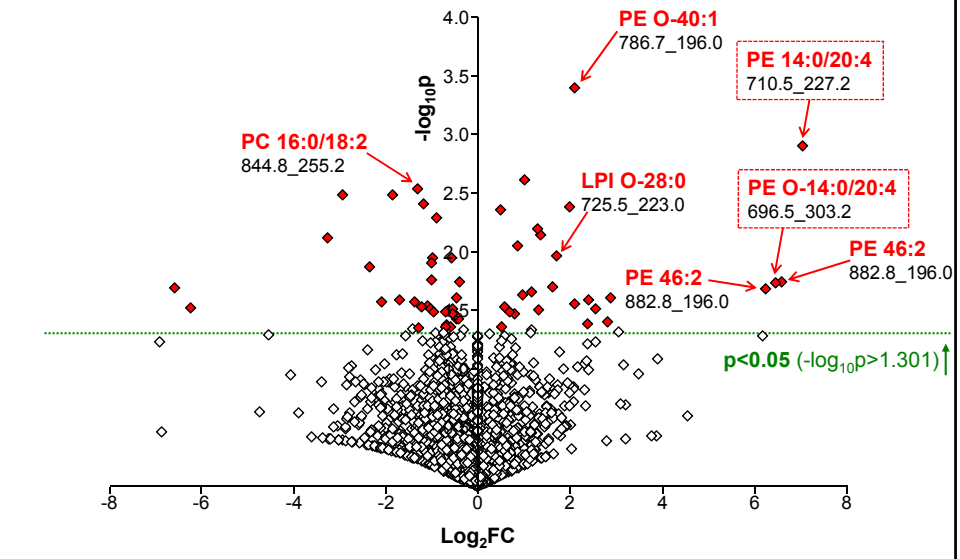
In vivo mouse model of AKI:



Mouse kidney lipids changed following IR (0.5/6hrs) (5600 Triple-TOF SWATH positive mode).

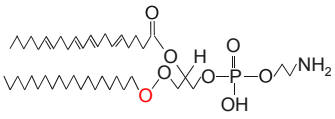


Mouse kidney lipids changed following IR (0.5/6hrs) (5600 Triple-TOF SWATH negative mode).

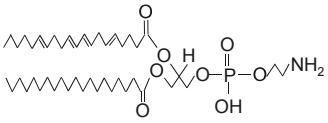


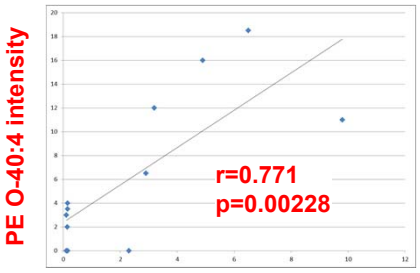
8-10 weeks old C57Bl6/J (n=6)

1-alkyl, 2-acyl PE

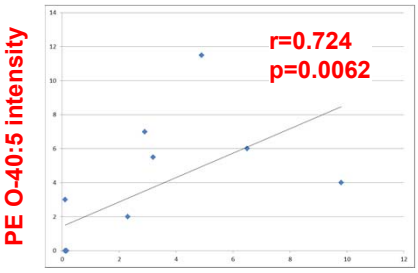


1-acyl, 2-acyl PE



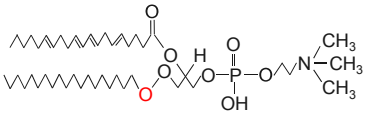


Plasma creatinine (IR 0.5/6hrs)

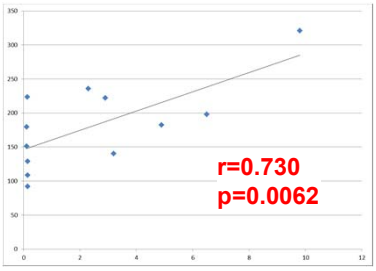


Plasma creatinine (IR 0.5/6hrs)

1-alkyl, 2-acyl PC

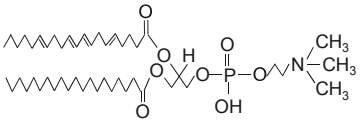


PC O-38:1 intensity

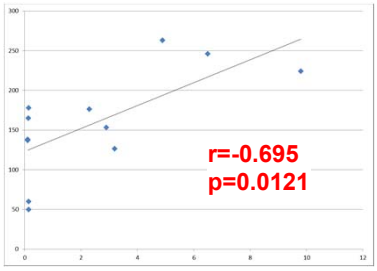


Plasma creatinine (IR 0.5/6hrs)

1-acyl, 2-acyl PC

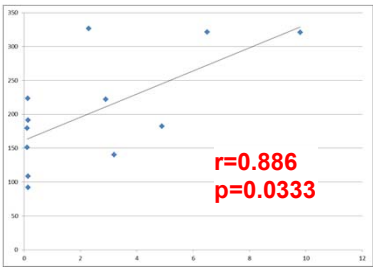


SM O-39:3;2 intensity

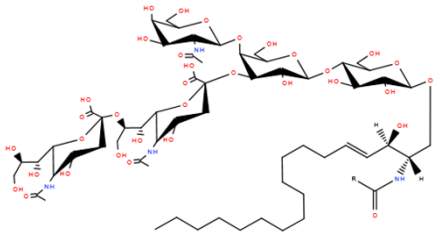


Plasma creatinine (IR 0.5/6hrs)

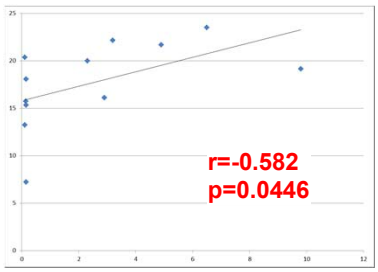
CE 27:3 +NH_4 (chol) intensity



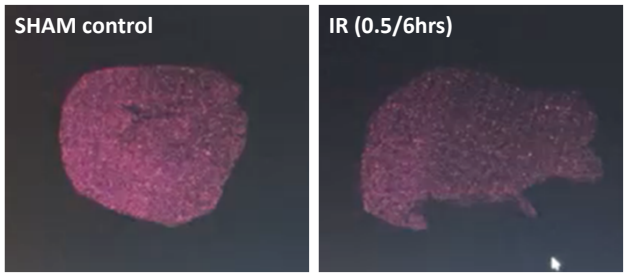
Plasma creatinine (IR 0.5/6hrs)



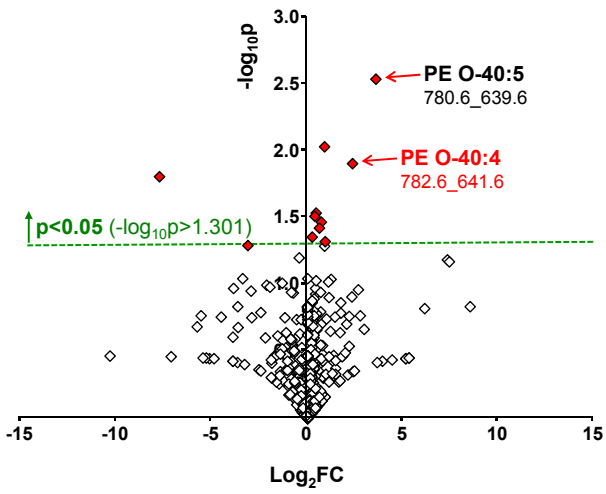
GD2 32:2;2 intensity

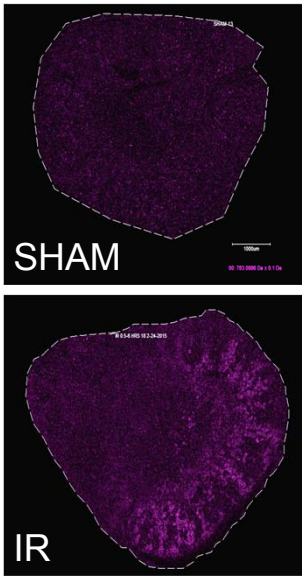


Plasma creatinine (IR 0.5/6hrs)



MALDI-IMS on PE O-40:4 (782.6_641.6)





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