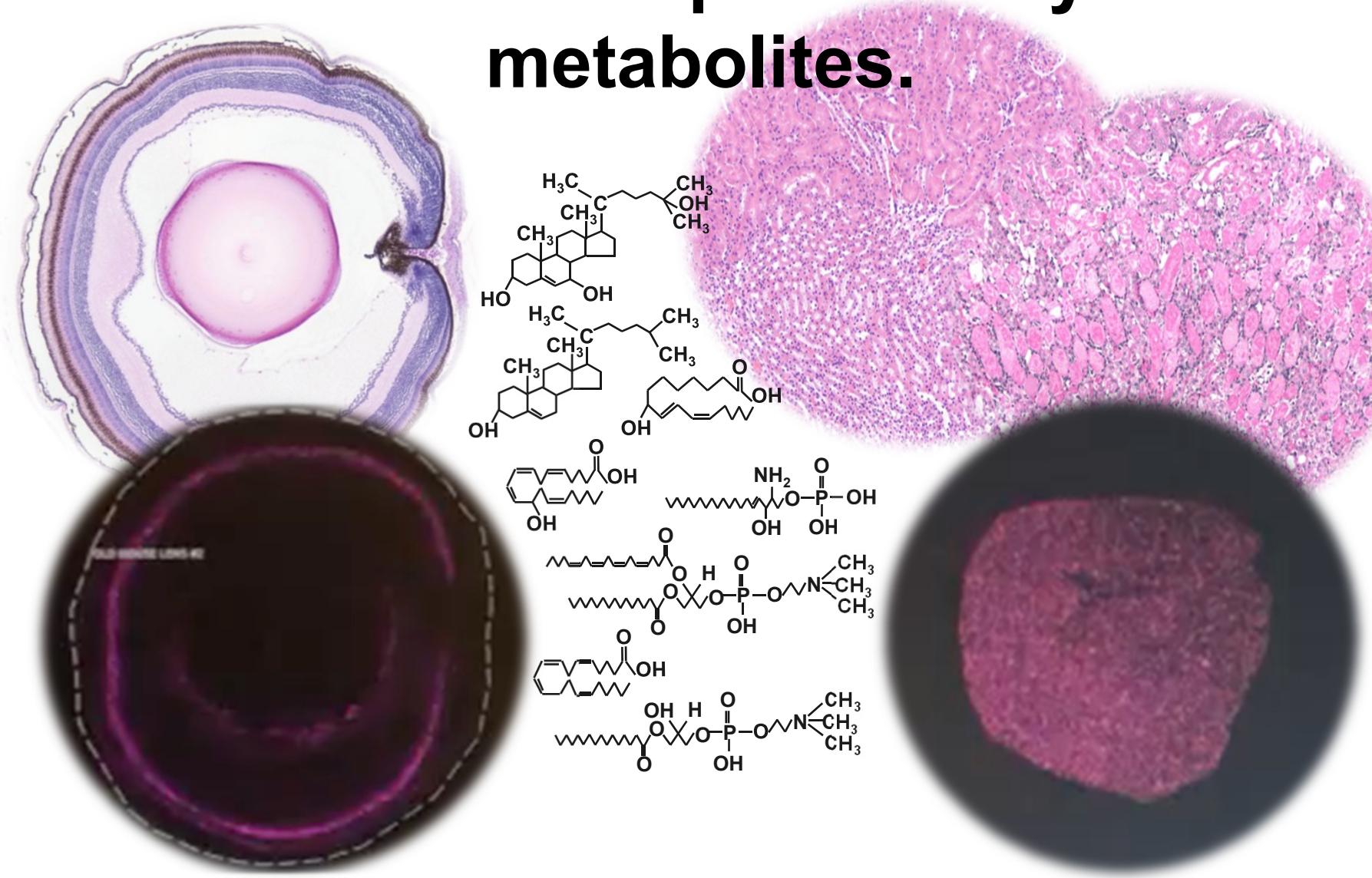
MALDI-IMS for spatial analysis of metabolites.



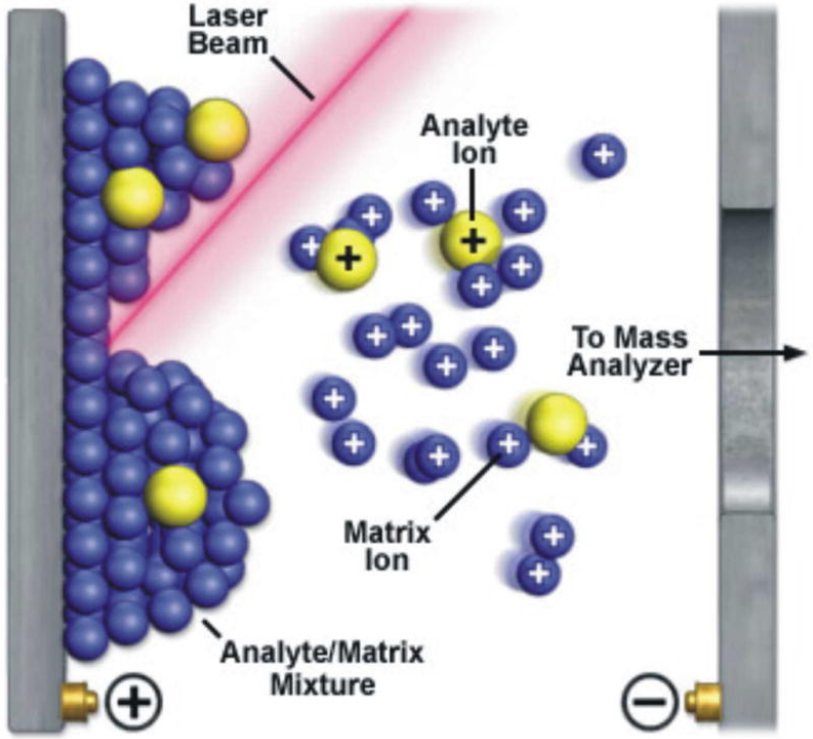
Metabolomics Workshop 6-15-2015

Janusz Kabarowski, Dept. 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.

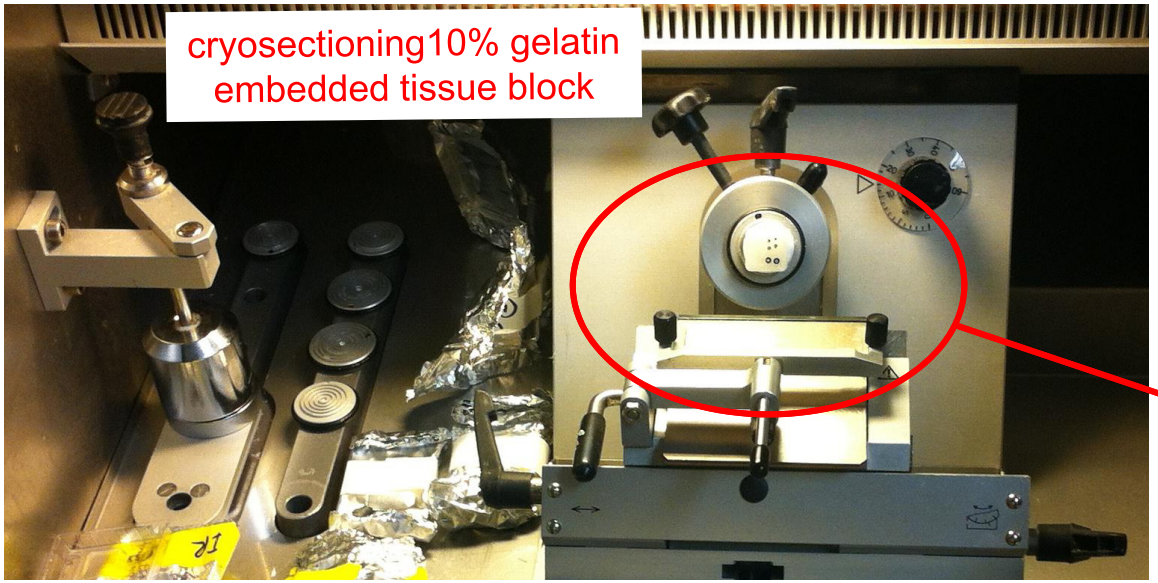
Conventional MALDI plate

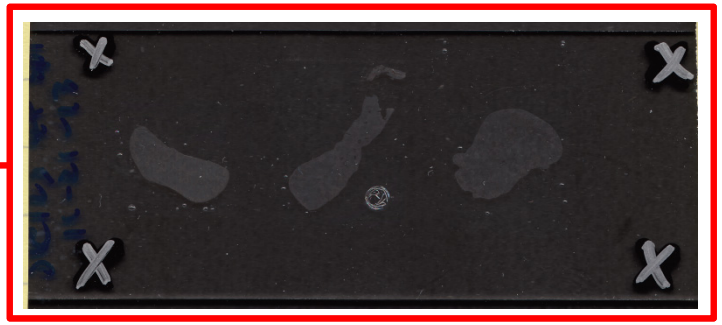
MALDI-TOF instrument



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.



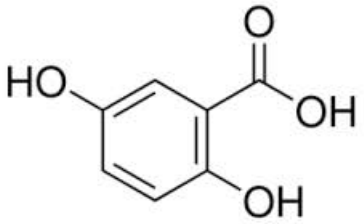


Matrix application

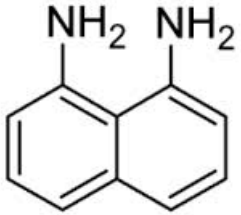
Vacuum sublimation is used to apply an even microscopically thin uniform layer of matrix compound onto tissue section without the need for solvents.

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 

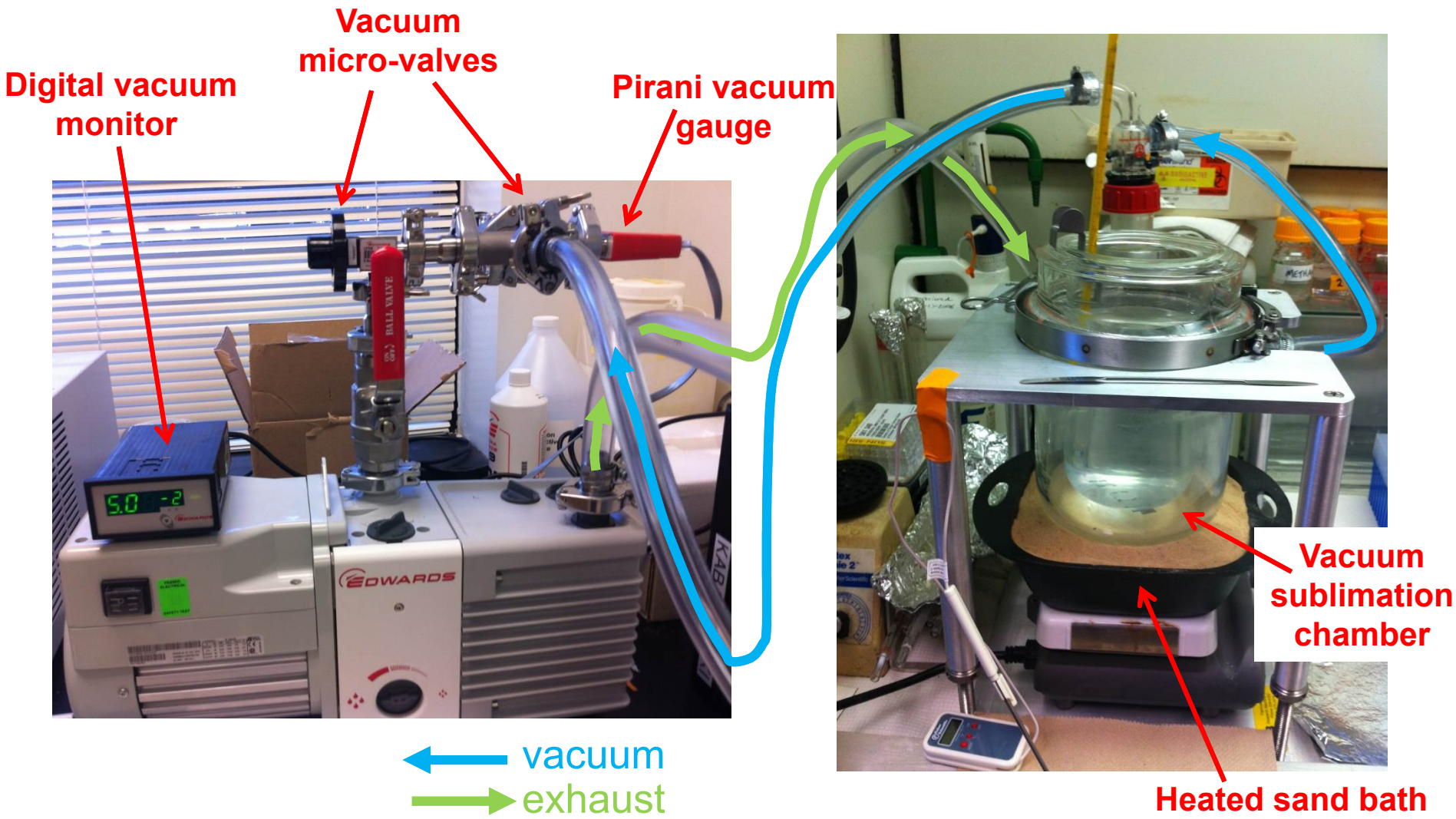
(+ve mode)

1,5-diaminonapthalene 

(-ve mode)

How do we apply matrix for MALDI Imaging?

We built a vacuum sublimation apparatus.

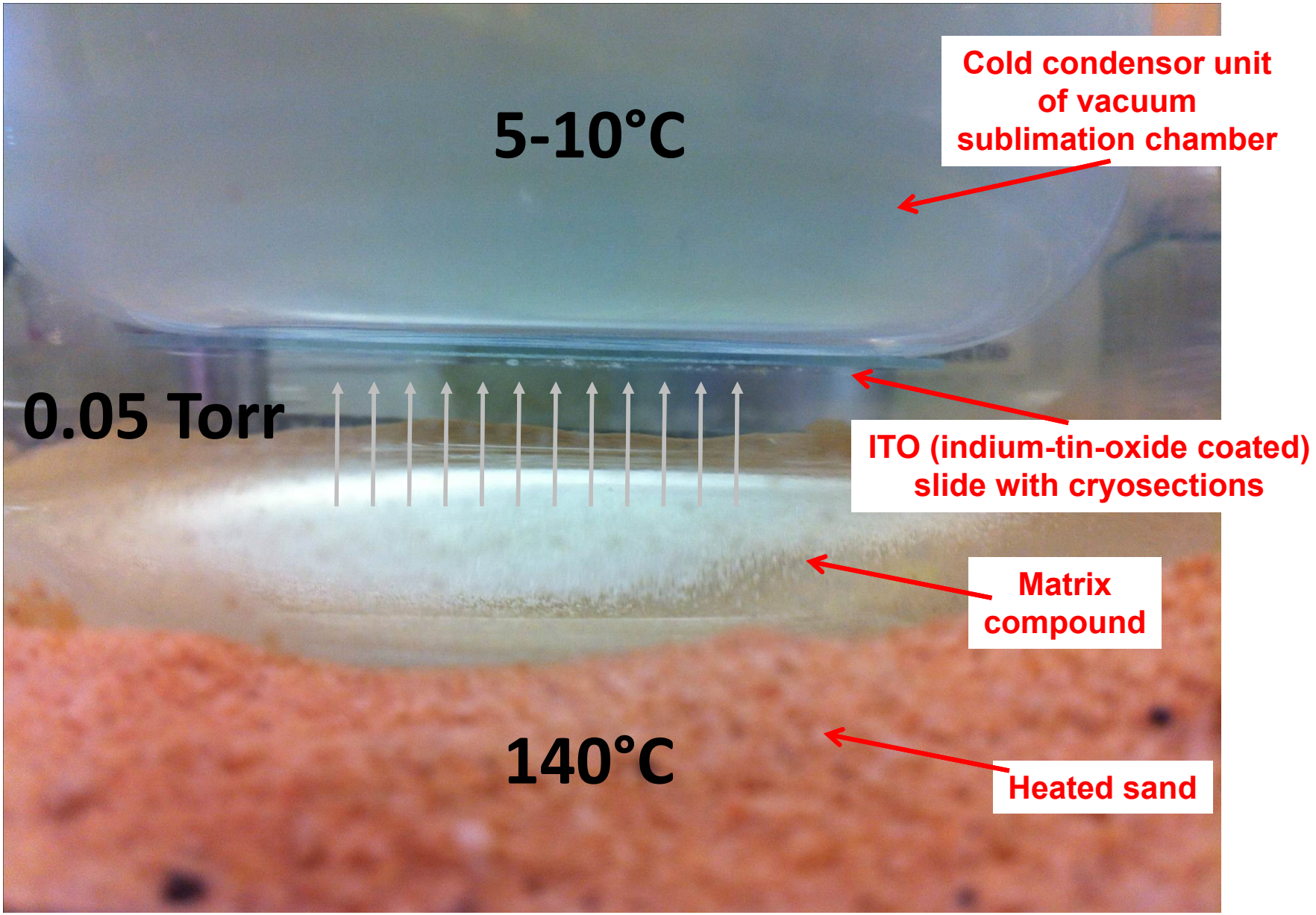


Vacuum at 0.05 Torr pressure is required in sublimation chamber and is monitored by electronic Pirani vacuum gauge.

750 Torr
(atmospheric pressure)

0.05 Torr

Matrix deposition by vacuum sublimation.



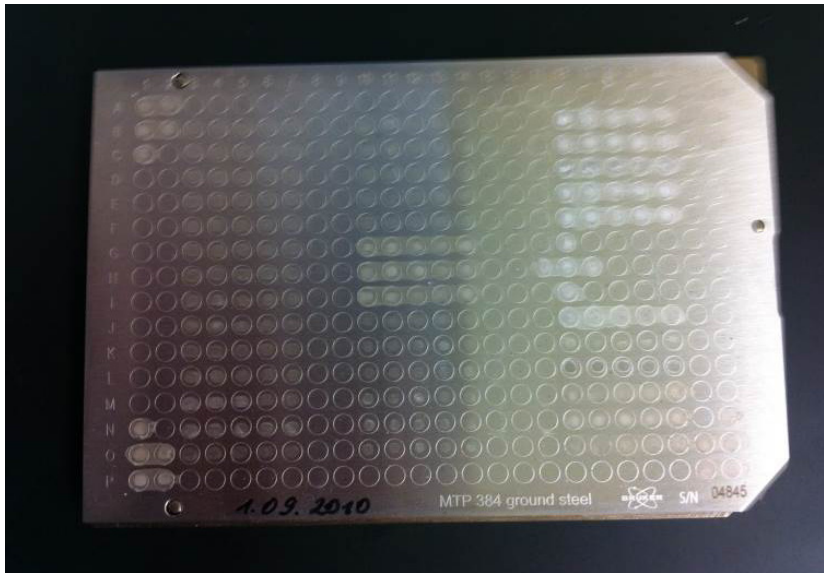
Slides with matrix applied by vacuum sublimation.

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.

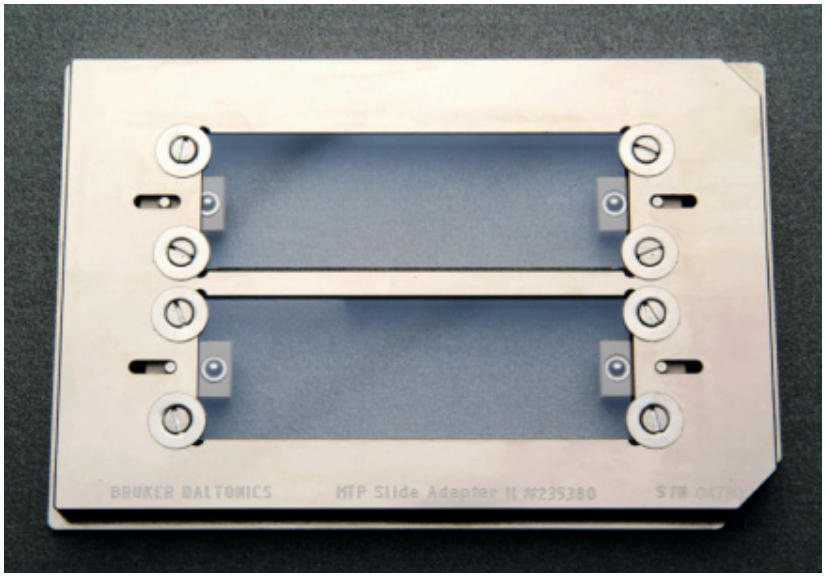
The uniformity of matrix deposition onto the slide attached to the cold condenser surface reflects the random Brownian motion of the released gaseous matrix molecules.

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

Conventional MALDI plate

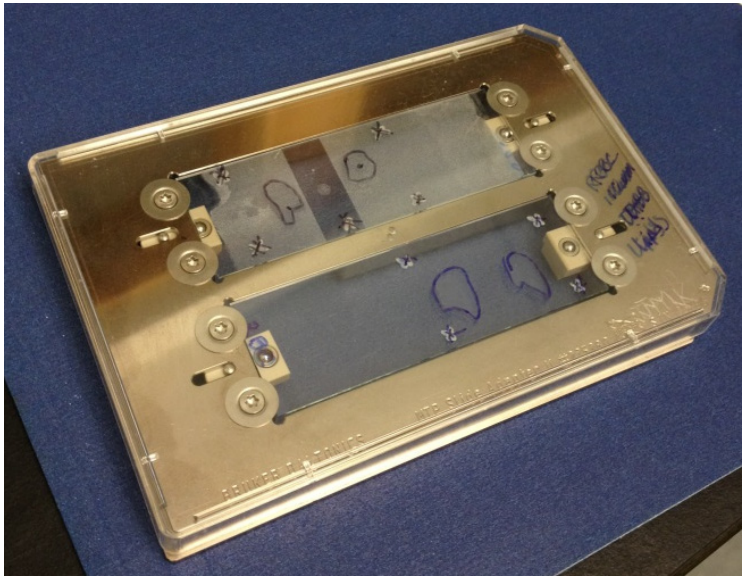


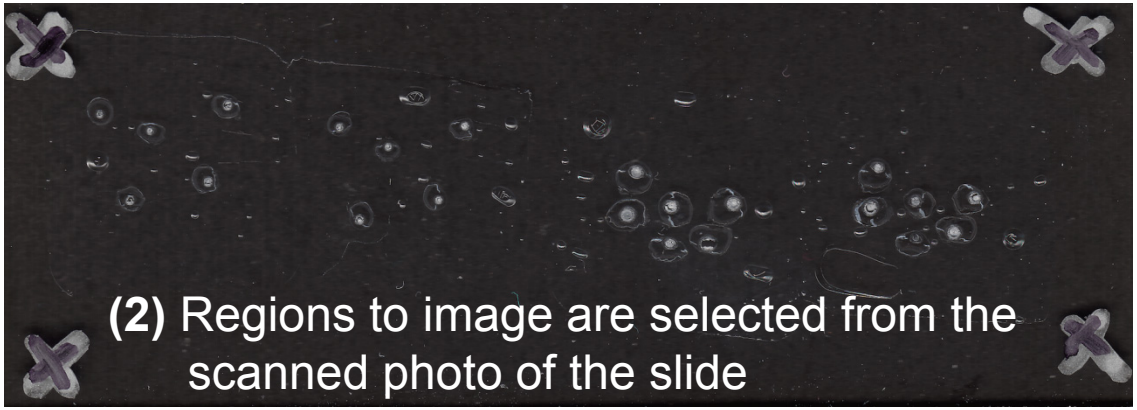
MALDI plate for cryosections



Setting up a MALDI-IMS run after matrix sublimation.

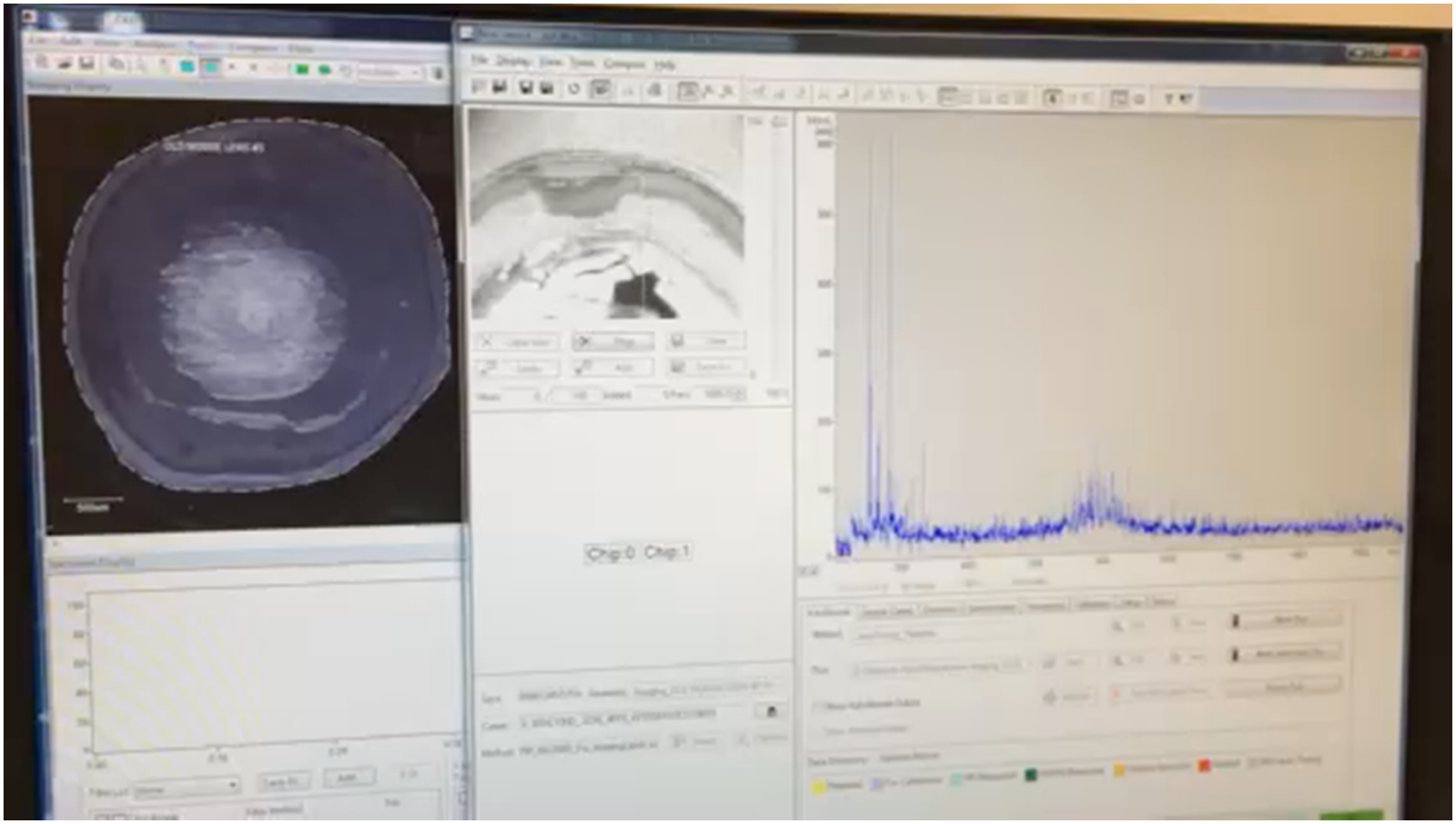
(1) The slide is placed into a Bruker slide adaptor and into the MALDI-TOF instrument



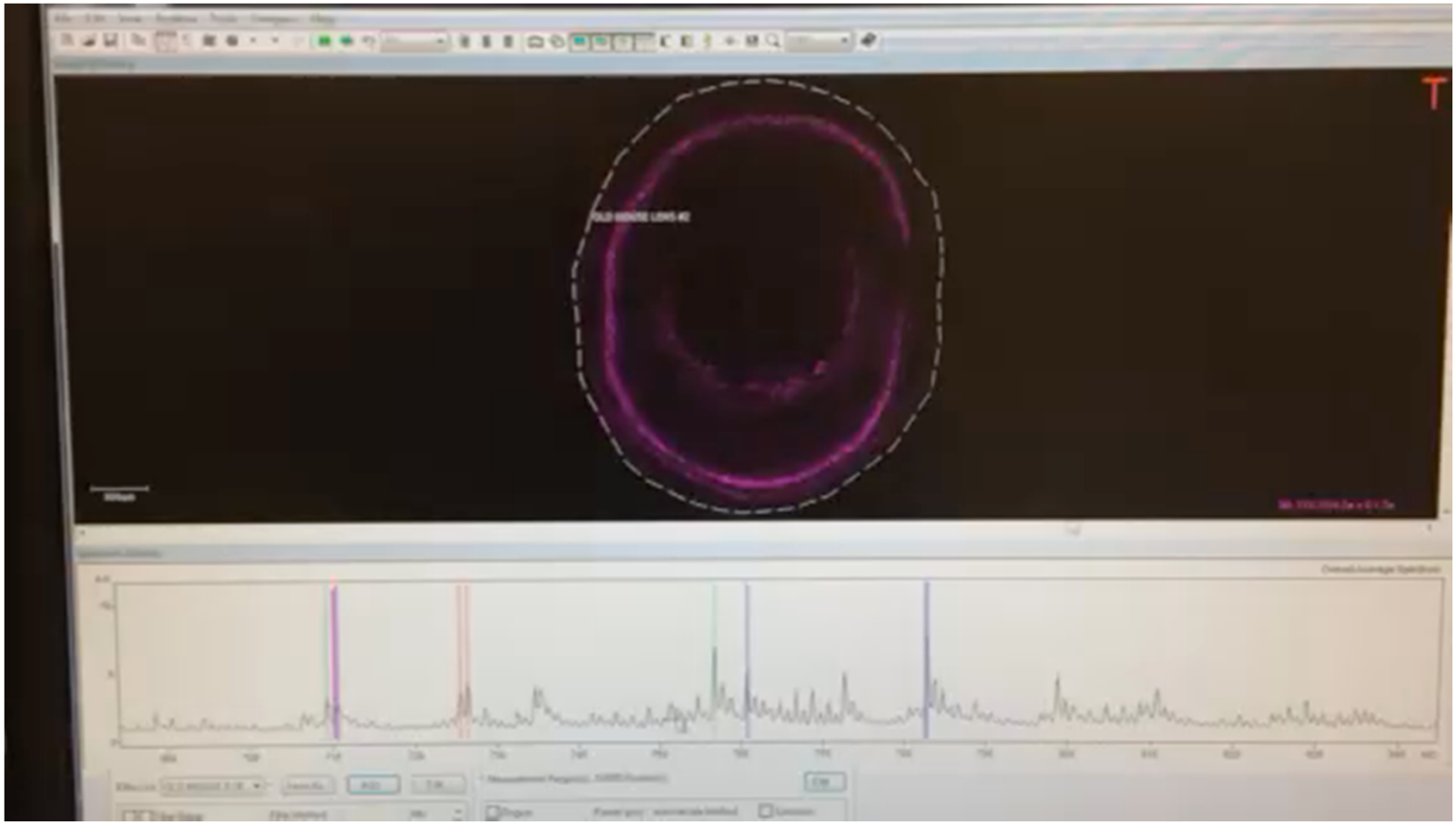


(2) Regions to image are selected from the scanned photo of the slide

MALDI-IMS in action.



After MALDI-IMS run is complete, cumulative spectra are uploaded for the entire tissue area imaged and individual lipids (peaks) can be selected to view their precise localization.



How are we applying MALDI-IMS to existing research projects?

Lipids as mediators of age-related changes in eye lens:
"Quantitative and Spatial Analysis of Lipids in the aging eye lens".

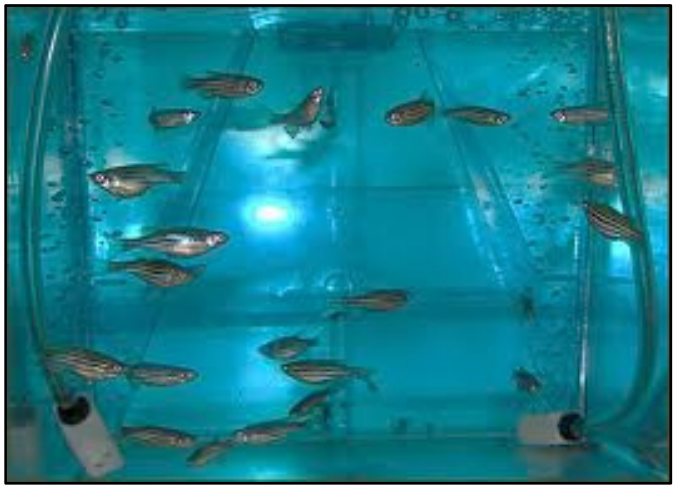
•Acute kidney injury (UAB/UCSD O'Brien Center):
"**Early lipid changes in ischemia reperfusion related acute kidney injury using SWATH lipidomics coupled with MALDI tissue imaging"**

•Lipid based mechanisms of immune suppression and anti-inflammatory action by HDL in Lupus:
"Modulation of HDL as a tool for the discovery of novel immune suppressive and anti-inflammatory lipids in Lupus"

Quantitative and Spatial Analysis of Lipids in the aging 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

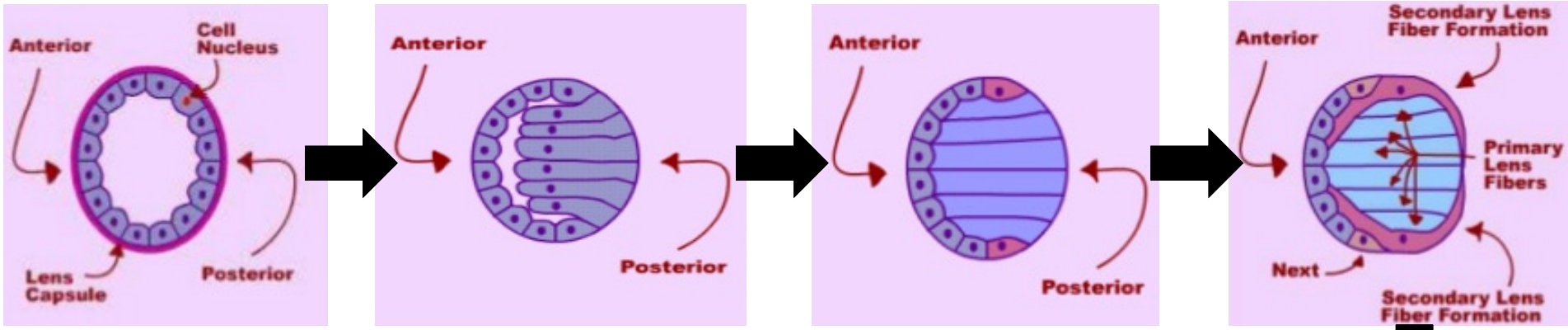


- $0.75/cage/day (max 4-5 mice per cage)
- 6-8 pups monthly per sexually mature female
- Functional vision by 2 weeks of age

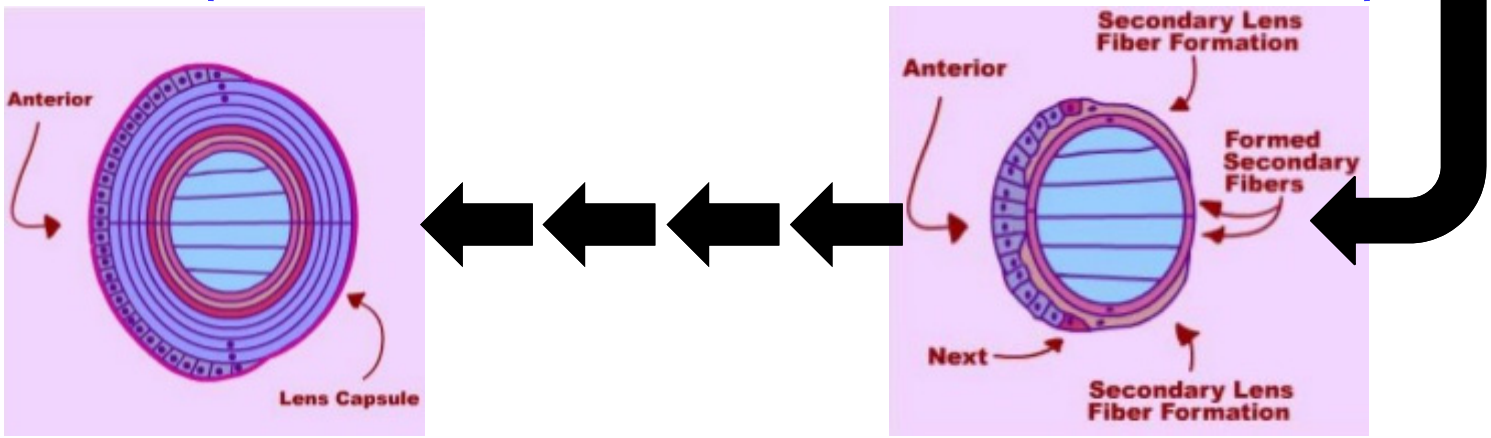
Stephen Watts, Ph.D., Department of Biology, Director UAB Aquatic Animal Research Core for NORC.

Elongation of posterior cells into cavity forming 1° fiber cells.

1° fiber cells lose nuclei/organelles, anterior cells divide, cells along equatorial edges forming 2° fiber cells (red).



2° fiber cells elongate along posterior surface of 1° lens fiber, forming rings around them. Cortex of 2° fibers increase in size, original 1° fibers persist to form lens nucleus. Only outermost 2° fibers contain nuclei/organelles. New 2° fibers continue to form throughout life.



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.

1 month → 6 x 12 lenses
12 months → 6 x 5 lenses

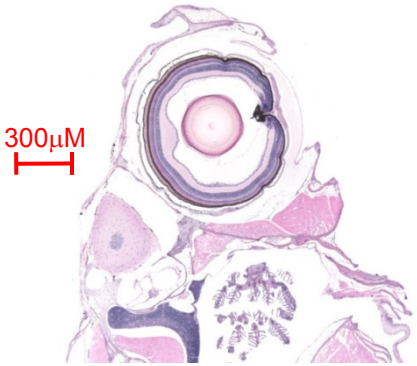
1 month → 6 x 3 lenses
12 months → 6 x 1 lens

Normalized weight

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

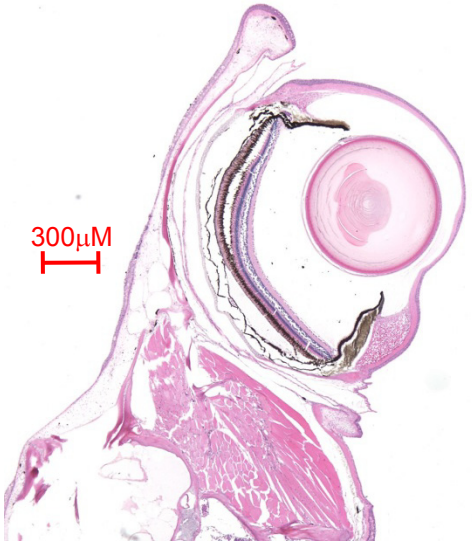
Stephen Barnes, Ph.D., Department of Pharmacology & Toxicology, Director UAB TMPL.

1 month-old Zebrafish eye

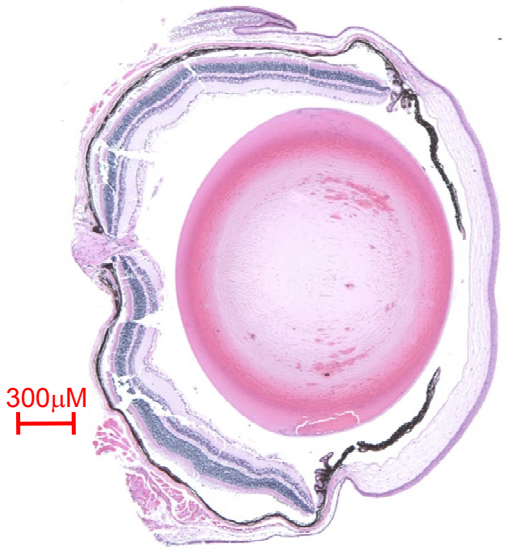


AGING

12 month-old Zebrafish eye

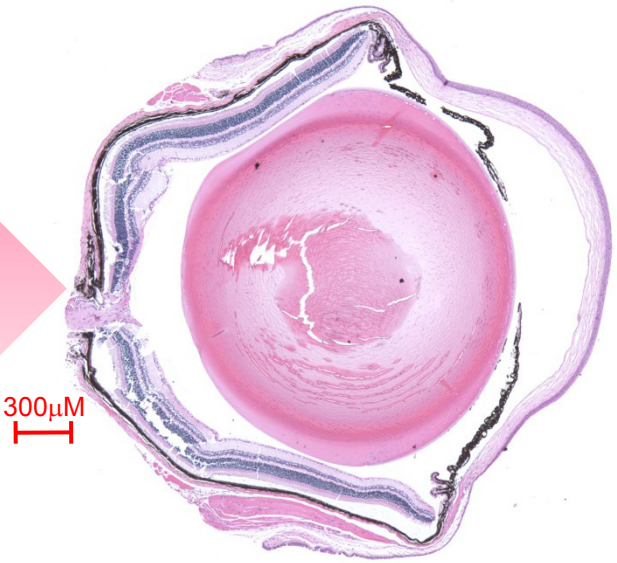


1 month-old Mouse eye

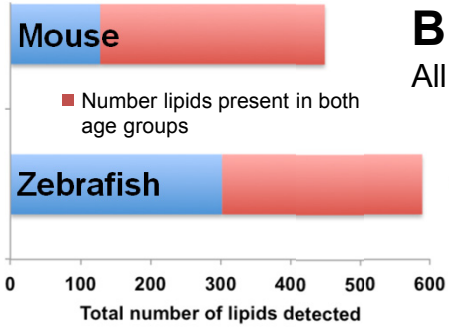


AGING

12 month-old Mouse eye

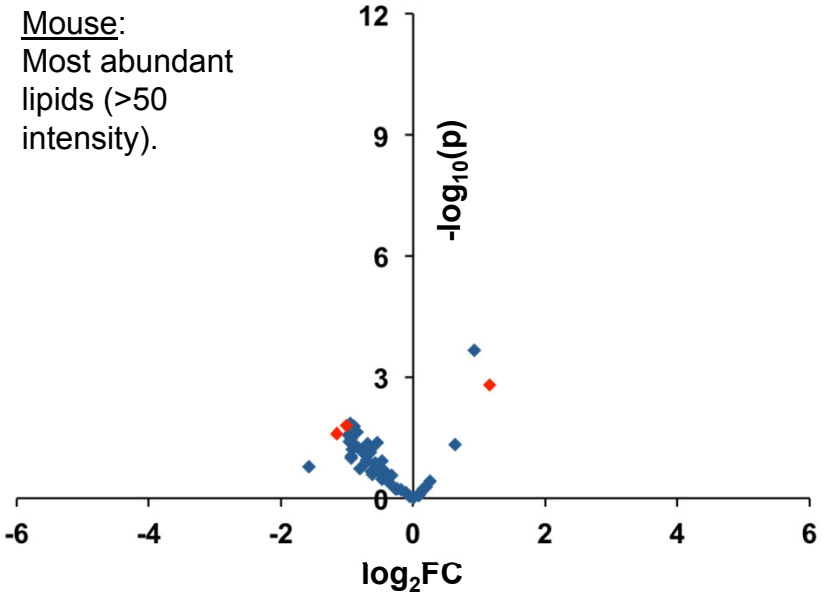


A Mouse:
All lipids.

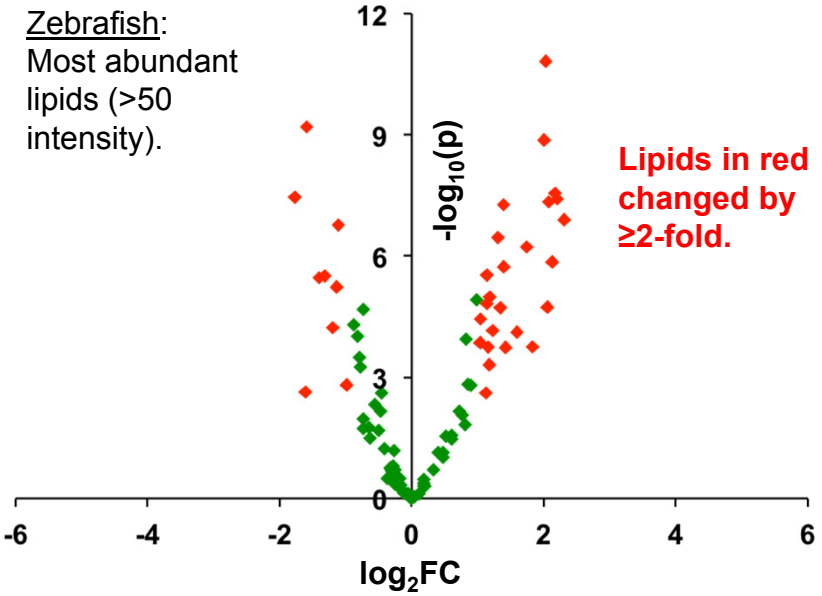


B Zebrafish:
All lipids.

Mouse:
Most abundant lipids (>50 intensity).



Zebrafish:
Most abundant lipids (>50 intensity).



Lipids in red changed by ≥2-fold.

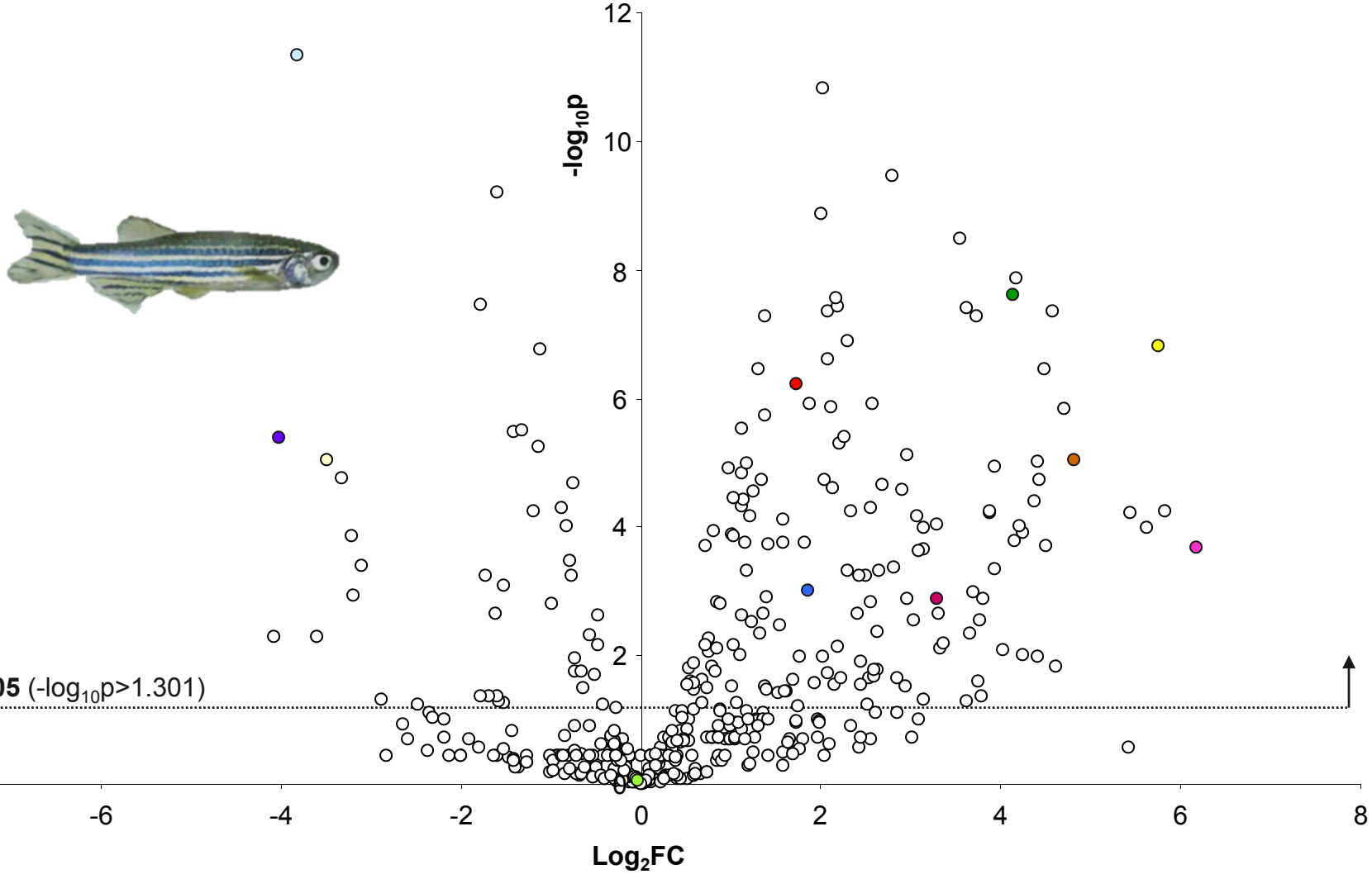
Mouse lens positive mode (Total lipids).

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).

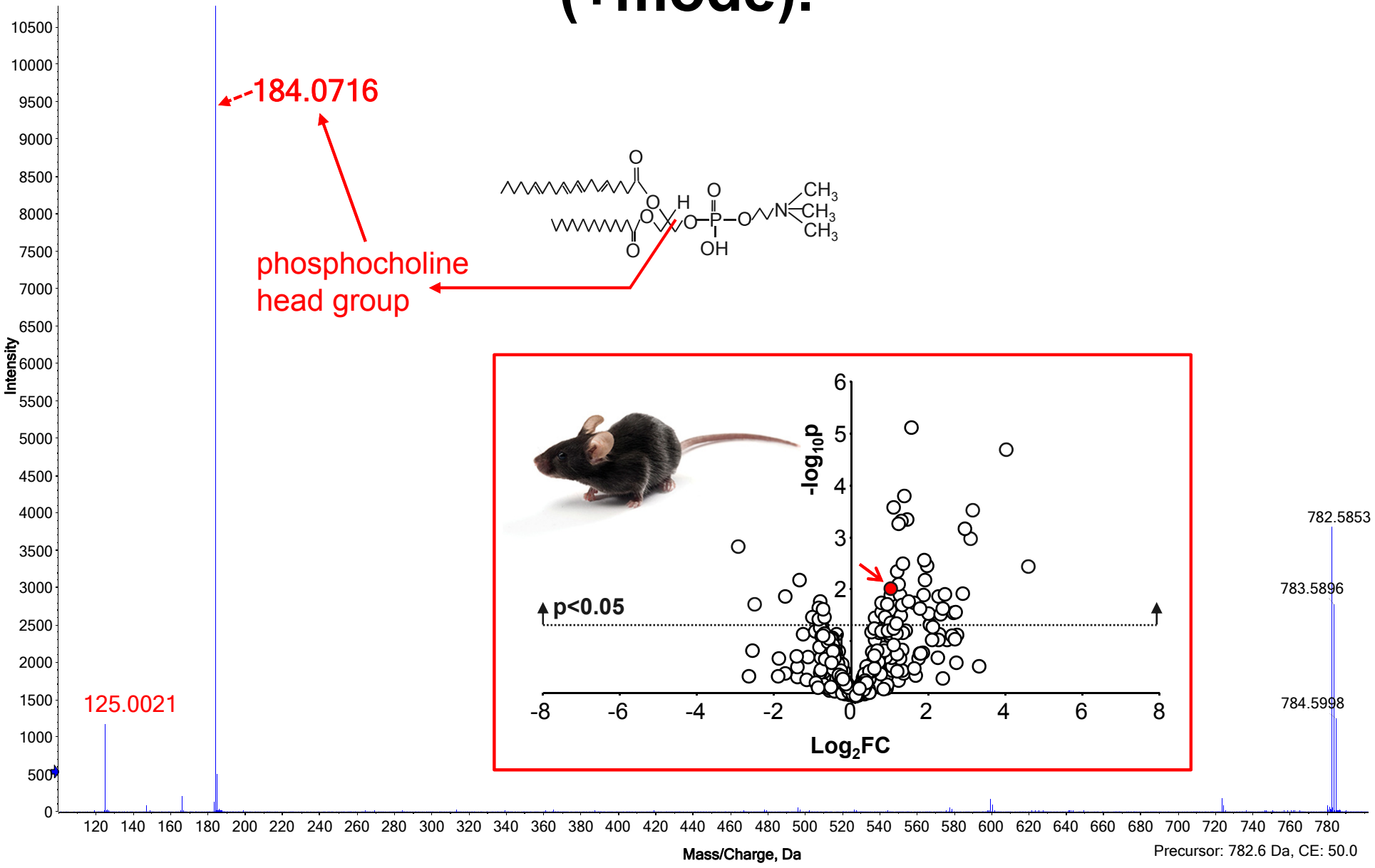


PG O-44:2 (-PG) | SM 38:4;2 (SM) | LPE 12:3 (LPE_p) | LPC 16:0 | PC 36:4 (16:0/20:4) | PC O-32:1 (PC) [alkyl link]

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)

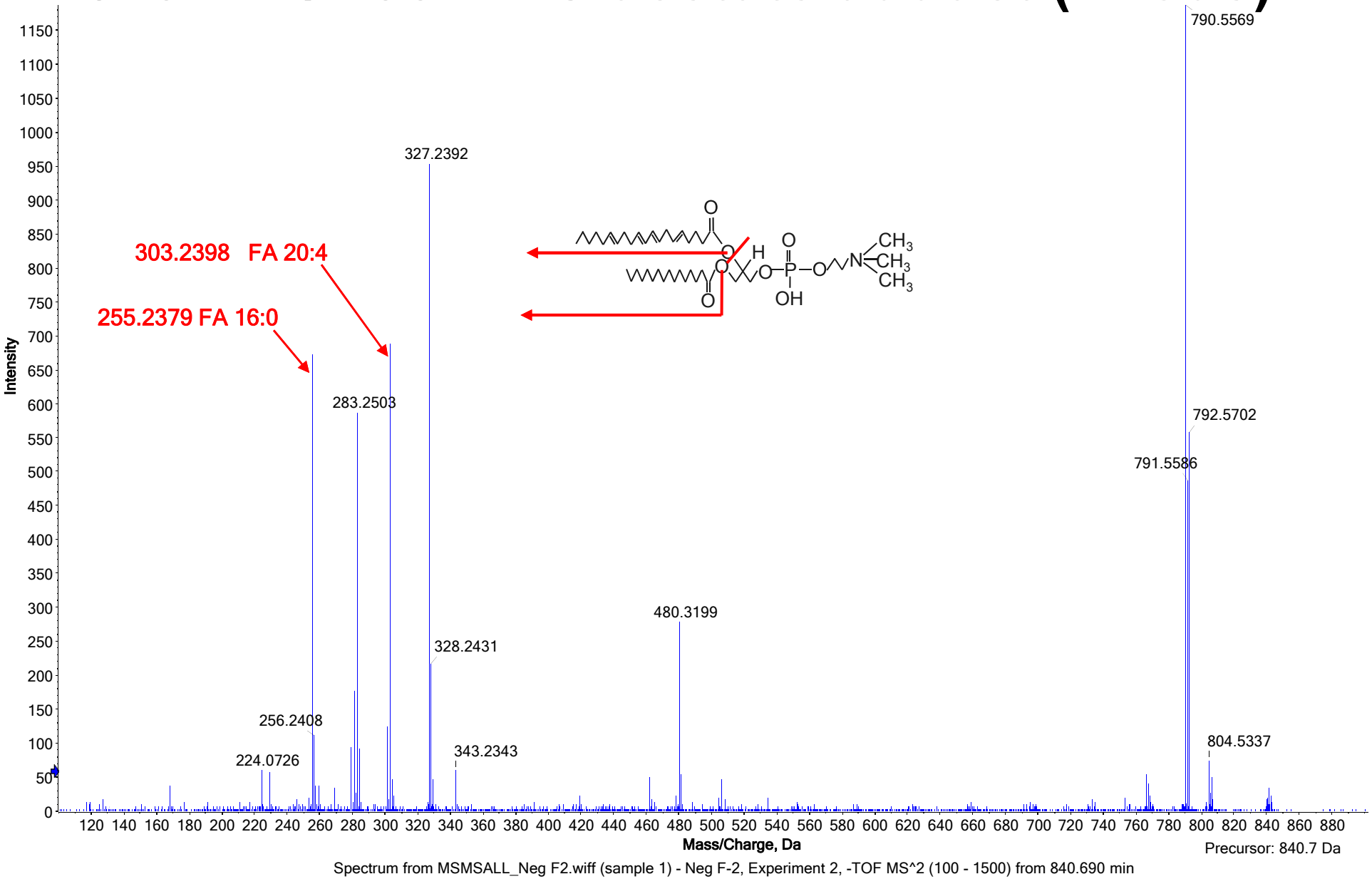
MS/MS fragments from782.6 m/z 36:4 PC (+mode).



Precursor: 782.6 Da, CE: 50.0

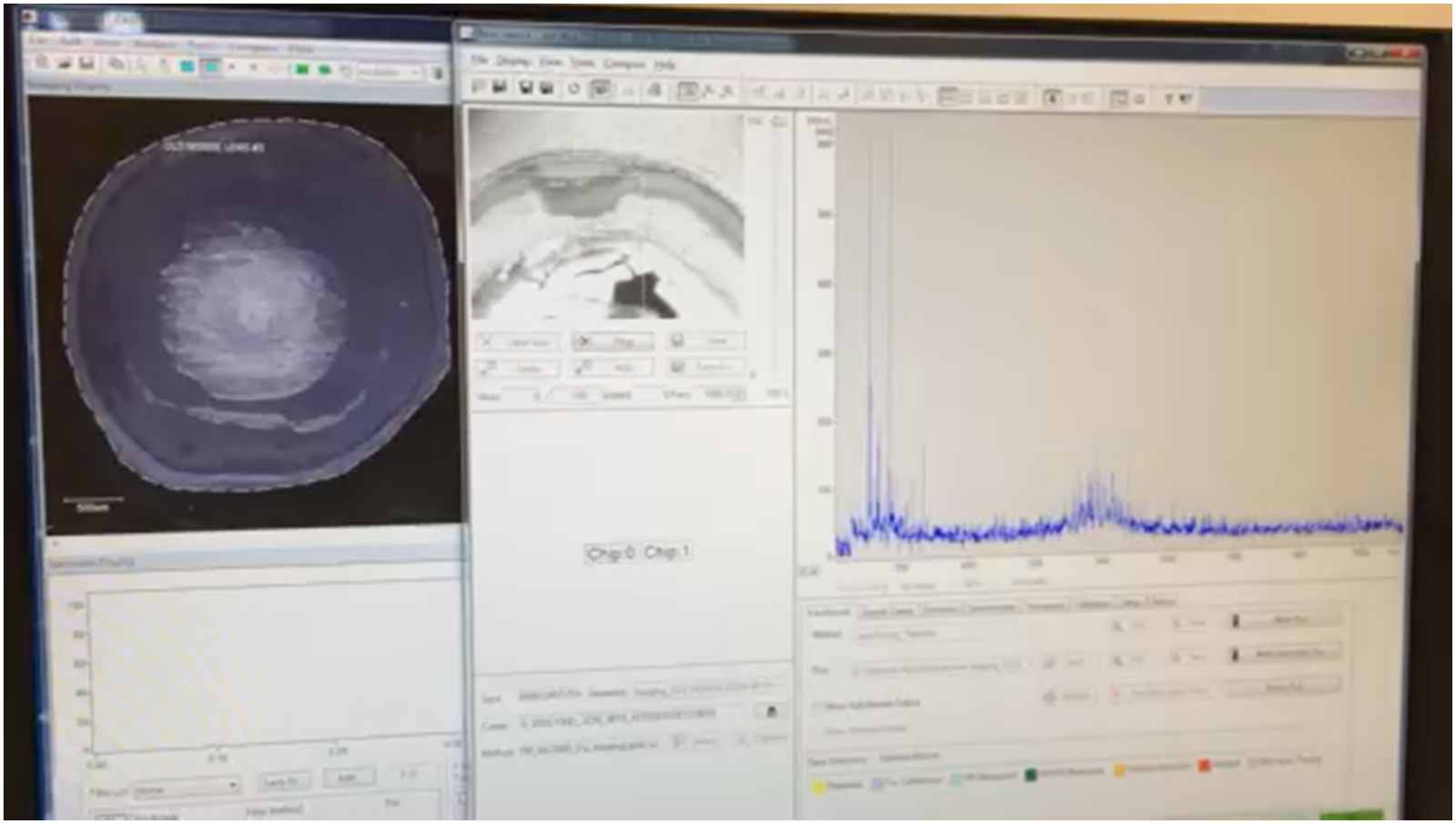
Spectrum from MSMSALL_Pos F2.wiff (sample 1) - Pos F-2, Experiment 2, +TOF MS^2 (100 - 1500) from 782.632 min

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

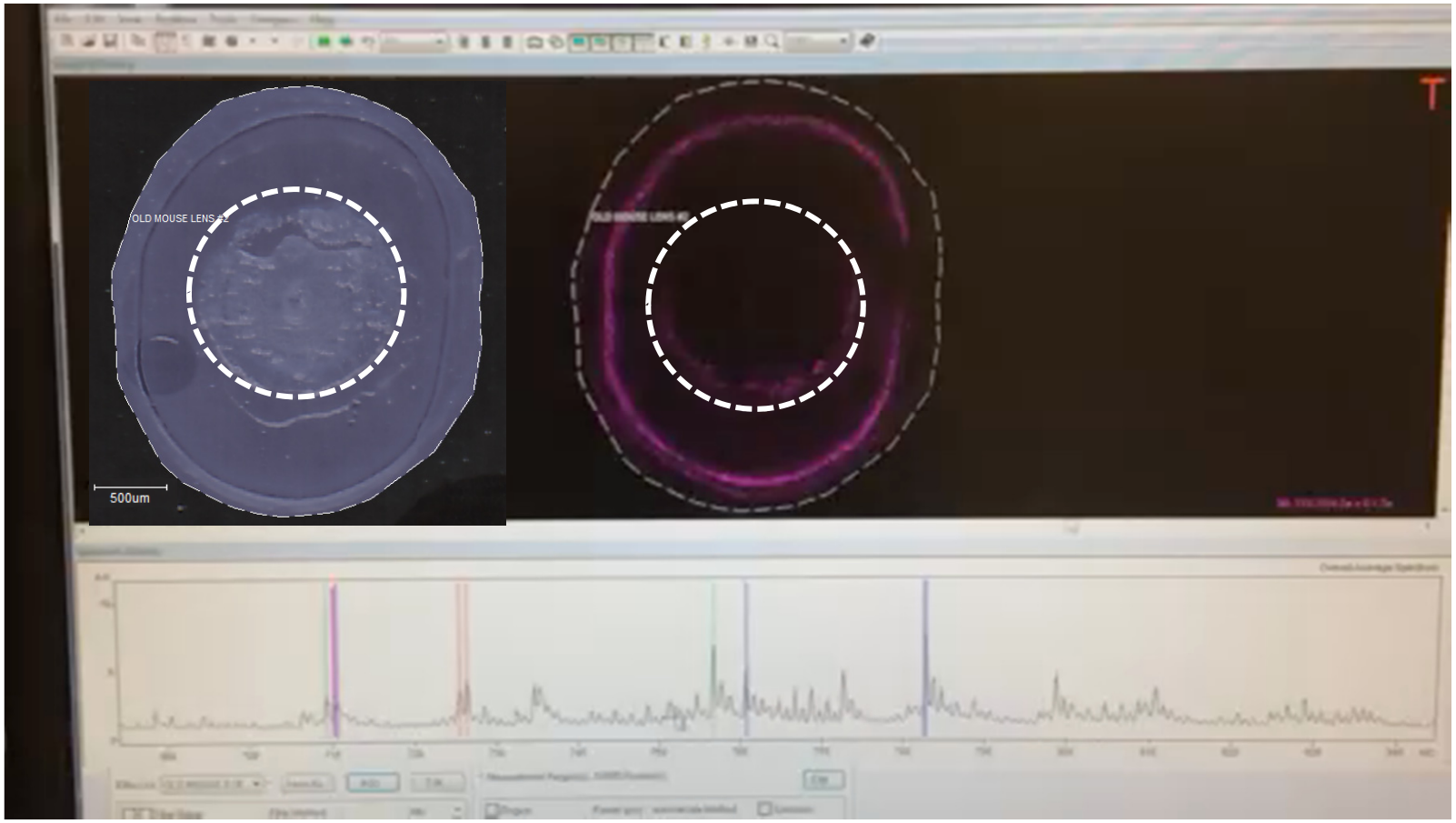


Spectrum from MSMSALL_Neg F2.wiff (sample 1) - Neg F-2, Experiment 2, -TOF MS^2 (100 - 1500) from 840.690 min

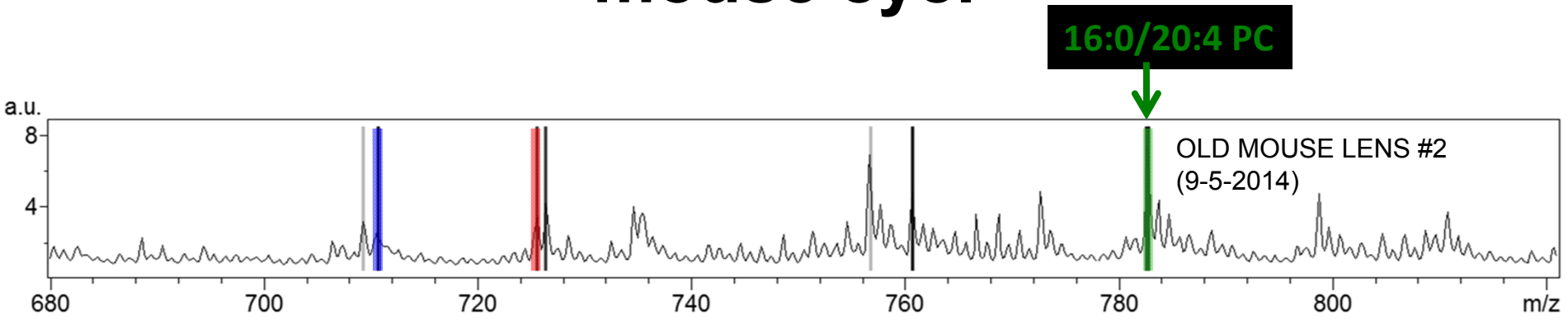
Where in the lens are the changes in 16:0/20:4 PC occurring?

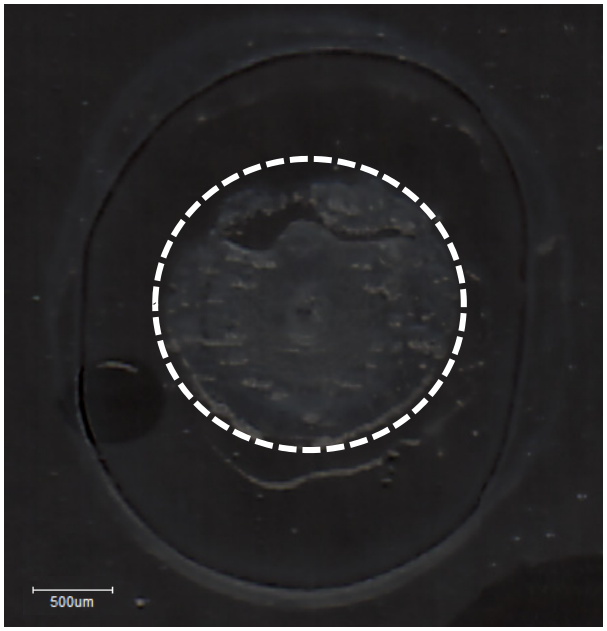


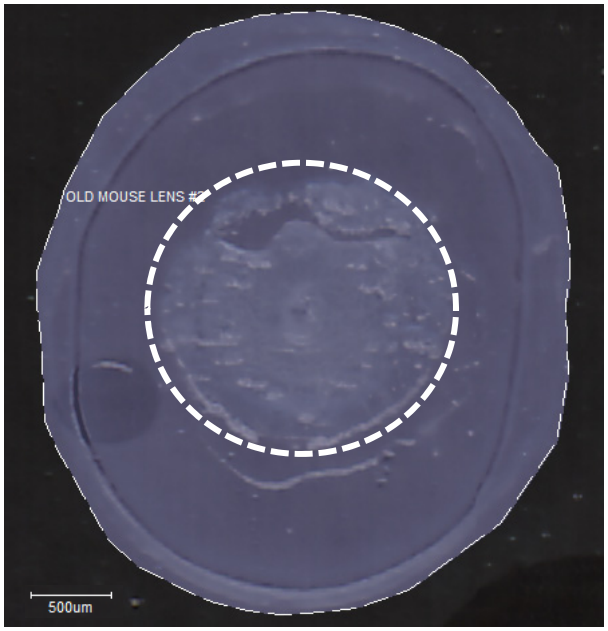
Spatial information on lens lipids provided by MALDI-Imaging MS.

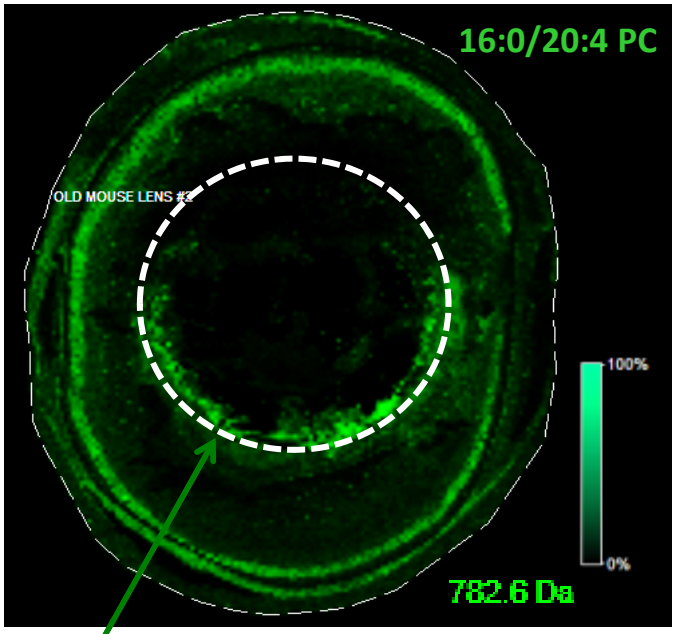


MALDI-IMS on 16:0/20:4 PC(36:4) in1 year old mouse eye.



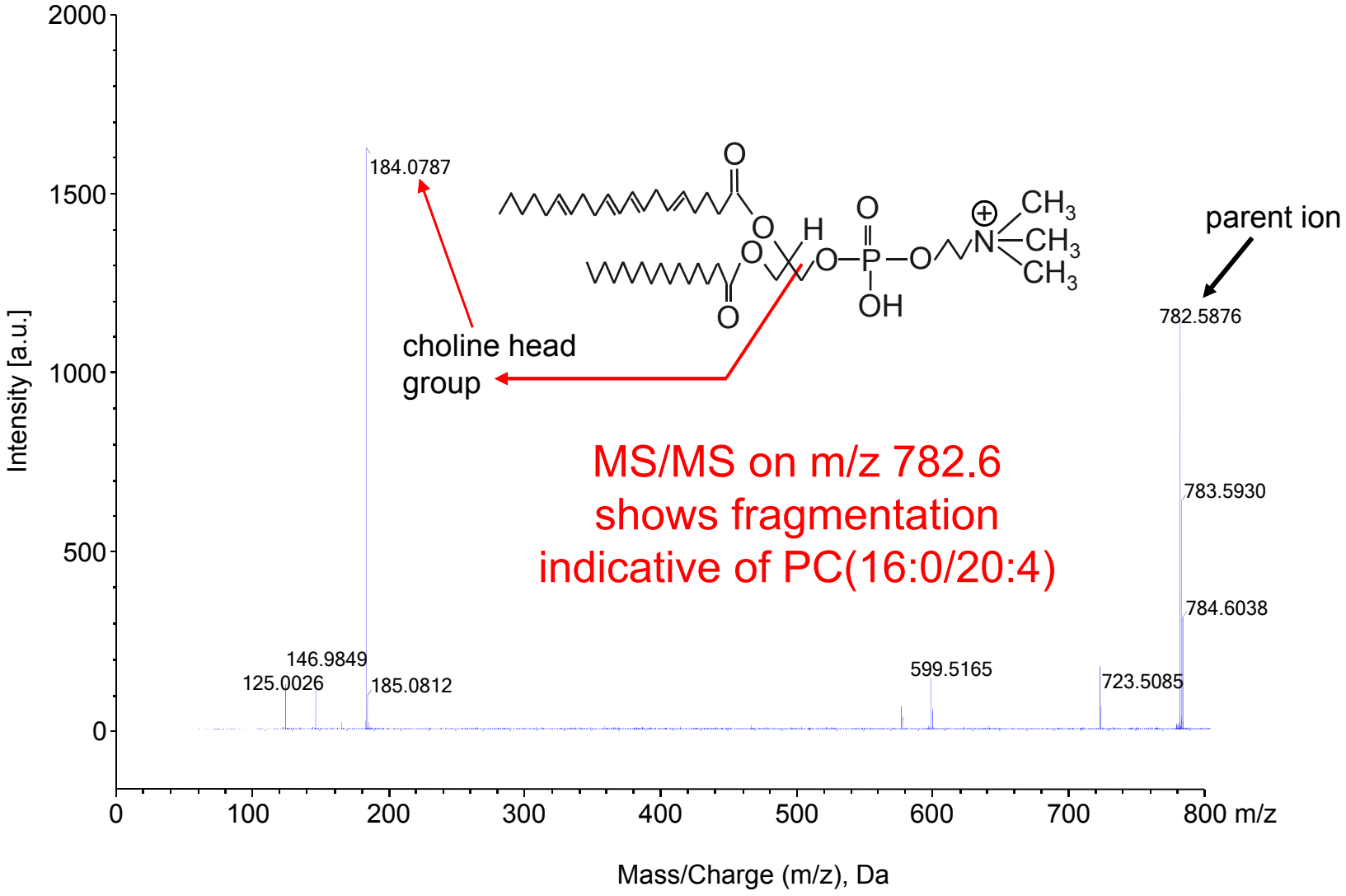




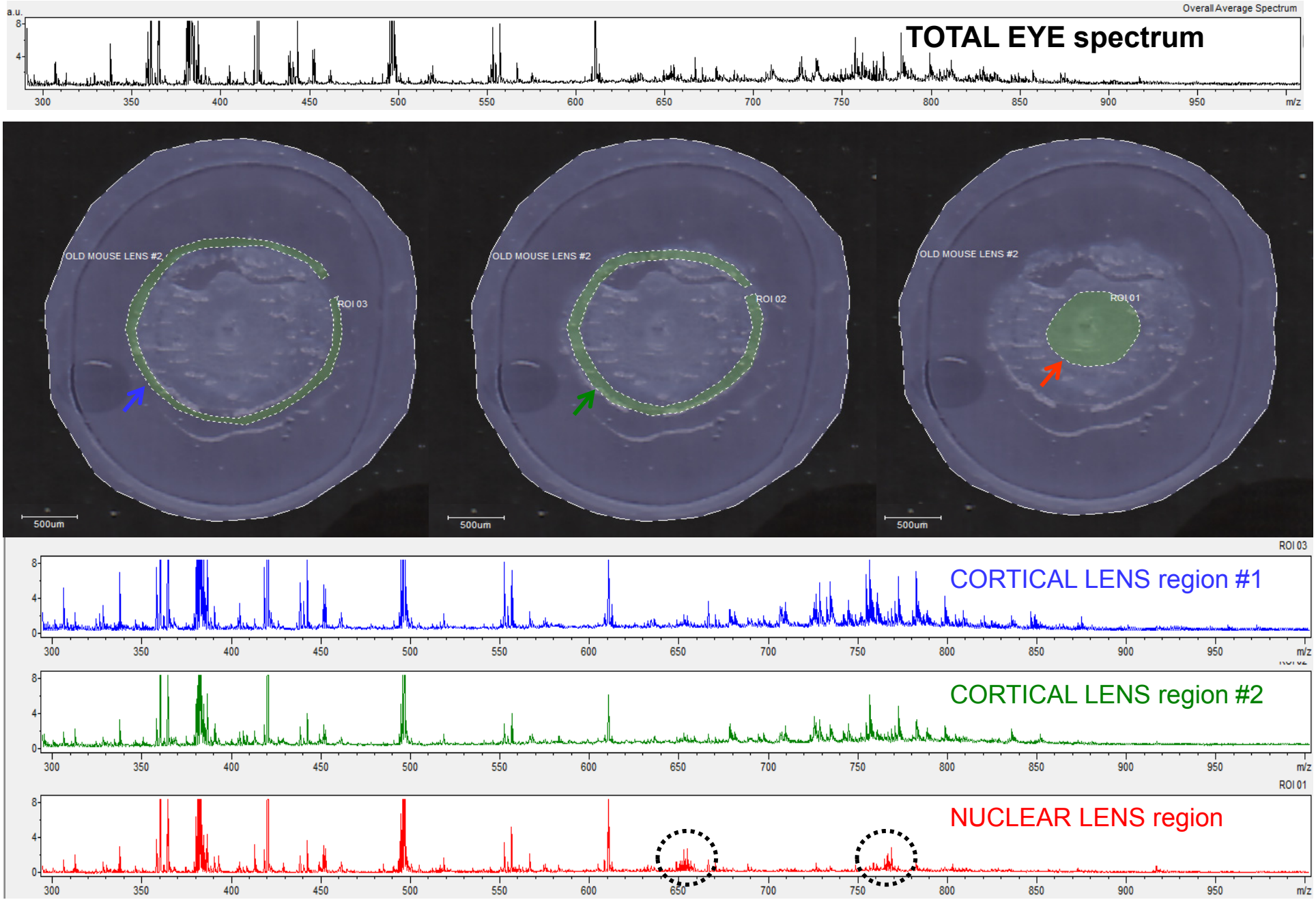


Lens 16:0/20:4 PC is localized exclusively to cortical region of the lens where metabolically active epithelial cells are present.

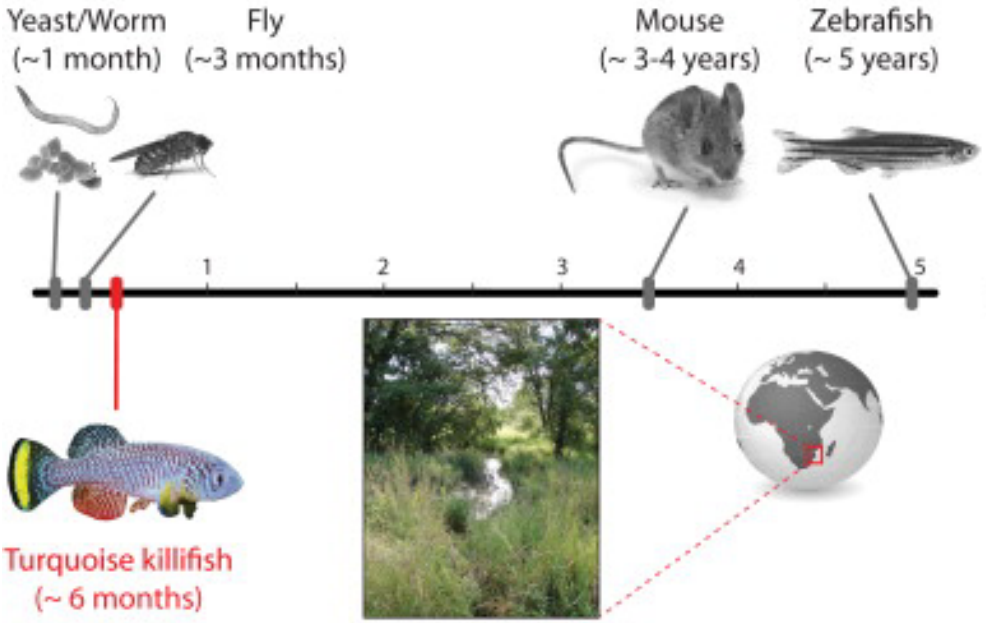
MS/MS of protonated PC(36:4) in eye using MALDI Imaging MS platform.

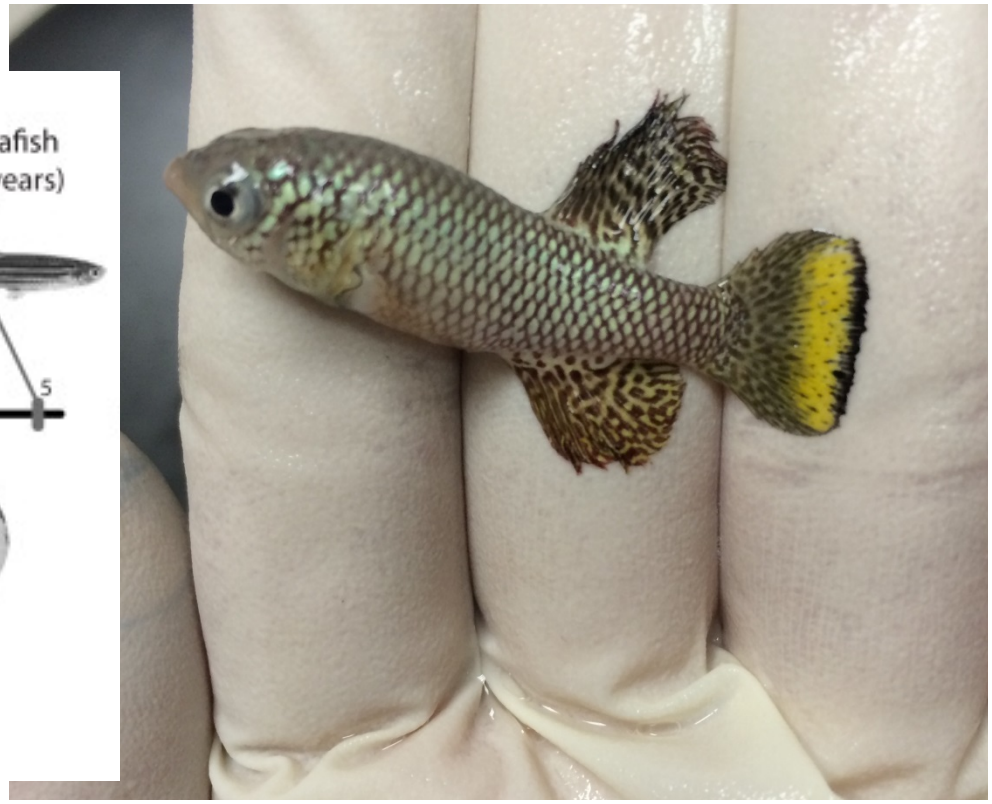


Reduced diacyl phospholipids in lens nucleus.



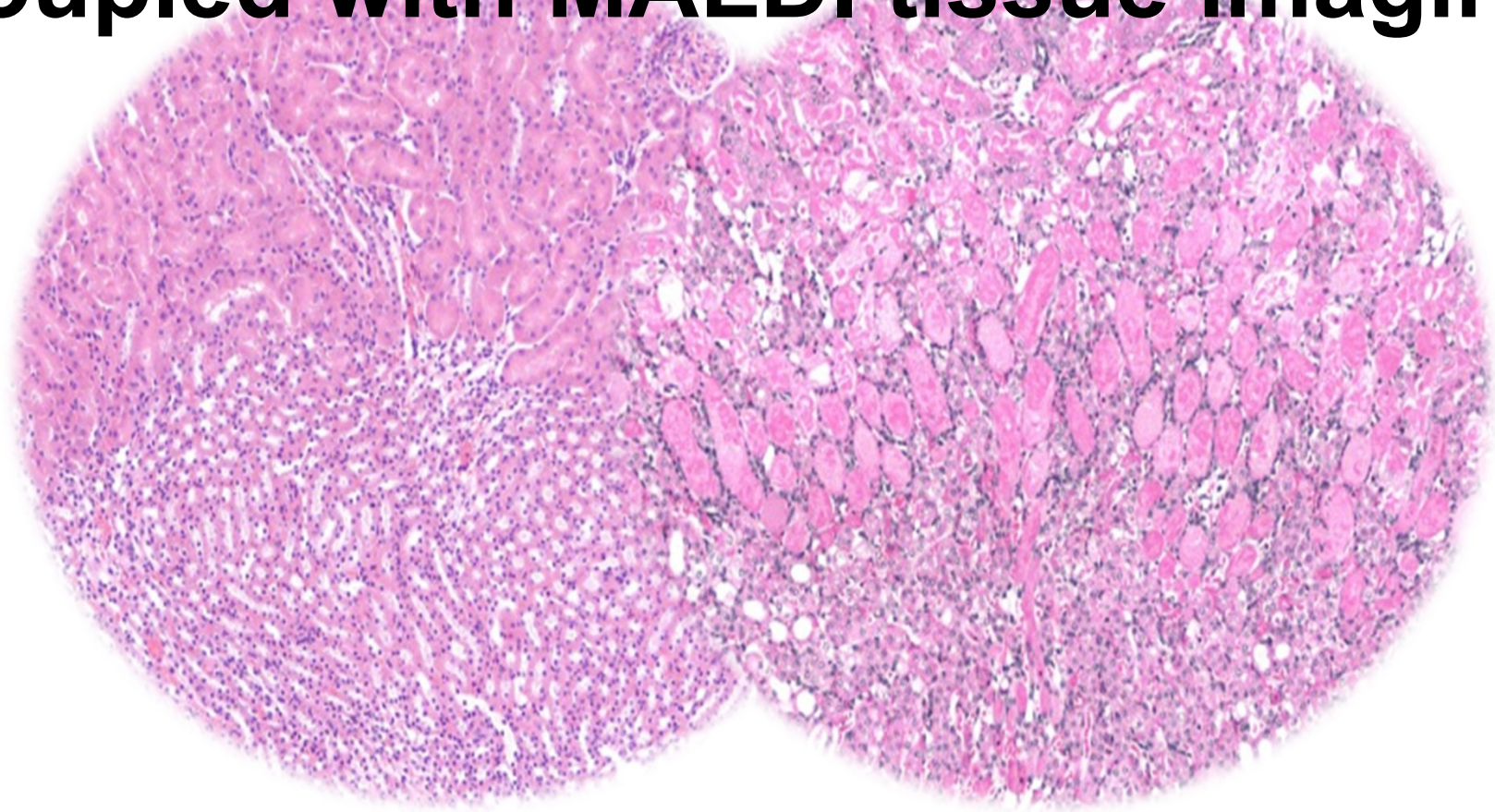
Nothobranchius fuzeri: A naturally short-lived (rapidly aging) fish model for aging research.





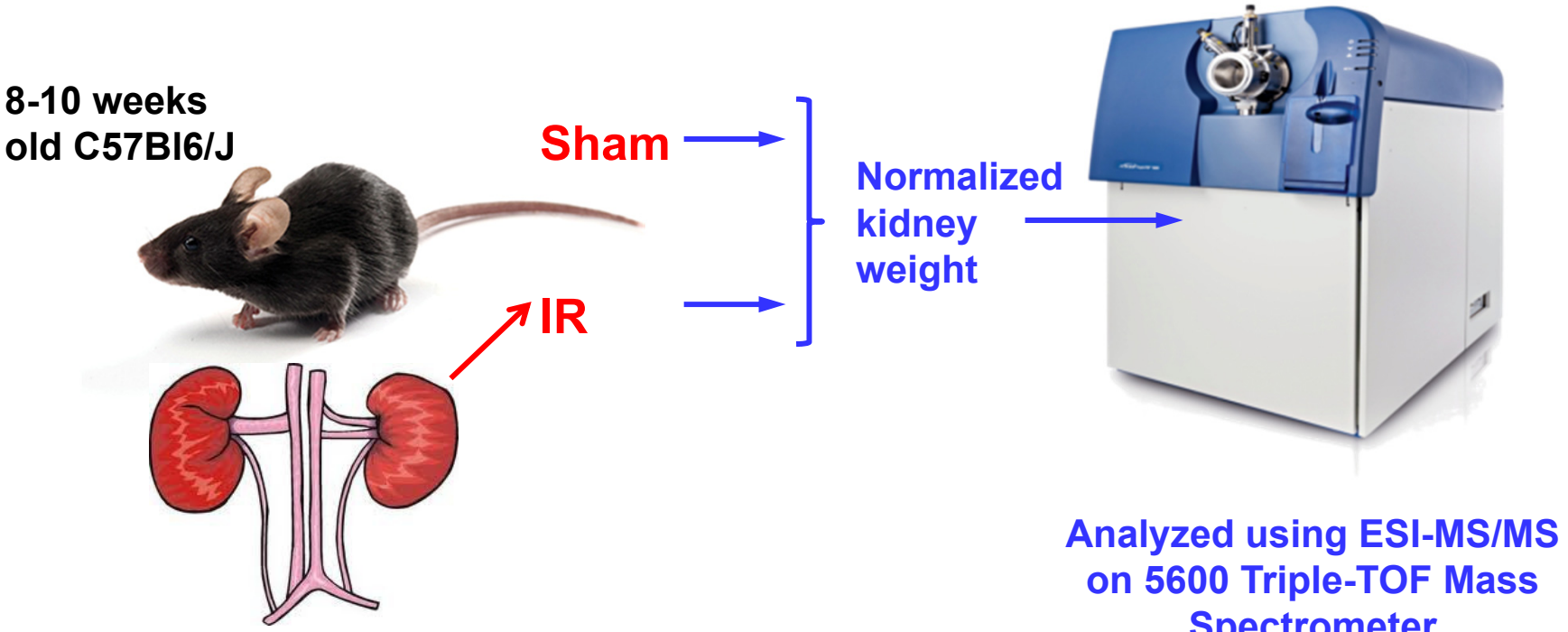
Itamar Harel , Bérénice A. Benayoun , Ben Machado , Param Priya Singh , Chi-Kuo Hu , Matthew F. Pech , Dario Ricca, *et al*. **A Platform for Rapid Exploration of Aging and Diseases in a Naturally Short-Lived Vertebrate.** Cell, Volume 160, Issue 5, 1013 – 1026, 2015.

Early lipid changes in ischemia reperfusion related acute kidney injury using SWATH lipidomics coupled with MALDI tissue imaging.

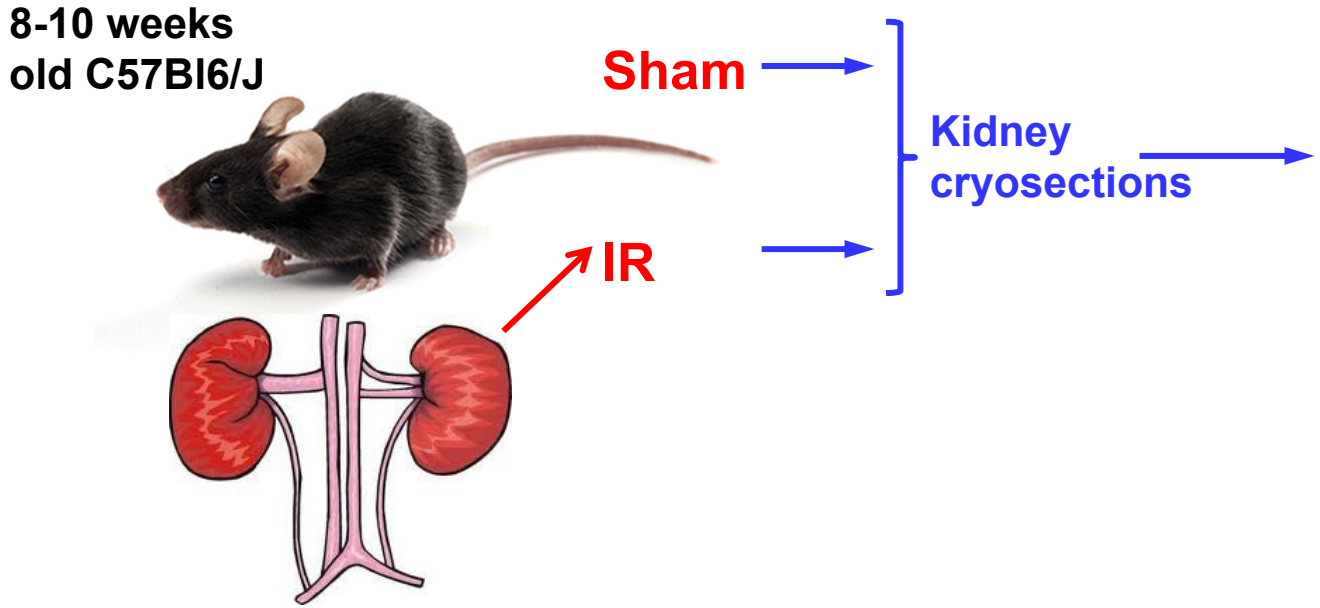


Specific aims of the study:

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

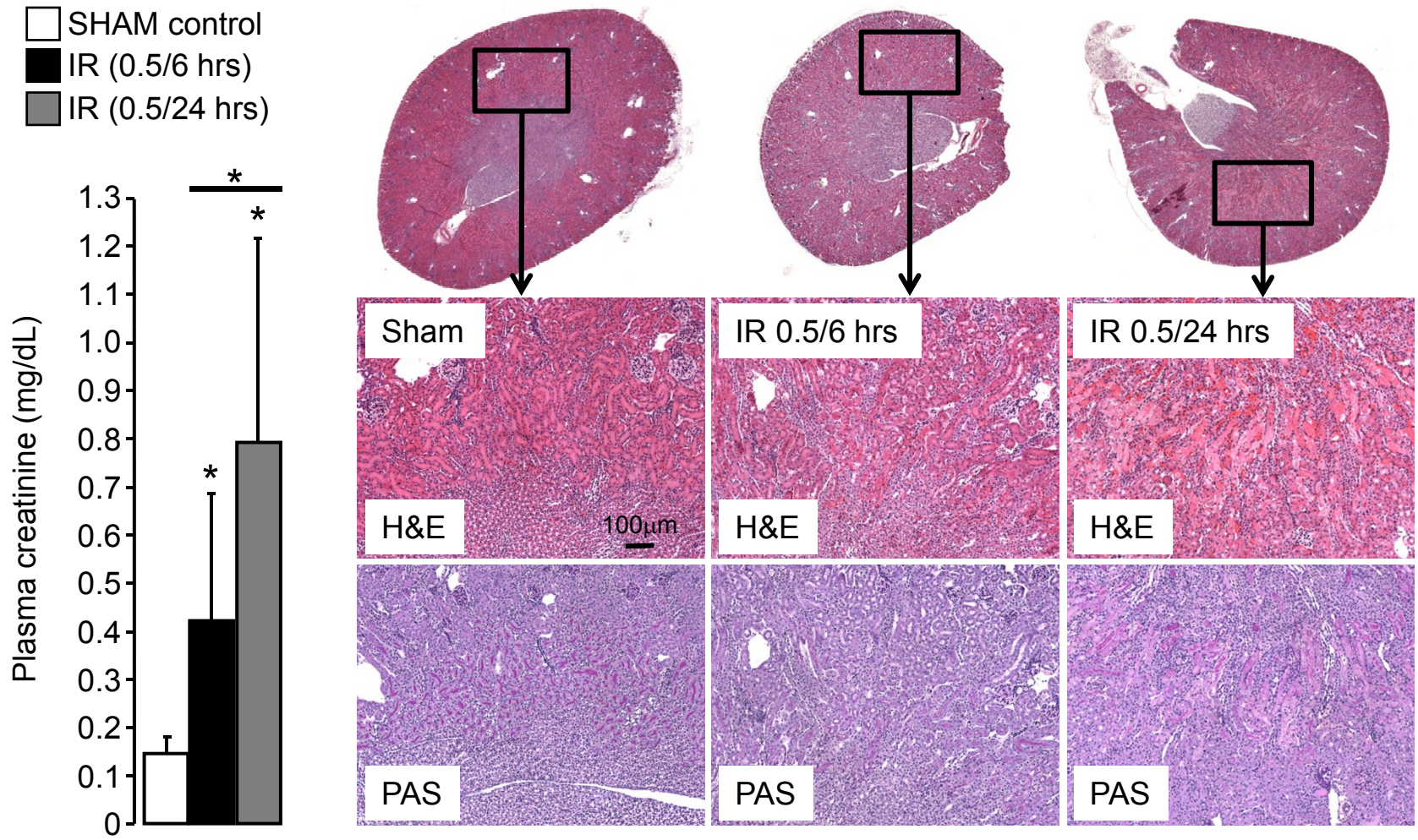


2) **To analyze where the changes in lipids occur in the kidney and to characterize those lipids that remain unchanged quantitatively but might be spatially altered.**

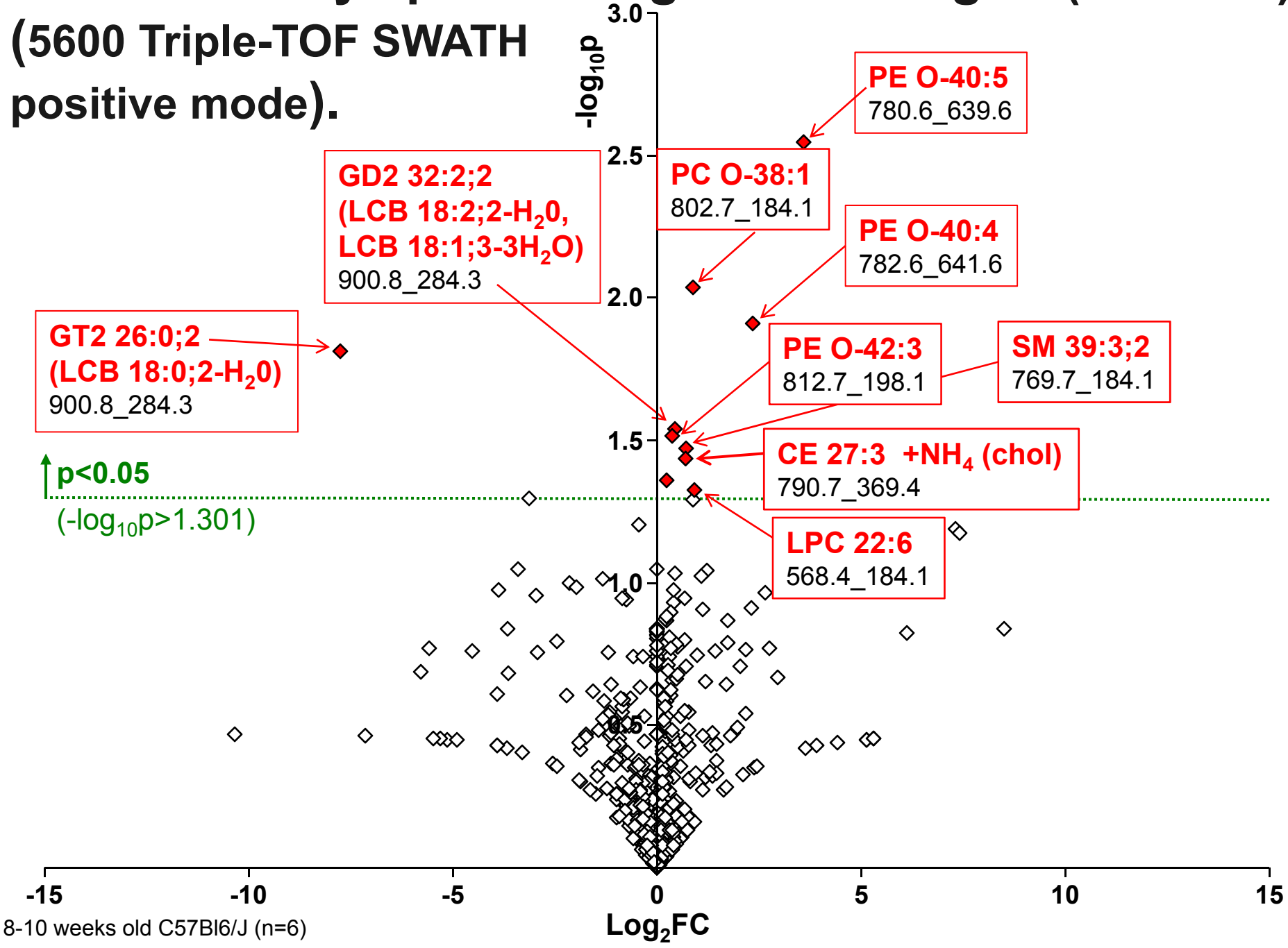


MALDI-Imaging MS on a Bruker-TOF Mass Spectrometer

Plasma creatinine and kidney histology in mice subjected to ischemia/reperfusion (IR) related kidney injury at early and late time-points.

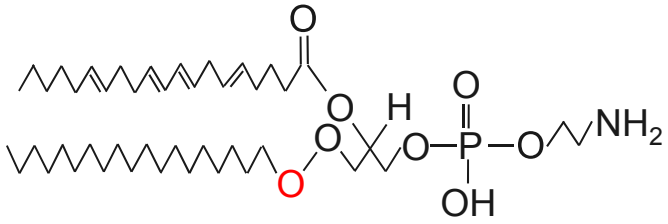


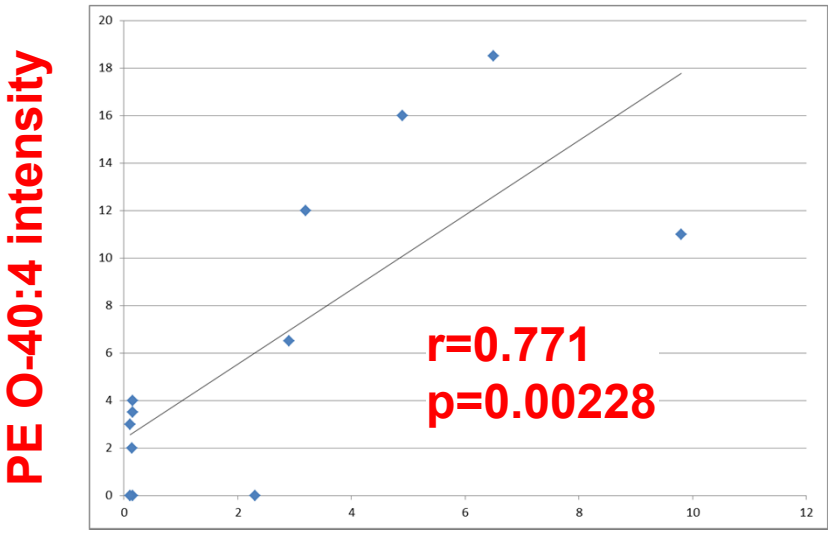
Mouse kidney lipids changed following IR (0.5/6hrs) (5600 Triple-TOF SWATH positive mode).

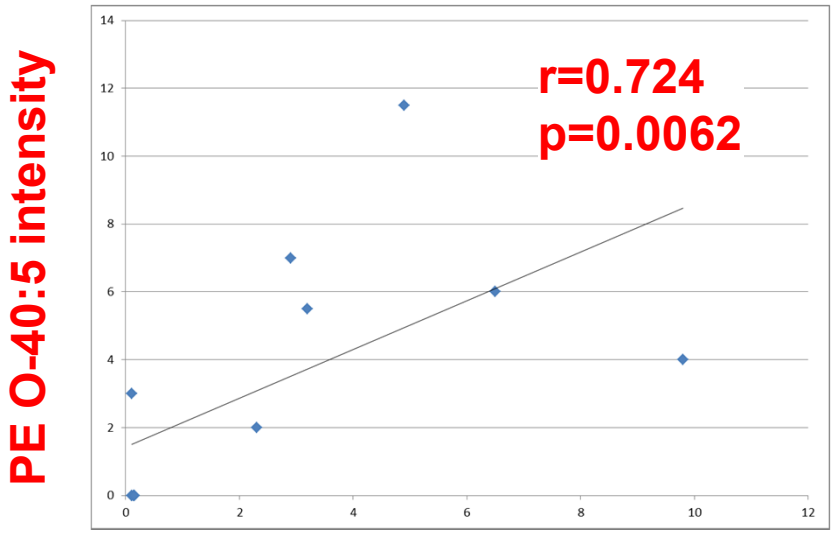


Increases in renal ether-PE correlate with severity of AKI (as measured by plasma creatinine level.

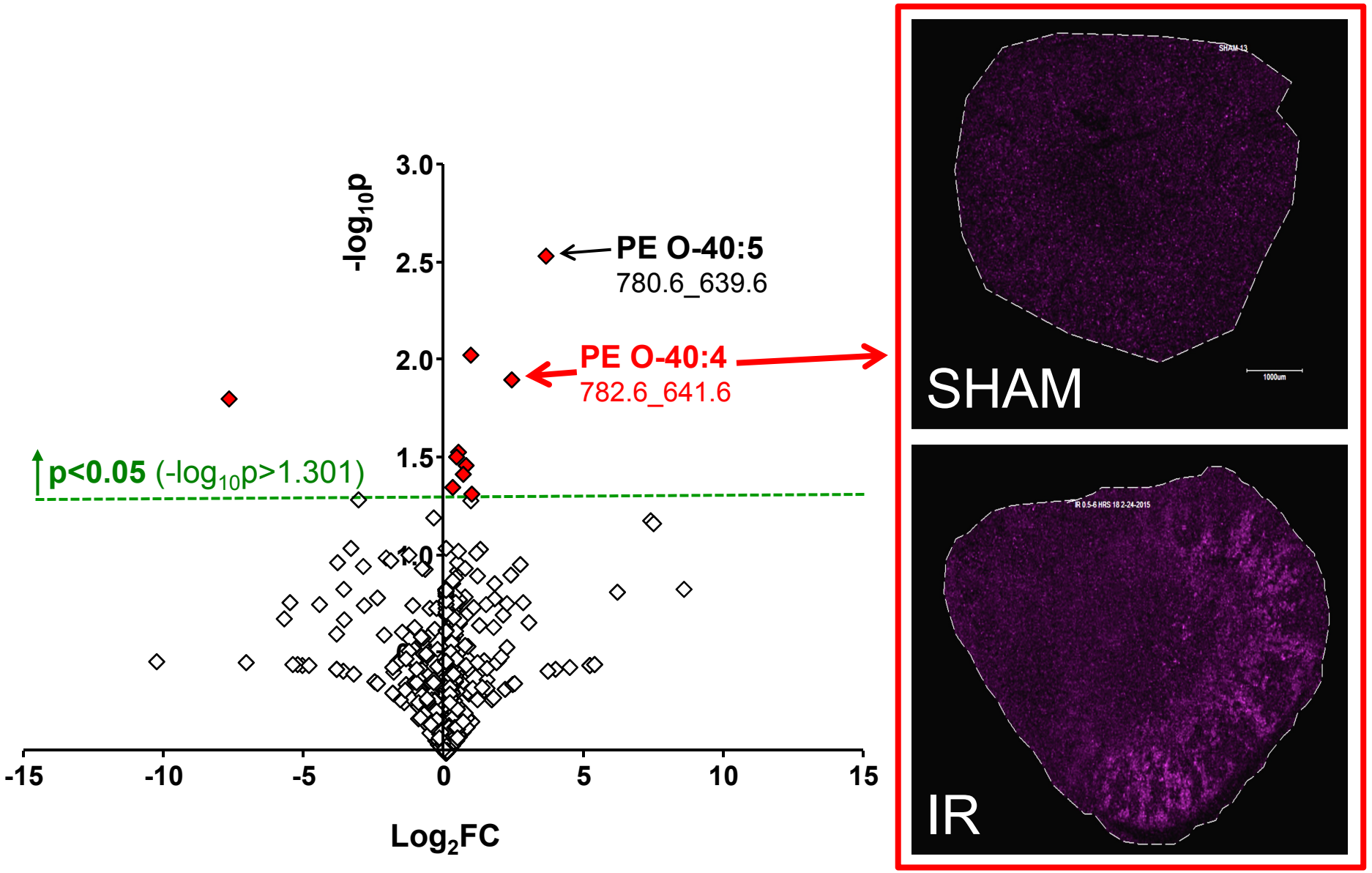
1-ether, 2-acyl PE







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



Janusz Kabarowski
UAB, Department of Microbiology
(205) 996 2082
janusz@uab.edu

Kelly Walters
Miranda Collier
Alex Johnson
UAB, Undergraduate Chemistry

Sangeetha Rao
UAB Resident

Stephen Barnes
Landon Wilson
UAB, Targeted Metabolomics and Proteomics Lab

David Graves
UAB, Chair Department of Chemistry

Anupam Agarwal
UAB, Dept Medicine, O'Brien Acute Kidney Injury Research Center

Steve Watts
UAB, Department of Biology, Dir. NORC Aquatic Research Core.

Trenton Schoeb
UAB, Dept Genetics, Dir. Comparative Pathology Core Laboratory

Steve Burgess
Dir. R&D, Avantipolar Lipids

U.S Department of Health and Human Services
National Institutes of Health
National Heart Lung and Blood Institute
People Science Health

Lupus Research Institute

O'Brien Center
UAB UCSD
Acute Kidney Injury Research

UAB School of Medicine
AMC21 Immunology, Autoimmunity & Transplantation Strategic Planning