



# Untargeted Lipidomic Analysis of Human Retinas using LC-MS/MS and MALDI Imaging Mass Spectrometry



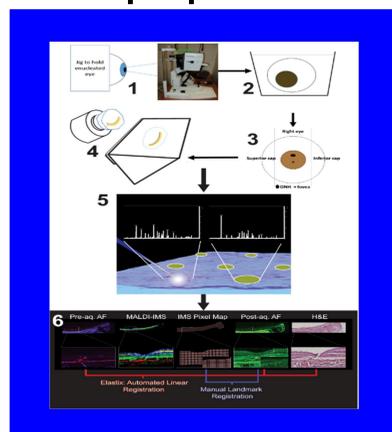
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## Introduction

Age related macular degeneration (AMD) is a major cause of legal blindness in older adults worldwide. Ample evidence supports a role of lipids and associated pathways in degeneration of vision critical cells of the neural retina. Imaging Mass Spectrometry (IMS) is a powerful analytical tool capable of untargeted mapping the spatial distribution of lipids at the single cell level with high spatial accuracy and chemical specificity. Liquid chromatography tandem mass spectrometry (MS/MS) provides molecular identifications of hundreds of lipids in an untargeted fashion. Combining accurate mass measurements from Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI IMS) and accurate mass measurements and fragmentation data from LC-MS/MS analysis provides spatially-resolved lipid identifications. The goal of this study was to compare two discovery lipidomic methods, LC-MS/MS and imaging mass spectrometry (IMS), in analyzing localized role of specific lipid classes in human RPE.

## Methods

**Figure 1: Sample procurement and preparation for MALDI-IMS**



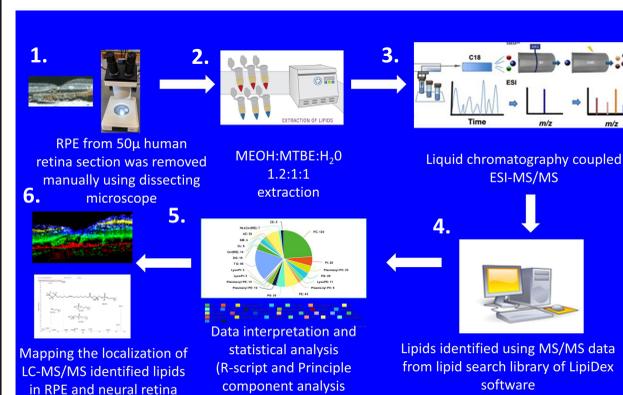
**Figure 1:** Workflow of sample preparation and multimodal imaging. 1. Whole eye mounted in jig to obtain OCT image. 2. The cornea is removed before fixation with 4% paraformaldehyde for 48 hours at 4°C. 3. The eye is placed in a dissection guide as described in the text to capture a belt with the optic nerve, macula, and temporal periphery. The belt is embedded in 2.6% CMC in a cryomold. The caps are discarded. 4. Cryosections at 12-14 µm throughout the entire eye are thaw-mounted on either glass or ITO slides. 5. ITO slides are imaged for autofluorescence (AF) before being coated with matrix via sublimation for acquisition of IMS data. 6. Highly accurate data registration is performed from IMS, post-acquisition AF, pre AF, and H&E stained tissue.

### Fixation conditions:

Donor eyes were obtained from Advancing Sight Network (formerly Alabama Eye Bank) <6 hours post mortem and immediately prepared for fixation and/or embedding. The anterior portion of the eye was removed before placing in paraformaldehyde for 48 hours at 4°C.

12-14 µm sections of fixed human donor tissue were cut and (DHA) or 1,5-diaminonaphalene (DAN) was applied using an in-house sublimation device for positive and negative ion mode analysis. MALDI IMS data were acquired with a 15 µm pixel size in full scan using solariX 9.4T FT ICR mass spectrometer (Bruker Daltonics) equipped with a modified source to provide higher spatial resolution.

### LC-MS/MS procedure:



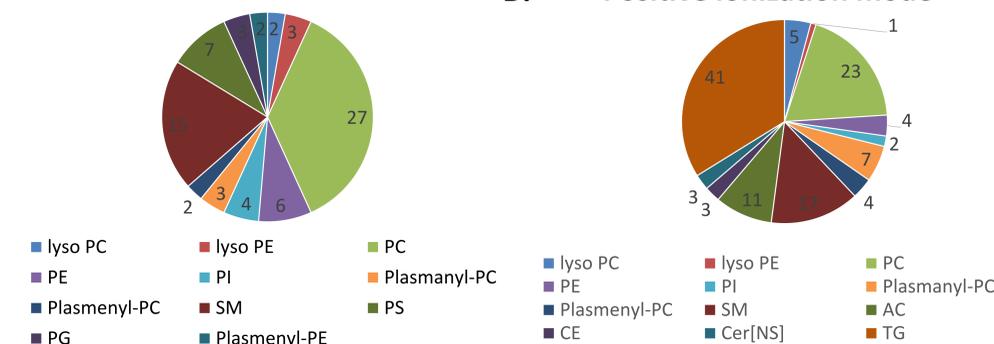
**Figure 2:** Workflow of sample preparation for LC-MS/MS. 1. Cryosections at 50 µm throughout the entire eye are thaw-mounted on plain glass slide. RPE was removed under dissecting microscope through surgical blades. 2. RPE tissue placed in HPLC glass vial, lipids were extracted using MEOH:MTBE:H<sub>2</sub>O(1.2:1:1) spiked with internal standard mixture and centrifuged for 1000 rpm for 10 minutes. 3. Samples were reconstituted in 30 µl methanol, 10 µl was injected from Vanquish autosampler (Thermo Scientific, San Jose, CA, USA) maintained at 4°C and ionized by a HESI heated ESI source in negative/positive polarities (Thermo Scientific), and analyzed using Q-Executive HF platform (Thermo Scientific, San Jose, CA, USA). 4. LC-MS/MS data were acquired using Xcalibur version 4.0 in both positive and negative ionization (ESI) modes and lipid identifications were made using LipiDex software ( Hutchins et al., 2018). 5. R-script and Principle component analysis were used for the statistical analysis. 6. LC-MS/MS identified lipids were mapped using IMS for their localization in RPE and neural retina.

### LC-MS/MS conditions:

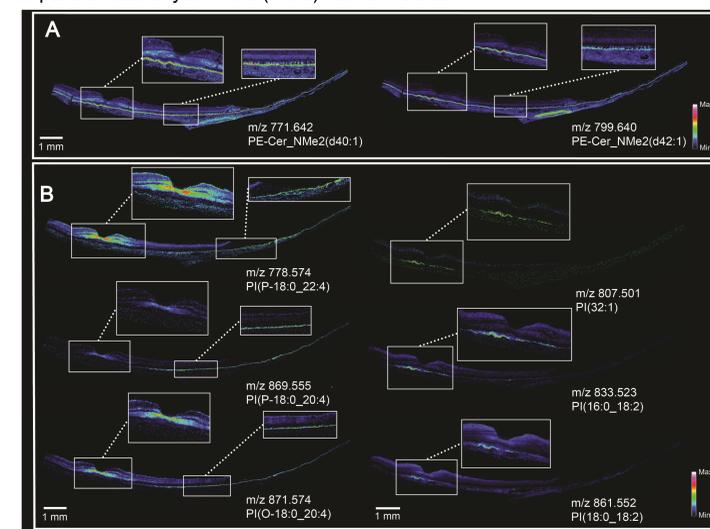
Column: BEH C-18, 1.7µ, 2.1 X 150mm  
 Mobile Phase:  
 A; 30% water, 70% acetonitrile, 10mM ammonium acetate, 0.1%FA  
 B; 90% isopropanol, 10% acetonitrile, 10mM ammonium acetate, 0.1% FA  
 Run type: Gradient; Run time: 55min; Flow: 250µL/min  
 Q-Exactive HF Mass spectrometer  
 Full MS/ dd-MS2 (TopN-5)  
 Polarity: negative/Positive  
 The SPLASH LIPIDOMIX standard from Avanti Polar Lipids, Inc. was spiked into each sample as an internal standard.

## Results

**A. Negative ionization mode** **B. Positive ionization mode**



**Figure 3:** Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) results using human donor retina with supporting tissues (≥80 years). In manually dissected RPE, 195 lipids were identified from 15 lipid classes. LC-MS/MS confirmed lipids identified in **A.** negative and **B.** positive ion modes from paraformaldehyde-fixed (fixed) human retina.



**Figure 4.** MALDI IMS from an 81 year old donor eye section from a region including the fovea and peripheral tissue, analyzed in negative ion mode. Fig 2A displays the distribution of 2 demethylated sphingomyelin species (PE-Cer\_NME2) localized with high abundance in the RPE region of the tissues. Fig 2B displays 6 images of phosphatidylethanolamine lipids (left side) that localize to the inner retina of the fovea and peripheral RPE regions. Phosphatidylinositols (right side) localize to the macular RPE region.

## Conclusions

Lipids identified included; LysoPC, LysoPE, PC, PE, PI, PG, PS, SM, Plasmanyl-PC, Plasmenyl PE, Plasmenyl PC, AC, CE, Cer and TG. SM species were localized to the RPE and Bruch's membrane in macular and peripheral regions while a number of PI and PE species are localized specifically in the macular RPE region. Further work using LC-MS/MS and IMS are in progress to identify lipid signals localized in RPE by IMS. "Untargeted lipidomic approach provide a comprehensive overview for the identification of biologically significant lipids in human retina to increase our understanding towards the pathophysiology of AMD".

### References

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