



# Lipid analysis from amphibian eggs, cell pellets and tissues by reactive LAESI-MS

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## Introduction

- Lipids have vital physiological functions and are routinely studied by mass spectrometry (MS) using soft ionization methods.<sup>1-3</sup>
- In Situ* direct ambient analysis of lipids in tissue minimizes artifacts resulting from sample preparation and postmortem tissue disintegration.
- Laser ablation electrospray ionization (LAESI) MS<sup>4</sup> was utilized for the direct analysis of lipids in variety of tissues and cells.
- LAESI-MS relies on the native water content of the tissue for sampling based on its strong absorption at mid-infrared wavelengths due the OH vibrations.
- Ablation for LAESI was carried out by a Nd:YAG laser-driven optical parametric oscillator. A homemade electrospray source was used to postionize the ablation plume. Positive ions were detected by an orthogonal acceleration time-of-flight mass spectrometer.

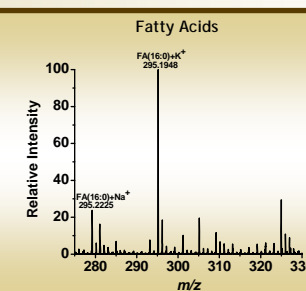
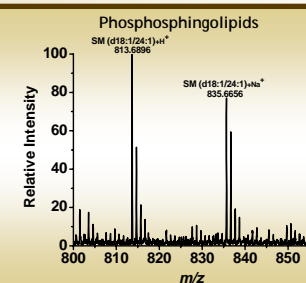
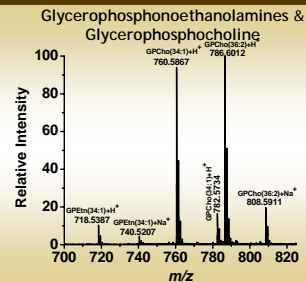
[1] Uphoff, A.; Hermansson, M.; Haimi, P.; Somerharju, P., "Analysis of complex lipidomes," In *Medical Applications of Mass Spectrometry*, Vekey, K.; Telekes, A.; Vertes, A., Eds. Elsevier Science: New York, NY, 2007; pp 217-243.

[2] Pulfer, M.; Murphy, R. C., "Electrospray mass spectrometry of phospholipids," *Mass Spec. Rev.* 2003, 22, 332.

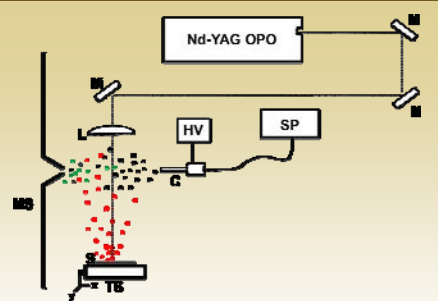
[3] Schiller, J.; Su, R.; Arnholt, J.; Fuchs, B.; Leig, J.; Muller, M.; Petkovic, M.; Spalteholz, H.; Zschornig, O.; Arnold, K., "Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research," *Progr. Lipid Res.* 2004, 43, 449.

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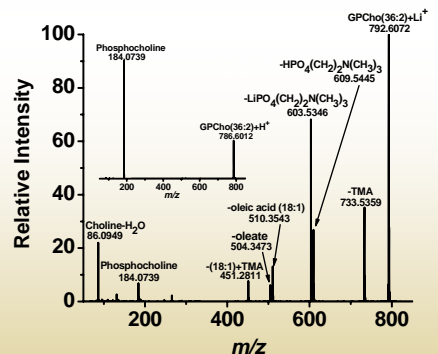
## LAESI-MS of Lipid Standards



## Reactive LAESI

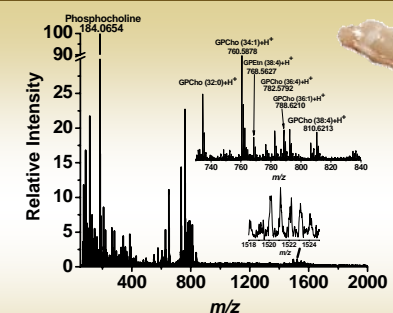


Reactive LAESI provides an opportunity to conveniently introduce liquid or gas phase reactions in the ion source by adding a reactant to the electrospray solution to react with the ablated analyte.



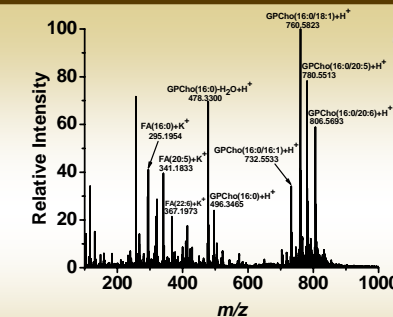
The collision-activated dissociation (CAD) of lithiated DOPC produced by reactive LAESI yields structural fragments identifying the molecule as GPCho (18:1/18:1), while CAD of the protonated DOPC ion (inset) produced only a single fragment, the phosphocholine ion.

## Mouse Brain



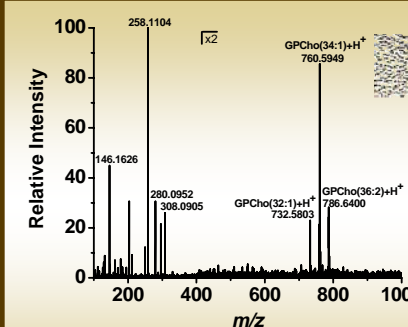
Positive ion LAESI mass spectrum from the transverse section through the middle of untreated mouse brain cerebrum shows predominantly phospholipids.

## Unfertilized *Xenopus* Egg



Positive ion LAESI mass spectrum from an untreated unfertilized egg of *Xenopus laevis* is rich in glycerophosphocholines, lyso-glycerophosphocholines, and fatty acids.

## Rat $\beta$ -Cell Culture



Analysis of B-cell pellets from RIN-5mF rat pancreatic insulinoma cell line by LAESI-MS showed the presence of glycerophosphocholines and small metabolites.

## Future Directions

LAESI Imaging: Utilize the imaging capabilities of LAESI-MS to perform ambient *two-dimensional* imaging of lipids and related metabolites in mouse brain sections.

Reactive LAESI: Explore other *in-plume* reactions to enhance the ion yields of poorly ionizable lipids (e.g., cholesterol).

Single Cell Analysis: We have demonstrated direct ambient analysis of unfertilized *Xenopus* egg. Extend to the analysis of lipids in other single mammalian cells.

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