All-in-one Data-Processing and Interactive Visualizations of Lipid LC-HRMS/MS Data using LipidMatch 4.0

Jeremy Koelmel[1]; Paul Stelben[1]; Bernard Brooks[2,3]; Jung Suh[4]; Mark Sartain[5]; Timothy J. Garrett[6]; John A. Bowden[6]; Emma E Rennie[5]; Krystal J Godri Pollitt[1]

[1]Yale University, New Haven, CT; [2]Innovative Omics, Sarasota, FL; [3]Mudai Studios, Sarasota, FL; [4]Denali Therapeutics, San Francisco, CA; [5]Agilent Technologies, Santa Clara, CA; [6]University of Florida, Gainesville, FL

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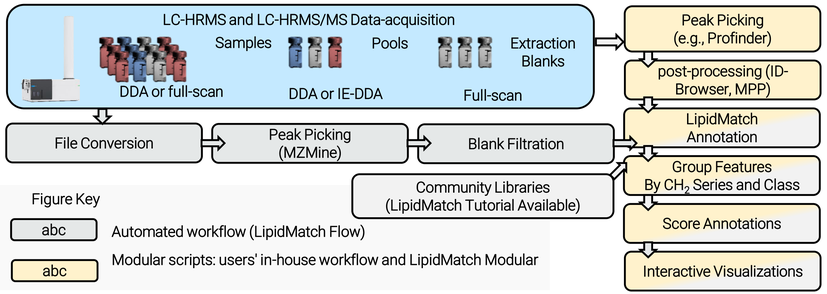
Introduction

Lipid pathways are altered in virtually all disease states, making lipidomics a valuable tool for biomarker discovery and understanding mechanisms of disease. As application of lipidomics gains prevalence, it is essential that software tools adapt to provide high-confidence and high-coverage annotations and be designed to be user friendly. Currently, manual review of lipidomics data is necessary for confident assignment. To address this need, LipidMatch 4.0 was developed. This software provides confident annotations which have been benchmarked against Lipid Annotator, MS-DIAL, and GREAZY, and to our knowledge, provides the most in-depth interface for validating annotations and discovering new lipid species.

Methods (see Application Note 5994-1356EN)

Lipidomics profiling workflow was used to analyze lipid alterations the Acute Myeloid Leukemia (AML) K562 cell line in response to different drug combinations. Data was acquired on a 6546 LC/Q-TOF with an Agilent 1290 Infinity II LC. Reverse phase chromatography was applied using an Agilent InfinityLab Poroshell 120 EC-C18 (3.0 × 100 mm, 2.7 µm) with a polar phase consisting of water:methanol (9:1) and non-polar phase consisting of acetonitrile:methanol:isopropanol (2:3:5) both with 10 mM ammonium acetate.

Entire Acquisition and Software Workflow



LipidMatch Flow and LipidMatch Modular (acquisition and data-processing workflow)

The LipidMatch software data analysis workflow starts by importing data collected using MS, and MS/MS data dependent (DDA), iterative exclusion MS/MS (IE-DDA), or targeted MS/MS modes from individual, pooled and blank samples. LipidMatch algorithms cover file conversion, blank filtering, feature annotation, and visualization. LipidMatch Software also directly imports data processed initially using Agilent's Mass Profiler software or other peak picking software.

Conclusions

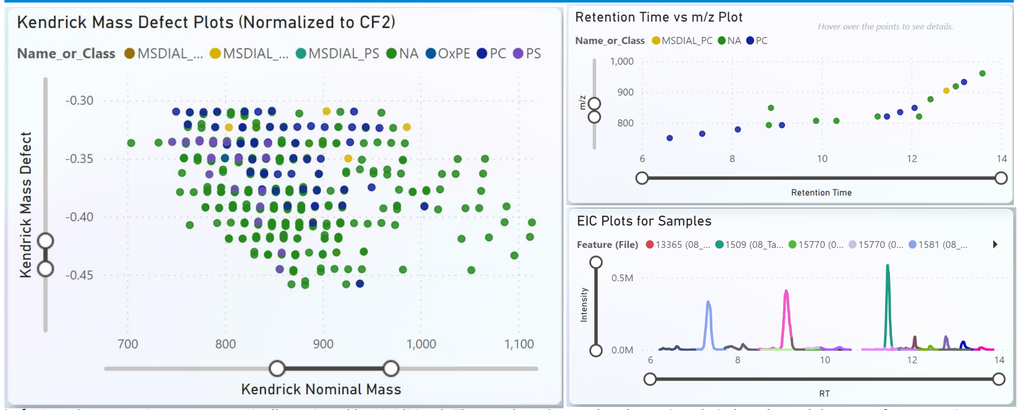
LipidMatch can be used to rapidly annotate lipids in an automatic fashion and determine unknowns and expand annotation using an interactive visualizer

- Incorporates MS/MS, MS, EICs, homologous series, and retention time
- Has over 300,000 species with fragmentation in libraries; fragment screening and substructure assignment for unknowns
- Many previously unidentified species exist in datasets which can be discovered using the visual interface
- < 5% False Positive

Innovative Omics

To install the software please visit:
Innovativeomics.com/software
Questions? Trainings? Collaboration?
Contact: jeremykoelmel@gmal.com

Visualizer Interface: Validation and Discovery using Homologous Series



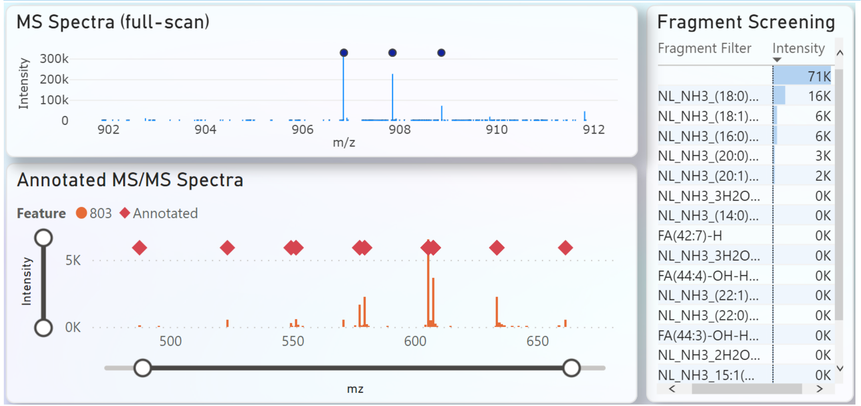
Left: **Homologous series are automatically assigned by LipidMatch Flow, and can be used to determine chain lengths and degrees of unsaturation not annotated using MS/MS.** This zoomed in region is for phosphatidylcholines in negative mode, green are unknowns, blue and gold are PCs assigned by MS/MS automatically, and purple are annotated PS species with overlapping masses

Right Top: **Each homologous series is defined by a specific lipid class and unsaturation can be viewed in retention time vs m/z plots where outliers (false positives) can easily be determined**

Right Bottom: **EICs of selected files can also be viewed simultaneously for each selected series, readily showing peak shape, any isomers, and the most dominant members of a class or series**

The interactive dataset can be review for lipidomics can be viewed at innovativeomics.com/datasets

Annotation Using Isotopic Pattern and MS/MS

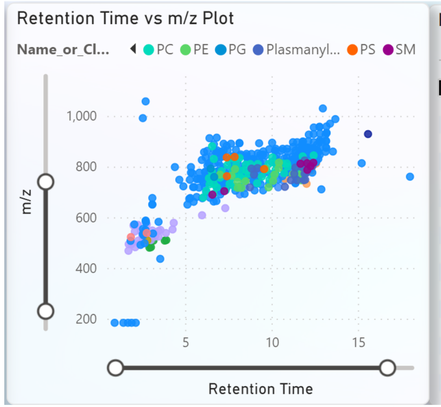


Left Top: **isotopic pattern for TG(54:1), all isotopic peaks are labeled. This can be especially helpful for validation and discovery when it comes to sulfur, chlorine, or other atoms with strong isotopic signals**

Left Bottom: **MS/MS spectra with NL annotations TG(16:0_18:0_20:1)+NH4, TG(18:0_18:0_18:1)+NH4, TG(16:1_18:0_20:0)+NH4, TG(16:0_18:1_20:0)+NH4, TG(14:0_18:0_22:1)+NH4, TG(12:0_18:0_24:1)+NH4, TG(14:0_20:0_20:1)+NH4, TG(14:0_18:1_22:0)+NH4, TG(14:0_16:0_24:1)+NH4, TG(16:0_16:0_22:1)+NH4, TG(16:0_16:1_22:0)+NH4, TG(12:0_20:1_22:0)+NH4, TG(12:0_20:0_22:1)+NH4, were all annotated with that rank, and MS/MS evidence shows why all species likely exist under the peak**

Right: **Annotations of NL peaks**

Discovery of Unknowns: Fragment Screening



***m/z* vs retention time plot filtered by observed fragment(s) in positive ion mode. In this case the phosphorylcholine *m/z* 184.073 fragment was used to filter features, indicating PC, SM, ether and oxidized derivates, and other species containing this head group.**

Light blue dots are unknowns showing that a significant number of species which were potentially polar lipids with a phosphocholine head group were left unidentified. This signifies the wealth of information which is missing in traditional lipidomics approaches without unknown discovery. It is important to note that some (but not all) of these unknown features, which overlap in *m/z* and retention time with PCs or SMs may be isobaric and hence the 184 peak may come from another species.