Single Cell Lipidomics

Highly Sensitive Targeted Method for Single Cell Lipidomics on Stellar MS – A Hybrid Nominal Mass Instrument

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Abstract

Purpose: This study aims to enhance lipidomics workflows for single cels leveraging the Thermo Scientific™ Stellar™ mass spectrometer-a to improve specificity, sensitivity, and throughput in lipid quantitation, addressing limitations in existing clinical and translational lipidomics methods.

Methods: Bulk Cell and Single Cell (RK13 and Vero Cells) lipid samples were extracted and separated using a Thermo Scientific™ Vanquish™ Neo UHPLC systems. Data-dependent acquisition (DDA) for untargeted lipidomics was performed on Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer. Targeted analysis of lipids was performed on Stellar MS, a nominal mass instrument.

Results: Using data obtained from bulk cell extracts, a targeted list of lipids was prepared to use on the nominal mass instrument. Single cell extracts showed differences in different cel types.

Introduction

Lipids play critical roles in multiple biological processes and their heterogeneous distribution among cells has significant biological implications.

Why single cell analysis?

Single-cell analysis offers a significantly higher resolution and deeper understanding of biological systems compared to bulk cell analysis.

- Resolving Cellular Heterogeneity
- Identifying Rare Cell Types
- Understanding Developmental Processes and Cell Lineage
- Dissecting Complex Biological Systems
- Revealing Hidden Differences and Subpopulations

Bulk analysis provides an "average" picture, single-cell analysis provides a "cellular census," allowing for a much more nuanced and comprehensive understanding of biological complexity.

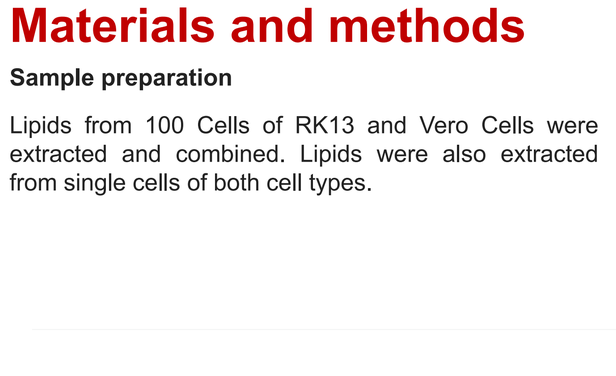


Figure 1. Single cell vs. Bulk cell analysis

Materials and methods

Sample preparation

Lipids from 100 Cells of RK13 and Vero Cells were extracted and combined. Lipids were also extracted from single cells of both cell types.

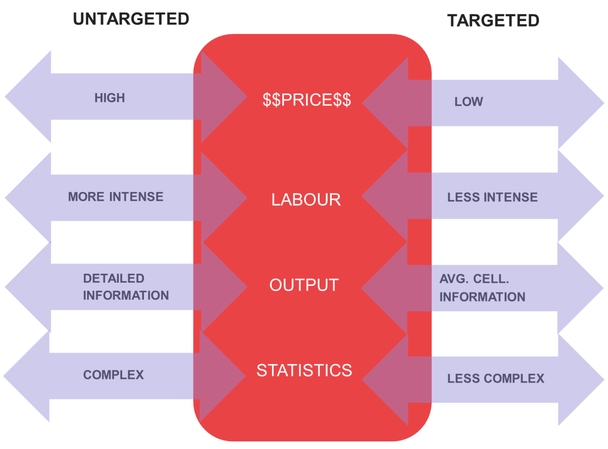


Figure 2. Targeted vs. Untargeted analysis

LC-MS method

Lipid extract was separated using Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC column (C18, 75µmx150mm, 2µm) connected to a Thermo Scientific™ Vanquish™ Neo UHPLC system.

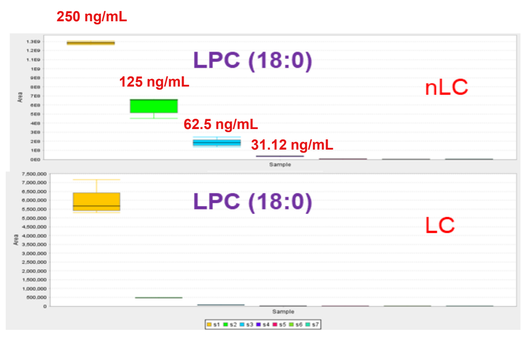
Data acquisition was carried out on the Orbitrap Ascend Tribrid MS and nominal mass Stellar MS. Both instruments are capable of alternate fragmentation techniques and multi-stage fragmentation.

Data analysis

Data processing, including quantitation of analytes and annotation of unknowns, was performed using Thermo Scientific™ Compound Discoverer™ 3.4 software, Thermo Scientific™ LipidSearch™ software and Skyline software.

Results

Increasing sensitivity for single cell analysis



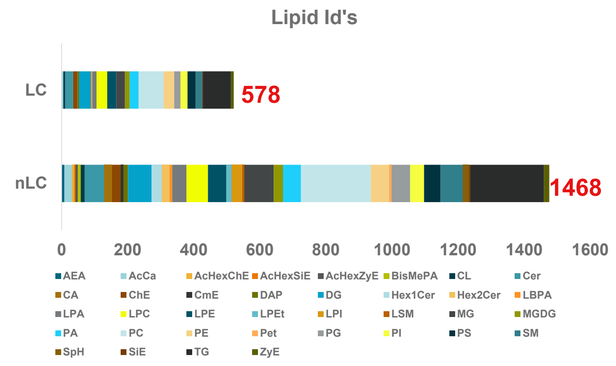


Figure 3. Use of nanoflow increases sensitivity and improves identification of lipid class

Confident and deep annotation of lipids

For creation of a targeted list, confident and deep annotations are a pre-requisite. Use of alternate fragmentation, multi-stage fragmentation and iterative data acquisition workflows can assist. LipidSearch 5.2 software can help to reduce false annotations and improve the confidence of annotations by utilizing the above tools.

Mass list from LipidSearch software

PRM targeted lipid mass list is generated from the output of LipidSearch software. LipidSearch software also gives the product ions for data processing.

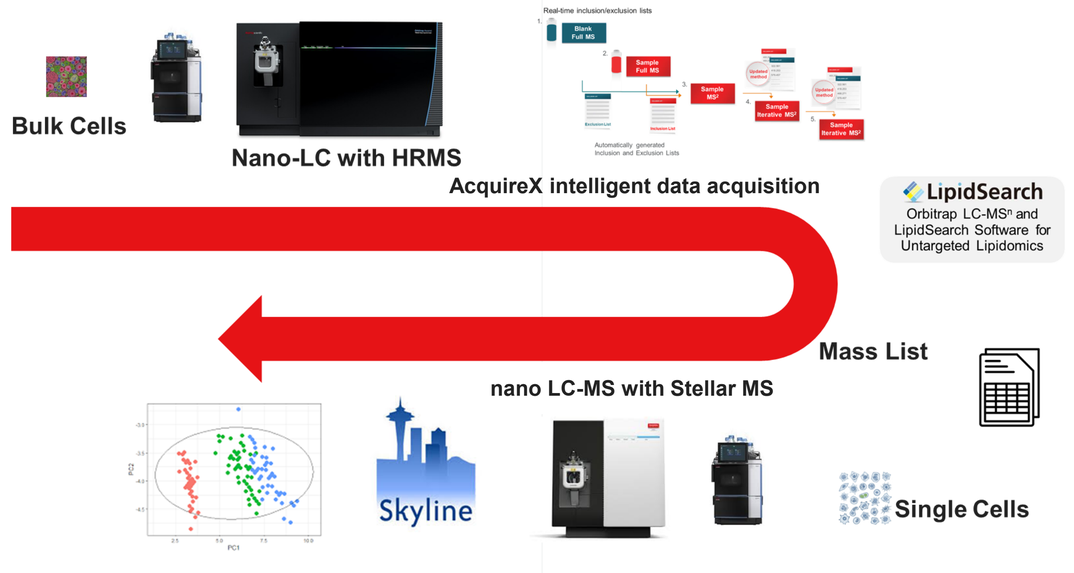


Figure 4. Workflow for omics-scale targeted analysis of single cell

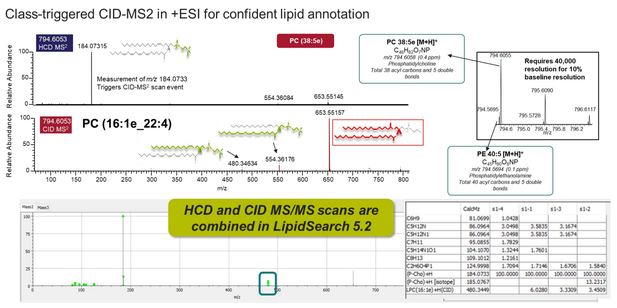


Figure 5. Alternate fragmentation enhances the confidence of annotation

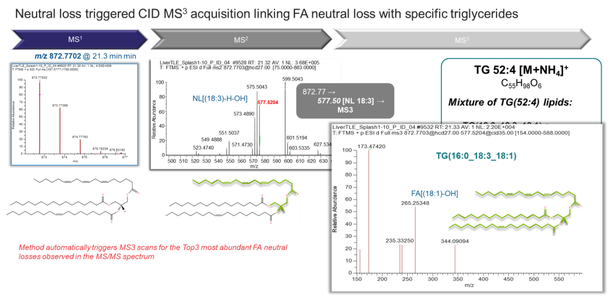


Figure 6. Use of MSⁿ can help decipher co-eluting isomers

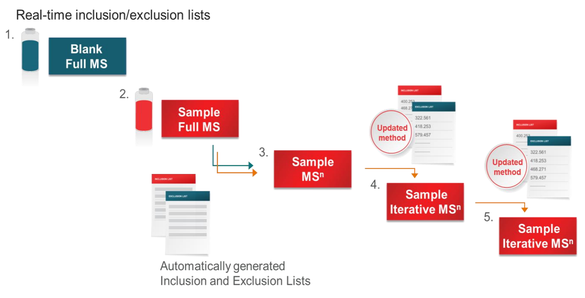


Figure 7. Use of Thermo Scientific™ AcquireX™ intelligent data acquisition workflow helps to increase the annotation coverage of the bulk cell samples.

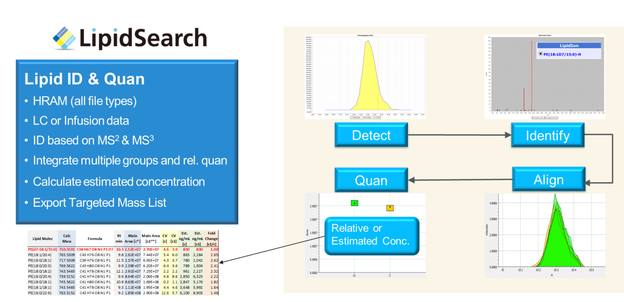
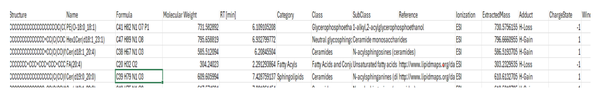


Figure 8. Use of LipidSearch 5.2 software to confidently annotate lipid and generate the targeted lipids mass list.



Mass list of 864 Lipids with confident annotation from bulk cell extracts

613 PRM List-Isomeric/Isobaric Precursors for Stellar

IonFormula	Class	Rt	LipidId	Q1	Q3_1	Q3_2	Q3_3
C38H73N1O8P1	PC	4.3017	PC(14:1_16:1)+H	702.5068	124.9998	184.0733	86.0964
C36H73N1O8P1	PC	4.3655	PC(14:0_14:0)+H	678.5068	124.9998	86.0964	184.0733
C44H77N1O8P1	PC	4.3854	PC(14:0_22:6)+H	778.5381	124.9998	450.2979	184.0733
C38H75N1O8P1	PC	4.5072	PC(14:0_16:1)+H	704.5225	124.9998	494.3241	184.0733
C40H77N1O8P1	PC	4.6102	PC(16:1_16:1)+H	730.5381	124.9998	86.0964	184.0733

For MRM/Data Processing From PRM

Targeted Analysis of Single Cels

Extracts from single cells were run on the Stellar MS with the targeted list generated.

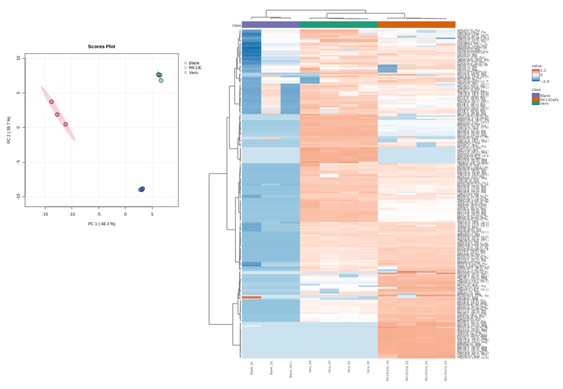


Figure 9. 258 lipids were identified across the single cell extracts from both cell types. Skyline software was used for data processing to quantify peak areas. PCA plot showed clear separation between the cell types.

Conclusion

We have successfully showed the use of targeted platform for single cell analysis. We hope that this can lead to more labs adapting this technology to reveal interesting biological insights.

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