# ENHANCED SINGLE-CELL SHOTGUN LIPIDOMICS WORKFLOW WITH THE SELECT SERIES CYCLIC IMS

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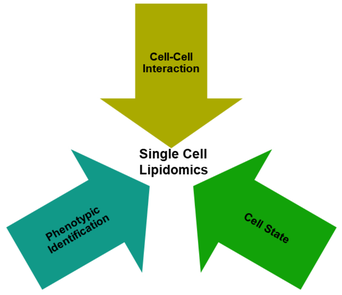
## INTRODUCTION

Traditional lipidomics often overlooks lipid diversity at the single-cell level. Recent advances enable detection of lipids in individual cells, offering insights into cell interactions, states, and phenotypes [1]. Due to minimal sample volumes, shotgun approaches are preferred over LC-based methods.

Ion Mobility Spectrometry (IMS), particularly the SELECT SERIES™ Cyclic™ IMS, improves lipid separation by shape, size, and charge—resolving complex mixtures and isomers. It enhances data clarity and identification confidence using CCS values matched to the Waters Lipids CCS library.

To enable aligned data matrices for multivariate analysis, and quantification across sample cohorts we have developed a new workflow integrating Cyclic IMS with LipidXplorer software.




## METHODS

THP-1 leukaemia cancer cell lines were used for a proof-of-concept (POC) study. The lipids were extracted using a simple protein precipitation [2] with organic solvent containing deuterated lipid standards in 96 well plates. Bulk cell extracts were also analysed for comparison. Bulk cells were extracted in IPA and Ammonium Formate buffer containing Avanti EquiSPLASH mix and diluted to 200, 40, 8 and 1.6 cell/ 100 nL. A robotic nanospray ion source using nanoelectrospray chips was used to infuse the lipid extracts (n=3) at 50 nL/min for 2 mins (100 nL total volume).

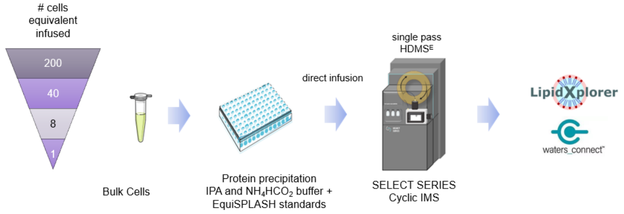



*Figure 1. Bulk cell equivalents from 200 down to 1 were infused via a well plate by nanospray.. Initially the Cyclic analysed these extracts in single pass mode*

The lipid extracts were analysed in positive ESI modes on the SELECT SERIES Cyclic IMS system using High Definition MS$^E$ (HDMS$^E$) single pass mode to enable the use of the Waters CCS library containing predicted CCS values [3] for identification. A *m/z* tolerance of 2 ppm for accurate mass matching and CCS tolerances of 3% compared to predicted values were used. Data were acquired using MassLynx™ and processed using waters_connect™ and LipidXplorer software.

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## HIGLIGHTING THE BENEFITS OF CYCLIC IMS

Volatile polydimethylcyclosiloxanes in the ambient laboratory air have been identified as source of extreme background signals in nanoelectrospray mass spectrometry [4]. These compounds are widespread in industrial products, e.g. in deodorants where they serve as a cosmetic vehicle. Since these cyclic silicones are volatile, they are ubiquitously present in ambient air. . An example spectra of a blank contain EquiSPLASH lipid standards is shown in Figure 2. The 3D viewer shows that the polydimethylcyclosiloxanes can be separated from the lipids by drift time.

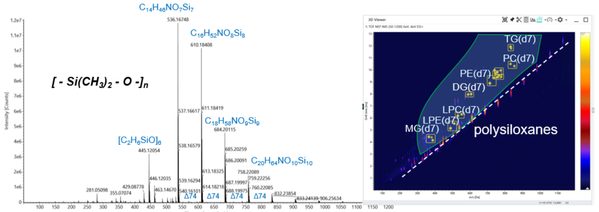


*Figure 2. Infusion nanospray spray spectra of IPA and EquiSPLASH showing intense common polycyclosiloxane peaks. The 3D plot shows these contamination peaks are separated from lipids in the drift time dimension*

**IMS can be used to clean up spectra** by selection the region for lipids and excluding the contamination as shown in Figure 3. Zooming into the phospholipid region (Figure 4) around the most abundant PCs e.g. PC(34:1) we can see that we an begin to **separate isobaric lipids** in the drift time domain. An example of this is the peak (s) at *m/z* 718.6. These isomer can be further separated using multipass experiments.

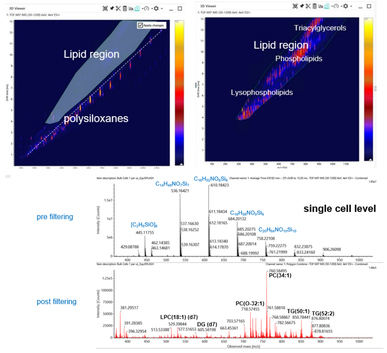


*Figure 3. Clean sample spectra can be generated by selecting a region on the 3D plot way from the contamination. The post filtered spectra highlights some abundant lipid signals*

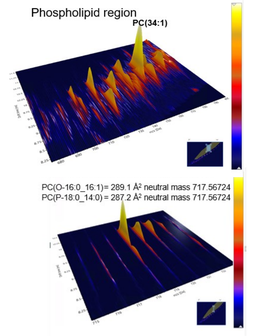


*Figure 4. By zooming into the Phospholipid region, some isobaric signals are separated in the drift time dimension. e.g. m/z 718.6*

## SINGLE CELL WORKFLOW

A waters_connect screening method using 3200 lipids with predicted CCS values was used to process triplicate bulk cell infusions. Approximately 295 lipids were identified at the single-cell level using a 2 ppm mass tolerance and 3% CCSΔ (Figure 5). However, this approach lacked blank subtraction and showed group fragmentation from multiple precursors, potentially leading to false positives.

Using the LipidXplorer workflow, blank subtraction and quantification via internal standards were incorporated. Lipid identifications were retained if the sample-to-blank average intensity ratio exceeded 10. Stable lipid profiles across major classes were observed (Figure 6), with class composition at the single-cell level shown as mol% in the pie chart. Some variation was noted due to improper adjustment of the PS internal standard. Lipids from the PC/PC-O class displayed consistent values across both low and high abundance species (Figure 7).

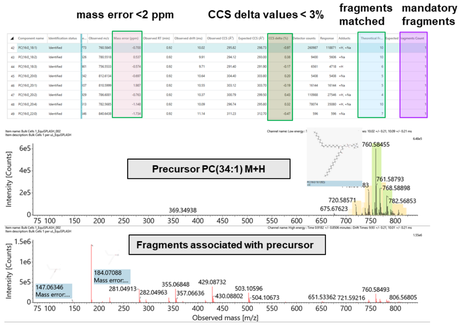


*Figure 5. Example identification PC(34:1) for a single cell equivalent sample shows 0.7 ppm mass error, CCS Δ 1% and matching fragments*

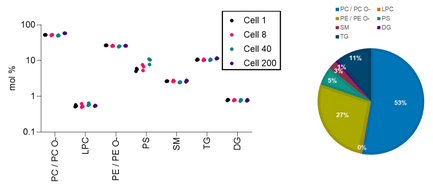


*Figure 6. Stable profile for major abundant lipid class / single cell to 200 cell (n=3). PS internal standard addition in positive ion mode not properly adjusted. Pie chart shows the class complement at single cell level in mol percentages*

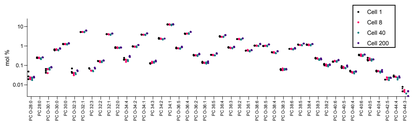


*Figure 7. PC species show similar values for low to high abundance species at 1,8,40 and 200 cell levels (n=3).*

## Key Points

**1** **Using Shotgun Lipidomics Enabled Comprehensive Characterization of Lipids at the Single Cell Level**

**2** **Cyclic IMS Improved confidence; Provided spectra clean-up, detection of isomers and CCS measurements**

**3** **LipidXplorer maximizes information gleaned from shotgun lipidomics experiments performed on Cyclic IMS enabling more confident assignments and quantification of endogenous lipids**

**References**

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2. Sarafian *et al* (2014) *Objective Set of Criteria for Optimization of Sample Preparation Procedures for Ultra-High Throughput Untargeted Blood Plasma Lipid Profiling by Ultra Performance Liquid Chromatography-Mass Spectrometry.* Anal Chem 86, 5766– 5774
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