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iprm-PASEF: an integrated workflow for the analysis and interpretation of spatial on-tissue tandem mass spectrometry of lipids

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Introduction

MALDI Imaging is now a commonplace analytical tool used to gain insight in the spatial molecular composition of tissues and to generate hypotheses in spatial biology studies. One of the open challenges for MALDI Imaging is the availability of an integrated method for MS/MS confirmation of ions of interest.

iprm-PASEF offers a new workflow for the timsTOF fleX, which includes CCS-enabled MALDI Imaging and a targeted MALDI prm-PASEF-based MS/MS analysis (Figure 1). Compared to other available workflows which rely on "profiling" MS/MS strategies, iprm-PASEF generates fragment ion images, which add an additional layer of confidence in the identification of unknown precursor ions.

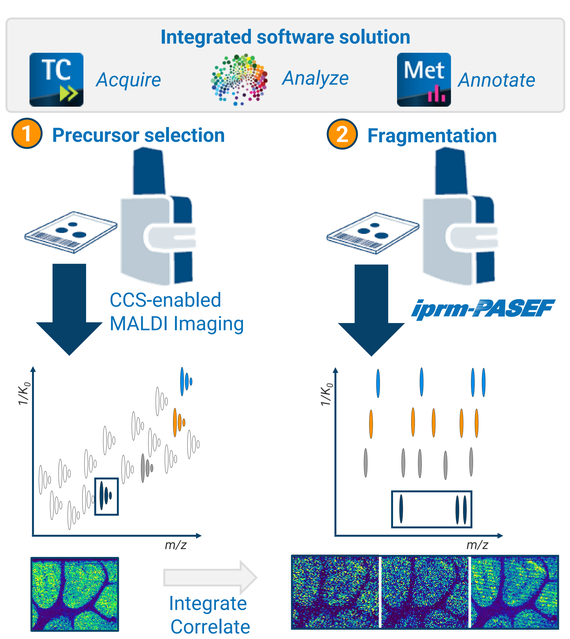

Figure 1. Schematic representation of iprm-PASEF. (1) Select precursors from CCS-enabled MALDI Imaging data in SCiLS Lab. (2) Acquire iprm-PASEF data, then analyze and annotate the fragment images and spectra using SCiLS Lab and MetaboScape.

Methods

CCS-aware MALDI Imaging (MS1), using a timsTOF fleX (Bruker Daltonics, Bremen, Germany), was performed on a 10 µm-thick tissue section from fresh frozen rat kidney. Prior to data acquisition, the sample was homogeneously sprayed with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDC) matrix using an HTX M3 plus (Chapel Hill, NC). Acquisition was performed in negative ion mode at a spatial resolution of 40 µm using a 20×20 µm² laser pitch.

MS1 data was imported into a prototypical version of SCiLS™ Lab 2025a. T-Rex³ Feature Finding and automatic CCS feature extraction was performed during import. The T-ReX³ Feature Finding result was filtered manually, and a list containing 13 mass-mobility features of interest was saved. Selection of these features was based on abundance in key morphological areas of the kidney (i.e., cortex, medulla, etc.). The precursor feature list, as well as the measurement area were exported to the new SCiLS prmPASEF parameter (.spp) file format. The iprm-PASEF (MS2) acquisition was set up in timsControl and flexImaging by setting up the acquisition method and loading the .spp file to transfer precursor mass-mobility windows, and measurement area. The MS2 acquisition covered the identical MS1 measurement region with a 20 µm X-offset. Following acquisition, iprm-PASEF data were imported into the prototypical SCiLS Lab software and merged with the MS1 precursor images using SCiLS Ion Image Mapper. Molecular Annotation, including MS2 spectral information, was performed using a prototypical version of MetaboScape® 2025, and the various fragment ion images were studied using SCiLS Lab's build-in tools.

Results

A list of 13 precursors obtained from the MALDI Imaging MS1 acquisition (Figure 2A) was fragmented using iprm-PASEF (Figure 2B). MS2 spectra were extracted and annotated using the Lipid Species annotation tool in MetaboScape (Figure 2C+D and Table 1). Based on the Molecular Annotation, *m/z* 885.54 was annotated as PI 18:0_20:4. The feature ion at *m/z* 766.54 however, was annotated as PE 18:0_20:4, PE 18:1_20:3, PE 18:2:20:2 and PE 16:0_22:4, of which the first was the dominant feature based on fragment ion feature intensities (and resulting image quality). Fractional contribution images showed the relative contribution and distribution of the 18:0 acyl chain fragment from the PI 38:4 and PE 38:4 lipids (Figure 3).

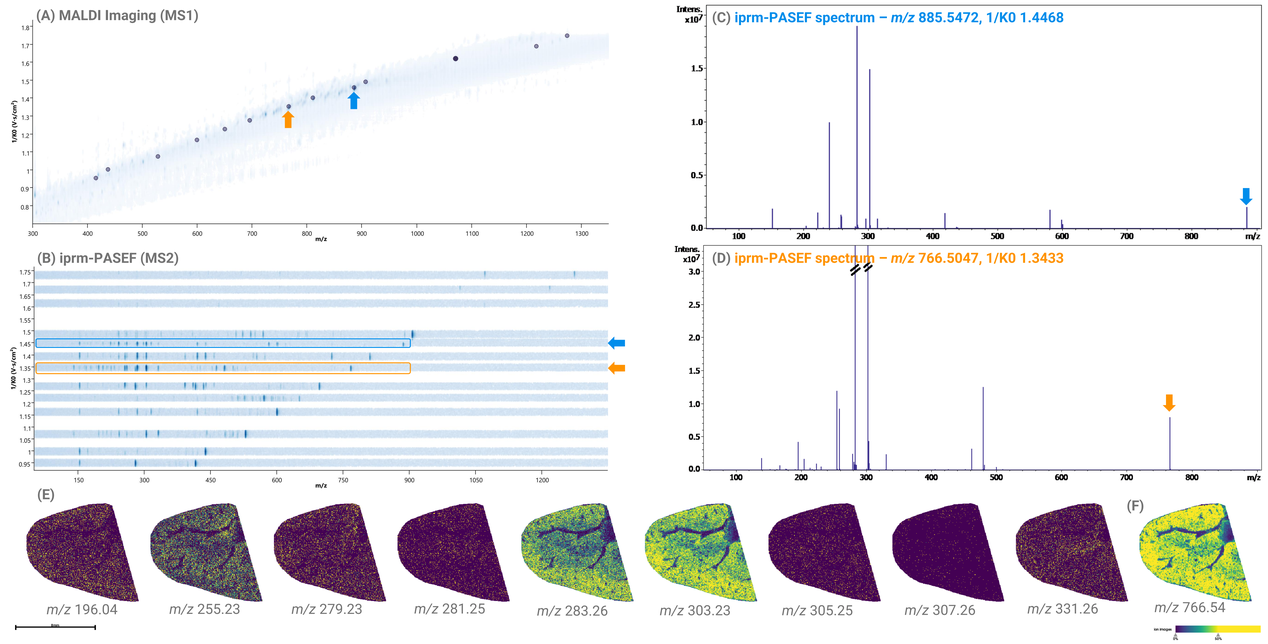

Figure 2. (A) Mass-mobility heatmap of the lipid MALDI Imaging MS1 data set, where the selected precursors are represented by blue dots. Blue and orange arrows indicate selected features for further visualization. (B) Mass-mobility heatmap of the iprm-PASEF MS2 data set. (C) and (D) show MS2 spectra of the highlighted precursors, (E) shows the ion images for the fragment ion peaks in (D), and (F) the MS1 precursor ion image. All images are based on non-normalized intensities.

Table 1. List of identified fragments visualized in Figure 2E.

m/z	Identity	ID	m/z	Identity	ID
766.54	Precursor ion	PE 38:4	283.26	18:0 (Acyl chain frag.)	PE 18:0_20:4
331.26	22:4 (acyl chain frag.)	PE 16:0_22:4	281.25	18:1 (Acyl chain frag.)	PE 18:1_20:3
307.26	20:2 (acyl chain frag.)	PE 20:2_18:2	279.23	18:2 (Acyl chain frag.)	PE 20:2_18:2
305.25	20:3 (acyl chain frag.)	PE 18:1_20:3	255.23	16:0 (Acyl chain frag.)	PE 16:0_22:4
303.23	20:4 (acyl chain frag.)	PE 18:0_20:4	196.04	Head group frag.	PE 38:4

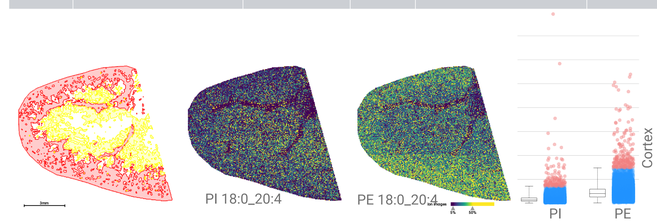

Figure 3. Fractional contribution images of the 18:0 (Acyl chain fragment) ions originating from PI 18:0_20:4 (m/z 885.54) and PE 18:0_20:4 (*m/z* 766.54). Boxplot shows intensity distribution of the cortex (red).

Summary

iprm-PASEF provides a full workflow for the generation of MS2 fragment ion imaging data sets, including acquisition, analysis and annotation of fragment ion features. The workflow relies on the CCS-enabled analysis offered by timsTOF fleX, timsControl, SCiLS Lab and MetaboScape.

Conclusion

- iprm-PASEF offers a full workflow for the acquisition, analysis and annotation of MS2 data with spatial fidelity.
- Using iprm-PASEF, *m/z* 885.54 (1/K0 1.45) was identified as PI 18:0_20:4.
- Using iprm-PASEF, *m/z* 766.54 (1/K0 1.34) was identified as PE 18:0_20:4. Additional evidence was found for PE 18:1_20:3, PE 18:2:20:2 and PE 16:0_22:4.

Imaging MS: Instrumentation

COI Disclosure All authors are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation and products, including mass spectrometers and software used in this study.