

# Automatic molecular annotation of mass spectrometry imaging data



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ASMS 2019, TP 409

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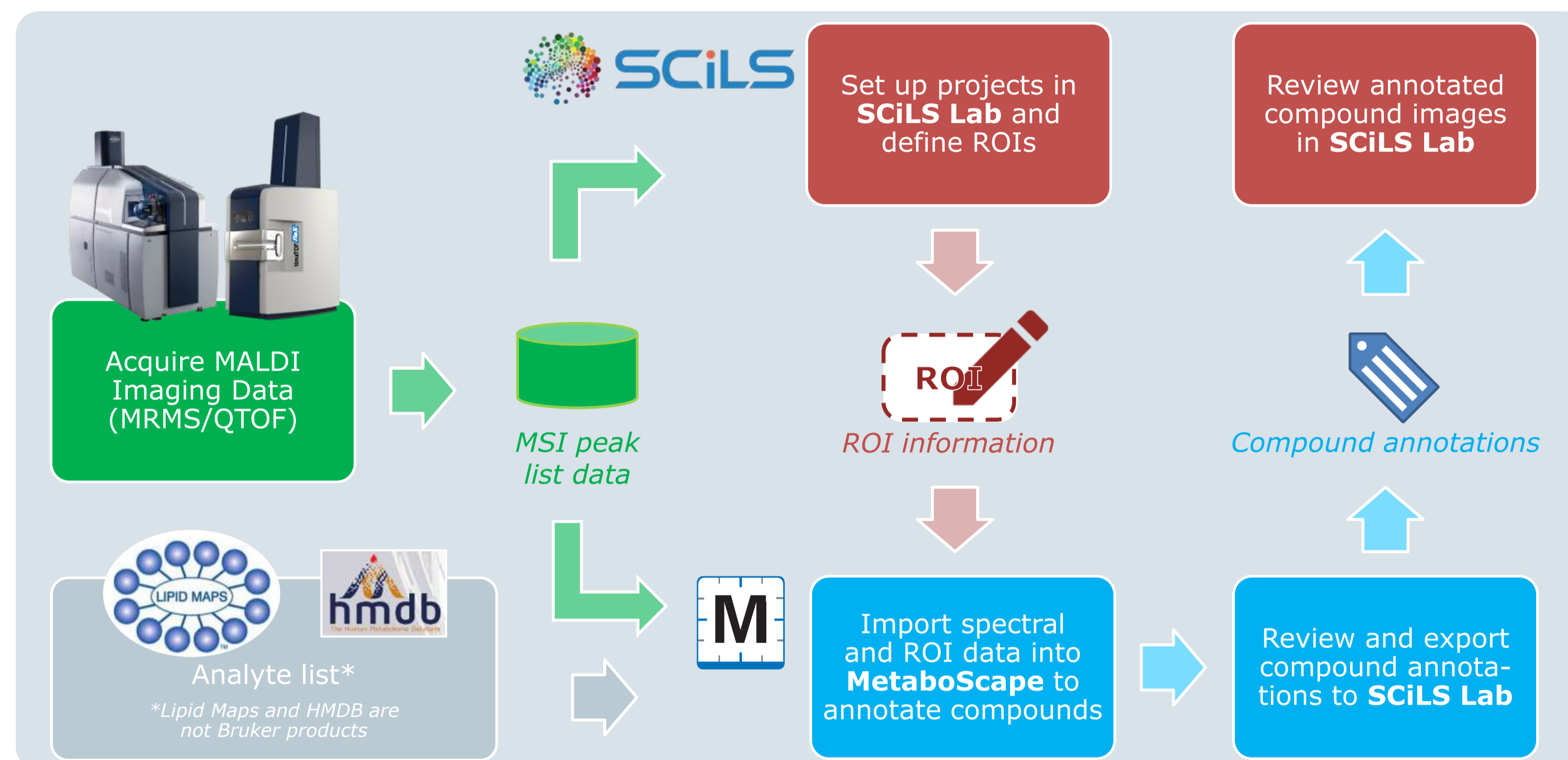
## Introduction

Mass spectrometry imaging (MSI) is used in untargeted metabolomics studies to investigate how compounds are localized across tissue samples. Modern MS instrumentation enables measurement of analytes at high mass resolving power with both high mass accuracy and high lateral resolution.

We present a novel software workflow for the identification of signals using data from the high-speed, high spatial resolution timsTOF fleX instrument (see Fig. 1).

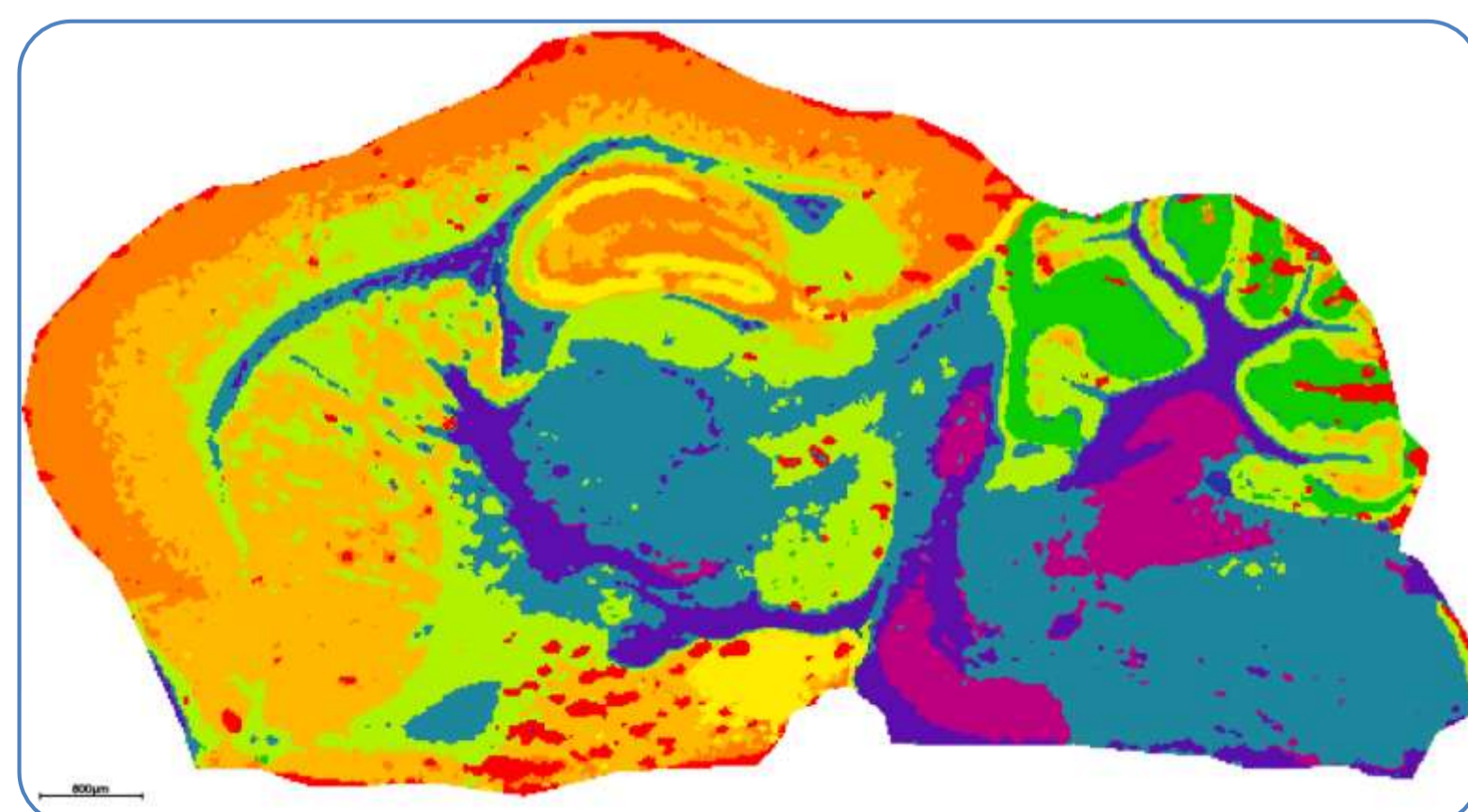
## Methods

- **Sample preparation:** Fresh frozen mouse brain specimen was sectioned to 10  $\mu\text{m}$  thickness, and mounted onto conductive glass slides. DHB matrix was applied using a HTX TM sprayer.
- **MSI acquisition:** Data were collected in positive ionization mode from 300 to 1000 Da at a pixel size of 20  $\mu\text{m}$  on a timsTOF fleX MALDI-QTOF instrument.
- **ROI information:** SCiLS Lab Pro (version 2020a) was used to automatically create segments based on molecular similarity with bisecting k-means.
- **Analyte list matching:** MetaboScape 5.0 was used to detect and combine isotope peaks and adducts with T-ReX<sup>2</sup> algorithm. Annotations were then assigned based on chemical formula and matched against LIPID MAPS Structure Database.



▲ **Fig. 1:** Workflow of automated molecular annotation in MS imaging experiments.

▼ **Fig. 2:** Segmentation map obtained with SCiLS Lab Pro; the regions cortex (orange), cerebellum (green), hippocampus (yellow), and mid- plus hindbrain (teal) were transferred to MetaboScape.



## Results

In total, the imaging experiment comprised of 123,300 pixels and 830 peaks. The spectra were segmented according to their molecular similarity. Representative segments matching anatomical features were converted into regions of interests (ROIs, see Fig. 2).

From these regions, 2500 spectra were averaged into 5x5 blocks yielding 100 spectra with average signal intensities. For feature extraction, deisotoping, and ion deconvolution, mass tolerances were set to 5 mDa and 250 mSigma for isotopic pattern fits.

The MetaboScape T-ReX<sup>2</sup> algorithm found 114 unique features. Chemical formula could automatically be assigned for 110 of these. Furthermore, 32 formulae could be matched against lipids in the LIPID MAPS Database.

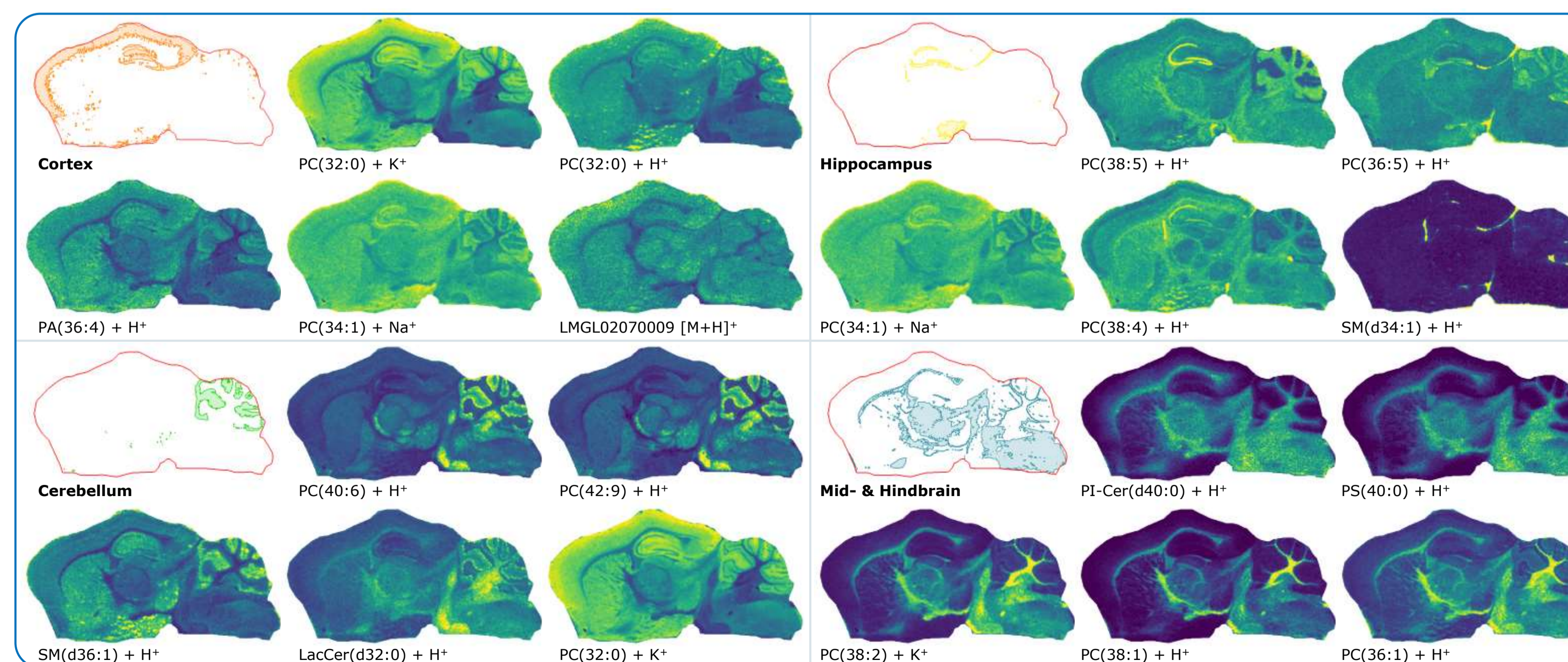
All found peaks were well within the quality range  $\pm 3$  mDa and less than 140 mSigma, providing confident compound annotations to transfer to SCiLS Lab Pro for further analysis.

Spatial correlation of the 32 tentatively annotated images against each the anatomical feature regions was computed. Those annotated images with highest correlation to the input regions are visualized in Fig. 3.

It is interesting to note that PC(34:1)+Na<sup>+</sup>, the annotation for m/z 782.568, yields high intensities for both Cortex and Hippocampus, similar to PC(32:0)+K<sup>+</sup> (m/z 772.525) for both Cortex and Cerebellum.

While not shown here, this untargeted metabolomics workflow is also applicable to MRMS instrumentation which provides even higher confidence of the annotations.

▼ **Fig. 3:** Distributions of tentatively annotated lipids matched to the anatomical regions.



## Conclusion

- This workflow provides annotations to the morphological topography in untargeted metabolomics studies
- Lipid annotation automated with MetaboScape 5.0 featuring T-ReX<sup>2</sup> feature extraction technology and confident annotation quality scoring
- Visualization of annotated signals with SCiLS Lab Pro completing the intuitive workflow and confirming lipid distributions map to the expected localizations

Imaging MS