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# Evaluation of an improved feature finding algorithm for QTOF and ion mobility imaging data in spatial lipidomics and metabolomics

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## Introduction

Feature detection is a critical pre-processing step in the analysis of any MS data. Algorithms such as T-ReX[2] and T-ReX[3] that operate on additional spatial information must be robust and reproducible to detect low S/N or highly localized features.

In this work, we evaluated the performance and reproducibility of the new feature finding tool that uses the improved T-ReX[3] algorithm (Oetjen et al. 2021) recently implemented in SCiLS Lab 2023b. This tool offers three input parameters – the relative intensity threshold, spatial smoothing and coverage – which have a significant effect on the number of features detected in a dataset. Using mouse testis (structurally heterogeneous) and liver (structurally homogeneous) tissue samples, we optimized these parameters for positive and negative ion mode imaging.

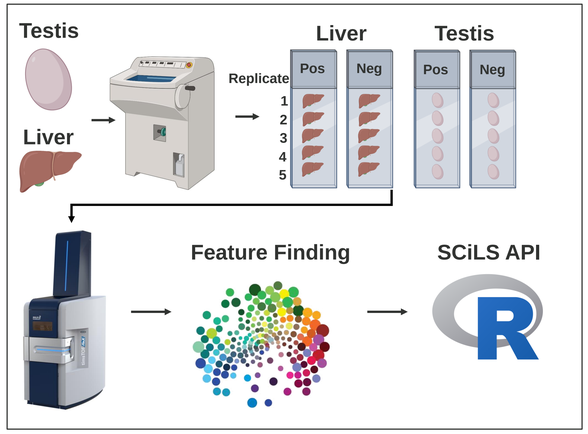


Fig. 1. Evaluation workflow of the feature finding algorithm in SCiLS Lab.

## Methods

Serial sections (n = 5 ) from fresh-frozen mouse liver and testis were coated with either 7.5 mg of DHB or 7 mg of Norharmane by sublimation for positive and negative ion mode TIMS-MSI on a Bruker timsTOF fleX. Data were acquired between *m/z* 300-1300 using a 20 µm pixel size with 250 shots/pixel at a 50% laser energy setting. Samples were obtained as scavenged tissue with institutional animal ethics committee approval.

The SCiLS Lab feature finding tool was used to detect and extract *m/z* and CCS features from each dataset, feature matching between replicates was performed in RStudio using the SCiLS API.

The user parameters were optimized in the order of 1) relative intensity threshold, 2) spatial smoothing and 3) coverage for each tissue type and ion mode by finding the parameter set that maximized the number of features detected and had a positive predictive value above 75%. True positives were classified as features detected in at least 3 replicates.

## Results

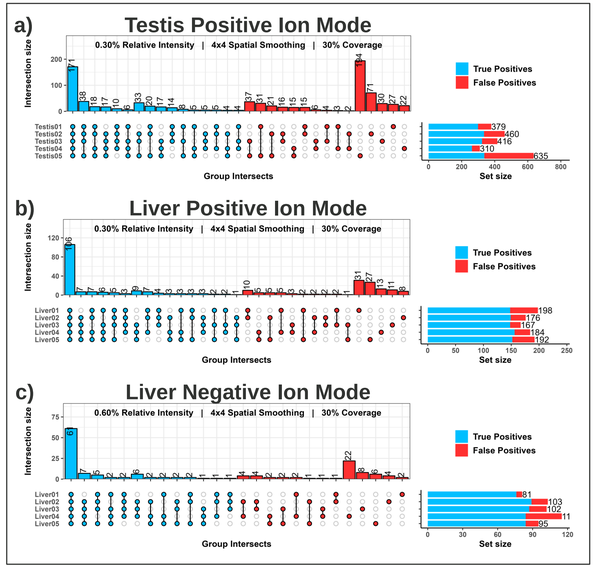


Fig. 2. Upset plots showing intersections between features detected in 5 technical replicates from the optimized parameter set from **a)** positive ion mode testis, **b)** positive ion mode liver and **c)** negative ion mode liver data. Vertical bars indicate numbers of features found in all contributing members of the group intersection. Horizontal bars indicate total numbers of features the sample replicate shared with other replicates.

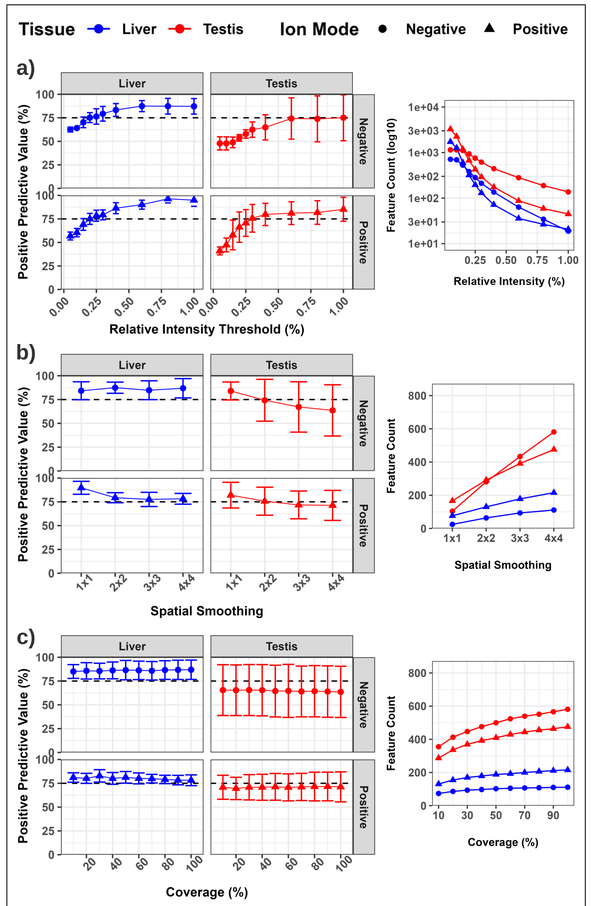


Fig. 3. Optimization of the **a)** relative intensity threshold (%) **b)** spatial smoothing and **c)** coverage (%) parameters for mouse testis and liver imaged in negative and positive ion mode. The positive predictive value (PPV) is the estimated probability a detected feature is a true feature. The horizontal dashed lines indicates a PPV of 75%. Data points are represented as the mean +/- SD of 5 replicates.

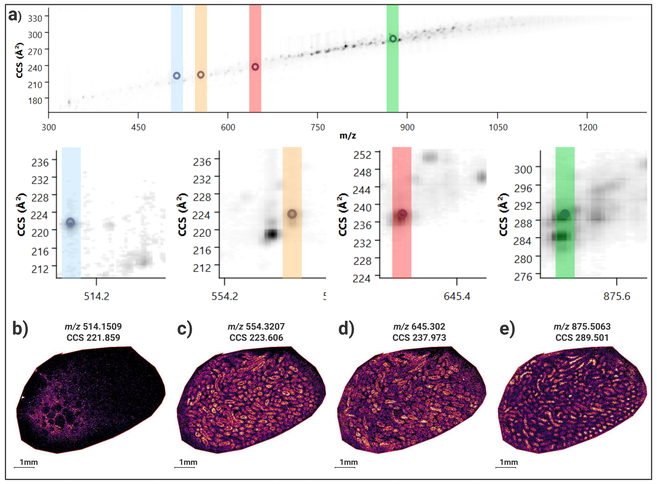


Fig. 4. **a)** *m/z* and CCS heat map with four low intensity features **b-e)** detected using a parameter set optimized for the negative ion mode testis data. All features were detected with an average peak area less than 1000 counts. **b)** Shows a highly localized low intensity ion that was detected using only a 30% pixel coverage.

## Summary

- Increasing the relative intensity threshold increased the PPV but significantly restricted the number of features extracted. An optimal threshold of 0.3% for positive ion and 0.6% for negative ion was selected.
- Increasing the spatial smoothing resulted in more features detected with only a small reduction in PPV that did not drop below 75% for 3 out of the 4 datasets. The recommended spatial smoothing for positive and negative ion mode datasets is 4x4.
- There was no significant change in the algorithm performance when changing the coverage percentage.
- Once optimized, the reproducibility of the feature finding tool between the 5 replicates was acceptable. However, outlier datasets such as Testis05 in positive ion resulted in a lower PPV as more features were detected compared to replicate tissues.
- **Detection of low S/N and highly localized features reproducibly is critical for MSI analysis. With an optimized set of parameters to use with the feature finding tool, more reliable features lists can be generated and be used confidently for further data processing.**

**References:**

1. Oetjen, J.; Meyer, S.W.; Marsching, C.; Henkel, C.; Koch, A.; Korf, A.; Kessler, N.; Timm, W.; Schweiger-Hufnagel, U.; Barsch, A.; Kobarg, J.H.; Trede, D.; Neuweger, H.; Hopf, C.: CCS-aware SpatialOMx® enables highly confident and automatic lipid annotations with regiospecific context. Bruker Application Note LCMS-177