

## Introduction

The lack of separation in mass spectrometry imaging (MSI) limits peak capacity, dynamic range and specificity. As a separation technique compatible with MSI workflow, ion mobility is critical to interpret MSI features. However there are no open-source tools for MSI analysis with ion mobility. Here we developed a open-source package that enables data exploration and feature preprocessing.

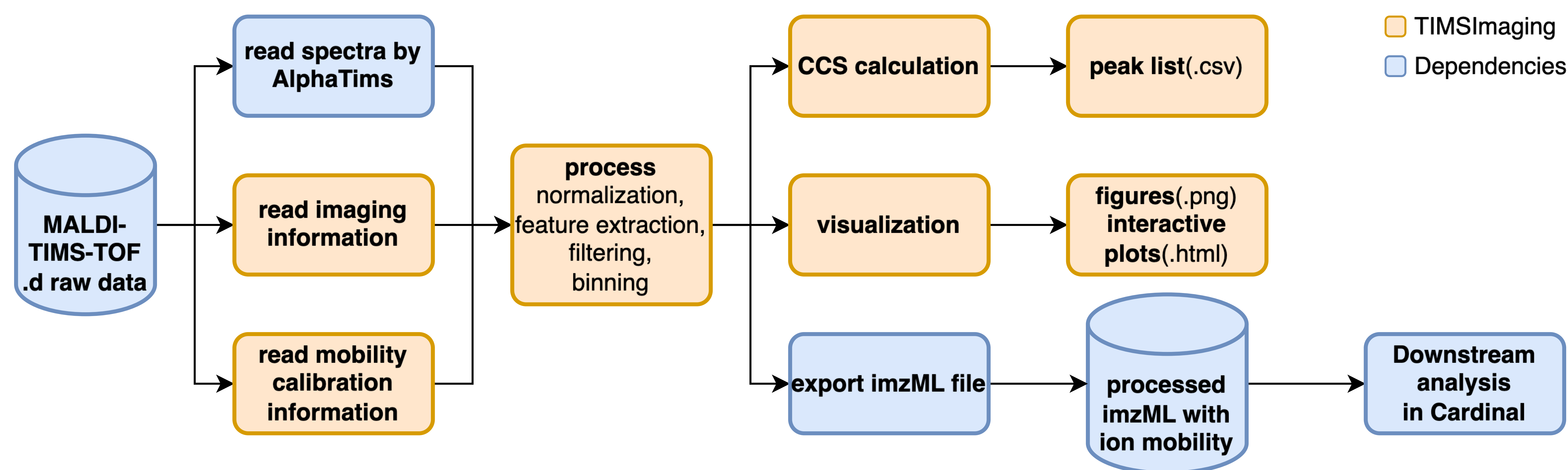


Figure.1 Overview of functionalities in TIMSIImaging

## Graph-based 2D peak-picking algorithm enables efficient feature extraction in sparse data

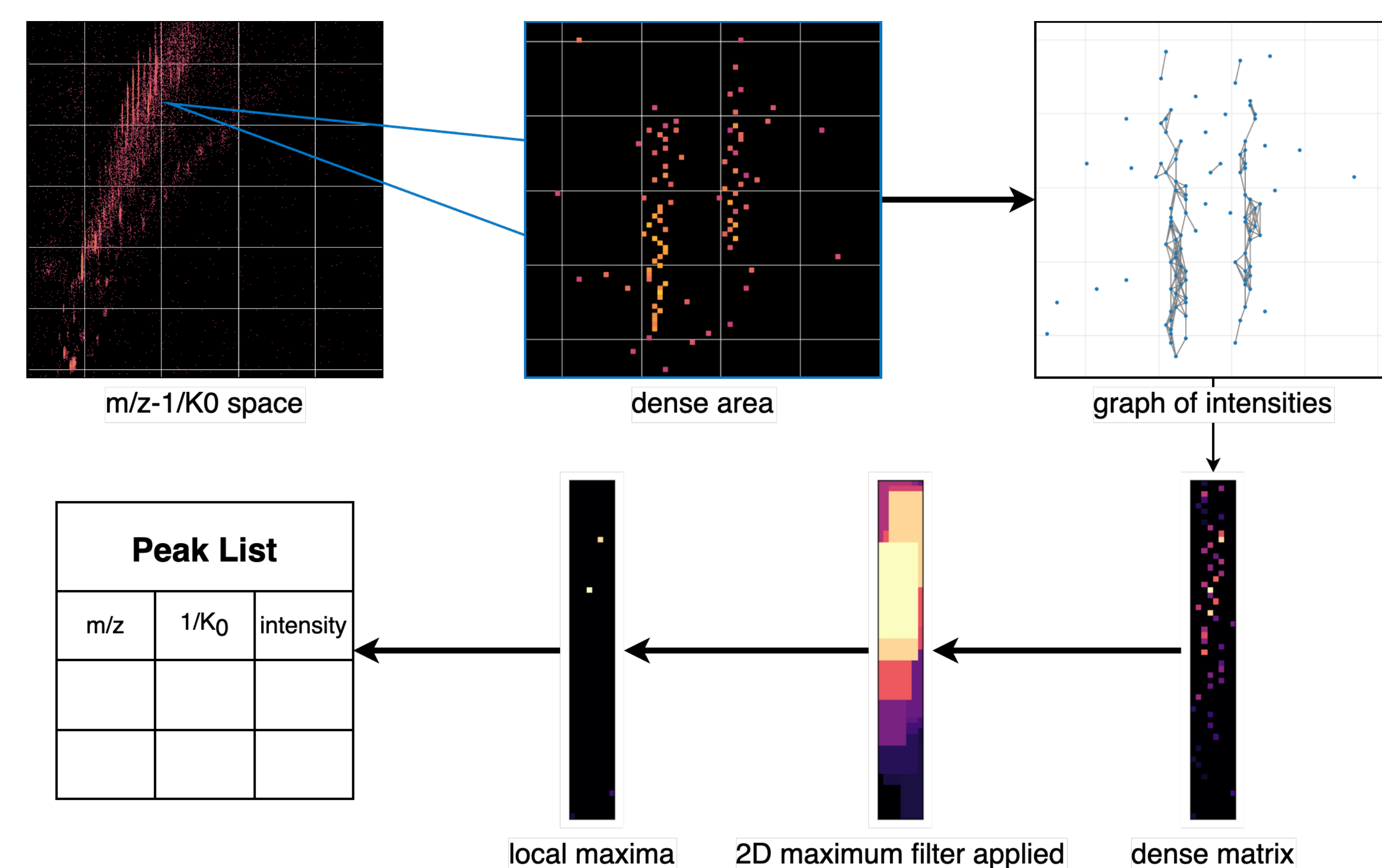


Figure.2 Overview of 2D peak picking algorithm  
First locate dense areas in two-dimensional m/z-ion mobility space by building a radius nearest neighbors graph, then detect local maxima within each area.

## Interactive visualization is friendly to non-programmer users for data exploration

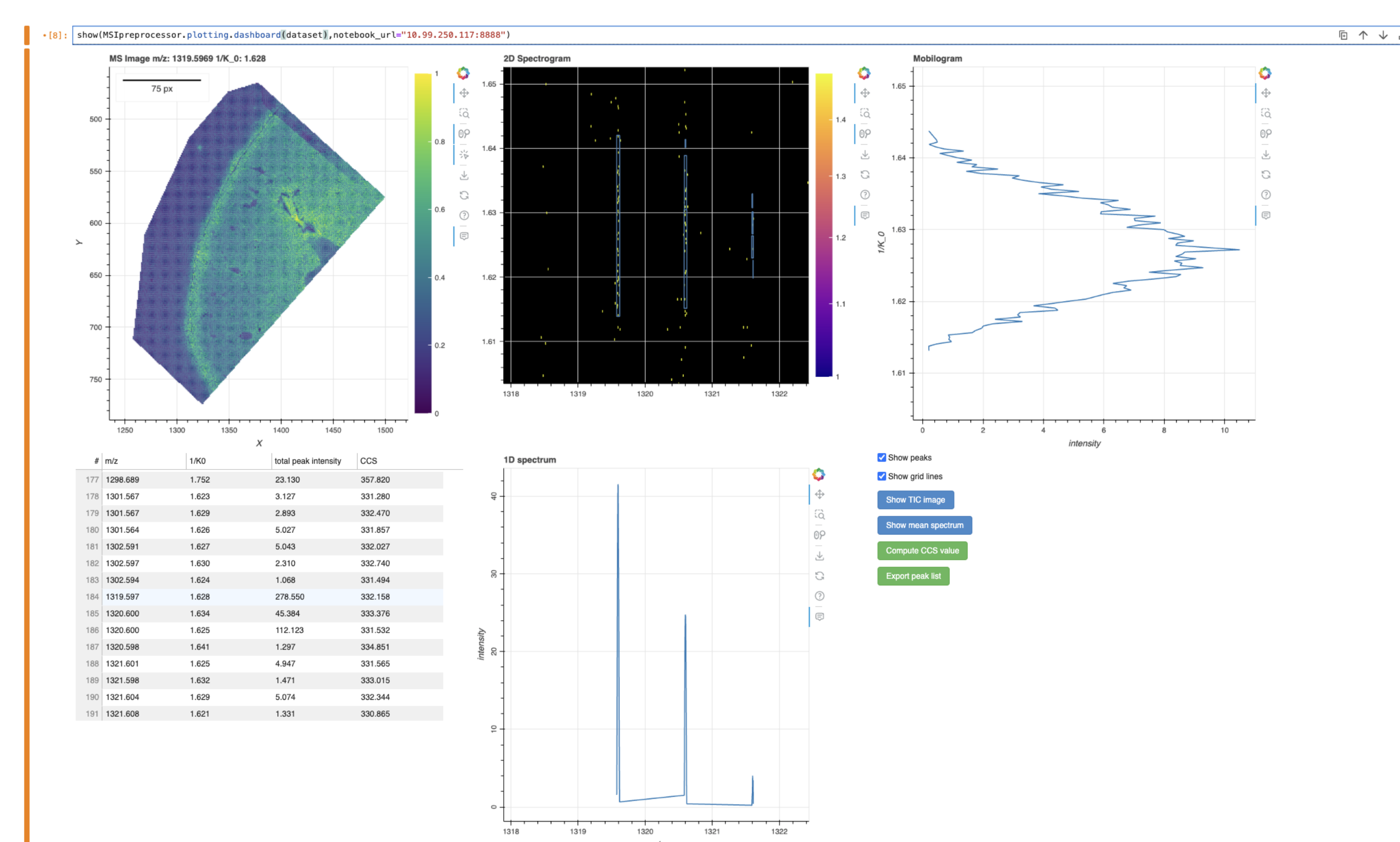


Figure.3 Screenshot of the GUI embedded in Jupyter Notebook  
By clicking pixels in the image (top left), users can view its mass spectrum (bottom middle), mobilogram (top right) and 2D spectrogram combining these two (top middle). Also, users can click entries in the peak list (bottom left) to investigate ion images and spectral details of that feature. The menu (bottom right) provides summarizing functions like TIC image and mean spectrum, as well as file export options.

## TIMSIImaging distinguishes ions with close m/z values by ion mobility

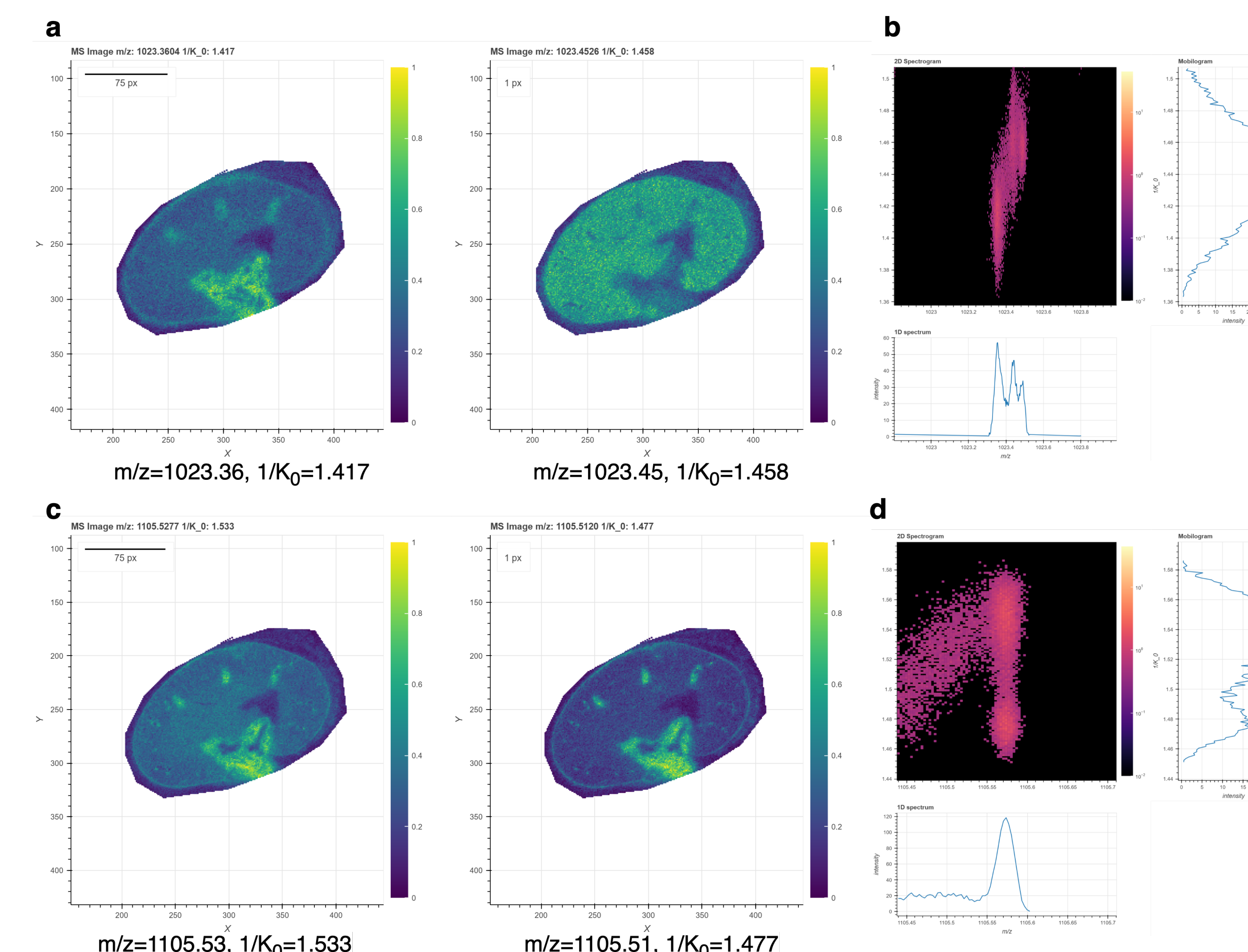


Figure.4 a) TIMSIImaging separates two ions at close m/z (b) but with very different spatial distributions; c) With 2D peak picking, TIMSIImaging is able to detect ions with the same m/z, which could not be distinguished by m/z, but separable on the mobilogram (d)

## Processed data is compatible with downstream spatial analysis in Cardinal

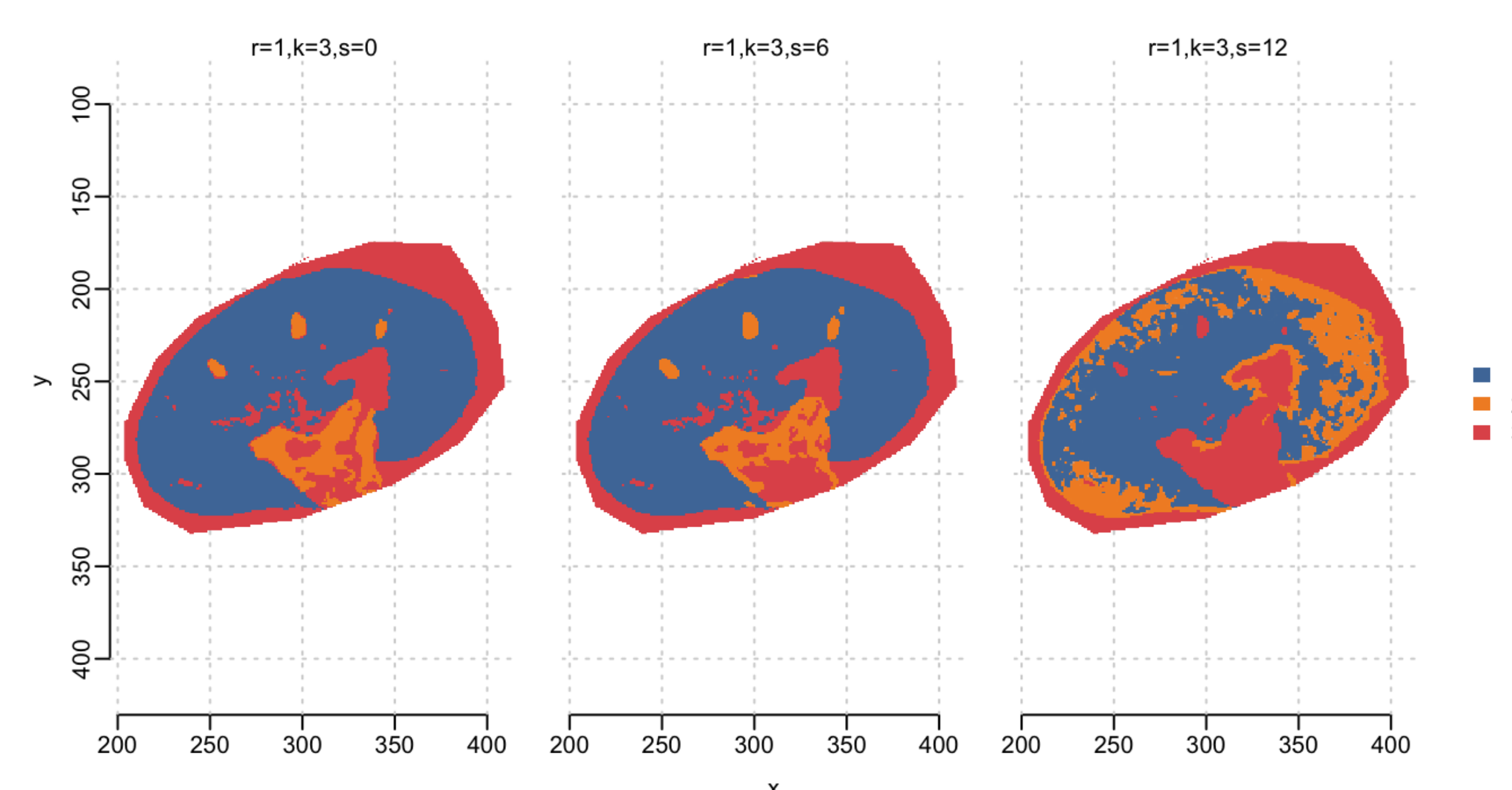


Figure.5 TIMSIImaging exports the processed data with ion mobility in open imzML format. The imzML could be read into Cardinal for spatial analysis like Spatial Shrunked Centroids segmentation.

## Conclusion

We developed TIMSIImaging, a open-source Python package, to preprocess and visualize MSI data with ion mobility. Our method could separate ions with similar m/z, detect more peaks and provide features more informative in biology to downstream MSI analysis.

**code availability:** <https://github.com/YinyueZhu/TIMSIImaging>

The authors declare no competing financial interest.