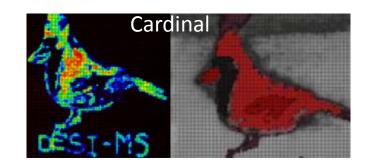
Mass spectrometry imaging analysis with Cardinal

Melanie C. Föll

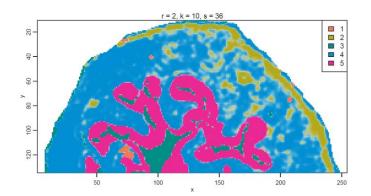
Northeastern University
Khoury College of Computer Sciences



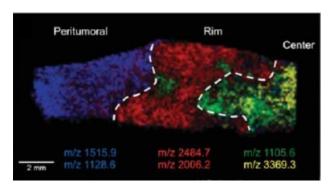


Mass spectrometry imaging

Tissue histology

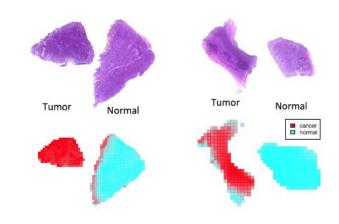


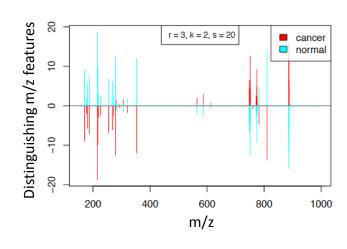
Tissue heterogeneity



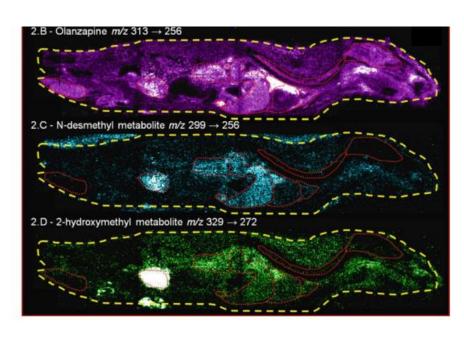
Turtoi 2014, Hepatology

Biomarker discovery



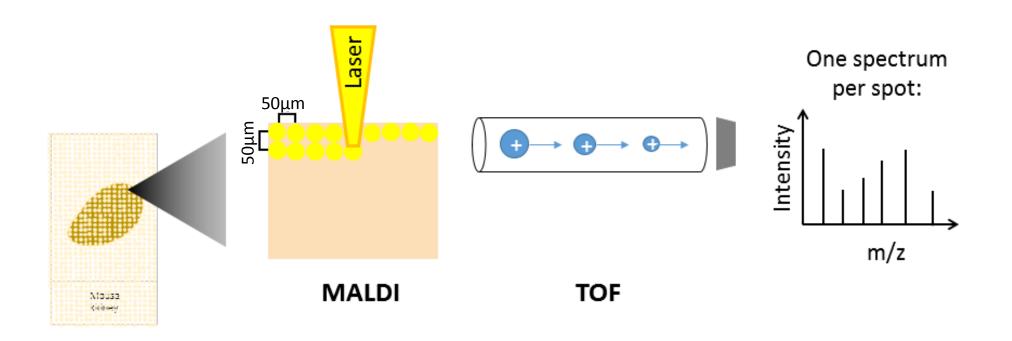


Drug imaging

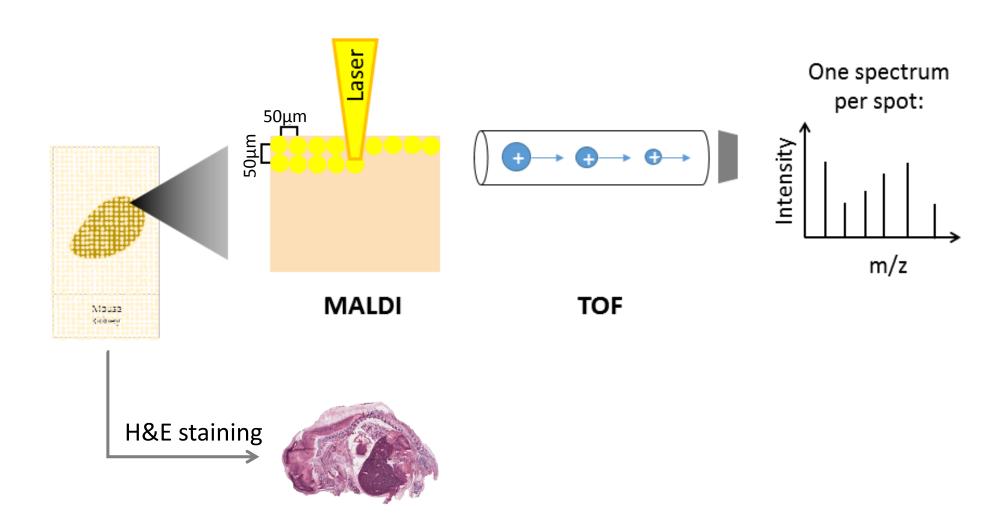


Khatib-Shahidi 2006, Anal. Chem.

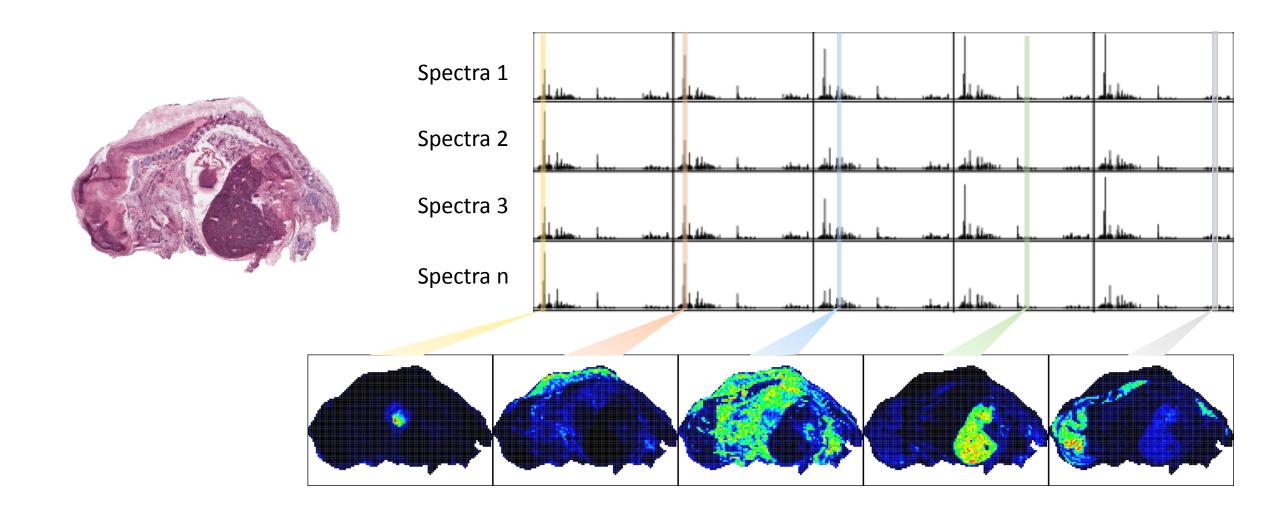
Data acquisition



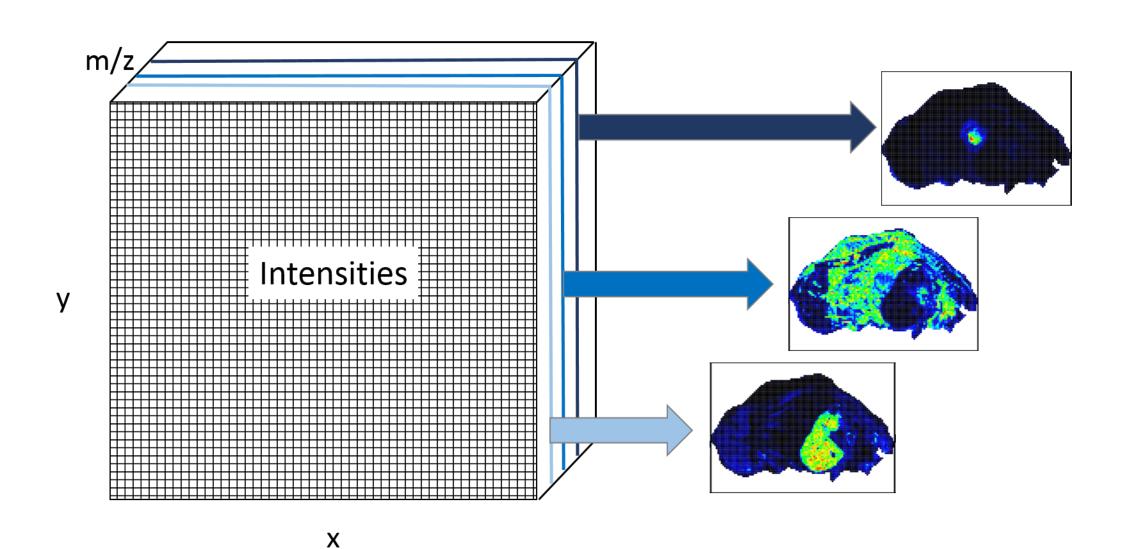
Data acquisition



From spectra to distribution profiles



From spectra to distribution profiles



Cardinal: open-source statistical software for MSI



- Free, open-source
- R-based
- Rich methods portfolio
- Compatible with larger than memory datasets
- Extensive documentation
- Active mailing list

- Over 7,000 unique downloads since public release in 2015
- 2015 John M. Chambers Statistical Software Award

Bioconductor



Software
Statistical methods
Large-than memory data

K. Bemis et al. Bioinformatics, 2015.

K. Bemis et al. Molecular and Cellular Proteomics, 2016.

K. Bemis et al. Bioinformatics, 2017.

Cardinal: Methods portfolio

Statistics & Al **Visualization Preparation Pre-processing Export Import** imzML Ion Images Annotate ROIs Normalization Colocalization imzML -Select ROI -Reference m/z –MSI data Singe m/z -MSI data Total ion current -ROI - Contrast enhancement -Annotate spectra Root-mean-square Analyze 7.5 Analyze 7.5 - Image smoothing Reference m/z Classification Subset & Filter -MSI data -MSI data - Scaling Baseline removal PLS-DA -m/z and mass range - color palette Text files Text files OPLS-DA Spectra and pixels -Median -Multiple m/z overlay Intensity matrix -Intensities -Spatial Shrunken Centroids Locmin Image files -Summary statistics -m/z features - Gaussian Combine (TIC, median, ...) **Smoothing** -Mass spectra - Adaptive **RData** -Samples Gaussian Pixel coordinates Mass spectra -Spectra Segmentation -Moving average Rscript Experimental metadata Single spectrum -Savitzky-golay Single ions Transformation Multiple spectra overlav Markdown files - Spatial-DGMM Image files Log-transform Spectral alignment Summary statistics -Multivariate lon images Replace values (Mean, sum, ...) -Mean - PCA -Mass spectra (NA, negative, ...) -Reference Statistical results - Spatial k-means Statistical results Re-calibration - Gaussian **RData** Adaptive Peak detection Stastistical models - Spatial Shrunken -Simple Software Centroids Rscript -Adaptive - Gaussian ∟MAD -R analysis Adaptive Peak alignment Markdown files Class comparison matter -Mean R analysis Reference Summary statistics Peak integration Peak height -Peak area Mass binning Parallel CPU computation

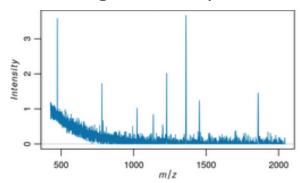
Out-of-memory computation

Visualizations

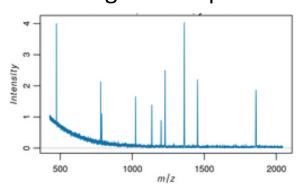
Mass spectra

plot(mse, ...)

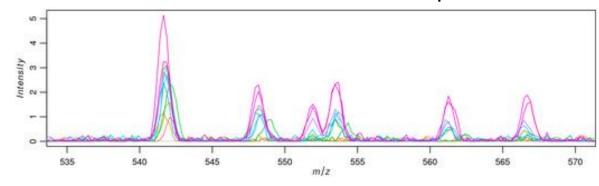
Single mass spectra



Average mass spectra

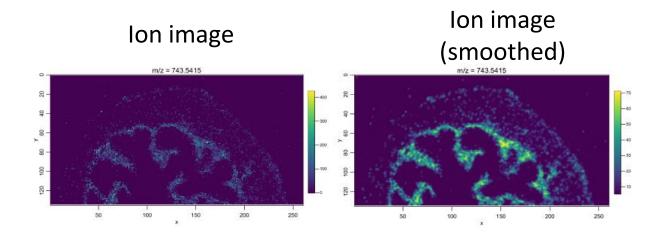


Zoomed in & overlaid mass spectra

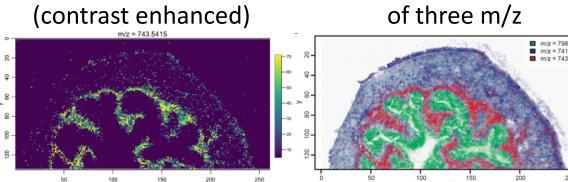


Ion images

image(mse, ...)



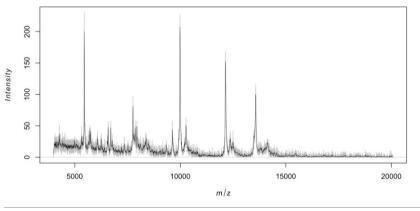
Ion image (contrast enhanced)



Ion image overlay of three m/z

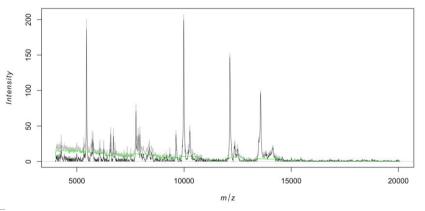
Pre-processing

Smoothing



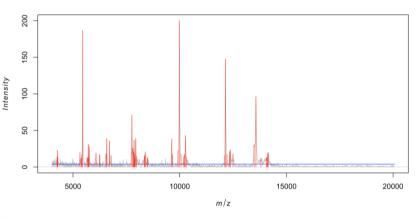
smoothSignal(mse, ...)

Baseline removal



reduceBaseline(mse, ...)

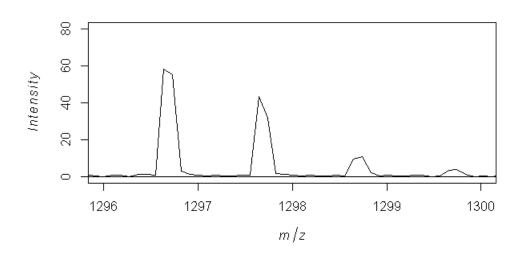
Peak detection



peakPick(mse, ...)

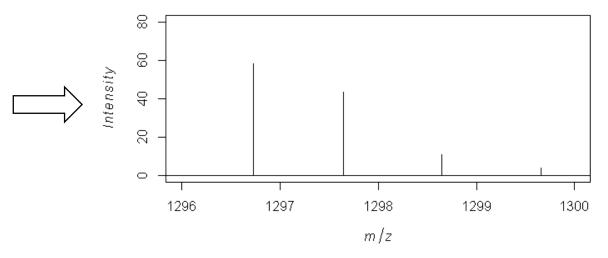
Pre-processing

Profile spectrum



centroided(mse) = FALSE

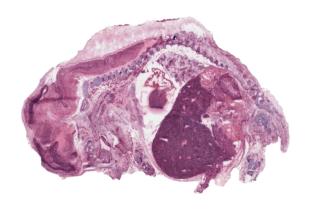
Centroided spectrum

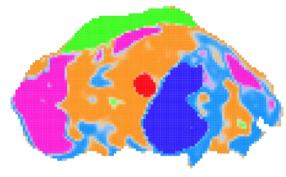


centroided(mse) = TRUE

Statistics & Al

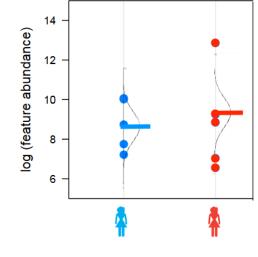
1. Class discovery (segmentation)

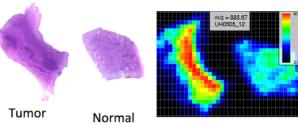




spatialShrunkenCentroids(mse, ...) spatialDGMM(mse, ...)

2. Class comparison (linear models)

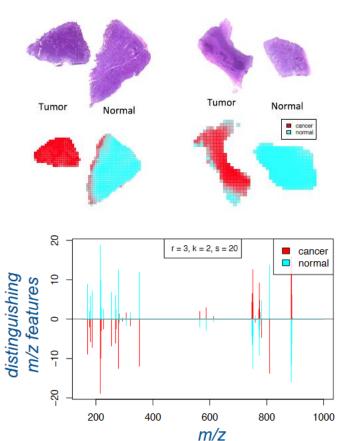




meansTest(mse, ...)
segmentationTest(mse, ...)

.

3. Class prediction (classification)



spatialShrunkenCentroids(mse, ...)

. . . .

MSI data import

& export

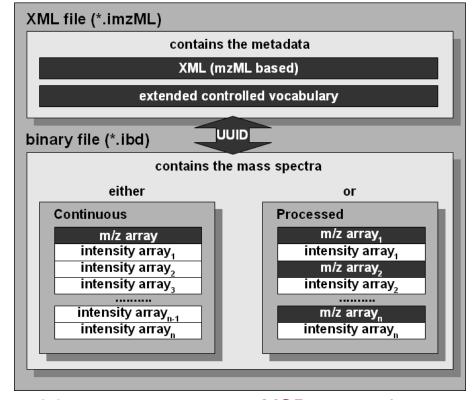
readImzML(mse, ...)

writeImzML(mse, ...)

imzML: open standard file format for MSI data

File1: .imzML

File2: .ibd



MSContinuous

MSProcessed

ImagingExperiment

ImagingExperiment

imzML converter:

https://www.cs.bham.ac.uk/~ibs/imzMLConverter

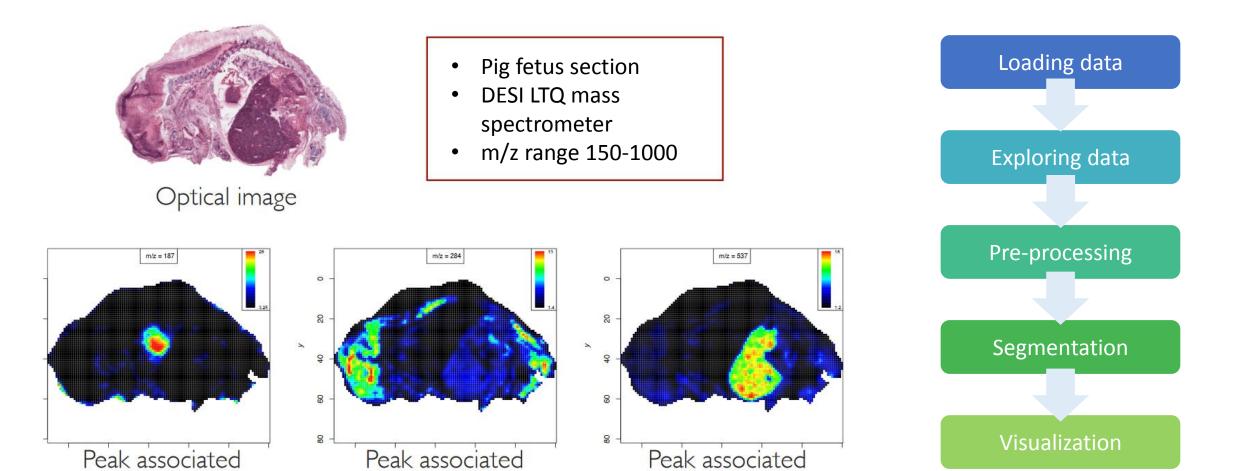
Race, Alan M., Iain B. Styles, and Josephine Bunch. "Inclusive sharing of mass spectrometry imaging data requires a converter for all." Journal of proteomics 75.16 (2012): 5111-5112.

imzML validator:

https://gitlab.com/imzML/imzMLValidator

Race, Alan M., and Andreas Römpp. "Error-Free Data Visualization and Processing through imzML and mzML Validation." *Analytical chemistry* 90.22 (2018): 13378-13384..

Today's dataset & analysis



Vignette:

with heart

http://bioconductor.org/packages/release/data/experiment/vignettes/CardinalWorkflows/inst/doc/MSI-segmentation.html

with liver

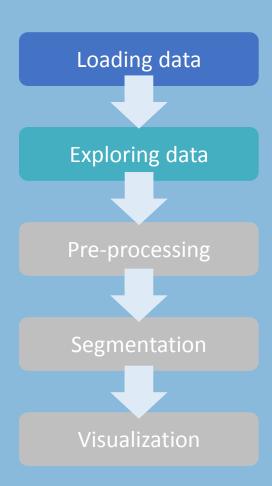
with brain

Part I: Loading & exploring MSI data

Loading: 75%







Importing MSI data

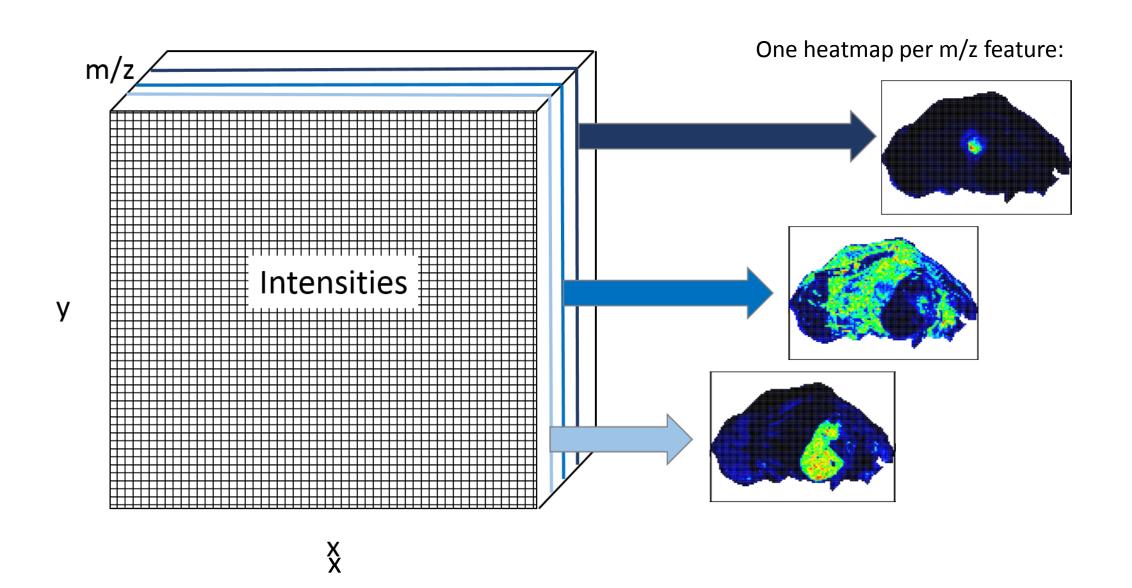
• From CardinalWorkflows package

Object type: MSImageSet (old format)

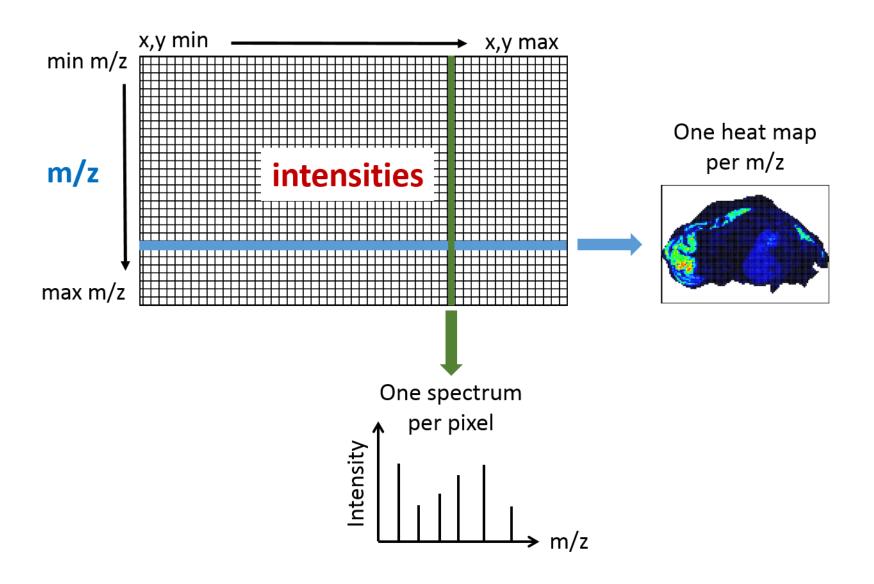
Coercion to MSImagingExperiment (current format)



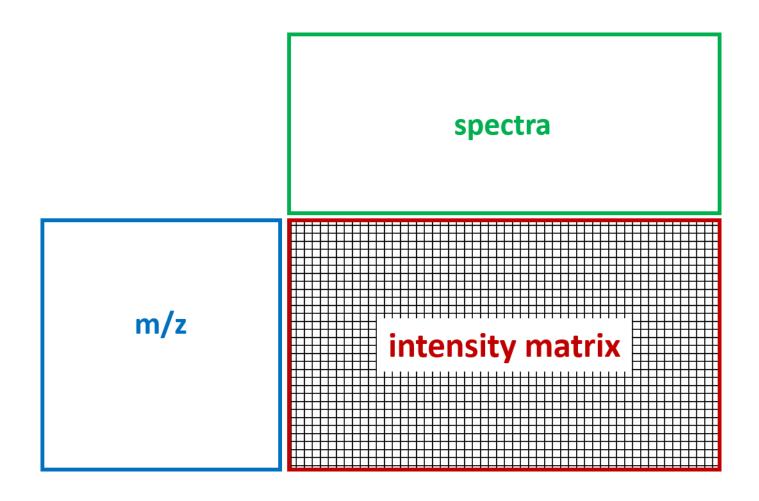
MSI data structure – 3D presentation



MSI data structure – 2D presentation



MSImagingExperiment – basic data structure



MSImagingExperiment – basic data structure

spectra

```
pixelData(mse) = pData(mse)
coord(mse)
run(mse)
```

m/z

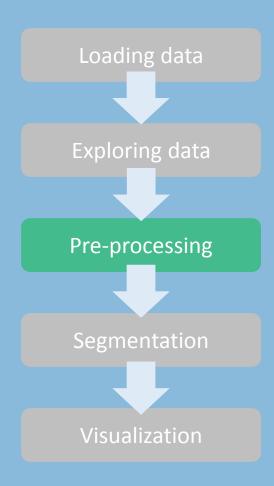
mz(mse) featureData(mse) = fData(mse)

intensity matrix

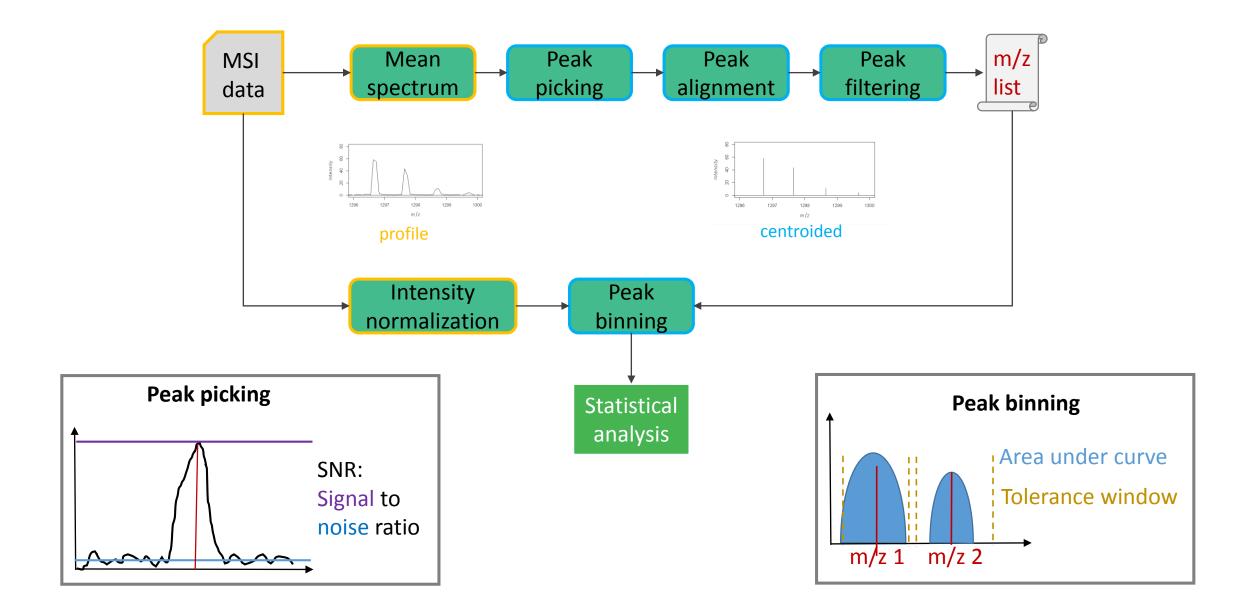
R

Part II: Pre-processing





Pre-processing workflow



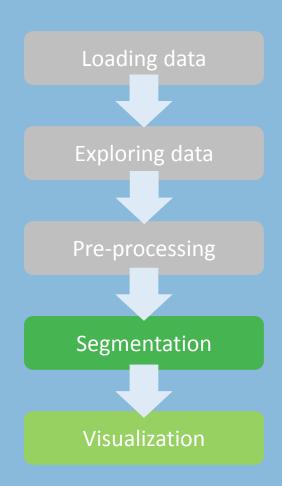
R

Part III: Segmentation & visualization







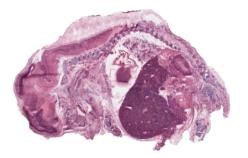


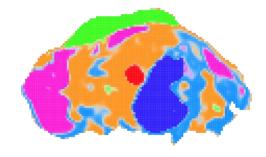
Unsupervised segmentation

Spatial shrunken centroids:

spatially-aware segmentation with feature selection

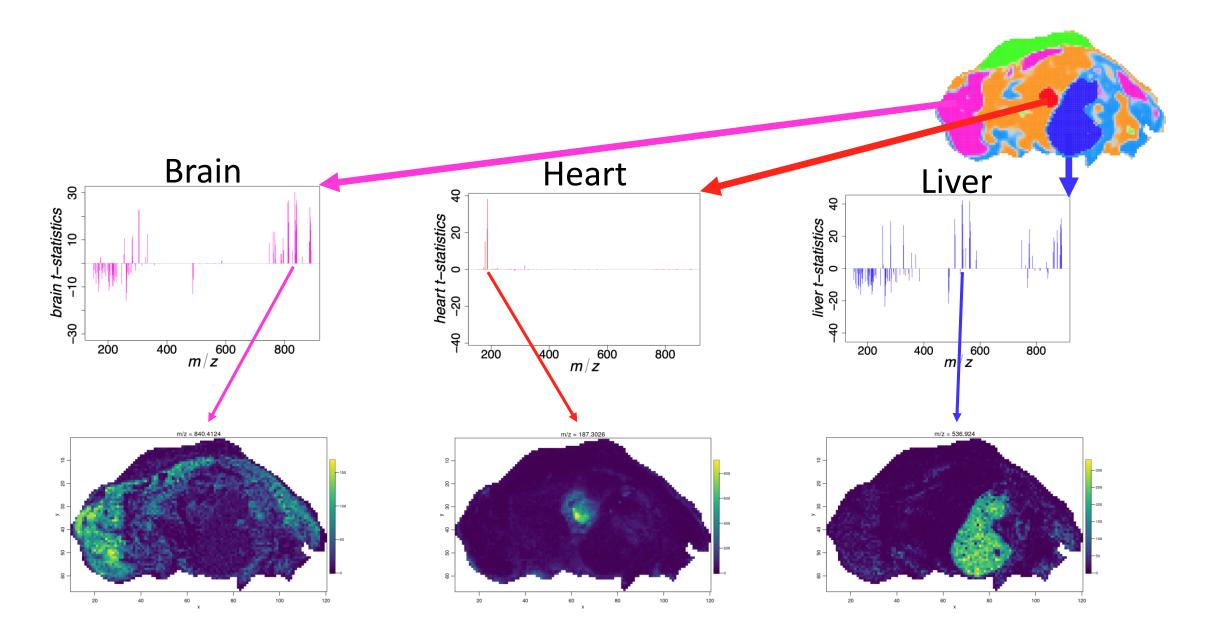
- Data-driven selection of appropriate number of segments
- Selects most important m/z for distinguishing segments
- Probablity model characterizes uncertainty



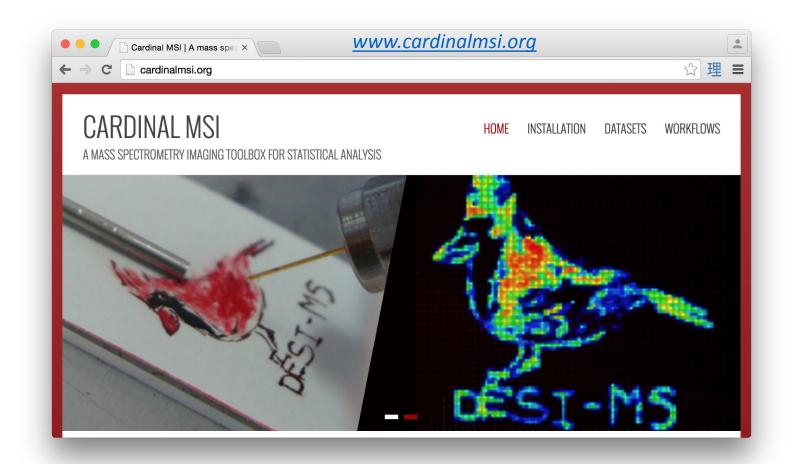


K. Bemis, et al. "Probabilistic segmentation of mass spectrometry (MS) images helps select important ions and characterize confidence in the resulting segments." *Molecular & Cellular Proteomics* 15.5 (2016): 1761-1772.

Visualization of most important m/z



Cardinal: resources & thank you





Kylie Bemis Olga Vitek





Dan Guo Sai Lakkimsetty

User guides/vignettes Videos May Institute Help forum

http://cardinalmsi.org/workflows/ https://www.youtube.com/c/MayInstituteNEU/playlists http://groups.google.com/d/forum/CardinalMSI