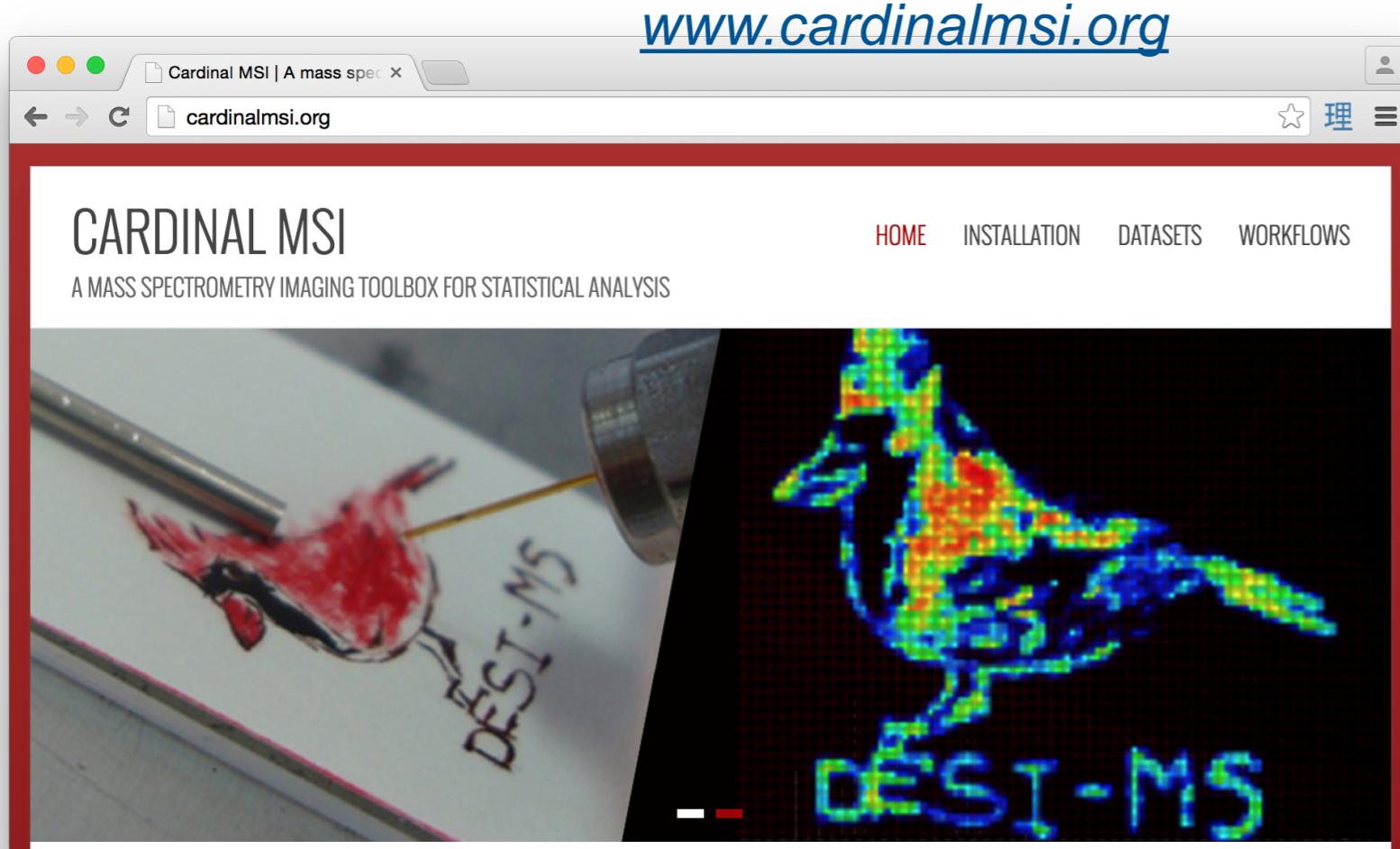


# CARDINAL

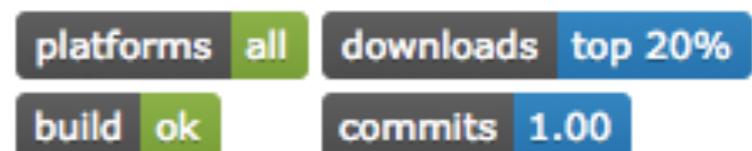
## Open-source statistical software for MSI



- Free, open-source
- R-based
- Data processing & visualization
- Image segmentation & classification
- Extensive documentation
- Active mailing list

- Over 6,000 unique downloads since public release in 2015
- 2015 John M. Chambers Statistical Software Award

### Bioconductor



### Software

### Statistical methods

### Larger-than memory data

K. Bemis et al *Bioinformatics*, 2015.

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

K. Bemis et al *Bioinformatics*, 2017.

# WHY R?

- *Researcher:*
  - Lightweight: minimal software/hardware requirements
  - Interactive: easy data exploration on a laptop
- *Developer:*
  - Large community: leverage state-of-the-art
  - Easy to customize/extend: e.g. include in existing pipelines
- *Science:*
  - Full transparency: open algorithms and code
  - Infrastructure for fully documentable workflows

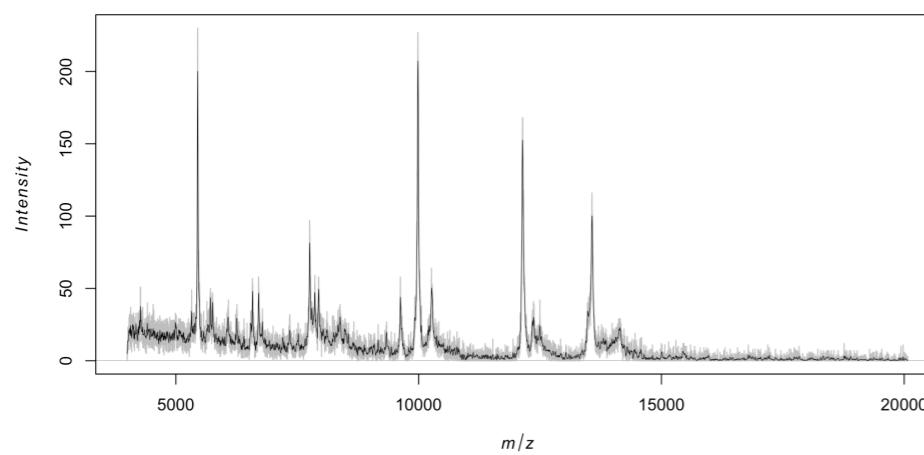
# SOFTWARE FOR MSI EXPERIMENTS

full workflows, focus on experiments, not just datasets

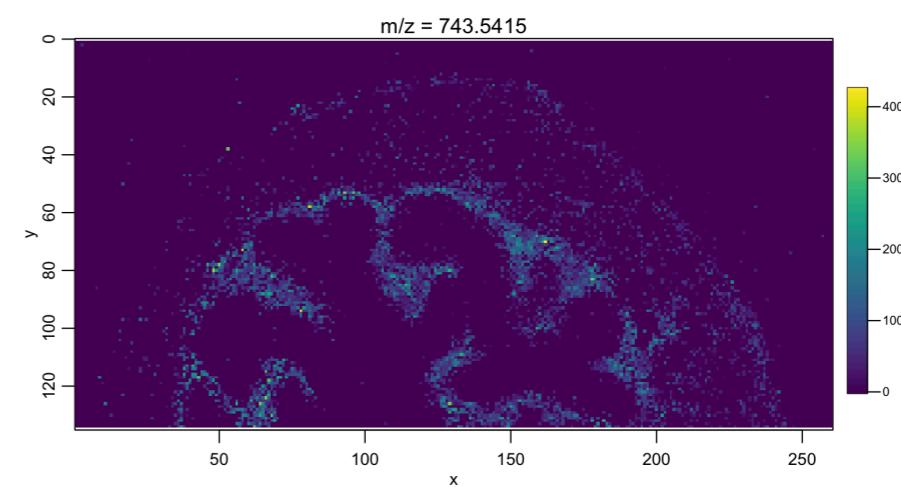
- Format support
  - imzML (continuous & processed) and Analyze 7.5
- Visualization
  - Plotting of mass spectra and molecular images
- Spectral processing
  - Normalization, smoothing, baseline reduction, peak picking
- Image processing
  - Contrast enhancement, spatial smoothing
- Statistical analysis
  - PCA, PLS, spatial shrunken centroids (classification & segmentation)

# SPECTRAL AND IMAGE PROCESSING

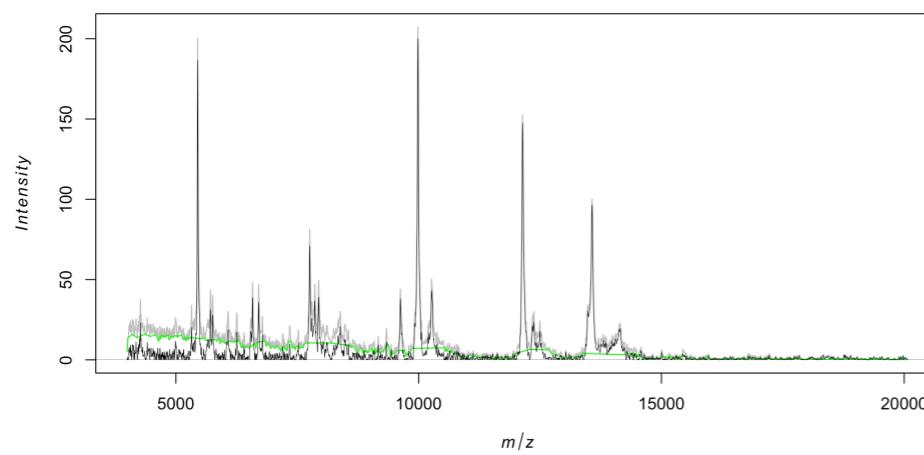
`smoothSignal(mse, ...)`



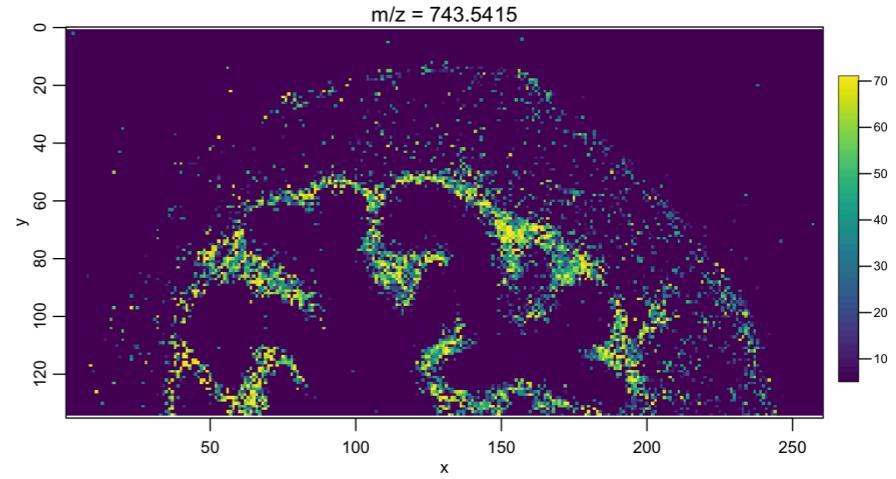
`image(mse, mz=743.5482)`



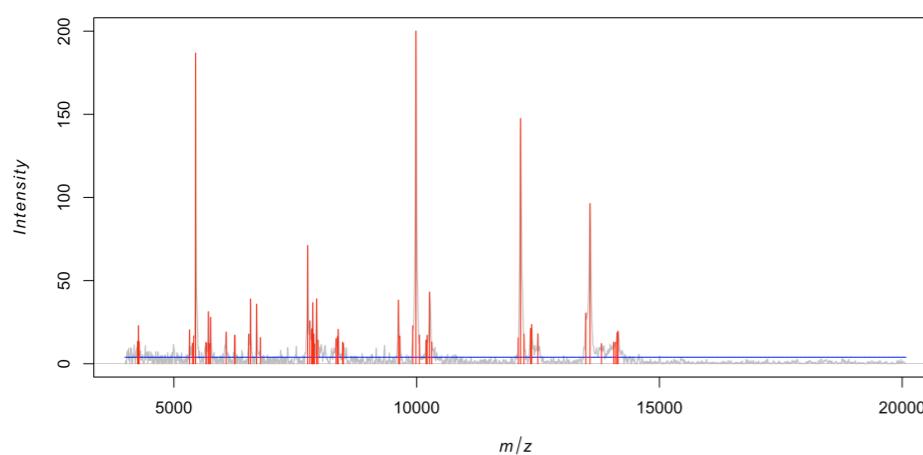
`reduceBaseline(mse, ...)`



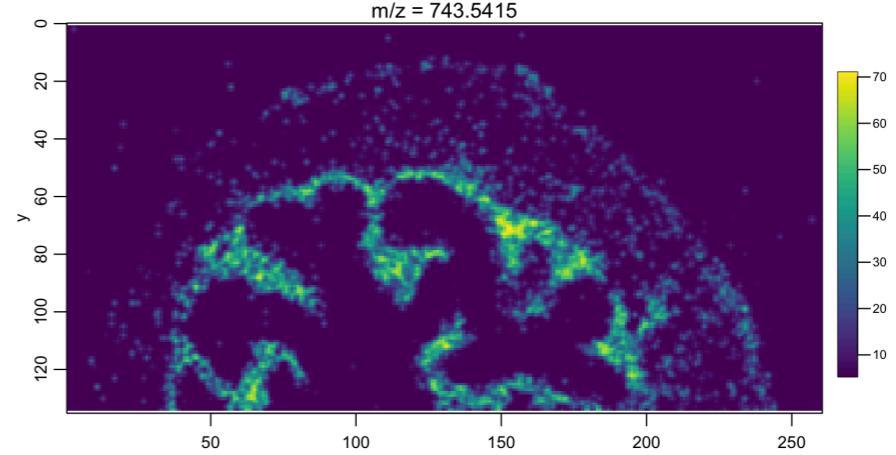
`image(..., contrast增强="histogram")`



`peakPick(mse, ...)`



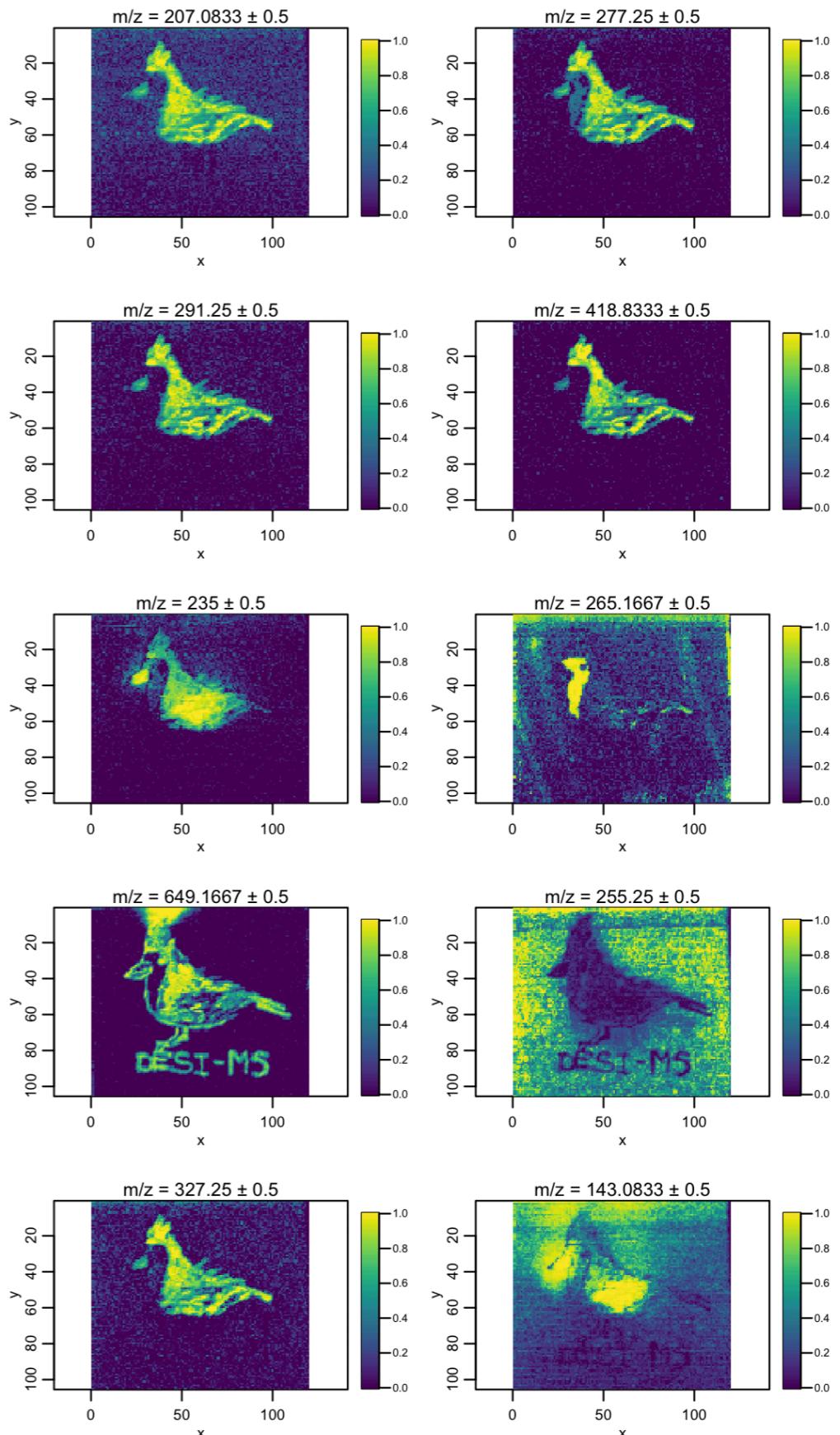
`image(..., smooth.image="gaussian")`



# VISUALIZATION TOOLS

```
library(CardinalWorkflows)  
  
data(cardinal, cardinal_analyses)  
  
top <- topFeatures(cardinal.sscg,  
                     model=list(r=1, k=10, s=3),  
                     n=10)  
  
cardinal <- as(cardinal,  
                 "MSImagingExperiment")  
  
image(cardinal,  
      mz=top$mz,  
      plusminus=0.5,  
      normalize.image="linear",  
      contrast.enhance="histogram",  
      layout=c(3,3))
```

*Plot top 10 ion images  
from spatial segmentation*



# VISUALIZATION TOOLS

```
image(cardinal,  
mz=c(207.08, 235, 255.25, 265.17, 649.17),  
plusminus=0.5,  
normalize.image="linear",  
contrast.enhance="histogram",  
col=c("red",  
      "darkred",  
      "gray",  
      "black",  
      "brown"),  
superpose=TRUE)
```

*Recreate painting from  
overlay of ion images*

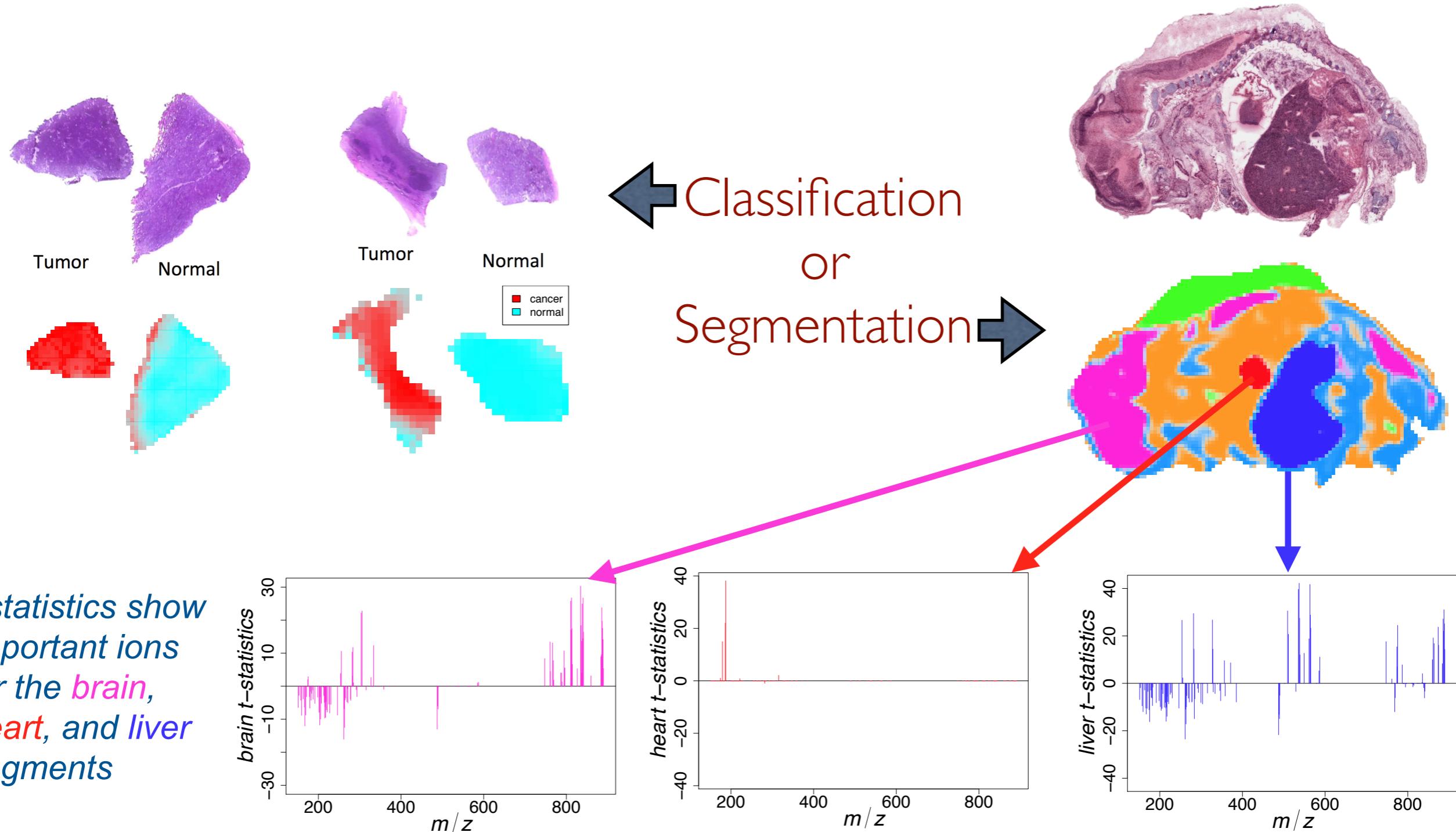


# STATISTICAL METHODS

- *Exploratory analysis*
  - Principal components analysis — **PCA()**
  - Feature colocalization — **colocalized()**
- *Image segmentation*
  - Spatial shrunken centroids clustering — **spatialShrunkenCentroids()**
  - Spatial Dirichlet Gaussian mixture modeling — **spatialDGMM()**
- *Classification and cross-validation*
  - Projection to latent structures — **PLS()** and **OPLS()**
  - Spatial shrunken centroids classification — **spatialShrunkenCentroids()**
- *Class comparison*
  - Group means-based testing — **meansTest()**
  - Segmentation-based testing — **segmentationTest()**

# SPATIAL SHRUNKEN CENTROIDS

## spatially-aware classification/segmentation with feature selection



- K. Bemis, A. Harry, L. S. Eberlin, C. Ferreira, S. M. van de Ven, P. Mallick, M. Stolowitz, O. Vitek. "Probabilistic segmentation of mass spectrometry images helps select important ions and characterize confidence in the resulting segments ". Molecular & Cellular Proteomics, 2016