Data analysis

Based on the R-package Cardinal, R, and the Galaxy platform (https://galaxy.uni-freiburg.de)

Prerequisites:
- Digestable control peptides spotted
- Internal Calibrants used
- For regions of interest (ROI):
  - Teachmarks on slide
  - H&E staining after MALDI imaging analysis
  - annotation of regions of interest by a pathologist

1. Quality control
   a. Control of equal matrix spray
   b. Control of digestion
   c. Control of general machine performance
   d. Descriptive statistics of data
2. Preprocessing options:
   a. Mass recalibration
   b. Normalization
   c. Baseline reduction
   d. Spectra smoothing
   e. Peak picking
   f. Peak alignment
   g. Resampling
   h. Binning
3. Normalization, transformation & filtering
   a. Log-transformation
   b. Normalization on patient/sample level
   c. Filtering for masses of interest (e.g. masses present in at least 5% of pixels)
   d. Filtering for pixels of interest (e.g. tumor regions of interest = ROI)
4. Visualization
   a. Heatmaps with ion images of interest
   b. Mass spectra of pixels of interest
5. Unsupervised analysis (spatial clustering):
   a. Principal component analysis
   b. K-means clustering
   c. Spatial shrunken centroids
6. Supervised analysis:
   a. Fold change ranking
   b. Regression analysis (LIMMA, Lasso, etc.)