Informed Identification and Quantitation of Peptides in LC-IMS-MS Based Proteomics Data

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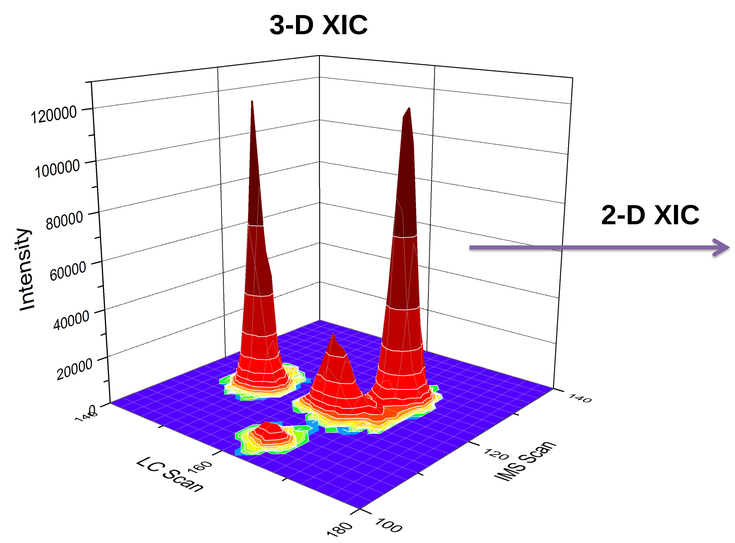
Introduction

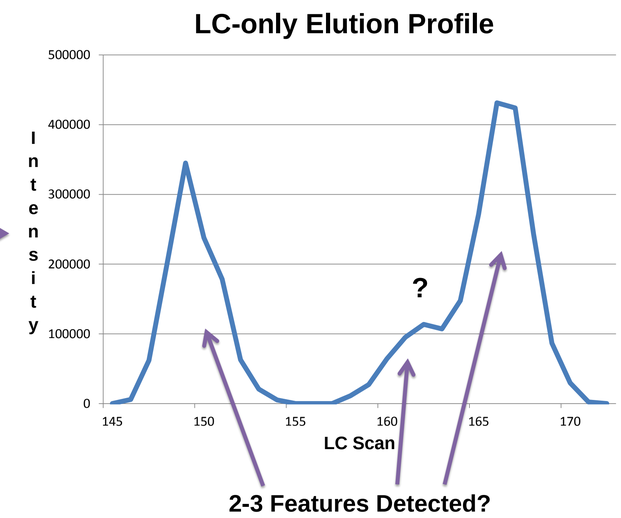
- Ion mobility provides an extra dimension of separation for greater confidence in peptide identifications
- Data processing algorithms for mass spectrometry data are numerous, slow, and prone to errors
- Informed analysis, using prior knowledge, is becoming a popular technique of data analysis
- Improvements to informed analyses such as faster processing algorithms are necessary to search all possible peptides (millions) in the genome

Algorithm Workflow

- Users enumerate target list (e.g. all tryptic peptides)
- For each peptide target
  - For each possible charge state
    - Generate theoretical isotopic profile
    - Grab 3-D Extracted Ion Chromatogram (XIC)
      - Elution time, drift time, intensity
    - Find individual 3-D features
    - For each feature
      - Find apex of LC and IMS separation and perform saturation correction if necessary
      - Extract observed isotopic profile and compare to theoretical
      - Filter bad results
        - Mass error, poor isotopic envelope quality, incorrect charge state, etc.
      - Quantify
  - Correlate charge states using elution time
  - Report results

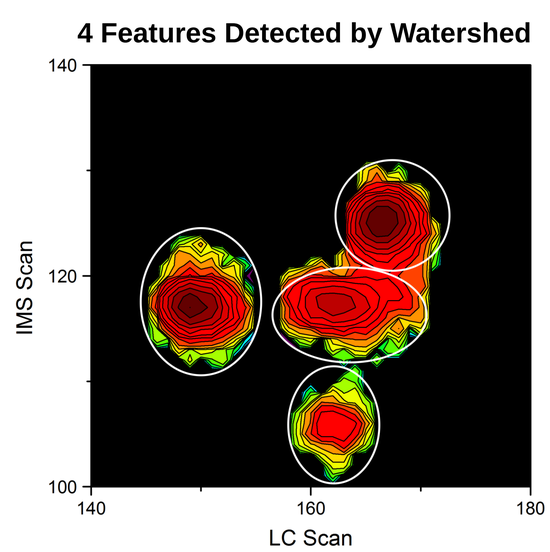
Feature Extraction and Detection





- XIC-based feature extraction instead of spectrum-based
  - Average of 0.217 ms per XIC extraction
- Uses a watershed algorithm for feature detection commonly used in image processing

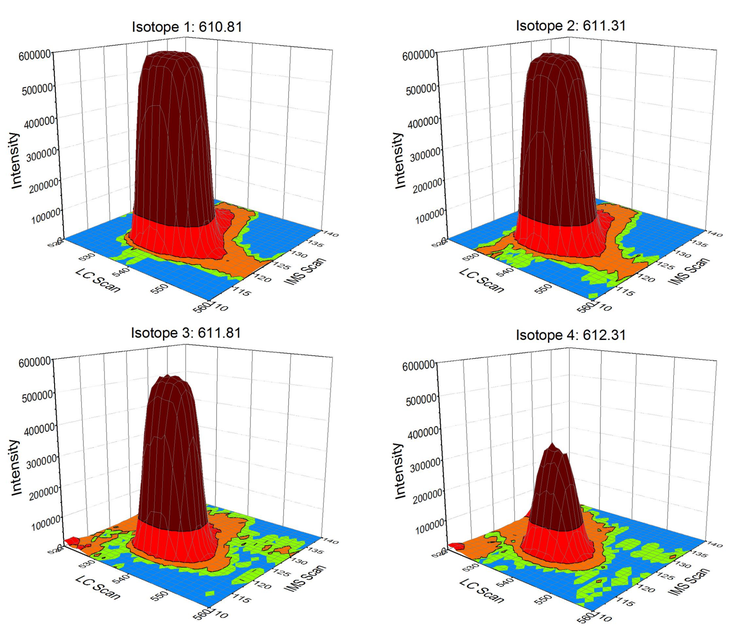
- Unable to detect all features using LC dimension
- Ion mobility dimension allows for detection of 4 features

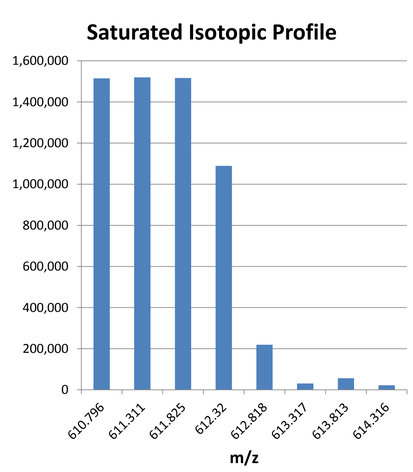


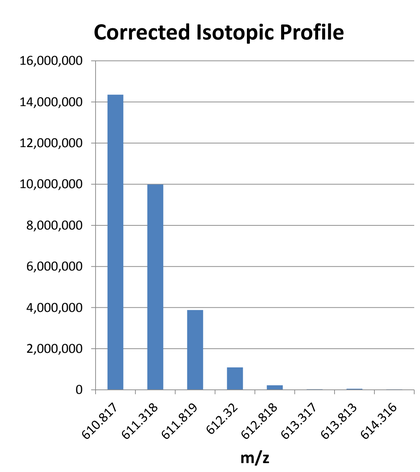
Watershed Algorithm

- The watershed algorithm is extremely fast
  - Average of 0.255 ms per 3-D XIC
- Commonly used for edge detection in images
  - Imagine 3-D features as an overhead heat map
- How to use the algorithm:
  - First apply Savitzky-Golay smoothing
  - Filter out low-level noise features if desired

Saturation Correction







- Find isotope that is not saturated (4th isotope in above case)
- Use unsaturated isotope to find accurate LC and IMS apex

- Saturation correction increases dynamic range
  - 1 order of magnitude added in above case
- Allows for accurate quantitation of very abundant peptides

Conclusions

- Algorithms developed and implemented for informed analysis of LC-IMS-MS data
- Uses prior knowledge about each peptide target to extract evidence for the peptide
- Replaces multiple levels of processing normally executed globally during data analysis such as deisotoping, feature finding, etc.
- Fast processing allows for targeting millions of peptides instead of thousands
- Saturation correction provides greater dynamic range
  - Essential for ToF-based instruments
- Similar approaches can be adopted to traditional LC-MS data

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