COMPUTATIONAL PROTEOMICS AND METABOLOMICS

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5. Quantification II: Label-free quantification, SILAC

Overview

- Label-free quantification
    - Definition of features
    - Feature finding on centroided data
    - Absolute quantification using label-free quantification
- SILAC quantification
    - Problem
    - Application of simple feature finding and linking
    - MaxQuant algorithm
    - Application examples

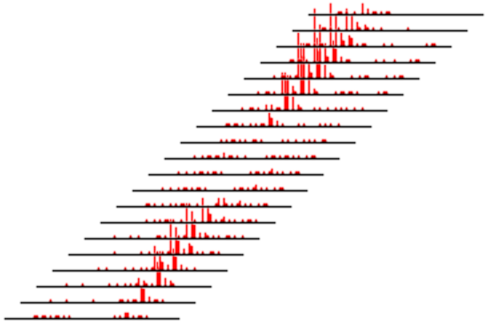
LEARNING UNIT 5A
FEATURE FINDING FOR LABEL-FREE QUANTIFICATION

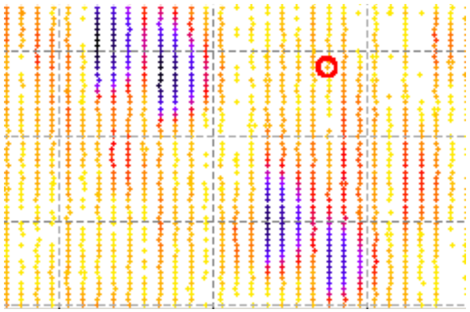
Feature-finding

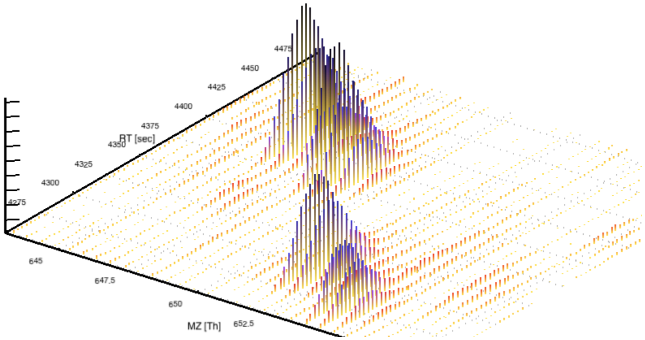
- Definition of terms (maps, features)
- Key concepts in label-free quantification
- Averagine model
- Feature finding on centroided data

Label-Free Quantification (LFQ)

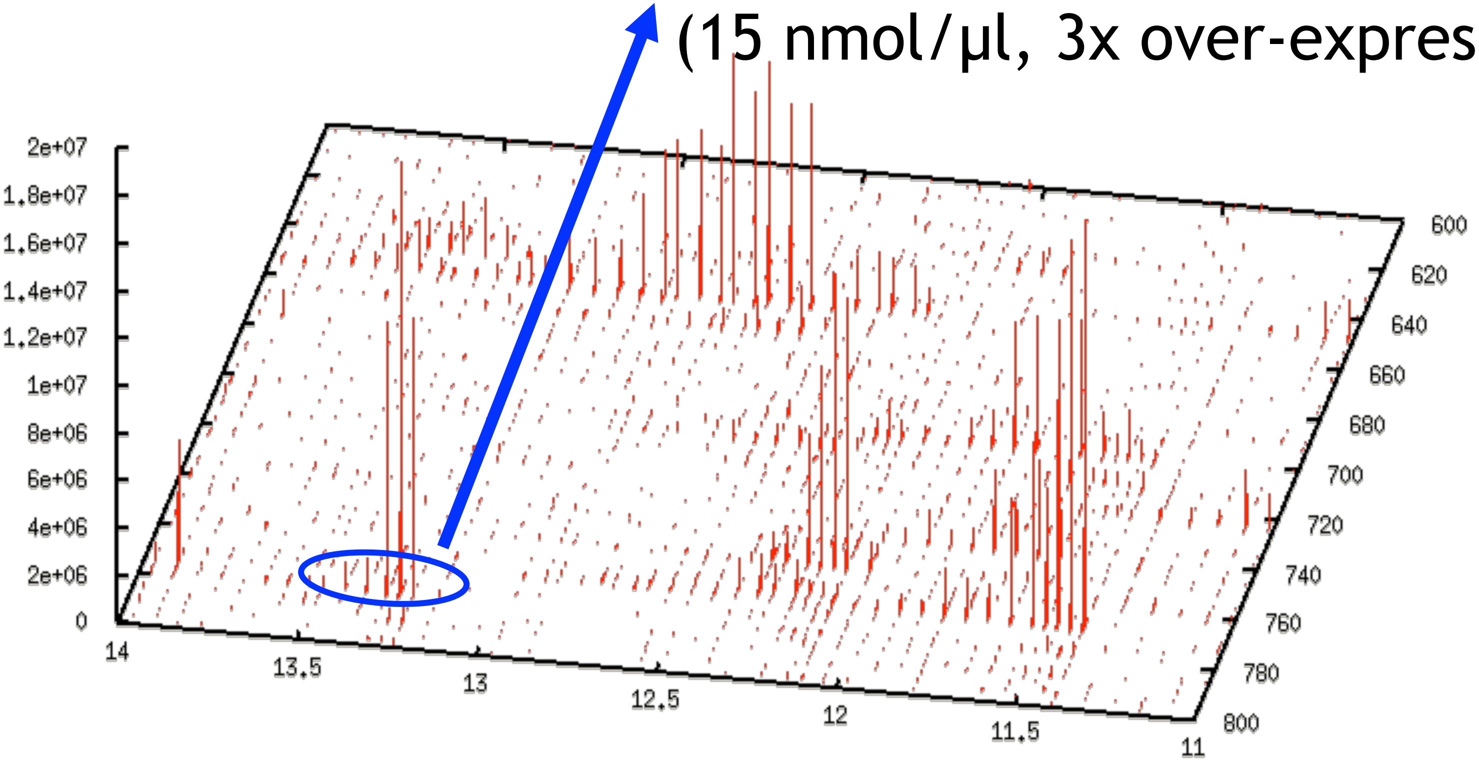
- **Quantification through the ion current in MS spectra**
- Key advantage: no labeling needed – cheap, scales well
- Key disadvantage: normalization tricky – direct comparison
- Based on the notion of **features** and **maps**
  - LC-MS data: 2D datasets of up to hundreds of GB per sample
  - **Raw data**: unmodified detector signal
  - **Centroided data**: peaks called on the MS level
  - **Features**: the stuff that matters in **maps**







LC-MS Data (Map)



Quantification

(15 nmol/µl, 3x over-expressed, …)

Feature Finding – Terms

Map:

Two-dimensional data set (RT, m/z) containing the MS signal from one LC-MS run.

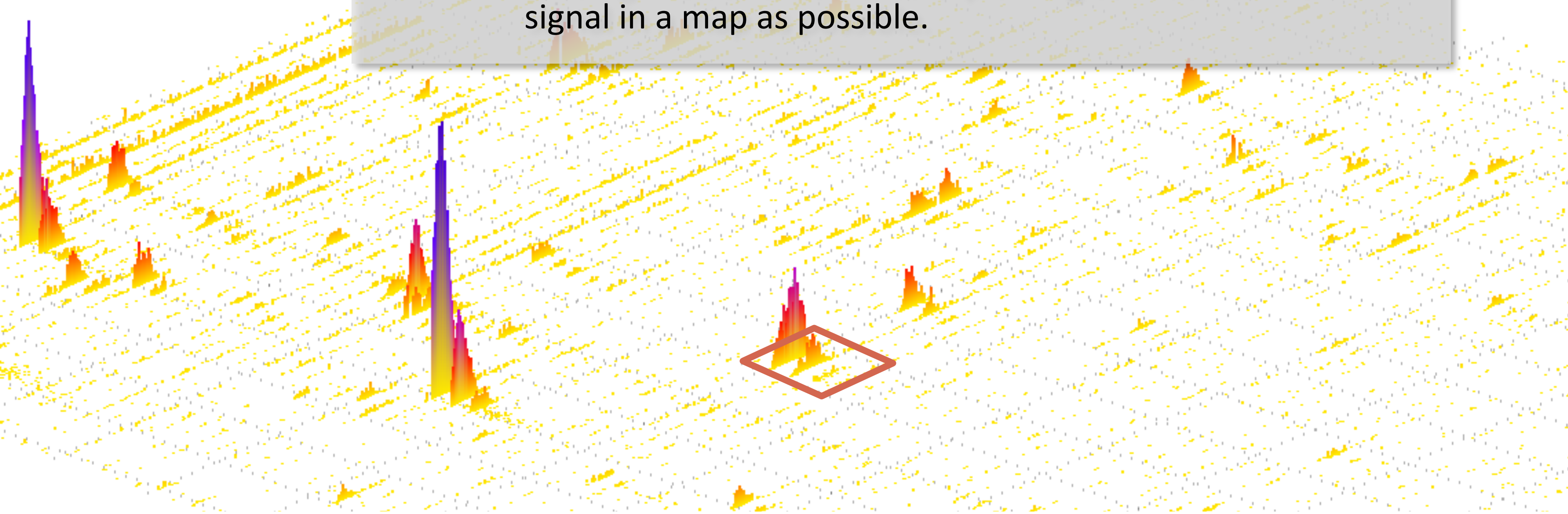
Feature:

The sum of all the MS signals caused by the same analyte in a specific charge state.

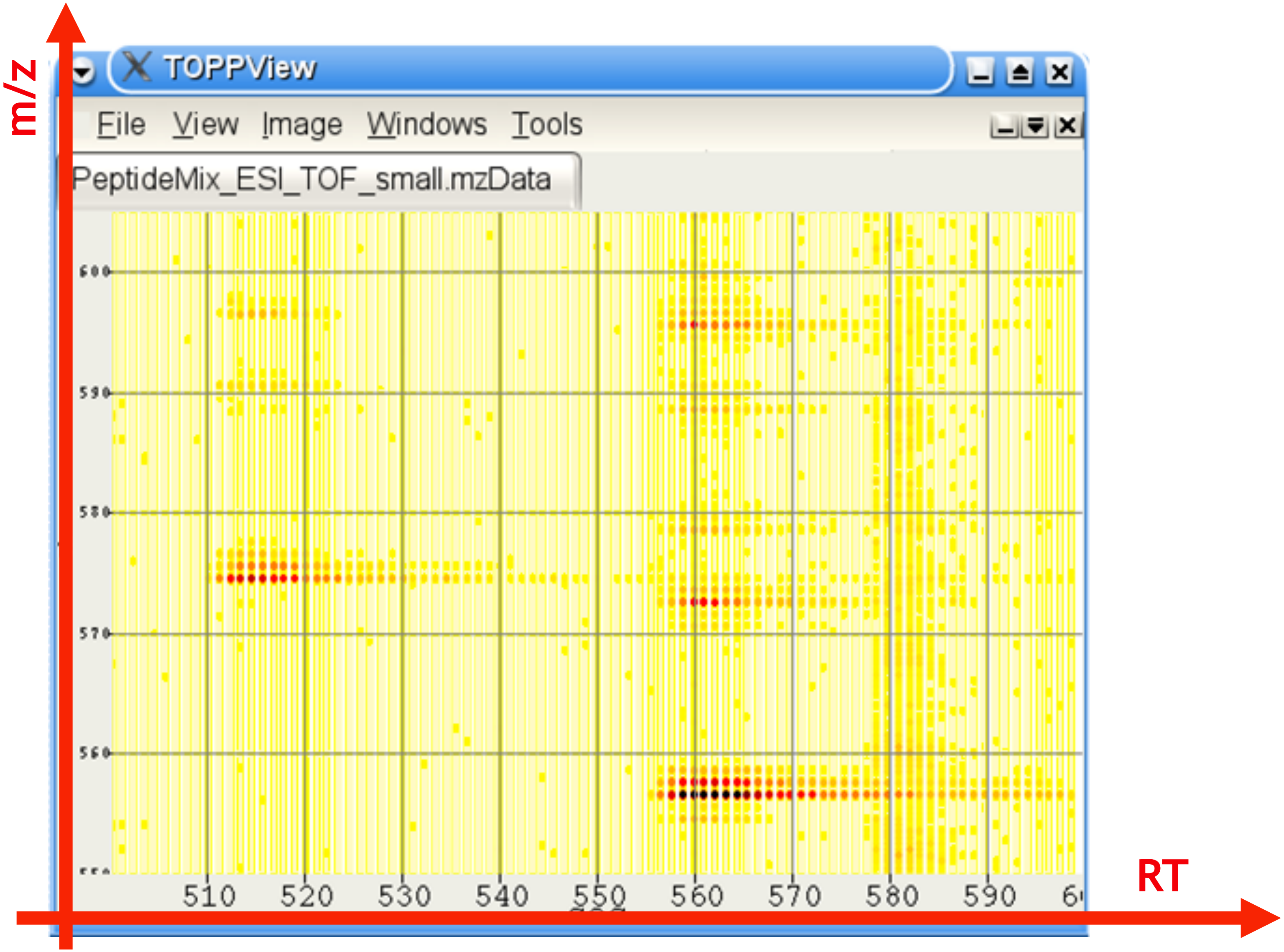
Different charge states or adducts will result in distinct features. Primarily characterized by RT, m/z, charge, intensity.

Feature finding:

Finding the set of features explaining as much of the signal in a map as possible.



Raw Map



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PeptideMix_ESI_TOF_small.mzData
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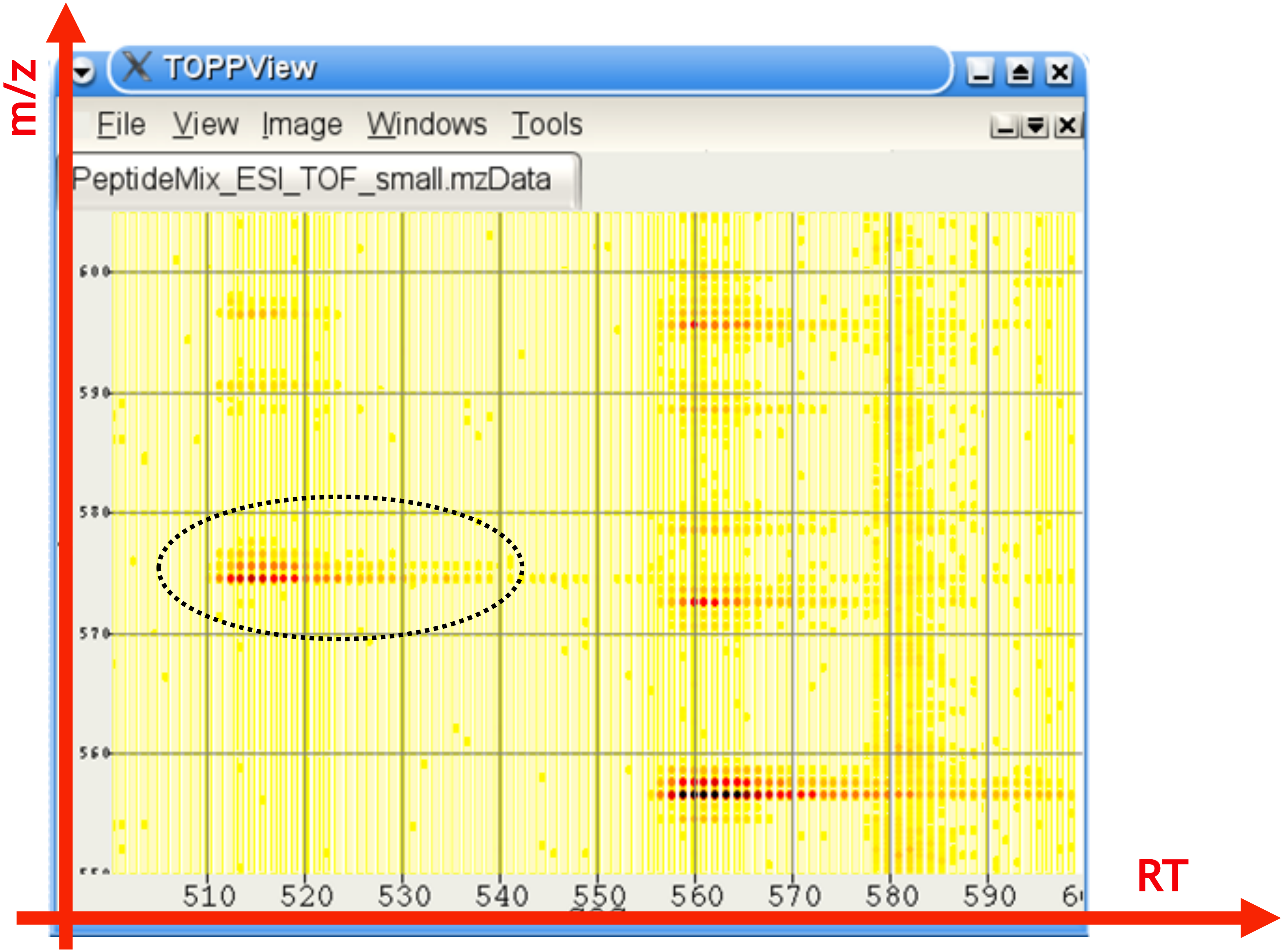
Raw Map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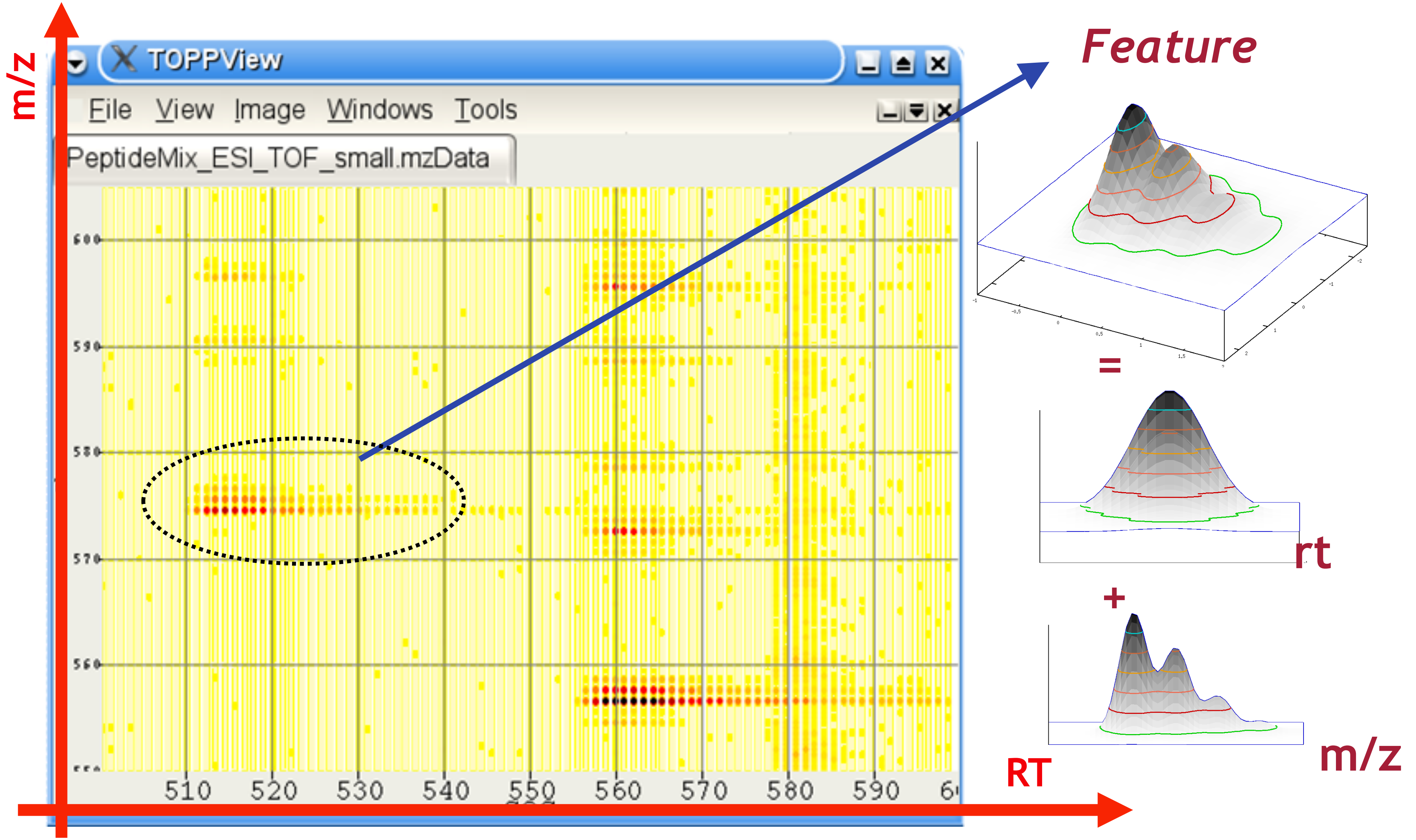
Raw Map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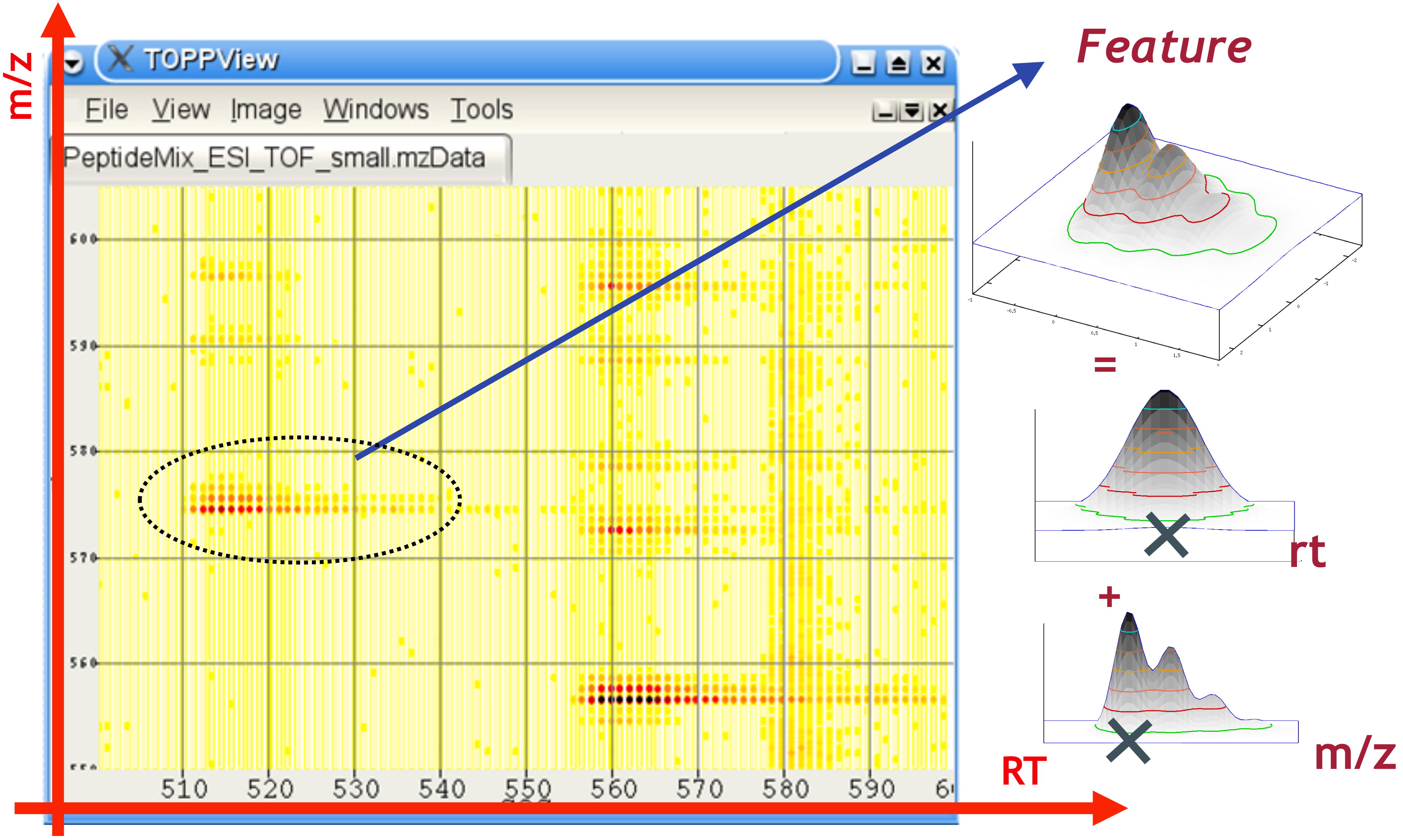
Raw Map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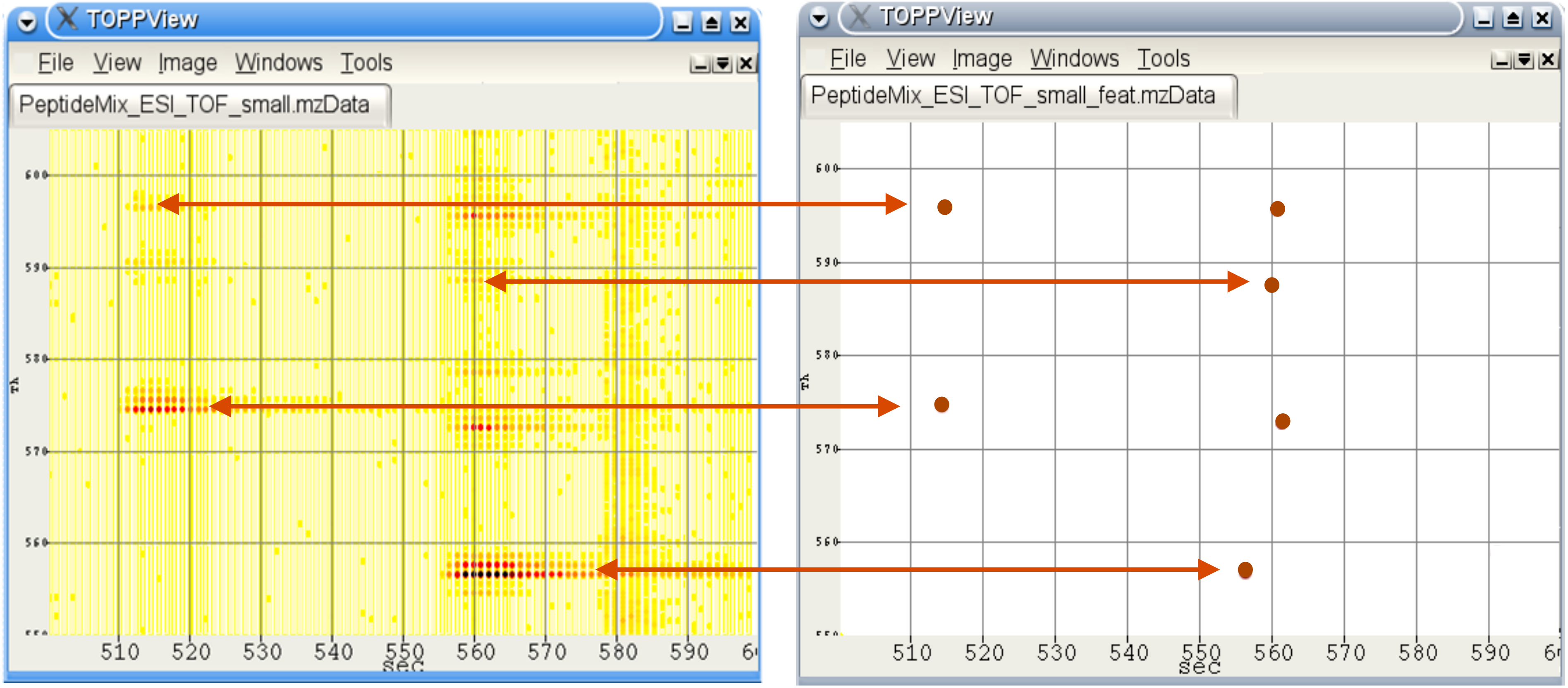
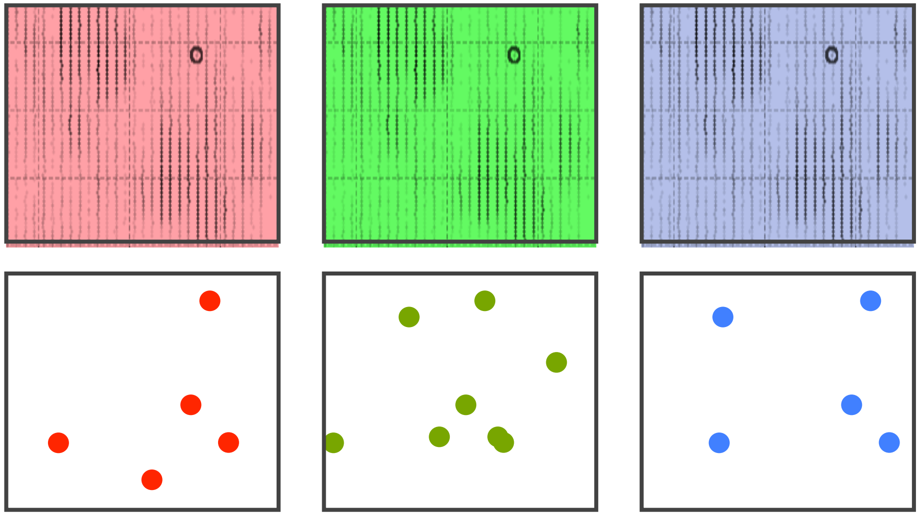
Raw Map → Feature Map



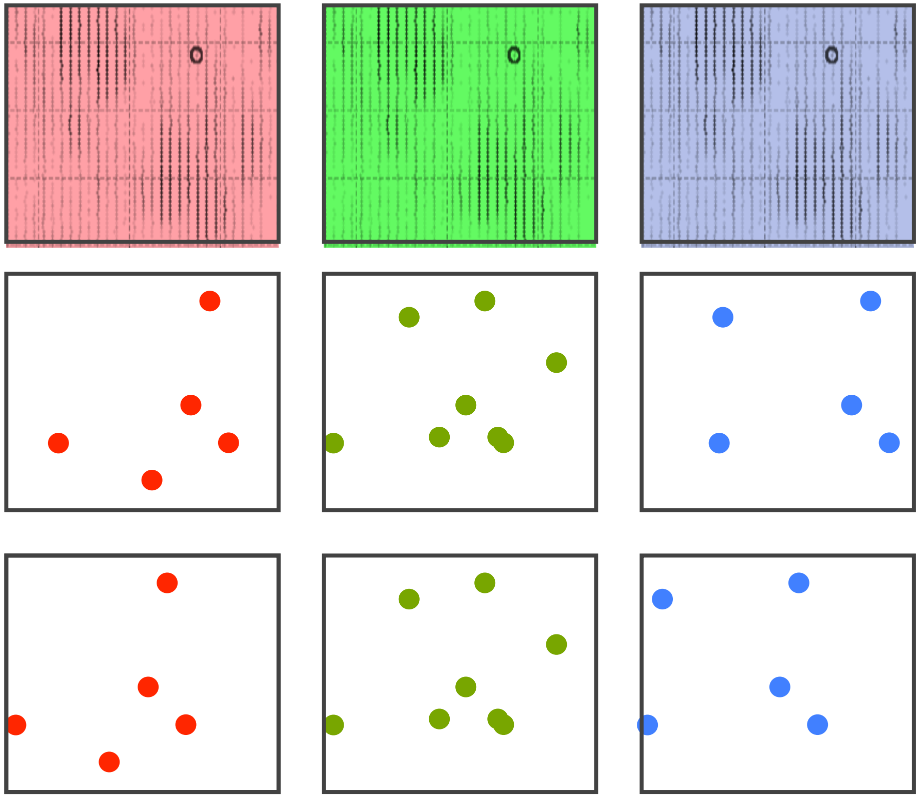
LFQ – Analysis Strategy

1. **Find** features in all maps



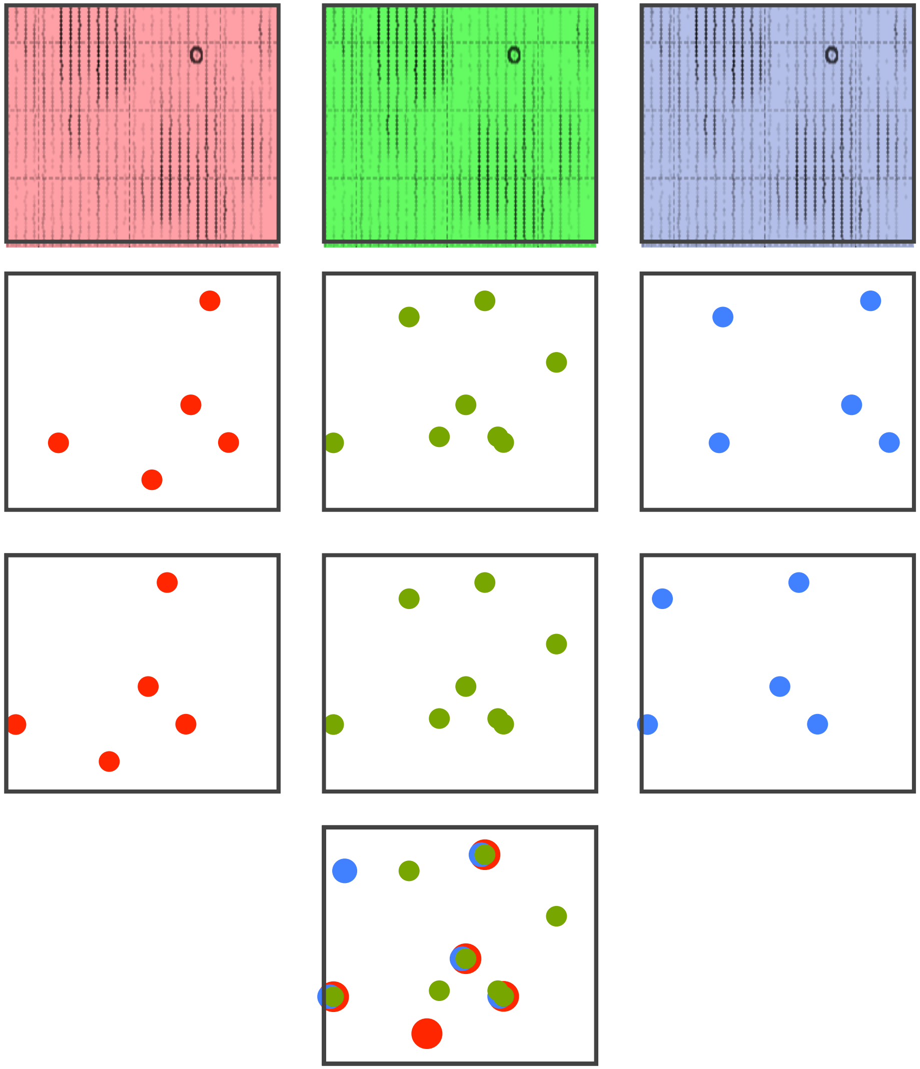
LFQ – Analysis Strategy

1. **Find** features in all maps
2. **Align** maps



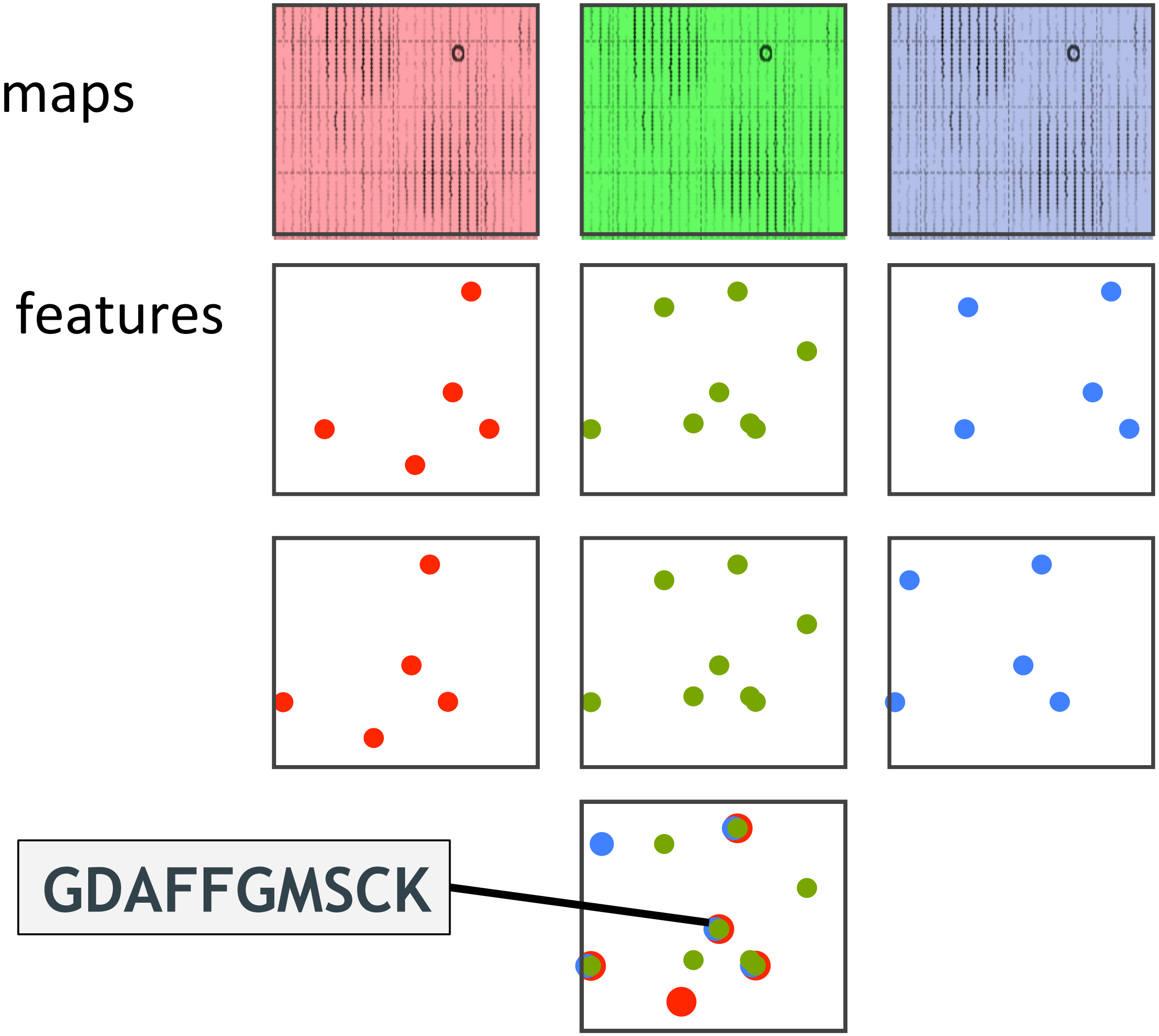
LFQ – Analysis Strategy

1. **Find** features in all maps
2. **Align** maps
3. **Link** corresponding features



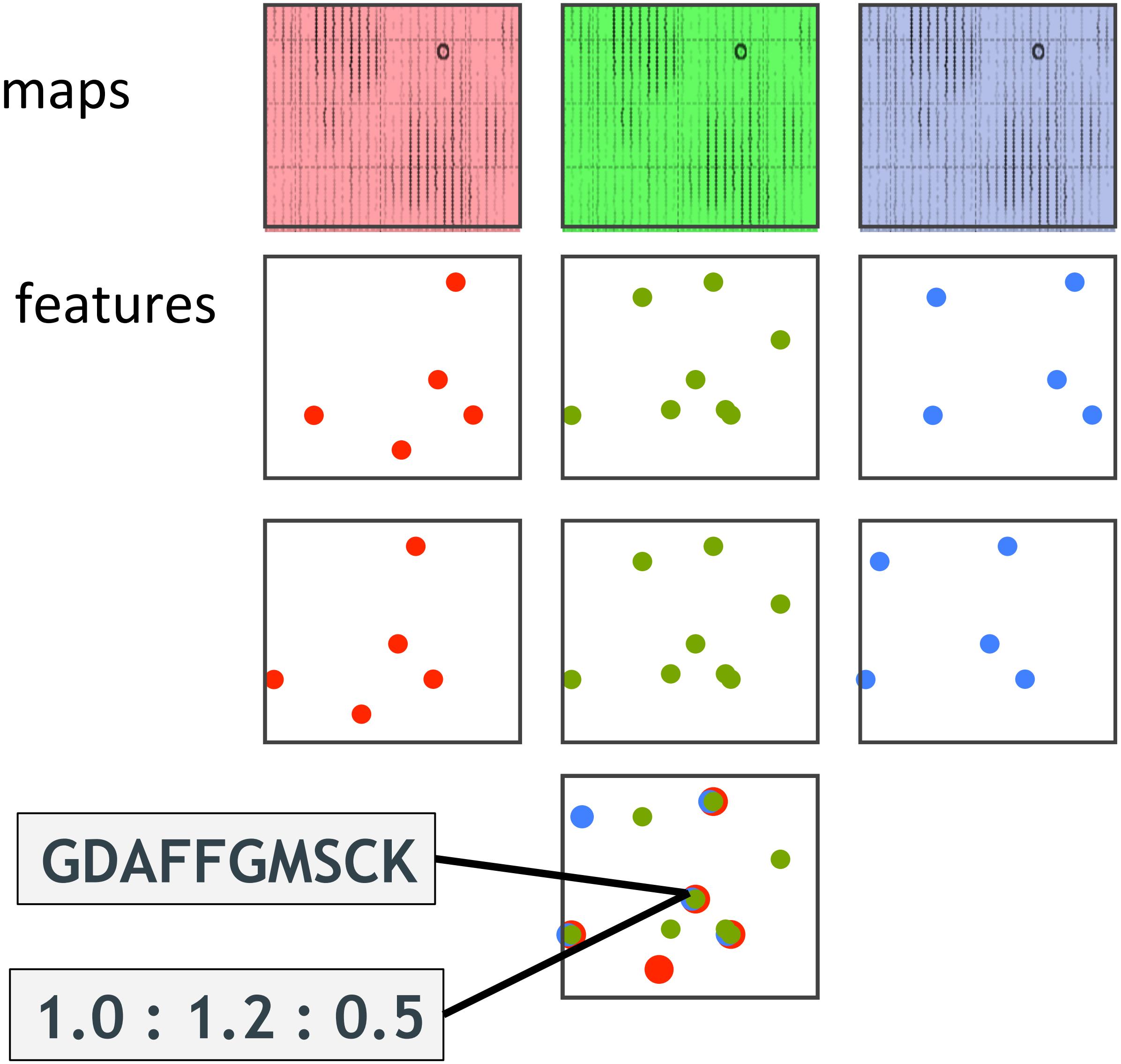
LFQ – Analysis Strategy

1. **Find** features in all maps
2. **Align** maps
3. **Link** corresponding features
4. **Identify** features



LFQ – Analysis Strategy

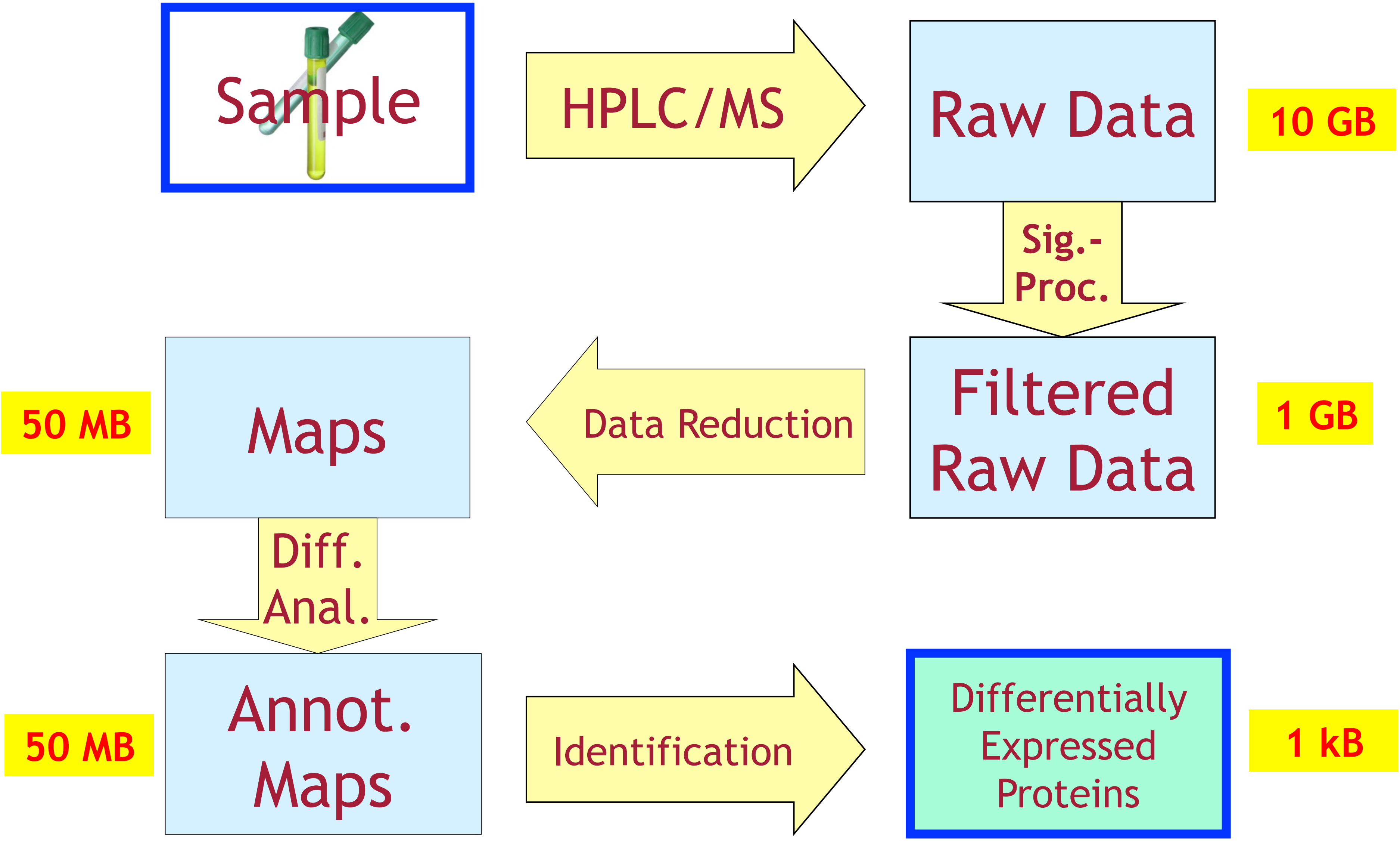
1. **Find** features in all maps
2. **Align** maps
3. **Link** corresponding features
4. **Identify** features
5. **Quantify**



GDAFFGMSCK

1.0 : 1.2 : 0.5

Feature Finding as Data Reduction



Sample

HPLC/MS

Raw Data

10 GB

Sig.-Proc.

Filtered Raw Data

1 GB

Data Reduction

Maps

50 MB

Diff. Anal.

Annot. Maps

50 MB

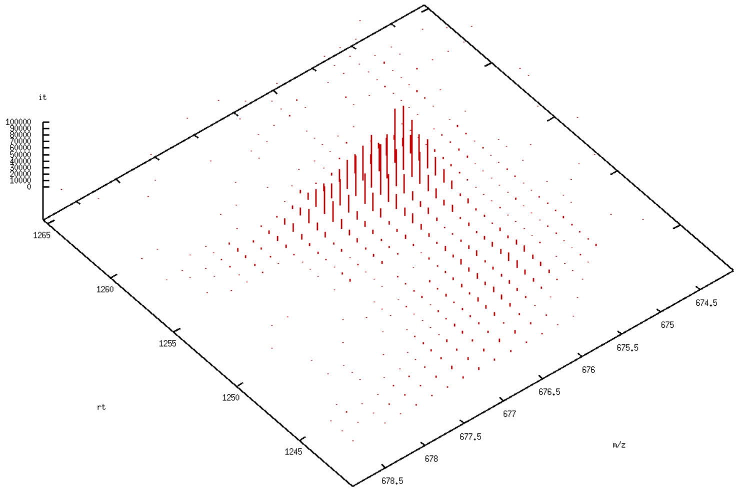
Identification

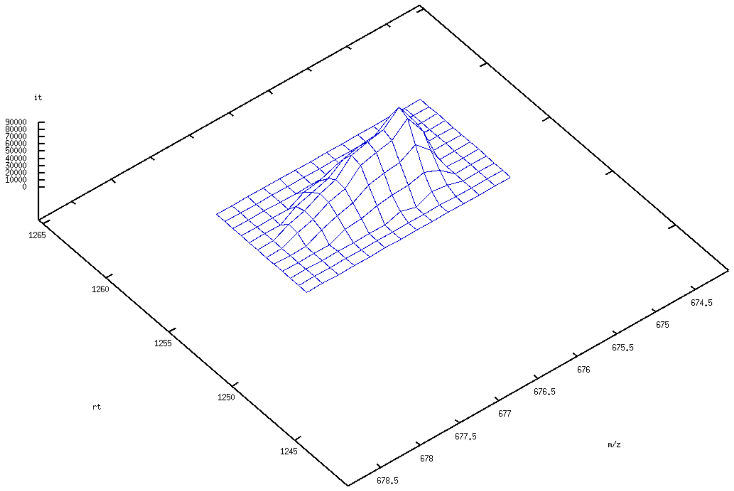
Differentially Expressed Proteins

1 kB

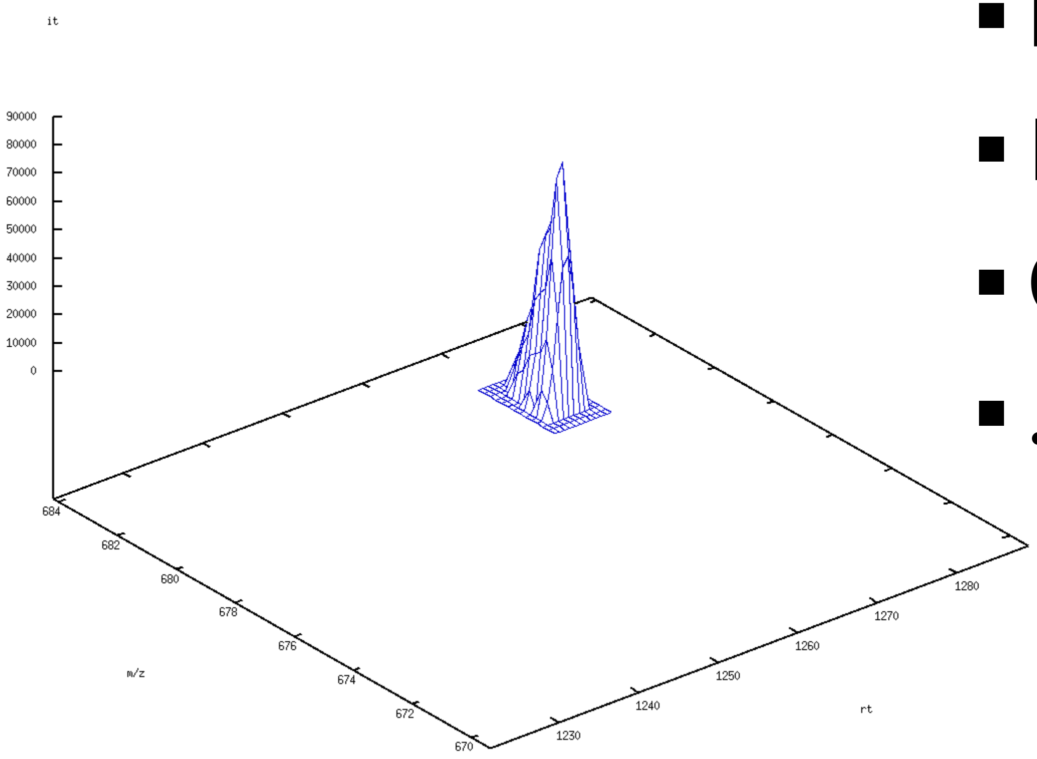
Feature Finding

- Identify all peaks belonging to one peptide
- Key idea:
  - Identify suspicious regions
  - Fit a two-dimensional model to that region





Feature Attributes

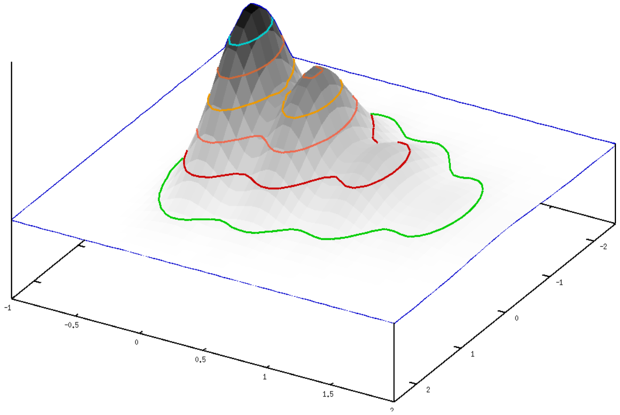


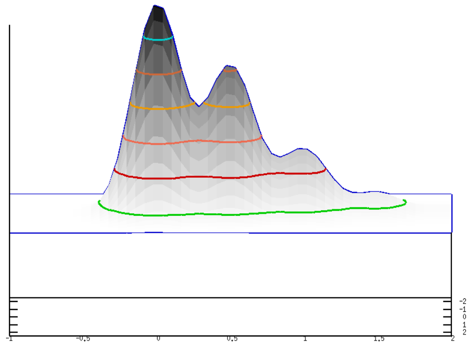
Attributes

- Position (*m*/*z*, *RT*)
- Intensity, volume
- Quality
- … … …

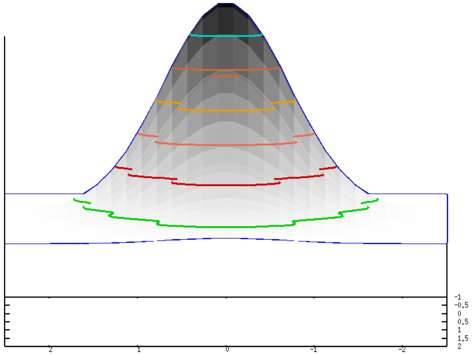
Feature Model

Feature model = Isotope pattern x Elution profile





m/z



rt

Feature Model

- Physical processes leading to the shape of a feature:
  - Chromatography
    - Elution profiles are (ideally) shaped like a Gaussian
    - Parameters: width, height, position
  - Mass spectrometry
    - Mass spectra of peptides are characterized by the isotope pattern
    - Modeled by a binomial distribution
- Both **separation processes are independent**
- A two-dimensional feature is then described by the product of two one-dimensional models

Averagine

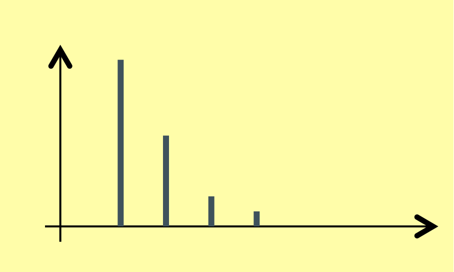
- Since the isotope pattern changes with the composition of the peptide, it is unknown which pattern should be fitted!
- Idea
  - We know the mass of the feature
  - Assume an average composition of an amino acid
  - Then we can estimate the composition
- The elemental composition of such an average amino acid, also called ‘averagine’, can be derived statistically:

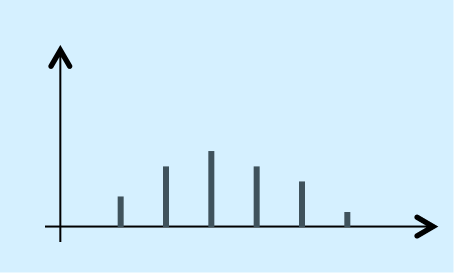
$$C_{4.94}H_{7.76}N_{1.36}O_{1.48}S_{0.04}$$

Isotope Patterns

- Based on averagine compositions one can compute the isotope patterns for any given *m/z*
- Heavier peptides have smaller monoisotopic peaks
- In the limit, the distribution approaches a normal distribution

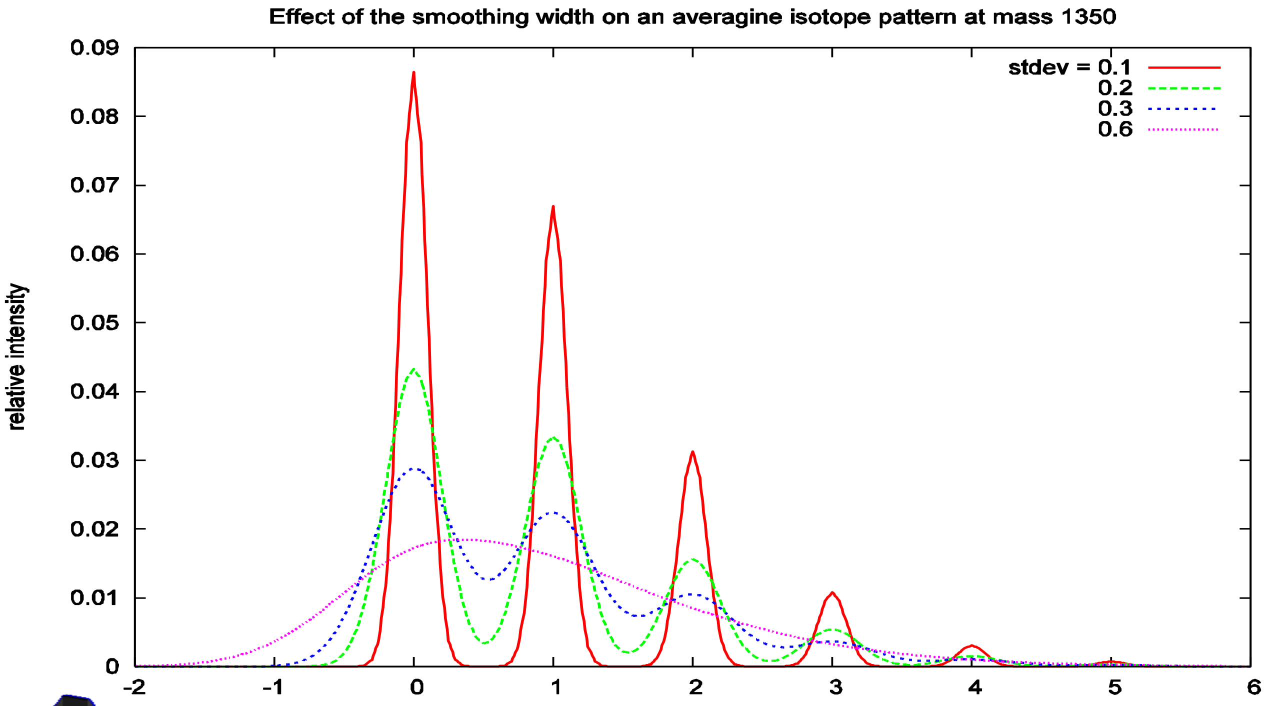
m [Da]	P (k=0)	P (k=1)	P (k=2)	P (k=3)	P (k=4)
1000	0.55	0.30	0.10	0.02	0.00
2000	0.30	0.33	0.21	0.09	0.03
3000	0.17	0.28	0.25	0.15	0.08
4000	0.09	0.20	0.24	0.19	0.12

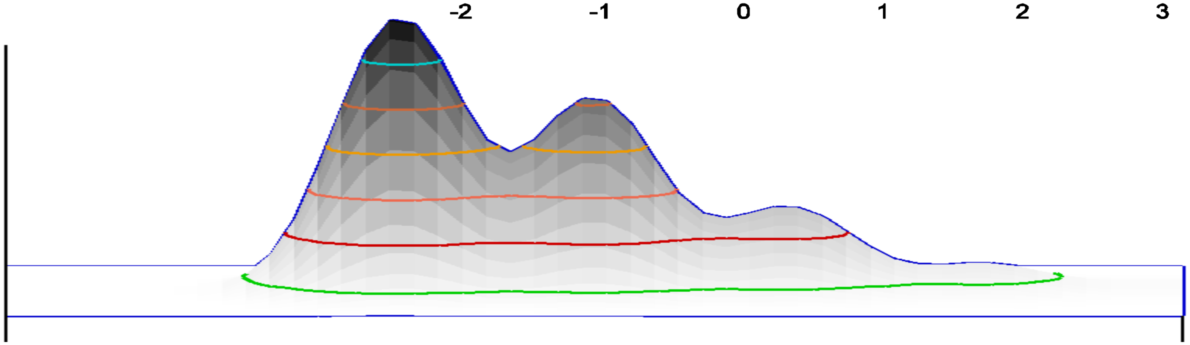




Feature Model – m/z

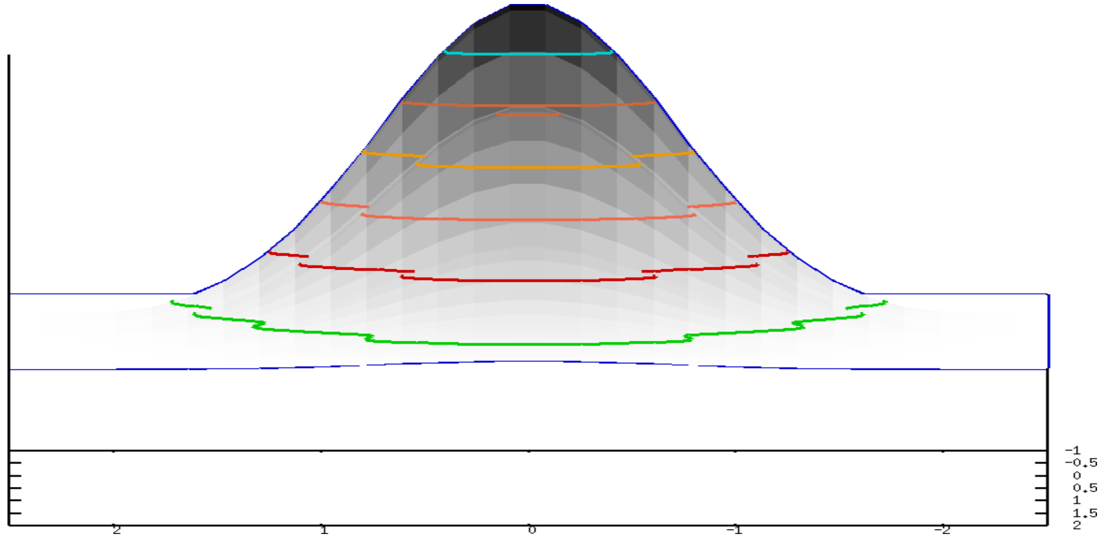
- Isotope pattern is also modulated by the **instrument resolution**
- We can assume a Gaussian shape for each of the peaks of the isotope pattern





Feature Model – RT

- Elution profile is typically assumed to be a Gaussian
- There are some variants that also allow for asymmetric peaks
- This defines the shape of a feature in in the RT dimension



Feature Finding – Algorithm

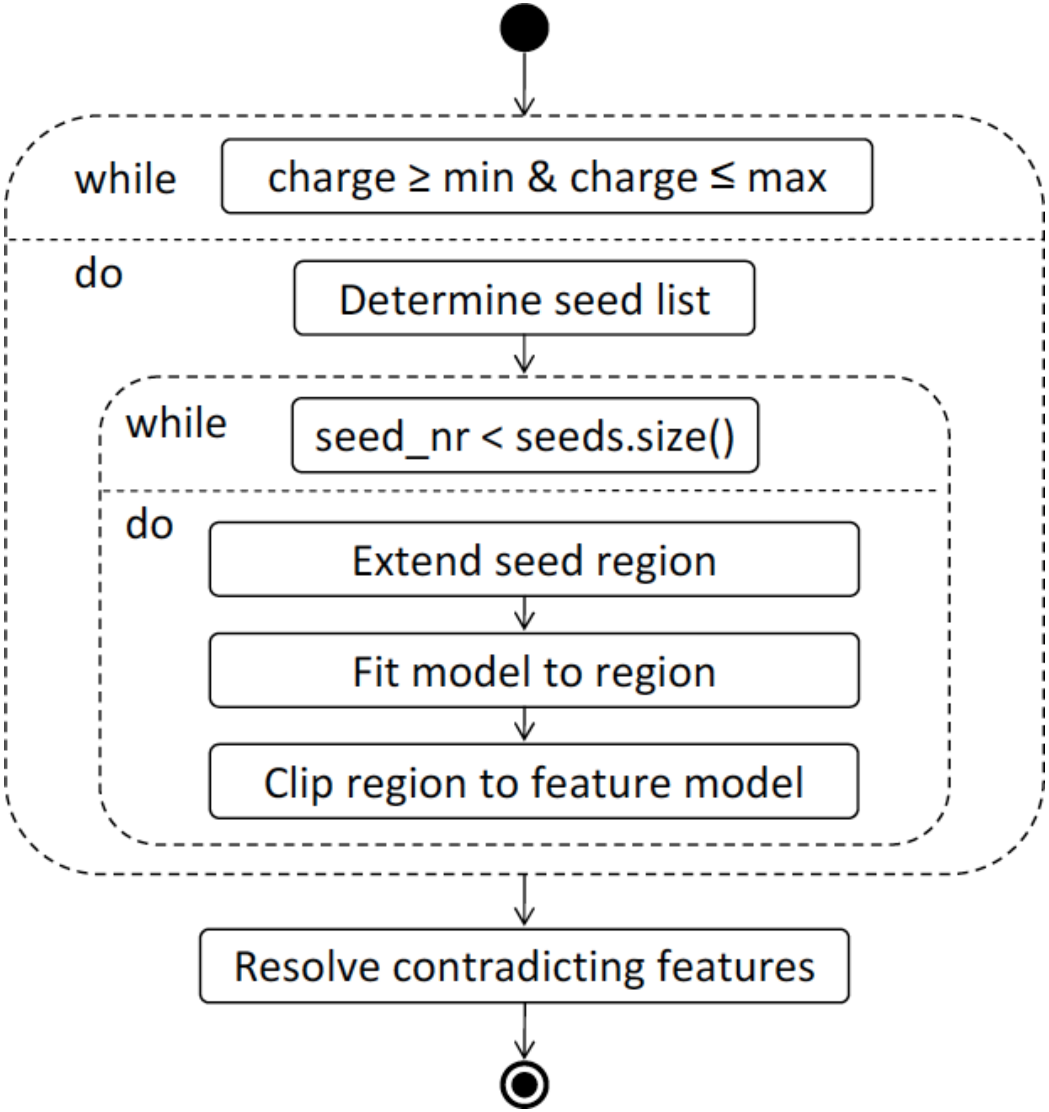
Most algorithms consists of four phases

1. ***Seeding.*** *Choose peaks of high intensities, as those are usually in features ("seeds").*
2. ***Extension.*** *Conservatively add peaks around the seed, never mind if you pick up a few peaks too many.*
3. ***Modeling.*** *Estimate parameters of a two-dimensional feature for the region.*
4. ***Refinement.*** *Optimally fit a model to the collected peaks. Remove peaks not agreeing with the model. Iterate until convergence.*

Algorithm: Seeding

- Start with the highest peaks in the map
- Pick only one seed per feature, thus exclude peaks of already identified features for later seeding
- More advanced variants of the algorithm use Wavelet techniques to detect the best seeds
- **Problems**
    - Low-intensity features have intensities barely above the surrounding noise
    - Choose a threshold based on the average noise
    - Dilemma:
        - threshold too high, features will not get seeded
        - Threshold too low, millions of noise peaks will be considered as seeds HUGE run times

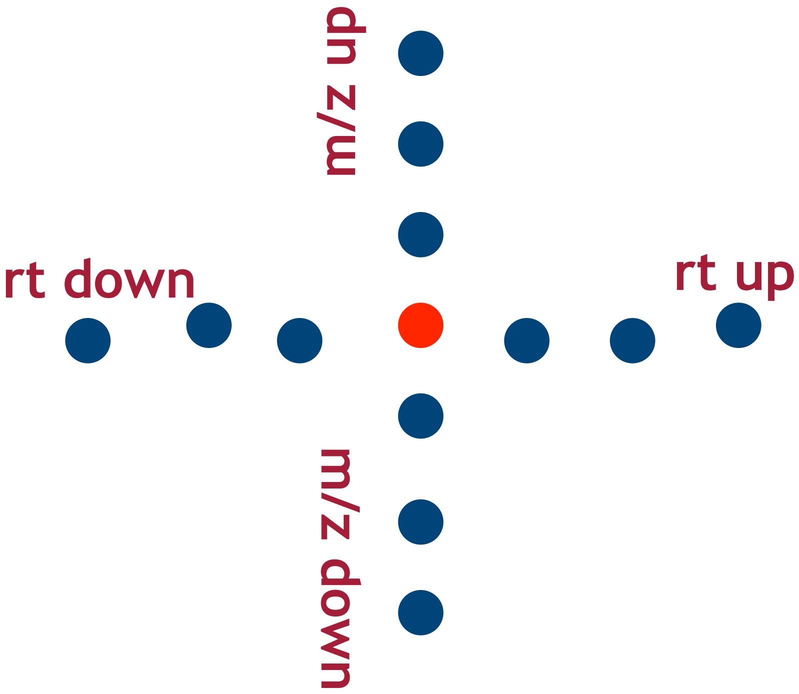
Feature Finding – Overview

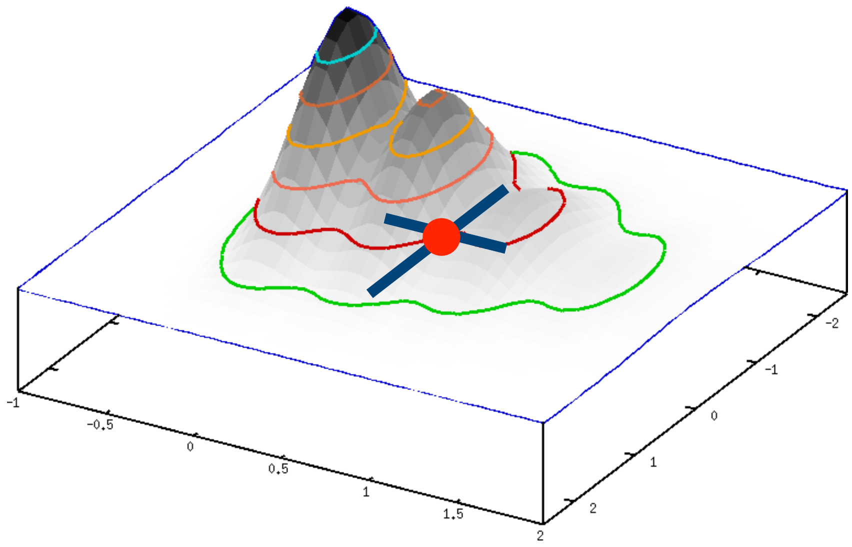


Sturm, OpenMS – A Framework for Computational Mass Spectrometry, Dissertation, Tübingen, 2010

Algorithm: Extension

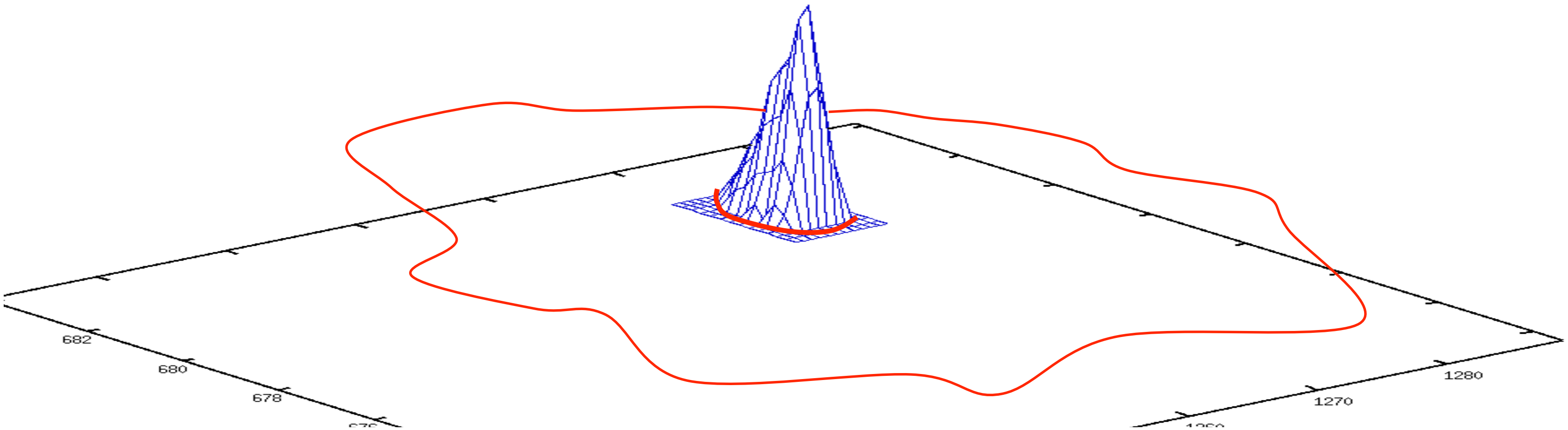
- Explore the peaks around the seed
- Add them to a set of relevant peaks
- Abort if the peaks are getting too small or too far away





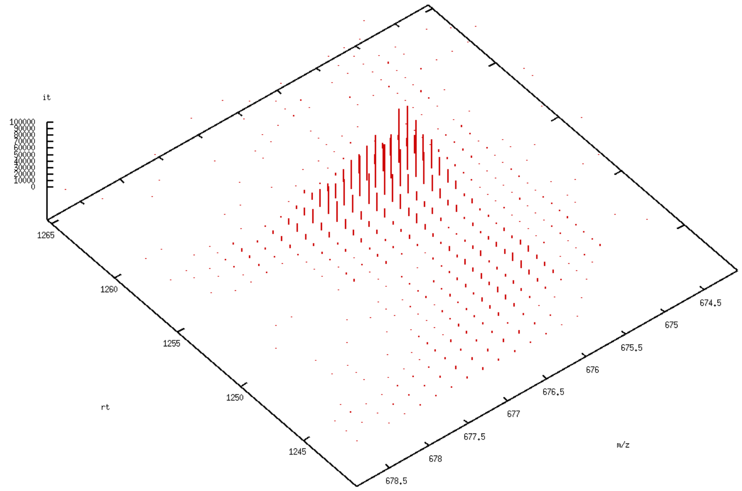
Algorithm: Refinement

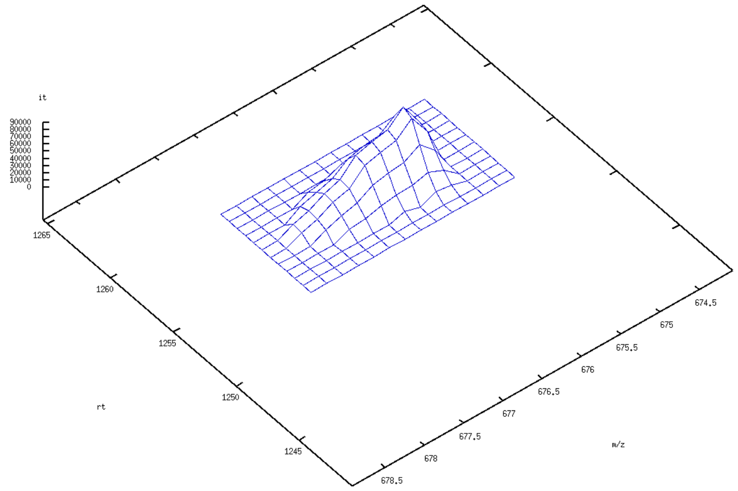
- **Remove peaks** that are not consistent with the model
- **Determine optimal model** for the reduced set of peaks
- Iterate this until no further improvement can be achieved
- Remove all peaks of this feature from potential seeds



Feature Finding

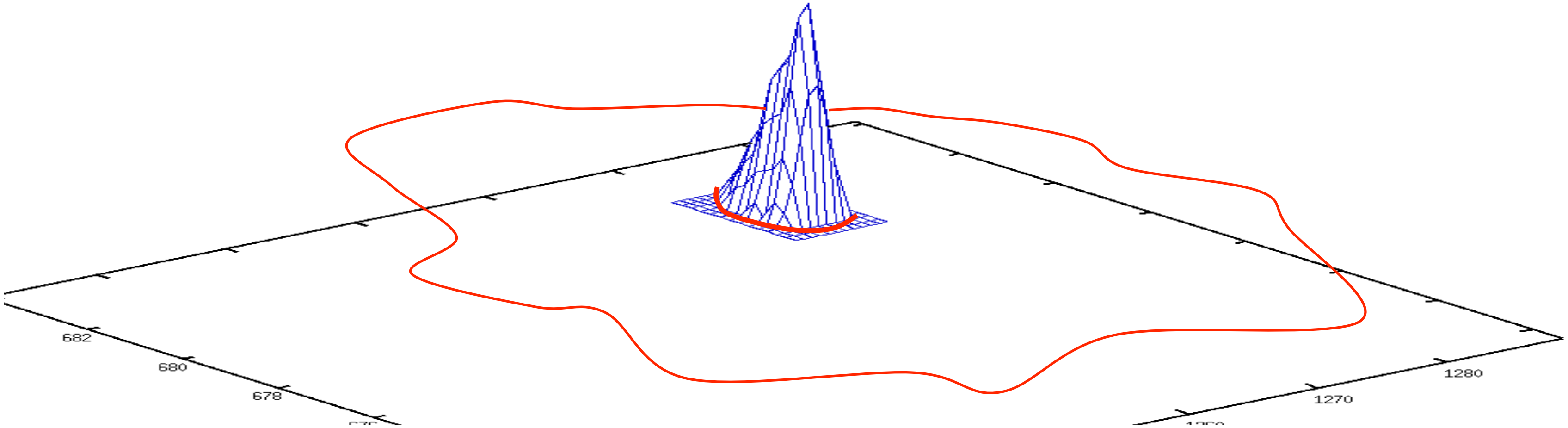
- Identify all peaks belonging to one peptide
- Key idea:
  - identify suspicious regions
  - Fit a **model** to that region and identify peaks are explained by it





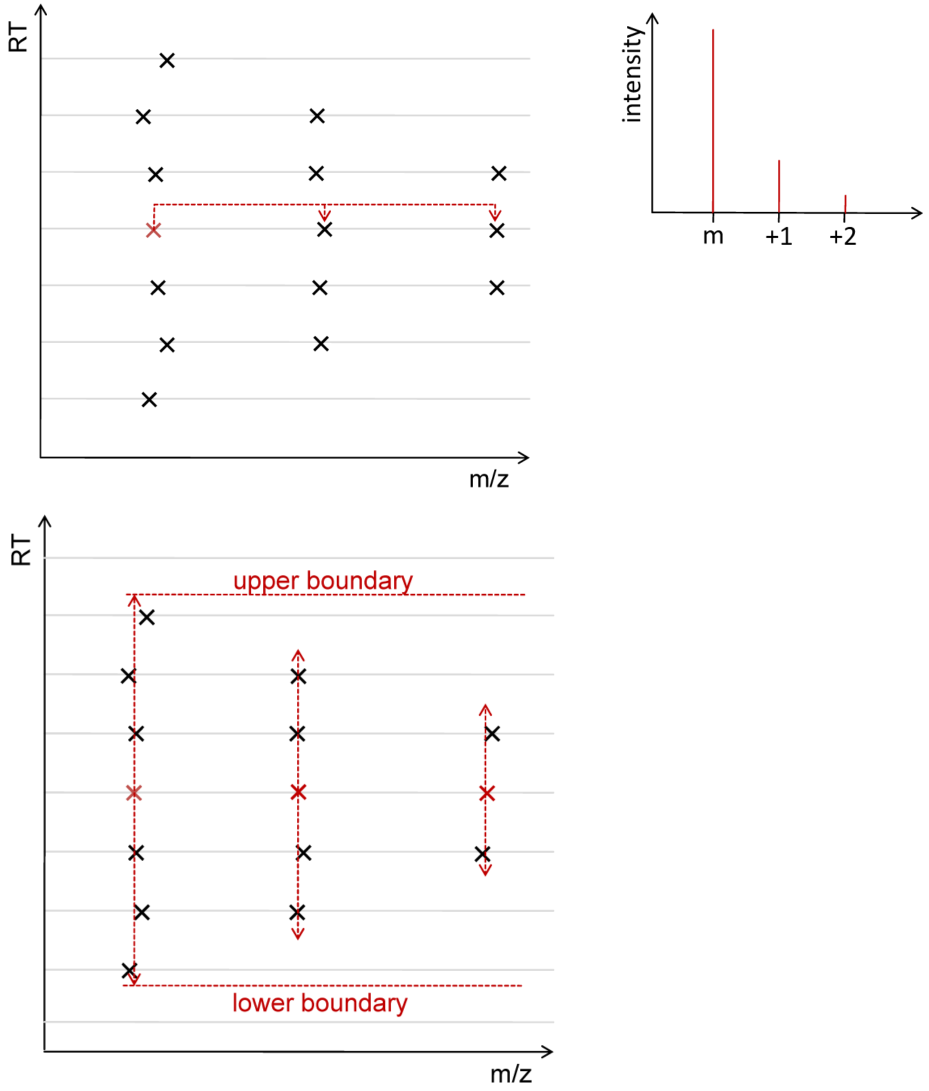
Feature Finding

- **Extension:** collect all data points close to the seed
- **Refinement:** remove peaks that are not consistent with the model
- **Fit an optimal model** for the reduced set of peaks
- Iterate this until no further improvement can be achieved



Collecting Mass Traces

- A mass trace is a series of peaks along the RT dimension with little variation in the m/z dimension
- Mass traces are found with a simple heuristic aborting the search if the peak intensity hits the local noise level
- Search for mass traces in the correct m/z distance
- Limit length of mass trace to the length of the most intense mass trace

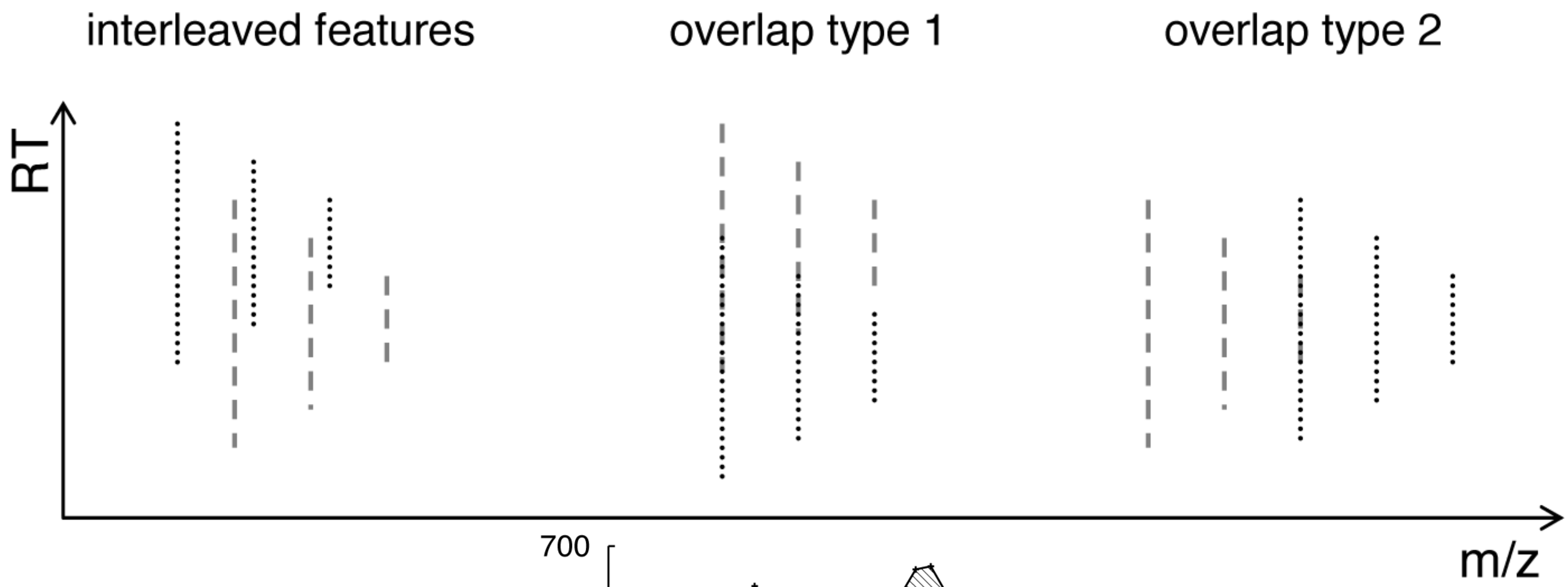


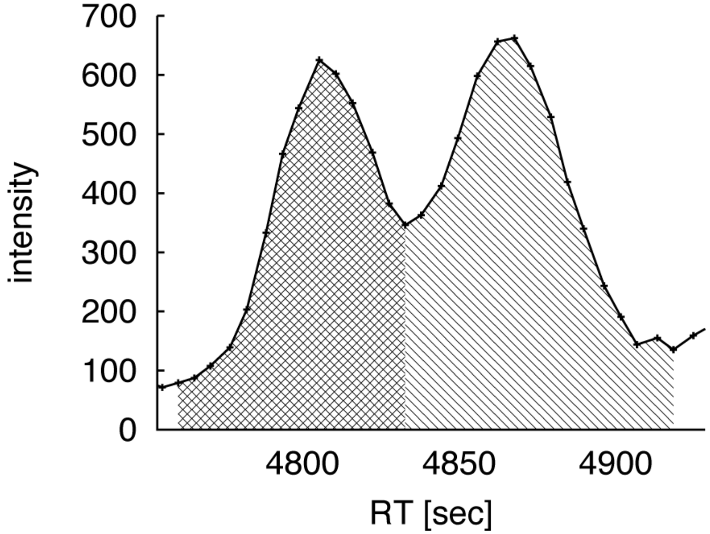
Sturm, OpenMS – A Framework for Computational Mass Spectrometry, Dissertation, Tübingen, 2010

Feature Deconvolution

- Features can overlap in various ways
  - Mass traces can contain more than one chromatographic peak (features not baseline-separated in RT dimension)
  - Mass traces can be interleaved between features in the m/z dimension
  - Co-eluting features can be sharing mass traces
- Resolving these conflicts is done in a feature deconvolution step by statistical testing:
  - Test several hypotheses that could explain the features
  - The most likely of all hypotheses will be identified through comparison with the data

Feature Deconvolution

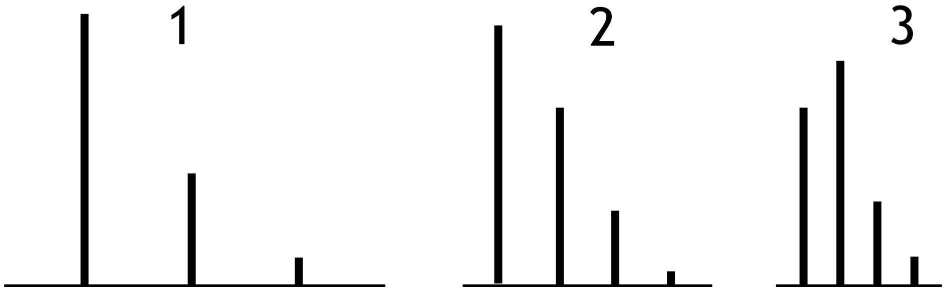


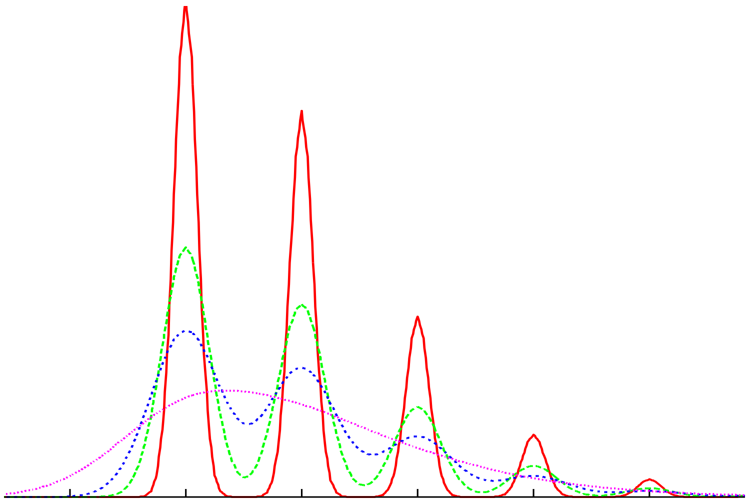


Sturm, OpenMS – A Framework for Computational Mass Spectrometry, Dissertation, Tübingen, 2010

Algorithm: Modeling

- Test all possible models for different charges states (charge +2, charge +3, …)
- Decide on the charge of the features based on the best fit for these models





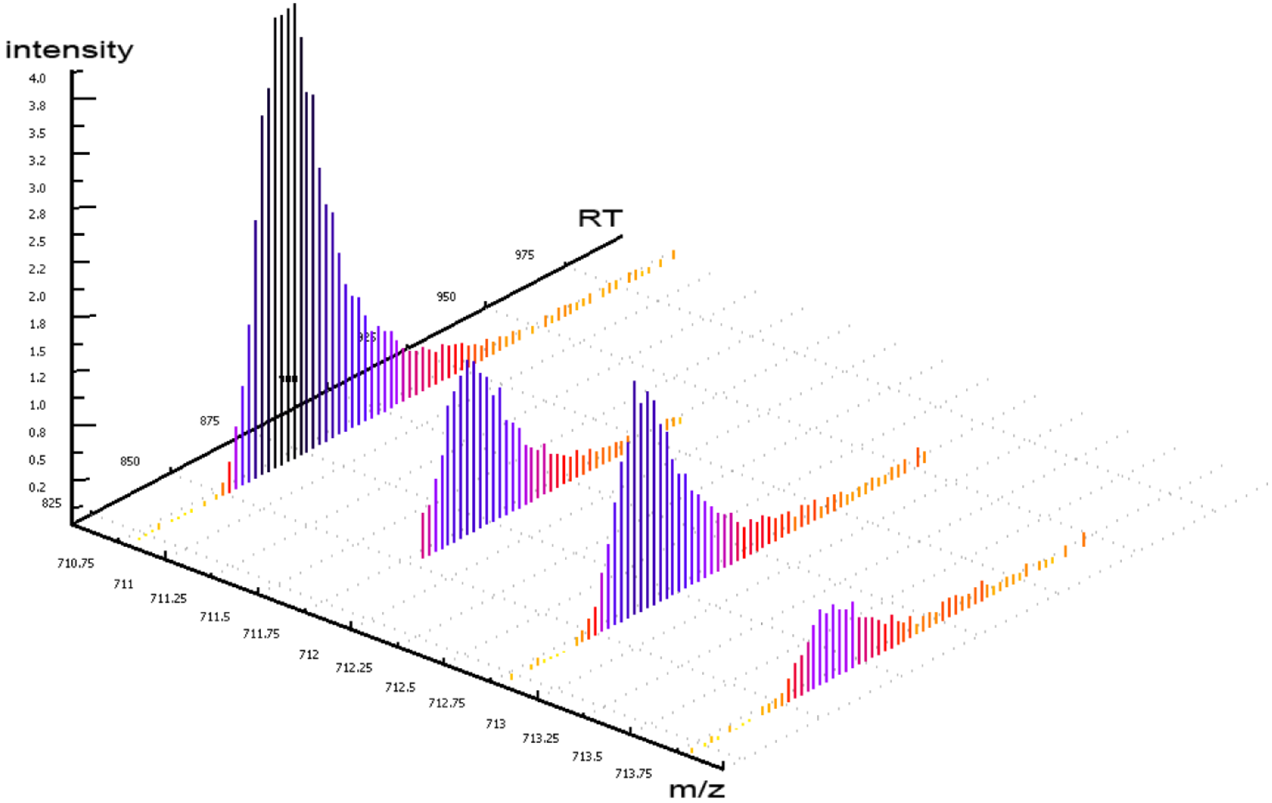
Algorithm: Modeling/Refinement

- Estimate **quality of fit** for model *m* and data d_i at positions r_i:

$$\text{fit}(m,d) = \frac{\left(\sum_i m(r_i)d_i\right)^2}{\sum m(r_i)^2 \sum d_i^2}$$

- Maximum Likelihood Estimator determines good **starting values for model parameters**
- **Further optimization of model parameters in refinement phase** (least-squares fit)

Feature Assembly



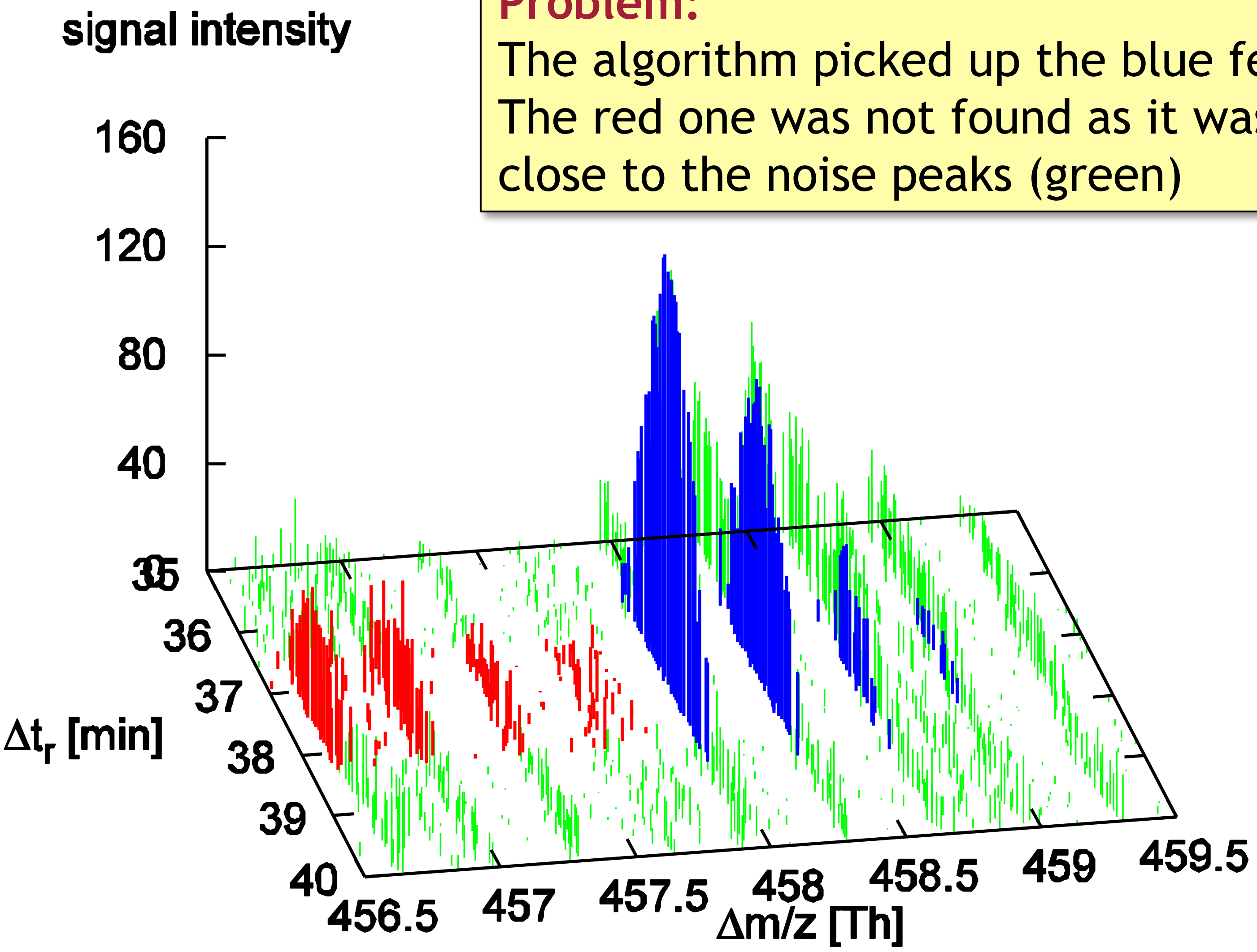
- Feature resolution is not always possible unambiguously

Feature Finding – Problems

Problems

- Low-resolution instruments might not yield good isotope patterns
- Peptides can overlap, in particular in complex samples
- Fitting of such overlapping patterns can yield bogus results
- Low-intensity features are hard to distinguish from noise peaks
- Isotope labels can skew the distributions or can lead to overlapping pairs

Still Difficult: Low-Intensity Features



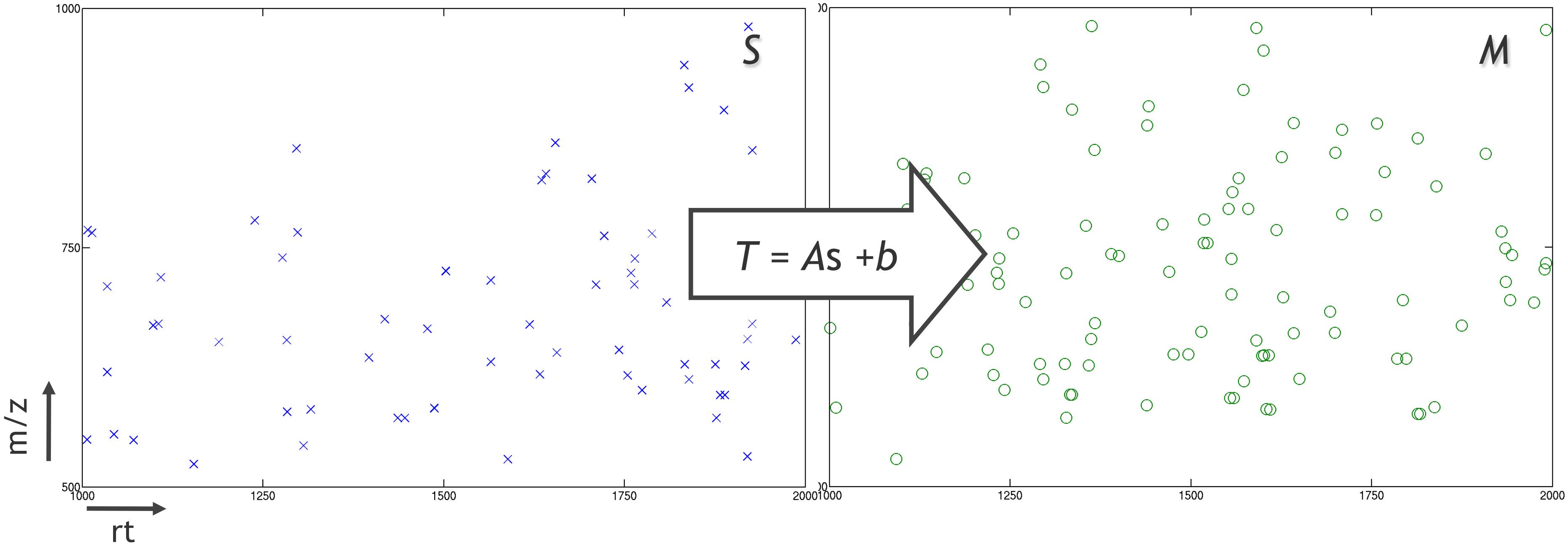
Problem:
The algorithm picked up the blue feature,
The red one was not found as it was too close to the noise peaks (green)

LEARNING UNIT 5B
MAP ALIGNMENT

Map alignment

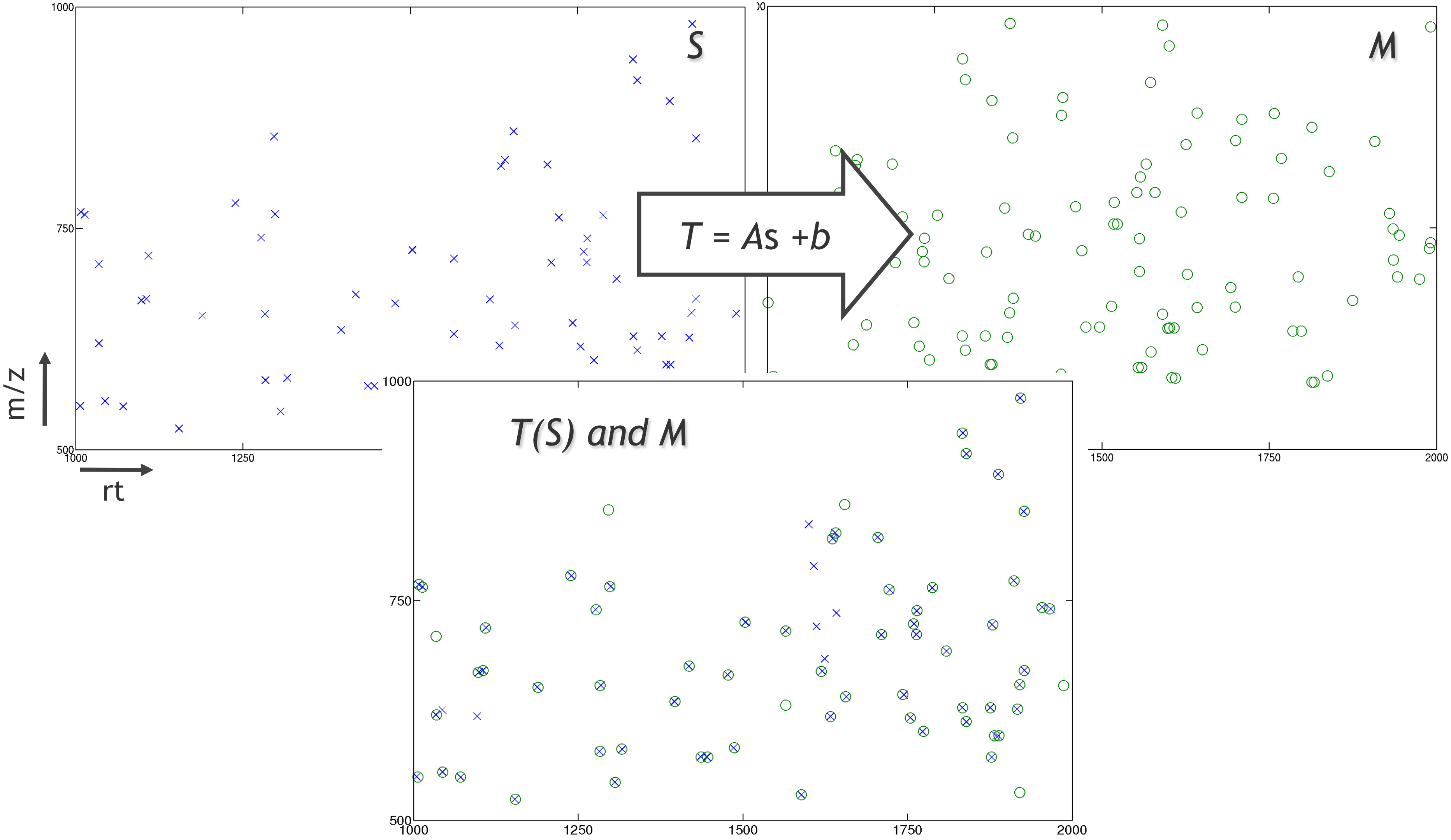
- Problem definition
- Pose-clustering algorithms
- Dynamic time-warping techniques
- Map alignment and feature linking
- Map normalization

Pairwise Alignment



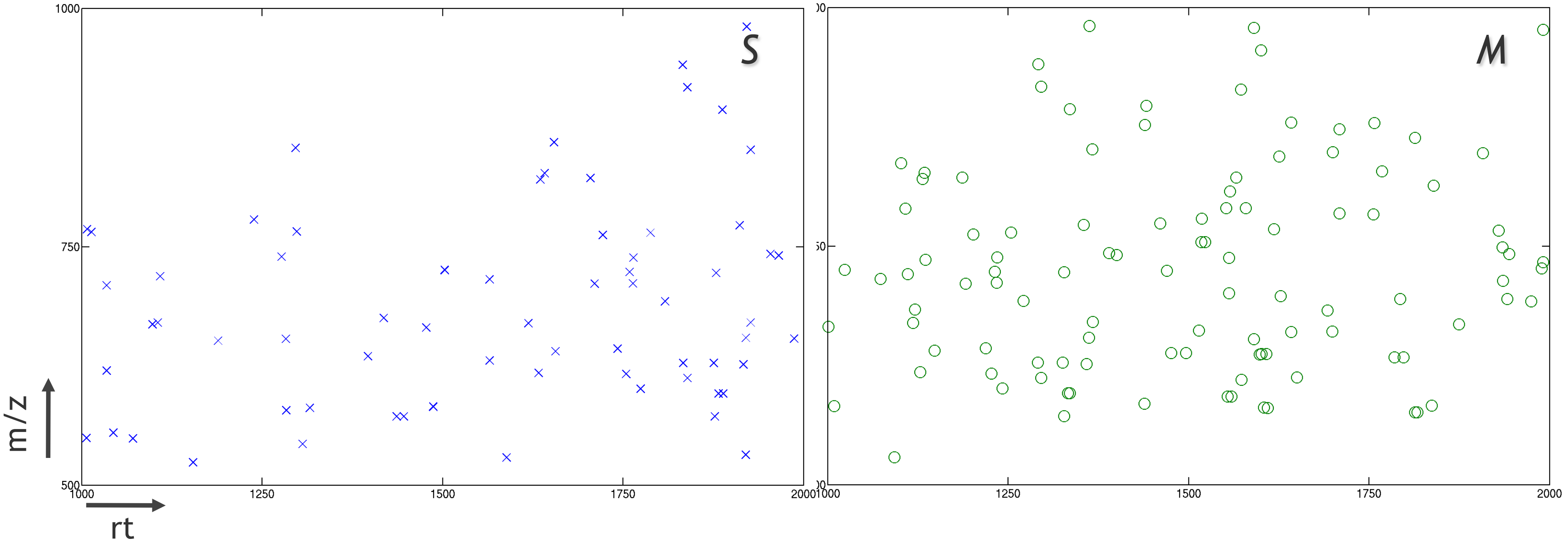
The problem is to find the affine transformation *T* that minimizes the distance between *T*(*S*) and *M*.

Pairwise Alignment



$T = As + b$

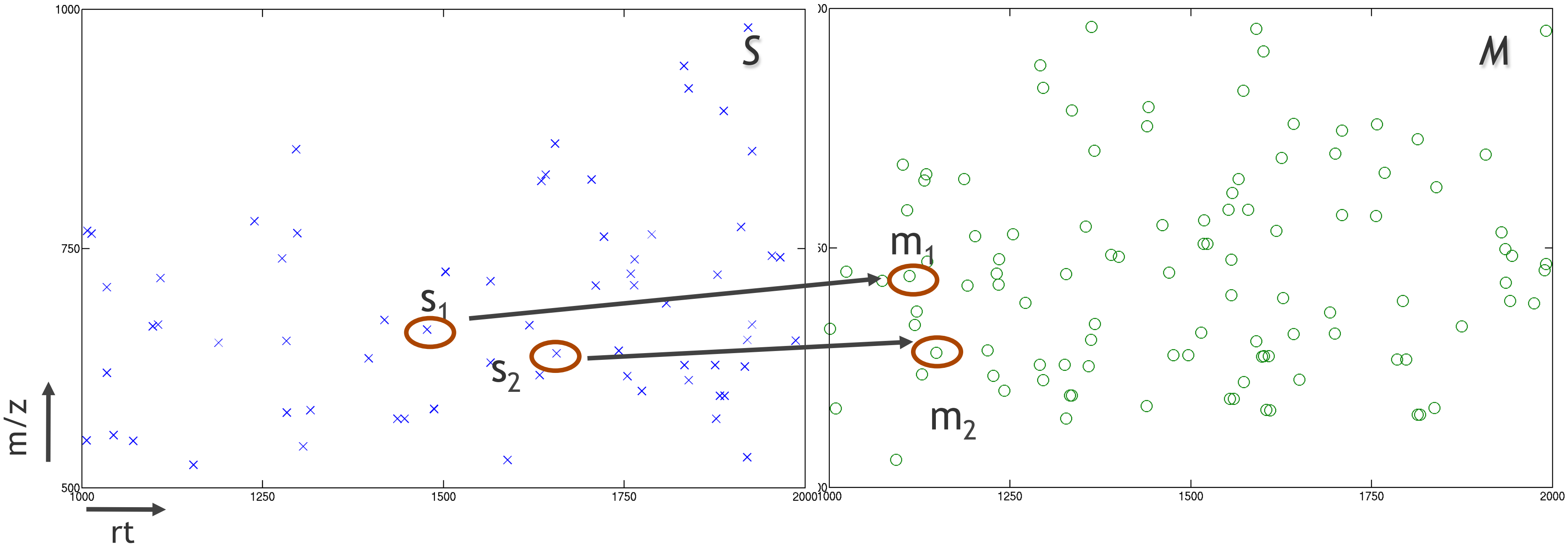
Pose Clustering

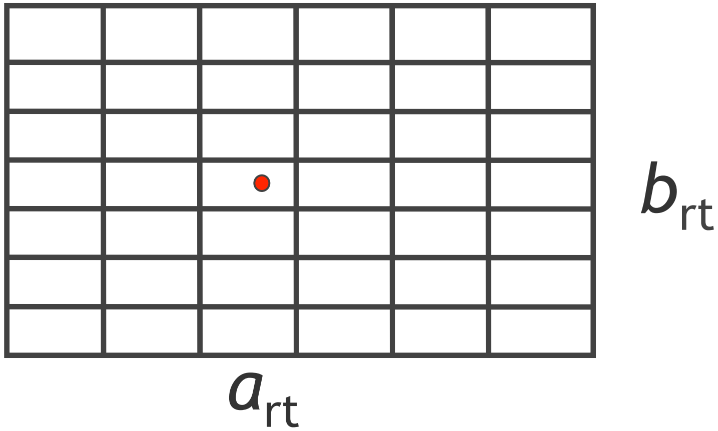


$$T_{rt}(s_{rt}) = a_{rt}s_{rt} + b_{rt}$$

$$T_{m/z}(s_{m/z}) = a_{m/z}s_{m/z} + b_{m/z}$$

Pose Clustering

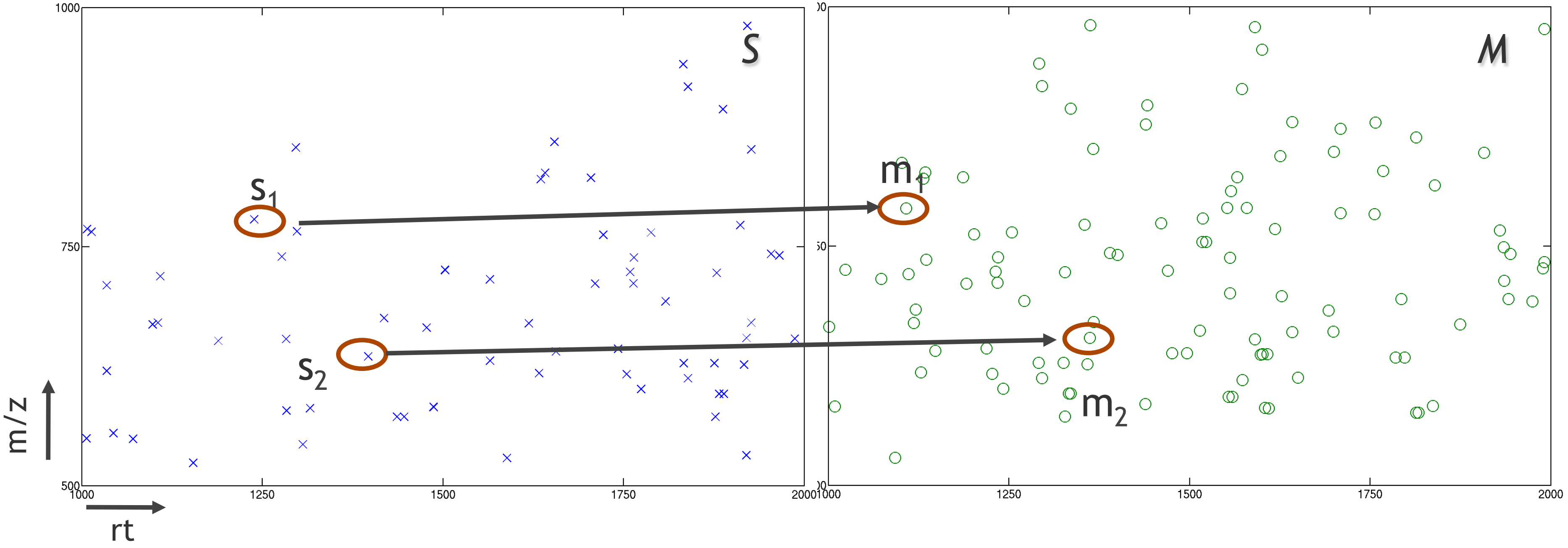


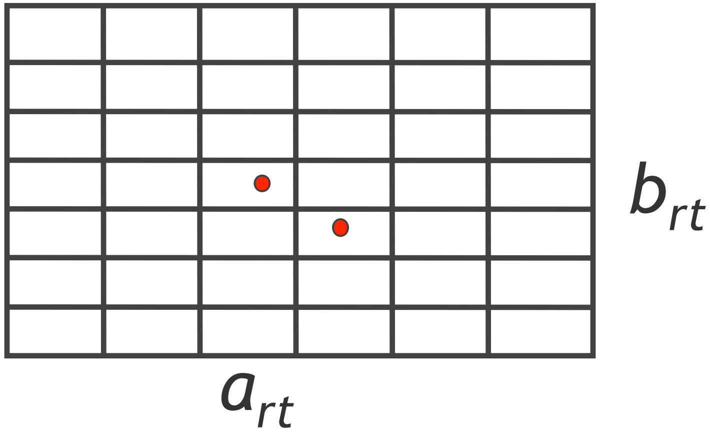


$$m_1 = a_{rt}s_1+b_{rt}$$

$$m_2 = a_{rt}s_2+b_{rt}$$

Pose Clustering

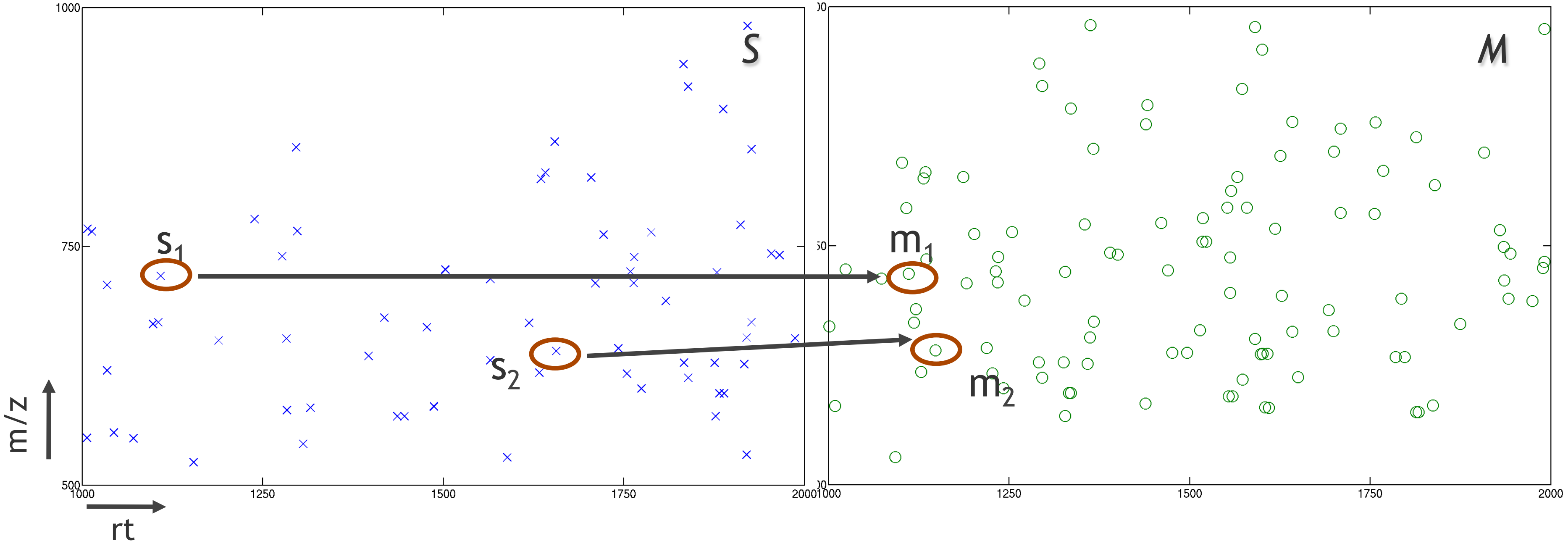


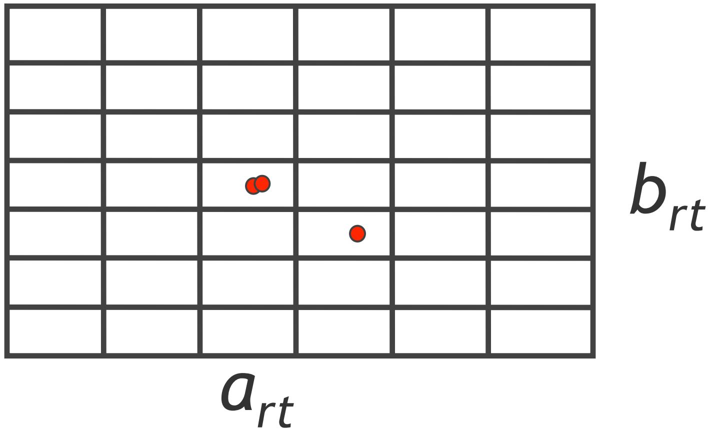


$$m_1 = a_{rt}s_1 + b_{rt}$$

$$m_2 = a_{rt}s_2 + b_{rt}$$

Pose Clustering

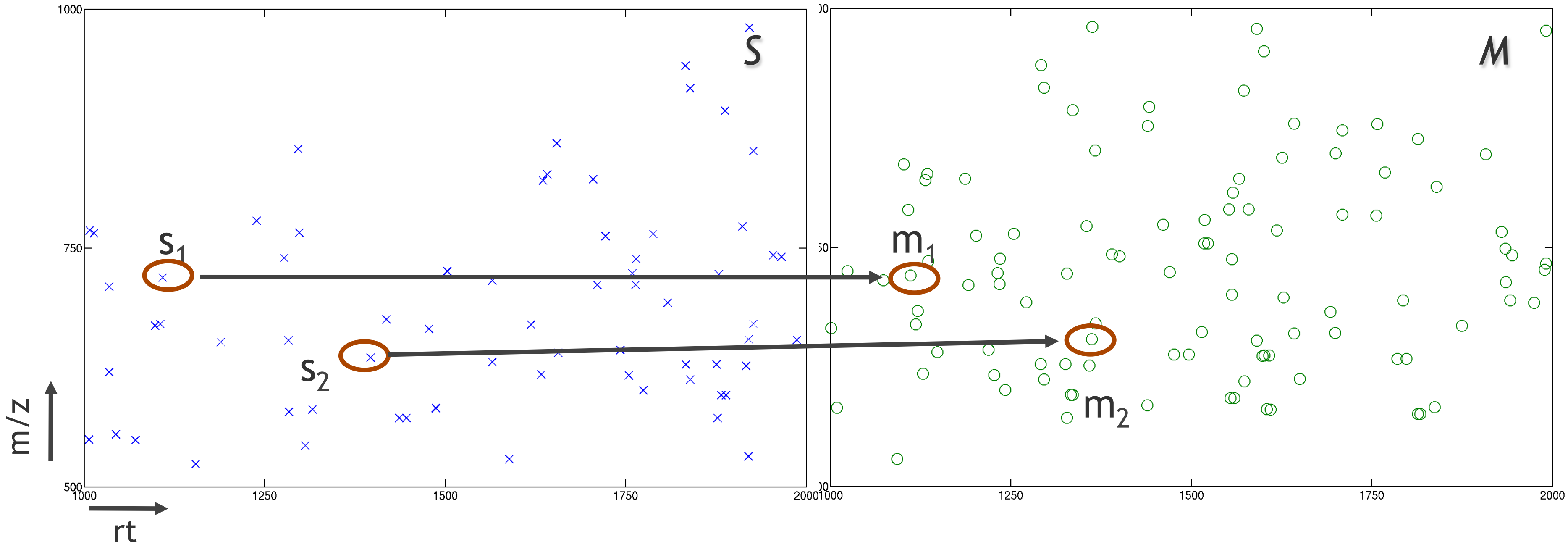


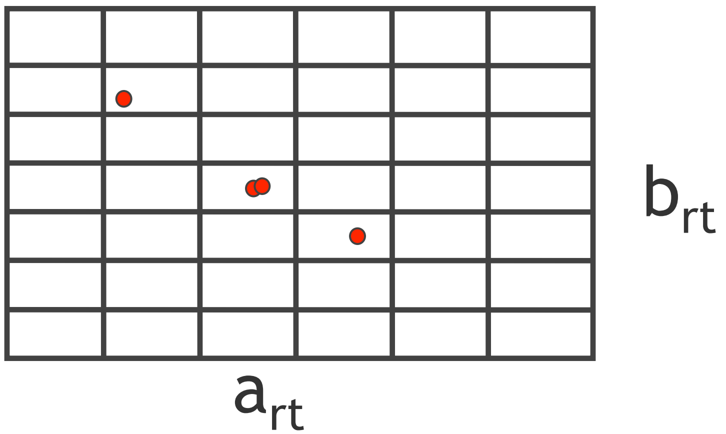


$$m_1 = a_{rt}s_1+b_{rt}$$

$$m_2 = a_{rt}s_2+b_{rt}$$

Pose Clustering

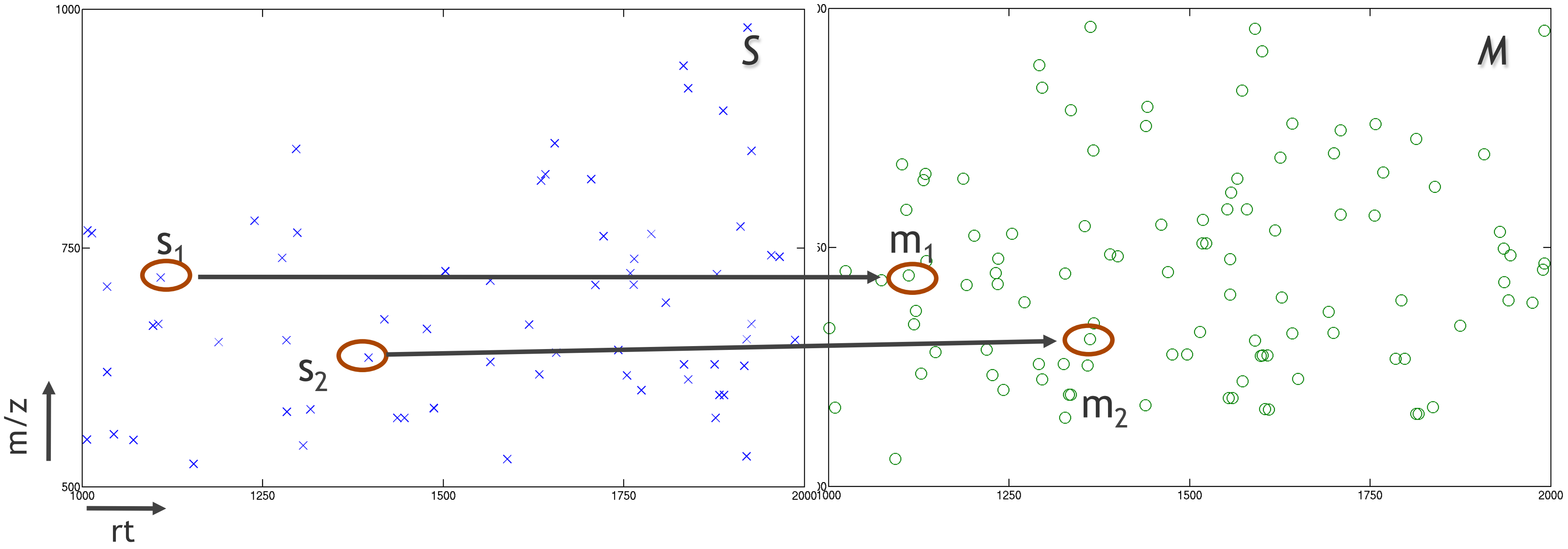


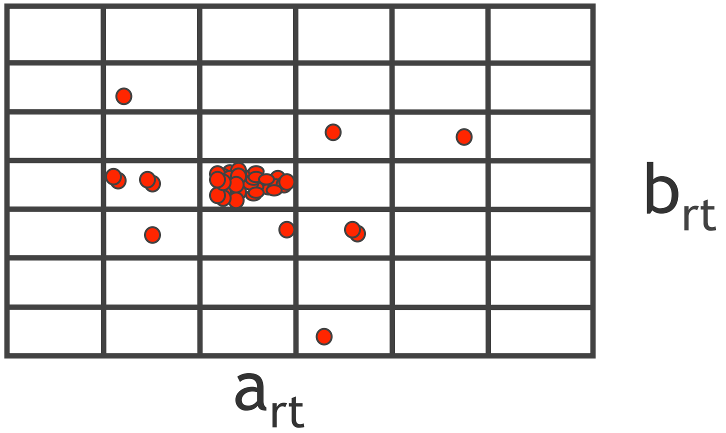


$$m_1 = a_{rt}s_1+b_{rt}$$

$$m_2 = a_{rt}s_2+b_{rt}$$

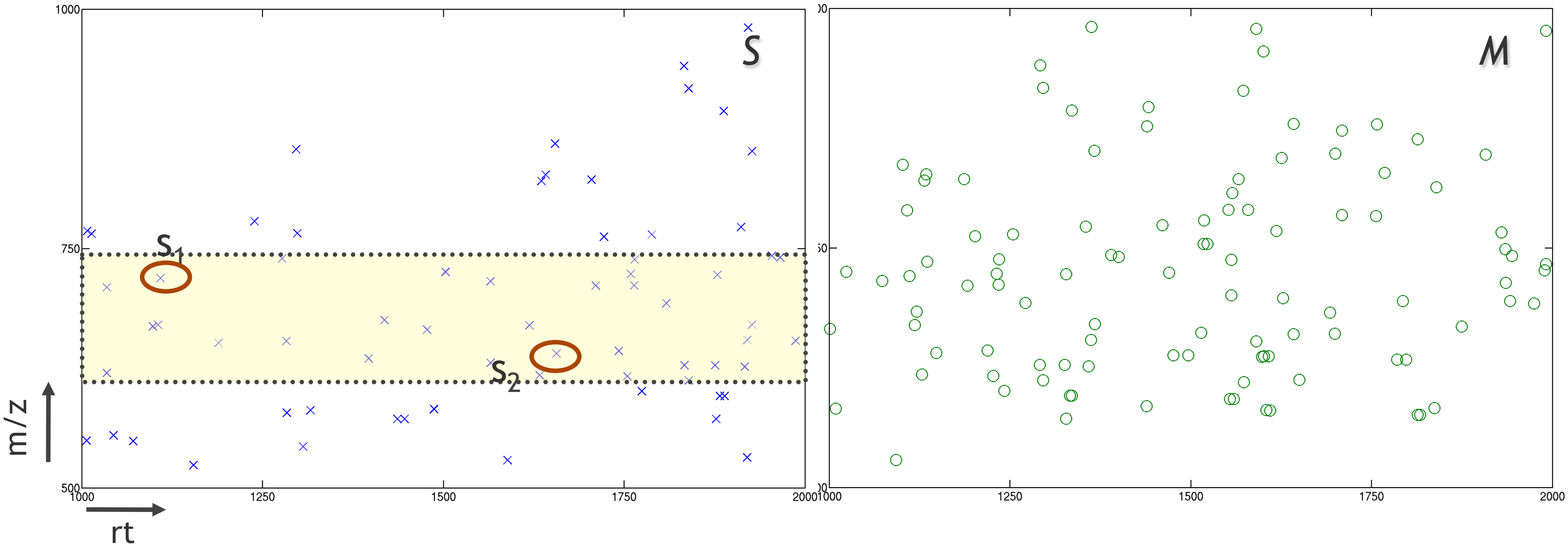
Pose Clustering





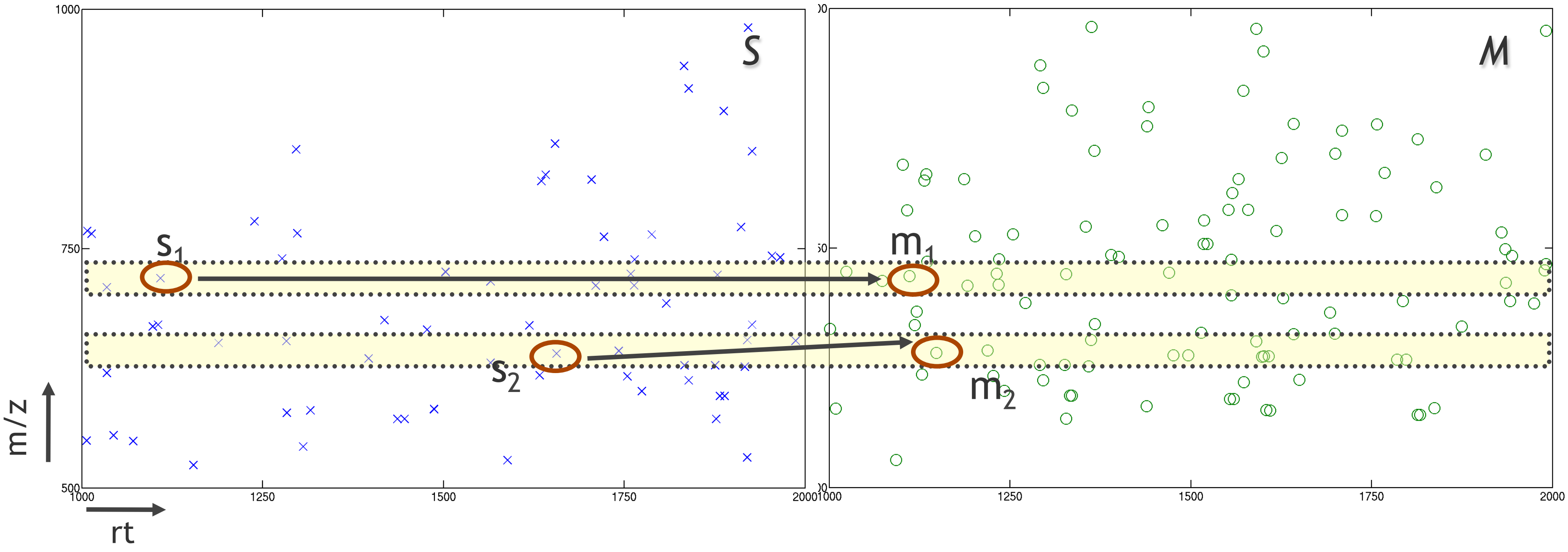
- Matching of corresponding pairs will result in the correct transformation
- These are more likely than random matches!

Speeding Things Up



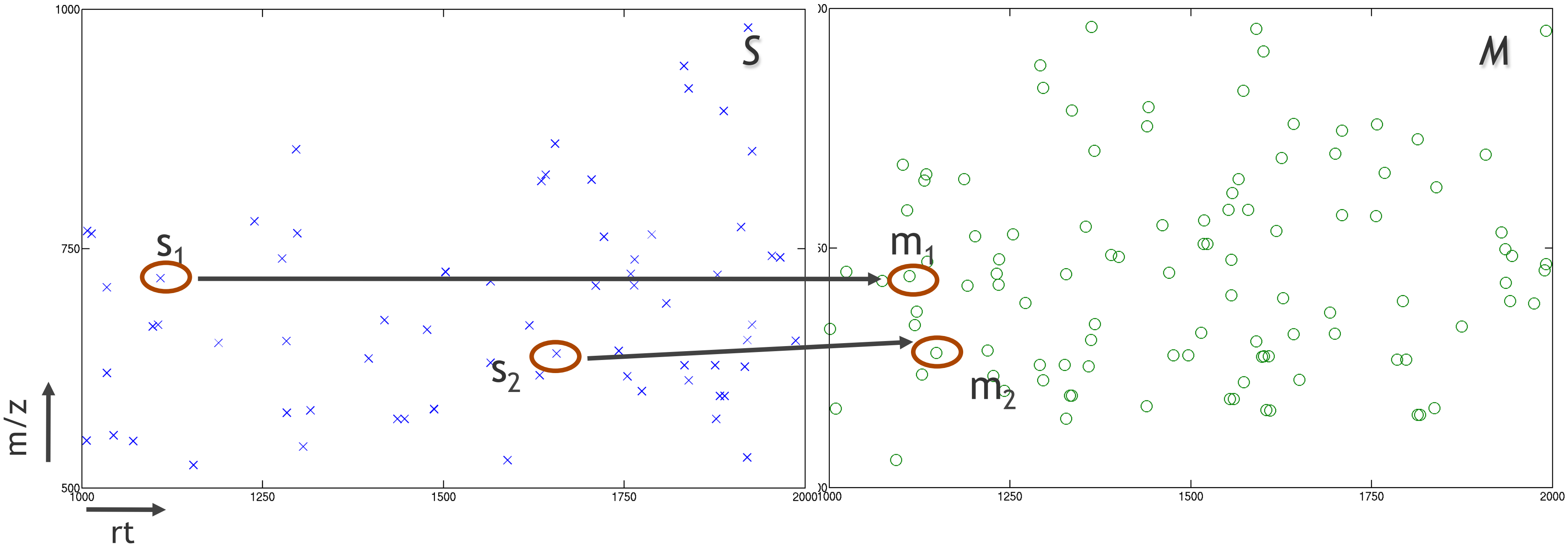
Only consider pairs (s_1,s_2) in *S* with s_1 having a small distance to s_2 in *m*/*z*.

Speeding Things Up



Only match
pair (s_1,s_2) onto pair (m_1,m_2)
if s_1 and m_1 as well as s_2 and m_2
lie close together in m/z.

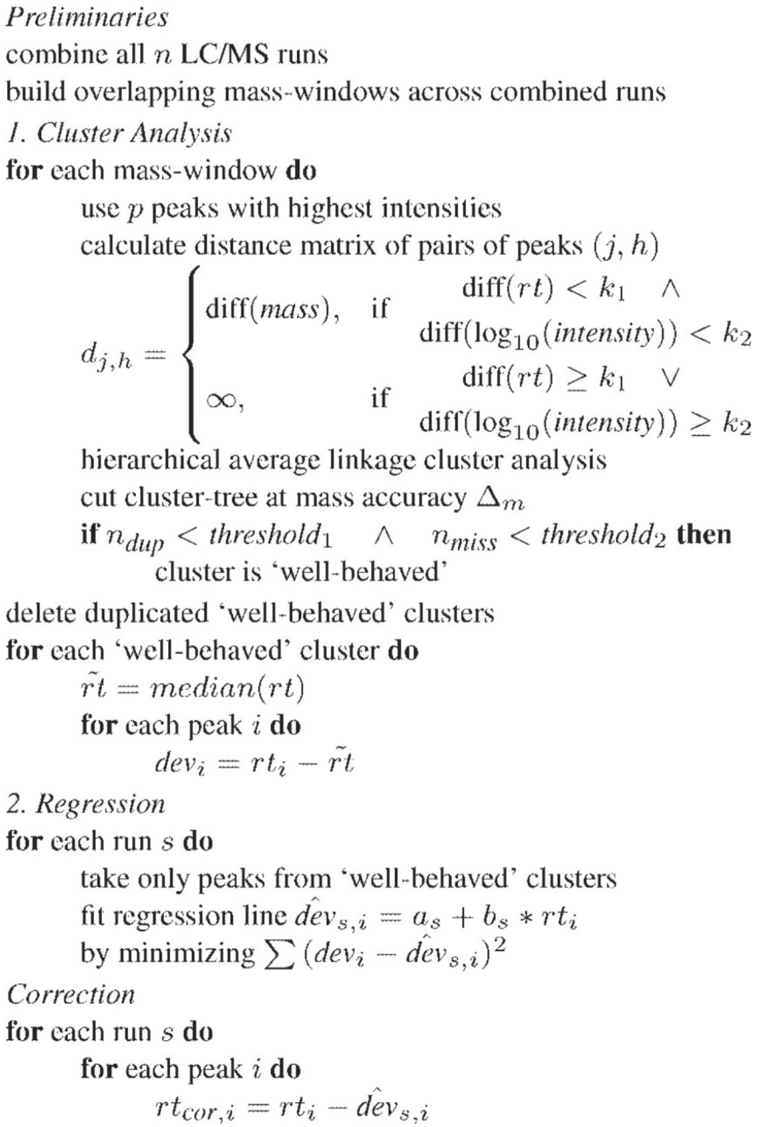
Improve Matching



Normalize intensities in M and S: weight the vote of each transformation by the intensity similarities of the point matches (s_1,m_1) and (s_2,m_2).

Linear Alignment

- Podwojski *et al.* proposed an alternative linear alignment method and also extended this to a nonlinear alignment
- The linear alignment is similar to the algorithm by Lange *et al.*
- It uses a different type of cluster analysis to determine a linear regression
- In contrast to the Lange algorithm, it generalizes nicely to multiple map alignment



```
*Preliminaries*
combine all $n$ LC/MS runs
build overlapping mass-windows across combined runs
*1. Cluster Analysis*
**for** each mass-window **do**
    use $p$ peaks with highest intensities
    calculate distance matrix of pairs of peaks $(j, h)$
    $d_{j,h} = \begin{cases} \text{diff}(mass), & \text{if} \quad \text{diff}(rt) < k_1 \quad \wedge \quad \text{diff}(\log_{10}(intensity)) < k_2 \\ \infty, & \text{if} \quad \text{diff}(rt) \geq k_1 \quad \vee \quad \text{diff}(\log_{10}(intensity)) \geq k_2 \end{cases}$
    hierarchical average linkage cluster analysis
    cut cluster-tree at mass accuracy $\Delta_m$
    **if** $n_{dup} < threshold_1 \quad \wedge \quad n_{miss} < threshold_2$ **then**
        cluster is 'well-behaved'
delete duplicated 'well-behaved' clusters
**for** each 'well-behaved' cluster **do**
    $\tilde{rt} = median(rt)$
    **for** each peak $i$ **do**
        $dev_i = rt_i - \tilde{rt}$
*2. Regression*
**for** each run $s$ **do**
    take only peaks from 'well-behaved' clusters
    fit regression line $\hat{dev}_{s,i} = a_s + b_s * rt_i$
    by minimizing $\sum (dev_i - \hat{dev}_{s,i})^2$
*Correction*
**for** each run $s$ **do**
    **for** each peak $i$ **do**
        $rt_{cor,i} = rt_i - \hat{dev}_{s,i}$
```

Podwojski et al., Bioinformatics (2009), 25:758-764.

Nonlinear Alignment

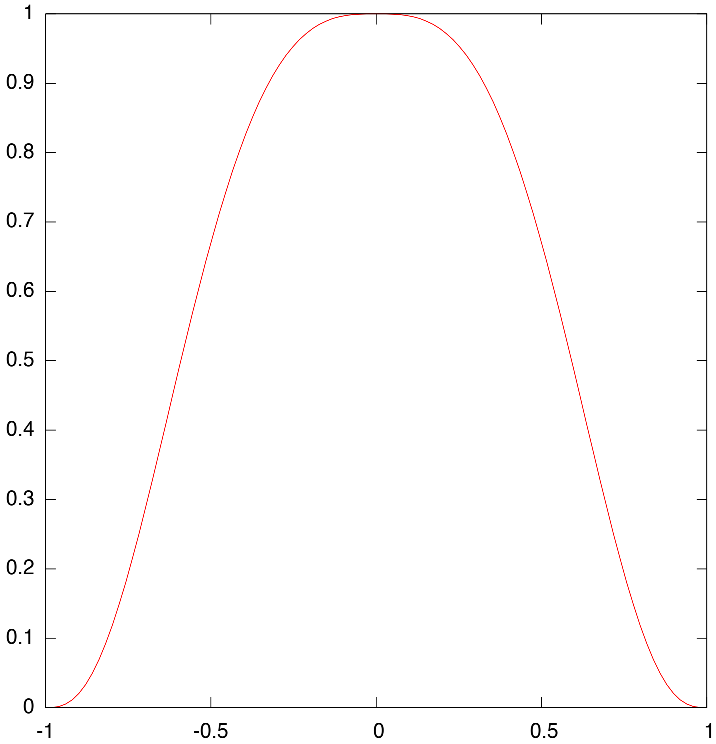
- **Idea**
  - Perform linear alignment (using pose clustering)
  - Compute a more accurate local alignment using LOESS regression
- **LOESS regression** (often also called LOWESS)
  - Locally weighted polynomial regression
  - Based on a pre-defined window size
  - Points within this window contribute to the local regression
  - Perform local regression (linear or quadratic, cubic) around the predicted coordinate

LOESS Regression

- Weighting is often performed by tricubic weighting function

$$w(z) = \begin{cases} (1 - |z|^3)^3 & if|z| < 1 \\ 0 & otherwise \end{cases}$$

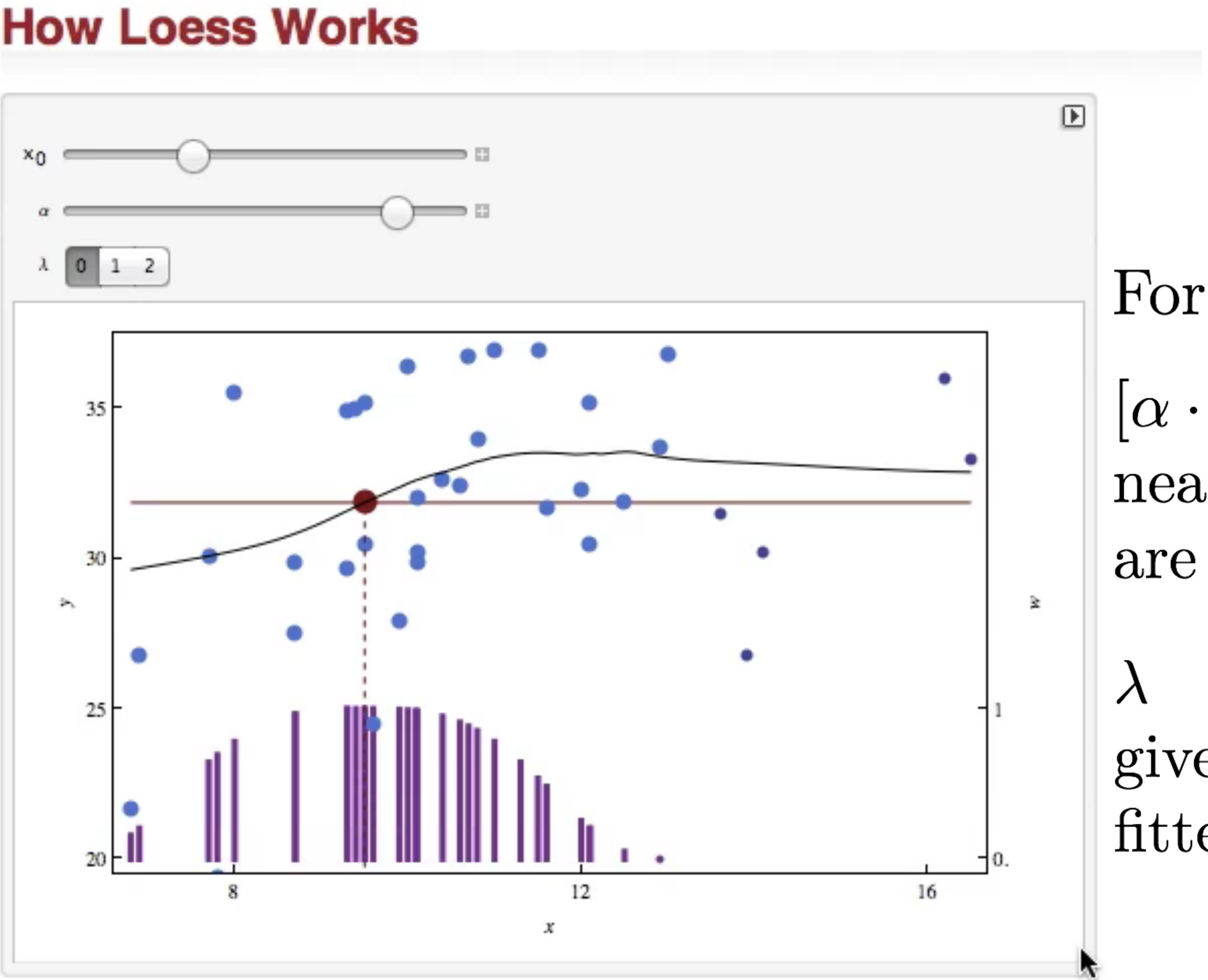
- Weighting function is applied to coordinates scaled into the chosen window (-1 · 0 · 1)
- Local regression (linear quadratic) needs to be recomputed around every point (computationally very expensive)



tricubic function

Cleveland, J. Am. Stat. Soc (1979), 74:829-836

LOESS Regression


How Loess Works

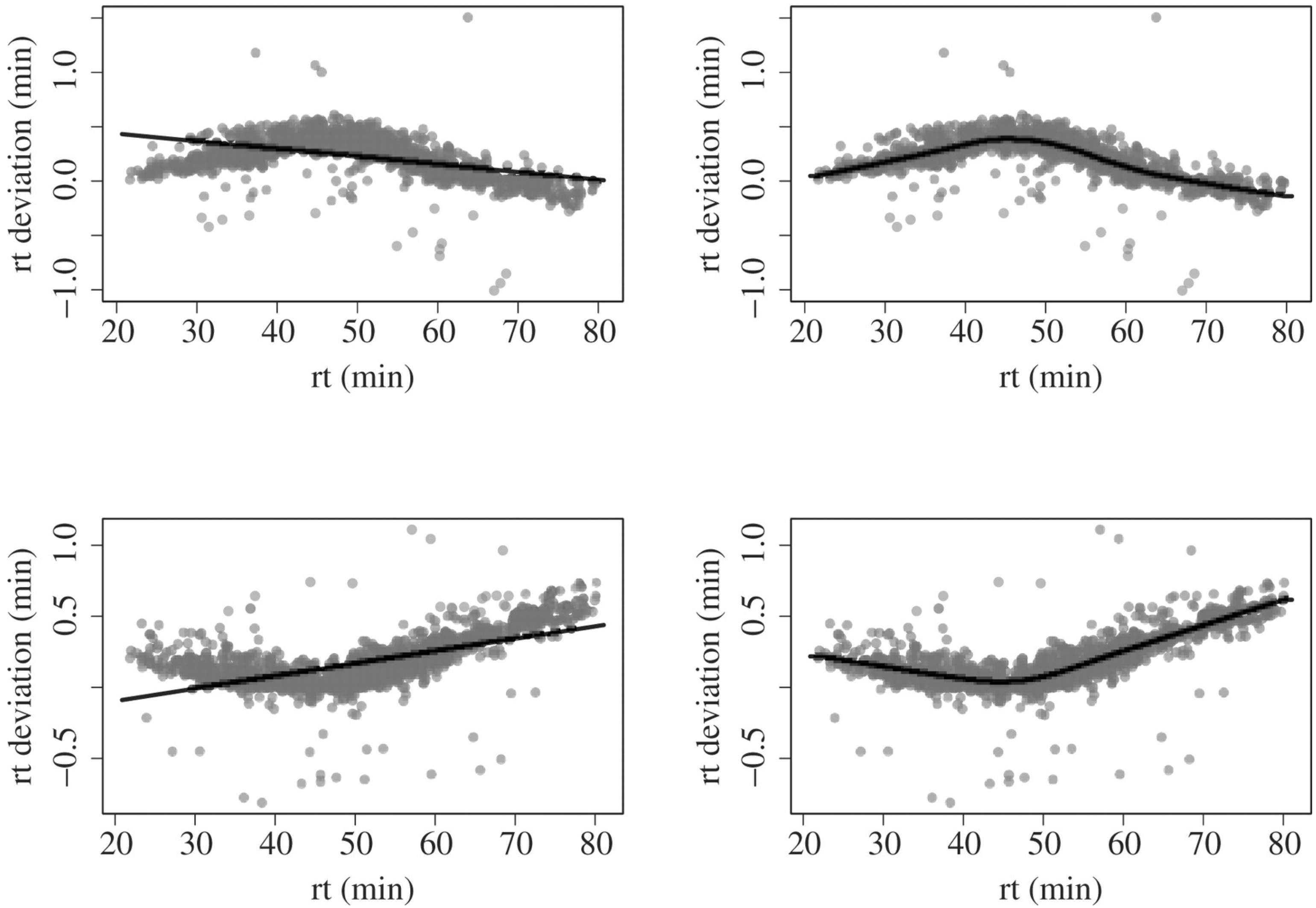
For $0 < \alpha \le 1$

$[\alpha \cdot n]$
nearest neighbours
are considered

λ
gives degree of
fitted polynomial

http://demonstrations.wolfram.com/HowLoessWorks

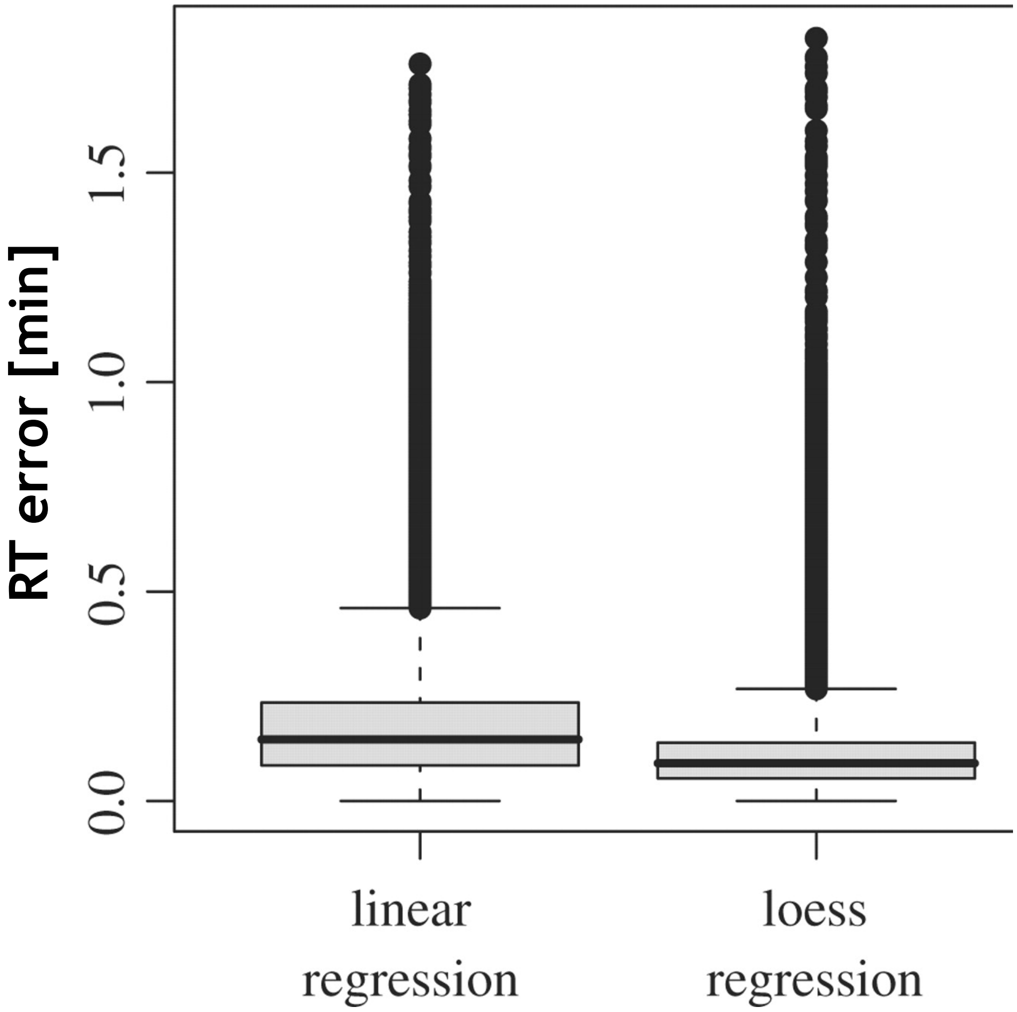
Nonlinear Alignment



Alignment of two different datasets (top/bottom). Left: linear, right: nonlinear. (around 30 k aligned peaks)

Podwojski et al., Bioinformatics (2009), 25:758-764.

Nonlinear Alignment



Comparison of median RT error for linear/nonlinear regression

Podwojski et al., Bioinformatics (2009), 25:758-764.

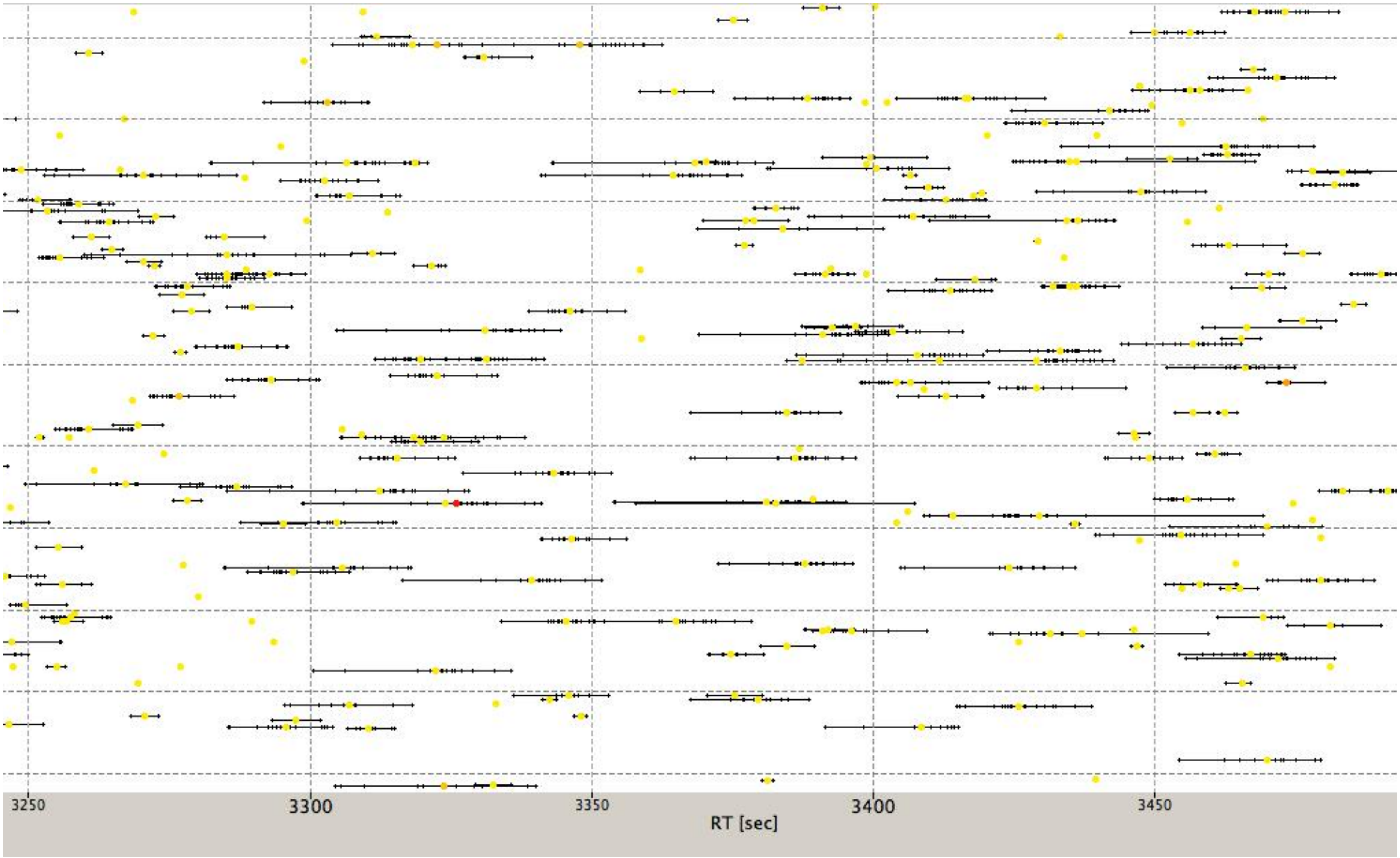
Feature Linking

- Map alignment does not yet create a direct correspondence (bijection) between the features!
- Feature linking pairs up features
  - **across maps** for label-free quantification
  - **within maps** for arbitrary labeling strategies (e.g., SILAC: link pairs 6 Da apart)
- A user-specified **mass tolerance** and **retention time tolerance** are required as input
- Labeled feature linking also requires the specification of the label distance (mass difference)
- The result are consensus features containing the original features as well
- Correctness of linked features can also be verified through identifications (if present)

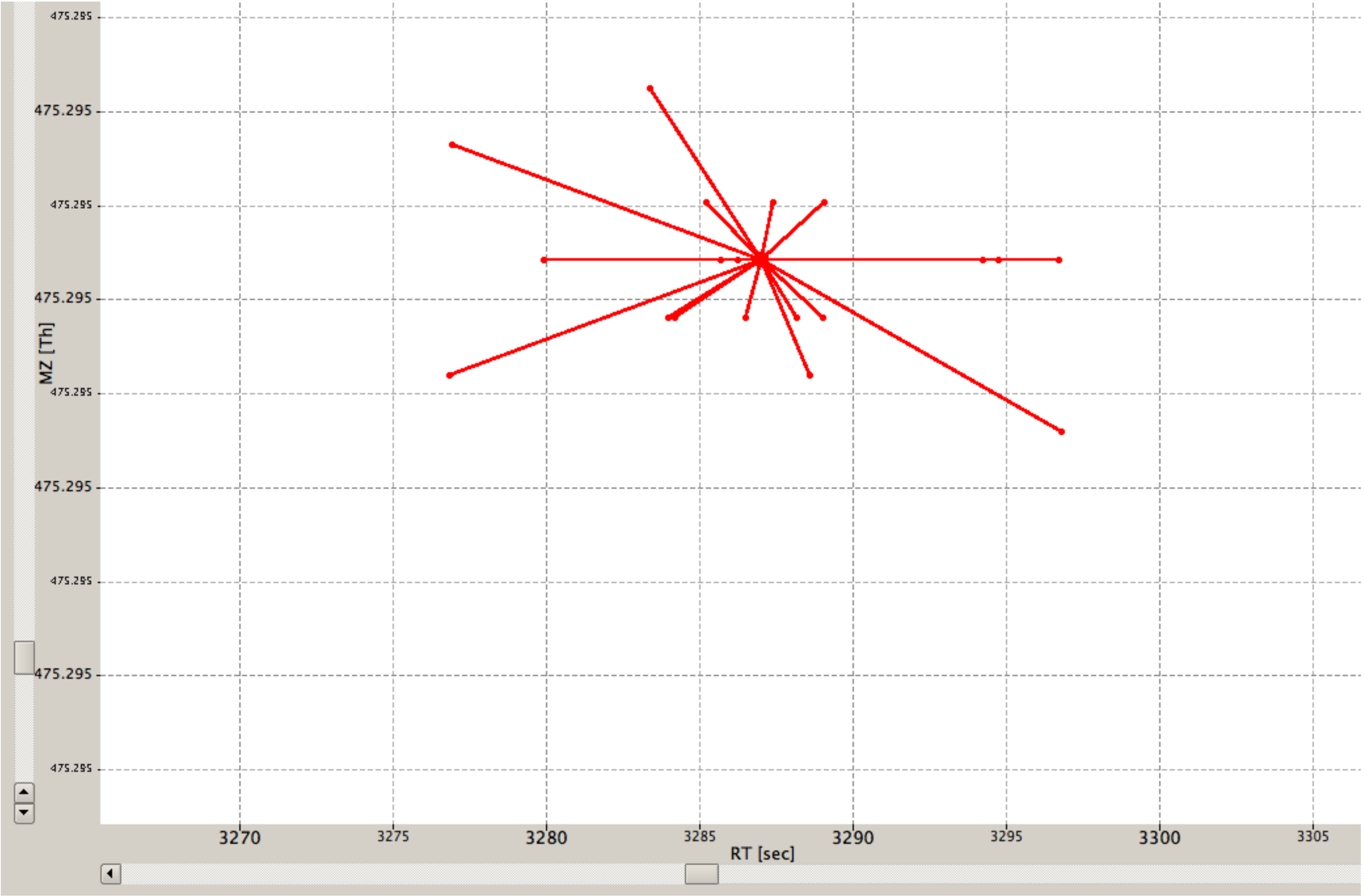
OpenMS/TOPP

- OpenMS implements the Lange et al. algorithm
- TOPP contains tools for map alignment and for feature linking
  - MapAlignerPoseClustering
    - Implements the pose clustering algorithm and computes the corresponding transformation
  - FeatureLinkerUnlabeledQT
    - Uses QT clustering to compute the best assignment of features across several maps
    - Result is a consensus map

Consensus Features

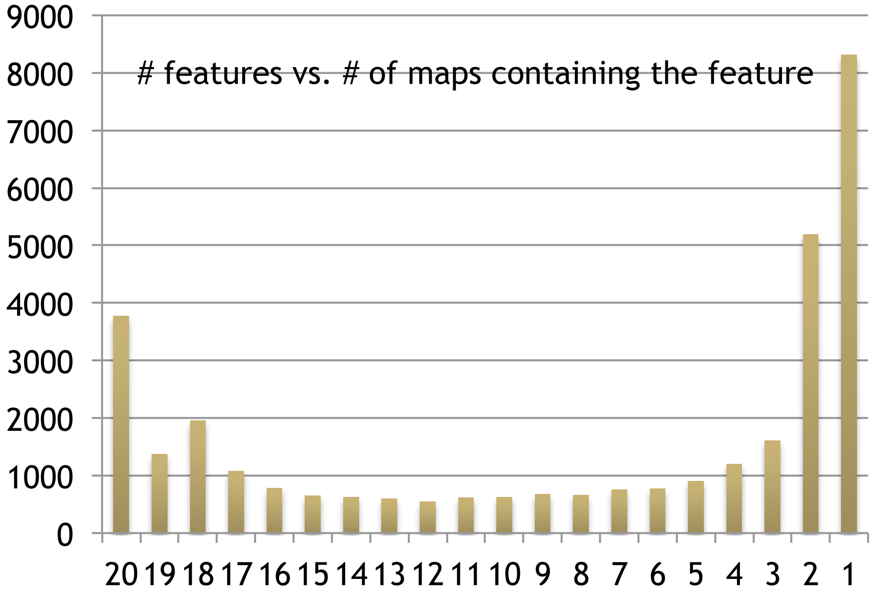


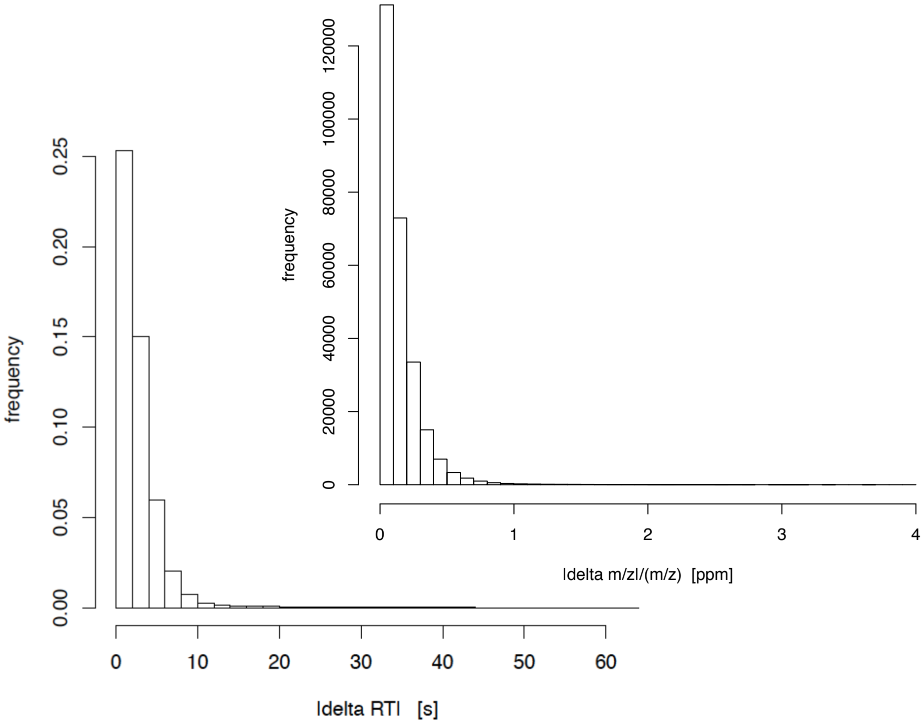
Consensus Features



Quality Control

- **MapStatistics**
  - Produces some descriptive statistics of a map for QC
    - Did feature finding and map alignment work properly?
    - Do all maps we aligned have roughly the same amount of features?
    - Check instrument calibration and stability of chromatography



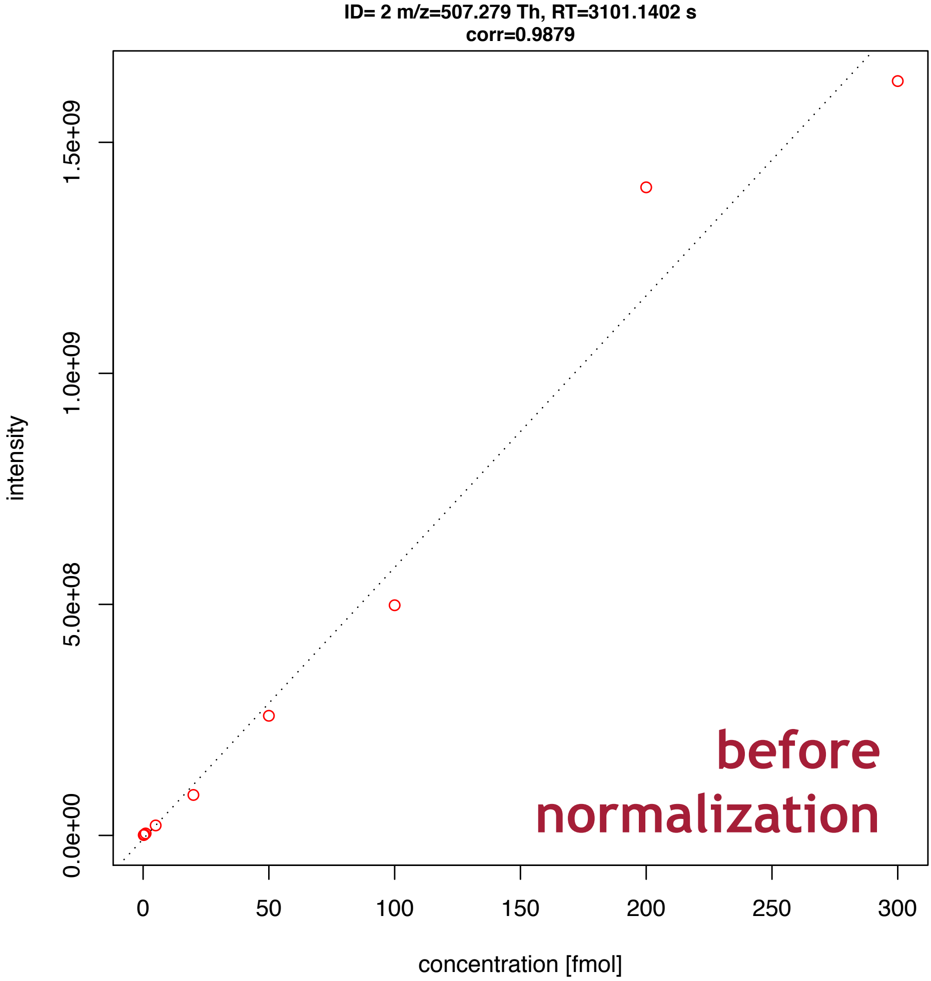


Map Normalization

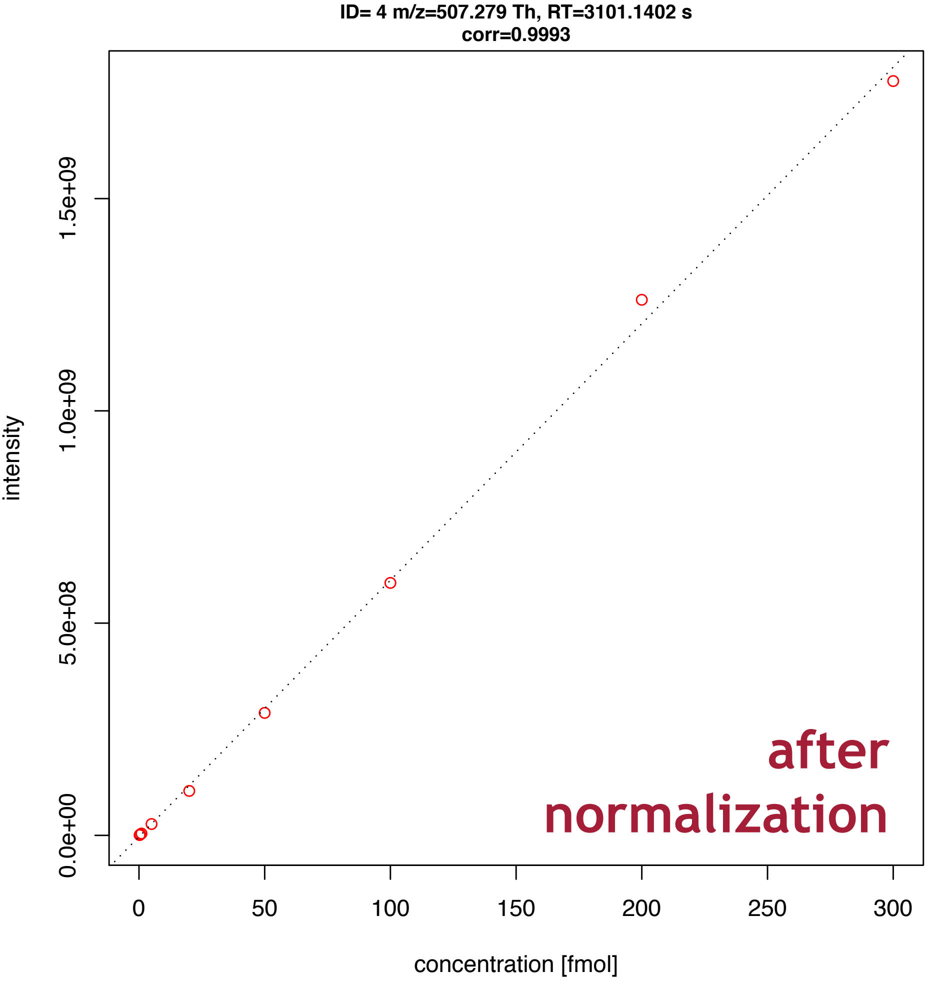
- For label-free quantification a normalization of features across maps is often helpful
- **Strategy 1: internal standards**
  - Spiked in peptides/proteins are used for normalizing maps
  - This is easily done in a statistics package or Excel after the analysis
- **Strategy 2: background normalization**
  - For a sufficiently complex background only a small number of features/ peptides will be differential
  - The background can be used to normalize maps with respect to each other (keeping the ration of unregulated background features at 1:1)
- **Idea**: 'robust regression'
  - Look at all the ratios
  - Remove outliers
  - Determine the normalization factor from the rest

Effect of Normalization

- Label-free quantification in a complex (platelet) background measured with a spiked in peptide


ID= 2 m/z=507.279 Th, RT=3101.1402 s
corr=0.9879

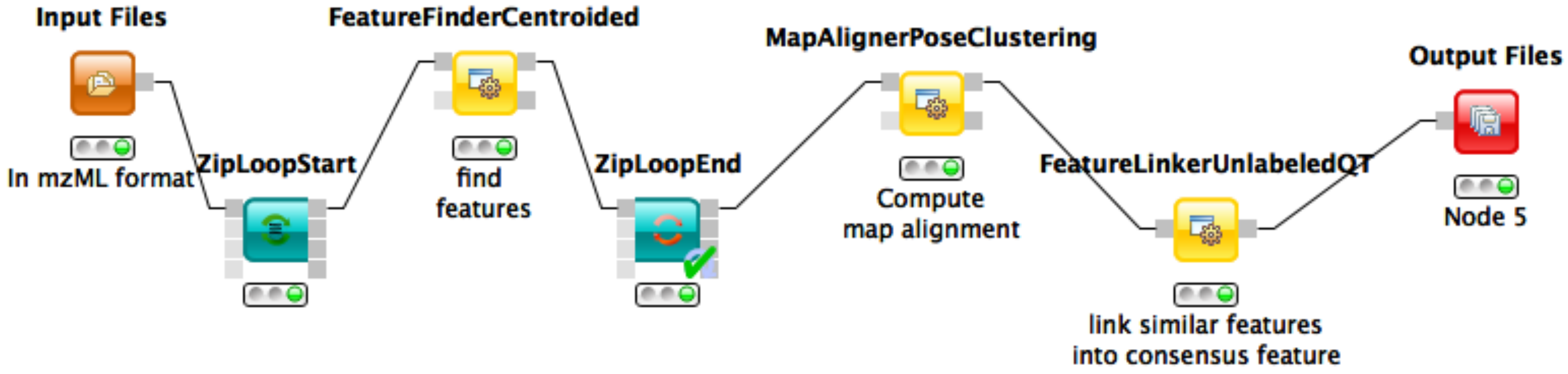
before normalization


ID= 4 m/z=507.279 Th, RT=3101.1402 s
corr=0.9993

after normalization

Feature Finding in KNIME

- TOPP tool FeatureFinder (FeatureFinderCentroided in OpenMS 1.11)
- Reads a centroided LC-MS map – so if data is available as raw data, it needs to be converted to centroided data using a peak picker
- Label-free workflows can get rather complicated and usually require identification steps as well (which we will discuss later in the lecture)

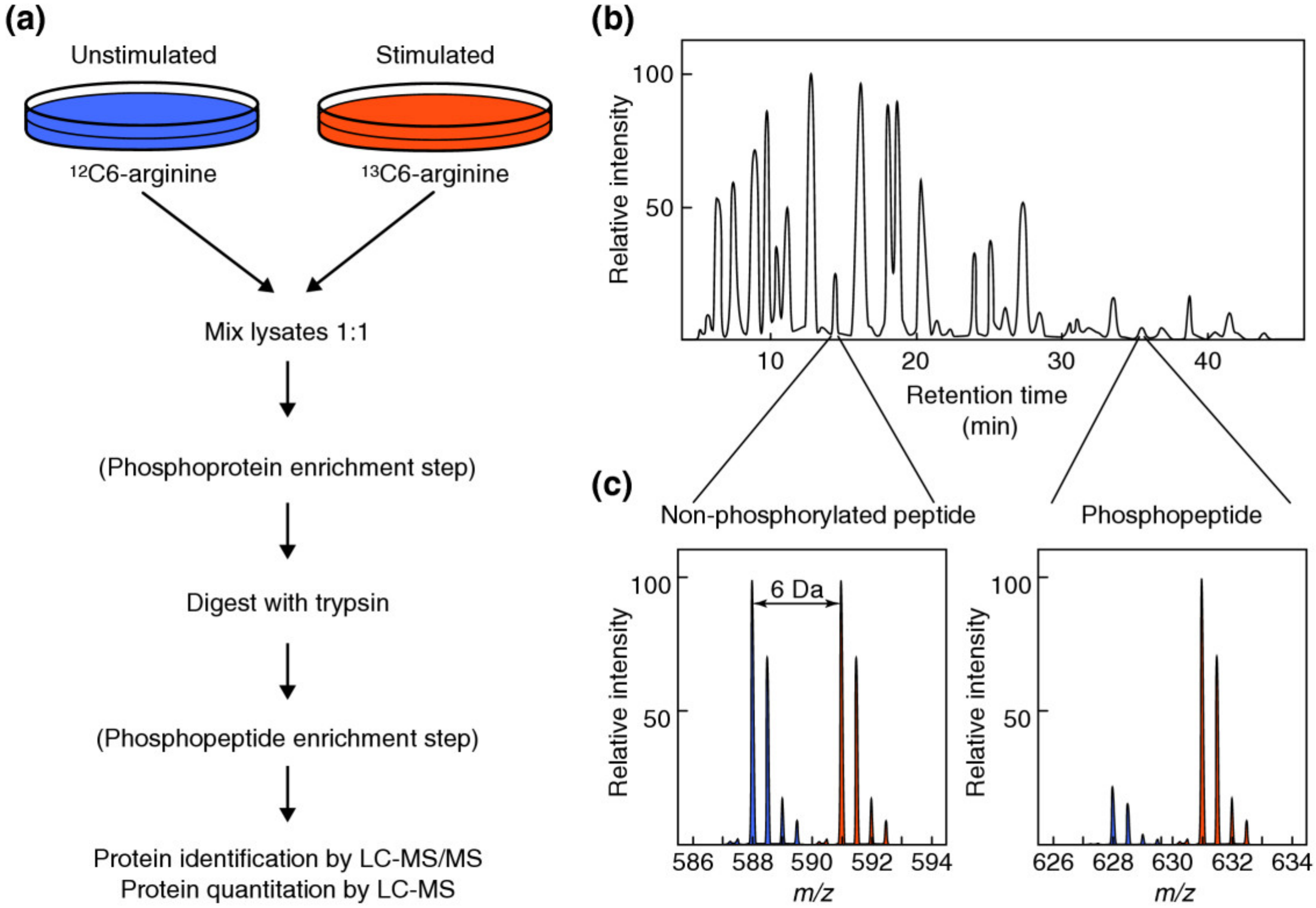


LEARNING UNIT 5C
SILAC QUANTIFICATION

SILAC Quantification

- Experimental techniques
- MaxQuant algorithm

SILAC



Unstimulated

Stimulated

^{12}C6-arginine

^{13}C6-arginine

Mix lysates 1:1

(Phosphoprotein enrichment step)

Digest with trypsin

(Phosphopeptide enrichment step)

Protein identification by LC-MS/MS
Protein quantitation by LC-MS

Mumby, Brekken, Genome Biol (2005), 6:230

SILAC Analysis

- In principle, SILAC pairs are regular features
- Note that isotopic labels shift the averagine model
- A standard analysis workflow could thus look like:
  - Feature finding
  - Linking of pairs with the proper distance (4/6/8/10 Da, depending on the experiment)
- Specialized SILAC analysis tools can make use of the additional information contained in pairs
  - Exact mass differences
  - Presence of a second pair can increase confidence in the detection
- Inclusion of this knowledge generally improves sensitivity of the feature/pair detection

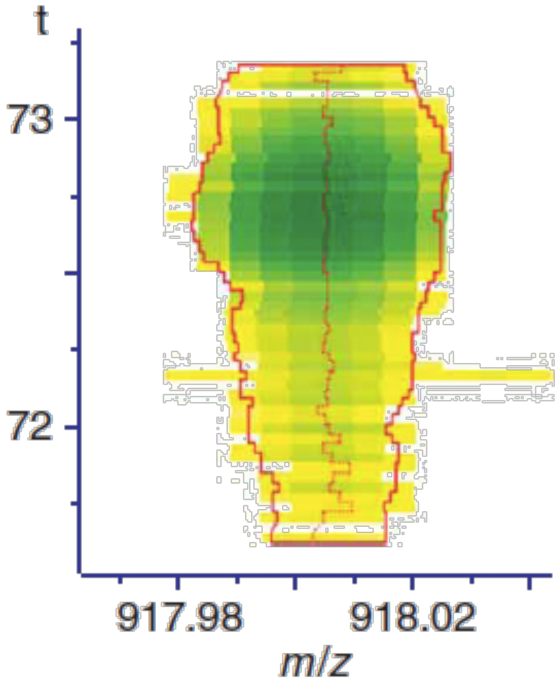
MaxQuant

- **Peak detection**
  - Identify chromatograpic peaks
- **De-Isotoping**
  - Construct features from the matching chromatographic peaks
- **Pair detection**
  - Identify SILAC pairs among the de-isotoped peaks
- **Ratio estimation**
  - Determine the ratio of the SILAC pair

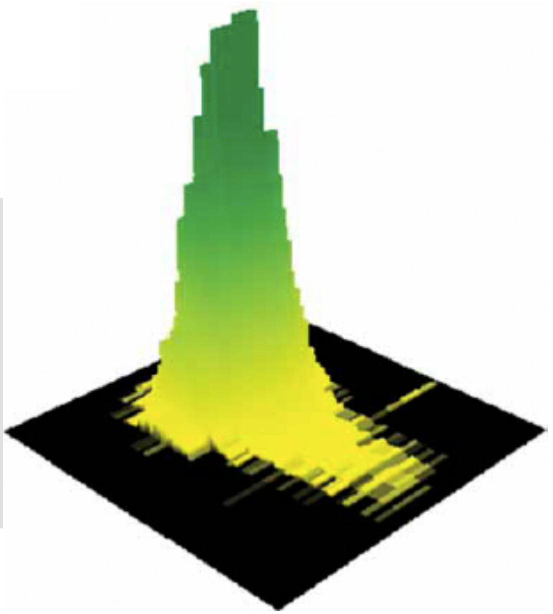
Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

Peak Detection

- MaxQuant uses the notion of **3D peaks** to describe the mass traces on the raw data (three dimensions: RT, m/z, intensity)
- 3D peaks can be defined as all the signal caused by one isotopic mass of an analyte – they correspond to mass traces in centroided feature finding
- Features are then defined as several of these 3D peaks



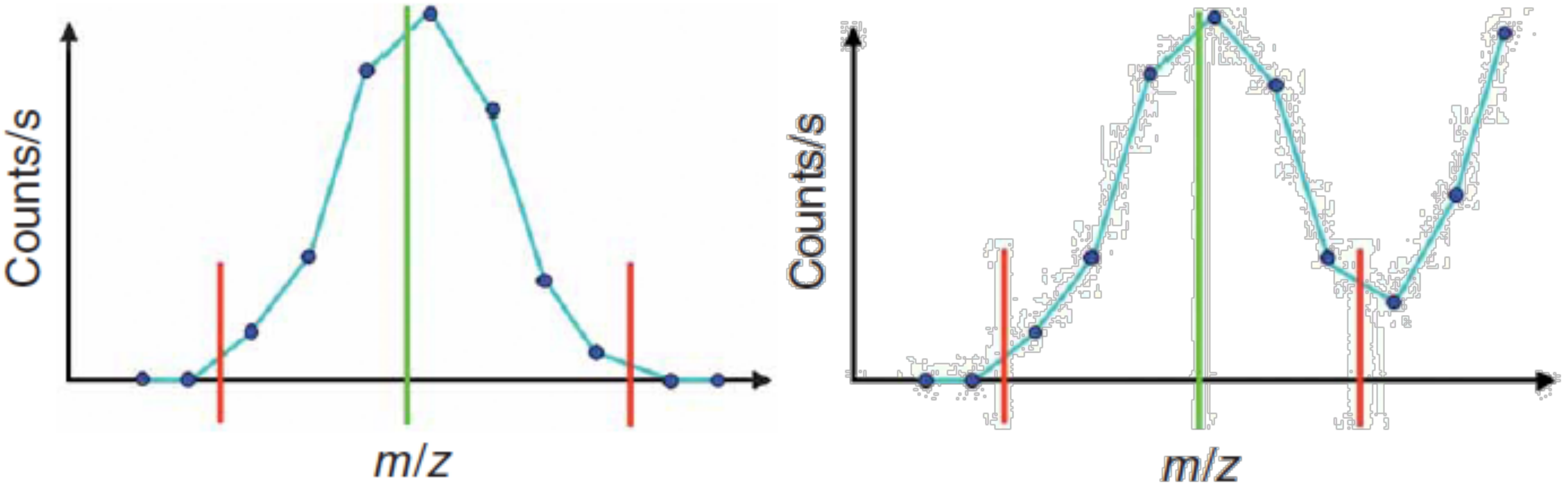
3D peak eluting over 1.5 min, m/z around 918 Da in 2D and 3D representation



Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

Peak Detection

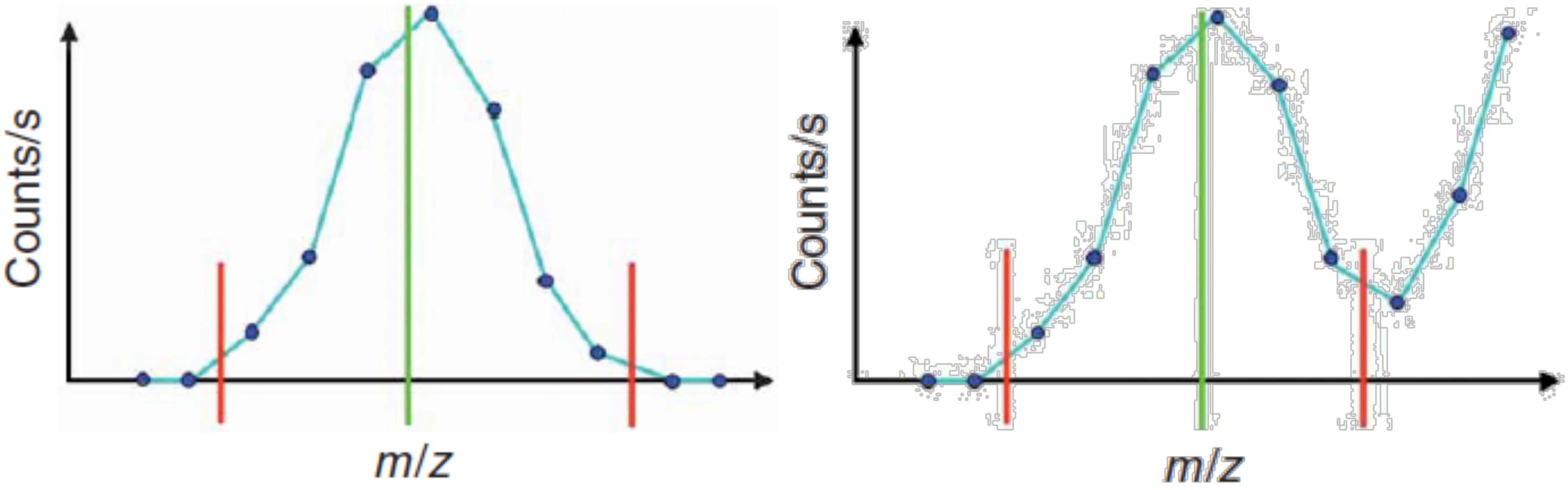
- 3D peaks are detected by detecting peaks within individual mass spectra first
- For high-resolution MS instruments (e.g., Orbitrap), peak detection is achieved by looking for local maxima
- 2D peaks are then determined as the range from the maximum until either zero or a local minimum has been reached



Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

Peak Detection

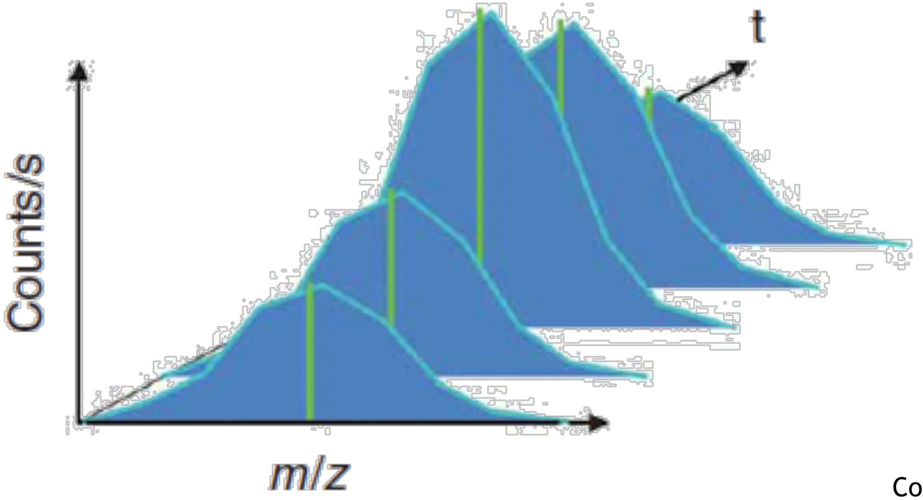
- If there are more than three data points to the peak, then the center of the peak (centroid) is determined as by a **Gaussian fit** to these three peaks
- Special treatment for peaks consisting of only one or two peaks
- Intensity of the peak is approximated by the **sum of the intensities of all raw data points** of the peak



Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

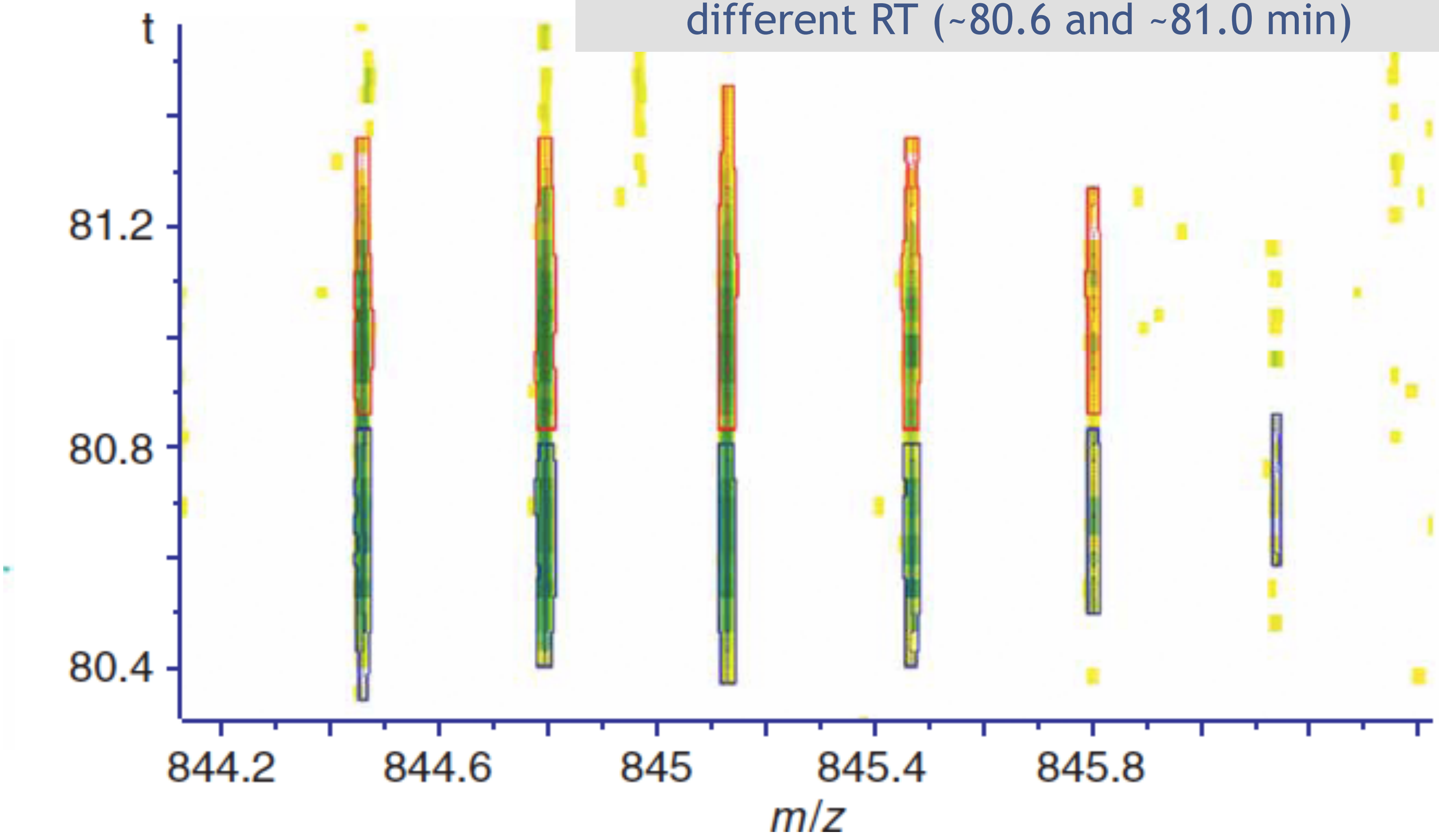
Peak Detection

- 2D peaks of adjacent scans are assembled into a 3D peak, if their centroid positions differ by less than 7 ppm
- 2D peaks may be missing in up to one scan (e.g., in case a 2D peak detection did not work well), 3D peak consists of the maximum number of 2D peaks that can be joined in this way
- Intensities of 2D peaks are smoothed and the 3D feature is split if there are local minima in the intensity
- The 3D peak mass the intensity-weighted average of its 2D peaks' masses



Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

Peak Detection



Two 3D peaks with identical masses, but different RT (~80.6 and ~81.0 min)

Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

De-Isotoping

- 3D peaks are aggregated to features
- To this end, a **compatibility graph** is constructed
- **3D peaks are represented by nodes**
- An **edge** is added between two nodes, if
  - Their masses match the distance within an isotope profile
  - Their elution profiles overlap (normalized inner product [cosine] of the two 3D peaks is greater than 0.6)
- **Connected components** of this graph are potential features, but can still contain 3D peaks from multiple features (overlapping features)

De-Isotoping

The mass criterion for an edge between the nodes representing two 3D peaks is fulfilled if the following holds:

$$\left|\Delta m - \frac{\Delta M}{z}\right| \leq \sqrt{\left(\frac{\Delta S}{z}\right)^2 + (5\Delta m_1)^2 + (5\Delta m_2)^2}$$

Where *m* is the mass difference between the peaks and ΔM is the mass difference between the monoisotopic and the ^{13}C satellite for an averagine of mass 1,500 Da (1.00286864 Da), *z* the charge.

Δm_1 and Δm_2 are the bootstrapped standard deviations of the two exact peak masses and

ΔS = 2 m(^{13}C) – 2 m(^{12}C) – m(^{34}S) – m(^{32}S) = 0.0109135 Da

Is the maximum mass shift caused by the incorporation of one sulphur atom.

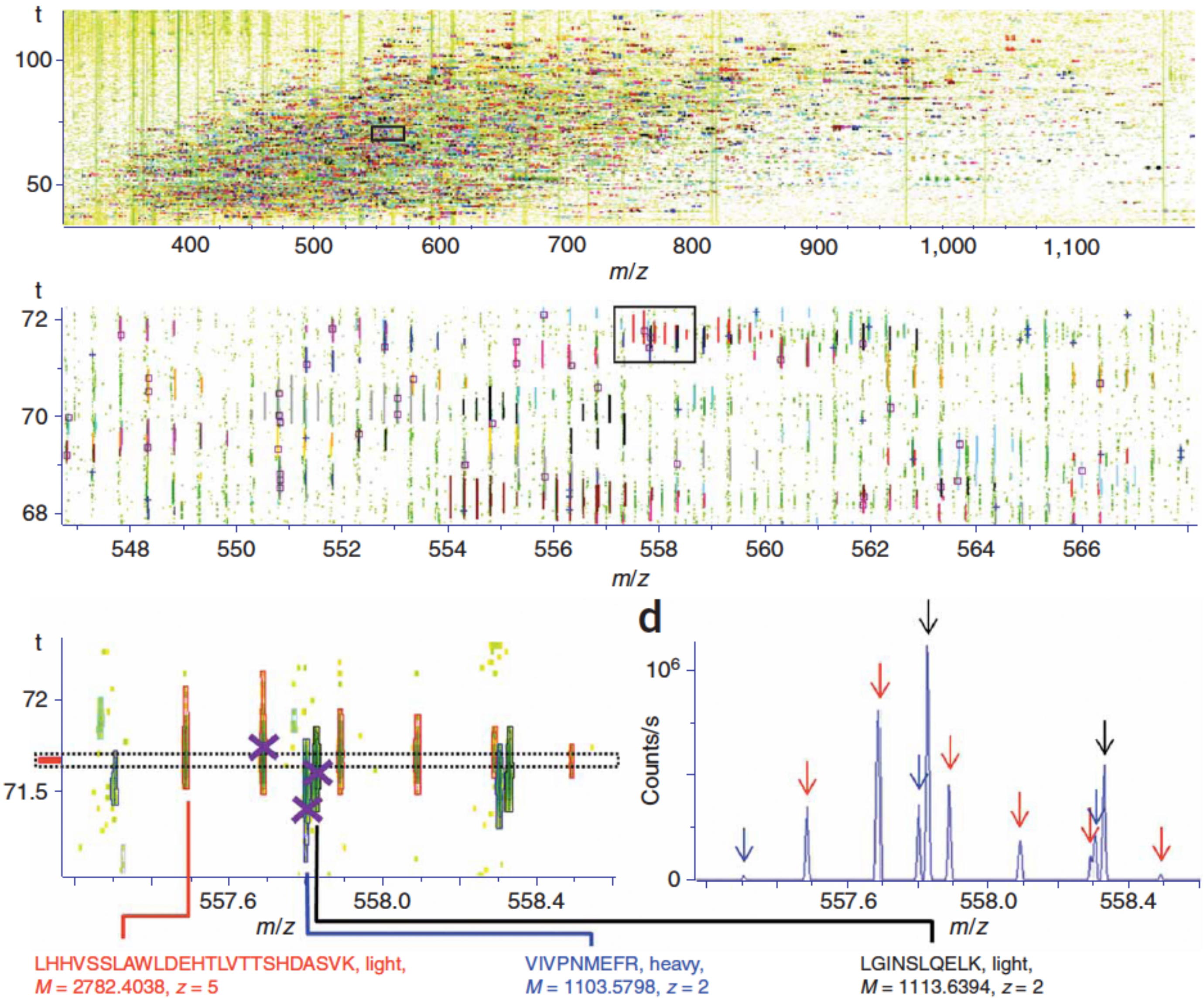
De-Isotoping

- Connected components of this graph correspond to sets of overlapping features and individual (noise) 3D peaks
- They are resolved by iteratively removing the largest set of 3D peaks that are consistent
- Consistency is defined by
    - Mutual consistency of all pairs of peaks with respect to their mass distances (similar to the above definition for an edge, but also between more distant peaks)
    - Correlation of 0.6 or better between all elution profiles
    - Correlation of 0.6 or better of the 3D peak distances with the isotope distribution of an averagine at mass 1,500 Da

Pair Detection and Ratio Estimation

- SILAC pairs are found through their distances by searching for pairs in the correct distance (for up to three labeled K or R in all possible combinations)
- Intensities of the two features have to have a correlation of 0.5 or better
- For each pair, the intensity ratios are determined as the slope of a regression line through the itensities of corresponding 3D peaks in the light and heavy feature

Result



SILAC pairs identified in a large-scale study of human HeLa cells. Over 5,000 SILAC pairs were found in one run.

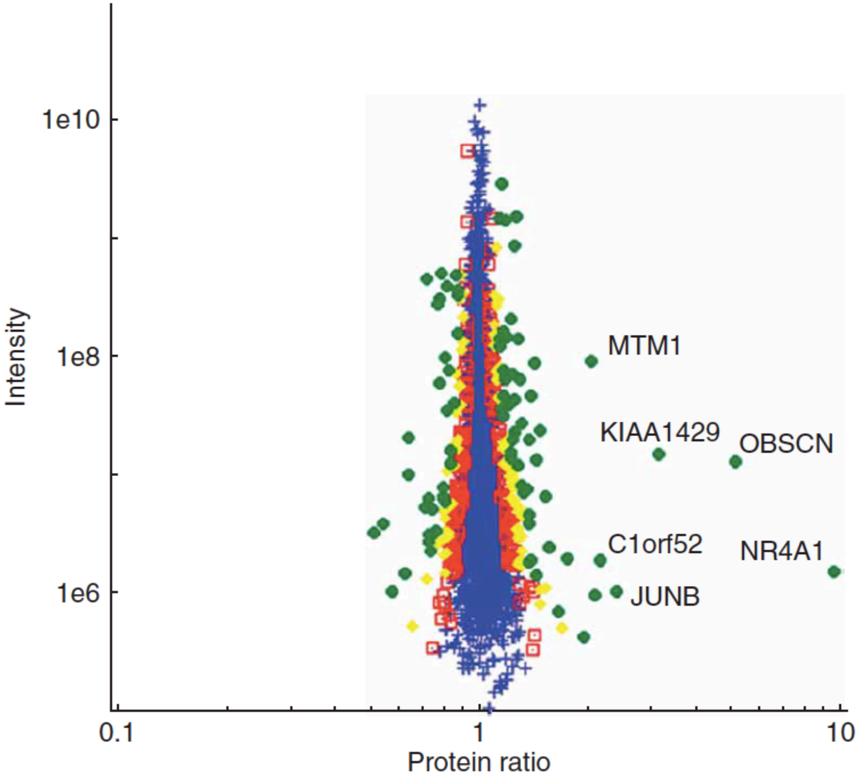
Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

MaxQuant

- MaxQuant implements the SILAC pair detection algorithm sketched here
- Later versions of MaxQuant can also be applied to label-free quantification
- MaxQuant is unfortunately restricted to a specific vendor format (ThermoFischer RAW format) and platform (Windows)
- The output consists of a text file, that can then be parsed and analyzed statistically with other tools

MaxQuant

- Differential quantification of protein ratios of HeLA cells after 2 h of EGF stimulation
- 99.3% of all proteins have a ratio of 1.0 (+/- 50%) and are thus not significantly regulated
- Transcription factor JunB and orphan nuclear receptor NR4A1 are both significantly upregulated
- Their upregulation by EGF has been found through other methods and described in literature as well



'christmas tree plot':
pair intensity as a function of the pair ratio (double logarithmic plot) reveals the distribution of ratios, accuracy, LOD, LOQ, LOL

Cox & Mann, Nat. Biotech. (2008), 26:1367-1372.

Original Papers

- Label-free feature finding (OpenMS feature finder)
  - Clemens Gröpl, Eva Lange, Knut Reinert, Oliver Kohlbacher, Marc Sturm, Christian G. Huber, Bettina M. Mayr, Christoph L. Klein: Algorithms for the Automated Absolute Quantification of Diagnostic Markers in Complex Proteomics Samples. CompLife 2005: 151-162.

    Online: http://www.springerlink.com/content/81lk5vjtxqwbflce/
  - Sturm, Marc: OpenMS – A framework for computational mass spectrometry, Dissertation, Tübingen (2010)

    Online: http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-51146
  - Website: http://openms.de
- SILAC feature finding (MaxQuant)
  - Cox, J. and Mann, M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol 26, 1367-72.

    (algorithm: see Supplementary Material at http://www.nature.com/nbt/journal/v26/n12/extref/nbt.1511-S1.pdf)
  - Website: http://maxquant.org

Materials

- Online Materials
  - Learning Unit 5[A,B,C],
  - Learning Unit 1C