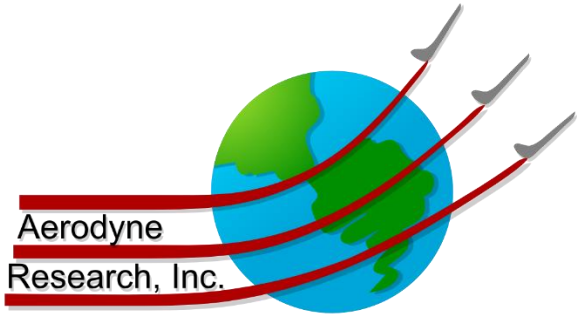
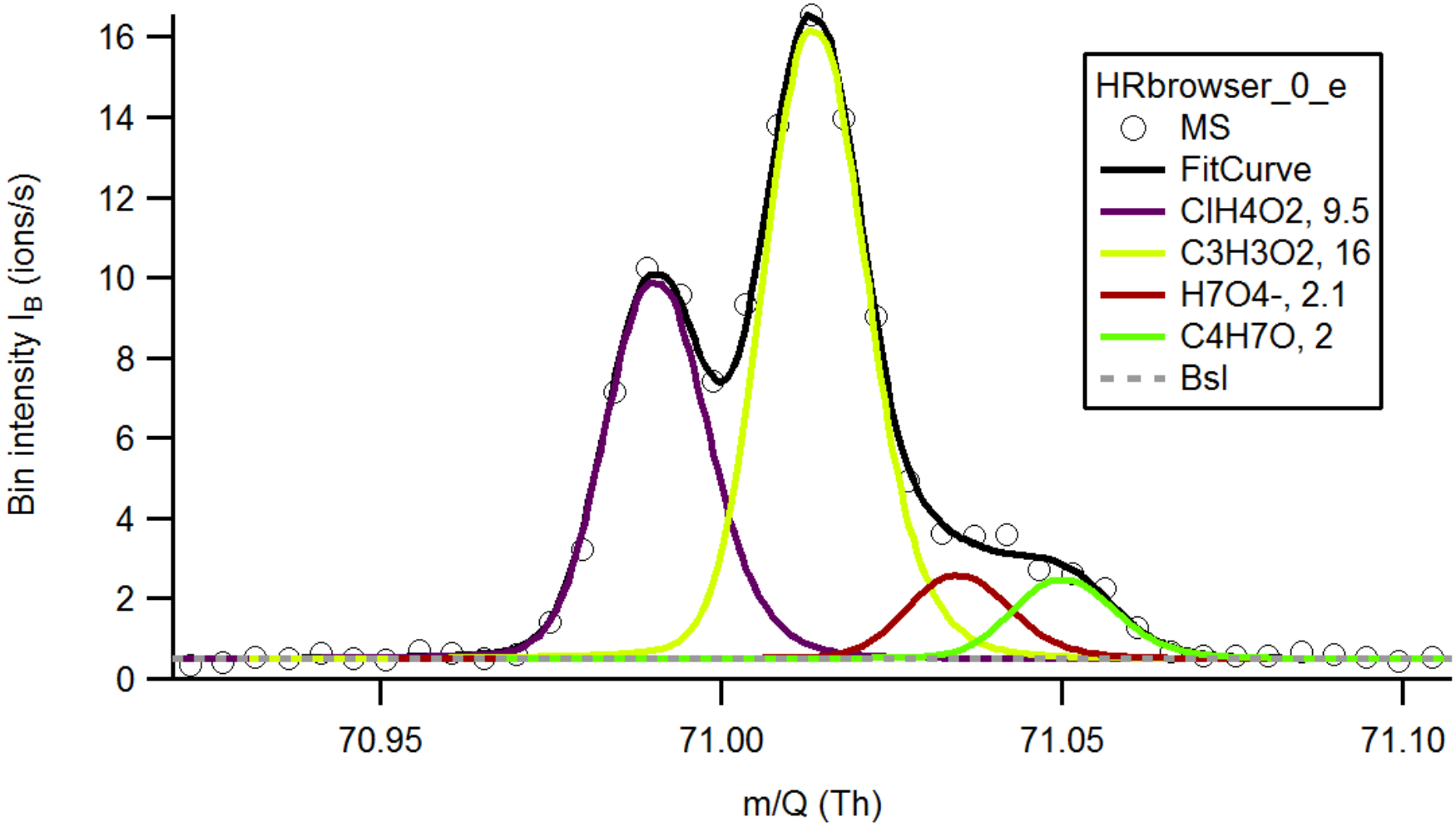


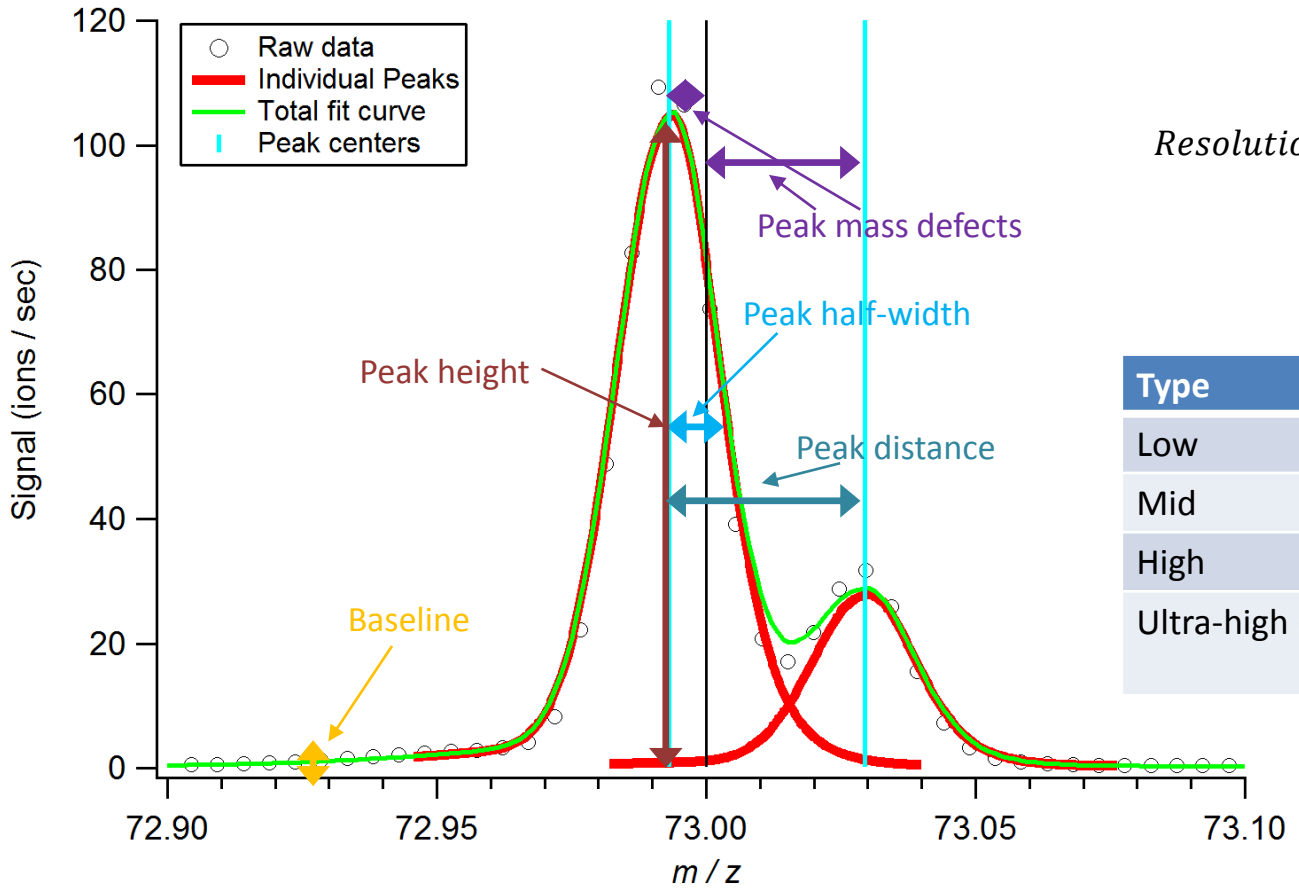
Peak fitting/assignment

Harald Stark

The task


HRbrowser_0_e
MS
FitCurve
ClH4O2, 9.5
C3H3O2, 16
H7O4-, 2.1
C4H7O, 2
Bsl
Bin intensity I_B (ions/s)
m/Q (Th)
0
2
4
6
8
10
12
14
16
70.95
71.00
71.05
71.10

Properties of a (multi-)peak



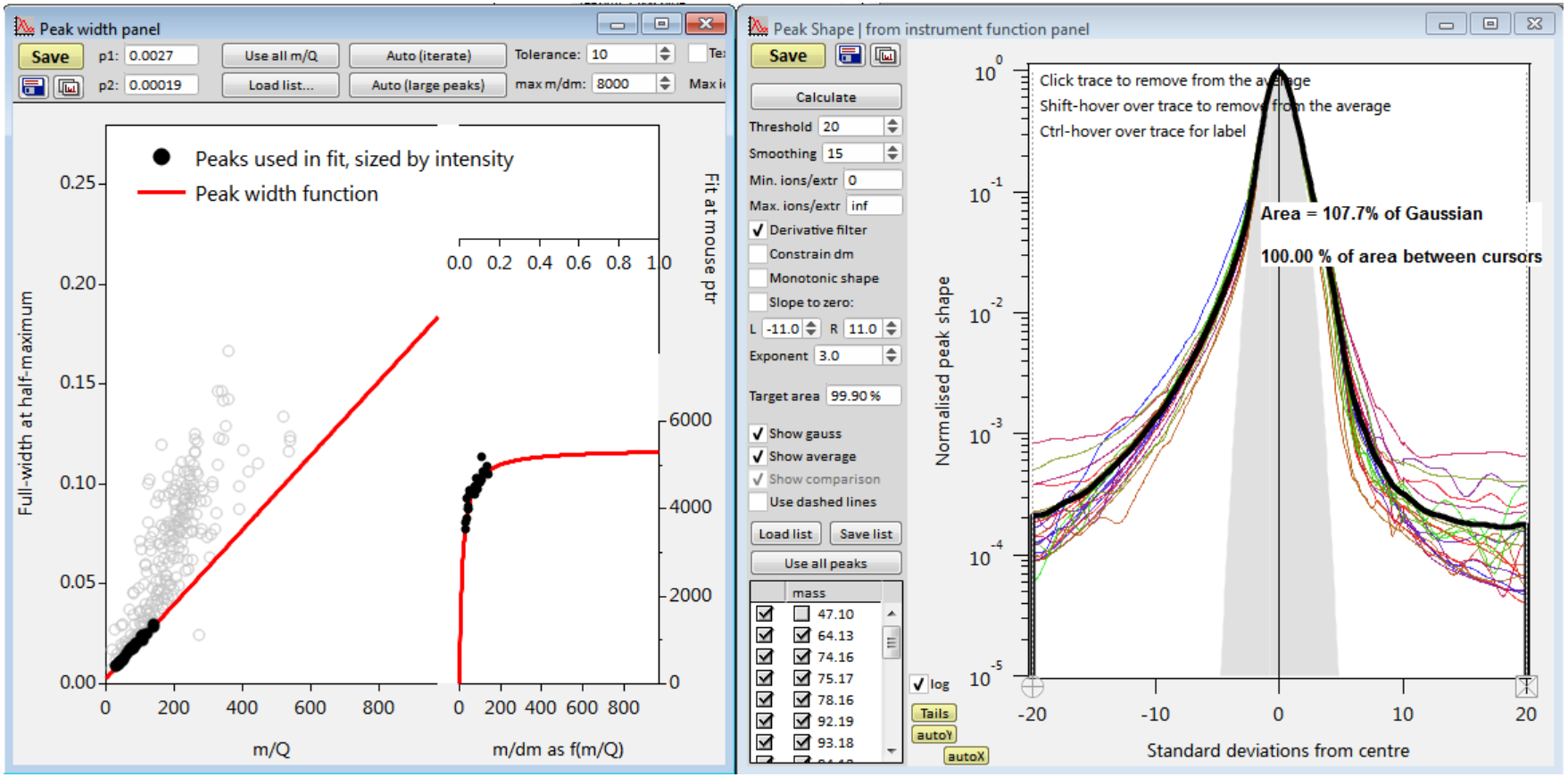
$$Resolution = \frac{m/z}{peak\ width}$$

Type	Resolution
Low	1-500
Mid	500-2,000
High	2,000-10,000
Ultra-high (UHR)	100,000-200,000

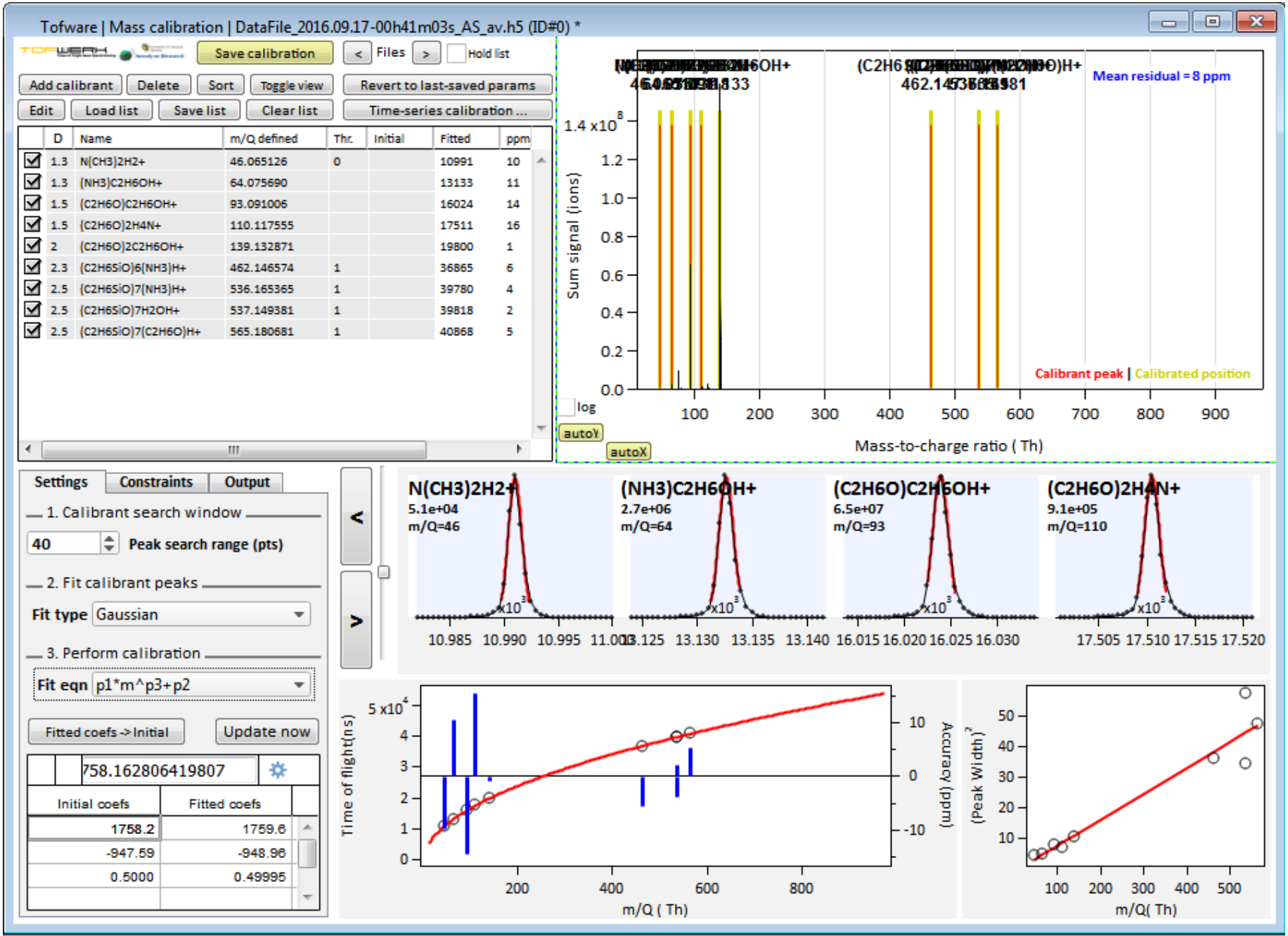
Tofware and Pika HR peak fitting idea

1. Determine “instrument functions” before fitting:
   - peak widths
   - peak shapes
   - peak positions (mass calibration and peak assignment)
   - baseline
2. Simultaneously fit all peak heights at one unit *m/z*

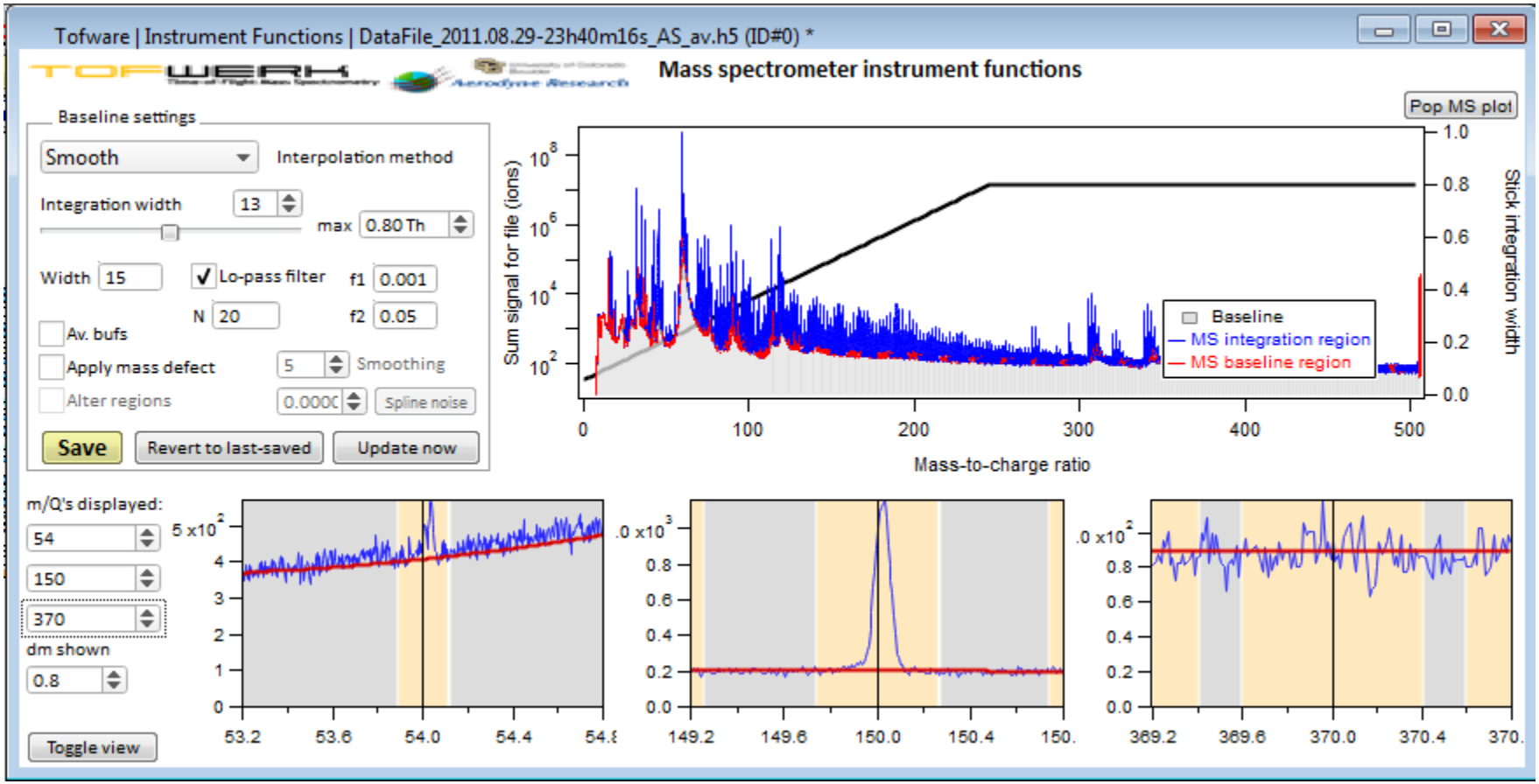
Peak shape and peak width in Tofware



Mass calibration



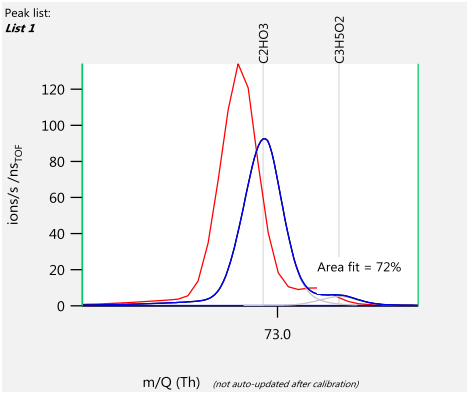
Baseline


Tofware | Instrument Functions | DataFile_2011.08.29-23h40m16s_AS_av.h5 (ID#0) *
Mass spectrometer instrument functions
Baseline settings
Smooth
Interpolation method
Integration width
13
max
0.80 Th
Width
15
Lo-pass filter
f1
0.001
N
20
f2
0.05
Av. bufs
Apply mass defect
5
Smoothing
Alter regions
Spline noise
Save
Revert to last-saved
Update now
Pop MS plot
Sum signal for file (ions)
Stick integration width
Mass-to-charge ratio
Baseline
MS integration region
MS baseline region
m/Q's displayed:
54
150
370
dm shown
0.8
Toggle view

Group exercise: Evaluating peak fits

- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

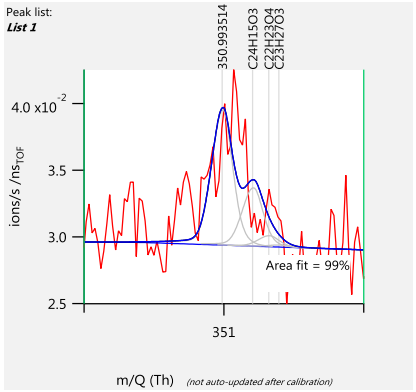
Example 1



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Bad mass calibration: peak areas too small or wrong altogether

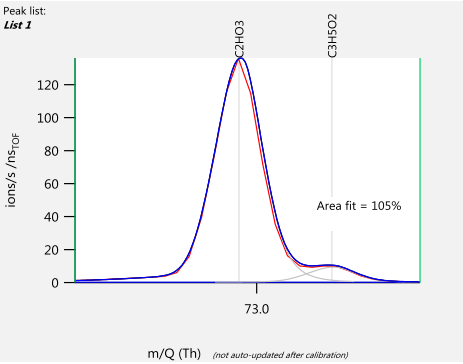
Example 2



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Not enough signal, expect large uncertainties and questionable peak assignment

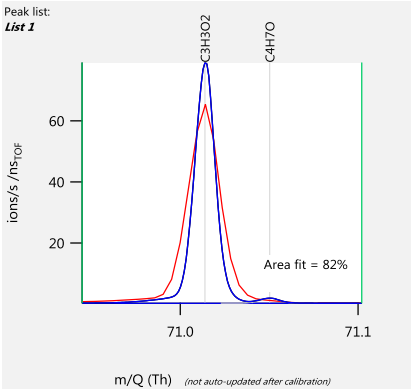
Example 3



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Good fit

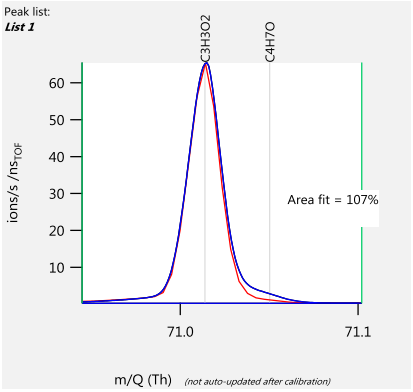
Example 4



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Wrong peak width: incorrect peak areas, especially for small peaks

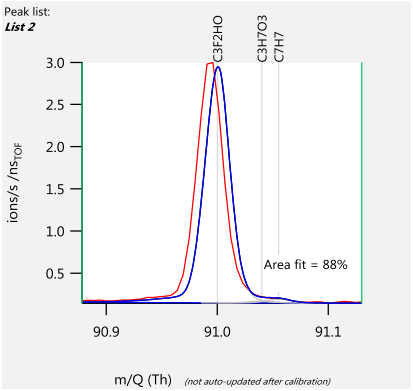
Example 5



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Wrong peak shape: small peak underestimated

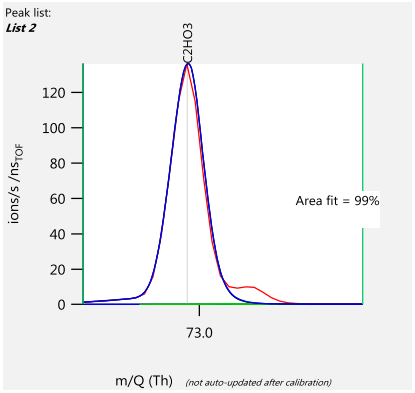
Example 6



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Wrong peak assignment

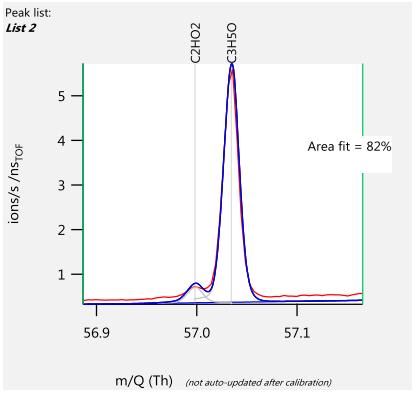
Example 7



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

- Missing peak: peak area for identified peak too large

Example 8



- Enough signal?
- Correct width?
- Correct mass calibration?
- Correct position/ion assignment?
- Correct shape?
- Correct number of peaks?

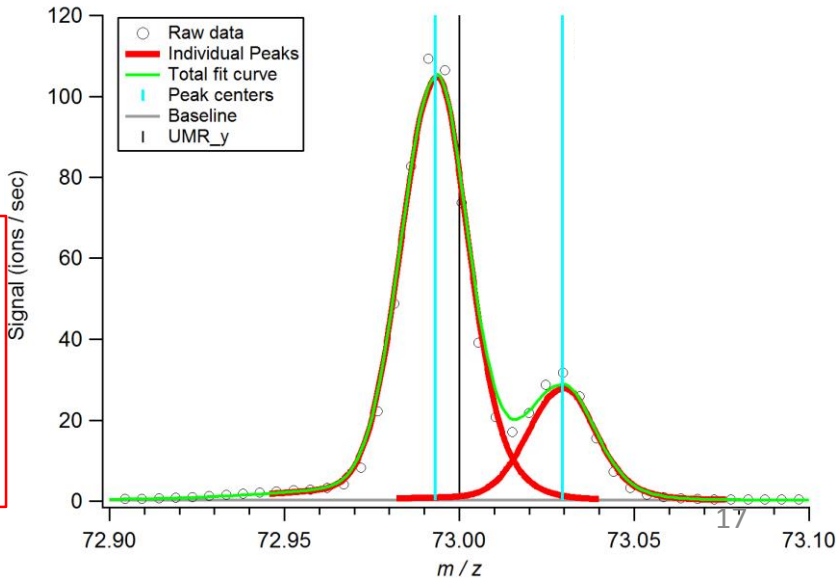
- Bad baseline: peak areas (for small peaks) too large

How precise are peak intensities when peaks overlap?

Simulating peak fits to a system of two overlapping peaks

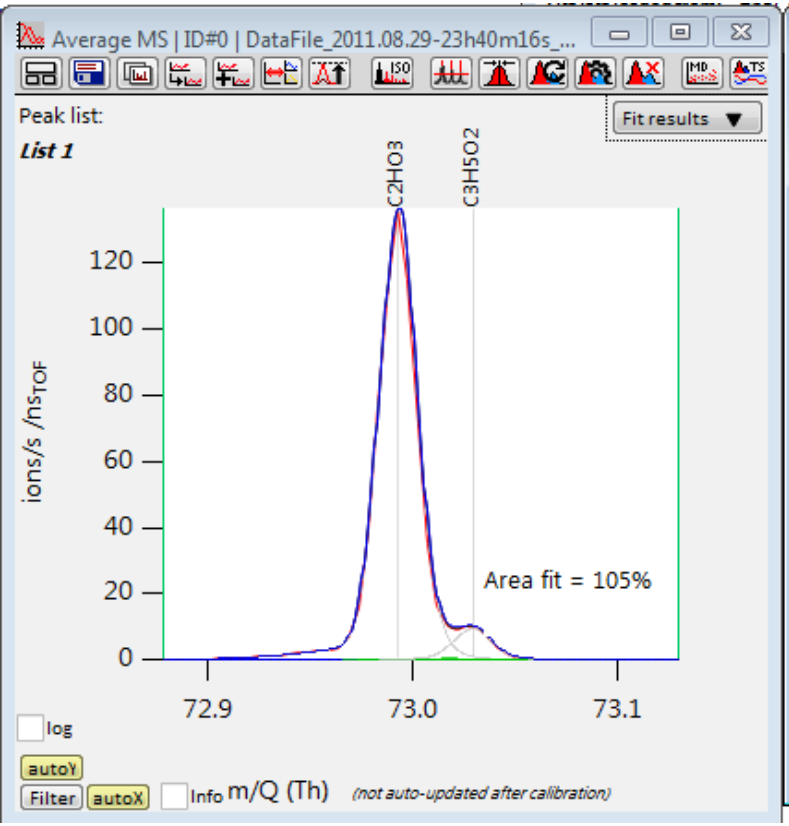
1. Generate synthetic distribution consisting of two Gaussian peaks
   - Fix peak position
   - Fix peak width
2. Apply counting noise to distribution
3. Perturb position of both peaks to simulate imperfect calibration
4. Apply Gaussian fits, holding constant
   - Peak position
   - Peak width
5. Compare fitted intensities with synthetic, known, values
6. Repeat a million times!
7. Build histogram of normalized deviation in fitted peak intensity
8. Width of this is precision in fitted intensity

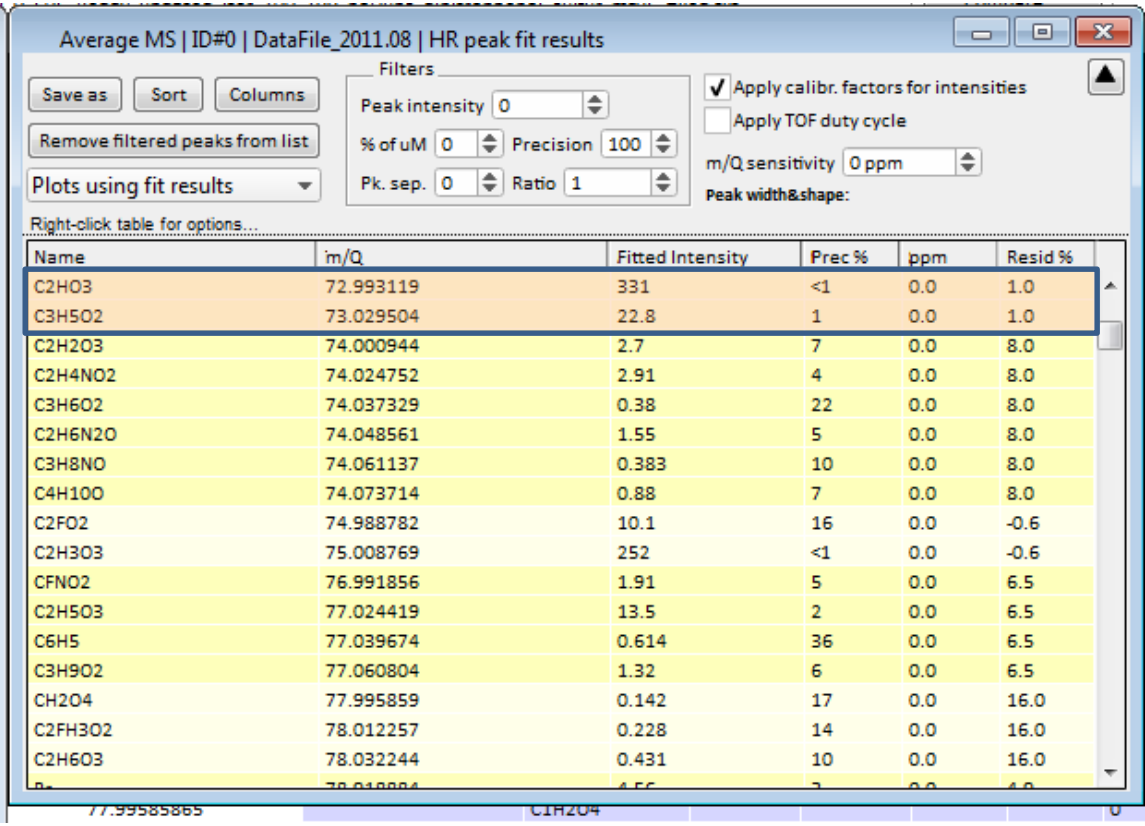
Can be parameterized and used to predict high-resolution peak height precisions!



Cubison & Jimenez, AMT, 2015

Example: *m/z* 73



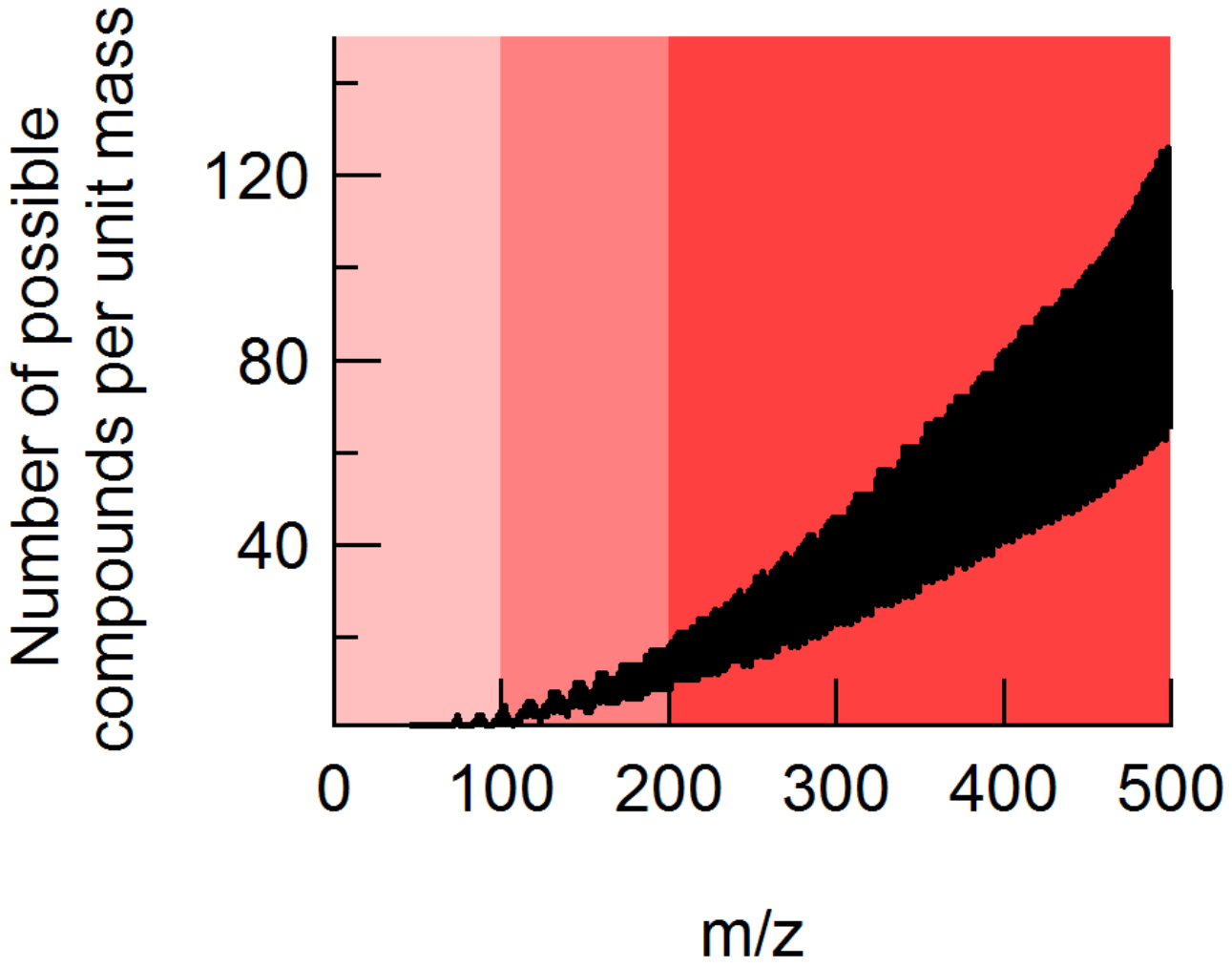


Name	m/Q	Fitted Intensity	Prec %	ppm	Resid %
C2HO3	72.993119	331	<1	0.0	1.0
C3H5O2	73.029504	22.8	1	0.0	1.0
C2H2O3	74.000944	2.7	7	0.0	8.0
C2H4NO2	74.024752	2.91	4	0.0	8.0
C3H6O2	74.037329	0.38	22	0.0	8.0
C2H6N2O	74.048561	1.55	5	0.0	8.0
C3H8NO	74.061137	0.383	10	0.0	8.0
C4H10O	74.073714	0.88	7	0.0	8.0
C2FO2	74.988782	10.1	16	0.0	-0.6
C2H3O3	75.008769	252	<1	0.0	-0.6
CFNO2	76.991856	1.91	5	0.0	6.5
C2H5O3	77.024419	13.5	2	0.0	6.5
C6H5	77.039674	0.614	36	0.0	6.5
C3H9O2	77.060804	1.32	6	0.0	6.5
CH2O4	77.995859	0.142	17	0.0	16.0
C2FH3O2	78.012257	0.228	14	0.0	16.0
C2H6O3	78.032244	0.431	10	0.0	16.0

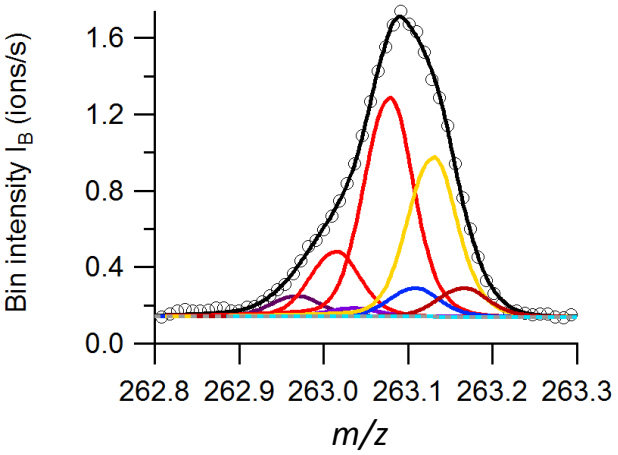
Peak shape and width effects can increase the uncertainty significantly!

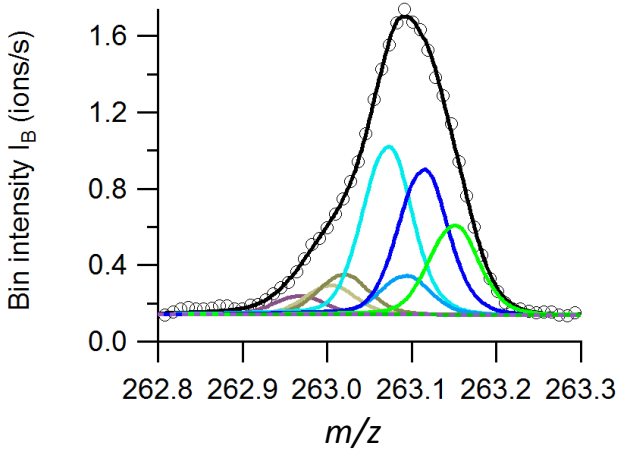
Limits of HR fitting

- Chemical space: C,H,O,N O>2, O:C<2, H:C>0.1



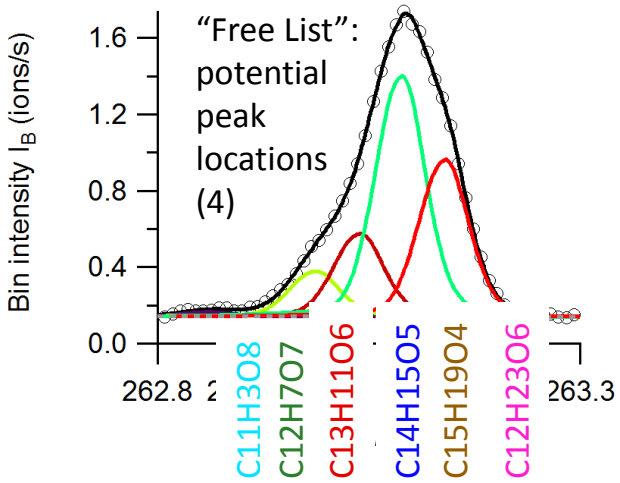
Iterative assignment method: example at *m/z* 263

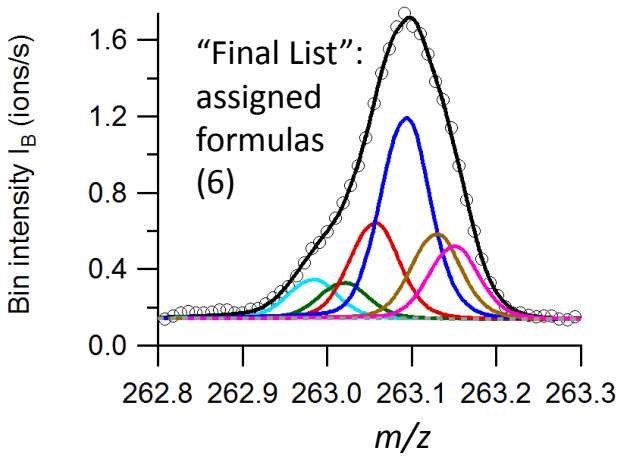




"Full List":
all possible formulas (18)

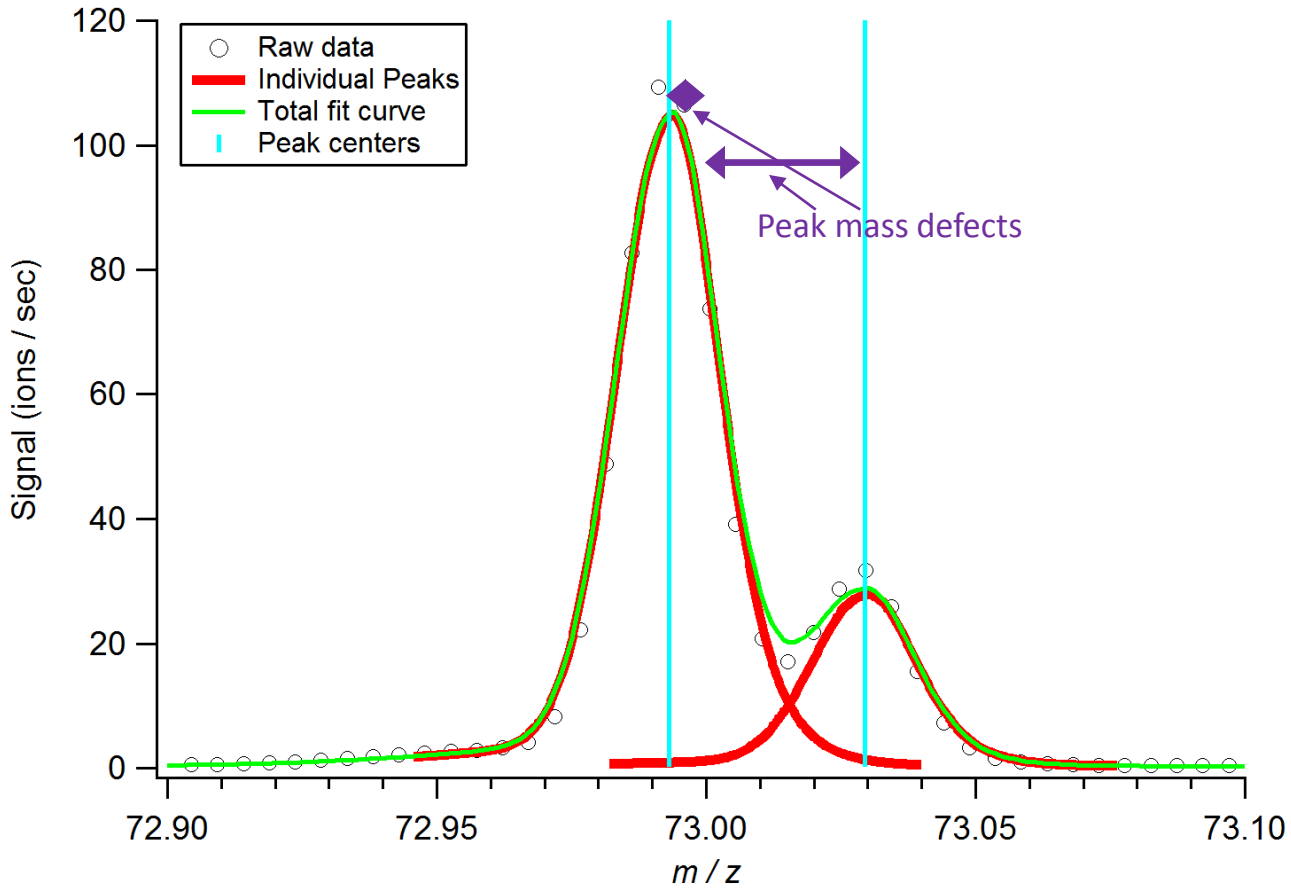
Formula	*m/z*
C7H3O11	262.9681
C11H3O8	262.9833
C15H3O5	262.9986
C8H7O10	263.0045
C19H3O2	263.0138
C12H7O7	263.0197
C16H7O4	263.035
C9H11O9	263.0409
C13H11O6	263.0561
C17H11O3	263.0714
C10H15O8	263.0772
C14H15O5	263.0925
C18H15O2	263.1078
C11H19O7	263.1136
C15H19O4	263.1289
C12H23O6	263.15
C16H23O3	263.1653
C17H27O2	263.2017



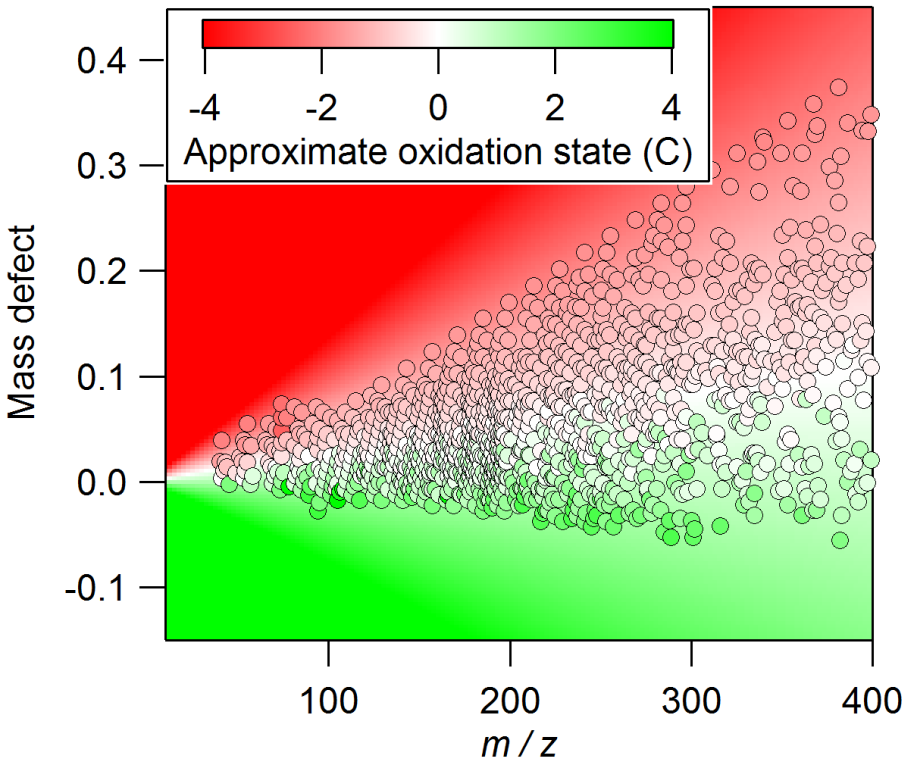


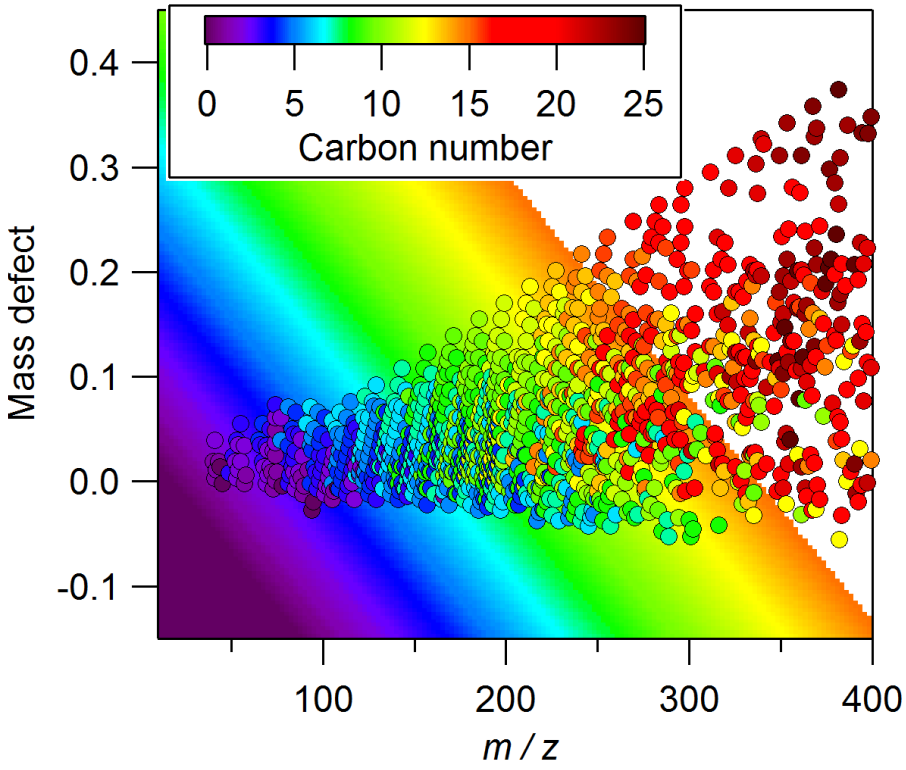
Stark et al., IJMS, 2015

Mass defect as chemical indicator



Oxidation state and carbon number as f(mass defect, *m/z*)





Stark et al., IJMS, 2015