UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

Reclining langourously on my Phase Longue

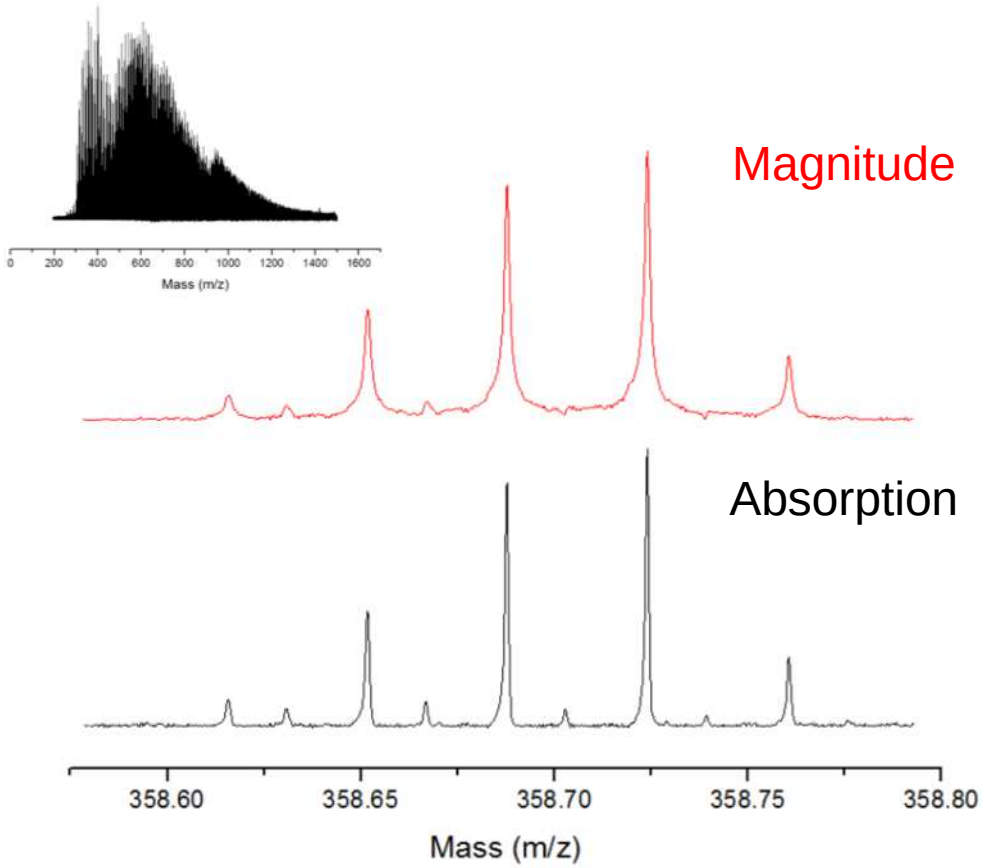
Phasing WTF?

- Phase corrected Absorption mode spectrum view offers an alternative to magnitude mode
- It’s a post processing trick you can play on FT-ICR MS data which brings some major benefits

- Benefits
- How and Why
- New Advances

Benefits of absorption mode

- Improved resolving power
- Sample
  - Pyrolysis fluid from waste organic matter feedstock



Data with permission from the University of Edinburgh

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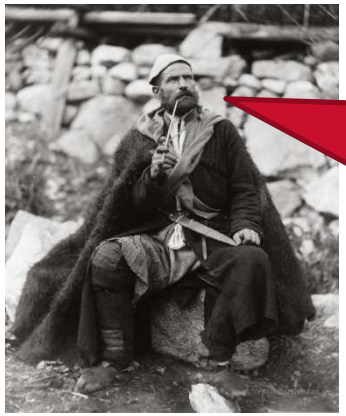
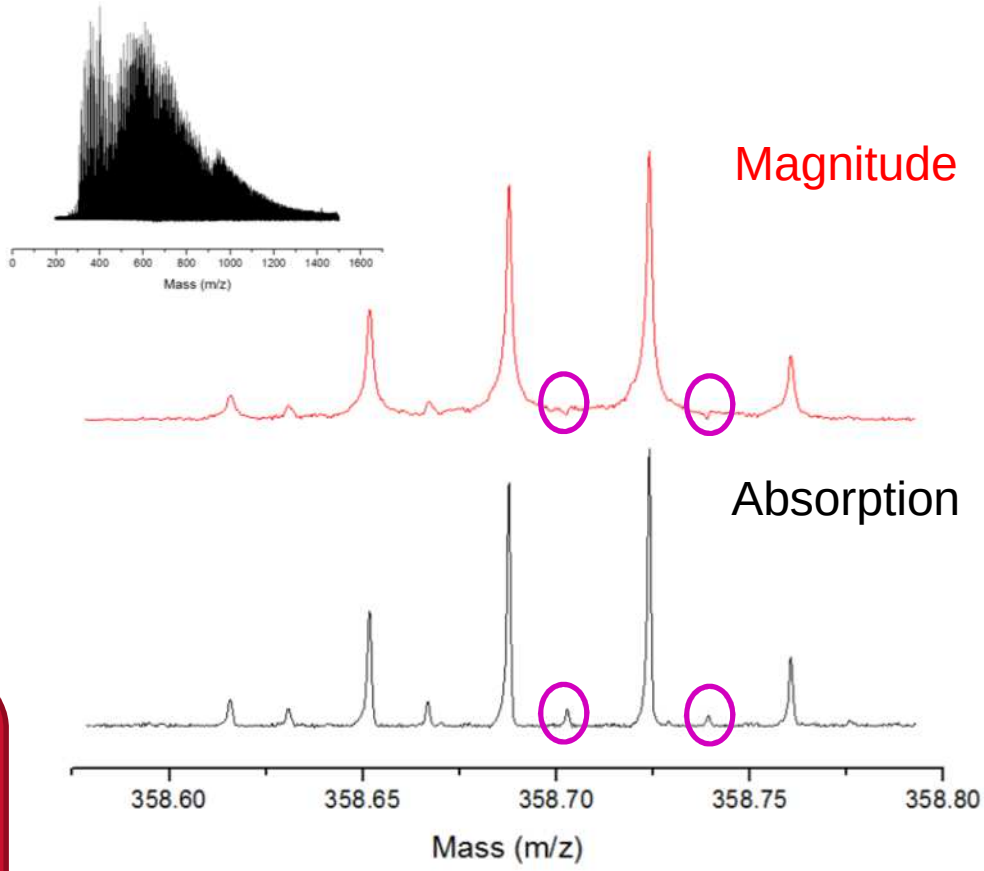


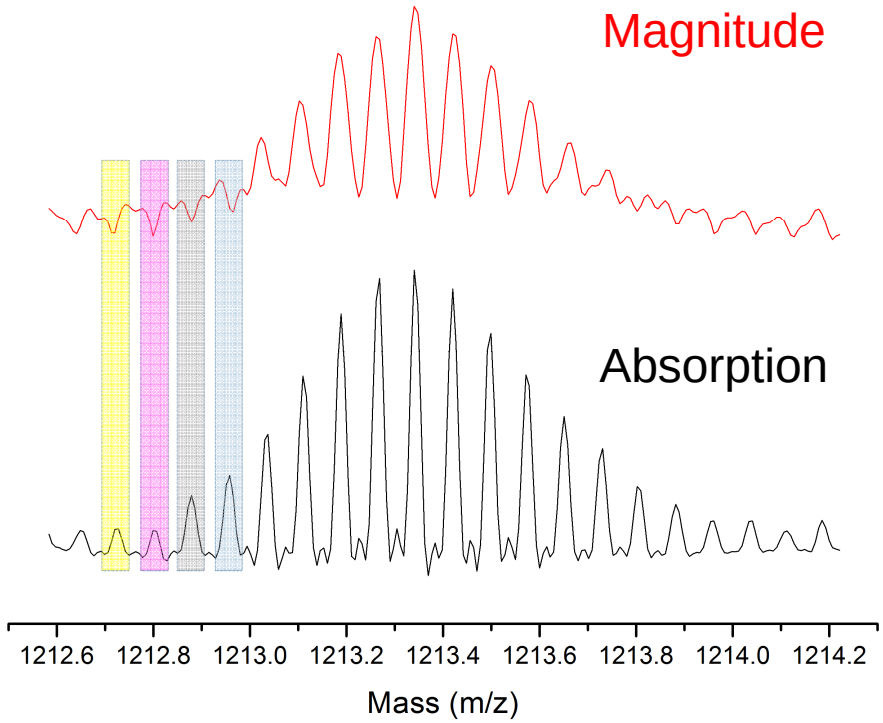
Been huntin' those peaks since 1850, dammit!



Data with permission from the University of Edinburgh

Benefits of absorption mode

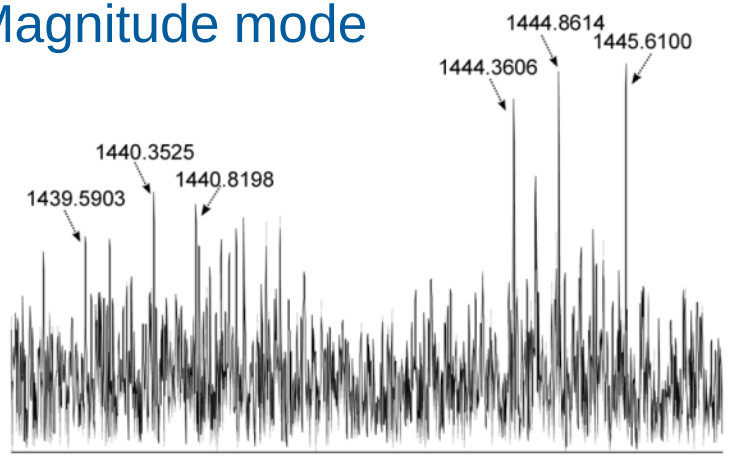
- Improved resolving power
- Same improvements also in protein spectra
  - More peaks detectable



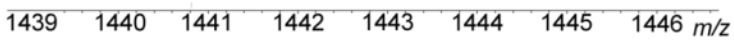
Protein coverage

- Improved signal to noise ratio
- Means
  - More fragments detected
  - More confident assignment
  - Better sequence coverage
  - More PTM information

Magnitude mode



- Cannot determine the charge state for these peak clusters



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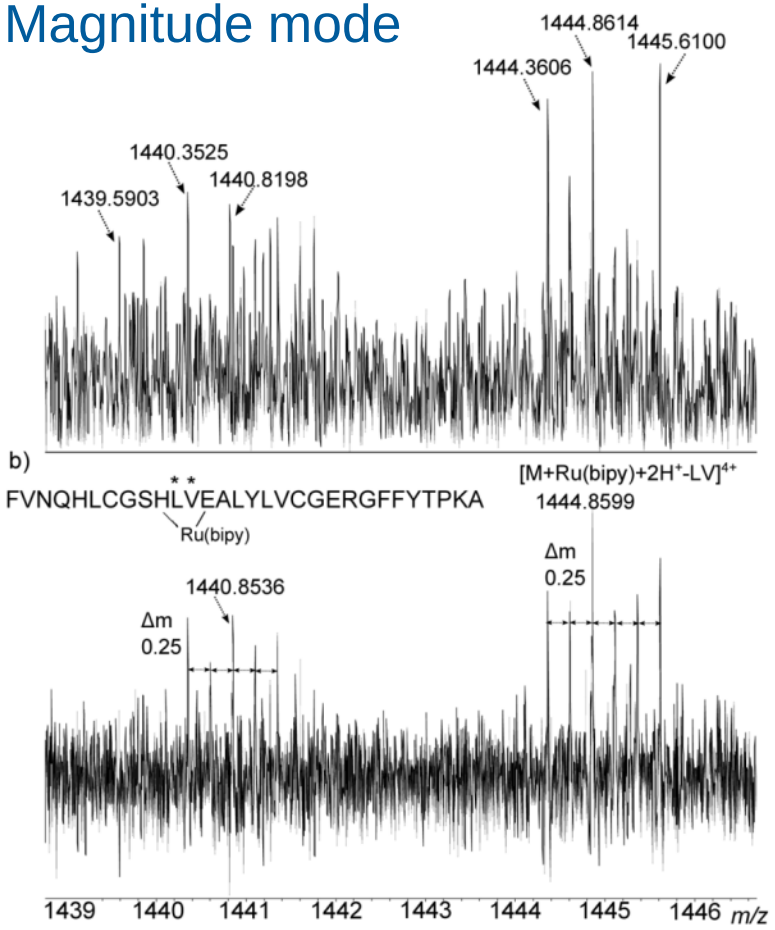
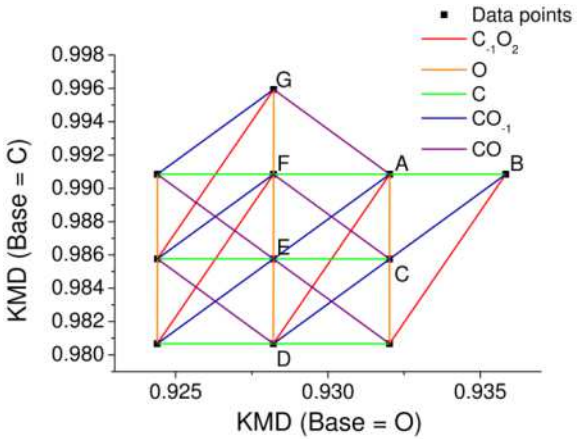


Absorption mode

Improved Mass Accuracy

- Sample
  - Talisker Malt Whisky
  - 12T Solarix, + mode ESI
- Peak Assignment by KMD algorithm[1,2]



- Assigned peak mass accuracy
  - Magnitude mode 0.080ppm ±0.104
  - Absorption mode 0.066ppm ±0.084

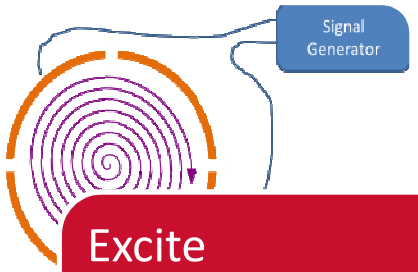
1.Kilgour, Mackay, Langridge-Smith, O'Connor. Anal Chem, 84(17), 7431-7435
2.Kilgour, Mackay, Langridge-Smith, O'Connor. Anal Chem, 84(17), 7436-7439

Benefits of absorption mode

- Features
  - Improved resolving power
  - Improved signal-to-noise
  - Better mass accuracy
- Benefits
  - You will be able to see more peaks
  - You can assign with more confidence

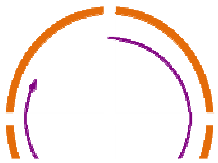
  - **It's free!!**

Phase shifts FT-ICR MS



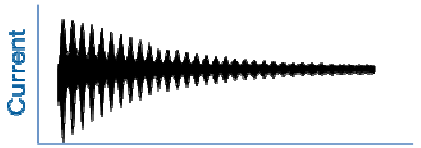
Excite

- Excitation waveform used to coherently excite ions
  - e.g. Frequency Sweep
  - or SWIFT



Delay

- To prevent detection circuitry being swamped by the excitation waveform
- Can last milliseconds

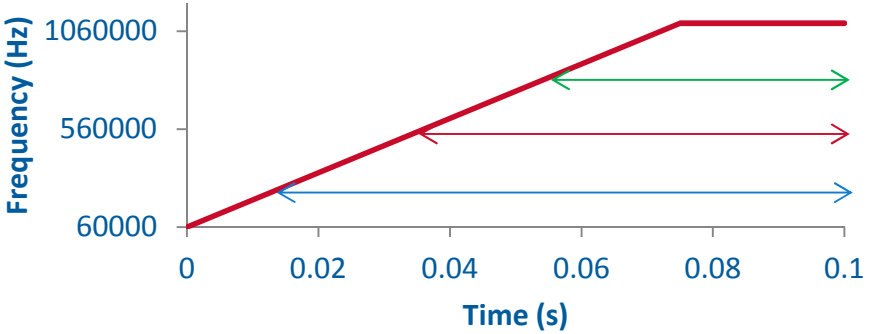


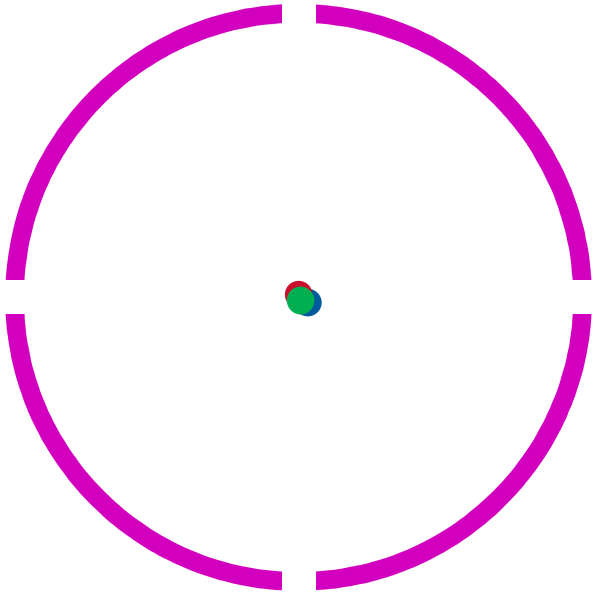
Detect

- Switch on amplifier
- Detect transient

Phase shifts FT-ICR MS

- Excite and delay cause phase shift

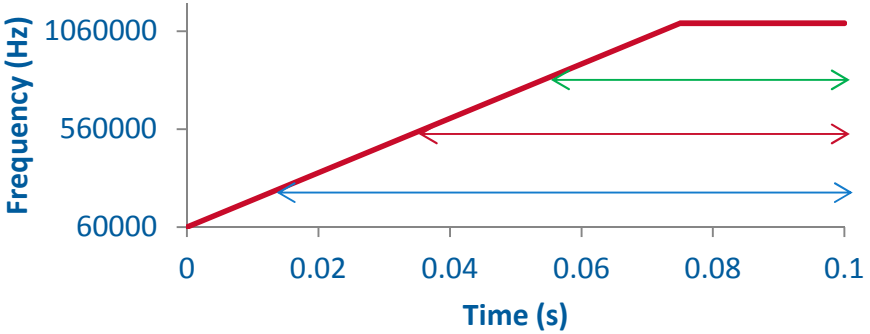


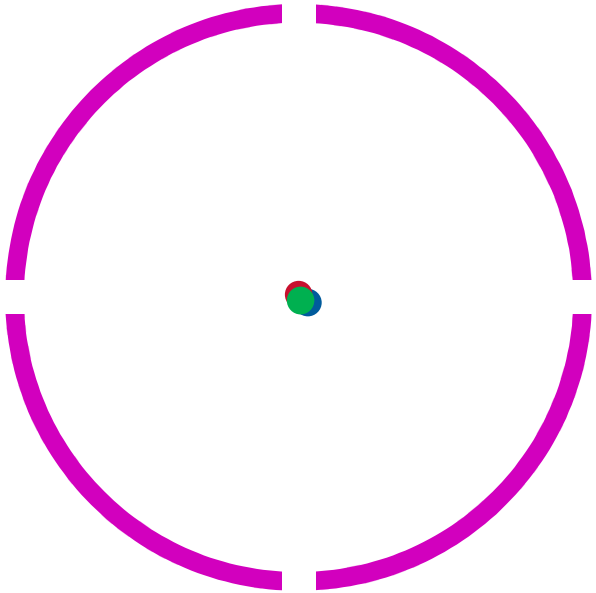


- By the beginning of detection
  - Ions with different frequencies have different starting angles
- This causes the peak distortion

Phase shifts FT-ICR MS

- Excite and delay cause phase shift





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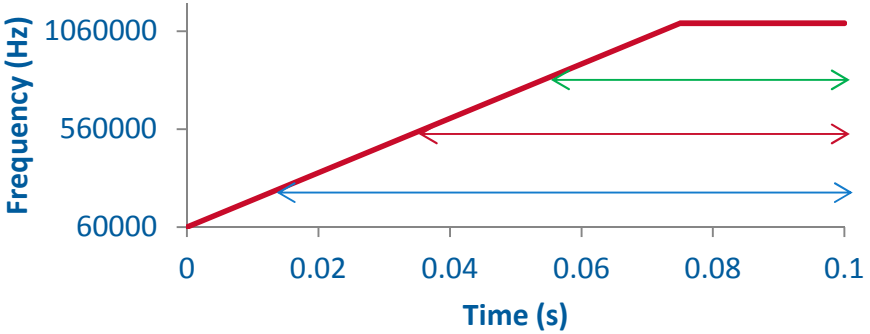

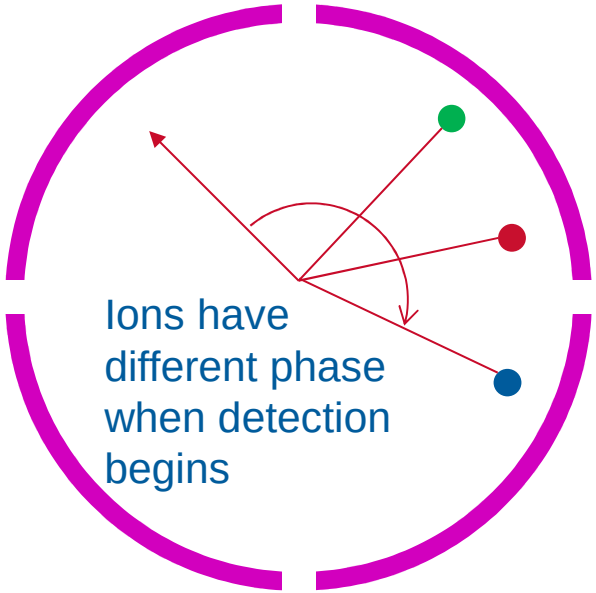

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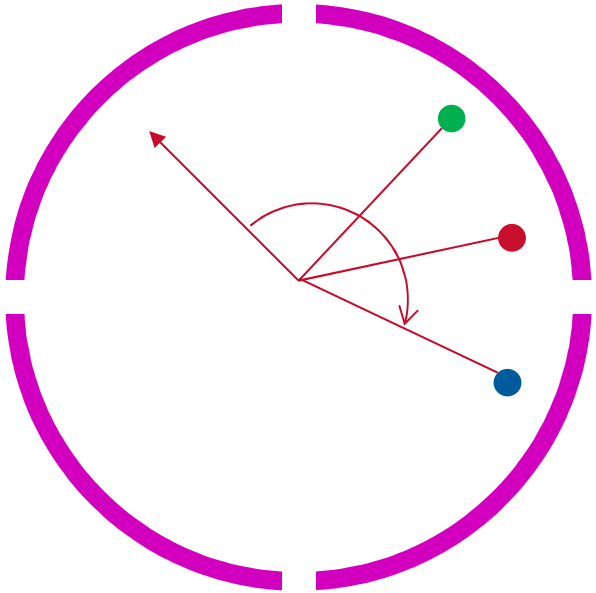
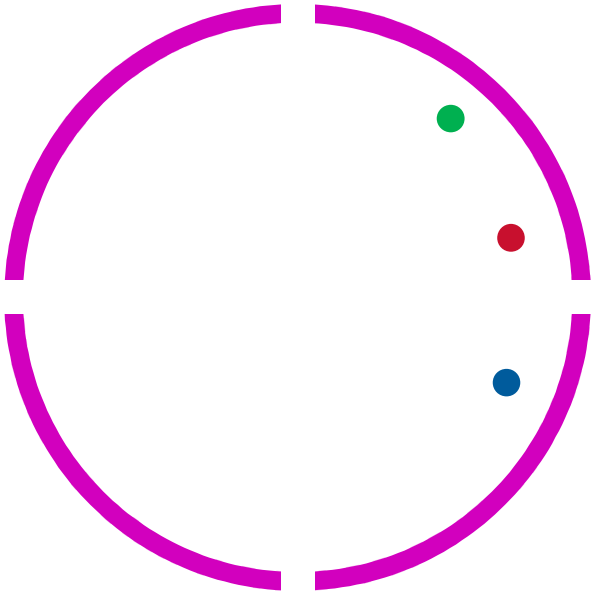
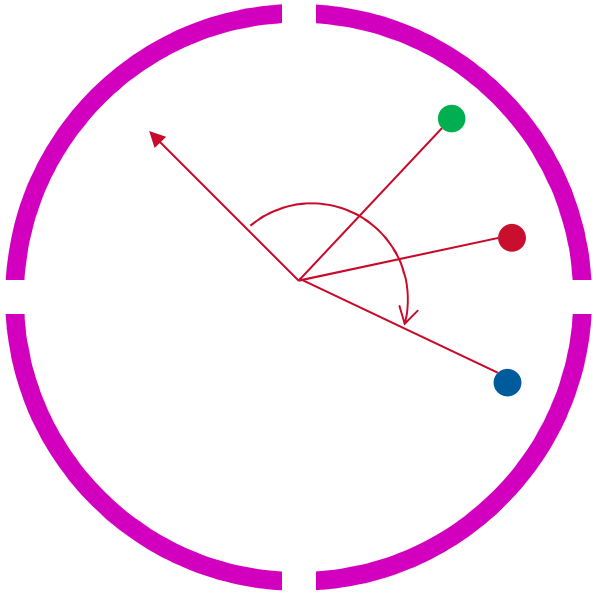


- Phase correction aim
  - Remove all accumulated phase



Phase shifts FT-ICR MS

- Excite and delay cause phase shift



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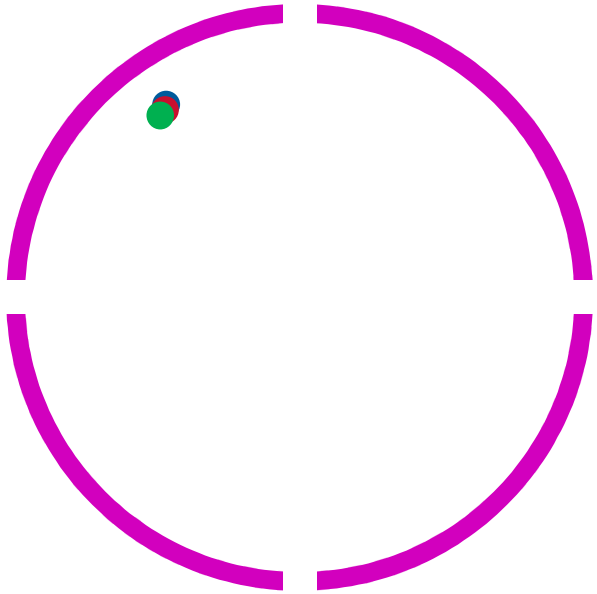
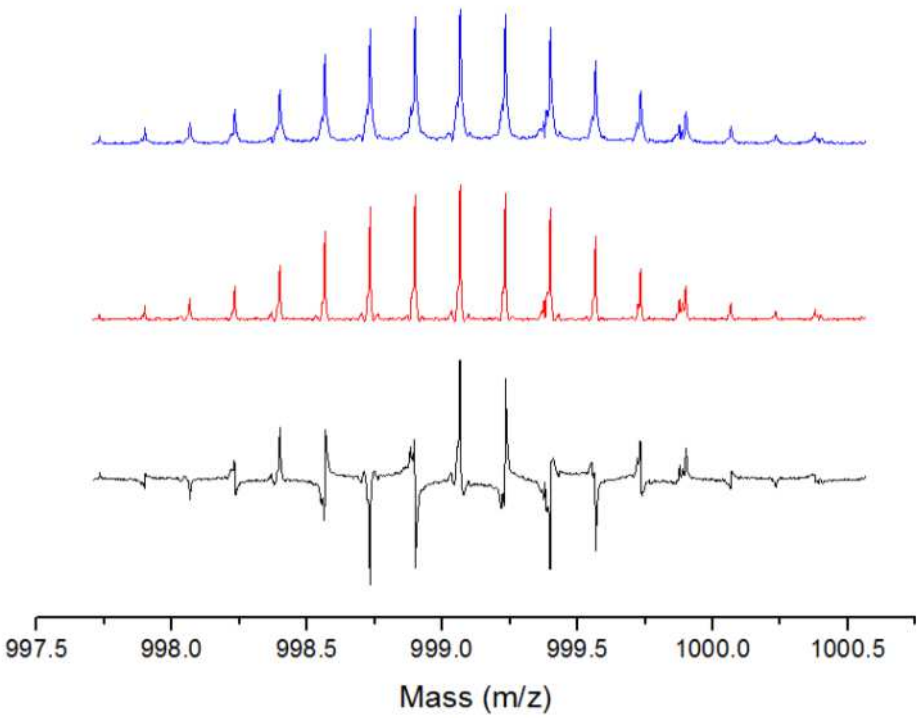


Makes it look like all ions started at the same place

The fly in the ointment

- Magnitude mode spectrum
- Phase corrected absorption mode
- Un-phase corrected absorption mode



How do you make an absorption mode spectrum

- Record ion transient
- FFT
  - Extract magnitude and phase angle of all ion frequencies
- Calculate phase angle correction function
  - Quadratic relationship
  - $\varphi_f = Af^2 + Bf + C$

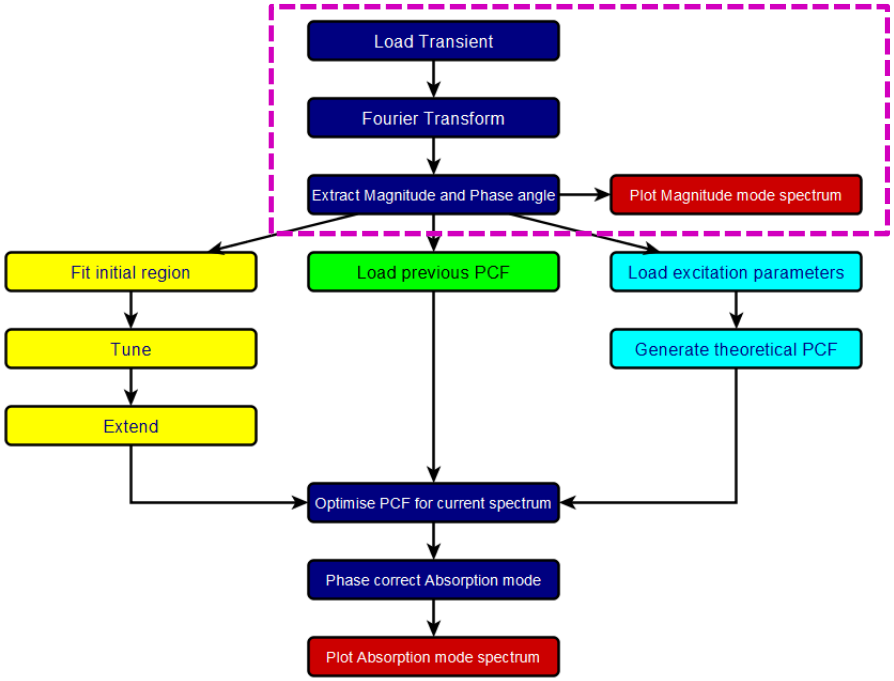
f – frequency
φ_f – phase angle correction
A, B & C – coefficients that are optimised

- Plot phase corrected absorption mode spectrum

Autophaser Method

- Simple method of producing Absorption mode spectra

- PCF
  - Phase Correction Function
  - $\varphi_f = Af^2 + Bf + C$

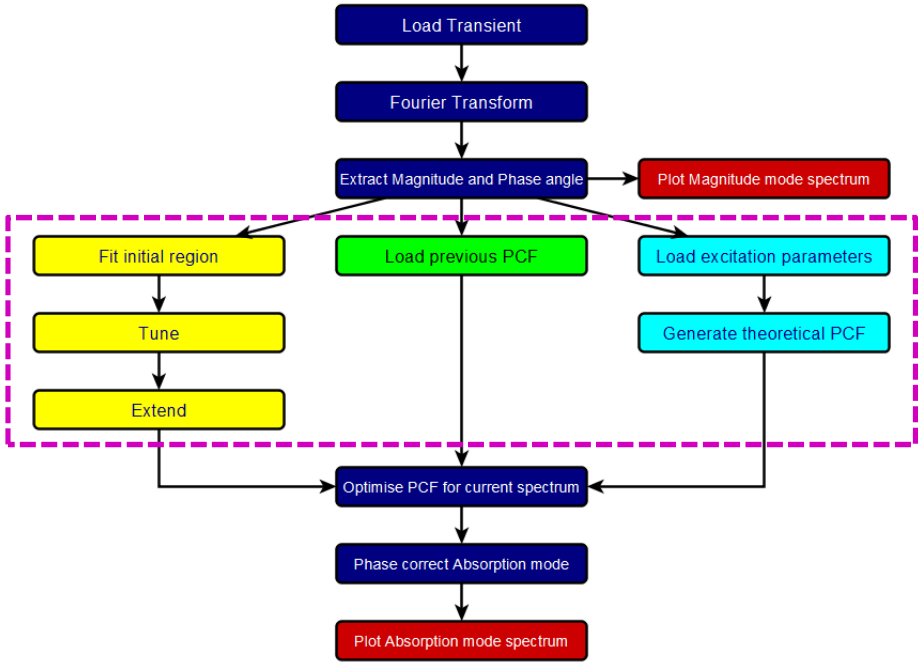


Autophaser

Absorption mode for all

Autophaser Method

- Simple method of producing Absorption mode spectra
- Basis Phase Correction Function
  - Estimate from spectrum*
  - Load for previous spectrum*
  - Generate from excite function
- *Also options in Bruker software

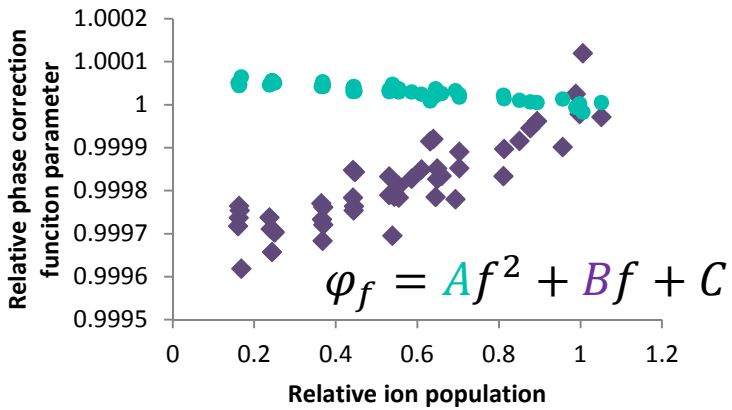


utophaser

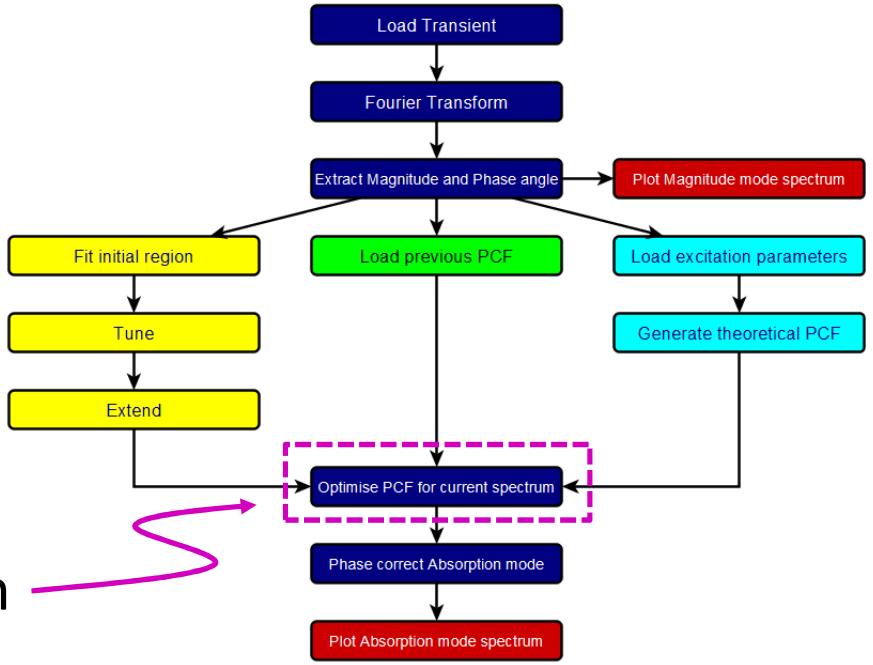
Absorption mode for all

Autophaser Method

- Simple method of producing Absorption mode spectra



- Optimise Phase Correction Function for current spectrum
  - Account for ion population related frequency shifts



utophaser

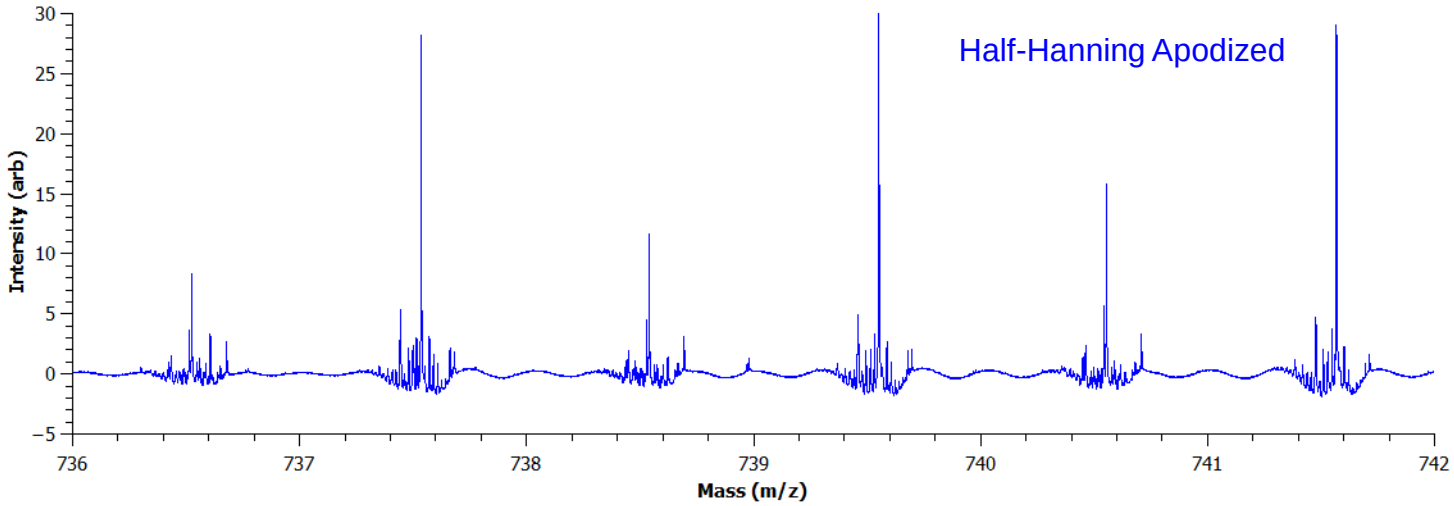
Absorption mode for all

So, What's new?

- The perfidious baseline correction
  - And how to avoid it
- Non-quadratic phase correction functions
  - When are they needed
  - What causes them

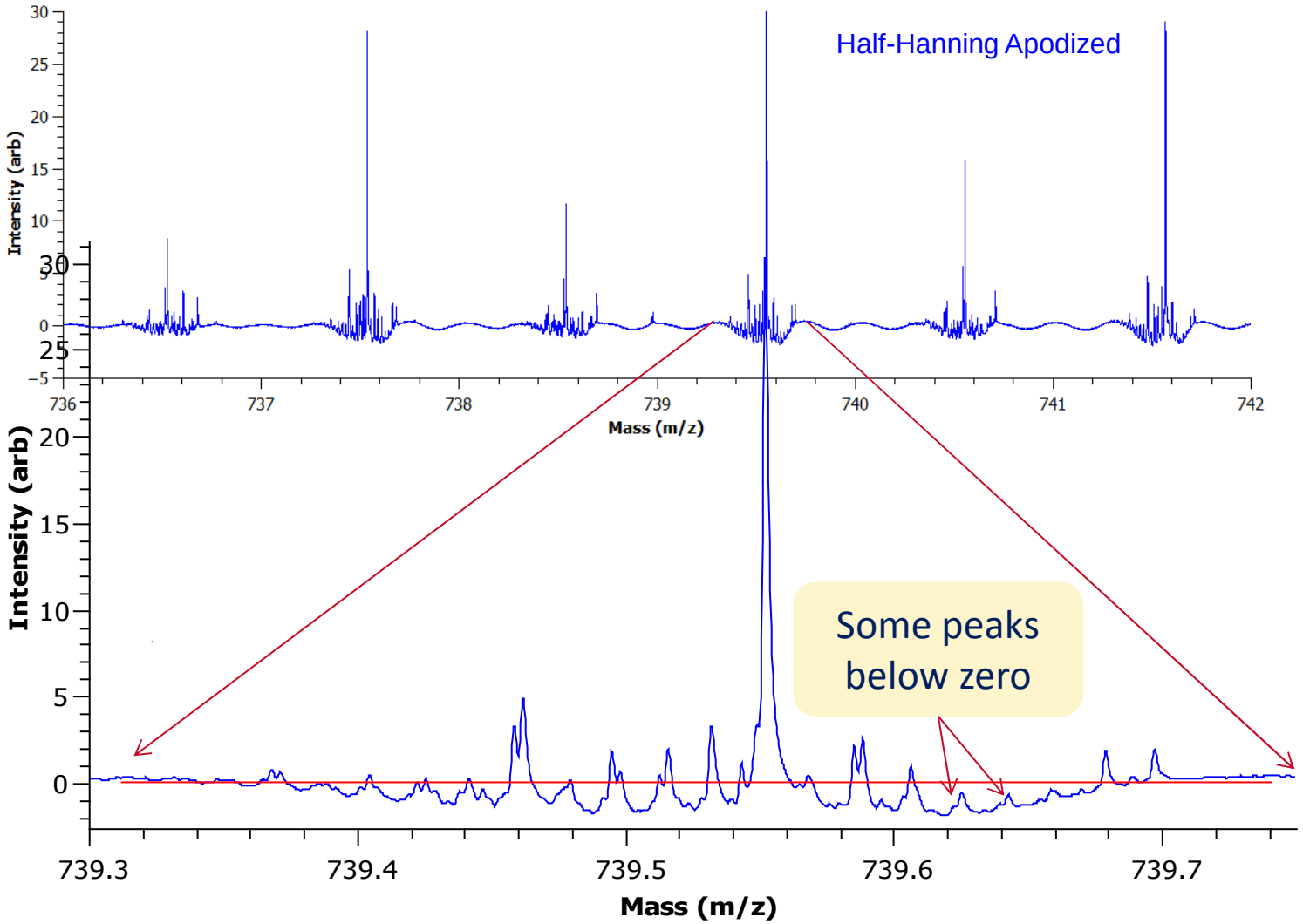
Baseline Correction

Raw absorption mode



Baseline Correction

Raw absorption mode



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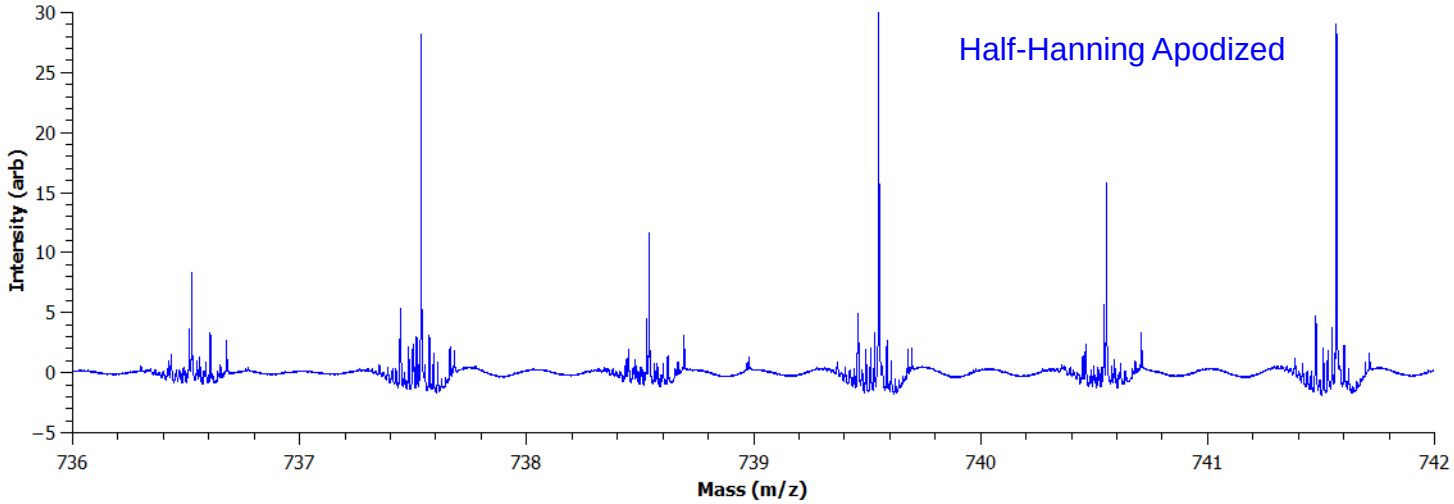
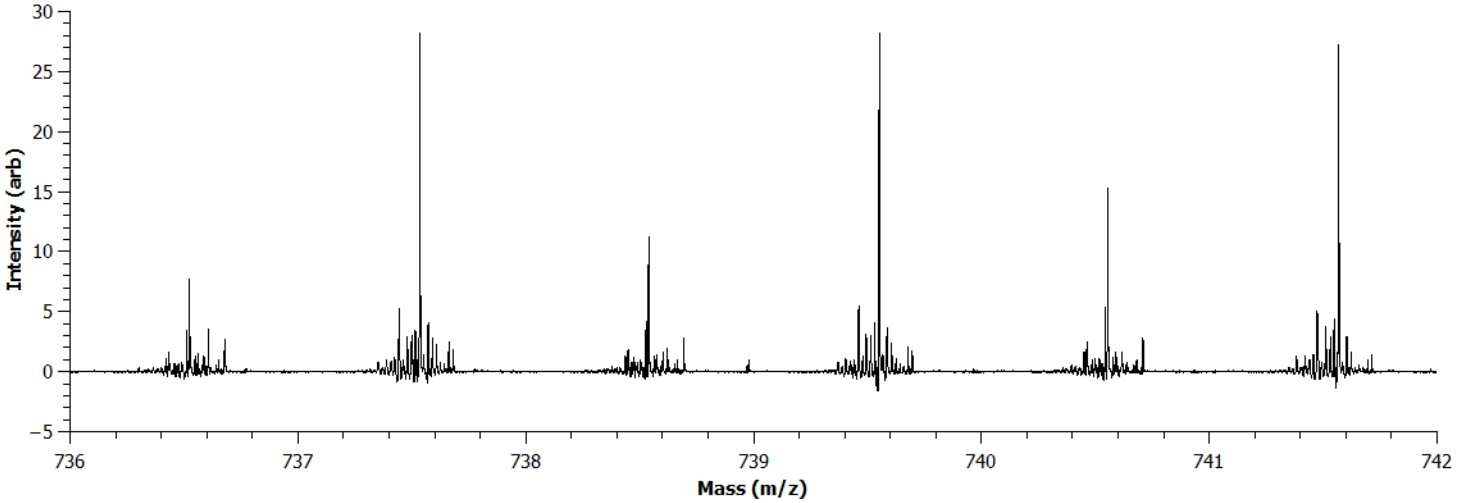


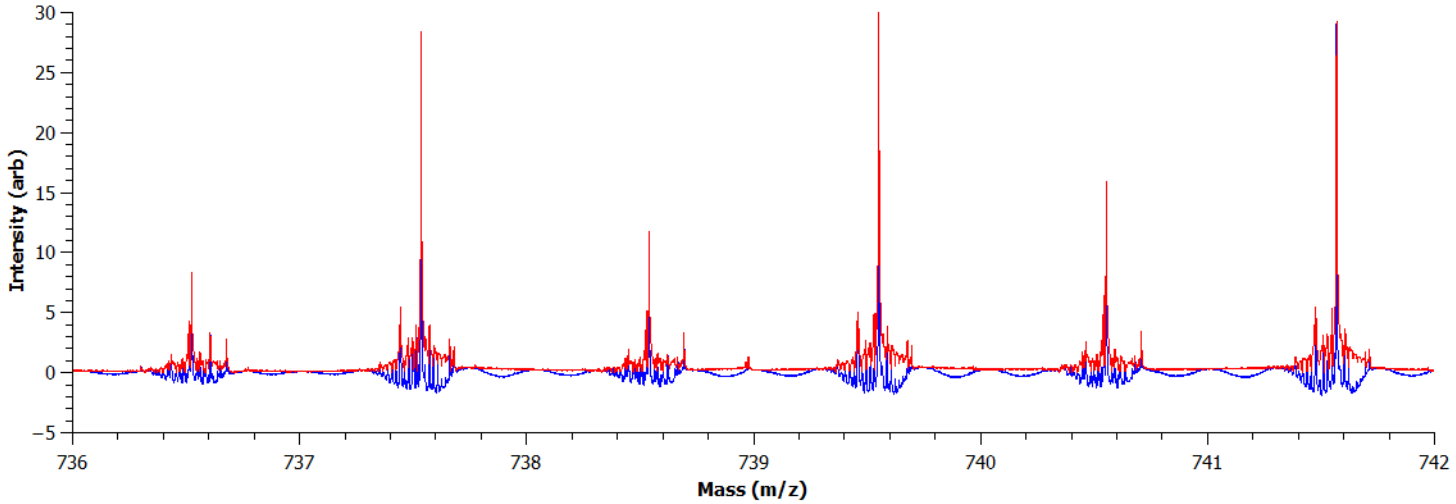
Baseline corrected



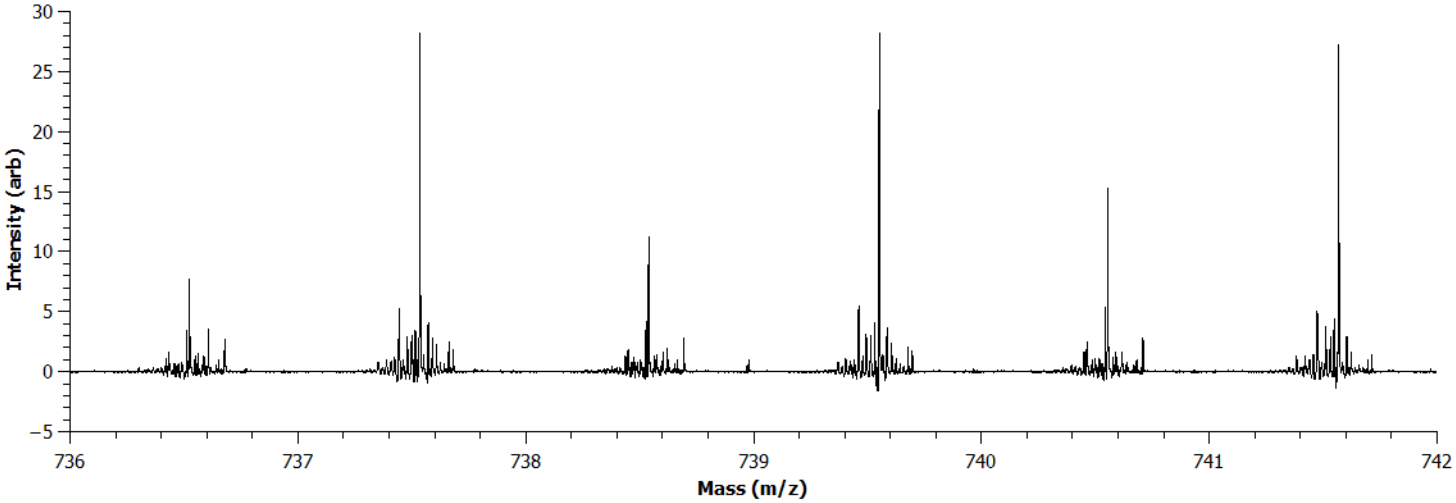
Baseline Correction

Raw absorption mode

Magnitude mode

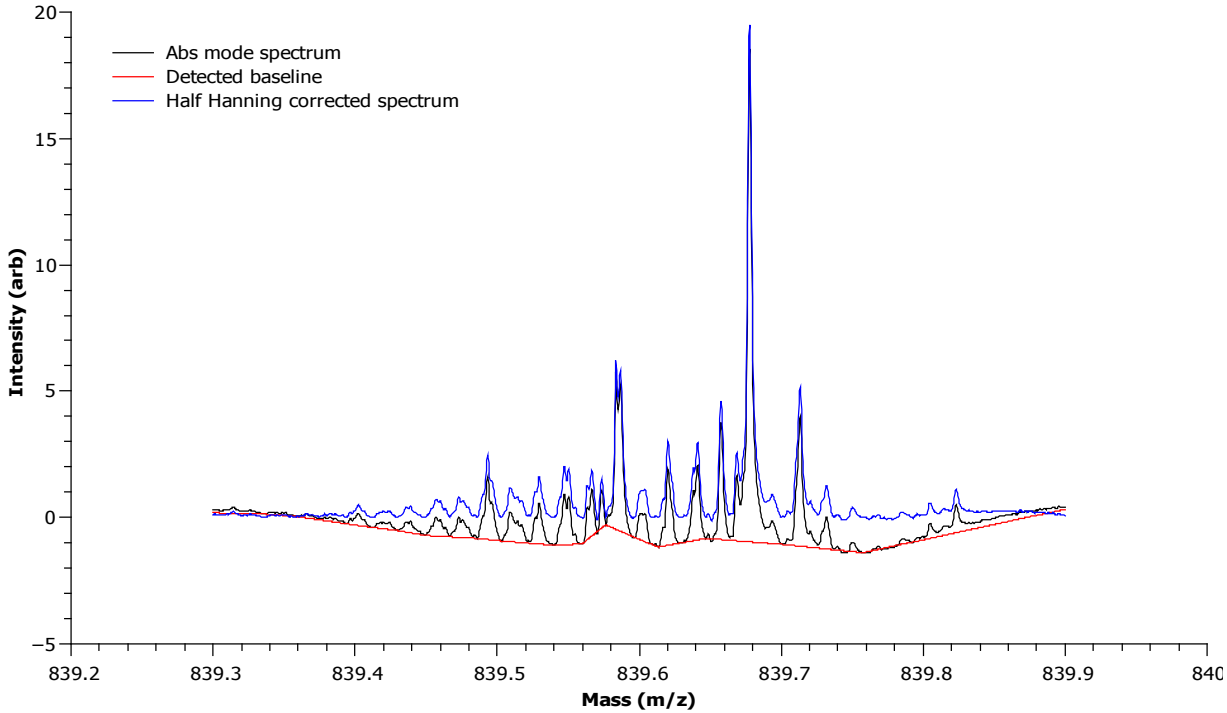


Baseline corrected



Relative peak height

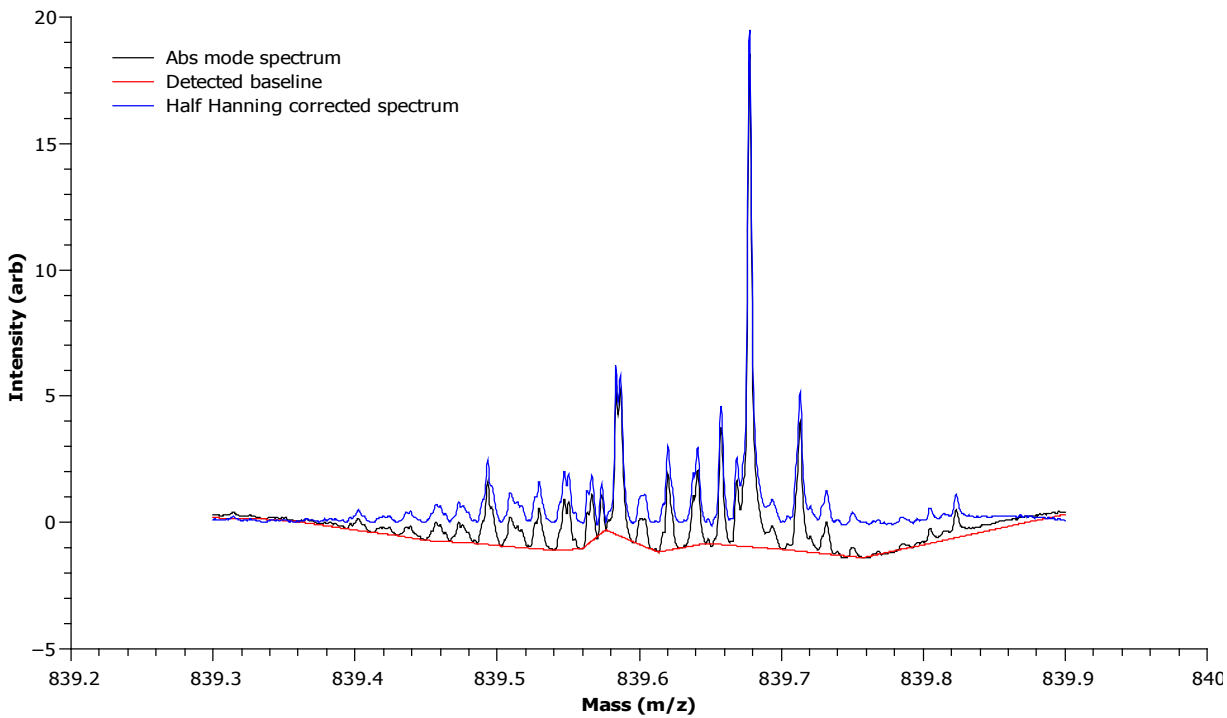
- No baseline correction
- Detected baseline
- After baseline correction



Very difficult to produce accurate baseline fit fully automatically, **reliably**

Relative peak height

- No baseline correction
- Detected baseline
- After baseline correction

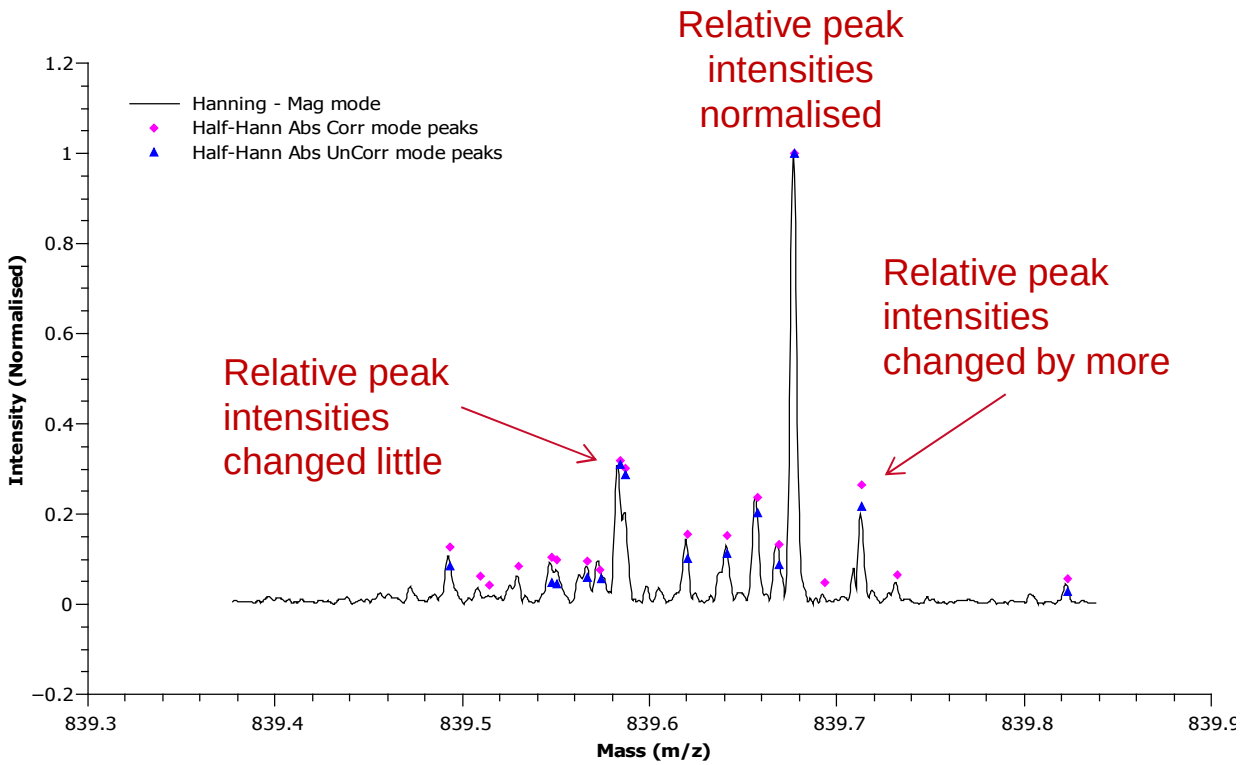


3s per pixel on a 50k pixel image = 42 extra hours of processing!

Takes ~3s (1M transient) – ~20 secs (8M transient) to compute

Relative peak height

- Magnitude mode
  - Hanning
  - As ref
- Absorption mode peaks
  - Before Corr
  - After Corr



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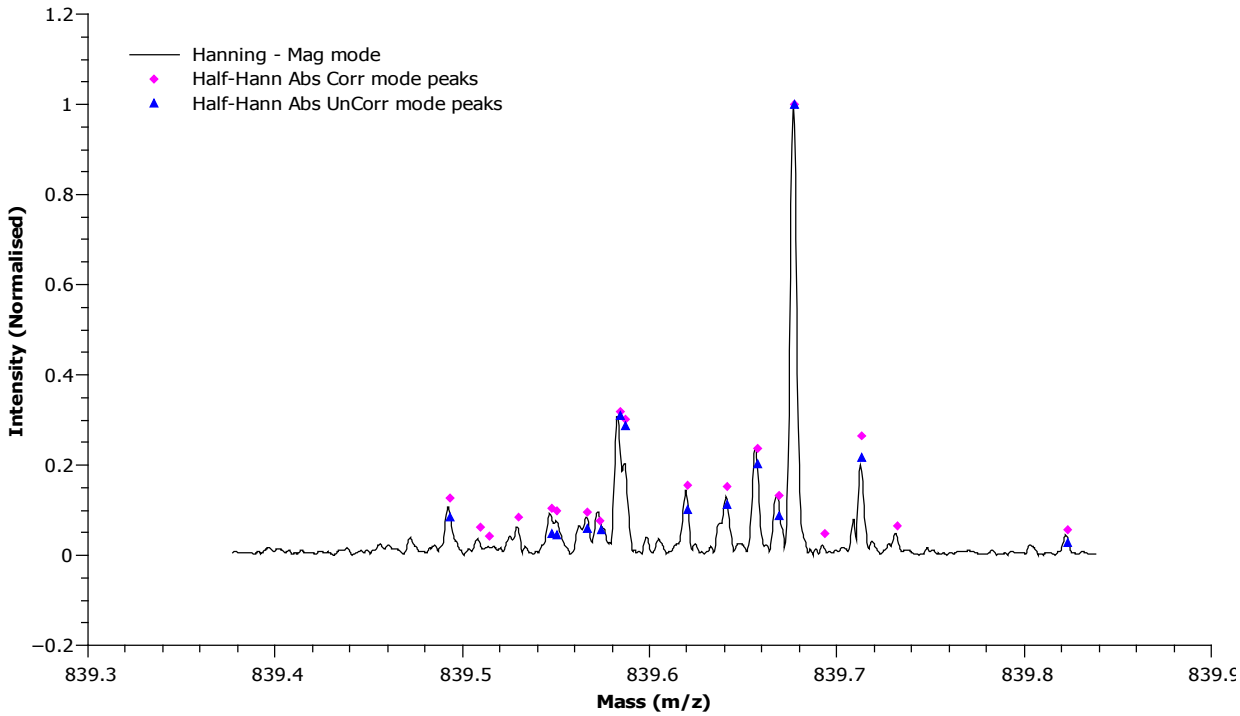
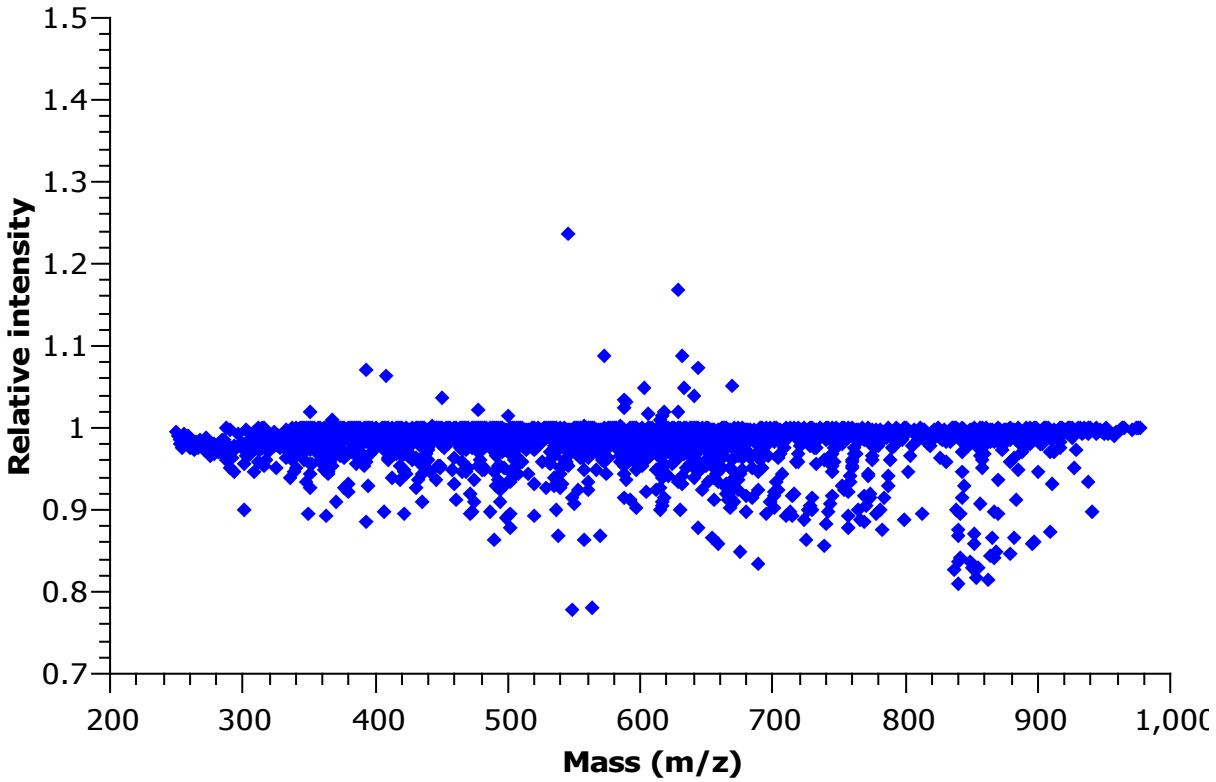


Baseline correction may perturb isotopic relative abundances

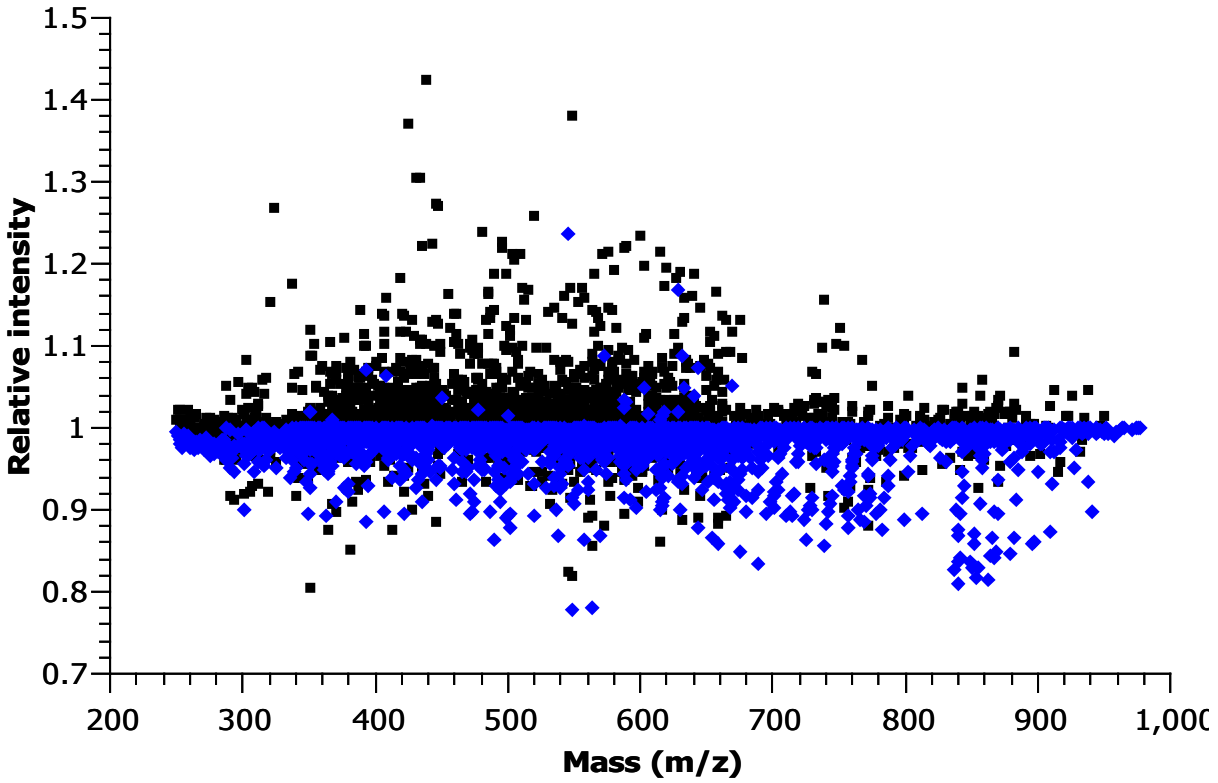
Relative peak height

- No baseline correction
  - Mean 0.98
  - SD 0.03



Relative peak height

- No baseline correction
  - Mean 0.98
  - SD 0.03
- After baseline correction
  - Mean 1.02
  - SD 0.05

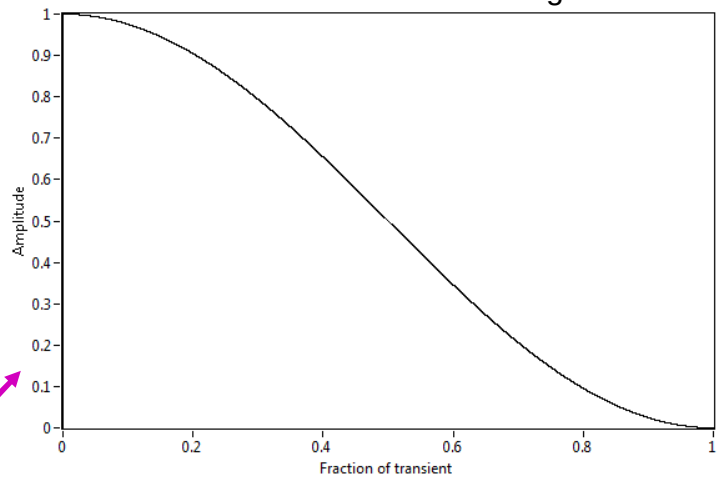


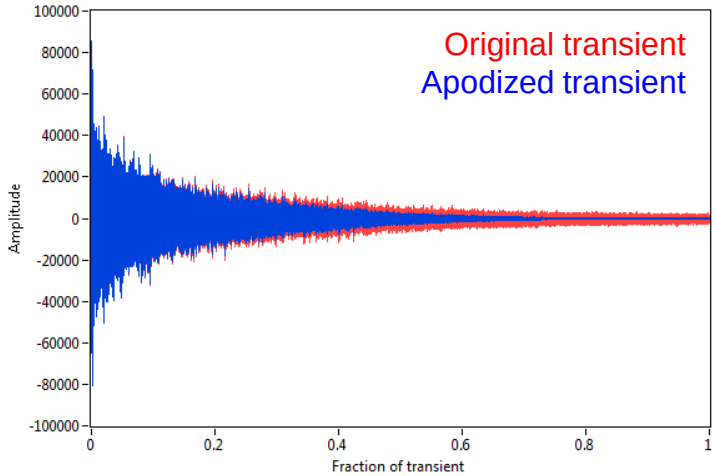
Baseline correction may cause more problems than it solves

Apodization games

- Fourier transform
  - Assumes the waveform is infinitely long
  - Generates spectral leakage on finite signals
- Apodization or windowing
  - Helps limit spectral leakage
- Half-Hanning function
  - Commonly used for Absorption mode spectra

‘Half-Hanning’ function





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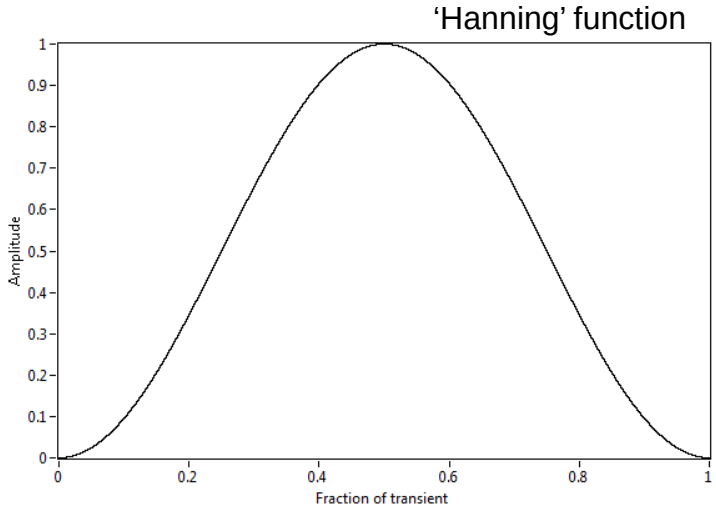
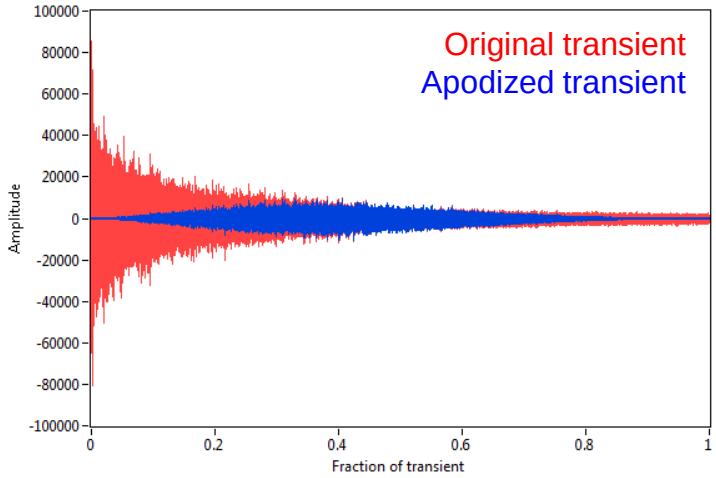
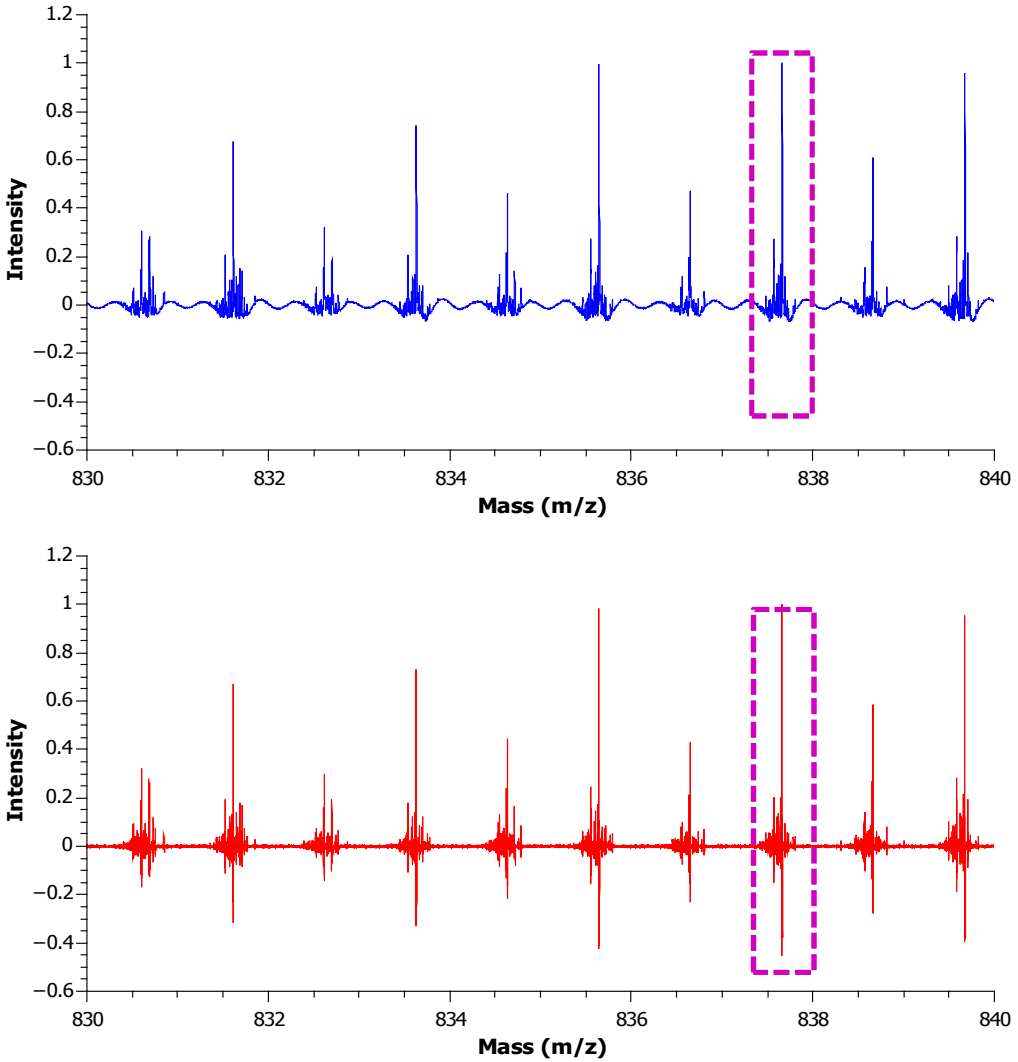




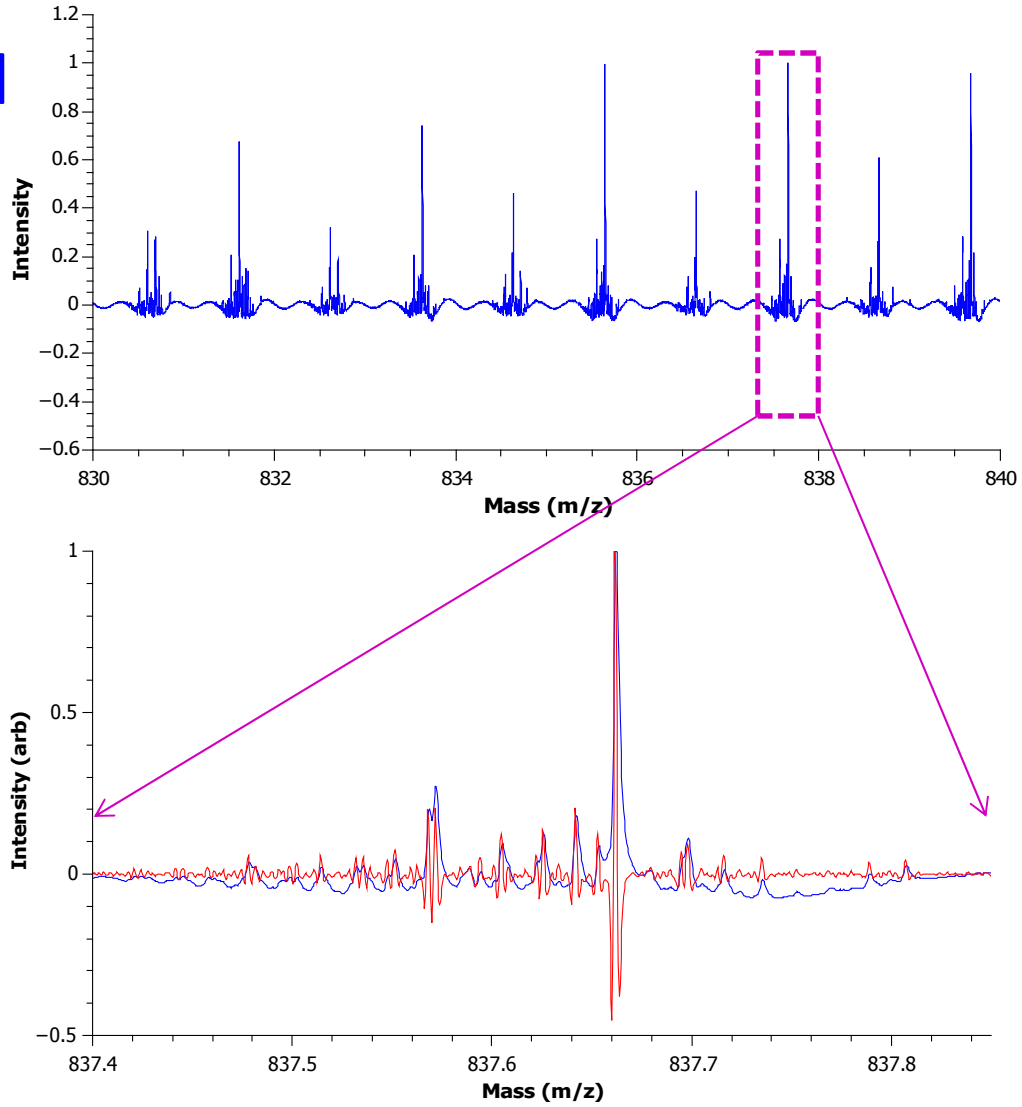
Results

- Half-Hanning apodized absorption mode
- Full Hanning apodized absorption mode
- Baseline is now flat
  - On the wide scale



Results

- Half-Hanning apodized absorption mode
- Full Hanning apodized absorption mode
- Baseline is now flat
  - On the wide scale
  - Also on zoom



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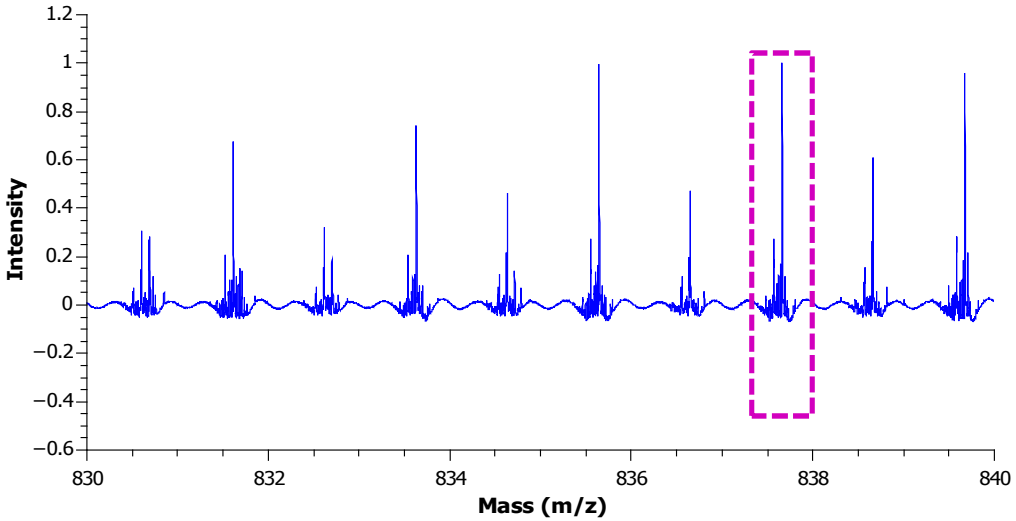
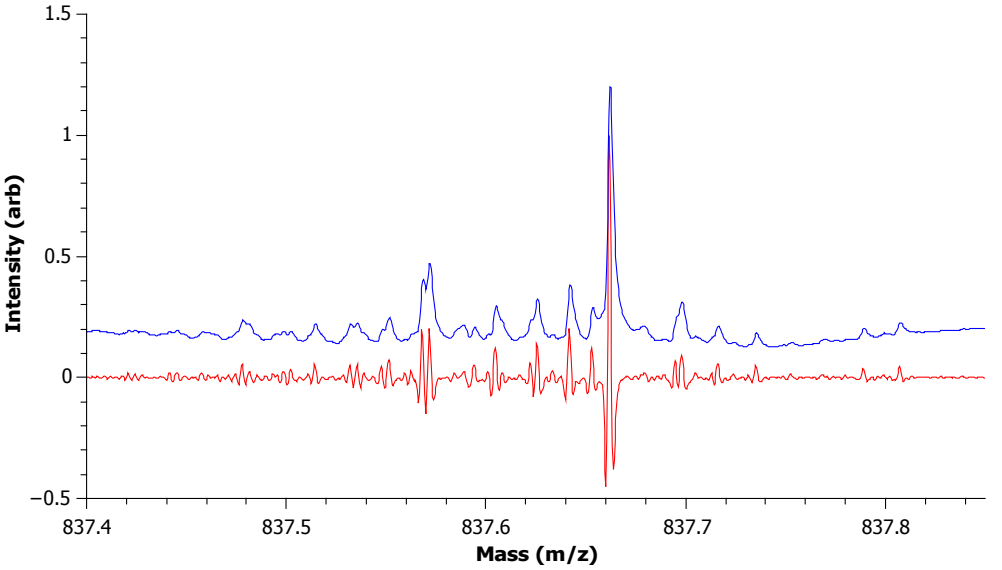
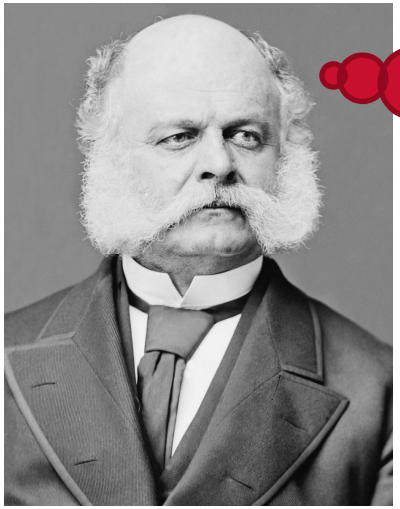




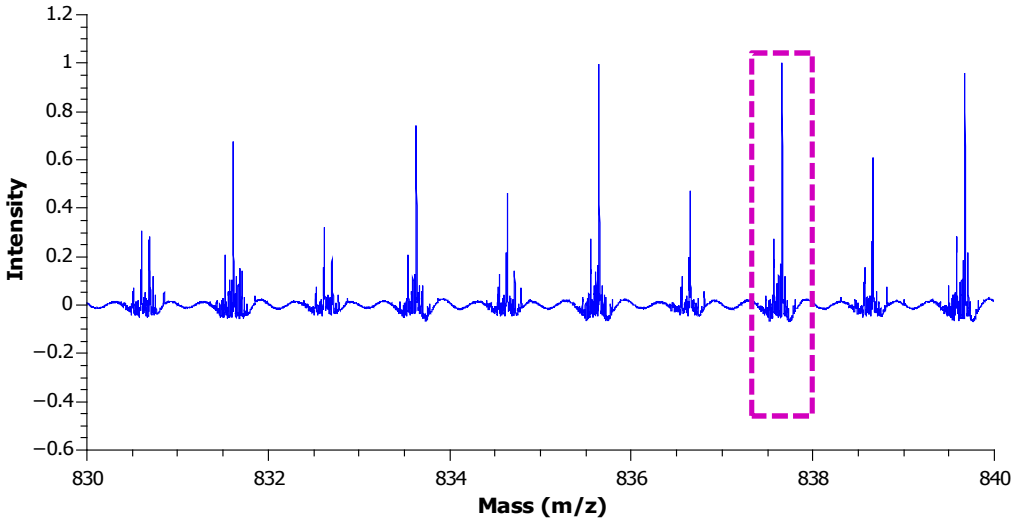
Results

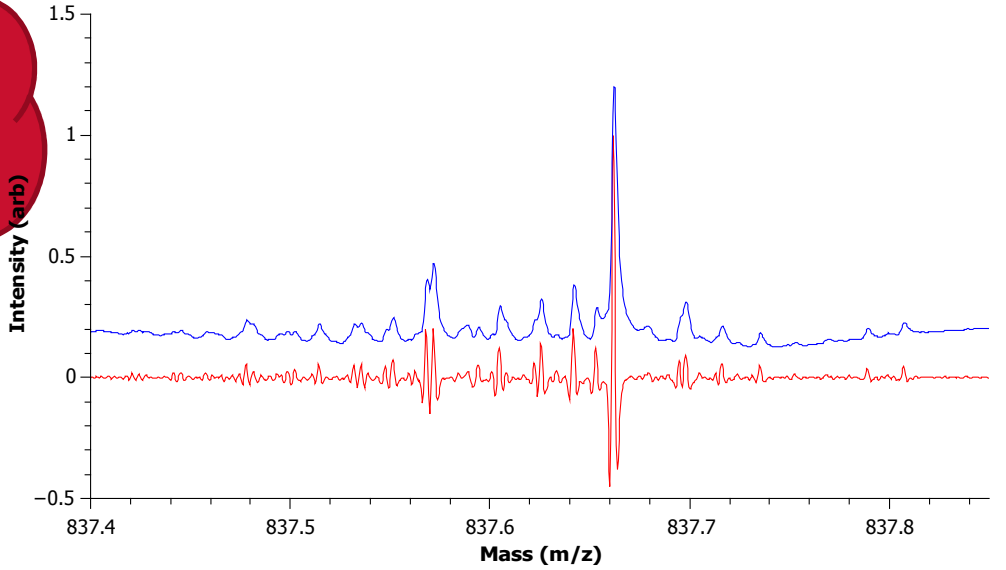
- Half-Hanning apodized absorption mode
- Full Hanning apodized absorption mode
- Concern



C'mon bruv!

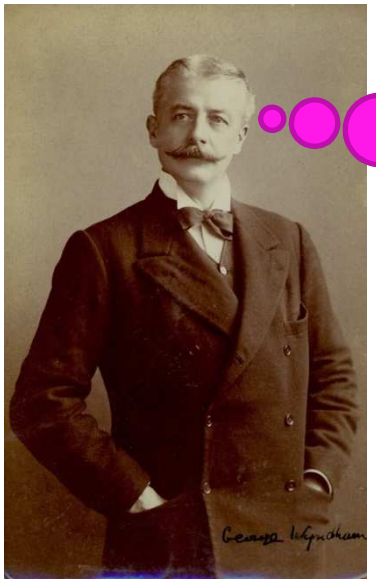
MS don't have no negative side lobes!



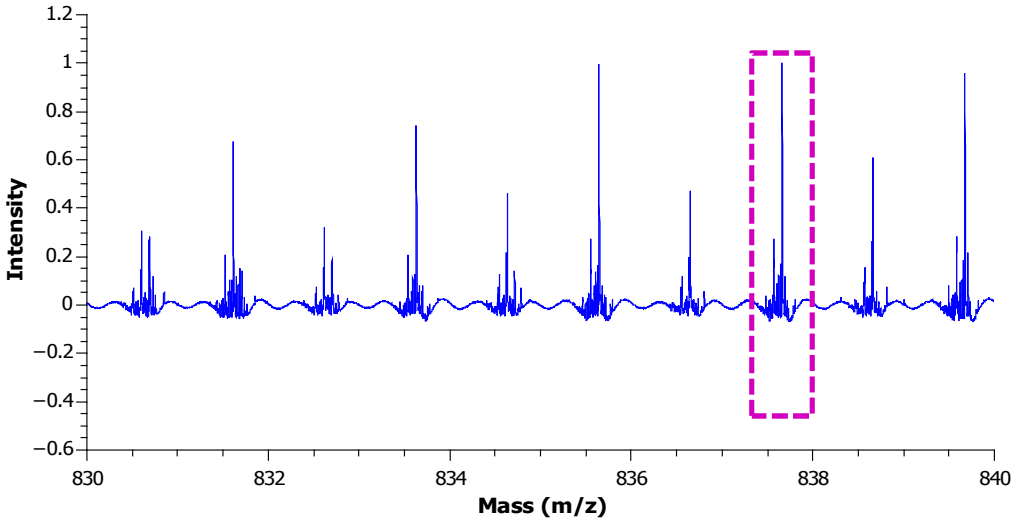


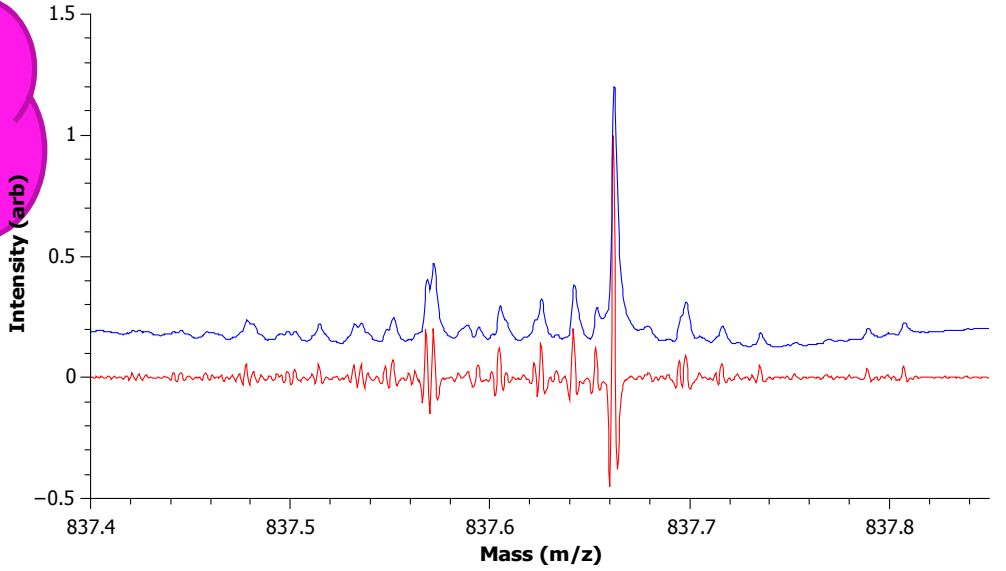
Results

- Half-Hanning apodized absorption mode
- Full Hanning apodized absorption mode
- Concern



No no, homeboy. The sidelobes are true, innit?





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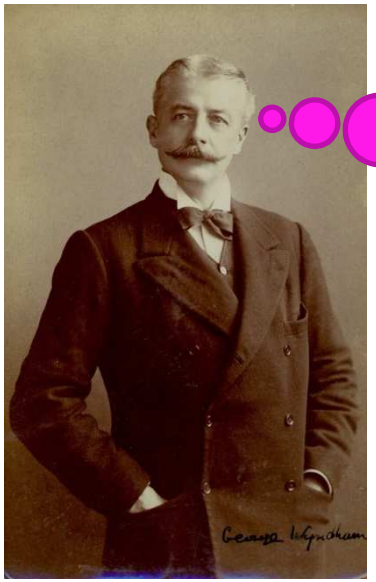
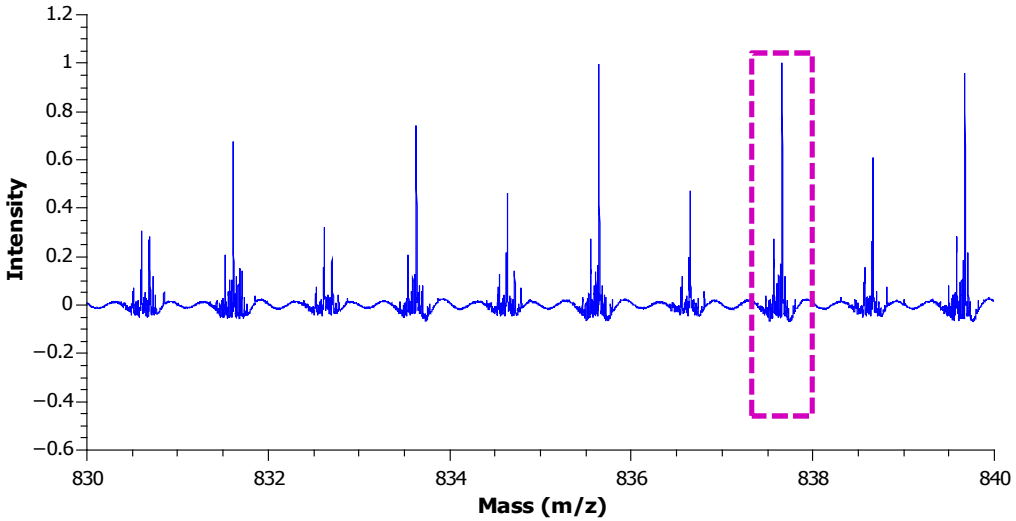
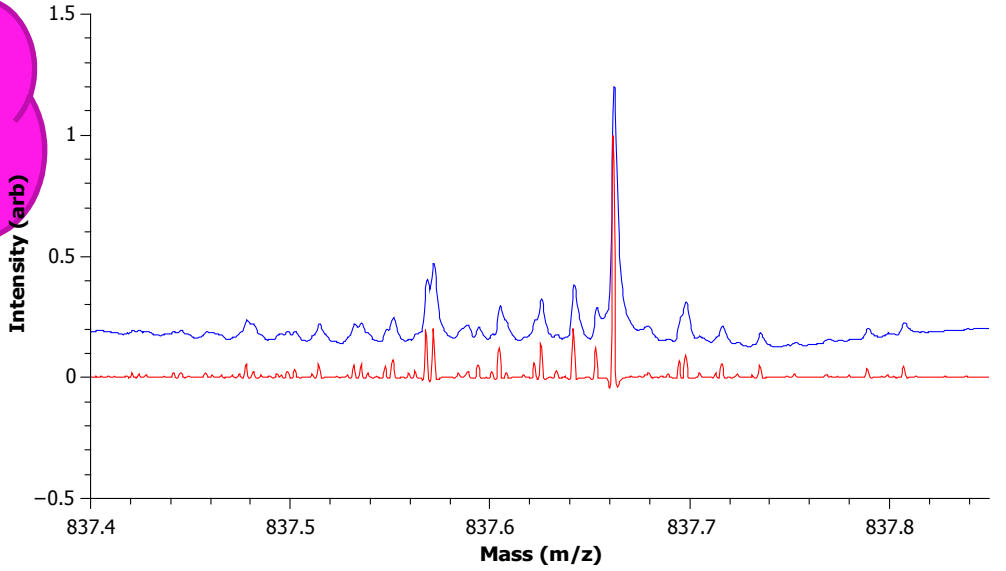


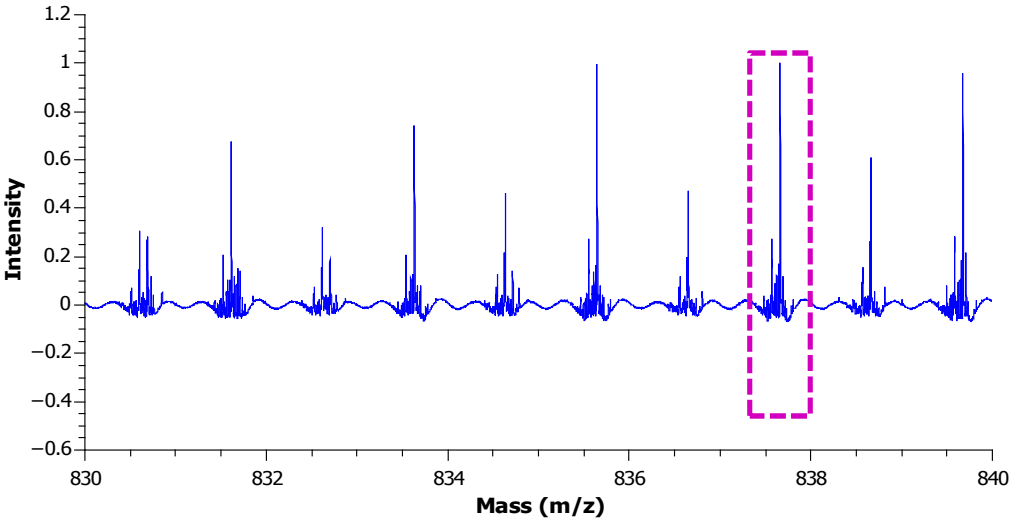
But you can use an adaptive filter to reduce them



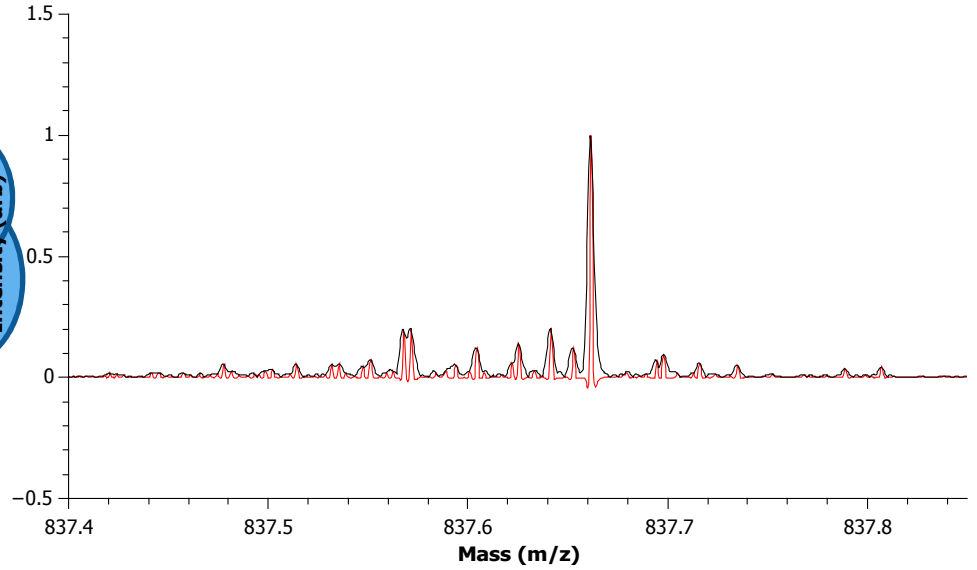


Results

- Half-Hanning apodized absorption mode
- Full Hanning apodized absorption mode
- Magnitude Mode

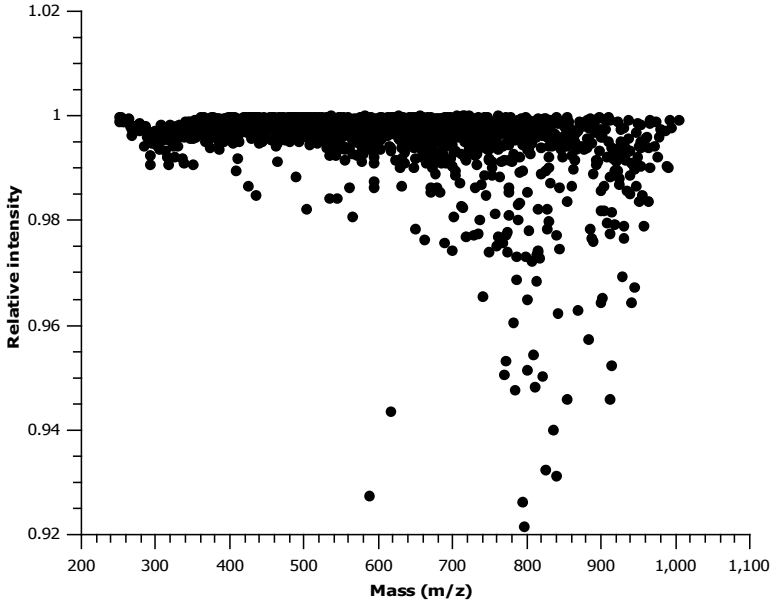


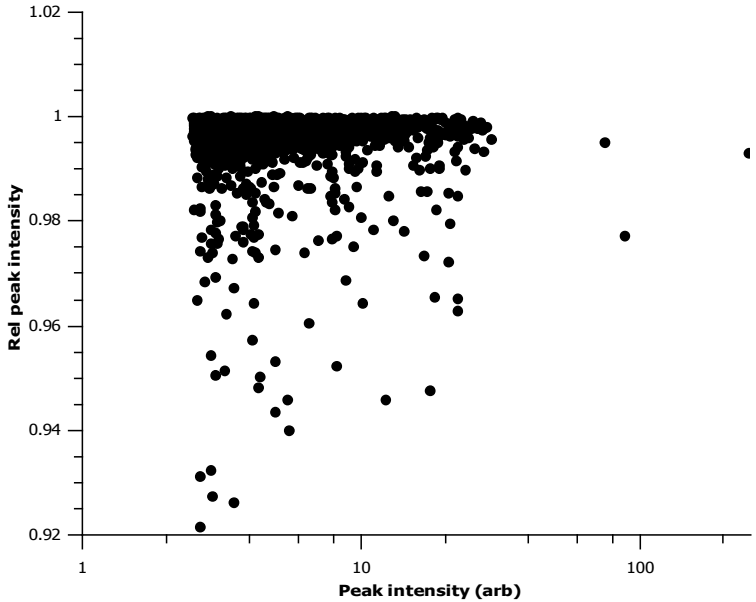
Gosh – that's much better than magnitude mode!



Results

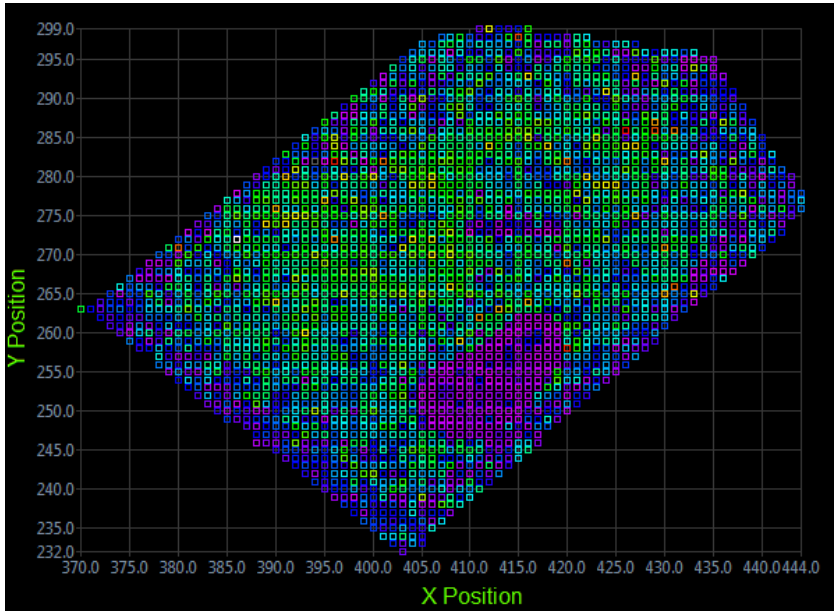
- Relative peak intensity
  - Absorption mode vs Magnitude mode
  - Mean – 1.00
  - SD – 0.01





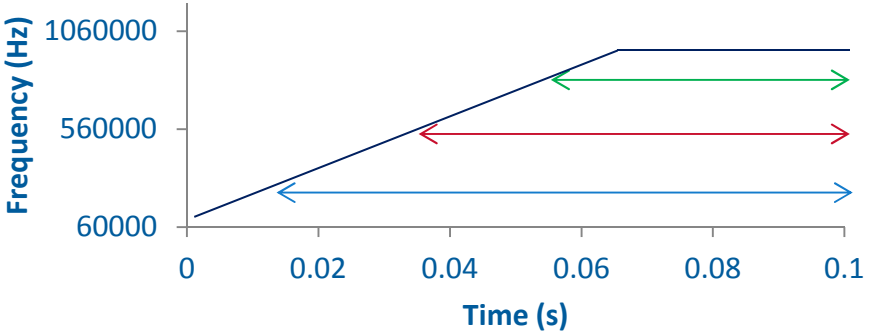
Benefits

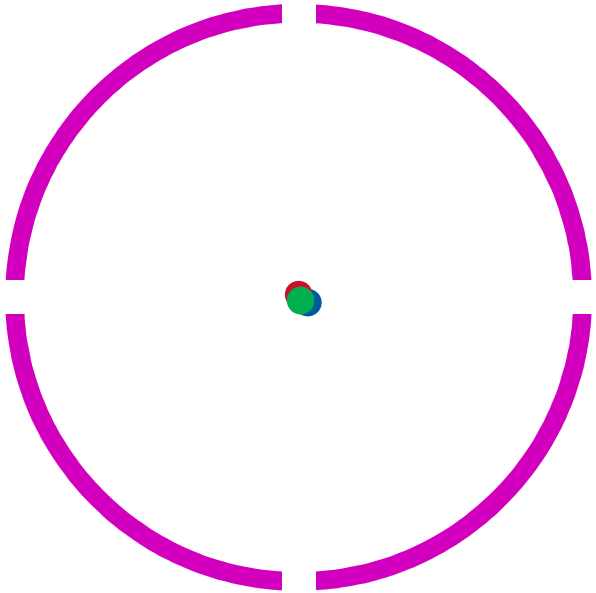
- Avoiding baseline correction
  - Speeds up processing
  - Improves relative abundance accuracy
- Should be very beneficial in FT-ICR imaging
  - Also makes peak detection easier



Phase shifts FT-ICR MS

- Excite and delay cause phase shift

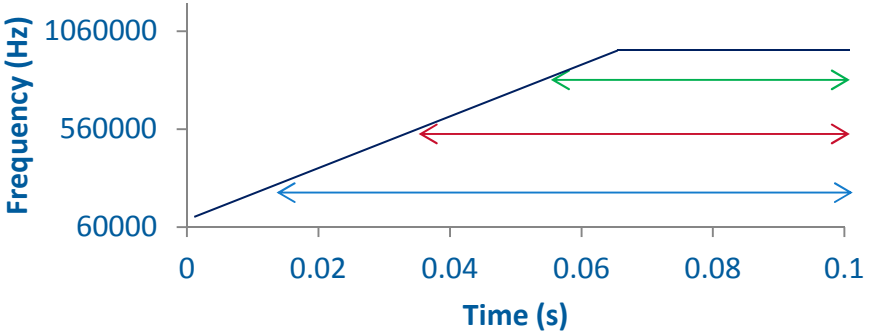


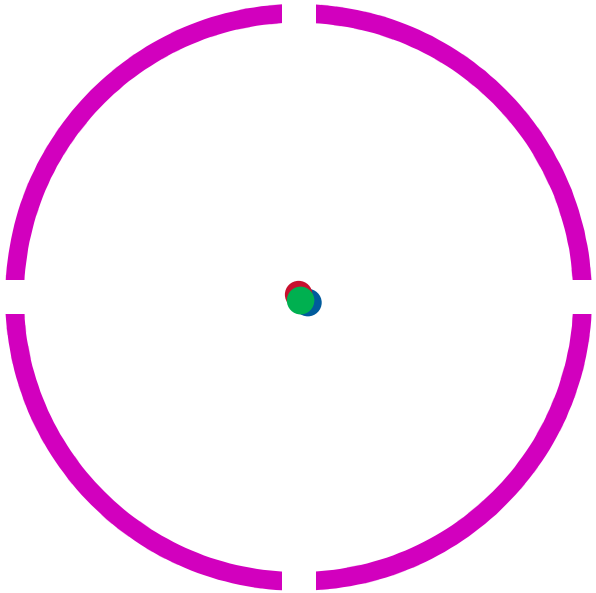


- By the beginning of detection
  - Ions with different frequencies have different starting angles
- This causes the peak distortion

Phase shifts FT-ICR MS

- Excite and delay cause phase shift





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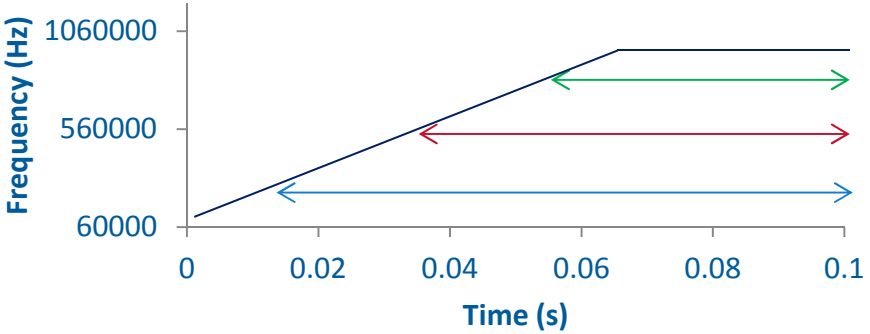

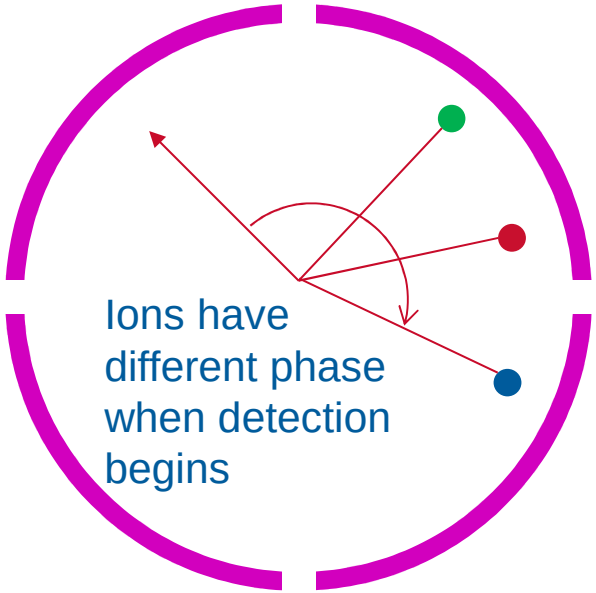

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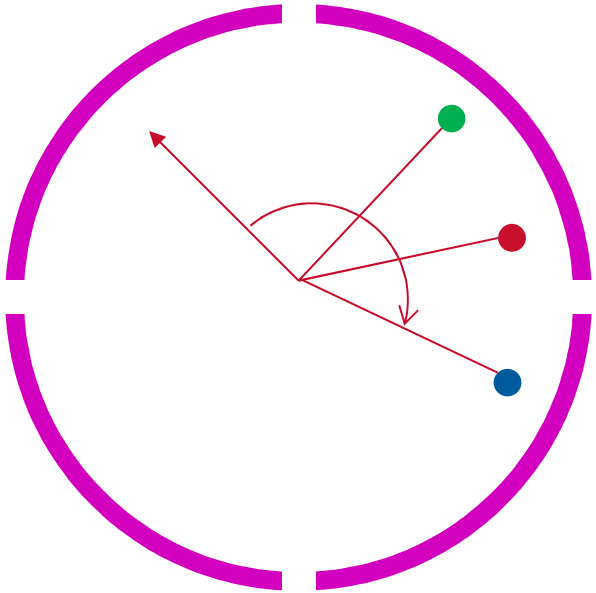
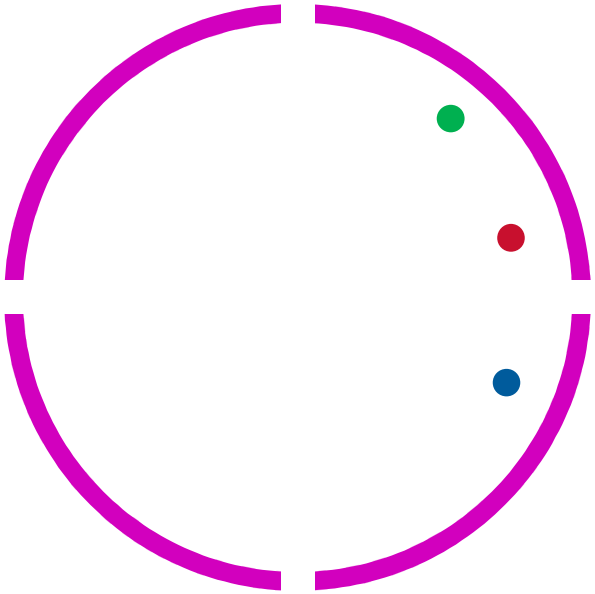
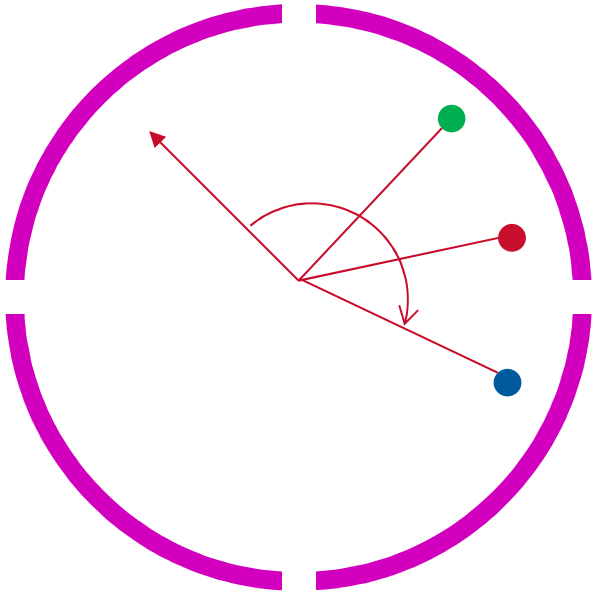


- Phase correction aim
  - Remove all accumulated phase



Phase shifts FT-ICR MS

- Excite and delay cause phase shift



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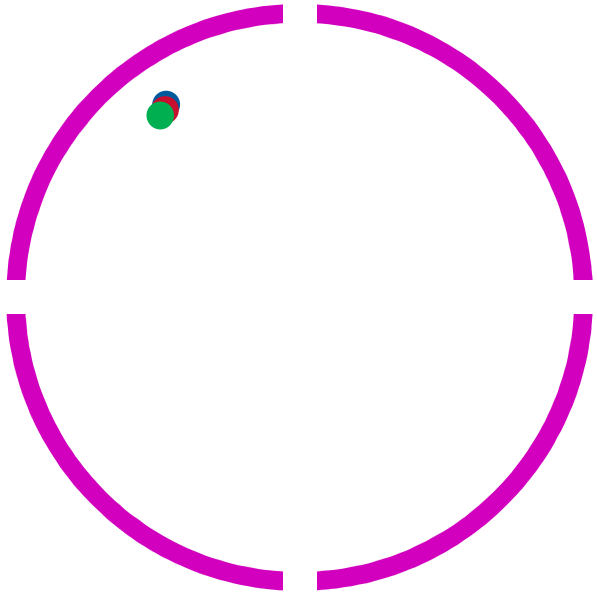
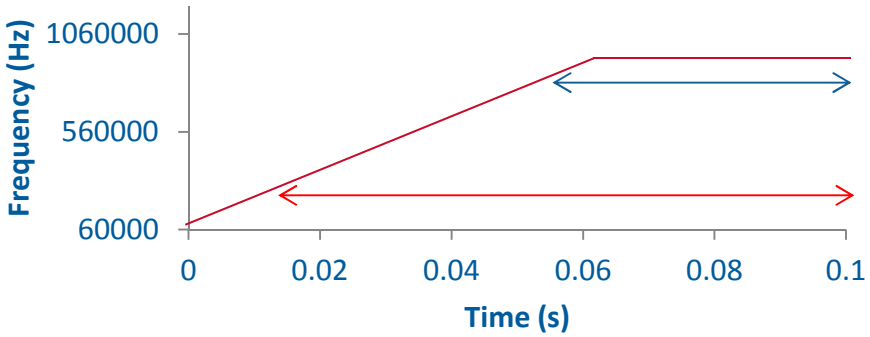


Makes it look like all ions started at the same place

Source of phase shifts

- Frequency sweep and delay times

$$\varphi = \frac{-\omega^2}{R} + \omega\left(\frac{\omega_{Final}}{R} + t_{Delay}\right)$$



- Legend
  - ϕ_i – initial phase angle (rad) of excite
  - ω_x – detected ion frequency (rad.s^{-1})
  - ω_0 – initial frequency (rad.s^{-1}) of excite
  - ω_{Final} – final frequency (rad.s^{-1}) of excite
  - R – excitation sweep rate
  - t_{Delay} – delay time (s)

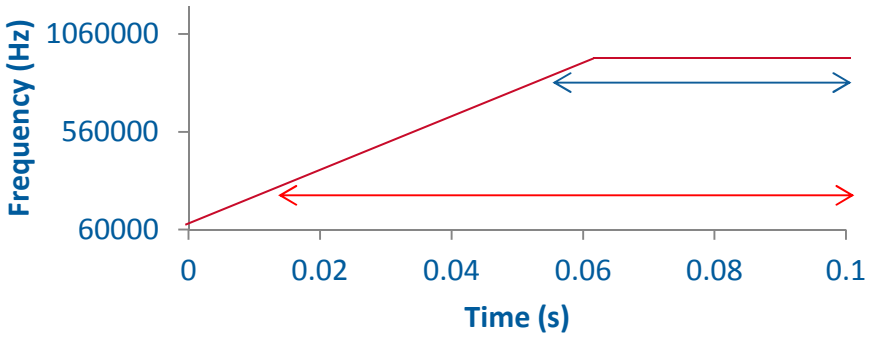
Source of phase shifts

- Initial phase

$$\varphi_t = \frac{\omega_t^2}{2R} + \frac{-\omega_0^2}{R} + \varphi_i$$

- Frequency sweep and delay times

$$\varphi = \frac{-\omega^2}{R} + \omega\left(\frac{\omega_{Final}}{R} + t_{Delay}\right)$$



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- Frequency sweep and delay times

$$\varphi = \frac{-\omega^2}{R} + \omega\left(\frac{\omega_{Final}}{R} + t_{Delay}\right)$$

- Total equation

$$\varphi_x = \frac{-\omega_x^2}{2R} + \omega_x\left(\frac{\omega_{Final} - \Delta\omega_K}{R} + t_{Delay}\right) + \left(\frac{-\omega_0^2}{2R} + \varphi_i\right) + \frac{\Delta\omega_K\omega_{Final}}{R}$$

You never really know this, accurately

- Complication
  - Frequency shifts
  - $\overline{\omega_i} = \omega_x + \Delta\omega_K$
- Legend
  - φ_i – initial phase angle (rad) of excite
  - ω_x – detected ion frequency (rad.s^{-1})
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Source of phase shifts

- Initial phase

$$\varphi_t = \frac{\omega_t^2}{2R} + \frac{-\omega_0^2}{R} + \varphi_i$$

- Frequency sweep and delay times

$$\varphi = \frac{-\omega^2}{R} + \omega\left(\frac{\omega_{Final}}{R} + t_{Delay}\right)$$

- Total equation

$$\varphi_x = \boxed{\frac{-\omega_x^2}{2R}} + \boxed{\omega_x\left(\frac{\omega_{Final} - \Delta\omega_K}{R} + t_{Delay}\right)} + \boxed{\left(\frac{-\omega_0^2}{2R} + \varphi_i\right) + \frac{\Delta\omega_K\omega_{Final}}{R}}$$

You never really know this, accurately

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  - R – excitation sweep rate
  - t_{Delay} – delay time (s)

Phase correction by fitting

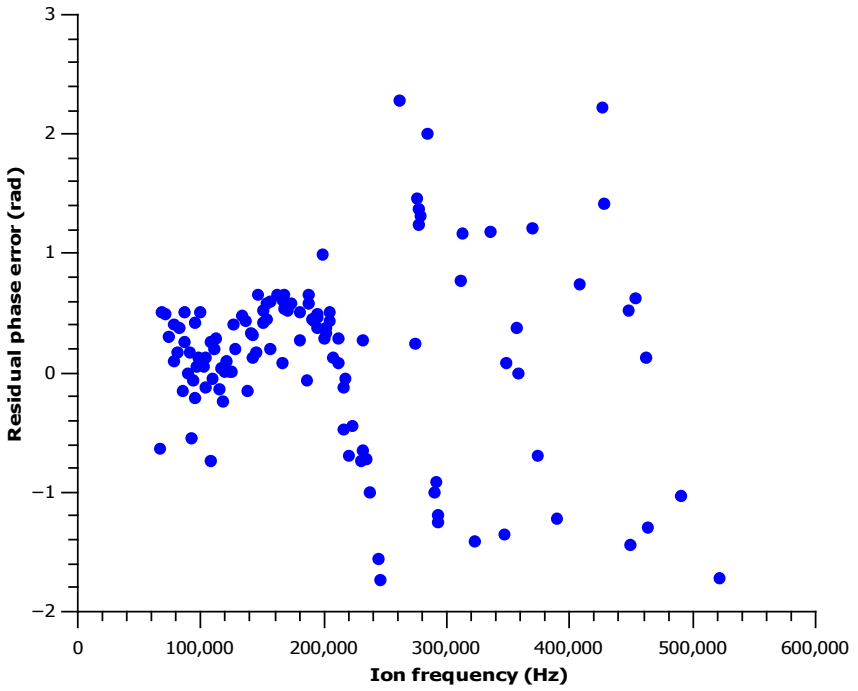
- Full quadratic fitting is required
  - To allow for $\Delta\omega_K$
- Phase correction function
  - $\varphi_f = Af^2 + Bf + C$
- Can solve for *A*, *B* and *C* (if you know pairs of φ and *f*)

- With no phase correction
  - $Re = Mag * \cos\theta$
- With phase correction
  - $\mathrm{Re}_f = \mathrm{Mag}_f \times \cos(\theta_f - \varphi_f)$
  - φ_f is the phase correction factor

An unexpected pleasure

- The file that wouldn’t phase correct …
- Residual phase error is not quadratic
  - Original phase error was of a higher order
  - Probably third order
- (In this case caused by a non-linear frequency sweep)

Residual phase error after optimization



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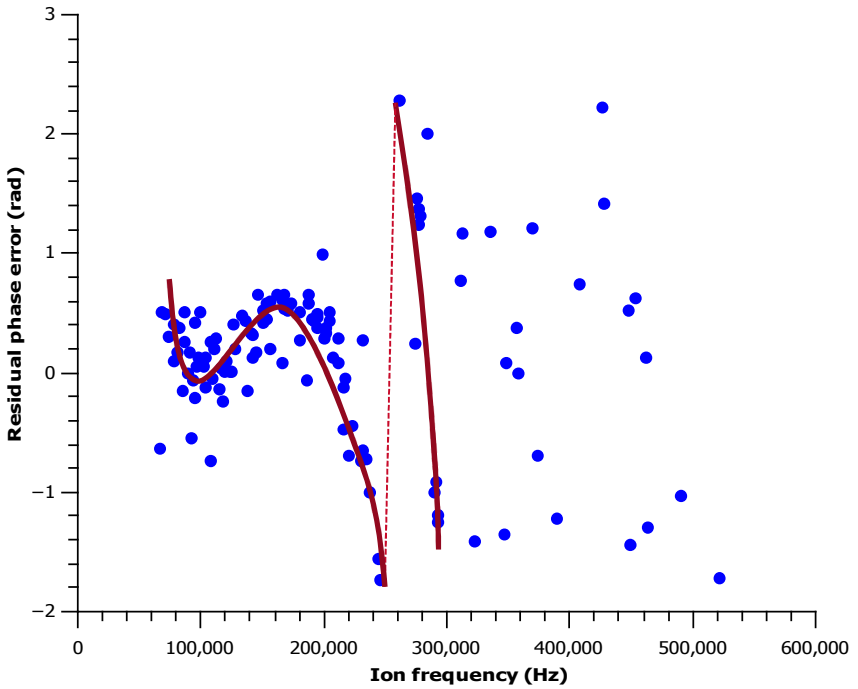
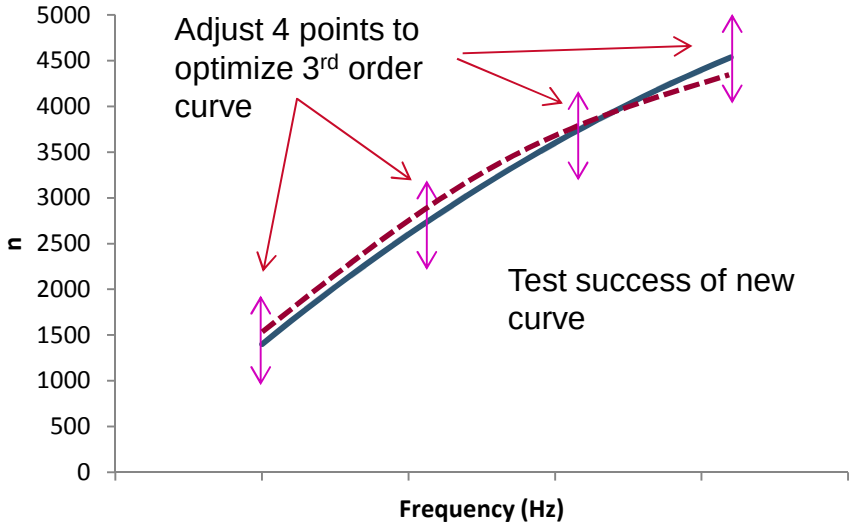
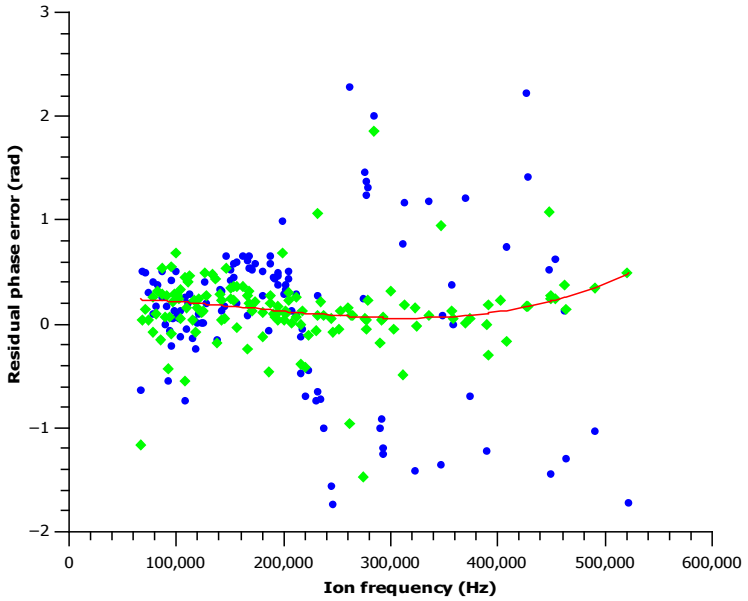


Solution

- Adapt Autophaser to solve n^{th} order PCFs
  - Simple adaptation to GA to fit curve to (n+1) nodes instead of 3





- 3rd order fitting actually makes it easier to phase correct other files too

Conclusions

- An new apodization approach
  - Removes the necessity of baseline correction
  - Better abundance sensitivity
  - Improved processing times
  - Will be key for absorption imagery
- n^{th} order PCFs
  - Can be solved!
  - May be caused by unusual excitation functions