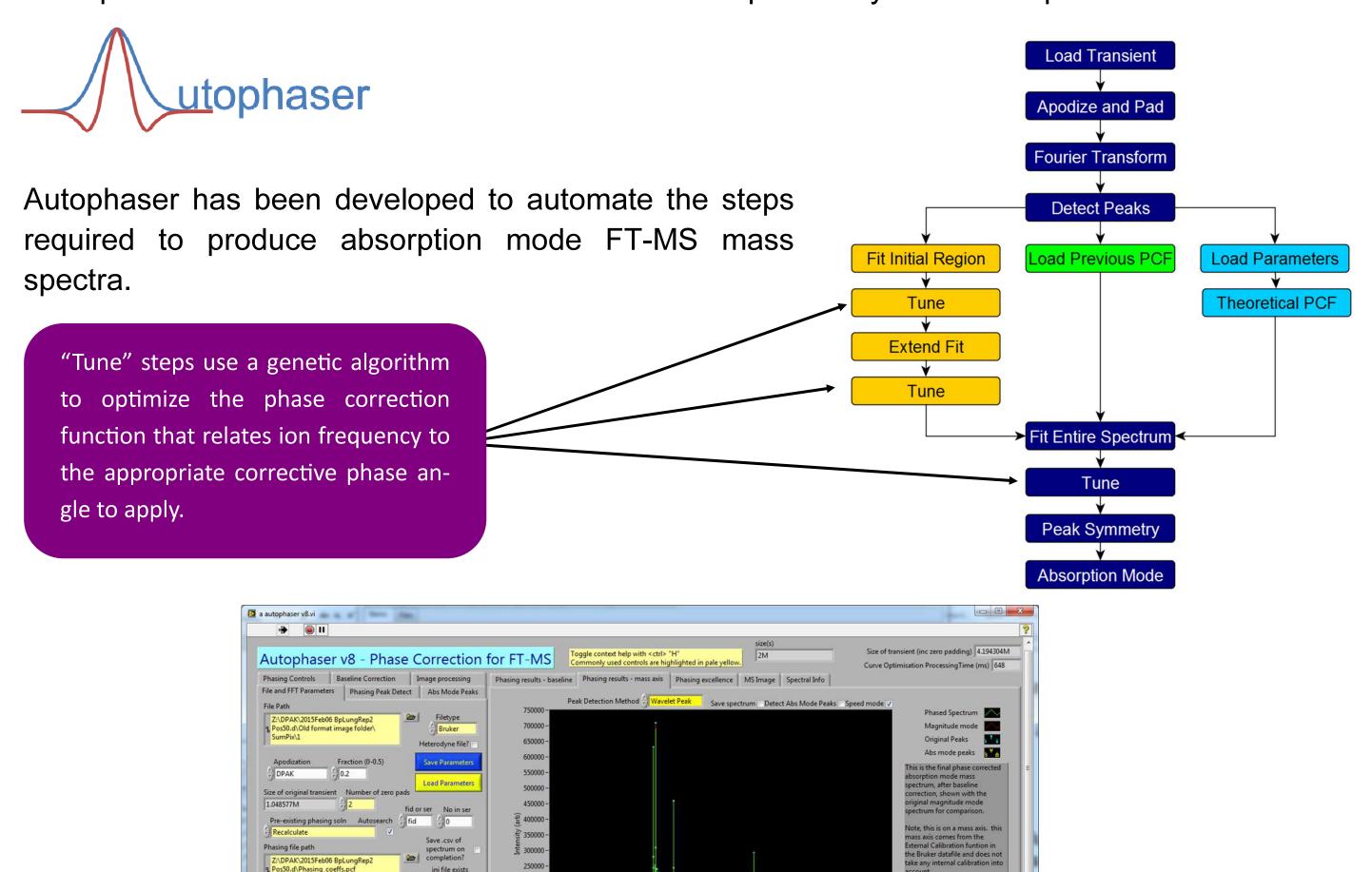
Absorption mode gets even better with its svelte new curves

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Introduction:

Absorption mode is a different way of viewing FT-MS data – giving improved resolution, signal-tonoise ratio and accuracy. You don't have to adapt your instrument, just process the data in a different way. Methods for producing absorption mode spectra have been around for nearly 10 years, but have a couple of weaknesses – the techniques have relied on the phase correction function being quadratic in nature and the spectra produced often exhibit a severe baseline deviation which must be corrected before the MS data can be interpreted. Making this correction is both computationally difficult and time-consuming. Here we show how these problems can be solved by the application of some well-chosen curves that improve resolving power, remove the necessity for baseline correction,[1] and provide absorption mode specific isotopic models.[2] We also show how some instruments can require a non-standard, higher order (>2) phase correction function in order to produce absorption mode spectra.[3] As a consequence of these developments you can benefit from faster, higher performance spectral processing and interpretation, and apply absorption mode to new instruments which would not previously have been possible.



Phase correction function:

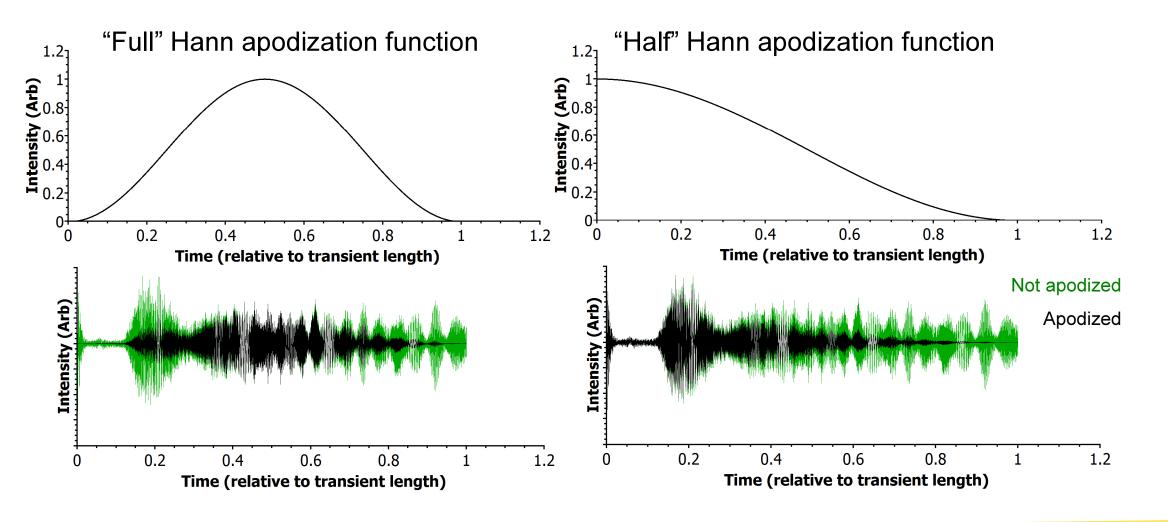
The key step in producing an absorption mode spectrum is optimizing the phase correction function. For both FT-ICR instruments and Orbitraps, the phase correction function is normally quadratic. For example, a linear frequency sweep excitation in an FT-ICR instrument will result in phase (φ_X) varying as a function of ion frequency (ω_X):

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$$\varphi_{x} = \frac{-\omega_{x}^{2}}{R} + \omega_{x} \left(\frac{\omega_{Final}}{R} + \frac{-2\Delta\omega_{K}}{R} + t_{Delay} \right) + \varphi_{0} + \frac{\Delta\omega_{K}\omega_{Final}}{R} + \frac{-(\Delta\omega_{K})^{2}}{R}$$

Apodization:

FT-MS transients are commonly apodized prior to Fourier transformation and further processing. Full function apodization (e.g. Hann or Sine bell) is commonly applied to magnitude mode spectra. Hitherto, a half Hann function apodization has been by far the type most commonly applied to absorption mode spectra



Comparison:

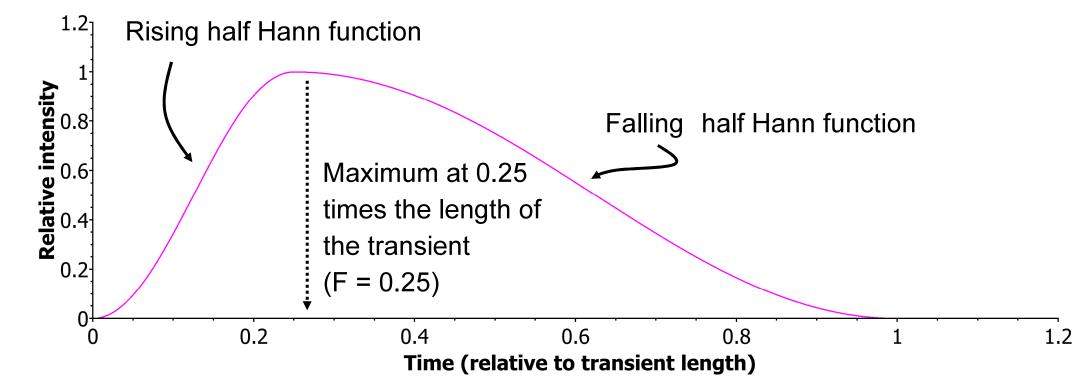
"Full" Hann apodization provides better reduction in peak side lobes by dropping to a minimum (zero) amplitude at both ends. Half Hann apodization provides better signal-to-noise because the apodized signal contains more of the earlier, higher intensity part of the transient. However half Hann apodized absorption mode spectra show significant peak side-lobes which result in troublesome spectral baseline deviations.

Baseline Deviation Problem:

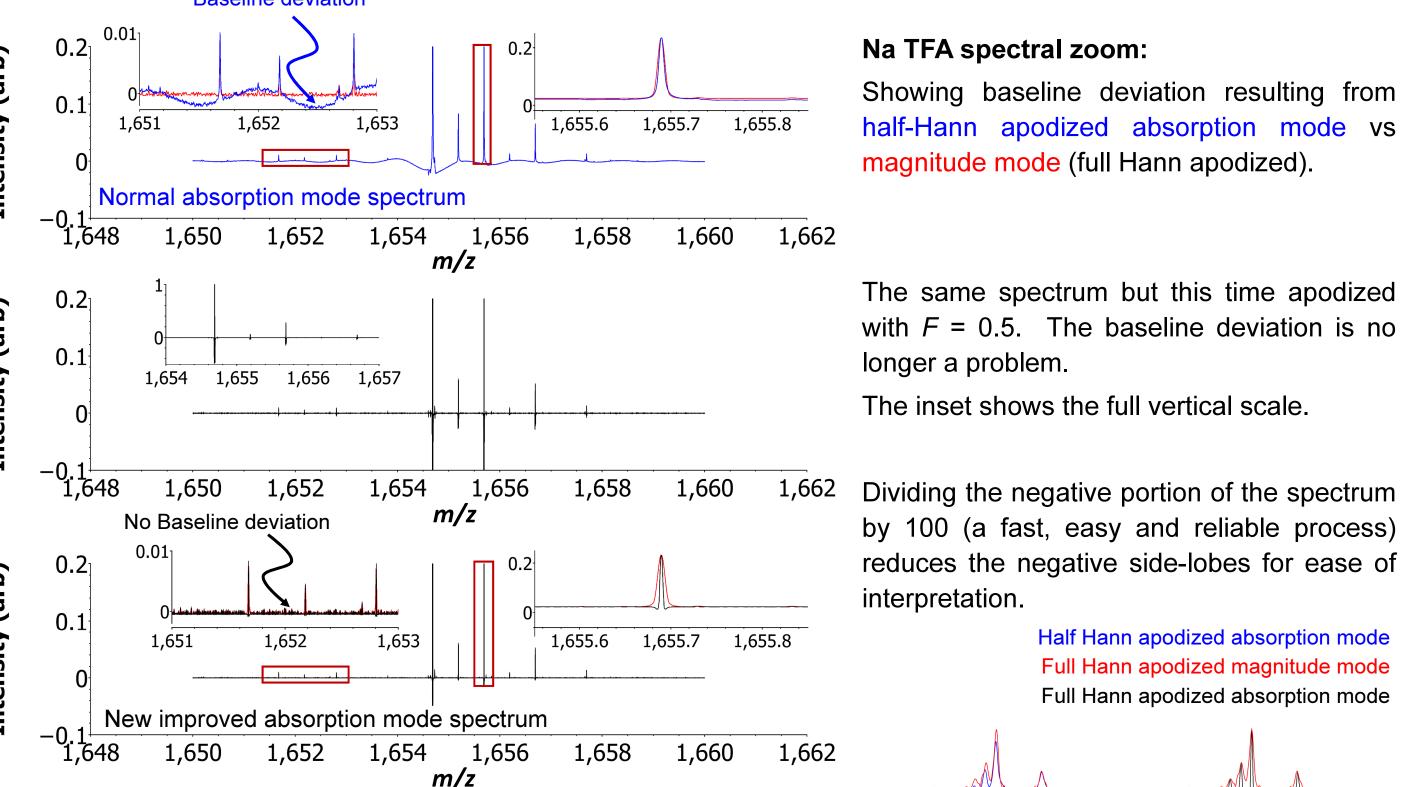
The baseline deviation issue can skew relative peak intensities and can also cause missed peaks. The baseline deviation (in red in the figure) could be removed if it could be reliably tracked, but we have found that this is both difficult to automate reliably and time consuming to apply. It would be better if the problem could be avoided.

Curing the deviation problem:

We have developed a new apodization approach to produce absorption mode FT-MS spectra which do not exhibit baseline deviation, whilst maintaining all the normal absorption mode benefits.[1]



This method involves the use of a new, asymmetric "full" apodization function, replacing the more common Hann or half Hann functions, and where the user can control the position of the function maximum expressed as a fraction (F) of the transient length. Using a full apodization function helps to remove the peak side lobes which are the cause of the baseline deviation. F can normally be varied from 0.0 to 0.5. Lower values of F retain more of the early part of the transient and therefore using a lower value of F can improve the signal-to-noise ratio of the spectrum if the spectrum is very weak. For most spectra, it is beneficial to use a higher value of *F* as this improves the resolving power.



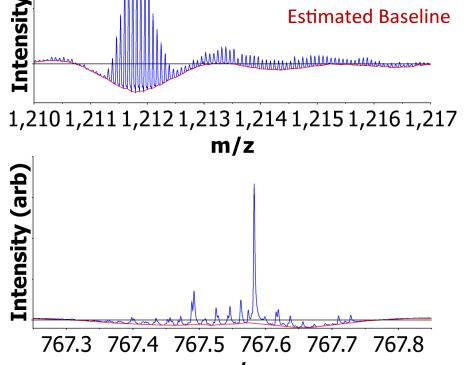
Here we see the same benefits, but this time in different views from a MALDI in-source decay spectrum of an IgG standard. In moving from F = 0.0 (half Hann apodization) to F = 0.5 (full Hann apodization) we see that the absorption No baseline deviation mode baseline deviation disappears and the spectral resolving power improves. Relative peak intensity maintained with no requirement for baseline correction.

R-MS: Ultrasonic Acoustic Wave Nebulization coupled with Proton-Transfer-Reaction Mass Spectrometer, Lucas Maerk MP076 Native MS using SAWN, a Novel Ionization Source for Waters SYNAPT G2, Gloria Yen MP155 Bacterial Glycolipids Characterized on an IMS-QExactive, Yue Huang

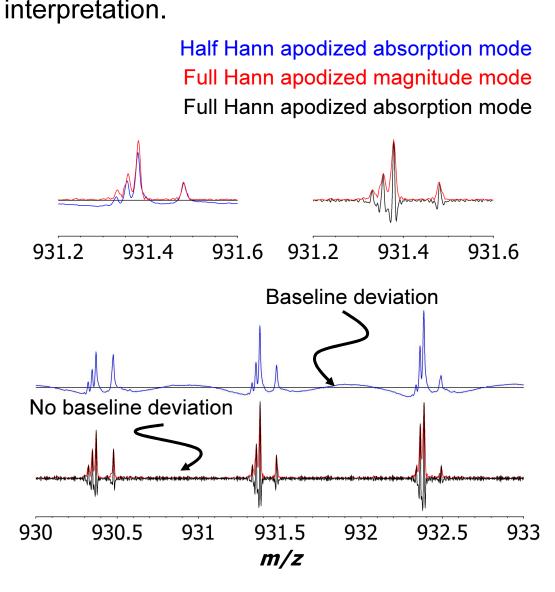
Posters from Goodlett Lab and Collaborators

MP217 Characterization of a Monoclonal Antibody (mAb) using Multiple Fragmentation Techniques and Novel FT Data Processing Software, Bao TP122 Absorption Mode Gets Even Better with its Svelte New Curves, David Kilgour

MP560 The Associations between Enterovirus Infections and Type 1 Diabetes, Niina Lietzen MP646 Characterization of Semi-Synthetic Motor Oil using FT-ICR, Sung Hwan Yoon

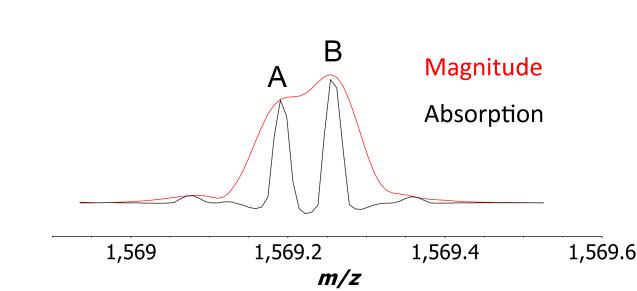


Half Hann Absorption Mo

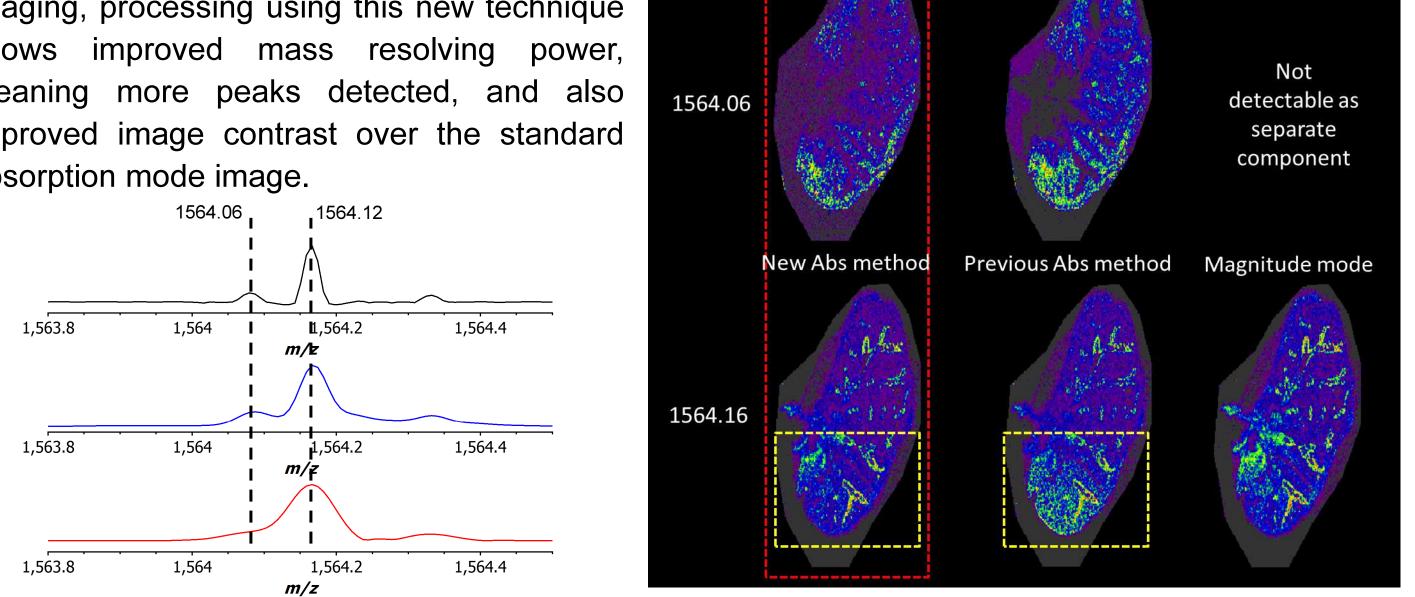


Application to MS Imaging:

The necessity for baseline correction was a major restriction on the application of absorption mode to FT-MS imaging. For an FT-ICR MS mass spectral imaging experiment gathering 50,000 pixels, and gathering a 1 million point transient at each pixel, this baseline correction step, summed for all pixels, would take more than 41 50 100 150 200 250 300 350 400 450 500 550 600 650 700 hours, and that is without any manual tuning of the baseline Two MSI of mouse lungs showing the correction for individual pixels. More information can be found on distribution of two peaks (A and B) only poster MP180. separable in the absorption mode.

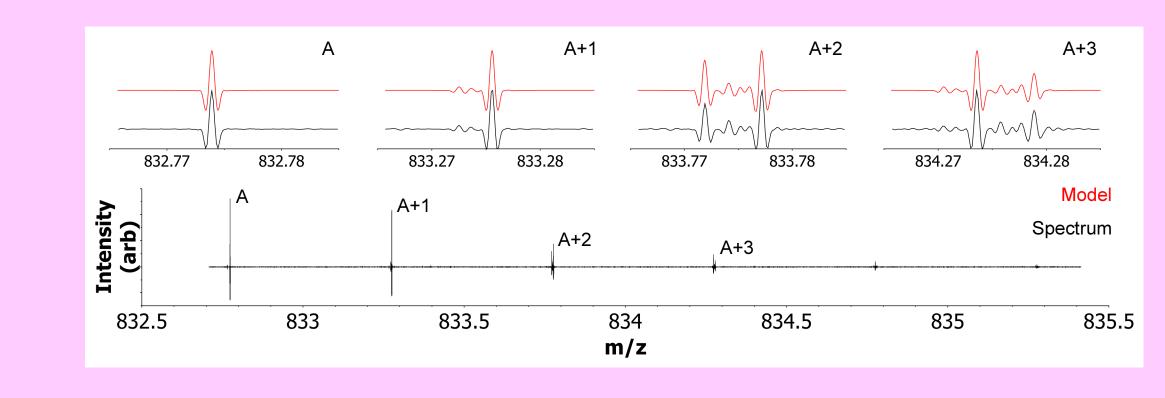


As with previously presented absorption mode imaging, processing using this new technique shows improved mass resolving power, improved image contrast over the standard absorption mode image.

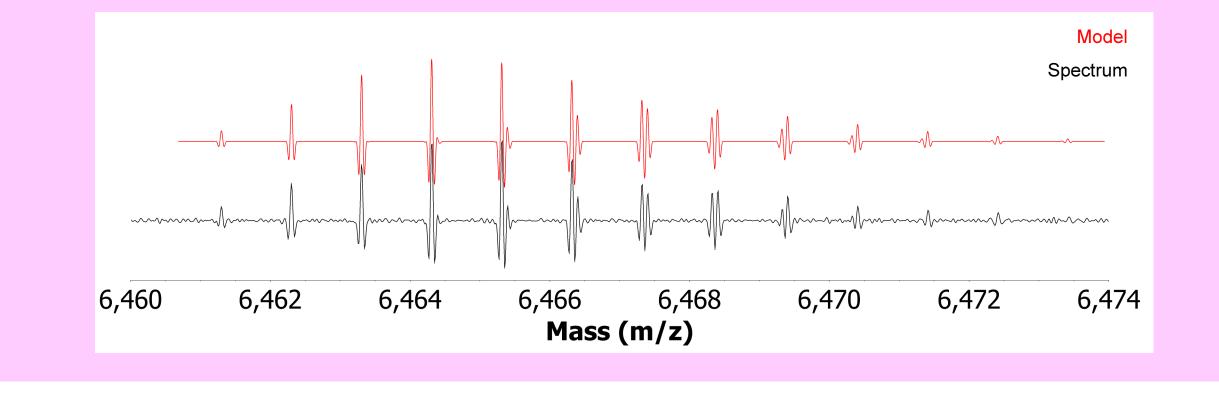


Isotopic modelling in absorption mode:

Conventional methods for modeling isotopic distributions are not applicable to fully apodized absorption mode spectra because the peak shapes in these spectra are distinctly different to those seen in normal (i.e. magnitude mode) spectra. We have adapted the Mercury algorithm approach to generate absorption mode specific isotopic models. [2]

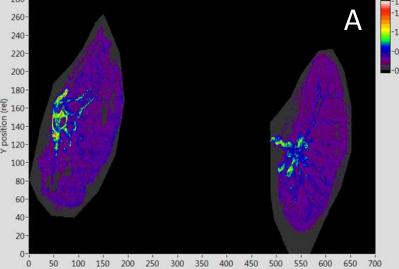


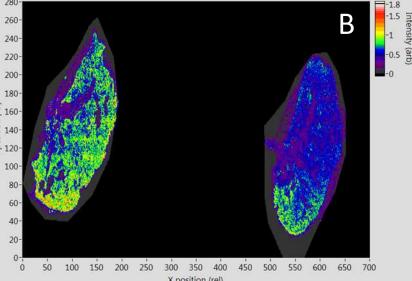
The spectrum above shows the isotopic distribution of thiostrepton, showing the isotopic fine structure, with zoomed portions to show detailed comparison of the spectrum and the isotopic model. The spectrum below shows a zoomed portion of the MALDI in-source decay spectrum of an IgG molecule showing the overlapping isotopic distribution of two fragments together with an isotopic model for comparison. This spectrum was provided by Magnus Palmblad.



MP180 Absorption Mode Analysis of FT-ICR Imaging Data Improves Peak Resolution in a Bordetella pertussis Infection of Mini Surface Acoustic Wave Nebulization Chip by Using Different Types of Mass Spectrometer, Tao Liang

WP233 Direct Beverage Analysis by SAWN MS, David Goodlett WP268 A Cartesian Product Approach To Lipid A Structure Identification, Lisa Leung WP450 Ultrasonic Acoustic Wave Nebulization-Mass Spectrometry (UltrAWN-MS) for Unconventional Explosives Characterization, Ben Oyler WP475 Bridging the Gap between Ion Mobility Spectrometry and an Orbitrap, Mike Belov WP595 Use of Native Mass Spectrometry for Quantification of Protein Complex, Wenjing Li

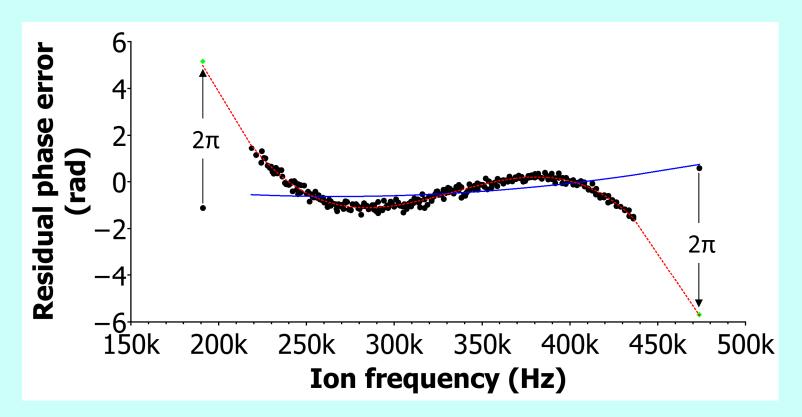




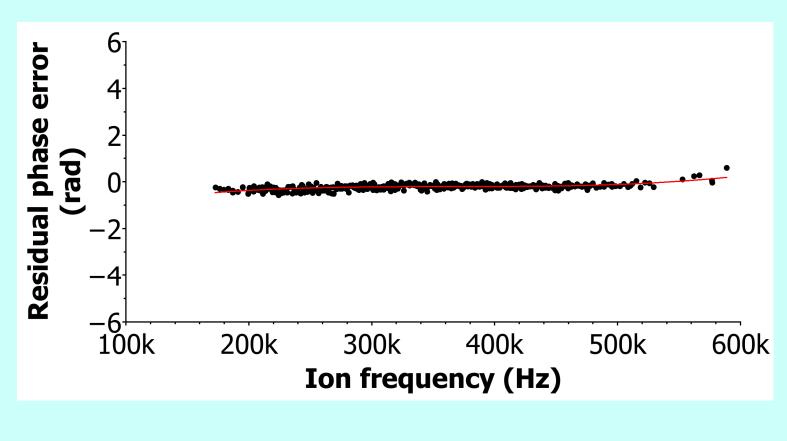
Non-quadratic phase correction functions:

Previously described methods for producing absorption mode FT-ICR MS spectra have all relied on the phase correction function being quadratic. [4,5] This assumption has been found to be incorrect for some instruments and spectra, and so it has not been possible to produce absorption mode spectra for these cases.

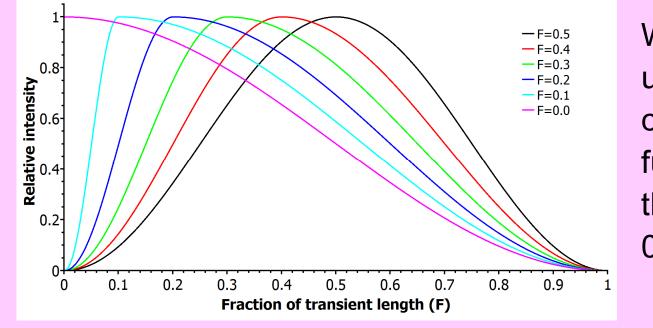
For example, we have processed data from a modified Thermo LTQ-FT-ICR MS, using ESI and a novel ICR cell design (NADEL)[6] for which a quadratic phase correction could not be optimized by the genetic algorithm in Autophaser. the figure, each point indicates the residual phase error (calculated from the absorpti mode peak asymmetry) that remains after attempting to optimize the phase correction



for a spectrum recorded on the modified LTQ-FT to a second order function. The residual phase error does not follow a quadratic trend (blue line) but can instead be fitted with a third order function (red line). This indicates that the true phase correction function for this spectrum cannot be quadratic and may in fact be third order. [3]

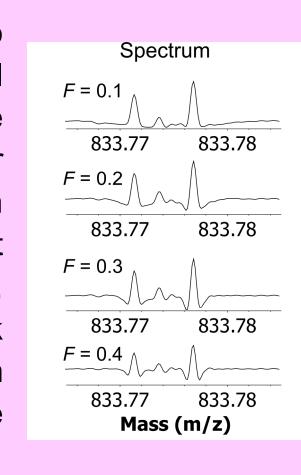


When the same spectrum is reprocessed, but where the optimization is set to use a third order function (left), the residual phase error now indicates that the spectrum has been properly "phased". The reason for this change to the phase correction function is not yet fully understood. It is likely to be related to some unknown non-linearity in the excitation signal.



When apodizing transients, prior to transformation, using the asymmetric apodization method, the user can set the position of the maximum of the apodization function to any position on the transient by adjusting the parameter F. F can be any fraction in the range 0.0 - 0.5.

As F varies, the peak shape seen in the spectrum also varies – for most spectra, the signal to noise ratio will increase as F decreases but the resolving power will be reduced. Of particular benefit is the fact that at higher values of *F*, the breadth of the negative baseline deviation around the peak is greatly reduced. The figure to the right shows an example demonstrating that the isotopic model, using the asymmetric apodization function for peak shaping, matches the changes in the mass spectrum profile as a result of changing F. The spectral data here comes from the thiostrepton spectrum shown up and left.

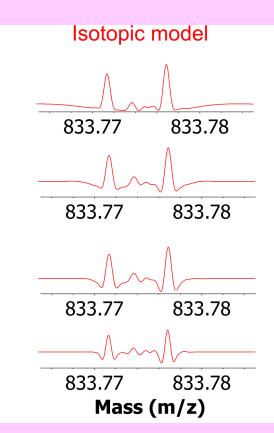


The closer an isotopic distribution spectral model matches the actual spectrum, the more confident an analyst may be in the assignment. The characteristic peak shapes seen in absorption mode, which introduce negative portions into the spectrum, are very different to those simple Gaussian or Lorentzian style peaks seen in magnitude mode mass spectra. The method we have developed produces isotopic distributions that closely match the peak profile seen in absorption mode FT-MS spectra and so make the comparison between modeled and spectral data much simpler. This approach is applicable to absorption mode spectra produced from all types of FT-MS system.

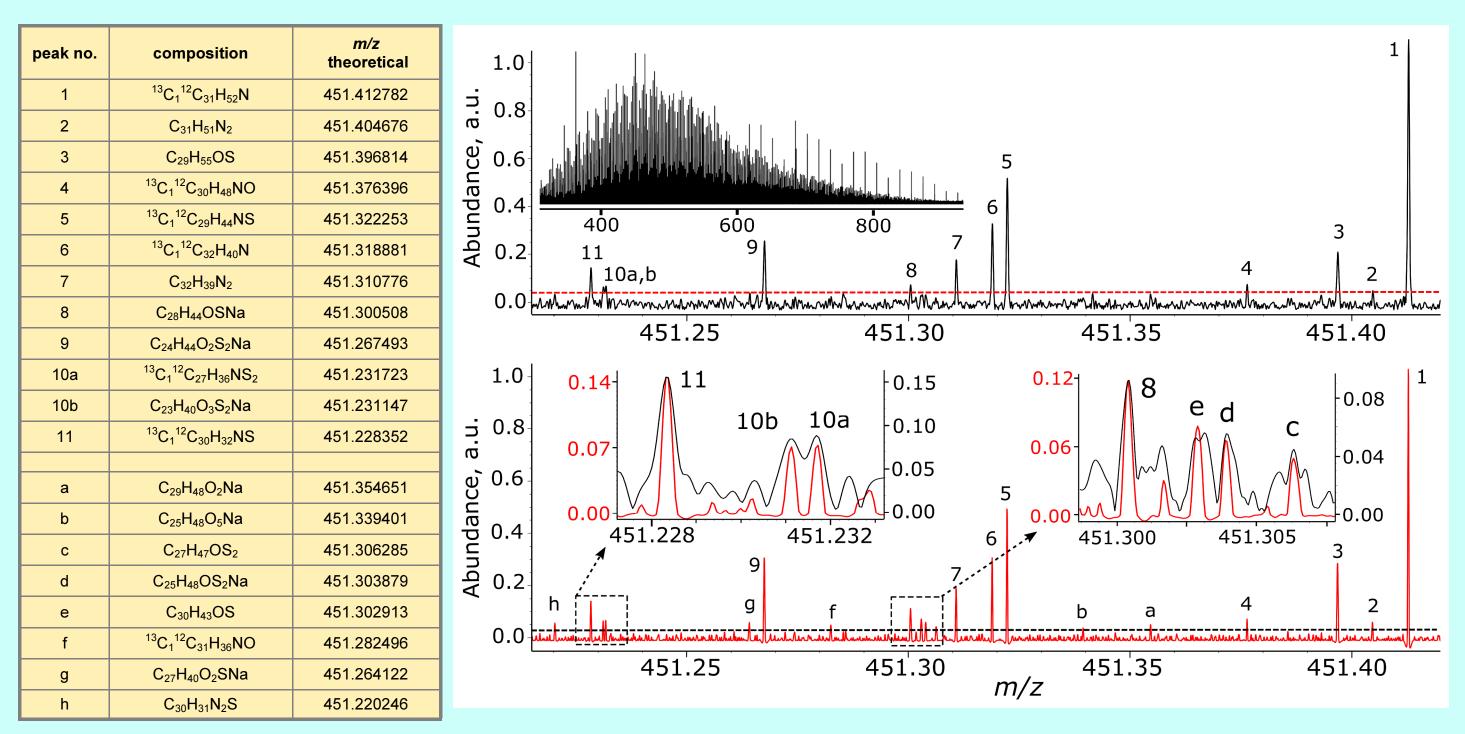




Nth order genetic algorithm:

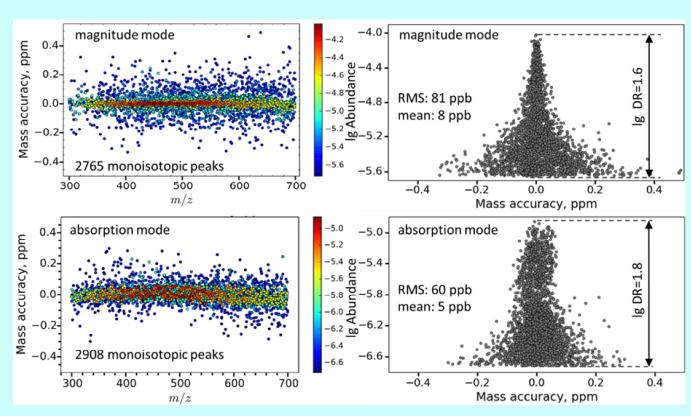


We upgraded Autophaser to allow the genetic algorithm to optimize *n*th order polynomial phase correction functions to allow these spectra to be presented in Absorption mode. [3]



For example, an ESI mass spectrum of a crude oil fraction obtained from the sum of 800 transients of 6.144 s in the "unperturbed" cyclotron frequency regime using a NADEL ICR cell on a 10 T LTQ FT-ICR MS. Magnitude mode data is shown in black and the absorption mode data is in red. Horizontal dotted lines show the noise cut-off thresholds determined following recommendations by Zhurov and co-workers.[7] Insets with expanded views into m/z 451 nominal mass are shown, to illustrate the improvement in the spectral quality. The absorption mode spectrum was apodized using an asymmetric Hann function (F = 0.2) [1] and the magnitude mode spectrum was full Hann apodized (F = 0.5).

Analysis of the peak assignments from the absorption mode spectrum generated through third order phase correction shows improvements both in the number of peaks identified and in the mass errors of those assignments.



Conclusions:

- New apodization function minimizes undesirable baseline deviation in absorption mode spectra and so avoids the difficult and time-consuming step of removing it.
- Also offers the user the opportunity to trade the spectral S/N ratio against the resolving power.
- Absorption mode specific isotopic models can result in higher confidence in peak assignments.
- Allowing non-quadratic phase correction functions can allow users to produce absorption mode spectra for systems which could not, previously have been phased.
- Future versions of Bruker FTMS control may incorporate these features for users of Bruker solariX FT-ICR MS instruments
- Spectroswiss are developing methods to allow these techniques to be applied to data collected on Orbitrap or LTQ-FT instruments.

Acknowledgments:

We would like to thank Alison Scott for the imaging data.

References:

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