

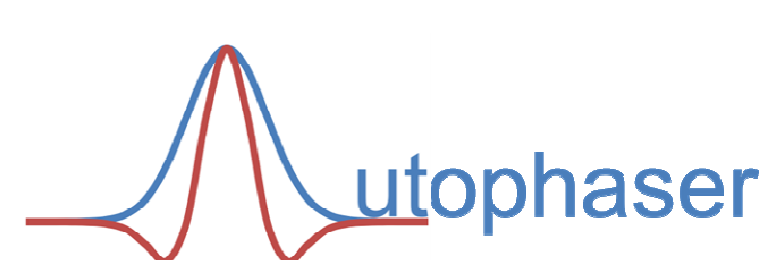
# Absorption mode takes on some new curves

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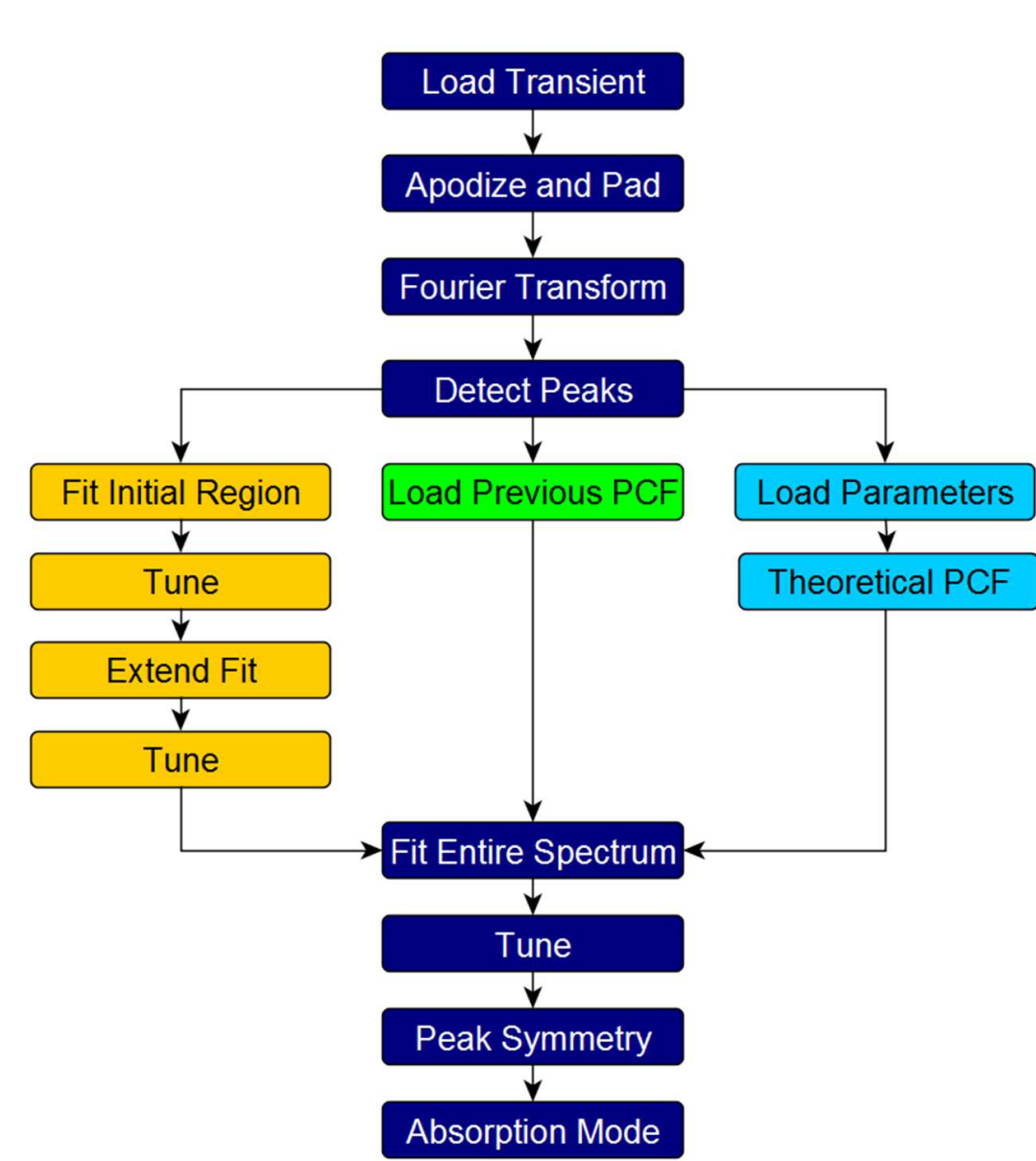
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## Introduction:

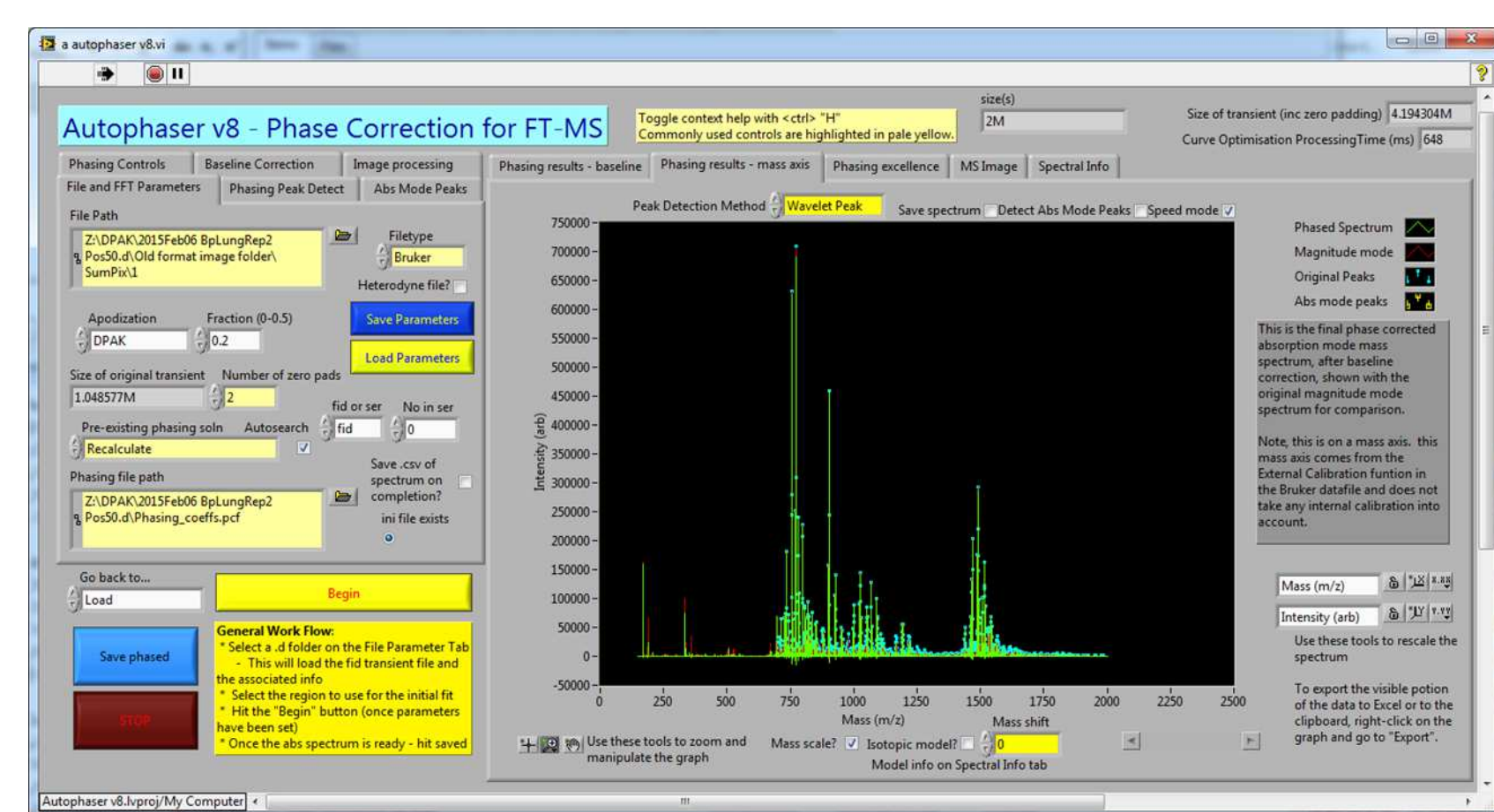
Absorption mode is a different way of viewing FT-MS data – giving improved resolution, signal-to-noise ratio and accuracy. You don't have to adapt your instrument, just process the data in a different way. Methods for producing absorption mode spectra have been around for nearly 10 years, but have a 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.



Autophaser has been developed to automate the steps required to produce absorption mode FT-MS mass spectra.



"Tune" steps use a genetic algorithm is used to optimize the phase correction function that relates ion frequency to the appropriate corrective phase angle to apply



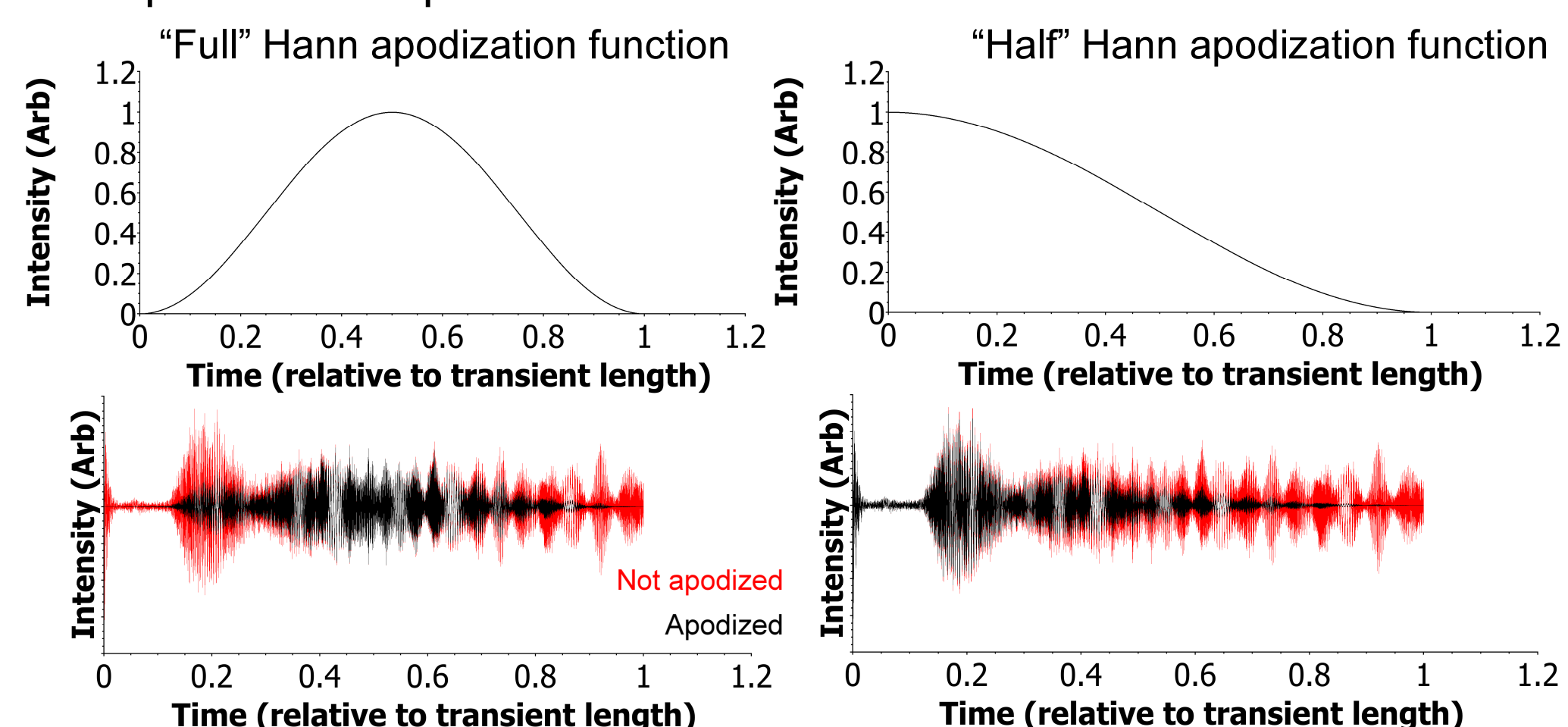
## Phase correction function:

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 ( $\varphi_x$ ) varying as a function of ion frequency ( $\omega_x$ ):

$$\varphi_x = \frac{-\omega_x^2}{R} + \omega_x \left( \frac{\omega_{Final}}{R} + \frac{-2\Delta\omega_K}{R} + t_{Delay} \right) + \varphi_0 + \frac{\Delta\omega_K \omega_{Final}}{R} + \frac{-(\Delta\omega_K)^2}{R}$$

## 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.

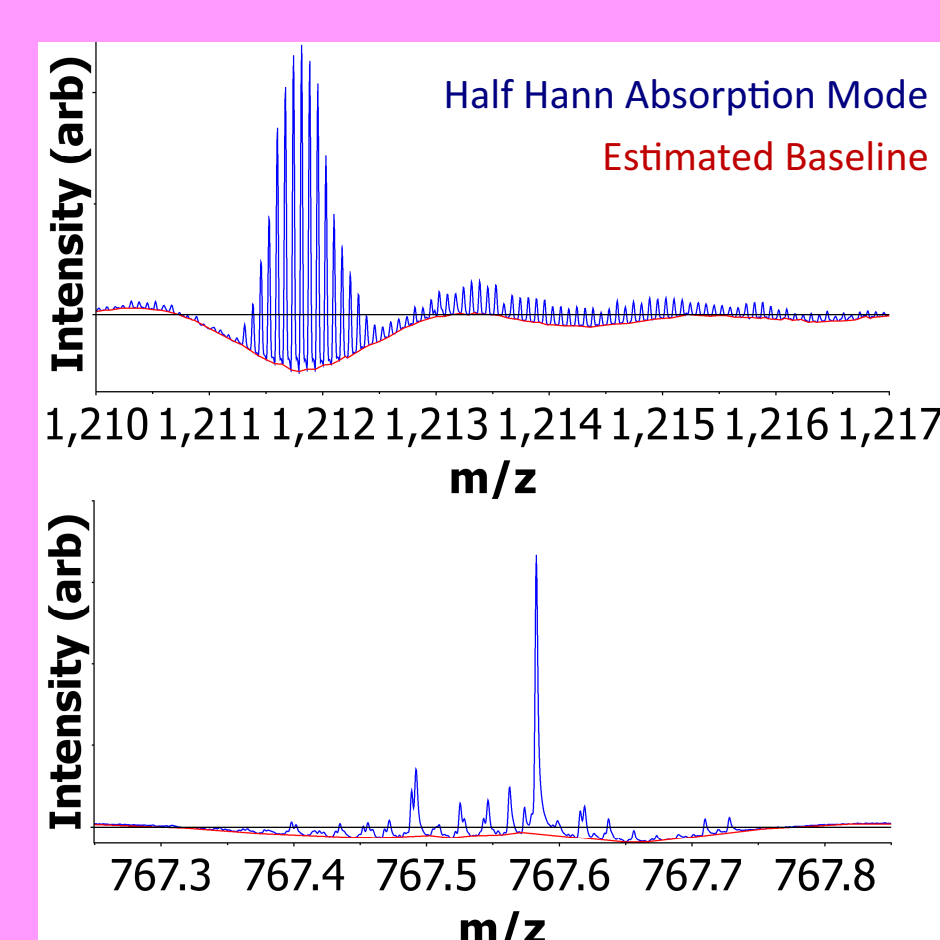


## Comparison:

"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.

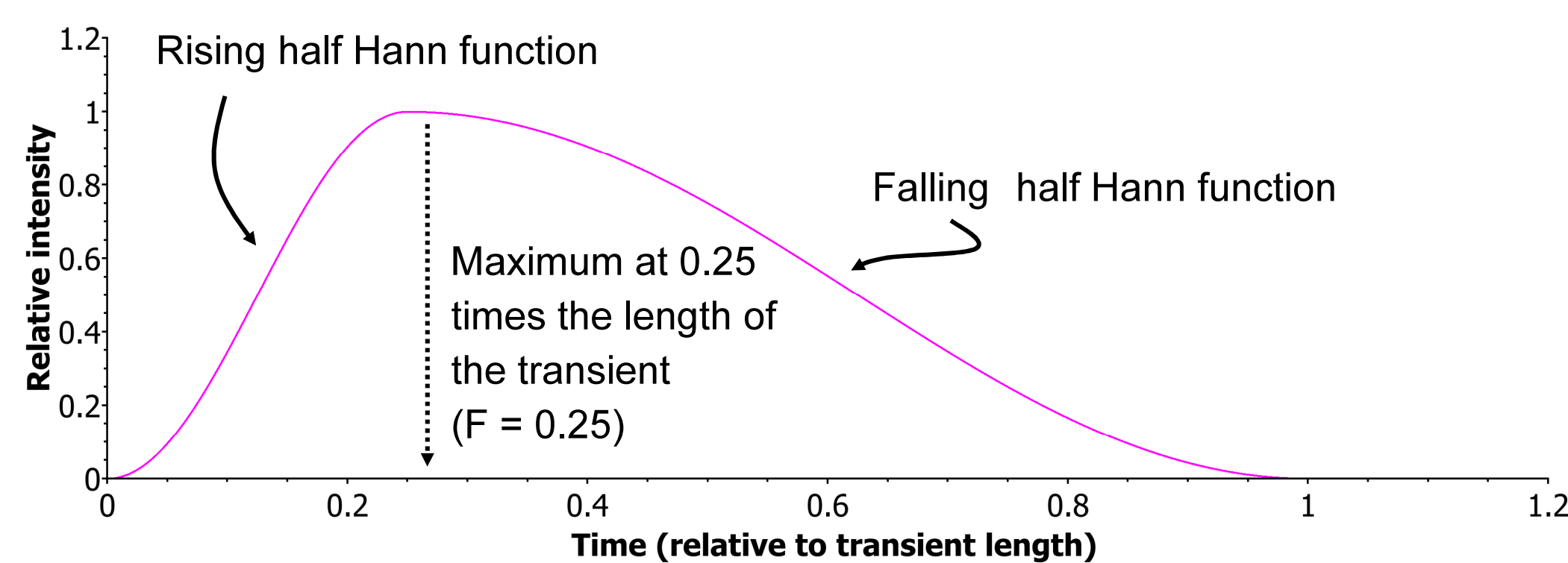
## Baseline Deviation Problem:

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.

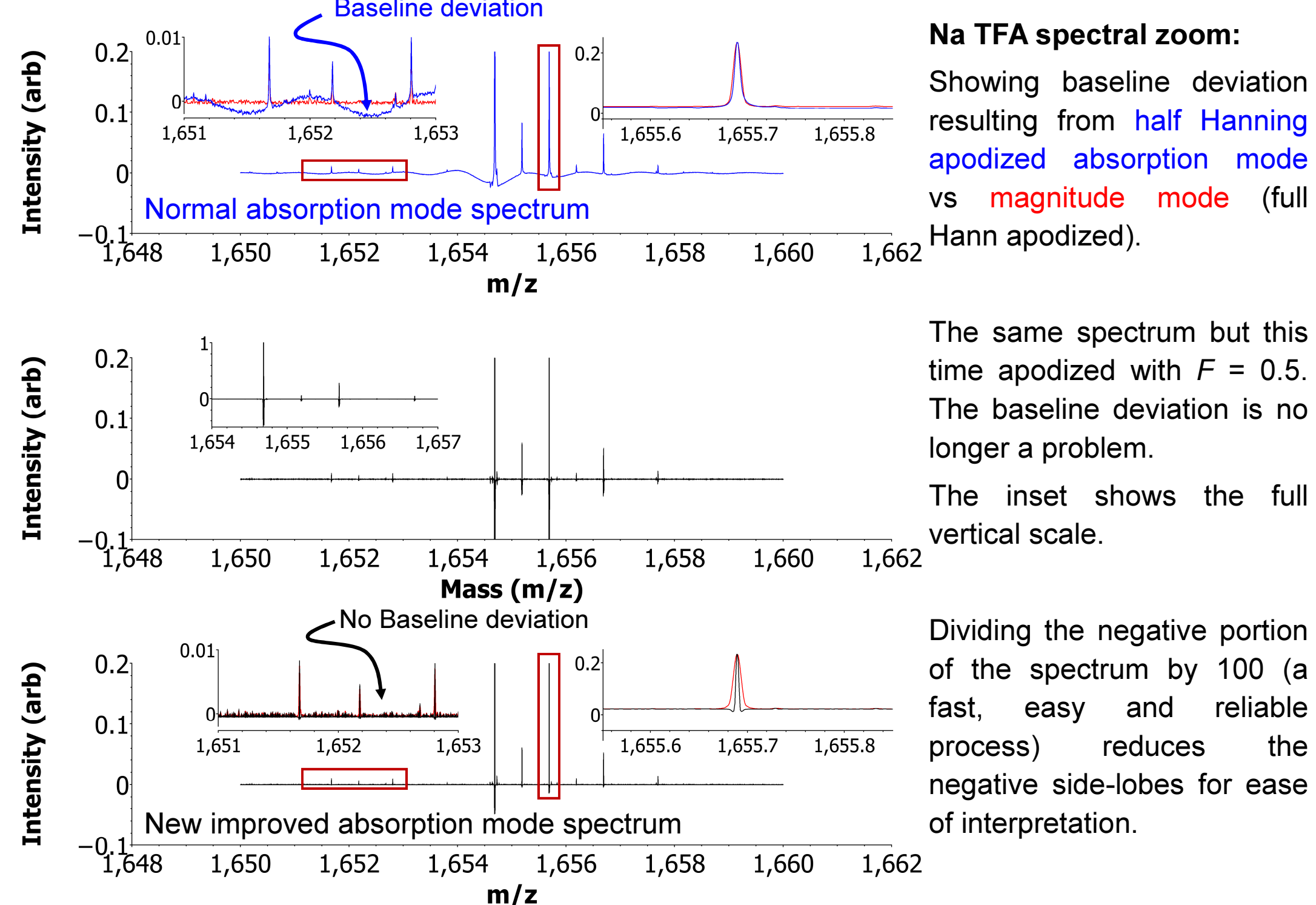


## Curing the deviation problem:

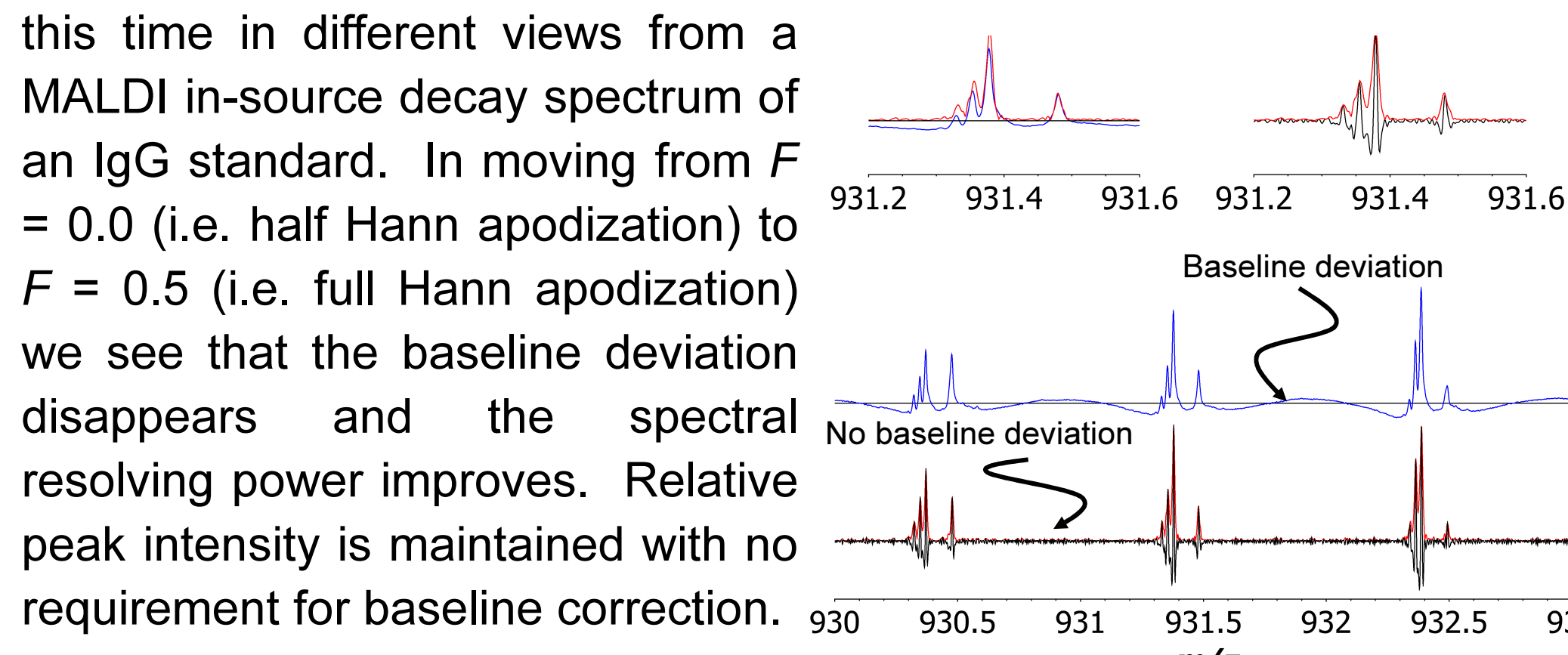
We have developed a new apodization approach to produce absorption mode FT-MS spectra which do not exhibit baseline deviation, whilst maintaining all the normal absorption mode benefits.[1] This method involves the use of a new, asymmetric "full" apodization function, replacing the more common Hann or 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.

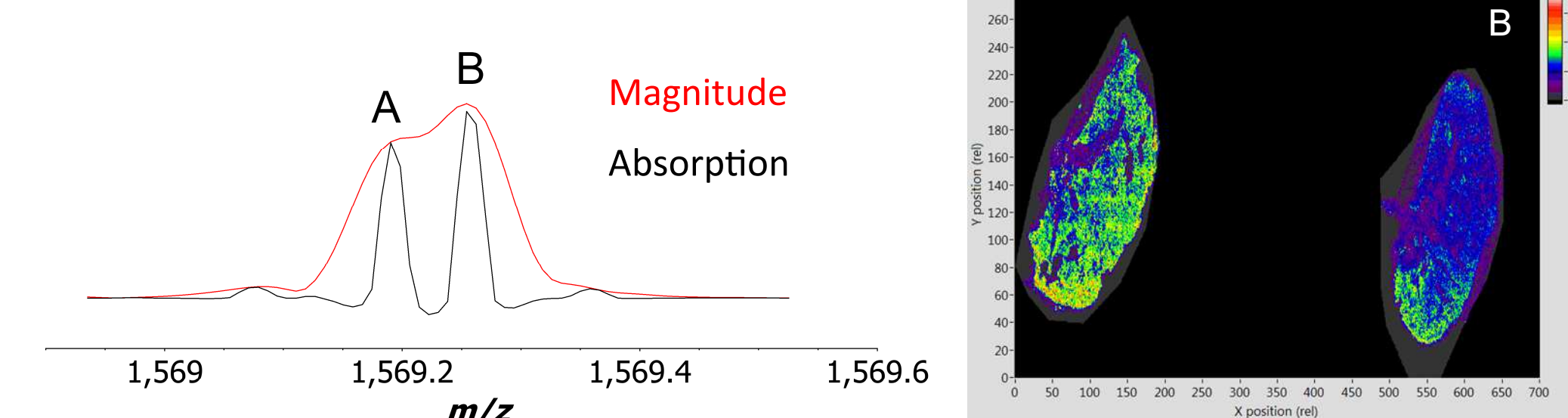


Here we see the same benefits, but this time in different views from a MALDI in-source decay spectrum of an IgG standard. In moving from  $F = 0.0$  (i.e. half Hann apodization) to  $F = 0.5$  (i.e. full Hann apodization) we see that the baseline deviation disappears and the spectral resolving power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



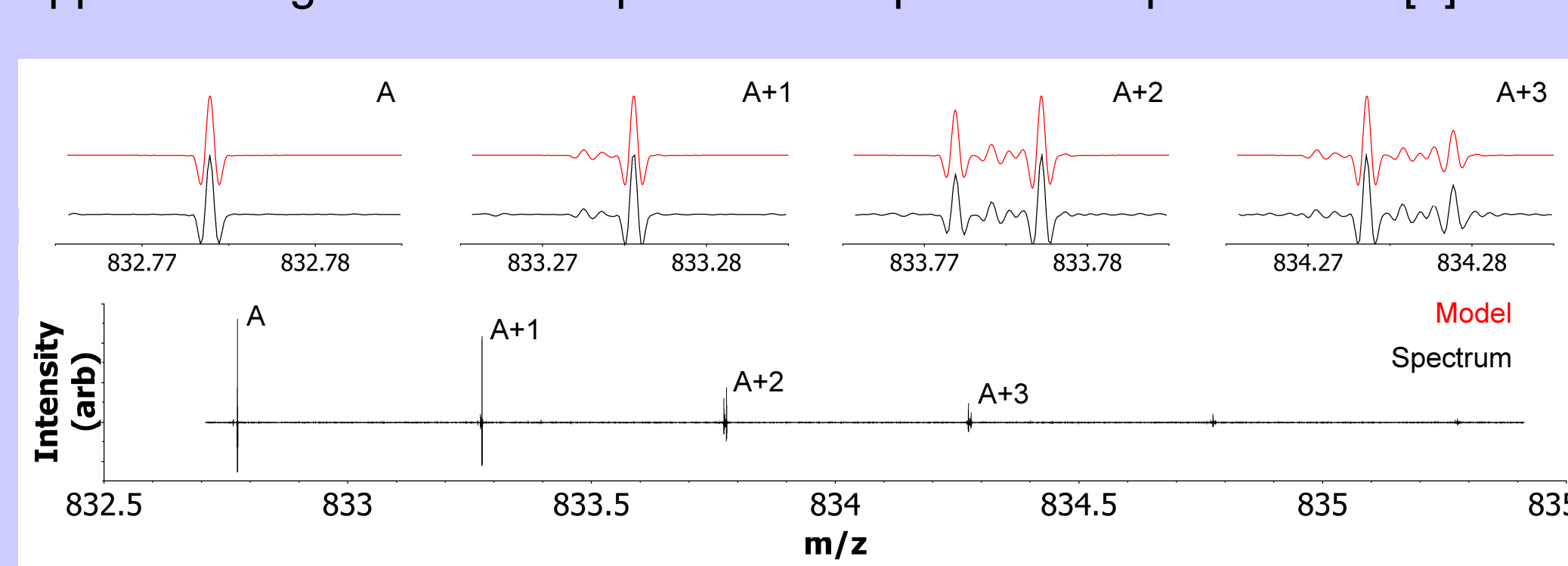
## Application to MS Imaging:

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 hours, and that is without any manual tuning of the baseline correction for individual pixels. Here we present early results showing absorption mode MS image processing now with no requirement for baseline deviation correction.



## 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]



## Non-quadratic phase correction functions:

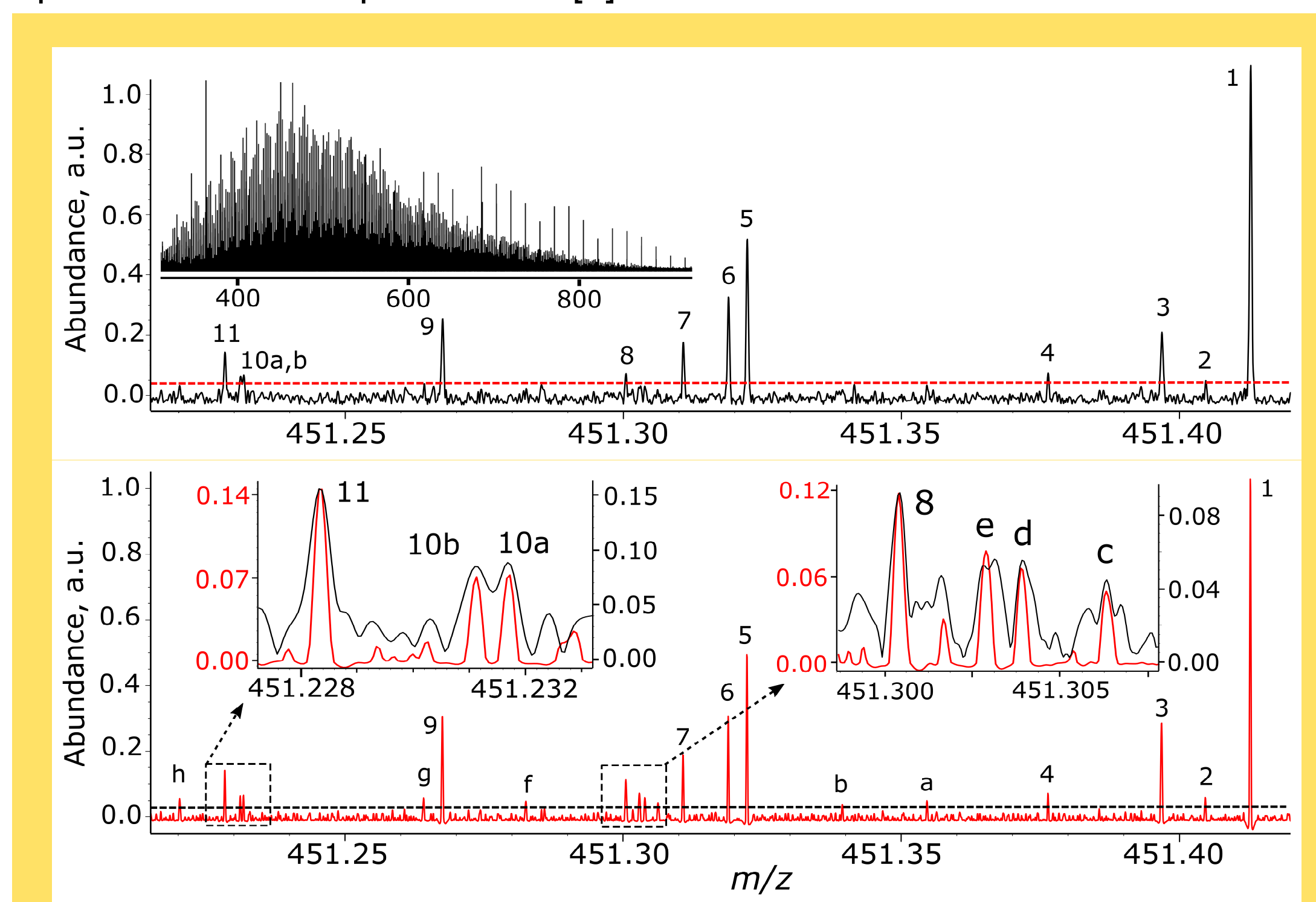
Previously described methods for producing absorption mode FT-ICR MS spectra have all relied on the phase correction function being quadratic. [4,5] This assumption has been found to be incorrect for some instruments and spectra and so it has not been possible to produce absorption mode spectra for these 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 function could not be optimized by the original genetic algorithm in Autophaser. The residual phase error (recorded from spectral peaks, in absorption mode, after optimization) suggested that the phase correction error was following a third order trend. [3]

The reasons 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.

## Mth 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 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 ( $F = 0.5$ ).

## Conclusions:

Application of a new apodization function results in a minimization of the undesirable baseline deviation in absorption mode spectra and so avoids the difficult and time-consuming step of removing it. This new apodization function also offers the user the opportunity to adapt the spectral S/N ratio against the resolving power to suit their application, in post processing. Generating isotopic models specifically adapted to the peak shapes seen in absorption mode allow users to have more confidence in peak assignments. Finally, optimizing 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.

## References:

- [1] Kilgour and Van Orden. *RCMS* in press 2015
- [2] Kilgour, Van Orden & Tran. submitted
- [3] Kilgour, Nagornov, Kozhinov, Zhurov & Tsybin. *RCMS*, in press 2015
- [4] Xian, et al. *Anal Chem* 2010, 82, 8807-8812.
- [5] Qi, et al. *JASMS* 2013, 24, 828-834.
- [6] Nagornov, Kozhinov, Tsybin & Tsybin. *JASMS* 2015, 1-11
- [7] Zhurov, Kozhinov, Fornelli & Tsybin. *Anal Chem* 2014, 86, 3308-3316

Below left is the isotopic distribution of thioestrepton, showing the isotopic fine structure, with zoomed portions to show detailed comparison of the spectrum and the isotopic model. Below right is 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.

