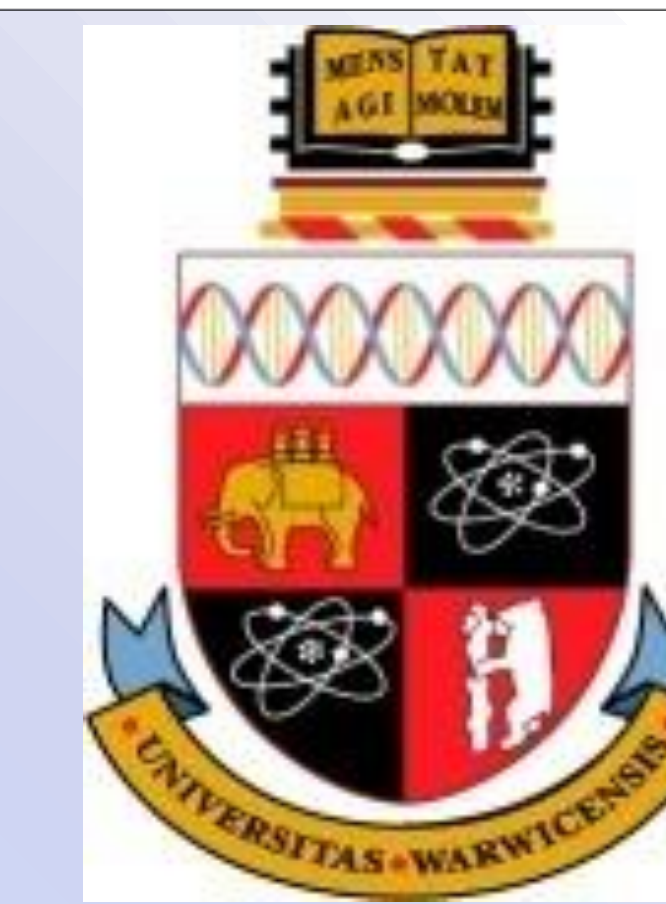


Broadband Phase Correction of FT-ICR Mass Spectra and the Parameters Controlling the Phase Function



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Overview

A discussion on the data processing to correct the phase of FTICR spectra based (Fig.1) on mathematic theory and experimental data:

- > Review the features of an absorption-mode FTICR spectrum
- > Parameterize the phase function with variation of experimental conditions
- > Determine the extent of improvement in mass resolving power and accuracy
- > Apply the phase function from scan to scan in routine phase correction

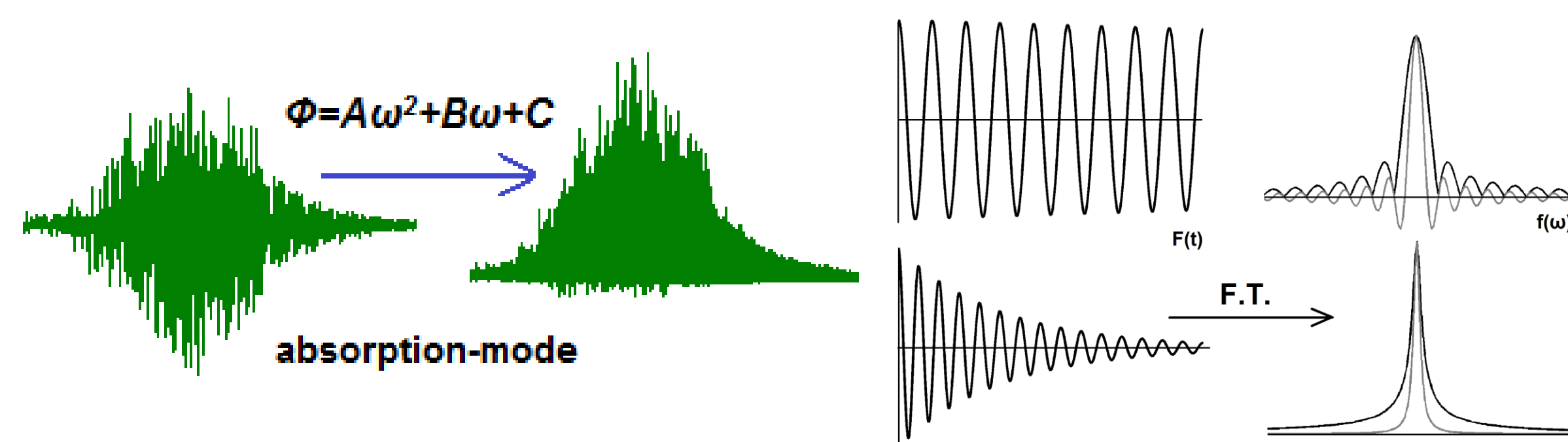


Figure 1. Left: phase correction of an absorption-mode spectrum using quadratic phase function. Right: simulated ICR signals in frequency-domain magnitude (black) and absorption-mode (gray) spectra, for $\gg t$, sinc function, for $\ll t$, lorentzian function. Note that the sinc function has negative sidelobes in the absorption-mode after Fourier transform.

Introduction

Phase correction, routinely done in FT-NMR, is still not used in FT-ICR due to the complexity of the phase function. As is known for 40 years, the resolving power for FT spectra can be enhanced by a factor up to two if the magnitude-mode spectrum is phase corrected into its corresponding absorption-mode. This topic has been recently resurrected by two publications, first by F. Xian et al.[1], using a detailed model of the excitation pulse from the experiment, and second by me[2], using a quadratic least-squares fit and iteration. The resulting work demonstrates an improvement in resolving power of about 1.7 (Fig. 2). Additionally, the 'tails' commonly observed in traditional FT spectra, which distort neighboring peaks and causing assignment errors, are greatly reduced. The advantage of this method is that the user doesn't need to know anything about the experimental pulse sequence. Here, we continue the research by investigating the factors that determine and modify the phase function in order to optimize and automatize the function from scan to scan.

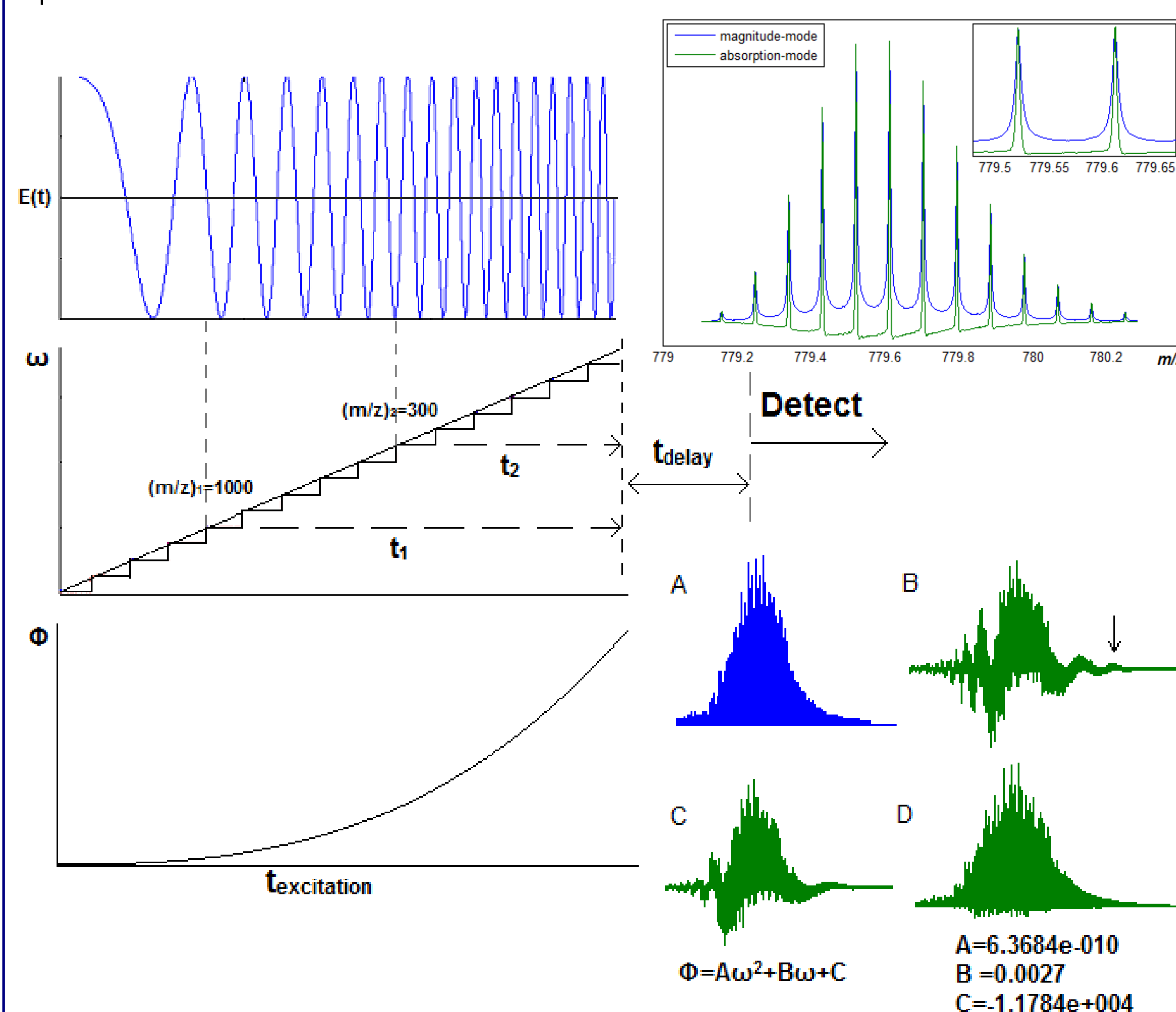


Figure 2. Left: time-domain profiles for frequency sweep excitation, frequency of the chirp and ions' quadratic phase accumulation. Right top: 11^+ charge isotope peaks of ESI ubiquitin spectrum to show magnitude-mode (green) and phase corrected absorption-mode. Right bottom: oil spectrum to show the phase correction by least square fit and iteration[2].

Theory

>Reduce Ion Cyclotron Frequency

$$\omega_+ = \frac{\omega_c}{2} [1 + (1 - 4k / \omega_c)^{1/2}], \quad k = \frac{qV\alpha}{ma^2} + \frac{q^2\rho G_i}{\epsilon_0 m}$$

trapping factor space-charge factor

>Variation of the Phase Function with Trapping Voltage

$$\omega_+ = \frac{qB_0}{m} - \frac{\alpha V}{a^2 B_0} - \frac{V^2 \alpha^2 m}{a^4 B_0^3 q}$$

$$\Phi(\omega) = A\omega^2 + B\omega + C - \frac{2\alpha A}{a^2 B_0} \omega V + \frac{\alpha^2 A}{a^4 B_0^2} V^2 - \frac{\alpha B}{a^2 B_0} V$$

>Variation of the Phase Function with Total Ion Number[4]

$$\omega_{new} = \frac{qB_0}{m} - \frac{\alpha V}{a^2 B_0} - \frac{q^2 \rho G_i}{\epsilon_0 B_0} = \omega - st, s = \frac{q^2 \rho G_i}{\epsilon_0 B_0 t}$$

$$\Phi(\omega) = A\omega^2 + (B - 2Ast)\omega + C + As^2 t^2 - Bst$$

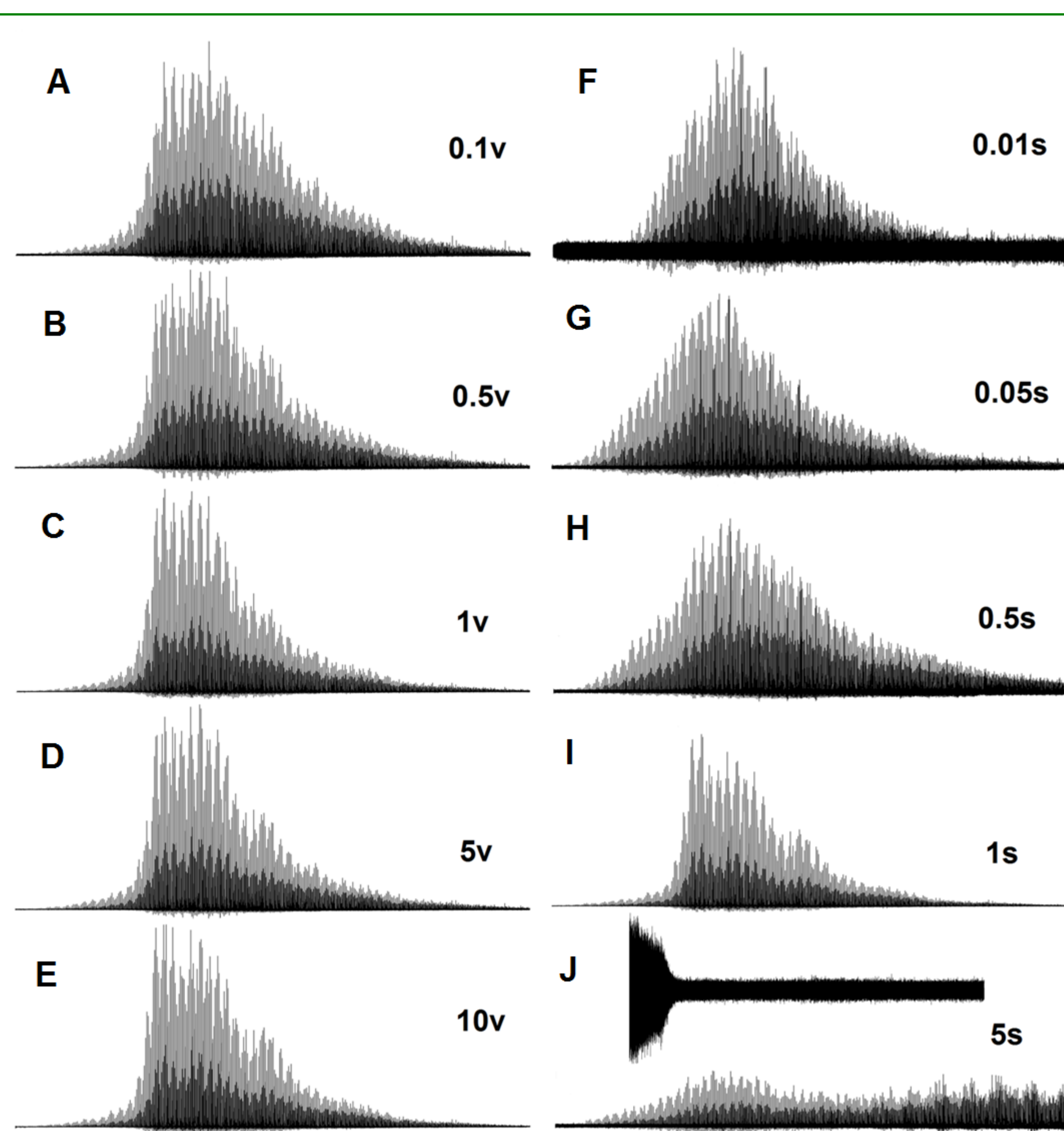


Figure 3. Left: phase corrected absorption-mode spectra with the trapping potential from 0.1-10 volt; Right: ion accumulation time form 0.01-5s (inset J with the transient, shows the spontaneous loss of coherence catastrophe of "nipple effect").

Method

The crude oil sample (SRM 2721) was purchased from NIST; ubiquitin was purchased from Sigma-Aldrich, samples were dissolved in 50:50:1 toluene: methanol: formic acid mixture. All spectra were recorded using a Solarix 12T FT-ICR mass spectrometer (Bruker, Bremen, Germany). Ions were excited using broadband frequency sweep (92-922 kHz corresponds to m/z 2000-200, at 125 Hz/ μ s sweep rate) (Fig.2), followed by a pre-scan delay of 3.5ms, and image current detection to yield 4 mega points fid time-domain data sets. The data sets were co-added (10 acquisitions each to increase the S/N), and the transient is zero-filled once, fast Fourier transformed without apodization, all data sets were then processed using MatLab. And a series of experiments were performed to test the variation of the phase function with particular experimental:

- >Trapping voltage in the ICR cell from 0.1 to 10 volt (gated trapping)
- >Ion accumulation time in the accumulation cell (hexapole) from 0.01 to 10s
- >Ion excitation power from 10 to 100 volts

Results and Discussion

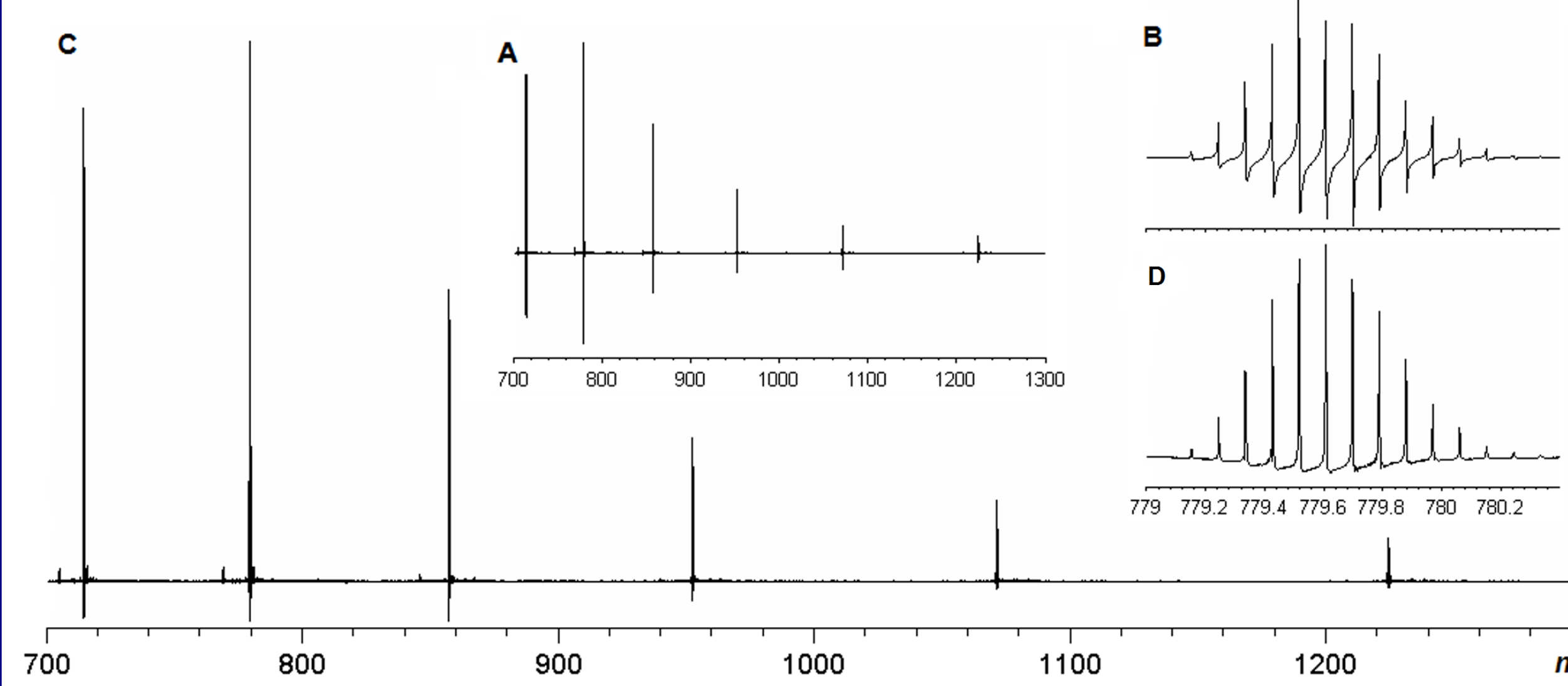


Figure 4. A: Ubiquitin charge state spectra in absorption-mode corrected by the phase function from crude oil spectrum (Fig. 3) using same instrument parameters; B: zoom in of the 11^+ charge isotope peaks in A; C: correct the phase value of the start and end points, the entire spectrum is then perfectly phased; D: zoom in of the 11^+ charge isotope peaks in C.

>Parameterize the phase function

The same instrument parameters of the oil spectrum were then applied to acquire an ubiquitin charge state spectrum, which clearly shows that computation of the phase function must be done only when the pulse sequence is changed – so that in the vast majority of cases, it only has to be calculated once whenever instrumental drift requires recalibration of the instrument. Subsequent experiments were performed to determine the phase function, and the variance of the phase function was applied to correct the phase of spectra using the predicted functions mentioned above.

1.Trapping Potential

Under the normal voltage ($<1v$), the phase shift is totally predictable, and as the magnetron expansion causes frequency shift is increased dramatically by the trapping potential, normally detection is performed with a trapping potential below 1v.

2.Number of Ions

As is pointed by Easterling et al.[3], the space-charge effect is linear with the ion population, moreover, the phase shift via ion accumulation time is independent of m/z . The modified phase function remains stable for the entire time scale of accumulation time, even in the extreme space-charge condition which already shows the spontaneous loss of coherence[4] in the transient (Fig.3). However, in such extreme case, the peak shift and artifacts caused from the space-charge effect are much severe than the phase correction itself.

3.Excitation Radius

The shift of ion's frequency was recorded which roughly follows a quadratic relation with the ion excitation potential; However, the data herein was generated from the infinity cell, in which the trapping field is not ideal in any way. Thus, frequency does change slightly with excitation Radius, and the resulting quadratic function varies for different ions, thus not sufficiently accurate to reach a reliable prediction for ions' frequency to be used for a proper evaluation of the phase function.

>Baseline and Phasing Artifact

In a real experiment, the peak shape of a spectrum is always a convolution of both a 'sinc' and a 'lorentzian' function(Fig.5); thus the negative wiggles are the nature of an absorption spectrum which cannot be eliminated except by apodization.

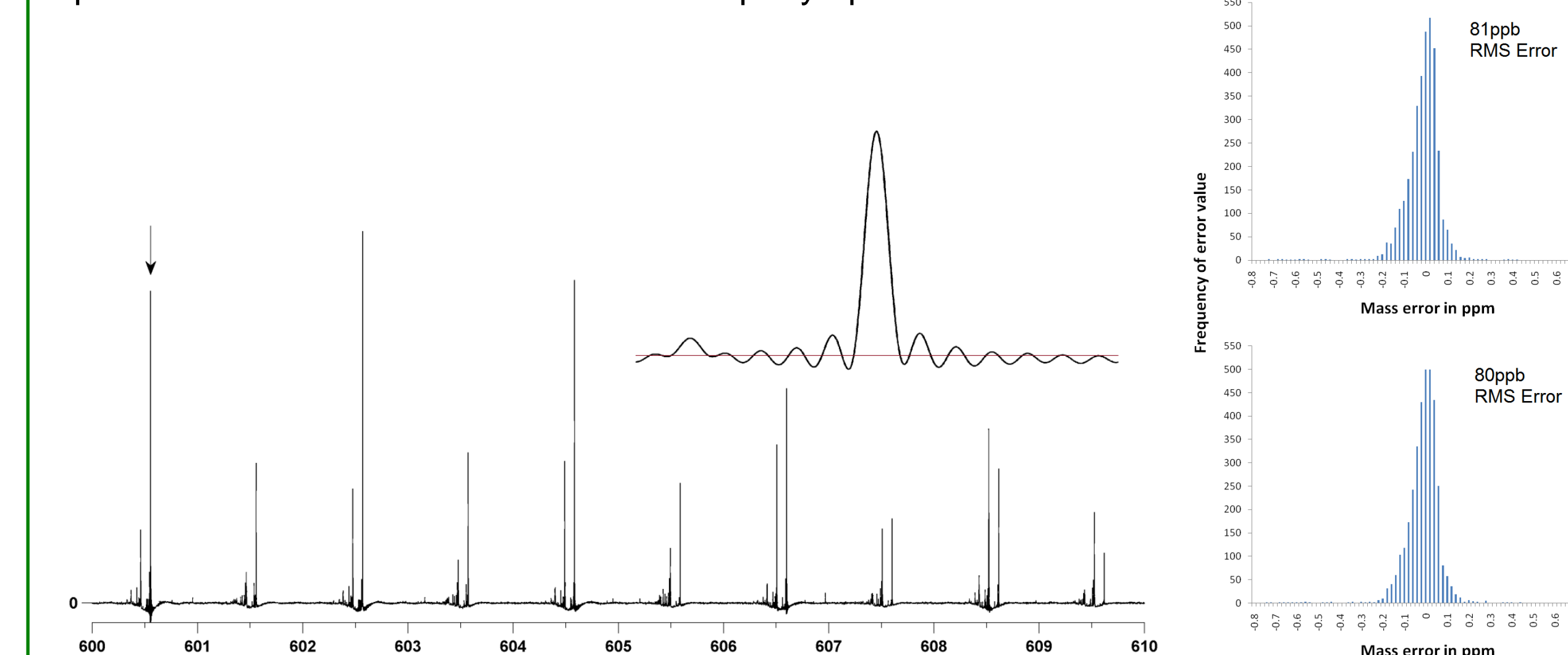


Figure 5. Left: a small segment from the absorption-mode crude oil spectrum, inset: zoom in for the labeled peak to show the negative blips of sinc function; Right: histogram showing the mass error of the crude oil with both magnitude-mode (top) and absorption-mode (bottom).

>Mass Resolving Power and Accuracy

The peak width at FWHM is narrower than its corresponding magnitude-mode by a factor depending on the damping of the transient, ranging from 1.7 ('lorentzian' peak shape) to 2 ('sinc' peak shape) (Fig. 1&5). Which means the mass resolving power, $m/\Delta m$ can be enhanced by the same factor.

~3500 peaks were assigned in the oil spectrum for both magnitude and absorption-mode display with a S/N over 8(Fig. 5,right). It is interesting to notice the absorption-mode doesn't improve the mass accuracy for these peaks. One possible reason for this surprising lack of improvement is that, the intensity of a perfectly phased peak is identical to its corresponding magnitude-mode (Fig. 1, right), therefore, the position of peaks picked by centroiding is almost same if the peak is intense and not distorted by neighboring peaks. However, noting the absorption-mode has much narrower baseline compared with magnitude-mode; this feature should more or less change the centroiding when peaks are sufficiently close to overlap in the baselines, and such improvement is considered to be systematic, depending on the degree of overlapping, and the corresponding research is still on the way.

Conclusion

This project shows that the phase function can be applied directly from scan to scan and from sample to sample in cases where 1) the pulse sequence doesn't change or 2) the pulse sequence change is limited to modest variation to trapping voltage or ion accumulation time. Thus, in the vast majority of cases, the phase function need to be calculated only once whenever the instrument calibration changes, and it can then be applied directly to all spectra with little effort to yield ~100% resolution increase at no cost. This broadband phase correction can be automated in routine work as is already done in NMR.

Reference

- [1] Xian, F.; Hendrickson, C. L.; Blakney, G. T.; Beu, S. C.; Marshall, A. G. *Anal. Chem.* **2010**, *82*, 8807.
- [2] Qi, Y.; Thompson, C.; Van Orden, S.; O'Connor, P. *Journal of The American Society for Mass Spectrometry* **2011**, *22*, 138.
- [3] Easterling, M. L.; Mize, T. H.; Amster, I. J. *Anal. Chem.* **1999**, *71*, 624.
- [4] Aizikov, K.; Mathur, R.; O'Connor, P. B. *Journal of The American Society for Mass Spectrometry* **2009**, *20*, 247.

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