ANALYSIS OF PHASE DEPENDENT FREQUENCY SHIFTS IN SIMULATED FTMS TRANSIENTS USING THE FILTER DIAGONALIZATION METHOD

RUNNING TITLE:

FDM ANALYSIS OF SIMULATED FTMS TRANSIENTS

(FOR SUBMISSION TO JASMS)

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ABSTRACT

Space-charge perturbs ion motion and affects mass accuracy in ion trapping mass spectrometers. In Fourier transform mass spectrometry (FTMS), both ion-ion and ion-image charge interactions have been examined by experiments and by multiparticle ion simulations using the particle-in-cell (PIC) approach, and the magnitude of observed frequency shifts as a function of ion number agrees with theoretical models. Frequency shifts due to ion-ion interactions have generally been treated in a time-integrated fashion, that is, for the duration of the transient signal. Aizikov and O'Connor have shown that there is a time-dependence for such interactions, with a periodicity that correlates to the beat period between isotope peaks. Here, we investigate such interactions using PIC simulations and the filter diagonalization method (FDM) for obtaining frequencies from very short duration s of the transient. Periodic decreases in observed frequency correlate with ion clouds of isotope peaks coming into phase in their cyclotron orbit. A similar phenomenon is observed in the simulations of ion motion in an Orbitrap mass analyzer, corresponding to the axial motion of isotope groupings moving in and out of phase.

INTRODUCTION

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) [1] provides the highest mass accuracy and highest mass resolution of any currently available mass spectrometer. The cyclotron motion of an ion arises due to a radially constraining magnetic field and the frequency of this motion can be defined as:

$$\omega_c = qB/m \qquad \text{(Equation 1)}$$

where B is the magnetic field, q is the ion's charge, and m is the ion's mass. Values of cyclotron frequency ($\omega_c/2\pi$) range from tens of kHz to MHz for most ions. The study of deviations from the expected cyclotron frequency has been of continued interest in FT-ICR MS [2-9] as the desire to reduce mass errors to the sub-ppm range has increased. In practice, the magnitude of the observed cyclotron frequency is slightly reduced from the value predicted by Equation 1 due to the radially-repulsive trapping electric field, by the repulsive electric field that exists between ions of like charge, also known as space-charge [10, 11], and by ion-image charge interactions [5, 6, 12-14]. The space-charge induced frequency shift is generally approximated by the following expression:

$$\Delta\omega_{sc} = q\rho G_i/\varepsilon_o B \qquad \text{(Equation 2)}$$

where q is the elementary unit of charge, ρ is the charge density, G_i is a geometrical description of the charge distribution, ε_o is the free permittivity of space, and B is the applied magnetic field, and $\Delta\omega_{sc}$ is the frequency reduction experienced by all ions, independent of their m/z value. Examination of these terms reveals that the effect is directly proportional to charge and ion density and inversely proportional to applied magnetic field. Space charge frequency shifts can be reduced experimentally by control

of ion number and excitation conditions [15, 16], which reduce ion density, and post-experiment by mass calibration [11, 17-22].

While space-charge frequency reduction is generally treated in a time-integrated fashion, it has also been examined in a time-dependent fashion. As ion packets move into and out of phase with each other, their mutual repulsion increases and decreases. The fast nature of such interactions requires a harmonic inversion technique that can operate on short time domain signals, as demonstrated in the work of Aizikov and O'Connor [8, 9], wherein the filter diagonalization method (FDM) [23] was utilized to dissect FT-ICR transients to reveal modulations in the orbital frequencies of ions during an experimental acquisition. The fast Fourier transform (FFT) [24] has limited value when applied to truncated time domain signals due to limited spectral resolution. Alternative inversion techniques such as the short-time Fourier transform [25] or linear prediction [26] can also be utilized but have their own limitations. FDM provides an attractive method due to high spectral resolution and moderate computational demands.

To examine the nature of dynamic phase-dependent space charge events during FTMS experiments in a controlled manner, particle-in-cell (PIC) ion trajectory calculations have been employed [27-29]. PIC calculations have been shown to accurately model experimental results in FT-ICR [14] and currently serve as a valuable tool to gain insight into fundamental aspects of ion behavior. A model has been developed to systematically examine the time-dependent space charge induced frequency shifts due to isotopes of a molecular ion. Extension of this model to selected peptides of a tryptic digest approach conditions of a typical MS experiment. Space charge effects are

present in any ion-trapping instrument and extension of FDM analysis to simulated

orbitrap time domain signals are also presented.

EXPERIMENTAL METHODS

Multi-particle ion trajectory simulations were conducted on Linux clusters located

at the Foundation for Fundamental Research on Matter- Institute for Atomic and

Molecular Physics (FOM-AMOLF) using a serial version of the PIC code and parameters

summarized in Table 1. Ion populations were constrained radially by a magnetic field of

7.0 T and trapped axially by 1 V. A cubic trapping potential and an idealized

quadrupolar trapping potential of a Penning trap were employed using particle-in-cell

methods to enable coulombic interactions on a finite grid 32 x 32 x 32 in dimension. The

analyzer geometry in all simulations was 5.08 cm x 5.08 cm x 5.08 cm, corresponding to

a two-inch cubic cell. Analyzer cells of arbitrary geometry will be implemented for

further studies. Prior to excitation, the ion cloud is generated as an ellipsoid (major axis

0.2 cm, minor axis 0.05 cm), with the major axis parallel to the magnetic field and a

uniform distribution of particles. The initial particle velocity distribution is Maxwellian

at 300 K. For each particle, the direction of the velocity vector is randomized. Prior to

image current detection, a chirp excitation (90 Vp-p) was utilized to produce an orbital

radius of approximately 35 %. The orbitrap geometry employed for PIC simulations was

mathematically derived from the analytical expression of the potential with relevant

element radii listed in Table C.1. Ions were initially accelerating by 1300 V towards the

central spindle, which was held at 3500 V.

For each computational experiment, a simulated time domain transient was generated and a frequency domain spectrum was derived using the fast Fourier transform (FFT) incorporated in FOM-AMOLF's AWE software [30]. The filter diagonalization method (FDM) was also imported in AWE. Frequency chasing experiments were performed using a segment of 15k-25k data points and incremented 10k data points to provide overlap and reduce boundary effects. Visualization of the simulated ion cloud was accomplished with in-house software (Particle Vis) developed at FOM-AMOLF.

RESULTS AND DISCUSSION

In the simple case of single ion detection in FT-ICR MS, the transient detection signal is a sinusoid generated as the particle passes near the surface of the detection electrodes in an alternating fashion. In the case of ions of two distinct m/z values, two sinusoids, with frequencies inversely proportional to each ion's m/z value are produced, with a periodically varying phase shift between the sinusoids due to the difference in cyclotron frequencies. As the phase approaches 0°, the ions are located on the same side of the analyzer cell and a maximum amplitude is observed in the time domain signal. When the phase approaches 180°, the two ions are located on opposite sides of the analyzer cell, and a node occurs due to deconstructive interference in the image current signal. This well-known behavior is the basis of isotopic beats in FTMS [31]. This illustrative example is difficult to generate experimentally due to the occurrence of a variety of isotopes, yet straightforward to generate via computer simulation.

To establish a model for the study of space charge interactions, simulations were conducted based on the isotopes of substance P. This ion was selected as a model based

on prior work by Aizikov and O'Connor, but could have been arbitrarily selected.   The monoisotopic ion is denoted as 'A' with additional peaks differing by one heavy isotope as 'A+1' and 'A+2'. At an applied magnetic field of 7.0 T, the cyclotron frequency difference between 'A' and 'A+1' or 'A+1' and 'A+2' are both approximately 60 Hz (16.7 ms) with the difference between 'A' and 'A+2' as 120 Hz (8.3 ms).

Simulation of 'A' only

PIC simulation of only the A or monoisotopic ion results in the time domain signal shown in Figure 1. A sinusoid of constant amplitude is generated, but is not entirely apparent due to the short period of oscillation. Due to the lack of additional isotopes, a beat pattern is not observed. FDM analysis of the signal results in a frequency that is stable to within 10 ppb. In the absence of space-charge interactions, an ion's cyclotron frequency is extremely stable and systematic examination of the addition of these interactions could provide more precise methods for accounting for induced perturbations to the cyclotron mode. The presented data was simulated with a perfectly quadrupolar trapping potential and produced variation of only 60 μHz. When compared to a simulation employing the trapping potential of a cubic cell, frequency variation ranged approximately 30 mHz, nearly three orders of magnitude larger. This result further validates that the incorporation of a more ideal trapping potential, through compensation or novel cell design, reduces the variation in measured frequency and results in a more accurate mass measurement.

Simulation of 'A' and 'A+1'

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Introduction of an ion corresponding to the 'A+1' peak differing only by the mass of one ^{13}C incorporation is shown to introduce the anticipated isotopic beat in Figure 2. The calculated cyclotron frequency difference between these two ions is approximately 60 Hz and corresponds to the beat period of 17.3 ms in the time domain. As the ions come into phase with one another, an amplitude maximum is observed in the transient, whereas a node is observed when ions are on opposite sides of the analyzer due to deconstructive interference. Considering the location of each ion, the maximum space charge interaction is anticipated to occur during a beat as the charge density reaches a maximum.

FDM analysis of the simulated transient produces two frequencies, also shown in Figure 2, the higher frequency due to the A peak and lower frequency due to the A+1. Each frequency displays variability of several Hz, but a periodic reduction of approximately 15 Hz is shown for the A peak and 30 Hz for the A+1. When the frequency analysis and time domain signal are aligned, the maximum frequency shift is shown to occur at the beat maximum.

Simulation of 'A' and 'A+2'

Elimination of the 'A+1' ion and incorporation of an ion two amu higher than the monoisotopic ion generates the time domain signal shown in Figure 3. Due to the difference in cyclotron frequency doubling, the isotopic beat now occurs twice as often in the time domain. As depicted in the corresponding frequency chase, the periodic frequency shift now occurs twice as often when compared to the previous data for the A and A+1 peaks and remains aligned with the transient maximum. Frequency reductions

of 15 Hz and 20 Hz are observed, respectively, for the A and A+2 peaks. Examination of further combinatorial possibilities due to isotopes is possible, but not necessary, as the method has shown validity when compared to theory and prior experimental result.

Simulation of a Protein Tryptic Digest

Simulations of the isotopes for a single charge state provide method validation and fundamental insight, but are of limited value. To extend this analysis to typical experimental conditions, simulations were conducted based on a more complex mixture of ions. The ion assemblage was based on the components of a MALDI FT-ICR MS spectrum acquired from a tryptic digest of BSA. Nine peptides and their corresponding isotopes were selected as an example (experimental acquisition and simulated peptide m/z values are included as supplementary material).

In Figure 4, the FDM analysis of the frequency corresponding to the monoisotopic ion (1567 m/z) for the most intense peptide is shown. A periodic reduction in frequency is shown to occur on an interval of 23 ms. The period corresponds to the frequency difference between the monoisotopic ion and its 'A+1' ion, which is approximately 44 Hz. This result is consistent with simplified simulation results for substance P discussed earlier as well as what is known experimentally in the literature. For comparison, the FFT of the time domain signal is shown. The FFT result is the apparent time average of the instantaneous variations in frequency during the experiment. A detailed examination of frequency shifts for other components of complex mixtures will be the subject of a future manuscript.

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Simulation of Ion Motion in an Orbitrap

Space charge effects have not been examined in depth for orbital FTMS but recent work has provided a mass calibration to account for these interactions [32]. The occurrence of isotope beating is not limited to FT-ICR, and is known to occur in orbital FTMS instruments such as the Orbitrap [33]. The question arises as to whether similar frequency shifts are present during an Orbitrap experiment. Unfortunately, public access is not provided to directly analyze real world time domain signals from the instrument, but we have been able to conduct ion trajectory calculations to simulate such an experiment.

Although isotope beating occurs in both types of FTMS instruments, the nature of the effect differs for the Orbitrap. After injection into the analyzer, ion clouds form discs which oscillate axially along the central electrode. As the analyzer is segmented to allow for image current detection, maximum amplitude of the time domain signal occurs when ions are on the same side of the analyzer whereas a node occurs when ions are on opposite sides. This behavior is displayed in Figure 5, where the sinusoids generated by monitoring the position of the two ions is displayed as a sum. Calculation of the beat period based on theory [33] is approximately 7 ms for isotopes differing by 2 amu and is consistent with our simulation. Corresponding FDM analysis of the image current signal reveals a periodic reduction in axial frequency of ~ 30 Hz for the A ion and ~ 40 Hz for the A+2 ion and corresponds to the beat, for 30k particles evenly divided between the two isotopes. The case of isotopes differing by only 1 amu has also been examined and is consistent with these results, differing only in the beat period.

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CONCLUSIONS

Space charge induced frequency shifts in FTMS instruments arise due to ion confinement required for image current detection. This effect can be localized or global in nature, but is greatest in the examination of a molecular ion and its isotopes due to closely spaced frequencies and therefore short interaction distances. Manipulation of ion population size and calibration can account for space charge, but the effects on an ion's frequency are difficult to completely characterize analytically due to the large number of interactions in a typical experiment. PIC simulation of a molecular ion's isotopes and a simple mixture of ions from a tryptic digest have provided simple models to examine these frequency perturbations through harmonic inversion of the time domain signal by filter diagonalization. Although FDM allows for examination of these fast interactions, the FFT is still a robust harmonic inversion method, which effectively averages the phase dependent frequency shifts due to space charge.

ACKNOWLEDGEMENTS

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TABLE CAPTIONS

1) Parameters utilized for PIC simulations.

TABLES

Table 1.

<u>FT-ICR Simulation</u>

Trapping Potential	1.0 V
Magnetic Field	7.0 T
Analyzer geometry	Cubic
Ion Cloud	Ellipsoid
Semi-major axis	0.2 cm
Semi-minor axis	0.05 cm
Trap Dimension	5.08 cm
PIC Grid	32x32x32
Simulated Particles	30,000 – 52,000
Ion m/z	

Excitation

Voltage	90 (Vp-p)
Excitation Steps	16383
Duration	90 μs
Time Step	0.005 μs / step

Detection

Detection Steps	1045876
Duration	97.5 ms
Time step	0.093 μs /step

<u>Orbitrap</u>

PIC Grid	32x32x64
Initial Ion Acceleration	1300 V
Characteristic Radius	22 mm
Shell Electrode Radius	15 mm
Spindle Electrode Radius	6 mm
Spindle Potential	3500 V
Detection Time Step	0.131 μs / step
Detection Duration	26.2 msec

FIGURE CAPTIONS

Figure 1. Simulated FT-ICR time domain signal for the monoisotopic ion (A peak) of substance P and corresponding FDM frequency analysis. No beat pattern or frequency shifts are observed due to the lack of adjacent ions due to isotopes.

Figure 2. Simulated FT-ICR time domain signal for the monoisotopic ion (A) and A+1 of substance P and corresponding FDM frequency analysis. A beat pattern with period of 16.7 ms is observed due to ~ 59 Hz difference between cyclotron frequencies.

Figure 3. Simulated FT-ICR time domain signal for the monoisotopic ion (A) and A+2 of substance P and corresponding FDM frequency analysis. A beat pattern with period of 8.3 ms is observed due to ~ 120 Hz difference between cyclotron frequencies.

Figure 4. FDM analysis of the frequency shifts in the simulated cyclotron frequency for most intense monoisotopic ion in a peptide mixture. For comparison, the FT frequency is shown.

Figure 5. Simulated orbitrap time domain signal generated by summing the Z-axial positions of the A and A+2 ions of substance P and corresponding FDM derived frequencies.
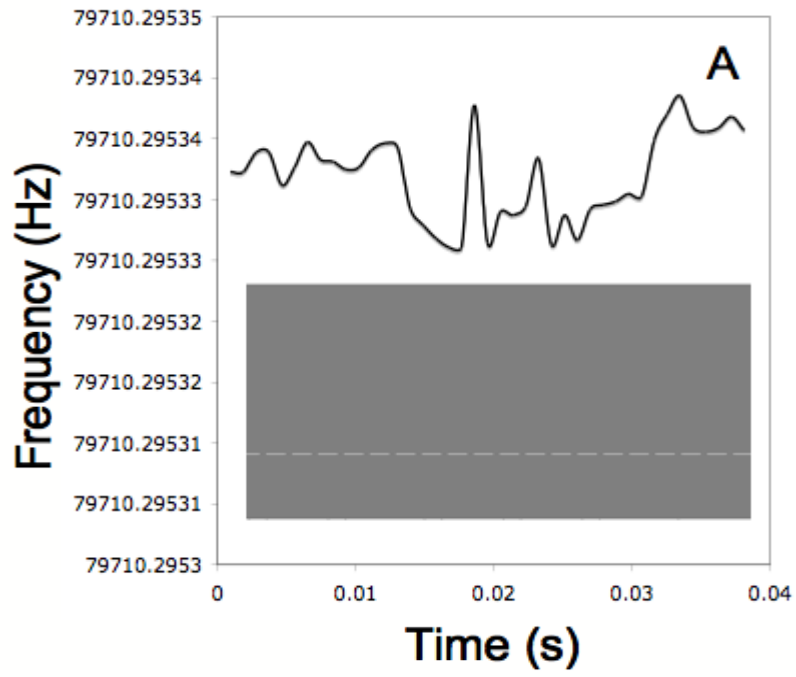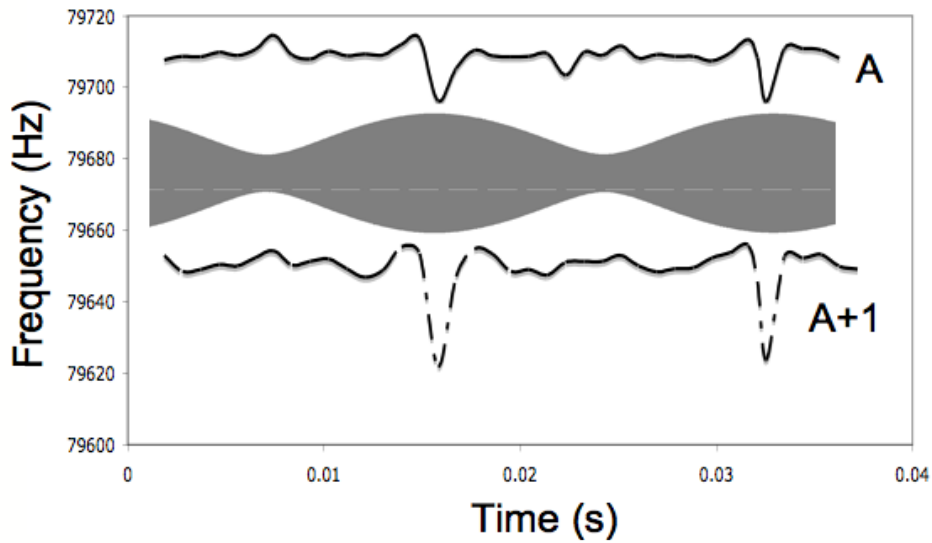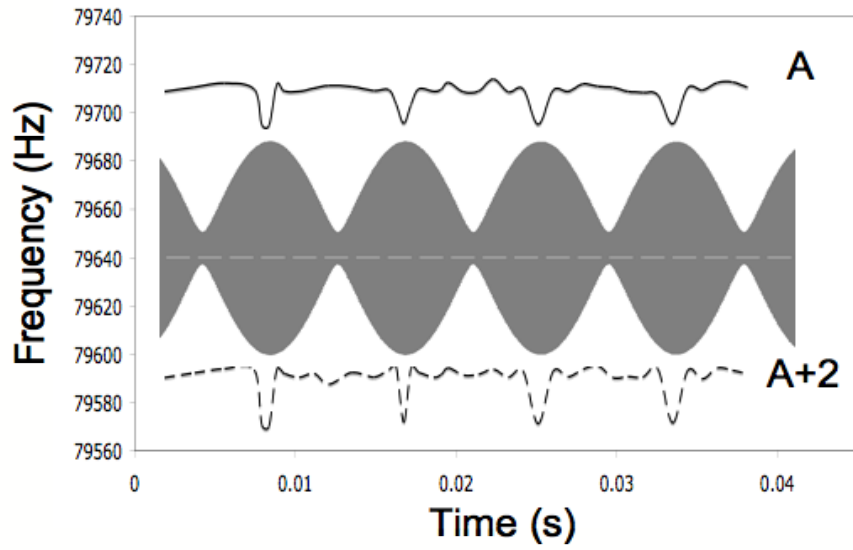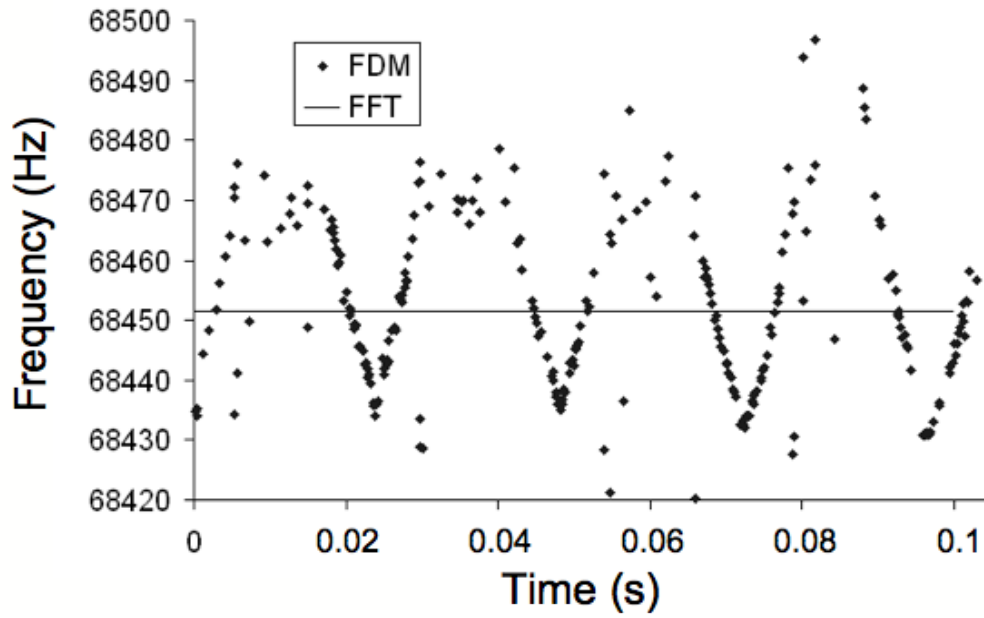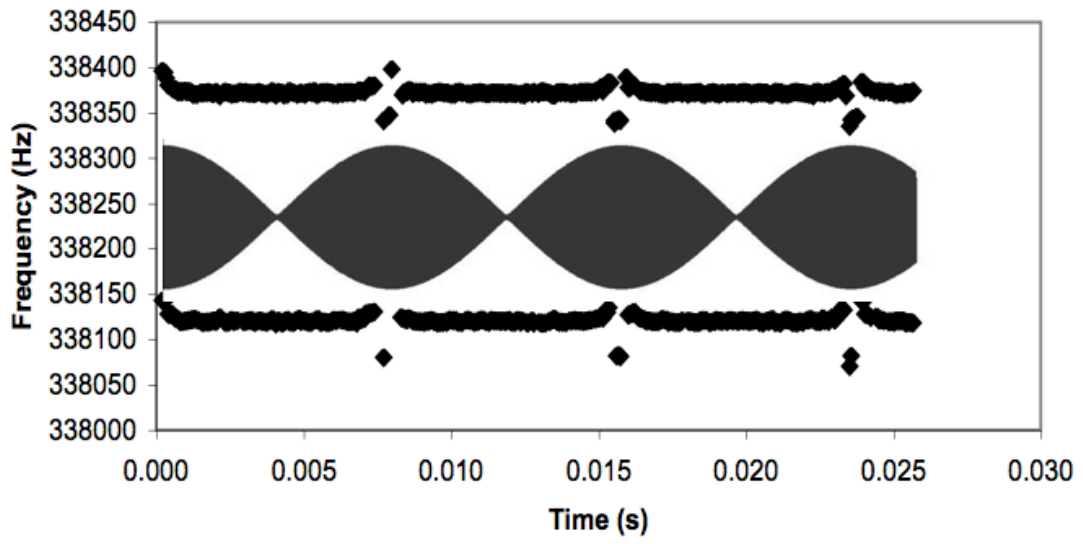
Figure 1.

Figure 2.

Figure 3.

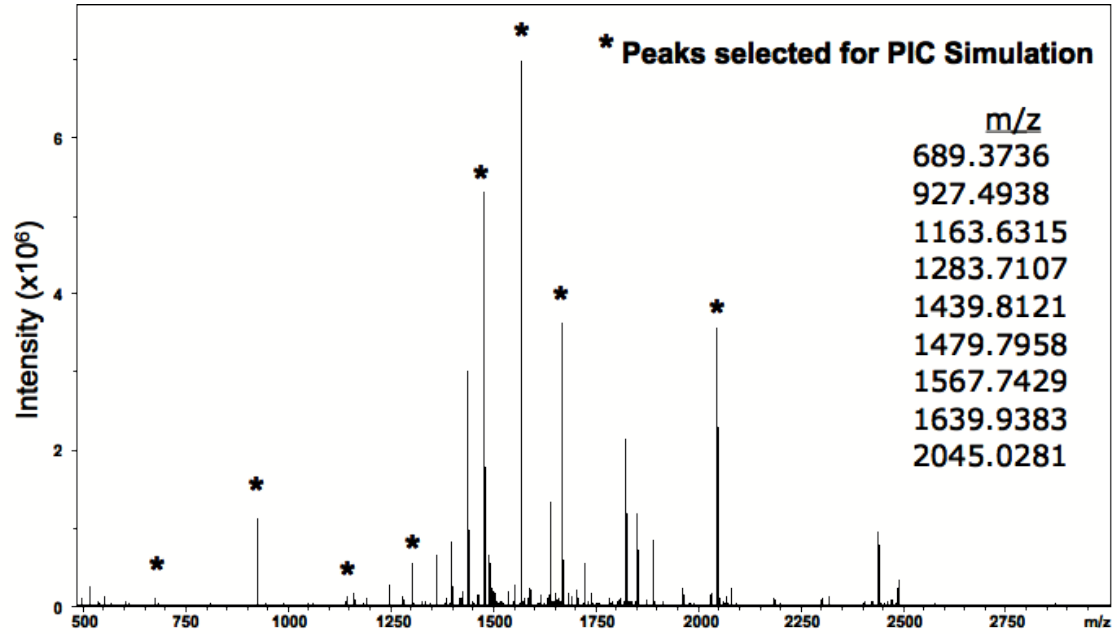**Figure 4.**

Figure 5.

Figure. Experimental MALDI spectrum and corresponding monoisotopic peaks selected for PIC simulation.