A Procedure for Tuning and the Preliminary Evaluation of an Electrically Compensated Cylindrical ICR Cell

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NIH/NCRR MS Resource

Overview

Purposes

- To tune a compensated trap and evaluate its performance at various masses.

Methods

- Sample empirically the cyclotron frequency surface at various cyclotron radii and z-mode amplitude distributions.
- Compare the mass resolving power and sensitivity at various *m/z's* achievable with a compensated trap compared to those of an uncompensated trap.

Results

- The frequency surface can be flattened by adjusting the compensated trap's auxiliary ring voltages.
- An experiment-based algorithm flattens the frequency surface in one step.
- The compensated trap offers a factor of two increase in apparent resolving power and a significant increase in sensitivity.

Introduction

- FTMS offers high sensitivity, high resolving power, and high accuracy in mass measurement. Mass accuracies of < 5 ppm [1] and sub ppm with careful recalibration [2][3] are commonly achieved.
- The electrostatic potential well created by the trapping electrodes, required to confine ions along the z-axis, introduces variations in the frequency surface that diminish the ultimate performance of the instrument.
- The performance of an FTMS instrument can be improved by correcting or compensating the electric trapping fields in the analyzer.
- Previous work in compensation of the Penning trap include, but are not limited to:
    - Van Dyck, who used traps of hyperbolic geometry in physics experiments [4][5];
    - Gabrielse who compensated a cylindrical Penning trap [6], and analyzed the cylindrical and hyperbolic geometries based [7] on polynomial expansions of the potential at the trap origin;
    - Inoue who compensated a cylindrical trap for application in FTMS by first considering concentrically divided trapping electrodes and then building a trap with three pairs of aperture electrodes arranged at the trap ends to reduce the radial electric field [8];
    - Jackson who constructed a matrix-shimmed trap to obtain a more globally quadrupolar trapping potential [9];
    - Knobeler who designed a quadratic z-axis electric trap potential in closed cylindrical trap by using a ring system [10];
    - Vartanian who minimized the radial electric field in an open cylindrical trap by use of one compensation ring pair [11] and by varying the aspect ratio of the trap [12];
    - Barlow who has built a cylindrical trap compensated to 4th order for the study of the effects of charge and cloud shape [13];
    - Gooden who has utilized compensation and an RF-only-mode event to improve the performance of a cubic trap [14].
- Phase locking obscures changes of peak shape owing to small variations in the frequency surface, so that tuning by peak shape is difficult.
- Thus the need for another tuning strategy.

Methods

Materials

- $[Arg]^8$-Vasopressin, insulin oxidized B-chain, TRF2, and cytochrome C were mixed with 2,5-dihydroxybenzoic acid at ratios from 1:1500 to 1:5000, prior to spotting.
- The above chemicals with the exception of TRF2, were obtained from Sigma/Aldrich (St. Louis, MO). The TRF2 was obtained from Satoko Akashi at Yokohama City University in Japan by overexpression in *E. coli*.

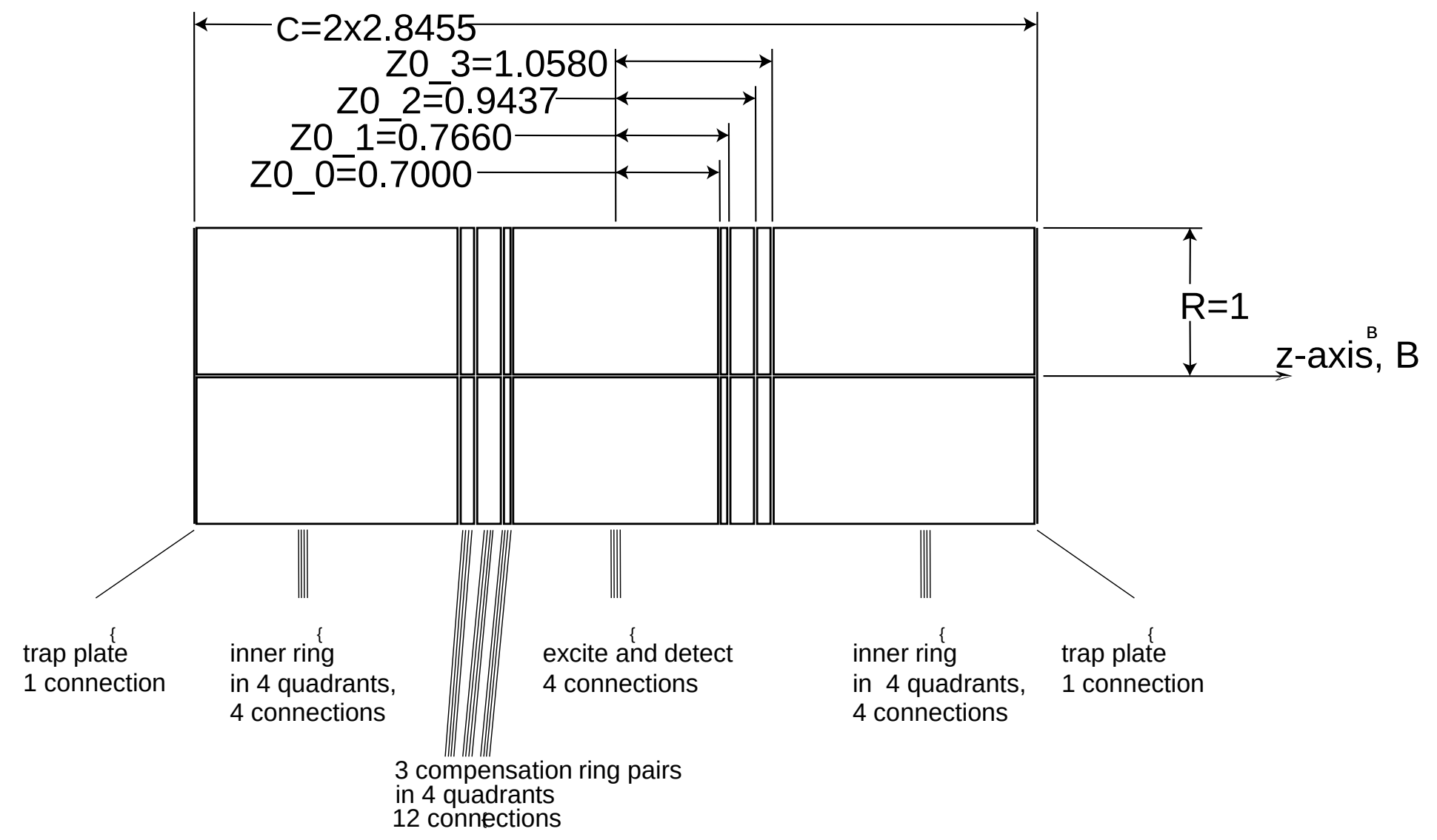
Data Treatment

- Transient data were imported into a Fortran program for calculation of the complex FFT, the subsequent calculation of the frequency centroid and resolving power was performed in a MathSoft Mathcad (Cambridge, Ma) worksheet.
- Peak intensities were taken from the vendor-supplied Omega8 software package.

Instrumentation

- 7.0-T IonSpec ProMALDI FTMS (Lake Forest, CA) equipped with an external hexapole for the accumulation of ions produced from multiple laser pulses;
- Ion transfer via a quadrupole ion guide into a custom-designed trap (Fig. 1);.
- Compensated and uncompensated modes implemented by turning on and off (zero volts) the voltages supplied to the auxiliary rings, respectively;
- Various cyclotron mode amplitudes produced by using different ARB waveform amplitudes.
- z-mode amplitude distribution manipulated by collisionally cooling the ions with pulses of N_2 gas, followed by z-mode re-excitation by pulsing the end caps for a short duration (±35 V for 300 μs).
- Narrowband detection parameters of a 1024K point transient with an ADC rate of 10 kHz (105-s transient)

Fig. 1



Tuning

- Auxiliary ring voltages were varied modestly with no noticeable effect on the peak shape, indicating the frequency surface is masked by *phase locking*.
- The compensation voltages were refined from a starting set of voltages by measuring the frequency centroid as a function of mode amplitudes.
- Three Δfs for each frequency surface were measured for frequency changes from a ref. point to three other points caused by stepping mode amplitudes (Fig. 2).
- A Δf set was collected for the starting voltages, as well as three Δf sets for when each ring voltage was individually perturbed by 150 mV.
- The difference between a Δf set for a perturbed voltage and the starting Δf set gives a ΔΔf set, corresponding to the change in the frequency surface due to a 150 mV perturbation of a single auxiliary electrode.
- A linear combination of ΔΔfs was then calculated that corrected for the starting Δf set.

Fig. 2

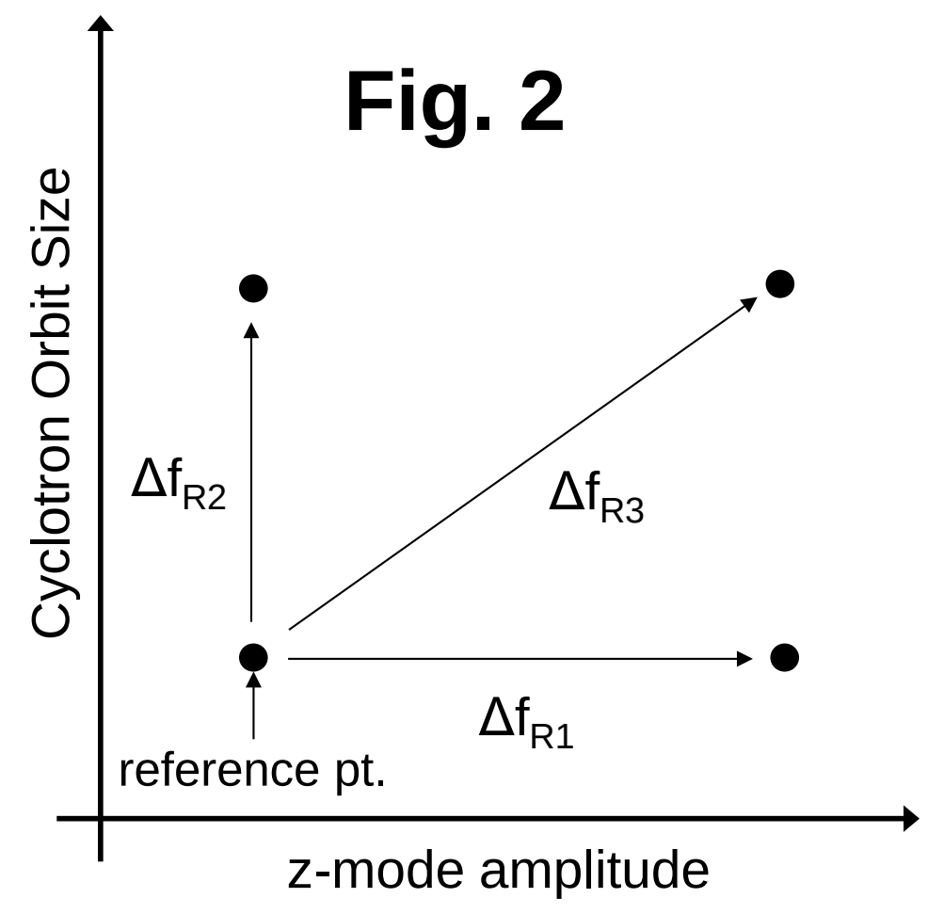


Fig. 3a Cyclotron Frequency vs ARB Amplitude (m/z 1084)
Before Tuning

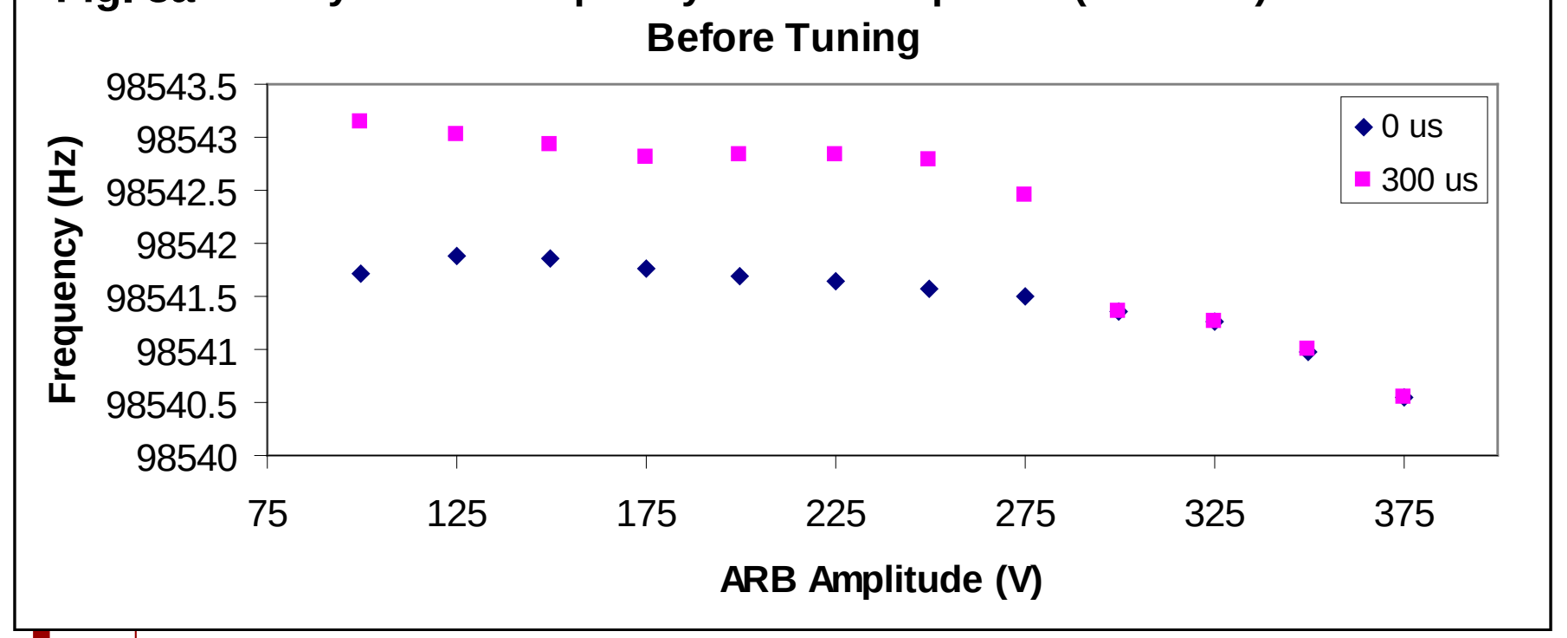
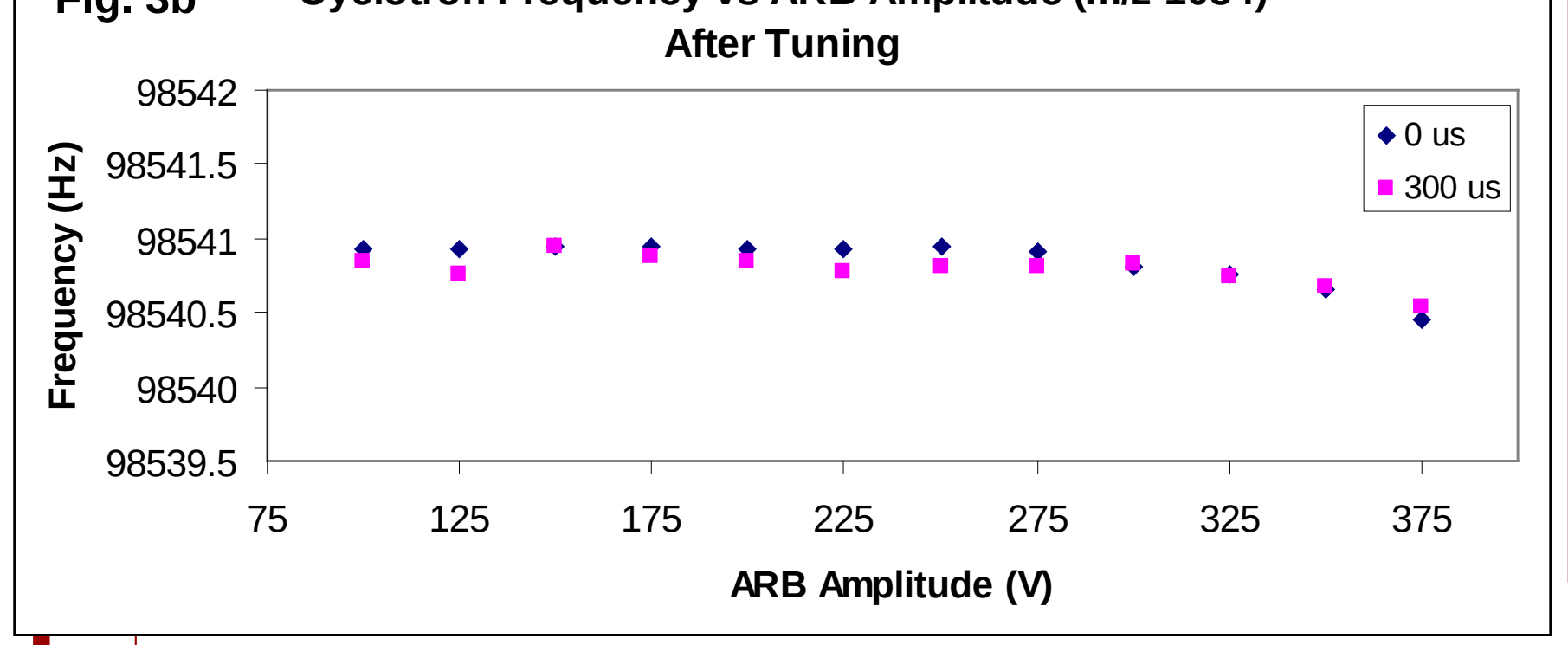


Fig. 3b Cyclotron Frequency vs ARB Amplitude (m/z 1084)
After Tuning

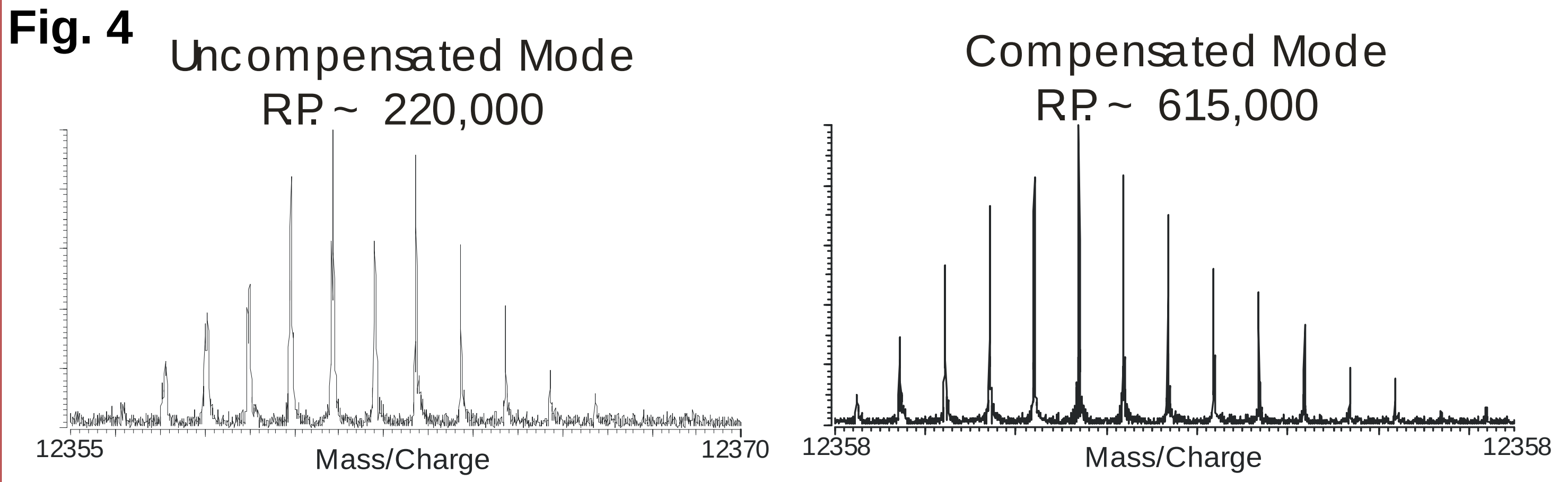


- The corrections were applied to the voltages in the starting set of voltages.
- These graphs (left) show the frequency centroid of vasopressin (*m/z* ~ 1084) at various cyclotron orbit sizes and at two different z-mode amplitudes before tuning and after one iteration of tuning.
- The average frequency difference between the high and low z-mode amplitude ions went from 10 ppm (Fig. 3a) to 1 ppm (Fig.3b).
- The frequency shift with increasing cyclotron orbit size also decreased after tuning, as evident from a decrease in the slope of the freq. vs ARB amplitude line.

Mass Resolving Power

- As the inhomogeneities of the frequency surface are smoothed out by compensation, a factor of two or more increase in mass resolving power over that of the uncompensated trap can be obtained for Cytochrome C. (Fig. 4)
- The reason that the increase is not greater is that part, if not all, of the ion cloud in the uncompensated trap is phase-locked, yielding a much higher apparent resolving power than expected by examination of the frequency surface.
- At higher *m/z*, the advantages of the compensated cell in resolving power should increase owing to a decrease in phase locking in the uncompensated cell [15] (i.e., larger *m/z* → larger thermal cloud → greater frequency spread → more ions required to phase lock)

Fig. 4



Sensitivity

- The compensated trap has a lower ion-number threshold than the uncompensated trap for phase locking because the frequency spread of the ions in the cloud has decreased.
- A smaller number of ions is needed to produce a high resolving power coherent signal in the compensated trap.
- As the *m/z* of the ion increases, so should the advantage of using the compensated cell over that of the uncompensated.
- Figures 5a and 5b illustrate the increase in sensitivity with the compensated trap using insulin oxidized B-chain and TRF2, respectively.

Fig. 5a Abundance vs Time of Acquisition
m/z ~3500 @ Laser Power 23%

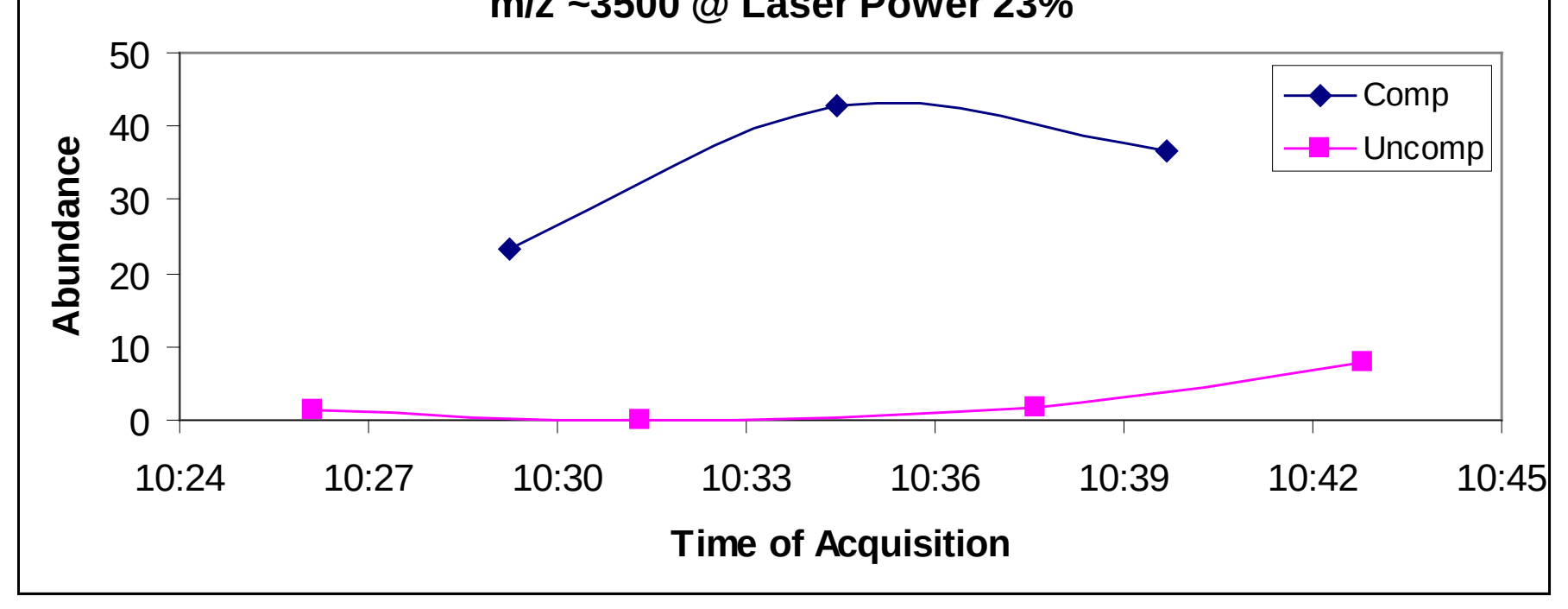
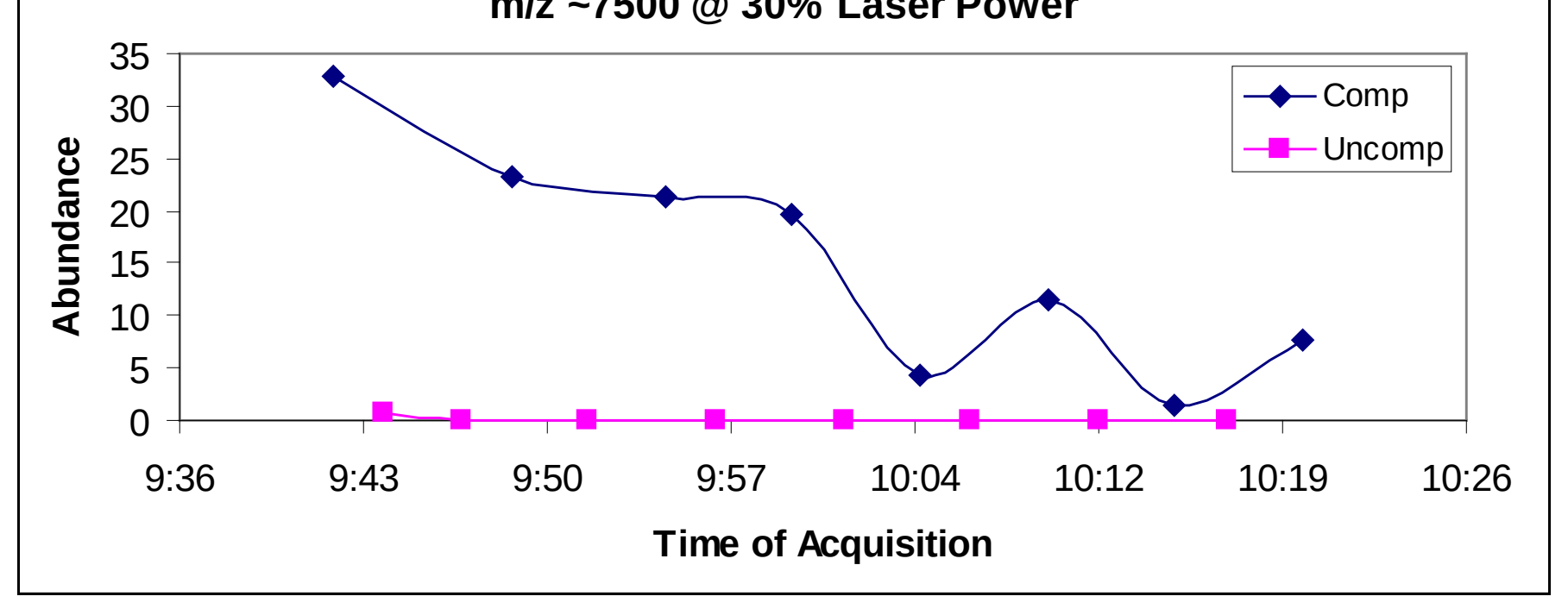


Fig. 5b Abundance vs Time of Acquisition
m/z ~7500 @ 30% Laser Power



Conclusions

- Phase locking masks the subtle variations in the frequency surface, so using peak shape as an evaluation of trap performance is impractical.
- Tuning of a compensated trap was accomplished using a experiment-based algorithm.
- The compensated trap has at least two fold better mass resolving power and ten fold increase in sensitivity for ions of *m/z* 1000-10,000.
- The advantages of the compensated trap over the uncompensated should increase for ions of m/z > 10,000.

Future Work

- Incorporate ionic liquid matrices to improve our quantification of increased sensitivity offered by the compensated trap.
- Investigate further the upper mass limit of the compensated trap.
- Apply cell compensation to our efforts to improve accurate mass measurement.

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