Interference of adjacent ion signals in CDMS: the effect on charge accuracy and potential correction algorithms

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Abstract

The Selective Temporal Overview of Resonant Ions (STORI) method improves the accuracy of charge assignment of ions in charge detection mass spectrometry (CDMS) by monitoring essential conditions of ions oscillating in the electric field over the time. The STORI method will lose the accuracy if there is another “interference” ion oscillating at a similar frequency as the target ion during the acquisition of the transient signal. Artificial transients, simulating different situations in which two ions close in *m/z* ratios coexist in the Thermo Scientific™ Orbitrap™ analyzer, are subjected to STORI analysis to demonstrate how this interference effect can be identified and influence the assignment. The existing interference-correction solution, that applies a window to filter out the interference in the frequency domain, is demonstrated, and the limitation of this solution is discussed. An interference subtraction loop is proposed as an alternative solution to correct the interference effect.

Introduction

Charge detection mass spectrometry (CDMS) measures the ion current of individual ions to provide the mass-to-charge ratio (*m/z*) and the number of charges (z) simultaneously. This technique has proven to be highly effective in accurately measuring the masses of macromolecular analytes, such as viruses. One of the main challenges in determining the charges of ions in CDMS is that ions trapped in the analyzer may shift in mass or vanish due to unintended collisions or abrupt losses during transient acquisition. These factors may lead to inaccuracies in charge measurements.

Fortunately, the Selective Temporal Overview of Resonant Ions (STORI) and its extension, misSTORI, allow for the monitoring of ion conditions throughout the entire period of transient acquisition. This enables further corrections during data processing based on four ion conditions monitored by STORI: the frequency of ion motion, the amplitude of the ion current, the disappearance of the ion, and the phase of the ion signal.

While reliable single-ion transients can be acquired with Automatic Ion Control (AIC) technology, it is inevitable to encounter ion signals that are extremely close to each other in the frequency domain spectra during CDMS analysis. These neighboring signals cause the STORI and misSTORI plots to exhibit a “ripple” pattern, which complicates the accurate acquisition of the four ion conditions and subsequently affects the accuracy of charge assignment.

Materials and methods

Artificial transients that simulate signals of one or two ions with controllable lifetimes and frequency jumps were generated using a custom Python script. STORI analysis can be performed in two ways: "brute-force STORI," which involves processing all points of the transient and resonating these points with a cosine and a sine wave to generate the real and imaginary traces of the STORI plot, and "efficient STORI," which is achieved by convoluting the frequency domain ion signal with the FT signal of a box function spanning from 0 ms to the corresponding time point.

The interference correction algorithm was developed using a custom Python script that can also generate STORI/misSTORI plots. This algorithm extracts rough survival times, amplitudes, and transient initial phases of adjacent ions from the STORI/misSTORI plots. Based on these rough parameters, the signal of one ion can be subtracted, making the information extracted from the STORI/misSTORI plots for the other ion more accurate. This process is cycled to minimize the interference effect.

Results

Monitoring Ions’ Conditions via STORI Over the Time Domain

When a single-ion transient is subjected to STORI analysis, a plot (STORI plot) is generated, as shown in Figures 1(b) and 2(b). The real and imaginary traces in the STORI plot reflect the amplitude accumulation when a cosine wave and a sine wave resonate with the transient signal. These two traces are expected to be linear during the ion's survival time if there is no change in the frequency of the ion's oscillation. Another trace on the STORI plot represents the magnitude of the real and imaginary parts, which is also expected to be linear, with the slope proportional to the amplitude of the ion current during the ion's survival time.

Figure 1(a). FT spectrum of a 1000 ms artificial transient (transient 1) simulating a 3.5 MDa ion with 150 charges which vanishes at 900 ms without frequency shifting

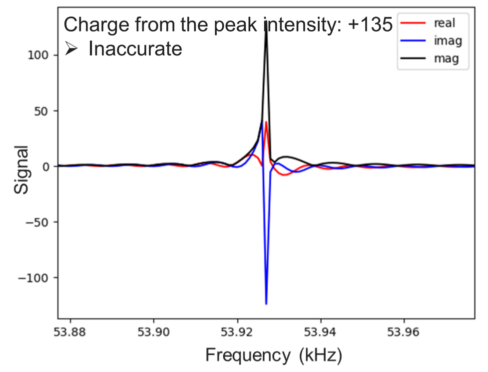


Figure 2(a). FT spectrum of a 1000 ms artificial transient (transient 2) simulating a 3.5 MDa ion with 150 charges which disappears at 900 ms with a 0.216 Hz frequency shift (28-Da mass change) at 500 ms

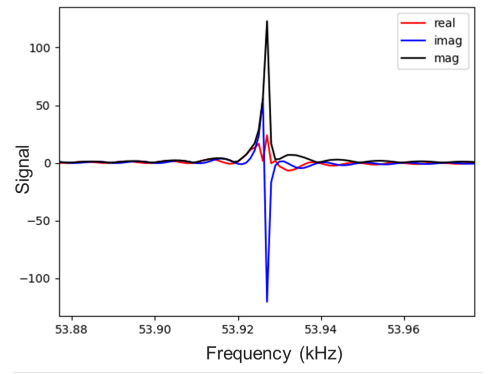


Figure 1(b). STORI plot of the artificial transient 1

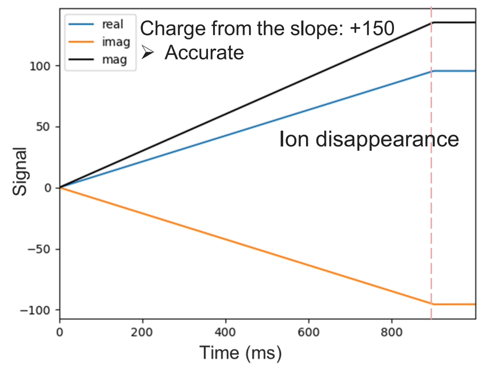


Figure 2(b). STORI plot of the artificial transient 2

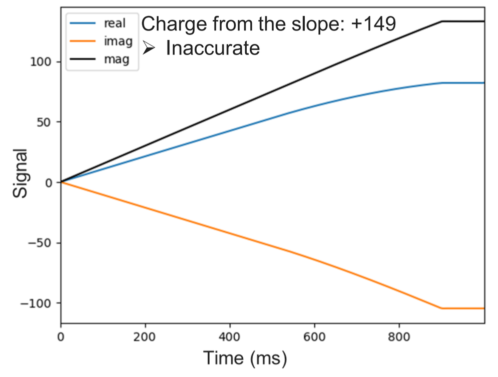


Figure 1(c). misSTORI plot of the artificial transient 1

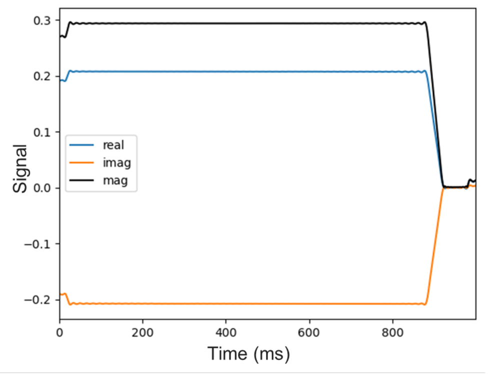
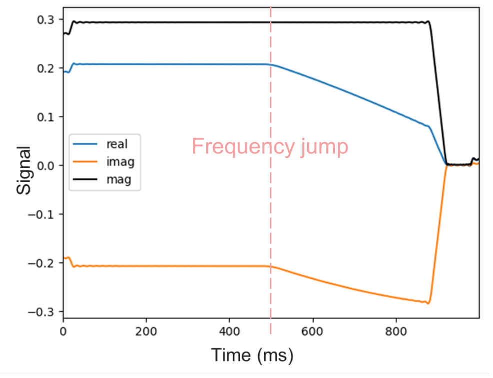


Figure 2(c). misSTORI plot of the artificial transient 2



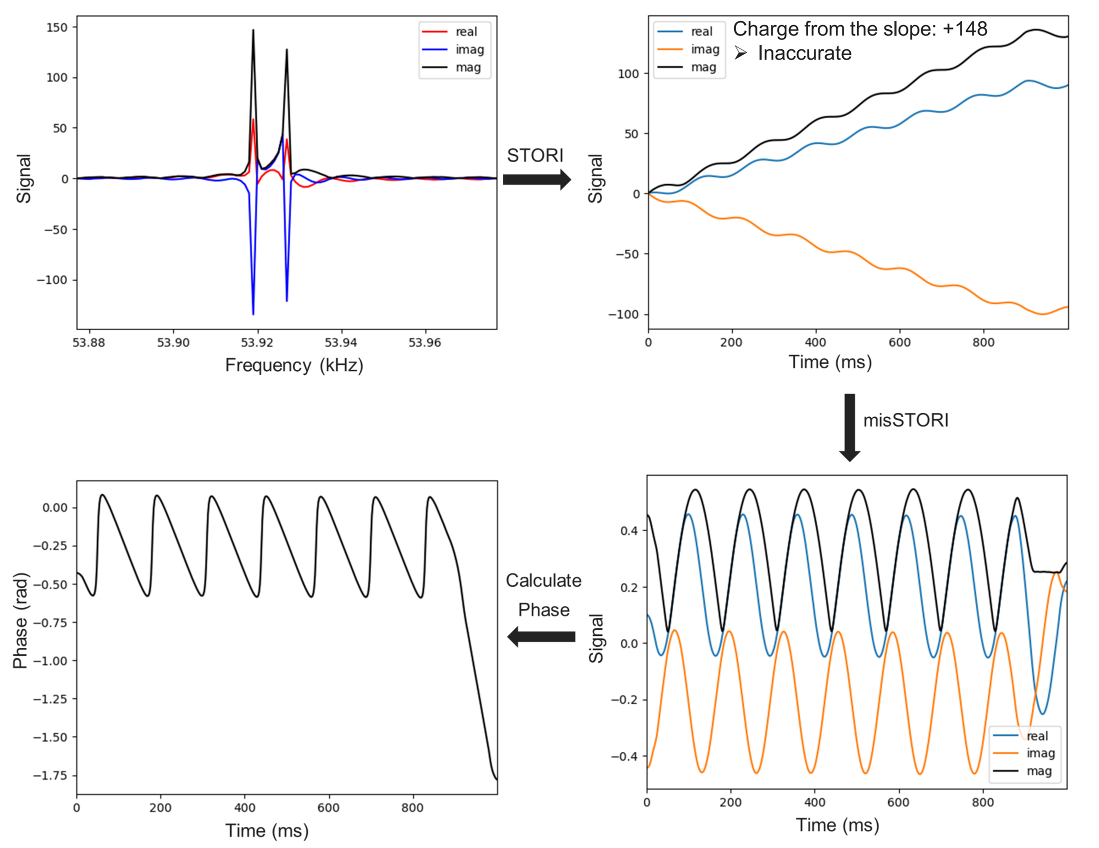
The most apparent advantage of STORI technology is its ability to accurately monitor the disappearance of the ion and use the slope of the magnitude trace to estimate the ion's charge. For example, the noise-free artificial transient 1 simulates the +150 charge state of a 3.5-MDa ion oscillating in the Orbitrap™ during a 1-second signal acquisition time, disappearing at 900 ms. The frequency spectrum of this transient is shown in Figure 1(a). The ion disappearance causes a loss of signal intensity of the main peak in the frequency spectrum, leading to an inaccurately lower charge estimate if ion death occurs. In the case of artificial transient 1, the charge would be mistakenly estimated to be approximately 135 from peak intensity. The STORI plot of artificial transient 1, shown in Figure 1(b), accurately reflects the ion disappearance at 900 ms by flattening all three traces, allowing the slope of the magnitude trace between 0 and 900 ms to be used to accurately estimate the charge as 150.

In addition to ion disappearance, changes in ion oscillation frequencies during transient acquisition can also cause inaccuracies in charge assignment in CDMS. Artificial transient 2 provides an example where an ion with the same conditions as artificial transient 1 experiences a mass shift of +28 Da, mimicking a nitrogen molecule capture at 500 ms. The misSTORI plot, shown in Figure 2(c), reflects this frequency shift by the "bending" in the real and imaginary traces at the corresponding time of the shift. A phase plot can be generated from the misSTORI plot to provide deeper information about the frequency change, with more details available in *Goodwin et.al 2024* [1].

Interference of Adjacent Ion Signal in CDMS

In circumstance in which an interfering ion, with a mass 1 kDa higher (7.7 Hz lower in oscillating frequency) than the target ion, coexists in the analyzer is simulated in artificial transient 3. As shown in Figure 3, the STORI plot of the target ion exhibits a “ripple” pattern rather than a straight line, bending at the time of ion disappearance. This pattern causes inaccuracies in the estimation of ion conditions over the transient. More importantly, it results in inaccuracies in the charge assignment based on the slope of the magnitude trace on the STORI plot. In the case shown in Figure 3, the charge is mistakenly assigned as +148 due to the interference.

Figure 3. STORI analysis of a 1000 ms artificial transient (transient 3) simulating a 3.5 MDa ion and a 3.501 MDa ion, both have 150 charges, coexisting in an Orbitrap™ analyzer, with the 3.5 MDa ion disappearing at 900 ms without frequency shifting



Interference Correction Methods – Windowing

One of the most straightforward methods to correct interference is to apply a window in the frequency domain to filter out the interfering signal and then subject the resulting frequency domain to STORI analysis. Various shapes of filters have been explored, including box, Gaussian, half-sine, and half-sine with a flat top. Among these, the half-sine filter with a flat top recovered the highest accuracy in charge assignment.

Figure 4 shows the application of a flat-topped half-sine window to the ion signal of the +150 charge state of the 3.5-MDa ion to filter out an interfering signal that is 26.94 Hz lower than the target (simulating an interfering ion that is 3.5 kDa higher in mass). The flat-topped half-sine window is in the width slightly narrower than the frequency gap and has a 50:50 flat to half-sine ratio. This filtering corrects the slope of the STORI plot, adjusting the charge assignment from +149 to the correct value of +150.

However, windowing has limited performance when the frequency of the interfering ion is very close to that of the target ion. A narrower window will filter out a wider range of signals in the frequency domain, causing a loss of information and thus impacting the accuracy of the data acquired. Transient 3 is a good example of this limitation, as the two signals are so close in the frequency domain that they overlap over a wide range of frequencies.

Figure 5 shows the result of STORI analysis after windowing. Although the “ripple” pattern is minimized in the STORI plot after windowing, the bend that reflects the ion disappearance time becomes a curve, and the slope inaccurately assigns the charge as +149.

Figure 4. STORI analysis of the +150 charge state of the 3.5-MDa ion disappears at 900 ms after filtering out an interference 26.94 Hz away with a flat-top half-sine window

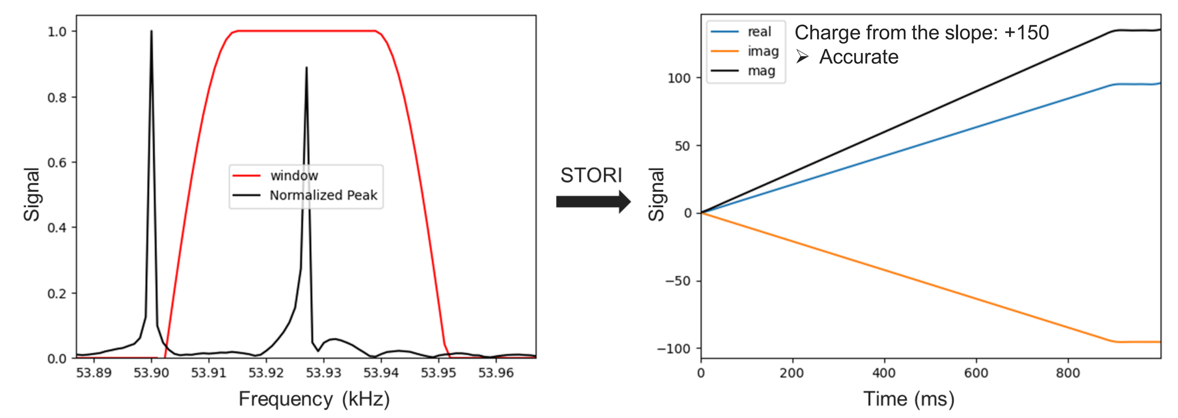
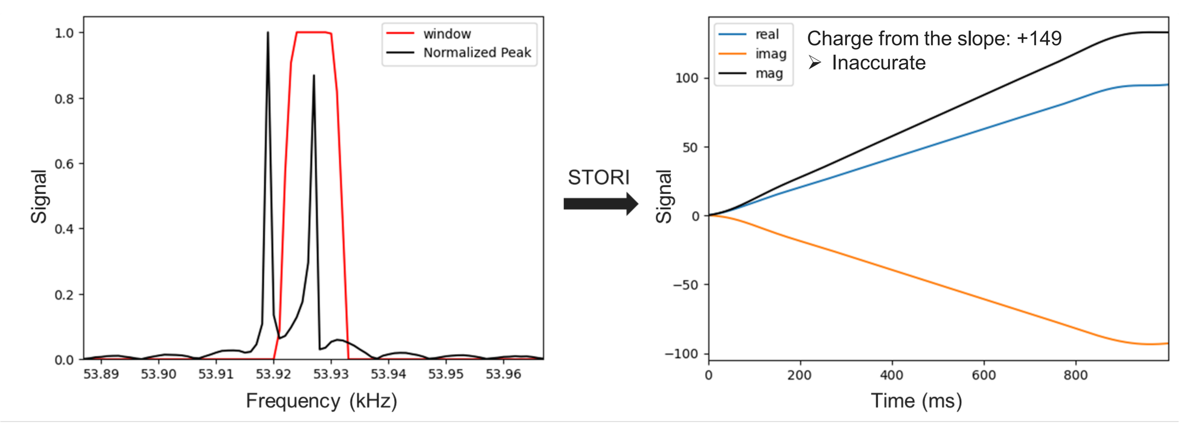


Figure 5. STORI analysis of the +150 charge state of the 3.5-MDa ion vanishes at 900 ms (artificial transient 3) after filtering out an interference 7.71 Hz (1kDa in mass) away with a flat-top half-sine window

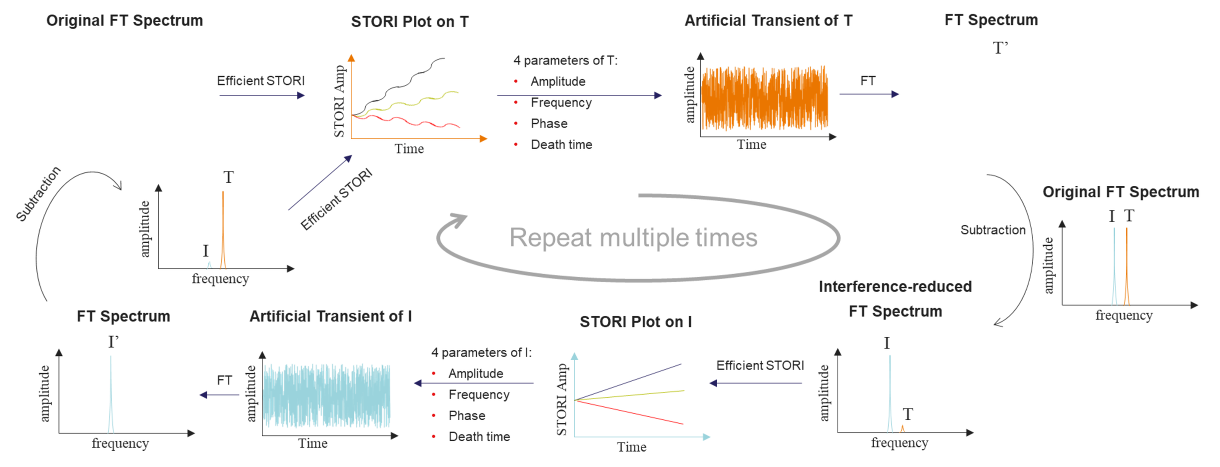


Future Development

Interference Signal Subtraction Method

A novel interference correction method is under investigation. In this method, artificial transients are generated based on four parameters: amplitude, frequency, time of disappearance, and initial phase. These parameters are obtained from the rough information derived from the STORI analysis of both the target and the interfering ions. The artificial transients are then subtracted from the original transient to produce an interference-reduced transient, thereby enhancing the accuracy of determining the four parameters. By iterating this process, it is theoretically possible to generate a reliable interference-free transient.

Figure 6. The flow chart of the transient subtraction method



Conclusions

In CDMS data analysis assisted by the STORI method, an ion signal with a frequency close to the target ion can interfere with the resulting STORI plot, causing a “ripple” pattern on the plot that can cause an inaccurate charge assignment. Thus, it is important to ensure that target ions subjected to STORI analysis is free from interfering signals. Apparently, reliable attenuation of ions with high-performance hardware is a key factor of reducing negative impacts of the interference effect, whereas interference-correction algorithms are available to reduce the impact to a lower level.

References

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2. Kafader, J. O.; Beu, S. C.; Early, B. P.; Melani, R. D.; Durbin, K. R.; Zabrouskov, V.; Makarov, A. A.; Maze, J. T.; Shinholt, D. L.; Yip, P. F.; Kelleher, N. L.; Compton, P. D.; Senko, M. W. STORI Plots Enable Accurate Tracking of Individual Ion Signals. J. Am. Soc. Mass Spectrom. 2019, 30 (11), 2200-2203.

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