thermoscientific

Improved Signal Processing for Orbitrap Charge Detection Mass Spectrometry

Michael P. Goodwin, Kyle P. Bowen, Dmitry Grinfeld, Ping Yip, Michael Senko

ABSTRACT

Many large, complex species undergo changes in m/z during mass analysis, which can lead to suppressed charge and mass estimations using conventional STORI processing. The STORI algorithm can be modified to track changes in m/z (and therefore changes in frequency). This information can be fed back into the curve generation, significantly improving the measurement accuracy when mass changes occur.

INTRODUCTION

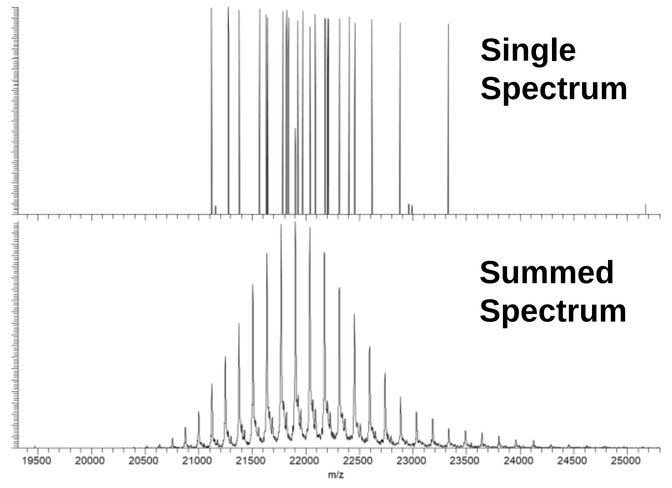
Orbitrap-based Charge Detection Mass Spectrometry (CDMS) has evolved as a powerful tool for studying large, heterogeneous species which are unresolvable by conventional mass spectrometry.[1] The STORI (Selective Temporal Overview of Resonant Ions) concept was developed to better discern charge for CDMS by measuring the rate at which an ion accumulates signal over a specified time interval.[2] Conventional STORI processing presumes a fixed frequency signal and monitors the rate at which signal builds over time for said frequency. If an ion changes mass/frequency during the detection period (e.g. from loss of non-covalently bound solvent inside the Orbitrap), the resultant frequency shift can significantly lower the charge estimate. Since many large species may lose mass during detection, it is beneficial to enhance STORI processing to accommodate variable frequency.

PRELIMINARY STORI ANALYSIS

A viral capsid sample, which was artificially constructed using only minor capsid protein VP3, was electrosprayed into a Thermo Scientific™ Q Exactive™ UHMR. Mass spectra were collected with 1 second acquisitions for ~85 minutes. Injection time was fixed at 20 milliseconds. The flux was such that any single spectrum contained several tens of ions (**Figure 1**).

As a baseline reference, STORI plots were generated for peaks that met the criteria in **Table 1**. Virtually all ions provided signal for the full length of the transient. Two example peaks of +160 charge will be used throughout to demonstrate the observed behaviors, complications and remedies.

Figure 1. Mass Spectra



Individual spectra contain tens of individual ions (top), while a summed spectrum shows a more traditional profile (bottom)

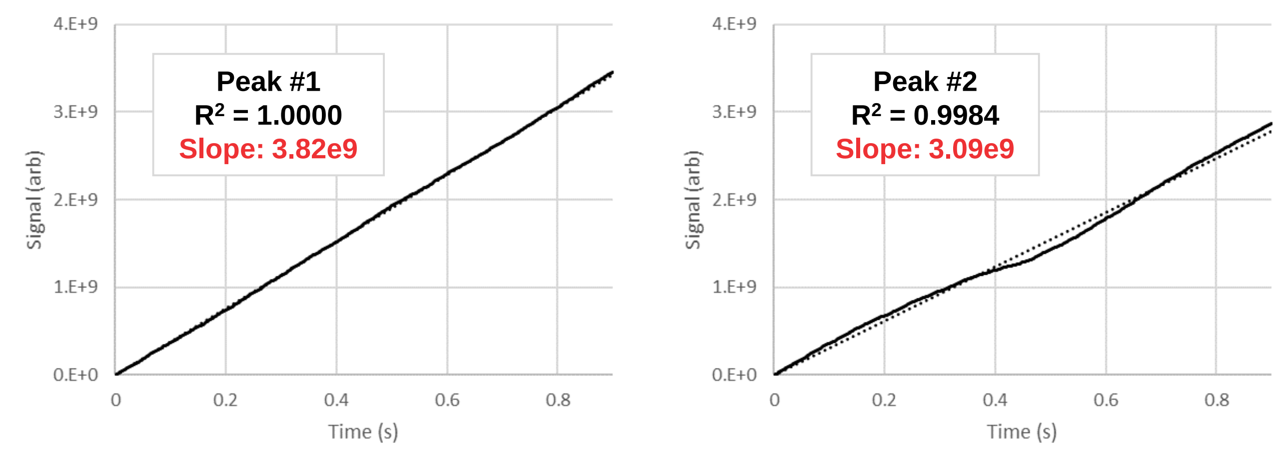
Table 1. Filtering Conditions

Condition	Value
S/N	> 3.0
R^2	>0.95
Duration	>0.5 seconds
Spacing	>100 Hz

Peaks/segments were filtered based on the following criteria.

Many of the filtered peaks produced traditional STORi plots with near-ideal straight lines (example Peak #1, **Figure 2, left**). However, many other peaks generated STORI plots with shapes that were curved and had reduced slopes, relative to other ions of the same charge state (example Peak #2, **Figure 2, right**). The reduced slope of such species would result in an underestimation of charge, and therefore an underestimation of absolute mass for such ions. It was speculated that this undesirable characteristic may result from ions undergoing changes in m/z (and therefore frequency) during transient acquisition. Such phenomena have been observed previously in Orbitrap mass analysis of large, complex species.[3]

Figure 2. Traditional STORI Plots From Two Peaks

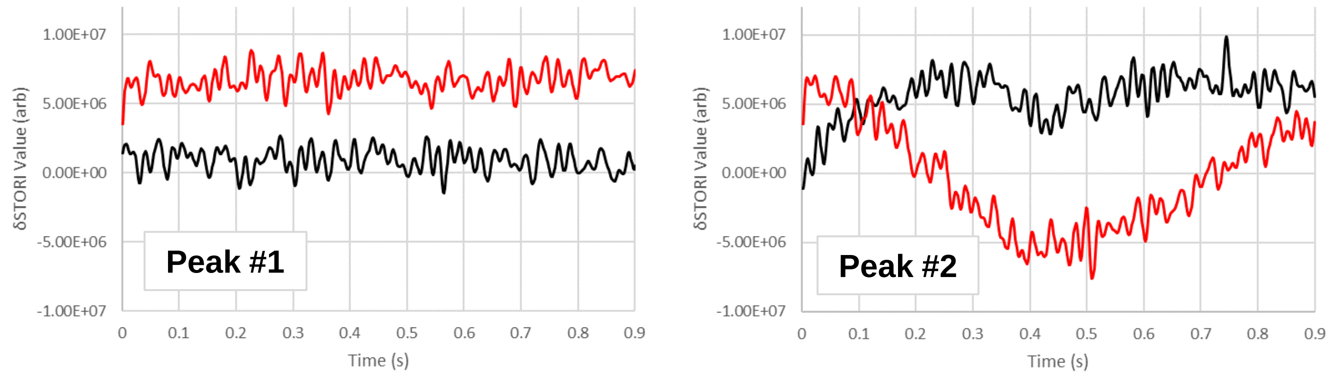


Standard STORI plots for two different peaks from the same charge state (+160). Both ions provide R^2 values >0.99, but peak #1 (left) is clearly straighter and has ~24% higher slope than Peak #2 (right).

δSTORI ANALYSIS

In order to understand the role of frequency changes in these signals, the time derivative of the complex STORI data was taken. This analysis, termed "δSTORI", generates a complex data set that represents the rate at which signal is building for cosine and sine waves (real and imaginary parts, respectively) of the STORI frequency over time. When the signal frequency and STORI frequency are well-matched, signal builds at a constant rate in both components, resulting in flat δSTORI plots, as see for Peak #1 (**Figure 3, left**). However, if there is a difference between the signal frequency and the STORI frequency during the acquisition, signal will not build at a constant rate for the cosine and sine waves, but rather transfers between the two over time. This results in non-flat δSTORI plots, which are evident for Peak #2 (**Figure 3, right.**)

Figure 3. δSTORI Plots from Two Peaks



δSTORI plots of Peaks 1 and 2 (black=real, red=imaginary). For peak #1 (left) the δSTORI plots are flat, indicating little difference between the signal frequency and STORI frequency. For peak #2 (right) the plots are not flat, indicating a difference between the signal frequency and STORI frequency.

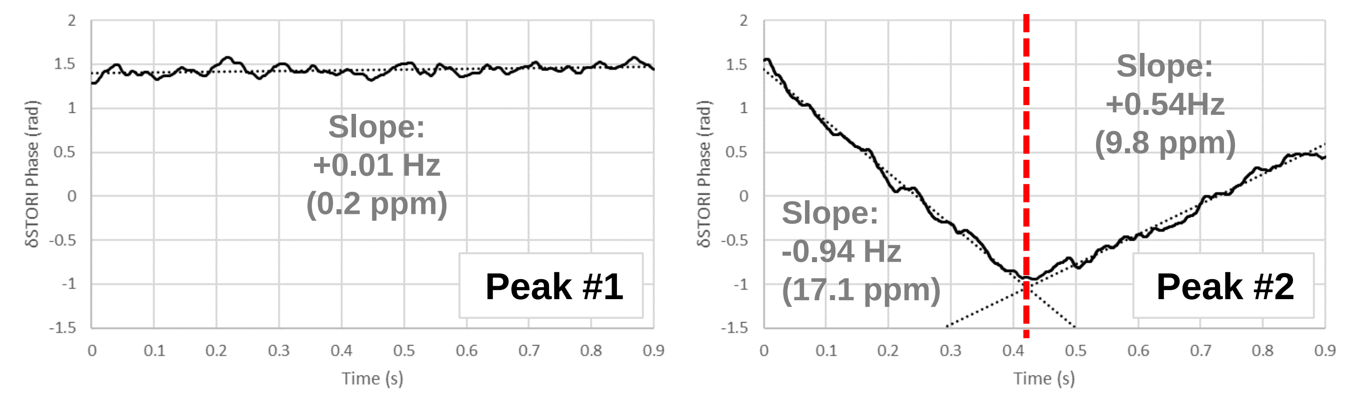
δSTORI PHASE AND FREQUENCY ERRORS

The rate at which signal is transferring between cosine and sine waves is evident in the phase angle of the δSTORI data. **The slope of the δSTORI phase angle is a direct measure of how far apart the signal frequency and STORI frequency are at any point in time.** Examples from Peak #1 and #2 are shown in **Figure 4** (both have +160 charge).

Peak #1's δSTORI phase plot is in **Figure 4, left**. The δSTORI phase slope is +0.01 Hz, meaning the true ion frequency is only ~0.01 Hz higher than the STORI frequency. This represents a difference of +0.23 ppm.

Peak #2's δSTORI phase plot is in **Figure 4, right**. Until 0.4 seconds, the slope is -0.94 Hz, meaning the ion frequency is 0.94 Hz (17.05 ppm) lower than the STORI frequency. After 0.4 seconds, the slope is +0.54 Hz, meaning the ion frequency is 0.54 Hz (9.80 ppm) higher than the STORI frequency. The inflection point at 0.4 ms indicates a discrete jump in frequency of +1.48 Hz (26.85 ppm). This translates to a 53.7 ppm drop in m/z (1.2 m/z). With a charge state of +160, this corresponds to a decrease of ~192 amu in absolute mass.

Figure 4. δSTORI Phase Plots from Two Peaks

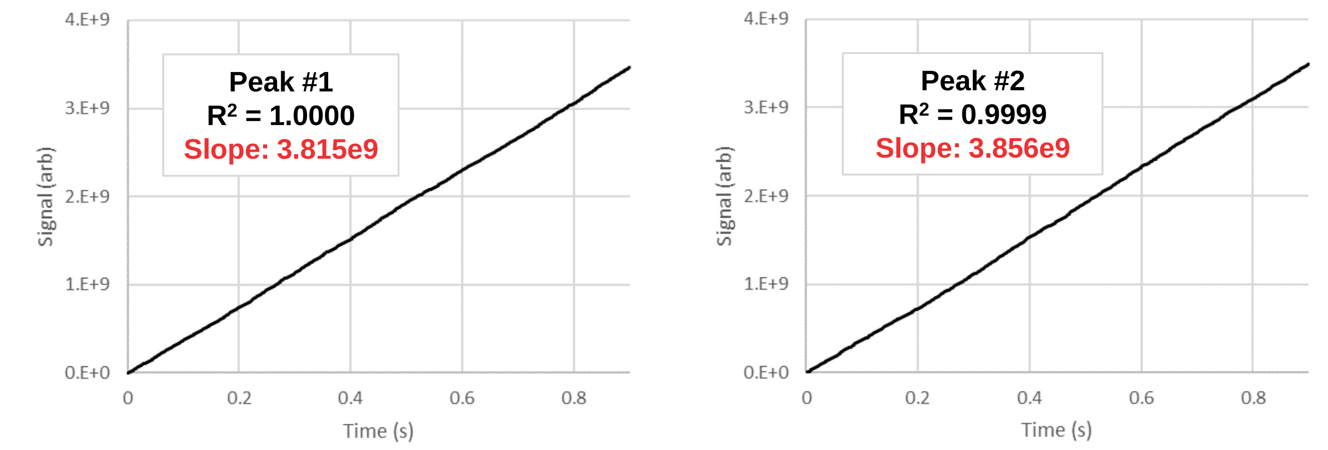


For Peak #1 (left) the δSTORI phase plot is virtually flat, indicating little difference between the STORI frequency and the signal frequency. For peak #2 (right) there are two distinct regimes, indicating a discrete jump in frequency at the inflection point.

FEEDING BACK THE FREQUENCY ERROR

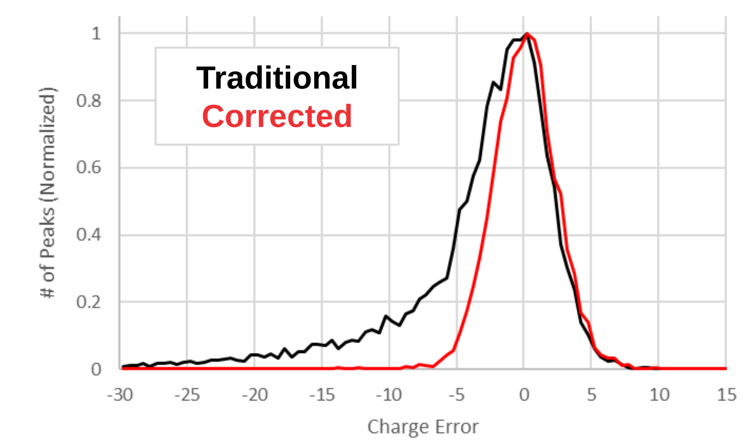
Since the slope of the δSTORI phase plot is a direct measure of the distance between the STORI frequency and the true signal frequency, that information can be fed back into the algorithm, dynamically adjusting the STORI frequency over time to match the true frequency of the ion as the transient progresses. This results in much straighter STORI plots for ions that undergo mass changes during the transient while maintaining accurate shapes for ions that didn't change (**Figure 5**). A histogram of charge error for both traditional and "corrected" STORI plots shows a dramatic improvement in accuracy when frequency shifts are encountered (**Figure 6**), essentially eliminating the fronting to low charge that stemmed from mass shifts.

Figure 5. "Corrected" STORI Plots



Corrected STORI plots for the two peaks. Both provide R^2 values >0.9998. While peak #1 (left) has an unchanged slope, peak #2 (right) is now within 0.9% of peak #1. This is in comparison to the ~24% difference of the uncorrected STORI plots.

Figure 6. Charge Error Histograms

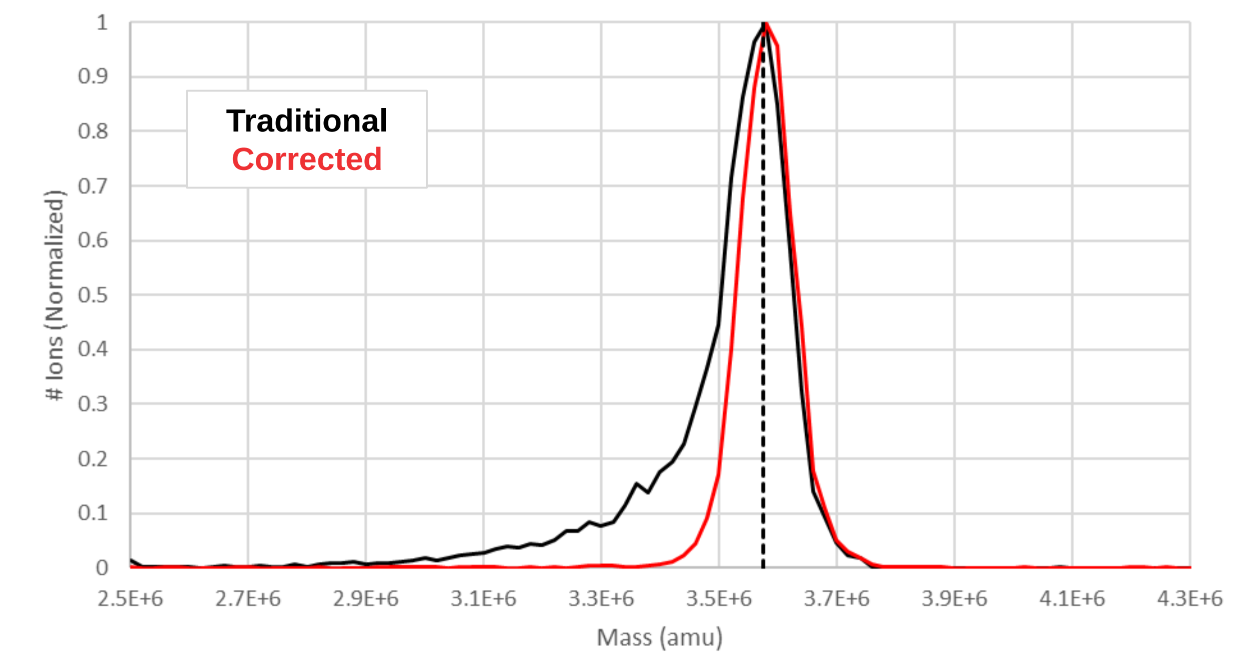


Charge error histograms. The traditional STORI processing has a long tail toward negative charge error, which is caused by shifting frequencies. The "corrected" data has a symmetric distribution around zero charge error, as it should be. Note that a significant fraction of ions underwent mass changes during acquisition. For longer transients, the likelihood of mass shifts would be even higher.

ABSOLUTE MASS SPECTRA

The absolute mass of each peak is estimated by rounding the estimated charge to the nearest integer and multiplying with m/z. The masses of these individual ions can then be binned to generate an absolute mass spectrum. Such spectra for the traditional and corrected STORI approaches are shown in **Figure 7**. The mass spectrum from traditional STORI processing has the tell-tale fronting that stems from reduced slope/charge assignments when mass shifting occurs. Conversely, the corrected STORI processing has a much more symmetric distribution. This improved precision should prove especially useful when more complex species, with many overlapping distributions, are analyzed.

Figure 7. Absolute Mass Spectrum



Absolute mass spectra. The expected mass of a VP3 capsid is indicated with a dashed line. Traditional STORI processing has a low mass tail, while corrected STORI processing is much more symmetric.

CONCLUSIONS

Mass shifts during transient collection are quite common for large, complex species (e.g. from continued desolvation inside the Orbitrap). Modifications to the STORI algorithm allow for accurate tracking and correction for any such mass/frequency shifts. This can potentially provide significant improvement in charge accuracy (and therefore mass accuracy) for many analyses. It should also allow for longer transient acquisitions to be performed without compromising charge precision, since the higher likelihood of shifting with longer transients can now be properly accounted for.

REFERENCES

1. US Patent No.11450520-B2
2. US Patent App. No. US-20220246414-A1
3. Warner TP, Aizikov K, Snijder J, Fort K, Makarov AA, Heck AJR, *Nature Chemistry*, 2022, 14, 515-522

TRADEMARKS/LICENSING

PO2023-43EN

ThermoFisher SCIENTIFIC