Encoded Frequent Pushing®

Improving Duty Cycle in the Folded Flight Path® High Resolution Time-of-Flight Mass Spectrometry

Introduction

Duty Cycle is a critical parameter affecting the sensitivity of analysis by Time-of-Flight Mass Spectrometry with Orthogonal Accelerator (OA-TOFMS). The Duty Cycle for OA-TOFMS is defined as the ratio between the time to fill the acceleration region with largest mass/charge ions and the time-of-flight (TOF) through the mass analyzer of these ions: Duty Cycle = T_{fill}/TOF[1]. For example, a typical time (T_{fill}) to fill the acceleration region with ions of m/z 1000 is 10us. The typical time-of-flight (TOF) for m/z 1000 ions in many OA-TOFMS is 50us. Thus, for this example, the Duty Cycle of the instrument is 10/50=20%. This means that 20% of ions with m/z =1000 entering OA are pushed into the mass analyzer for TOF analysis, and the remaining 80% are completely lost for analysis. Even more severe, the Duty Cycle for ions with lower m/z will be less than 20%, because the fill time is shorter (T_{fill}~$\sqrt{m/z}$), while the sampling period (TOF) stays the same, since it corresponds to the TOF of the maximum m/z of interest. A duty cycle of 20% is considered as a high value and quite typical for low resolution (<1,000) and medium-high resolution (<15,000) TOFMS. Of course, there are many other factors affecting instrument sensitivity (i.e. ionization efficiency, interface and mass analyzer transmission, detection efficiency, etc.), but the Duty Cycle is one of the most significant factors.

The resolving power in TOFMS is defined according to the well-known formula: R=TOF/(2·ΔT), where TOF is the time-of-flight of the corresponding ion and ΔT is the ion's peak width. Multi-Reflecting (MR) TOF mass analyzers in general and the Folded Flight Path® (FFP®) mass analyzers in particular, provide ultra-high resolving power by increasing TOF while maintaining a narrow peak width due to low aberration ion optics. However, long TOF reduces the Duty Cycle and thus could affect ultimate sensitivity of the *FFP* based mass spectrometers. The LECO Pegasus® GC-HRT$^+$ mass spectrometer uses an *FFP* analyzer to reach a resolving power above 25,000 (@FWHH of the peak with m/z 219) and an LOD of 1pg of OFN. The TOFMS technology with *FFP* achieves high resolution and sensitivity independent of mass range and data acquisition speed, unlike some other types of high resolution mass analyzers.

Increasing sensitivity, while providing high resolution and fast data acquisition, is a very desirable trait in modern mass spectrometry. It's been long recognized that the sensitivity of OA-TOFMS may be increased with higher frequency OA pulsing (extraction frequency). However, at higher frequency, spectral overlap can occur as light (and fast) ions from the later "pushes" catch up with the heavy slower ions from the earlier "pushes" still traveling through the analyzer. This results in mass spectra that are not very useful for interpretation unless an efficient way of decoding such overlaps is proposed.

Several methods were suggested in the past for encoding and decoding multiplexed overlapped spectra. The methods based on analysis of peak width[2] or single shift of pulsing frequency[3] will only work for high intensity peaks and thus will miss low intensity peaks, which essentially defeats the purpose of multiplexing for increasing sensitivity. The Hadamard transformation (HT), initially developed for IR spectroscopy[4], was proposed for mass spectrometry[5,6]. This method of multiplexing provides a simple encoding and fast decoding, and the signal-to-noise ratio of the healthy signals improves as √N, where N is the number of multiplexed pushes. However, the spectra variations observed in TOFMS produce artifacts (false peaks) in the decoded spectra making the Hadamard approach unsuitable for efficient sensitivity improvements. Systematic overlaps where a small peak from one push is interfered by a more intense peak from a different push can occur due to the encoding method that uses equal intervals between the pushes 50% of the time. This has the effect of decreasing the multiplexing sensitivity of the lower intensity peak by two times when these overlaps occur.

Encoded Frequent Pushing (EFP®)

LECO has developed[7] *Encoded Frequent Pushing (EFP)*, a method of pulsing an orthogonal accelerator multiple times per transient with unique time intervals between each push pulse. This method is particularly well suited for improving the duty cycle of an MR-TOFMS with *FFP,* where the highly resolved ion peaks are sparsely populated across the TOF spectrum. The key concept is that within overlaid mass spectra produced by the multiple pushes, some overlaps are likely to occur between peaks of various ion species. However, if the time intervals between the OA pushes are unique, the overlaps will not be systematic! In addition, the decoding method departs from a traditional 'inverse method' used by Hadamard which is responsible for creating mass spectral artifacts. Instead, a proposed decoding method[7] uses a logical and statistical analysis of spectra overlaps to prevent the production of these spectral artifacts. With *EFP,* the duty cycle is improved proportionally to the increased pushing frequency, meaning that the continuous ion beam is sampled much more efficiently. For example, if the typical non-multiplexed operation of the GC-HRT$^+$ mass spectrometer uses 2 kHz pushing frequency, a multiplexed acquisition with 20 push pulses per transient will increase the pushing frequency to approximately 20 kHz, and in *EFP* mode the sensitivity will also grow by up to 10 times.

The concept diagram in Figure 1 shows how the multiplexed spectrum is composed of multiple push pulses, each with multiple ion species. The resulting multiplexed spectrum includes ion peaks from the many overlapped push pulses and is hard to comprehend. However, an appropriate decoding algorithm takes into account the knowledge of the pushing time sequence such as Ti=TD*i*(i-1)/2, which is one encoding option that ensures unique intervals between any pair of pushes. By knowing the expected push pulse intervals, the decoding (de-multiplexing) algorithm collects the signals from throughout the multiplexed spectrum that relate to the same flight time relative to the multiple push pulses. These signals are numerically processed to resolve overlaps and produce a decoded spectrum.

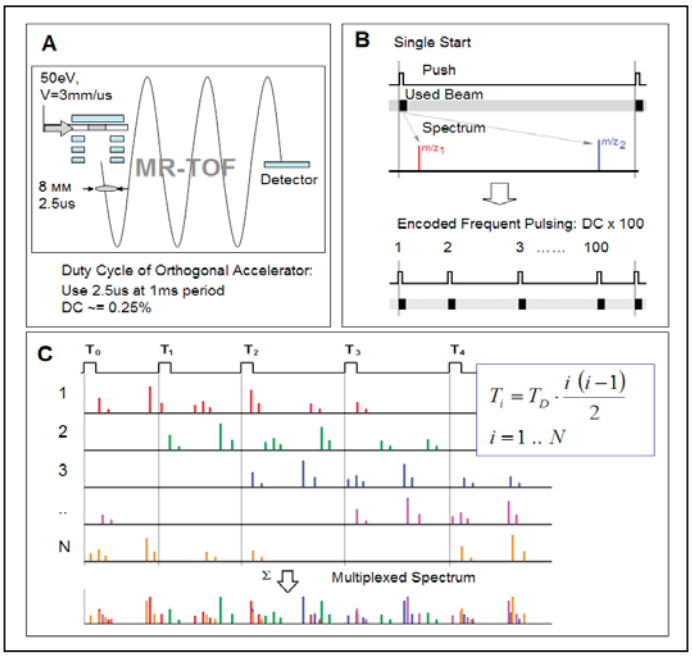

Figure 1. (A) Duty Cycle estimation in the MR-TOFMS; (B) Encoded Frequent Pulsing diagram; (C) concept of *EFP* multiplexing.

The decoding process illustrated in Figure 2 occurs in real time during data acquisition and is fully automated. Note the increase of the ion peaks intensity in *EFP* decoded spectrum.

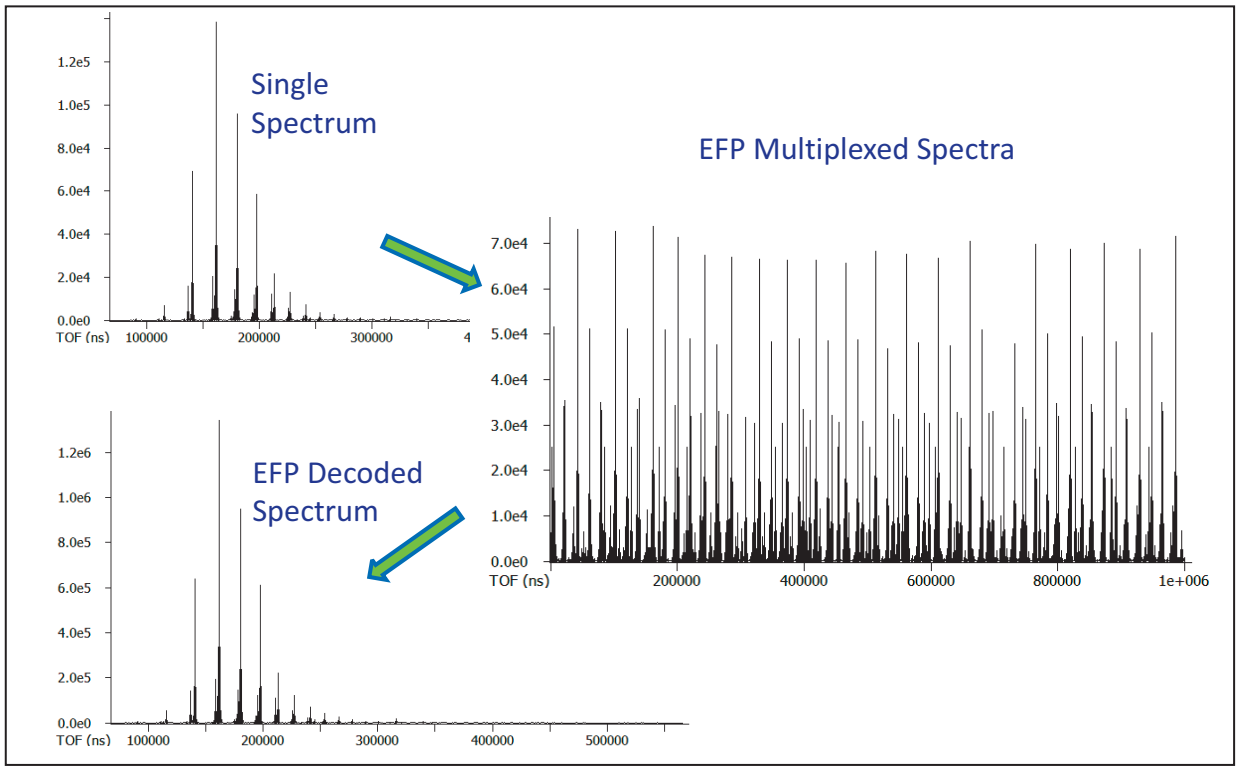

Figure 2. Illustration of the *EFP* workflow: single spectra are acquired (left top) and summed into the multiplexed mass spectrum (right), which is then decoded in real time into the de-multiplexed mass spectrum (left bottom).

EFP Application in High Resolution TOFMS

Sensitivity Gain and Low Abundance Signal Recovery. Figure 3 presents an example of the sensitivity gain provided by implementing *EFP* during GC-HRT$^+$ operation. A trace amount of the analyte is detected in presence of a rich matrix and the results were compared between a single-push analysis data and data acquired with *EFP.* Expectedly, the absolute signal from the analytes grows proportionally to the number of push pulses per transient (~20 in this example), as shown on the Total Ion Current (TIC) traces. Figure 4 and 5 illustrates that more of the low abundant isotopes are reliably detected with *EFP* as compared to with a single push pulse analysis (i.e. without *EFP)* resulting in a much wider dynamic range. As a result, the detection and identification of the trace compounds is much more reliable.

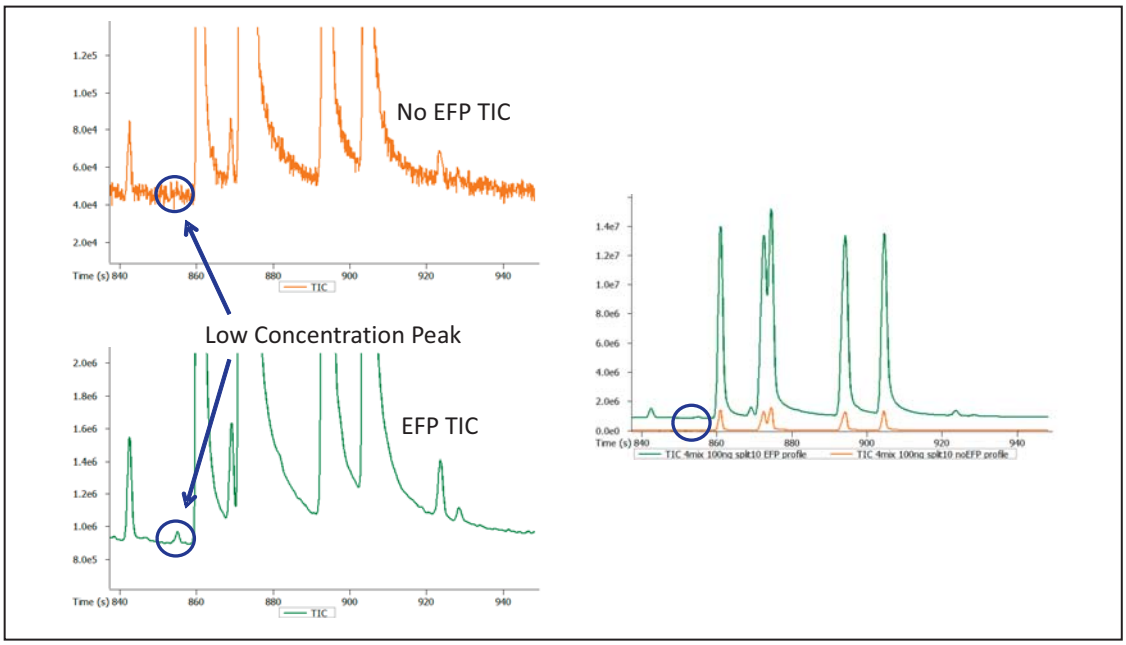

Figure 3. The run without *EFP* (left top, orange trace) doesn't allow detecting of the trace amount analyte, while the analysis with *EFP* (left bottom, green trace) clearly shows that low level analyte is detected.

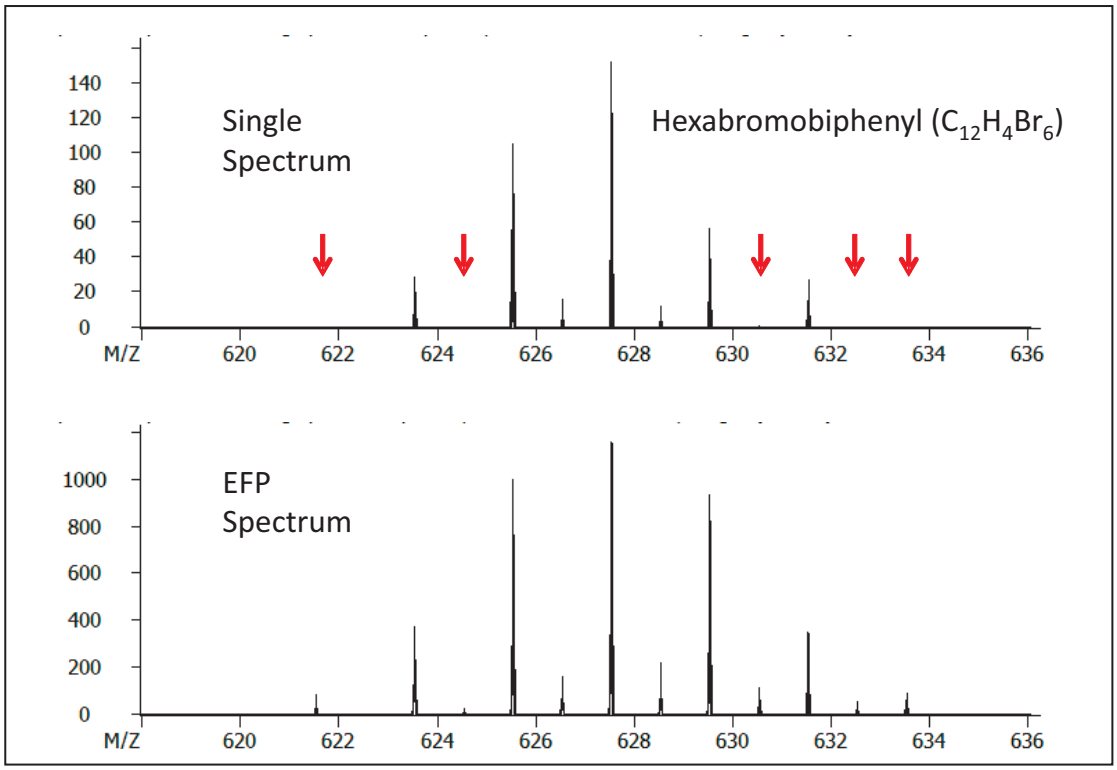

Figure 4. Some isotope peaks in the HBB mass spectrum were not detected due to signal low abundance (top); however the *EFP* runs allows detecting low abundance signals from all isotopes (bottom).

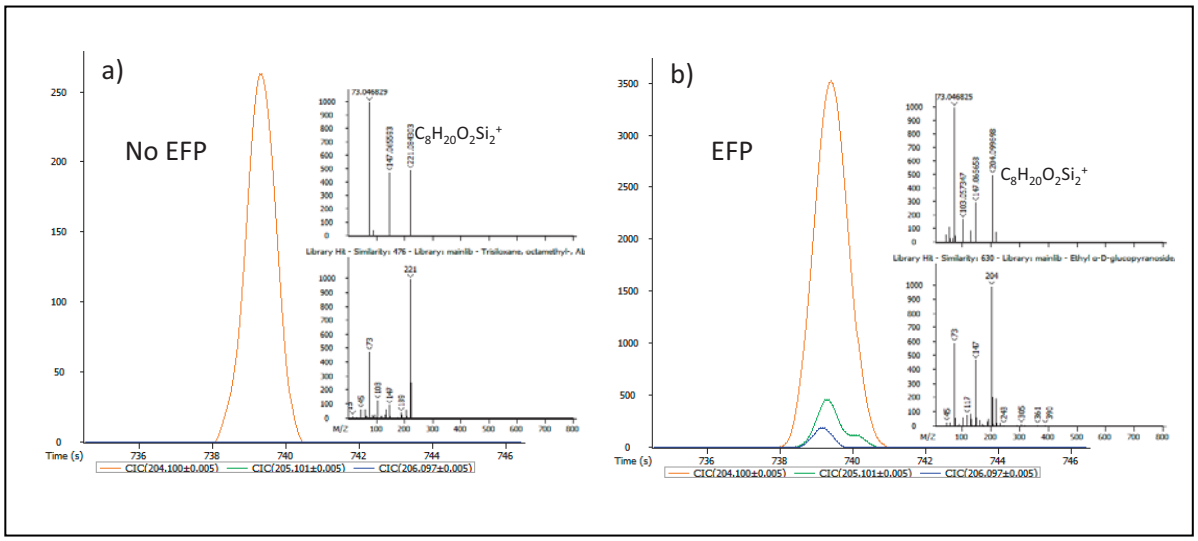

Figure 5. The peak trace of $C_8H_{20}O_2Si_2^+$ (a) was acquired without *EFP,* but no isotopes M+1 and M+2 were detected. The *EFP* run allows detecting main peak of $C_8H_{20}O_2Si_2^+$ (b) with the two following isotopes.

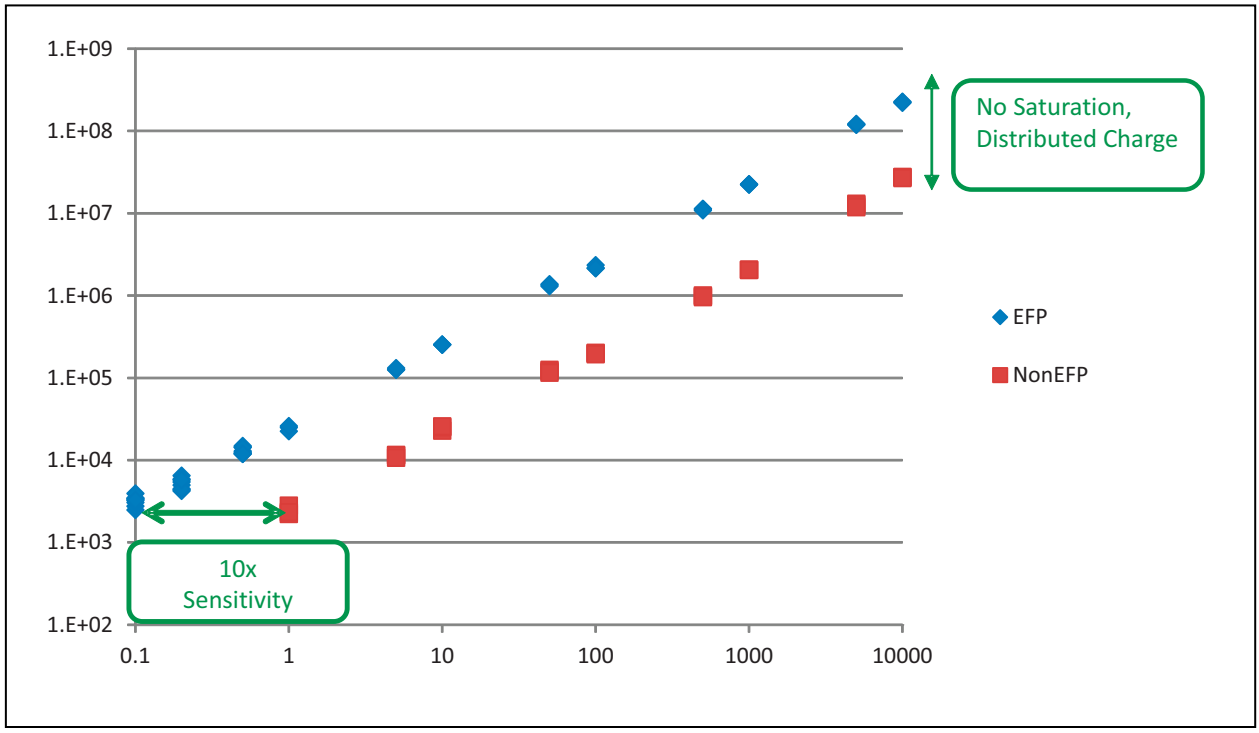

Figure 6. Linear dynamic range (OFN, m/z 271.9866) is expanded towards lower concentrations for *EFP* runs (blue dots), and stays the same at the high concentration end, comparing to analysis without *EFP.*

Linear Dynamic Range Increase. All ion optical components of the mass spectrometer (source, interface, orthogonal accelerator, analyzer, and detector) are operating essentially the same with or without the *EFP.* The only difference is a significantly higher extraction rate—the orthogonal accelerator pulser is working at a higher pushing frequency in *EFP* mode (2 kHz – no *EFP;* 20 kHz – with *EFP).* The sensitivity increase is achieved due to a more efficient use of the ions generated in the ion source. Hence the low end of the linear dynamic range is extended, but the high end of the range stays the same as ion source load was not changed (Figure 6). More ions are entering the mass analyzer, but they are re-distributed throughout the flight path and thus the detector is also not overloaded on a per peak basis. The overall number of ions arriving on the detector is increased, thus a new higher charge capacity detector is used with *EFP* operation.

Reduction of the Electrical and Random Chemical Noise in the Data. The *EFP* decoding algorithm passes signals that indicate the coherence of ions between the multiple push pulses, while non-coherent signals are removed from the data. This feature results in a significant reduction of noise in the data—both electrical and chemical (Figure 7)—reducing data file size and making detection of low-level signals more efficient. The chemical background ions which are persistently present in the mass spectra remain in the data after decoding and the user could use this background information to assess the presence of air leaks and other parameters important for appropriate instrument functioning.

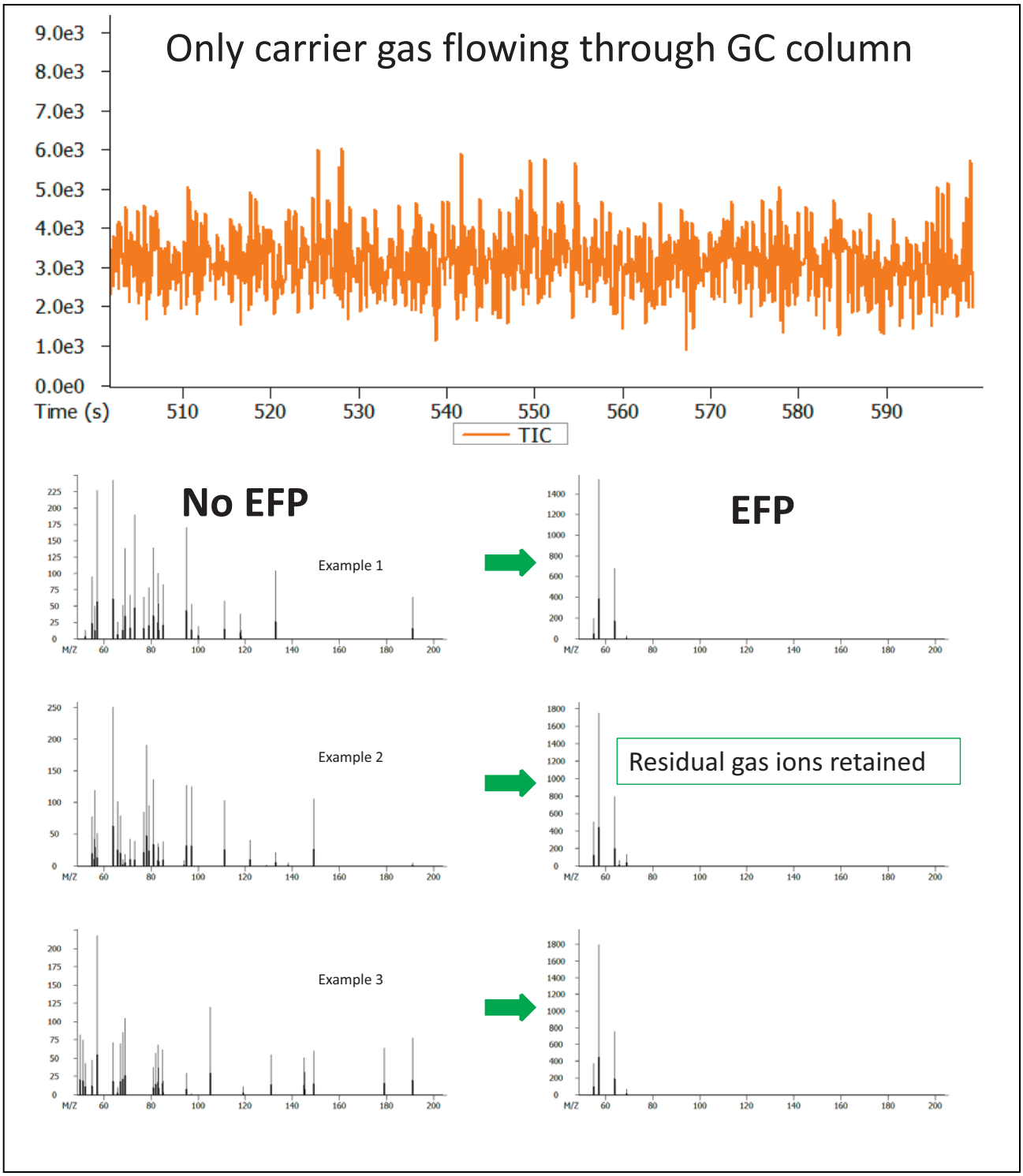

Figure 7 (bottom). *EFP* removes the non-coherent signals created by electrical and chemical noise from the data.

Wide Mass Range—All the Time. In the single spectrum (non *EFP)* mode of TOFMS operation, increasing the extraction frequency is a common way of improving duty cycle and sensitivity. When increasing the extraction frequency, the user has to consider the effect of the reduced TOF period on the maximum mass range of the acquired ions. *EFP* operation removes this limitation and the data can be acquired at the maximum mass range (10-1500 m/z) while enjoying the sensitivity improvement.

Improved Mass Accuracy. The summation of signals from a higher number of push pulses during the *EFP* mode of operation increases the number of ions included in the decoded peaks and thus improves the statistics of the peaks. Mass accuracy depends on resolution and ion statistics (MA~1/(R·√N), where MA – mass accuracy, R – resolution, N – number of ions in the peak). The GCxGC operation of GC-HRT$^+$ requires very high data acquisition speed – up to 200 spectra per second to quantify and deconvolute very narrow (~50 ms) chromatographic peaks. At a 200 spectra per second acquisition rate and 1kHz OA extraction frequency, each data point includes only 5 summed transients, which is very low for achieving high mass accuracy especially for low concentration species. The *EFP* mode of operation increases ion statistics 10 times, which significantly improves mass accuracy for low concentration analytes and in GCxGC mode of acquisition (Figure 8).

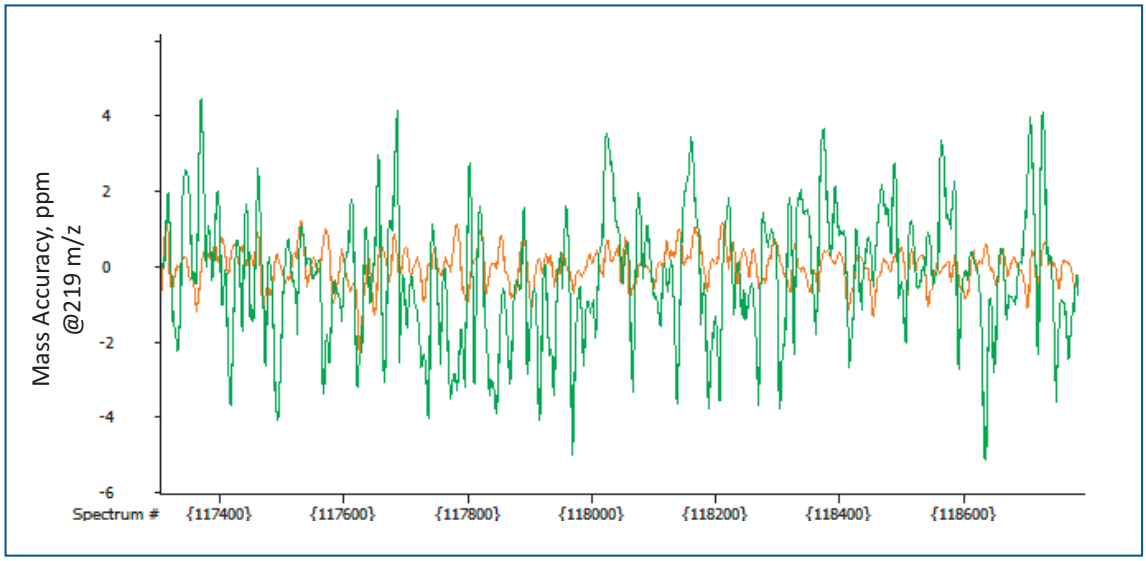

Figure 8. Mass accuracy plots for m/z 219 (PFTBA, Low Flow) at 200 spectra/s: *EFP* (orange) and non-*EFP* (green).

Limitations of the Applicability of the EFP Acquisition Method. The *EFP* decoding algorithms depend on a minimum coherence from the consecutive push pulses. Each mass spectrum is expected to be sparsely populated. This is usually the case with GC-HRT$^+$ operations as high resolution provides ample space between the ion peaks. The samples introduced with GC and ionized in an electron impact source usually do not create very rich spectra and have low chemical noise. However, it is possible to create conditions in GC-HRT analysis when spectra will be highly populated if an extremely high concentration complex mixture is analyzed with minimum chromatographic separation. In this hypothetical case, the *EFP* decoding algorithm will experience difficulties and results might be not as expected. However, in such cases, a good chromatographic separation, and GCxGC separation in particular, will benefit the results of analysis of complex highly concentrated mixtures when using *EFP.*

Conclusions

The *Encoded Frequent Pushing* technology, as it applied to high resolution *FFP* TOFMS, expands the analytical capabilities of the LECO GC-HRT$^+$ and GCxGC-HRT$^+$ instruments, demonstrating the following advantages:

- Significant increase of sensitivity
- Expanded dynamic range
- Wide mass range—all the time
- Improved mass accuracy for low concentration analytes
- Low levels of chemical and electrical noise in the acquired data

References

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