

Alignment and Filtering Tools for Enhanced Differencing of Two-Dimensional Chromatograms

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Introduction

Comparative analysis of two-dimensional chromatograms can reveal subtle differences between samples, including peaks present in one chromatogram but absent in another. Such comparisons are essential not only for sample classification, but also for evaluating method robustness and supporting method development and transfer across instruments, detectors, and operating conditions.

Challenges

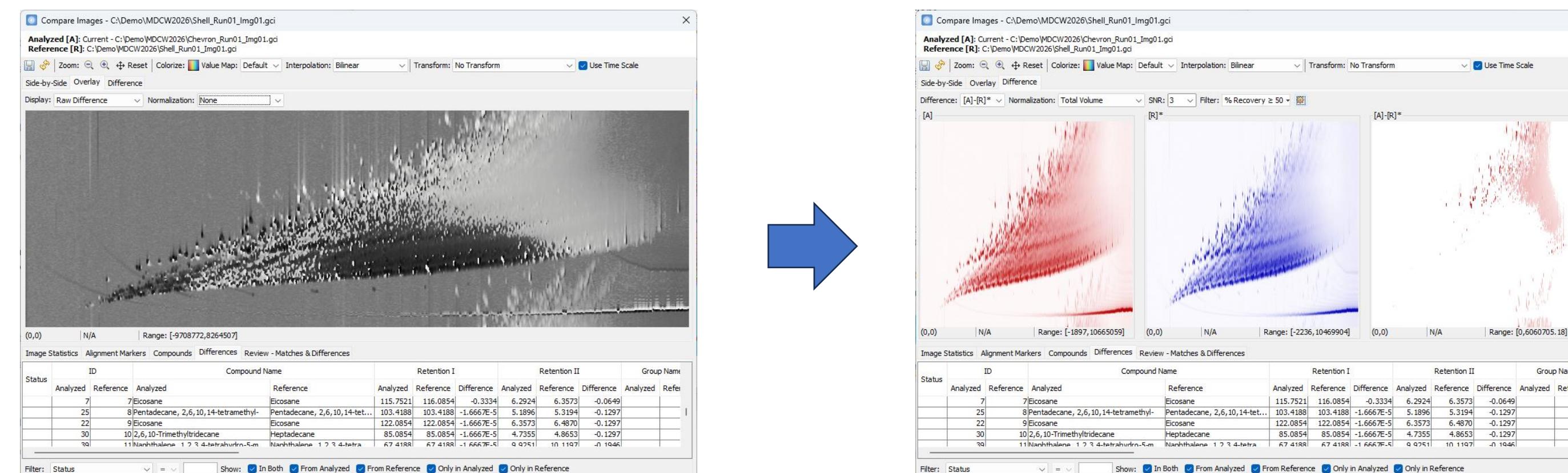
Chromatogram-like visual differencing is intuitive, yet simple pixel-to-pixel subtraction often produces noisy artifacts, fails to correct retention-time shifts, and can obscure low-intensity but meaningful differences.

Previously, we developed comparative visualization methods [1] based on classic image-comparison techniques, including:

- Alignment with specialized color maps to visually highlight discrepancies between chromatograms
- Interactive matching across two chromatograms [2], enabling both qualitative and quantitative side-by-side assessment at the individual-peak level

We extend this work by presenting a more robust chromatogram differencing visualization method designed to overcome these limitations.

Enhanced Differencing Tools



Our enhanced differencing tool highlights what is present in the first chromatogram but absent or diminished in the second. It goes beyond standard subtraction methods, integrating advanced alignment and transformation capabilities to deliver highly accurate results, including:

- **Alignment:** Leverages alignment markers and transformations to ensure a reliable side-by-side comparison.
- **Fuzzy Radius:** Optimizes pixel correspondence by accommodating local retention-time and intensity variations.
- **Normalization Options:** Adjust how the second chromatogram is normalized relative to the first for improved comparison clarity.
- **Filtering:** Applies specified % Recovery or Fold Change to refine difference results while accounting for baseline fluctuations measured via SNR.

References

1. B. Hollingsworth et al., Comparative Visualization for Comprehensive Two-Dimensional Gas Chromatography. *J Chromatogr A*. 2006 Feb 10;1105(1-2):51-8.
2. Q. Tao et al., New Peak-Based Differencing Tools for Side-by-Side Comparison of Two Samples with GCxGC-MS. GCxGC Symposium, May 2018.
3. B. Wiegler et al., 2020, "Benchmark GCxGC Data, Chocolate", <https://doi.org/10.7910/DVN/AKT6BH>, Harvard Dataverse, V1.
4. S. Krieger, Fingerprinting Analysis of Different Types of Beer. *LC GC Europe*. 2015, 28, 7, 414-414.

❖ Data Set: GCxGC data of a diesel fuel sample

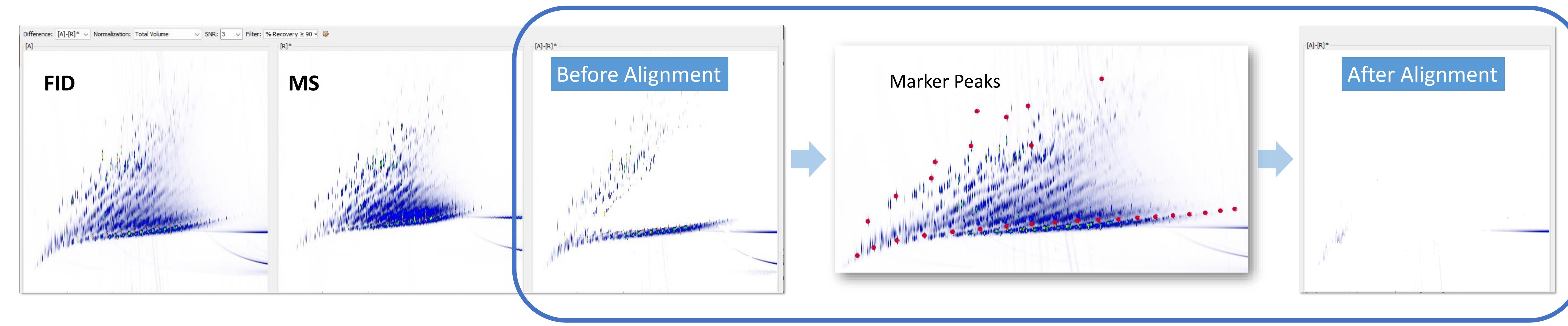
❖ Instruments: Agilent 7890A GC/InfoMass L10 thermal modulator/Hexin TOFMS

- Detectors: FID (350 C, 200 Hz), MS (45-450 m/z, 50 Hz)
- Modulation: 6 seconds with programmable cold jet gas flow (15L/min to 6 L/min)

❖ Data Processing: Align FID and MS data to enable meaningful data fusion (e.g., combining MS selectivity with FID quantitation)

- Alignment: An affine transform calculated from 29 matched marker peaks is applied.
- Normalization: Both chromatograms are normalized to their total response prior to differencing, compensating for the large response-magnitude differences between FID and MS detectors.
- Filters: SNR = 3, % Recovery \geq 90%, Col II Fuzzy Radius = 0

Example 1: Alignment for Data Fusion (FID vs MS)



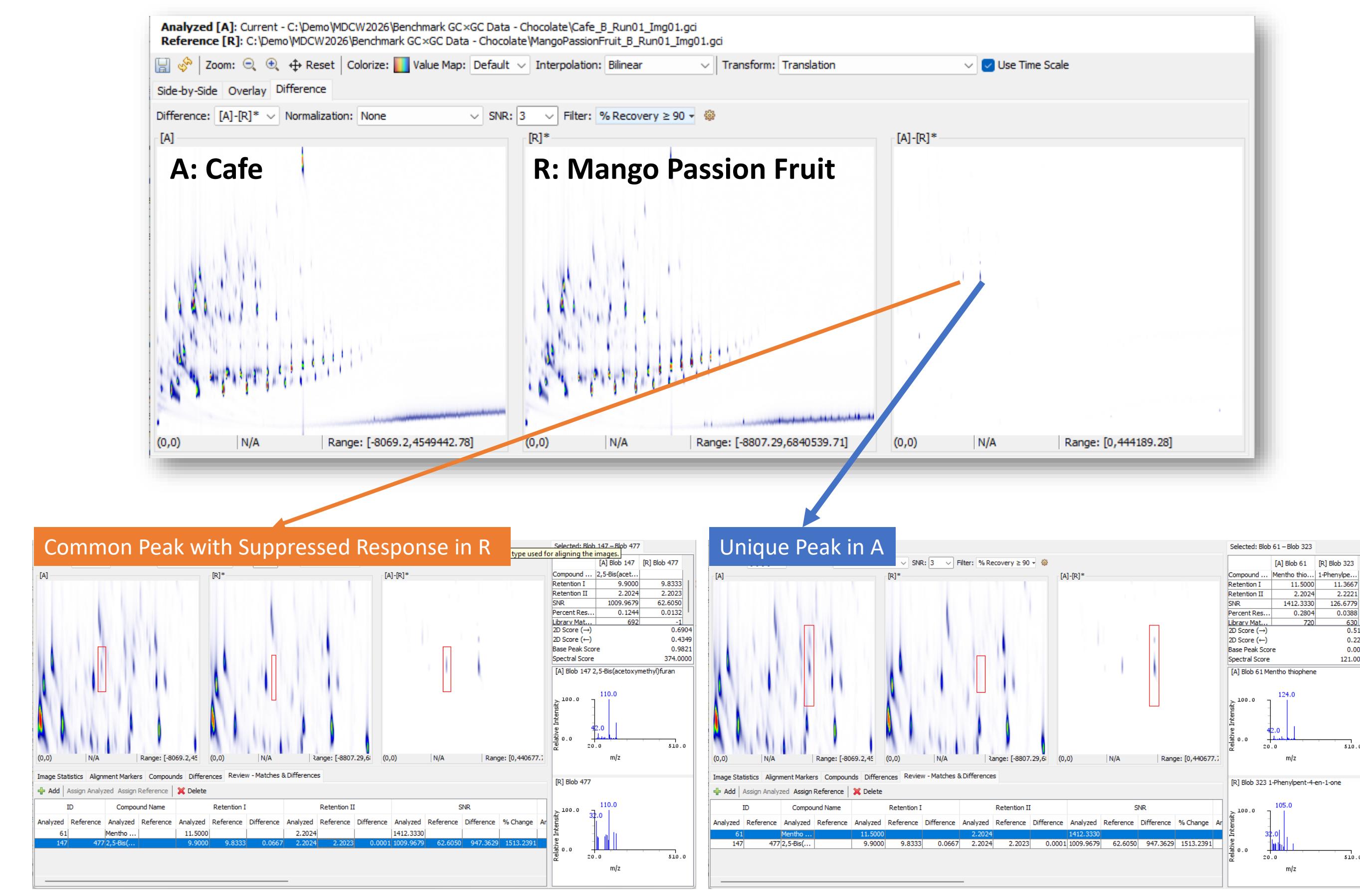
Example 2: Differencing (GCxGC-MS)

❖ Data Set: GCxGC-TOFMS data set of different dark chocolates [3].

❖ Instruments: JEOL AccuTof GC mass spectrometer with an Agilent 7890 GC

❖ Data Processing:

- Alignment: Automatic pairing peaks with identical compound names from library search
- Filters: SNR = 3, % Recovery \geq 90%, Col II Fuzzy Radius = 2



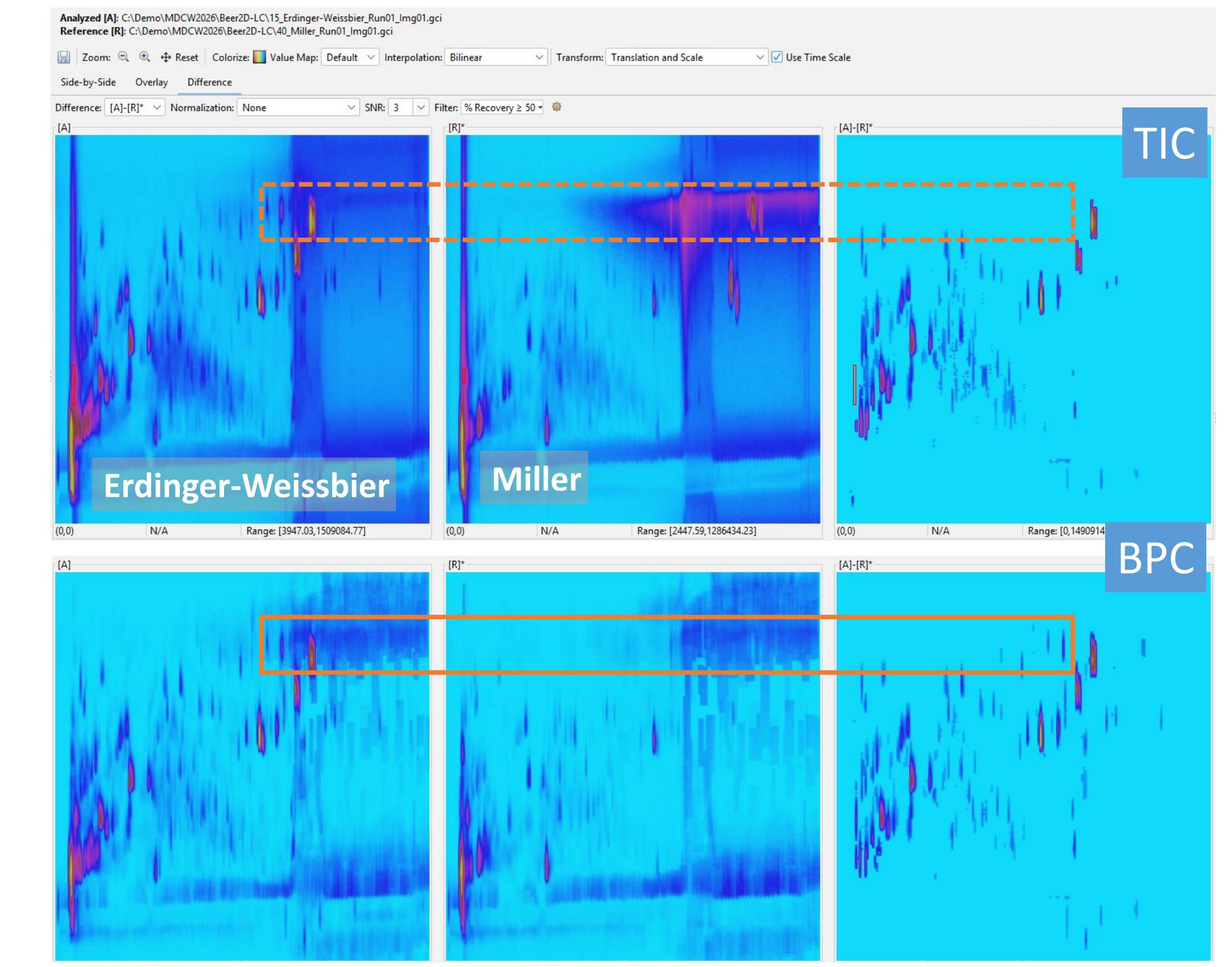
Example 3: Differencing with BPC (LCxLC-MS)

❖ Data Set: LCxLC-MS data of different types of beer [4].

❖ Instruments: Agilent 1290 Infinity 2D-LC solution coupled with an Agilent 6530 Accurate-Mass Q-TOF LC/MS system

❖ Data Processing:

- Preprocessing: An ion-peak-based BPC mask is extracted from one chromatogram and applied to the other, effectively filtering dominant background and improving SNR.
- Filters: SNR = 3, % Recovery \geq 90%, Col II Fuzzy Radius = 0



GC Image
Software for Multidimensional Chromatography

InfoMass LLC