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

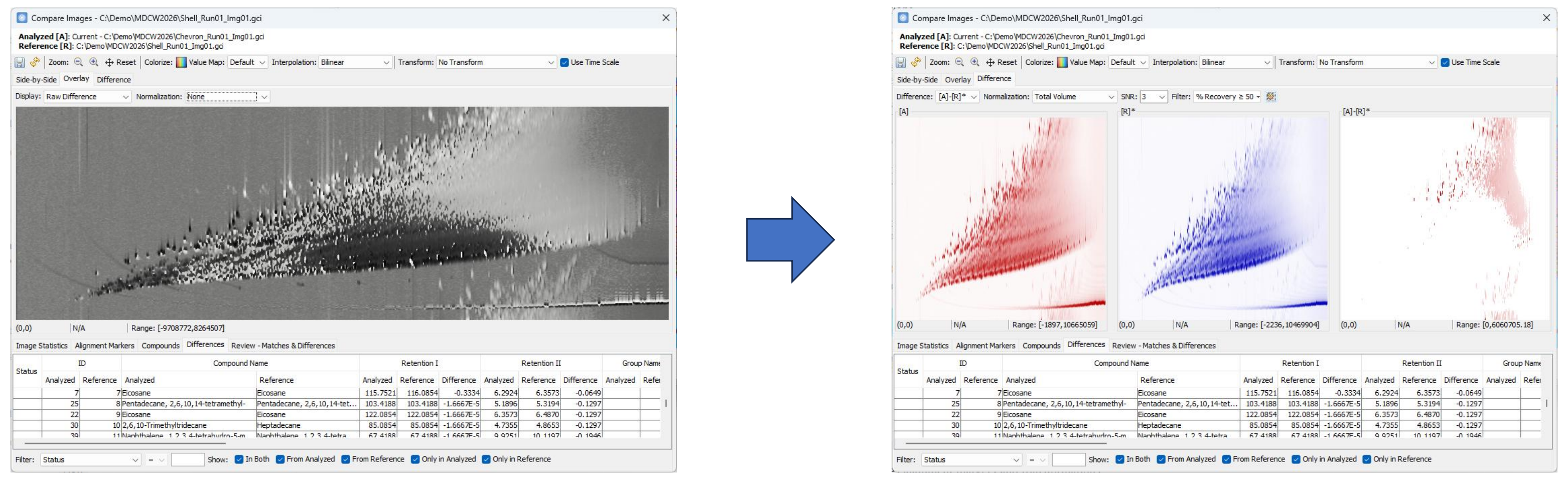
Chromatogram-like visual differencing is intuitive, yet simple pixel-to-pixel subtraction often produces noisy artifacts, fails to correct retention-time shifts, and can obscure low-intensity but meaningful differences.

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

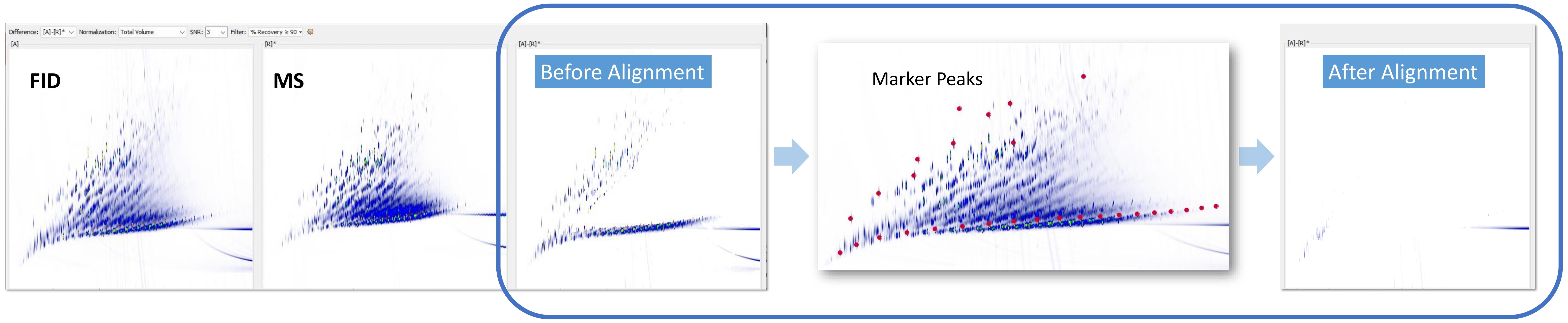


Our enhanced differencing tool highlights what is present in the first chromatogram but absent or diminished in the second. It goes beyond standard subtraction methods, integrating advanced alignment and transformation capabilities to deliver highly accurate results, including:

- **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.

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

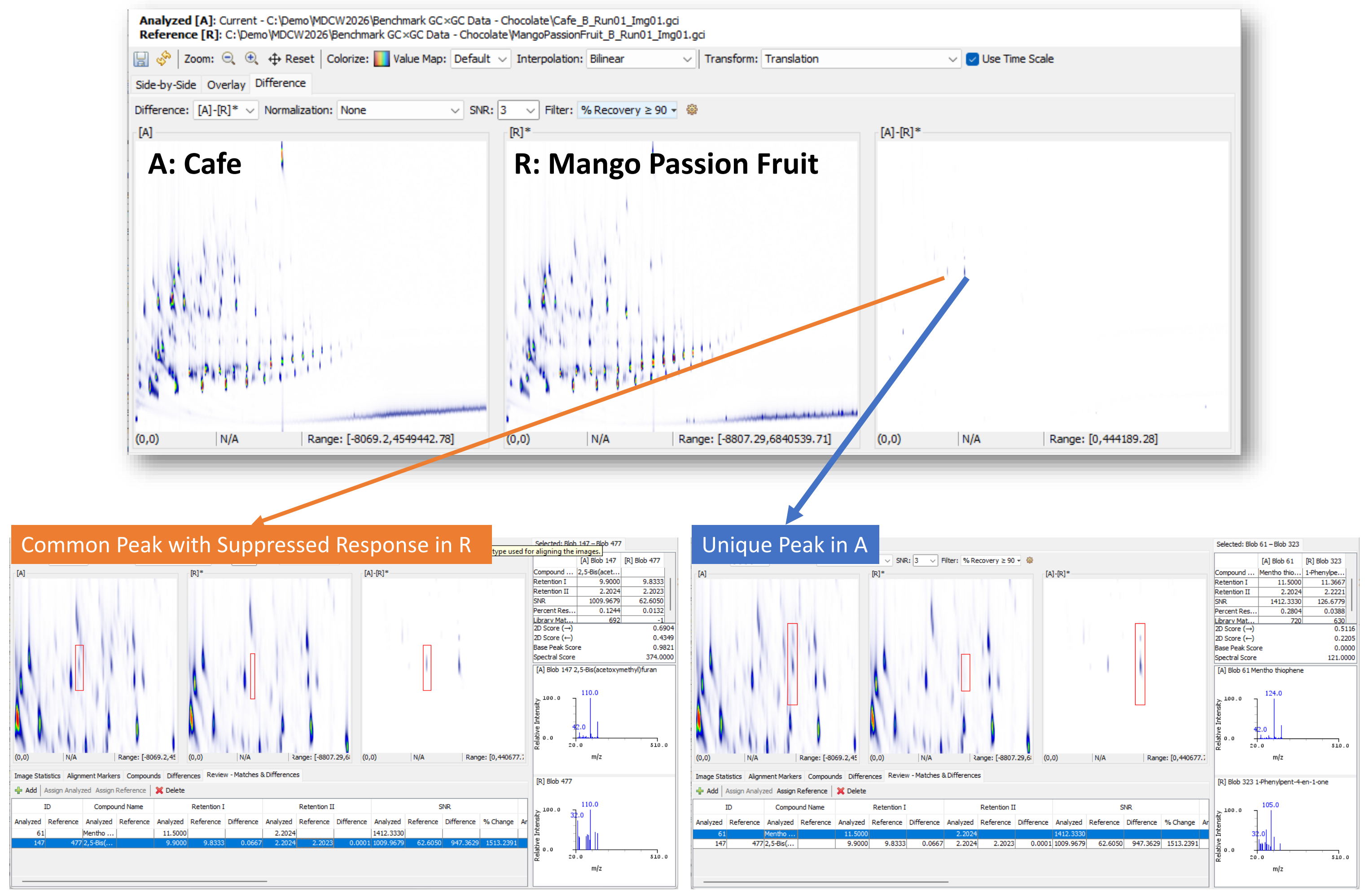
- ❖ Data Set: GCxGC data of a diesel fuel sample
- ❖ Instruments: Agilent 7890A GC/InfoMass L10 thermal modulator/Hexin TOFMS
  - Detectors: FID (350 C, 200 Hz), MS (45-450 m/z, 50 Hz)
  - Modulation: 6 seconds with programmable cold jet gas flow (15L/min to 6 L/min)
- ❖ Data Processing: Align FID and MS data to enables 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 ≥ 90%, Col II Fuzzy Radius = 0



Difference: [A]-[R]* Normalization: Total Volume SNR: 3 Filter: % Recovery ≥ 90

Example 2: Differencing (GC×GC-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 ≥ 90%, Col II Fuzzy Radius = 2



Analyzed [A]: Current - C:\Demo\MDCW2026\Benchmark GC×GC Data - Chocolate\Cafe_B_Run01_Img01.gci
Reference [R]: C:\Demo\MDCW2026\Benchmark GC×GC Data - Chocolate\MangoPassionFruit_B_Run01_Img01.gci

Value Map: Default Interpolation: Bilinear Transform: Translation ☑ Use Time Scale

Difference: [A]-[R]* Normalization: None SNR: 3 Filter: % Recovery ≥ 90

[A] Range: [-8069.2,4549442.78]
[R]* Range: [-8807.29,6840539.71]
[A]-[R]* Range: [0,444189.28]

Selected: Blob 147 – Blob 477

	[A] Blob 147	[R] Blob 477
Compound ...	2,5-Bis(acet...	
Retention I	9.9000	9.8333
Retention II	2.2024	2.2023
SNR	1009.9679	62.6050
Percent Res...	0.1244	0.0132
Library Mat...	692	-1
2D Score (→)		0.6904
2D Score (←)		0.4349
Base Peak Score		0.9821
Spectral Score		374.0000

[A] Blob 147 2,5-Bis(acetoxymethyl)furan

[R] Blob 477

Selected: Blob 61 – Blob 323

	[A] Blob 61	[R] Blob 323
Compound ...	Mentho thio...	1-Phenylpe...
Retention I	11.5000	11.3667
Retention II	2.2024	2.2221
SNR	1412.3330	126.6779
Percent Res...	0.2804	0.0388
Library Mat...	720	630
2D Score (→)		0.5116
2D Score (←)		0.2205
Base Peak Score		0.0000
Spectral Score		121.0000

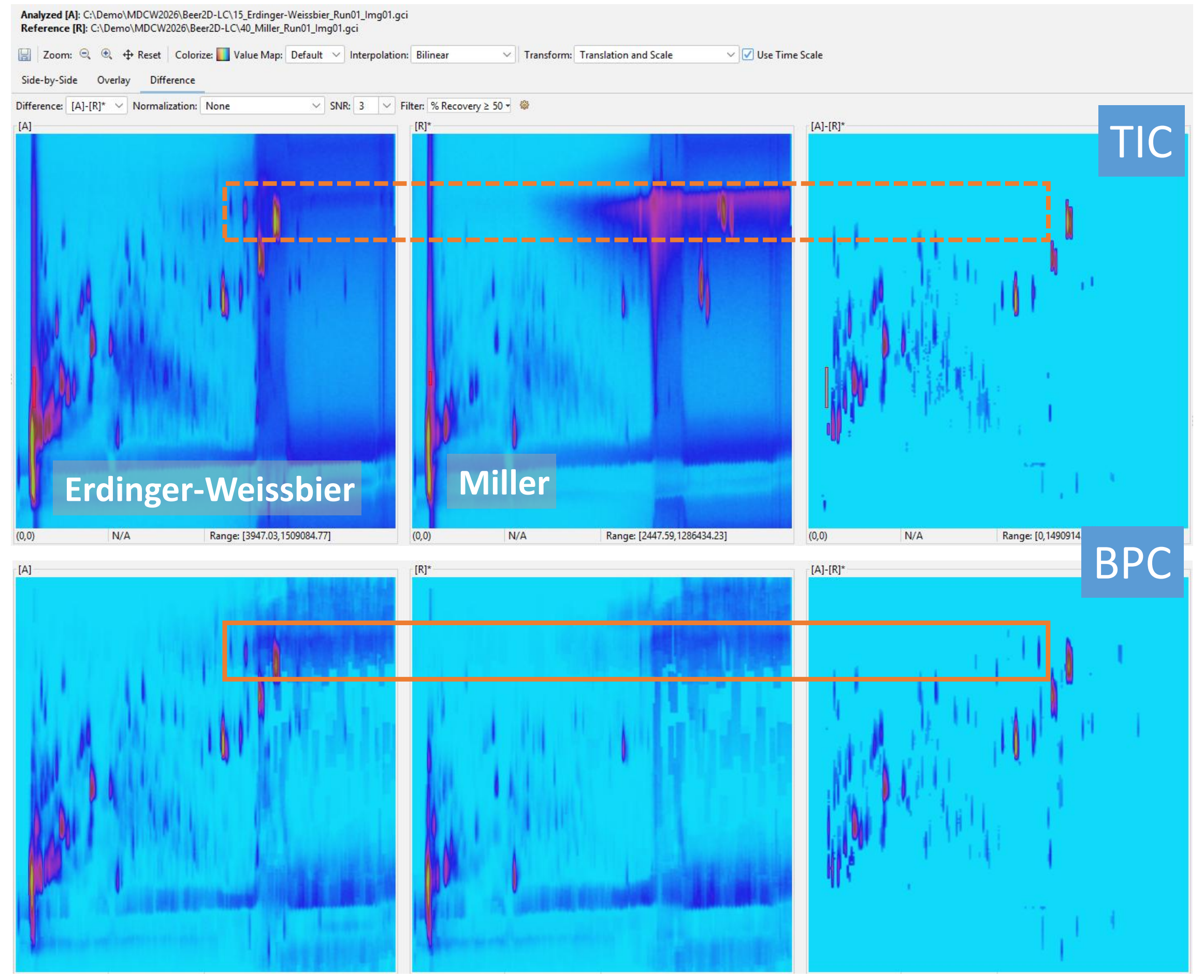
[A] Blob 61 Mentho thiophene

[R] Blob 323 1-Phenylpent-4-en-1-one

ID		Compound Name		Retention I			Retention II			SNR			
Analyzed	Reference	Analyzed	Reference	Analyzed	Reference	Difference	Analyzed	Reference	Difference	Analyzed	Reference	Difference	% Change
61		Mentho ...		11.5000			2.2024			1412.3330			
147	477	2,5-Bis(...		9.9000	9.8333	0.0667	2.2024	2.2023	0.0001	1009.9679	62.6050	947.3629	1513.2391

Example 3: Differencing with BPC (LC×LC-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 ≥ 90%, Col II Fuzzy Radius = 0



Analyzed [A]: C:\Demo\MDCW2026\Beer2D-LC\15_Erdinger-Weissbier_Run01_Img01.gci
Reference [R]: C:\Demo\MDCW2026\Beer2D-LC\40_Miller_Run01_Img01.gci

Value Map: Default Interpolation: Bilinear Transform: Translation and Scale ☑ Use Time Scale

Difference: [A]-[R]* Normalization: None SNR: 3 Filter: % Recovery ≥ 50

[A] Range: [3947.03,1509084.77]
[R]* Range: [2447.59,1286434.23]

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. Weggler 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 processes and screenshots for this publication are from an alpha version of GC Image v2026 (Visit www.gcimage.com for current v2025 releases)

GC Image
Software for Multidimensional Chromatography

InfoMass LLC