NEW PEAK-BASED DIFFERENCING TOOLS FOR SIDE-BY-SIDE COMPARISONS OF TWO SAMPLES WITH GCXGC-MS

Qingping Tao;[1] Stephen E. Reichenbach;[1,2] Chase Heble;[1]
[1]GC Image, LLC, Lincoln NE; [2]University of Nebraska, Lincoln NE

Introduction

Identifying chemical differences among samples is useful for process monitoring, sample classification or identification, correlative determinations, and other important tasks. Comprehensive two-dimensional gas chromatography (GCxGC) is a powerful technique for highly effective chemical separations of complex mixtures. Coupled with mass spectrometry, GCxGC-MS offers analytical advantages of chromatographic resolution, peak capacity, and mass spectral identification, but it also produces highly complex data that require both interactive and automated comparative analysis methods.

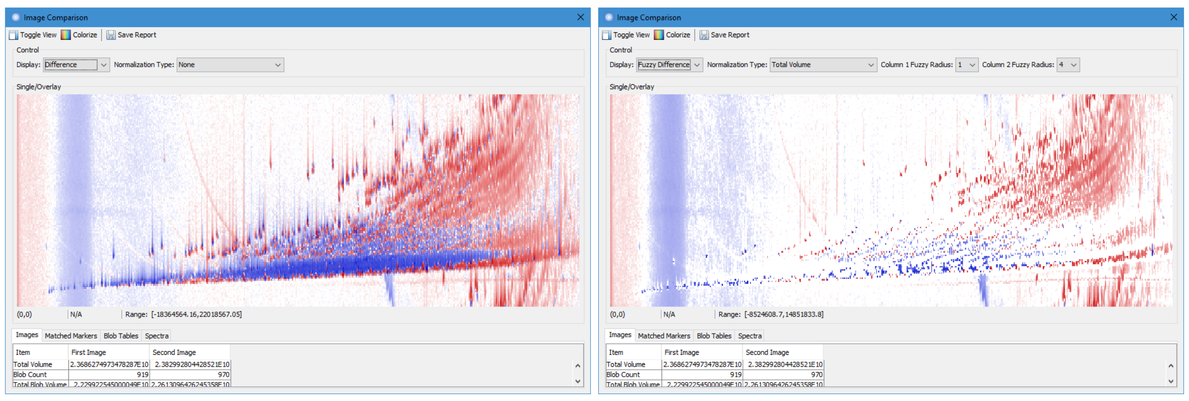
One common task in GCxGC analysis is to compare two chromatograms to determine differences or changes due to chemical components or experimental conditions. A new comparative tool provides a detailed comparison of two chromatograms, utilizing advanced matching techniques and informative visualizations.

Challenge 1 – Raw Data Alignment

With two samples/chromatograms involved, there are many challenges to obtaining a detailed comparison. These challenges include:

- Aligning the two chromatograms;
- Normalizing intensities to remove sample amount variations;
- Tolerating small misalignments and differences in peak shapes in local regions.

A simple side-by-side or overlay visualization does not fully address these challenges. Our previous comparative visualization methods apply our template matching technology for alignment and present difference chromatograms with specialized color maps to visualize differences.



Difference vs. Fuzzy Difference with Normalization

Challenge 2 – Peak Matching

Furthermore, in order to characterize chemical differences between two samples, both qualitatively and quantitatively, it is necessary to compare individual compounds detected in both samples. This kind of comparison is often non-targeted and requires:

- Detecting all peaks from two chromatograms;
- Matching all detected peaks between two chromatograms.

An ideal tool should provide not only comparative visualization, but also comprehensive characterization of sample differences down to individual peaks.

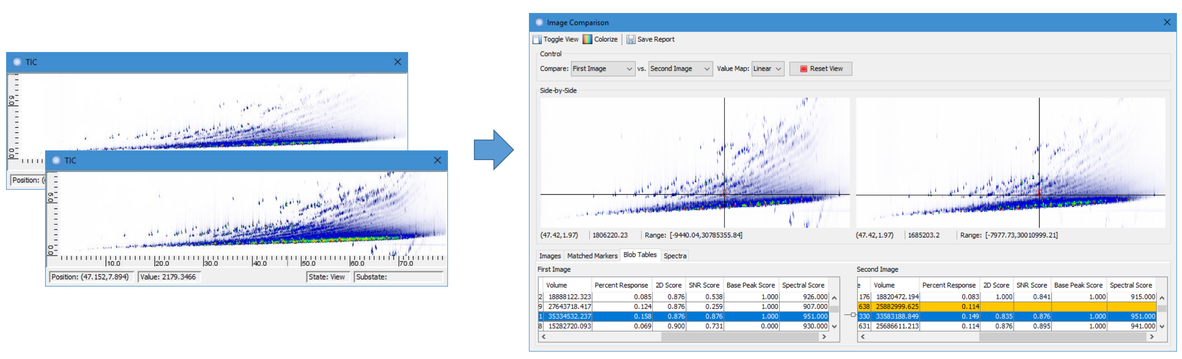
Our Approach

The new side-by-side differencing tool adapts the reliable peak and peak region alignment methods from our Investigator™ framework, enables comprehensive peak matching between two chromatograms using both chromatographic and spectral information, and automates difference detection.

The approach includes the following steps:

1. Detect peaks and their regions in each chromatogram.
2. Determine an alignment transform based on auto-generated reliable peaks or user-specified marker peaks.
3. Match peaks between the two chromatograms using an alignment transform and the intersections of peak apexes and regions.
4. Calculate match scores and differences of the matched peaks.

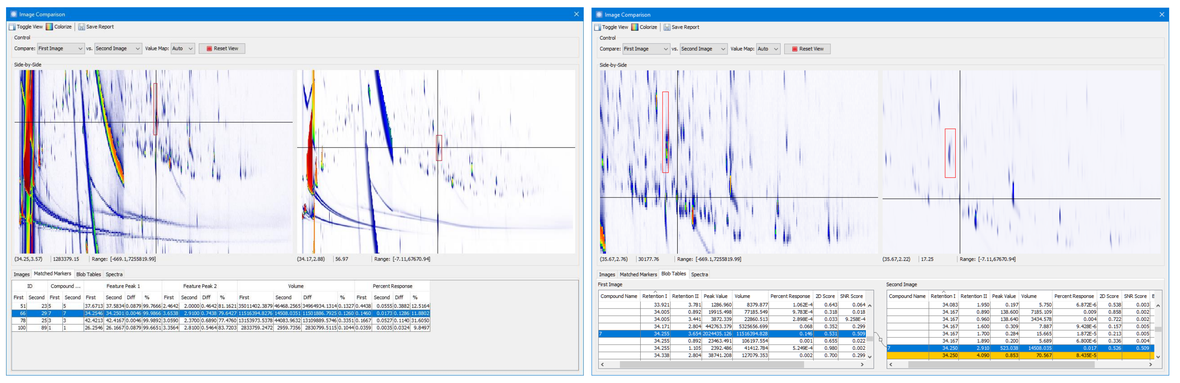
In addition, the differencing tool provides a set of visualizations that allow users to interactively review the chromatograms, the alignment transformation, and peak matches.



Application 1: MS vs FID Comparison

- Samples: Urine samples of adults with type 2 diabetes **[1]**.
- Instruments: Agilent 6890 GC with a Zoex thermal modulator and a microfluidic splitter from the ^{1}D column into two parallel ^{2}D columns coupled with FID and MS detectors. The modulation cycle was 5 seconds. The FID sampling rate was 100 Hz and the MS performed full scans (50−350 m/z) at 24 Hz.

Alignment is provided by a simple transform using translation and scaling calculated from 4 manually selected marker peaks.

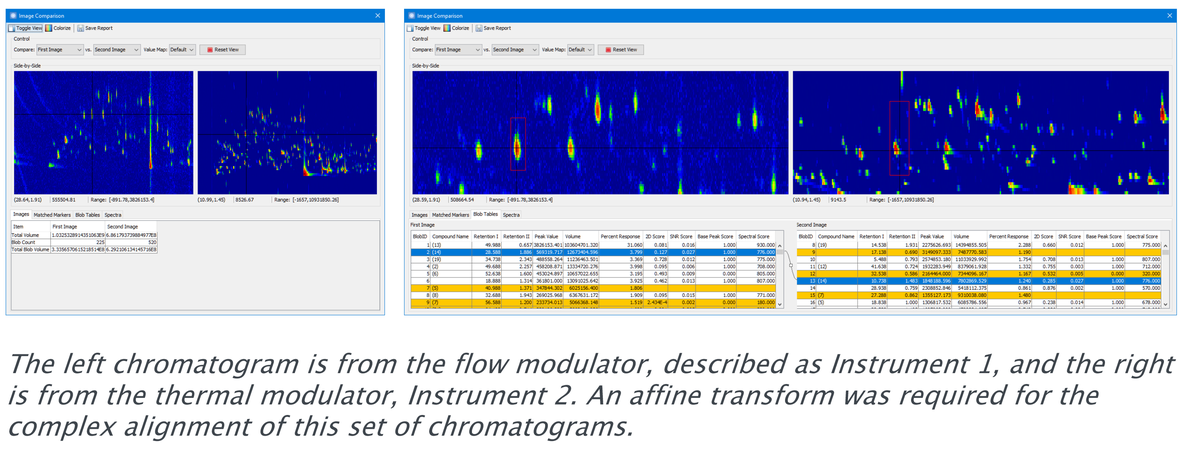


The left chromatogram is the from the MS detector and the right is from the FID. The results can be reviewed using the Matched Markers table shown in the left picture. In the right picture, a matched peak is shown in the Blob Tables and zoomed into on the chromatogram view.

Application 2: Flow vs Thermal Comparison

- Samples: Multiple runs of a single sample of roasted cocoa from Ecuador **[2]**.
- Instrument 1 – Reverse-inject differential flow modulator (Agilent): Agilent 7890B GC unit coupled to an Agilent 5977A fast quadrupole MS detector with modulation of 3 seconds and sampling rate of 35Hz.
- Instrument 2 – Thermal modulator (Zoex): Agilent 6890 unit coupled to an Agilent 5975C MS detector with modulation of 3 seconds and sampling rate of 29 Hz.

Alignment is provided by an affine transform calculated from 20 manually selected marker peaks.

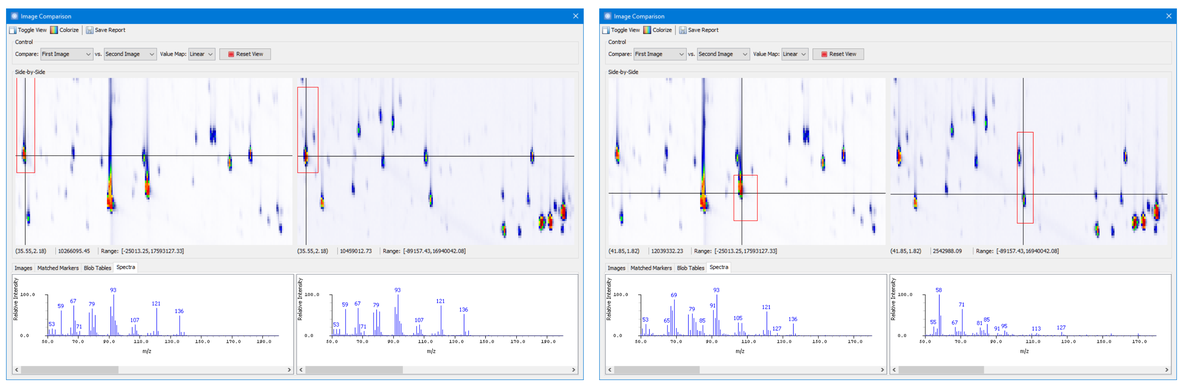


The left chromatogram is from the flow modulator, described as Instrument 1, and the right is from the thermal modulator, Instrument 2. An affine transform was required for the complex alignment of this set of chromatograms.

Application 3: Sample Comparison

- Samples: Non-targeted analysis of 10 essential oils **[3]**.
- Instrument: Agilent 7890A/ZOEX ZX2 thermal modulation system coupled with Agilent 7200 Q-TOF

Alignment is provided by a simple transform using translation and scaling calculated from 3 common peaks automatically detected by Investigator.



The left chromatogram is Lavender and the right is Ginger. Spectral graphs were used to review pairs of peaks in the matched RT regions. The left pair have two similar spectra, while the right pair have two spectra that do not match.

References

1. S. E. Reichenbach, D. W. Rempe, Q. Tao, D. Bressanello, E. Liberto, C. Bicchi, S. Balducci, and C. Cordero, "Alignment for Comprehensive Two-Dimensional Gas Chromatography with Dual Secondary Columns and Detectors," Analytical Chemistry, vol. 87, issue 19, 10056-10063, 2015.
2. C. Cordero, P. Rubiolo, S. E. Reichenbach, A. Carretta, L. Cobelli, M. Giardina, and C. Bicchi, "Method translation and full metadata transfer from thermal to differential flow modulated comprehensive two dimensional gas chromatography: Profiling of suspected fragrance allergens." Journal of Chromatography A, 1480, 70-82, 2017.
3. Q. Tao, S. E. Reichenbach, C. Heble, and Z. Wu. "New Investigator Tools for Finding Unique and Common Components in Multiple Samples with Comprehensive Two-Dimensional Chromatography." Chromatography Today, 11, 13-18, 2018.
4. Data processes and screenshots for this publication are from a beta version of GC Image 2.8 GCxGC-HRMS. (Visit www.gcimage.com for the current R2.7 public release and additional information.)

GC IMAGE
Software for Multidimensional Chromatography

UNIVERSITY OF Nebraska Lincoln