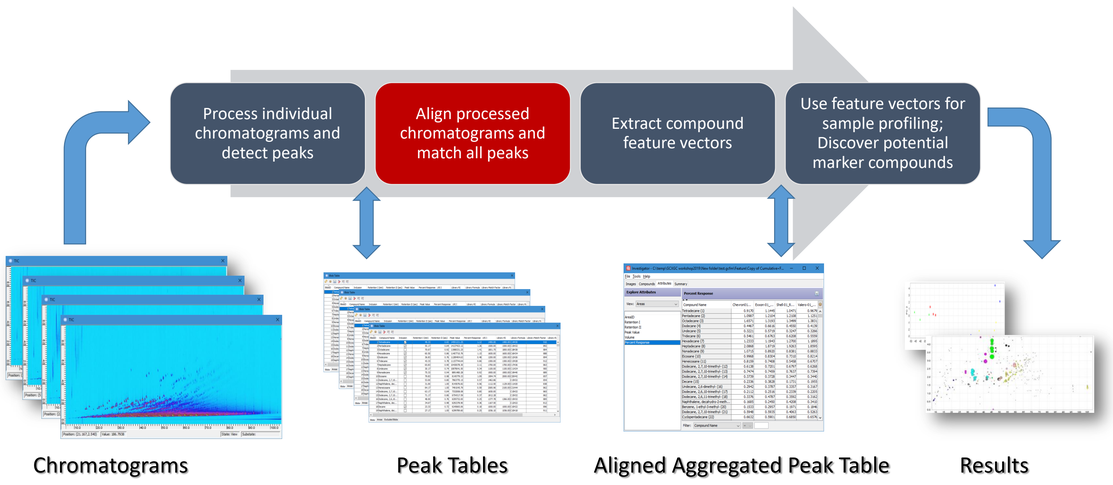
Extended Investigator Workflow and Tools for Directly Aligning Peak Tables of GCxGC-MS Data

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

Introduction

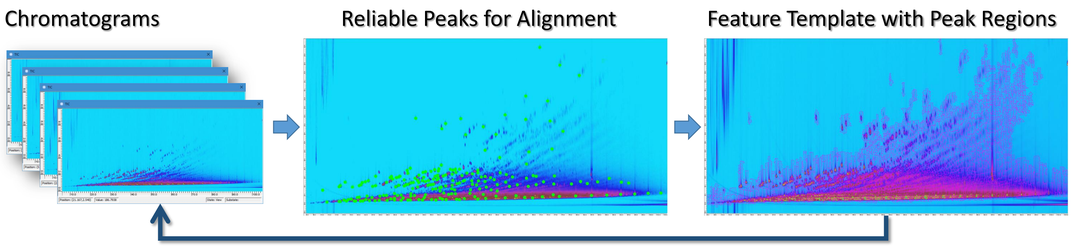
Comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GCxGC-MS) is a powerful technique for highly effective chemical separations of complex mixtures, and is increasingly used for cross-sample analyses such as sample classification and biomarker discovery. For these analyses, it is necessary to have a data analysis workflow that can compare multiple samples to determine similarities and differences. The center of this workflow is to align peaks across all chromatograms and extract an aggregated peak table, which continues to be one of the most difficult problems.



Investigator Workflow

Our Investigator framework **[1]** analyzes data from multiple samples to extract a peak-region based feature template that comprehensively captures the pattern of peaks detected in the retention-times plane. Then, for each sample chromatogram, the template is transformed to align with the detected peak pattern and generate a set of feature measurements for cross-sample analyses.

Example: Non-targeted analysis of 4 brands of diesel fuel **[2]**.
- ❖ Instrument: Shimadzu 2010 Ultra GC/MS with Zoex ZX2 thermal modulator
- ❖ 10 samples were collected for each brand, for a total of 40 chromatograms.

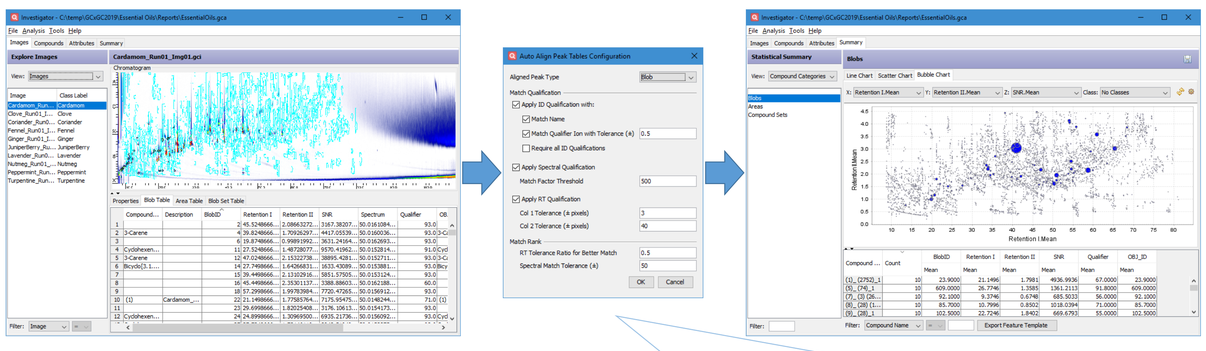


Pros: The approach avoids the intractable problem of comprehensive peak matching. No need to match every peak.
- ➢ Aligning a peak region is more robust than just a peak location. It is a natural tolerance window itself.
- ➢ Peak regions capture any compound as long as it can be detected in at least one sample.

Cons: The approach previously has not utilized peak information from the source chromatograms. Peaks originating from different analytes in different samples may be contained in the same peak-region feature due to close proximity.

Extended Workflow: Peak-Region Features + Peaks

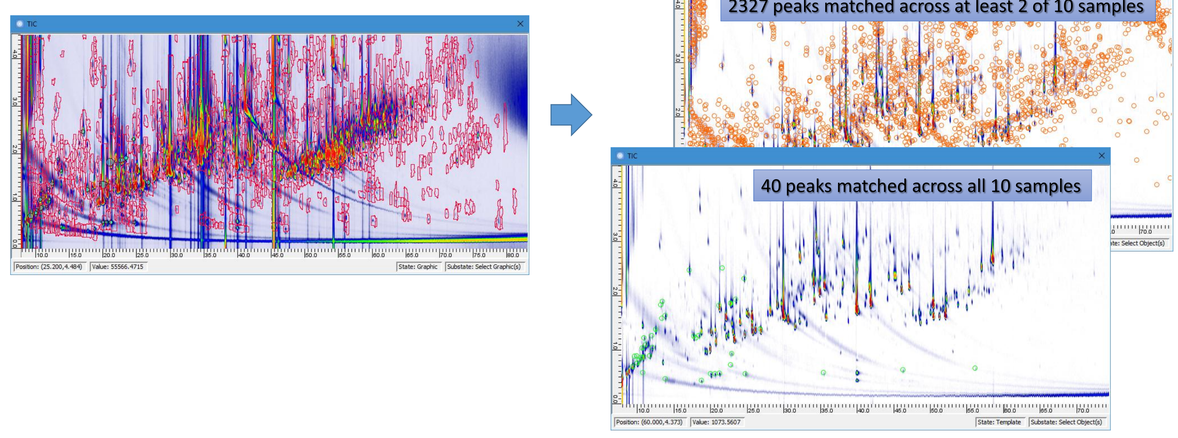
This new workflow extends the Investigator framework to use peak-region features along with ID and spectra matching to align peaks from the source chromatograms.



Example 1: Essential Oils

- ❖ Samples: 10 samples of different types of essential oils **[3]**.
- ❖ Instruments: Agilent 7890A/ZOEX ZX2 thermal modulation system coupled with Agilent 7200 Q-TOF.
- ❖ Data Processing: Peak detection, library search, peak alignment were all performed with an alpha version of GC Image v2.9.

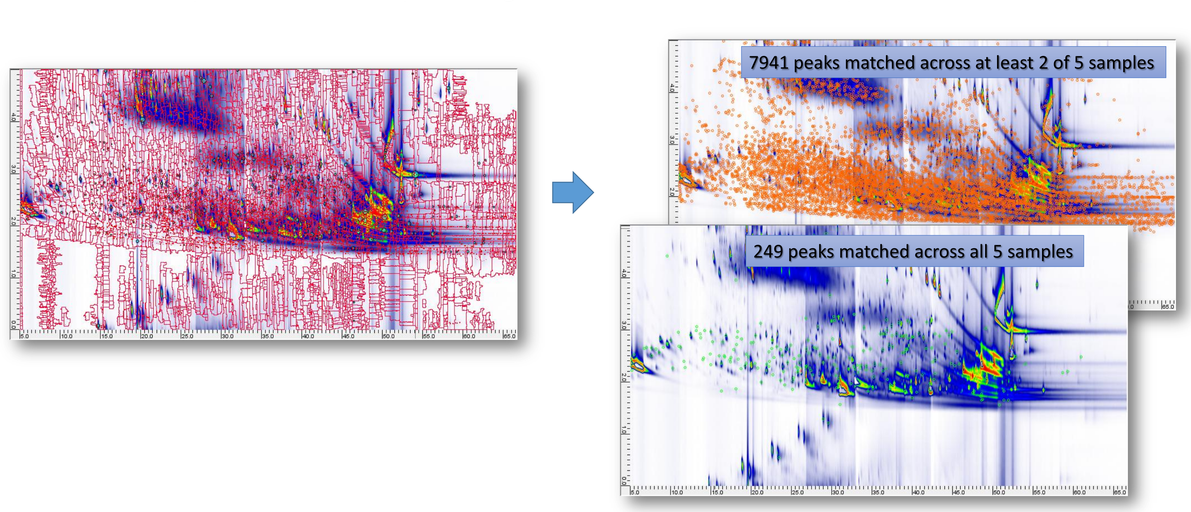
Alignment Results:
- More than 1000 peaks were detected in each sample chromatogram excluding column bleeds, streaks, and other background artifacts.
- 35 reliable peaks and 1374 peak-region features were extracted automatically.



Example 2: Fish Extracts

- ❖ Samples: 5 samples of Fish Extracts from the Great Lakes **[4]**.
- ❖ Instruments: LECO Pegasus 4D GC×GC-HRT.
- ❖ Data Processing: Peak detection and library search were performed with vendor software. Peak alignment was performed with an alpha version of GC Image v2.9.

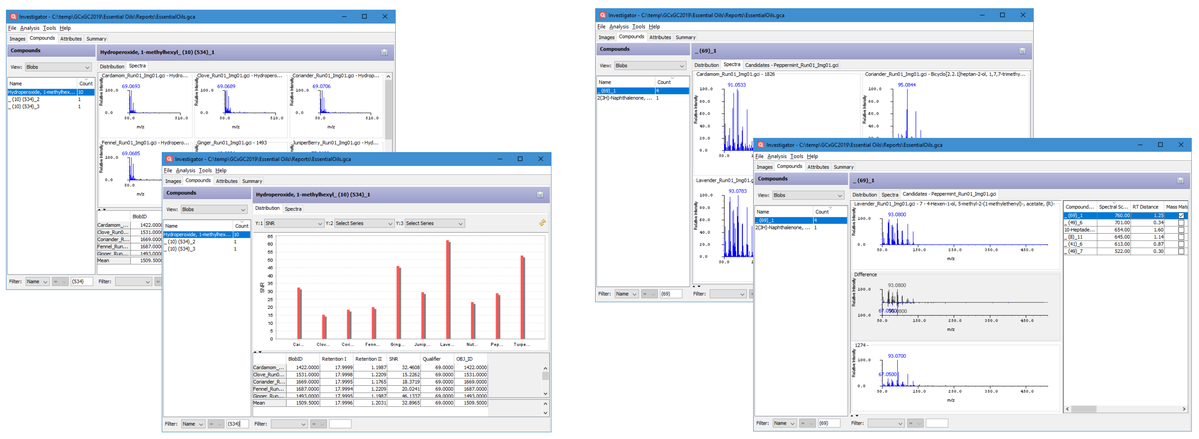
Alignment Results:
- A TIC and a peak table with more than 6000 peaks were exported from each processed chromatogram data file.
- 385 reliable peaks and 3752 peak-region features from the composite TIC



Tools for Validation

Result validation is crucial due to unavoidable problems with comprehensive peak matching.
- ➢ Variations: RT, spectra;
- ➢ Peak Detection: Split peaks, noise artifacts;
- ➢ The more strict matching constraints are, the fewer peaks can be matched;
- ➢ The less strict matching constraints are, the more mismatches can occur.

The new approach ranks and tags all peaks with the peak-region features, which allows easier and quicker verification assisted by search tools.



Check Spectra and Responses of Matched Peaks

Check the List of Possible Matches of a Peak

References

1. S. Reichenbach, X. Tian, Q. Tao, E. Ledford, Z. Wu, O. Fiehn. "Informatics for Cross-Sample Analysis with Comprehensive Two-Dimensional Gas Chromatography and High-Resolution Mass Spectrometry (GCxGC-HRMS)". Talanta, 83(4):1279-1288, 2011.
2. Z. Wu, J. Coleman, Q. Tao. "Distinguishing Commercial Diesel Fuel Brands Using Comprehensive Two-Dimensional Gas Chromatography/Mass Spectrometry". The International Symposium on Capillary Chromatography (ISCC), Riva del Garda, Italy, May 2018.
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. S. Fernando, A. Renaguli, M.S. Milligan, J.J. Pagano, P.K. Hopke, T.M. Holsen, B.S. Crimmins. "Comprehensive Analysis of the Great Lakes Top Predator Fish for Novel Halogenated Organic Contaminants by GC×GC-HR-ToF Mass Spectrometry". Environ. Sci. Technol., 52(5):2909–2917, 2018.
5. Data processes and screenshots for this publication are from an alpha version of GC Image v2.9 GCxGC-HRMS (Visit www.gcimage.com for current v2.8 releases).

GC IMAGE
Software for Multidimensional Chromatography

University of Nebraska Lincoln