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Robust Algorithm for Aligning Two-Dimensional Chromatograms

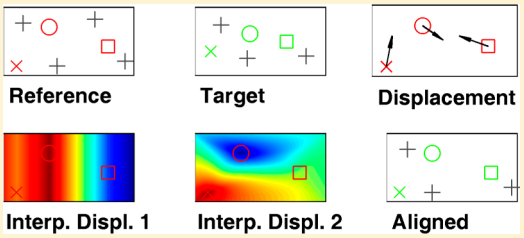
Jonas Gros, Deedar Nabi, Petros Dimitriou-Christidis, Rebecca Rutler,† and J. Samuel Arey*

Environmental Chemistry Modeling Laboratory (LMCE), GR C2 544, Swiss Federal Institute of Technology at Lausanne (EPFL), Station 2, CH-1015 Lausanne, Switzerland

Department of Environmental Chemistry, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

Supporting Information

ABSTRACT: Comprehensive two-dimensional gas chromatography (GC × GC) chromatograms typically exhibit run-to-run retention time variability. Chromatogram alignment is often a desirable step prior to further analysis of the data, for example, in studies of environmental forensics or weathering of complex mixtures. We present a new algorithm for aligning whole GC × GC chromatograms. This technique is based on alignment points that have locations indicated by the user both in a target chromatogram and in a reference chromatogram. We applied the algorithm to two sets of samples. First, we aligned the chromatograms of twelve compositionally distinct oil spill samples, all analyzed using the same instrument parameters. Second, we applied the algorithm to two compositionally distinct wastewater extracts analyzed using two different instrument temperature programs, thus involving larger retention time shifts than the first sample set. For both sample sets, the new algorithm performed favorably compared to two other available alignment algorithms: that of Pierce, K. M.; Wood, Lianna F.; Wright, B. W.; Synovec, R. E. *Anal. Chem.* **2005**, *77*, 7735−7743 and 2-D COW from Zhang, D.; Huang, X.; Regnier, F. E.; Zhang, M. *Anal. Chem.* **2008**, *80*, 2664−2671. The new algorithm achieves the best matches of retention times for test analytes, avoids some artifacts which result from the other alignment algorithms, and incurs the least modification of quantitative signal information.



Comprehensive two-dimensional gas chromatography (GC × GC) is widely used to analyze complex mixtures based on two sequential separation steps: material eluting from a first chromatographic column is trapped and then released at discrete intervals into a second column. Transport in the second column is fast compared with the first column, which allows the compounds to be separated further without significant loss of resolution from the first separation. Compared to one-dimensional gas chromatography, GC × GC improves the signal-to-noise ratio[1] and allows the separation of thousands of compounds.[2] Since its inception two decades ago,[3] this technique has proven effective in domains involving very complex mixtures, such as petroleum, volatile organic compounds in air, fragrances, plant and animal extracts, food, alcoholic beverages, and pesticides in environmental samples.[4−6]

Good retention time reproducibility between GC × GC chromatograms is broadly important for practical applications. For example, difference chromatograms,[2,7] which selectively show only the compositional differences between two samples, require well-aligned chromatograms. Similarly, automated peak-tracking tools can quantify abundance changes for thousands of compounds, and this requires good reproducibility of retention times between chromatograms.[2] However, some uncontrollable processes, such as small pressure and temperature variations and column degradation,[8] lead to variations of analyte retention times from run to run. This variability complicates the ability to make effective and automated comparisons between samples. Therefore, chromatogram alignment is of interest to diverse scientific fields that apply GC × GC, including metabolomics,[9,10] oil weathering, and oil spill forensics.[2,7,11−14] This is also an interesting step prior to the application of chemometric techniques[15,16] and more widely for any kind of analysis implying comparisons between different samples.[17−19]

Alignment algorithms have been developed in order to improve the agreement of retention times between whole GC × GC chromatograms of compositionally similar samples. To do this, a *target chromatogram* is modified to improve the similarity of its peaks' retention times with respect to those in a *reference chromatogram*. Ni et al.[20] developed an algorithm to correct deformations due to controllable parameter variations, e.g., intended pressure or temperature program modifications, using a global affine transformation which is calibrated by a set of predefined alignment points. However, this algorithm is not designed for correcting the shifts resulting from uncontrollable variations. The first published alignment algorithm for the correction of shifts resulting from uncontrollable variations for whole GC × GC chromatograms was developed by Pierce et al.,[8] extending their previous 1-D GC alignment algorithm.[21] Their algorithm divides the target chromatogram into windows of a user-defined size and then shifts each window such that the extent of shift optimizes the correlation with the corresponding window on a reference chromatogram. This shifting is then

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applied to the center of the window, and the remaining values are interpolated. A second alignment algorithm called two-dimensional correlation optimized warping (2-D COW) was introduced by Zhang et al.,[22] and it also represents an extension of a 1-D method.[23] Like the algorithm of Pierce et al.,[8] 2-D COW relies on the division of chromatograms into windows, but instead of shifts, warping (i.e., stretching or compression) is applied to the target chromatogram within limits set by the user. Maximum correlation is used to optimize the extent of deformation that is applied. Later, Vial et al.[24,25] applied dynamic time warping, a one-dimensional method, as a correction to GC × GC second dimension shifts, but disregarded first dimension shifts. More recent work proposes to diminish retention time shifts at the measurement stage.[26] Other tools have been developed for GC × GC coupled to mass spectrometry,[27−29] but these are not directly applicable to GC × GC coupled with other types of detectors.

As part of the analysis of GC × GC chromatograms acquired from environmental samples in our laboratory, we tried the different alignment algorithms currently available. We experienced the following limitations and shortcomings, depending on the method used: unrealistic distortions of the peaks, unwanted modification of the reference chromatogram, lack of access to the raw code of the alignment tool, and limited possibilities to improve the alignment if results were not satisfactory. Therefore, we decided to develop a new alignment algorithm according to the following principles. The user selects a set of alignment points that correspond to compound peaks found in both the reference chromatogram and the target chromatogram. These points are aligned, and deformations between these points are interpolated. With this new algorithm, we aimed to satisfy the following criteria: (a) significant improvement in the alignment of a target chromatogram with respect to the reference, (b) ease of use, (c) straightforward strategies to improve unsatisfactory results, (d) avoidance of unrealistic artifacts, (e) robustness to differences of composition between samples, and (f) modification of only the target chromatogram and not the reference. We evaluated the performance of the new algorithm using several GC × GC chromatograms of environmentally relevant complex mixtures, and we compared this to the performance of other previously developed algorithms.

EXPERIMENTAL AND COMPUTATIONAL METHODS

Sample Analysis. The algorithm was tested using two sets of samples. In the first test set, twelve differently weathered, compositionally distinct oil spill samples were analyzed using the same GC × GC instrument parameters, and these chromatograms exhibited some retention time shifting due to normal variations in instrument stability. These samples were obtained from a 2009 oil spill experiment on the North Sea. The neat (unweathered) oil was chosen as the reference, and eleven weathered oil sample chromatograms were aligned to the chromatogram of this sample. Instrument parameter details (program C) are given in section S-3 of the Supporting Information.

In the second test set, two compositionally distinct wastewater sample extracts were both analyzed twice using two different temperature programs, thereby inducing more significant retention time shifts compared to those of the first test set. One temperature program (program A, 3.5 °C/min) was arbitrarily chosen as the reference, and the two chromatograms resulting from analysis of the two samples with the second temperature program (program B, 4 °C/min) were aligned to the chromatograms acquired with the first temperature program. Two different cases were then studied. In the first case, a chromatogram of each sample was aligned to the chromatogram of the same sample analyzed with the other temperature program. In the second and more challenging case, the chromatogram of one sample was aligned to a chromatogram of the other sample, each analyzed using different temperature programs. See the Supporting Information for complete instrument and analysis details (sections S-1, S-2, and S-3).

Data Pretreatment. Prior to alignment, all chromatograms were baseline-corrected using GC Image with default parameters.[30] The signal in each oil spill chromatogram was then normalized (rescaled) such that the sum of the 5 integrated *n*-alkanes peaks, octocosane (n-C_{28}) to dotriacontane (n-C_{32}), was the same as in the neat oil, for all samples. Wastewater extract chromatograms were normalized such that the total signal was the same in the target and reference chromatograms, for the cases where a chromatogram was aligned to a chromatogram of the same sample analyzed using a different temperature program. These data pretreatment steps represent typical protocols used in the analysis of real samples. Although not required for application of any of the alignment algorithms, we considered these steps likely to improve the realism of the results.

Alignment Algorithm. The new alignment algorithm is designed to improve the similarity of peak retention times in a target chromatogram with respect to those in a reference chromatogram. The algorithm alters the target chromatogram, whereas the reference chromatogram remains unchanged. Chromatogram alignment is conducted in three steps. First, alignment points selected by the user are assigned on both the target chromatogram and the reference chromatogram. Second, a retention time shift, or displacement, is estimated for each pixel in the reference chromatogram with respect to the target chromatogram, based on information gained from the alignment points. Third, the signal values of all target chromatogram pixels are reinterpolated on the basis of nearby points and corrected for stretching or compression. These steps are described in detail below.

An alignment point is the pixel with the maximal signal value of a peak which is believed to have the same chemical identity in both the reference chromatogram and the target chromatogram. The positions of each alignment point, both in the target and reference chromatograms, are supplied by the user. In the first step of the algorithm, these points are perfectly aligned. In other words, the location of the alignment point in the aligned chromatogram is made identical to its location in the reference chromatogram. The signal value of the alignment point remains unchanged from its value in the target chromatogram.

In the second step of the algorithm, the displacement of each reference chromatogram pixel is estimated on the basis of the displacements of nearby alignment points. First dimension displacements and second dimension displacements were computed differently. First dimension displacements are independent of second dimension elution time, and therefore they are linearly interpolated between alignment points. This was considered physically reasonable; for example, a linear temperature ramp produces a nearly linear sequence of retention times for a homologous set of compounds.[31] Hence, in the case where two different linear temperature ramp programs would be used, the discrepancies in first dimension retention times from one chromatogram to the other would be approximately linear with respect to retention time. First dimension displacements outside of the region of the chromatogram bounded by alignment points

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are estimated using a linear extrapolation based on the first and last alignment points in the first dimension.

To calculate displacements in the second dimension, the two-dimensionality of shift variation must be taken into account. The second dimension displacement is estimated using a Sibson natural-neighbor interpolation, which is based on Voronoi diagrams,[32] as explained in Figure 1. This interpolation is

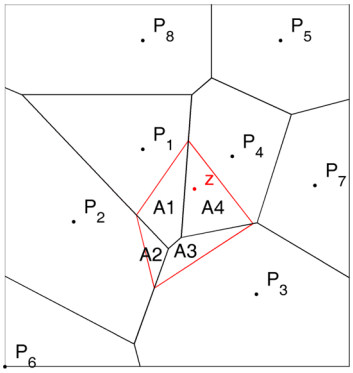

Figure 1. Explanation of the Sibson natural-neighbor interpolation. In the example schematic above, a Voronoi decomposition of a GC × GC chromatogram space is shown, based on the positions of a hypothetical set of alignment points, P_1 to P_8. In the example above, the Voronoi diagram segregates the plane into a set of 8 convex polygons, where each associated polygon is defined as the region of space that is nearer to point P_k than to any other alignment point. We denote this set *basis polygons* (black solid lines). Now consider any pixel z. The appropriate second dimension shift for pixel z is calculated as a weighted average of second dimension shifts from nearby alignment points. For each pixel z, a new polygon is temporarily constructed, which is given by the Voronoi diagram of the combined set of points $\{P_1, P_2, ..., P_8, z\}$. The new polygon adopts some area (A_1, A_2, A_3, A_4 in the example above) from the set of basis polygons. The second dimension shift for pixel z is computed as the weighted average of second dimension shifts of nearby alignment points (P_1, P_2, P_3, P_4) according to weights A_1, A_2, A_3, A_4 (see Supporting Information section S-4 for formula).

applicable only within the convex hull of the alignment points. In order to apply the algorithm to the whole chromatogram, an additional alignment point is imposed at each corner of a rectangle 10% bigger than the reference chromatogram. The displacements assigned to these four added alignment points are extrapolated from a weighted average of displacements of the interior set of alignment points, each weighted by the square of the inverse of the distance from the corner point (see section S-5 in Supporting Information).[33] Additionally, the second dimension interpolation scheme could, in principle, depend upon the way that the distances are computed. Distances expressed in pixels are dependent on the modulation period and sampling rate, and this may lead to arbitrary bias in the interpolation. In order to ensure a consistent treatment across different chromatograms obtained using different instrument parameters, interpeak distances are renormalized with respect to a single *typical peak width* (tpw) value in both the first and second dimension. In other words, distances in first and second dimensions were defined so that a typical peak has a width equal to approximately one in both dimensions. The tpw parameter, defined as the number of pixels corresponding to approximately two standard deviations of a typical peak signal (assumed Gaussian-shaped), is supplied by the user for each dimension. We chose values which correspond to a typical peak eluting close to the middle of the run. Although this definition is somewhat subjective, in practice we found that algorithm results are insensitive to the tpw parameter. See Figure S-1 in Supporting Information for an example of the pixel displacements generated for sample OS1.

In the third step of the algorithm, the signal values of all pixels are reinterpolated. Displacements are not applied directly to the pixels of the target chromatogram because it would be inconvenient to work with a pixel grid having irregular spacings. Instead, to account for the displacement, the pixel grid in the aligned chromatogram remains identical to that in the target chromatogram, but each pixel signal value is reinterpolated using a bicubic convolution interpolation of the sixteen pixels surrounding that pixel (see section S-7 of the Supporting Information for more details).[34] Wrap-around resulting from deformations in the aligned chromatogram is also considered: transfer of a pixel to the next or previous modulation period is allowed if required by the displacement estimate. Finally, peak volumes (i.e., the sum of pixel signal values for a peak) should not be modified by the alignment. Hence, pixel signal values are corrected for the extent of deformation resulting from alignment: the signal value at each pixel after alignment is divided by one plus the fraction change in the adjacent interpixel spacing through alignment. This correction is applied in both dimensions. Our alignment technique is the first to correct chromatogram signals for the extent of deformation incurred.

The alignment algorithm was implemented in Matlab,[35] using its implementation of the Sibson natural-neighbor and bicubic convolution interpolations. To aid the identification of alignment points, we also developed a tool which proposes alignment point positions in target chromatograms based on their positions in the reference chromatogram (see section S-8 of Supporting Information). The codes are freely available upon request from the authors.

Parameters Used for the Different Alignment Algorithms. For chromatograms of oil spill samples, we used 7 to 14 alignment points, which included the analytes *n*-C_{12}, *n*-C_{18}, *n*-C_{24}, *n*-C_{29}, *n*-C_{36}, naphthalene, biphenyl, and 17α,21β (H) hopane. For wastewater sample chromatograms, 10 alignment points were applied. Supporting Information Figure S-2 shows the positions of all alignment points for all chromatograms. Alignment points were chosen according to the following criteria: (a) they correspond to analyte peaks having confirmed presence in both the target and reference chromatograms; (b) they bound the interesting sample region of the chromatogram, where possible; and (c) they remain sufficiently few as to be easily managed by the user.

Parameter values chosen with the Pierce et al.[8] and 2-D COW alignment codes were those which appeared to give the best results after testing different sets of model input parameters. For oil spill chromatograms, the following values were applied: maximum warping (2-D COW) or shifting (Pierce et al.[8]) of 75 pixels in second dimension and 5 pixels in first dimension, and window size of 100 pixels in the second dimension and 50 pixels in the first dimension. Larger values were also tested, because these parameter values did not allow sufficient shifting correction for two of the alignment points used with our code for the sample OS7. However, using larger values did not globally improve alignment for all the samples. For wastewater chromatograms, the values of the four parameters listed above were: 115, 35, 150, and 50 pixels, respectively.

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Table 1. Statistics of Absolute Deviations[a] of Test Point Retention Times, Using Different Alignment Algorithms, in Units of Typical Peak Width (tpw)[b]

		mean		median		spread[c]		maximum value	
		I	II	I	II	I	II	I	II
oil spill samples	unaligned	0.19	0.17	0.03	0.10	0.27	0.19	1.67	2.28
	Gros et al. (this work)	0.11	0.11	0.06	0.05	0.15	0.14	1.00	1.72
	2-D COW	0.19	0.19	0.00	0.03	0.36	0.40	4.33	1.80
	Pierce et al.[8]	0.13	0.11	0.05	0.04	0.19	0.17	1.33	2.14
wastewater, same samples	unaligned	18.75	0.98	18.50	0.89	10.65	0.71	34.00	2.83
	Gros et al. (this work)	0.75	0.48	0.75	0.24	0.95	0.57	4.00	1.63
	2-D COW	2.50	0.49	0.00	0.12	6.14	0.92	20.00	2.95
	Pierce et al.[8]	2.89	1.33	1.80	1.11	4.06	1.18	14.00	3.55
wastewater, different samples	unaligned	18.75	0.98	18.50	0.83	10.66	0.72	34.00	2.60
	Gros et al. (this work)	0.80	0.50	0.75	0.24	0.97	0.58	4.00	1.63
	2-D COW	5.28	0.71	3.25	0.18	6.80	1.08	20.00	3.18
	Pierce et al.[8]	3.40	1.34	2.00	1.21	4.04	1.12	14.00	3.25

[a]Values for first (I) and second (II) dimension are presented separately. In some cases of application of the 2-D COW and Pierce et al.[8] codes, a peak was so distorted by alignment that it was no longer recognizable as a test point. These test points are not considered in the statistics above, except for the median statistic, in which case the value for the affected peak was considered as a very large number. Such distortions eliminated four points on oil spill chromatograms aligned using 2-D COW, three points on wastewater chromatograms aligned using the Pierce et al.[8] code, and one point on a wastewater chromatogram aligned using 2-D COW. [b]A typical peak width (tpw) is equal to 0.625 min and 0.6 s in first and second dimensions, respectively, for oil spill chromatograms, and 0.25 min and 0.8 s in first and second dimensions, respectively, for wastewater chromatograms. [c]The spread is defined as the square root of the variance of mean-subtracted retention time deviations.

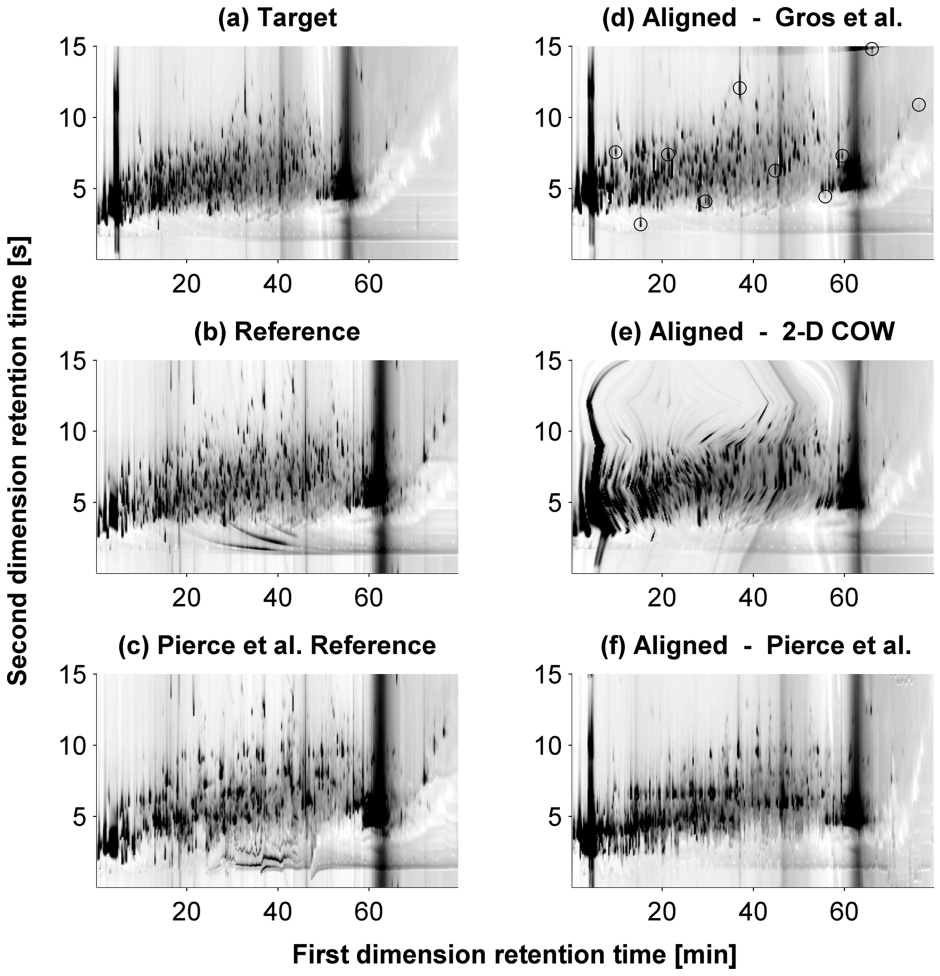

Figure 2. Alignment results for sample WW2B. (a) The chromatogram of wastewater sample WW2B (target) is aligned to (b) the chromatogram of the sample WW1A (reference), which is a compositionally distinct sample analyzed using a different temperature program, or (c) to a modified version of this chromatogram for the Pierce et al.[8] code. This leads to different results, depending on the alignment code used: (d) our code; (e) 2-D COW; (f) the Pierce et al.[8] code. The ten black circles indicate the positions of the alignment points used with our algorithm.

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RESULTS AND DISCUSSION

Extent of Misalignment Observed in the Original, Unaligned Chromatograms. To evaluate the extent of misalignment in target chromatograms prior to application of any alignment algorithm, we evaluated the retention time shifts of test points identified in both target and reference chromatograms (Supporting Information Figure S-2 shows test point positions for all chromatograms). For oil spill chromatograms, the mean deviations were 0.19 and 0.17 typical-peak-widths (tpw) in the first and second dimensions, respectively (Table 1). The shifts exhibited by wastewater chromatograms acquired using two different temperature programs were much larger, with mean retention time deviations of 18.75 and 0.98 tpw in the first and second dimensions, respectively (Table 1). For both oil spill and wastewater samples, retention time shifts were not systematic throughout each chromatogram. Some regions were much more prone to deformations than others, as indicated by the observed "spread" and maximum value statistics (Table 1).

Visual Comparison of Alignment Results Using Different Algorithms. The alignment algorithm proposed here was compared with the two other available alignment algorithms for whole two-dimensional chromatograms: 2-D COW developed by Zhang et al.[22] and the alignment algorithm developed by Pierce et al.[8] For oil spill samples, all chromatograms were aligned to the neat oil chromatogram, because the latter represents the native state of the spilt oil. On visual inspection, the Pierce et al.[8] algorithm produces slightly improved alignment of the target chromatograms and the reference chromatogram. The version of the code that we obtained also modifies the reference chromatogram, and for some chromatograms, this led to excessive and unrealistic distortions in the second dimension (Figure 2c,f and Supporting Information Figure S-5c,f). Results obtained with 2-D COW were better for chromatograms that are highly similar to the reference chromatogram, but unrealistic distortions nonetheless appear in some regions where only a few compounds eluted (Supporting Information Figure S-3c).

Additionally, when the target chromatogram contains peaks not present in the reference chromatogram, 2-D COW leads to unrealistic peak distortions (Supporting Information Figure S-4c). Our algorithm avoids these artifactual distortions and visibly improves the alignment in every case, regardless of the degree of signal similarity of the samples (Supporting Information Figures S-3b and S-4b). In other words, our algorithm is robust against composition differences between the reference and target chromatograms, assuming that at least some common analytes can be identified and employed as alignment points.

Similar conclusions are reached for the wastewater chromatograms (Figure 2 and Supporting Information Figure S-5). Our code leads to significant improvement without apparent unrealistic distortions of peaks. 2-D COW gives good results when it is used to align together two chromatograms of the same sample (Supporting Information Figure S-5e), but considerable artifacts arise when it is applied to aligning different samples analyzed with different temperature programs; some peaks of the aligned chromatograms exhibit zigzag-shaped deformations (Figure 2e). The Pierce et al.[8] code behaves poorly by unrealistically modifying both peak shapes and positions, even when applied to alignment of two chromatograms of the same sample analyzed using different temperature programs (Figure 2c,f and Supporting Information Figure S-5c,f).

Improvement in Peak Retention Time Deviations after Alignment, Using Different Algorithms. In order to assess quantitatively the performance of the different alignment codes, we conducted several tests. In the first test, we measured the deviations of test points both before and after alignment. To evaluate our own code, we performed a leave-one-out test of the alignment points. In other words, for each chromatogram, we applied our alignment code using all alignment points except one, which we treated as a test point. We repeated this procedure, each time leaving out a different alignment point, until we had tested all alignment points. This reflects a conservative estimate of the performance of our code compared to what it actually achieves. To conduct comparable tests of the Pierce et al.[8] and 2-D COW codes, we evaluated their abilities to properly align the set of points that we had used as alignment points. Although the data-withholding procedure applied to our code gives it a slight disadvantage, this approach enables a direct comparison of the three codes for the same set of test points (Table 1).

The new alignment code obtains the best global results. Among the three alignment codes, ours produces the most improvement in mean absolute retention time deviations in both the first and second dimension, for both oil spill chromatograms and wastewater chromatograms (Table 1). The Pierce et al.[8] code worsens mean absolute second dimension shifts for both sets of wastewater chromatograms. 2-D COW improves the mean absolute retention time deviations in both dimensions for wastewater chromatograms, but it does not significantly improve this statistic for oil spill chromatograms. As shown by the median absolute retention time deviation (Table 1), 2-D COW is able to achieve good alignment for most of the test points in oil spill chromatograms. However, misalignments for some test points remain significant, as shown by the spread and maximum value statistics. Our code has higher median absolute deviation values for oil spill chromatograms in comparison to the two other codes, but it is the most successful at limiting the most egregious shifts, as shown by low values for both the spread and maximum value statistics.

The code by Pierce et al.[8] sometimes suffers from inconsistent treatment of first and second dimension deviations: in cases where it brings improvement in the first dimension, it often worsens the situation in the second dimension. In such cases, peak shapes are badly distorted by alignment, leading to difficulties in identifying the peaks studied. 2-D COW works well for very similar chromatograms but often performs poorly for samples having significant compositional differences, and this is frequently associated with distortions of the aligned chromatogram. By construction, our code avoids unrealistic distortion of the aligned chromatogram, and peaks are always easily identifiable after alignment.

In additional tests of our code, we evaluated the dependence of alignment quality on the number of alignment points used (section S-14 in Supporting Information). For the samples considered here, the use of 8 or more alignment points gave reliably improved alignments.

Improvement in Quality of Difference Chromatograms after Alignment, Using Different Algorithms. In a second quantitative test, we compared the evolution of the sum of the absolute values of the pixel intensities of the difference chromatograms. A "difference chromatogram" represents the difference, pixel by pixel, between two chromatograms.[7] In principle, chromatogram alignment should decrease the total sum of pixel absolute values of the difference chromatogram of the reference minus the target. This test gives weight to large-valued pixels. Therefore, we excluded column bleed and solvent signal, because this signal would obscure that of the relevant

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Table 2. Peak Volume Bias Brought by Alignment, For Different Codes

sample name	baseline method (number of peaks)[a]	alignment code	mean absolute deviation[b]	mean absolute percent deviation, %
OS5 aligned to neat oil	GC Image (37 peaks)	Gros et al. (this work)	0.6	3.2
		2-D COW	1.9	12.0
		Pierce et al.[8]	3.5	23.6
	Eilers (112 peaks)	Gros et al. (this work)	0.2	1.7
		2-D COW	1.0	8.6
		Pierce et al.[8]	5.6	29.6
OS8 aligned to neat oil	GC Image (10 peaks)	Gros et al. (this work)	0.8	8.5
		2-D COW	4.4	21.5
		Pierce et al.[8]	3.8	16.9
	Eilers (56 peaks)	Gros et al. (this work)	0.2	2.5
		2-D COW	3.9	20.9
		Pierce et al.[8]	4.0	20.4
WW2B aligned to WW1A	GC Image (10 peaks)	Gros et al. (this work)	2.9	8.6
		2-D COW[c]	8.0	28.8
		Pierce et al.[8,d]	5.3	20.0
	Eilers (10 peaks)	Gros et al. (this work)	2.8	8.6
		2-D COW[c]	10.5	29.0
		Pierce et al.[8,d]	5.0	19.9

[a]Shown in parentheses is the number of peaks that were successfully matched across all four alignment cases: unaligned, using our algorithm, 2D-COW, and Pierce et al.[8] [b]Values given in arbitrary units. [c]One alignment point not detectable using GC Image; statistics computed with the 9 remaining values. [d]One alignment point not recognizable; statistics computed with the 9 remaining values.

sample region. Using our algorithm, the sum of the absolute values of the pixel intensities of the difference chromatograms shows improvement for all samples except one (sample OS5) (Supporting Information Table S-3). Of the three algorithms, ours results in the lowest absolute values for difference chromatograms, overall. 2-D COW obtains globally better results for the oil spill chromatograms; in principle, this should correspond to the best alignment, in contradiction to results shown in Table 1. The apparent success of 2-D COW for difference chromatograms may result partly from unrealistic peak distortions which apparently maximize correlation and thereby also decrease the total signal of the corresponding difference chromatogram. The code of Pierce et al.[8] did not give better global results than the other two codes for this test. Finally, in cases where samples are most dissimilar, our new code obtains favorable results compared to the other two.

Peak Volume Alterations Induced by Alignment, Using Different Algorithms. In a third quantitative test of the alignment codes, we evaluated peak volume changes arising from alignment. Ideally, the alignment should leave integrated peak volumes unchanged, so that the aligned chromatograms can be used in subsequent analysis without undue bias in chromatogram information. To conduct this test, we employed a previously developed peak-tracking code[2] which identifies matching peaks in different chromatograms, based on an analysis of the respective "blob tables" (i.e., tabulated lists of integrated peak retention times). The peak-tracking match criteria were parameterized very conservatively, so as to minimize or eliminate the possibility of wrongly assigned matches. We studied two oil spill samples: OS5 and OS8. For each sample, we used the peak-tracking code to identify peaks that could be matched in all four of the following chromatograms: the unaligned target chromatogram and the three aligned chromatograms obtained with the three alignment codes. Using this set of matched peaks, we computed peak volume changes induced by alignment, for each sample (Table 2). It was not possible to apply the peak tracking code to the severely shifted wastewater chromatograms; therefore, we evaluated peak volume changes observed in the 10 alignment peaks for the case of sample WW2B aligned to WW1A. Because integrated peak volumes are sensitive to the baseline delineation method, we decided to report results for chromatograms baseline-corrected using the relatively conservative delineation method of Reichenbach et al.[30] and also using a more aggressive delineation method developed by Eilers.[36] Peak footprint areas were delineated and integrated using GC Image.[37]

Among the three alignment codes, our code always induced the smallest changes in integrated peak volumes, regardless of the sample considered and regardless of the baseline delineation method applied (Table 2). For all cases studied, our alignment code induced <10% average change in peak volume. This peak volume bias is sufficiently small to allow further quantitative analysis of the data. By comparison, 2-D COW and the Pierce et al.[8] code both led to >20% average change in peak volume in 2/3 of the cases, which limits the usefulness of these aligned samples for further quantitative analysis. Unlike the other two algorithms, our code incorporates a correction for the local deformation applied during the reinterpolation step, and this limits the peak volume bias induced by alignment. Additional analysis of total chromatogram signal bias (Supporting Information section S-13) further corroborated these results.

All three alignment procedures led to modest increases in the total number of apparent chromatogram peaks that were detectable using the GC Image[37] peak delineation tool. Additional analysis revealed that all three alignment procedures also caused increases in the footprint area of delineated peaks, on average. We think these artifacts arise from the effective smoothing caused by signal reinterpolation during alignment. Subsequent changes in integrated peak volumes should thus depend on the way that the baseline is delineated. When used with our alignment method, the (more aggressive) Eilers baseline correction led to lower peak volume bias than did the (more conservative) Reichenbach baseline correction. To minimize peak volume bias caused by alignment, we recommend the use of our alignment procedure in combination with the baseline correction of Eilers.

CONCLUSIONS

We present a new alignment algorithm for 2-D chromatograms. In this study, the algorithm is applied to real samples analyzed using GC × GC, but in principle, it could also be applied to other types of 2-D chromatograms. In comparison to other available algorithms, our algorithm performs the best overall in terms of decreased retention time deviations of matching analytes. Additionally, the new alignment algorithm performs better than the two previously published algorithms according to three more criteria: it is insensitive to differences in composition between the target and reference chromatograms; it avoids unrealistic distortions of the aligned chromatograms; and it leads to only limited modification of the peak volumes. Moreover, our tests with wastewater chromatograms demonstrate that the algorithm can align chromatograms acquired under different chromatographic conditions. The algorithm modifies only the target chromatogram, perfectly aligns designated alignment points, and uses displacement interpolation for aligning the other pixels, thereby avoiding distortion artifacts that arise with the other two algorithms. Unlike previous algorithms, we apply a correction of pixel intensity values to account for the applied alignment deformation, and this limits the extent of modification of peak volumes induced by alignment. This enables further quantitative analysis to be applied to aligned chromatograms, whereas previously developed algorithms result in large peak volumes bias in certain cases. Although not discussed in this Article, our algorithm can also be applied directly to the alignment of peak tables, comparable to a previously developed method.[38]

Unlike the other two algorithms considered, our algorithm requires the a priori knowledge of alignment points. This may be viewed as a limitation. However, user-assigned alignment points confer important advantages in terms of flexibility and control, compared to the other two codes. Our code enables the user to choose appropriate points leading to proper alignment of two chromatograms, whereas the previous two codes determine points considered similar in an automated way. The previously developed codes thus leave the user blind to occasional false assignments of corresponding information between chromatograms. This leads to inferior performance of the previous codes compared to our algorithm, despite that our approach employs significantly less alignment information.

How can the user ensure that alignment points are properly assigned? In cases where sample compositions are similar or where retention time shifts are small, matching peak positions are often identifiable by visual inspection, because peak patterns remain similar.[39] This depends partly on the user's familiarity with the samples' compositions. If in doubt, a user could also add or ascertain alignment points using added internal standards. To help support the identification of alignment points, we developed a tool which proposes matches of alignment point positions in the target chromatograms based on their positions in the reference chromatogram (see section S-8 in the Supporting Information).

The algorithm presented here is systematically improvable. Alignment points are intuitive to use, and the improvement of the alignment is straightforwardly achievable by changing the number and positions of alignment points. We recommend that alignment points are chosen in each part of the chromatogram that exhibits shifting trends different from neighboring regions and that the most interesting sample information of the chromatogram is situated within the convex hull of the alignment points retained. The use of 8 to 10 well-distributed alignment points appears sufficient to produce reliable improvements in alignment. In cases where the choice of reference sample is not dictated by the study aim, the reference chromatogram should be selected as that which contains the largest number of well-distributed analytes employable as alignment points.

In practice, the algorithm could be applied to large sample sets. Once alignment points have been assigned in a reference chromatogram, an experienced practitioner typically can locate alignment points and perform alignment of a target chromatogram in 15 min or less, using a typical desktop computer. The new algorithm is expected to increase the possibilities available for scientists to use more deeply and quantitatively the information contained in two-dimensional chromatograms.

ASSOCIATED CONTENT

Supporting Information

Details about samples and their analysis; detailed explanations of the weighted average; formulas for displacement estimate at the four added pixels around the chromatogram; displacement estimates for OS1; explanation of the bicubic convolution interpolation; description of a tool designed to find the positions of the alignment points in a target chromatogram based on their positions in a reference chromatogram; depiction of alignment points positions; additional visual comparisons of the algorithms' abilities; numerical results for the sums of the absolute values of the difference chromatograms; statistics about alteration of total signal volumes through alignment; analysis of the improvement of alignment in function of the number of alignment points used. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: samuel.arey@epfl.ch. Fax: +41 21 693 80 70.

Present Address

[†]Cimo Compagnie industrielle de Monthey SA, Station d'épuration, Case postale, CH-1870 Monthey, Switzerland.

Notes

The authors declare no competing financial interest.

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