# Comprehensive Two-Dimensional Gas Chromatography

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**Comprehensive two-dimensional gas chromatography (GC×GC) has attracted much attention recently. As the latest newcomer to the collection of GC techniques, GC×GC delivers more GC information in a shorter time than do other methods; however, the complexity of GC×GC might be delaying its entrance to the GC application mainstream.**

It has been approximately 12 years since the initial description in chromatographic literature of the technique now called comprehensive two-dimensional (2D) gas chromatography (GC) or GC×GC.[1] Subsequently, the number of publications in the field has grown rapidly as researchers have explored GC×GC, its variants and related techniques. The interest level remains high today, as demonstrated by the large portion of this past year's GC papers and presentations that address GC×GC and related areas such as GC×GC–mass spectrometry (MS). Applications for complex samples and difficult matrices abound, and the increased volume of chromatographic information provides impetus to revisit or reformulate existing separations solutions.

Although conceptually elegant, the practical details of the chromatographic modulation techniques that enable comprehensive separations can be somewhat complex, and they require a level of customization that most GC users had dismissed as belonging to the 1950s and 1960s. The original oscillating thermal discontinuity, driven by gears or belts, has been displaced primarily by thermal gas jets, fluidic switches, various valving arrangements and their combinations, all of which offer significant improvements in reliability and reproducibility, as well as allow a better degree of control of the GC×GC process. But the requirement for modulation followed by synchronous reconstruction of the detector signal will always remain, along with requirements for tuning the system properly to avoid artefacts.

These technical hurdles are barriers to the widespread acceptance of GC×GC, but the technology development and acceptance cycle that is underway is no different than it was for capillary GC columns or even temperature-programmed GC ovens. History shows that when given an application-driven need for a separation technique and a clearly defined set of desirable outcomes, multiple research groups that are actively pursuing the technical developments in industry and academia will eventually find a way to put an initially obscure or difficult new technology into general use.

It is impossible to include sufficient references in this "GC Connections" column to do justice to the very large number of researchers who have made significant contributions in the various subfields of multidimensional GC. Interested readers should read the excellent two-part review of the subject by Bertsch,[2,3] which is still very relevant today.

## Multidimensional GC

GC×GC, at its roots, is a multidimensional separation technique in which the resolving power of two or more different columns is applied to some or all of the components in a sample. Multidimensional GC is not a new concept — it is almost as old as GC itself. Chromatographers have applied multiple columns to difficult separation problems in various ways. During the beginning years of GC, separations relied more heavily upon a much larger variety of stationary phases than is now the case; hundreds of phases were used then, in contrast to the 20 or so commonly used with modern capillary columns. Packed columns dominated the field, but they required more selectivity to overcome their lackluster efficiencies relative to capillary columns. Analysts needed a unique stationary phase for almost every separation class, and they soon turned to mixing column packings coated with different stationary phases as a means to fine-tune difficult separations. The resulting chromatograms exhibited separation behaviours that were the combination of separations on the individual phases; the contribution of stationary phases could be controlled by changing the proportions of each packing. Strictly speaking, these blended separations were not multidimensional because only one column was used.

**Series-coupled columns:** Other researchers soon realized that placing two columns in series — each with a different stationary phase — would yield results equivalent to mixing the packings, after the effects of the pressure drops throughout the column series were taken into account. Again, the results were proportional to the

contribution of the individual packings, but in this situation they were in relation to the lengths of the columns connected in series. By attaching shorter or longer columns in series, gas chromatographers could also fine-tune their separations, and these manipulations became — and remain today — standard fare in dedicated GC systems such as those found in process-control or on-line monitoring applications. With two columns in series, these systems produced the first multidimensional separations.

**Selectivity tuning:** Series column operation can be easily extended to capillary columns. In addition, positive control of the pressure at the midpoint between two columns yields a dynamically tunable separation system. Conveniently, the contribution of each column to the overall separation is proportional to the fraction of the total unretained peak time contributed by either column. Instead of adjusting column lengths — a time-consuming and costly optimization procedure — chromatographers can simply adjust the linear carrier-gas velocities through the columns to achieve a selectivity-tuning effect. The polarity range of these systems is curtailed by the limited range of efficient carrier-gas velocities: too-high or too-low velocities yield reduced overall efficiencies.

Figure 1 shows a schematic representation of a selectivity-tuned system. Peaks from the first column, usually non-polar, pass through a midpoint splitter that continuously routes a fraction of the column effluent to the first detector. The bulk of the effluent passes into the second column and on to a second detector. The carrier-gas velocity in the second column is set by the midpoint pressure, and the velocity through the first is determined by the pressure difference between the first column inlet pressure and the midpoint pressure. In practice, a variable restrictor is usually placed between the midpoint splitter and the first detector to balance the flows between the columns.

The two representational chromatograms in Figure 1 show the effect of a series-column separation on relative peak positions for a hypothetical sample. In chromatogram (a), peaks 1 and 2 are coeluted and peaks 4, 5 and 6 are poorly resolved after they have passed through column 1. As the peaks pass through column 2, their positions shift according to the strength of their retentions on the second column. Peak 1 is somewhat strongly retained and it shifts to a later position. Peak 2 is weakly retained and doesn't shift much. Peak 3 is less retained and is eluted relatively earlier. Peak 4 is also eluted earlier, but it interferes with peak 1 after the two peaks pass through column 2, as is also the situation with peaks 6 and 8.

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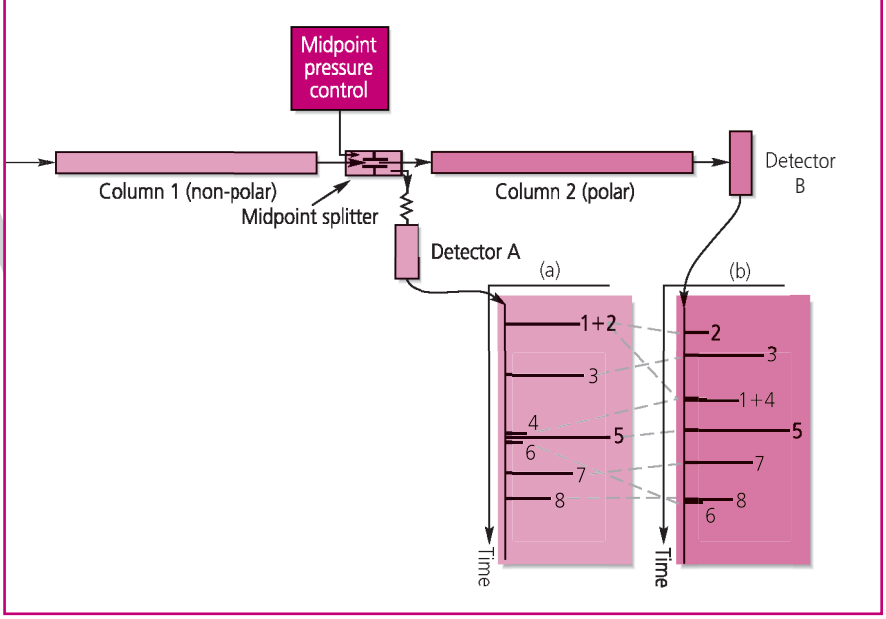
Series columns are the simplest embodiments of multidimensional chromatography. The effects they produce are limited by carrier-gas velocities and because all the solutes transit both columns in a single continuous stream. When working with complex samples, chromatographers might observe that peaks that are well separated after passing the first column might come back together or might interfere with other peaks as they pass through the second column. A peak-delay technique can help control this effect by momentarily time-shifting one peak at the column juncture so that another peak, with which the delayed peak would otherwise interfere, can move along the column and be eluted before the delayed peak.

Series-coupled column systems by themselves do not yield comprehensive separations: the results are a convolution of the two columns' separations. The first separation couples into and strongly influences the second. The number of theoretical plates and basic resolving power are the same as if a single column with the length of the two individual coupled columns had been prepared with a stationary phase of equivalent selectivity — as when earlier researchers mixed coated packings together in a single column. As I will demonstrate in the next section, comprehensive 2D GC means that the separation power of each column applies fully to each analyte independently to eliminate the dependencies of the second column's separation on the relative position of peaks from the first column.

**Heartcutting:** In heartcutting systems, one or several discrete portions of a separation are directed from the first column to the second. Because only a few selected peaks enter the second column at a time, interference from other nearby peaks that precede or follow the heartcut is eliminated, and the second column's separation becomes largely independent of the first's. Figure 2 illustrates the effect of passing a limited portion of the column 1 effluent onto column 2 for the same hypothetical separation as shown in Figure 1.

**Figure 1:** Selectivity-tuned GC system: Representative relative peak positions (a) at the column 1–column 2 juncture and (b) after transiting the second column.



In Figure 2, the pneumatic splitter device is replaced with a pneumatic switch that can direct flow from column 1 to detector A or to the entrance of column 2. The switch works by slightly increasing or decreasing the relative pressures on the two midpoint pressure control lines by a few tenths of a pound per square inch. A slightly higher pressure on the column 1 side of the switch will direct column 1 effluent through the switch's middle passage and into column 2. Alternatively, a slightly higher pressure on the column 2 side will reverse the flow through the switch, sending only pure carrier gas into column 2 and forcing the effluent from column 1 into the detector.

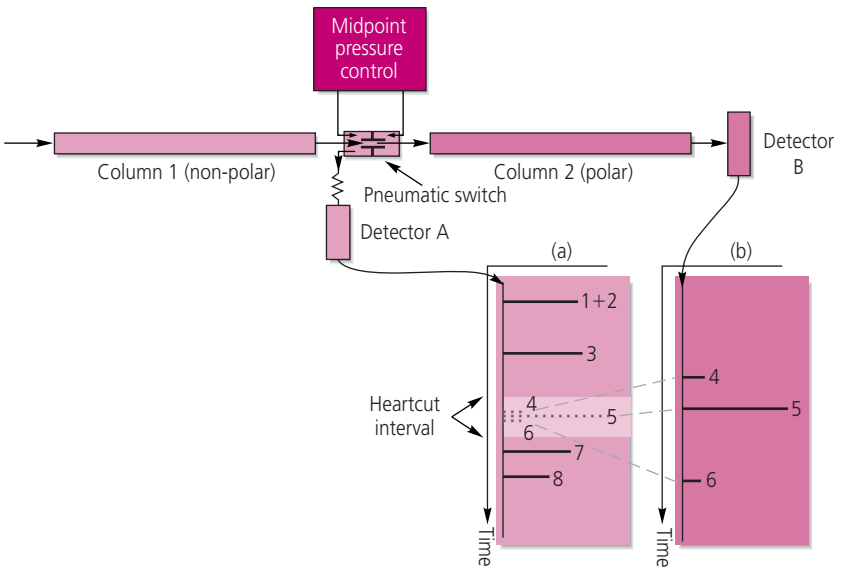
In chromatogram (a), after peaks 1, 2 and 3 go to detector A, the pneumatic switch is set to send peaks 4, 5 and 6 directly to the second column. Next, the pneumatic switch is reset and the remainder of the column 1 effluent goes to detector A. Peaks 4, 5 and 6 don't appear in chromatogram A (they're shown as grey dotted lines for reference): all of the column effluent goes to column 2 during the heartcutting period. Because only these three peaks enter column 2, no possibility of interference from peaks 1 and 8 exists.

**Trapping:** As they transit into the second column, the heartcut peaks could occupy a relatively large volume of carrier gas and the switching device could introduce some extra carrier gas or broadening as well. Cold-trapping or otherwise concentrating the transferred peaks into a smaller volume after they leave the first column can narrow the peaks' profiles at the onset of the second separation. This trapping collapses the localized separation of the selected peaks from each other and results in the removal of any contribution from the first column to the immediate separation of the selected peaks on the second column.

However, the number of heartcuts from the first column that can be analysed by a second column is limited by the analysis and recovery times of the second column before each subsequent heartcut cycle. Peaks that are eluted from the first column while the second column is actively analysing a previously sampled heartcut do not benefit from separation in the second column and are sent to the first detector instead. One solution for this problem of peaks queuing up and waiting for the second column to finish a separation is to use more than one secondary column. Another solution, which is the essence of comprehensive chromatography, is to make the secondary separation faster so that queuing peaks don't accumulate too much during each secondary separation pass.

Thus at one dual-column extreme — selectivity tuning and its variants — all the peaks enter the second column separated just as they are eluted from the first. Some manipulation and delay of the peaks is possible as they transit the column juncture, but these fully coupled systems produce a net result that is a convolution of both separations, peaks that are separated at the exit of the first column might recombine as they pass through the second column. In the intermediate heartcutting situation, only selected fractions from the first column enter the second column. Those components that do pass through the second column are separated largely independently of their behaviour on the first column, as if they had been injected independently of the rest of the sample. Heartcutting is very useful for directed pinpoint separations of a few selected peak groups but fails to produce a complete separation that combines all the resolving power of both columns for the entire sample.

**Figure 2:** Heartcut GC system: Representative relative peak positions (a) at the column 1–column 2 juncture and (b) for heartcut section after passing alone through the second column.




## Comprehensive Separations

As the name implies, comprehensive separations apply all the available resolving power of both columns to all the peaks in a sample. In GC×GC each peak transits the first column, is trapped at the end of the first or the beginning of the second column and is released onto the second column. The major difference between GC×GC and heartcutting is that for GC×GC the trapping time and second column separation speeds are fast enough so that many heartcuts can be analysed in the second column during the course of a single first-column run. The heartcuts can be taken rapidly enough that individual peaks from the first column are sliced across several sequential secondary column runs, but going much faster than that has no advantage.

**High-speed:** Achieving this level of performance requires a high-speed separation for the secondary column and encourages ordinary speeds for the first column. Faster separations on the first column can drive the second-column speed requirement higher than what is practical. Typical secondary column analysis times used today are on the order of 0.1 min or less with very short (1–2 m) narrow-bore (100–180 mm i.d.) dimensions and linear velocities several times the optimum. Hydrogen carrier gas is advantageous as it maintains column efficiency at higher linear velocities better than helium does.

A secondary column with those dimensions yields few theoretical plates by the usual standards for capillary columns, but it doesn't have to, because it's only being asked to handle the few peaks at a time that coexist in any one heartcut from the first column. This distinction is essential to understanding GC×GC. Gas chromatographers are not used to thinking that short, narrow-bore capillary columns are practical for anything. Even at linear

velocities well above optimum, however, these columns can deliver 3000–5000 theoretical plates, which is sufficient for the purpose at hand.

**Modulation:** Comprehensive GC separations can be accomplished many ways. The most prevalent methods in use today involve some form of thermal modulation at the juncture of columns 1 and 2. Figure 3(a) illustrates the main principles of GC×GC operation with a thermal modulator. The simplest arrangements intermittently blow cooled nitrogen or air over a section of the beginning of column 2. When the cooling flow is turned off, the cooled column section rapidly reheats from circulating hot oven air. Some arrangements positively heat the cooled section to release the trapped peaks more rapidly.

While cooling is applied, peaks eluted from column 1 are trapped in place at the beginning of column 2. When heated, the peaks are released and begin to move again in a narrow band to start the secondary separation. However, during the heating stage, any new peak material exiting column 1 will not be trapped and will enter column 2 along with the already trapped material. This situation creates some undesirable overlapping and smearing of peak bands between adjacent heartcut sections.

A second thermal modulator solves this problem, as Figure 3(a) shows. The modulators are heated and cooled out of phase with each other. Peaks are trapped at the first modulator when it is cooled down. The second modulator is also cooled down and then the first modulator is heated. The trapped peaks move to the cooled second modulator zone along with any material that leaks through while the first modulator is hot. When the first modulator is cooled off again, the two trapping zones are effectively isolated from each other. The second modulator is heated, and the peaks trapped within are released into the secondary column. Because the first modulator is still cooled, any new material coming from column 1 is trapped inside and does not enter the second column until the next secondary analysis is ready to start. This scheme effectively isolates the two columns from each other for the purposes of GC×GC.

Thus, a series of rapidly repeating injections are performed effectively onto the secondary column with slices trapped off the first column. What does the resulting chromatogram look like? Figure 3(b) shows a representation of a raw GC×GC chromatogram section that is 2.5 min long. I have used the same set of eight sample peaks to represent the separation from column 1 as in the previous series-coupled and heartcut examples. For GC×GC, the chromatogram from the secondary column appears with discrete groups or bands of peaks. Each secondary column run is represented by the 0.1-min-long bands along the baseline.

Peaks 1 and 2, which are coeluted from column 1, are separated in the sixth secondary column run. Peaks 4, 5 and 6, which were poorly resolved on column 1, are also separated, but the separation occurs in the 16th secondary column run. Significantly, none of these peaks interfere with each other as they did with the series-coupled system. Peak 1, which is coeluted with peak 4 on the secondary column, does not interfere in GC×GC because it passes through the secondary column 10 cycles before peak 4 enters the column; peaks 6 and 8 behave similarly. It is this result — that the separation of the peaks on the first column is maintained — that gives GC×GC its tremendous resolving power.

**Data reduction:** Additional relationships between peaks in GC×GC exist, but they do not become apparent until some data manipulation is performed. The most common data transformation is the construction of a 2D representation in which one axis represents the separation on the first column and the other axis represents the secondary column separation. A contour plot using elevation lines or colour coding represents the signal intensity. A third dimension can be added optionally by making the *z*-axis proportional to the signal intensity.

To generate the reconstructed 2D plot, chromatographers use the timing of the thermal modulators to locate the start of each secondary column run in the raw data stream. The raw data stream is sliced into multiple subchromatograms that represent each of the secondary runs. As Figure 3(c) shows, these slices can be assembled side by side to produce a two- or three-dimensional (3D) map of the overall separation. The map is very useful because it shows relationships between groups of peaks in the primary and the secondary column separations. Both types of plot are shown in Figure 4.

## Example — Light Cycle Oil

GC×GC has been applied to hundreds of complex and challenging samples. One

**Figure 3:** GC×GC system: (a) Schematic diagram with dual thermal modulators, (b) representation of a raw GC×GC chromatogram showing positions of individual slices, and (c) representation of a 3D chromatogram reconstructed from the raw chromatogram.

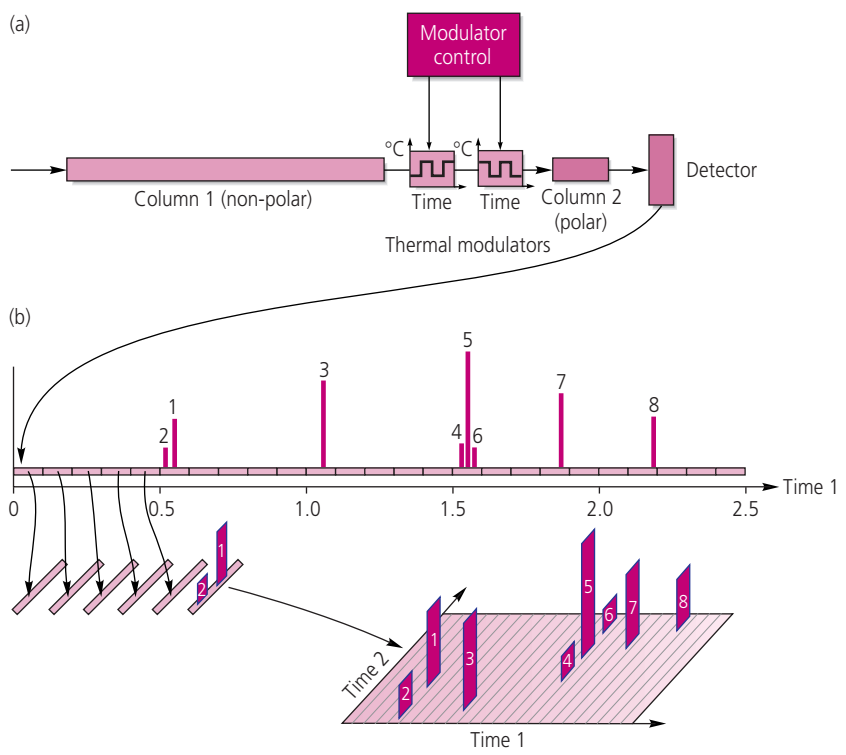
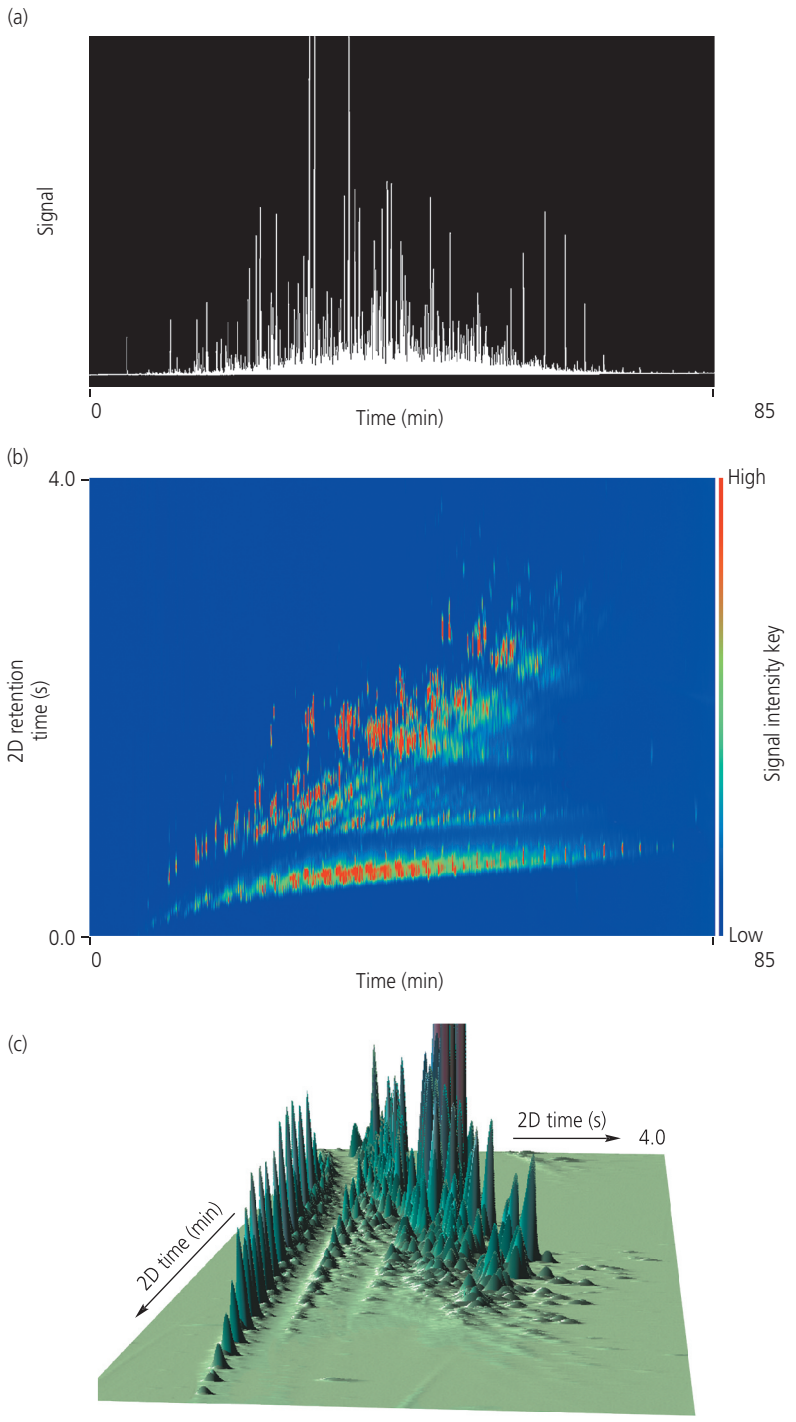


**Figure 4:** GC×GC analysis of light cycle oil: (a) raw GC×GC data; (b) a reconstructed 2D chromatogram plot; and (c) a reconstructed 3D chromatogram. Column 1: 30 m × 0.32 mm, 0.25 µm $d_f$ RTX-5; Column 2: 1 m × 0.1 mm, 0.1 µm $d_f$ BPX-50; oven temperature programme: 50 °C (1 min isothermal) to 300 °C at 3 °C/min; modulation cycle time: 4 s; injection mode: 0.4 µL, split ratio 200:1; flame ionization detector sampling rate: 200 Hz; instrument: Trace GC Ultra in 2D GC configuration with dual-stage carbon dioxide modulator, split–splitless injector and fast flame ionization detector; data system: HyperChrom SW for qualitative and quantitative analysis of bidimensional GC×GC separation patterns. Peak groupings: 1 = paraffins; 2 = mono-aromatics; 3 = naphthenic mono-aromatics; 4 = di-aromatics; 5 = naphthenic di-aromatics; 6 = tri-aromatics. (Courtesy of Thermo Electron Corp., Waltham, Massachusetts, USA.)




area in which GC×GC excels is the elucidation of petroleum samples. Oils and other related materials of natural origin contain homologous series of hydrocarbon compounds: normal paraffins, isoparaffins and cycloparaffins; unsaturates; and one-, two- and multiple-ring aromatic compounds. A non-polar column provides separations by order of increasing carbon number for such samples. Usually, the normal paraffins dominate the mixture and produce a recognizably spaced series of peaks from the beginning to the end of a one-dimensional chromatogram.

Figure 4(a) shows the raw data from a GC×GC analysis of a sample of light cycle oil. The hydrocarbons stand out as large, regularly spaced peaks. Superimposed over the normal hydrocarbon series are the other series of isoparaffins, unsaturates and aromatic compounds. With many thousands of compounds present, a single-column chromatogram suffers from peak overlap, often to the point of completely merging the peaks into one or more lumps. When these samples are run on polar columns, the peak groups are sorted by degree of unsaturation and branching more than by the number of carbons, but the result is similar for complex samples — a large degree of overlap occurs.

GC×GC, with its independent-but-combined non-polar and polar separations, can resolve an astonishing number of substances. Even so, the total number of peaks in complex petroleum samples usually exceeds even this enhanced resolving power. Figure 4(b) illustrates a 2D contour plot constructed from the raw data in Figure 4(a) for the light cycle oil example. The colour of the plot represents the signal intensity. In particular, note the distinct grouping of the various functional-group homologous series. The horizontal group of peaks running from left to right closest to the *x*-axis are the paraffins, and the groupings higher on the plot are various types of aromatic compounds. Figure 4(c) shows the same data in a 3D representation, as viewed from the right-hand side of Figure 4(b).

**GC×GC–MS:** This example clearly demonstrates the potential of GC×GC to generate a wealth of additional information about a complex sample. But even so, it is obvious that much more information is present. One way to add yet another dimension to the information is to tack a mass spectrometer onto the end of a GC×GC system. In doing so, chromatographers can make additional demands of the MS system for scan speed

and data storage beyond conventional or even so-called high-speed GC systems that require rethinking of how such systems are designed. The amount of data generated by a GC×GC–MS system is truly large, and reducing those data to meaningful results presents yet another challenge for analysts.

## Conclusion

The power of GC×GC lies in its ability to pull the polar-column information into the second-dimension axis while retaining the first-dimension non-polar column information. The results are striking in their visual appearance — patterns formed by the various compound groups seem to leap off the page. Clearly, GC×GC offers tremendous potential for the further elucidation of thousands of GC sample types that will occupy researchers for a long time to come. But this technology is not one that is easily accessible to average gas chromatographers. A successful GC×GC system or accessory involves both hardware and software developments that must be well integrated, which essentially requires the resources of an instrument company along with academic participation. Thus, this newcomer must follow the classic path of earlier major developments in chromatographic instrumentation.

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