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An Introduction and Overview on Comprehensive Two-Dimensional Gas Chromatography (GCxGC):

New Opportunities for Unresolved Complex Mixtures

Daniela Cavagnino
GC Product Manager
Thermo Fisher Scientific, Italy

Outlines

- Why GCXGC
  - Limitation of 1D chromotography
- How to accomplish GCxGC
  - Principle of operation
- HW/SW requirements
- MS coupling
- Further analytical perspectives

Limitations of conventional (1D) GC (HRGC!)

- 1D separation process is statistically limited in case of mixtures exceeding 50-60 compounds
  Davis J.M., Giddings J. Anal Chem (1983), 55, 418-424

- How to handle samples like:
  - Petrochemical samples
  - PCB's (209 congeners, 46 isomers)
  - Toxaphene (= chlorinated boranes, 32768 congeners)
  - Flavours (coffee aroma: >> 700 compounds)
  - Target analytes in complex matrices (soil, fruit, biological)
  - ....

Sample Dimensionality

- A mixture's dimensionality is the number of independent chemical properties that are required to specify the compounds of the mixture
  J. Calvin Giddings, J. of Chrom. A, 703 (1995), 3-15

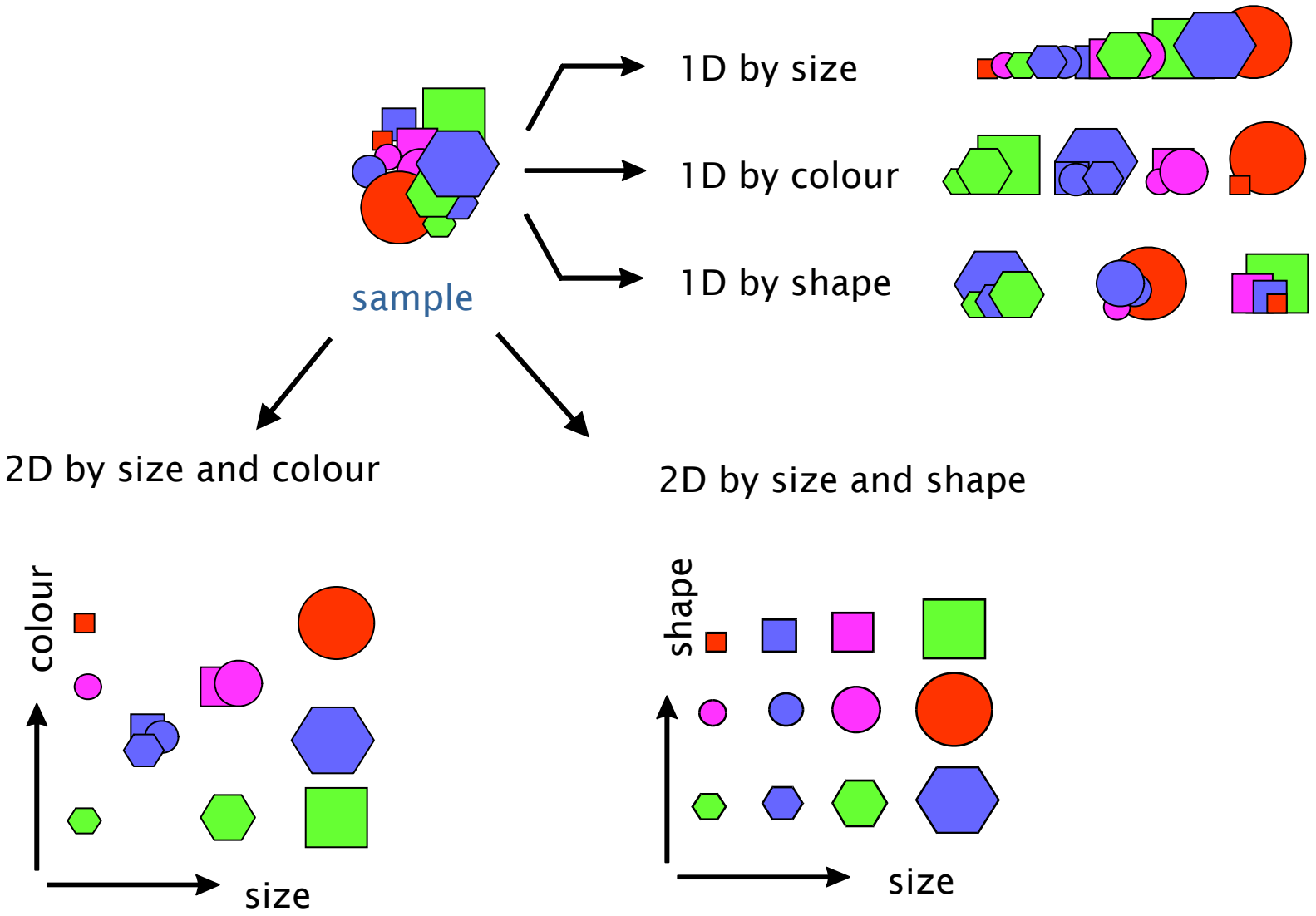
Fundamental problem

Dimensionality mismatch between the analytical system and the analytical sample

Solutions

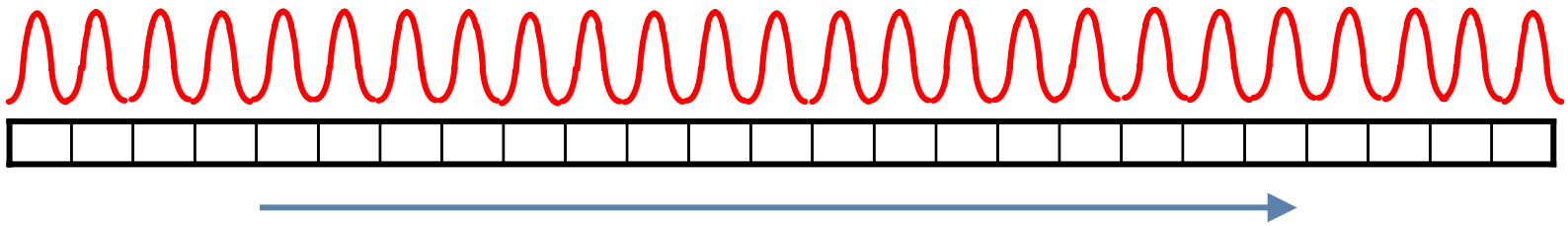
- Reduce the sample dimensionality (sample clean-up)
- Increase the separation system dimensionality

The use of sample dimensionality



Multidimensional GC separation

1D-chromatography GC

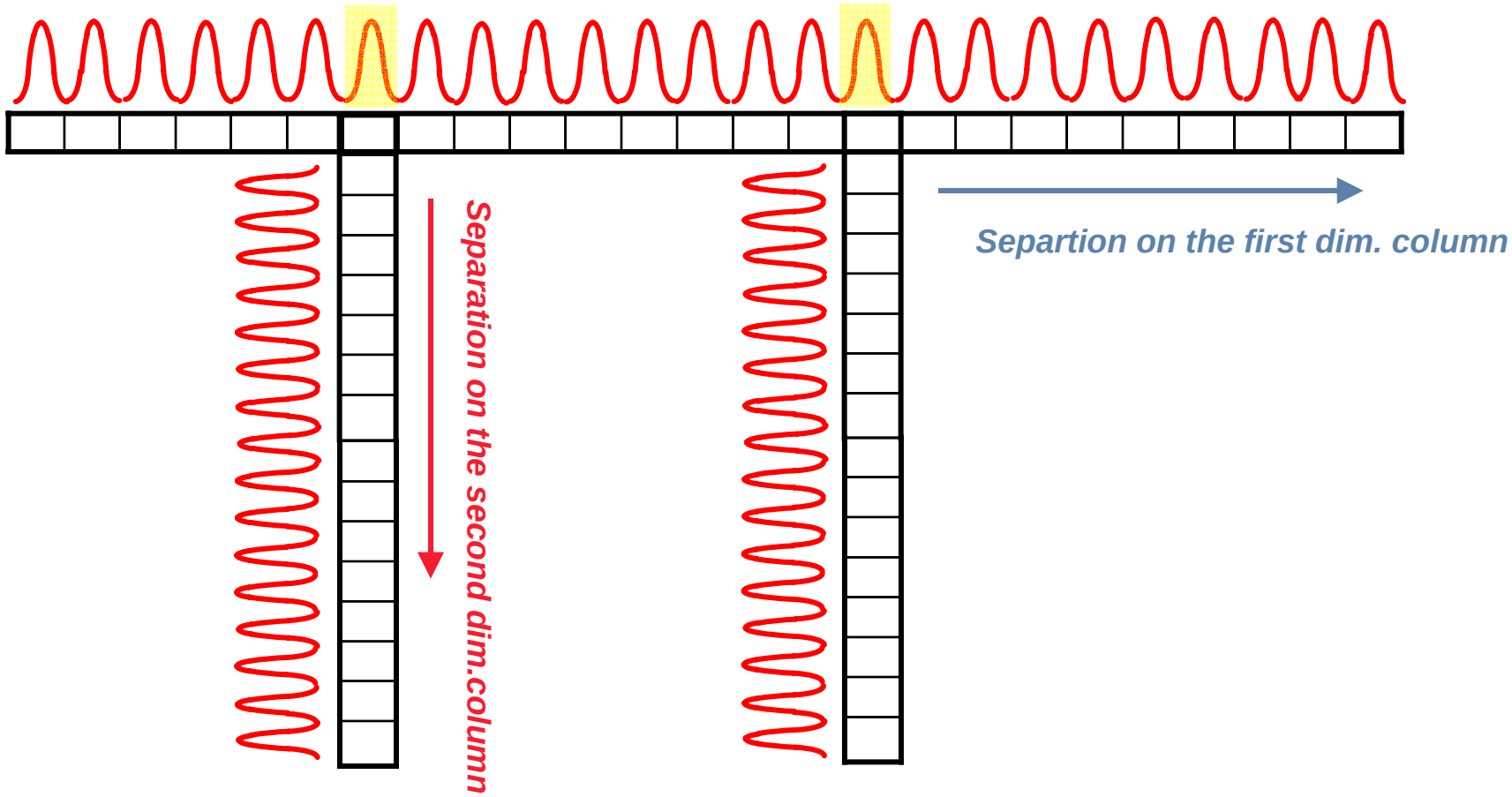


Separation on a single column

peak capacity (n) ranges between 500 – 1000

Multidimensional GC separation

Heart-cut 2D-chromatography GC-GC



peak capacity $(n) = n_1 + (n_2 \times x)$

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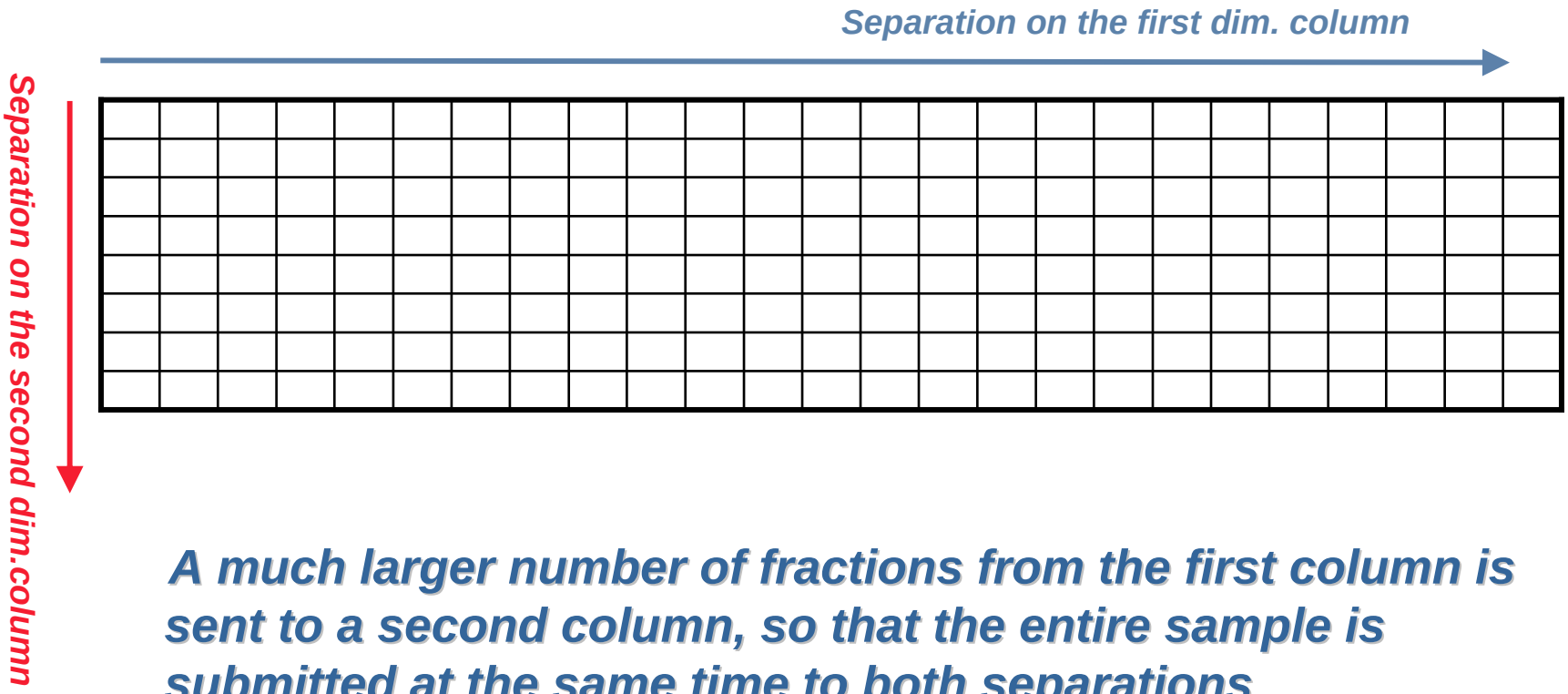
Multidimensional GC separation

Comprehensive 2D-chromatography GCxGC



A much larger number of fractions from the first column is sent to a second column, so that the entire sample is submitted at the same time to both separations

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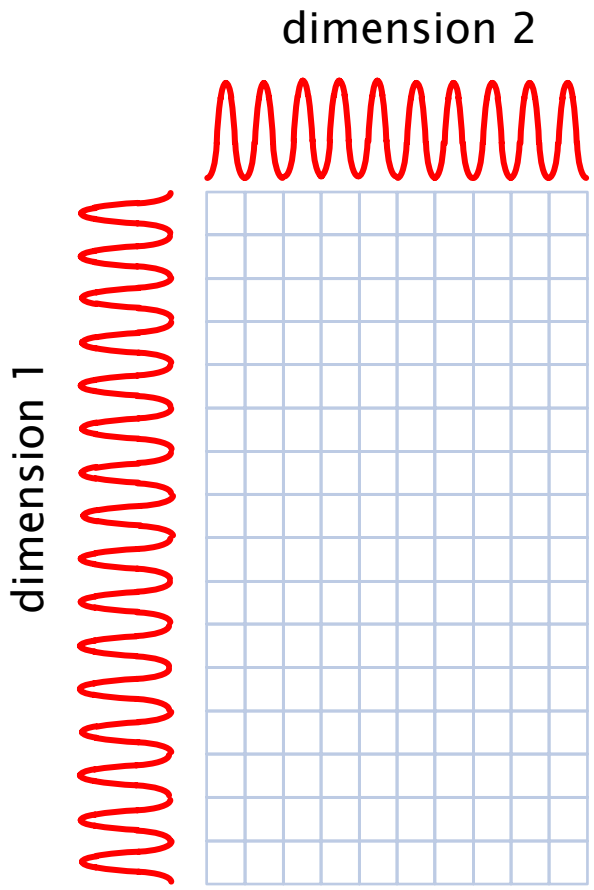
Enhanced Peak Capacity

Much higher peak capacity for enhanced separation capabilities



peak capacity ~ n_1 x n_2

1st dim conventional GC Column n = 1000

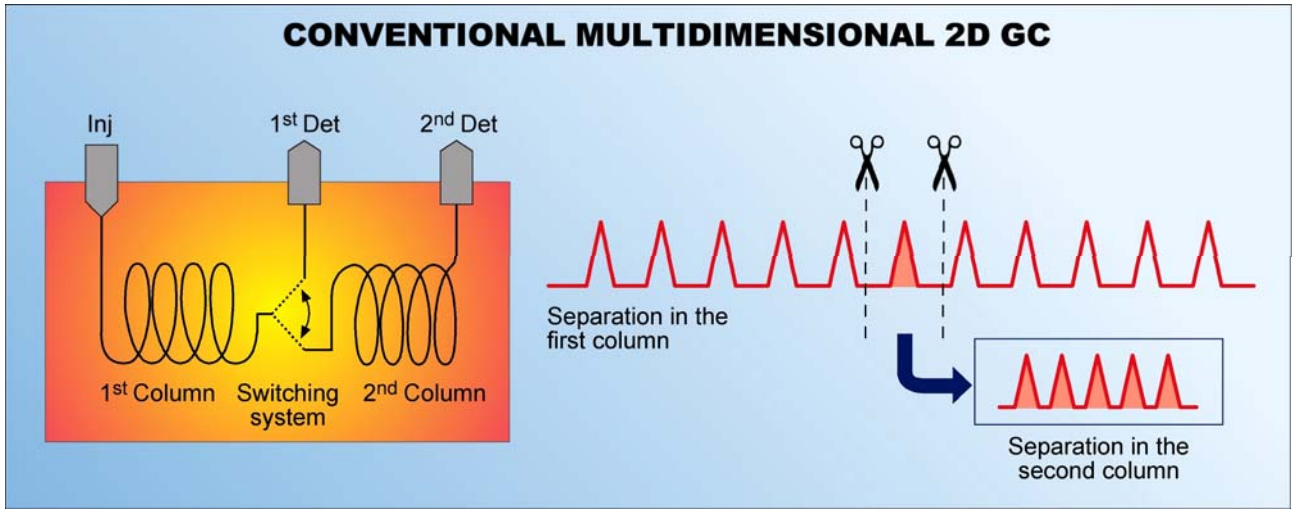
2nd dim fast GC column n = 30

→ **Multidimensional GC-GC**
$n_{GC\text{-}GC}$ = 1000 + 1000 = 2000

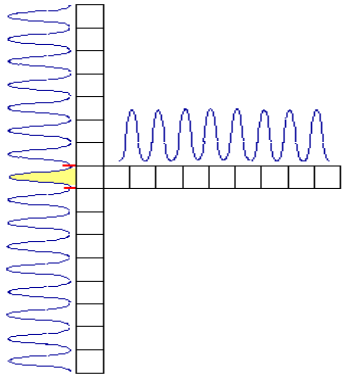
→ **Comprehensive 2D GC**
n_{GCxGC} = 1000 x 30 = 30000

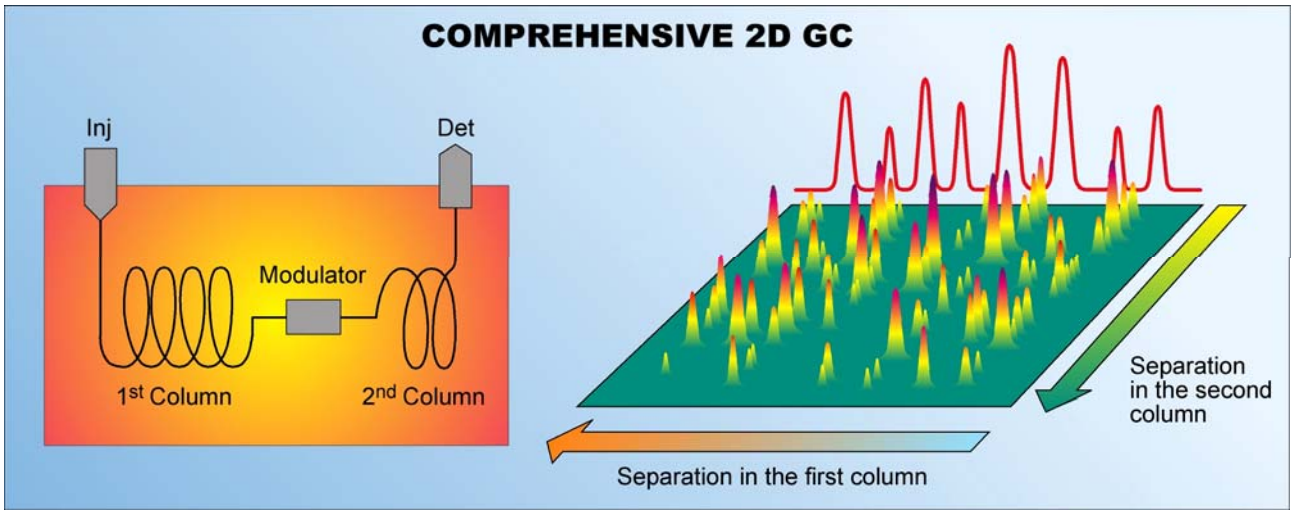
L = 12 Km t_0 = 10 h t_R= 1.5 years

Multidimensional GC-GC vs GCxGC

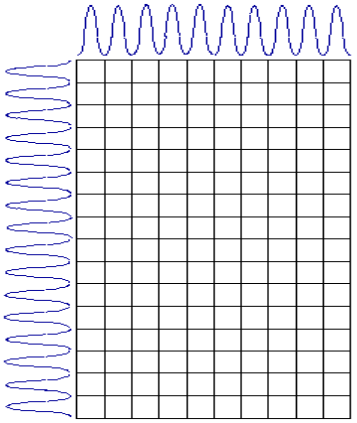


GC-GC





GCxGC



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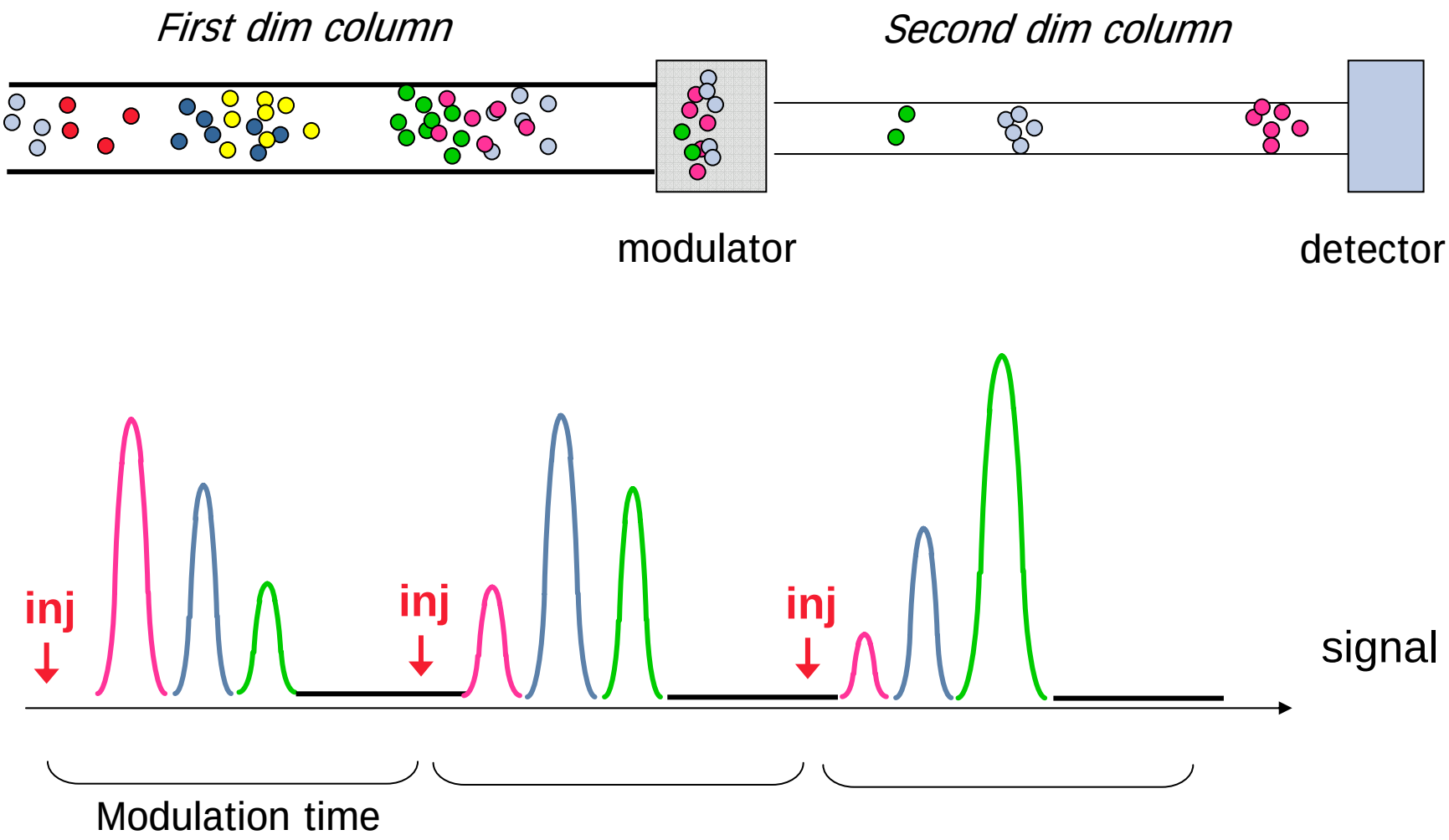
How the modulator works



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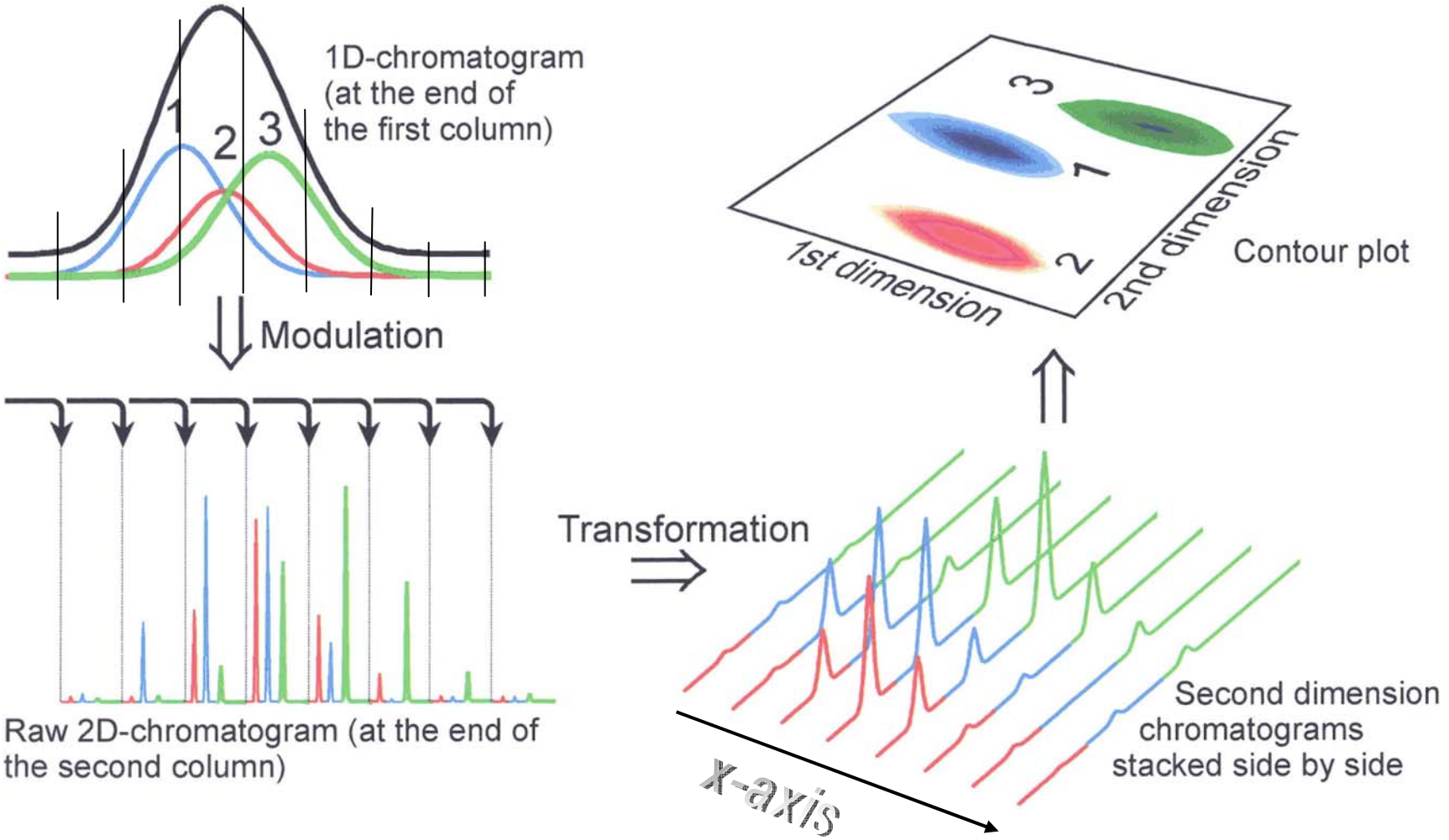
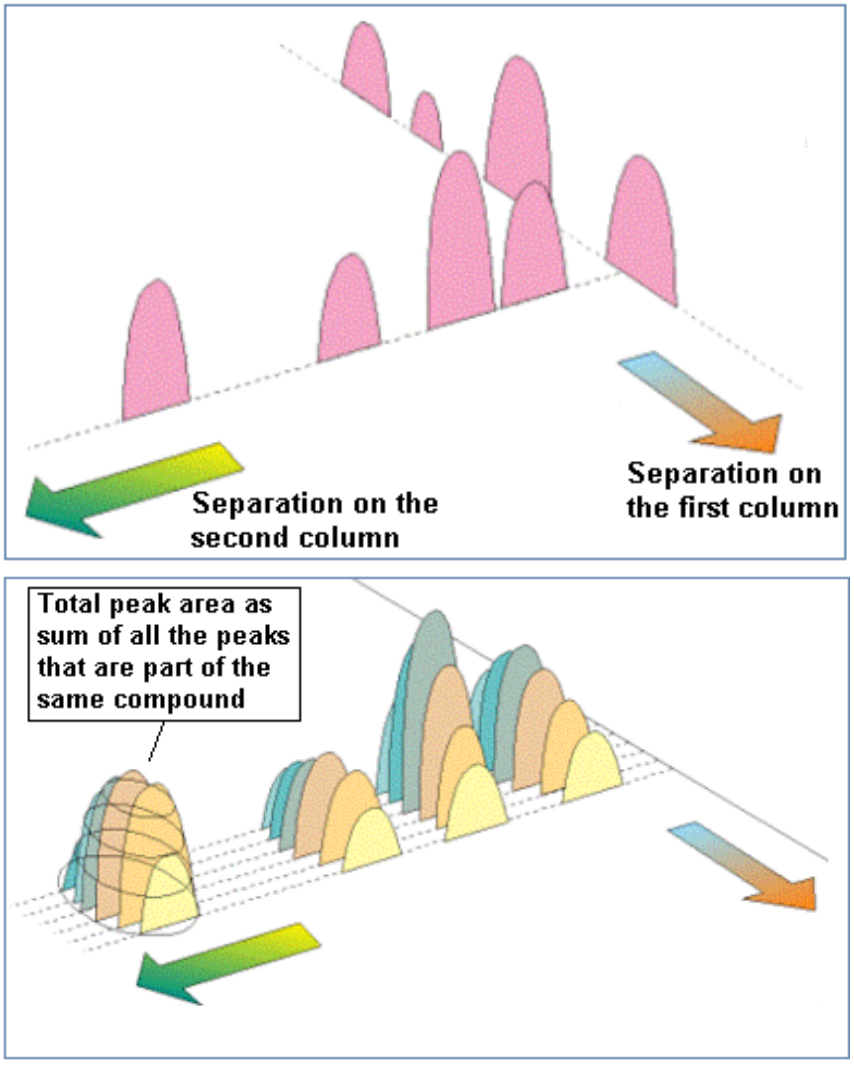
Data conversion for visualization



Quantitative Approach



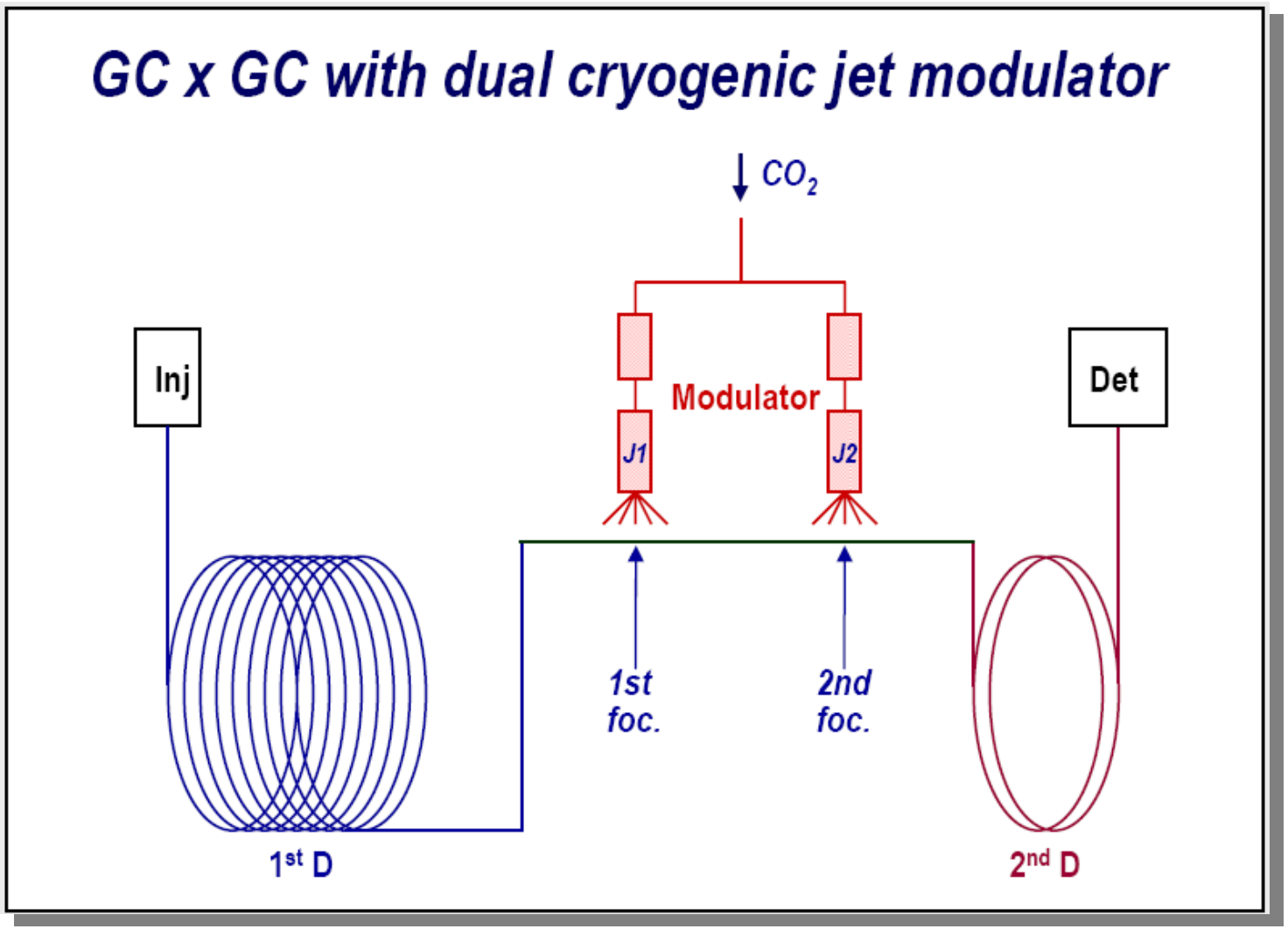
- Conventional integration of raw data (detector data stream)
- Grouping of fast peaks generated by the modulation and belonging to the same compound
- Summation of the peak areas to get a total peak area proportional to the analyte concentration
- Conventional calibration method set up

Modulators

- Dual stage Thermal Modulator
  - Dual-Jet LCO2
  - Quad-Jet LN2
  - Loop Type

- Flow Modulator
  - Valves system
  - Capillary Flow Technology device

Dual-Jet CO2 modulator



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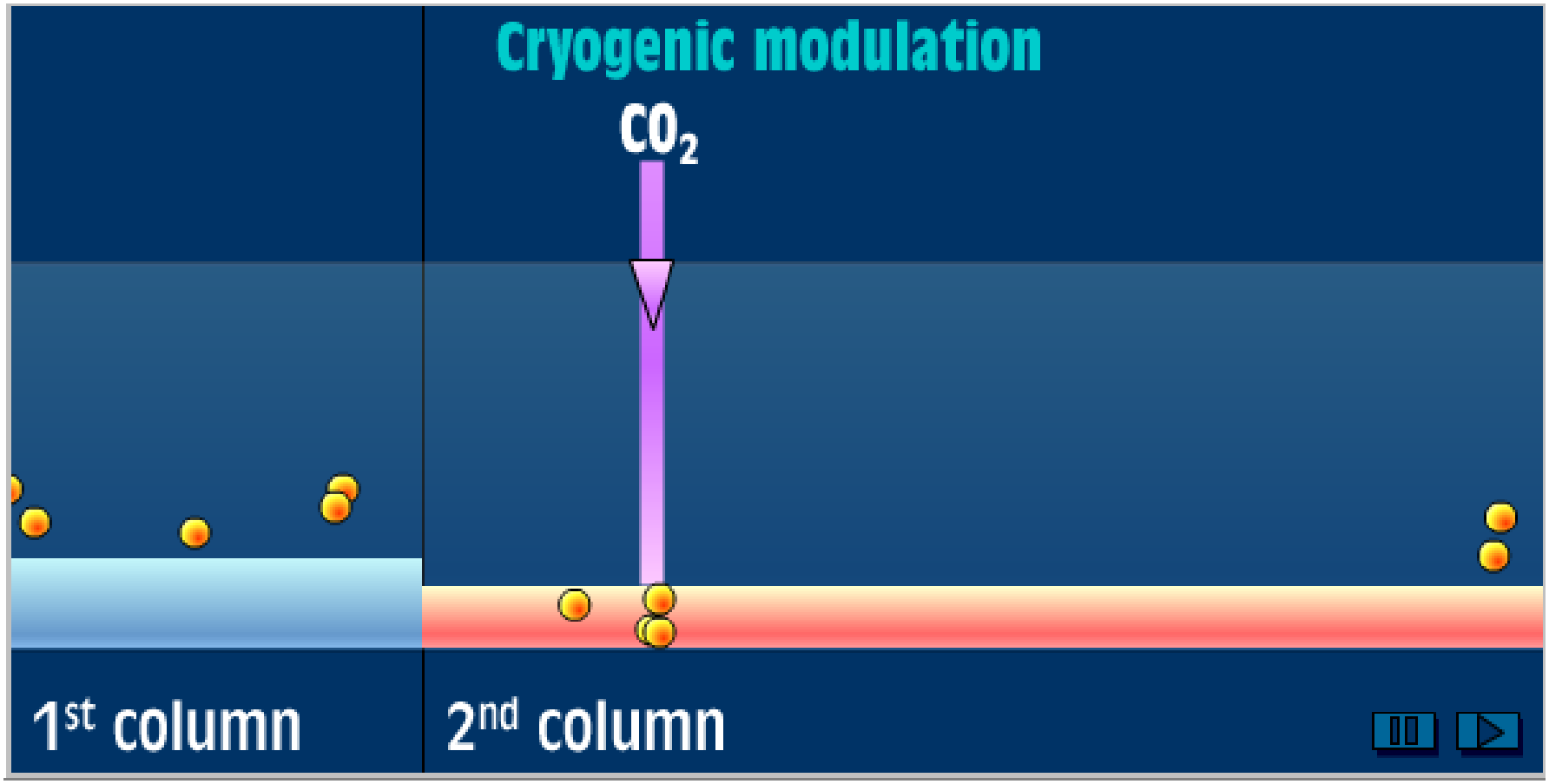
Dual-Jet CO2 modulator



Cryo Modulation

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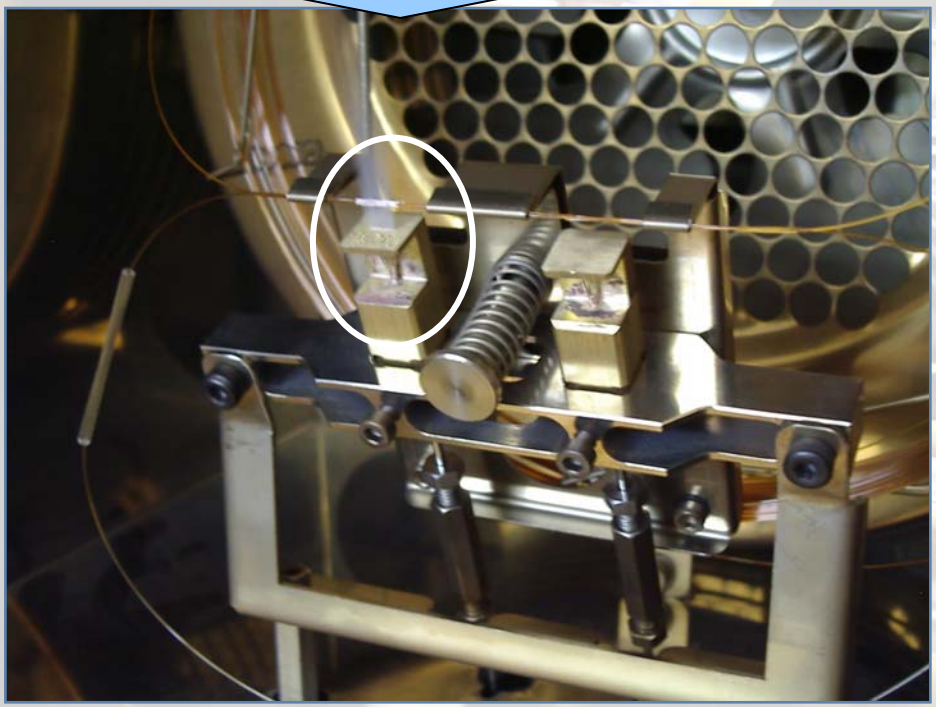
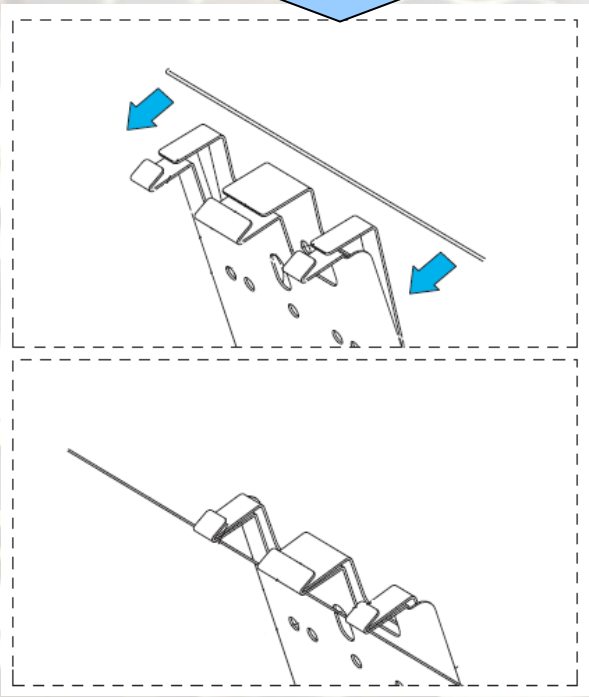
Dual-jet CO_2 modulator

Cryo-trapping



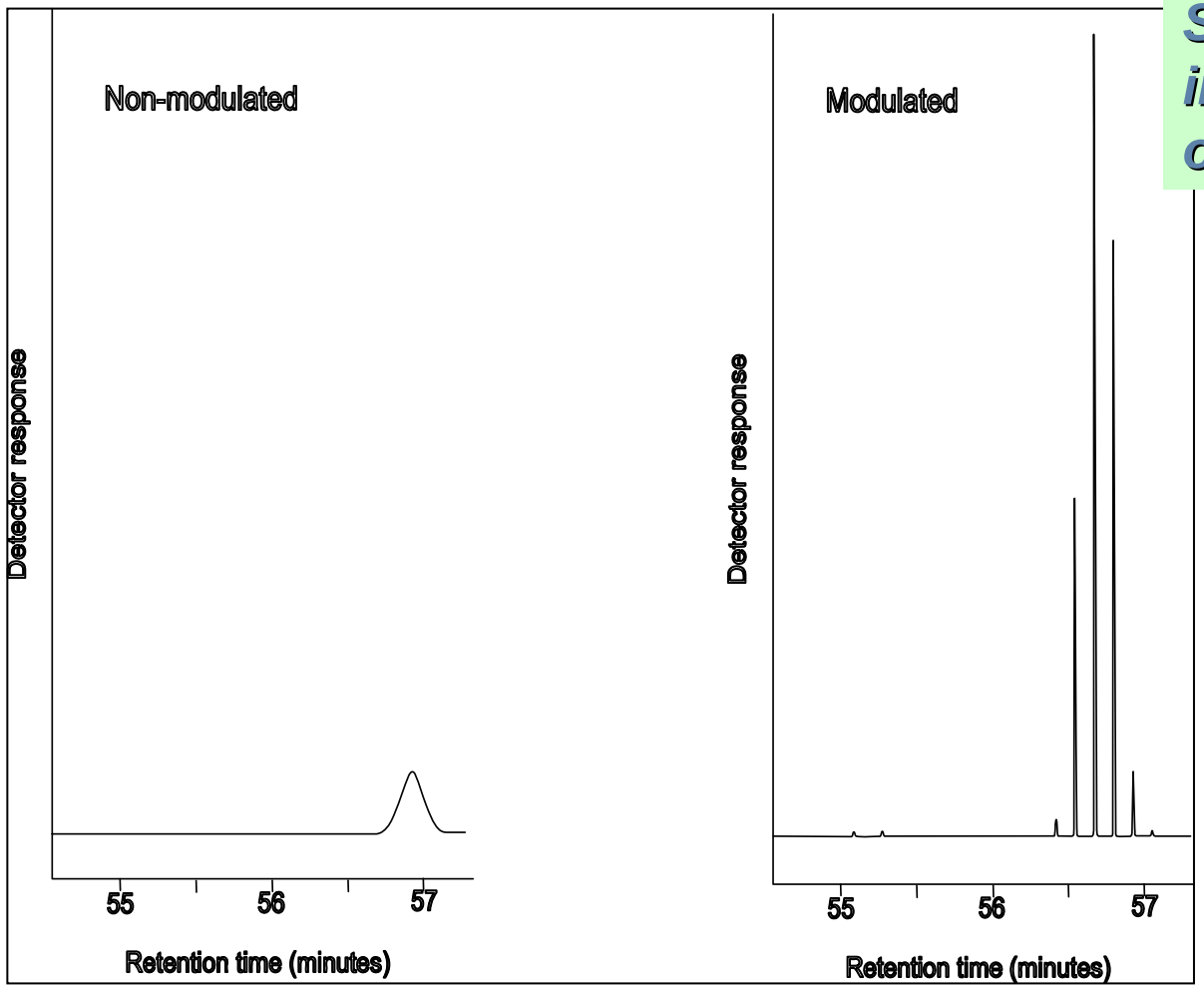
Fast and easy column installation



Thermal Modulation Licensed by Zoex Corporation

CO_2 cooling

Sensitivity enhancement



Significant improvement of S/N ratio

peaks are immobilised by switching on the cryogen

remobilised injection band is approximately 10ms wide

peaks at the detector are typically between 80 and 200 ms wide

Rules of GCxGC

➢ ***Rule 1***

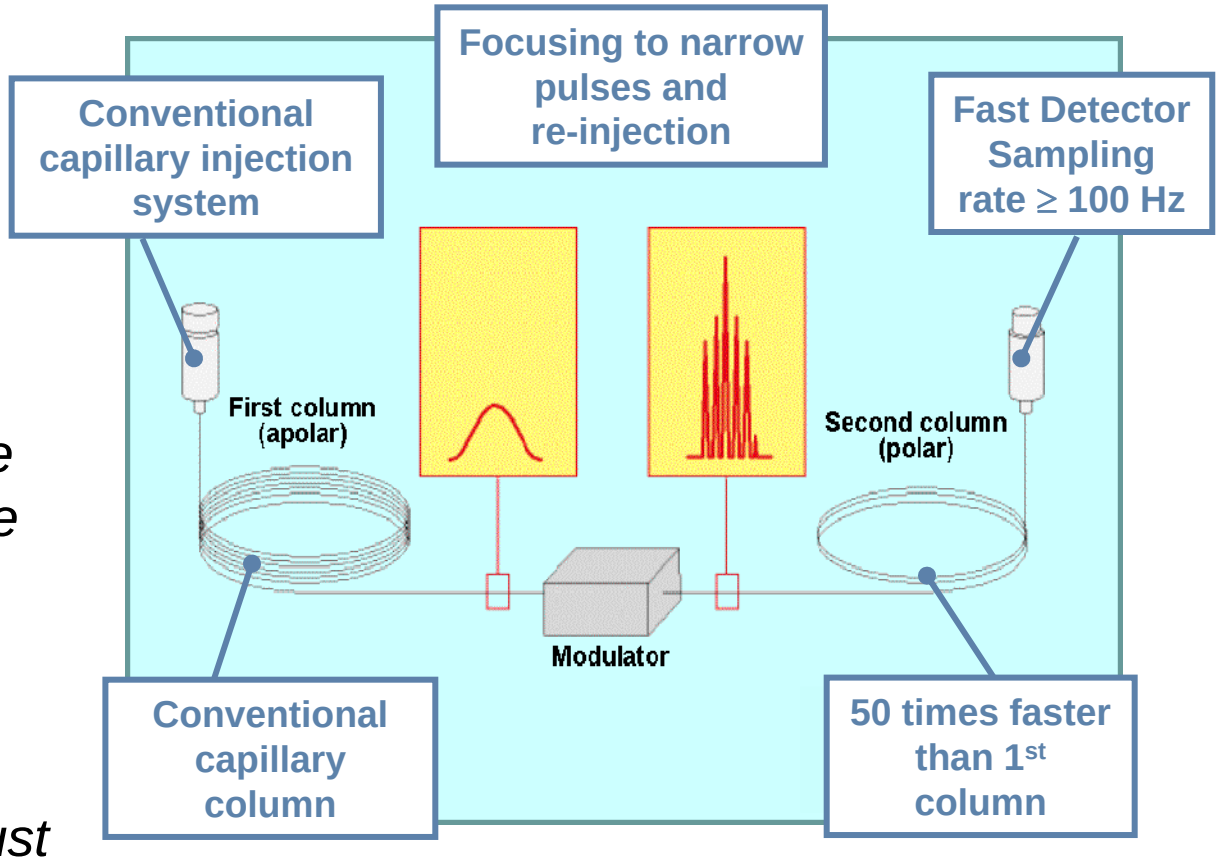
Sample analytes must undergo two discrete separation mechanisms

➢ ***Rule 2***

Separation achieved in the first dimension must not be destroyed in the second dimension

➢ ***Rule 3***

The second dimension must be significantly faster than the first dimension



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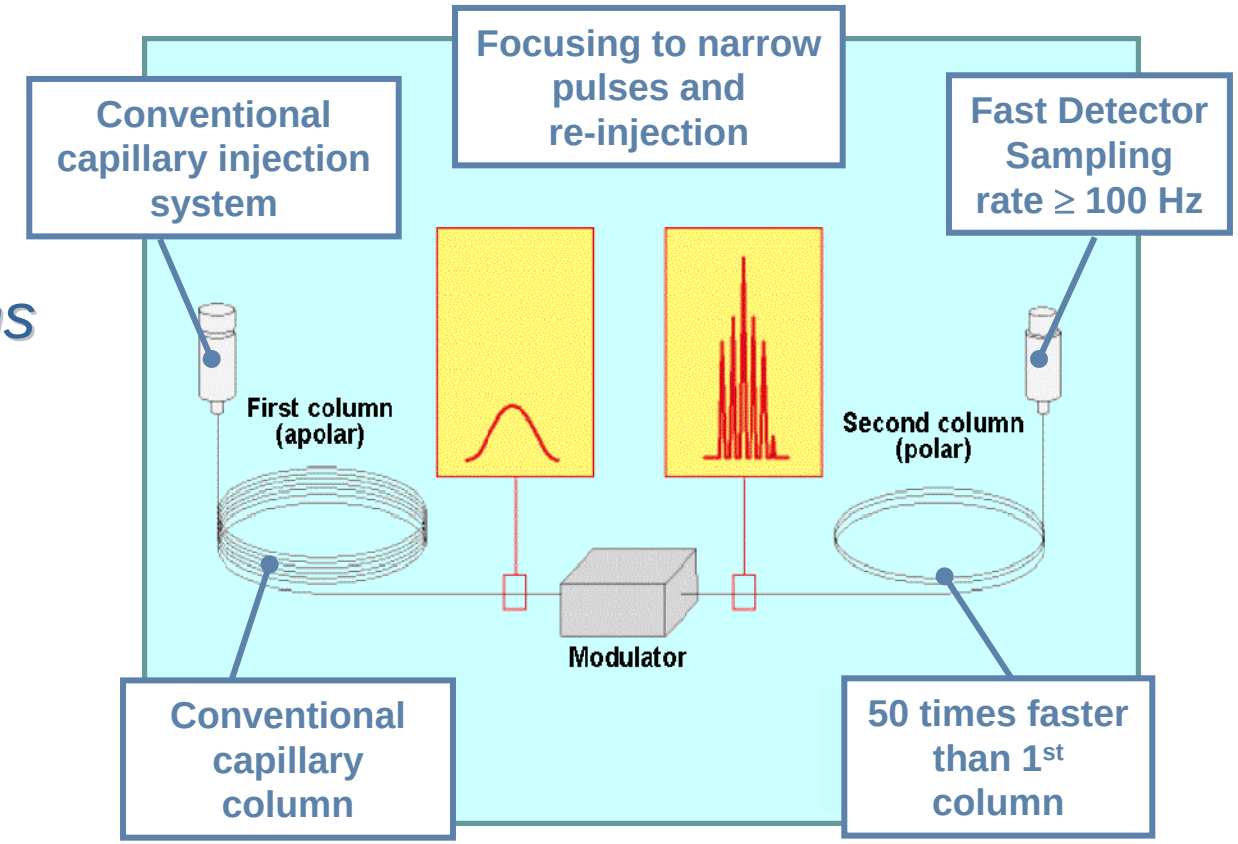
GCxGC system: schematic diagram and set up

- ✓ *Stationary phase chemistry*
- ✓ *Column dimensions*
- ✓ *Modulation Time*
- ✓ *Gas Flow*
- ✓ *Temperature Program*
- ✓ *Detector setting*



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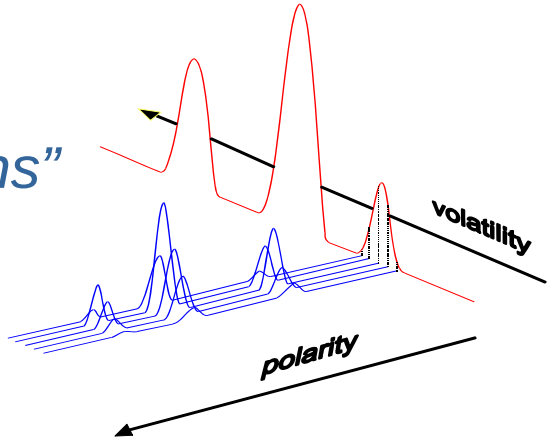
Orthogonality in GCxGC

"The absence of a correlation between retention behaviour on the two dimensions"

Retention $\propto 1/p_i^0 \gamma_i^\infty$

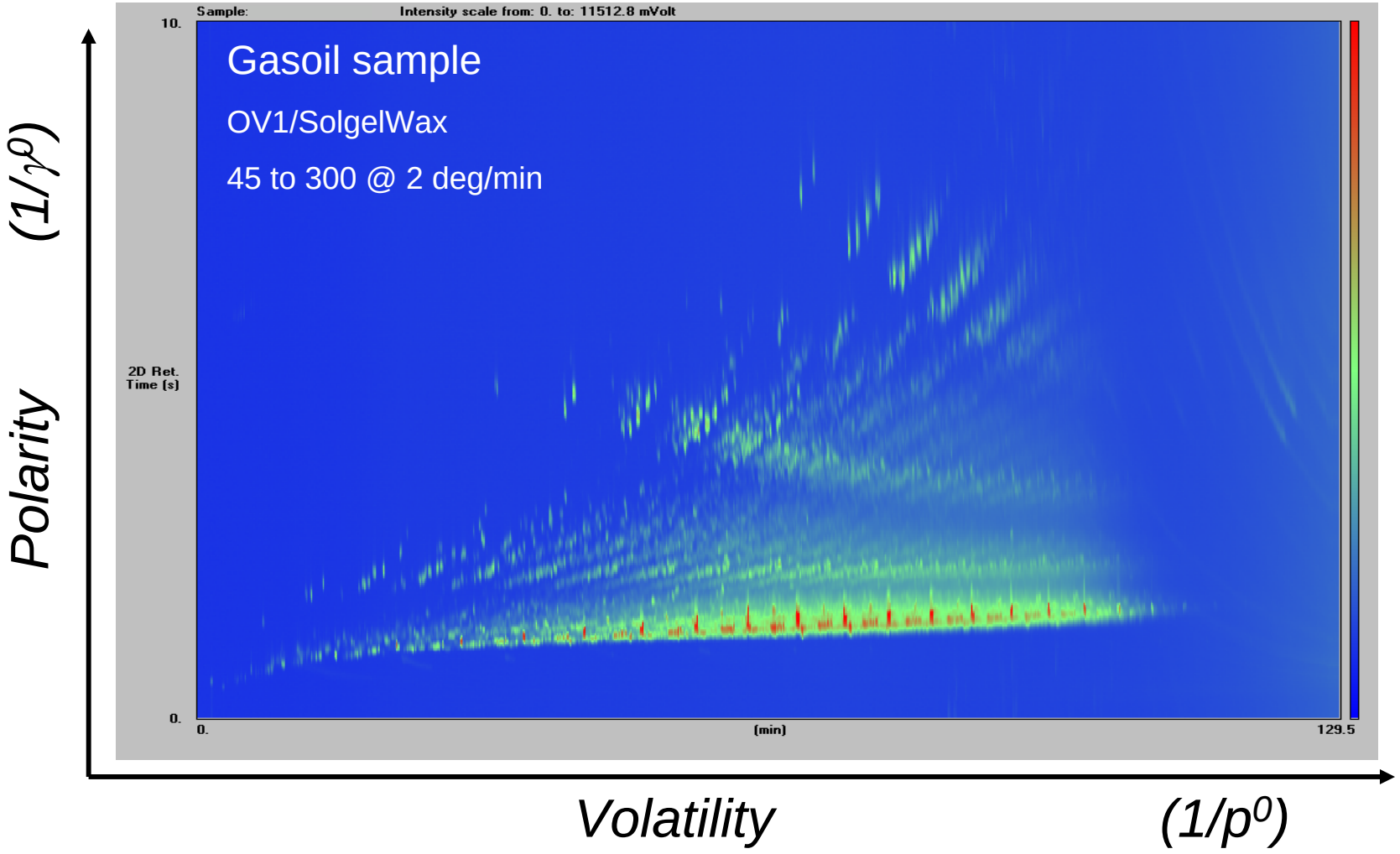


1st column : non-polar, boiling point separation	*Retention* $\propto 1/p_i^0$
2nd column: (medium) polar/shape selective, polarity/shape selectivity separation	*Retention* $\propto 1/\gamma_i^\infty$

So, first and second separation independent: orthogonal

Structured chromatograms

Separation according volatility and polarity in one spot



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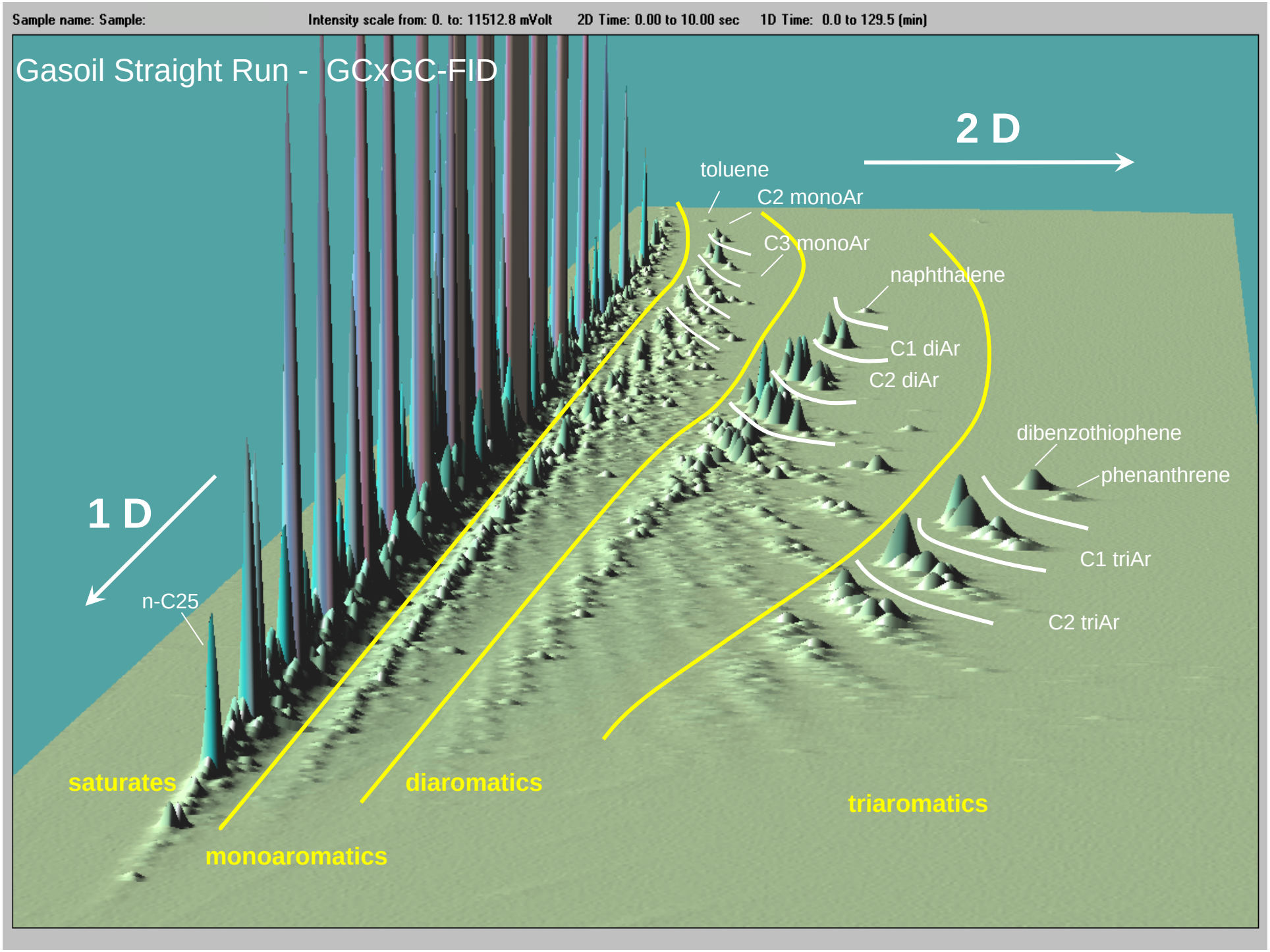
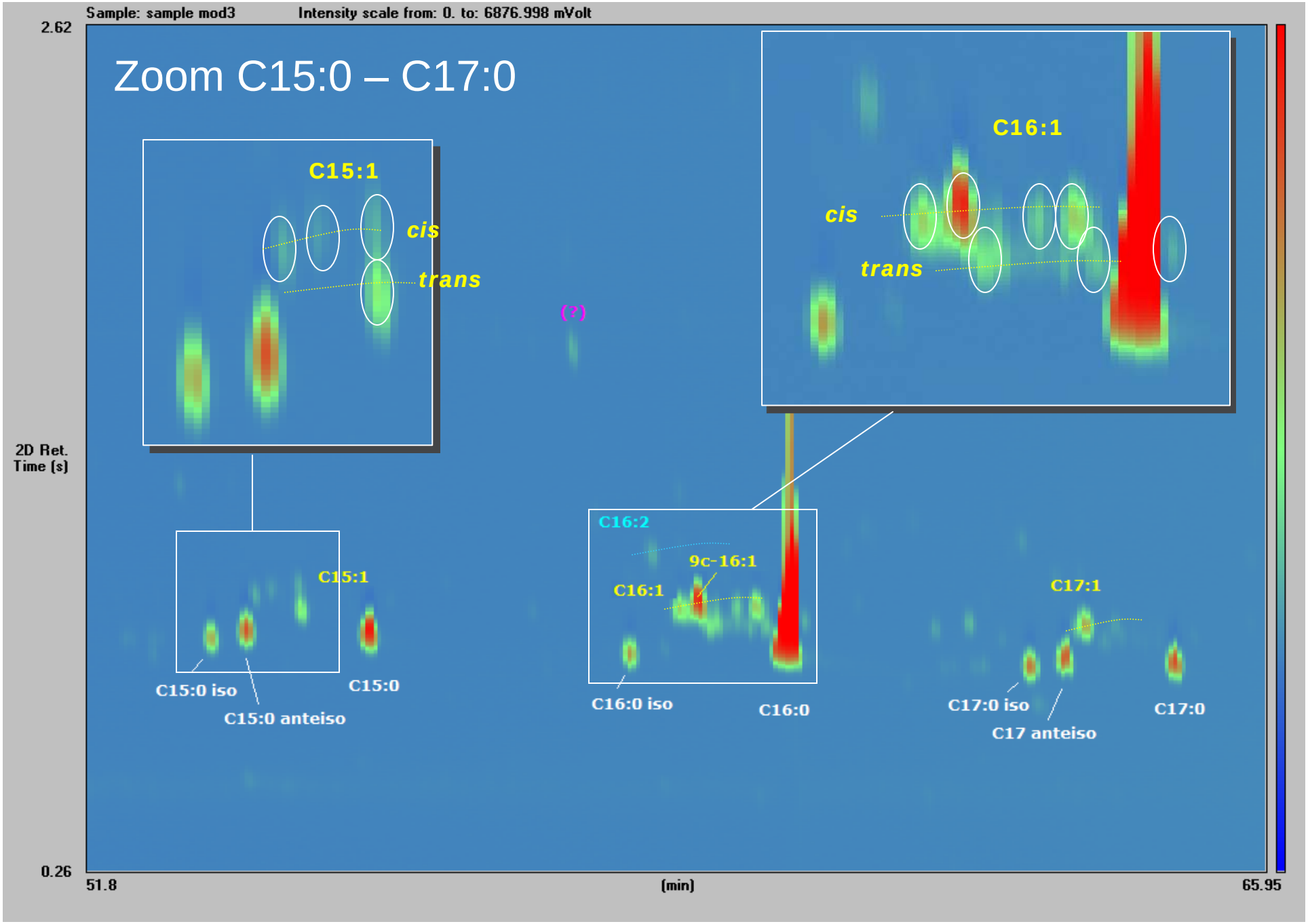
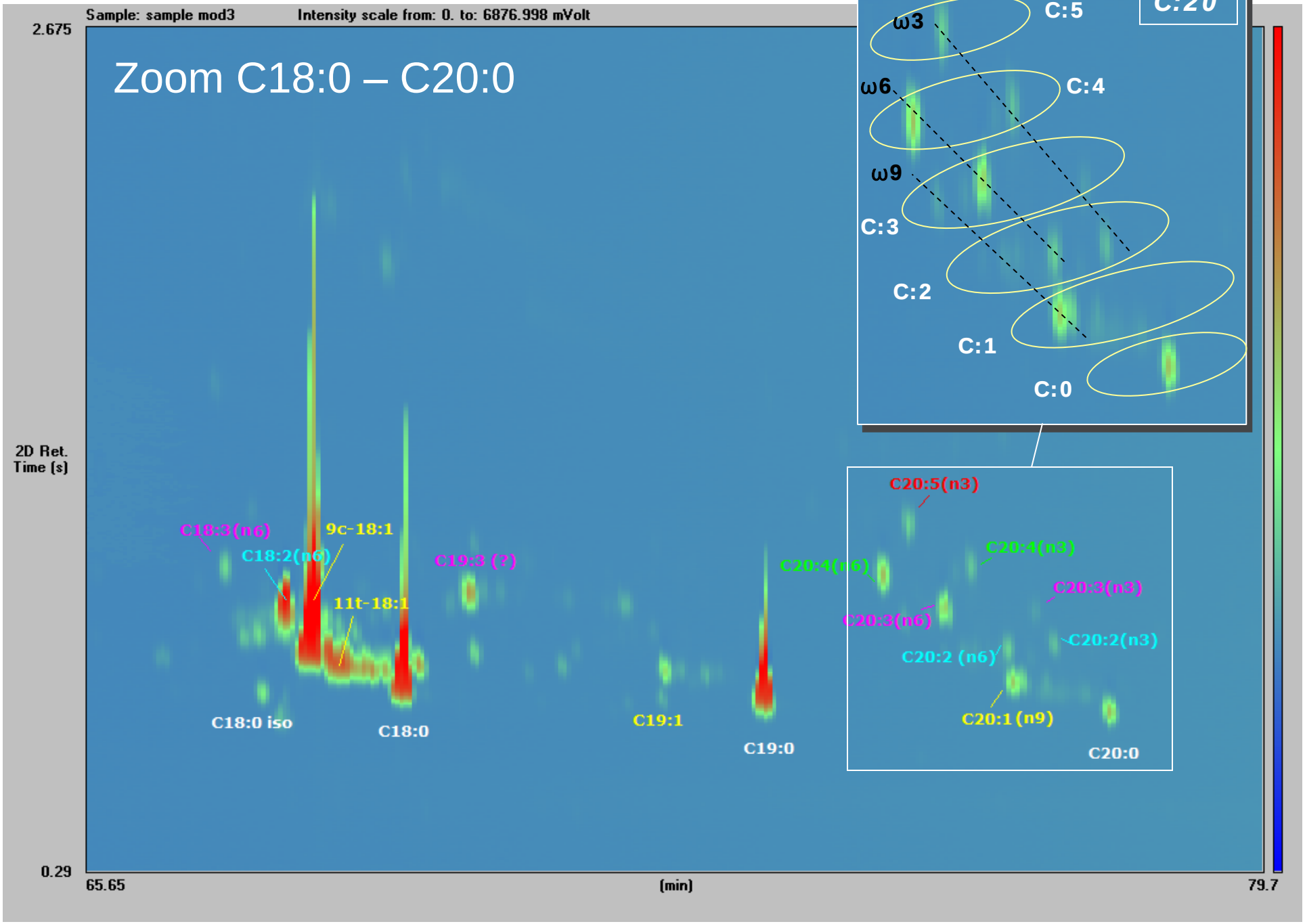

Sample name: Sample:
Intensity scale from: 0. to: 11512.8 mVolt
2D Time: 0.00 to 10.00 sec
1D Time: 0.0 to 129.5 (min)
Gasoil Straight Run - GCxGC-FID
2 D
1 D
toluene
C2 monoAr
C3 monoAr
naphthalene
C1 diAr
C2 diAr
dibenzothiophene
phenanthrene
C1 triAr
C2 triAr
n-C25
saturates
monoaromatics
diaromatics
triaromatics

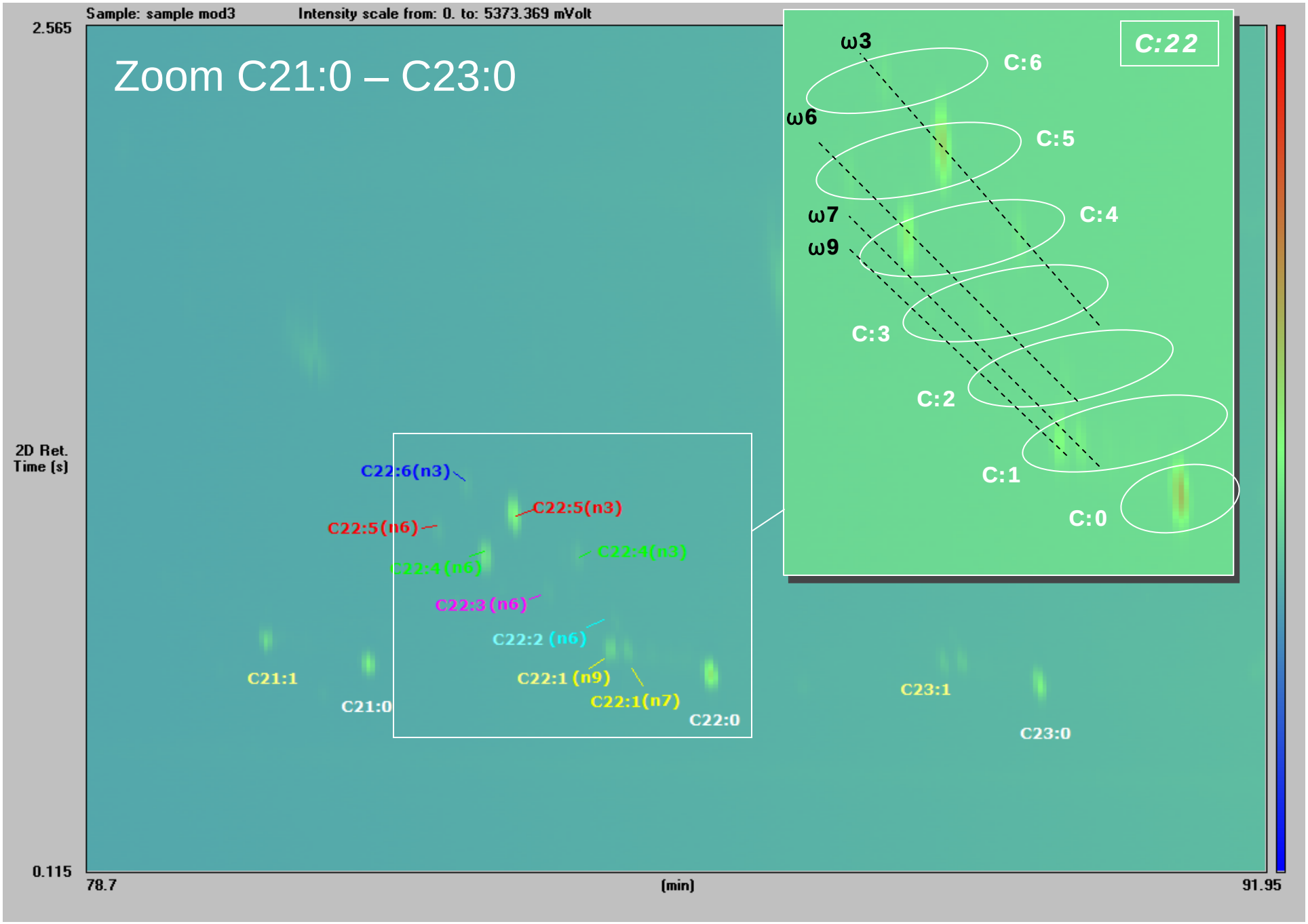
Cis/Trans FAMEs in Milk



Cis/Trans FAMEs in Milk



Cis/Trans FAMEs in Milk



Advantages of Comprehensive 2DGC

1. The separation capabilities of GCxGC is considerably higher than conventional 1D capillary GC and GC-GC

2. GCxGC offers better sensitivity than conventional 1D capillary GC and GC-GC due to the peak compression during modulation process.

3. GCxGC generates structured chromatograms which make the technique more suitable for sample screening than conventional 1D GC as it gives considerably more information about the sample in comparable analysis times

Advantages of Comprehensive 2D GC

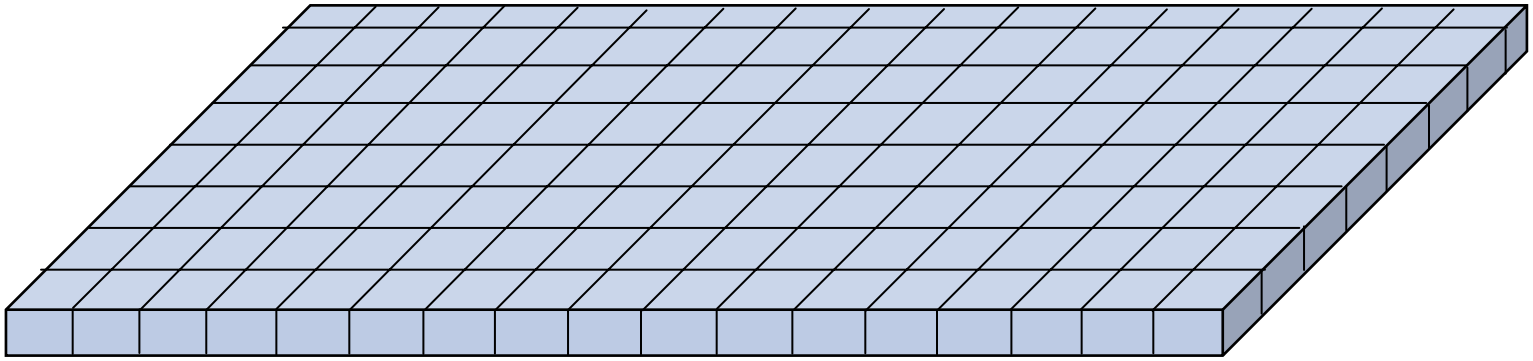
…. and more

3. GCxGC separation permits more reliable peak identification compared with conventional 1DGC as the peak elution is characterised by two retention times

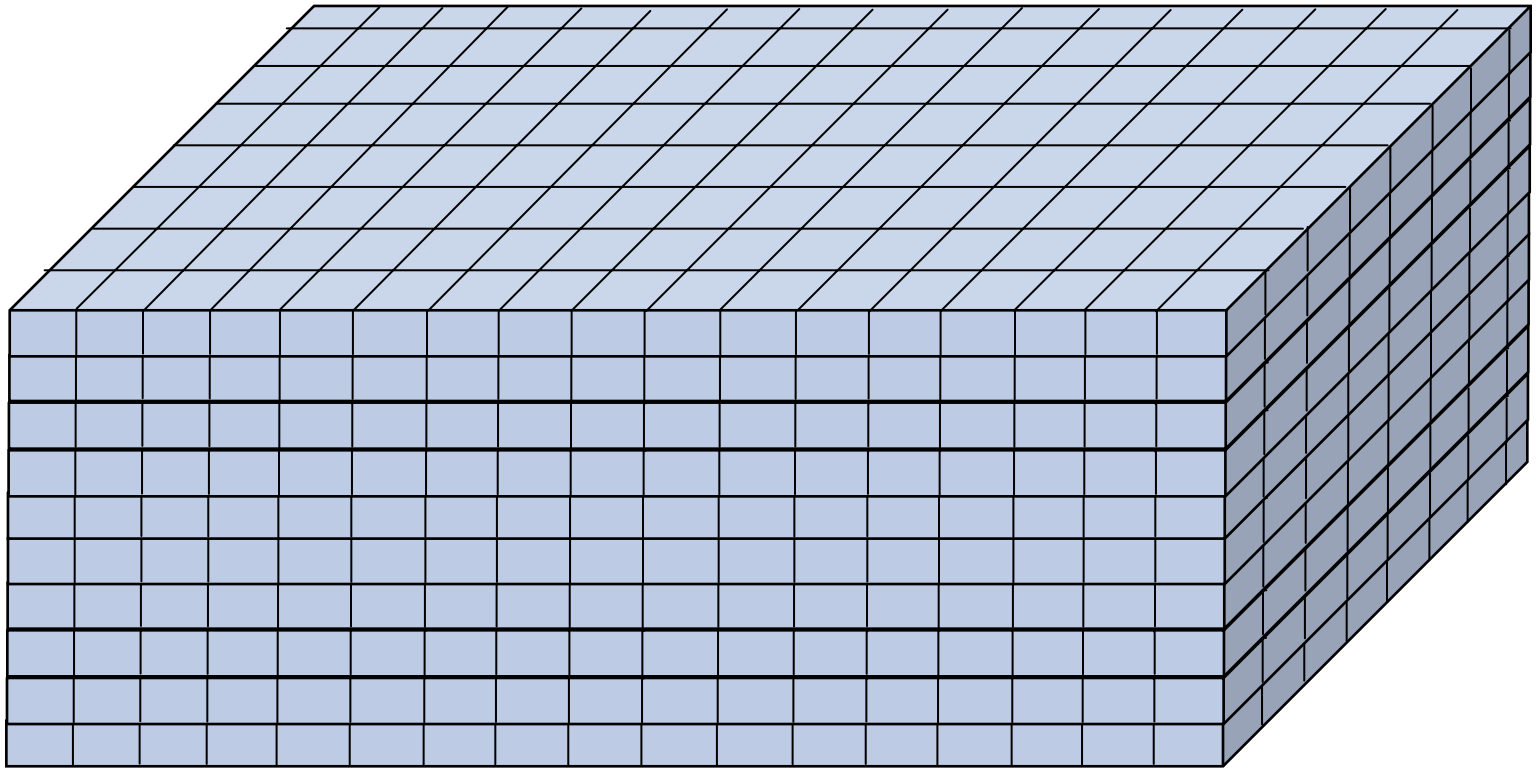
4. GCxGC technique is compatible with all type of injection systems and sample handling techniques used in GC

5. GCxGC can reduce the sample clean up procedures as the high separation capability of the technique allows to reduce matrix interferences on target compounds

MS coupling for extended information capacity



MS coupling for extended information capacity



MS coupling for extended information capacity

Most used mass spectrometers coupled with GCxGC

- TOF-MS
  - Optimum acquisition speed up to 500 spectra/s
  - Deconvolution possibility
  - Heavy data files and long data processing time

- Single Quad MS
  - Acceptable acquisition speed with new generation of rapid-scanning qMS up to 50 scan/s on limited mass range with reduced spectral skewing
  - Faster data reprocessing
  - Cheaper solution
  - Very suitable for qualitative purposes and ID confirmation
  - Suitable for quantitative analysis on a limited mass scan range or in SIM mode

MS coupling for extended information capacity

Extended capability with more performant MS spectrometers

- GCxGC-HRMS
  - Compensate the low acquisition speed with slower chromatography
  - Ultimate sensitivity for target compounds when coupled with thermal modulation process

- GCxGC-TripleQ MS
  - Transition speed compatible with GCxGC
  - Ultimate selectivity through MS/MS

MS coupling for extended information capacity

Challenging sample characterizations need both chromatographic separation and MS detection to be exploited at the maximum of their capability

- Environmental forensic
  - Ultra traces POPs
  - Fingerprinting of contaminants source
- Biomonitoring
  - Ultra traces toxic compounds in human matrices
  - Single run analysis for more families of toxicants
- Food safety
  - Ultra traces of toxic compounds in biological matrices
  - Resolution of targets from co-extracted interfrences
- Petrochemical biomarker

Conclusion

- The advent of GCxGC has enabled a deeper insight into several matrices and revealed unexpected complexity for several samples
- Despite this technique is known for more than 15yr it is far from being fully established and exploited
- Coupling the MS detection as third dimension, unprecendent amount of information can be obtained from a single analysis
- New opportunites for even more performant analytical approaches can be found by coupling the GCxGC to high performing MS as HRMS and Triple quadMS