Comprehensive two-dimensional gas chromatography a gestalt in separation science

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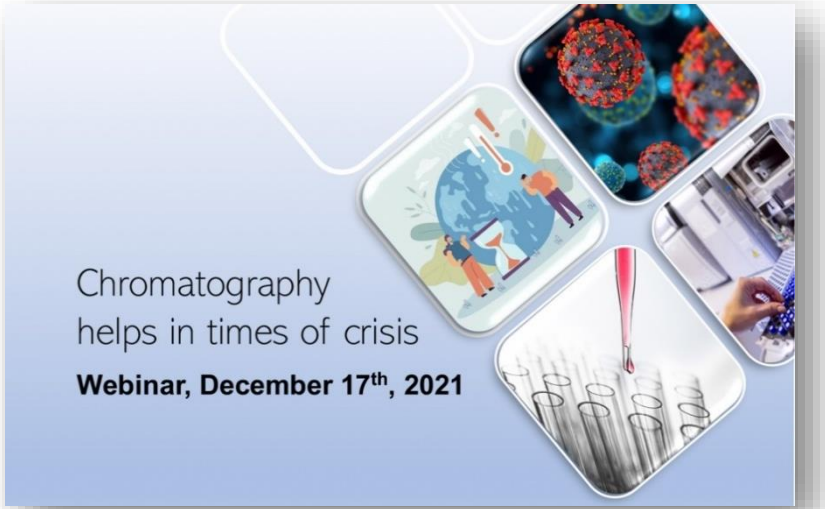

Chromatography helps in times of crisis ….

…. Comprehensive two-dimensional gas chromatography
a *gestalt* in separation science”

Chiara Cordero;[1] Simone Squara;[1] Andrea Caratti;[1] Carlo Bicchi;[1] Nicola Spigolon;[2] and Stephen E. Reichenbach [3,4]

1: Dept. of Drug Science and Technology, University of Turin, Turin, Italy
2: Soremartec Italia, Ferrero Group, Alba-CN, Italy
3: GC Image LLC (Lincoln NE, USA)
4: Dept. of Computer Science and Engineering, University of Nebraska, (Lincoln NE, USA)

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Foreword

Is comprehensive two-dimensional gas chromatography worthy to be adopted in food chemical characterization?

Opinions...

Gestalt: *a configuration or pattern of elements so unified as a whole that it cannot be described merely as a sum of its parts*

Platforms: dimensions, information space, configurations, instrumentation
Data processing: new perspectives for fingerprinting

- ✓ Challenge 1: Cocoa origins *identitation* - thermal *vs.* flow modulation
- ✓ Challenge 2: Hazelnuts volatiles and spoilage patterns - Computer vision
- ✓ Challenge 3: Extra-virgin olive oil aroma blueprint - AI *Smelling machines*

Combine challenges in a single step analytical process

Conclusive remarks

GC: The State of the Art

Chairperson: Pat Sandra
Participants: Steven Lehotay
Hans-Gerd Janssen
Chiara Cordero
Frank David
John Hinshaw

GC: The State of the Art

November 01, 2017

By **Chiara Cordero**, **Pat Sandra**, **John Hinshaw**, **Hans-Gerd Janssen**, **Frank David**, **Steven Lehotay**

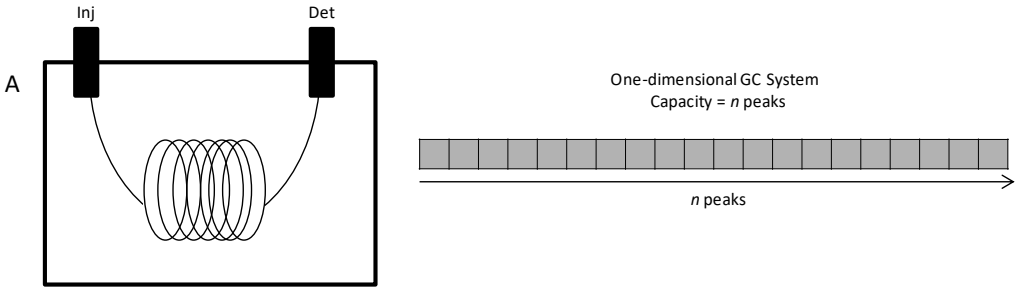
Pat Sandra: Comprehensive GC×GC has gained prominence at international meetings and in the literature in recent years. Do you expect a breakthrough in the coming years for routine analyses? Will modulation by temperature or by flow be mostly applied? Is the data handling sufficiently developed in terms of accuracy and speed for routine applications?

Steven Lehotay: GC×GC provides greater selectivity in separations, but as it is commonly used now, it adds too much time to the analysis. Another major problem is that a microbore second-dimension column is easily overwhelmed by high concentration matrix components, which is nearly always the case in real-world samples. GC×GC is overkill in common applications and fails in many difficult ones, thus, it needs to be used in a different way to provide faster separations with more sample capacity. I think a breakthrough in GC×GC would have been possible many years ago if the drivers of the technology had decided to overcome its practical limitations, including excessive liquid nitrogen usage for cryogenic modulation, rather than demonstrate niche applications.

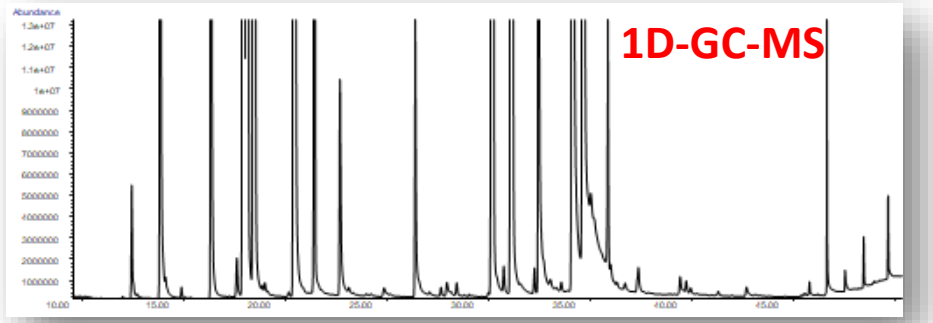
Frank David: GC×GC will definitely find its way to routine application, mainly in petrochemical analysis. All types of modulators can be used, but easier, user-friendly, intuitive software and data handling are needed. Moreover, the application potential of GC×GC should not be overestimated. One-dimensional GC and GC–MS are able to cover most GC-amenable applications.

Hans-Gerd Janssen: GC×GC is already routinely used in the mineral oil area and in the flavour and fragrance industry, simply because one-dimensional GC cannot do the job. For many other applications we are forced, by government policies or for company-internal reasons, to stick with one dimensional GC. I do not expect a dramatic breakthrough for GC×GC, but it could evolve to 10–15% of the GC market.

…a configuration or pattern of elements so unified as a whole that it cannot be described merely as a sum of its parts…



- ✓ Separation power (peak capacity) is given by the product of the two chromatographic dimensions (GC×GC);
- ✓ Independent (almost) displacement in both dimensions produces rational retention patterns for homologue series
- ✓ Band compression (in space - for thermal modulators) produces Signal-to-Noise ratio enhancement - sensitivity
- ✓ Bi-dimensional peak patterns exploits a 3D space where fingerprinting could be more accurate that in a 2D space (as for 1D-GC profiles)



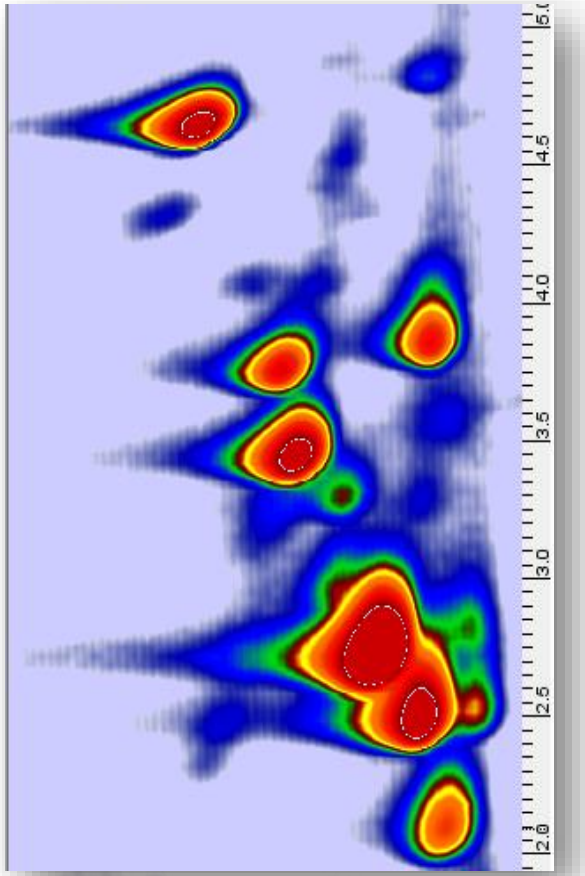
Profiling[1]
detailed analysis of the chemical pattern

Targeted - Untargeted profiling[2]
multidimensional platforms provide data on analytes identity (MS signatures) and amount in the sample

Fingerprinting[1]
general and rapid high-throughput screening
-> discriminate and classify samples

[1] Harrigan G., Goodacre R. (2003) Metabolic profiling: its role in biomarker discovery and gene function analysis. Kluwer Academic Publishers: Boston
[2] S.E. Reichenbach et al. J. Chromatogr. A 1226 (2012) 140– 148

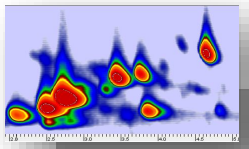
Comprehensive 2D GC
unified multi-dimensional platform



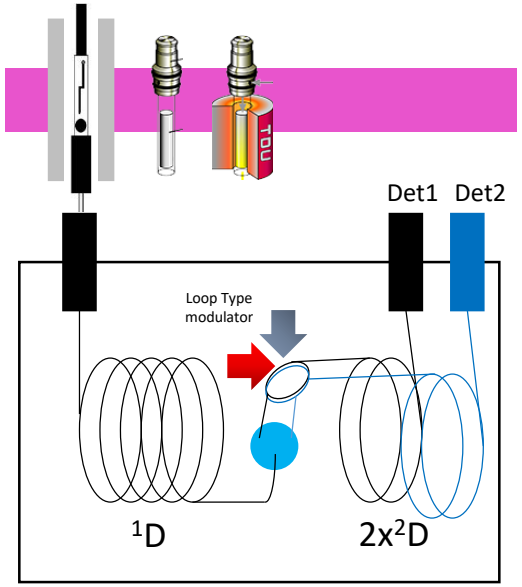
Chromatographic fingerprinting[2]
pattern recognition
extends samples comparison to all detectable analytes

"High resolution" profiling
GC×GC separation power enables accurate quantitative profiling in complex samples

Rationalized patterns of chemical classes
Group-Type Analysis
Ordered elution patterns for chemically correlated analytes

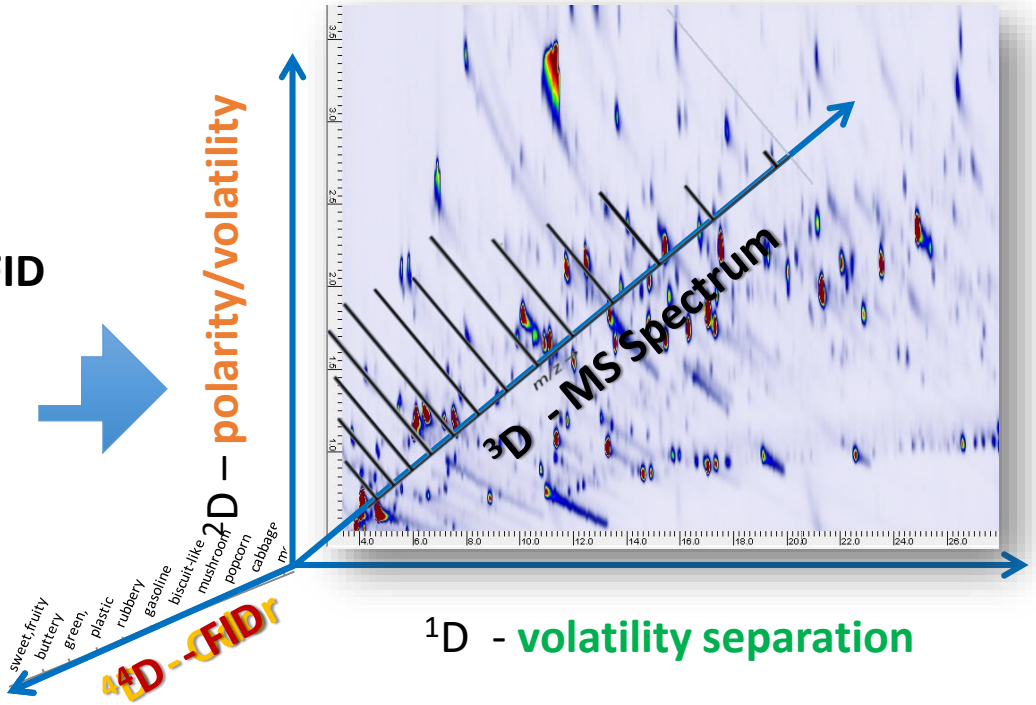


Platforms



Sample prep - GC×2GC-MS/FID
Sample prep - GC(O)×GC-MS

Information dimensions
spectral signature (identity)
volatility/polarity
sensory descriptor (bio-assay)



[1] J.C. Giddings, Sample dimensionality: a predictor of order-disorder in component peak distribution in multidimensional separation, J. Chromatogr. A 703(1995) 3–15.

Rational information space

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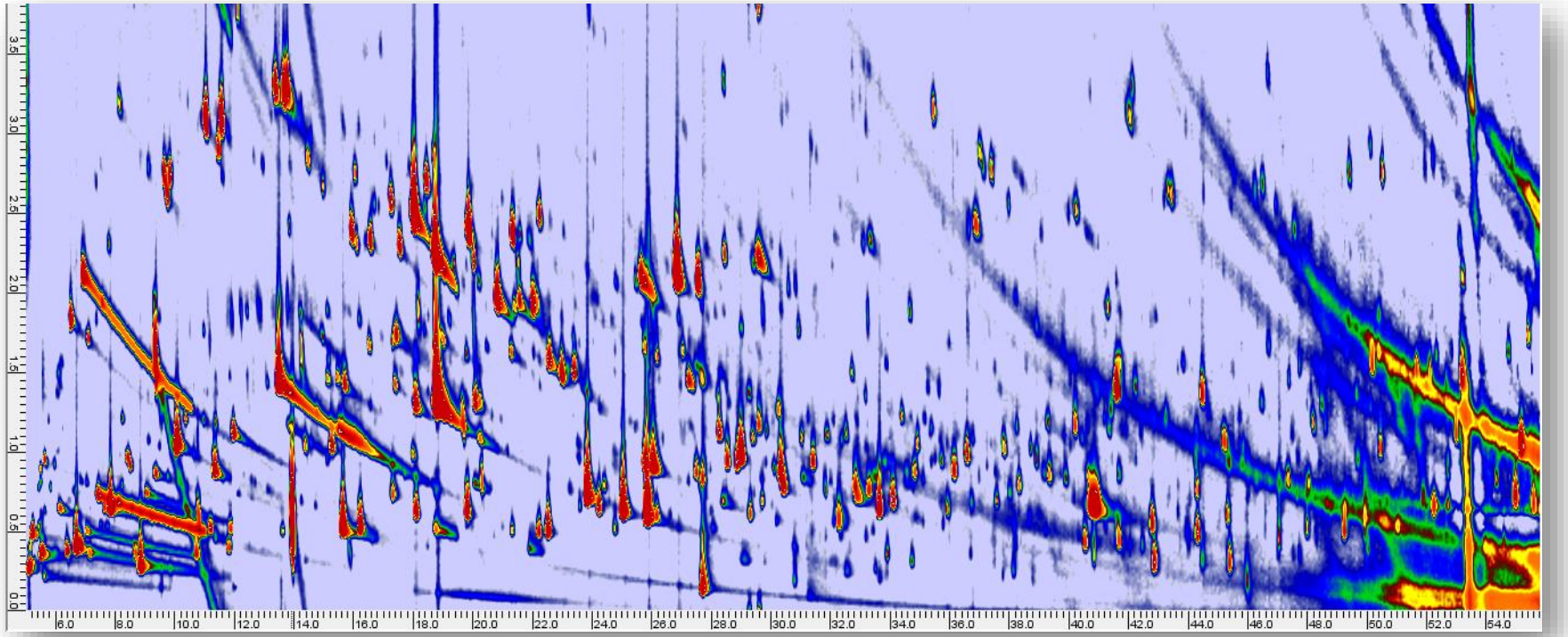
Extra Virgin Olive oil volatiles - Italian origin
HS-SPME (CAR/PDMS/DVB) - 500 mg - 50°C/50 min

Chemical dimensions

^{2}D - volatility separation



^{1}D - polarity/volatility separation

Targeted peaks over more than 800 detectable analytes

255 reliably identified by 70 eV spectrum and I^T coherence

Various chemical classes highly correlated with autoxidation processes , enzymatic peroxidation, aroma compounds and potent odorants

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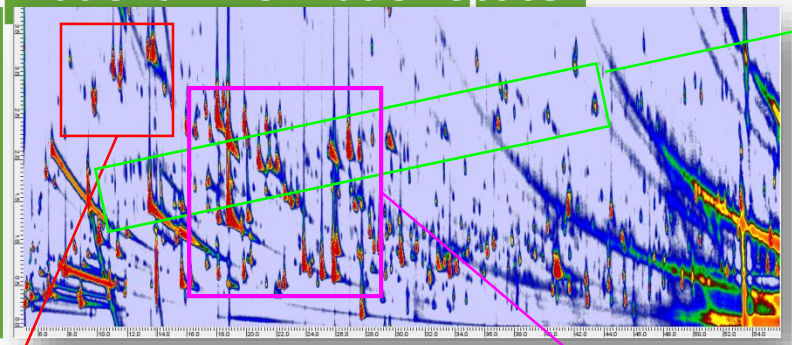
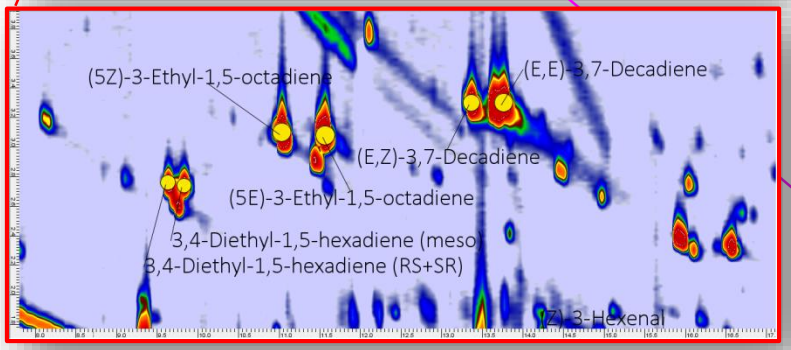
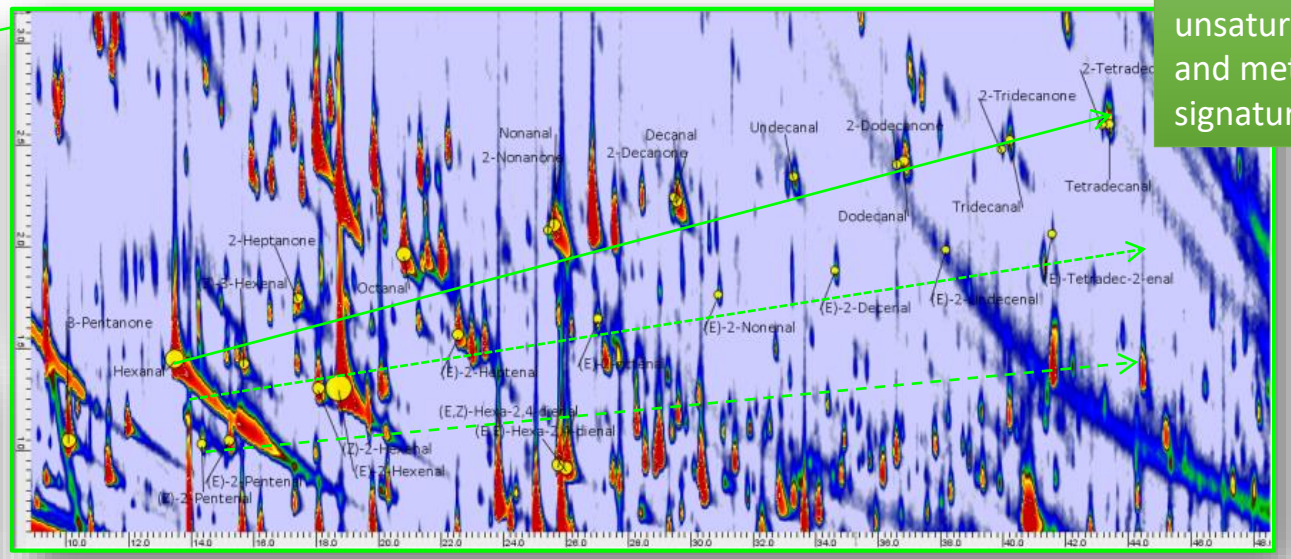
Rational information space

Chemical dimensions

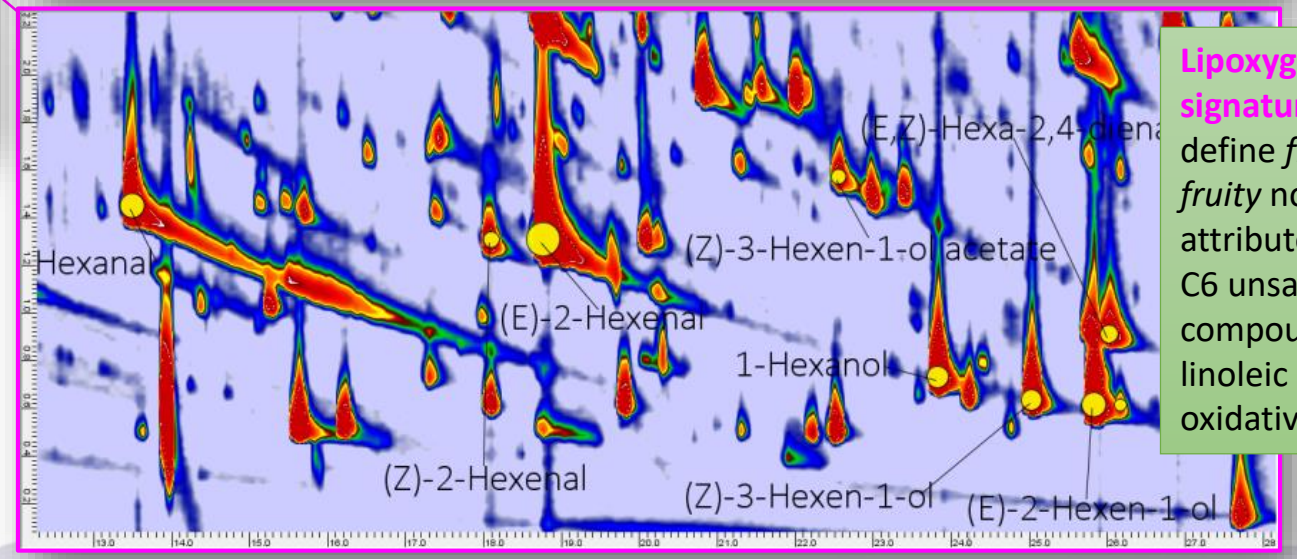




Unsaturated hydrocarbons: distinctive for earlier harvest stages: 3,4-diethyl-1,5-hexadiene (*RS* þ *SR*), 3,4-diethyl-1,5-hexadiene (meso), (5*Z*)-3-Ethyl-1,5-octadiene, (5*E*)-3-Ethyl-1,5-octadiene, (*E,Z*)-3,7-decadiene, (*E,E*)-3,7-decadiene, and (*E*)-4,8-Dimethyl- 1,3,7-nonatriene



Linear saturated and unsaturated aldehydes and methyl-ketones signatures



Lipoxygenase (LOX) signature: fundamental to define *fresh-green* and *fruity* notes (positive attributes) -> biogenesis of C6 unsaturated compounds derived from linoleic and linolenic acids oxidative cleavage.

Orthogonal information space

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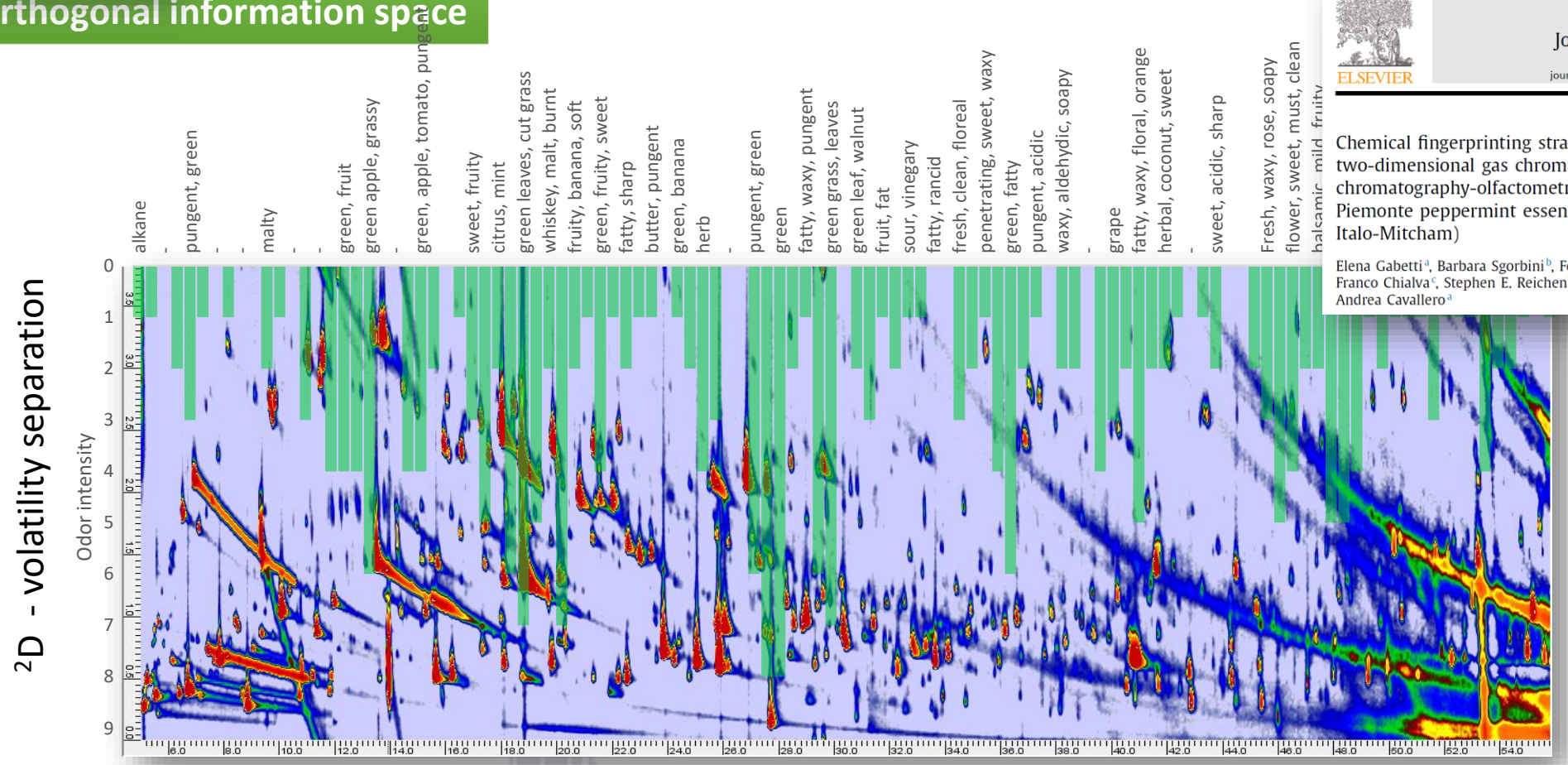
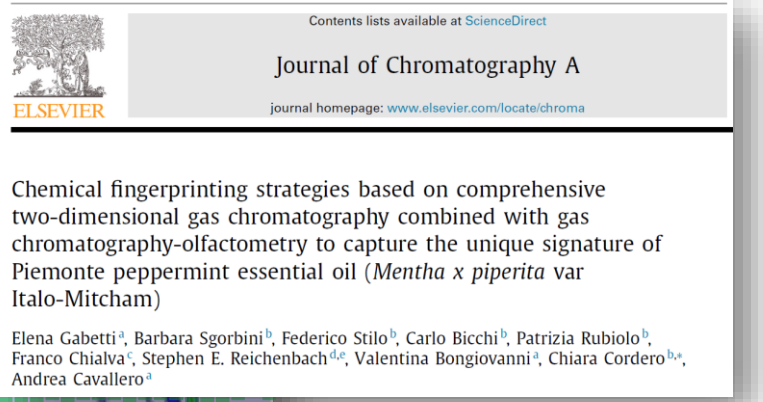


^{2}D - volatility separation

^{1}D - polarity/volatility separation



Contents lists available at ScienceDirect

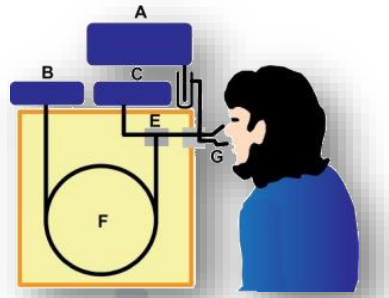
Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

ELSEVIER

Chemical fingerprinting strategies based on comprehensive two-dimensional gas chromatography combined with gas chromatography-olfactometry to capture the unique signature of Piemonte peppermint essential oil (*Mentha x piperita* var Italo-Mitcham)

Elena Gabetti[a], Barbara Sgorbini[b], Federico Stilo[b], Carlo Bicchi[b], Patrizia Rubiolo[b], Franco Chialva[c], Stephen E. Reichenbach[d,e], Valentina Bongiovanni[a], Chiara Cordero[b,*], Andrea Cavallero[a]



Picture from:
TrAC (2011) 30(11) :1756–1770

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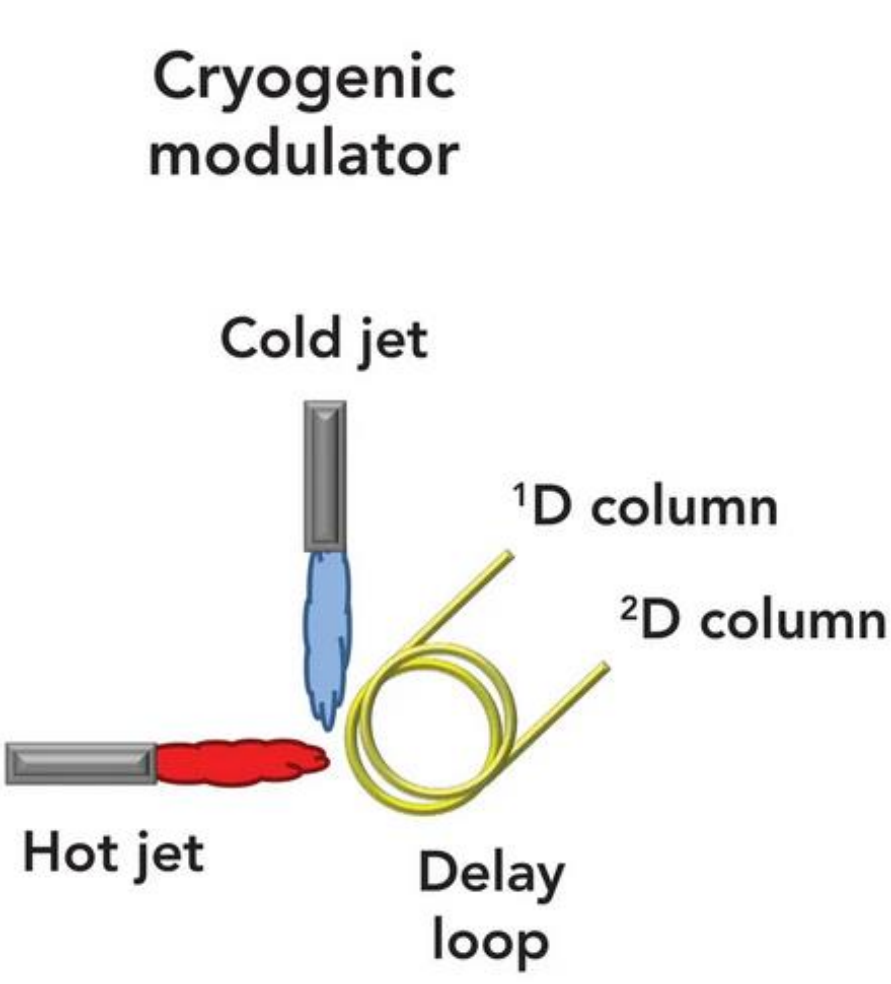
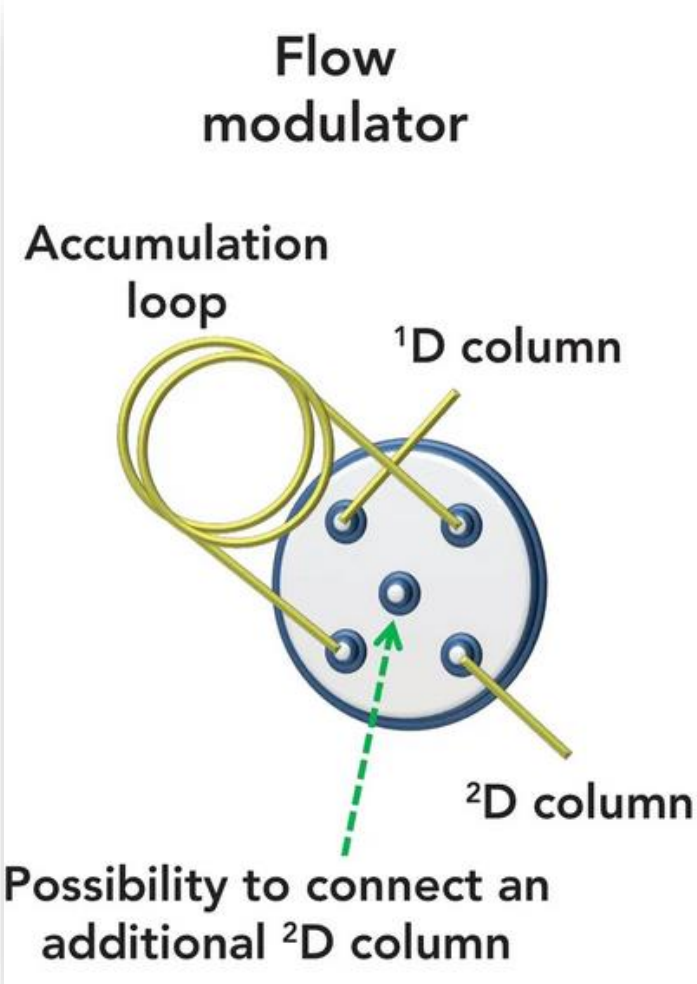
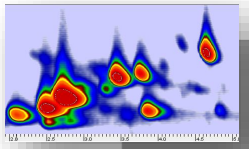
Thermal/cryogenic modulation



Differential-flow modulation



https://www.chromatographyonline.com/



Platforms

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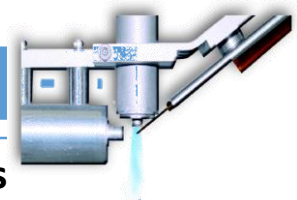
Thermal/cryogenic modulation



Successful **"high resolution" investigations**

✓in-depth sample characterization
✓adulterations - origin
✓classification based on chemical signatures

- *food metabolomics*
- *sensomics*
- *food safety*
- *emerging issues (MOH)*

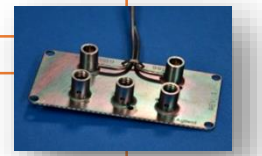
Careful tuning of experimental conditions
Expert analysts (separation science)
Data processing - new concepts

Rather high instrumental costs
High **operational costs** (cryogenics)
Trained analyst

Differential-flow modulation

Careful tuning of experimental conditions
Expert analysts (separation science)
Data processing - new concepts

Moderate instrumental costs
No additional costs for operation
Trained analyst

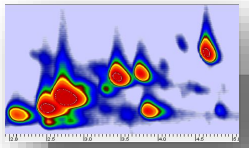


Method Translation[1,2]
Context of Fast GC - speed - capacity - resolution
Carrier gas conditions (p_i, p_0 and F), temp. programming

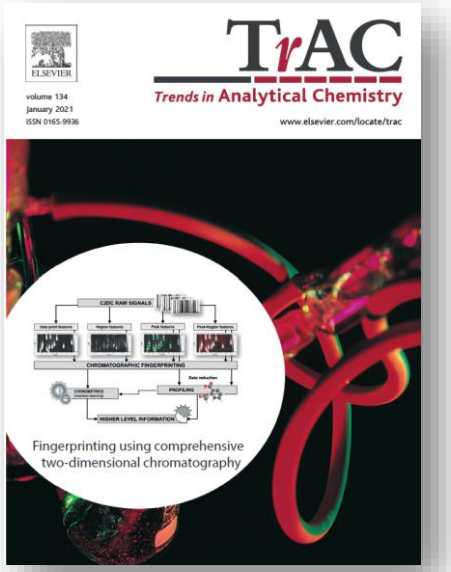
…by translating

Preserve the **elution order**
Keep **coherent elution pattern, and resolution**
Exploit all **information dimensions**
Speed-up the analysis

1. L. Blumberg and M. Klee Anal Chem 1998 70: 3828-3829
2. M. Klee and L. Blumberg J. Chrom Sci 2002 40: 234-247



Data processing



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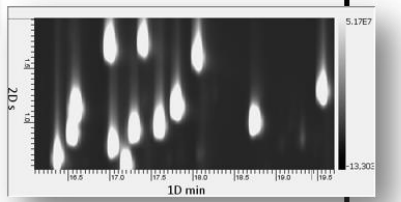
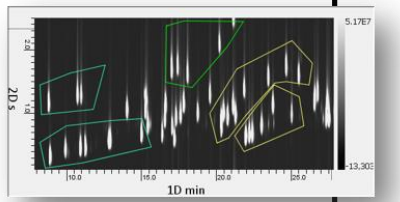
Data processing

C2DC RAW SIGNALS

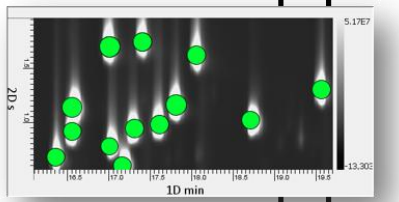
Data point features



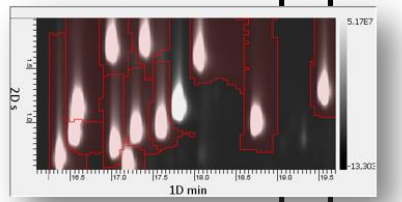
Region features



Peak features



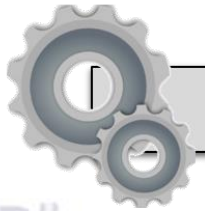
Peak-Region features



CHROMATOGRAPHIC FINGERPRINTING

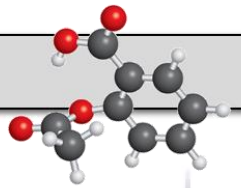
Data analysis

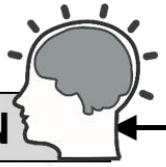
Data reduction



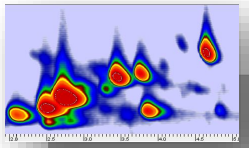
CHEMOMETRICS
(machine learning)

PROFILING





HIGHER LEVEL INFORMATION



Data processing

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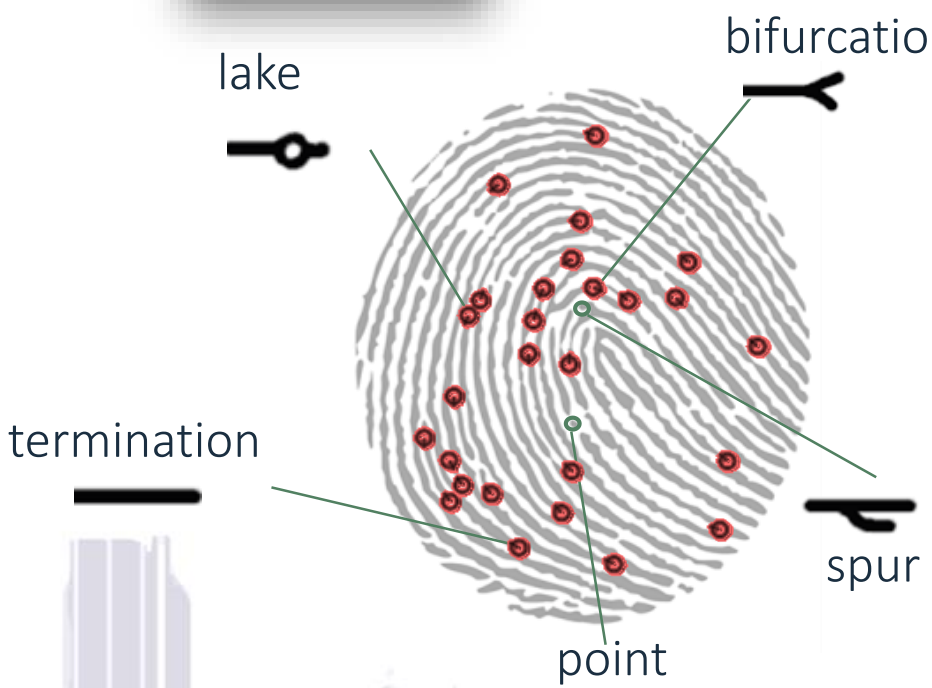
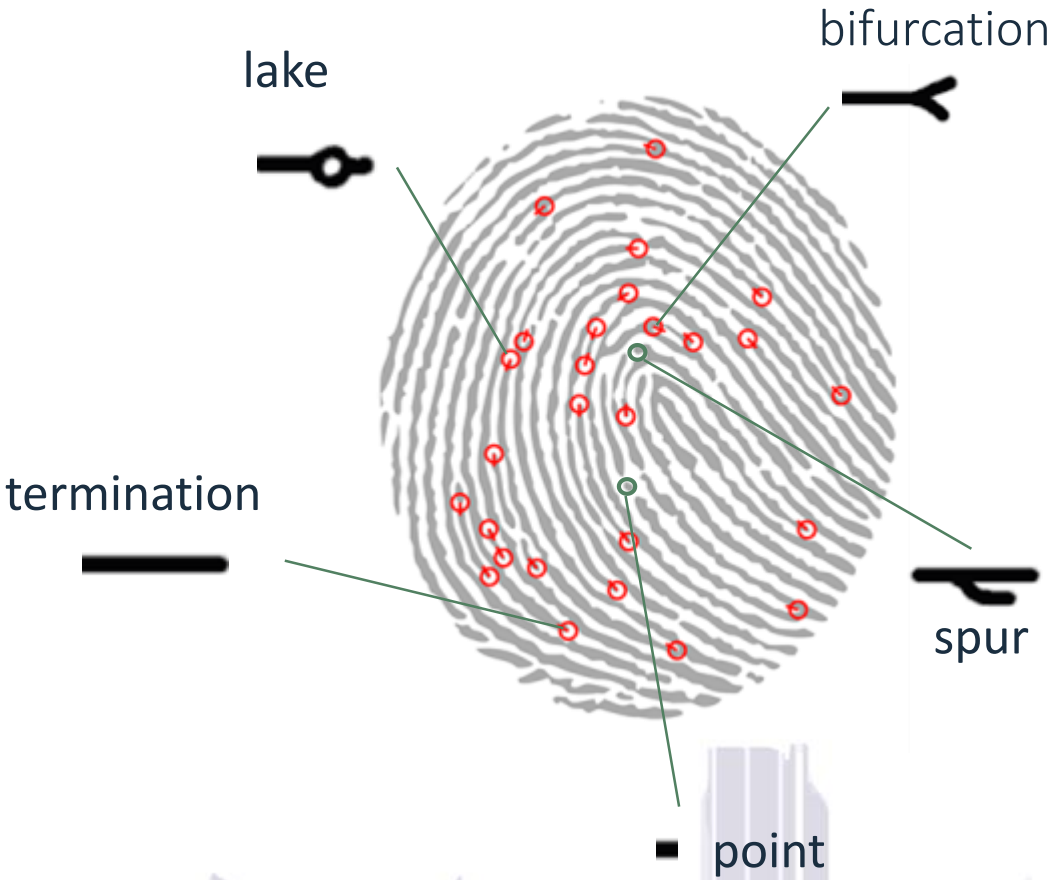
A **fingerprint** is the pattern of ridges and valleys on the surface of a fingertip
-> Everyone has unique fingerprints

Crime scene fingerprint

Database fingerprints

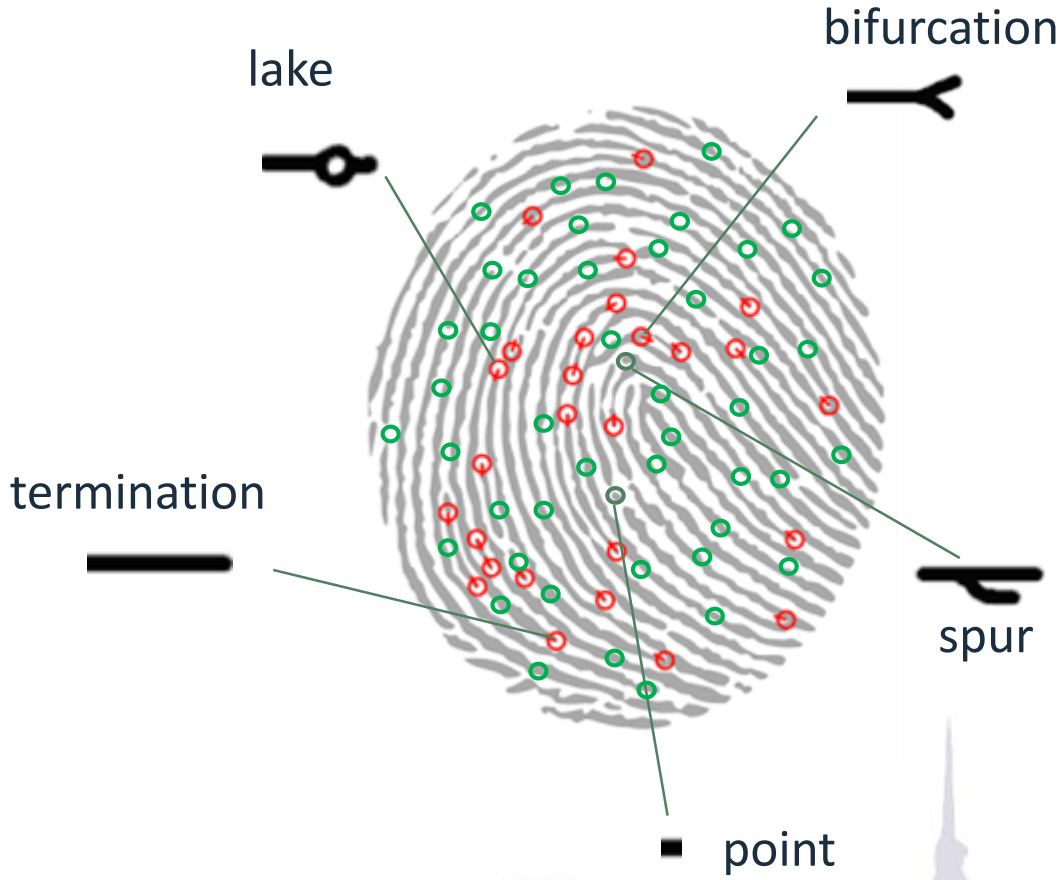


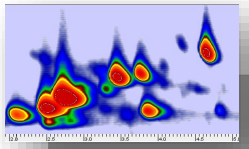
Targeted - minutiae



Untargeted - Targeted

UT - extended investigation





STEP 1

Data processing

Untargeted/*Targeted* Fingerprinting - comprehensive mapping

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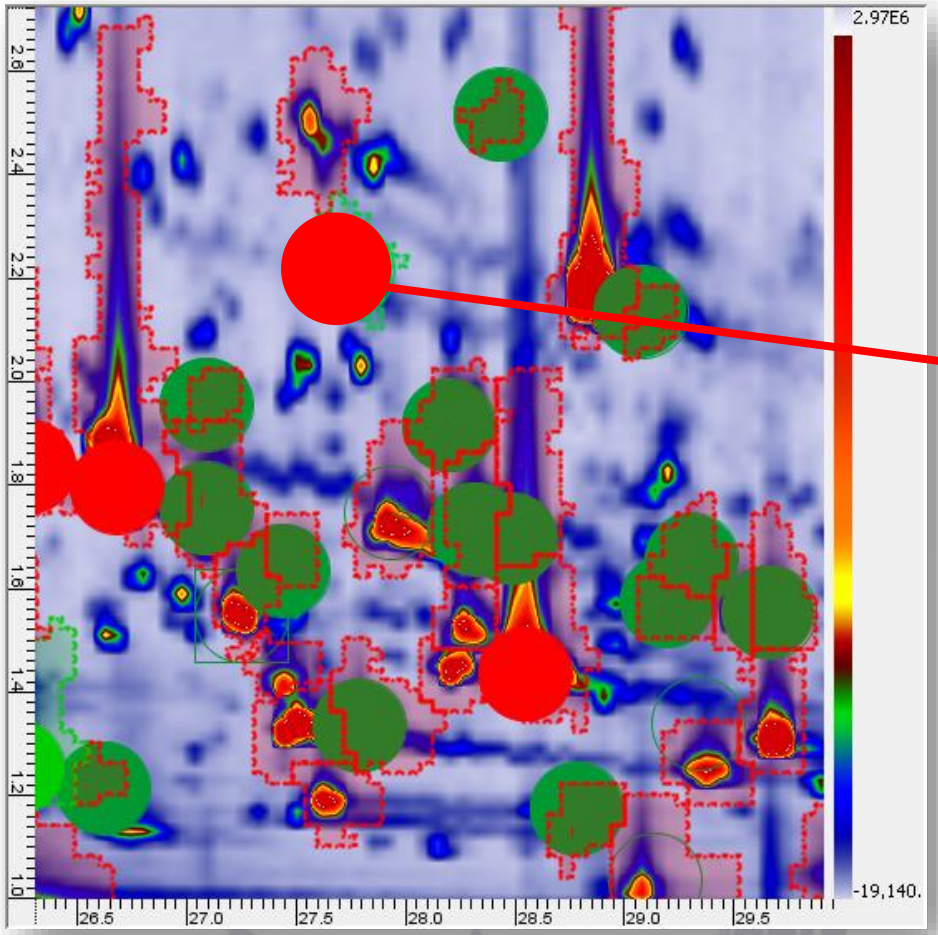

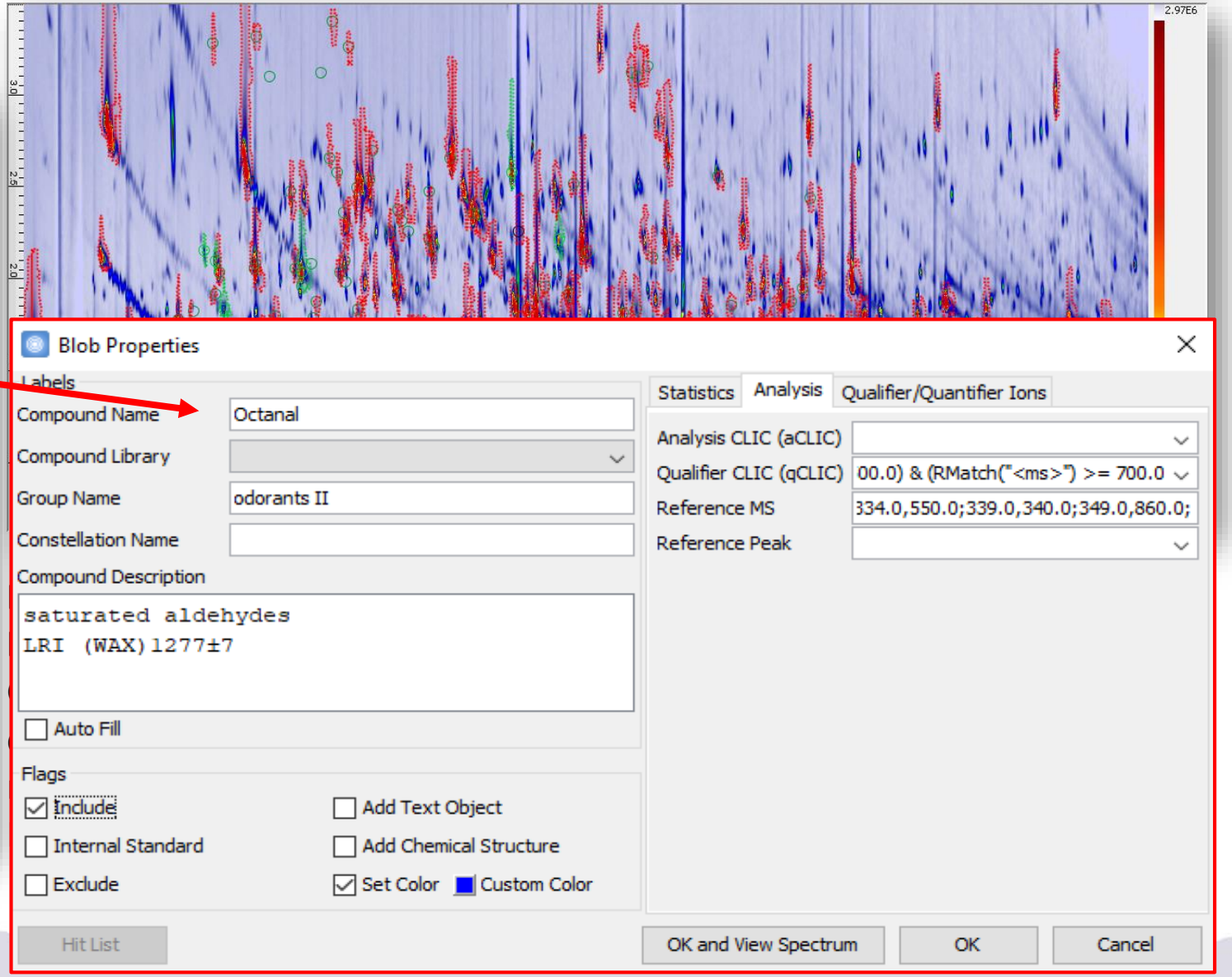

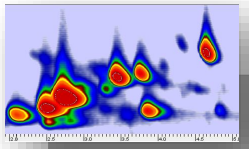

STEP 2

Data processing

Untargeted/Targeted Fingerprinting - comprehensive mapping

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Untargeted Template Construction (GC Investigator™)

Beginning with pre-targeted chromatograms:

– comprehensive pair-wise peak matching

– determination of **reliable registration peaks**

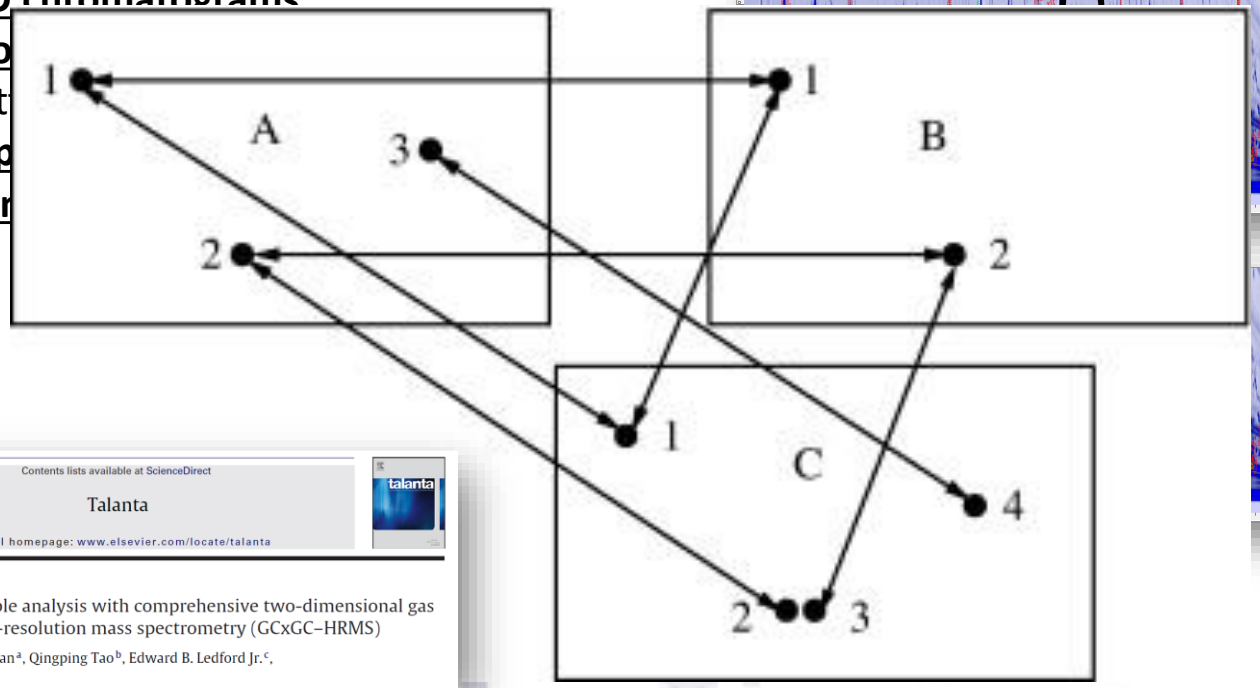
– **alignment of 2D chromatograms**

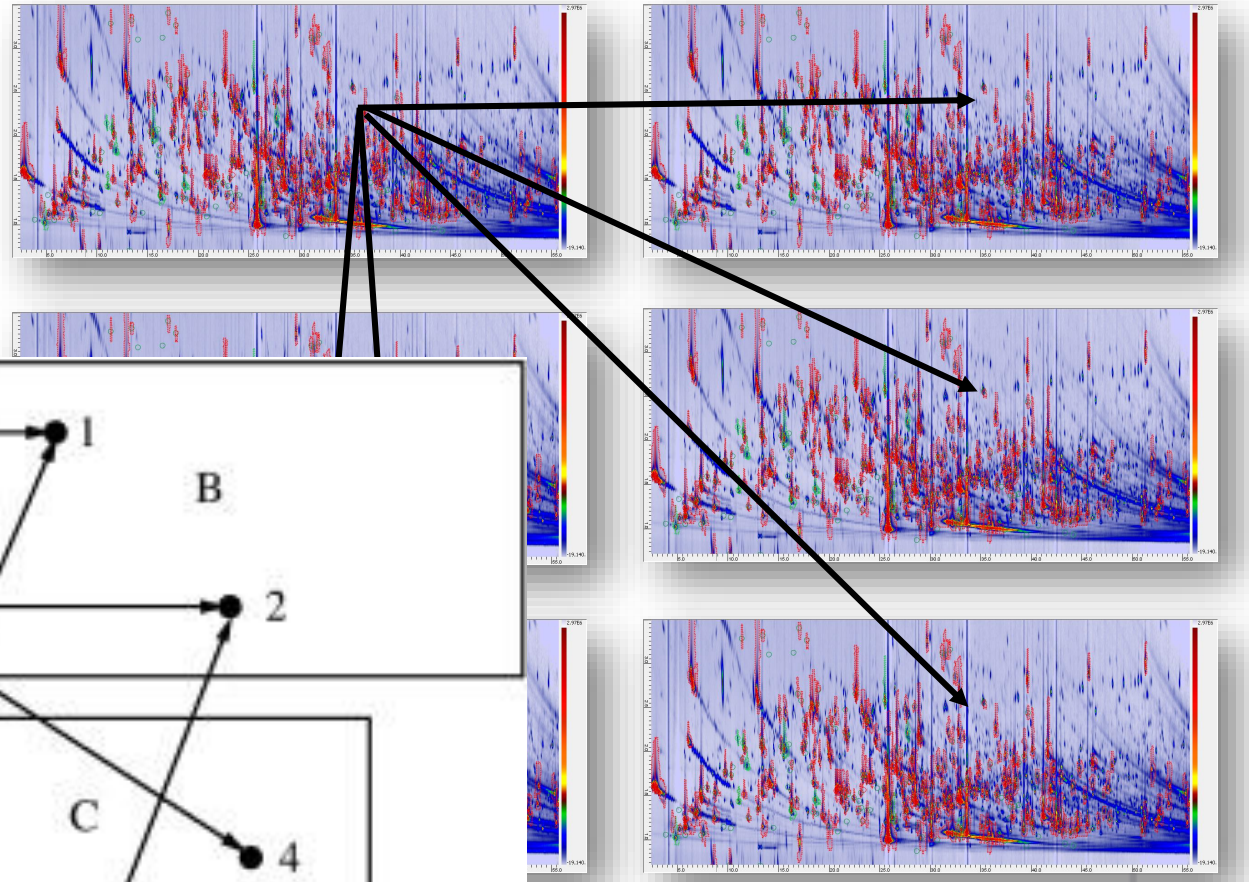
– generation of **co**

– definition of pat

– building of **temp**

and peak-regio





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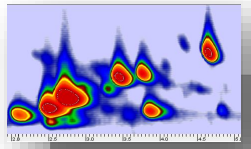
Talanta

journal homepage: www.elsevier.com/locate/talanta

Informatics for cross-sample analysis with comprehensive two-dimensional gas chromatography and high-resolution mass spectrometry (GCxGC–HRMS)

Stephen E. Reichenbach[a,*], Xue Tian[a], Qingping Tao[b], Edward B. Ledford Jr.[c], Zhanpin Wu[c], Oliver Fiehn[d]



STEP 2

Data processing

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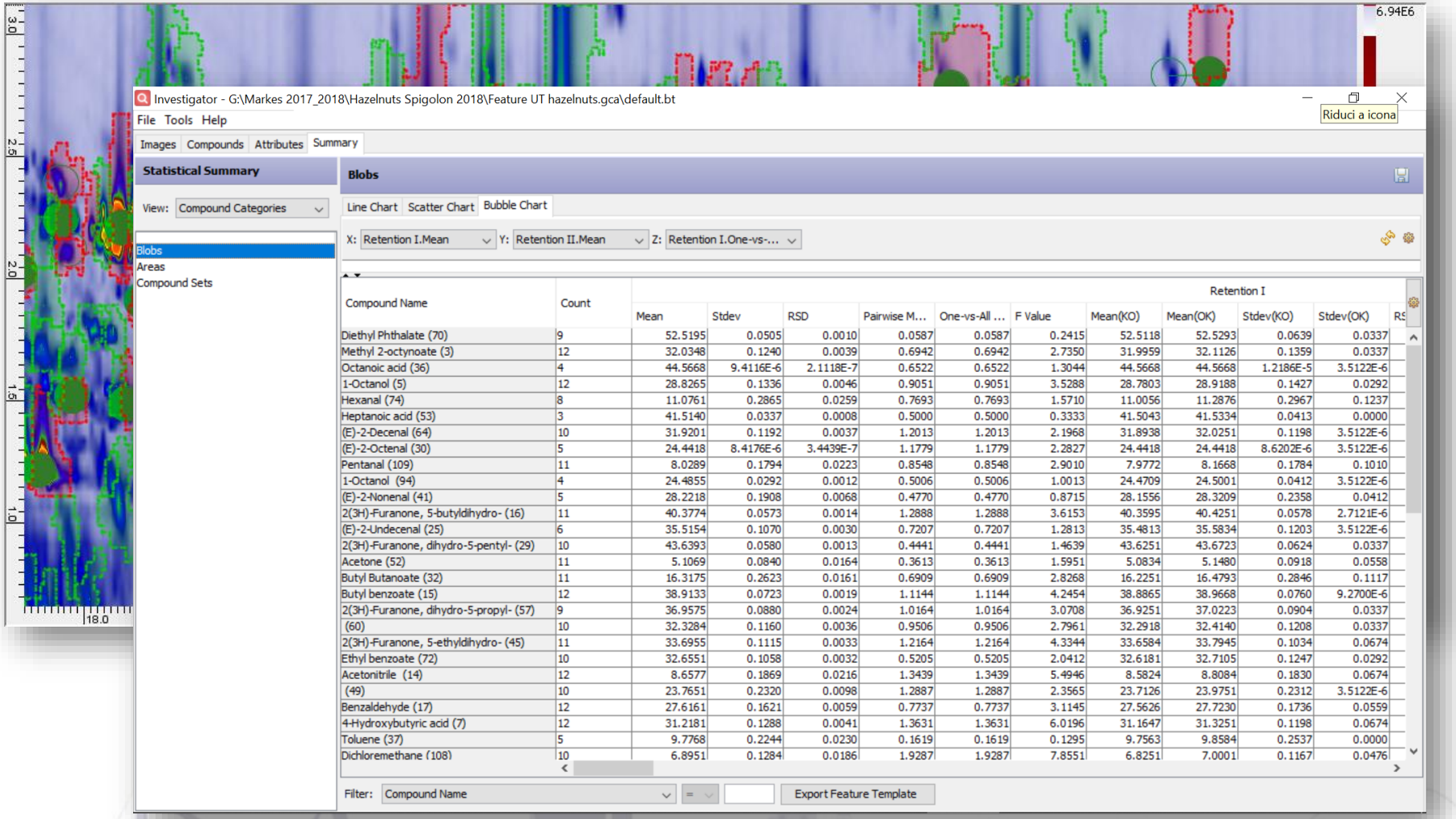


Compound Name	Count	Retention I										
		Mean	Stdev	RSD	Pairwise M...	One-vs-All ...	F Value	Mean(KO)	Mean(OK)	Stdev(KO)	Stdev(OK)	RS
Diethyl Phthalate (70)	9	52.5195	0.0505	0.0010	0.0587	0.0587	0.2415	52.5118	52.5293	0.0639	0.0337	
Methyl 2-octynoate (3)	12	32.0348	0.1240	0.0039	0.6942	0.6942	2.7350	31.9959	32.1126	0.1359	0.0337	
Octanoic acid (36)	4	44.5668	9.4116E-6	2.1118E-7	0.6522	0.6522	1.3044	44.5668	44.5668	1.2186E-5	3.5122E-6	
1-Octanol (5)	12	28.8265	0.1336	0.0046	0.9051	0.9051	3.5288	28.7803	28.9188	0.1427	0.0292	
Hexanal (74)	8	11.0761	0.2865	0.0259	0.7693	0.7693	1.5710	11.0056	11.2876	0.2967	0.1237	
Heptanoic acid (53)	3	41.5140	0.0337	0.0008	0.5000	0.5000	0.3333	41.5043	41.5334	0.0413	0.0000	
(E)-2-Decenal (64)	10	31.9201	0.1192	0.0037	1.2013	1.2013	2.1968	31.8938	32.0251	0.1198	3.5122E-6	
(E)-2-Octenal (30)	5	24.4418	8.4176E-6	3.4439E-7	1.1779	1.1779	2.2827	24.4418	24.4418	8.6202E-6	3.5122E-6	
Pentanal (109)	11	8.0289	0.1794	0.0223	0.8548	0.8548	2.9010	7.9772	8.1668	0.1784	0.1010	
1-Octanol (94)	4	24.4855	0.0292	0.0012	0.5006	0.5006	1.0013	24.4709	24.5001	0.0412	3.5122E-6	
(E)-2-Nonenal (41)	5	28.2218	0.1908	0.0068	0.4770	0.4770	0.8715	28.1556	28.3209	0.2358	0.0412	
2(3H)-Furanone, 5-butyldihydro- (16)	11	40.3774	0.0573	0.0014	1.2888	1.2888	3.6153	40.3595	40.4251	0.0578	2.7121E-6	
(E)-2-Undecenal (25)	6	35.5154	0.1070	0.0030	0.7207	0.7207	1.2813	35.4813	35.5834	0.1203	3.5122E-6	
2(3H)-Furanone, dihydro-5-pentyl- (29)	10	43.6393	0.0580	0.0013	0.4441	0.4441	1.4639	43.6251	43.6723	0.0624	0.0337	
Acetone (52)	11	5.1069	0.0840	0.0164	0.3613	0.3613	1.5951	5.0834	5.1480	0.0918	0.0558	
Butyl Butanoate (32)	11	16.3175	0.2623	0.0161	0.6909	0.6909	2.8268	16.2251	16.4793	0.2846	0.1117	
Butyl benzoate (15)	12	38.9133	0.0723	0.0019	1.1144	1.1144	4.2454	38.8865	38.9668	0.0760	9.2700E-6	
2(3H)-Furanone, dihydro-5-propyl- (57)	9	36.9575	0.0880	0.0024	1.0164	1.0164	3.0708	36.9251	37.0223	0.0904	0.0337	
(60)	10	32.3284	0.1160	0.0036	0.9506	0.9506	2.7961	32.2918	32.4140	0.1208	0.0337	
2(3H)-Furanone, 5-ethyldihydro- (45)	11	33.6955	0.1115	0.0033	1.2164	1.2164	4.3344	33.6584	33.7945	0.1034	0.0674	
Ethyl benzoate (72)	10	32.6551	0.1058	0.0032	0.5205	0.5205	2.0412	32.6181	32.7105	0.1247	0.0292	
Acetonitrile (14)	12	8.6577	0.1869	0.0216	1.3439	1.3439	5.4946	8.5824	8.8084	0.1830	0.0674	
(49)	10	23.7651	0.2320	0.0098	1.2887	1.2887	2.3565	23.7126	23.9751	0.2312	3.5122E-6	
Benzaldehyde (17)	12	27.6161	0.1621	0.0059	0.7737	0.7737	3.1145	27.5626	27.7230	0.1736	0.0559	
4-Hydroxybutyric acid (7)	12	31.2181	0.1288	0.0041	1.3631	1.3631	6.0196	31.1647	31.3251	0.1198	0.0674	
Toluene (37)	5	9.7768	0.2244	0.0230	0.1619	0.1619	0.1295	9.7563	9.8584	0.2537	0.0000	
Dichloremethane (108)	10	6.8951	0.1284	0.0186	1.9287	1.9287	7.8551	6.8251	7.0001	0.1167	0.0476	

Targeted and **untargeted** peak(-region) features are cross-aligned between all samples and metadata collected for further processing

Challenge 1

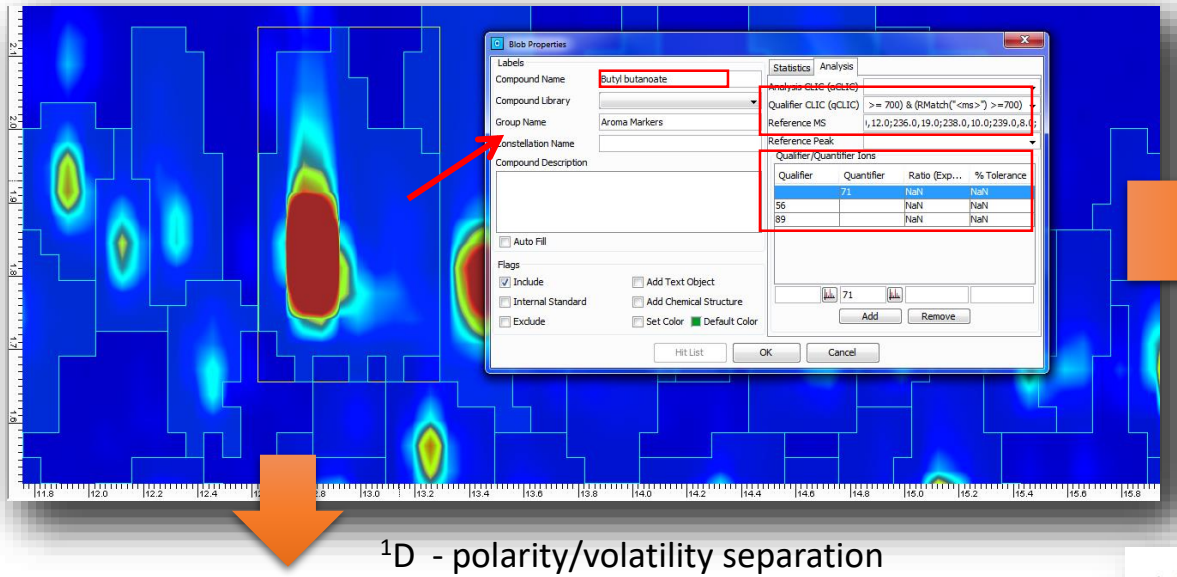
Cocoa *identitation*

UNIVERSITÀ DEGLI STUDI DI TORINO

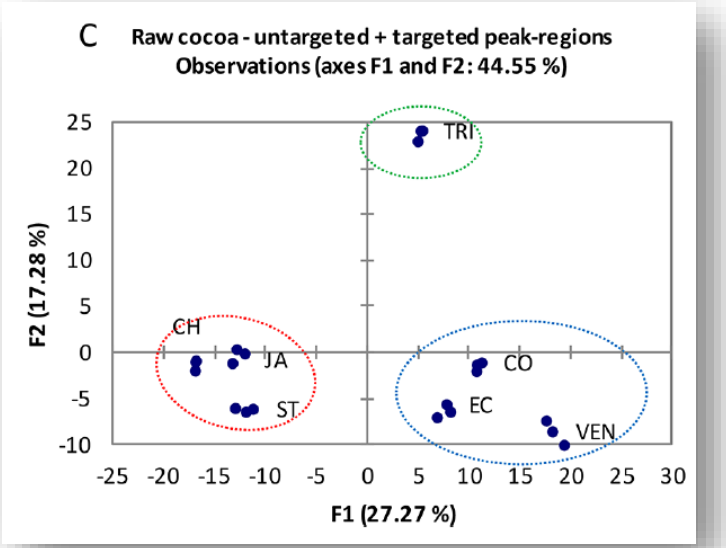
Cocoa roasted stage volatiles - Mexico Chontalpa origin
HS-SPME (CAR/PDMS/DVB) - 500 mg - 50°C/50 min
^{1}D CW 20 m × ^{2}D OV17O1 - thermal modulation LN_2

Theobroma cacao volatiles encrypt information on quality - origin - processing impact

Study on seven origins from South America, Africa, Sao Tomè and Java



^{1}D - polarity/volatility separation



A **template** consisting of **all detected peak-regions** **595 peak-regions** including **130 targeted analytes** and **17 key-odorants** eliciting characteristic cocoa notes

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Comprehensive Chemical Fingerprinting of High-Quality Cocoa at Early Stages of Processing: Effectiveness of Combined Untargeted and Targeted Approaches for Classification and Discrimination

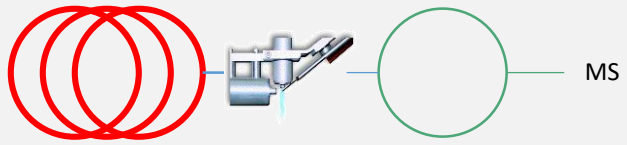
Federico Magagna,[†] Alessandro Guglielmetti,[†] Erica Liberto,[†] Stephen E. Reichenbach,[‡] Elena Allegrucci,[§] Guido Gobino,[§] Carlo Bicchi,[†] and Chiara Cordero*[,†]

Challenge 1

Cocoa *identitation*

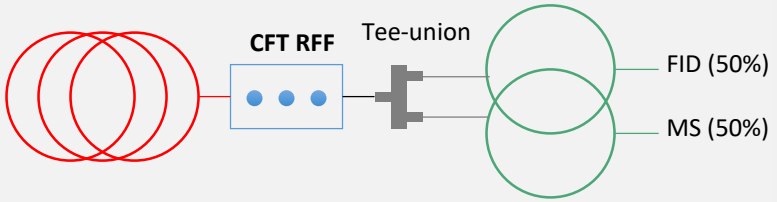
UNIVERSITÀ DEGLI STUDI DI TORINO

Thermal Modulation
Agilent 7890-5975C with Zoex KT 2004 loop-type thermal modulator
Optimode v2.0 - Cryogenic liquid nitrogen



^{1}D - Polar PEG (Sol-gel WAX)
30 m × 0.25 mm × 0.25µm
He carrier @ **1.5 mL/min**

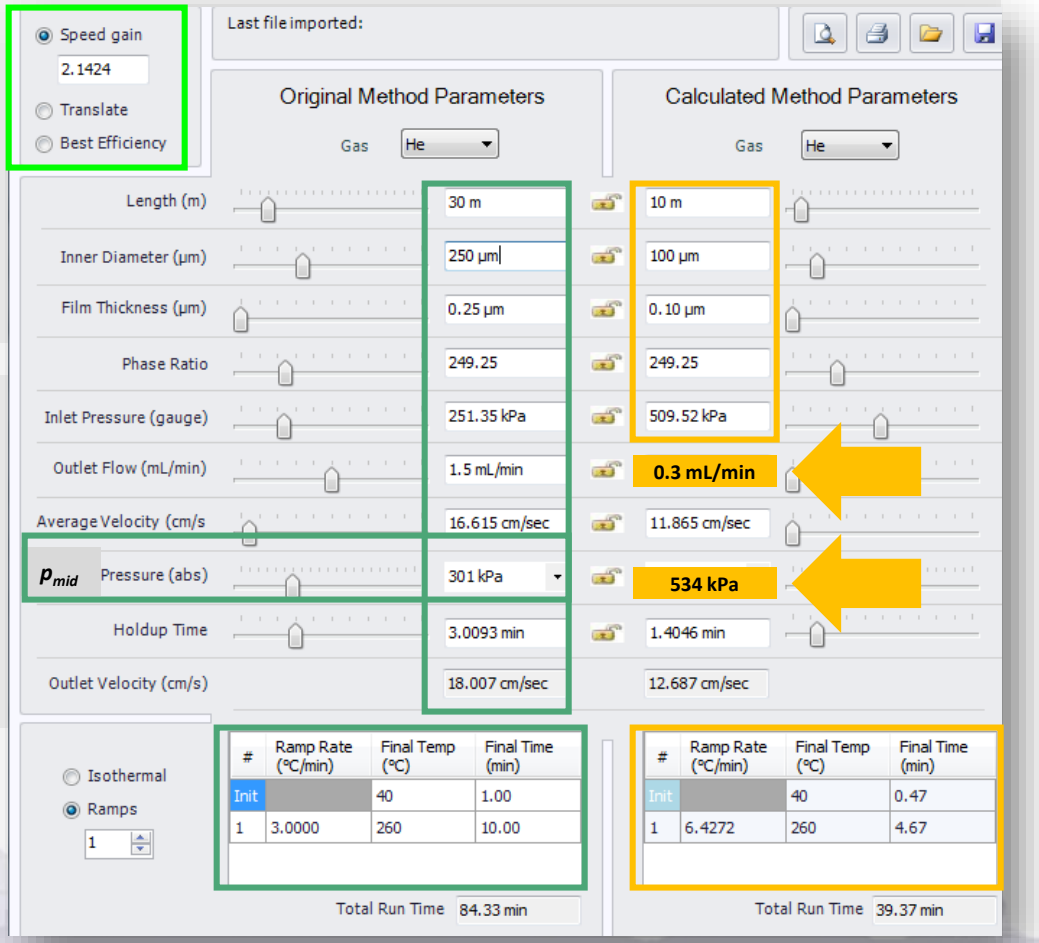
^{2}D - Medium polarity OV1701
1.0 m × 0.10 mm × 0.10µm
He carrier @ **1.5 mL/min**



^{1}D - Polar PEG (Sol-gel WAX)
10m×0.10mm×0.10µm
He carrier @ **0.30 mL/min**

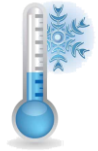
^{2}D - Medium polarity OV1701
two parallel 1.5m×0.10mm×0.10µm
He carrier @ **4 mL/min**

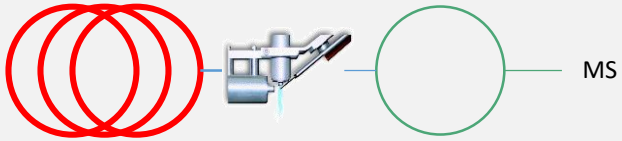
Differential Flow Modulation with Reverse Inject
Agilent 7890-5977B HES with CFT plate for RFF DFM



UNIVERSITÀ DEGLI STUDI DI TORINO

Thermal Modulation
Agilent 7890-5975C with Zoex KT 2004 loop-type thermal modulator
Optimode v2.0 - Cryogenic liquid nitrogen



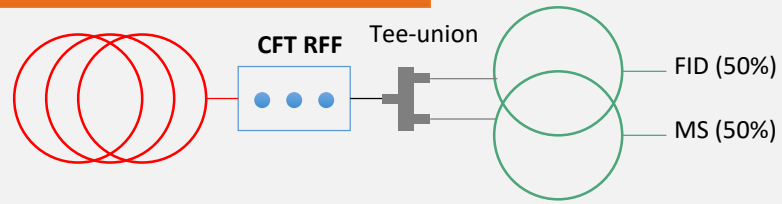


^{1}D - Polar PEG (Sol-gel WAX)
30 m × 0.25 mm × 0.25µm
He carrier @ **1.5 mL/min**

^{2}D - Medium polarity OV1701
1.0 m × 0.10 mm × 0.10µm
He carrier @ **1.5 mL/min**

GC Oven programming: 40°C(1') to 260°C (10') @ 3°/min
S/SL injector: 270°C, split mode, split ratio 1:20
MS Transfer line: 270°C MS in EI mode, 70 eV Full Scan acquisition
Loop dimensions: 1.0 m × 0.10 mm deactivated silica
MS Tune: Atune option; Scan range 40-240 m/z; scan rate 12,500 amu/s
Optimode settings: modulation period **3s**, hot-jet pulse time 250 ms
cold jet stream MFC from 35% to 5% in 60 min

Translated conditions



^{1}D - Polar PEG (Sol-gel WAX)
10m×0.10mm×0.10µm
He carrier @ **0.30 mL/min**

^{2}D - Medium polarity OV1701
two parallel 1.5m×0.10mm×0.10µm
He carrier @ **4 mL/min**

GC Oven programming: **40°C (0.47') to 260°C (4.67') @ 6.4272°/min**
S/SL injector: 260°C, split mode, split ratio 1:20
MS Transfer line: 260°C MS in EI mode, 70 eV Full Scan acquisition
MS Tune: **HES Etune**; Scan range 40-240 m/z; scan rate 12,500 amu/s
FID: 150 Hz acquisition frequency
Modulation valve settings: modulation delay: 0.2 s, **modulation period 2s injection time 200 ms**

Differential Flow Modulation with Reverse Inject
Agilent 7890-5977B HES with CFT plate for RFF DFM

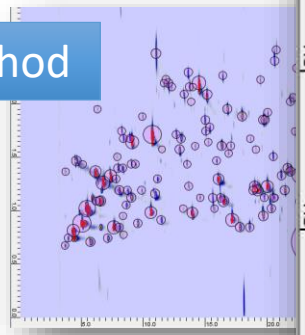
Challenge 1

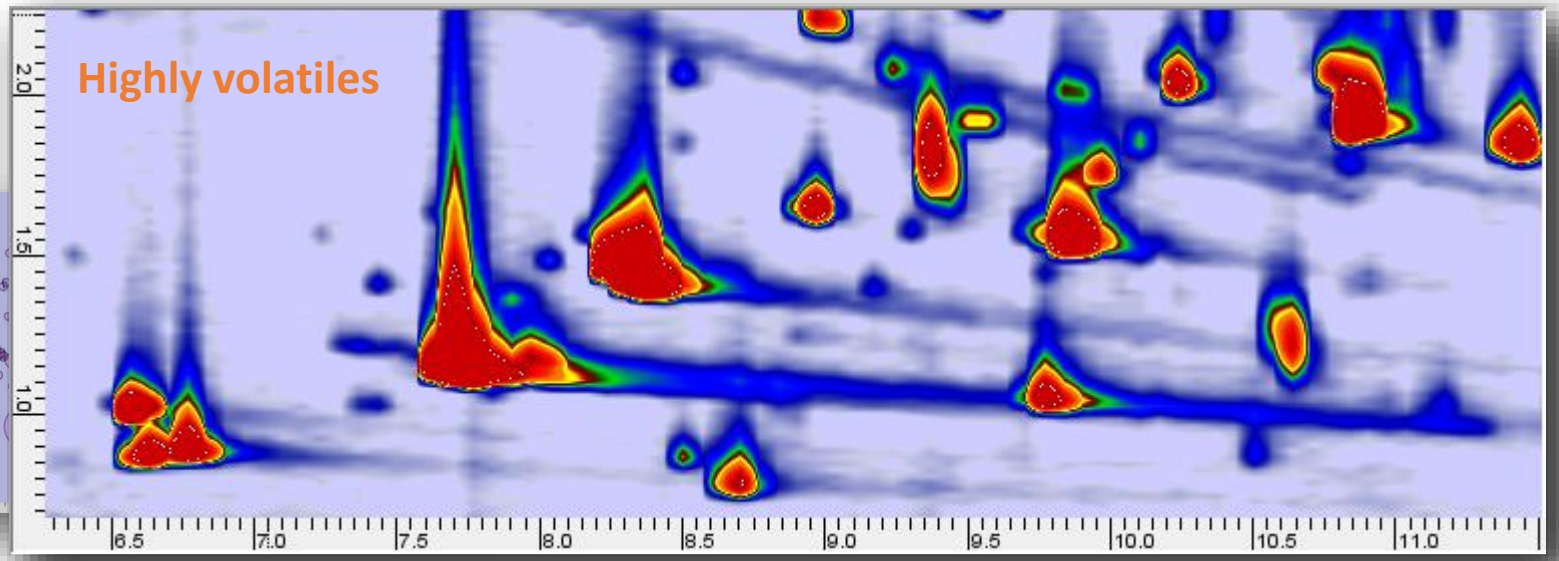
Cocoa *identitation*

UNIVERSITÀ DEGLI STUDI DI TORINO

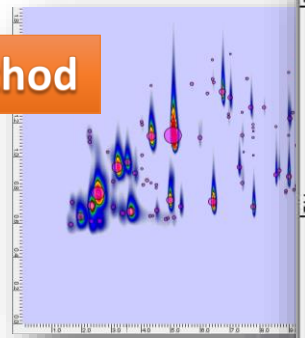
Cocoa roasted stage volatiles - Mexico Chontalpa origin
HS-SPME (CAR/PDMS/DVB) - 500 mg - 50°C/50 min
^{1}D CW 20 m × ^{2}D OV17O1 - method translation

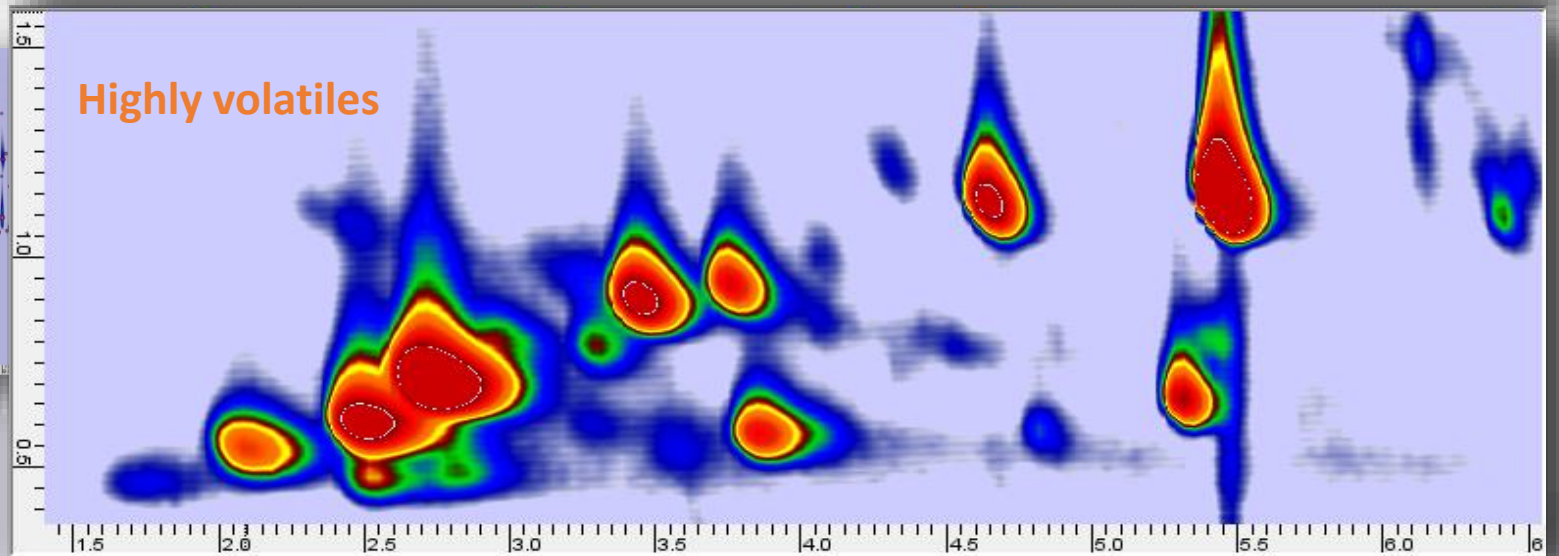
Original method





Translated method





Challenge 1

Cocoa *identitation*

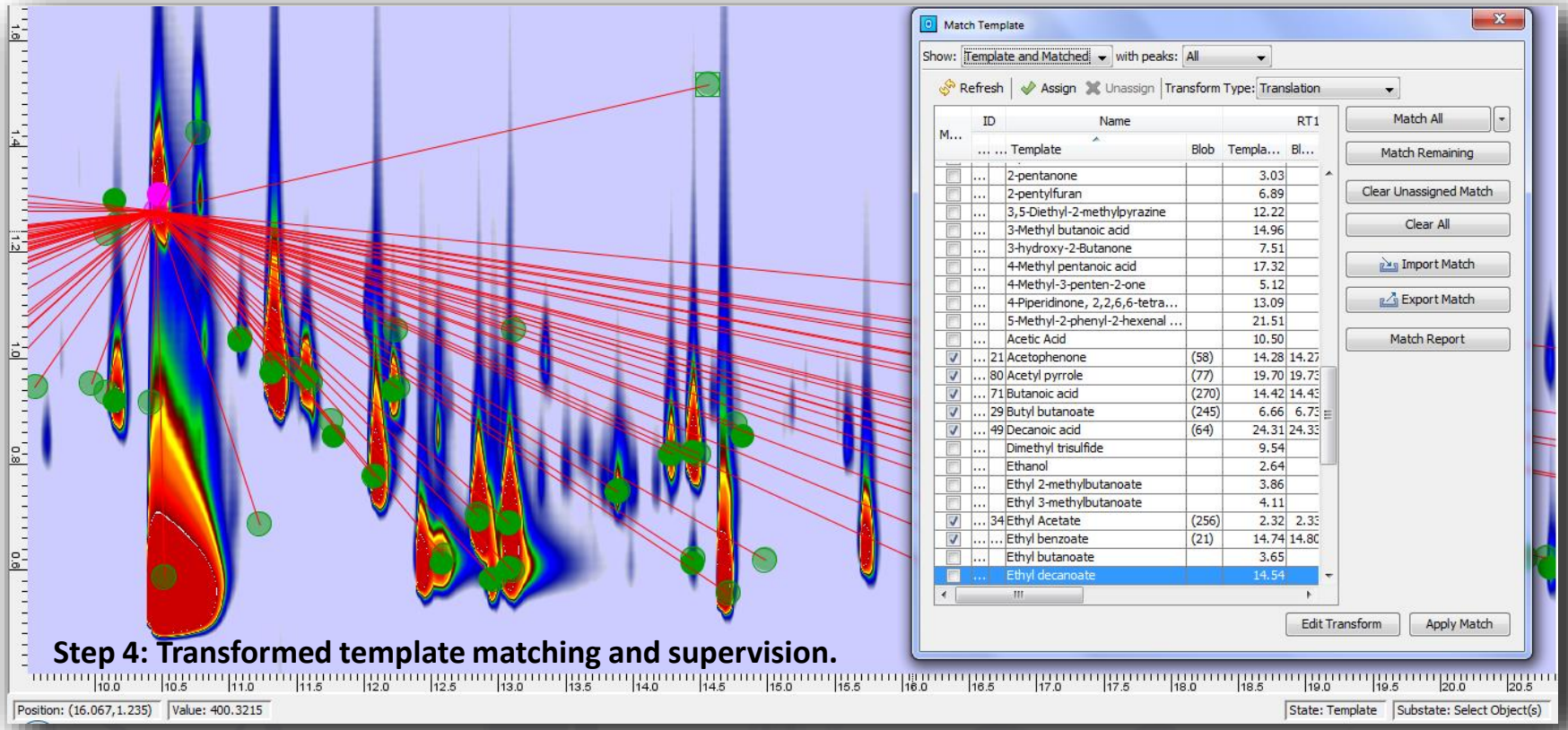
UNIVERSITÀ DEGLI STUDI DI TORINO

Target analysis

Adaptation of peaks pattern

Template transformation

Second-degree polynomial global transformation algorithms[1,2]



Step 4: Transformed template matching and supervision.

Cocoa roasted stage

volatiles - Mexico

Chontalpa origin

HS-SPME

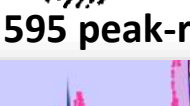
(CAR/PDMS/DVB) - 500 mg

- 50°C/50 min

1. S.E. Reichenbach et al. Anal. Chem. 87 (2015) 10056–10063
2. D.W. Rempe et al. Anal. Chem. 88 (2016) 10028–10035

Challenge 1

Cocoa *identitation*



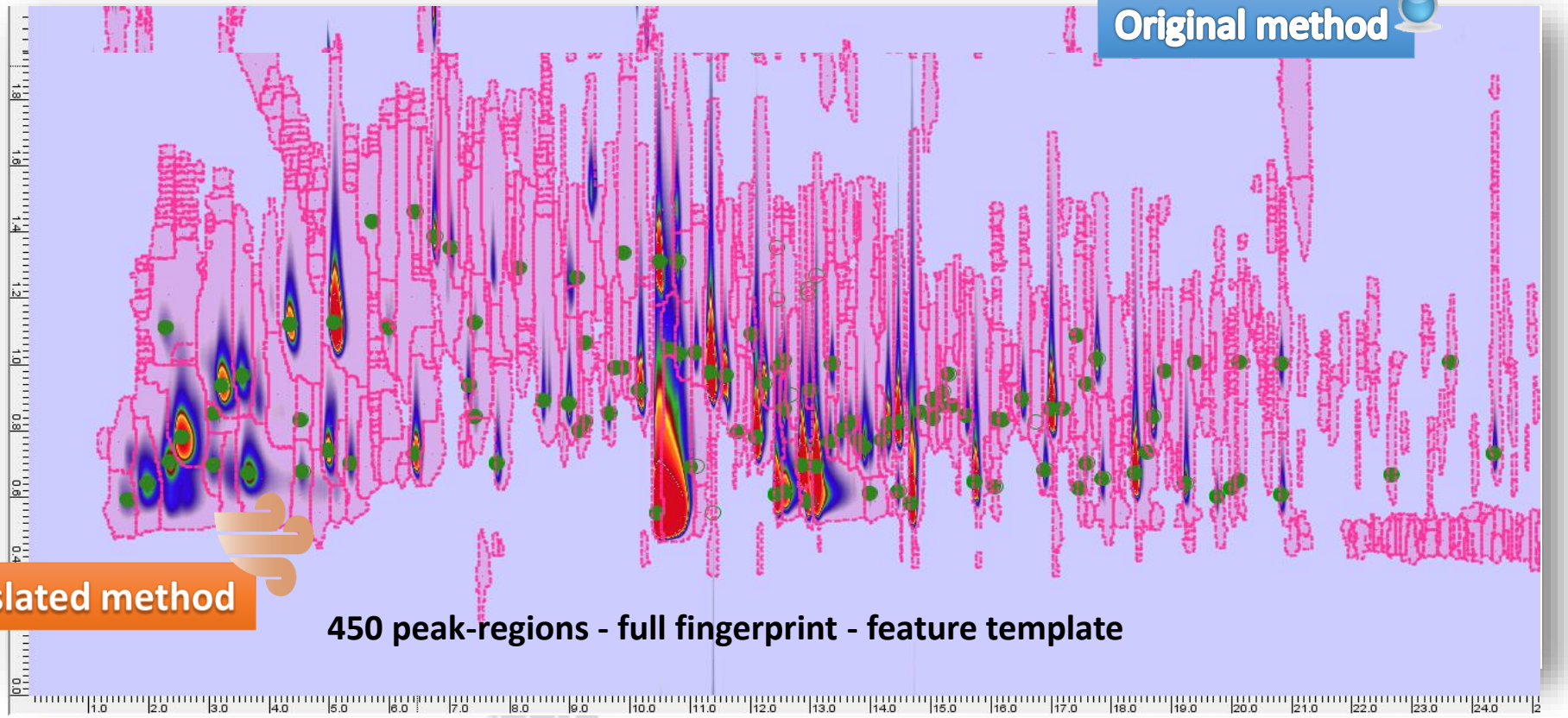
UT fingerprinting

Peak-regions (targeted and untargeted)

UNIVERSITÀ DEGLI STUDI DI TORINO

595 peak-regions - full fingerprint - feature template

Original method



Translated method

450 peak-regions - full fingerprint - feature template

Cocoa roasted stage

volatiles - Mexico

Chontalpa origin

HS-SPME

(CAR/PDMS/DVB) - 500 mg

- 50°C/50 min

Independent processing - UT fingerprinting

2D peaks have different σ in both dimensions

tailored peak-regions

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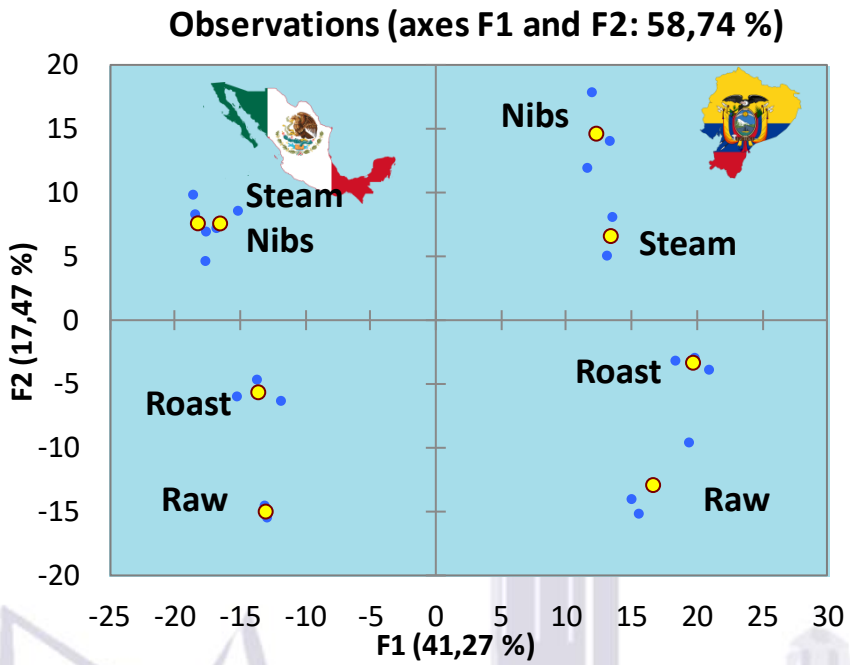
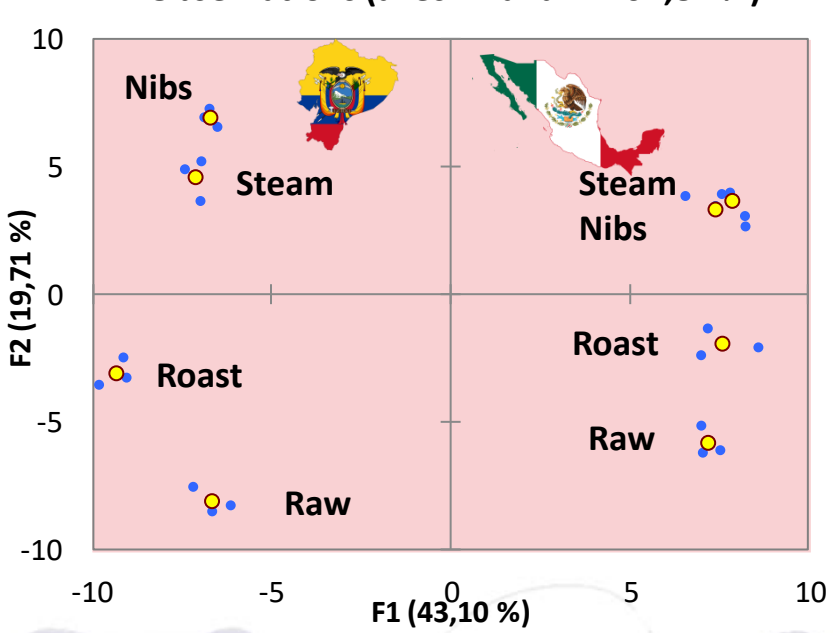
Original method

HS-SPME - GC×GC-MS
Loop-type Thermal Modulation



Translated method

HS-SPME - GC×2GC-MS/FID
Differential Flow Modulation with Reverse Inject



Challenge 1

Cocoa *identitation*

UNIVERSITÀ DEGLI STUDI DI TORINO

UT fingerprinting

Peak-regions (targeted and untargeted)

Original method

HS-SPME - GC×GC-MS
Loop-type Thermal Modulation

Translated method

HS-SPME - GC×2GC-MS/FID
Differential Flow Modulation

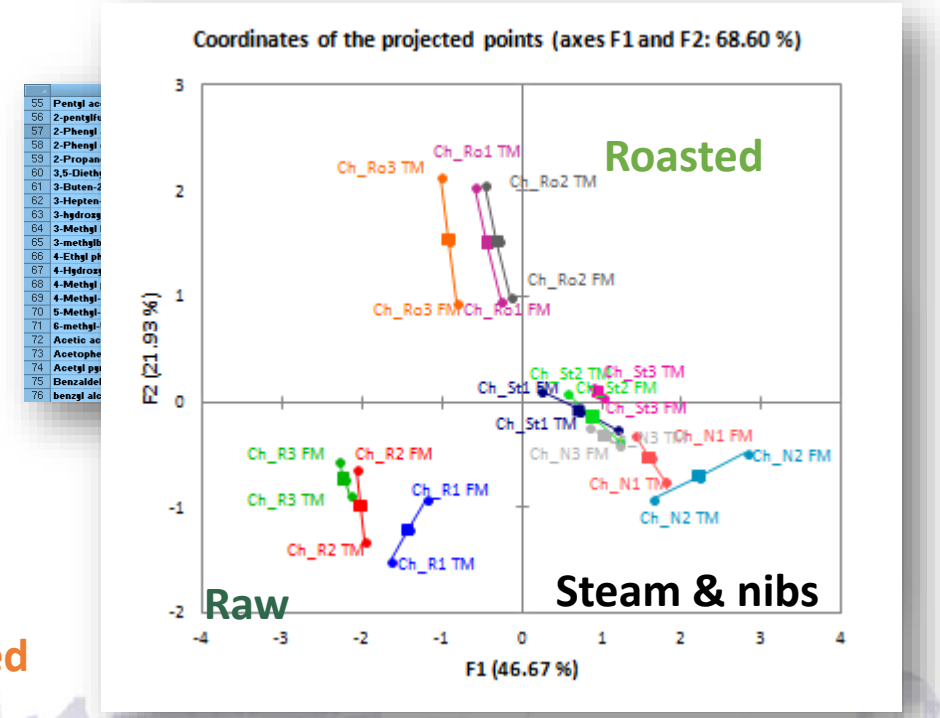
Multiple factor analysis
Targeted 2D peaks
Chontalpa Mexico

Loss of sensitivity

130 *vs.* 75 reliably identified targets
595 *vs.* 450 reliable peak-regions
17 *vs.* 14 key-aroma compounds

✓^{2}D two parallel columns
✓higher outlet flows toward MS

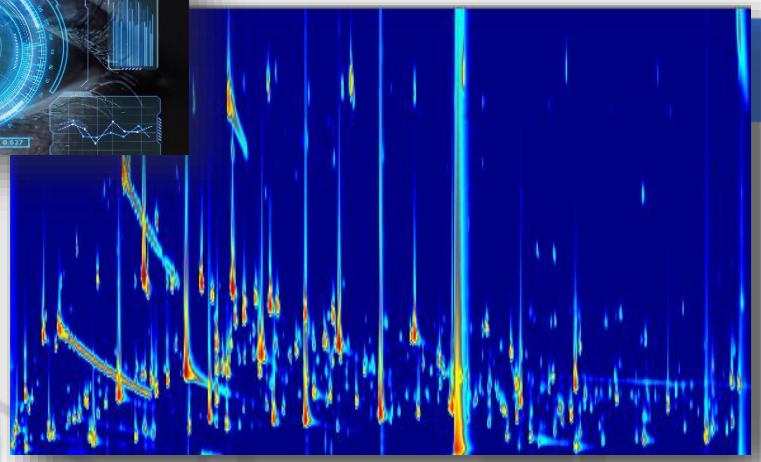
Fingerprint informative role is preserved



Challenge 2

Computer vision

Quality assessment at **industrial level** focuses on morphological aspects, presence of damaged kernels, perceivable sensory defects (mould, rancid, *cimiciato*, stale etc..)



Step-ahead in quality assessment *molecular resolution* probes:

1. Cuadros-Rodríguez, L.; Ruiz-Samblás, C.; Valverde-Som, L.; Pérez-Castaño, E.; González-Casado, A. *Anal. Chim. Acta* **2016**, *909*, 9–23.
2. Cialiè Rosso, M.; Mazzucotelli, M.; Bicchi, C.; Charron, M.; Manini, F.; Menta, R.; Fontana, M.; Reichenbach, S. E.; Cordero, C. J. Chromatogr. A **2020**, *1614* (460739)

Challenge 2

Computer vision

Computer Vision strategy
Classification trees

Volatiles patterns diagnostic of spoilage

Computer Vision tools Prompt identification of non-conform samples and confident rejection

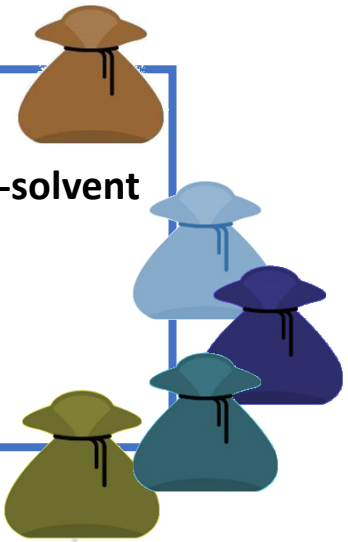
Good (OK) Rancid (KO) Spoiled (KO)

Collection of defected hazelnuts

- ✓ harvest years
- ✓ origin
- ✓ shelf-life stage

Flash profiling

Mould
Mould-rancid-solvent
Rancid
Rancid-stale
Solvent
Uncoded KO



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Challenge 2

Computer vision

Platform

p_i p_{Aux} p_{Tee}

Tee-union

FID (70%)

MS (30%)

^{1}D - Polar Heavy Wax (J&W)
20 m × 0.18 mm × 0.18 µm
He carrier @ **0.4 mL/min**

^{2}D - Medium polarity OV17
1.8 m × 0.18 mm × 0.18 µm
He carrier @ **8 mL/min**

Tools

Headspace SPME

ISTDs Pre-loading

α and β-Thujone
100 mg/L (5.0 µL)

Methyl-2-octynoate
100 mg/L (5.0 µL)

5 min
50°C

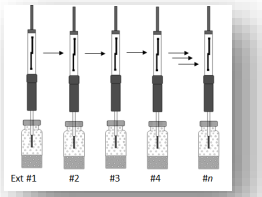
Hazelnuts
powder
0.100 g

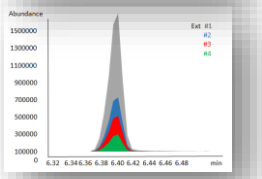
50 min
50°C

Multiple Headspace SPME - quantitation

MHS-SPME enables **accurate quantitation** of **several markers (ESTD and response factors)**

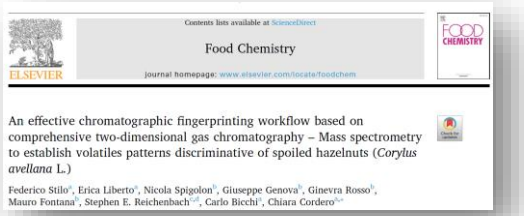
secondary products of lipid oxidation (hexanal, heptanal, octanal, nonanal, (E)-2-octenal, (E)-2-nonenal);
key-aroma compunds (3-methylbutanal, ethyl 2-methylbutanoate, (E)-β-damascenone, 2-nonanone, heptanoic acid etc);
markers of defected hz (nonanoic acid, butyric acid, 4-heptanol, 1-pentanol, propanoic acid, 2-heptanol, pentanoic acid etc)





Challenge 2

Computer vision



Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

An effective chromatographic fingerprinting workflow based on comprehensive two-dimensional gas chromatography – Mass spectrometry to establish volatiles patterns discriminative of spoiled hazelnuts (*Corylus avellana* L.)

Federico Stilo[a], Erica Liberto[a], Nicola Spigolon[a], Giuseppe Genova[b], Ginevra Rosso[b], Mauro Fontana[b], Stephen E. Reichenbach[c,d], Carlo Bicchi[a], Chiara Cordero[a,*]

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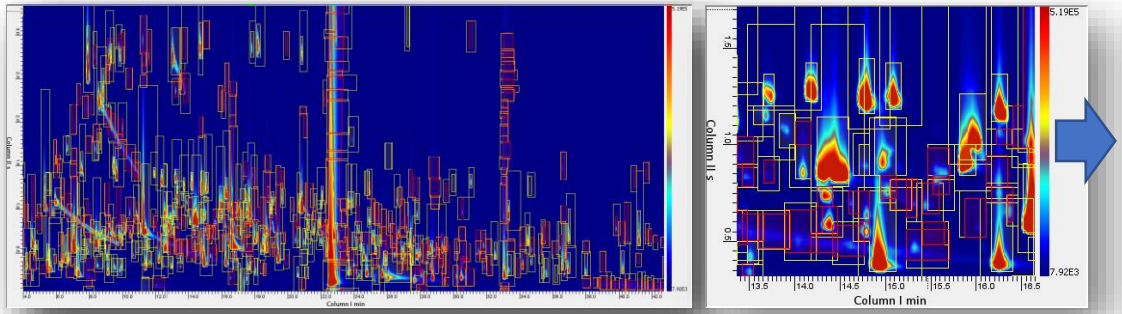
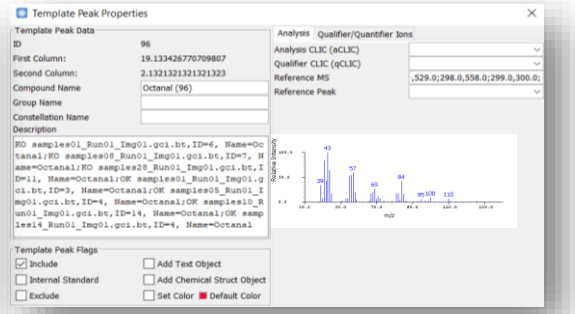
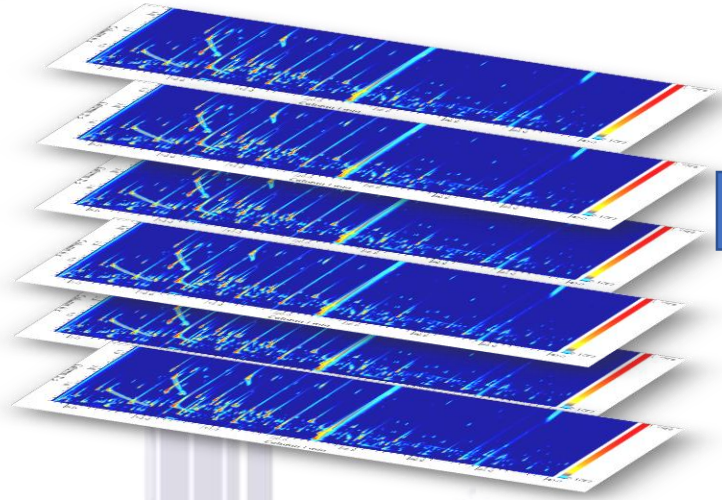
Untargeted/Targeted (UT) fingerprinting on single chromatograms



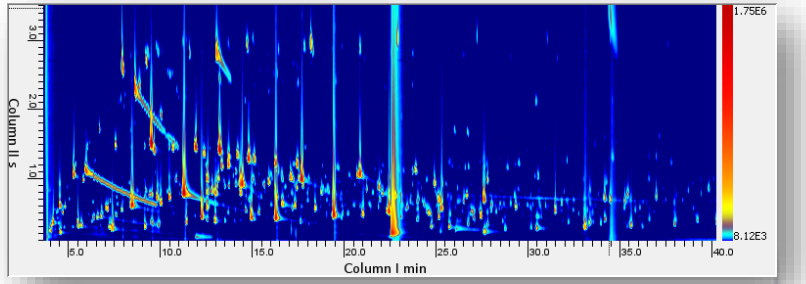


Generation of composite class-images from samples groups - one for each sensory defect

✓ patterns re-alignment by reliable 2D peaks and raw data summation (composite image)



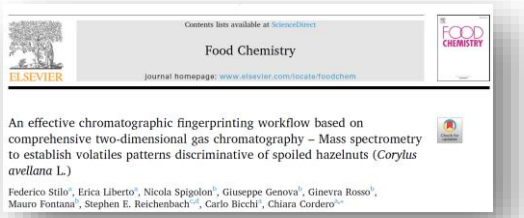
Cumulative class-image "Mould"



The effect of dominant variables (origin, harvest year, cultivar, shelf-life etc..) is minimized while the "signature" of *mold* sensory defect emphasized - easier detection

Challenge 2

Computer vision



Contents lists available at ScienceDirect

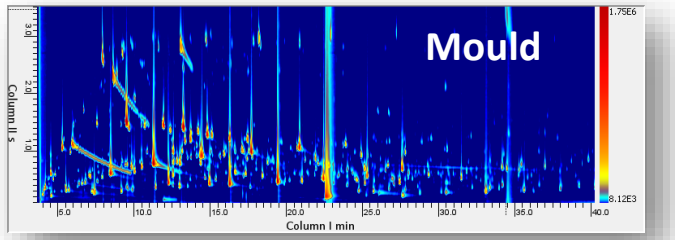
Food Chemistry

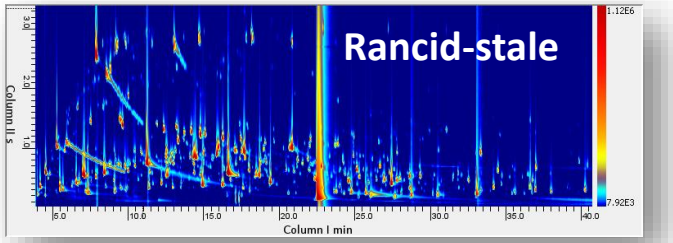
journal homepage: www.elsevier.com/locate/foodchem

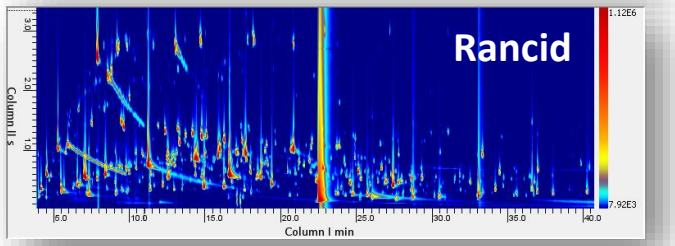
An effective chromatographic fingerprinting workflow based on comprehensive two-dimensional gas chromatography – Mass spectrometry to establish volatiles patterns discriminative of spoiled hazelnuts (*Corylus avellana* L.)

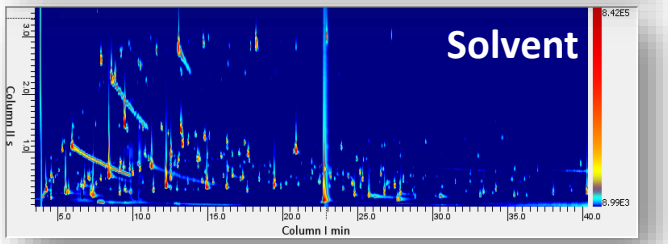
Federico Stilo[a], Erica Liberto[a], Nicola Spigolon[a], Giuseppe Genova[a], Ginevra Rosso[b], Mauro Fontana[a], Stephen E. Reichenbach[c,d], Carlo Bicchi[a], Chiara Cordero[a,*]

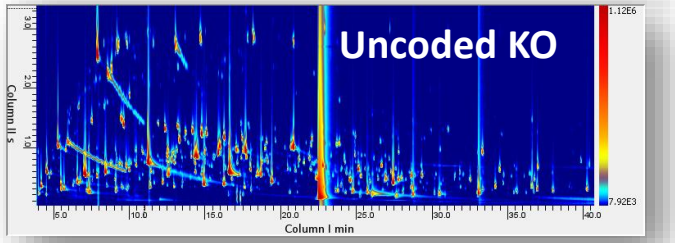
Composite class-images from samples groups - one for each sensory defect

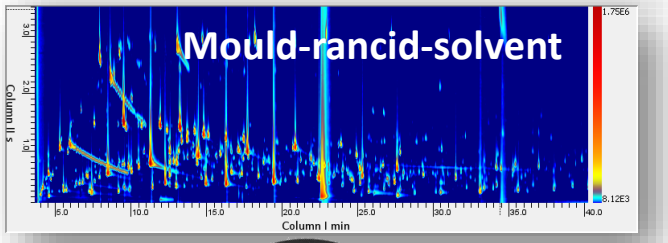


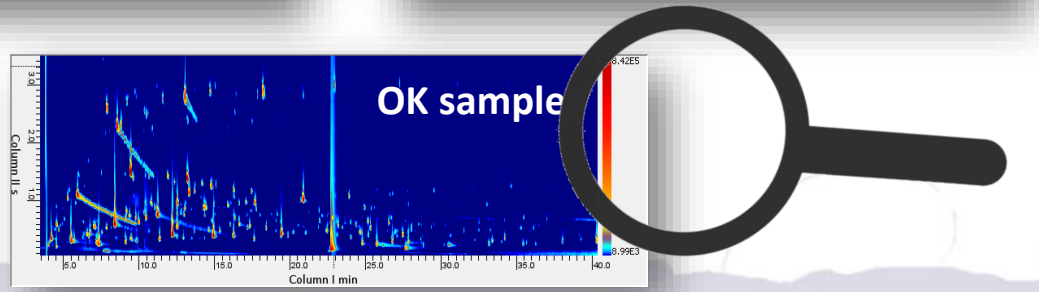










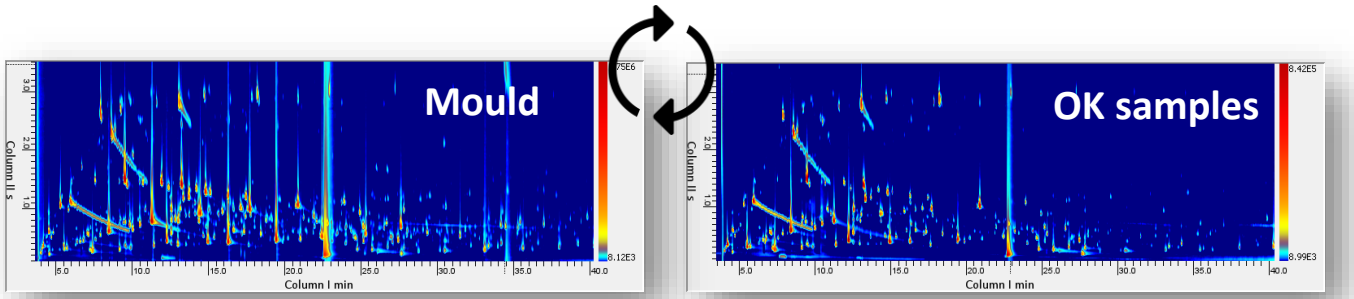


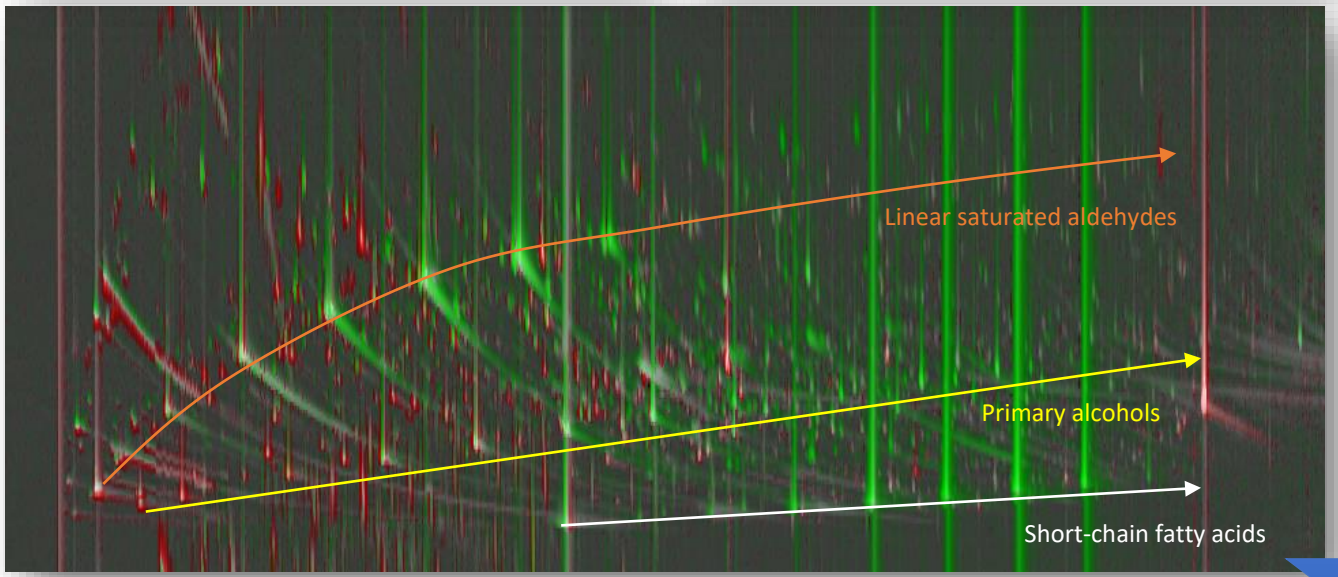
Mould
Mould-rancid-solvent
Rancid
Rancid-stale
Solvent
Uncoded KO

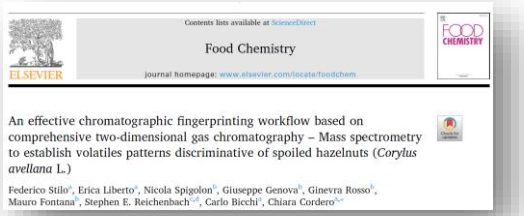
Reference OK samples

Challenge 2

Computer vision







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Datapoint features fingerprinting combined to peak-regions UT fingerprinting

Computer vision and chemical patterns

First Image

BlobID	Compound ...	Retention I	Retention II	Peak Value
9	Heptanoic acid	41.417	0.661	1027913.00
10		10.908	1.351	2002502.00
11		14.642	1.562	1919573.00
12	Nonanoic acid	47.367	0.721	1150889.00
13		21.000	0.931	1722252.00

Second Image

BlobID	Compound ...	Retention I	Retention II	Peak Value
97	Heptanoic acid	41.533	0.631	165949.000
98	1-Decene	10.208	2.883	165851.000
99	3-Penten-2-...	12.658	1.021	163385.000
100	Oxirane, pe...	13.592	1.682	162084.000
101	1-Nonene	7.467	2.132	161864.000

Challenge 2

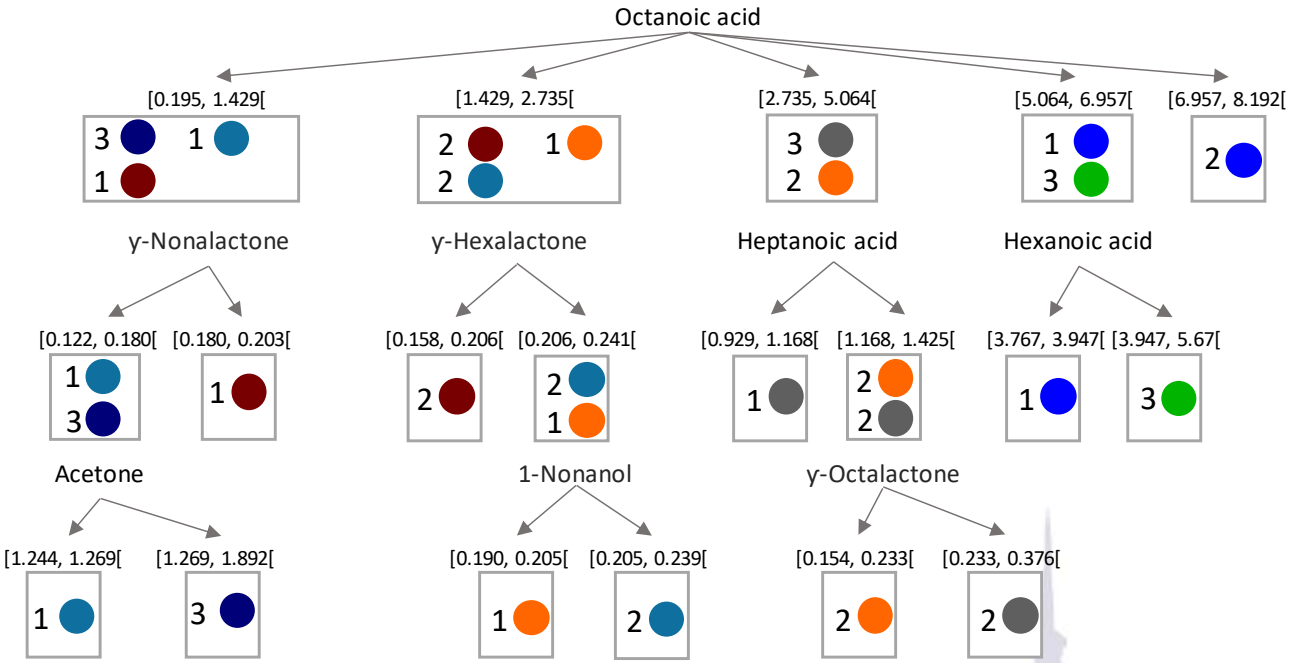
Computer vision

VOCs patterns distinctive of spoiled hazelnuts guide effective classification into seven classes.

- ✓ **Octanoic acid** guides the classification tree being positively correlated to **mould**;
- ✓ **y-nonalactone**, **y-hexalactone**, **acetone**, and **1-nonanol** are decisive to classify OK and rancid samples;
- ✓ **heptanoic** and **hexanoic** acids and **y-octalactone are** present in high relative abundance in rancid-solvent and rancid-stale samples

OK *Rancid* *Rancid-stale* *Mould-rancid-solvent*

Uncoded KO *Rancid-solvent* *Mould*



Challenge 3

AI *smelling*

UNIVERSITÀ DEGLI STUDI DI TORINO

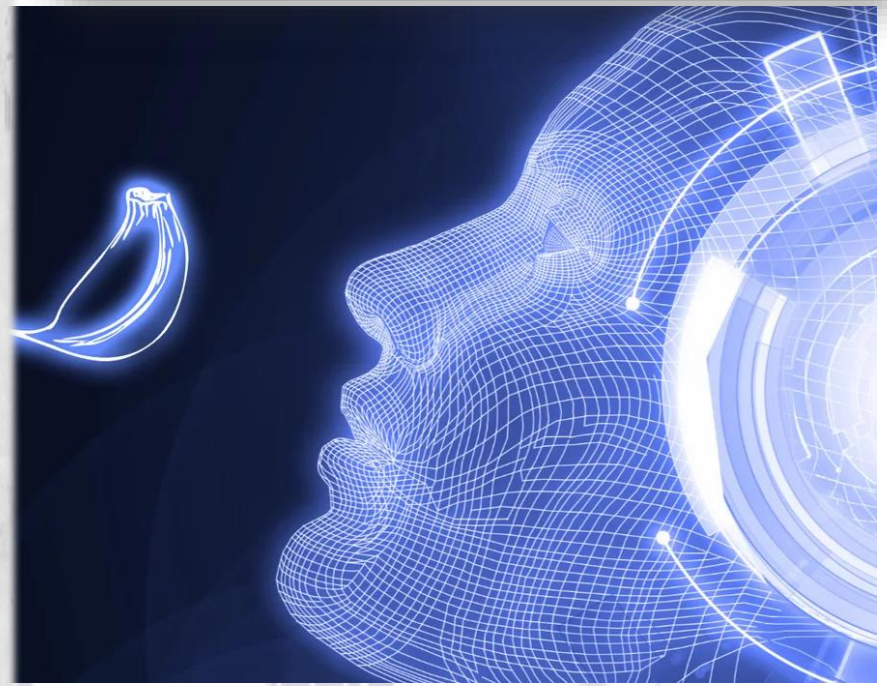
Our noses are busy beasts. At any given moment, multiple smells are competing for our attention, and somehow the brain can tell when it's smelling an orange even against a backdrop of other scents, say perfume or soap. The brain's olfactory bulb has hundreds of receptors tracking odors all the time, and yet somehow keeps everything straight. Scientists at Cornell University working with researchers at Intel have just created an AI algorithm trained to recognize 10 scents by mimicking the mammalian olfactory bulb (MOB). Give the algorithm a computer chip to run on and it can learn to identify new odors.[1]

[1] https://bigthink.com/

nature machine intelligence
ARTICLES
https://doi.org/10.1038/s42256-020-0159-4
Check for updates

Rapid online learning and robust recall in a neuromorphic olfactory circuit

Nabil Imam[1] and Thomas A. Cleland[2]



Artificial Intelligence *Smelling*[2]

Context: *Sensomics*[3]

Principle: key-odorants and odorants patterns evoke specific smells/aroma qualities while contributing to define the overall flavor perception of a food

Methods: extract, isolate, quantify potent odorants by reliable methods

Outcome: *Sensomics*-based expert system (SEBES)[2] capable to predict key-aroma signatures of food without using human olfaction

2. Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann, T. Angew. Chemie - Int. Ed. 53 (28) (2014) 7124–7143.
3. Nicolotti, L.; Mall, V.; Schieberle, P. J. Agric. Food Chem., 67 (2019) 4011–4022

Challenge 3

AI *smelling*

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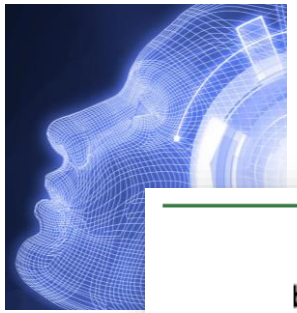

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Characterization of the Key Odorants in High-Quality Extra Virgin Olive Oils and Certified Off-Flavor Oils to Elucidate Aroma Compounds Causing a Rancid Off-Flavor

Anja Neugebauer, Michael Granvogl, and Peter Schieberle*

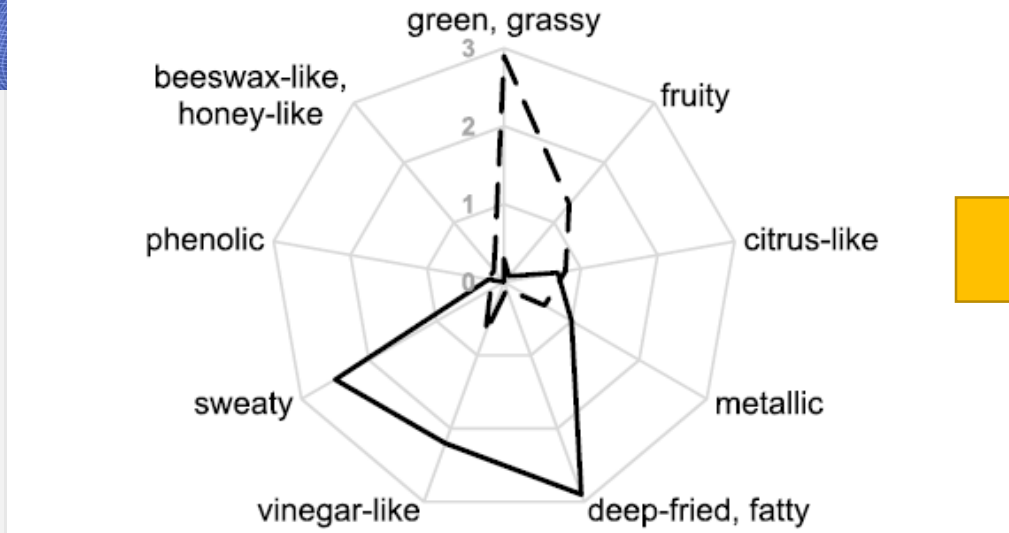

Figure 1. Aroma profile analysis of the rancid olive oil (*RanOO*1, solid line) and the premium extra virgin olive oil (*PreOO*1, dashed line).

aroma compound	OT_{oil}[a] (μg/kg)	OAV[b] *PreOO*1	OAV[b] *RanOO*1
(*E*,*Z*)-2,4-decadienal	4[c]	<1	3480
hexanoic acid	460	<1	3070
octanal	140	<1	2590
hexanal	300	4	1090
(*E*)-2-octenal	120[d]	<1	1000
(*E*,*E*)-2,4-decadienal	66	<1	971
butanoic acid	34	<1	821
acetic acid	350	10	614
3-ethylphenol	8[d]	<1[i]	516
pentanoic acid	400	<1	480
(*E*)-2-nonenal	140	<1	385
(*E*,*E*)-2,4-nonadienal	30	<1	325
(*Z*)-2-nonenal	4	<1	144
heptanal	500	<1	89
trans-4,5-epoxy-(*E*)-2-decenal	13	23	78
(*E*)-2-heptenal	1200	<1	66
(*E*)-2-decenal	2200[d]	n.c.[k]	33
(*E*,*Z*)-2,4-nonadienal	30[e]	n.c.[k]	33
nonanal	610[d]	<1	27
3-methylbutanoic acid	11	<1	20
(*E*,*Z*)-2,6-nonadienal	65	<1	8
(*E*)-2-undecenal	7700[d]	<1	8
(*E*,*Z*)-2,4-heptadienal	55[f]	<1[i]	5

Challenge 3

AI *smelling*

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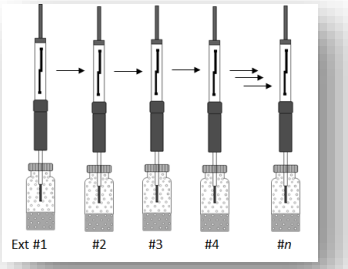
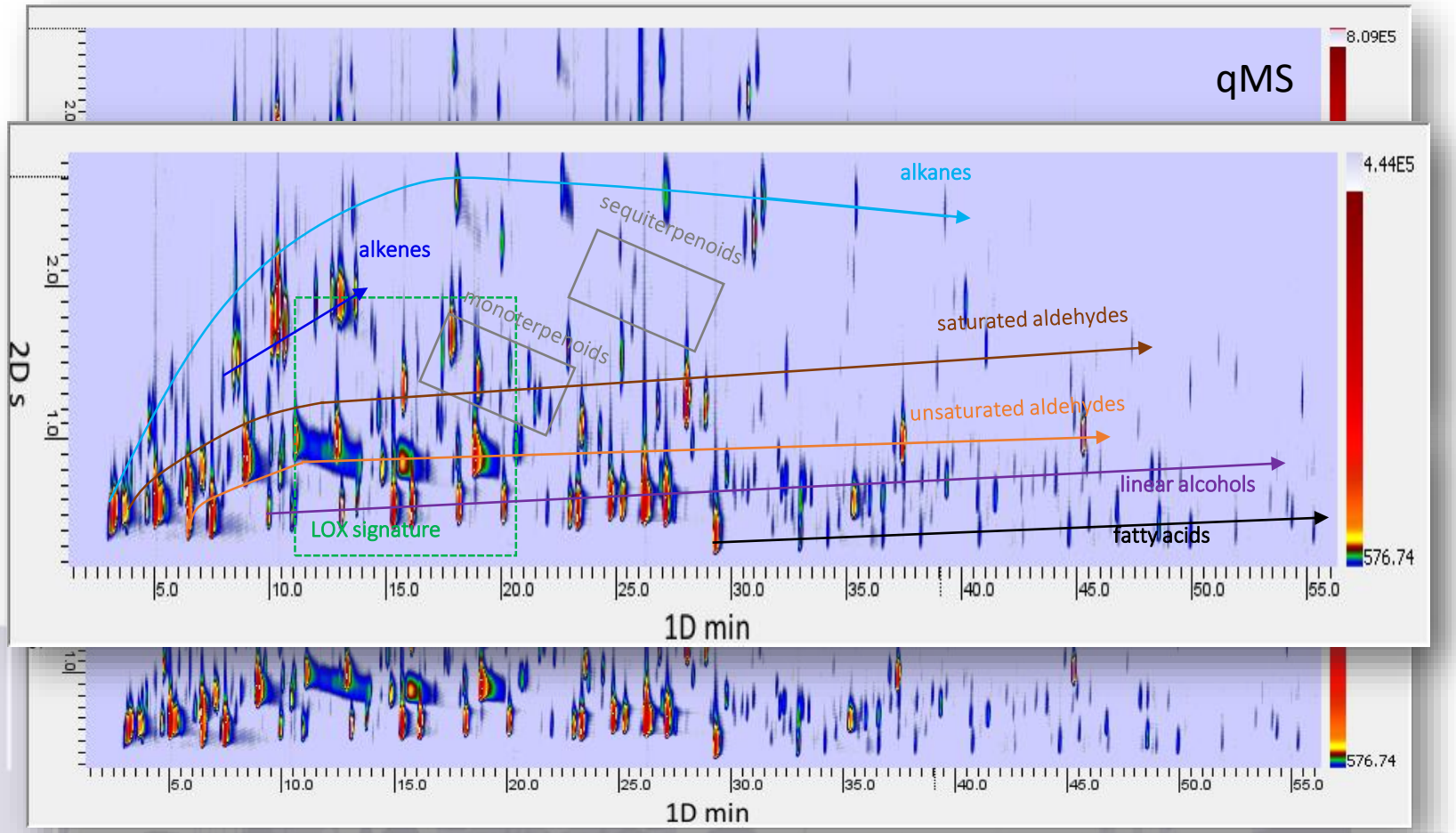
Multiple Headspace SPME
Accurate quantification / ESTD and RF



42 analytes
key-aromas
markers

Differential-flow modulator
parallel detection qMS/FID

Develop a sensomics-based expert system acting as AI *smelling machine*



Challenge 3

AI *smelling*

UNIVERSITÀ DEGLI STUDI DI TORINO

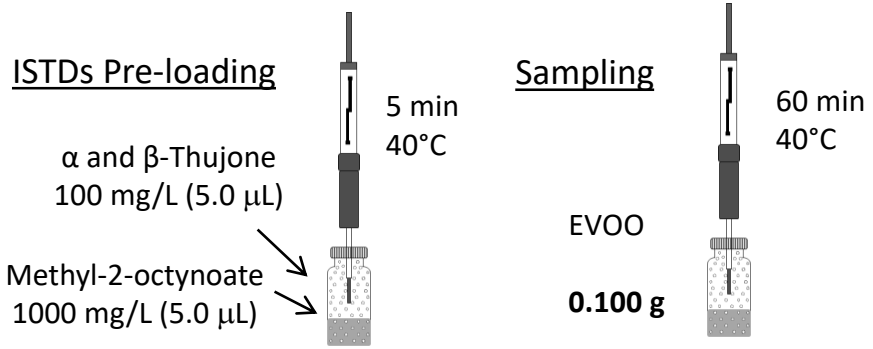
Extra virgin olive oil - EVOO

Identification of **markers of origin** and of **sensory quality** (*i.e.*, positive attributes and defects)
AI *smelling*

Italy - Regional discrimination

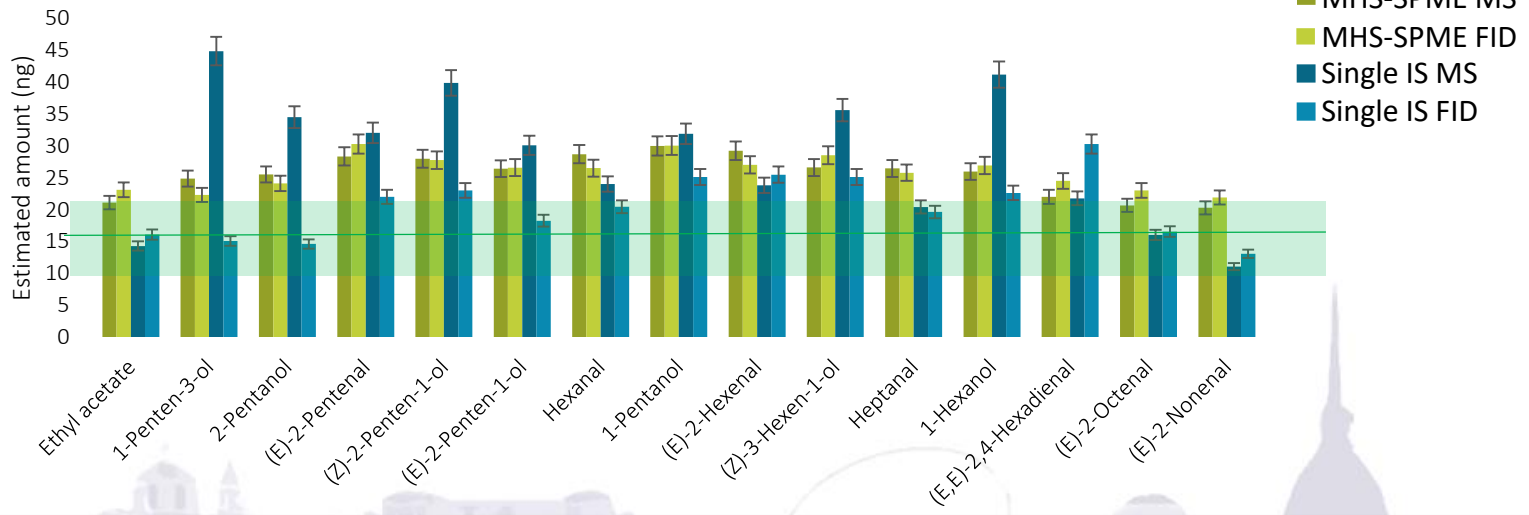
Italy *vs.* Brazil

HS SPME - HS linearity conditions



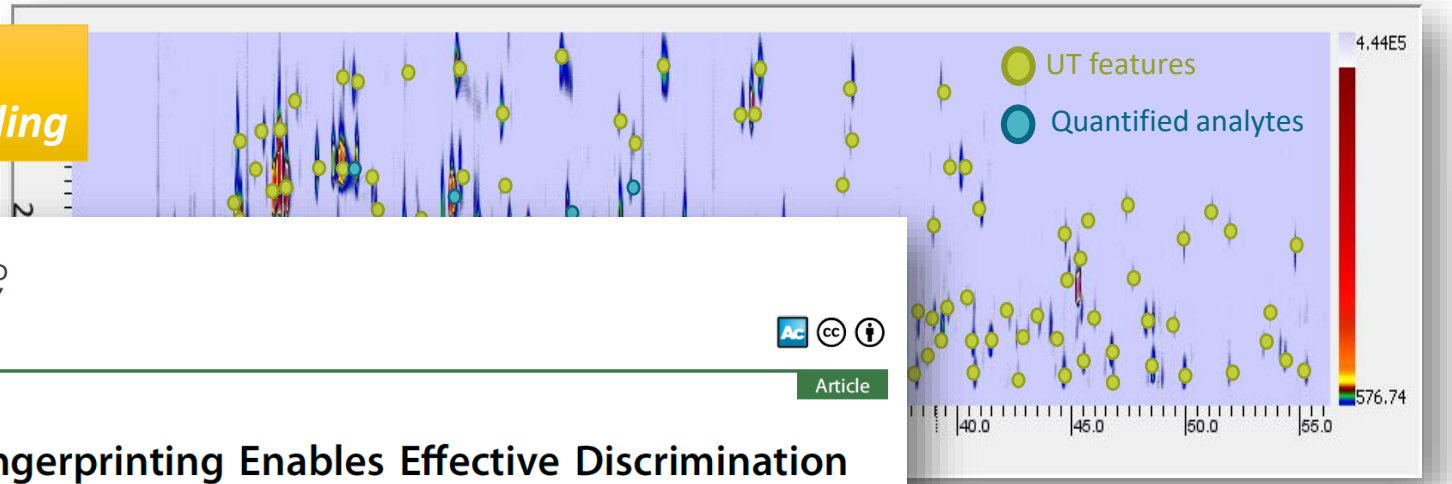
Predicted Relative Response Factors (RRFs) based on combustion enthalpies and molecular structure - FID quantification without ESTD

Accuracy results on 25 ng calibration solution



Challenge 3

AI *smelling*



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Article

Chromatographic Fingerprinting Enables Effective Discrimination and Identitation of High-Quality Italian Extra-Virgin Olive Oils

Federico Stilo, Ana M. Jiménez-Carvelo,* Erica Liberto, Carlo Bicchi, Stephen E. Reichenbach, Luis Cuadros-Rodríguez, and Chiara Cordero*

1-Penten-3-one -

Contents lists available at ScienceDirect

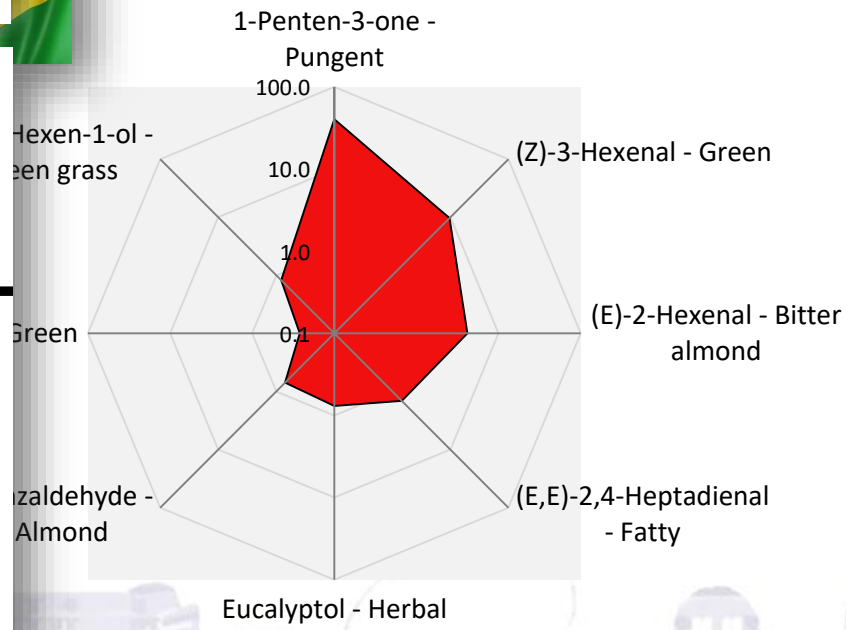
Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Delineating the extra-virgin olive oil aroma blueprint by multiple headspace solid phase microextraction and differential-flow modulated comprehensive two-dimensional gas chromatography

Federico STILO[a], Maria del Pilar SEGURA BORREGO[b], Carlo BICCHI[a], Sonia BATTAGLINO[b], Raquel Maria CALLEJÓN FERNADEZ[b], Maria Lourdes MORALES[b], Stephen E. REICHENBACH[c,d], James MCCURRY[e], Daniela PERONI[f], Chiara CORDERO[a,*]

Brazil blueperint



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Gestalt

…a configuration or pattern of elements so unified as a whole that it cannot be described merely as a sum of its parts…

Accurate quantitative fingerprinting/profiling

Chromatographic Fingerprinting and Computer vision

Artificial Intelligence smelling machine molecular resolution tool

UNIVERSITÀ DEGLI STUDI DI TORINO

GESTALT

noun \ gə-'stält

understanding the whole,
not merely the sum of
its parts.

GC: The State of the Art

Chairperson: Pat Sandra
Participants: Steven Lehotay
Hans-Gerd Janssen
Chiara Cordero
Frank David
John Hinshaw

GC: The State of the Art

November 01, 2017

By **Chiara Cordero**, **Pat Sandra**, **John Hinshaw**, **Hans-Gerd Janssen**, **Frank David**, **Steven Lehotay**

Chiara Cordero: GC-GC cannot be an alternative to GC×GC! They are both capable of expanding the potentials of 1D-GC where a single dimension is not sufficient or selective enough to solve an analytical challenge. However, one is still a multiple 1D-GC approach (that is, GC-GC) and does not require a change of mindset or skills for new users while, as already mentioned, GC×GC requires a "jump" towards new measurement concepts. Once we overcome this gap, we cannot turn back!

Chiara Cordero: I see GC×GC growing in core application areas, including petrochemical, environmental, food and flavours, natural products, and metabolomic studies, and in my research activity I've met new users approaching this technique with curiosity but also with many prejudices and false convictions. My feeling is that we still are in the "induction period".

The possibility of applying dedicated pattern recognition approaches to the analysis of 2D chromatographic data opens new perspectives for fingerprinting studies. This last aspect is a key feature of the technique and it will soon trigger the widespread use of GC×GC in many fields. As experts and passionate chromatographers we have to continue research in the direction of making this technique more intuitive and easy to use with new data analysis tools and approaches to create a "toolbox" for various applications.

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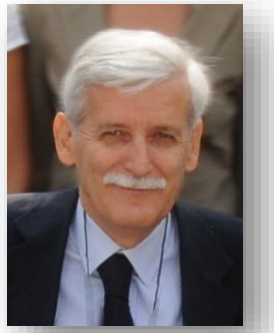
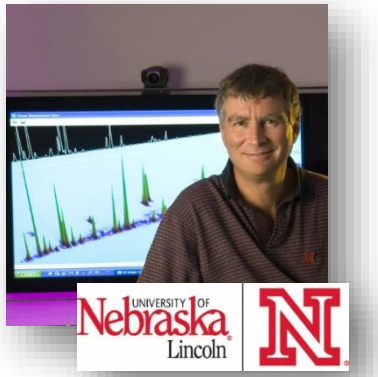
Thank you for your attention

Acknowledgments

Prof. Carlo Bicchi



Prof. Stephen E Reichenbach



Dr. Andrea Caratti

Dr. Simone Squara

Agilent

Applications and Core Technology University Research (ACT-UR) Project #4294

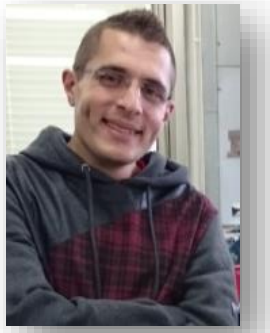
SRA INSTRUMENTS ANALYTICAL SOLUTIONS

GC IMAGE Software for Multidimensional Chromatography

Dr. Qingping Tao

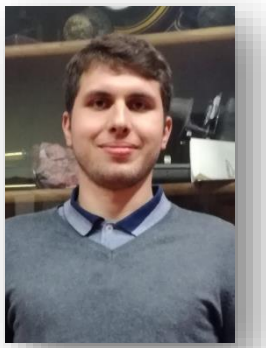
Dr. Federico Stilo PhD

Dr. Marta Cialiè Rosso PhD



Dr. Federico Magagna PhD

Dr. Alessandro Guglielmettti PhD



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https://www.gcxgc-symposium.com/