



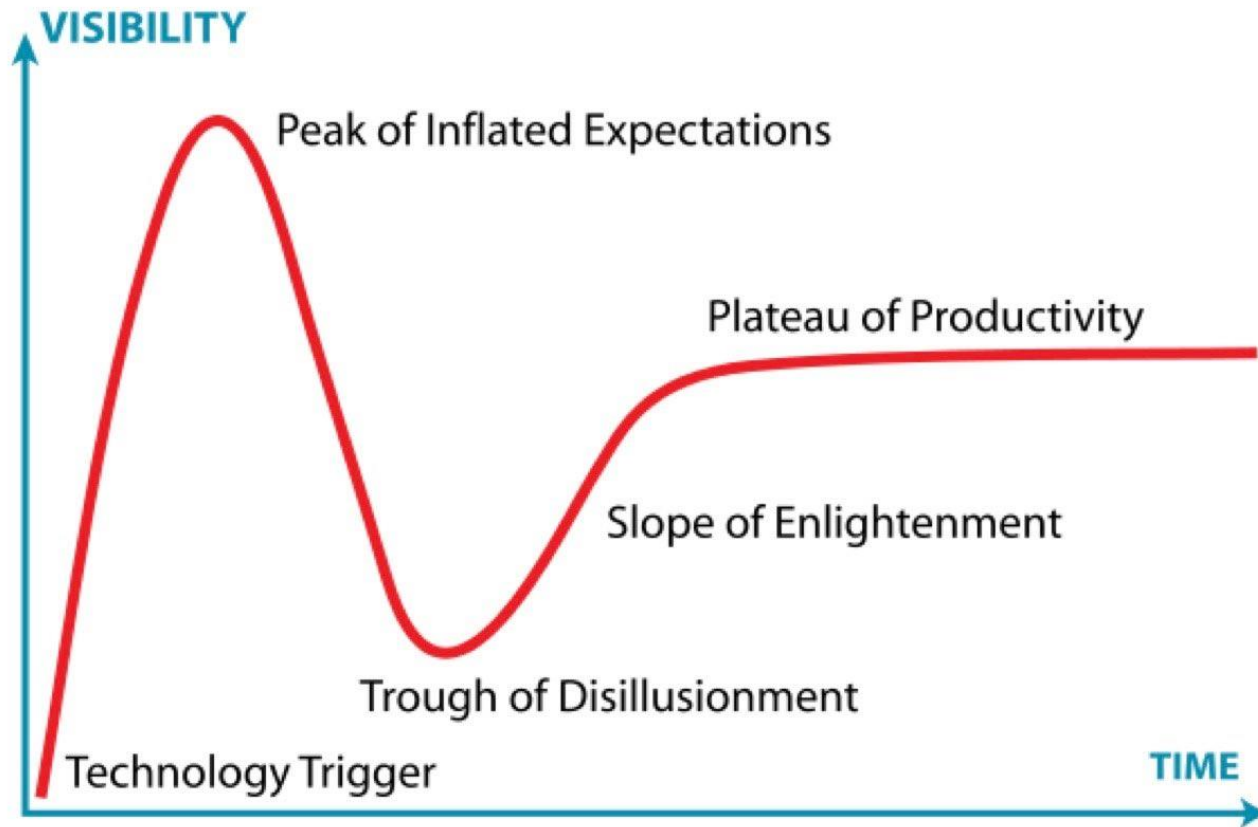
# MULTI-OMICS WORKFLOW FOR BLOOD SERUM SCREENING USING GC×GC-TOFMS

Kinjal Bhatt, T. Massenet, T. Dejong and Jean-François Focant, **Pierre-Hugues Stefanuto**

Molecular System, Organic & Biological Analytical Chemistry Group, Liège University, Belgium



# Gartner Hype Cycle



*Where is GCxGC-MS?*

*Where is GCxGC-HRMS?*

*Where is Metabolomics?*


*Where are "omics"?*

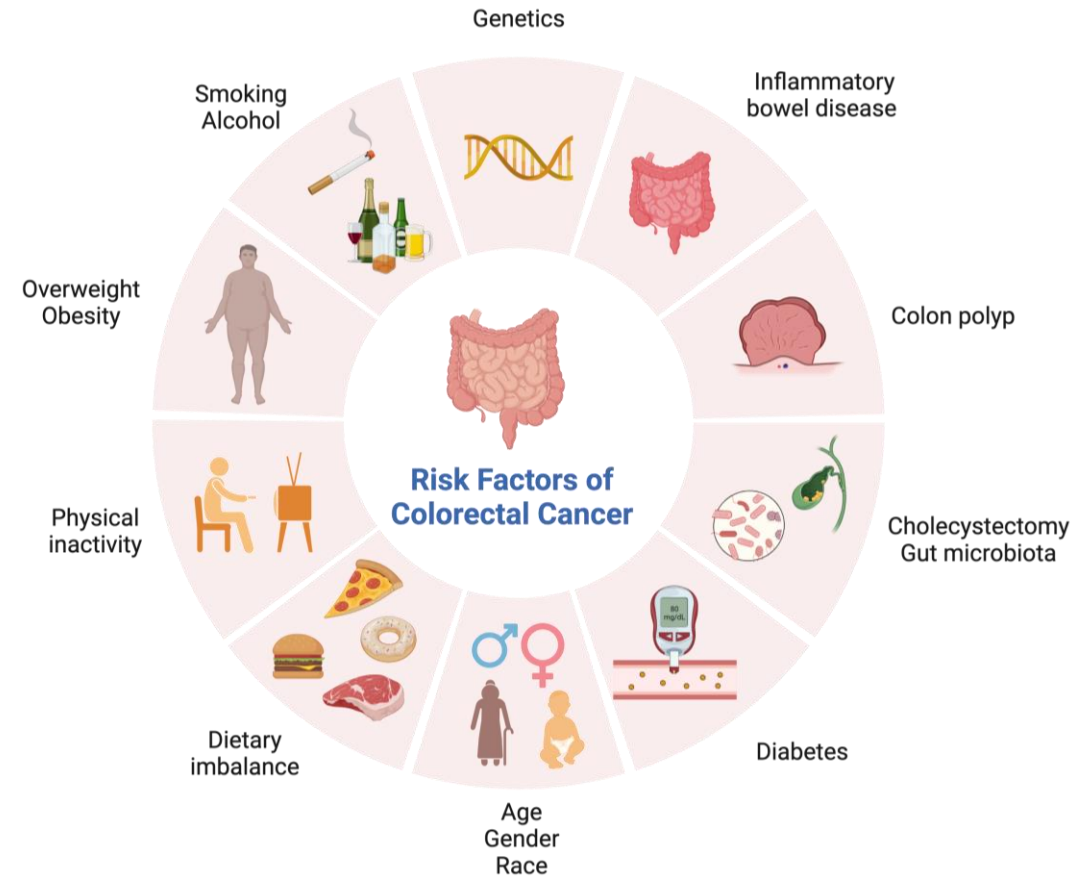
# **Omics, a world to explore for separation science**

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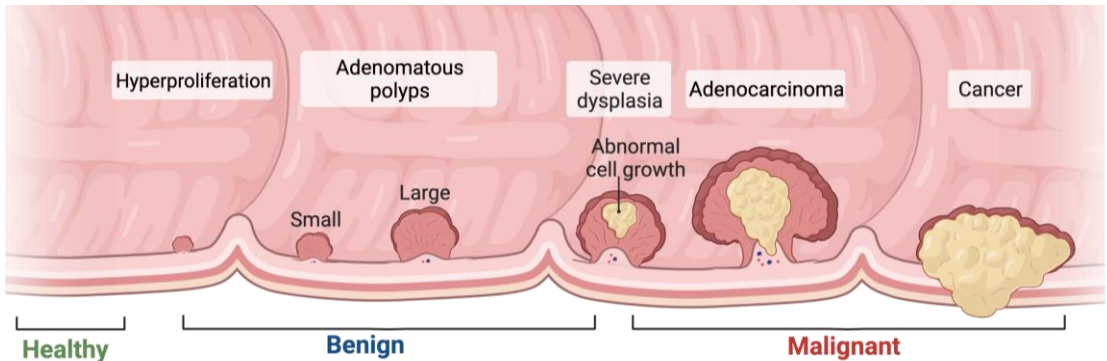
# Specific focus on colorectal cancer

## Estimated New Cases

			Males	Females			
Prostate	191,930	21%		Breast	276,480	30%	
Lung & bronchus	116,300	13%		Lung & bronchus	112,520	12%	
<b>Colon &amp; rectum</b>	<b>78,300</b>	<b>9%</b>		<b>Colon &amp; rectum</b>	<b>69,650</b>	<b>8%</b>	
Urinary bladder	62,100	7%		Uterine corpus	65,620	7%	
Melanoma of the skin	60,190	7%		Thyroid	40,170	4%	
Kidney & renal pelvis	45,520	5%		Melanoma of the skin	40,160	4%	
Non-Hodgkin lymphoma	42,380	5%		Non-Hodgkin lymphoma	34,860	4%	
Oral cavity & pharynx	38,380	4%		Kidney & renal pelvis	28,230	3%	
Leukemia	35,470	4%		Pancreas	27,200	3%	
Pancreas	30,400	3%		Leukemia	25,060	3%	
<b>All Sites</b>	<b>893,660</b>	<b>100%</b>		<b>All Sites</b>	<b>912,930</b>	<b>100%</b>	



# Classification and monitoring methods



Screening Methods approved by EU



Colonoscopy



Flexible Sigmoidoscopy



gFOBT FIT

	Endoscopy based	Stool based
Invasive	✓	✗
High sensitivity	✓	✗
<hr style="border-top: 1px dashed black;"/>		
Non-invasive	✗	✓
Low sensitivity	✗	5 ✓

*There is space for alternative low invasive techniques!*

# Analytical strategy



Minimally invasive  
sampling

*Global metabolomics*

*Semi-targeted  
lipidomics*

Analysis

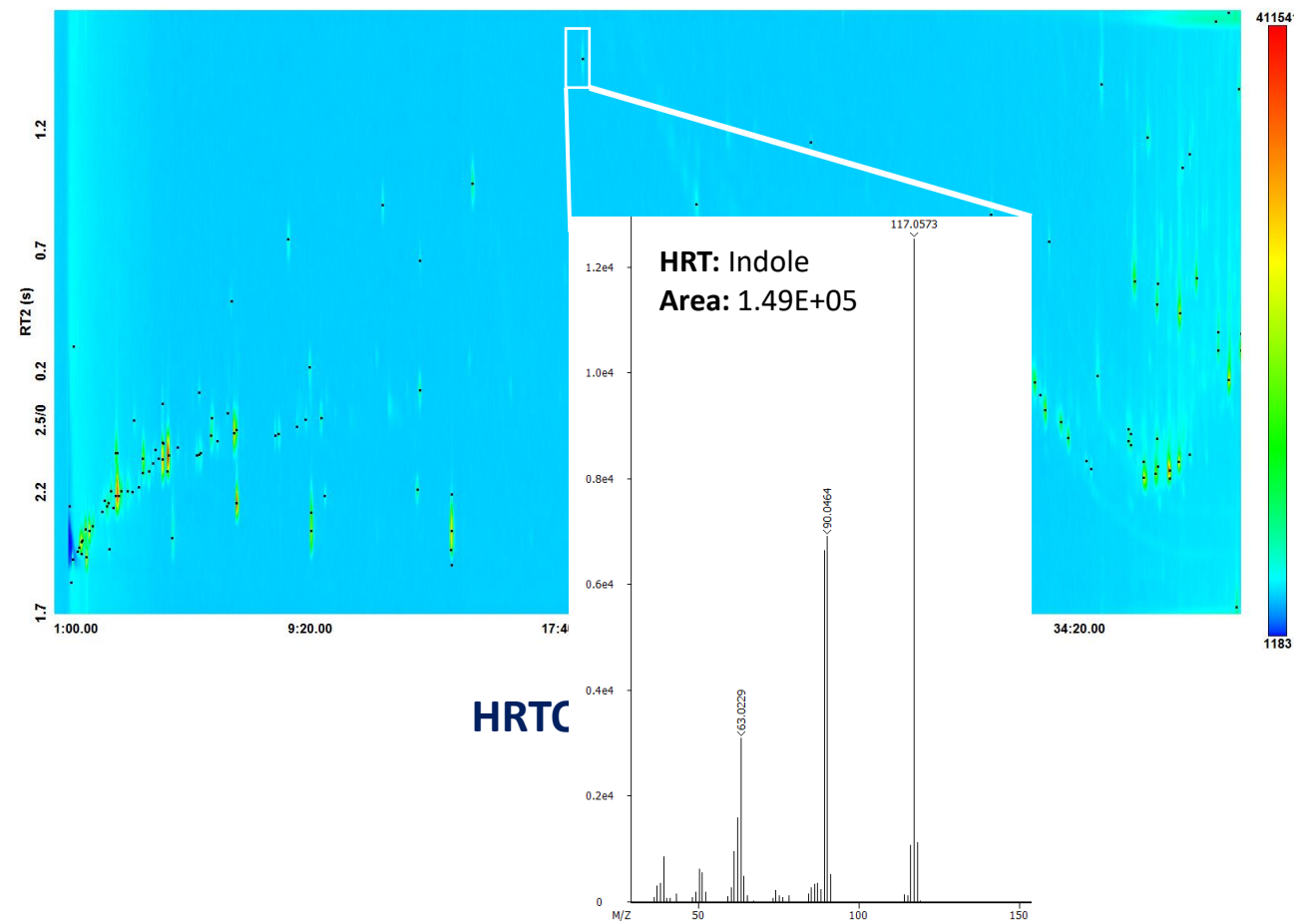
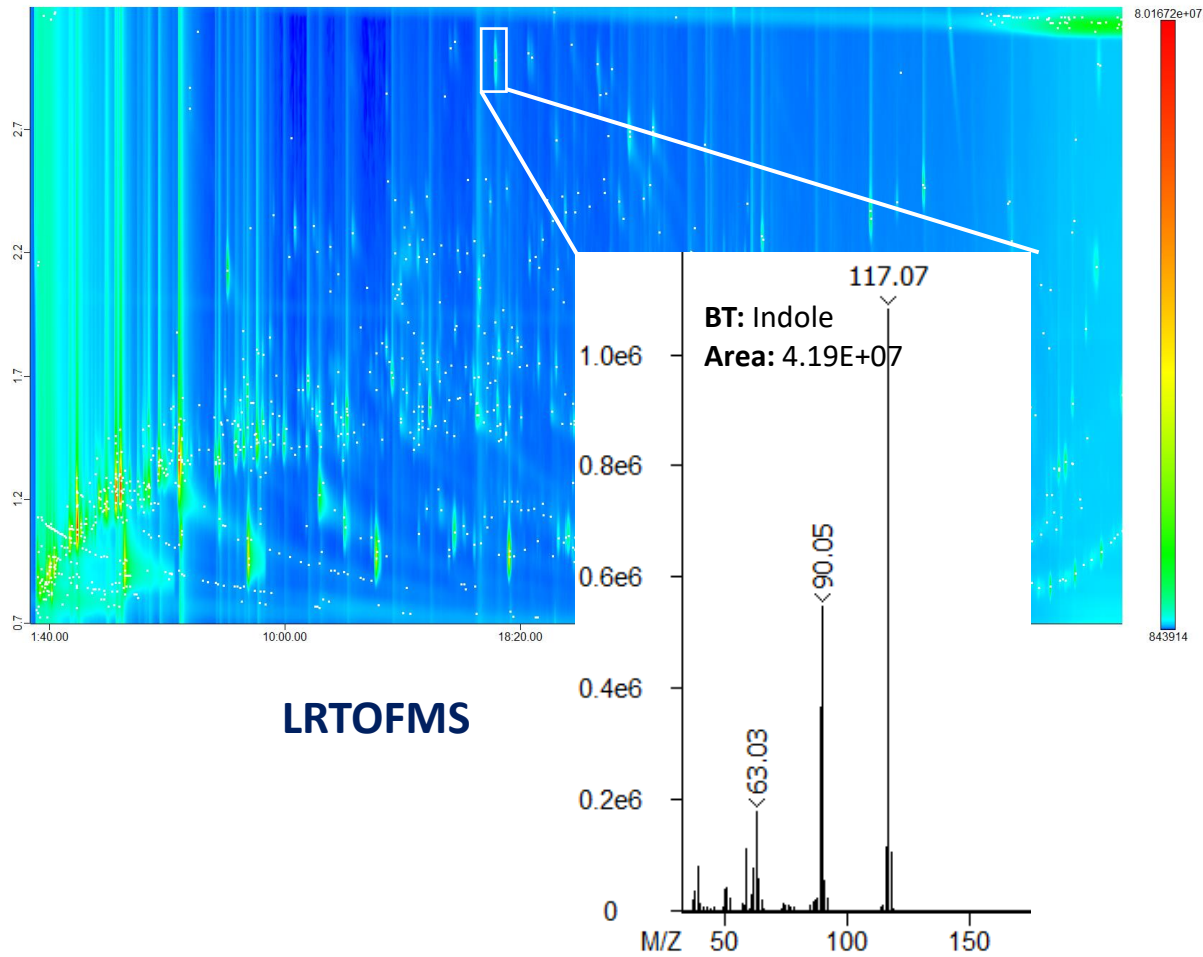


Large scale screening



Identification and annotation

# Separation translation





# Analytical strategy and demographics

Metabolomics (2022) 18: 70  
<https://doi.org/10.1007/s11306-022-01926-3>

REVIEW ARTICLE



## Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management

How

Jennifer A. Kirwan<sup>1,2,3</sup> · Helen Gika<sup>4,5</sup> · Richard D. Beger<sup>6</sup> · Dan Bearden<sup>7</sup> · Warwick B. Dunn<sup>8</sup> · Royston Goodacre<sup>8</sup> · Georgios Theodoridis<sup>9,5</sup> · Michael Witting<sup>10</sup> · Li-Rong Yu<sup>6</sup> · Ian D. Wilson<sup>8,11</sup> on behalf of the metabolomics Quality Assurance and Quality Control Consortium (mQACC)

64 Samples  
9 "bad"



10 baseline QC  
19 QCs



UNIST  
SRM1950



UNIST  
standards



14  
injections

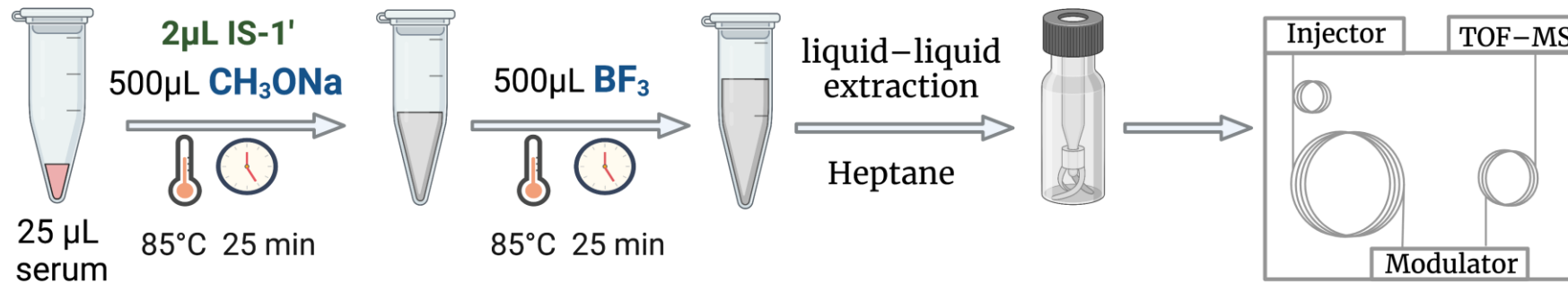
**2 methods → 228 injections**



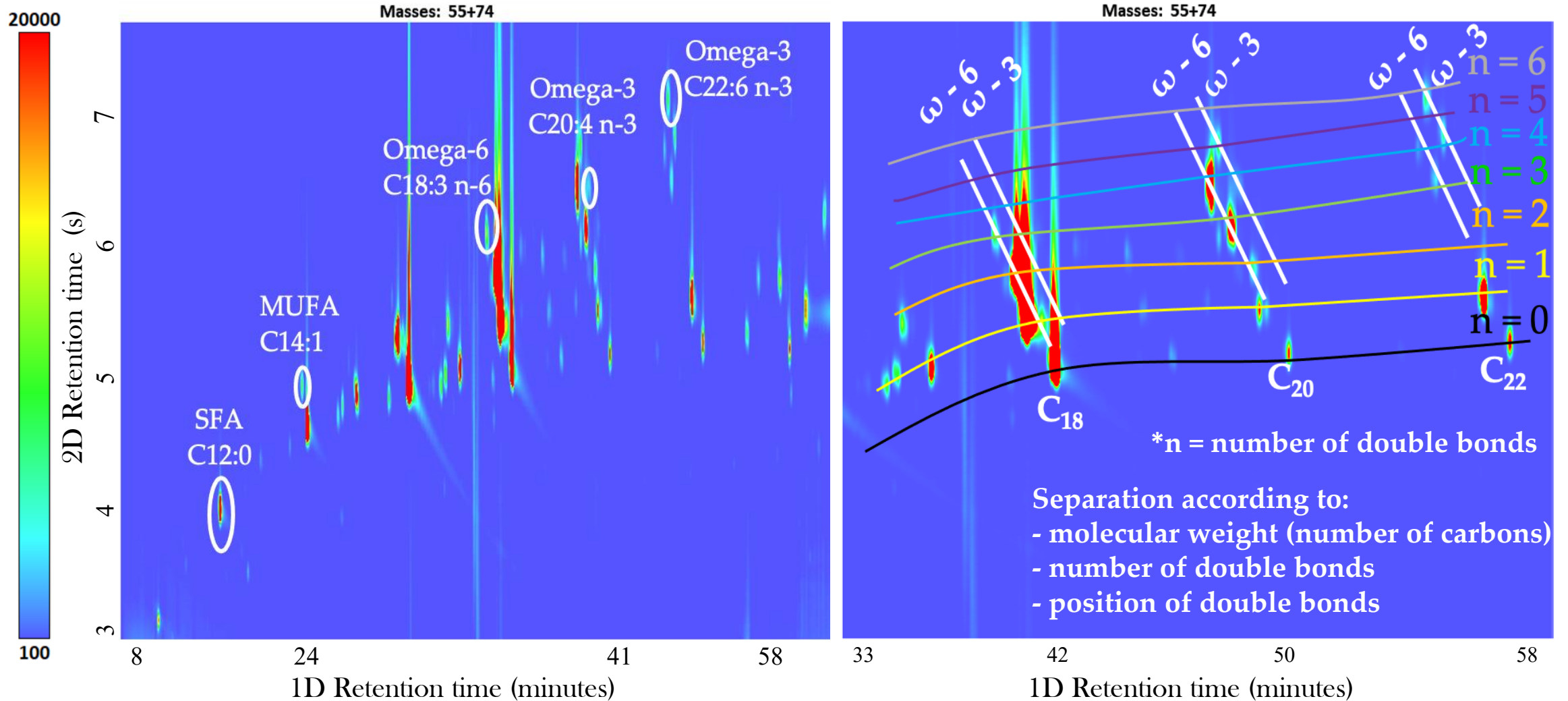
# **Lipidomics screening**

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# Tailored and optimized approach

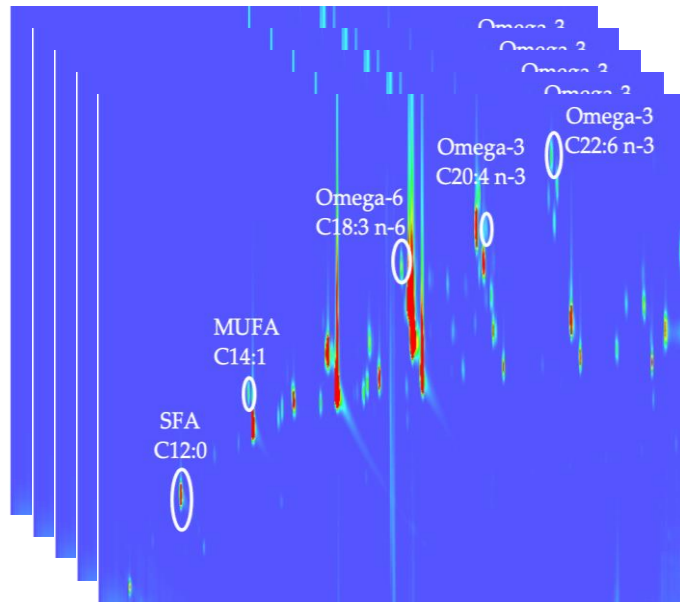


# Structured and informative separation

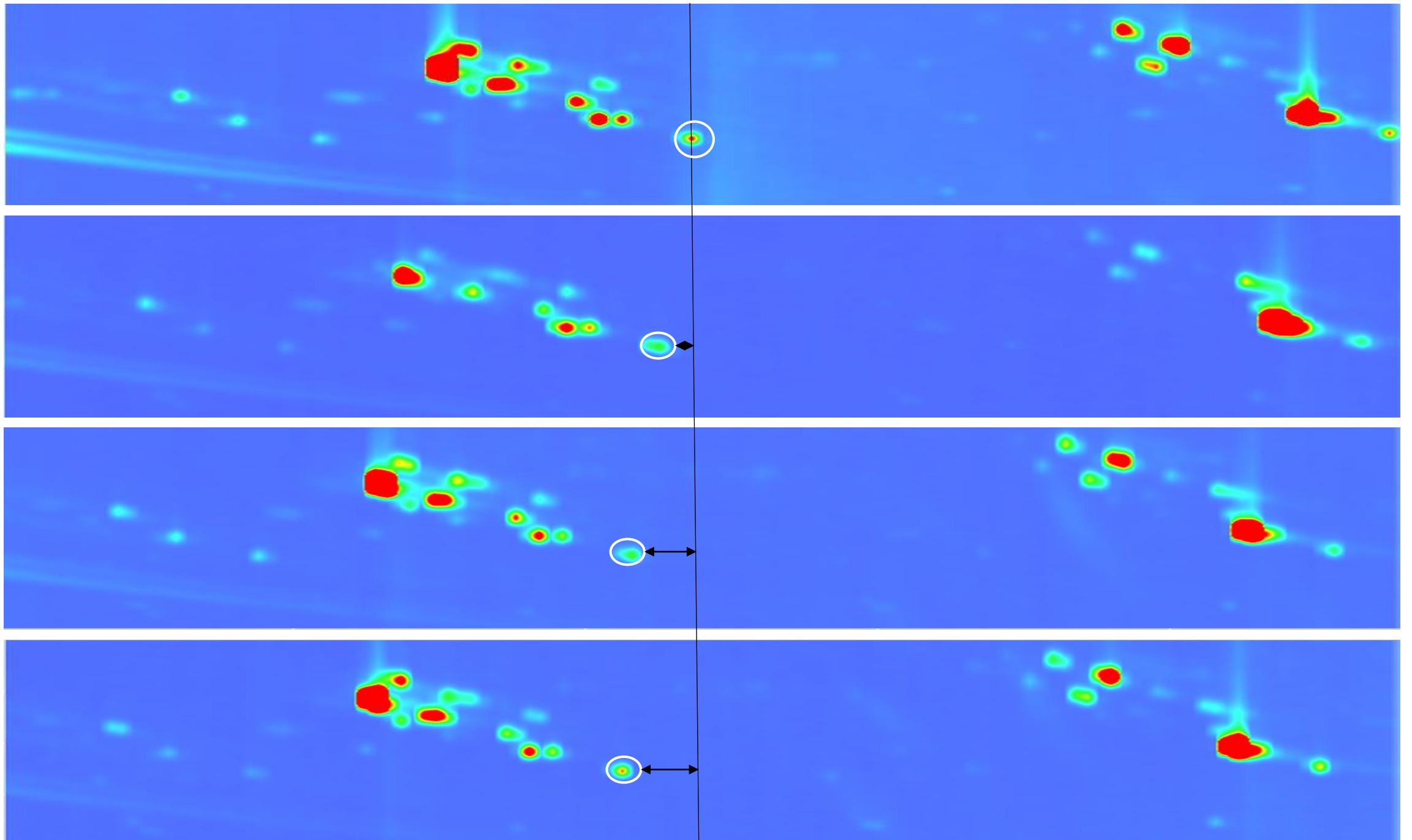


# Data processing workflow

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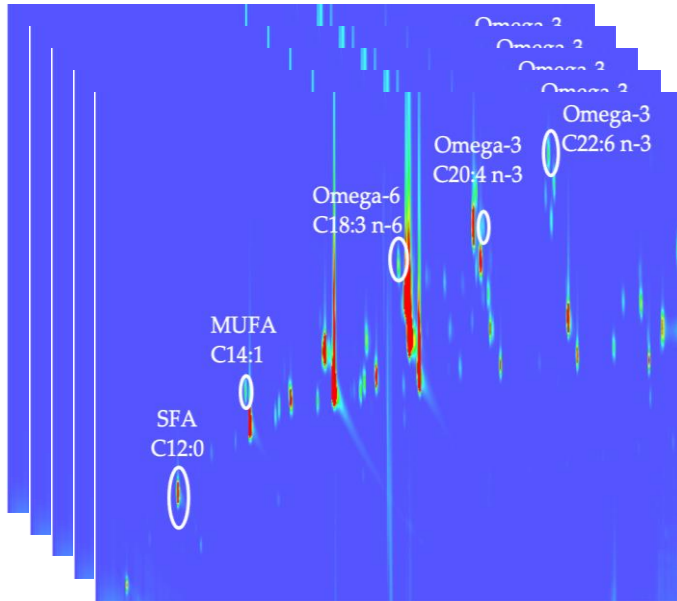


*Alignment and pre-processing*



# Data processing workflow

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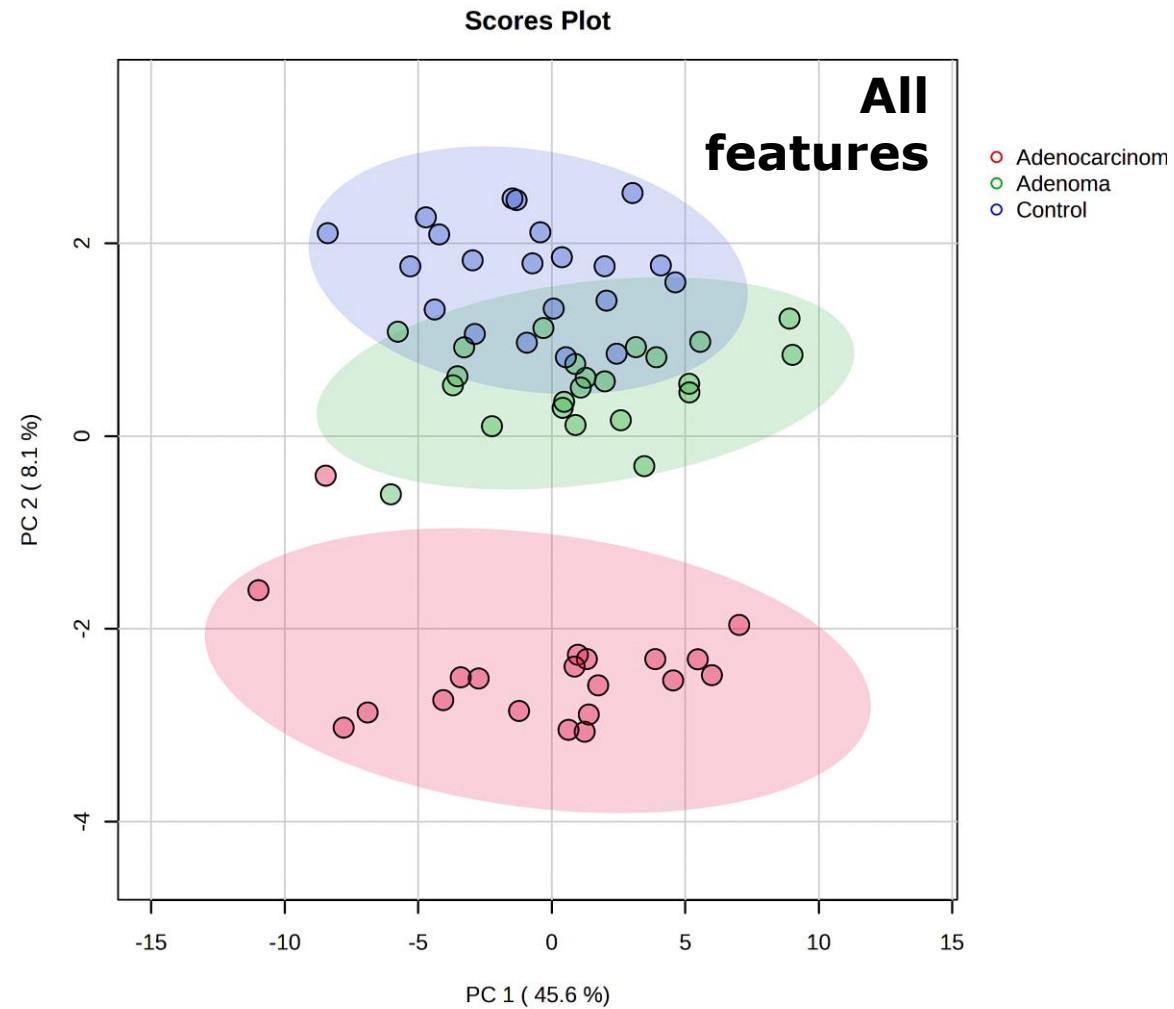


*Alignment and pre-processing*



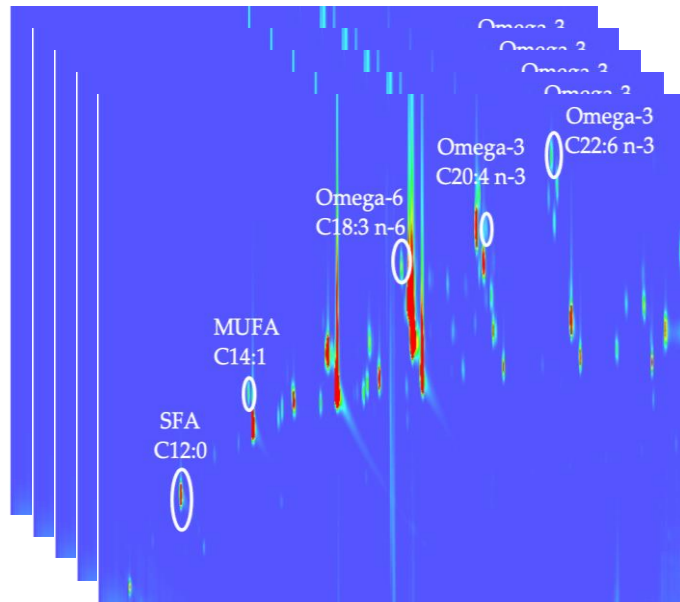
*Unsupervised screening*

# Unsupervised screening - PCA





# Data processing workflow



*Alignment and pre-processing*

*Unsupervised screening*

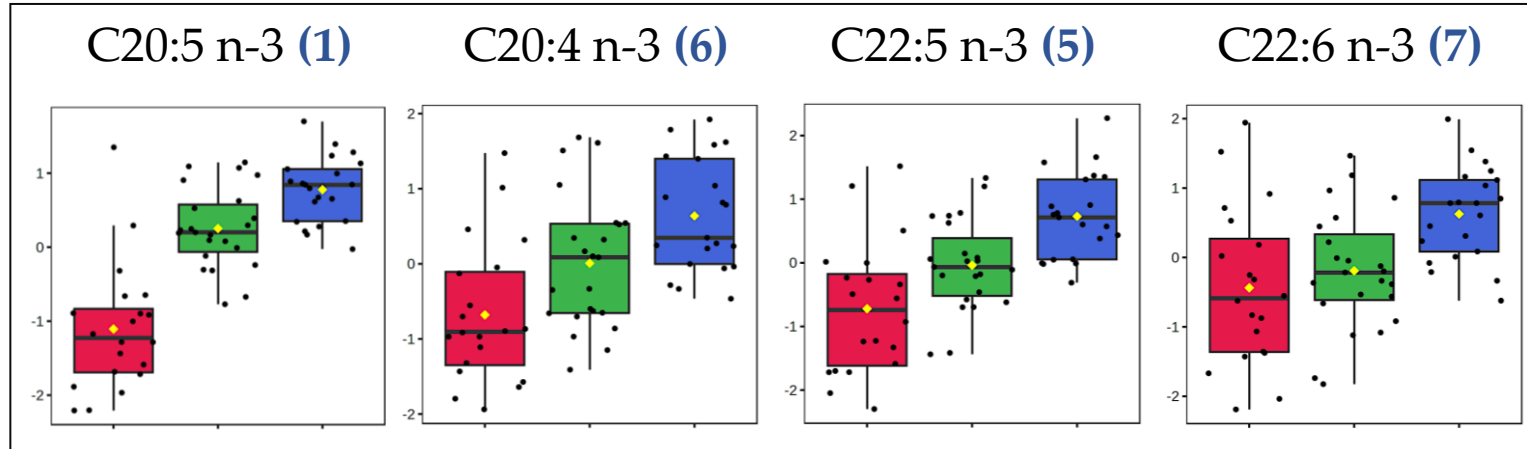
*Feature selection approaches*

*Metabolic pathways*

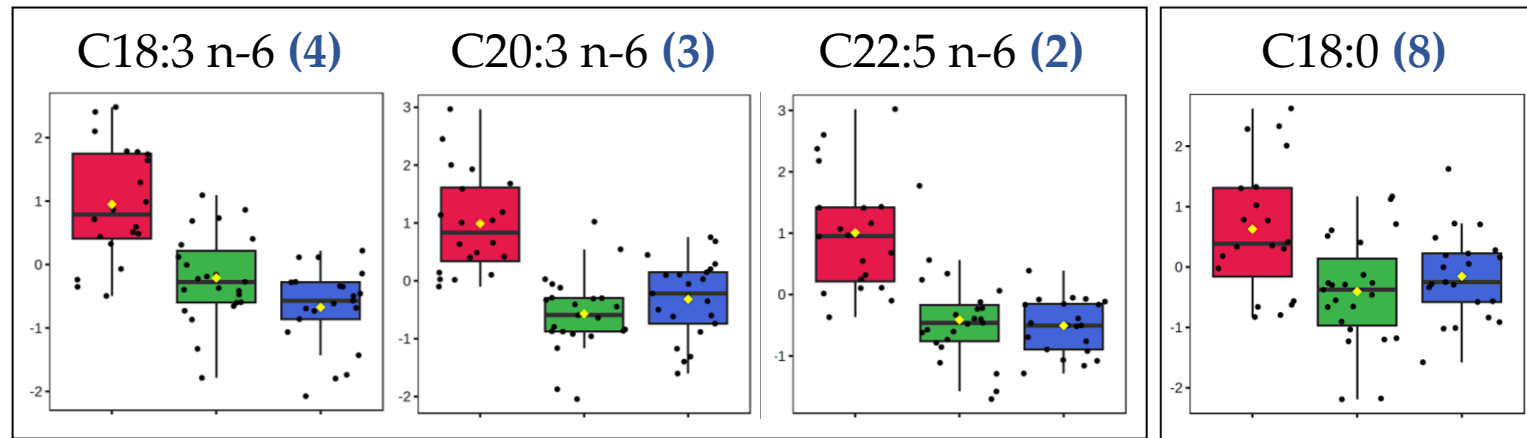
# Top 8 features across ANOVA and RF

Downregulated  
in CRC

↓  
PUFA ( $\omega$ -3)



PUFA ( $\omega$ -6)



SFA

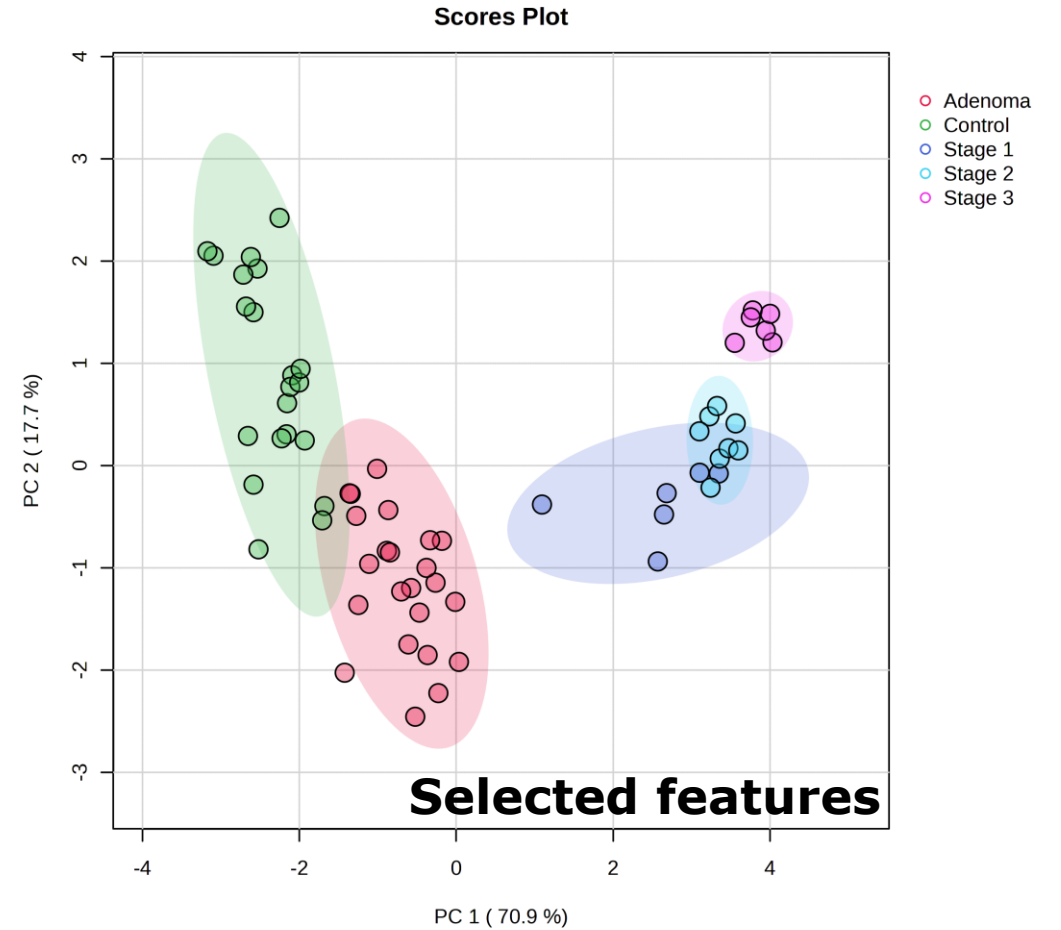
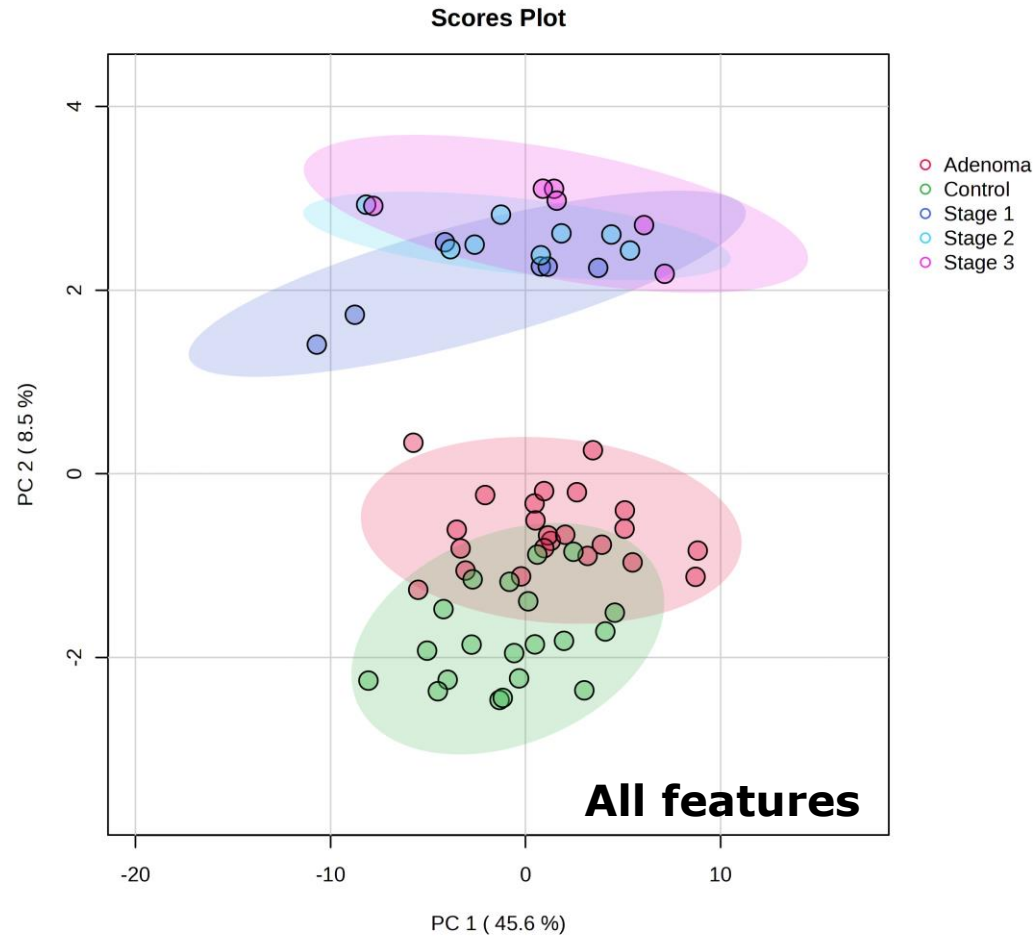
↑  
Upregulated  
in CRC



# Top 8 features ID metrics

Potential ID	Class	CAS	Similarity	Reverse	Probability (%)	$\Delta$ LRI	Mass accuracy (ppm)	(FDR)<0.05	VIP Score (>1)	RF, MDA (>0.008)
C20:4 n-3	PUFA ( $\omega$ -3)	132712-70-0	882	875	30.1	12	-	$2.6 \times 10^{-4}$	1.9847	0.019331
<b>C20:5 n-3*</b>	PUFA ( $\omega$ -3)	2734-47-6	890	892	58.5	8	-1.17	$1.1 \times 10^{-11}$	2.8251	0.079084
C22:5 n-3	PUFA ( $\omega$ -3)	108698-02-8	851	851	74.1	13	-	$5.3 \times 10^{-3}$	2.1896	0.032492
<b>C22:6 n-3*</b>	PUFA ( $\omega$ -3)	2566-90-7	900	910	72.9	16	-1.01	$2.0 \times 10^{-3}$	1.6093	0.019366
<b>C18:3 n-6*</b>	PUFA ( $\omega$ -6)	16326-32-2	875	875	56.3	11	0.74	$9.7 \times 10^{-8}$	2.4366	0.046362
<b>C20:3 n-6*</b>	PUFA ( $\omega$ -6)	21061-10-9	919	907	69.7	12	-1.03	$7.5 \times 10^{-8}$	1.9479	0.036985
C22:5 n-6	PUFA ( $\omega$ -6)	-	897	883	28.6	18	-	$7.5 \times 10^{-8}$	2.2631	0.044829
<b>C18:0*</b>	SFA	112-61-8	925	955	84.3	1	0.37	$7.0 \times 10^{-3}$	1.1598	0.0087911

# Supervised screening - PCA



# Take home message

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- Multidimensional chromatography has its seat at the omics table
- The QA/QC aspects are keys
- Lipidomics: high classification between classes + stages information
  - Simpler sample prep and chromatograms
  - A lot of information for pathway analysis

# Acknowledgement





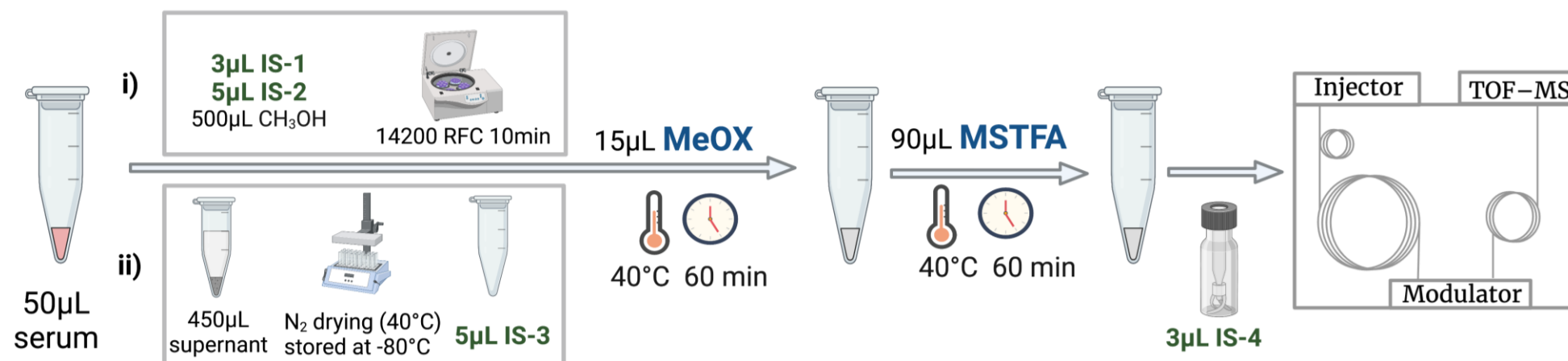




# Metabolomics screening

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# General optimized workflow



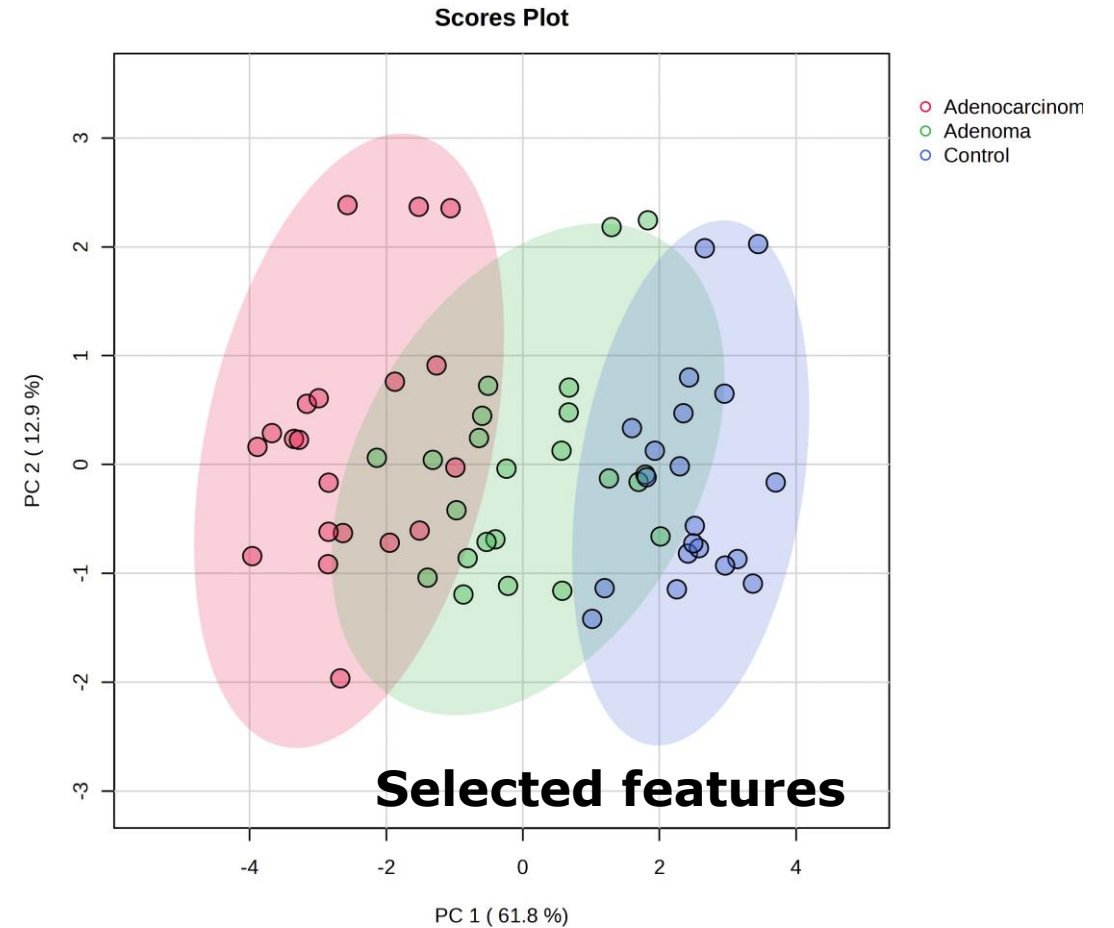
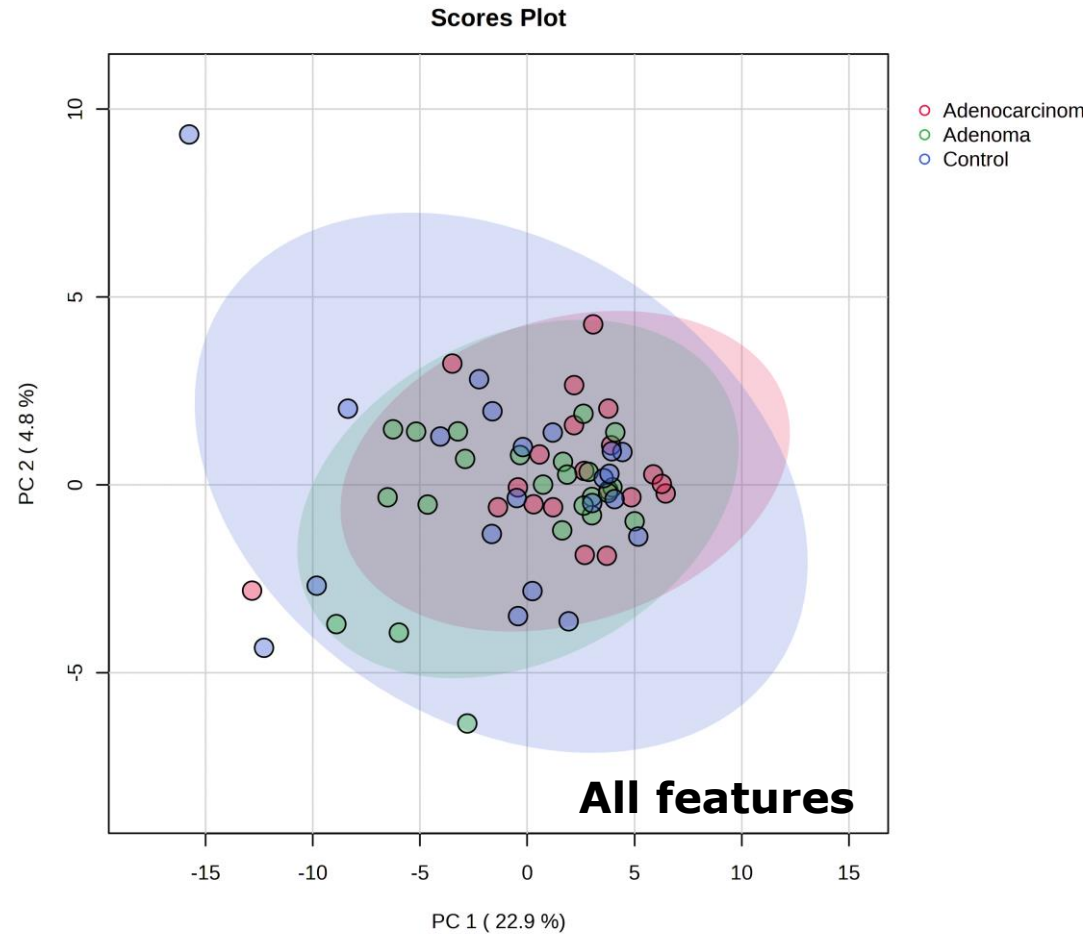
## Untargeted Serum Metabolic Profiling by Comprehensive Two-Dimensional Gas Chromatography–High-Resolution Time-of-Flight Mass Spectrometry

Nicolas Di Giovanni,<sup>†</sup> Marie-Alice Meuwis,<sup>‡</sup> Edouard Louis,<sup>‡</sup> and Jean-François Focant<sup>\*,†</sup>

<sup>†</sup>Department of Chemistry, Organic and Biological Analytical Chemistry Group, Quartier Agora, University of Liège, Allée du Six Août, B6c, B-4000 Liège (Sart Tilman), Belgium

<sup>‡</sup>GIGA institute, Translational Gastroenterology and CHU de Liège, Hepato-Gastroenterology and Digestive Oncology, Quartier Hôpital, University of Liège, Avenue de l'Hôpital 13, B34-35, B-4000 Liège, Belgium

# Unsupervised and supervised PCA analysis



## Summary for lipid serum profiling for CRC

1) The long and very long chain PUFA ( $\omega$ -3) and PUFA ( $\omega$ -6) are majorly influenced in progression of CRC

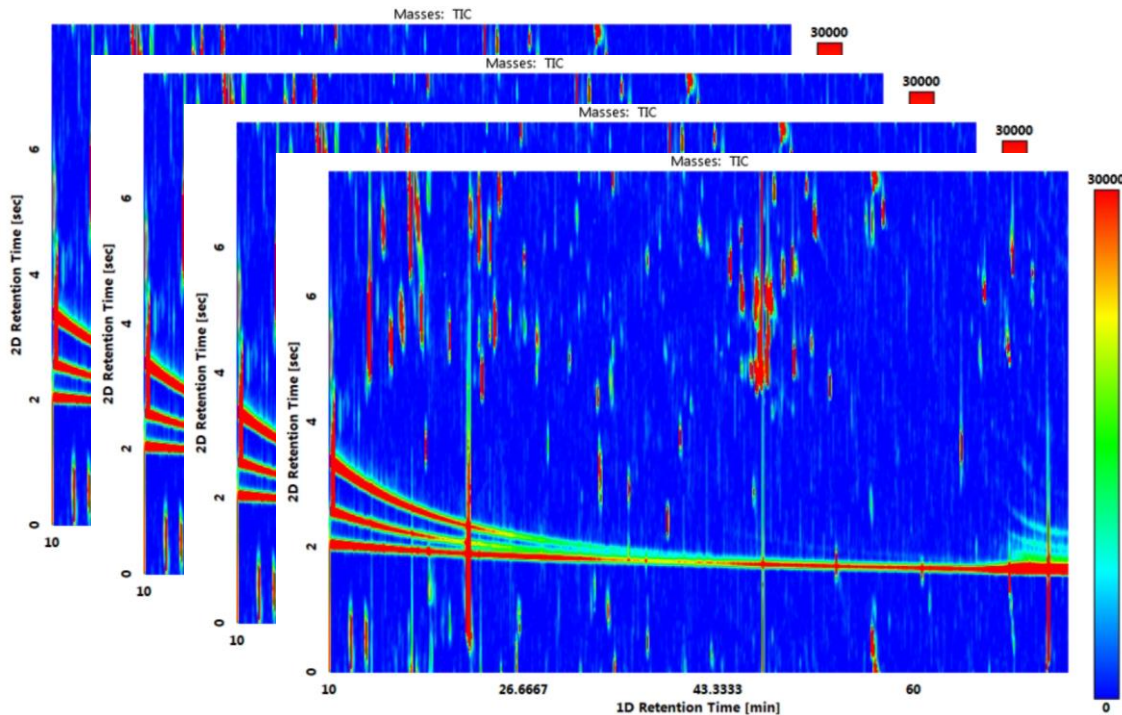
- PUFA ( $\omega$ -3) are downregulated in CRC [EPA (C20:5 n-3), DPA (C22:5 n-3), C20:4 n-3, and DHA (C22:6 n-3)]
- PUFA ( $\omega$ -6) are upregulated in CRC [C22:5 n-6, C20:3 n-6, and C18:3 n-6]
- SFA (C18:0) is upregulated in CRC

2) Alpha Linolenic Acid and Linoleic Acid Metabolism is majorly involved metabolic pathway in CRC.

## Data processing

Data export (.csv)  
\*with baseline correction

Data alignment, feature identification



64 Samples

total  
features

480

identified  
features

230

105

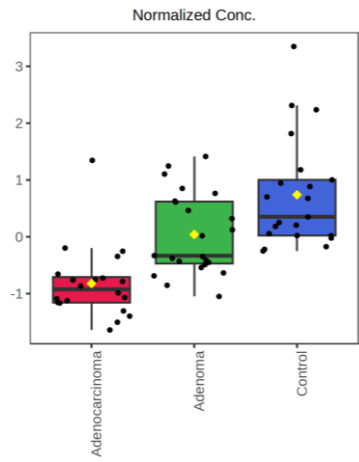
solvent artifact,  
column bleeding

## Class

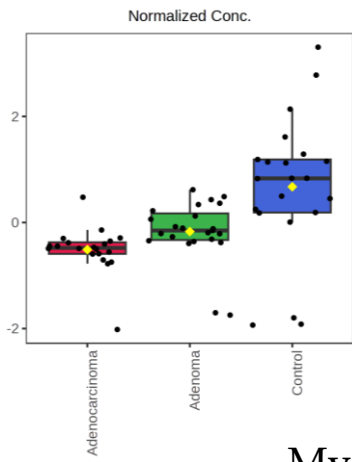
Amino acids  
Fatty acids  
Organic acids  
Methyl xanthine,  
purine,  
pyrimidine nucleoside, etc.

# Normalized concentration of top 8 significant features

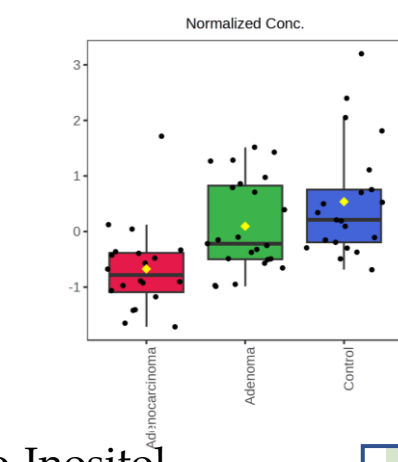
L-Valine, 2TMS derivative



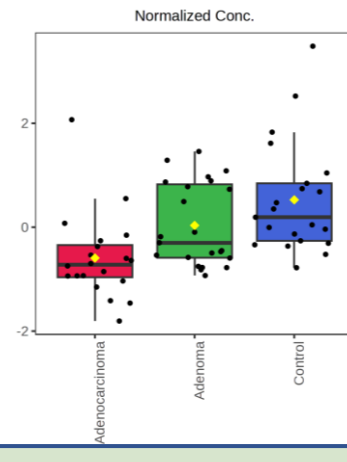
L-Glutamic acid, 3TMS derivative



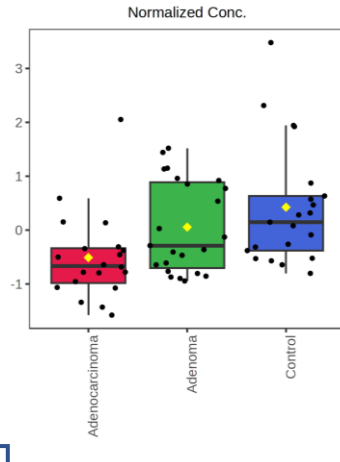
L-Tryptophan, 3TMS derivative



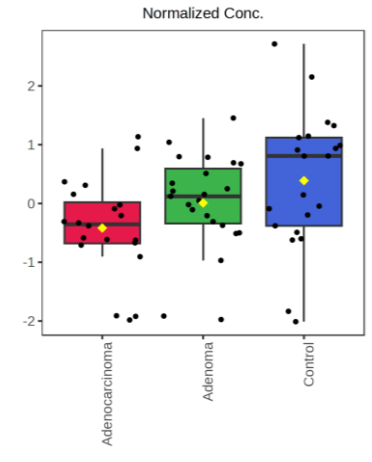
Tyrosine, 2TMS derivative



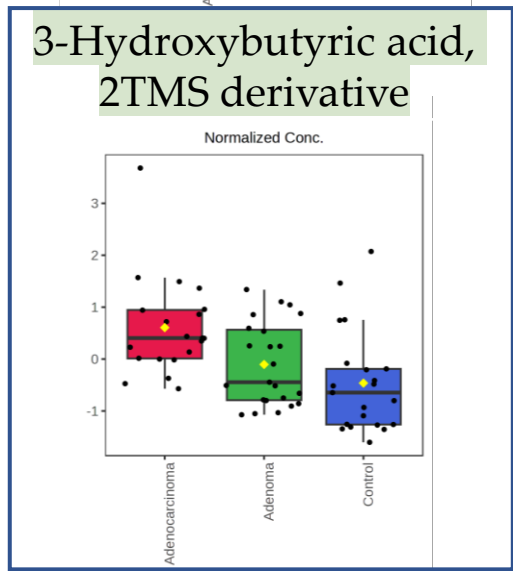
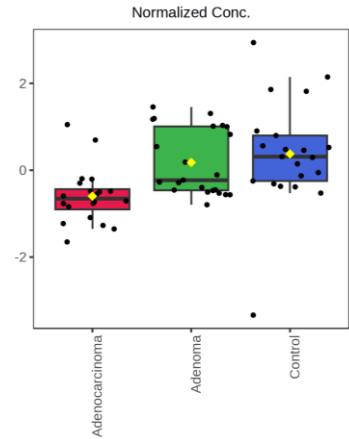
L-Alanine, 2TMS derivative



L-Methionine, 2TMS derivative



Myo-Inositol, 6TMS derivative



Adenocarcinoma Adenoma Control



**Table 3. Annotation of the Candidate Biomarkers<sup>a</sup>**

candidate	ID	match	probability	$\Delta RT$	$\Delta$ (ppm)	criteria met
2	erythronic acid/threonic acid	<b>902</b>	49	57	<b>0.1</b>	<b>2</b>
7	aspartic acid	<b>758</b>	<b>82</b>	<b>19</b>	<b>0.2</b>	<b>3</b>
8	threonolactone*	<b>808</b>	57	40	<b>0.6</b>	2
9	glutamic acid	<b>853</b>	<b>78</b>	<b>5</b>	<b>0</b>	<b>3</b>
10	xylose	<b>757</b>	6	28	<b>0.7</b>	<b>2</b>
13	methionine	<b>678</b>	<b>86</b>	<b>0</b>	<b>0.1</b>	<b>3</b>
14	2-hydroxybutyric acid	<b>846</b>	27	<b>1</b>	<b>0.1</b>	<b>3</b>
17	1,5-anhydroglucitol	<b>912</b>	<b>66</b>	<b>11</b>	<b>0.4</b>	<b>3</b>
18	citric acid	<b>872</b>	<b>95</b>	<b>12</b>	<b>0.2</b>	<b>3</b>
19	galactose	<b>739</b>	11	<b>10</b>	34	<b>2</b>
23	erythrose/threose	572	12	<b>24</b>	<b>0.4</b>	<b>2</b>
26	capric acid	<b>795</b>	<b>93</b>	<b>0</b>	<b>0.3</b>	<b>3</b>
31	erythritol/threitol	<b>823</b>	23	<b>16</b>		<b>2</b>
33	myristic acid	<b>879</b>	<b>96</b>	<b>14</b>	<b>0.1</b>	<b>3</b>
34	glucose	<b>899</b>	15	7	<b>0.6</b>	<b>3</b>
36	lauric acid	<b>806</b>	<b>90</b>	<b>8</b>	<b>0.1</b>	<b>3</b>
37	terephthalic acid*	644	0	10	0.2	2

<sup>a</sup>Candidates highlighted in the CD vs HC separation (top) and candidates highlighted in the three CD subgroups separation (bottom). The values that meet the significance thresholds are in bold. \*Threonolactone and terephthalic acid are probable artifacts.