

Analysis and Discovery of Breath-Based Biomarkers for Performance and Health Metrics

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SPACE SCIENCE

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Conflict of Interest Statement

- "I have no financial relationships with, or financial interests in, any commercial companies pertaining to this research."

Motivation and Problem - OEH

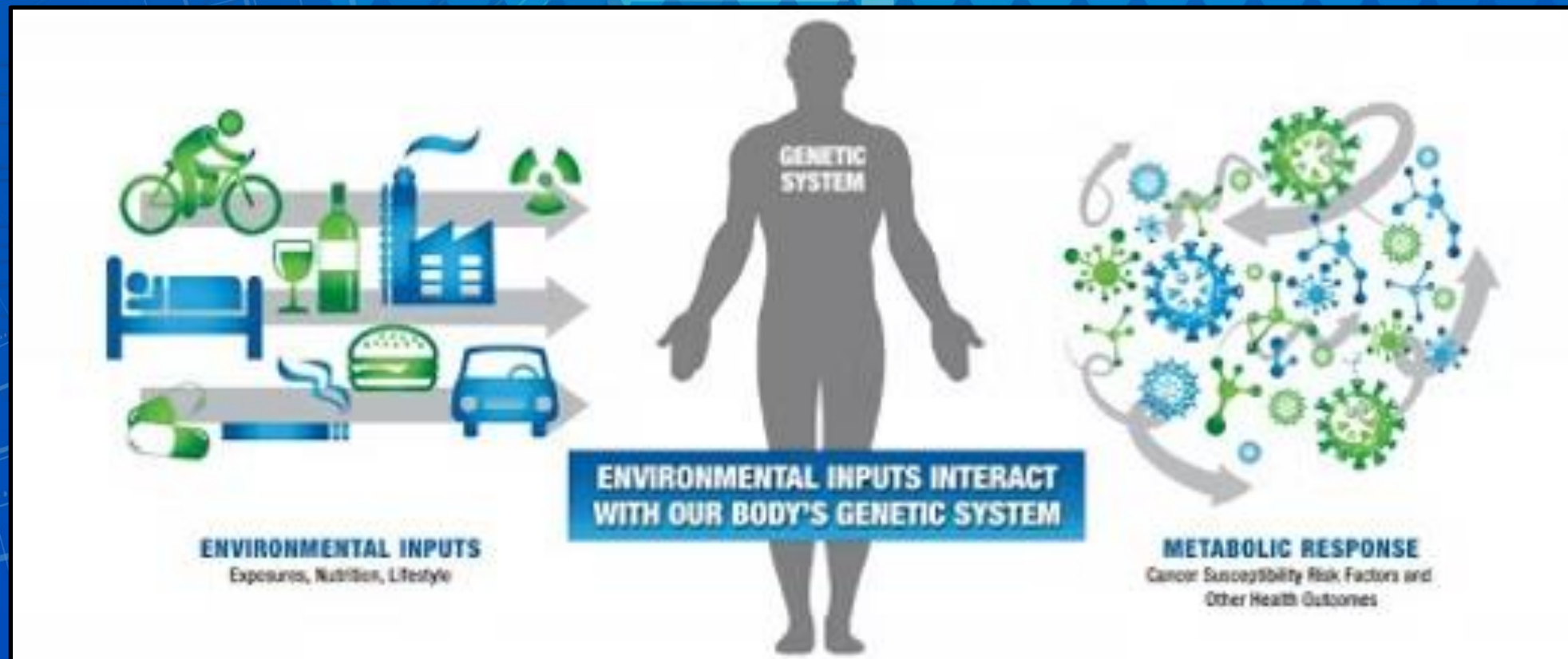
- Occupational and Environmental Health (OEH) chemicals can pose toxic, physical, and long-term health effects.

Major Chemicals include:

- Fuels, Solvents, and Degreasers (JP-8, Kerosene, Benzene, TCE, PCE)
- Combustion and Air Pollutants (Particulates, PAHs, Dioxins, VOCs)
- Per- and Polyfluoroalkyl Substances (PFAS)
- Historical Tactical Agents and Pesticides (Agent Orange, OPP, CWAs)
- Heavy Metals and Munitions Constituents (Pb, Hexavalent Chromium)

OEH Exposure Tracking

- A continued need for Real-Time, Non-Invasive, and Field Deployable instrumentation.
- Breath Analysis is an underutilized, but information rich matrix that can be easily collected, analyzed, and used to determine OEH exposure levels.



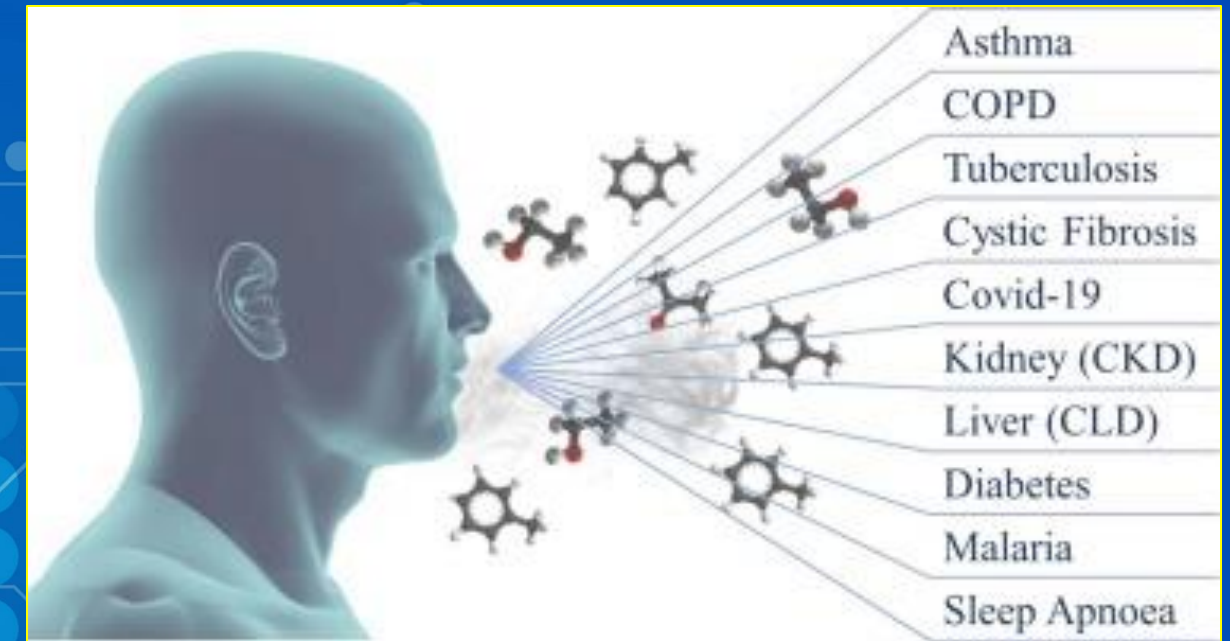
<https://www.mindfulpediatricskc.com/post/environmental-exposures-overview>

Human Breath Analysis

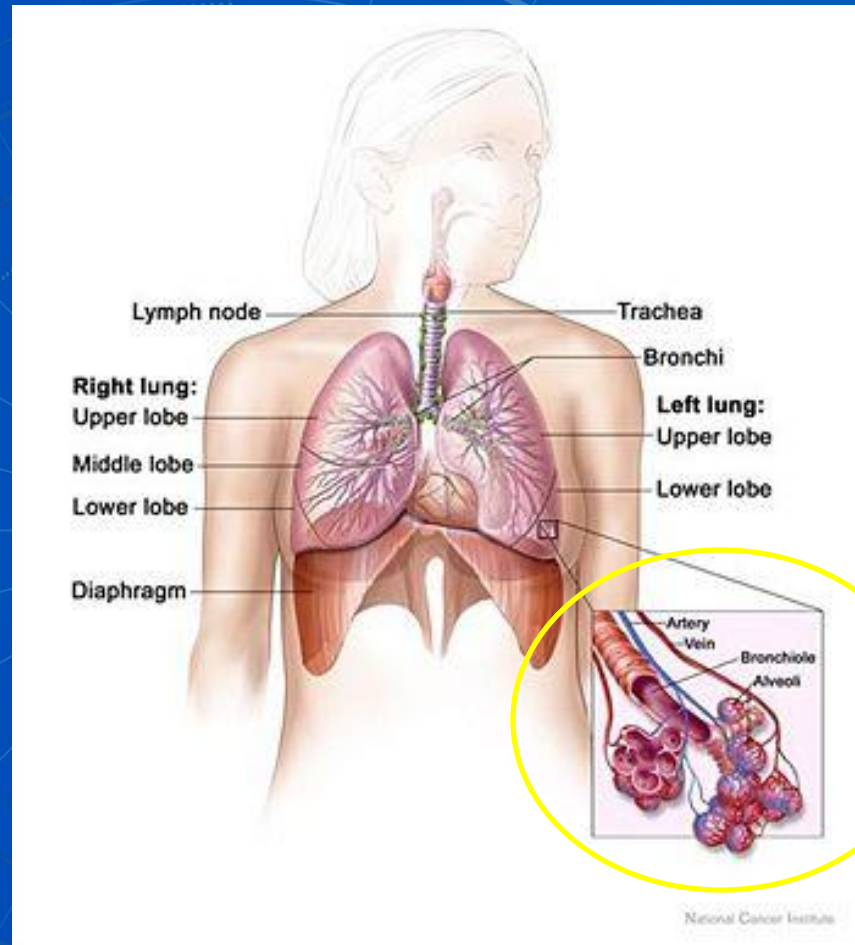
I. Noninvasive technique

II. Signature “markers” – linked to metabolic states and health related diseases

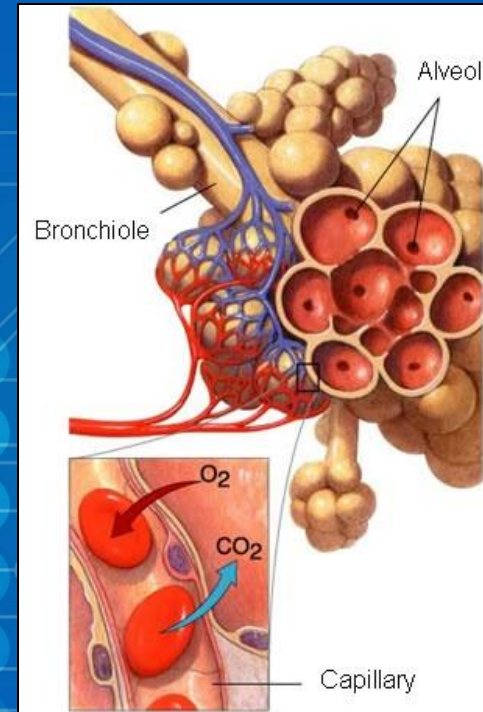
- Acetone for diabetes
- Isoprene for hypercholesterolemia
- Straight chain hydrocarbons related to lipid peroxidation of polyunsaturated fatty acids found in cellular membranes
- Increased hydrocarbons related to pulmonary, liver, bowel and neurological diseases



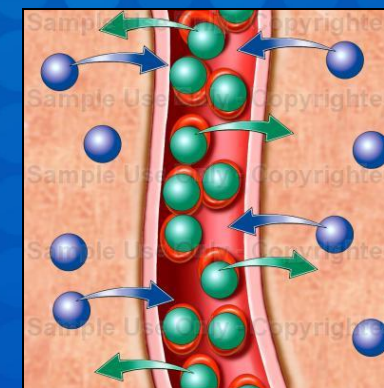
Human Breath Analysis



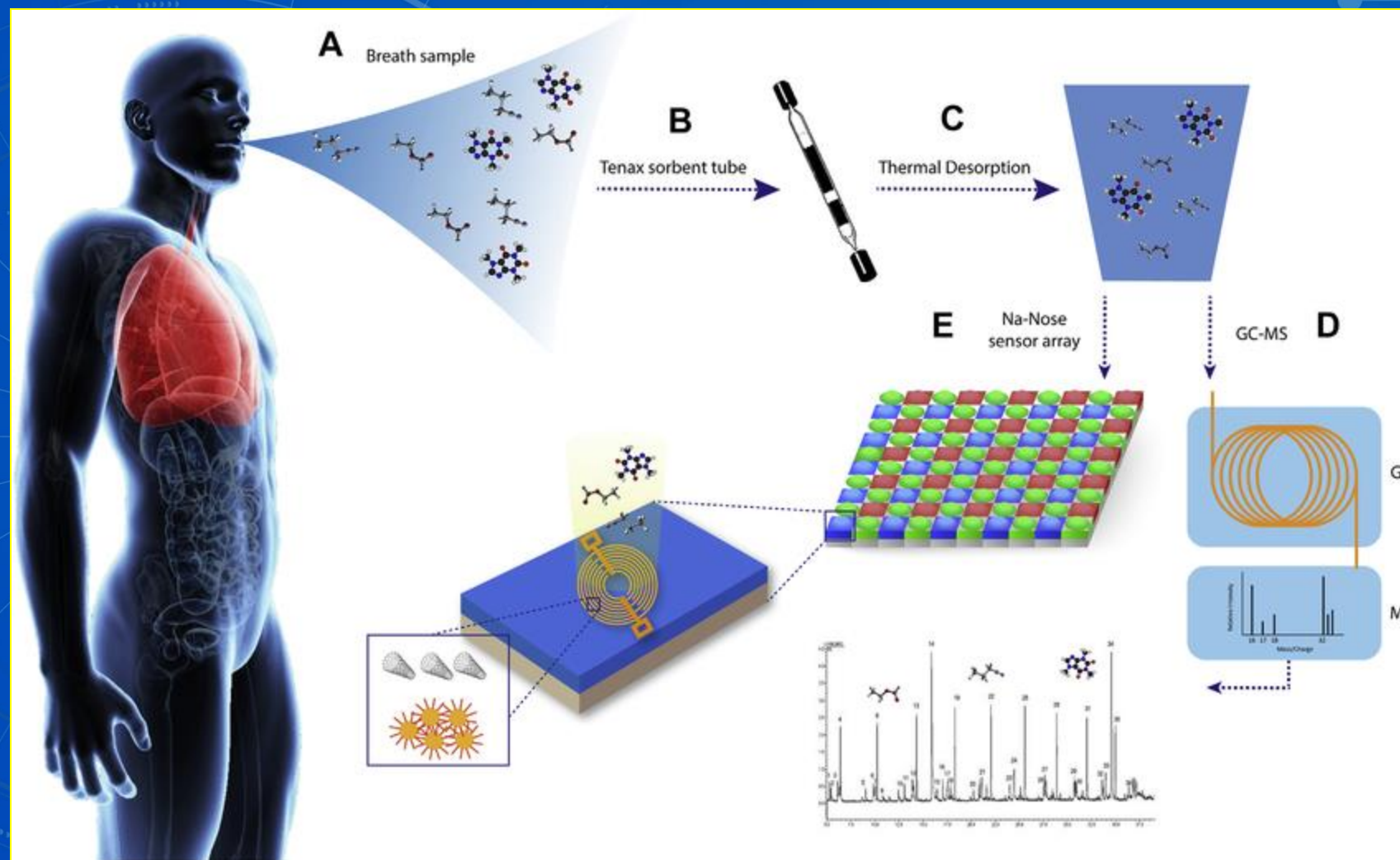
Physiology of the Human Lung



- Biomarkers are exchanged between the blood and lung through the Alveoli
- The ability to collect and analyze these trace level (sub-ppb) VOCs and SVOCs is the principle for breath-based correlation



Breath-Based Methodology



Sciencedirect.com

A – Collection of expired breath

B – Transfer 1-L of breath to a multi-bed sorption trap

C – Desorb the sorption trap onto a GCMS and/or sensor based device

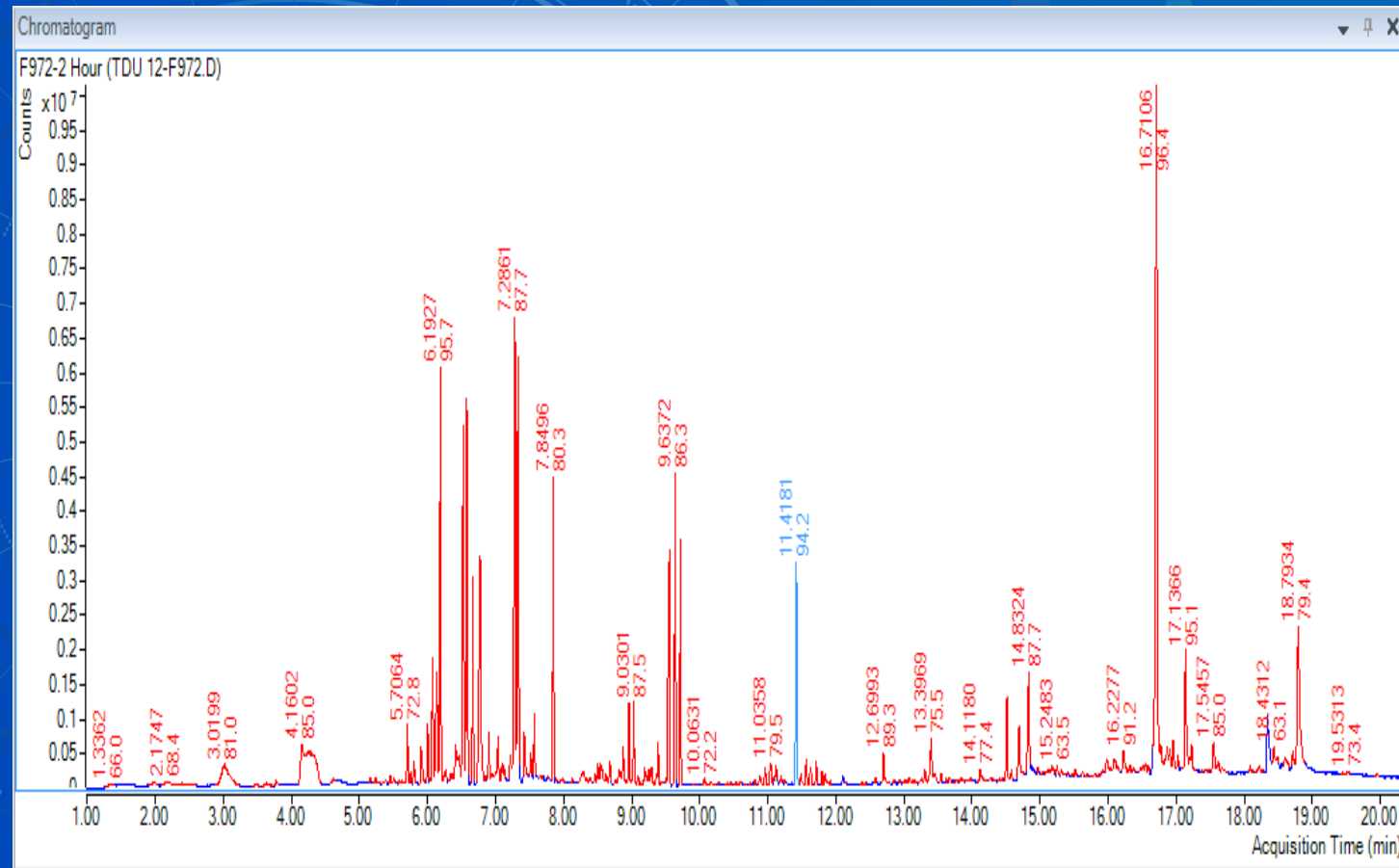
D – GCMS and/or GC×GC-MS analysis

E – Analysis via a chemical sensor for targeted applications

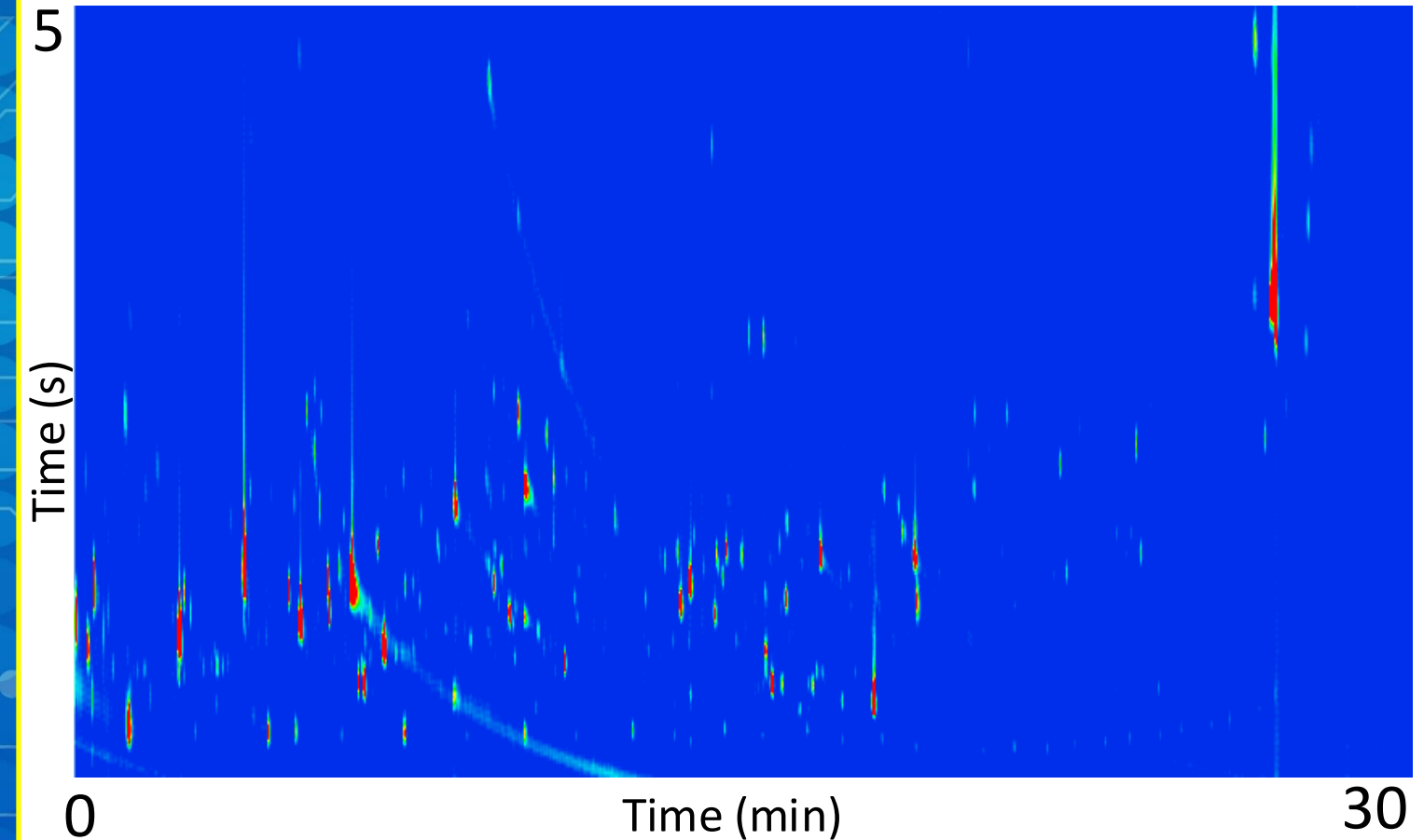
Human Breath Chromatograms

GCMS Analysis of Human Breath

GC×GC-MS Analysis of Human Breath

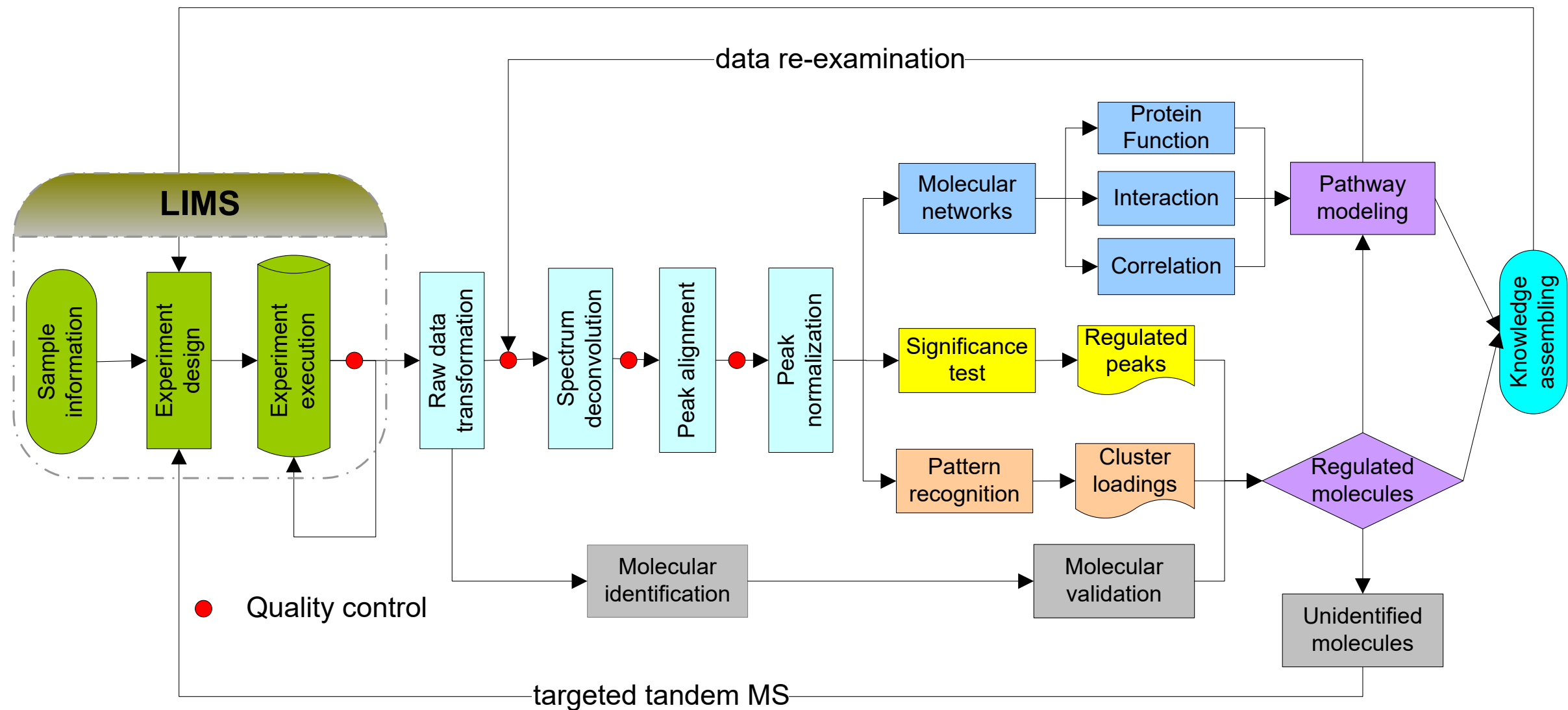


100-500 organic compounds detected



1000 – 1800 identified compounds

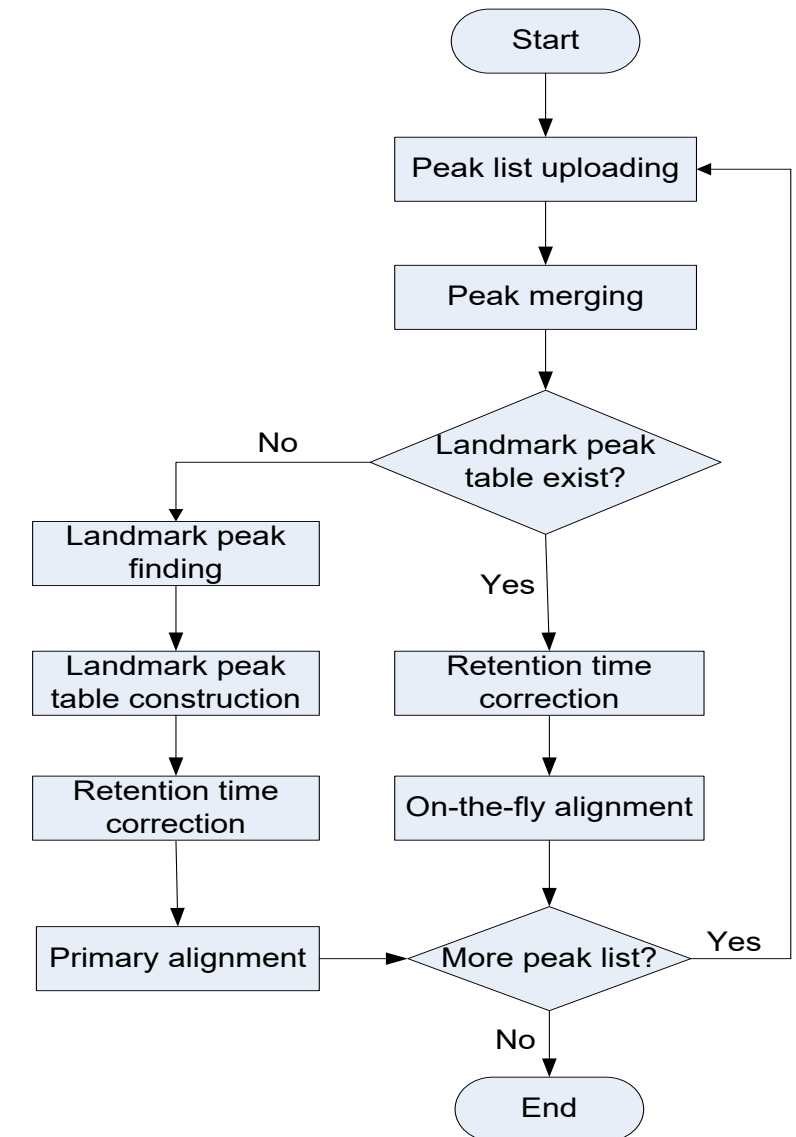
Project Methodology



Data Analysis Methodology

1. The raw data are first processed using instrument software to generate peak lists.
2. Peak alignment is accomplished by correcting retention time shifts of the same metabolite detected in different samples.
3. The only difference between the primary alignment and the on-the-fly alignment is that the landmark peaks in primary alignment is not fixed, but the on-the-fly alignment uses a list of fixed landmark peaks.

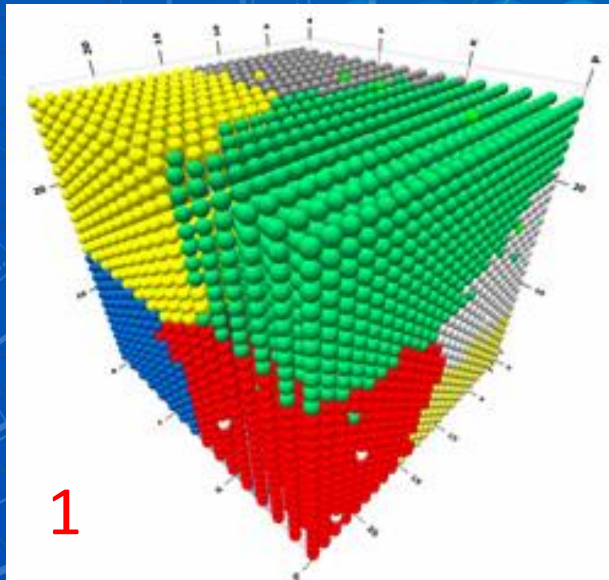
Analytical Chemistry. **2010**, 82, 5069-5081.



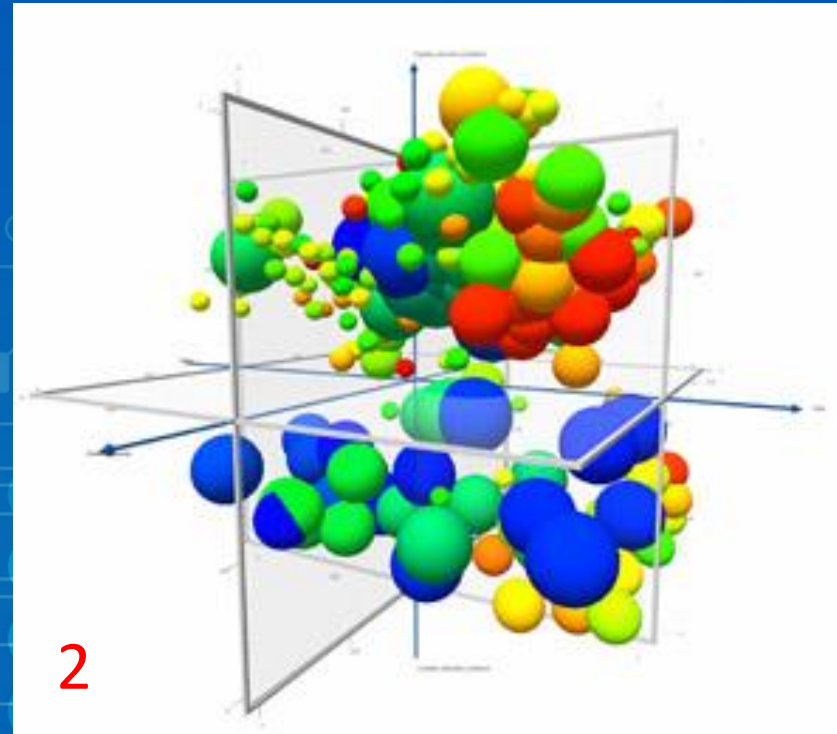
Statistical Analysis

Methods

Fisher Ratio	(variance)
Pair-wise t-test	(diff. mean)
Mann-Whitney U test	(diff. median)
Kolmogorov-Smirnov test	(diff. population)
Kruskal-Wallis	(variance)

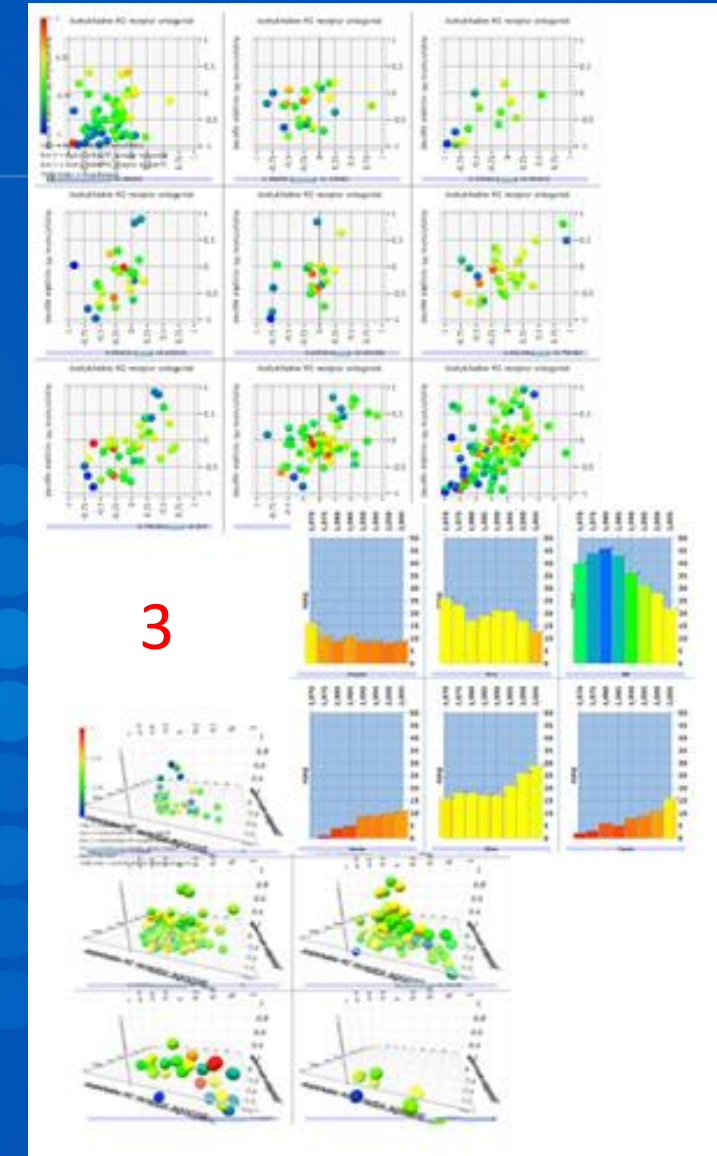


K-means clustering : data reduction



PCA – Principal Component Analysis

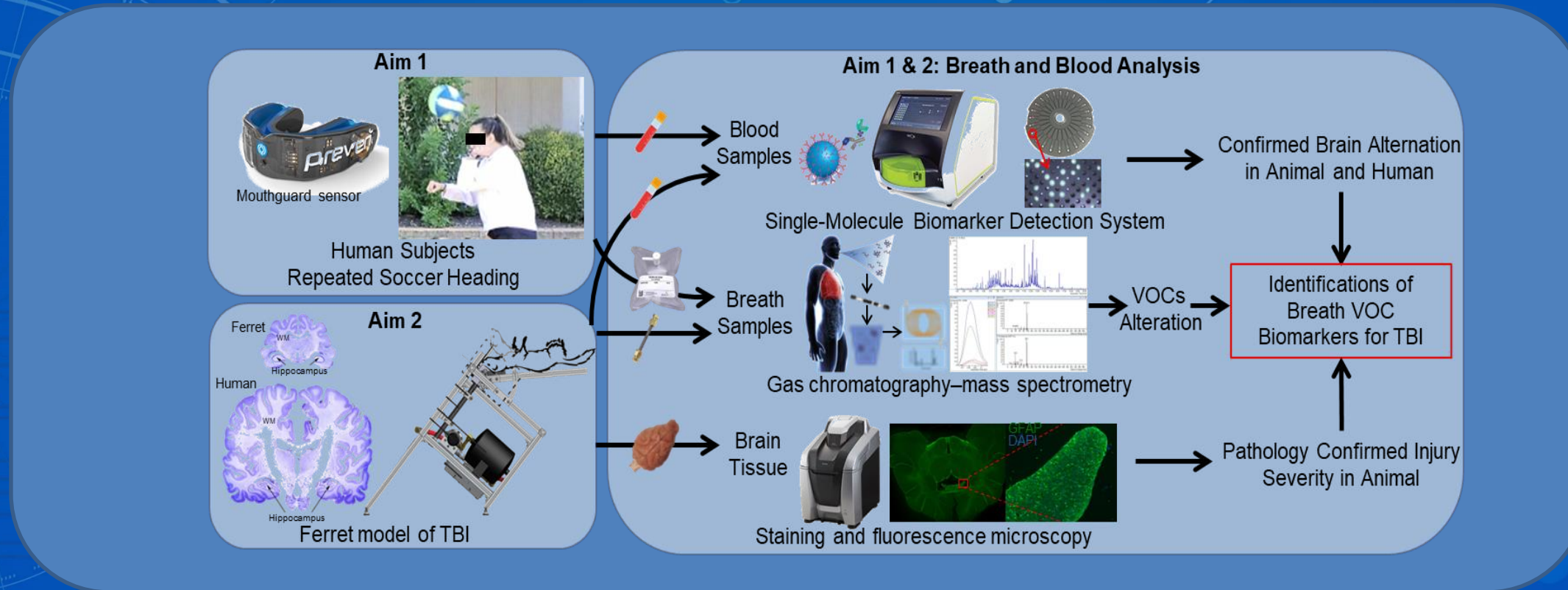
Independent and uncorrelated variables that help explain the observed variability



Trellis Charts

Breath Analysis Pilot Study (TBI)

Collaboration between SwRI (Dr. Libardoni) and UTSA (Dr. Memar)

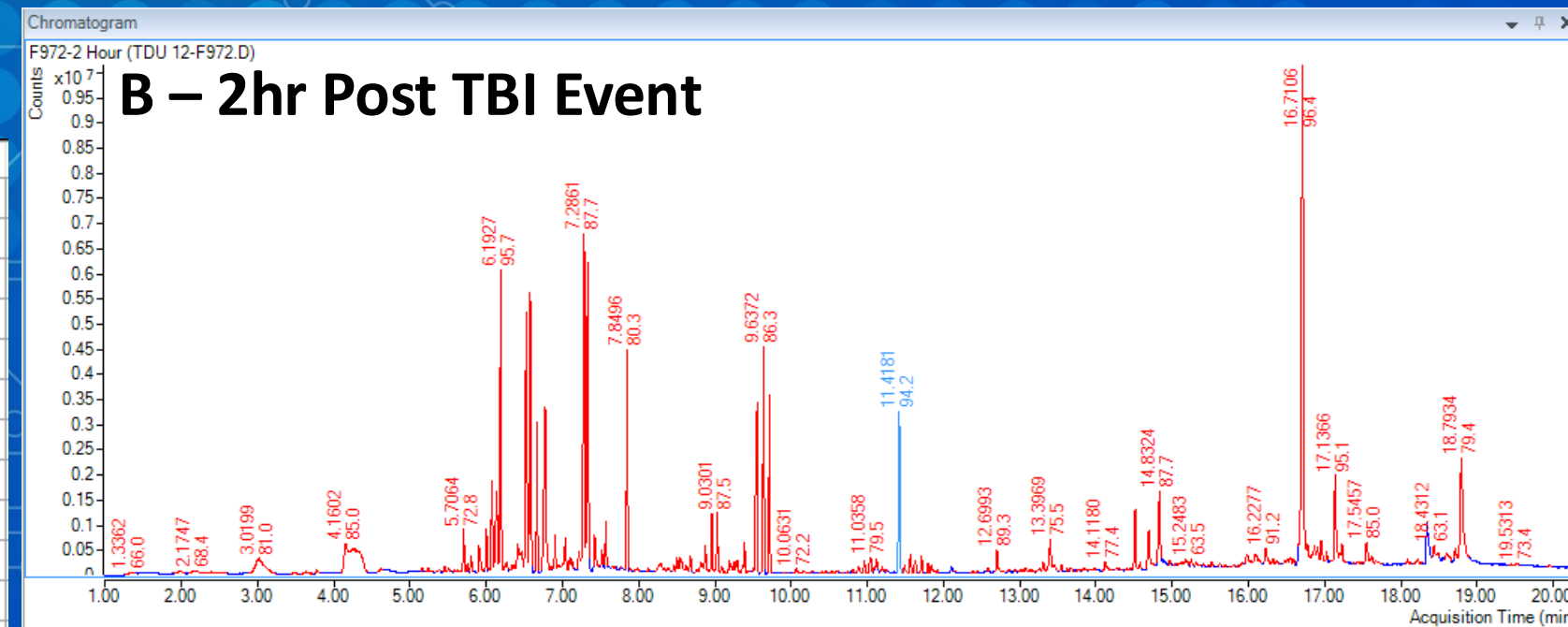
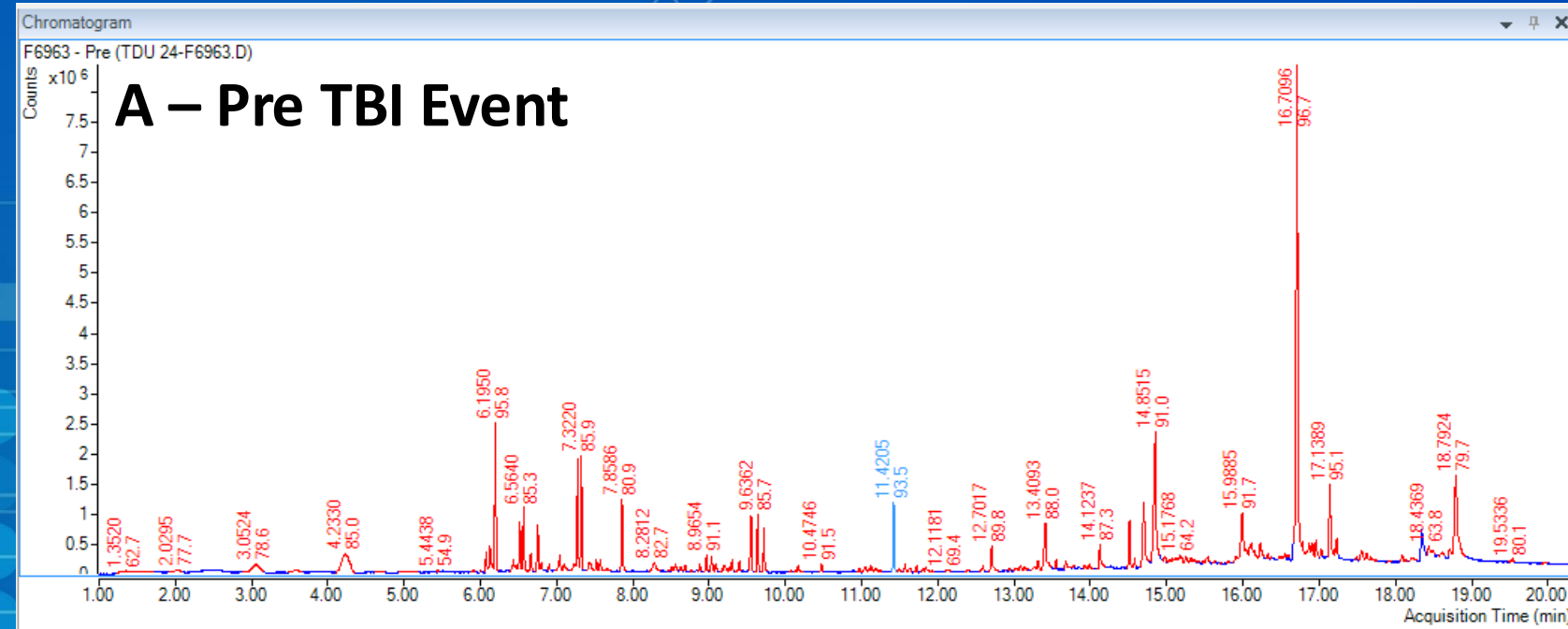


- **Aim 1-** Identify EB VOC alterations following sport-related RSC. Human subjects (18-25 age, 8 females and 8 males) go under controlled repeated (10 times) soccer headings, relevant to on-field ones, while wearing head kinematic sensors and blood and EB-VOC biomarkers will be measured prior to and at 1-hour (immediately), 1-day (acutely) following head exposures.
- **Aim 2-** Discover EB biomarkers due to mild-TBI. Ferrets (6-month-old, 16 males) will be assigned to TBI, sham, and control groups. TBI group goes under repeated closed head impacts and biomarkers in blood and VOCs in EB will be measured prior and at 1-hour, 1-day post-TBI. The EB VOC biomarkers will be correlated with blood biomarkers and neuropathology outcomes.

Pilot Study (TBI)

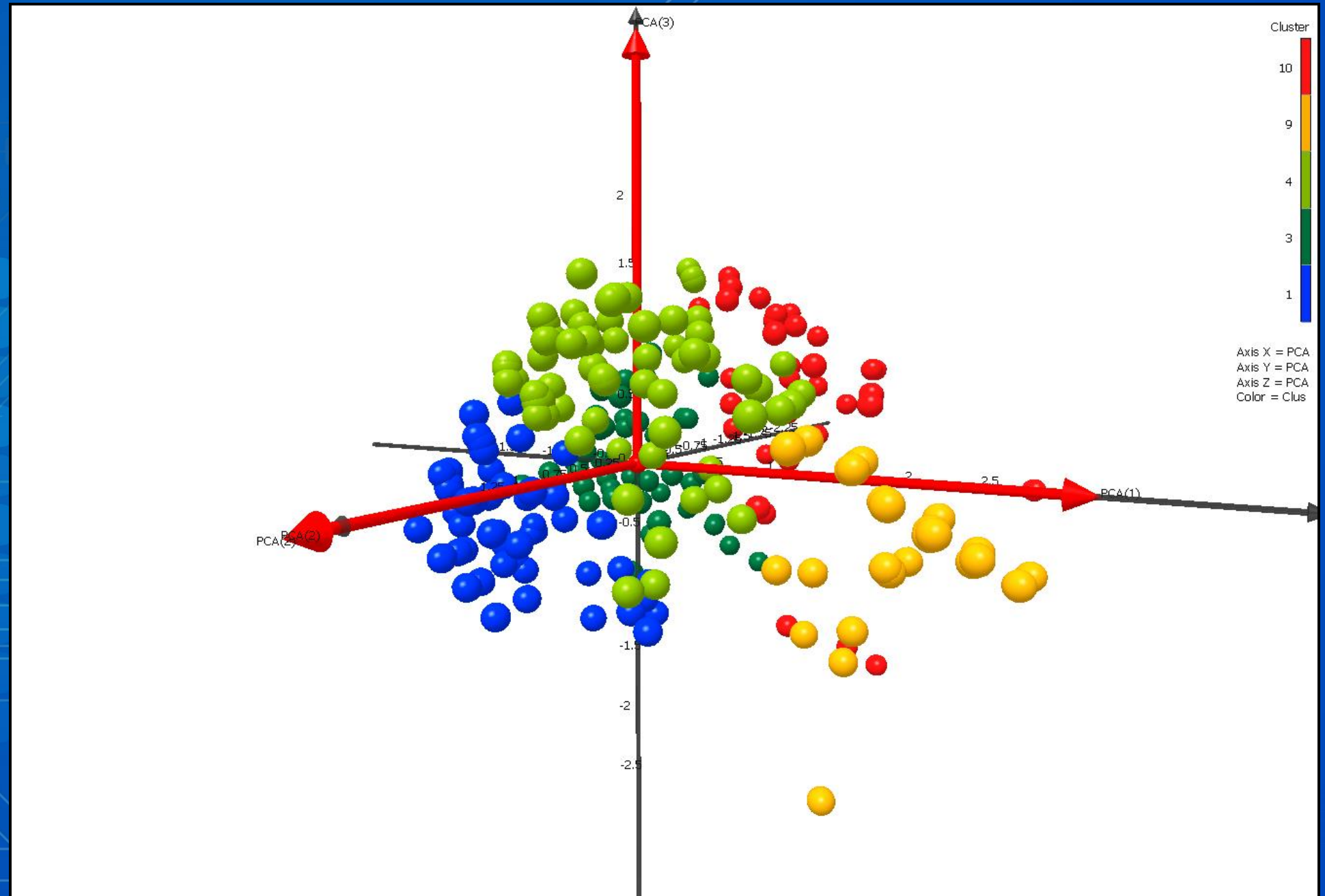
- Visual differences between pre and post samples
- Visual fold-change differences across time points
- High-degree of similarity between animal and human subjects
- High-degree of confidence in targeted biomarkers

Component RT	Compound Name	Match Factor	Formula	Component Area	Component Height	CAS#
8.6986	Carbonic acid, eicos...	85.9	C23H44O3	110347.2	89193.6	1000382...
8.7892	1,3-Dioxane, 4-(hex...	72.8	C35H70O3	22294.7	20385.1	56599-4...
8.8932	Dodecane, 4,6-dime...	86.3	C14H30	120463.9	108765.2	61141-7...
8.9804	Dodecane, 4,6-dime...	91.0	C14H30	259247.0	230036.5	61141-7...
9.0474	Hexadecyl octyl ether	84.4	C24H50O	260531.5	203497.7	1000406...
9.1011	Octadecane, 1-chloro-	67.1	C18H37Cl	51995.6	30393.6	3386-33-2
9.1883	Octadecane, 3-ethyl...	79.4	C26H54	24411.0	29005.4	55282-1...
9.2151	Oleic Acid	72.4	C18H34O2	172422.4	84979.4	112-80-1
9.2755	1-Hexadecanol, 2-m...	77.9	C17H36O	72139.5	50688.1	2490-48-4
9.3158	Dodecane, 4,6-dime...	86.4	C14H30	188030.8	150131.6	61141-7...
9.4063	1-Hexadecanol, 2-m...	81.4	C17H36O	215229.3	128604.1	2490-48-4



Statistical Analysis

- Post processing of the data allows for deeper correlation
- Machine learning systems significantly improve analytical results by eliminating false positives
- Identified compounds are then re-studied using MS-MS capabilities and imported into a user library for future identification

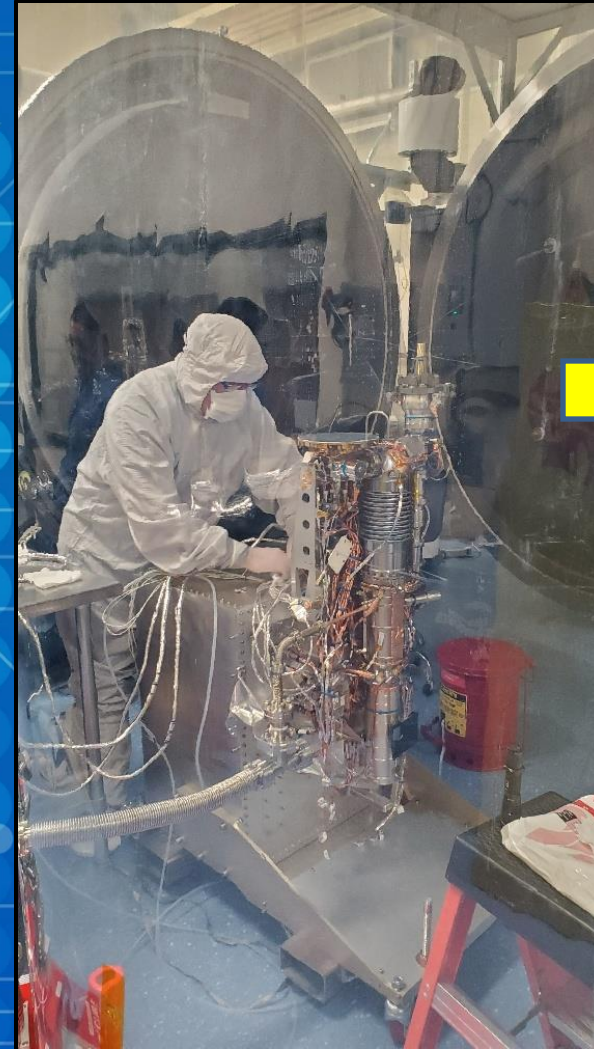


Mass Spectrometry and MEMS Technology

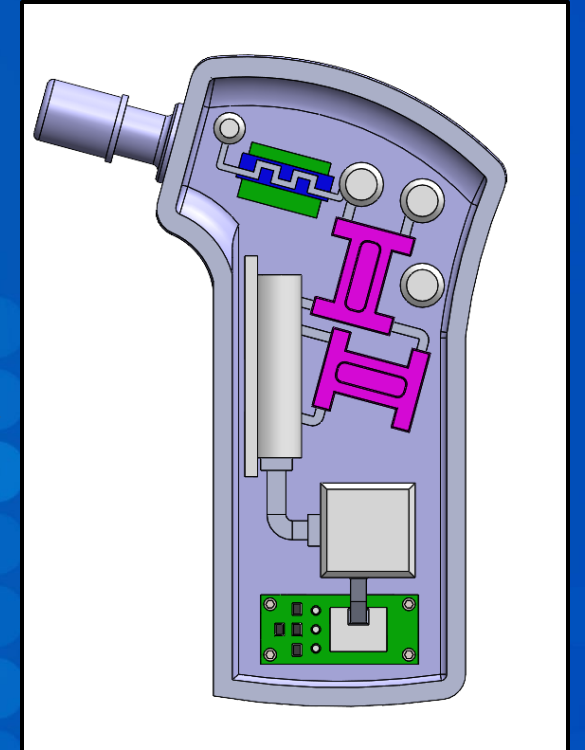
Heritage in Spaceflight Instrumentation has allowed key technology pass-through into the Health and Human Performance Discipline.



Commercial MS Instrumentation



Spaceflight MS Instrumentation



Handheld and Portable MS systems

Conclusions

- Breath contains hundreds to thousands of volatile organic compounds (VOCs) that represent individual metabolic states, health status, and exposure levels.
- Utilizing breath analysis for OEH exposure/assessment is non-invasive and provides real-time data.
- Continued improvement in instrumentation will lead to commercially available and field deployable devices.

Acknowledgments

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Separation Science and Mass Spectrometry Group