Breath Analysis and Biomarker Discovery–Leveraging Separation Space for Disease Interrogation

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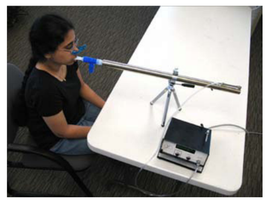
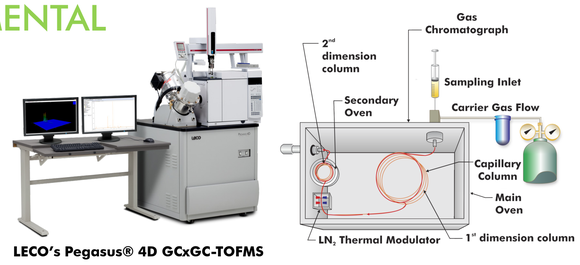
INTRODUCTION

Breath analysis has been performed for many decades using a wide range of techniques. Better understanding of breath capture, and advances in the techniques to do so, have resulted in more meaningful and controlled samples. Through these developments GCMS has been a stalwart technique and has received considerable attention. Many analytes have been detected in the "volatome" and correlated with disease states. The challenge has been in the broad range of analyte concentrations and in the limited ability of GC to provide adequate separation space for these compounds of similar volatility and high structural homology. The advent of two-dimensional GC interfaced to high speed time-of-flight mass spectrometry (GCxGC-TOFMS) has provided a new tool with which to address these challenges. By comparison, the number of analytes detected in a GCxGC-TOFMS analysis of a breath sample provides 3 to in excess of 10 fold more analytes of similar confidence. This advanced technique has been applied to several disease states, and in monitoring the difference between healthy and affected populations. Here, examples from selected studies are provided and include tuberculosis, radiation exposure, and cancers. Selected examples are shown and provide representative capabilities for the extreme information content provided by GCxGC-TOFMS in this type of study. The data provided show meaningful biological correlation with disease or treatment. In select instances the analyses are compared and contrasted between traditional one-dimensional GCMS analyses, and GCxGC-TOFMS.

EXPERIMENTAL



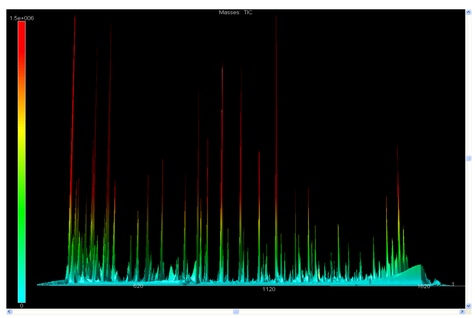
Samples were collected using proprietary Menssana technology (New Jersey, USA). Both deep volume exhalate and control ambient air were collected on thermal desorption tubes. The adsorbed analytes were released for analysis using a custom-interfaced Markes unit Representative conditions for collection and desorption can be found as published previously (see below). StatCompare (LECO or MetPP (Courtesy of Prof. Xiang.



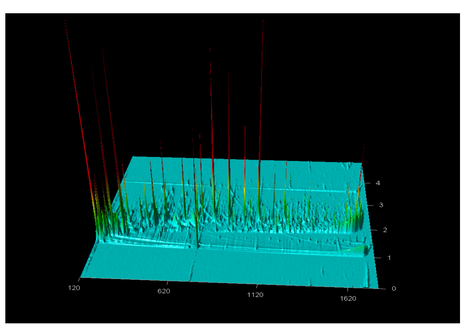
LECO's Pegasus® 4D GCxGC-TOFMS

The volatome, as released analytes from the tibes, was analyzed using a Pegasus 4D GCxGC Time-of-Flight mass spectrometer (LECO, Michigan, USA). The system was operated in two-dimensional mode with subtle differences in details among the different studies mentioned here. Data processing was achieved using ChromaTOF® software (version 4.5; LECO, Michigan, USA) with post-acquisition processing Zhang, University of Louisville, USA).

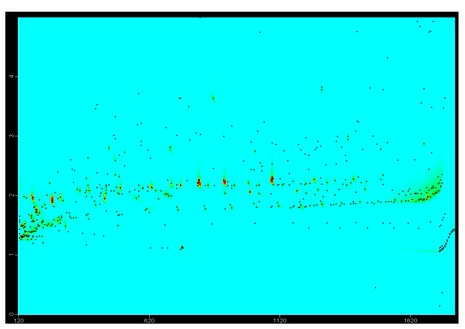
General methodology for GCxGC analysis of breath volatile analytes is provided in: Michael Phillips, Renee N. Cataneo, Anirudh Chaturvedi, Peter D. Kaplan, Mark Libardoni, Mayur Mundada, Urvish Patel, Xiang Zhang, PLOS ONE, Volume 8 (9), 1-8.



A one-dimensional depiction of a typical breath volatome analysis. The experiment was performed in two-dimensional mode. Even in this one-dimensional presentation, a rich body of analytes is apparent.



A two-dimensional depiction of the same typical breath volatome analysis. This view shows the full two-dimensions of separation. The first dimension (long column) time scale is left-to-right, and the second dimension (short column) is front-to-back. There are a significant number of analytes which align in the first dimension and would have gone undetected or misidentified in a 1D experiment.

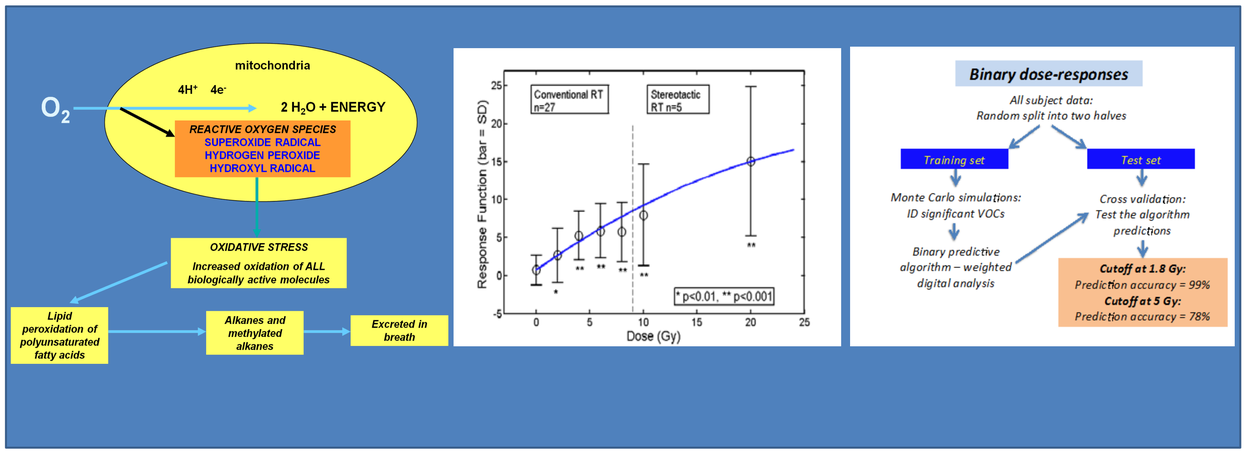


A different view of the 2D separation is shown here. In this planar view individual analytes are shown as black dots indicative of analytes found by ChromaTOF deconvolution. The increase in number of detected analytes in 1D versus 2D experiments varies but ranges from 2-10 fold increases in the 2D experiment increasing the data available in the differential analyses undergone in the following experiments.

RERESENTATIVE STUDY EXAMPLES

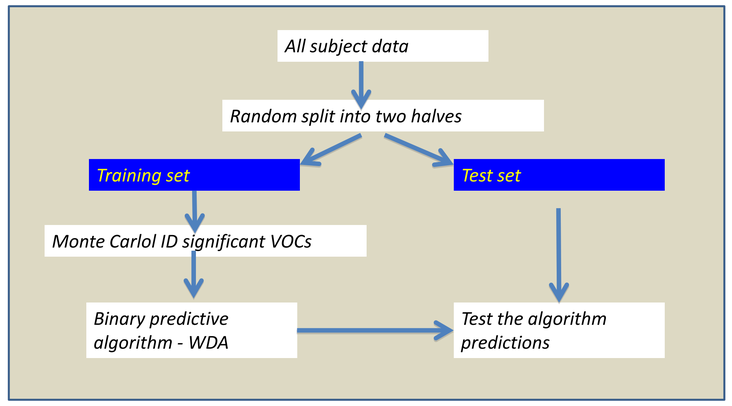
1

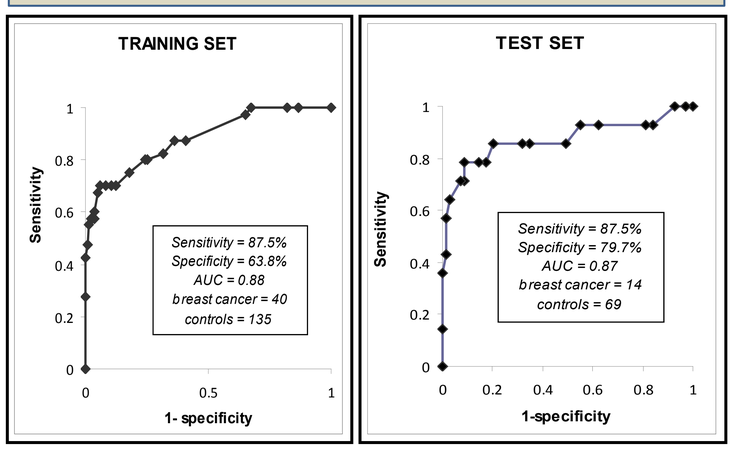
The first application of two-dimensional GC-TOFMS in exploring the use of breath as a source of biomarker identification and disease correlation is with radiation exposure. Breath was collected from human subjects after exposure to low doses of radiation. The hypothesis is that oxygen-radical chemistry will induce the breakdown of biologics (left). The smaller products could be volatile and emitted in the breath. The analysis provided more than 20 correlated metabolites. In the learning set (middle) using known exposures; the Test set then used the same set of compounds and successfully predicted exposure ranges as 1.8 and > 5 Gy. Biological variability at higher doses created issues.



2

The second example explores the use of two-dimensional GC-TOFMS in the differential analysis of breast cancer using the volatome as a source of potential biomarkers. Here 204 controls and 54 carcinoma patients provided breath (and control air) samples (*Phillips M., et. al., J Breath Res 2010;4:026003*). The data were split randomly into a training set and a test set. A comparison of the area under the curve (AUC) between the two sets indicates that it is a consistent model and that the AUC indicates a good predictive capability.





CONCLUSIONS

The results provided here indicate the following:

1) GCxGC offers the ability to resolve additional analytes compared to the one-dimensional experiment.
2) The additional analytes provide greater opportunity to identify "panels" of volatile metabolites which might be predictive of disease states.
3) GCxGC-TOFMS has been successfully applied to the differential analysis of breath in clinically-relevant studies, including radiation exposure and breast cancer.
4) There is more promise ahead for the use of GCxGC-TOFMS in the analysis of breath as a diagnostic and prognostic tool in medical and health research.