

Towards an Online Breath Cancer Diagnostic Tool.

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Objectives

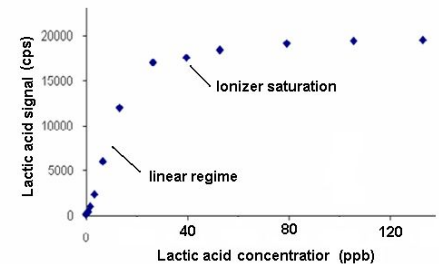
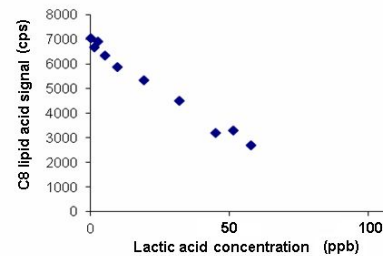
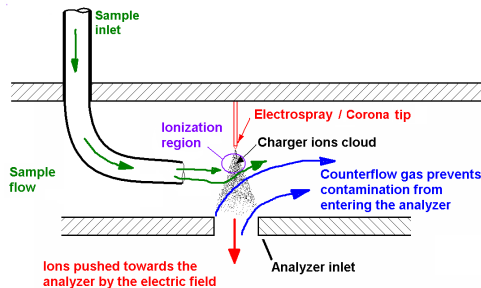
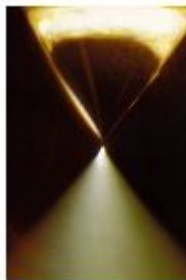
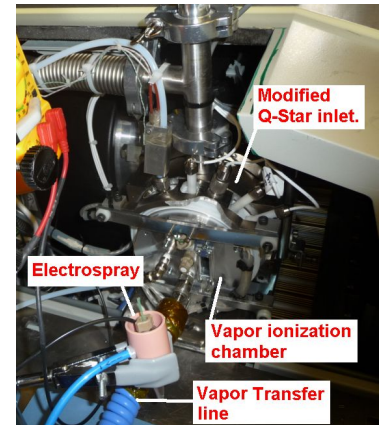
- Find reliable cancer biomarkers in breath.
 - Trained dogs show that there are biomarkers in breath.
- Develop a SESI-MS On-line breath analyzer.
 - Based on previous work by P.Martinez-Lozano and J. F. de la Mora [1,2].
 - Sensitivity below 1 ppt (measured with QStar and TNT vapors).
 - Capable of handling big populations.
 - Reducing contamination (real sensibility limiting factor).
 - Reducing required flow of breath.
- Study Healthy vs Cancer breath differences.
 - Once found, biomarkers could be tested with more specific analyzers.

[1] P. Martinez-Lozano and J. F. de la Mora, "Electrospray Ionization of volatyles in breath" Aug 2007.

[2] P. Martinez-Lozano and J. F. de la Mora, "Direct analysis of fatty acid vapors in brath by electrospray ionization and atmospheric pressure ionization-mass spectrometry" Nov. 2008

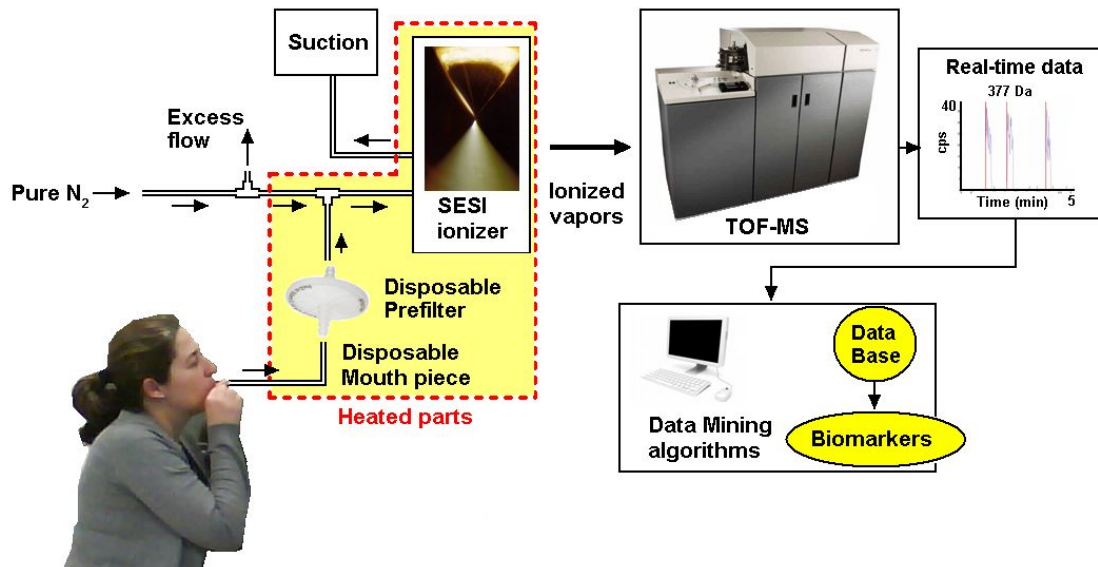
SESI Ionizer

- Noted by J. Fenn et al and used for breath ionization by P. Martinez-Lozano and J. F. de la Mora
- Electro spray cloud produces OH⁻ ions.
 - Soft ionization technique at atmospheric pressure. Ions transfer Charge to acidic molecules.
 - Ionizes polar molecules.
 - Ionization efficiency: 10⁻³ ion/molecule.
 - Sensibility measured with TNT vapors and Q-Star XL: 0.8ppt
- Improved fluid configuration reduces required flow rate (from 5 lpm to 1.5 lpm) and increases ionization efficiency.
- Humidity increases ionization efficiency
 - Contaminant signals rise with breath though not included in breath.
 - System contamination measurement requires introducing humid air.
- High ionization efficiency permits eliminating the preconcentration step and allows On-line analysis.



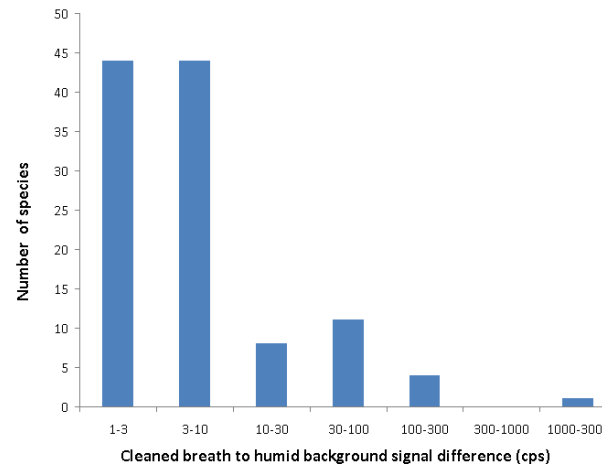
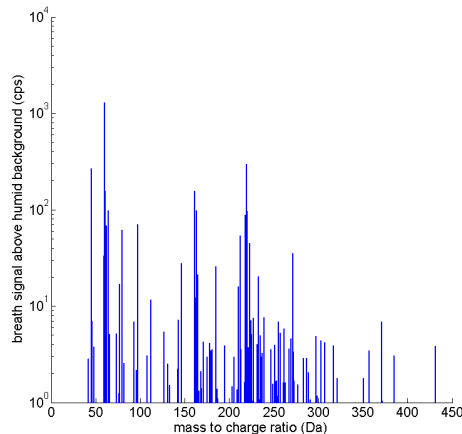
System architecture.

- Problems to be addressed:
 - Heavier species are typically more specific, but less volatile. VOCs are typically in the ppt level and below.
 - Breath is a complex process affected by many confounding variables (inhaled air, spirometric profile, state of humor, bacterial metabolism, food, ... and health)
- Requirements:
 - High sensitivity required to detect more specific VOCs.
 - On-line system helps scientists to understand dynamic processes.
 - High selectivity required to distinguish biomarkers from confounding species.
 - Easy and safe for patients.



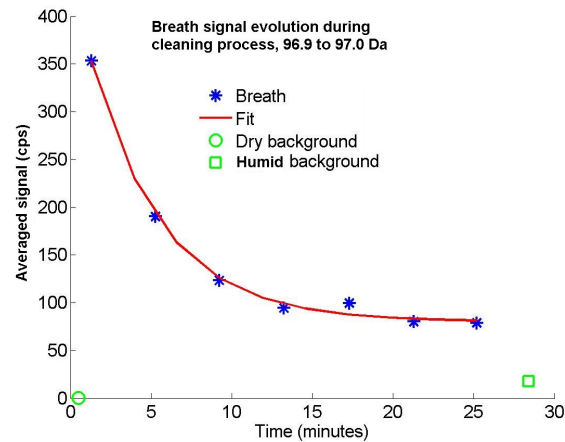
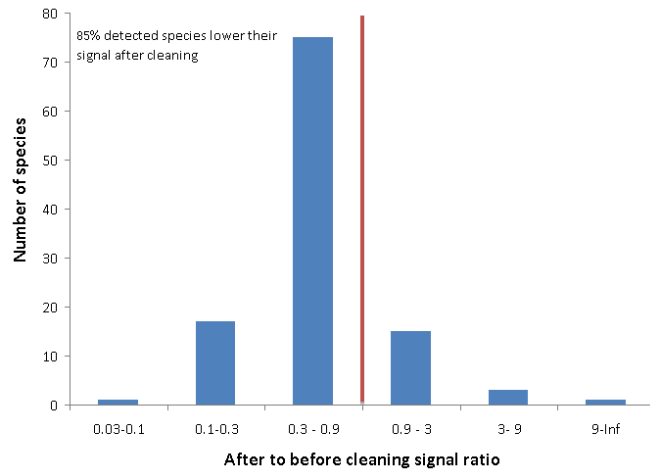
First tests. Detected species.

- How to differentiate real breath signals from contamination signals?
 - To eliminate room air contamination:
 - Subject breaths purified medical grade air.
 - To eliminate equipment and medical grade air contaminants:
 - Background measured by introducing humidified medical grade air at the same flow rate as breath.
 - Signals are accounted for after 'cleaning process' if:
 - Breath signal rises more than 3 times humid background variance above humid background and
 - Breath signal rises more than 1 cps above humid background.
- Heaviest detected specie: 431 Da
- Around 110 detected species coming from breath.



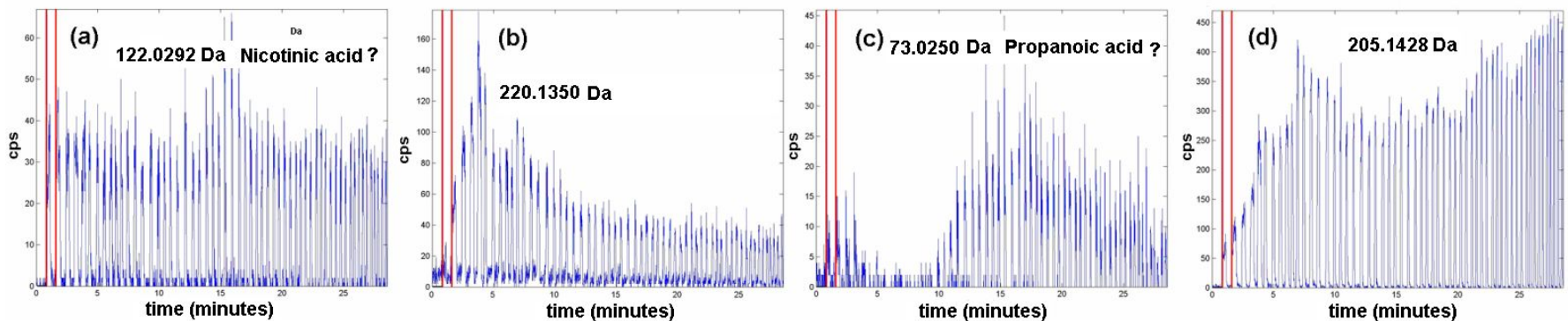
First tests. Room air effect and cleaning.

- Room air is a known confounding variable in breath analysis.
- Subject starts breathing medical grade pure air and breath is analyzed every 5 minutes.
- Most species reach an stationary state after 10 minutes.
- Around 85% of detected species are also present in room air.



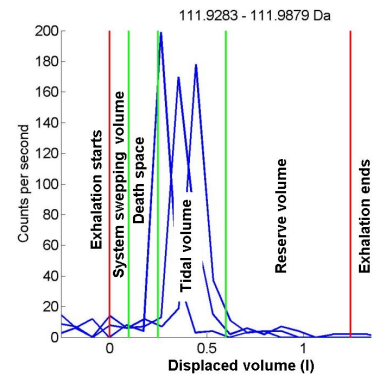
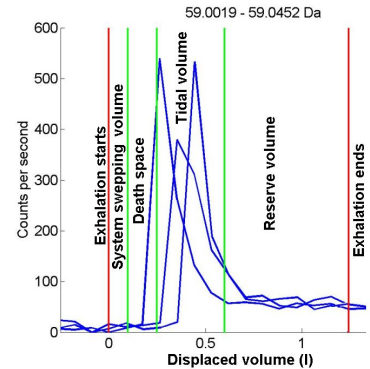
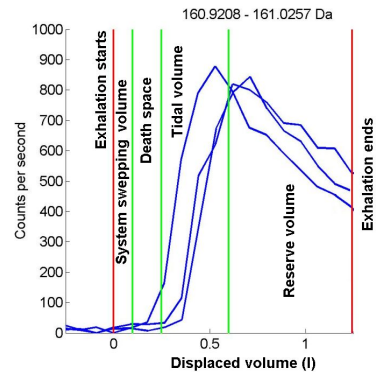
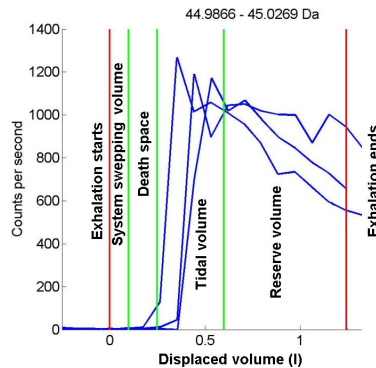
First tests. Mouth bacterial metabolism

- Subject rinses mouth with water and glucose and breathes normally exhaling through the analyzer.
- Results show the behavior of different substances:
 - Constantly produced substances (a), immediate short response (b), delayed response (c) and Immediate and long lasting response (d).
- This experiment illustrates the complexity of the dynamics of breath.



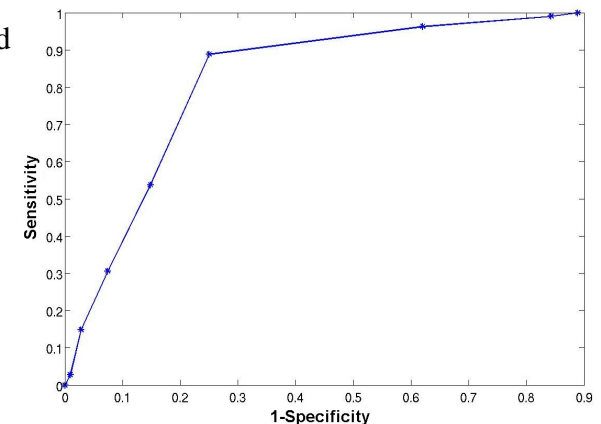
First tests. Spirometric profiles.

- A healthy subject inspires normally and exhales through the analyzer at constant flow rate until depletion.
- Spirometric profile:
 - shows different patterns for different species belonging to different parts of the lungs.
 - could be used as separation technique in breath analysis to improve species separability.
 - Permits differentiating mouth originated VOCs (associated to bacterial metabolism) from deeper lungs VOCs.



First Colon Cancer Diagnosis Campaign.

- Population:
 - Colon Cancer Diagnosed Patients (Colonoscopy diagnosed) CCDP - 36 people.
 - Healthy Diagnosed Patients (from Early Detection Preventive Program, Colonoscopy diagnosed) HDP 36
 - No breath cleaning procedure available for this campaign.
- Variable pre-selection:
 - Averaged signal during exhalation.
 - Only signals rising above dry background when blowing.
 - Only signals found in at least 25% of HDP or CCDP
 - Signals having coherent time evolution during campaign eliminated.
 - Circadian cycles? Long term system contamination or cleaning process? environmental changes?
- Algorithm
 - Training Set: CCDP and HDP
 - Empirical optimization or ROC curves and signal thresholds and Forward characteristics.
 - Sensitivity: 88.9%, Specificity: 75%
- Conclusions:
 - Results are promising.
 - But couldn't find obvious differences.
 - Uncontrolled confounding variables are blurring the results.
 - Inhaled air is very variable during campaign.
 - Long term transient effects produce time varying signals that blind the algorithms if CCDP and HDP are appointed at separated periods.



Conclusions

- High sensitivity SESI based analysis allows On-line analysis without a pre-concentration step.
- Sampling system precludes room air from contaminating the analyzer.
- The system permits spirometric analysis of exhaled breath.
- Spirometric analysis allows for separation of species.
- Inhaled air effect characterized in laboratory environment.
 - It can be one of the main components of exhaled breath.
 - Most detected species are cleaned after 10 minutes breathing pure medical grade air.
- Bacterial mouth metabolism could be a potential confounding variable if subjects didn't fasten before test.
- A test on Cancer and Healthy subjects:
 - Shows promising results.
 - But also the need for cleaning confounding variables (Specially inhaled air.)
- Inhaled air is one of the most important confounding variables found in our research.
 - Cleaning the breath seems to be a necessary step to find biomarkers.

Future work.

- Prototype 2:
 - Ionizer and sampler temperature increased to prevent deposition and long term contamination.
 - Positive and negative mode analysis using two Electro-Sprays in parallel.
- Campaign 2:
 - Breath cleaning procedure implemented during next campaign.
- Spirometric analysis to be implemented in postprocessing algorithms.



Thanks for your attention

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