Real-time connection of Mass Spectrometry with Medicine and Surgery

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1913 Mass spectrometry
Stable isotopes
Metabolism
NIH
Enzymology
Proteomics
Transcriptomics
Genomic sequencing
Gene cloning

Fundamental physics
Nuclear weapons

Clinical Chemistry, Medicine & Surgery
Metabolomics Lipidomics

GBS 724
March 24, 2017
Dissociative research

• Samples are collected and stored for analysis at a “later” time
• “Later” can be months or years after sample collection
  – Of little direct benefit to the patient
  – Although may influence the community of patients
  – True of many analyses

Real time analysis

• Existing, familiar applications
• Gases!
• The iknife
  – GI surgery
  – Cancer margins
  – Pathology
• DESI
• CARS
Real-time analysis

- We see the real-time use of MS when we go through security checks at the airport
  - Checks for ion signatures of explosives

- Other devices are used to check for specific volatiles in the breath

Noses and smell – real time analysis

The superior volatile metabolite detector
Gases produced in the GI tract

- H₂, CO₂ and CH₄ from carbohydrates
  - *Firmicutes*
  - From pyruvate and NAD(P)H/FADH₂
  - H₂ used by sulfate-reducing bacteria (SRBs), methanogenic Archaea, and acetogens
- SRBs produce H₂S
- NO from nitrates

Methods for measuring gases

<table>
<thead>
<tr>
<th>Technology</th>
<th>Operation mode</th>
<th>Target intestinal gas</th>
<th>Detection limit</th>
<th>Cross-sensitivity</th>
<th>Response time</th>
<th>Life time</th>
<th>Estimated cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spectrometry based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>GC-MS</td>
<td>Off line</td>
<td>All gases</td>
<td>ppt to ppb</td>
<td>Low</td>
<td>~Several minutes</td>
<td>Long</td>
<td>~US$300k</td>
</tr>
<tr>
<td>IMS</td>
<td>Real time</td>
<td>All gases</td>
<td>ppb</td>
<td>Low</td>
<td>&lt;1 min</td>
<td>Long</td>
<td>~US$100k</td>
</tr>
<tr>
<td>PTR-MS</td>
<td>Real time</td>
<td>All gases</td>
<td>ppt</td>
<td>Low</td>
<td>&lt;1 min</td>
<td>Long</td>
<td>~US$400k</td>
</tr>
<tr>
<td>SIFT-MS</td>
<td>Real time</td>
<td>All gases</td>
<td>ppb</td>
<td>Low</td>
<td>&lt;1 min</td>
<td>Long</td>
<td>~US$400k</td>
</tr>
<tr>
<td>LS</td>
<td>Real time</td>
<td>Most gases except H₂</td>
<td>ppt to ppb</td>
<td>Low</td>
<td>&lt;1 min</td>
<td>Long</td>
<td>~US$50k</td>
</tr>
<tr>
<td><strong>Sensor based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Real time</td>
<td>H₂, H₂S, NO, and CO₂</td>
<td>ppm</td>
<td>Medium</td>
<td>~30 s</td>
<td>Short</td>
<td>~US$100k</td>
</tr>
<tr>
<td>Calorimetric</td>
<td>Real time</td>
<td>H₂, CH₄, and CO₂</td>
<td>ppt</td>
<td>High</td>
<td>&lt;10 s</td>
<td>Medium</td>
<td>~US$100k</td>
</tr>
<tr>
<td>NDIR</td>
<td>Real time</td>
<td>CO₂, CH₄, and VDO₅</td>
<td>ppm to ppt</td>
<td>Low</td>
<td>&lt;20 s</td>
<td>Long</td>
<td>~US$300k</td>
</tr>
</tbody>
</table>

GC-MS           | gas chromatography-mass spectrometry
IMS             | ion mobility mass spectrometry
PTR-MS          | proton transfer reaction mass spectrometry
SIFT-MS         | selection ion flow tube-mass spectrometry
LS              | laser spectrometry

*Jian Zhen Ou et al., Trends Biotech, 2015*
Device for measuring fecal gas production

Jian Zhen Ou et al., Trends Biotech, 2015

Fecal gas production (ex vivo)

Jian Zhen Ou et al., Trends Biotech, 2015
Real-time *in situ* monitoring gas production

- The device is swallowed
- Completes full mouth-to-anus transit, reporting data as it goes
- Also provides positional information
- Operates at 405, 433, and 915 MHz
- Uses Lithium batteries!!

Jian Zhen Ou et al., Trends Biotech, 2015

Real time intestinal gas production

Jian Zhen Ou et al., Trends Biotech, 2015
The Challenge for Mass Spec

How to get the mammoth into the gas phase for analysis?

Droplet principle of electro spray

Droplet spray
- Sneeze
- Lung motion
- Surgical knife
- Other vapors

MS inlet
iKnife device

Solvent
25 μl/min
Infusion pump

Venturi pump
Veriflo VAC 100

N₂ gas

Teflon transfer tubing

Mass spectrometer

RadioSurg 220

Link to videos by James Kinross

Colorectal surgeon from Imperial College, London
Plenary Speaker at the UAB 2016 Metabolomics Workshop

http://www.uab.edu/proteomics/metabolomics/workshop/2016/videos/kinross_day2.html

http://www.uab.edu/proteomics/metabolomics/workshop/2016/videos/kinross2_day2.html
Mass spectrum of canine stomach
Predominantly phospholipids

Balog et al
Anal Chem
82:7343, 2010

Phospholipid patterns are characteristic of cells and tissues

- Single items are not sufficient as biomarkers
- The classes of phospholipids and their fatty acid composition contain pattern discriminators
- In the absence of known classifiers, principal components analysis looks for groups of components that have the larger sources of variation
  - An individual sample’s contributions to these groups are plotted in a 2D or 3D manner
Principal components analysis of ions from surgical “smoke”

Balog et al.
Anal Chem
82:7343, 2010

Differentiation of brain tumors
Changing lipids across cancer margin

Computer-driven, Rapid Evaporative Imaging MS (REIMS) for tissue sections
Examining tissue (slices) by REIMS

Modes of data acquisition for REIMS

Golf et al., Anal Chem 2015
Optimizing data acquisition for REIMS

PCA analysis of REIMS data from tissue sections
Desorption electrospray ionization (DESI)

- Works by directing an electrical fine spray at a tissue target – does not require deposition of a matrix

The IDH story of brain and other tumors

- IDH1 (isocitrate dehydrogenase) is mutated in position 132 in a GWAS study of patients with glioblastomas
- IDH1 catalyzes the conversion of isocitrate to alpha-ketoglutarate (αKG) which is a two-step reaction
- Mutant IDH1 catalyzes the first step – to 2-hydroxyglutarate (2HG), but not the second one to αKG
- 2HG is considered to be an onco-metabolite
- What follows is a study from a group at Harvard – performed in the Advanced Multimodality Image Guided Operating Suite at Brigham and Women’s Hospital
Whither 2-hydroxyglutarate?

- Tissue expressing wild type IDH1
- MSMS of m/z 147 - not 2HG
- Tissue expressing mutant IDH1
- MSMS of m/z 147 is 2HG

Santagata et al. PNAS 2014

Value of exact mass – “147” vs “147”

Monoisotopic mass of 2HG

\[ \text{Monoisotopic mass of 2HG} = 148.037 \]

\[ \text{[M-H]- ion} = 148.037 - 1.00725 \]

\[ = 147.030 \]

This is not 2HG

Santagata et al. PNAS 2014
Tumor xenograft imaging and 2HG

The ion at m/z 146.9 was subjected to MSMS to measure 2HG

Application to human glioblastoma

Santagata et al. PNAS 2014
Comparative imaging of normal-tumor tissue transition

Golf et al., Anal Chem 2015

Banerjee et al. 2017
Red = elevated in cancer
Blue = down in cancer
Distribution of glucose/citrate ratio of some representative prostate tissue specimens showing significant elevation of the glucose/citrate ratio in cancer. The Top of each panel (A–O) shows the histopathological evaluation (H&E) of the corresponding tissue, where cancer areas have been demarcated by red, benign areas by black, stroma areas by green, and inflammation areas by blue.
Use of Raman spectroscopy
Real-time imaging of metabolites in skin

- [http://bernstein.harvard.edu/research/cars-why.htm](http://bernstein.harvard.edu/research/cars-why.htm)

Sunny Xie, PhD - Harvard

The future of medicine and surgery

[http://www1.imperial.ac.uk/phenomcentre/](http://www1.imperial.ac.uk/phenomcentre/)
Publications


