THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

GBS 724
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Real-time connection of Mass Spectrometry with Medicine and Surgery

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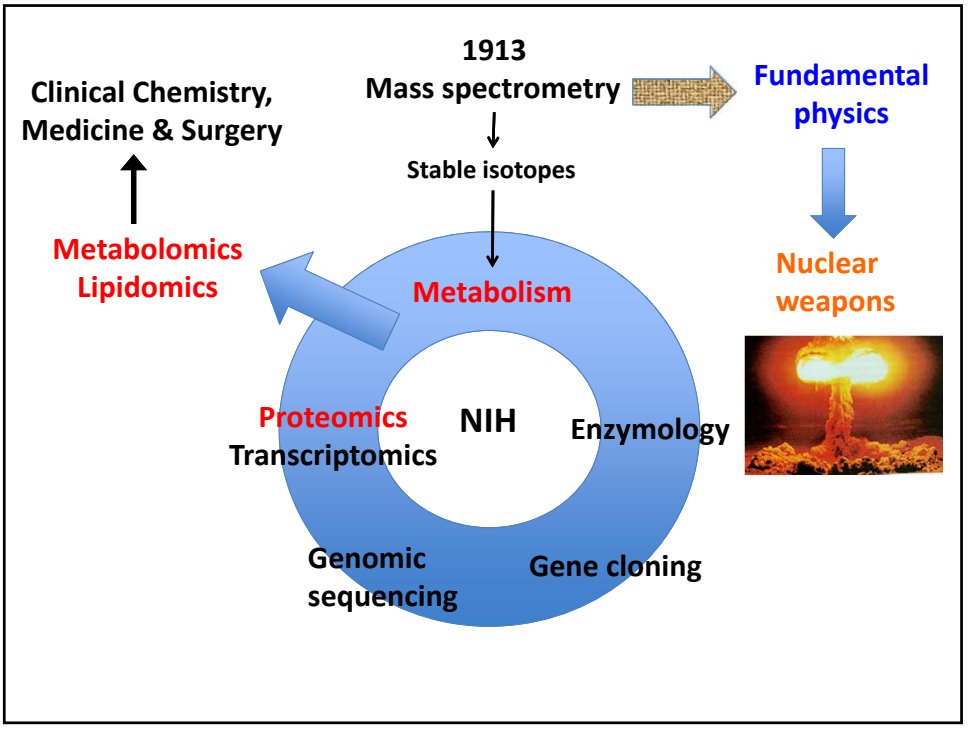
Targeted Metabolomics & Proteomics Laboratory



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

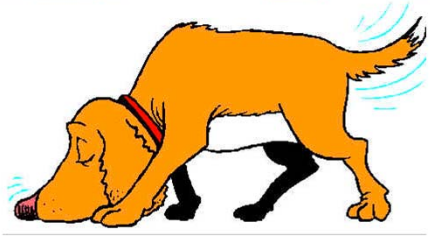
- Existing, familiar applications
- Gases!
- The iknife
  - GI surgery
  - Cancer margins
  - Pathology
  - Bacterial masses
- DESI
- CARS

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 metabolite detector

Gases produced in the GI tract

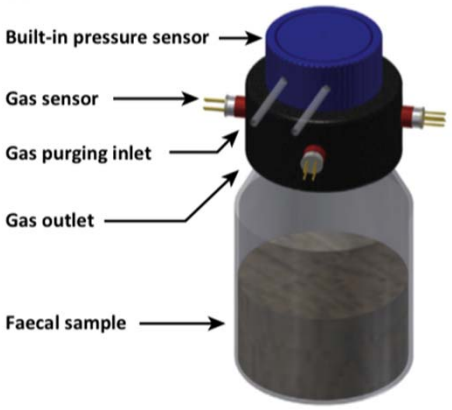
- H_2, CO_2 and CH_4 from carbohydrates
  - Firmicutes
  - From pyruvate and NAD(P)H/FADH2
  - H_2 used by sulfate-reducing bacteria (SRBs), methanogenic Archaea, and acetogens
- SRBs produce H_2S
- NO from nitrates

Methods for measuring gases

Technology	Operation mode	Target intestinal gas	Detection limit	Cross-sensitivity	Response time	Life time	Estimated cost
Spectrometry based[a]							
GC-MS	Off line	All gases	ppt to ppb	Low	~Several minutes	Long	>US$300k
IMS	Real time	All gases	ppb	Low	<1 min	Long	>US$100k
PTR-MS	Real time	All gases	ppt	Low	<1 min	Long	>US$400k
SIFT-MS	Real time	All gases	ppb	Low	<1 min	Long	>US$400k
LS	Real time	Most gases except H_2	ppt to ppb	Low	<1 min	Long	<US$50k
Sensor based[b]							
Electrochemical	Real time	H_2, H_2S, NO, and CO_2	ppm	Medium	<30 s	Short	<US$100
Calorimetric	Real time	H_2, CH_4, and CO_2	ppt	High	<10 s	Medium	<US$100
NDIR	Real time	CO_2, CH_4, and VOCs	ppm to ppt	Low	<20 s	Long	<US$300

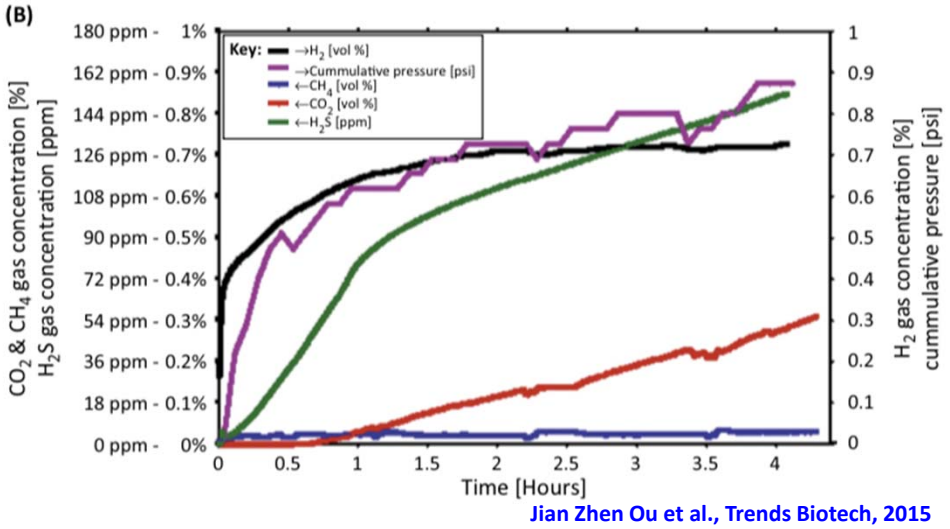
Jian Zhen Ou et al., Trends Biotech, 2015

Device for measuring fecal gas production



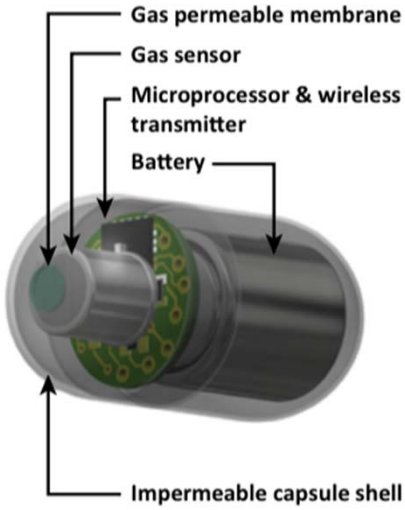
Jian Zhen Ou et al., Trends Biotech, 2015

Fecal gas production (ex vivo)



Jian Zhen Ou et al., Trends Biotech, 2015

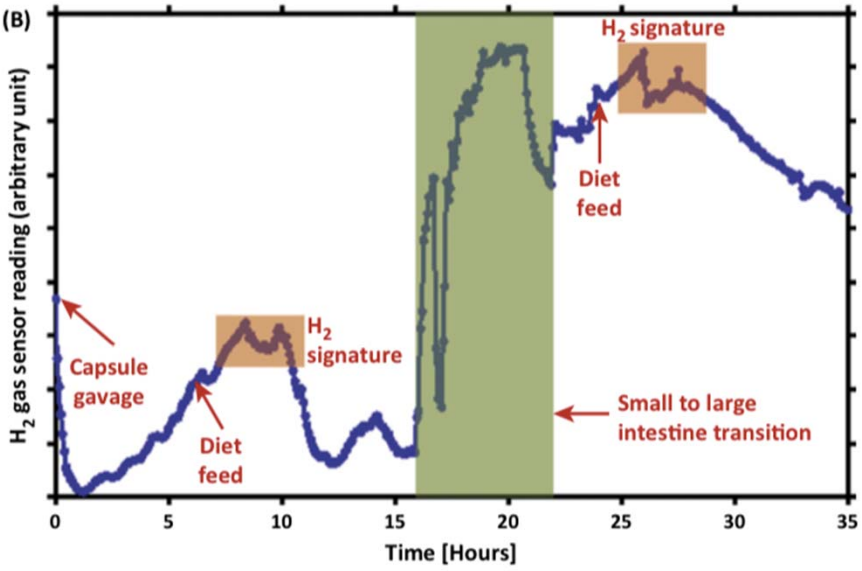
Real-time *in situ* monitoring gas production



- 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

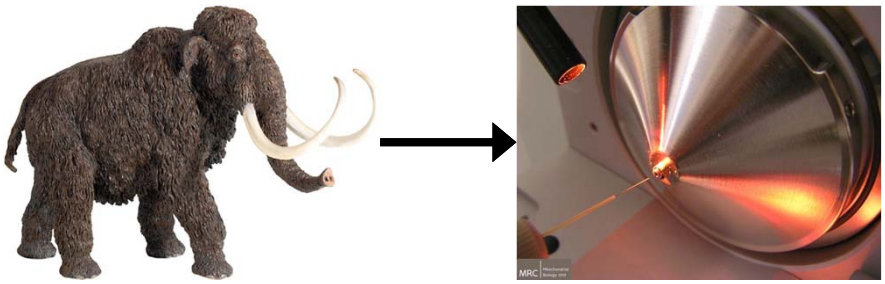
Jian Zhen Ou et al., Trends Biotech, 2015

Real time intestinal gas production



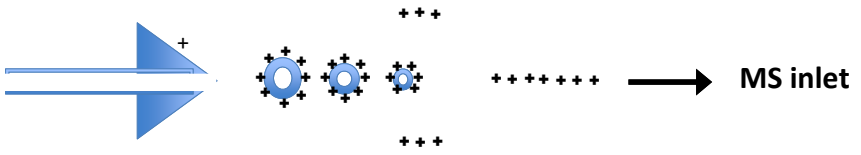
Jian Zhen Ou et al., Trends Biotech, 2015

The Challenge for Mass Spec



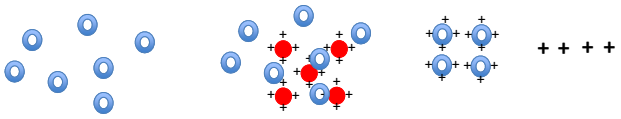
How to get the mammoth into the gas phase for analysis?

Droplet principle of electrospray

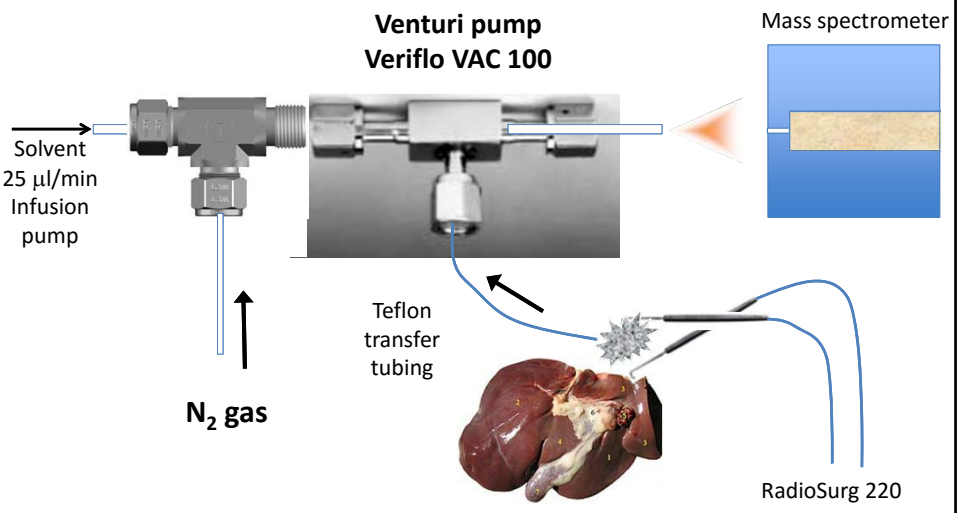


Droplet spray

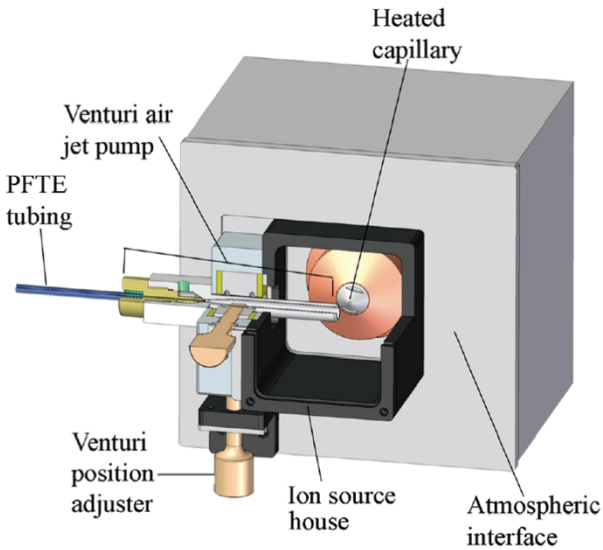
- **Sneeze**
- **Lung motion**
- **Surgical knife**
- **Other vapors**



iKnife device



Need to rebuild the interface



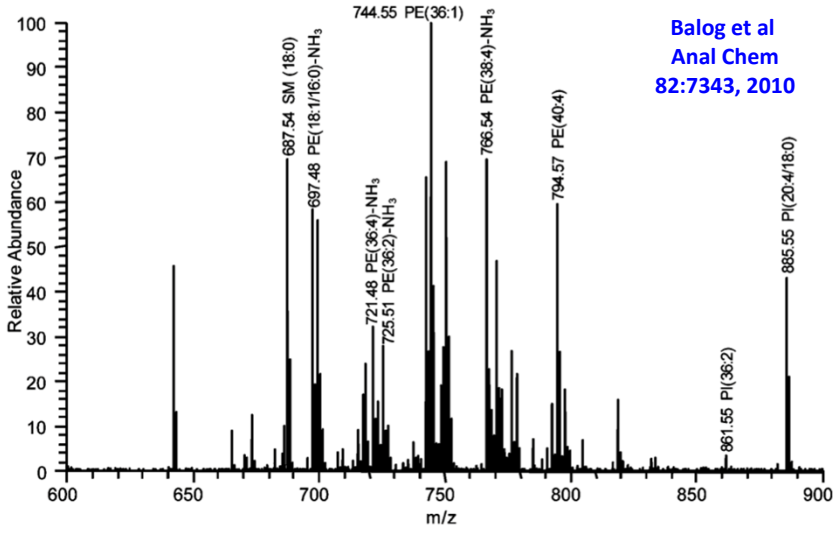
Balog et al
Anal Chem
82:7343, 2010

Link to video

http://www.smh.com.au/technology/sci-tech/doctors-praise-new-surgical-knife-that-diagnoses-as-it-cuts-20130718-2q72c.html

Mass spectrum of canine stomach

Predominantly phospholipids



Balog et al
Anal Chem
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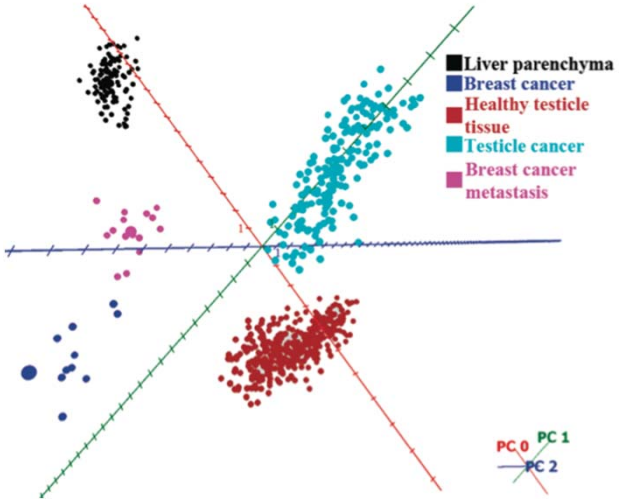
Fatty acid profiles of bacteria and other microorganisms

- Gas chromatography of fatty acid methyl esters distinguishes organisms
  - Used in Pathology Labs
- In 1990-93 my lab supported early engineering work on the International Space Station (subcontract from Boeing Corp.)
  - To determine the microorganisms that grow in a water treatment unit in microgravity

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
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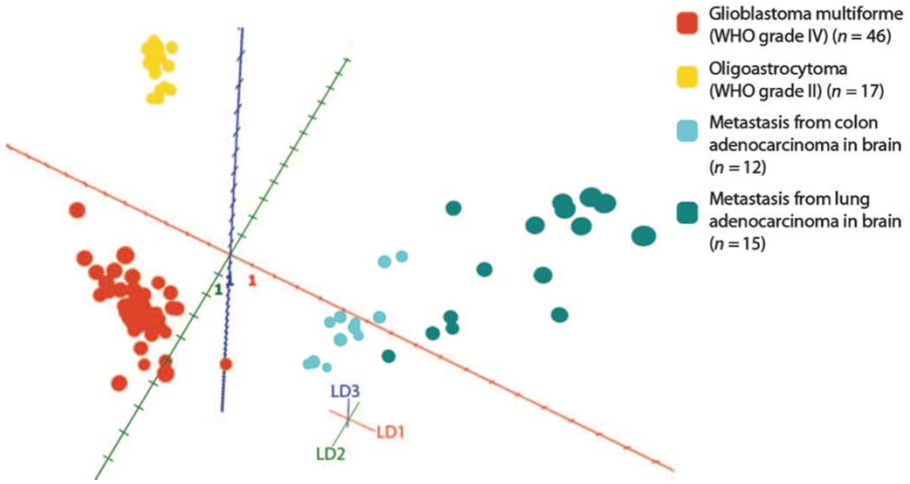
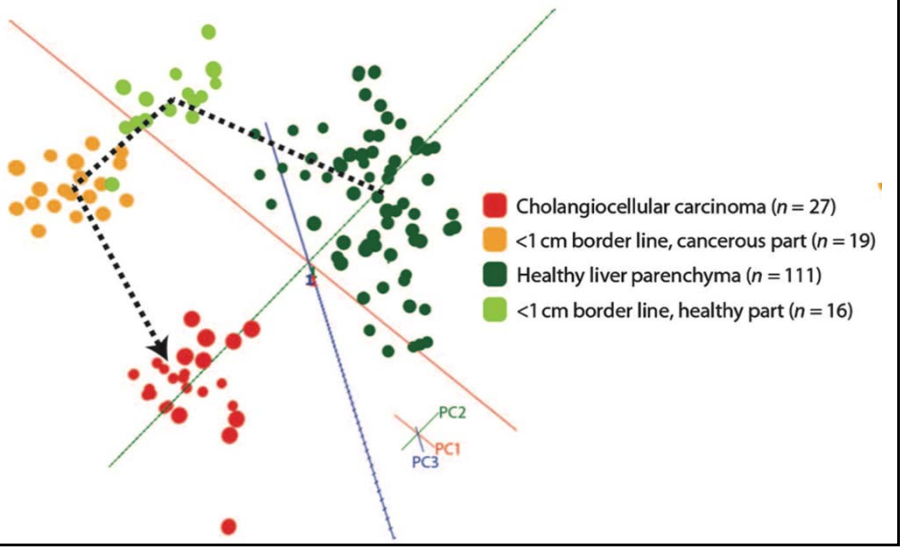
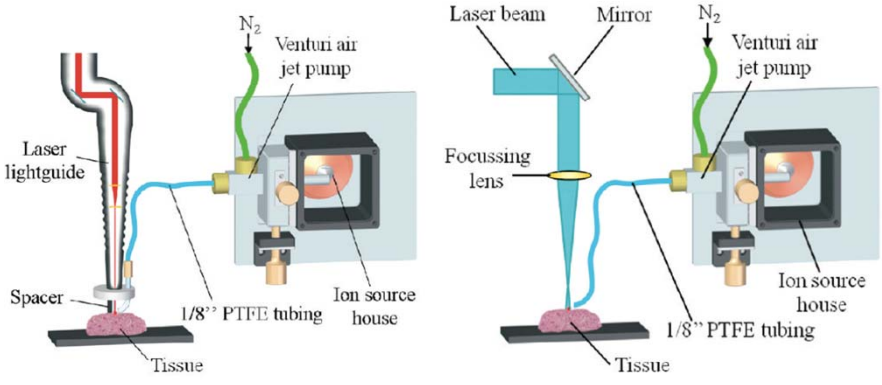
Differentiation of brain tumors



Changing lipids across cancer margin

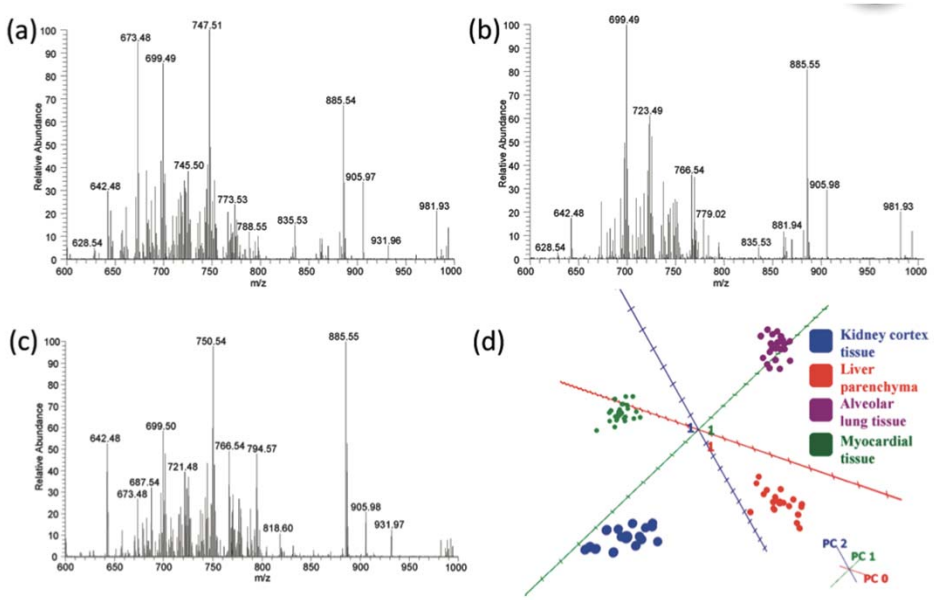


Laser-driven direct mass spectrometry



Schafer et al., Anal Chem 59:1632, 2011

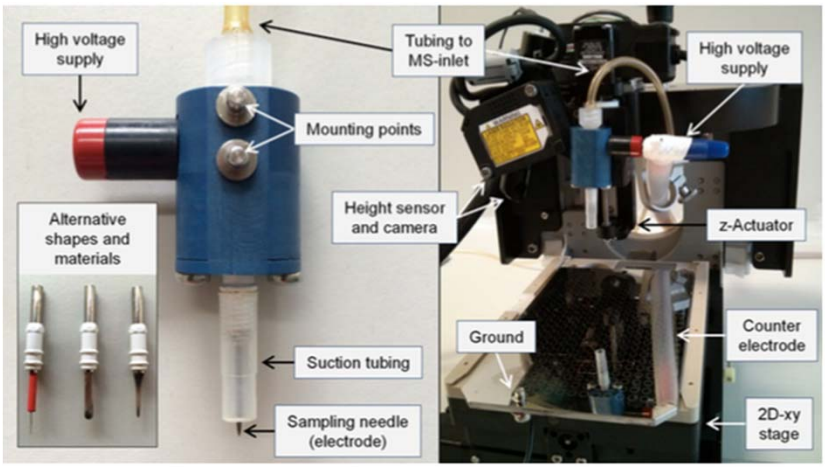
Differentiation of tissues



Schafer et al., Anal Chem 59:1632, 2011

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

Examining tissue (slices) by REIMS



Golf et al., Anal Chem 2015

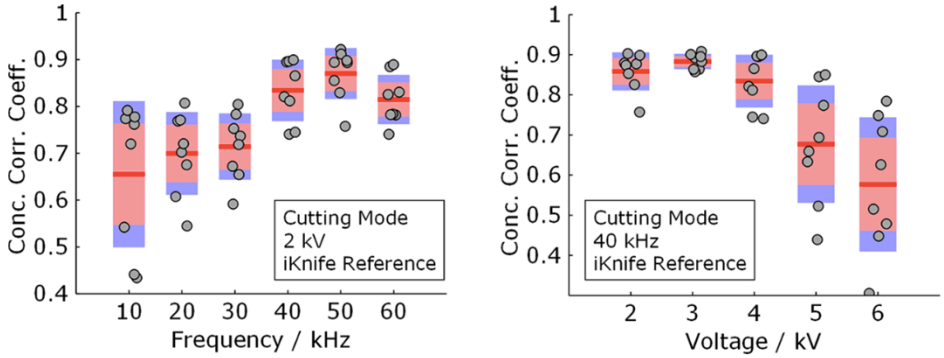
Modes of data acquisition for REIMS

Line Scans:
Cutting Mode

Individual Pixels:
Pointing Mode

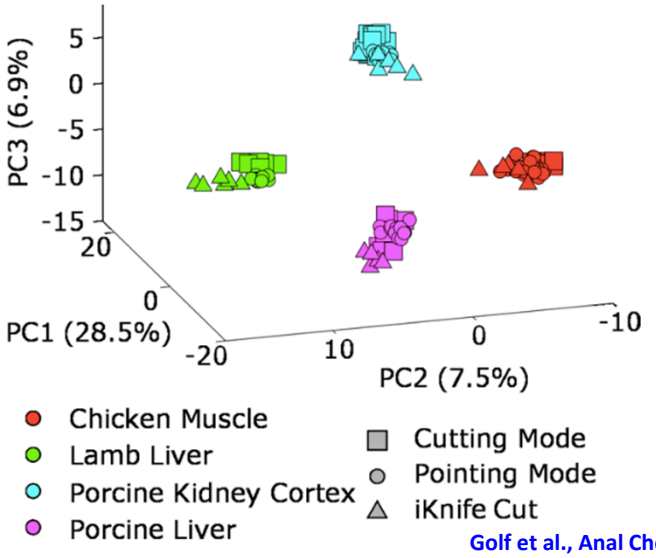
Golf et al., Anal Chem 2015

Optimizing data acquisition for REIMS



Golf et al., Anal Chem 2015

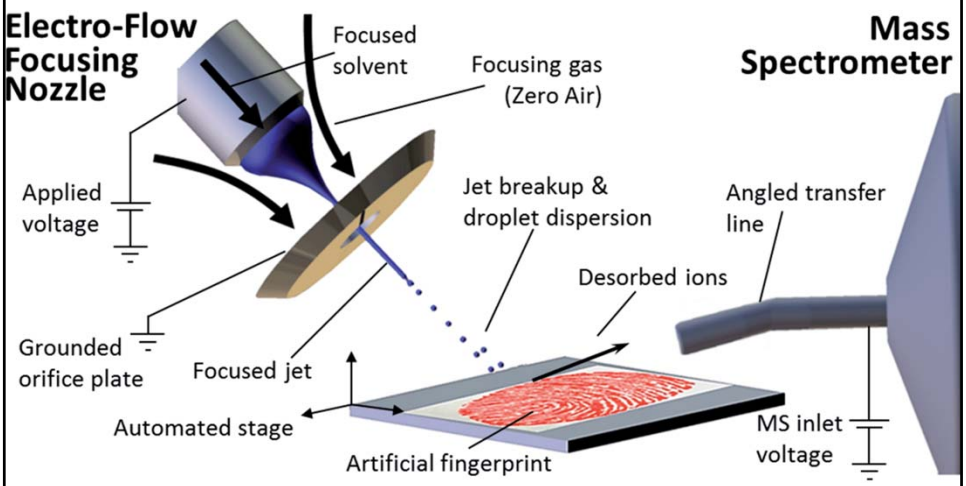
PCA analysis of REIMS data from tissue sections



Golf et al., Anal Chem 2015

Desorption electrospray ionization (DESI)

- Works by directing an electrical fine spray at a tissue target

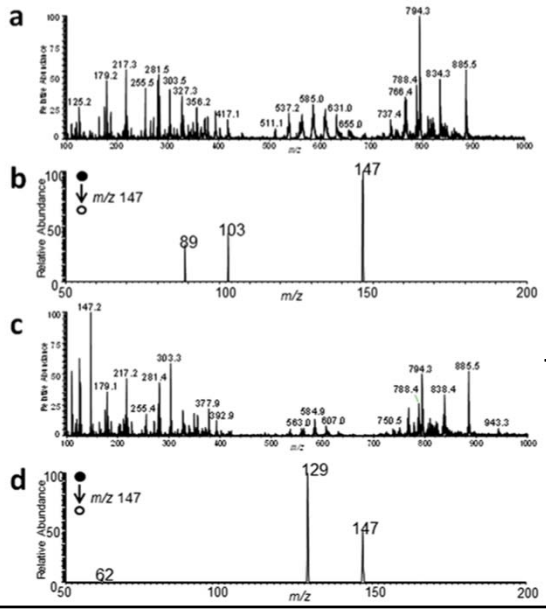


http://pubs.rsc.org/services/images/RSCpubs.ePlatform.Service.FreeContent.ImageService.svc/ImageService/Articleimage/2014/AN/c4an00172a/c4an00172a-f1_hi-res.gif

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 oncometabolite**

Whither 2-hydroxyglutarate?



Tissue expressing wild type IDH1

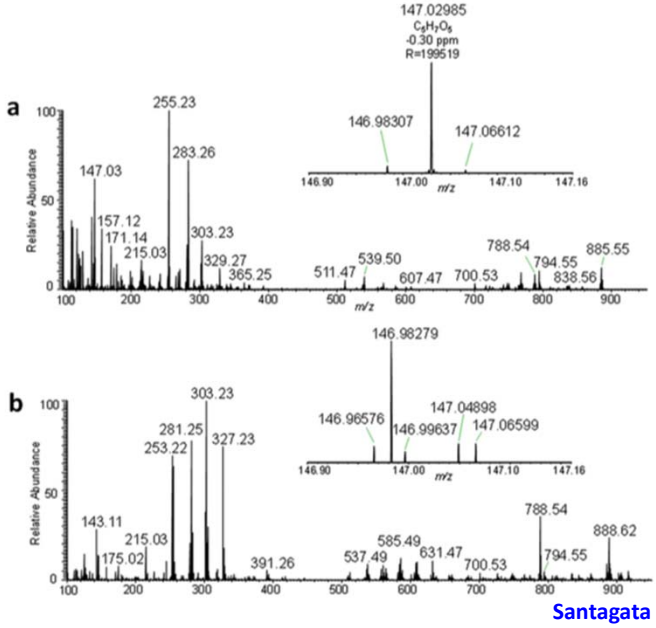
MSMS of *m/z* 147 - not 2HG

Tissue expressing mutant IDH1

MSMS of *m/z* 147 is 2HG

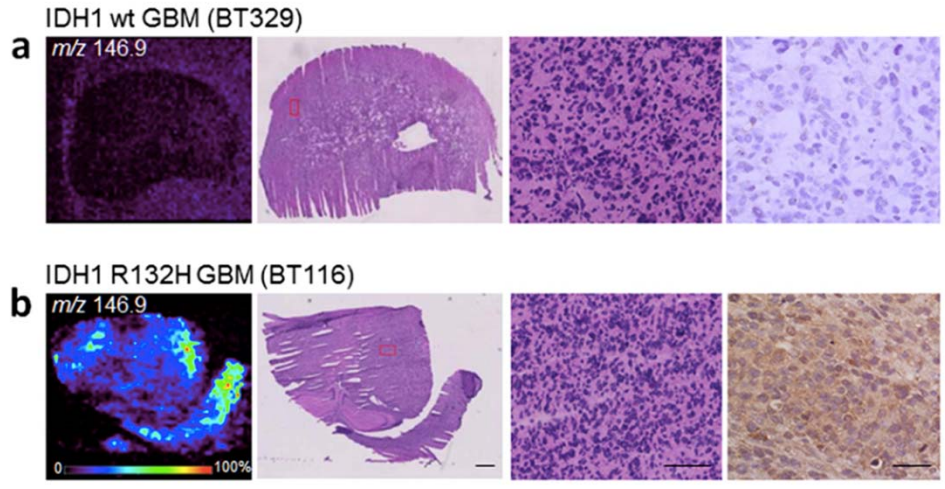
Santagata et al. PNAS 2014

Value of exact mass – "147" vs "147"



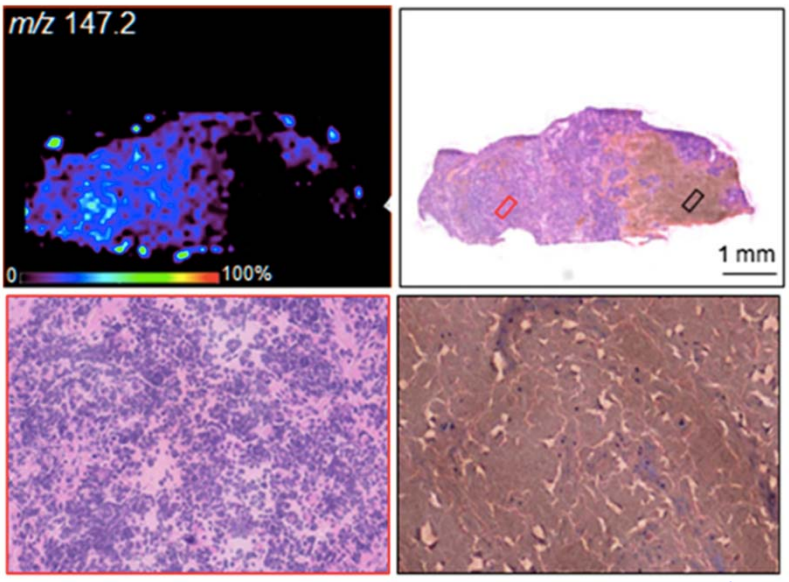
Santagata et al. PNAS 2014

Tumor xenograft imaging and 2HG



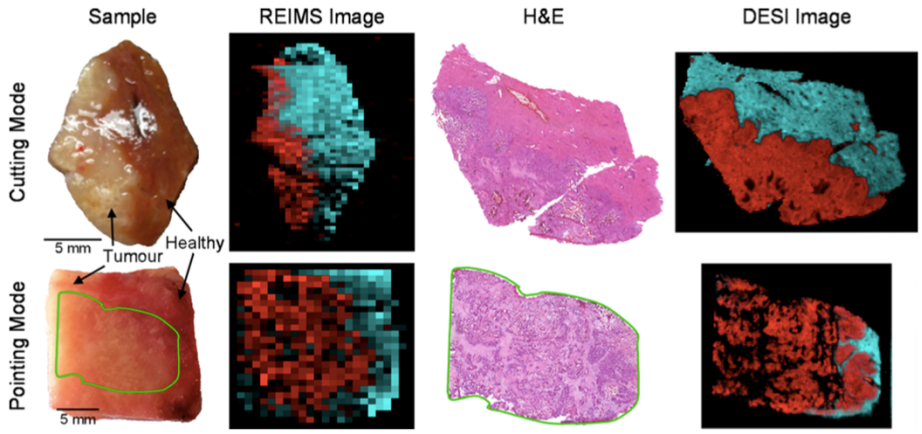
Santagata et al. PNAS 2014

Application to human glioblastoma



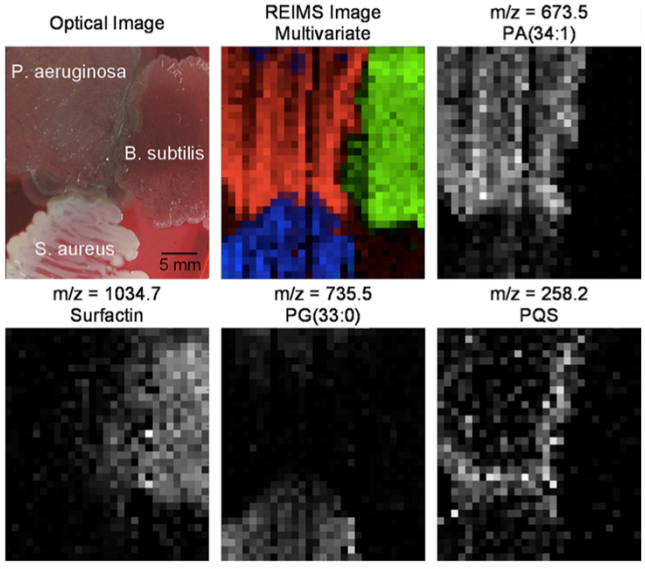
Santagata et al. PNAS 2014

Comparative imaging of normal-tumor tissue transition



Golf et al., Anal Chem 2015

Distinguishing bacterial populations



Golf et al., Anal Chem 2015

Use of Raman spectroscopy

Real-time imaging of metabolites in skin

- http://bernstein.harvard.edu/research/cars-why.htm

Sunny Xie, PhD - Harvard

The future of medicine and surgery

http://www1.imperial.ac.uk/phenomecentre/