THE UNIVERSITY OF
ALABAMA AT BIRMINGHAM
Knowledge that will change your world

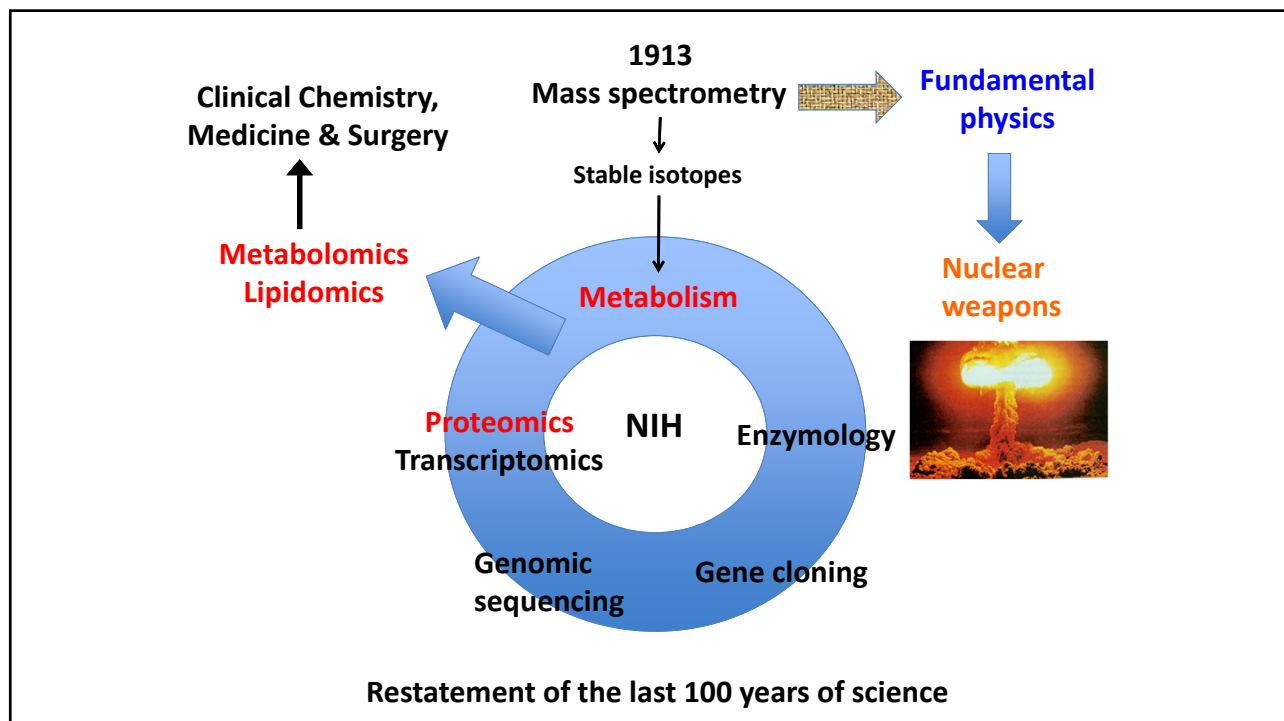# Real-time connection of Metabolomics with Medicine and Surgery and the rest of life

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Director, Targeted Metabolomics and Proteomics Laboratory

Targeted
Metabolomics &
Proteomics
Laboratory

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1913
Mass spectrometry → **Fundamental physics**

Clinical Chemistry, Medicine & Surgery

Stable isotopes

Metabolomics Lipidomics

Nuclear weapons

Metabolism

Proteomics
Transcriptomics

NIH

Enzymology

Genomic sequencing

Gene cloning

Restatement of the last 100 years of science

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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**

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Real time analysis

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

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Real-time analysis

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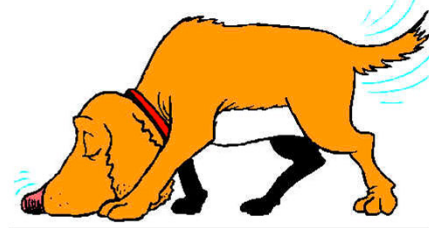 - **Checks for ion signatures of explosives**

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

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Noses and smell – real time analysis

The superior volatile metabolite detector

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Gases produced in the GI tract

- H_2, CO_2 and CH_4 from carbohydrates
  - *Firmicutes*
  - From pyruvate and NAD(P)H/FADH$_2$
  - H_2 used by sulfate-reducing bacteria (SRBs), methanogenic Archaea, and acetogens
- SRBs produce H_2S
- NO from nitrates

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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

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
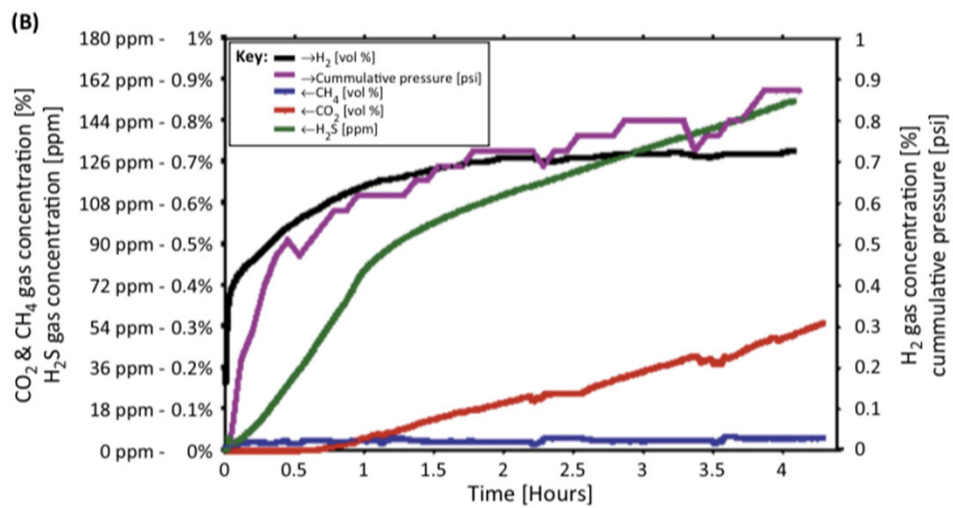
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Device for measuring fecal gas production
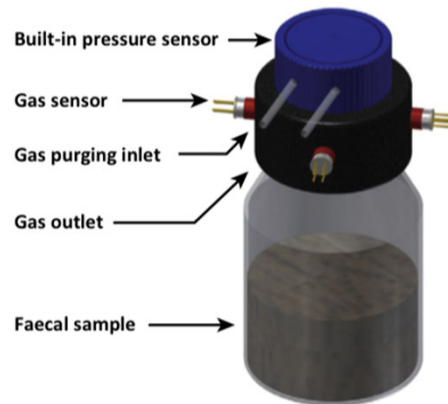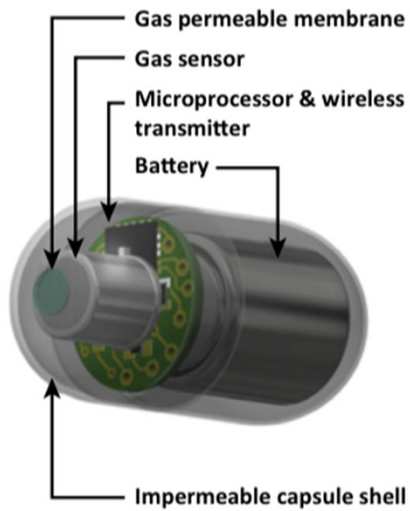

Jian Zhen Ou et al., Trends Biotech, 2015

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Fecal gas production (ex vivo)

Jian Zhen Ou et al., Trends Biotech, 2015

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Real-time *in situ* monitoring gas production

Gas permeable membrane
Gas sensor
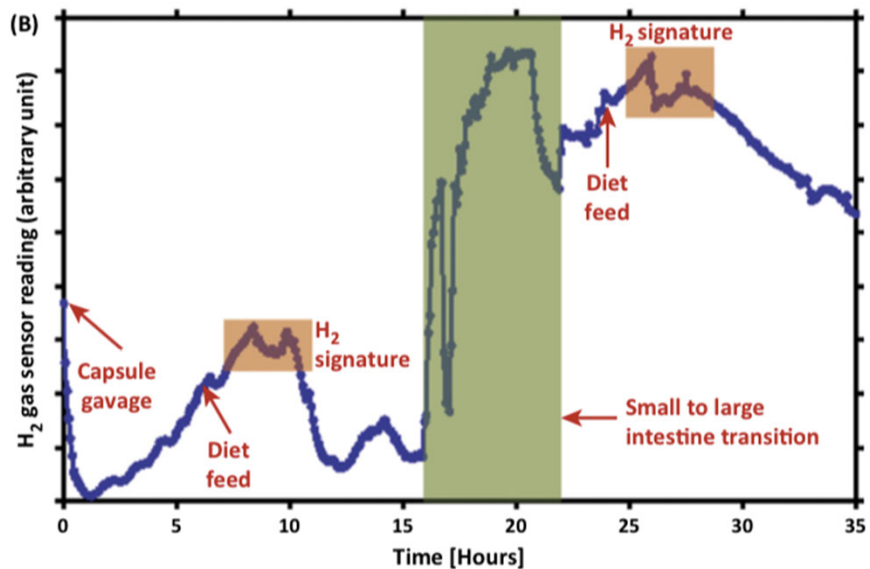Microprocessor & wireless transmitter
Battery

Impermeable capsule shell

- 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!!

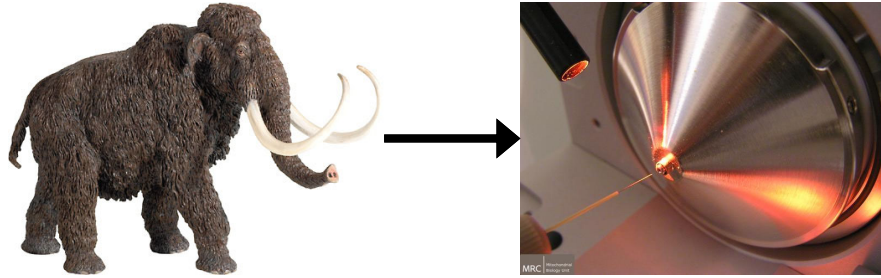Jian Zhen Ou et al., Trends Biotech, 2015

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Real time intestinal gas production

(B) H₂ gas sensor reading (arbitrary unit) vs Time [Hours]

Capsule gavage, Diet feed, H₂ signature, Small to large intestine transition, Diet feed, H₂ signature
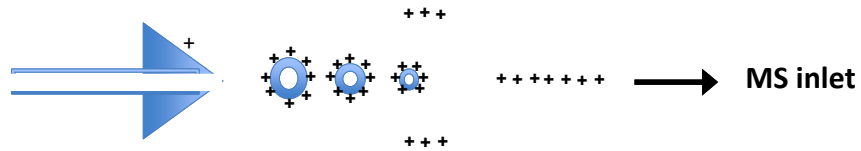
Jian Zhen Ou et al., Trends Biotech, 2015

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The Challenge for Mass Spec

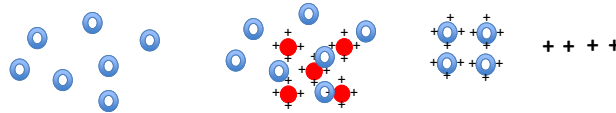

How to get the mammoth into the gas phase for analysis?
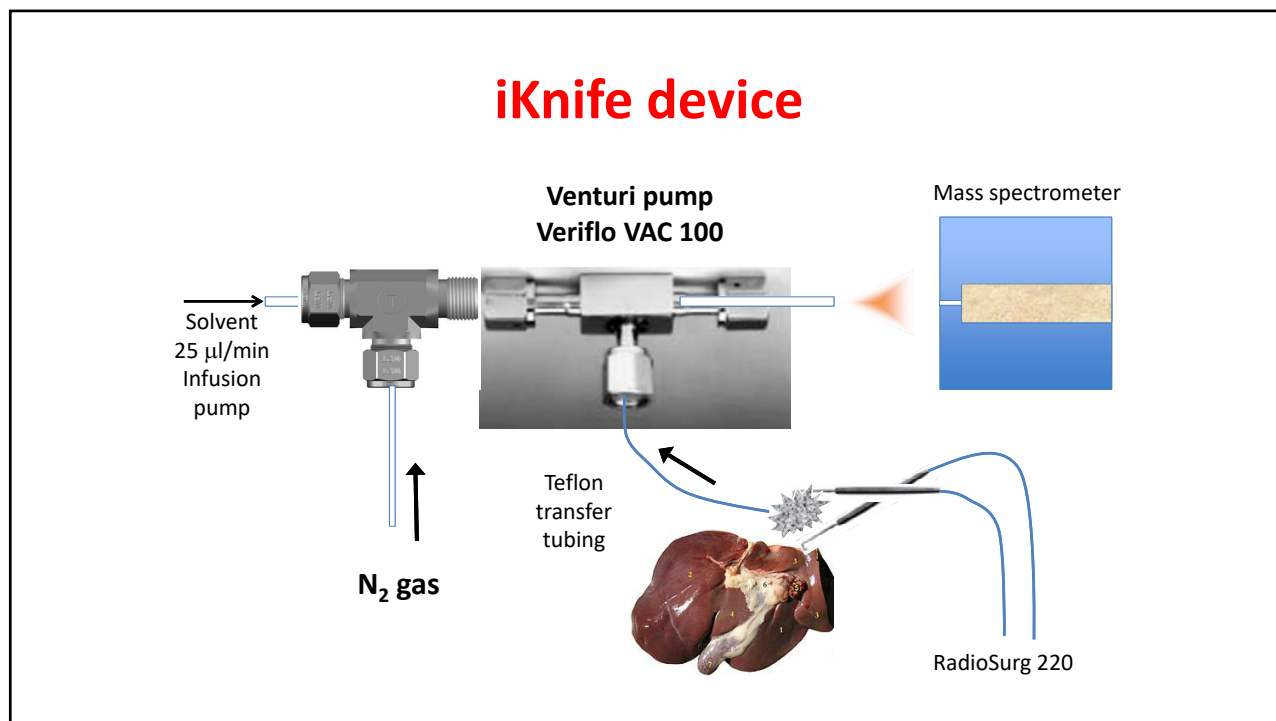
13

Droplet principle of electrospray
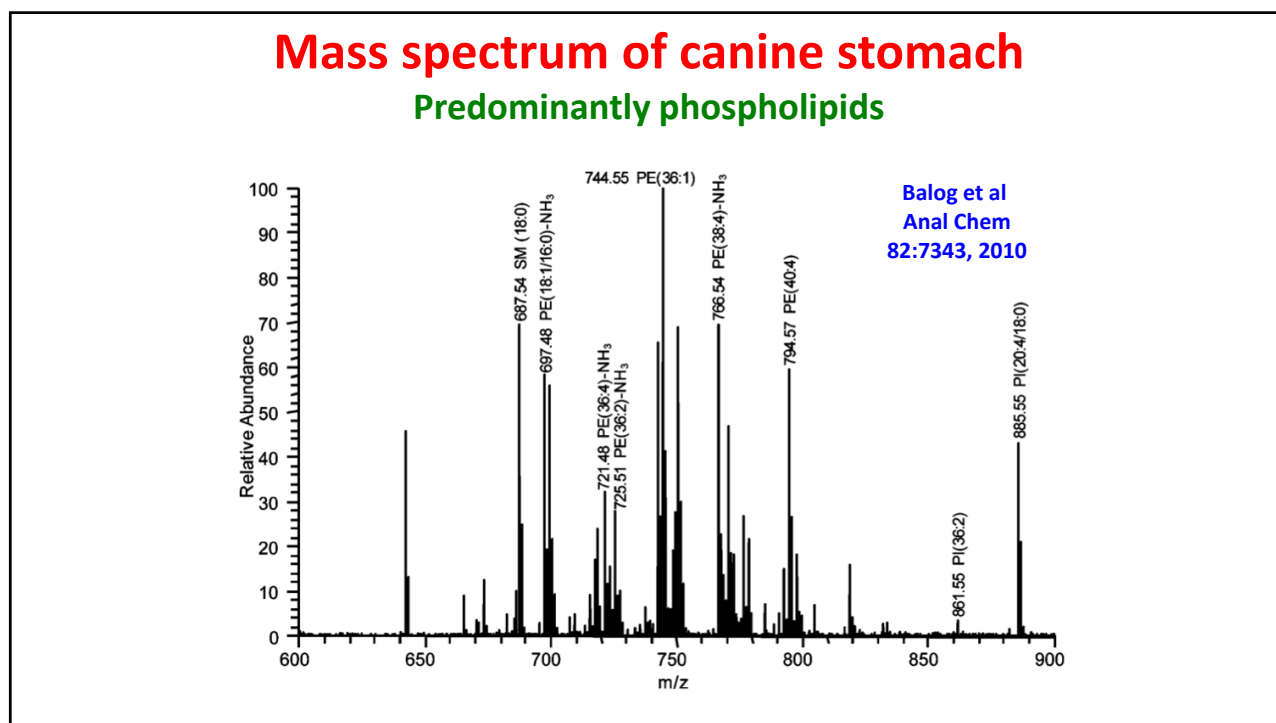

MS inlet

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

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iKnife device

**Venturi pump
Veriflo VAC 100**

Mass spectrometer

Solvent
25 µl/min
Infusion
pump

N₂ gas

Teflon
transfer
tubing

RadioSurg 220

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Mass spectrum of canine stomach
Predominantly phospholipids

**Balog et al
Anal Chem
82:7343, 2010**

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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**
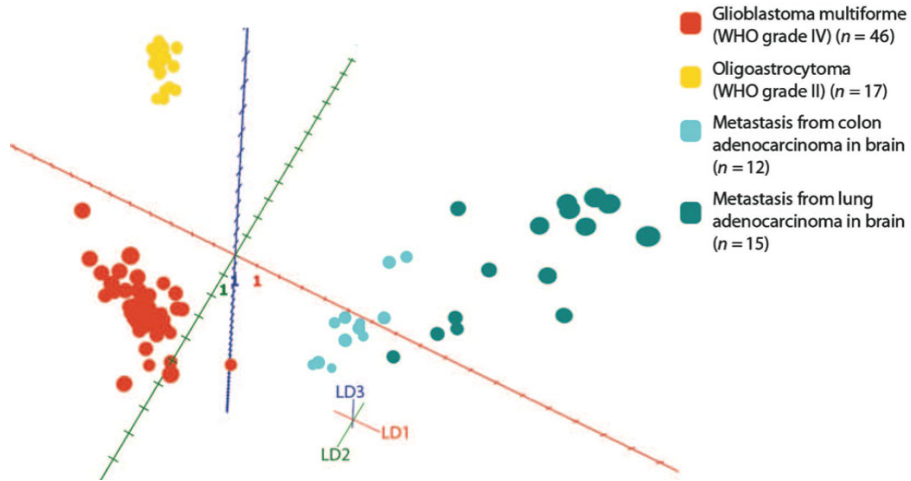
17

Principal components analysis of ions from surgical "smoke"

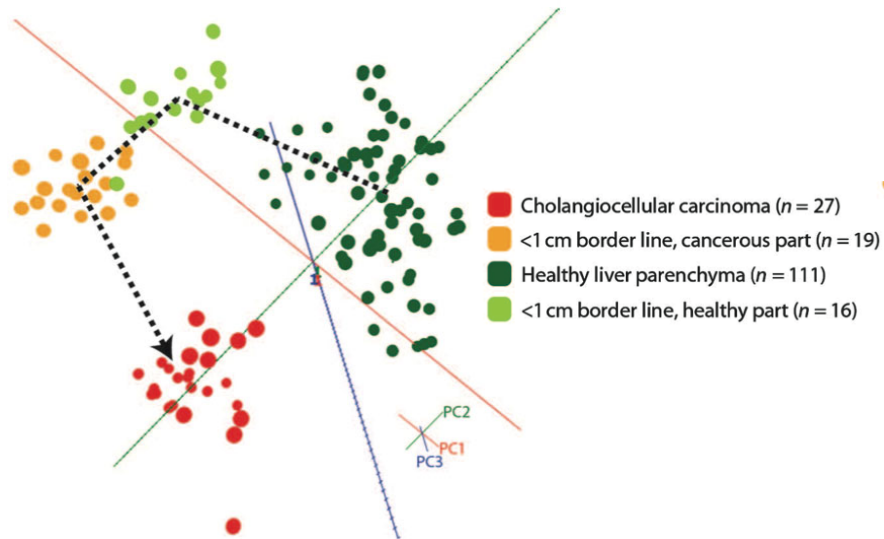**Balog et al
Anal Chem
82:7343, 2010**
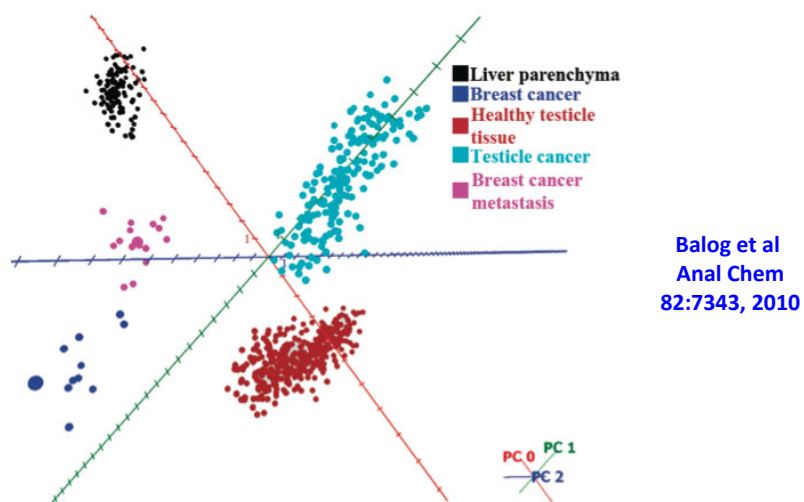
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Differentiation of brain tumors

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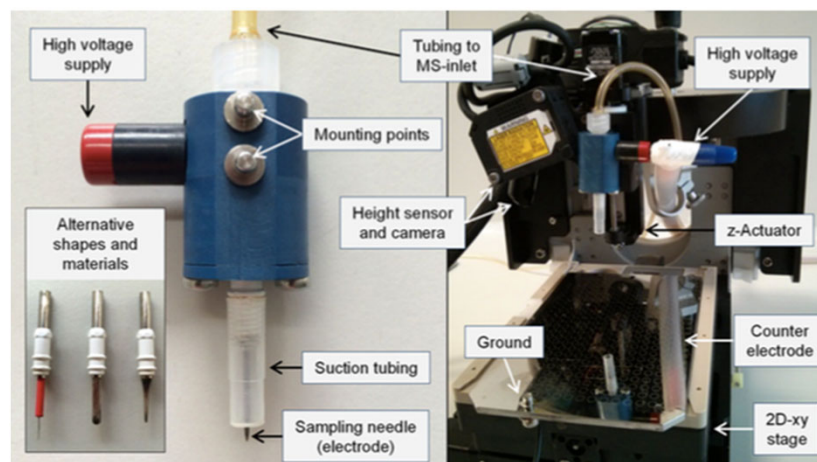# Changing lipids across cancer margin

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

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Examining tissue (slices) by REIMS
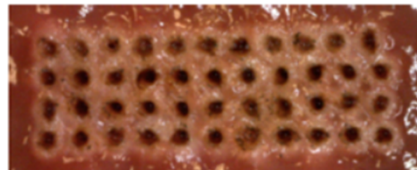

Golf et al., Anal Chem 2015

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Modes of data acquisition for REIMS
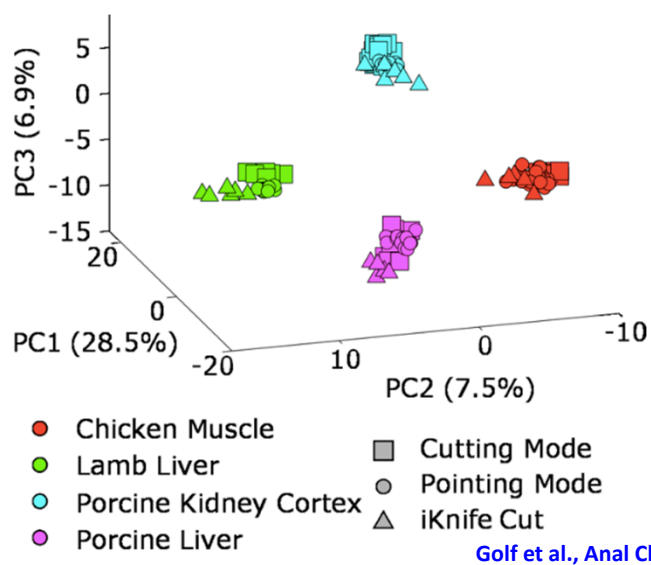
Line Scans: Cutting Mode

Individual Pixels: Pointing Mode

2 mm

Golf et al., Anal Chem 2015

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PCA analysis of REIMS data from tissue sections

PC3 (6.9%)

PC1 (28.5%)

PC2 (7.5%)

- Chicken Muscle
- Lamb Liver
- Porcine Kidney Cortex
- Porcine Liver

- Cutting Mode
- Pointing Mode
- iKnife Cut

Golf et al., Anal Chem 2015

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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

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Desorption electrospray ionization (DESI)

- Works by directing an electrical fine spray at a tissue target – does not require deposition of a matrix
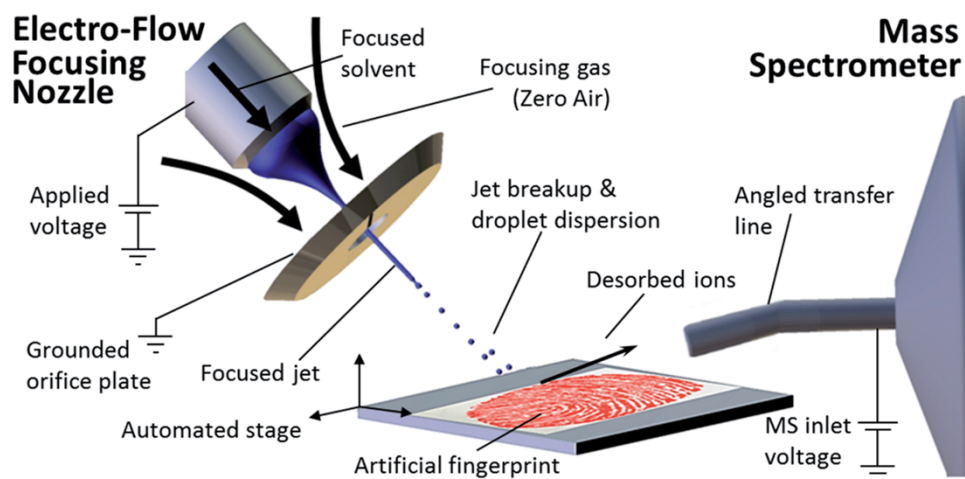
http://pubs.rsc.org/services/images/RSCpubs.ePlatform.Service.FreeContent.ImageService.svc/ImageService/Articleimage/2014/AN/c4an00172a/c4an00172a-f1_hi-res.gif
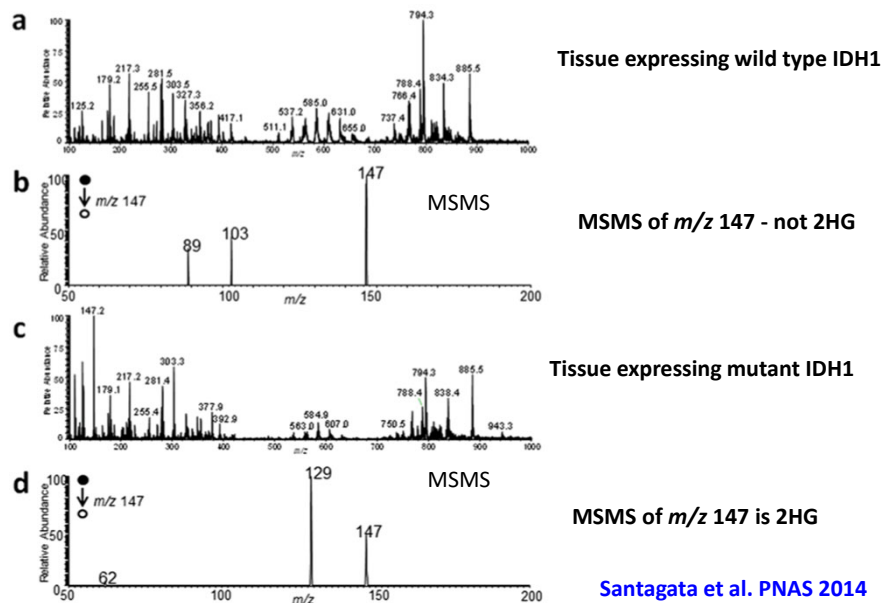
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The IDH story of brain and other tumors

- **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**

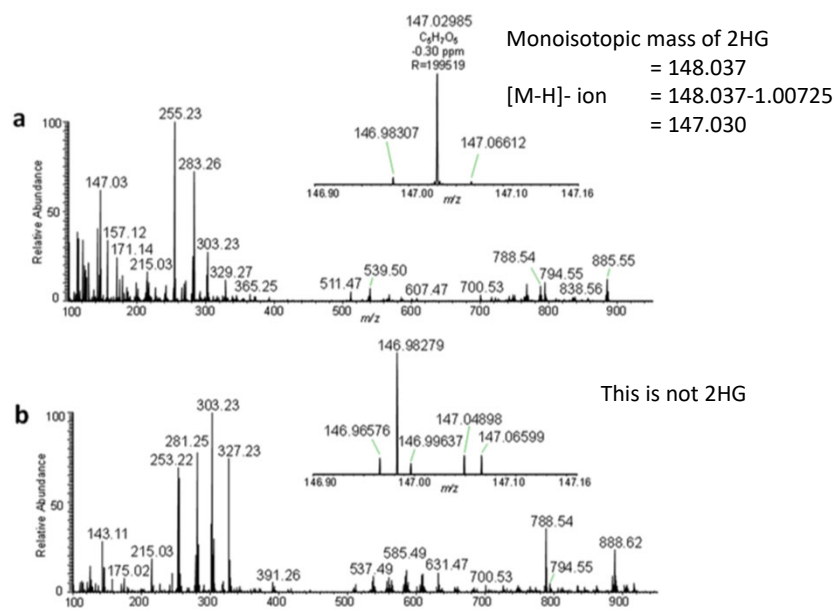27

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

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Value of exact mass – "147" vs "147"

147.02985
C5H7O5
-0.30 ppm
R=199519

Monoisotopic mass of 2HG
= 148.037

[M-H]- ion = 148.037-1.00725
= 147.030

This is not 2HG

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Tumor xenograft imaging and 2HG
The ion at *m/z* 146.9 was subjected to MSMS to measure 2HG

IDH1 wt GBM (BT329)
a *m/z* 146.9

IDH1 R132H GBM (BT116)
b *m/z* 146.9
0 — 100%

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15
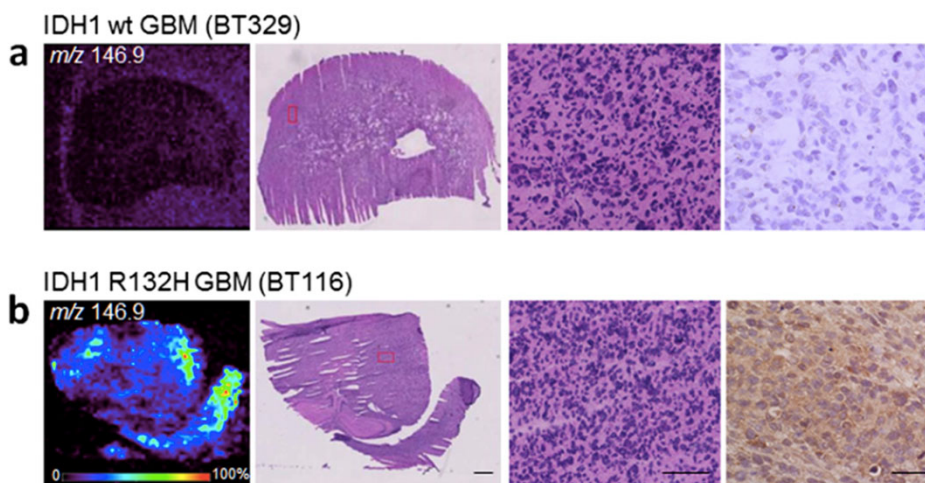
Application to human glioblastoma
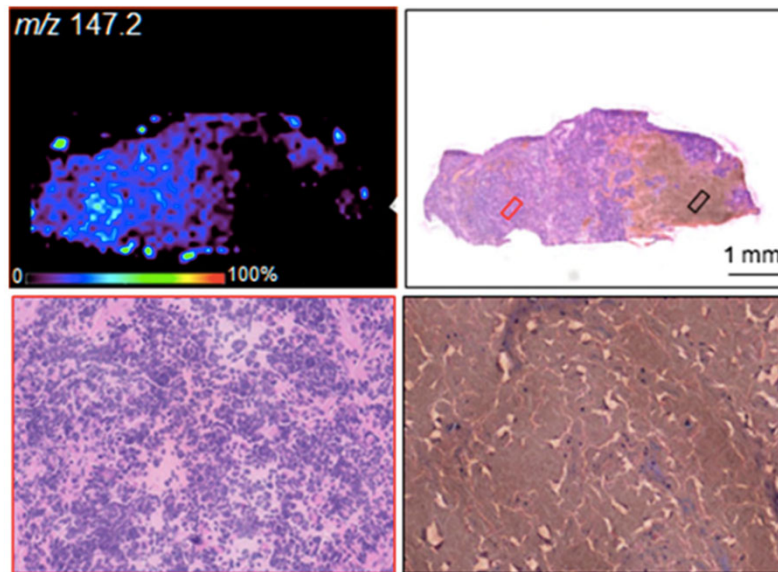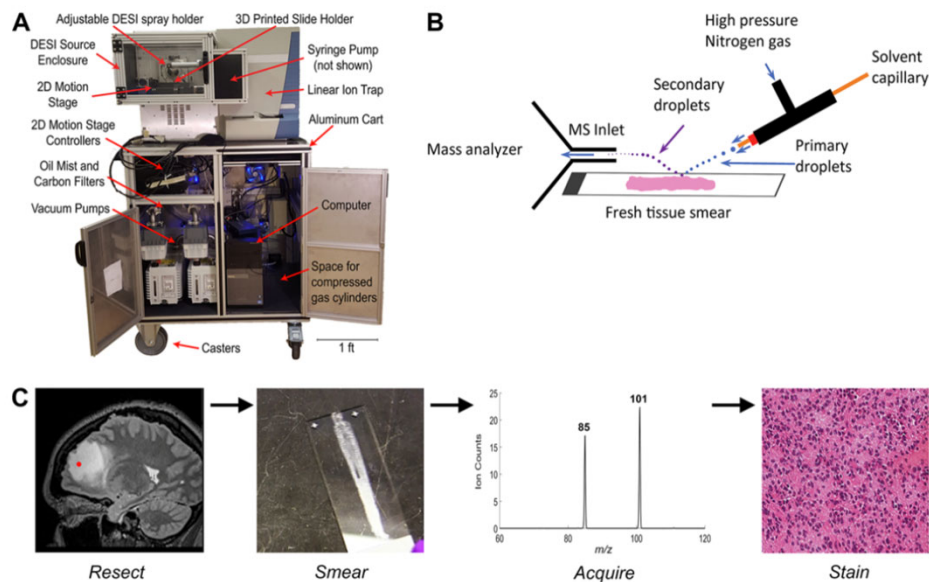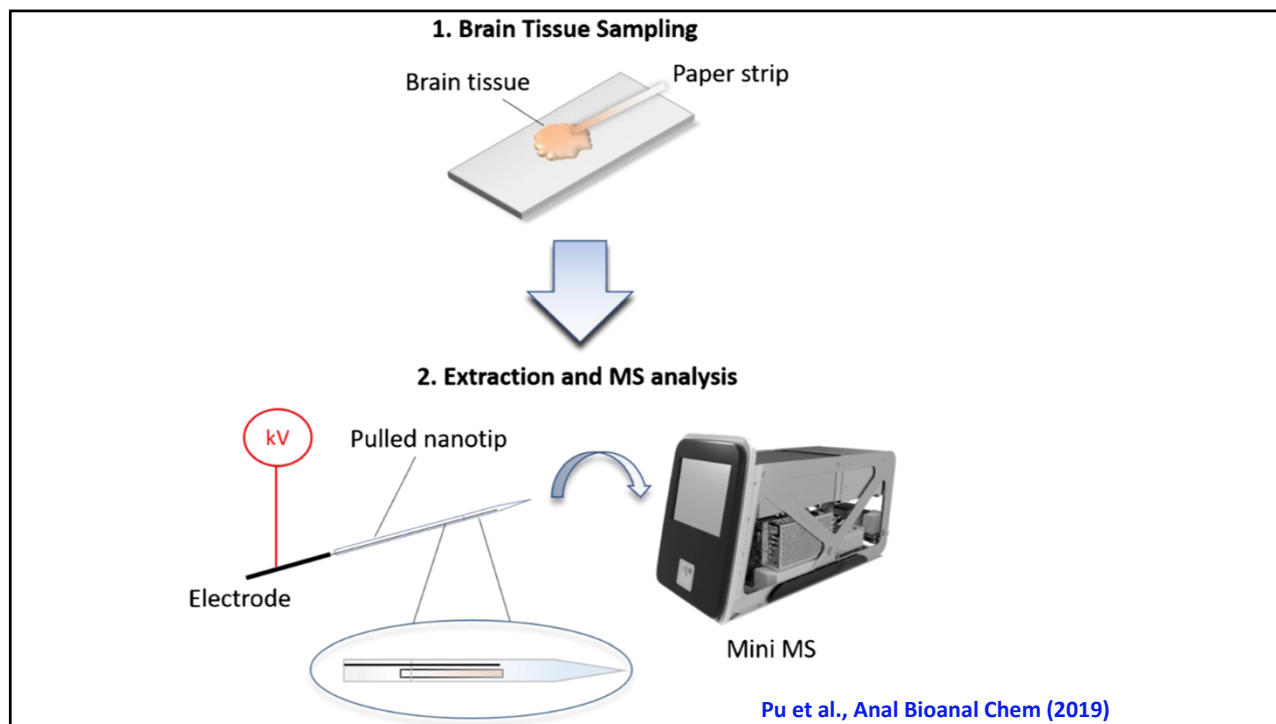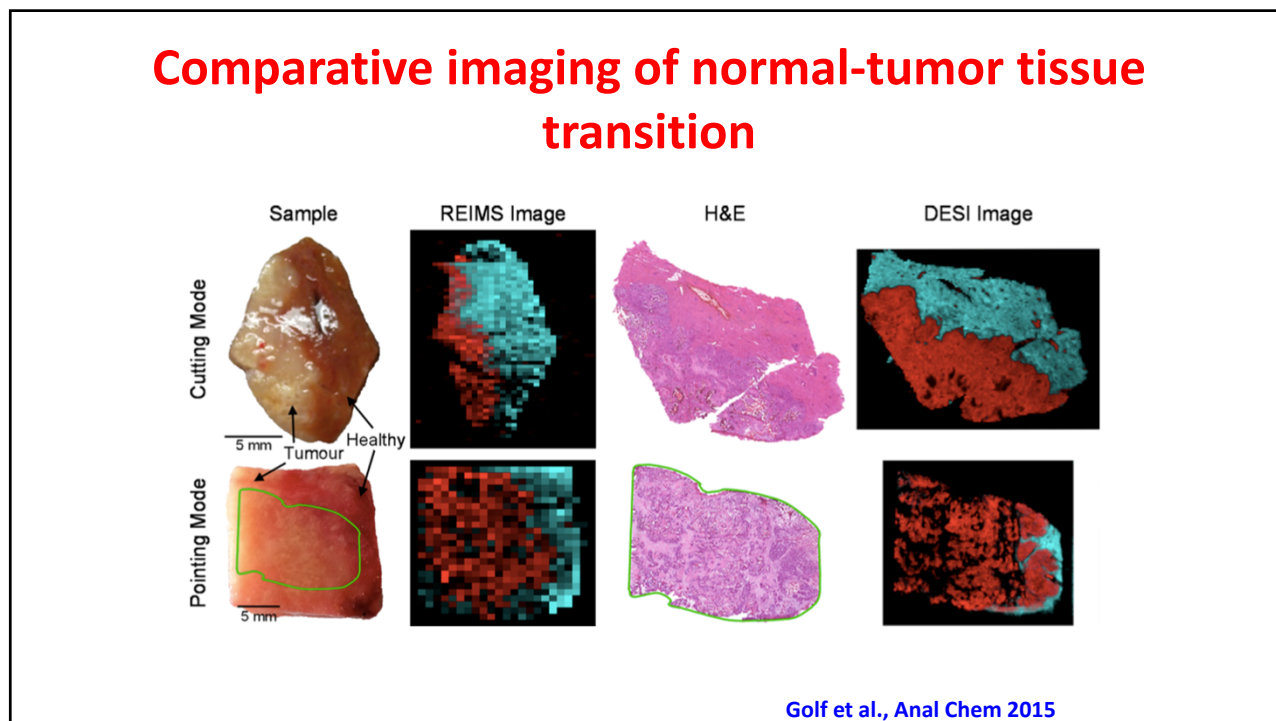

Santagata et al. PNAS 2014

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FIG. 1. DESI-MS method overview. **A:** Image of the custom-made intraoperative DESI-MS system for intraoperative analysis of tissue biopsies. **B:** Diagram of the DESI process as described by Takáts et al. **C:** Workflow of intraoperative analysis protocol consisting of tissue collection (*red spot*) and smearing, DESI-MS analysis, and post hoc histopathological staining. Original magnification ×20. Figure is available in color online only.
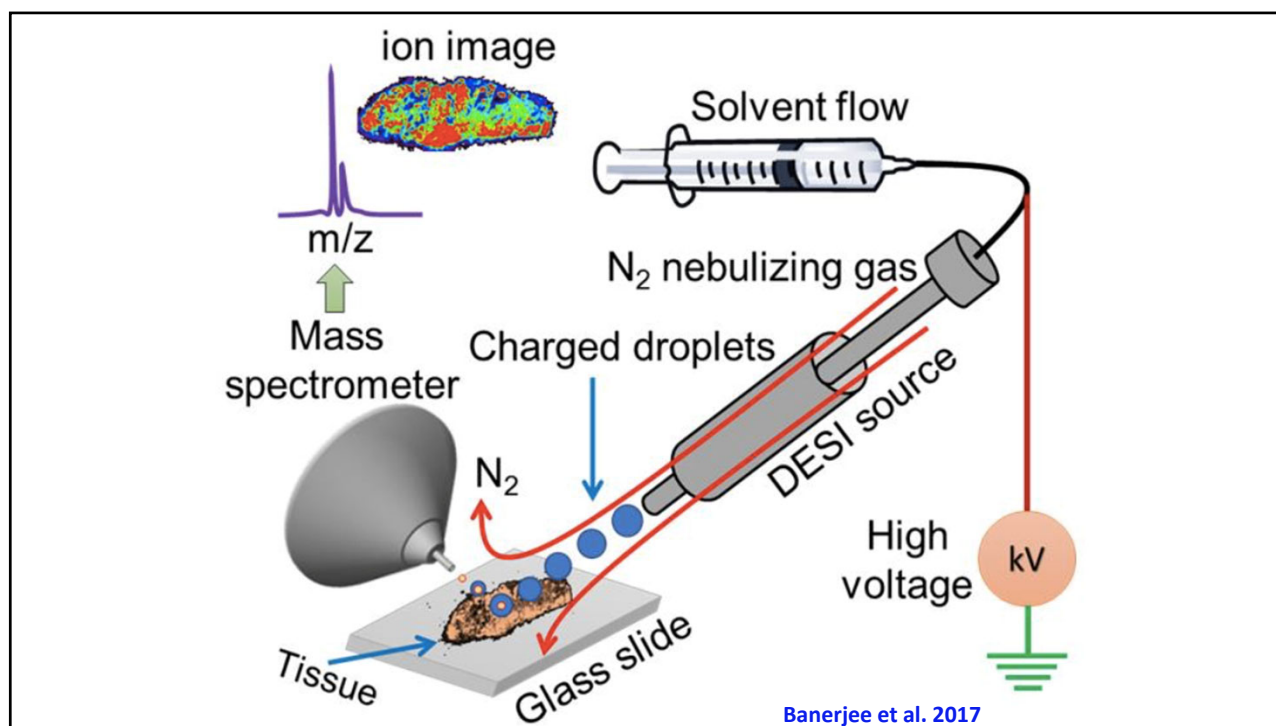
Alfaro et al., J Neurosurg (2019) Jan 4 1-8

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1. Brain Tissue Sampling

Brain tissue   Paper strip

2. Extraction and MS analysis

kV   Pulled nanotip

Electrode   Mini MS

Pu et al., Anal Bioanal Chem (2019)

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Comparative imaging of normal-tumor tissue transition

| | Sample | REIMS Image | H&E | DESI Image |

Cutting Mode

Tumour   Healthy
5 mm

Pointing Mode
5 mm

Golf et al., Anal Chem 2015

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Banerjee et al. 2017

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Banerjee et al. 2017

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Banerjee et al. 2017

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Red = elevated in cancer
Blue = down in cancer

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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**.

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Raman spectroscopy

NMR
Non-destructive
*highly reproducible
Low sensitivity
Moderate resolution

GC-MS
Destructive
Reproducible
*High sensitivity
X – complex lipids
Moderate resolution

LC-MS
Destructive
Reproducible
High sensitivity
*Complex lipids!
High resolution

When photons strike a molecule, in the great majority of cases they are scattered elastically (Rayleigh scattering). Raman spectroscopy is about the inelastic scattering that occurs when the photon's energy matches that of a process that the molecule is undergoing, typically vibrational energy.

The ideal method should be
Non-destructive
Highly reproducible
Highly sensitive
Measurable *in situ*
Measurable in real time
Subcellular

Raman spectroscopy fulfills each of the above

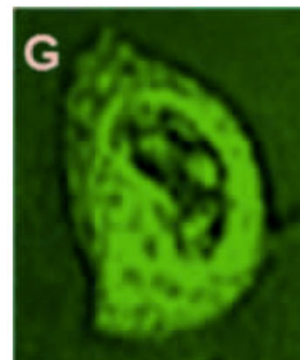https://commons.wikimedia.org/wiki/File:Ramanscattering.svg

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Where to next?

**MALDI-Imaging of a phospholipid
Janusz Kabarowski/Kelly Walters**

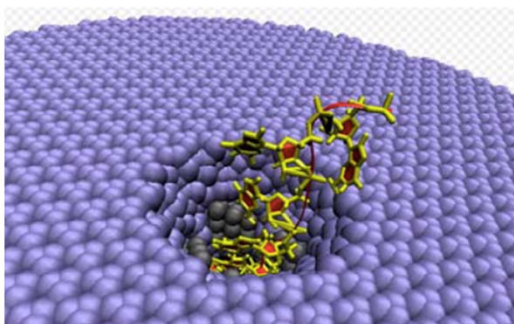**Multiple sampling single cells – Nemes group**

**CARS imaging of a cancer cell –
spectroscopic, real time
Raman imaging**

OR, two people with disparate abilities and insights will create something we've never heard of (yet)

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What might it be?

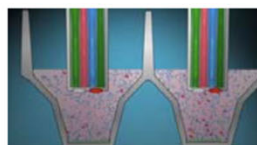**Nanoprobe inserted into the wall of a cell
recording changes in metabolism in real time –
sub nl sampling/analysis**

TOMORROW?

**Measuring O_2 uptake using a
Warburg apparatus – 10 ml
incubations**

YESTERDAY

**The O_2 and pH probes of a
Seahorse™ apparatus – 7 µl
incubations**

TODAY

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Publications

- Santagata S, Eberlin LS, Norton I, Calligaris D, Feldman DR, Ide JL, Liu X, Wiley JS, Vestal ML, Ramkissoon SH, Orringer DA, Gill KK, Dunn IF, Dias-Santagata D, Ligon KL, Jolesz FA, Golby AJ, Cooks RG, Agar NY. Intraoperative mass spectrometry mapping of an onco-metabolite to guide brain tumor surgery. PNAS 2014;111(30):11121-6.
- Banerjee S, Zarea RN, Tibshirani RJ, Kunder CA, Nolley R, Fan R, Brooks JD, Sonn GA. Diagnosis of prostate cancer by desorption electrospray ionization mass spectrometric imaging of small metabolites and lipids. PNAS early edition, March 2017
- Golf O, Strittmatter N, Karancsi T, Pringle SD, Speller AV, Mroz A, Kinross JM, Abbassi-Ghadi N, Jones EA, Takats Z. Rapid evaporative ionization mass spectrometry imaging platform for direct mapping from bulk tissue and bacterial growth media. Anal Chem. 2015 Mar 3;87(5):2527-34.
- Balog J, Kumar S, Alexander J, Golf O, Huang J, Wiggins T, Abbassi-Ghadi N, Enyedi A, Kacska S, Kinross J, Hanna GB, Nicholson JK, Takats Z. In vivo endoscopic tissue identification by rapid evaporative ionization mass spectrometry (REIMS). Angew Chem Int Ed Engl. 2015 Sep 14;54(38):11059-62.
- Alfaro CM, Pirro V, Keating MF, Hattab EM, Cooks RG, Cohen-Gadol AA. Intraoperative assessment of isocitrate dehydrogenase mutation status in human gliomas using desorption electrospray ionization-mass spectrometry. J Neurosurg. 2019 Jan 4:1-8.
- Pu F, Alfaro CM, Pirro V, Xie Z, Ouyang Z, Cooks RG. Rapid determination of isocitrate dehydrogenase mutation status of human gliomas by extraction nanoelectrospray using a miniature mass spectrometer. Anal Bioanal Chem. 2019 Feb 2. doi: 10.1007/s00216-019-01632-5.
- Lima C, Muhamadali H, Goodacre R. The Role of Raman Spectroscopy Within Quantitative Metabolomics. Annu Rev Anal Chem. 2021 Jul 27;14(1):323-345.

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