

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

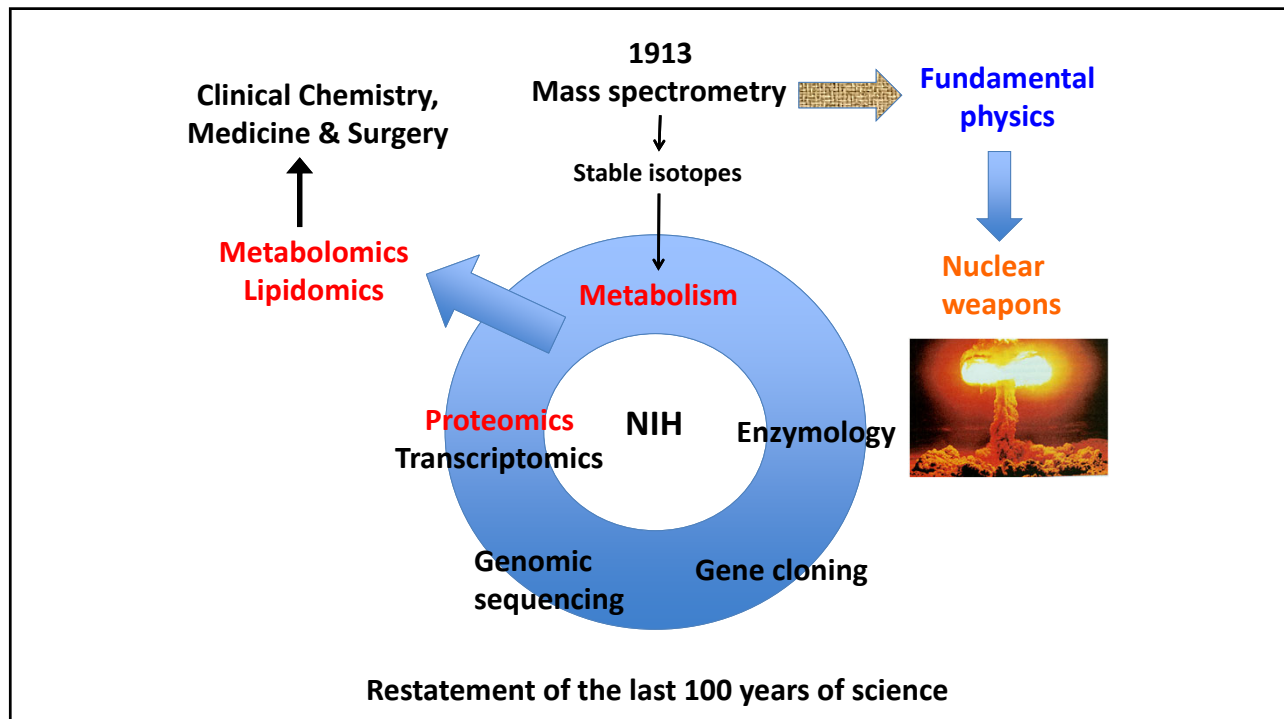
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Director, Targeted Metabolomics and
 Proteomics Laboratory

Targeted
Metabolomics &
Proteomics
Laboratory

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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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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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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

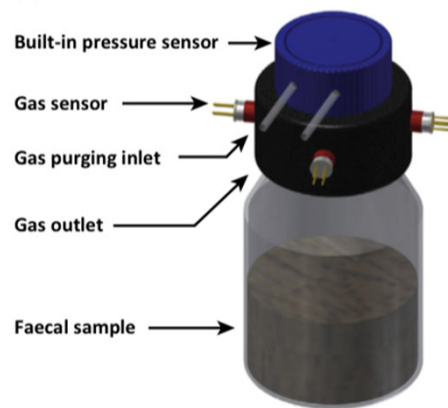
Technology	Operation mode	Target intestinal gas	Detection limit	Cross-sensitivity	Response time	Life time	Estimated cost
<i>Spectrometry based^a</i>							
GC-MS	Off line	All gases	ppt to ppb	Low	~Several minutes	Long	>US\$300k
IMS	Real time	All gases	ppb	Low	<1 min	Long	>US\$100k
PTR-MS	Real time	All 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
<i>Sensor based^b</i>							
Electrochemical	Real time	H ₂ , H ₂ S, 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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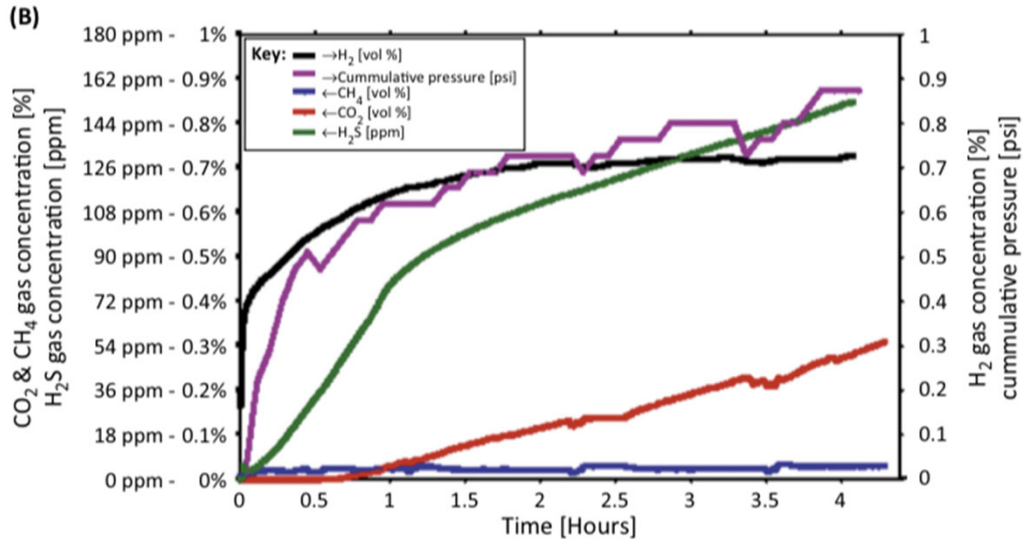
Device for measuring fecal gas production



Jian Zhen Ou et al., Trends Biotech, 2015

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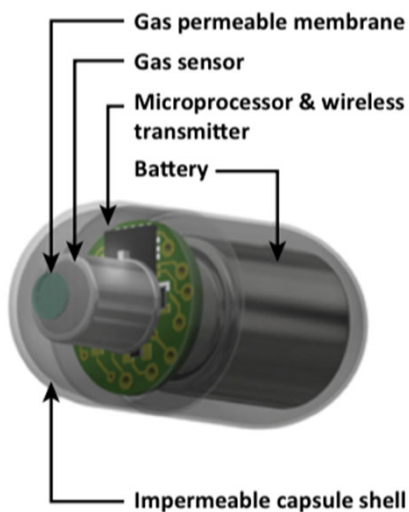
Fecal gas production (ex vivo)



Jian Zhen Ou et al., Trends Biotech, 2015

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Real-time *in situ* monitoring gas production

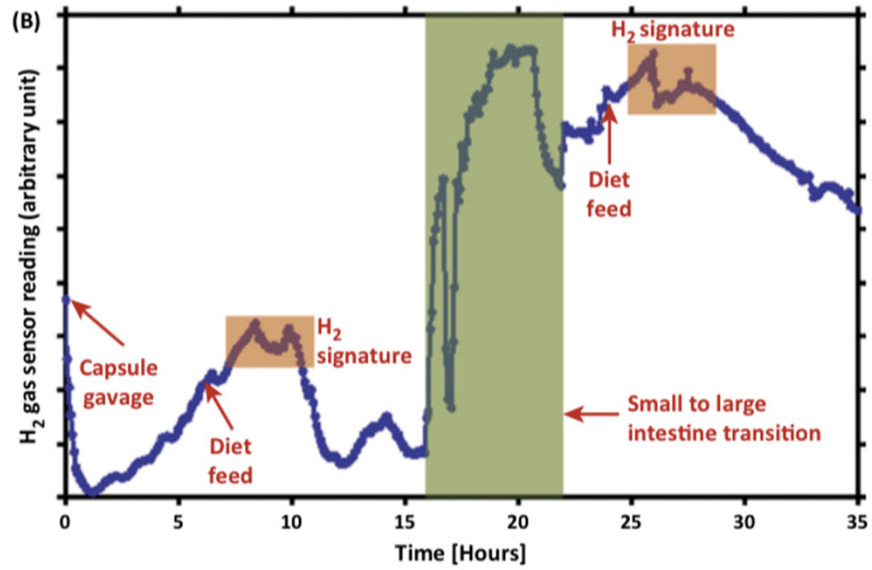


- The device is swallowed
- Completes full mouth-to-anus transit, reporting data as it goes
- Also provides positional information
- Operates at 405, 433, and 915 MHz
- Uses Lithium batteries!!

Jian Zhen Ou et al., Trends Biotech, 2015

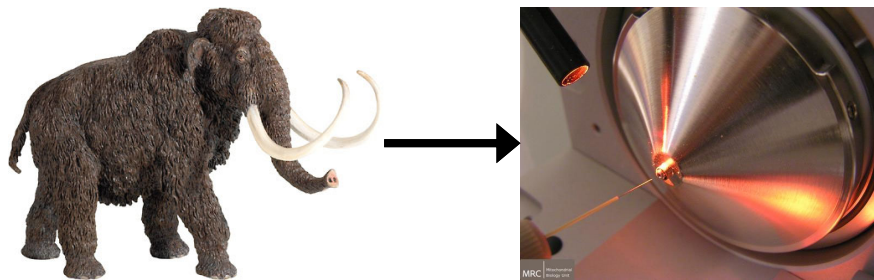
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Real time intestinal gas production



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The Challenge for Mass Spec



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

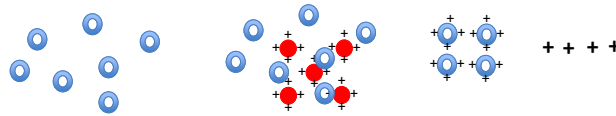
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Droplet principle of electrospray



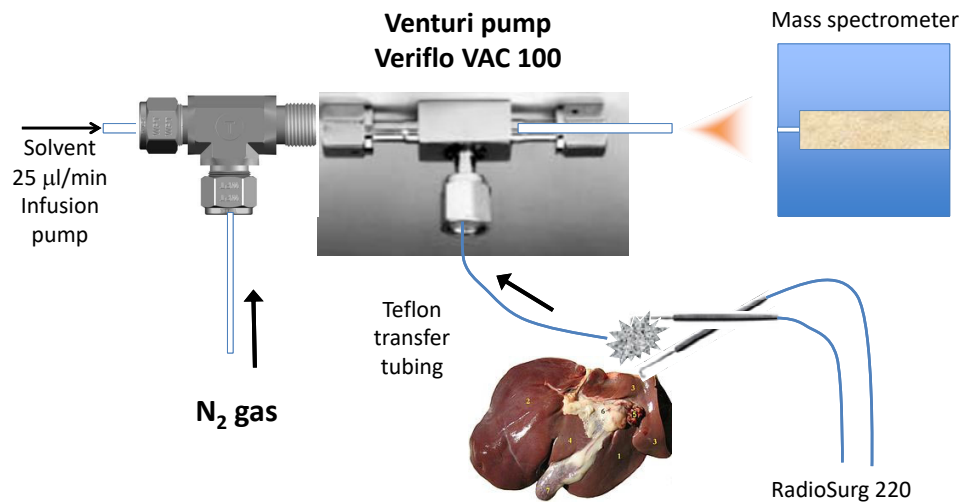
Droplet spray

- Sneeze
- Lung motion
- Surgical knife
- Other vapors



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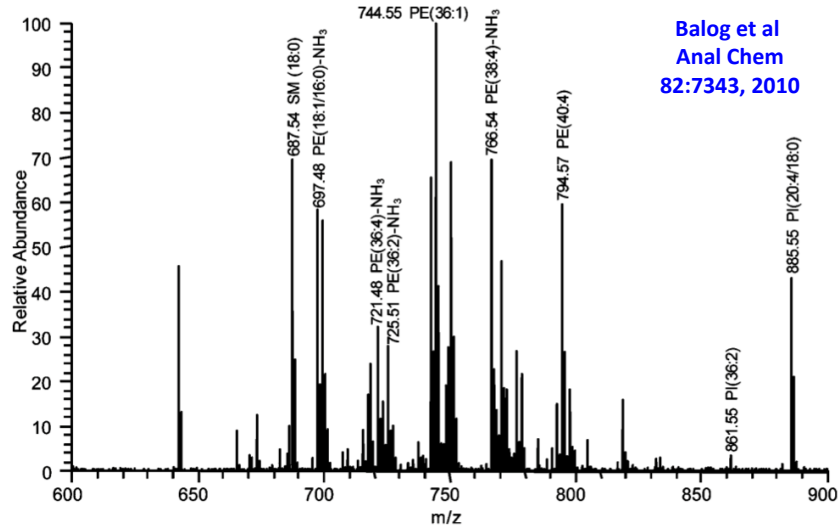
iKnife device



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Mass spectrum of canine stomach

Predominantly phospholipids



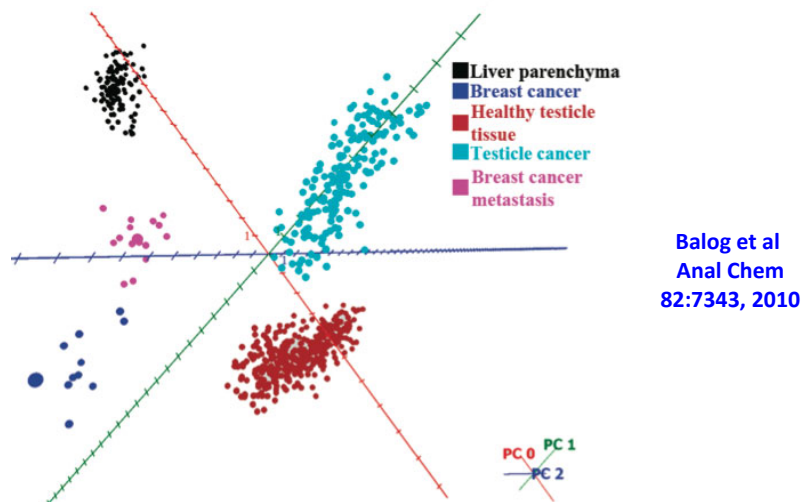
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Phospholipid patterns are characteristic of cells and tissues

- 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

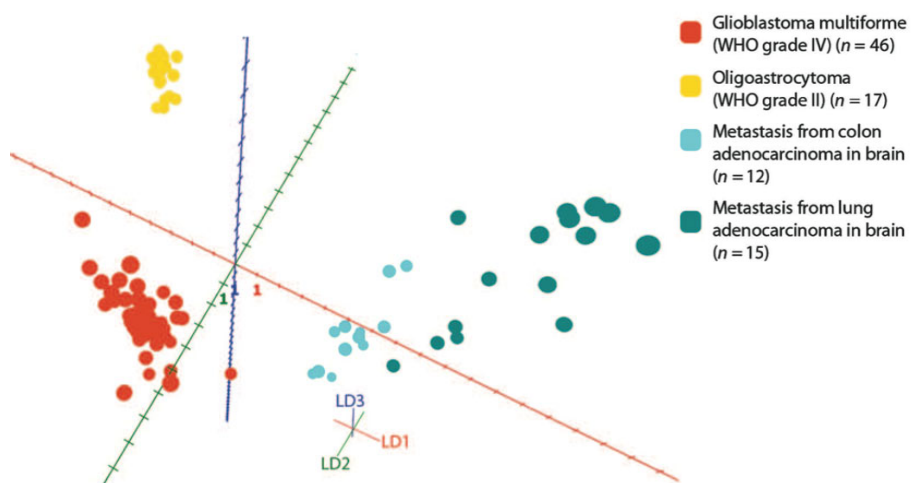
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Principal components analysis of ions from surgical "smoke"



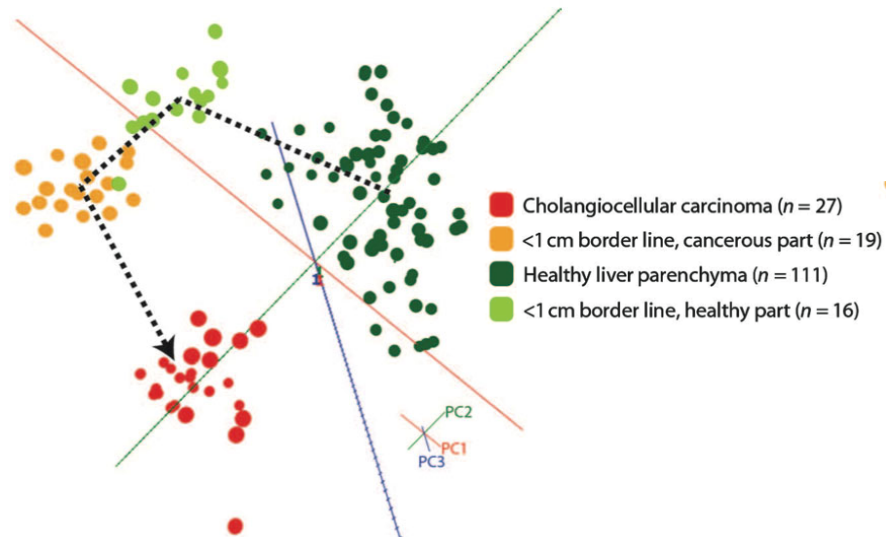
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Differentiation of brain tumors



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Changing lipids across cancer margin



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Adding real-time imaging – breast cancer

Scan no.	Video snapshot	Mass spectrum	Classification
38			 Normal 89.44% ●●●●
52			 Tumour 99.97% ●●●●
61			 Normal 99.88% ●●●●

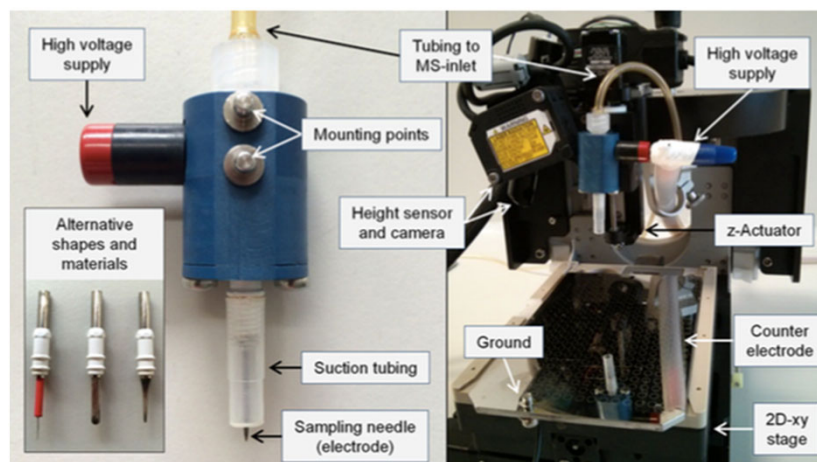
Fig. 7 Ex-vivo validation case study. An electrosurgical hand-piece was moved through the mastectomy specimen in *coag* mode from normal breast tissue, into tumour and out through normal tissue. A simultaneous video recording reveals the position of the hand-piece in relation to the specimen and the generated spectra and demonstrates good correlation with the recognition software compared to macroscopic findings

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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



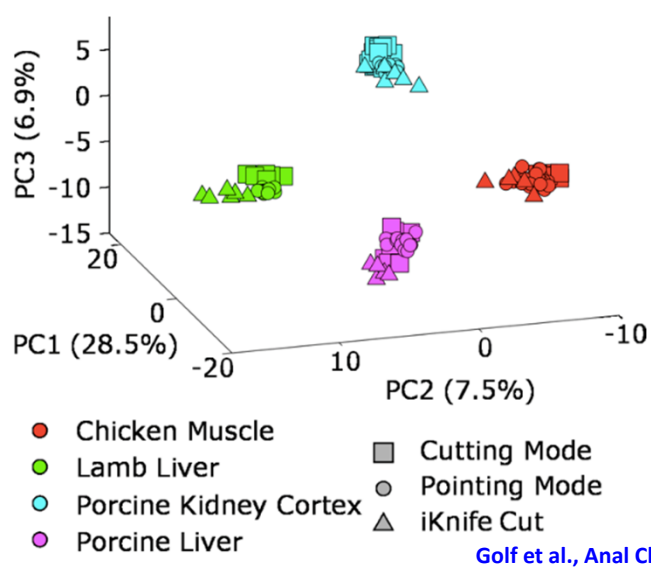
Individual Pixels:
Pointing Mode



Golf et al., Anal Chem 2015

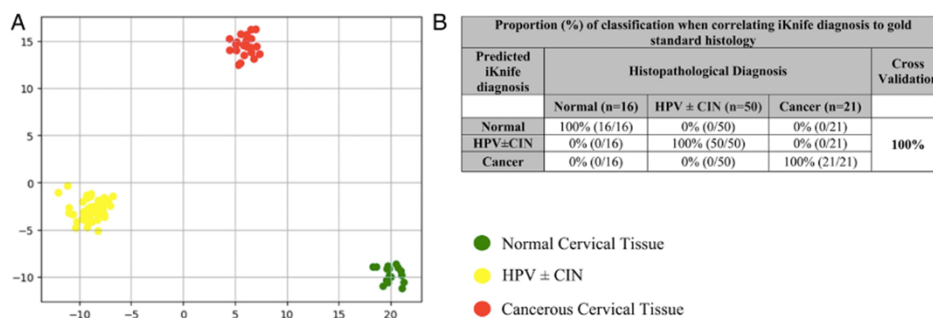
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PCA analysis of REIMS data from tissue sections



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Application of iKnife to HPV and cancer

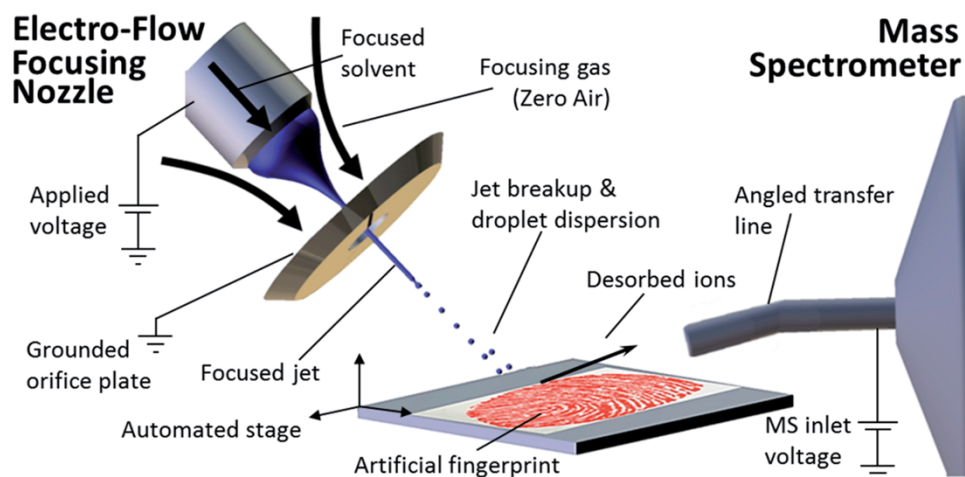


Tzafetas M et al., PNAS, March 2020

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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

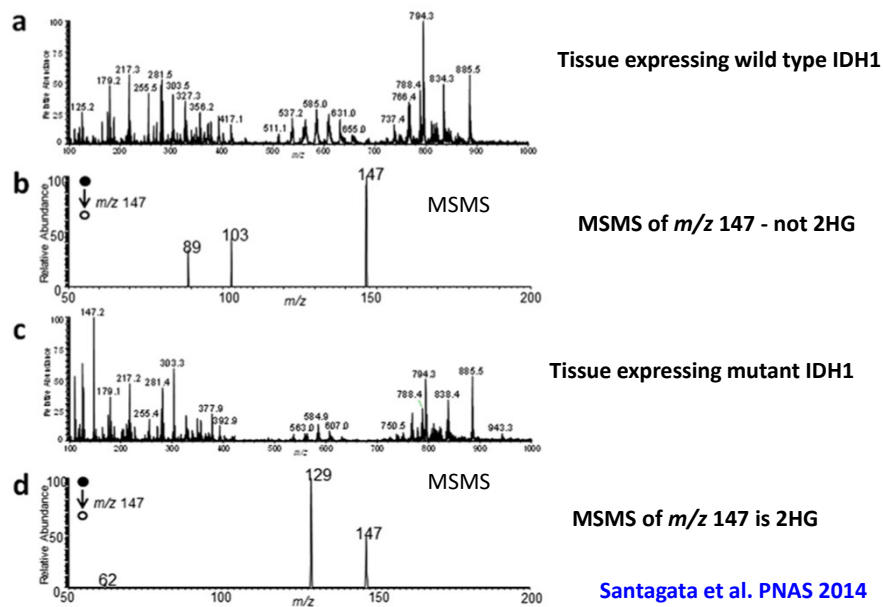
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The IDH story of brain and other tumors

- 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 (α KG) which is a two-step reaction
- Mutant IDH1 catalyzes the first step – to 2-hydroxyglutarate (2HG), but not the second one to α KG
- 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](#)

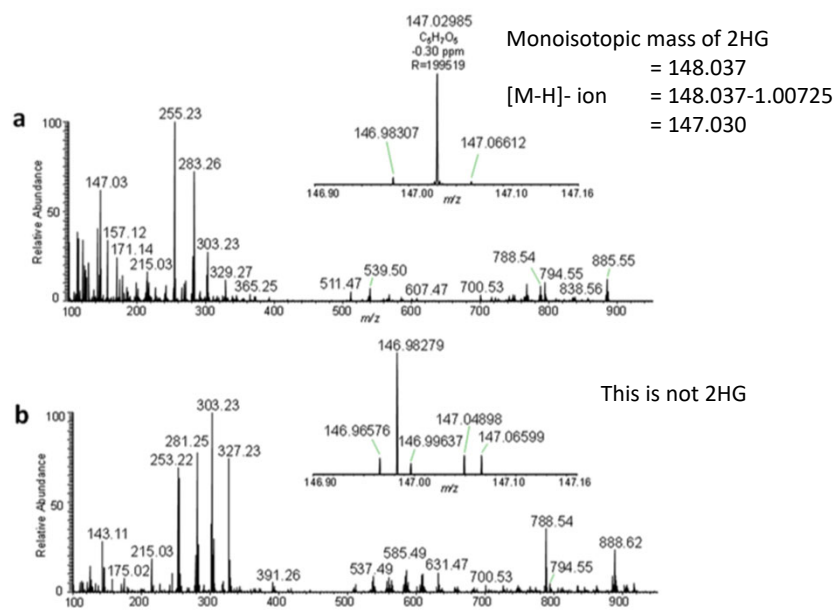
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Whither 2-hydroxyglutarate?



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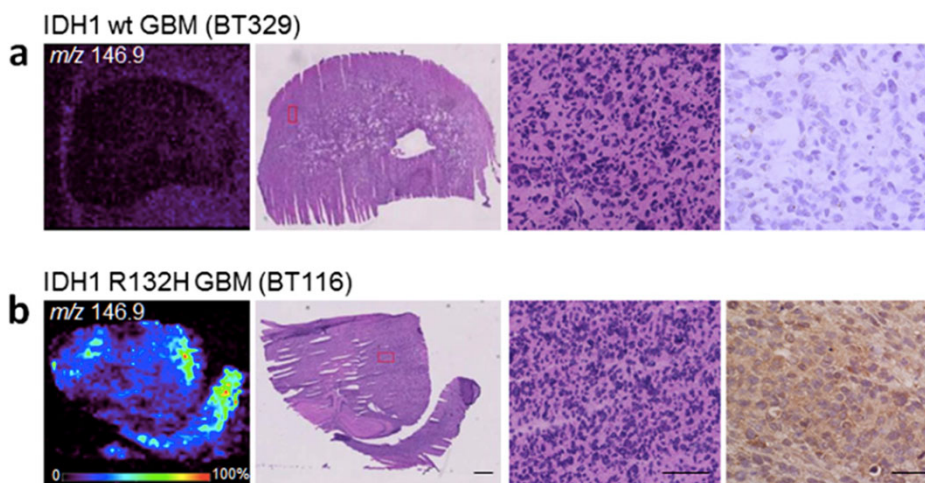
Value of exact mass – “147” vs “147”



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Tumor xenograft imaging and 2HG

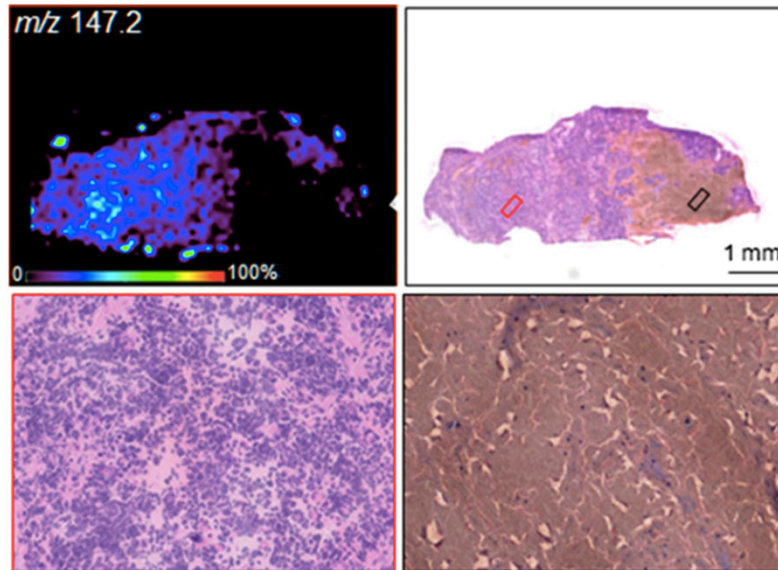
The ion at m/z 146.9 was subjected to MSMS to measure 2HG



Santagata et al. PNAS 2014

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Application to human glioblastoma



Santagata et al. PNAS 2014

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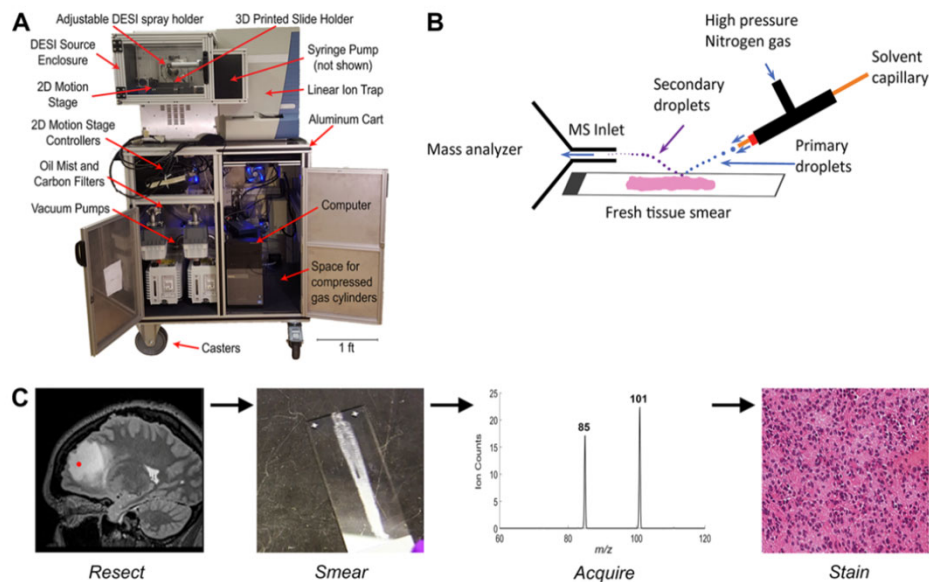
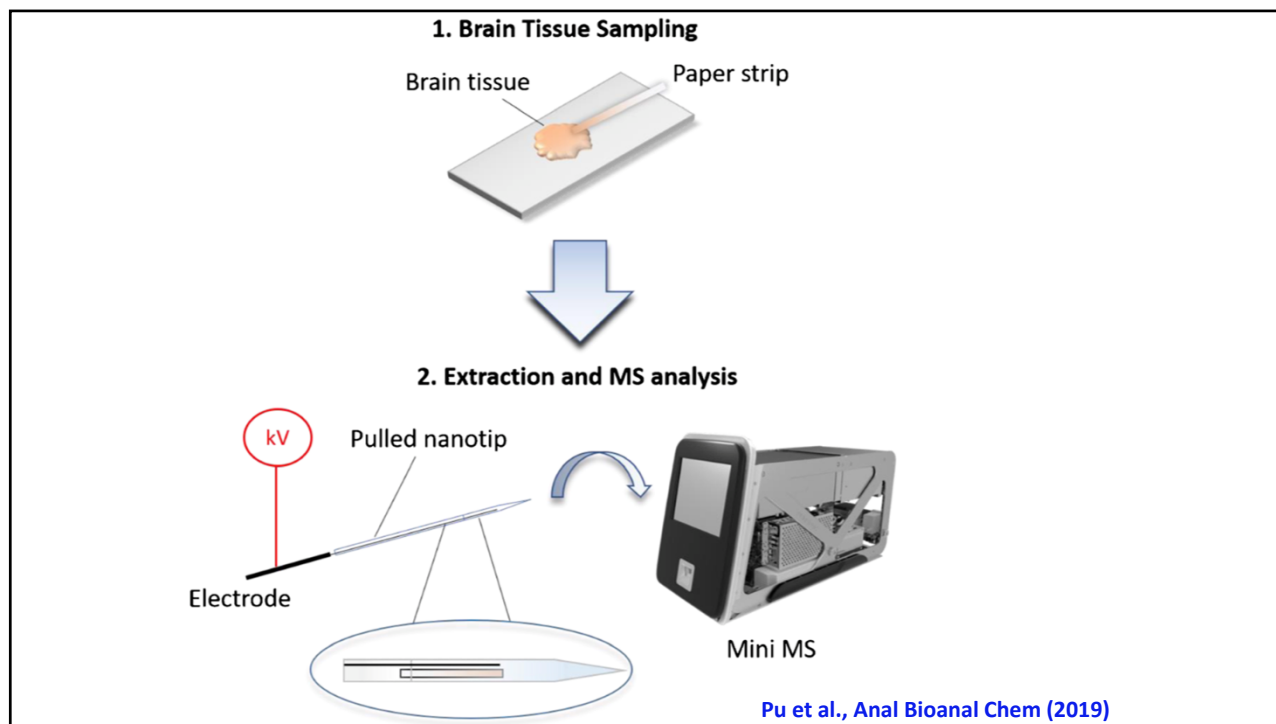


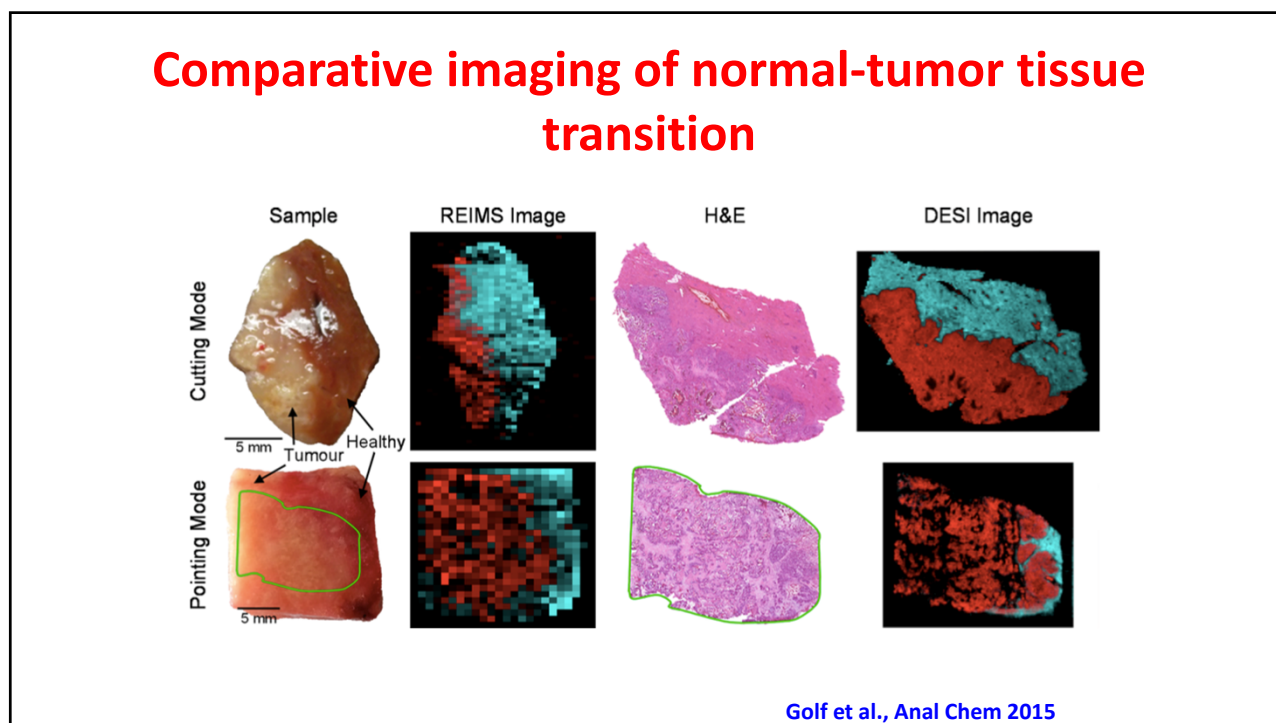
FIG. 1. DESI-MS method overview. **A:** Image of the custom-made intraoperative DESI-MS system for intraoperative analysis of tissue biopsies. **B:** Diagram of the DESI process as described by Takáts et al. **C:** Workflow of intraoperative analysis protocol consisting of tissue collection (*red spot*)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

Alfaro et al., J Neurosurg (2019) Jan 4 1-8

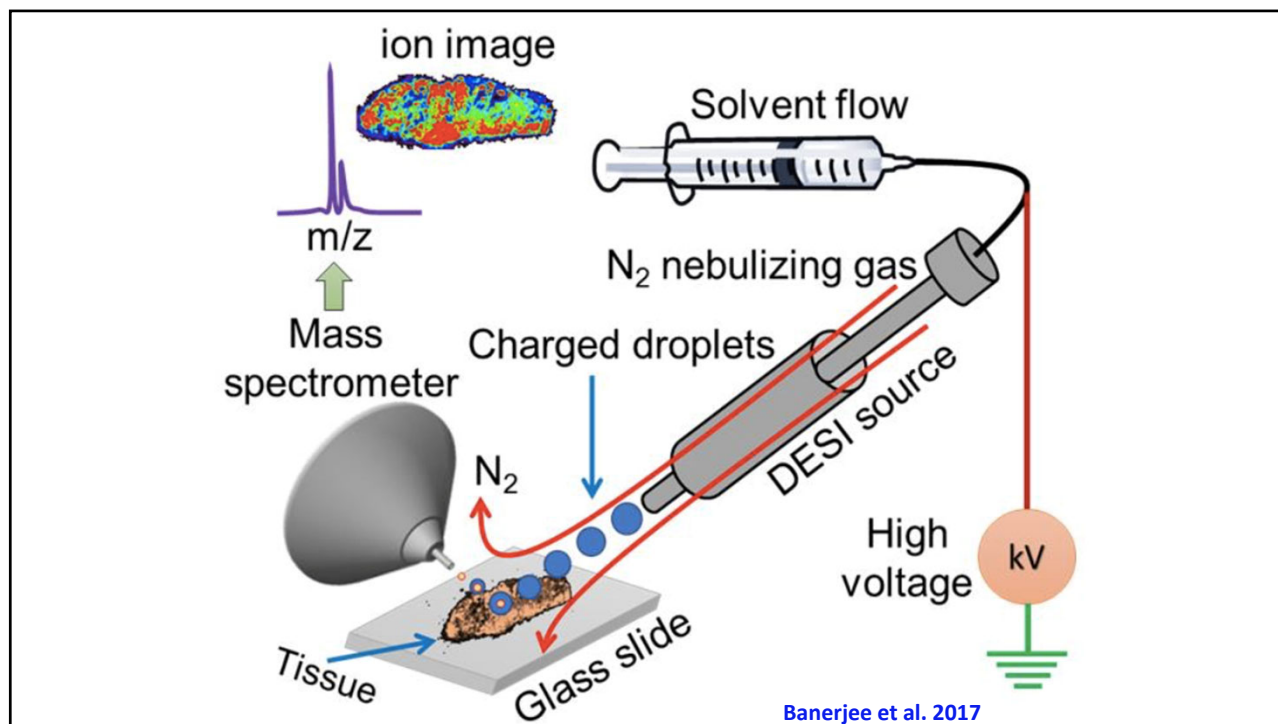
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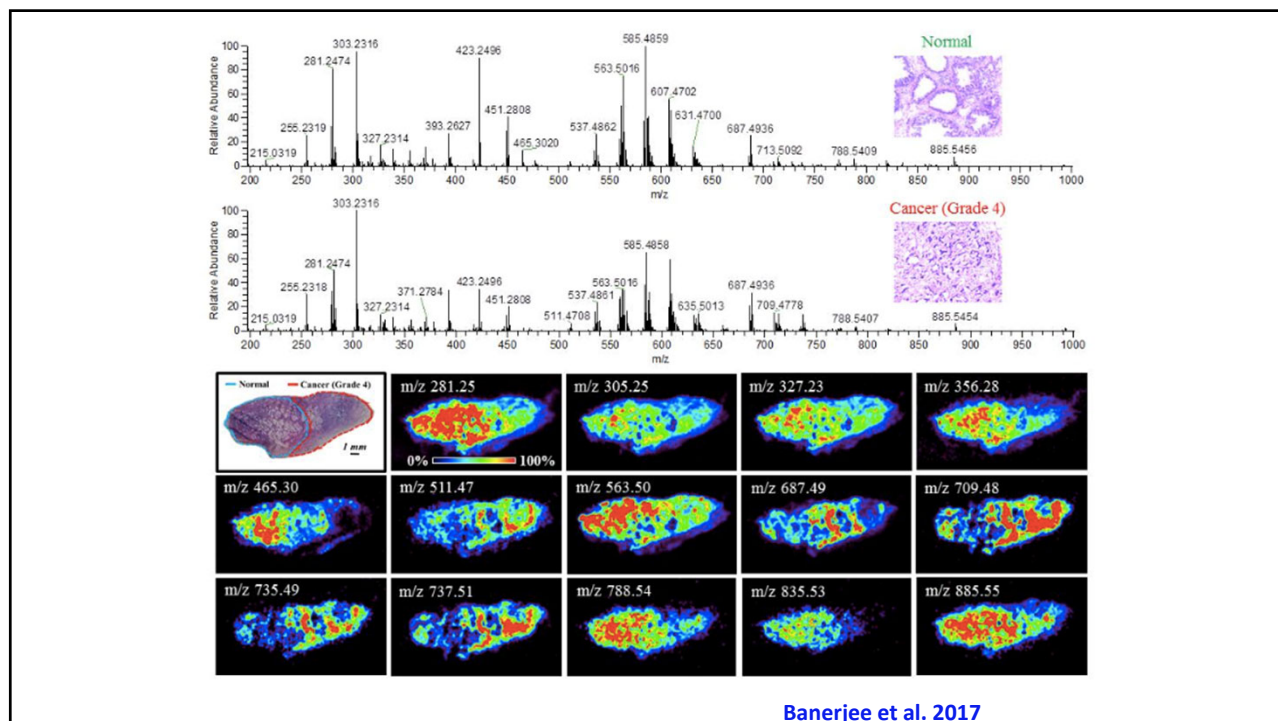
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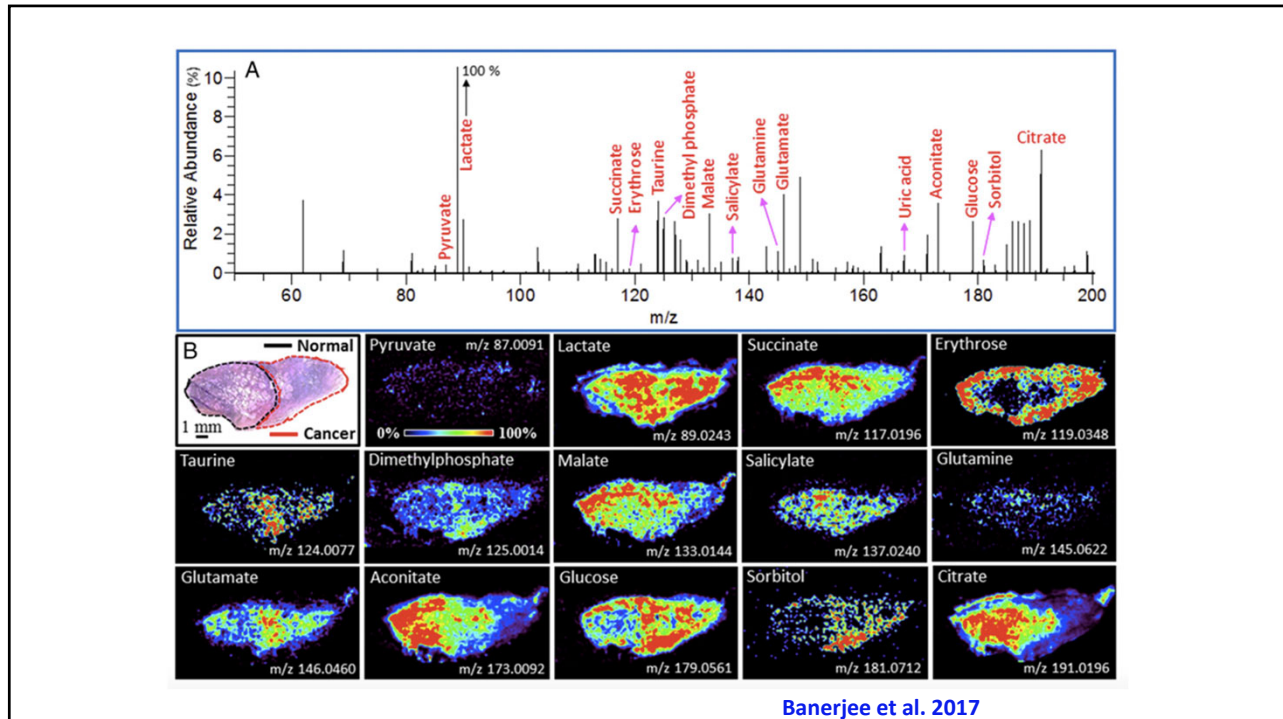
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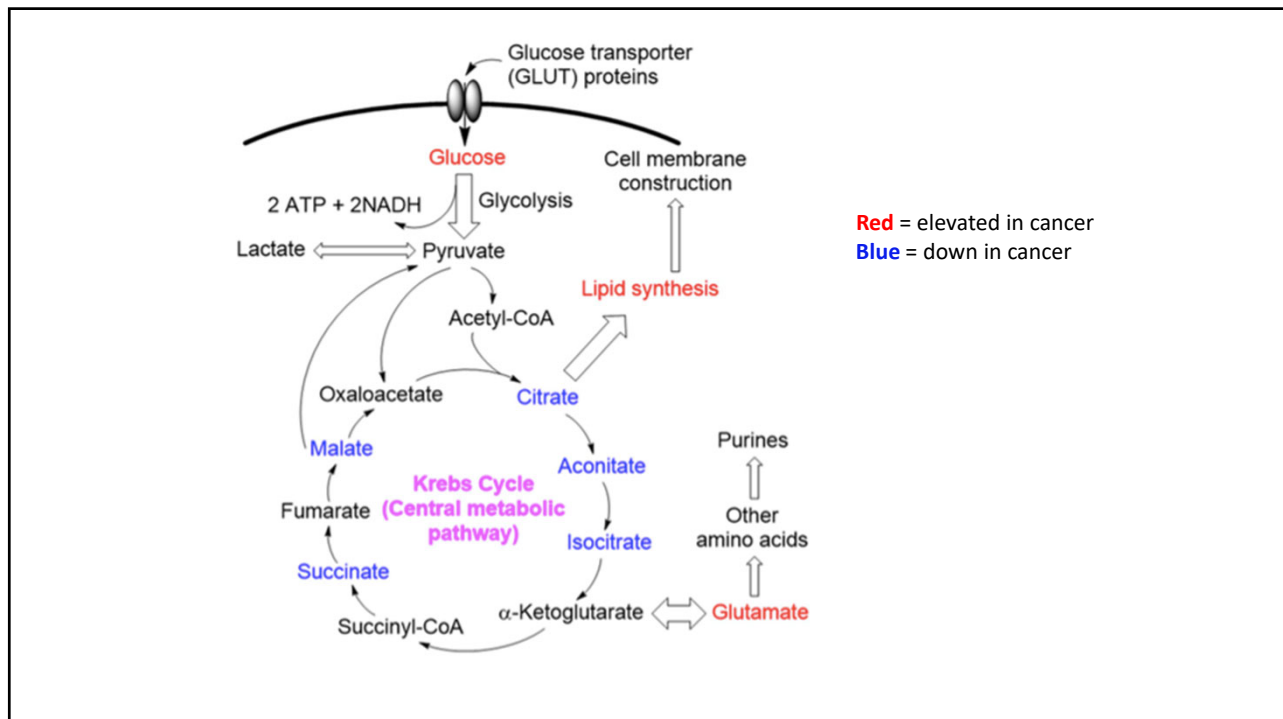
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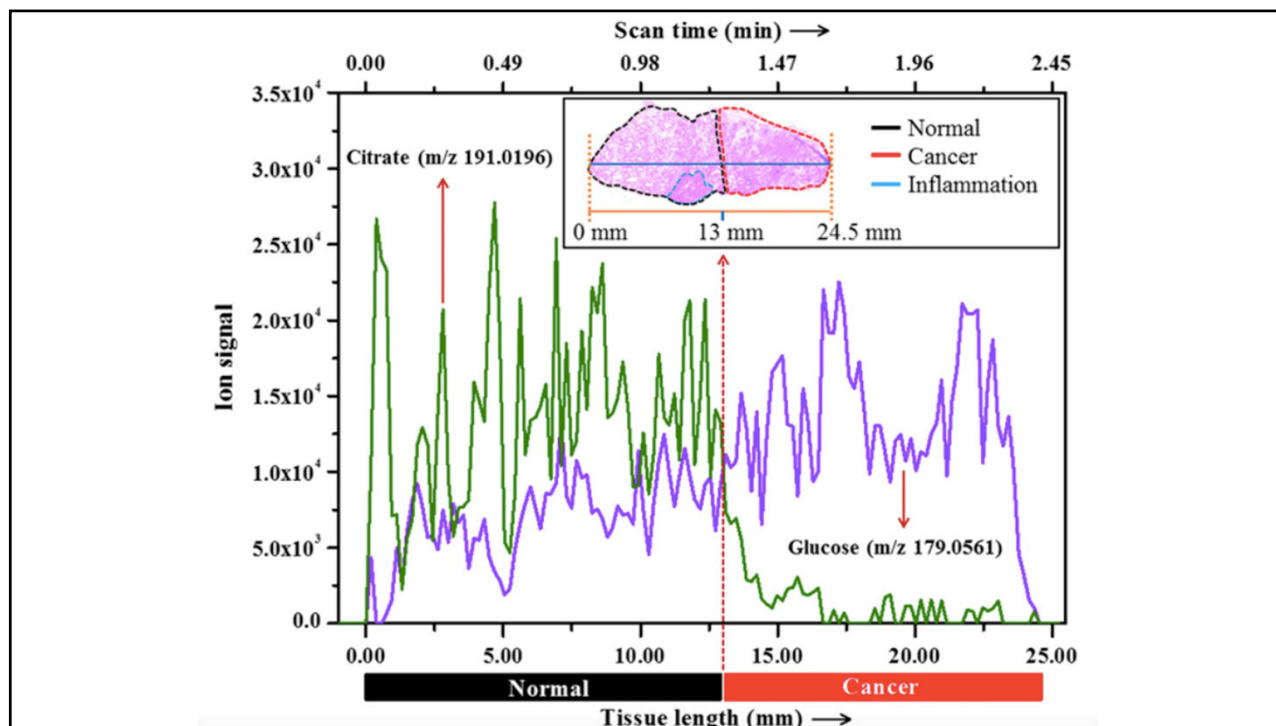
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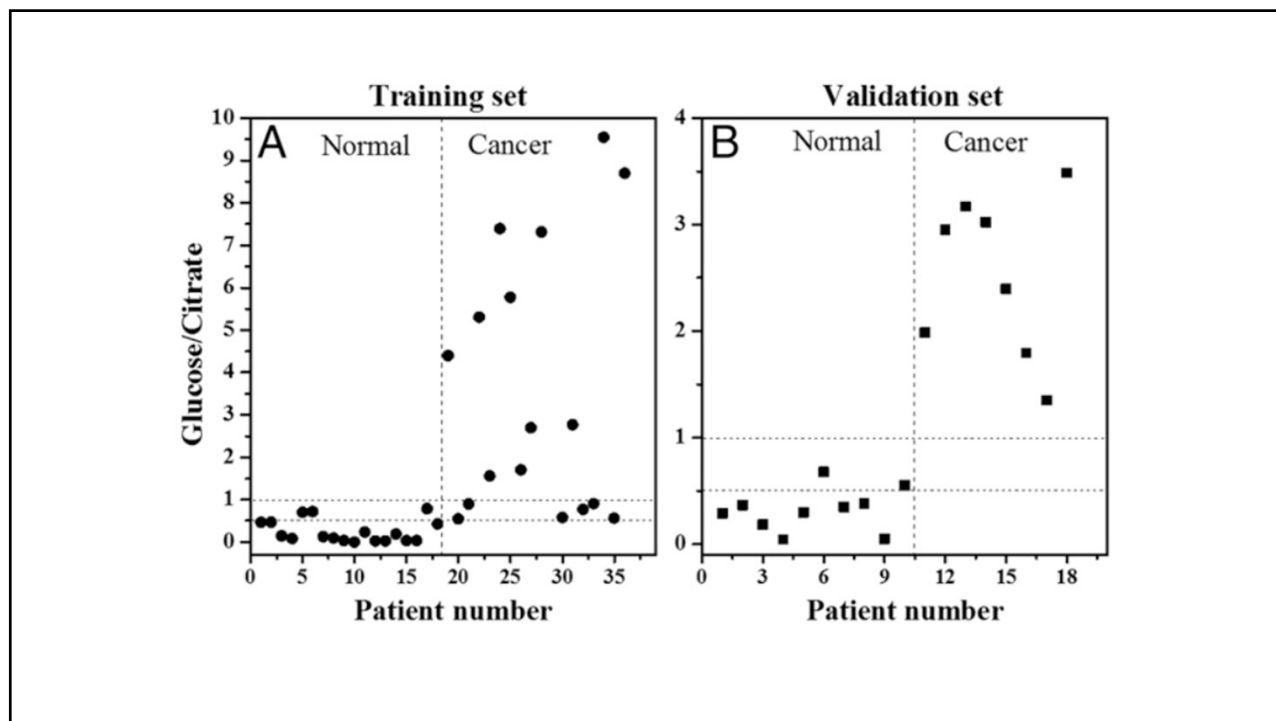
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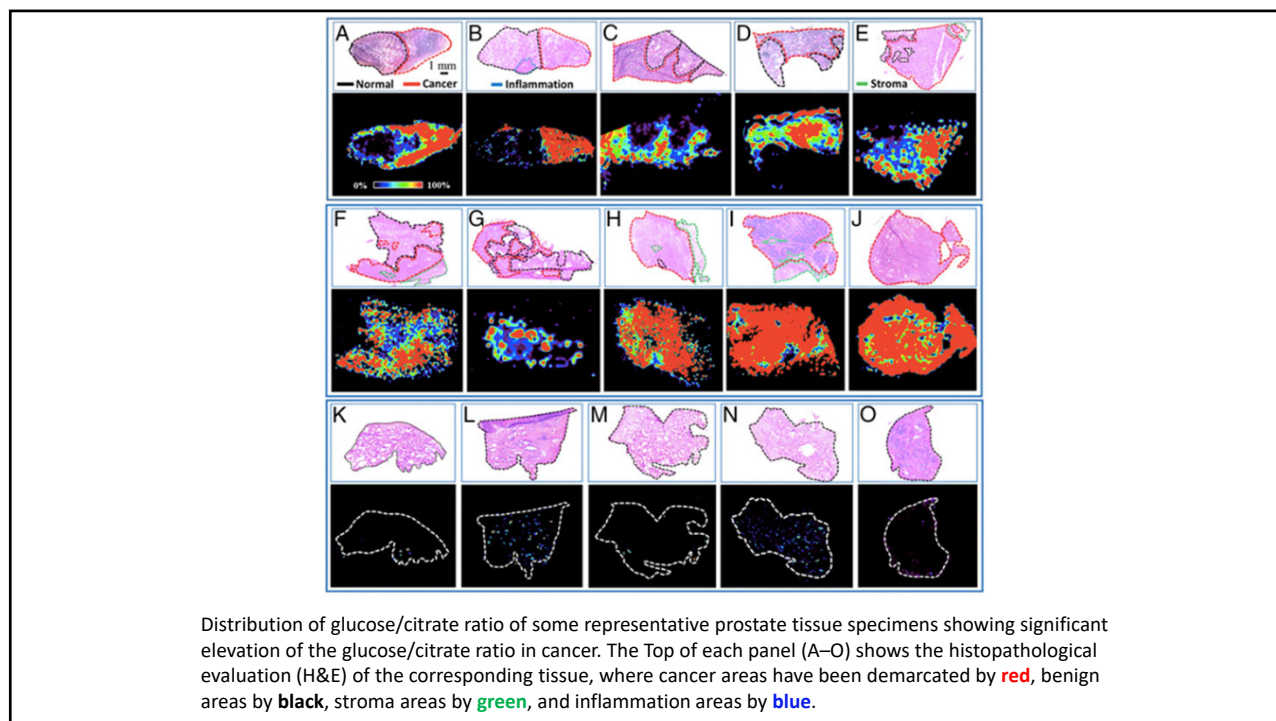
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Use of Raman spectroscopy Real-time imaging of metabolites in skin

- <http://harvard.sunneyxielab.org/research/carstechniques.htm>



Sunny Xie, PhD - Harvard

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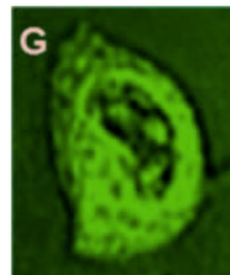
Where to next?



MALDI-Imaging of a phospholipid
Janusz Kabarowski/Kelly Walters



Multiple sampling single cells – Nemes group

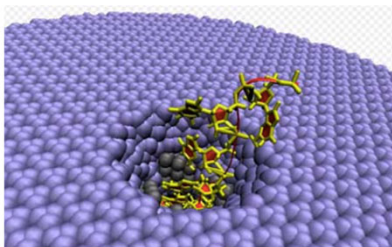


CARS imaging of a cancer cell –
spectroscopic, real time
Raman imaging

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?



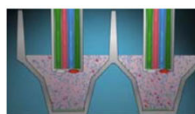
Nanoprobe inserted into the wall of a cell
recording changes in metabolism in real time –
sub nl sampling/analysis

TOMORROW?



Measuring O₂ uptake using a
Warburg apparatus – 10 ml
incubations

YESTERDAY



The O₂ 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.
- 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](#).
- 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](#)
- St John ER, Balog J, McKenzie JS, Rossi M, Covington A, Muirhead L, Bodai Z, Rosini F, Speller AVM, Shousha S, Ramakrishnan R, Darzi A, Takats Z, Leff DR. Rapid evaporative ionisation mass spectrometry of electrosurgical vapours for the identification of breast pathology: towards an intelligent knife for breast cancer surgery. [Breast Cancer Res. 2017 May 23;19\(1\):59](#).

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More publications

- Phelps DL, Balog J, Gildea LF, Bodai Z, Savage A, El-Bahrawy MA, Speller AV, Rosini F, Kudo H, McKenzie JS, Brown R, Takats Z, Ghaem-Maghami S. The surgical intelligent knife distinguishes normal, borderline and malignant gynaecological tissues using rapid evaporative ionisation mass spectrometry (REIMS). [Br J Cancer. 2018 May;118\(10\):1349-1358](#).
- 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;132\(1\):180-187](#).
- Pu F, Alfaro CM, Pirro V, Xie Z, Ouyang Z, Cooks RG. Rapid determination of isocitrate dehydrogenase mutation status of human gliomas by extraction nano-electrospray using a miniature mass spectrometer. [Anal Bioanal Chem. 2019 Feb 2. doi: 10.1007/s00216-019-01632-5](#).
- Hänel L, Kwiatkowski M, Heikaus L, Schlüter H. Mass spectrometry-based intraoperative tumor diagnostics. [Future Sci OA. 2019 Mar 7;5\(3\):FSO373](#).
- Tzafetas M, Mitra A, Paraskevaïdi M, Bodai Z, Kalliala I, Bowden S, Lathouras K, Rosini F, Szasz M, Savage A, Balog J, McKenzie J, Lyons D, Bennett P, MacIntyre D, Ghaem-Maghami S, Takats Z, Kyrgiou M. The intelligent knife (iKnife) and its intraoperative diagnostic advantage for the treatment of cervical disease. [Proc Natl Acad Sci U S A. 2020 Mar 16](#).

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