THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

4th UAB Metabolomics Workshop
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Future of Metabolomics

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Targeted Metabolomics & Proteomics Laboratory

Issues in metabolomics research

- Standards and methods standardization
- Derivatives and Isotope labeling
- Spatial distribution
  - Imaging mass spectrometry
  - Single cell analysis
- Capillary electrophoresis
- Other localized metabolomics
  - Head space GC-MS
    - Breath and other body odors
  - iKnife-MS
    - Metabolome of the "smoke" created with cauterized surgical knife
- Big picture - Phenomics
- Where next?

Standards

- The majority of compounds that are detected have not been fully characterized and don't exist in pure standard form
- The LipidMaps endeavor (www.lipidmaps.org) has gone a long way to improve the state of that field
- A heavy investment in chemical synthesis of other metabolites is needed

http://www.metabolomicsworkbench.org/standards/nominatecompounds.cgi

Nominate Compounds for Synthesis by the Metabolite Standards Synthesis Core (MSSC)

The NIH Common Fund's Metabolomics program aims to increase national capacity in metabolomics by supporting the development of next generation technologies to enhance the sensitivity and speed with which specific elements of the cellular metabolome can be identified and quantified, providing training and mentoring opportunities, increasing the inventory of chemically identifiable metabolites through the synthesis and availability of high quality reference standards, and by promoting data sharing and collaboration.

The form should be completed by researchers wishing to nominate a compound for synthesis by the NIH Common Fund's Metabolite Standards Synthesis Cores (MSSC). Nominated compounds will be reviewed by the NIH Common Fund's executive committee, and prioritized for synthesis.

Standardized methods

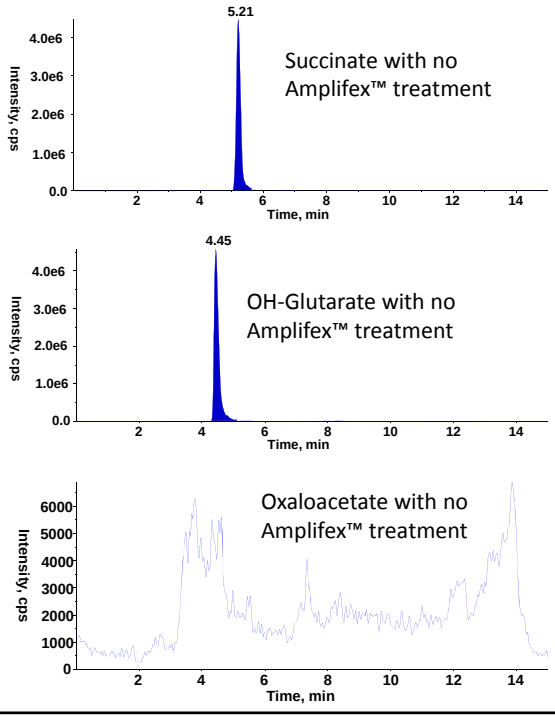
- Importance of standardized operating procedures and recording keeping of any deviations from them – use of LIMS
  - http://www.metabolomicsworkbench.org/
  - The protocols used in the studies deposited on the metabolomics workbench
  - They are often sample and/or compound-specific
    - plasma versus urine versus cells versus tissue
    - untargeted versus a specific class, e.g., bile acids

Choice of platform

- NMR yields absolute, quantitative data, but for a limited number of metabolites
- Whereas LC-MS is very sensitive, quantification is a challenge
- For primary untargeted metabolomics data collection, the Qtof instrument is both the fastest and most robust
  - The UK NIHR Phenomics Center is using a single manufacturer's Qtof - harmonization

Back to derivatization in metabolomics

- A great advantage of LC-MS is its ability to measure compounds "as is"
- However, there are many compounds that either weakly form ions or are found in multiple chemical forms (e.g., steroids, ketosteroids, keto acids, fatty acids)
- The reagents used to overcome this problem can be isotopically labeled (iMetab)
- Could be applied to NMR, too (^{13}C- and 15N-labeled)



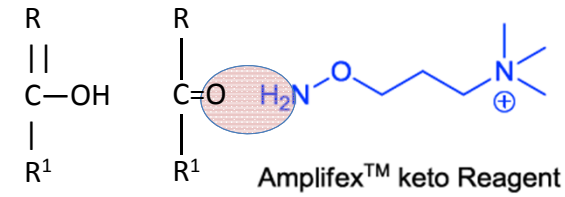
Individual MRM channels for citric acid cycle intermediates

For most compounds, the MRM signal yields a single peak

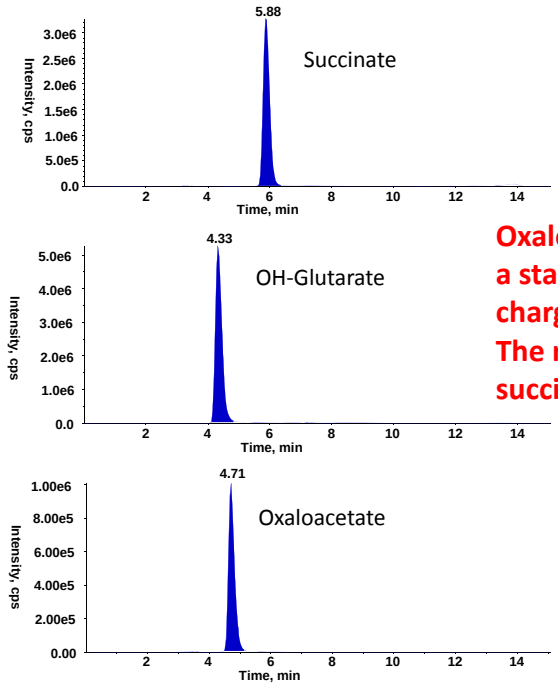
Note that the signal for oxaloacetate is weak and "ugly" – keto acids can undergo keto-enol tautomerism

Modifiers to the keto group

- **Methoxylamine – CH_3ONH_2 (from GC-MS)**
- **Biotin hydrazide – $RN.NH_2$**
- **Amplifex™-Keto Reagent (ketosteroids)**

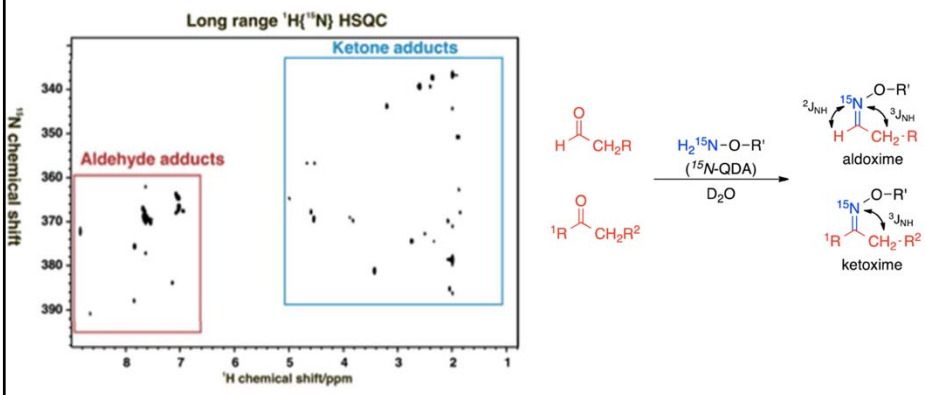


Enol Keto



Oxaloacetate is converted to a stabilized, positively charged oxime.
The reagent does not alter succinate or 2-OH glutarate

NMR ketone/aledehyde reagent



Lane et al. Magn Reson Chem 53:337 (2015)

Isotopic derivatization

- investigators are exploring reagents that can be coupled to $-NH_2$ and –COOH groups
  - c.f., ICAT, iTRAQ and TMT reagents for proteomics
- These may allow one sample of a pair to be labeled with a "light" reagent and another sample with the same reagent, but one incorporating ^{2}H, ^{13}C or ^{15}N atoms and therefore being "heavy".
  - The two samples can then be mixed and run at the same time
  - Multiplexed reagents are also possible
- Software to sort this out has not yet been written

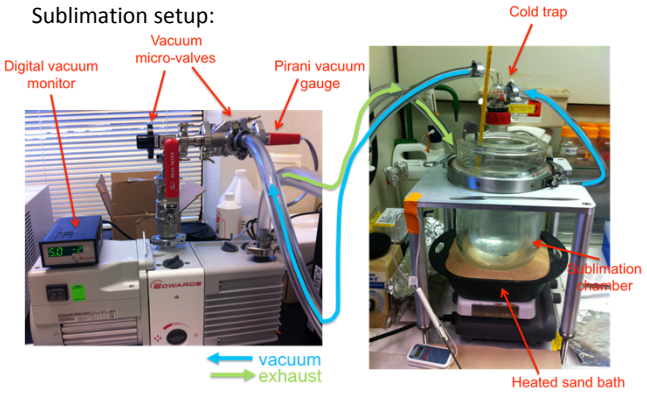
Where are the metabolites?

Understanding where metabolites are

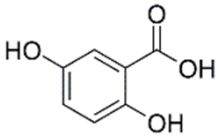
- When we collect the metabolites from tissue or from cells, we destroy their localization information
- We assume that all metabolites are in contact with each other
- But there as many as 15 distinct compartments in cells
- Some cells rely on neighboring cells for specific metabolites

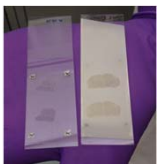
Matrix application – vacuum sublimation

Sublimation setup:



2,5-dihydroxybenzoic acid

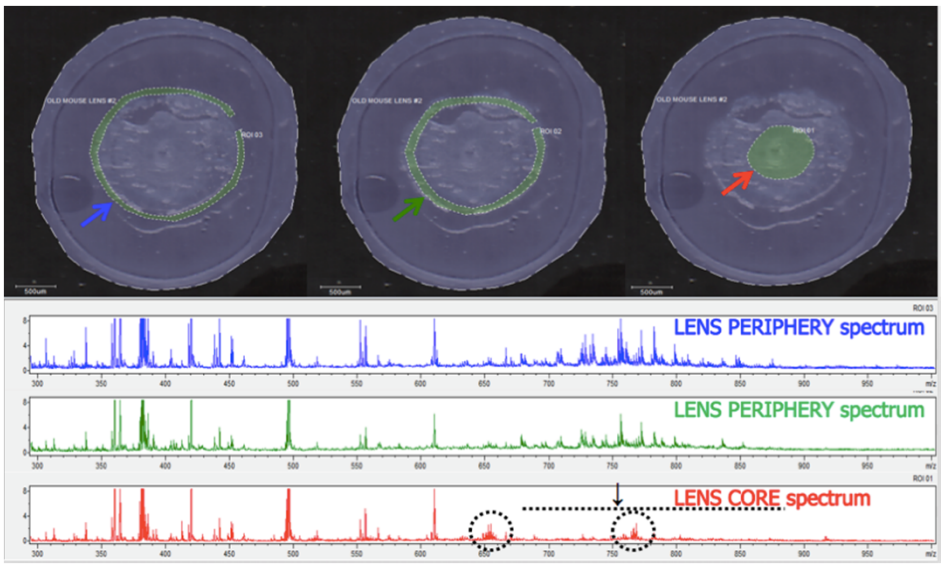




Miranda Collier/Janusz Kabarowski

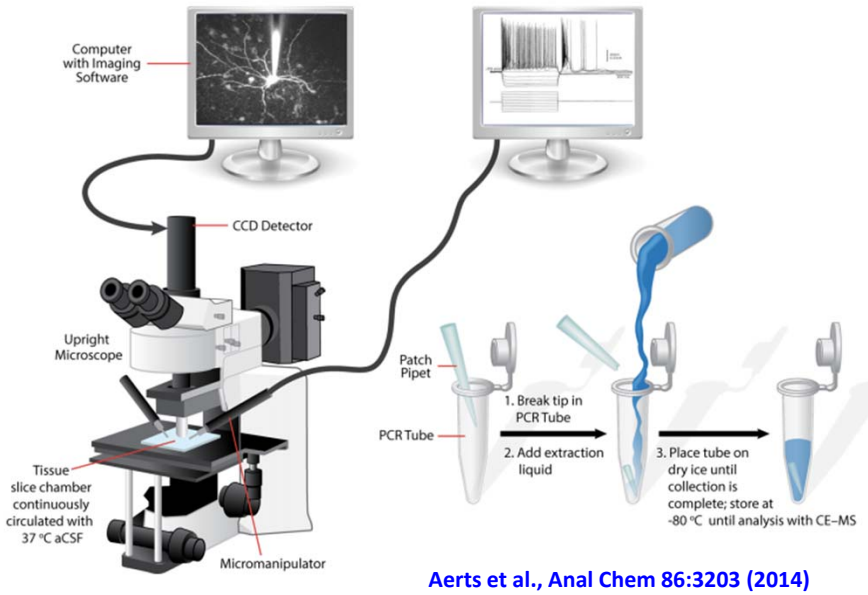
Power of imaging-MS

(Kabarowski, Walters and Barnes)

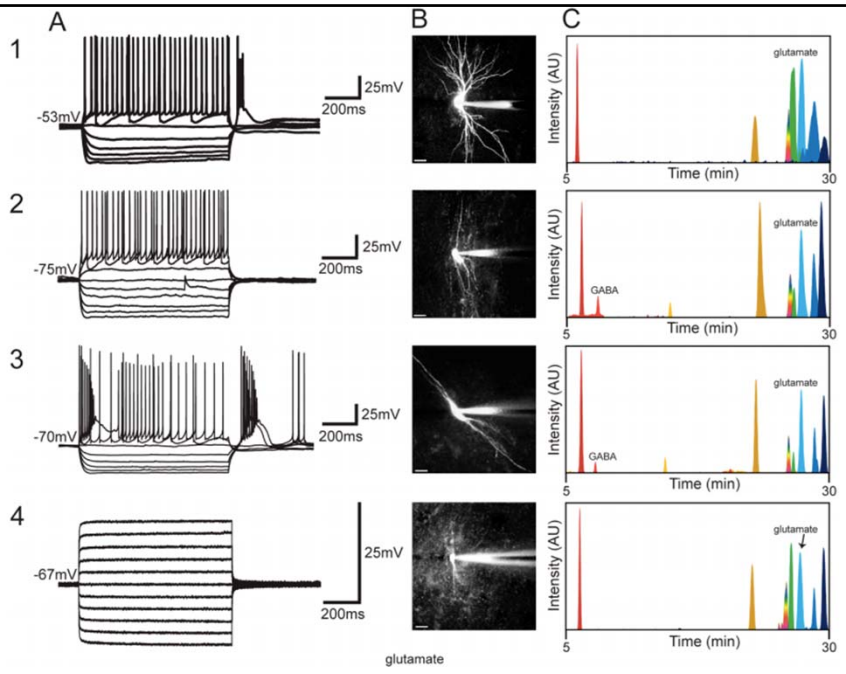


This is a section of a 12-month old mouse eye. The inner region is the lens. Spectra from the newly formed lens epithelial cells contain phospholipids. In the core of the lens they are gone.

Single cell sampling



Aerts et al., Anal Chem 86:3203 (2014)



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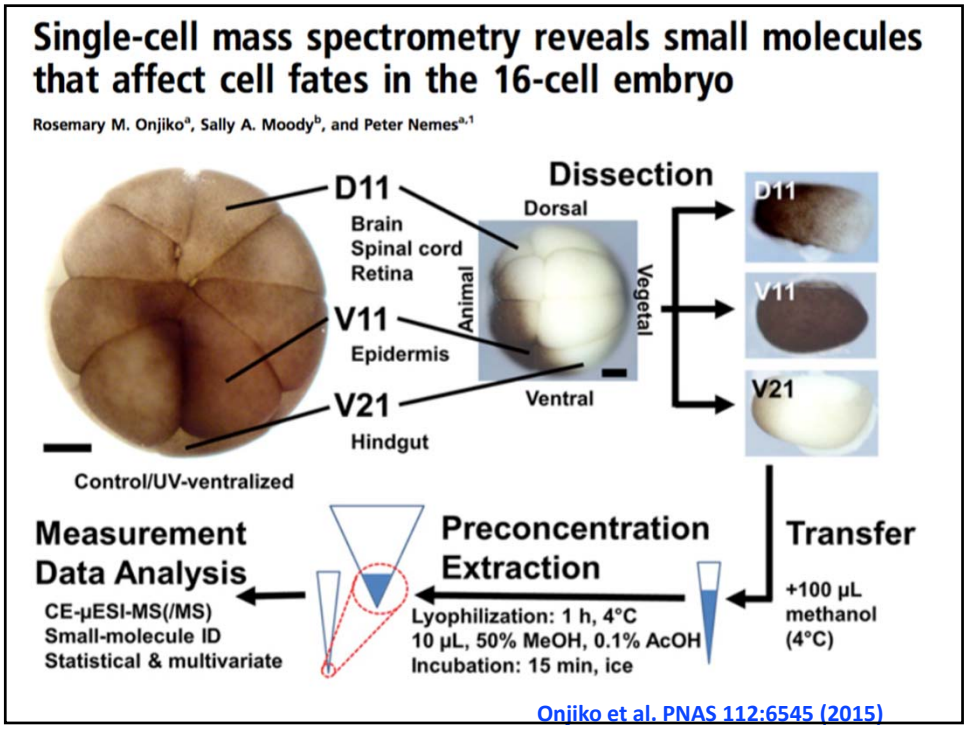
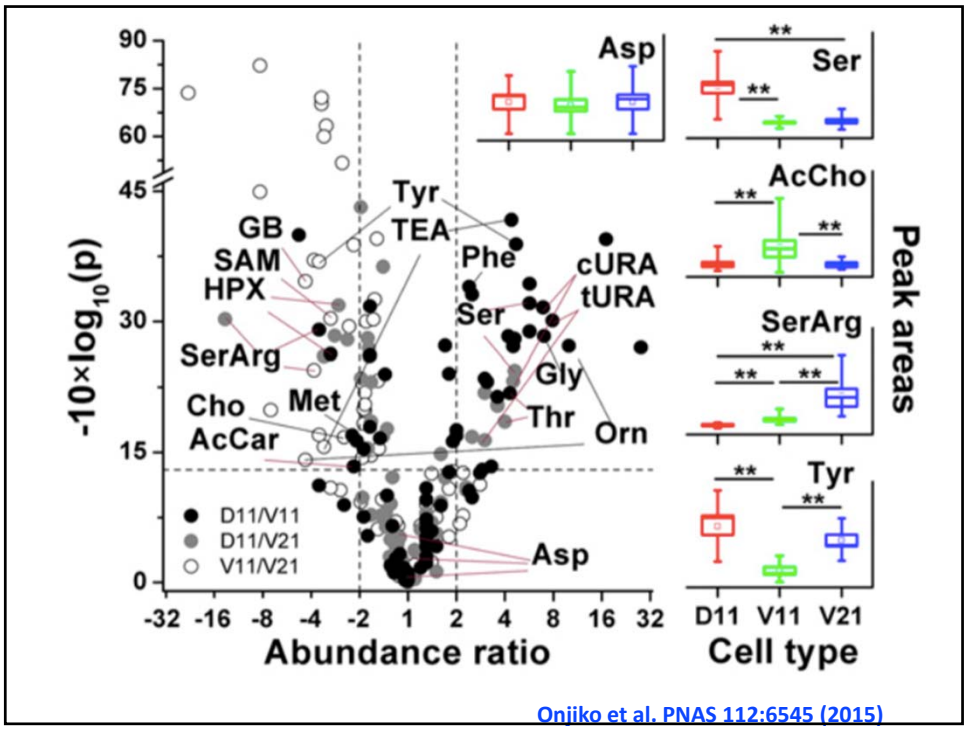
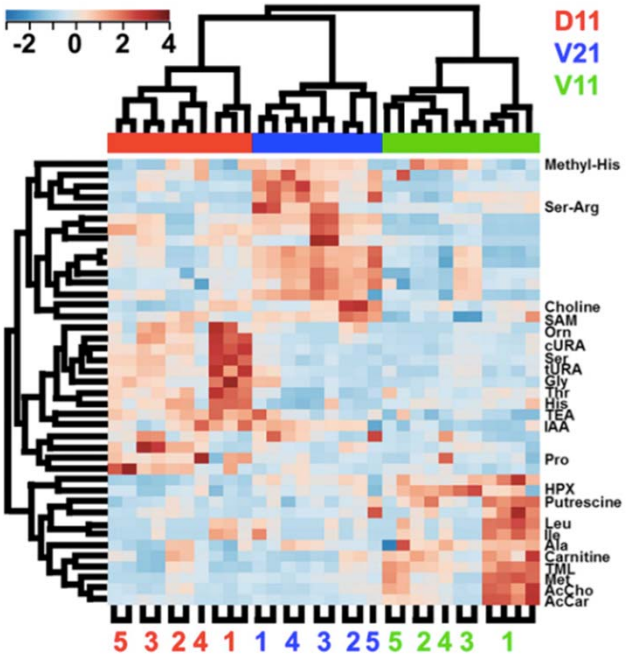

Single-cell mass spectrometry reveals small molecules that affect cell fates in the 16-cell embryo
Rosemary M. Onjiko[a], Sally A. Moody[b], and Peter Nemes[a,1]
D11
Brain
Spinal cord
Retina
V11
Epidermis
V21
Hindgut
Control/UV-ventralized
Dissection
Dorsal
Animal
Vegetal
Ventral
D11
V11
V21
Transfer
+100 µL
methanol
(4°C)
Preconcentration
Extraction
Lyophilization: 1 h, 4°C
10 µL, 50% MeOH, 0.1% AcOH
Incubation: 15 min, ice
Measurement
Data Analysis
CE-µESI-MS(/MS)
Small-molecule ID
Statistical & multivariate
Onjiko et al. PNAS 112:6545 (2015)


-10×log10(p)
Abundance ratio
Cell type
Peak areas
D11/V11
D11/V21
V11/V21
Tyr
TEA
GB
SAM
HPX
SerArg
Cho
AcCar
Met
Phe
Ser
cURA
tURA
Gly
Thr
Orn
Asp
AcCho
SerArg
Tyr
D11
V11
V21
Onjiko et al. PNAS 112:6545 (2015)



Onjiko et al. PNAS 112:6545 (2015)

Capillary electrophoresis-MS and metabolomics

- "*Capillary electrophoresis and MS is a marriage made in heaven, but not on earth*" (Richard Smith, 1994)
- Can CE-MS provide for metabolomics what CE has done for DNA deep sequencing?
- CE has many forms and can accommodate a wide variety of even hydrophilic compounds
- The interface is the key component

Why CE-MS?

- Commercialized, new version of CE-MS has been developed
  - https://vimeo.com/121631544
  - No dilution and compatible across different instruments
- The sheer number of metabolites demands a better chromatographic solution
  - CE-MS is one answer
- Advantages of CE-MS is that it is an open tubular design where the capillary is also the emitter
  - Particularly effective for hydrophilic compounds

Improving LC in LC-MS

- Taking a leaf from the gas chromatography community and CE-MS, LC needs to move towards open tubular columns
  - The back pressure of smaller and smaller particles is limiting
  - So, get rid of the particles
  - Engineering challenges
- The very sharp peaks (width 1 sec or less) not compatible with ion motion Orbitrap or FT-ICR analyzers – need Qtof mass spectrometers

Metabolism and time

- Not only should metabolites appear in the right place, there is also the question of the importance of the timescale
- Metabolism defects in the heart may be only seconds away from death – rogue wave in metabolism??
- Irreversible damage to the brain may occur in minutes
- Go/No-Go decisions for a cell to divide or apoptose may occur in a similar timescale

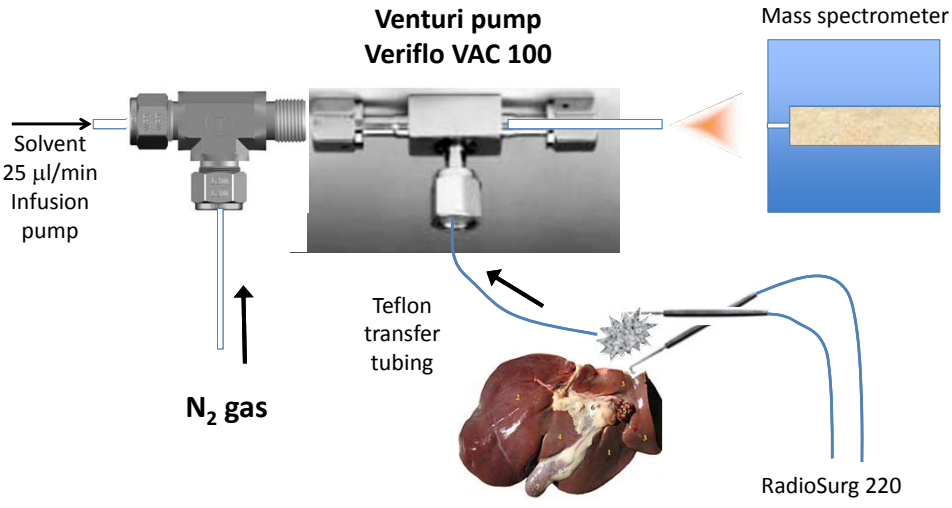
Detecting smells

- Unlike dogs, humans have relatively few olefactory receptors (dogs have lousy taste!)
  - We can detect certain "bad smells" (and cover these by washing and deodorants and fragrances)
- The reality is that we all emit gases that can be diagnostic
  - GC-MS using a headspace technique can be used
    - Breath hydrogen is diagnostic for lactase insufficiency

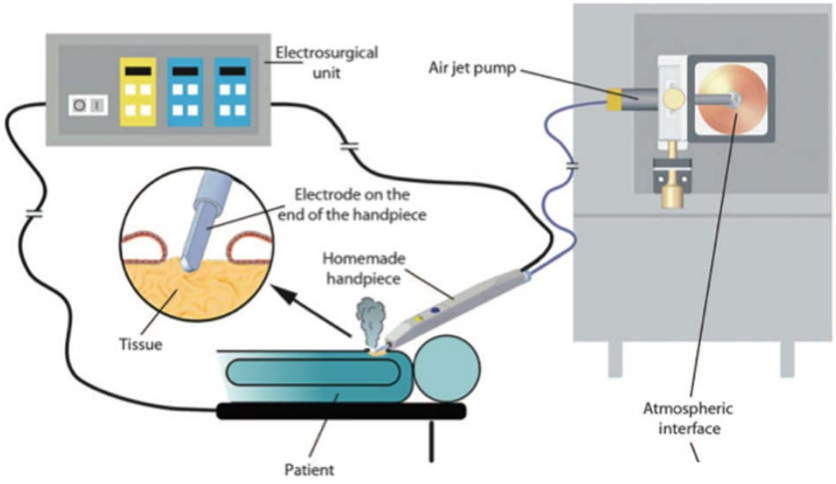
Portable Metabolomics Devices

David Wishart

iKnife device

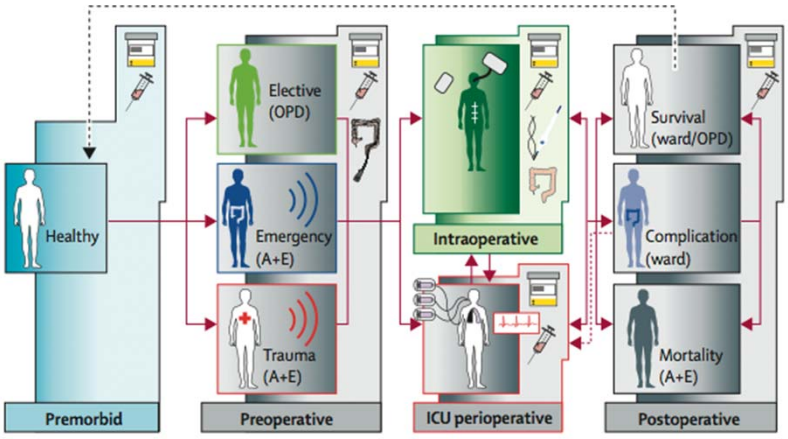


The iKnife – real time metabolomics



Balog et al. Nature Med 5; 194r93

Clinical applications of metabolomics



Concepts of Jeremy Nicholson

Hardware/software innovation

- **Acquiring data compatible with the surgical/clinical timescale**
  - iKnife (2-3 sec)
  - Rapiflex™, a MALDI-TOF-MS with a 10 kHz laser
    - Allows for collection of images of frozen sections (2 x 2 cm) with 20-30 μm resolution in <15 min
- **Requires efficient software**
- **Parallels to other -omics**

International Phenome Centers

The UK NIHR Phenome Center (2012)
http://www1.imperial.ac.uk/phenomecentre/

University of Birmingham Phenome Center (2016)
http://www.birmingham.ac.uk/research/activity/phenome-centre/index.aspx

Professor Sir Mark Wolpert, Chief Government Scientific Officer
https://www.youtube.com/watch?v=fcSp2N_qLCA&feature=youtu.be

Professor Mark Viant
https://www.youtube.com/watch?v=sOEMN-gIWGE&feature=youtu.be

Singapore Phenome Center (2015)

Can existing techniques provide answers?

- **Mass spec is mostly a destructive method**
  - **However, it can measure volatiles rapidly on a sec timescale**
- **NMR can provide spatial and descriptive information on living or unextracted materials**
  - **However, sensitivity and speed of data acquisition are extremely limiting**
- **Future in other spectroscopic techniques?**

Where next?

- **"Whatever you're using now to measure metabolites, in 10 years it will be something different"**
  - *Stephen Barnes, Metabolomics Society 2013*
- **"Throughout history, people with new ideas—who think differently and try to change things—have always been called troublemakers."**
  - *Richelle Mead, Shadow Kiss*

Questions