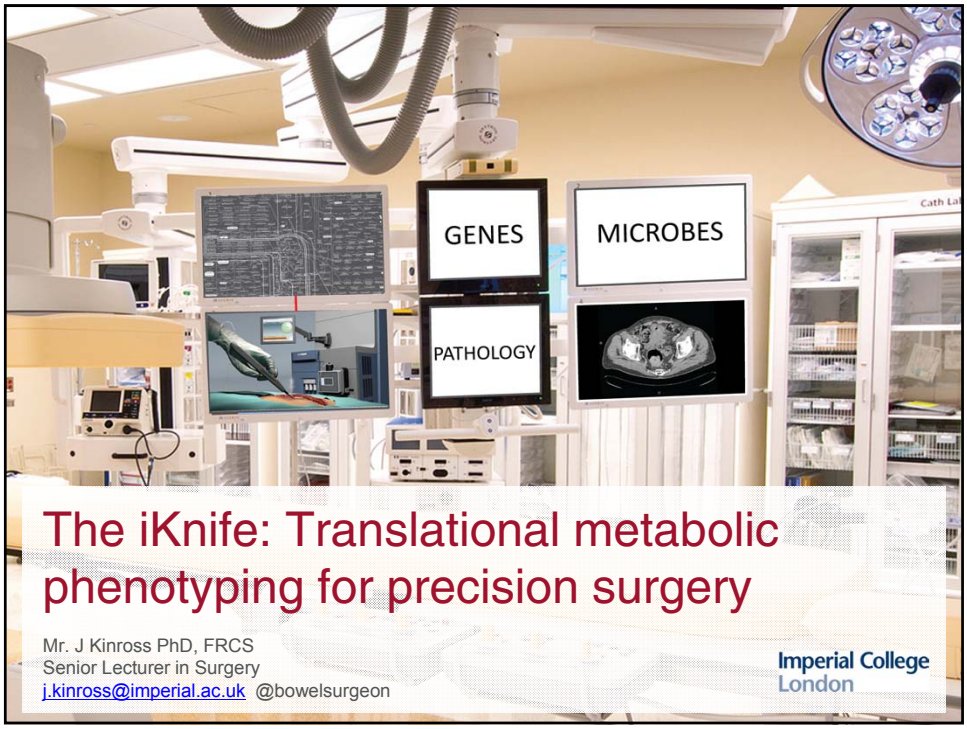

The iKnife: Translational metabolic phenotyping for precision surgery

Mr. J Kinross PhD, FRCS
Senior Lecturer in Surgery
j.kinross@imperial.ac.uk @bowelsurgeon

Imperial College London

Introduction: Overview

- Why do we need precision surgery? And what is it?
- Why can't we sequence our way out of poor cancer outcomes?
- What is a phenome?
- What is ambient mass spectrometry?
- DESI imaging – Why is it relevant to surgery?
- REIMS – how does it work?
- Current overview of work:
  - Ovarian
  - Breast
  - Colorectal
  - Microbiology in chronic disease
- Future vision – Supersystem surgery

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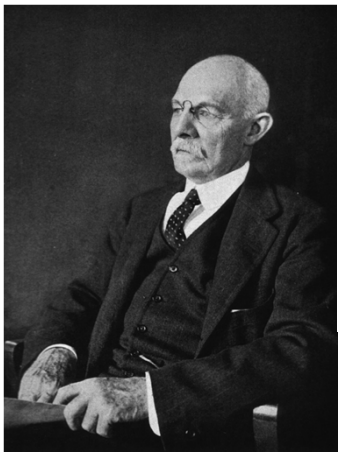
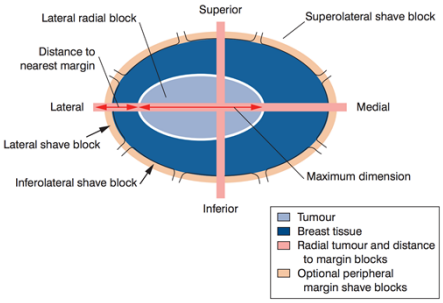
Surgical Oncology: History





PATHOLOGY REPORTING OF BREAST DISEASE A Joint Document Incorporating the Third Edition of the NHS Breast Screening Programme's Guidelines for Pathology Reporting in Breast Cancer Screening and the Second Edition of The Royal College of Pathologists' Minimum Dataset for Breast Cancer Histopathology. 2005.

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Evolution in minimally invasive oncological surgery

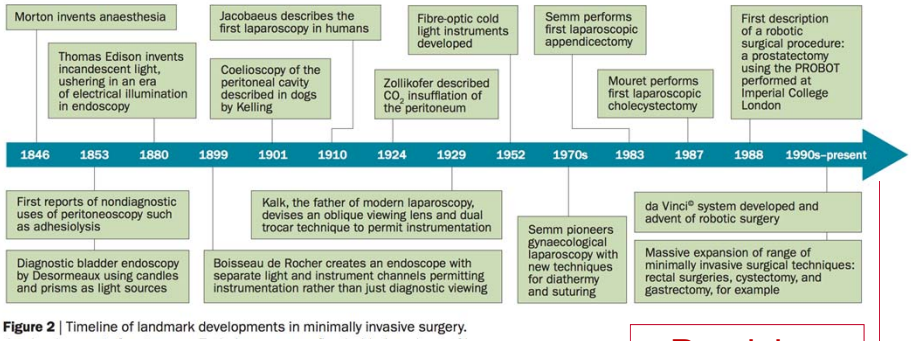

Figure 2 | Timeline of landmark developments in minimally invasive surgery.

Precision Surgery

- The maintenance of form and function
- The preservation of quality of life

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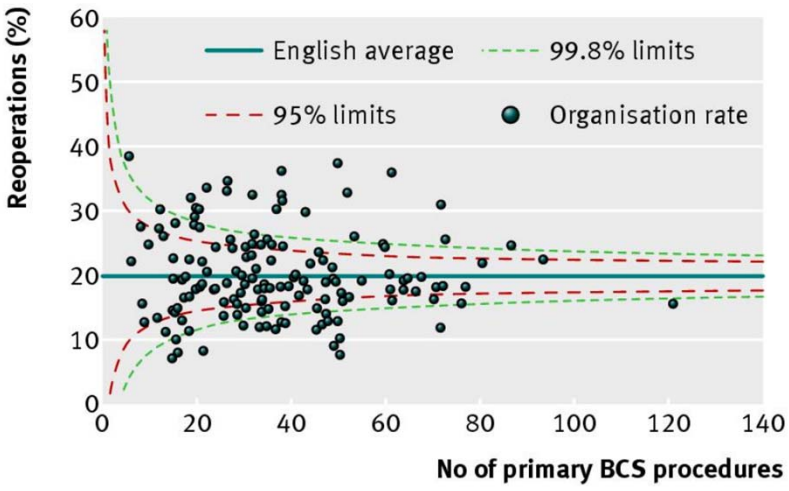
Not all units created equally: Systems failure



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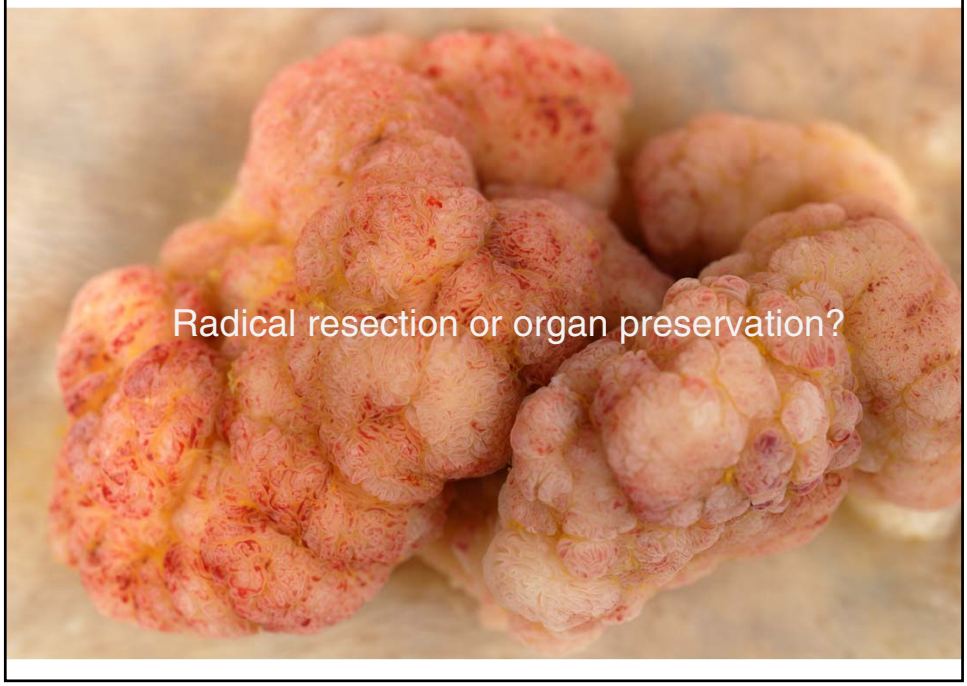
BMJ 2012;345:e4505 doi: 10.1136/bmj.e4505 (Published 12 July 2012)

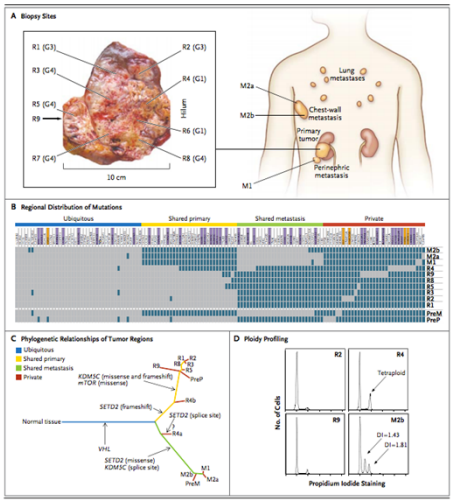


Precision oncology: Genomics


The NEW ENGLAND JOURNAL of MEDICINE

MARCH 8, 2012

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing



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Precision oncology: Molecular phenotyping

nature medicine

CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

Gunny et al. The consensus molecular subtypes of colorectal cancer subtypes. Nature medicine. 2015. doi:10.1038/nm.3967

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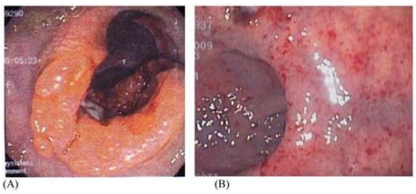
Stratified Surgery: Stratified therapy



"At present no robust markers of prediction of pCR have been identified and the topic remains an area for future research."

Ryan, E.J. et al. Predicting pathological complete response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer: A systemic review. Colorectal disease. 2015.

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Precision Medicine

"Coupling established clinical–pathological indexes with state-of-the-art molecular profiling to create diagnostic, prognostic, and therapeutic strategies precisely tailored to each patient's requirements".

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What is a Phenome?

Analytical

An integrated set of measurable physical and clinical features coupled to chemical, metabolic and physiological properties that define biological sub-classes

Philosophical

The direct product of gene-environment (exposome) interactions on an individual or group operating throughout development and life - a *dynamic* property

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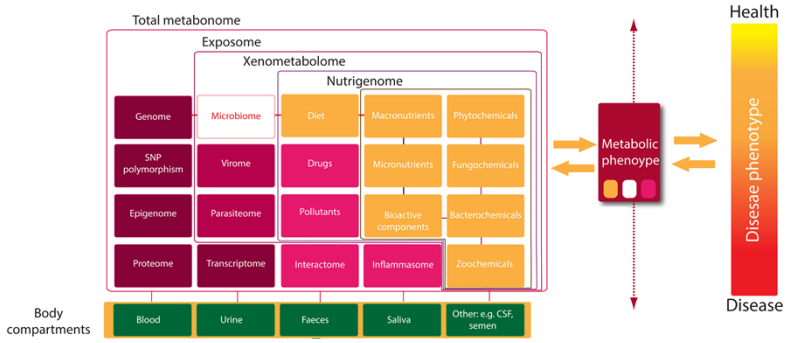

Nutritional modulation of the metabonome: applications of metabolic phenotyping in translational nutritional research

James Kinross[a], Jia V. Li[b], Laura J. Muirhead[a], and Jeremy Nicholson[a,b]

Volume 30 • Number 2 • March 2014

Precision = Systems Surgery

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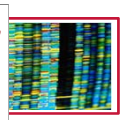
Precision Surgery: The patient journey

Clinical data integrati

Integration clinical to



ics integration/ networks

Computing / big data

SCALABLE AND TRANSLATABLE MODELS:

CANCER CHEMOTHERAPY

CARDIOVASCULAR

NEUROENDOCRINE DISEASE

RARE DISEASES

GUT SURGERY, SURGICAL ONCOLOGY

CRITICAL CARE

LIVER DISEASES

RENAL TRANSPLANTATION

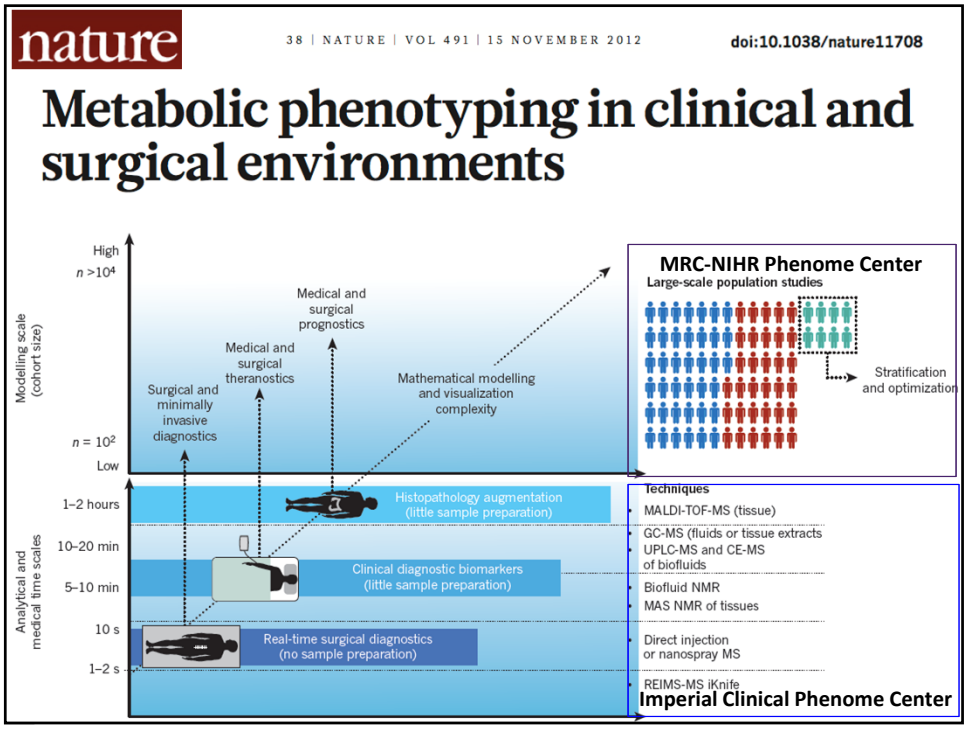
nature

NHS National Institute for Health Research

Waters THE SCIENCE OF WHAT'S POSSIBLE.™

REVIEW

Metabolic phenotyping in clinical and surgical environments


nature
38 | NATURE | VOL 491 | 15 NOVEMBER 2012
doi:10.1038/nature11708
Metabolic phenotyping in clinical and surgical environments
Modelling scale (cohort size)
High
n >10^4
n = 10^2
Low
Medical and surgical prognostics
Medical and surgical theranostics
Surgical and minimally invasive diagnostics
Mathematical modelling and visualization complexity
MRC-NIHR Phenome Center
Large-scale population studies
Stratification and optimization
Analytical and medical time scales
1–2 hours
10–20 min
5–10 min
10 s
1–2 s
Histopathology augmentation (little sample preparation)
Clinical diagnostic biomarkers (little sample preparation)
Real-time surgical diagnostics (no sample preparation)
Techniques
MALDI-TOF-MS (tissue)
GC-MS (fluids or tissue extracts
UPLC-MS and CE-MS of biofluids
Biofluid NMR
MAS NMR of tissues
Direct injection or nanospray MS
REIMS-MS iKnife
Imperial Clinical Phenome Center


FLEMING
DISCOVERED
PENICILLIN
BRUKER
AVANCE III
SAMPLEJET

Ambient Mass Spectrometry: Theory

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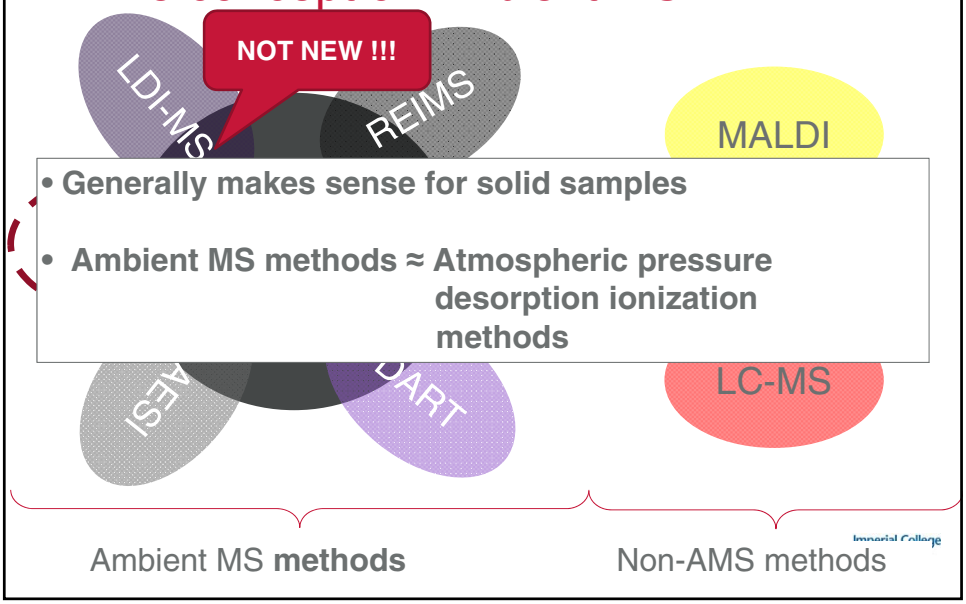
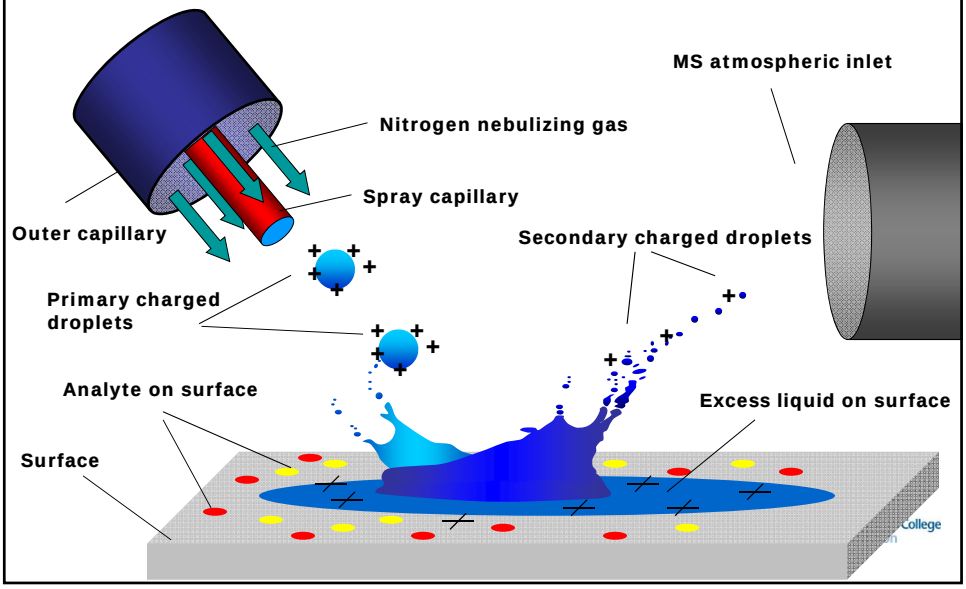
The concept of Ambient MS



Desorption Electrospray Ionization (DESI)



Advantages of Ambient MS Profiling

- Low $H_{desorption}$
- Ionic character

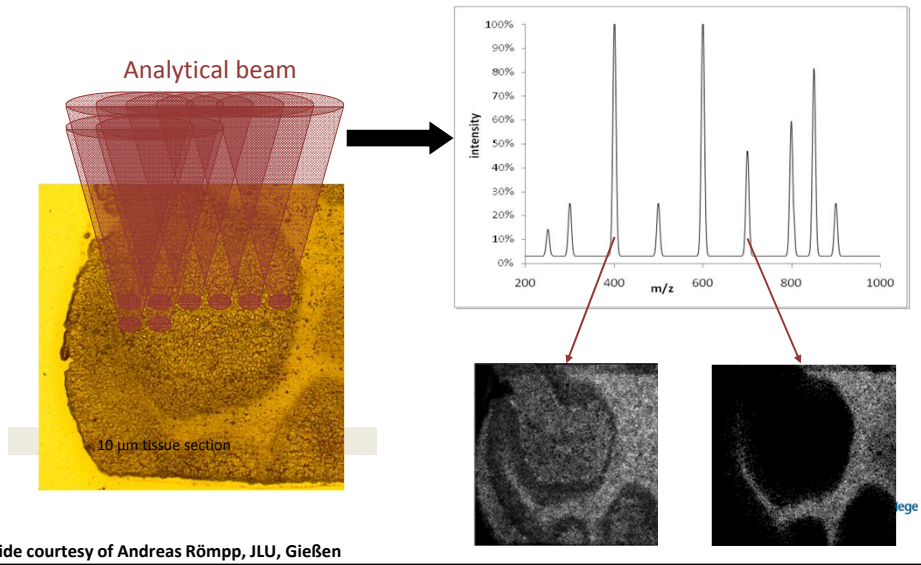
Ambient MS

>95% lipidome
15% metabolome
in a **few seconds**
Chemical interpretation is relatively simple

METABOLOME
LIPIDOME
PROTEOME
GENOME

Integration of various methods at the level of well-defined chemical species (e.g. DESI-REIMS-LC/MS)

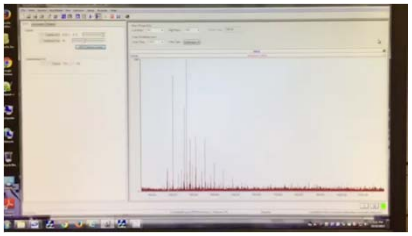
Mass Spectrometric Imaging (MSI)



Slide courtesy of Andreas Römpp, JLU, Gießen

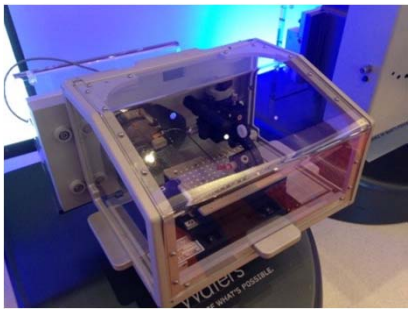
Rapid DESI with new sprayer

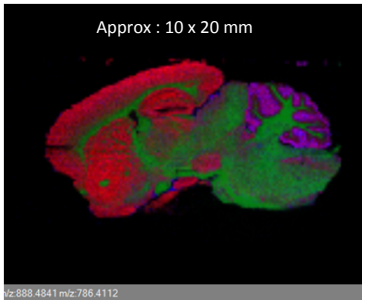
Emrys Jones



Waters Xevo G2-XS Q-TOF
OmniSpray source
30 scans per second
100µm x 100µm pixel

Total time 23 minutes
Analysis time 6 minutes





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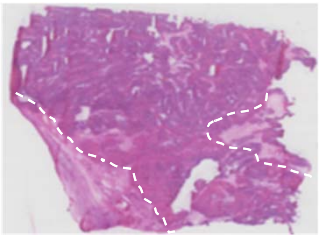
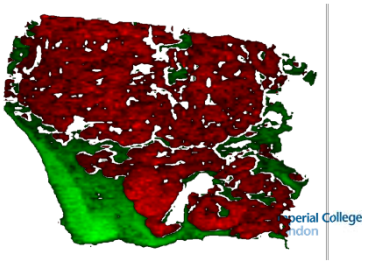
DESI Imaging - Characteristics

- Resolution 20 – 500 µm
- Adjustable
- Non destructive analysis
- Multiple consecutive DESI analysis is possible
- Staining after imaging
- Good co-registration

Ovarian cancer at 80 µm





Analysis: univariate

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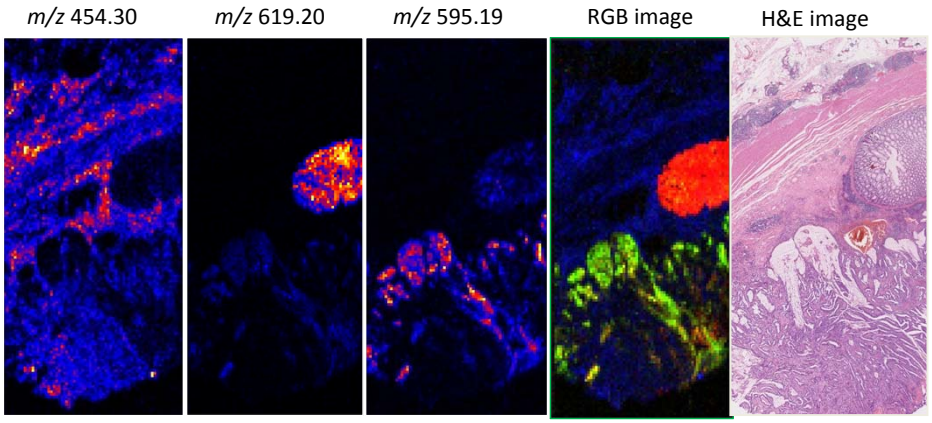
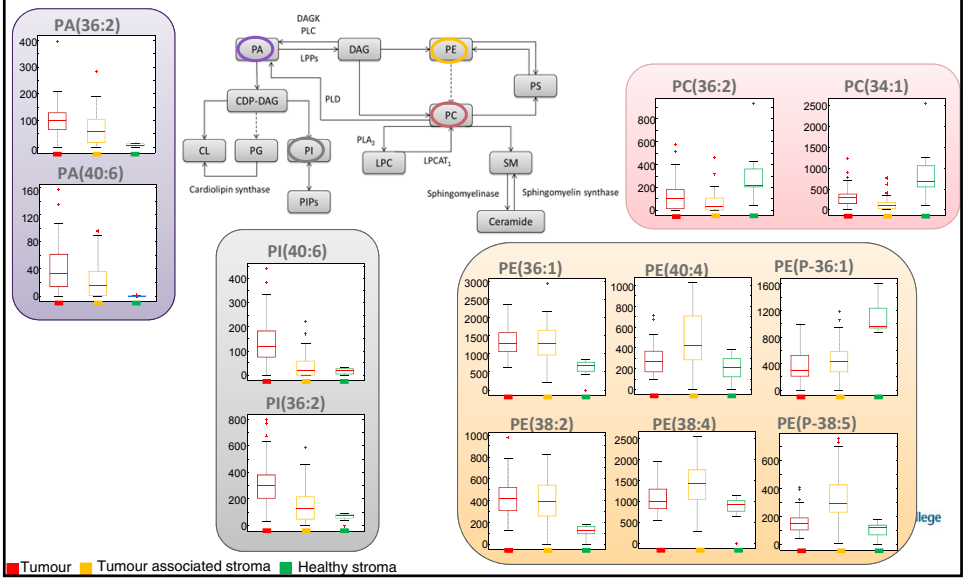
Ion intensity distributions

- Ion intensity distribution ~ Species concentration distribution
- 500 – 5000 species in a single experiment !



Example: Lipid metabolism of ovarian cancer



Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis

Javier A. Menendez* and Ruth Lupu†

Lipid metabolism in cancer

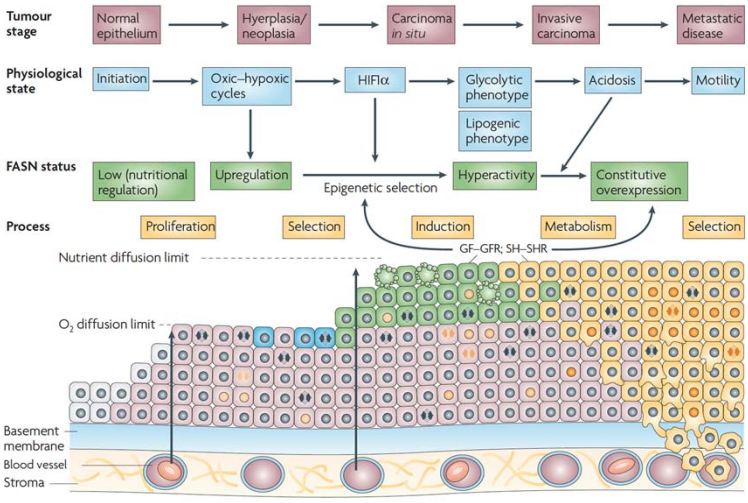


Figure 4 | **The 'lipogenic phenotype' and cell–environment interactions in carcinogenesis: a working model.**

erial College
don

Analysis: Multivariate

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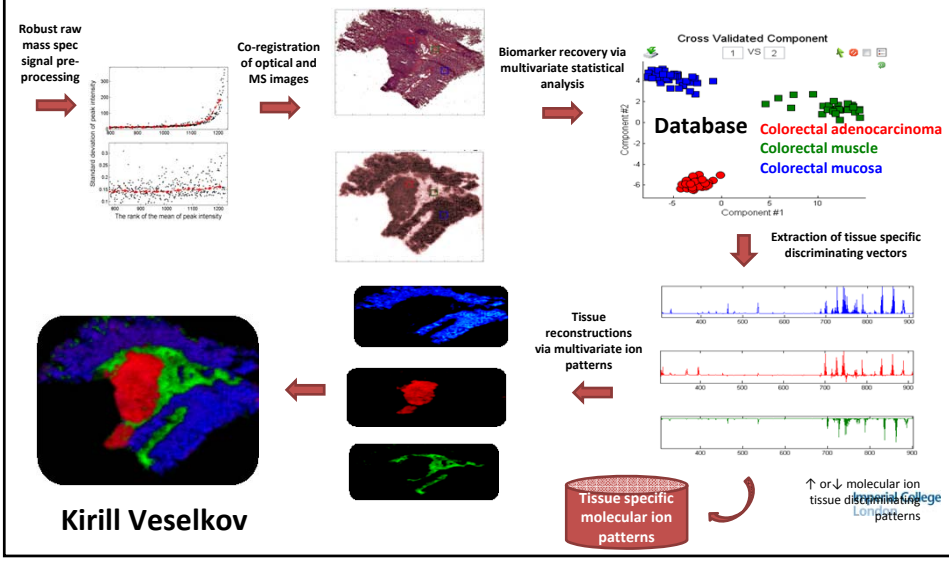
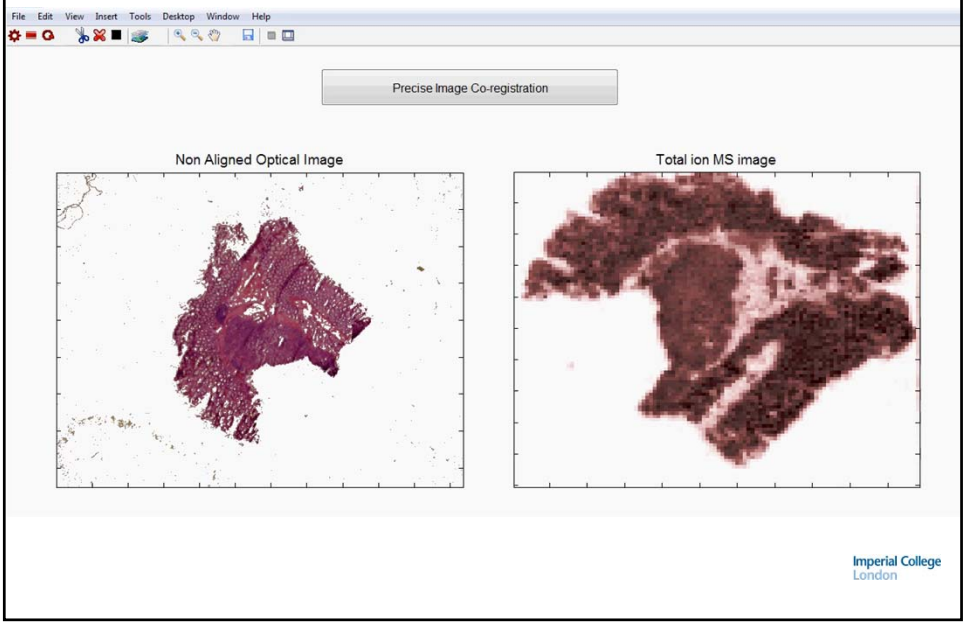
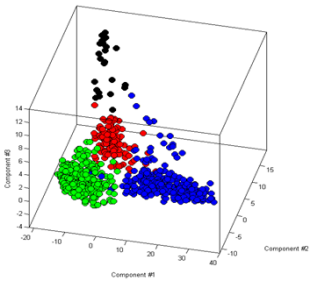
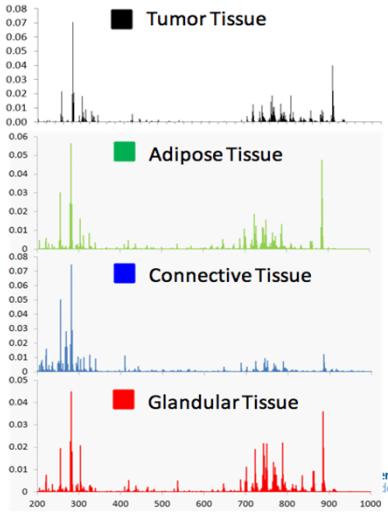
Multivariate Analysis of Mass Spectrometry Imaging Datasets





Identification of histology - Statistical analysis (PLS)





cross validation: 98.2% accuracy

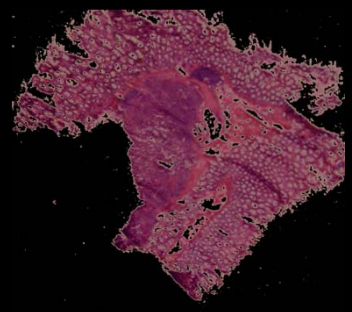
Target Class

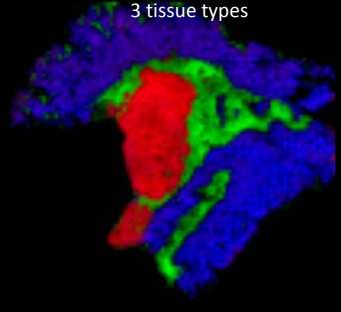
Predicted class	Adipose Tissue	Connective Tissue	Glandular Tissue	Tumor tissue
Adipose Tissue	99.3%	0.0%	0.7%	0.0%
Connective Tissue	1.6%	97.8%	0.4%	0.4%
Glandular Tissue	1.7%	3.5%	94.8%	0.0%
Tumor tissue	0.0%	0.0%	0.0%	100.0%

Highly accurate reconstruction of different tissue types based on molecular ion patterns extracted *via* supervised machine learning

Optical image of colorectal tissue types

RGB image of multivariate ion patterns of 3 tissue types

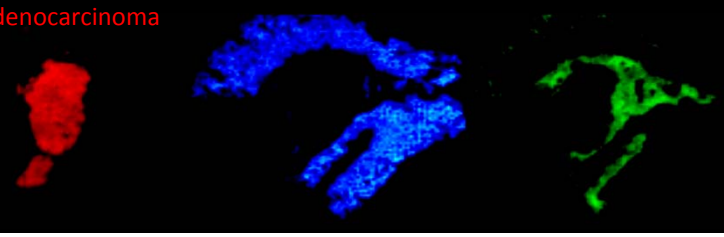




Multivariate ion patterns for adenocarcinoma

Adjacent mucosa

Muscle

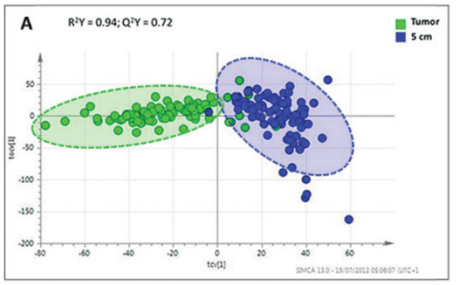


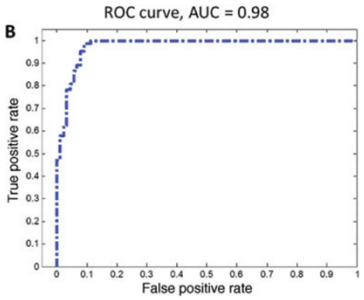
1HNMR – Colorectal cancer

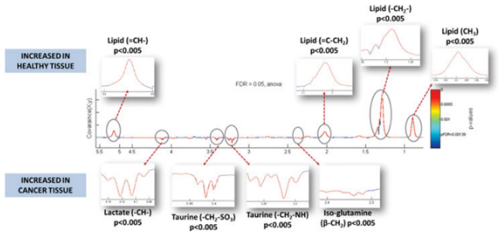
Journal of proteome research

¹H HR-MAS NMR Spectroscopy of Tumor-Induced Local Metabolic "Field-Effects" Enables Colorectal Cancer Staging and Prognostication

Beatriz Jiménez,[†] Reza Mirnezami,[‡] James Kinross,[‡] Olivier Cloarec,[†,§] Hector C. Keun,[‡] Elaine Holmes,[†] Robert D. Goldin,[∥] Paul Ziprin,[⊥] Ara Darzi,[‡] and Jeremy K. Nicholson*[,†]







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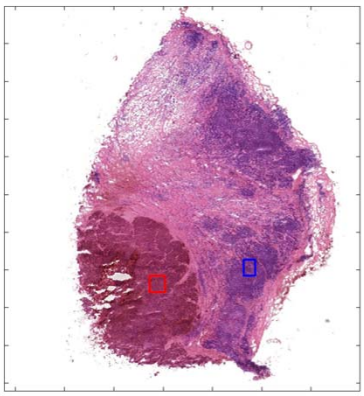
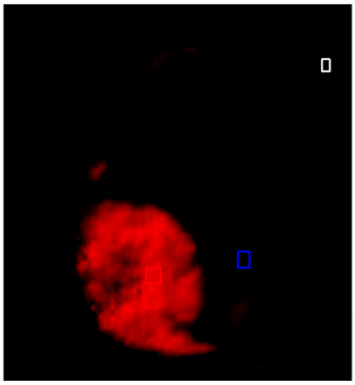
Virtual Immunohistochemistry



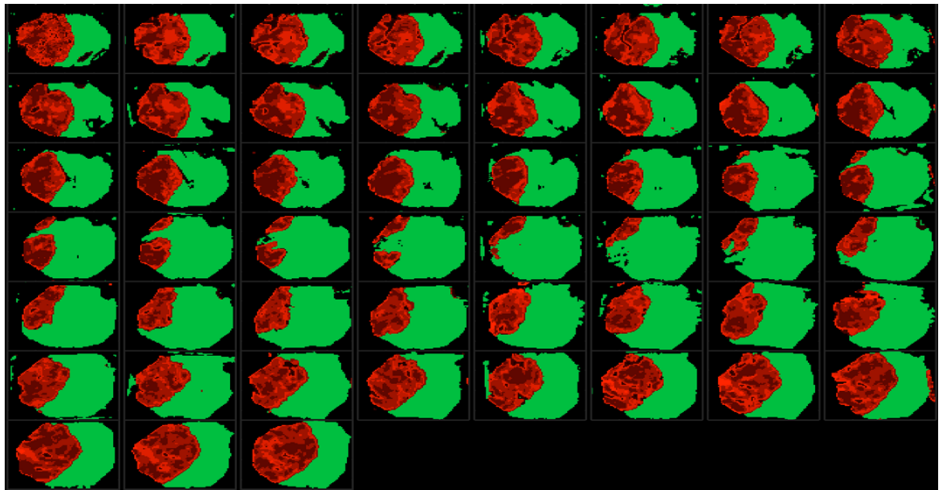
Anti-cytokeratin AE1/AE3 stain



In-silico Visualization of tumour tissue using DESI data

Imperial College London

3D DESI-MS hyperspectral imaging: Tumor heterogeneity using statistical biomarker mapping



Statistical segmentation:
- tumor
- normal

51 biopsy slices in m/z range [600, 950] ppm
290,000 spectra and 2,500 metabolites
= 725 million metabolic parameters dispersed in 3D

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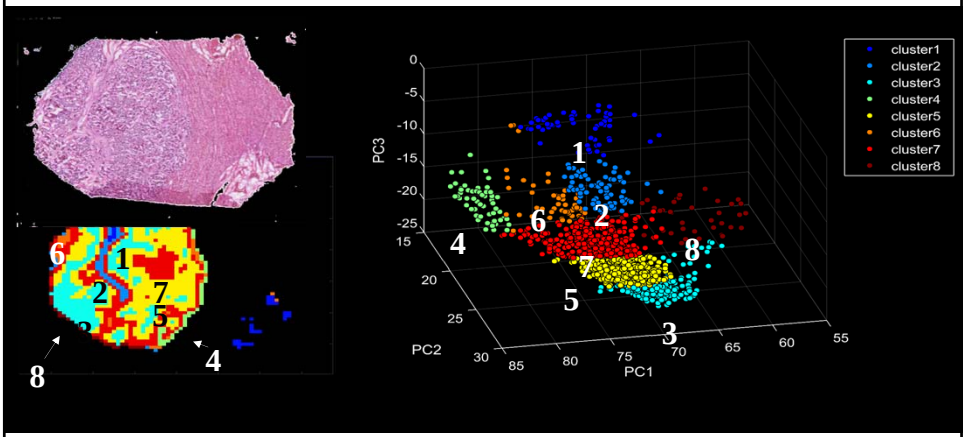
Heterogeneity of liver tumour molecular phenotypes



8 metabolic phenotypic sub-classes (Veselkov et al.)

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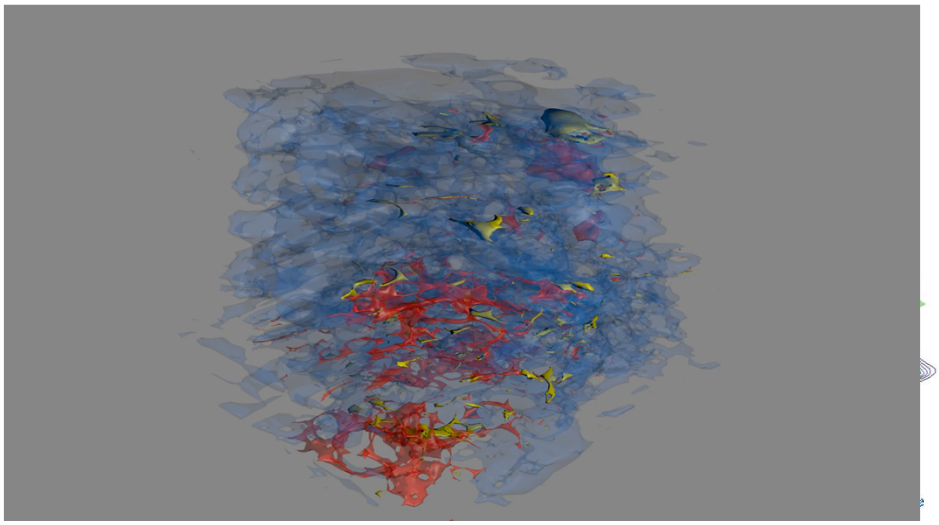
Heterogeneity of tumor molecular phenotypes



The iKnife: REIMS

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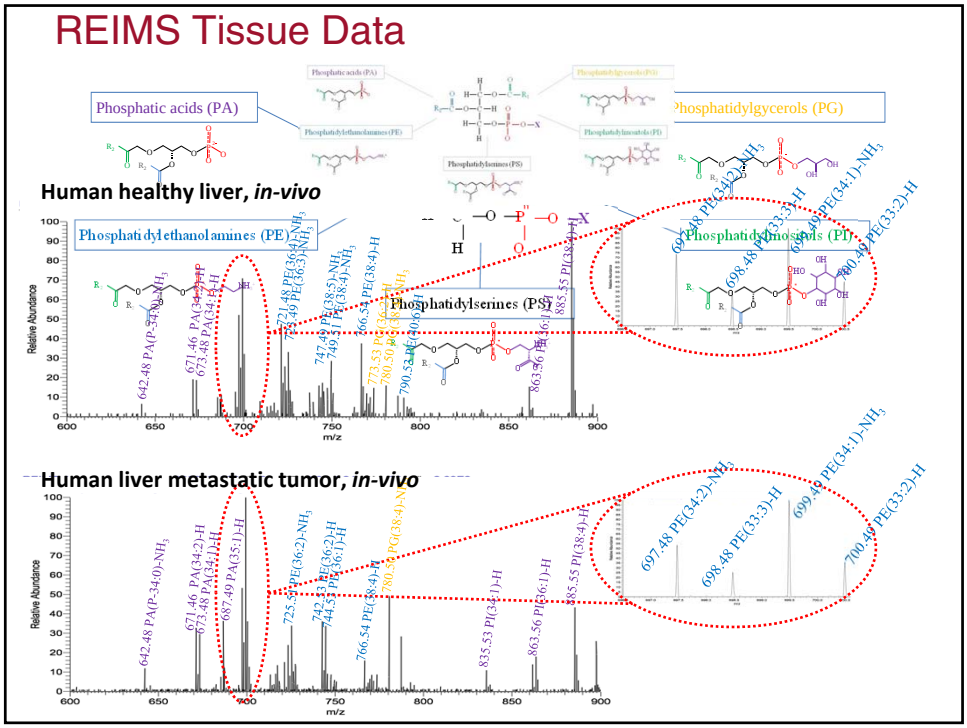
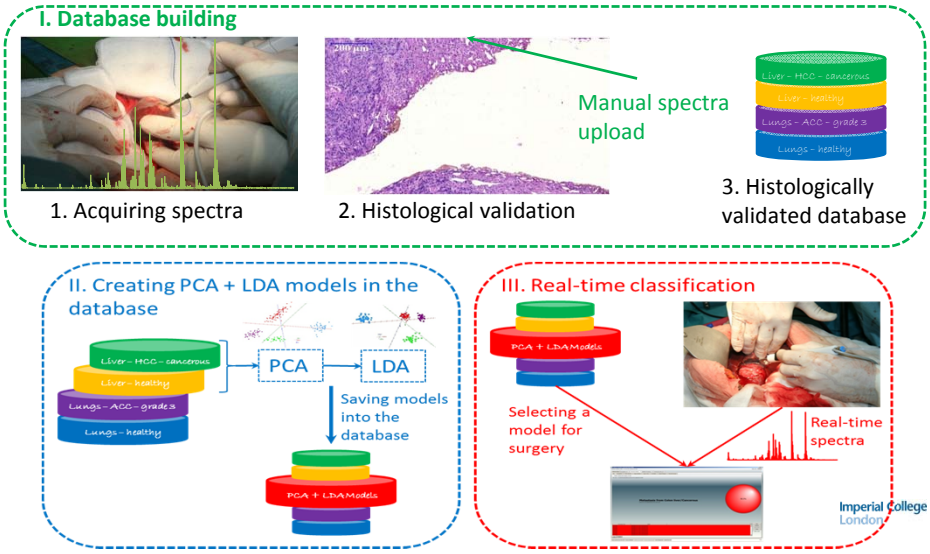
iKnife

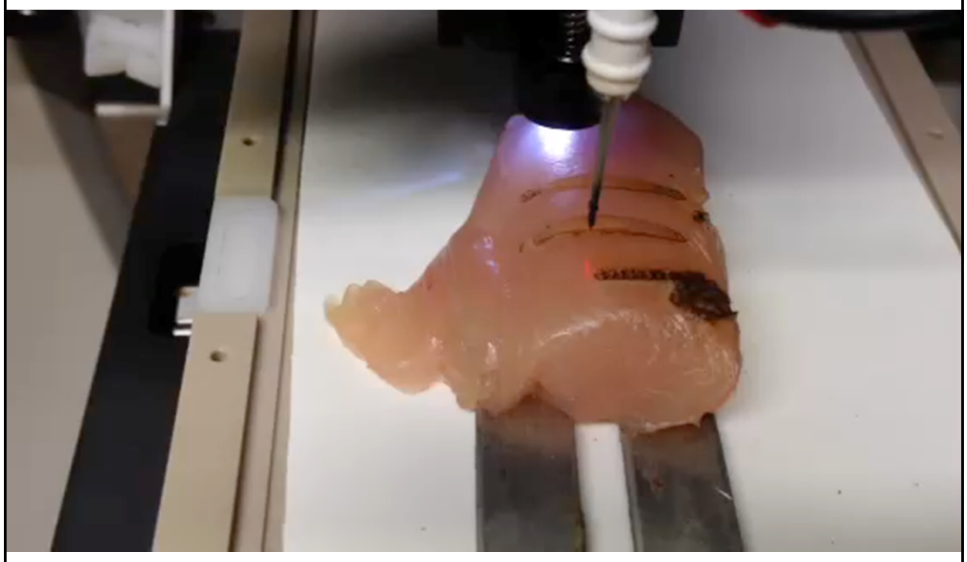
REIMS Tissue Data



Data Analysis – Scheme



Systematic data collection



The Concept of Profiling - Practice

Method capable of the parallel determination of thousands of system parameters

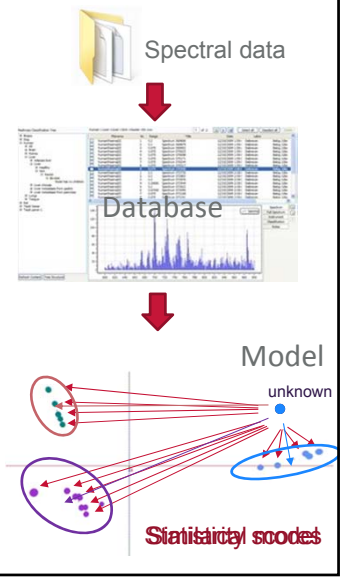
- Highly reproducible

Construction of authentic database

- Identification of elements by accepted, alternative method
- Statistically relevant numbers

Identification of unknowns by similarity to database elements

- Similarity scores
- Multivariate statistical models



Initial Results

RESEARCH ARTICLE
CANCER DIAGNOSTICS
Intraoperative Tissue Identification Using Rapid Evaporative Ionization Mass Spectrometry

Science Translational Medicine
AAAS

Organ	Tumor type	Number of patients	Number classified	Correct classification
Gastric	Adenocarcinoma	37	37	100%
	Mucinous	14	14	100%
Large intestine/colon	Adenocarcinoma	85	85	98.24%
Large intestine rectum, border	Adenocarcinoma	72	72	100%
Liver	HCC	14	14	100%
	Metastasis	38	38	100%
Lungs	Adenocarcinoma	52	52	100%
	SCC	16	16	100%
	Other	9	6	76.67%
Breast	Lobular	23	18	100%
Brain	Mixed	43	23	100%

Overall 525 interventions, tumor was identified in 417 cases, 2 misclassifications (other type of cancer)

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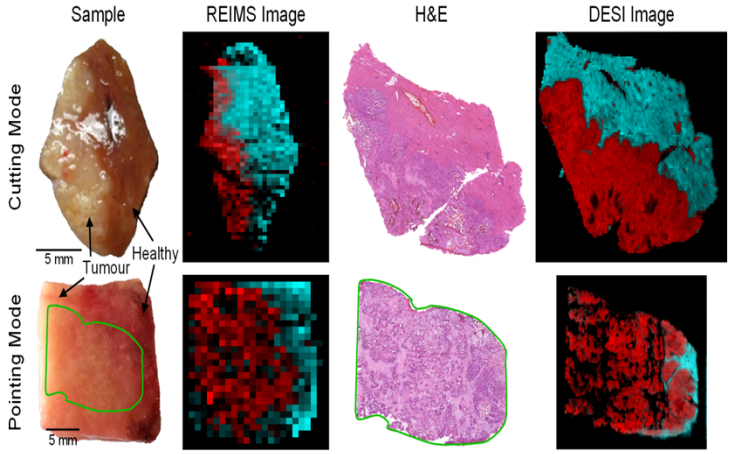
DESI and REIMS are complimentary



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Ovarian

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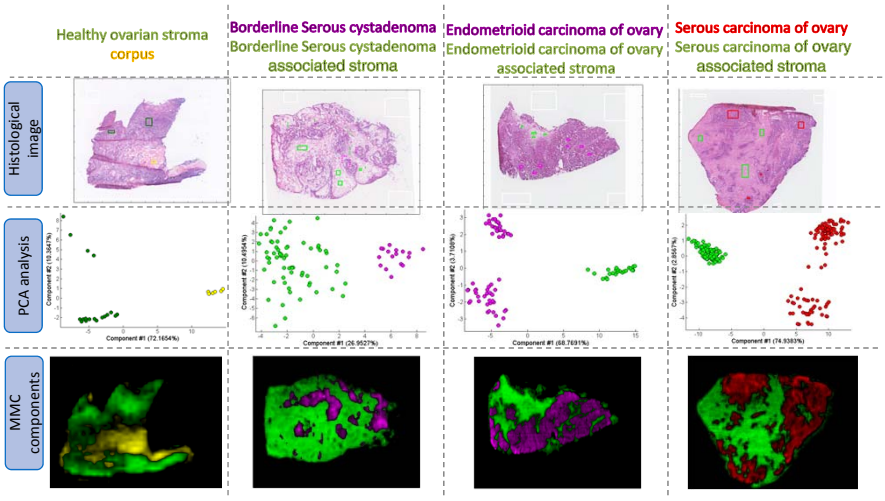
DESI-MSI : Identification of Different Ovarian Tissue Types



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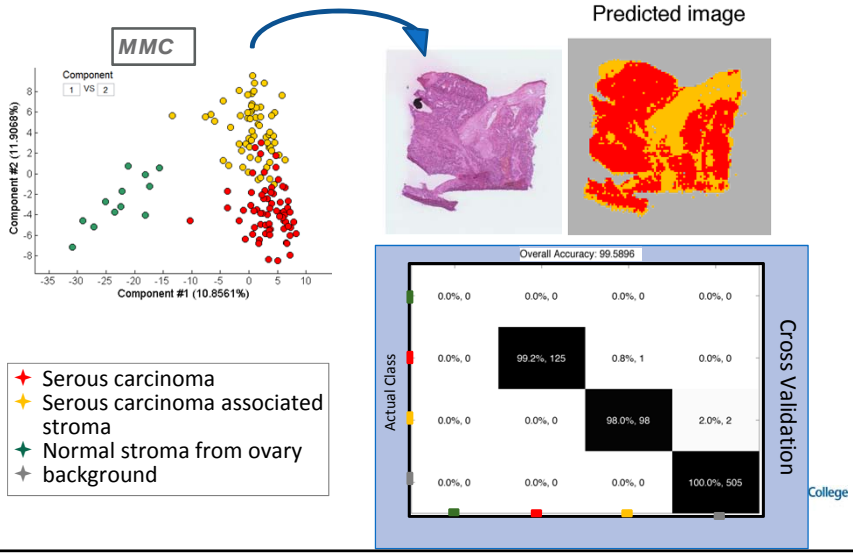
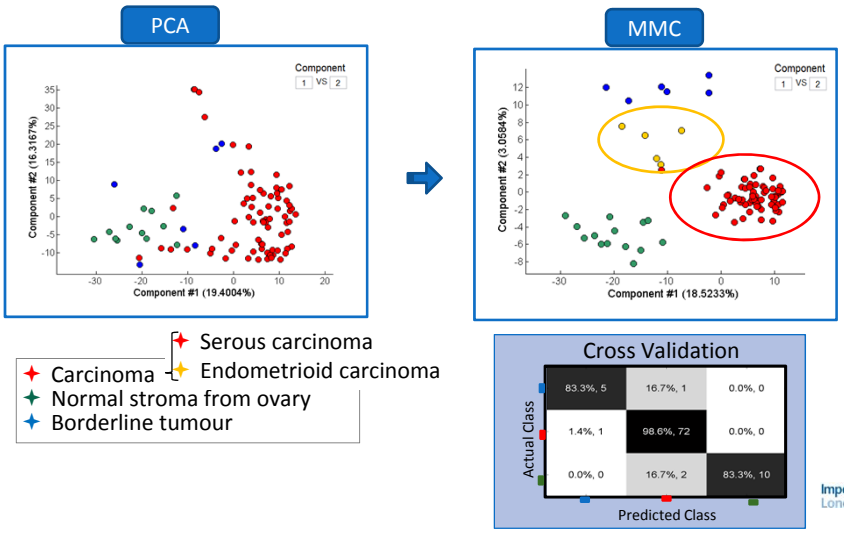
n_{total}= 91

DESI - Histological classification of unknown samples



College

DESI - Tumour classification



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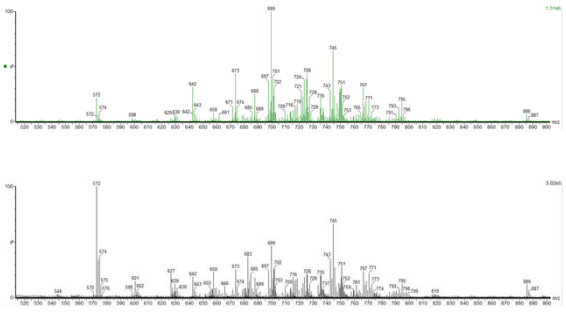
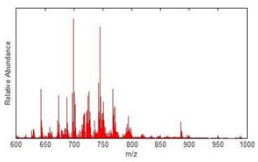
n= 91

REIMS (iKnife) – Ovarian raw data

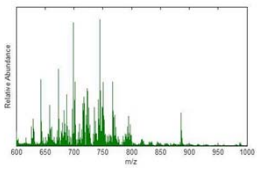
546 sampling points (cuts) – average 3.7 cuts per sample
Species within fatty acid, phospholipid and triglyceride ran
Phospholipid (600-900 *m/z*) spectra yielded unique fingerp



Spectra average of all serous adenocarcinoma



Spectra average of all normal ovary samples



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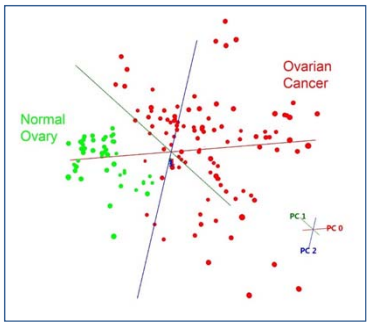
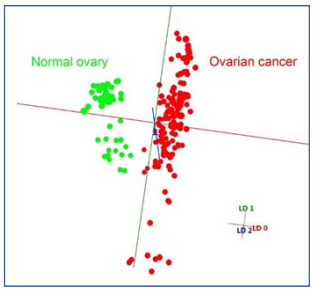
REIMS (iKnife) – Ovarian cancer vs normal

Normal Ovary versus Ovarian Cancer
Principal component analysis (unsupervised)



Normal Ovary versus Ovarian Cancer
Linear discriminant analysis (supervised)



LOOCV multivariable analysis:
N=189, 100% specificity, 100% sensitivity

Ovarian cancer tumour content 100% in analysed samples

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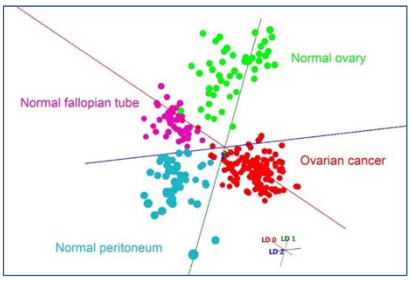
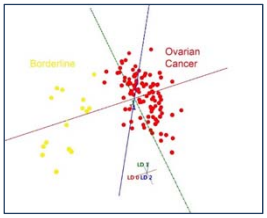
REIMS (iknife) – Ovarian tissue types

Ovarian Cancer versus Normal Gynaecological Tissue
Linear discriminant analysis (supervised)

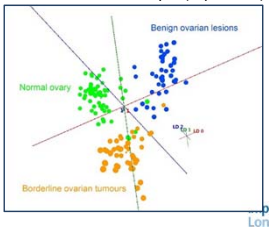


LOOCV multivariable analysis:
N= 291, 100% specificity, 100% sensitivity
Correct tissue classification 92.7%

Ovarian Cancer versus Borderline tumours
Linear discriminant analysis (supervised)



Normal, Benign, Borderline tissue
Linear discriminant analysis (supervised)



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c

Breast

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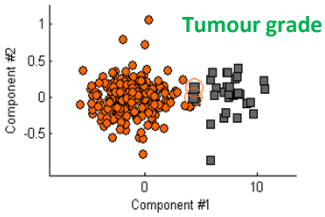
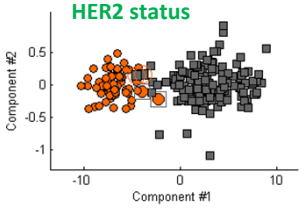
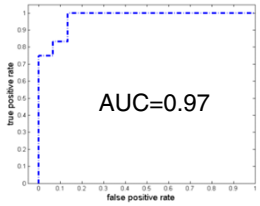
DESI: Depth of information in breast cancer



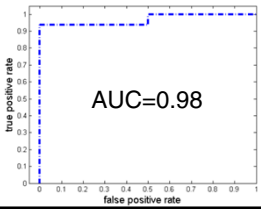
Tumour pixels only



	Grade II	Grade III
Grade II	100.0%	0.0%
Grade III	6.3%	93.8%



	HER2 +	HER2 -
HER2 +	97.0%	3.0%
HER2 -	1.3%	98.7%



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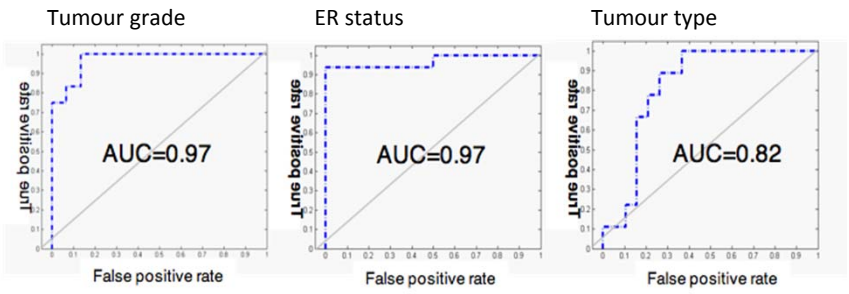
DESI Diagnostic value in breast cancer



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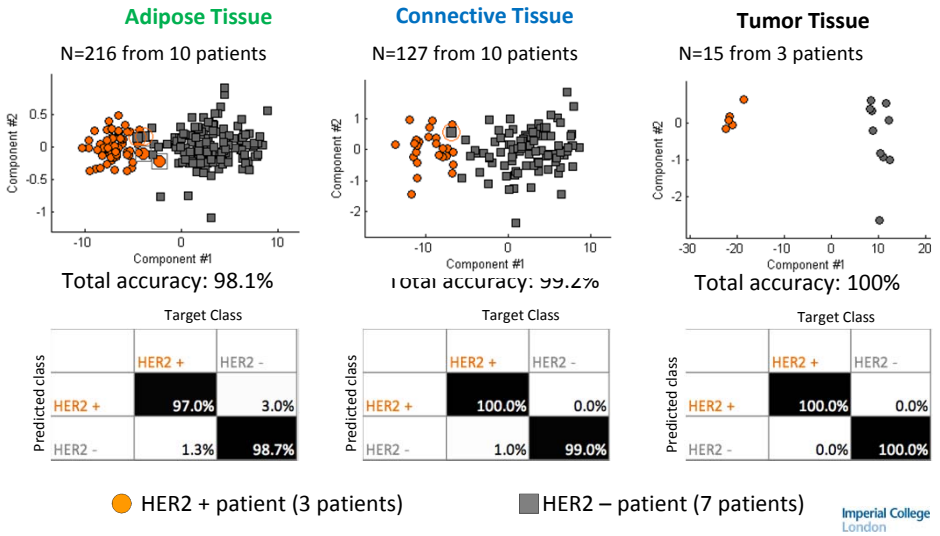
DESI Breast TISSUE Phenotyping status – HER2



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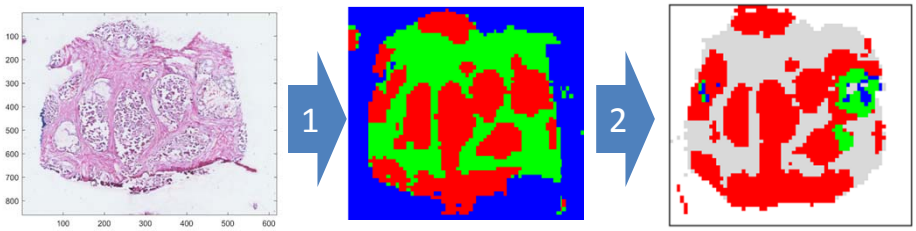
DESI - Breast Cancer Tumour heterogeneity

1. Supervised segmentation
2. Unsupervised segmentation of the tumour



Clonal populations ?

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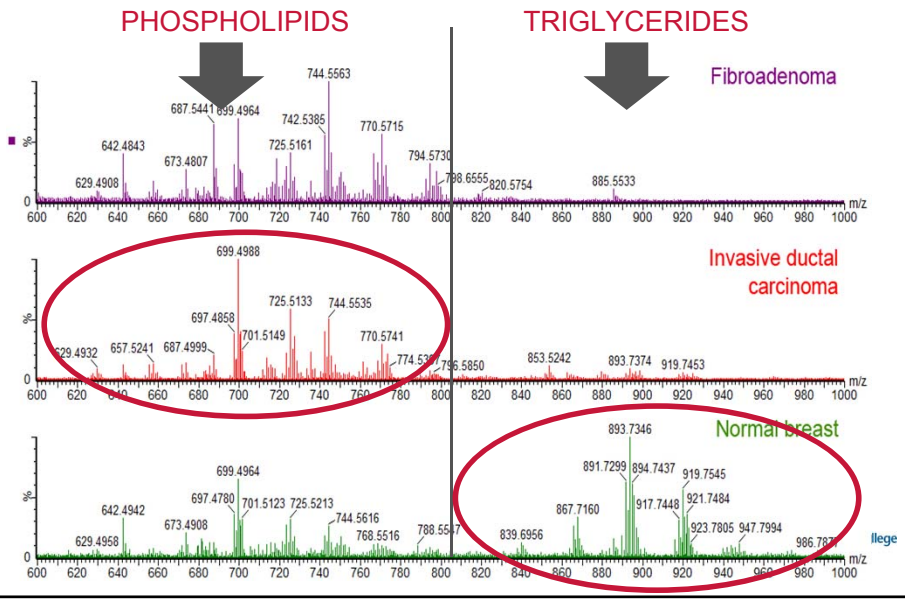
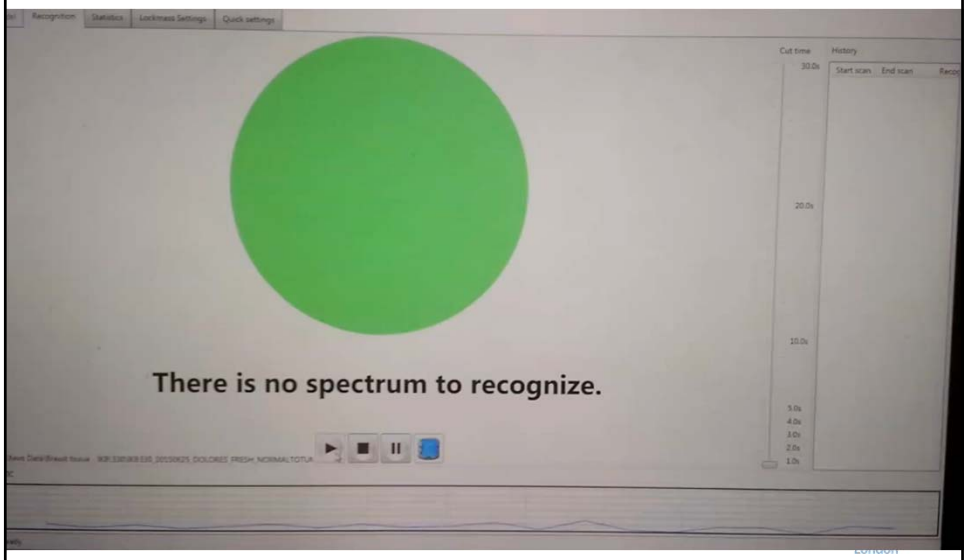
REIMS: Typical Breast Spectra (600-1000m/z)

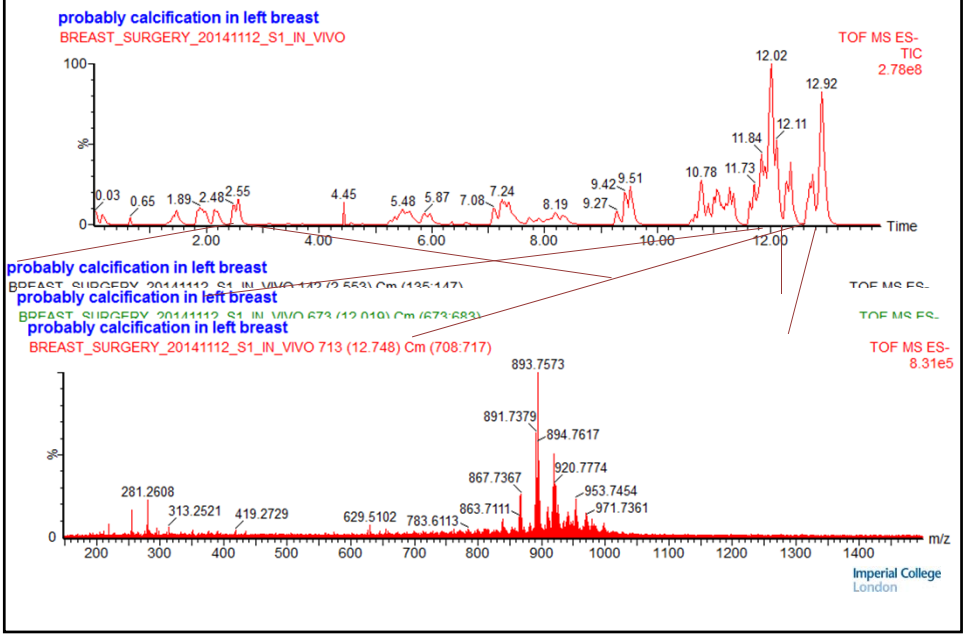


REIMS Margin test – Normal through Tumour



REIMS Intra-Operative Method

Intraoperative Breast surgery



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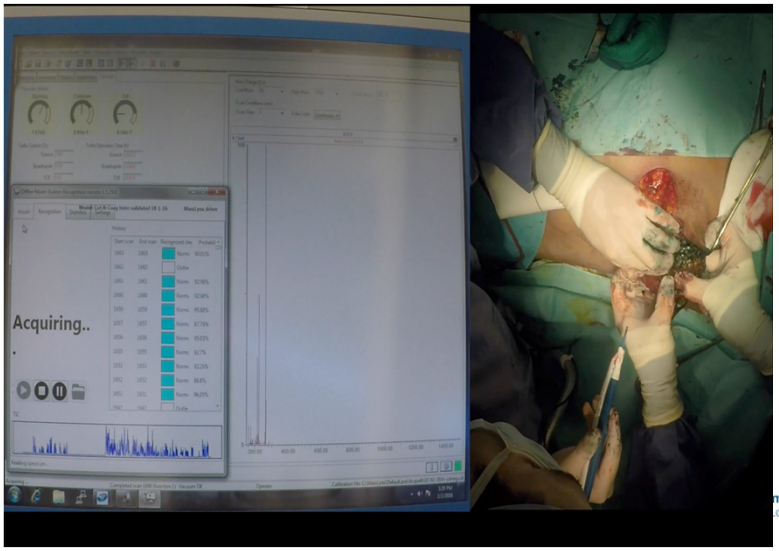
ASSOCIATION OF BREAST SURGERY

INTRA-OPERATIVE INTELLIGENT KNIFE ANALYSIS



Phospholipids identified in both REIMS MS/MS and DESI-MS

Green = more abundant in Normal in DESI
Yellow = more abundant in Tumour in DESI

671.47	673.48	699.5	713.51	714.51
PA(16:0/18:2) M-H	PA(16:0/18:1) M-H	PA(18:1/18:1) M-H	PE(20:2/18:1) M-H	PE(16:0/18:2) M-H PE(16:1/18:1) M-H
716.52	**735.47**	**742.54**	**744.55**	**747.51**
PE(18:0/16:1) M-H PE(16:0/18:1) M-H	PA(P-20:0/20:4) M-H PA(P-20:1/20:3) M-H	PE(18:0/18:2) M-H PE(18:1/18:1) M-H	PE(18:0/18:1) M-H	PA(18:1/22:5) M-H PA(16:1/18:1) M-H PA(18:2/22:4) M-H PA(18:0/16:1) M-H
766.54	**768.55**	**770.57**	**772.58**	
PE(18:0/20:4) M-H	PA(18:0/18:2) M-H PE(18:0/20:3) M-H	PE(18:0/20:2) M-H	PE(18:0/20:1) M-H PE(22:1/16:0) M-H	

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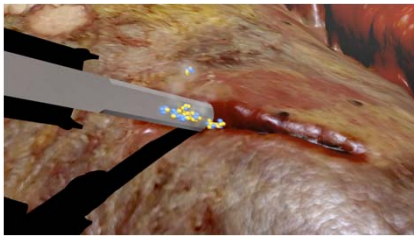
Summary

- MS optimised for rapid analysis of hetrogenous breast tissue
- High accuracy for identification of ex-vivo breast tissue
- Intra-operative spectra obtained throughout entire operation

Procedure	Sensitivity	Specificity
Frozen Section	65-78%	98-100%
Touch imprint cytology	70-80%	85-100%
Digital Specimen X-ray	54%	87%
iKnife (REIMS)	92%%	96%



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Colorectal

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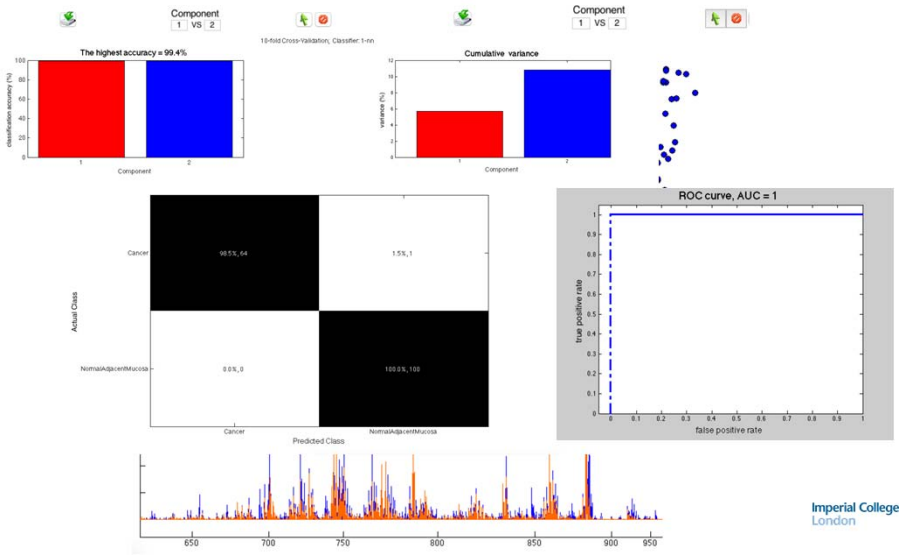
Results: DESI-MSI shows cancer harbors topographically discrete regions of lipids that are diagnostic of CRC



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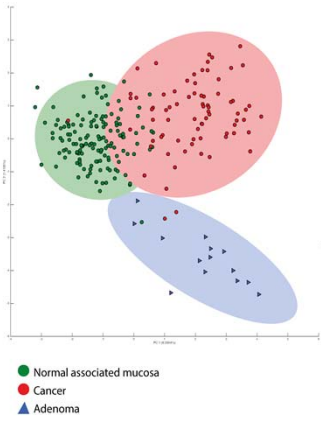
REIMS: Diagnostic accuracy



a)

Linear Discriminant (LDA) scores plot

Results: Lipid Chemistry

m/z	Putative Molecular structure	P value	Cancer	Adenoma	Normal
633.465	PA(31:0)	0.0001			
886.565	PS (44:6)	1.11E-16			
886.565	PS(44:8)	1.11E-16			
888.75	PS(41:0)	1.38E-12			
702.625	Cer(D18:0/H24:0)	2.28E-11			
775.545	PG(36:1)	0.0001			
673.485	PA(34:0)	3.33E-16			
642.495	GlcCer(30:1)	2.47E-12			
880.665	PS (43:4)	1.86E-07			
699.505	PA	1.12E-07			
710.485	PE(34:4)	6.73E-08			
718.615	Cer(T18:0/24:0(2OH))	9.30E-10			
653.495	1,2-DG(36:3)	0.0002			
797.535	PG(38:4)	0.004			
864.675	Plasmalogen	0.0004			
891.725	TG (54:0)	1.63E-08			

Metabolites were over expressed (red) or under expressed (green) in specific histological states of cancer, adenoma or normal associated mucosa.

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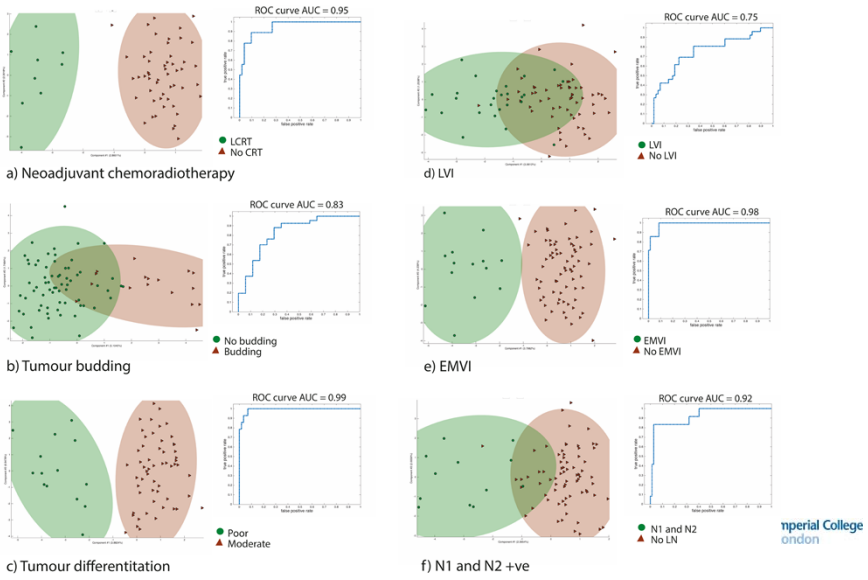
Results: Rectal Cancer Lipidomic Phenotypes



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Results: Summary accuracy

	Spectra n =	Accuracy	True Positive	True Negative	False Positive	False Negative	AUC
Diagnostic markers All							
Cancer vs. NAM	220	90.5%	86.7%	92.4%	13.3%	7.6%	0.96
Cancer vs. Adenoma	89	94.4%	78.6%	97.3%	2.7%	21.4%	0.99
Adenoma vs. NAM	159	97.5%	85.7%	98.6%	1.4%	98.6%	0.99
Histological subtype (Mucinous vs. Adenocarcinoma)	75	90%	94.2%	83.3%	16.7%	5.8%	0.96
Prognostic performance – whole model							
Tumour differentiation (Mod vs. poor)	183	83.1%	68.3%	87.3%	12.7%	31.7%	0.88
Tumour budding	234	78.2%	80.6%	74.4%	25.6%	19.4%	0.87
LVI	234	73.9%	71.6%	75.3%	24.7%	28.4%	0.83
EMVI	234	73.5%	65.3%	77.2%	22.8%	34.7%	0.81
+ve Nodes	234	77.4%	69.0%	81.0%	19.0%	31.0%	0.81
Rectal cancer prognostic factors							
Differentiation (Mod vs. poor)	84	94.4%	78.6%	98.2%	1.8%	21.4%	0.99
Tumour Budding	84	84.5%	88.1%	70.6%	29.4%	11.9%	0.82
LVI	84	71.4%	72.4%	30.8%	69.2%	27.6%	0.75
EMVI	84	96.4%	85.7%	98.6%	14.3%	1.4%	0.98
+ve Nodes	84	92.9%	83.3%	94.4%	5.6%	16.7%	0.92
LCRT vs. None	75	96%	95.7%	96.2%	3.8%	4.3%	0.99
cPR vs. NAM	52	100%	100%	100%	0%	0%	1

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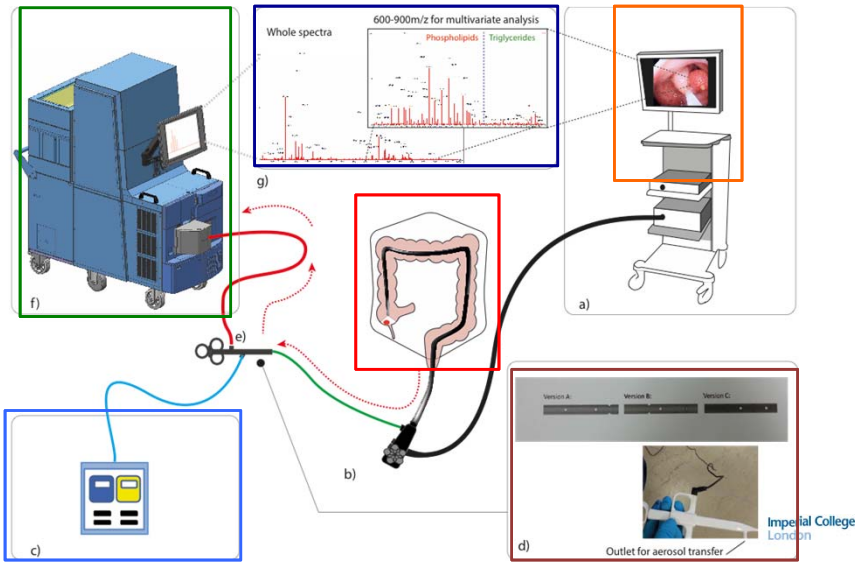
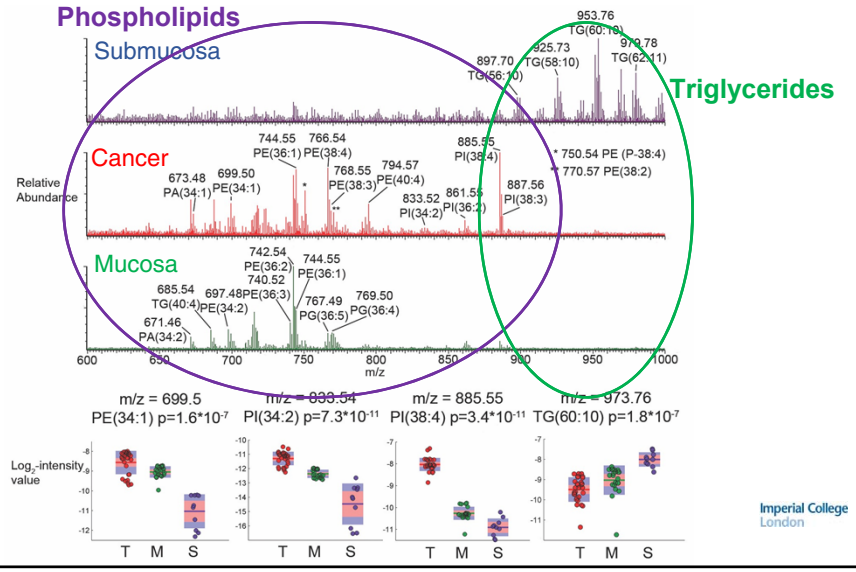
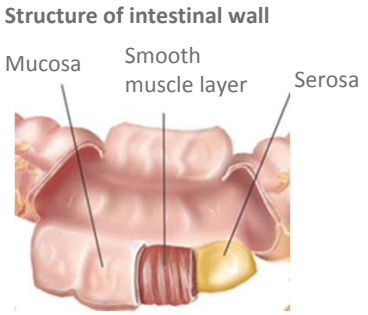
iEndoscope: Chemically enhanced endoscopy

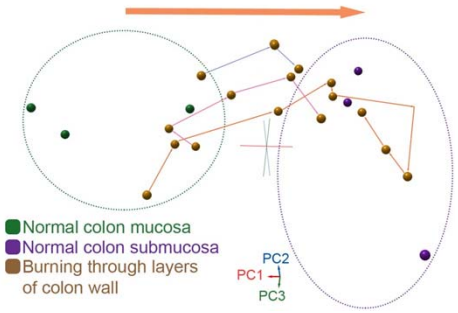


Real-time MS during endoscopic resection



iEndoscope (REIMS) Safety signal – submucosal cutting





- Incidence of bowel perforation is ~ 1-3% in electrosurgical polypectomy major complication
- On-line REIMS monitoring can give warning signal when smooth muscle layer is dissected

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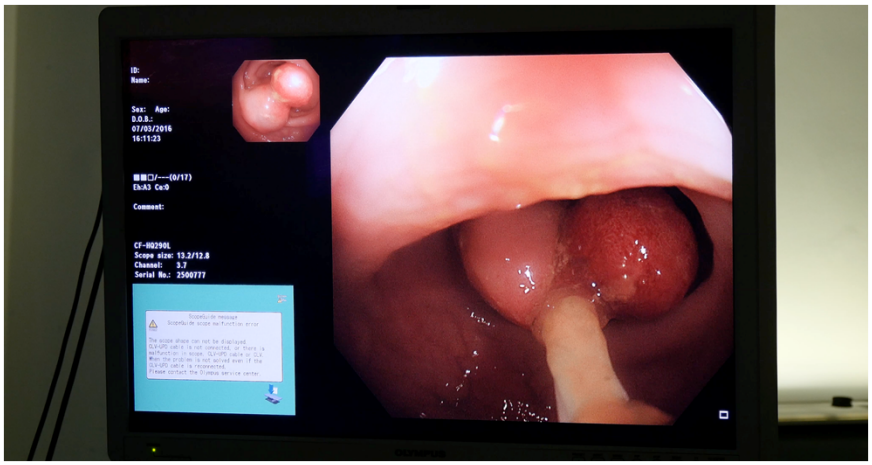
In-vivo REIMS endoscopy



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Microbiology

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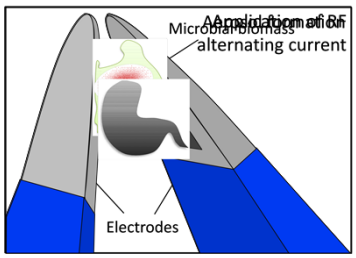
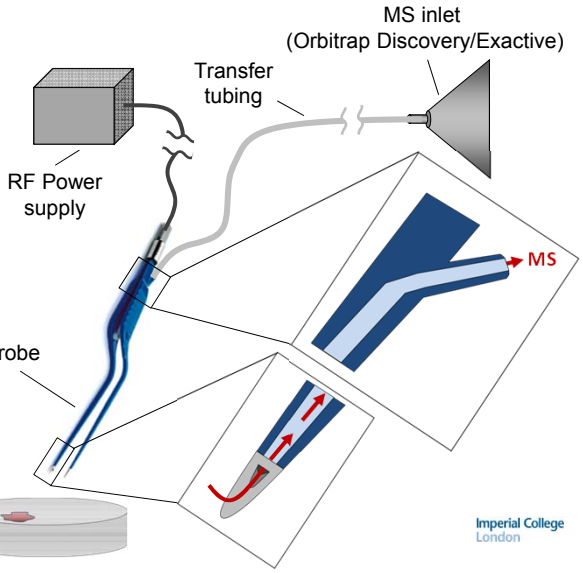
Identification of Bacteria using Rapid Evaporative Ionisation Mass Spectrometry (REIMS)





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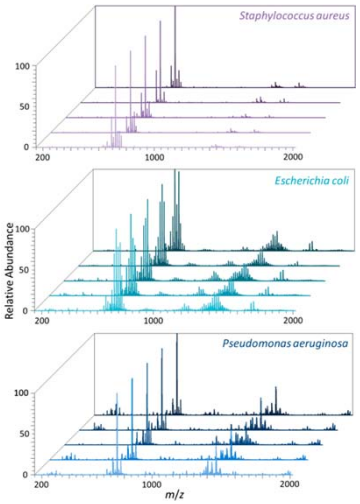
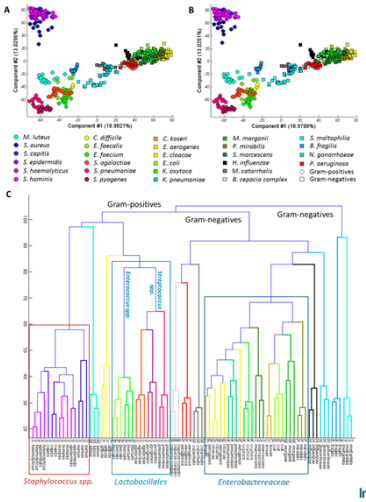
Identification of Bacteria using Rapid Evaporative Ionisation Mass Spectrometry (REIMS)





Strittmatter et al. Analytical Chemistry 2014, 86, 6555−6562

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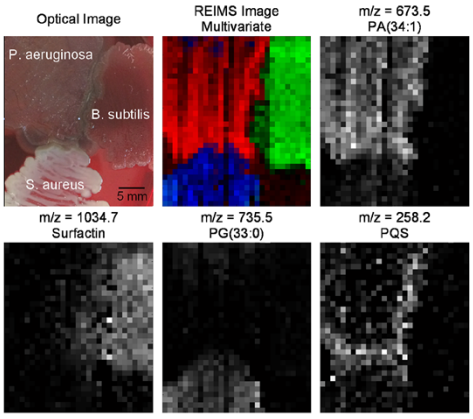
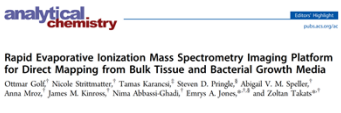
Real time functional analysis of microbial signalling





REIMS spectrum indicating five QSM: -Heptylquinoline-4(1H)-one, 2-Heptyl-3-hydroxy-4(1H)-quinolone (PQS), Hydroxynonenylquinoline, Hydroxynonylquinoline and Hydroxyundecenylquinoline identified from *P. aeruginsoa* isolates.

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Automated REIMS microbiology

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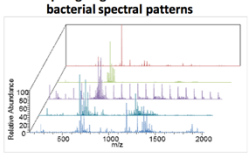
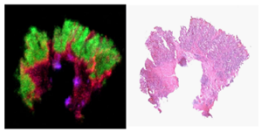
REIMS-DESI-MSI integration

Compiling large-scale database of bacterial spectral patterns



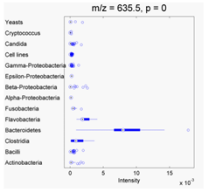
Dataset comprising 596 bacterial strains (228 species, 80 genera), cultured under various conditions.

Histologically assigned DESI imaging dataset



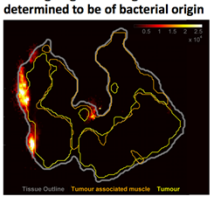
Dataset consisting of 48 human colorectal tissue samples (16 cancerous, 32 healthy) from 16 different patients. Results of sequencing-based community analysis available.

Extracting taxon-specific markers



Anova test followed by Tukey's HSD test performed on different taxonomical levels. Identification of markers with specificity on taxon-level.

Generating single ion images for markers determined to be of bacterial origin



Generation of single ion images including predicted tissue outlines. Bacterial distribution to be non-identical with tissue distribution.

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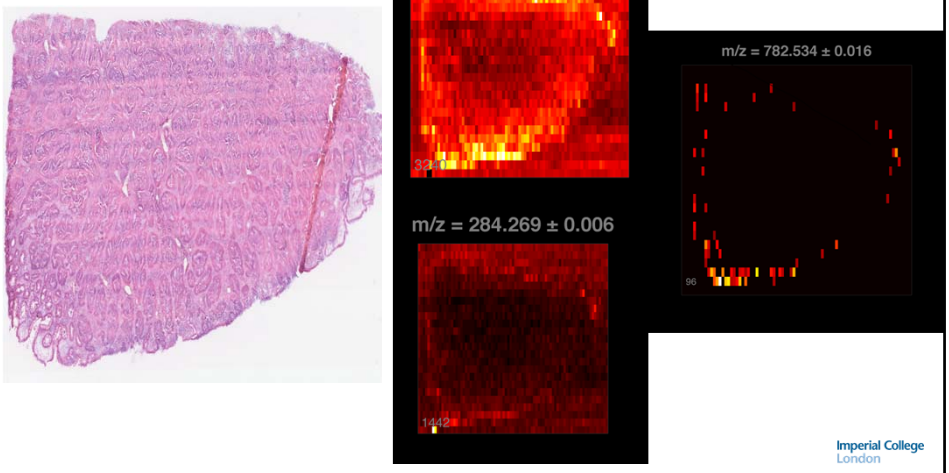
LCFAs coalesce with markers of Proteobacteria in the discrete regions of tumour



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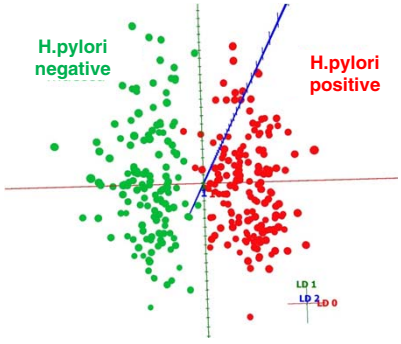
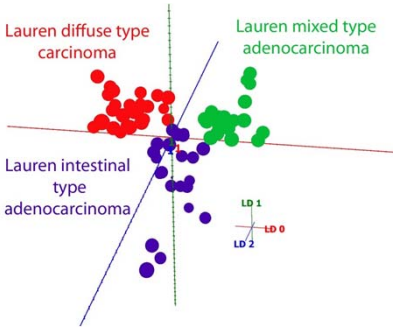
Clinical applications? iENDOSCOPE!!



- 3s response time
- 98.3 % agreement with H. pylori urease test
- 94.6% agreement with histology



- 600-900 m/z negative ion mode
- 0.1 bin
- Linear discriminant analysis

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Summary: Chemically augmented precision surgery

- Ambient spectroscopy flexible and highly amenable to challenging clinical environments.
- Precision ‘surgery’ means the right operation at the right time in the right person with the BEST outcome.
- REIMS and DESI are highly complimentary
- REIMS optimised for rapid analysis of heterogenous tissue
- Lipidome analysis robust and repeatable – NOT A BLACK BOX
- REIMS also provides phenotypic data on tumours.
- REIMS can AUGMENT current and future surgical and imaging technologies
- Analytical tool for studying cancer lipidome

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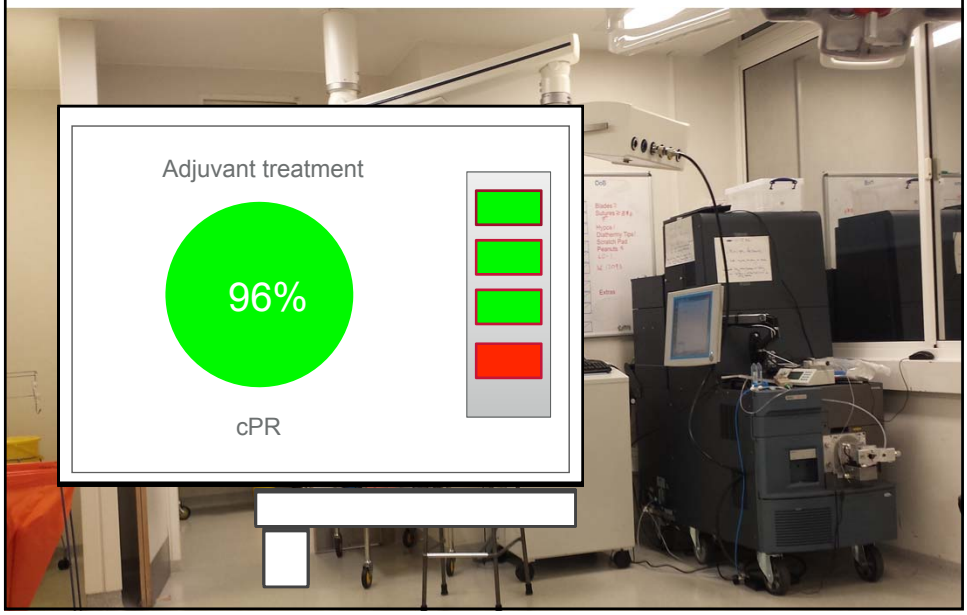
REIMS: Multiple clinical functions



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