The road map towards providing a robust Raman spectroscopy-based cancer diagnostic platform and integration into clinic

Riccardo Tagliapietra
Renishaw SpA, via dei Prati 5 – 10044 Pianezza (TO)
riccardo.tagliapietra@renishaw.com

Raman spectroscopy (RS) can distinguish cancer types/subtypes

- Raman spectroscopy (RS) has been demonstrated to:
  - distinguish healthy and cancerous tissues
  - identify cancer stages and disease subtypes
  - high accuracy
  - a number of organs, e.g. gastrointestinal (GI) tract, breast, brain, lymph nodes

Why is Raman spectroscopy not adopted for routine cancer diagnostics yet?

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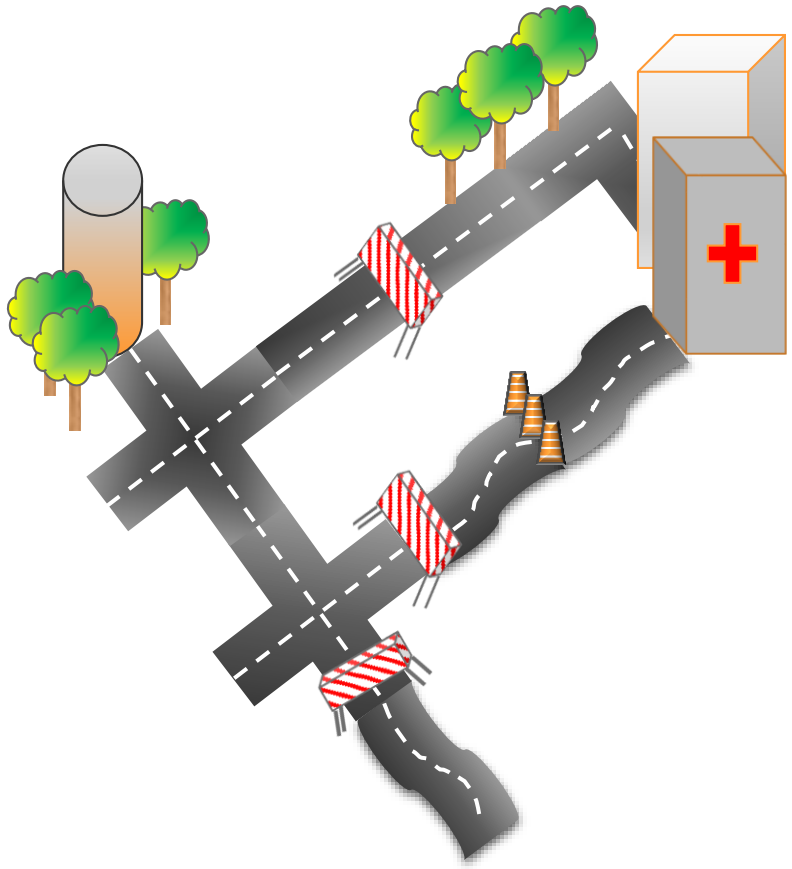
Reasons RS is not adopted for routine cancer diagnostics

- Awareness/acceptance by clinicians
- Solutions to current unmet needs
- Practicality of integrating the technology into their clinical workflow
  - Sample preparation
- Availability of a dedicated pathology diagnosis instrument
  - Robust platform without extensive optimisation
- Maturity of the technology
  - Data reproducibility
  - Data transferability
- Availability of a robust, validated and distributable disease classification model



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An industry-academic collaborative consortium to overcome challenges

Biophotonics Research Unit

Gloucestershire Hospitals NHS

NHS Foundation Trust

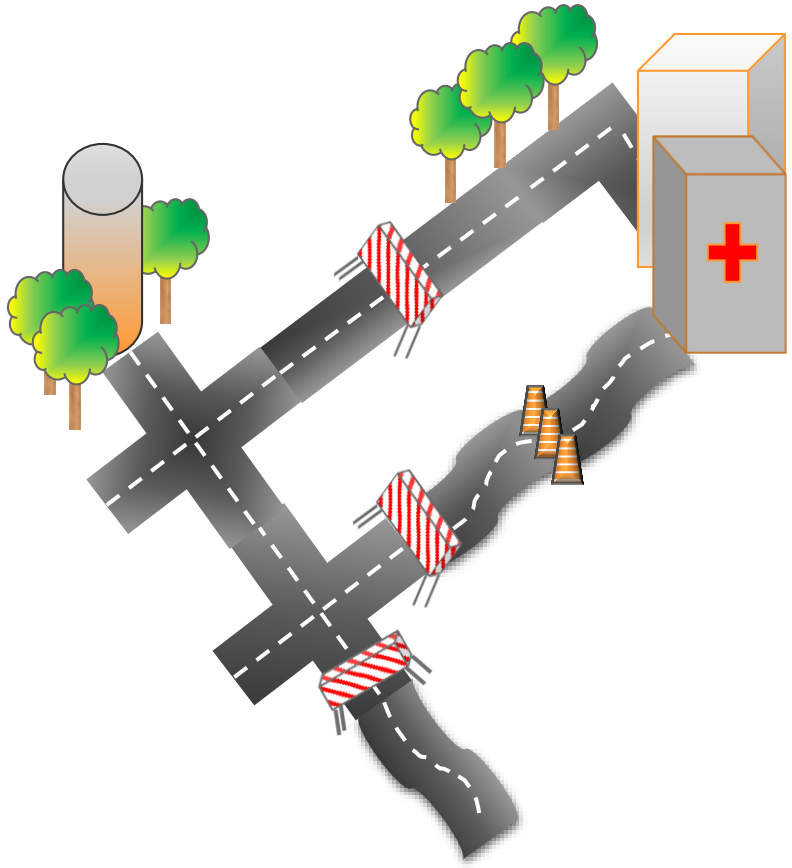
UCL

Stratified Medicine through Advanced Raman Technologies (SMART)

University of Exeter

BIOSPEC Biomedical Spectroscopy Labs

Innovate UK Technology Strategy Board



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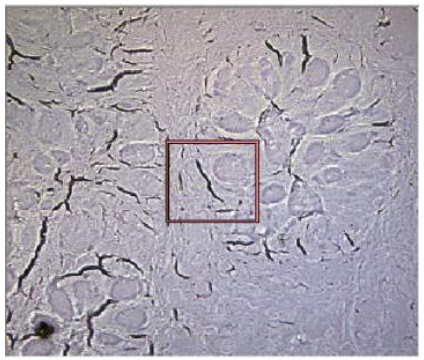
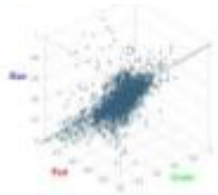
Propose Raman imaging as a diagnostic tool

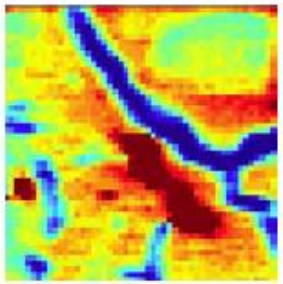
- Identify cancer stage/subtype through their spectral fingerprints
- Extract chemical information from spectra and possible biomarker discovery
- Spatial information, e.g. tumour margin analysis



Human oesophageal tissue section



Non-squamous
Barrett's mucosa
Low grade dysplasia
High grade dysplasia
Adenocarcinoma



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Aims of SMART: clinical integration of RS-based cancer diagnosis

Increase awareness and acceptance by clinicians

Identify unmet needs and provide tailored solutions

Optimised protocols

- Tissue preparation compatible with current clinical workflow
- Optimised mapping parameters
- Data pre-processing to ensure transferability

Provide a robust RS-based dianostic platform

- Hardware
  - Tissue Raman scanner
  - Fast Raman imaging
- Software
  - User friendly
  - Enables model building and classification

Provide a robust GI tract cancer classification model

- Validated
- Distributable

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Increase awareness and acceptance by clinicians

Identify unmet needs and provide tailored solutions

Engage with clinicians

- Interview pathologists and surgeons
- Histopathology is the current gold standard for diagnosing cancer and identifying the cancer stage
  - Gain a better understanding of the current histopathology routine
  - Observe the current practice at histopathology laboratories

uclh NHS

St James's University Hospital

Oxford University Hospitals NHS
NHS Foundation Trust

University Hospitals Bristol NHS
NHS Foundation Trust

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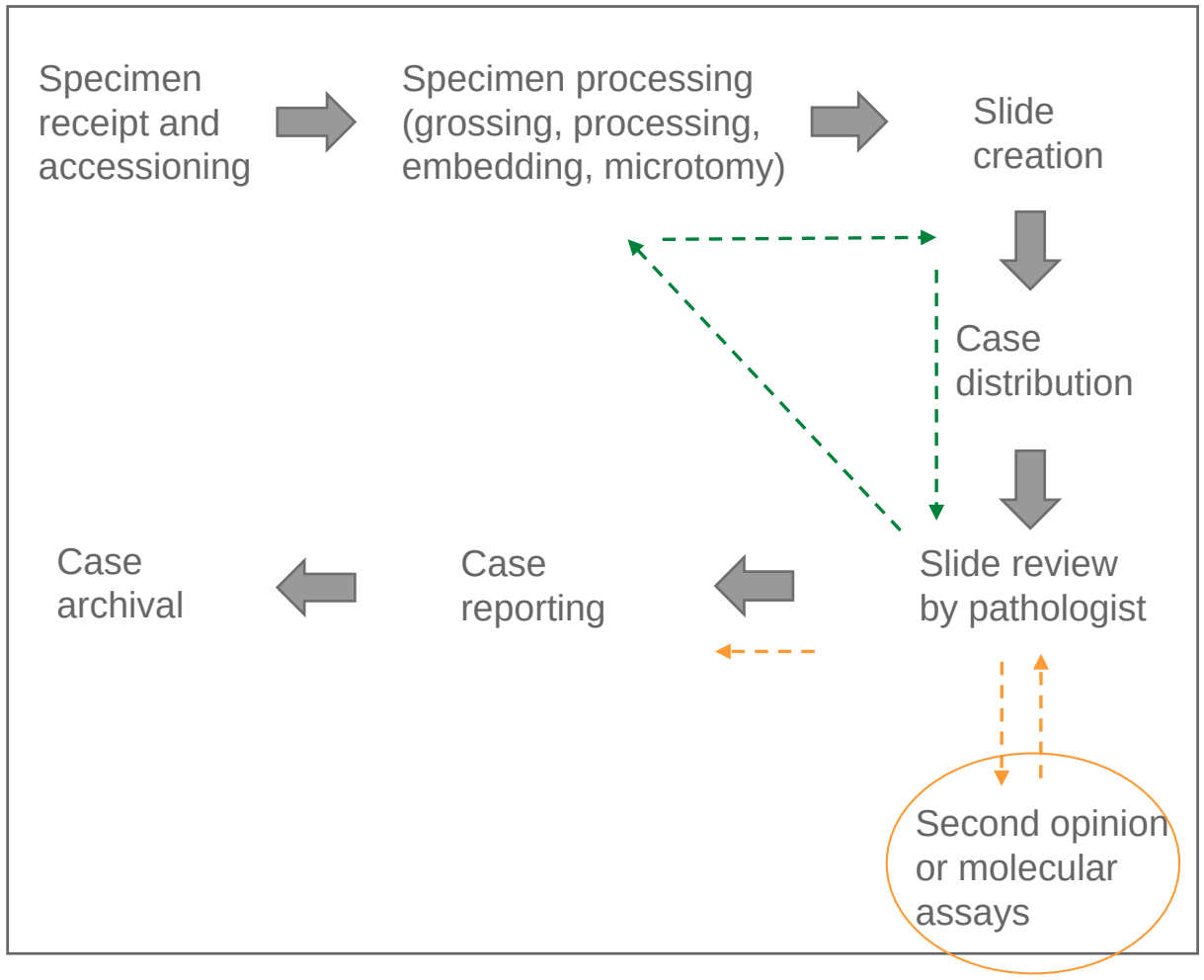
Histopathology routine and RS integration

Increase awareness and acceptance by clinicians

Identify unmet needs and provide tailored solutions



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Tissue preparation compatible with current histopathology workflow

Optimised protocols

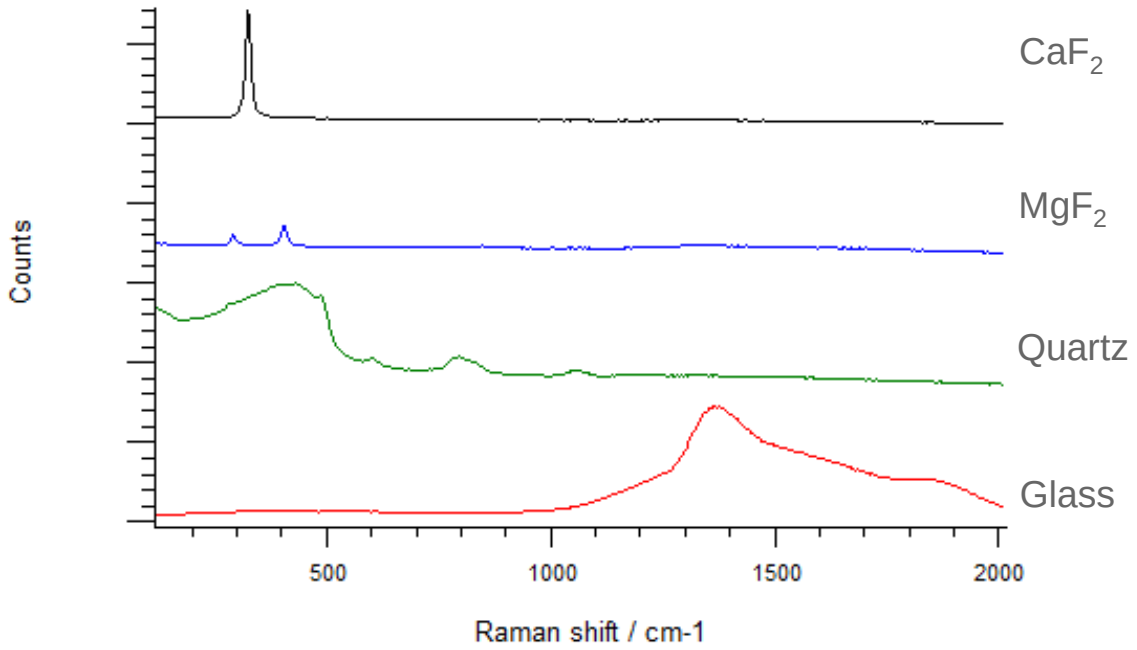
Raman imaging

- CaF_2 and MgF_2 are popular substrates but expensive
- Snap frozen
- Cryosections

Routine histopathology in the hospitals (UK)

- Glass slides (cheap)
- Formalin fixed paraffin embedded (FFPE)
- Microtomed

Substrate spectra collected at 785 nm



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Tissue preparation compatible with current histopathology workflow

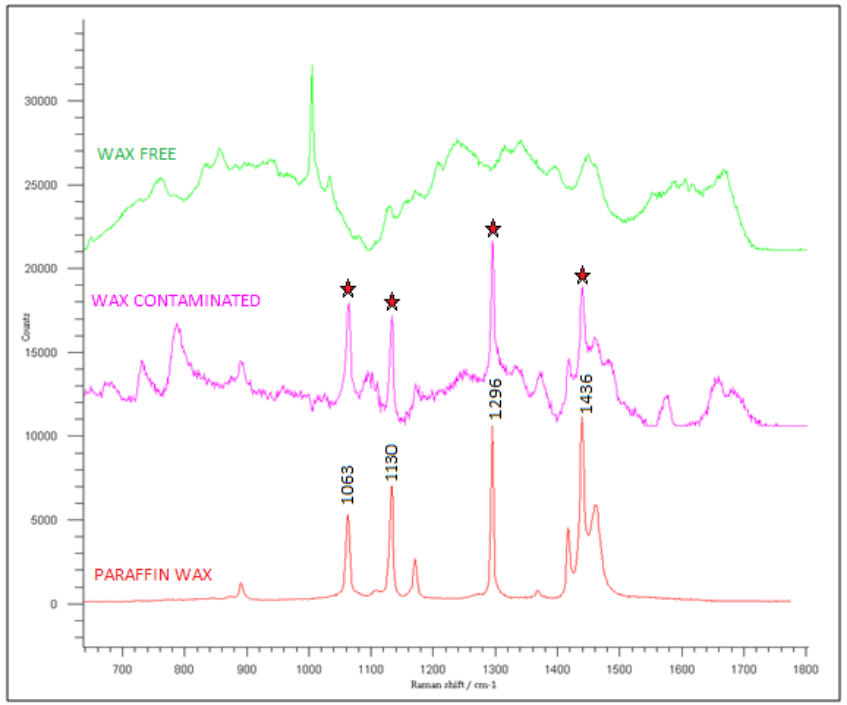
Optimised protocols

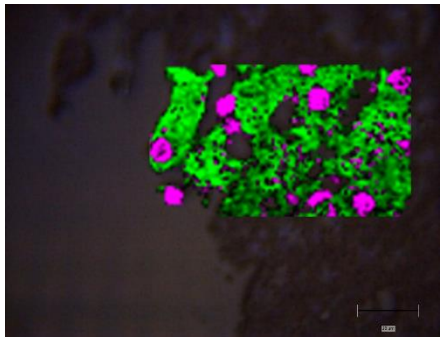
SMART:

- Snap frozen cryosections and FFPE samples
- CaF_2 and an alternative Raman-friendly substrate

Sample preparation optimisation:

- Complete deparaffination
- Choice of substrate:
  - No interfering Raman background signals
  - Cheap
  - Enable tissue region selection





MCR-ALS image of a rat liver section

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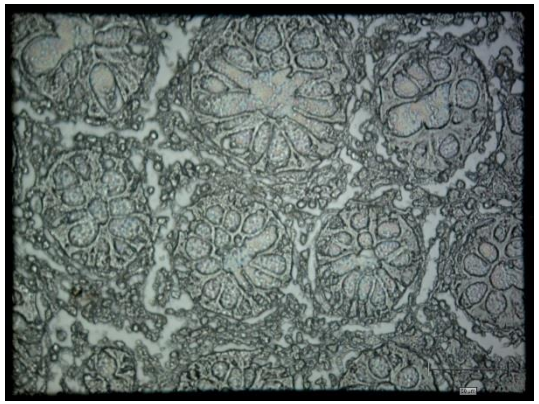
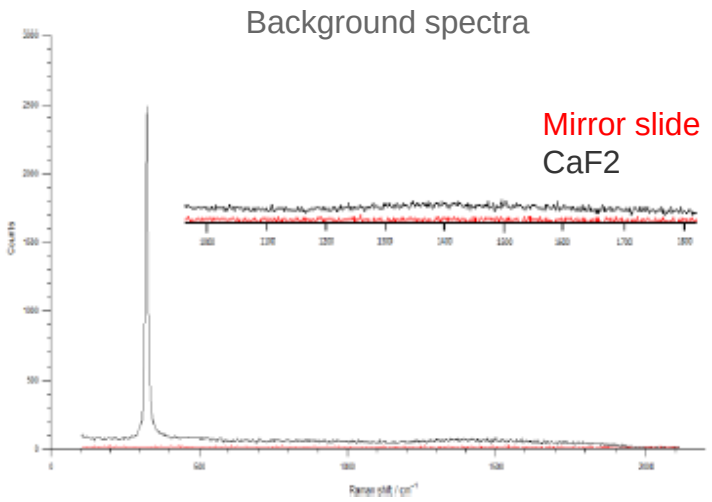
Mirror slides

Optimised protocols

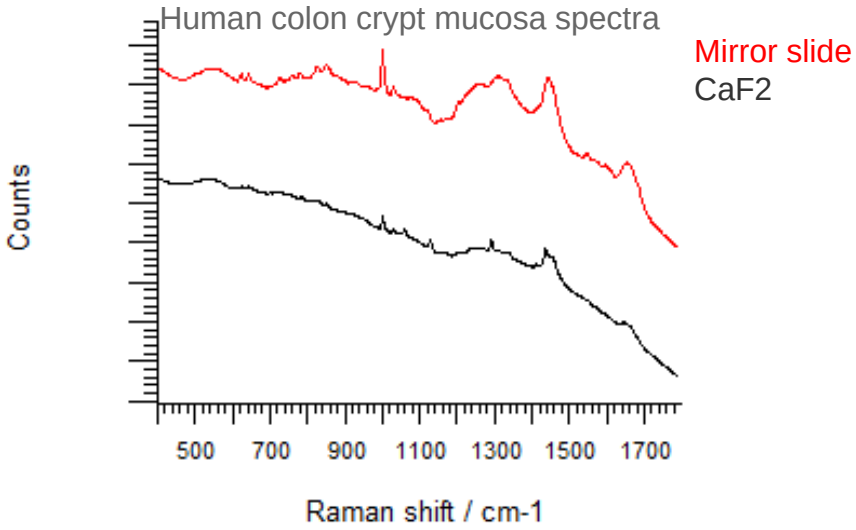
- Mirror finish – reflective, high contrast even for unstained tissue sections
- No Raman background
- Higher signal-noise-ratio (SNR) spectra
- Complete deparaffination
- Significantly lower cost



Reflective white light image – human colon crypts cross section on mirror slide



Lewis *et al* 2016, Analytical Chemistry (manuscript submitted)



Gaifulina *et al* 2016 (manuscript in preparation)

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Provide a robust RS-based diagnostic platform

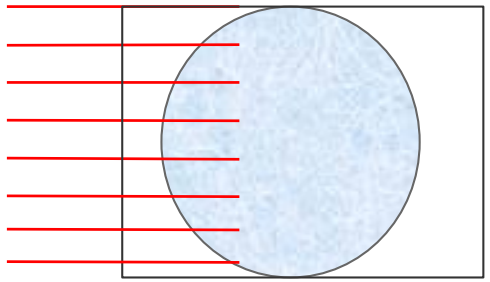
Optimised protocols

Mapping parameters

Laser wavelength: 785 nm

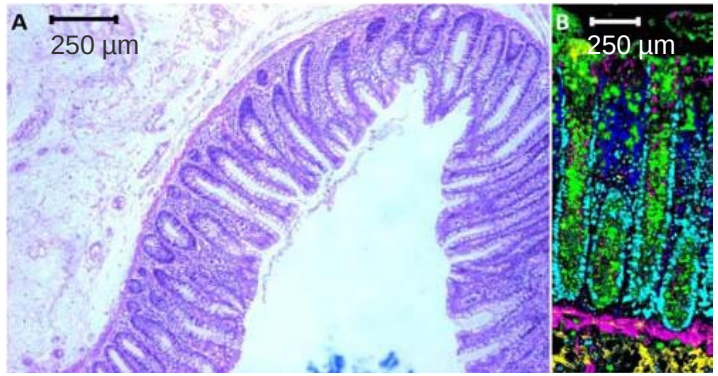
StreamLine™ imaging:

- Laser line geometry
- Fast imaging
- Automates collection of 1000's of spectra
- Slalom mode (covers the gaps between data points when large step sizes are used)



Step size:

- High spatial resolution image
  - 1.1 µm step size
  - Images more familiar to pathologists
  - Lacks nuclear-to-cytoplasmic ratio information



A. H&E stained human colon. B. PCA image.
Gaifulina *et al* 2014, Austin J Clin Pathol 1(5): 3

- Lower spatial resolution
  - 10 µm – 40 µm step size (Slalom mode)
  - Include nuclear-to-cytoplasmic ratio information

*Gavin Lloyd, Paper 9703-5 15 Feb 9:30

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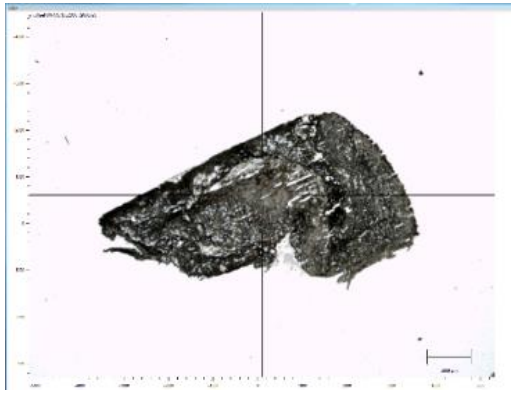
Facilitating the identification of diagnostically relevant regions

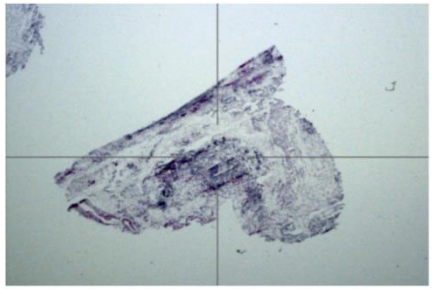
Provide a robust RS-based diagnostic platform

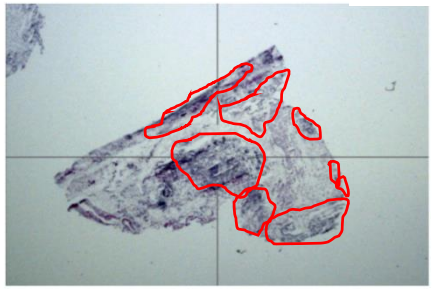
RA800 series tissue scanner

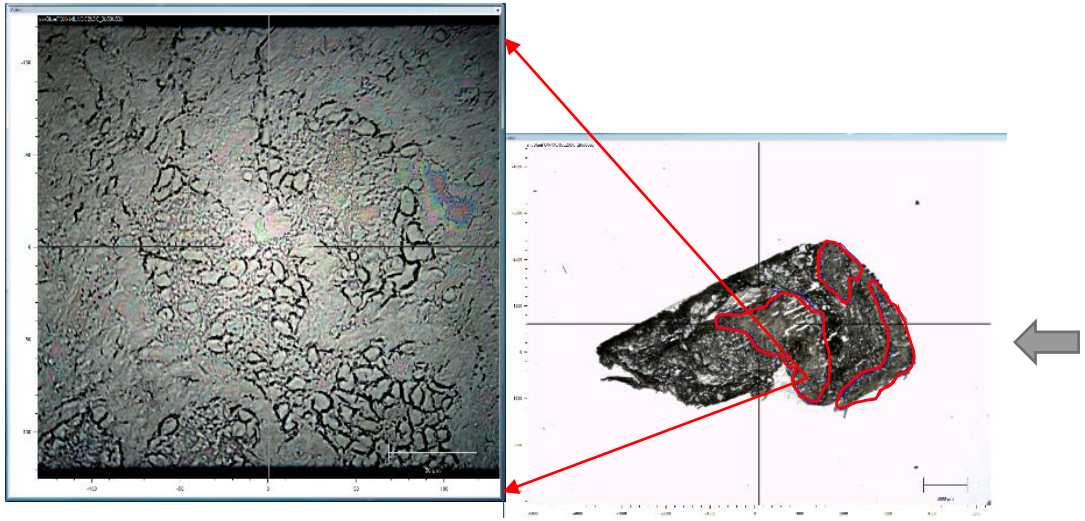


0.4× magnification
Reflective white light

H&E adjacent section







25× magnification

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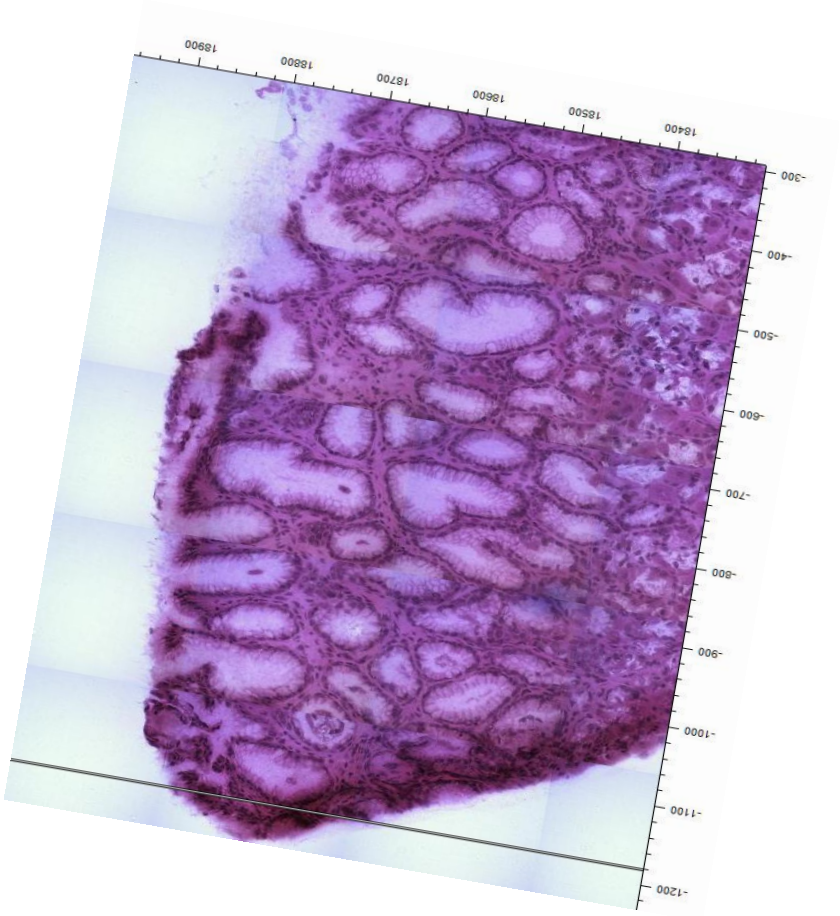
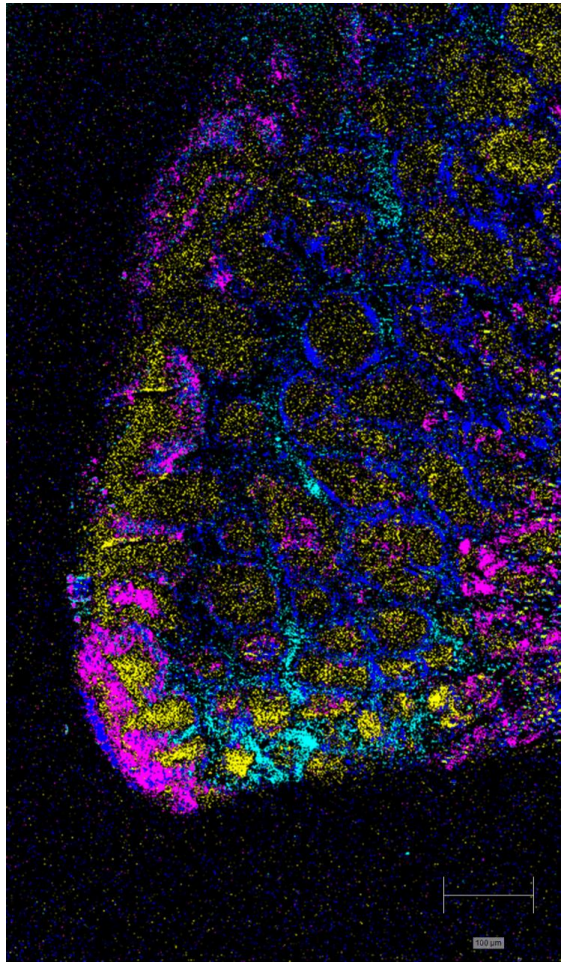
Raman imaging to discern different anatomical layers

- H&E stained Barrett's mucosa (human oesophagus) section



- Raman image generated by PCA
- inVia 785 nm StreamLine



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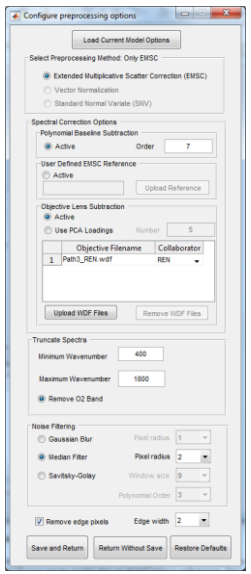
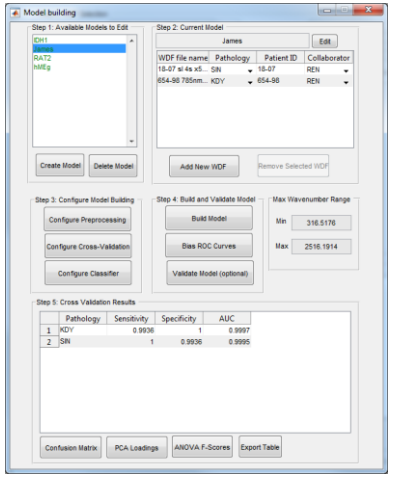
Classification model building

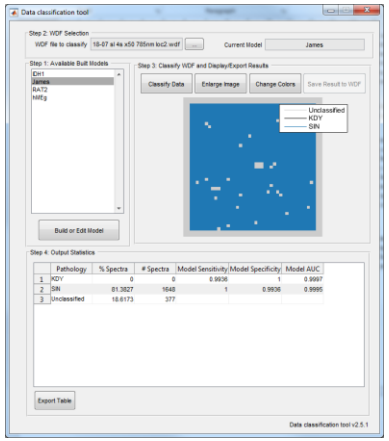
Provide a robust RS-based diagnostic platform

Optimised protocols

- A classification model building tool will be provided
- Direct import of maps generated using RA800 tissue analyser
- Data pre-processing algorithms are implemented
- Ensuring data transferability







- Multi-centre study currently in place
- Human oesophageal sections
  - 10x intraepithelial metaplasia (IM)
  - 10x adenocarcinoma (AC)
- Algorithms in the final product will depend on the outcomes

Disease stage		No.of spectra	
IM		114,456	
AC		104,911	
Site 1 as training set (site 2 as test set)		**Site 1 as training set (site 3 as test set)**	
Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
95.7	76.9	78.8	80.8

Isabelle *et al* 2016, Faraday Discussions, published online

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Distributable GI tract cancer classification model

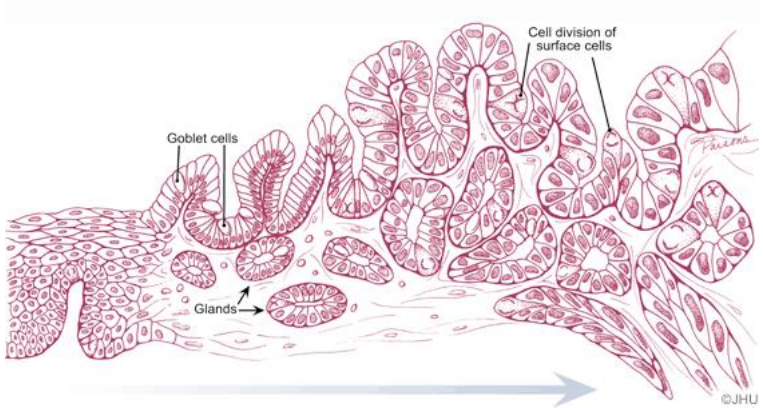
Provide a robust GI tract cancer classification model

- To be used by clinicians for diagnosis
- Trialled as part of the histopathology review routine
- Evaluate practicality

- Oesophgeal cancer
- 5th largest cancer killer in the UK
- 5 year survival rate = only 15%
- Early diagnosis = better survival
- RS-based identification to improve accuracy and objectivity

Model:

- >250 healthy/cancer samples
- Confirmed by consultant pathologist with second opinion
- Stages:
  - Normal squamous
  - IM (non-dysplastic/indefinite for dysplasia)
  - Dysplasia (low to high grade)
  - AC



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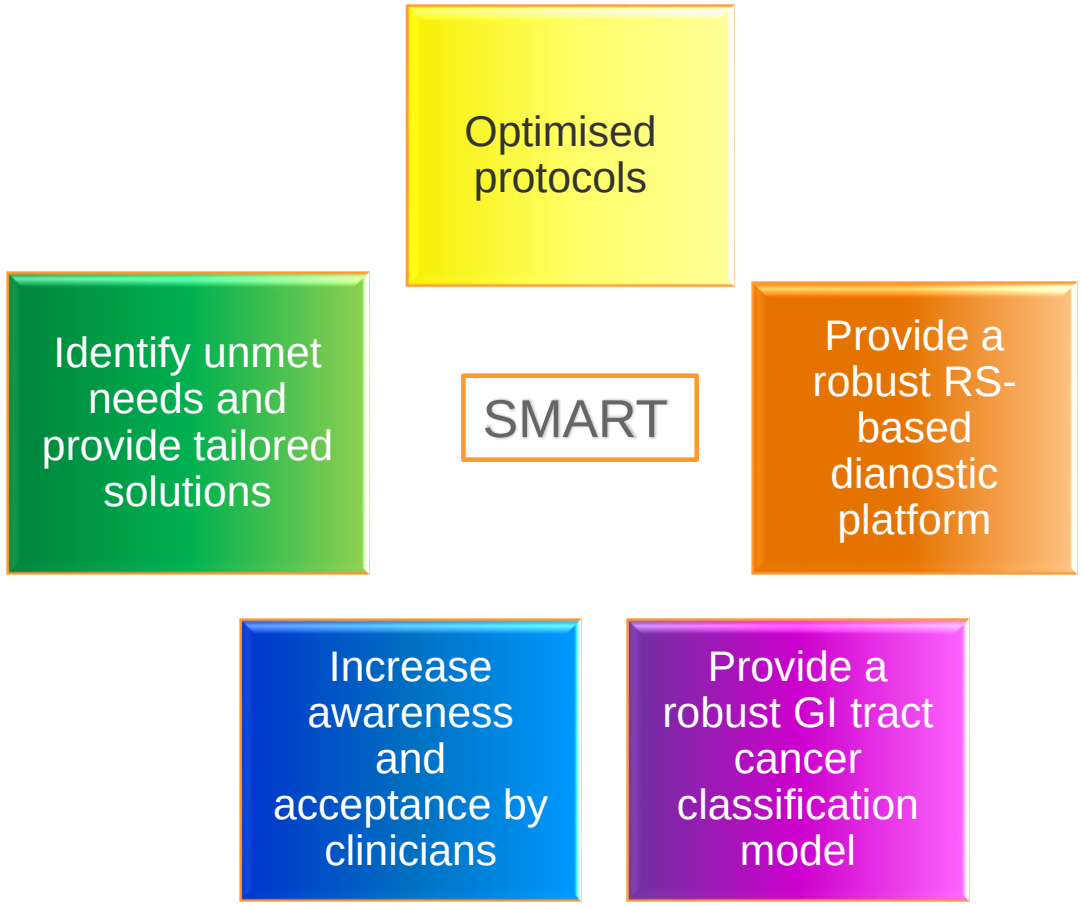
Conclusion

- The hurdles towards the clinical adoption of RS for routine histopathology review have been discussed
- The SMART consortium is addressing each of the hurdles
- By Oct 2016, SMART aims to deliver:
  - Increased awareness and acceptance of Raman technologies among clinicians
  - Optimised sample preparation, mapping and data analysis protocols
  - A robust Raman platform for building disease classification models and classifying unknowns
  - A distributable, robust and validated GI tract cancer model



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Acknowledgments

SMART Consortium

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- Dr Gavin Rhys Lloyd
- Dr Catherine Kendall
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- Dr Manuel Rodriguez-Justo
- Prof Geraint Thomas

Innovate UK

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University College London, UK

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- Mr Robert Brown
- Miss Stephanie Ohrel
- Miss Ruth Lawson
- Mr Tim Smith
- Mr Steve Ritchings
- Mr Marek Synowiec
- Mr Tim Mills
- Dr Brian Smith
- Mr David Reece
- Mr Simon Holden

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Thank you!

Morning 

SATELLITE EVENTS
Workshops

WS.V	Raman imaging techniques demonstrated on an inVia Reflex micro-Raman system: examples and discussion	Sep 22 09:00 - 11:00
Co-organized and Sponsored by: **RENISHAW** *Abstract*		RENISHAW
WS.VI	Optimising, redesigning and preparing to build: steps to additively manufacture mechanical parts	Sep 22 15:00 - 17:00

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Raman imaging to discern different anatomical layers

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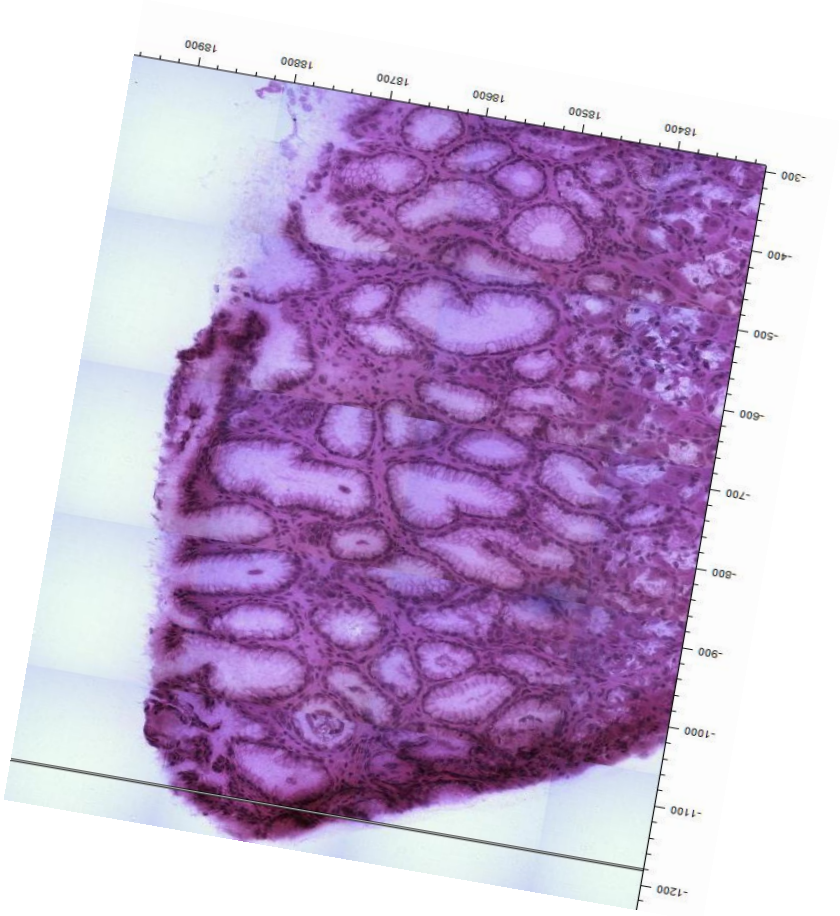

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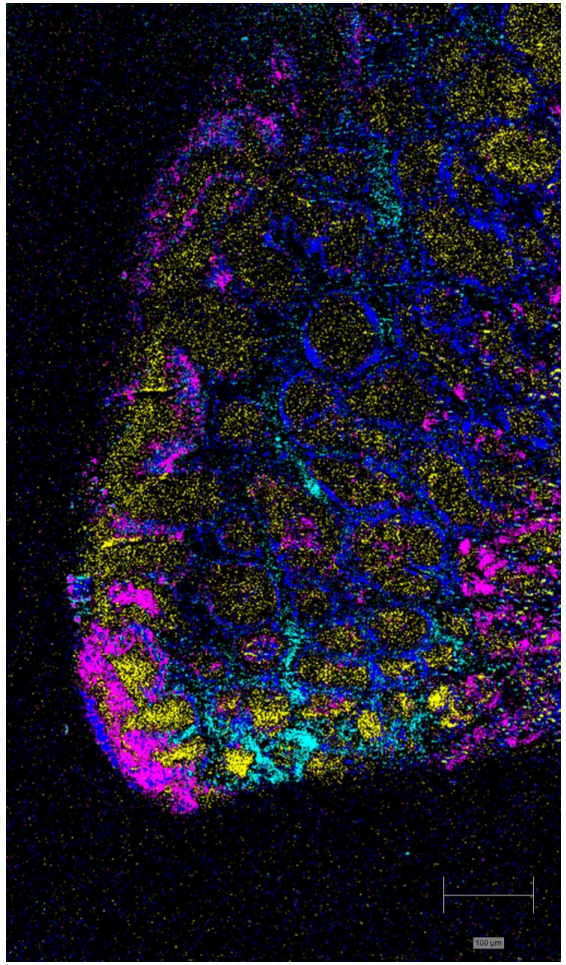

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