



# Intra-operative assessment of surgical margins and sentinel lymph nodes by Fast Raman spectroscopy

**Ioan Notingher**

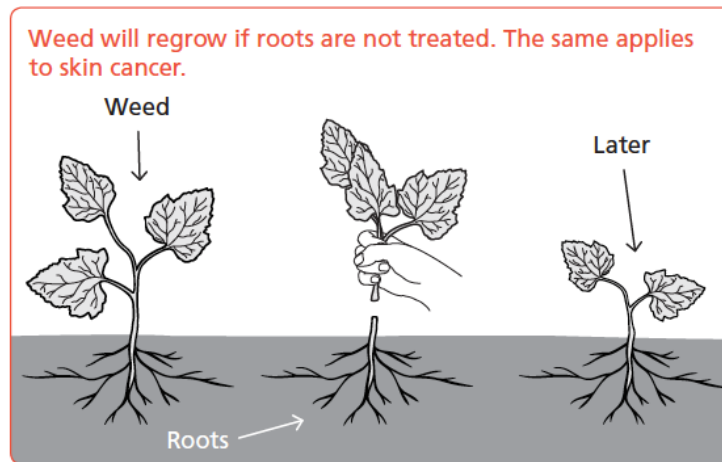
*School of Physics and Astronomy,  
University of Nottingham, UK*

# Basal Cell Carcinoma (BCC) Surgery



The commonest human cancer with around 150,000 cases each year in the UK.

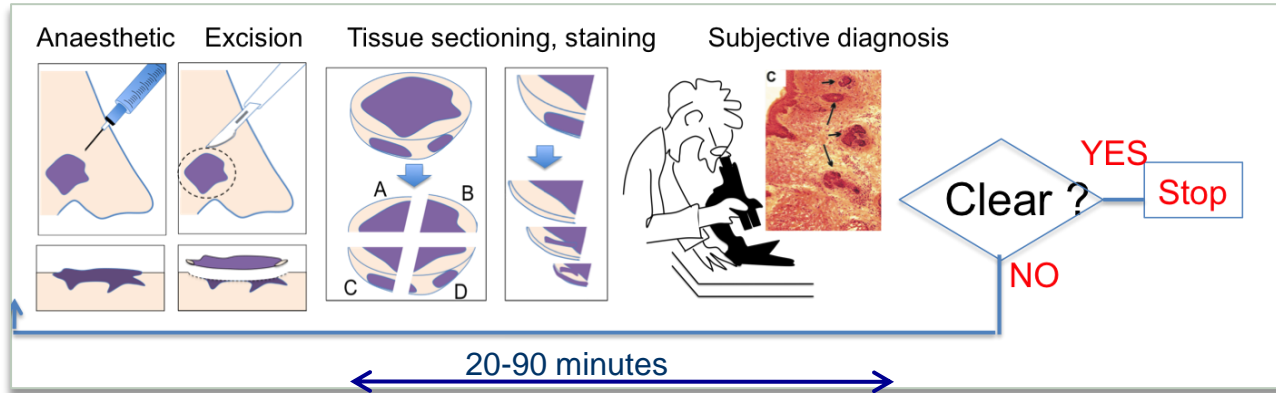
Challenge:  
cut all cancer out  
spare healthy tissue



Incomplete margins: 11% (19.9% for BCC around eyes)

# Mohs micrographic surgery

(invented 1936)

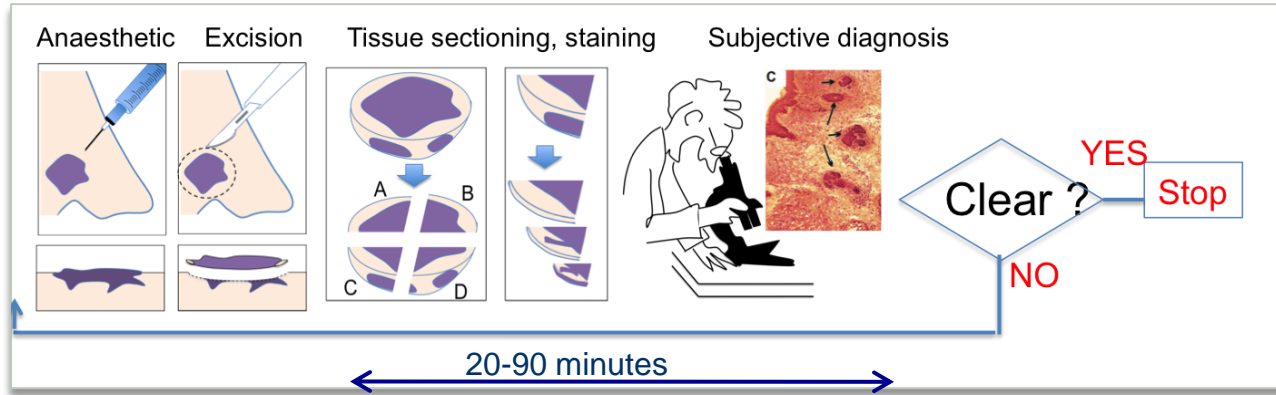


Frederic Edward Mohs  
(1910 – 2002)

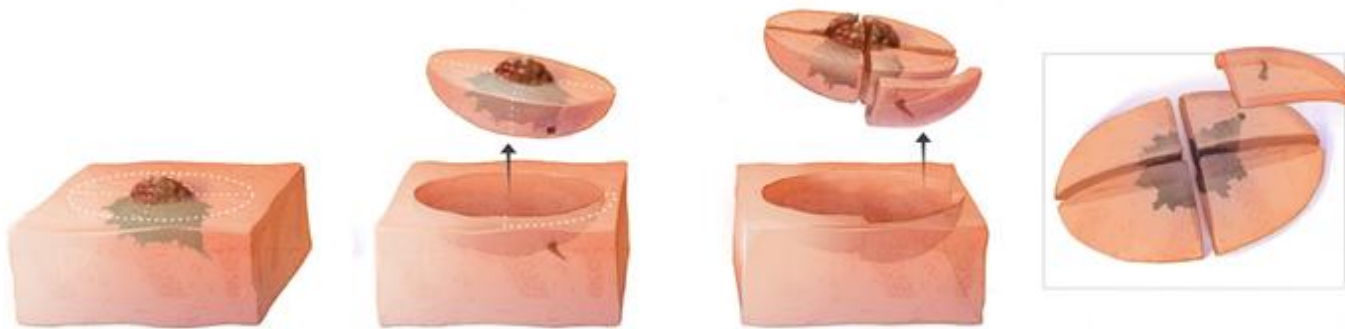
100% of resection  
surface  
recurrence rates <2%  
at 5 years

# Mohs micrographic surgery

(invented 1936)



Frederic Edward Mohs  
(1910 – 2002)



100% of resection  
surface  
recurrence rates <2%  
at 5 years

Frozen tissue



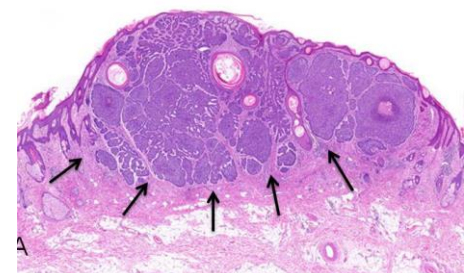
Sectioning



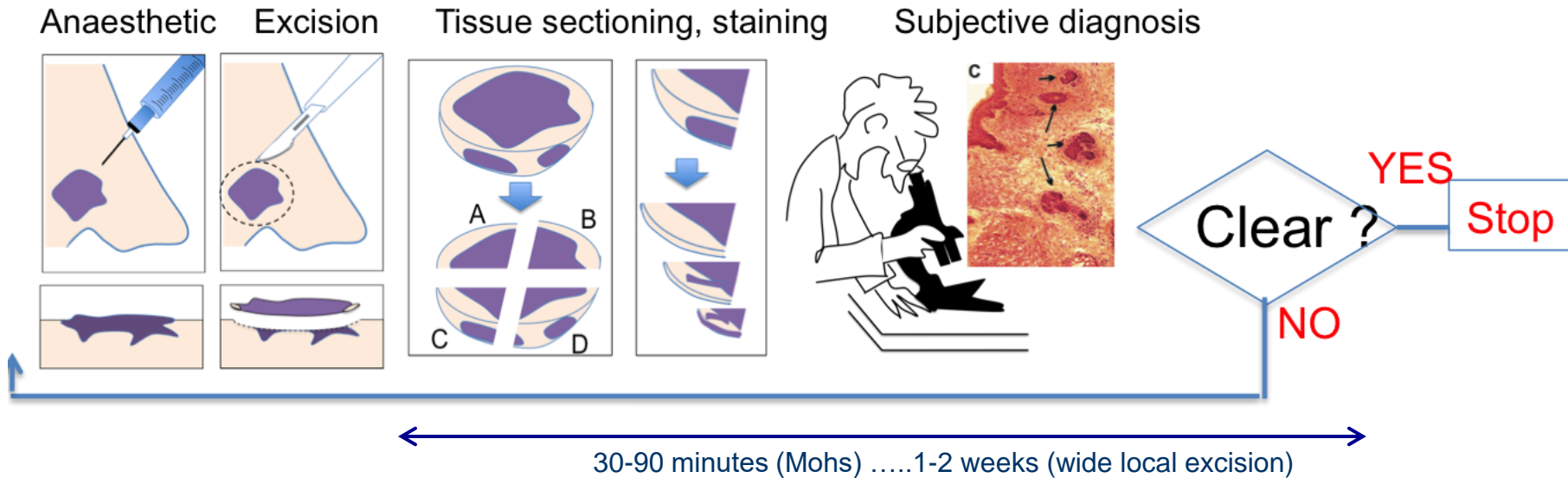
Staining



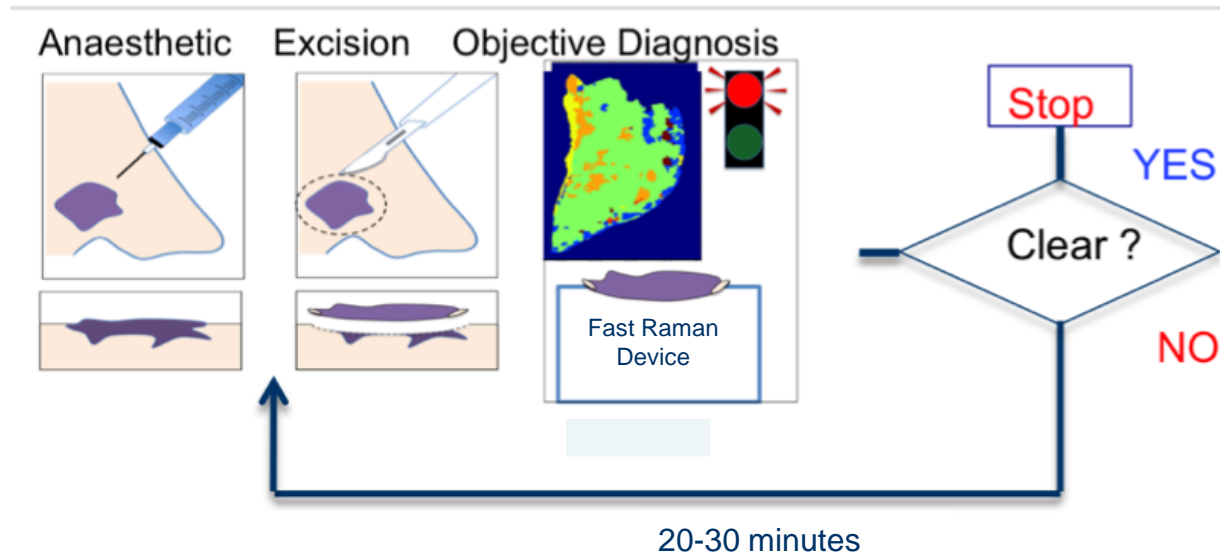
Diagnosis by Mohs surgeon

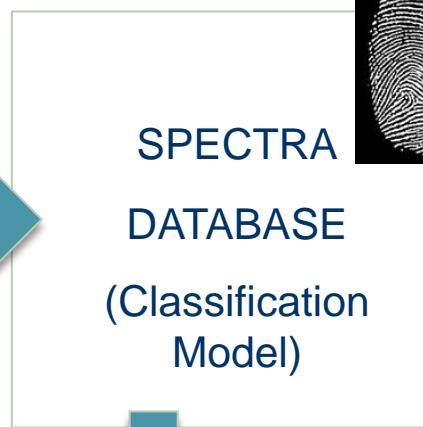
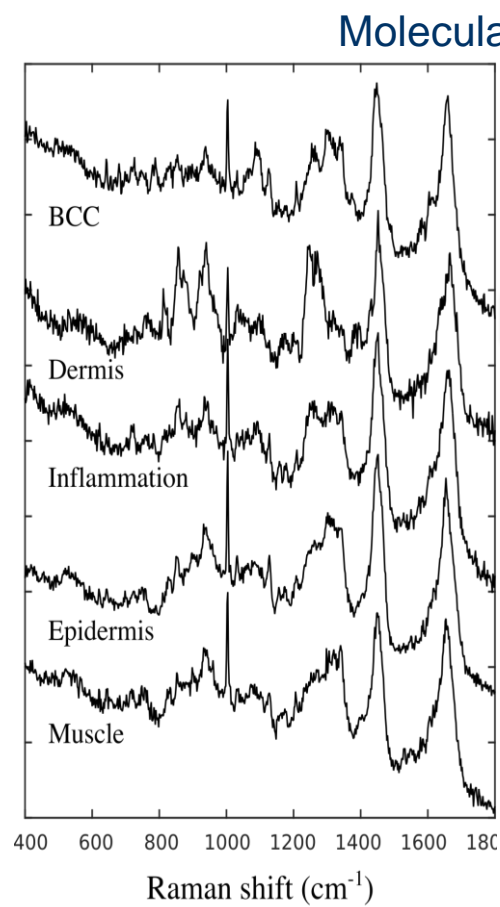
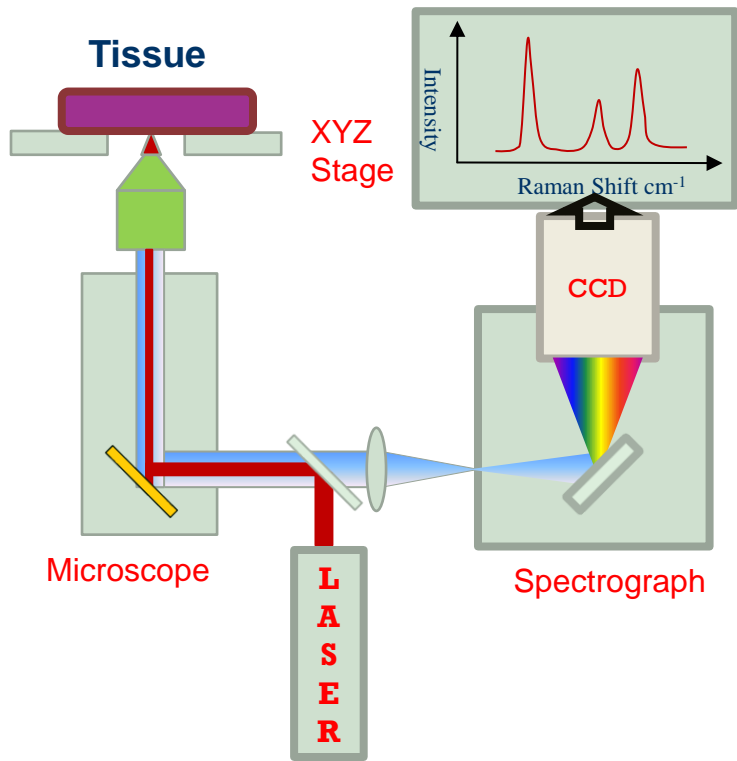


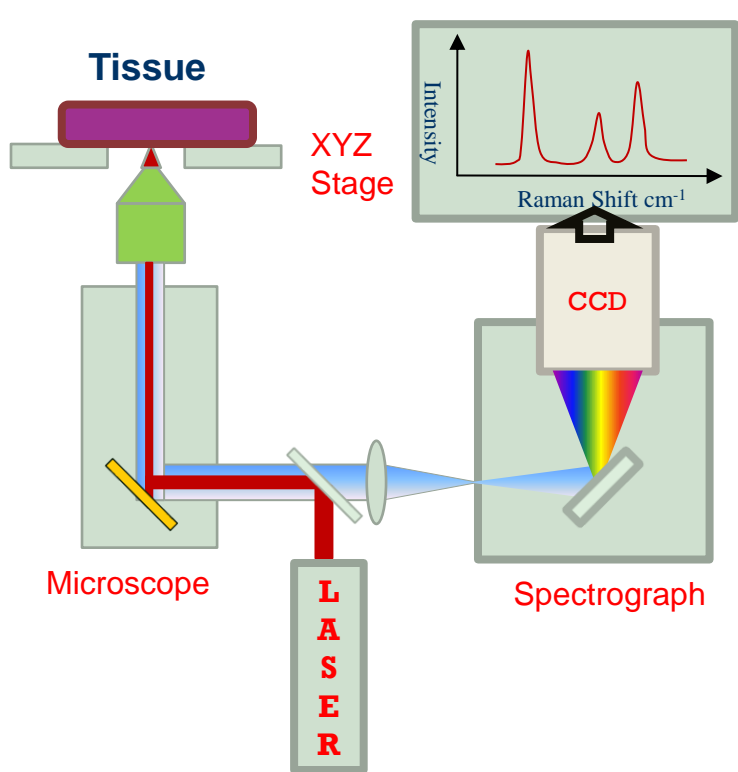
# Current surgery



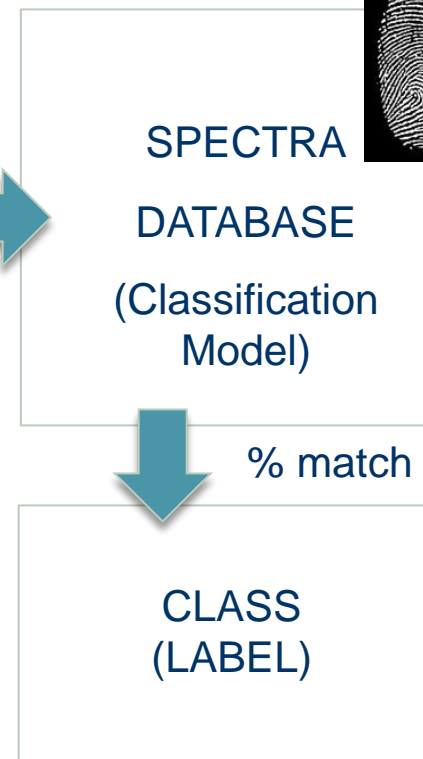
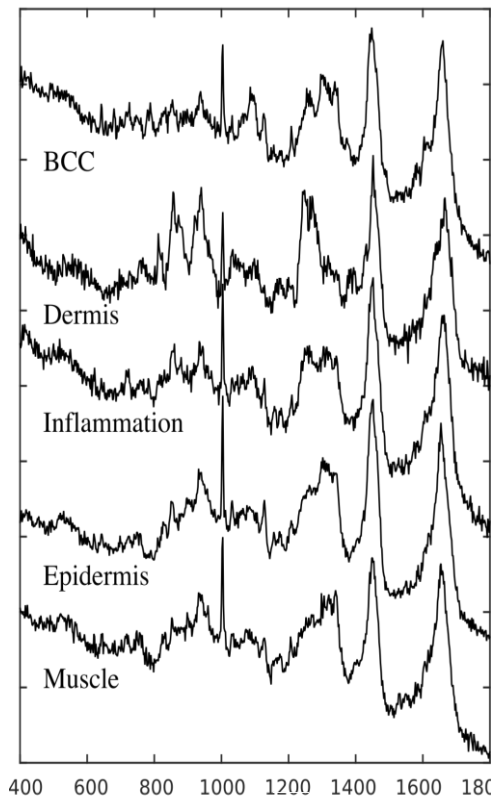
# Future surgery?



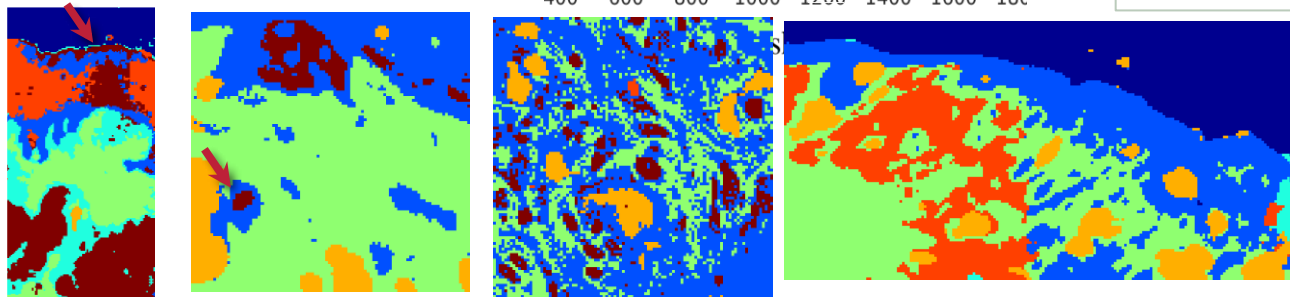




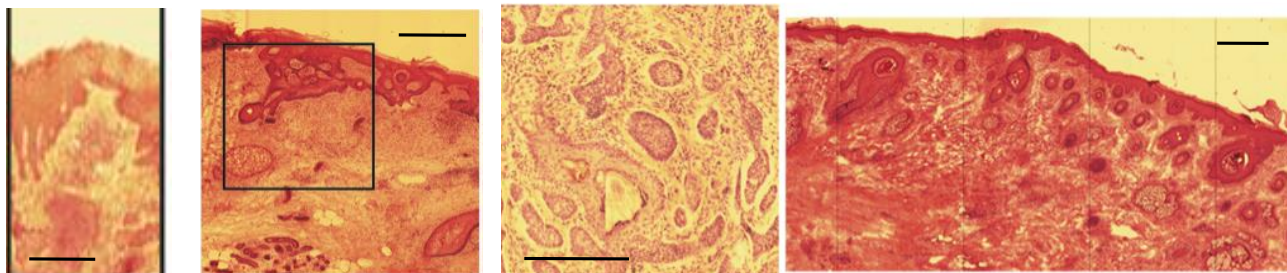
## Molecular Fingerprints



RMS Diagnosis



H&E histopathology

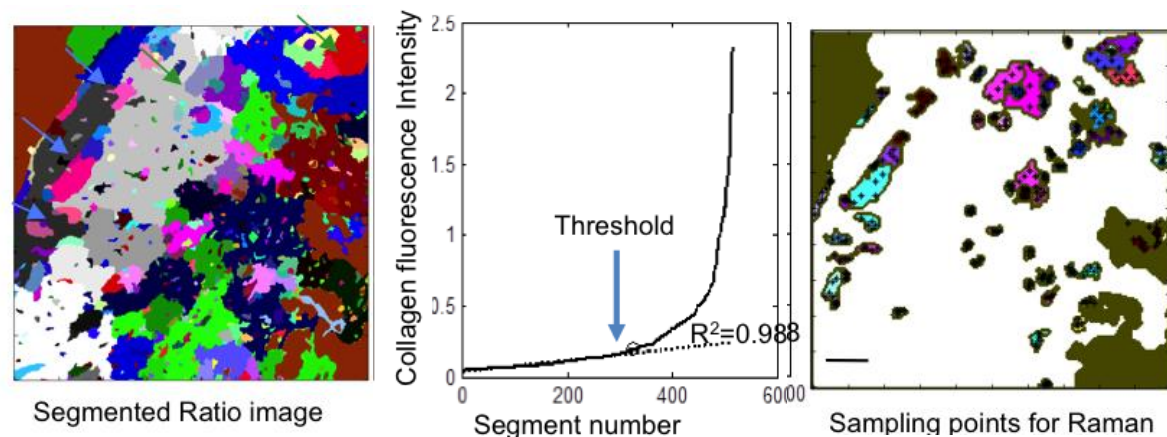
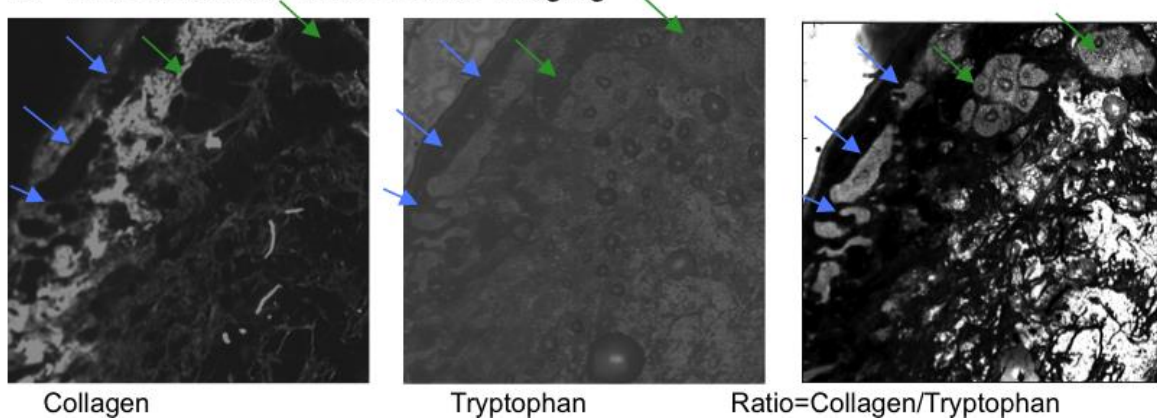


- BCC
- Muscle
- Fat
- Dermis
- Inflamed D.
- Epidermis
- Substrate
- Unknown

Scale bars:  
400  $\mu\text{m}$

# Multi-modal spectral imaging

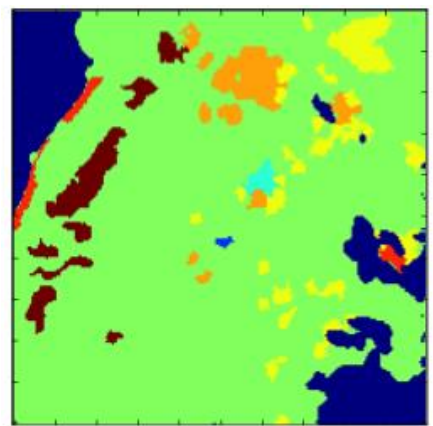
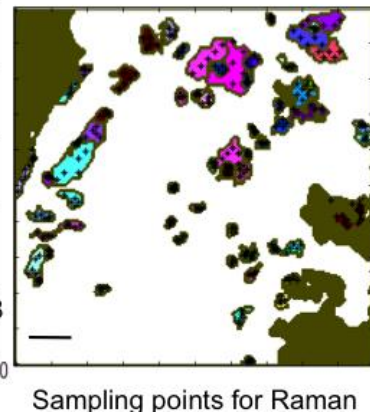
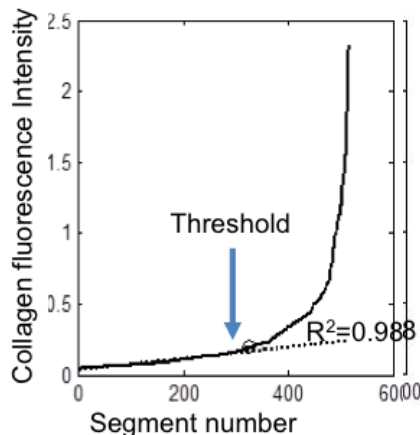
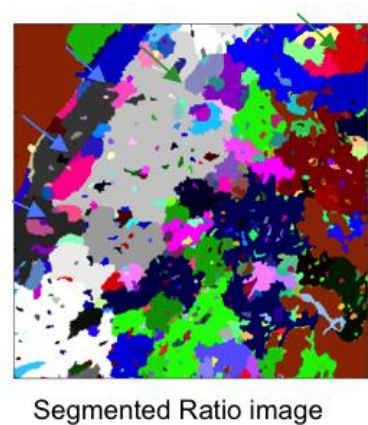
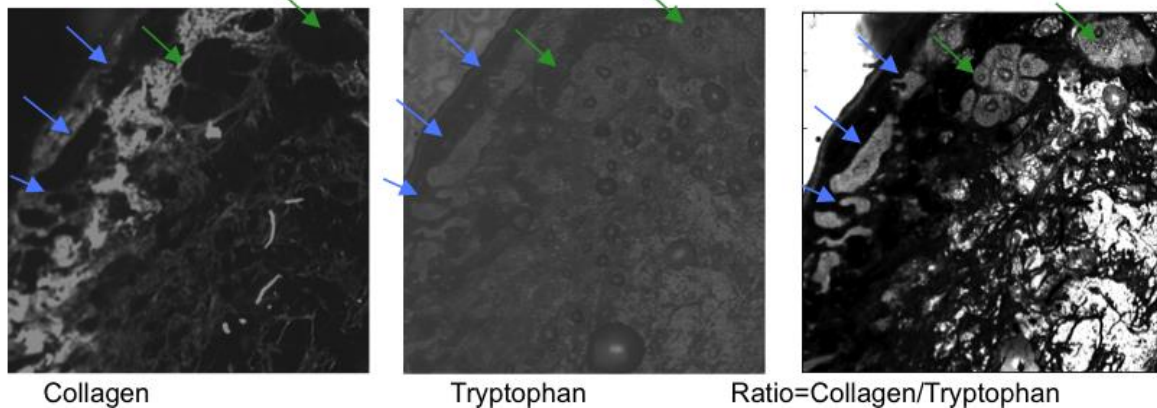
**a** Wide-field auto-fluorescence imaging





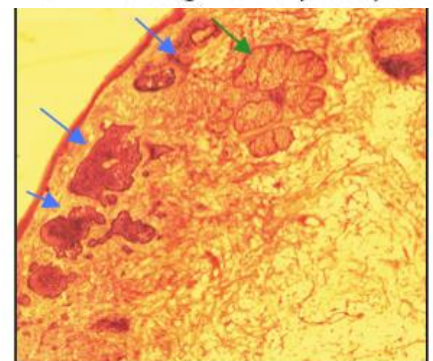
# Multi-modal spectral imaging

**a** Wide-field auto-fluorescence imaging



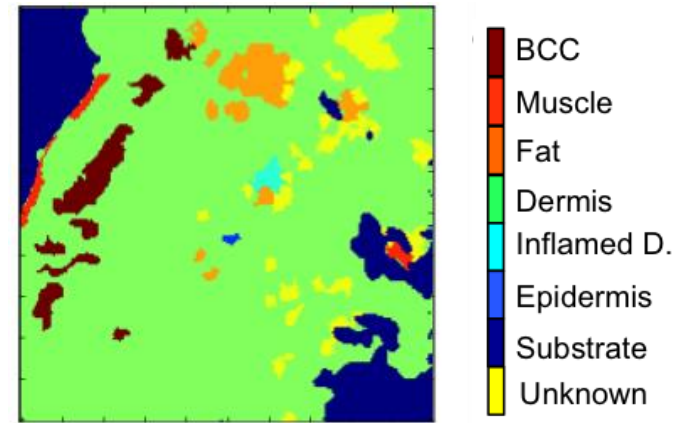
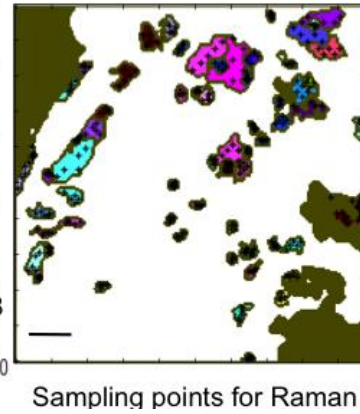
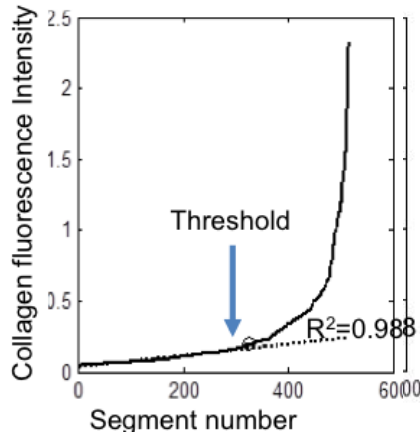
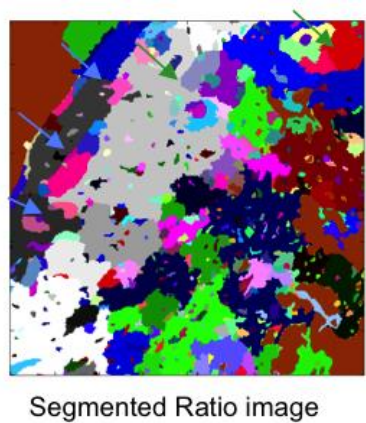
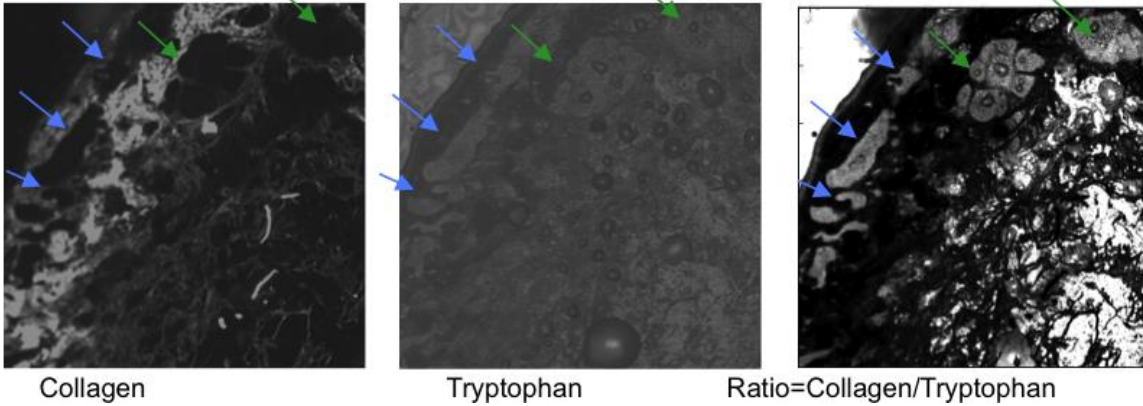
- BCC
- Muscle
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- Substrate
- Unknown

MSH 70 segments (350spectra)

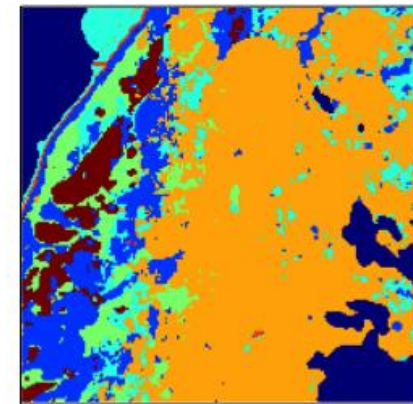
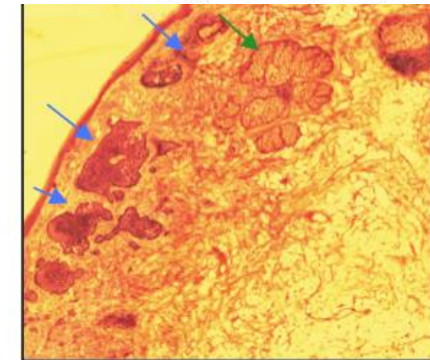


# Multi-modal spectral imaging

## a Wide-field auto-fluorescence imaging



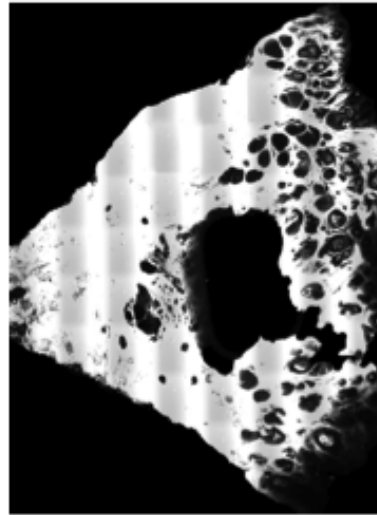
MSH 70 segments (350spectra)



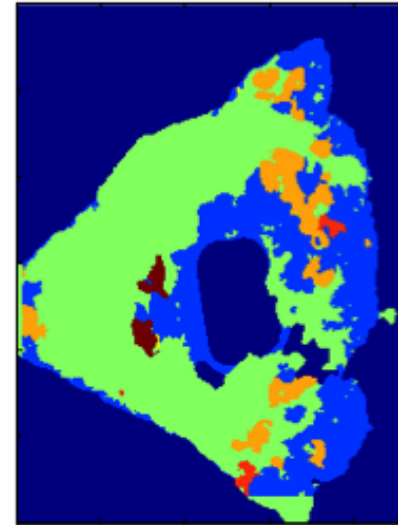
Raster-scanning (40,000spectra)



**Auto-fluorescence**



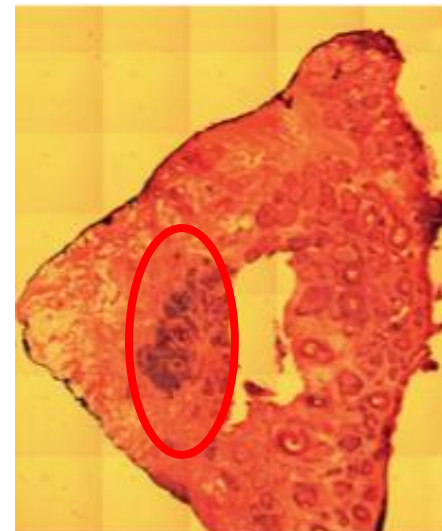
**Raman**



- BCC
- Muscle
- Fat
- Dermis
- Inflamed D.
- Epidermis

**Outcome**

Diagnosis 1cm<sup>2</sup> tissue:  
1500 spectra (20-60 min)





Gerwin Puppels





Gerwin Puppels



National Institute for  
Health Research



*Mohs surgeons:*  
Sandeep Varma  
Sunita Oedra  
Asish Sharma  
Anand Patel  
Richard Jerrom

**Primary outputs:**

- (i) integrate the AF- Raman instrument into clinical pathway (Mohs surgery).
- (ii) evaluate validity (sensitivity/specificity) and reliability (inter- and intra-user variability) in order to plan a definitive national diagnosis test accuracy study.

# Step 1: Raman analysis



Immediately after excision

Time limit: 40 minutes

# Step 1: Raman analysis

The screenshot displays a software interface for Raman analysis. At the top left is the logo for The University of Nottingham, with the text "UNITED KINGDOM · CHINA · MALAYSIA". The interface is divided into several sections:

- Patient Information:** A table with the following data:

Date	05-Oct-2023
Name	patient
ID	test
- Status:** A section containing the text:

Result:  
Analysis completed  
The sample is: positive  
0.1 perc is BCC positive (red)  
0.3 perc is covered by blood (yellow)  
0.3perc is unsampled (cyan)

This text is enclosed in a red rounded rectangle.
- Analysis Results:** Two Raman spectra plots are shown side-by-side. Each plot has a red circle highlighting a specific region of interest.
- Controls:** Three buttons are located on the right side: "Start" (blue), "Stop" (grey), and "Load / Unload" (grey).
- Measurement Progress:** A progress bar at the bottom right is labeled "Measurement" and shows a full bar with "100 %" next to it.

Immediately after excision

Time limit: 40 minutes

# Proof-of-concept study

Recruitment period: March 2020 – July 2021

Split layers: 30 layers: 9 BCC +ve  
21 BCC –ve

	<b>Sensitivity</b>	<b>Specificity</b>
AF-Raman instrument	89%	81%
Mohs surgeons:	88%	93%
<i>Br. J Dermatol</i> 2016 175: 549-554	88-92%	58-85%

ORIGINAL ARTICLE

 **JEADV**  
CLINICAL PRACTICE OPEN ACCESS  
JOURNAL OF THE  
EUROPEAN ACADEMY  
OF DERMATOLOGY &  
VENEREAL DERMATOLOGY

**Ex vivo assessment of basal cell carcinoma surgical margins in Mohs surgery by autofluorescence-Raman spectroscopy: A pilot study**

Received: 29 August 2023

Revised: 5 October 2023

Accepted: 7 November 2023

DOI: 10.1002/jvc2.336



# Diagnostic test accuracy study: Recruitment Sept 2022 – May 2023

Full-face layers: 125 patients (1 layer/patient): (56% female and 44% male)

	Sensitivity	Specificity
Raman vs. reference	67%	73%
Raman vs reference (exclude out-of-focus images)	96%	73%
Mohs surgeon vs. reference	86%	89%
Mohs surgeon vs. reference (EMC Rotterdam study 2016)	92-88%	58-85%

Reference = consensus panel 3 dermatopathologists

# Breast conserving surgery (BCS)



UK: 50,000 new patients per year  
(300,000 in the USA)  
BCS: 60-70% patients



BMJ

## Reoperation rates after breast conserving surgery for breast cancer among women in England: retrospective study of hospital episode statistics

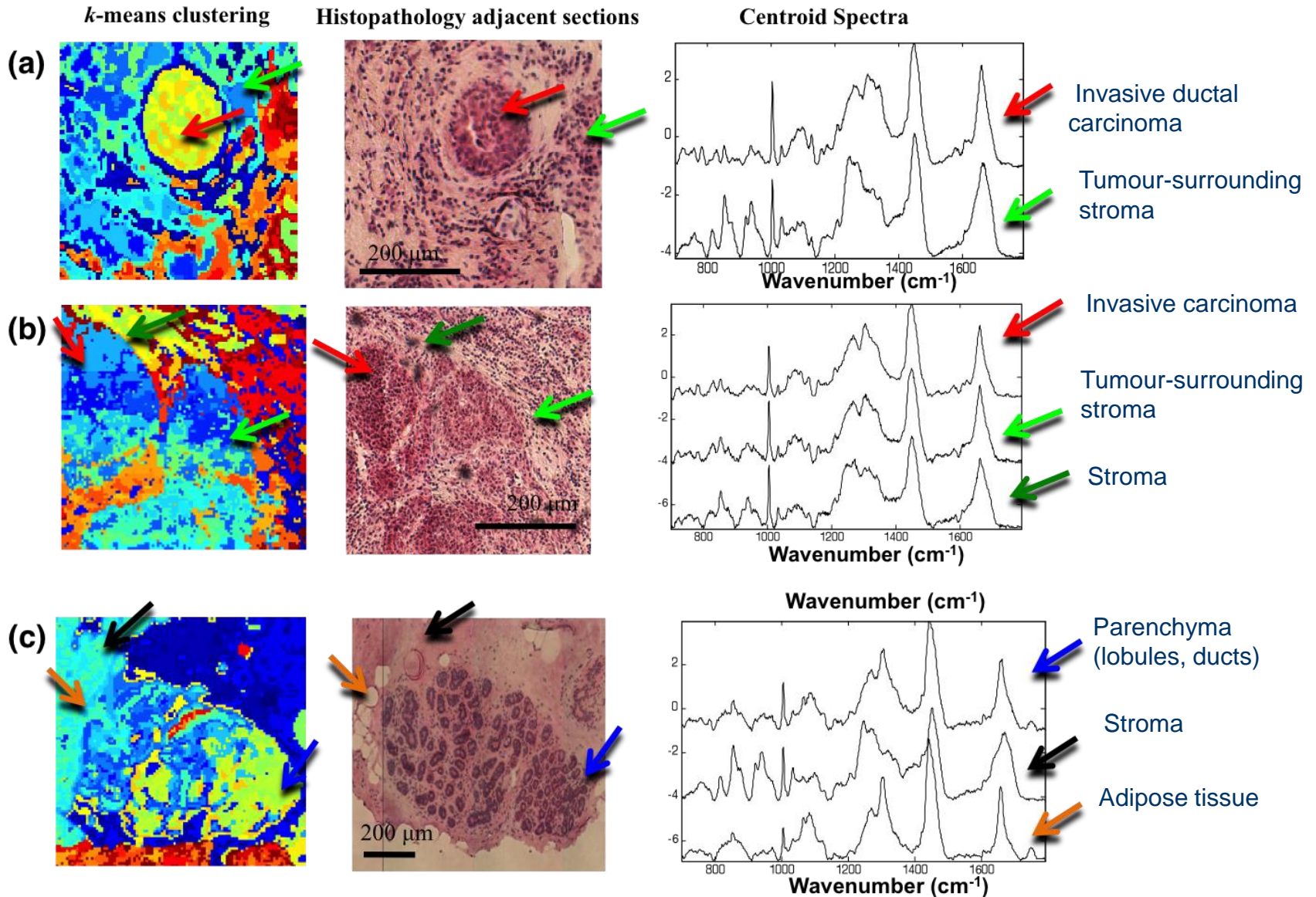
OPEN ACCESS

**Conclusion:** One in five women who had breast conserving surgery in England had a reoperation. Reoperation was nearly twice as likely when the tumour had a carcinoma in situ component coded. Women should be informed of this reoperation risk when deciding on the type of surgical treatment of their breast cancer.

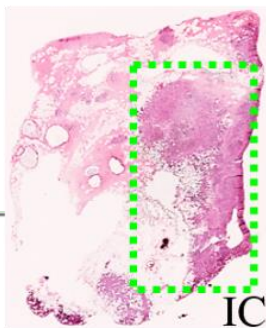
~5000 re-operations per year  
(these patients are ~4 times more likely to die)

BMJ 2012;345:e4505

# Invasive Breast Cancers

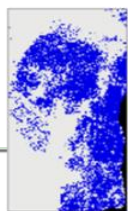


H&E



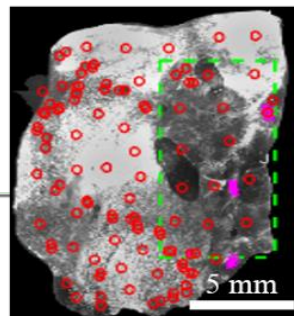
IC

Raman  
raster scan

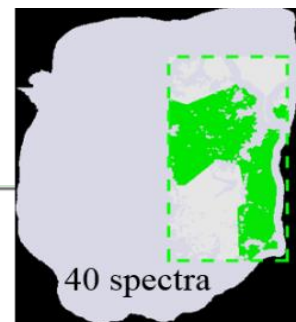


11,300  
spectra

AF

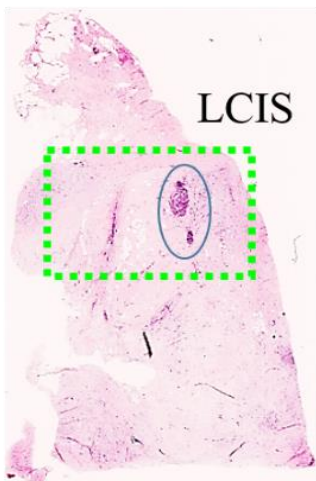


AF-Raman

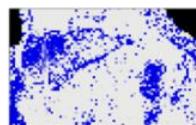


40 spectra

(A)

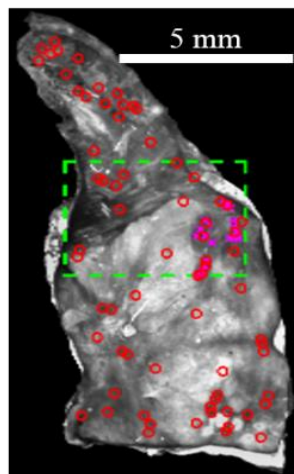


LCIS

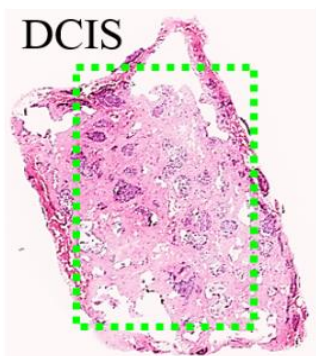


5,000 spectra

(B)

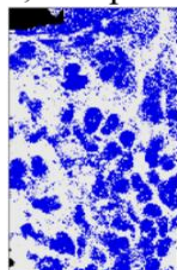


46 spectra

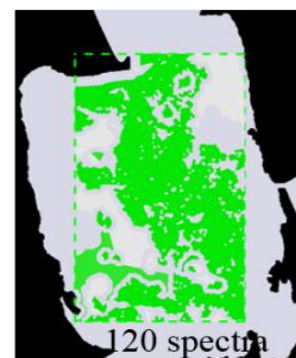
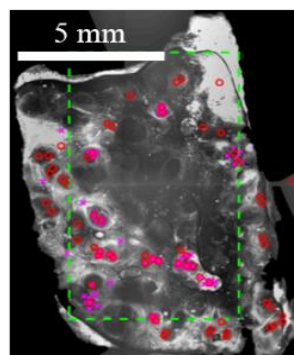


DCIS

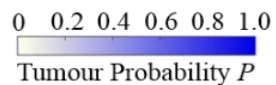
24,000 spectra



(C)

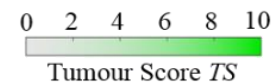


120 spectra



Tumour Probability  $P$

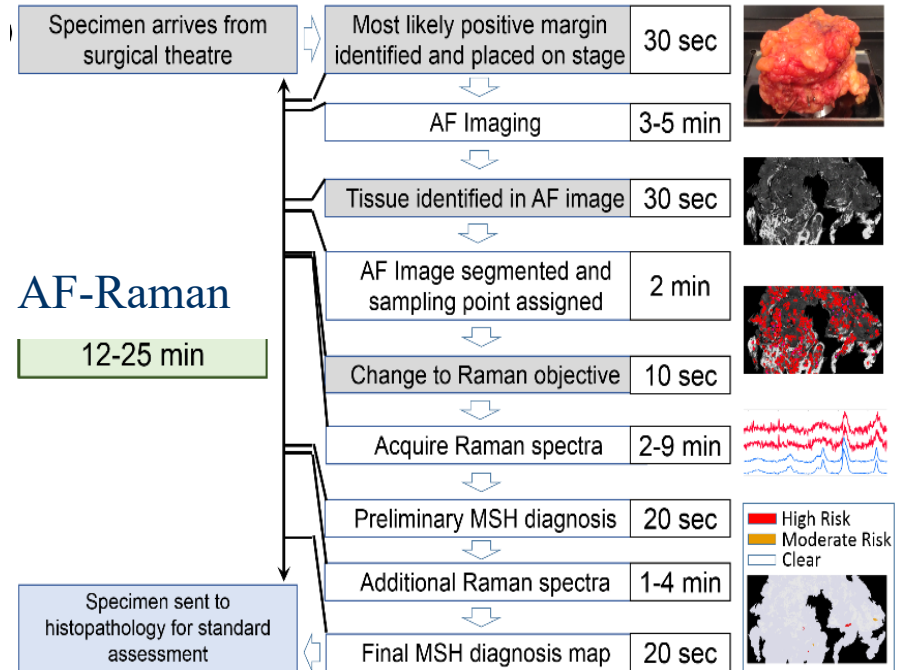
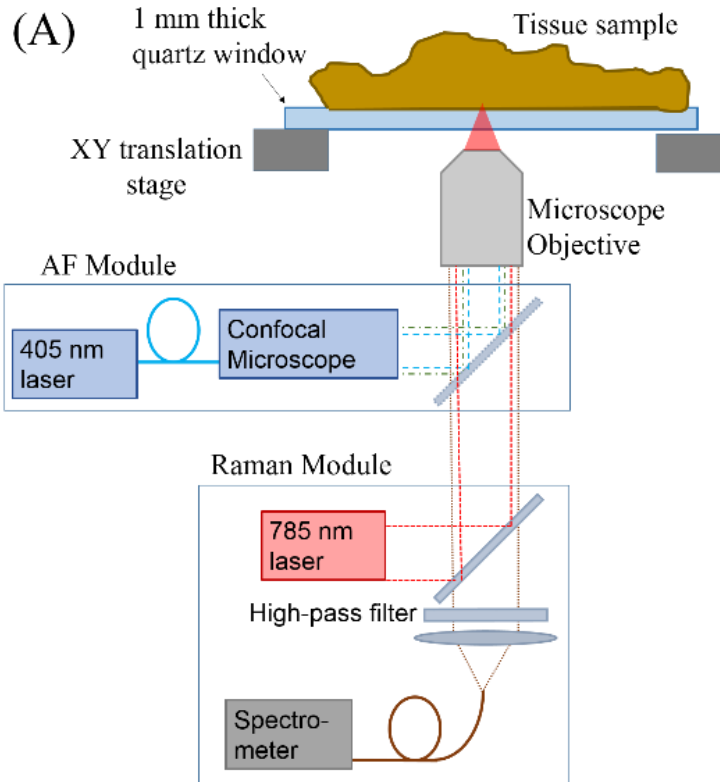
$$TS = 10 \left[ 1 - \prod_i (1 - P_i) \right]$$



Tumour Score  $TS$

# Integrated auto-fluorescence imaging and Raman Spectroscopy Imaging

*PNAS* 2013, 110 (38), 15189-15194.

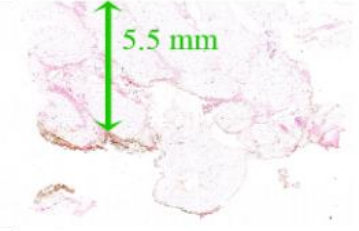
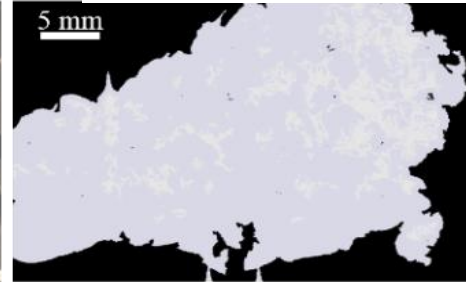
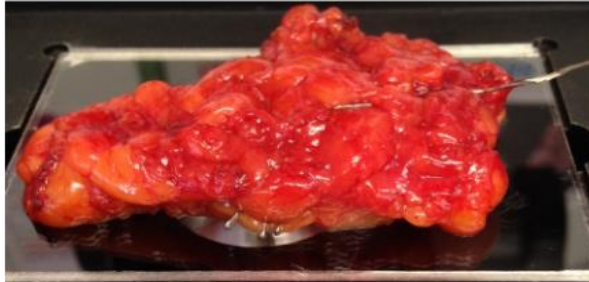


# Independent validation using whole lumpectomy specimens (51 samples from 51 patients)

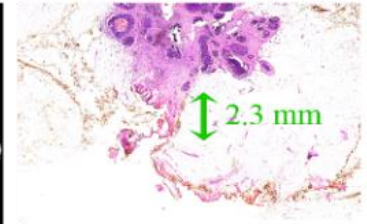
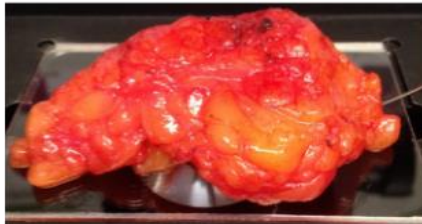
AF-Raman

H&E

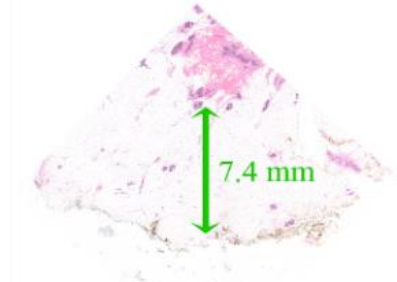
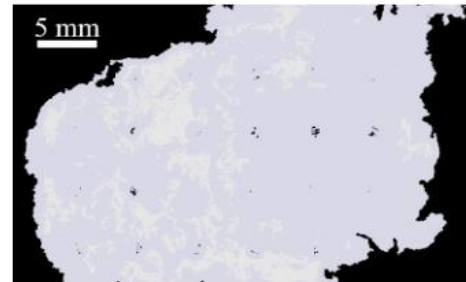
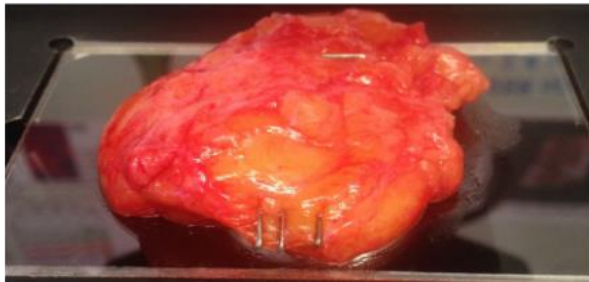
(A)



(B)

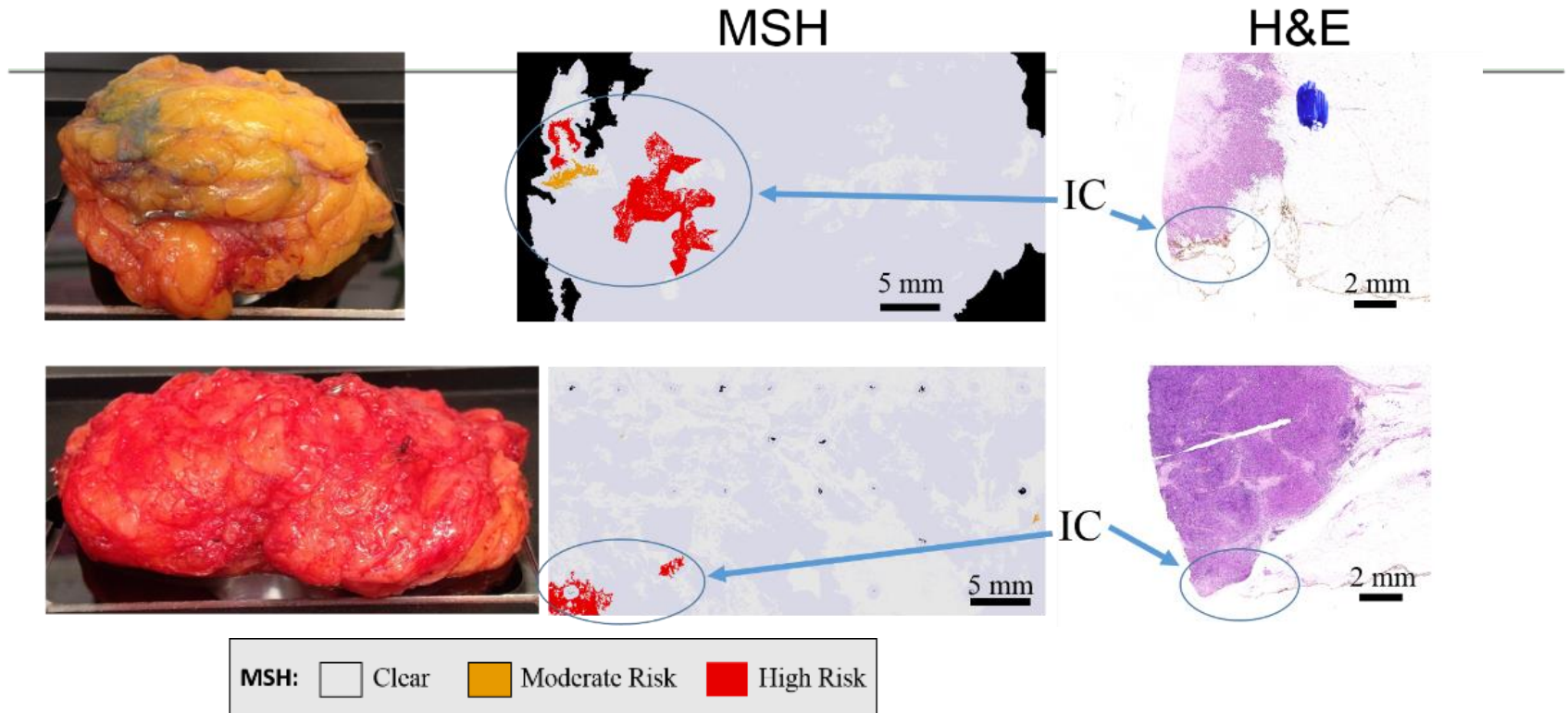


(C)



MSH:  Clear  Moderate Risk  High Risk

# Independent validation using whole lumpectomy specimens (51 samples from 51 patients)

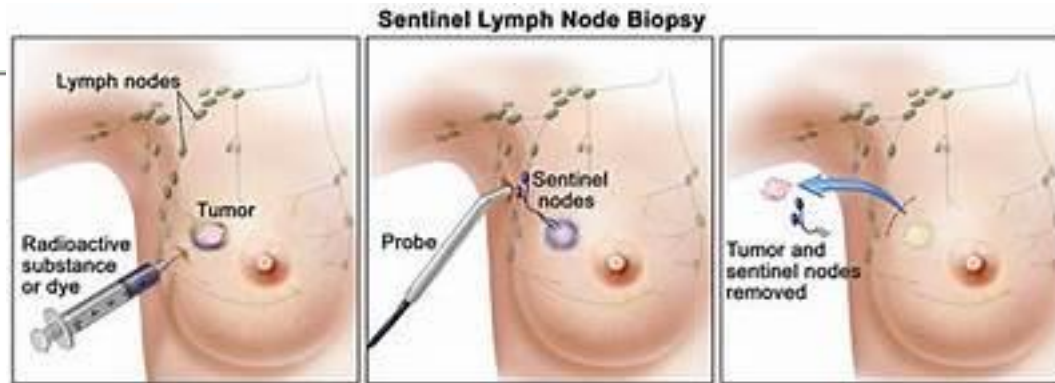


10 lumpectomy samples histology “positive margins”  
AF-Raman “Positive” diagnosis for 10 specimen

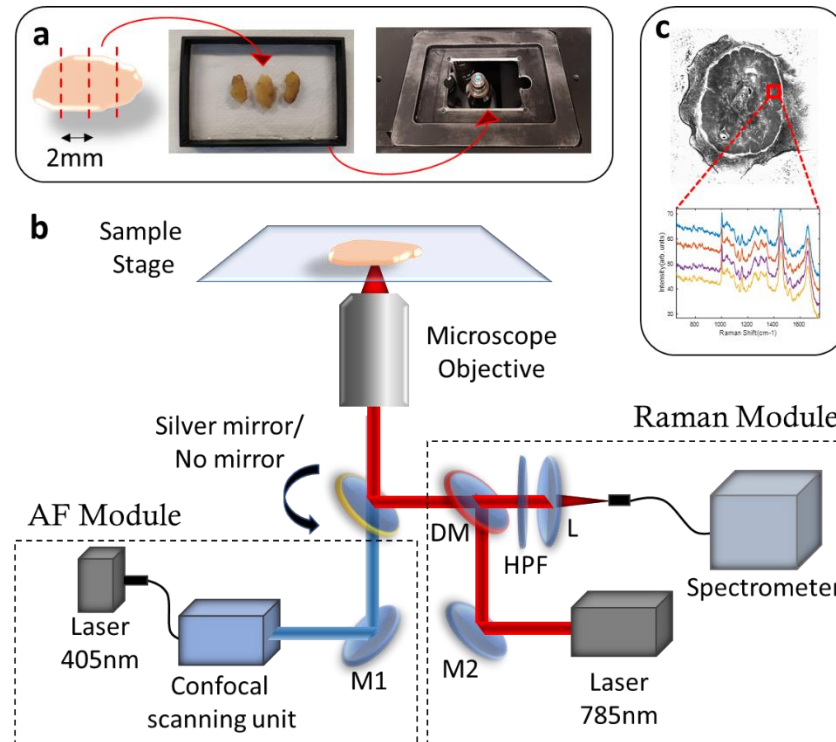
100% sensitivity , 78% specificity

Tissue analysis time:  
Sample size: 4 x 6.5 cm<sup>2</sup>;  
12-24 minutes.

# Intra-operative assessment of lymph nodes

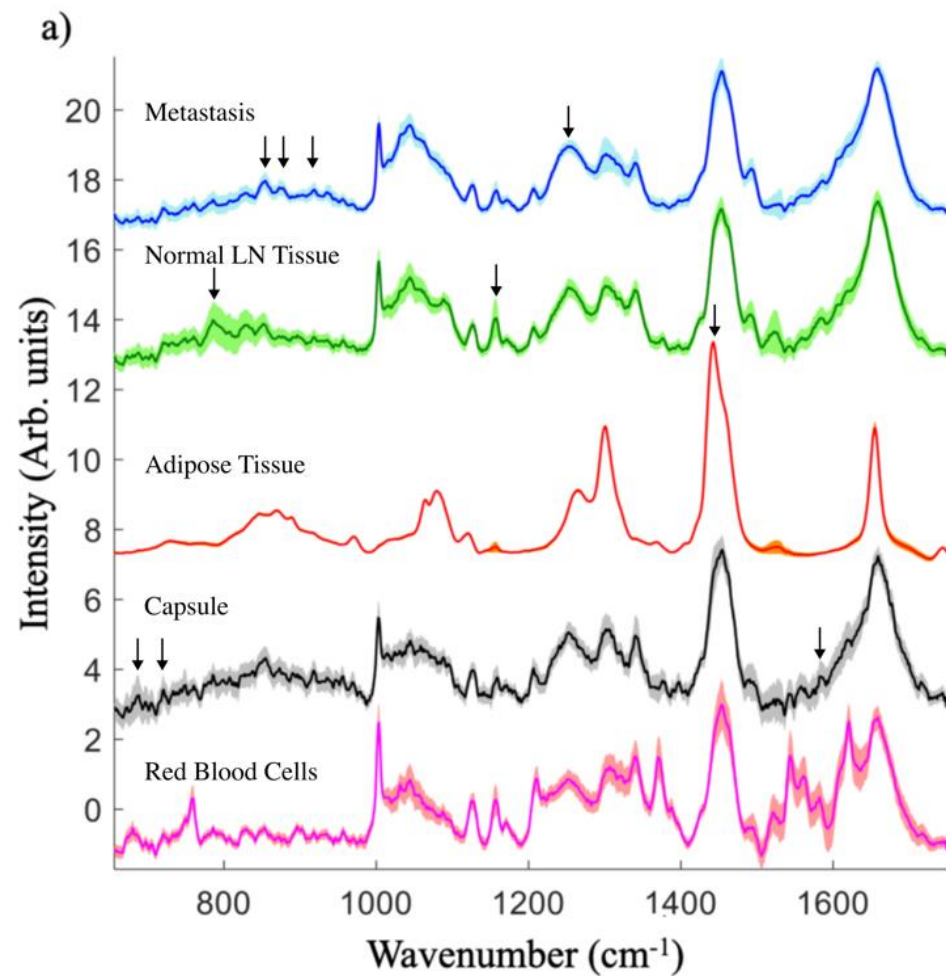
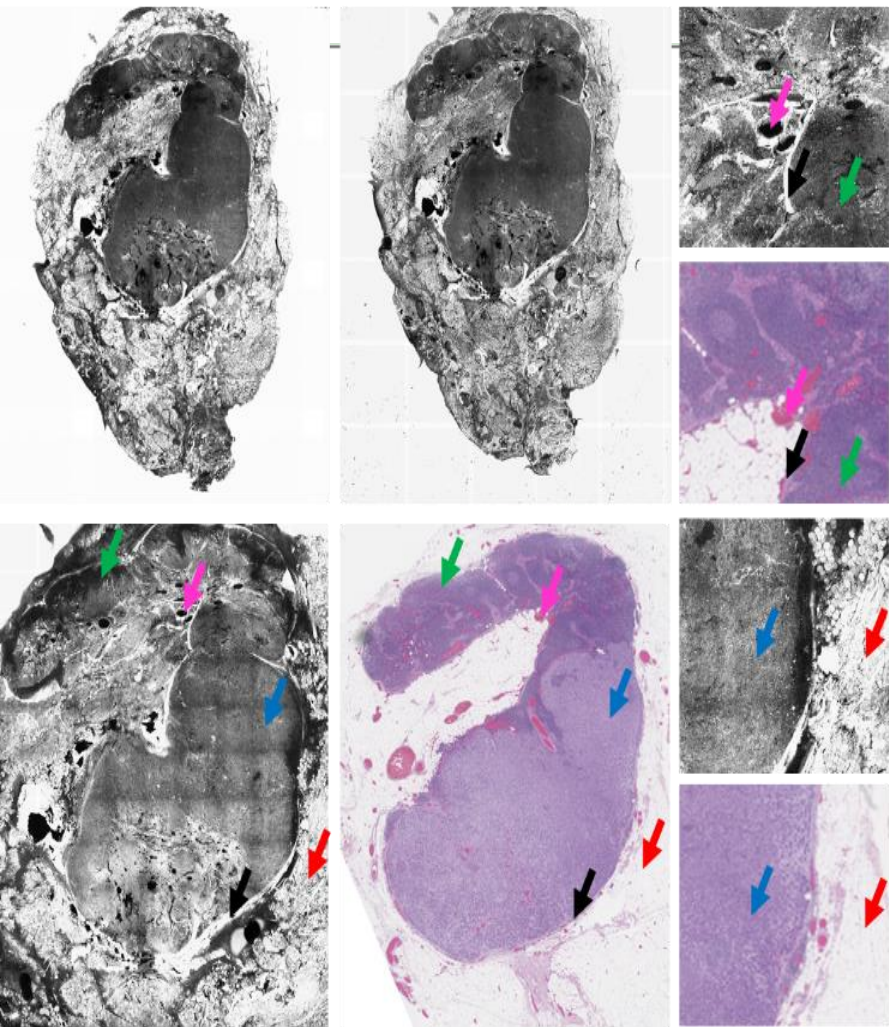


© 2010 Tenax Wireless  
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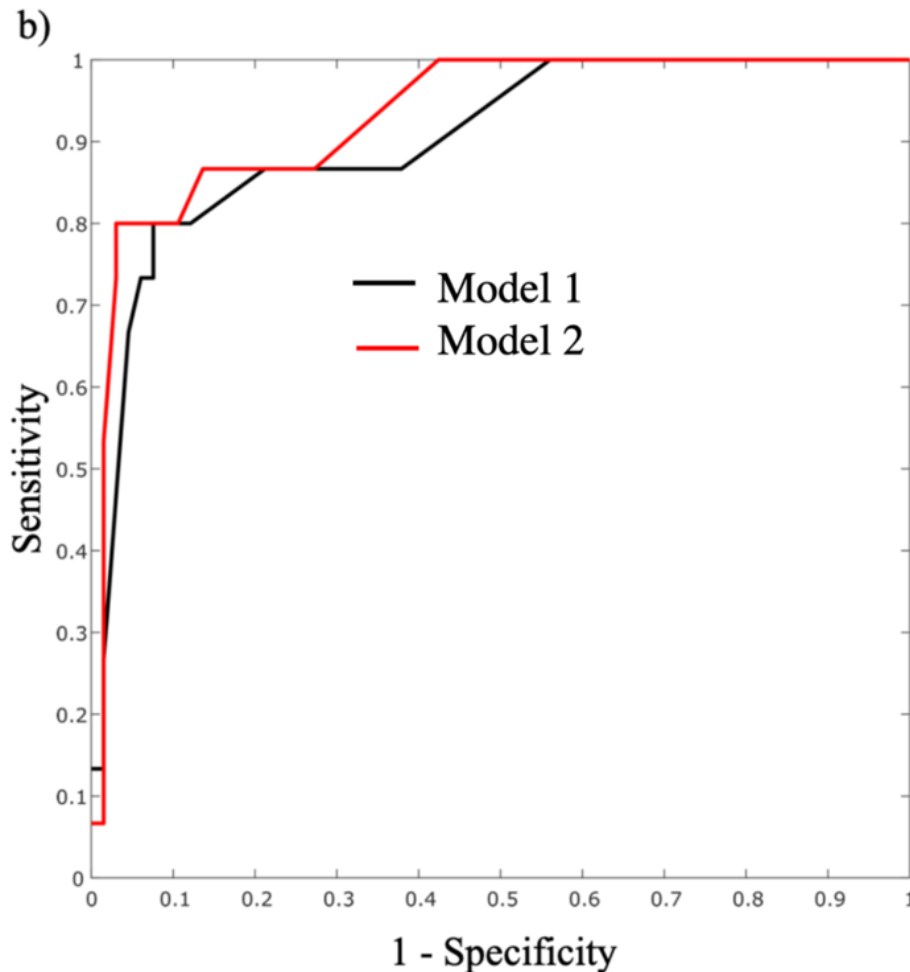
# Annotation of the Raman spectra



# Machine learning classification of whole lymph nodes

## Combined AF-Raman scanning/classification

81 LNs (78 new patients):  
15 +ve, 66 -ve



**Model 2:** SLN +ve: one segment  $>350\ \mu\text{m}$  or two or more segments (regardless of size)

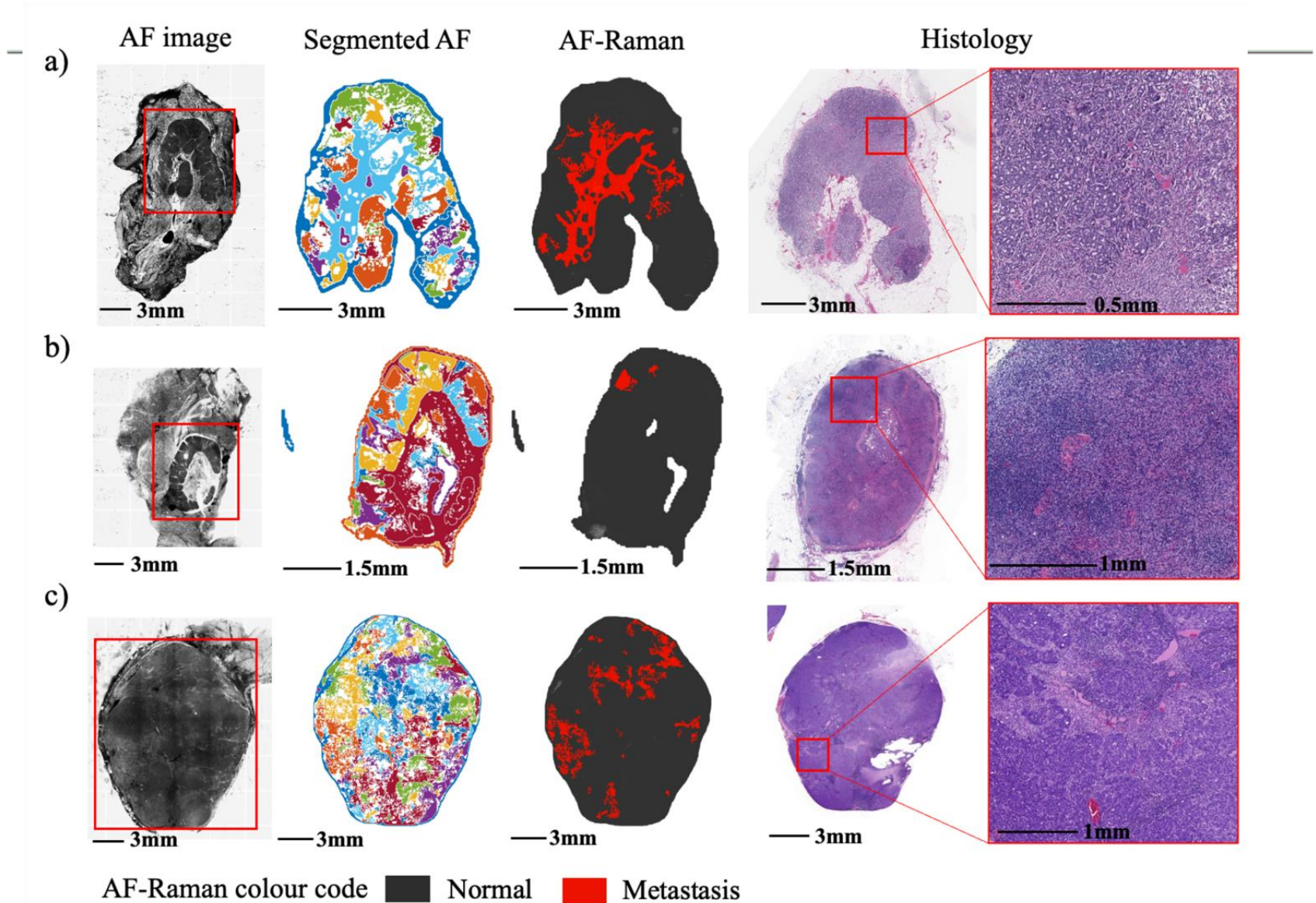
**96.97% specificity**  
[95% CI 95.82-97.59]

**80% sensitivity**  
[95% CI 75.38-83.16]

13 true +ve, 64 true -ve  
2 false -ve ; 2 false +ve

# Representative examples of true positive

(Model 2 operating regime: 96.97% specificity)



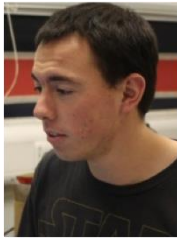
# Conclusions

- Integrated Raman spectroscopy and auto-fluorescence imaging can be used to detect BCC in whole resected tissue layers within 20-30 minutes
- No user training needed for diagnosis (objective diagnosis)
- Platform technology (“molecular fingerprinting”):
  - Basal cell carcinoma: diagnostic test accuracy (125 patients in the clinic)
  - Breast cancer surgical margins: prototypes + proof-of-concept
  - Sentinel lymph nodes (breast cancer): prototype + proof-of-concept

# Acknowledgments



Dr K Kong



Dr C Rowlands



Dr D Shipp



Dr S Barkur



A Ghita



M Larraona-Puy



Dr Radu Boitor



Dr A. Koloydenko



Prof H Williams  
Dermatology



Dr S Varma  
Mohs surgeon



Dr S Elsheikh.  
Pathologist



H Khout  
Breast surgeon



Prof E Rakha  
Pathologist



Dr S Koljenovic.  
Pathology



G Puppels  
(RiverD team)

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