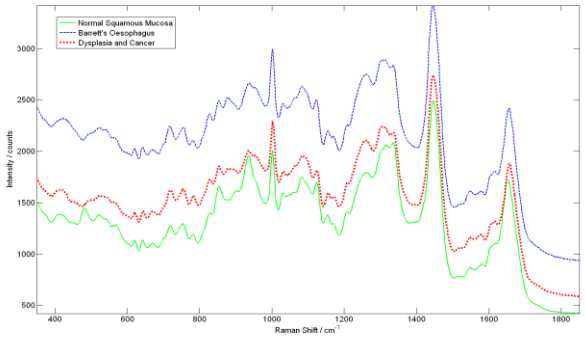
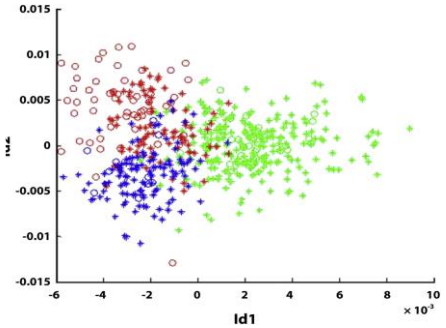
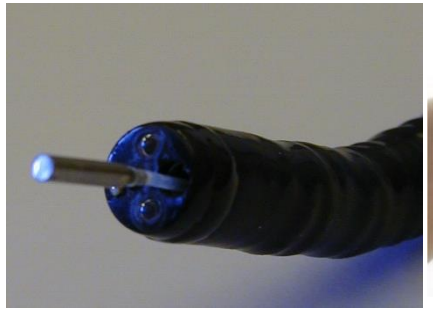
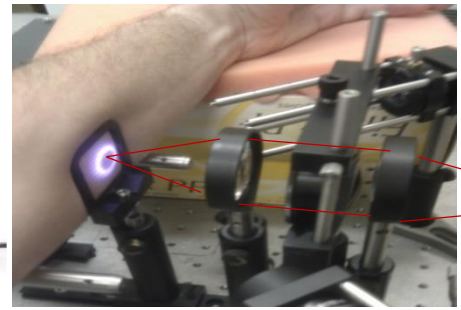








Clinical Raman Spectroscopy
– going beyond diagnosis…

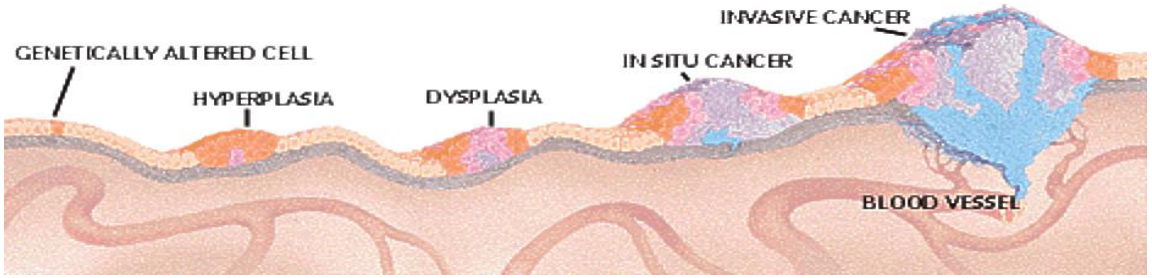
Prof Nick Stone

Chair of Biomedical Imaging and Biosensing
NHS Consultant Clinical Scientist

BIOSPEC Biomedical Spectroscopy Labs

University of Exeter Biomedical Physics

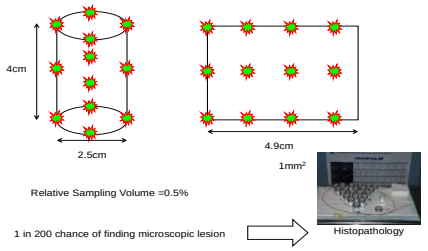
Early diagnosis is vital

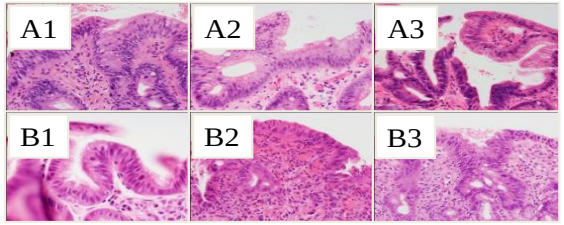


Survival can be 10% at 5 years for advanced disease.

1 in 2 will get Cancer
1 in 4 will die from Cancer

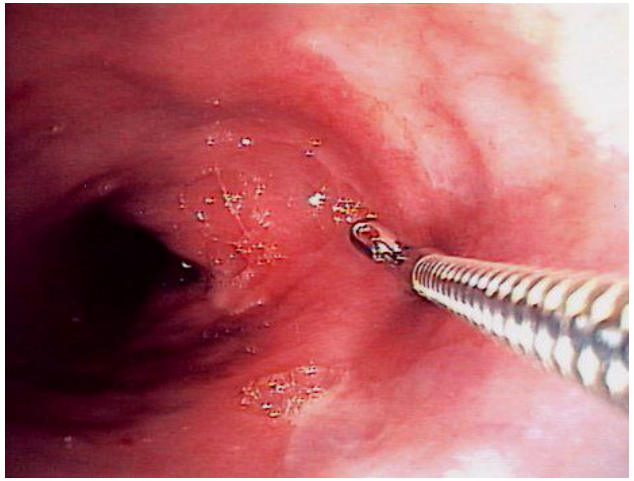
Histopathology – 50% agreement LGD/HGD

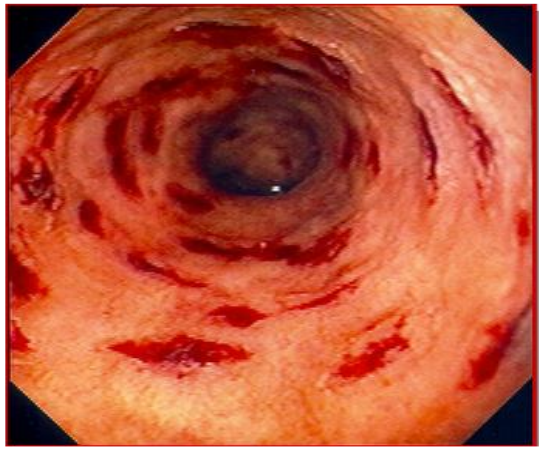




	A1	A2	A3	B1	B2	B3
Path 1	No dys	No dys	Indef	Indef	Indef	Indef
Path 2	Indef	Indef	LGD	Indef	Indef	Indef
Path 3	LGD	LGD	LGD	LGD	LGD	Indef
Path 4	No dys	No dys	Indef	Indef	Indef	Indef

Barrett's oesophagus… Dysplasia… Cancer…





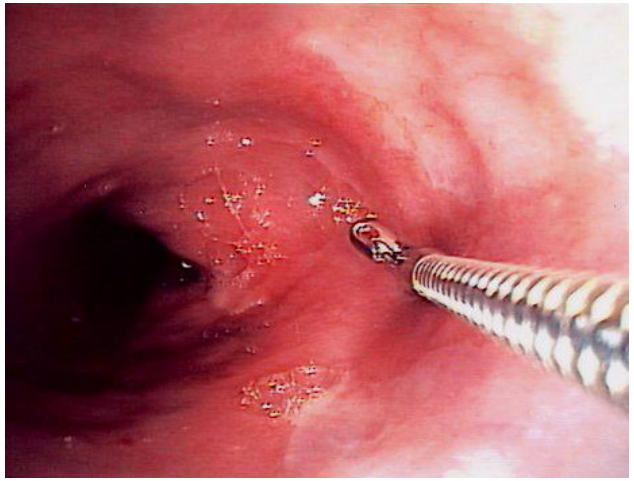
- Biopsy protocols sample <5% of mu
- Biopsies may miss to 50% of dysplasi
- Pathology subjecti

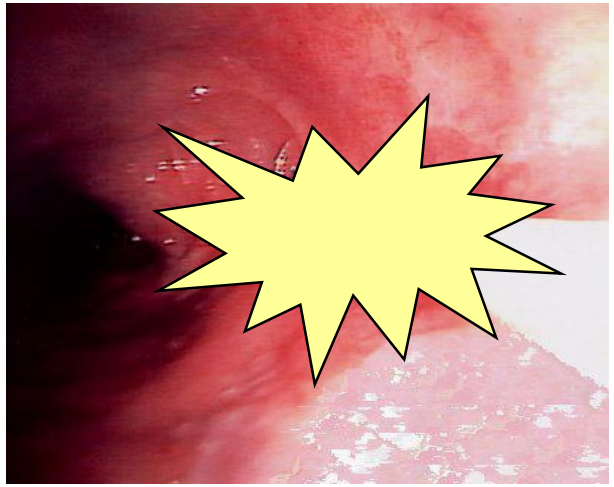
The proposed solution:

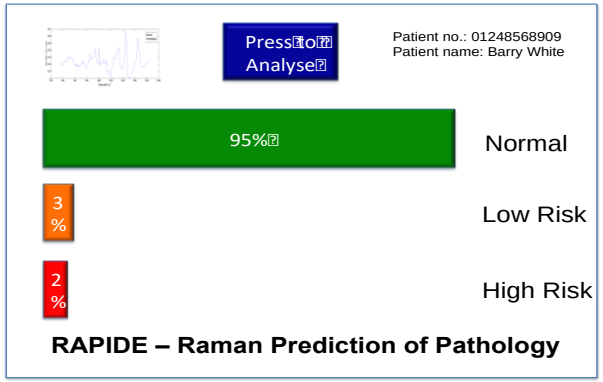
- Objective measure of disease specific molecular changes using light.
- In vivo, rapid, non-destructive.

Will provide:

- Biopsy targeting
- Potential for targeted therapy of dysplastic lesions
- Real-time diagnosis and detection



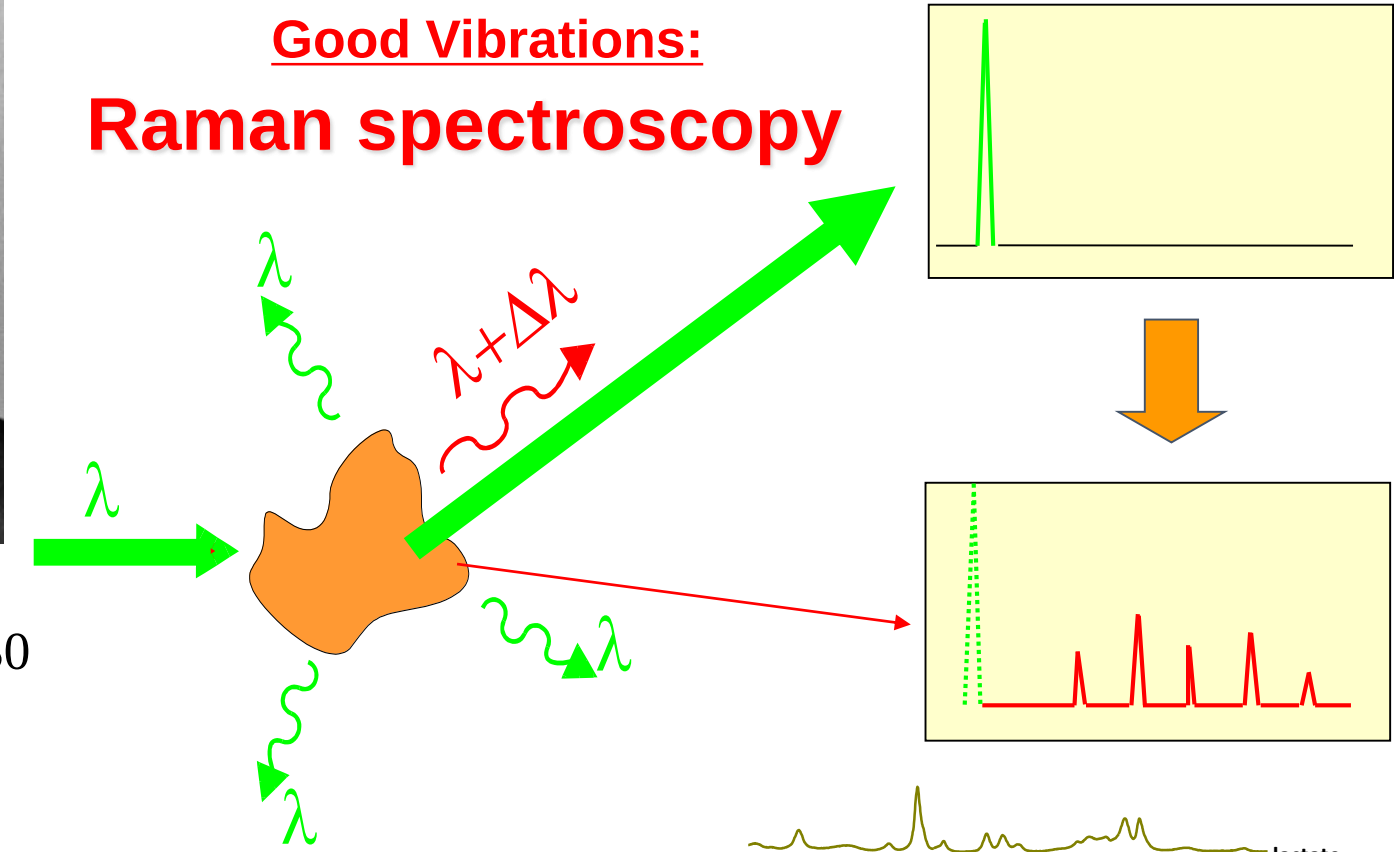


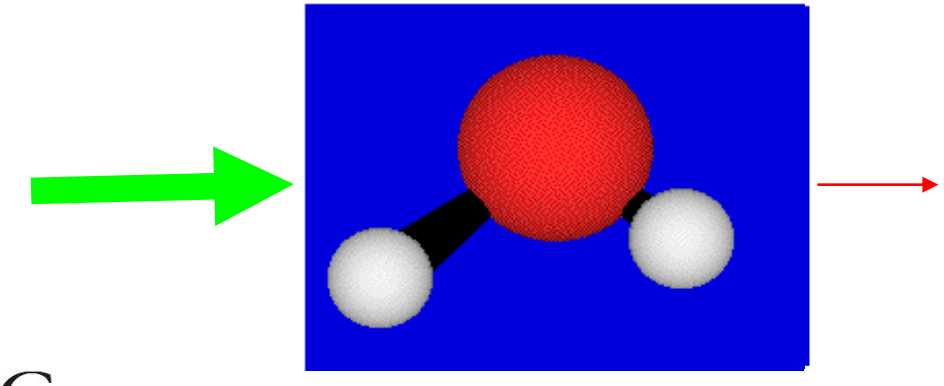


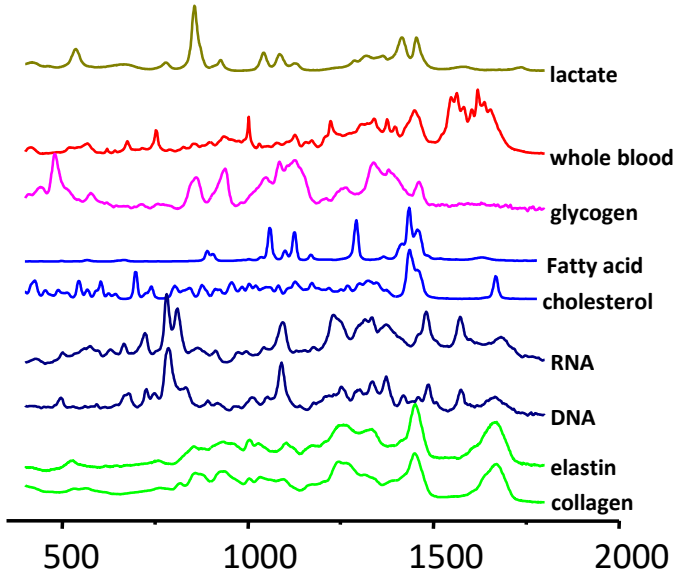
Good Vibrations:

Raman spectroscopy

Sir CV Raman
Nobel prize 1930







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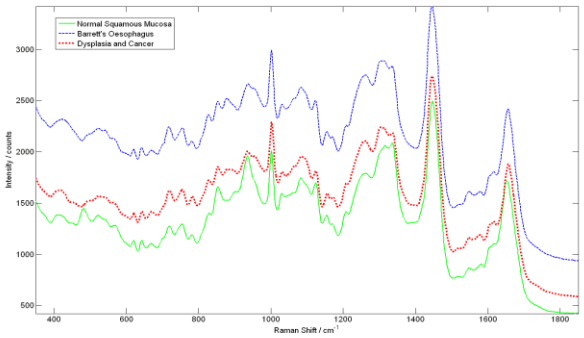
RAMAN DIAGNOSTIC PLATFORM TECHNOLOGY

- Molecular fingerprint of cells or tissues.
- Rapid, non-destructive
- Reproducible: systems / centres / users.
- Can exceed performance of independent pathologists
- Prognosis possible [Kendall 2011, Crow 2004]



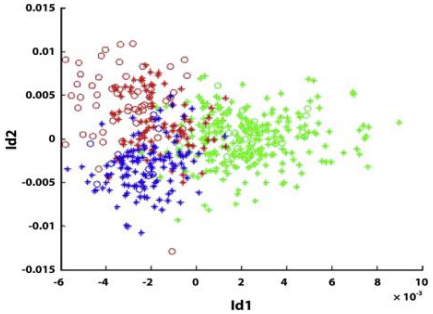
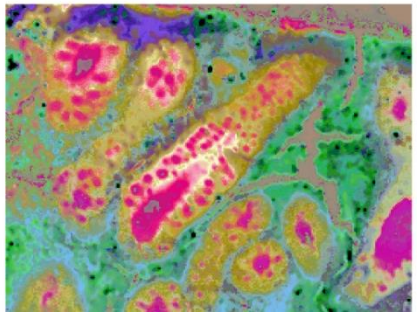
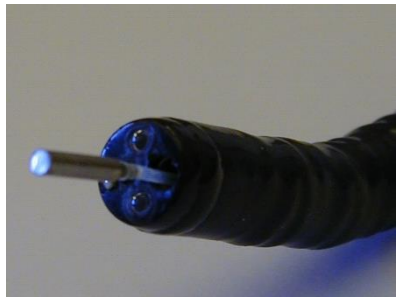


Figure 2. Three-group linear discriminant score prediction scatterplot identifying samples that were measured from fresh (stars) and from fresh-frozen (circles) tissue samples.





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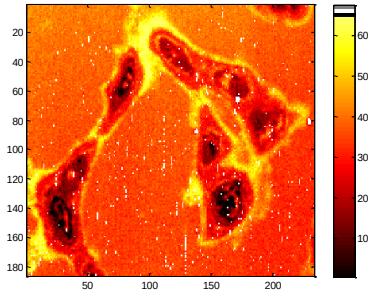
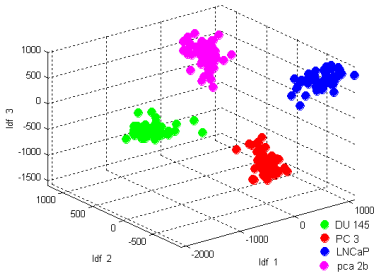
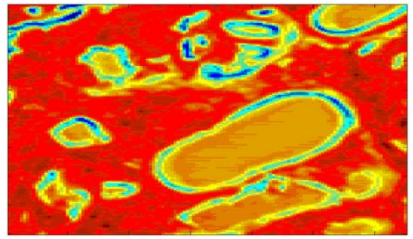
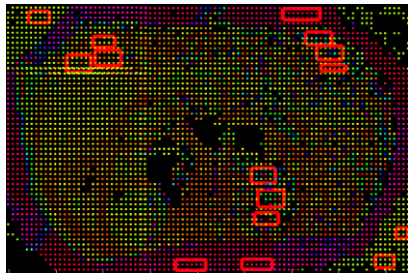
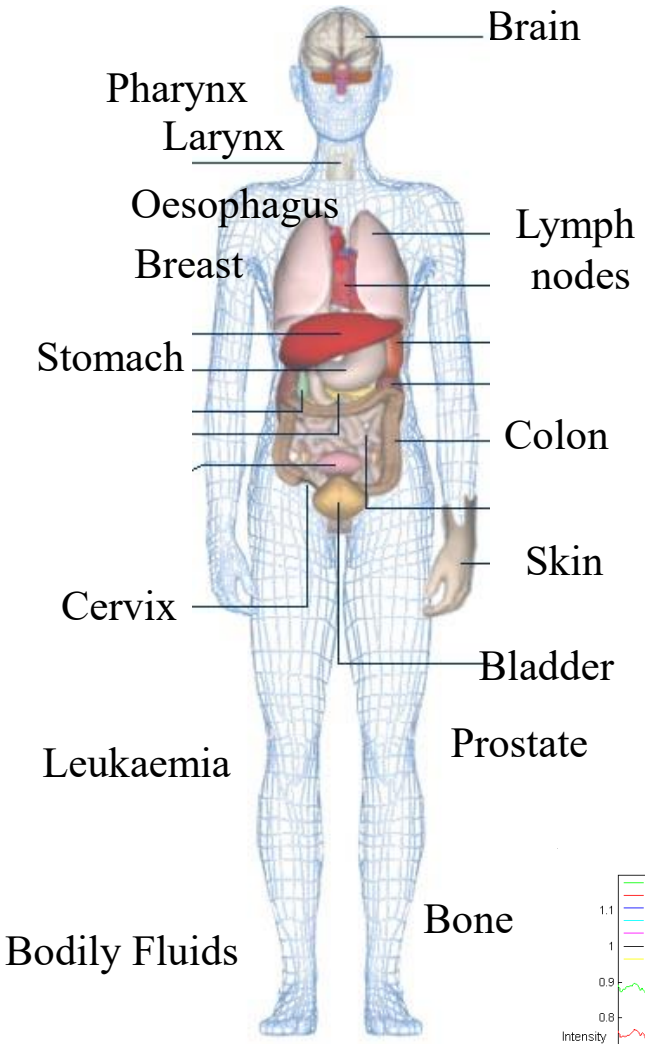
Many cells and tissues characterised:

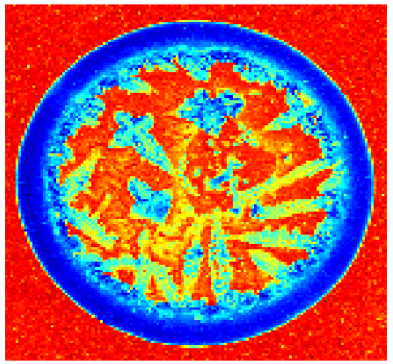


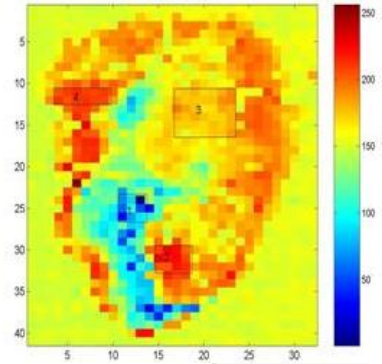


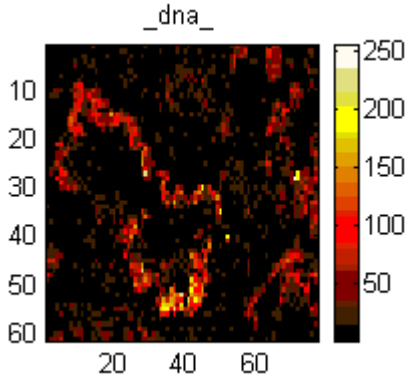


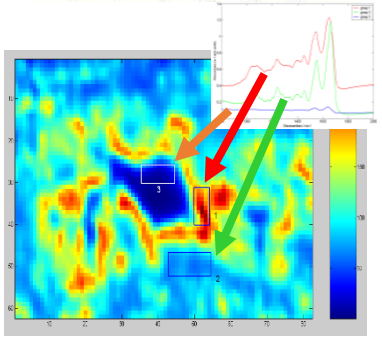


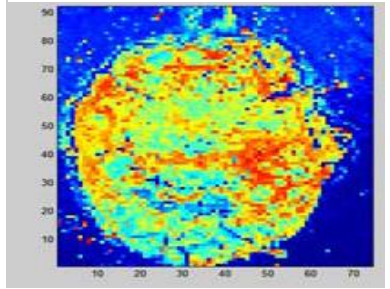


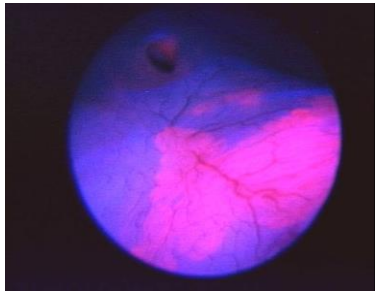


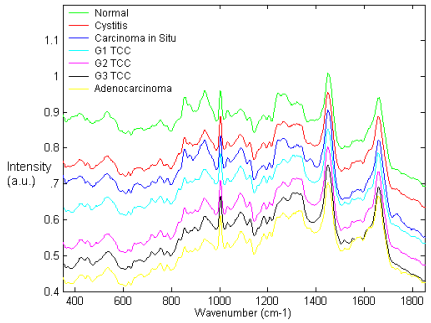


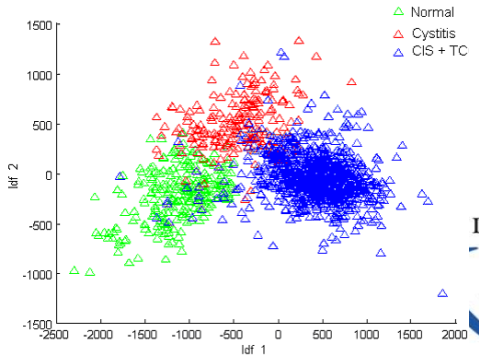












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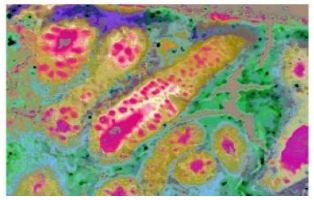
Raman diagnostics

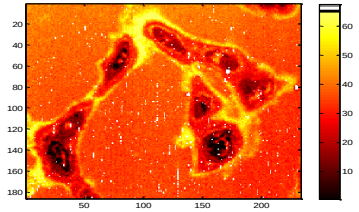
Lab based

Point of care
Near patient

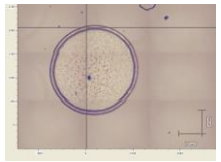
Spectral
Histology



Spectral
Cytology



Biofluid
DCDRS

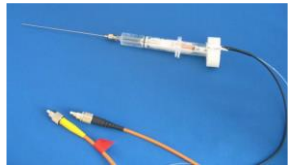


Ex vivo
tissue

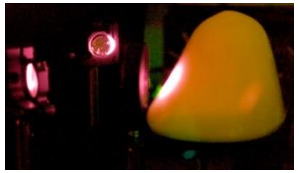
In vivo

Endoscopic

Needle



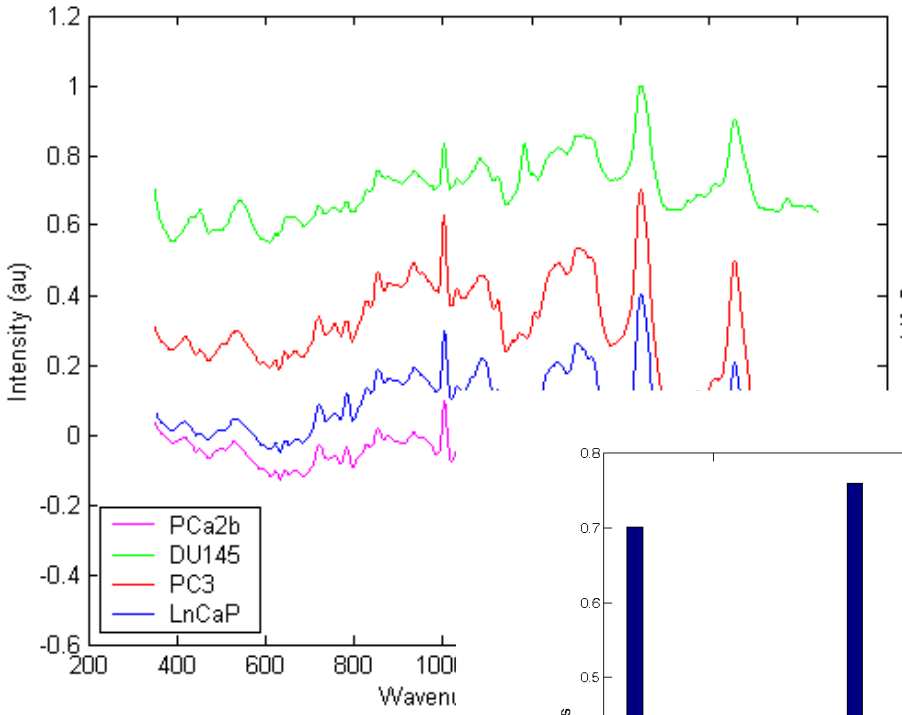
SORS / SESORS

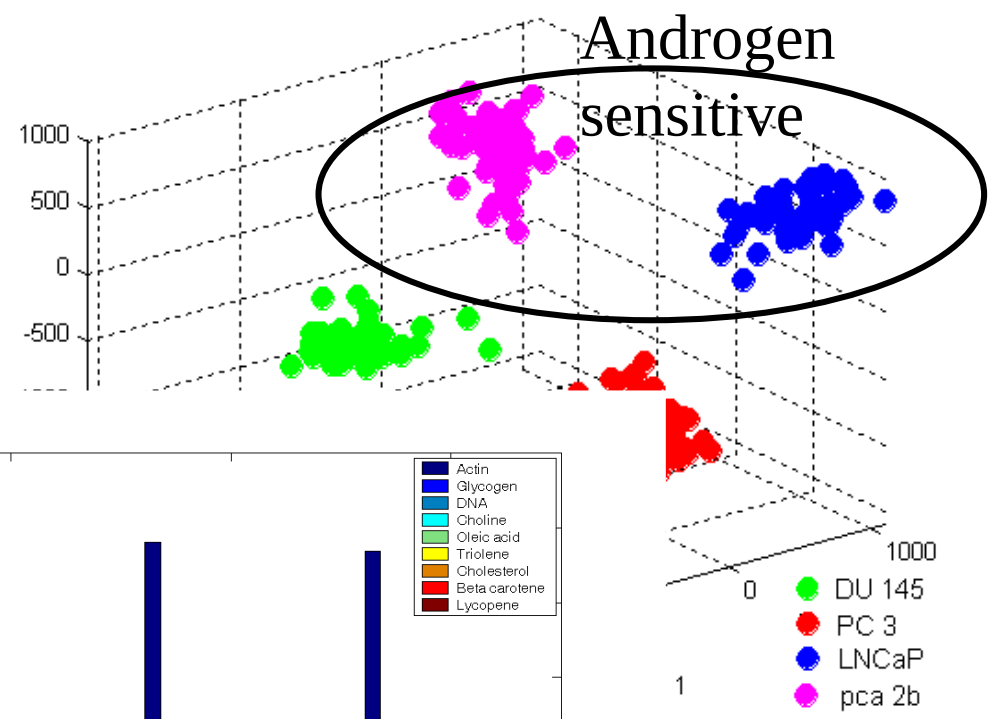


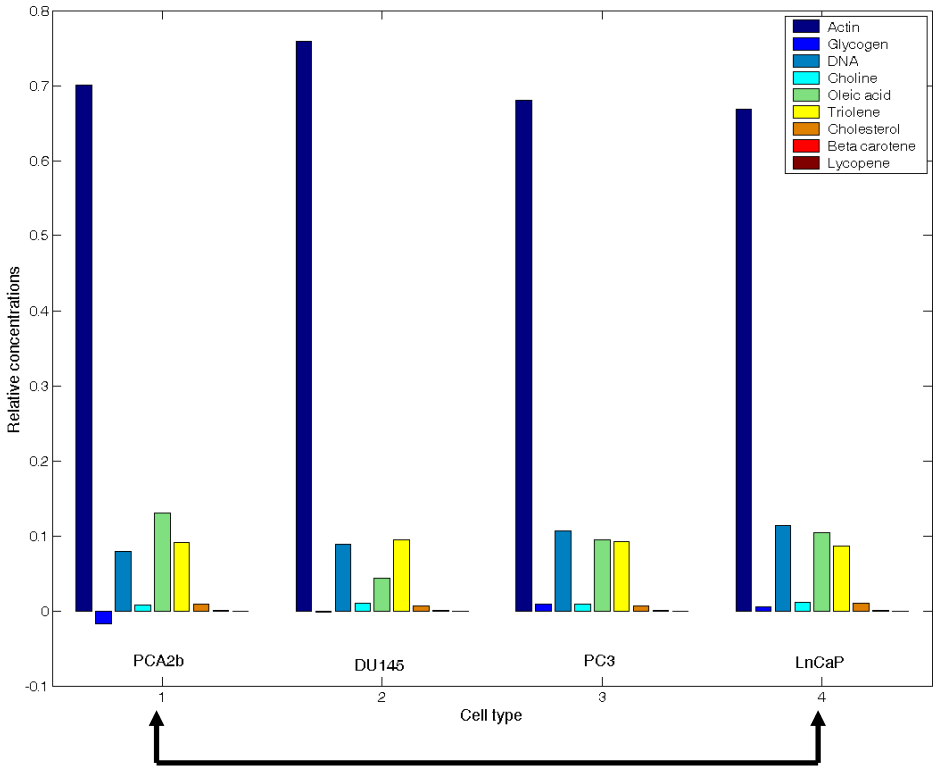
Prostate cell lines

P Crow*,[1], B Barrass[2], C Kendall[1], M Hart-Prieto[1], M Wright[2], R Persad[2] and N Stone[1]

British Journal of Cancer (2005) 92, 2166–2170







Androgen sensitive

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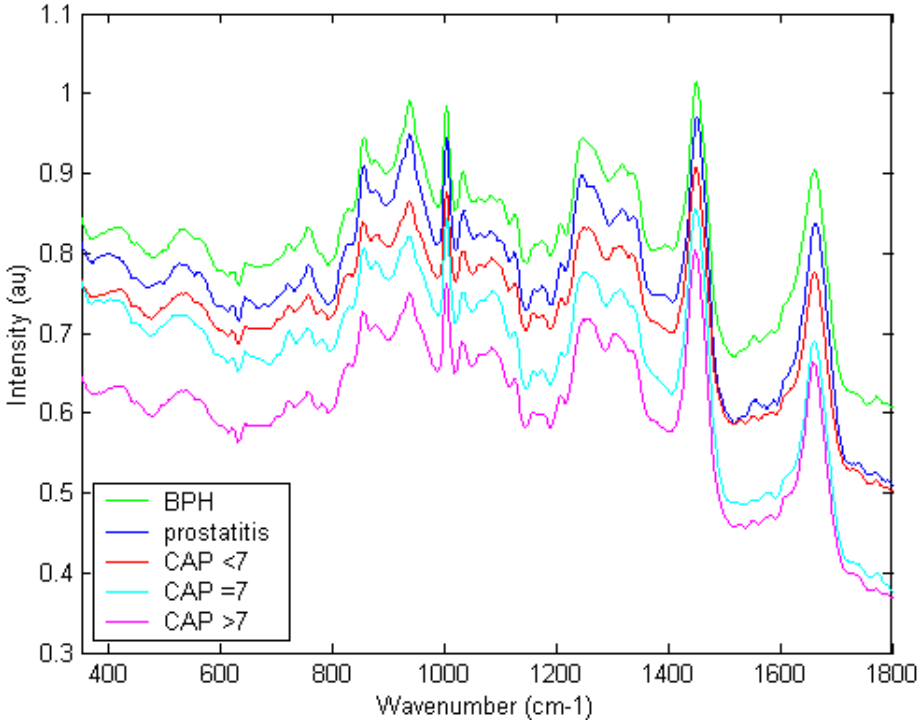

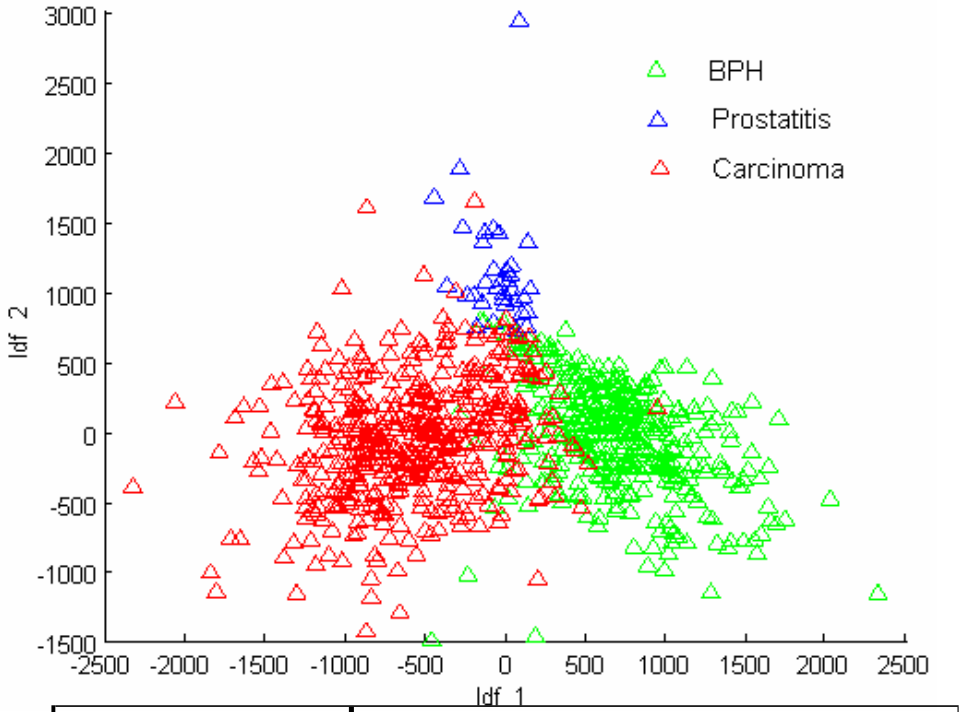

In vitro prostate model – grading disease

		Raman Prediction		
		BPH	**Prostatitis**	**Carcinoma**
Confirmed Histology	**BPH**	**350**	21	10
	Prostatitis	0	**34**	0
	Carcinoma	34	33	**389**

	BPH	Prostatitis	CaP (GS<7)	CaP (GS=7)	CaP (GS>7)	Totals
No. of Samples	33	2	11	5	5	**56**
No. of Spectra	381	34	231	111	114	**871**

	BPH	Prostatitis	Cancer
Sensitivity	92%	100%	85%
Specificity	93%	94%	98%

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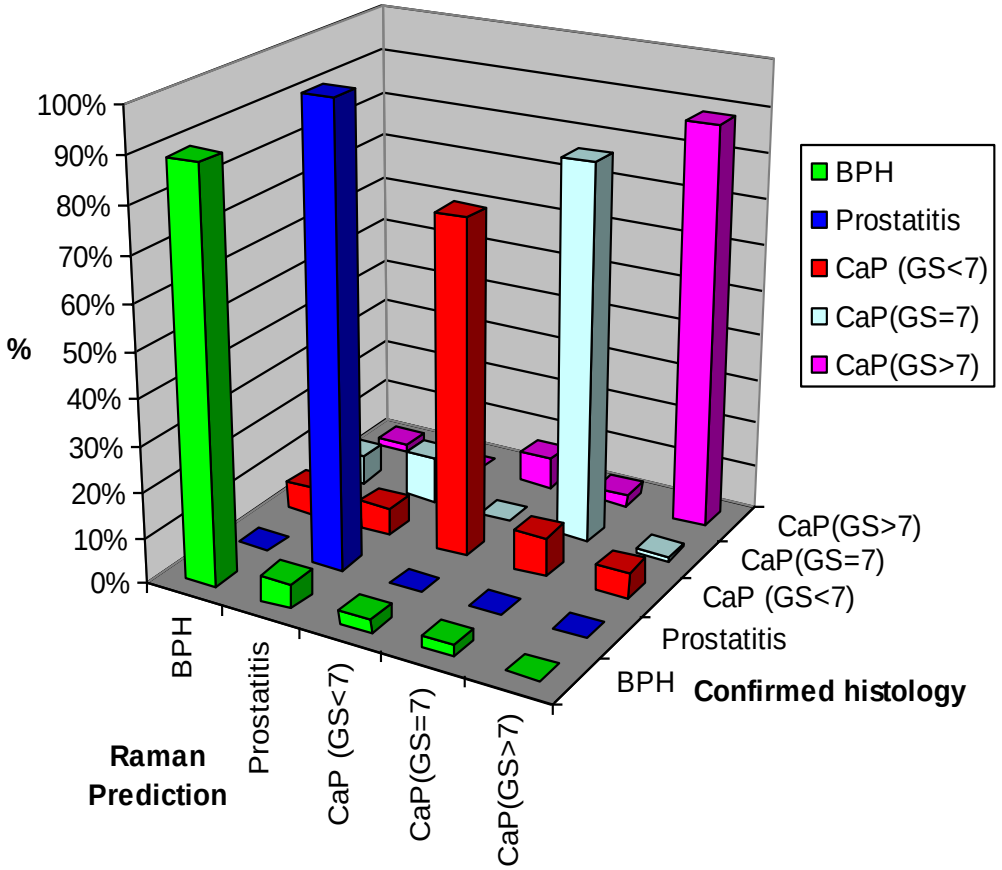
The Prediction Power of the Five Group Algorithm



	BPH	Prostatitis	Gleason Score <7	Gleason Score =7	Gleason Score >7
Sensitivity	89%	100%	74%	83%	89%
Specificity	95%	95%	97%	96%	98%

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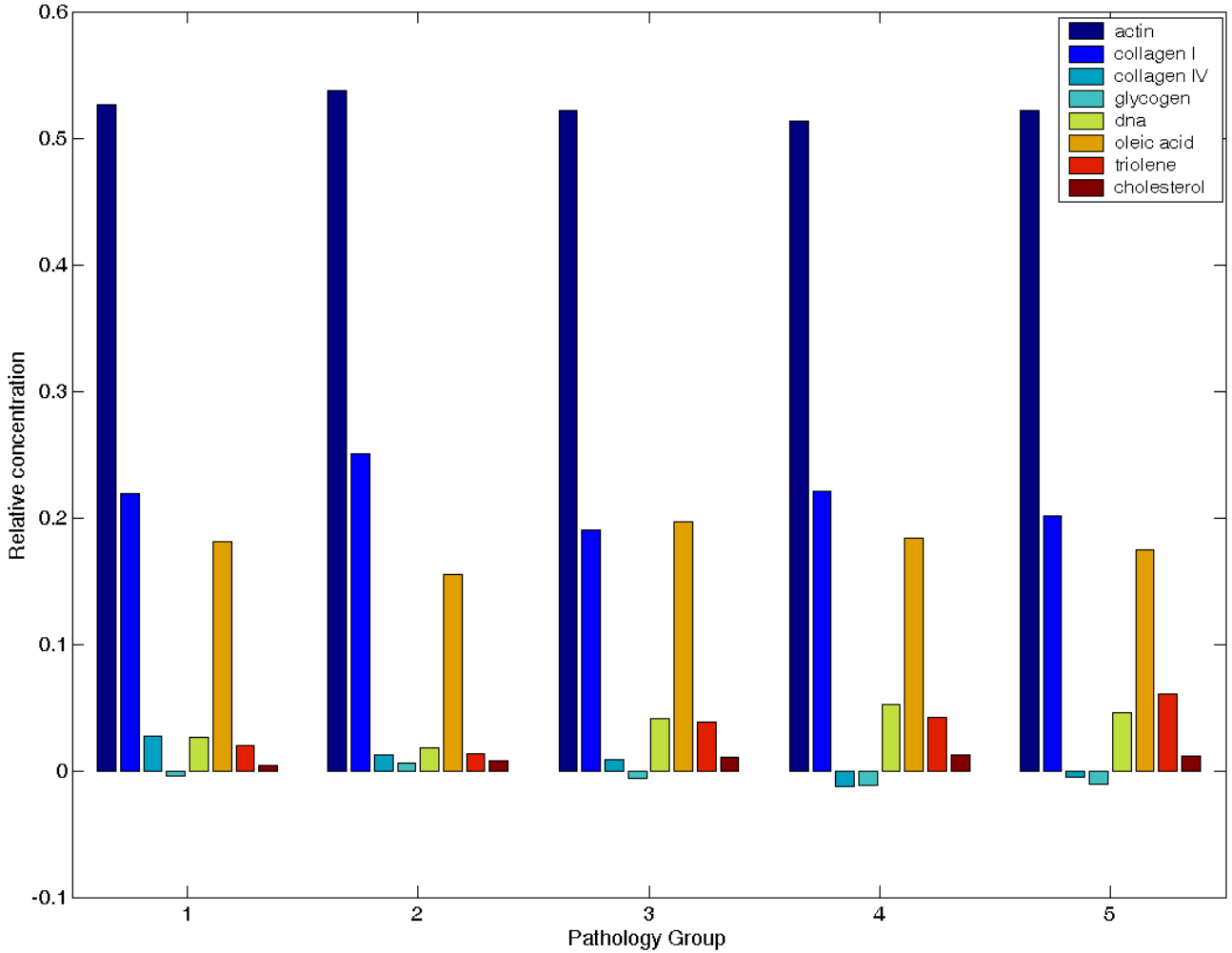
Anal Bioanal Chem (2007) 387:1657–1668
DOI 10.1007/s00216-006-0937-9

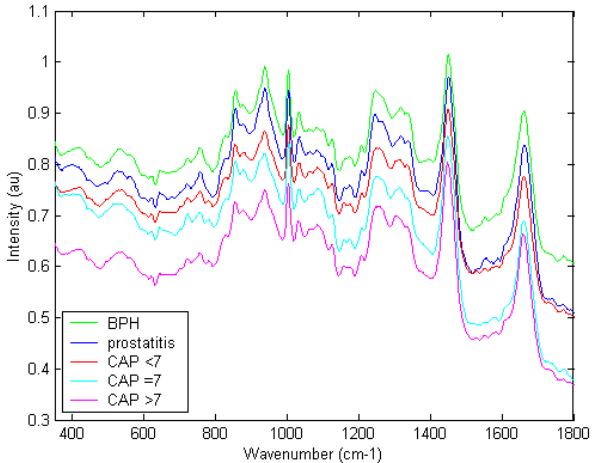
ORIGINAL PAPER

The use of Raman spe
an estimation of the g
with urological pathol

Nicholas Stone · Maria Consuelo Hart
Paul Crow · Jeremy Uff · Alistair Willi



BPH P'titis CaP: <7 =7 >7



Gross biochemistry of sample volume
- Can be fractions of cells to many cells

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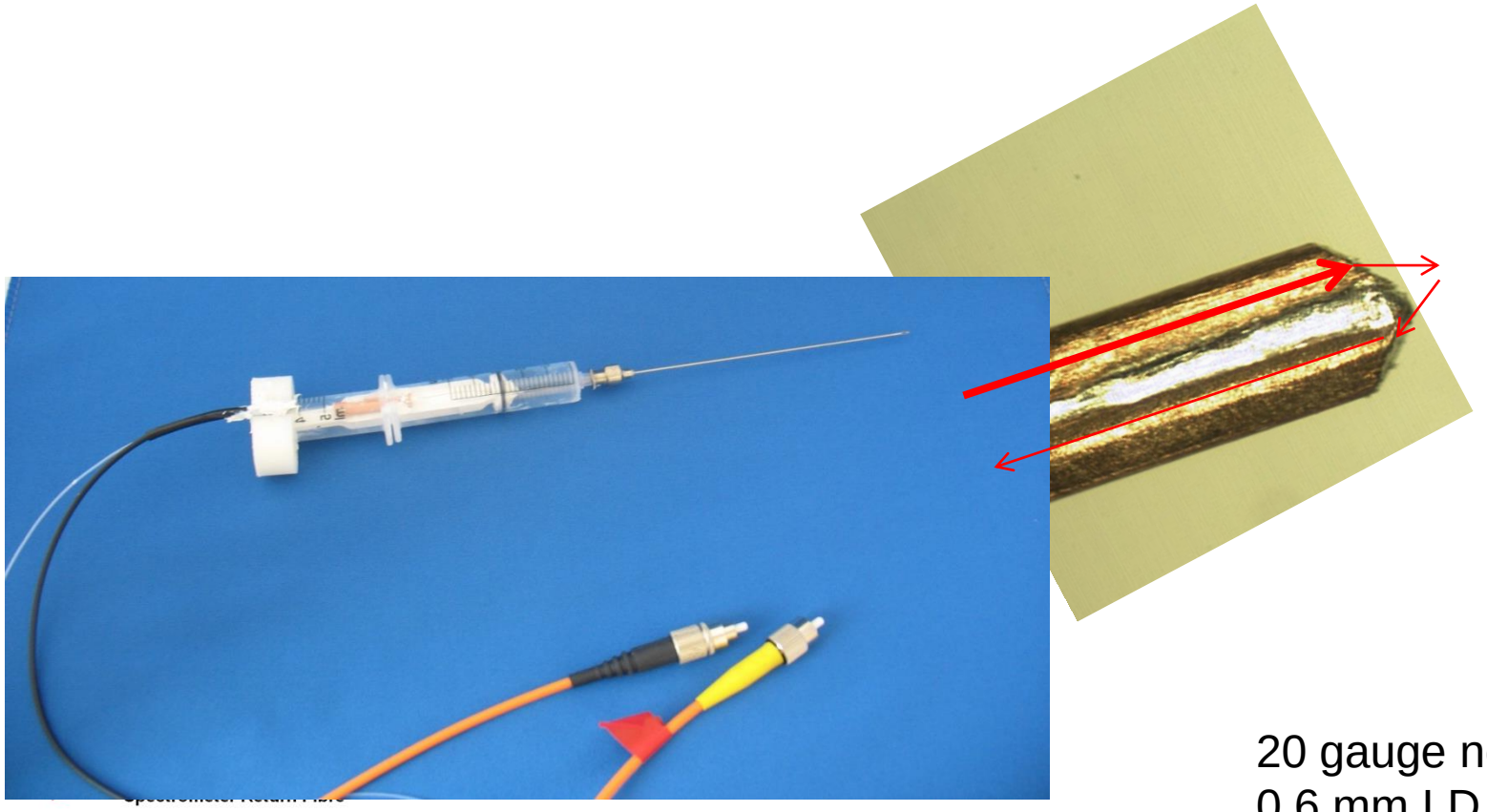
Smart Raman Needle

Day and Stone, *Applied Spectroscopy* 2013.



20 gauge needle
0.6 mm I.D.
0.9 mm O.D.

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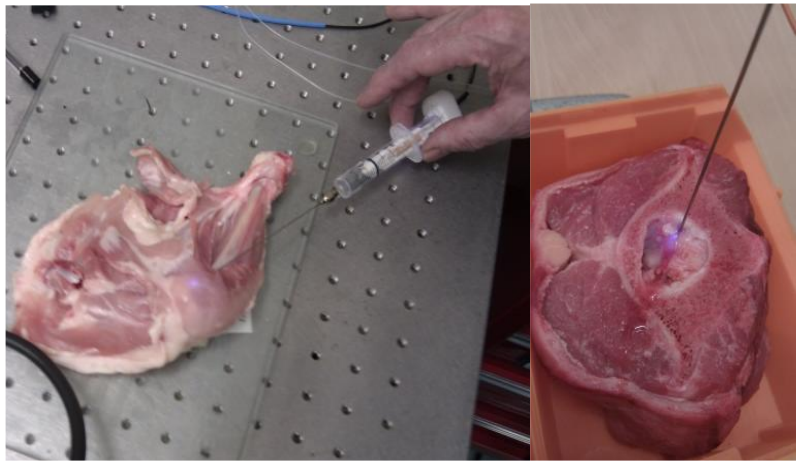
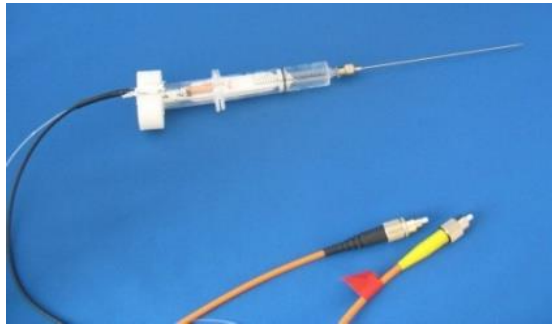
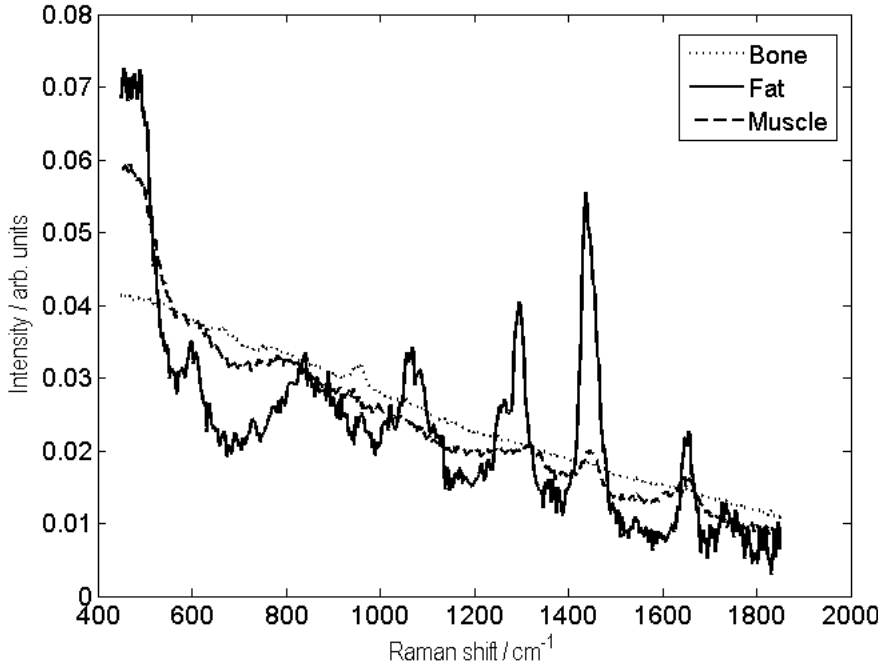
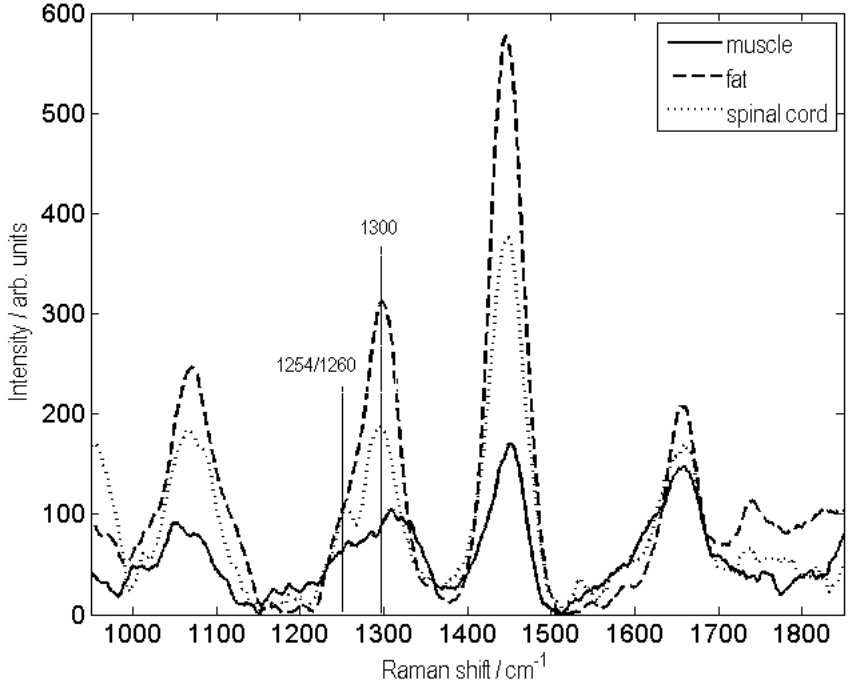
i4i Feasibility study
12 months 2011



Day and Stone, *Applied Spectroscopy* 2013.







Mean corrected spectra obtained from lamb and chicken tissues using the 100 µm probe in t=10s (24mW).

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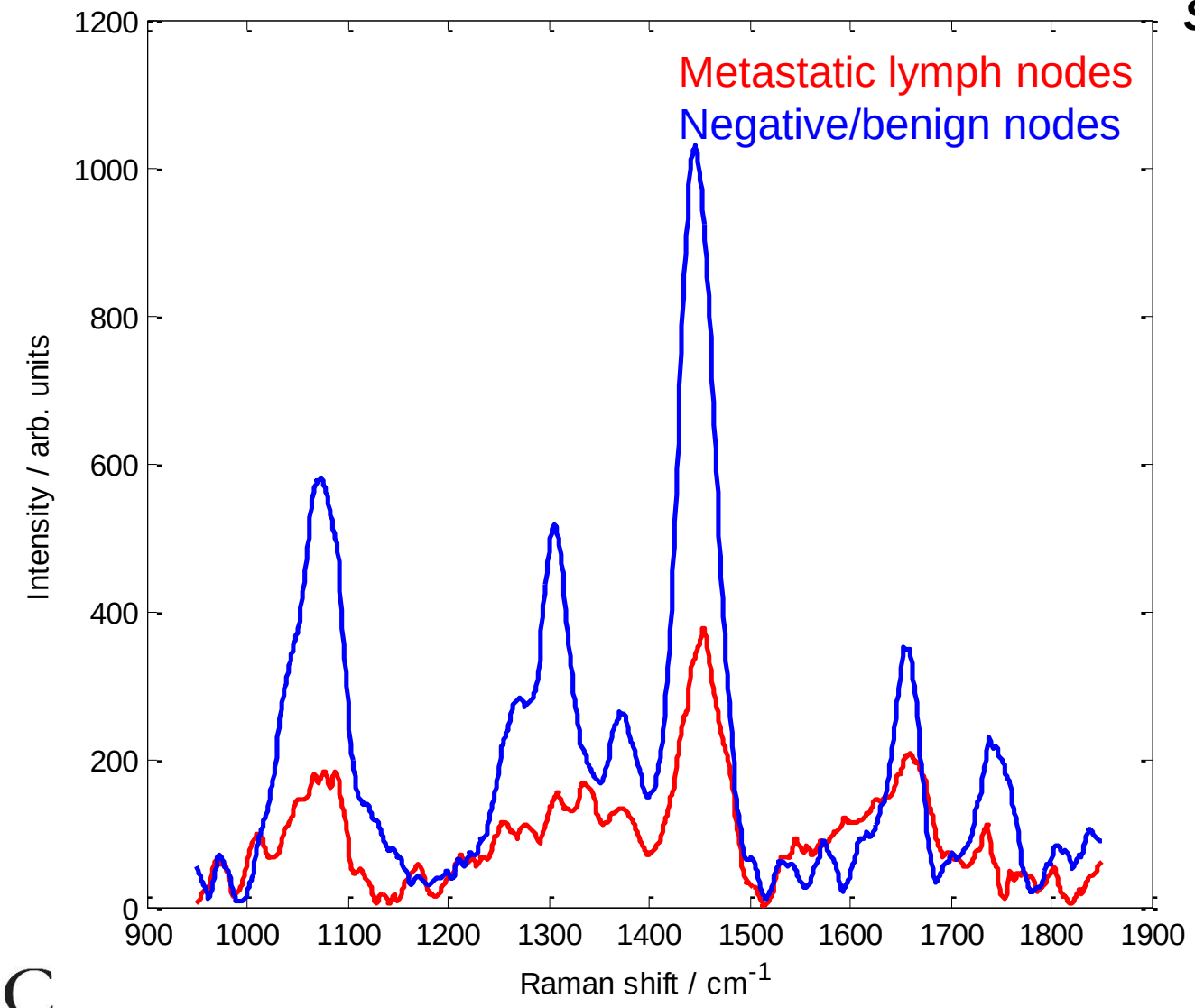
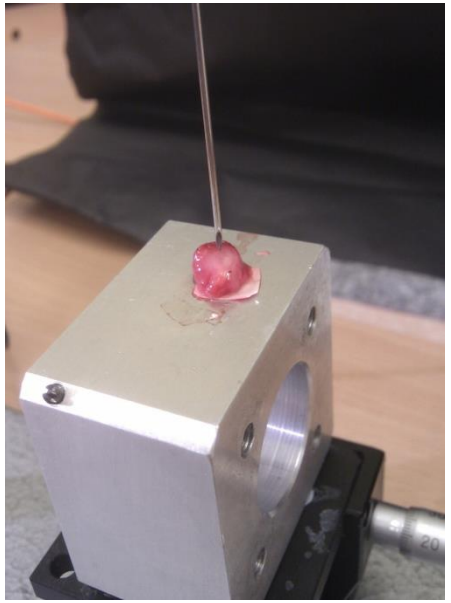
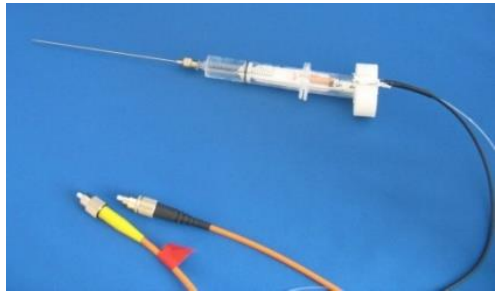
Mean spectra from
4 locations within 2 nodes of +ve
4 locations within 2 nodes of –ve



Day and Stone, *Applied Spectroscopy* 2013.





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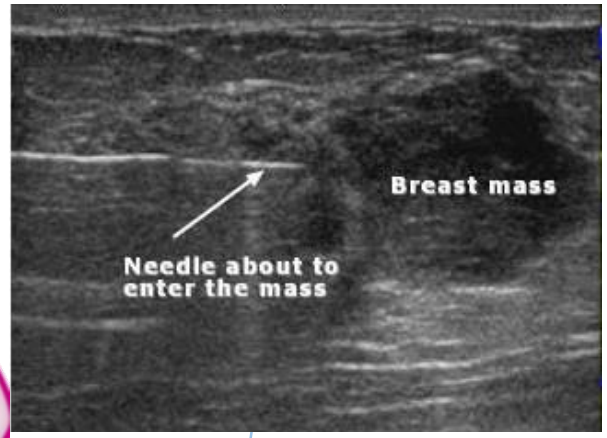
The Raman smart needle

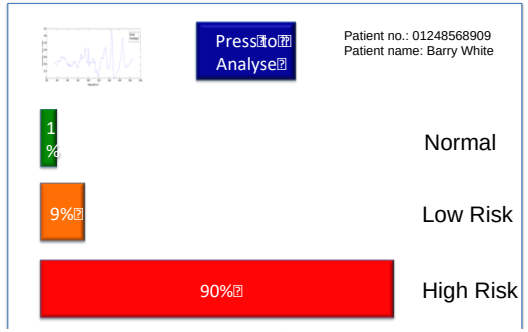
"an intelligent optical biopsy at the tip of a needle, in real-time"

Ultrasound

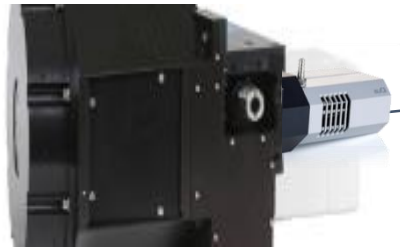


Needle probe

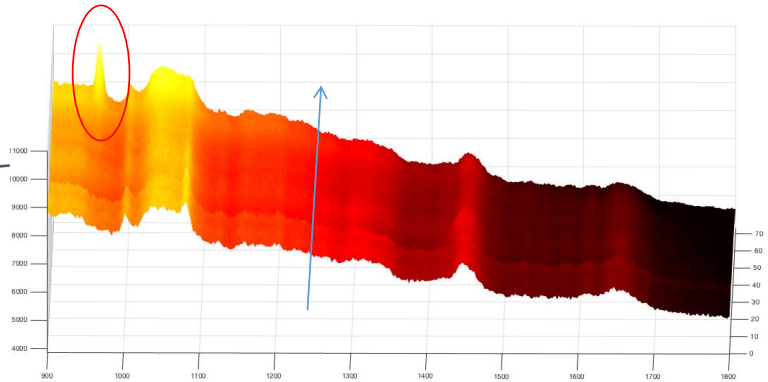
Interpretation software



Laser



Spectrometer



BIOSPEC Biomedical Spectroscopy Labs

Ok – what about beyond diagnosis?

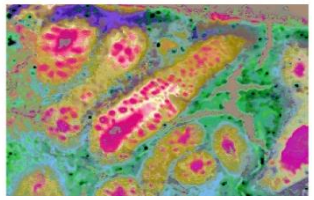
Surgical decision making / Prognosis / Monitoring treatments…

Raman diagnostics

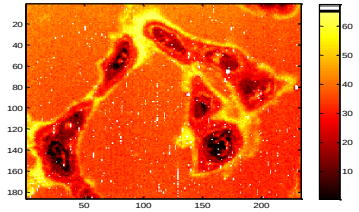
Lab based

Point of care
Near patient

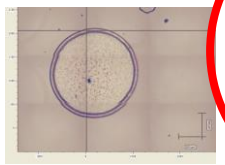
Spectral
Histology



Spectral
Cytology



Biofluid
DCDRS

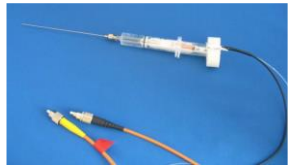


Ex vivo
tissue

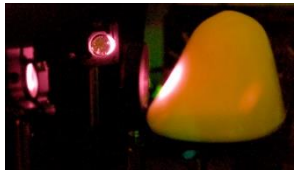
In vivo

Endoscopic

Needle



SORS / SESORS



Raman probe for axillary nodes

Dudley Hospital

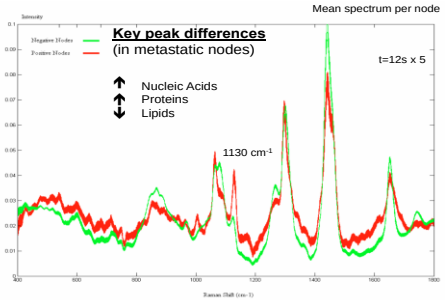
Gloucester Royal

Standard
Assessment
Clinical Mx

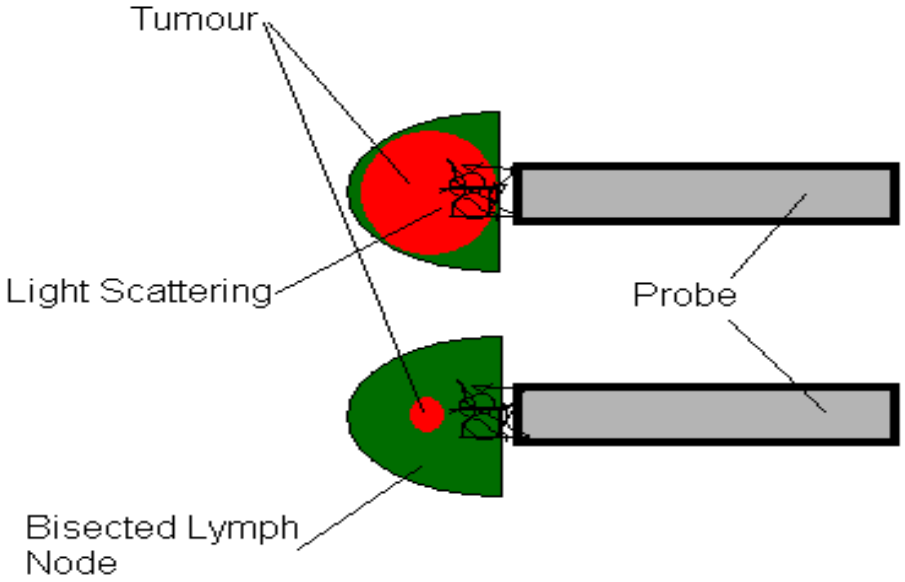
Pathology

Raman

Recruit 300 patients – sentinel+ lymph nodes

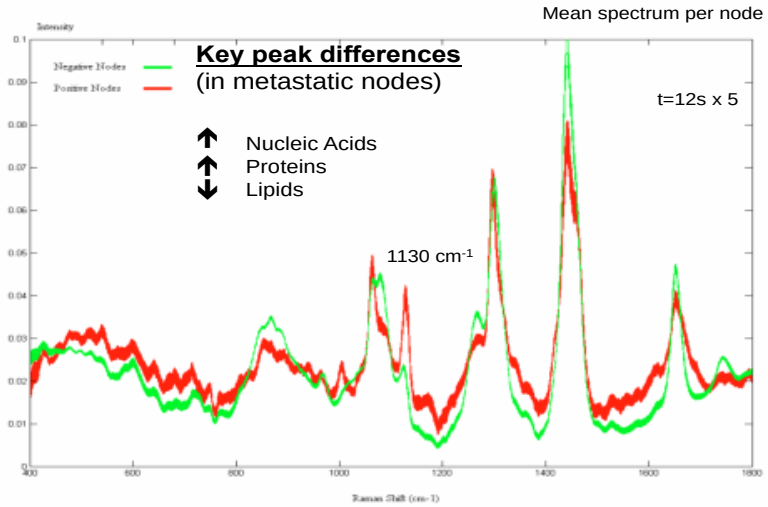


Horsnell et al., Analyst, 2010, 135, 3042–3047

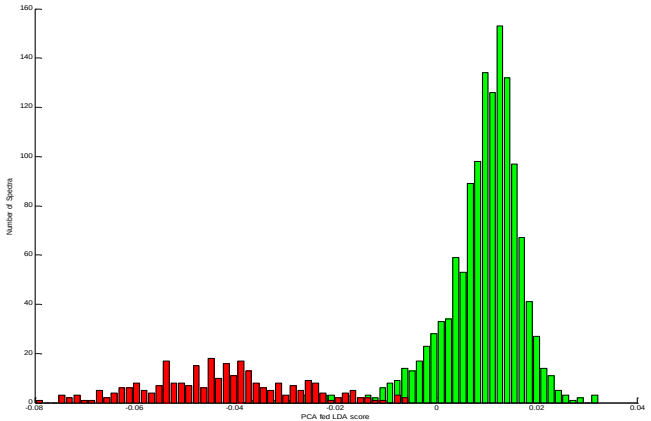


a. Analysis of a Node with a Macrometastases

b. Analysis of a Node with a Micrometastases



Clinical context: Improve surgical decision making, specificity must be near 100% and sensitivity good to enable the majority of metastatic nodes to be identified during surgery and those that are missed will be picked up with later histopathology and require reoperation.



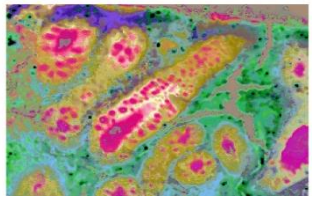
Technique	Sensitivity	Specificity
Frozen Section Analysis	57-76%	99%
Touch Imprint Cytology	33-81%	95-99%
Molecular Assays	87-96%	92-97%
Raman BWTEK probe	**85-94%**	**96-99%**

Raman diagnostics

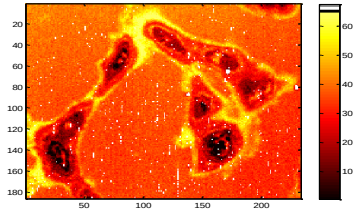
Lab based

Point of care
Near patient

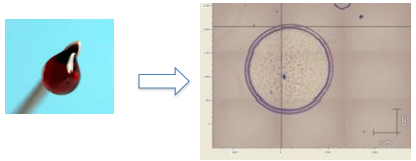
Spectral
Histology



Spectral
Cytology



Biofluid
DCDRS

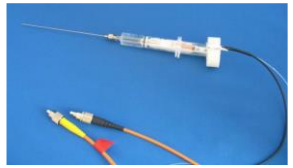


Ex vivo
tissue

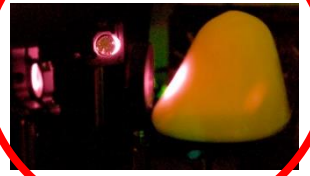
In vivo

Endoscopic

Needle



SORS / SESORS

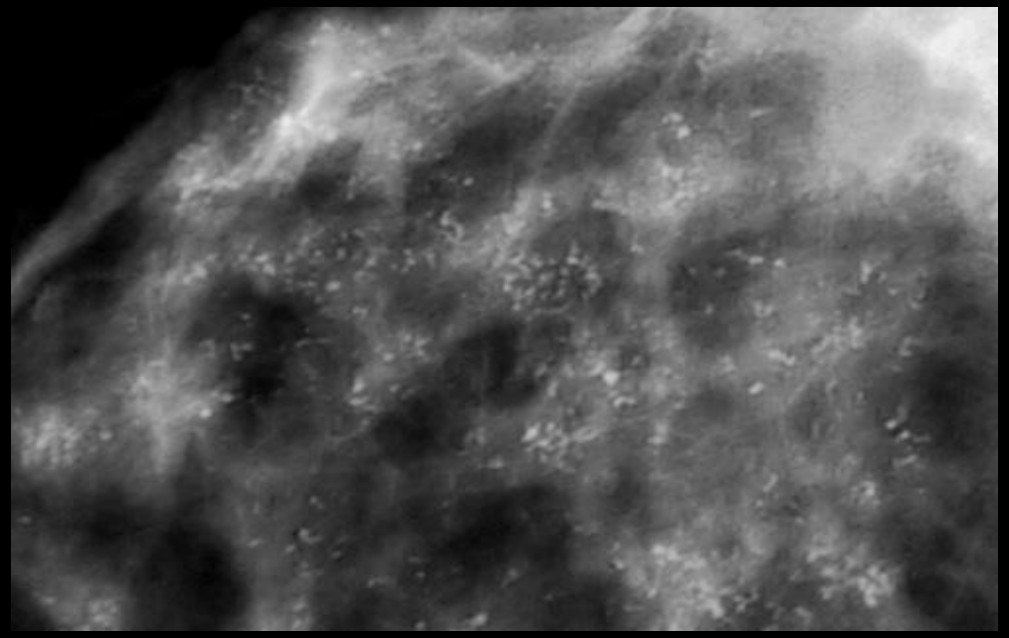


Mammogram – calcifications

Often the only sign of malignancy

Benign

Malignant

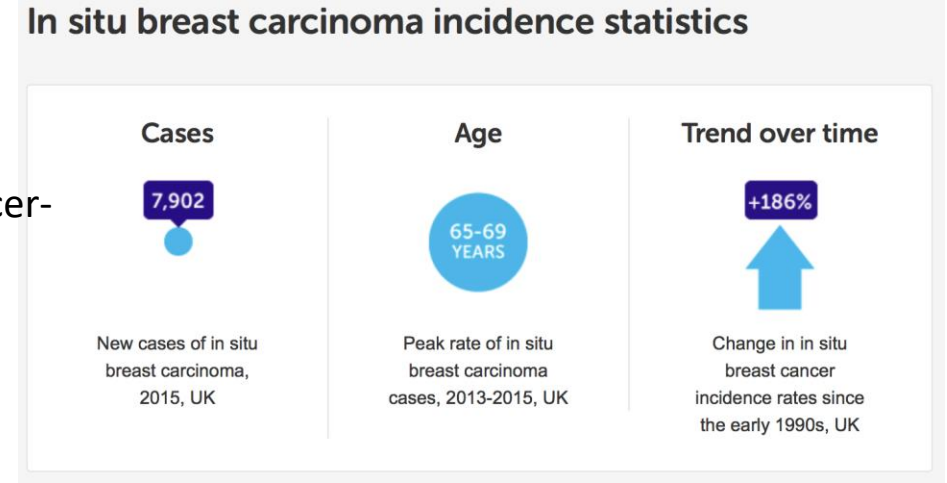


They are found in: **10%** of women aged 25-29 years
86% of women aged 76-79 years.

Breast cancer – who should we treat?

- 55000 cancers per annum UK - 464K EU / 1.68M worldwide (2012)
- 15K Cancers detected with mammographic screening – from >2M screening tests per year
- 40K Cancers detected by 'finding lumps' from 400K women presenting with lumps
- **95% (>7500) of DCIS identified with mammography**
- Incidence rates for breast cancer in the UK are highest in people aged 90+ (2013-2015).
- 87% of women survive 5 years / 11,400 breast cancer deaths in the UK per year (2014-16).
- Around 491,300 women who had previously been diagnosed with breast cancer were alive in the UK at the end of 2010.

https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer#heading-Five



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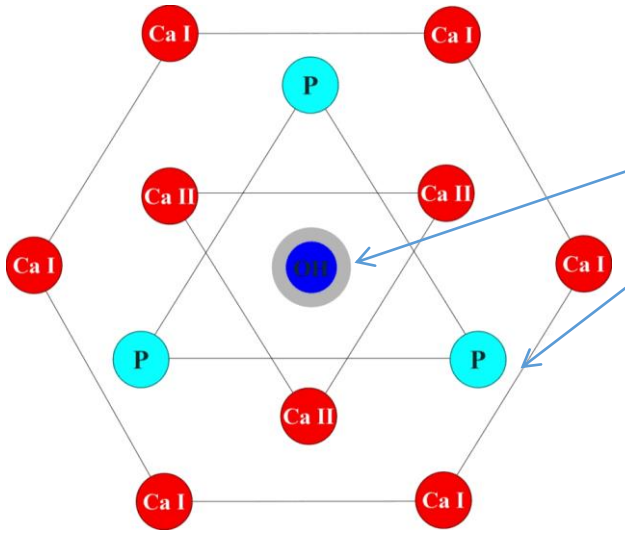
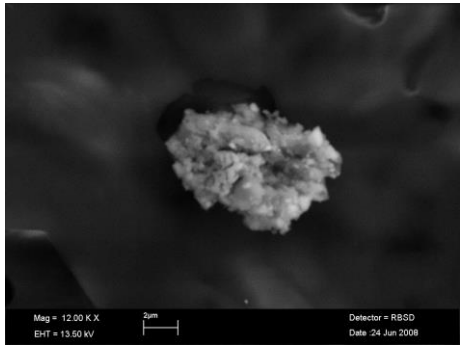
Type II calcifications – calcium hydroxyapatite



Carbonate ions

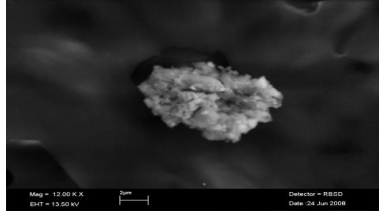
A-type – OH^- substitution

B-type – PO_4^{3-} substitution



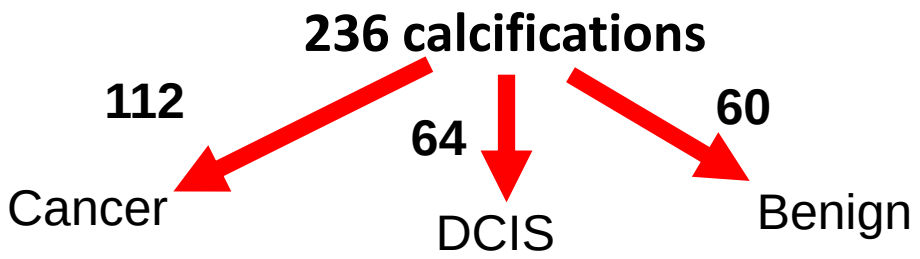
Hydroxyapatite lattice structure showing arrangement of the two types of calcium ions (Ca^{2+} I and II), phosphate (PO_4^{3-} ions(P) and hydroxyl ions (OH^-))

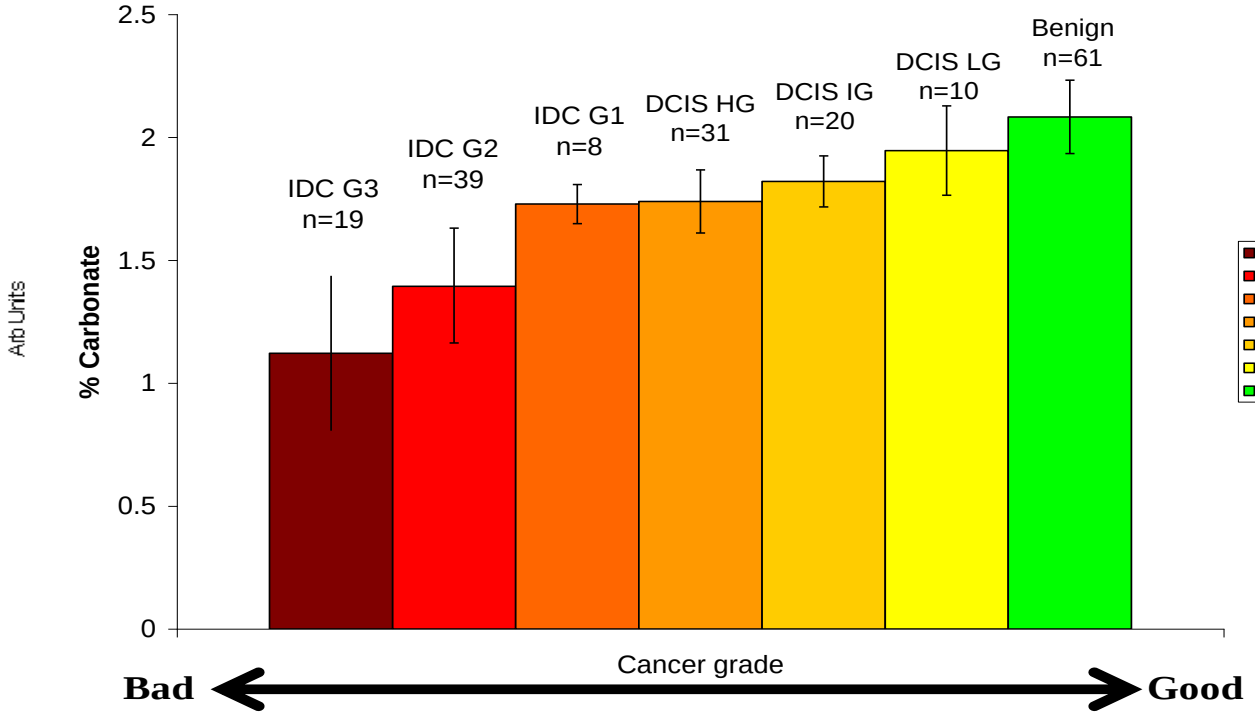
Mid-FTIR spectroscopy of Breast Calcifications: Composition of calcifications varies with pathology

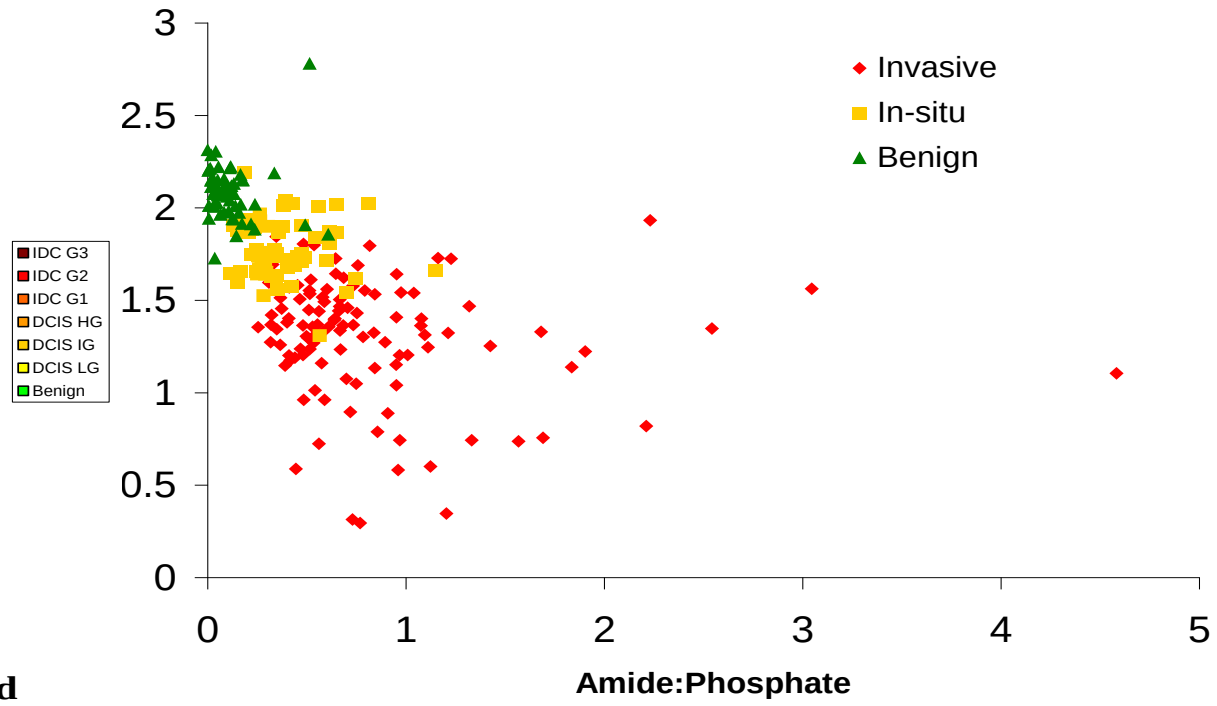


- Paraffin-embedded sections (110 patients) and deparaffinized (15 of the same patients): samples collected from archives of patients undergoing biopsy
- Blocks cut to 7µm thickness and mounted onto CaF_2 slides

Baker et al., BJC 2010

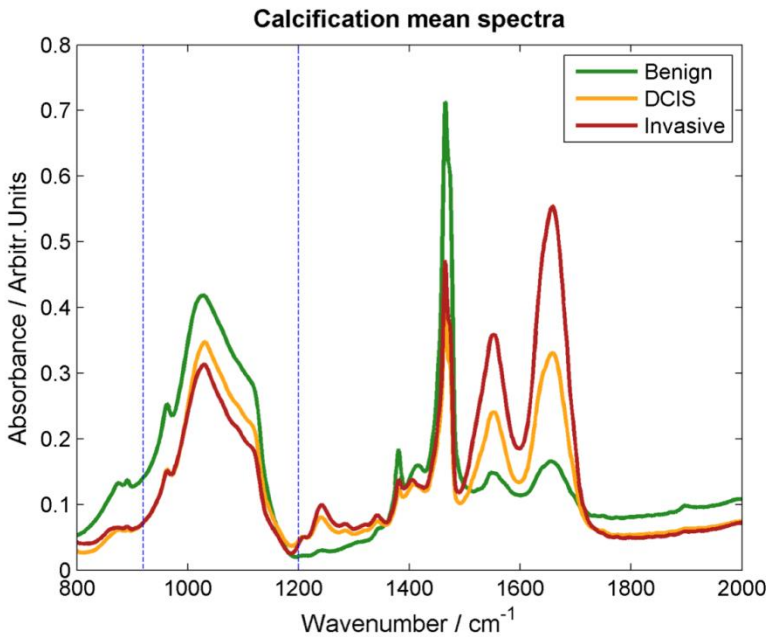


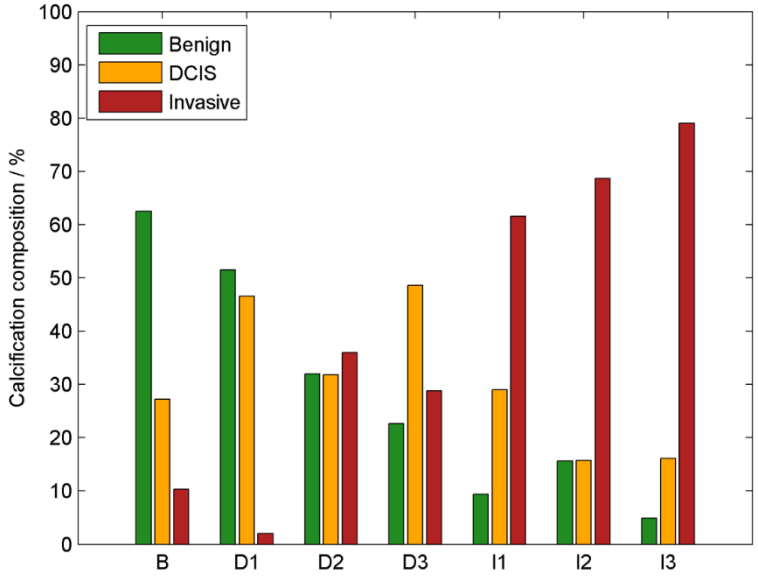


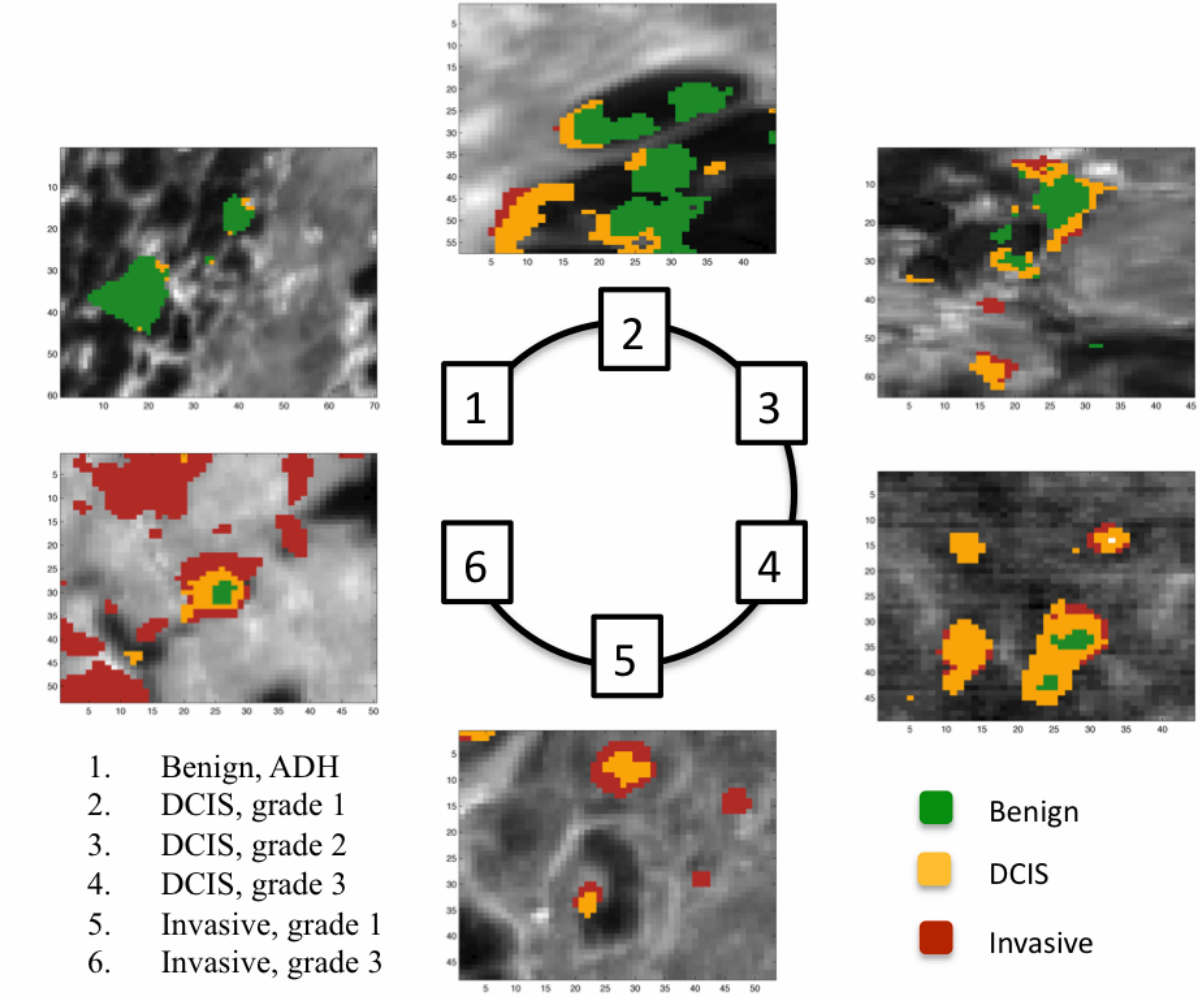


--- Malignant --- Benign

SVM image prediction – trained on mean spectra







Sample illustration of transformation of calcifications during disease progression.
→transformation to advancing pathology grades starts in the periphery.

CANCER RESEARCH UK

Let's beat cancer soo

HOME | ABOUT CANCER | SUPPORT US | OUR RESEARCH | FUN

Home › Funding for researchers › How we deliver research ›

GRAND CHAL

THE TOUGHEST PROBLEMS NEED

OVERVIEW | ROUND 1 AWARDS ▼ | A

Tackling the toughest challenges in cancer

In 2015 we launched a series of £20m awards for researchers attempting game c research.

These are the most ambitious grants in the world allowing international research t take on the biggest problems in cancer research, our Grand Challenges.

Last year, we set seven Grand Challenges and asked multidisciplinary teams fror the world to submit proposals to tackle them – nine pioneering teams were shortli 56 bids.

Our independent scientific advisory panel were so excited by the potential of thes shortlisted teams, they urged us to increase our investment from one award.

Thanks to the generous support of partners and donors we are able to fund not ju but four of these exceptional teams.

"Millions of women attend breast screening every year, but often lesions show up that will never go on to turn into full-blown breast cancer. Because doctors don't know which of these lesions could be dangerous, they often treat women just in case. In our project we will study thousands of these lesions in great detail to understand which are low- and high- risk and help doctors to make more informed decisions about treatment."

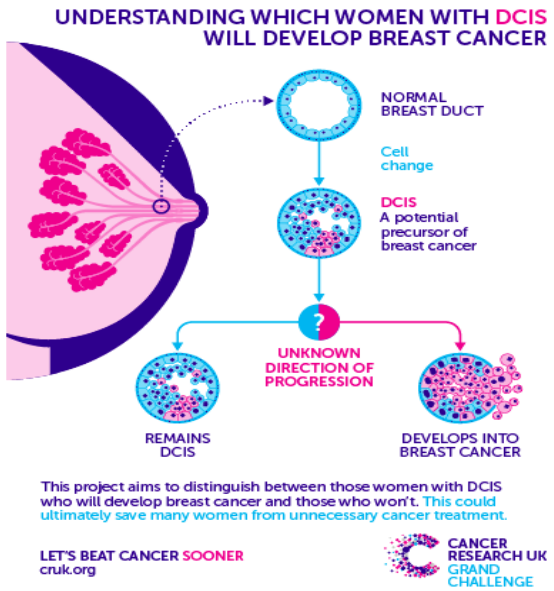
Dr Jelle Wesseling, Principal Investigator

Their project

Ductal carcinoma in situ (DCIS) is a condition that can sometimes develop into breast cancer. Each year it affects more than 6,300 women in the UK, and thousands more worldwide.

But right now, doctors can't tell whether women with DCIS will go on to develop breast cancer. This means that, unfortunately, some women with DCIS undergo hospital visits, surgery and even chemotherapy and radiotherapy that they don't need, while also causing them unnecessary stress and anxiety.

Dr Jelle Wesseling and his team of scientists from the UK, Netherlands and the US want to change this, and stop women getting treatment they won't benefit from.



The Research

To achieve their aim, Wesseling's team will study tissue samples taken from women with DCIS during surgery. These samples will come from women living in the UK, US and the Netherlands.

They will look at these samples in great detail, studying their characteristics, including their genetic make-up and what kind of immune cells they contain. Alongside this, they will gather clinical information about these women, recording whether their DCIS came back, if they later developed breast cancer, and if so, whether it spread.

The team will then combine all of this information and use mathematical modelling to search for clues (biomarkers) in the DNA of women who have had DCIS, that could indicate how likely they are to develop breast cancer later on.

Once they have identified potential biomarkers, they will test them in larger clinical trials for women with DCIS. Their goal is to find out whether these biomarkers can accurately and reliably distinguish between women with DCIS who will likely develop breast cancer and should be treated, and those who can safely avoid treatment.

The Impact

By identifying biomarkers that can distinguish DCIS patients with a low risk of developing cancer from patients with a high risk, this project has the potential to reduce over-treatment of DCIS patients.

Ultimately, Wesseling's team hopes to spare thousands of women unnecessary treatment while making sure those who need it, get it.

The Dutch Cancer Society

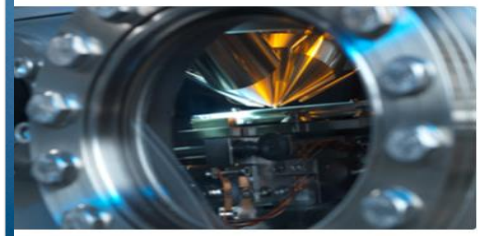
we're able to fund four remarkable Grand Challenge

ting virtual reality maps of ours

ng established techniques with new technology, Professor s team will build 3D tumours containing every cell in ich can be studied using virtual reality. This new way of breast cancer could change how the disease is d, treated and managed.

more about the team's research

ying tumour metabolism from y angle



rious new mass spectrometry imaging techniques, the by Dr Bunch will develop a new way to map tumours in ented detail – from the whole tumour to the molecules in cells. The work could lead to new ways to and treat cancer.

more about the team's research

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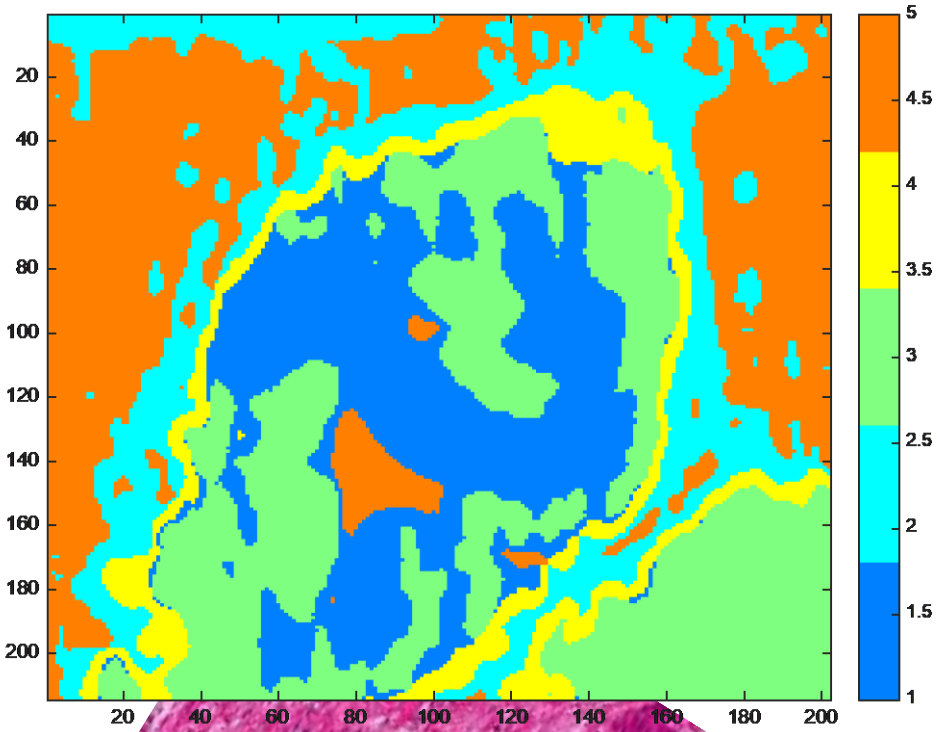
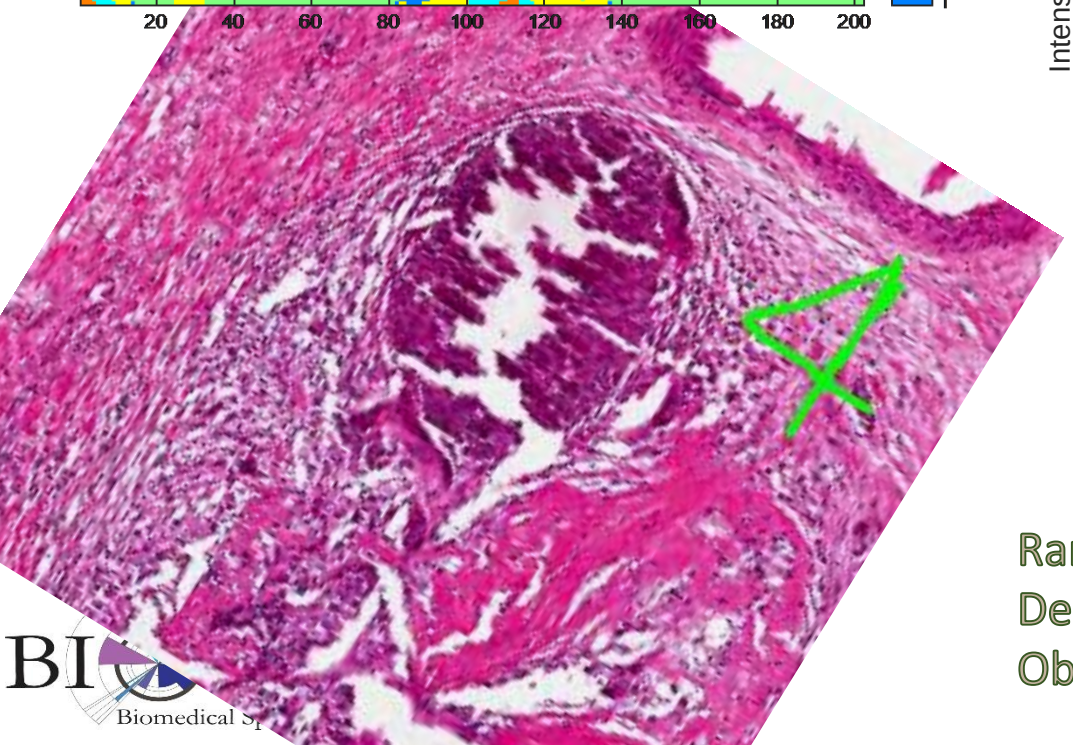
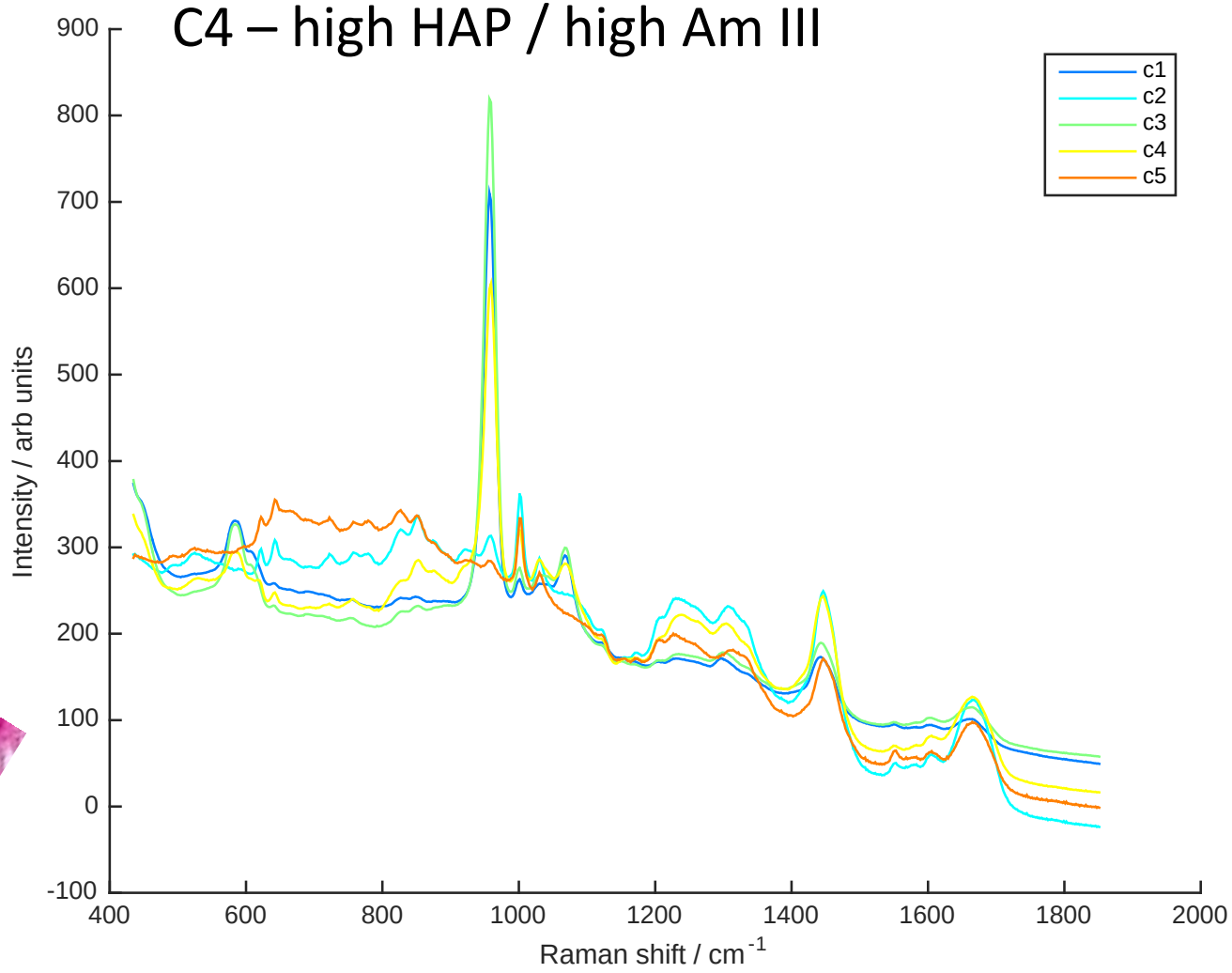
Biomedical Spectroscopy Labs





C1/C3 – high HAP / low Am III
C4 – high HAP / high Am III



Raman maps with Renishaw in Via
Deparaffinised on polished stainless steel
Obj 50x, 830 nm, 1.4 µm steps

BIO Biomedical Sp

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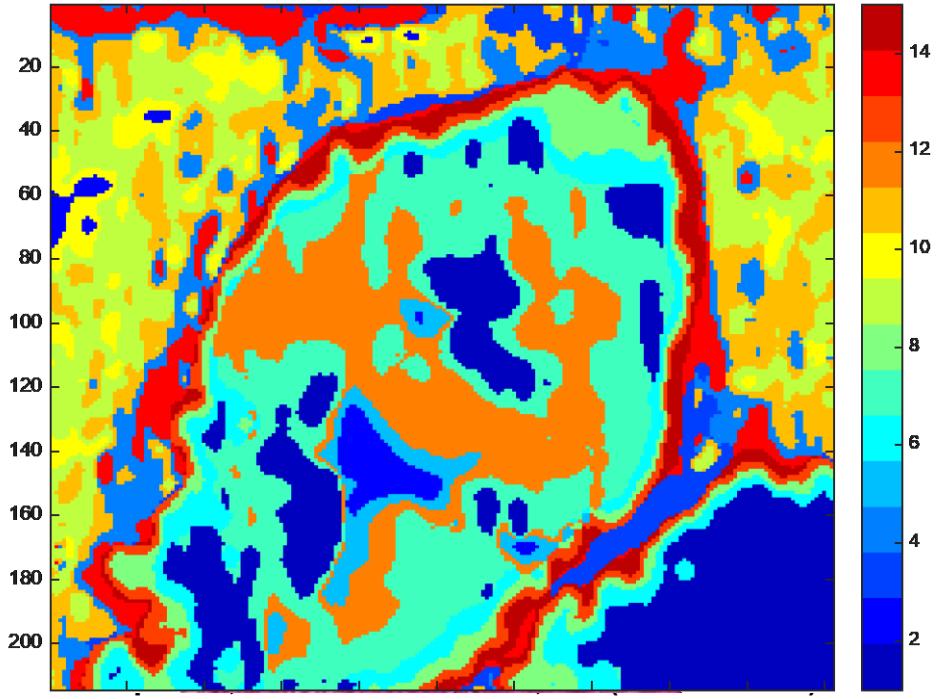

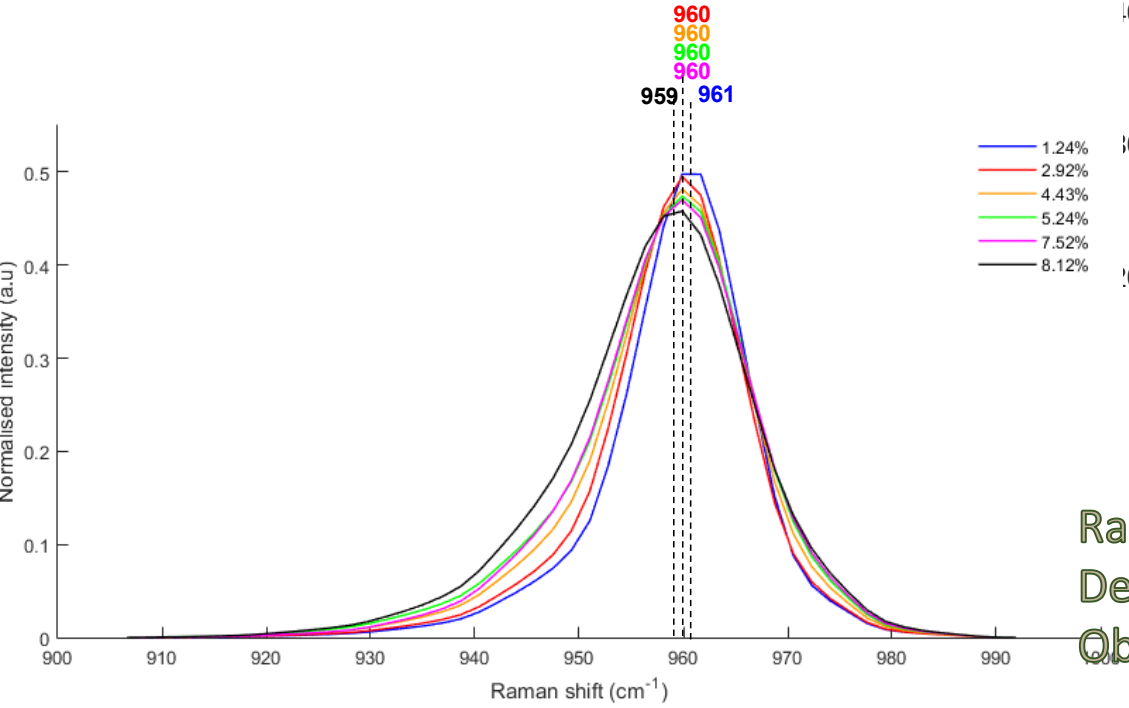

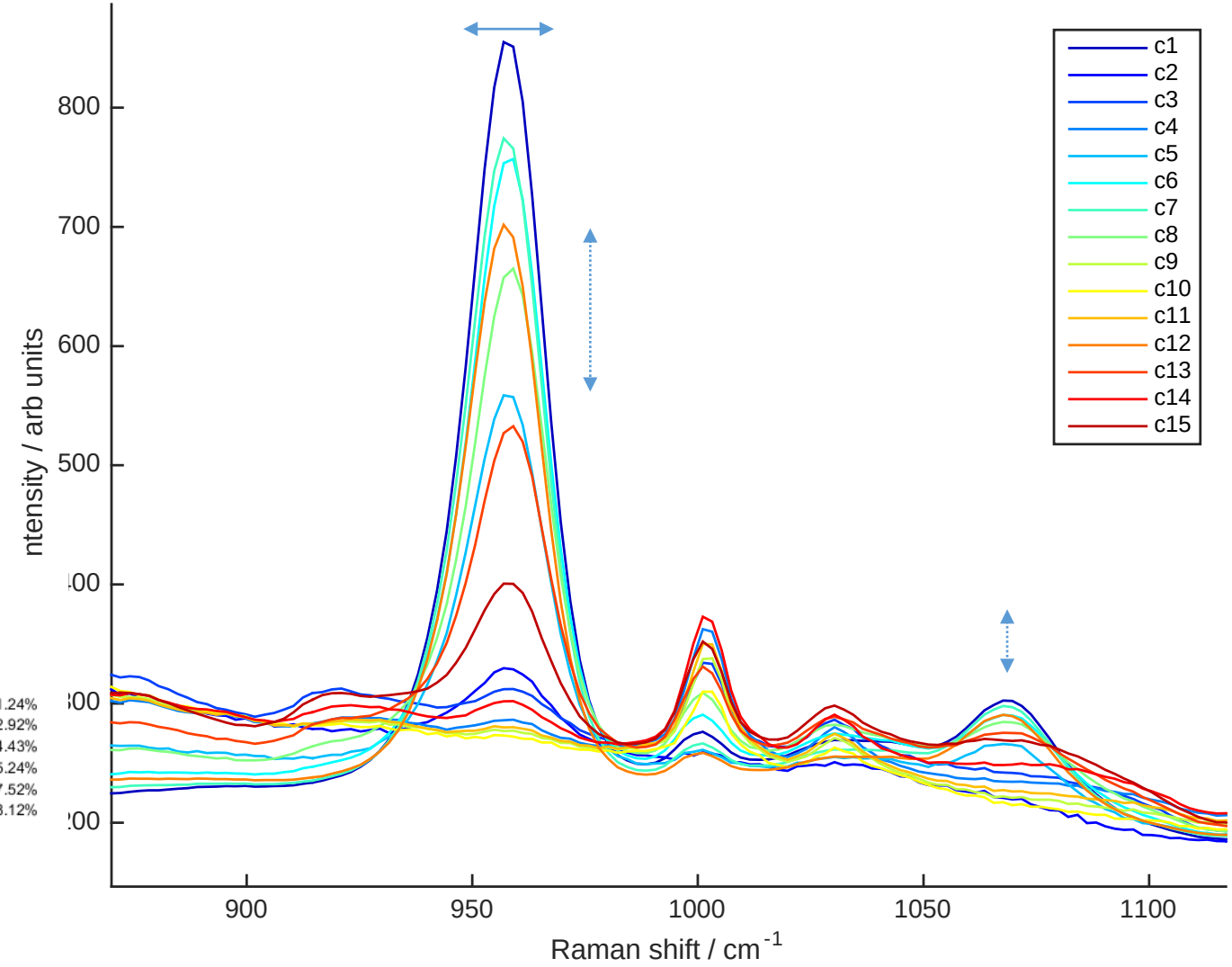

Raman maps with Renishaw in Via
Deparaffinised on polished stainless steel
Obj 50x, 830 nm, 1.4 µm steps

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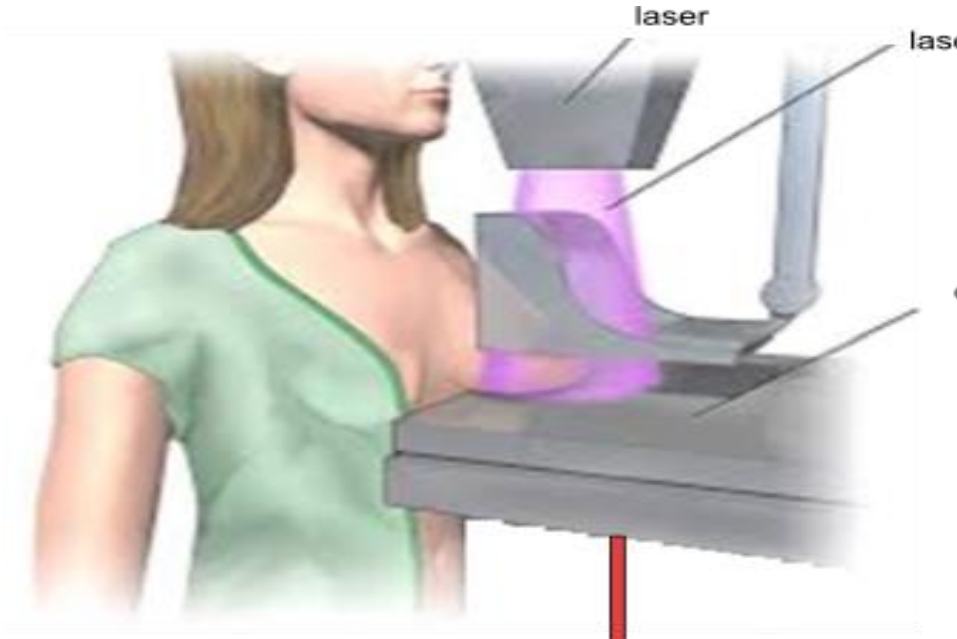
Biomedical Physics

Exploring Deep Raman for Advanced Screening for breast cancer

- Improve the rate of detection
- Improve survival rate
- Reduce overdiagnosis
- Enable non-invasive monitoring of those with early disease
- Enable non-invasive monitoring of those undergoing treatment

Dr Ben Gardner

Dr Adrian Ghita

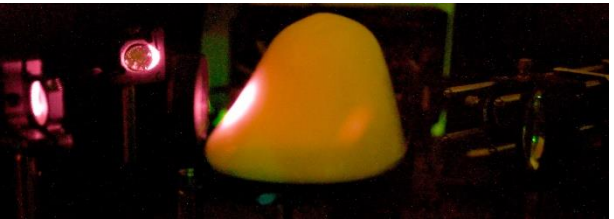


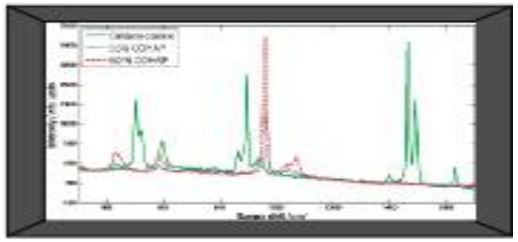
£1.2M EPSRC

Mr Douglas Ferguson
& Miss Charlotte Ives
RD&E NHS FT

Prof Pavel Matousek

EPSRC
Engineering and Physical Sciences Research Council





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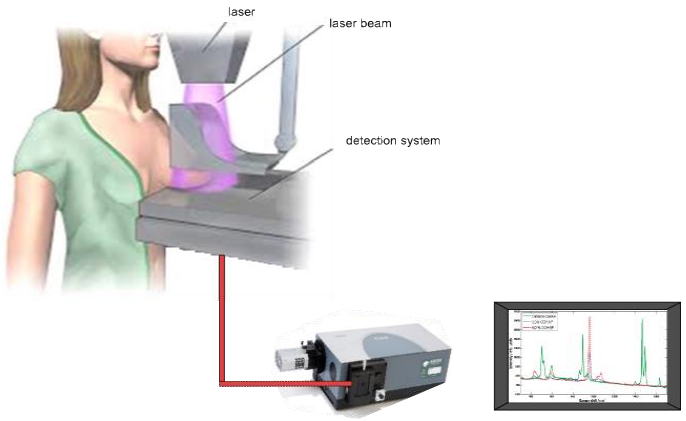
How can we use this for in vivo diagnostics?

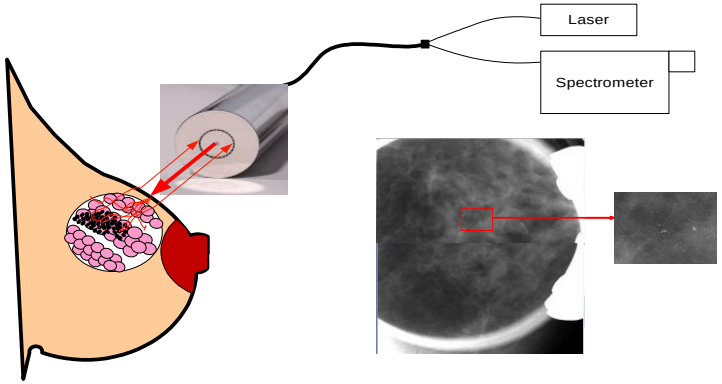
Research Article

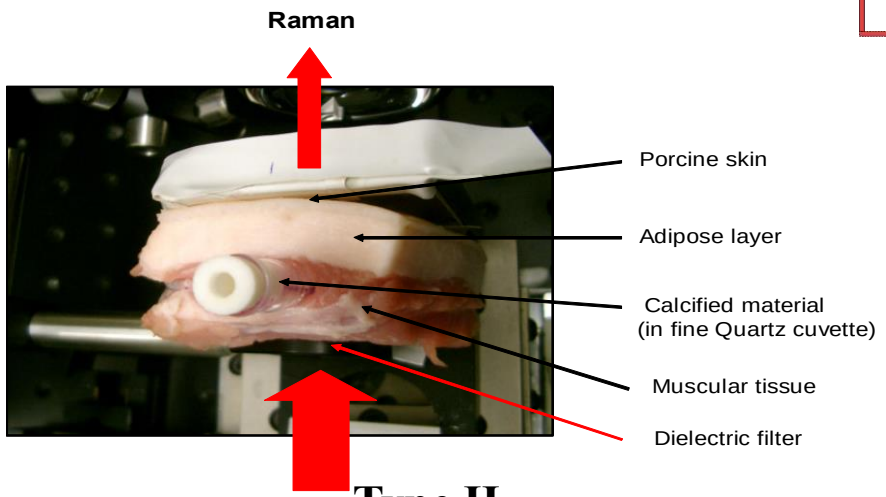
Advanced Transmission Raman Spectroscopy: A Promising Tool for Breast Disease Diagnosis

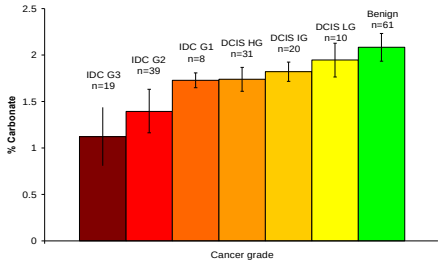
Nicholas Stone[1] and Pavel Matousek[2]

Cancer Res 2008; 68: (11). June 1, 2008 4424 www.aacrjournals.org



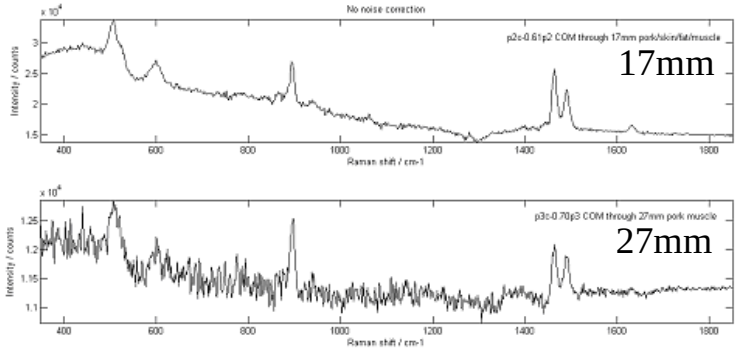




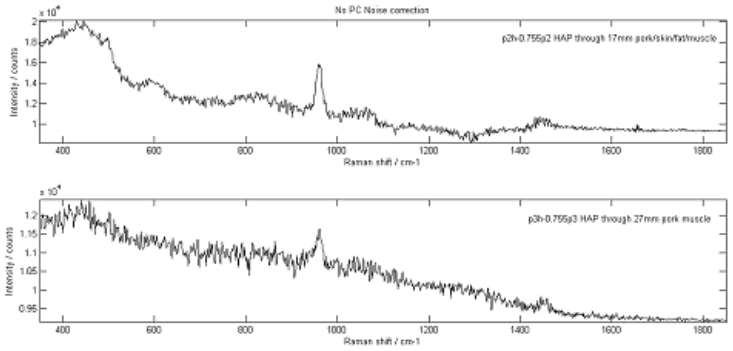


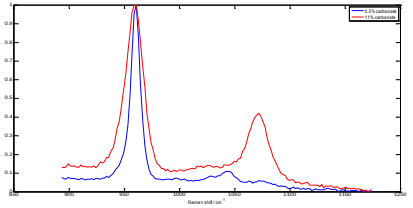
Baker et al., 2010
British Journal of Cancer

Type I



Type II





Kerssens et al., 2010
Analyst

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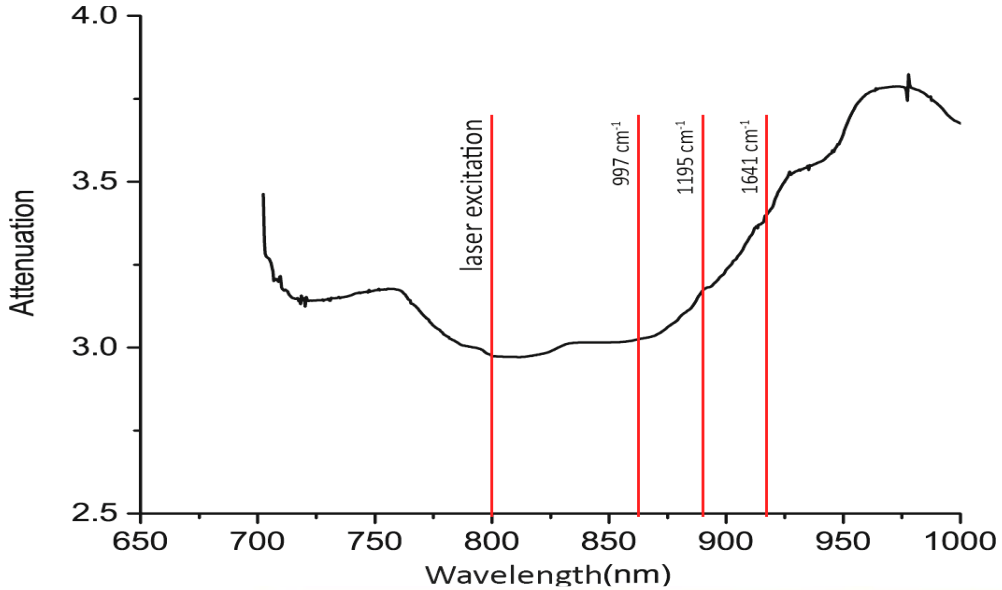
Optimising Wavelength

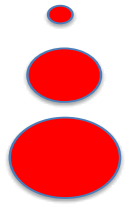


Extrapolate power density (x20)

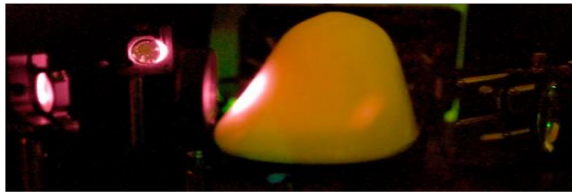
- 100mW in 2mm spot = 31mW.mm^{-2}
- 900mW in 6mm spot = 31mW.mm^{-2}
- 2000mW in 9mm spot = 31mW.mm^{-2}

Open up slits (x10) and change grating

Not actual spot size!

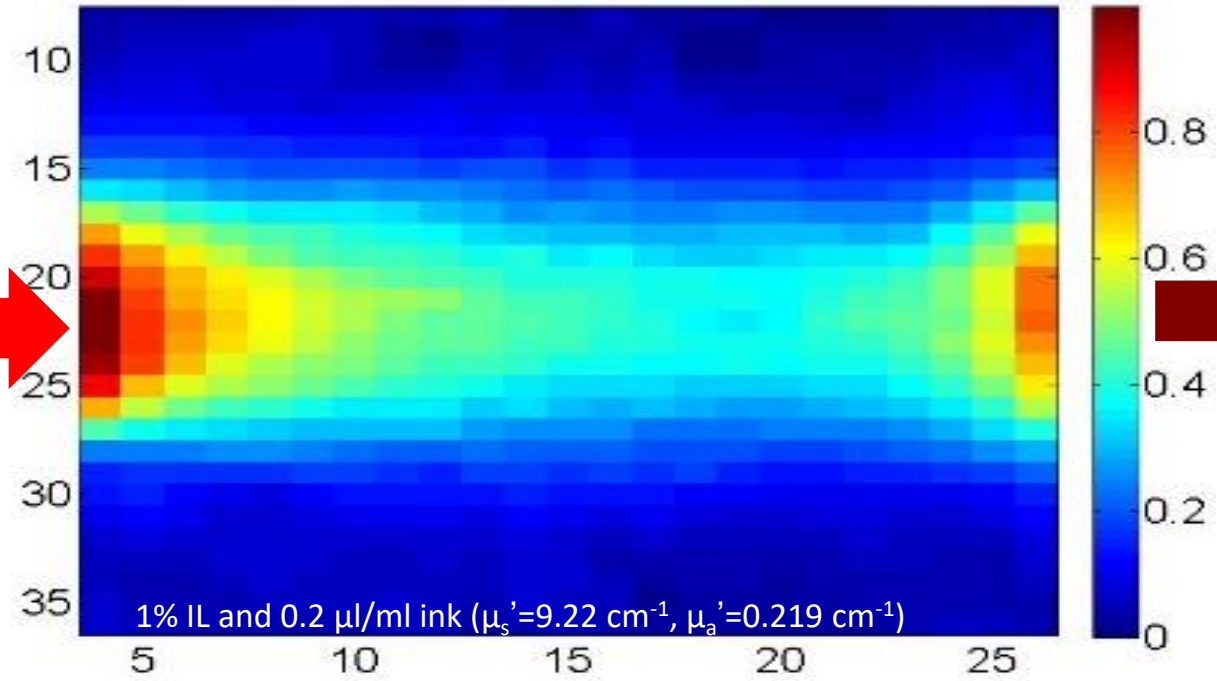


Mark II: x200 throughput
x14 SNR
→ 3x2s acquisition?



Assessing the sampling regions

Laser



Raman

scattering

Distance from the illumination side in mm

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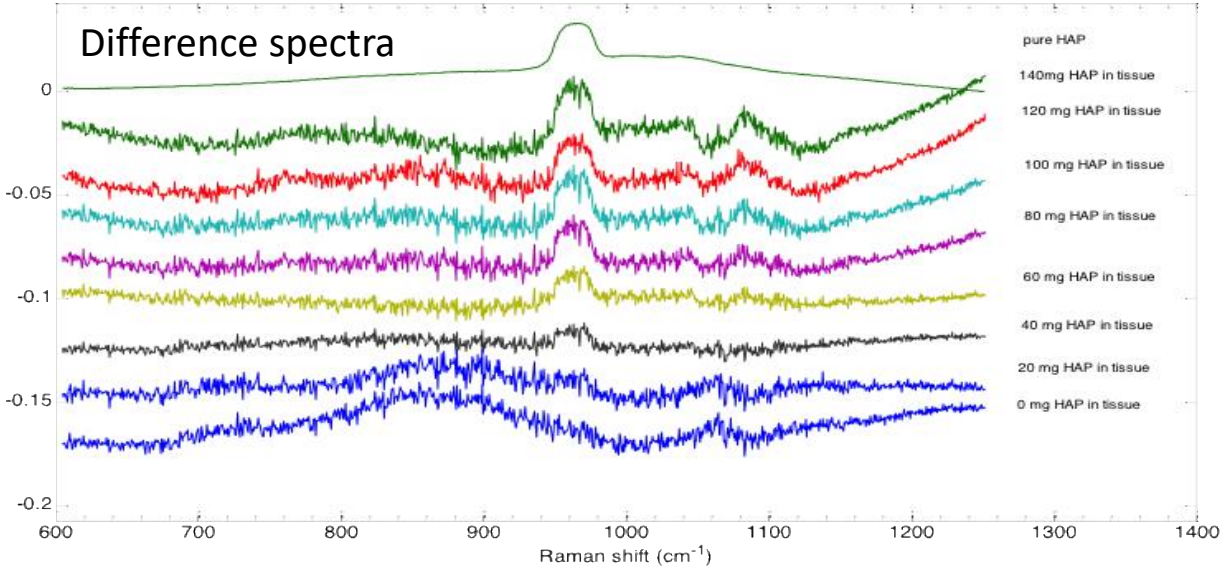
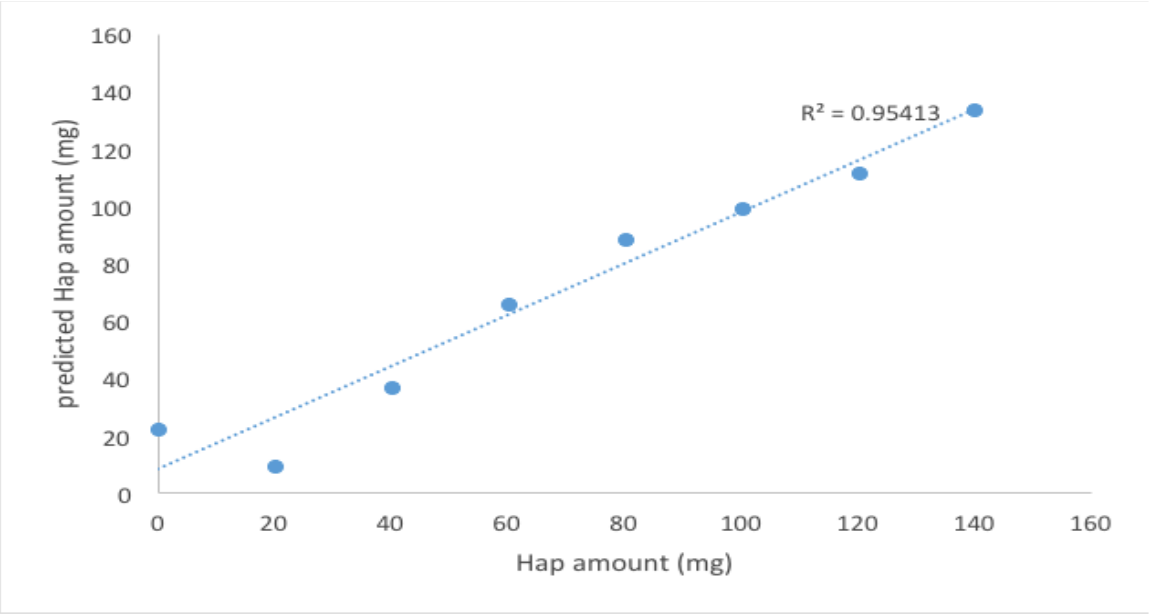
Sample No.3: 4-4.2cm , 2W laser power

New optimised system @ 808nm

HAP detection limit in 4 cm porcine tissue

Ghita, Matousek, Stone, J Biophotonics 2017





L.O.D.=3.3×S/σ (where S is slope of the plot and σ standard fit residuals.)

38.28 mg or translating this back to relative volume (using HAP density) we get: **a relative volume L.O.D. of 0.08% through 40 mm vs 0.125% in 20 mm (Cancer Research 2008)**

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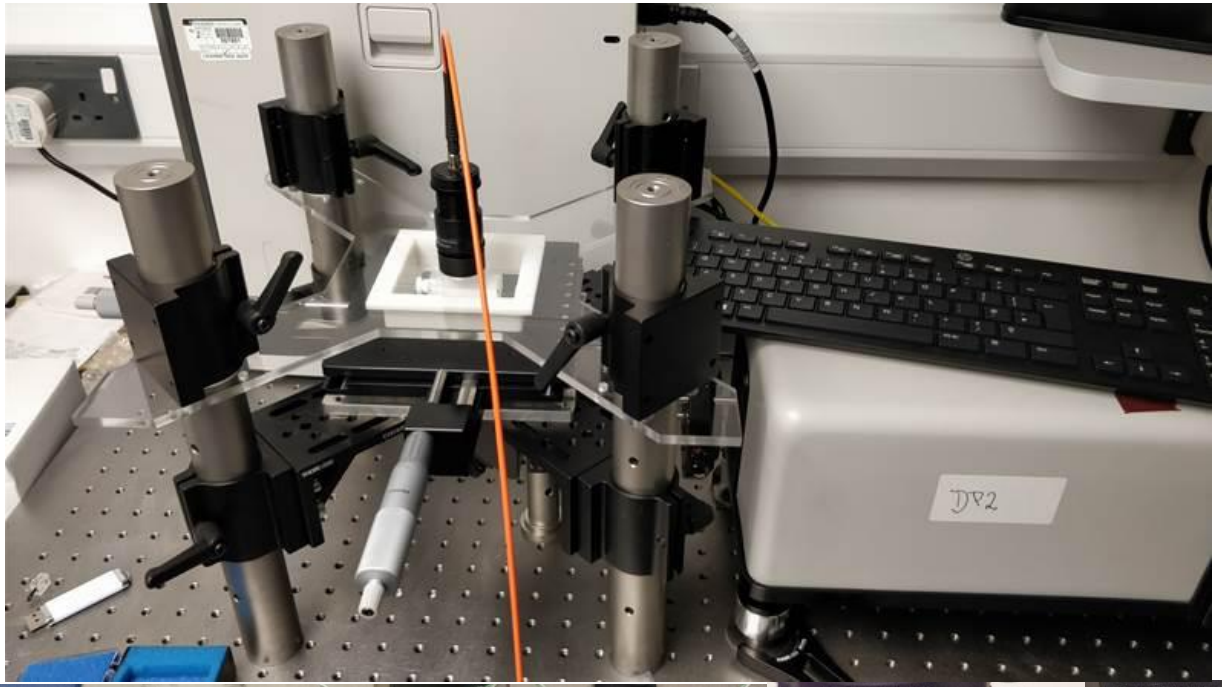

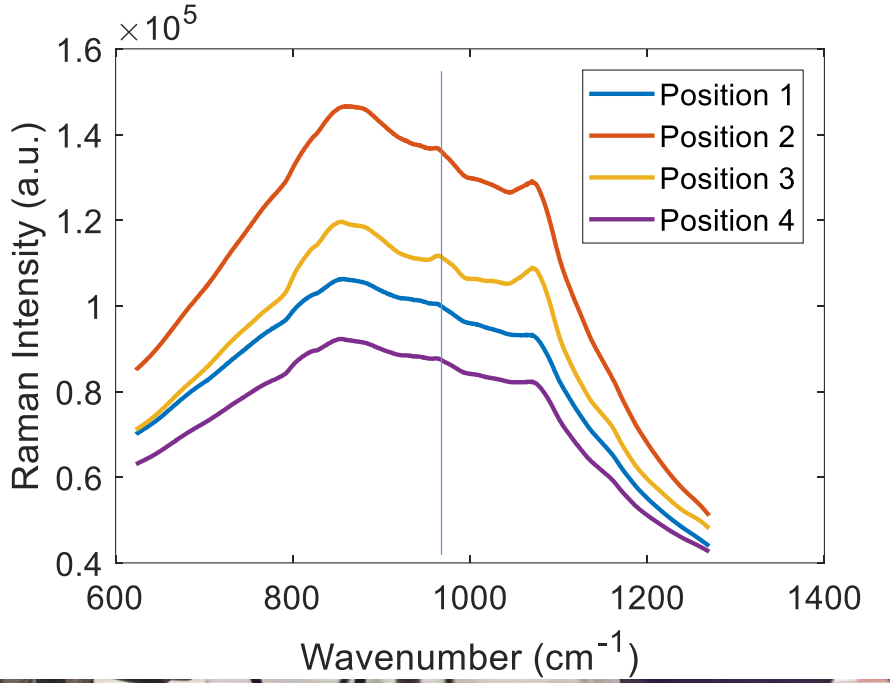

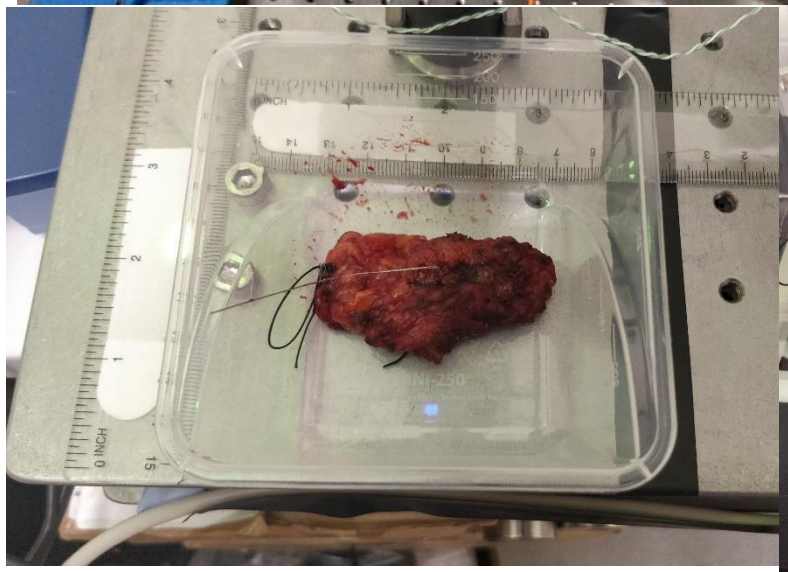

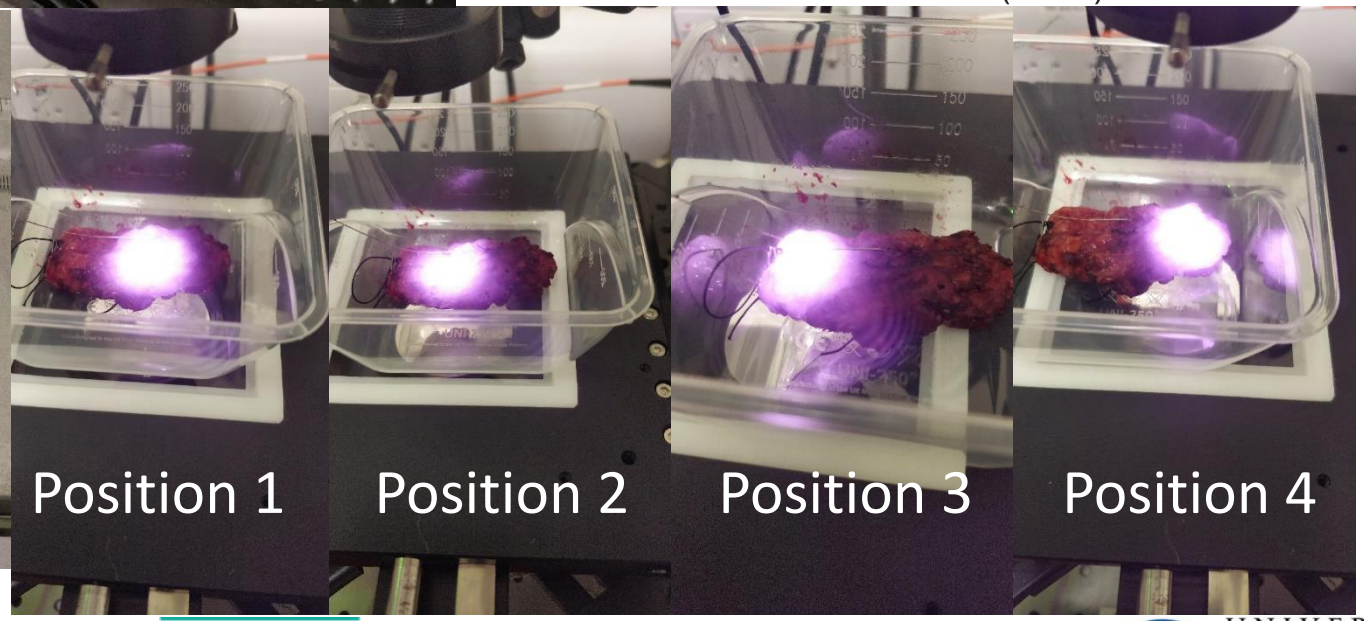

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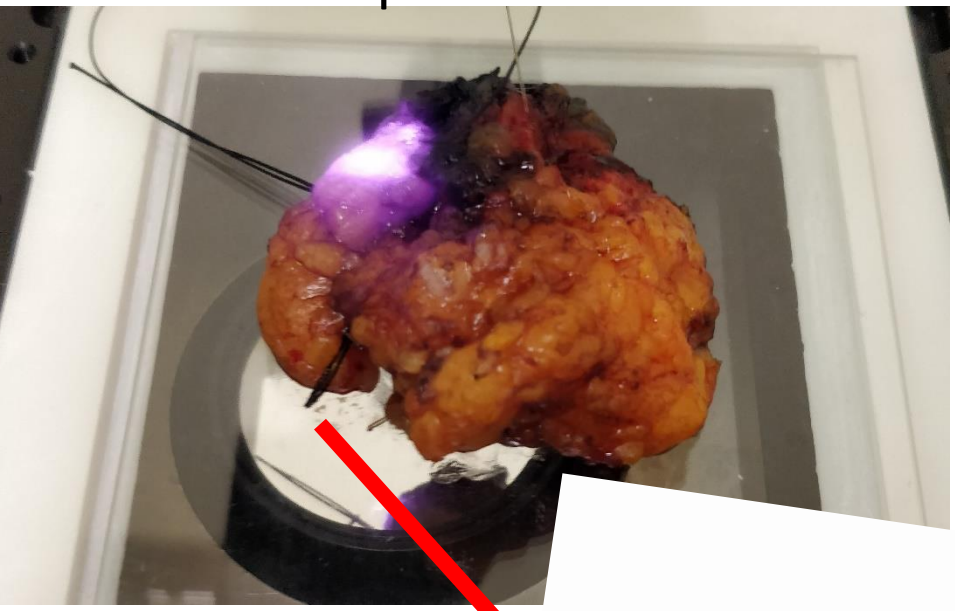
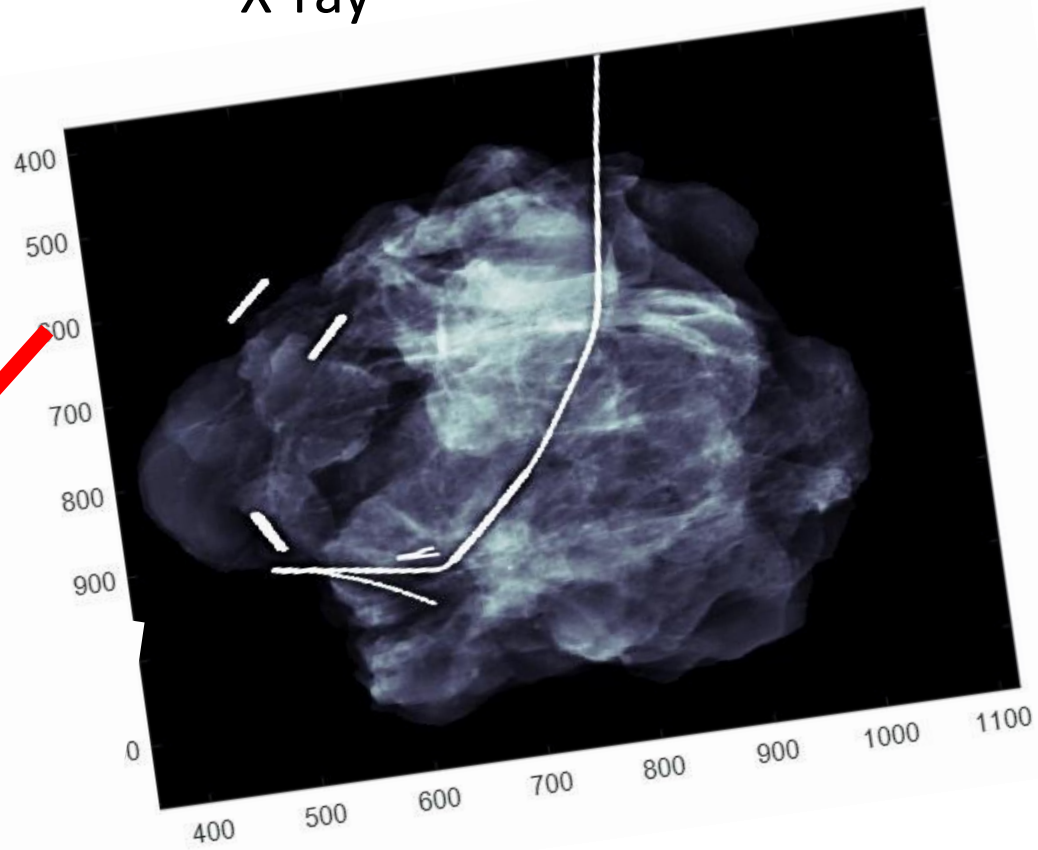
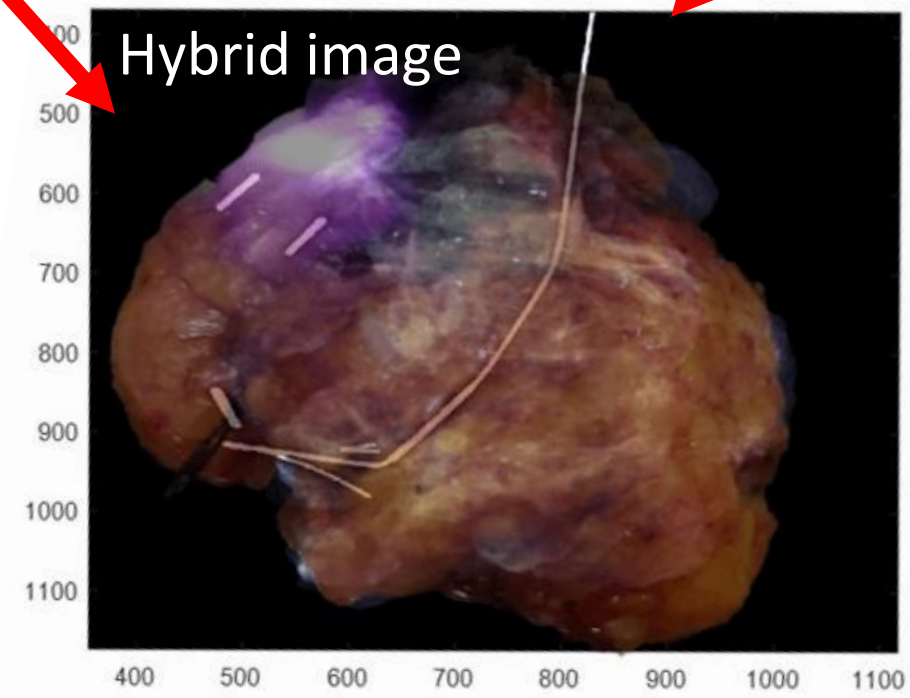
Colour photo



X-ray



Hybrid image



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Progress – Deep Raman Breast (RD&E)

- Optimised system for ex vivo samples
- Ethics approved
- Ex vivo sample collection ongoing @ RD&E
  - around 120 to date.
  - Aim for n=200.
- Develop in vivo device based on findings
- MHRA/ethics
- Recruit patients for first in human studies to measure Raman non-invasively in vivo

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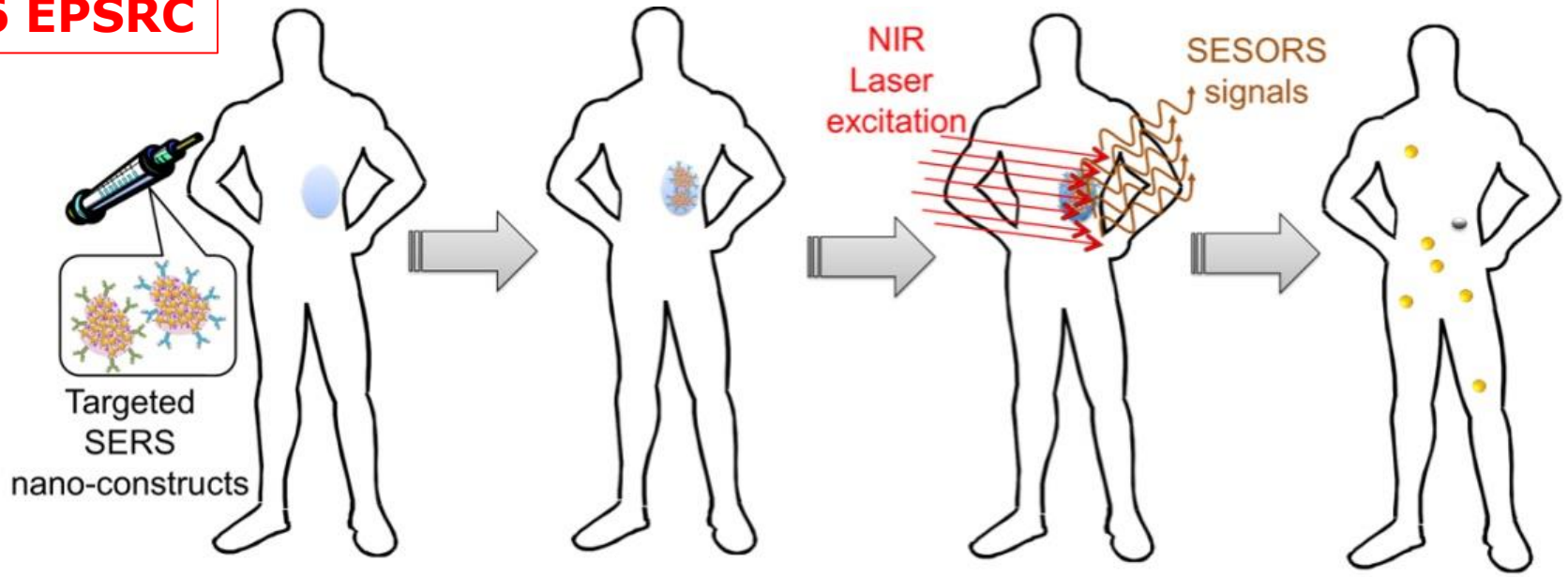
Raman Nanotheranostics – RaNT

developing the targeted diagnostics and therapeutics of the future by combining light and functionalised nanoparticles.

£5,752,646 EPSRC



Targeted SERS nano-construct injected into patient's body

Targeted SERS nano-construct accumulate in specific tumour site

SORS set-up employed to detect SERS signals, SESORS diagnostics

After Hyperthermic therapy, SERS nano-construct distingerate into single smaller NPs and can be safely cleared from the body *via* the excretion system

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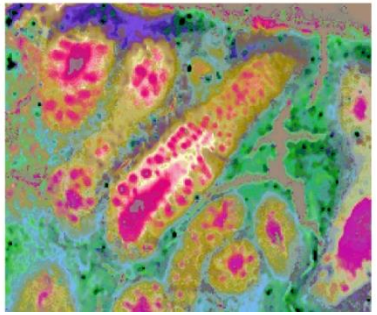
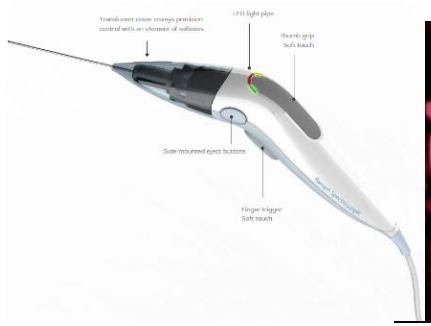
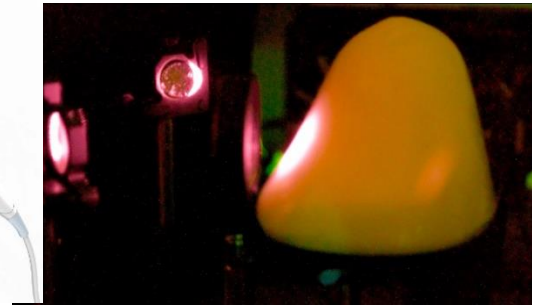
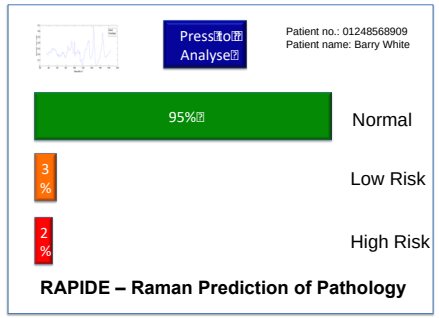
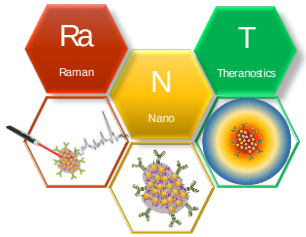




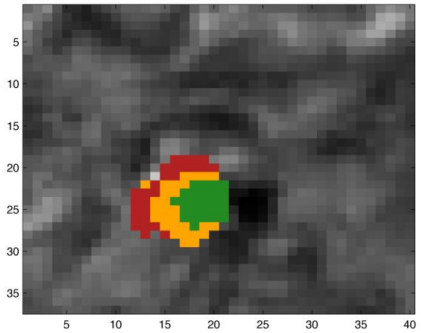


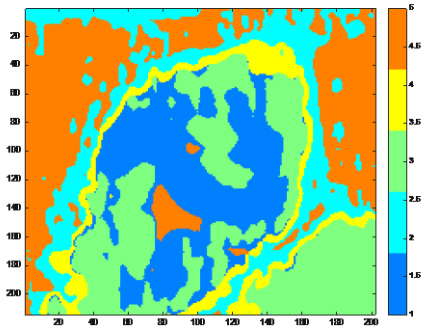




Summary

- **The future is bright for light based cancer diagnostics and targeted therapies.**
- Raman can provide real-time, minimally invasive, objective, molecular analysis of disease & monitoring of treatments.
- Calcification composition and microstructure may indicate more susceptibility to progression.
- **Can they predict a patient's future? – watch this space…**





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Acknowledgements

Calcifications

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CLIRSPEC

The International Society for Clinical Spectroscopy

Promoting the translation of vibrational spectroscopies into the clinical arena

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