BIOMEDICAL APPLICATIONS OF NANO-SPECTROSCOPY

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early diagnosis of cancer and desease (Oesophagous, Cervical, ALS, ...) Acticity at ALICE Daresbury, micro-Raman

Perspective with fs lasers

Prevention of cancer related death by early diagnosis is estimated at 90%, strongly motivating the continuous development of better diagnostic techniques.

Problem of resolution with standard infrared microscopy

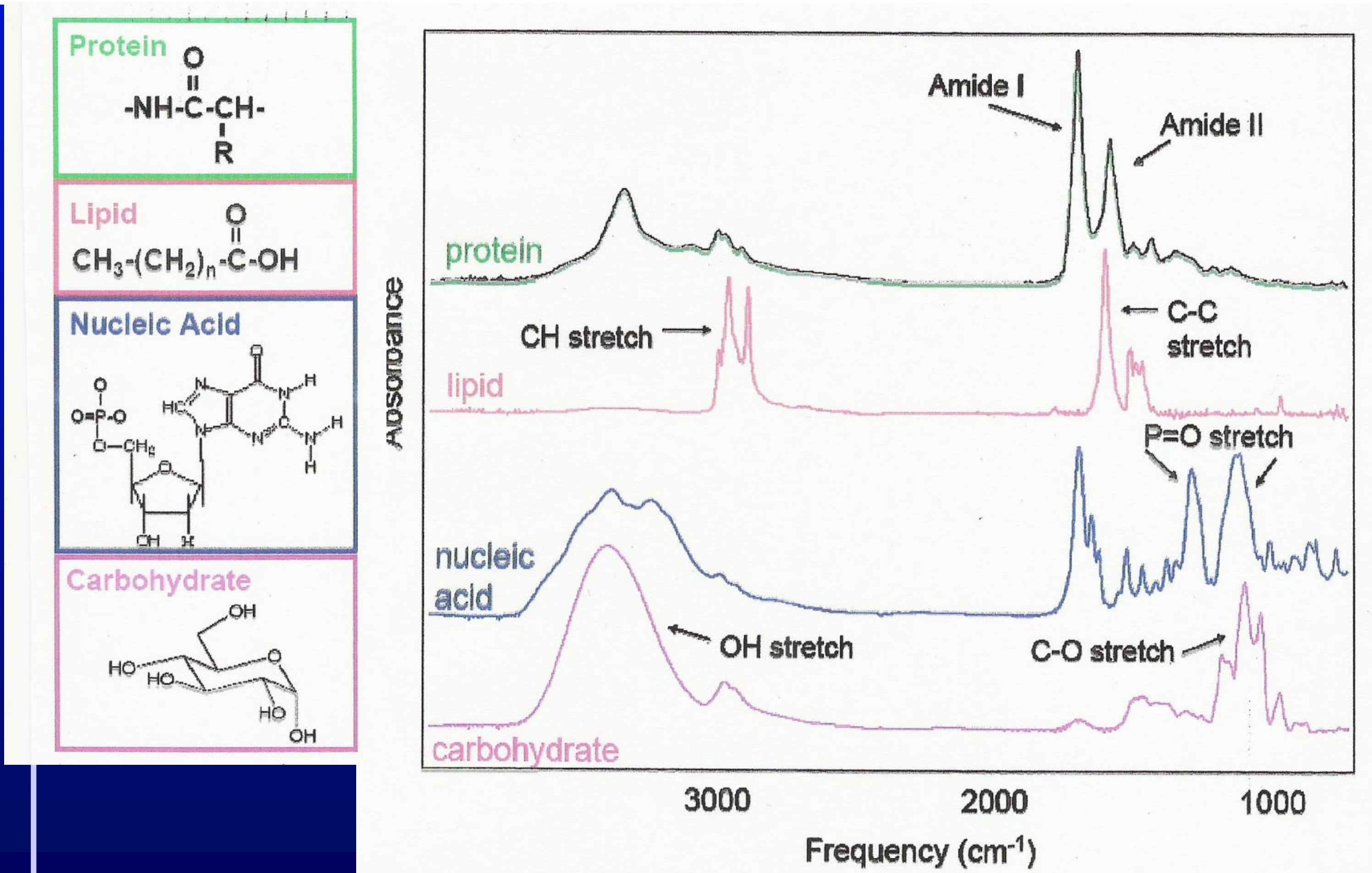
The tumor cells are localized in different and small areas (hundreds of nanometers), so if we want to prevent the desease we must look at single area: average techniques are not good enough tissue imaging by nano-spectroscopic methods to detect alterations of the biochemical composition (modifications in lipid components, protein aggregations or conformational changes, disruption of nucleic acids organisation, etc.…) with unprecedented resolution.

These molecular changes will be designated as diagnostic markers of cancer and diseases in general. Monitoring the impact of advanced therapeutic treatments (deriving from the related nanotechnology field) is a further opening.

In parallel, the development of ultrasensitive biosensors based on an enhanced optical response and targeting specific pathogens leading to the occurrence of cancer will be supported.

Spectroscopy and microscopy in the infrared

Strength: Spectral fingerprints of molecules

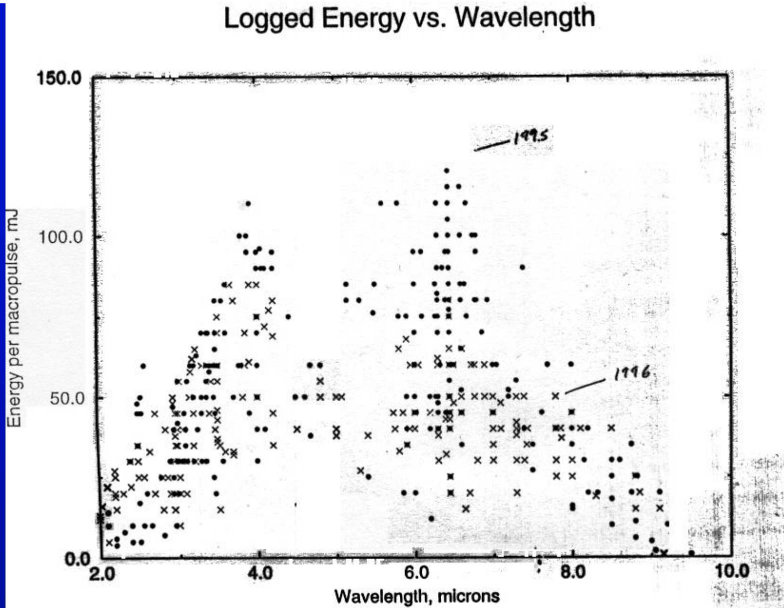


Weakness: Long wavelengths Spatial Resolution ~ λ/2

Solution: Near field optics ----> SNOM ---> needs high intensity

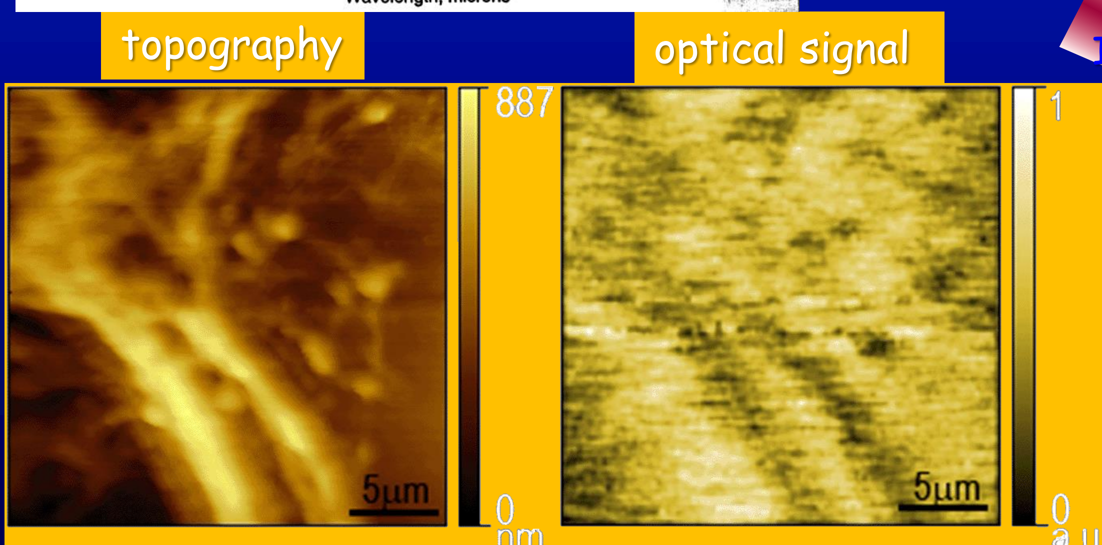
Combine Spectroscopy and SNOM ---> needs very high intensity ---> IR FEL

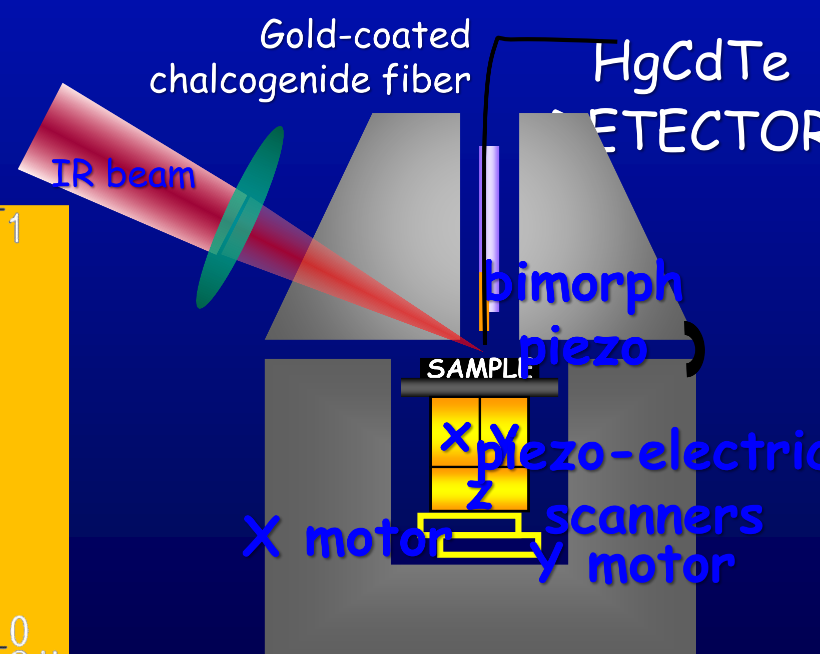
Free Electron Laser (FEL)

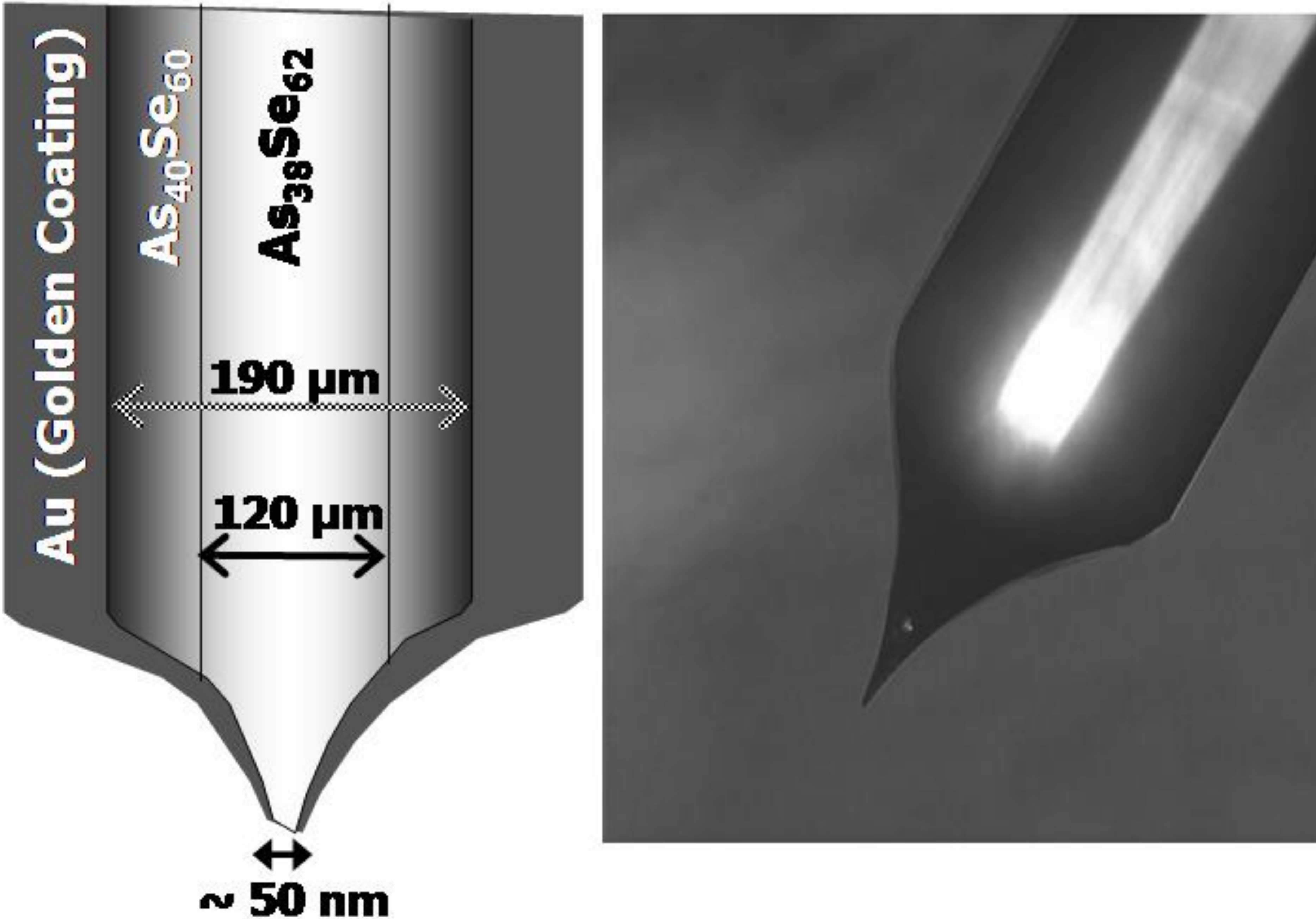


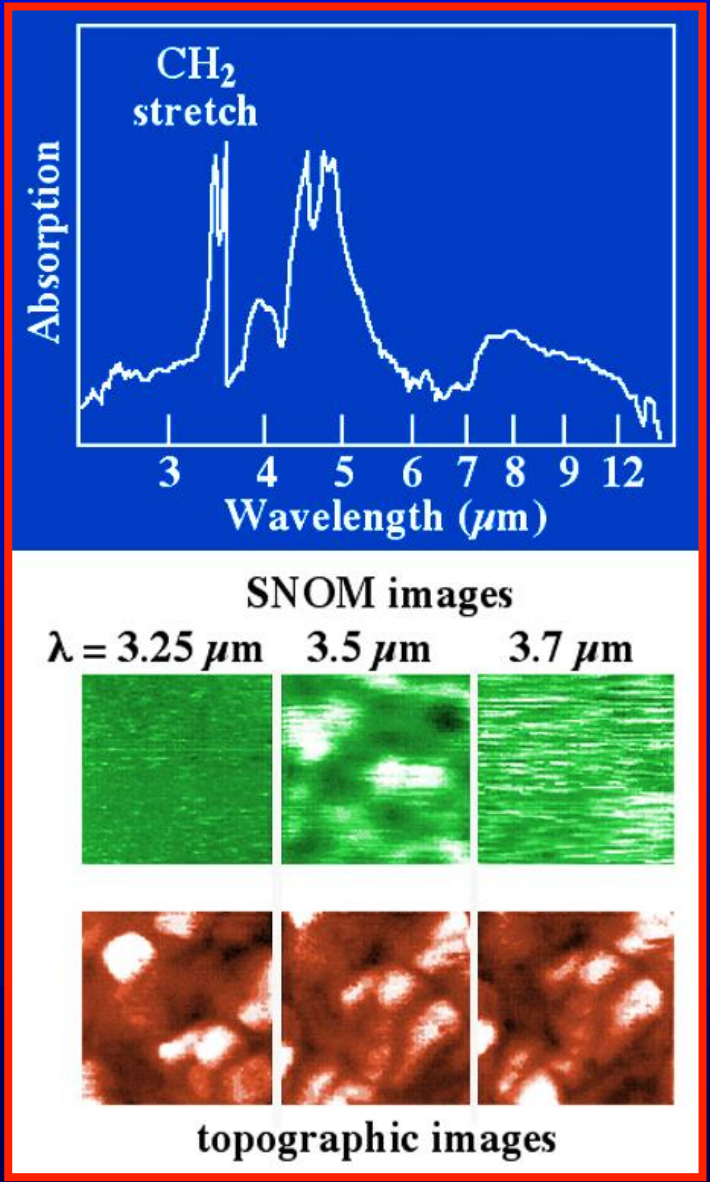
Chose wavelengths corresponding to vibrational modes of the interested chemical bonds

obtain a map of sample's chemical contents






Au (Golden Coating)
$As_{40}Se_{60}$
$As_{38}Se_{62}$
190 µm
120 µm
~ 50 nm



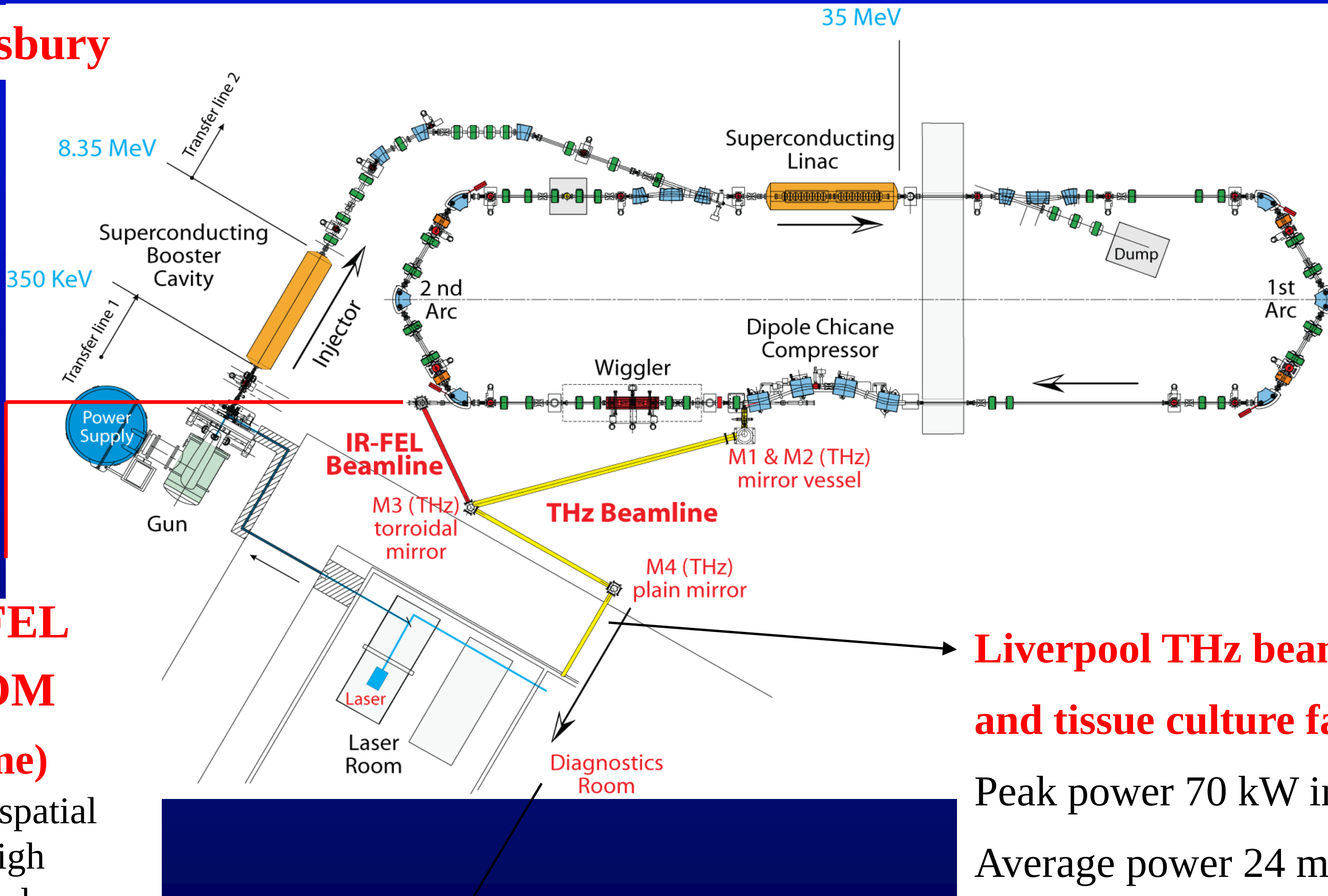
example of IR Spectroscopic SNOM: diamond films

J. Microscopy 202, 446 (2001).

Diagnosis of cancer at Daresbury 2011-

THz and IR Research Cancer Programme on ALICE

Daresbury



IR FEL SNOM (Rome)

High spatial and high spectral resolution imaging

Liverpool THz beamline and tissue culture facility

Peak power 70 kW in 0.6 psec

Average power 24 mW

No thermal effects

Tissue Culture Facility on 2nd floor

EPSRC: "The mechanism of biological organisation"

Oesophageal Adenocarcinoma

Oesophageal cancer is the fastest rising incidence of cancer in the western world.

Most patients have distant metastases on diagnosis and are not suitable for surgery.

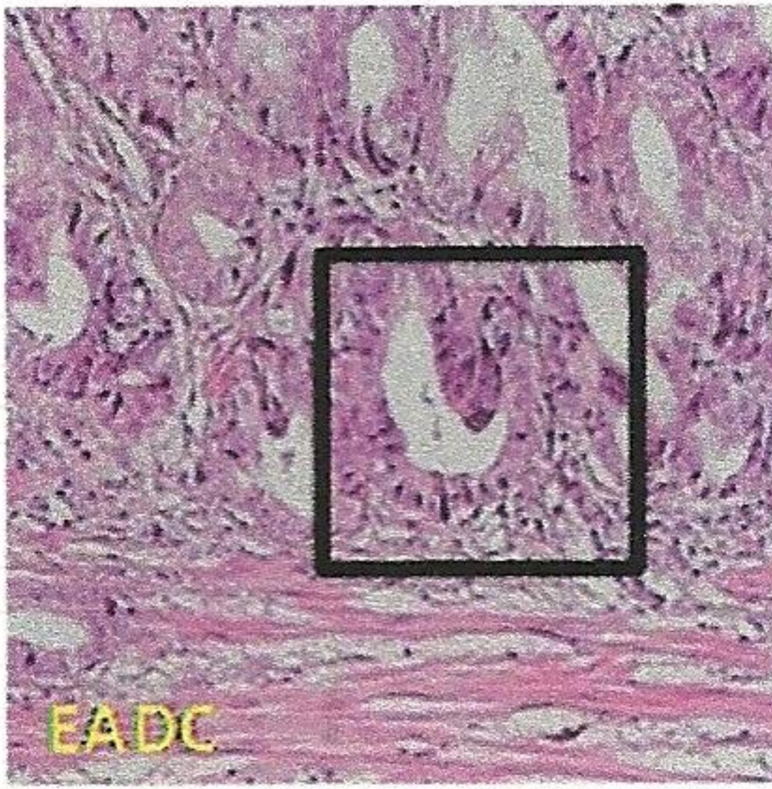
Surgery is the only potentially curative treatment so survival rates are very poor.

However, very low percentage (8%) in identify it

The challenge is to do early diagnosis on patients who can develop oesophageal cancer.

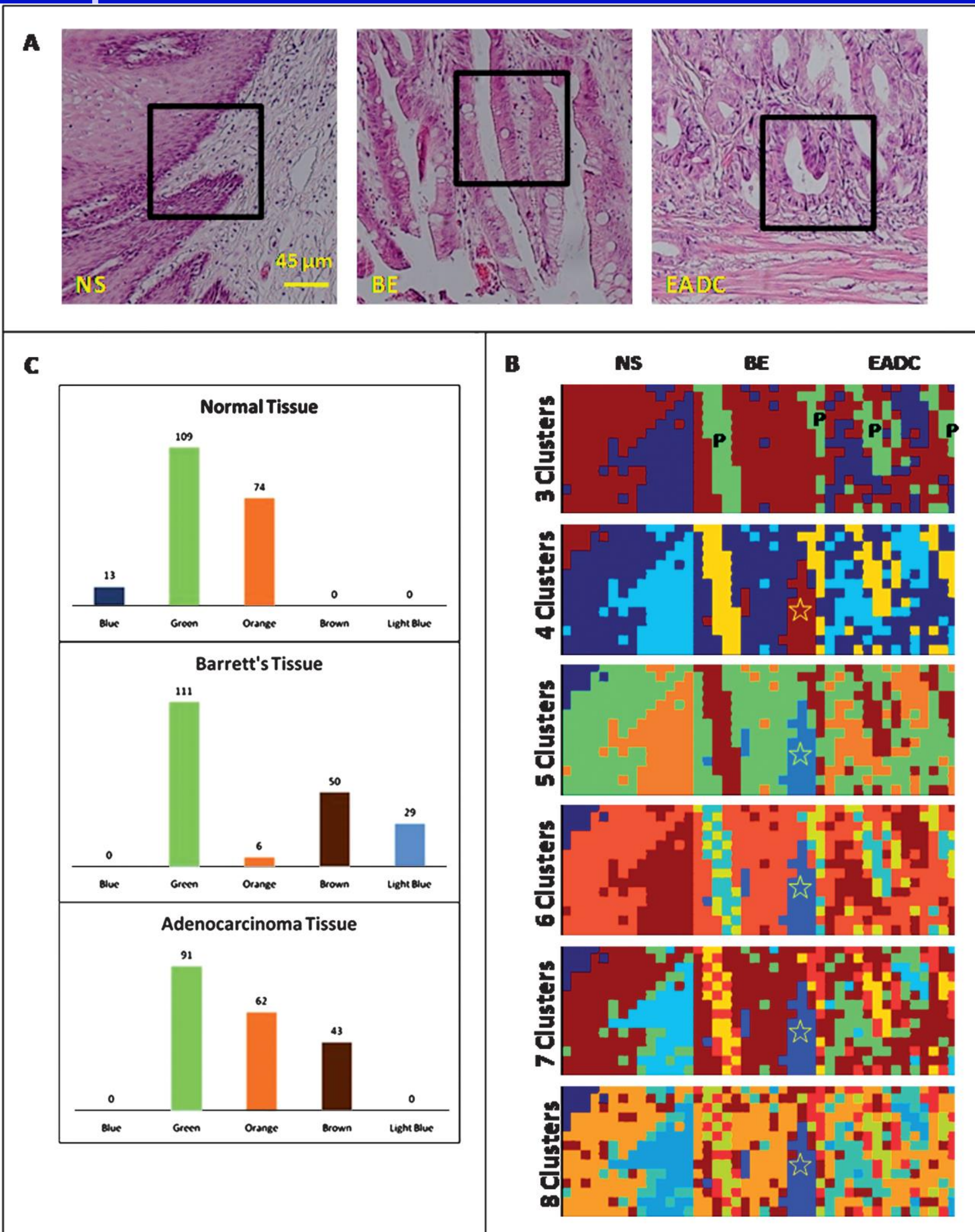
Detail: Stroma

Oesophageal cancer consists of cancer cells surrounded by stroma made up of various (non-cancer) cell types and extracellular matrix (ECM) proteins.



Visible light image of specimen of Barrett's oesophagus.

Previous work: FTIR on a Synchrotron



Quaroni et al. 2009
Esophagous cells
Synchrotron IR
15 micron step
900-1300 cm-1

Blue bands of glicogen

Number of pixels in a given region of normal, Barrett's and adenocarcinoma tissue in particular regions of the infrared spectrum

Problem of resolution with standard infrared microscopy

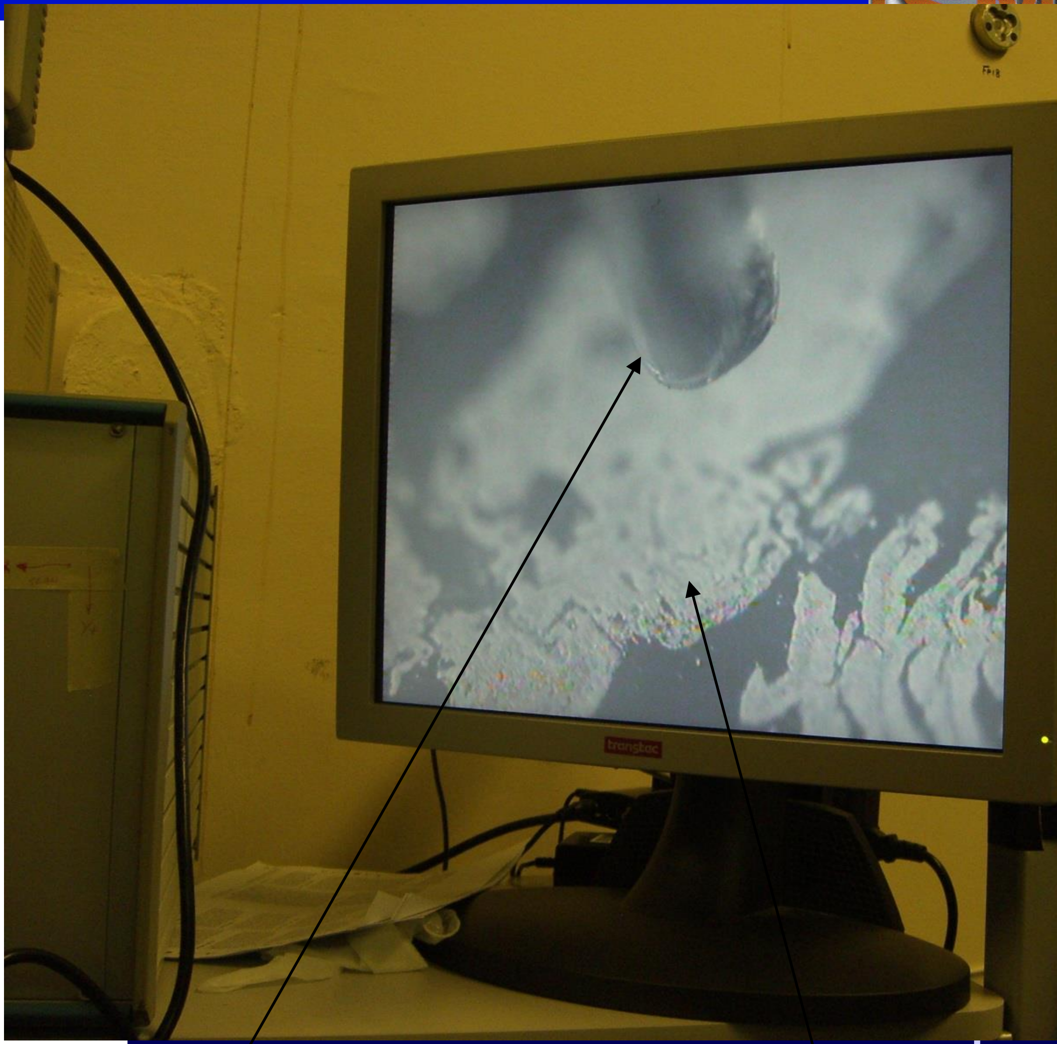
The tumor cells are localized in different and small areas (hundreds of nanometers), so if we want to prevent the desease we must look at single area: average techniques are not good enough

Technical Issues

Accelerator and IR FEL
Optimise performance of accelerator for IR FEL characteristics
Stability: intensity, frequency
Tuning: 4μm to 10μm
Macro bunch structure 10 Hz
ALICE 100 μs
Vanderbilt 25 μs

SNOM
Electronics matched to macro bunch
Control of scan
Optics: fabrication of tips
Performance
Images at different λ
10 μm x 10 μm at
0.1 μm spatial resolution
10000 points IR Intensity/stability is key

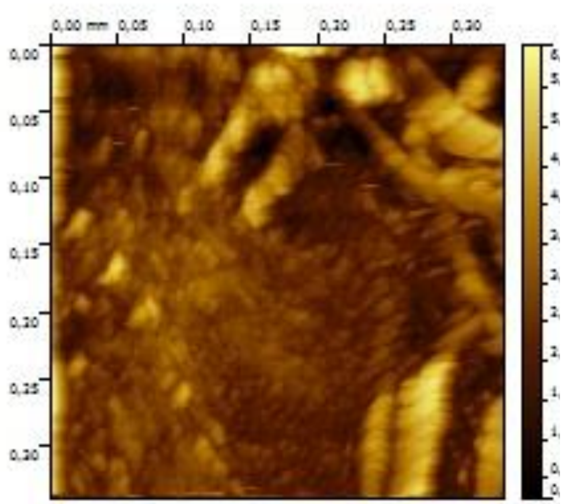
Beamline: Commissioned



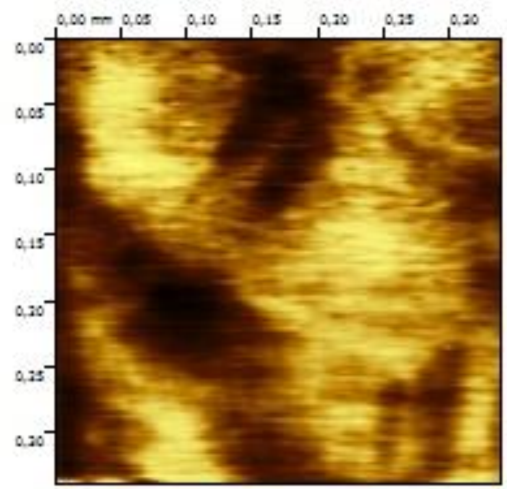
Tip
Diameter 300 μm
Aperture ~ 1 μm

Specimen
Scan 50 μm x 50 μm

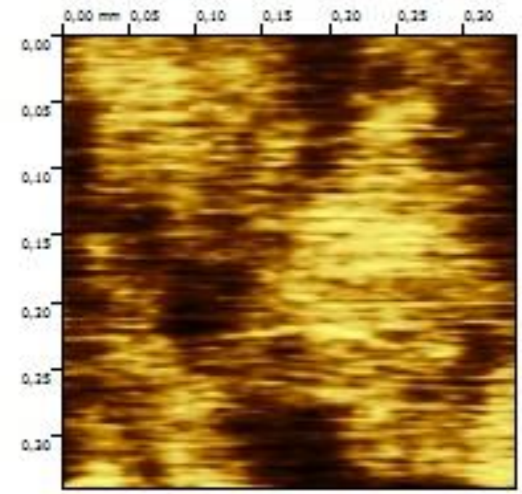
Benign cells



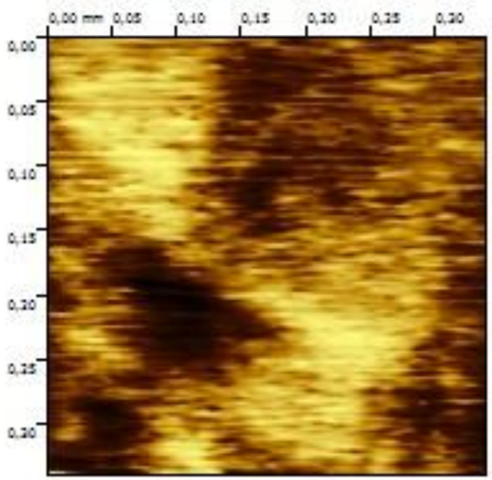
Topography



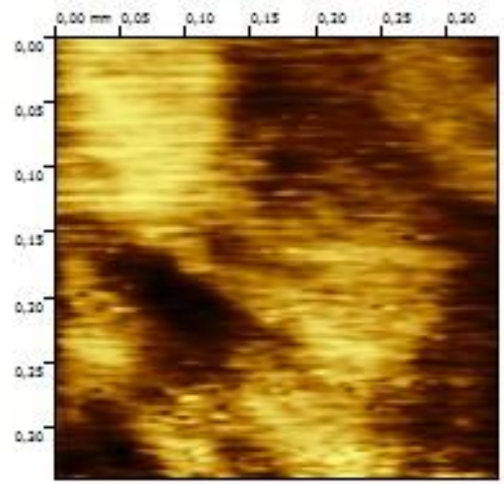
λ=8,60μ



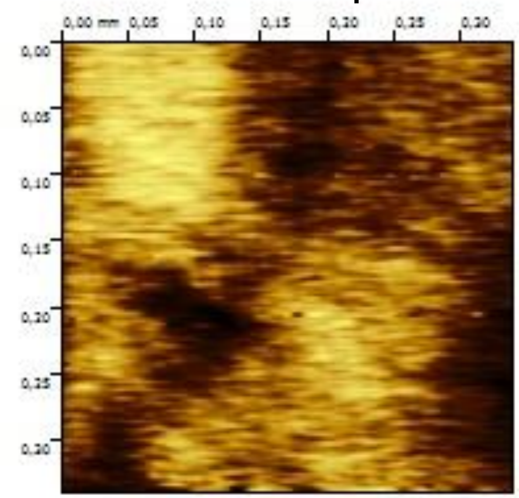
λ=8,05μ



λ=7,30μ

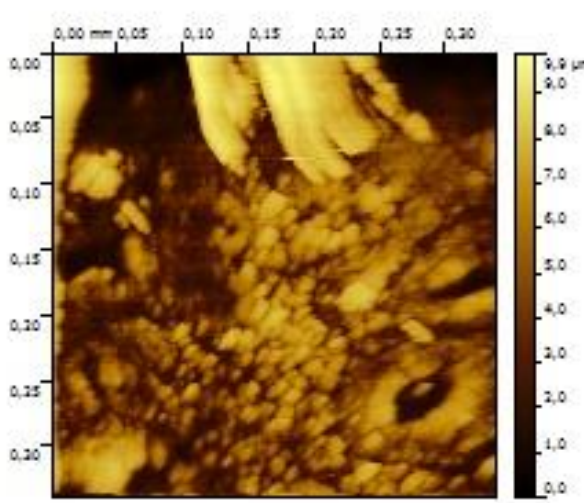


λ=7,00μ

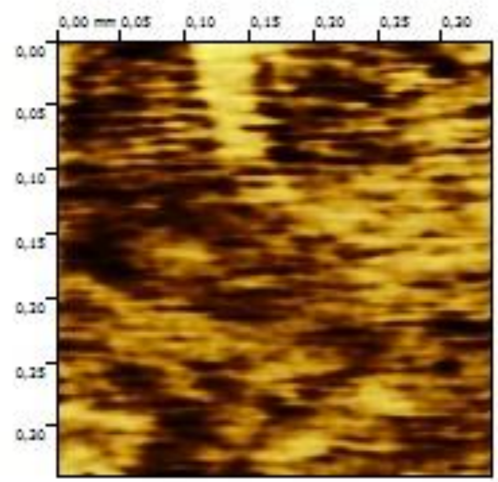


λ=6,50μ

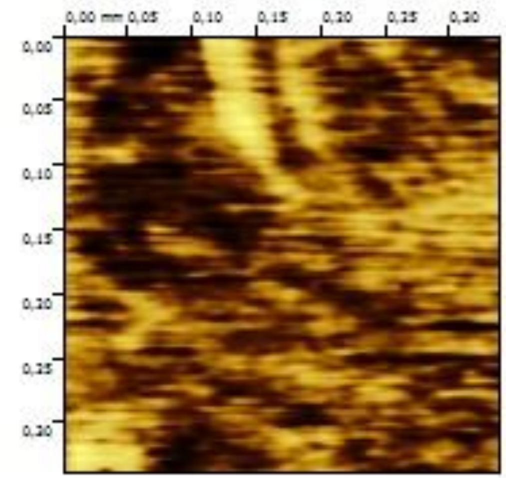
Cancer cells



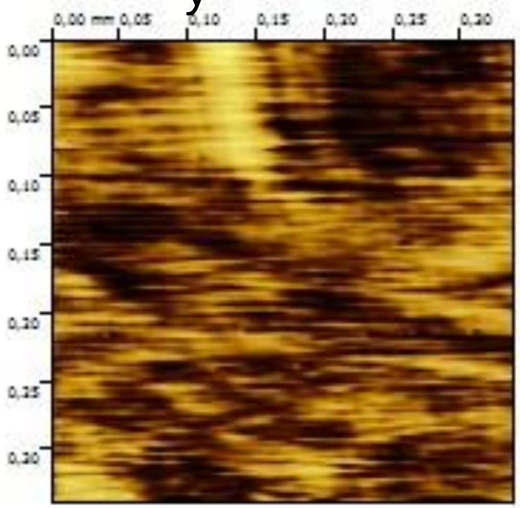
Topography



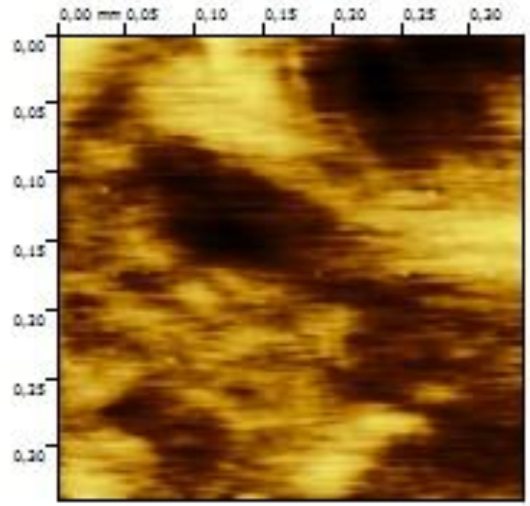
λ=8,60μ



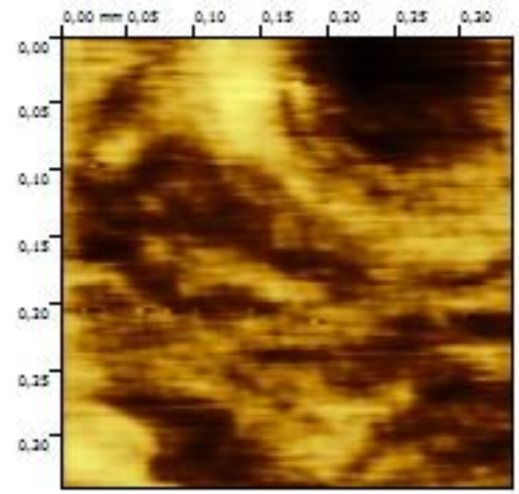
λ=8,05μ



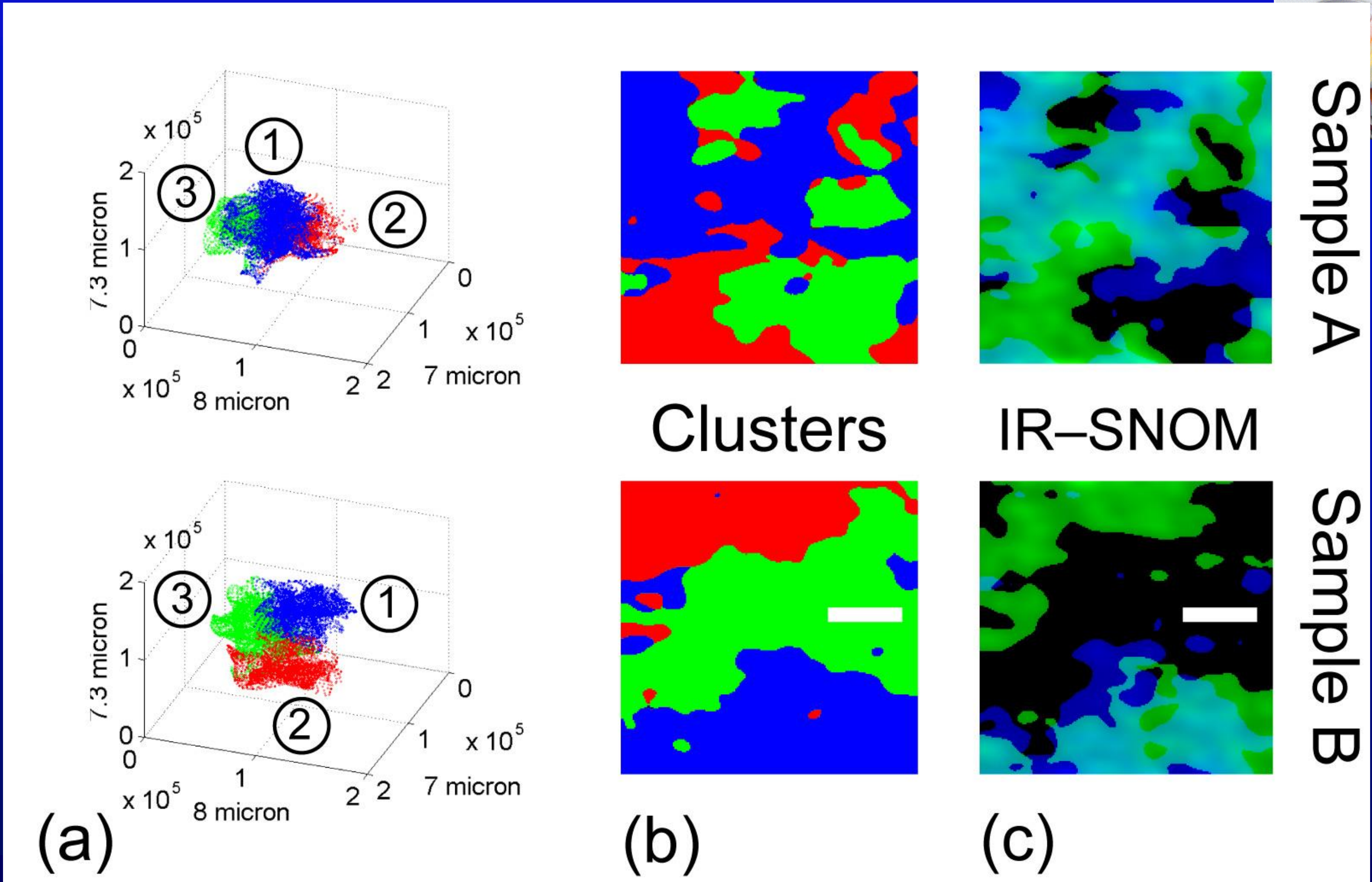
λ=7,30μ



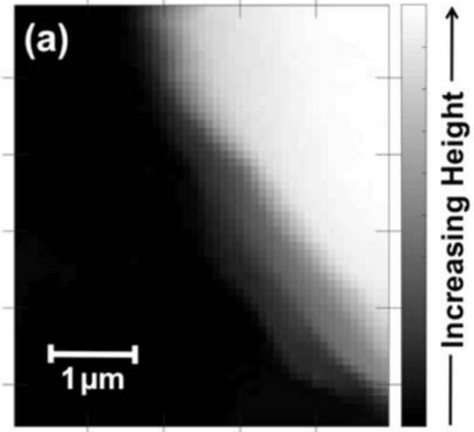
λ=7,00μ



λ=6,50μ

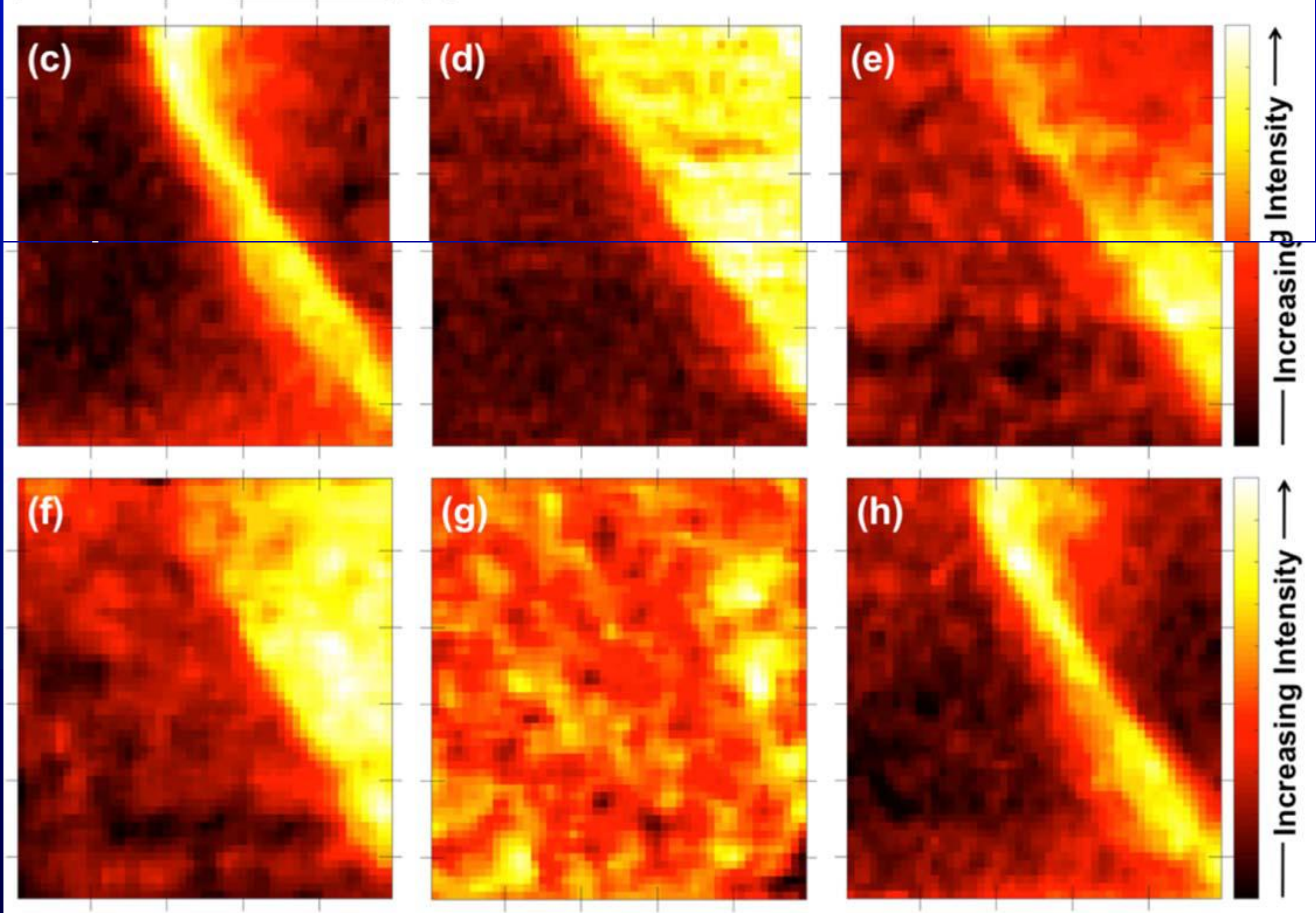


Smith AD, Siggel-King MRF, Holder GM, Cricenti A, Luce M, Harrison P, Martin DS, Surman M, Craig T, Barrett SD, Wolski A, Dunning DJ, Thompson NR, Saveliev Y, Pritchard DM, Varro A, Chattopadhyay S, Weightman P; Near-field optical microscopy with an infra-red free electron laser applied to cancer diagnosis; Applied Physics Letters; 102 (2013) 053701

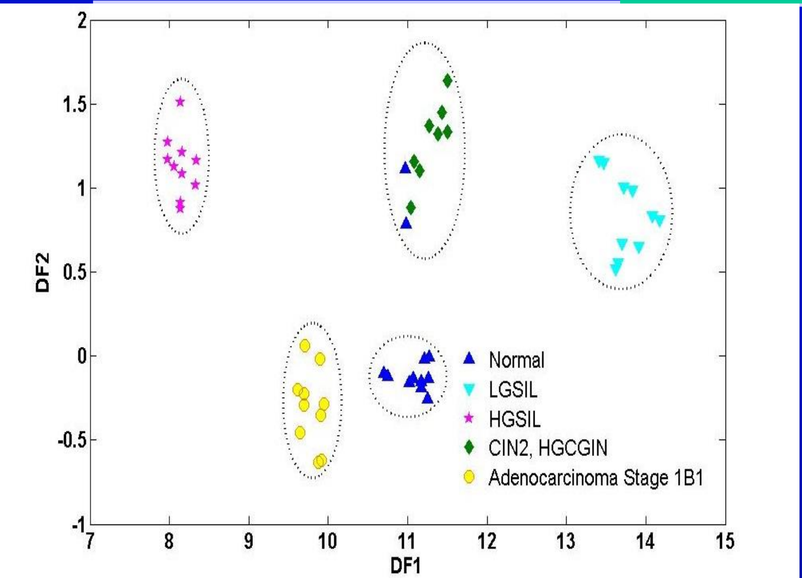


(b)

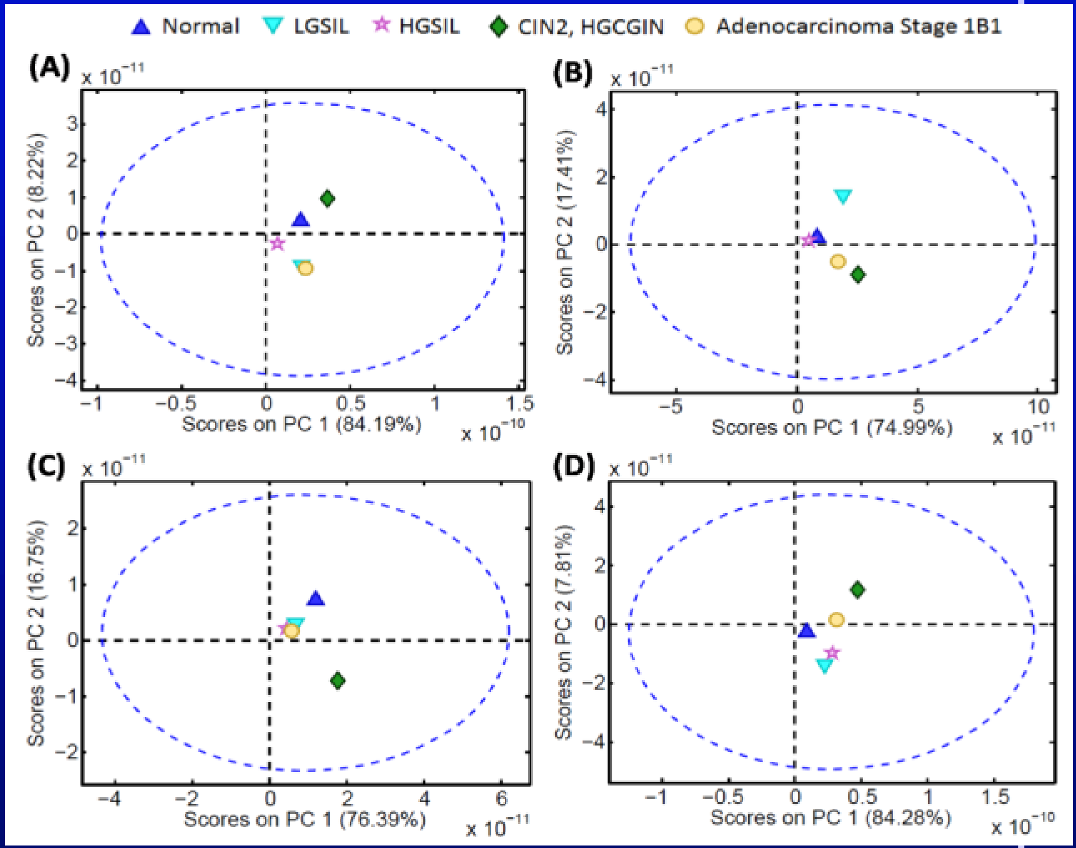
λ (µm)	Fig. 2 Pane	Biomarker
8.05	(c) and (h)	Relating to DNA
7.30	(d)	Control
6.50	(e)	Amide II (Largely ß-sheet)
6.06	(f)	Amide I (Largely α-helix)
5.71	(g)	Lipids



Cervical Cancer

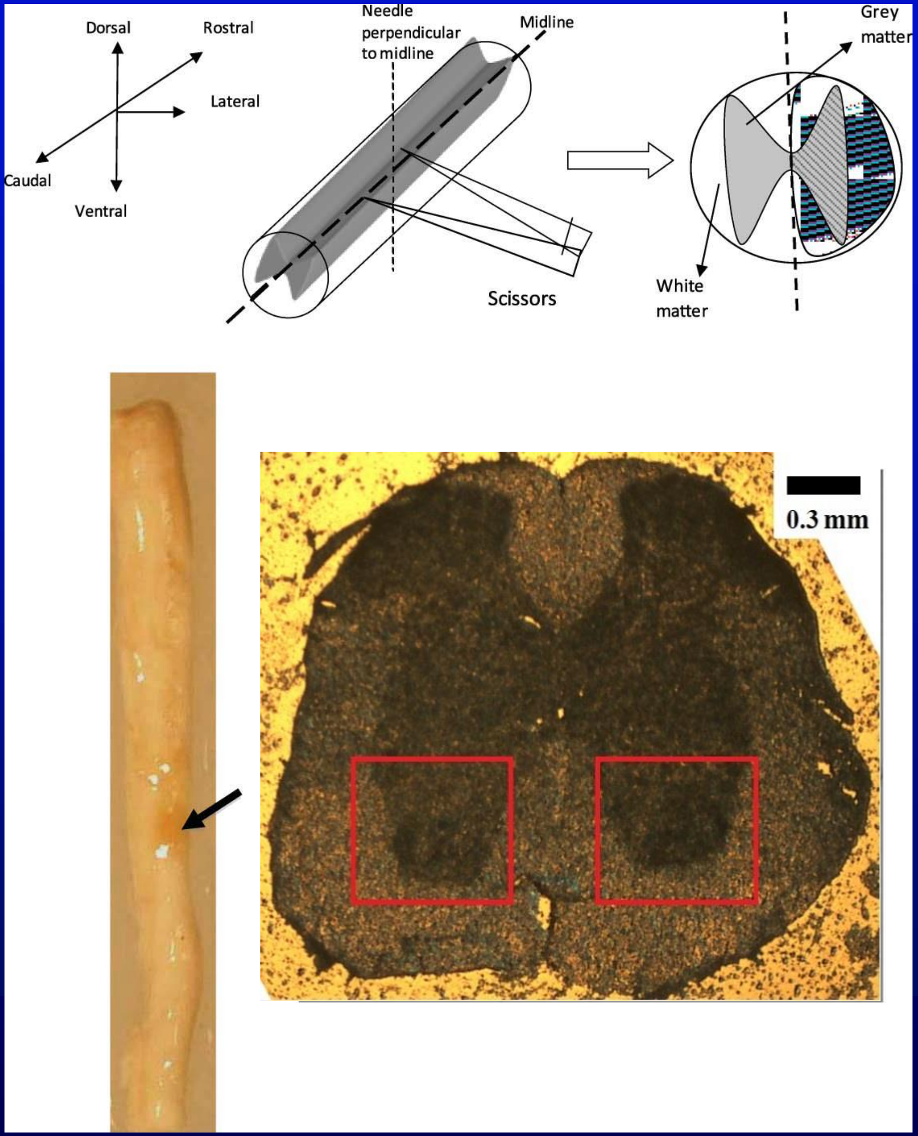


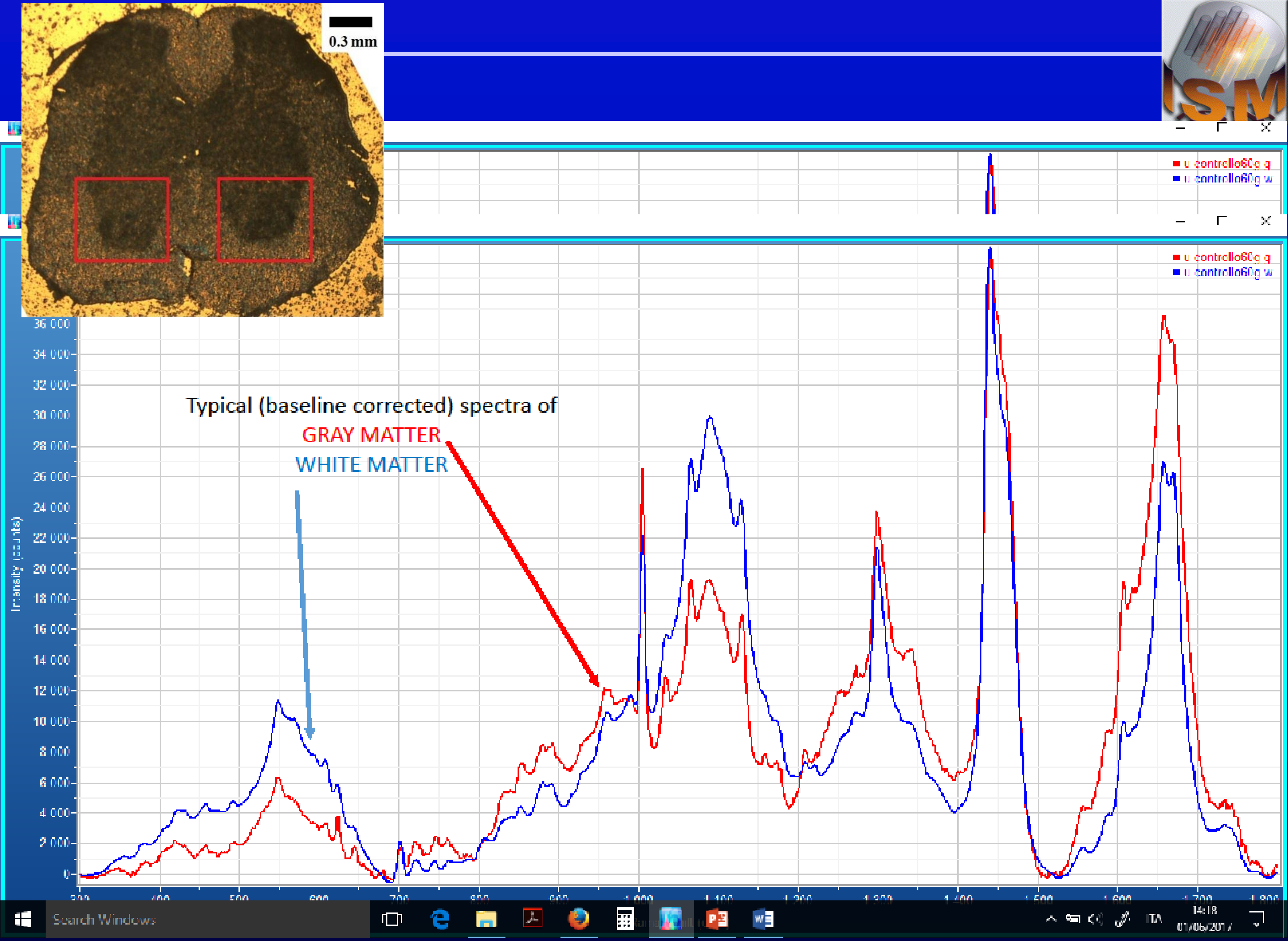
ATR-FTIR Spectroscopy



SNOM-IR-FEL: Principle Component Analysis

Halliwell Diane E, Morais Camilo LM, Lima Kássio MG, Trevisan Julio, Siggel-King Michele RF, Craig Tim, Ingham James, Martin David S, Heys Kelly A, Kyrgiou Maria, Mitra Anita, Paraskevaidis Evangelos, Theophilou Georgios, Martin-Hirsch Pierre L, Cricenti Antonio, Luce Marco, Weightman Peter, Martin Francis L; Nature Scientific Reports; 6 (2016) 29494.


Dorsal
Rostral
Lateral
Caudal
Ventral
Needle
perpendicular
to midline
Midline
Scissors
Grey
matter
White
matter
0.3 mm


0.3 mm
Typical (baseline corrected) spectra of
GRAY MATTER
WHITE MATTER
Intensity (counts)
36 000
34 000
32 000
30 000
28 000
26 000
24 000
22 000
20 000
18 000
16 000
14 000
12 000
10 000
8 000
6 000
4 000
2 000
0
Search Windows
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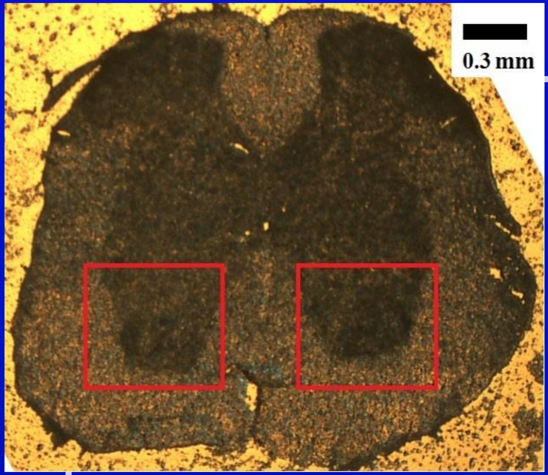
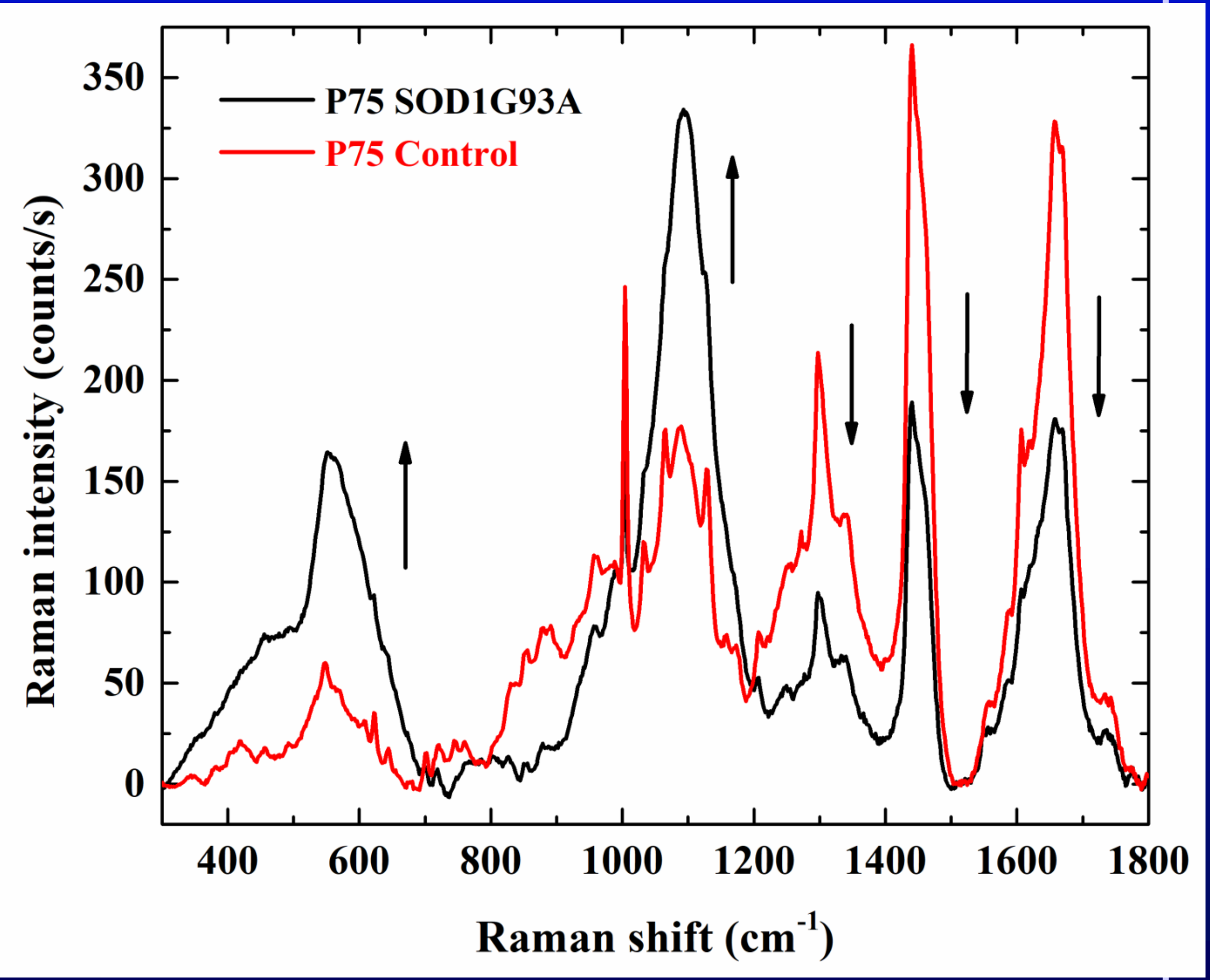
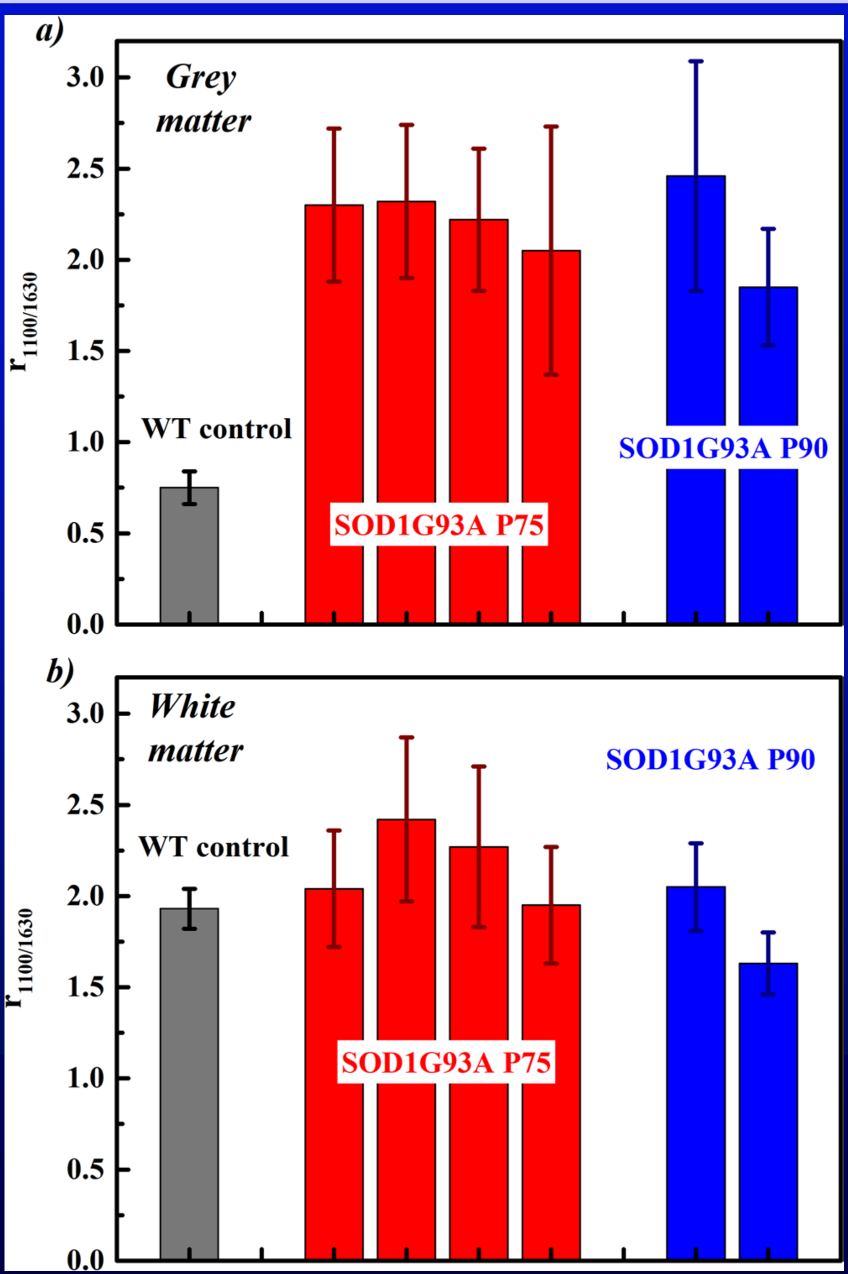
14:18



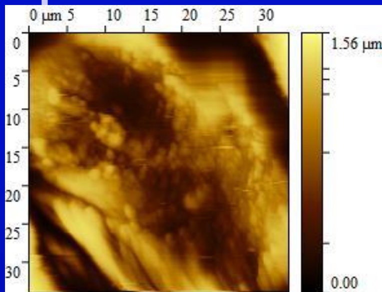


Desease detectable at 75 days from infection   Scientific Report 2018

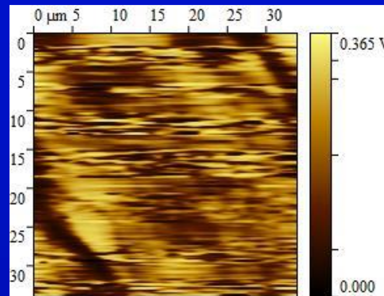


Histogram reporting the Raman signal intensity ratio between the spectral envelopes centred at 1100 cm−1 and at 1630 cm−1

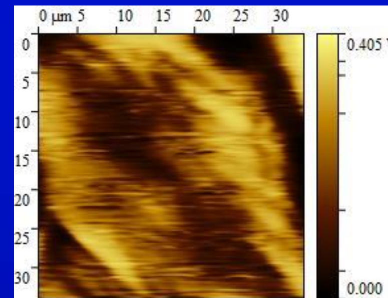
Osteoblast cells observed with SNOM coupled with 30 fs laser



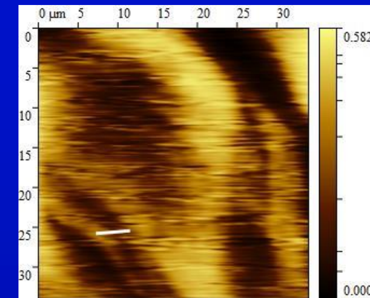
Topography



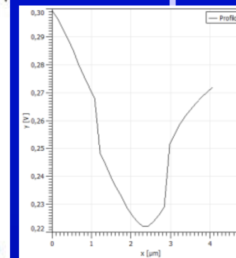
λ=2,5µ

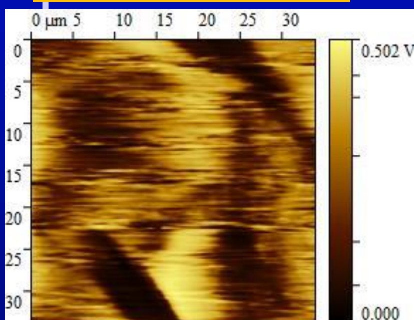


λ=3,3µ

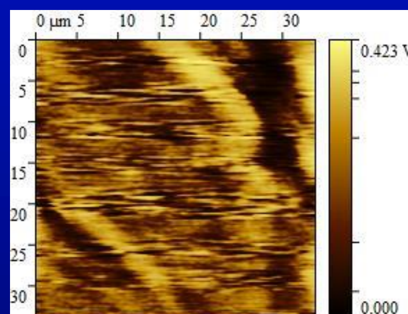


λ=5,7µ

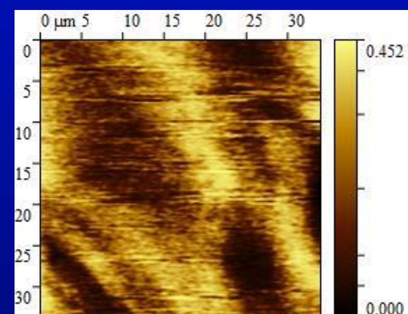




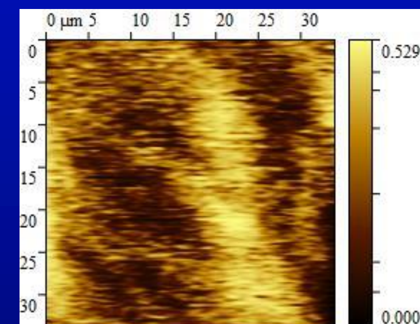
λ=6,1 µ



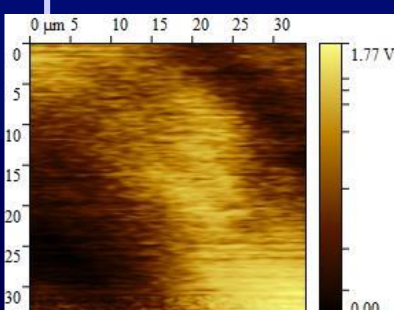
λ=6,45 µ



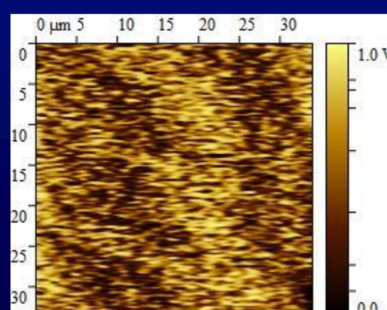
λ=7,0 µ



λ=7,5 µ



λ=8,05 µ



λ=8,6 µ

Conclusions and perspectives

IR, micro-Raman (TERS), AFM and SNOM (aperture, scattering,nano-IR) can be used for early diagnosis of cancer and deseases (ALS)

The advancement in technology give us a good possibility to be able to perform statistics for an early diagnosis of cancer

Collaborators

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ASTeC

David Dunning, Andy Smith, Mark Surman, Neil Thompson

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Andy Wolski, Swapan Chattopadhyay, Amy Schofield,

Rachel Williams (Eye and Vision Science, University of Liverpool)

Royal Liverpool and Broadgreen Hospital and University of Liverpool

Prof. Mark Pritchard, Head of Gastroenterology

Prof. Andrea Varro, Professor of Physiology