The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

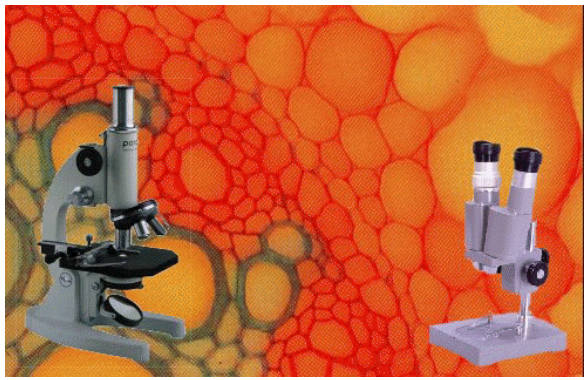
1936-43

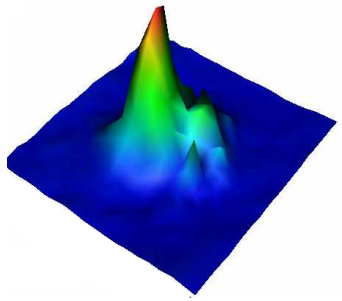
Advanced School on Synchrotron and Free Electron Laser Sources and their Multidisciplinary Applications

7 - 25 April 2008

Infrared spectrometry and microscopy in Biology and Biochemistry.

Paul Dumas
SOLEIL Synchrotron (France)

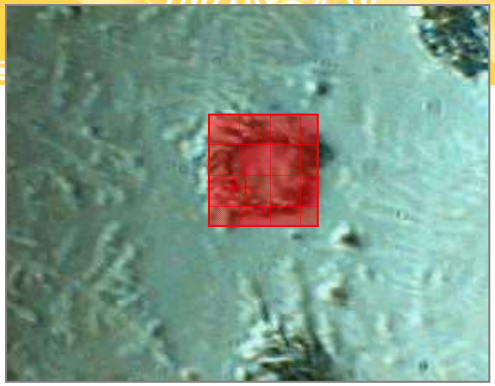


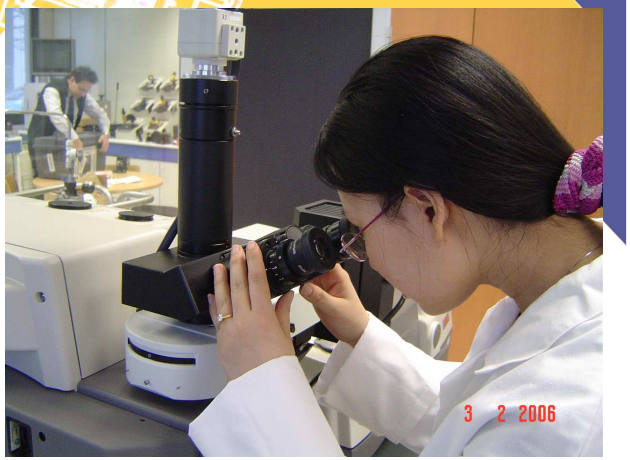


Infrared spectrometry and microscopy in Biology and Biochemistry

Paul Dumas
SOLEIL Synchrotron-France
paul.dumas@synchrotron-soleil.fr

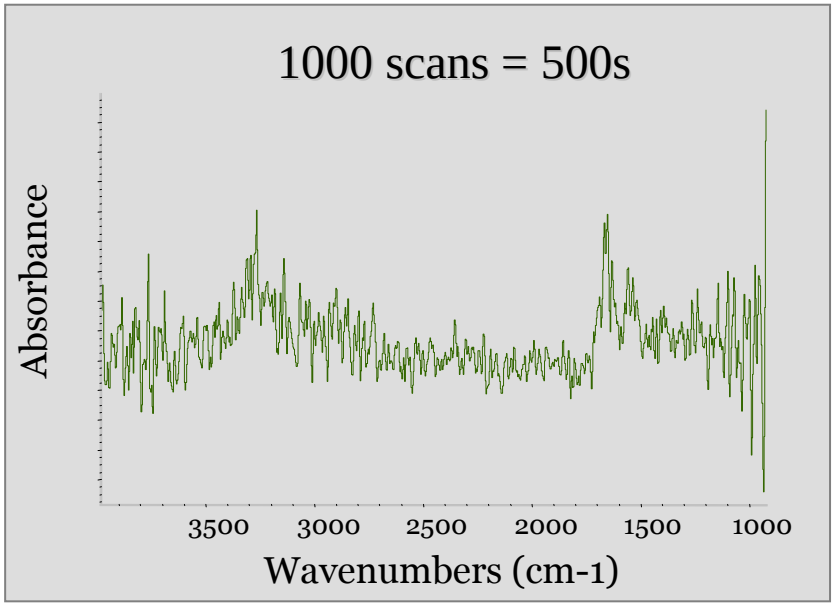
Exploiting the source brightness



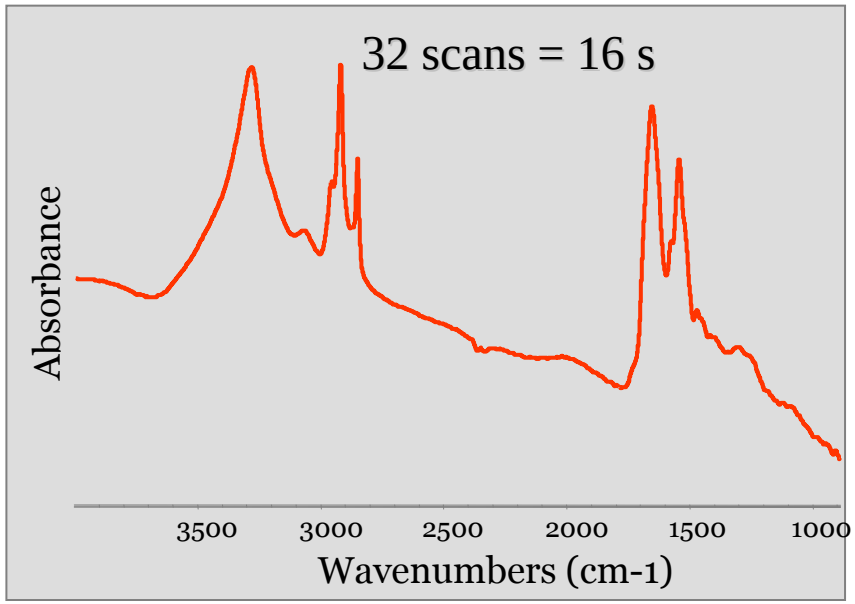


6X6 µm² aperture, confocal

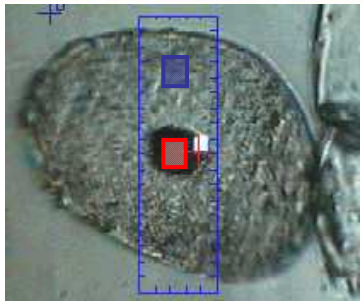
With the globar source

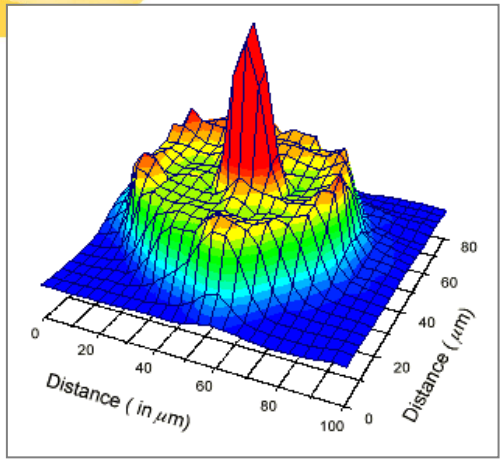


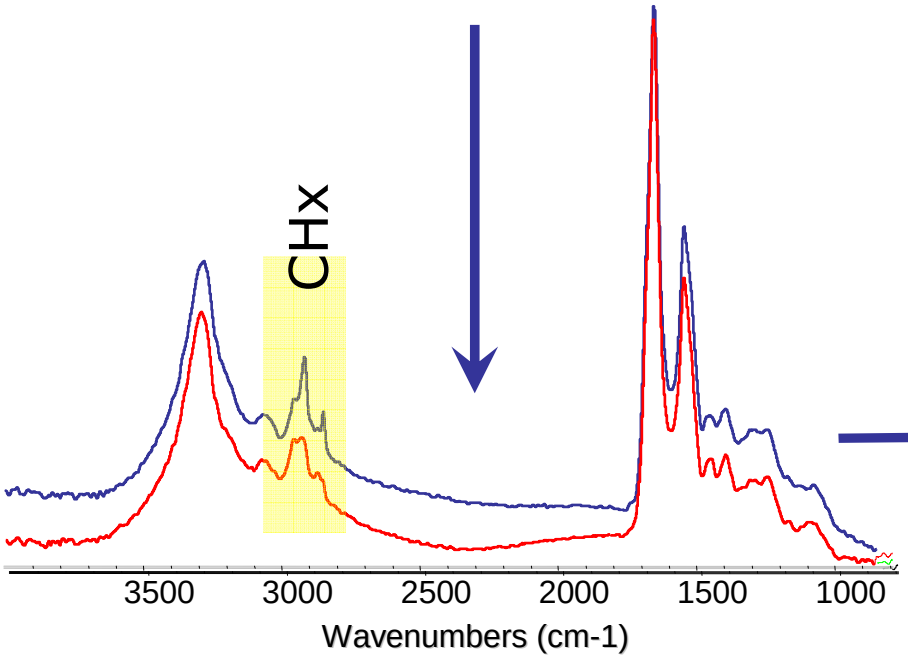
With the synchrotron source

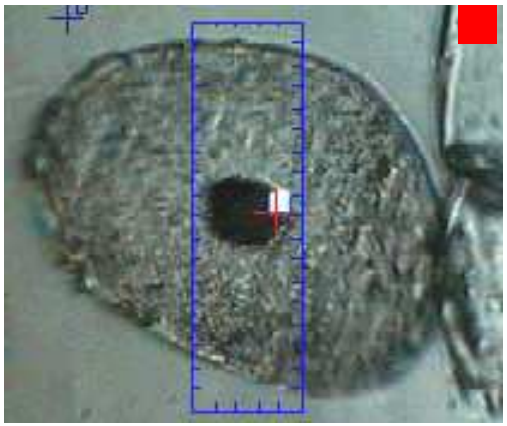


Generating a chemical image

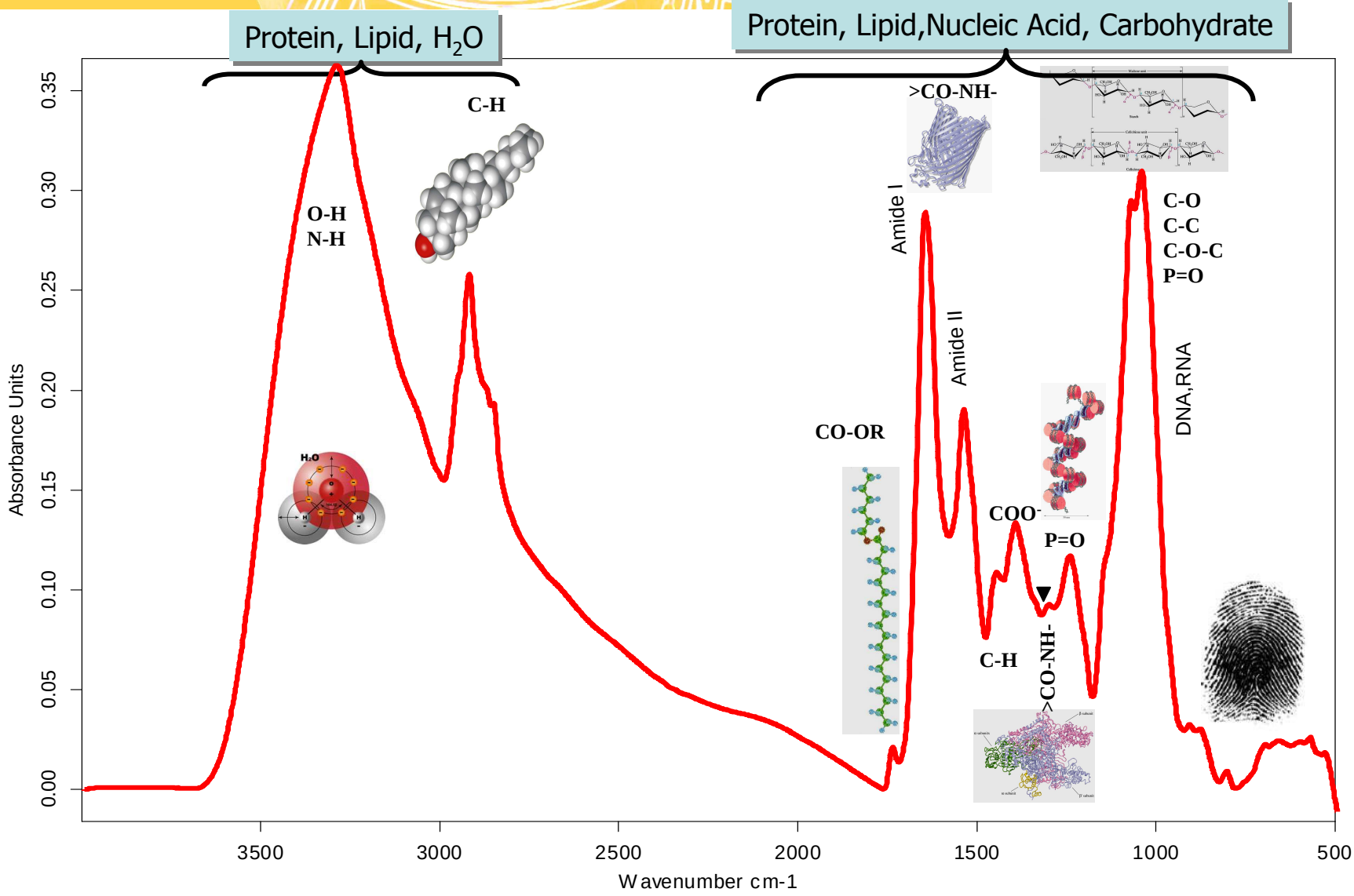






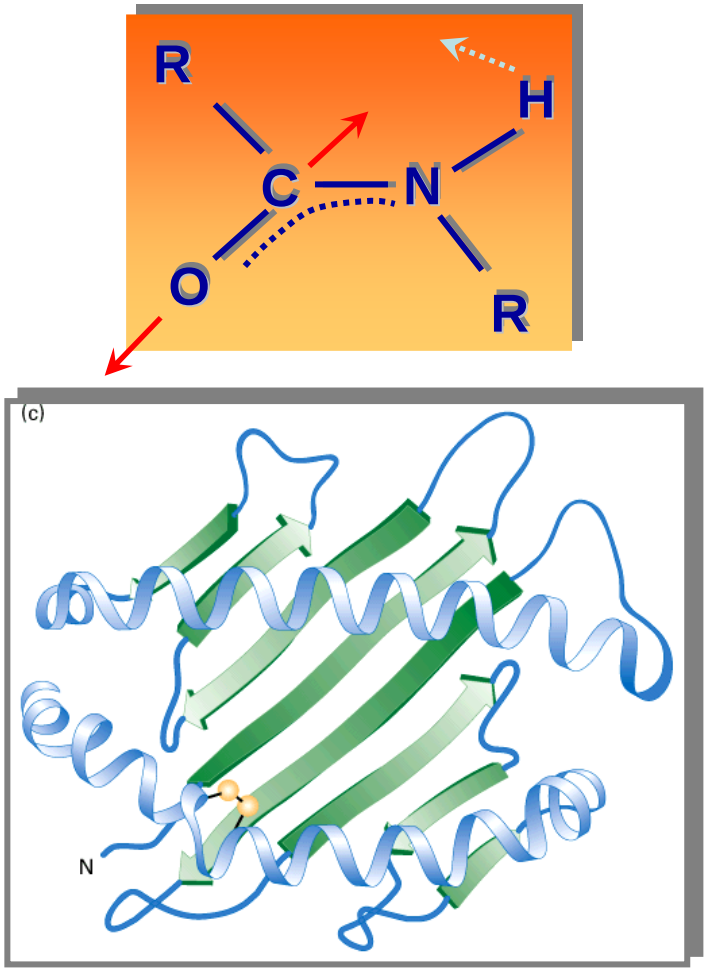


Exemple of IR spectrum : biological sample

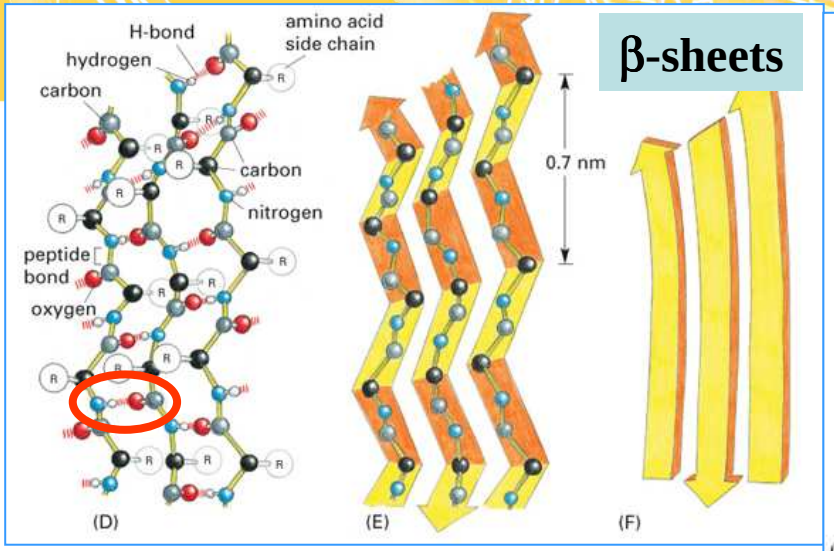


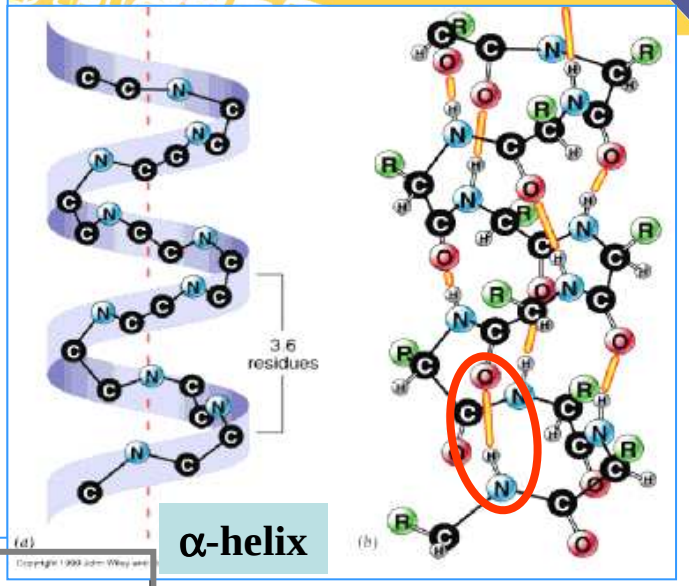
Structural informations?

Lineshape of Amide I allows to determine the relative secondary structure composition



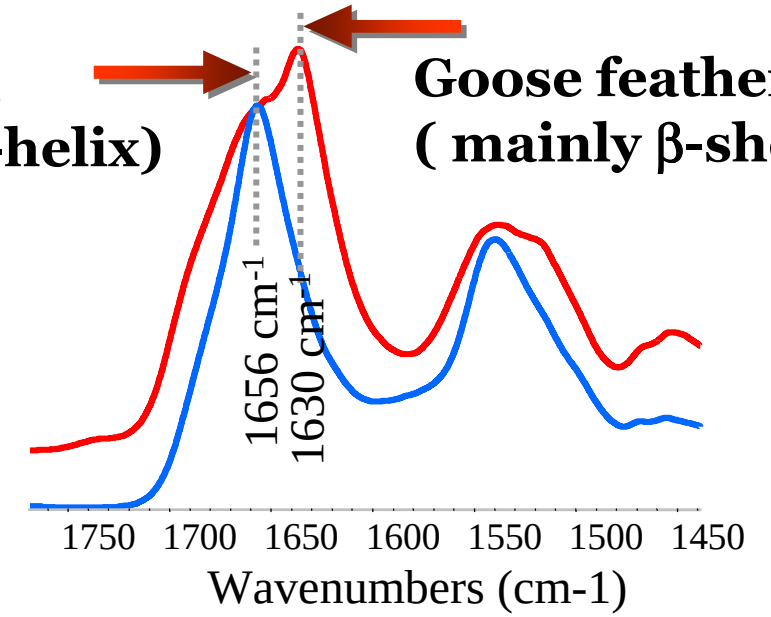
Structural informations?



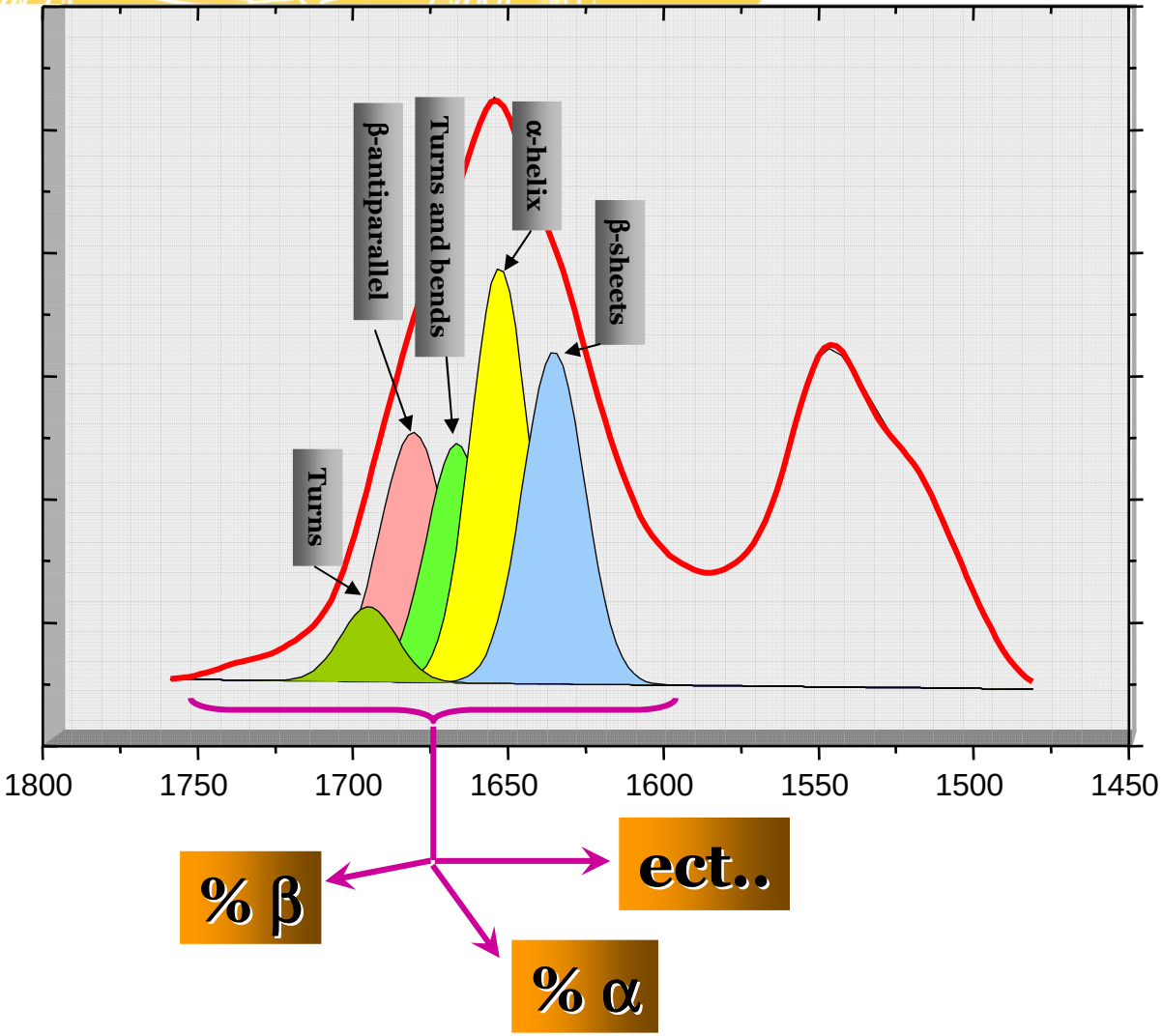


Myoglobin
(mainly α-helix)

Goose feather
(mainly β-sheets)



Semi-quantitative analysis using deconvolution

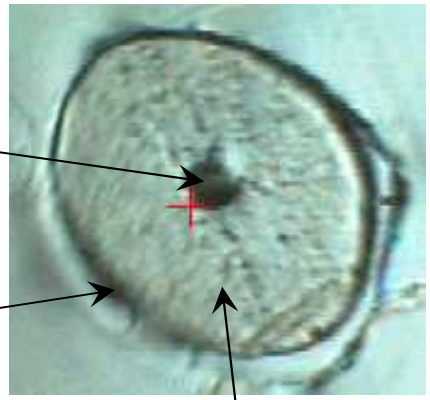


Synchrotron IR microscopy of human tissues

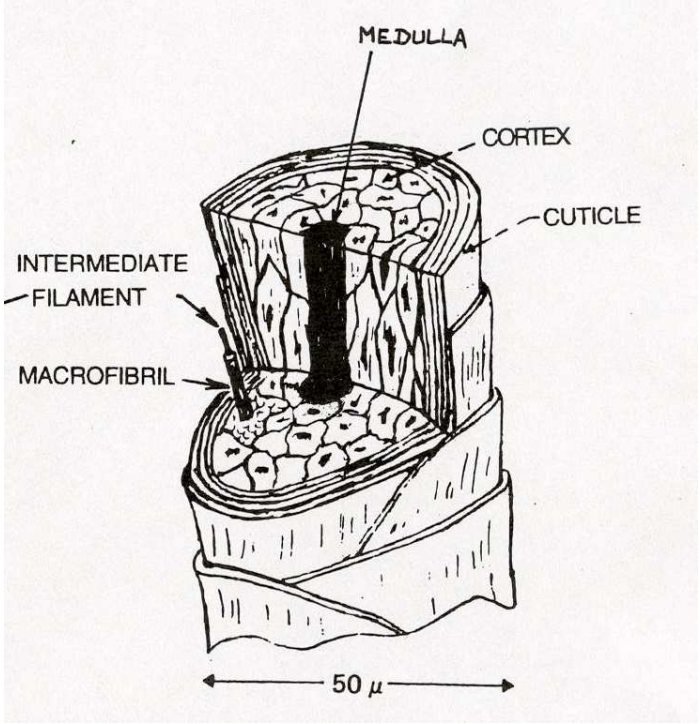
Studying hair with synchrotron IR microscopy

Medulla
(about 10 µm)

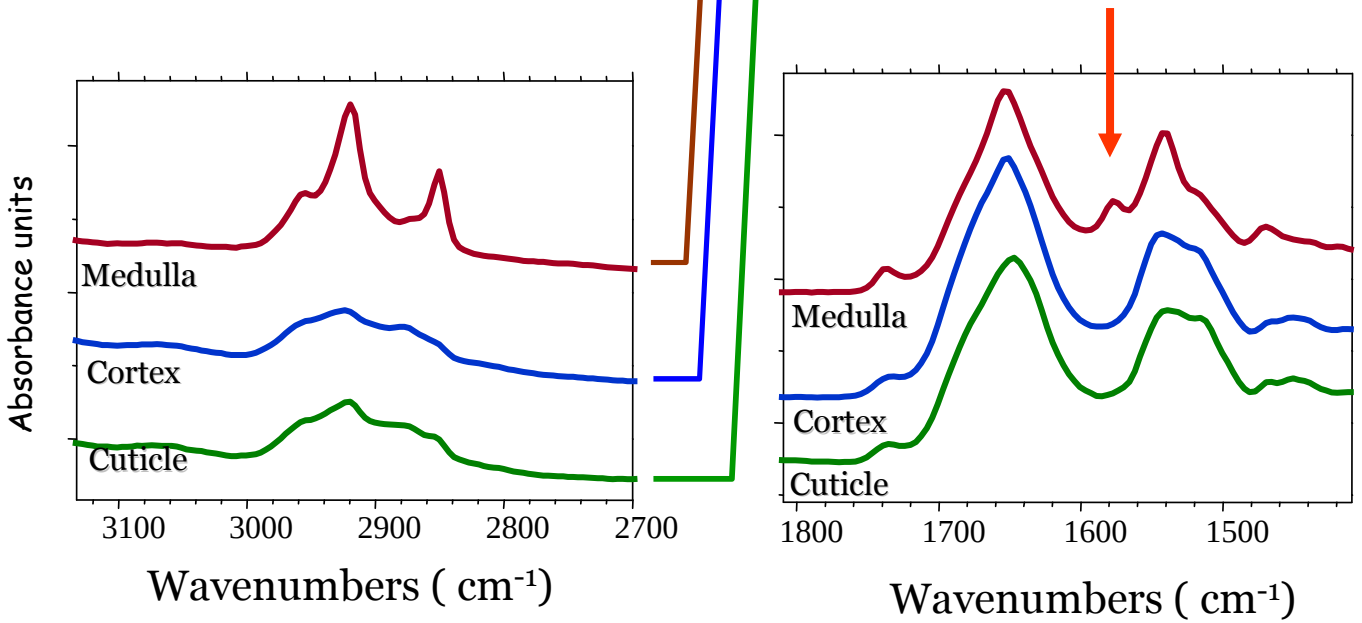
Cuticle
(about 5 µm)



Cortex
(about 40-50 µm)



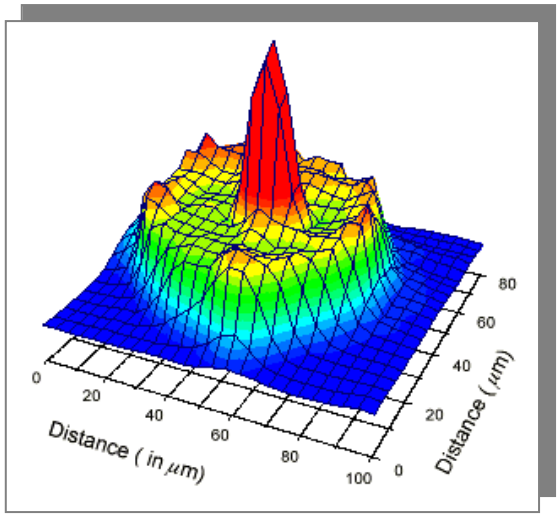
Apt. Size= 6x6 µm²



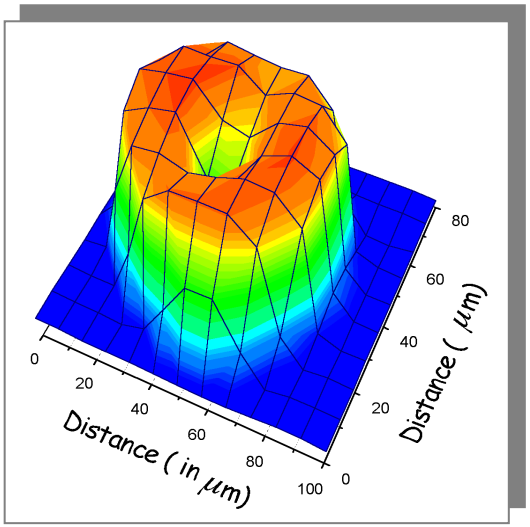
L. Kreplak, et al. … Int. J of Cometics **23** *1-6* (2001) 369-374

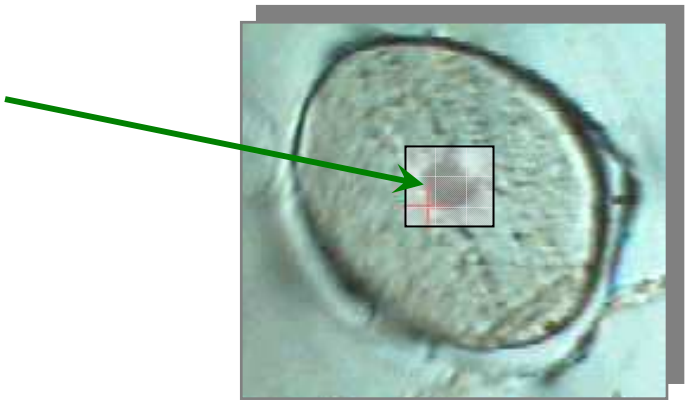
Intern.J. of Cosmetics Science. **23** *1-6* (2001) 369-374

Lipid profile

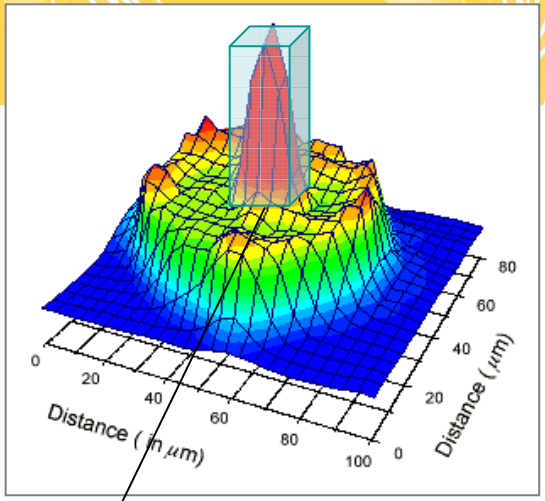


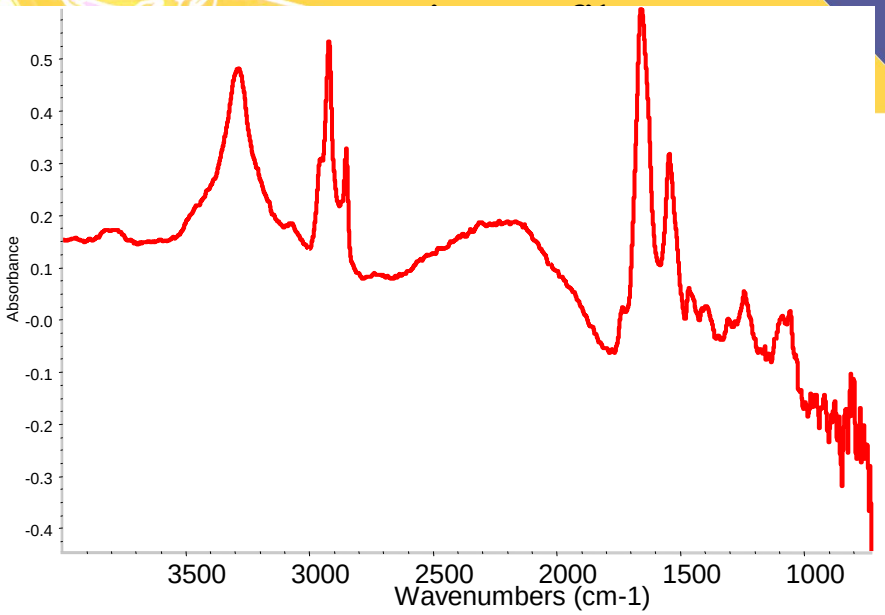
Protein profile



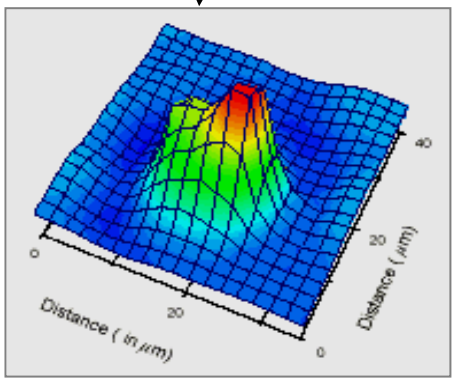


Lipid profile

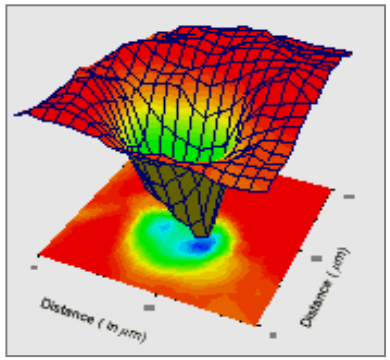




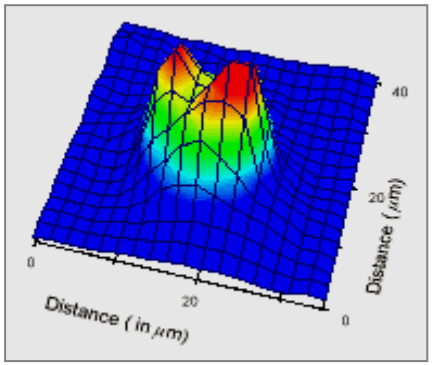
3x3 µm^2 aperture
1 µm step



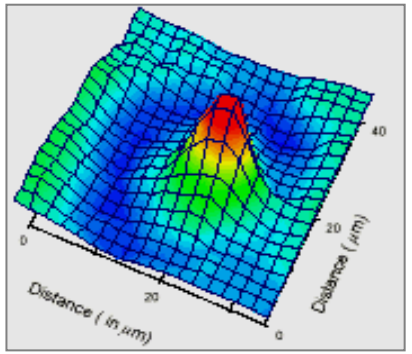
CH_2



Amide I



C=0



carboxylate

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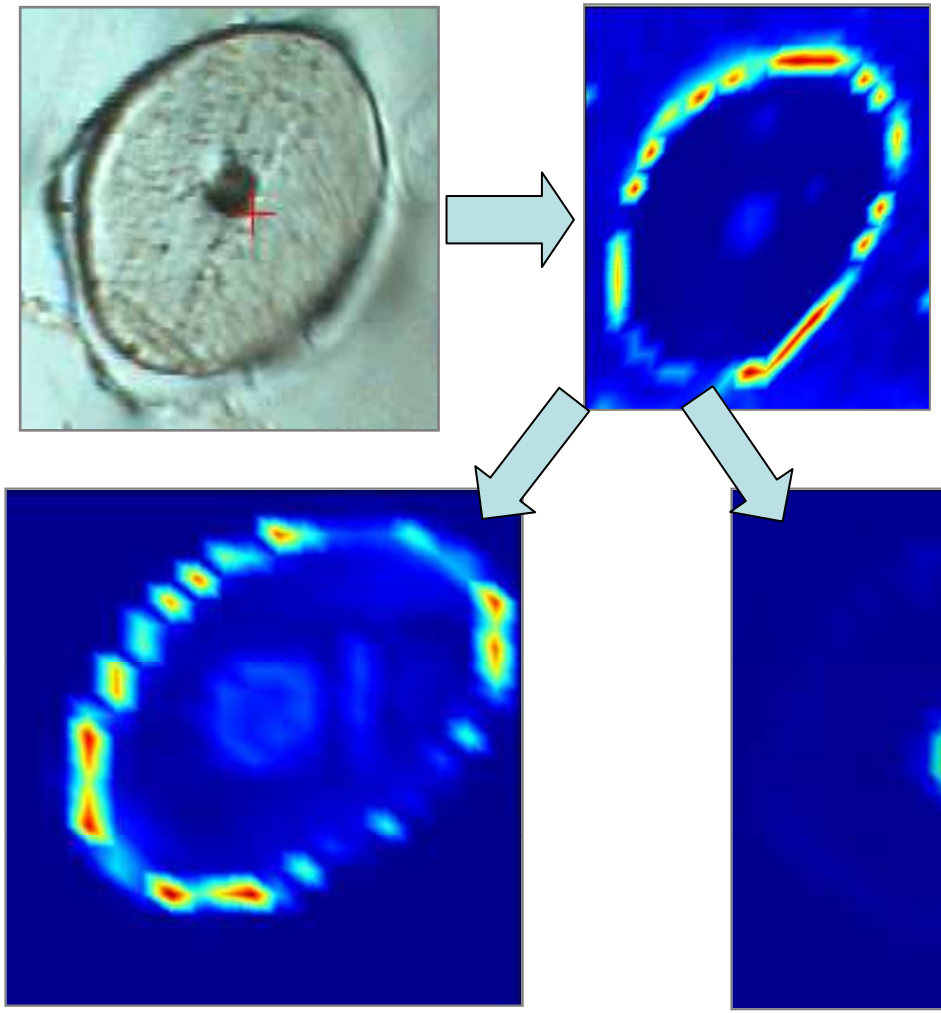
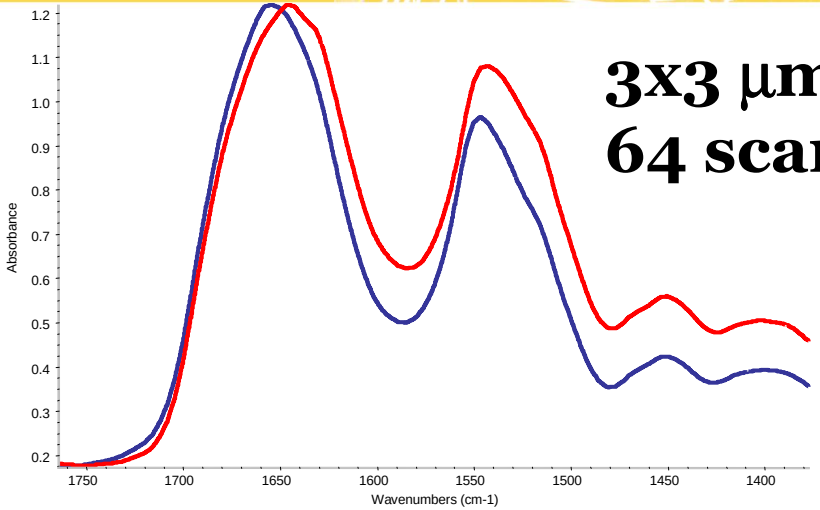
Beside chemical imaging: multivariate…



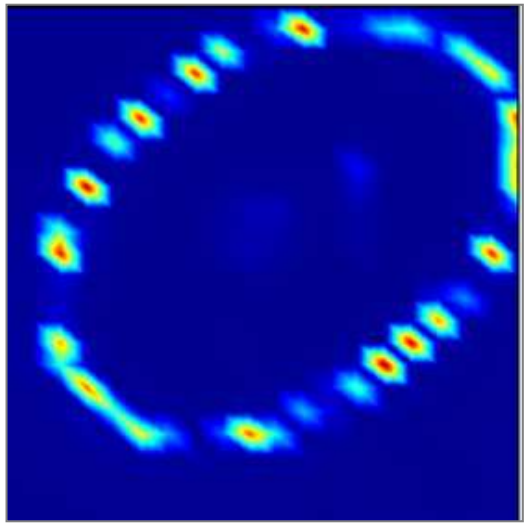
Two types of lipids identified across hair section

Two types of lipids identified by HCA (Hierarchical Cluster Analysis)

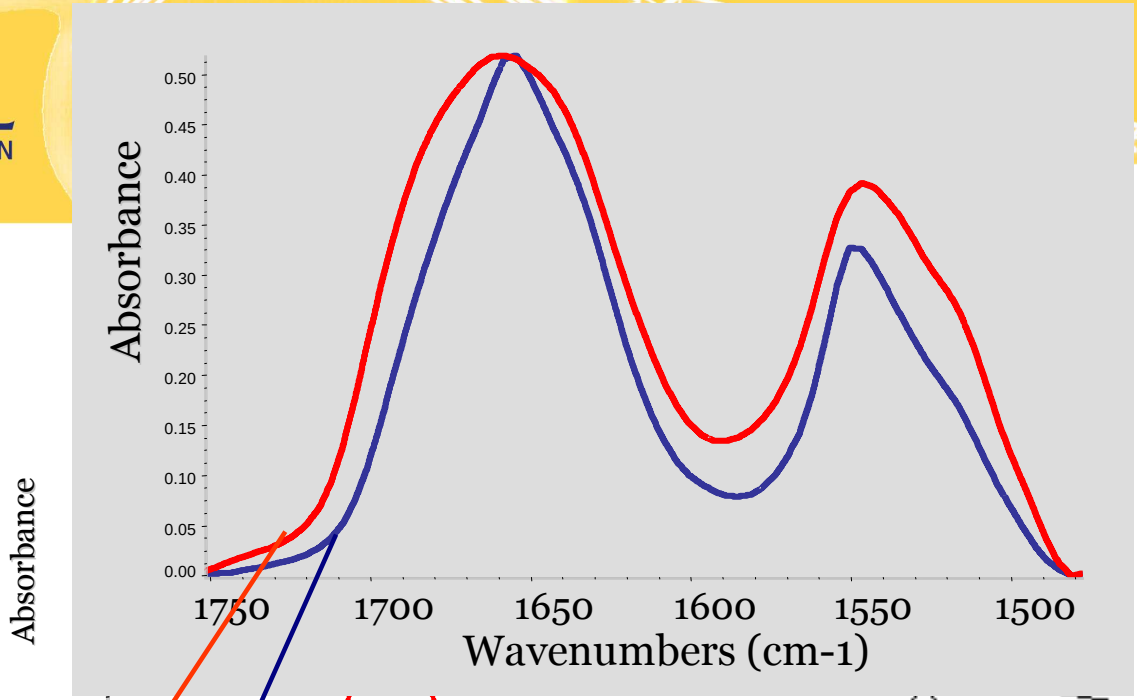
Secondary structure of proteins



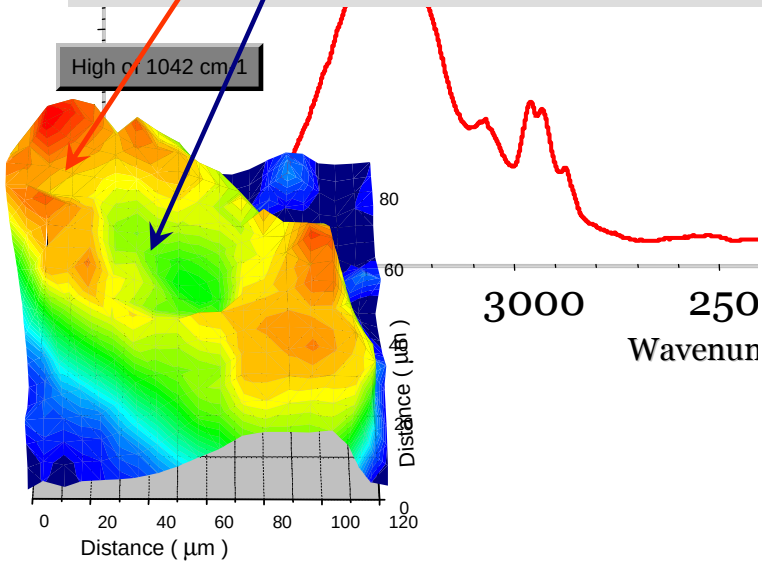
3x3 μm²
64 scans

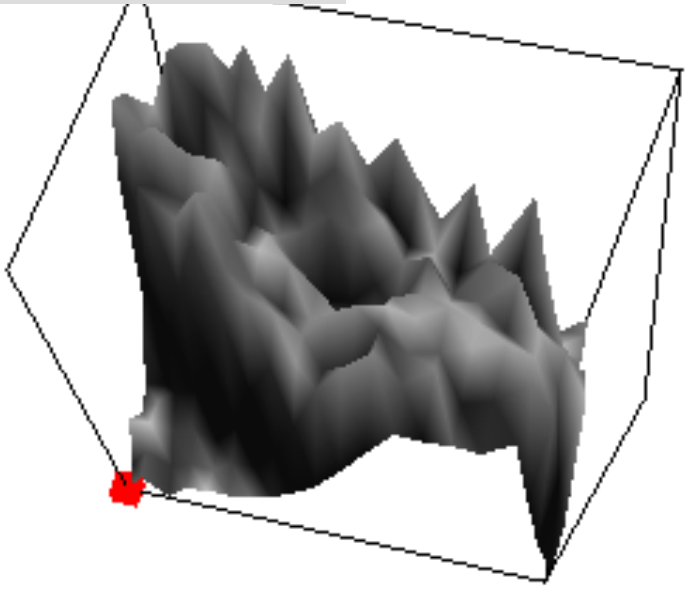


Higher concentration in β Sheets in the cuticle.

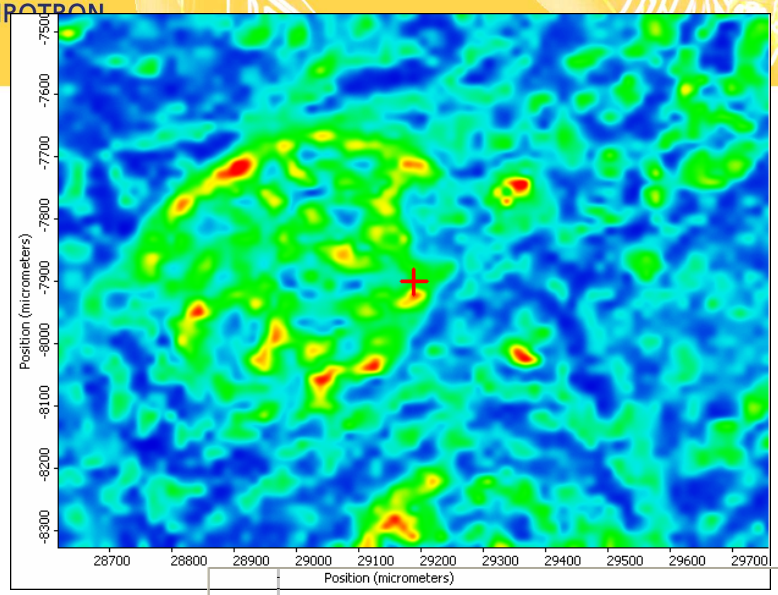


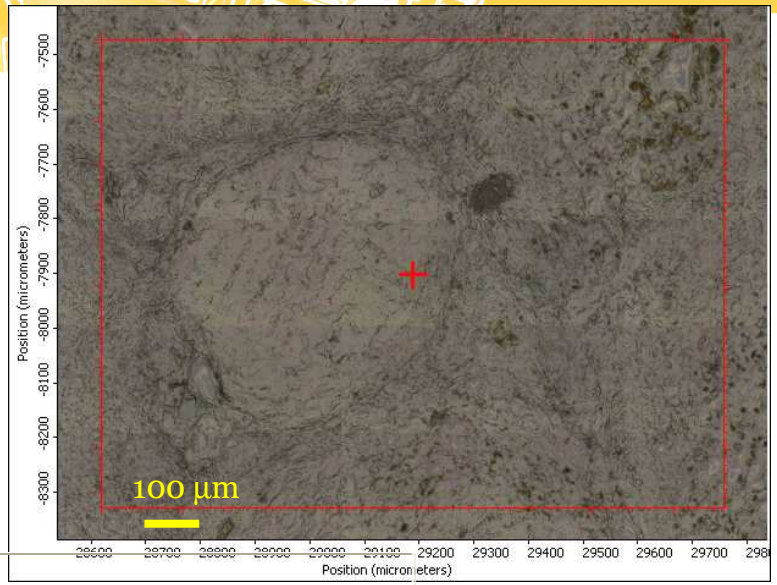
νS=O
sulphonate

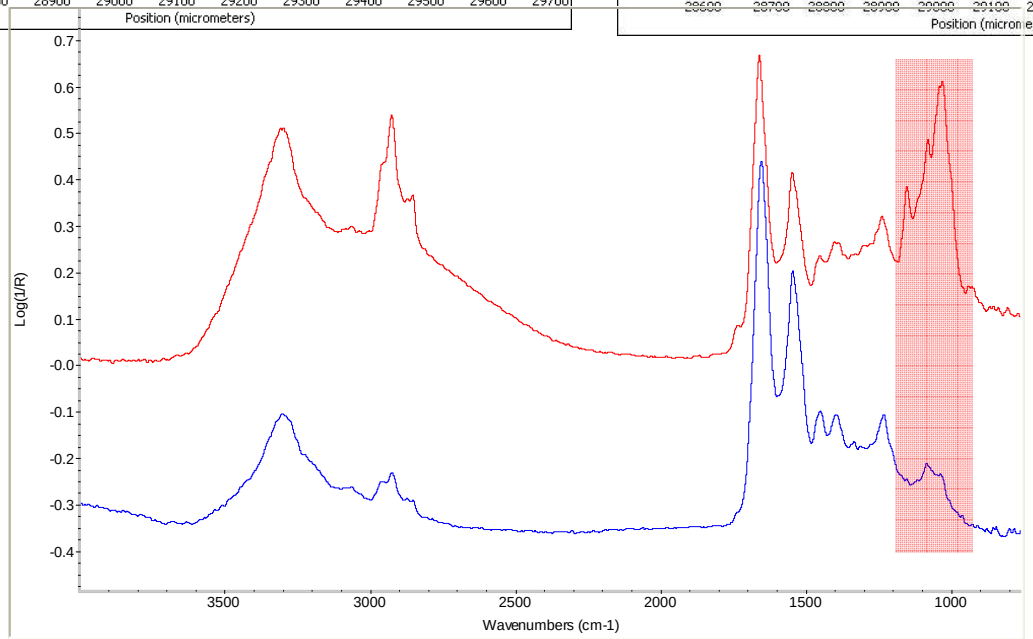


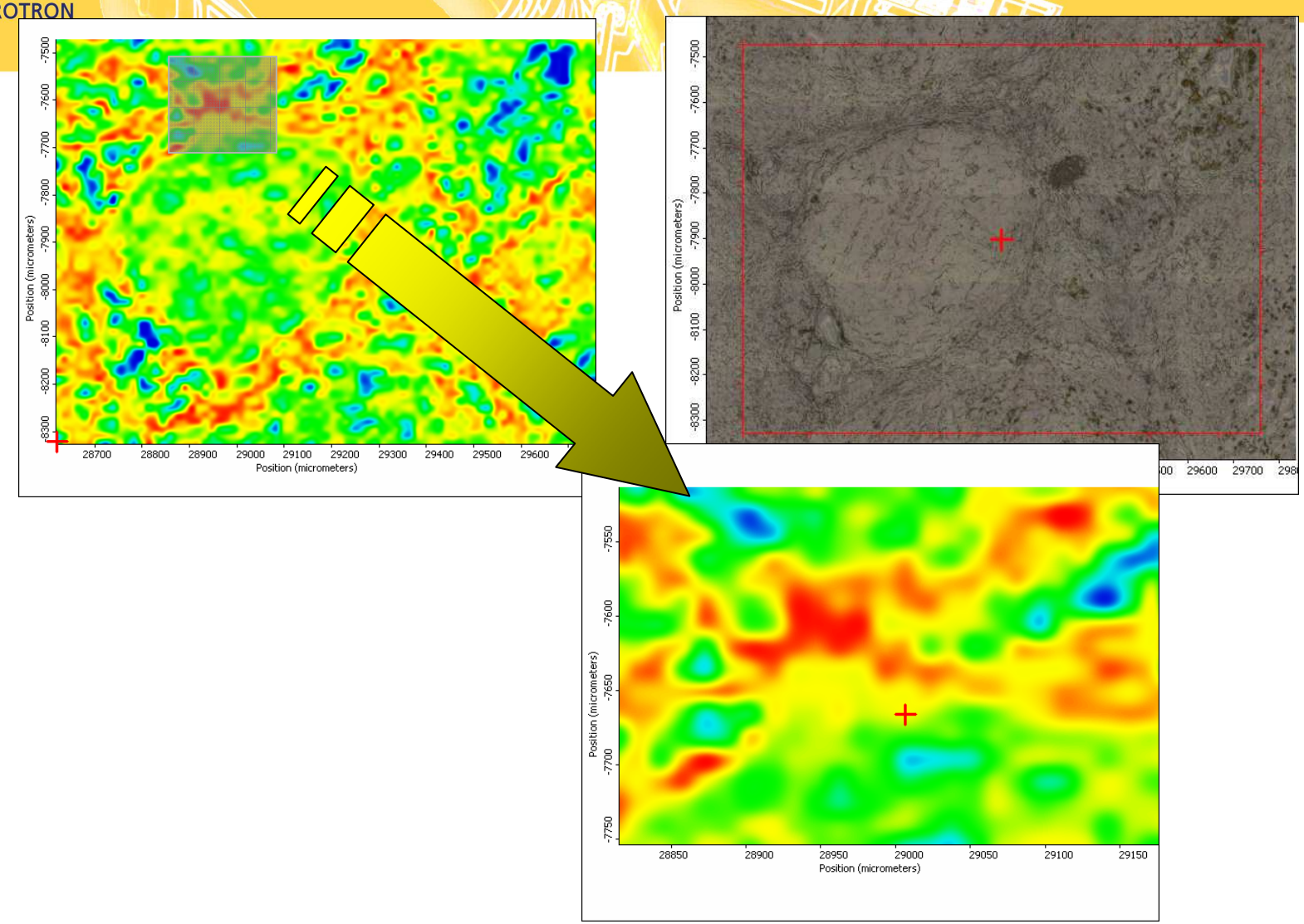


Cancer and cirrhosis (20x20 microns)



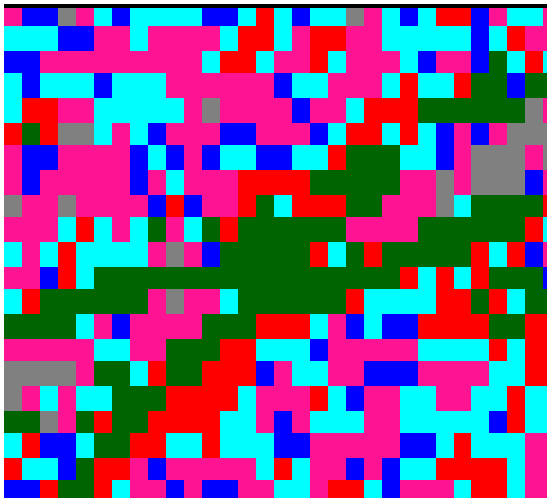


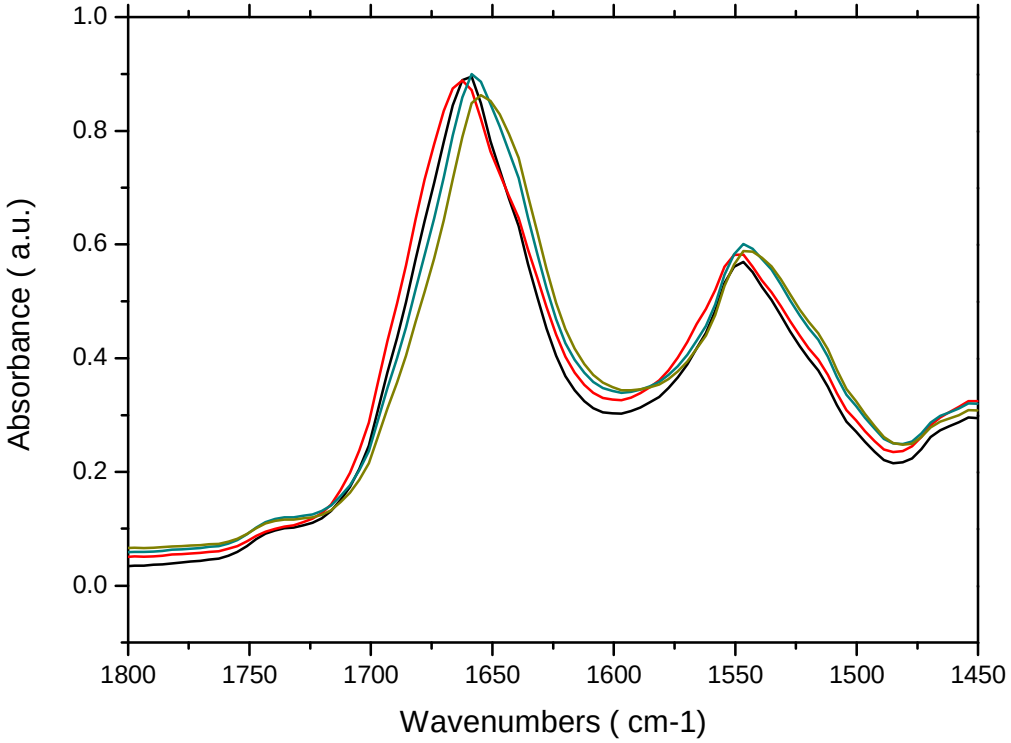



Position (micrometers)
-7500
-7600
-7700
-7800
-7900
-8000
-8100
-8200
-8300
28700
28800
28900
29000
29100
29200
29300
29400
29500
29600
Position (micrometers)
-7500
-7600
-7700
-7800
-7900
-8000
-8100
-8200
-8300
29600
29700
Position (micrometers)
-7550
-7600
-7650
-7700
-7750
28850
28900
28950
29000
29050
29100
29150
Position (micrometers)

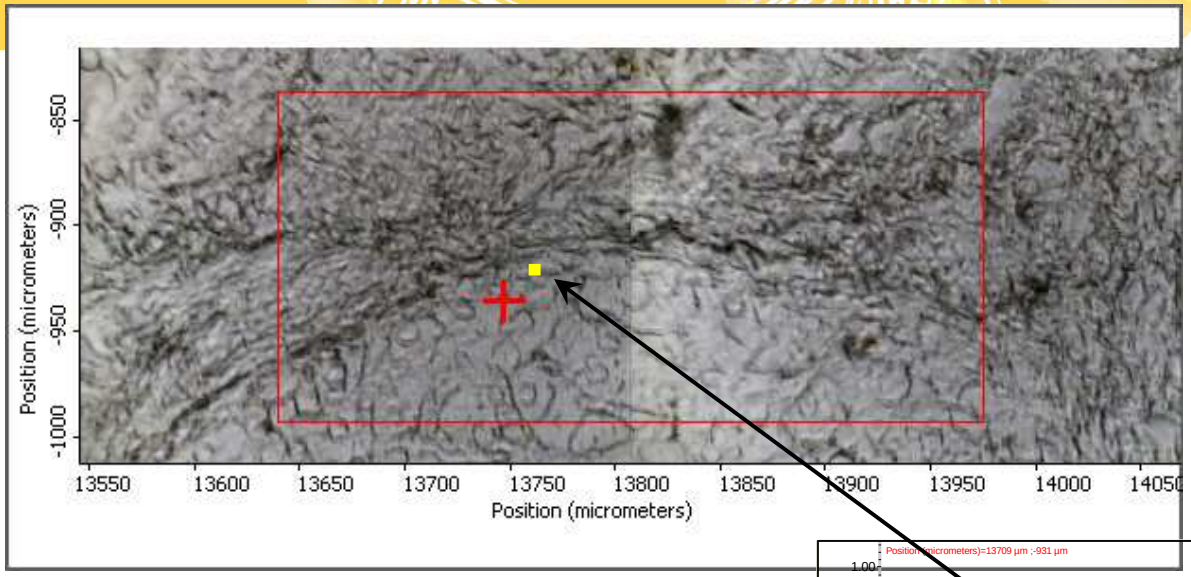
HCA= Cluster hierarchique

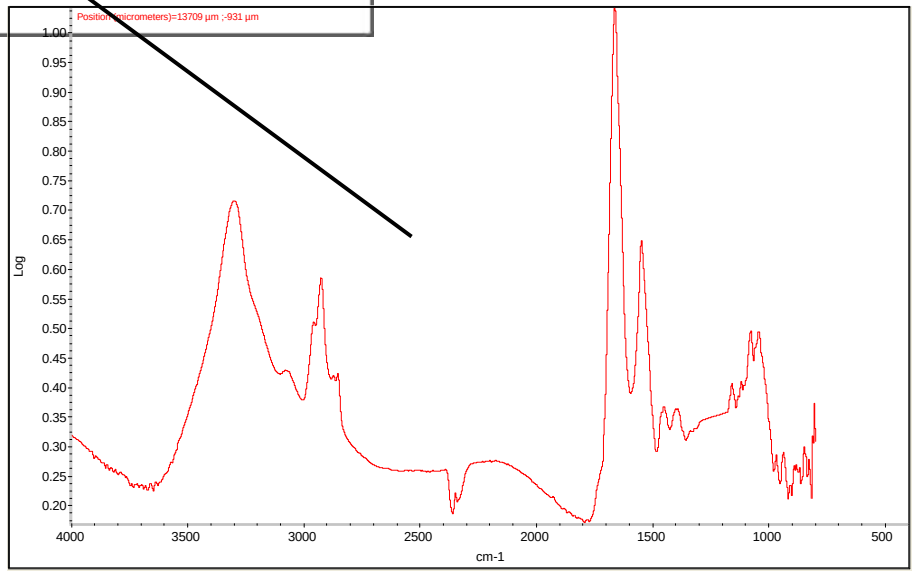
Little to learn at the interface

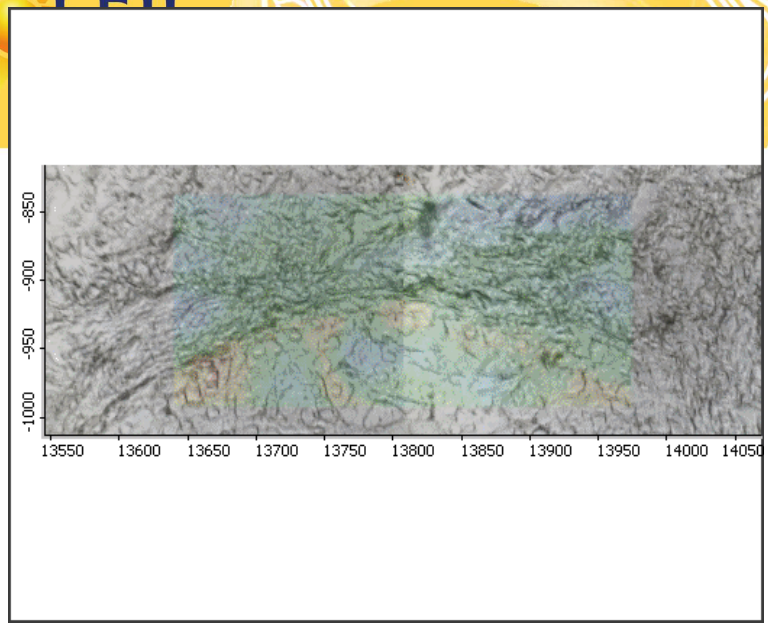


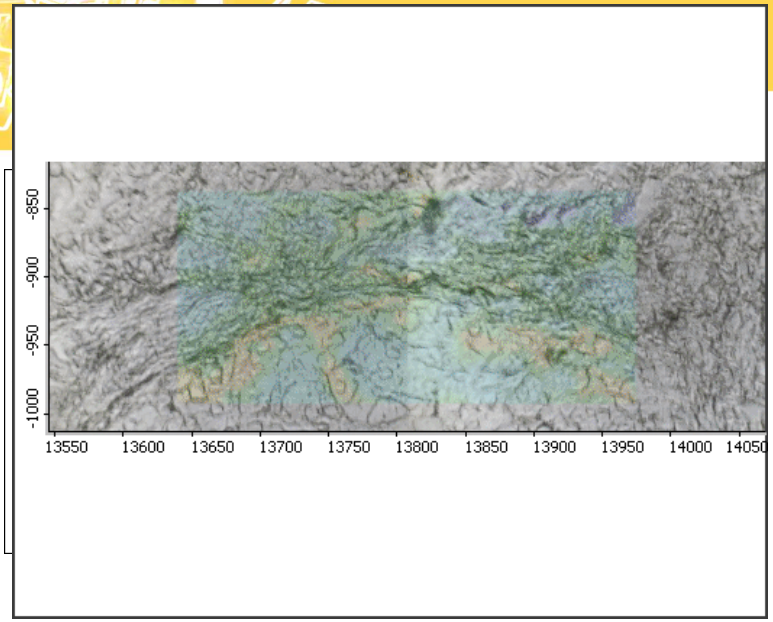


Synchrotron study with a 4x4 µm²

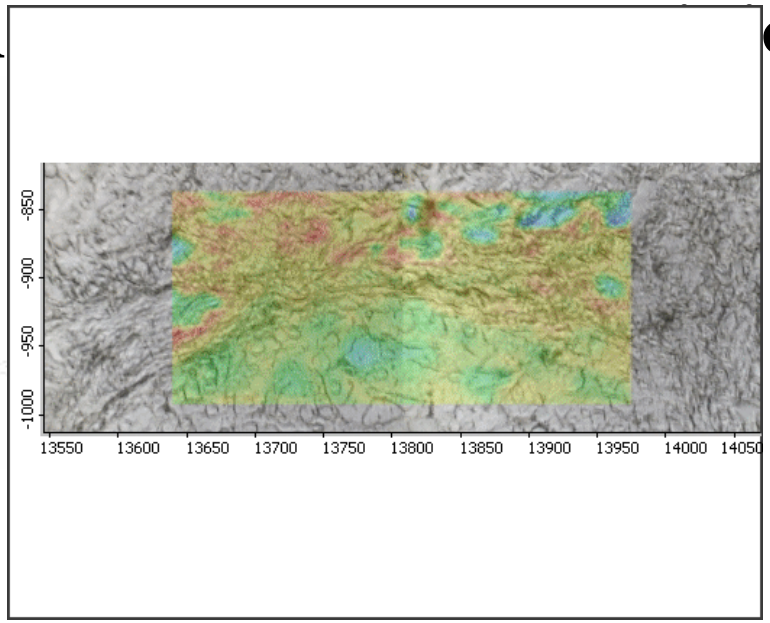








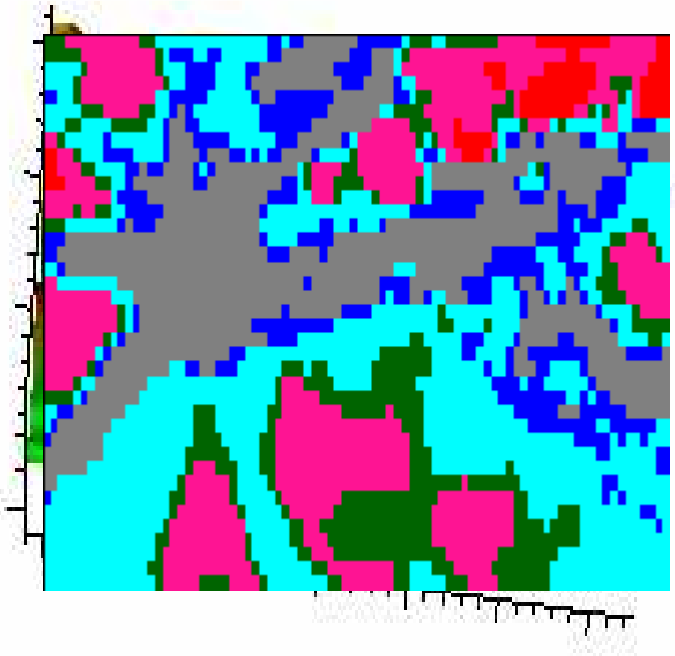
DNA im ds Image

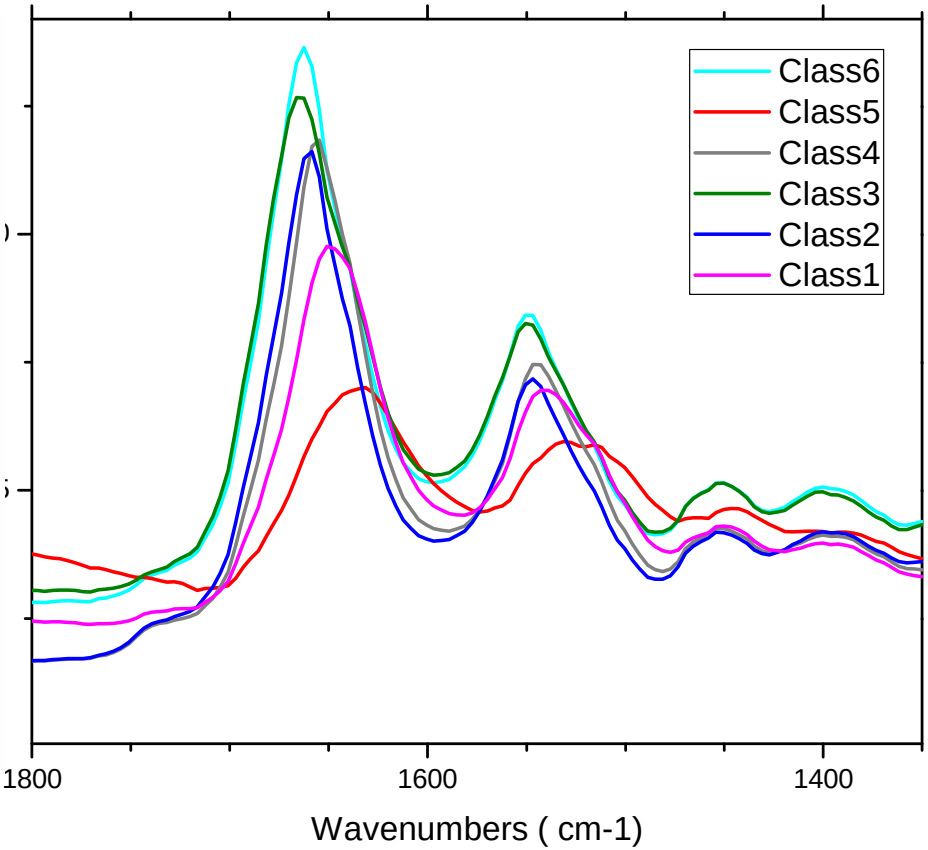


Proteins Image

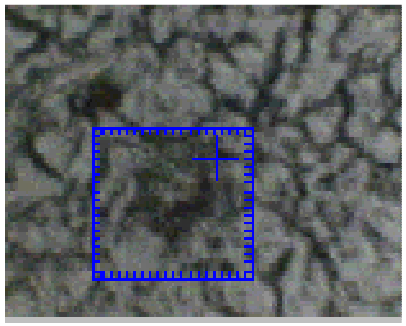
Statistical imaging

Hierarchical cluster analysis



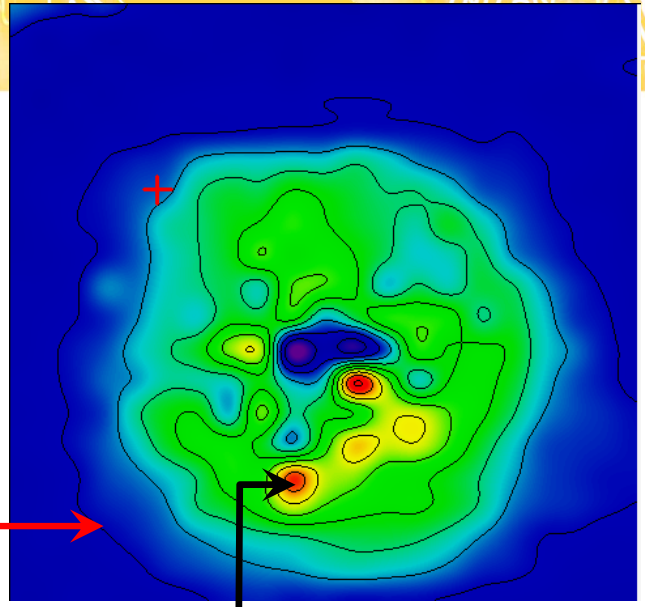


Plaque in brain tissue

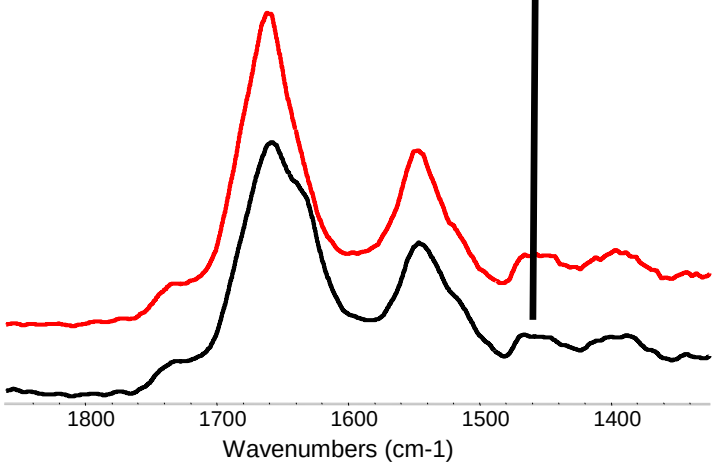


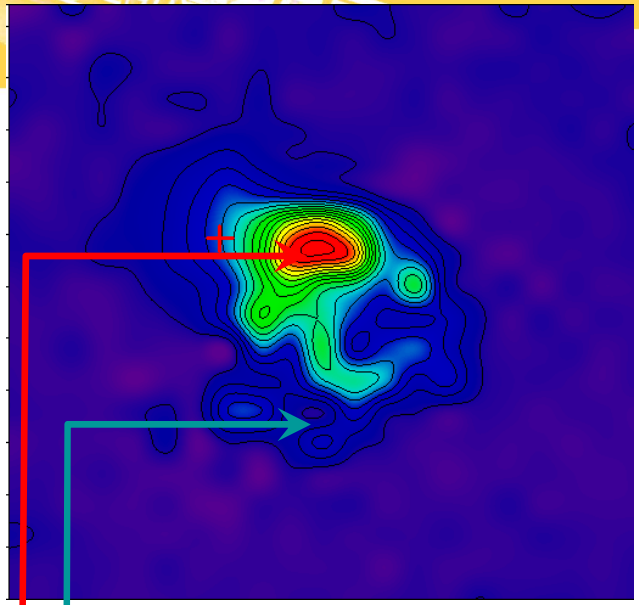
3x3 μm²

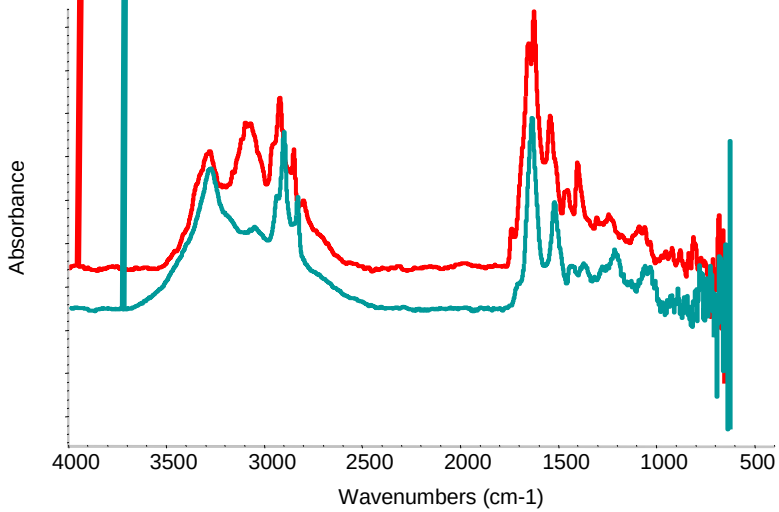
Step: 1 μm



10 μm

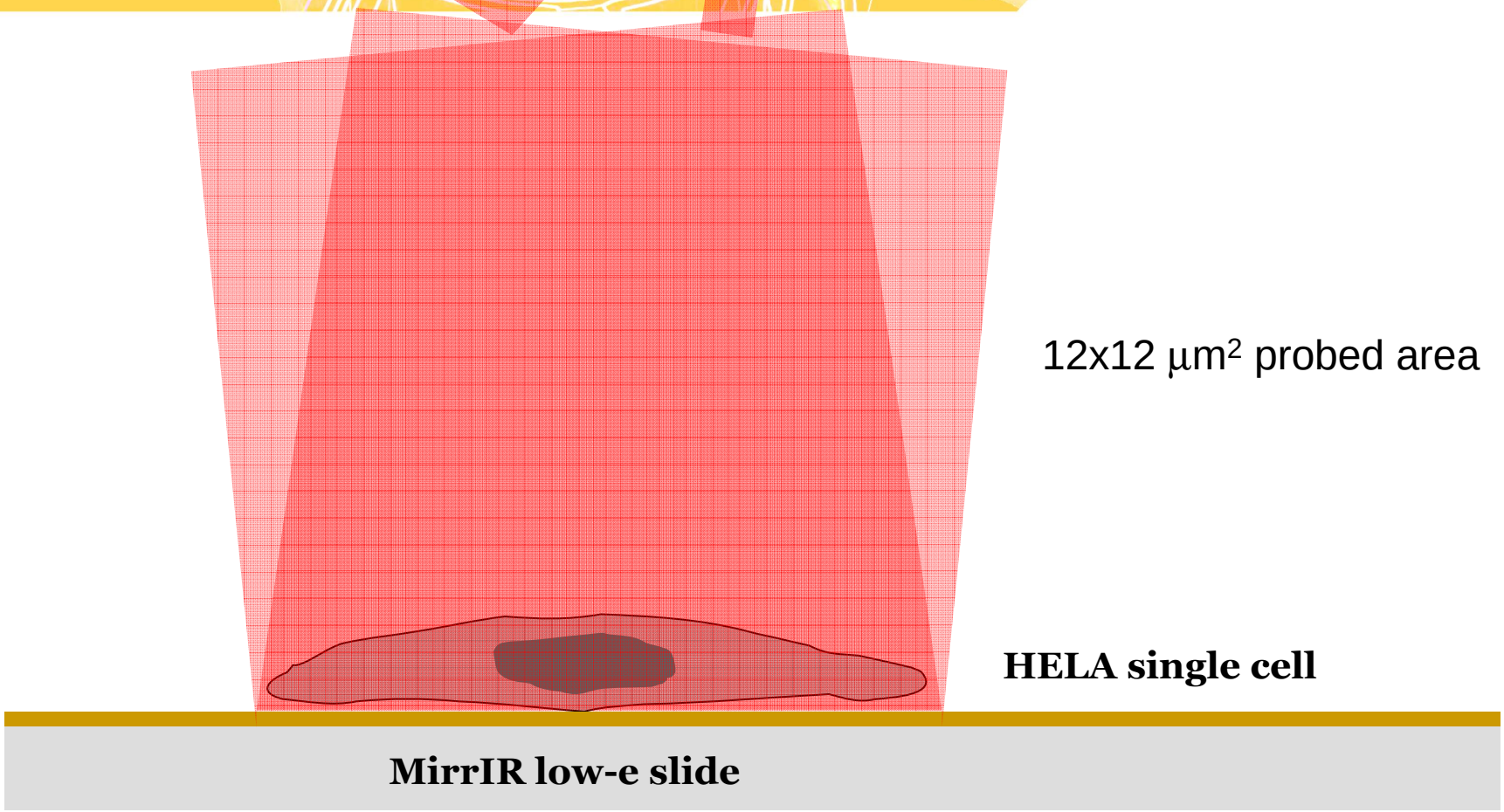


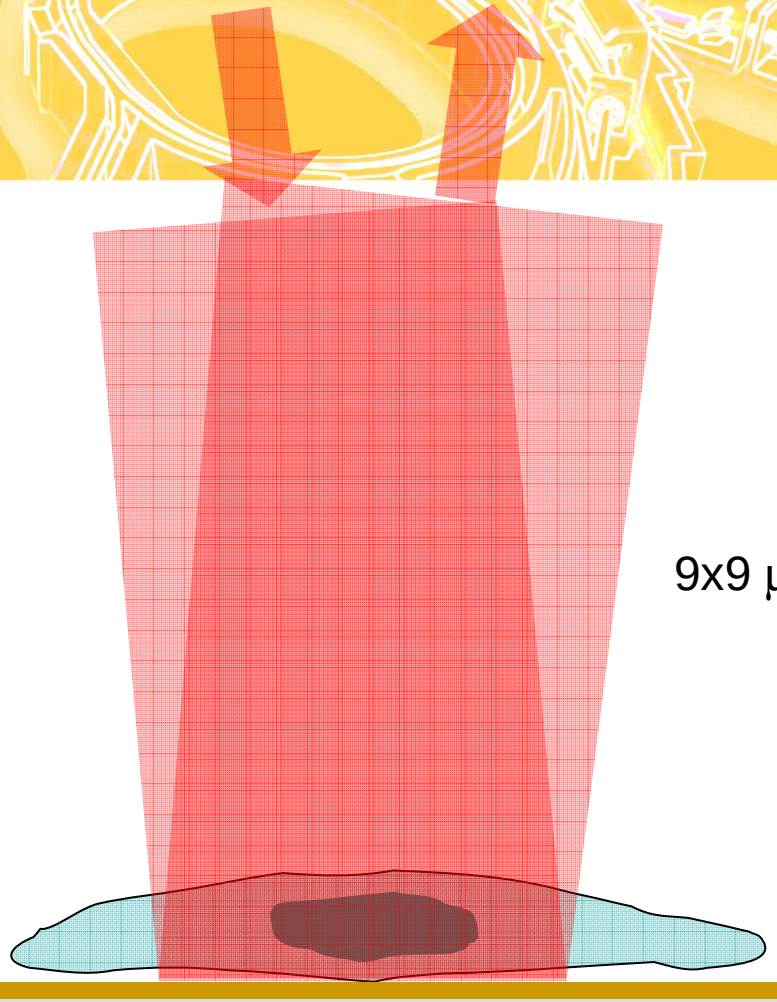




MIRAGE beamline LURE **From K. Gough , P. Dumas unpublished**

Subcellular analysis using synchrotron infrared

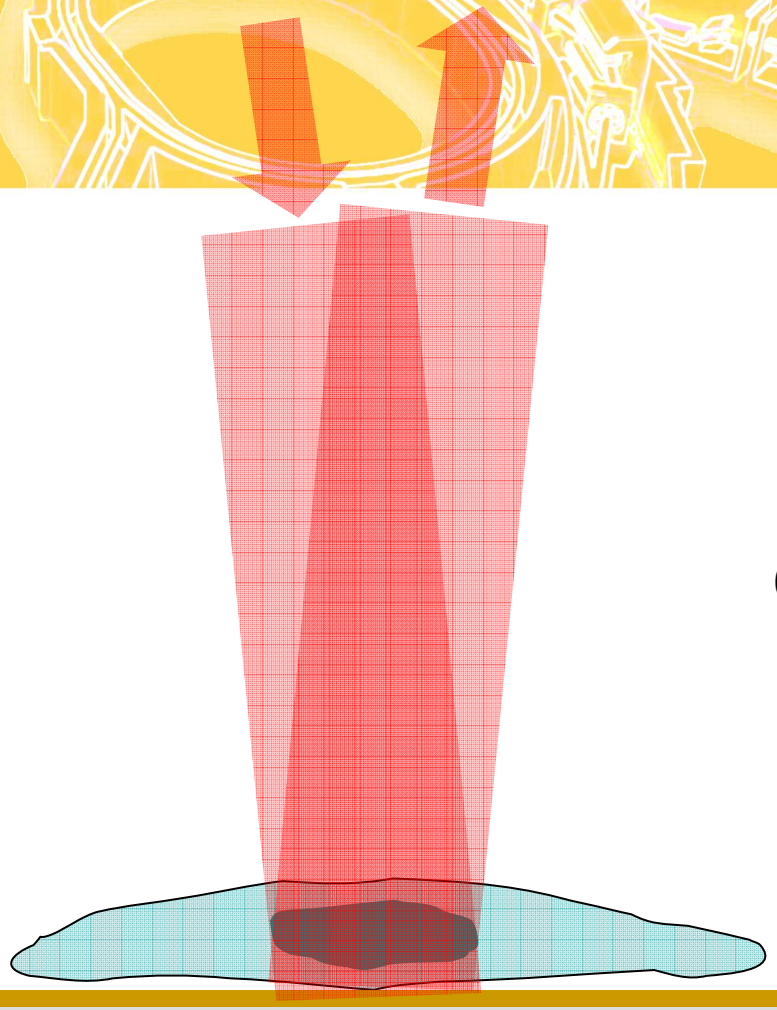

12x12 µm² probed area
HELA single cell
MirrIR low-e slide



9x9 μm^2 probed area

HELA single cell

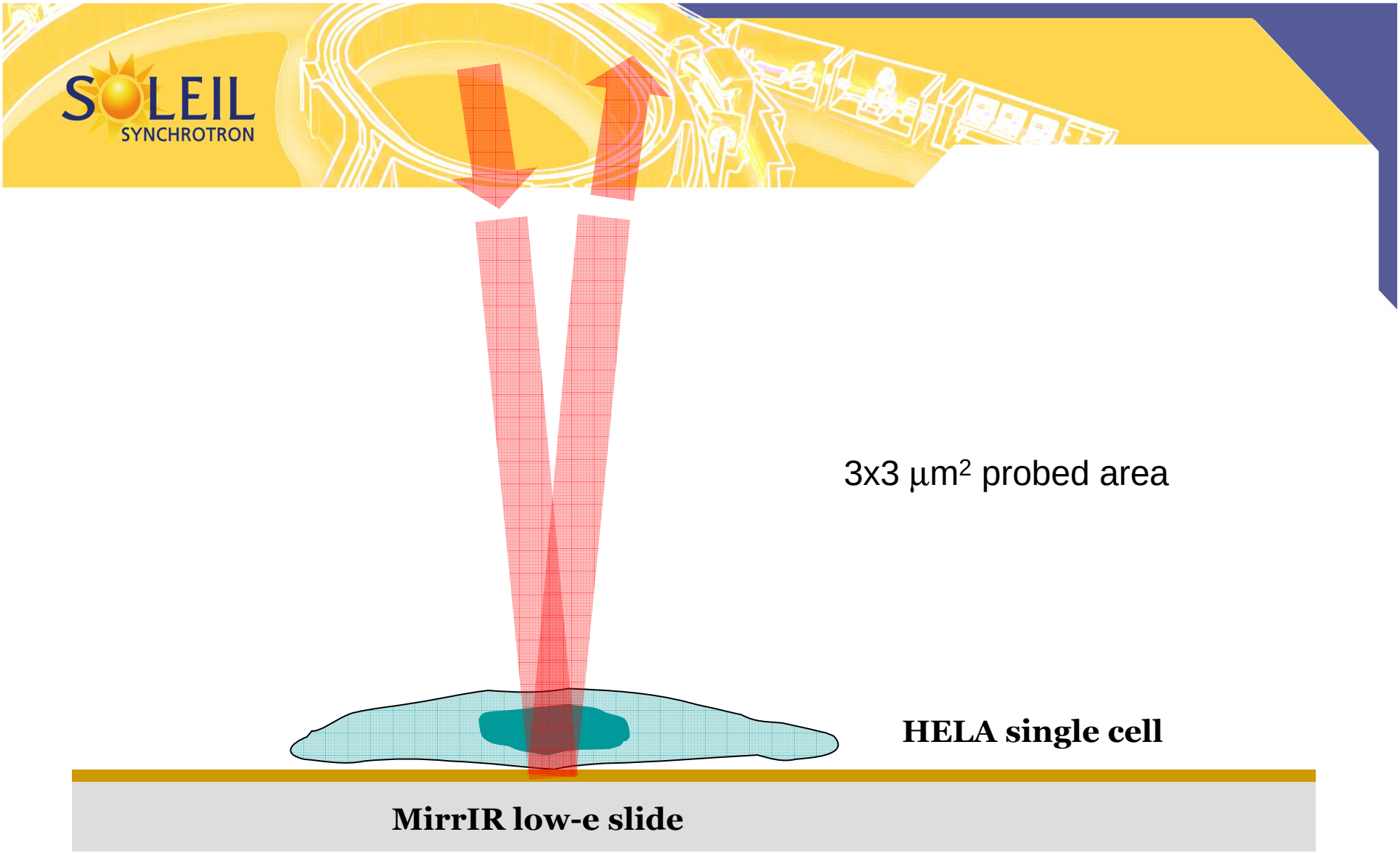
MirrIR low-e slide



6x6 μm^2 probed area

HELA single cell

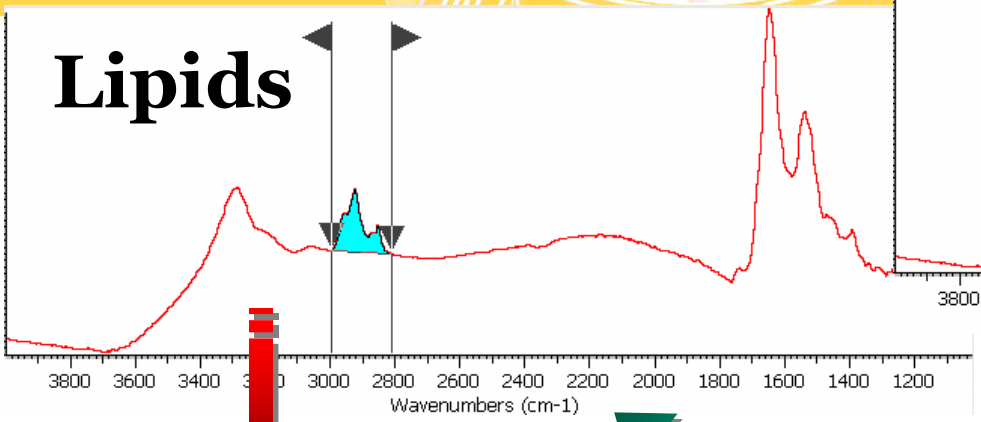
MirrIR low-e slide


3x3 µm² probed area
HELA single cell
MirrIR low-e slide

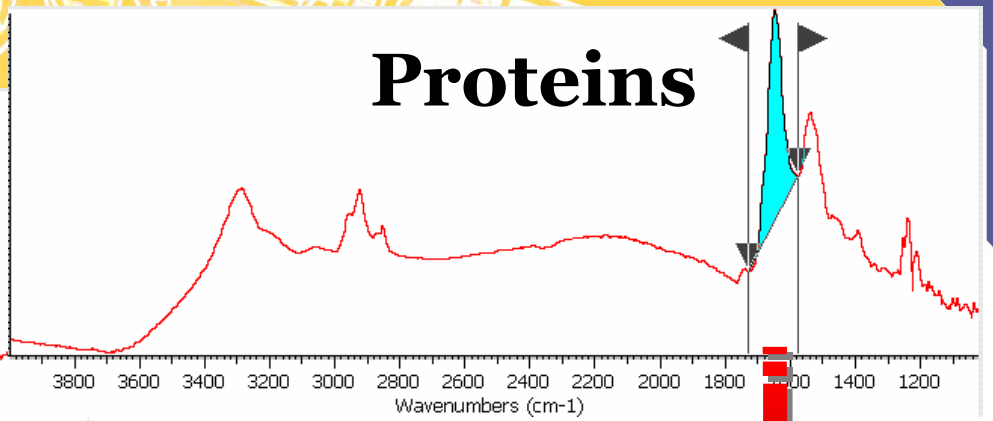
From imaging to spectroscopy

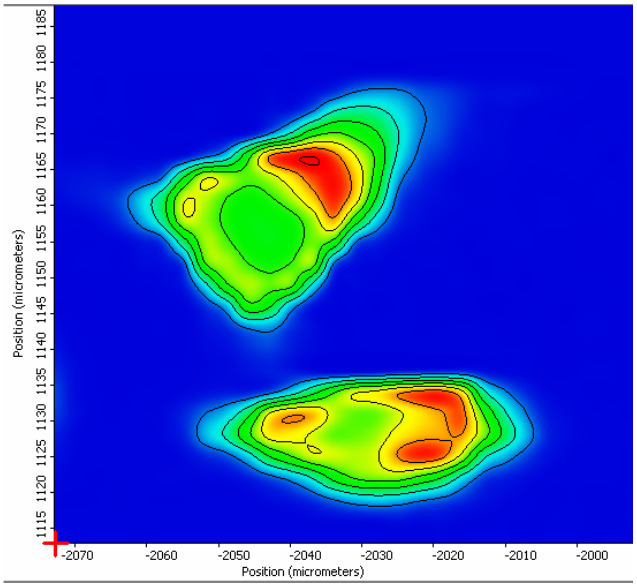
SOLEIL SYNCHROTRON

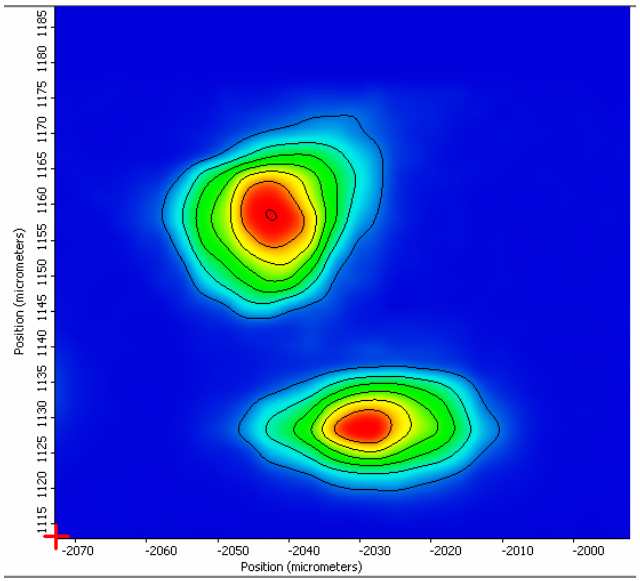
Lipids



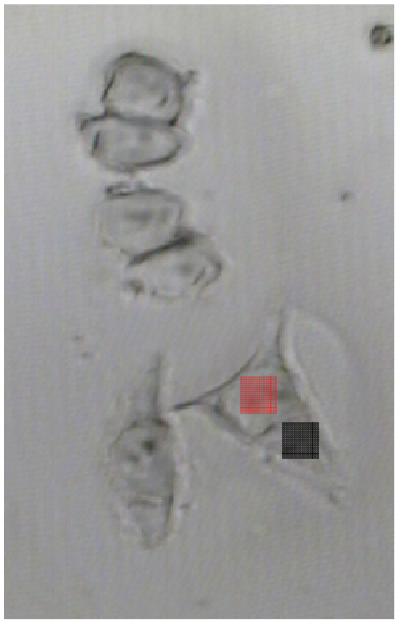
Proteins

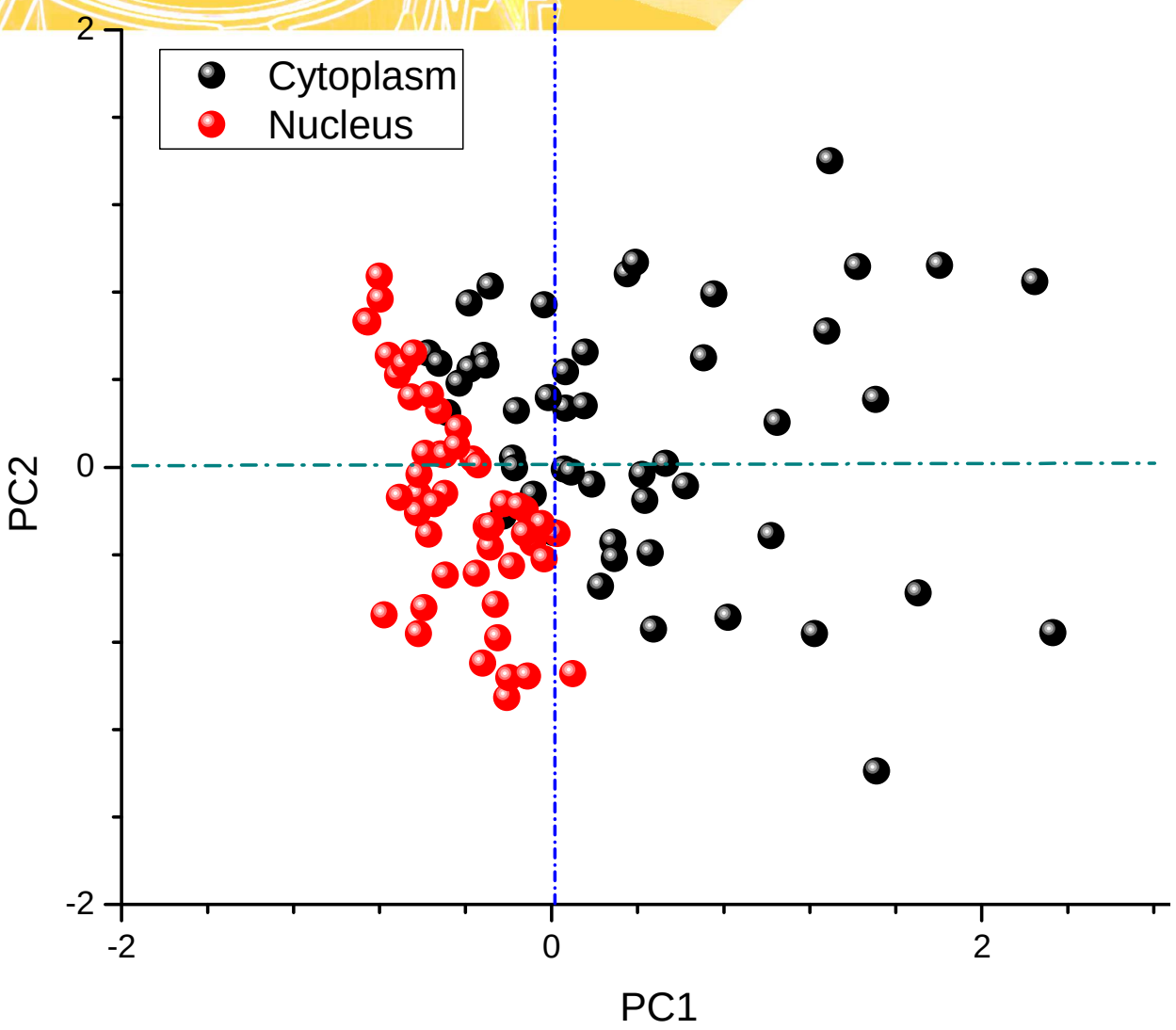


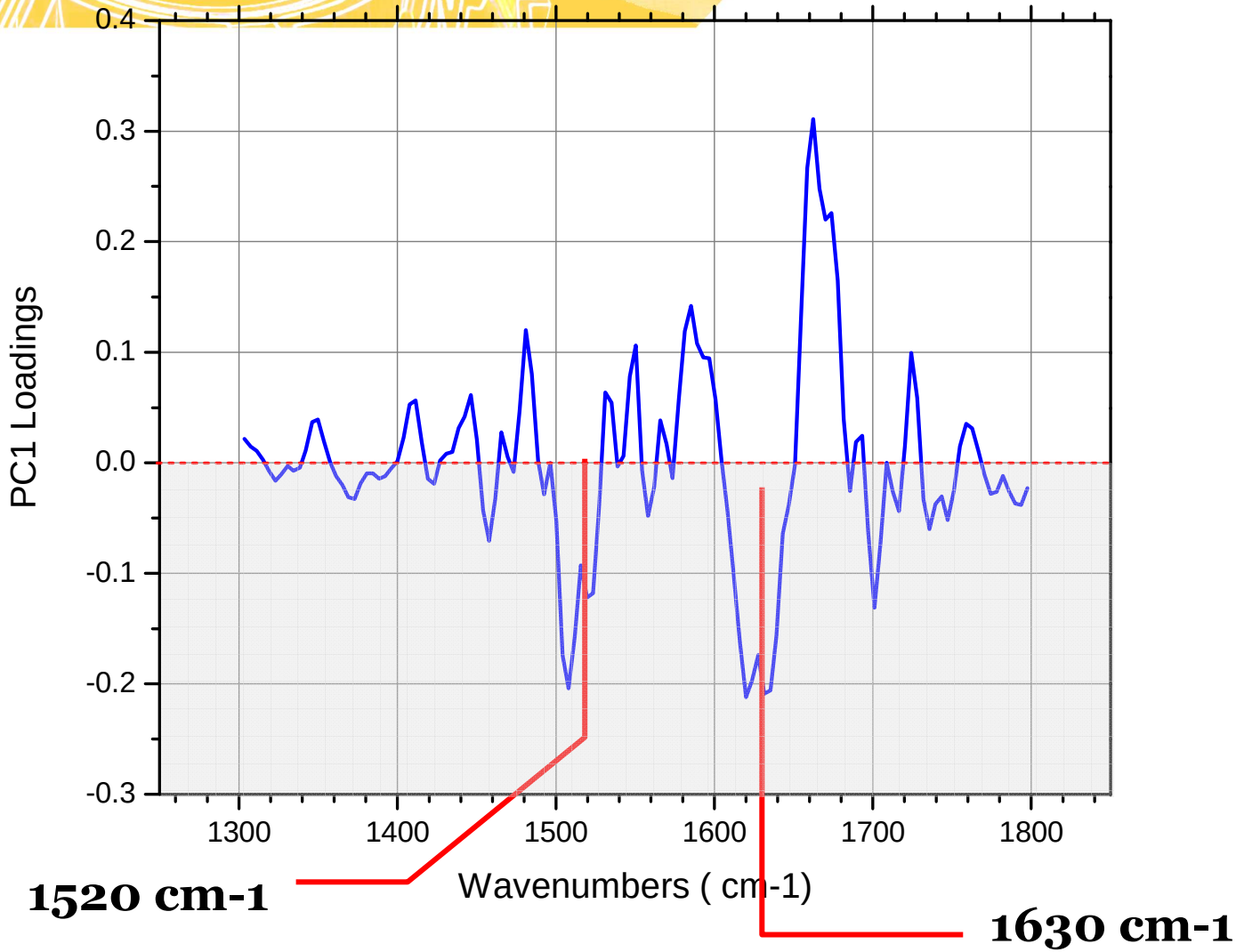




Unscramber software





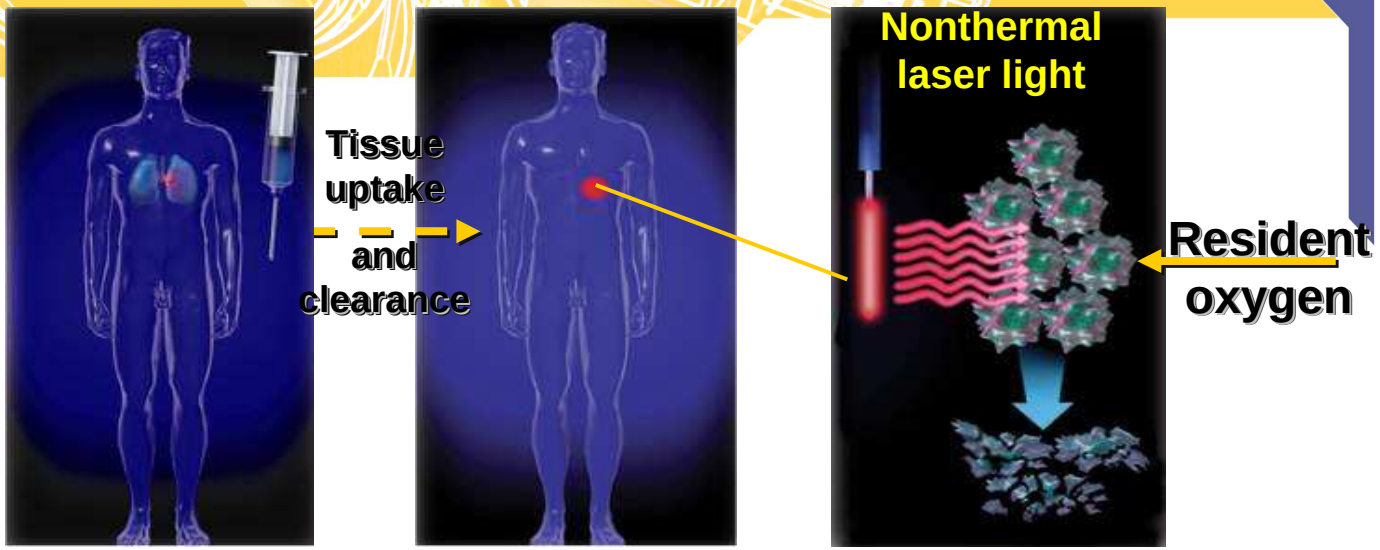

0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
PC1 Loadings
1300
1400
1500
1600
1700
1800
Wavenumbers (cm-1)
1520 cm-1
1630 cm-1

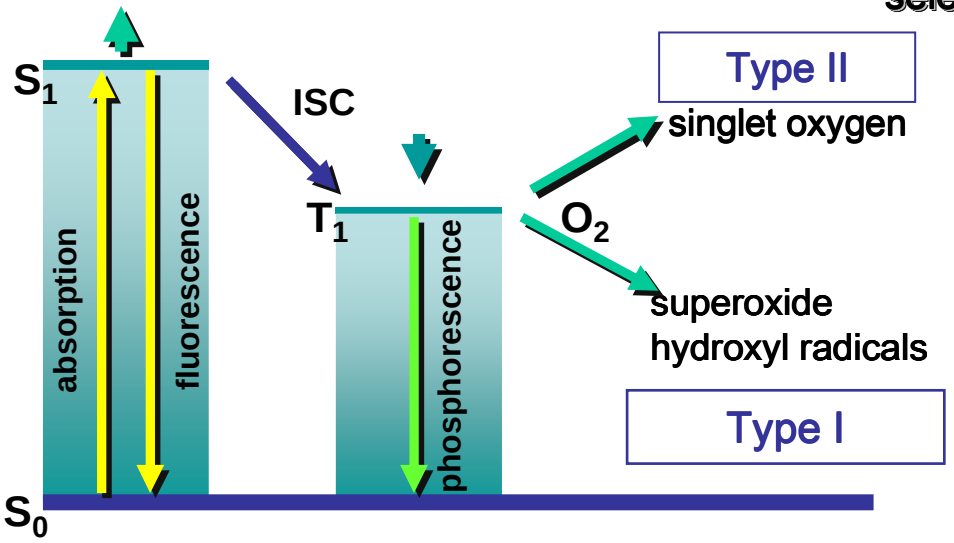
Aperture= 3x3 µm²
Step size= 2 microns

Diagnosis Capabilities at Cellular Level

Photodynamic therapy of cancer cells

Photodynamic therapy : principles

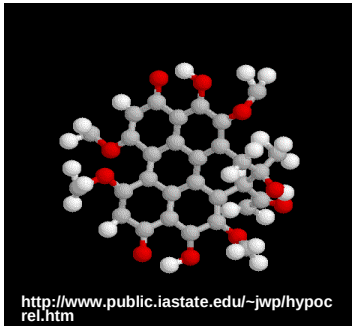


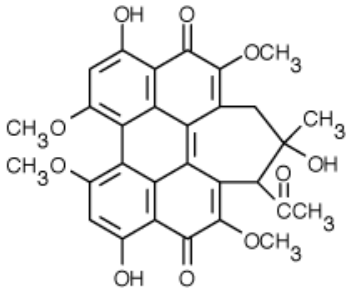


http://www.photofrin.com

Photosensitizer: Hypocrellin A

Hypocrellin A (HA), a lipid-soluble peryloquinone derivative isolated from natural fungus sacs of *Hypocrella bambusae*





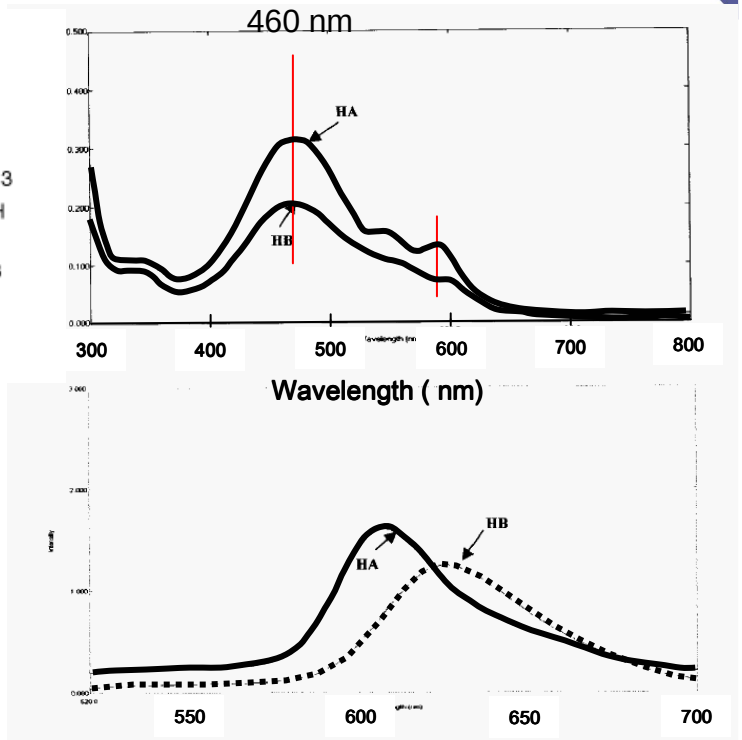
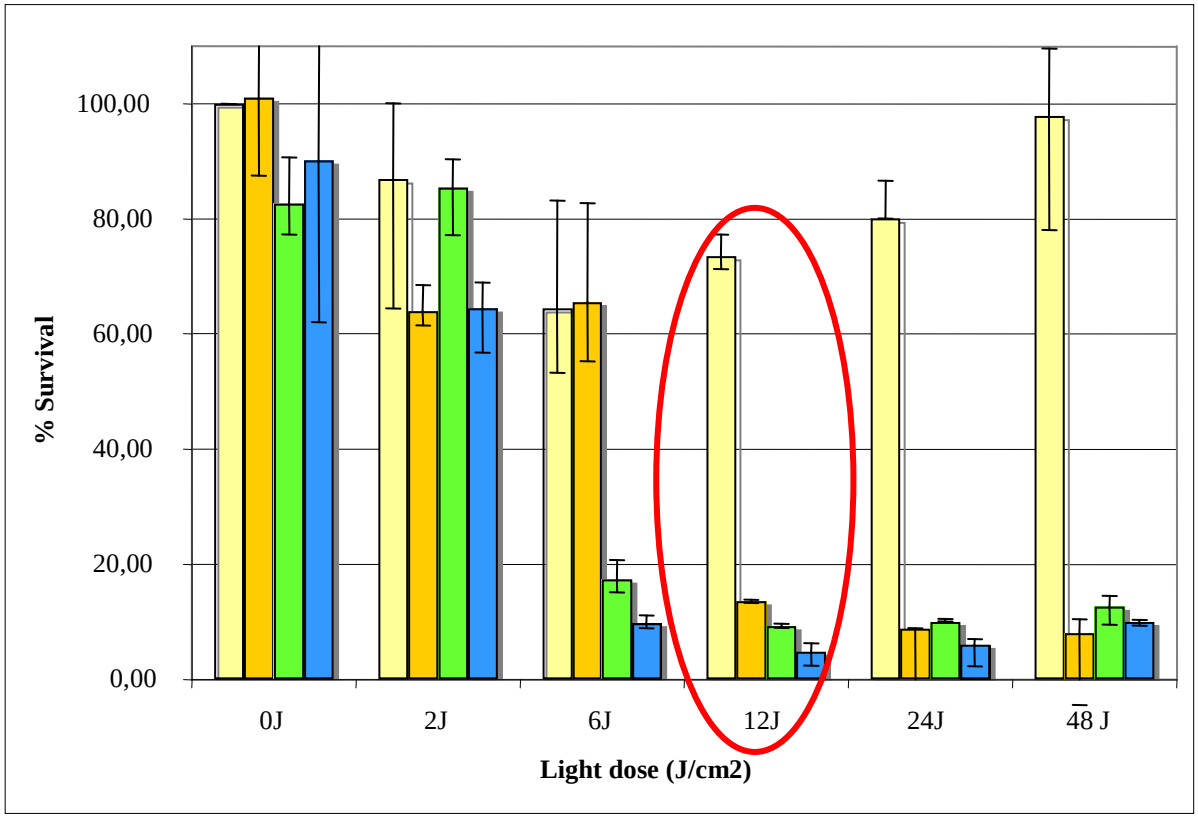


Figure 1. Molecular structures of photosensitizers HA, HB and their absorbance and fluorescence emission spectra measured in DMSO solution.

HA absorption mainly in the 400-650 nm energy domain.

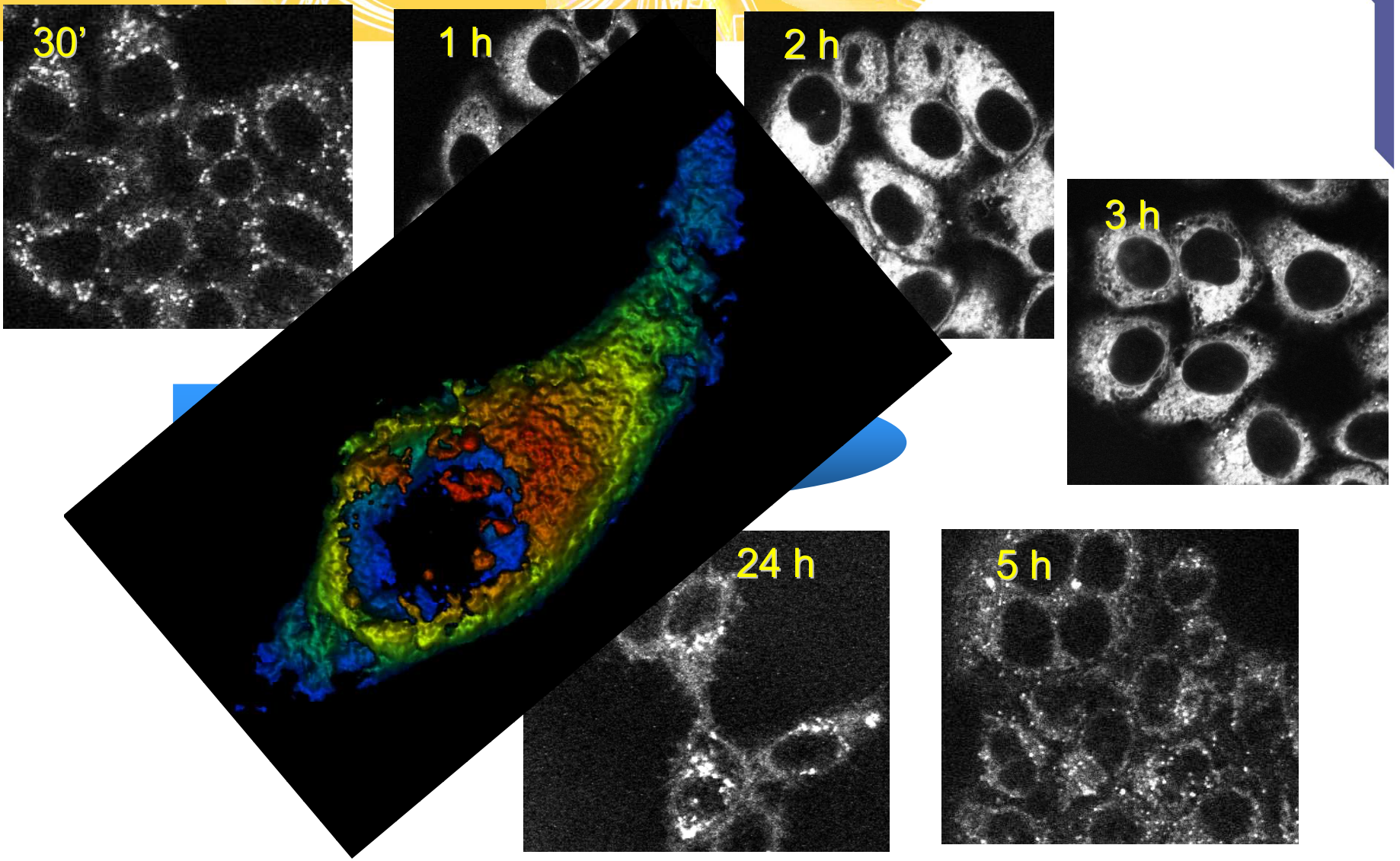
Fluorescence emission between 580 and 700 nm

Viability assays : HELA cells



S. Srichan,et al. submitted

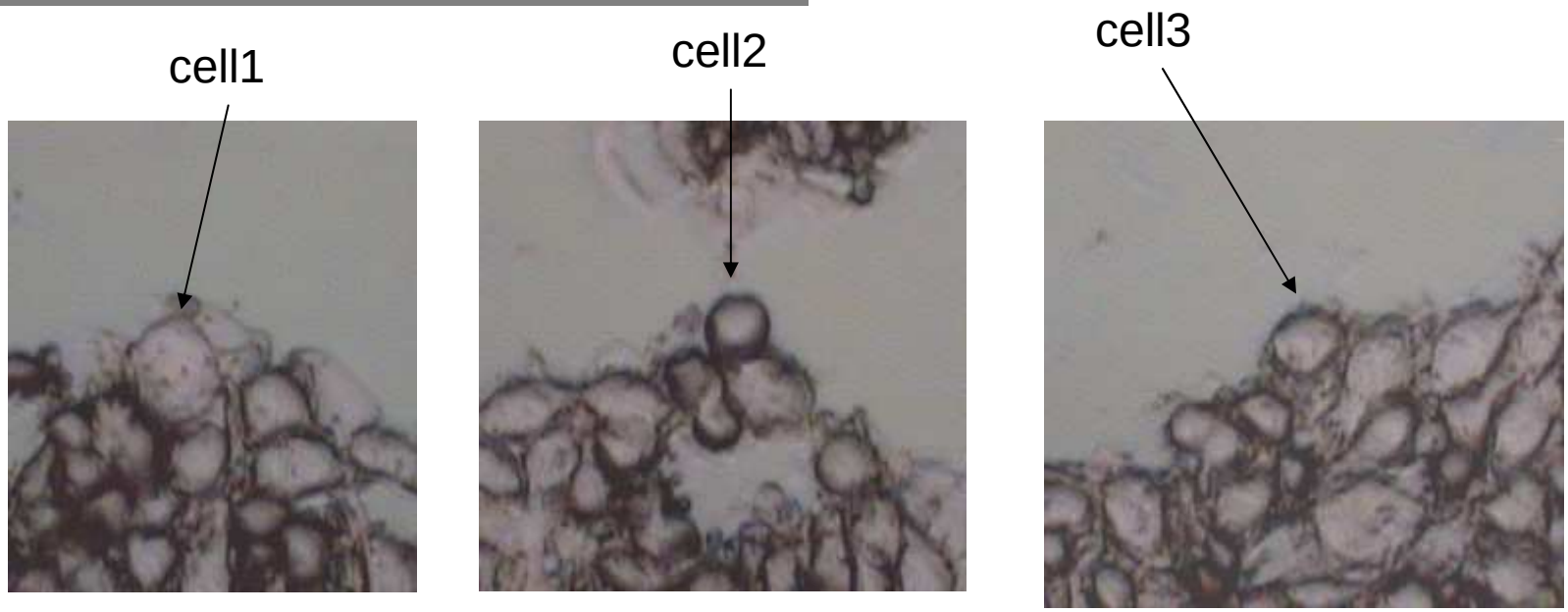
HA location inside cells



Individual cell probing with synchrotron IR microscopy

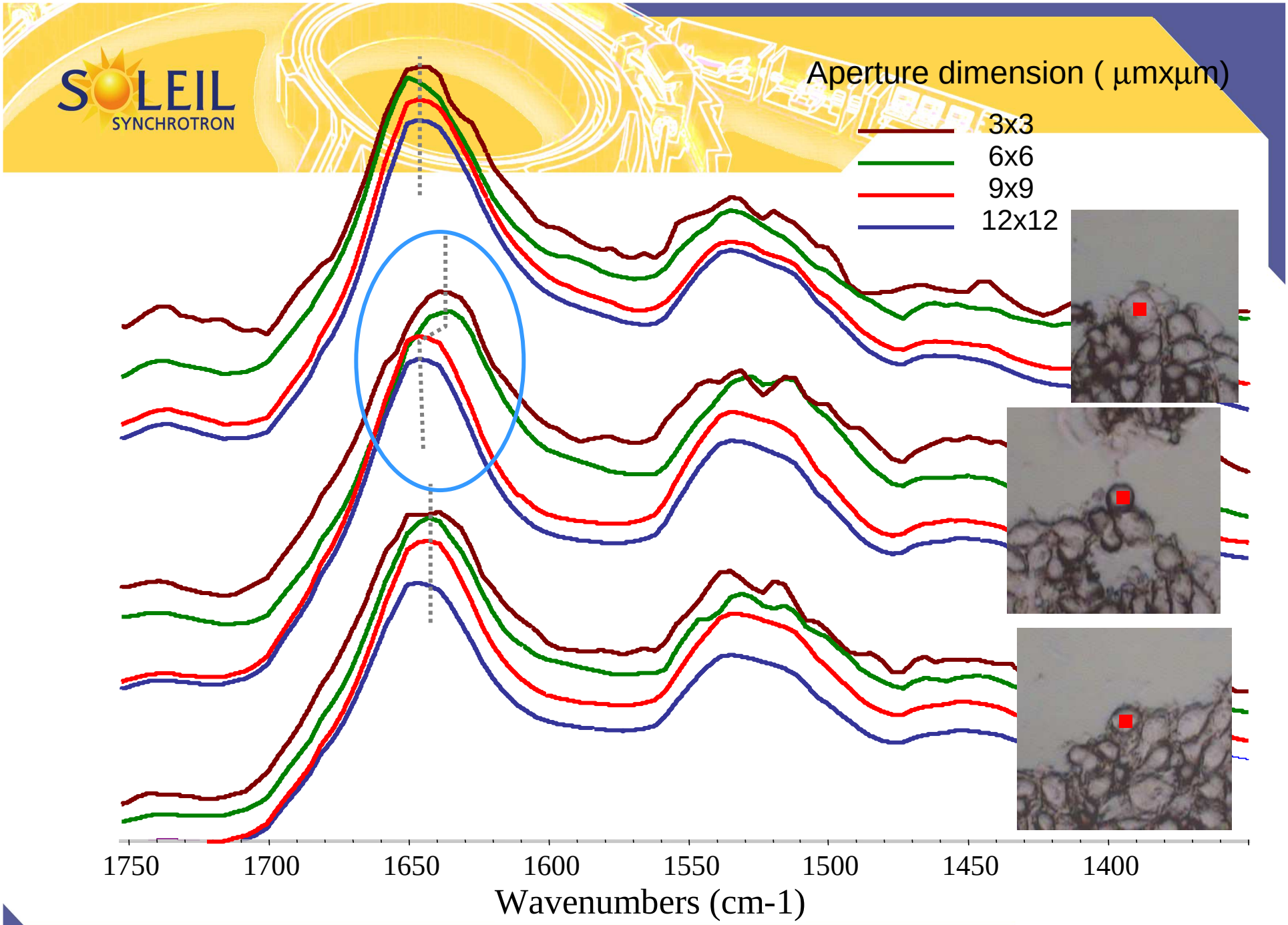
HELA cells
treated 10 minutes with
Hypocrelline , with irradiation

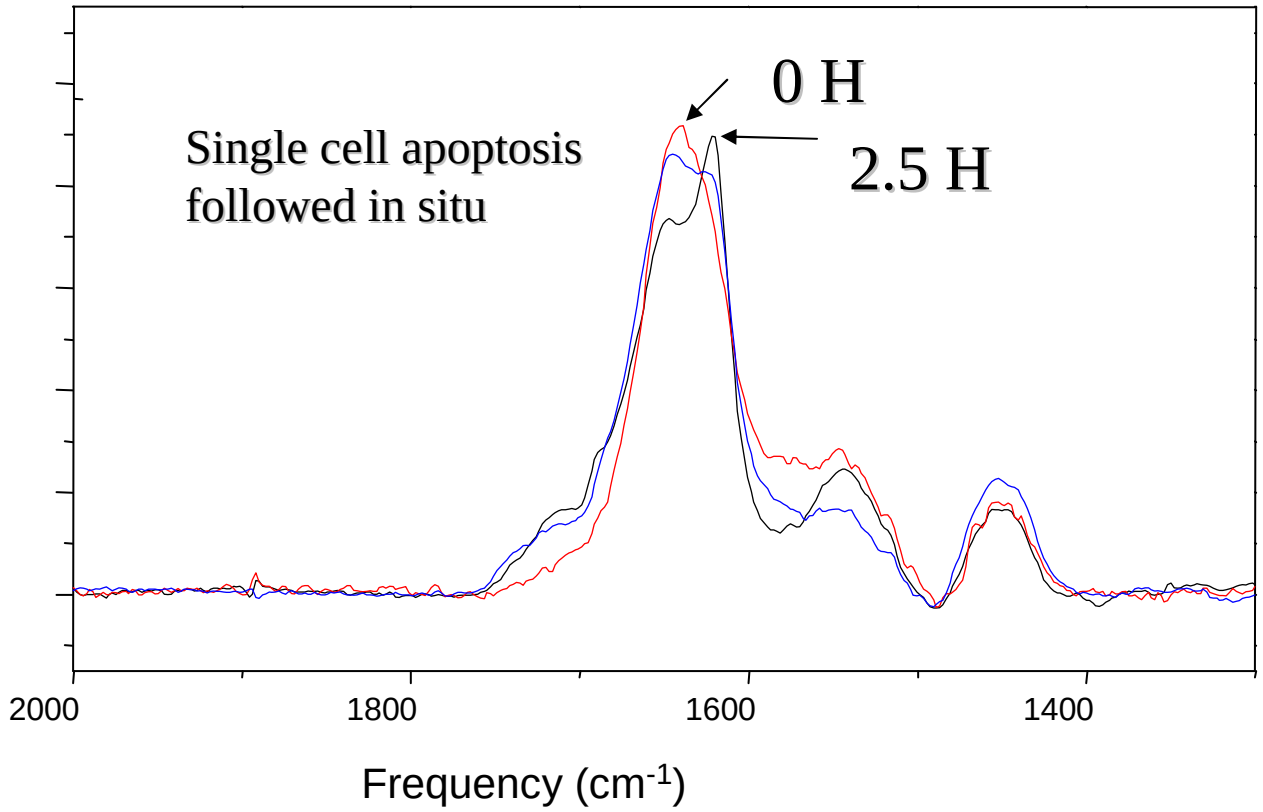
20% mortality, according to MTT assay



@ ESRF IR Beamline

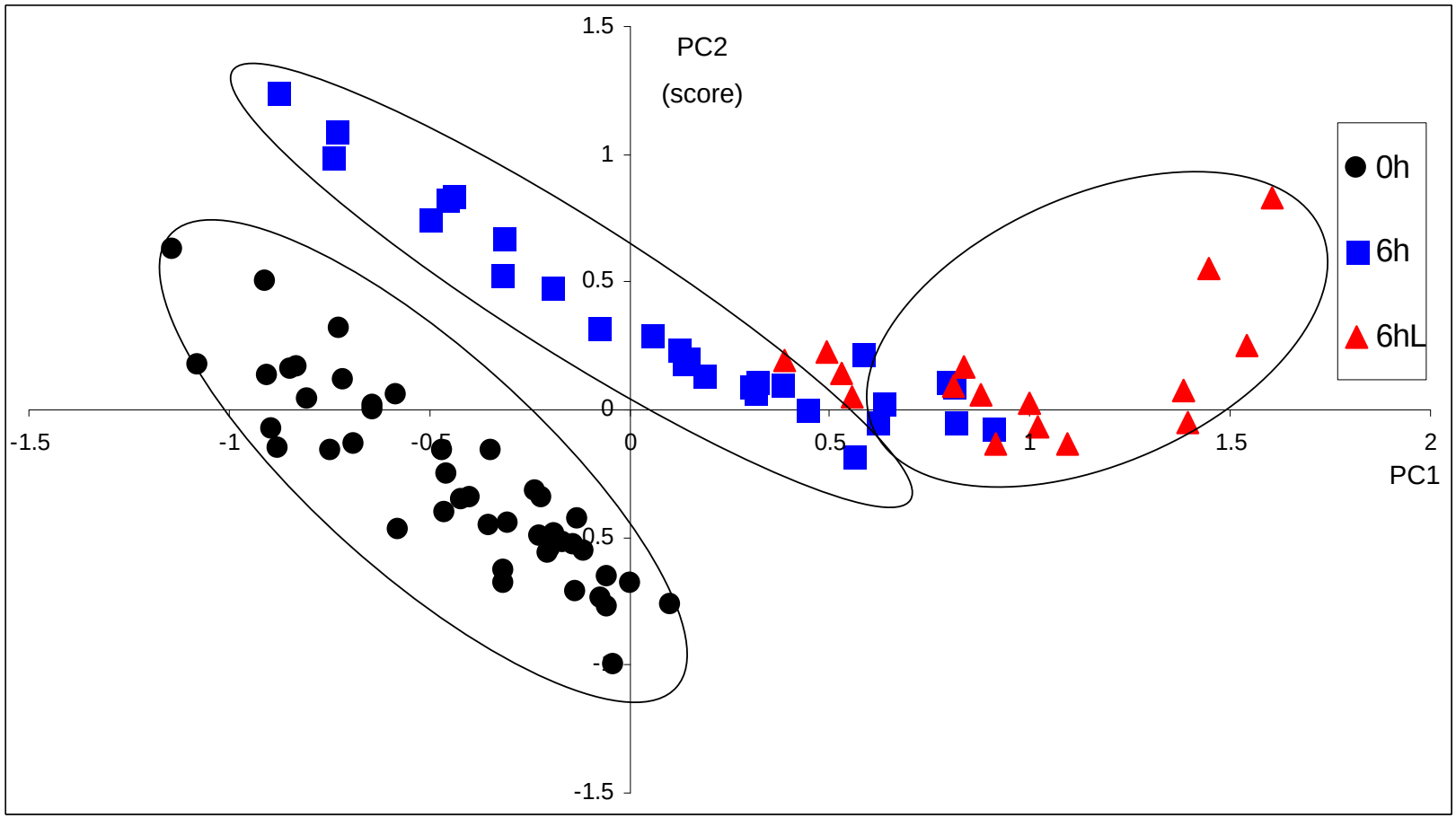
S. Srichan,et al.


Aperture dimension (µmxµm)
3x3
6x6
9x9
12x12
1750
1700
1650
1600
1550
1500
1450
1400
Wavenumbers (cm-1)

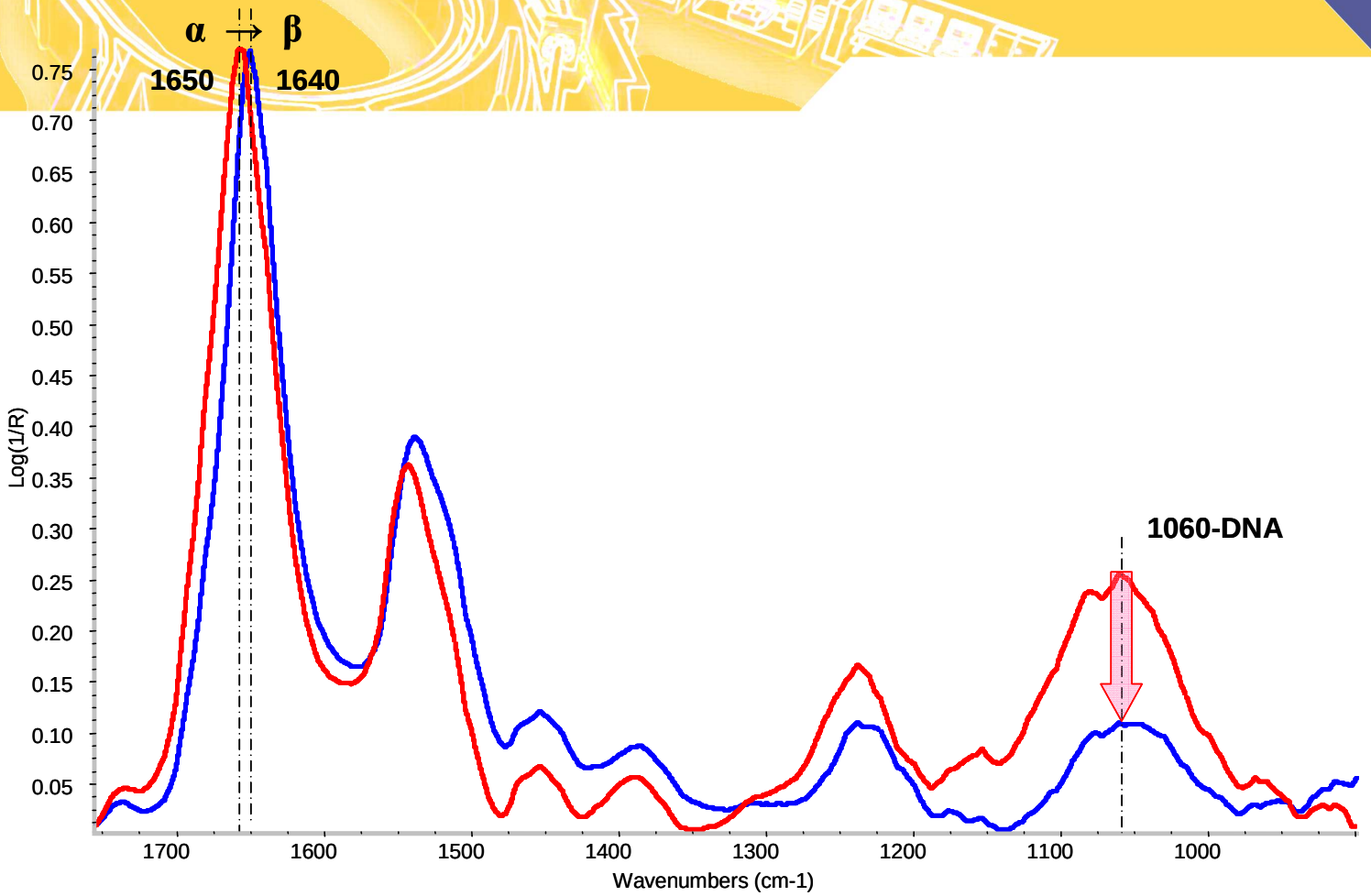


N. Jamin, J.L. Teillaud,L.M. Miller, P. Dumas,G.P. Williams

Statistical analysis of large data sets: exemple PCA



S. Srichan,et al.

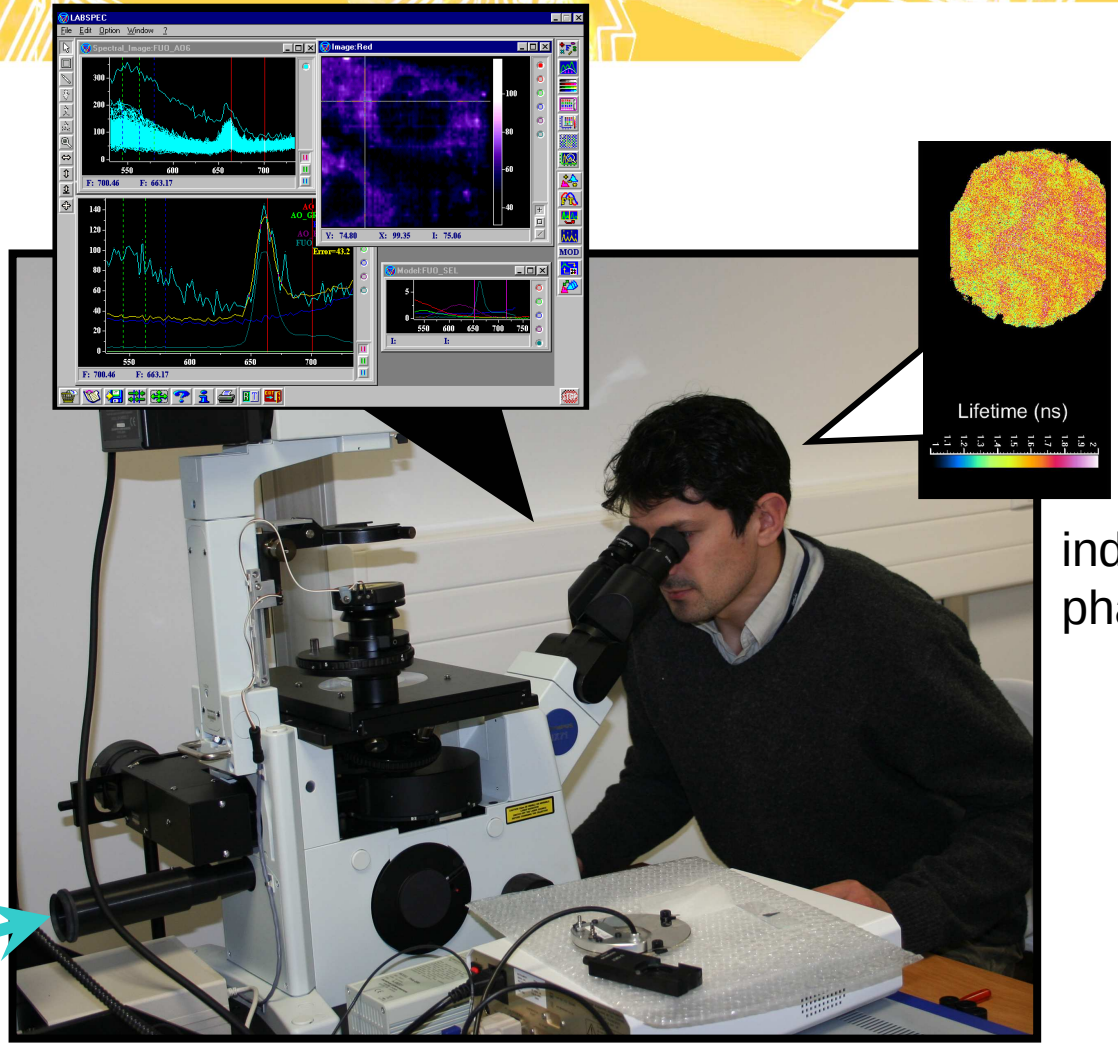


PCA analysis of 2µM HA treated HeLa cells after 6h incubation with/without 12Jcm^{-2}irradiation.

S. Srichan,et al.

Combining synchrotron IR microscopy with other synchrotron-based techniques?

Visible and UV Microscopy

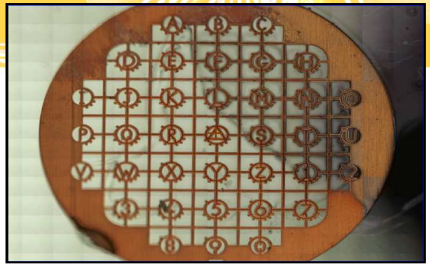


induced phase-shift

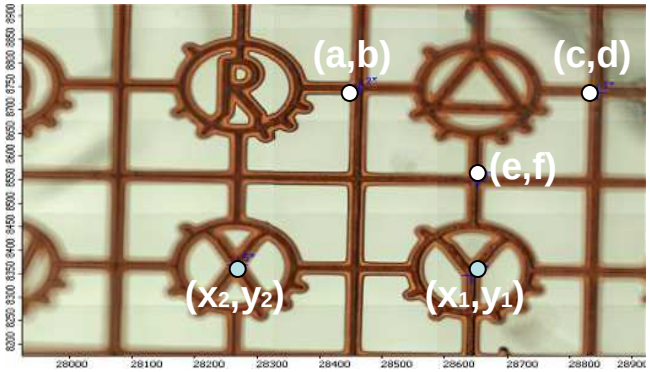
synchrotron radiation beam

from 180 to 700 nm

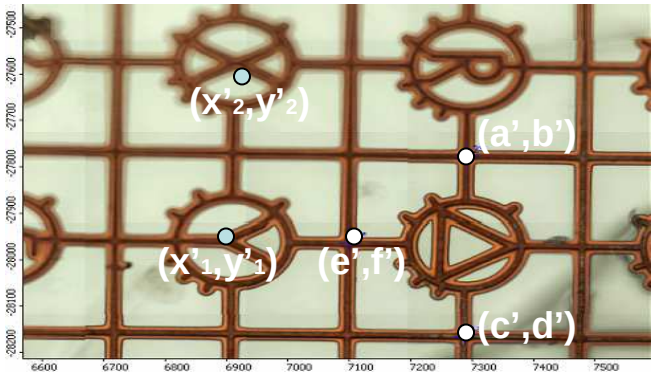
References= electron microscope grids

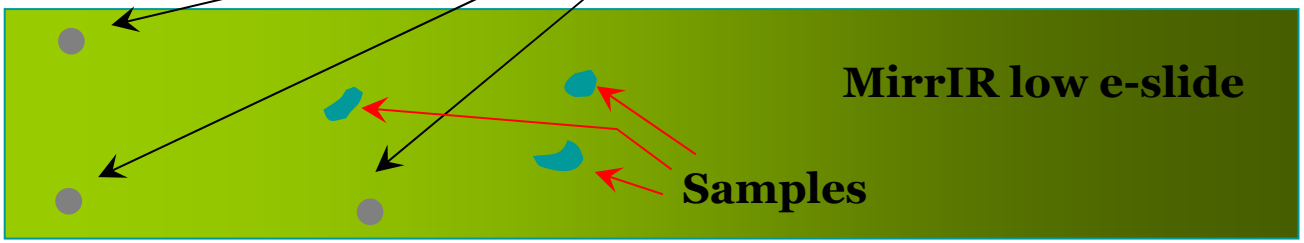


locations of references and the samples.



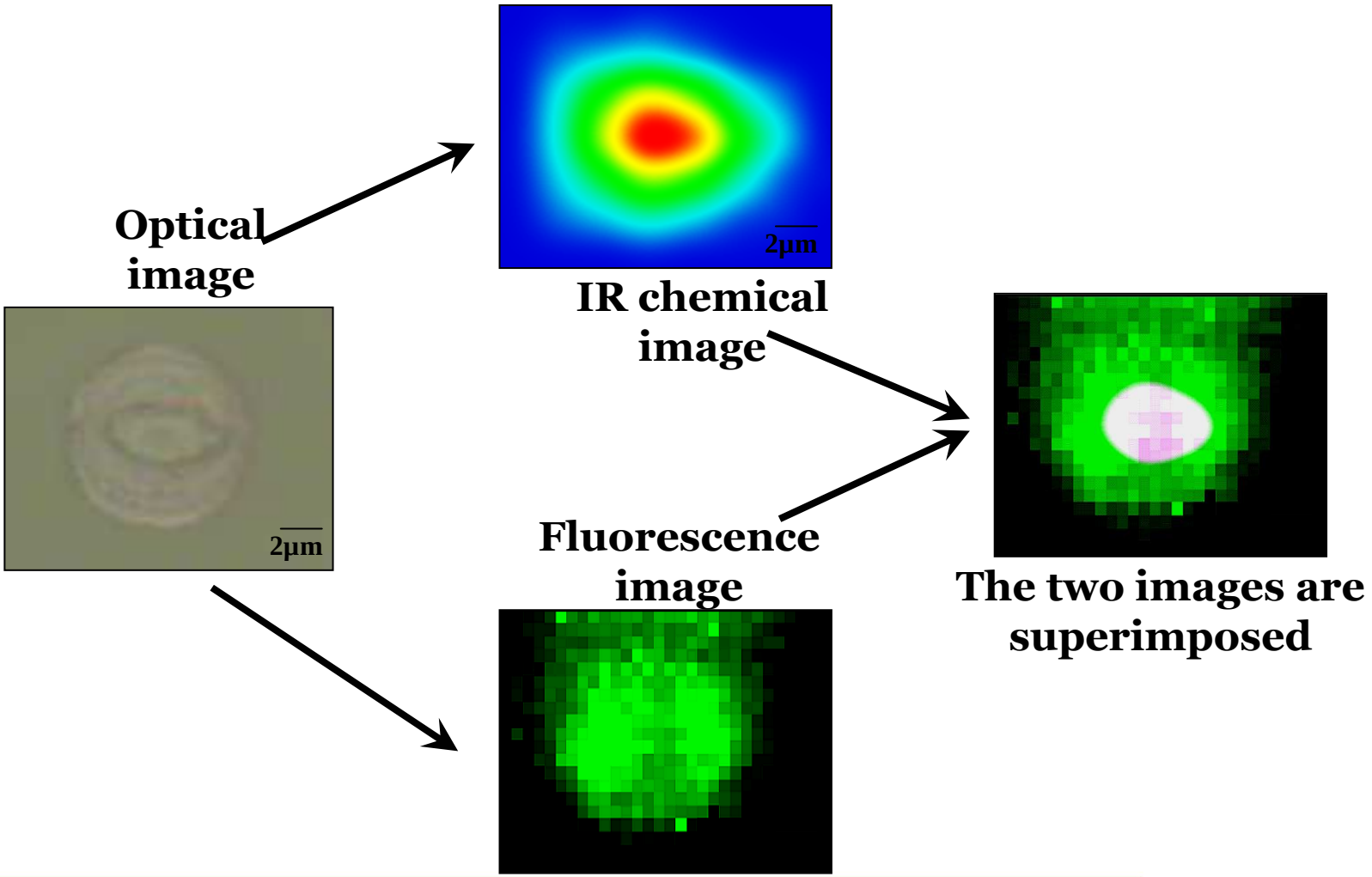
locations of the samples after rotated the slide.





Human cervical cancer (HELA) cell

Infrared and fluorescence images of 10µM hypocrellin A and light(48J/cm²,λ = 470nm) treated HeLa cells .

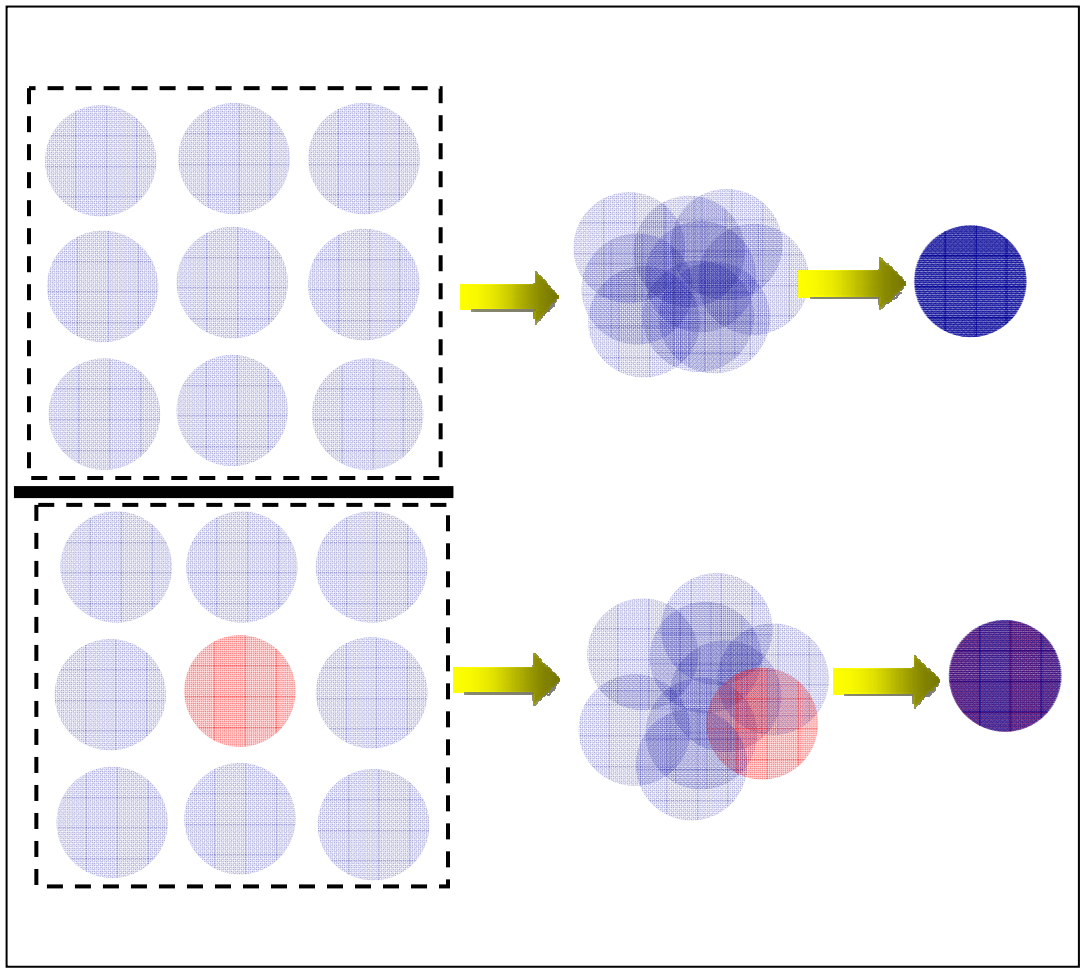


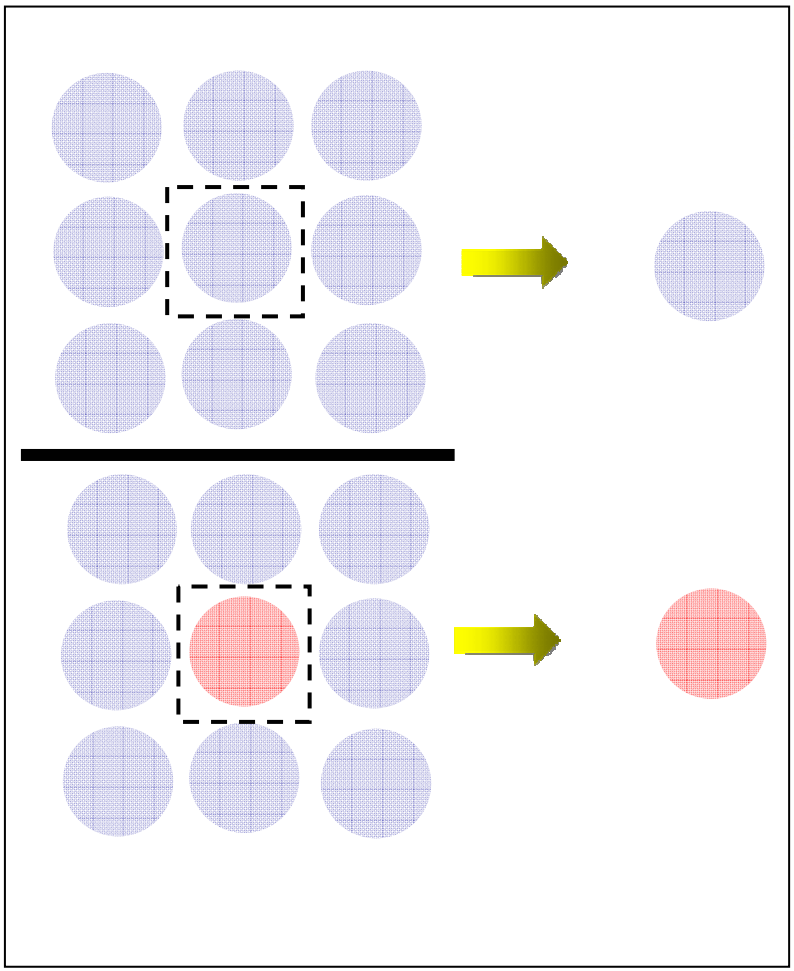
Summary

- The results from this study show the capability of FTIR microspectroscopy to demonstrate the effects of PDT on HeLa cells, showing indirect effects of the drug at individual cell level.

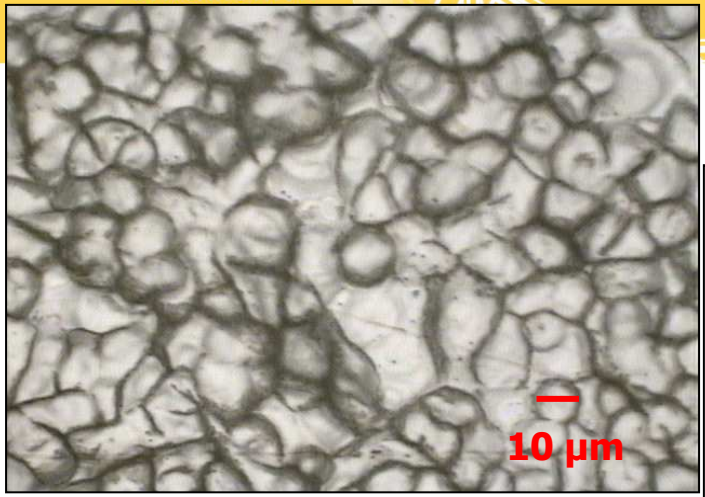
- Complementary study using fluorescence microscopy shows the intracellular distribution of HA in the cells. The combination with FTIR microspectroscopy at the cellular level will play an important role in the diagnosis of cancer cell responses to treatments.

Diagnosis? Pre-diagnosis?

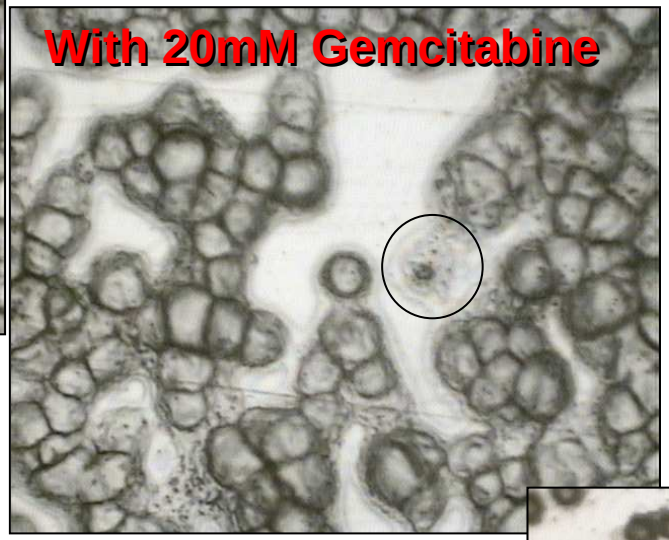


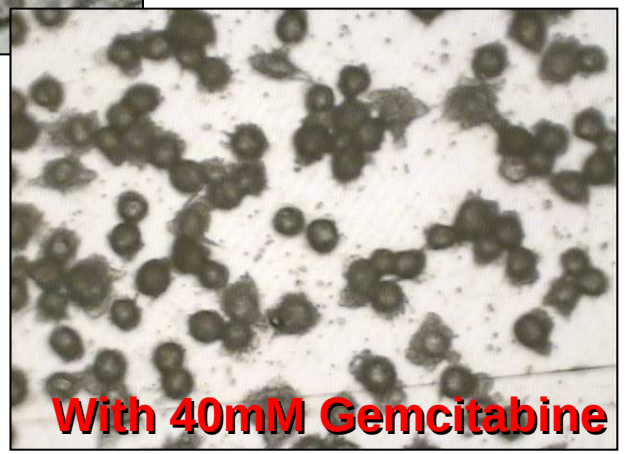


SKMES lung cancel cells line



Gemcitabine= a chemotherapeutic drug



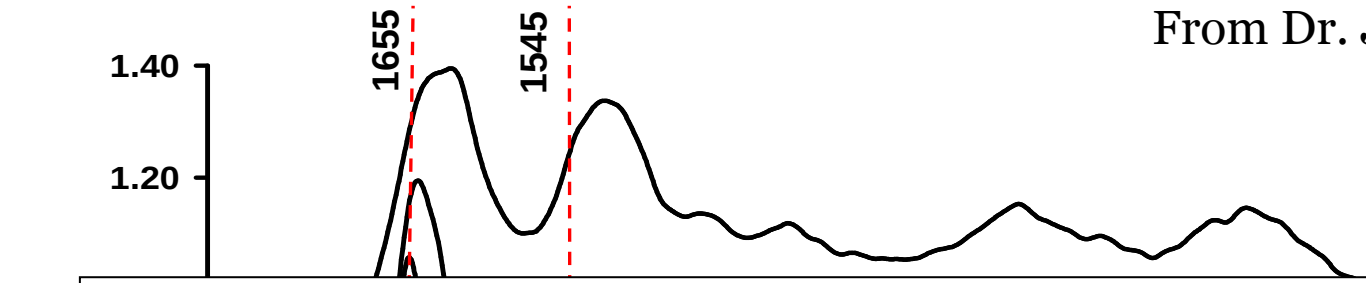


Are these cells sensitive to the drug?

From Dr. Josep Sulé-Suso

SKMES lung cancel cells line

From Dr. Josep Sulé-Suso



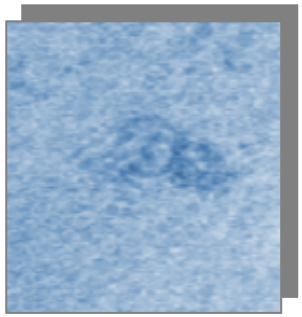
the biochemical changes in these cells measured with synchrotron IR micro-spectroscopy could help us to better clarify the state of a single cell, i. e.,

- ***alive without damage,***
- ***alive with damage but able to recover,***
- ***alive but entering a death pathway and unable to recover,*** **or**
- ***dead.***

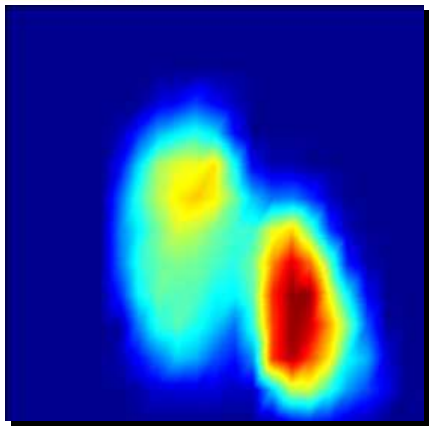
Cell differentiation

Induced by Phorbol Myristate Acetate (PMA)
(morphology and activity changes)

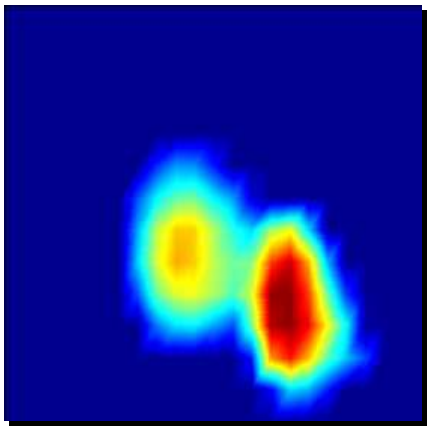
HL-60 few minutes after « activation »



20 µm

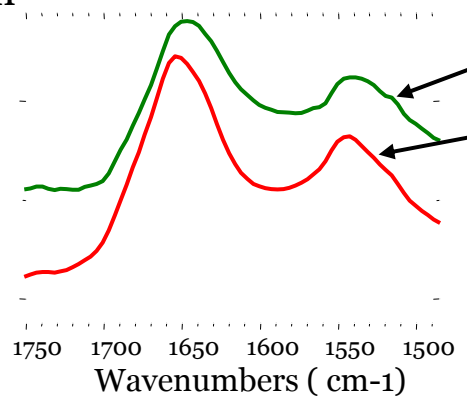


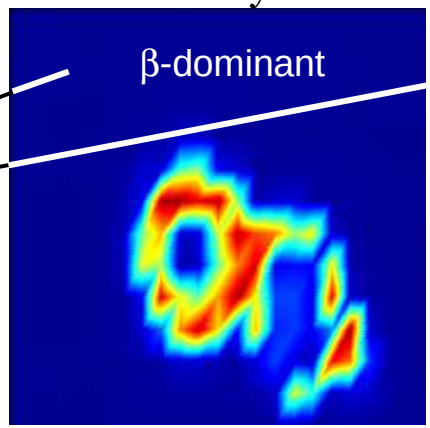
Lipids profile

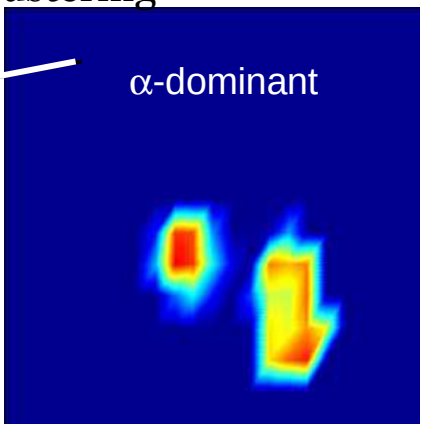


Protein profile
(Amide I)

Fuzzy-c-means clustering







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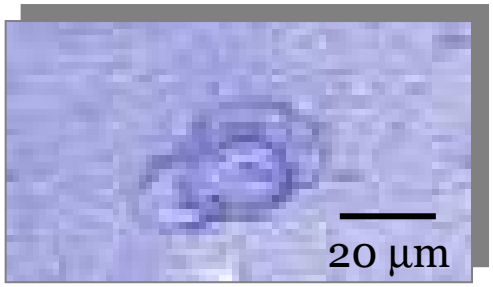
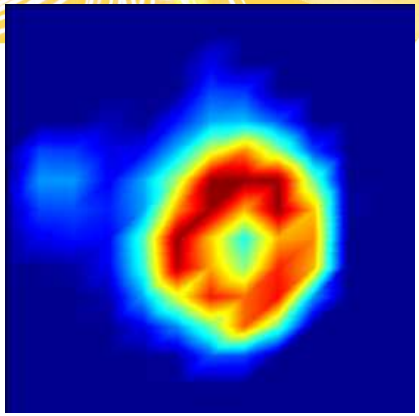
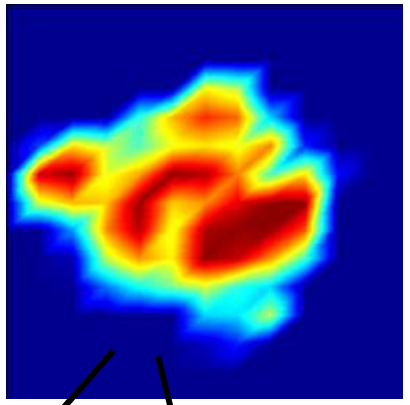
48 Hours after PMA...

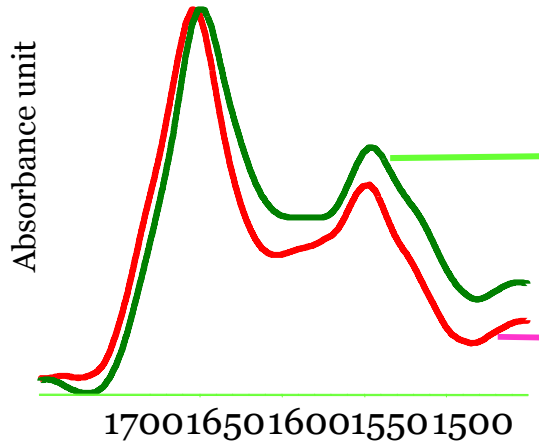


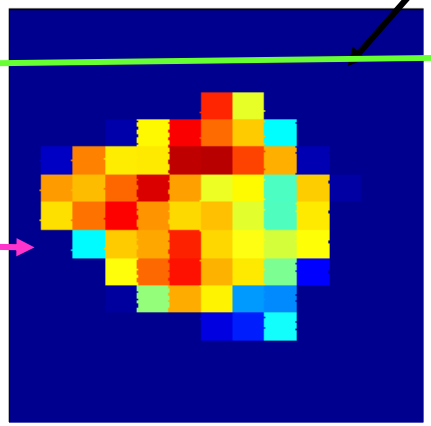


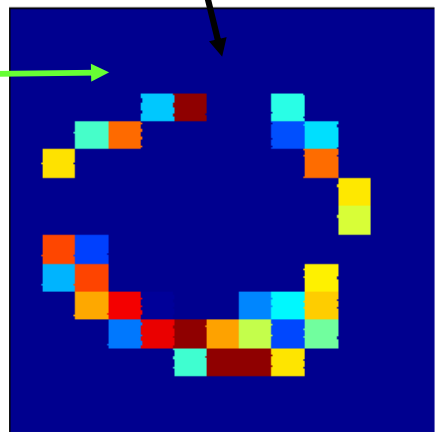


Intensity profile of lipids

Nucleus (Amide I)







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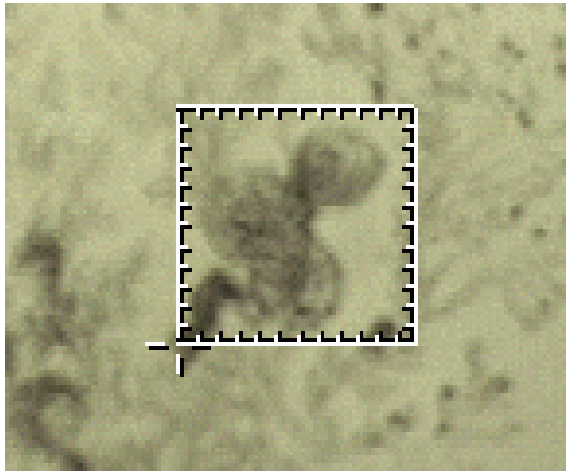
Imaging the Response of Prostate Cancer Cells to Chemical Treatments

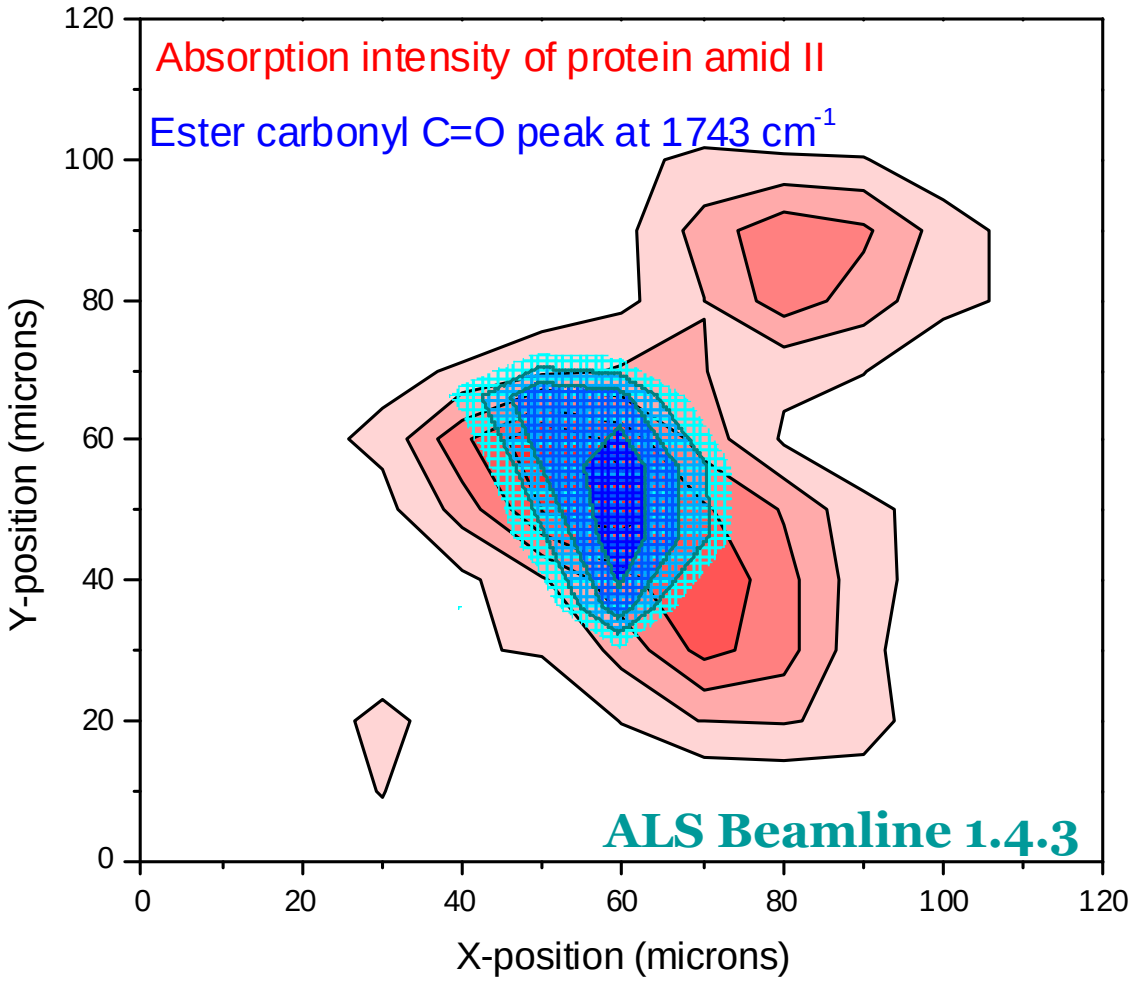
ERNEST ORLANDO LAWRENCE
BERKELEY NATIONAL LABORATORY

With Harvard Medical School



4 hour treatment with a model chemotherapy. IR maps are measured in-vitro, in real time

From M.Martin (Berkeley)

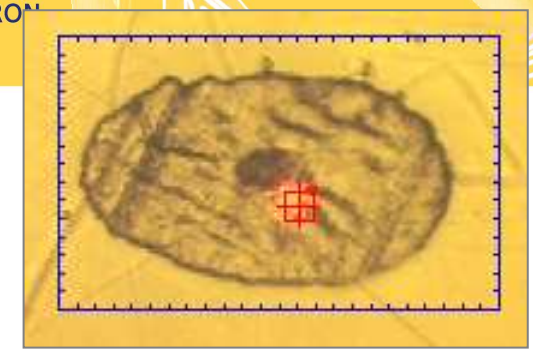


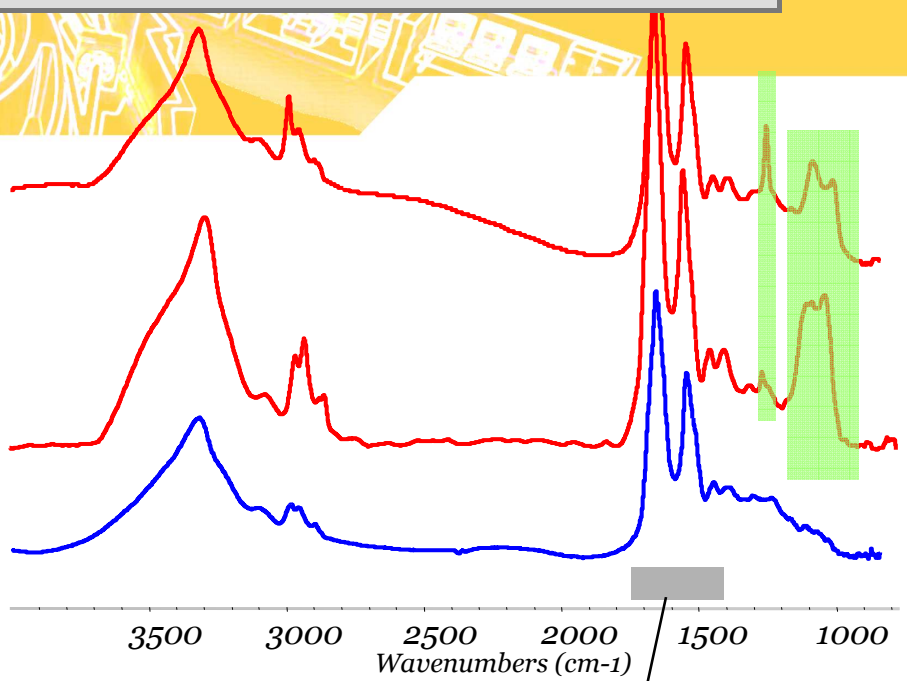
Human tissues from mummy

Mummy from Taklamakan desert

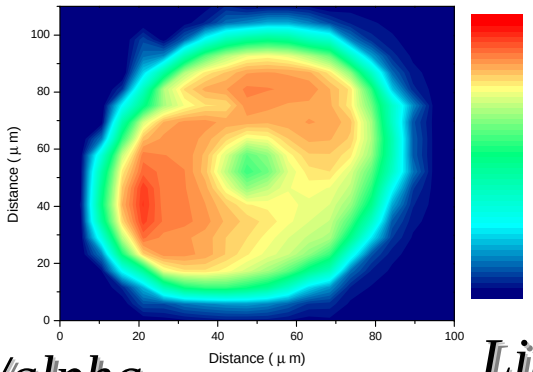
M. Cotte, Ph. Walter and P. Dumas

Mummy from Taklamakan desert: hair

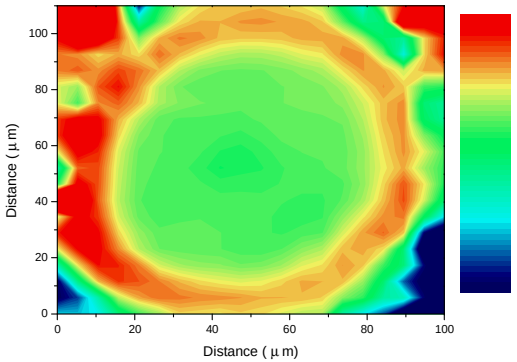




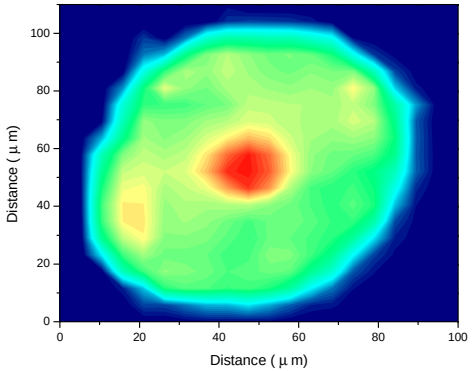
Proteins

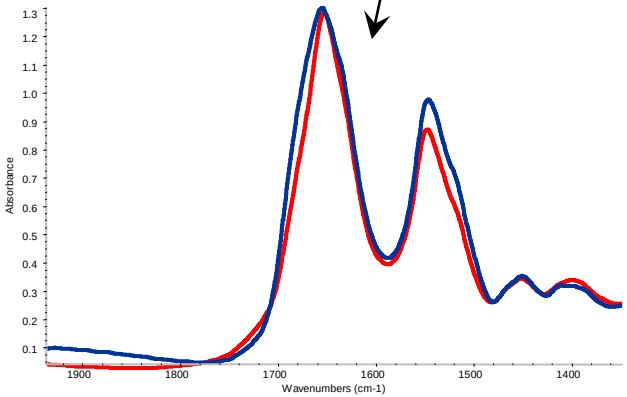


Beta/alpha

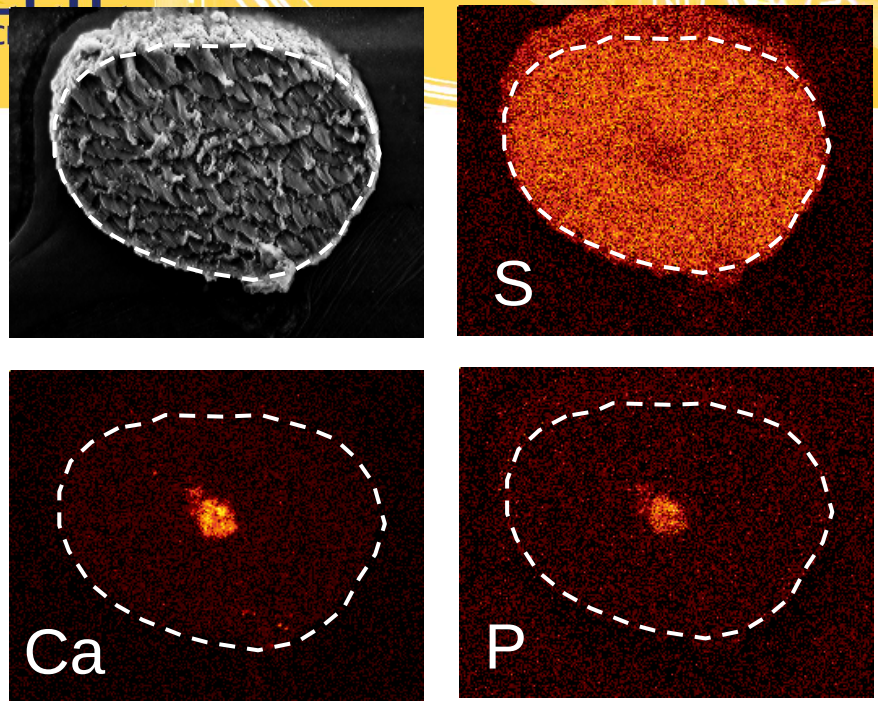


Lipids

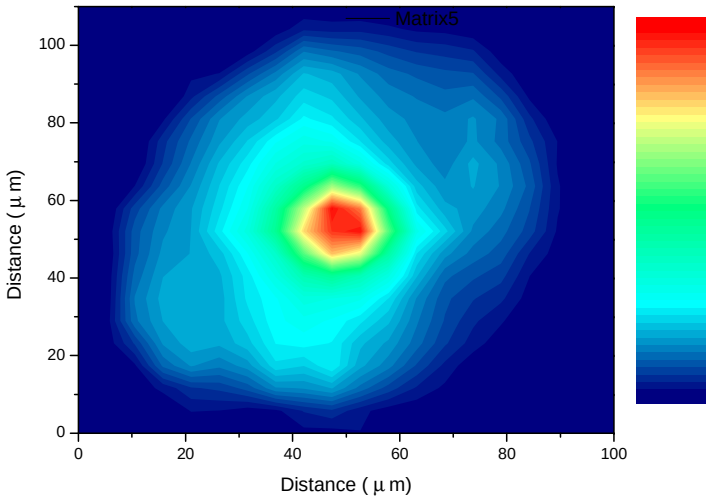


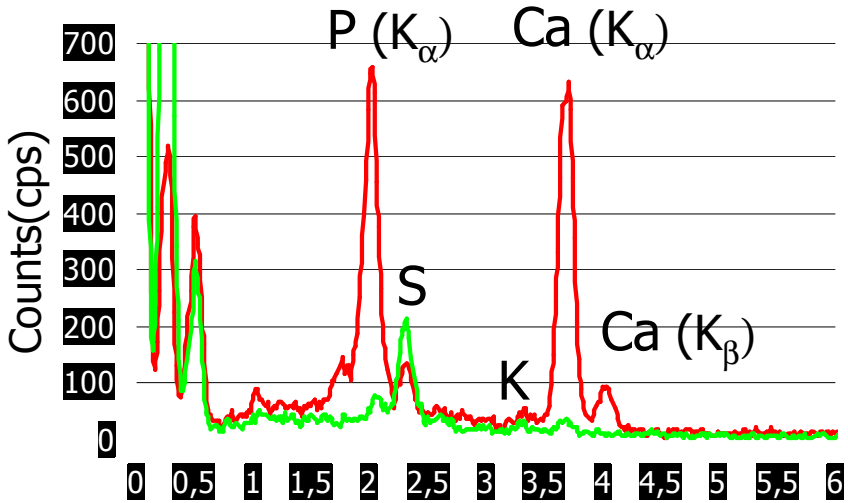


Mummie from Taklamakan desert:hair

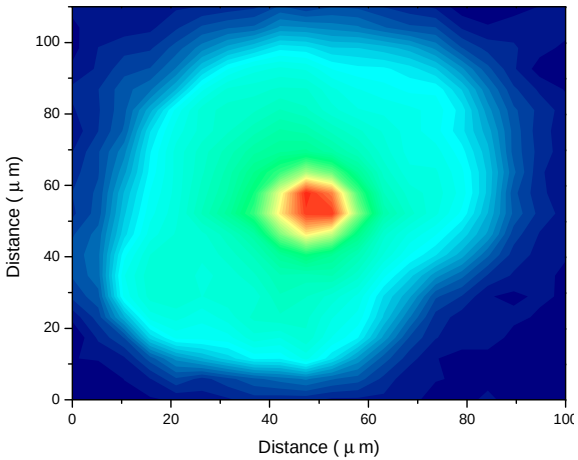


« Phosphate » band at 1250 cm-1





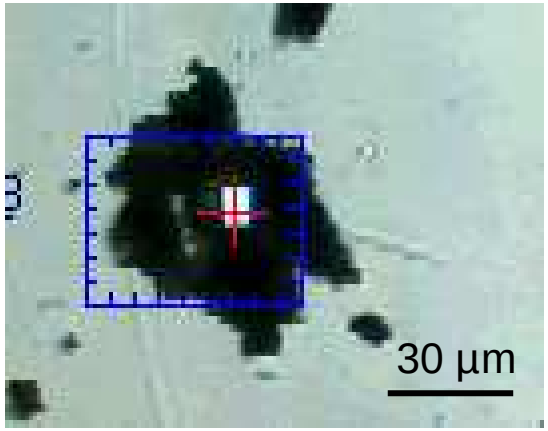
«Hydroxyapatite » band 1000-1100 cm-1



Ancient cosmetics:

Greek pyxide 3rd BC

Reed from Egypt,
13th BC

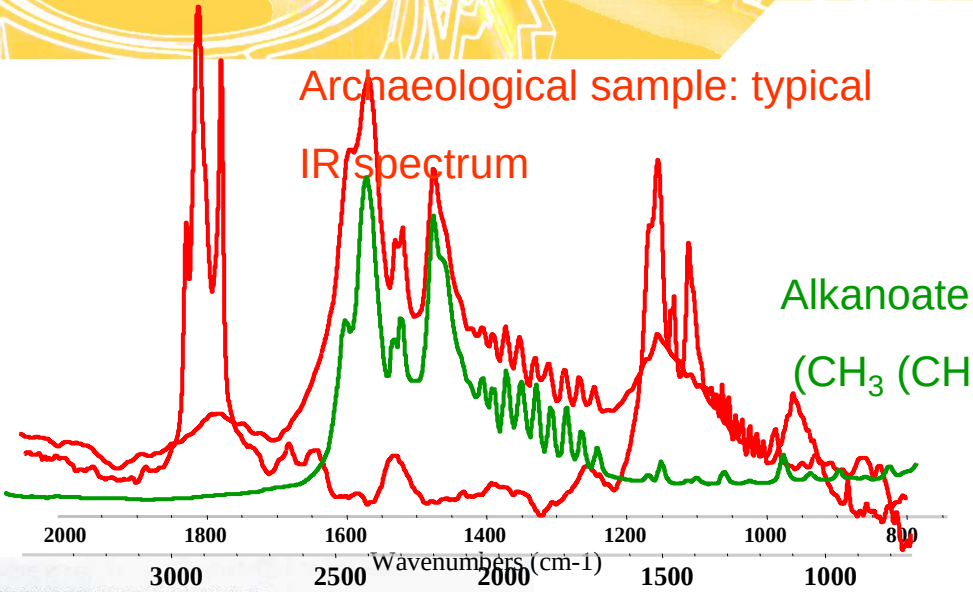


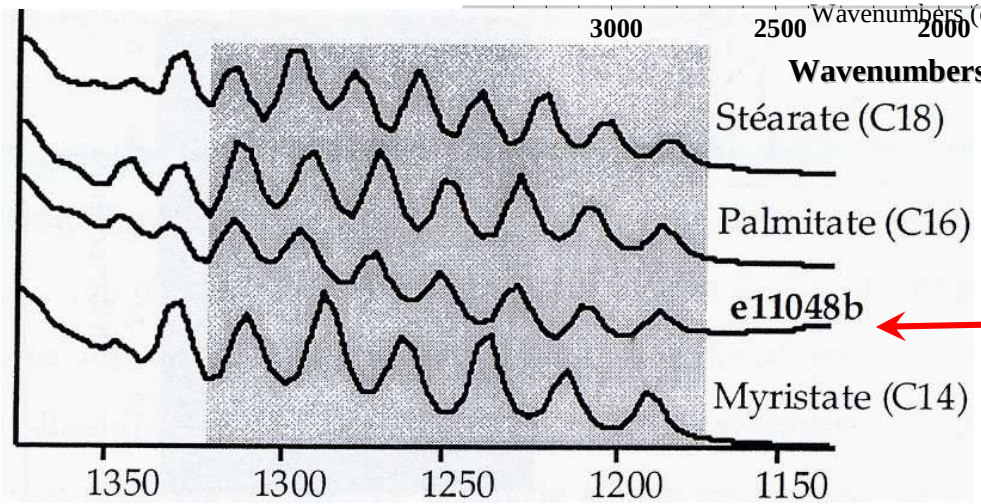
Ancient cosmetics:

Archaeological sample: typical IR spectrum

Alkanoate

$(CH_3 (CH_2)_n CO_2 X)$





8 bands

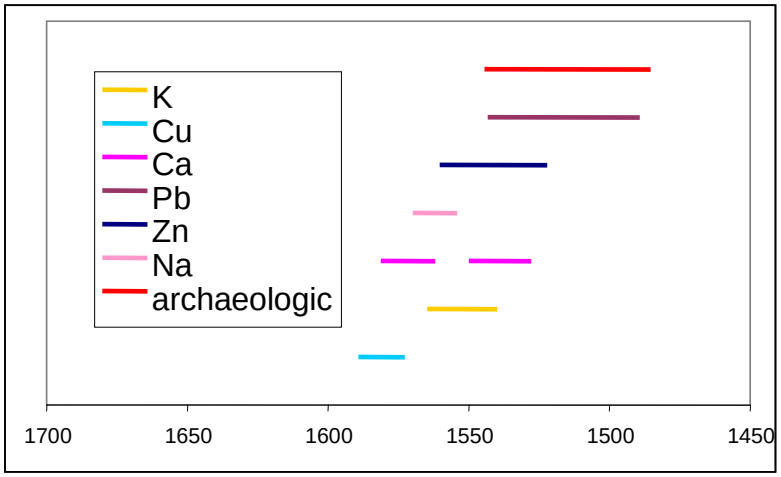
7 bands

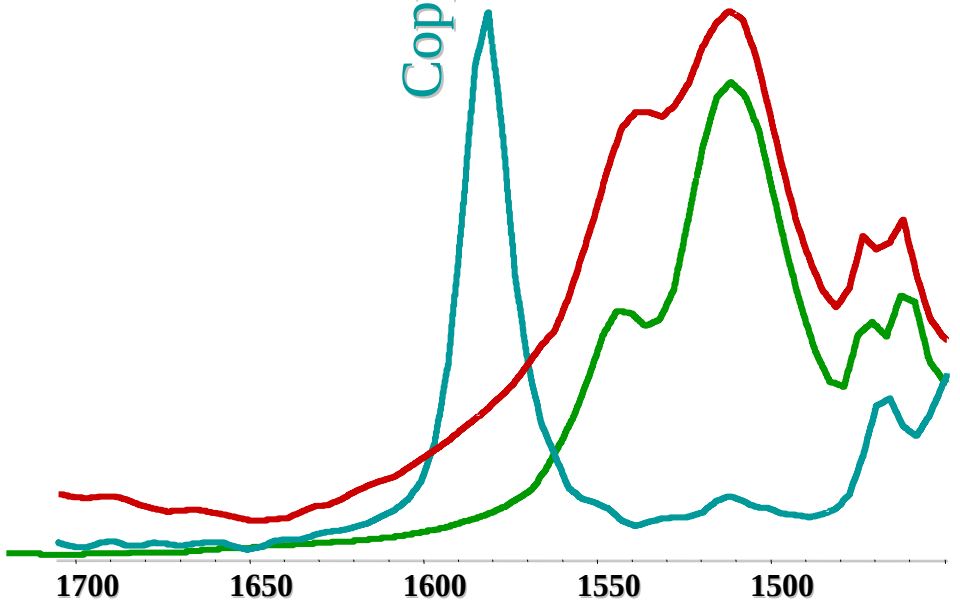
7 bands

6 bands

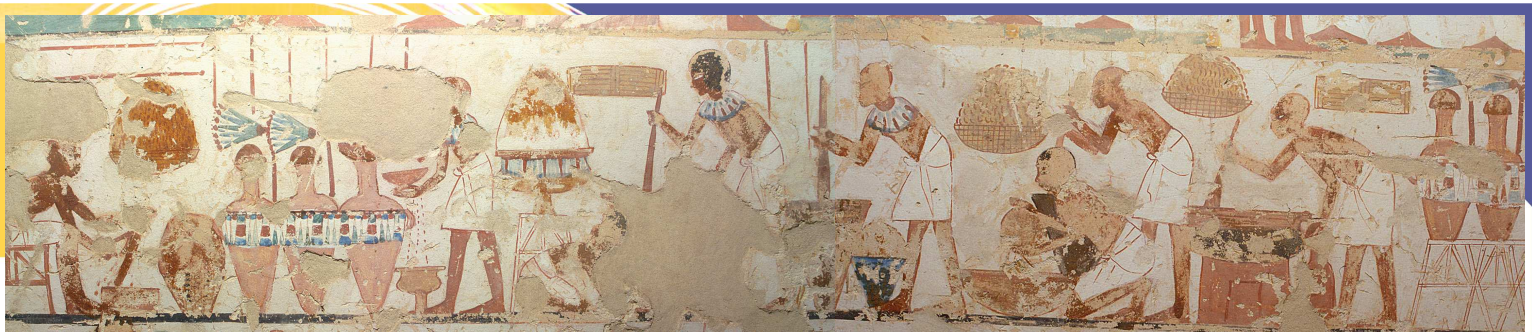
Wavenumbers (cm-1)

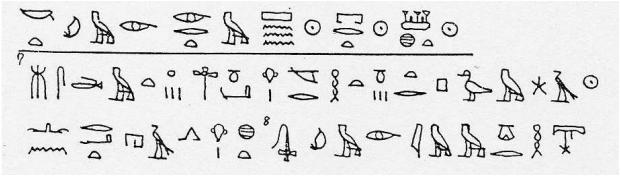
Focus on cation



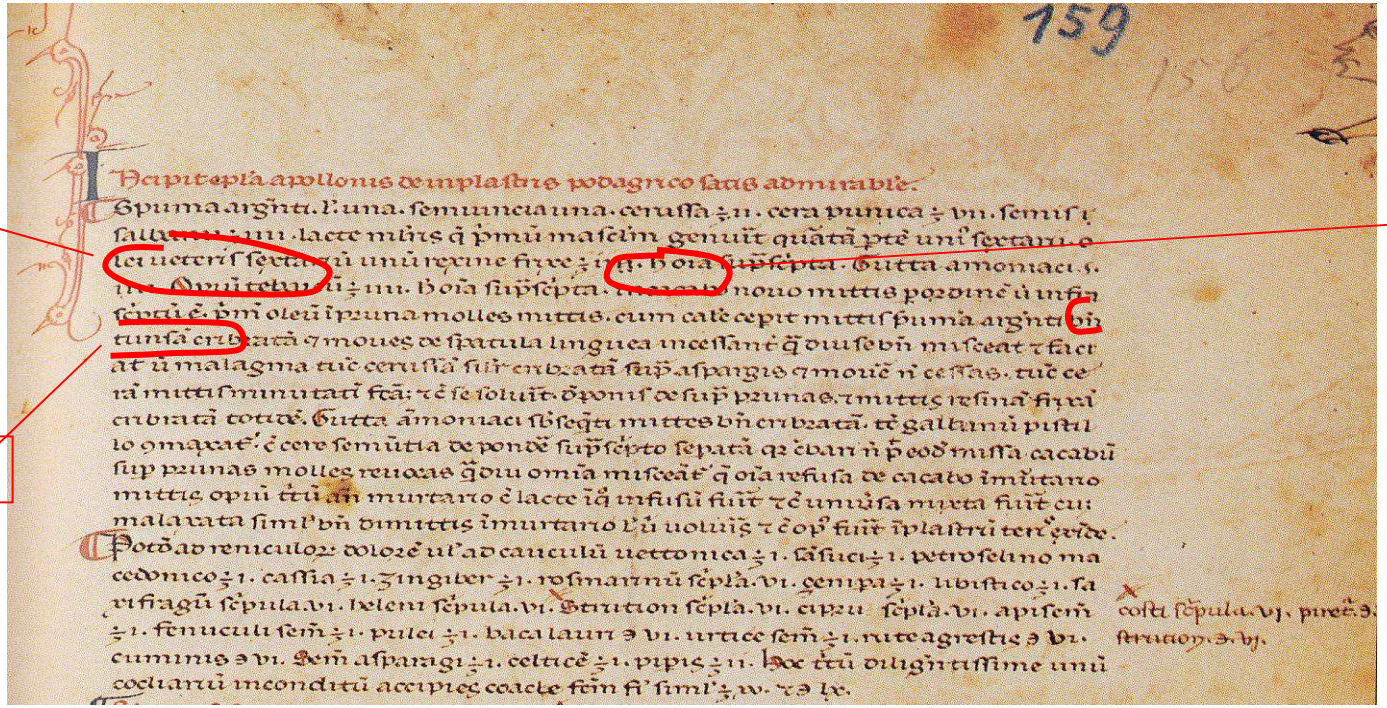


M. Cotte, P. Dumas, G. Richard, R. Breniaux, Ph. Walter
Analytica Chemical Acta 2005 (in press)

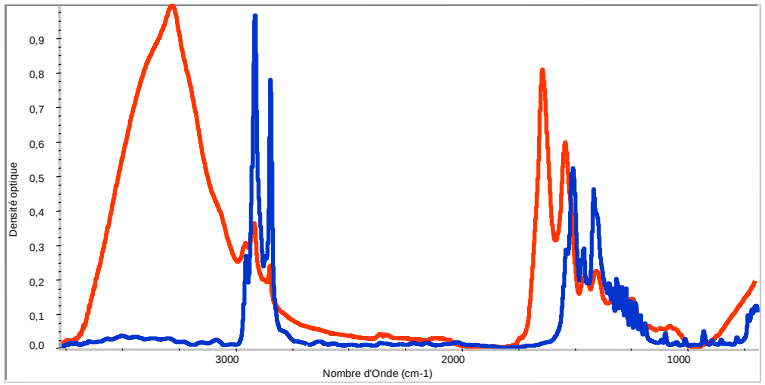




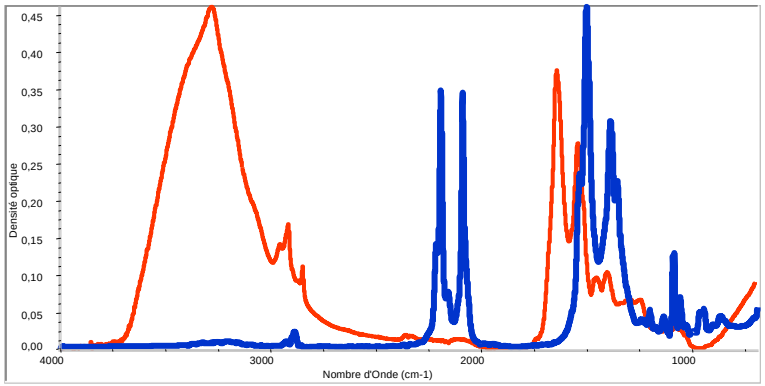
Ebers 389 *make-up to be prepared during summer, winter and flooding season*: galena. It will be ground in goose-*terep* fat, in the morning, without permitting it to drop on fire. Make-up for the night.



Penetration of lipidic solution into skin

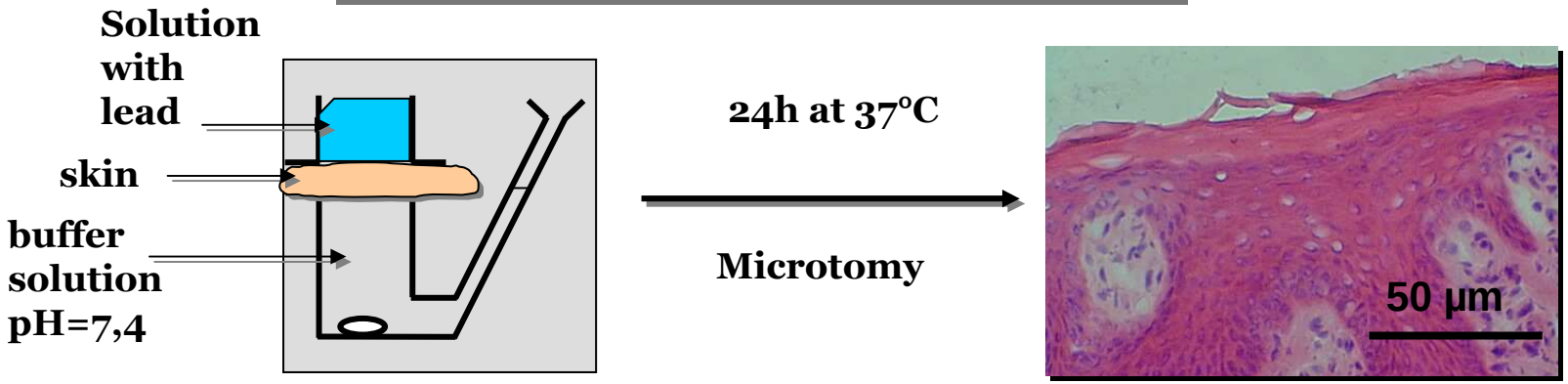


Protonated lipidic chain

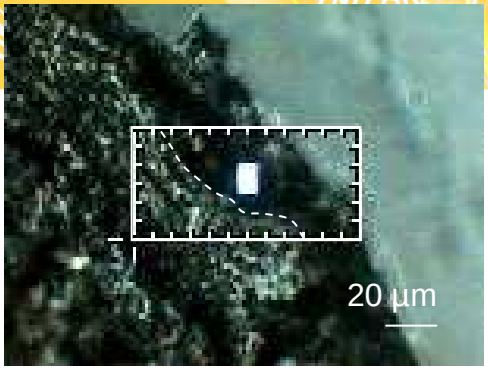


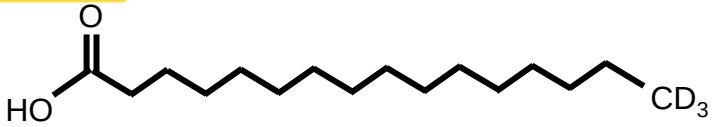
Deuterated lipidic chain

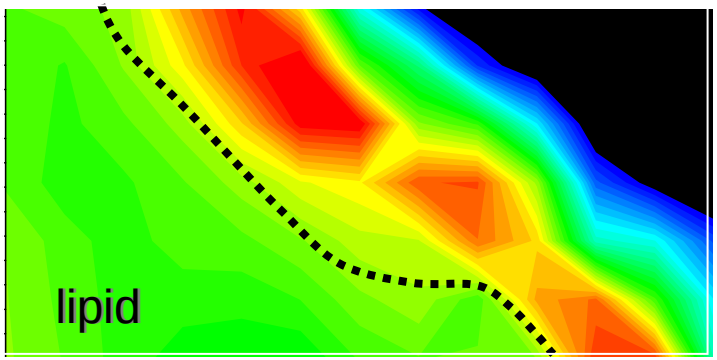
Diffusion in a Frantz-type cell

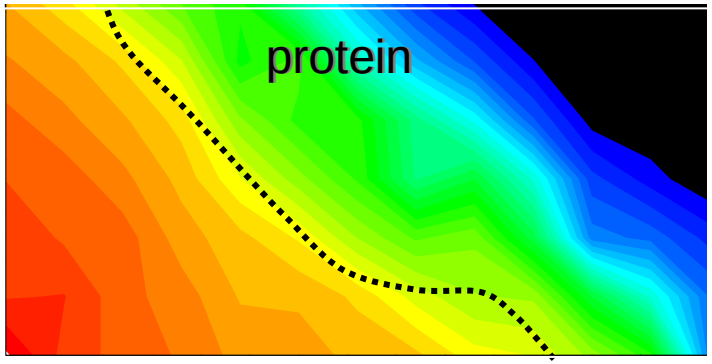


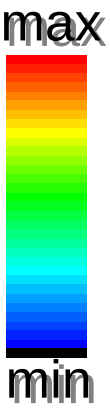
With palmitic acid (16C)…

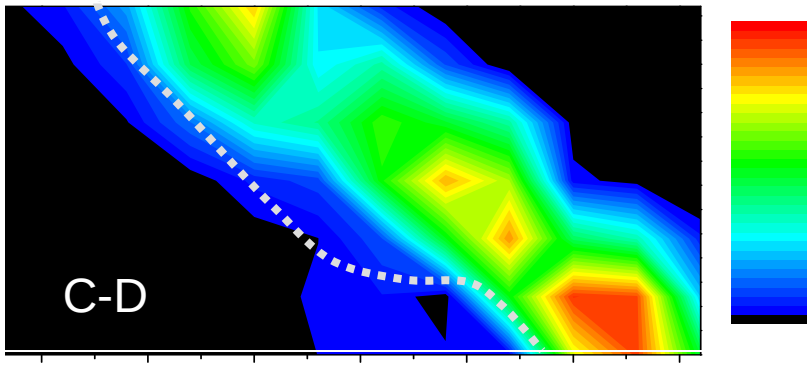




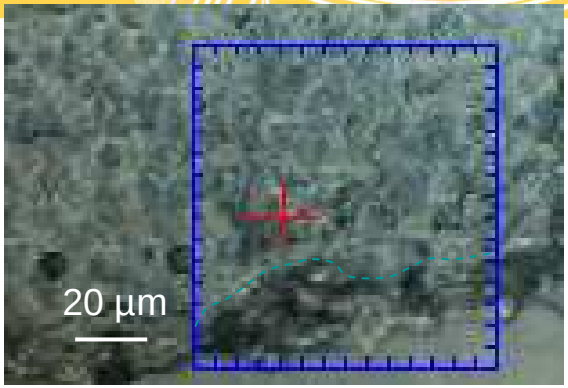


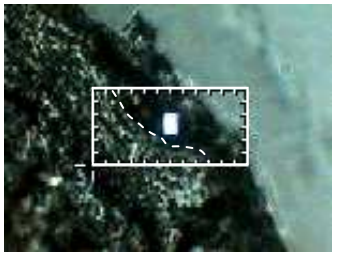


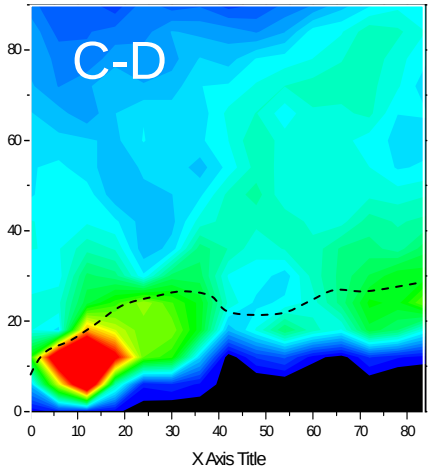




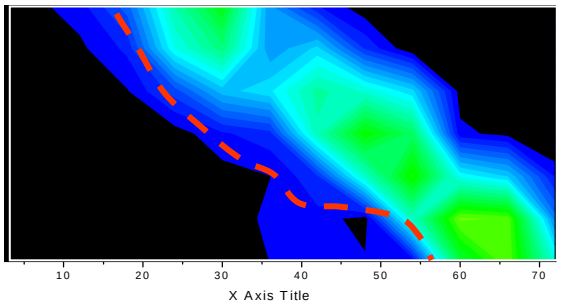
With Myristic acid (14C)…







← same scale →



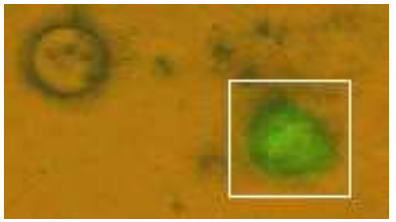
palmitic acid

myristic acid penetrates deeplier than palmitic acid

Summary

- Synchrotron IR microscopy has became an important analytical tool in synchrotron facilities

- Association with fluorescence is desirable



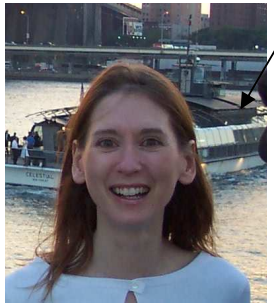
- Good S/N and higher spatial resolution…. Statistical treatment (unsupervised or supervised)

- Complementarities with other synchrotron based techniques are very potential especially if combined studies are performed on the same sample.

Acknowledgments

Special thanks to my long term collaborators:

- G.Larry Carr (NSLS)
- Gwyn P. Williams (JLab)
- Lisa M. Miller (NSLS)
- O. Chubar (SOLEIL)
- F. Polack (SOLEIL)



And

J. Susini (ESRF)
P. Elleaume (ESRF)
K. Scheidt (ESRF)

And many others…