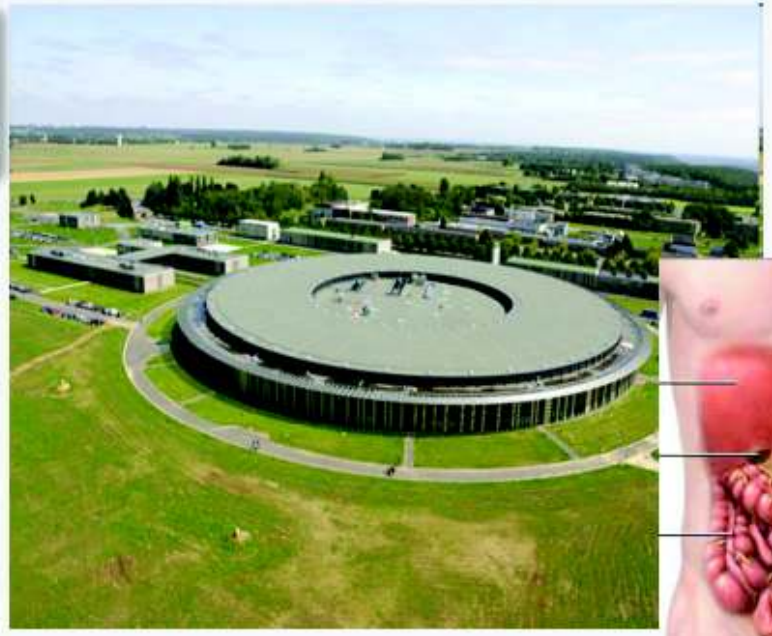
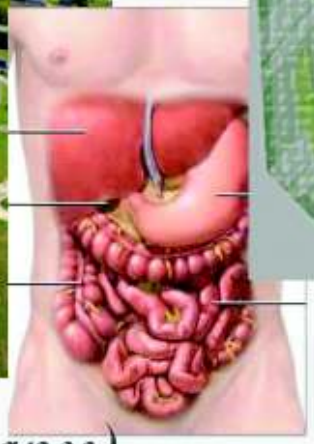
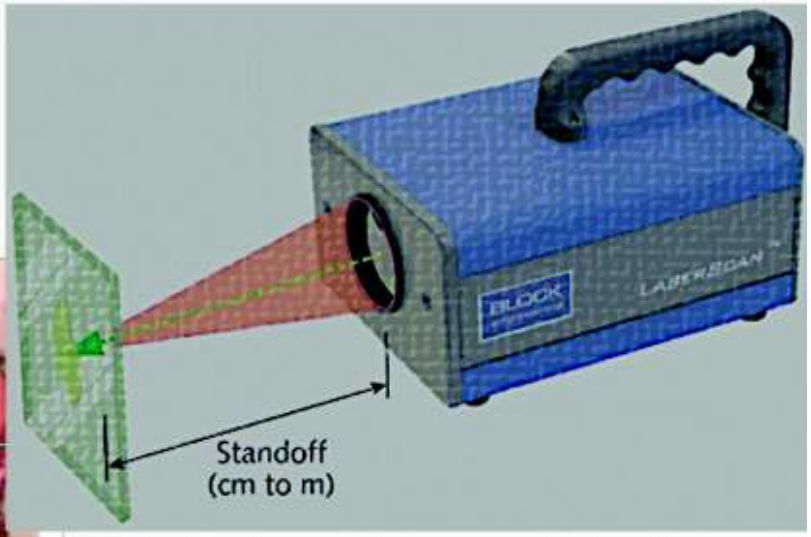




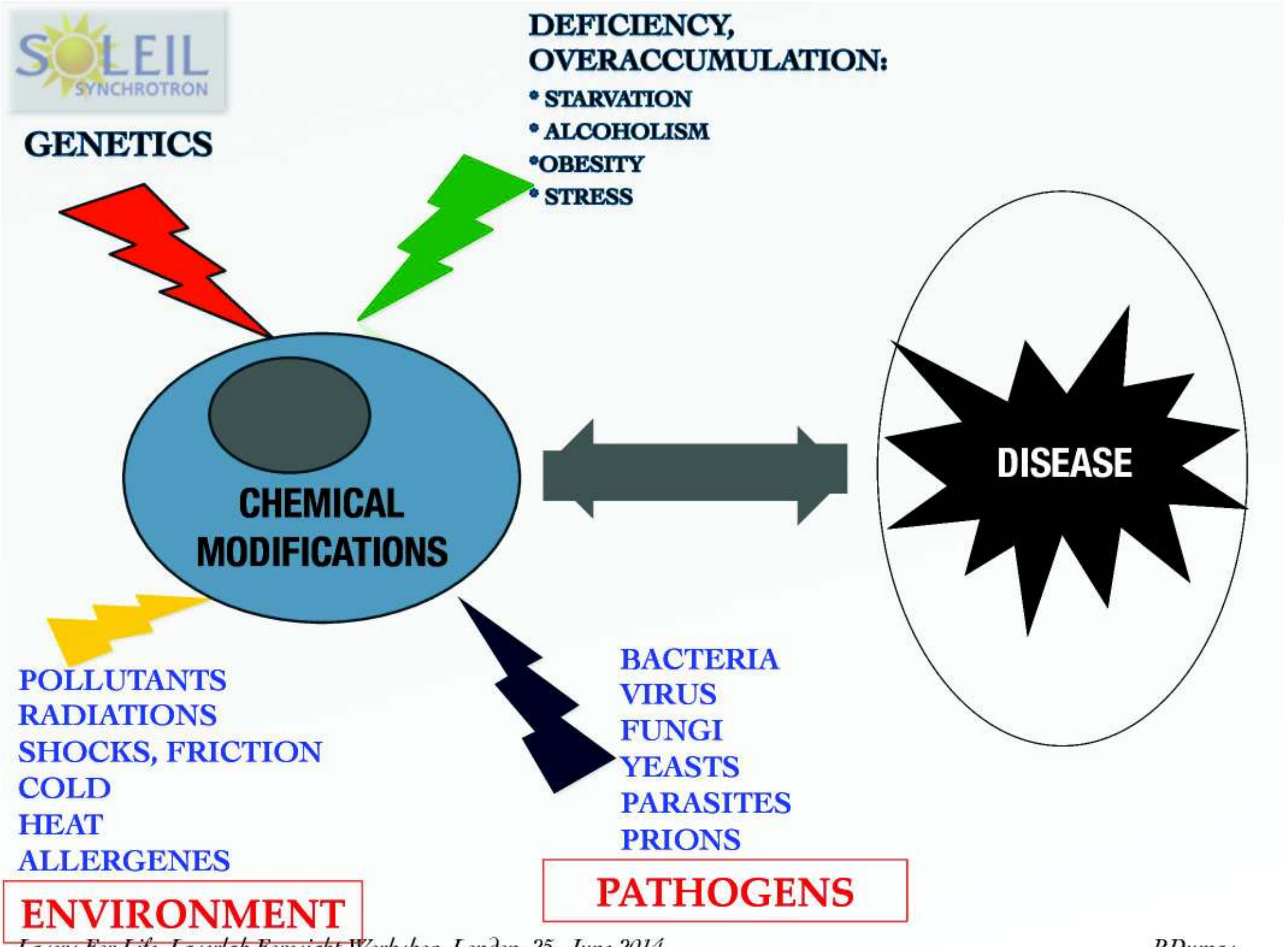


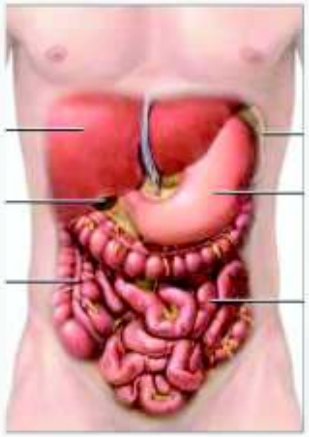
Synchrotron Facility (SOLEIL-France)

IR laser

INFRARED SYNCHROTRONS AND LASERS FOR BIOMEDICAL-RELATED APPLICATIONS

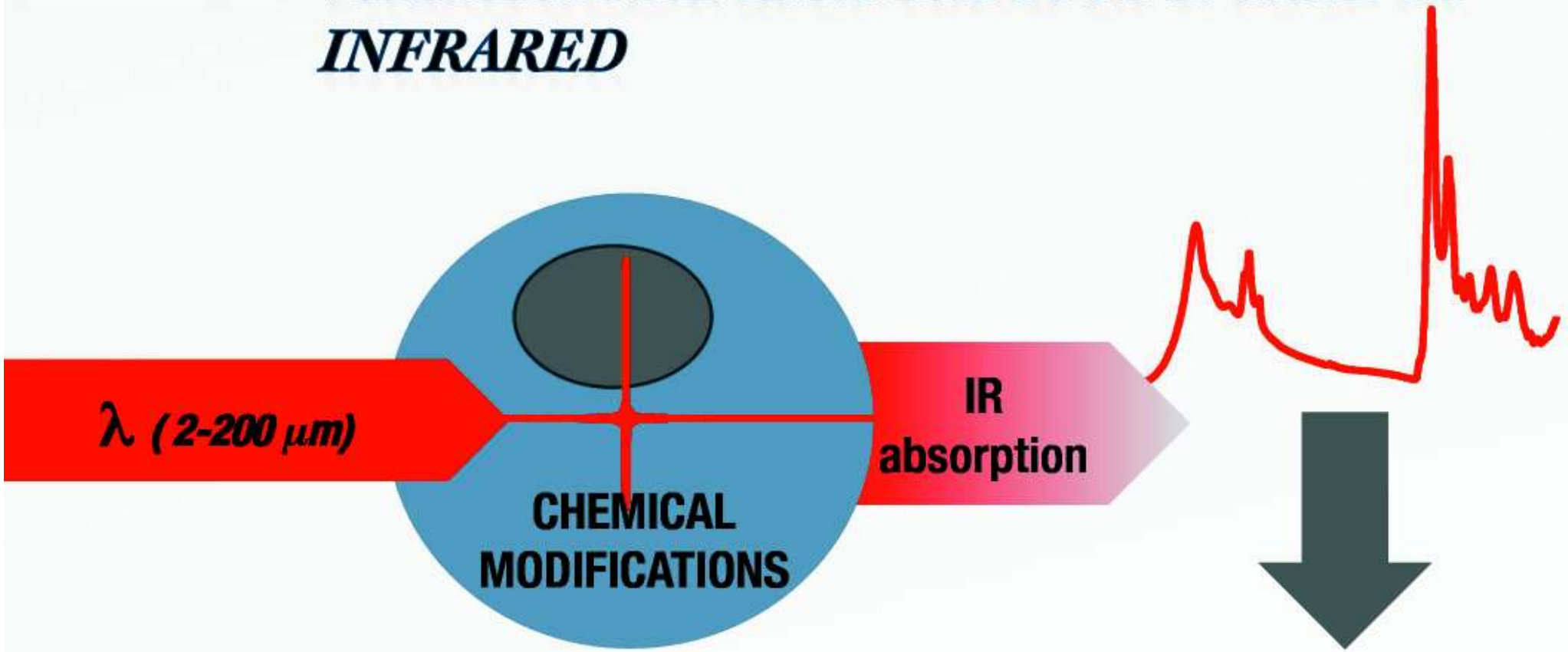
P. Dumas
SOLEIL Synchrotron (France)
dumas@synchrotron-soleil.fr


SOLEIL
SYNCHROTRON
GENETICS
DEFICIENCY,
OVERACCUMULATION:
• STARVATION
• ALCOHOLISM
•OBESITY
• STRESS
CHEMICAL
MODIFICATIONS
DISEASE
POLLUTANTS
RADIATIONS
SHOCKS, FRICTION
COLD
HEAT
ALLERGENES
ENVIRONMENT
BACTERIA
VIRUS
FUNGI
YEASTS
PARASITES
PRIONS
PATHOGENS



BIOCHEMICAL COMPOSITION AND ITS DISORDERS… SPECTROSCOPY TOWARDS DIAGNOSTIC

PROBING MOLECULAR MOTIONS BY VIBRATIONAL SPECTROSCOPY: CASE OF INFRARED

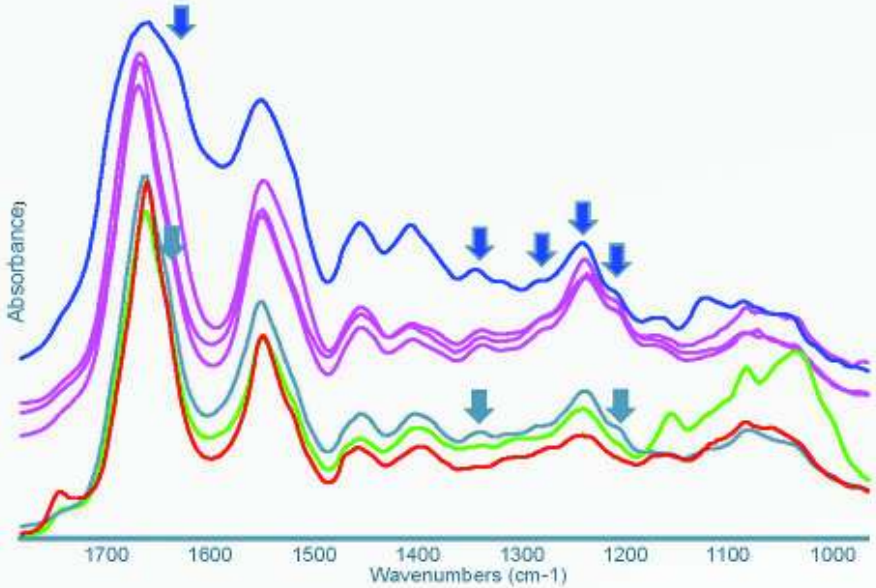


AS TISSUES ARE VERY HETEROGENEOUS, ONE NEED CELLULAR, SUB-CELLULAR PROBE

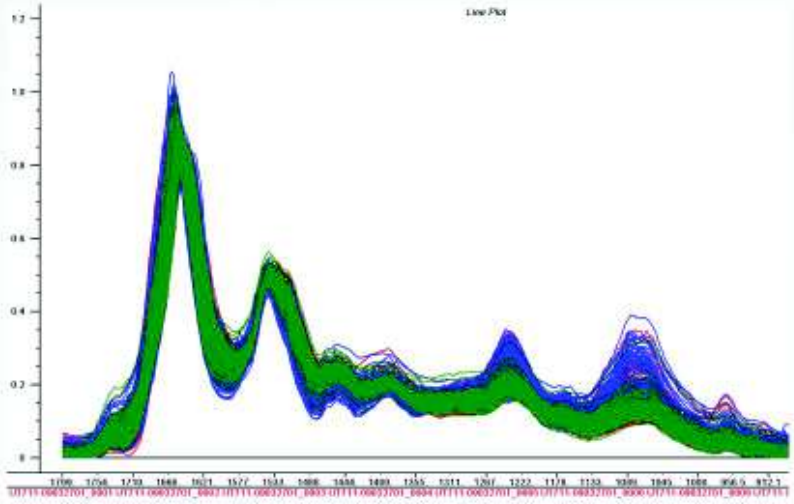
DIAGNOSIS ?
PROGNOSIS ?

INFRARED SPECTROSCOPIC PROBE: THE ESSENTIAL

- Excellent spectral quality (high S/N): tiny changes , but important to detect



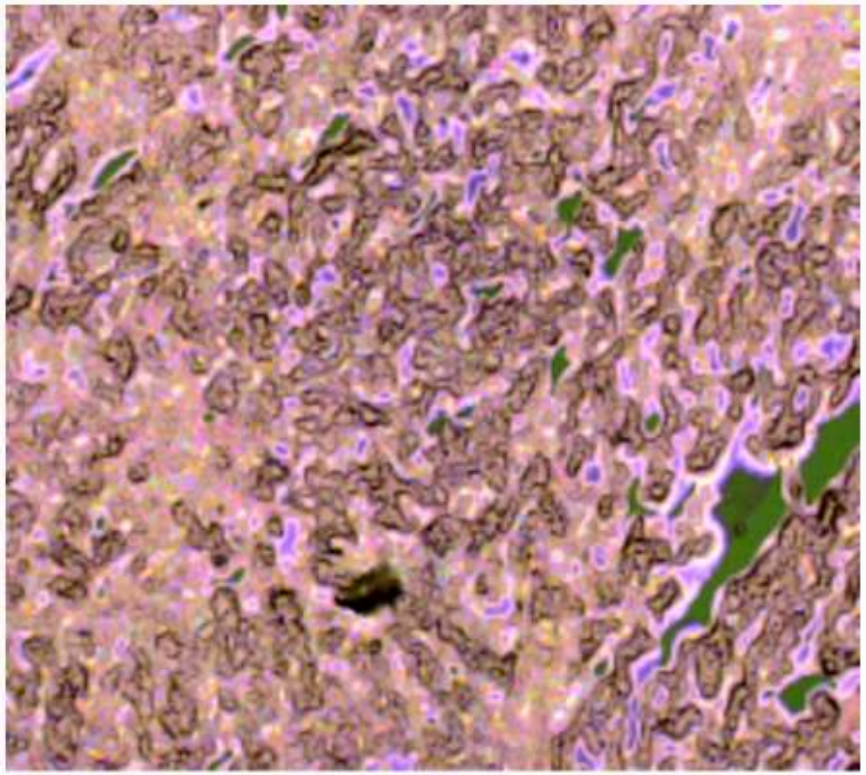
- Data recording as fast as possible: need statistical analysis due to the biological heterogeneity of the analysis



- Tunability (wavelength range)!

INFRARED SPECTROSCOPIC PROBE: THE ESSENTIAL

- Highest spatial resolution possible: require a microscopic analysis, at the limit of the diffraction or better



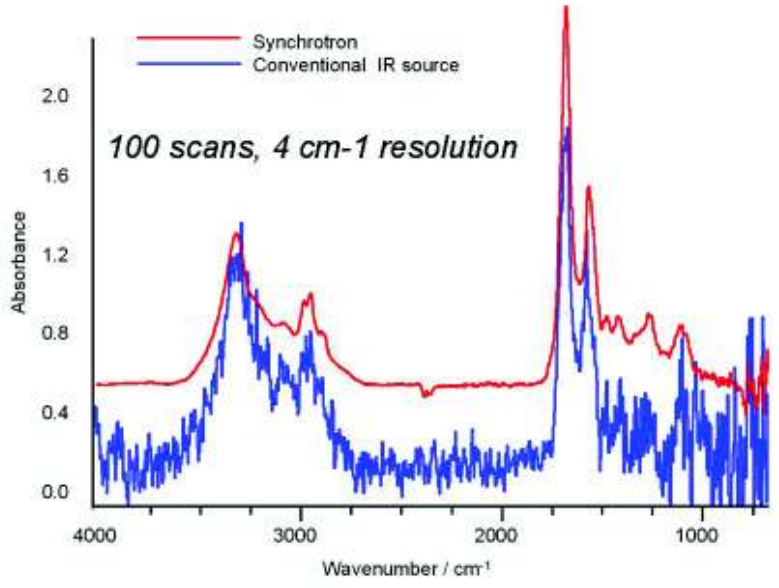
180 µm

- Infrared useful range for chemical analysis = 1 to 50 microns

INFRARED PROBE: NEED HIGH BRILLANCE SOURCE

Synchrotron in infrared frequency domain :

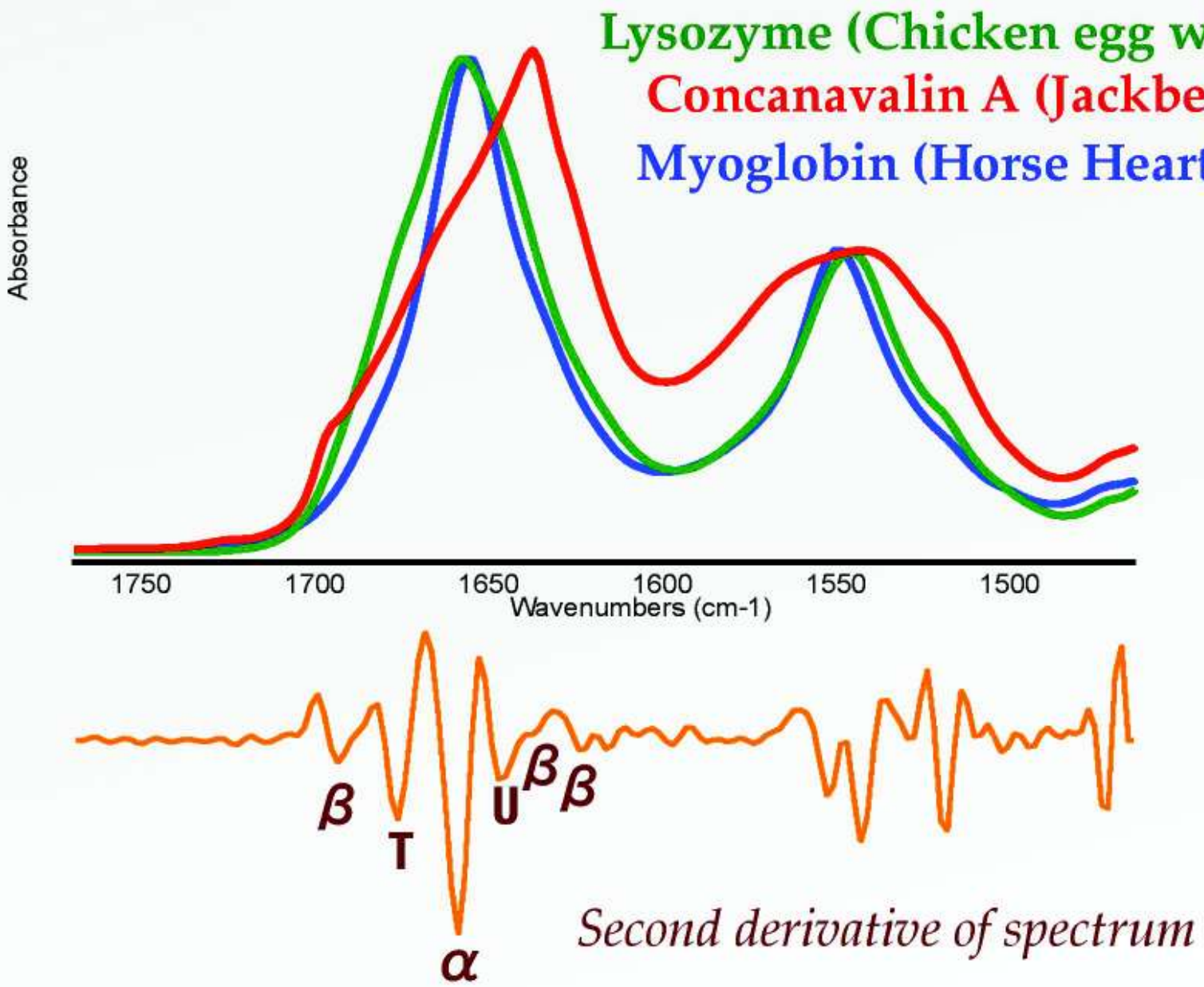
For diagnostic purpose, probably the best appropriate at the moment . Source is very stable, broad band, about two to three orders of magnitude better than laboratory source (globar, or also called thermal source)



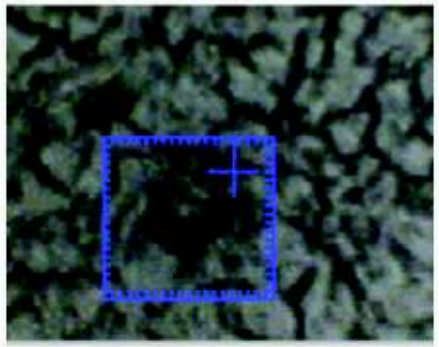
Infrared lasers:

They are becoming more and more available and tunable in a wide energy domain (OPO, QCL). Brighter than synchrotron, they are less stable ... But the best has to come!

SECONDARY STRUCTURE AND INFRARED SIGNATURE

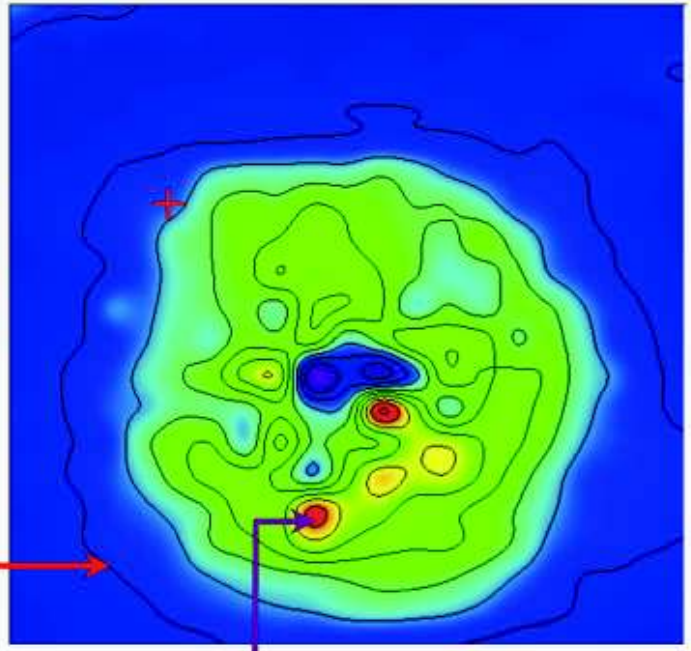


IMAGING SECONDARY STRUCTURE BY SYNCHROTRON-IR MICROSPECTROSCOPY: ALZEIHMER PLAQUE IN BRAIN TISSUES

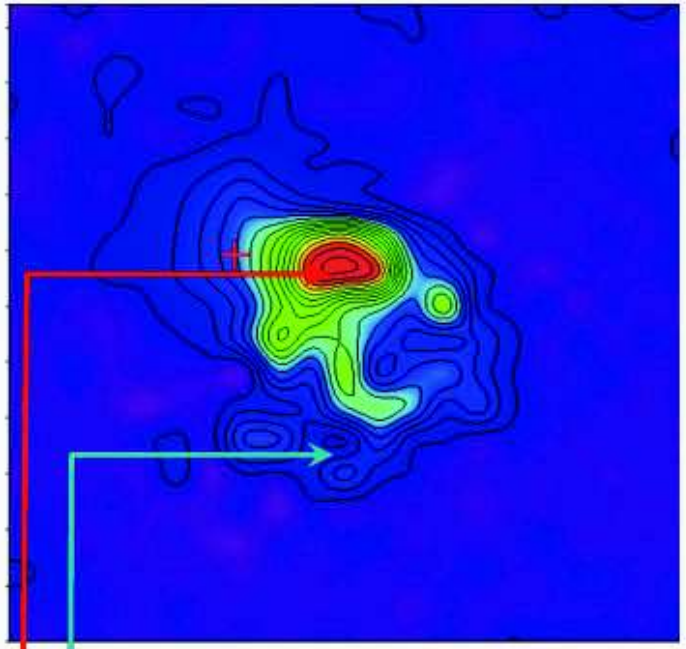


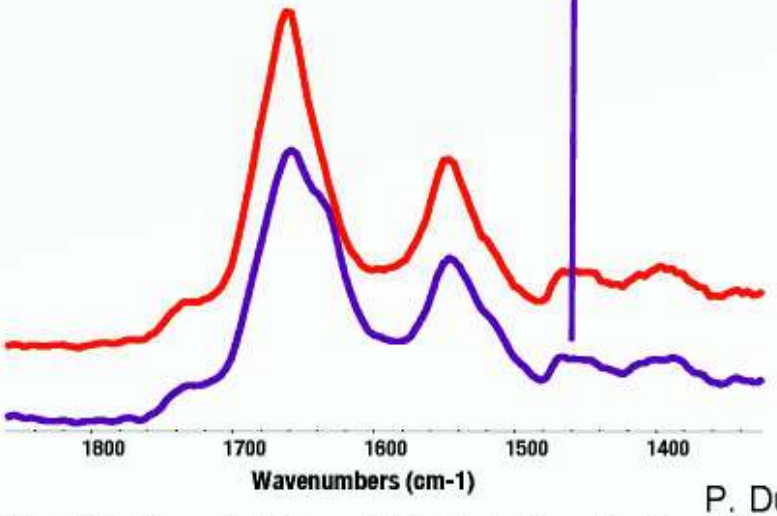
3x3 μm^2

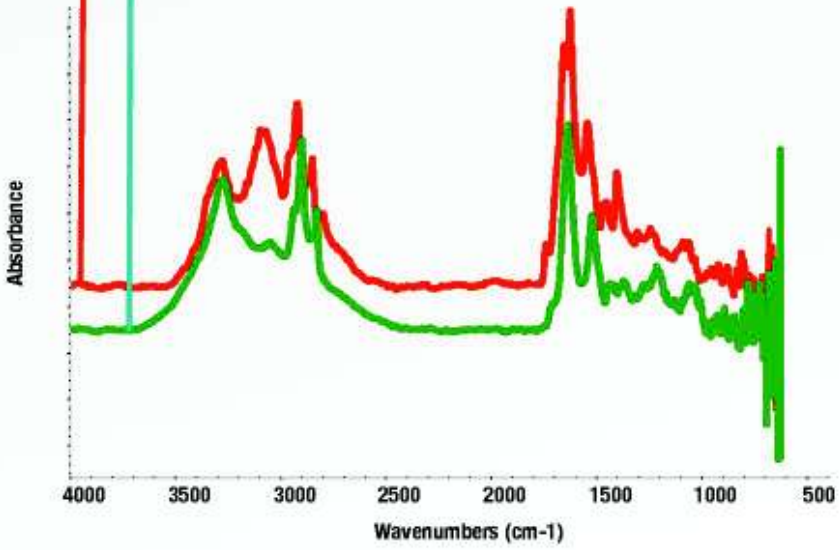
Step: 1 μm



10 µm

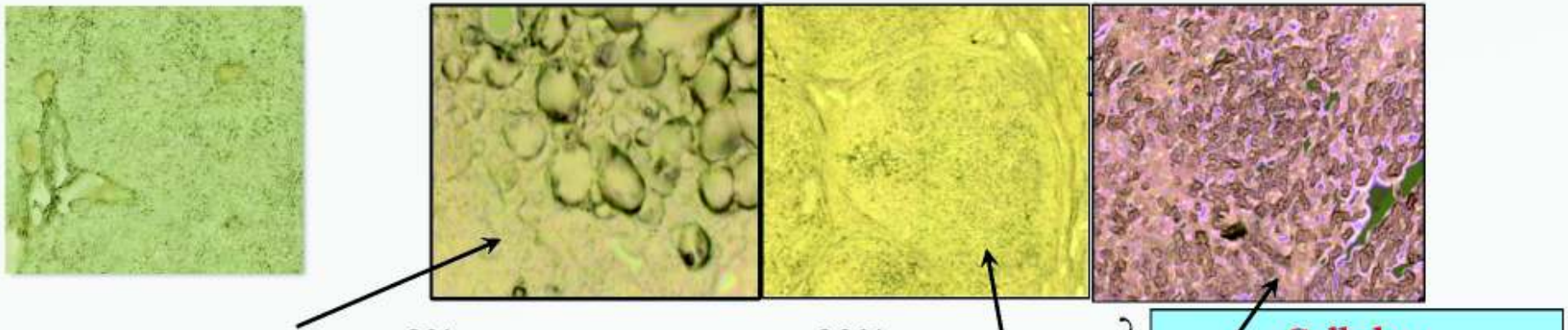






P. Dumas et al.

DISEASES IN LIVER, SYNCHROTRON IR MICROSCOPY A POTENTIAL PROBE?



Steatosis —3%→ Steatohepatitis —26%→ Cirrhosis —?→ Cellular Hepatocarcinoma (CHC)

Accumulation of lipids

+ inflammation NASH/ ASH

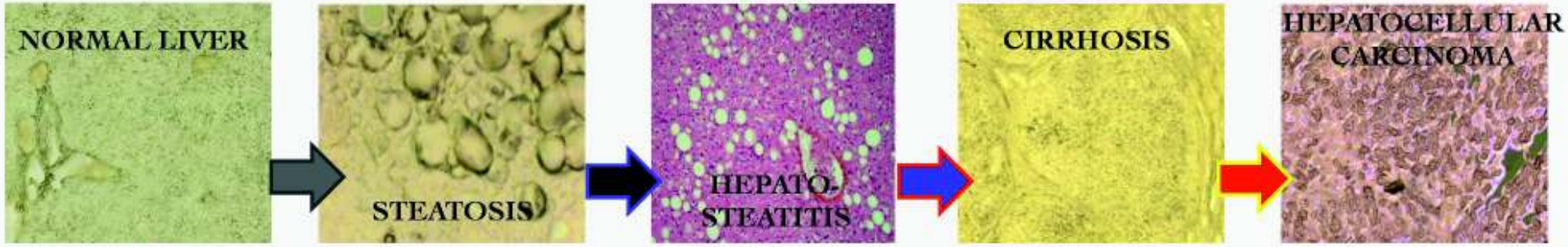
Fibrosis + regenerating nodules

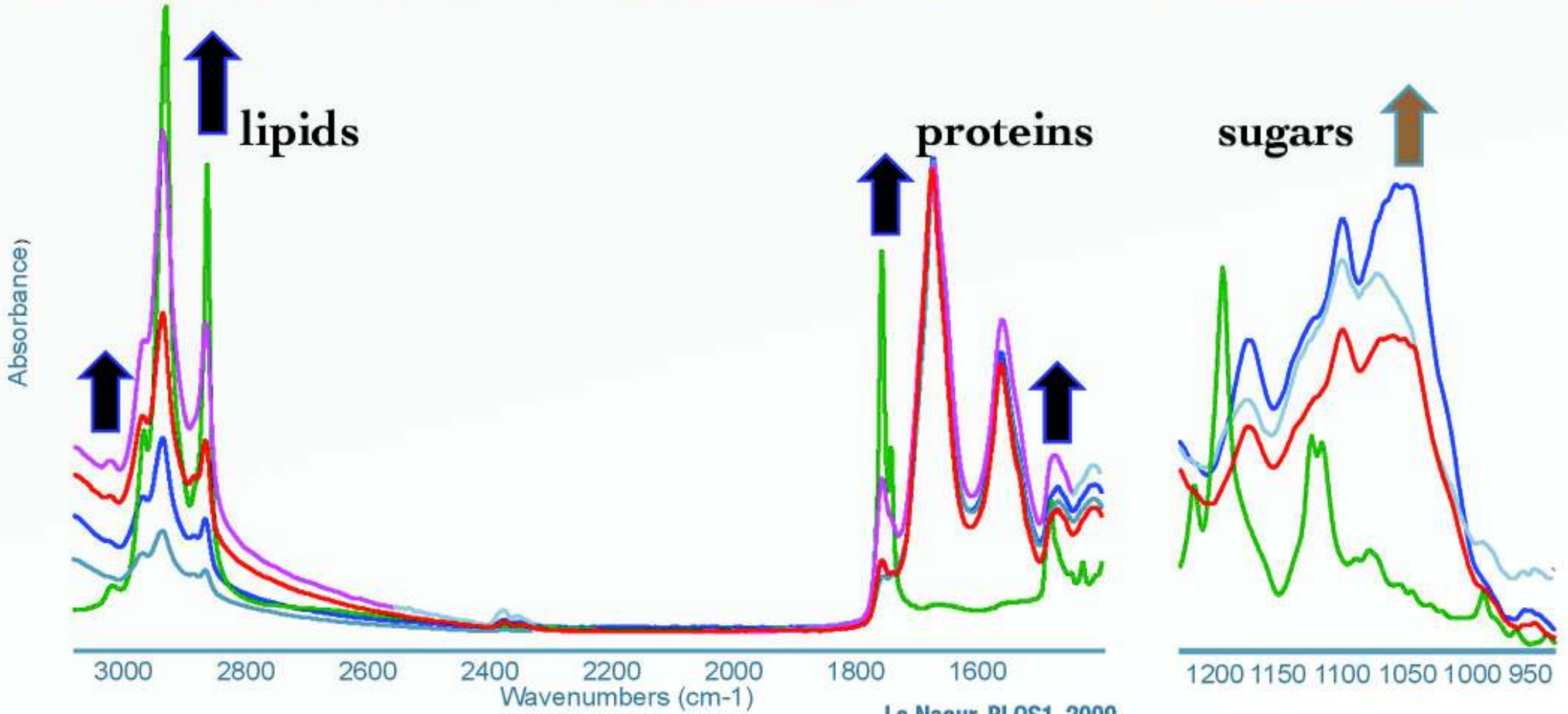
Obesity / Diabete / Drugs

Alcohol

Viruses (HBV, HCV)

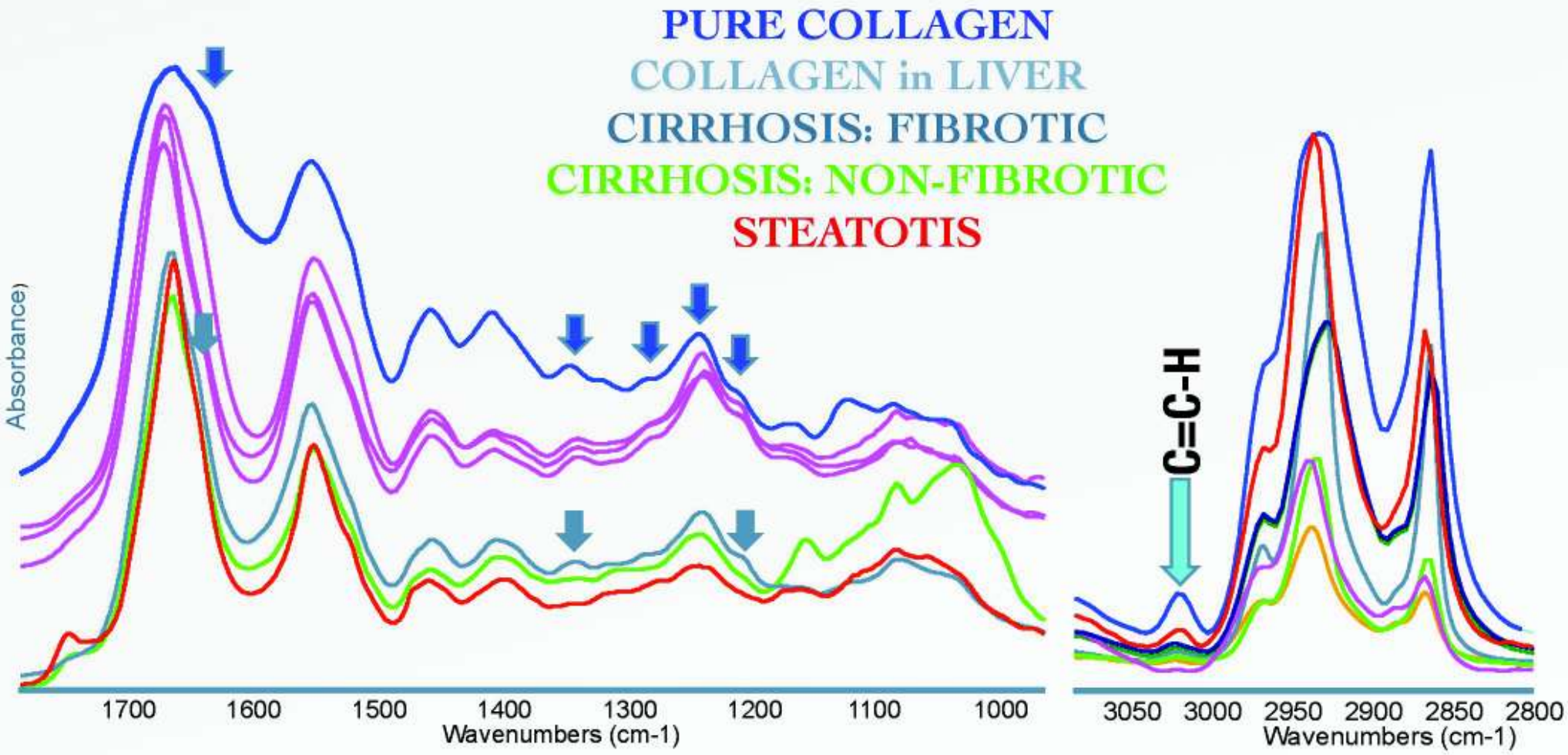
INFRARED SIGNATURES OF LIVER DISEASES



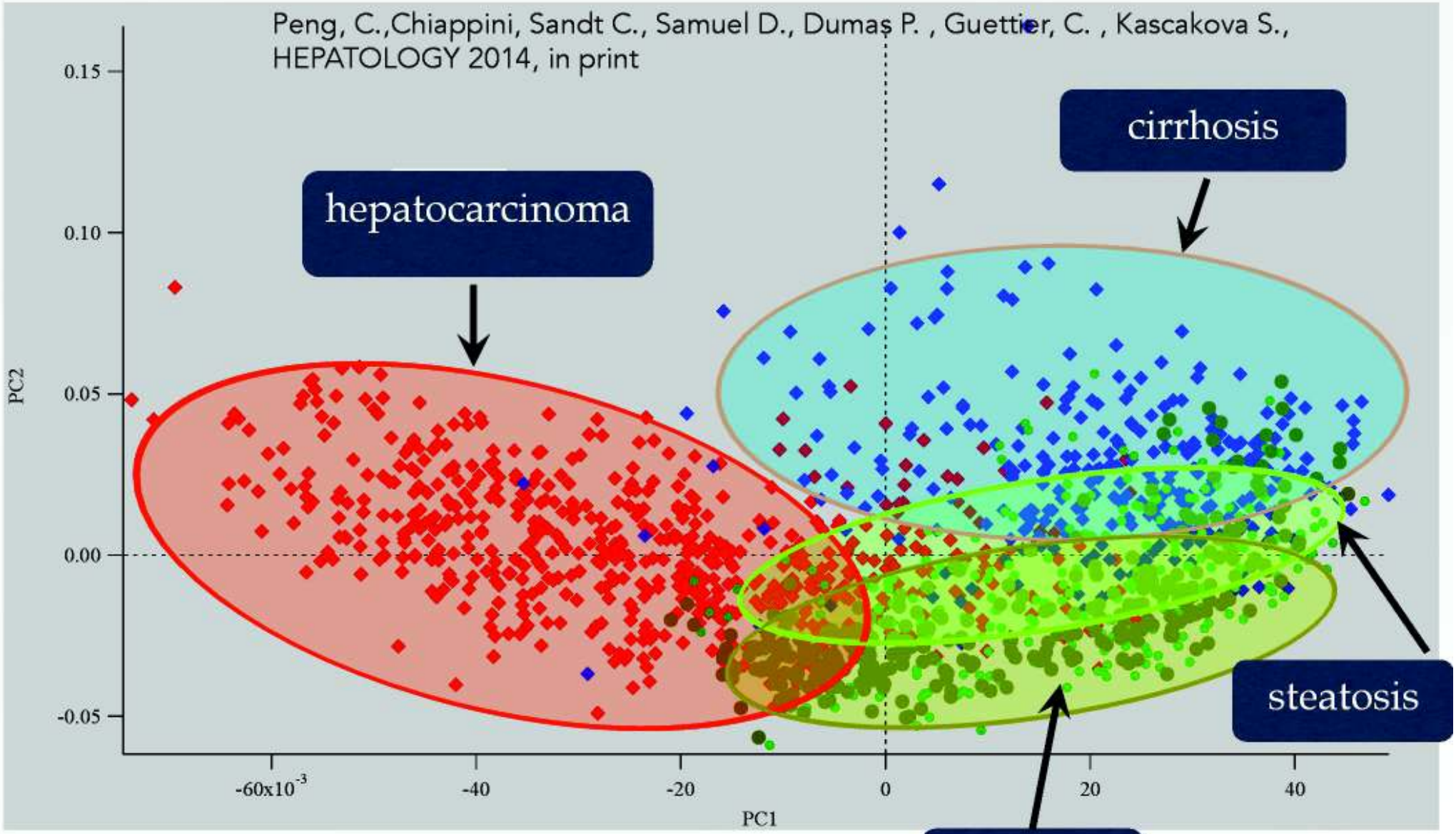


Le Naour, PLOS1, 2009

MORE DETAILS WITH CLOSE LOOK AT SPECTRA



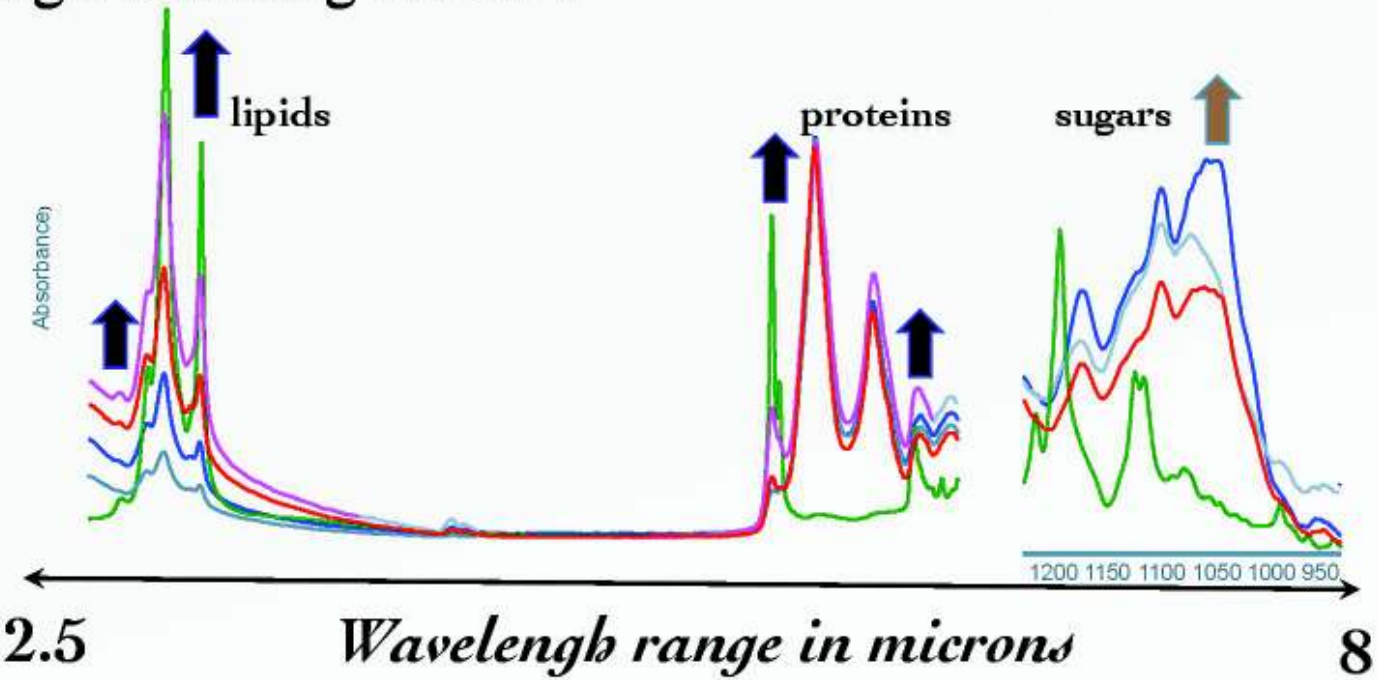
SYNCHROTRON IR DO PROVIDE FINGERPRINT OF VARIOUS DISEASE



IR LASER IMPACT FOR SUCH DIAGNOSTIC?

Not very favorable, even though such analysis in Hospital will speed up the diagnostic.

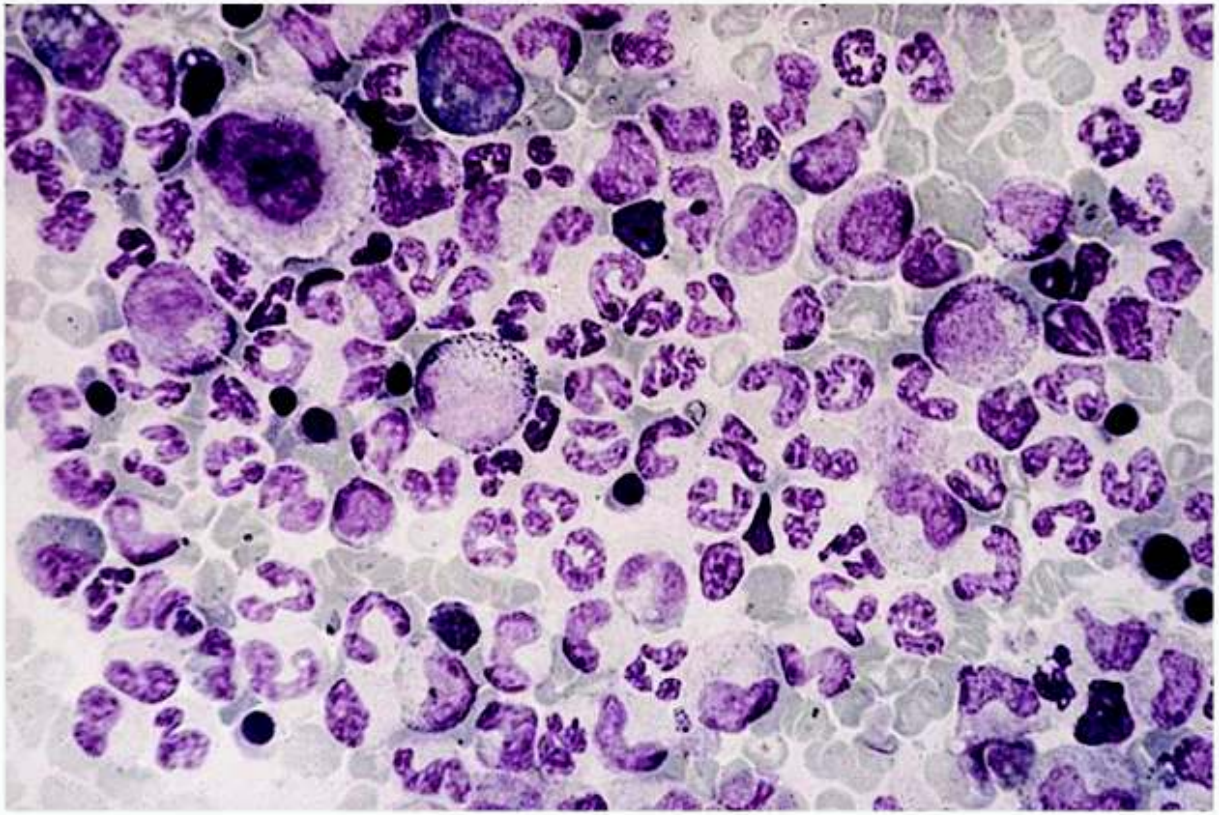
Too wide spectral range scanning needed!



②High S/N spectral, so high stability!

③Highest spatial resolution mandatory? Satisfactory discrimation with thermal source identified

CHRONIC MYELOID LEUKEMIA: IR SYNCHROTRONS AND LASERS CONTRIBUTION?



Professor Ali Turhan
Head of Hematology and Oncology Department , Poitiers Hospital

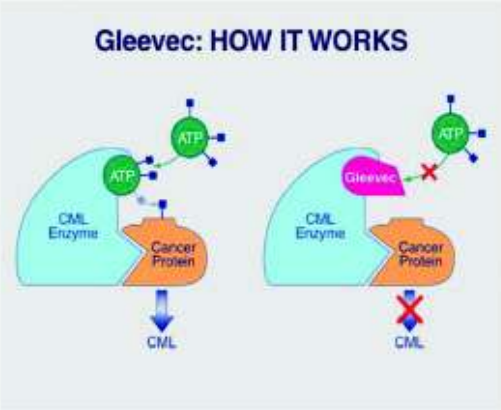
CHRONIC MYELOID LEUKEMIA ?

* Cancer of white blood cells
* Clonal bone marrow stem cell disorder
* Caused by reciprocal translocation of a piece of chromosome 22 on chromosome 9 =>
  - -Phildaelphia chromosome (Ph+)
  - - *bcr-abl* fusion gene

* Bcr-abl is a tyrosine kinase (TK) that activates a cascade of proteins by unregulated phosphorylations => Increased proliferation , diminution of DNA repair => increased DNA damages and mutations

CHRONIC MYELOID LEUKEMIA TREATMENT ?

- Inhibitors of tyrosine kinase (imatinib, dastinib, nilotinib)



- Resistance: the T315I mutation (in the imatinib fixation site) renders bcr-abl resistant to all first generation TK inhibitors

  The frequency of the mutation can be as low as 1 cell in 100,000 and as high as 40%

CHARACTERIZATION OF A DRUG RESISTANCE PHENOTYPE

3 CELL MODELS STUDIED :

- MURINE EMBRYONIC STEM CELLS (PROGENITOR CONTEXT)
- GENETICALLY ENGINEERED UT7 CELLS; STABLE BCR-ABL EXPRESSION
- GENETICALLY ENGINEERED UT7 CELLS: REPRESSIBLE BCR-ABL EXPRESSION

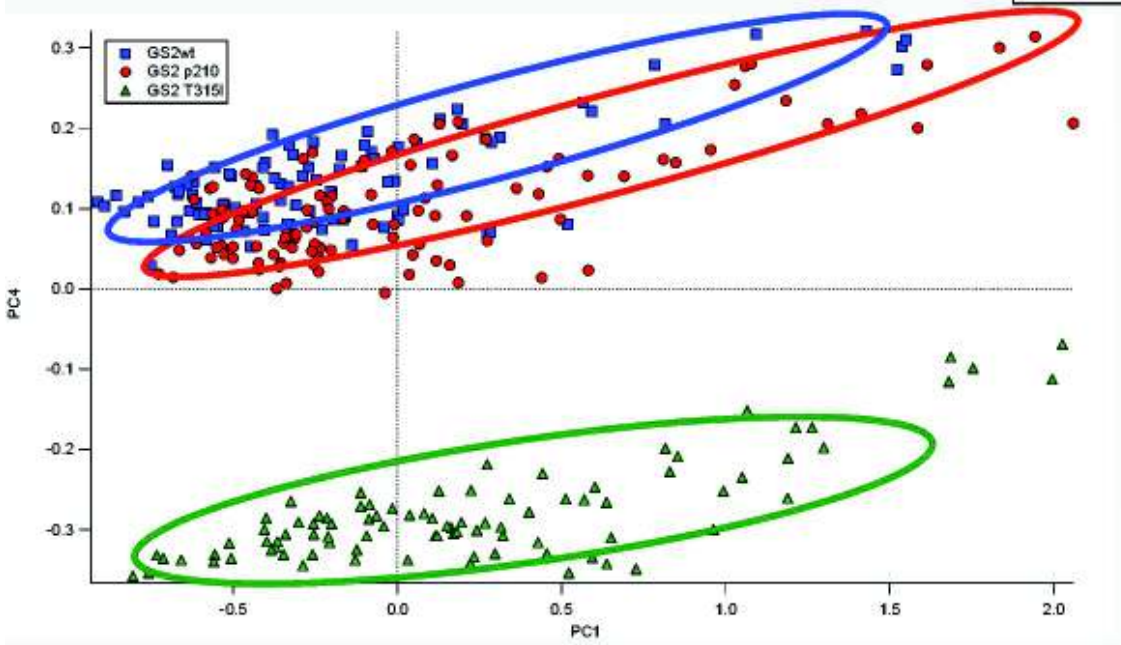
Féraud O., Turhan A., Sandt C., Dumas P.
INSERM, CNRS, SOLEIL

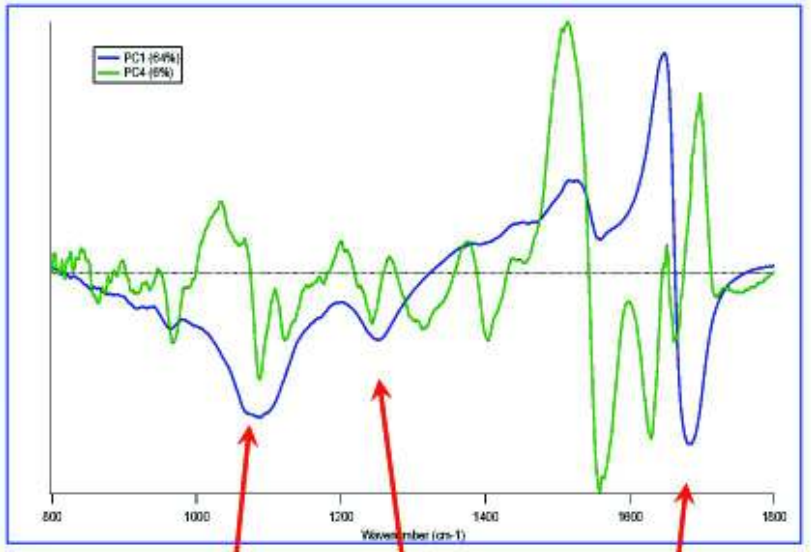
MURINE EMBRYONIC STEM CELLS GS2

GS2

GS2 transfected by the wild-type bcr-abl p210

GS2 transfected by the mutant bcr-abl T315I





Sugar, DNA?

Phosphate

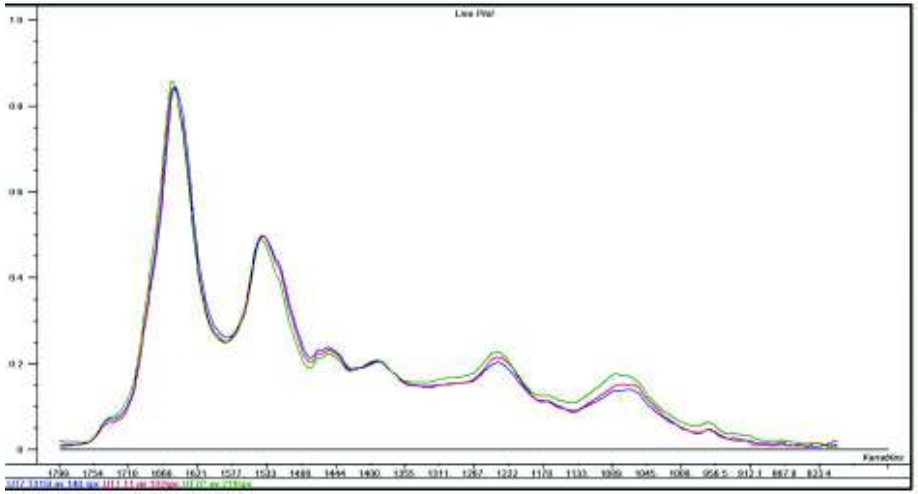
Secondary structure

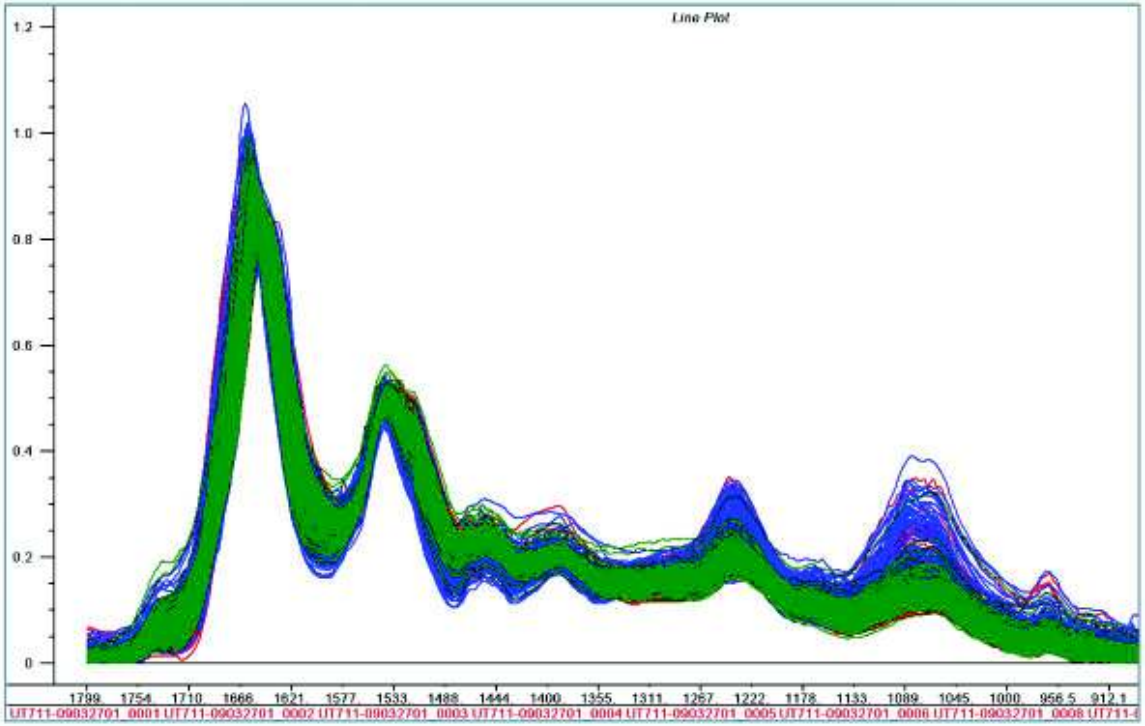
WITH HAEMATOLOGICAL CELL LINE UT7

UT7P: NO *bcr-abl* EXPRESSION

UT7-11: STABLY TRANSFECTED WITH WILD TYPE *bcr-abl*

UT7T315I: STABLY TRANSFECTED WITH T315I *bcr-abl* MUTANT





Ensemble of IR spectra

WHAT CAN WE CONCLUDE FROM SYNCHROTRON IR?

- IT IS POSSIBLE TO DETECT A SPECIFIC SIGNATURE OF *bcr-abl* EXPRESSION IN DIFFERENT CELL CONTEXTS (MURINE, EMBRYONIC STEM CELLS, HUMAN, HAEMATOPOIETIC CELLS)

- SPECIFIC SIGNATURES OF THE T315I MUTANT IS DETECTABLE

- THIS SUGGESTS HIGHER PHOSPHORYLATION RATE OR DIFFERENT TARGETS FOR T315I

- IT IS POSSIBLE TO DETECT THAT SPECIFIC MUTATION IN HAEMATOPOIETIC PROGENITORS FROM CML PATIENTS IN SINGLE CELLS => DIAGNOSTIC TOOL ? (FIRST RESULTS ARE PROMISING)

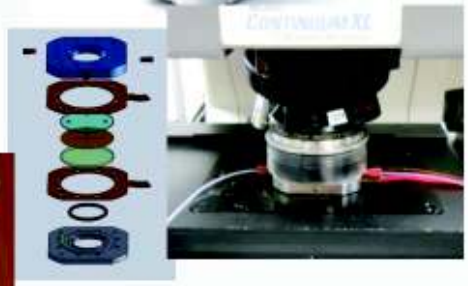
CAN LASER IR PLAY AN IMPORTANT ROLE?

- Signatures are in three spectral domain: Sugar (around 10 microns wavelength),Phosphate (around 8 microns) and amide I (around 6 microns)
- Probably one could use only one probe (the strongest = amide I) @ 6 microns
- Tunable IR laser around 6 μm, linear array detectors seem one way to bring the diagnostic to Hospital. Laser and spectral quality ? Cell sorter and fast data recording? Quick diagnostic on circulating blood cells from patients?

HOW LASER IR CAN BE IMPLEMENTED?

Infrared laser

Microscope



Circulating microfluidic cells

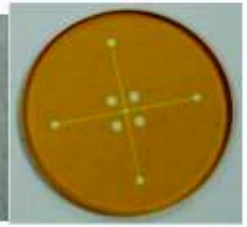
Linear array detector

ONE CRUCIAL ENVIRONMENT FOR DIAGNOSTIC…

In-situ studies, both for spectro-microscopy and imaging of fully hydrated cells at sub-celullar resolution

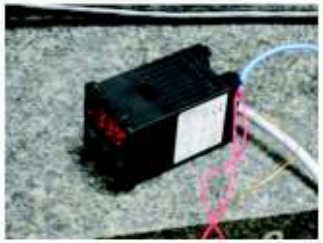
MICROFLUIDIC DEVICE

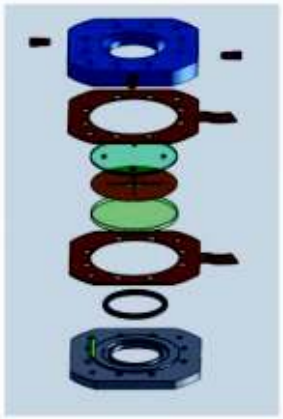
PTFE SPACERS FOR SIMPLE APPLICATIONS

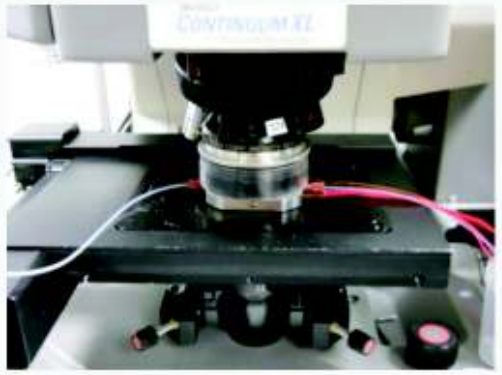


LITHOGRAPHIED SPACERS FOR MORE COMPLEX APPLICATIONS

Temp. control

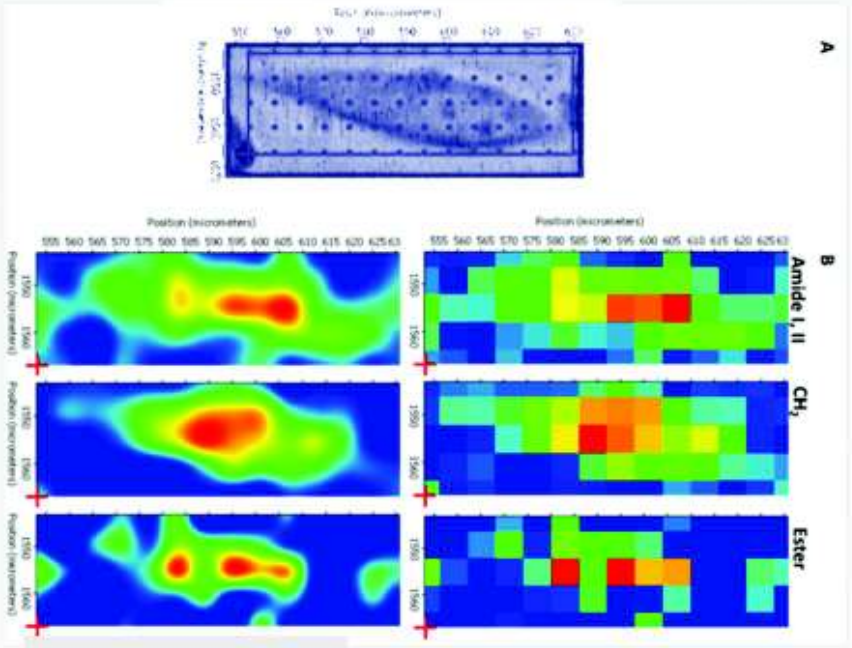


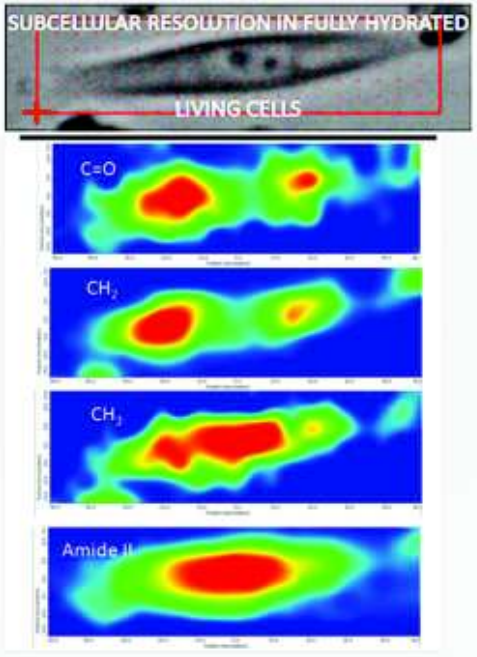




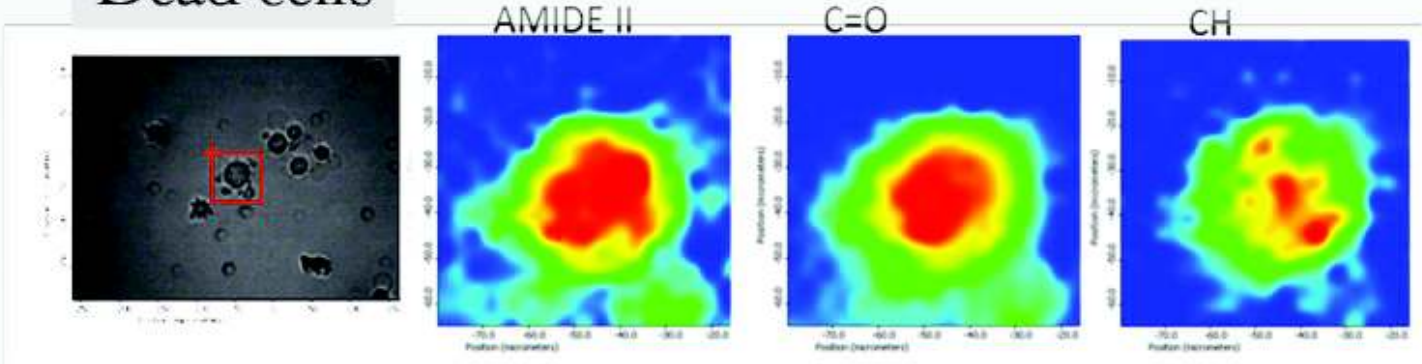
FLOW CONTROL

Sub-cellular imaging can be achieved in fully hydrated environment…. Allow in-situ kinetics…

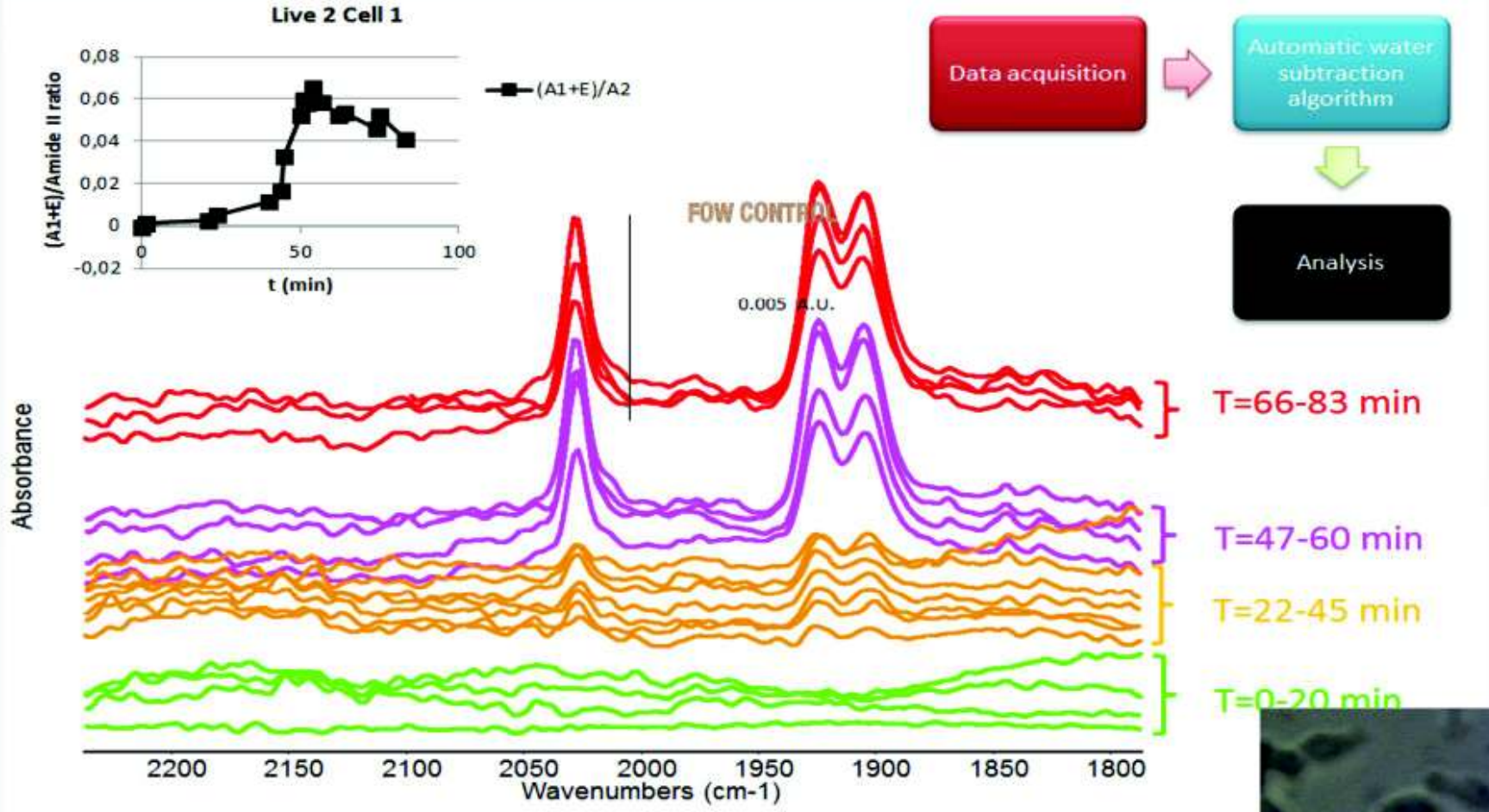


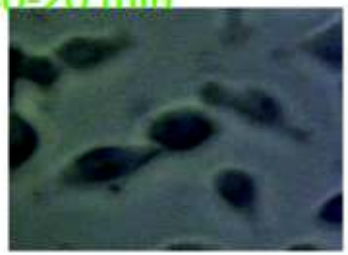


Dead cells



PENETRATION KINETIC OF RHENIUM CARBONYL TAGGED LIPIDS IN LIVING CELLS





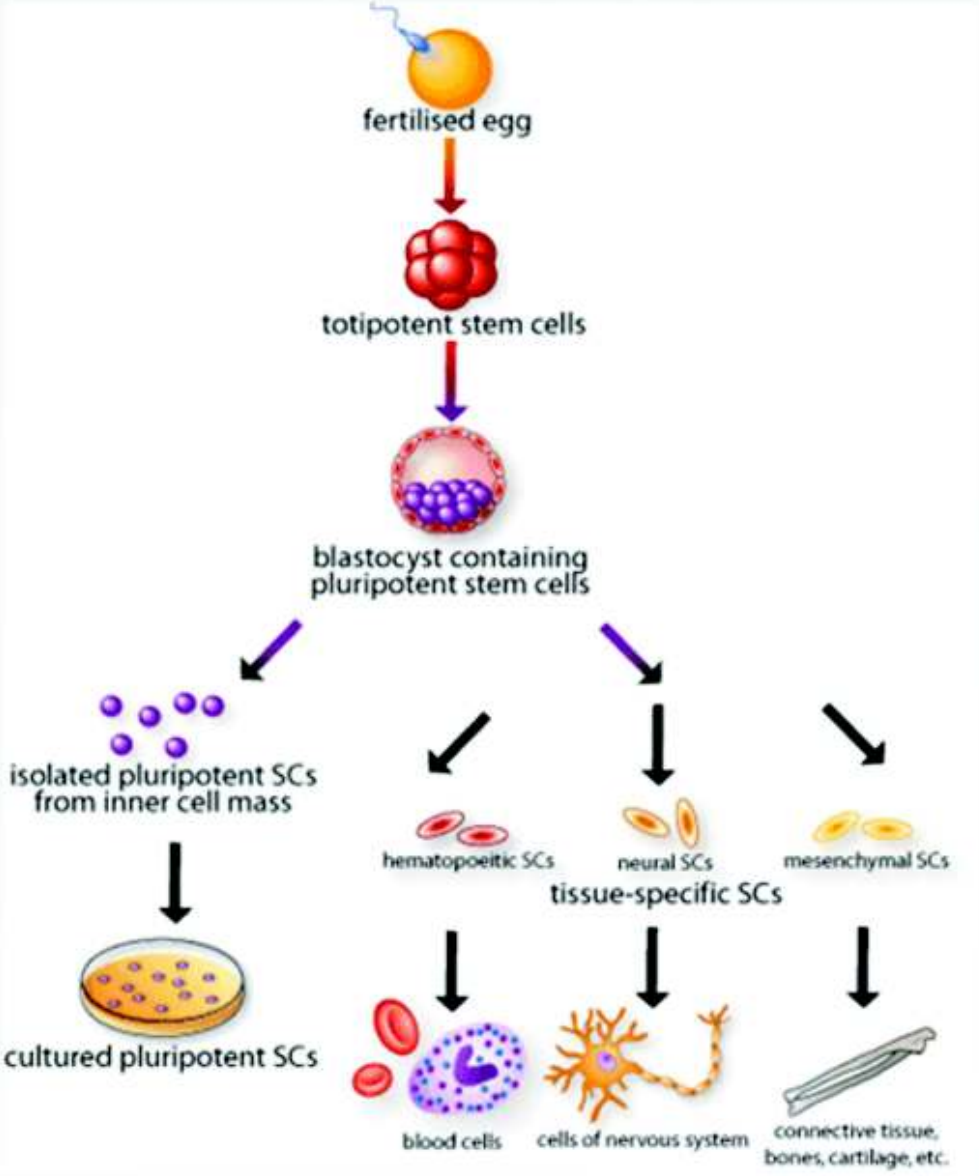
Limited output using the simple microfluidic device (3-4 cells/day).
Perspective: increase the output with litpoghraphied spacer (with 4 channels per chip, 12-16 cells per day could be measured)

C. POLICAR, S. CLEDE, C. SANDT ET AL.. TO BE PUBLISHED

STEM CELLS RESEARCH: IR SYNCHROTRONS AND LASERS CONTRIBUTION?

EMBRYONIC STEM CELLS

- UNDIFFERENTIATED CELLS
- TOTIPOTENT: CAN GIVE ANY CELL TYPE
- IMMORTAL: SELF-RENEWAL CAPACITY



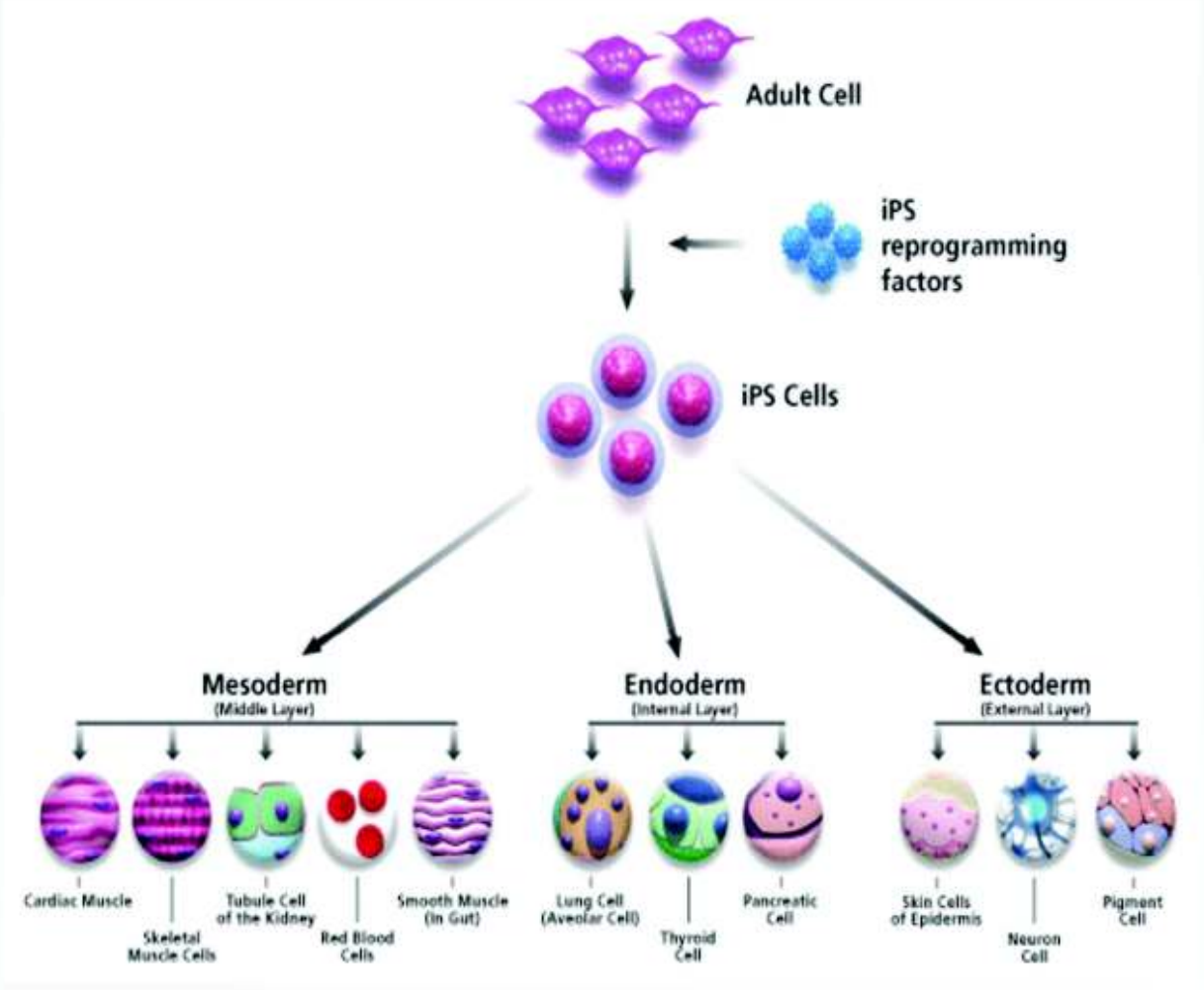
INDUCED PLURIPOTENT STEM CELLS

INDUCED PLURIPOTENT STEM CELLS

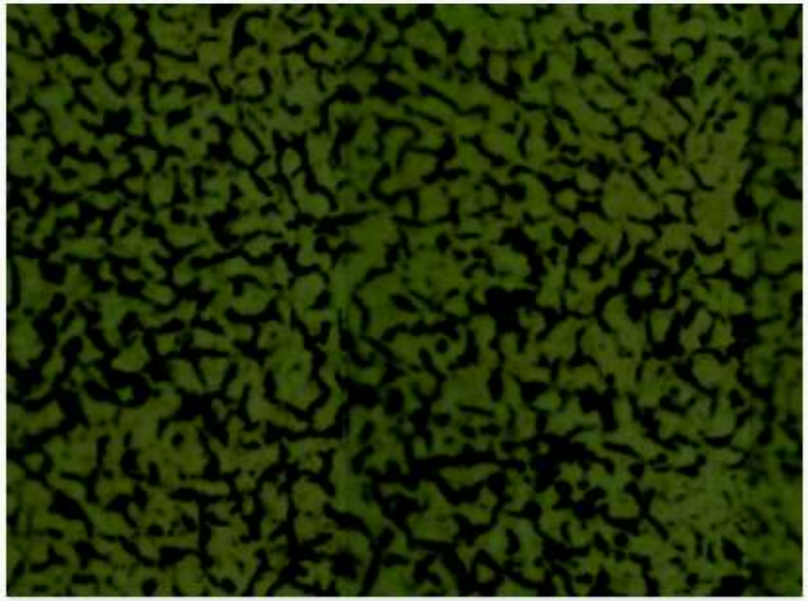
*REPROGRAMMED FROM ADULT CELLS BY TRANSFECTION OF 4 GENES (EMBRYONIC TRANSFECTION FACTORS among Oct-4, c-Myc Sox-2, Klf-4, Nanog, Lin-28…)

*PLURIPOTENT: POSSIBLY TOTIPOTENT

*IMMORTAL: COULD BE MALIGNANT

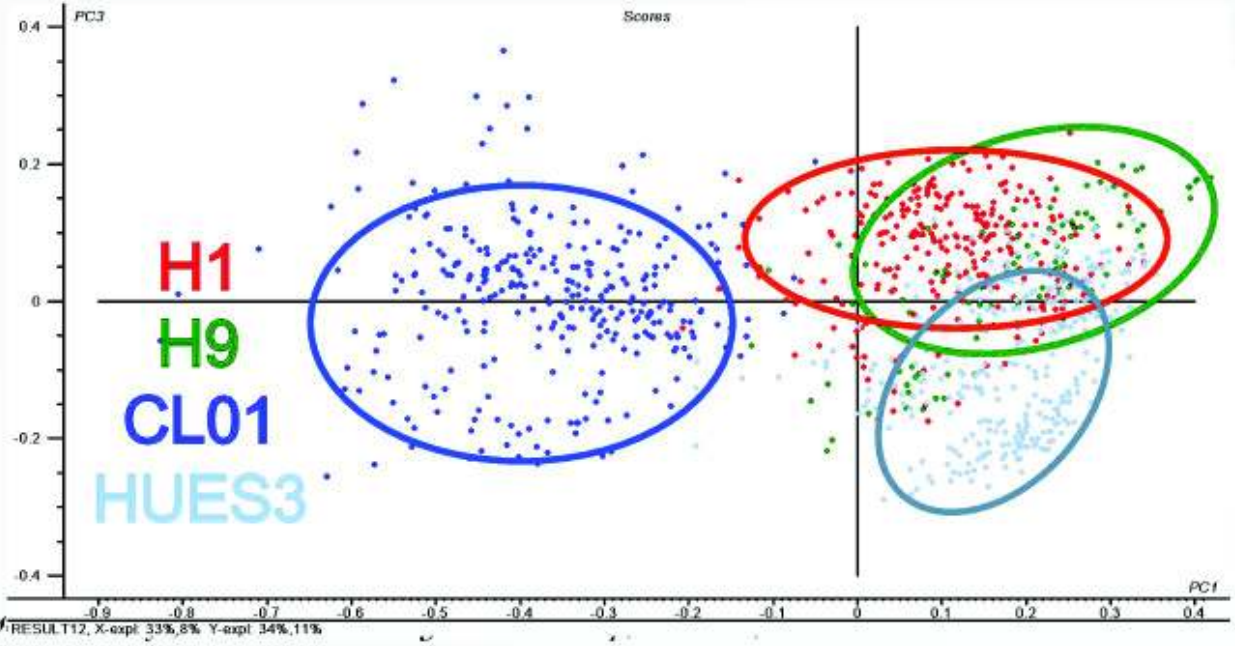


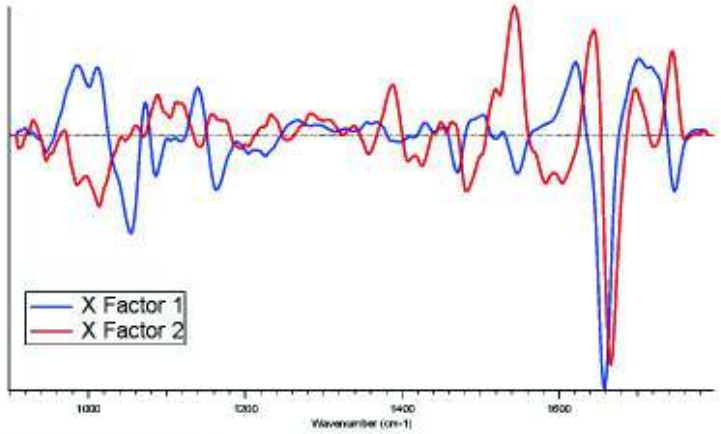
ADRESSING IDENTITY WITH SYNCHROTRON IR



H1 & H9 ARE VERY SIMILAR (THEY WERE ISOLATED IN THE SAME LAB)

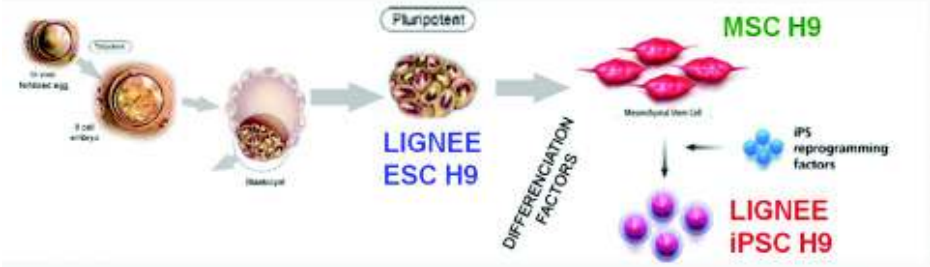
CL01 IS DIFFERENT (IT CARRIES GENETIC ABNORMALITIES: TRISOMY 1)

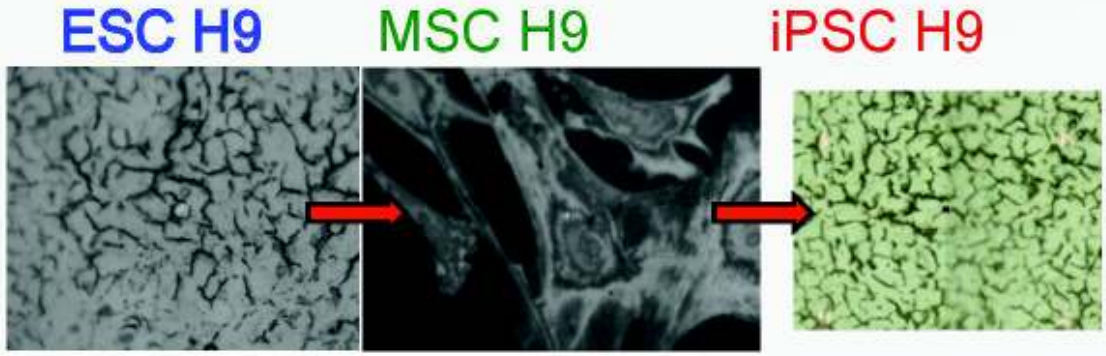


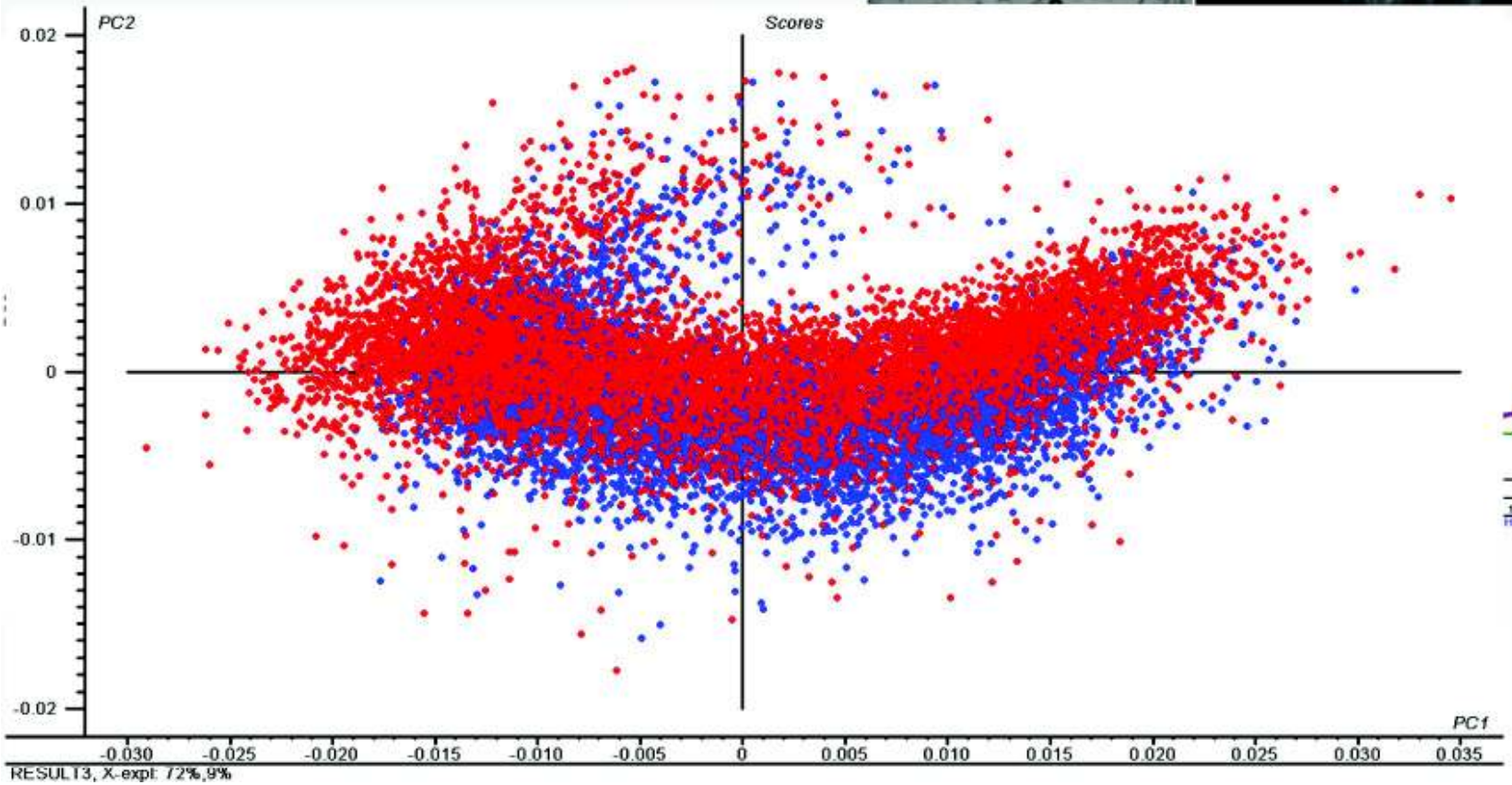


P.Dumas

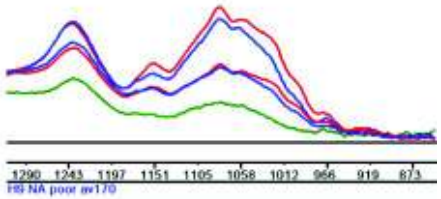
ADRESSING REPROGRAMMATION STATUS





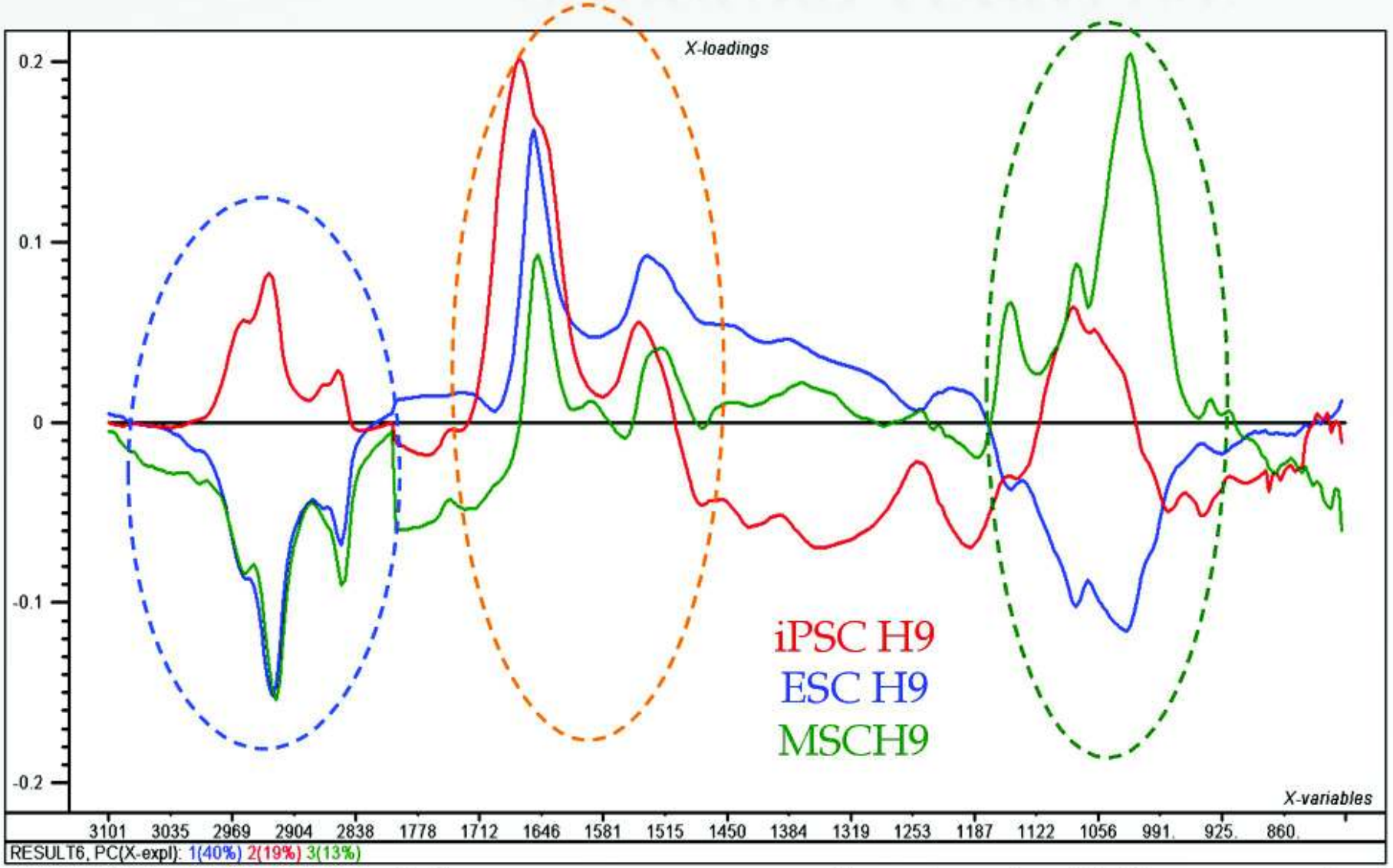


iPSC H9
ESC H9
MSCH9



P.Dumas

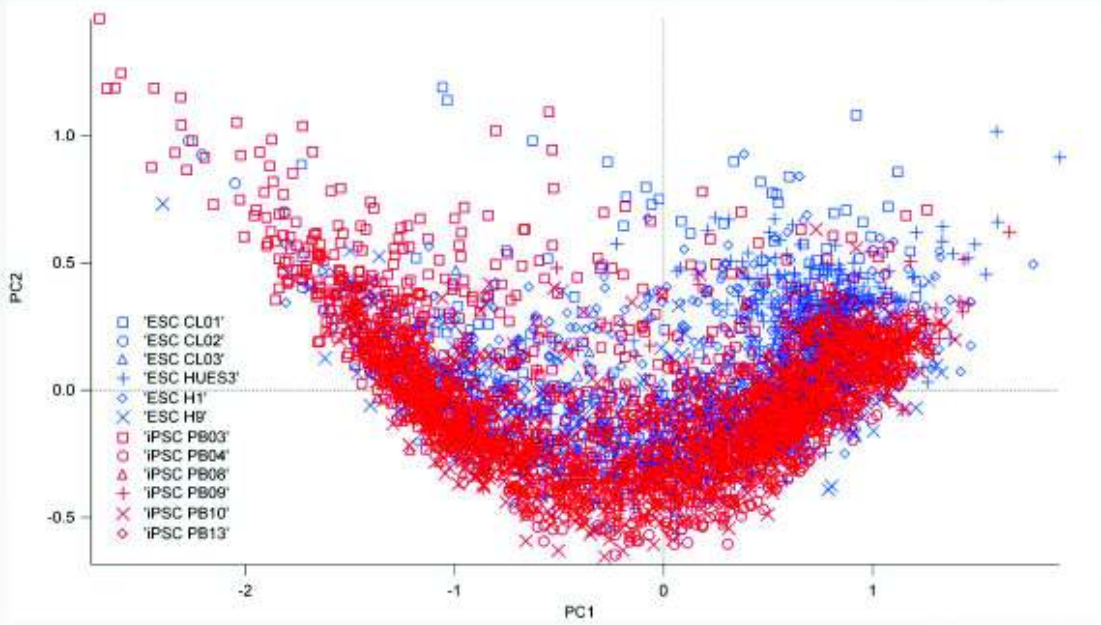
WHAT THE STATISTICAL DATA ANALYSIS TELLS US?



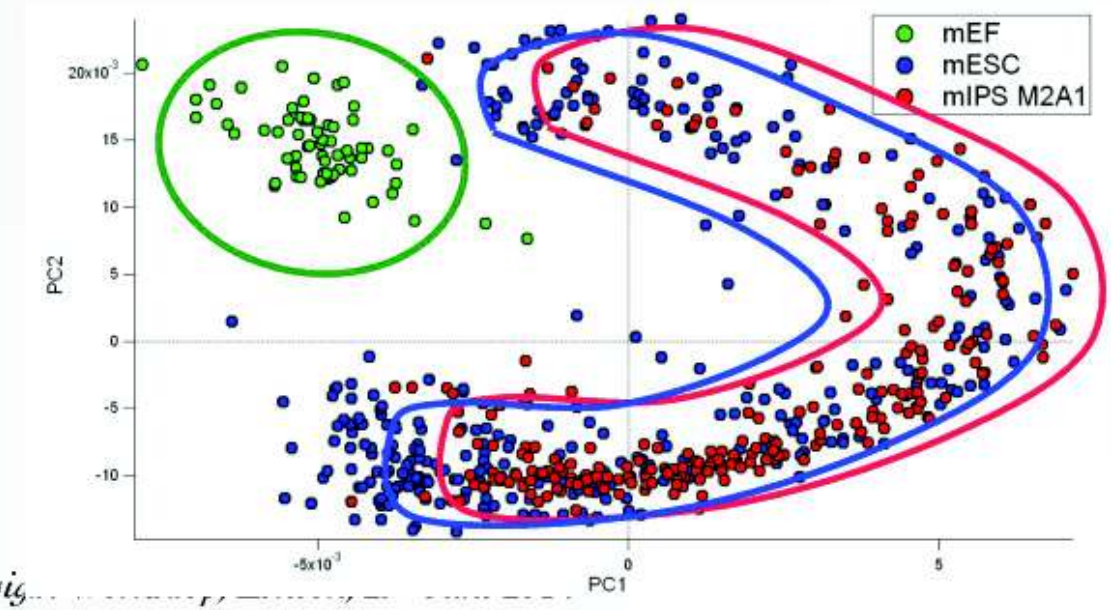
Consistent with more sugar production in iPS… metabolic activity !

VALID ALSO FOR HUMAN, MOUSE STEM CELLS

IN HUMAN



IN MOUSE

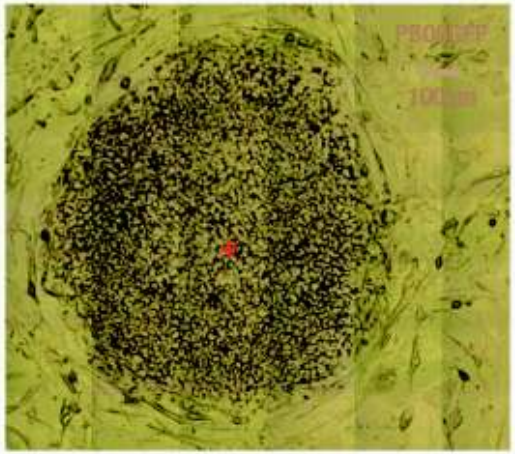


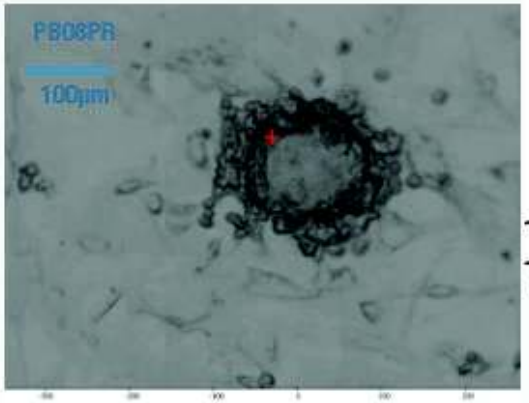
DOUBLE CHECKING WITH PARTIALLY REPROGRAMMED CELLS

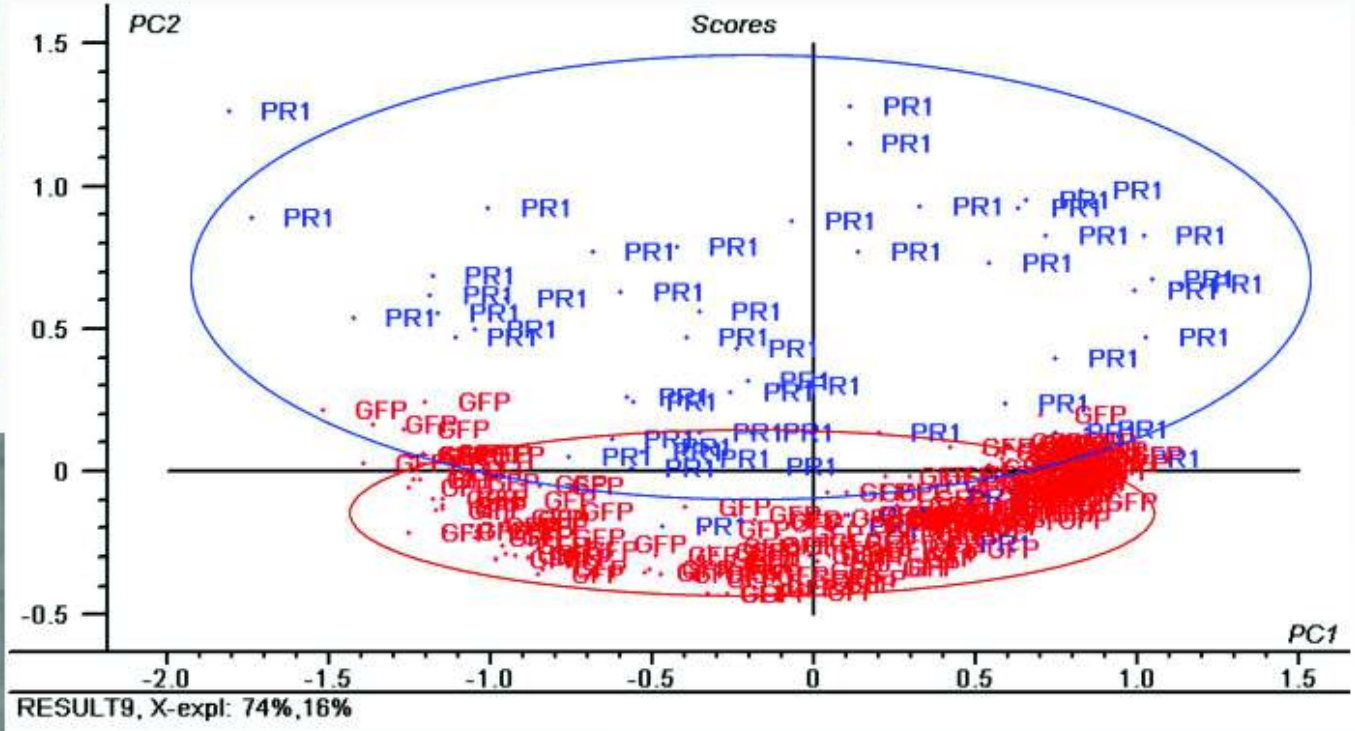
Comparison of fully reprogrammed and partially reprogrammed iPSC cells

PB08PR (partially reprogrammed)

PB08GFP (fully reprogrammed)







PlosONE , April 2012 , Volume 7 , Issue 4 , e30743

ROLE OF LASER IR?

- IR microscopy has a great potential to become a diagnostic tool for stem cell reprogrammation and stemness.

- Signatures are in two spectral domain: sugar (around 10 microns wavelength), and amide I (around 6 microns)

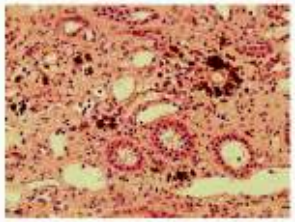
- IR lasers be fastly tuned around each of these wavelengths , may be in using several of them . Spectral quality needed. Instrument development for this specific purpose?

SYNCHROTRON IR IS PROBABLY A TESTBED …

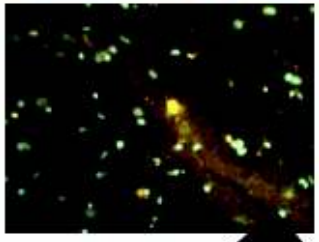
FOR OTHER BIOMEDICAL-RELATED STUDIES..

AT SMIS, THE FRENCH SYNCHROTRON IR BEAMLINE:

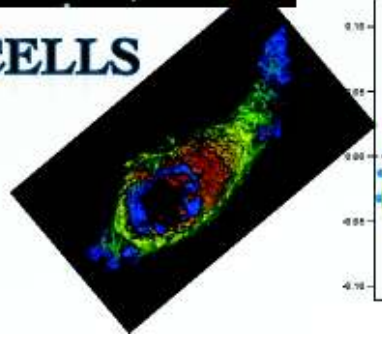
- **RAPID IDENTIFICATION OF CALCIFICATION IN KIDNEY (HOPITAL NECKER PARIS)**

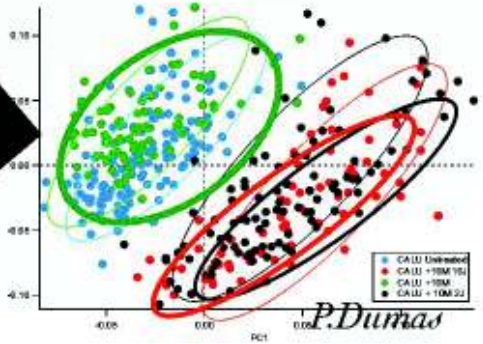


- **PRECISE DETERMINATION OF BETA AMYLOID IN HUNGTINTON INCLUSIONS (JUVENILE AND ADULT)**



- **PDT EFFECTS ON INDIVIDUAL CELLS**





P.Dumas

THE STEP FURTHER…

SYNCHROTRON IR HAS ADVANTAGES AND DRAWBACKS:

- NOT EASILY ACCESSIBLE
- LIMITED BEAMTIME ACCESS
- NOT REALLY APPROPRIATE FOR RAPID DIAGNOSTICS
- BRIGHT BUT NOT ENOUGH FOR FASTER DATA RECORDING
- REMAINS AN IMPORTANT TESTBED NOT ONLY FOR LASER BUT ALSO FOR LAB-BASED THERMAL SOURCE
- SPATIAL RESOLUTION ?

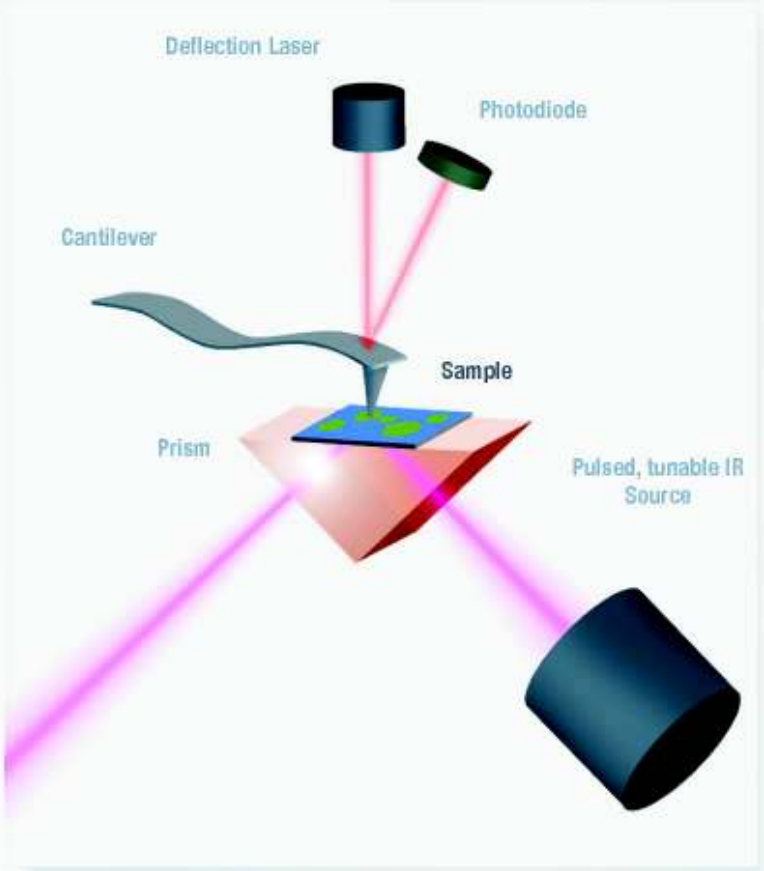
THE STEP FURTHER…

- LASER IR HAS GREAT POTENTIAL:
- - CAN BE IMPLEMENTED IN LABORATORIES AND HOSPITAL
- - HIGHTER BRIGHTNESS
- - SAMPLE DAMAGE?
- - COHERENCE?
- - STABILITY, HIGH QUALITY DATA?... GREAT PROGRESSES HAS TO COME
- - 1D, 2D DETECTORS WILL BECOME AFFORDABLE, EVEN WITHIN « LAB BUDGET »
- - COMPLEMENTARY TO SYNCHROTRON IR

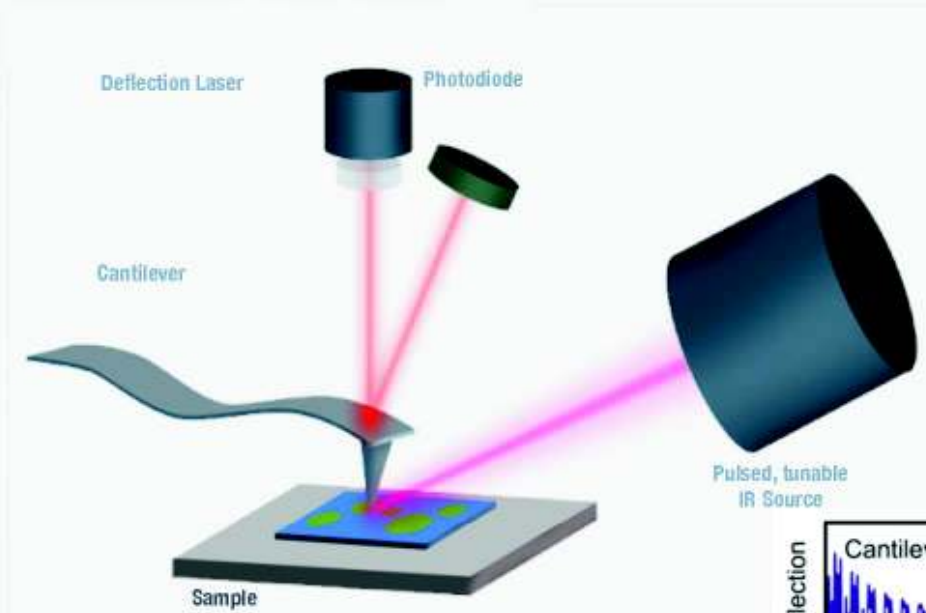
PUSHING ANALYSIS DOWN TO THE NANOSCALE?

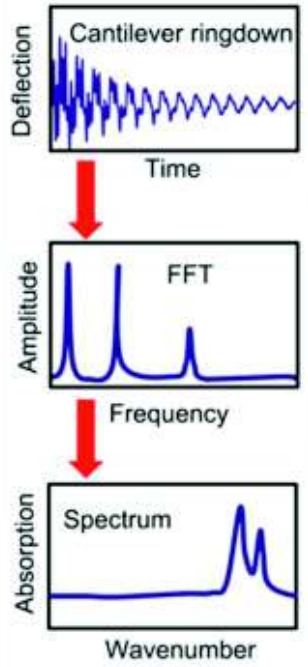
Anasys inch
ttp://www.anasysinstruments.com/

Bottom side illumination



Top side illumination

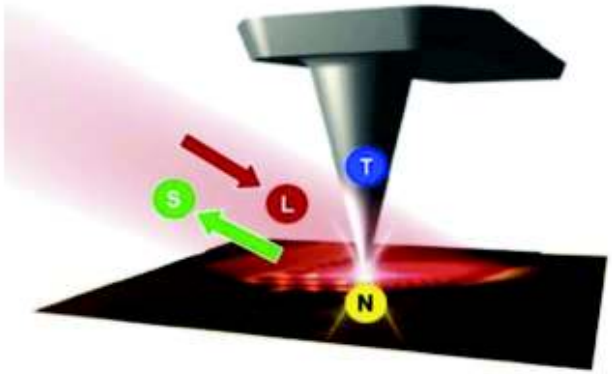




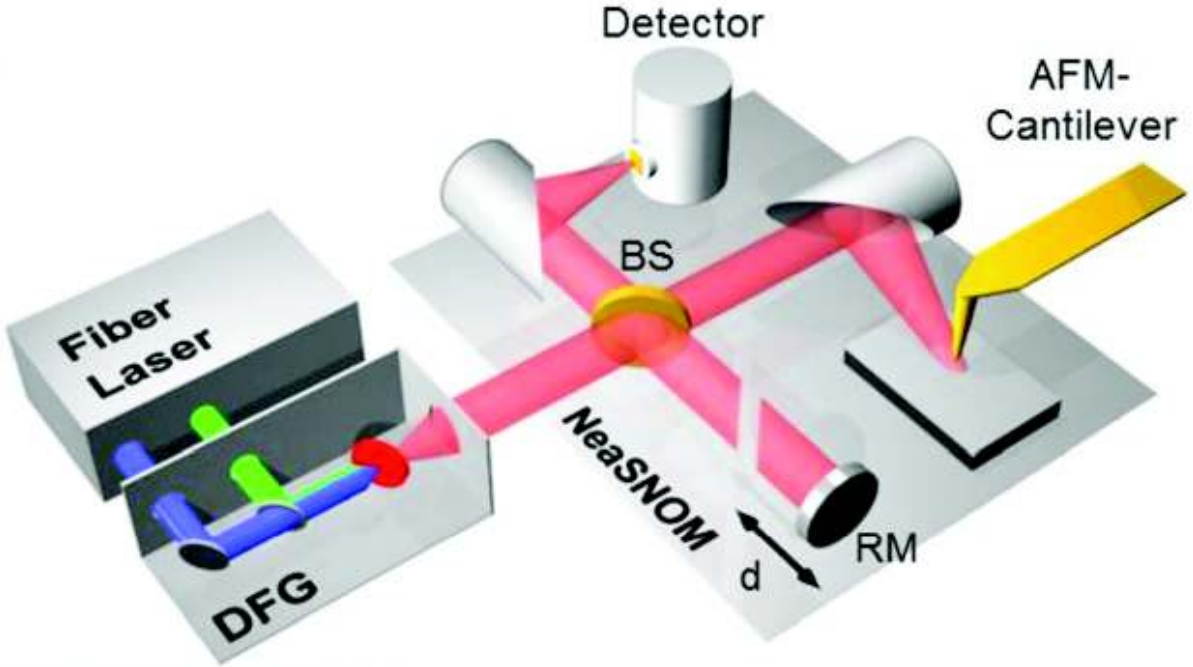
Laser are curretly used to acheive resolution down to about 20-100 nm

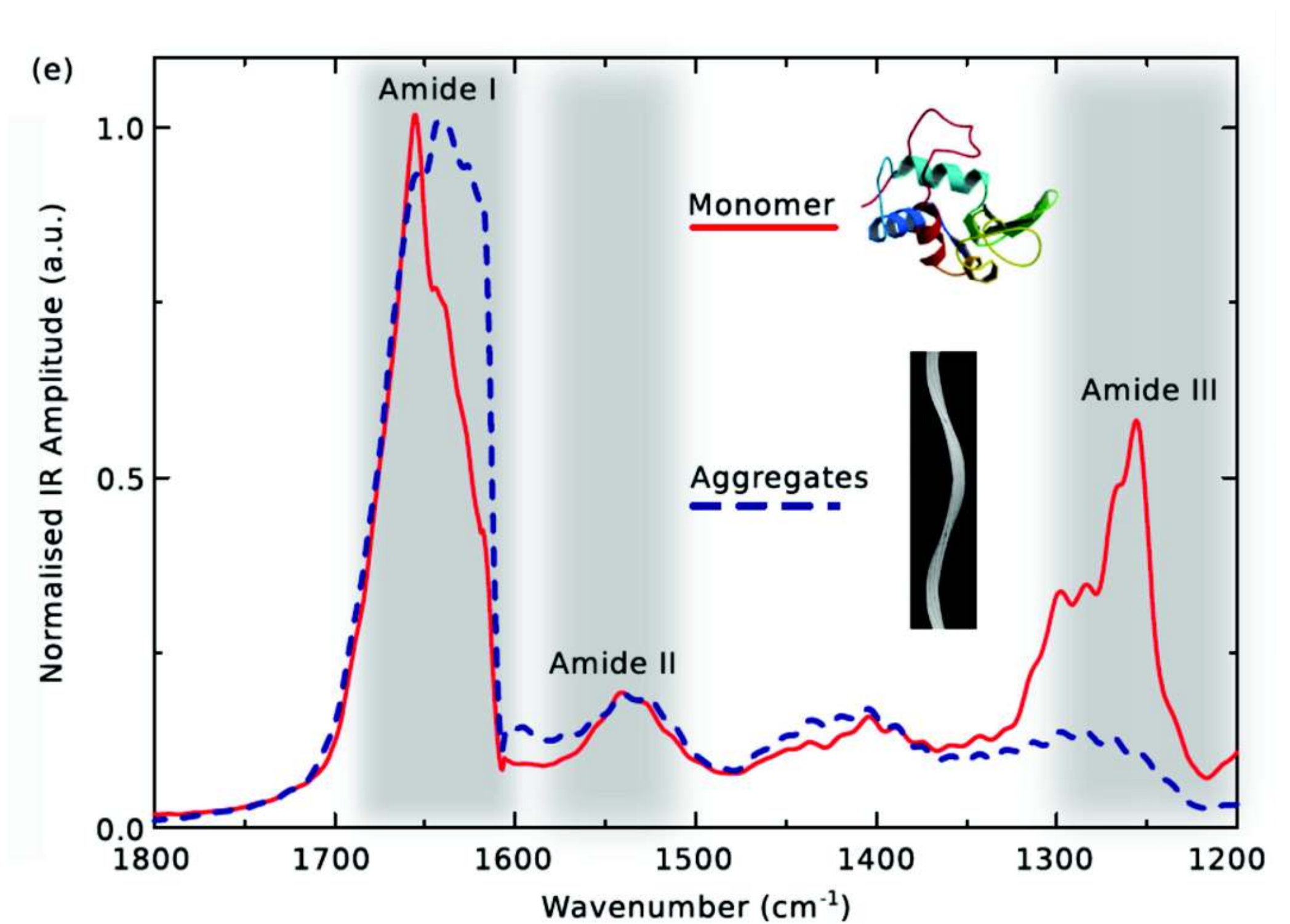
Sample geometry no longer a big issue

NaNo-IR with IR laser

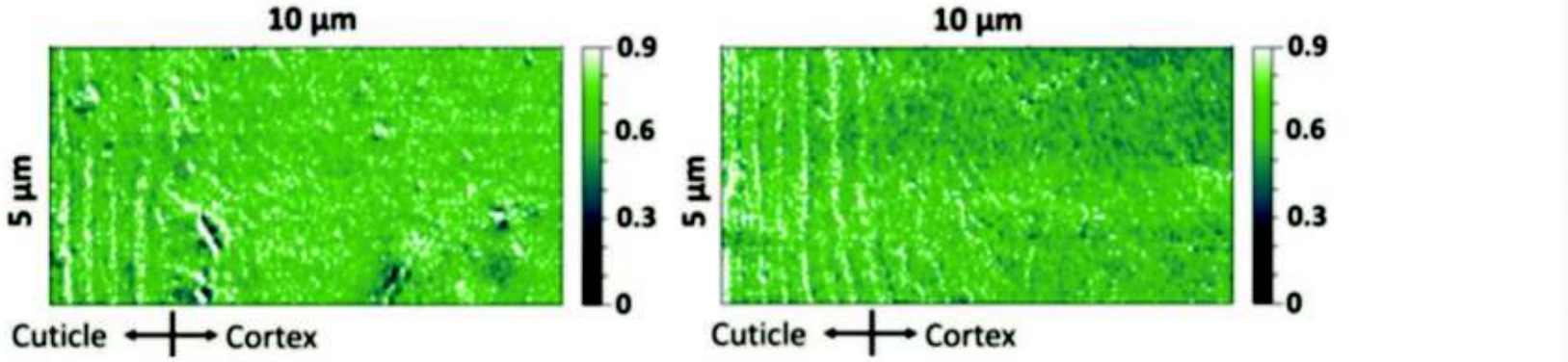


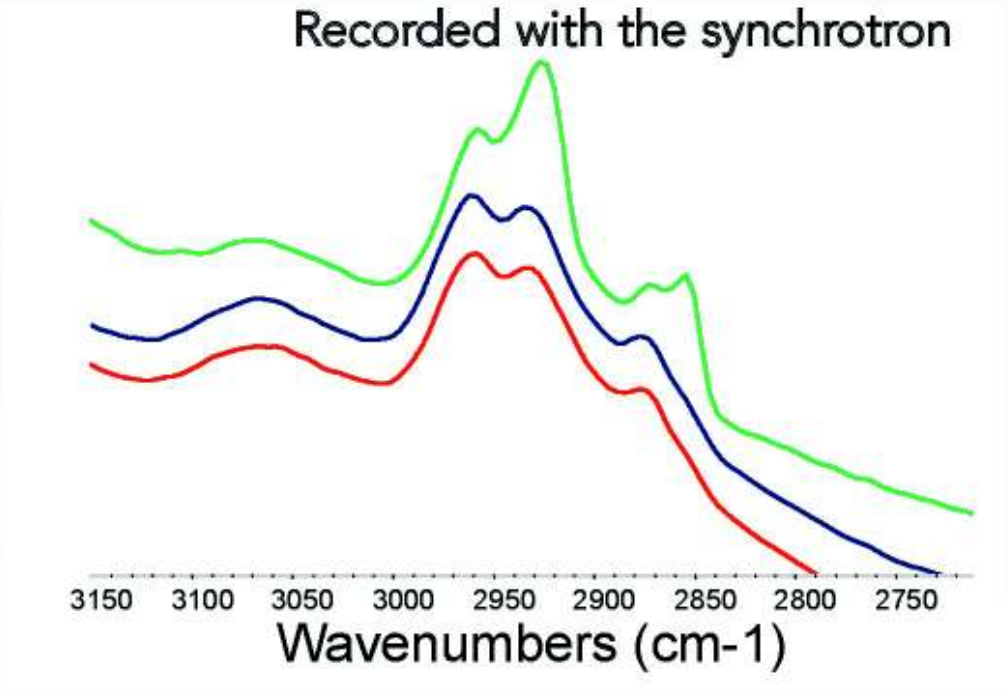
http://www.neaspec.com/

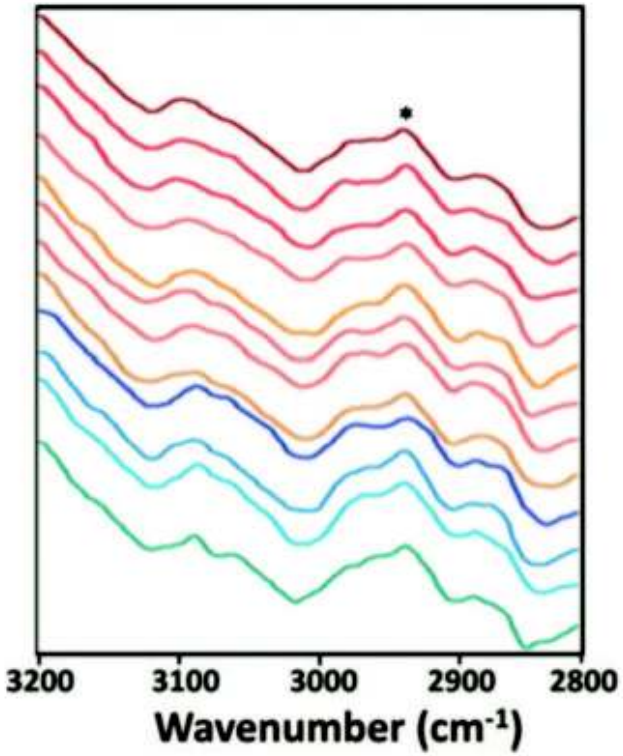



(e)
Amide I
Amide II
Amide III
Monomer
Aggregates
Normalised IR Amplitude (a.u.)
1.0
0.5
0.0
1800
1700
1600
1500
1400
1300
1200
Wavenumber (cm-1)

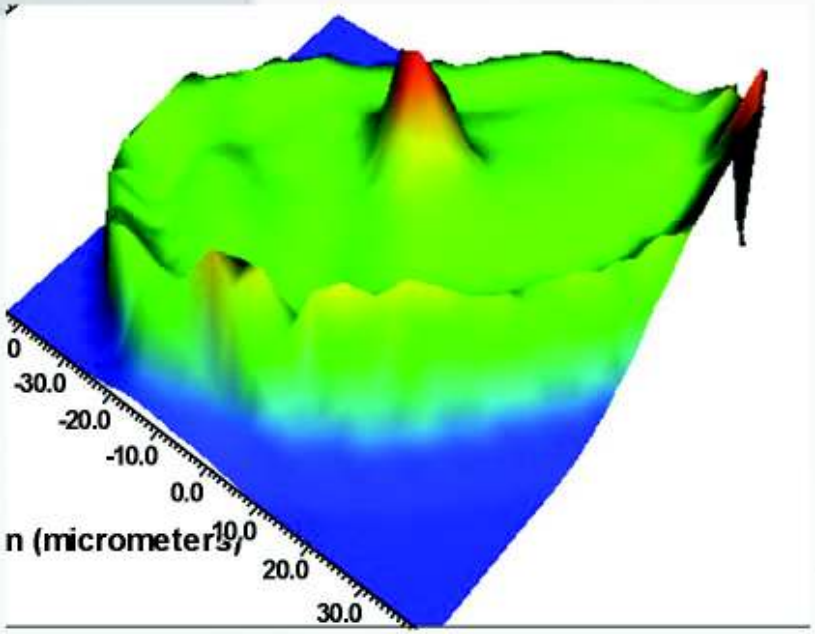
APPLIED SPECTROSCOPY Volume 68,
Number 5, 2014 , pp 564

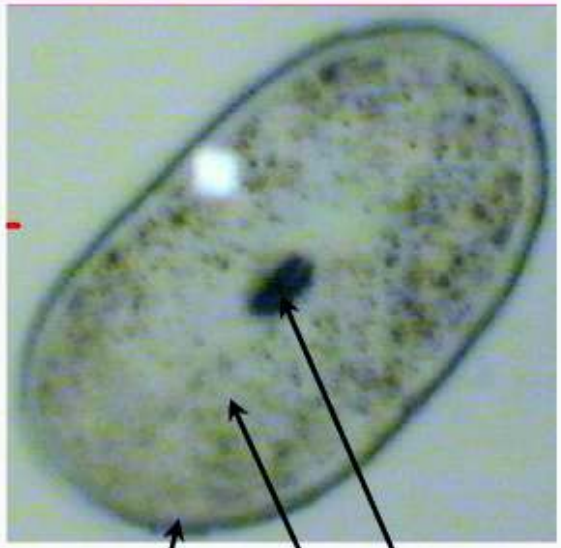


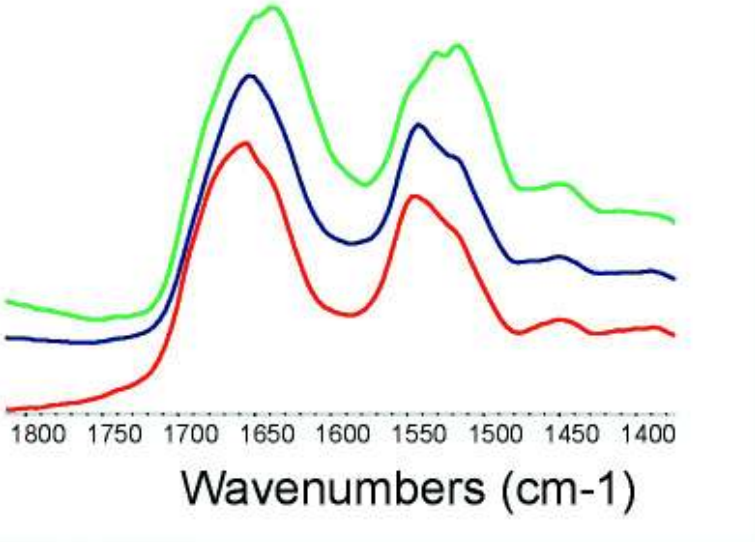


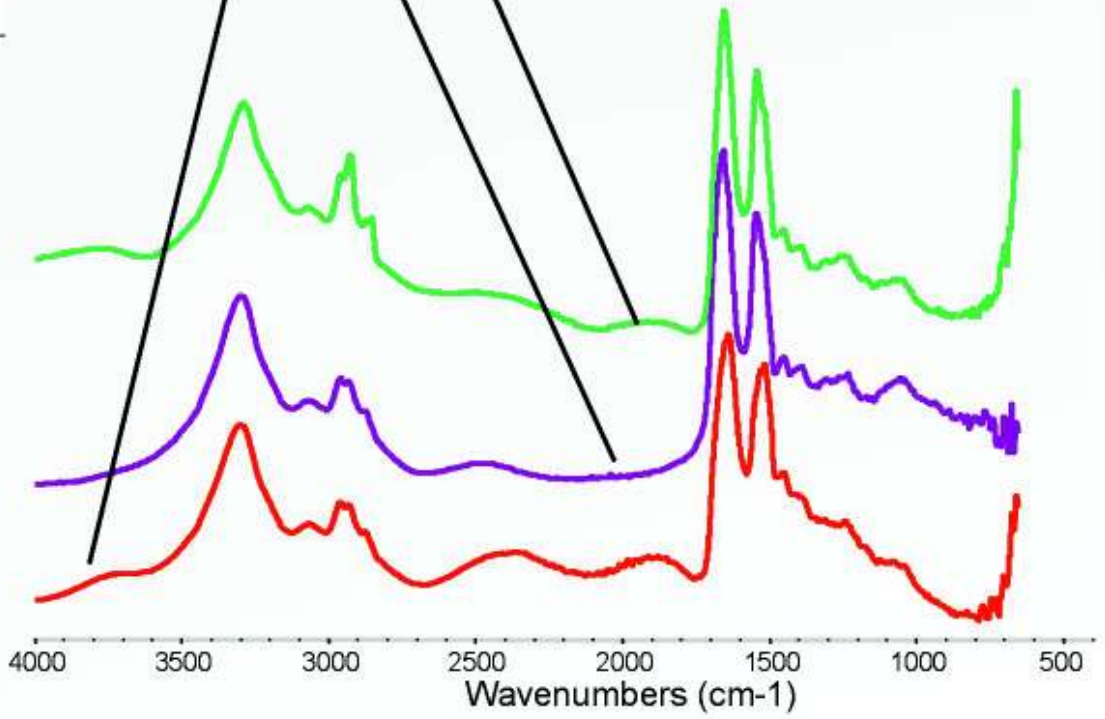


Synchrotron IR @ SOLEIL

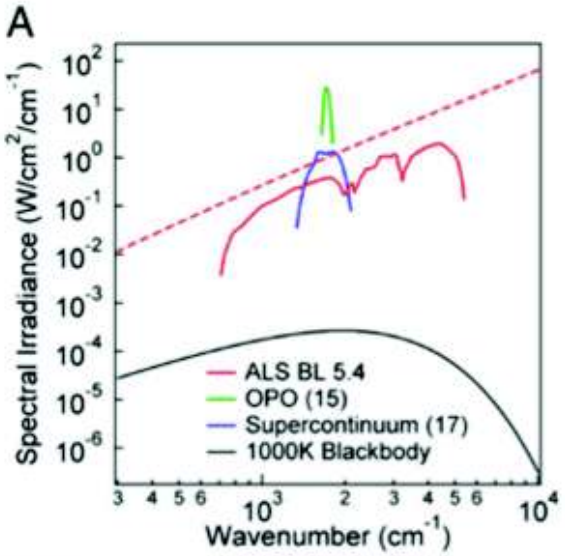


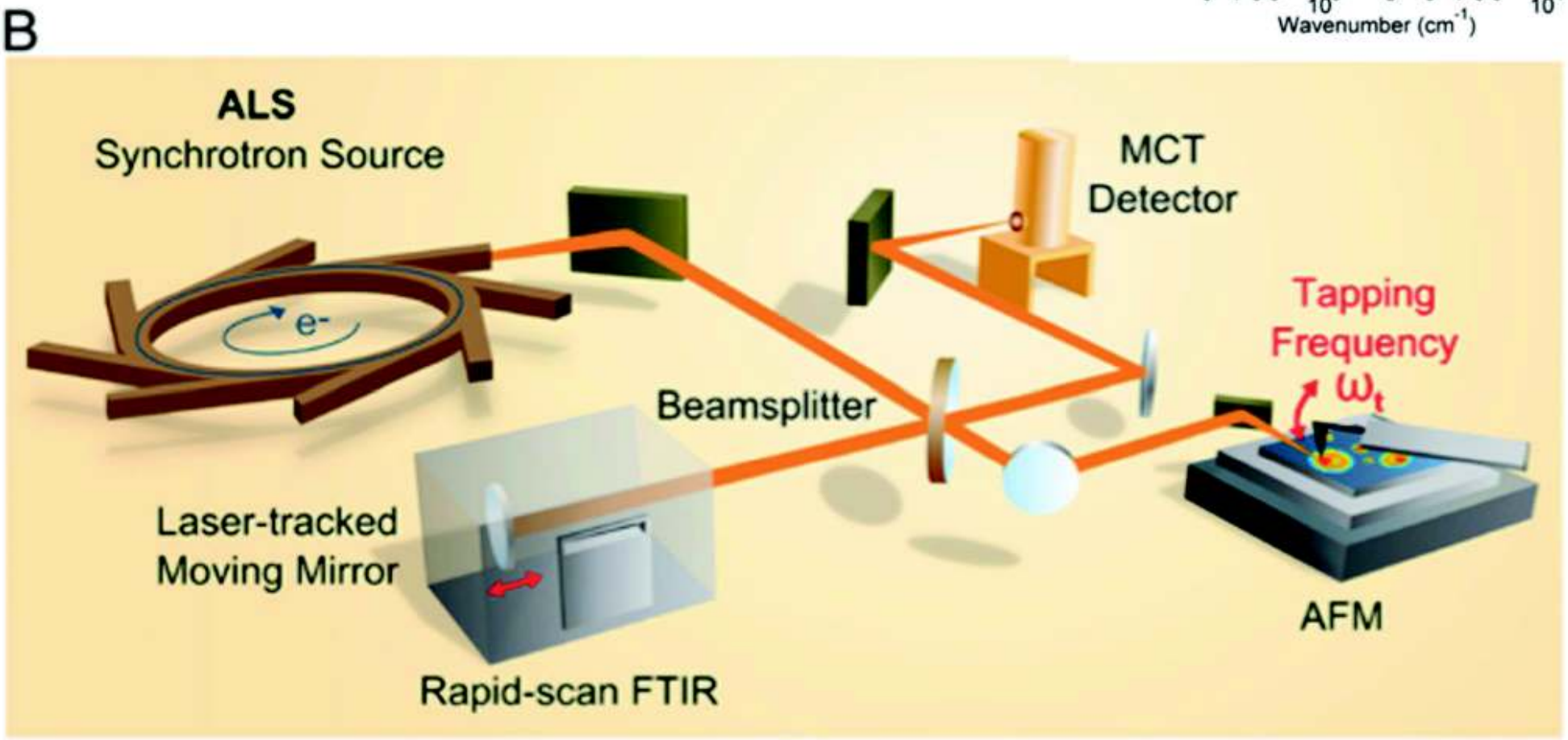






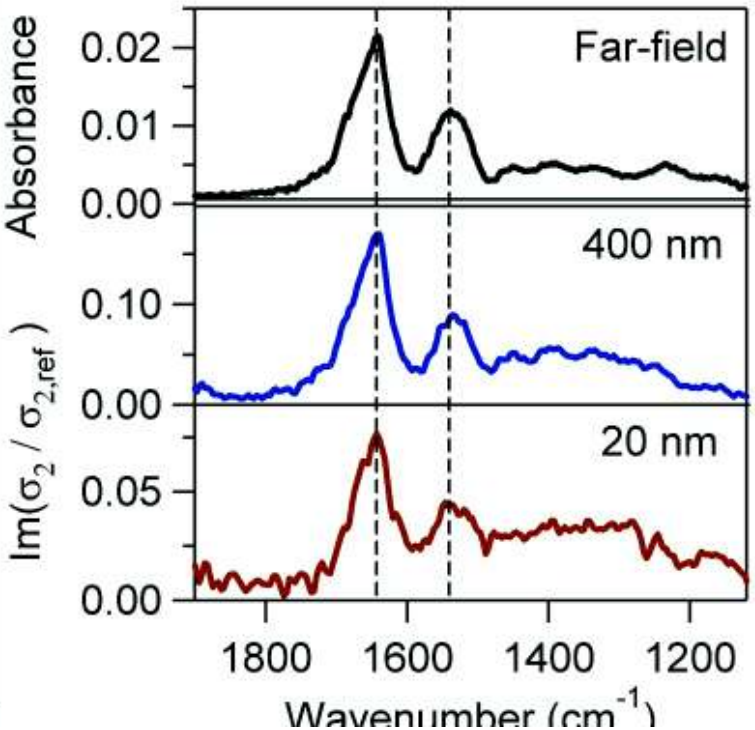
NaNo-IR with IR synchrotron

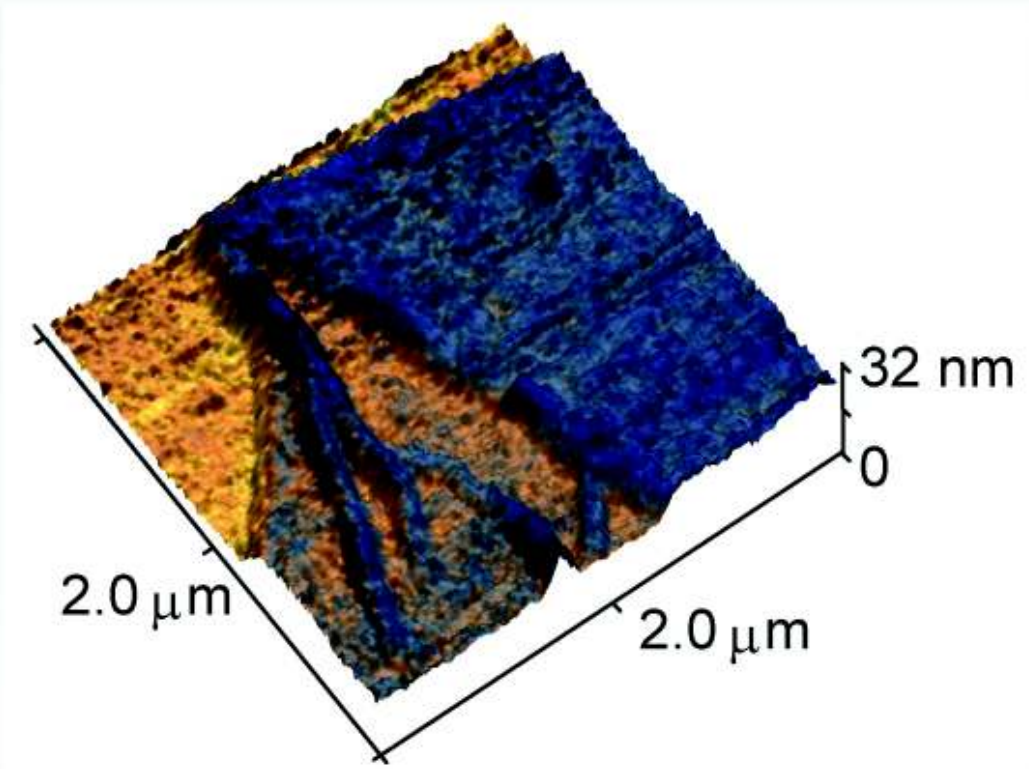




NaNo-IR with IR synchrotron

Ultrabroadband infrared nanospectroscopic imaging
Hans A. Bechtela, Eric A. Muller, Robert L. Olmon, Michael C. Martina, and Markus B. Raschke
PNAS (May 2014)Early Edition





Also at LNLS (Brazil)
Raul de oliveira Freitas, H. Westfahl, P. Dumas and Y. Petroff (to be published)

SUMMARY

- *Synchrotron IR has a great potential for biomedical –related studies and applications*

- *It is a test bed for future applications in lab-environment, and a provider for useful wavelength domain to be probed*

- *Laser and fast data recording in a wider frequency domain possible is the next step*

- *High quality spectra is crucially important, and shot-to-shot stability of lasers is one issue which is going to improve in a near future*

ACKNOWLEGMENTS

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(SMIS beamline)