

# Infrared spectroscopy with synchrotron and FEL radiation

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SISSI-Bio & TeraFERMI



1st on-line School on Synchrotron Radiation "Gilberto Vlaic":  
Fundamentals, Methods and Application



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Sincrotrone  
Trieste

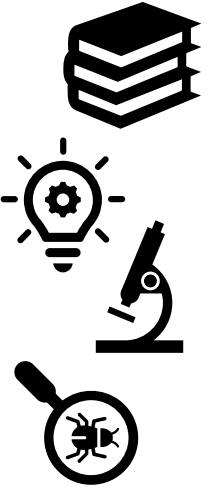
# Outlines

## Part 1\_ Infrared spectroscopy with synchrotron radiation

- Infrared Synchrotron Radiation: production and properties
- Far-field FTIR microscopy
- Near-field FTIR nanoscopy

## Part 2\_ THz studies with SR and FEL radiation

- The THz spectral range
- THz spectroscopy with Synchrotron Radiation
- THz non-linear studies





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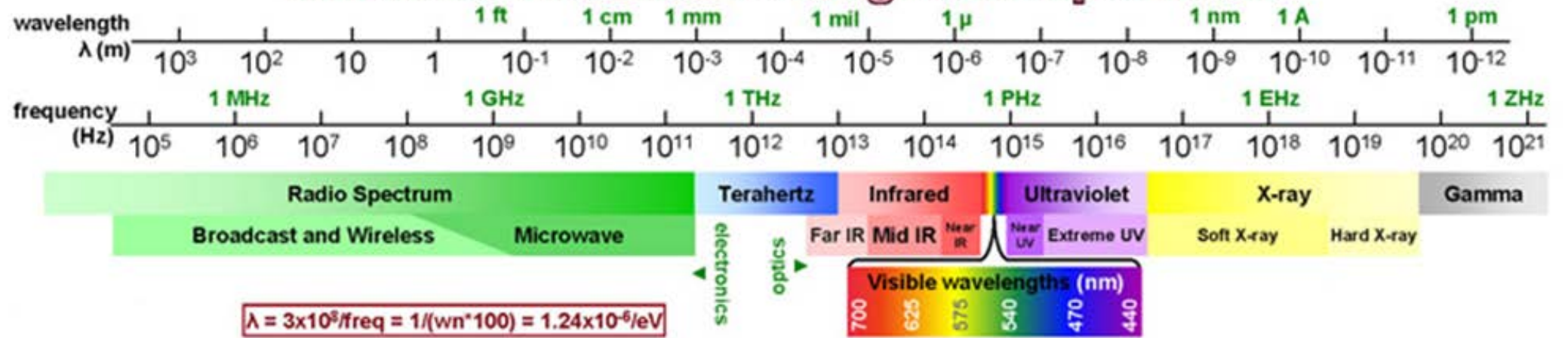
# Infrared Spectroscopy with Synchrotron Radiation



# Electromagnetic Spectrum

## A closer view into the IR-THz spectral range

### Chart of the Electromagnetic Spectrum



	NIR	MIR	FIR	THz
λ (μm)	0.74	3	30	3000
$\bar{\nu}$ (THz)	400	100	10	0.1
$\nu$ (cm <sup>-1</sup> )	~13000	~3333	~333	~0.33
E (eV)	1.65	0.413	0.041	0.0004
E (Kcal/mol)	37	10	1	0.01

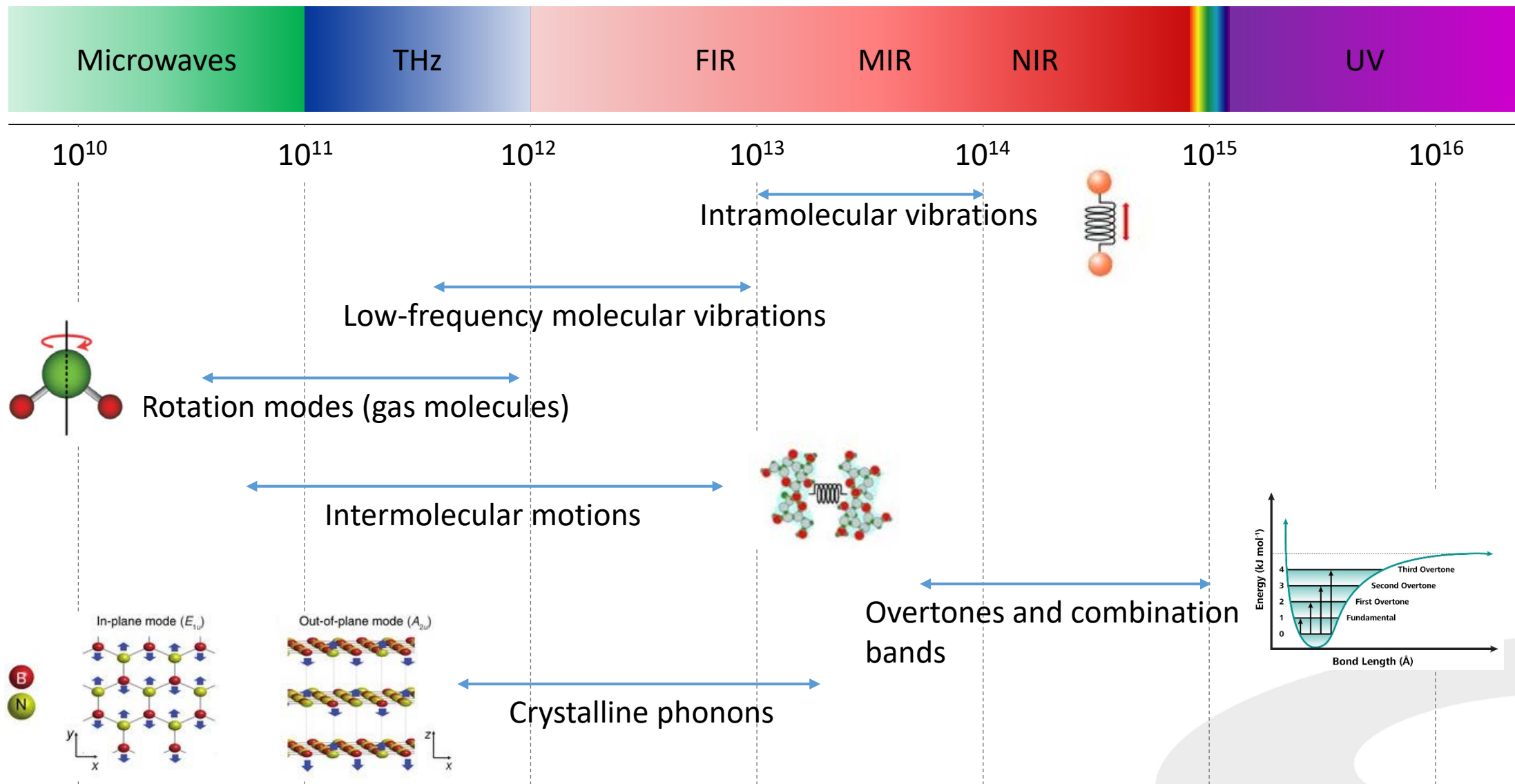






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# What is IR spectroscopy used for?

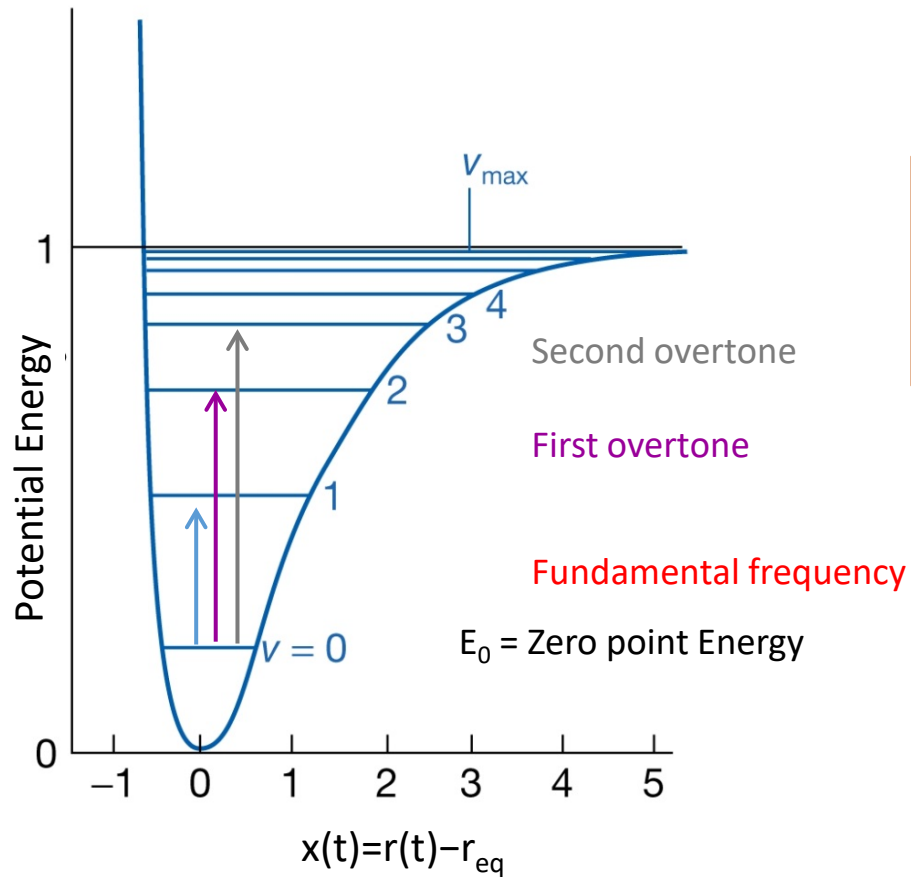




# Infrared vibrational spectroscopy: Basic concepts on theory



$$V(x) = D_e(1 - e^{-ax})^2$$



## Quantum mechanical Model of anharmonic oscillator

$$E_{vib} = h\nu_e[(n + 1/2) - x_0(n + 1/2)^2 + \text{higher terms}]$$

$\nu_e$  = harmonic frequency  $x_0$  = anharmonic constant

### Selection Rules

$$\mu_{trans} = \left( \frac{d\mu}{dx} \right) \langle \psi_n | x | \psi_{n'} \rangle$$

$$\mu_{trans} \neq 0$$

$$\left( \frac{d\mu}{dx} \right) \neq 0$$

$$\langle \psi_n | x | \psi_{n'} \rangle \neq 0$$

$$\Delta n = \pm \text{integer}$$

Overtone bands are observed, with frequencies usually lower than the whole multiples of fundamental.

Combination bands are also allowed (two vibrational quantum number changes at the same time)



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# Infrared vibrational spectroscopy: Basic concepts on theory

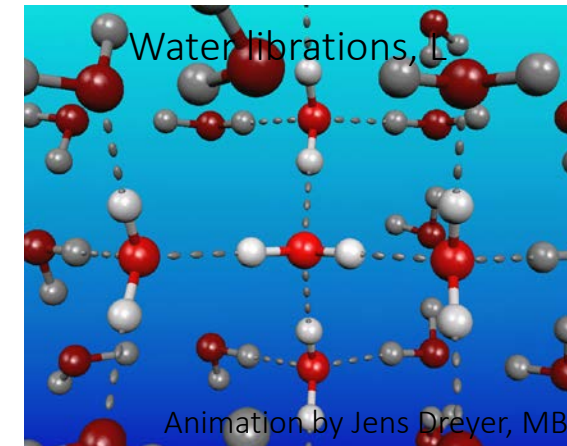
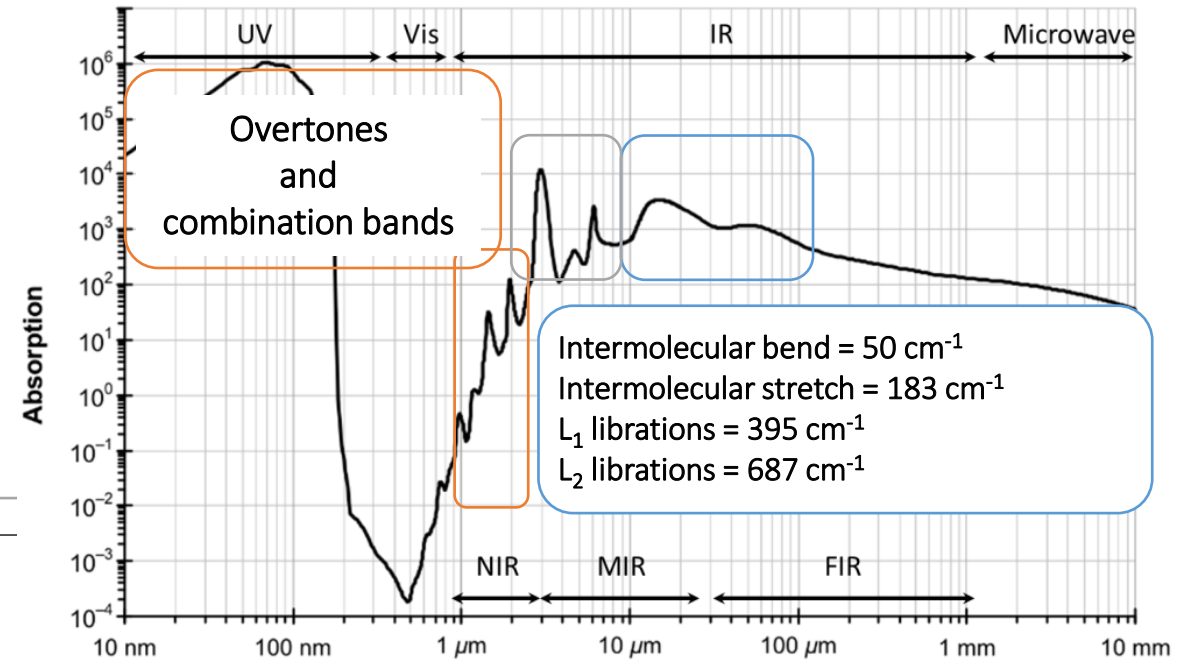
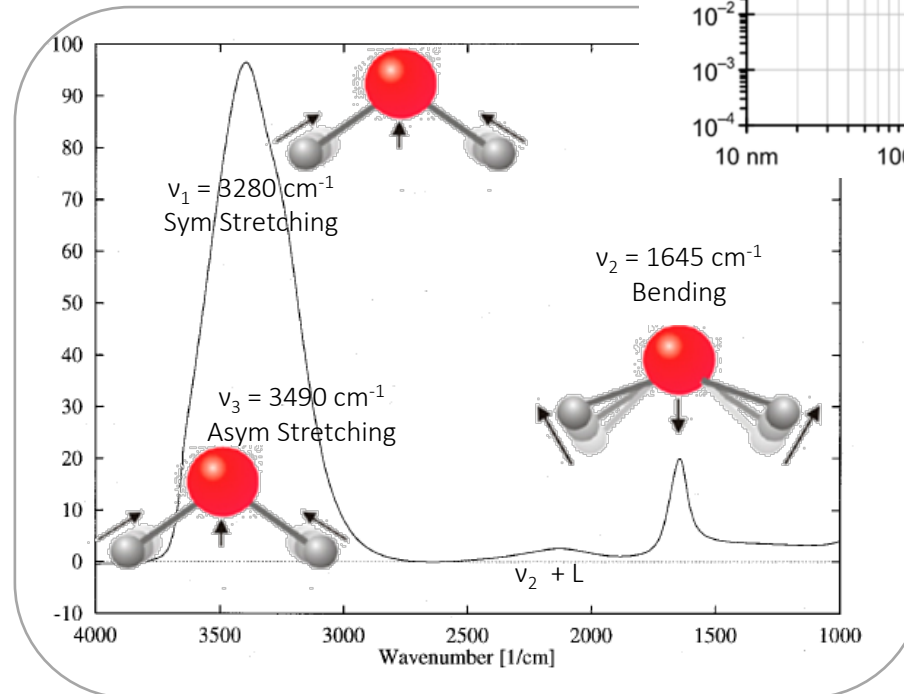


Polyatomic molecules

Normal modes of vibrations

3N-6 (5)

Vibrational  
Spectrum of  
liquid water





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# Infrared Spectroscopy: a brief history



Volume 1.

July-August, 1893.

Number 1.

## THE PHYSICAL REVIEW.

### A STUDY OF THE TRANSMISSION SPECTRA OF CERTAIN SUBSTANCES IN THE INFRA-RED.

BY ERNEST F. NICHOLS.

WITHIN a few years the study of obscure radiation has been greatly advanced by systematic inquiry into the laws of dispersion of the infra-red rays by Langley,<sup>1</sup> Rubens,<sup>2</sup> Rubens and Snow,<sup>3</sup> and others. Along with this advancement has come the more extended study of absorption in this region. The absorption of atmospheric gases has been studied by Langley<sup>1</sup> and by Ångström.<sup>4</sup> Ångström<sup>5</sup> has made a study of the absorption of certain vapors in relation to the absorption of the same substances in the liquid state, and the absorption of a number of liquids and solids has been investigated by Rubens.<sup>6</sup>

In the present investigation, the object of which was to extend this line of research, the substances studied were: plate glass, hard rubber, quartz, lamp-black, cobalt glass, alcohol, chlorophyll, water, oxyhæmoglobin, potassium alum, ammonium alum, and ammonium-iron alum.

<sup>1</sup> Report on Mt. Whitney Expedition, Profess. Papers, U. S. Signal Service, XV.

<sup>2</sup> Annalen der Physik und Chemie, N. F. XLV., p. 238.

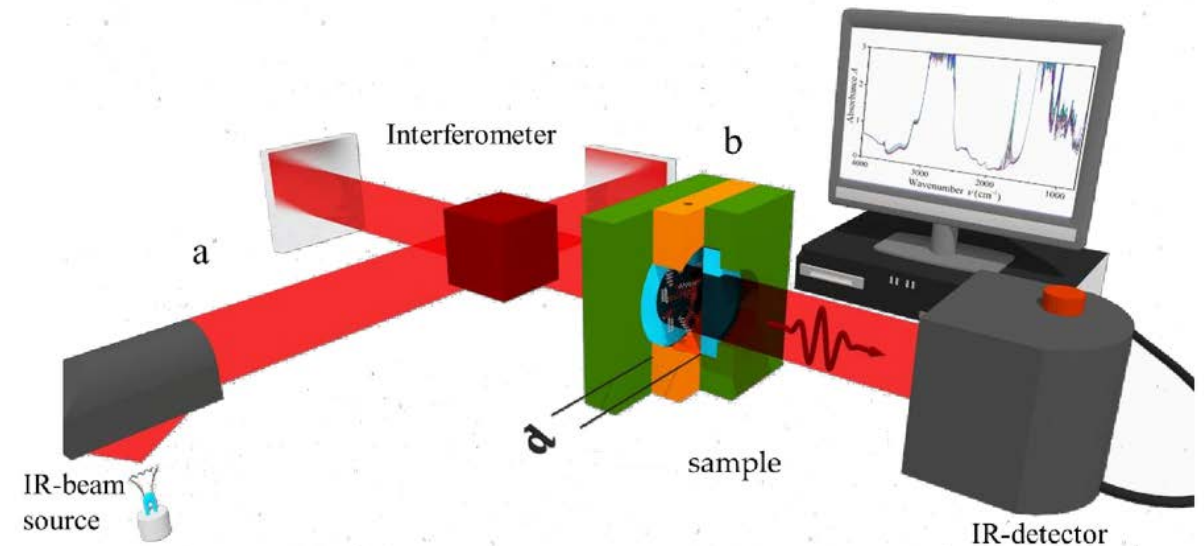
<sup>3</sup> Annalen der Physik und Chemie, N. F. XLVI., p. 529.

<sup>4</sup> Bihang till K. Svenska Vet.-Akad. Handlingar, Band 15, Afd. 1, No. 9.

<sup>5</sup> Öfversigt af Kongl. Vetenskaps-Academiens Forhandlingar, 1890, No. 7, Stockholm.

<sup>6</sup> Annalen der Physik und Chemie, N. F. XLV., p. 258.

Despite born in the 1800's, infrared spectroscopy became popular only in the second half of the 20<sup>th</sup> century thanks to advent of Fourier Transform InfraRed (FTIR) interferometer.



Alex Risos, Nicholas Long, Arvid Hunze, Gideon Gouws, "Mid-IR absorbance and its relation to static permittivity as a robust in-field tool tracking oil deterioration," Proc. SPIE 10215, Advanced Environmental, Chemical, and Biological Sensing Technologies XIV, 1021500





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# IR beamlines: The Cinderella Story

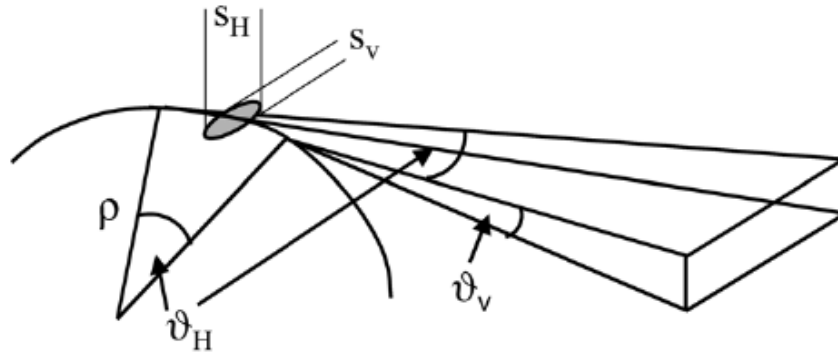


- 1976 Meyer and Lagarde (LURE, Orsay) published the first paper on IRSR
- 1981 Duncan and Yarwood observed at Daresbury the first IRSR emission
- 1985 The first IRSR spectrum (on N<sub>2</sub>O) is collected at Bessy (Berlin)
- 1986 The first beamline was opened to users at UVSOR (Japan)
- 1987 Started the brilliant story of IR-beamlines at NSLS Brookhaven (USA)
- 1992 In Europe: Orsay (France), Lund (Sweden), Daresbury (GB)
- 1995 First international workshop on IRSR, Rome (Italy)
- 2001 First IR beamline in Italy (SINBAD@DAΦNE)
- 2006 Second beamline in Italy (SISSI@Elettra)
- ⋮
- Today Many mores





# IRSR Generation\_Bending Magnet IRSR



$$P_{BM}(\lambda) = 4.38 \times 10^{14} \times I \times \theta_H \times bw \times \left(\frac{\rho}{\lambda}\right)^{1/3} \text{ photons} \cdot \text{s}^{-1} \quad [1]$$

$I$  = Current (A)

$\theta_H$  = Horizontal Collection Angle (rads)

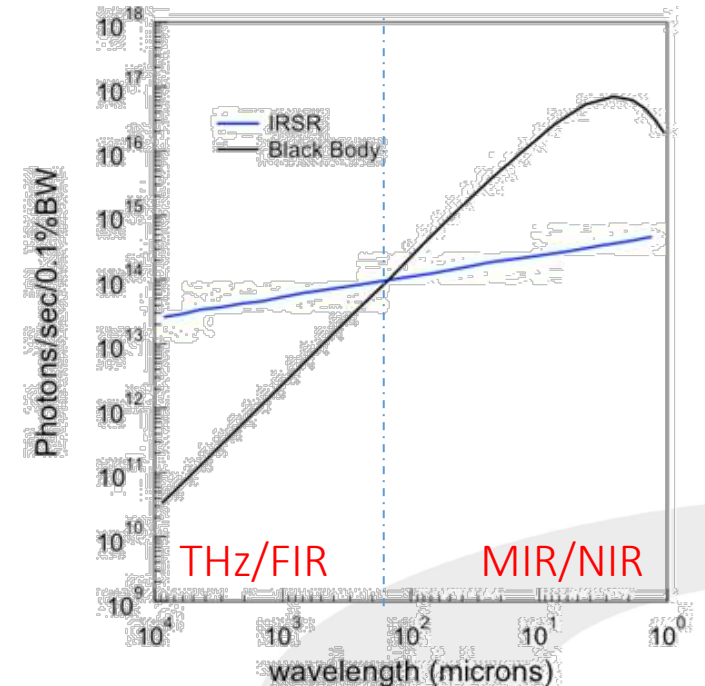
$bw$  = Bandwidth (%)

$\rho, \lambda$  = Radius of the ring, Wavelength (same units)

$P(\lambda)$  as obtained in [1], in the spectral range 1 to  $10^4 \mu\text{m}$  ( $10^4$  to  $1 \text{ cm}^{-1}$ ), for a current of **1 A**, a horizontal angle  $\theta_H = 100 \text{ mrad}$ s and  $\rho = 5 \text{ m}$ . Comparison with the emission for a BB source at 2000K.

Extrapolation of the Schwinger equations (1949) by WD Ducan and GP William (1980s)

*Infrared synchrotron radiation from electron storage rings; Appl Opt. 1983 22(18):2914.*

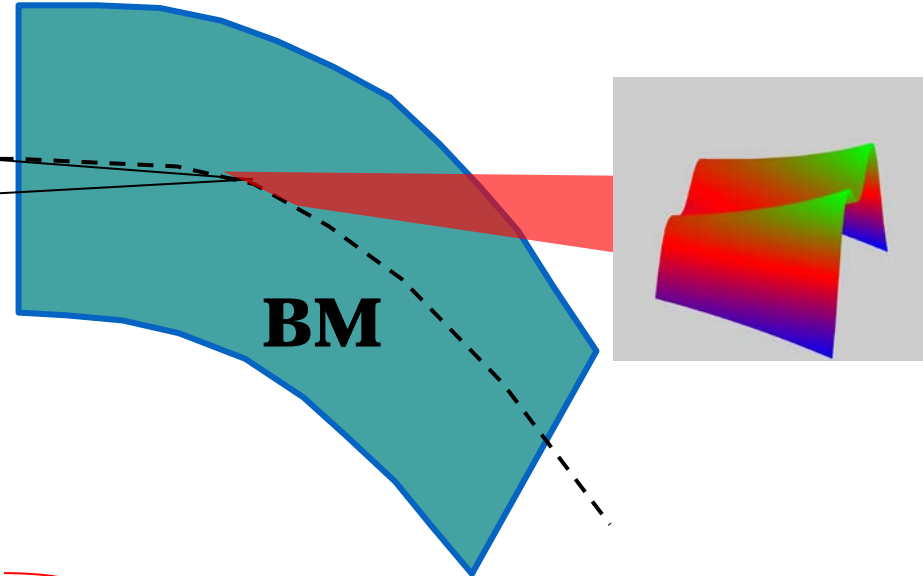
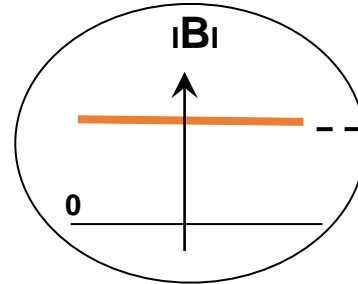




# IRSR Generation\_Bending Magnet IRSR



Constant Field  
Emission



$$\theta_{V-Nat} = 1.66 \left( \frac{1000 \times \lambda}{\rho} \right) \quad [\lambda] = \mu\text{m}; [\rho] = \text{m}$$

Angular range into which 90% of the emitted photons travel

	$\lambda$ [ $\mu\text{m}$ ]	$\nu$ [ $\text{cm}^{-1}$ ]	THz	$\theta_{V-Nat}$
NIR	1	10000	300	9.2
	10	1000	30	19.8
	100	100	3	42.2
FIR	1000	10	0.3	90.3

Calculated for Elettra  $\rho = 5.5$  m.

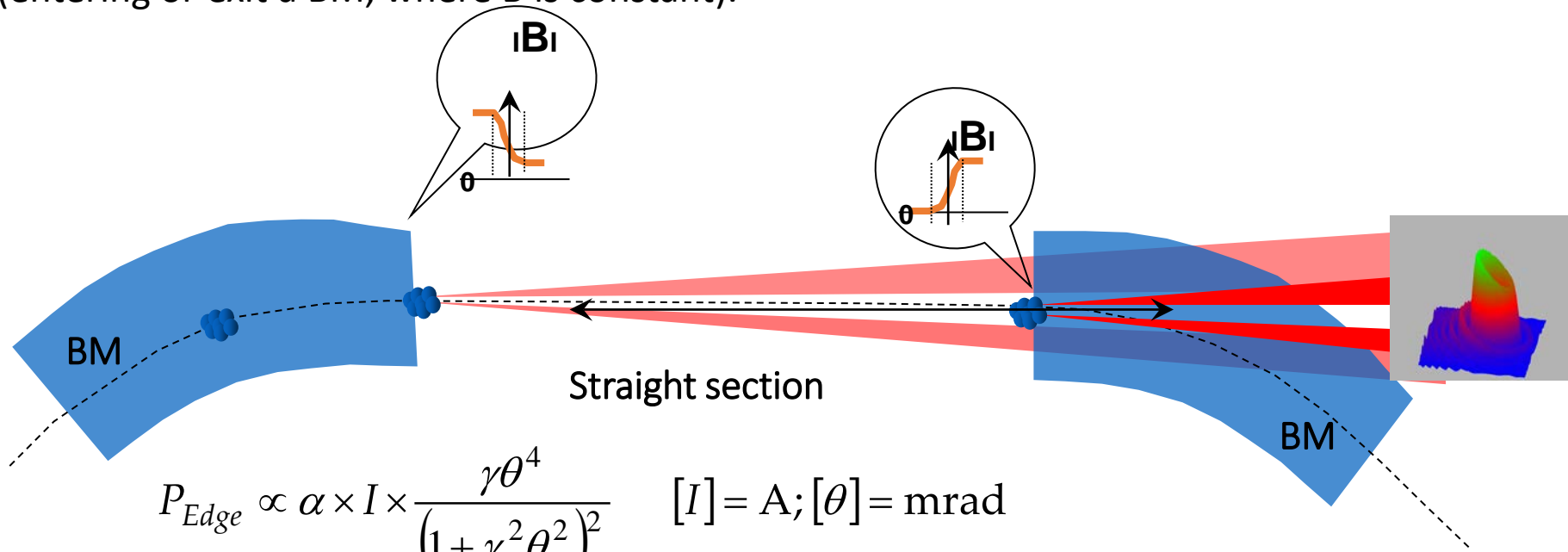
Very large extraction apertures are needed for IR beamlines for:

- Maximizing the flux ( $\theta_H$ )
- Allowing efficient extraction of lower energy components of IR synchrotron emission ( $\theta_V$ )



# IRSR Generation\_IR Edge Radiation

Edge radiation is produced when electrons experience a changing magnetic field (entering or exit a BM, where B is constant).



$$P_{Edge} \propto \alpha \times I \times \frac{\gamma \theta^4}{(1 + \gamma^2 \theta^2)^2} \quad [I] = A; [\theta] = \text{mrad}$$

$\gamma$  = Lorentz Factor;  $\alpha$  = Fine Structure Constant

- Edge radiation has a ring structure characterized by interference pattern
- Being  $\Theta_{max} \sim 1/\gamma \sim 10$  mrad, it is spatially confined and intrinsically bright
- It is radially polarized





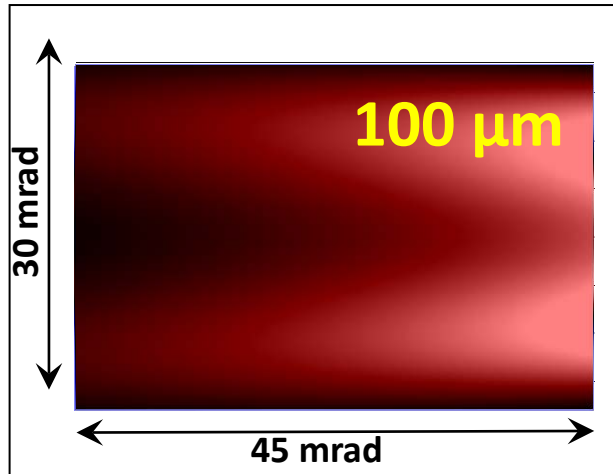


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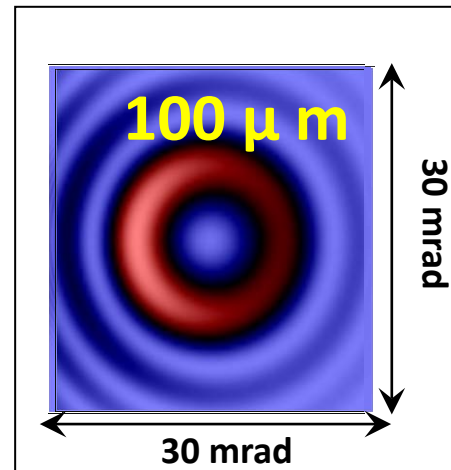
# IRSR Generation\_IR BM and Edge Radiation



**SOLEIL**  
2.75 GeV  
45 mrad H X 30 mrad V  
BM

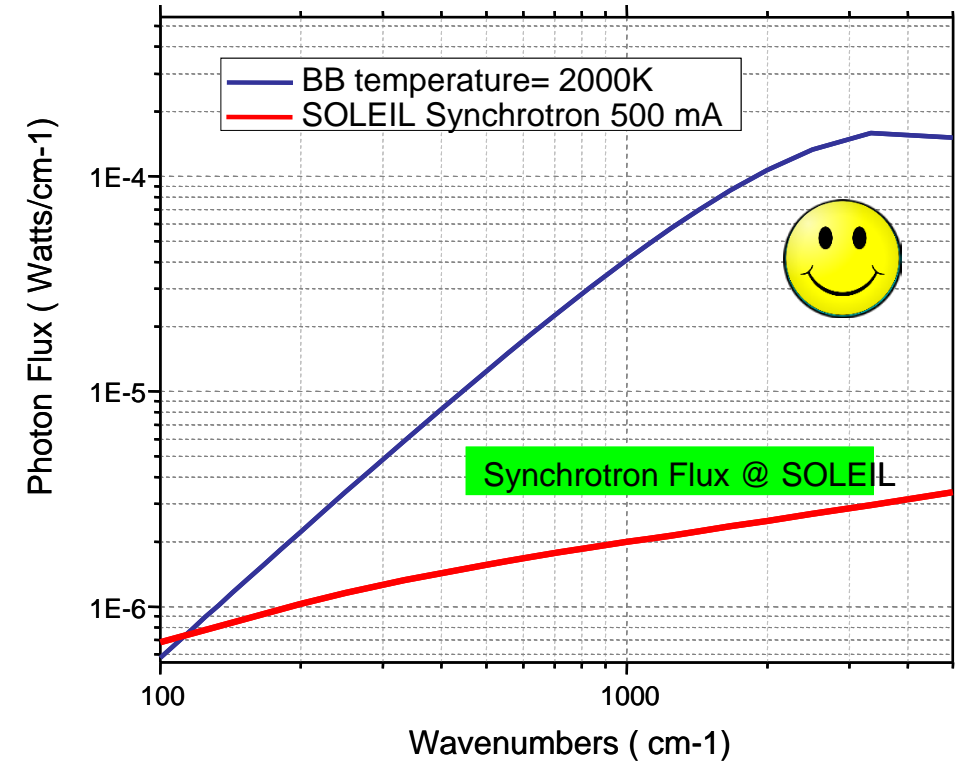


**SOLEIL**  
2.75 GeV  
30 mrad H X 30 mrad V  
BM



## IRSR

### The brightness advantage



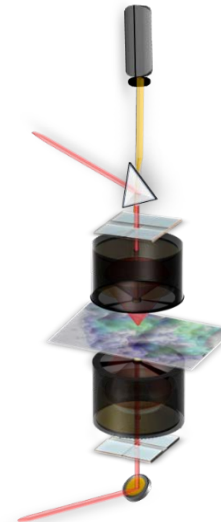
# The space domain of infrared

Vibrational information on the sample can be achieved at different level of detail, depending on instrumentation and IR source (IR conventional, IR-Synchrotron radiation, IR laser)



IR spectroscopy

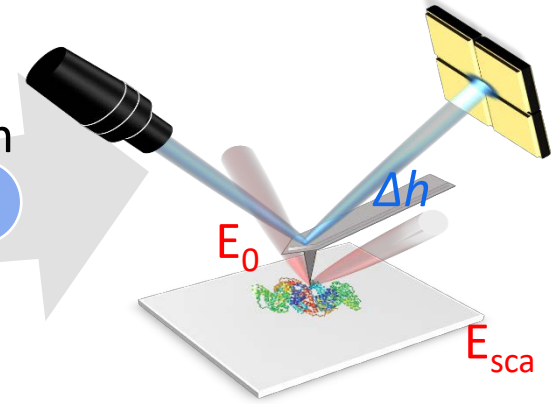
mm



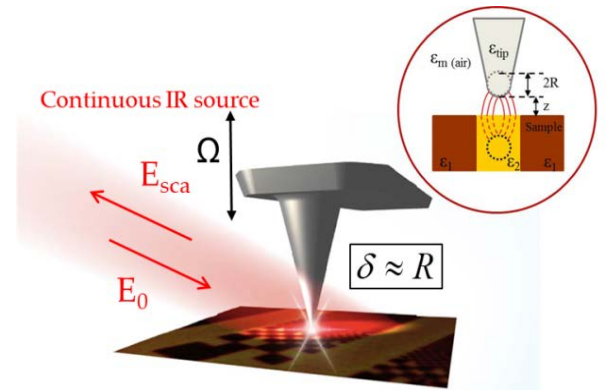
Far Field  
IR microscopy

$\mu\text{m}$

nm



Near field  
IR nanoscopy





## Exploitation of IRSR advantages



### Advantage

#### Flux Advantage in FIR and THz

- Higher S/N
- Faster data collection

#### Broad band nature

- Complete data collection

#### Polarization

- BM Linear polarization
- ER circular polarization

#### Brightness advantage

- Higher S/N ratio
- Faster data collection

### Application

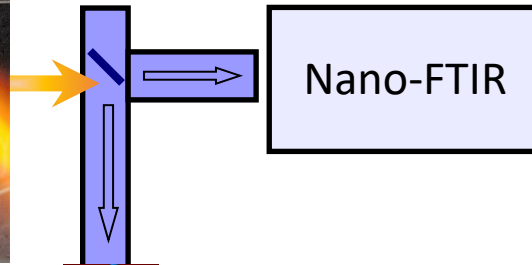
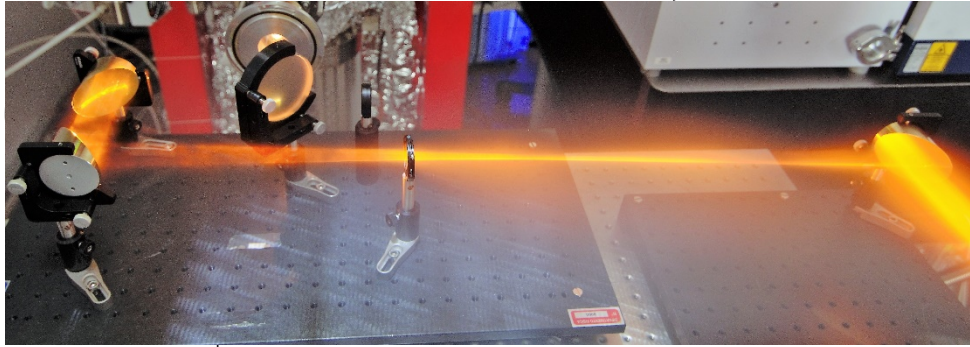
FIR and THz spectroscopy

Spectroscopy, Microscopy,  
Nanoscopy

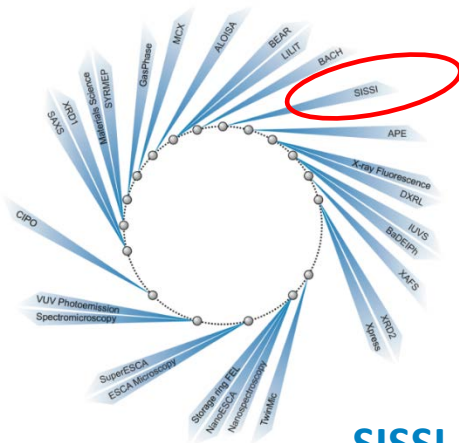
Spectroscopy and Microscopy

Microscopy and Nanoscopy

# SISSI-Synchrotron Infrared Source for spectroscopy and imaging

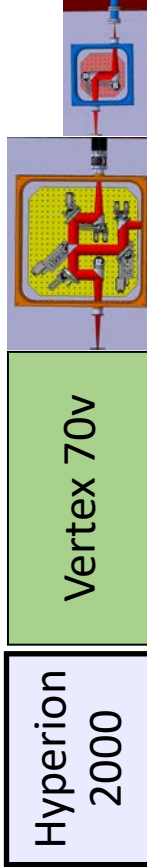


**SISSI-Nano**  
The new endstation for  
IR nanoscopy  
**IRSR & Lasers**



**SISSI-MAT**  
Material Sciences branch

**FIR and  
THz**



Vertex 70v FM

Hyperion  
3000

**SISSI-BIO**  
Chemical and Life Sciences branch



FTIR spectroscopy in the  
FIR-MIR regime, FTIR  
imaging and microscopy  
in the MIR  
**IRSR & Conventional IR  
source**

SISSI-Bio @Elettra - Photo courtesy of  
CERIC-ERIC , Photographer: Roberto  
Barnabà



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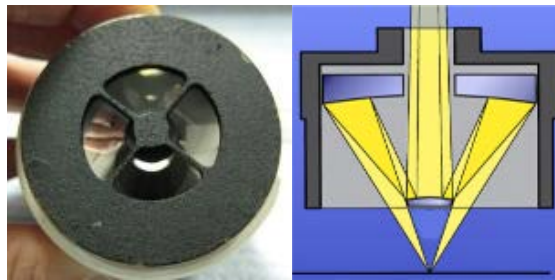
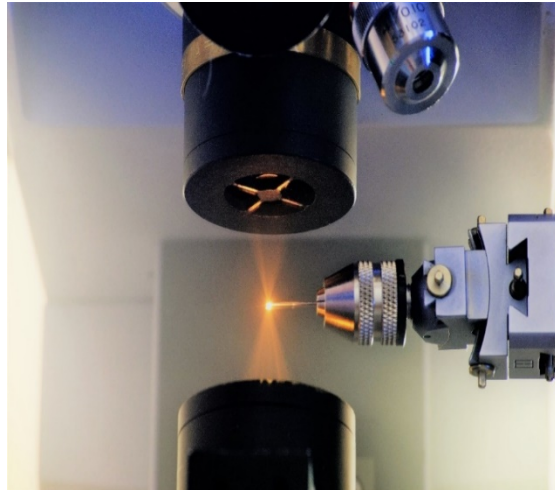
# Far-field FTIR microscopy for biospectroscopy



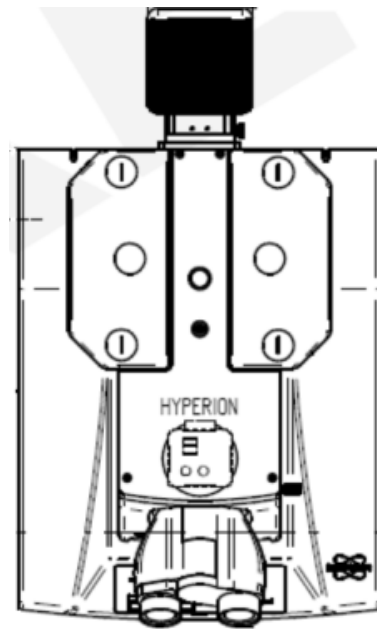


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# FTIR microscopy\_Instrumentation

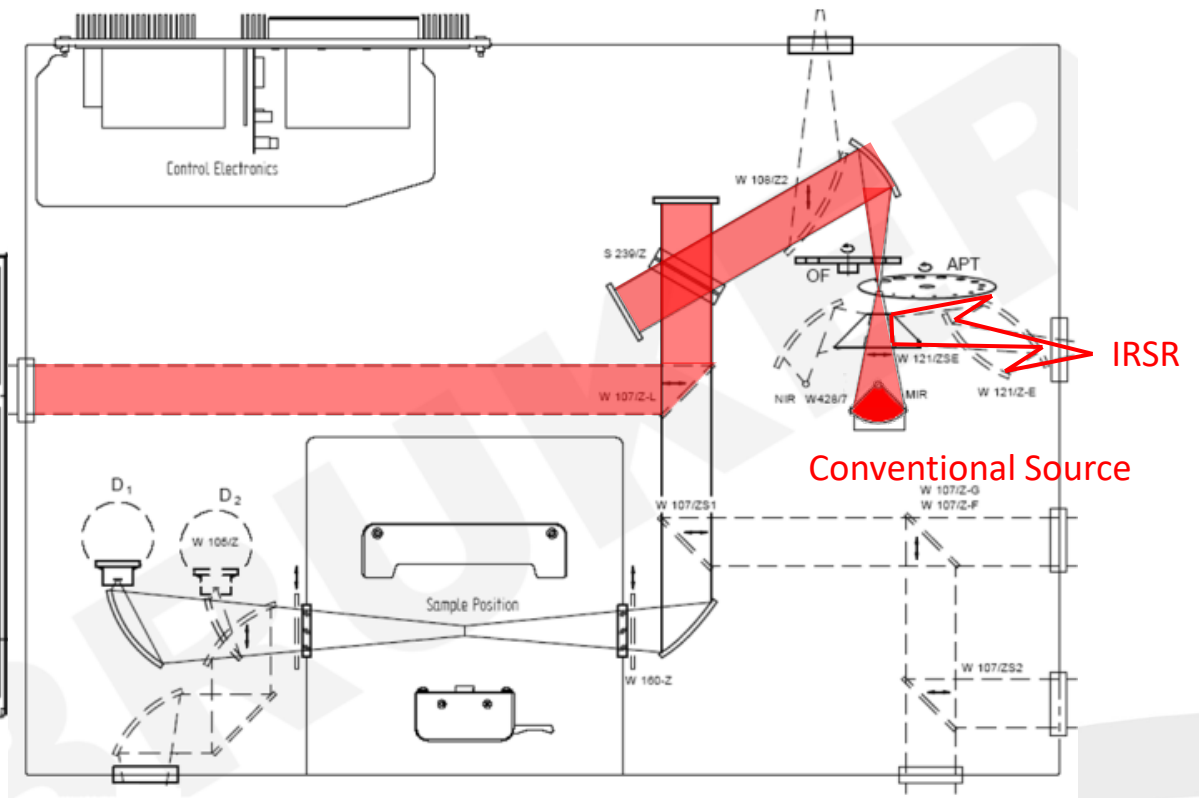


Schwarzschild/Cassegrain objective



Bruker Hyperion 3000 microscope

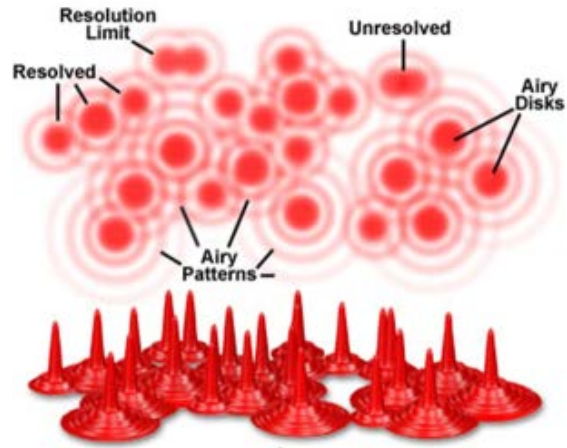
Bruker 70v interferometers





# FTIR microscopy\_Lateral resolution

## The Rayleigh criterion

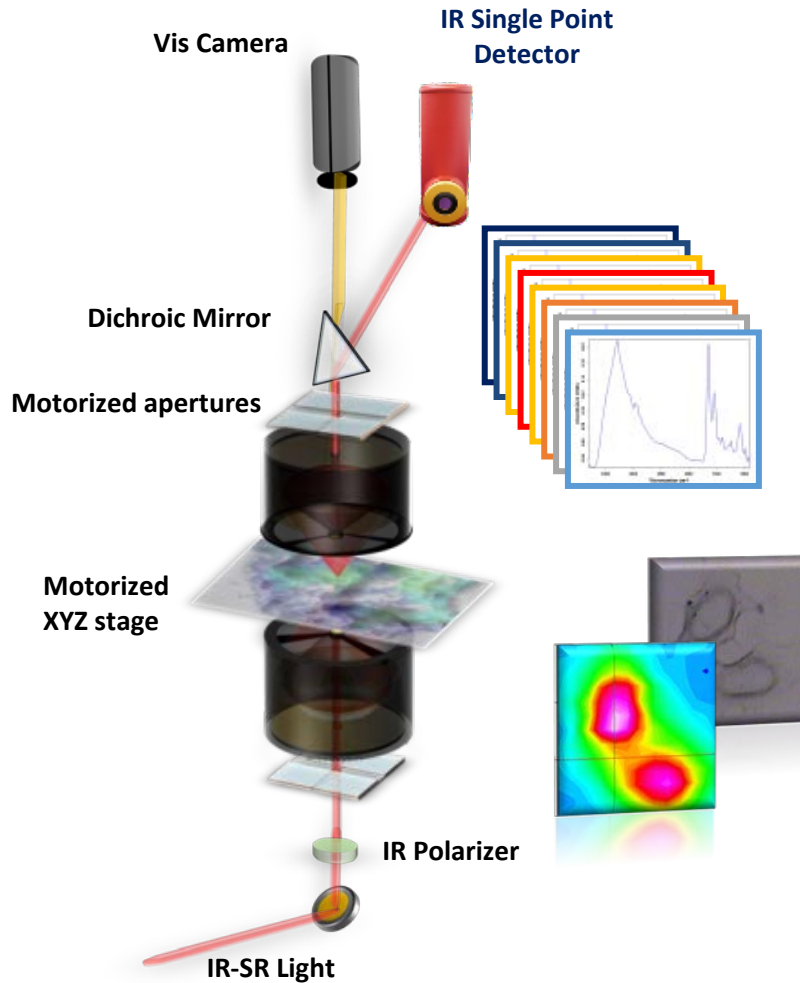


$$\delta = 0.66 \frac{\lambda}{nNA}$$

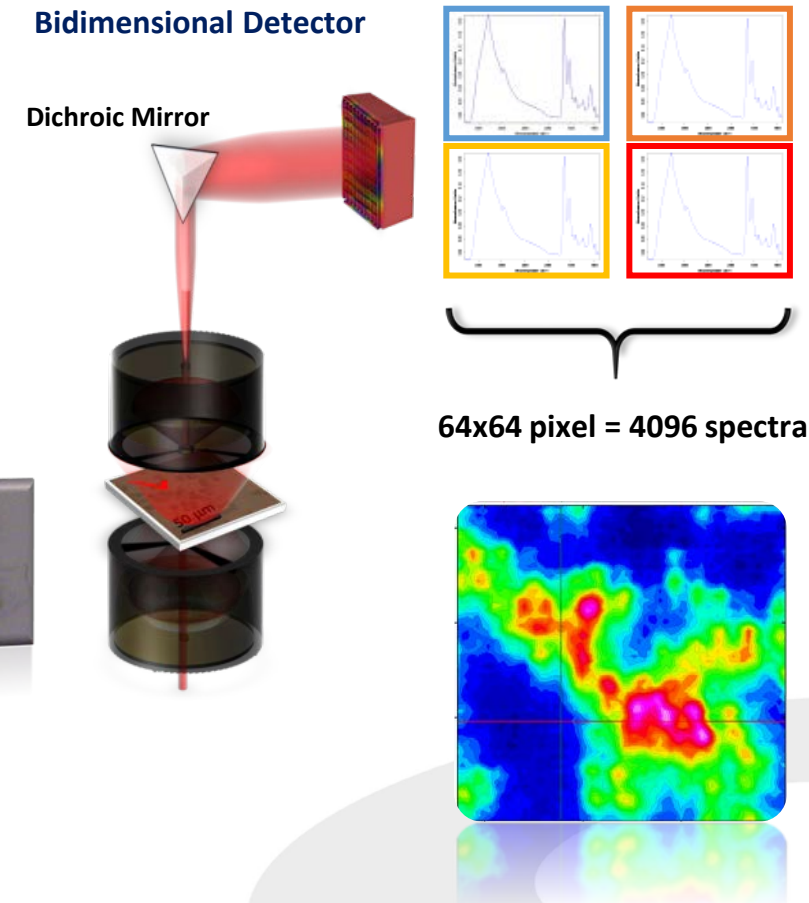
Far field microscopy  
Achievable lateral resolution

Wavelength	$\delta$ (NA=0.65)
10 mm (1 cm <sup>-1</sup> )	~ 10 mm
100 $\mu$ m (100 cm <sup>-1</sup> )	~ 100 $\mu$ m
10 $\mu$ m (1000 cm <sup>-1</sup> )	~ 10 $\mu$ m
2.5 $\mu$ m (4000 cm <sup>-1</sup> )	~ 2.5 $\mu$ m

## FTIR mapping



## FTIR imaging

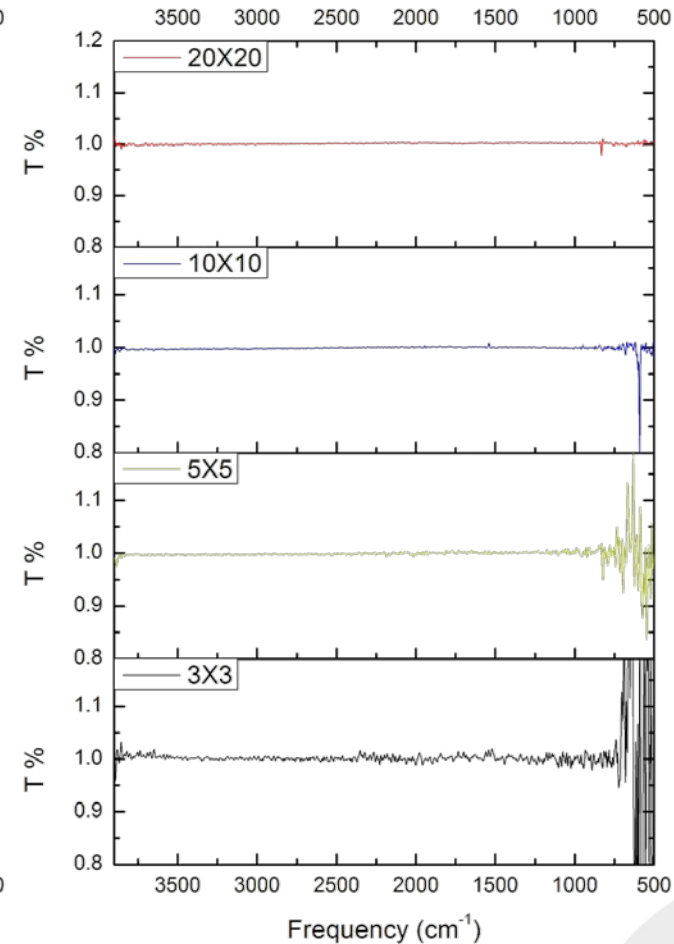
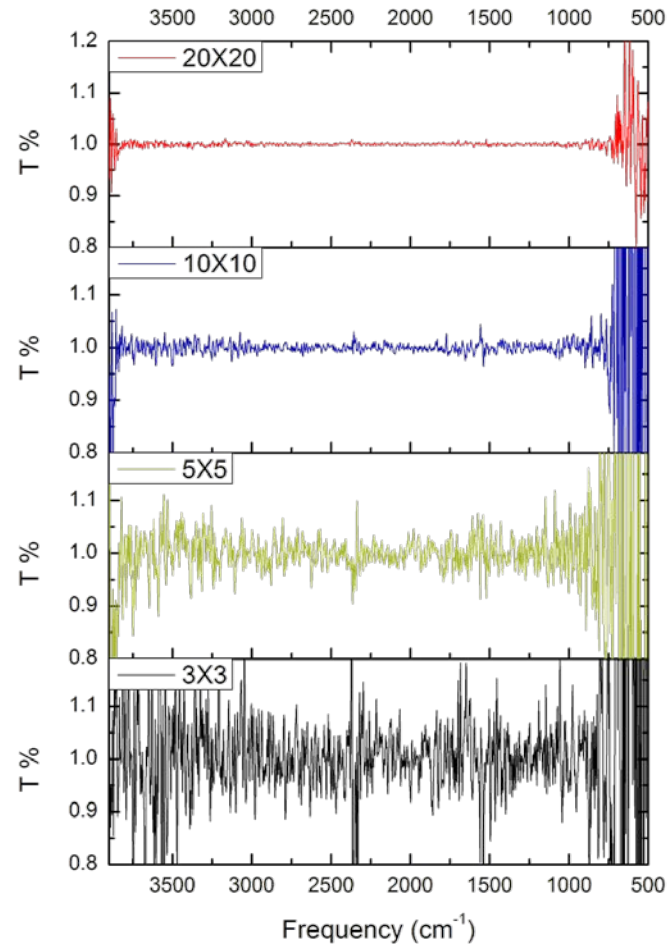
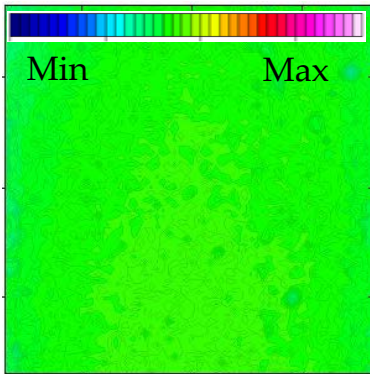


# IRSR advantage for FTIR microscopy

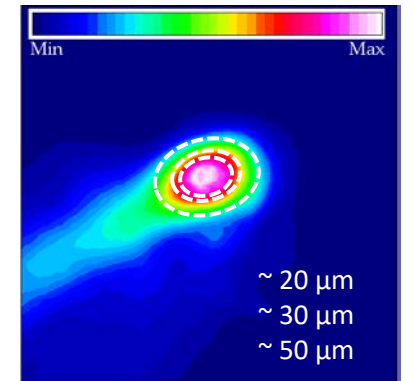
Diffraction Limited FTIR Microscopy is practically achievable only with IRSR



Conventional  
Source



IRSR



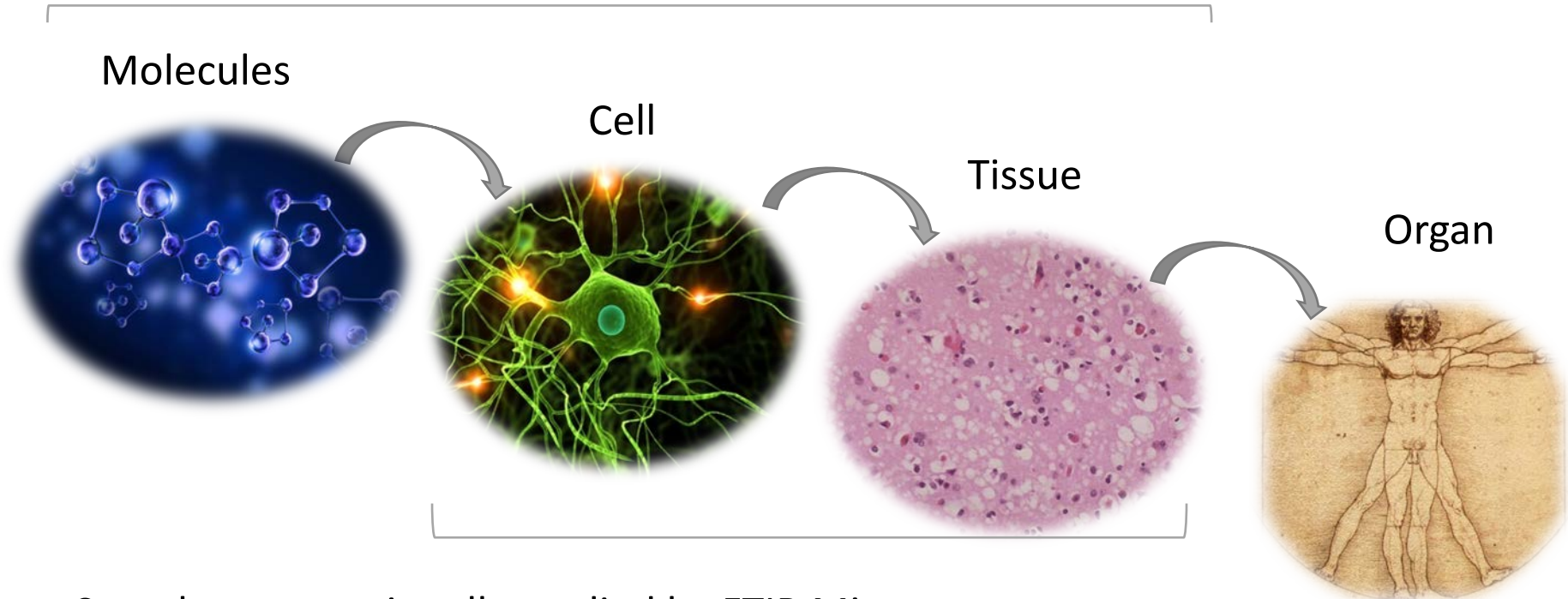
S/N ratio at SISSI for diverse knife-edge aperture settings (lateral resolution)





# A few words on bio-spectroscopy

Samples conventionally FTIR Bio-spectroscopy



Samples conventionally studied by FTIR Microspectroscopy

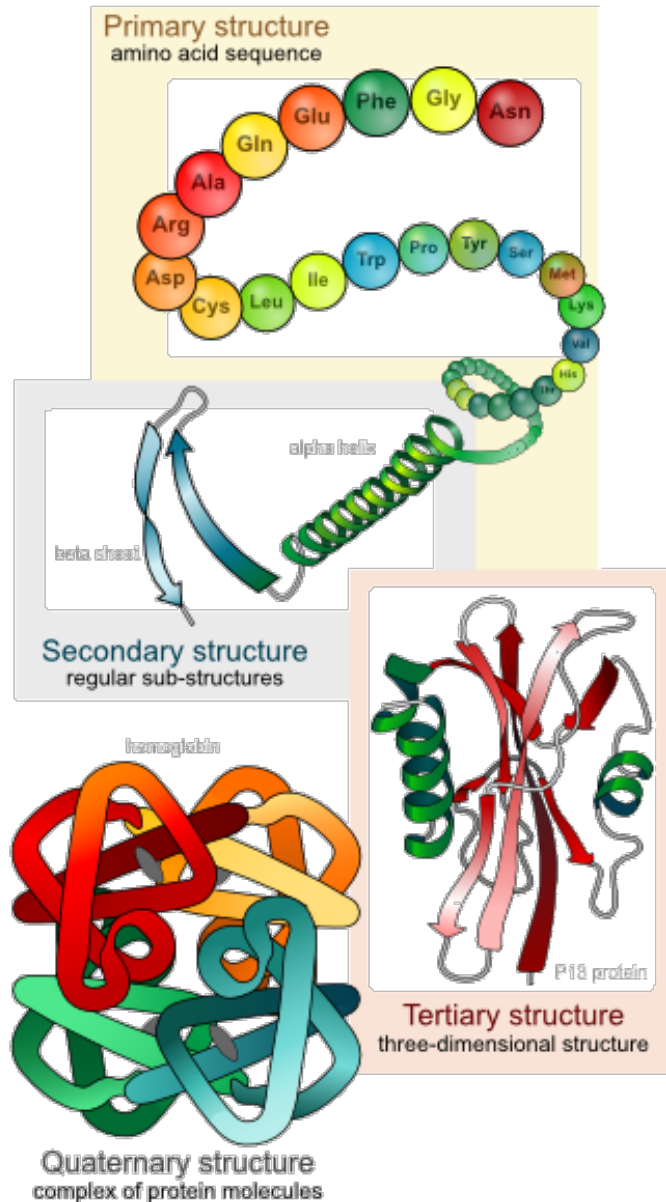
FTIR Microscopy allows to investigate several aspects of the **sample biochemistry**, from individual molecules to cells and tissues.

On the most relevant biomolecules (proteins, lipids, nucleic acids, carbohydrates), information can be retrieved on their relative proportion, conformation, state of order, dynamics and much more....

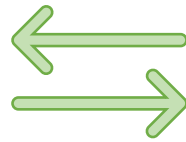




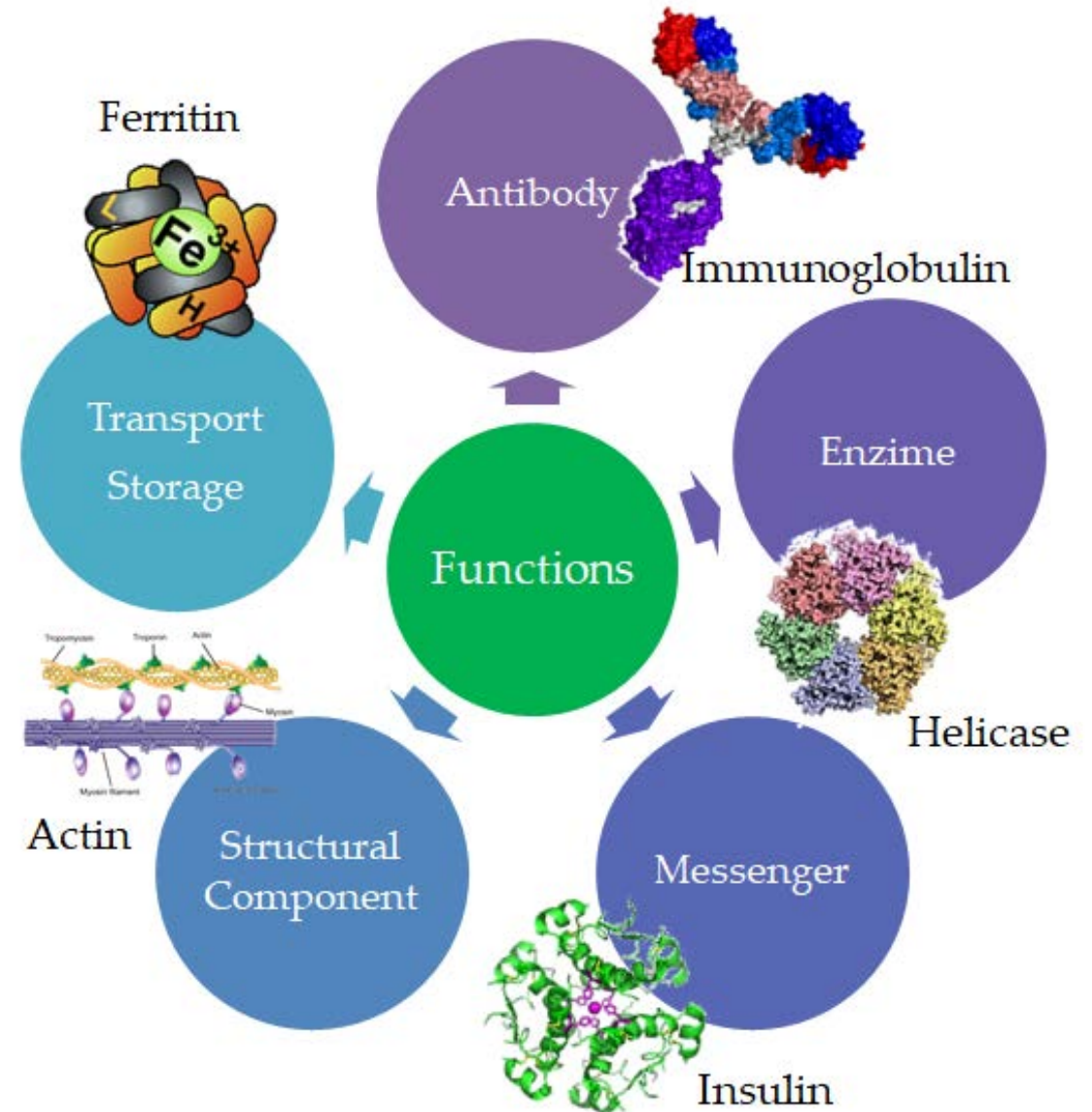
# A quick dip in protein structure and function



Structure



Activity



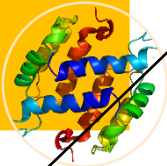


# Drug discovery and development process




- **Cellular and genetics targets**
- Bioinformatics
- Genomics
- Proteomics

Target selection




- **Synthesis and isolation**
- Combinatorial Chemistry
- High throughput screening
- Biophysical methods

Lead discovery



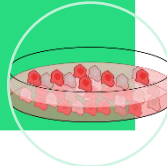
- **Library development**
- Structure Activity studies
- Chemical Synthesis

Medical chemistry



- **Drug Affinity & Selection**
- Cellular disease models
- Mechanisms of actions
- Lead Candidate refinement

In vitro studies




**Does the drug bind?**

Biophysical methods are fast, unexpensive, requires minimal sample preparation but they are blind to the protein structure


- **Animal Models and Disease States**
- Behavioural studies
- Functional Imaging
- Ex vivo studies

In vivo studies



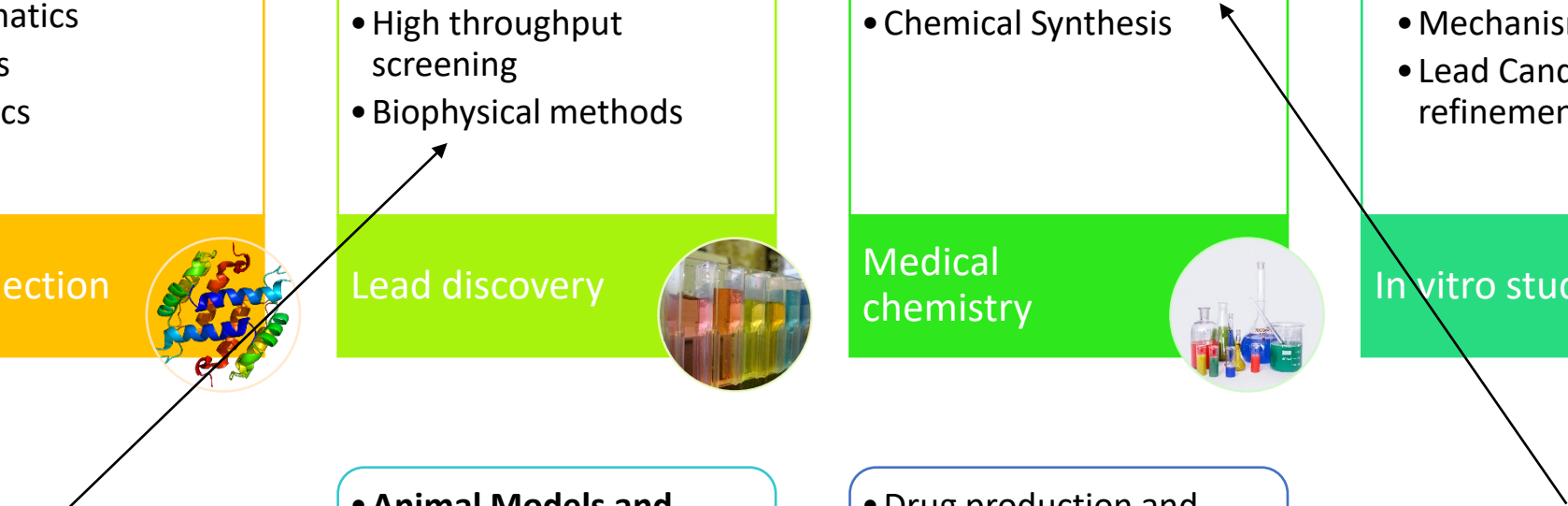
- Drug production and marketing

Clinical trials and Therapeutics



**What's the drug effect on the protein structure?**

Conventional technique for protein structural analysis may present some drawbacks





# Conventional techniques for protein structural analysis



X-ray crystallography

Nuclear Magnetic Resonance (NMR)

Cryo Electron Microscopy (Cryo-EM)\*

**Does exist a method able to bridge biophysical and structural protein studies?**

- ✓ About 90%\* of protein structures
- ✓ 3D structure
- ✓ Atomic resolution (Å)
- ✓ Protein-ligand interaction

- x Samples preparation (crystalization)
- x Not native conditions
- x No protein dynamic behavior

- ✓ About 8%\* of protein structures
- ✓ 3D structure
- ✓ Atomic resolution
- ✓ Measure in buffer
- ✓ Protein dynamic behaviour

- x Expensive sample preparation
- x Time and material consuming
- x Limited for little proteins (less 30kDa)

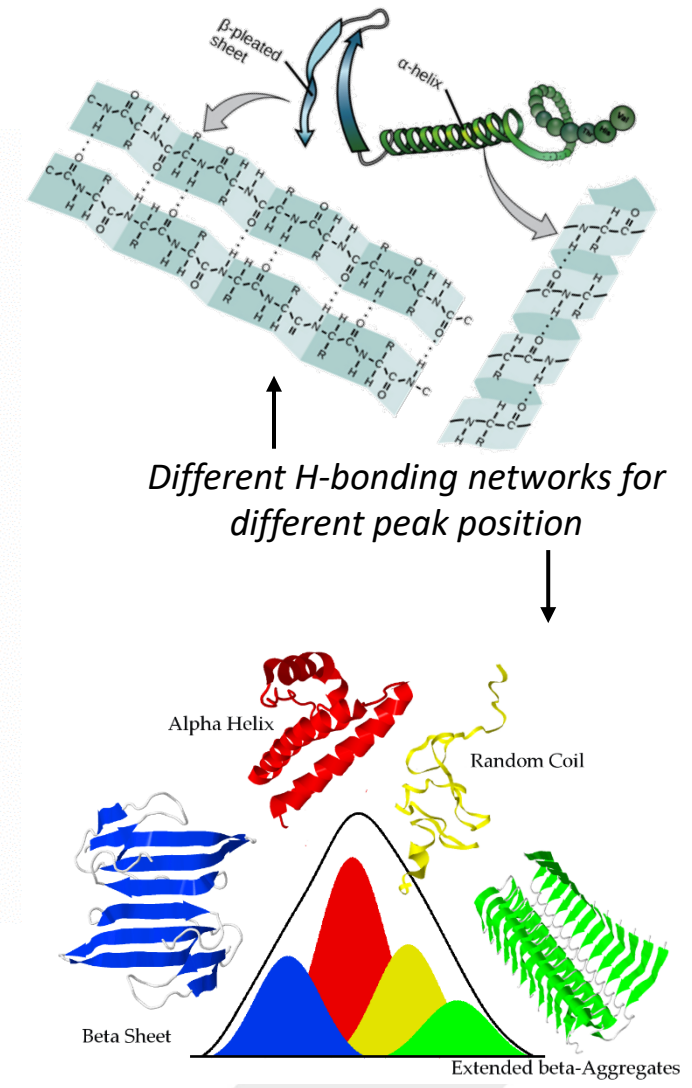
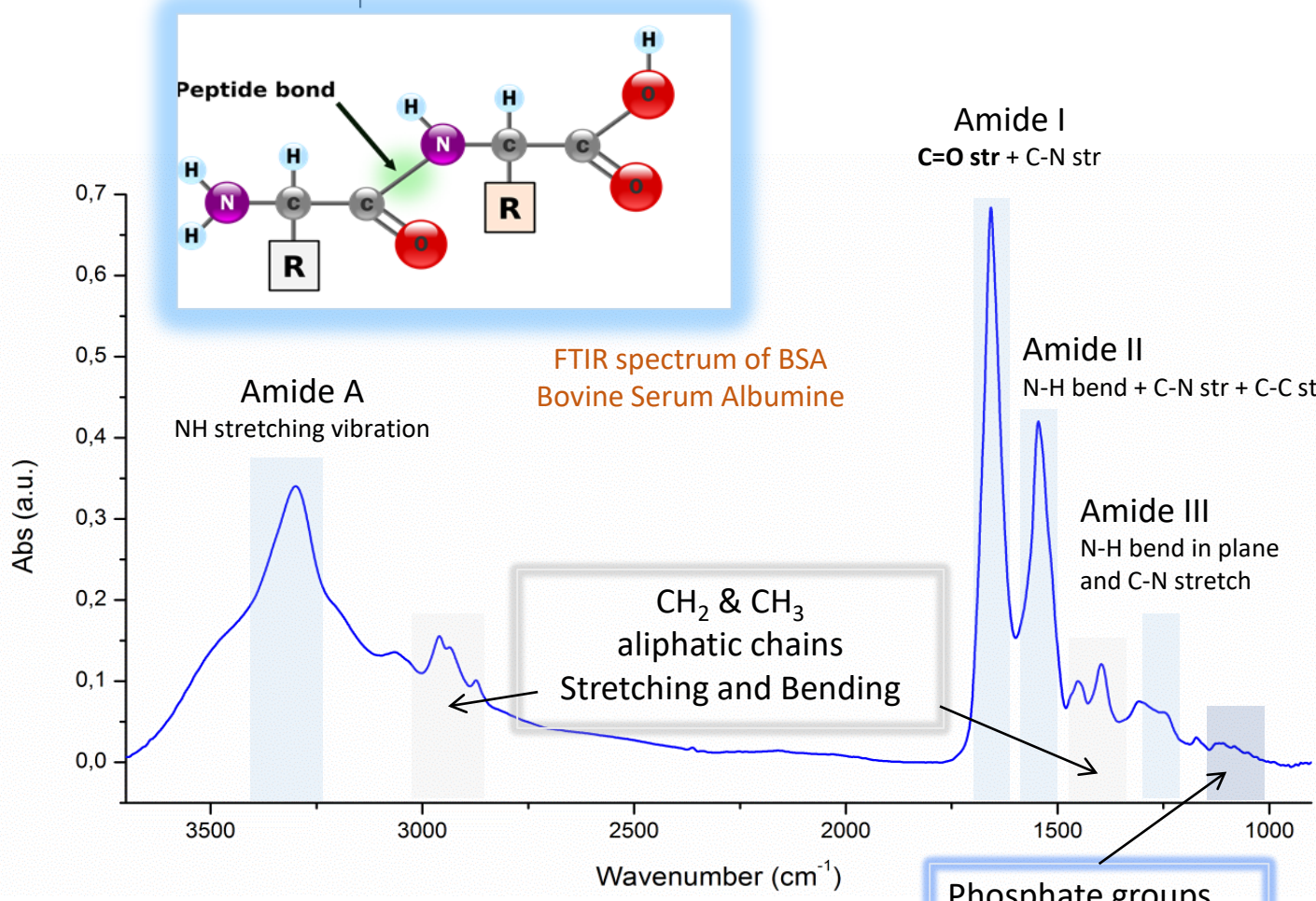
- ✓ About 2%\* of protein structure
- ✓ 3D structure
- ✓ Near-atomic resolution
- ✓ Fast sample preparation: froze samples in their NATIVE form

- x Only larger proteins (more than 50-75 Kda)
- x No protein dynamic behavior





# FTIR spectroscopy for protein conformational studies



## Advantages:

Relevant also for non-crystallizable proteins

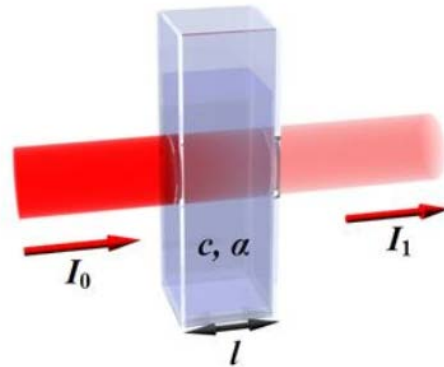
## Limitations:

**Sensitivity Limit**

**Water barrier**

# Sensitivity limit and water barrier

## Sensitivity Limit



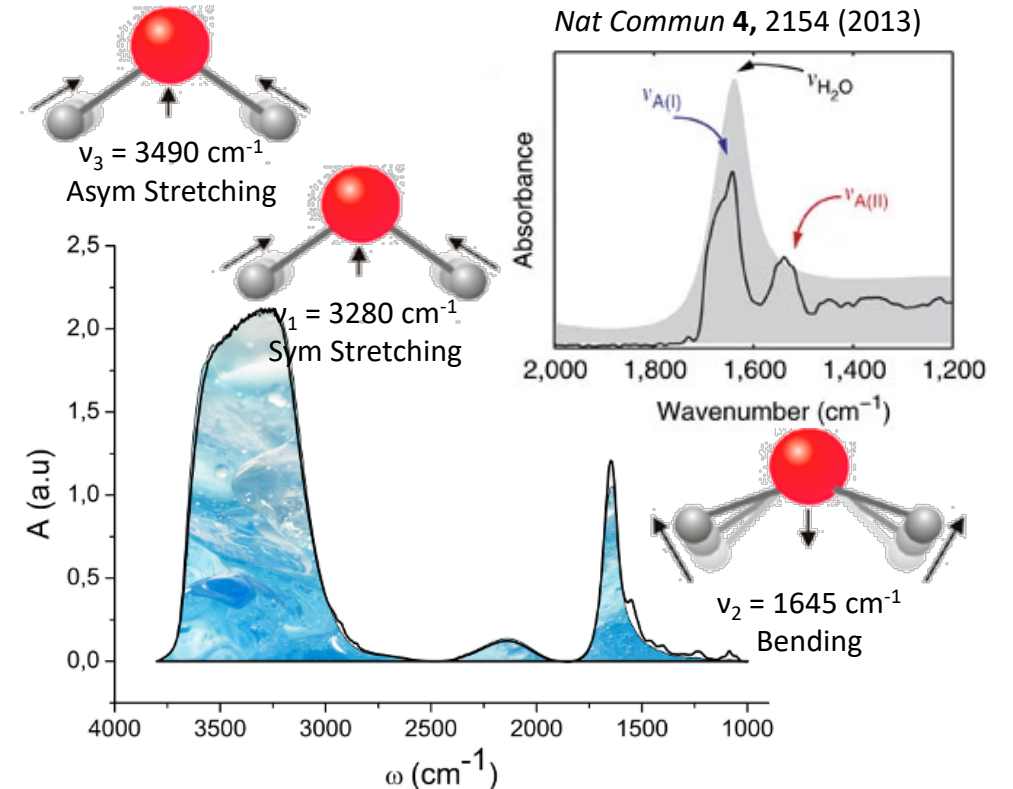
### Beer's Law

$$T = \frac{I}{I_0} \approx e^{-\alpha d}$$

- Requires extremely high concentrations, in the mM regime
  - These concentrations are often not relevant in physiological conditions
- Prohibits measurements from monolayers with nanometer thickness

## Water Barrier

Adato, R., Altug, H.  
*Nat Commun* **4**, 2154 (2013)



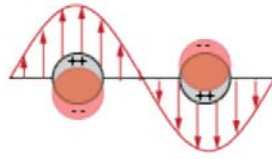
- Water bands overlaps with the vibrational features of biomolecules
  - Requires dried samples or D2O, that are not compatible with biomolecules



# Defeat the sensitivity limit: the plasmonic approach

$$IR \text{ absorbance} \propto \text{Oscillator strength of molecular bond} \times |Electric\ field|^2$$

## Plasmonic nanostructure

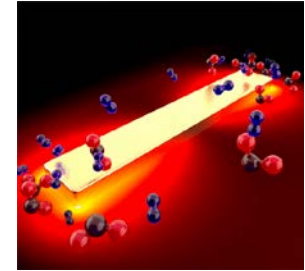


Adato, R., et al.

*Materials Today*, **18 (8)**, 436-446 (2015)

## Optical nano-resonators

- Focus light into nanoscale volumes
- Enable strong light-matter interaction
- Ideal to achieve ultra-high sensitivity

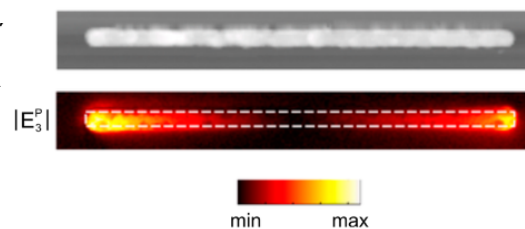
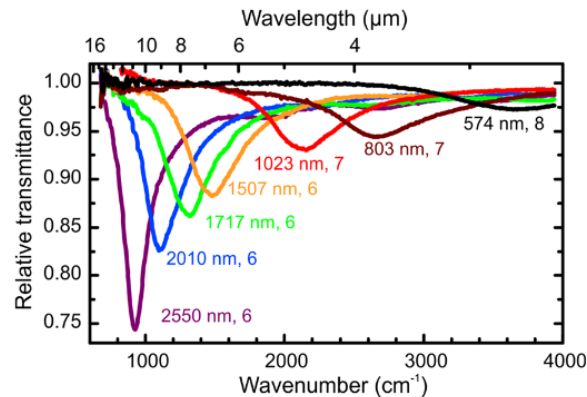


Neubrech, F., et al.

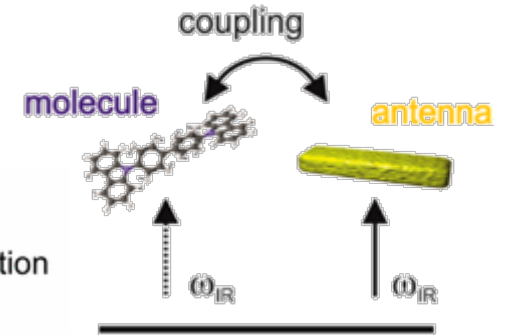
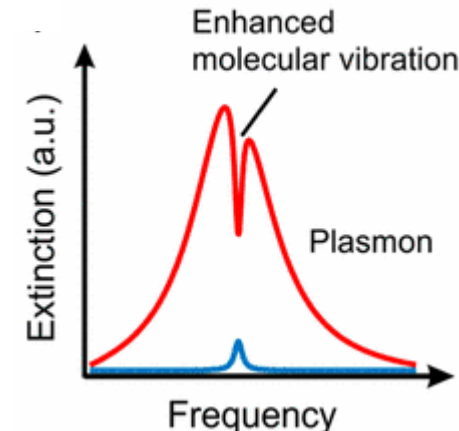
*Chem. Rev.*, **117 (7)**, 5110-5145 (2017)

## Resonance tuning

Antenna's material, shape and dimension are the key parameters for tuning position and bandwidth of the antenna response



## Spectral coupling



Signal enhancement of several order of magnitude can be achieved

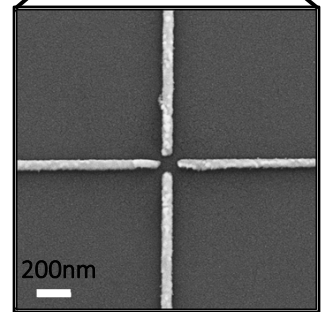
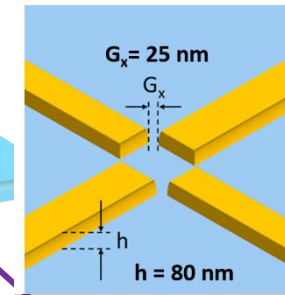
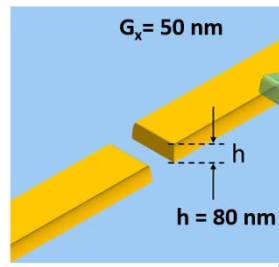
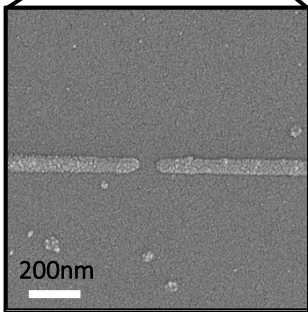
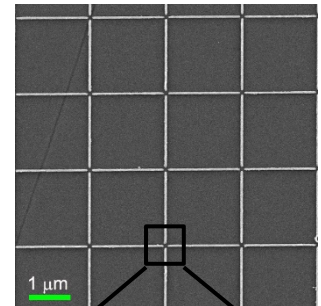
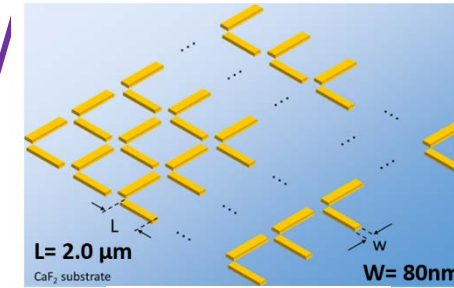
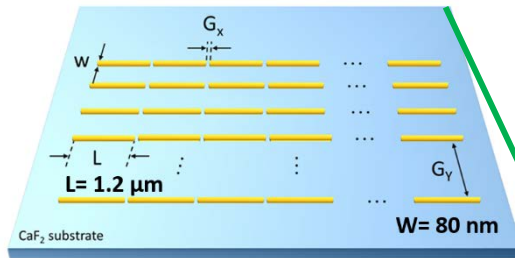
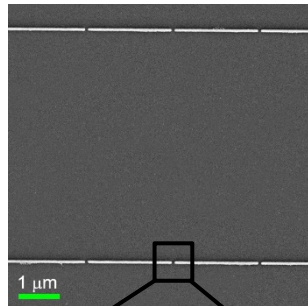




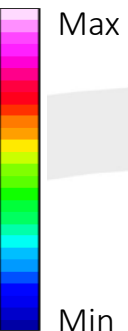
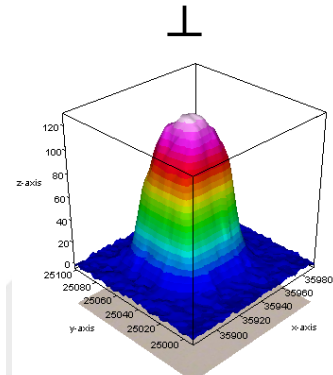
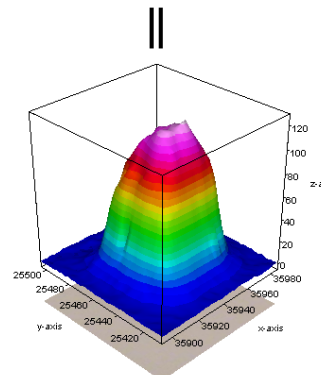
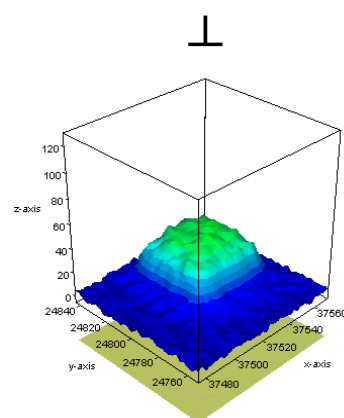
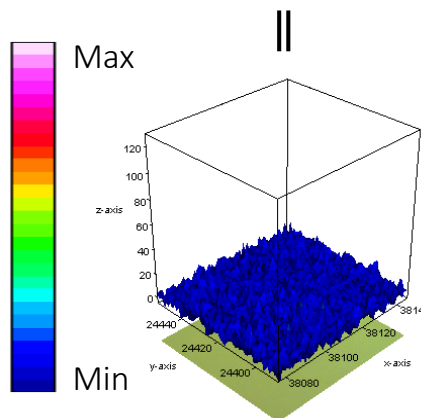
Elettra  
Sincrotrone  
Trieste

# Defeat the sensitivity limit: the plasmonic approach

## Surface Enhanced Infrared Absorption Spectroscopy (SEIRA) on protein monolayers



IR beam



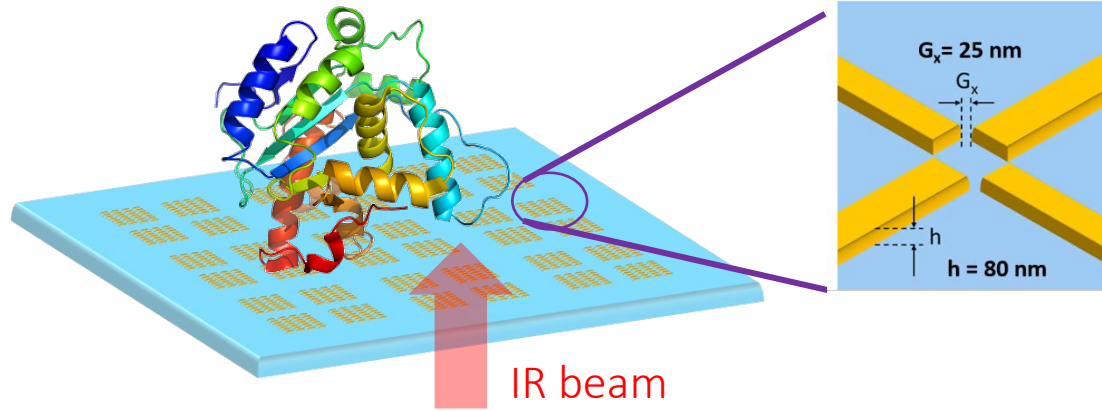




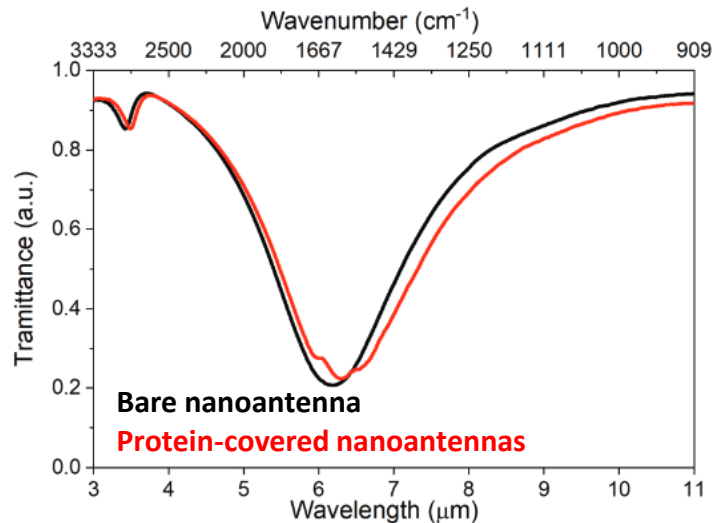
# Defeat the sensitivity limit: the plasmonic approach

## Surface Enhanced Infrared Absorption Spectroscopy (SEIRA) on protein monolayers

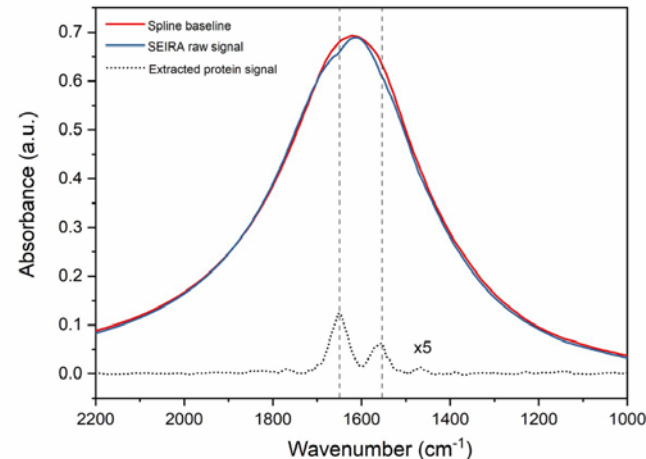
### 1\_Protein layer formation



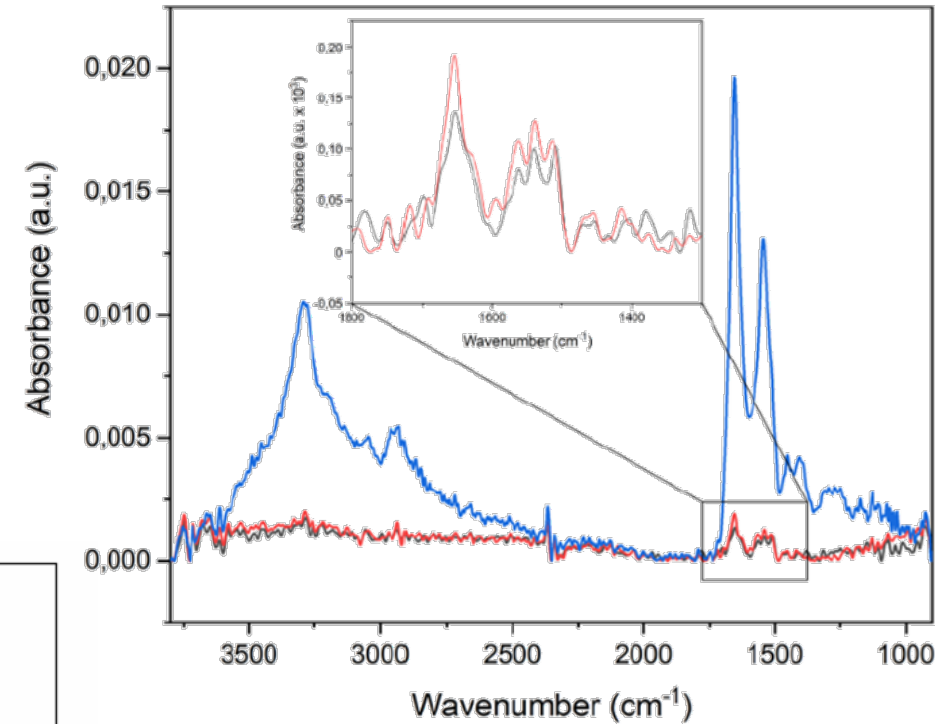
### 2\_Plasmonic response



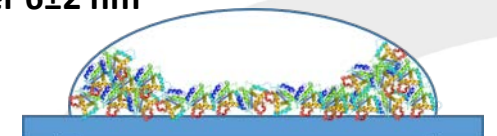
### 3\_Signal Extraction



### Conventional FTIR vs SEIRA



Protein layer  $6 \pm 2$  nm



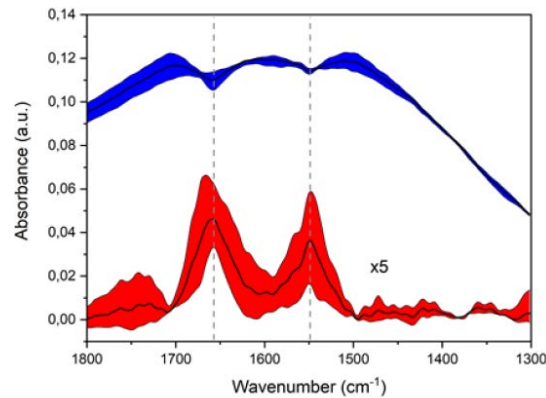
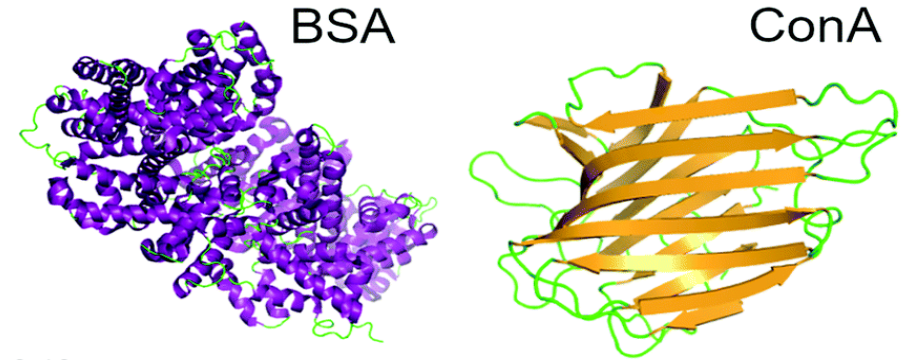
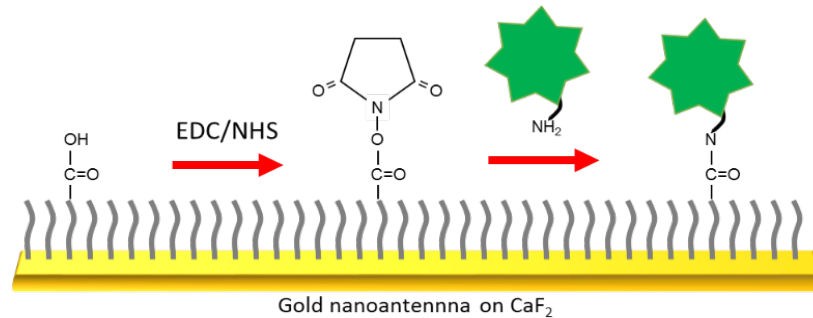


# Defeat the sensitivity limit: the plasmonic approach

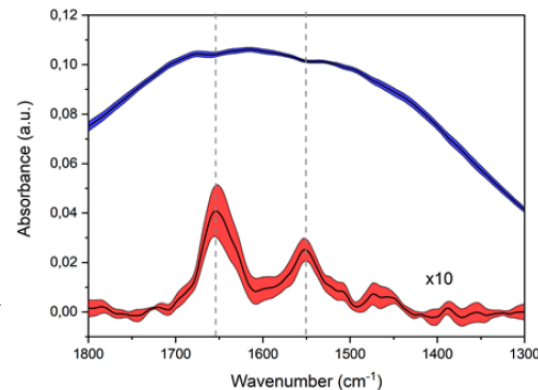
## SEIRA on BSA and ConA protein monolayers

### Protein monolayer formation

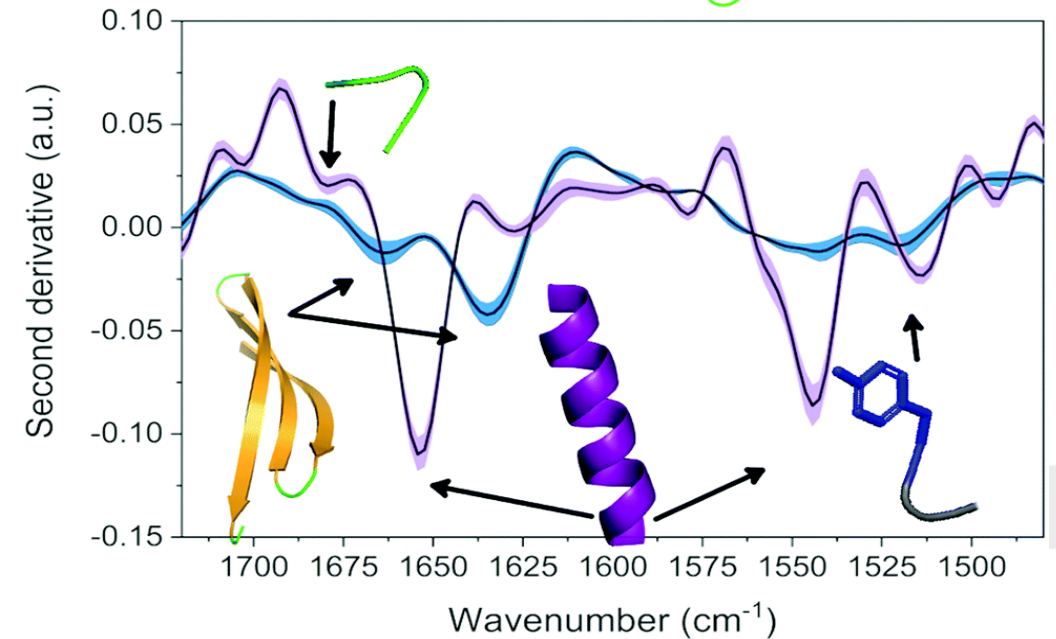
#### Amine coupling



#### Drop casting vs Amine coupling



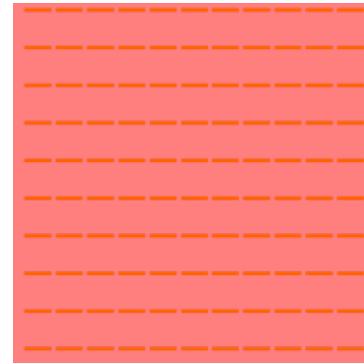
Standard deviation  
is much reduced



# Defeat the sensitivity limit: the plasmonic approach

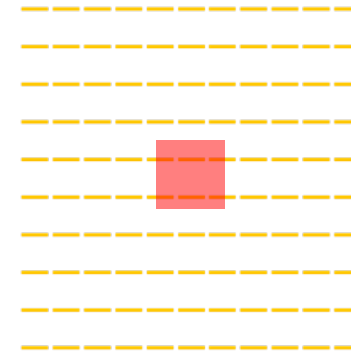
## Protein Signal enchantment in dried conditions: Conventional source vs IRSR

Globar  
Conventional  
Source  
(50x50)  $\mu\text{m}^2$

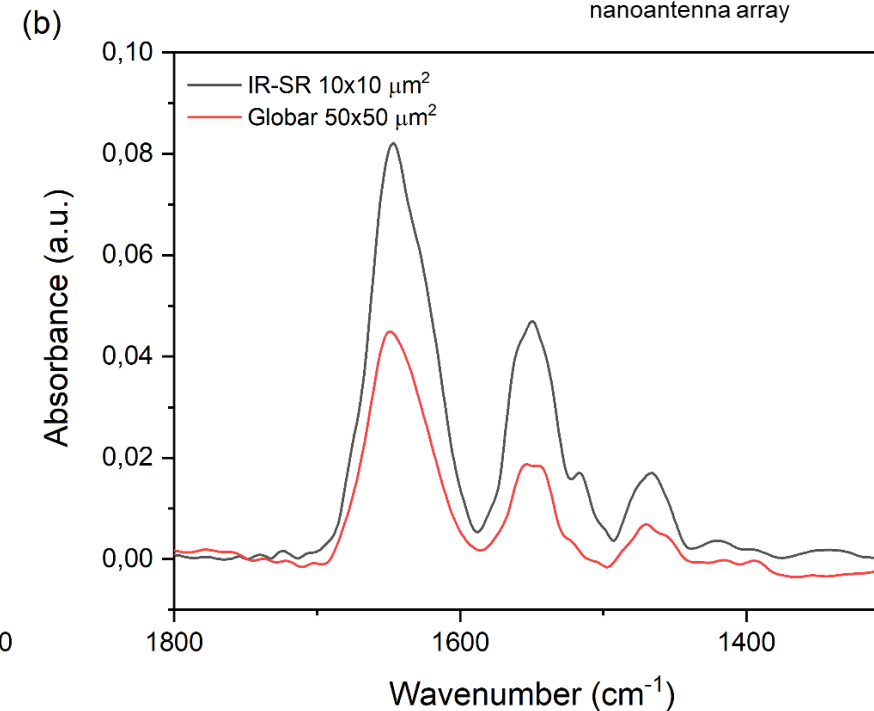
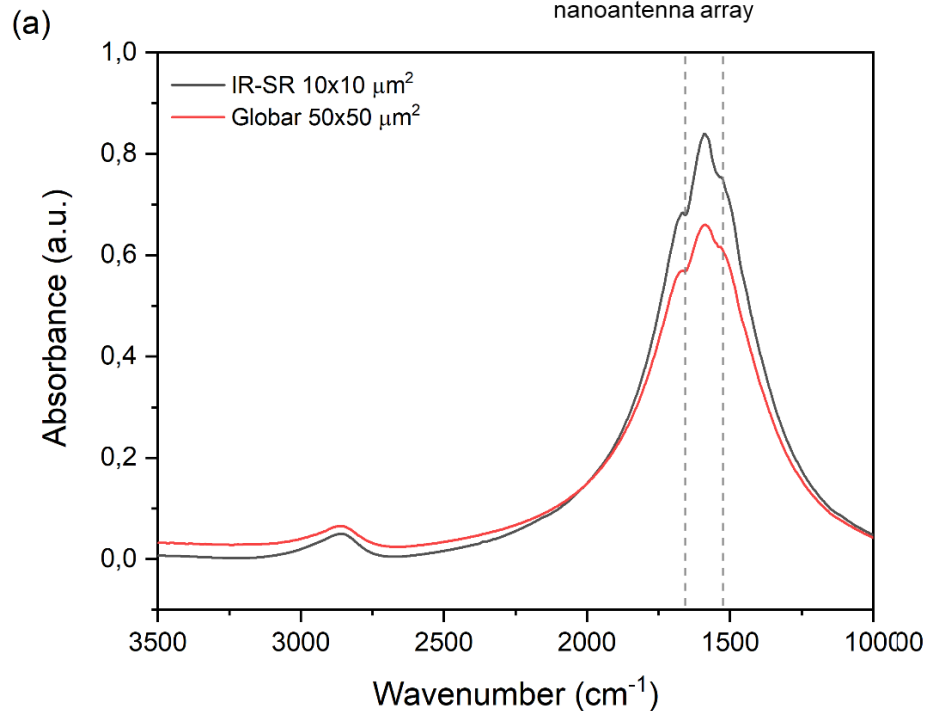


Single ordered  
nanoantenna array

InfraRed  
Synchrotron  
Radiation  
(10x10)  $\mu\text{m}^2$



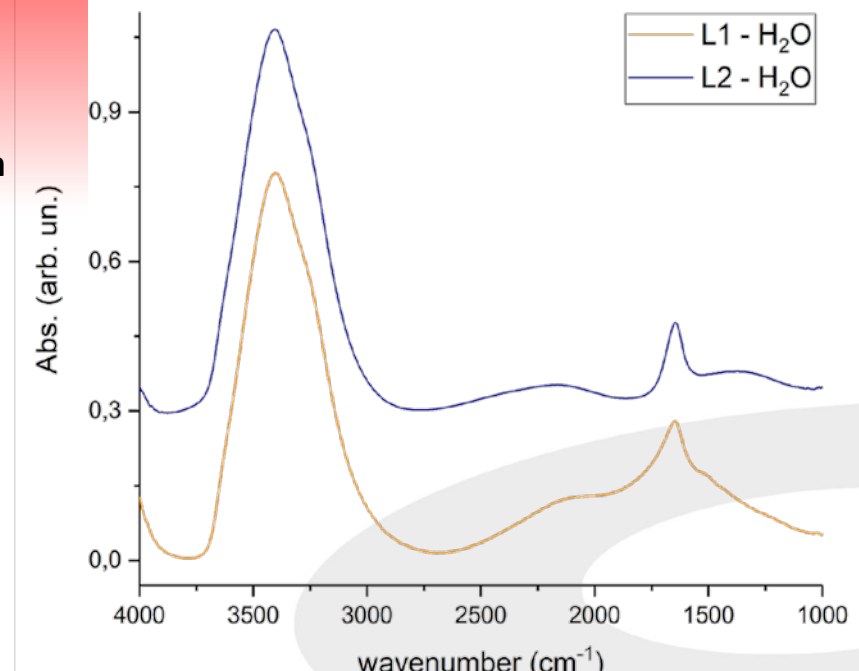
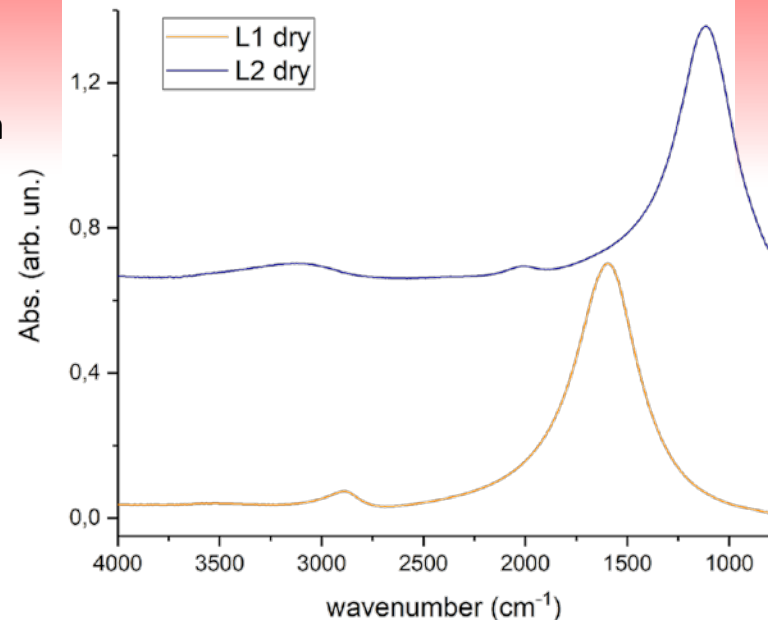
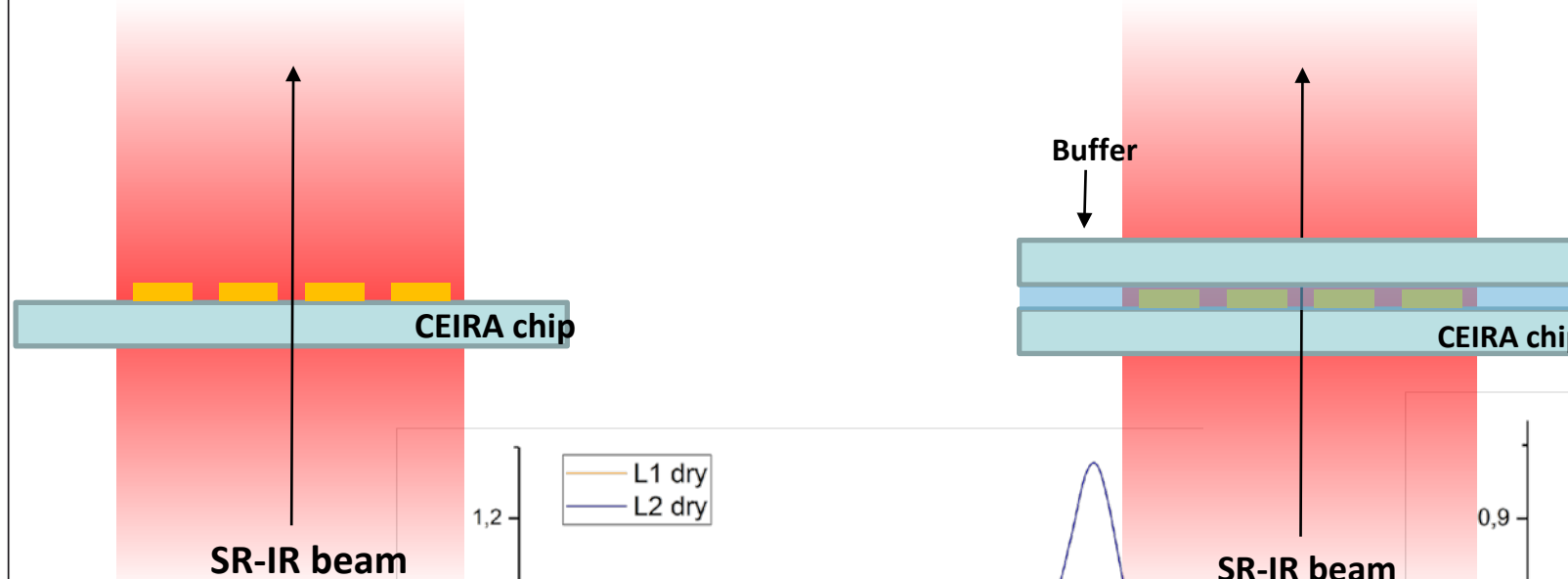
Single ordered  
nanoantenna array





# Defeat the water absorption barriers: the PIR approach

## SEIRA measurements in transmission mode in dried and wet conditions

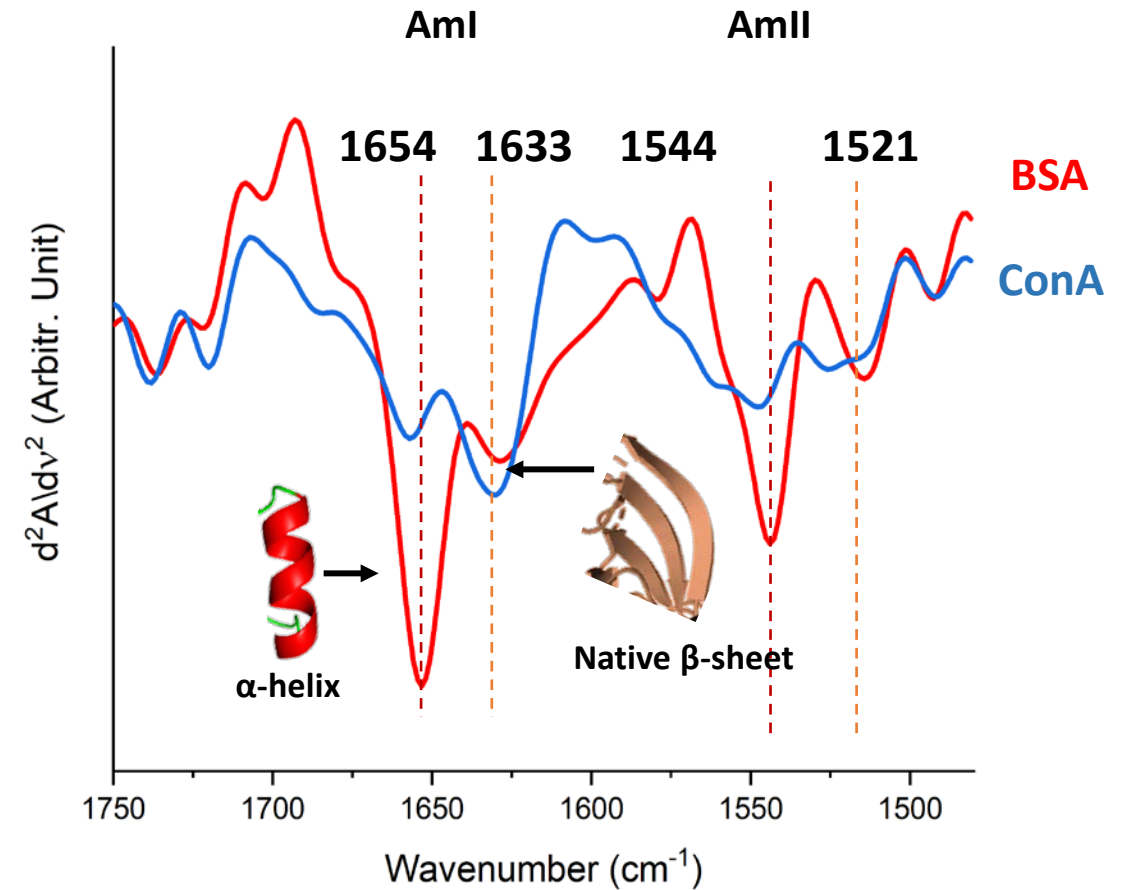
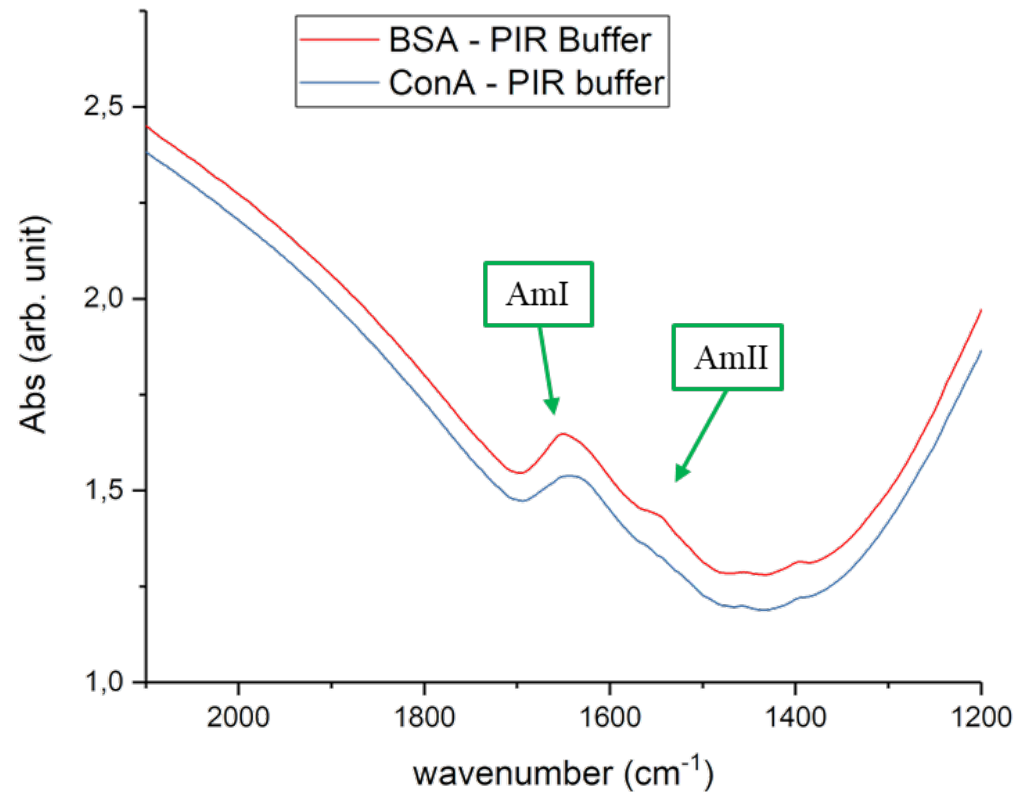




# Defeat the water absorption barriers: the PIR approach

## SEIRA measurements PIR geometry

### Plasmonic Internal Reflectance PIR







# Bridging biophysical and structural studies with PIR-SEIRA

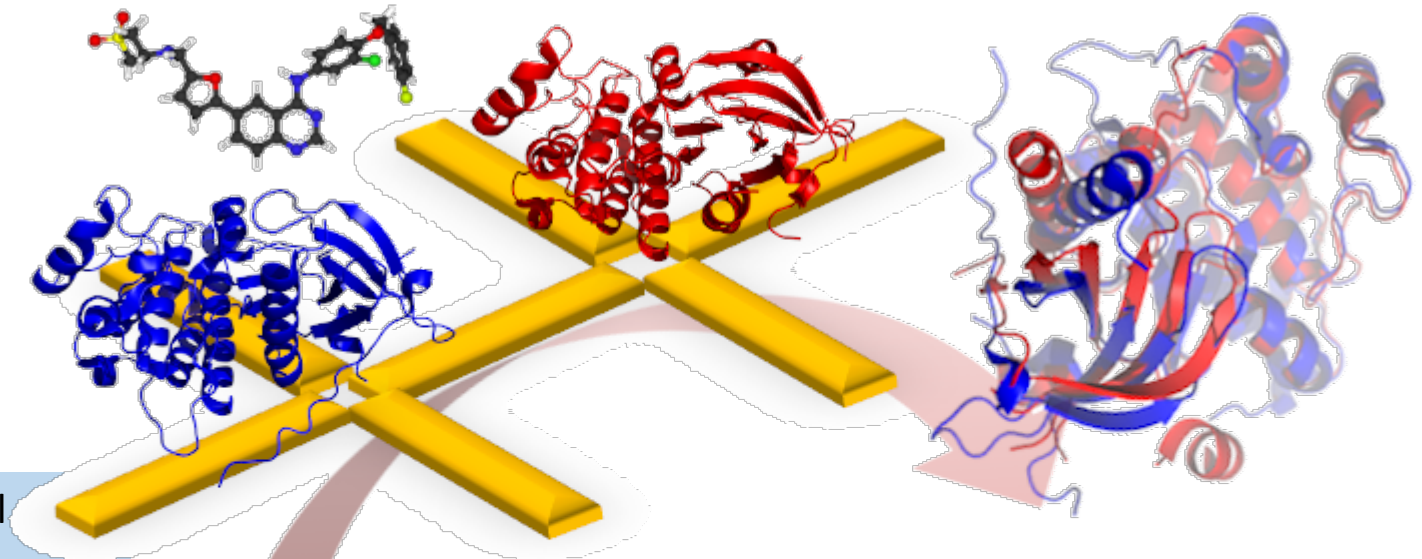
## EGFR-KD Lapatinib binding and conformational changes detected by PIR-SEIRA

### Kinase domain of Epidermal Growth Factor Receptor (EGFR-KD)

Receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands.

Mutations that lead to EGFR overexpression (upregulation) or over-activity have been associated with squamous-cell carcinoma of the lung (80% of cases).

Drug-target for anticancer therapies (Lapatinib, Gefatinib etc...).



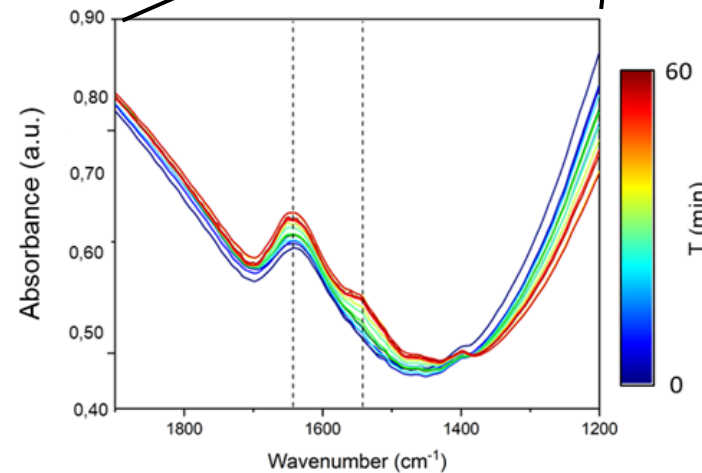
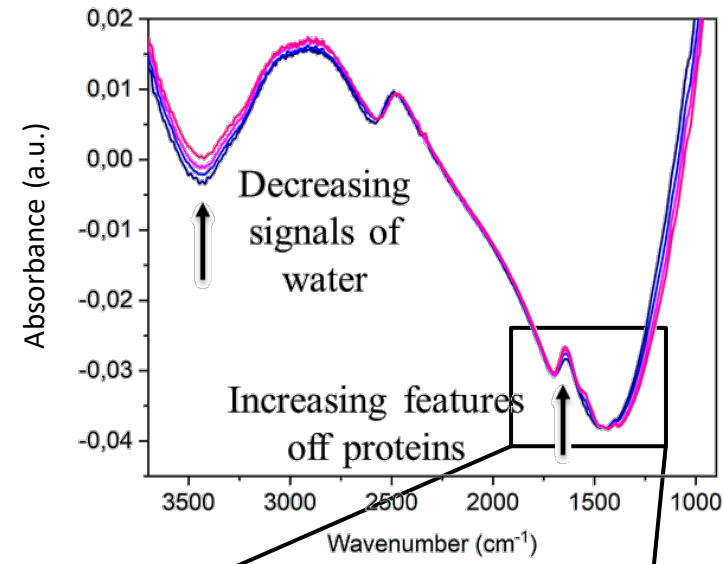
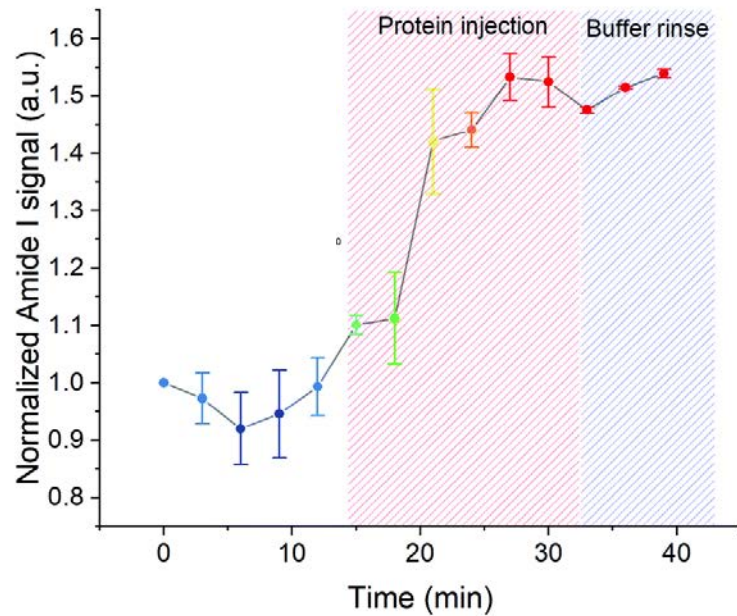
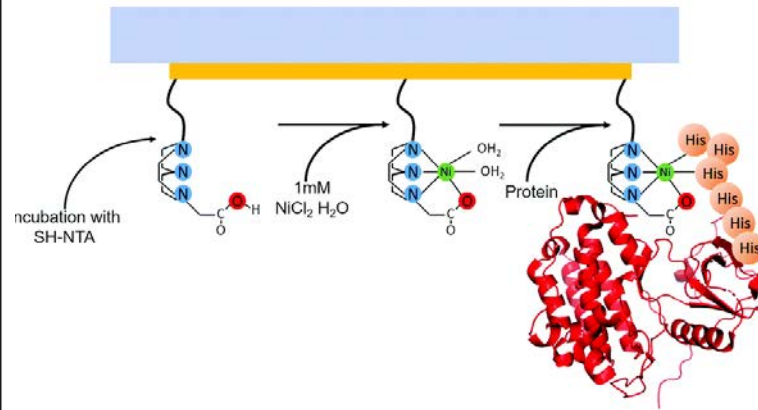
Mid-IR

EGFR-KD	Lapatinib/EGFR-KD
36% helices (both alpha and 3 <sub>10</sub> )	32% helices (both alpha and 3 <sub>10</sub> )
15% beta sheet strands	14% beta sheet strands.

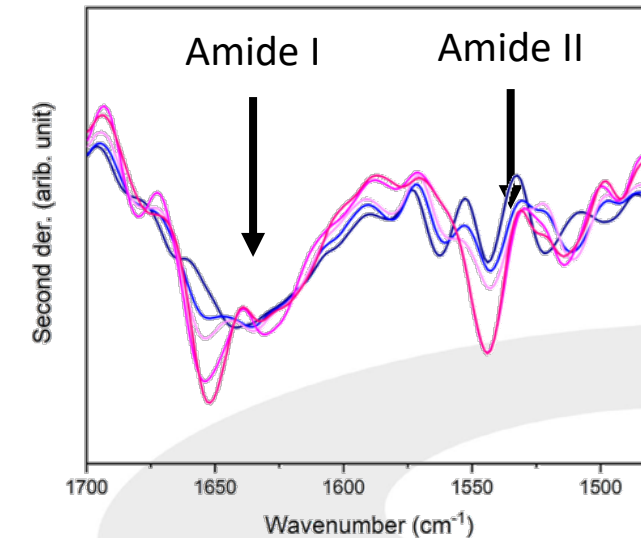


# Bridging biophysical and structural studies with PIR-SEIRA

## EGFR-KD Lapatinib binding and conformational changes detected by PIR-SEIRA



## Dynamic EGFR-KD protein binding

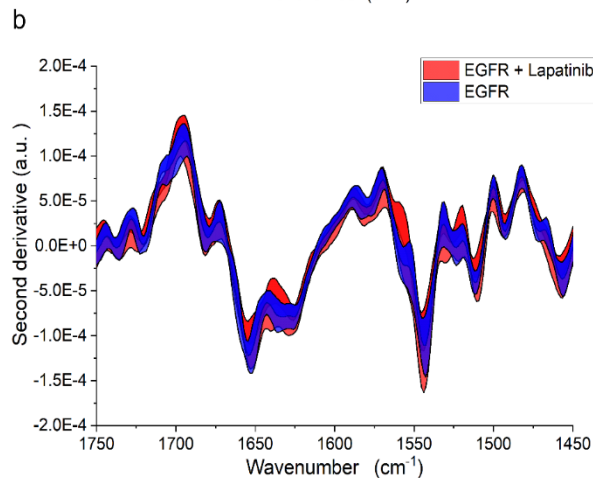
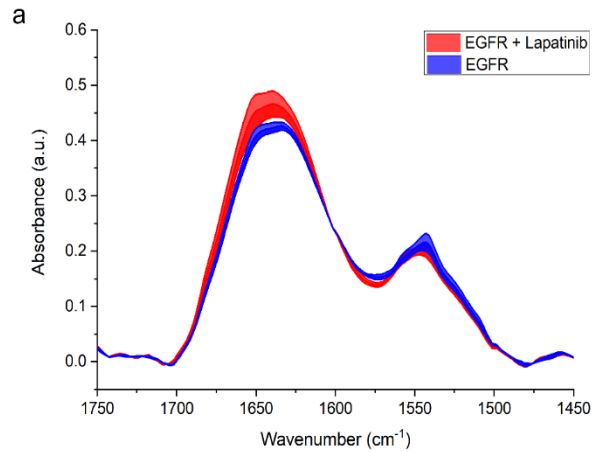




# Bridging biophysical and structural studies with PIR-SEIRA

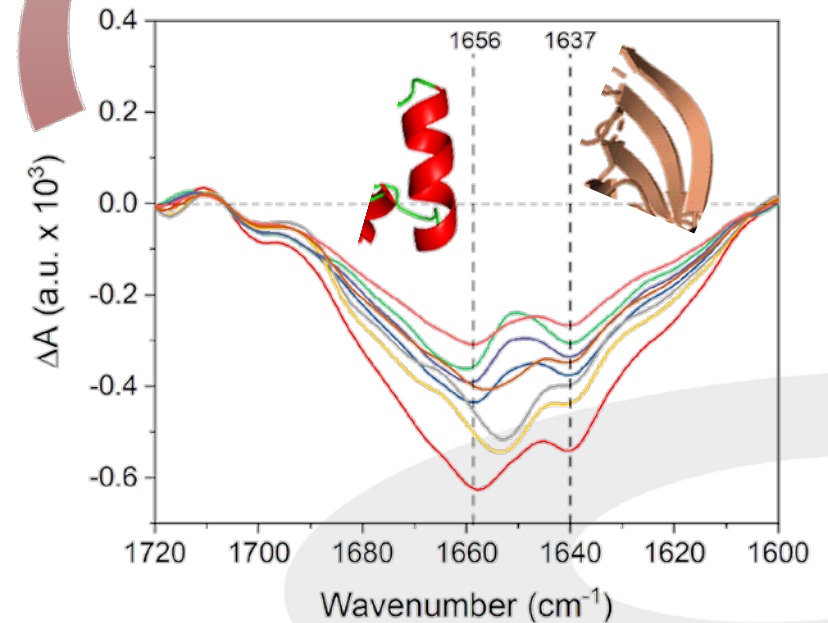
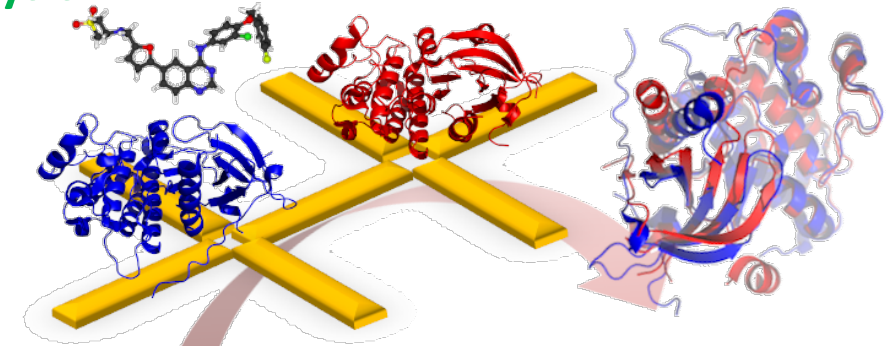
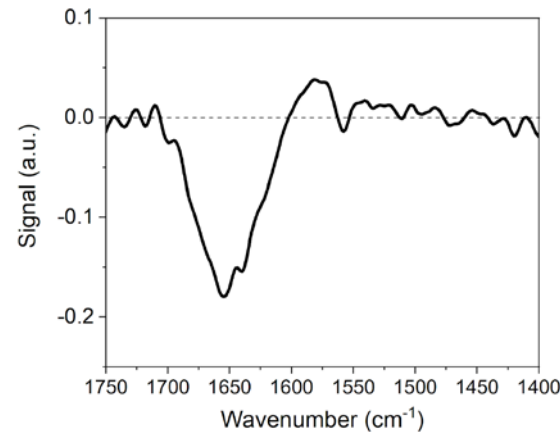
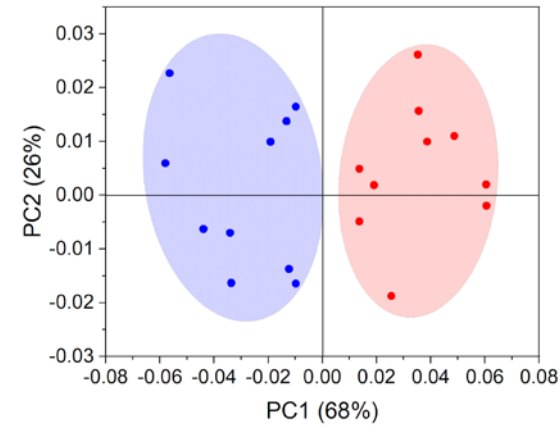
## EGFR-KD Lapatinib binding and conformational changes detected by PIR-SEIRA

### Raw spectra comparison



### PCA

### Principal component Analysis







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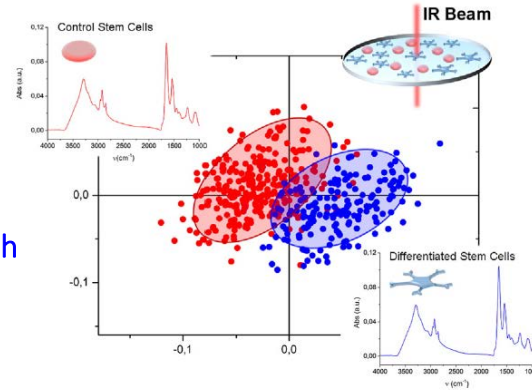
# Further application of IRSR for Life Sciences



## Cell differentiation

Prof. Alessandro Vindigni

Fourier transform  
infrared  
microspectroscopy  
reveals biochemical  
changes associated with  
glioma stem cell  
differentiation

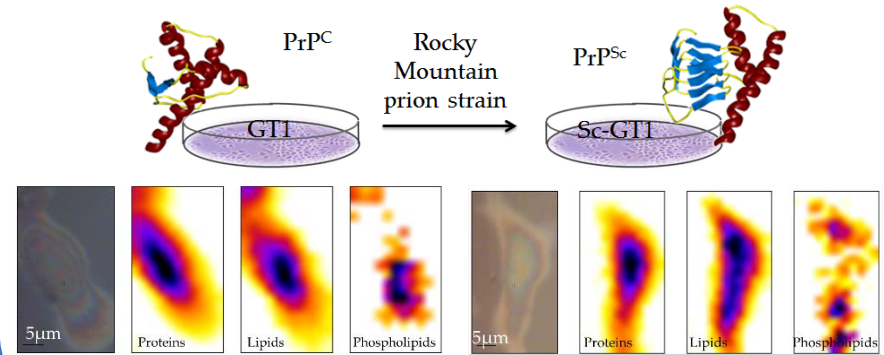


S. Kenig, et al., *Biophysical Chemistry*, (2015); **207**:90-96



## Prion Disorders

Prof. Giuseppe Legname



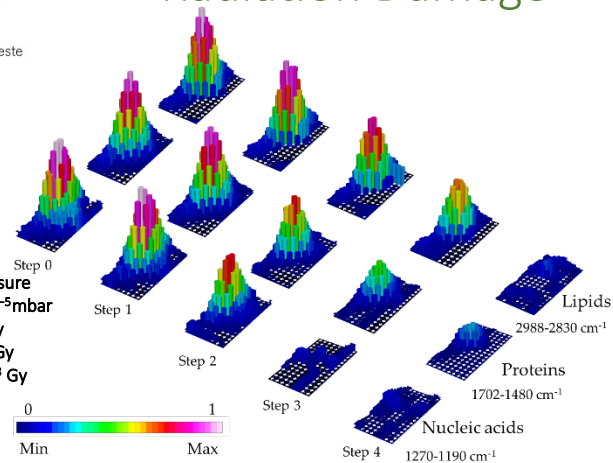
Didonna A, et al., *ACS Chemical Neuroscience*, (2014); **2**(3): 160-174



Elettra Sincrotrone Trieste

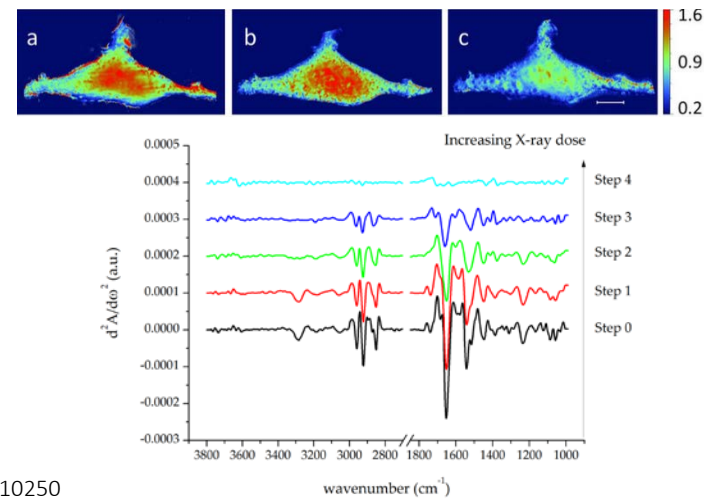
## Radiation Damage

- 0 - Ambient pressure
- 1 - Pressure  $5 \cdot 10^{-5}$  mbar
- 2 - Dose:  $2 \cdot 10^6$  Gy
- 3 - Dose:  $2.2 \cdot 10^7$  Gy
- 4 - Dose:  $6.22 \cdot 10^8$  Gy



A. Gianoncelli, L. Vaccri et al, *Scientific Reports* (2015); **5**:article number 10250

## Density maps at the 3 dose levels





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Sincrotrone  
Trieste

# Further application of IRSR for Life Sciences



**Rare disease**  
Prof. Annalisa Santucci

**A synchrotron light on Alkaptonuria: a comparative XRF and FTIR study on a rare disease**

Extended beta-aggregates, accumulation of lipids and proteoglycans, accumulation of bio-calcite

Mitri E. et al., *BBA Genral Subjects* **2016**, doi.org/10.1016/j.bbagen.2017.02.008

**Fertility and sterility**  
Prof. Oliana Carnevali

**Vibrational characterization of female gametes**

IR Microspectroscopy on GCs: a new non-invasive oocyte assessment

Giorgini E. et al., *Anal Bional Chem* **2010**, 398, 3063–72  
 Gioacchini G. et al., *Biology of Reproduction* **2012**, 86(3):65, 1–11  
 Giorgini E. et al., *Vibrational Spectroscopy* **2012**, 62, 279–285  
 Gioacchini G et al., *Fertility and Sterility*, **2014**, 101(1): 120–127  
 Giorgini E et al., *Analyst*, **2014**, 139(20):5049-60

**Nanotoxicology**

University of Ljubljana Prof. Damjana Drobne

Azienda per l'Assistenza Sanitaria n.2 Bassa Friulana-Isontina

Dr. Lorella Pascolo

**Differential protein folding and chemical changes in lung tissues exposed to asbestos or particulates**

T. Romih et al., *Nanotoxicology*, **2016**, 10(4):462-70  
 S. Novak et al., *Environmental Science and Technology*, **2013**, 57: 11284-11292  
 S. Novak et al., *Nanotoxicology*, submitted 2017

L. Pascolo et al. *Scientific Reports* **2015**, 5, Article number 12129



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Sincrotrone  
Trieste

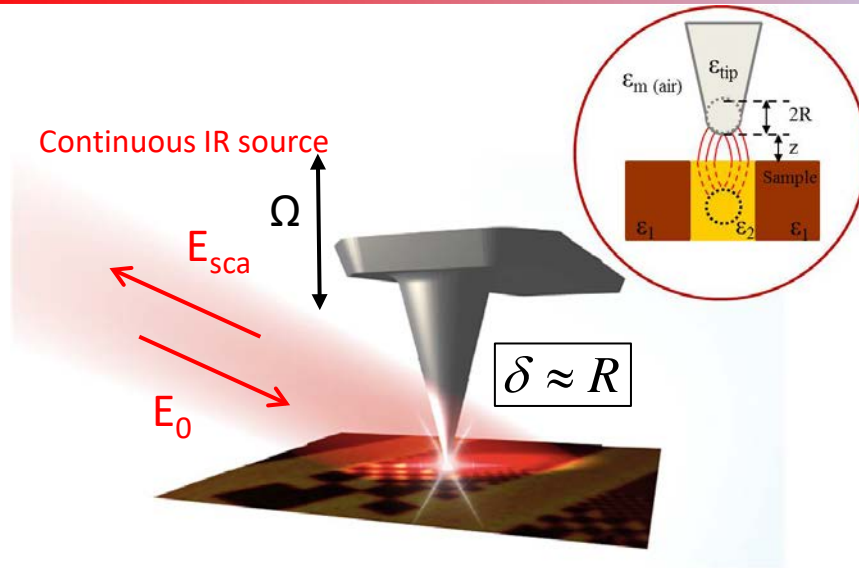


# FTIR nanoscopy



## Scattering-type Scanning Near-field Infrared Microscopy: s-SNIM

## Photo-Thermal Expansion: PTE



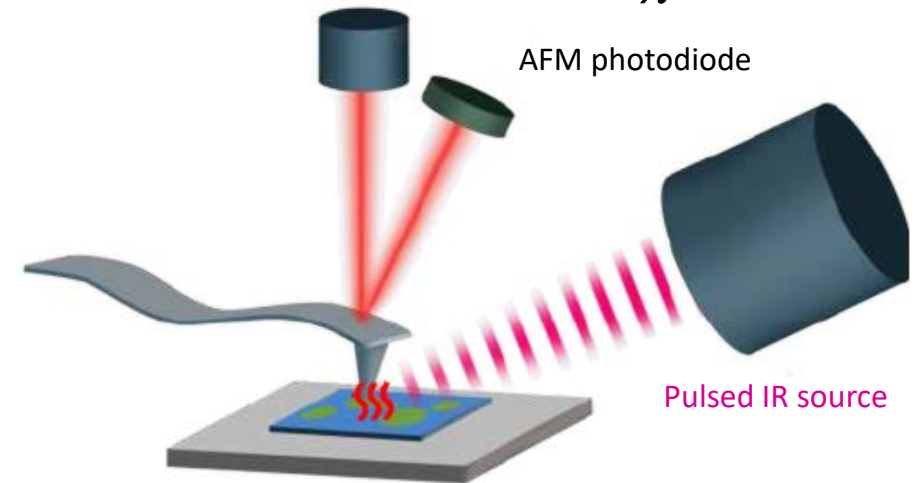
$$E_{sca} \propto \alpha_{eff} E_0 = s e^{i\phi} E_0$$

$$\alpha_{eff} = \alpha_{eff}(\epsilon_{medium}, \alpha_{tip}, R, z, \epsilon_{sample})$$

Fritz Keilmann and Rainer Hillenbrand

*Optical oscillation modes of plasmon particles observed in direct space by phase-contrast near-field microscopy, Applied Physics B 73, 239 (2001)*

$$\Delta h \propto T_{max} \propto P_{abs} \propto \frac{\text{Im}(n)}{\lambda} \propto Abs$$



$$\delta \approx \frac{1}{\text{Thermal diffusivity}}$$

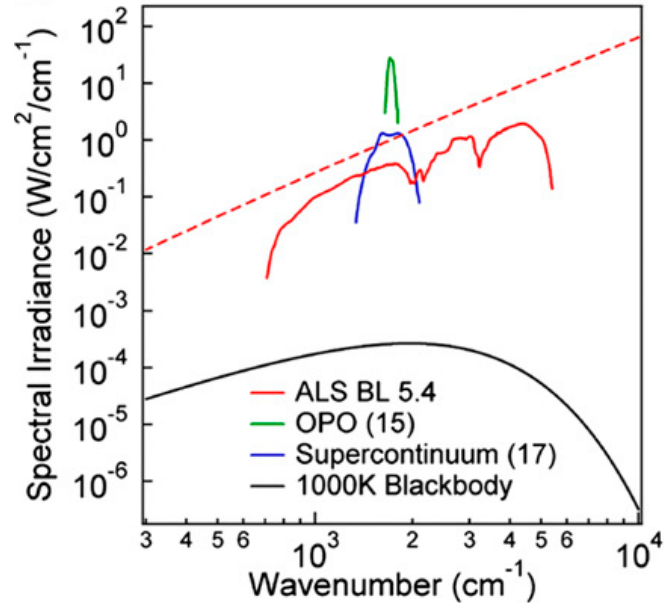
A. Dazzi, R. Prazeres, F. Glotin, and J. M. Ortega

*Local infrared microspectroscopy with subwavelength spatial resolution with an atomic force microscope tip used as a photothermal sensor, Optics Letters, 30(18), pp. 2388-2390 (2005)*





## IR Nanoscopy – IRSR advantage



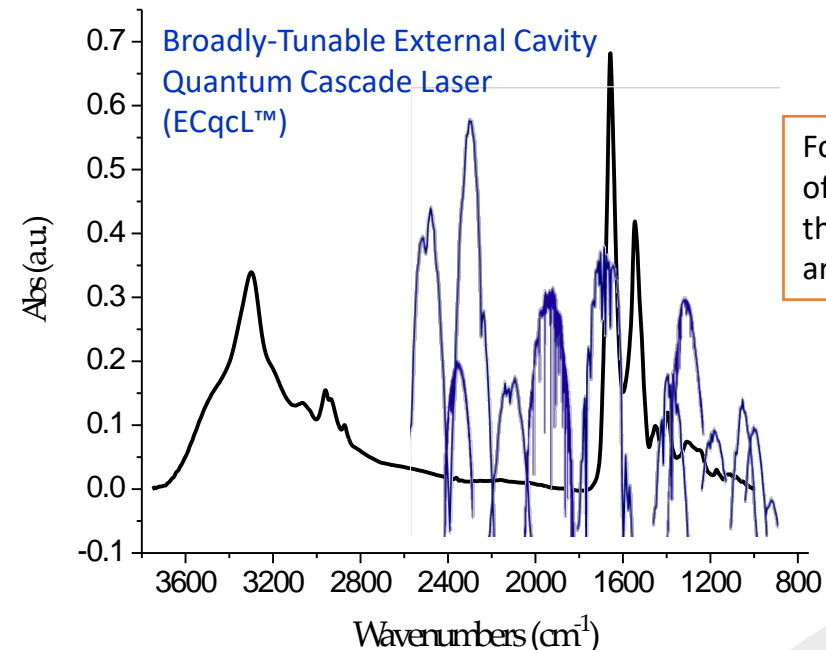
Hans A. Bechtel, Eric A. Muller, Robert L. Olmon, Michael C. Martin and Markus B. Raschke, *Proceedings of the National Academy of Sciences*, 111, 7191–7196 (2014)

S/N ratio is the key parameter for vibrational analysis.

The superior stability of IRSR compensate for the lower spectral density, without inducing radiation damage

- Ultra-broadband nature
- Superior density of power for spectral interval
- Superior spectral stability

The ultra-broadband nature of IRSR makes it the ideal source for IR nanospectroscopy



For barely covering almost half of the interesting MIR part of the spectrum, several QCL chips are needed.

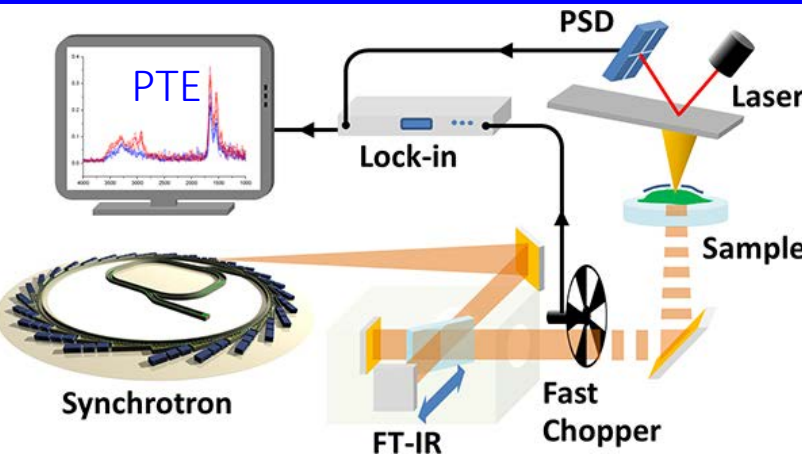
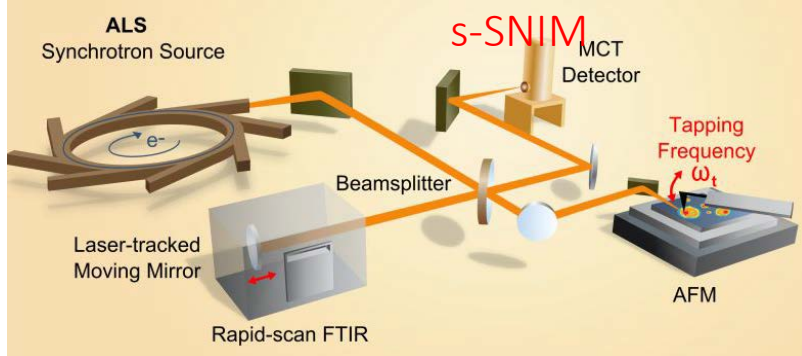
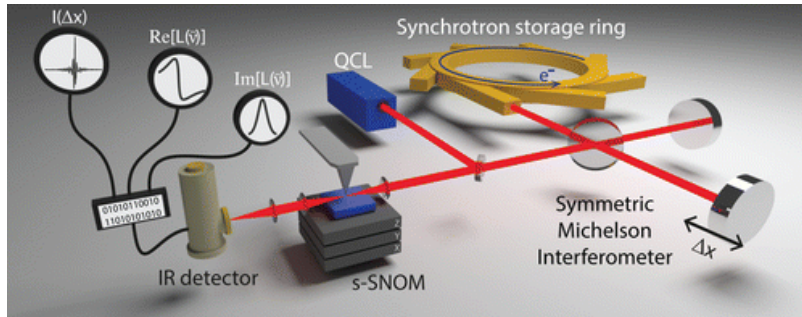
Only the ultra-broadband nature of IRSR can guarantee the **selectivity** requirements for chemical and biochemical analysis





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Trieste

# IR Nanoscopy at SR facilities worldwide



P. Hermann, A. Hoehl, A. Patoka, F. Huth, E. Rühl, and G. Ulm, *Optics Express* 21, 2913 (2013)

Not user dedicated



Benjamin Pollard, Francisco C. B. Maia, Markus B. Raschke, and Raul O. Freitas *Nano Lett.*, 16 (1), 55–61 (2016)

Open to users since 2015



Hans A. Bechtel, Eric A. Muller, Robert L. Olmon, Michael C. Martin and Markus B. Raschke, *Proceedings of the National Academy of Sciences*, 111, 7191–7196 (2014)

5.4 open to users since 2014; Second endstation opening soon



Projects just started



Commissioning phase



End of commissioning phase

Paul M. Donaldson, Chris S Kelley, Mark D. Frogley, Jacob Filik, Katia Wehbe, and Gianfelice Cinque, *Optics Express* 24, 1852–1864 (2016)





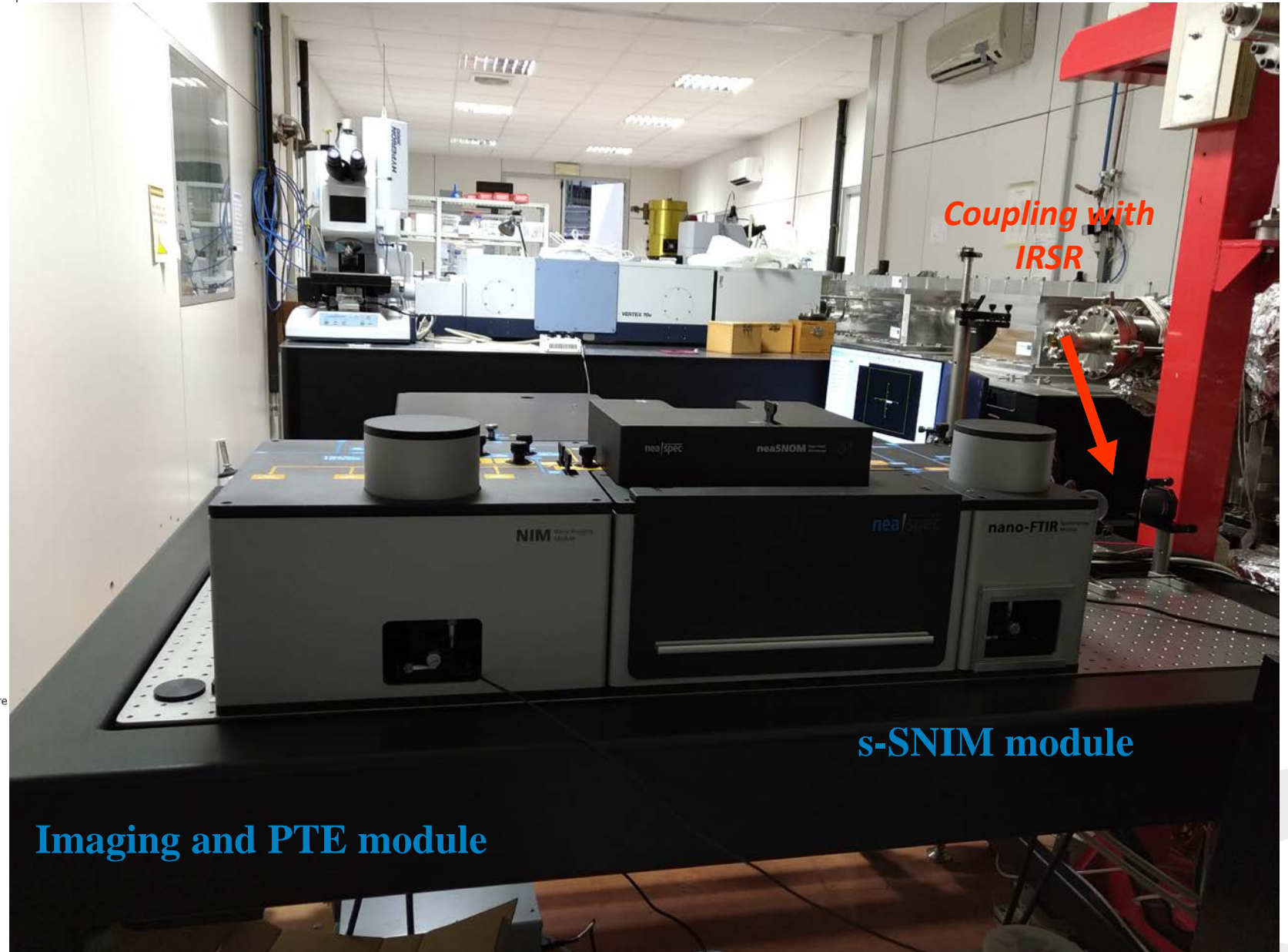
Elettra  
Sincrotrone  
Trieste



**Integra**

**CERIC** Central European  
Research Infrastructure  
Consortium

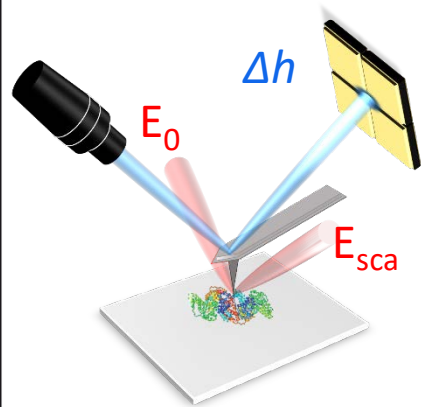
## IR Nanoscopy endstation at SSSI-Bio





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Sincrotrone  
Trieste

# IR Nanoscopy at SSSI-Bio: Opportunities for Life Sciences



$\delta$   
 $\lambda$  independent

Single  
Molecule

1nm

Molecular  
clusters

Large Molecular  
aggregates

Prokaryotic cells

Eukaryotic  
cells

Tissue

Human  
body

Nano-FTIR

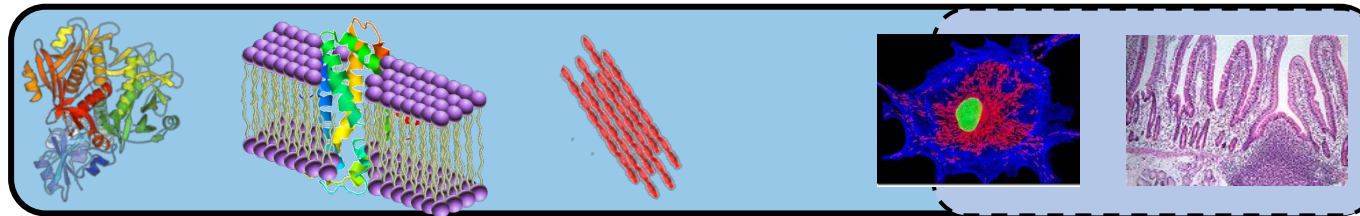
1 $\mu$ m

Micro-FTIR

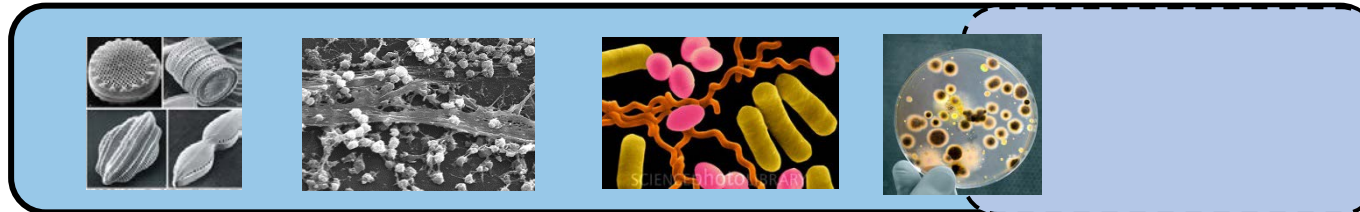
1mm

Vis Camera

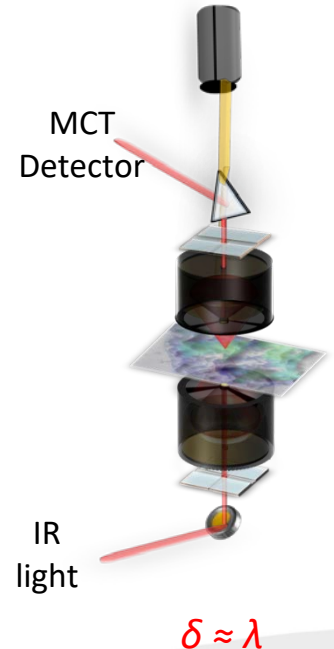
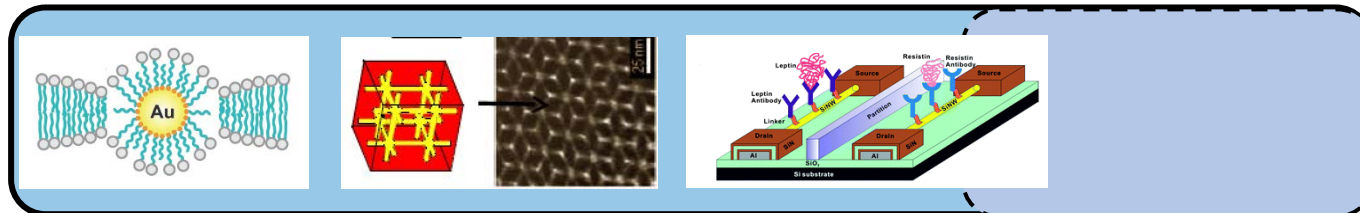
From Protein Science to Cellular



From a Single Organism to Cell Community



Chemical heterogeneity at the nanoscale



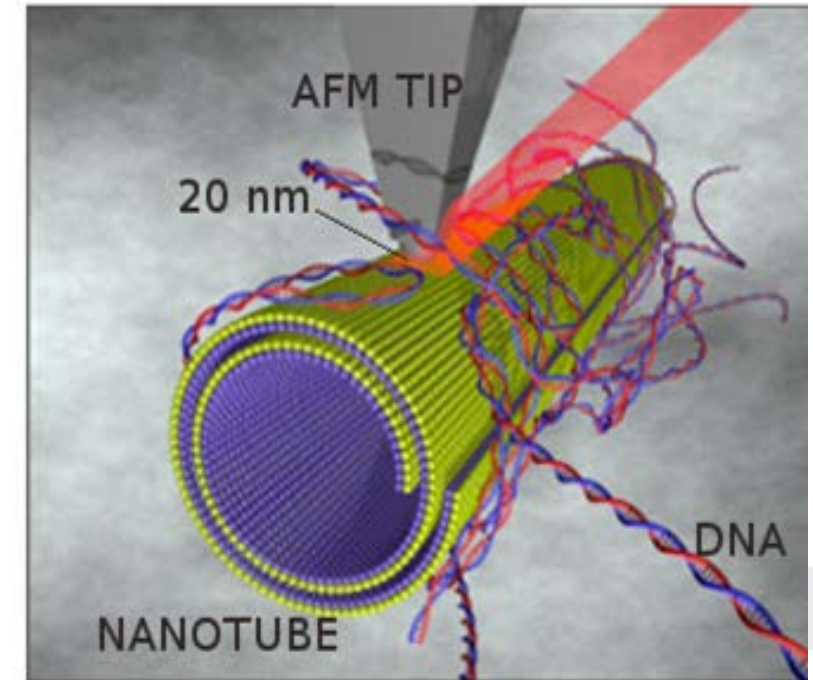
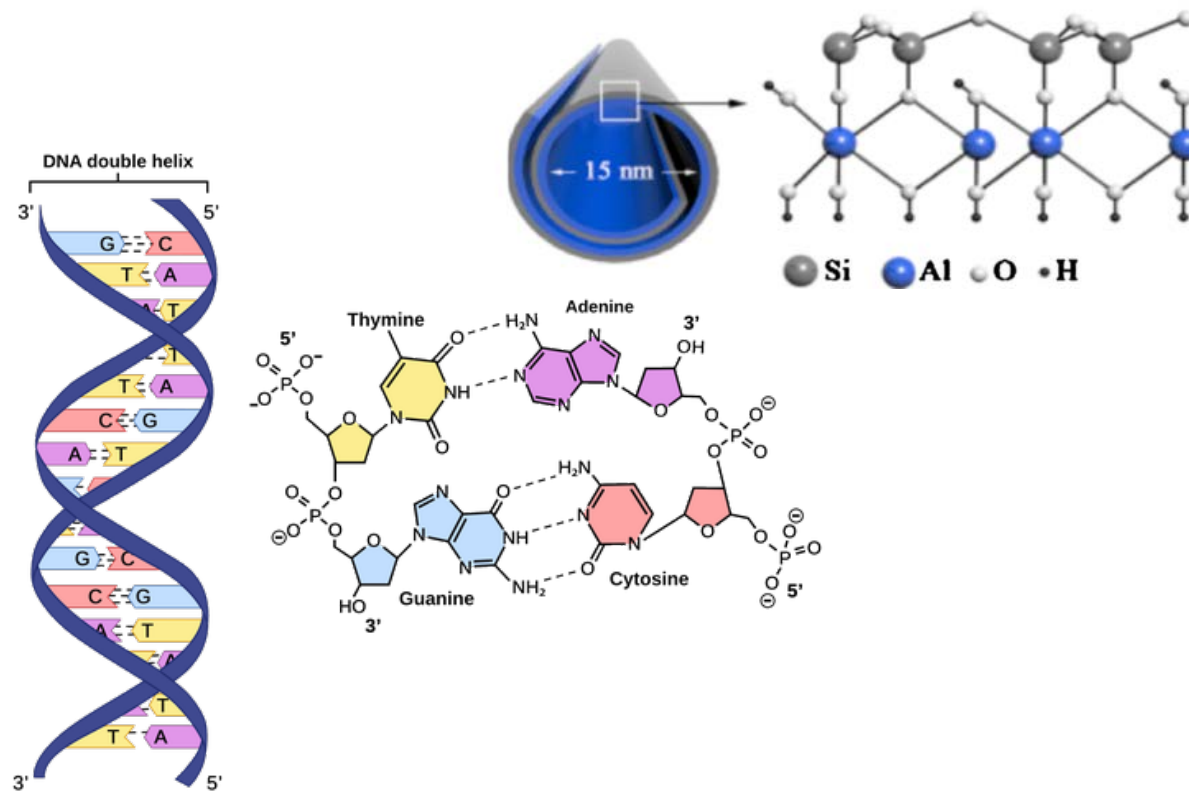
$\delta \approx \lambda$

# Probing DNA molecules on clay nanotubes

Halloysite nanotubes are natural biocompatible structures with high affinity for loading biomolecules, thus they are good candidates for drug delivery and gene transfer

.siloxane external surface **-**

.aluminum oxide internal surface **+**

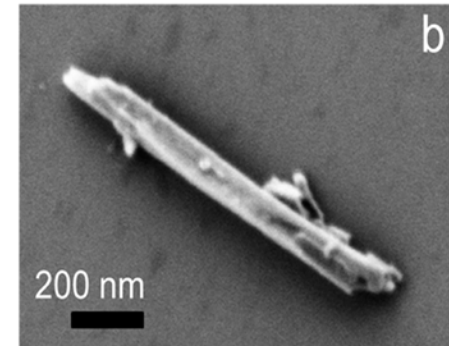
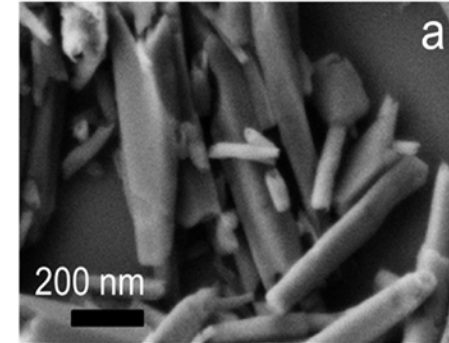
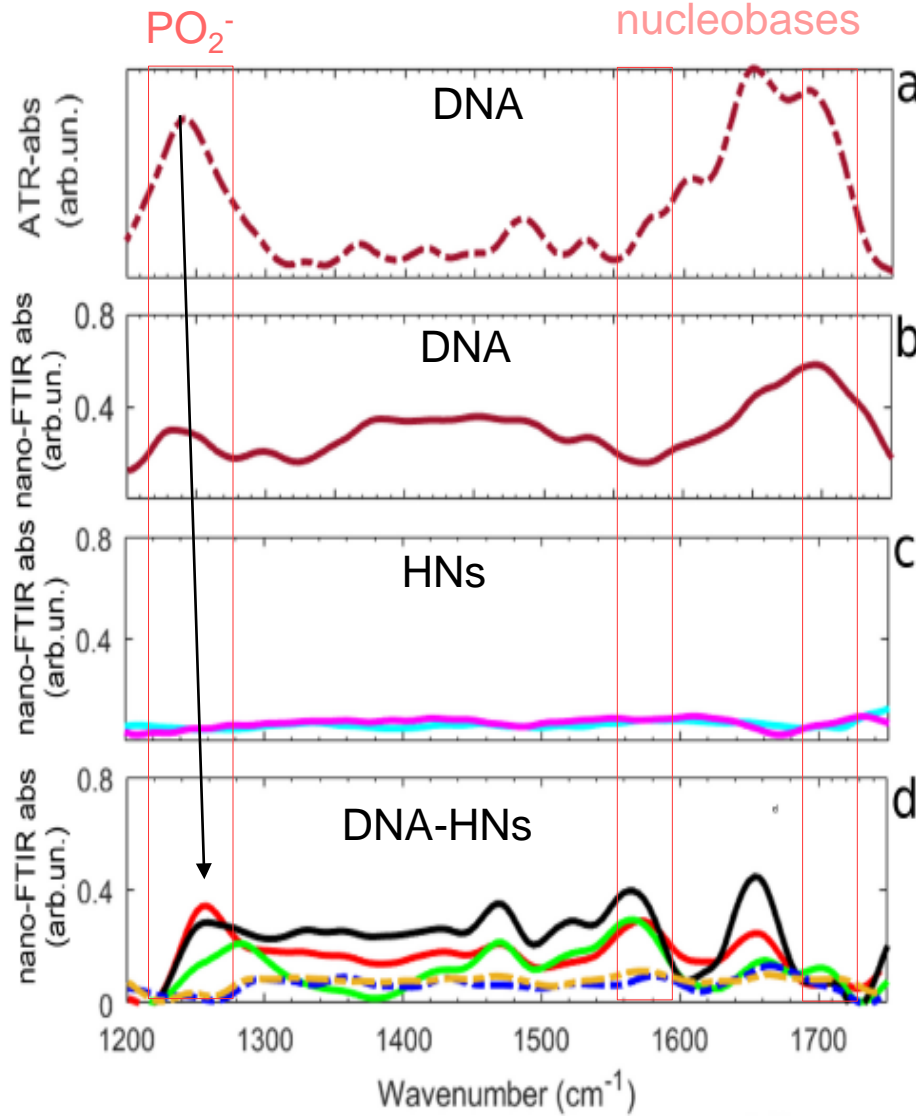
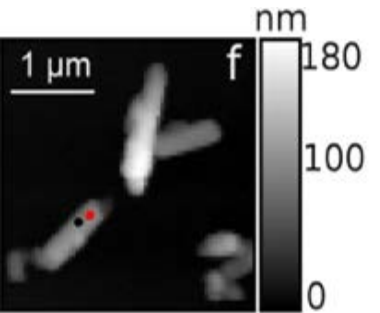
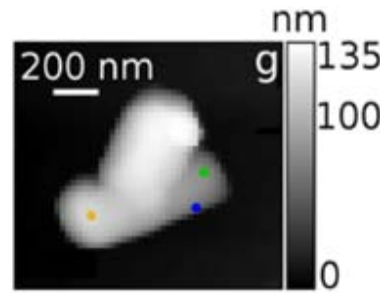
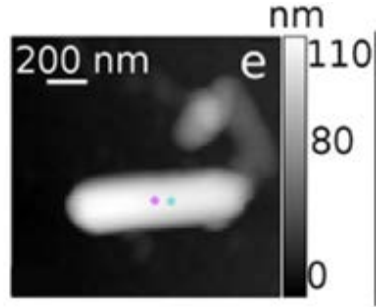


**DUE TO ELECTROSTATIC FORCES DNA SPONTANEOUS ADSORPTION ON HALLOYSITE NANOTUBES IS COUNTERINTUITIVE!**



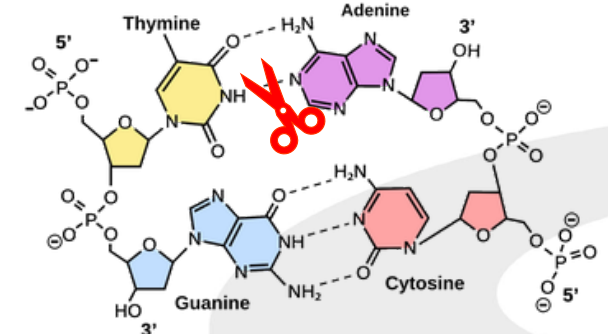


# Probing DNA molecules on clay nanotubes



**a** Pristine nanotubes

**b** Nanotube with DNA



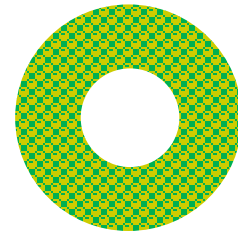
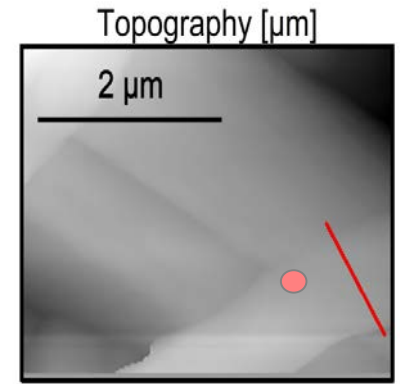
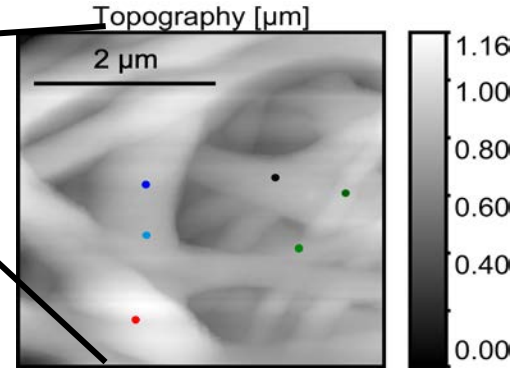
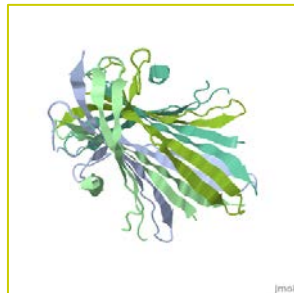
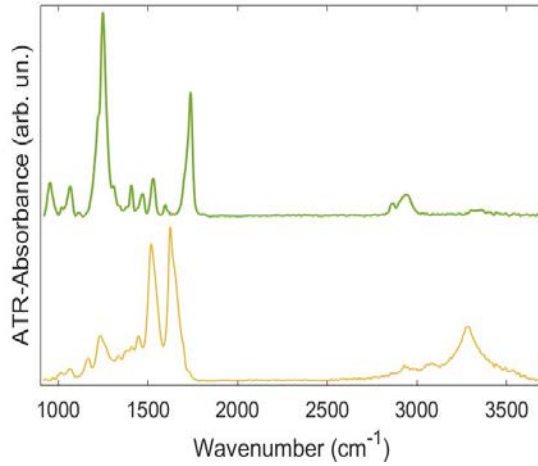
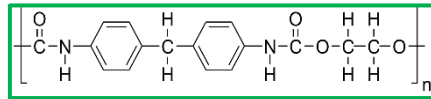
**DNA modify its structural arrangement when loaded onto nanotubes**

# Relating morphology to chemical composition in composite matrices for in situ vascular tissue engineering

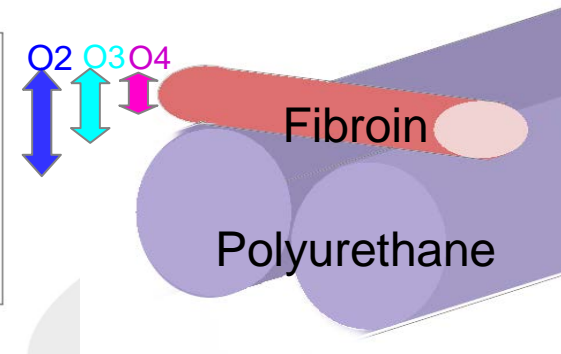
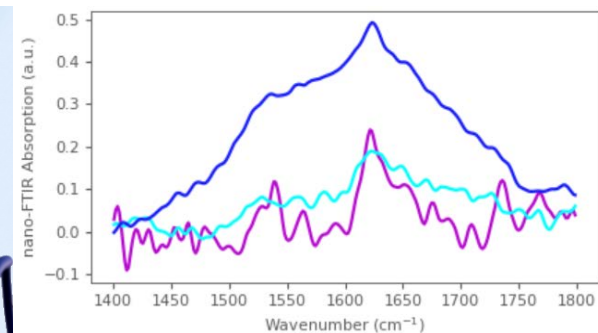
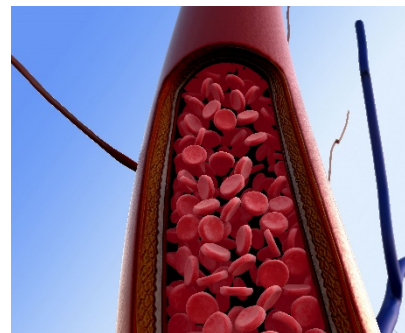
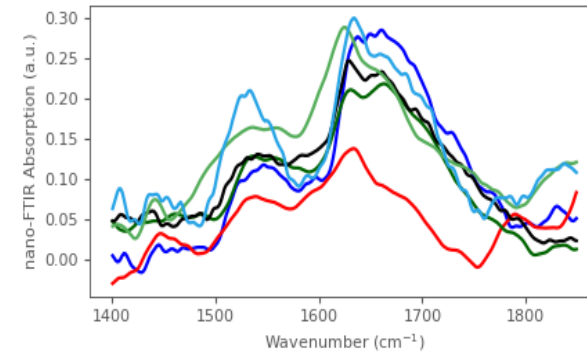


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*PU polyurethane*  
*SF Silk fibroin*







Elettra  
Sincrotrone  
Trieste

# *Thank for your attention!*

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