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Infrared micorscopy

From macro to nano scale on the molecules of life

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SISSI beamline manager

School on Synchrotron and Free-Electron-Laser Methods for Multidisciplinary Applications

ICTP

Outlines

- Infrared Spectroscopy
  - Basic Concepts on Theory and Instrumentation
- A brief history of IR spectroscopy at SR facilities
- IRSR: Generation and properties
- Infrared bio-spectroscopy
- From macro to nanoscale on the molecules of Life
  - Soft X-ray radiation damage
  - SR Collective Enhanced IR Absorption microscopy for protein conformational studies
  - Vibrational spectroscopy at the nanoscale

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Infrared Spectroscopy
Basic Concepts on Theory and Instrumentation

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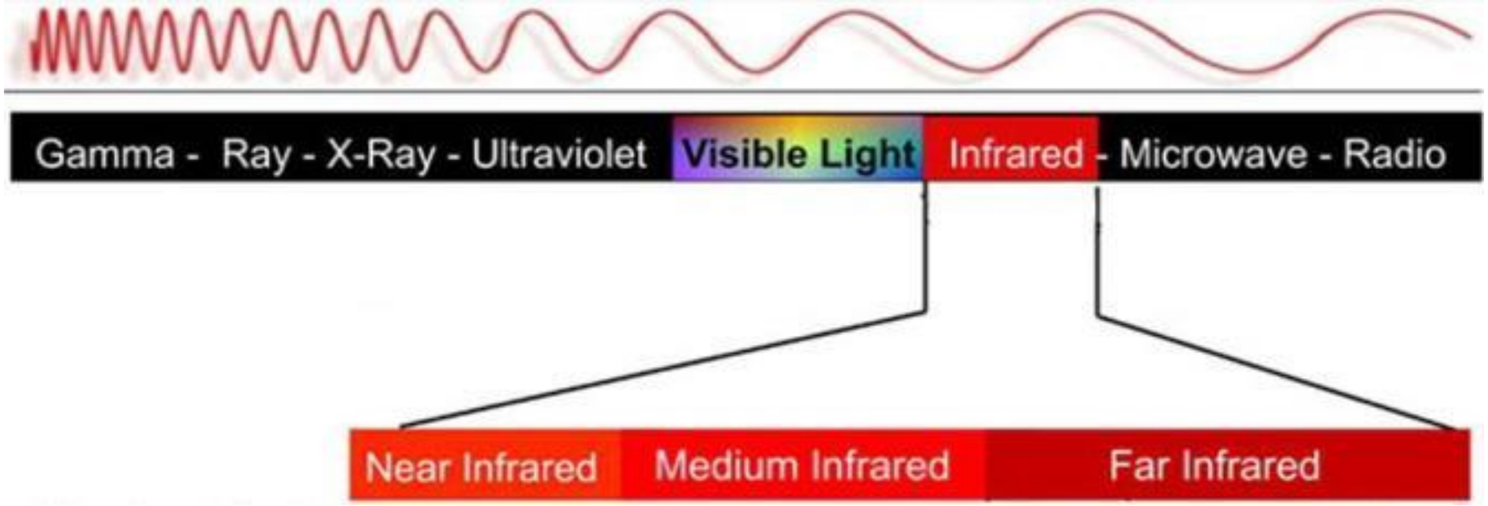
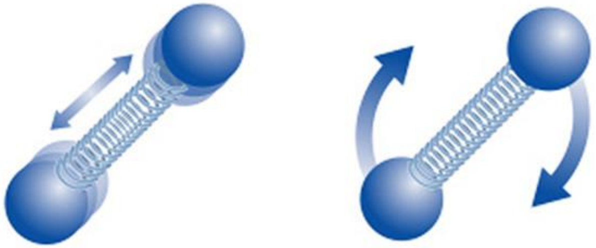
Electromagnetic Spectrum: a closer view into the IR spectral range



	NIR	MIR		FIR
λ (μm)	0.74	3	30	300
ν (THz)	400	100	10	1
$\overline{\nu}$ (cm^{-1})	~13000	~3333	~333	~33
E (eV)	1.65	0.413	0.041	0.004
E (Kcal/mol)	37	10	1	0.1

Molecular Vibrations



Molecular Rotations

Infrared Spectroscopy

Basic concepts on Theory

The Born-Oppenheimer Approximation

1- Electronic motion and nuclear motion in molecules can be separated and independently considered

$$\Psi molecule^{(\vec{r_i},\vec{R_j})} = \Psi electrons^{(\vec{r_i},\vec{R_j})} \cdot \Psi nuclei^{(\vec{R_j})}$$

The electronic wavefunction depends upon the nuclear positions but not on nuclei velocities → The nuclear motion is so much slower than electron motion that nuclei can be considered to be fixed.

Electronic transitions (10^{-15} s) are at least 10^2 times faster than nuclear transitions and involve energies 10 to 50 times greater

Degree of freedom

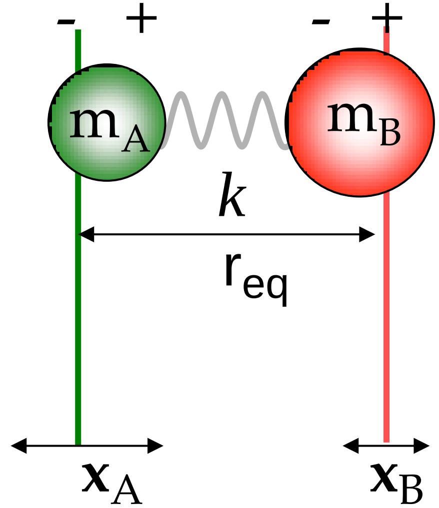
Degree of freedom is the number of variables required to completely describe the motion of a particle/molecule. For a molecule made by N atoms (ions) moving in 3-dimensional space, the degree of freedom becomes 3N. For non-linear molecules, all translational/rotational motions can be described in terms of translation/rotations along/around 3 axes. The remaining 3N-6 degrees of freedom constitute vibrational motion. For a linear molecule however there are only 2 rotational degrees of freedom for any linear molecule leaving 3N-5 degrees of freedom for vibration.

2- Vibrational and rotational motion can also be considered independently

- The energies involved in rotational transitions (10^{-10} s) are about 10^3 times smaller than the ones involved in vibrational transitions (10^{-13} s). Pure vibrational transitions falls in the MIR-FIR regime, while pure rotational transition in the FIR-THz regime

The classical description of vibrational motion

The simplest example: a diatomic heteronuclear molecule AB



$$\mu_{AB} = \frac{m_A m_B}{m_A + m_B}$$ Reduced Mass of AB molecule

The equilibrium internuclear distance is denoted by r_{eq}. However as a result of molecular vibrations, the internuclear distance is continuously changing; let this distance be called r(t).

Let $x(t)=r(t)-r_{eq}$

When x is non-zero, a restoring force F exists which tries to bring the molecule back to x=0 , that is equilibrium. For small displacements this force can be taken to be proportional to x .

F(restoring force) = $-k\cdot x$

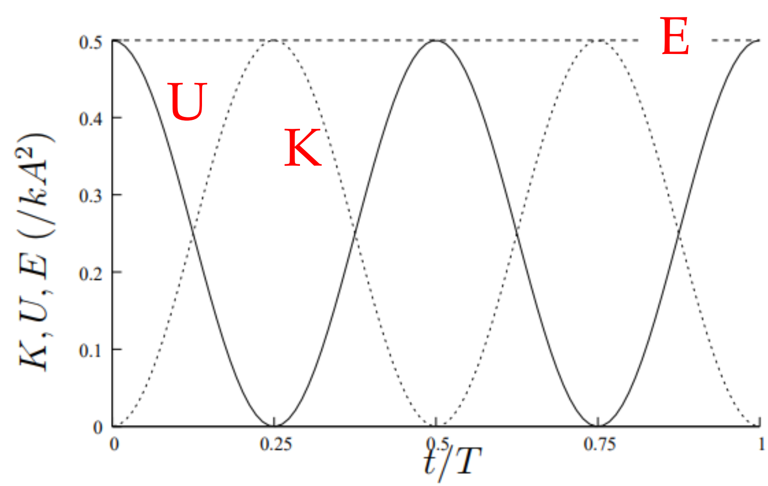
[The Hooke's law]

k = *Force constant* [Nm^{-1}]

$$x(t) = A\sin(2\pi\nu t)$$

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu_{AB}}}$$

$$E = K + U = \frac{1}{2}kA^2$$



Infrared Spectroscopy: Basic concepts

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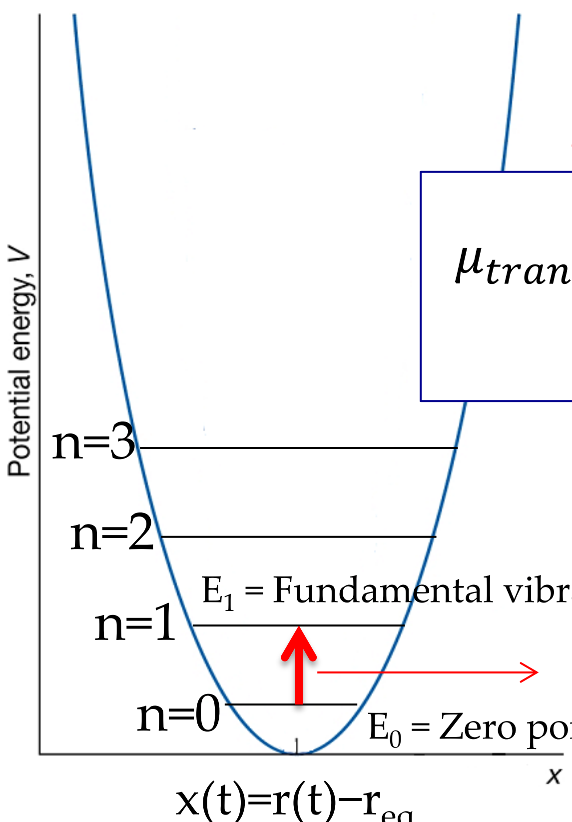
$$-\frac{h^2}{8\pi^2\mu_{AB}}\frac{d\psi}{dx^2}+\frac{1}{2}kx^2\psi = E\psi$$

Quantum mechanical Model of harmonic oscillator

$$E_{vib} = h\nu\left(n + {}^{1}/_{2}\right)$$

n: Vibrational quantum number (0,1,2,3,…)

A series of equally spaced never ending vibrational levels



Selection Rules

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

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

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

Vibrations that do not induce variation of the dipole moment of the molecule are forbidden

For a homonuclear molecule AA there are not vibrational transitions allowed

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

$\Delta n = \pm 1$

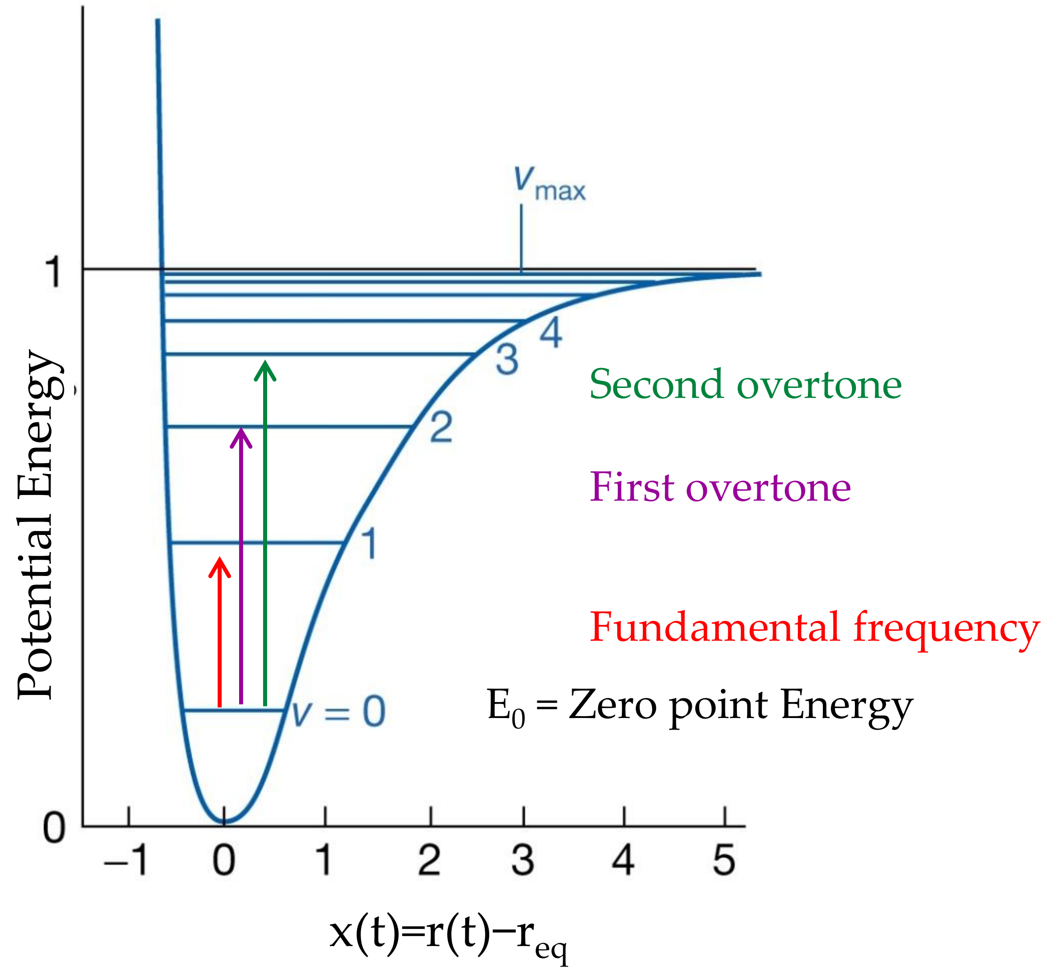
Infrared Spectroscopy: Basic concepts

Quantum mechanical Model of anharmonic oscillator

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

$$\nu_e = \text{harmonic frequency} \quad x_0 = \text{anharmonic constant}$$

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



Selection Rules

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

$\Delta n = \pm$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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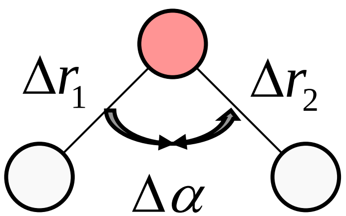
Infrared Spectroscopy: Basic concepts

Normal modes of vibration

- A normal mode is a molecular vibration where some or all atoms vibrate together at the same frequency.
- The number of "normal modes" is equal to the vibrational degree of freedom available
- Each mode has a definite frequency of vibration. Sometimes 2 or 3 modes may have the same frequency but that does not change the fact that they are distinct modes; these modes are called degenerate.

The 3 normal modes of vibratine of a triatomic molecule , defined by 3 normal coordinates (Q_1, Q_2, Q_3) may be defined in terms of internal coordinates



$$Q_1 = l_{11}\Delta r_1 + l_{21}\Delta r_2 + l_{31}\Delta\alpha \qquad \nu_1$$

$$Q_2 = l_{12}\Delta r_1 + l_{22}\Delta r_2 + l_{32}\Delta\alpha \qquad \nu_2$$

$$Q_3 = l_{13}\Delta r_1 + l_{23}\Delta r_2 + l_{33}\Delta\alpha \qquad \nu_3$$

$$Evib = \sum_{i=1}^{3N-6}\left(ni + \frac{1}{2}\right)h\nu_i$$

$$E_0 = \frac{1}{2}\sum_{i=1}^{3N-6} h\nu_i$$

3 quantum numbers: n_1, n_2, n_3

3 fundamental vibrations :

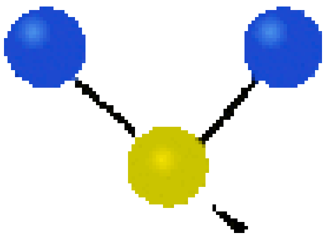
E(0,0,0) → E(1,0,0) ν_1
E(0,0,0) → E(0,1,0) ν_2
E(0,0,0) → E(0,0,1) ν_3

Overtones and combinations bands

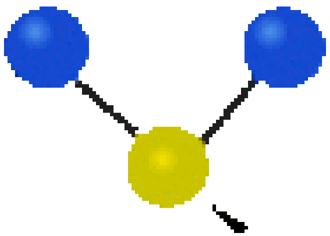
(000) → (020) $2\nu_2$
(000) → (110)

Stretching modes (ν)

Symmetric Stretching

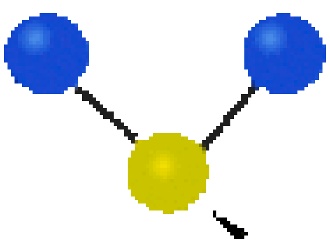


Antisymmetric Stretching

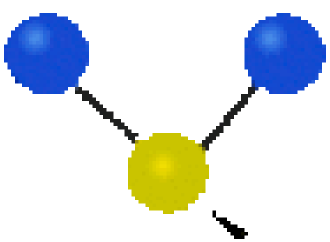


Deformation modes

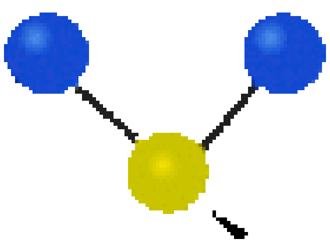
Scissoring (δ)



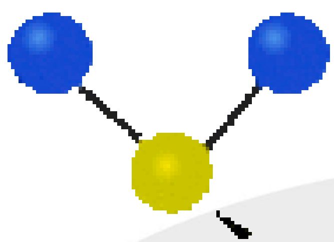
Rocking (r or ϱ)



Wagging (ω)



Twisting (τ)



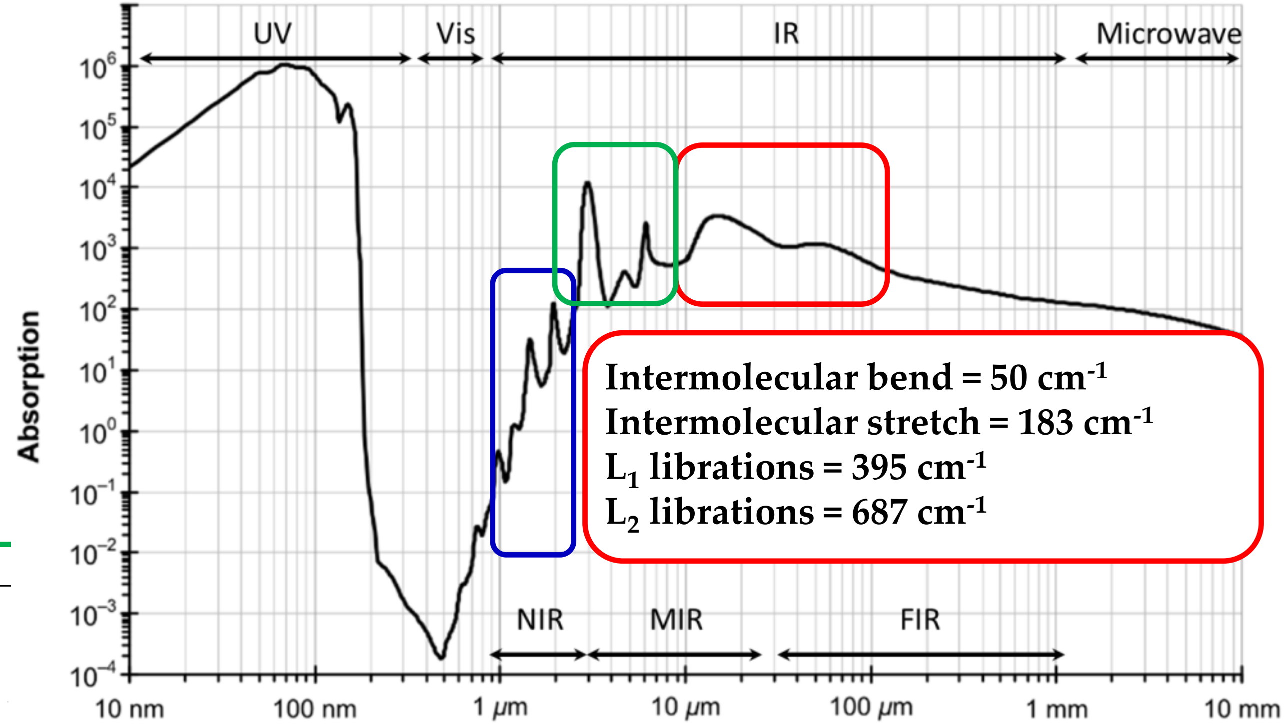
In plane deformations

Out plane deformations

Infrared Spectroscopy

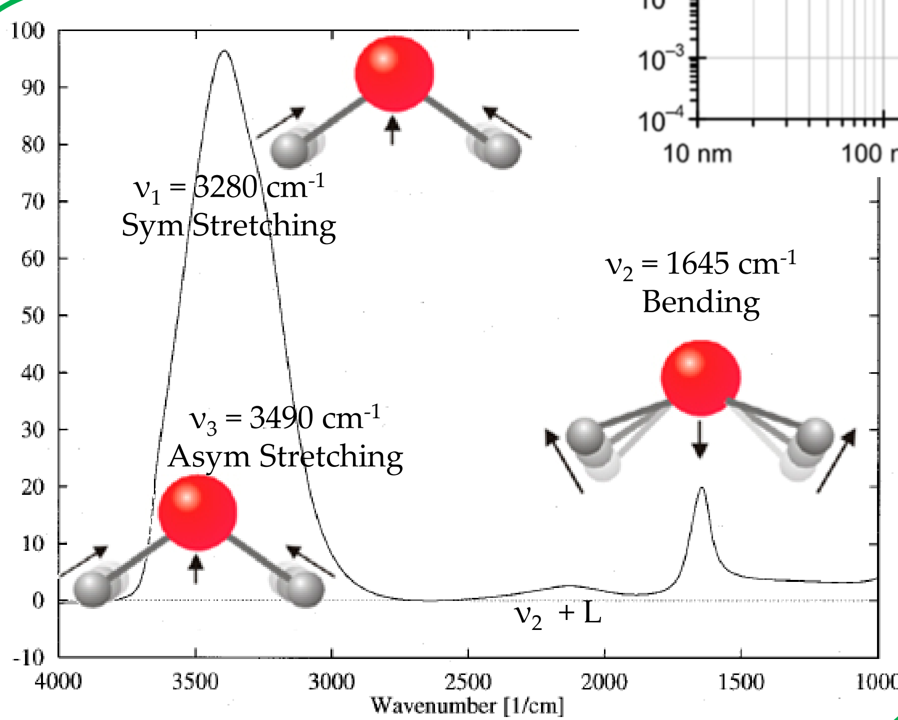
Basic concepts on Theory

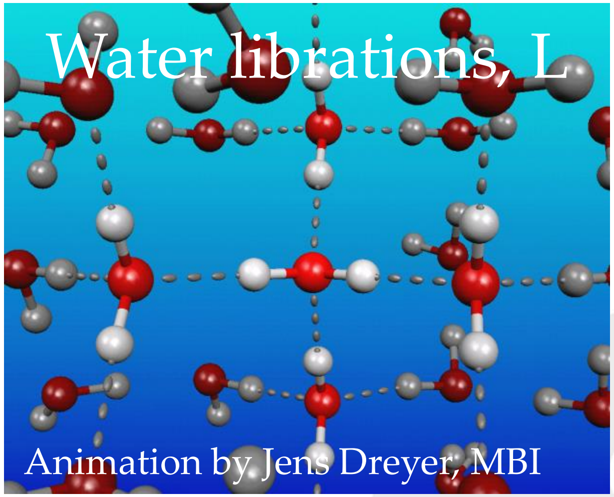
Vibrational Spectrum of liquid water



Overtones and combination bands

Intermolecular bend = 50 cm^{-1}
Intermolecular stretch = 183 cm^{-1}
L_1 librations = 395 cm^{-1}
L_2 librations = 687 cm^{-1}



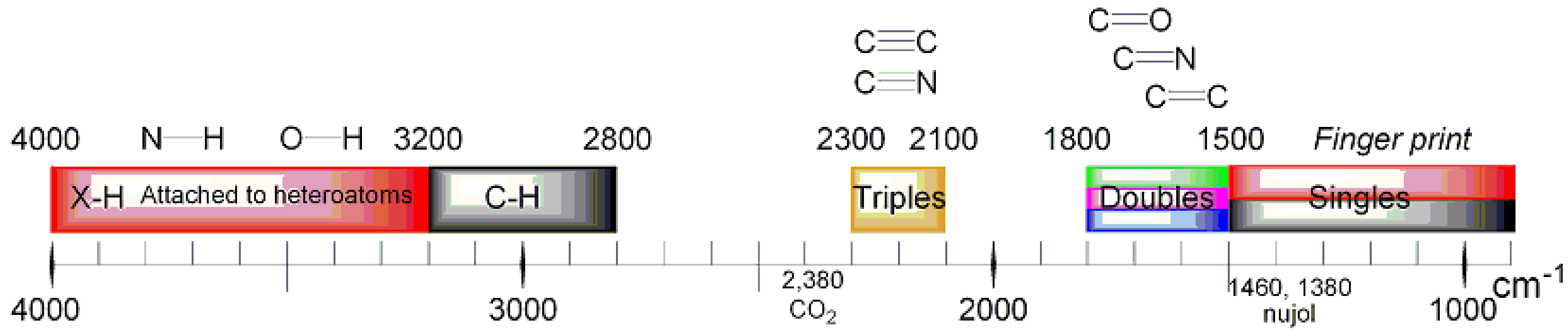


Infrared Spectroscopy

Basic concepts on Theory

FROM PEAK POSITION, INTENSITY AND WIDTH

NATURE OF ATOMS INVOLVED IN THE SPECIFIC VIBRATION
PARAMETERS OF THE ATOMIC BOND : BOND STRENGTH AND LENGHT
BOND CONFORMATION: DOUBLE BOND CIS/TRANS, ……
CHEMICAL ENVIRONMENT (THROUGH MODULATION OF THE DIPOLE MOMENT)
ROTATIONAL MODES IN THE FIR REGION



FROM WHOLE SPECTRUM

NATURE OF THE MOLECULE: SPECTRAL FINGERPRINT=> MOLECULAR IDENTIFICATION
SAMPLE INTERACTIONS: FREE/BOUND WATER …
SAMPLE EVOLUTION: REACTION KINETIC, AGING, PHYSICO CHEMICAL TREATMENT, CONSTRAINTS (PRESSURE, TEMPERATURE, pH) …

QUANTITATIVE or SEMI-QUANTITATIVE ANALYSIS

SIMPLE MIXTURES: BEER LAMBERT BOUGUER LAW

Infrared Spectroscopy

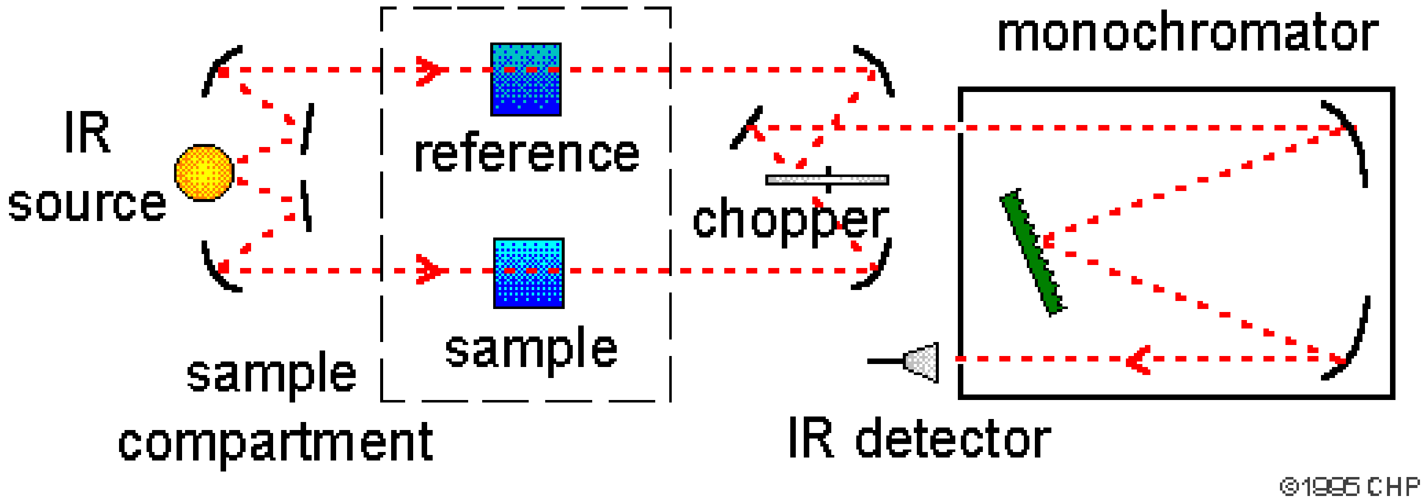
Basic concepts on Instrumentation

When dealing with molecular species (normal modes of vibration 3N-6), the absorption profile at a single frequency (or limited spectral range) is scarcely useful. Only a multi-frequency profile can account for the system complexity and its interaction with the environment

↓

An FTIR spectrum needs to be energy resolved over a large spectral range

The past instrumentation: Dispersive Interferometers



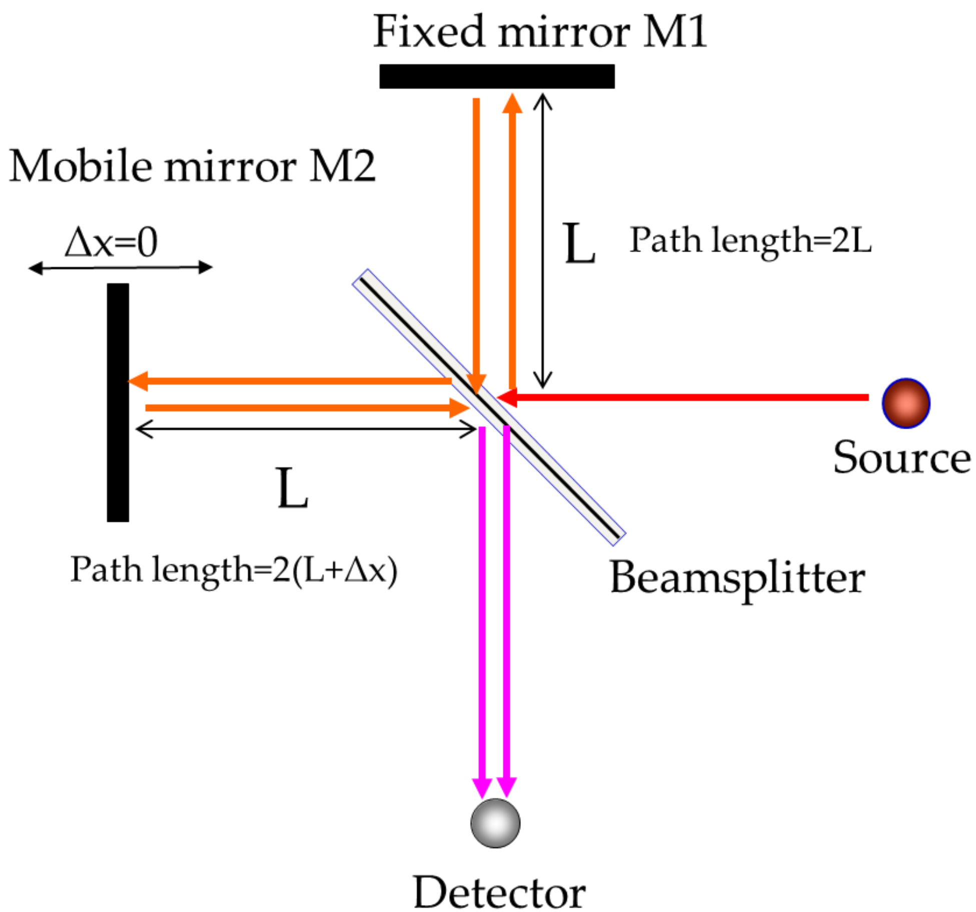
http://www.chemicool.com/definition/fourier_transform_infrared_spectrometer_ftir.htm

This slow acquisition time limited the wide spreading of infrared spectroscopy until 1960s', when Fourier Transform Interferometer have been first proposed.

Infrared Spectroscopy

Basic concepts on Instrumentation

The present instrumentation: Fourier Transform InfraRed Interferometers



Optical Path Difference _ OPD

$2\Delta x = 2vt$ $\qquad$ v = *mirror velocity*

Conventional sources

NIR: Tungsten lamp
MIR: Glow bar (SiC)
FIR: Hg-Arc

Beamsplitters

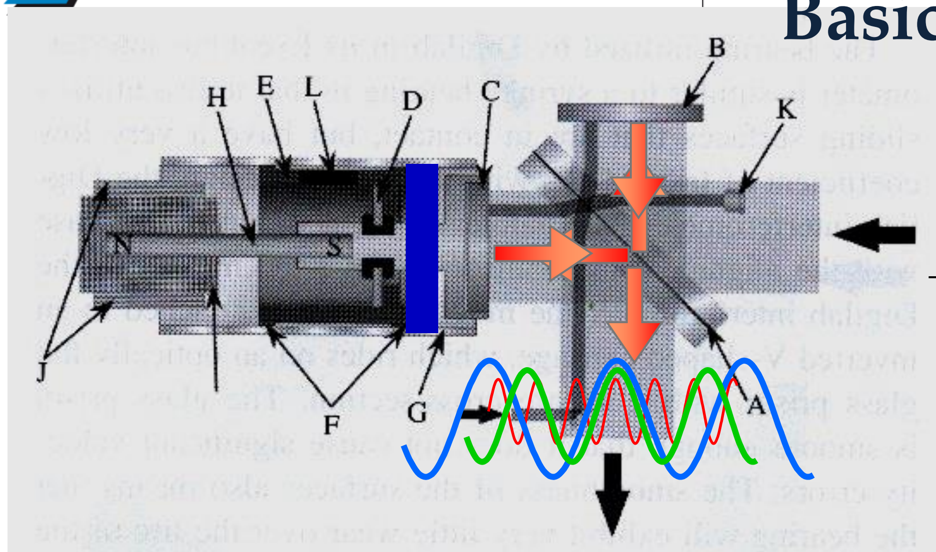
NIR: CaF_2
MIR: KBr
FIR: Mylar, Silicon

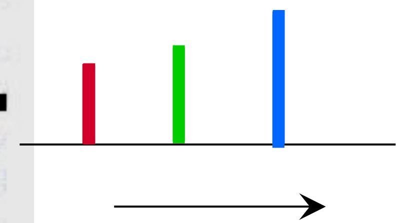
Detectors

NIR – InGaAs, InSb, Ge, Si room temperature detectors
MIR: Room temperature DLaTGS
Nitrogen cooled MCT
FIR – He Cooled Silicon Bolometer
Room temperature DLaTGS

Infrared Spectroscopy

Basic concepts on Instrumentation





For a single wavelength

$$I(x) = I(\tilde{\nu})[1 + \cos(2\pi x \tilde{\nu})]$$

For a polychromatic source

$$I(x) = \int I(\tilde{\nu}) d\tilde{\nu} + \int I(\tilde{\nu}) \cos(2\pi x \tilde{\nu}) d\tilde{\nu}$$

$$I(ZPD) = 2\int I(\tilde{\nu}) d\tilde{\nu} = I_0$$

$$I(x) = \frac{1}{2} I_0 + \int I(\tilde{\nu}) \cos(2\pi x \tilde{\nu}) d\tilde{\nu}$$

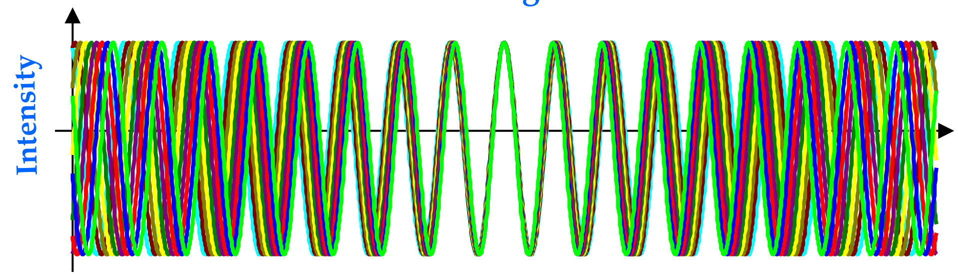
$$I(x) - \frac{1}{2} I_0 = I'(x) = \int I(\tilde{\nu}) \cos(2\pi x \tilde{\nu}) d\tilde{\nu}$$

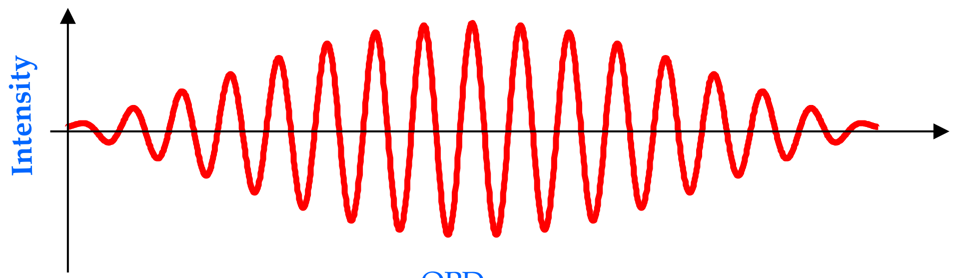
Fourier Transform (FT) →

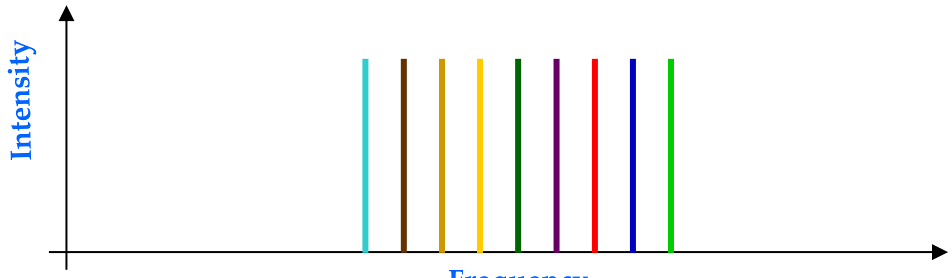
$$I(\tilde{\nu}) \propto \int_{-\infty}^{+\infty} I'(x) \cos(2\pi x \tilde{\nu}) dx$$

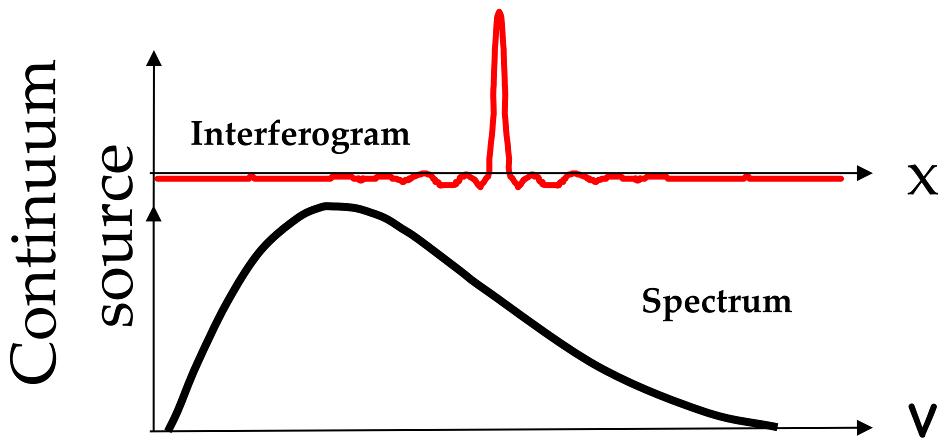
Infrared Spectroscopy

Basic concepts on Instrumentation









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A brief history of IR spectroscopy at SR facilities

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Once upon a time…..

Volume I. *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,[1] Rubens,[2] Rubens and Snow,[3] 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[1] and by Ångstrom.[4] Ångstrom[5] 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.[6]

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.

[1] Report on Mt. Whitney Expedition, Profess. Papers, U. S. Signal Service, XV.
[2] Annalen der Physik und Chemie, N. F. XLV., p. 238.
[3] Annalen der Physik und Chemie, N. F. XLVI., p. 529.
[4] Bihang till K. Svenska Vet.-Akad. Handlingar, Band 15, Afd. 1, No. 9.
[5] Ofversigt af Kongl. Vetenskaps-Academiens Forhandlingar, 1890, No. 7, Stockholm.
[6] Annalen der Physik und Chemie, N. F. XLV., p. 258.

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_2O) 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

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SR-IR beamlines

More than 40 IRSR beamlines worldwide

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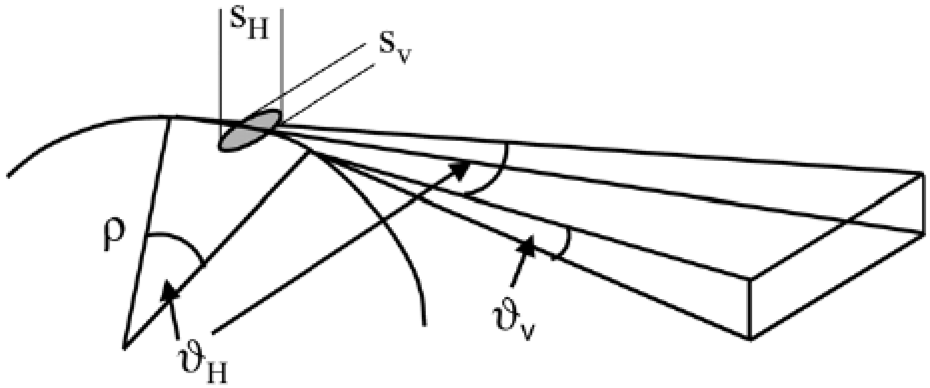
IRSR: Generation and properties

IRSR Generation

Bending Magnet IRSR



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.

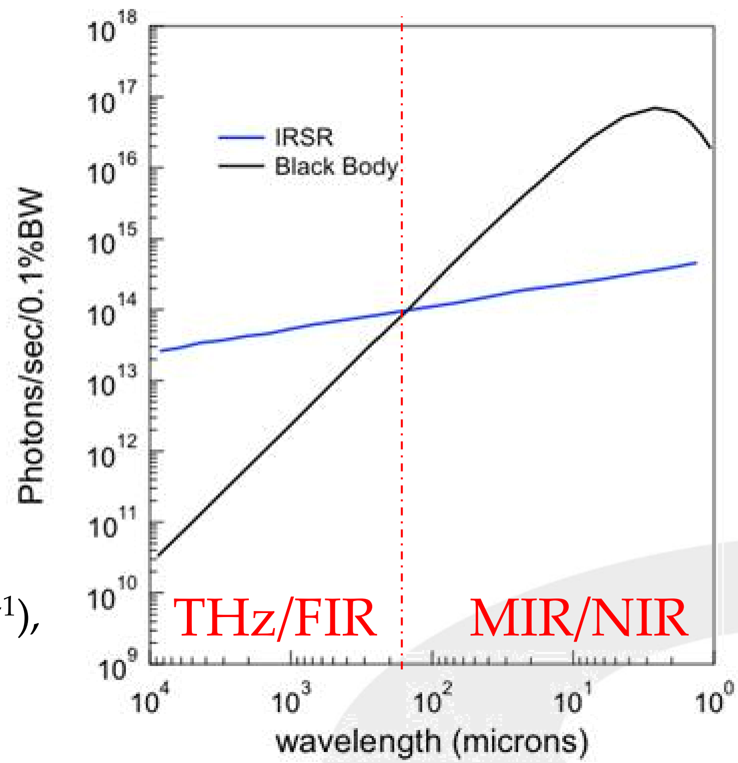
$$P_{BM}(\lambda) = 4.38\times10^{14}\times I\times\theta_H\times bw\times\left(\frac{\rho}{\lambda}\right)^{1/3} \quad \text{photons}\cdot s^{-1} \quad [1]$$

$I =$ Current (A)

$\theta_H =$ Horizontal Collection Angle (rads)

$bw =$ Bandwidth (%)

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

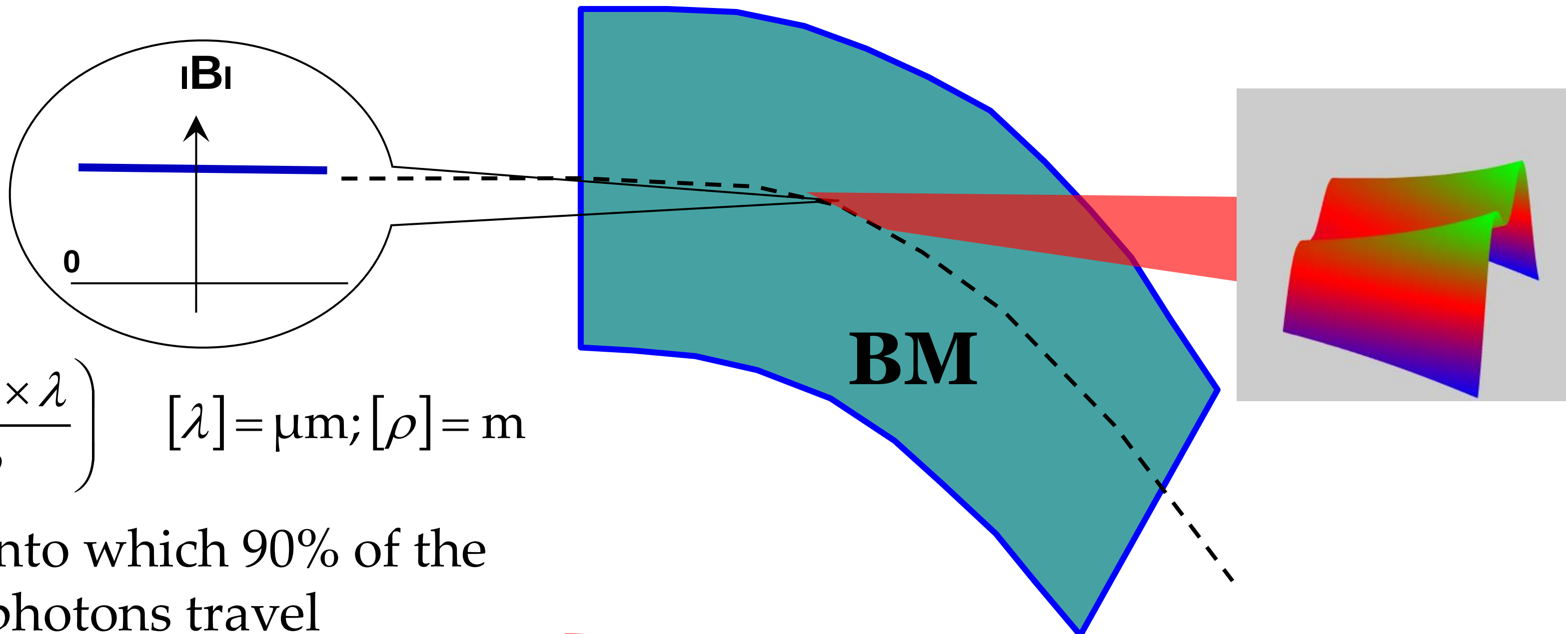


P(λ) as obtained in [1], in the spectral range 1 to 10^4 μm (10^4 to 1 cm^{-1}), for a current of **1 A**, a horizontal angle $\boldsymbol{\theta_H}$ **= 100 mrads** and $\boldsymbol{\rho}$ **= 5 m**. Comparison with the emission for a BB source at 2000K.

IRSR Generation

Bending Magnet IRSR

Constant Field Emission



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

Angular range into which 90% of the emitted photons travel

	λ [μm]	υ [cm⁻¹]	THz	$\theta_{V\text{-}Nat}$
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 ρ = 5.5 m.

Very large extraction apertures are needed for IR beamlines for:

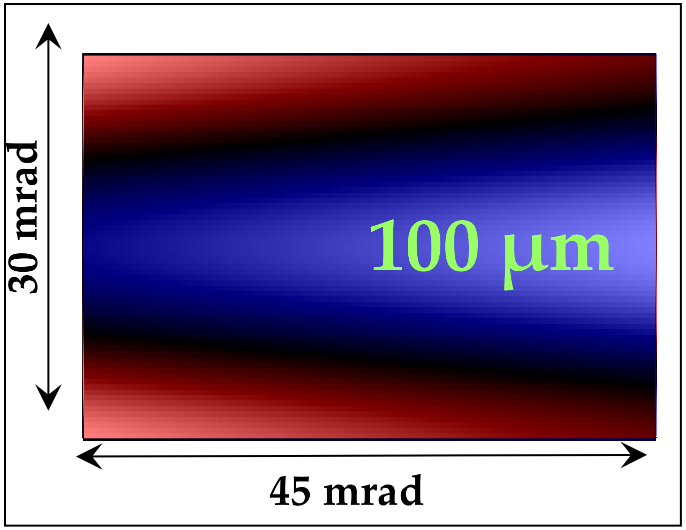
- Maximizing the flux (θ_H)
- Allowing efficient extraction of lower energy components of IR synchrotron emission (θv)

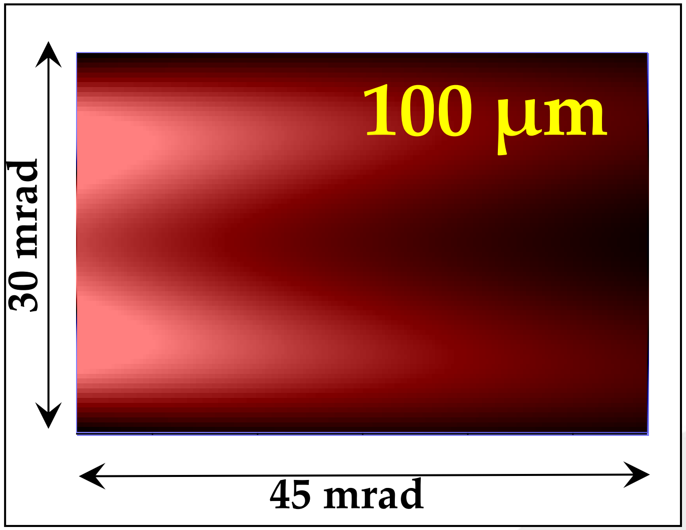
IRSR Generation

Bending Magnet IRSR

Vertical opening angle depends on the electron energy (through bending magnet radius)

INDUS	*DIAMOND*
0.45 GeV	**3.0 GeV**
45 mrad H X 30 mrad V	**45 mrad H X 30 mrad V**
BM	**BM**

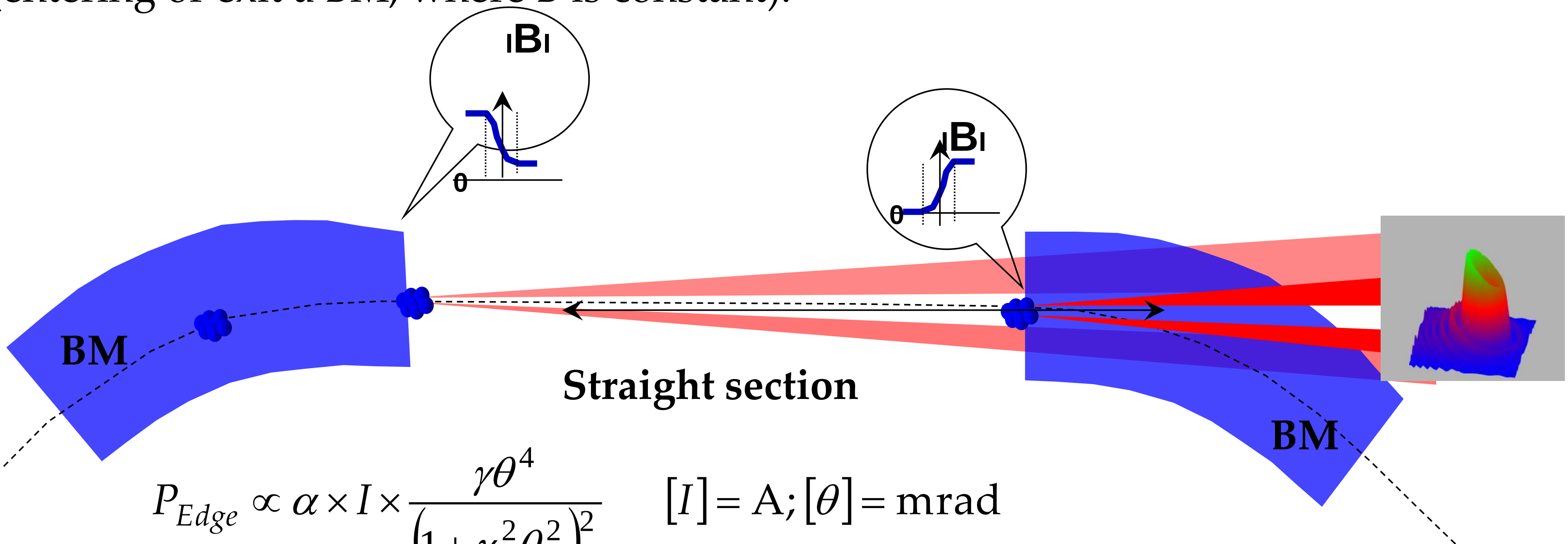




By courtesy of Paul Dumas, SOLEIL

IRSR Generation
Edge Radiation

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



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

$$\gamma = \mathrm{Lorentz\,Factor}; \alpha = \mathrm{Fine\,Structure\;Constant}$$

- Edge radiation has a ring structure characterized by interference pattern
- Being $\mathbf{\Theta}_{\mathbf{max}} \sim 1/\gamma$ ~10 mrads, it is spatially confined and intrisically bright
- It is radially polarized

By courtesy of Paul Dumas, SOLEIL

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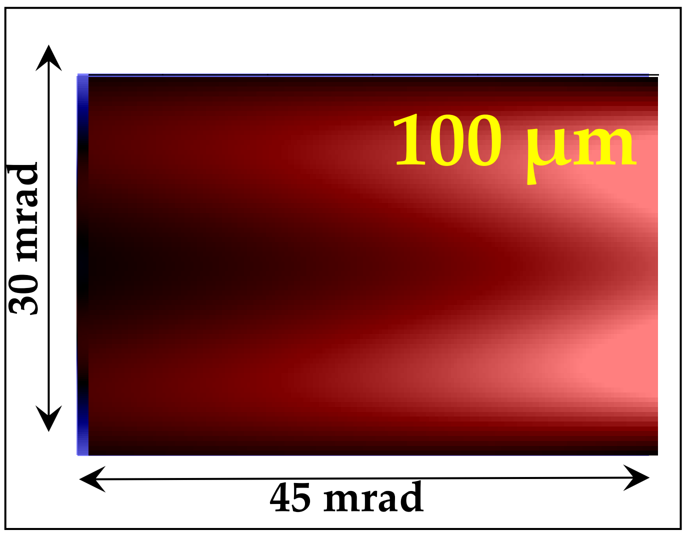
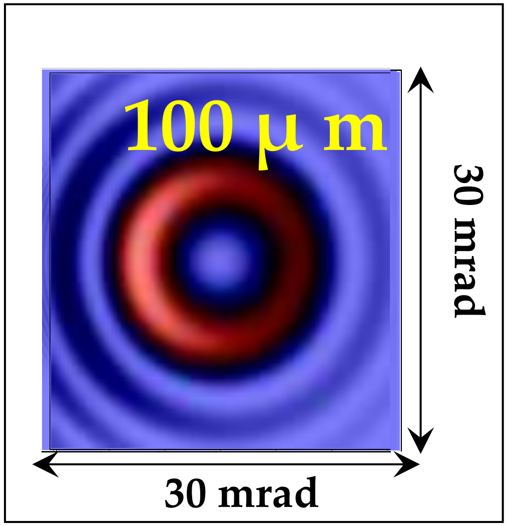
IRSR Generation

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

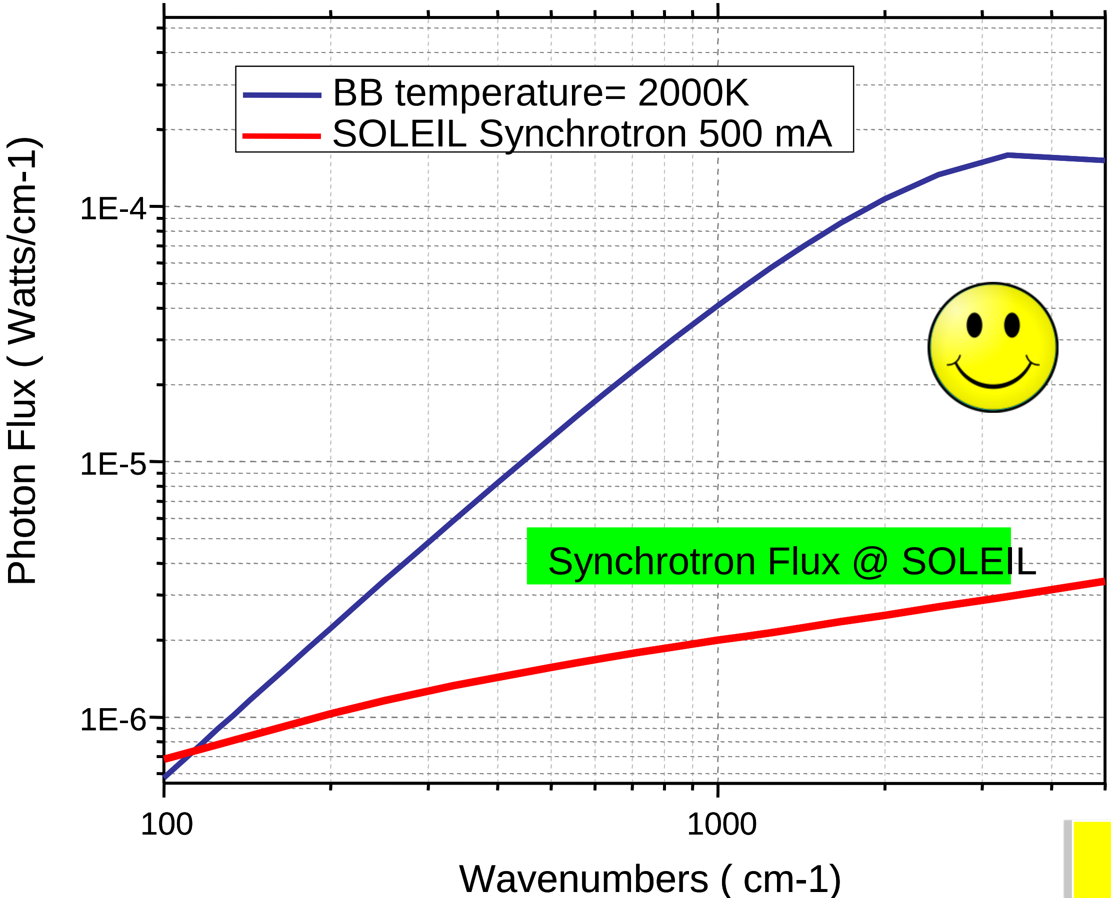




By courtesy of Paul Dumas, SOLEIL

IRSR

The brightness advantage



Synchrotron IR is much brighter !

$$B(\lambda) \approx \frac{3.8 \times 10^{20} \times bw \times I}{\lambda^2}$$

$[\lambda] = \mu\text{m}; [I] = \text{A}; [bw] = \%$

By courtesy of Paul Dumas, SOLEIL

Exploitation of IRSR advantages

Advantage	Benefit	Application
Flux Adavantage in FIR and THz	• Higher S/N • Faster data collection	FIR and THz spectroscopy
Broad band nature	• Complete data collection	Spectroscopy and Microscopy
Polarization	• BM Linear polarization • ER circular polarization	Spectroscopy and Microscopy
Brigthness advantage	• Higher S/N ratio • Faster data collection	Microscopy

SISSI

Synchrotron Infrared Source for Spectroscopy and Imaging

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

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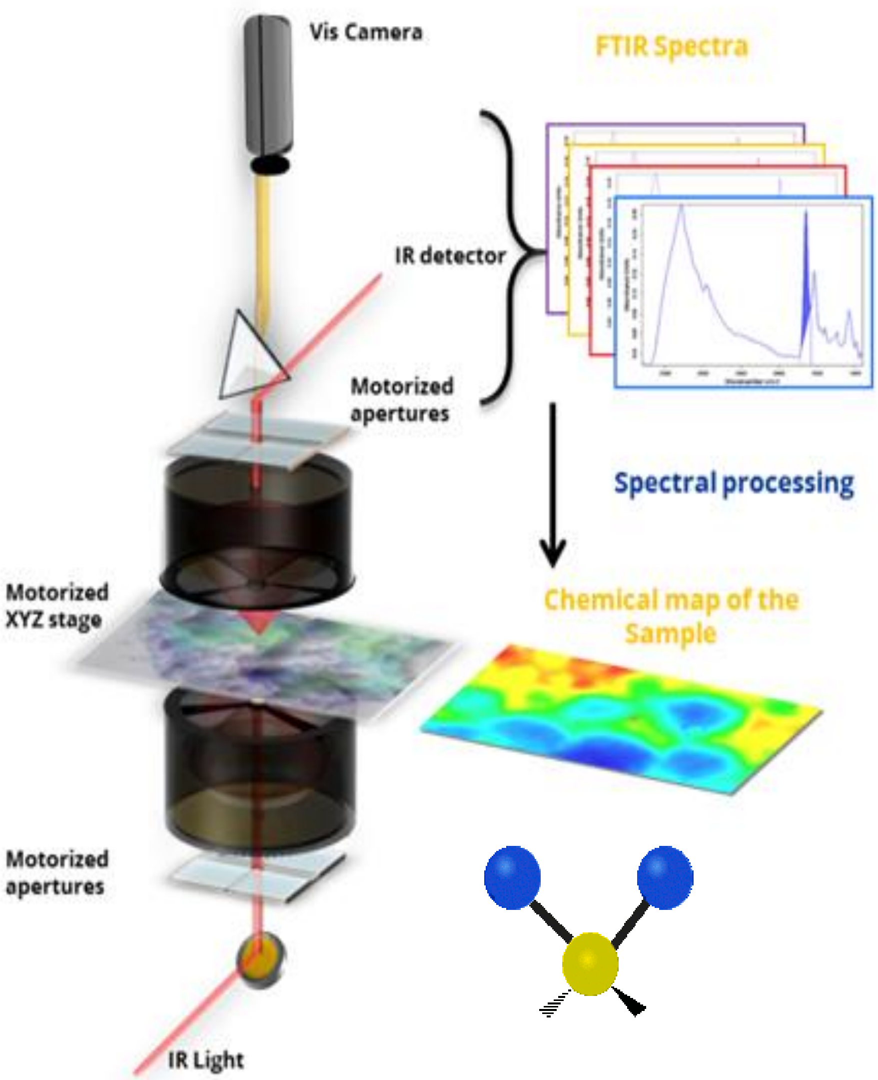
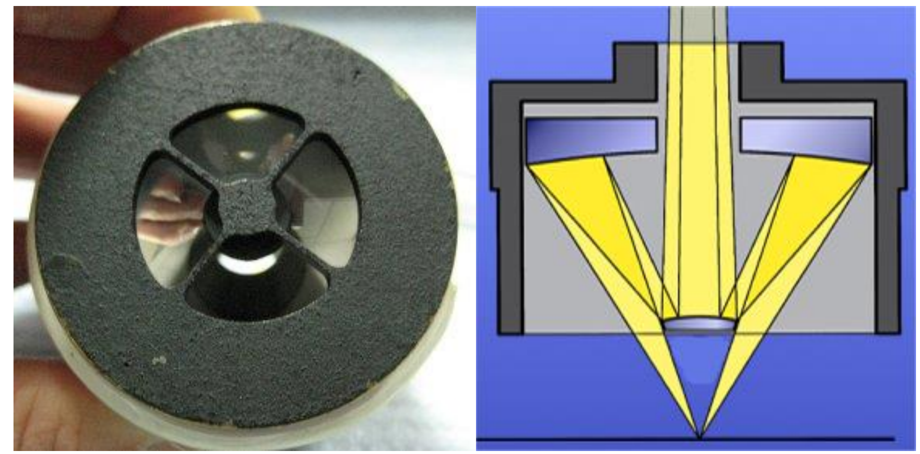
FTIR Microspectroscopy



Schwarzschild objective



FTIR microscopy is a far–filed microscopy

Lateral resolution, δ, is diffraction limited

$$d \approx 0.61\ \lambda\ /\ NA$$

Objective NA	Wavelength	δ
0.4	10 μm (1000cm^{-1})	~15 μm
	2.5 μm (4000cm^{-1})	~ 4 μm
0.65	10 μm (1000cm^{-1})	~ 9,5 μm
	2.5 μm (4000cm^{-1})	~ 2,5 μm

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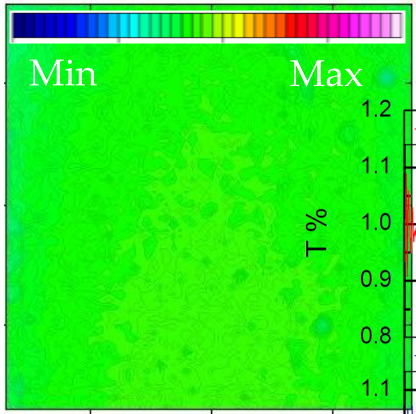
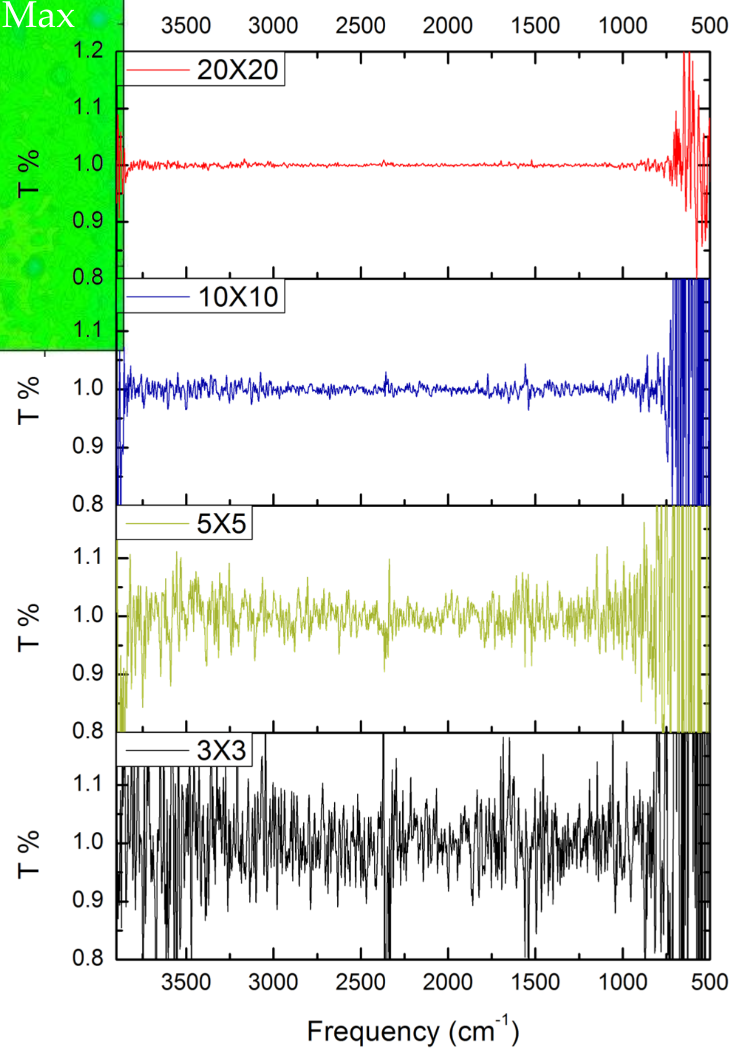
FTIR Microspectroscopy

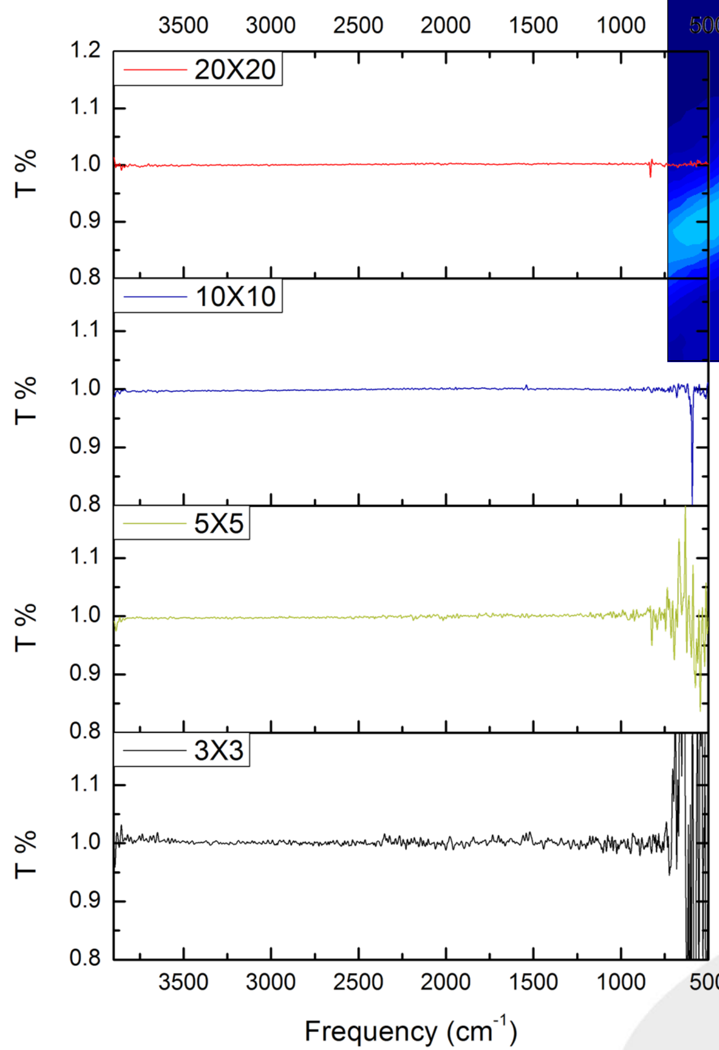
Diffraction Limited FTIR Microscopy is practically achievable only with IRSR

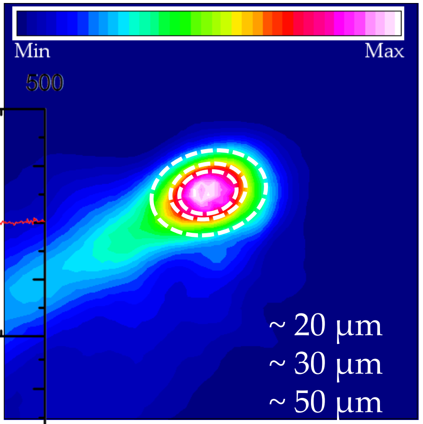
Conventional Source





IRSR





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

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Infrared bio-spectroscopy
From macro to nanoscale on the molecules of Life

School on Synchrotron
and Free-Electron-Laser
Methods for Multidisciplinary
Applications

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Biospectroscopy

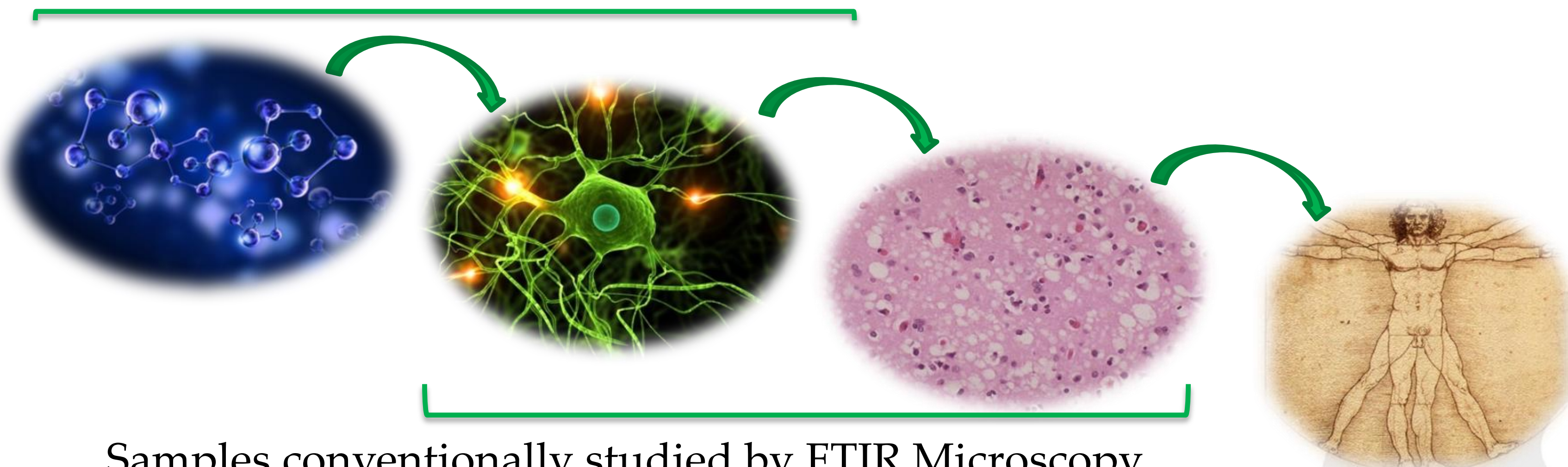
Biospectroscopy is the spectroscopy of the **Molecule of Life**

Organic molecules are the Molecules of Life.
They are built on chains of carbon atoms, usually very long (bio-macromolecules)

There are four main groups of bio-macromolecules to build sub-cellular structure, cells, tissue, organs up to living beings:

Proteins; **Lipids**; **Nucleic Acids**; **Carbohydrates**

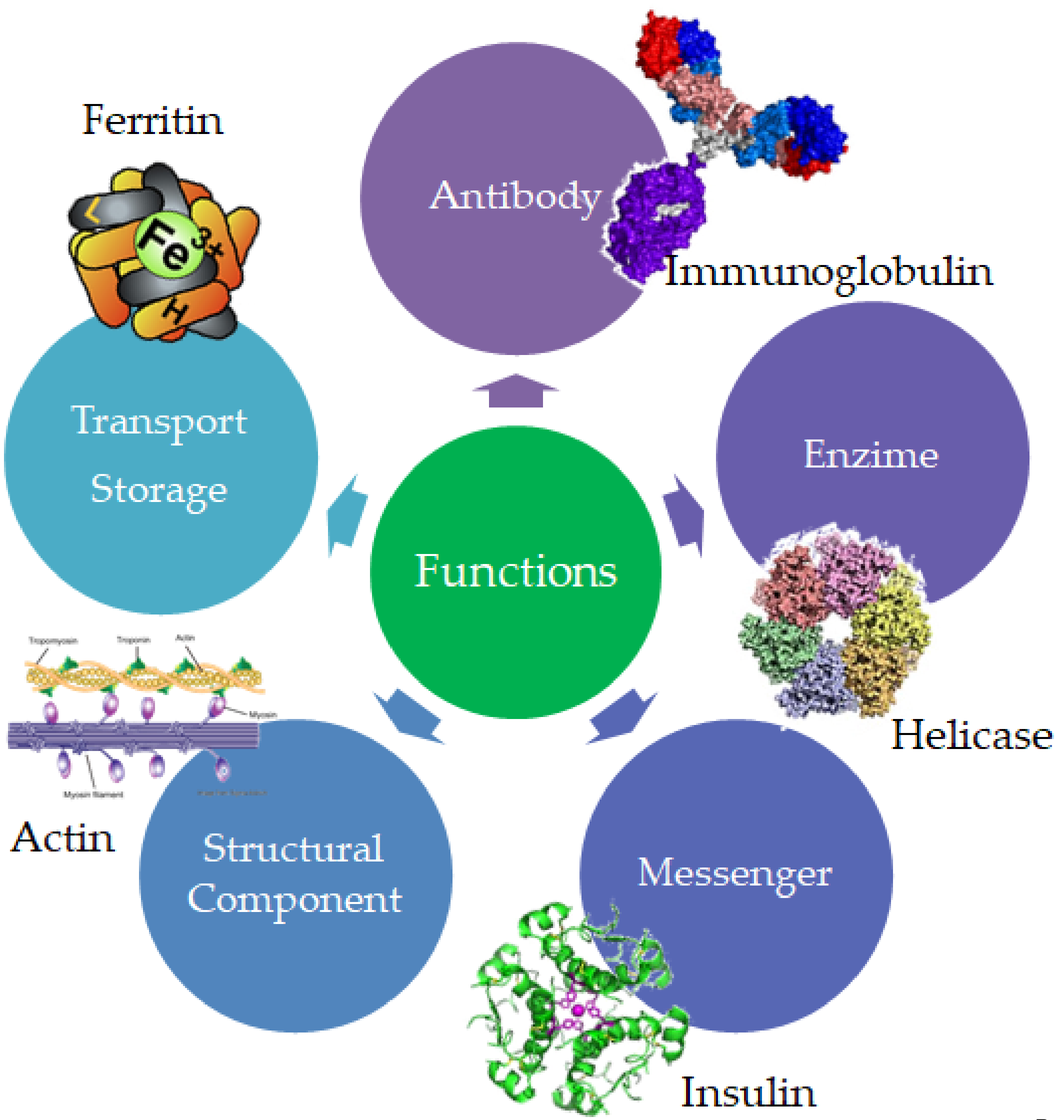
Samples conventionally studied by FTIR Spectroscopy

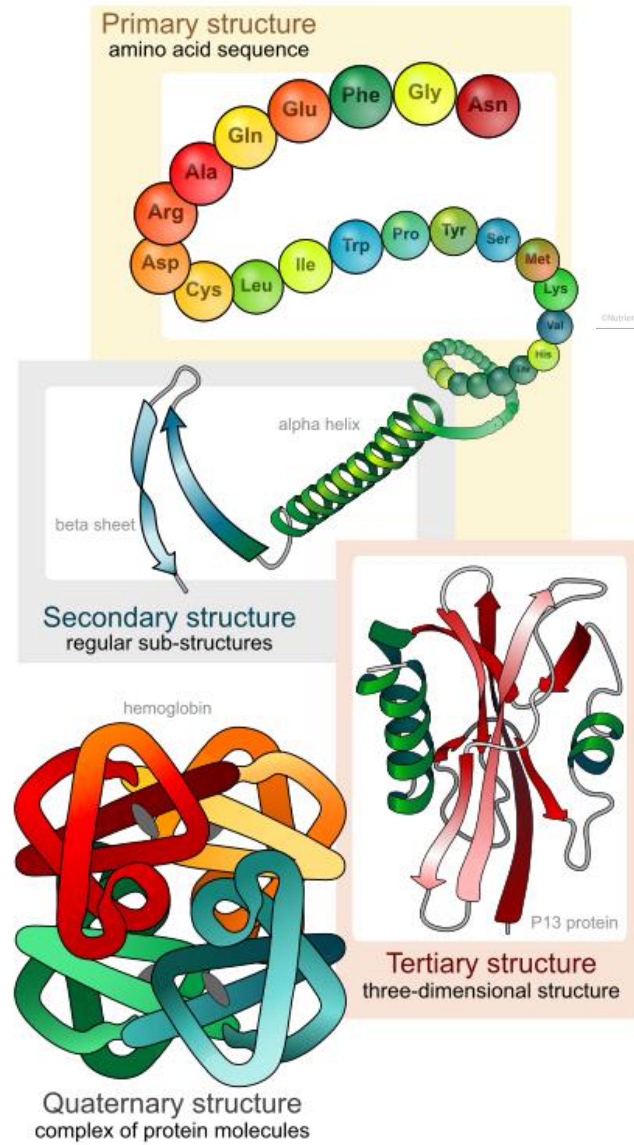


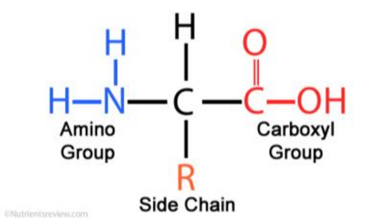
Samples conventionally studied by FTIR Microscopy

Proteins
Functions and Structure

Proteins perform a vast array of functions within organisms, exhibiting activity strictly related to their structure (<u>Structure-Activity relationship</u>)



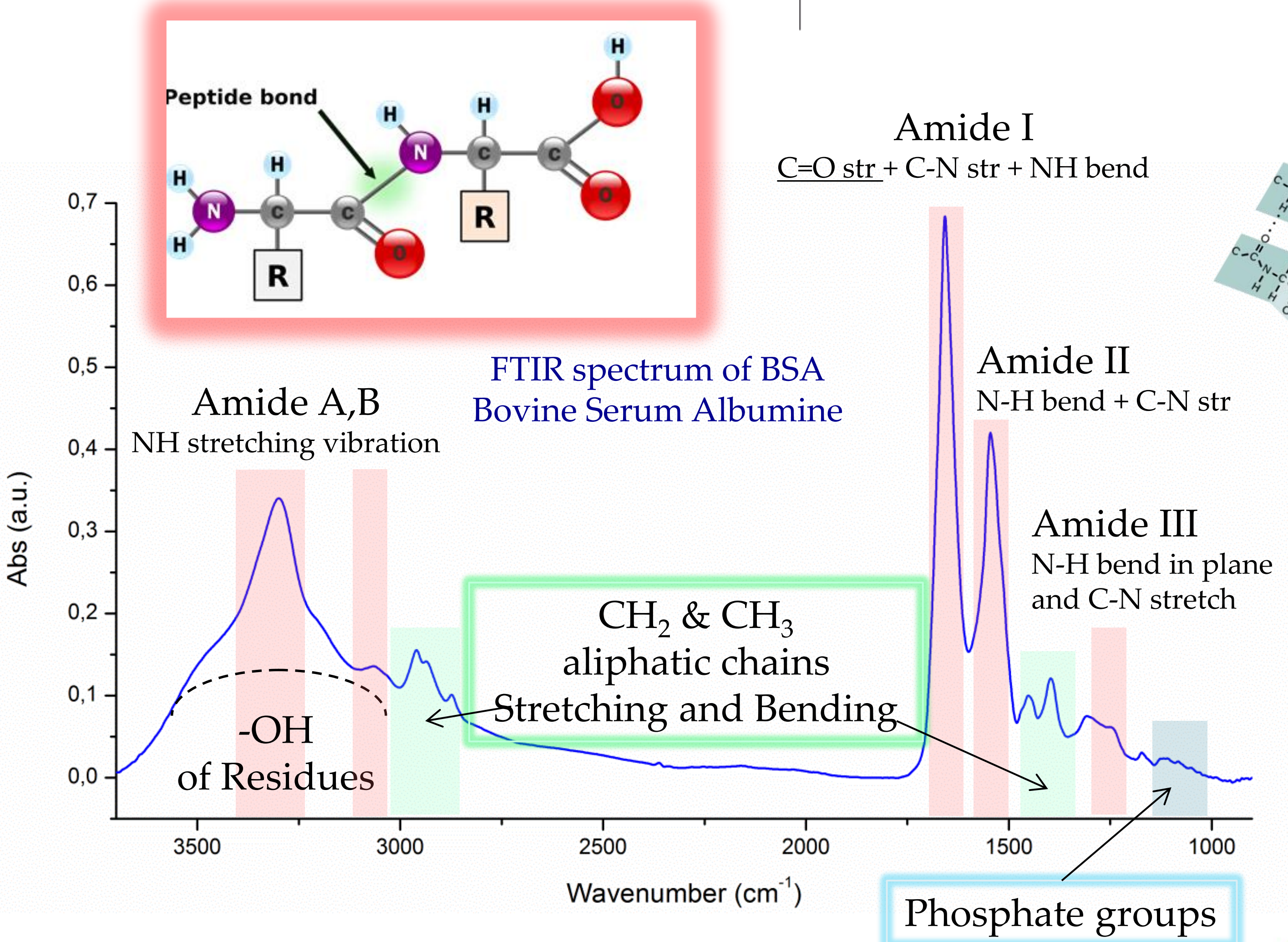


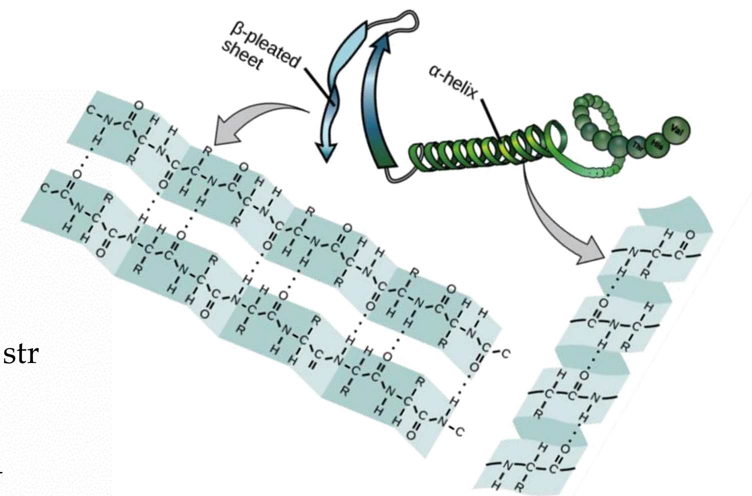


By LadyofHats - Public Domain, https://commons.wikimedia.org/w/index.php?curid=4945067

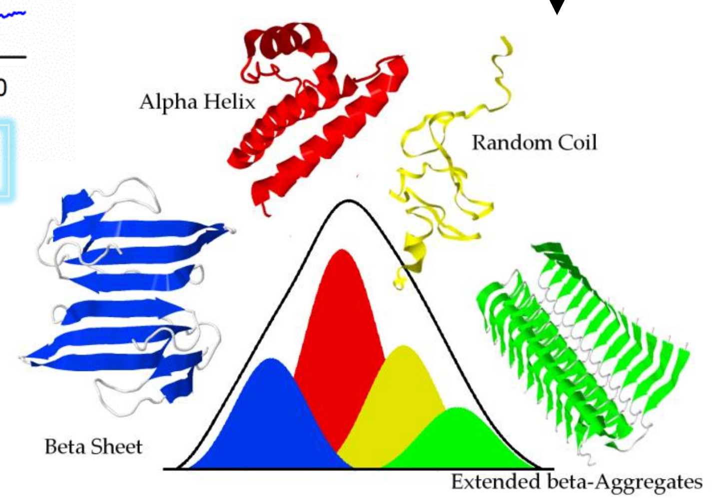
FTIR spectroscopy for protein conformational studies

Image credit: OpenStax Biology.





Different H-bonding networks for different peak positions



Amide I band is particularly sensitive to protein secondary structure, and conventionally employed for protein conformational studies

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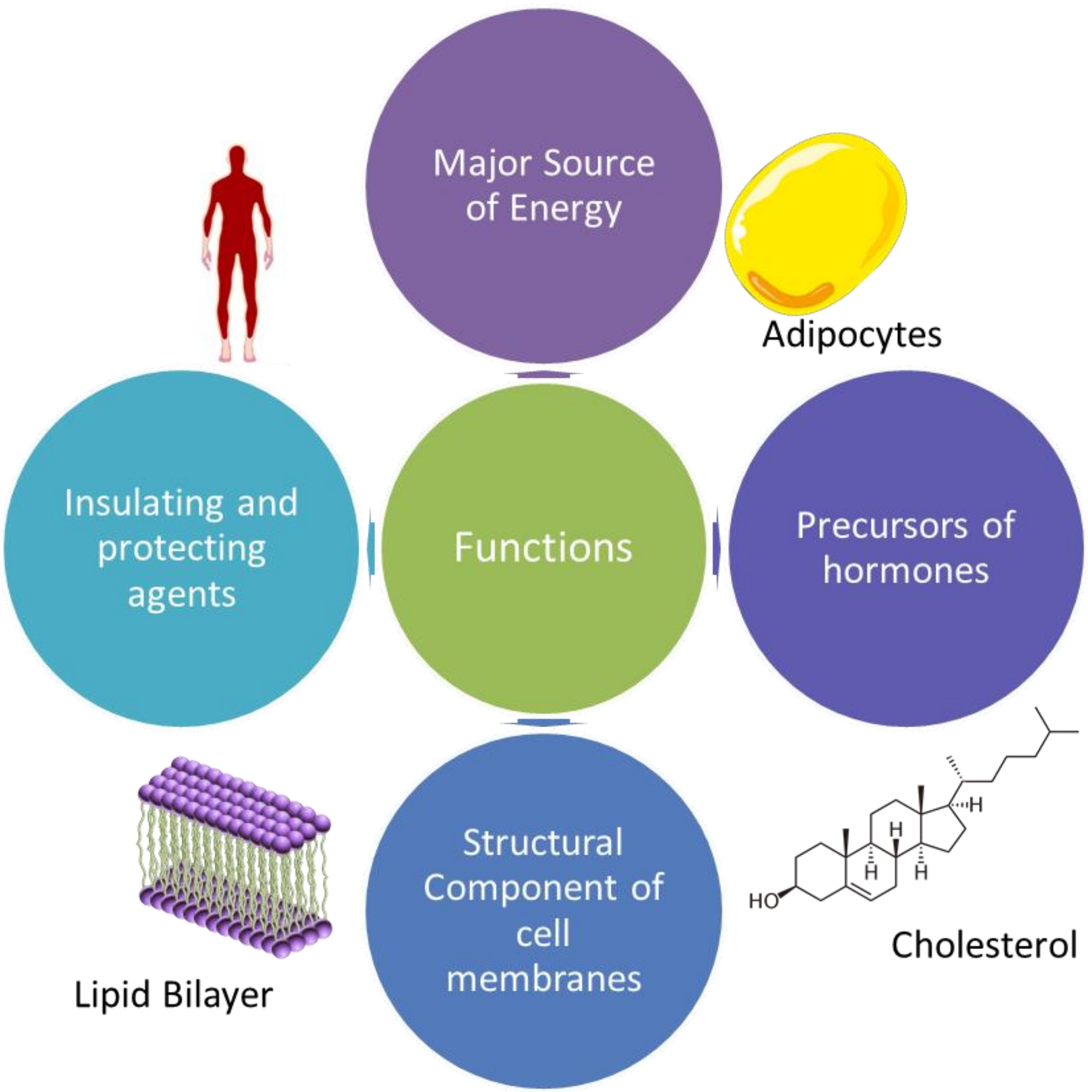
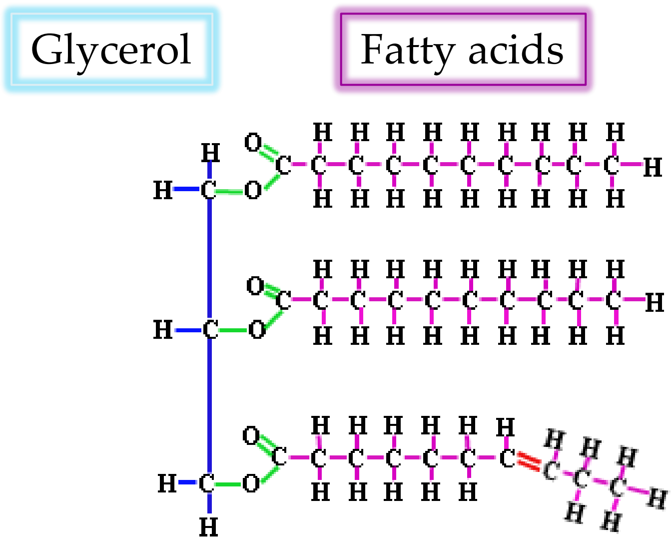
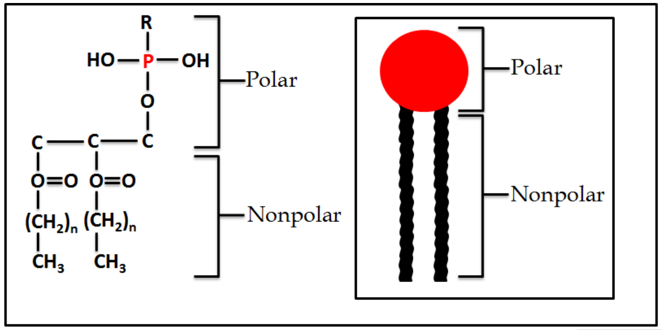
Lipids
Functions and Structure





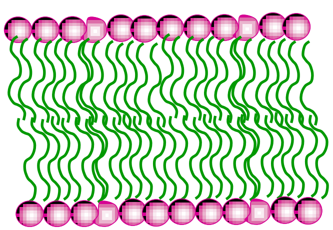
Triglyceride



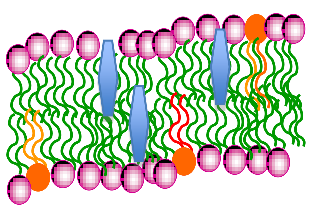
Phospholipids

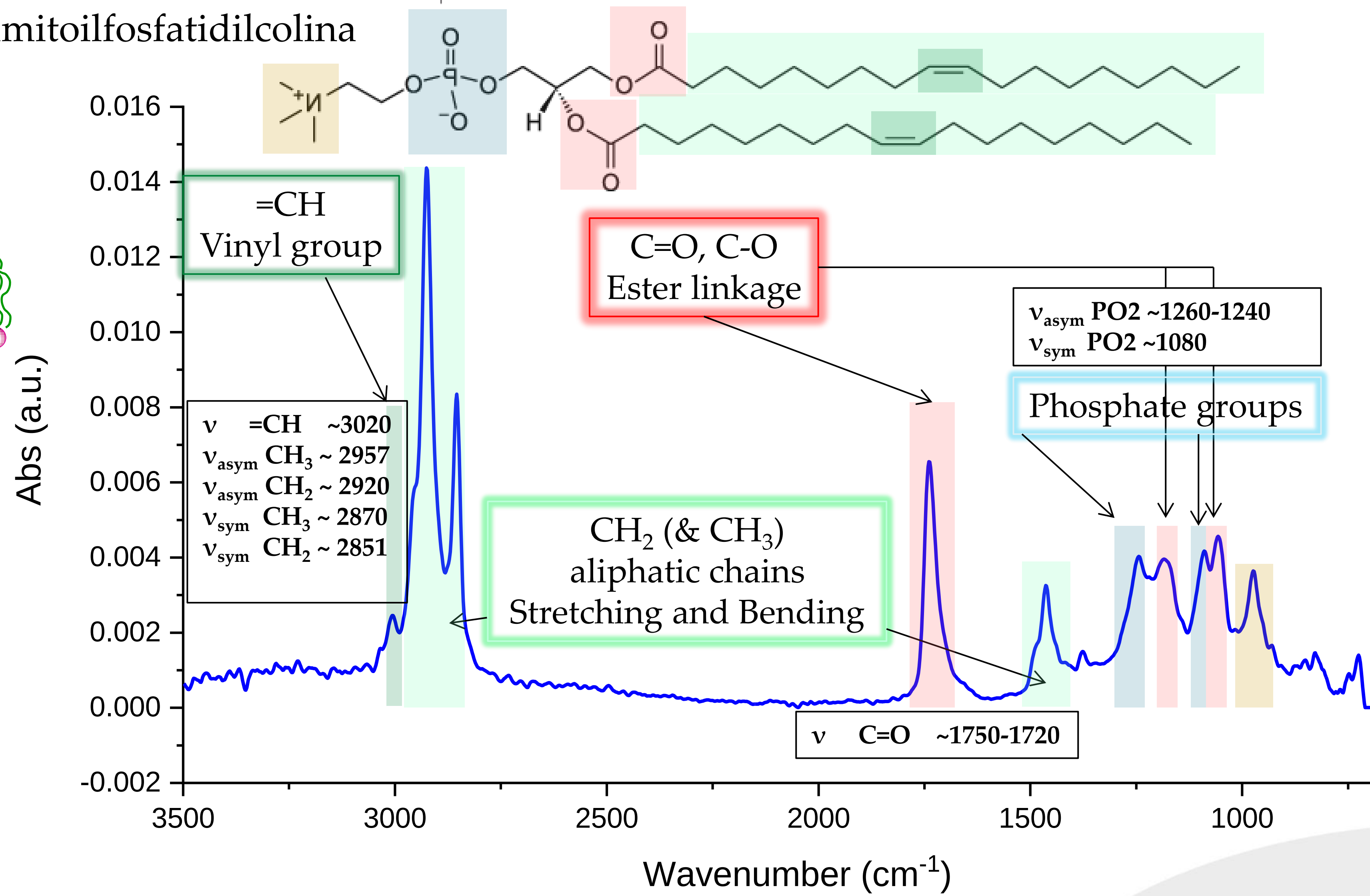
FTIR spectroscopy of lipid

DOPC - Dipalmitoilfosfatidilcolina



Viscous
To
Fluid





CH_2/CH_3 ratio: methyl-branched fatty acids increase membrane fluidity

=C-H: Unsaturated fatty acids increase membrane fluidity

Shifts and broadening of the methyl and methylene bands are indicative of increased lipid disorder/fluidity

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Nucleic acids
Structure and Function

DNA stores information

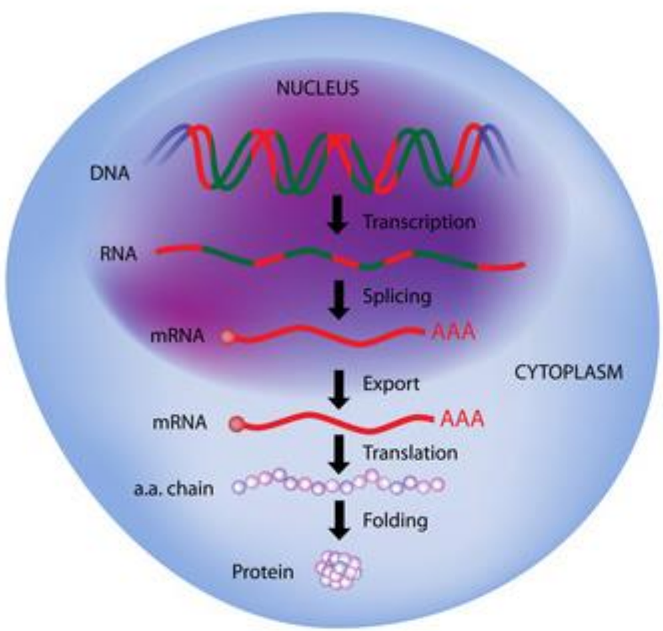


Image credit
https://www.wonderwhizkids.com/gene-expression

RNA transfer information

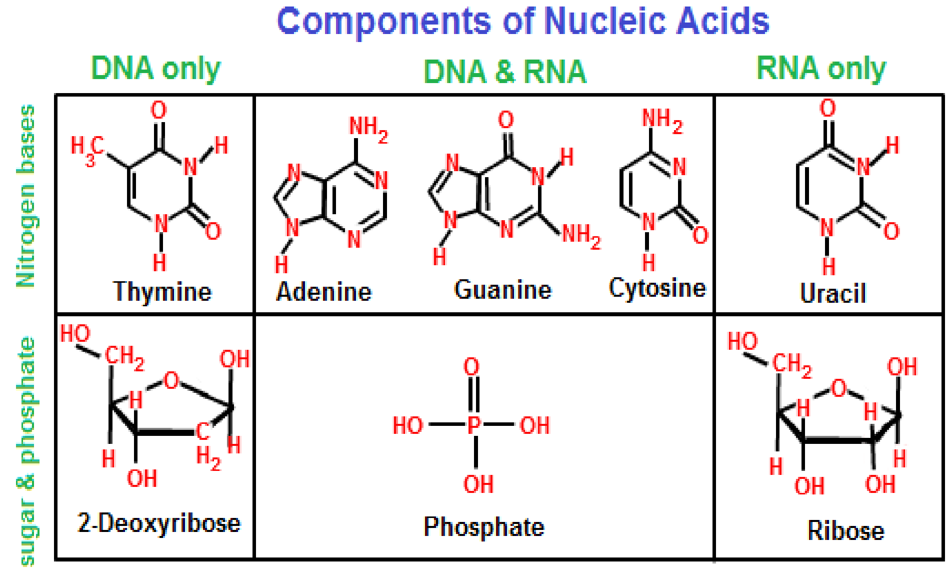
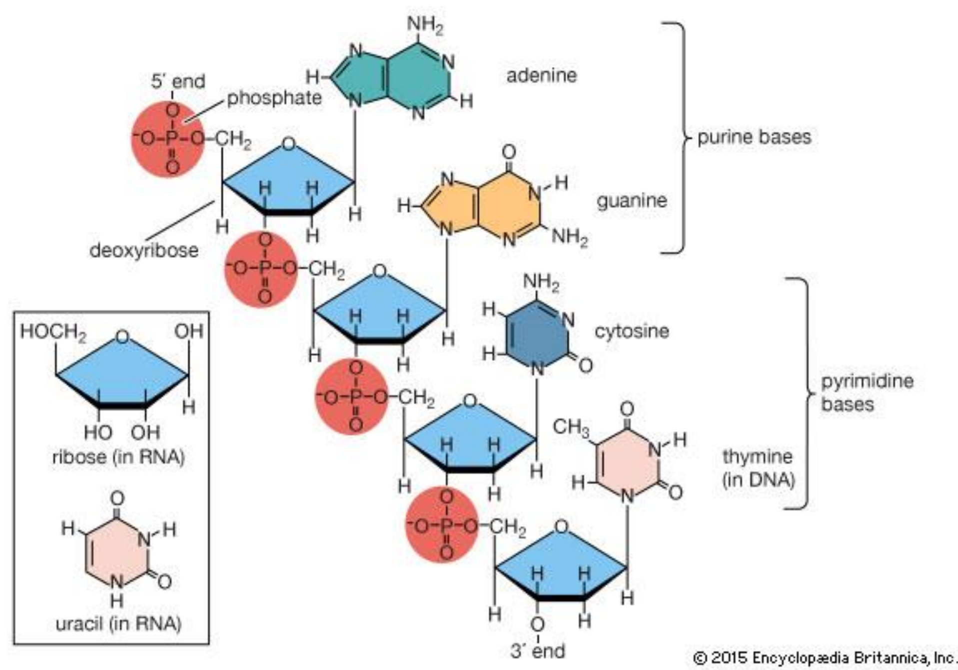


Image credit
https://biology.tutorvista.com/biomolecules/nucleic-acids.html



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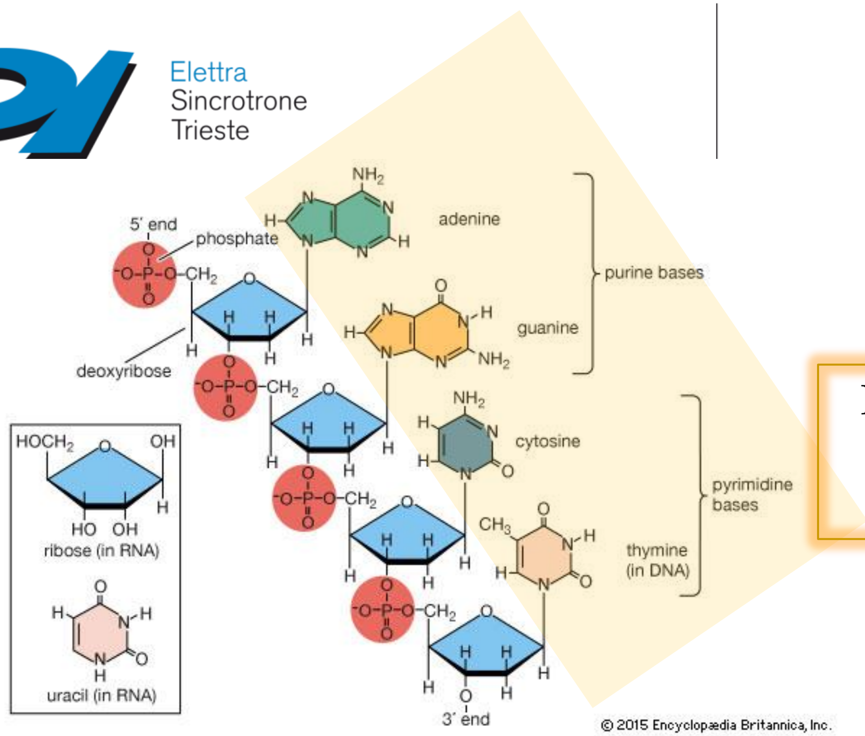
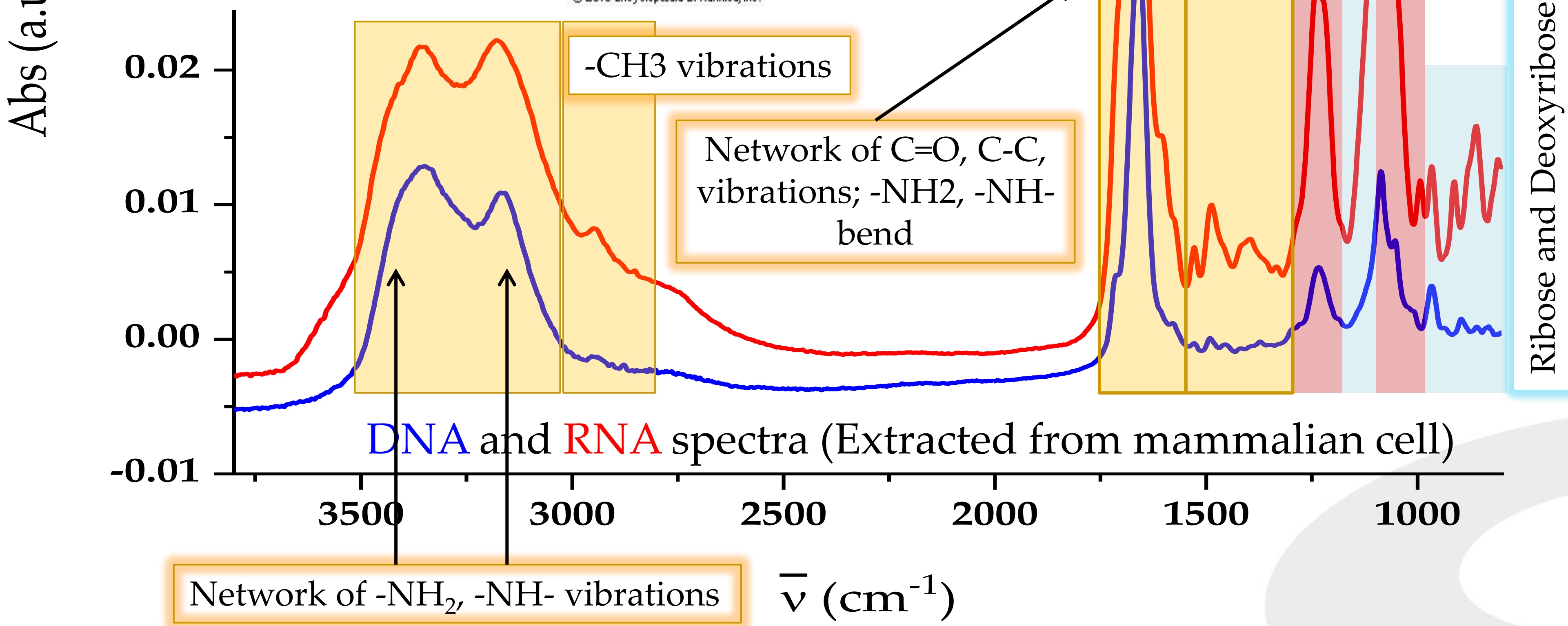
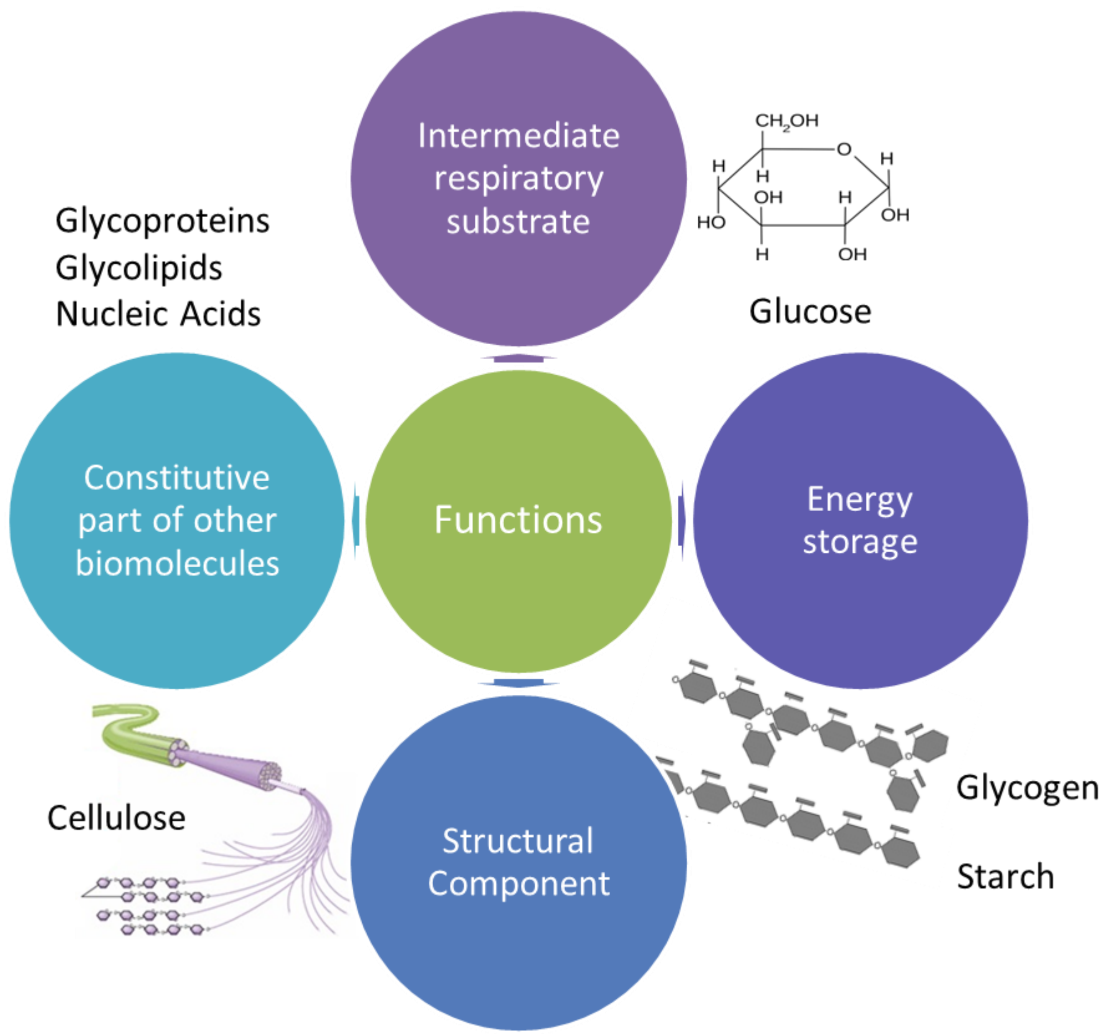
FTIR spectroscopy for Nucleic acids conformational studies

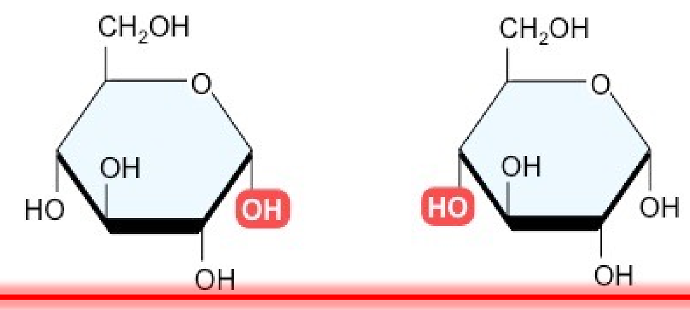




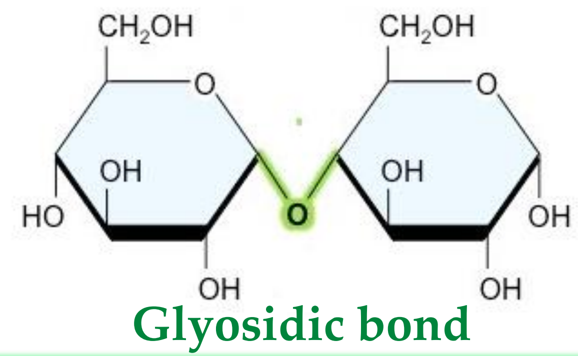
Carbohydrates
Structure and Function

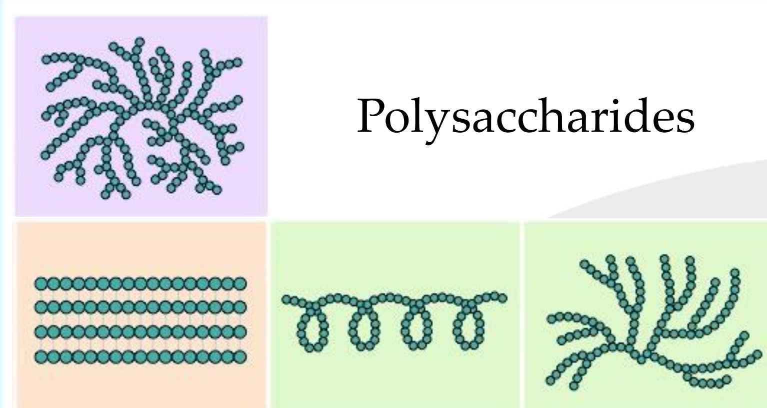


Monosaccharides



Disaccharides





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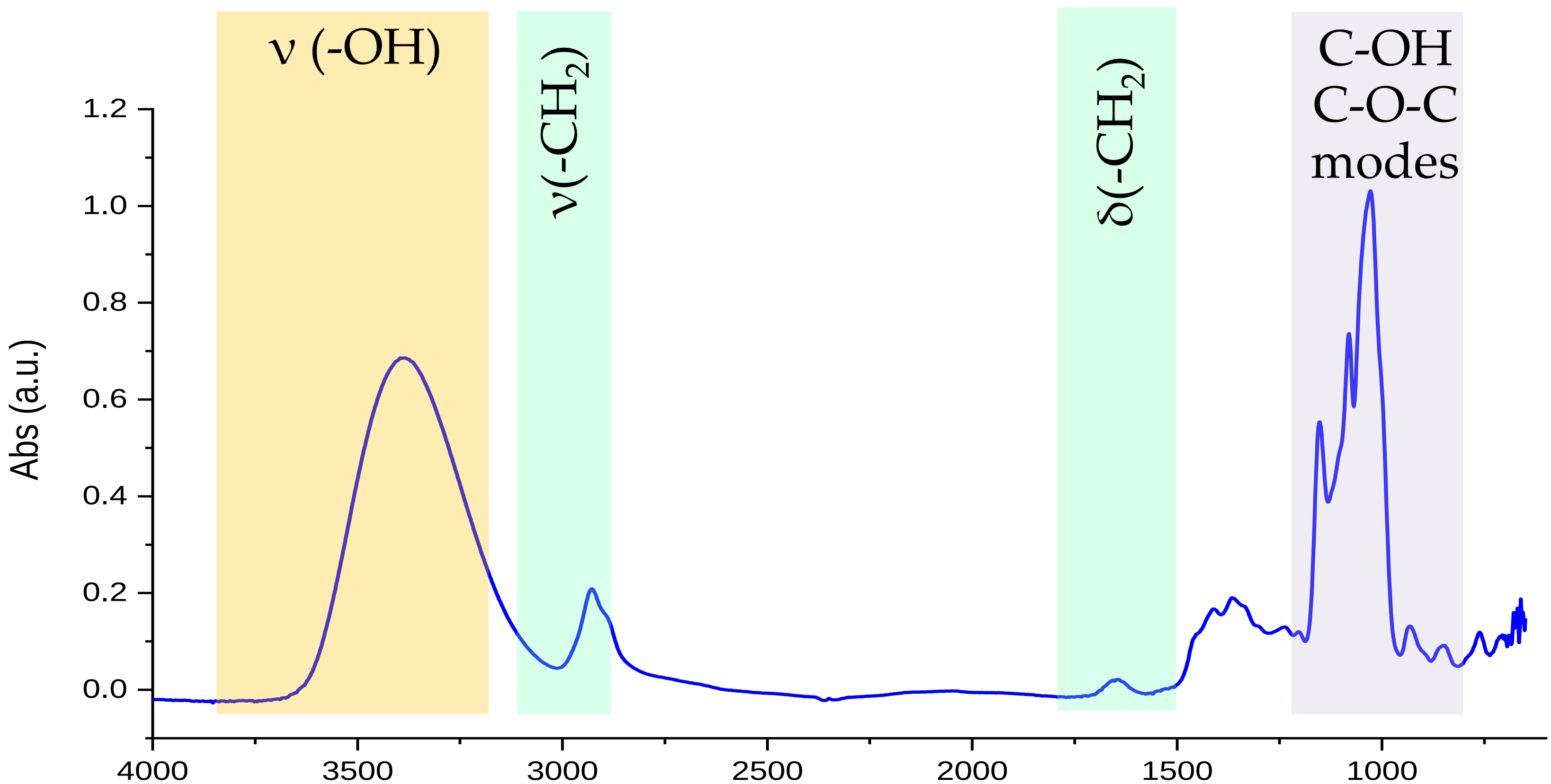
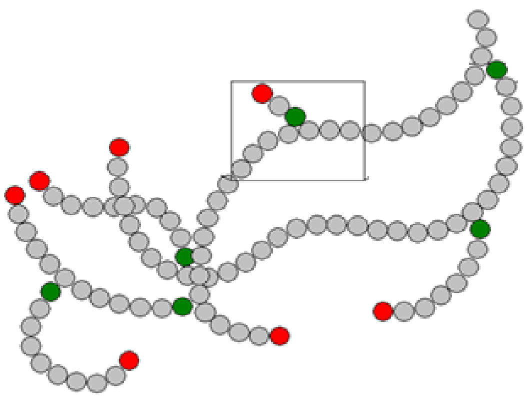
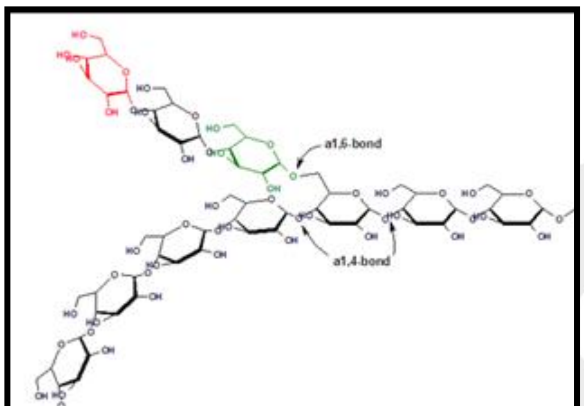
Carbohydrates
Structure and Function



GLYCOGEN

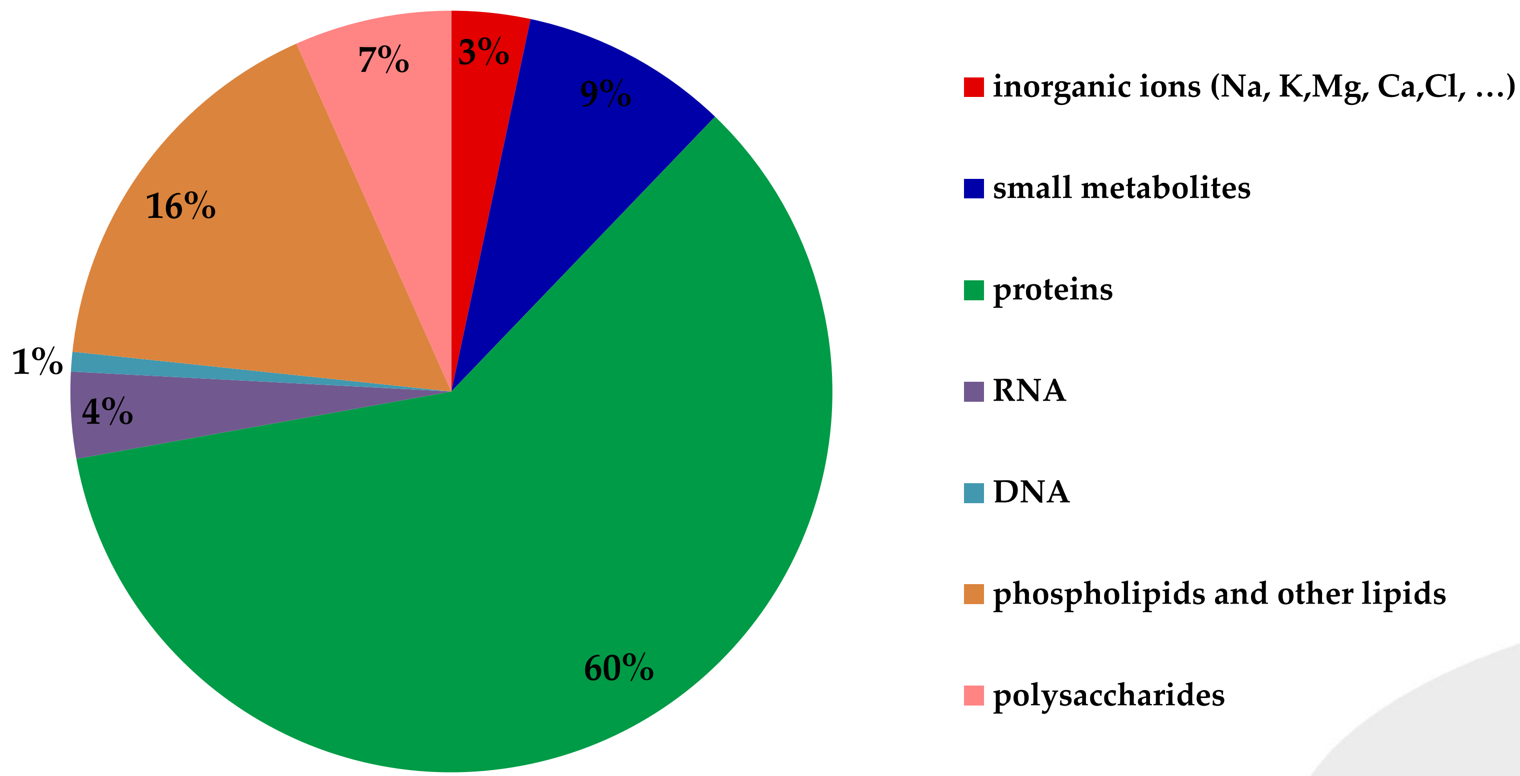




Mammalian cells

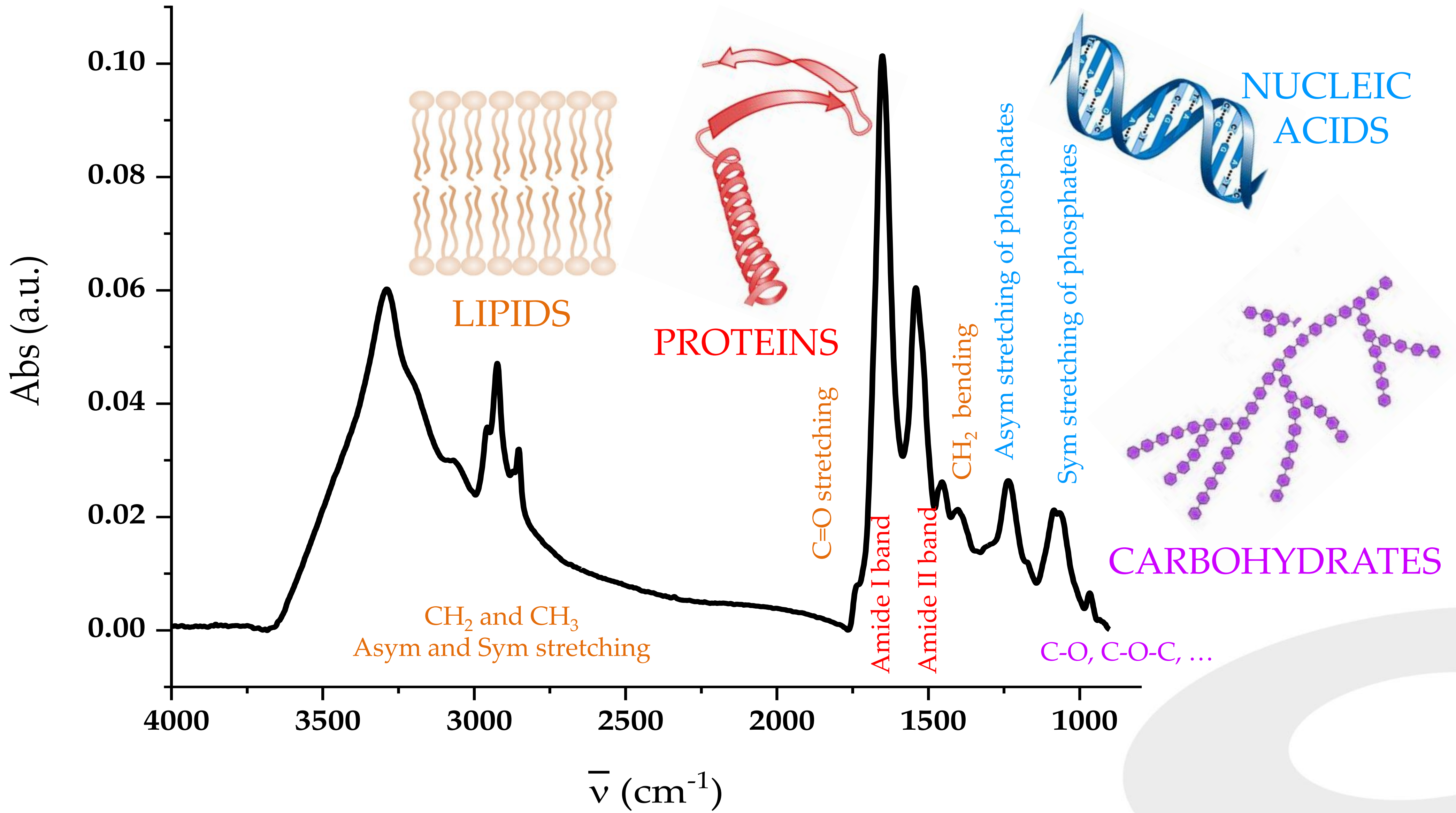
At a first glance, the FTIR spectrum of a mammalian cell can be viewed as the over imposition of the diverse spectral contribution of each individual components

Typical mammalian dried cell chemical composition (component percent of total cell weight)



Mammalian cells

Band intensity, position, width and shape (band components) are sensitive to subtle biochemical changes of bio-specimens.



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Infrared bio-spectroscopy
From macro to nanoscale on the molecules of Life

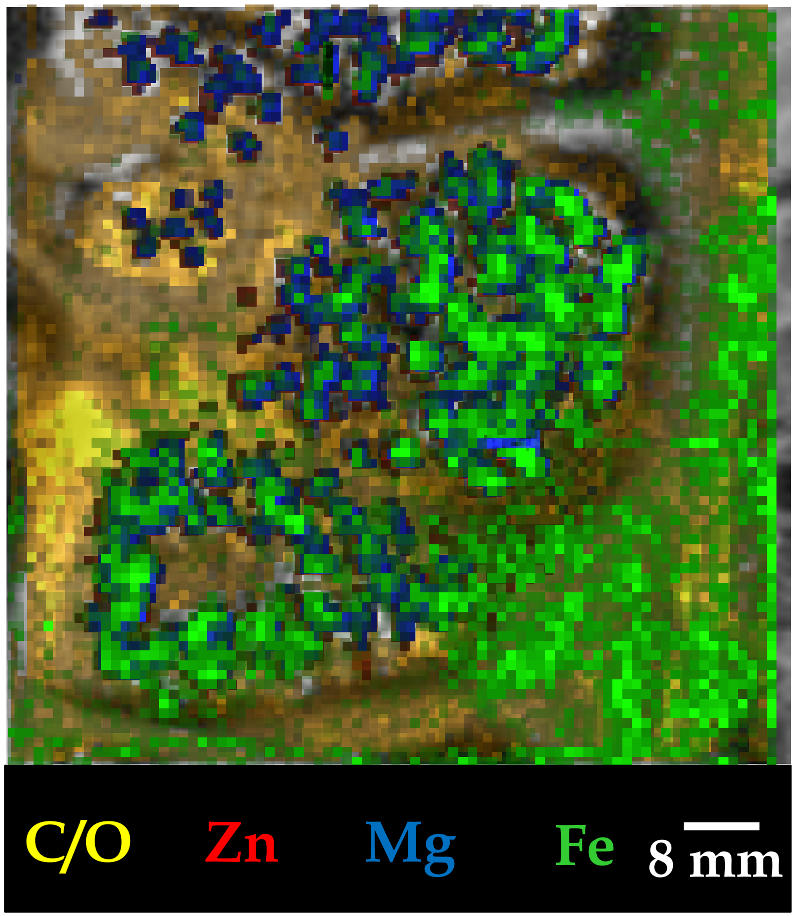
Soft X-ray Radiation damage

School on Synchrotron
and Free-Electron-Laser
Methods for Multidisciplinary
Applications

ICTP

TwinMic Beamline at Elettra

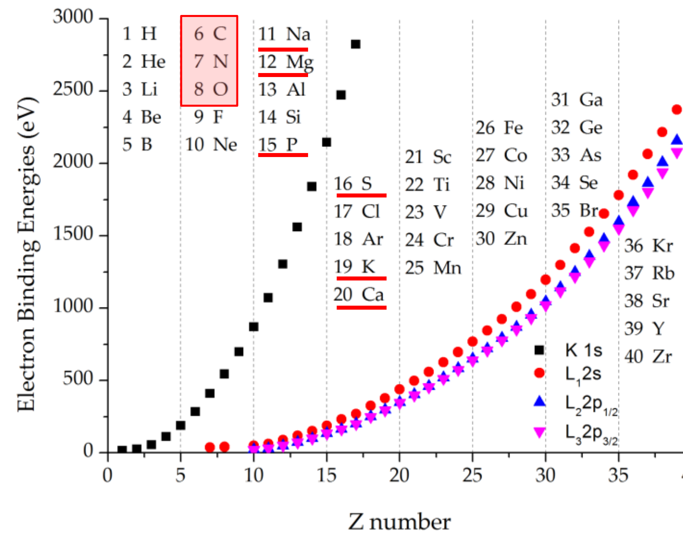
Functionality and toxicity of Zn in wheat



- Soft X-ray Radiation Damage

• Radiation damage induced by X-rays on biological samples is one of the remaining bottlenecks for their ultrastructural characterization by X-ray microscopy techniques

• X-ray nanofocusing is a today reality but the extent to which the lateral resolution can be pushed without unacceptable bio-sample degradation is still an open question



M. Regvar, D. Eichert, B. Kaulich, A. Gianoncelli, P. Pongrac, K. Vogel-Mikus, I. Kreft, New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron soft X-ray microscopy, Journal of Experimental Botany, Vol. 62, No. 11, 3929–3939, 2011.

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Soft X-ray Radiation Damage

Literature Survey

The very same radiation that induces damage is exploited for probing it

Radiation damage is dose dependent

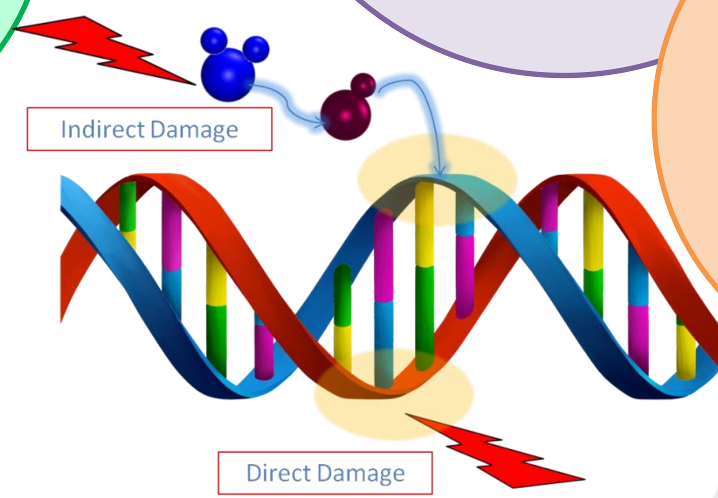
Hydrated samples undergo more relevant changes with respect to fixed ones

Radiation damage depends on sample preparation

Cryo-XRM better preserves the structural integrity of bio-samples

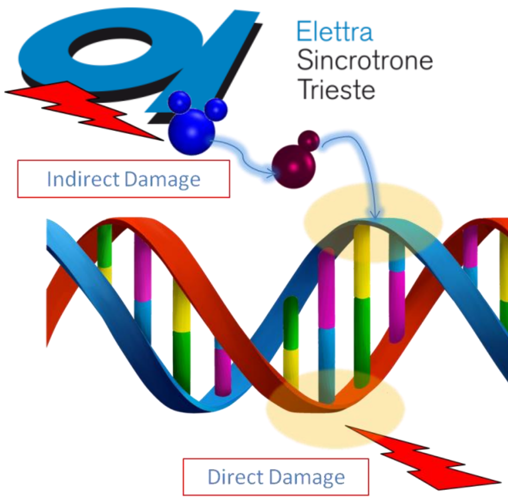
Radiolytic effects undergone by a specific molecule strongly depend on the sample architecture

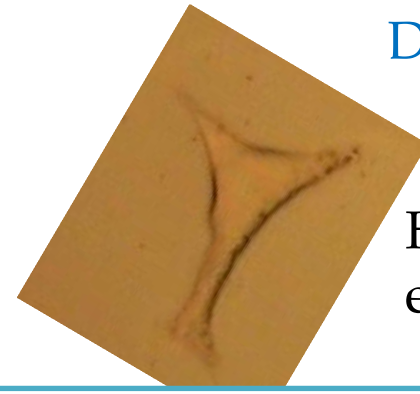
Genetic material is extremely sensitive to ionizing radiation



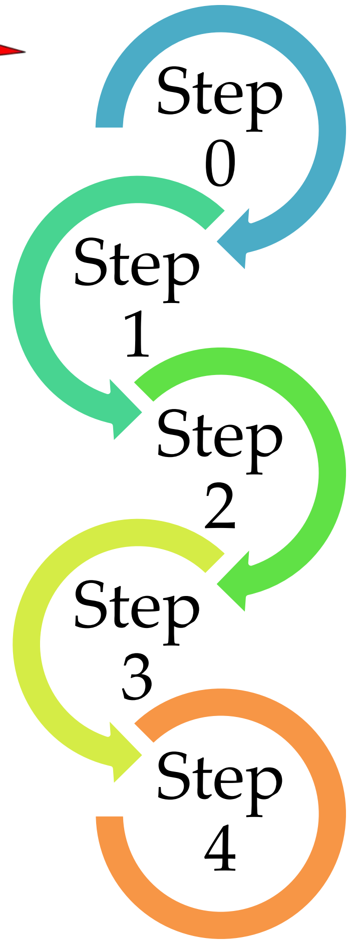
- ## Soft X-ray Radiation Damage

Design of Experiment





Hek293T cells (human embryonic kidney)



Cell growth on 100 nm Si_3N_4 membranes
Cell fixation with PFA 3.7% and overnight air drying

Cell drying in vacuum @ TwinMic
($p < 10^{-5}$ mbar) for 1:30 hour

Low Dose STXM mapping @ TwinMic 1 keV
Estimated dose: $2*10^6$ Gy

High Dose STXM mapping @ TwinMic 1 keV Estimated dose: $2*10^7$ Gy
Cumulative estimated dose: $2.2*10^7$ Gy

Very high dose STXM mapping @ TwinMic 1 keV
Estimated dose: $6*10^8$ Gy
Cumulative estimated dose: $6.2*10^8$ Gy

A. Gianoncelli, L. Vaccari, G. Kourousias, D. Cassese, DE Bedolla, S. Kenig, P. Storici,. M. Lazzarino and M. Kiskinova. **Soft X-Ray Microscopy Radiation Damage On Fixed Cells Investigated With Synchrotron Radiation FTIR Microscopy.** *Scientific Reports* **2015** *5*, article number 10250

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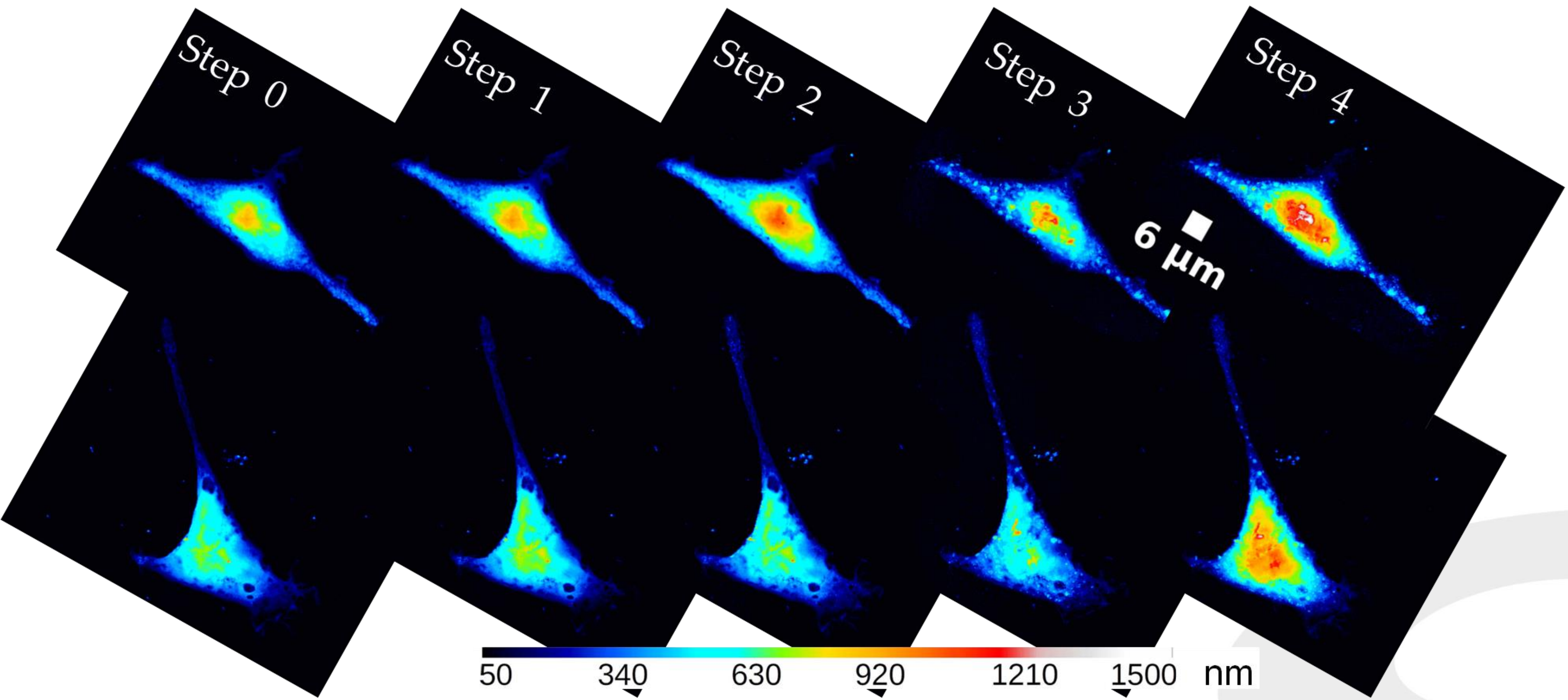
Soft X-ray Radiation Damage

AFM outcomes

- Minimal cell shrinkage
- Evident degradation/thinning of pseudopodia terminations
- Appreciable thickness variations, especially on the nuclear region at Step 4
- Outstanding topographical changes: nanometric pits and bulges increase in number and size when increasing dose

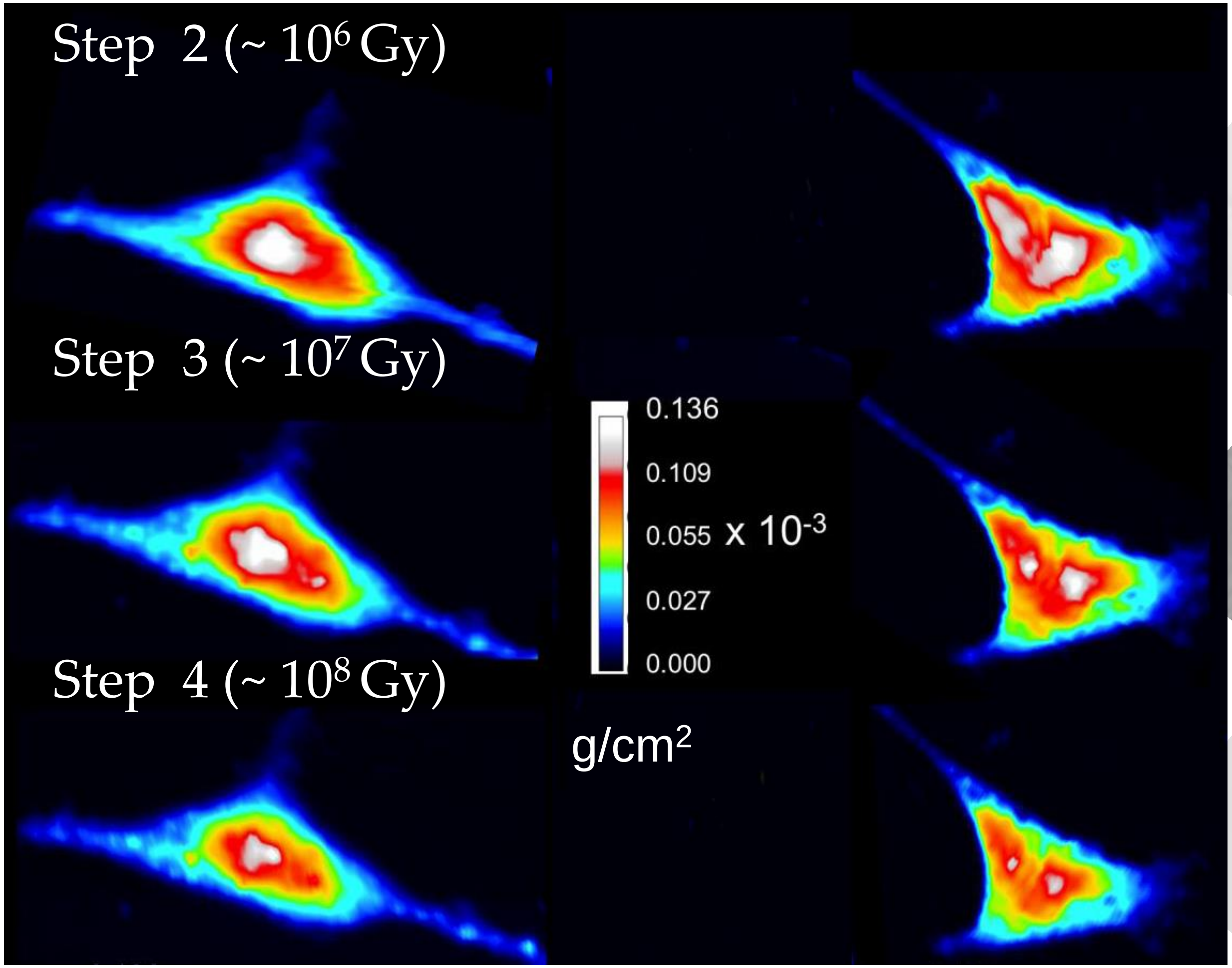


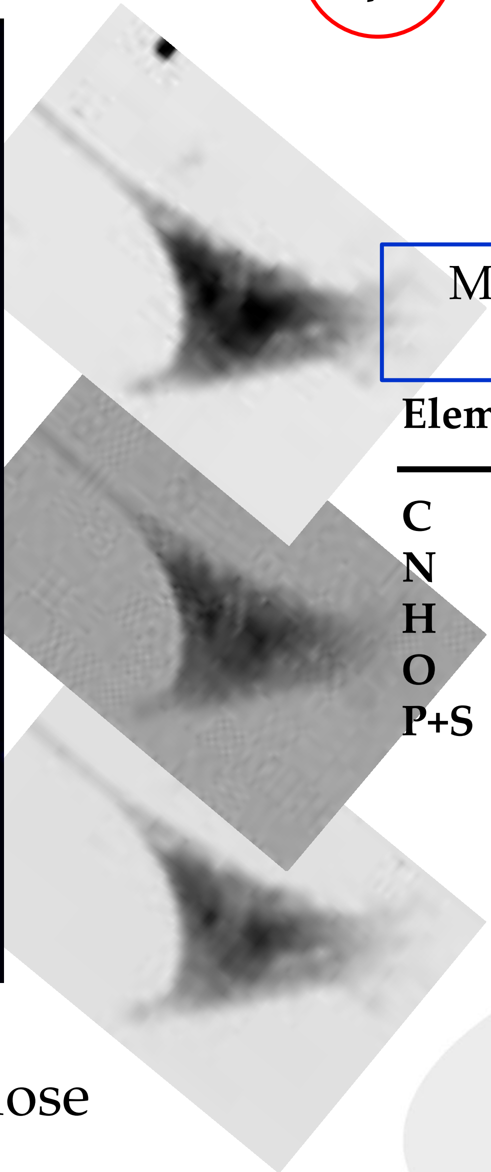
- # Soft X-ray Radiation Damage

X-ray Microscopy Outcomes

Mass Thickness $$\rho t = -\ln \frac{(I/I_0)}{\mu^*}$$

Mass absorption coefficient



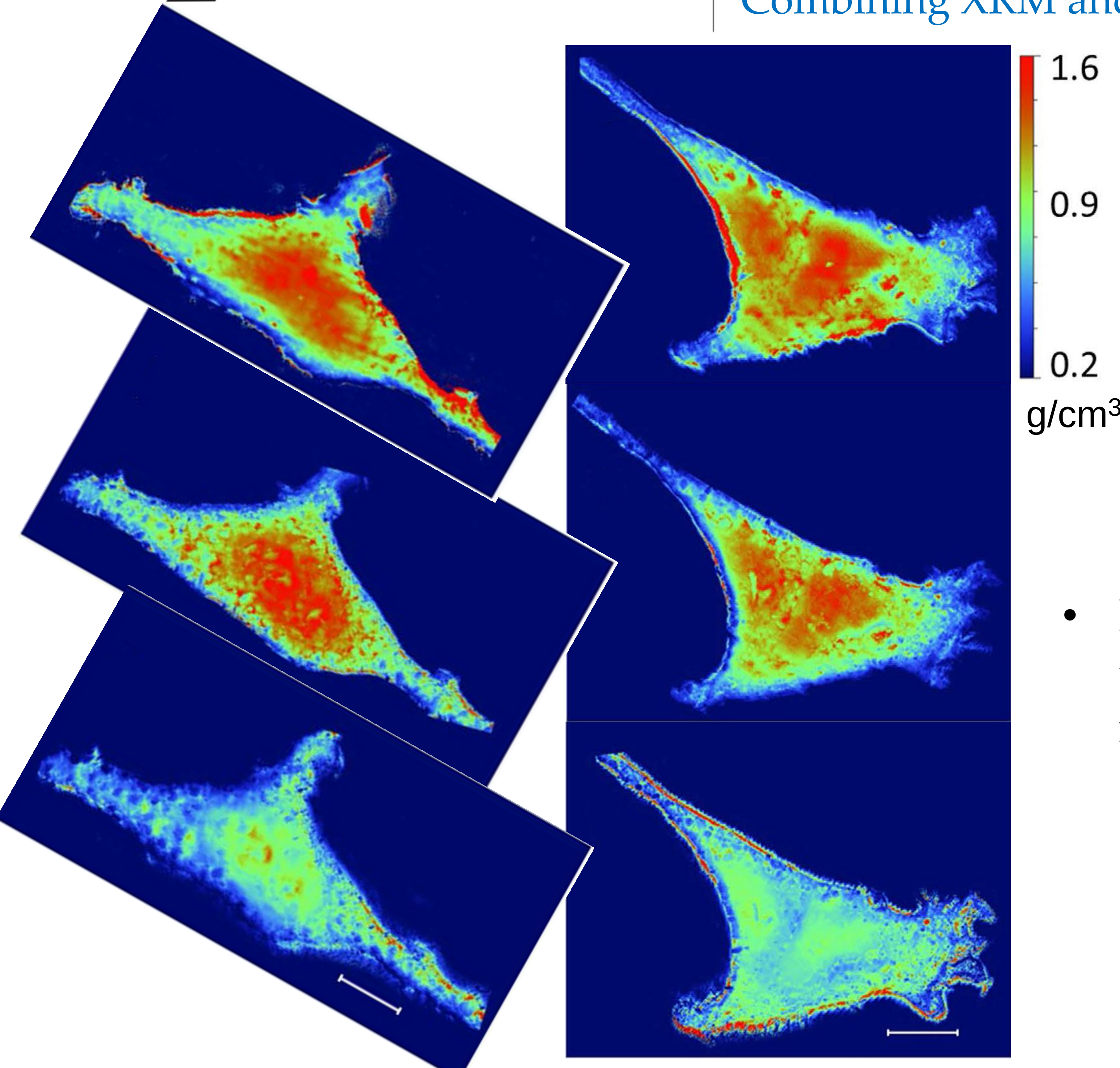


Element	Mass fraction
C	0.5
N	0.16
H	0.07
O	0.25
P+S	0.02

- Mass Thickness decreases with increasing dose

• Soft X-ray Radiation Damage

Combining XRM and AFM



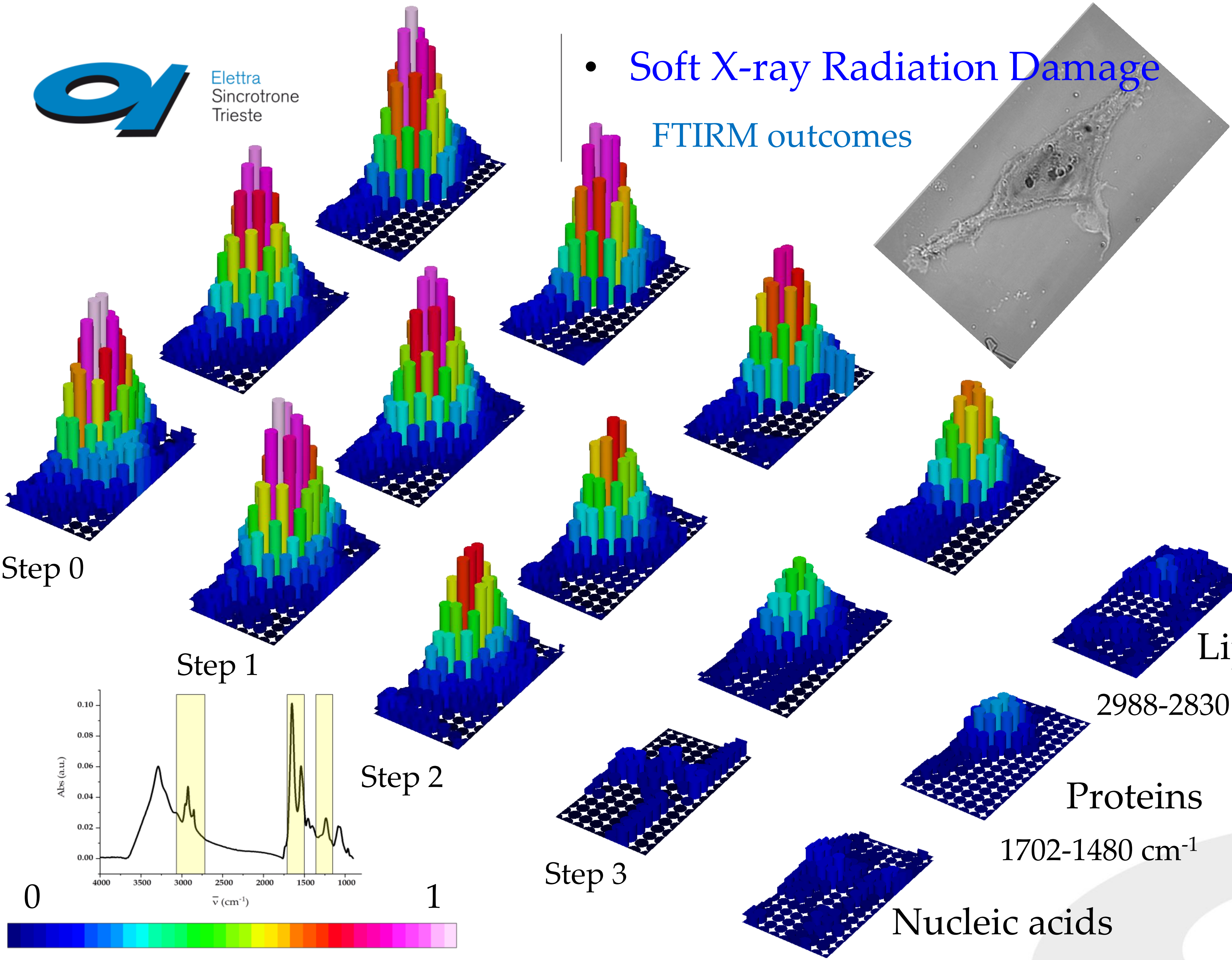
XRM cell images normalized over AFM cell thickness

$$\rho = -\ln \frac{(I/I_0)}{\mu^* t}$$

- Progressive reduction of the cell density with increasing X-ray dose

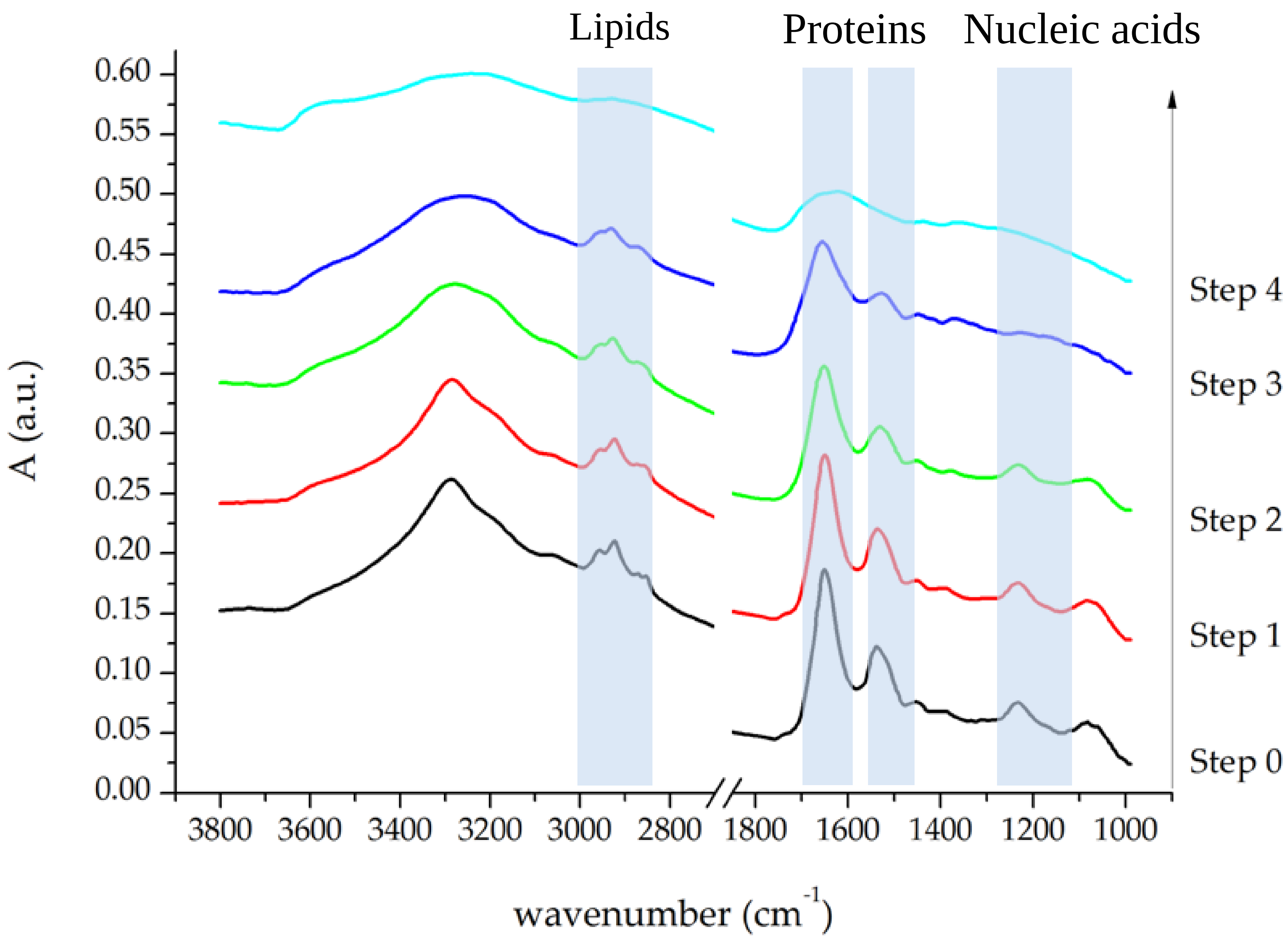
- Soft X-ray Radiation Damage

FTIRM outcomes



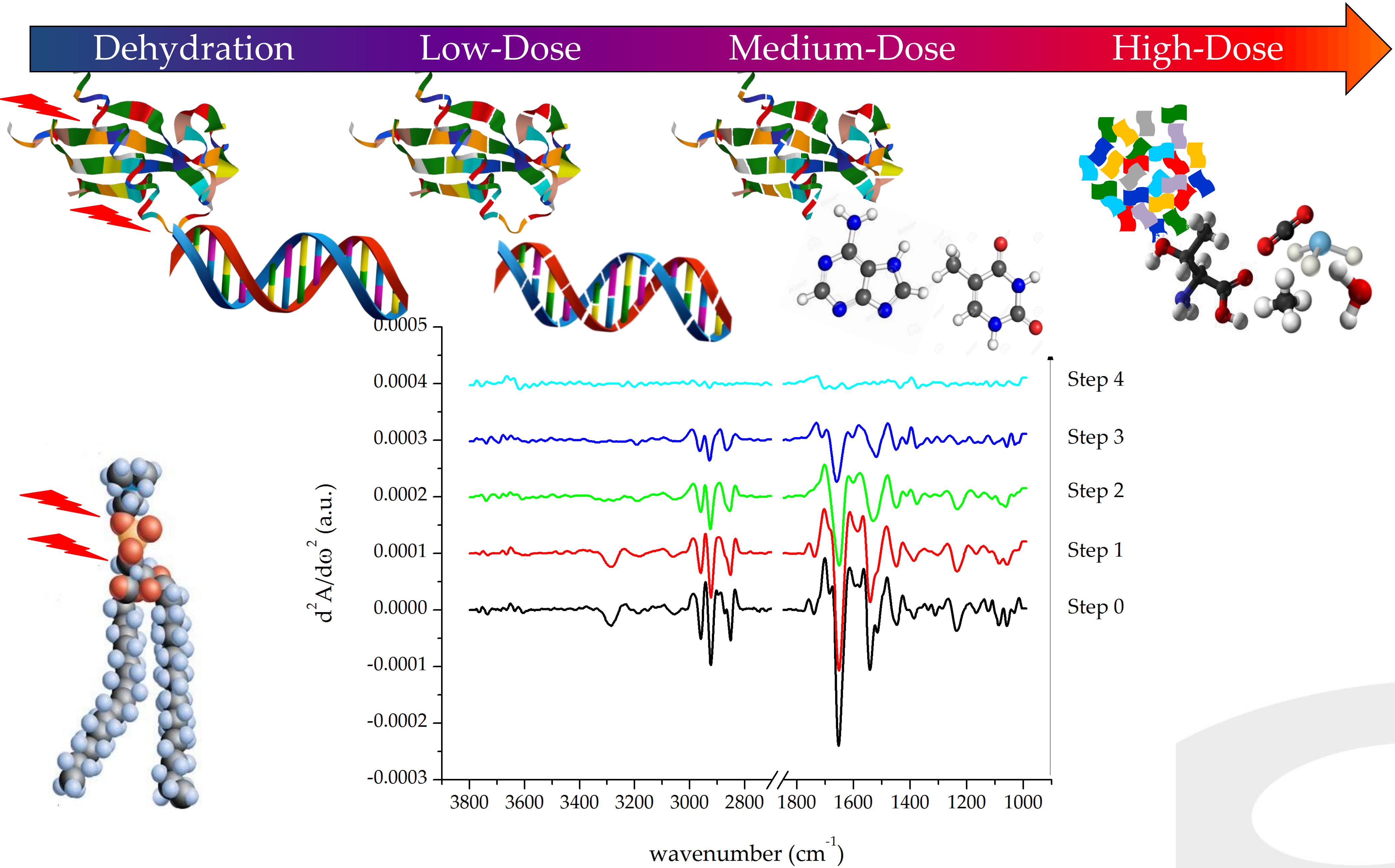
- Soft X-ray Radiation Damage

FTIRM outcomes



- Soft X-ray Radiation Damage

FTIRM outcomes



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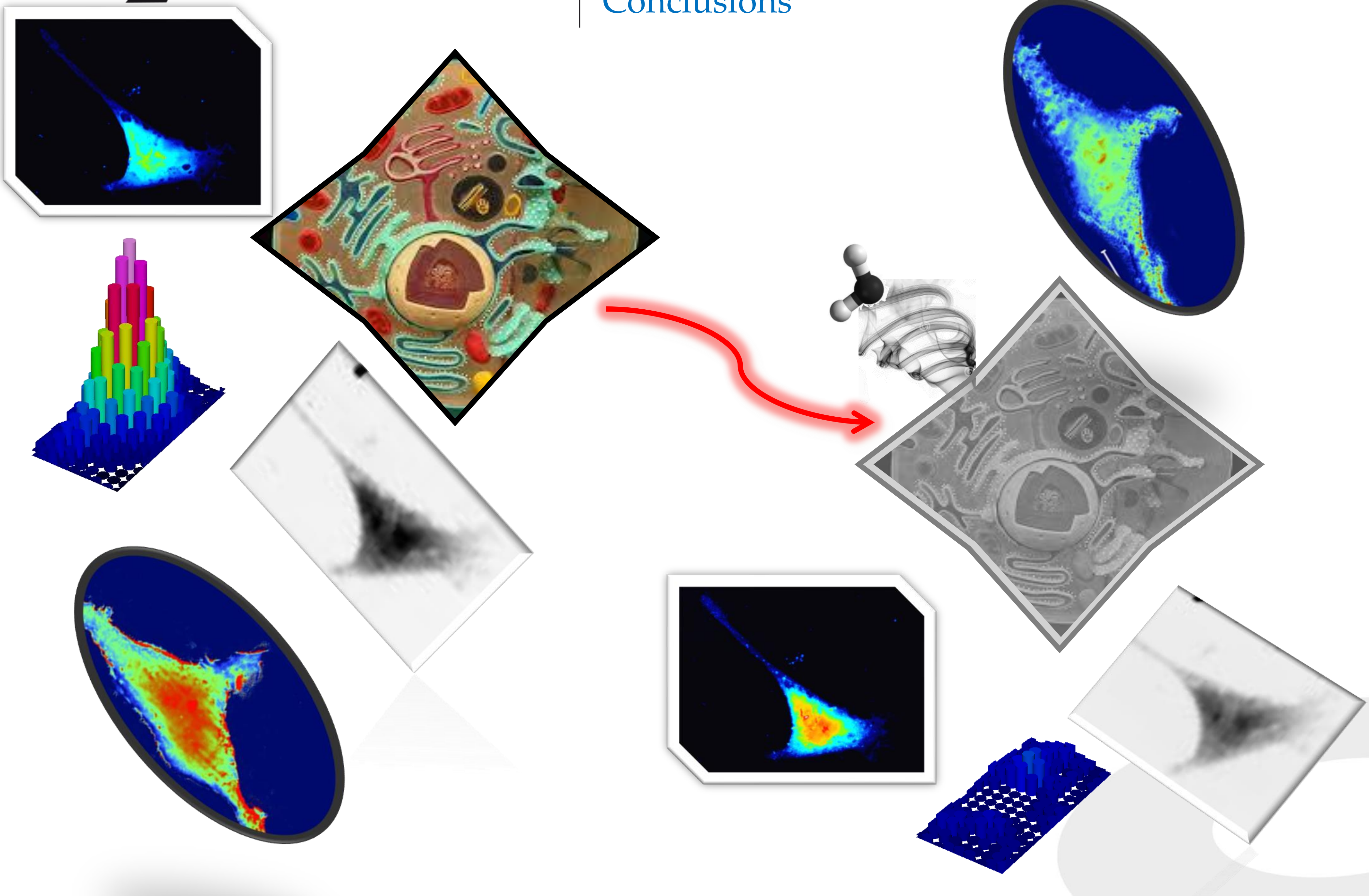
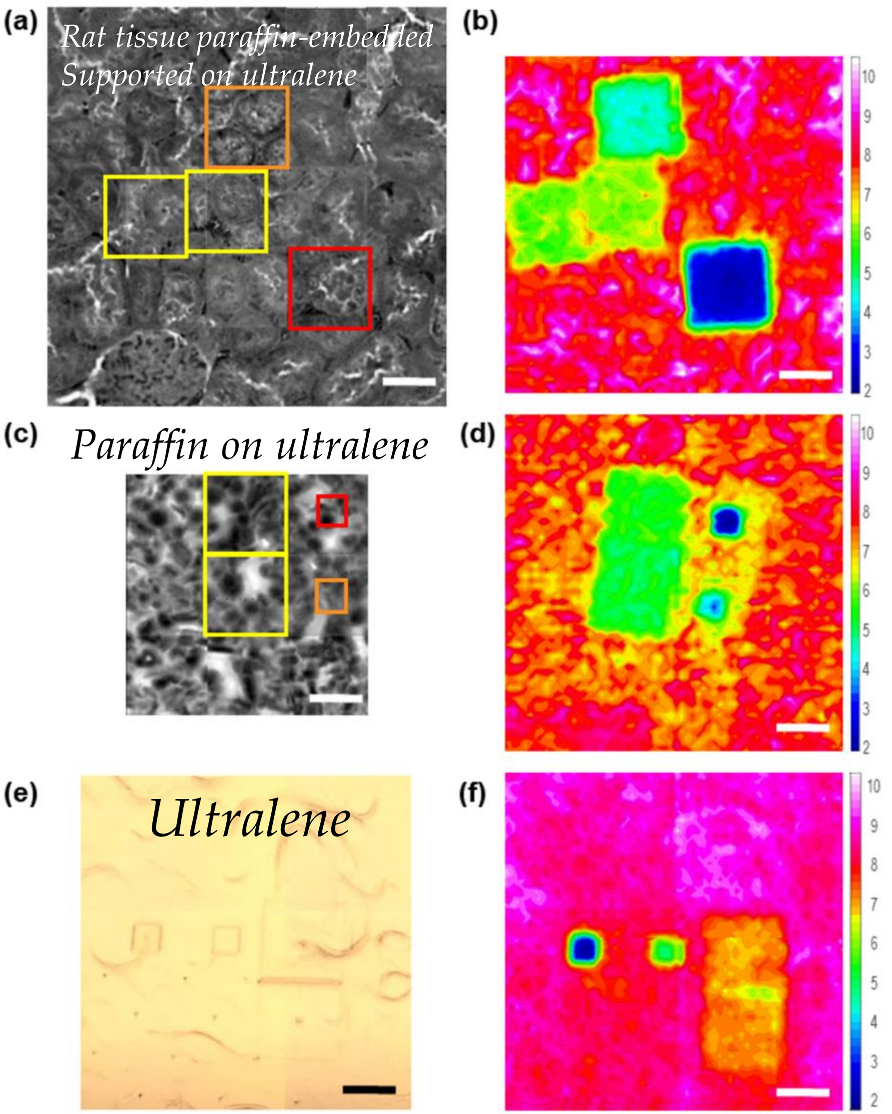
- Soft X-ray Radiation Damage

Conclusions



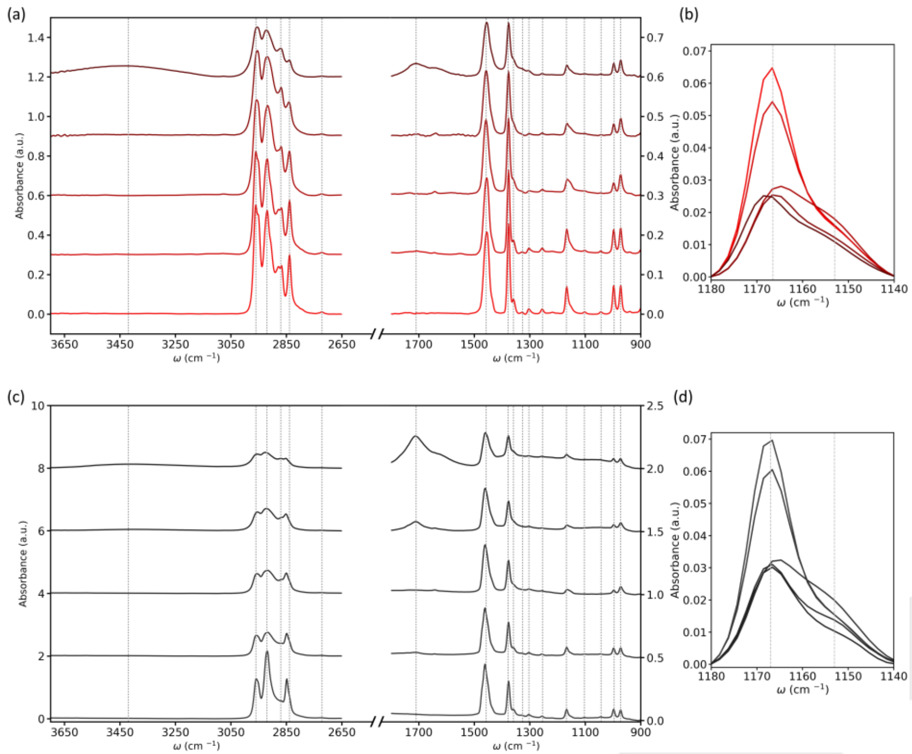
• Soft X-ray Radiation Damage

The role of substrate and embedding media



Polymeric substrates, such as ultralene, and embedding media, such as paraffin, degrade under X-ray exposure.

X-ray effects on bio-matter MUST be decoupled from oxidative damage induced on supporting/emedding materials.



D.E. Bedolla, et al. **Effects of soft X-ray radiation damage on paraffin-embedded rat tissues supported on ultralene: a chemical perspective.** *Journal of Synchroyron Radiation* **2018** 25, 1-9

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Infrared bio-spectroscopy
From macro to nanoscale on the molecules of Life

SR Collective Enhanced IR Absorption microscopy for protein conformational studies

School on Synchrotron
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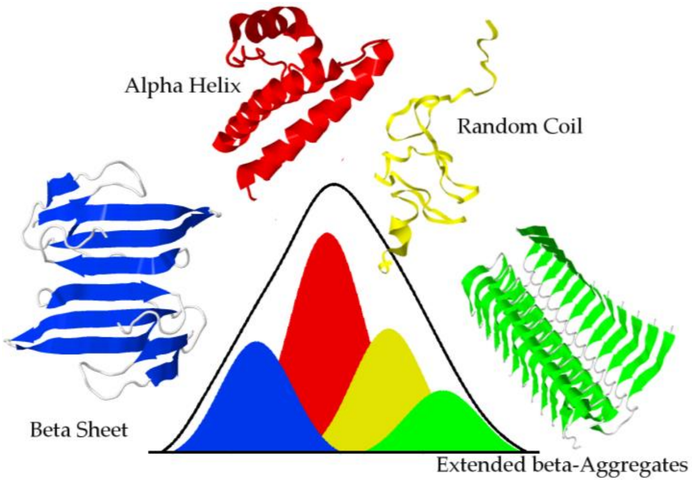
ICTP

How to defeat the detection limit



FTIR spectroscopy is largely employed for protein conformational studies

The vibrational approach can be very useful for proteins that are not crystallizable or available in limited quantities, as usually protein of biological relevance are.

Moreover, the possibility to detect protein conformation and conformational variations in liquid environment can provide data of outstanding biological relevance.

The major problem is represented by the detection limit of FTIR spectroscopy for the investigation of protein in solutions, that sets in the low mM range

More sensitivity is required in order to investigate protein conformation in liquid environment at concentrations biologically relevant (in the nM range)

Plasmonic help us!

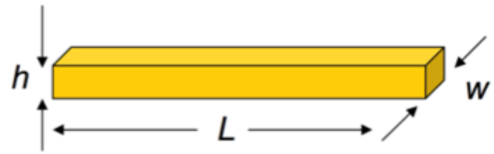
Plasmonic structures are metallic/semiconducor patterned structures that relies on Surface Plasmons.
Surface Plasmons are coupled oscillations that arise from the interaction between light and the conduction electrons in a metal or semiconductor.
A surface plasmon can effectively squeeze light into tiny, sub-wavelength volumes. Within these volumes, the optical fields can be strongly enhanced--well beyond that of the incident wave used to create the excitation--effectively magnifying the light-matter interaction.

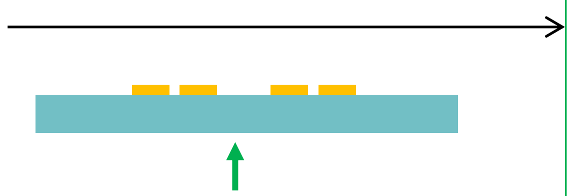
The detection limit of the technique is improved of several orders of magnitude!

How to defeat the detection limit

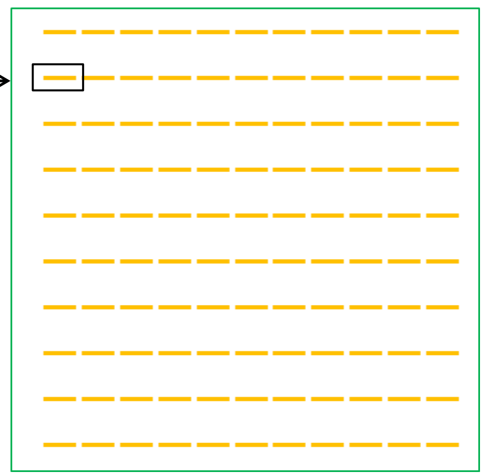
Surface-Enhanced InfraRed Absorption (SEIRA)

Collective Enhancement InfraRed Absorption (CEIRA)





CaF_2 windows



h=w=100nm

L=1900 nm

+

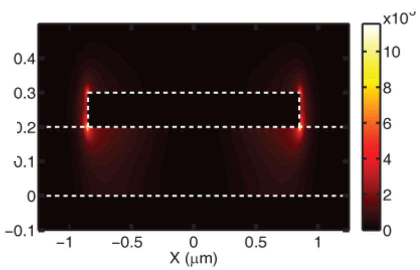
Nanontenna gap=50nm

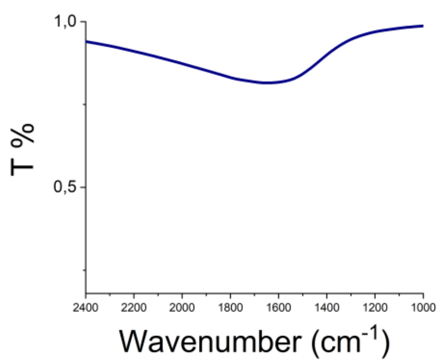
Linear array gap=5mm

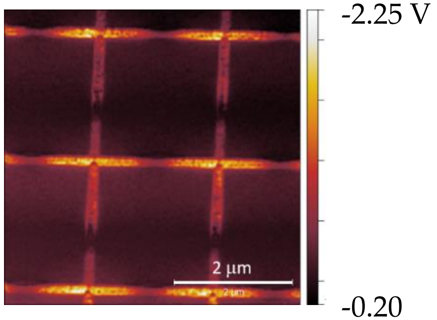
CEIRA devices design, simulation and fabrication developed in collaboration Dr. Andrea Toma of IIT in Genova

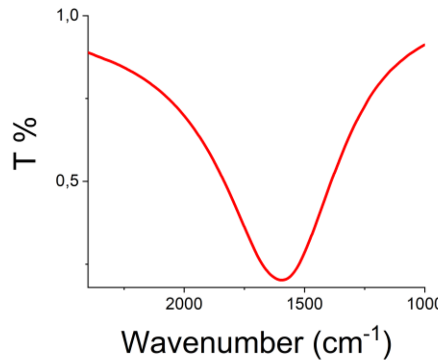
Single nanoantenna Signal enhncement ~10^3

CEIRA substrate Signal enhncement ~10^5

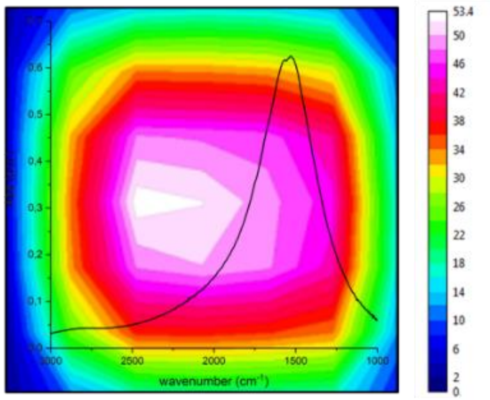


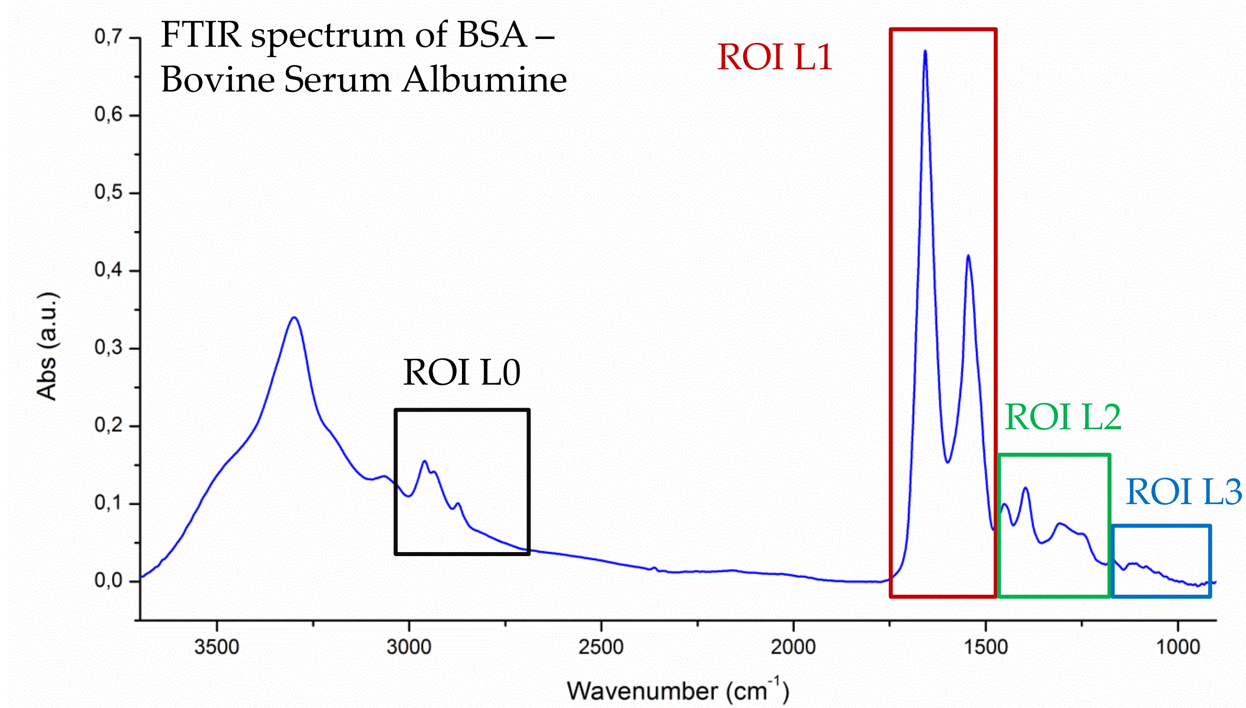


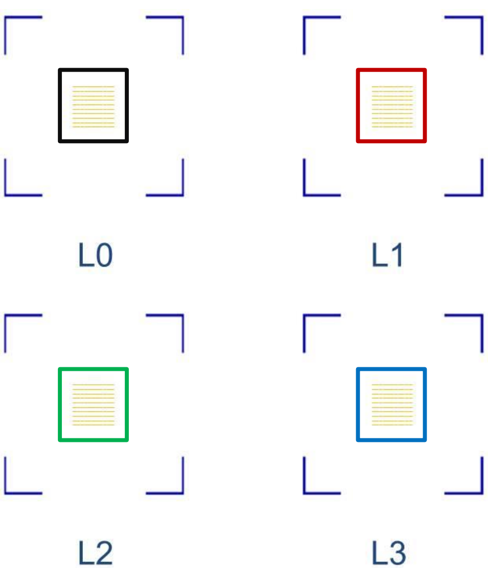


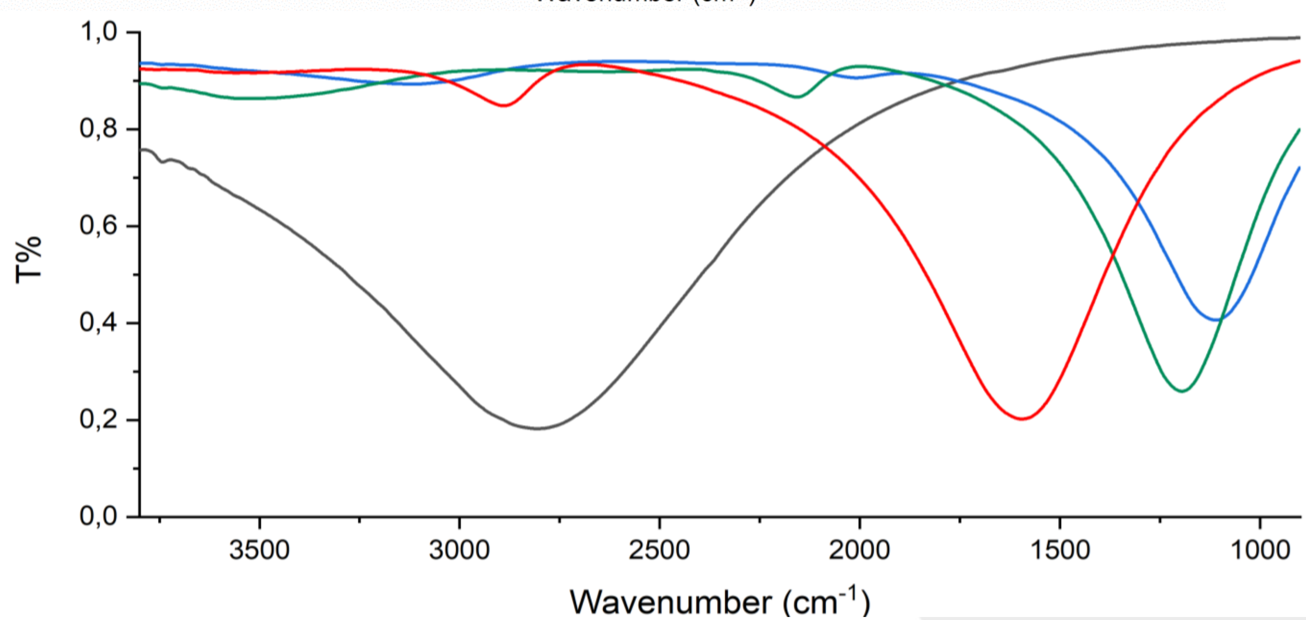


How to defeat the detection limit



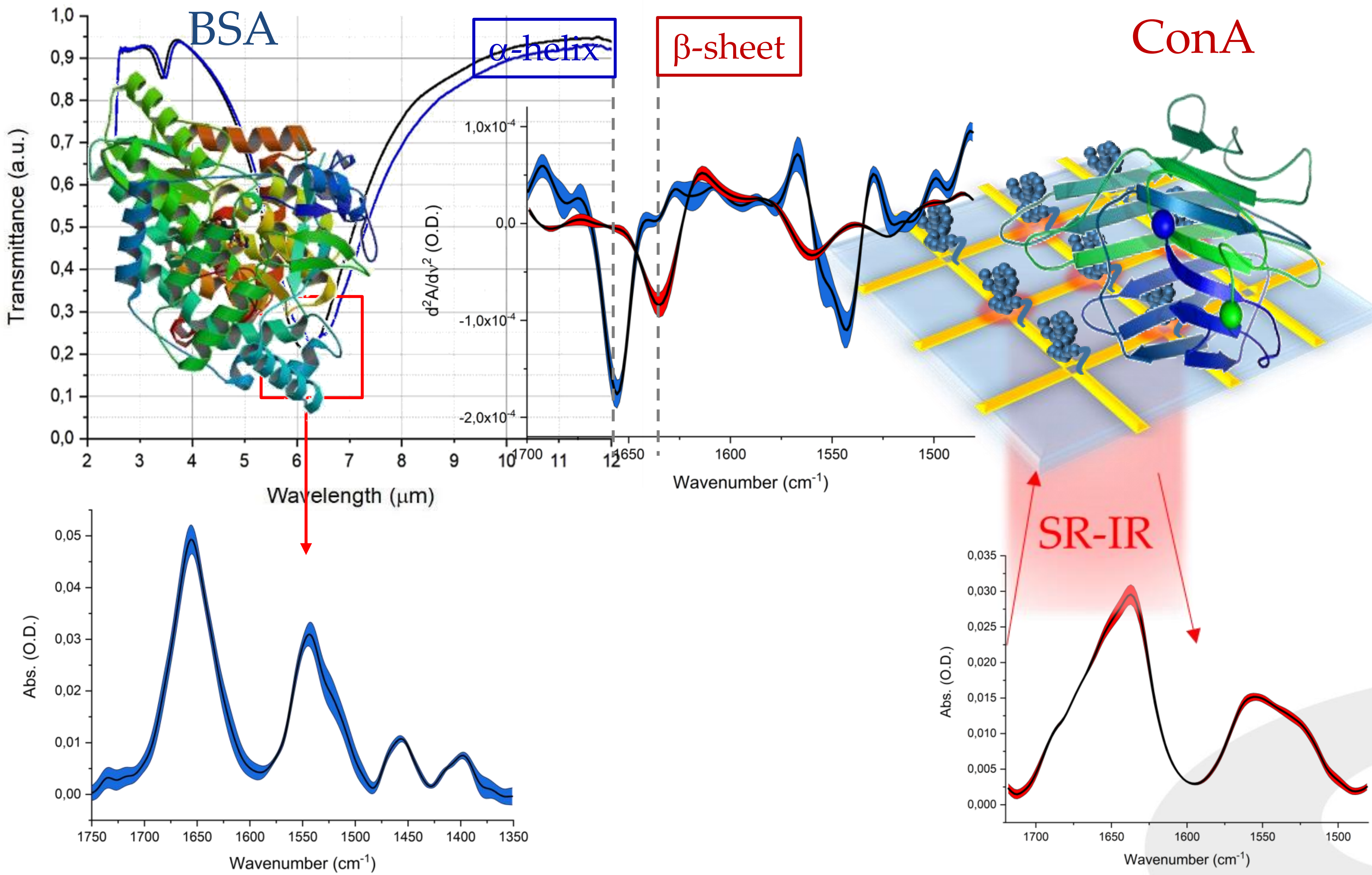






How to defeat the detection limit

Resonance response L1
Resonance response L1+BSA



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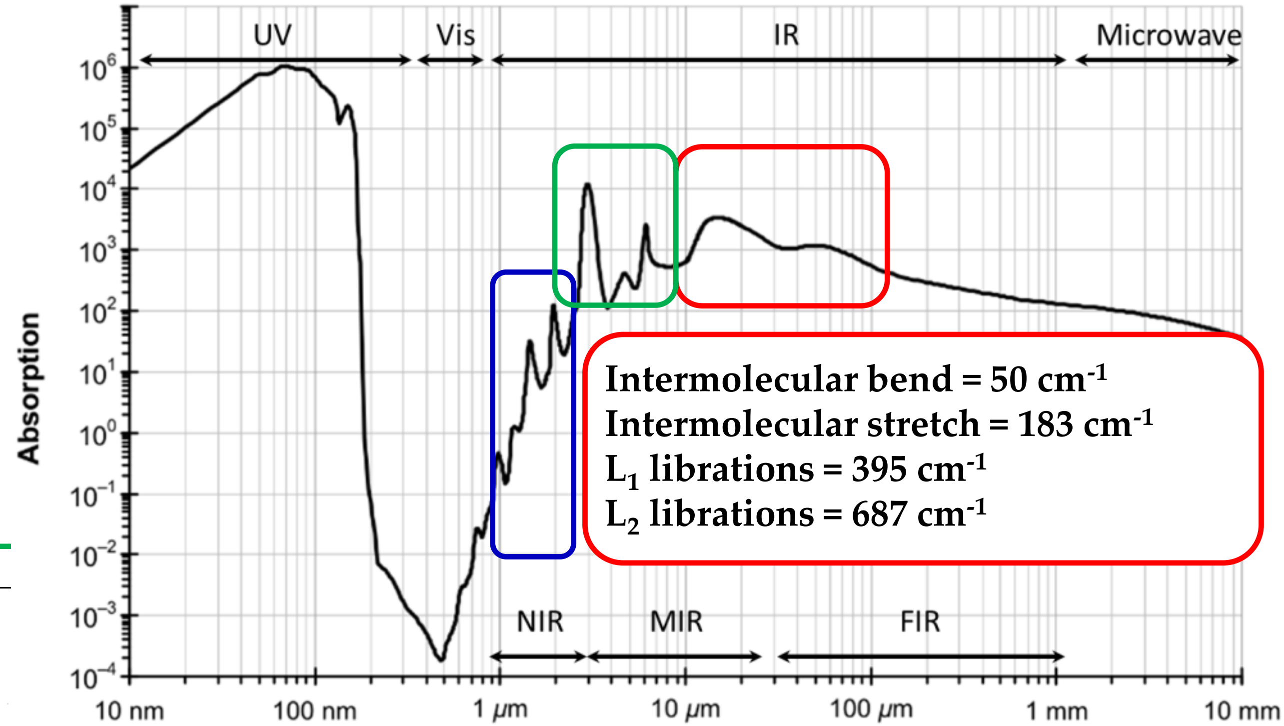
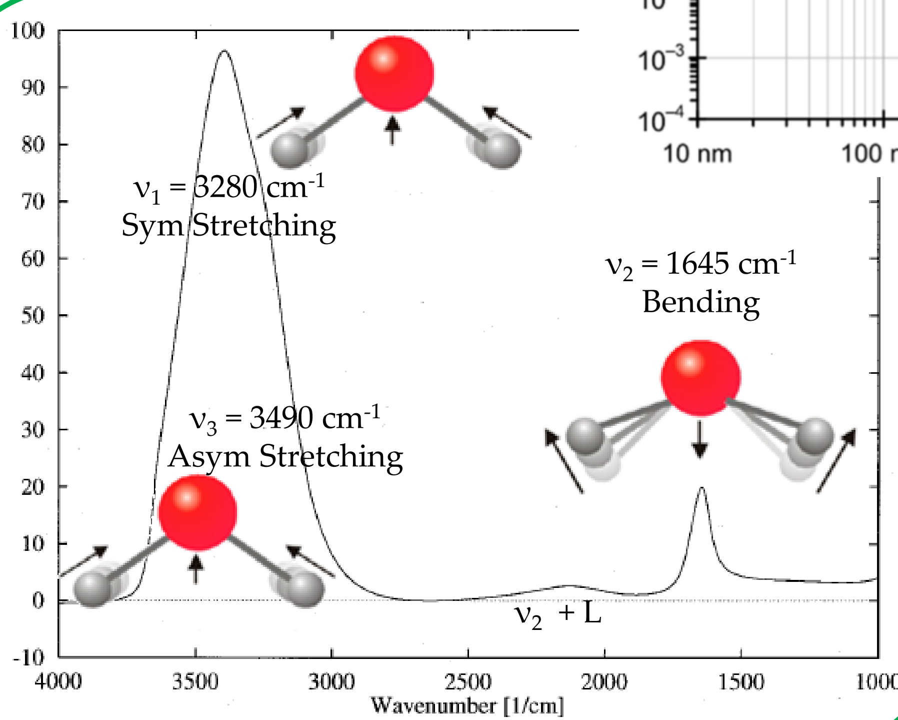
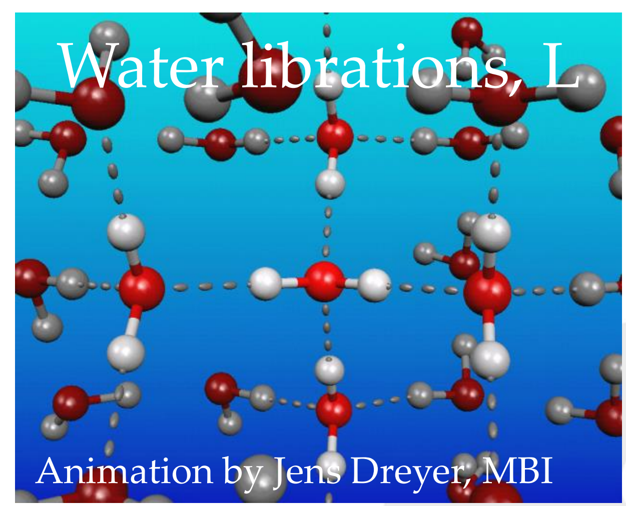
How to defeat the water limit

Vibrational Spectrum of liquid water

Overtones and combination bands



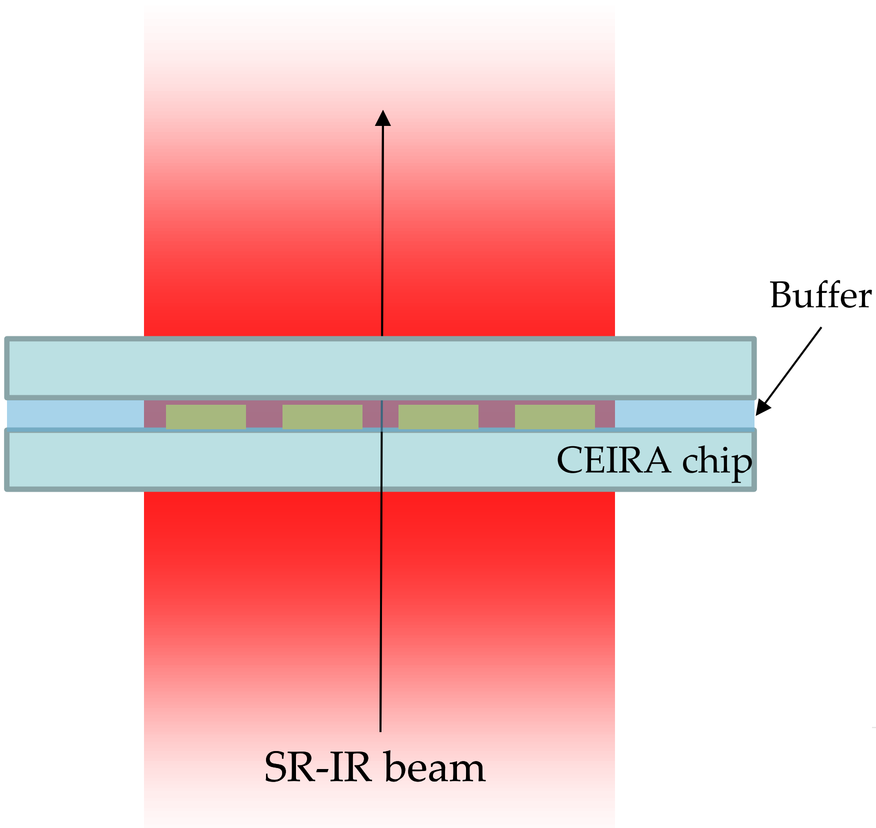




How to defeat the water limit

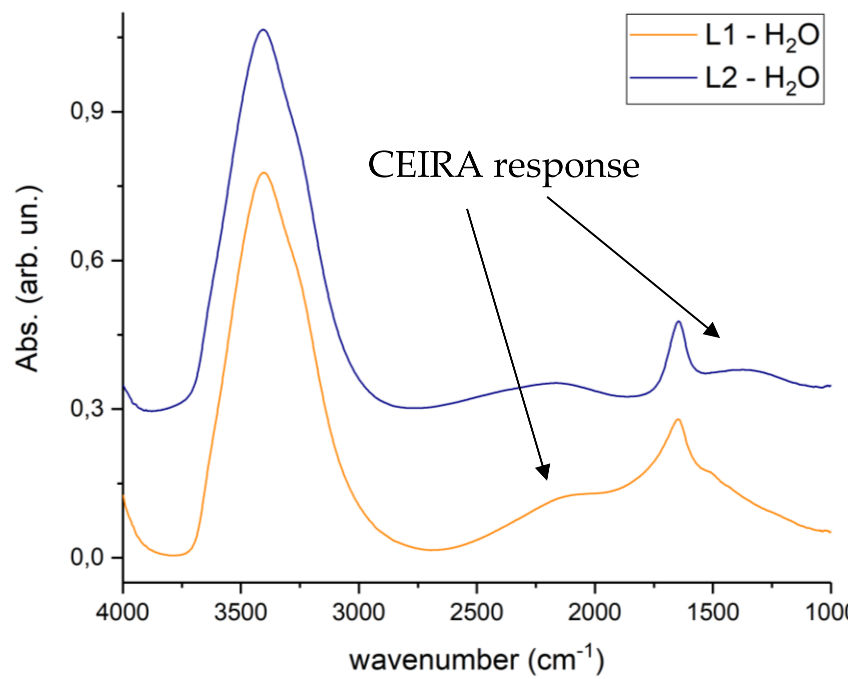
CEIRA measurements in transmission mode in water condition

in dry condition



CEIRA substrate response in H_2O

CEIRA substrate response in dry condition



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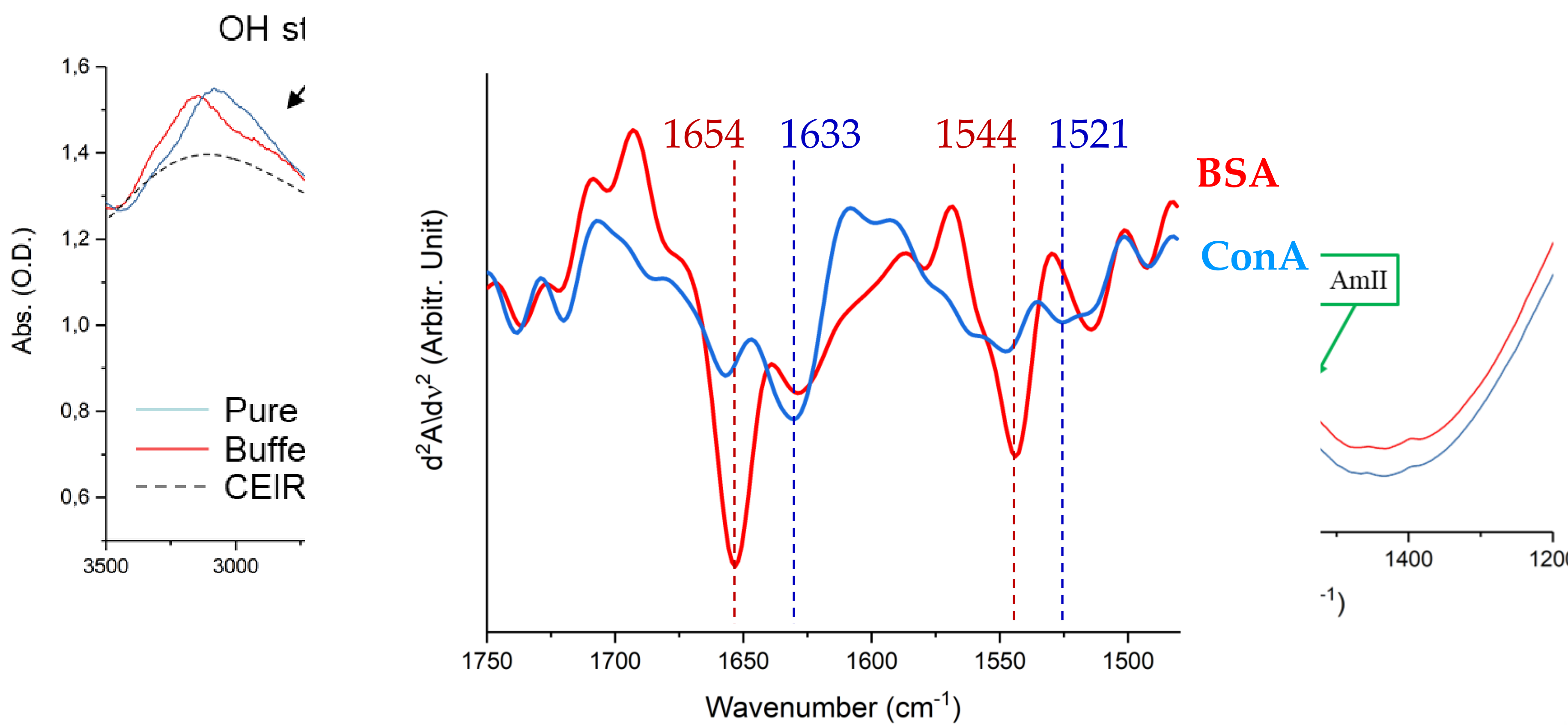
How to defeat the water limit

Plasmonic Internal Reflection (PIR)



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

Elettra
Sincrotrone
Trieste

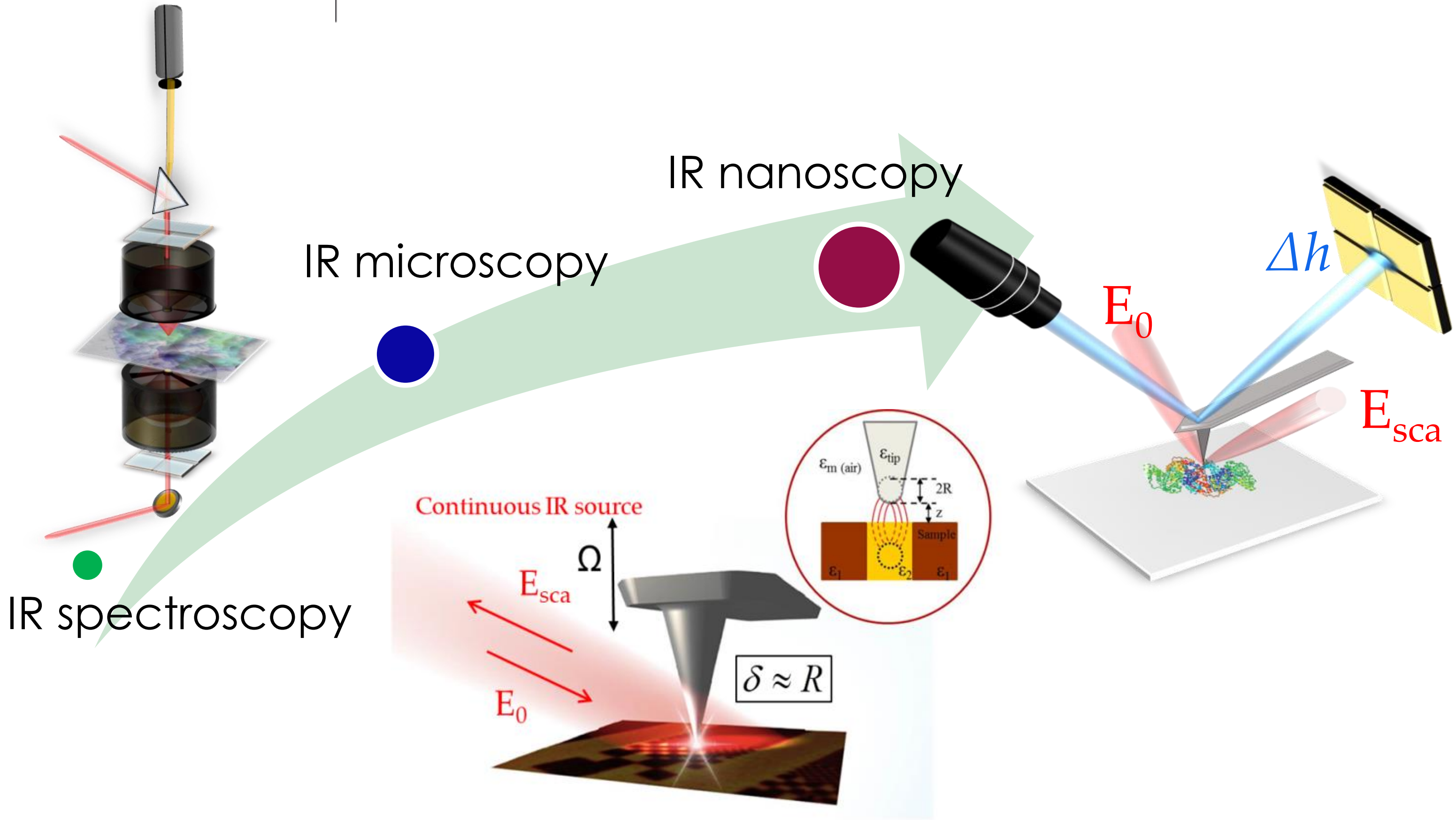
Infrared bio-spectroscopy
From macro to nanoscale on the molecules of Life

Vibrational spectroscopy at the nanoscale

School on Synchrotron
and Free-Electron-Laser
Methods for Multidisciplinary
Applications

ICTP

Vibrational characterization at the nanoscale



IR nanospectroscopy allows ***non-damaging vibro-electronic nano-resolved characterization*** *of materials with a lateral resolution wavelength independent, namely limited by the tip-size.*

IR nanospectroscopy opens a unique opportunity for ***covering the gap in lateral resolution*** *existing today between X-ray and IR microscopies, opening unique prospects for* ***understanding the complexity of matter at nano- micro- and macro- scale****, for both condensed matter and bio-oriented researches.*

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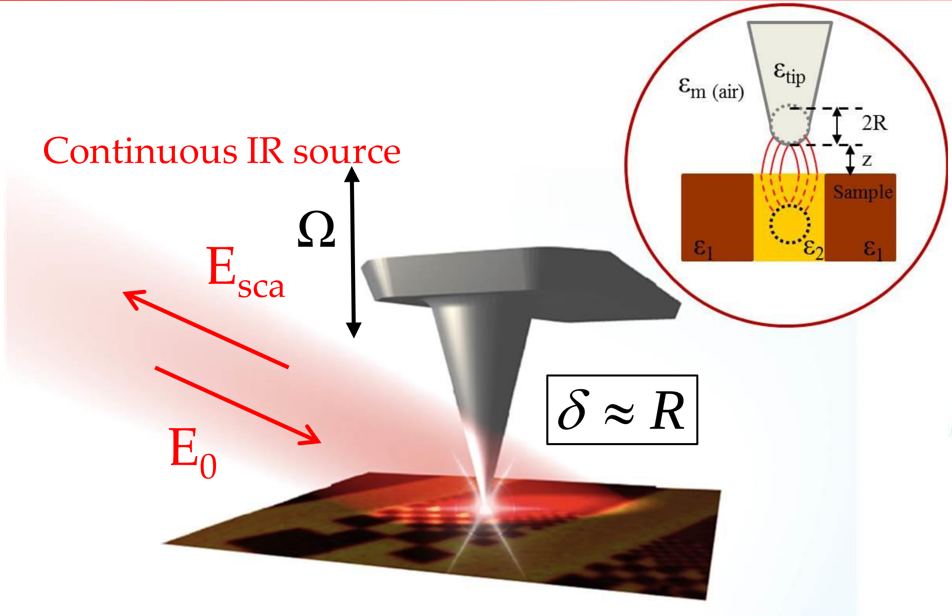
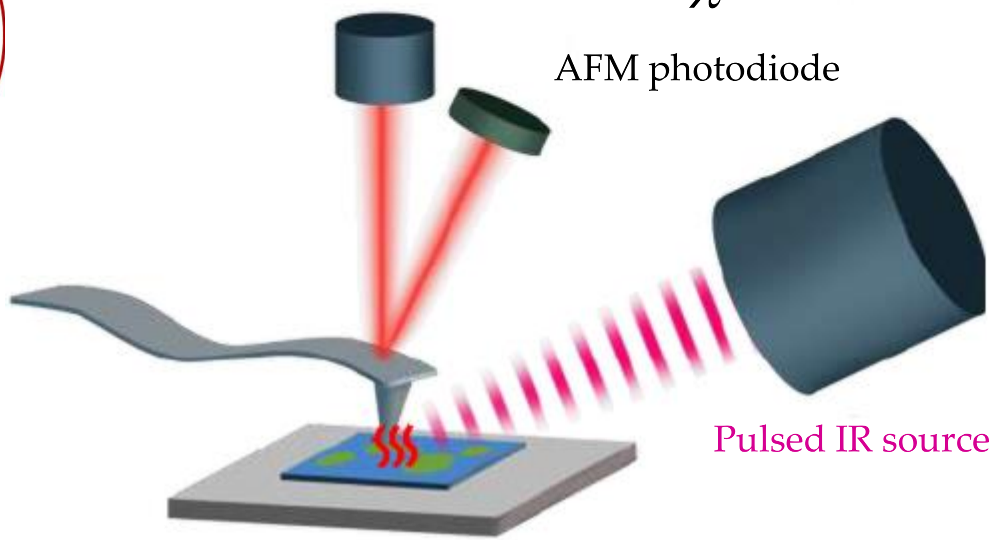
Near-field infrared Methods

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

Photo-Thermal Expansion: PTE



$$\Delta h \propto T_{max} \propto Pabs \propto \frac{\mathrm{Im}(n)}{\lambda} \propto Abs$$



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

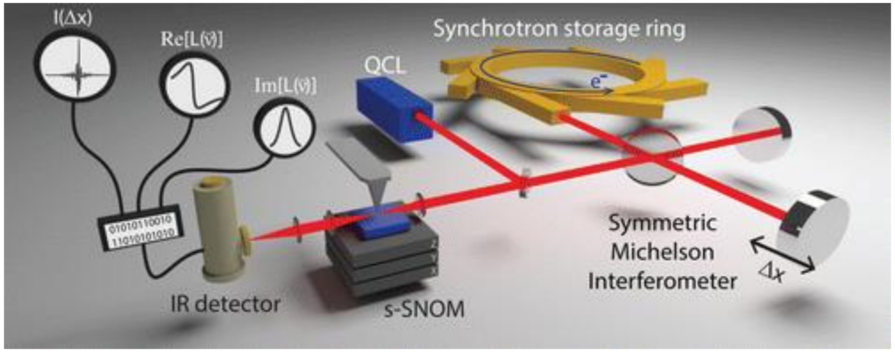
$$\alpha_{eff} = \alpha_{eff}(\varepsilon_{mdium}, \alpha_{tip}, R, z, \varepsilon_{sample})$$

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

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)

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)

Near-field IRSR programs worldwide

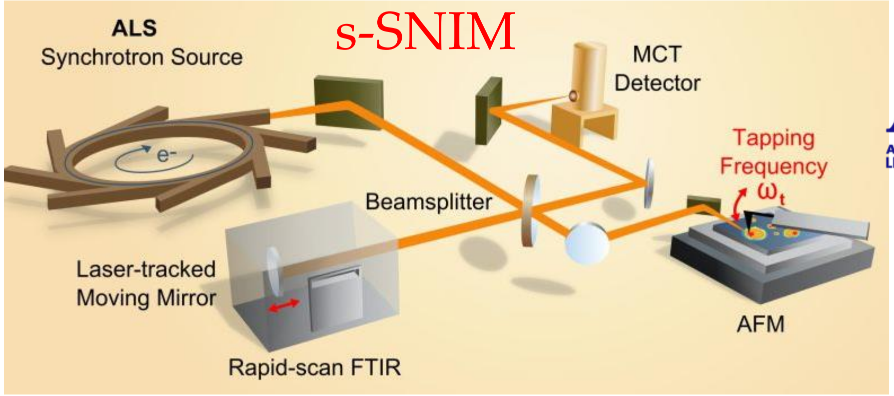


P. Hermann, A. Hoehl, A. Patoka, F. Huth, E. Rühl, and G. Ulm, *Optics Express* 21, 2913 (**2013**)

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

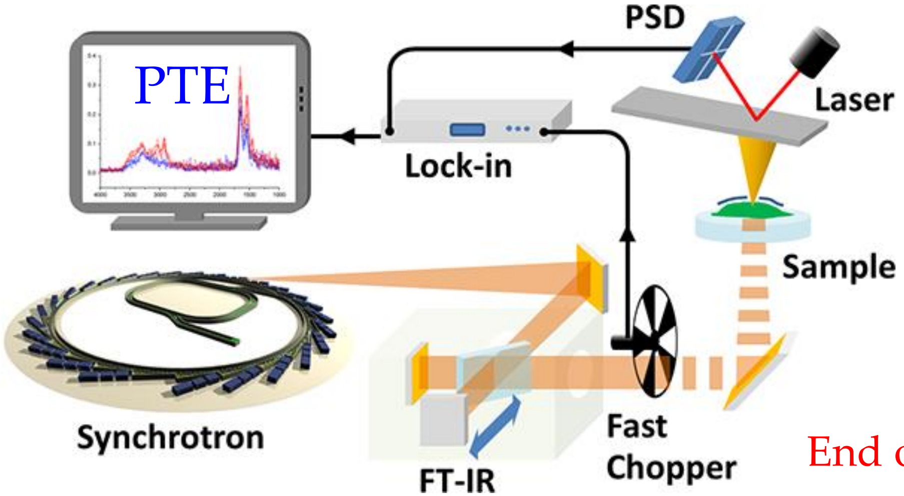
POHANG ACCELERATOR LABORATORY

Shanghai Synchrotron Radiation Facility

Projects just started

SOLEIL SYNCHROTRON

Commissioning phase



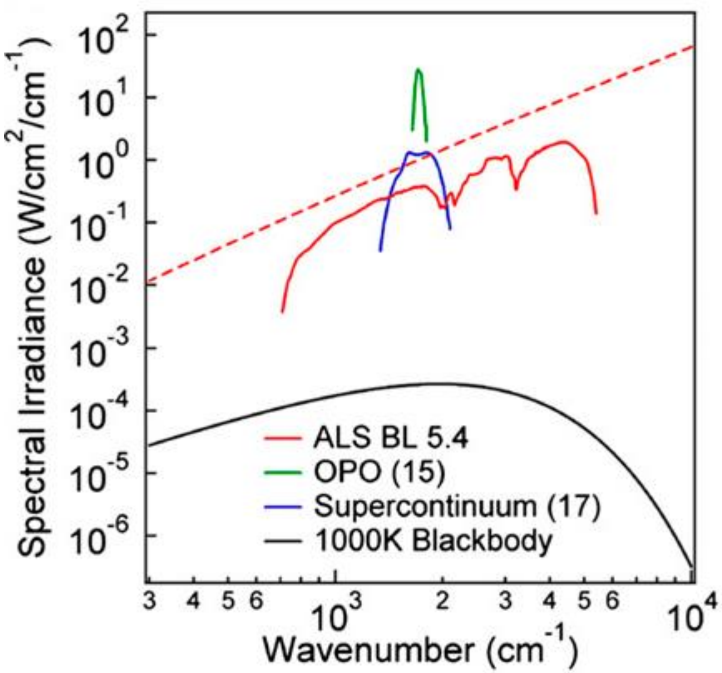
diamond

End of commissioning phase

Paul M. Donaldson, Chris S Kelley, Mark D. Frogley, Jacob Filik, Katia Wehbe, and Gianfelice Cinque, *Optics Express* 24, 1852-864 (**2016**)

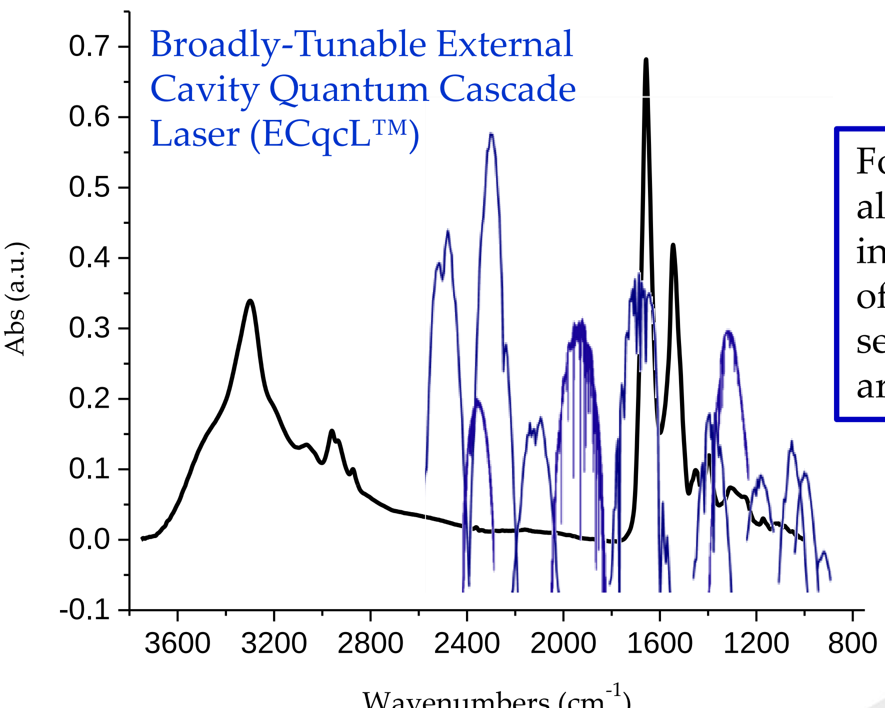
Opportunities offered by IRSR

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



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**)

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



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

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

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

Elettra Sincrotrone Trieste

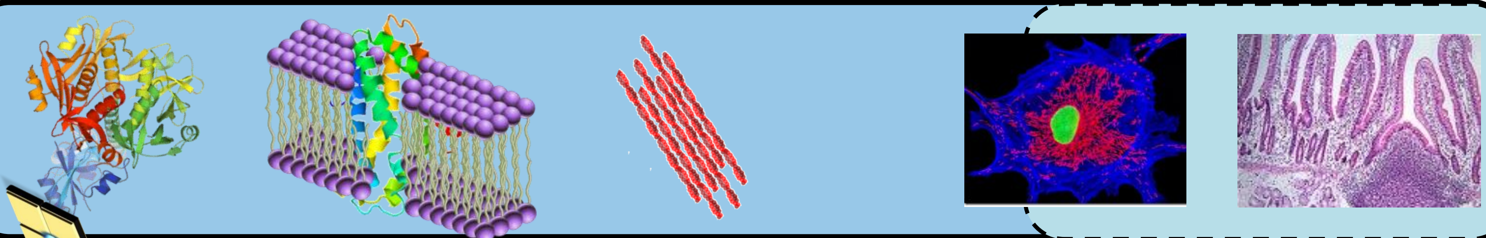
New fields of Life Sciences

Molecular clusters | Large Molecular aggregates | Prokaryotic cells | Eukaryotic cells | Tissue

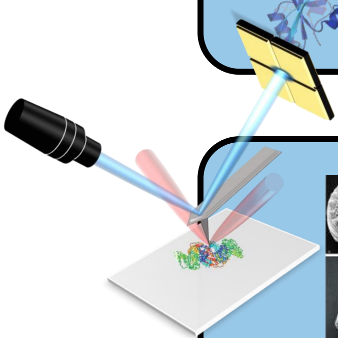
nm — 1μm — 1mm

Future Capabilities | **Present Capabilities**

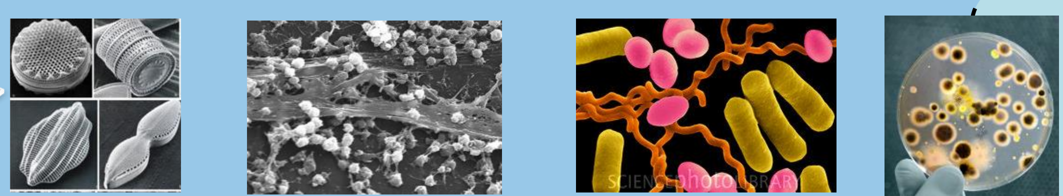
From Protein Science to Cellular and Tissue Biology



Biophysics
Biotechnology
Biology
Biomedicine

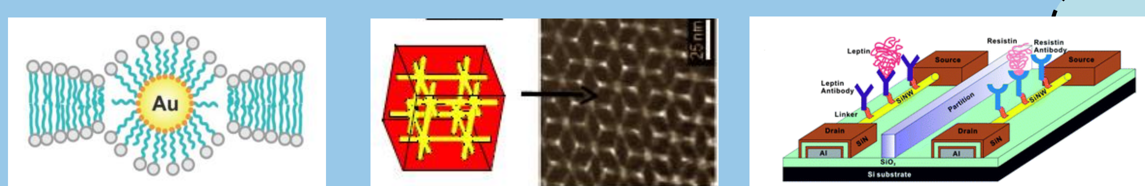


From a Single Organism to Cell Community



Antibiotic Resistance
CO_2 sequestration
Bio-mineralization

Functional chemistry of smart materials



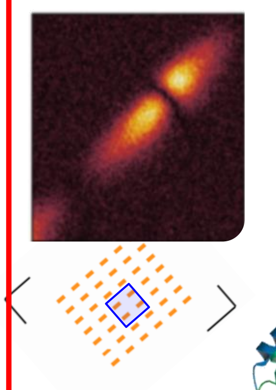
Smart materials for electronics, for energy, medicine, and bio-sensor

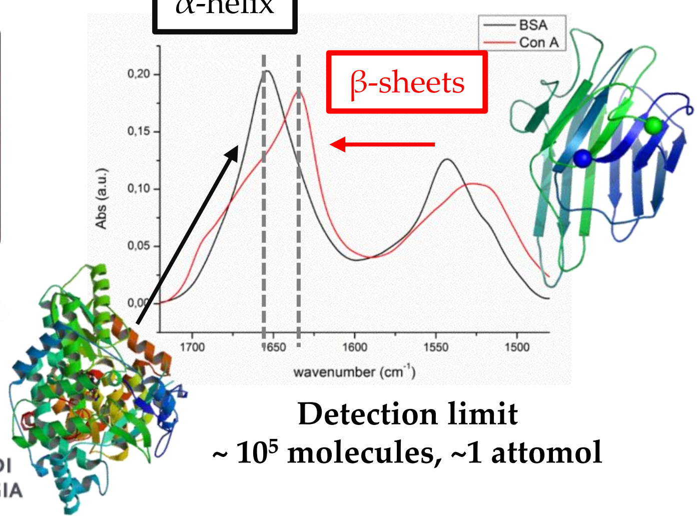
New fields of Life Sciences

Elettra Sincrotrone Trieste

Plasmonic devices for SR-CEIRA microscopy (Collective enhanced IR Absorption)

n-IR signal

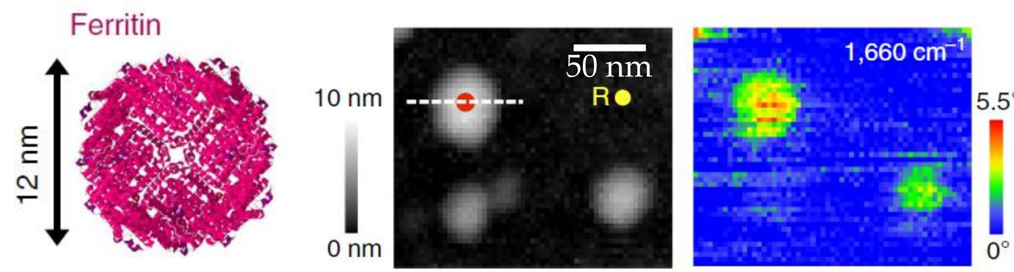




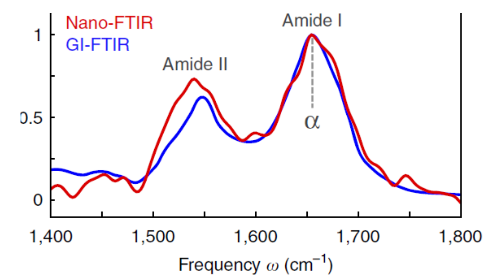
Detection limit
~ 10^5 molecules, ~1 attomol

ISTITUTO ITALIANO DI TECNOLOGIA

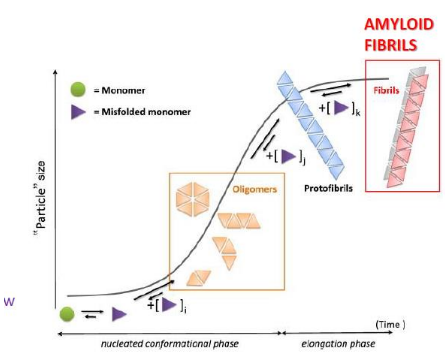
Structural Analysis of individual protein complexes by n-IR

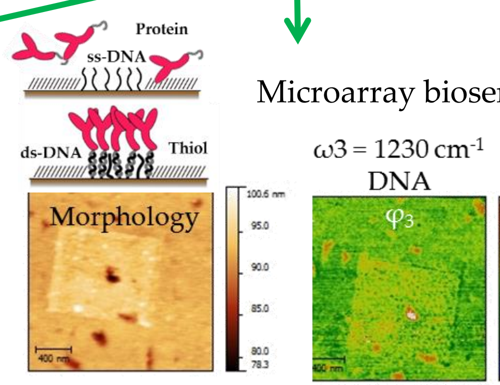


Iban Amenabar et. al, Nature Communications 4, Article number: 2890 (**2013**)



Amyloidogenic disorders

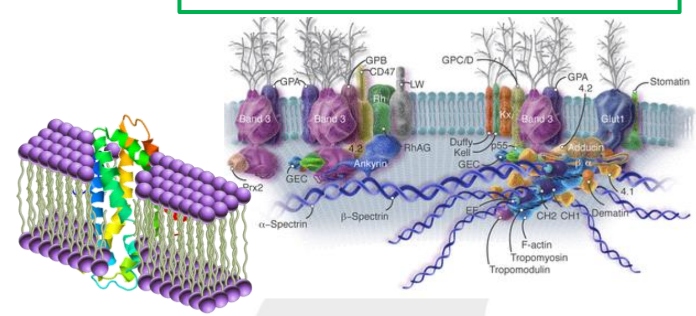




Microarray biosensor

Biophysics, biotechnology, Thin films

Protein membranes



Transmembrane protein models

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Thank you for your attention

www.elettra.eu