Elettra
Sincrotrone
Trieste

Infrared spectroscopy with synchrotron and FEL radiation

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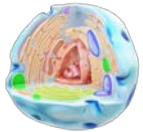
"Gilberto Vlaic" XVII School on Synchrotron Radiation: Fundamentals, Methods and Applications

Muggia (Italy), 16 - 26 September 2024

SILS Società Italiana Luce di Sincrotrone

Outlook

- ✓ Pills of IR spectroscopy
  - IRSR properties and instrumentation: from macro to nanoscale



- ✓ IRSR Cytology and Histology
  - Soft X-ray radiation damage

- ✓ Biochemistry and Biophysics with IR light
  - Plasmon enhanced IR microscopy
  - Halosite Nanotubes for drug delivery

- ✓ Polymers and soft matter
  - Polyurethane – Fibroin hybrid meshes for tissue engineering

IR spectroscopy

IR spectroscopy is a vibrational spectroscopy method for the characterization of any type of material

It enables label-free and non-damaging extraction of chemical information on (bio-macro)-molecules on diverse class of samples and at different spatial resolution from their roto-vibrational profile.

An IR spectrum contains info on the sample composition (**chemical identity**), and finer details such as molecular order or molecular structure, molecular network, etc…(**holistic identity**) depending on the sample under investigation and on the considered IR spectral domain

IR spectroscopy

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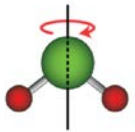
Microwaves | THz | FIR | MIR | NIR | UV

[m] 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}

[Hz] 10^{10} 10^{11} 10^{12} 10^{13} 10^{15} 10^{16}

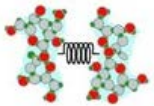
Intramolecular vibrations

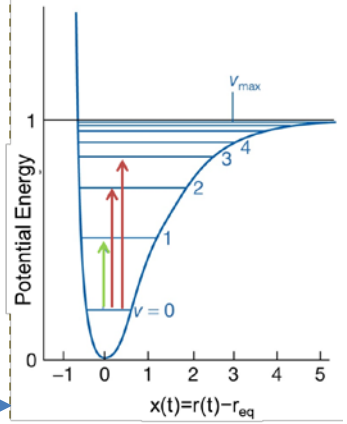
Low-frequency molecular vibrations



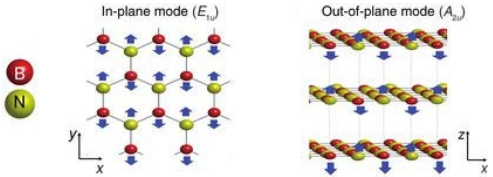
Rotation modes (gas molecules)

Intermolecular motions





Overtones and combination bands



Crystalline phonons

Chemical and holistic identity

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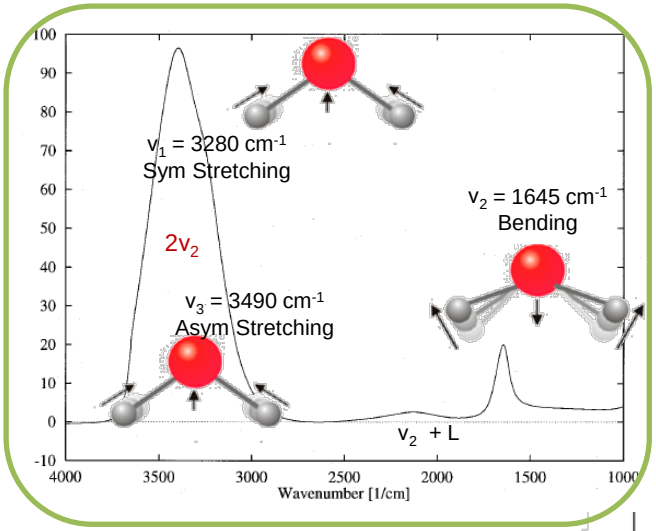

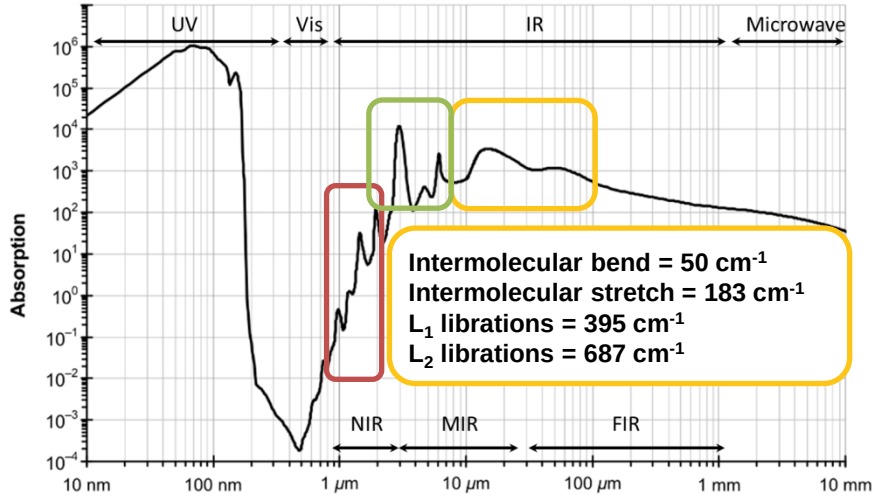

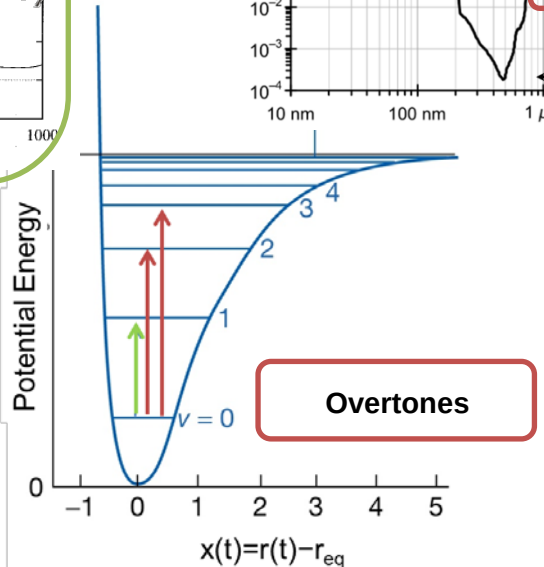

	NIR	MIR		FIR
λ (μm)	0.74	3	30	300
ν (cm^{-1})	~13000	~3333	~333	~33
E (eV)	1.65	0.413	0.041	0.004

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IR spatial domains

SISSI-Bio Instrumentation

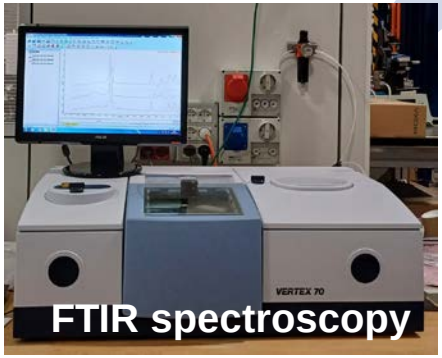
Synchrotron Infrared Source for spectroscopy and Imaging

Nano

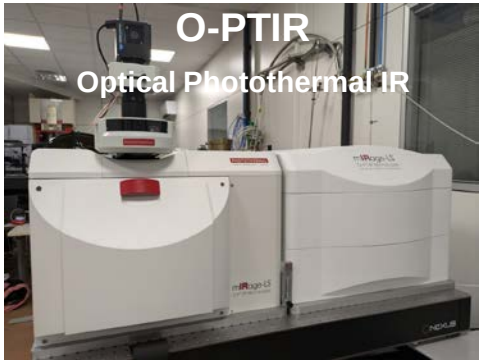
Submicron

Micro

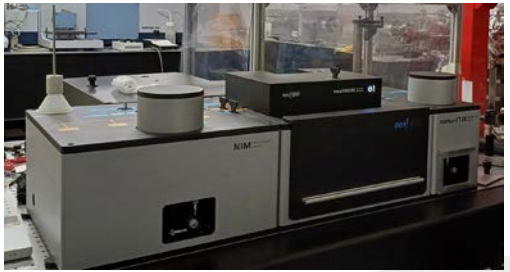
Macro



Far Field microscopy



Near Field microscopy



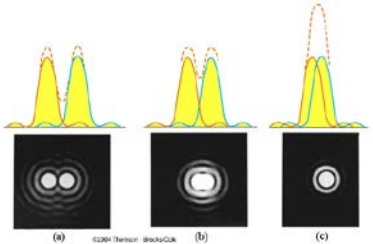
IR s-SNOM

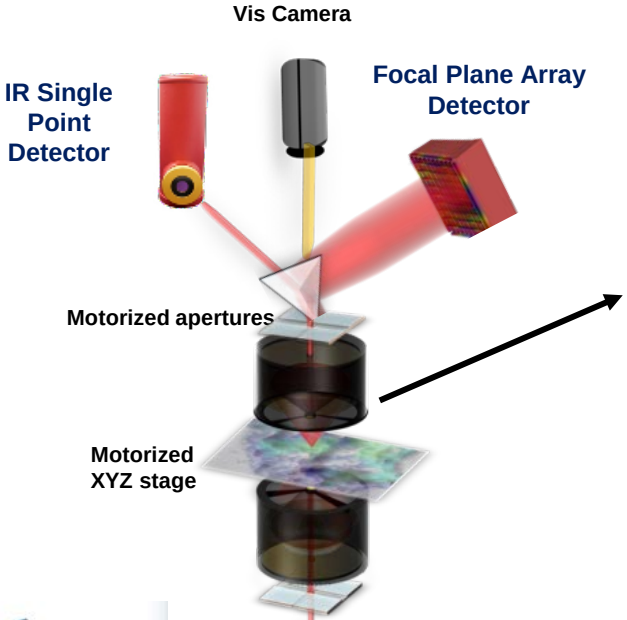
IR scattering-type scanning near-field optical microscopy

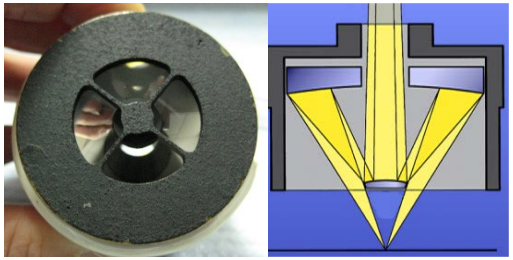
Far Field FTIR microscopy & imaging

The Rayleigh Criterion

$$\delta = 0.61\frac{\lambda}{nNA}$$



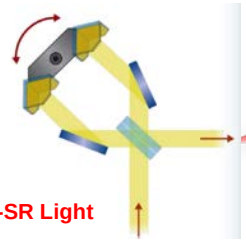




Schwarzschild
or Cassegrain objective
Reflective Achromatic Optics

Fourier Transform Interferometer

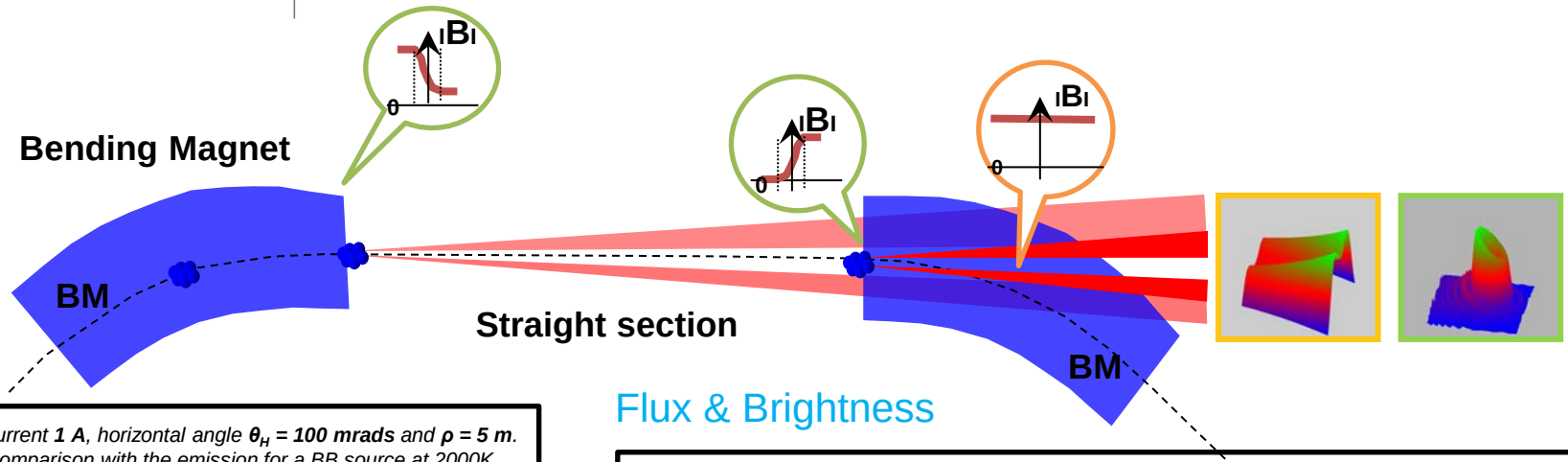
Cube-Corner Mirrors in a ROCKSOLID-Interferometer



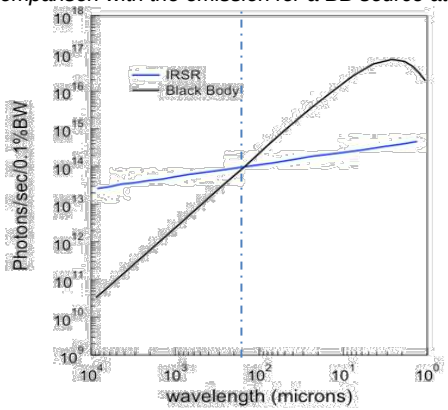
Conventional Source and/or IR-SR Light

NA	Wavelength	δ
0.65	10 μm (1000cm^{-1})	~ 9.5 μm
	2.5 μm (4000cm^{-1})	~ 2.5 μm

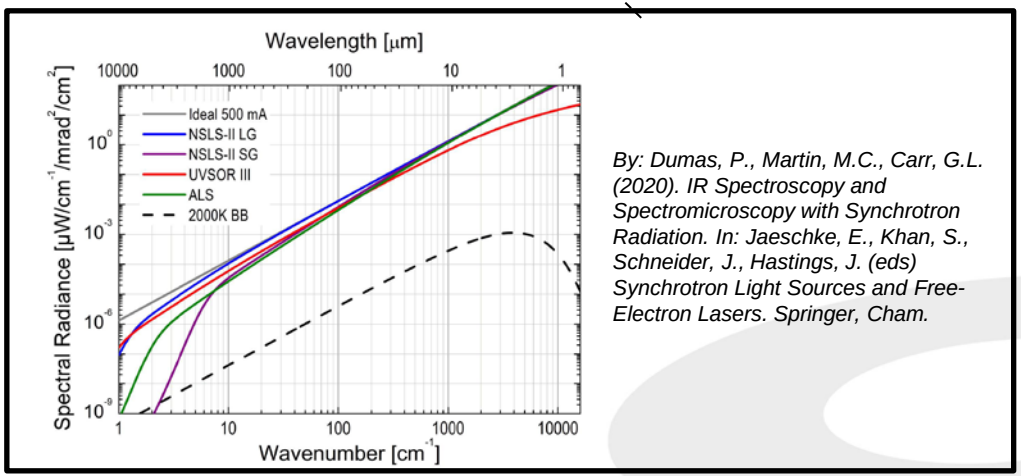
IR Synchrotron Radiation



*current **1 A**, horizontal angle **θ_H = 100 mrads** and **ρ = 5 m**. Comparison with the emission for a BB source at 2000K.*



Flux & Brightness



By: Dumas, P., Martin, M.C., Carr, G.L. (2020). IR Spectroscopy and Spectromicroscopy with Synchrotron Radiation. In: Jaeschke, E., Khan, S., Schneider, J., Hastings, J. (eds) Synchrotron Light Sources and Free-Electron Lasers. Springer, Cham.

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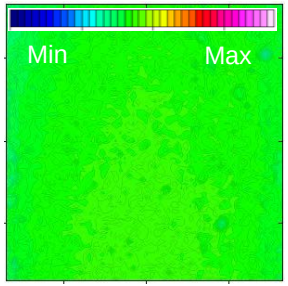
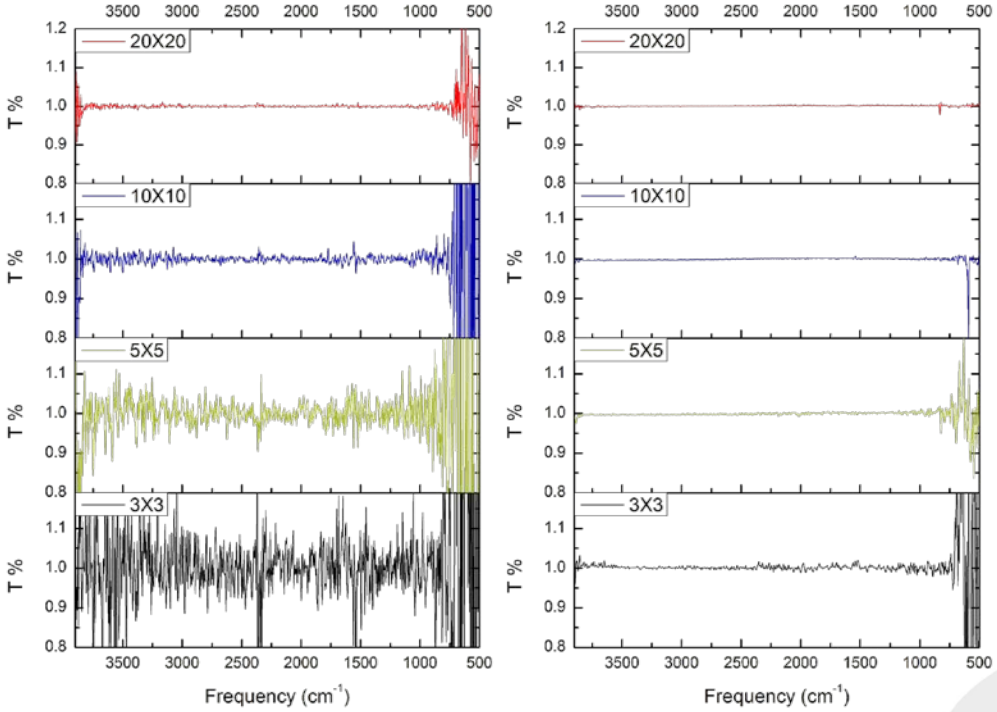
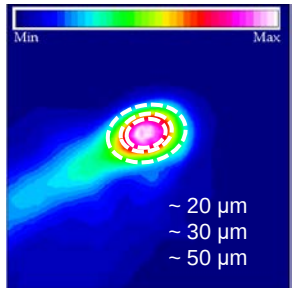
Far Field FTIR microscopy
SR brightness advantage in practice



Conventional Source





IRSR

Diffraction Limited FTIR Microscopy is practically achievable only with IRSR

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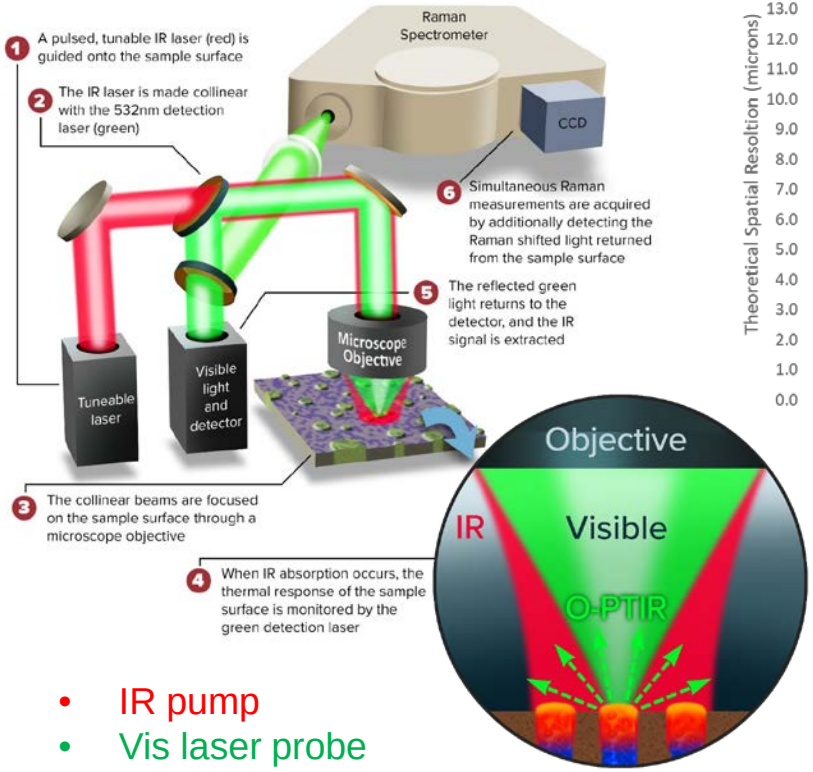
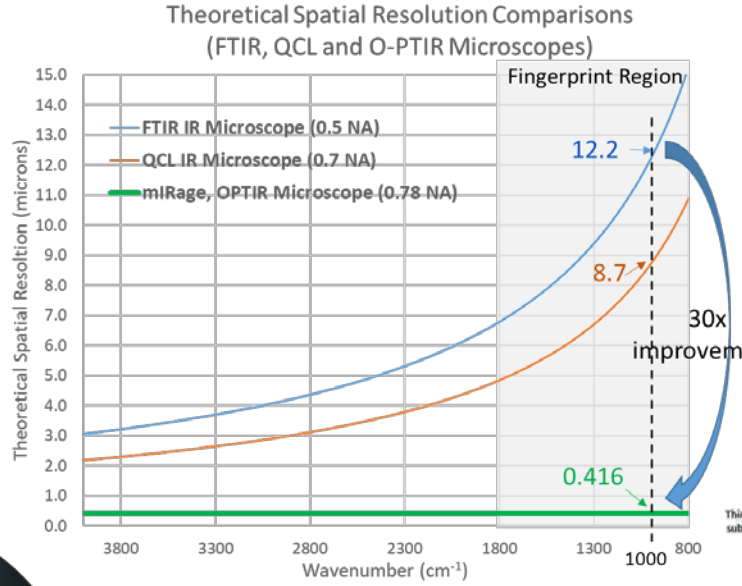
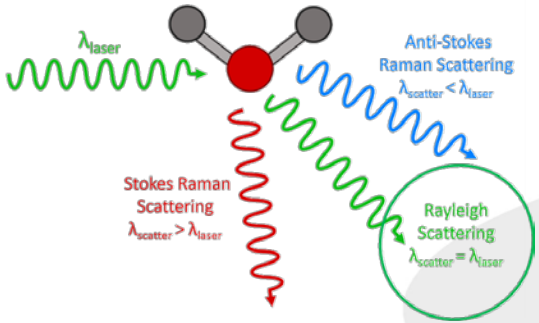
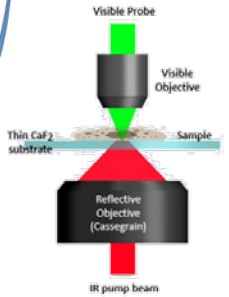
Far Field Optical PhotoThermal IR









- IR pump
- Vis laser probe

Near field scattering-type scanning near field optical microscopy

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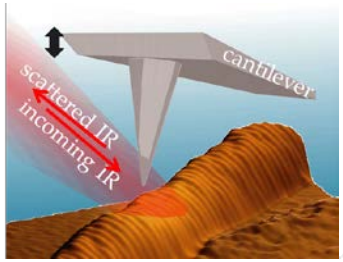
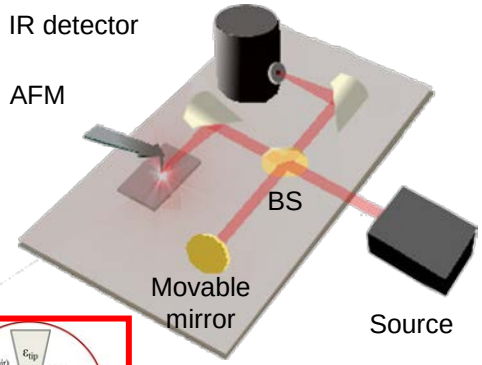
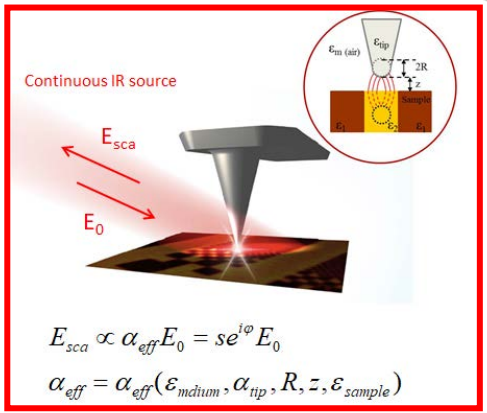
Near field microscopy $\delta \neq \lambda$



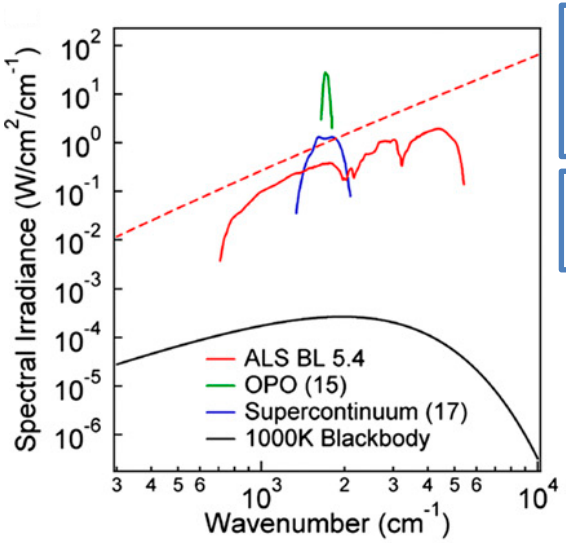




s-SNOM

- Resolution ~20 nm (tip curvature radius)
- Penetration depth ~100 nm
- Suitable for thin samples and surface analysis
- Allows sample depth profiling
- Spectroscopy -> SR-IR, broadband IR laser
- Imaging -> Tunable IR laser

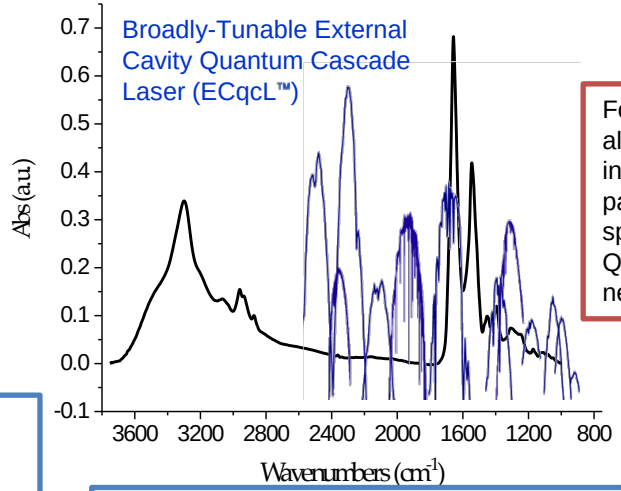
IRSR advantages for IR nanoscopy



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**)

- 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 nanospectrsocopy



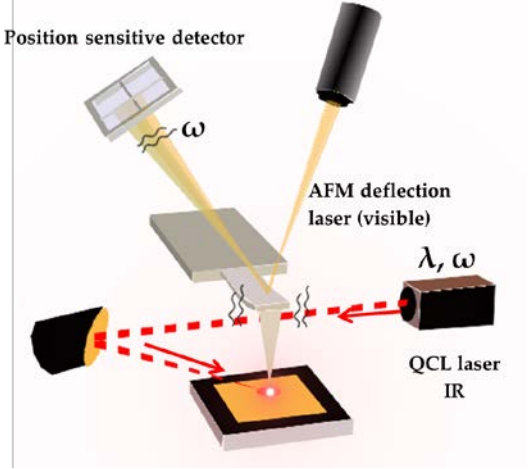
For barely covering almost half of the interesting MIR part of the spectrum, several QCL chips are needed.

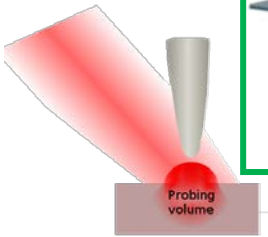
S/N ratio is the key parameter for vibrational analysis.
The superior stability of IRSR compensate for the lower spectral density, without inducing radiation damage

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

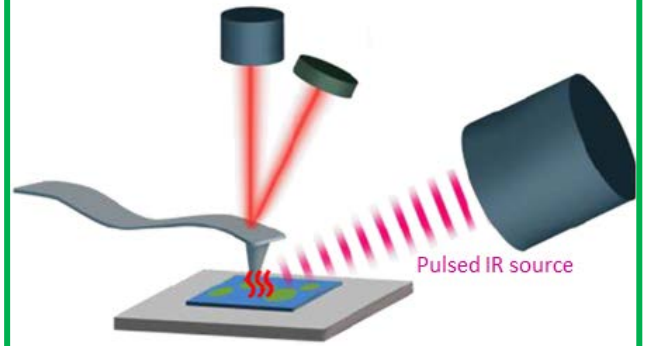
Near field PhotoThermal Expansion

Near field microscopy $\delta \neq \lambda$



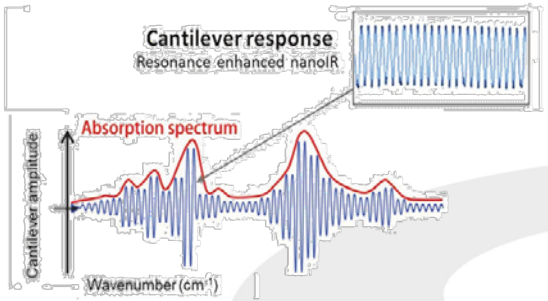


$$\Delta h \propto T_{max} \propto Pabs \propto \frac{\mathrm{Im}(n)}{\lambda} \propto Abs$$



PTE

- resolution > 20 nm (tip diameter)
- samples with high thermal expansion coefficient
- penetration depth > 100 nm
- Spectroscopy, Imaging -> Tunable IR laser & Chopped IRSR + step scan interferometer

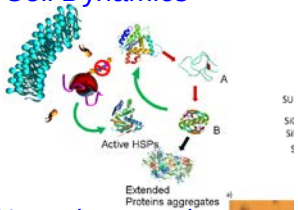


SISSI-Bio – Fields of application

(Dry) Cell and tissue mapping

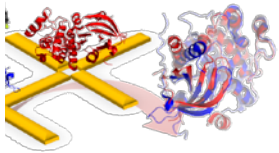
From diag

Cell Dynamics



Neurodegenerative disorders
Rare diseases

Biochemistry and Biophysics

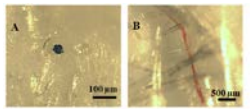


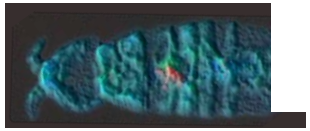
-IR

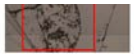
smonic for bio-
sical studies

Envi

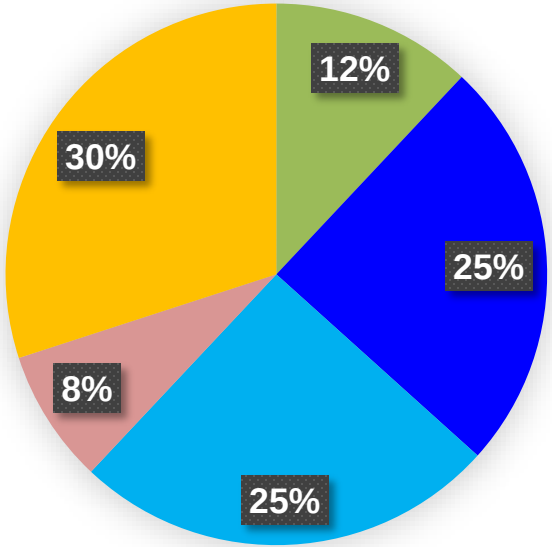
Microplastics in Antarctic





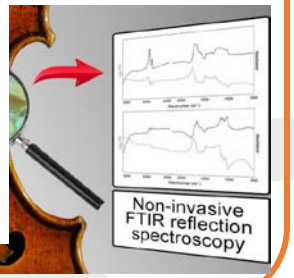


2020-2024

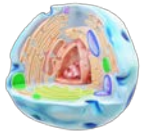


- Environmental Science
- Molecular and Cellular Biology
- Biochemistry&Biophysics
- Chemistry
- Cultural heritage
- Instrumentation / Method Development

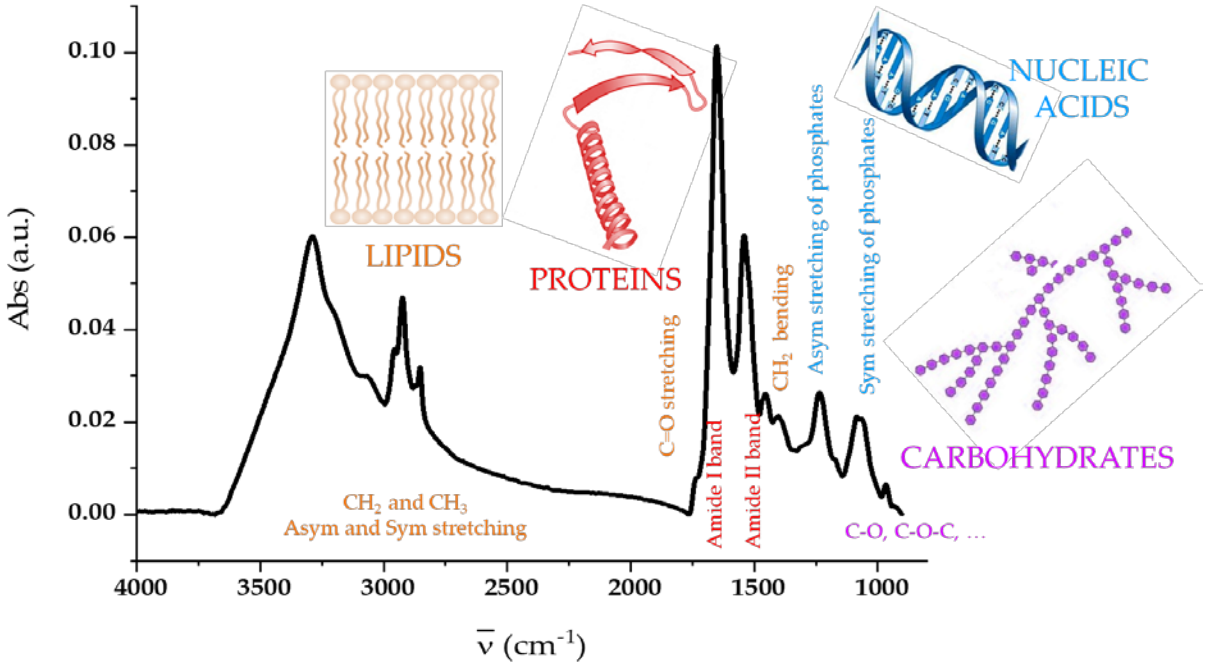
**wed string
struments**

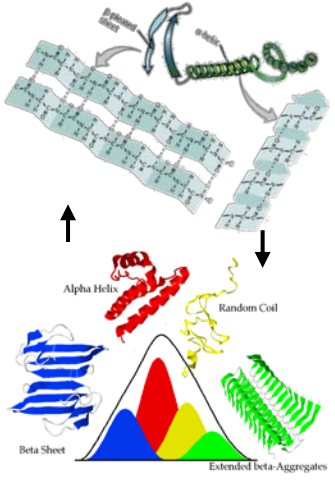


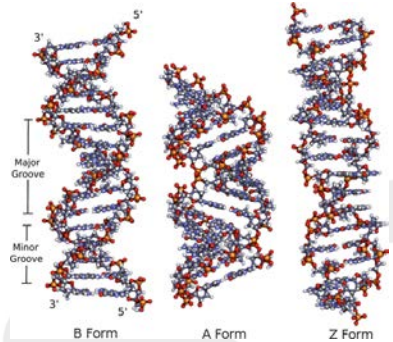
IRSR Cytology and Histology



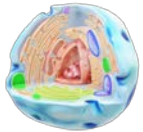
Single Cell IR micro-spectrum



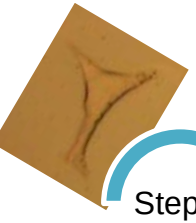




IRSR Cytology
Soft X-ray radiation damage



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



Hek293T cells
(human embryonic kidney)

Step 0
Cell growth on 100 nm Si_3N_4 membranes
Cell fixation with PFA 3.7% , air drying

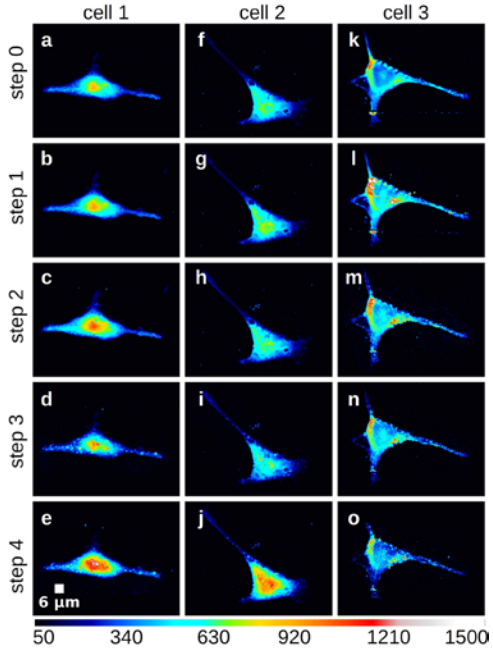
Step 1
Cell drying in vacuum @ TwinMic
($p < 10^{-5}$ mbar) for 1:30 hour

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

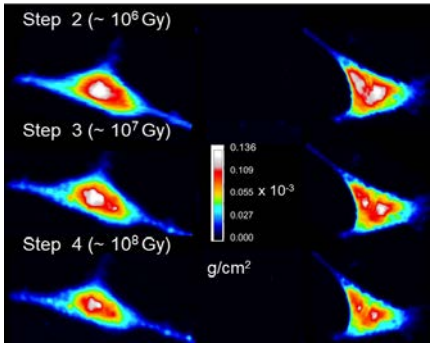
Step 3
High Dose STXM mapping @ TwinMic 1 keV
Estimated dose: $2*10^7$ Gy
Cumulative estimated dose: $2.2*10^7$ Gy

Step 4
Very high dose STXM mapping @ TwinMic 1 keV
Estimated dose: $6*10^8$ Gy
Cumulative estimated dose: $6.2*10^8$ Gy

AFM

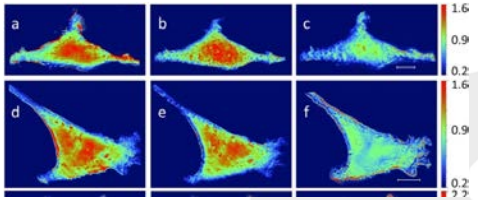


STXM



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

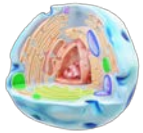
STXM & AFM

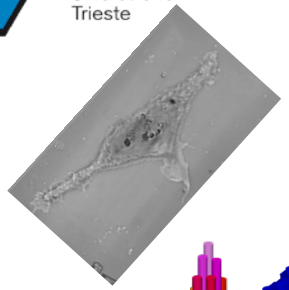


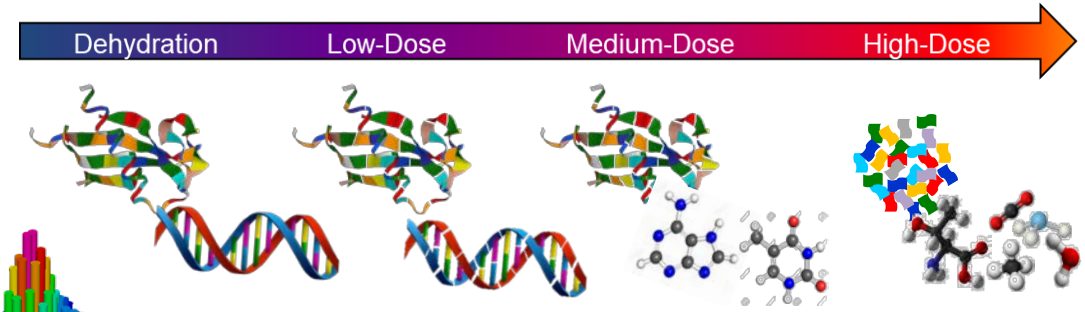
$$\rho = -\ln\frac{(I/I_0)}{\mu^* t}$$

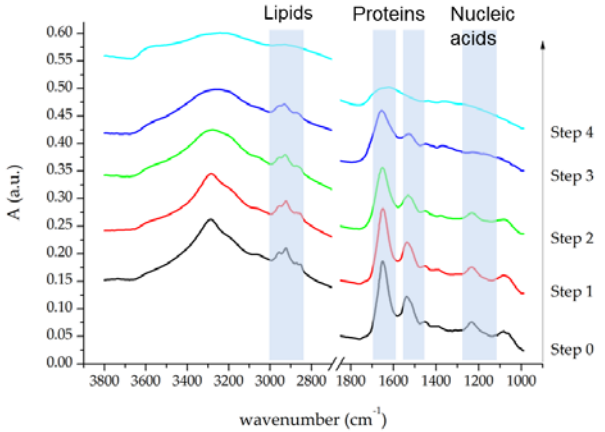
Elettra
Sincrotrone
Trieste

IRSR Cytology
Soft X-ray radiation damage









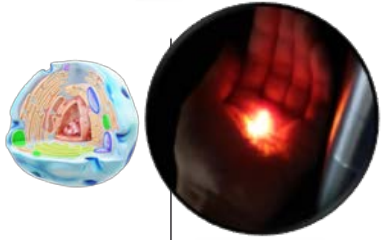
Scientific Reports volume 5, Article number: 10250 (2015)

Journal of Synchrotron Radiation. 2020 ;27, pp. 1218-1226.

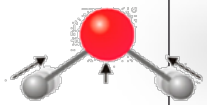
Journal of Synchrotron Radiation, 2021, 28, pp. 231–239

IRSR Cytology and Histology

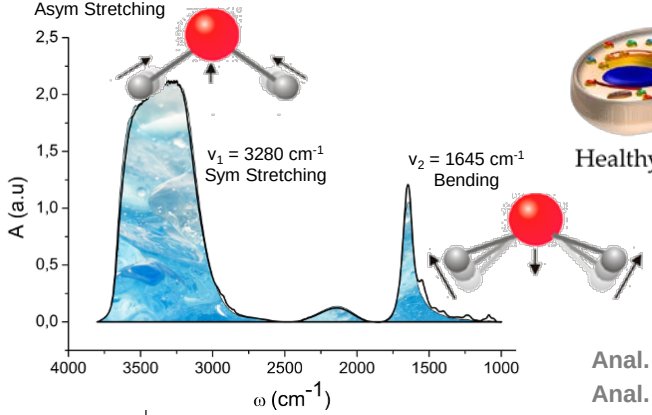
Elettra Sincrotrone Trieste



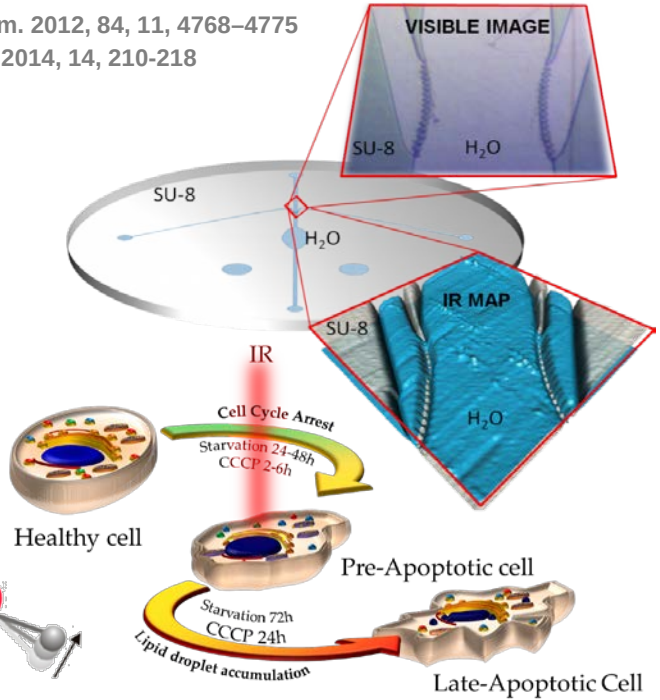
Water Barrier

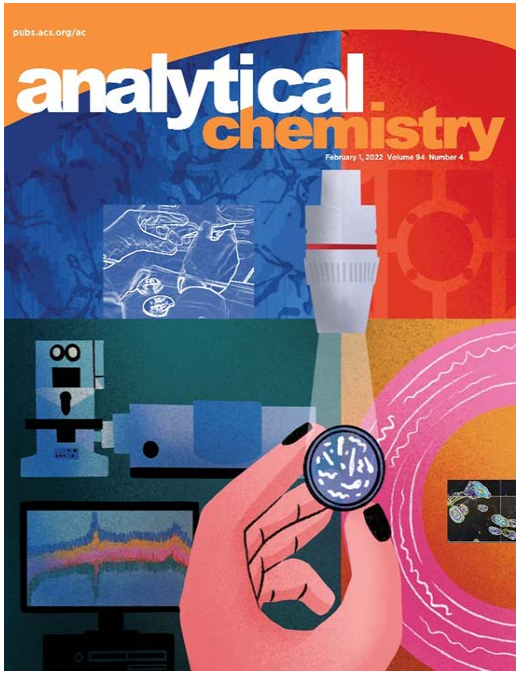


v_3 = 3490 cm^{-1}
Asym Stretching



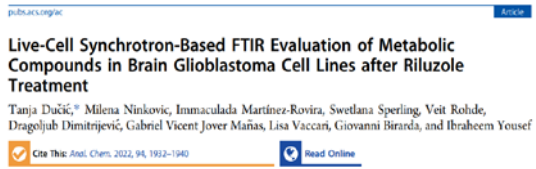
Anal. Chem. 2012, 84, 11, 4768–4775
Lab Chip, 2014, 14, 210-218





Anal. Chem. 2015, 87, 7, 3670–3677
Anal. Chem. 2016, 88, 24, 12090–12098
Analyst, 2013,138, 4015-4021
Vibrational Spectroscopy, 2014, 75, 127-135
Anal. Chem. 2016, 88, 24, 12090–12098

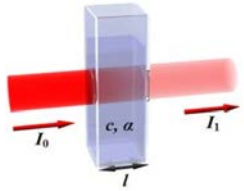
ALBA



Biophysics and Biochemistry with IR

Elettra Sincrotrone Trieste

Sensitivity Limit

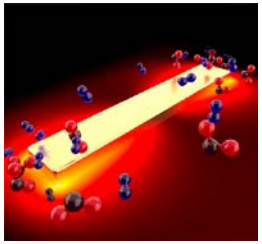


Beer's Law
[mM]

IR absorbance ∝ Oscillator strength of molecular bond x |Electric field|²

Optical nano-resonators

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

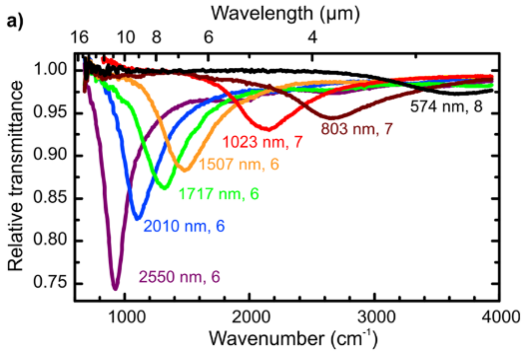


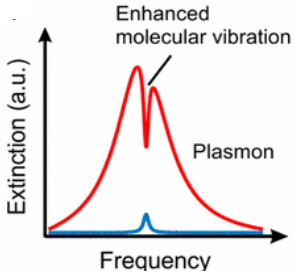
Adato, R., et al.
Materials Today, **18 (8),** 436-446 (2015)

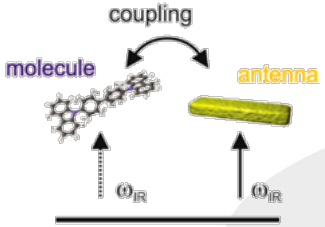
Neubrech,F., et al.
Chem. Rev., **117 (7),** 5110-5145 (2017)

Resonance tuning & Spectral coupling

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







Signal enhancement of several order of magnitude can be achieved

iit
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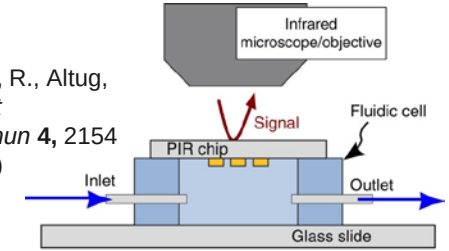
Biophysics and Biochemistry with IR

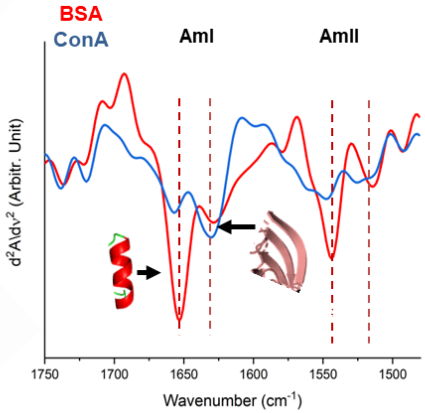
Elettra Sincrotrone Trieste

Plasmonic internal reflection (PIR)

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



Without water substraction!

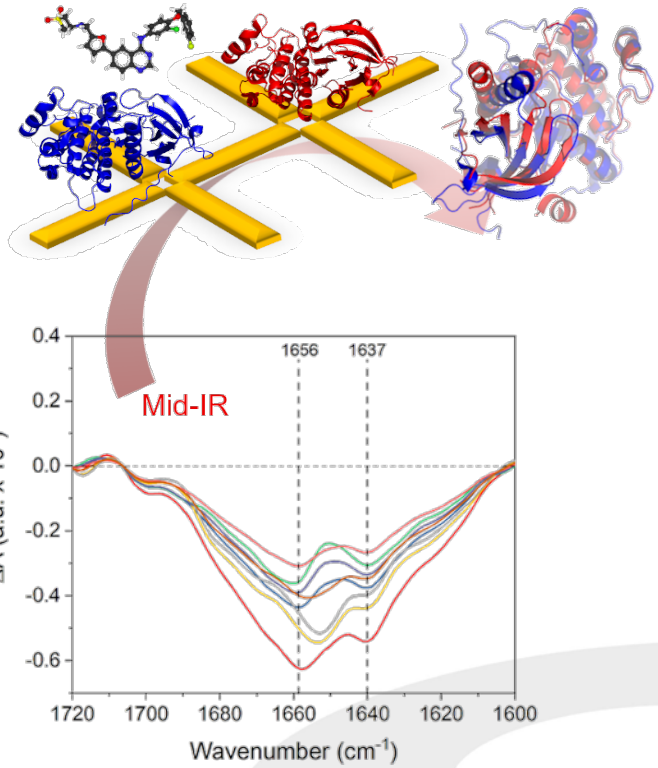


Binding of tyrosine kinase inhibitor Lapatinib to EGFR-KD

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

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

EGFR-KD	Lapatinib/EGFR-KD
36% helices (both alpha and 3_{10})	32% helices (both alpha and 3_{10})
15% beta sheet strands	14% beta sheet strands.



P. Zucchiatti et al., "Binding of tyrosine kinase inhibitor to epidermal growth factor receptor: surface-enhanced infrared absorption microscopy reveals subtle protein secondary structure variations", *Nanoscale*,. 2021. 13. 7667-7677

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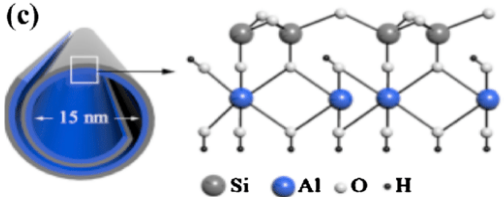
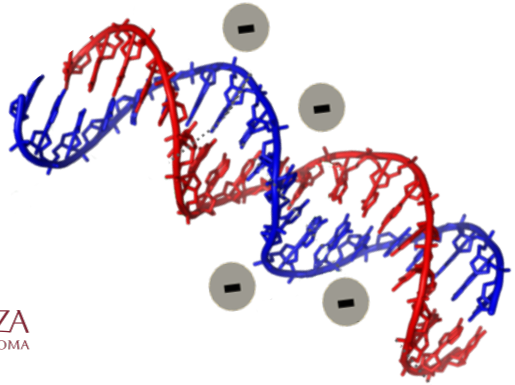
DNA Structural Modifications upon Immobilization onto Clay Nanotubes

Halloysite Nanotubes (NHs) are natural biocompatible structures with high affinity for loading biomolecules thus they are good candidates for drug delivery and gene transfer

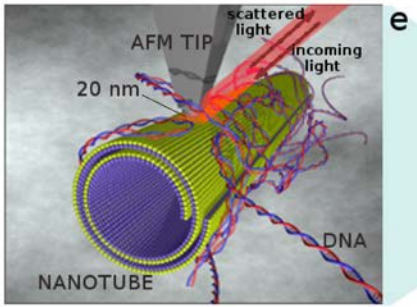


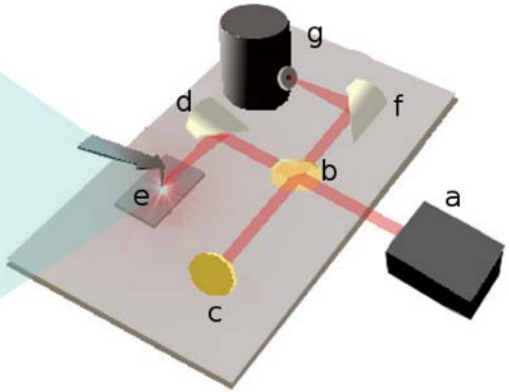
- **siloxane external surface**
+ **aluminum oxide internal surface**

Why DNA tends to aggregate onto HNs?



?





THE EXPERIMENTAL EVIDENCE OF THE DNA SPONTANEOUS ADSORPTION ON HNs IS COUNTERINTUITIVE!

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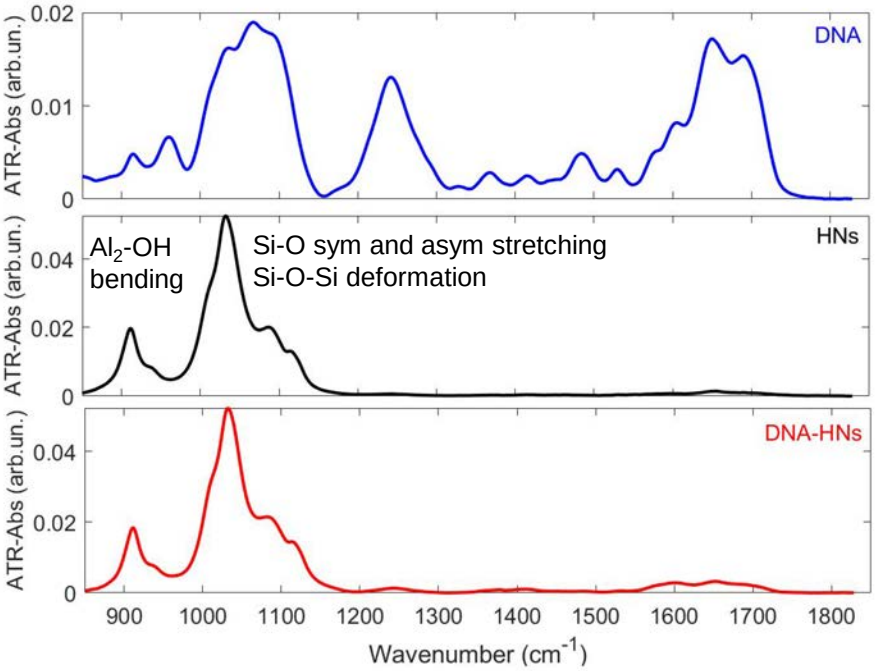
DNA Structural Modifications upon Immobilization onto Clay Nanotubes

Elettra Sincrotrone Trieste

ATR-FTIR spectroscopy



Chemical Assignment	DNA cm^{-1}	HNs cm^{-1}	DNA–HNs cm^{-1}
Si-O (amorphous) , PO_2^- as	1242	1243	1243
C-N s (A,T)	1330	-	-
C-N s (C,G)	1368	-	1366
Base in-plane vibration (C,G)	1415	-	1415
C-C C-N (G,C)	1485	-	1484
Base in-plane vibration (C,G)	1530	-	1530
C-C C-N (C), N-H (A)	1575	-	-
(A)	1602	-	1602
OH (G,C,T,A)	1650	1650	1650
C=O (T)	1698	-	1698
C=O of nucleobases	1707	-	1710

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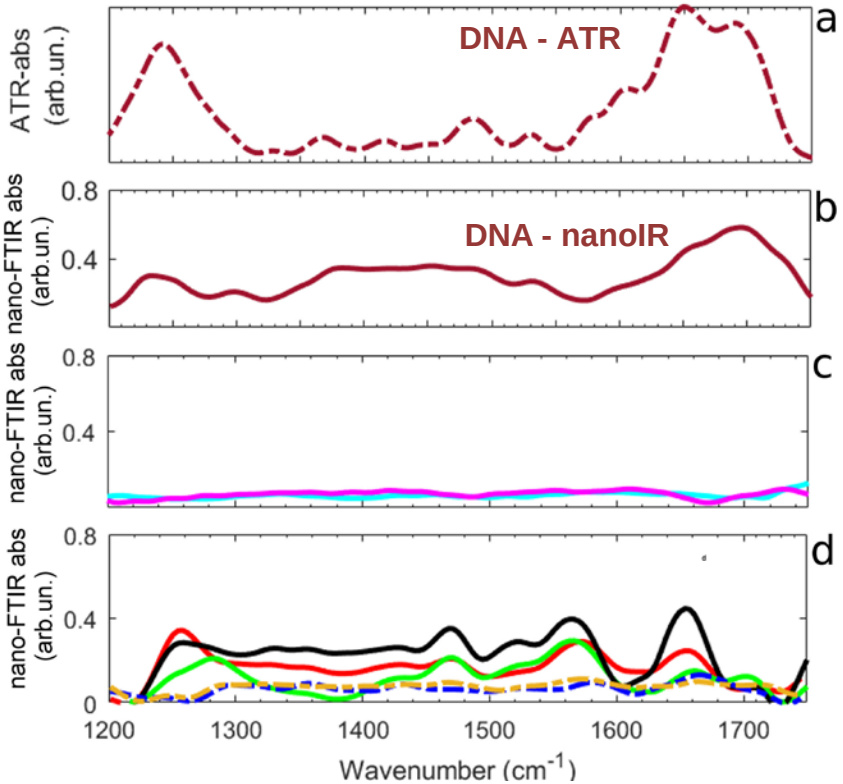
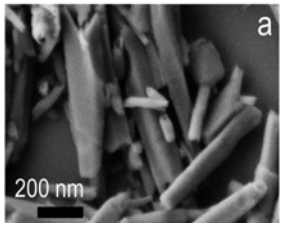
DNA Structural Modifications upon Immobilization onto Clay Nanotubes

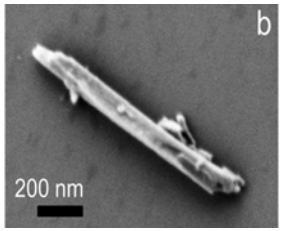
Elettra Sincrotrone Trieste

nanoFTIR spectroscopy



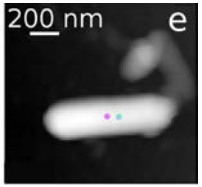


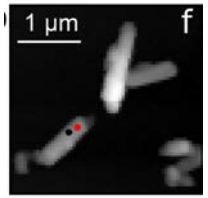
Pristine HNs [SEM]

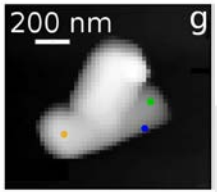


DNA-HNs [SEM]

AFM







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DNA Structural Modifications upon Immobilization onto Clay Nanotubes

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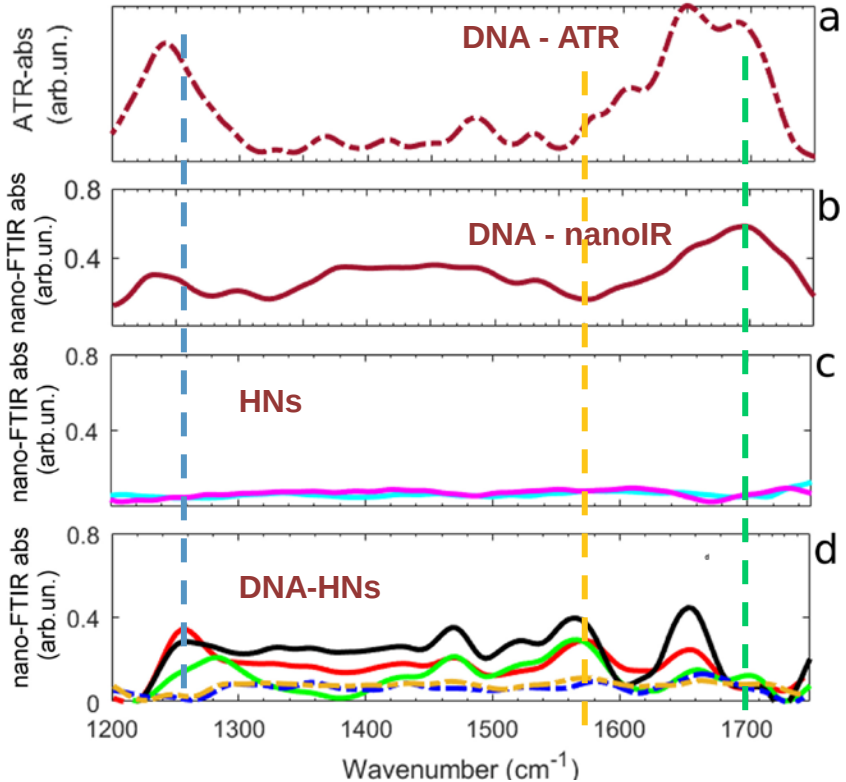
nanoFTIR spectroscopy



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Base in-plane vibration (C,G)	1530	1530	1520
C-C C-N (C), N-H (A)	1575	-	1570
(A)	1602	1602	-
OH (G,C,T,A)	1650	1650	1652
C=O (T)	1698	1698	-
C=O of nucleobases	1707	1710	1706

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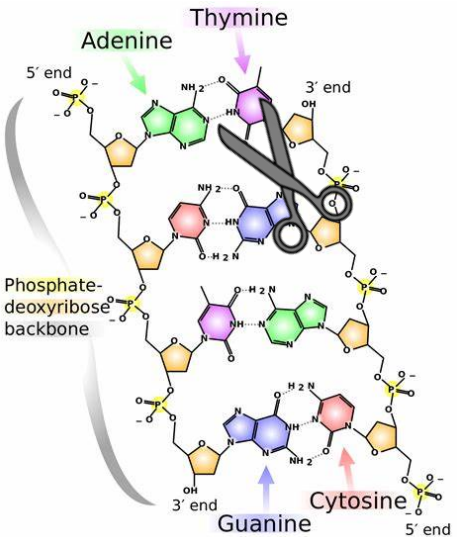
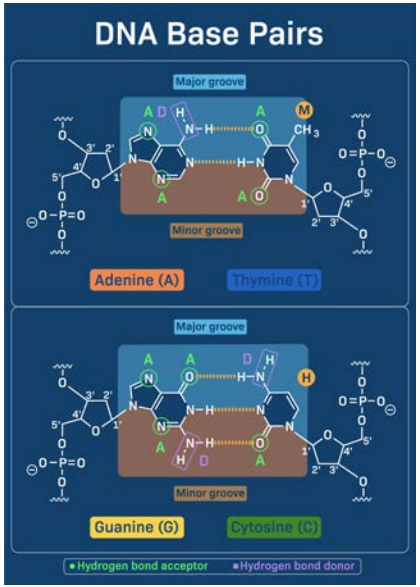
DNA Structural Modifications upon Immobilization onto Clay Nanotubes





- DNA – NHs interaction drives the break of base pairing between Thymine and Adenine
- Positive charges (possibly on N-H groups of Adenine) can more easily be attracted by the anionic surface of nanotubes
- Electrostatic interaction between positively charged edges of DNA and nanotubes surface constitute a further effect contributing to DNA adsorption

- Nanocarriers should guarantee optimal cargo molecules functionality
- Relevant structural modifications in DNA adsorbed on NHs are reported
- There are constraints for the use of nanostructured clays as DNA carriers
- Super-resolved infrared spectroscopy is an effective and versatile tool for the evaluation of immobilization processes in the context of drug delivery and gene transfer

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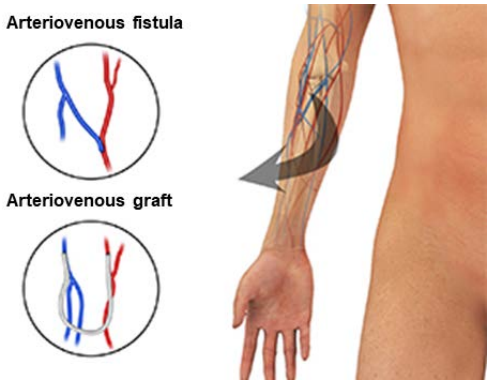
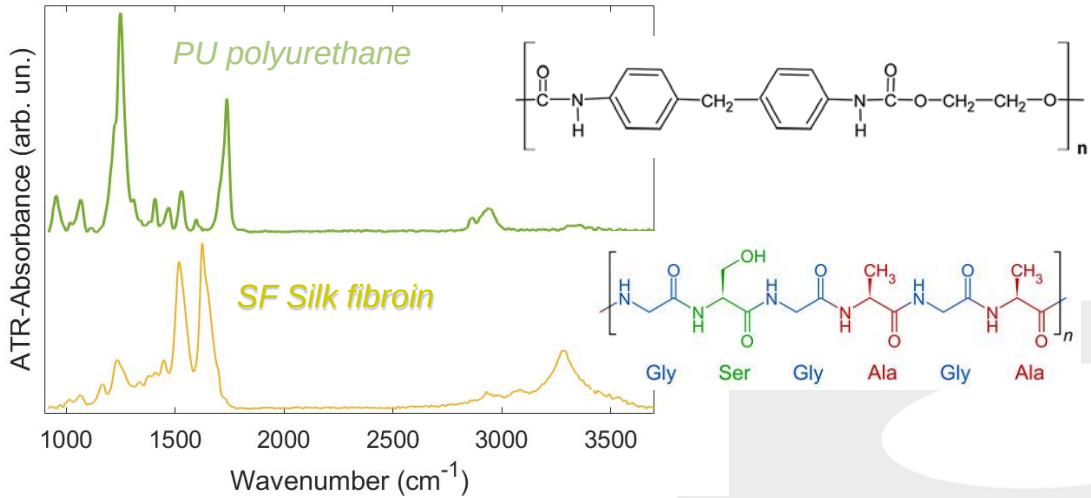
Silk Fibroin – Polyurethane meshes

Hybrid silk fibroin (SF) – Polyurethane (PU) meshes are innovative hybrid materials for arteriovenous grafts in hemodialysis



SF is a biodegradable and biocompatible protein with remarkable mechanical properties (*i.e.* stiffness)

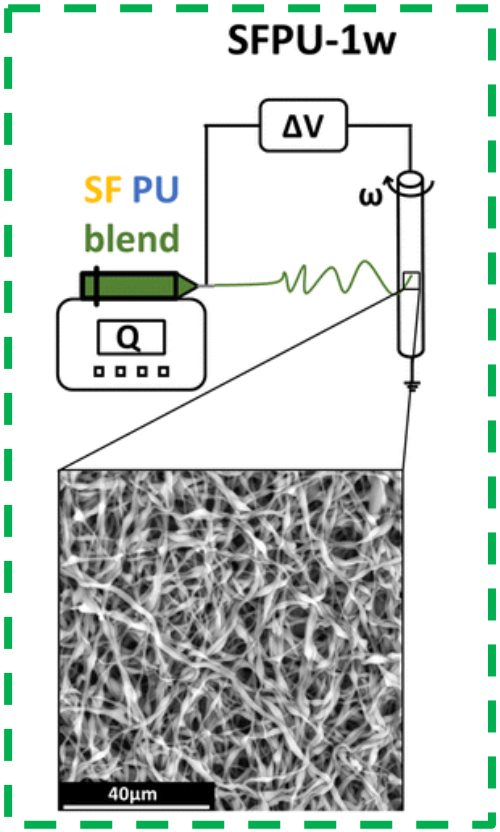
PU is characterized by good deformability in response to external solicitations

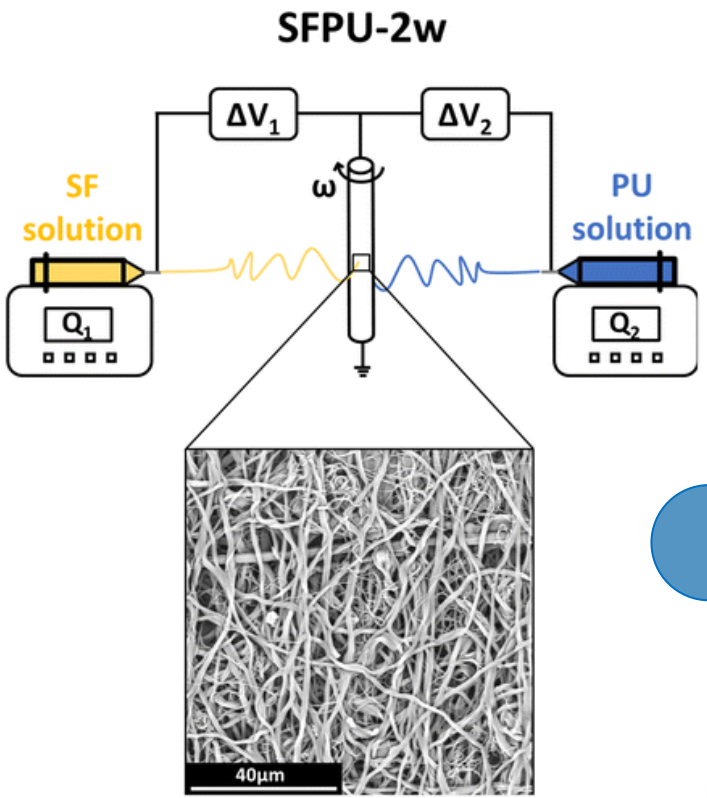


SF-PU meshes production by electrospinning

Elettra Sincrotrone Trieste

Silkothane® production





The chemical nature of individual threads and the arrangement of the two constituents in Silkothane® remains still elusive

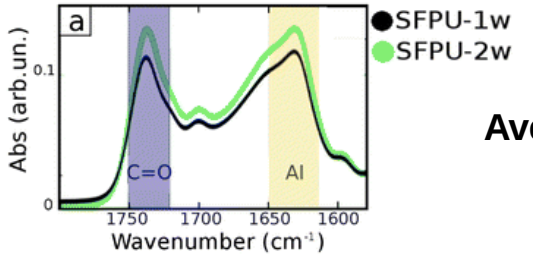
?

BEL

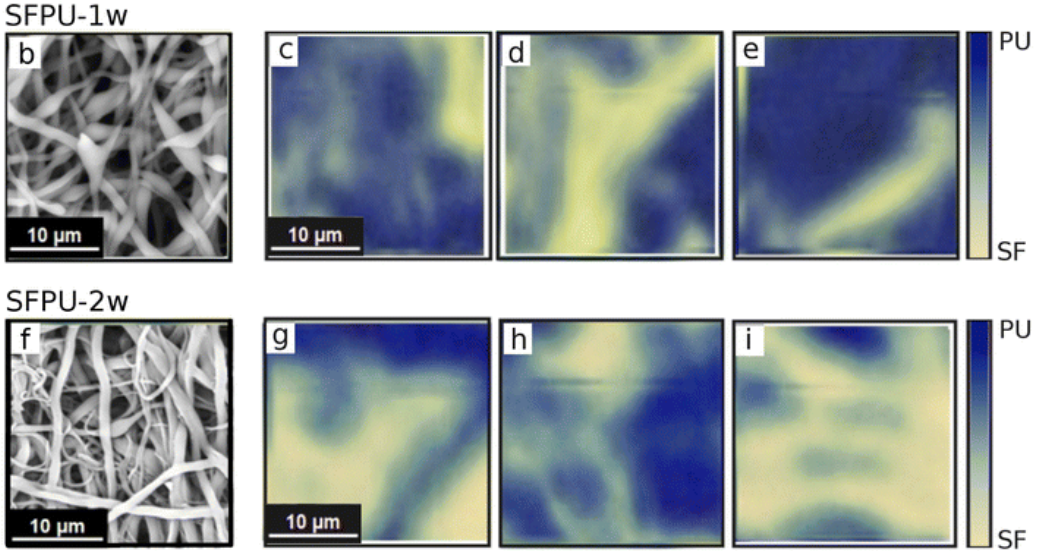
DIALYBRID

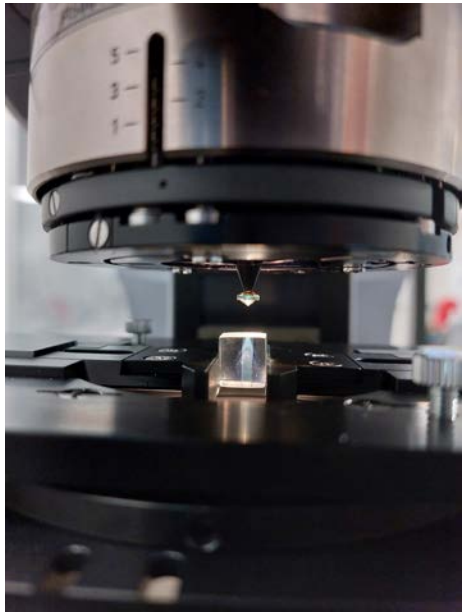
POLITECNICO MILANO 1863

Limits of conventional FTIR ATR-Imaging



Average micro-ATR spectra



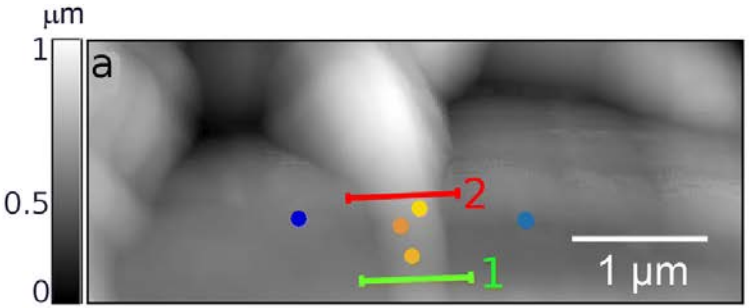


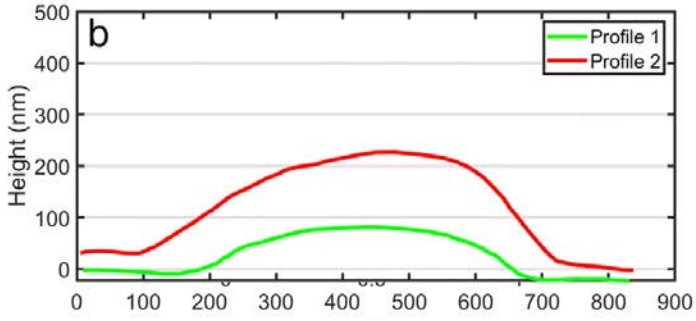
The mismatch between SEM and micro-ATR lateral resolution is clear and due to the axial pressure applied by the ATR-IRE (~5000 Pa)

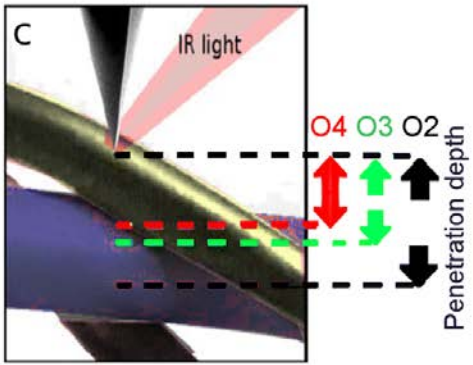
Beyond diffraction limit: IR s-SNOM data interpretation

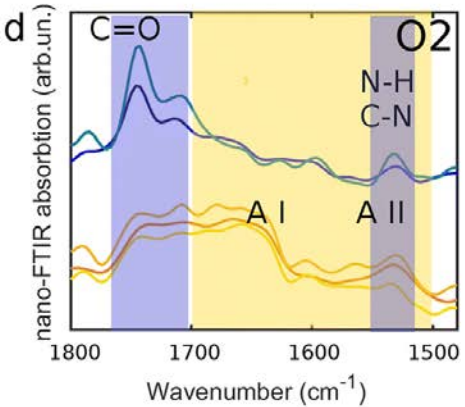
SFPU-2w

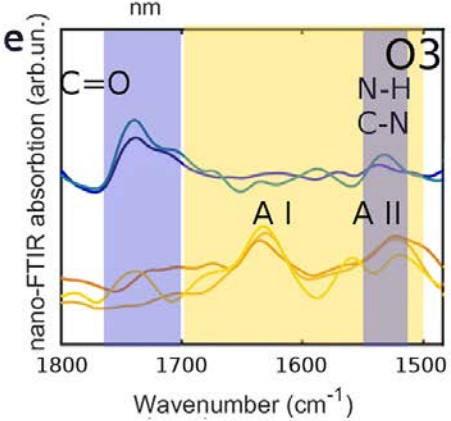
Depth dependence of IR s-SNOM versus harmonic order demodulation of IR s-SNOM







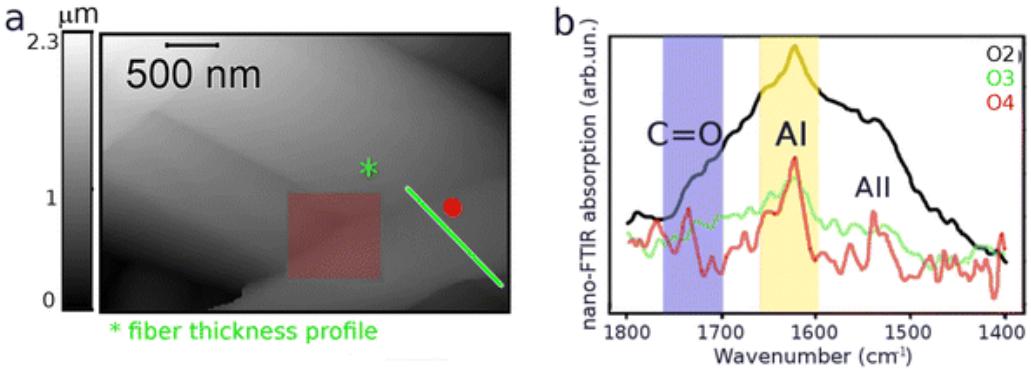


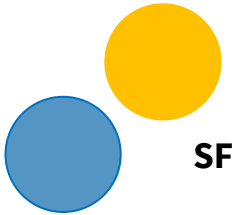


Beyond diffraction limit: IR s-SNOM data interpretation

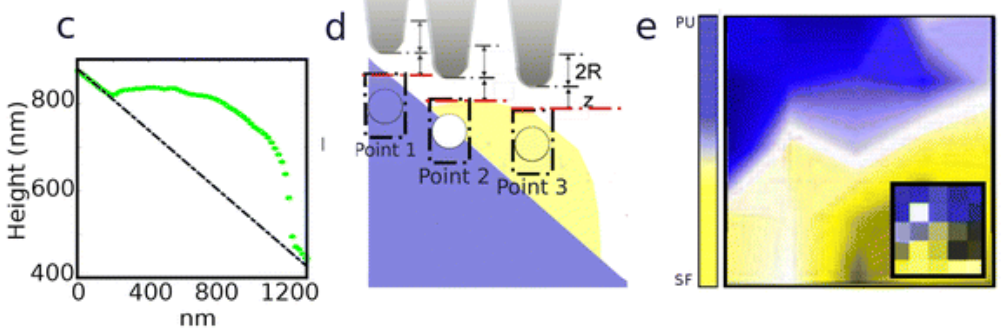
SFPU-1w

Depth dependence of IR s-SNOM versus harmonic order demodulation of IR s-SNOM





SF wire on PU wire



White areas does not reflect a SFPU mixture, while morphological situations comparable to point 2 in (d)

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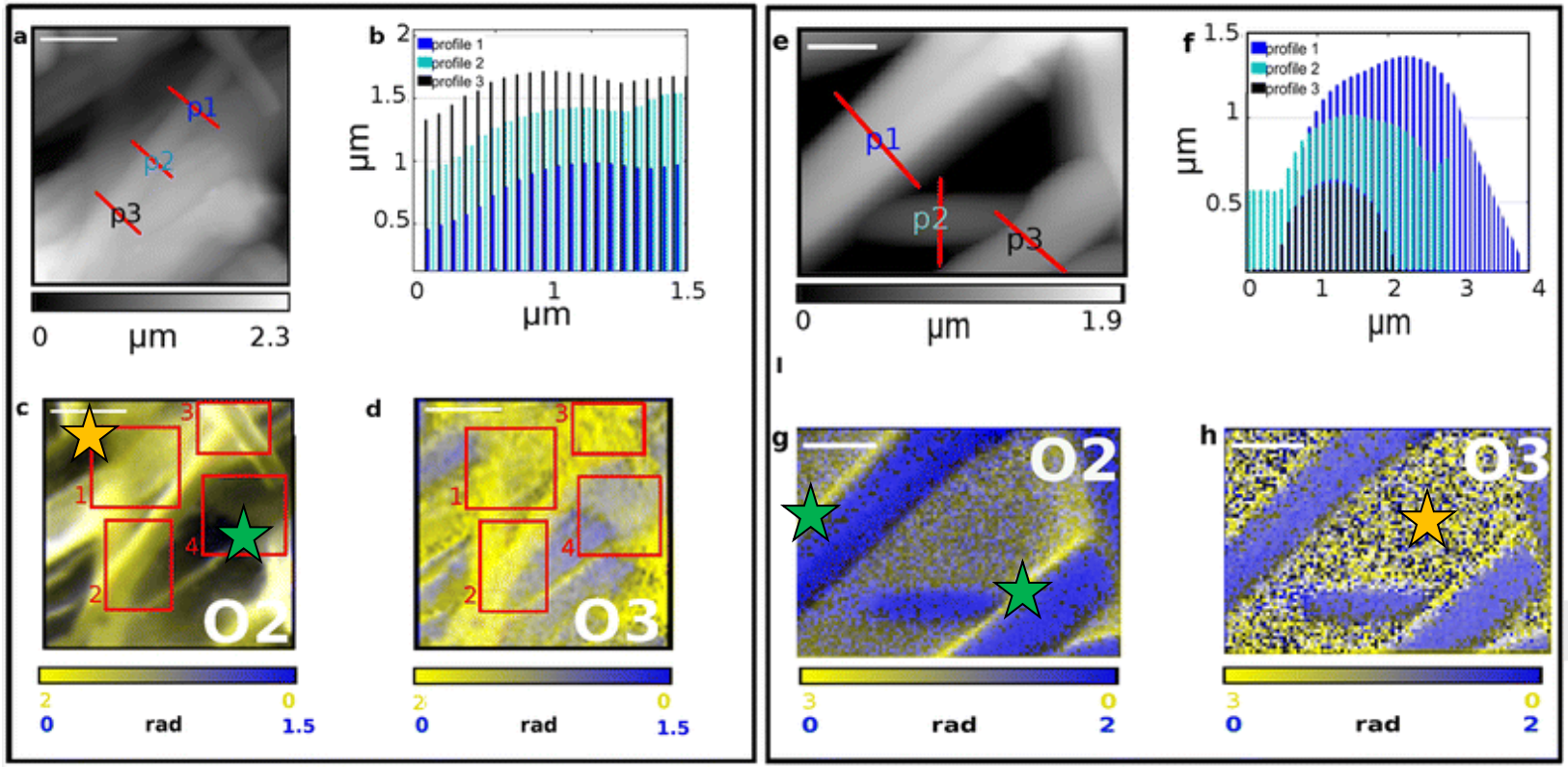
Beyond diffraction limit: nanoIR single frequency IR imaging

SFPU-2w



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POLITECNICO
MILANO 1863

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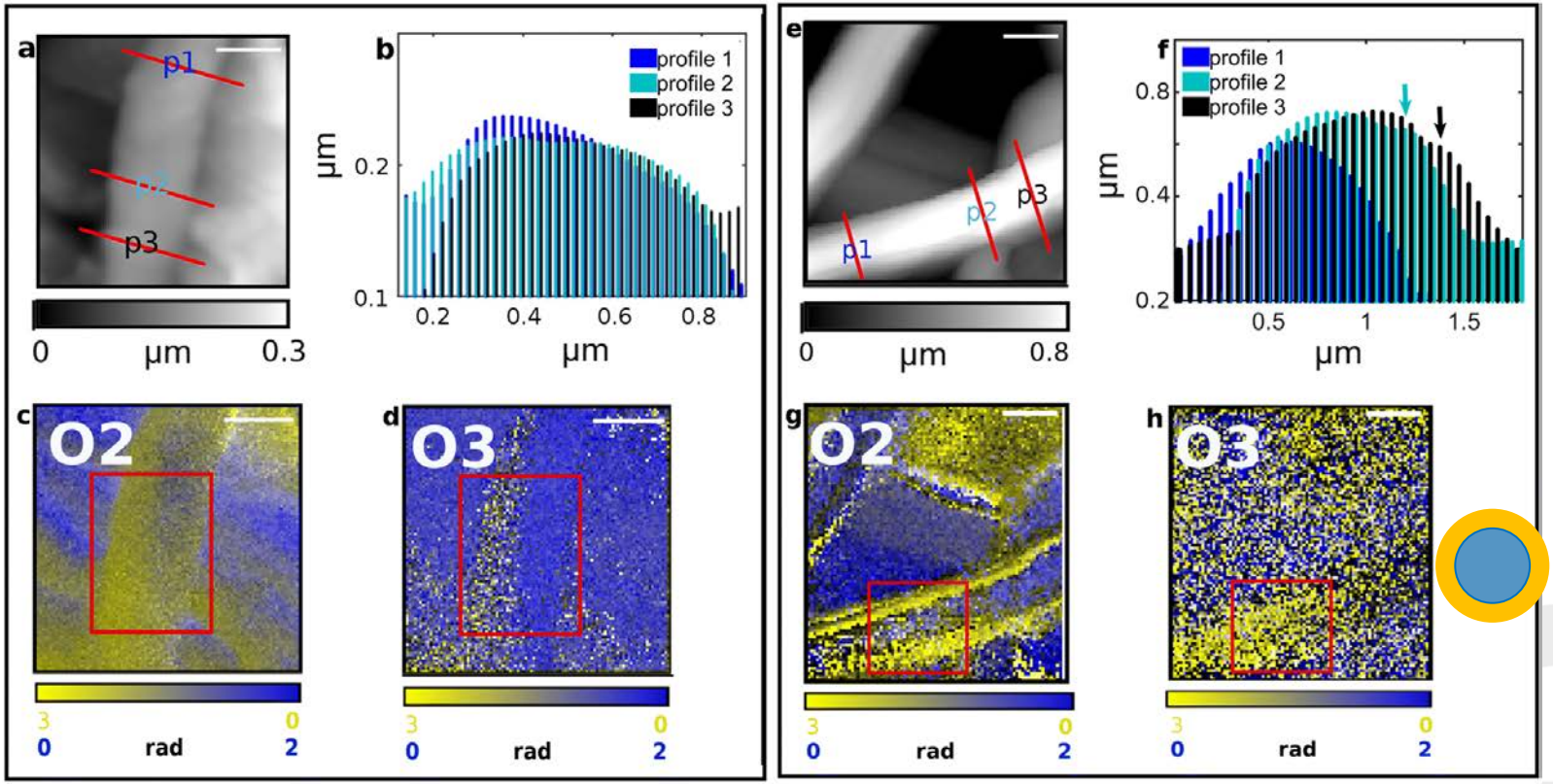
Beyond diffraction limit: nanoIR single frequency IR imaging

SFPU-1w



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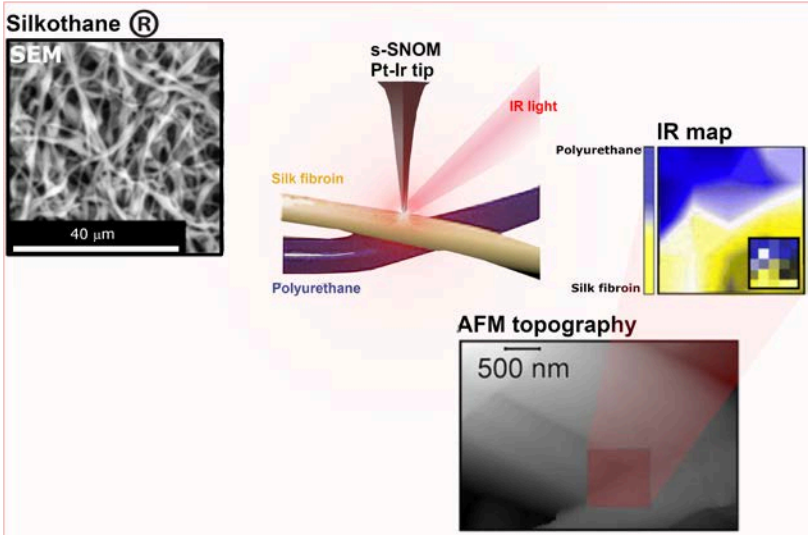
Conclusions



IR s-SNOM can be applied for the nanoscale morpho-chemical **profiling** of the corona the fibers constituting the hybrid meshes, up to a depth of around 100 nm.

- The surface of the fibers exposed to the environment is not a mixture of the two fiber constituents, even when SF and PU are spun as a blend.
- The two meshes are characterized by the co-existence of main fibrillar domains made of individual fibers, either completely made by a single component [or with a corona of about 100 nm made by a pure component].
- Only occasional the presence of core–shell surface has been detected.

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A Nanofocused Light on Stradivari Violins: Infrared s-SNOM Reveals New Clues Behind Craftsmanship Mastery

Chiaramaria Stani, Claudia Invernizzi, Giovanni Birarda, Patrizia Davit, Lisa Vaccari,* Marco Malagodi,* Monica Gulmini, and Giacomo Fiocco

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San Lorenzo 1718 violin, Stradivari

Toscano 1690 violin, Stradivari

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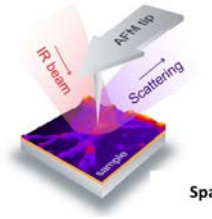

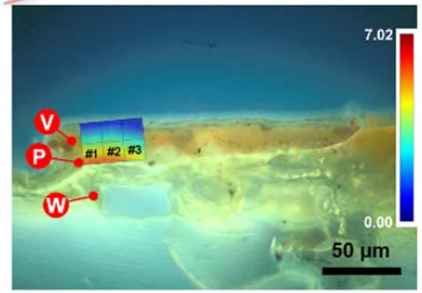

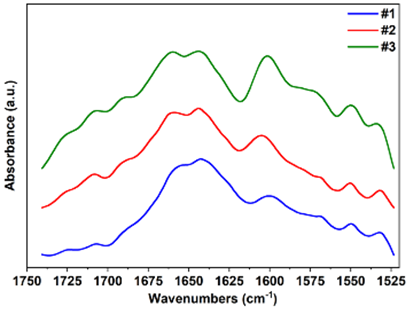

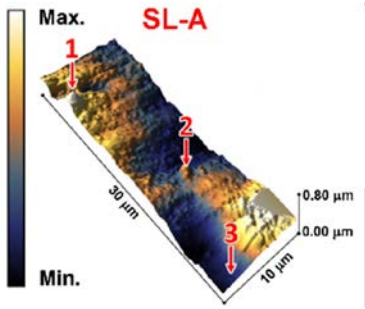

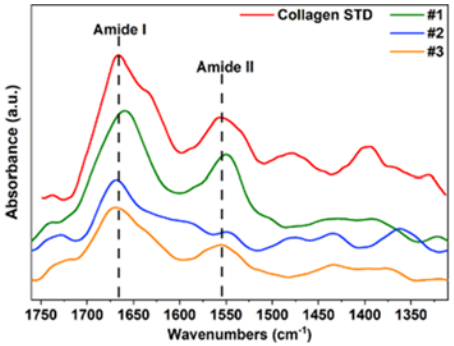

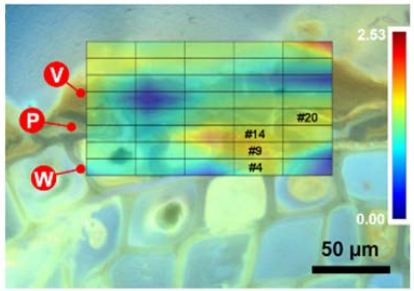

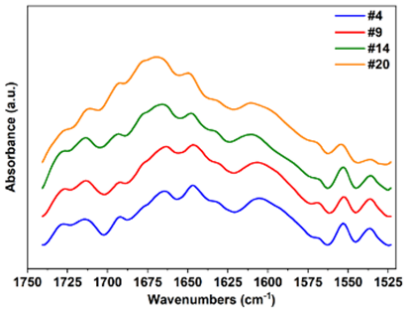

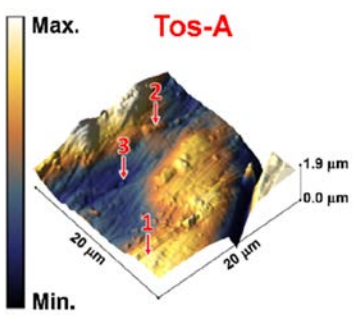

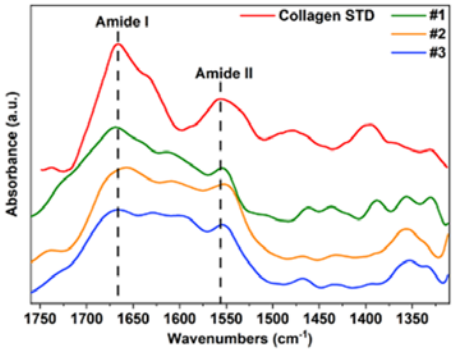

Stani et al. - A Nanofocused Light on Stradivari Violins: Infrared s-SNOM Reveals New Clues Behind Craftsmanship Mastery. Analytical Chemistry. 2022, 94, 43, 14815–14819 - doi.org/10.1021/acs.analchem.2c02965

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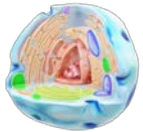
Thank you all



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