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# Infrared spectroscopy with synchrotron and FEL radiation

Lisa Vaccari

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

21 settembre 2026 a 1 ottobre 2026
Muggia (Italy)

Società
Italiana
Luce di
Sincrotrone

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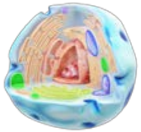
# Outlook

✓ Pills of IR spectroscopy

- IRSR properties and instrumentation: from macro to nanoscale



✓ IRSR Citology and Histology

- Soft X-ray radiation damage

✓ IR for cultural heritage

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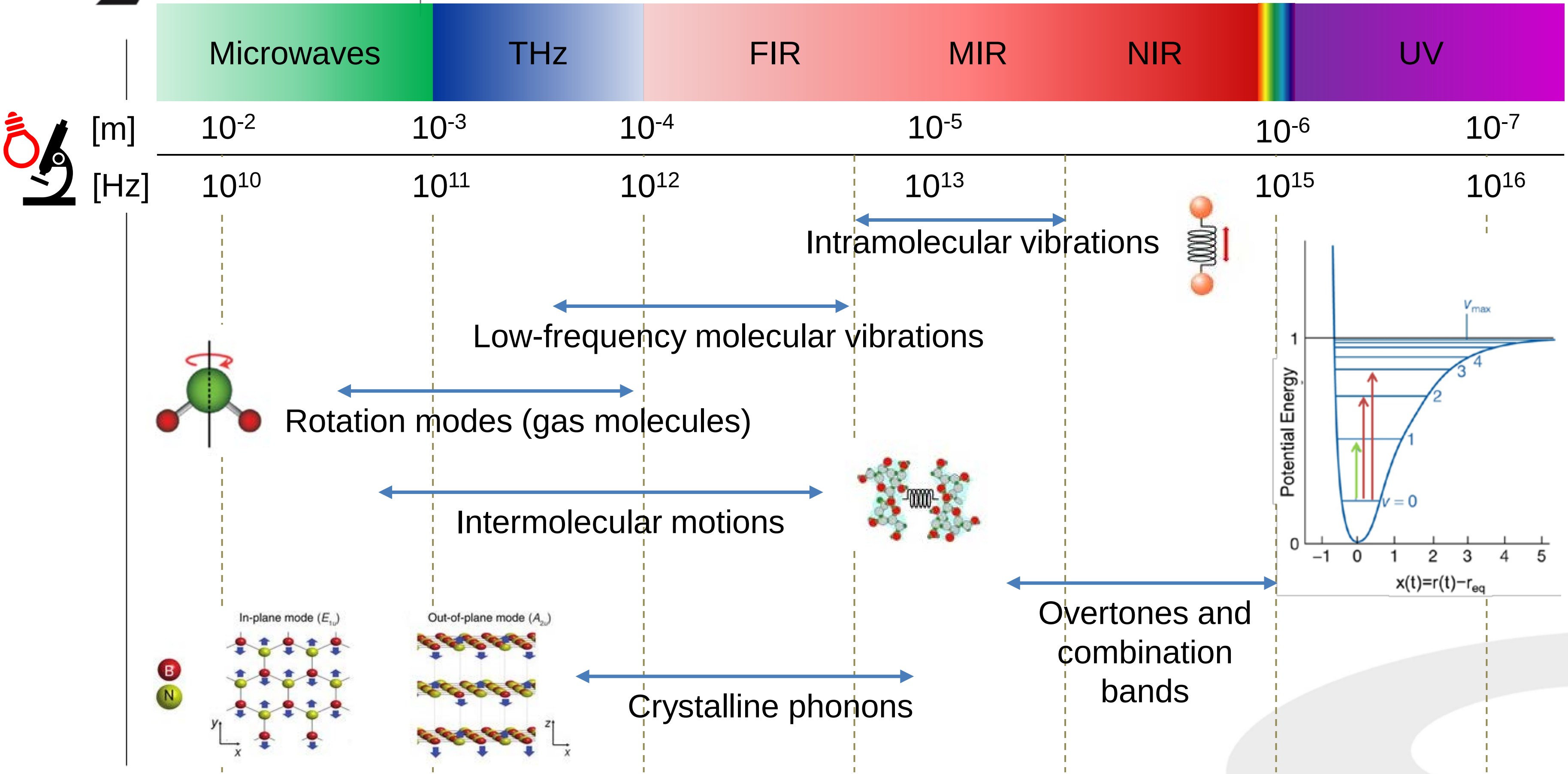
# IR spectroscopy

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

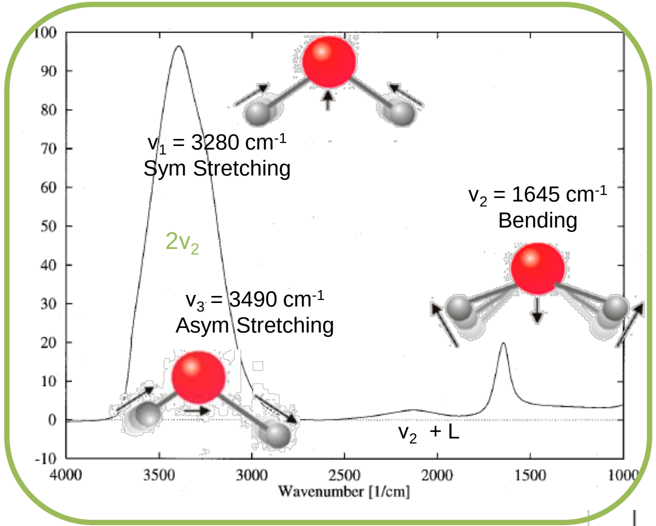
It enables label-free and non-damaging extraction of chemical information on (bio-macro)-molecules on diverse class of samples and at different spatial resolution from their roto-vibrational profile.

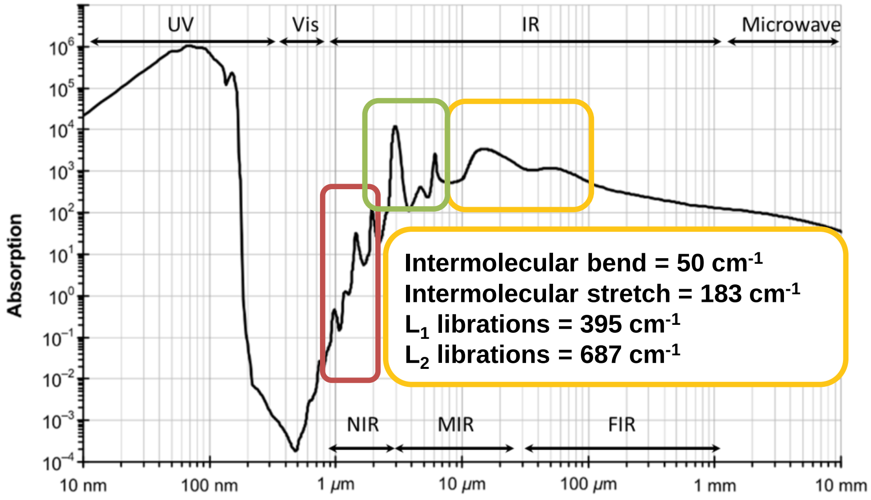
An IR spectrum contains info on the sample composition (**chemical identity**), and finer details such as molecular order or molecular structure, molecular network, etc…(**holistic identity**) depending on the sample under investigation and on the considered IR spectral domain

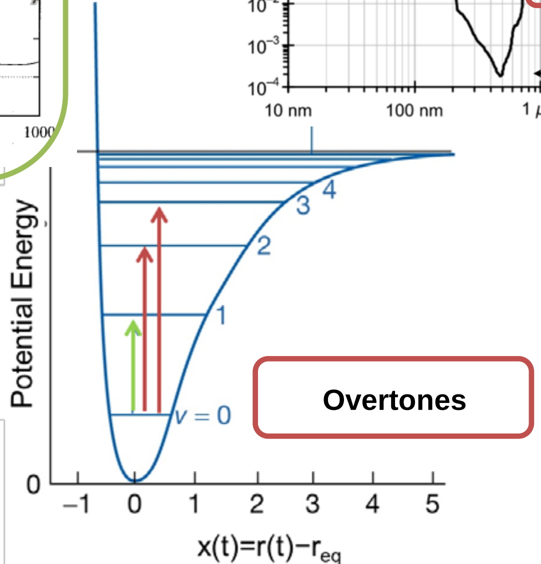
# IR spectroscopy



# Chemical and holistic identity





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

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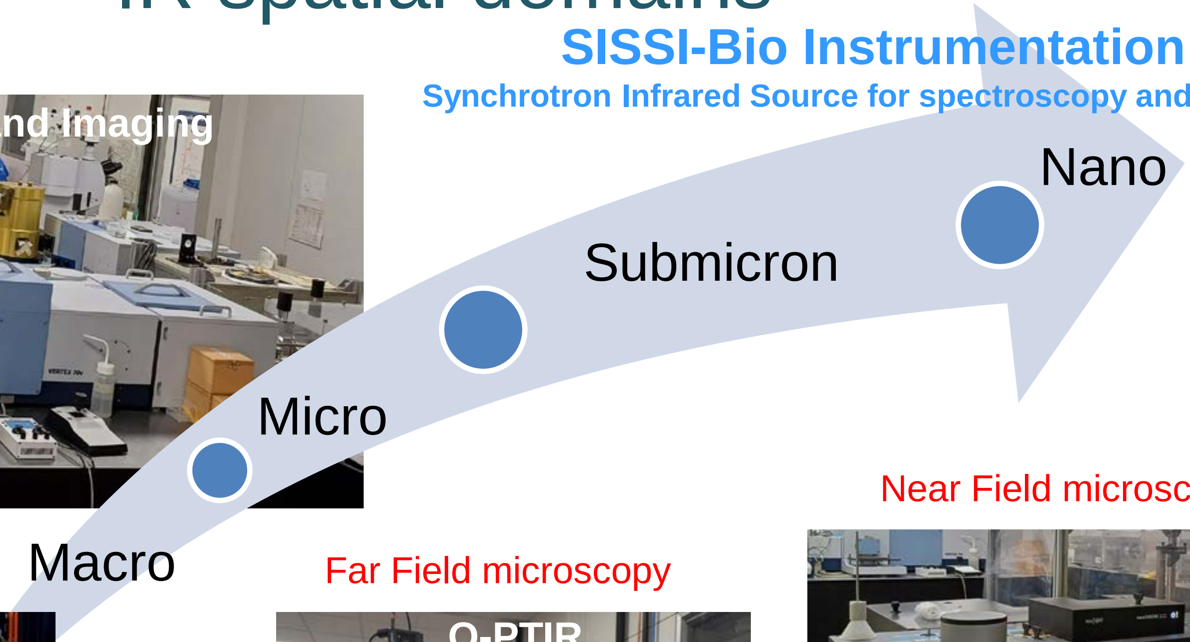
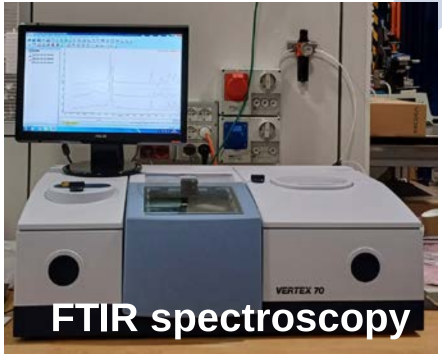
# IR spatial domains

## SISSI-Bio Instrumentation

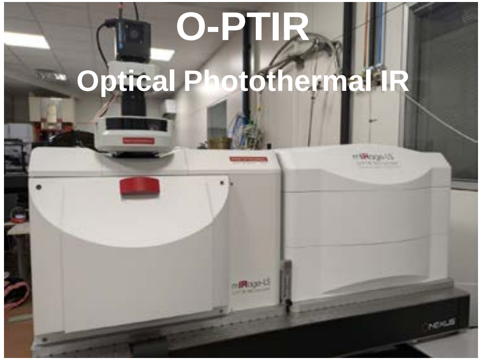
**Synchrotron Infrared Source for spectroscopy and Imaging**








Far Field microscopy




Near Field microscopy

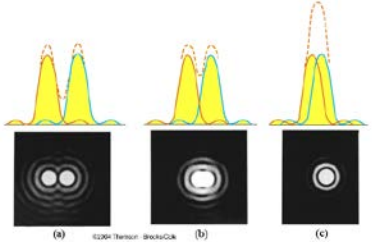
**IR s-SNOM**

**IR scattering-type scanning near-field optical microscopy**

# Far Field versus Near Field IR microscopy

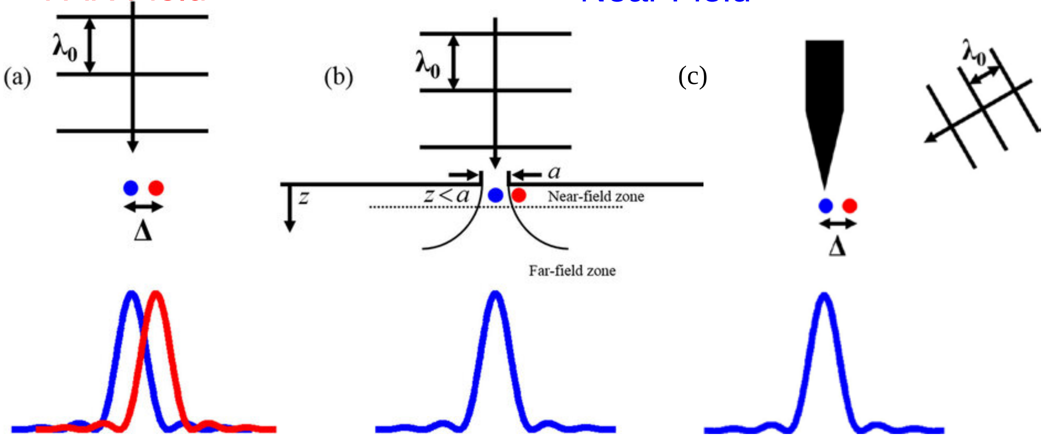
## The Rayleigh Criterion

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




FAR Field

Near Field




**Review of near-field optics and superlenses for sub-diffraction-limited nano-imaging**

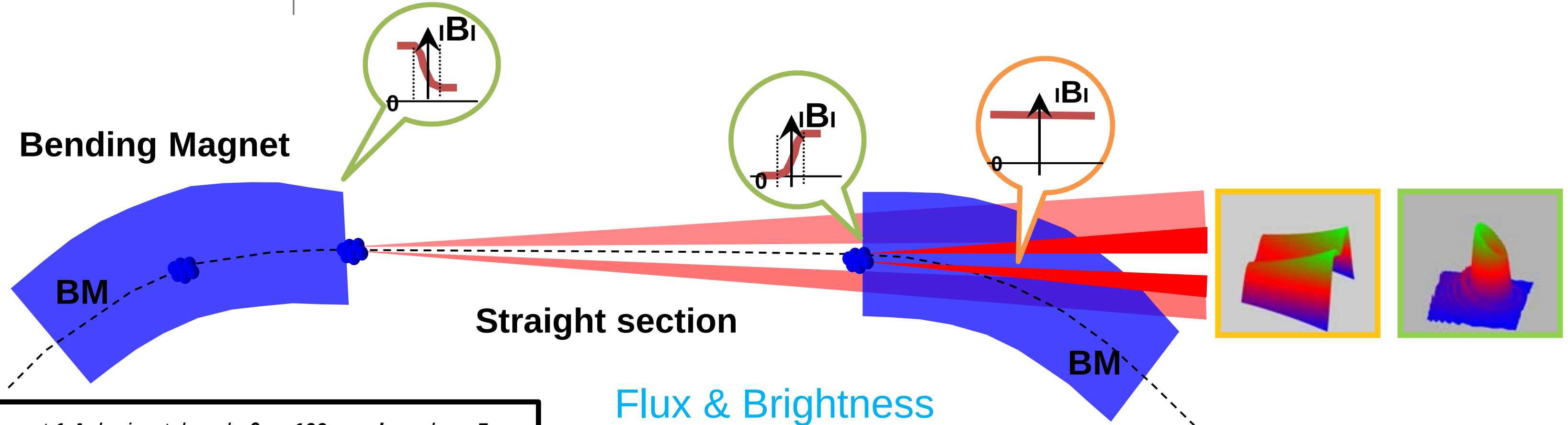
[https://doi.org/10.1063/1.4964498](https://doi.org/10.1063/1.4964498)

(a) Two features of a specimen in free space are separated by a distance **$\Delta<\lambda_0/2$**. When illuminated by a **plane wave**, the resulting intensity pattern in the far-field is unresolved due to the diffraction limit.

(b) **A small aperture of diameter $a\ll\lambda_0$ under the same illumination is brought within a distance $z<a$ to a specimen and scanned in the lateral direction**. Since the near-field radiation pattern is localized to the aperture itself, each feature is illuminated separately provided that *a* should be smaller than **$\Delta$** . After the full scan is completed, the result is a resolved image.

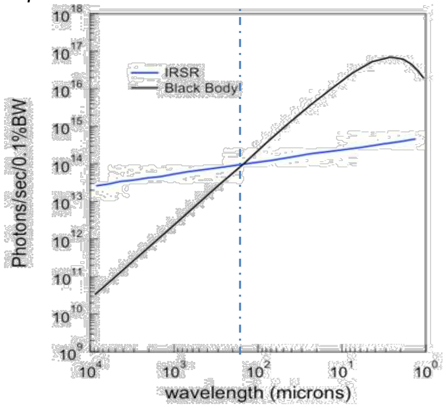
(c) Illustration of the **apertureless near-field concept**. Two features of a sample separated by a distance $\Delta<\lambda_0/2$ can be resolved with local excitation by a sharp probe.

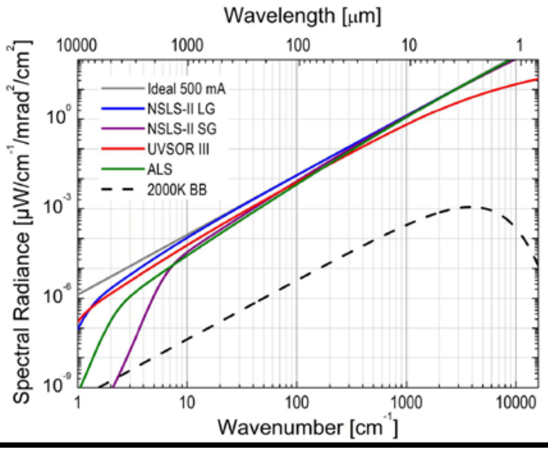
# IR Synchrotron Radiation




## Flux & Brightness

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



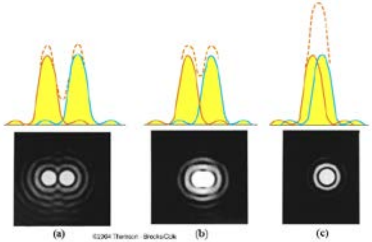



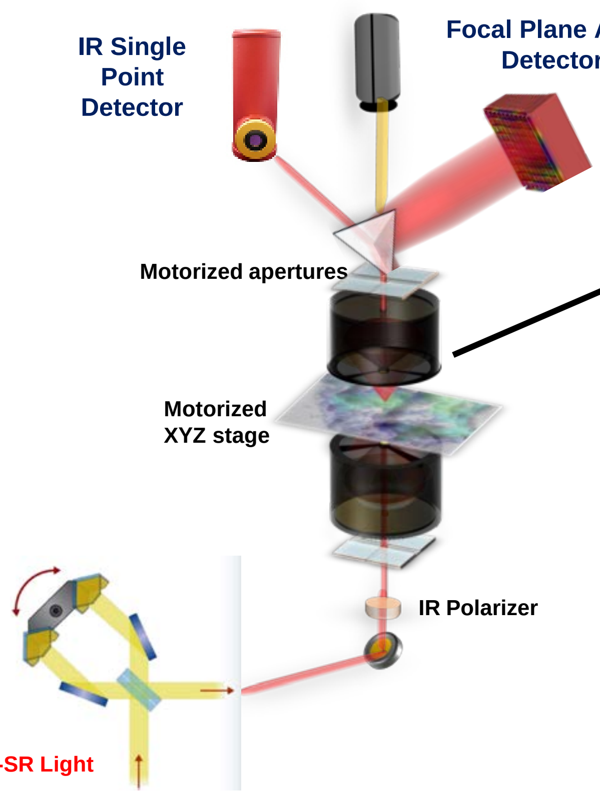

*By: Dumas, P., Martin, M.C., Carr, G.L. (2020). IR Spectroscopy and Spectromicroscopy with Synchrotron Radiation. In: Jaeschke, E., Khan, S., Schneider, J., Hastings, J. (eds) Synchrotron Light Sources and Free-Electron Lasers. Springer, Cham.*

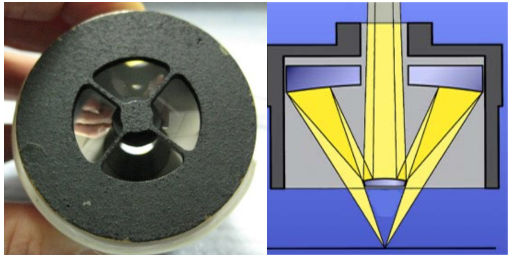
# Far Field FTIR microscopy & imaging

## The Rayleigh Criterion

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



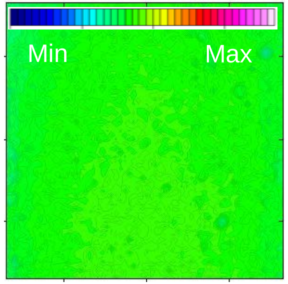



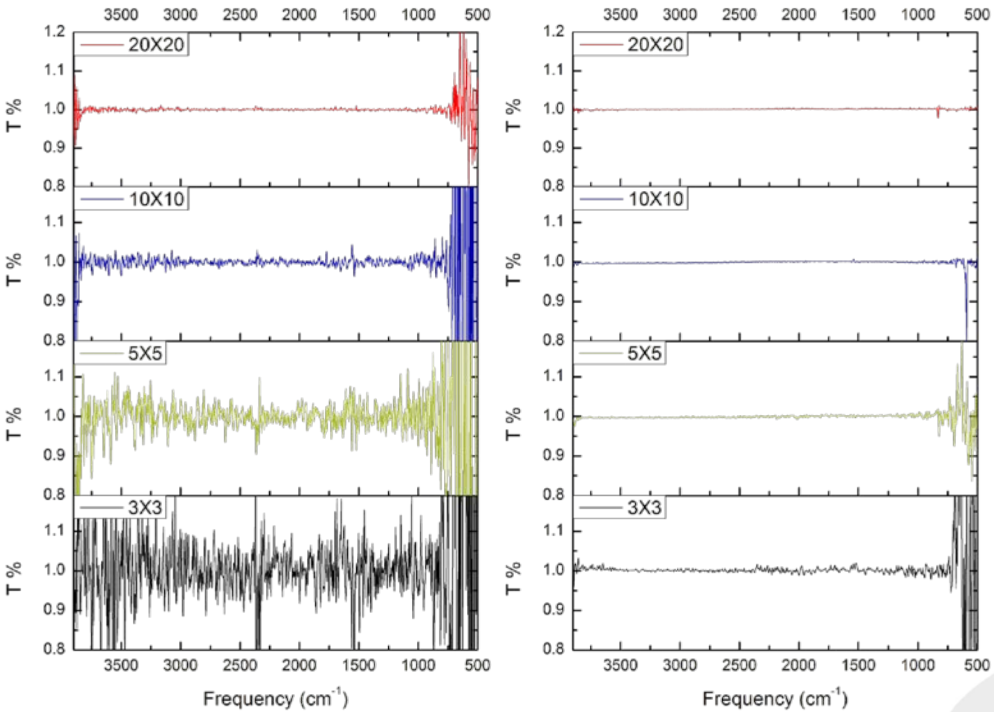
Schwarzschild
or Cassegrain objective
**Reflective Achromatic Optics**

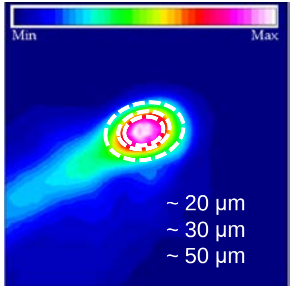
| NA | Wavelength | δ |
|---|---|---|
| 0.65 | 10 μm (1000cm$^{-1}$) | ~ 9.5 μm |
| | 2.5 μm (4000cm$^{-1}$) | ~ 2.5 μm |

# Far Field FTIR microscopy
# SR brightness advantage in practice




Conventional Source



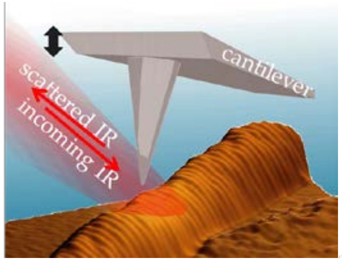



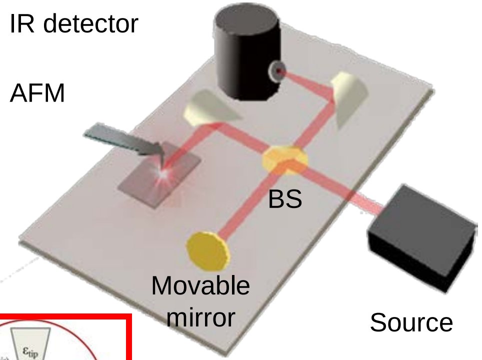

IRSR

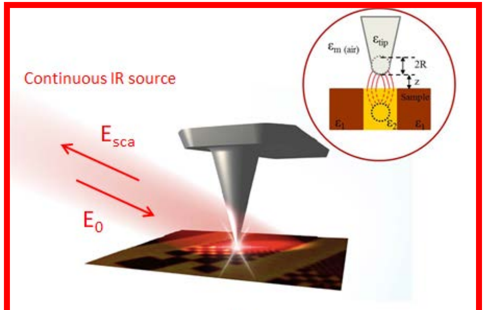
Diffraction Limited FTIR Microscopy is practically achievable only with IRSR

# Near field scattering-type scanning near field optical microscopy

## Near field microscopy $\delta \neq \lambda$




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

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

### 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

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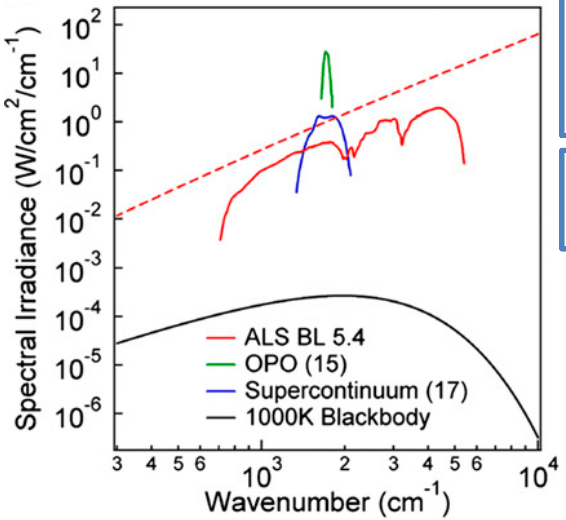
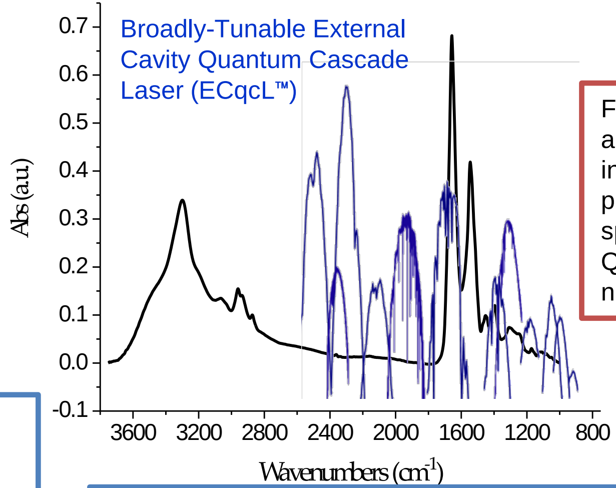



Hans A. Bechtel, Eric A. Muller , Robert L. Olmon, Michael C. Martin and Markus B. Raschke, *Proceedings of the National Academy of Sciences*, 111, 7191–7196 (**2014**)

- 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

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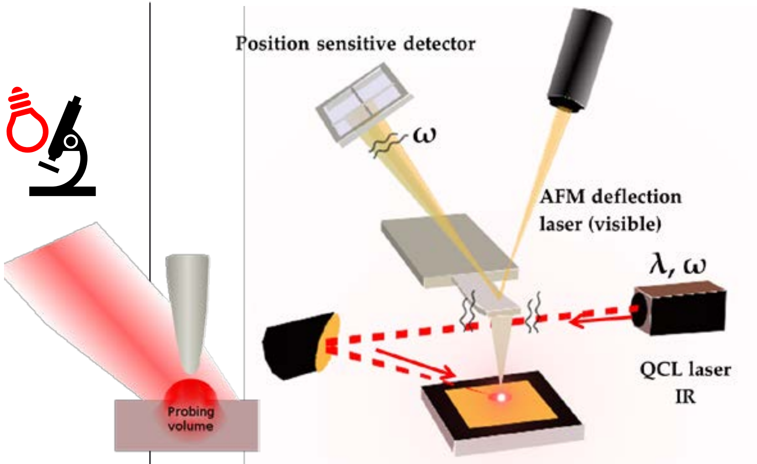
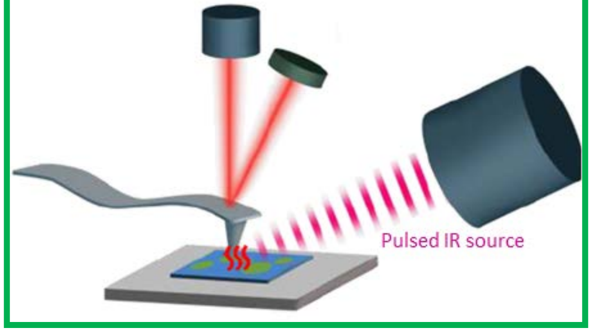



Near field microscopy $\delta \neq \lambda$

## PTE

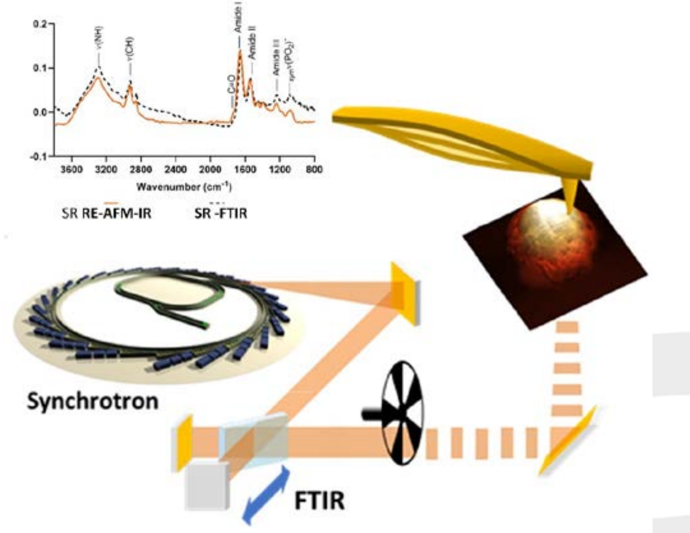
- resolution > 20 nm (tip diameter)
- samples with high thermal expansion coefficient
- penetration depth > 100 nm
- Spectroscopy, Imaging -> Tunable IR laser & Chopped IRSR + step scan interferometer

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

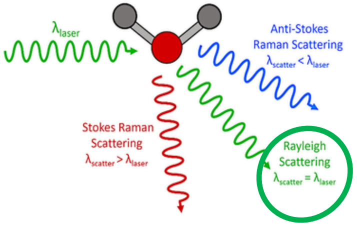


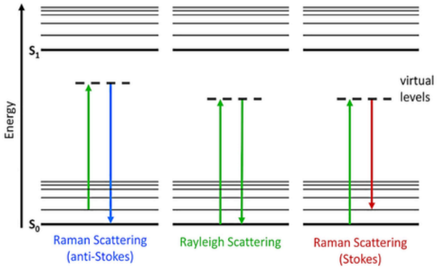

***Synchrotron Photothermal Infrared Nanospectroscopy of Drug-Induced Phospholipidosis in Macrophages***

K.L.A. Chan, et al., *Anal. Chem.* (2020) 92 (12): 8097–8107.(**2020**)

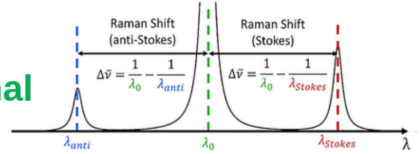


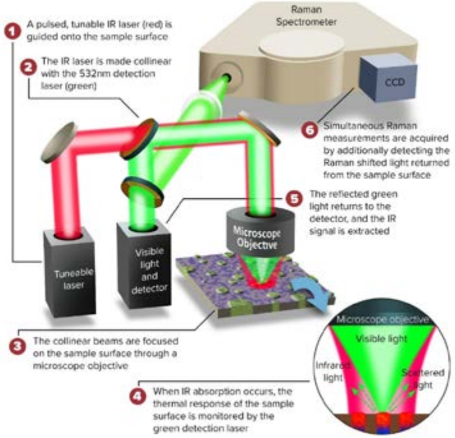
# Far Field Optical PhotoThermal IR



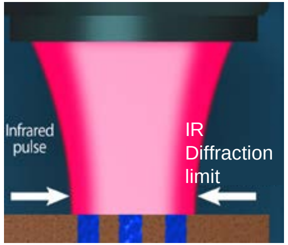




**O-PTIR Signal**

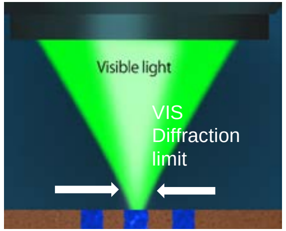


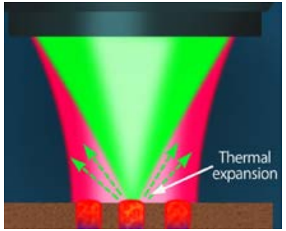

- IR pump
- Vis laser probe



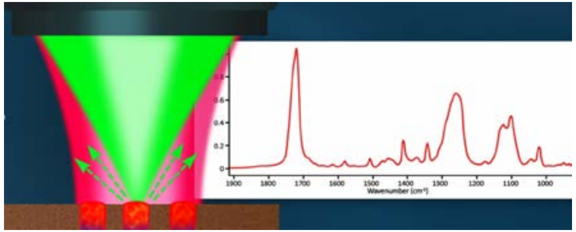

Step 1.
Pulsed, tunable, infrared laser source (pump) is focused through a reflective (Cassegrain) objective

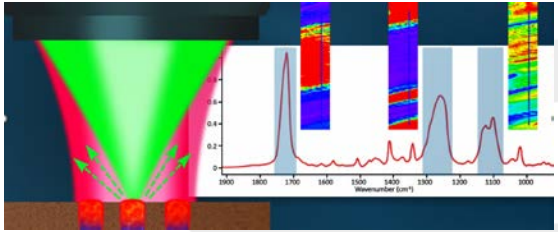



Step 2.
Colinearly introduce a visible laser, typically a 532 nm green Raman excitation laser (probe) through same objective

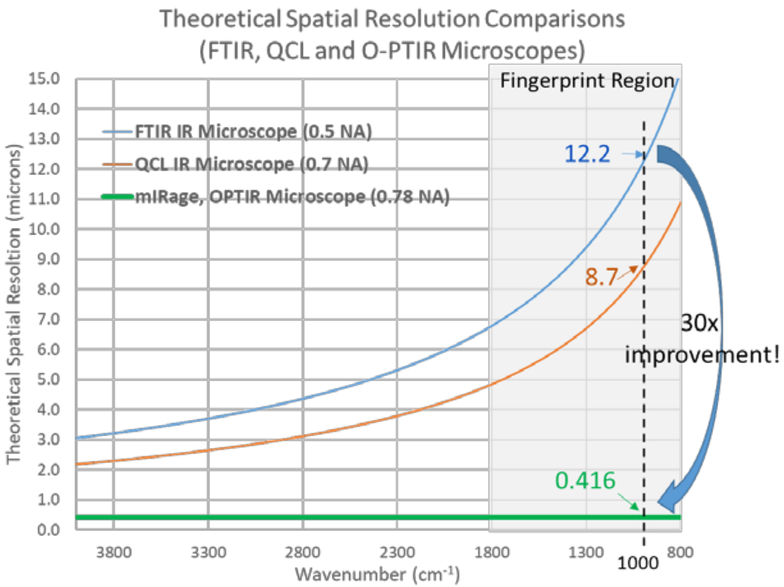



Step 3.
Detect green reflected (or transmitted) as a function of IR laser wavelength tuning
When the wavelength matches an absorption band in the sample the IR energy is absorbed causing a **photothermal response** (Expansion, refractive index variation) in the sample, thus changing its green reflectivity





# Far Field Optical PhotoThermal IR



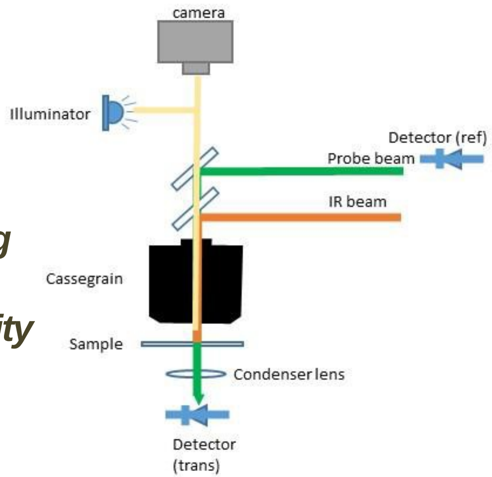

For typical FTIR Microscope (@ 1000cm$^{-1}$, 10micron);
Spatial Resolution = 0.61 x 10/ 0.5 = **12.2 microns**

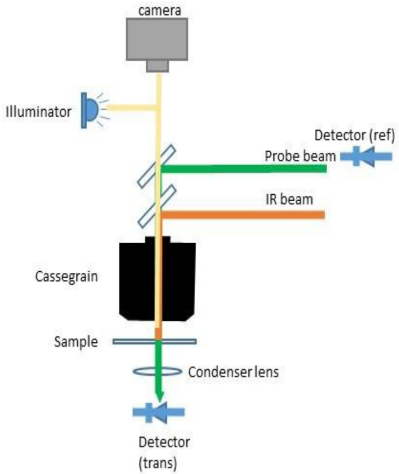
For the mIRage® O-PTIR (across entire spectrum);
Spatial Resolution = 0.61 x 0.532/ 0.80 = **406nm**

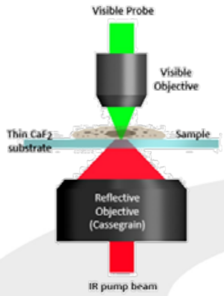
O-PTIR has up to **30x** better spatial resolution

***Co-propagating***
***&***
***Counter-propagating***

***Versatility and flexibility***



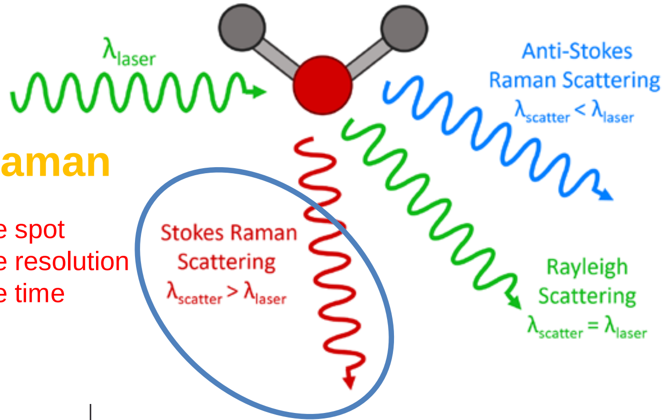



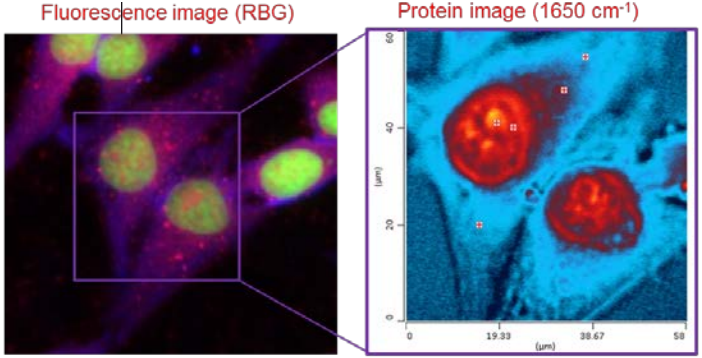
# O-PTIR Correlative measures

## IR+Raman

- Same spot
- Same resolution
- Same time

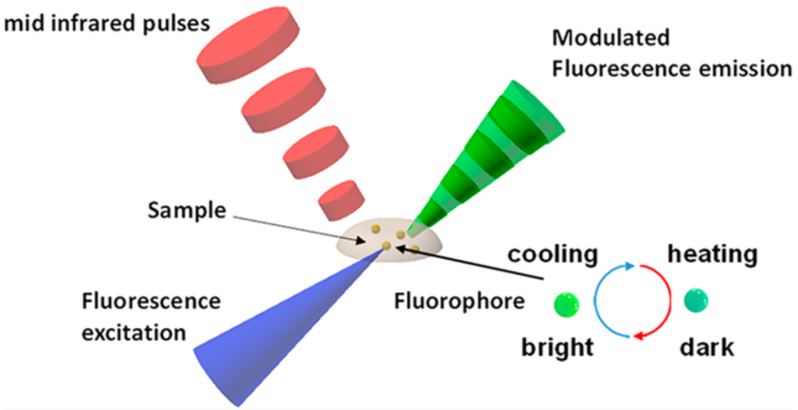



## IR + Visible Fluorescence




DAPI - nucleic acid, AF488 – lipid, AF647 - protein

## Widefield O-PTIR (Fluorescence detected-PTIR)




Y. Zhang, et al., *JACS* 143 (30): 11490 – 11499 .(**2021**)

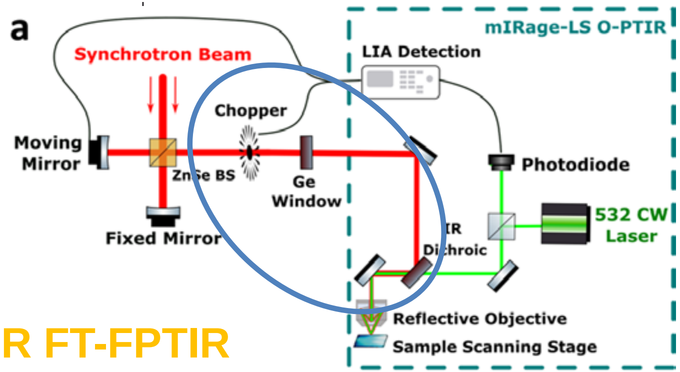
Conventional O-PTIR effect induces only a tiny change in the intensity and angular distribution of the scattered probe light due to the weak thermal dependence of the particle size and refractive index. Typical fractional change is on the order of **$10^{-4}$/K**.

The temperature dependent modulation of the (auto-)fluorescence emission roughly **100X larger** than the typical photothermal sensitivity of biological materials based on thermal expansion and/or index of refraction changes (of order $10^{-4}$/° C). This strong temperature dependence makes it possible to use (auto-)fluorescent emission as a reporter of temperature increases associated with infrared absorption,

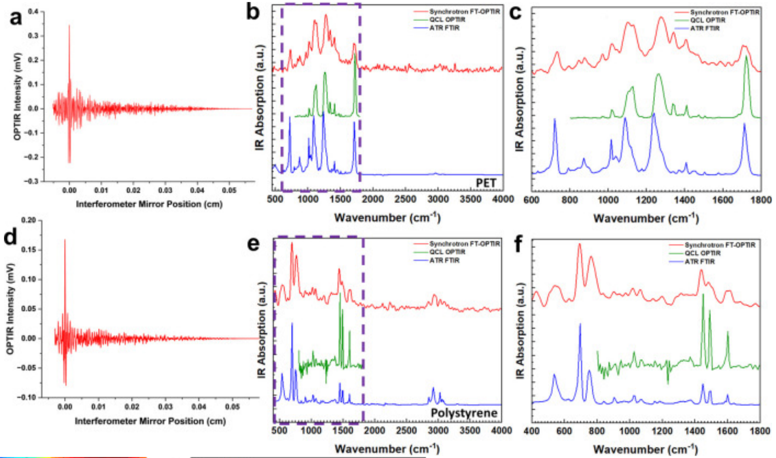
# SR FT-PTIR & SR FT-FPTIR

**Broadband Fourier-Transform Optical Photothermal Infrared Spectroscopy and Imaging**
A. RazumtcevZhang, et al., *Anal. Chem.* 97 (37): 20117 - 20126 (**2025**)

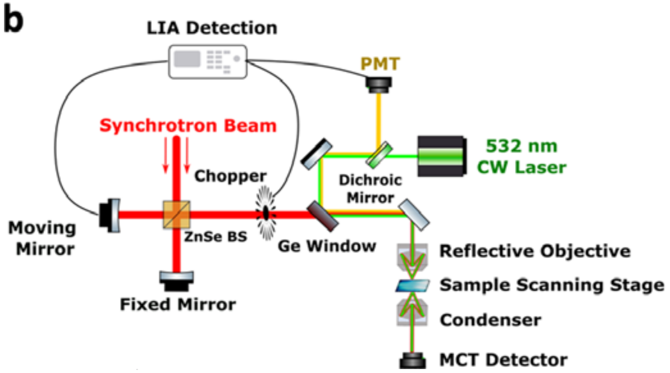
## SR FT-PTIR

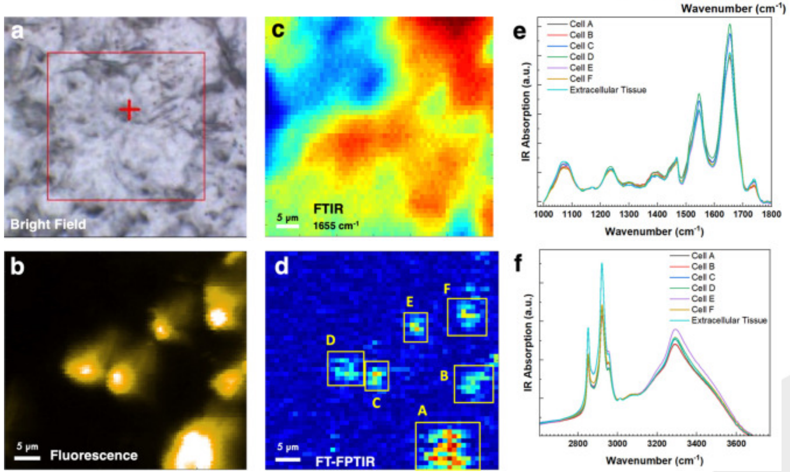



SR FT-PTIR, QCL OPTIR and ATR FTIR of PET (a–c) and PS (d–f)




## SR FT-FPTIR




Cell-specific broadband FT-FPTIR chemical imaging of a fluorescently labeled mouse tissue section.

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# SISSI-Bio – Fields of application

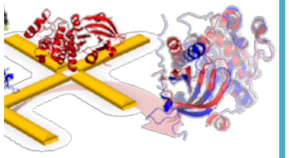
## *(Dry) Cell and tissue mapping*

***From diag***

Cell Dynamics

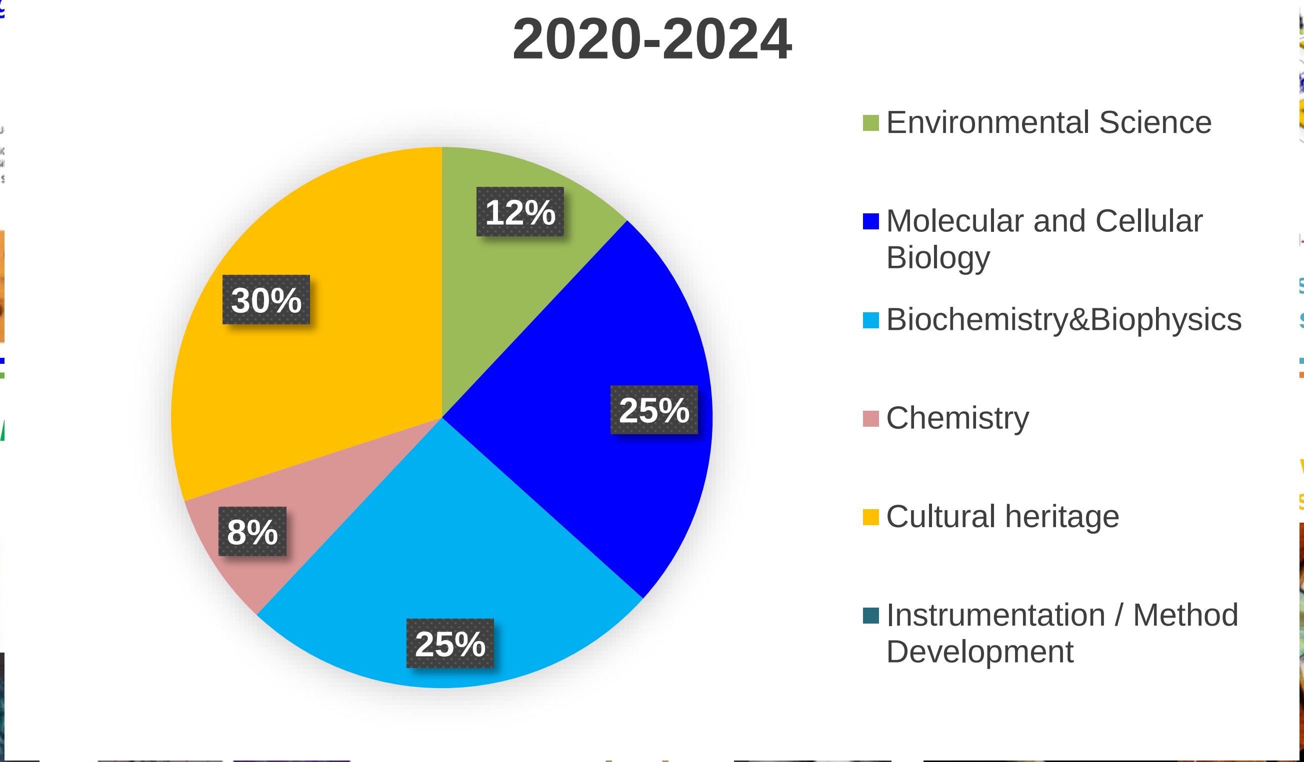

Neurodegenerative disorders
Rare diseases

## *Biochemistry and Biophysics*



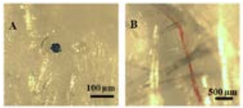
-IR

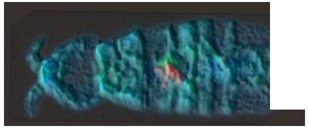
smonic for bio-
sical studies

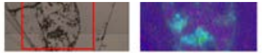



## *Envi*

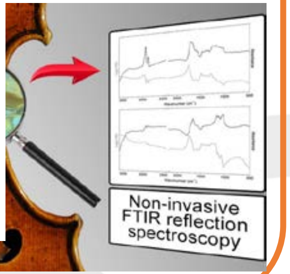
**Microplastics in Antarctic**





wed string
struments

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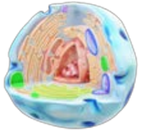
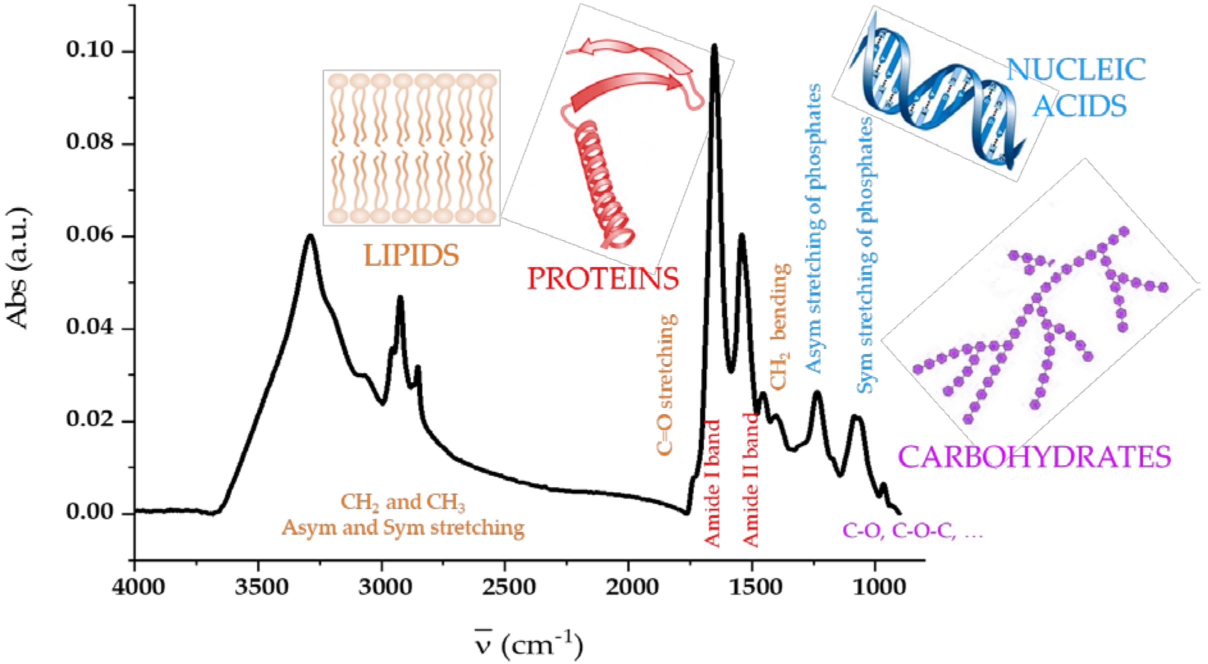
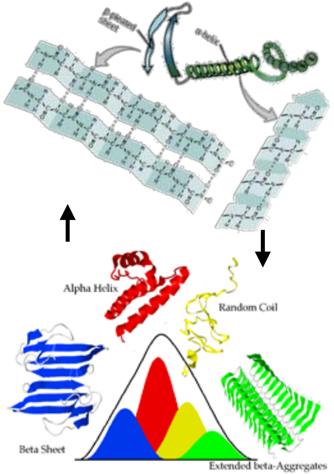
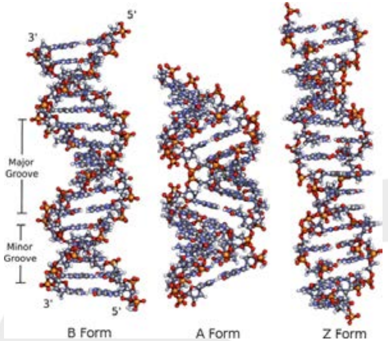
# IRSR Cytology and Histology

*Single Cell IR micro-spectrum*



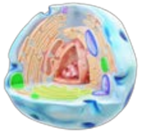


# IRSR Cytology
# Soft X-ray radiation damage

*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

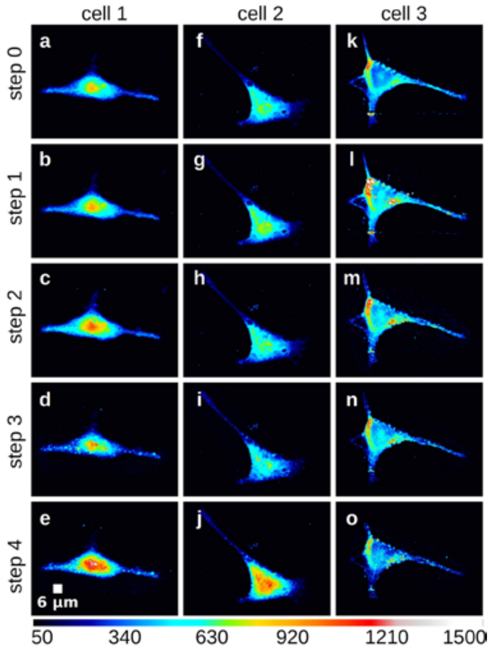
**Step 1** — Cell drying in vacuum @ TwinMic
($p < 10^{-5}$ mbar) for 1:30 hour

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

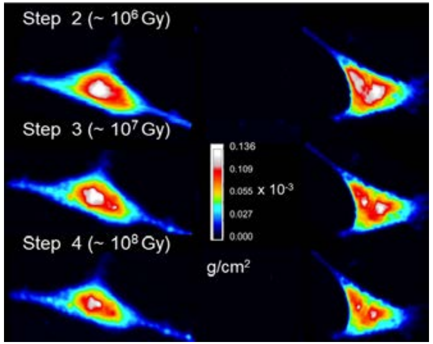
**Step 3** — High Dose STXM mapping @ TwinMic 1 keV
Estimated dose: $2{*}10^7$ Gy
Cumulative estimated dose: $2.2{*}10^7$ Gy

**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

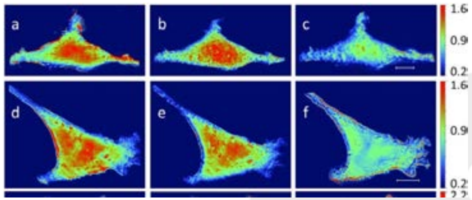



## STXM




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

## STXM & AFM




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

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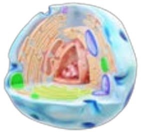
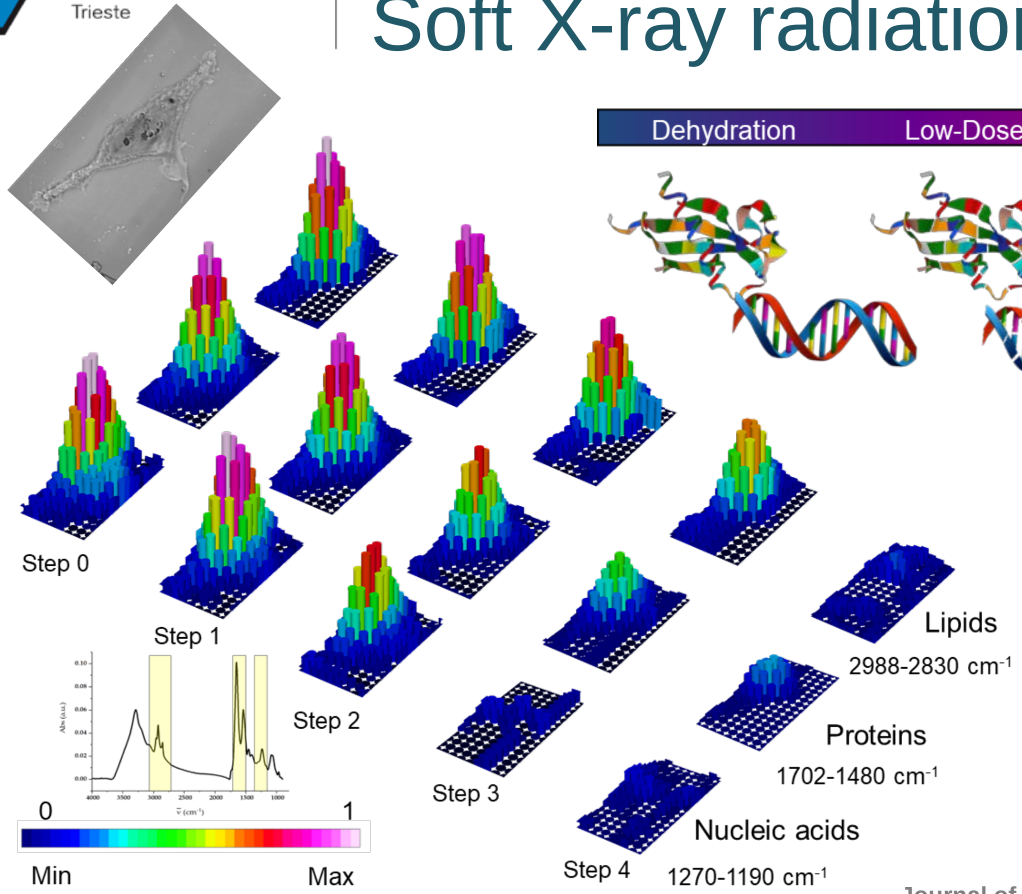
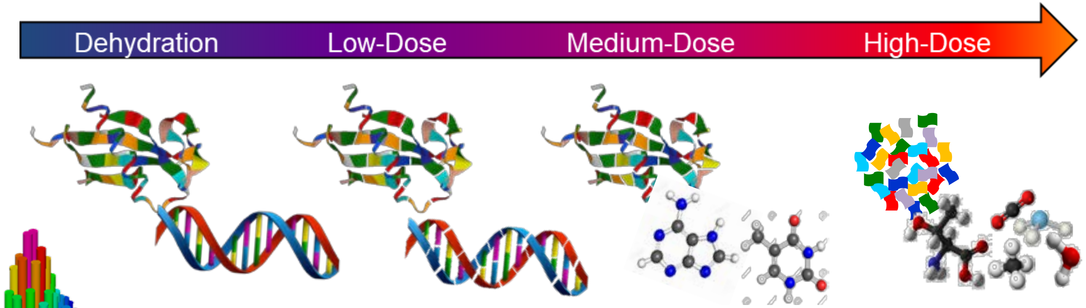
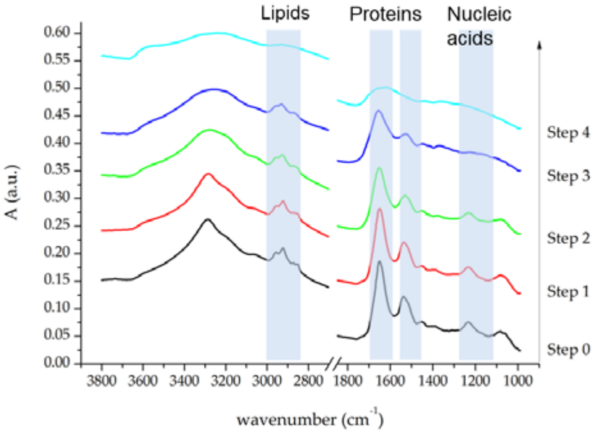
# IRSR Citology
# Soft X-ray radiation damage



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

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

Elettra Sincrotrone Trieste

analytical chemistry

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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

Cite This: https://doi.org/10.1021/acs.analchem.2c02965 Read Online


San Lorenzo 1718 violin, Stradivari


Toscano 1690 violin, Stradivari

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# IR for Cultural Heritage

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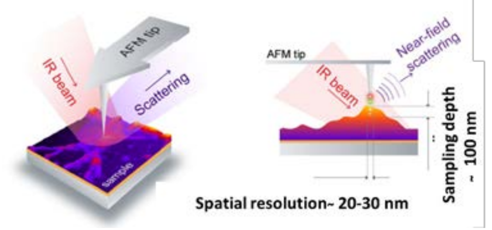


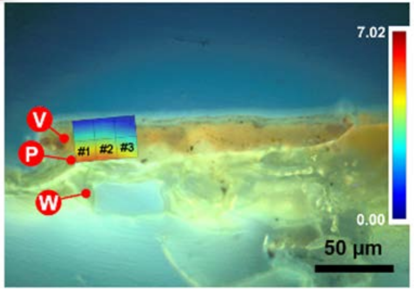


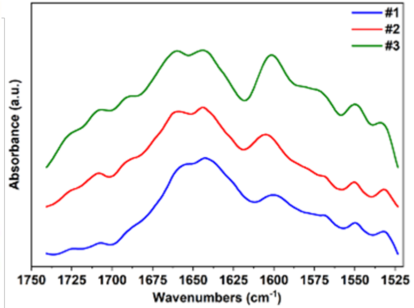


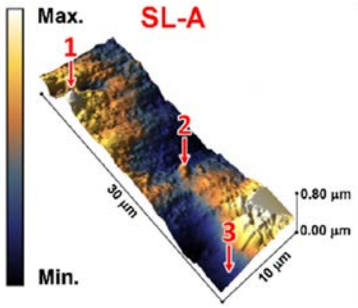


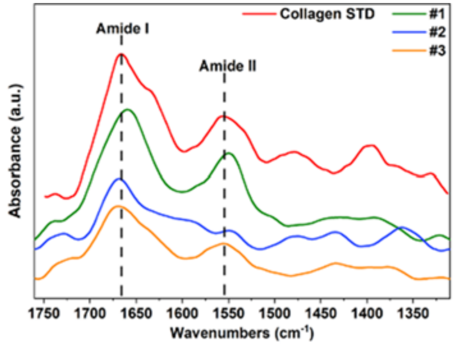


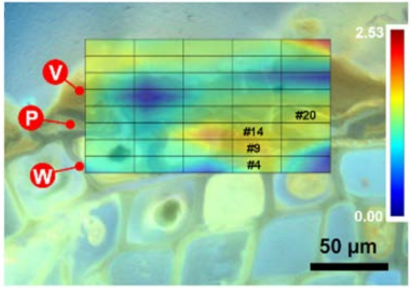


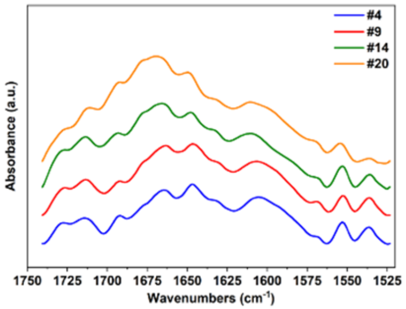


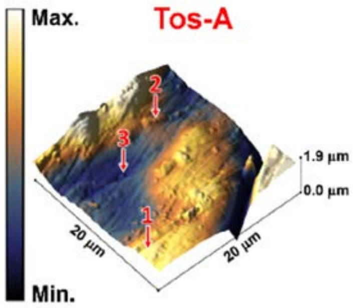


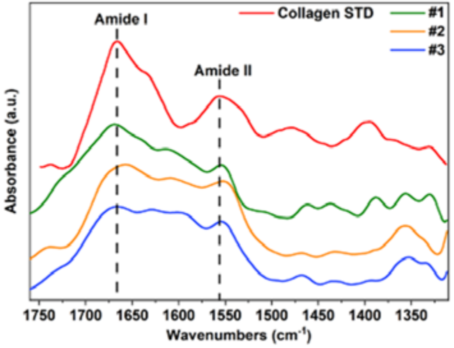


Stani et al. - A Nanofocused Light on Stradivari Violins: Infrared s-SNOM Reveals New Clues Behind Craftsmanship Mastery. Analytical Chemistry. 2022, 94, 43, 14815–14819 - doi.org/10.1021/acs.analchem.2c02965


# Thank for your attention

lisa.vaccari@elettra.eu