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Optical Biosensors: Vibrational Spectroscopy – based Detection

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Content

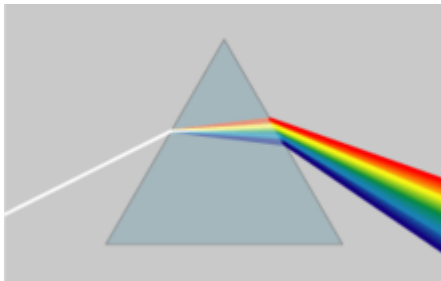
- **Implementation for fingerprinting of molecular species, complementarity of Raman and infrared absorption spectroscopy.**
- **Optical configurations used for the IR absorption.**
- **Amplification of weak IR absorption signal - SEIRA.**
- **Raman scattering, Amplification of weak Raman signal by using metallic nanostructures.**
- **Raman labels serving in assays.**
- **Optical configurations used for the Raman scattering detection.**

Refractive Index

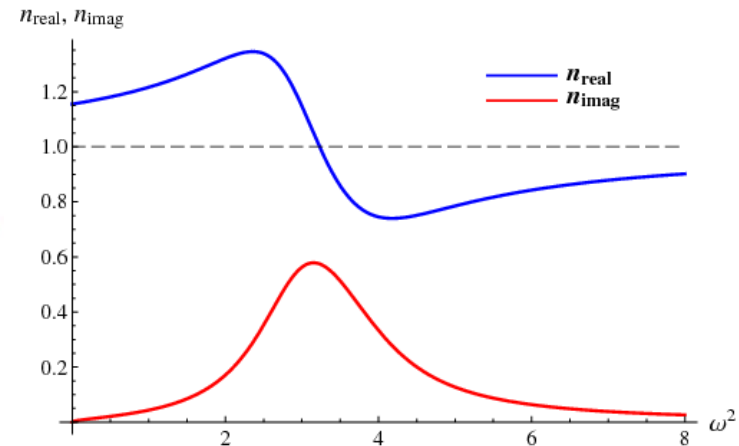
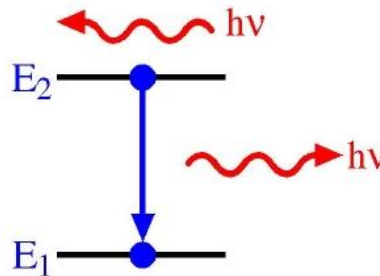
Parameter that describes interaction of light with matter composed of elements (e.g. atoms) that are $\ll \lambda$ and exhibit polarizability. By averaging over many atoms that are be polarized by the oscillating electric field.

$$\tilde{n} = n + ik$$

Real part – refraction



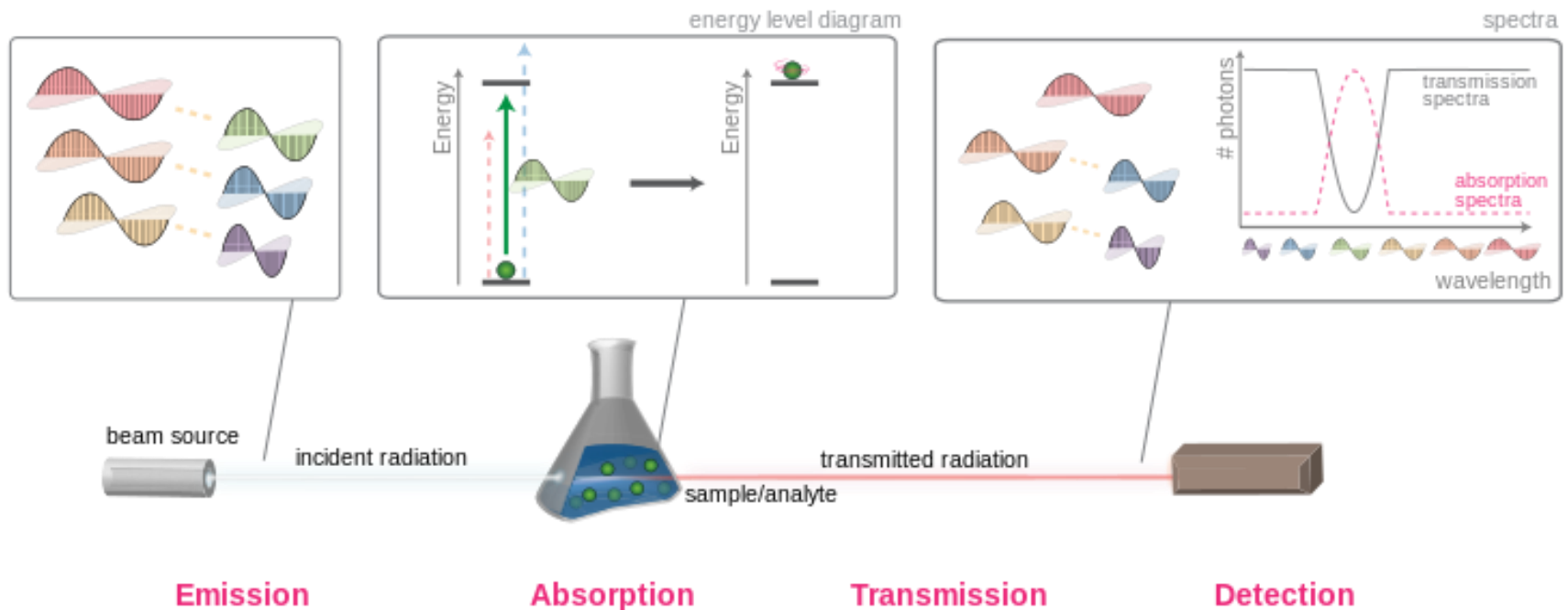
Imaginary part - absorption



For more details, see Lorenz Lorenz or Clausius-Mossotti theories.

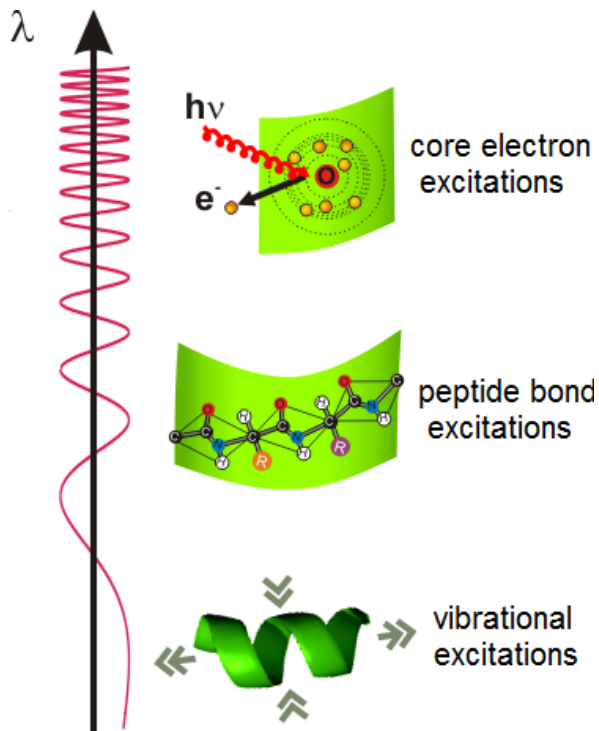
Absorption of Molecules

Absorption of light by molecules is accompanied with their transition from a ground state to excited state (followed by a relaxation). It typically occurs at distinct energies leading to specific bands in absorption spectrum.



E.g. is routinely used for measuring total concentration of proteins – UV absorption spectroscopy.

Absorption of Molecules - Wavelength Spectrum



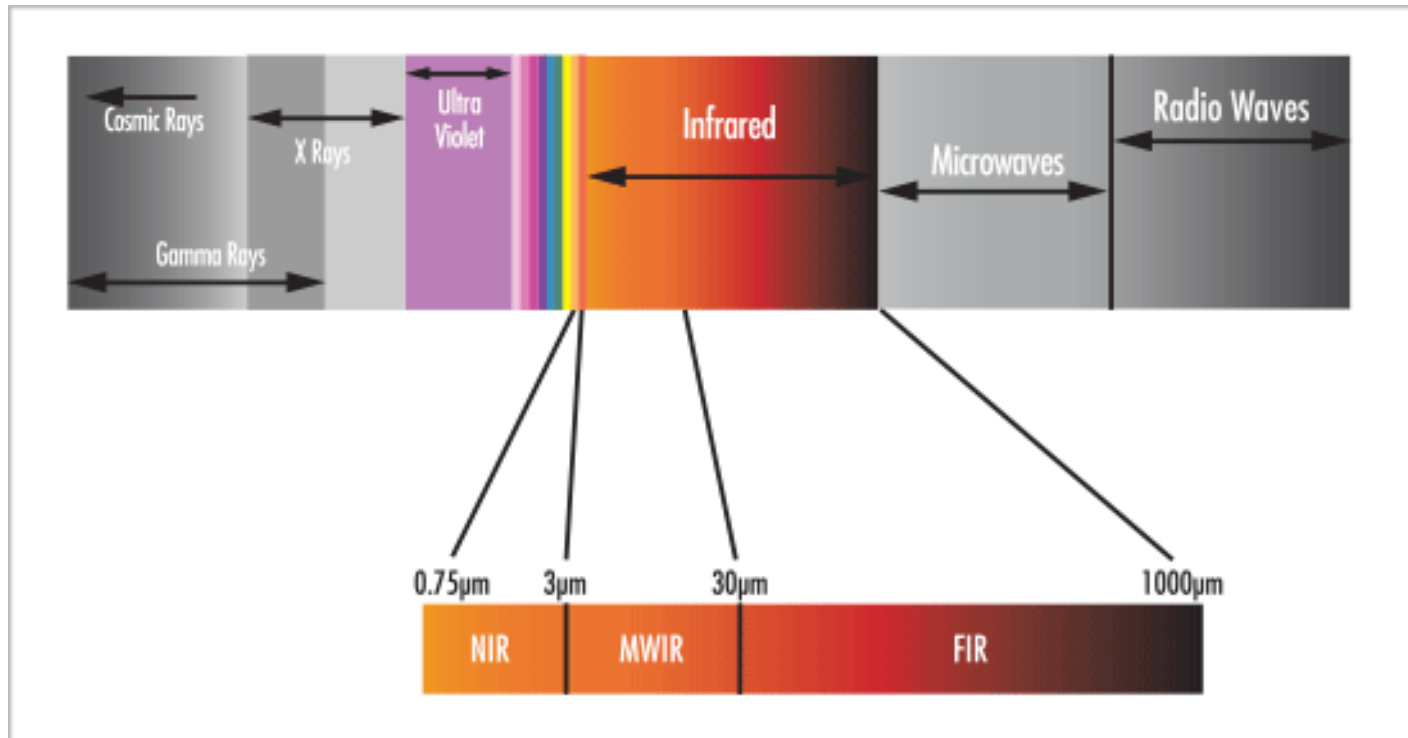
Electronic lines correspond to a change in the electronic state of an atom or molecule. Typically UV-Vis.

Vibrational lines correspond to changes in the vibrational state of the molecule and are typically found in the infrared region.

Rotational lines, for instance, occur when the rotational state of a molecule is changed. Rotational lines are typically found in the microwave spectral region.

Combination of above can lead to rather complex spectra.

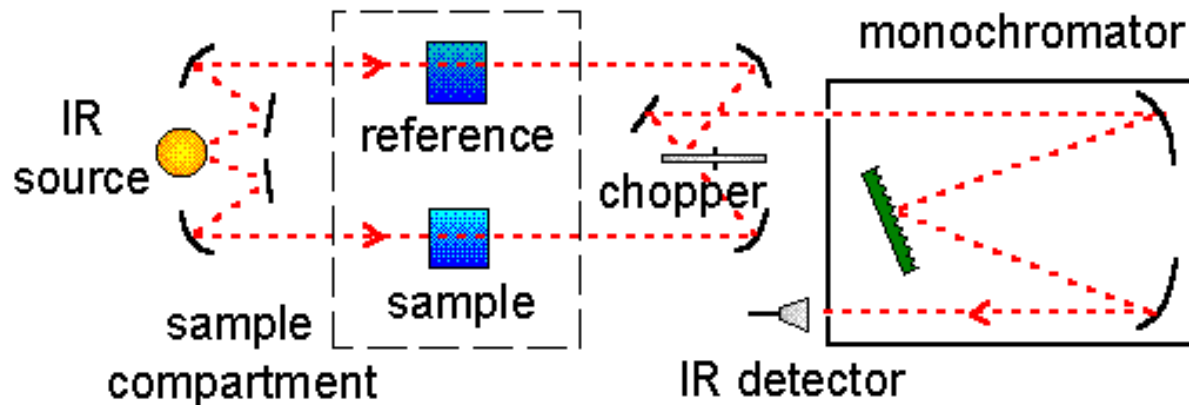
IR Spectral Range



www.edmundoptics.com

➡ Measuring in the spectral range of several – tens of μm

IR Absorption Spectroscopy

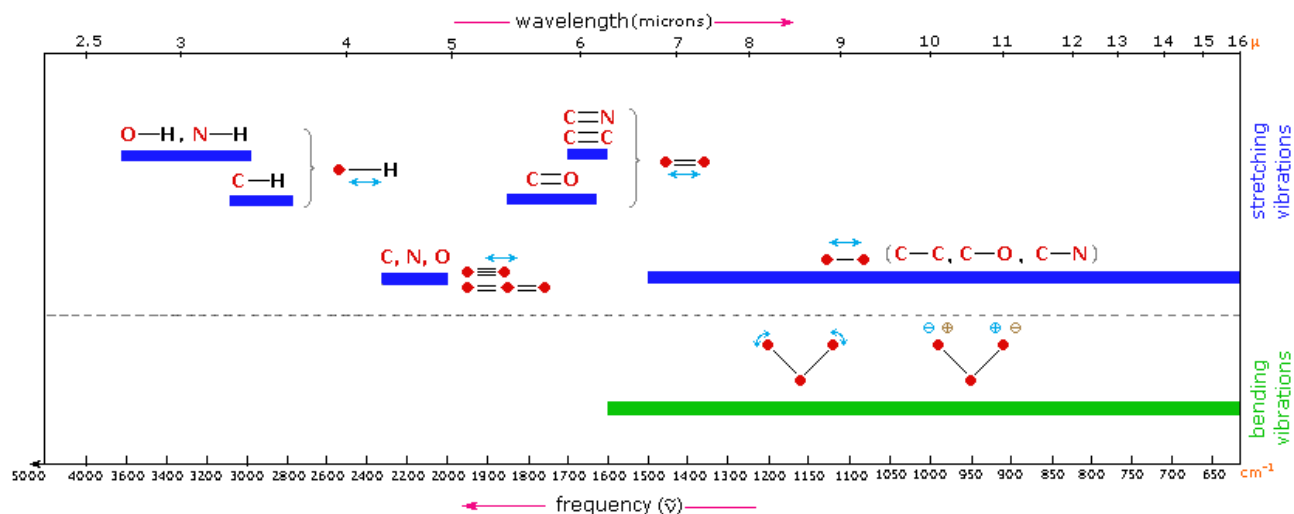
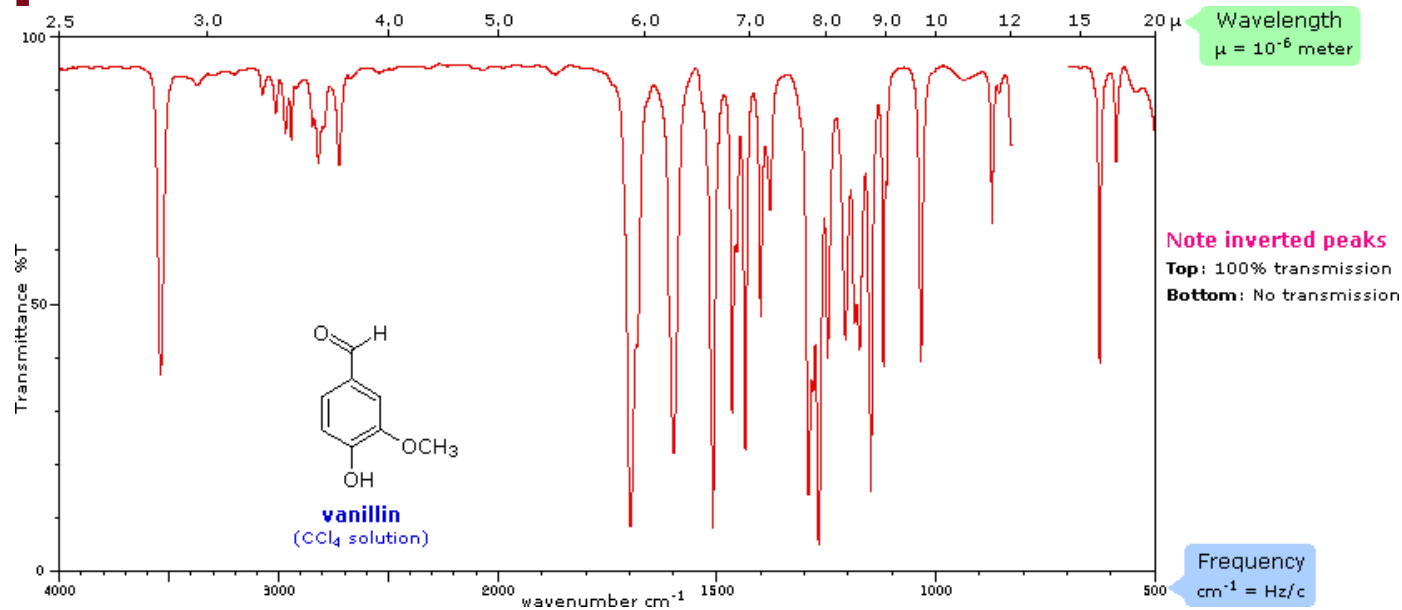


©1995 CHP

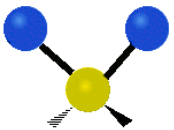
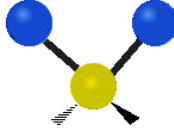
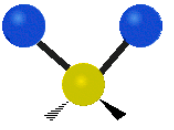
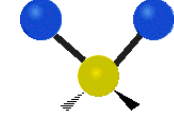
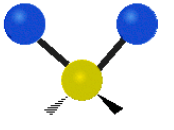
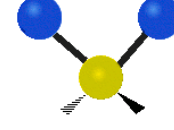
http://www.pci.tu-bs.de/aggericke/PC4/Kap_1/ir-instr.htm

- ➡ As a light-source, a polychromatic beam can be used on conjunction with a spectrometer (typically FTIR) or a monochromatic beam at a wavelength tuned for selected bands (quantum cascade lasers, monochromator)
- ➡ Detectors based on semiconductors with low bandgap (PbS, InGaAs,) or bolometers.

IR Spectra

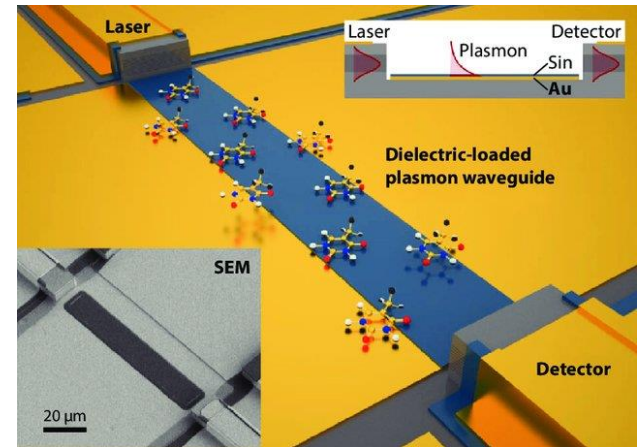
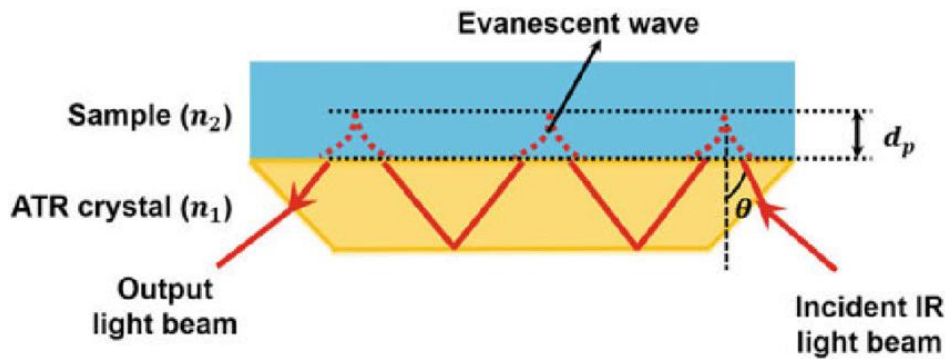


Examples of Vibration Modes

Symmetry Direction	Symmetric	Antisymmetric
Radial	 <p>Symmetric stretching (ν_s)</p>	 <p>Antisymmetric stretching (ν_{as})</p>
Latitudinal	 <p>Scissoring (δ)</p>	 <p>Rocking (ρ)</p>
Longitudinal	 <p>Wagging (ω)</p>	 <p>Twisting (τ)</p>

- ➔ IR bands arise with the transition from a ground state to higher vibrational state of specific modes.
- ➔ Symmetry matters and some transitions are not allowed (symmetrical molecules seen in Raman, not in IR). Selection rules.
- ➔ The bigger molecule, the more complex is the spectrum.

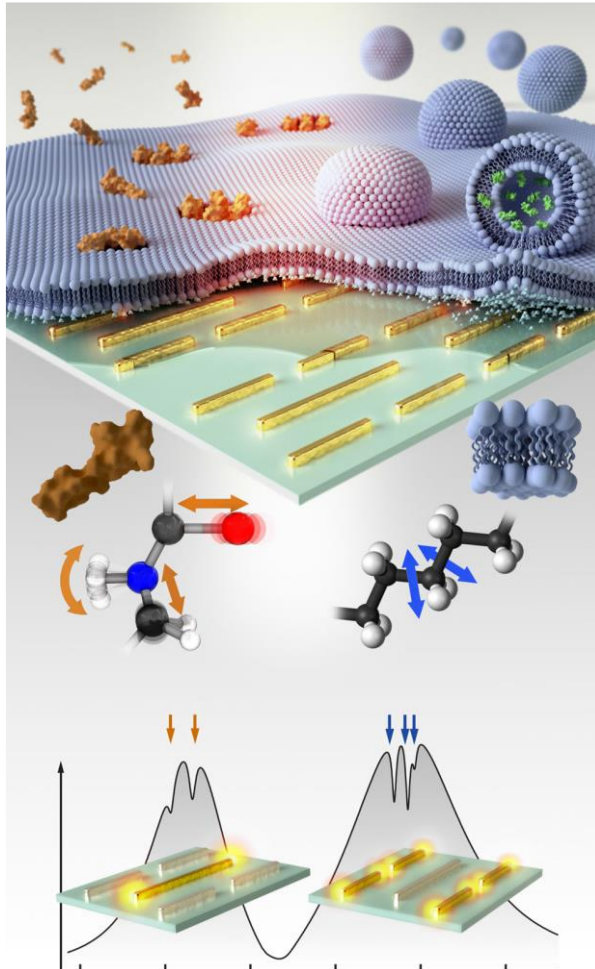
Multiple Reflection IR Absorption Spectroscopy



Strasser Lab.

- ➡ IR absorption from monolayers is possible to measure by using evanescent field.
- ➡ Multiple reflections allow to increase the sensitivity, alternatively a monolithic integration is possible by using surface plasmons

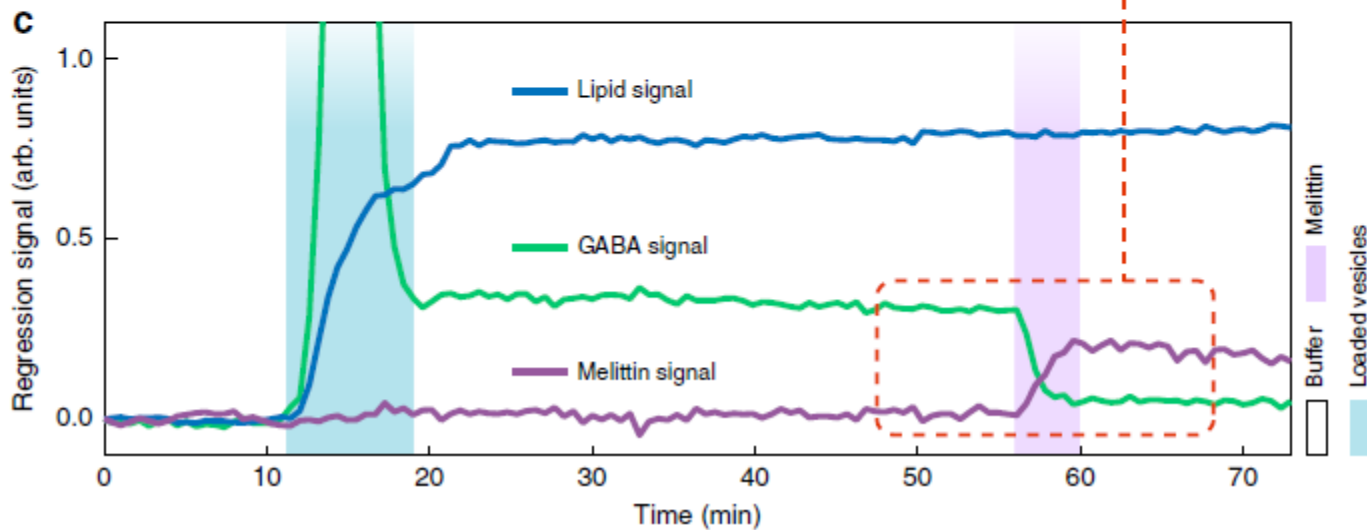
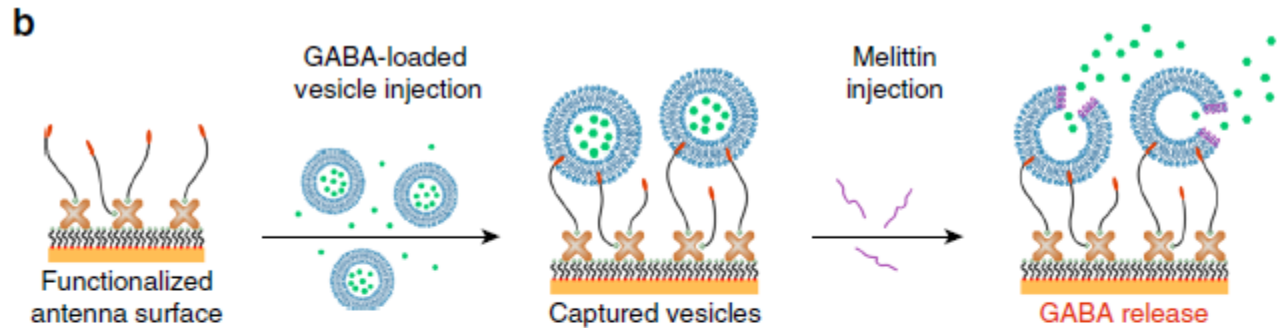
SEIRA on Engineered Antennas



- ➔ Arrays of plasmonic antennas tuned to excite localized surface plasmons at specific spectral bands.
- ➔ Antenna resonance positions are engineered to simultaneously overlap with the vibrational signatures of both the amide I, II, and the CH_2 , CH_3 absorption bands, allowing for the simultaneous enhancement and detection of lipid- and protein-induced absorption changes.

<https://www.nature.com/articles/s41467-018-04594-x>

SEIRA on Engineered Antennas



➡ Monitoring of cargo release from tethered lipid vesicles.

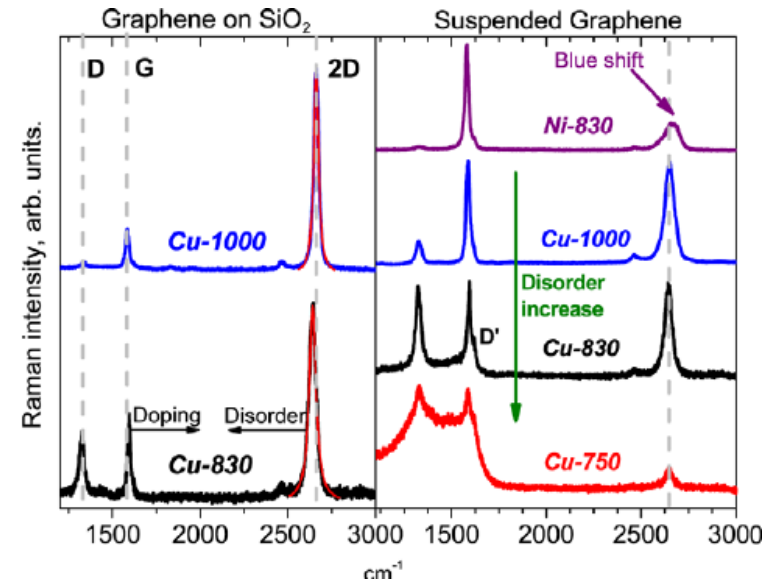
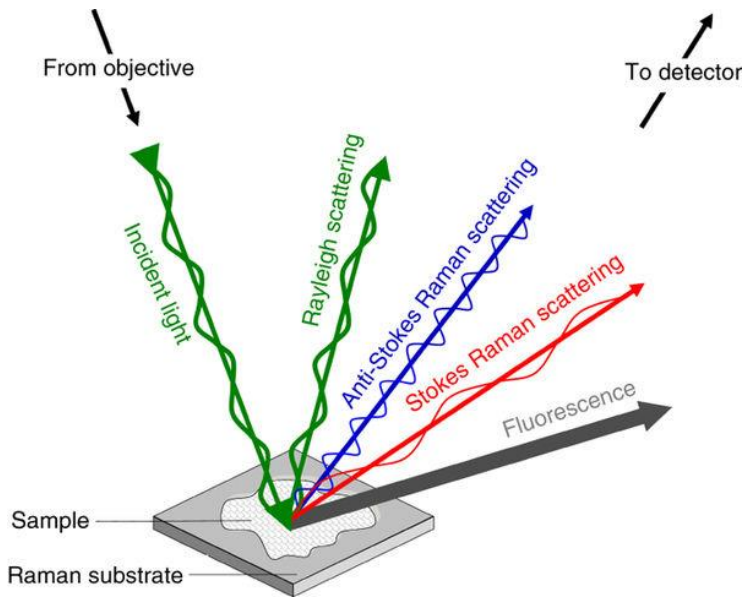
Raman Scattering

Raman scattering probability $\sim E^4$ Field intensity

IR absorption rate $\sim E^2$



Raman is much weaker effect (often masked by fluorescence) compared to IR.



DOI: 10.1088/0957-4484/22/27/275716

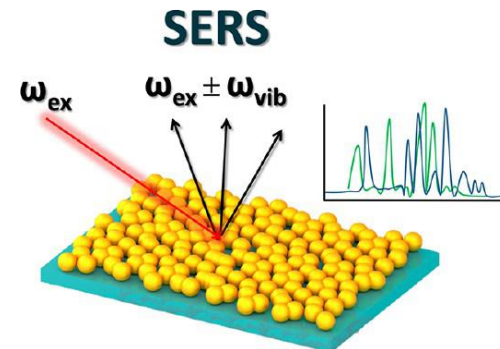
High EM Field Intensity

The description of refractive index independently on the electromagnetic field is valid for weak optical fields. For high field intensities E the polarizability can be treated in form of Taylor series:

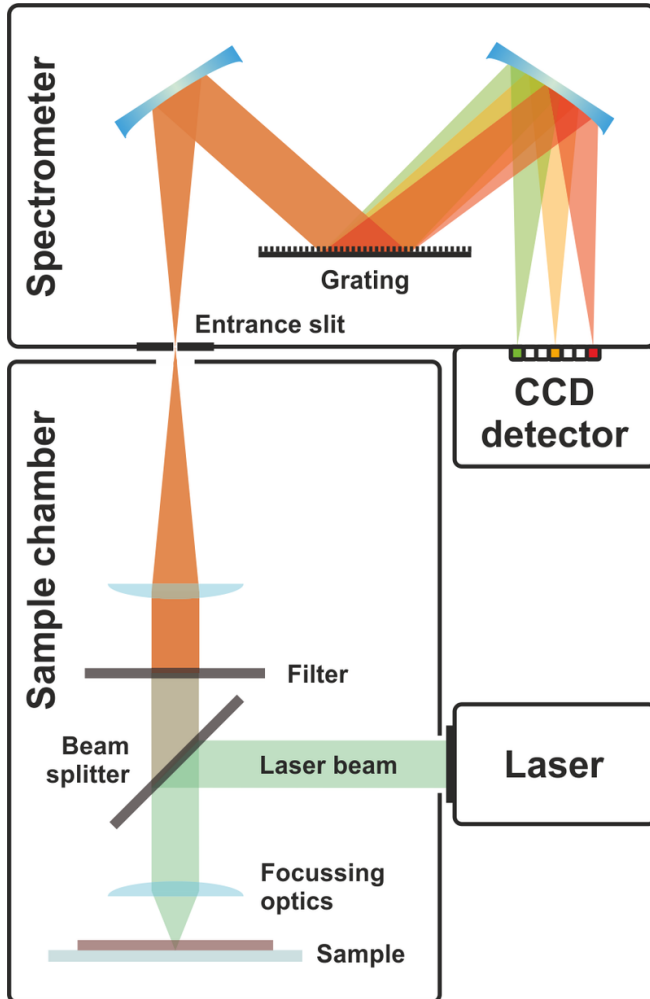
$$P_i = \varepsilon_0 \left[\sum_j \chi_{ij}^{(1)} E_j + \frac{1}{2} \sum_{j,k} \chi_{ijk}^{(2)} E_j E_k + \frac{1}{6} \sum_{j,k,l} \chi_{ijkl}^{(3)} E_j E_k E_l + \dots \right]$$

The higher order susceptibilities $\chi^{(2),(3)\dots}$ lead to non-linear optical effects such as:

- Second harmonic generation $\chi^{(2)}$
- Raman scattering $\chi^{(3)}$
-



Raman Spectroscopy



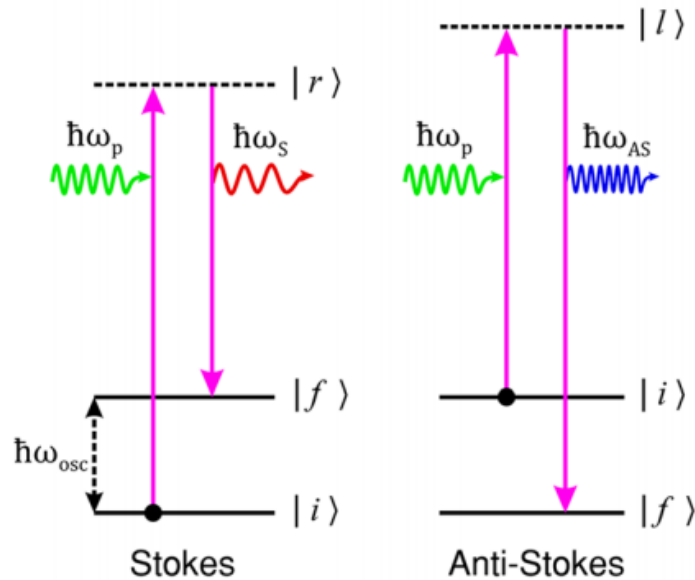
Raman scattering probability $\sim \lambda^{-4}$

Weak intensity – using of single photon detectors like cooled CCD.

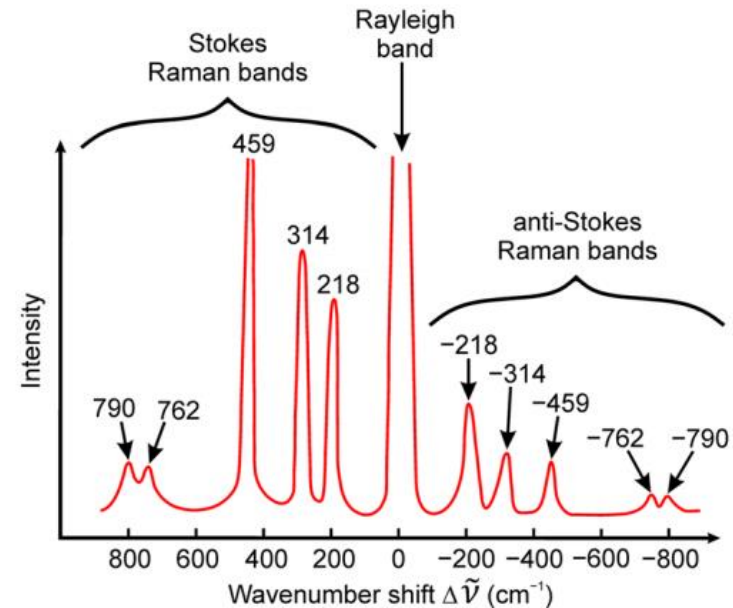
Efficient filtering of the background signal – using of notch filters, confocal configuration.

Typically long integration times are needed to collect a signal in counts per second, operation in a dark room...

Raman Spectra



<https://doi.org/10.1186/s11671-019-3039-2>



Ratio of Stokes and anti-Stokes band intensity:

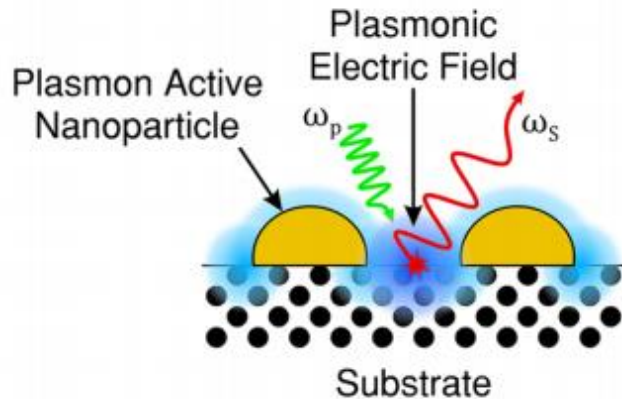
$$\frac{I_{AS}}{I_S} = \left(\frac{\omega_p + \omega_{osc}}{\omega_p - \omega_{osc}} \right)^4 e^{\left(-\frac{\hbar\omega_{osc}}{kT} \right)}$$

The spectra of plotted as depending on energy shift in cm^{-1} :

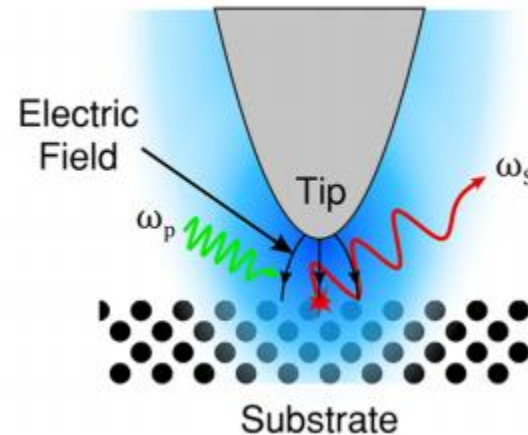
$$E = \hbar\omega = h\nu = h \frac{c}{\lambda} \quad \Delta E \sim \frac{1}{\lambda_1} - \frac{1}{\lambda_2}$$

Amplification of Raman Scattering

<https://doi.org/10.1186/s11671-019-3039-2>



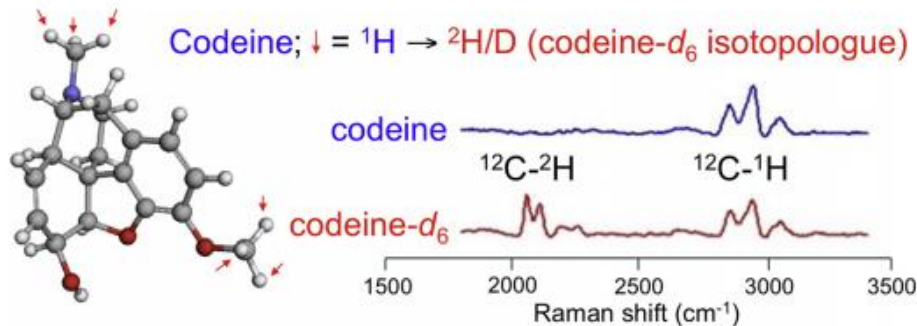
Surface-enhanced Raman spectroscopy (SERS)



Tip-enhanced Raman spectroscopy (TERS)

Analyzed molecules are adsorbed at the surface of plasmonic nanostructures with tuned resonant properties with respect to ω_p and ω_s .

SERS Chemosensors

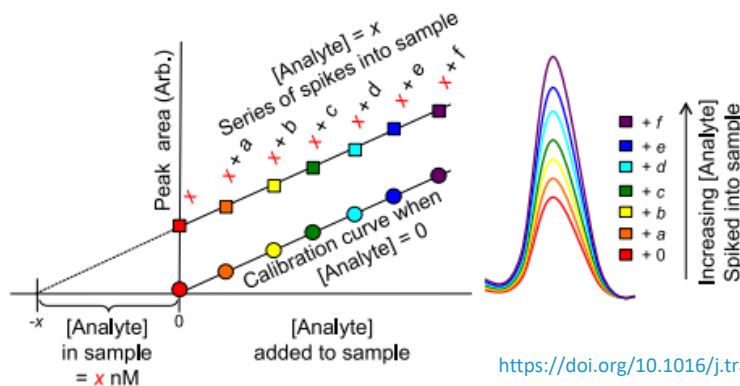


$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad \rightarrow \quad \mu = \frac{m_1 m_2}{m_1 + m_2}$$

Where: ν = vibrational frequency
 k = force constant
 μ = reduced mass

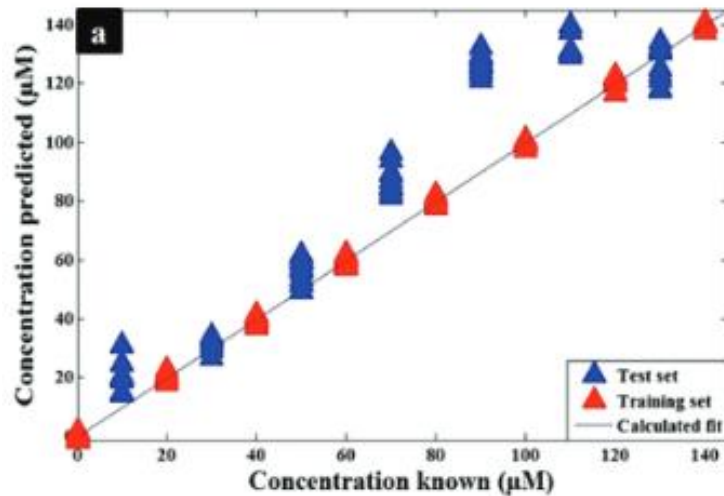
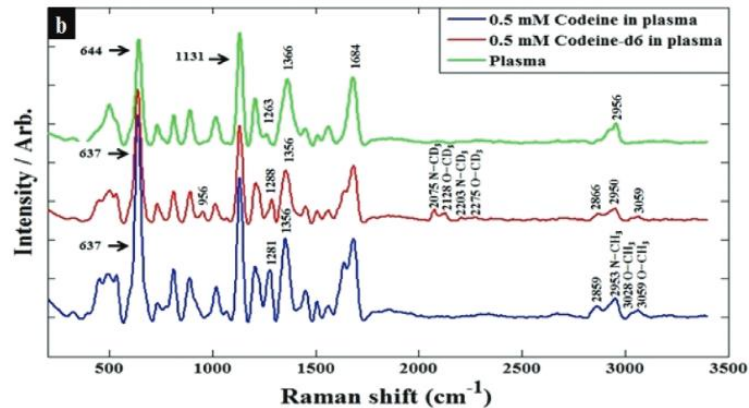
\therefore $^{12}\text{C-}^1\text{H}$, $\mu = 12/13 = 0.92$
 $^{13}\text{C-}^1\text{H}$, $\mu = 13/14 = 0.93$
 $^{12}\text{C-}^2\text{H}$, $\mu = 24/14 = 1.71$

- ➔ Direct finger-printing approach for detection of target analytes suitable for low molecular weight analytes.
- ➔ Quantitative SERS is possible to construct by appropriate calibration, e.g. by using the isotopologues.



<https://doi.org/10.1016/j.trac.2018.03.005>

SERS Chemosensors - Codein



- ➔ Implementation by using synthetic Ag nanoparticles mixed with plasma for detection of codeine.
- ➔ Using of miniature portable Raman spectrometers possible.

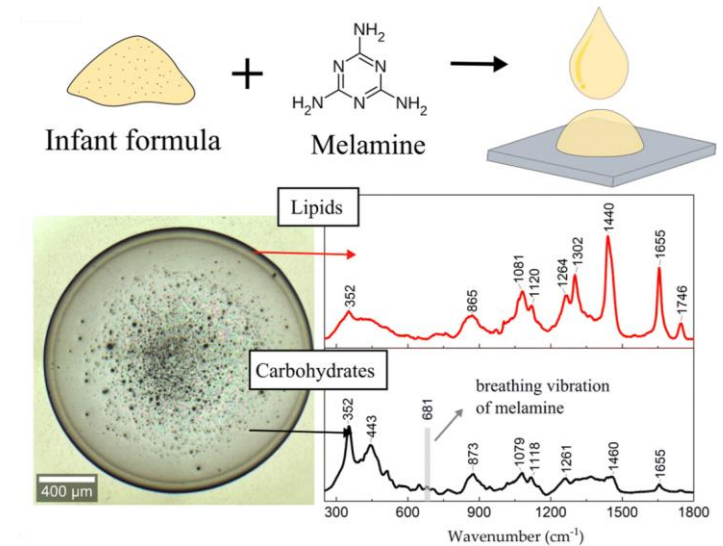
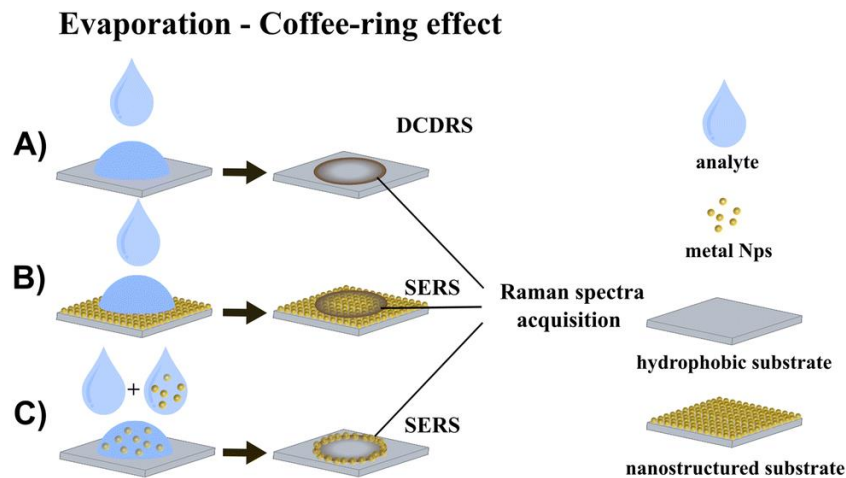


Ahura Scientific

SERS Chemosensors - Droplet Sample Drying

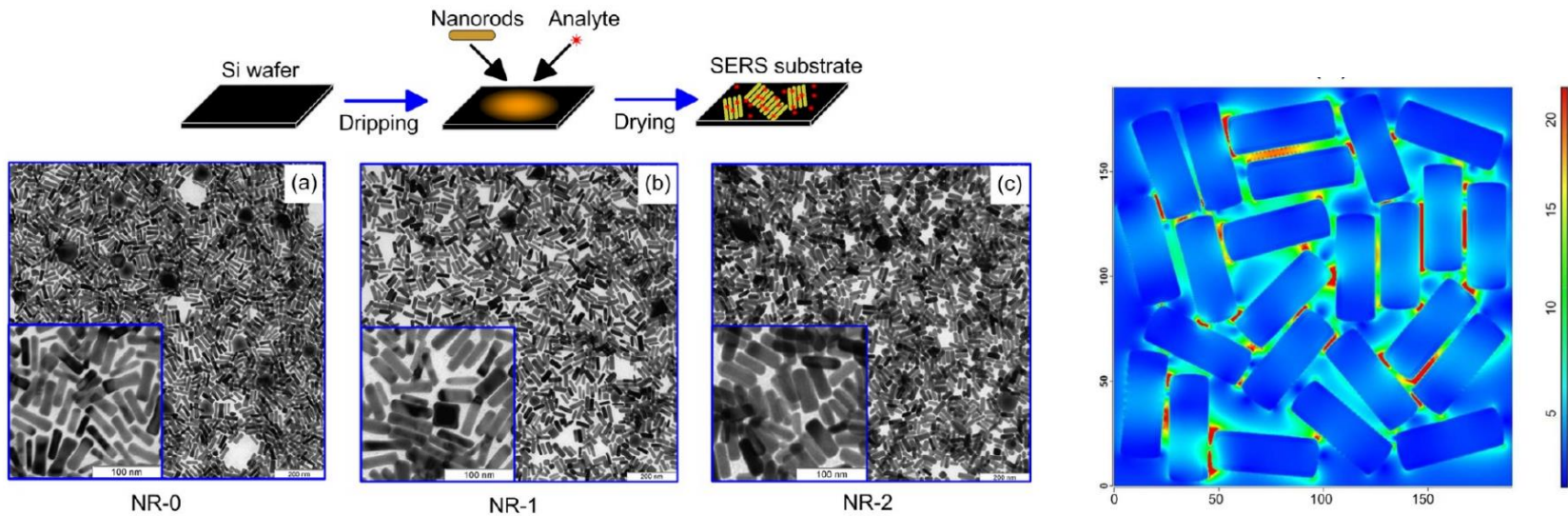
Marek Prochazka lab.

[10.1039/D4AN00336E](https://doi.org/10.1039/D4AN00336E)



➔ Analyte preconcentration by the use of sample drying atop the SERS plasmonic substrate.

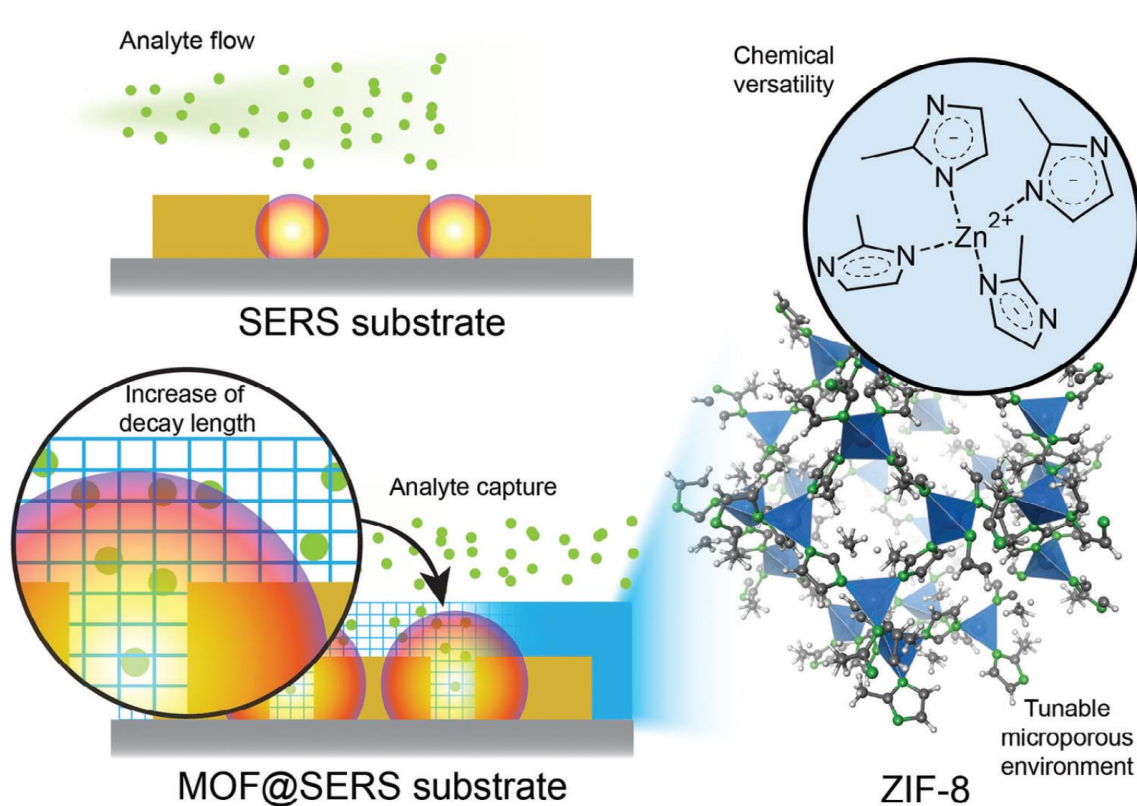
SERS Chemosensors - Droplet Sample Drying



- Analyte preconcentration by the use of drying a sample pre-mixed with plasmonic nanoparticles forming a plasmonic hotspot.

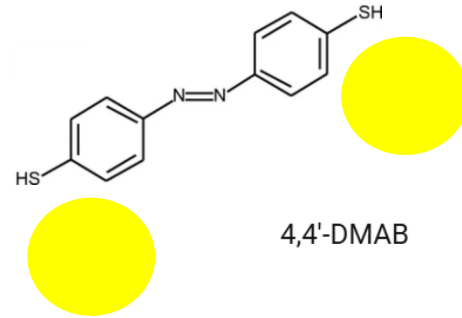
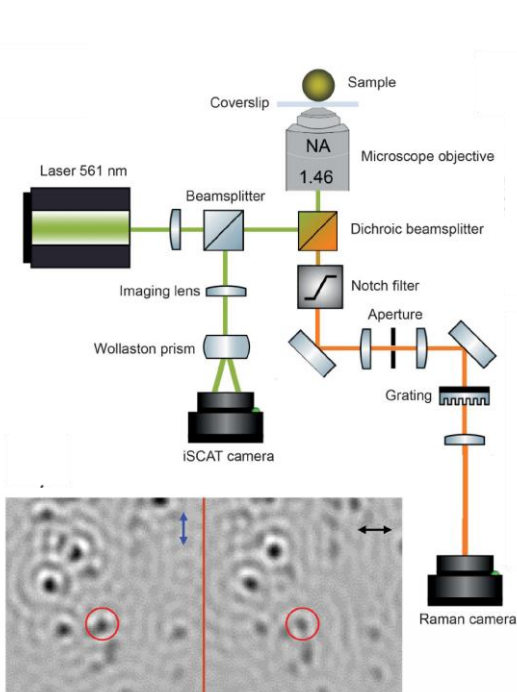
SERS Chemosensors - Preconcentration by MOF

DOI: 10.1002/adv.202401437

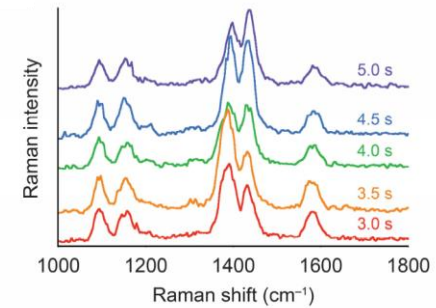
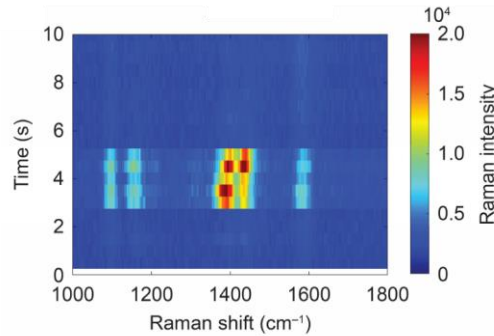


- ➡ Metal organic frameworks are combined with plasmonic nanostructures for preconcentration of target analyte species on the substrate.

SERS Chemosensors - Single Molecule Detection

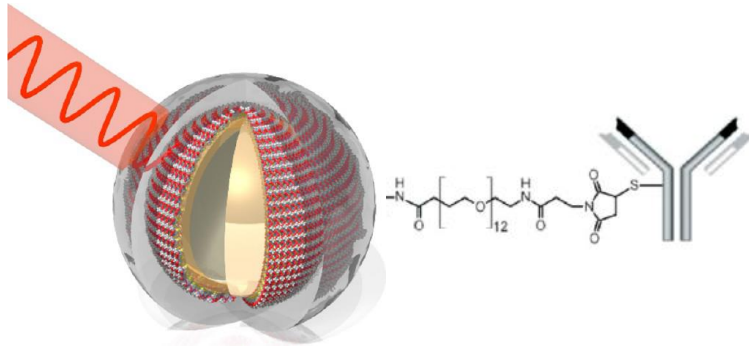


Ivan Kopal, dissertation VSCHT, 2026, Marek Piliarik group, UFE.

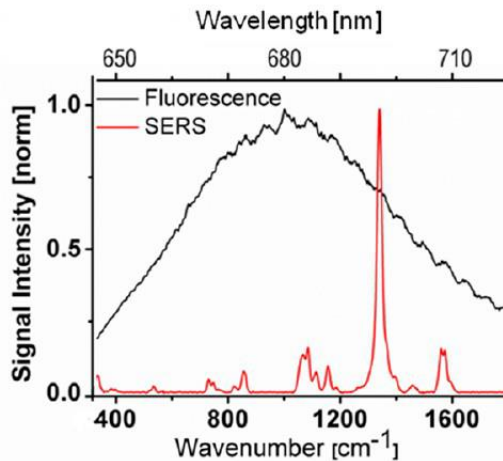


➔ Single molecule conformation changes with plasmonic dimer configuration tracked with SERS.

SERS Tags



Schlücker, S. Surface Enhanced Raman Spectroscopy: Analytical, Biophysical and Life Science Applications; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2011.



Salehi, M.; Schneider, L.; Ströbel, P.; Marx, A.; Packeisen, J.; Schlücker, S. Two-Color SERS Microscopy for Protein Co-Localization in Prostate Tissue with Primary Antibody-Protein A/G-Gold Nanocluster Conjugates. *Nanoscale* 2014, 6, 2361–2367.

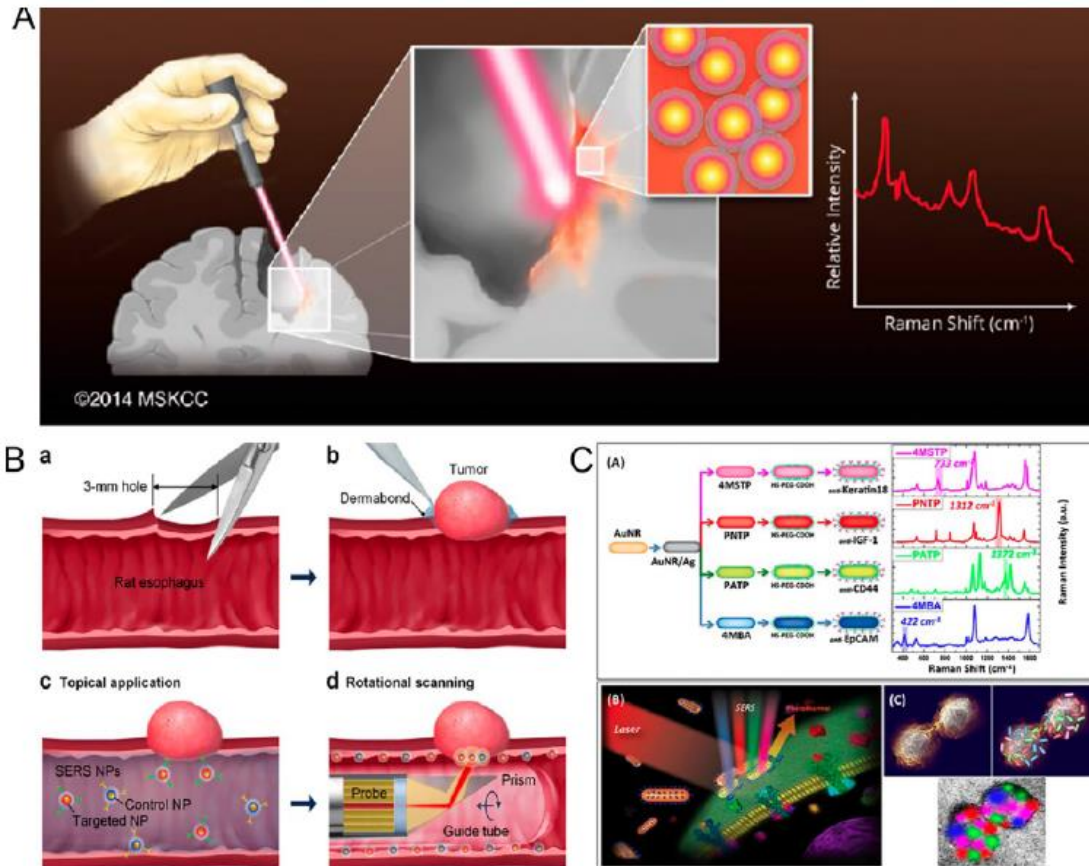
Assays employing labels (e.g. dyes) with SERS readout were pursued for the detection of protein and DNA biomolecules. The advantage over traditional fluorescence is narrow Raman spectra which are better suited for multiplexing and thus enabling detection of multiple reactions in parallel.

- Fluorescence emission band width for dyes >50 nm
- Fluorescence emission band width for quantum dots ~30 nm
- Raman peaks ~ 1 nm

Potential advantages:

- Multiplexing
- No photobleaching
- Single wavelength excitation
- Suppression of autofluorescence
- SERS can provide similar brightness to fluorescence tags

SERS Tags – Immunoassay Readout



➔ SERS-based imaging of cancer tissue with the use of SERS-tags conjugated with antibodies specific to a panel of biomarkers.