Biomolecules

ELEC-E3260

20th January

Raman-InfraRed Spectroscopy techniques

Summary

- Introduction of both techniques
- Raman Spectroscopy
- InfraRed Spectroscopy
- Difference between Raman and IR Spectroscopy
- In-class activity

Introduction

Brief History

- **Dr. Raman:** Discovery of the Raman effect (1928).
- **Nobel Prize of Physics** in 1930
- **Abney and Festing:** First infrared spectra measured in 1881.
- **William W. Coblentz:** Pioneer of IR spectroscopy (1905).
- **First Fourier Transform InfraRed Spectrometer** in the late 1960s.

Dr. Raman

William W. Coblentz

Raman and IR as complementary techniques

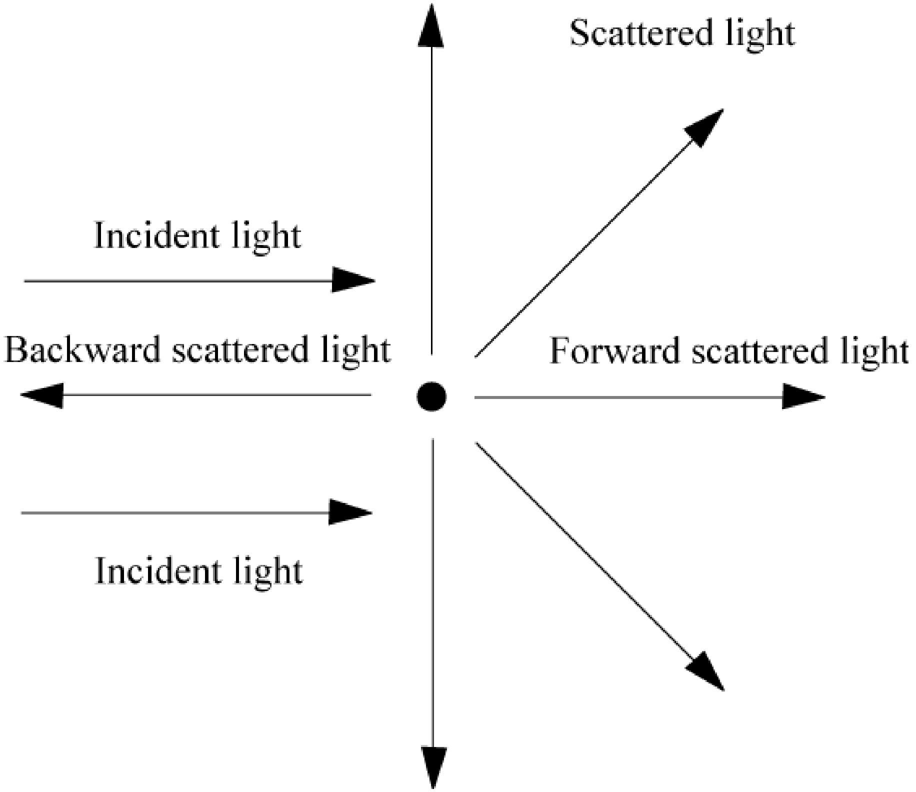
- Identify a molecule thanks to its vibrational modes

- Vibrational modes can be IR-active, Raman-active or both

- Raman active if Polarizability α changes
- IR active if dipole moment μ changes

- $Q=3N-6$ Number of Normal Mode of Vibration in general
- $Q=3N-5$ Number of normal mode for a linear molecule

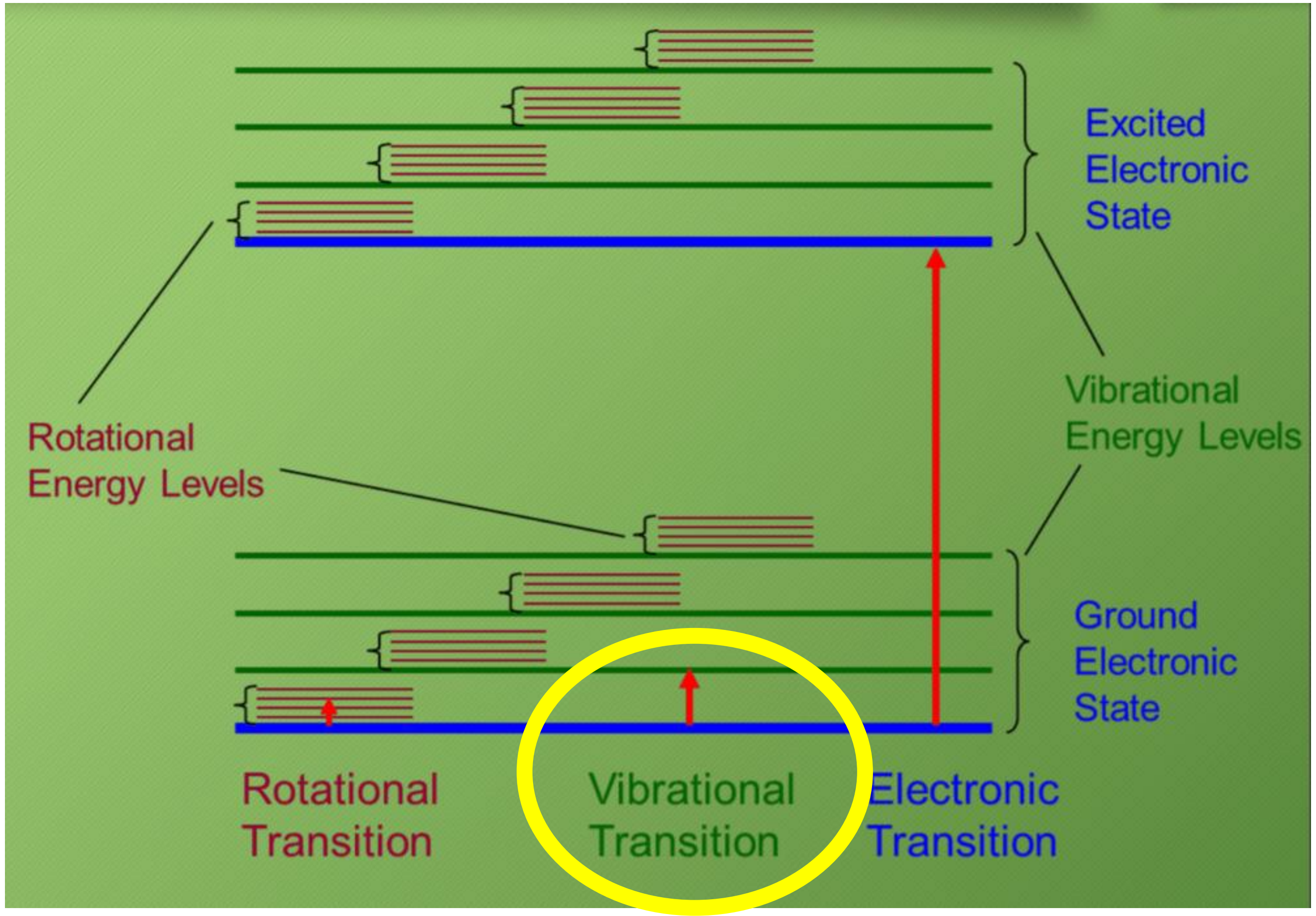
Where Q is the Number of Normal mode of vibration and N is the number of atoms in the molecule



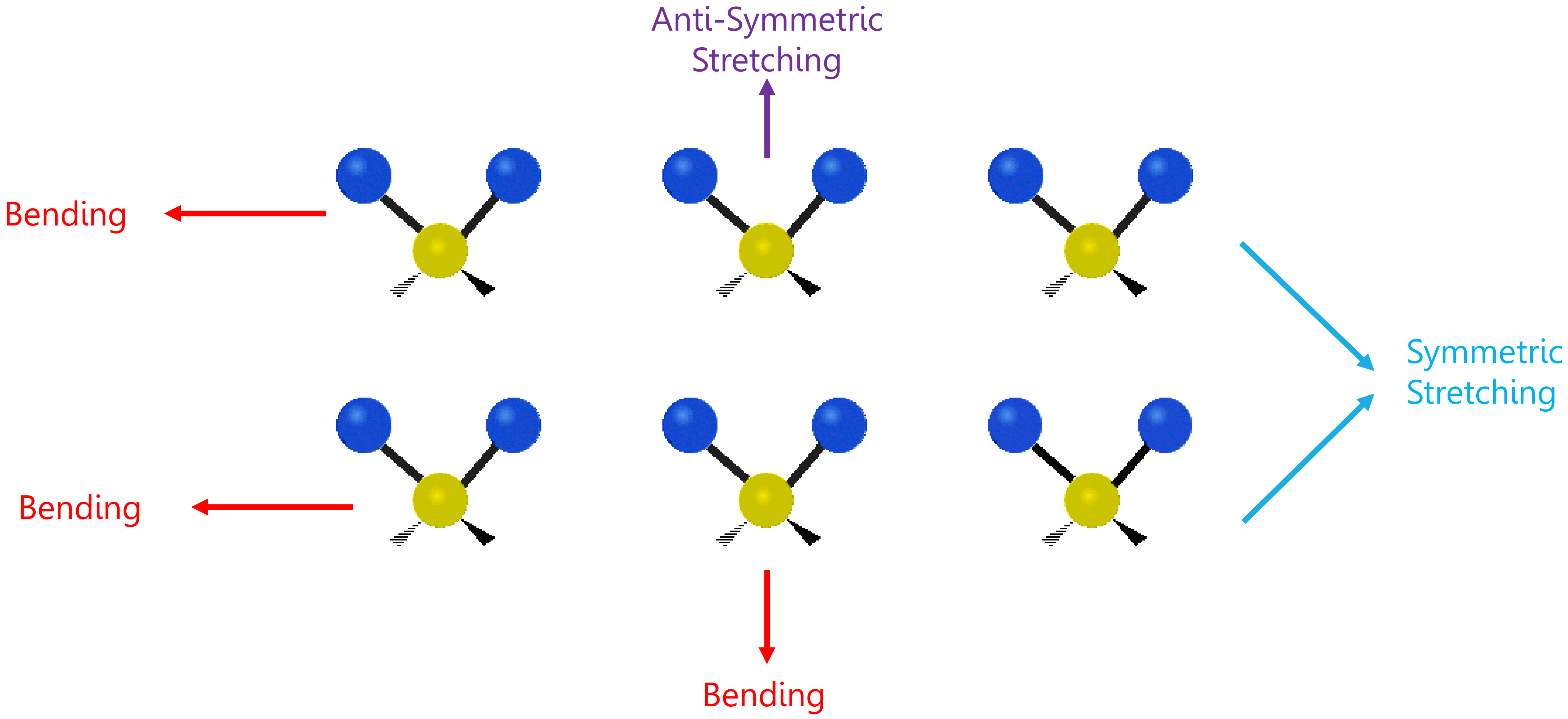
Vibrational transition

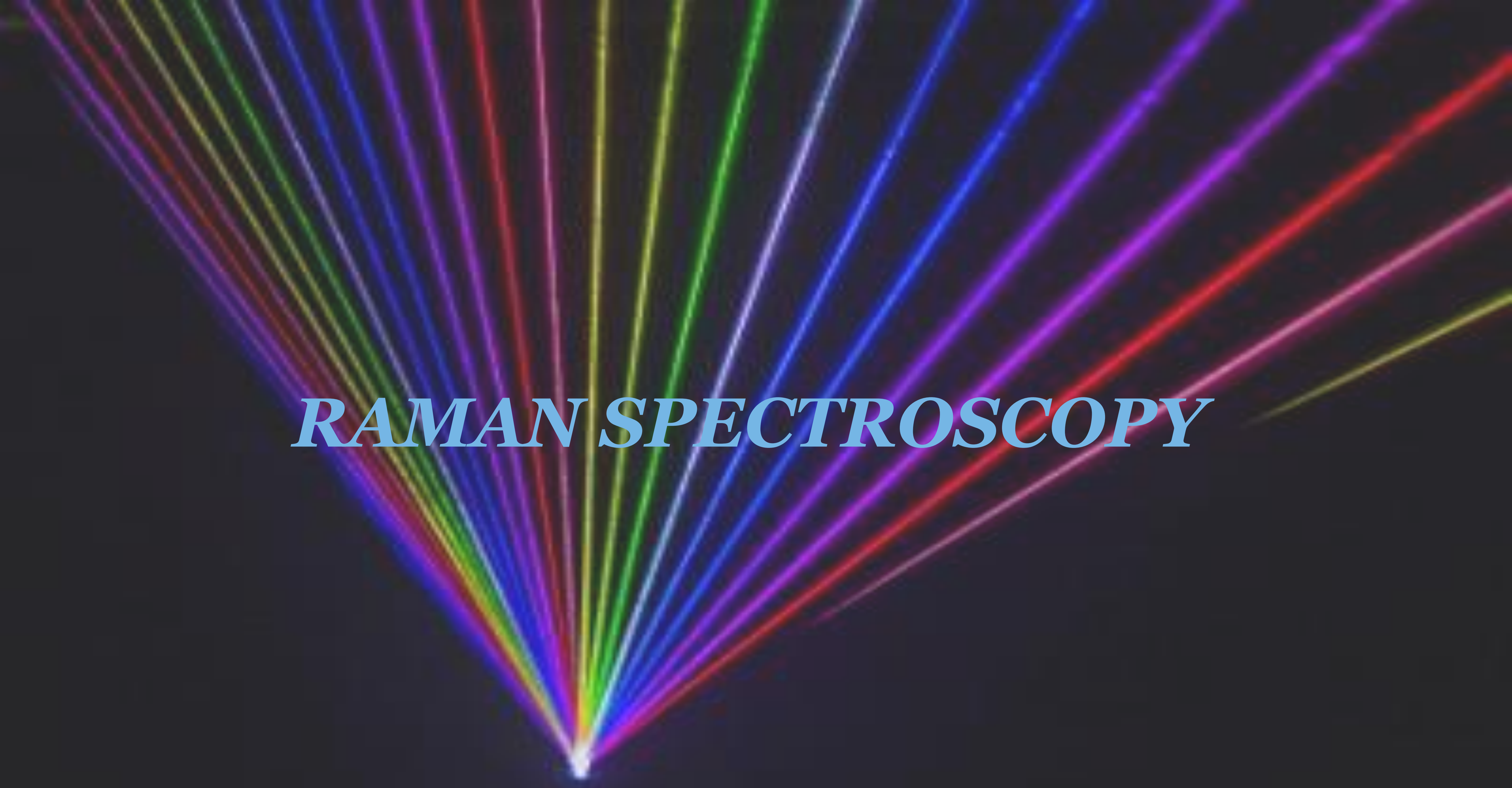
- $\Delta E_{el} >> \Delta E_{vib} >> \Delta E_{rot}$

- NIR, MIR and FIR : Investigate about Vibration Transition



Vibrational Mode





RAMAN SPECTROSCOPY

What is it ?

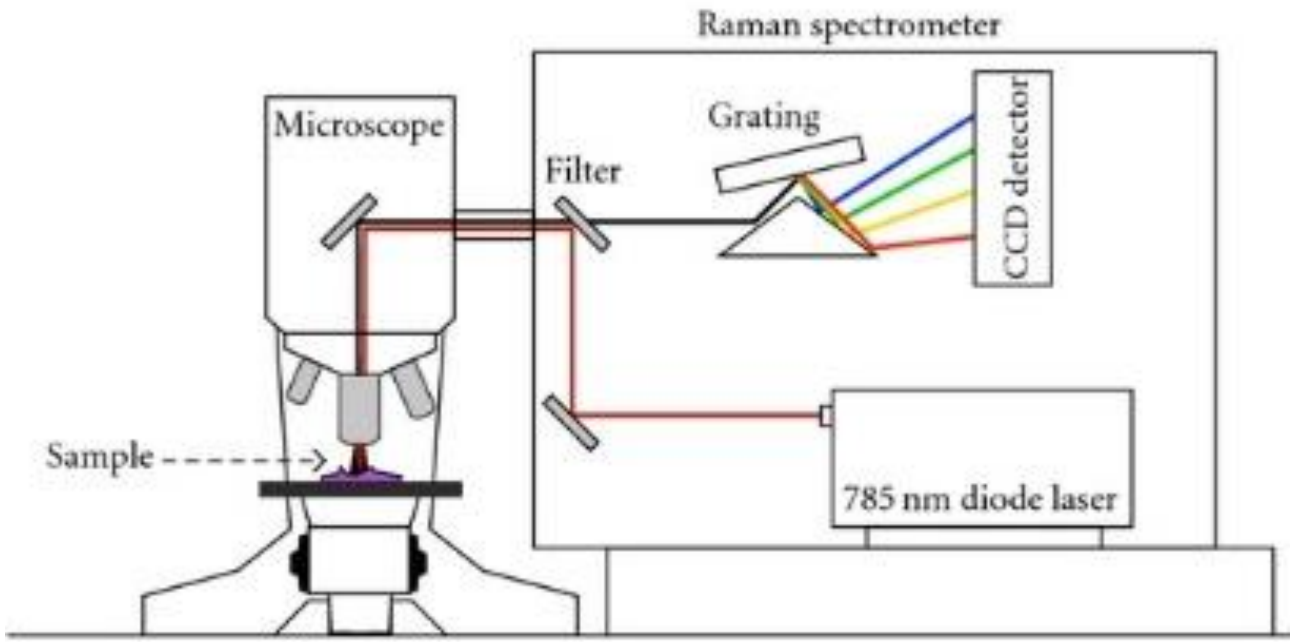
- Elastic Scatter Radiations = **Rayleigh Scattering** => No Information

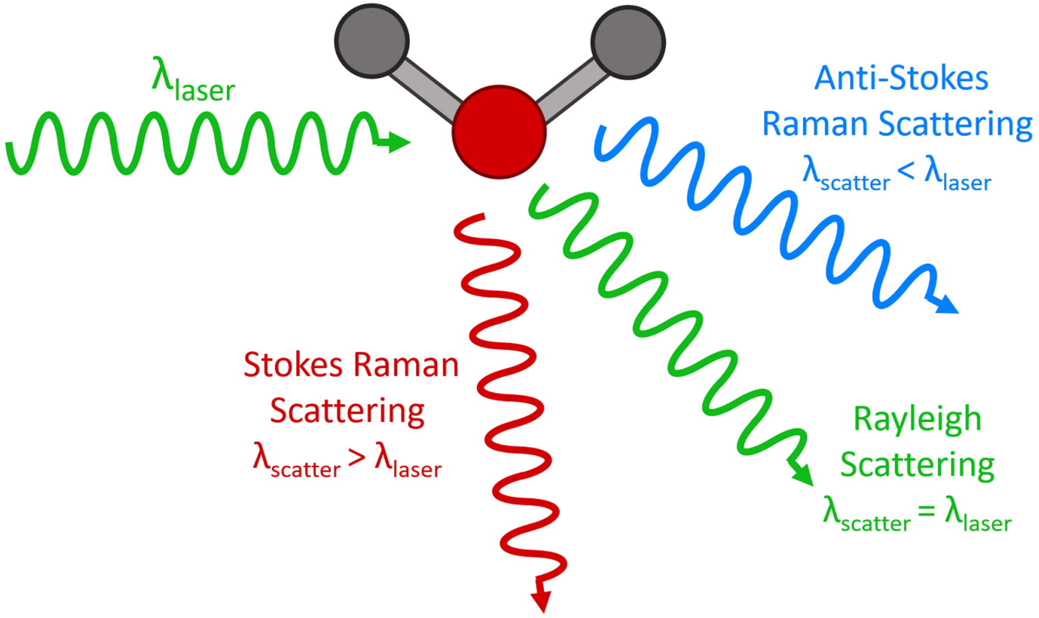
- Inelastic Scatter Radiations = Raman Scattering (Represents only 0.0000001% of the light !!) => information about the vibrational modes of the analyte.

  - Raman Scattering

Stokes

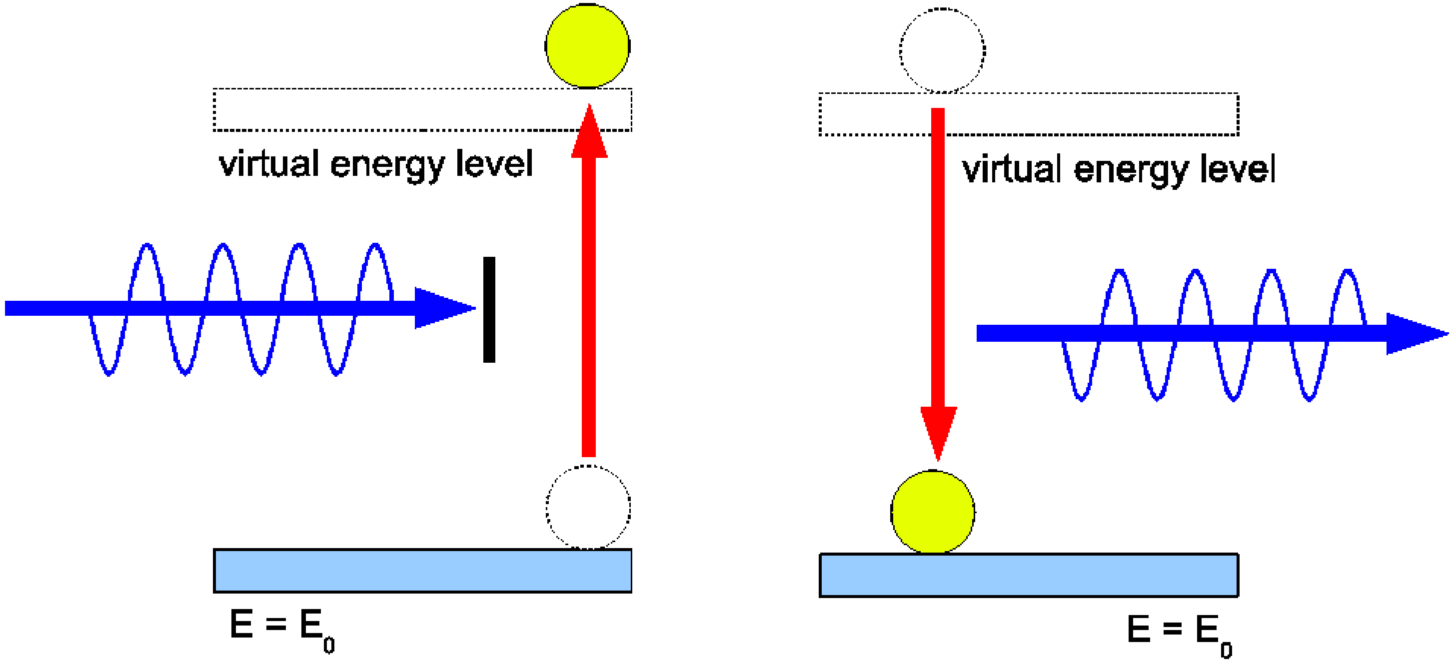
Anti-Stokes



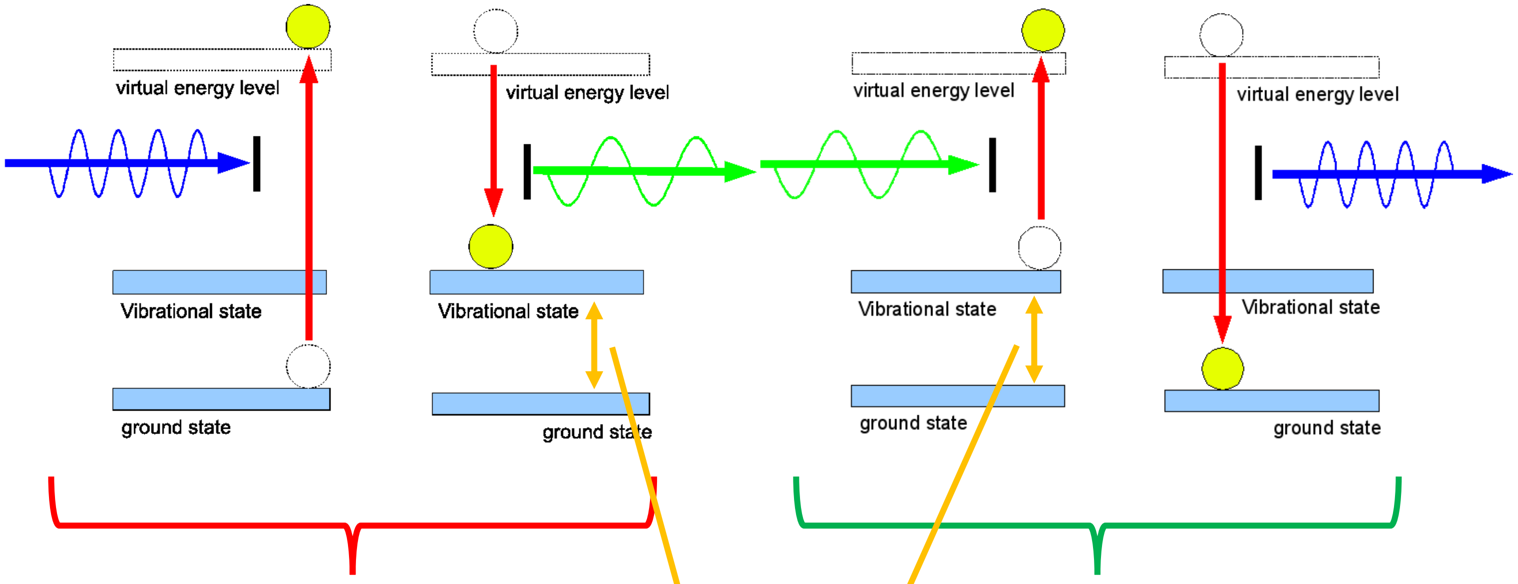


Virtual Energy Level

- Time-energy uncertainty principle $\Delta E \Delta t \geq \frac{\hbar}{2}$
- Very short lifetime $\sim 10^{-16} s$
- Rayleigh scattering



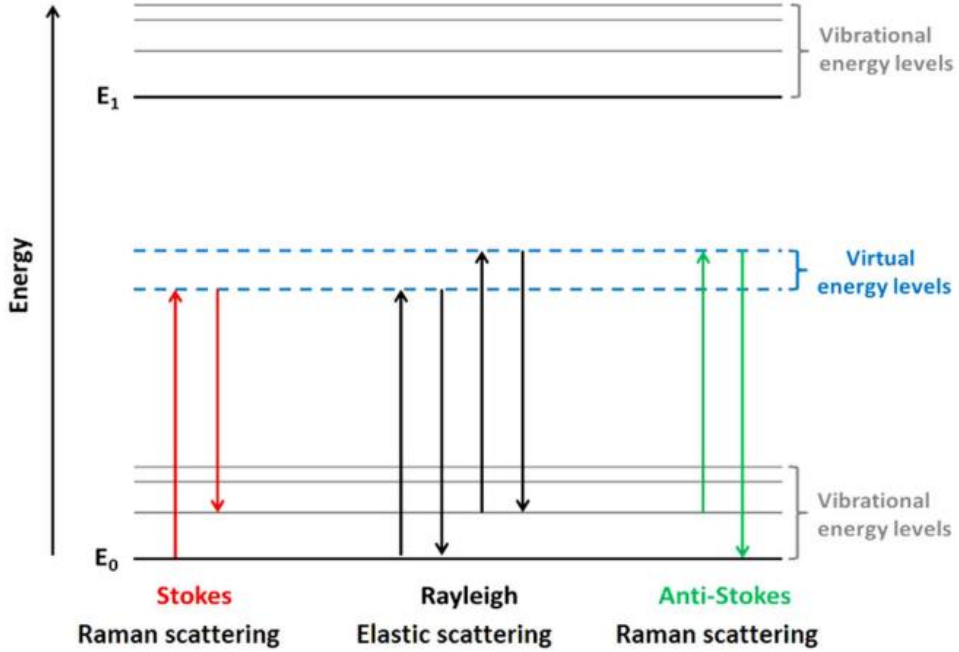
Stokes, Anti-Stokes Raman



Stokes Scattering
λscatter>λlaser

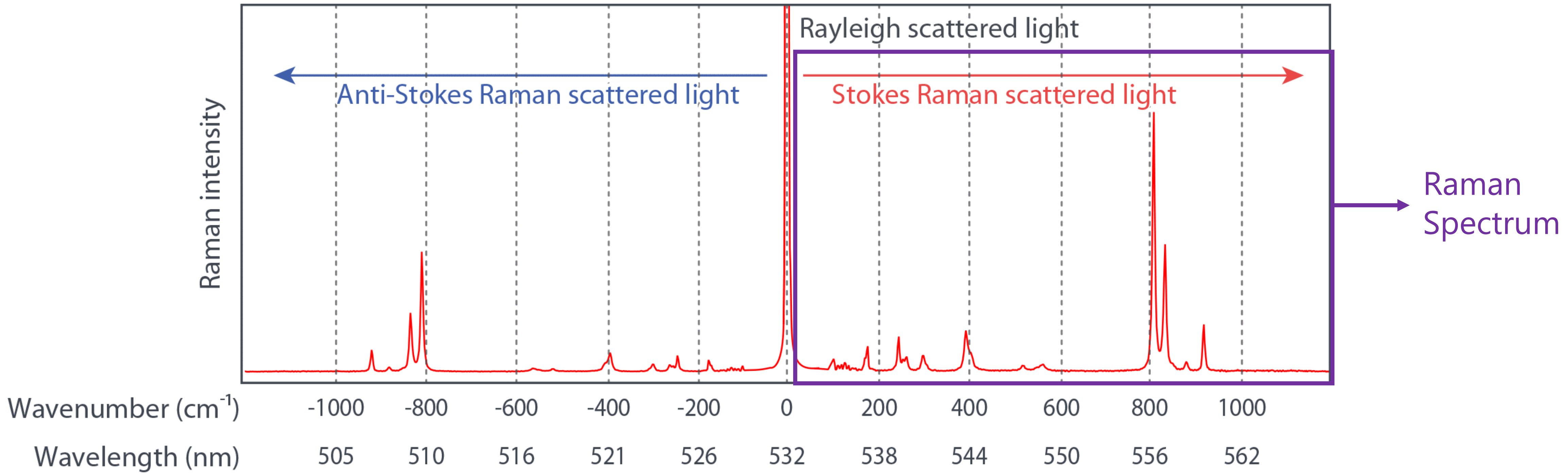
Anti-Stokes
Scattering
λscatter<λlaser

ΔE measured



Raman Scattering

Raman Spectrum: Ethanol

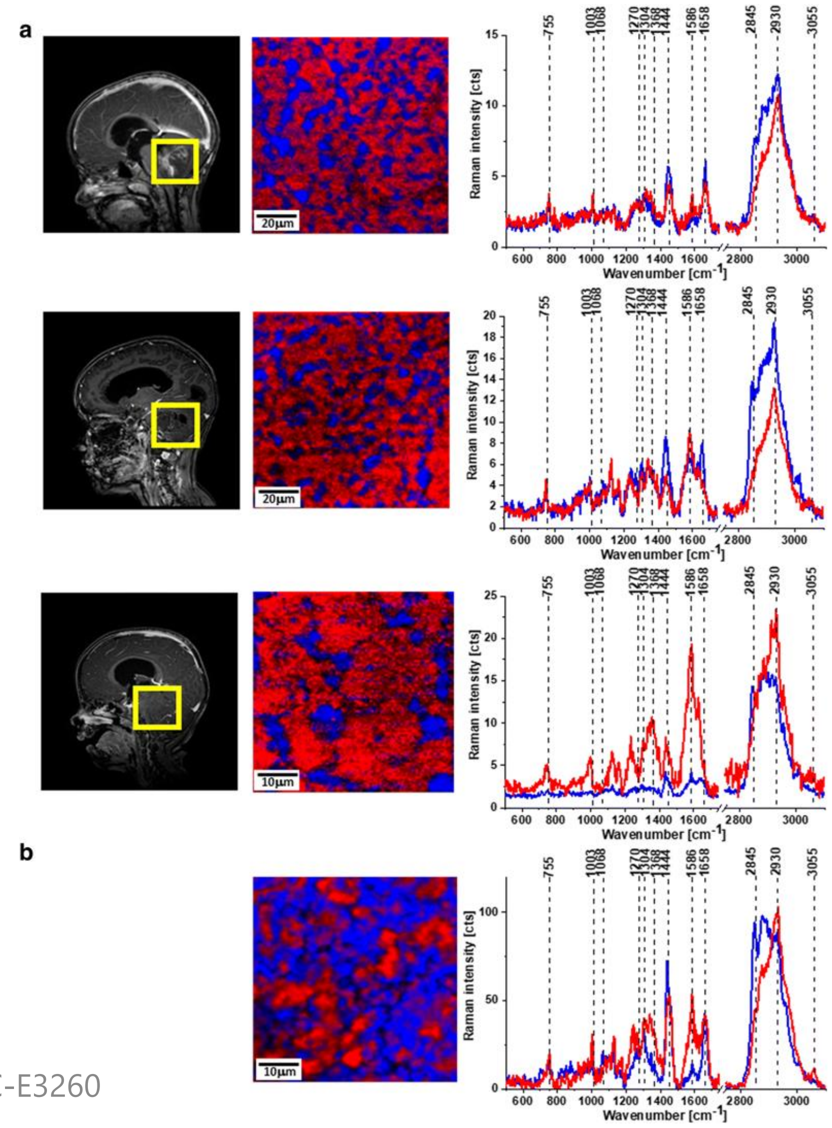


- Peak positions symmetric, but intensities vary
- Spectrum provides a unique chemical fingerprint
- Wavelength separation depends on excitation wavelength!
- Intensity of the peak is directly proportional to the concentration

Raman and Biomolecules

- Comparison between medulloblastoma(brain tumor) and normal brain tissue

- Figure:
  Blue – lipids
  Red - protein



Applications, Advantages and Limitations

Applications:

- Identify Chemical structure
- Purity of the sample
- Reaction monitoring
- Study single cells and tissues
- Study drug interactions
- Disease's diagnosis

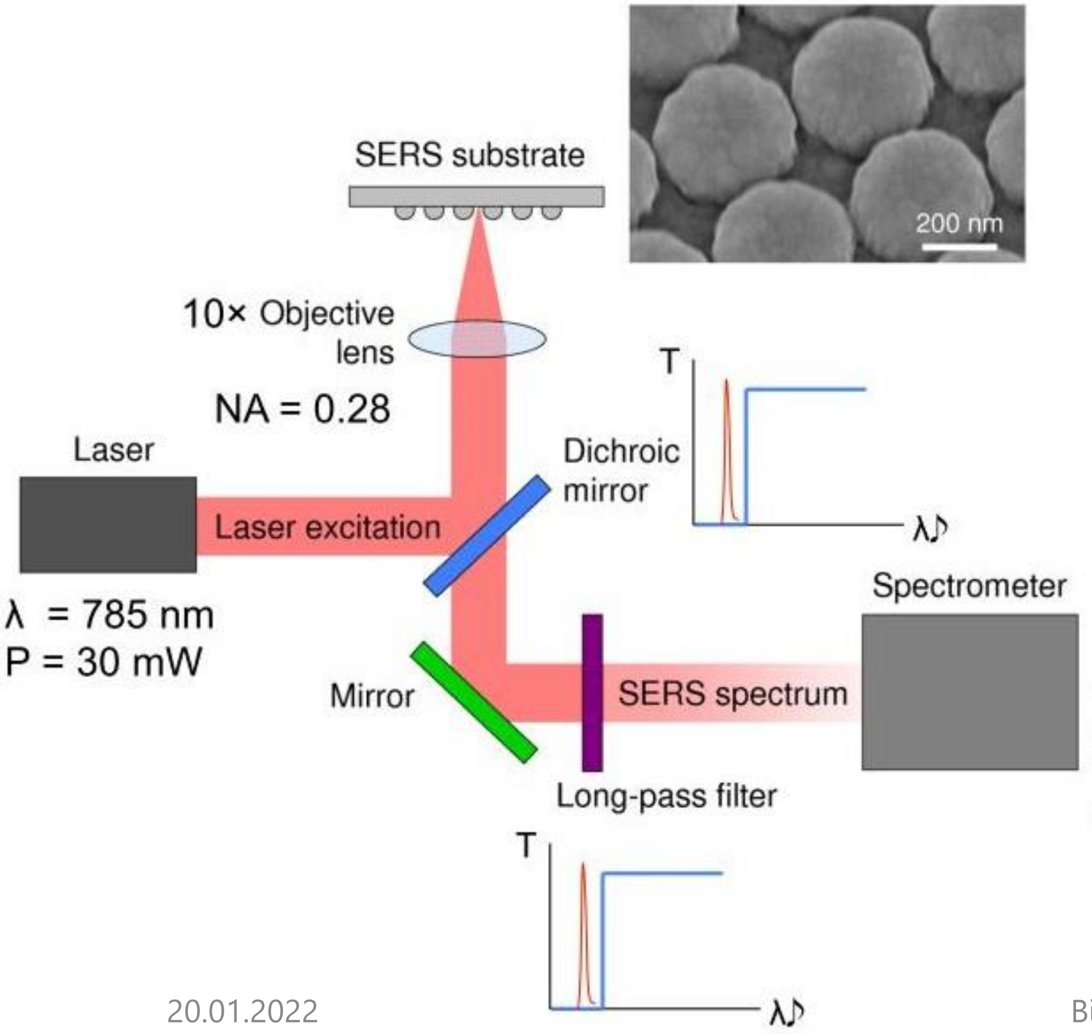
Advantages:

- Non-destructive
- Low wavenumber regions and high temperature
- Preparation of sample easy
- Low volume
- Molecular structure of biological samples without any labeling
- Combine with microscope and fiber for better focus and spectral resolution

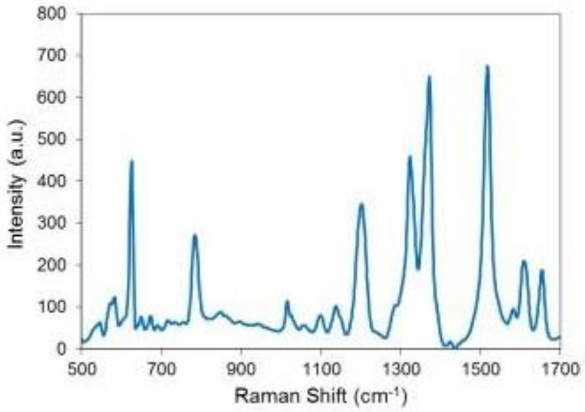
Limitations:

- Unfavorable process
  - Signals are weak

Surface Enhanced Raman Spectroscopy: SERS



- Increasing the intensity by a factor $10^{14\text{-}15}$
- Enhancement takes place at a metal surface
- Gold or silver metals are used
- Spectra can differ from a "normal" Raman spectrum

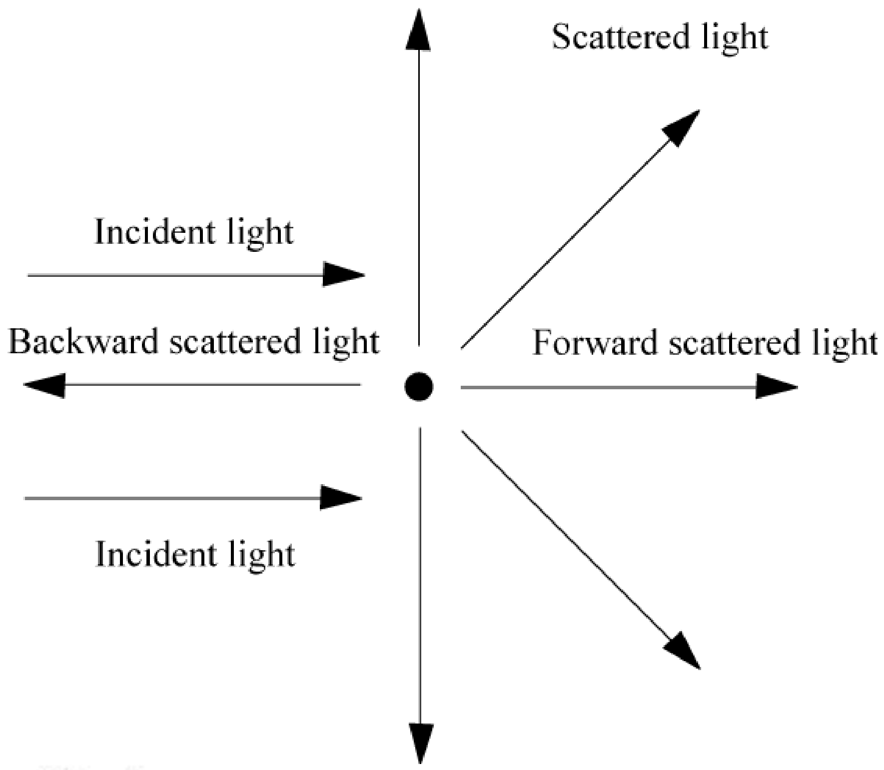


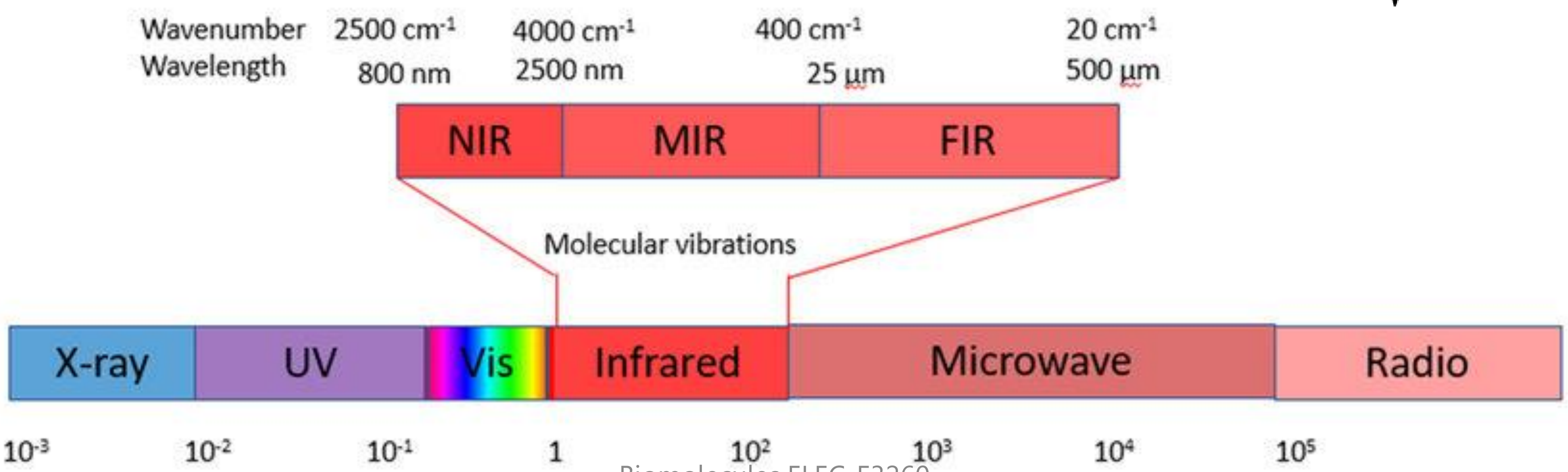
Fourier Transform InfraRed: FTIR

What is it ?

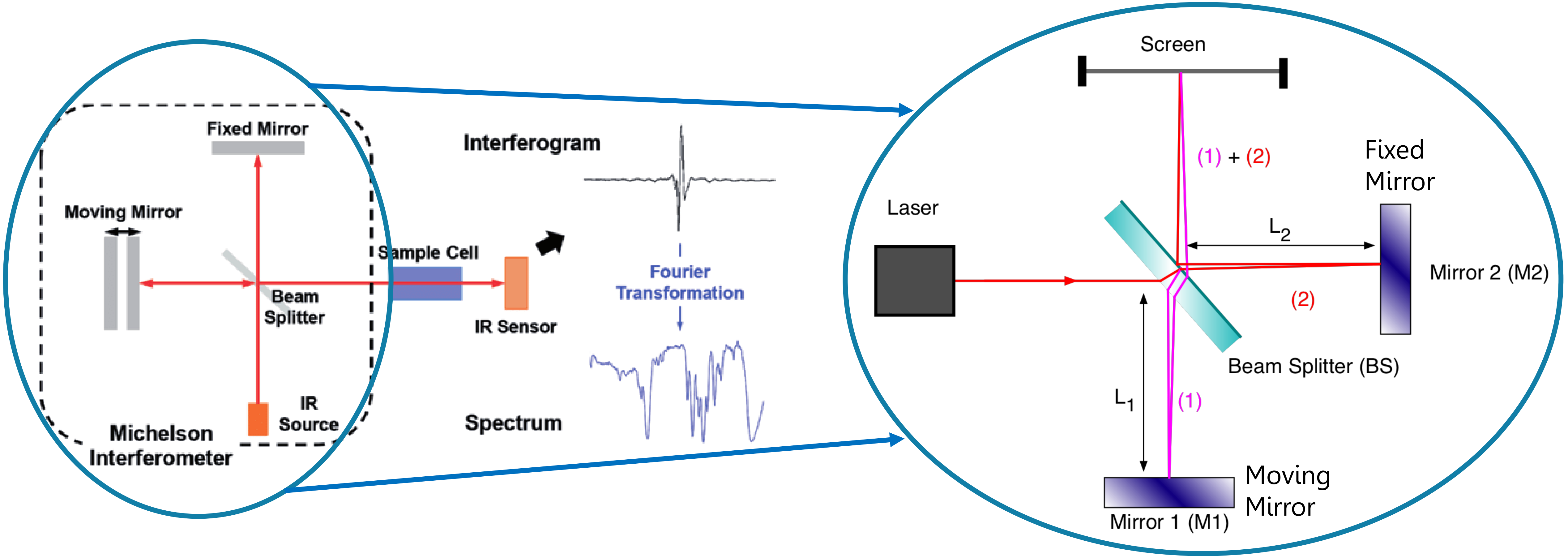
•IR active if dipole moment μ changes

•*Q=3N-6* number of normal mode of vibration in general

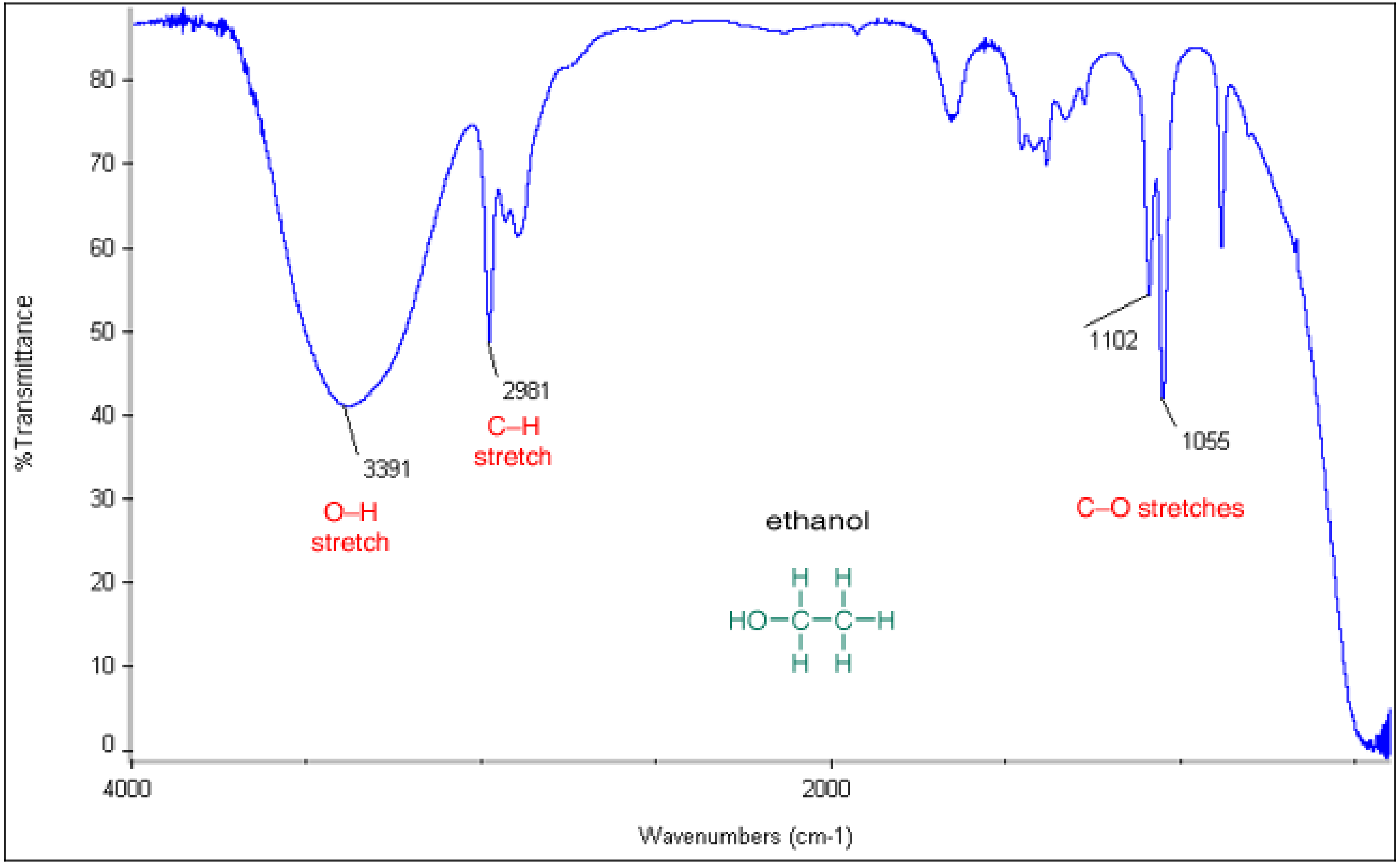




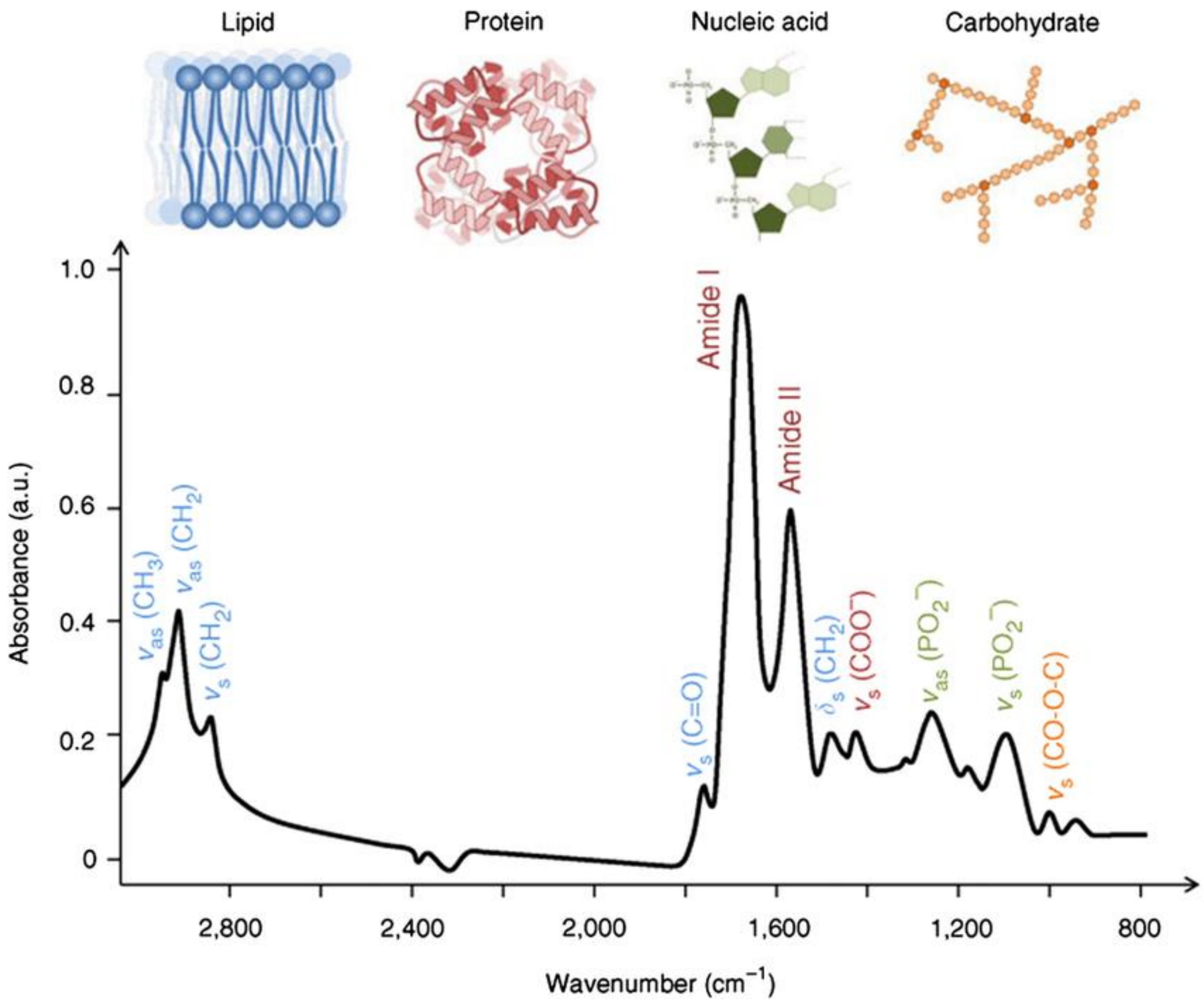
Michelson Interferometer



FTIR Spectrum



FTIR and Biomolecules



Applications, Advantages and Limitations

Applications:

- Identify Chemical structure
- Purity of the sample
- Basic drug research and structural elucidation
- Formulation development and validation
- Quality control processes for incoming and outgoing materials
- Packaging testing for drug

Advantages:

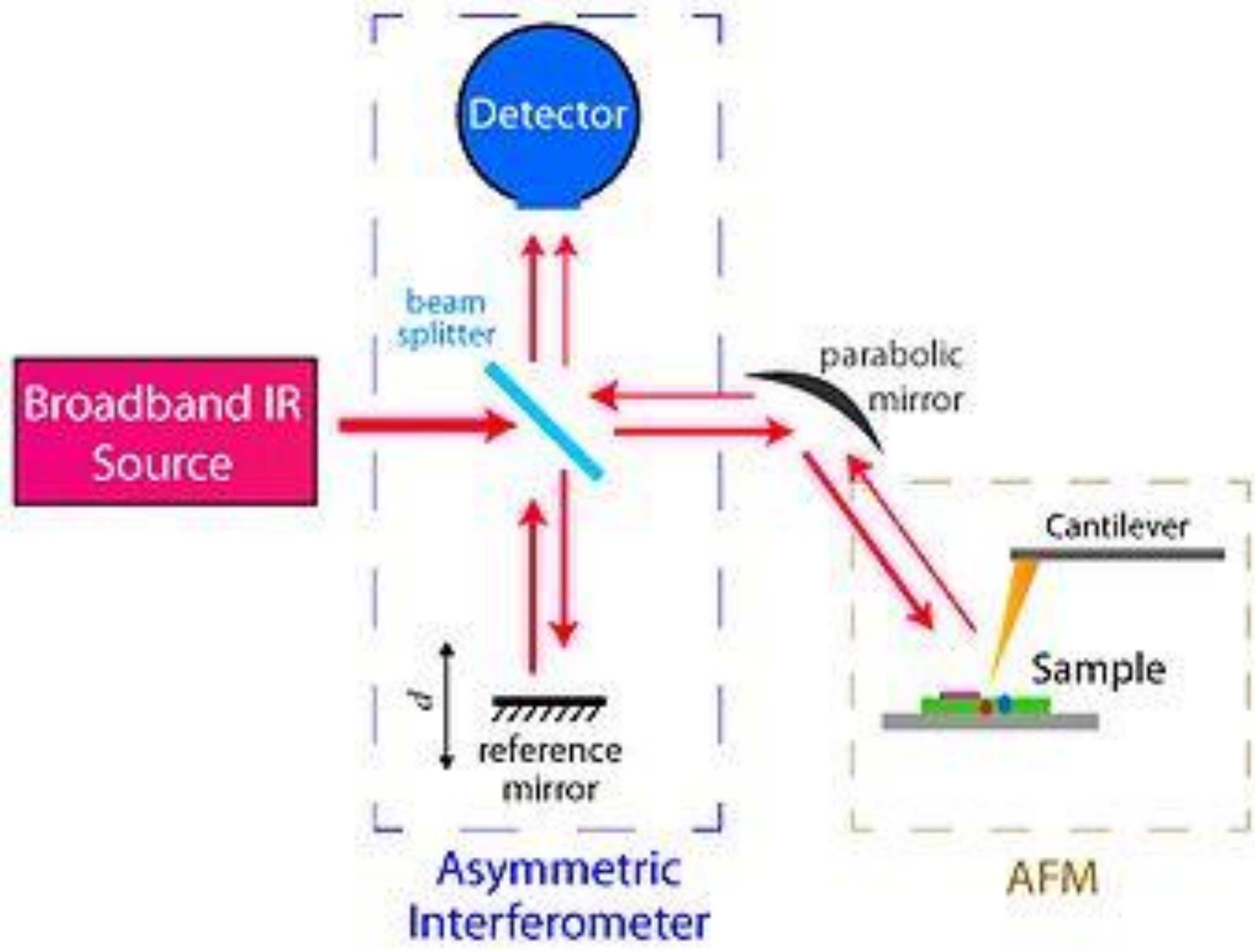
- Non-destructive
- All type of samples
- Spectral quality,
- Data collection speed,
- Reproducibility of data,
- Ease of maintenance and use

Limitations:

- strong IR absorption of water molecules
- Diffraction limited

Nano-FTIR

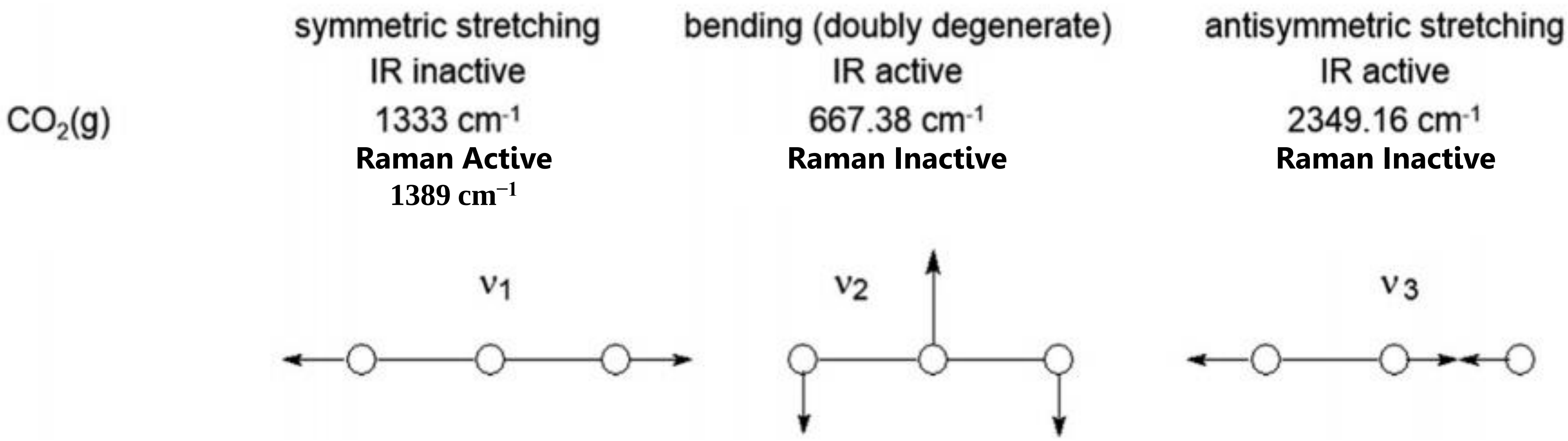
- Spatial Resolution on the order of 10-20 nm.
- Nano-FTIR (2005) for:
  - Secondary Structures of Proteins
  - Nanocomposites
  - Organic thin-film materials



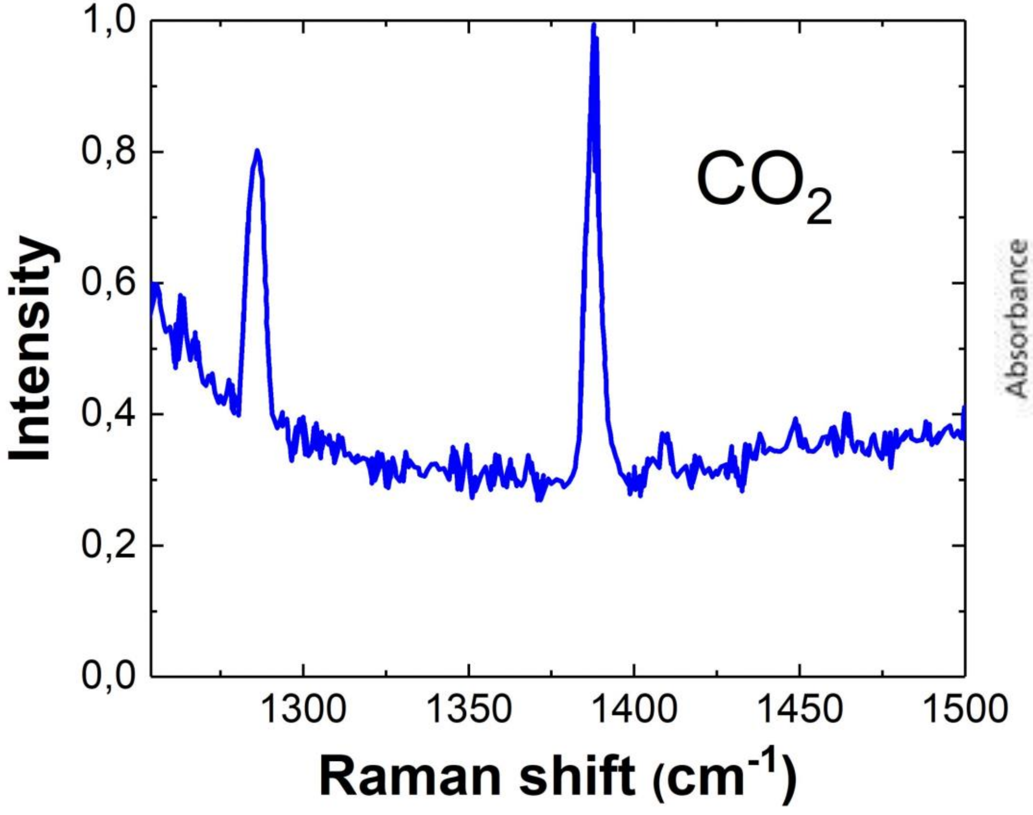
Concrete example for both technique: CO2 molecule

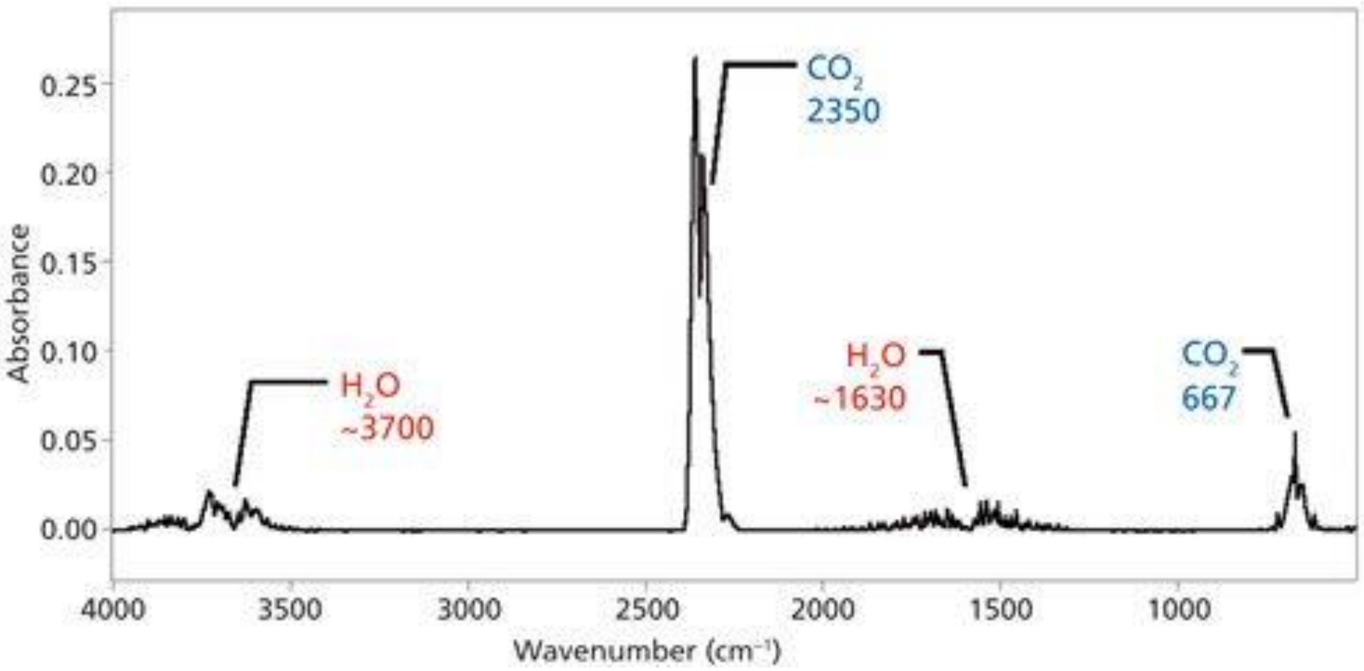
$$Q = 3N - 5 \qquad for\ a\ linear\ molecule$$
$$Q = 3N - 6 \qquad for\ a\ nonlinear\ molecule$$

So, for CO_2 we have a linear molecule with Q=4 Normal Vibration Modes.



Concrete example for both technique: CO2 molecule





Infrared spectrum

Raman vs. InfraRed Spectroscopy

Raman

- inelastic scattering phenomenon
- change in polarizability
- measures relative frequencies
- sensitive to homo-nuclear molecular bonds
- little to no sample preparation
- Fluorescence may interfere

Spectroscopy

- Relies on the absorbance, Transmittance or reflectance
- Change in the dipole moment
- Measures absolute frequencies
- Sensitive to hetero-nuclear functional group vibrations and polar bonds
- Sample thickness, uniformity and dilution

Any Questions ?

In-Class Activity

1. Give me the number of Normal Vibrational Mode of the molecule of water
2. How many peaks do we expect in the
    a. IR Spectrum
    b. Raman Spectrum
3. What would be the effect of raising the temperature on the intensity of Stokes and anti-Stokes lines?
4. Here you have the Raman and IR spectrum of the Acetylene (H-C≡C-H)
    a. Explain why we observed those peaks in both spectrum
    b. Try to find at which vibrational modes correspond the peaks in each spectrum

In-Class Activity