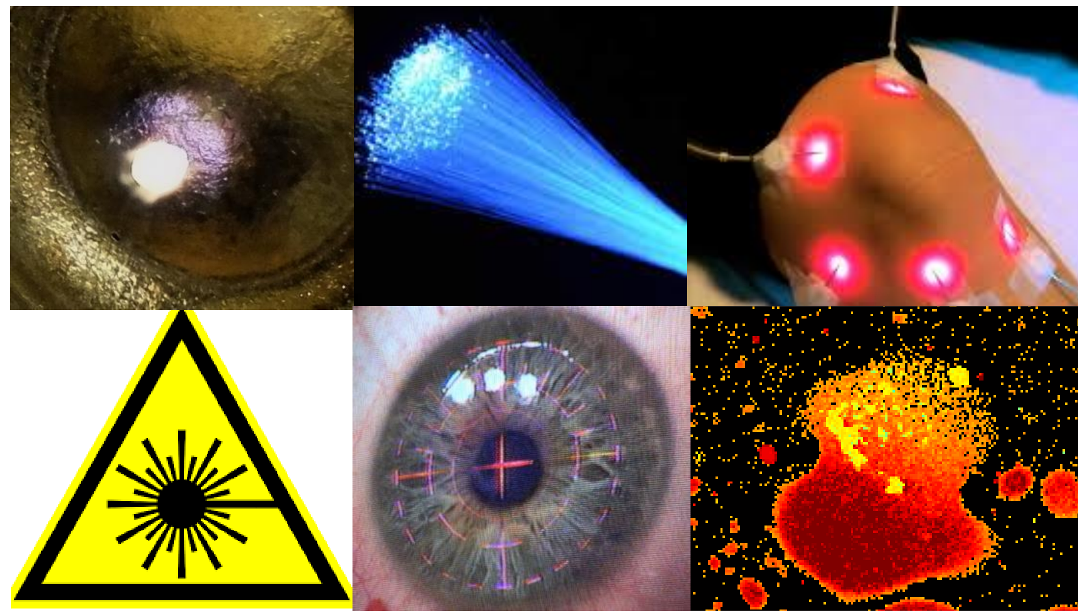
Lasers e Ótica Biomédica



Pedro Jorge

Non linear Effects

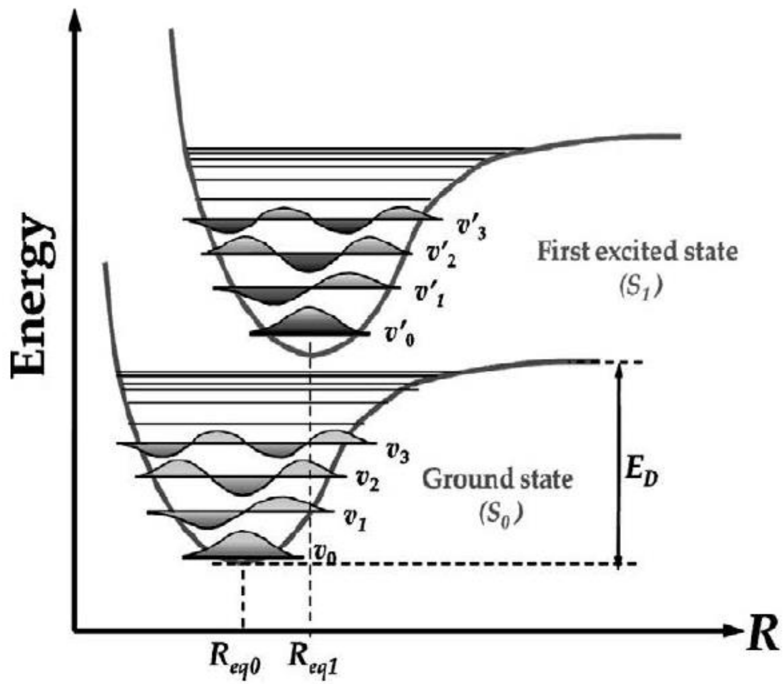
- Non Linear Effects;
  - Vibrational Spectroscopy
  - Raman Spectroscopy;
  - Principles and applications
- Optical Micromanipulation (optical trapping)
  - Basic principles
  - Applications
  - Manipulation using optical fibers
  - Principles and applications

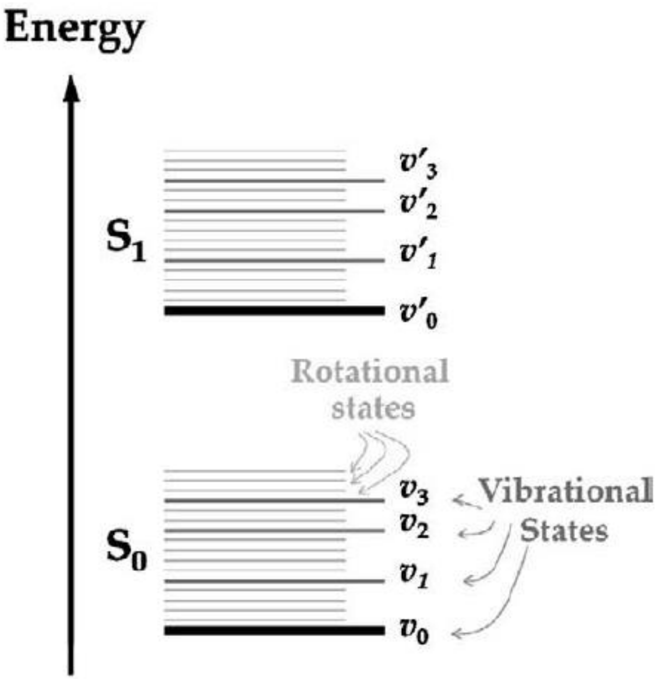
Molecular Orbitals: vibrational levels

Internuclear equilibrium distance.

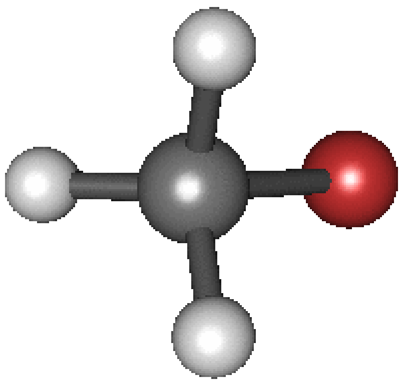
Dissociation Energy E_D

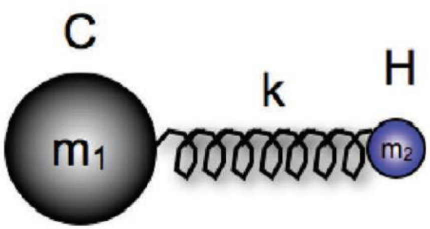
Each electronic level supports a set of **vibrational** and **rotational sub levels**





Vibrational Spectroscopy

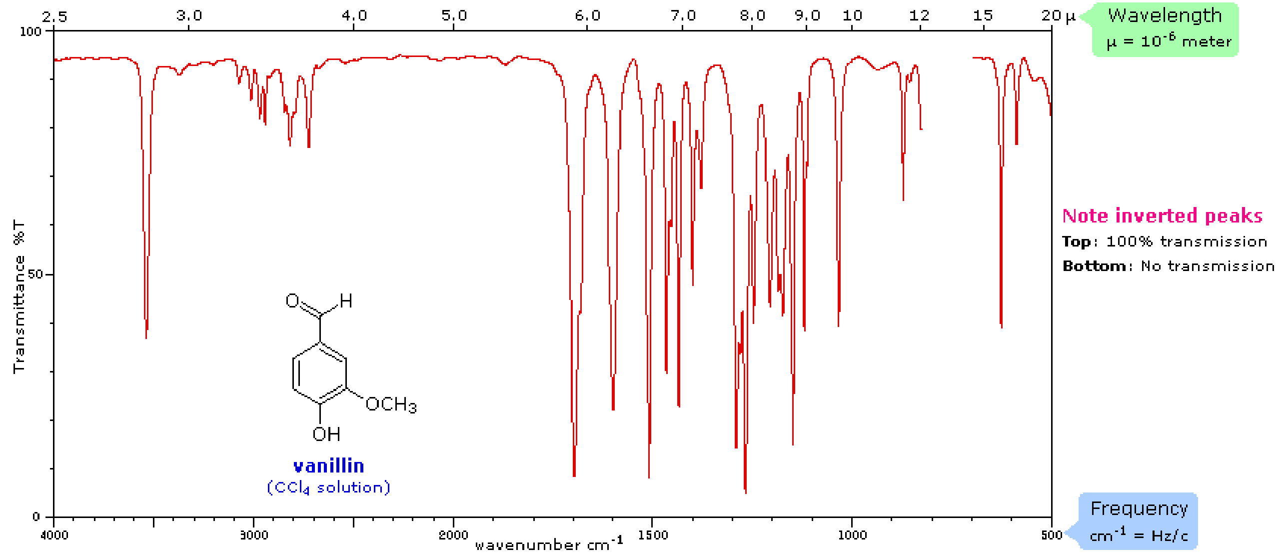




$$\frac{1}{\lambda} = \frac{\omega}{2\pi c} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$

Vibration frequency depends on the "Spring constant" k

$$\upsilon = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

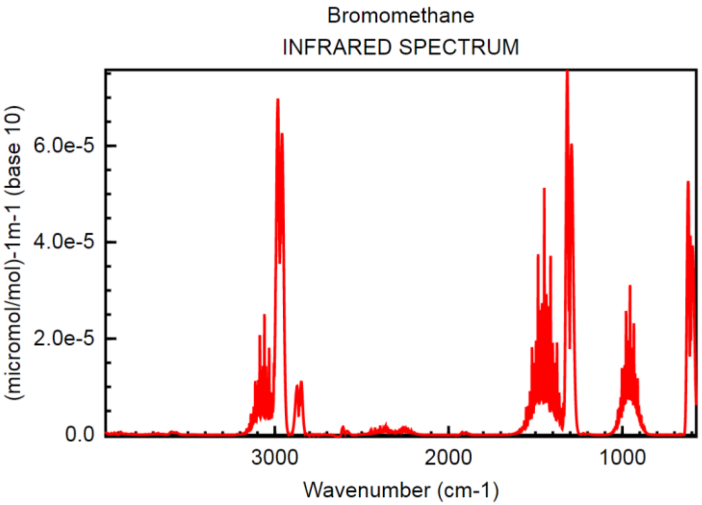


Units cm^{-1}

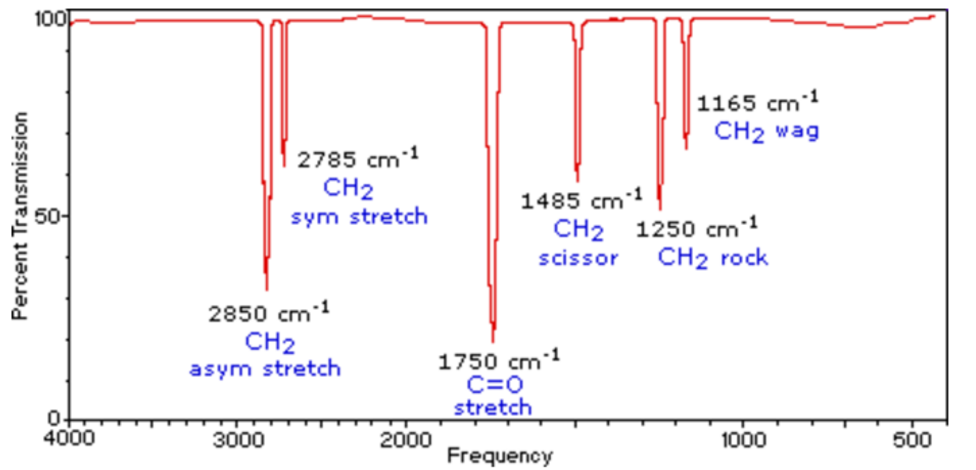
$$\upsilon[Hz] = \tilde{\nu}\left[cm^{-1}\right]c\left[cm/s\right]$$

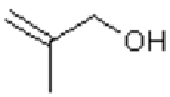
IR absorption spectrum, carries information of the structure of molecular bonds, allowing chemical analysis of the samples.

Vibrational Spectroscopy

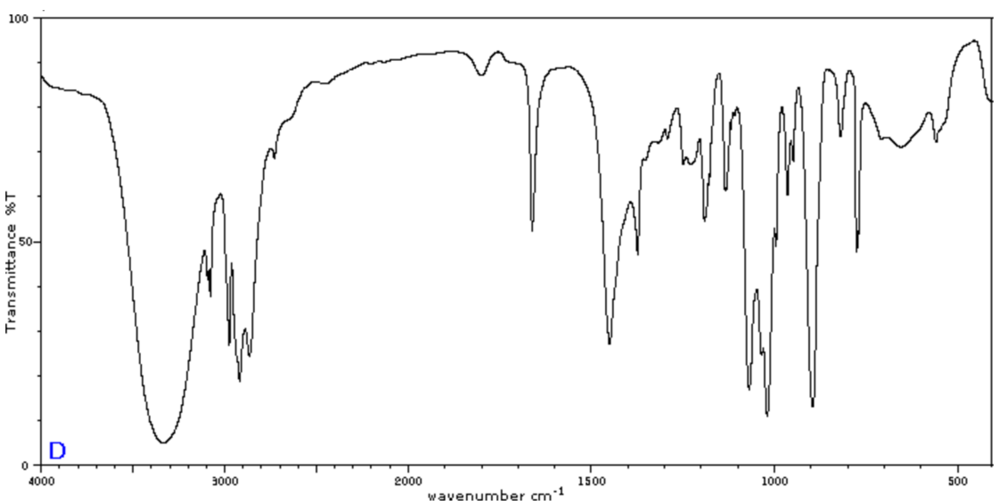


Gas Phase Infrared Spectrum of Formaldehyde, $H_2C=O$





2-methyl-2-propen-1-ol



https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Spectrpy/InfraRed/infrared.htm

Vibrational Spectroscopy

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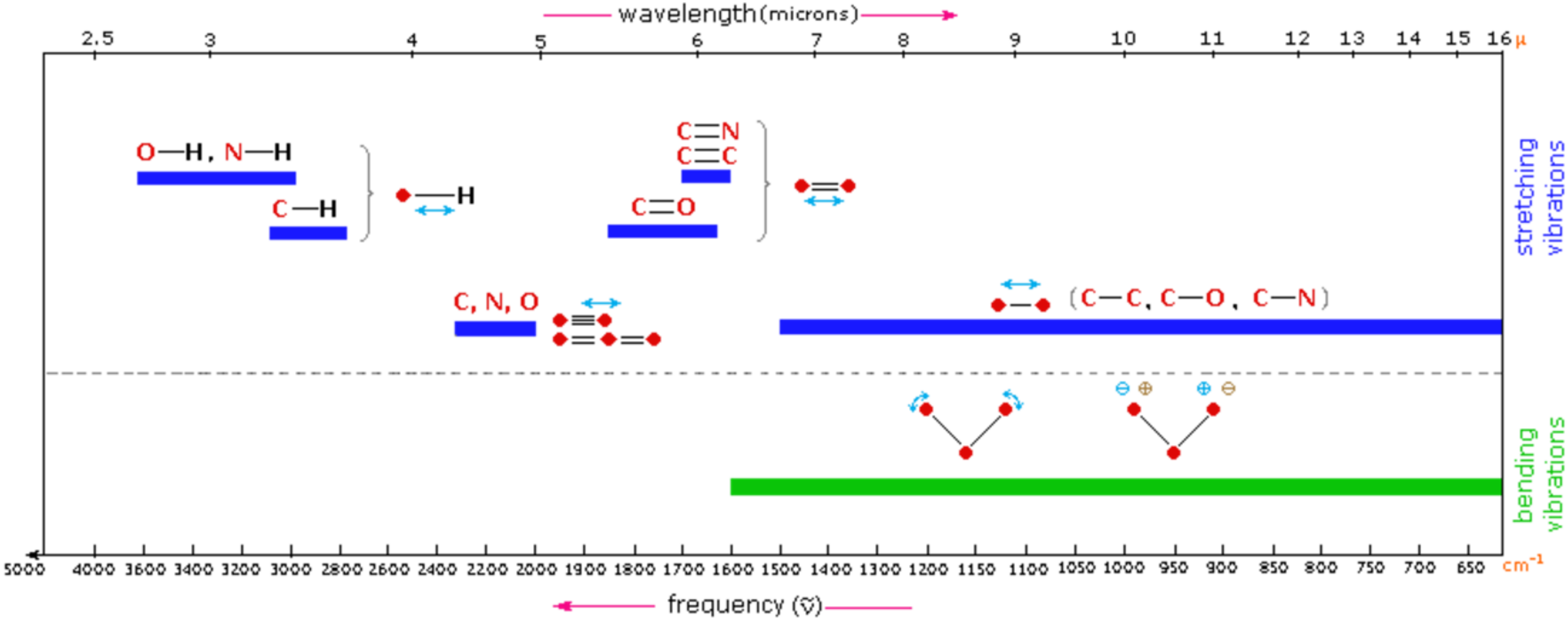
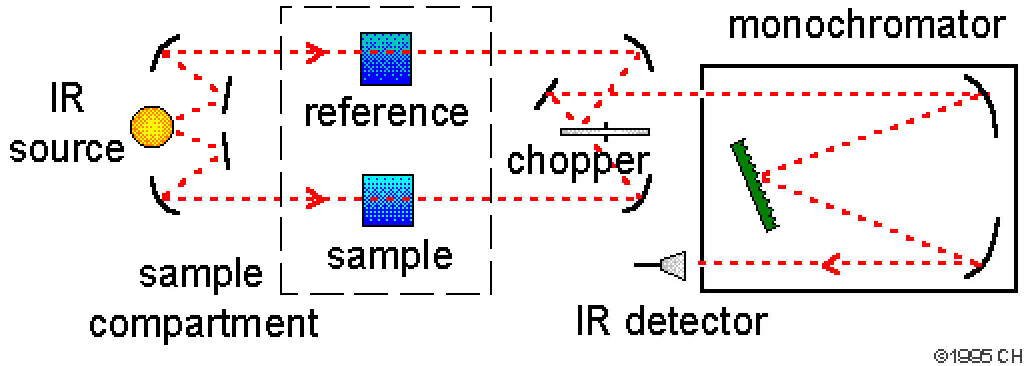


Vibrational Spectroscopy

Dispersive Absorption spectrometer with reference channel

- Low energy signals difficult detection
- Need for reference channel
- Long optical path needed
- Or else high concentration required



IR Optical sources

- tungsten lamps,
- Nernst glowers,
- glowbars.

IR Detectors

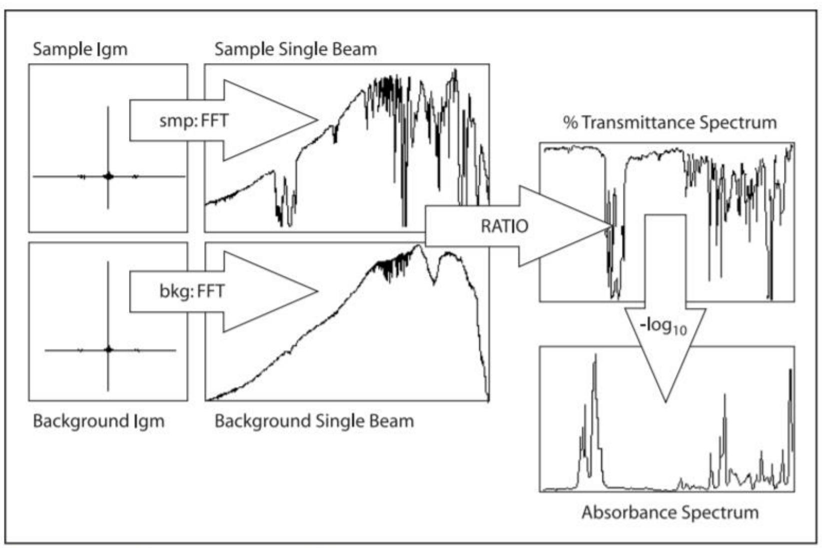
- Semiconductors (PbS)
- liquid-nitrogen-cooled HgCdTe

Vibrational Spectroscopy

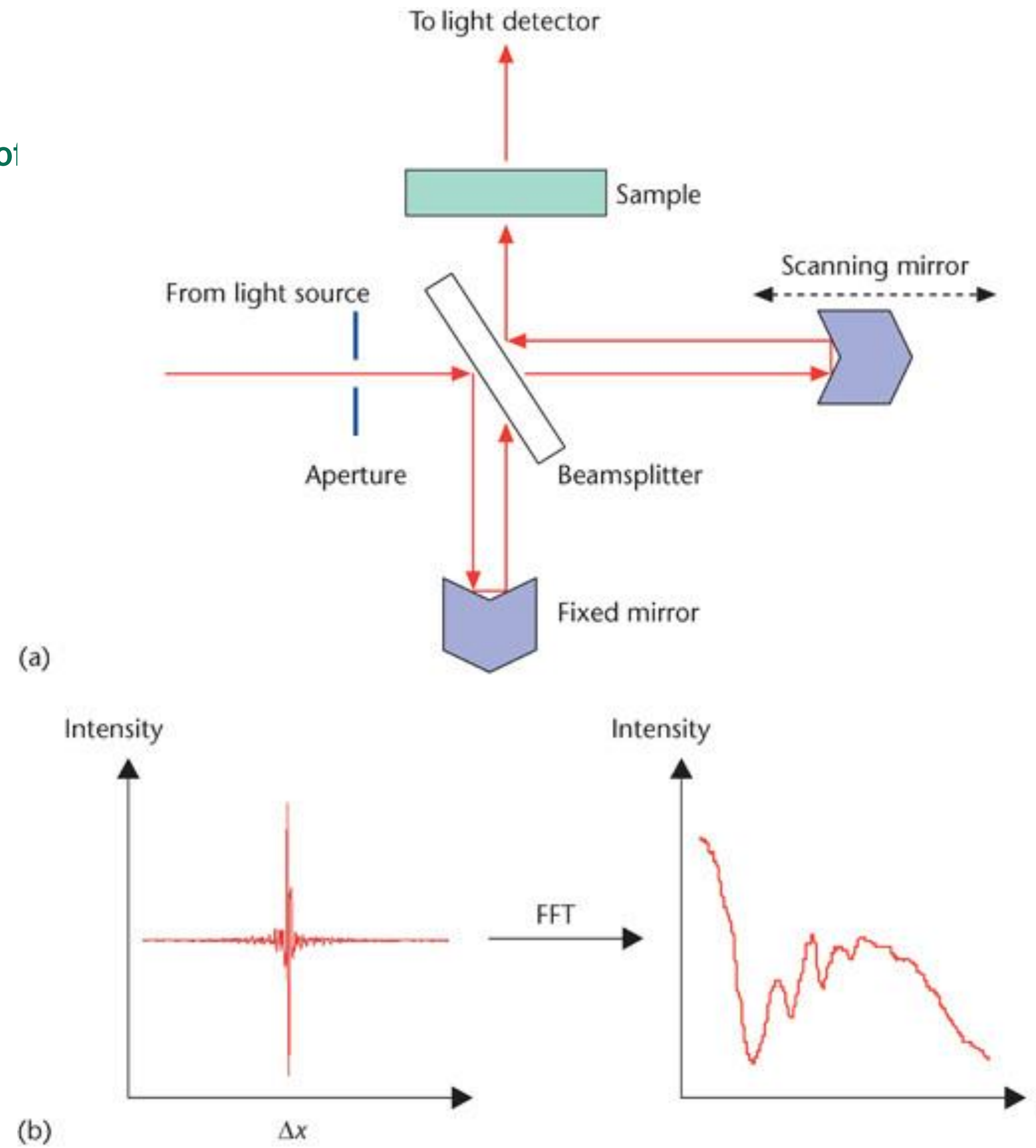
FTIR Spectrometer

- Interferometric scheme
- Spectrum inferred from the Fourier Transform of the Interference signal
- Detector reads the information over all wavelengths simultaneously
- Laser accurately measures mirror position serving as reference

- Faster
- Better SNR
- Higher precision and resolution



The process of collecting an infrared spectrum in an FT-IR spectrometer



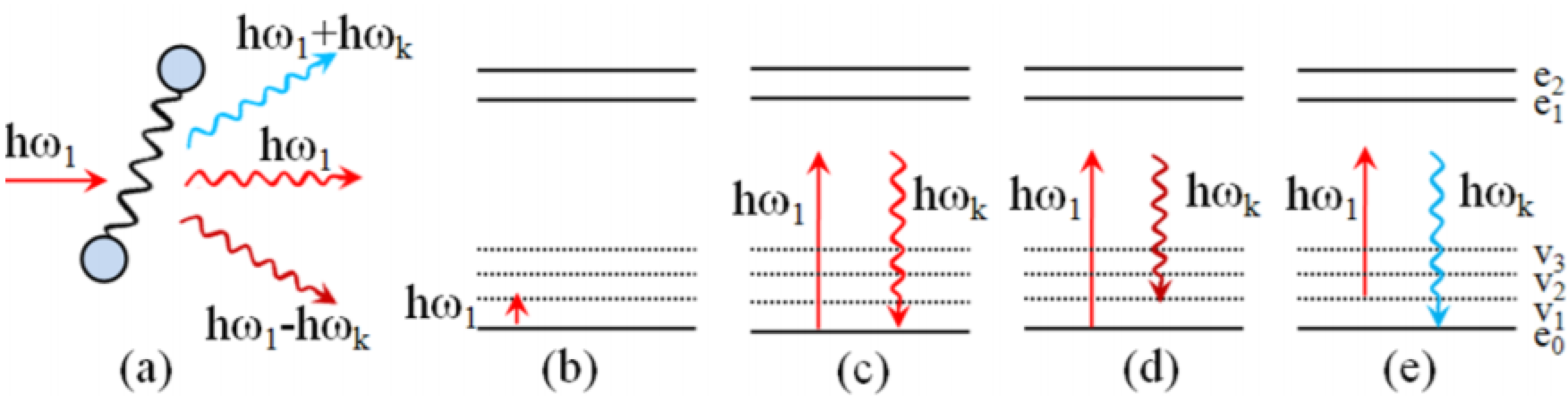
Nanometers, wavenumbers and relative wavenumbers

Absolute wavenumbers:

$$\sigma[in\ cm^{-1}] = \frac{10^7}{\lambda\,[in\ nm]}$$

Example: 500 nm corresponds to 20000 cm^{-1}

Raman scattering

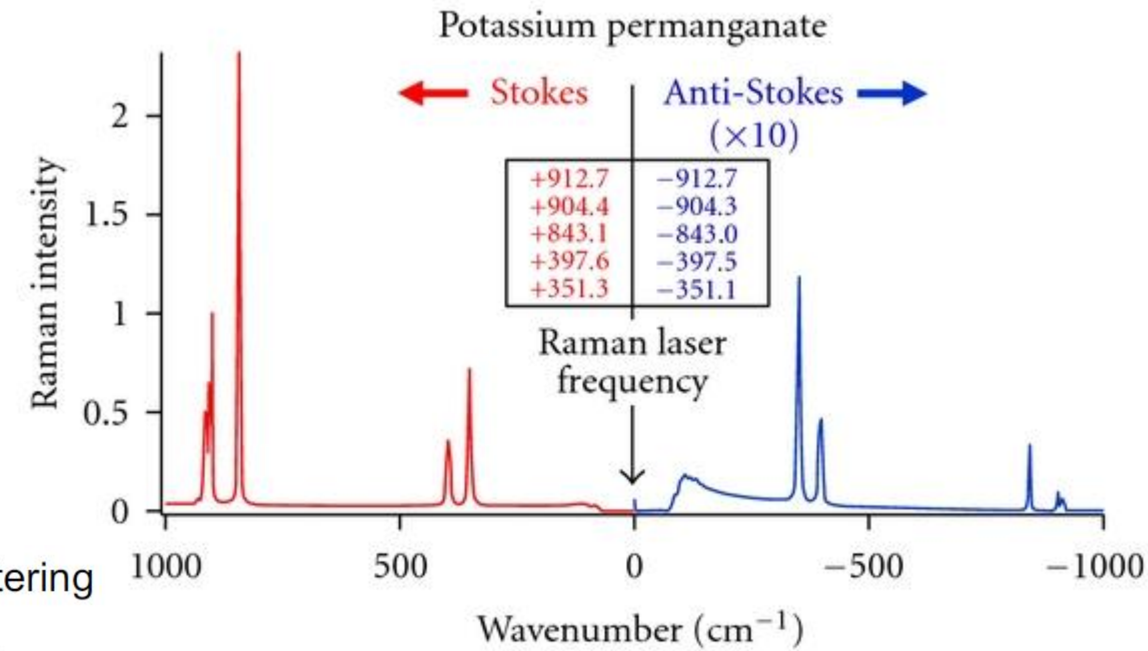


IR absorption spectroscopy	Light scattering	Stokes Raman scattering	anti-Stokes Raman scattering
1-photon effect	2-photon effect		

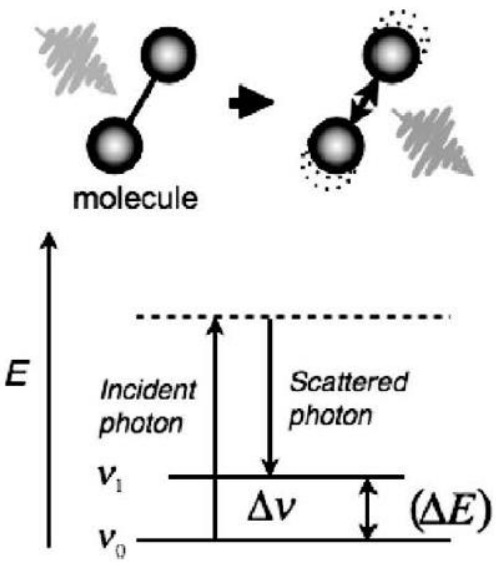
Inelastic Scattering of the incident radiation.

Interaction of photons with vibrational energy levels.

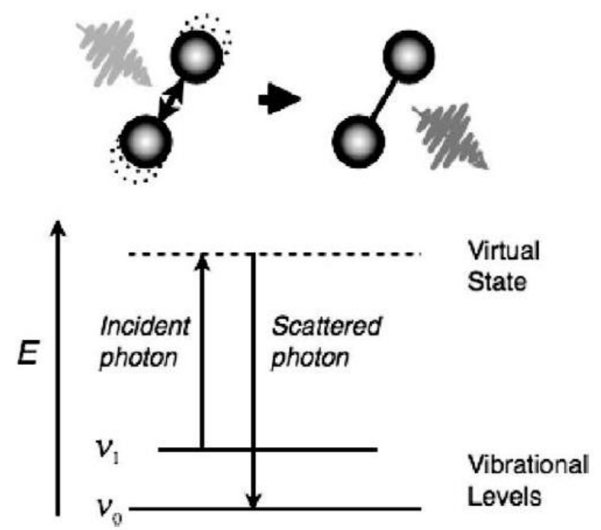
Raman Spectroscopy



(a) Stokes Raman Scattering



(b) Anti-Stokes Raman Scattering



Raman Spectroscopy

$\vec{p} = \alpha \vec{E}$ Incident optical field polarizes the molecules

$$\alpha = (\alpha)_0 + \left(\frac{\partial \alpha}{\partial r}\right)_0 r + \frac{1}{2}\left(\frac{\partial^2 \alpha}{\partial r^2}\right)_0 r^2 + \ldots$$

Taking the linear terms, considering an harmonic oscillator, that responds sinusoidally to the stimulus

$$\alpha = (\alpha)_0 + \frac{1}{2}\left(\frac{\partial \alpha}{\partial r}\right)_0 r_0 \sin(\omega_k t + \delta)$$

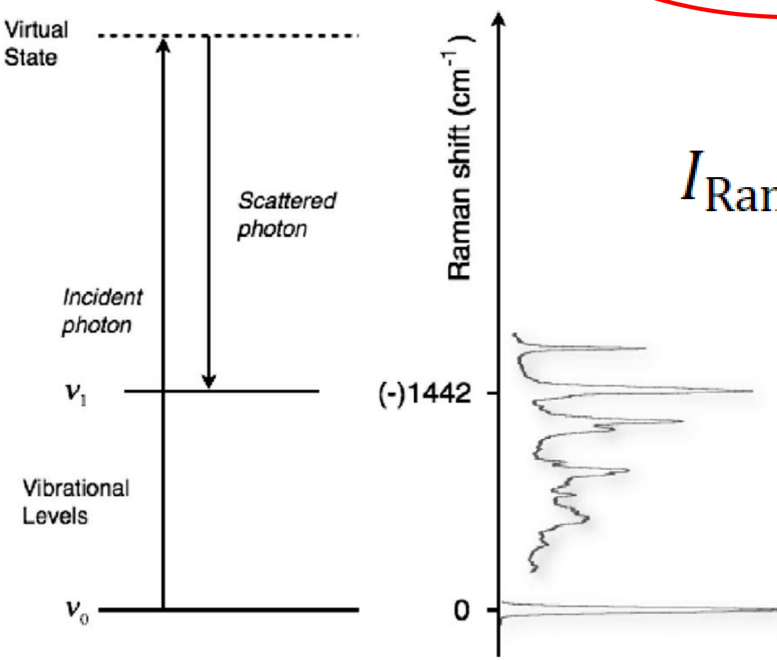
Raleigh **Raman**

$$p = \underbrace{(\alpha)_0 E_0 \sin(\omega_l t)}_{\text{Raleigh}} + \underbrace{\frac{1}{2}\left(\frac{\partial \alpha}{\partial r}\right)_0 r_0 E_0 \sin(\omega_k t + \delta) \sin(\omega_l t)}_{\text{Raman}}$$

Raman Spectroscopy

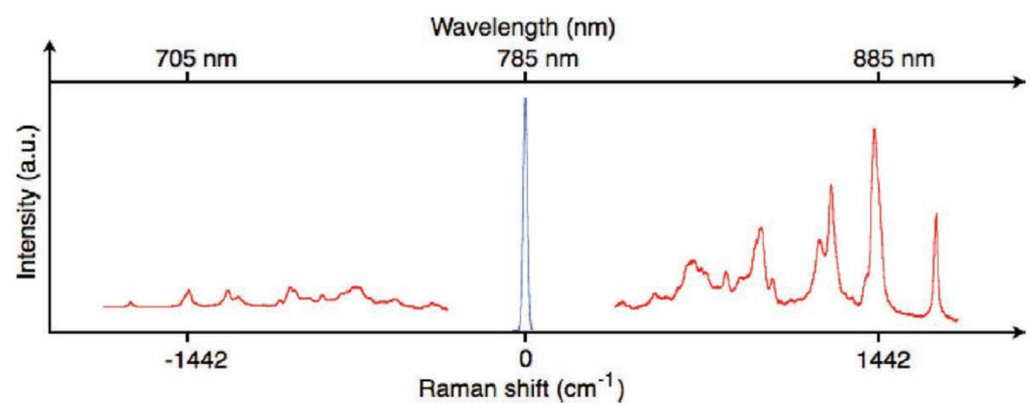
Stokes **Anti stokes**

$$p_{\text{Raman}} = \frac{1}{4}\left(\frac{\partial \alpha}{\partial r}\right)_0 r_0 E_0[\cos((\omega_{\text{l}} - \omega_{\text{k}})t + \delta) - \cos(\omega_{\text{l}} + \omega_{\text{k}})t + \delta]$$



$$I_{\text{Raman}} = N L \Omega \frac{\hbar}{2 m \omega_k}\left(\frac{\partial \alpha}{\partial r}\right)^2 \frac{\omega_i^4}{c^4} I_l$$

Raman signal, is always relative to the incident laser frequency.



Raman Spectroscopy

$$I_{\text{Raman}} = NL\Omega \frac{\hbar}{2m\omega_k} \left(\frac{\partial \alpha}{\partial r} \right)^2 \frac{\omega_i^4}{c^4} I_l$$

- Weak effect (small cross section)
- Use of lower wavelength is limited by fluorescence and phototocicity

Laser sources

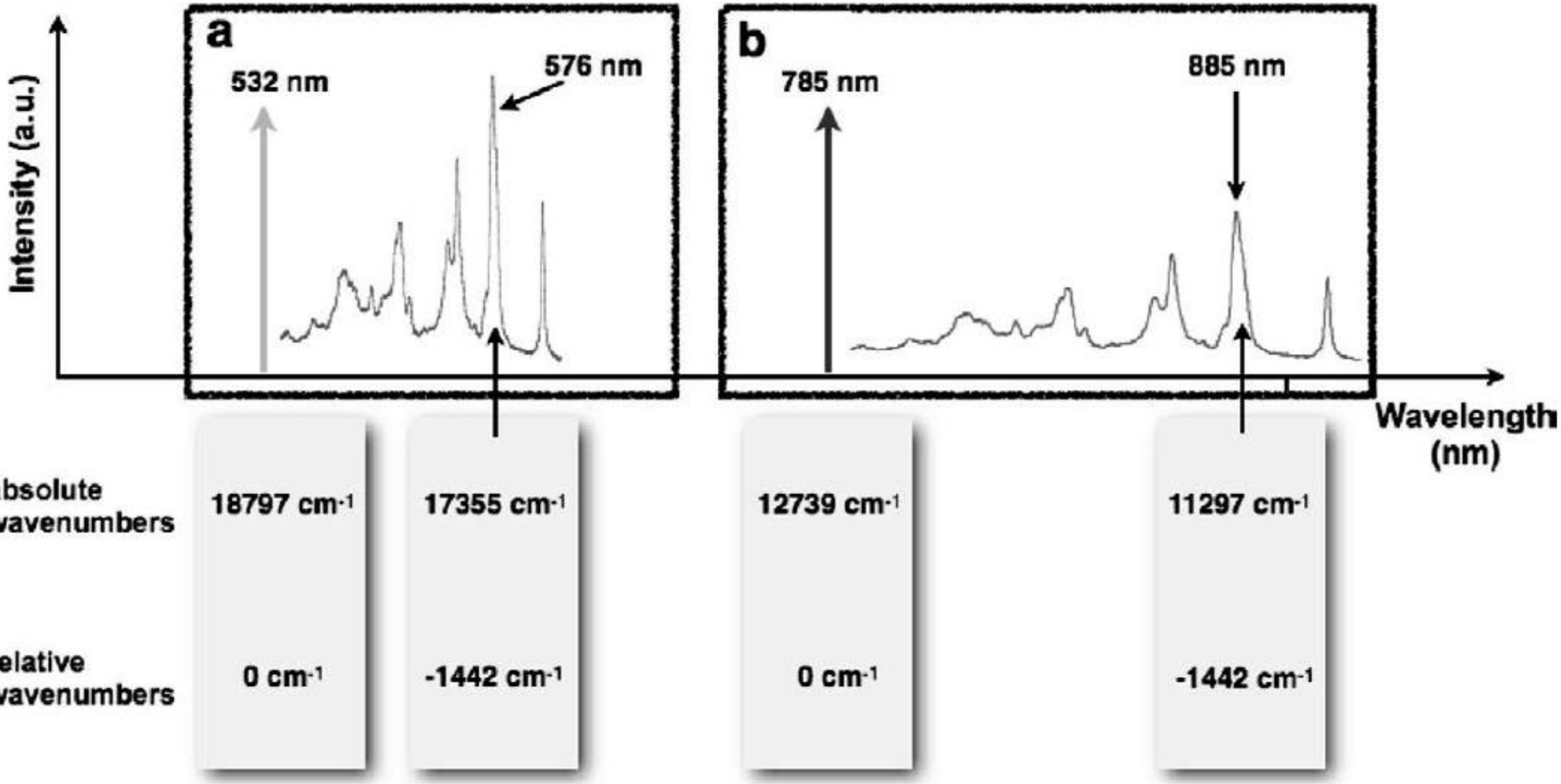
488 nm
532 nm
632 nm
785 nm
830 nm
1064 nm

A comparison between cross sections

Electronic (UV-Vis) Absorption spectroscopy:	10^{-20} m^2
Fluorescence spectroscopy:	Q x 10^{-20} m^2
Vibrational (IR) absorption spectroscopy:	10^{-23} m^2
Resonance Raman spectroscopy:	10^{-29} m^2
Non-resonant Raman spectroscopy:	10^{-33} m^2
Surface Enhanced Raman Scattering:	$10^{-?}$ m^2

Absolut Stokes shift, given in wavelength scale.

Raman Spectroscopy



To avoid distortions, spectrum are normalized to the excitation wavelength. The shift relative to the excitation wavelength is given in cm^{-1}.

Nanometers, wavenumbers and relative wavenumbers

Absolute wavenumbers:

$$\sigma[in\ cm^{-1}] = \frac{10^7}{\lambda\ [in\ nm]}$$

Example: 500 nm corresponds to 20000 cm^{-1}

Relative wavenumbers:

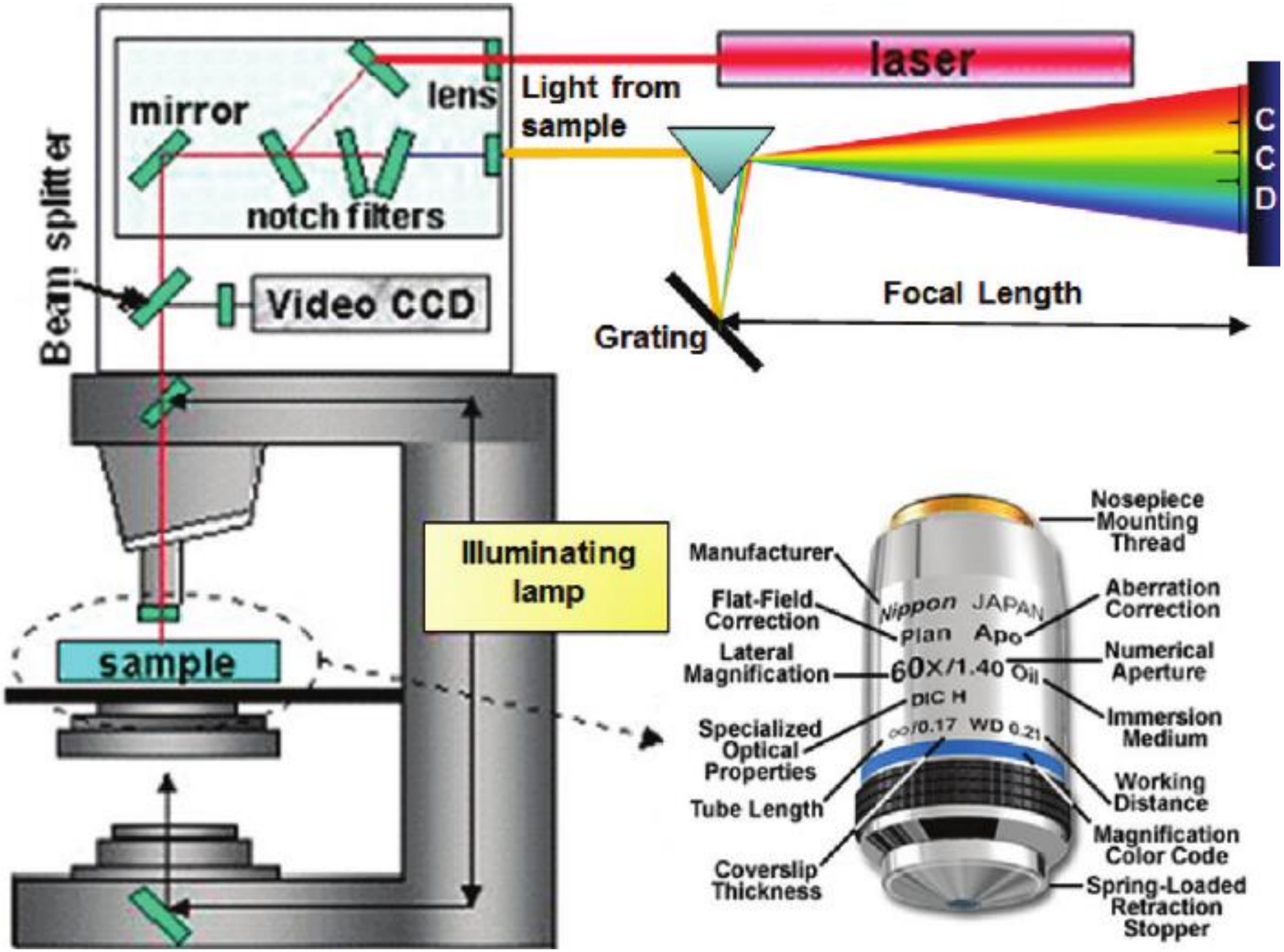
$$\sigma_{Raman}[cm^{-1}] = \frac{10^7}{\lambda_{exc}\ [in\ nm]} - \frac{10^7}{\lambda_{sc}\ [in\ nm]}$$

At each wavelength absorbs, a RAMAN transition at 1020 cm^{-1}

If we use a laser at 500 nm, at which wavelength is the light scattered by this transition?

Raman Microscopy

Cellular chemical analysis



Cellular chemical analysis

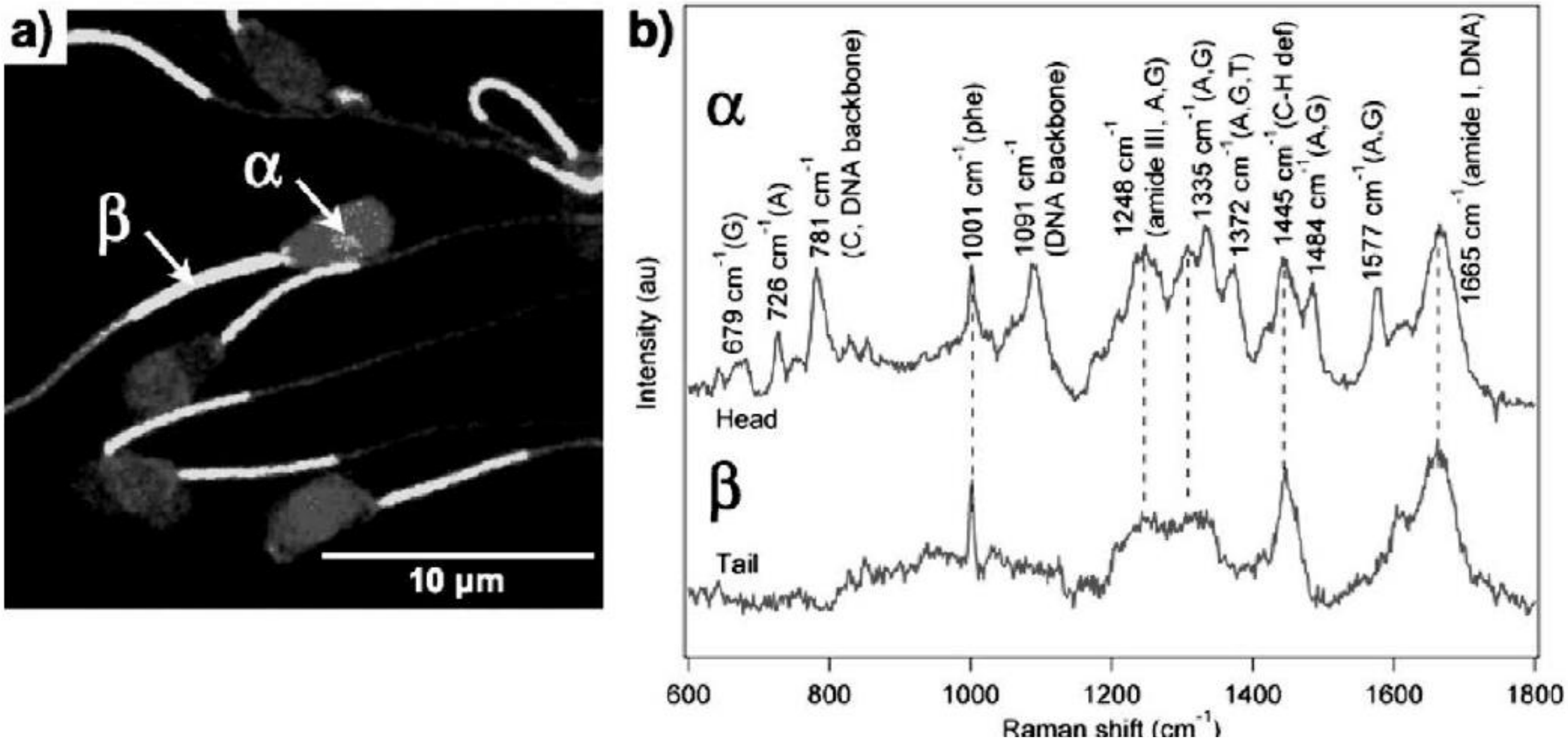
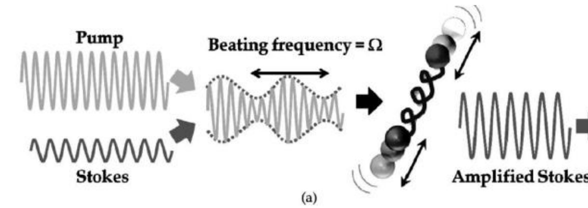


Figure 5.6 Raman spectra obtained from bovine sperm cells. (a) Intact, whole sperm cells are imaged in a confocal Raman microscope based on their autofluorescence. Specific areas, i.e., the sperm head (α) or sperm tail (β), can then be addressed by point spectroscopy. The resulting spectra and their assigned bond vibrations (see (b)) show that the sperm head is mostly composed of DNA and proteins (α), while the major constituent of the tail is mostly protein (β).

Stimulated RAMAN scatering

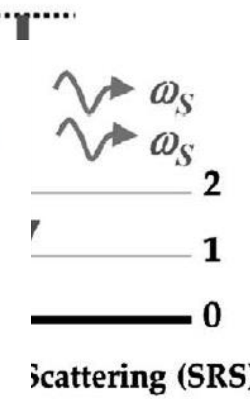
Several methods can be used to ehance Raman signals
Stimulated Raman Scatering (SRS)



$\Omega = \omega_i - \omega_s$

Cohere

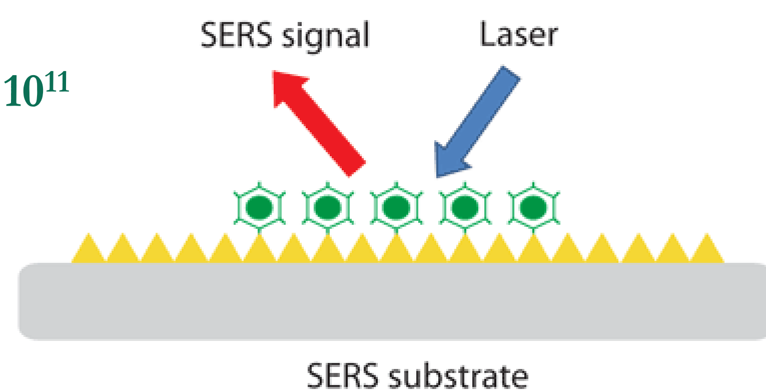
Electronic (UV-Vis) Absorption spectroscopy:	10^{-20} m^2
Fluorescence spectroscopy:	Q x 10^{-20} m^2
Vibrational (IR) absorption spectroscopy:	10^{-23} m^2
Resonance Raman spectroscopy:	10^{-29} m^2
Non-resonant Raman spectroscopy:	10^{-33} m^2
Surface Enhanced Raman Scattering:	$10^{-?}$ m^2

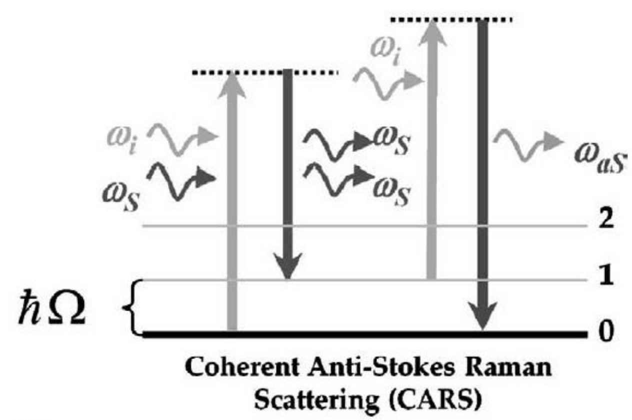


ω_{aS} =

Surfaced ehanced Raman Scatering

$10^{10} \sim 10^{11}$





Raman spectroscopy for medical diagnostics — From in-vitro biofluid assays to in-vivo cancer detection☆

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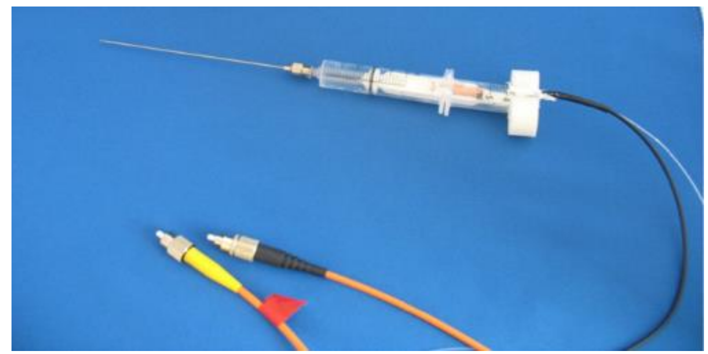

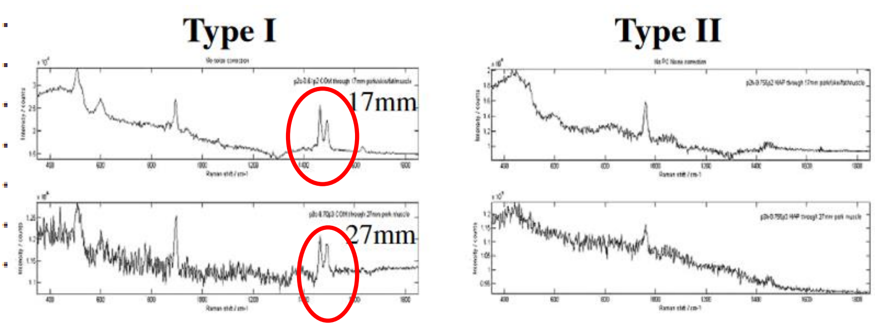

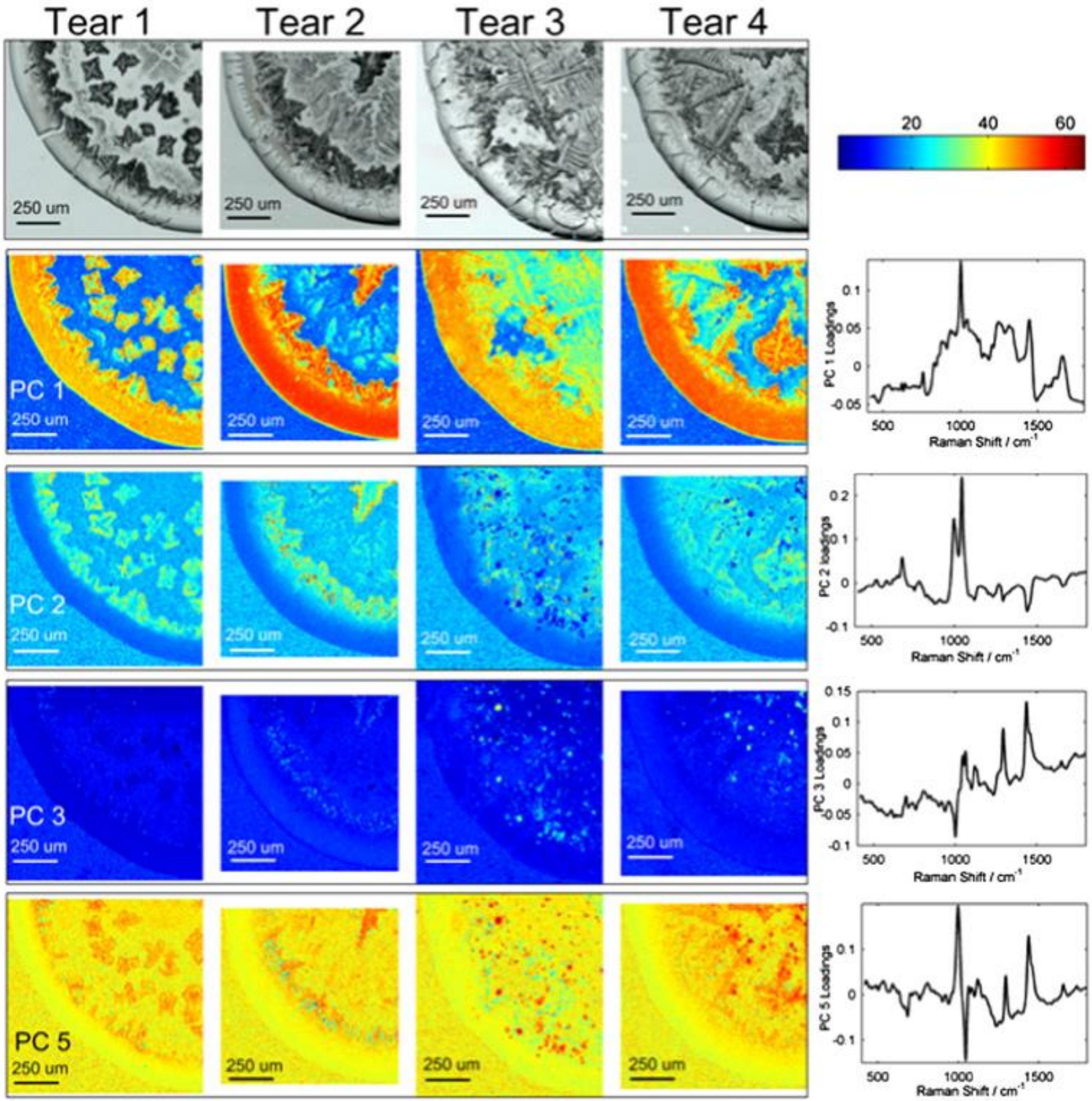

Tear 1
Tear 2
Tear 3
Tear 4
250 um
20
40
60
PC 1
PC 2
PC 3
PC 5
PC 1 Loadings
PC 2 loadings
PC 3 Loadings
PC 5 Loadings
Raman Shift / cm-1
500
1000
1500

	FTIR and Raman Spectroscopy. Optical trapping. Medical Applications	*2.9 2.11.4 2.11.5(Raman microscopy)*		