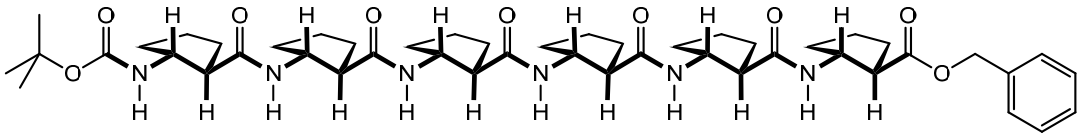
Rotational Frame Nuclear Overhauser Effect Spectroscopy (ROESY)

NOESY works well with small or large molecular weights.
For intermediate (1000-5000 Da) molecular weights, alternate ROESY pulse sequence is used to obtain nearly identical data.

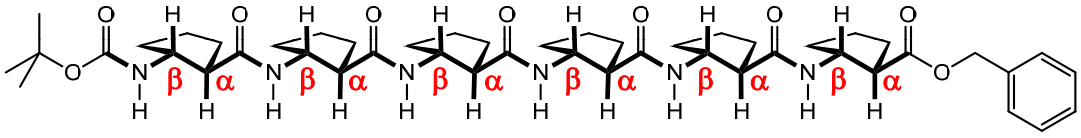
Example: Sam Gellman's β-peptides.

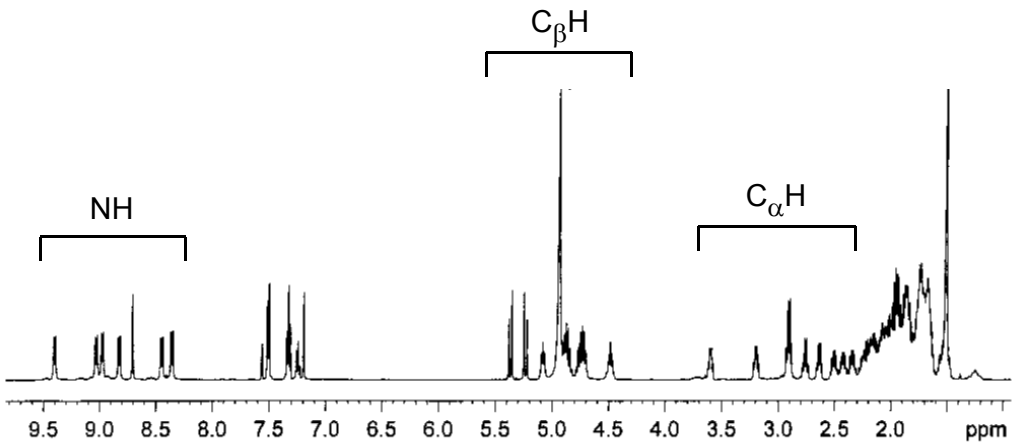


Gellman's question: Does β-"proline" enforce a turn, like proline?

Ref: Barchi, J. J.; Huang, X.; Appella, D. H.; Christianson, L. A.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc*. **2000**, *122*, 2711.

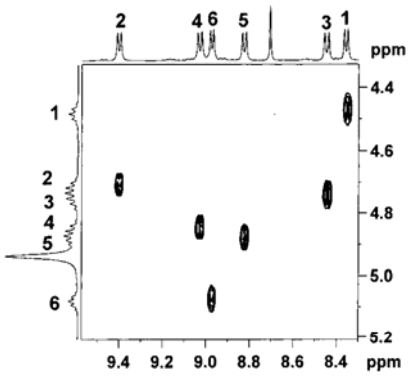
Rotational Frame Nuclear Overhauser Effect Spectroscopy (ROESY)





Rotational Frame Nuclear Overhauser Effect Spectroscopy (ROESY)

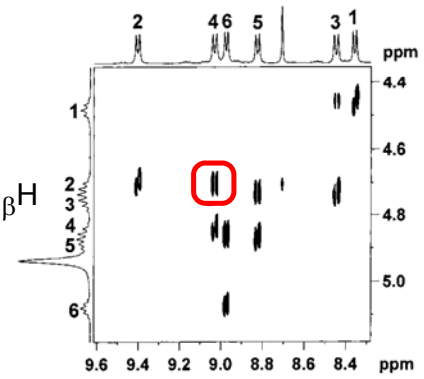
^{1}H TOCSY



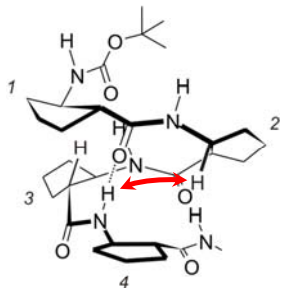
NH

$C_\beta H$

^{1}H ROESY



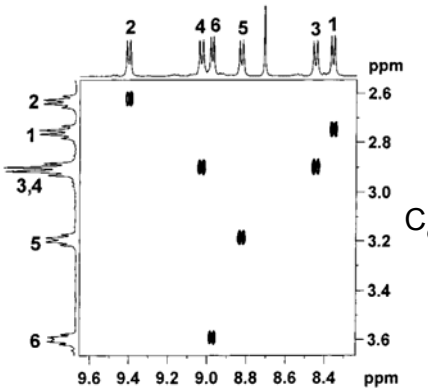
NH



As with NOESY, ROESY spectra are compared with TOCSY spectrum to rule out scalar coupling artifacts.

Rotational Frame Nuclear Overhauser Effect Spectroscopy (ROESY)

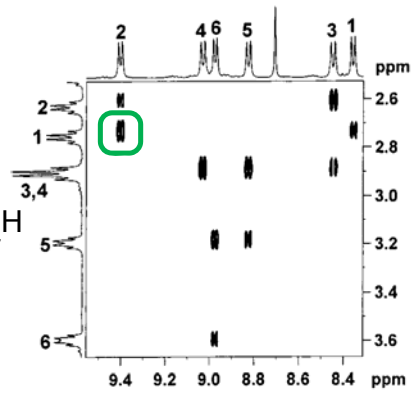
^{1}H TOCSY



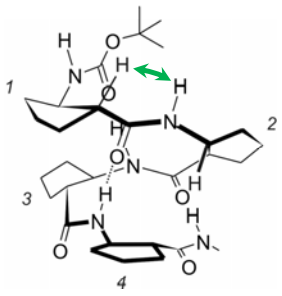
NH

$C_\alpha H$

^{1}H ROESY

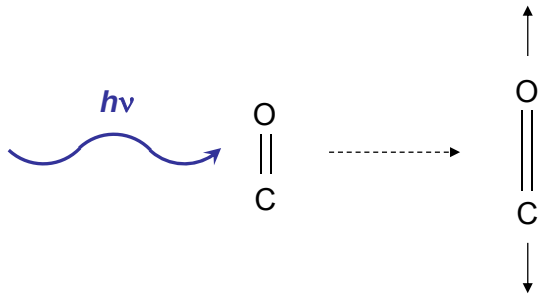


NH

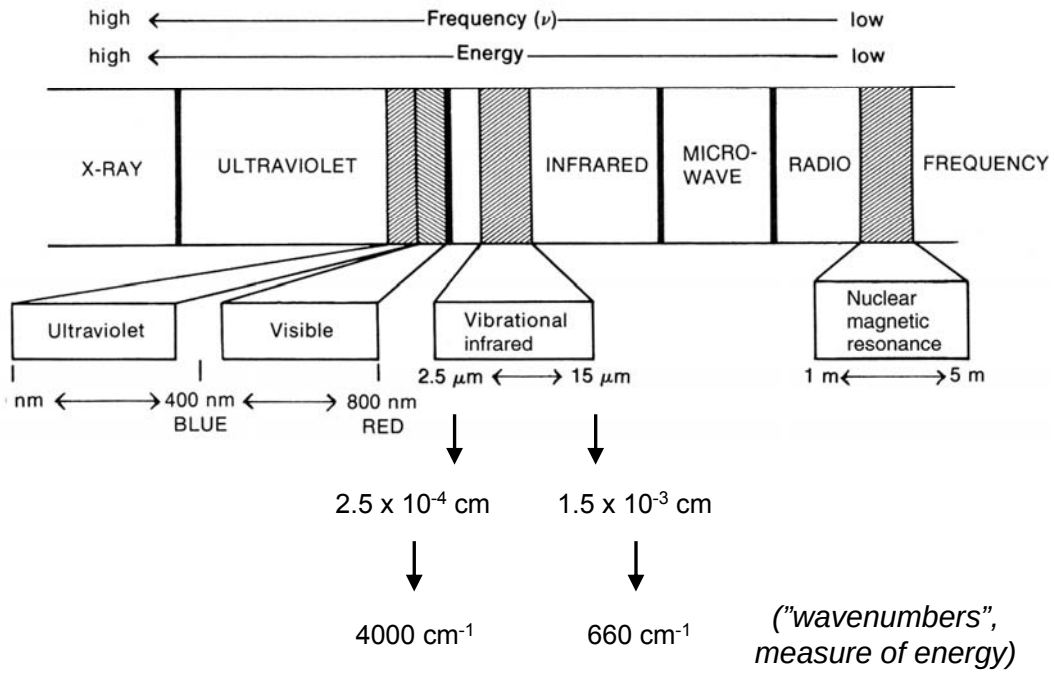


Infrared (IR) Spectroscopy

- Used to investigate specific functional groups
- No information on total structure, connectivity
- Direct absorption spectroscopy; involves excitation of a vibrational mode with an IR photon



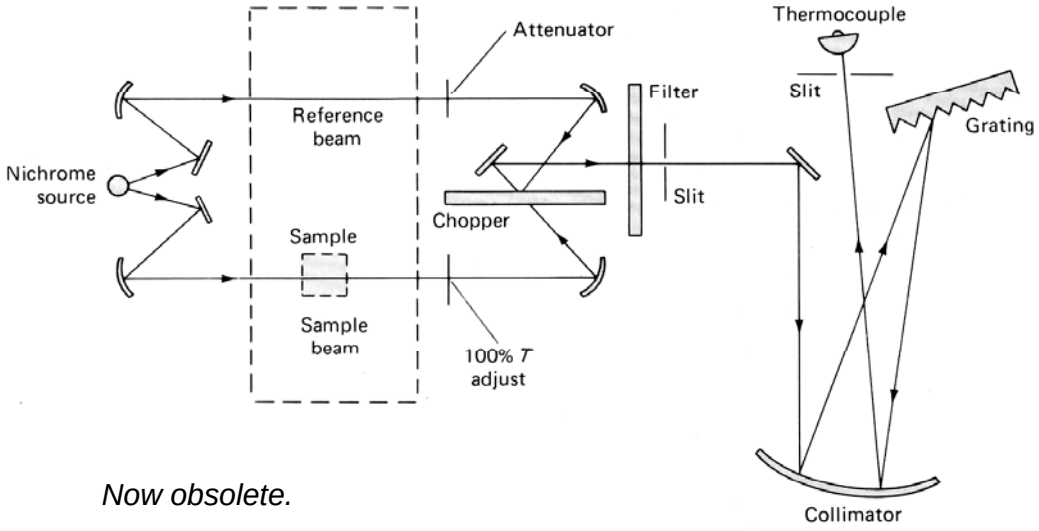
IR Spectroscopy



("wavenumbers", measure of energy)

IR Spectroscopy: Methods

Dispersive instrument:
Uses monochromator grating to scan wavelength.



Now obsolete.

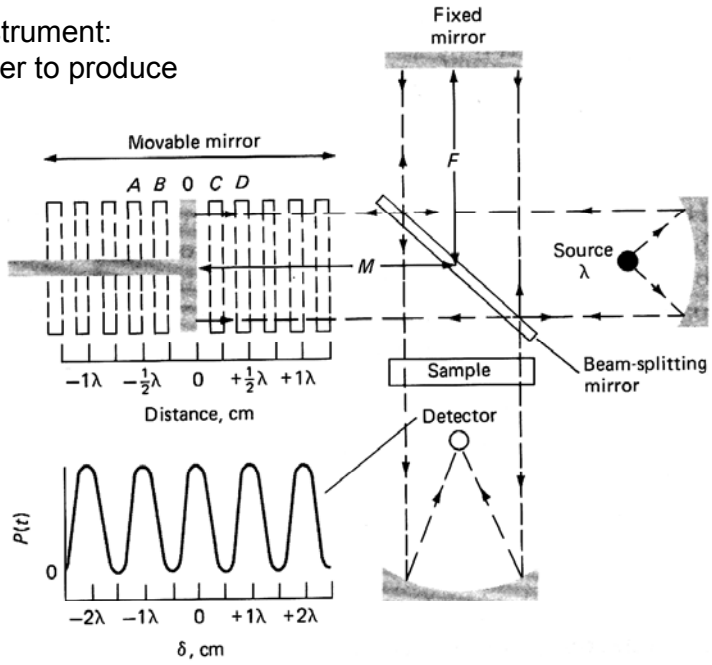
IR Spectroscopy: Methods

Fourier-transform (FT-IR) instrument:
Uses Michelson interferometer to produce full spectrum.

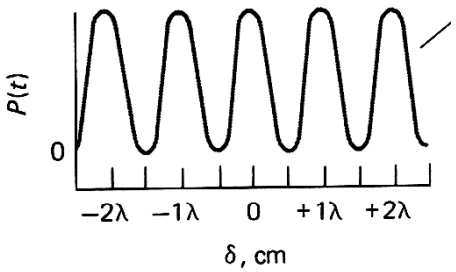
Source is broadband IR lamp.

As mirror is moved, frequency components of source constructively and destructively interfere, producing an *interferogram*.

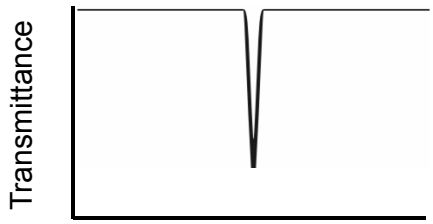
(Red HeNe laser is used to determine mirror position. Is not used for measuring spectrum.)



IR Spectroscopy: Methods

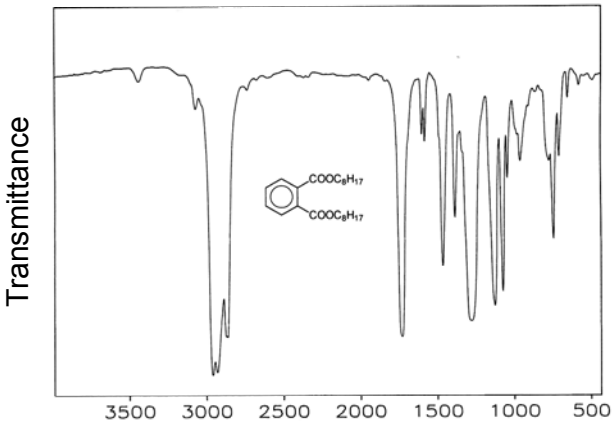


Fourier transform



Wavenumbers (cm^{-1})

Sum of interferogram components, Fourier-transformed, gives frequency-domain spectrum.



Wavenumbers (cm^{-1})

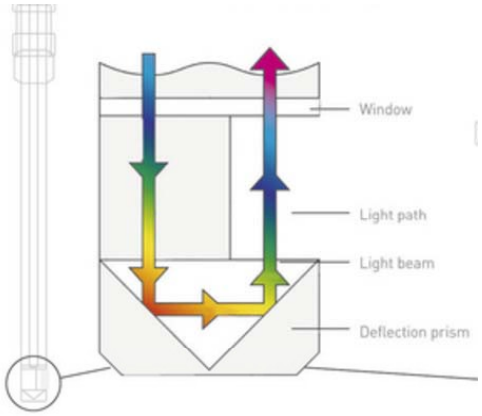
IR Sampling Formats: Pressed Salt Window (KBr Pellet)

Material can be combined with an IR-transparent solid (e.g., KBr) and pressed w/ high pressure into a clear window.

IR Sampling Formats: Salt Plate Deposition, Solution-Phase

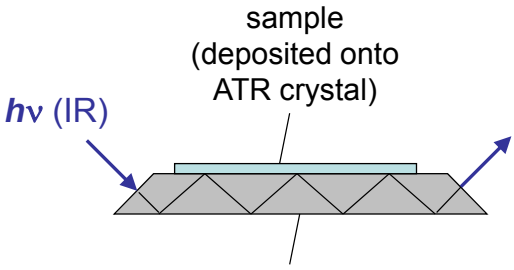
Material can be deposited as a solution, and then dried, onto an IR-transparent window material (e.g., NaCl).

Or, measurement can be performed on solution, either in a liquid cell (w/ IR-transparent windows) or with an immersion probe.



IR Sampling Formats: Attenuated Total Reflectance (ATR)

Method for looking at surfaces, films, small sample quantities.



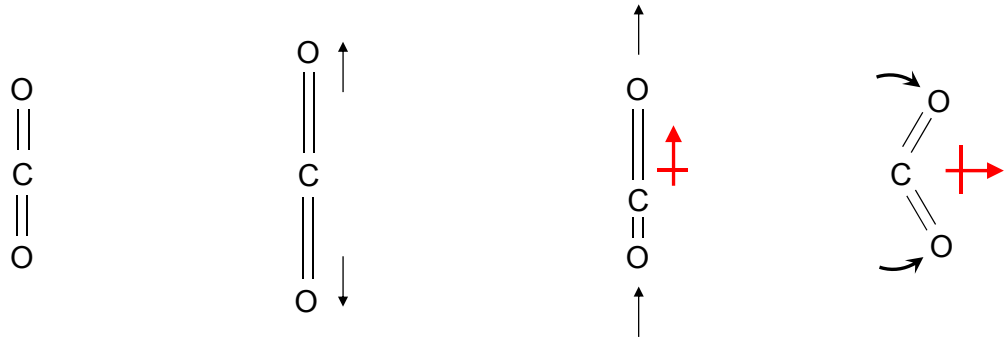
Bouncing light beam interacts multiple times with material deposited onto surface.

Straightforward to analyze μg of material.

IR Spectroscopy: Quantum Limitations

IR-absorbing transitions are allowed only when dipole moment changes during vibrational motion.

Modes can be combinations of bond vibrations.



symmetric stretch:
IR forbidden
(1340 cm^{-1})

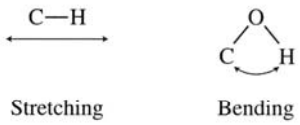
asymmetric stretch:
IR allowed
(2350 cm^{-1})

bend:
IR allowed
(660 cm^{-1})

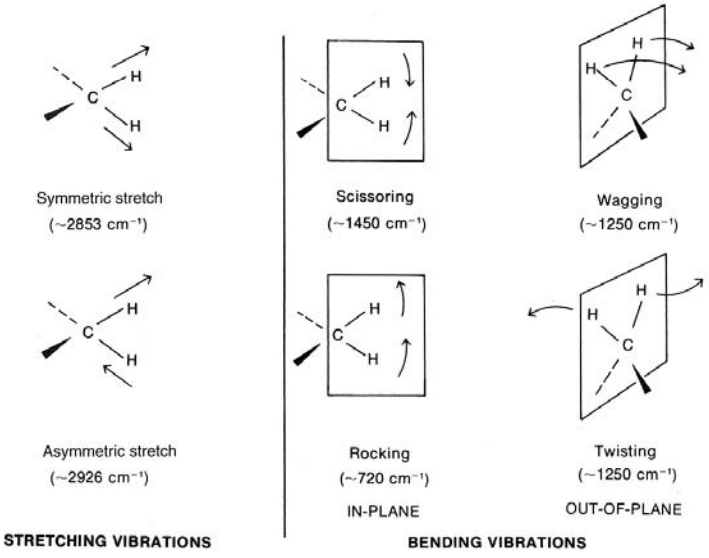
As a result, IR instruments sometimes purged with N_2 to get rid of CO_2.

IR-Allowed C-H Vibrations

single modes

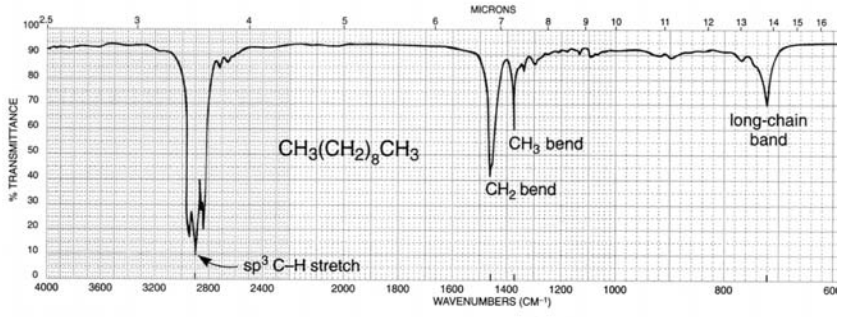


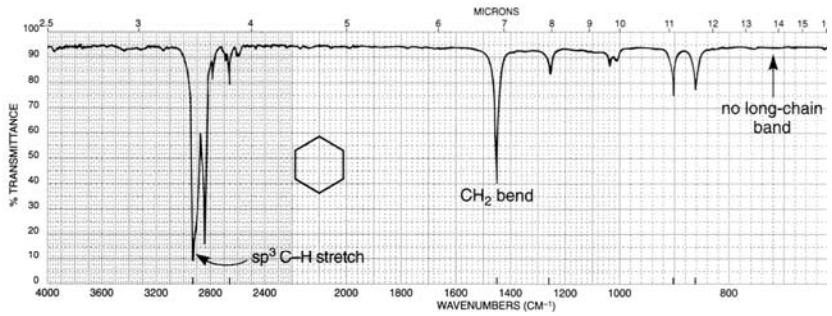
combination modes



Characteristic Features in IR: Alkanes

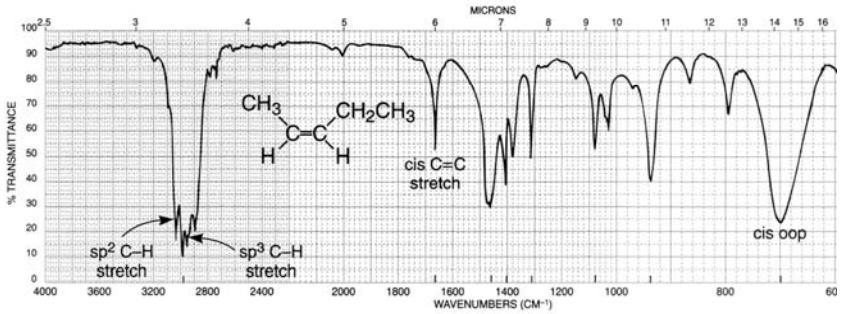
C-H stretch **below** 3000 cm^{-1}.



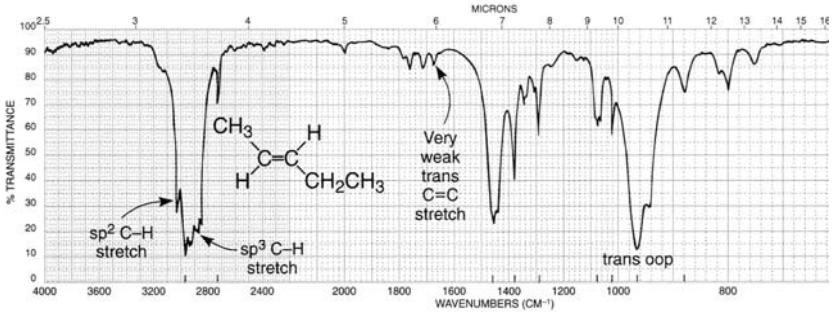


Characteristic Features in IR: Alkenes

C-H stretch **above** 3000 cm^{-1}.



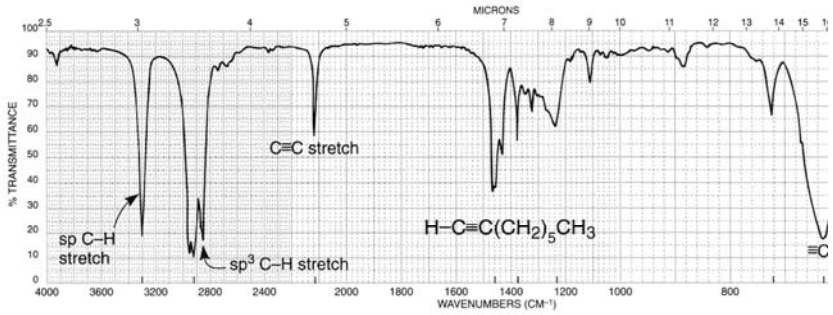
C=C stretch ~ 1600-1660 cm^{-1}; affected by bond stereochemistry.

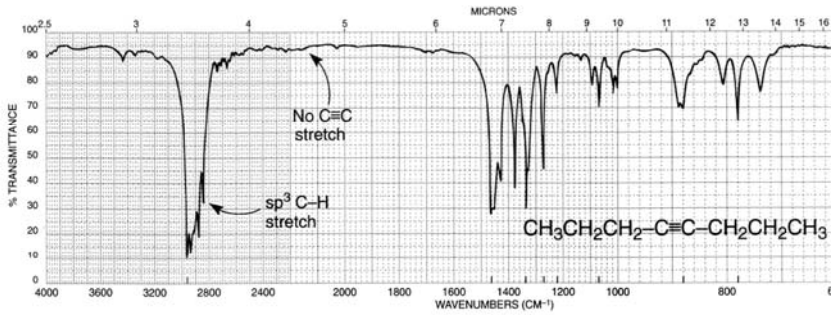


Characteristic Features in IR: Alkynes

C-H stretch
~ 3300 cm^{-1}.

C≡C stretch
~ 2150 cm^{-1},
but only when
asymmetric.

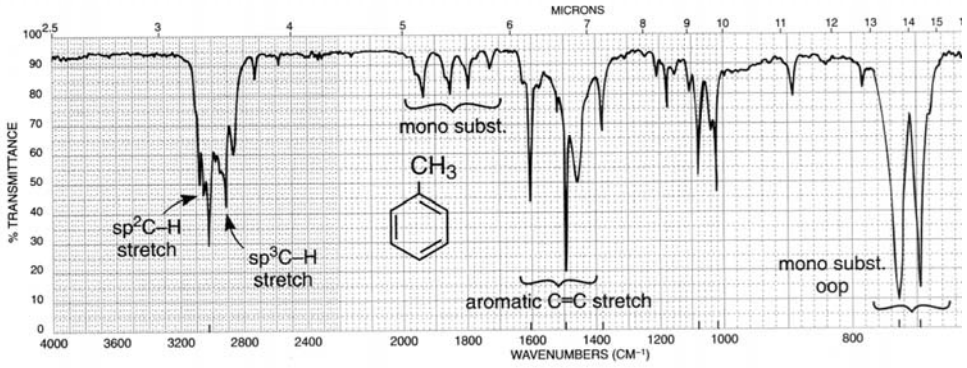




Characteristic Features in IR: Aromatics

C-H stretch **above** 3000 cm^{-1}.

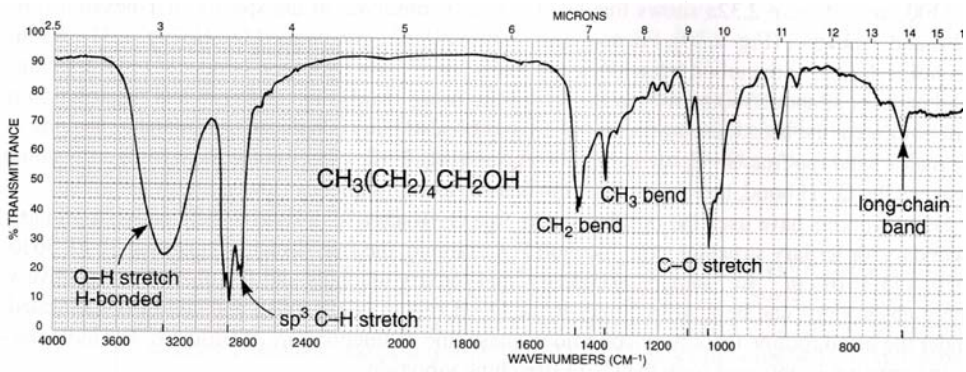
C=C stretch in pairs, ~ 1600 and 1475 cm^{-1}.



Characteristic Features in IR: Alcohols

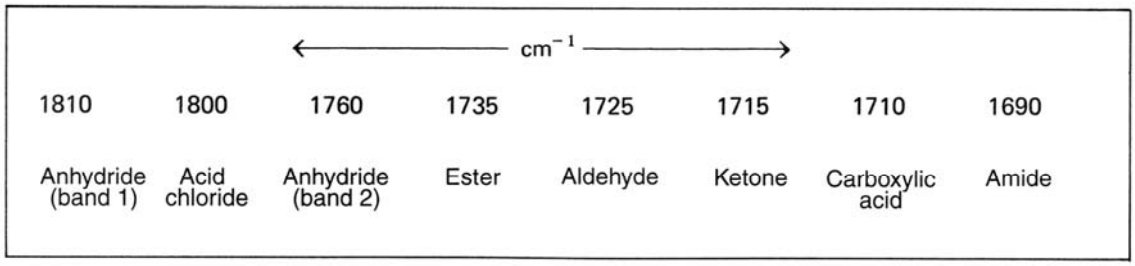
O-H stretch 3650-3600 cm^{-1} if dilute (no H-bonding).

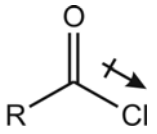
O-H stretch 3400-3300 cm^{-1} if H-bonded.



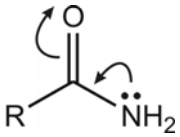
Characteristic Features in IR: Carbonyls

C=O stretch 1800-1600 cm^{-1}; typically sharp and strong.
Very diagnostic.
Frequency strongly affected by substituents:



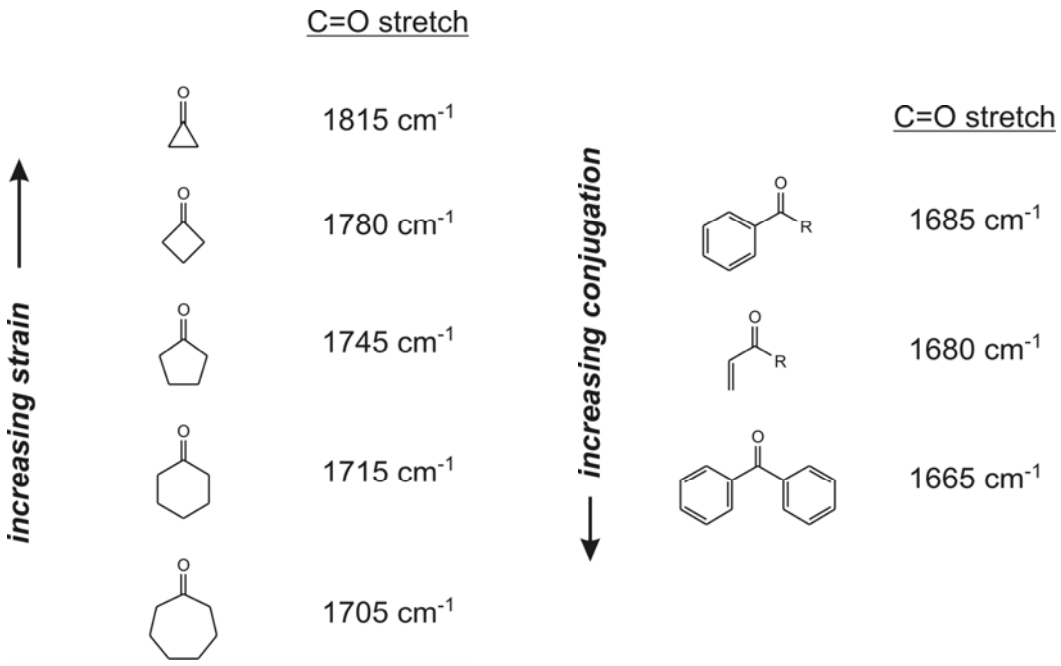


inductive effect
raises bond frequency

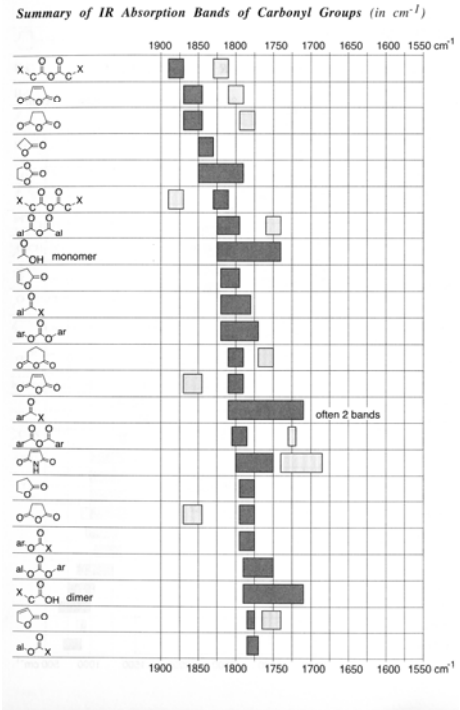


resonance effect
lowers bond frequency

Characteristic Features in IR: Carbonyls



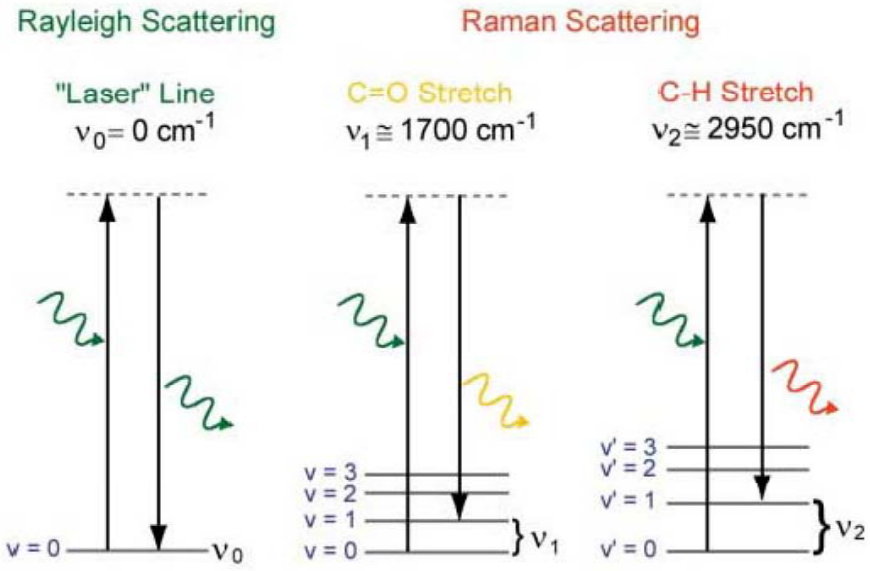
Characteristic Features in IR: Carbonyls



Tables & examples in Pretsch are the most helpful.

Raman Spectroscopy

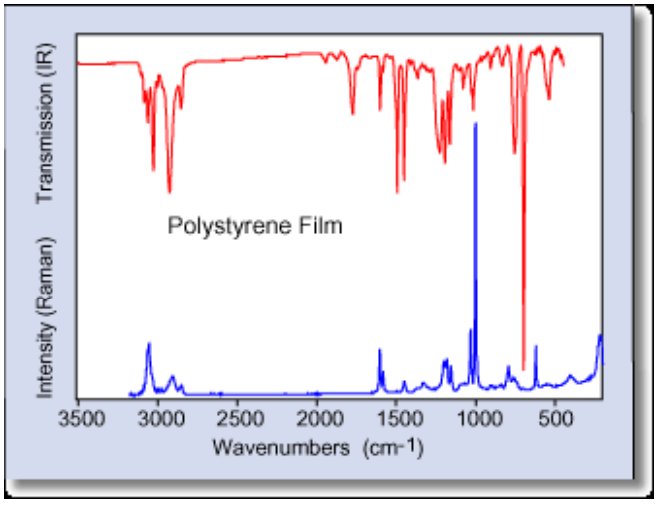
Vibrational frequencies can also be probed by change in wavelength of scattered, visible light.

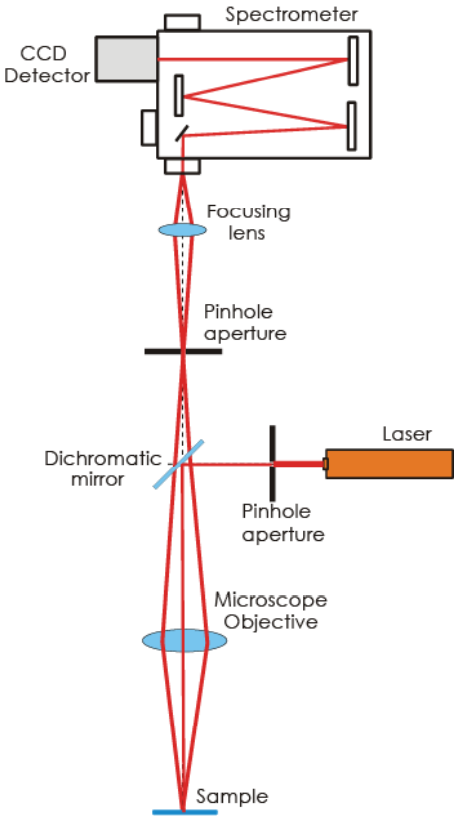


Raman Spectroscopy

Technically, quantum selection rules for Raman scattering are opposite those of IR absorbance; Raman probes symmetric modes rather than asymmetric ones.

Practically speaking, many modes in complex organic molecules are probed by both methods.



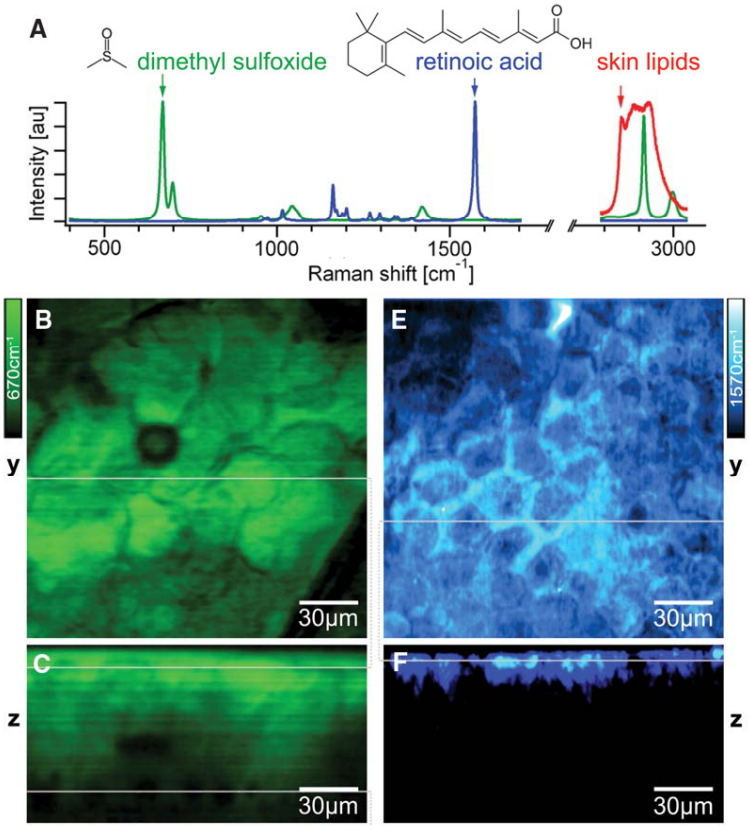


Confocal Raman Microscopy

Because light source in Raman spectroscopy is a laser, beam can be focused onto a sample or surface to do Raman "microspectroscopy".

Confocal Raman Microscopy

Allows for "functional group imaging".



Freudiger, C. W.; Min, W.; Saar, B. G.; Lu, S.; Holtom, G. R.; He, C.; Tsai. J. C.; Kang, J. X.; Xie, X. S. *Science* **322**, 1857 (2008).