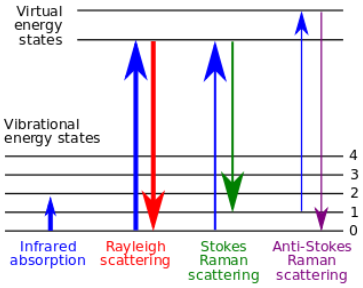
Chem 524 Lecture Notes –Raman (Section 17)— 2013

For HTML of 2005 notes, click here

XIII. Molecular Light Scattering and Raman Spectroscopy (Read Ch. 16)

A. Elastic Scattering $\lambda_o = \lambda_s$ - basis for Dynamic Light Scattering (DLS) experiments (see Notes 19)



1) Raleigh Scattering — scattering centers small compared to λ
λ_s - same frequency, Intensity $\sim\lambda^{-4}$, $\sim\alpha^2$ (polarizability)

2) Debye/Mie — more anisotropic, larger particles
spatial variation indicates size and shape

B. Inelastic - $\lambda_o \neq \lambda_s$

1) Brillouin — scatter from phonons (thermal density fluctuation) - not analytical use

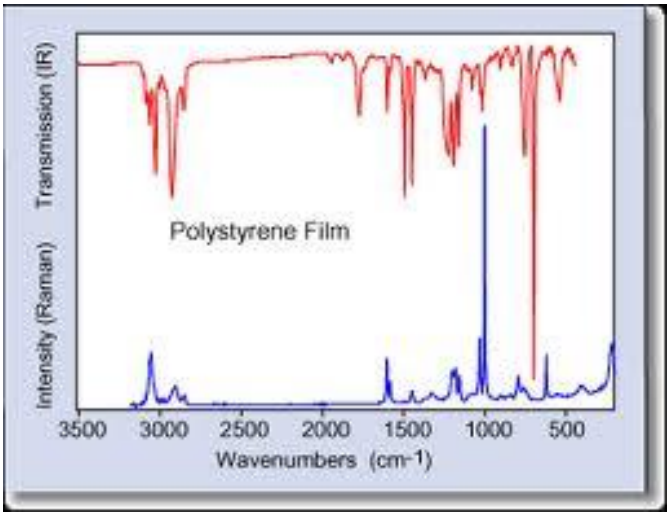
2) Raman — scatter from molecular excited states – high qualitative analysis use
— vibrations most often (can also be from rotations, low lying electronic states)

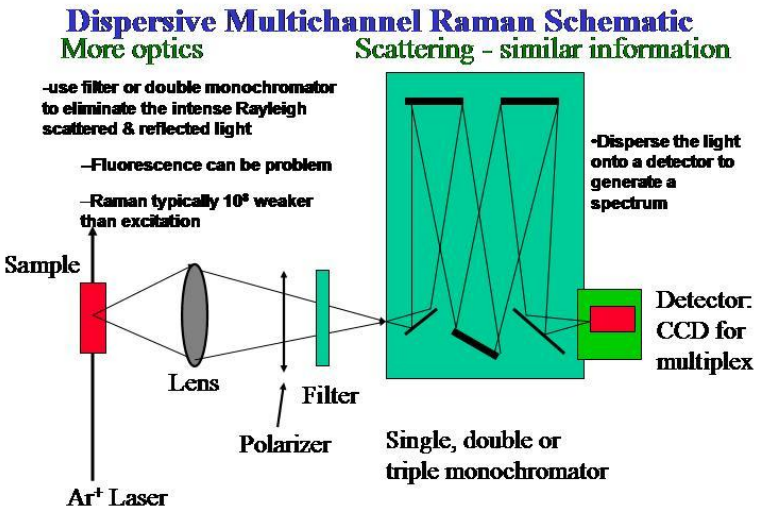
C. Raman — $\nu_s = \nu_o \pm \nu_{vib(rot)}$ (+) = anti-Stokes, (-) = Stokes (energy into molecule)

-- qualitative - big use (like IR) identify/characterize

-- quantitative - particularly difficult — intensity standards needed, vary across spectrum

-- single beam experiment, intensity depend on excitation (I_0) and geometry, focus plus environment, concentration, polarization – instrument dependent



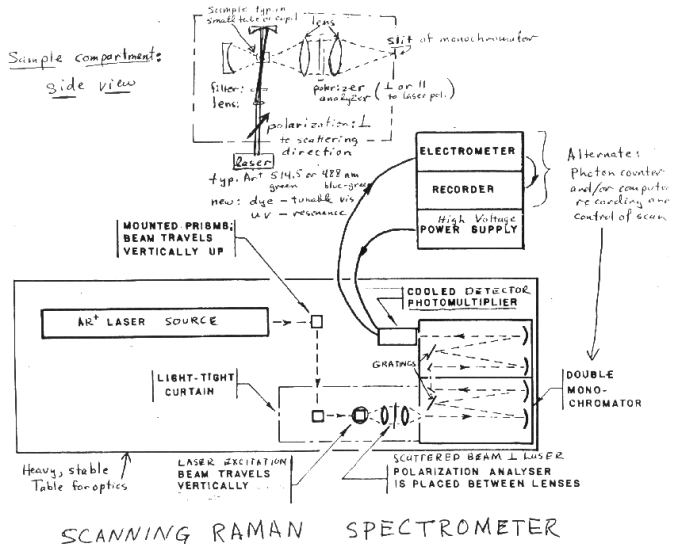


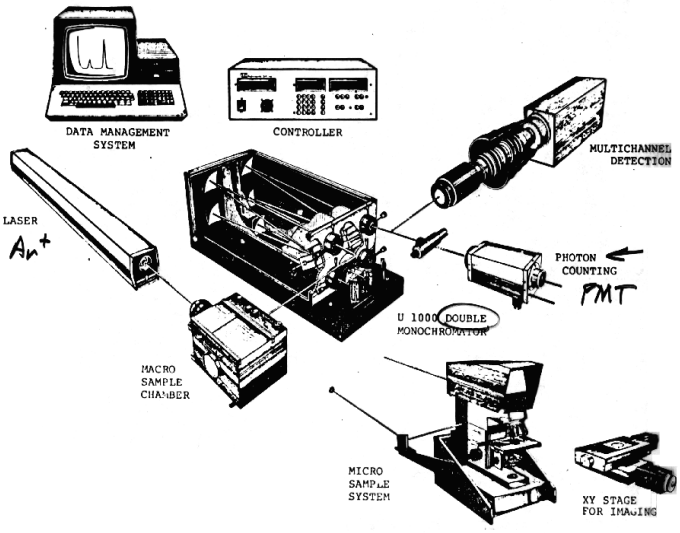
- capable of small volumes/but require relatively high concentration

--Complementary to IR, tend to opposite selection rules, different intensities $\sim[\partial\alpha/\partial Q]^2$
Often plot Raman – IR same spectrum one above other showing comparison

--Instrumentation vary depend on source (excitation), optics, dispersion and detector -- all alter character and use. Normally excite visible, avoid absorbance, if absorb can get resonance (enhance), if fluorescence is issue, can excite in red, near IR, may need alternate detector for this

1. Instruments – many used to be homemade, construct from components, still do in many labs





Commercial components

a. Concept same as emission spectrometer — higher res needed/very high sensitive?

-- laser excite (no monochromator) – Ar ion -visible, 488 or 514 nm (traditional, stable, narrow, powers up to W) – problems: very expensive, need high power use and water cooling

now often YAG (doubled at 532, green) or diodes various wavelengths viss-nearIR,

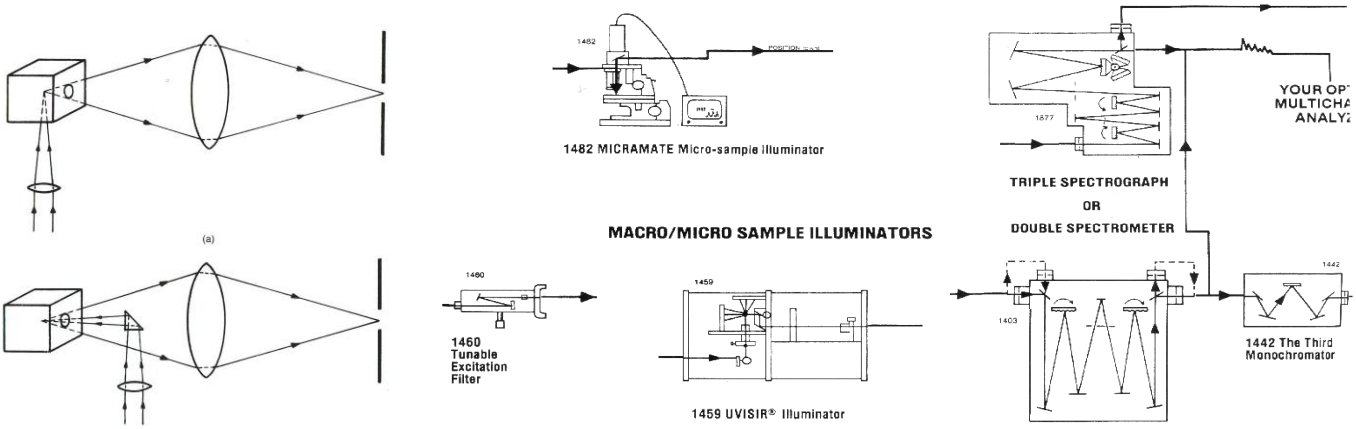
expensive option - special FRED lasers, double to UV, still cw, low power but OK

-- detect: PMT and photon count original method (scanning monochromator, one λ at time)

now CCD universal with spectrograph – simultaneous detection spectrum

-- 90º Scatter typical (include some opposite-reflection, “270º” with back mirror),

180º back-scatter sometimes more efficient, better for imaging or microscopy



Traditional: scan monochromator–double (even triple) ½ → 1m typical (reduce scatter light)

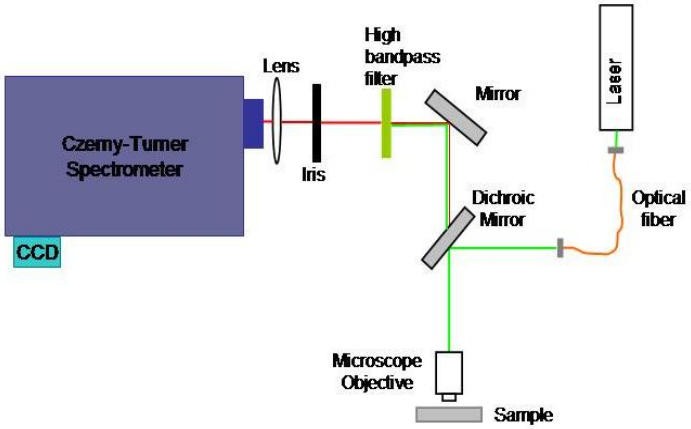
now multiplex: single spectrograph + CCD + holographic filter for laser blocking

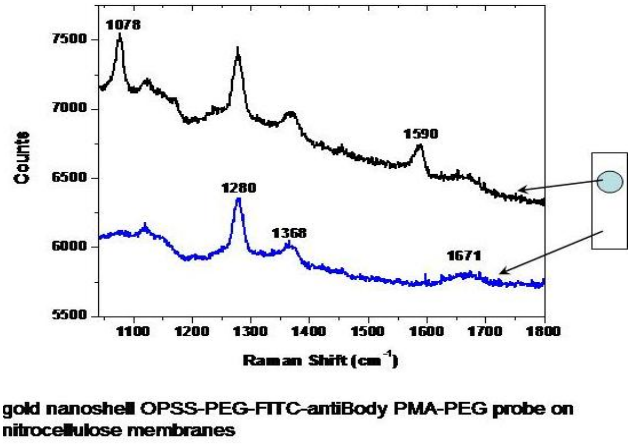
can be short or long focal length, depend on desired resolution/throughput

fast collection (f/1.8), with lens based spectrographs, transmission or reflection grating

Optics all glass/quartz + fibers - even handheld devices with very small optical packages

-- microscope (180º often), fine focus, high res image, possible due to shorter wavelength, λ_0





gold nanoshell OPSS-PEG-FITC-antiBody PMA-PEG probe on nitrocellulose membranes

-- polarization important (polarized, ||, and depolarized transitions, $\perp$)

Raman is 2-photon, so relative polarization of λ_{ex} and λ_s beams important

Polarization ratio: $\rho = I_\perp/I_{||}$ -- $\rho < 7/8$ (or $\rho < ¾$ for different excitation geometry)

means mode is *polarized*, can tell symmetry of transition (e.g. a_1 vs. low sym.)

crystals have polarization tensor, 9 components

b. Multichannel systems — fixed resolution

-- go for speed/ S/N by averaging

-- diode array works/CCD can be better, bigger slit image

-- can do time dependence with gate $\tau < \mu s$

-- alternative do pump-probe kinetics. Time depend on delay

Commercial spectrometer

Hand held

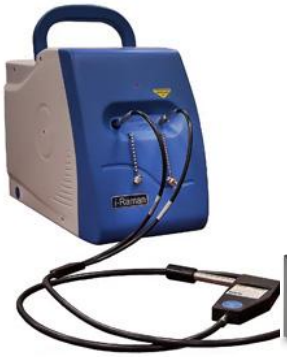
Inphotonics

B&W Tek

SciAps – DeltaNu models (see Specs) Rigaku – First Guard, Handheld

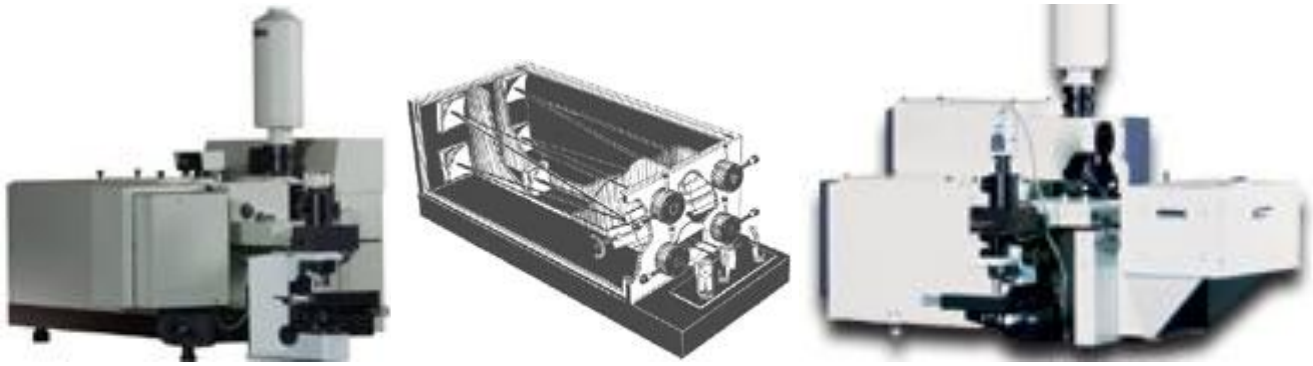
	ReporteR	Inspector 300	Inspector 500
Laser Power	120 mW	300 mW	300 mW
Laser Wavelength	785 nm	785 nm	1030 nm
Detector	Standard CCD	Low Background CCD	
Resolution (cm-1)	10	9	8-10
Spectral Range (cm-1)	300 – 2500	175 – 3200	100 – 2500
Software, Display	Monochromatic Standard matching of unknowns to spectral libraries	Color Touchscreen, on board libraries and analysis	Color Touchscreen, on board libraries and analysis
Weight	0.8 lb	3.7 lb	3.7 lb
Dimensions (in)	6 x 3 x 1.75	7.5 x 6.9 x 1.7	7.5 x 6.9 x 1.7
Battery Life	>4 hrs, rechargeable	4 hrs, removable	4 hrs, removable

Compact/portable



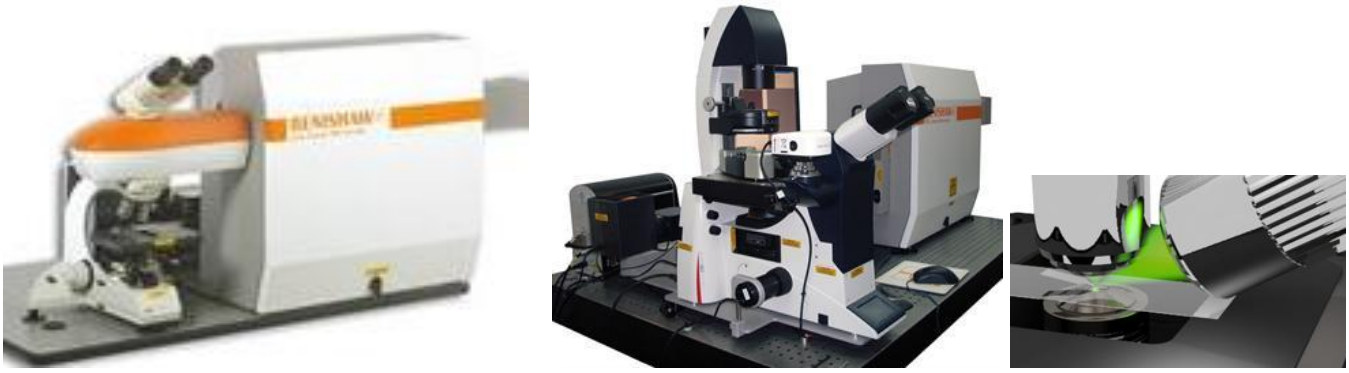
B&W Tek DeltaNu and SciAps StellarNet Rigaku – Xantus, dual freq, InGaAs

Commercial setups



Horiba (J-Y) - U1000 double 1m mono. Raman w/ CCD & sampling – triple mono version

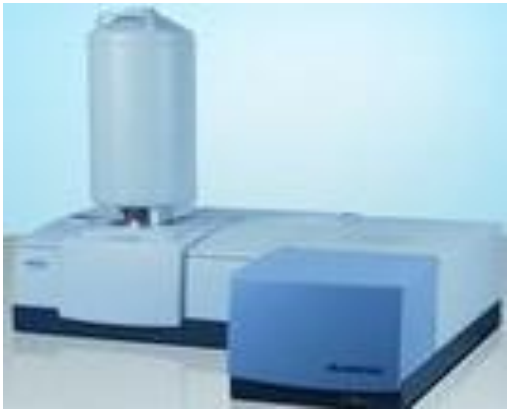
Horiba – modular, fiber coupled, small mono. CCD, microscope sampling – JASCO micro-Raman

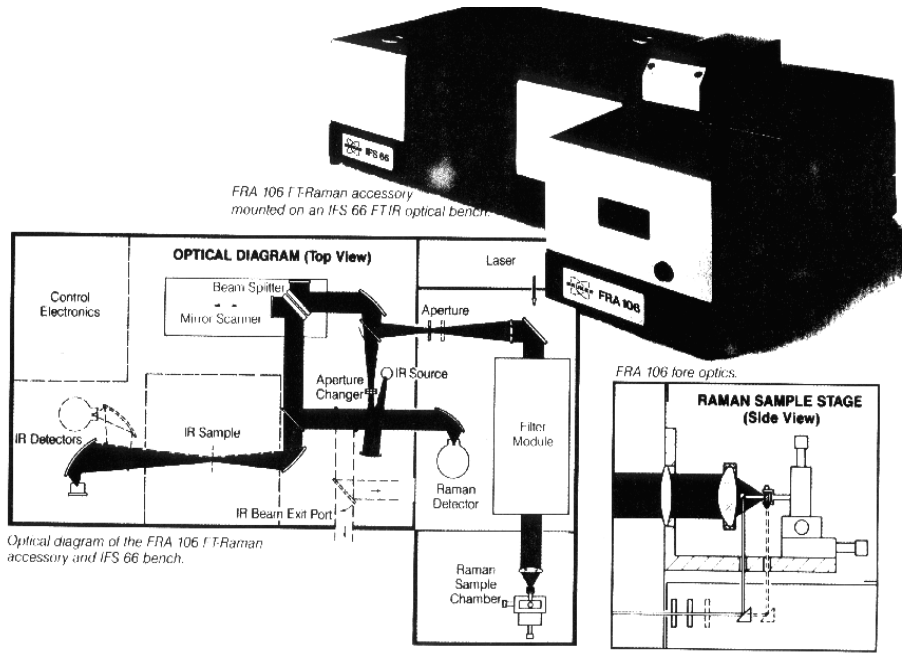


Renishaw microscope-Raman

Combine with Bruker AFM (parallel or TERS measurement)

c. FT Raman — near IR laser (700 nm → 1μ) -- lose as λ^4 -- gain from multiplex





Bruker examples Reduction in fluorescence, red excite Nicolet NXR model

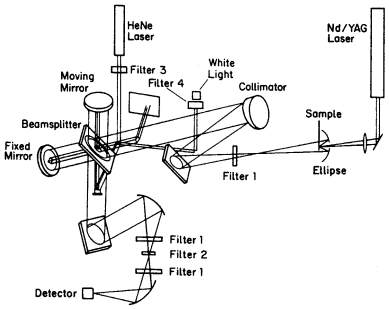
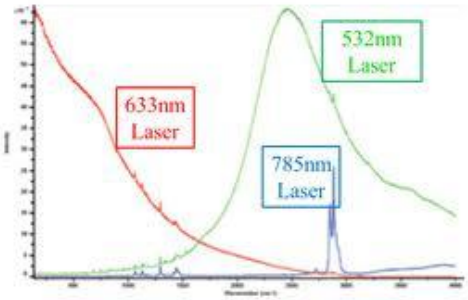


Figure 2.4. Instrumental layout for FT-Raman spectroscopy.



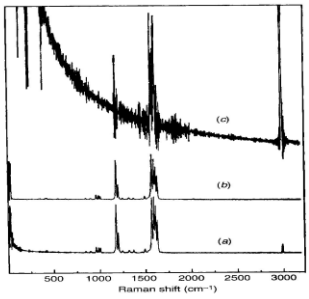


Figure 18.6. FT-Raman spectra of bis-methyl styryl benzene showing the shot noise introduced when the peak absorbance of the notch filter is decreased from (*a*) 10 to (*b*) 9 to (*c*) 8. (Reproduced from [6], by permission of the American Chemical Society; copyright © 1986.)

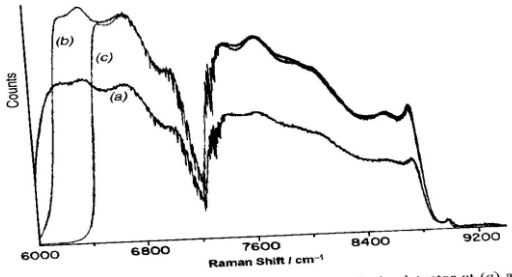


Figure 18.7. Spectra of white-light source measured with an InGaAs detector at (*a*) ambient temperature, (*b*) 198 K, and (*c*) 77 K. A filter to remove all radiation of shorter wavelength than 1064 nm is mounted in the optical path. (Reproduced from [8], by permission of Springer-Verlag, Vienna; copyright

Filter to eliminate ν_0 needed for shot noise InGaAs extend Raman past 3000 cm^{-1} if *not* cool 77K

-- big advantage — eliminate. Fluorescence from "dirty" samples

-- big application — materials/bio/complex sample / no preparation

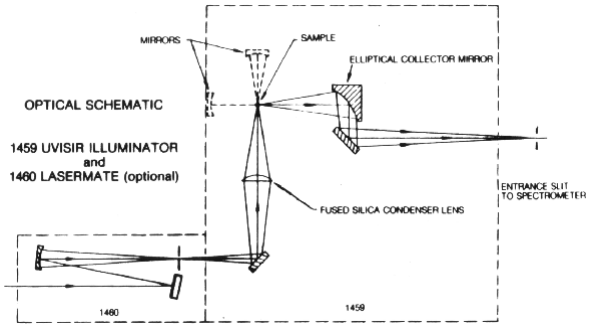
-- YAG: need InGaAs (~1.8 μ) on Ge (~1.6 μ) detector — limit $\Delta\nu$

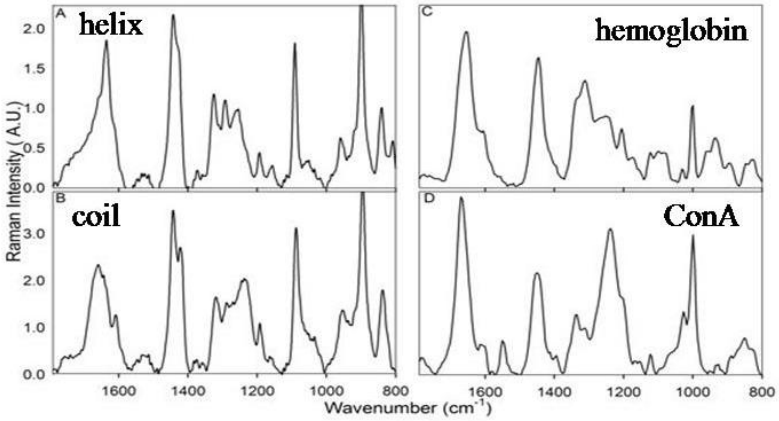
-- if Ti: Sapphire (or red diode) — GaAs PMT works, but still could get significant fluorescence

-- back scatter fits design, illuminate round pattern – match aperture
-- filtering Rayleigh line (laser) is important, i.e. inelastic scatter must dominate interferogram
-- as multichannel IR detectors become available, this will probably not be competitive

See old review of FT-Raman by Henry Bjuis, comparison dispersive and FT-Raman from Renishaw,

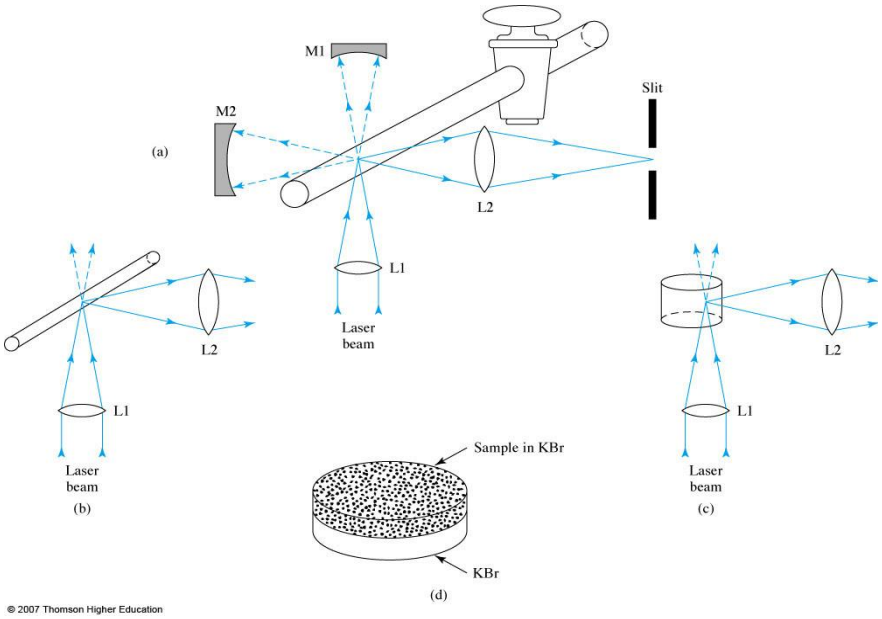
2. Sampling, multiple styles



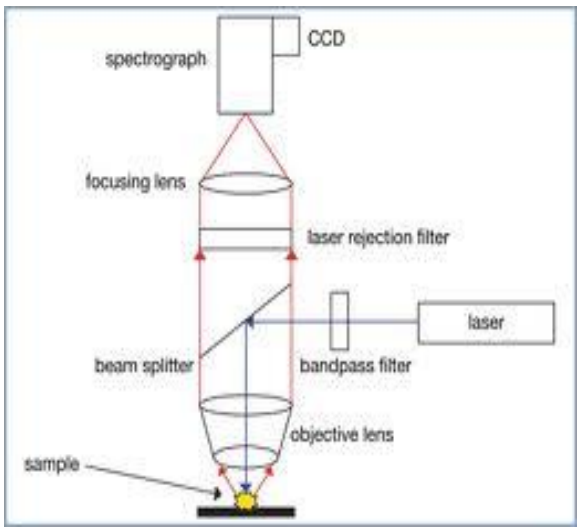


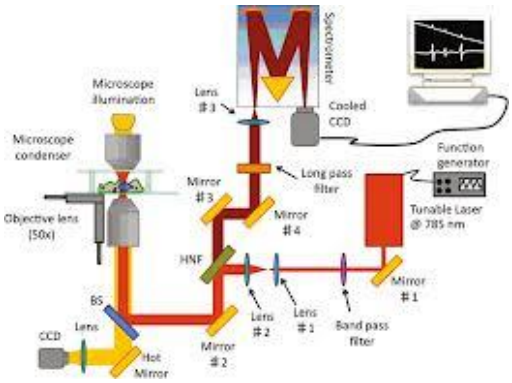
a) 90º — capillary or tube is fine/optical quality -- example: neat liquids, solutions,organics
can do aqueous biopolymers: nucleic acids, peptides, proteins
b) 180º — back scatter, from front surface of sample or bottom (liquid) cuvette - polish bottom,
or from top onto solid/film/powder even liquid – collect same direction
c) flow — narrow device/jet or channel
d) cool — stir/spin/blow cold N_2 (must avoid heating sample with laser, control temp)
-- crystal or other solid sample mount in dewar, on a cold finger (platform)

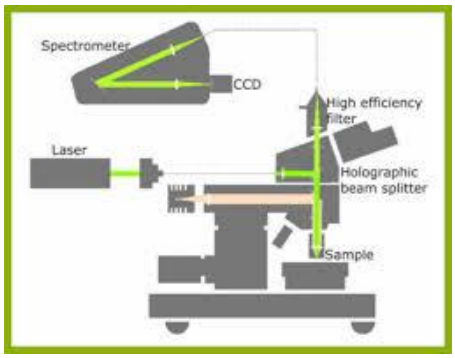
Variety of conventional 90° sampling



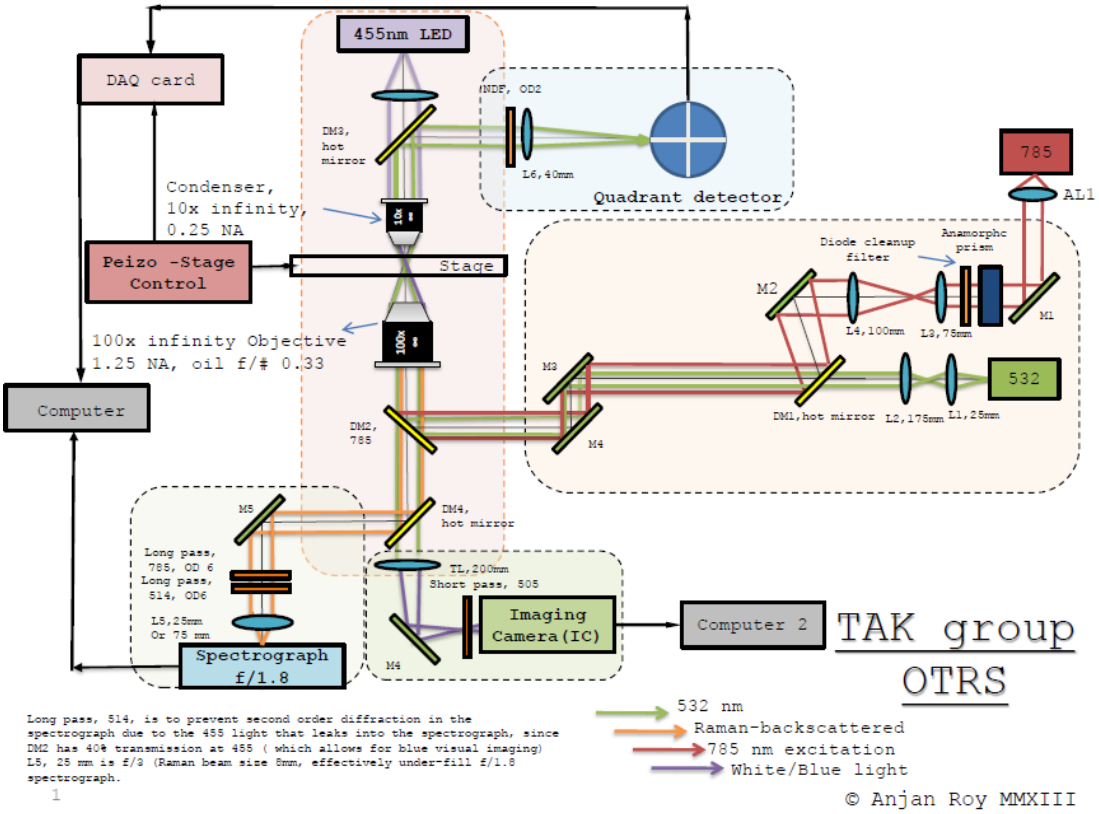
Microscope Raman sampling now big, including imaging





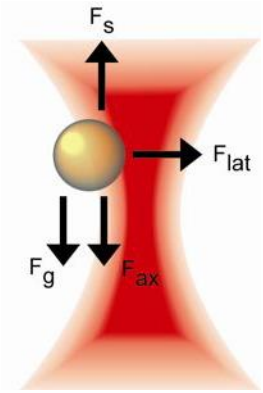


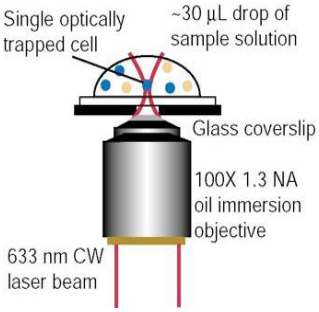
Home made Raman microscope/laser tweezer setup

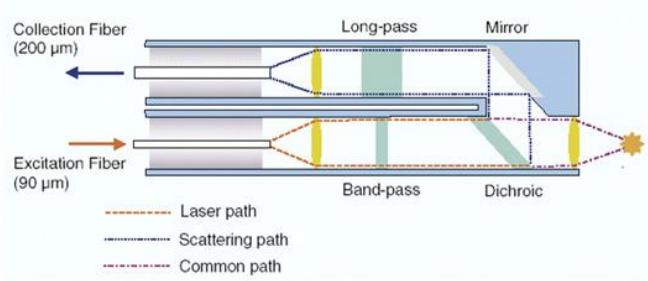


Long pass, 514, is to prevent second order diffraction in the spectrograph due to the 455 light that leaks into the spectrograph, since DM2 has 40% transmission at 455 (which allows for blue visual imaging)
L5, 25 mm is f/3 (Raman beam size 8mm, effectively under-fill f/1.8 spectrograph.

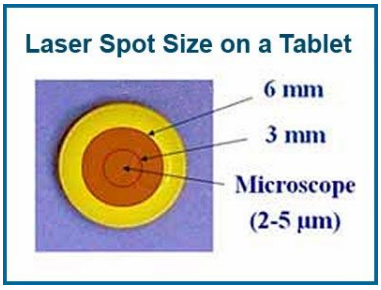
1







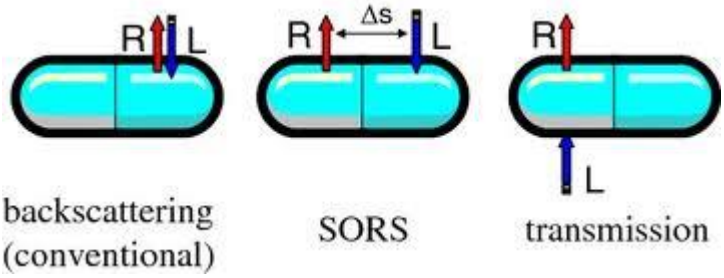
Fiber probes



Excite, detect fiber, dichroic mirror separate

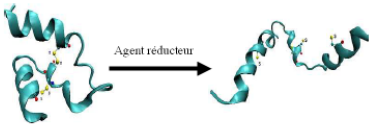
Kaiser PhAT probe - solids, large area, also liquid, gas probes

See Raman Application notes, Cornell, and PerkinElmer top 20 questions



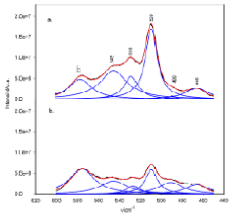
backscattering (conventional)	SORS	transmission
Samples spot	spatial offset/diff.	samples bulk
near surface	sample depth	no sample prep
small area	good for tissue	drug/tablet control

- Example of the disulfide bridge breaking in proteins

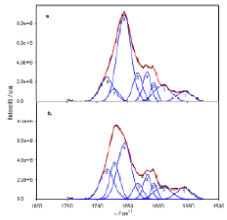


- Disulfide bridge:
  - stabilization of the protein structure
  - denaturation of the protein when broken
- Protein used: albumine (BSA)

- Example of the disulfide bridge breaking in proteins



S-S bond before/after reaction



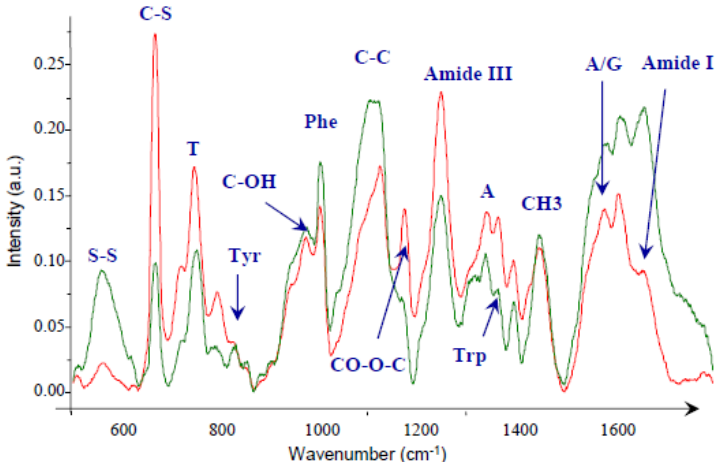
Amide I bond before/after reaction

Diagnosis and tissue analysis

HORIBA Scientific

Characterization of human thyroid tumor tissue

- Peak identification comparison: goiter and carcinoma.



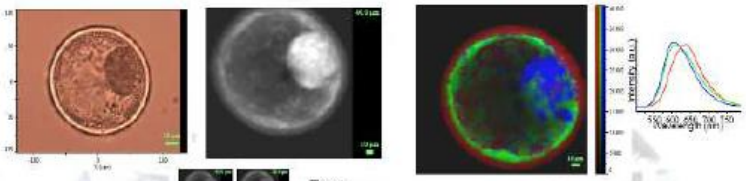
T: thymine
Tyr: tyrosine
Phe: phenylalanine
Trp: tryptophan
A: adenine
G: guanine

Cells analyses

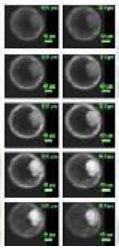
Video capture

Band-pass confocal microscopy mode

Spectral imaging mode : fluorescence, Raman



Bovin embryo incubated with Nile Red fluorochrome



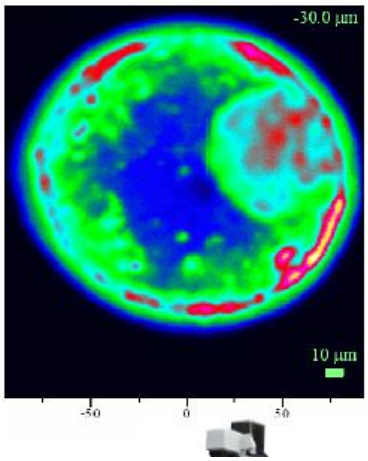
Fast selection of slice of interest

Spectral analysis reveals lipidic content of the sample

400x400 points
4 sec per slice

~35000 spectra recorded in 3 min, (SWIFT™ acquisition mode, Horiba Jobin Yvon)

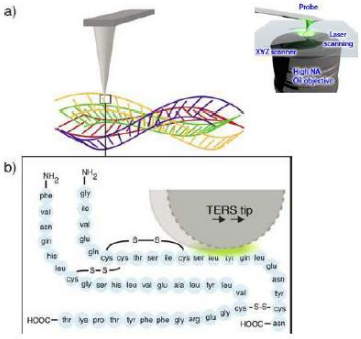
20 slices, with 400 x 400 data points (over a 203um x 185um area)

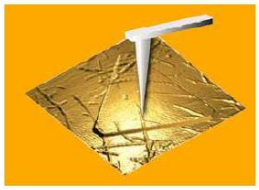


Tip Enhanced Raman Spectroscopy

HORIBA Scientific

- TERS profile across a single amyloid fibril



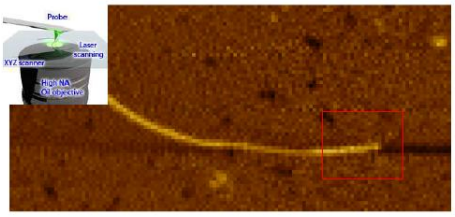


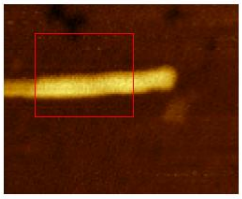
Deckert et al. J. Biophotonics 1–5 (2012)
DOI 10.1002/jbio.201100142

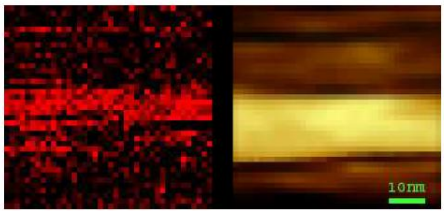
TERS Imaging results

HORIBA Scientific

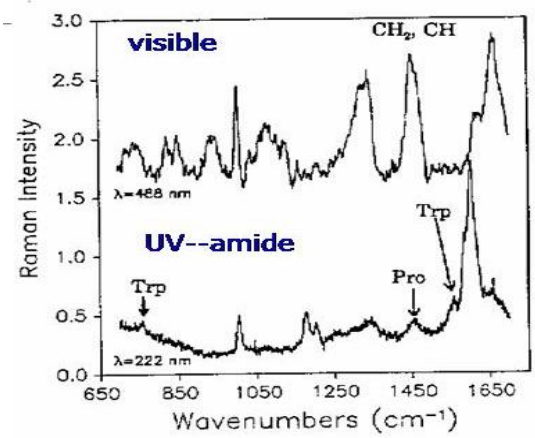
- DNA: Raman resolution <15nm
- Inverted microscope
- Oil immersion



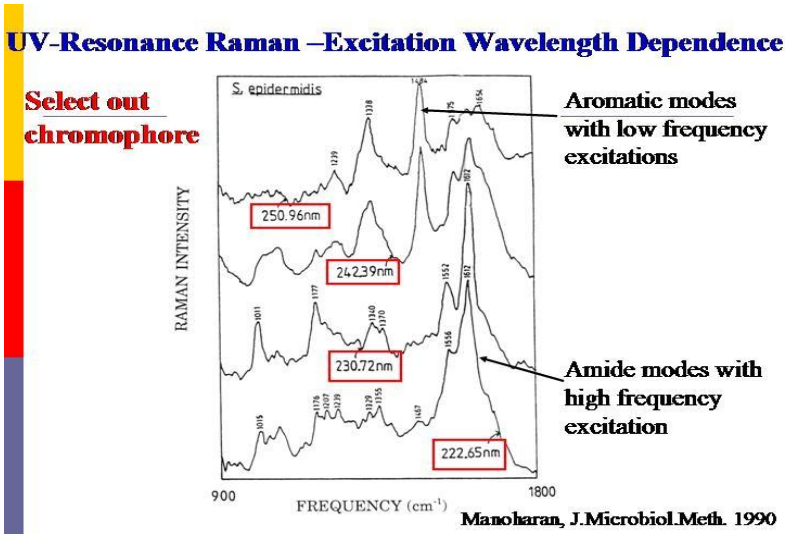




3. Resonance Raman — less analytically important

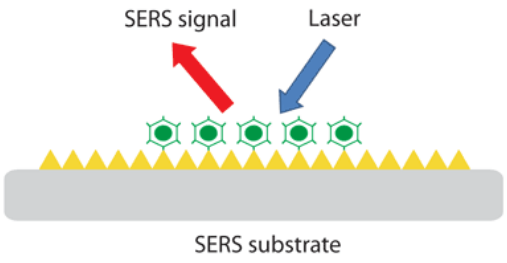


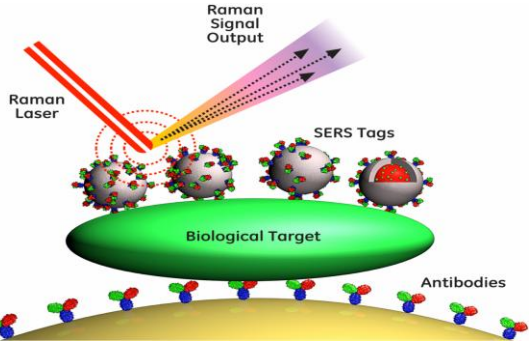
1. Resonance (λ = 222 nm) and nonresonance (λ = 488 nm) Raman spectra of bGH. Nonresonance spectrum is of a lyophilized solid, while the UVRR spectrum is of a 1 mg/mL solution at pH 8.

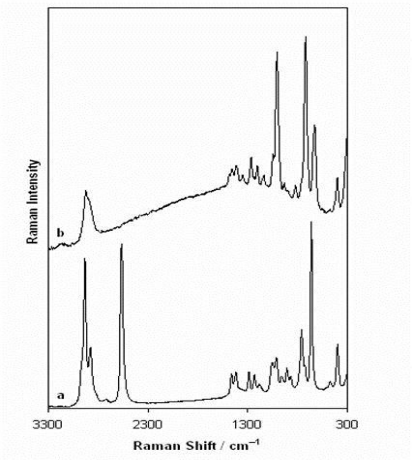


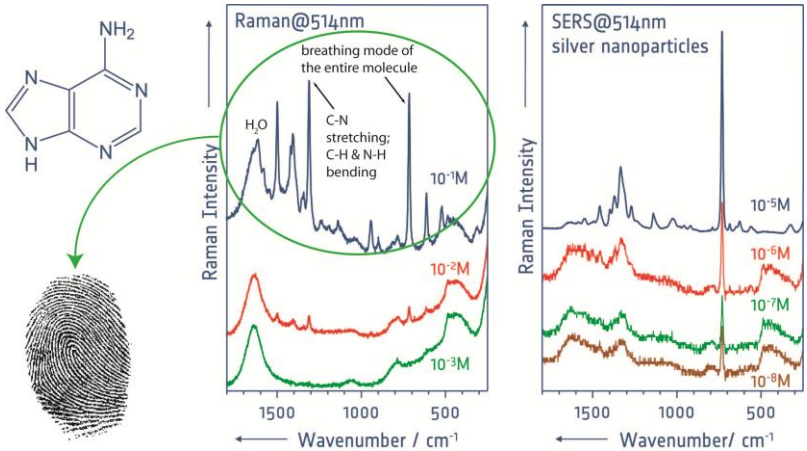
a) excite a real absorption state
b) seek information about vibrations and excited state properties
c) enhance intensity — allows study of more dilute samples (often not practical signal effect)

4. SERS — surface enhancement by analyte on metal (Au or Ag typical) surface — roughen or colloid
Growing analytical applications, problem - reproducibility, plasmon resonance enhance scatter
Pattern surface - more predictable results, Gold and other nanoparticles work,
flow analyte over and bind, then release major enhancement from “hot spots” –particles touch



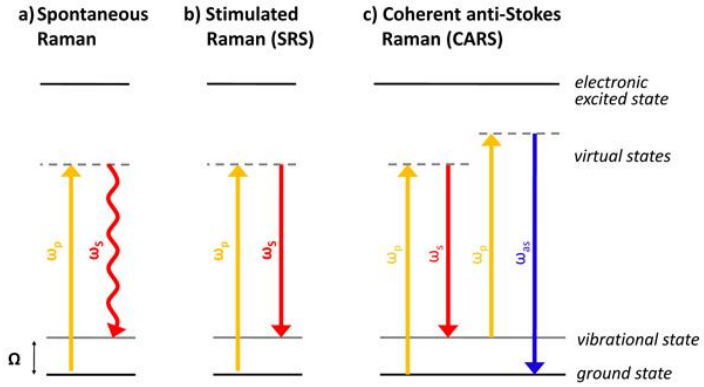






SERS (top) change from solution (bottom) enhancement neat ↑ compare to add ↑ nanoparticles

Many non-linear Raman methods utilize multiple photon excite



5. CARS —is non-linear phenomenon, 4-wave mix

3 input beams, one out, pump up population of ν_{vib}

Result - Raman output is a beam, intense

Problem is non-resonant background

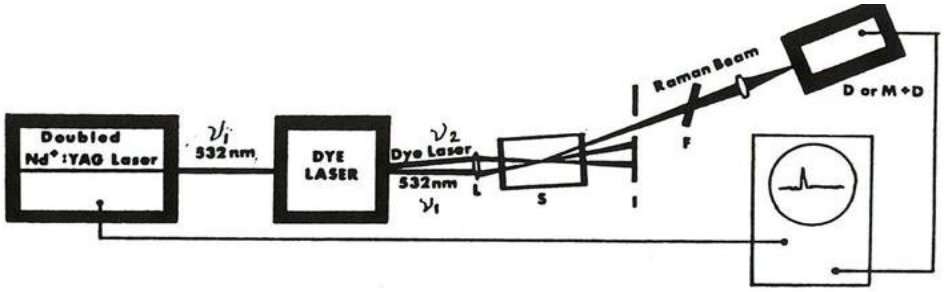
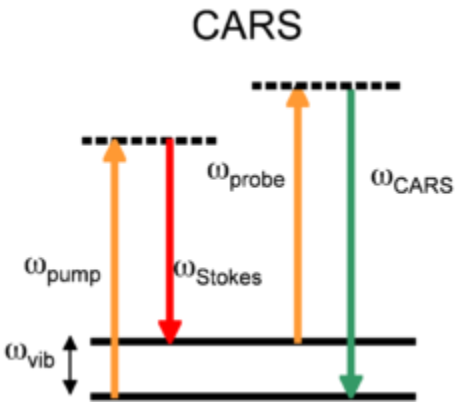
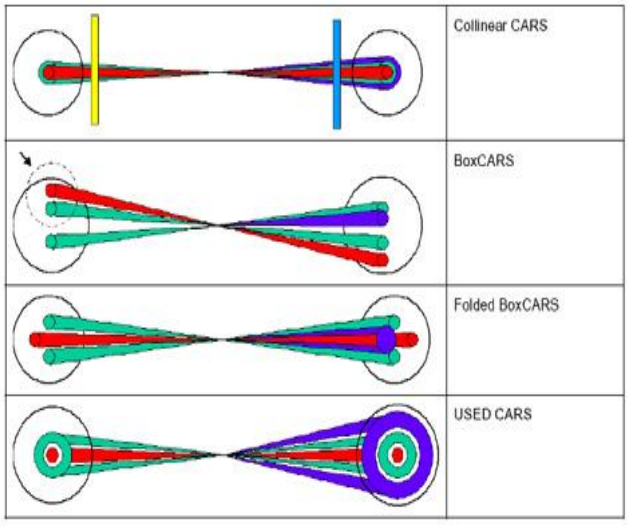
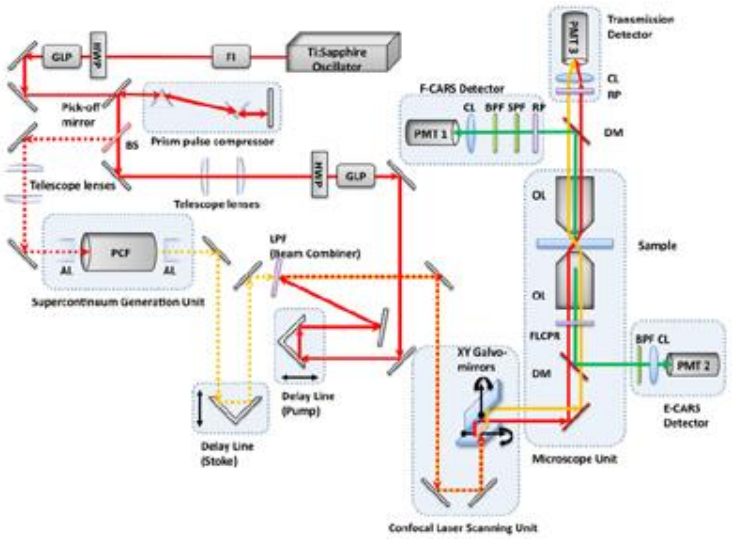


Figure 3-3 Initial apparatus for measuring anti-Stokes emission using a frequency-doubled Nd:YAG pumped dye laser. L is a short focal lens (3–4 cm); S is the sample; I is an iris for spatially filtering the two exciting beams; F is a wideband interference filter; D is the detector (usually a PIN diode); M is a monochromator (not usually necessary). Not shown are the PAR-160 box car integrator, chart recorder, and dye laser scan drive used to record spectra

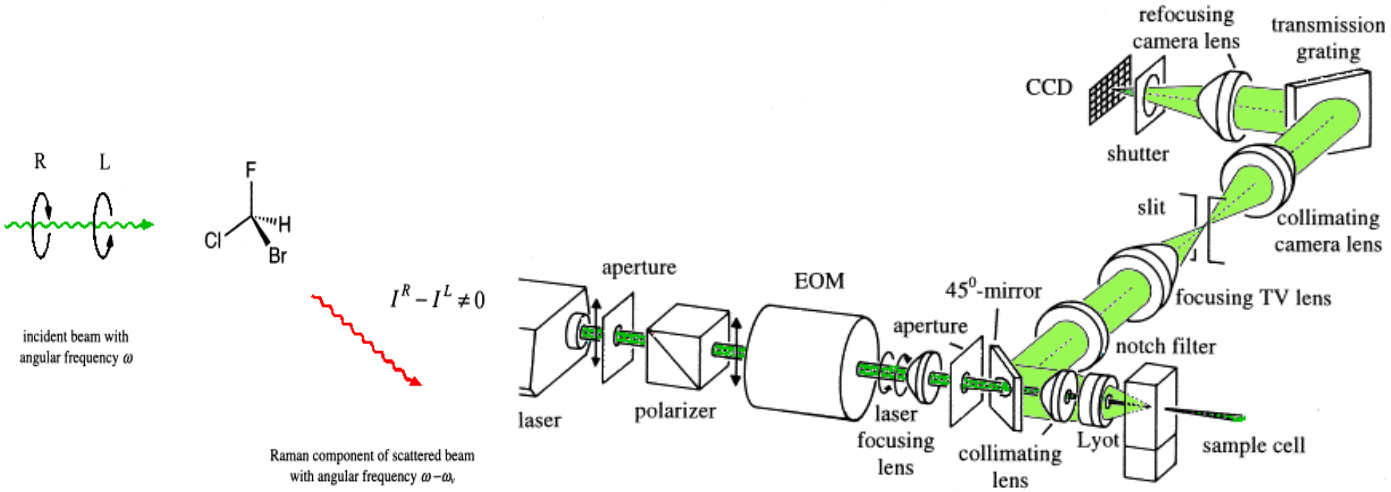




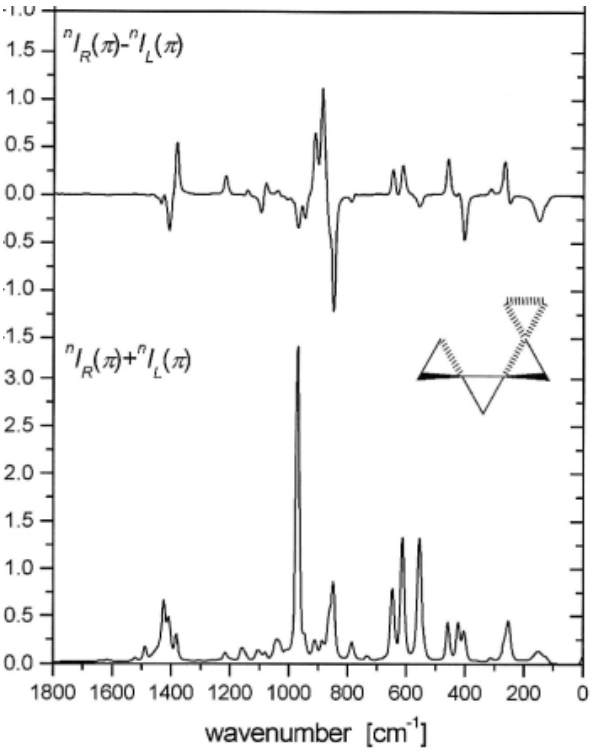


6. Time dependent — if signal enhanced can excite w/ps or ns laser and see time dependent processes

7. [Raman optical activity](#) — differential scatter of left and right circular polarized light (only for molecules that are chiral) -- instrument figures, 180° back scatter, transmission spectrograph, very good conventional design: L.D. Barron (note: W.Hug developed a more complex design, currently marketed by BioTools that has improvements, particularly with regard to artifacts)



Data: helicene (W. Hug below)



Lysozyme in water (BioTools)