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

A. Elastic Scattering $\lambda_0 = \lambda_s$ - basis for Dynamic Light Scattering (DLS) experiments (see Notes 19)

1) Raleigh Scattering — scattering centers small compared to $\lambda$
   
   $\lambda_s$ - 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 - $\lambda_0 \neq \lambda_s$

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 — $\nu_s = \nu_0 \pm \nu_{vib(rot)}$  

   (+) = 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 \( \lambda \) 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) \( \frac{1}{2} \rightarrow 1 \text{m} \) 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** (180° often), fine focus, high res image, possible due to shorter wavelength, \( \lambda_0 \)

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

Raman is 2-photon, so relative polarization of \( \lambda_{ex} \) and \( \lambda_s \) beams important

Polarization ratio: \( \rho = I_\perp/I_{||} \) -- \( \rho < 7/8 \) (or \( \rho < 3/4 \) for different excitation geometry)

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

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)

<table>
<thead>
<tr>
<th>Laser Power</th>
<th>ReporteR</th>
<th>Inspector 300</th>
<th>Inspector 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Wavelength</td>
<td>120 mW</td>
<td>300 mW</td>
<td>300 mW</td>
</tr>
<tr>
<td>Laser Wavelength</td>
<td>785 nm</td>
<td>785 nm</td>
<td>1030 nm</td>
</tr>
<tr>
<td>Detector</td>
<td>Standard CCD</td>
<td>Low Background CCD</td>
<td>Standard CCD</td>
</tr>
<tr>
<td>Resolution (cm-1)</td>
<td>10</td>
<td>9</td>
<td>8-10</td>
</tr>
<tr>
<td>Spectral Range (cm-1)</td>
<td>300 – 2500</td>
<td>175 – 3200</td>
<td>100 – 2500</td>
</tr>
<tr>
<td>Software, Display</td>
<td>Monochromatic</td>
<td>Color</td>
<td>Color</td>
</tr>
<tr>
<td>Weight</td>
<td>0.8 lb</td>
<td>3.7 lb</td>
<td>3.7 lb</td>
</tr>
<tr>
<td>Dimensions (in)</td>
<td>6 x 3 x 1.75</td>
<td>7.5 x 6.9 x 1.7</td>
<td>7.5 x 6.9 x 1.7</td>
</tr>
<tr>
<td>Battery Life</td>
<td>&gt;4 hrs, rechargeable</td>
<td>4 hrs, removable</td>
<td>4 hrs, removable</td>
</tr>
</tbody>
</table>

Rigaku – First Guard, Handheld

<table>
<thead>
<tr>
<th>Laser Power</th>
<th>ReporteR</th>
<th>Inspector 300</th>
<th>Inspector 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Wavelength</td>
<td>120 mW</td>
<td>300 mW</td>
<td>300 mW</td>
</tr>
<tr>
<td>Laser Wavelength</td>
<td>785 nm</td>
<td>785 nm</td>
<td>1030 nm</td>
</tr>
<tr>
<td>Detector</td>
<td>Standard CCD</td>
<td>Low Background CCD</td>
<td>Standard CCD</td>
</tr>
<tr>
<td>Resolution (cm-1)</td>
<td>10</td>
<td>9</td>
<td>8-10</td>
</tr>
<tr>
<td>Spectral Range (cm-1)</td>
<td>300 – 2500</td>
<td>175 – 3200</td>
<td>100 – 2500</td>
</tr>
<tr>
<td>Software, Display</td>
<td>Monochromatic</td>
<td>Color</td>
<td>Color</td>
</tr>
<tr>
<td>Weight</td>
<td>0.8 lb</td>
<td>3.7 lb</td>
<td>3.7 lb</td>
</tr>
<tr>
<td>Dimensions (in)</td>
<td>6 x 3 x 1.75</td>
<td>7.5 x 6.9 x 1.7</td>
<td>7.5 x 6.9 x 1.7</td>
</tr>
<tr>
<td>Battery Life</td>
<td>&gt;4 hrs, rechargeable</td>
<td>4 hrs, removable</td>
<td>4 hrs, removable</td>
</tr>
</tbody>
</table>

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 → 1 μ) -- lose as λ⁴ -- gain from multiplex

---

Filter to eliminate ν₀ needed for shot noise  

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

---

InGaAs extend Raman past 3000 cm⁻¹ if *not* cool 77K
-- 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

Variety of conventional 90° sampling

Microscope Raman sampling now big, including imaging
Home made Raman microscope/laser tweezer setup

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

- **Raman Application** notes, Cornell, and PerkinElmer top 20 questions

- Example of the disulfide bridge breaking in proteins
  - Disulfide bridge:
    - Stabilization of the protein structure
    - Denaturation of the protein when broken

- Protein used: albumin (BSA)

**Diagnosis and tissue analysis**

**Characterization of human thyroid tumor tissue**

- Peak identification comparison: goiter and carcinoma.
Cells analyses

Tip Enhanced Raman Spectroscopy

TERS profile across a single amyloid fibril

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

DOI 10.1002/jbio.201100142
3. Resonance Raman — less analytically important
   
   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, flow analyte over and bind, then release
   
   Gold and other nanoparticles work, 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 $\nu_{vib}$
Result - Raman output is a beam, intense
Problem is non-resonant background

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)