

Spectroscopy: Molecular Luminescence Spectrometry

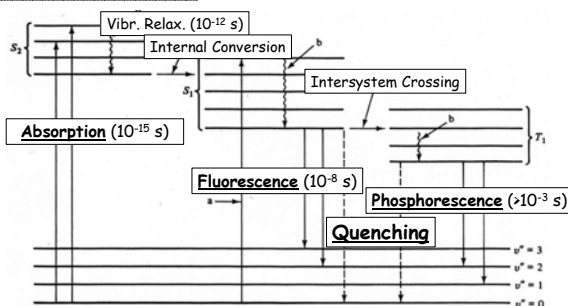
Chem 221
Instrumental Analysis
Spring 2005

Overview

- We will be concerned with:
 - Photon-excited emission
 - Decay from singlet state
 - *Fluorescence*
- Electronic Transitions (molecular)
 - UV/Vis
- Very low detection limits
 - ppb and below

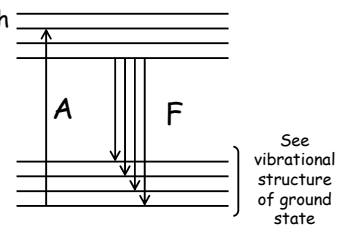
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Remember Jablonski!



Fluorescence Spectrum

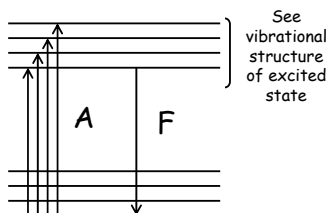
- *Excitation* at a single wavelength (λ_{ex})
- *Emission* at a range of wavelengths (longer λ)



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Excitation Spectrum

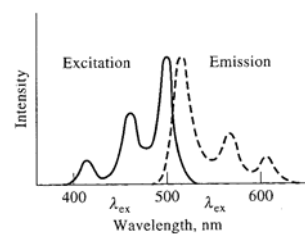
- *Excitation* at a range of wavelengths
- *Emission* at a specific wavelength (λ_{em})



➤ *Excitation spectrum should mimic absorption spectrum* (assuming vibrational relaxation is complete) 5

Emission/Excitation Spectra

- *Excitation spectrum* like absorption spectrum
- *Emission (fluorescence) spectrum* at lower energy (longer wavelength)



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Fluorescence Quantum Efficiency

Not all molecules *fluoresce* - **Why?**

➤ Reflected in Quantum Efficiency (Φ):

$$\Phi = k_f / (k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d)$$

fraction of excited molecules that fluoresce

rate constants for deactivation processes (lowest excited singlet state)

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Deactivation Rate Constants

- k_i - intersystem crossing
 - k_{ec} - external conversion
 - k_{ic} - internal conversion
- mostly affected by *environment*
- k_{pd} - predissociation
 - k_d - dissociation
 - k_f - fluorescence
- mostly affected by *structure*

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Factors Affecting Fluorescence Intensity

➤ Absorption Transitions

$\sigma \rightarrow \sigma^*$: too energetic (bonds break - k_d big)
(limits absorption to $\lambda > \sim 250\text{nm}$)

$n \rightarrow \pi^*$: low molar absorptivity (ϵ)
= long π^* lifetime (10^{-5} - 10^{-7} s)
(more chance for non-fluor deactivation)

$\pi \rightarrow \pi^*$: high molar absorptivity (ϵ)
= short π^* lifetime (10^{-7} - 10^{-9} s)
(greatest k_f)
Also: smaller k_i (less singlet/triplet overlap)

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More FAFI: Molecular Structure

➤ Low energy $\pi \rightarrow \pi^*$ transitions preferred

- aromatic functional groups
- highly-conjugated double-bond structures
- fused-ring structures

➤ Substitution Effects

- Heavy atom substitution: increases k_i
✓ Example: halogen substitution on Benzene
- Substitutions that decrease energy of $n \rightarrow \pi^*$
✓ Heterocyclic rings (not fused)
✓ COOH or C=O on aromatic rings

➤ Rigid Structures preferred

- likely due to decreased k_{ic}

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Still More FAFI: Environment Effects

➤ Solvent

- \uparrow viscosity, \downarrow k_{ec}
- heavy atom effect (solutes and solvent)

➤ Temperature

- \uparrow temp, \uparrow k_{ec}

➤ pH

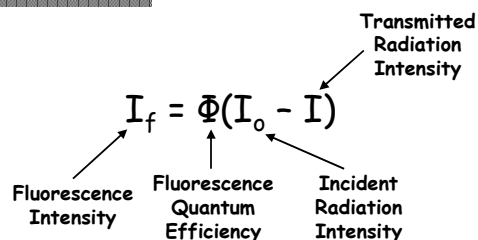
- affects electronic structure of acidic or basic substituents

➤ Dissolved Oxygen

- paramagnetic - \uparrow k_i

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Effect of Concentration



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Substitute and Simplify

Beer's Law: $I = I_0 e^{-\epsilon bc}$

Gives: $I_f = \Phi I_0 (1 - e^{-\epsilon bc})$

Expand:

$$I_f = \Phi I_0 \epsilon bc [1 - (\epsilon bc/2!) + ((\epsilon bc)^2/3!) \dots]$$

If ϵbc is small (< 0.05), then:

$$I_f = \Phi I_0 \epsilon bc$$

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Analytical Implications

$$I_f = \Phi I_0 \epsilon bc$$

➤ At specific λ_{exc} with constant I_0 :

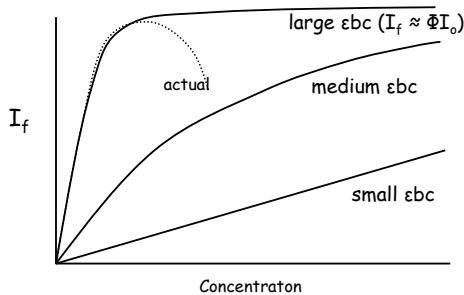
$$I_f = Kc$$

➤ $I_f \propto I_0$

- So, signal will increase with increased source intensity

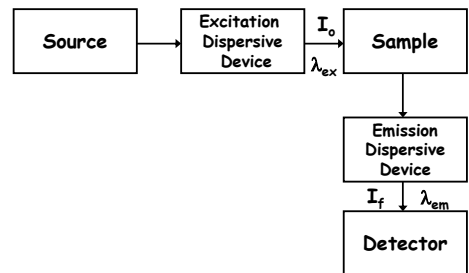
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Quantitative Considerations



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Instrumentation



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Sources

Continuum

- **Xe Arc lamp**
 - Intense UV/Vis source

Line

- **Hg Vapor Lamp**
- **Laser**
 - N_2 - or Nd:YAG-pumped tunable dye laser
 - Ar^+ or Kr^+ laser

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Dispersive Devices

➤ Excitation

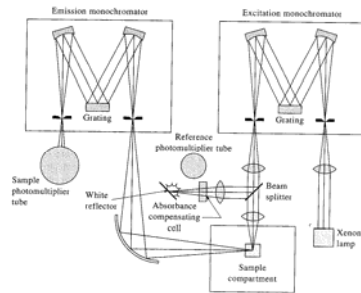
- Only needed for *continuum sources*
- modest resolution, high light throughput

• Emission

- modest resolution
- high stray light rejection (*double-monochromator*)

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Typical Spectrofluorometer



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Applications

➤ Quantitative Analysis

- Need molecule with high fluorescence quantum efficiency *OR* one that can react quantitatively to form a highly fluorescent compound
- Linear relationship with concentration only at low concentrations
- Fluorescence signal directly proportional to *source intensity*:

Laser sources should give best results

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Laser-Excited Fluorescence

Why use Laser Sources?

➤ Saturation Effects

- I_f indep of I_o
 - variation of source intensity not observed in signal
 - decreased noise
- I_f is maximized
 - increased signal
 - best detection limits (ppt and lower)
- Extends LDR
 - by lowering detection limits

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Still More "Why Lasers?"

➤ Temporal Resolution

use *pulsed* lasers (μs - ps)

- temporal discrimination between scatter and fluorescence
- measure fluorescence lifetimes

➤ Spatial Resolution

- focus laser to μm spot size
- efficient measurements with microsamples
- spatial mapping of sample surfaces

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Ultra-Trace Analysis via LEMFS

Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

- **PAH's:** large ϵ , $\Phi \approx 0.2 - 1$, 10-100 ns fluor. lifetime
- **Example** (using N_2 -pumped dye laser, time-resolved):

Detection Limits (PAHs in water):

- ✓ Benzene - 19 ppb (10^{-7} M)
- ✓ Naphthalene - 1.3 ppt (10^{-11} M)
- ✓ Anthracene - 8.9 ppt (10^{-11} M)
- ✓ Pyrene - 0.5 ppt (10^{-12} M)
- Limited by background fluorescence

- 6 decades LDR

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Emission Versus Absorbance?

- Let's assume a measurement uncertainty of **0.001**

For an emission measurement:

Noise = 0.001

Blank = 0.000

Signal at detection limit = $3N = \mathbf{0.003}$

Is this signal detectable via absorbance measurement?

For an absorbance measurement:

$s_T = 0.001$

Blank: Transmittance = 1.000

Comparable Signal = 1.000 - **0.003** = **0.997**

Is this above the detection limit?

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Is it Detectable?

Signal = Absorbance (= 0.0013)

Noise = s_A

$$\begin{aligned} S/N &= A/s_A = \frac{|s_T \text{Log } T|}{0.434 s_T} \\ &= \frac{|(0.001)\text{Log}(0.997)|}{(0.434)(0.001)} \\ &= (1.3 \times 10^{-6}) / (4.34 \times 10^{-4}) \\ &= \mathbf{0.003} (\ll 3) \end{aligned}$$

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Qualitative Analysis

➤ As in UV/Vis absorption, **broad emission bands** provide limited selectivity:

- multicomponent analysis requires separation
- excellent detection method for HPLC of compounds that fluoresce (or are tagged with fluorescent labels)

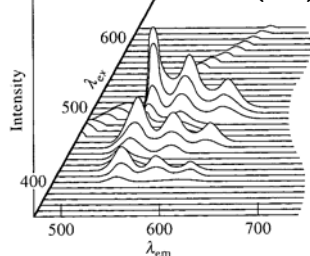
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More Qual Analysis

➤ **What about extra dimension of excitation?**

- emission spectrum changes with changing excitation wavelength

Emission-Excitation Matrix (EEM)

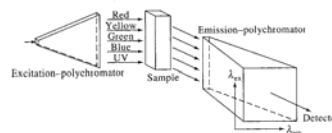


Measuring an EEM

The Hard Way

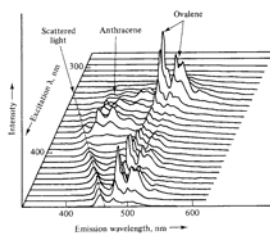
- Acquire emission spectra as λ_{ex} is sequentially changed

Or: can be done *simultaneously* using a **Video Fluorometer**



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Multi-component EEM



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Summary

- Not universally applicable
- Better detection limits than UV/Vis absorbance
 - nature of emission versus absorbance measurement
 - signal dependence on source intensity
- Limited qualitative analysis capability

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Spectroscopy: Raman Spectroscopy

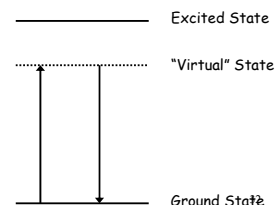
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Raman Scattering

➤ Discovered in 1928 by C.V. Raman (received Nobel Prize in 1930)

First, let's look at Elastic (Rayleigh) scattering (no change in energy of light):

EMR induces oscillating electric field in electron cloud around molecule - which is then re-emitted as a photon



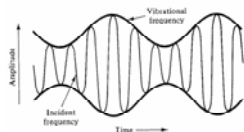
Inelastic Scattering

- If there is a *vibration* which results in a **change in the net polarizability** of the electron cloud:

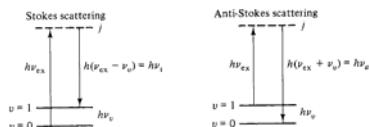
Scattered photon will be at an energy either greater than or lower than the original energy by an amount equal to the energy of the vibration:

Inelastic Scattering

(Only about 1 molecule in $10^8 - 10^9$ will inelastically scatter photons)



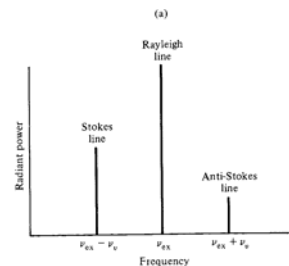
Quantum View



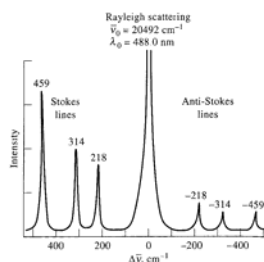
Stokes Lines:
longer wavelength
(more intense)

Anti-Stokes Lines:
shorter wavelength

$$\Delta\nu = \nu_{\text{Raman}}$$



Sample Spectrum: CCl₄



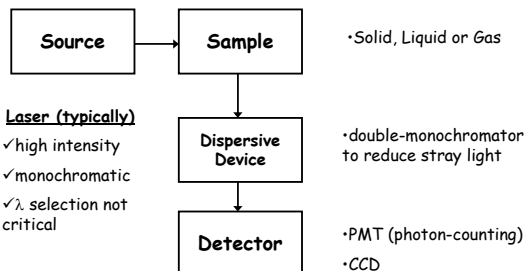
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Factors Affecting Raman Scatter Intensity

- **Concentration**
 - directly proportional
- **Source Intensity**
 - directly proportional
- **Source Wavelength**
 - $1/\lambda^4$ dependence

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Instrumentation



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Resolution?

Not straightforward - varies with source wavelength . . . why?

• $\Delta\lambda_{\text{eff}}$ is λ -independent, but $\Delta\bar{\nu}_{\text{eff}}$ varies with λ

For a 1-m monochromator, 1200 g/mm grating, and 100- μm slits: $\Delta\lambda_{\text{eff}} = 8 \text{ \AA}/\text{mm} \times 0.100 \text{ mm} = 0.8 \text{ \AA}$

So:

$$\lambda_{\text{source}} = 300 \text{ nm}; \text{ gives } \Delta\bar{\nu} = 8.9 \text{ cm}^{-1}$$

$$\lambda_{\text{source}} = 500 \text{ nm}; \text{ gives } \Delta\bar{\nu} = 3.2 \text{ cm}^{-1}$$

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Raman Properties

➤ Get vibrational spectrum

- ✓ complementary with IR
- ✓ can use UV/Vis instrumentation
- ✓ aqueous solutions are accessible to study

➤ Problems

- ✓ Low efficiency of effect = poor sensitivity
- ✓ Competition from fluorescence for highly fluorescent species

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Solving Problems

➤ **Resonance Raman**

- ✓ Selectivity and Detectability

➤ **Surface-Enhanced Raman**

- ✓ Selectivity and Detectability

➤ **Multi-Channel Detection**

- ✓ Detectability

➤ **Near-IR Excitation**

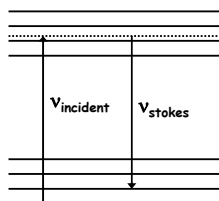
- ✓ Fluorescence Rejection

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Resonance Raman

- If $\lambda_{\text{incident}}$ corresponds to a strong absorption band, I_{Raman} enhanced by $10^2 - 10^6 \times$

• Enhancement only for vibrational modes associated with portion of molecule involved in electronic transition.



How does this differ from fluorescence?

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Surface Enhanced Raman Spectroscopy (SERS)

➤ Raman signal enhancement occurs if sample is on an "active metal" surface

- "active metal" = Ag, Au, Cu, and others
- a generalizable phenomenon (SEIRA)
- For ultimate in detectability (near unity "Raman Quantum Yield"), couple with:
 - resonance enhancement
 - sample adsorbed onto metal nano-particles

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Near-IR Excitation

➤ Can eliminate fluorescence background by using *low energy* excitation λ

- use $\lambda = 1.06 \mu\text{m}$ (Nd:YAG Laser)

➤ **Problem:** $I_{\text{Raman}} \propto \lambda^{-4}$

- 16x decrease in signal from $\lambda = 500 \text{ nm}$

How can we enhance S/N to make measurement possible?

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FT-Raman

➤ **Remember:** detector noise predominates, so *multiplex advantage* applies

➤ **How Implement?**

- ✓ CW Nd:YAG laser source
- ✓ Michelson Interferometer instead of monochromator
- ✓ Filter Rayleigh scatter (minimize *multiplex DIS* advantage)

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FT-Raman Instrument

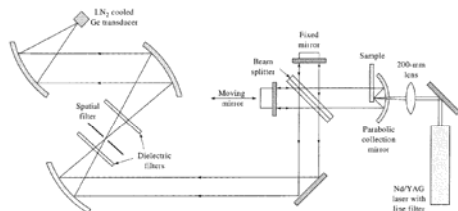


Figure 18-9 Optical diagram of an FT-Raman spectrometer (LN₂ = liquid nitrogen).
From B. Chao, *Anal. Chem.*, 1987, 59, 884A. (with permission.)

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It works!

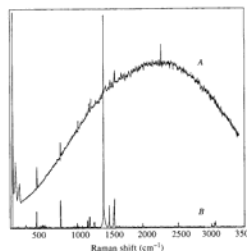


Figure 18-5 Spectra of anthracene: A: Conventional instrument, 5145 Å excitation; B: FT instrument, 1.064 μm excitation. From B. Chao, *Anal. Chem.*, 1987, 59, 885A. (with permission. Copyright 1987 American Chemical Society.)

• Outstanding fluorescence rejection

- ✓ can get Raman spectra of PAH's, etc.

• Precise λ -calibration

- ✓ spectral subtraction

• No S/N advantage

- ✓ need to increase laser power to get same S/N

• Can't do resonance Raman

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Multichannel Detection

➤ Another way to get *multiplex advantage*: use *multichannel detector (CCD)*

- **Example:** with low-power UV excitation:
 - ✓ ppb detection limits for PAHs
 - ✓ surface studies: *sub-monolayer* detection
 - ✓ speed (30 secs to acquire spectrum that would have taken 5 hours with scanning instrument)
 - ✓ ns time-resolution

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Near-IR Excitation: Revisited

Goal: Raman for the masses!

➤ **Near-IR excitation**

- ✓ fluorescence gone
- ✓ inexpensive diode laser source

➤ **Multichannel detection**

- ✓ gives S/N boost
- ✓ fast

➤ **Fiber Optic probe "sample cell"**

- ✓ eliminates laser/sample alignment issues

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Simple, Low-Cost Raman?

• Source:

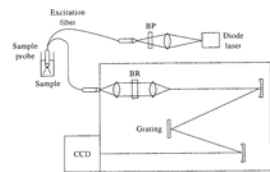
30-mW, 783-nm diode laser

• Dispersion:

$\frac{1}{4}$ -m, 600-g/mm grating spectrograph ($D^{-1} = 50 \text{ \AA}/\text{mm}$)

• Detector:

1152 pixel CCD (22.5 $\mu\text{m}/\text{pixel}$)



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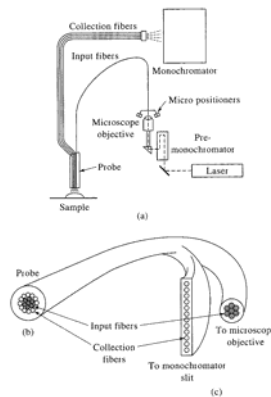
Fiber Optic Sampling

“User-Friendly Sampling”

• eliminates need for precise alignment of laser and sample

• place *directly in solution* containing the sample

• place *directly above solid* sample

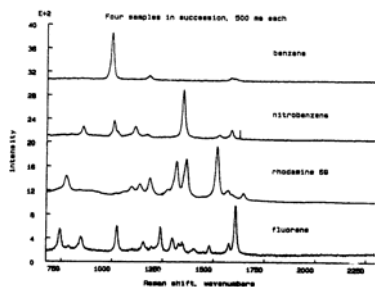


Spectral Results

• rapid spectral acquisition (500 ms)

• easy sampling of solutions and solids

• no fluorescence background



Limits? Tradeoffs?

➤ **No Resonance Enhancement**

➤ **Resolution versus Range:**

• 1.6 $\text{cm}^{-1}/\text{pixel}$ with 2000 cm^{-1} range

• *Higher resolution, decreases range:*

• $D^{-1} = 4 \text{ \AA}/\text{mm} \rightarrow 0.13 \text{ cm}^{-1}/\text{pixel}$

• 1152 pixels $\rightarrow 150 \text{ cm}^{-1}$ range

(technological limitation, not fundamental)

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Raman: What is it good for?

➤ **Vibrational Spectral Analysis**

- ✓ complementary with IR
- ✓ molecular structural info
- ✓ easy and fast!

➤ **Quantitative Analysis**

- ✓ can be as sensitive as fluorescence
- ✓ more selective than fluorescence

Raman as a detection method for separations? See:
<http://www.umich.edu/~morgroup/virtual/labelled/virtual.html> 53