1. Skoog problem 15-1 (omit terms (j), (k) and (m)). Draw diagrams as necessary.

   a) **fluorescence**  Relaxation of an excited state by emission of a photon without a change in electron spin. This satisfies the selection rule $\Delta S=0$, and so is allowed, rapid and fairly probable, depending on the relative rates of competing relaxation pathways. Note that this often involves singlet->singlet transitions in neutral molecules, since typically all electrons are paired in the ground state. Strictly speaking, though, this could involve any multiplicity (as long as it is conserved in the process) for free atoms, ions, molecular ions, molecular radicals, etc.

   b) **phosphorescence**  Relaxation of an excited state by emission of a photon with a concurrent change in electron spin. This breaks the selection rule $\Delta S=0$, and so is forbidden, slow and improbable. Often for neutral molecules this is a triplet->singlet transition, but it need not be, generally speaking.

   c) **resonance fluorescence**  Relaxation of an excited state by emission of a photon of the same frequency as the excitation frequency. This is more common in atoms than in molecules, which have vibrational relaxation pathways that may dissipate a portion of the excitation energy before fluorescence occurs.

   d) **singlet state**  A many-electron state in which all electron spins are paired. Total spin angular momentum $S=0$. This is commonly the multiplicity of neutral molecules, both in the ground state and in excited electronic states reached by spin-allowed optical transitions.

   e) **triplet state**  A many-electron state in which two electron spins are parallel. Total spin angular momentum $S=1$. Typically not accessible by *allowed* optical transitions from the ground state for neutral molecules.

   f) **vibrational relaxation**  Dissipation of excess energy in a molecular excited state among the vibrational degrees of freedom. This is the means of the Stokes shift in molecular fluorescence (emitted light appears at longer wavelength than the excitation wavelength).

   g) **internal conversion**  Intramolecular processes which allow an excited state to relax to another electronic state or the ground state without emission of a photon and without a change in multiplicity. This is a consequence of overlap of the vibrational manifolds of the electronic states.

   h) **external conversion**  Relaxation of an excited electronic state without emission of a photon due to intermolecular processes (collisions with other
molecules). This competes with fluorescence, reducing fluorescent quantum yield, and is often referred to as quenching.

i) **intersystem crossing** Processes in which the spin of an excited electron flips. For neutral molecules this is typically a singlet->triplet conversion.

l) **quantum yield** The ratio of the number of molecules that luminesce to the number of excited molecules. A quantification of the efficiency of luminescence.

2. How do you collect a fluorescence emission spectrum? A fluorescence excitation spectrum? An absorption spectrum? Which two most closely resemble each other?

   A fluorescence emission spectrum is collected by illuminating the sample with a single wavelength and collecting a scan of the intensities of the different wavelengths emitted by the sample. A laser would be an excellent excitation source, and the emitted light should be collected at 90° from the excitation and directed into a monochromator for scanning the emission.

   A fluorescence excitation spectrum is collected by measuring the intensity of a single emitted wavelength over a scan of excitation wavelengths. Typically the source would be a continuous source with a monochromator (or the harmonics of a tunable dye laser, if it covers the required wavelength range) and the emitted light should be collected at 90° from the excitation and directed into a monochromator fixed at a specific detection wavelength.

   An absorption spectrum is collected by measuring the difference in intensity between the light incident on the sample and the light passed by the sample, and measuring this difference (absorption) as a function of wavelength. Typically the source would be a continuous source with a monochromator (or the harmonics of a tunable dye laser, if it covers the required wavelength range).

   The fluorescence excitation and absorption spectra would closely resemble each other, as both are based on scanning the excitation wavelength to detect those that lead to excited electronic states.

3. Skoog problem 15-3. **Why is spectrofluorometry potentially more sensitive than spectrophotometry?**

   The key difference is that spectrofluorometry measures the intensity of fluorescence, while spectrophotometry (absorption spectroscopy) measures the ratio of the intensities of two beams (incident and passed beams). The fluorescence signal is directly proportional to the intensity of the excitation source.
We can increase the intensity of the excitation source (use a laser!) and gain a subsequent increase in the fluorescence signal—potentially leading to greater sensitivity. Spectrophotometry, on the other hand, is an absorption technique. Absorption depends on the ratio of incident to passed light:

\[ A = \log \left( \frac{P}{P_0} \right) \]

so simply increasing \( P_0 \) also increases \( P \).


The name gives it away—fluorescein should be expected to have a greater quantum fluorescence yield than phenolphthalein. Both have similar structures with aromatic and conjugated double bond chromophores; thus, both absorb strongly in the visible/UV. Fluorescein, however, has a more rigid fused-ring structure which hampers vibrational dissipation of excited state excess energy, making it more likely that the molecule remains in the excited state long enough to fluoresce. This should result in a greater fluorescent quantum yield for fluorescein than phenolphthalein.

5. Skoog problem 15-11. Predict the part of the quinine molecule that is most likely to behave as the chromophore and fluorescent center.

The fused aromatic ring portion is most likely to act as a chromophore (large conjugated pi-electron systems tend to show absorption in the UV/vis) and fluorophore (rigid ring structures hinder vibrational relaxation pathways for internal conversion, which competes with fluorescence).

6. Should water absorb strongly in the infrared? How many vibrational modes are expected? Draw the normal vibrational modes of water and comment on whether they are expected to be IR or Raman active. Comment on the impact that this may have for the use of water as a solvent for IR vs. Raman studies of analytes in solution.

Water has three atoms, so there are 3N-6=3 vibrational modes. The normal modes look like:
All modes result in a change in dipole moment, so they are all expected to be IR active. Raman intensity depends on change in induced dipole moment during a vibration, which can roughly be correlated to change in bond lengths. The antisymmetric stretch involves lengthening one bond while shortening another; as a result, the effects cancel each other out and this mode is expected to be Raman inactive. The other two modes do involve net bond lengthening, so they are likely Raman active. Even though two out of three modes are Raman active, we may expect the Raman intensities to be fairly low, since H is not very polarizable (recall from General Chemistry that large atoms with orbitals lying far from the nucleus are more polarizable than more compact atoms). The strong IR absorption makes water a poor solvent for IR studies of analytes in solution. The relatively weak Raman activity of water, however, means that Raman techniques are compatible with aqueous samples, which include biological samples.

7. How many vibrational modes are expected for HCN? Draw the normal vibrational modes. How many unique bands are expected in the IR spectrum?

HCN has three atoms and is linear, so there are \(3N-5=4\) vibrational modes. The normal modes look like:

\[
\text{H} \equiv \text{C} \equiv \text{N} \quad \text{H} \equiv \text{C} \equiv \text{N}
\]

\[
\text{H} \equiv \text{C} \equiv \text{N} \quad \text{H} \equiv \text{C} \equiv \text{N} \quad \text{H} \equiv \text{C} \equiv \text{N}
\]

The two stretching look more nearly like “pure” localized C-N and H-C stretches because of the mass differential between H and N. Try this on the ComSpec3D simulator. This is in contrast to the collective symmetric and antisymmetric modes of water. The two bending modes (remember, the “+” and “-” signs mean that the atoms are coming out of or going into the page) are degenerate and all modes are expected to be IR active (result in a change in the dipole moment), so only three unique bands would be expected in the IR spectrum.

8. Skoog problem 16-8. What are the advantages of a Fourier transform infrared spectrometer compared with a dispersive instrument?

The main advantage a FT spectrometer is the speed of acquisition—since all spectral components are sampled simultaneously. This is in direct opposition to the basis of a dispersive instrument, which selectively isolates one narrow spectral band at a time. A consequence of the speed of FT acquisition is the
ability to achieve higher S/N through multiple averaging of scans. Thus, FTIR can attain high sensitivity. Additionally, FTIR instruments are smaller than dispersive instruments and are now relatively cheap (cheap enough that the additional cost is justified by the superior performance for all but the crudest of analyses).

9. Skoog problem 18-1. Why does the ratio of anti-Stokes to Stokes intensities increase with sample temperature?

The scattering processes leading to anti-Stokes lines begin from vibrationally excited initial states, while those producing Stokes lines originate from the ground vibrational state. See Figure 18-2 in Skoog. Since the ratio of the population of excited vibrational levels to the population of the ground vibrational state increases with increasing T (by the Boltzmann distribution), the intensities of anti-Stokes lines consequently increase relative to the Stokes lines.

10. The use of Raman spectroscopy has historically not been as prevalent as IR spectroscopy. Describe two advantages that Raman spectroscopy holds over IR spectroscopy. What technological advance was the key to making Raman a feasible instrumental technique?

Raman spectroscopy often allows access to vibrational modes that may not be IR active. It is a great advantage that the Raman intensities of the vibrational modes of water are relatively small, and aqueous samples may be analyzed (water absorbs strongly in the IR). This allows analysis of a wide range of chemical and biological samples (even in vivo). Raman measures vibrational mode energies, but uses a visible wavelength light source. Optics and detectors for visible wavelength are generally well-developed, cheaper and easier to work with than IR optical components. Additionally, the requirement for a narrow linewidth, bright light source makes the use of a LASER ideal as an incident light source. In fact, it was the development of the LASER that made Raman spectroscopy feasible as a technique for widespread use.