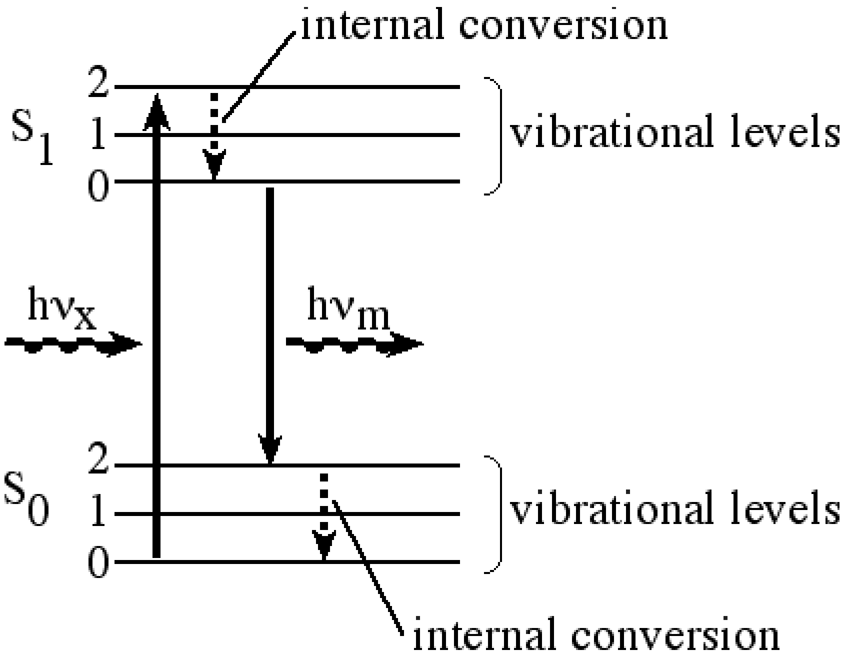
www.wehi.edu.au/.../exciting_fluorescence.html

Exciting Fluorescence

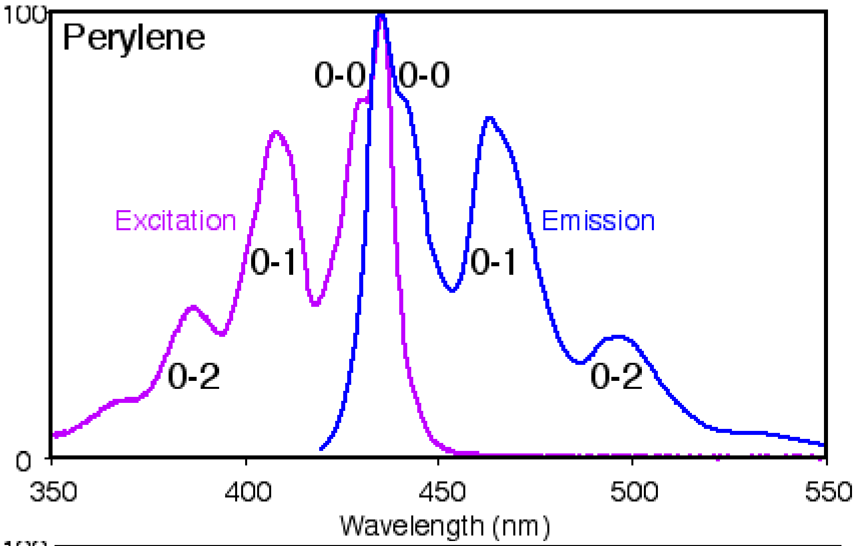
The fluorescence event can be described semi-classically as arising when a molecule is first excited to a higher electronic state by a sufficiently energetic photon and then falls back to its lower electronic state by photon emission. Obviously, the energy of the emitted photon will not be greater than that of the exciting photon but, because of interaction with vibrational states of the molecule, the emitted photon energy can be much less. The disparity is known as Stoke's shift. We observe this energy shift as a colour or wavelength shift of the emitted fluorescent light compared to the exciting light. The energy (E) and wavelength (λ) are, of course, related by $E = hc/\lambda$. The process can be depicted in a schematic energy level diagram:

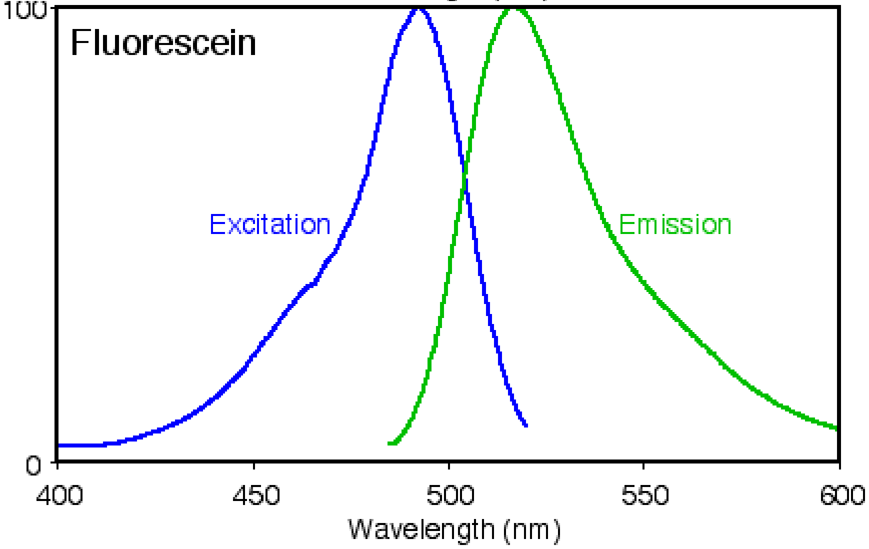


The solid arrows depict photon excitation and emission; the dashed arrows the molecule's return to lower vibrational levels by "internal conversion". One possible event is shown: the molecule is excited from ground state to a high vibrational level above its first excited electronic state, internal conversion returns it to the lowest vibrational state, it returns to the highest vibrational level above its electronic ground state by photon emission and finally returns to vibrational ground state by internal conversion. Note the photon energy disparity between excitation and emission. Several factors conspire to make this a preferred sequence in useful fluorescent molecules:

- Thermal energy is usually not enough to excite to higher vibrational levels. Before excitation, molecules are normally in their lowest electronic and vibrational states.
- Excitation commonly leads to a molecule at a high vibrational level.
- Lifetimes of excited vibrational states (~10^{-12}sec) are much shorter than fluorescence lifetimes (~10^{-8}sec). Thus internal conversion to the lowest vibrational level precedes fluorescence emission.
- Probability of photon emission leading to a high vibrational level is similar to the probability of arriving at that level upon excitation.
- The energy separations of vibrational levels are not much changed at higher electronic states.

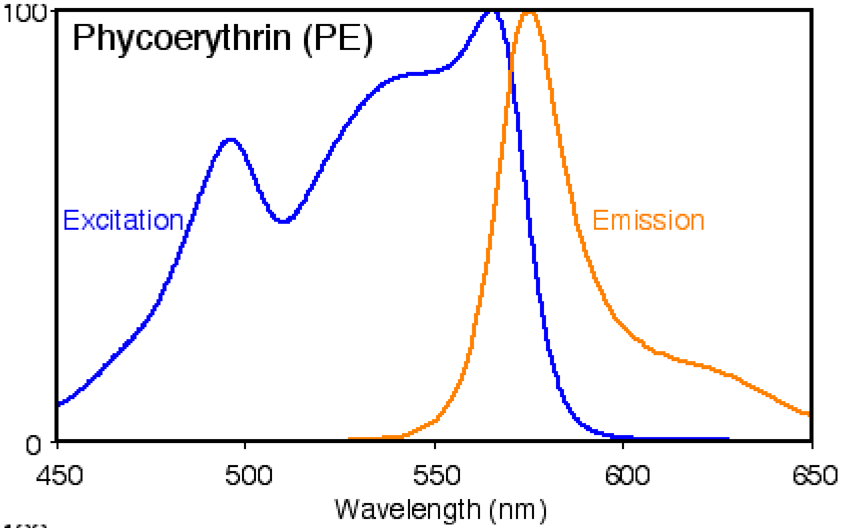
These factors also give rise to a striking mirror image symmetry between excitation and emission spectra. See the following figure for Perylene as an example.

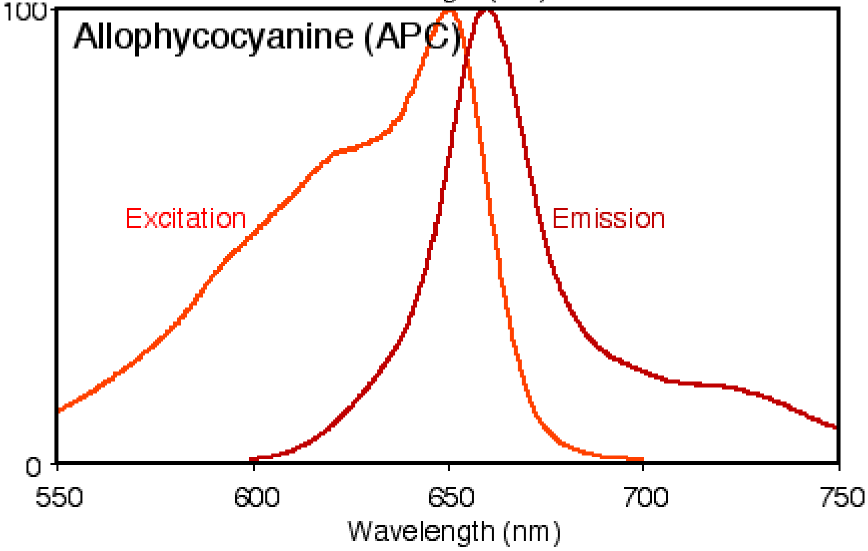


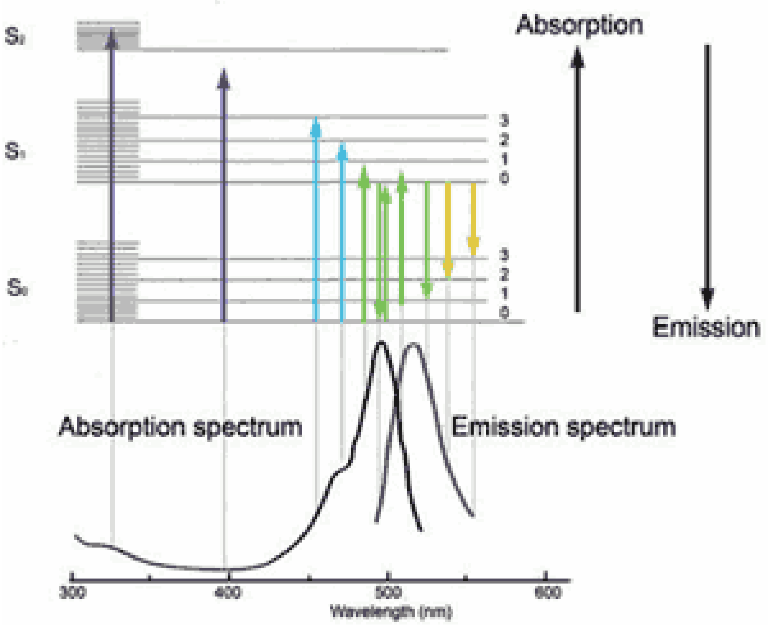


The figure is marked to relate structures in the curves to the above energy level diagram in a notional way only. Fluorescein, the most common fluorochrome in cytometry, also shows such symmetry.

Exceptions can arise where the molecule can undergo nuclear displacement or rearrangement during the process. The phycobili proteins PE and APC are two complex molecular structures that show departures from mirror image symmetry.






S_2
S_1
S_0
3
2
1
0
3
2
1
0
Absorption
Emission
Absorption spectrum
Emission spectrum
300
400
500
600
Wavelength (nm)