APh 162 - Fluorescence – what is it?

It is quite simple. A molecule absorbs a photon and emits a photon of lower energy. A photon excites an electron in the singlet ground state to a higher electronic singlet excited state (10^{-15} sec) where it relaxes (10^{-12} sec) to the lowest energy singlet excited state (vibrational relaxation). As the electron falls back into the ground state, it releases a photon of energy (10^{-9} sec).

Phosphorescence is related: Instead of vibrational relaxation after excitation, the electron undergoes a spin conversion into a triplet state. This is known as intersystem crossing. Electronic relaxation occurs, producing phosphorescence. Since the triplet state has lower energy than the lowest energy singlet state, the emitted photon is of longer wavelength than in fluorescence.

Note that when an electron is in the excited singlet state, energy may be transferred to nearby molecules, resulting in such phenomena as Forster resonance energy transfer, wherein the fluorescence in one molecule induces fluorescence in a molecule nearby, or fluorescence quenching, wherein (usually) oxygen interacts with the excited species to reduce fluorescence – hence some researchers add oxygen scavengers to get better signal. Photobleaching is the result when the fluorophore no longer emits photons – perhaps due to oxidation. However, this, too is the basis for various localization techniques and study of dynamics, especially through monitoring the recovery of fluorescence after photobleaching.

Other commonly used terms:
Singlet: paired spin states such that total spin is 0.
Triplet: paired spin states such that total spin is 1.
Quantum yield is the ratio of photons absorbed to photons emitted.
Fluorescence lifetime is the characteristic relaxation time of an excited state.

Other interesting facts:
One can “squeeze” the fluorescence to produce theoretically infinite resolution in fluorescence images by quenching the fluorescence with a laser pulse that eliminates the electronic relaxation. To wit: Laser illumination can only achieve diffraction limited resolution due to the finite aperture of a lens. Thus, dense fluorescent sample are limited in resolution by optics. One can do better by using few fluorophores and then localizing the source fitting by a Gaussian and finding the mean. However, intrinsic error to the localization is resultant from finite quantum yield before photobleaching. However, one can return to using dense samples by providing a secondary excitation source that “ablates” the surrounding fluorescence (through a ring configuration around the fluorescent excitation laser, for example), thus bypassing resolution limits imposed by optics and uncertainty due to limited number of photons. This is called STED – stimulated emission depletion.

Sources:
http://micro.magnet.fsu.edu/primer/java/jablonski/lightandcolor/index.html
http://en.wikipedia.org/wiki/Fluorescence
http://www.mpibpc.gwdg.de/groups/hell/STED.htm