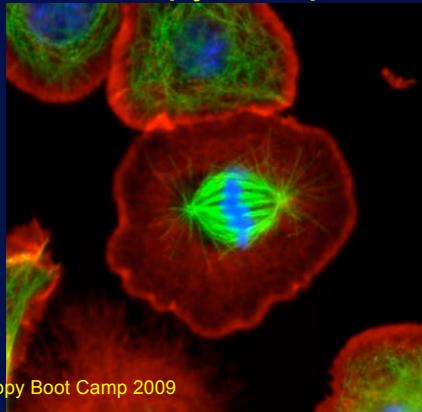
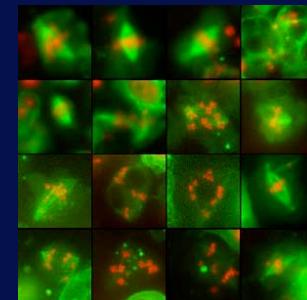


Fluorescence, Fluorescent microscopy and probes



Nico Stuurman
UCSF Microscopy Boot Camp 2009

Fluorescence in live cells

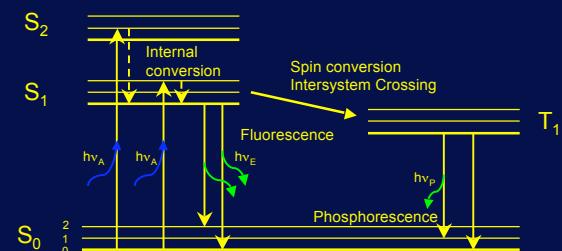


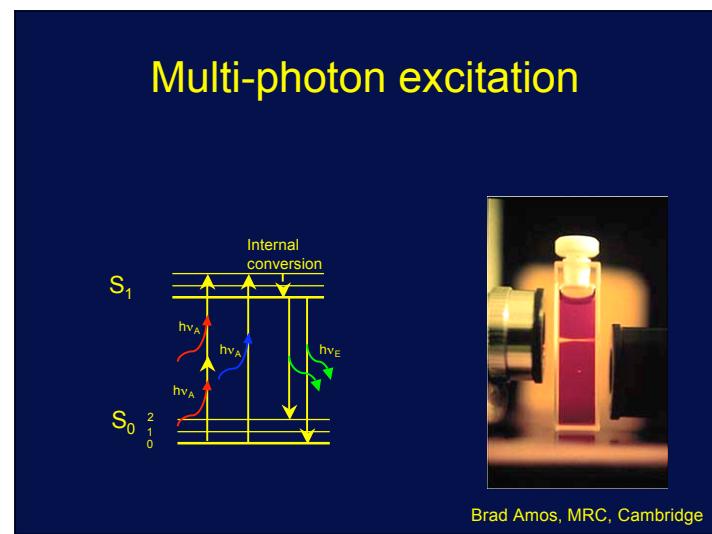
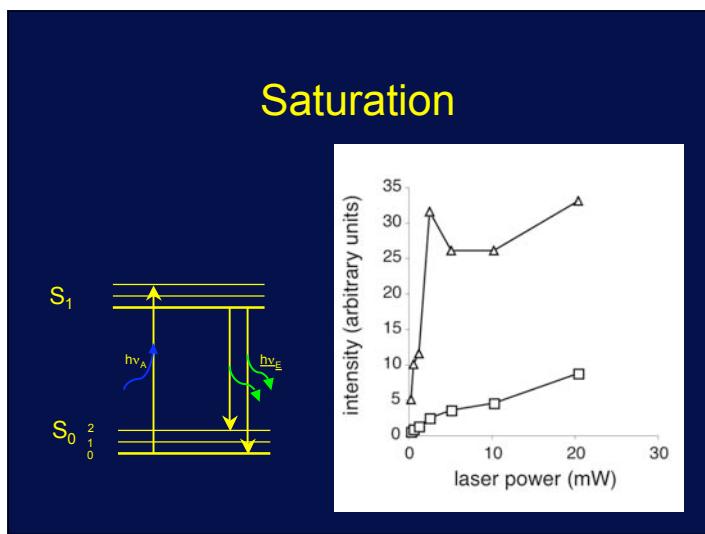
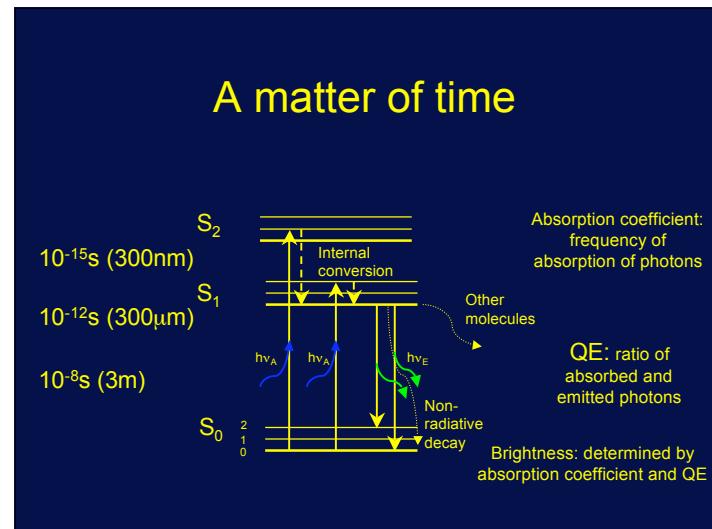
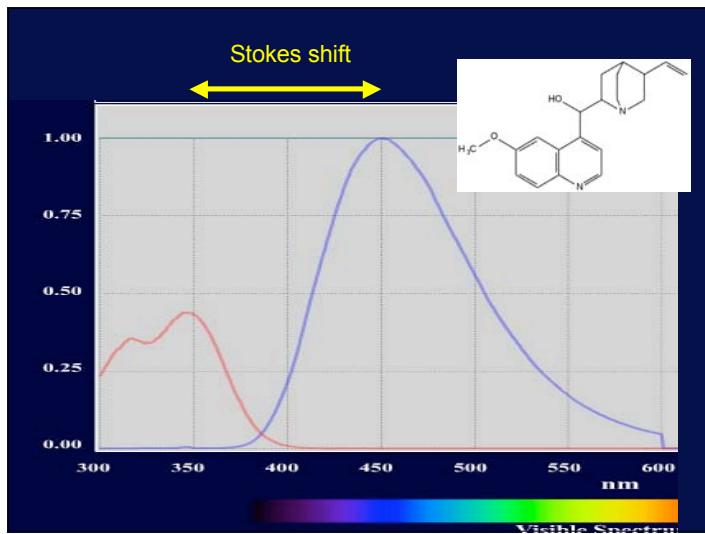
What is it?

Sir John Frederick William Herschel, 1854: Though perfectly transparent and colorless when held between the eye and the light, or a white object, it yet exhibits in certain aspects, and under certain incidences of the light, an extremely vivid and beautiful celestial blue colour, which, from the circumstances of its occurrence, would seem to originate in those strata which the light first penetrates the liquid.....

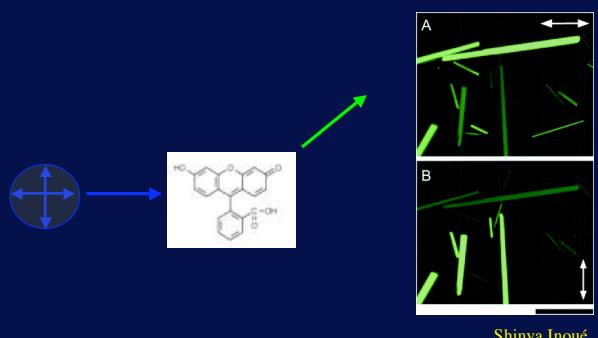
Fluorescence

Jablonski diagram



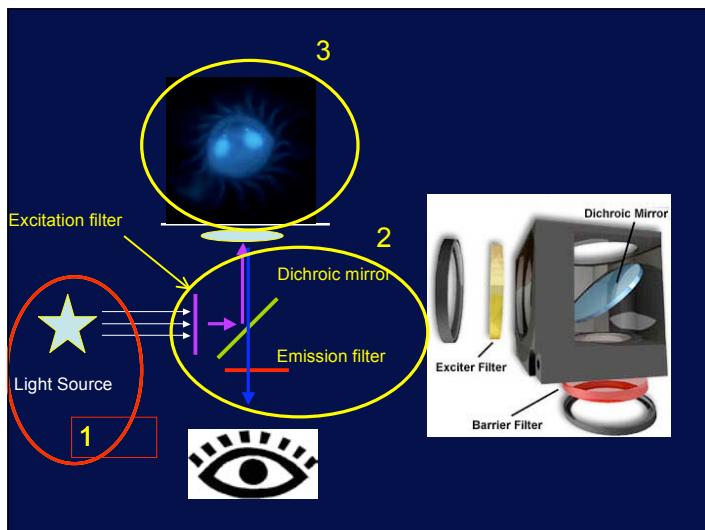


Polarization/Anisotropy



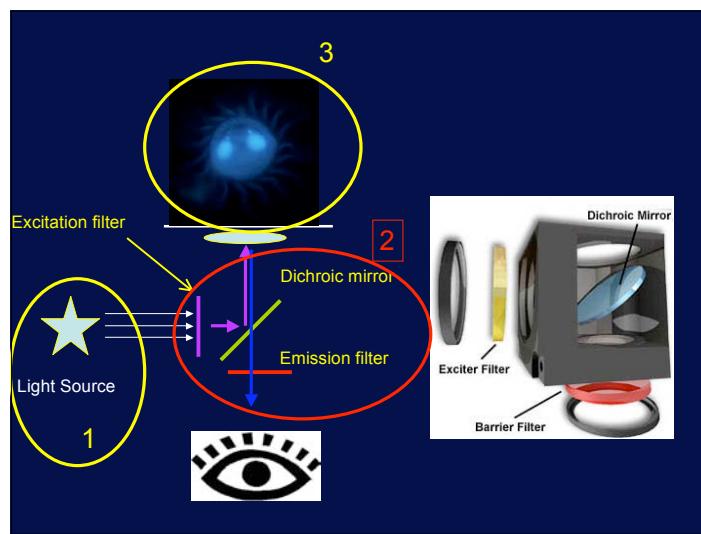
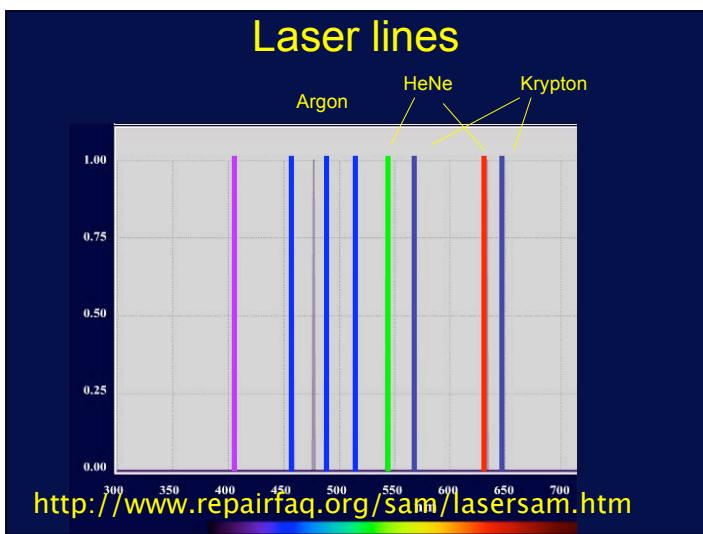
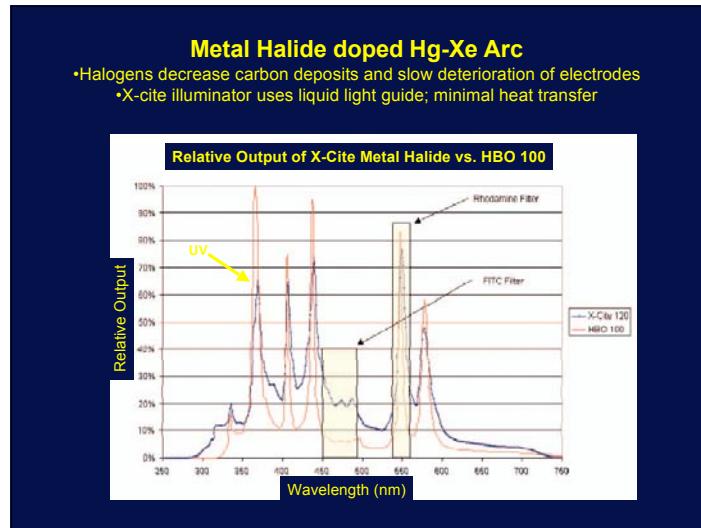
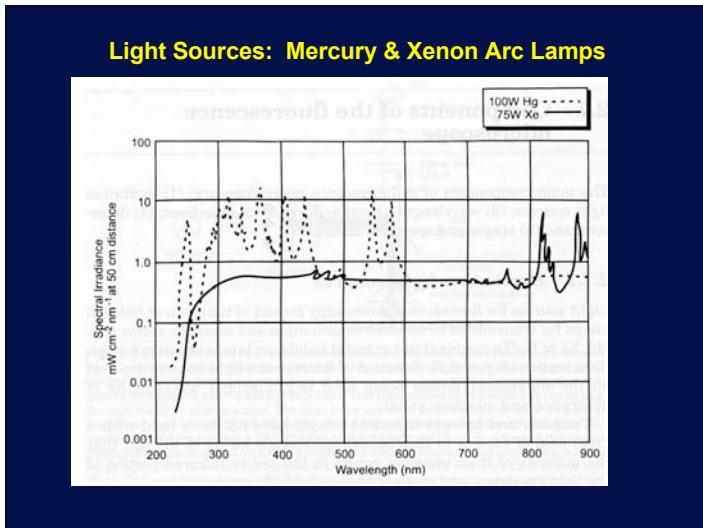
Why use Fluorescence probes?

- Sensitivity
- Specificity
- Analysis of location and quantity of a single component in a complex mixture
- Detection of small quantities of fluorophores and fluorescent objects below the resolution limit
- Environmental sensitivity
- Does not rely on physical properties of the specimen for contrast generation



Commonly Used Light Sources for Fluorescence Microscopy

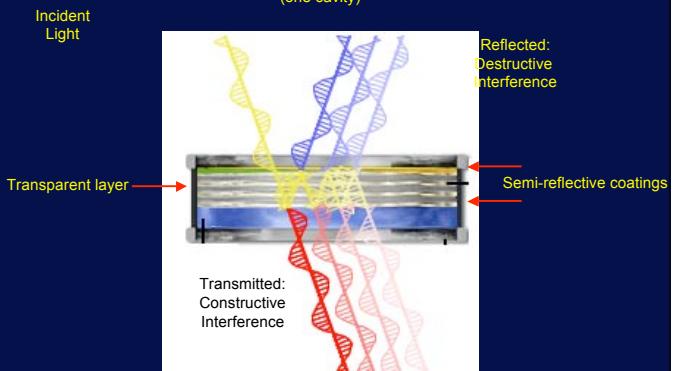
- Mercury arc lamp
- Xenon arc lamp
- Metal Halide doped Hg-Xe Arc
- LASERs
(most often used for confocal, TIRF)



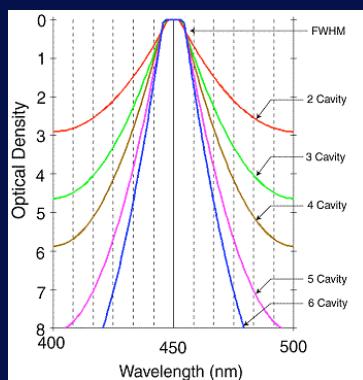
Filters

- Need to reject excitation light completely
- Need to be transparent for emitted light
- Need to match spectra of dyes
- Spectra of dyes:
www.zeiss.com/micro
probes.invitrogen.com/resources/spectraviewer/
www.mcb.arizona.edu/ipc/fret/

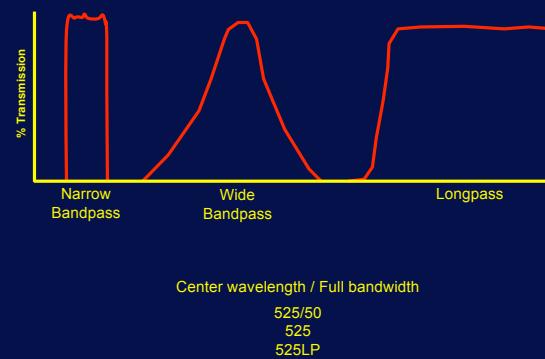
Interference Filter Design (one cavity)

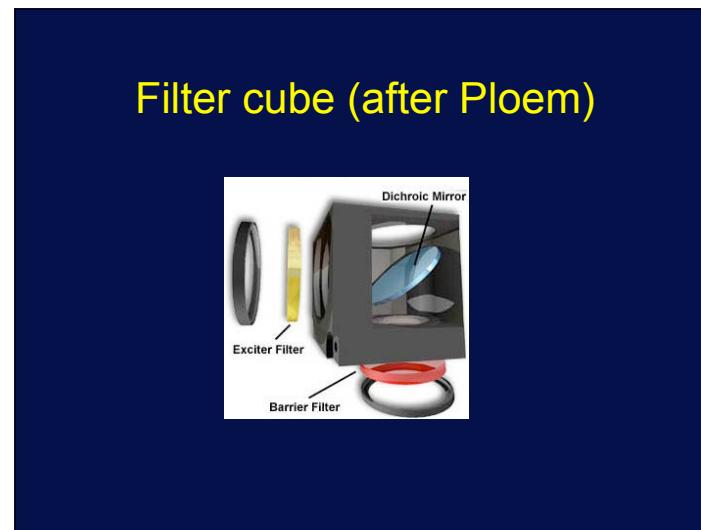
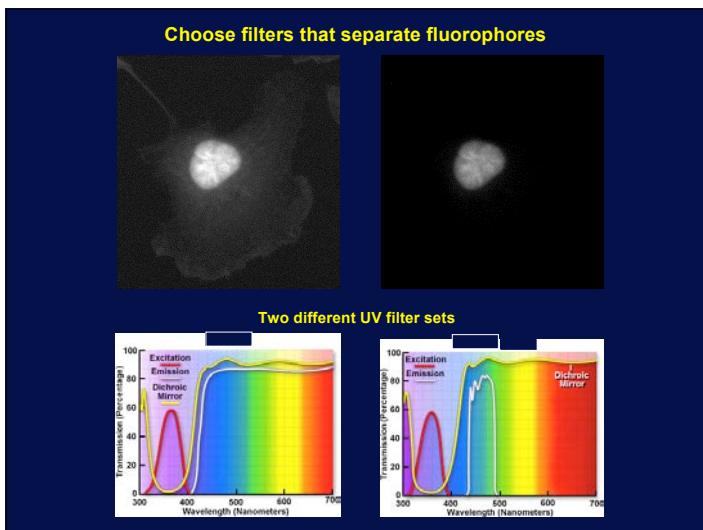
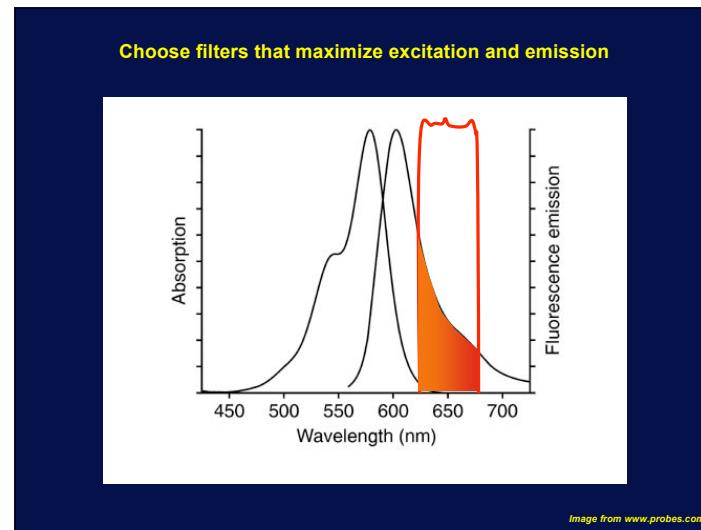
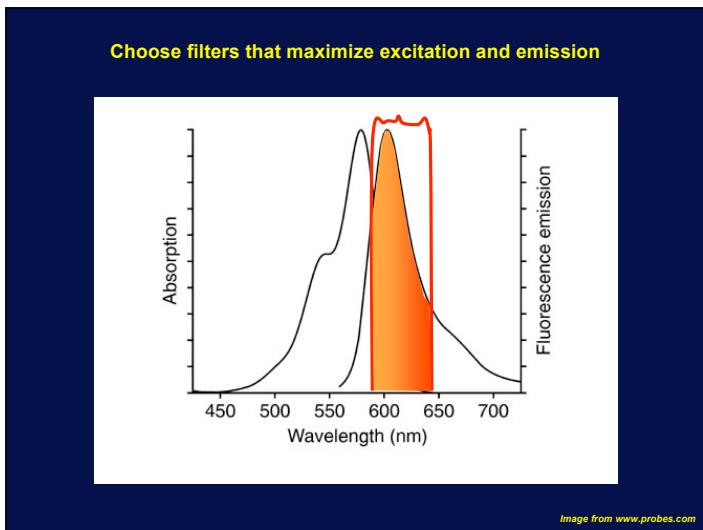


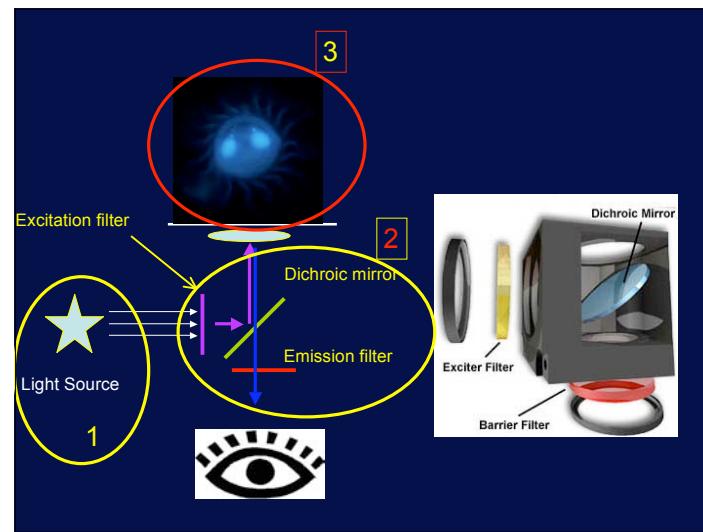
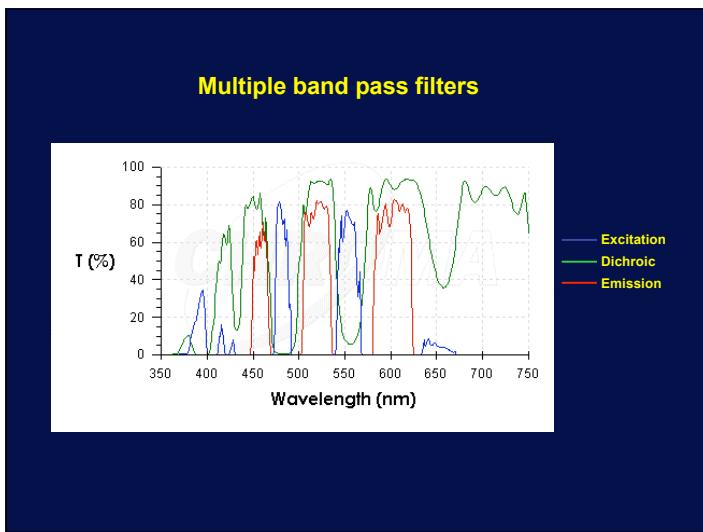
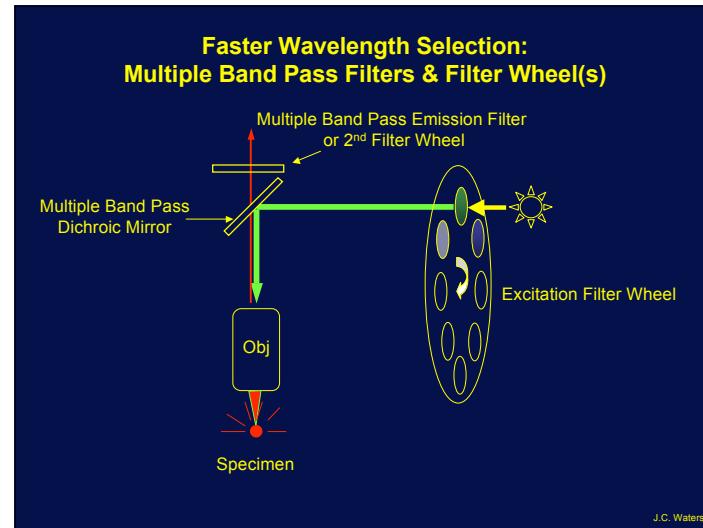
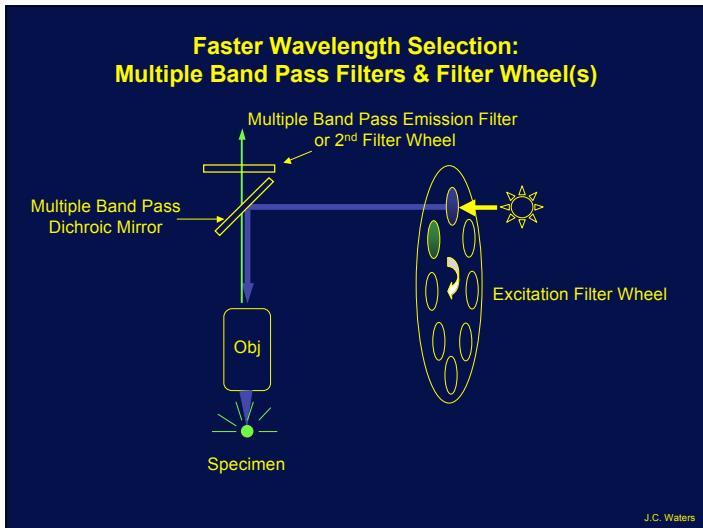
Interference Filter Design (multiple cavities)



Filter Terminology



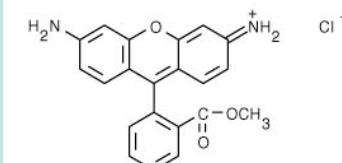
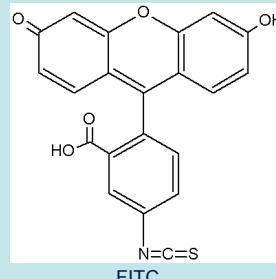




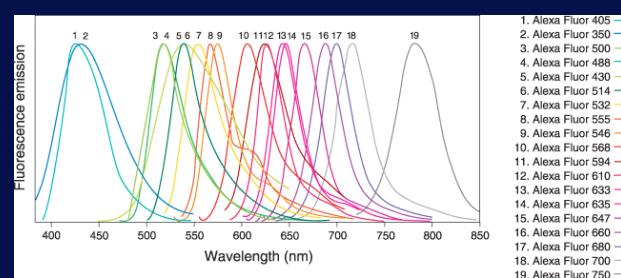
Types of fluorescent probes

- Immunofluorescence
- Fluorescent small molecules that bind specific cellular structures
 - DNA intercalating dyes (DAPI)
- Fluorescently labeled small molecules that bind specific cellular structures
 - Fluorescent phalloidin or taxol
- Fluorescently labeled proteins
- Fluorescent proteins (GFP)
- Genetically encoded tags binding fluorescent small molecules

The ‘classic’ dyes...



The Alexa Series Emission Spectra



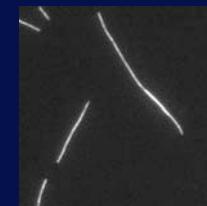
Molecular Probes (www.probes.com)

Conjugation of organic dyes

Chemistry/Method

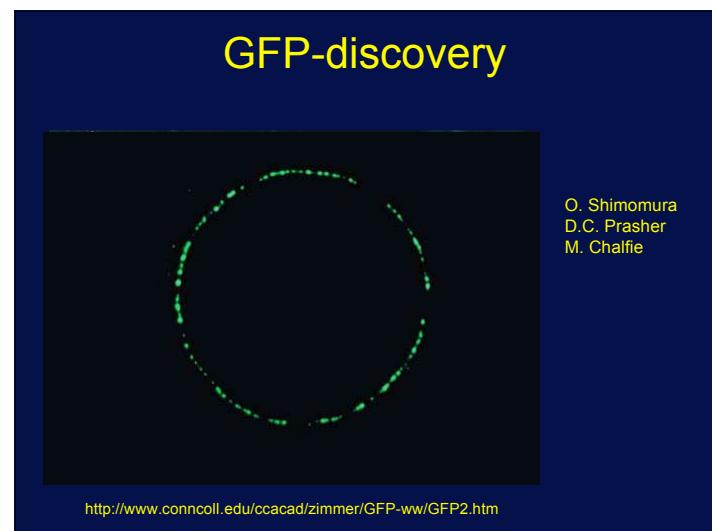
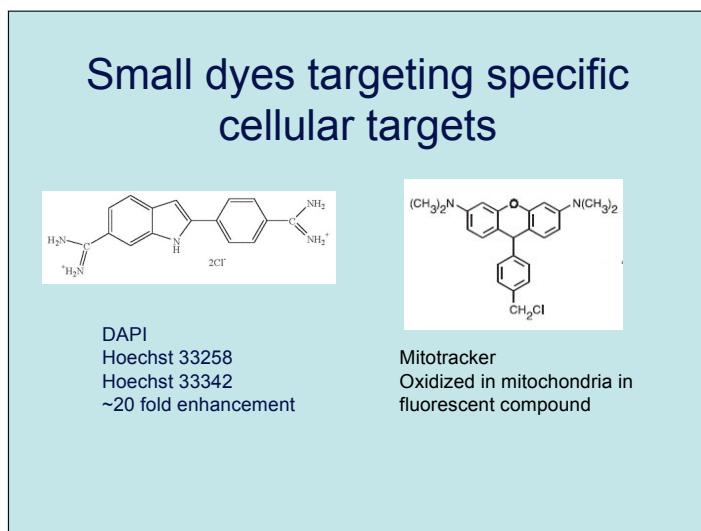
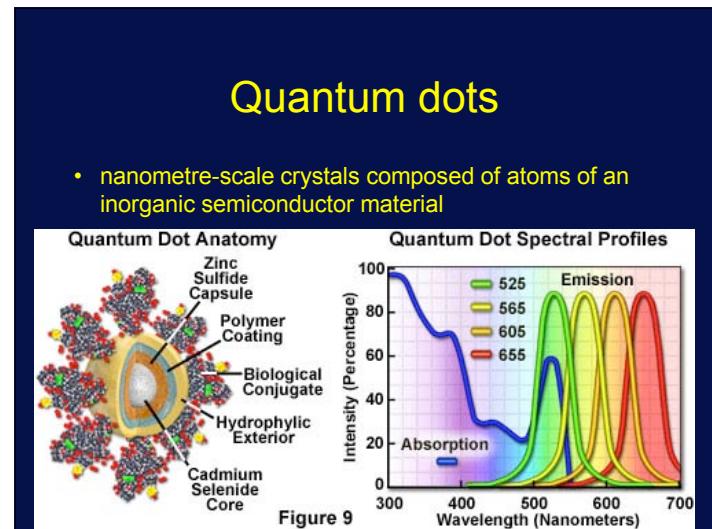
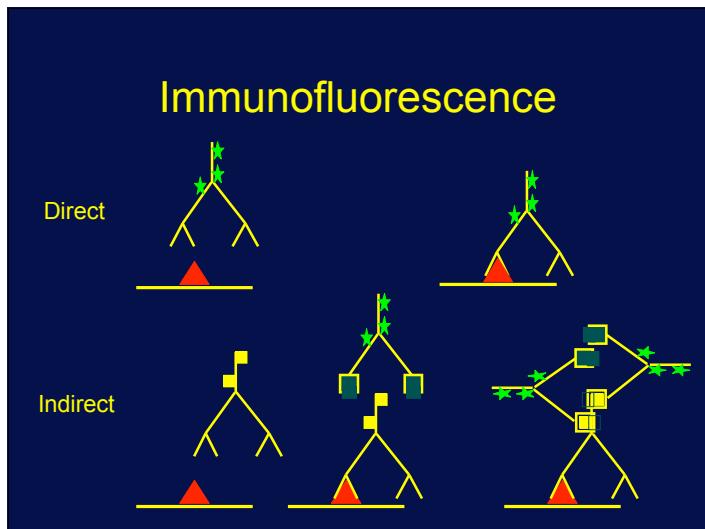
Amino groups (lysine): succinimidyl ester or isothiocyanate

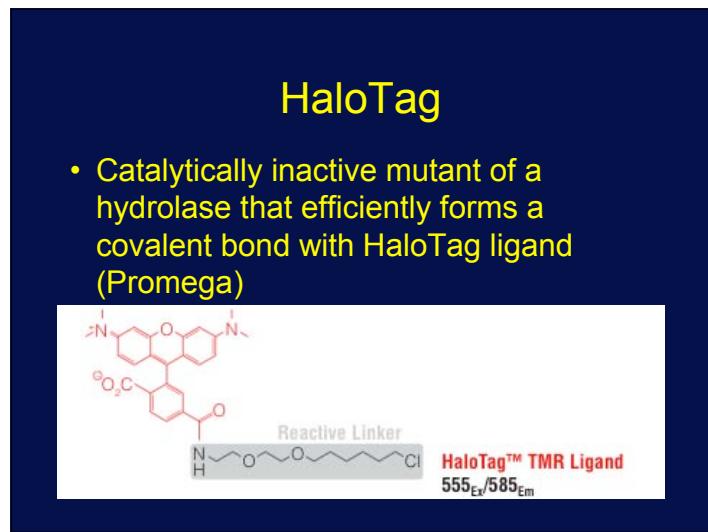
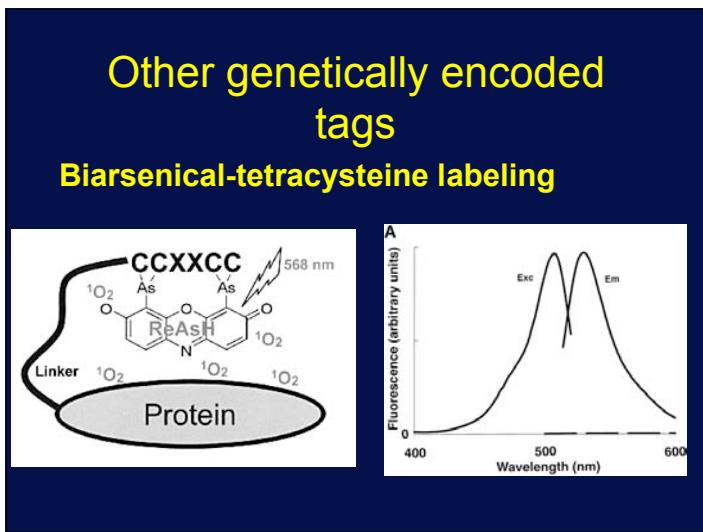
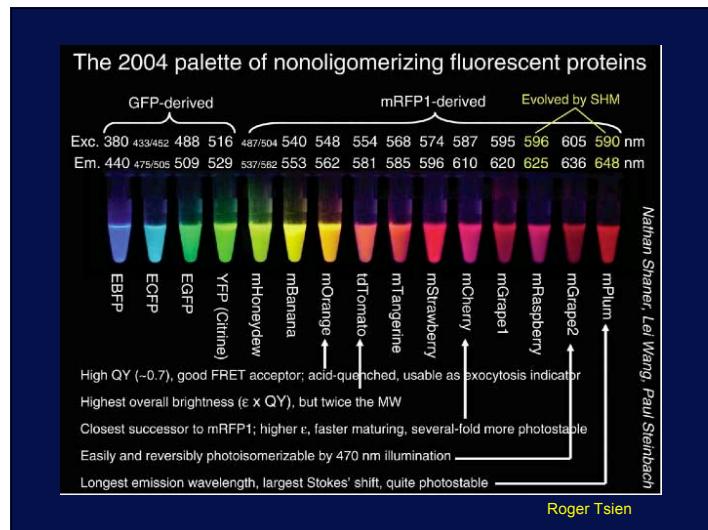
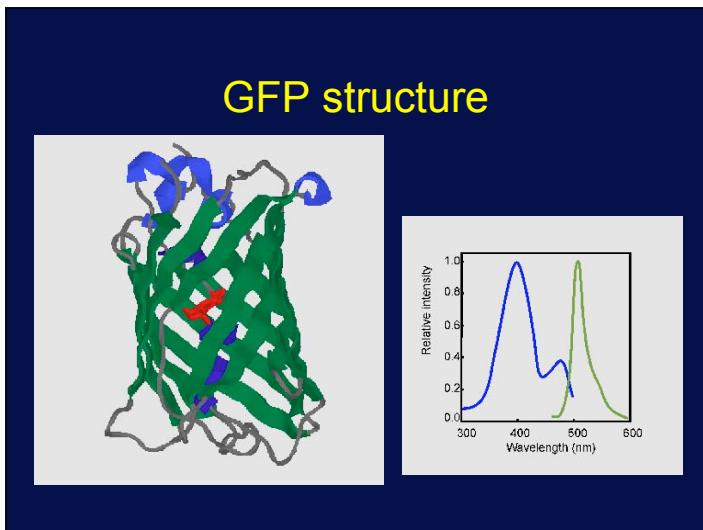
Example:
Dynein driven gliding of microtubules labelled with TMR on lysine residues.



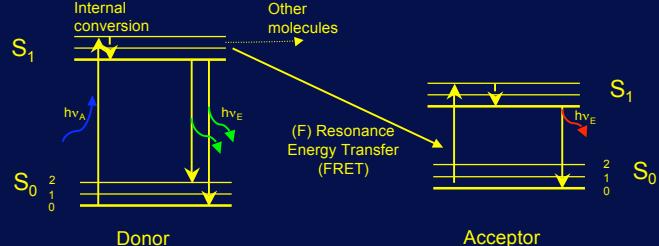
Targets

- Antibodies: direct/indirect labeling (Label density)
- Proteins: labeling site unspecific
- Small molecules, i.e. phalloidin, taxol

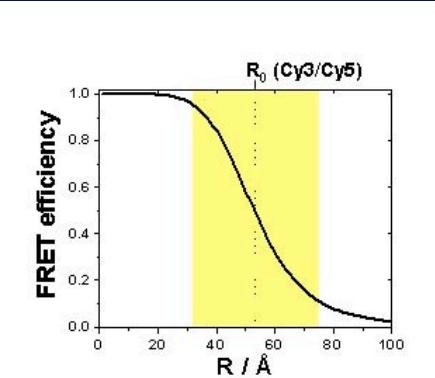




Quenching and FRET



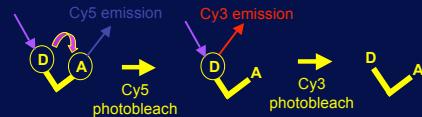
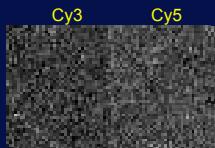
Use FRET as molecular ruler
 $E = R_0^6 / (R_0^6 + R^6)$



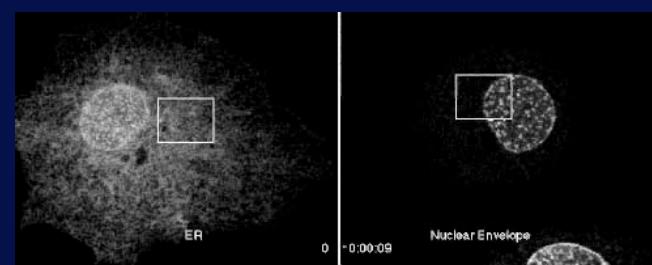
Evidence for single dye pair FRET

When Cy5 bleaches, Cy3 emission recovers

Kinesin showing
~100% FRET

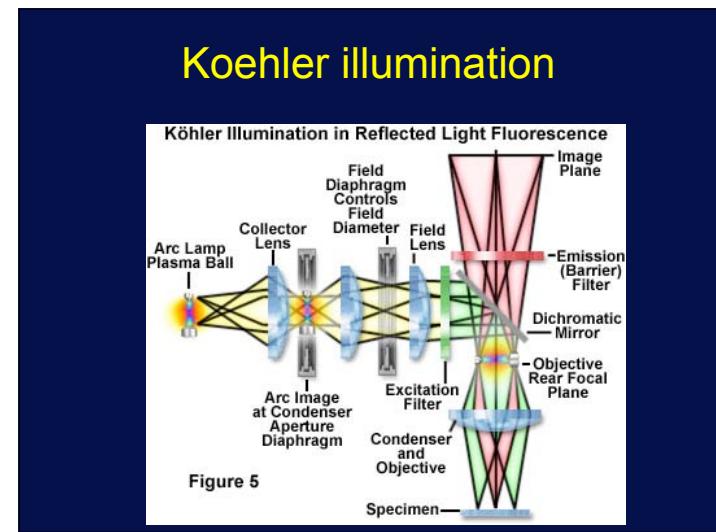
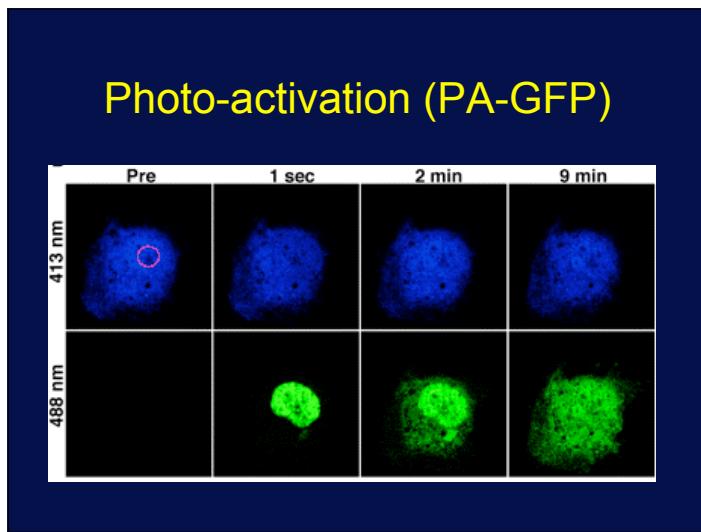
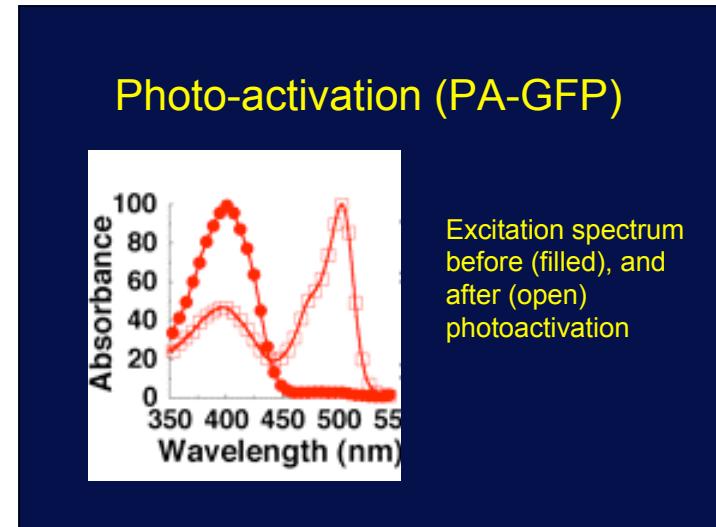
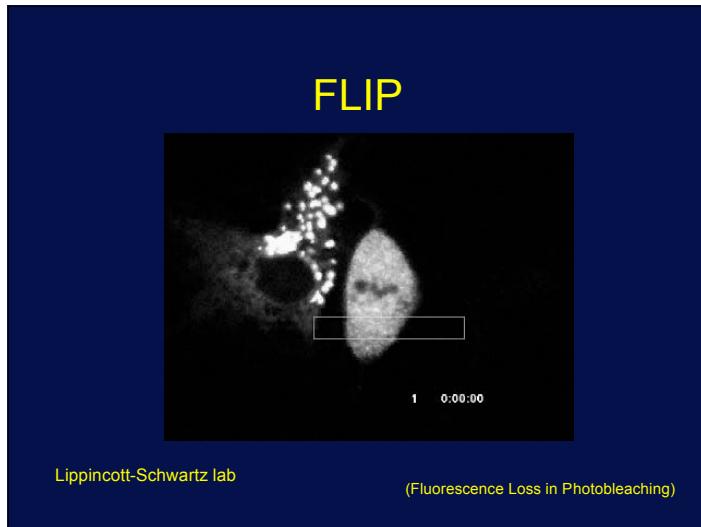


FRAP



Ellenberg et al., 1997 (Lippincott-Schwartz lab)

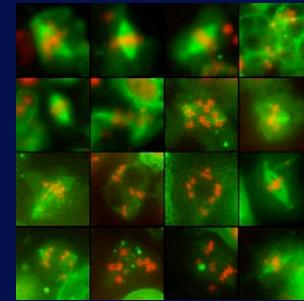
(Fluorescence Recovery After Photobleaching)



Lab samples:

- Drosophila S2 cells with various GFP-fusion proteins
- Stained tissue culture cells (multi-channel fluorescence)
- Fluorescent beads (visualize point spread function, registration shift)

THANKS!



Jennifer Waters

www.micro.magnet.fsu.edu

www.mcb.arizona.edu/ipc/fret/