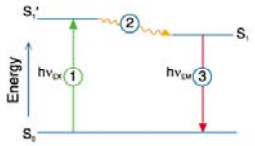
Md. Shahidul Islam, M.D., Ph.D.
Department of Clinical Research and Education
Karolinska Institutet
Forskningscentrum, Södersjukhuset 118 83 Stockholm, Sweden
Shaisl@ki.se

Calcium signaling course
Laboratory demonstration
May 2011

Measurement of parameters by microfluorometry

- Ions
  - Ca2+
  - Mg2+
  - Na+
  - H+
  - Cl-
  - Zn2+

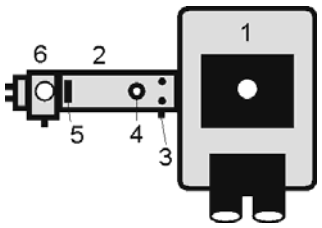
  Metabolic Parameters
  - NADH
  - FAD
- Membrane Potential
- Signaling molecules



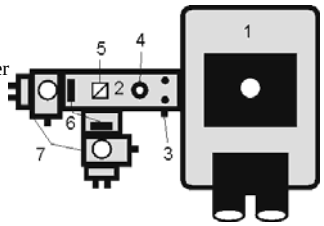
Stokes shift

Quantum yeild

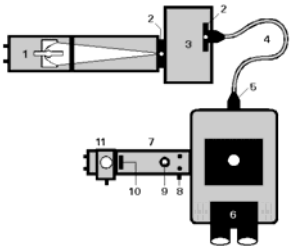
1. Microscope
2. Microscope photometer
3. Adjustable iris
4. Viewing eyepiece
5. Filter holder
6. PMT detector



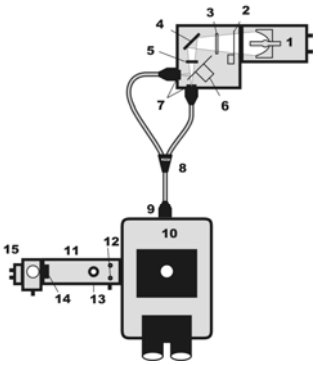
1. Microscope
2. Microscope photometer
3. Adjustable iris
4. Viewing eyepiece
5. Dichroic cube
6. Filter holder
7. PMT detectors

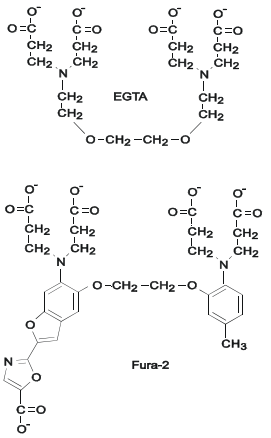


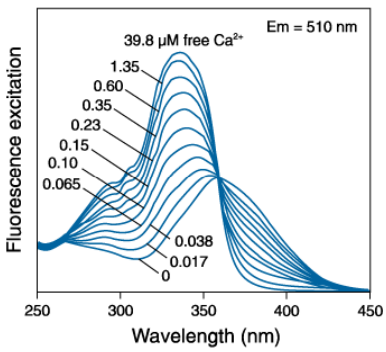
1 Arc lamp housing
2 Adjustable slits
3 Excitation monochromator
4 Liquid light guide
5 Liquid light guide adapter
6 Microscope
7 Microscope photometer
8 Adjustable iris
9 Viewing eyepiece
10 Filter holder
11 PMT detector

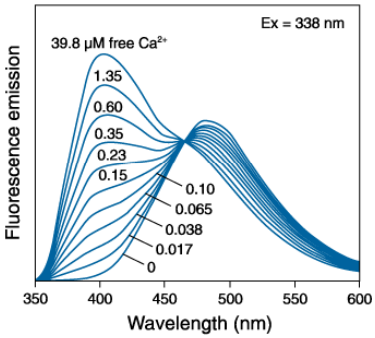


1 Arc lamp housing
2 Computer-controlled shutter
3 Iris
4 IR Trap
5 Broadband Filter
6 Optical Chopper
7 Filters
8 Bifurcated Fiber Optic Cable
9 Fiber optic adapter
10 Microscope
11 Microscope photometer
12 Adjustable iris
13 Viewing eyepiece
14 Filter holder
15 PMT detector









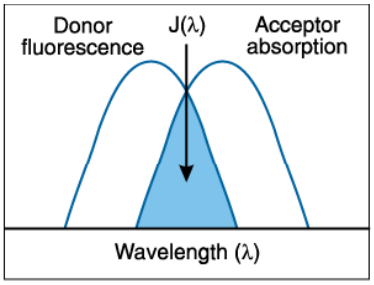
Fluorescence Resonance Energy Transfer

What is FRET

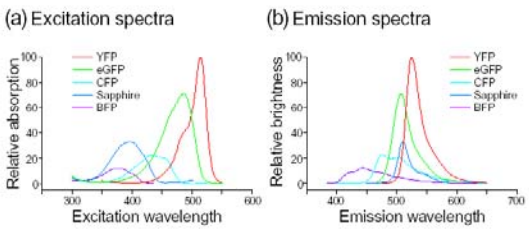
It is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule *without emission of a photon.*

Conditions for FRET

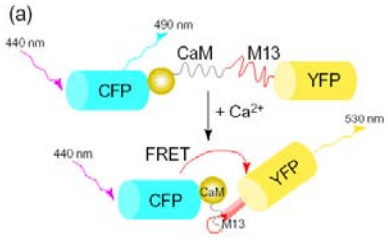
- Proximity (10-100Å)
- Absorption spectrum of acceptor overlaps emission spectrum of donor
- Donor and acceptor transition dipole orientation is parallel

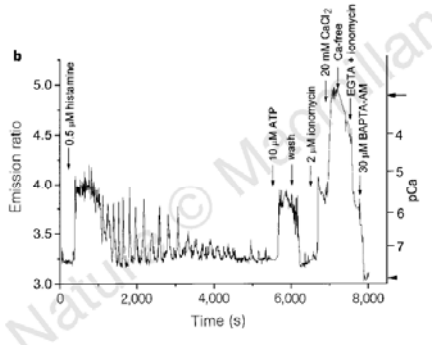


Excitation and emission spectra of different ”GFP”



Cameleons





Miyawaki A. 1997, Nature 388:6645