How to visualize (the dynamics of) molecular interactions?

**Förster or Fluorescence Resonance Energy Transfer (FRET)**

### Intramolecular FRET (biosensors)

**METHOD**

<table>
<thead>
<tr>
<th>Ratiometric imaging approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Donor/Acceptor)</td>
</tr>
</tbody>
</table>

**MICROSCOPES**

- confocal: Nipkow/spinning disk, Nikon A1r spectral/CLSM
- widefield: Bioflux

**SOFTWARES**

| NIS-Elements, Metafluor, Metamorph, ImageJ |

**CONDITIONS**

- Distance A-D <100 Å (Ro)
- Overlap λem(Donor) - λex(Acceptor)
- Fluorophore orientation
- Biological context
- Transfection level

**PROTOCOLE**


http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/spectralfret.html

### Intermolecular FRET

**METHODS**

- FRET by intensity measurement
- FRET by Acceptor photobleaching
- FRET by Donor photobleaching
- FRET by lifetime measurement (TCSPC, Time Correlated Single Photon Counting)
- FRET by fluorescence anisotropy

**MICROSCOPES**

- Confocal: Nikon A1r spectral/CLSM
- FLIM (Fluorescence Lifetime Imaging Microscopy) by single-photon counting
- FCS/ FCCS (Fluorescence Cross- Correlation Spectroscopy)

**SOFTWARES**

| NIS-Elements, ImageJ |

**CONDITIONS**

- Distance A-D <100 Å (Ro)
- Overlap λem(Donor) - λex(Acceptor)
- Stoichiometry D-A 1:1
- Fluorophore orientation
- Biological context
- Transfection level

**PROTOCOLE**

https://www.unige.ch/medecine/bioimaging/en/information/tutorials/ and go to: "F-techniques> * How to perform FRET experiments?"

Broussard et al. (2013). Fluorescence resonance energy transfer microscopy as demonstrated by measuring the activation of the serine/threonine kinase Akt. Nat. Protoc. 8, Jan 10, 265-281.

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**Common Fluorescent Protein FRET Biosensor Strategies**

- Sensory Domain
  - Substrate
  - No FRET
  - FRET
  - Sensory Domain

- Sensory Domain
  - Substrate
  - No FRET
  - FRET

**Figure 9**

- Ligand
  - No FRET
  - FRET

- Protease
  - Cleavage
  - No FRET
  - FRET

**Correct orientation**

- No FRET
  - Donor emission
  - Acceptor excitation

- Overlap
  - Donor emission
  - Acceptor excitation

- FRET
  - 405 nm
  - 477 nm

- >10 nm
  - No FRET

- <10 nm
  - 405 nm
  - 477 nm

- 528 nm
  - 406 nm

- 528 nm
  - 477 nm

- 477 nm
  - 405 nm

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