VisiFRET

Fluorescence Resonance Energy Transfer

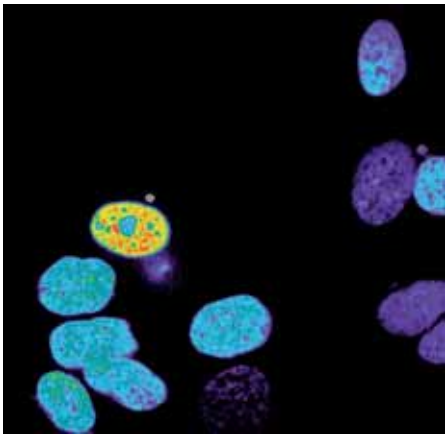
VisiFRET Imaging System

FRET in Live Cell Imaging

Förster (fluorescence) resonance energy transfer (FRET) is the process of radiation-free energy transfer between two spatially close fluorophores called donor and acceptor. In FRET condition, photoexcitation of the FRET-donor molecule leads to a decreased donor fluorescence and induces fluorescence of the FRET acceptor.

DualCam with two sCMOS edge cameras.

Axio Examiner with VisiChrome illumination and DualView Imager.



Quantitative Imaging

Using our VisiFRET fluorescence imaging system you can obtain quantitative temporal and spatial information about the binding and interaction between proteins, lipids, enzymes, DNA and RNA in vivo.
These processes are usually below the resolution of a light microscope. Because of the development of a number of green fluorescent proteins, it is possible to measure the integration of intracellular molecules.

VisiFRET Imaging System

The FRET efficiency decreases with the sixth power of the distance between acceptor and donor. Thus, FRET can be used as a distance measure and can indicate the interaction of biomolecules. The FRET signal is determined by exciting the donor molecule and measuring the acceptor emission.

VisiFRET

Fluorescence
Resonance
Energy
Transfer

FRET Theory

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A couple of years ago, variants of the green fluorescent protein (GFP) have been engineered, which can due to their overlapping excitation/ emission spectra serve as donor / acceptor pair in biological FRET experiments; e.g. ECFP/EYFP or EBFP/EGFP. The critical distance is about 6 nm.

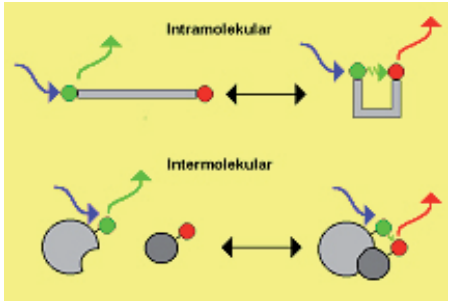
For time resolved measurements of the energy transfer the donor emission as well as acceptor signal is recorded at every time point and a ratio is built (donor emission/FRET signal). Using advanced FRET detector molecules, this method allows for example to study changes in second messenger concentration. Miyawaki and colleagues (1997) have developed a FRET based calcium sensor called Cameleon. Zaccolo and colleagues (2000) generated a cAMP sensitive FRET detector (Epac) by fusing GFP variants with protein kinase A sub-units.

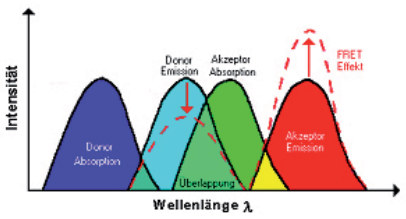
Another FRET application is the determination of the spatial arrangement of two molecules towards each other. Damelin and Silver (2000), for example, explored the interaction of nuclear transport receptors with the nuclear pore complex utilizing FRET microscopy.
In order to quantify FRET the ratio of the FRET- and the donor-emission is built.

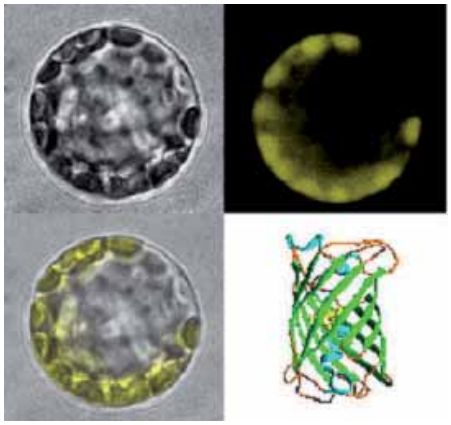
The FRET value is calculated by the following equation:

$$FRET\ value = \frac{mean\ ratio\ (donor-acceptor) - mean\ ratio\ (donoronly)}{mean\ ratio\ (donor\ only)}$$

donor-acceptor: cell line, expressing both donor and acceptor molecules
donor only: cell line, expressing just the donor molecules







Localization of FRET: DIC-Image (top left), FRET-signal (top right) and overlay image (down left). Schematic of GFP-molecule (down right).