Intelligent BioMed-Solutions from Visitron Systems GmbH

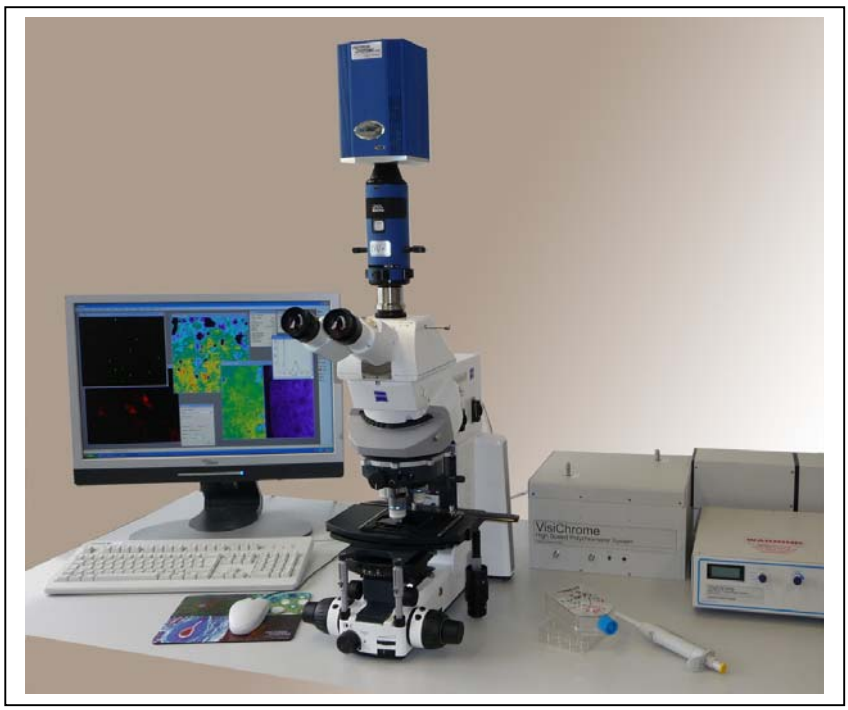
VisiFRET Imaging System

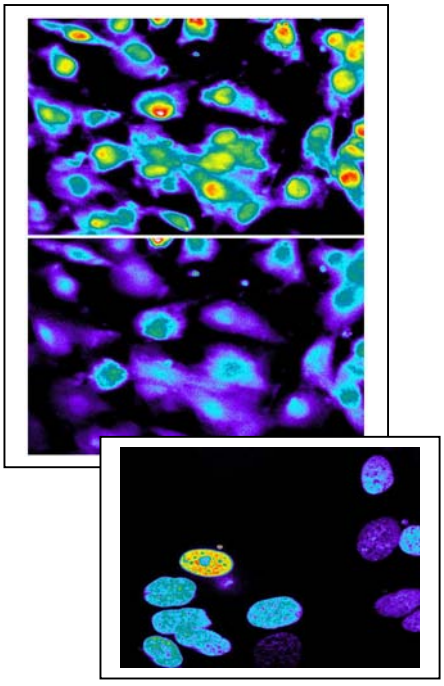
VisiFRET

Fluorescence Resonance Energy Transfer Imaging System

FRET in Live Cell Imaging

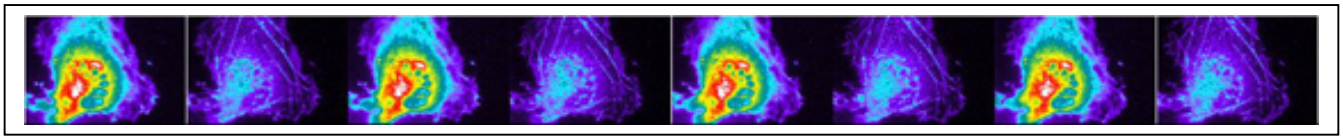
Förster (fluorescence) resonance energy transfer **(FRET)** is the process of radiation free energy transfer between two spatially close fluorophores called donor and acceptor. In FRET condition photoexcitation of the FRET-donor molecule leads to a decreased donor fluorescence and an induced fluorescence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





Using VisiFRET fluorescence imaging system you can obtain quantitative temporal and spatial information about the binding and interaction between protein, 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 fluorescence proteins, it is possible to measure the integration of intracellular molecules.



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Theory

The FRET efficiency decreases with the sixth power of the distance between acceptor and donor. Thus FRET can be used as distance measure and can indicate the interaction of bio-molecules. The FRET signal is determined by exciting the donor molecule and measuring the acceptor emission. 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 5nm.

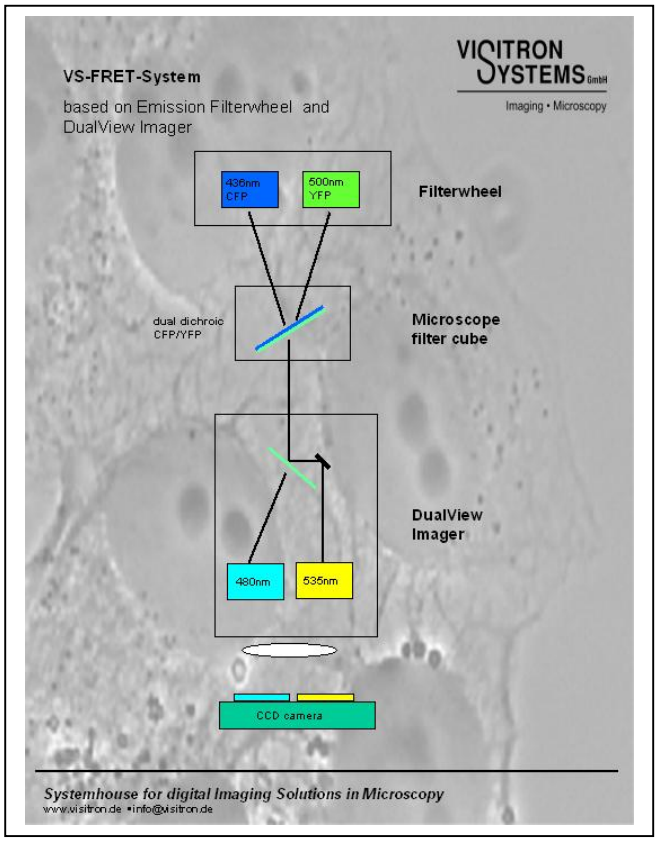
For time resolved measurements of the energy transfer the donor emission as well as acceptor signal is recorded at every time point and a ratio is build (donor emission/FRET signal). Using advanced FRET detector molecules this method allows for example to study changes in second messenger concentration. 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.
I order to quantify FRET the ratio of the FRET- and the donor-emission is build.

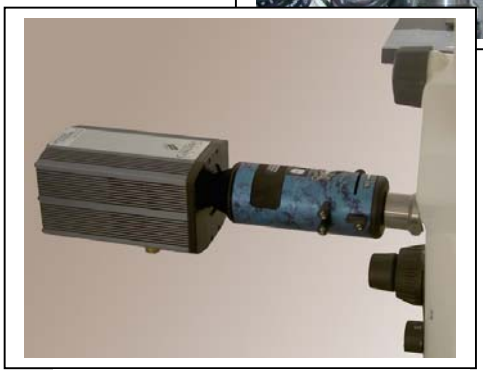
The FRET value is calculated by the following equation:

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

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



CSU10 confocal with emission-filterwheel



DualView imager with Cascade

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