Milestones of FRET (Förster Resonance Energy Transfer) Imaging

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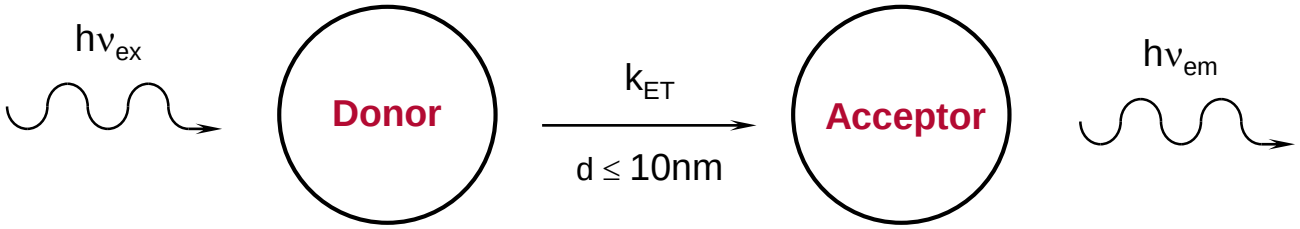
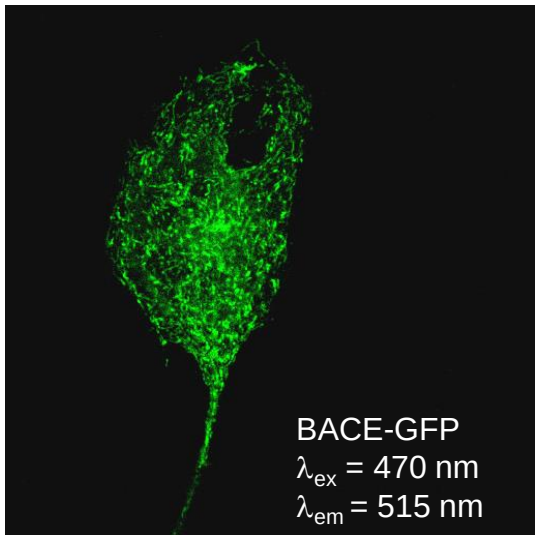
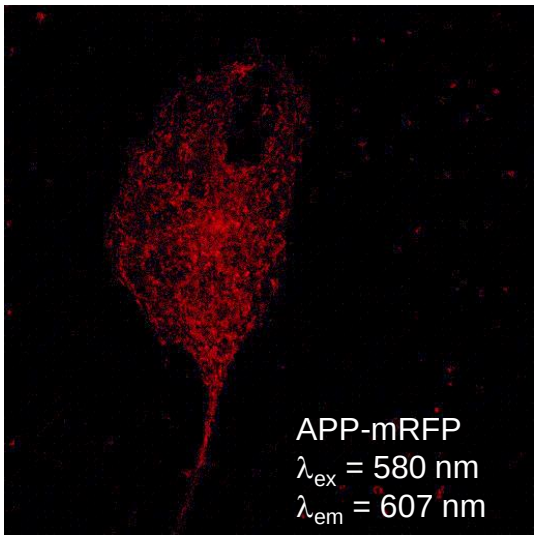
Förster Resonance Energy Transfer (FRET)

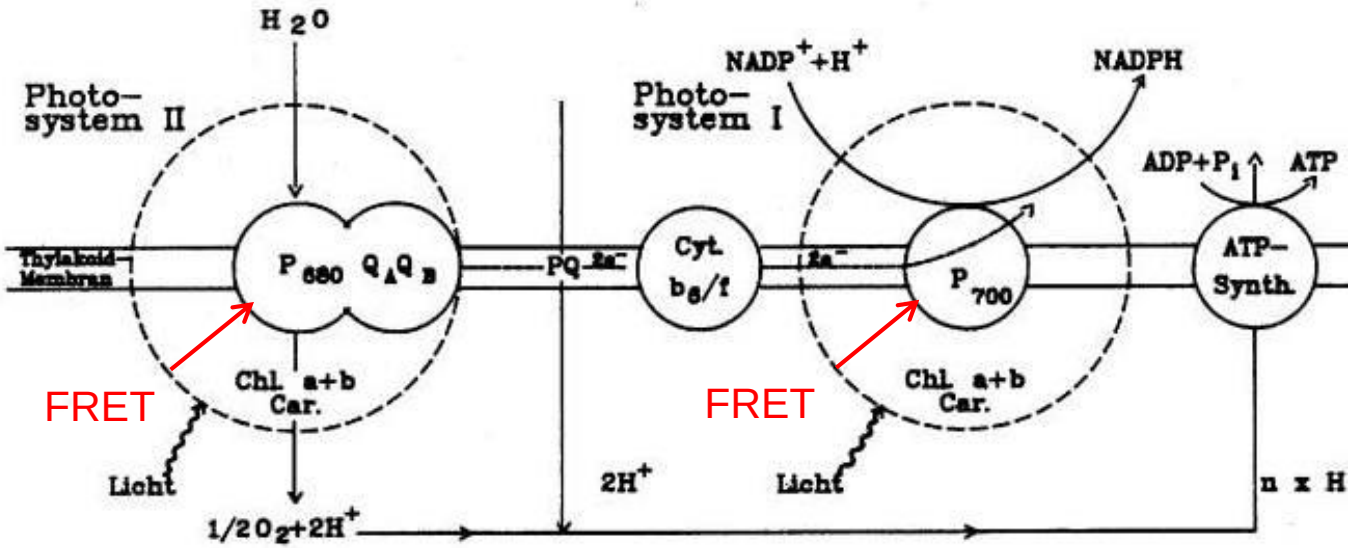
example: fluorescent proteins





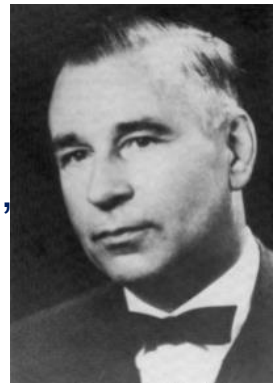


FRET History: Photosynthesis



First reports on photosynthesis: 3.8 billion years ago
Cyanobacteria with light harvesting complex: 2.4 billion years ago
Oxygenic photosynthesis: 2.3 billion years ago

Theodor Förster (1910 – 1974)



T. Förster: Zwischenmolekulare Energiewanderung und Fluoreszenz, Ann. Phys. 437 (1948) 55-75

Donor – aceptor energy transfer rate

(interaction between optical transition dipoles with Förster radius R_0 depending on spectral overlap and dipole orientation):

$$k_{ET} = \frac{1}{\tau_D^0} \cdot \left(\frac{R_0}{R}\right)^6$$

Experimental parameters:

- Stationary measurements:

$$\frac{I_A}{I_D} = \tau_D^0 \cdot \frac{\eta_A}{\eta_D} \cdot k_{ET}$$

- Time-resolved measurements:

$$k_{ET} = \frac{1}{\tau_D} - \frac{1}{\tau_D^0}$$

Competitive Processes of Intermolecular Energy Transfer

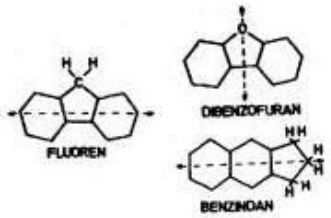
	Molecule A		Molecule B	
Re-absorption:	$S_1 \rightarrow S_0$	radiative	$S_0 \rightarrow S_1$	low analytical relevance
Förster:	$S_1 \rightarrow S_0$	non-radiative	$S_0 \rightarrow S_1$	spatial and spectral sensitivity
(dipole-dipole interaction)				
Energy exchange	$T_1 \rightarrow S_0$	non-radiative	$S_0 \rightarrow T_1$	limited spatial sensitivity
			$T_0 \rightarrow S_1$	

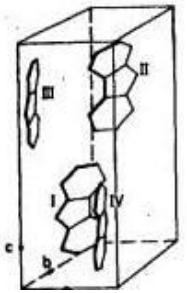
Post – Förster Period (1974 – 1994)

- Molecular physics and physical chemistry,

H. Port, H. Schneckenburger and H.C. Wolf: Dipole-forbidden energy transfer between host and guest in fluorene: dibenzofuran mixed crystals, Chem.Phys. Lett. 61 (1979) 503-508.

(Comment: „Very nice work, but you will never need it in your life“)





- Cell and membrane biology

Resonance Energy Transfer Microscopy: Observations of Membrane-bound Fluorescent Probes in Model Membranes and in Living Cells

Paul S. Uster and Richard E. Pagano

Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210. Dr. Uster's present address is Liposome Technology, Inc., Menlo Park, California 94025.

Ann. Rev. Biochem. 1978. 47:819-46
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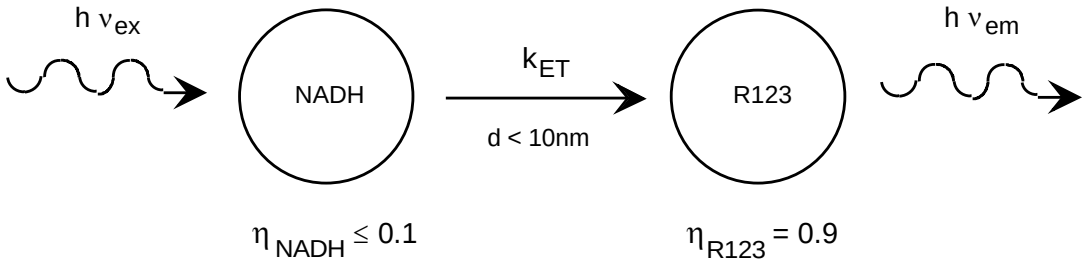
FLUORESCENCE ENERGY TRANSFER AS A SPECTROSCOPIC RULER[1]

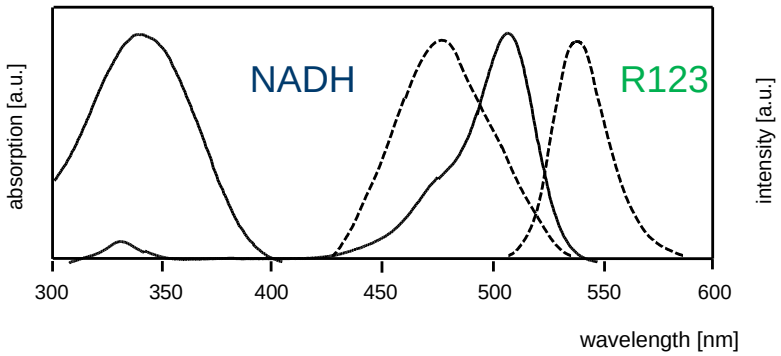
Lubert Stryer

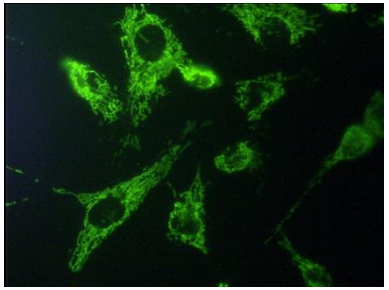
Department of Structural Biology, Sherman Fairchild Center, Stanford University School of Medicine, Stanford, California 94305

Post – Förster Period (1994 – 1997)

- Selective measurement of mitochondrial NADH for probing mitochondrial respiratory function







H. Schneckenburger, M.H. Gschwend, W.S.L. Strauss, R. Sailer, M. Kron, U. Steeb, R. Steiner: „Energy transfer spectroscopy for measuring mitochondrial metabolism in living cells“, Photochem. Photobiol. 66 (1997) 34-41.

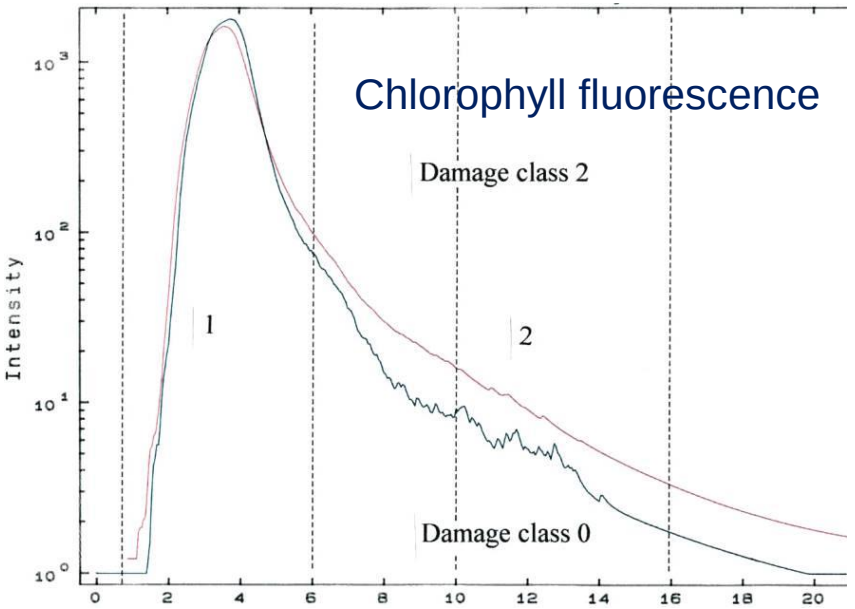
Post – Förster Period (1985 – 1997)

Picea abies (L.) Karst, Freudenstadt, Black Forest

Forest decline

Full sunlight (yellowing)

Reduced sunlight



ns

Fertilization with Mg^{2+}

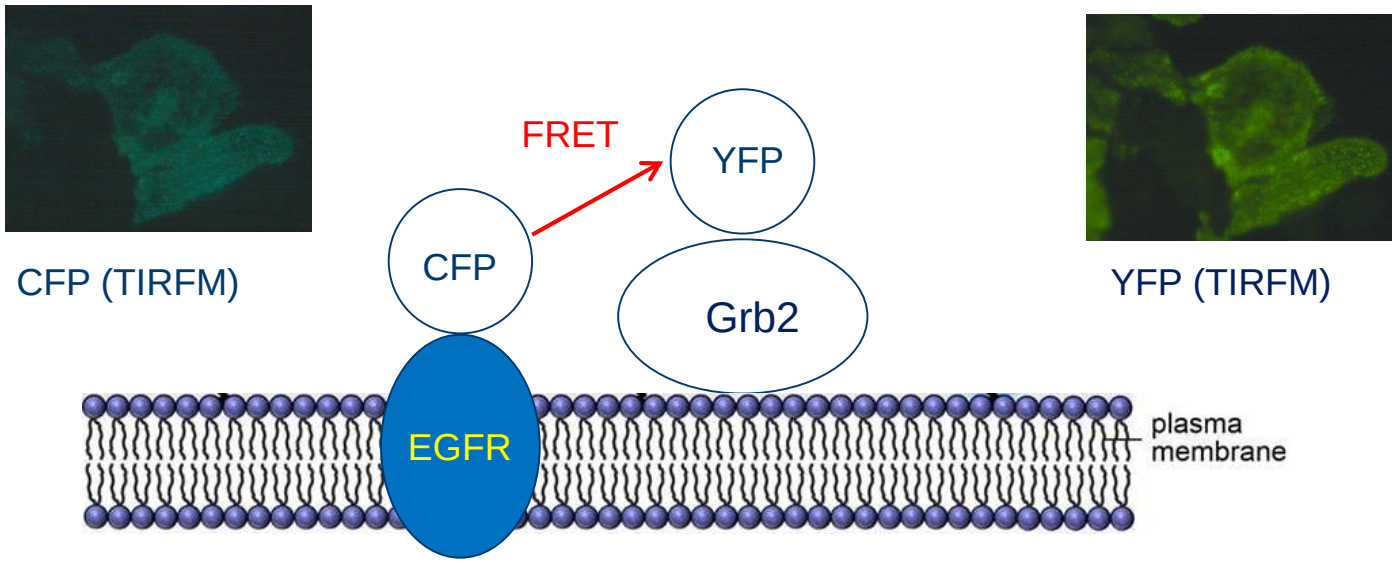
Reduced energy transfer from light harvesting complex to reaction centre of PS II in declined needles → prolonged fluorescence decay

H. Schneckenburger and W. Schmidt: Time-resolved chlorphyll fluorescene of spruce needles after different light exposure. J.Plant Physiol. 148 (1996) 593-598.

Milestone (1993 ff.): Fluorescent Proteins *(Aequorea victoria)*

R. Heim, R.Y. Tsien: Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer, Curr. Biol. 6 (1996) 178-182.

Subsequent applications, e.g.



A. Sorkin, M. McClure, F. Huang, R. Carter: “Interaction of EGF receptor and Grb2 in living cells visualized by fluorescence resonance energy transfer (FRET) microscopy”, Curr. Biol. 10 (2000) 1395–1398.

Milestone (1995 ff.): Single Molecule FRET

Detection of single molecules requires
- excitation of thin layers (TIRFM, Confocal LSM)
- ultra-sensitive low noise detection (e.g. EMCCD cameras[1])
- special single molecule techniques (blinking, correlation, antibunching)

[1]C.G. Coates, D.J. Denvir, N.G. McHale, K.D.Zhornbury, M.A. Hollywood: Optimizing low-light microscopy with back-illuminated electron multiplying charge-coupled device: enhanced sensitivity, speed and resolution, I. Biomed. Opt. 9 (2004) 1244-1252.

Applications include
- DNA and RNA structures
- Protein dynamics, e.g. protein folding, molecular motors
- Membrane dynamics

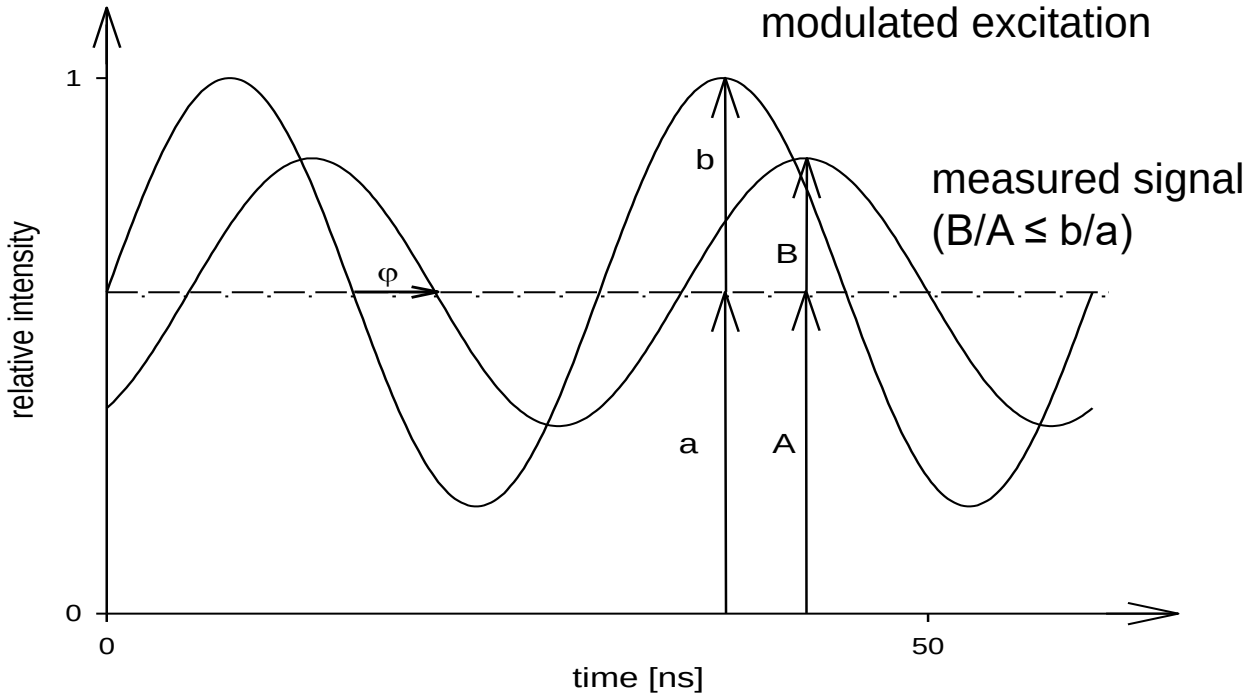
More than 90% of single molecule FRET applications are intensity based, not lifetime based.

Milestone (1993 ff.): Time-resolved FRET Imaging

Phase fluorometry
(Lakowicz et al.; Jovin & Clegg et al.)

$\tan \varphi = \omega \tau_p$

$m = [1 + \omega^2 \tau_m^2]^{-1/2}$



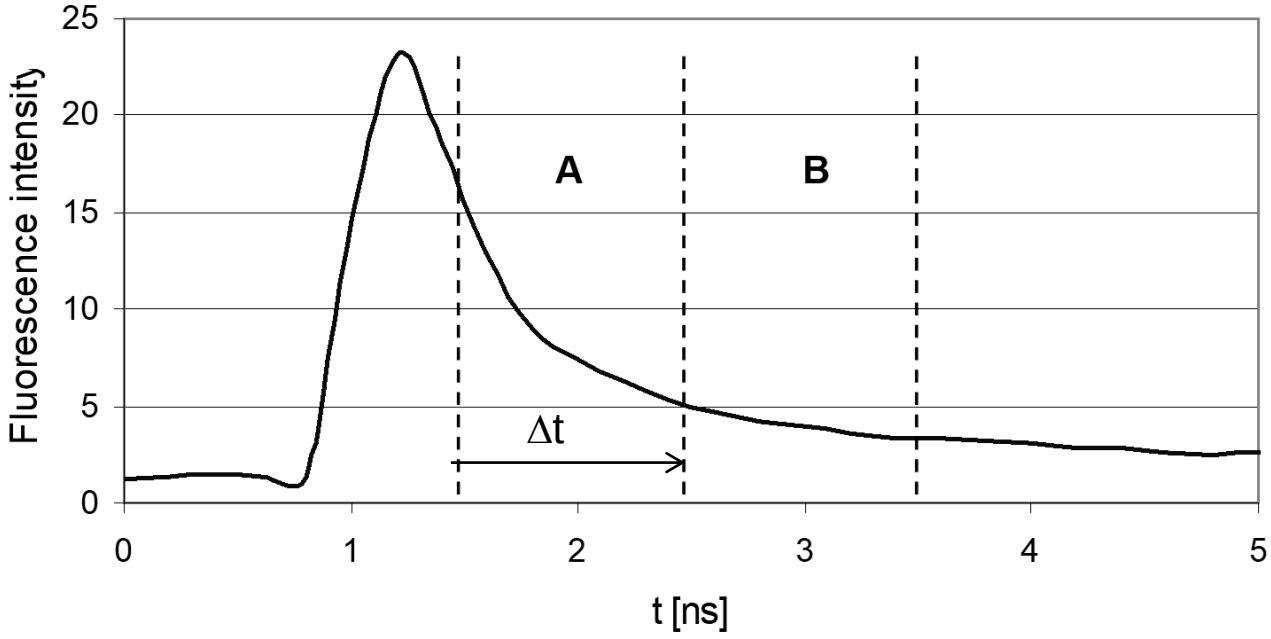
Milestone (1993 ff.): Time-resolved FRET Imaging

Laser scanning microscopy
(Buurman & Gerritsen et al.,)

$I(t) = \Sigma A_i e^{-t/\tau i}$

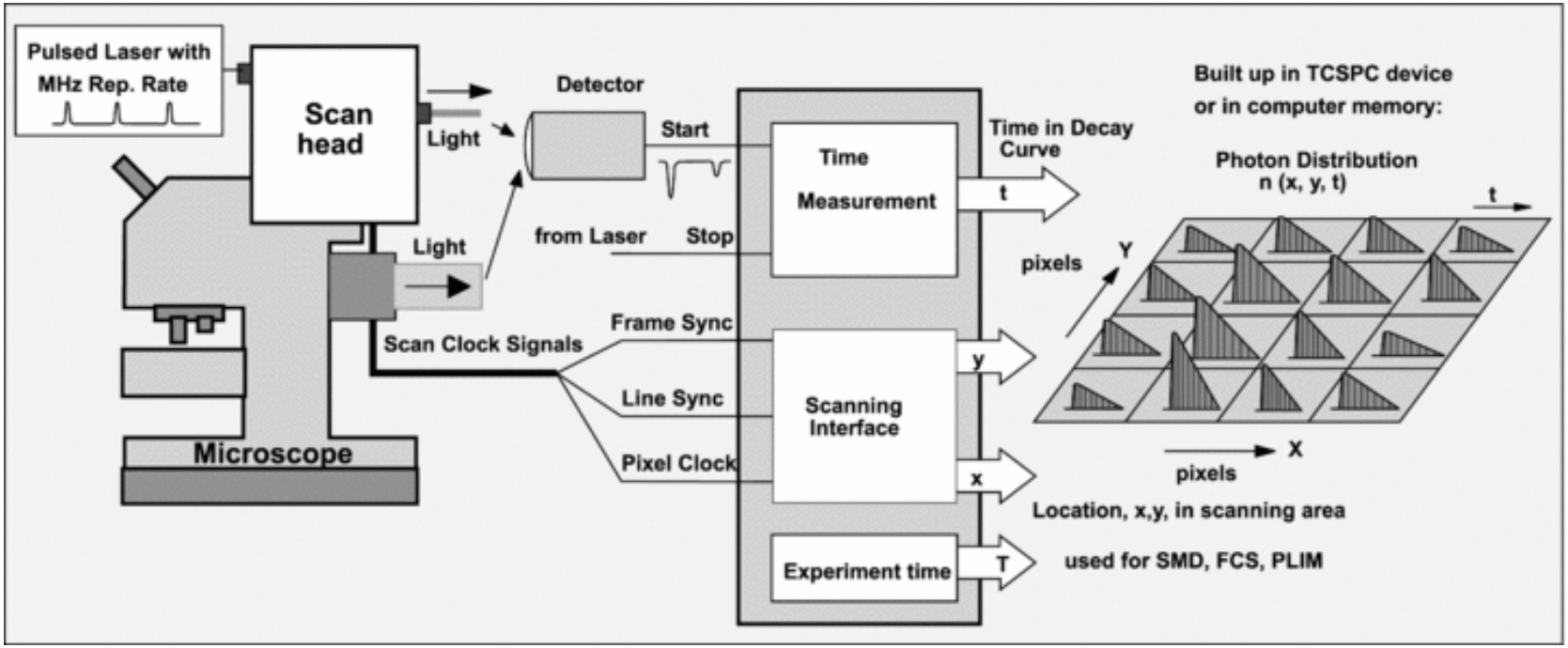
$\tau_{eff} = \Delta t / \ln (I_A/I_B)$

Fast camera technology
(Lakowicz et al., French et al., Herman et al., Biskup et al., Schneckenburger et al.)



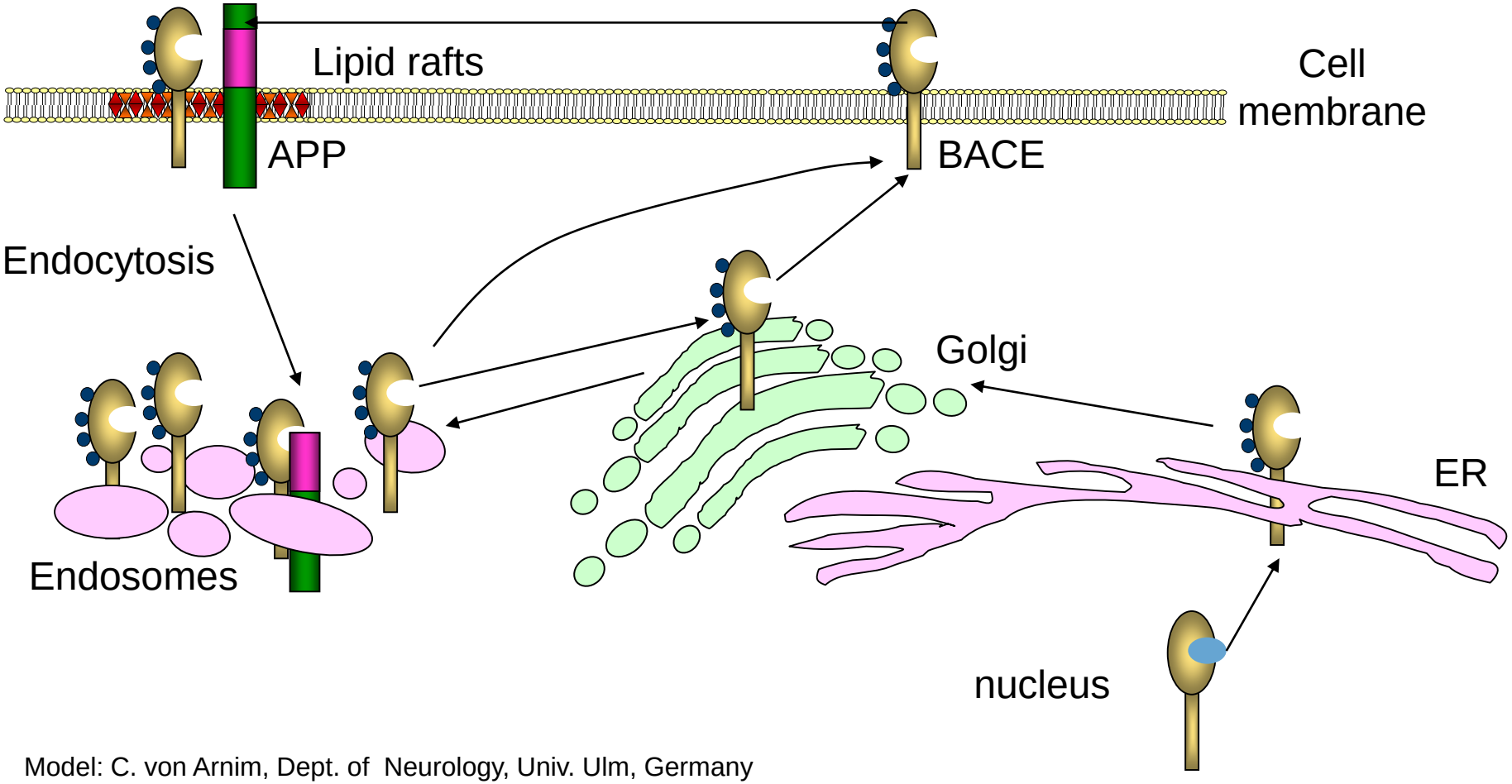
Milestone (2003 ff.): Time-correlated Single Photon Counting

Wahl et al. (2004); Biskup et al. (2004), Ameer-Berg et al. (2004), Suhling et al. (2005), Duncan (2006), Bereiter-Hahn et al. (2007), Gratton et al. (2008), French et al. (2010)



W. Becker: Fluorescence lifetime imaging – techniques and applications, J. Microsc. 247 (2012) 119-136

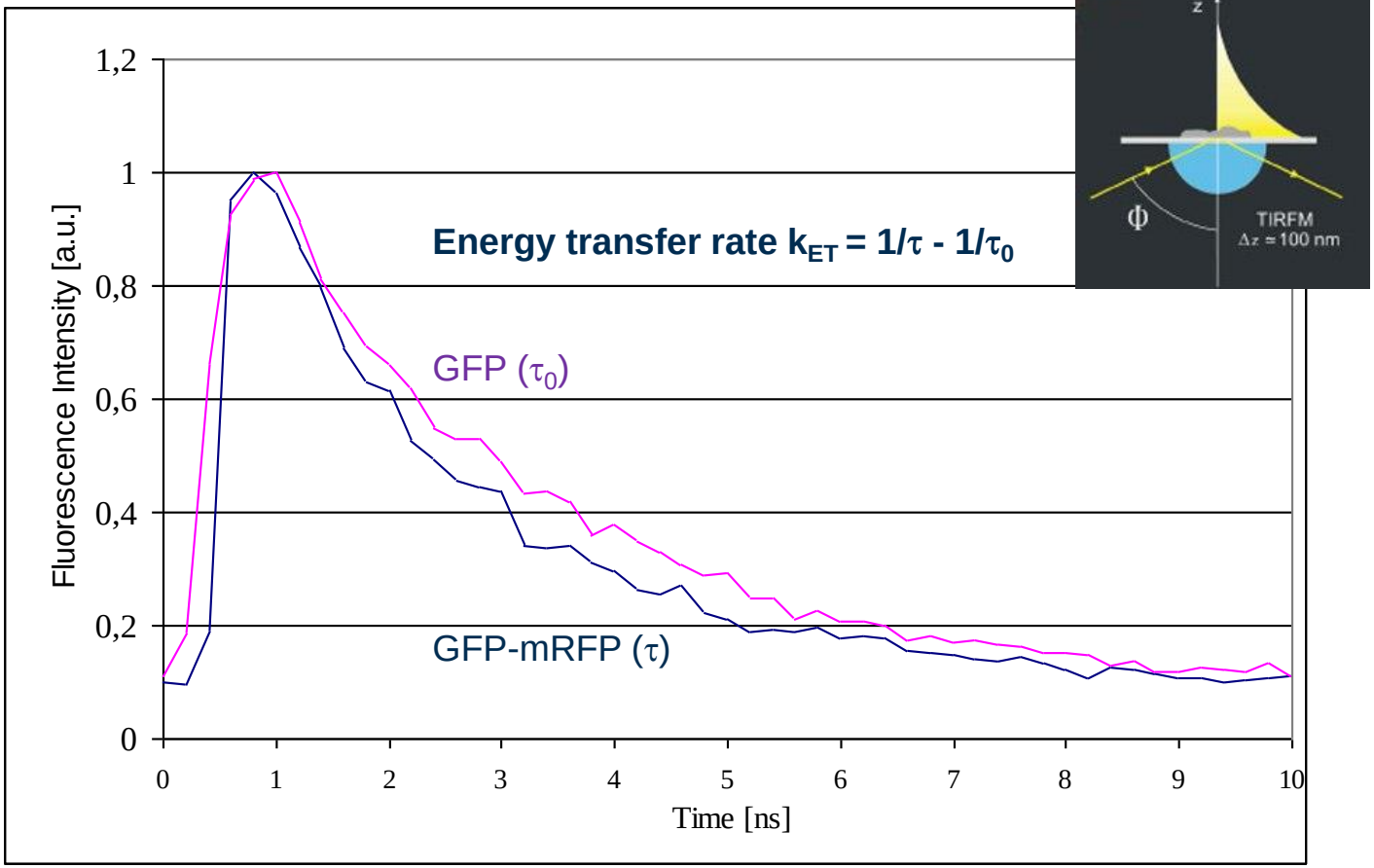
Alzheimer Research: BACE Trafficking (2006 – 2012)



Model: C. von Arnim, Dept. of Neurology, Univ. Ulm, Germany

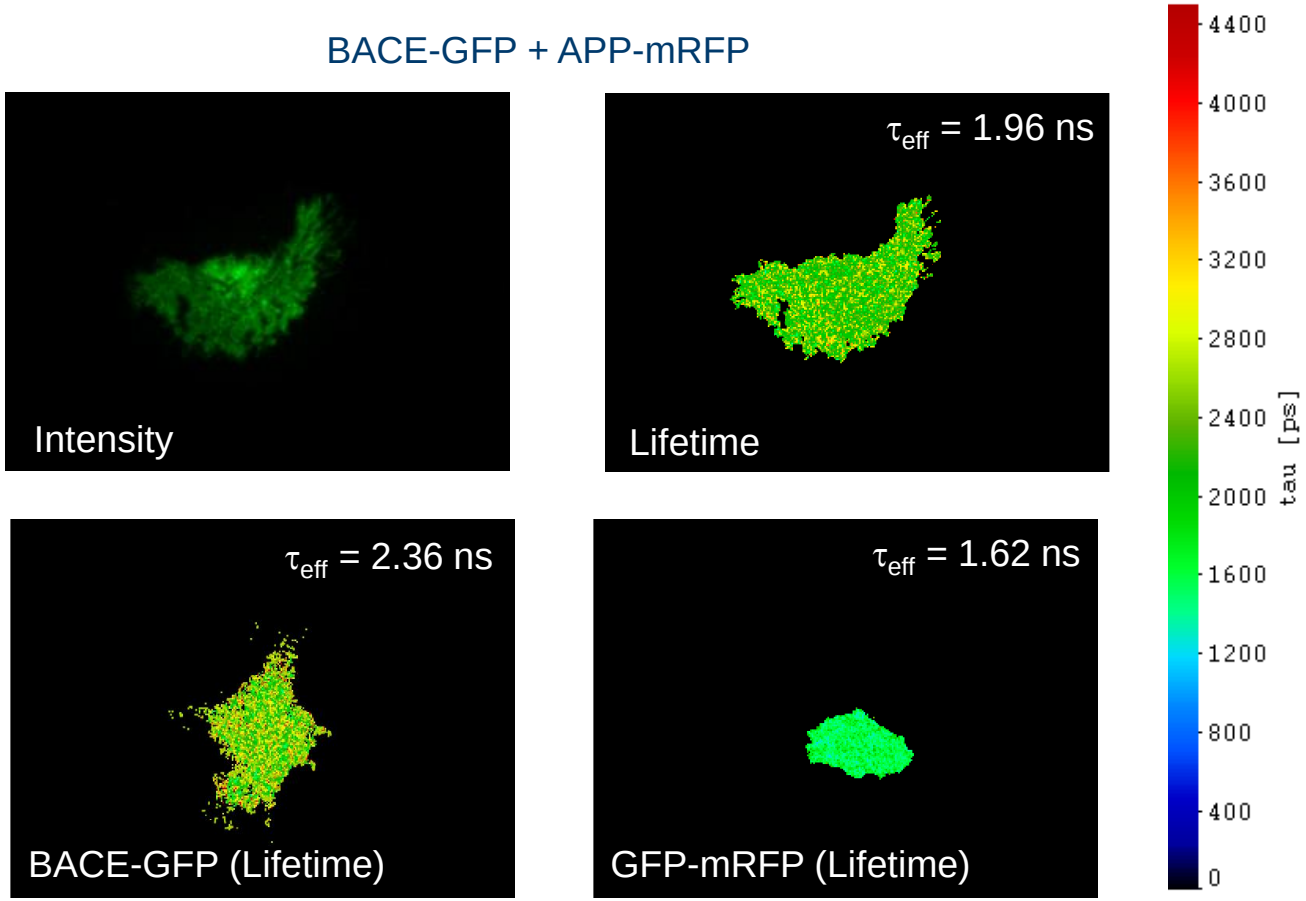
U373 MG Glioblastoma Cells: TIR-FRET Microscopy

Bace-GFP → APP-mRFP: Fluorescence Decay Kinetics of the Host



U373-MG Glioblastoma Cells: TIR-FRET Microscopy

BACE-GFP → APP-mRFP: Effective Fluorescence Lifetimes

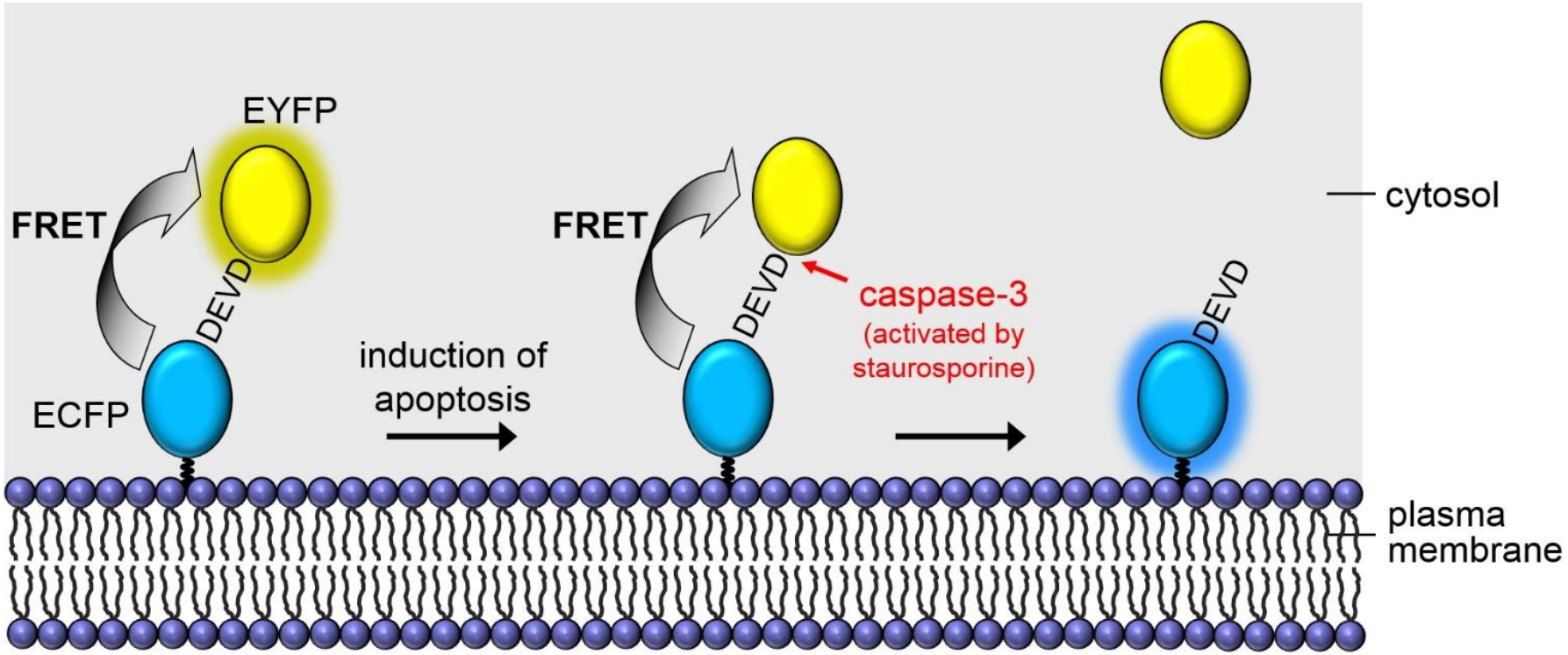


C.A.F. von Arnim, B. von Einem, P. Weber, M. Wagner, D. Schwanzar, R. Spoelgen, W.S.L. Strauss, H. Schneckenburger: “Impact of cholesterol level upon APP and BACE proximity and APP cleavage“, Biochem. Biophys. Res. Commun. 370 (2008) 207 − 212.

Results on Molecular Interactions in Alzheimer Research

- Interactions of amyloid precurser protein (APP) and ß-secretase (BACE) favour the formation of amyloid peptides;
- Interactions are significant in the cytoplasm and occasional in the plasma membrane;
- Interactions depend on the intracellular amount of cholesterol;
- Low density receptor-related protein (LRP) may serve as a competitive substrate of APP for BACE (von Einem et al., Exp. Neurol. 225 (2010) 85–93).
- Cholesterol-dependent energy transfer BACE-GFP → APP-mRFP also occurs in membranes of Niemann-Pick Type C disease cells, (von Einem et al., Int. J. Mol. Sci. 13 (2012) 15801-15812).

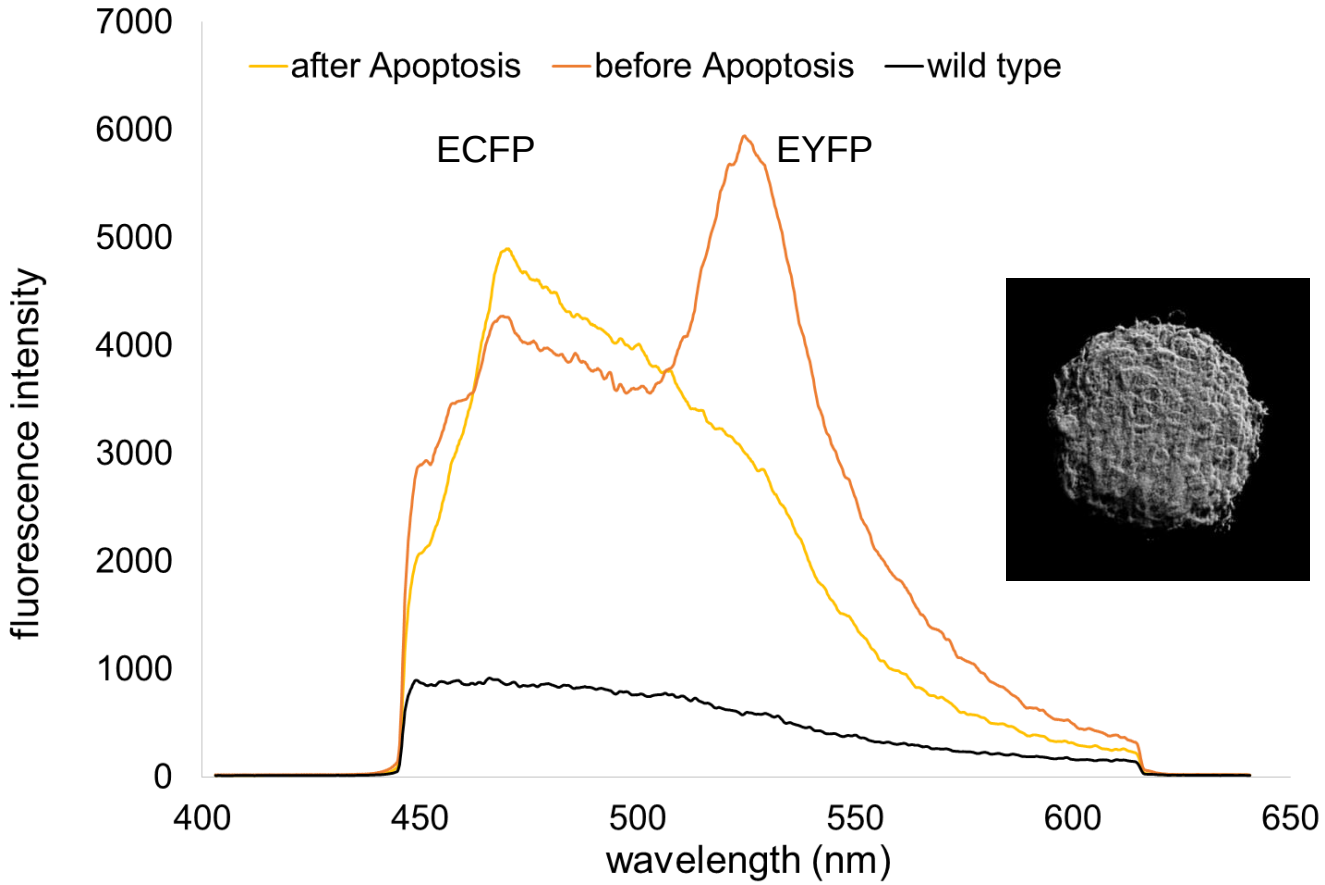
FRET-Based Sensor for Apoptosis (2008 – present)
Cleaving of a Membrane Associated ECFP – EYFP Fusion Protein



B. Angres, H. Steuer, P. Weber, M. Wagner, H. Schneckenburger: A membrane-bound FRET-based caspase sensor for detection of apoptosis using fluorescence lifetime and total internal reflection microscopy, Cytometry 75A (2009) 420–427

3D Multicellular Spheroids: FRET-Based Sensor for Apoptosis

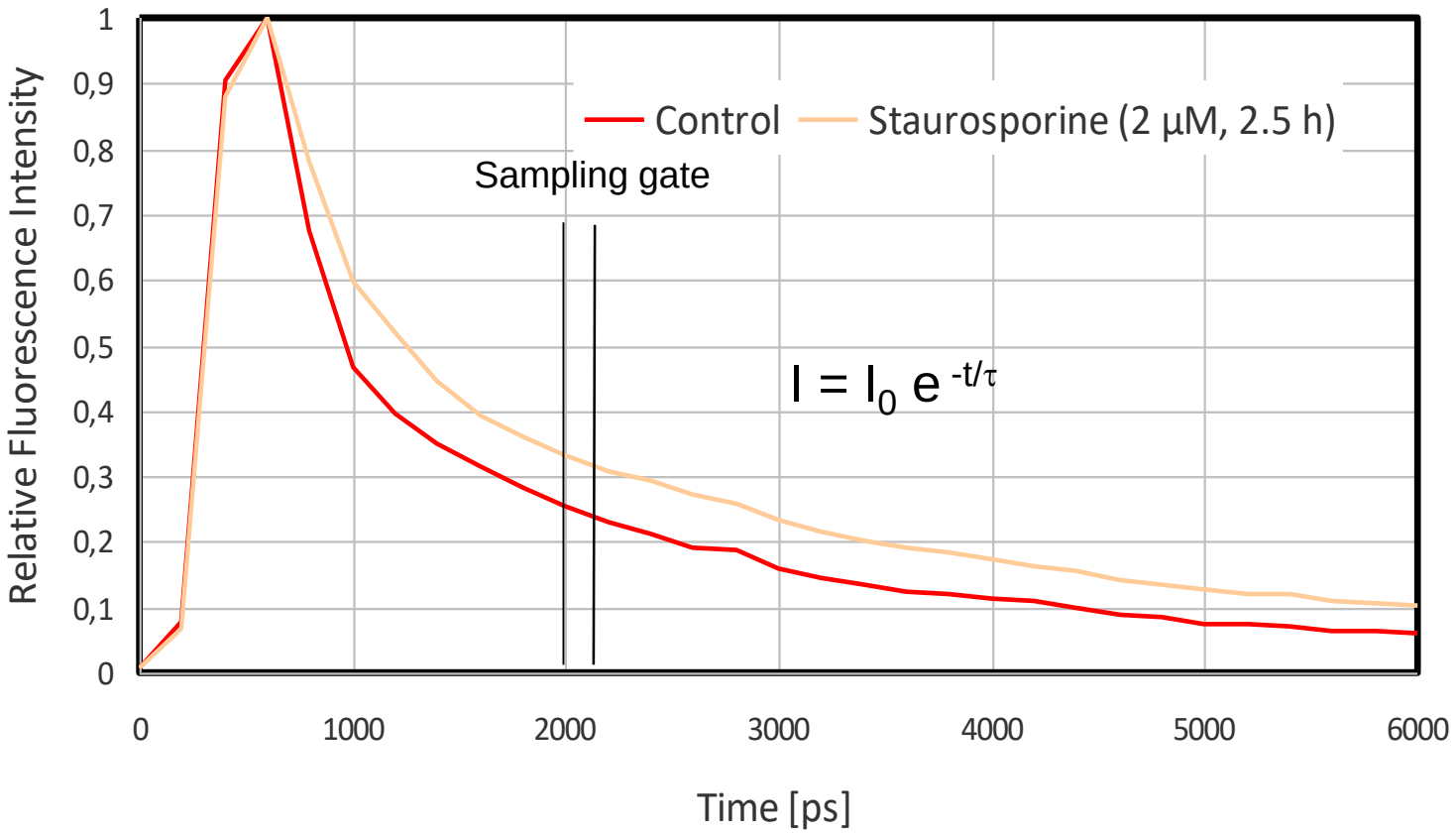
- Fluor. spectra prior to and subsequent to apoptosis; λ_{ex} = 391 nm -



P. Weber, S. Schickinger, M. Wagner, B. Angres, T. Bruns, H. Schneckenburger: Monitoring of apoptosis in 3d cell cultures by FRET and light sheet fluorescence microscopy, Int. J. Mol. Sci. 16(3) (2015) 5375–5385

3D Multicellular Spheroids: FRET-Based Sensor for Apoptosis

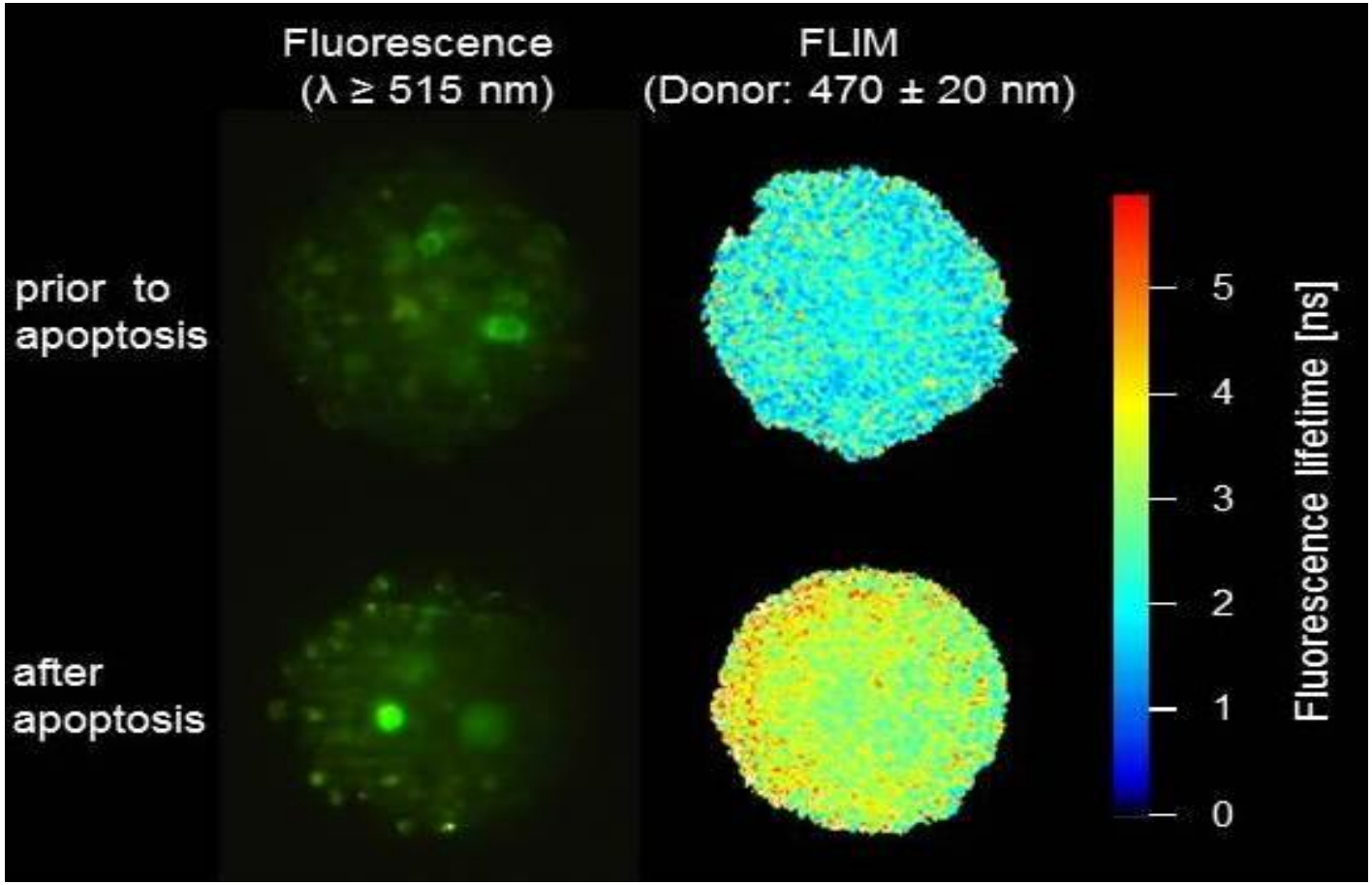
- Fluorescence decay kinetics of the donor ECFP; λ_{ex} = 391 nm -



Energy transfer rate: $k_{ET} = 1/\tau - 1/\tau_0$

3D Multicellular Spheroids: FRET-Based Sensor for Apoptosis

- Light sheet microscopy / FLIM of ECFP in HeLa Cells; λ_{ex} = 391 nm -

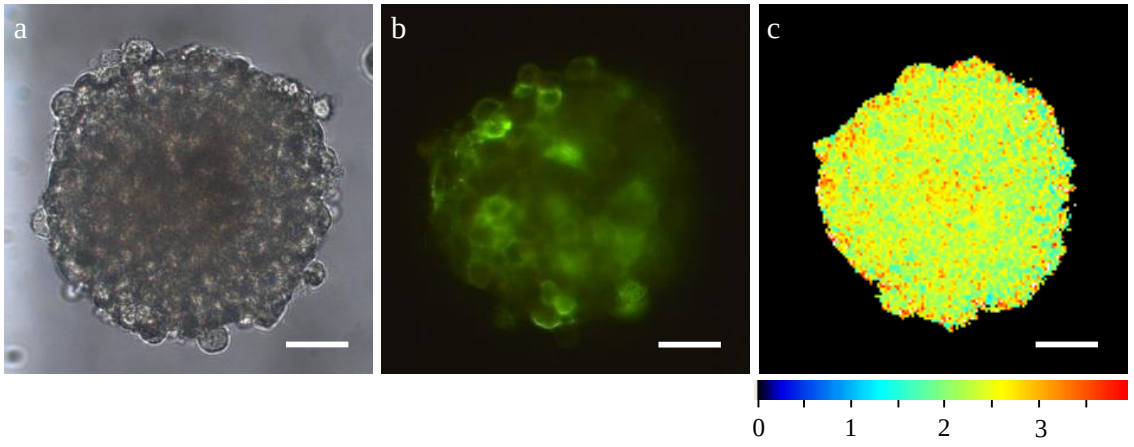


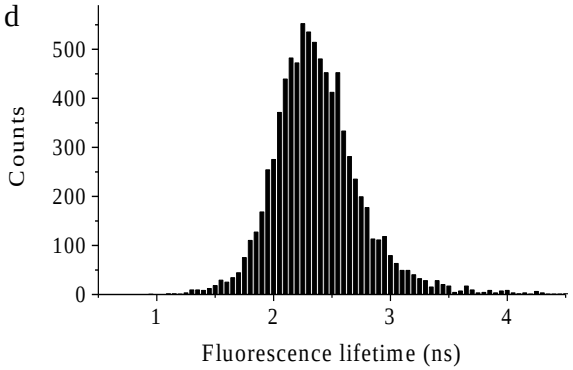
Fluorescence (Lifetime) Imaging after Application of PMA

- Mem-ECFP-DEVD-EYFP (cleavable) vs. Mem-ECFP-DEVG-EYFP (non-cleav.) -

A

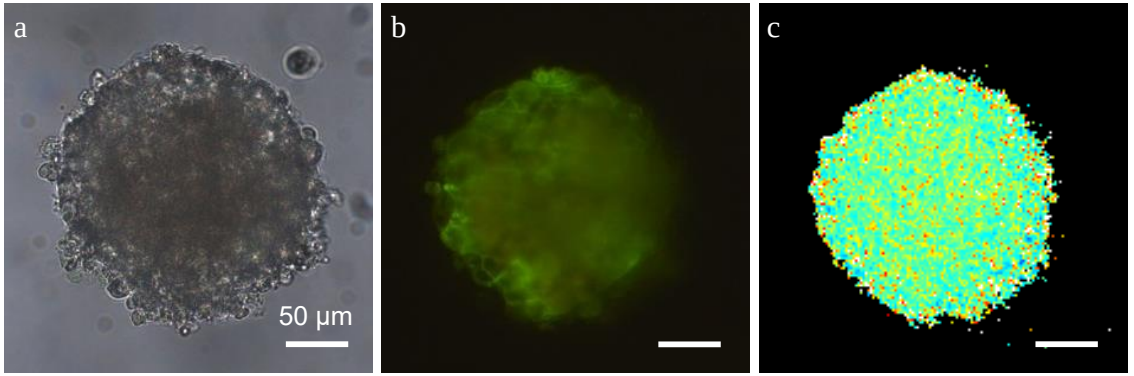
Hela-Mem-ECFP-DEVD-EYFP + PMA (250 nM, 48 h)

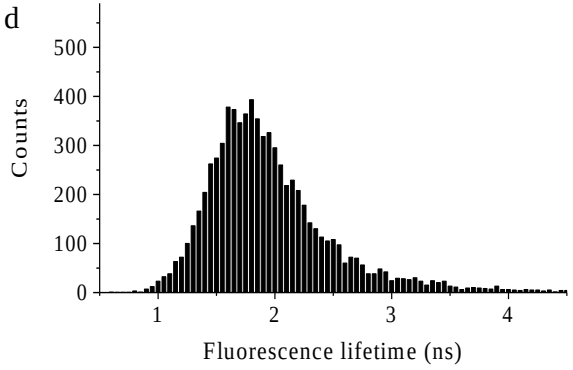




B

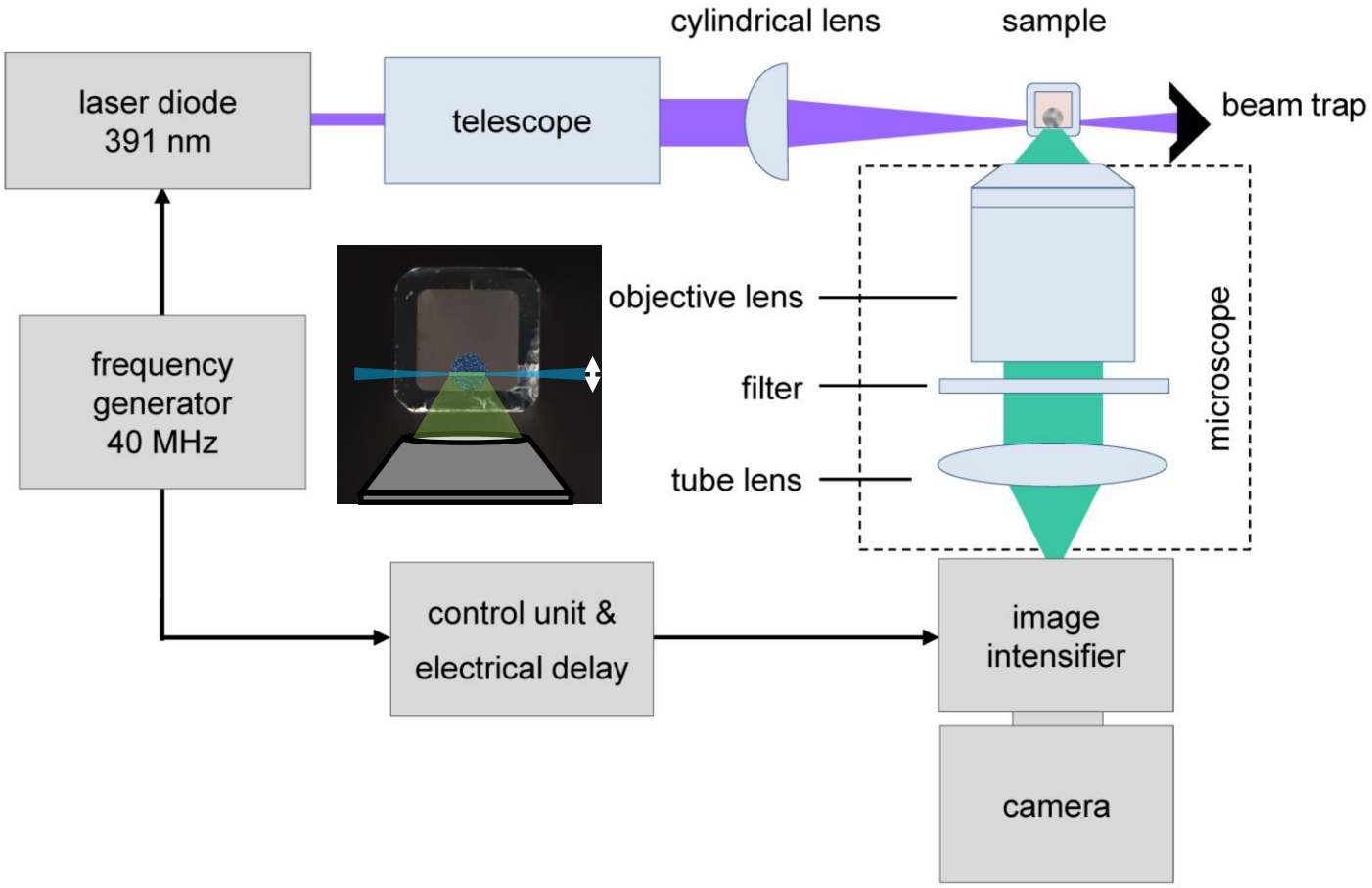
Hela-Mem-ECFP-DEVG-EYFP + PMA (250 nM, 48 h)



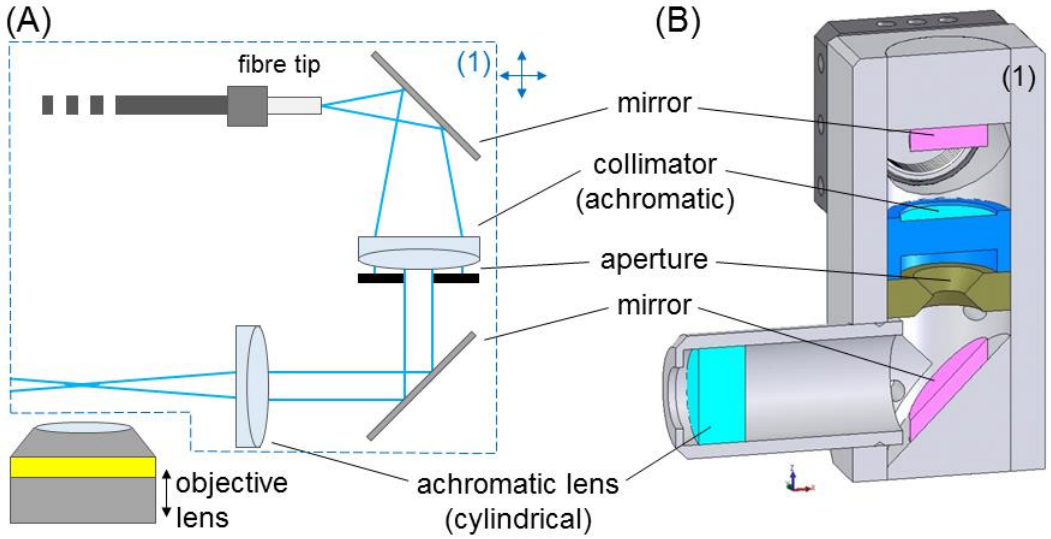


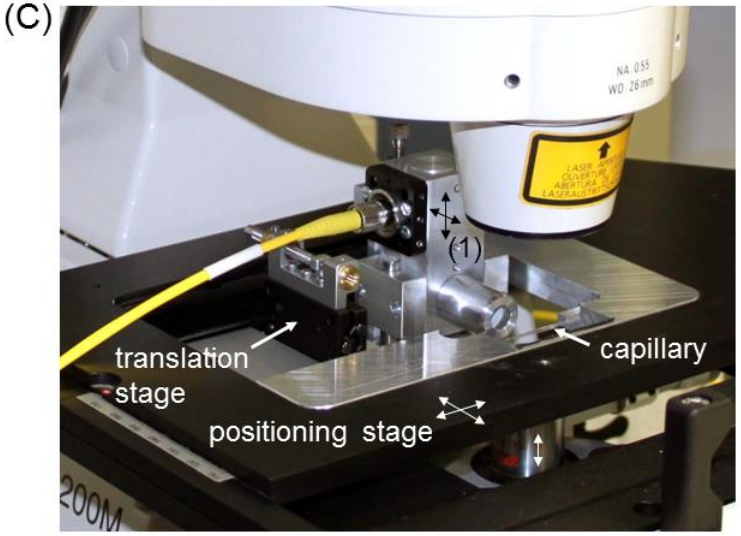
Transillumination EYFP fluorescence ECFP lifetime Lifetime histograms

Light Sheet Based Fluorescence Lifetime Imaging Microscopy (LS-FLIM) – Application for 3D FRET

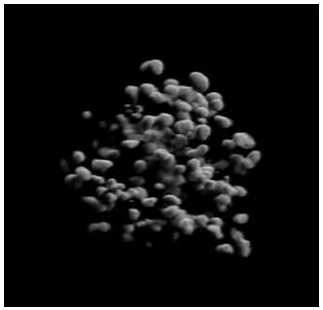


Miniaturized Light Sheet Module for Fluorescence Microscopy





- Light sheet created by achromatic cylindrical lens (f‘ = 25 mm)
- Beam waist: 6 µm
- Focal depth: ≤ 200 µm
- Simultaneous shift of light sheet and detection lens for 3D imaging
- Very low light exposure



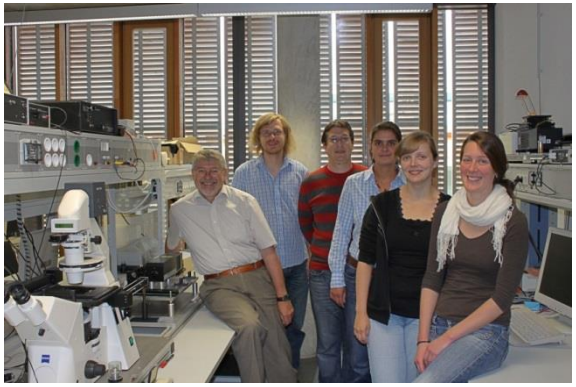
T. Bruns, M. Bauer, S. Bruns, H. Meyer, D. Kubin, H. Schneckenburger: “Miniaturized modules for light sheet microscopy with low chromatic aberration”, J. Microsc. 264(3) (2016) 261-267.

Milestones of FRET Imaging

- 2.4 billion years ago: FRET in photosynthesis
- 1948: FRET described theoretically by T. Förster
- 1978: First biological applications
- 1993: First applications of fluorescent proteins
- 1993 ff: Time-resolving FRET imaging
- 1995 ff: Single molecule FRET
- 2010 ff: FRET in 3D cell (or tissue) systems

Challenges of FRET Imaging

- Distinguish FRET from competitive processes
- Separate donor and acceptor fluorescence spectrally
- Avoid cross talk (e.g. direct excitation of the acceptor)
- Avoid high light exposure in 3D imaging (confocal, structured illumination)



Aalen University, Biophotonics group

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