Light Exposure in Microscopy – How can Cell Survival be Increased?

Herbert Schneckenburger, Sarah Schickinger, Petra Weber,
Michael Wagner, and Thomas Bruns

Aalen University, Institute of Applied Research,
73428 Aalen, Germany

Fluorescence Microscopy of Living Cells

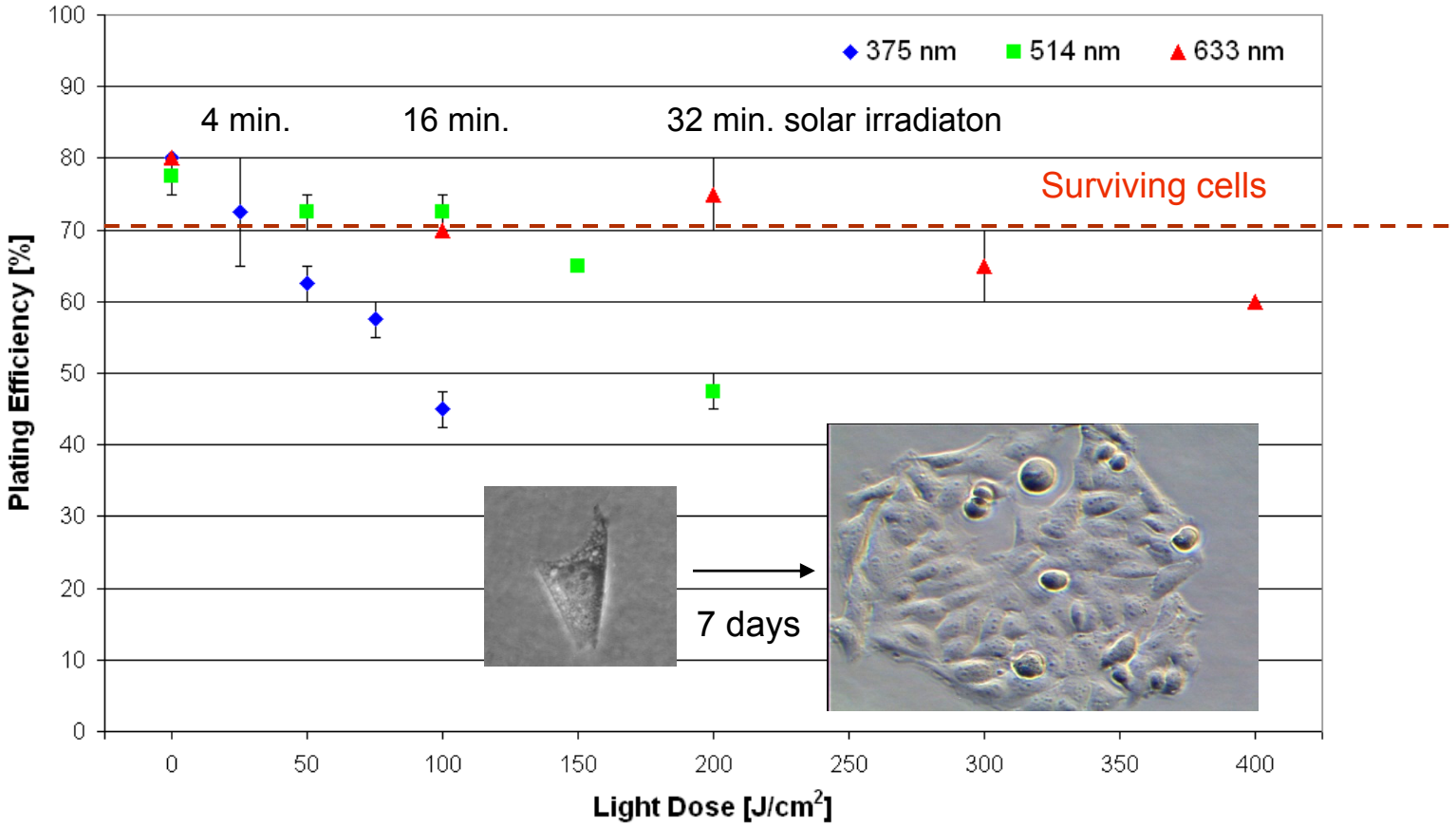
- How much light do we need for microscopy and how much light can we apply to living cells?

- Can we use or even exceed solar irradiance?

- How long will cells endure this irradiation?

Solar irradiance:
1 kW/m² = 100 mW/cm² = 1 mW/mm² = 1 nW/µm²
1 J/cm² = 10 s of solar irradiation

Viability of U373-MG Glioblastoma Cells upon Irradiation
(colony formation assay; native cells)

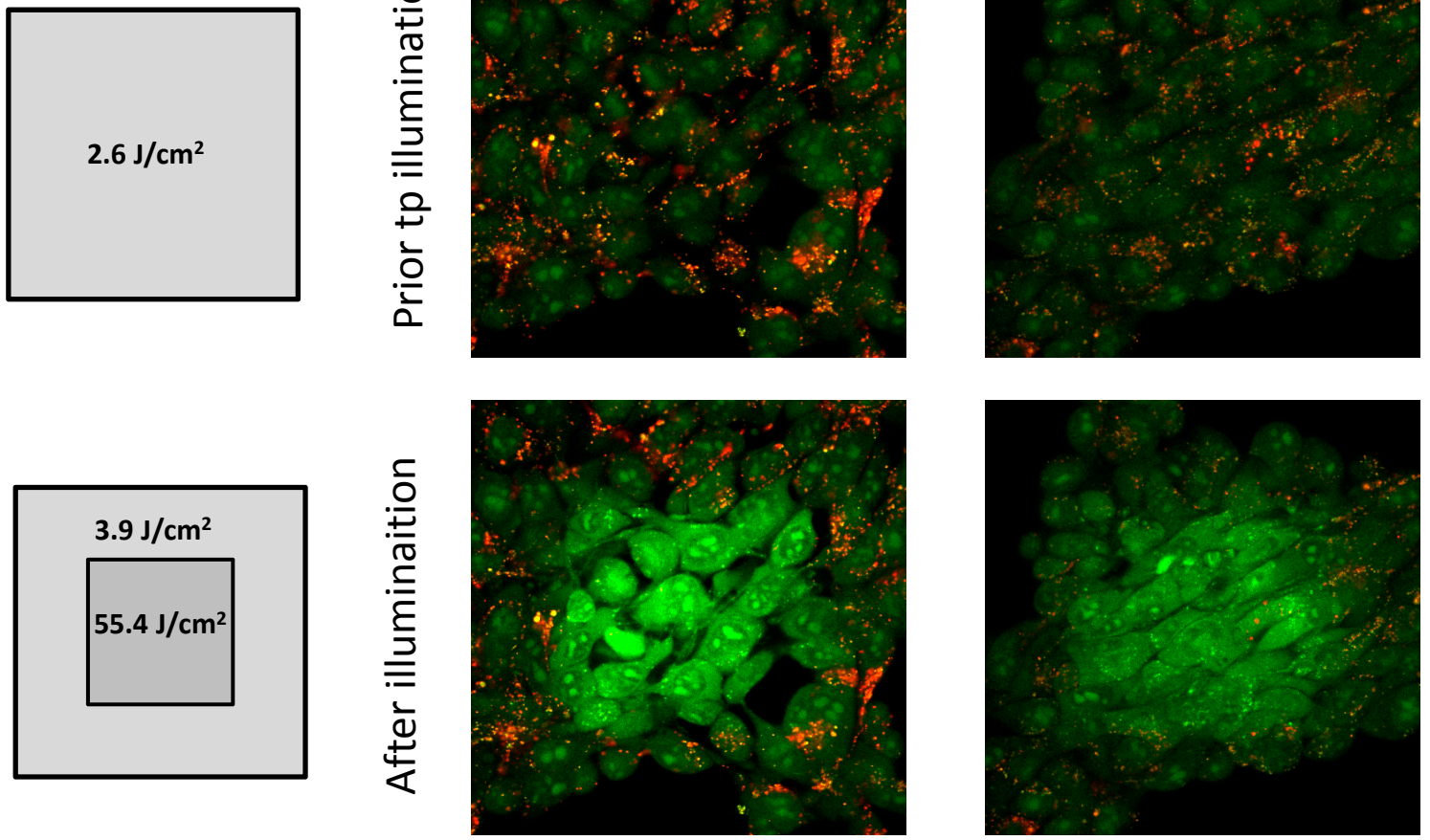


Cell Viability upon Irradiation

- native cells and fluorescent markers -

Cell line	Marker	Conc. [µM]	λ_{ex} [nm]	Max. light dose [J/cm²]	Solar exposure time [s]
U373-MG	–		375	25	250
U373-MG	–		514	100	1000
U373-MG	–		633	200	2000
U373-MG	Laurdan	8	391	10	100
CHO-K1	DiA	5	488	10	100
CHO-K1	DiO	5	488	10	100
CHO-K1	GFP-Mem		488	10	100
CHO-K1	R 123	5	488	5–20	50–200
CHO-K1	MTO	0.05	514	50	500
CHO-K1	GFP-Mito		488	5	50

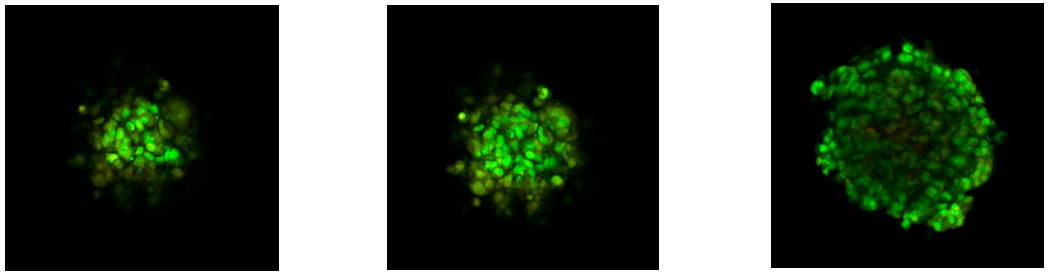
Example: 3T3 Fibroblasts + Acridine orange (5 µM, 30 min.)



Fluorescence Microscopy with Axial Resolution

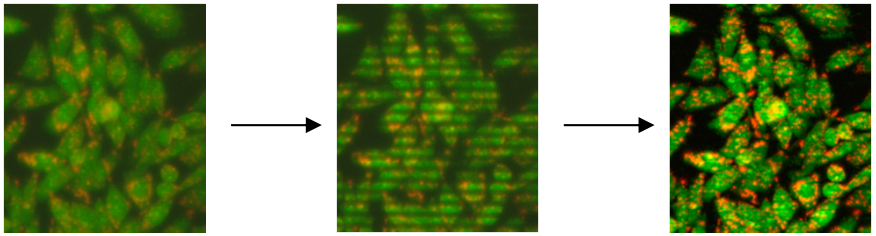
Methods:

- Laser Scanning Microscopy (LSM)

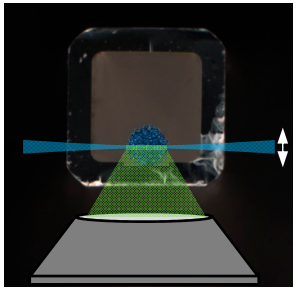


z = 10 µm z = 15 µm z = 60 µm

- Structured Illumination Microscopy (SIM)

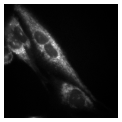
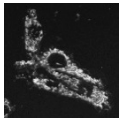
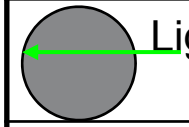


- Light Sheet Fluorescence Microscopy (LSFM)



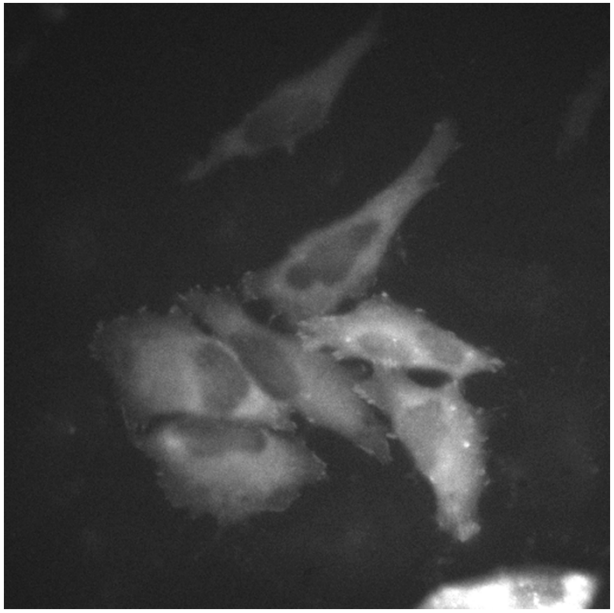
Only planes under investigation are illuminated
→ minimum light exposure

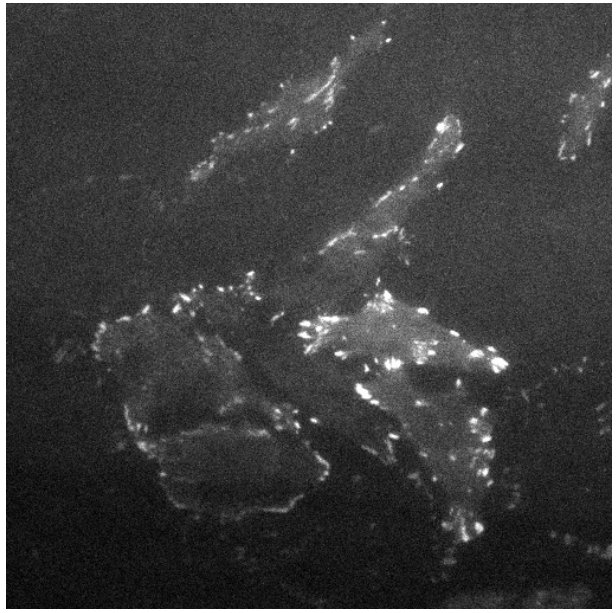
Maximum Light Exposure to Living Cells in 3D Microscopy

Method	Experiment	Max.light dose [nJ/μm²]	Irradiance [nW/μm²]	Max. no. of images
 Widefield microscopy	Autofluor.	250	1	250
 LSM	Fluor. marker	100	1	20
 Light Sheet (N layers)	Fluor. marker	100	1	N × 100
 TIRFM	Fluor. marker	300	1	100–300
Single Molecule Methods	Fluor. Marker (low conc.)	2,000	500	≤ 1
STED (650 nm)	Fluor. Marker	100–500	30,000	not relevant

Total Internal Reflection Fluorescence Microscopy (TIRFM)

Membrane Associated Paxilline (Pax-EYFP) / Focal Adhesions

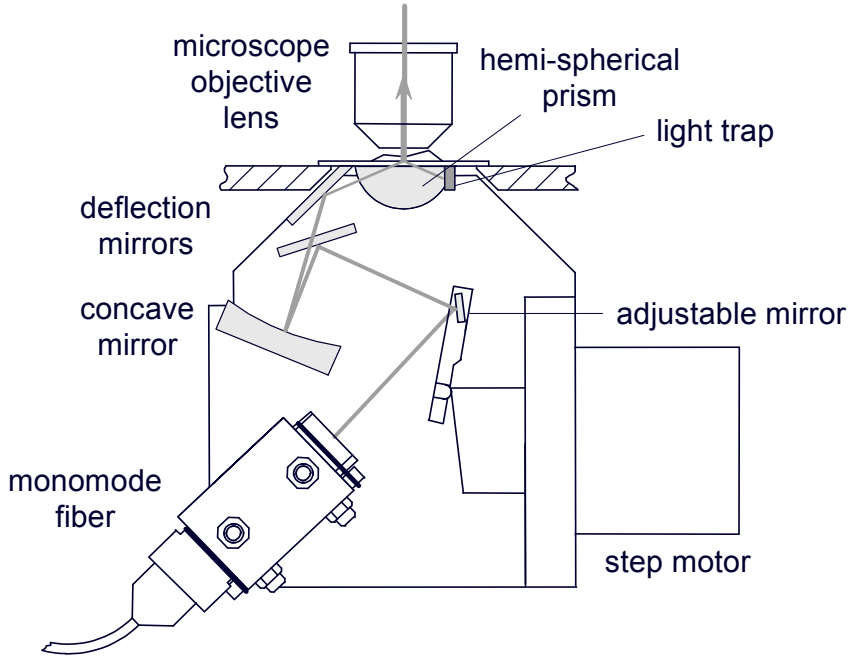


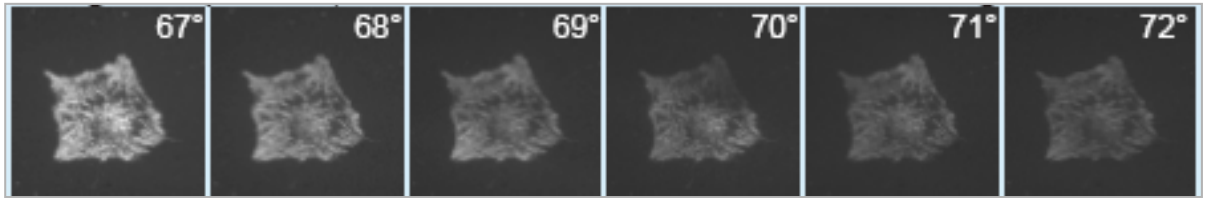


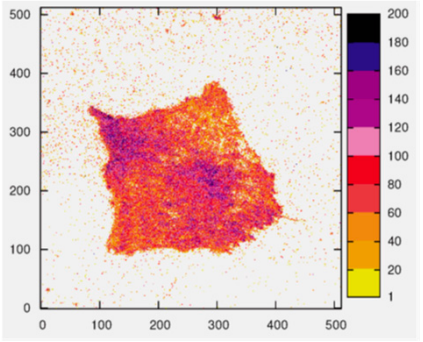
Conventional fluorescence microscopy TIRFM

λ_{ex} = 470 nm; $\lambda_d \geq$ 530 nm

Variable-angle Total Internal Reflection Fluorescence Microscopy (TIRFM)





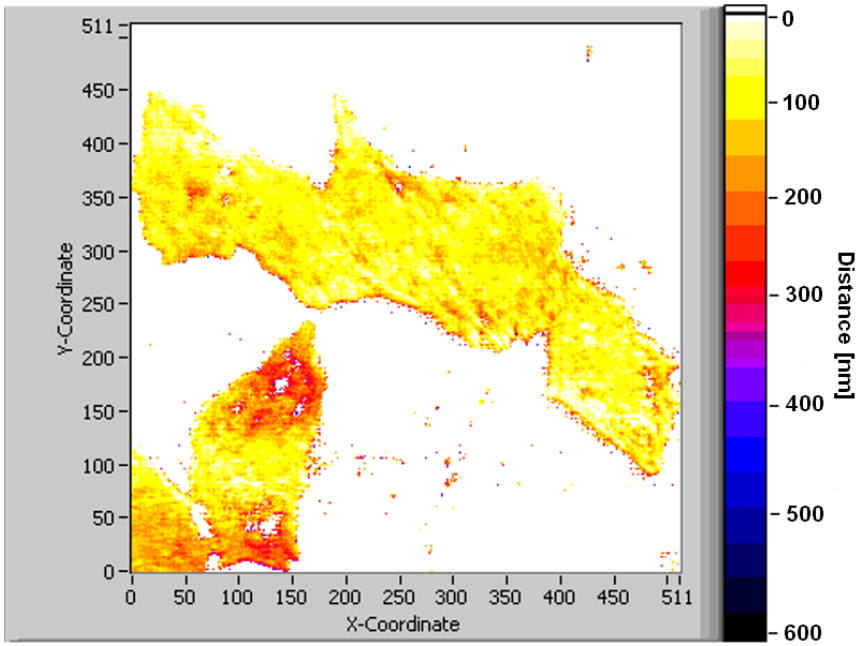


Δ (nm)

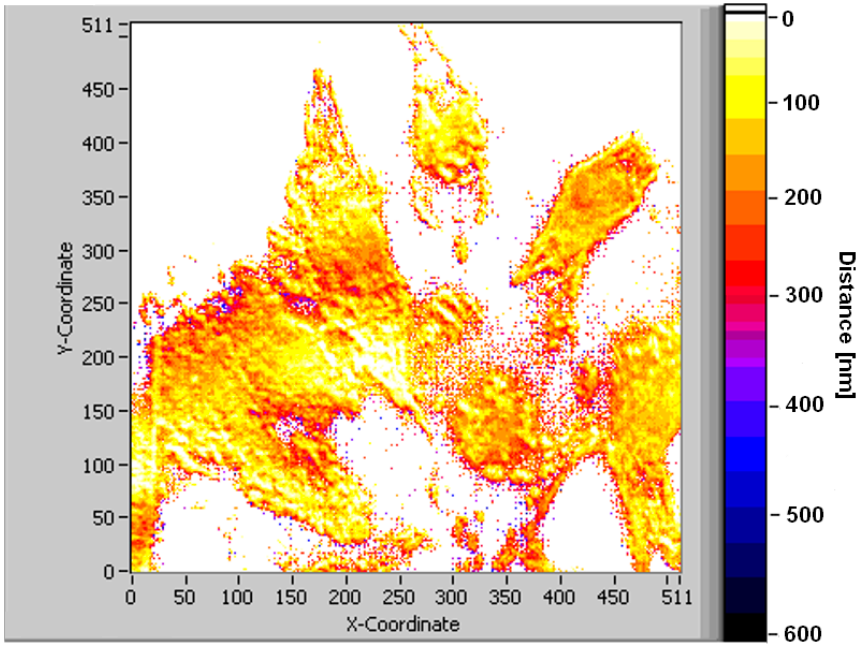
Hochschule Aalen

Nanometre Cell-Substrate Topology of Glioblastoma Cells

- using the fluorescent membrane marker laurdan -



U251-MG tumour cells

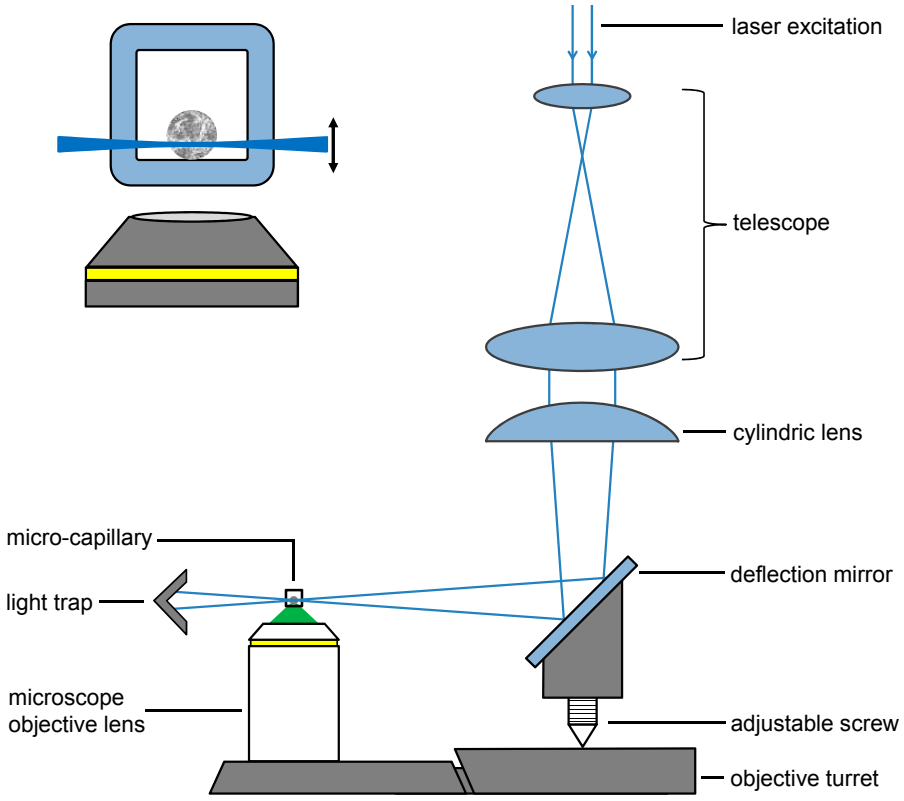


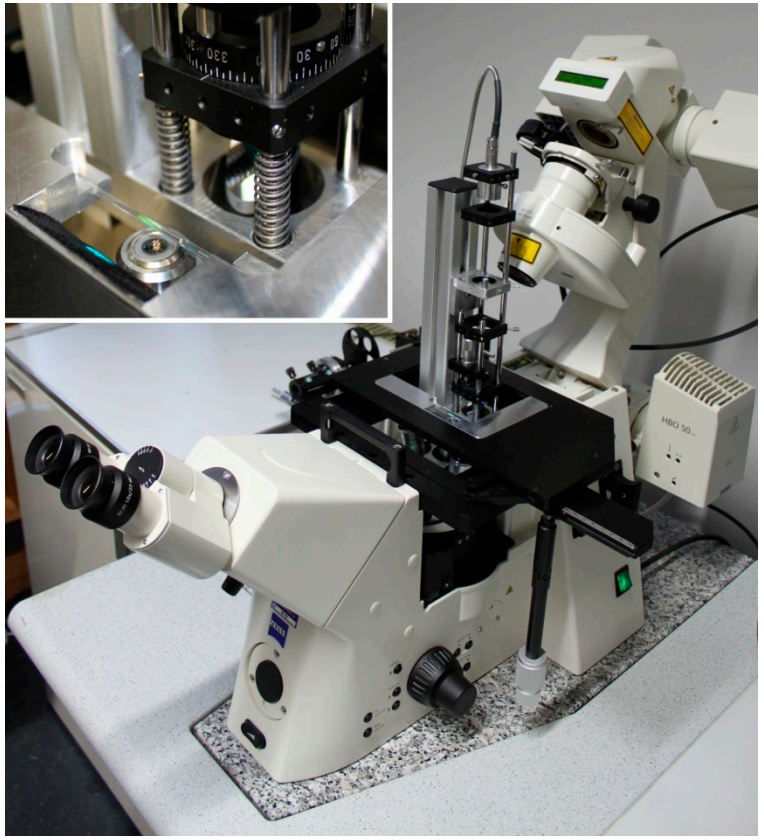
U251-MG with tumour suppressor gene TP53

Cell-substrate topology offers a criterion to distinguish tumour cells and less malignant cells

Cells provided by J. Mollenhauer, Dept. of Molecular Oncology, University of South Denmark, Odense

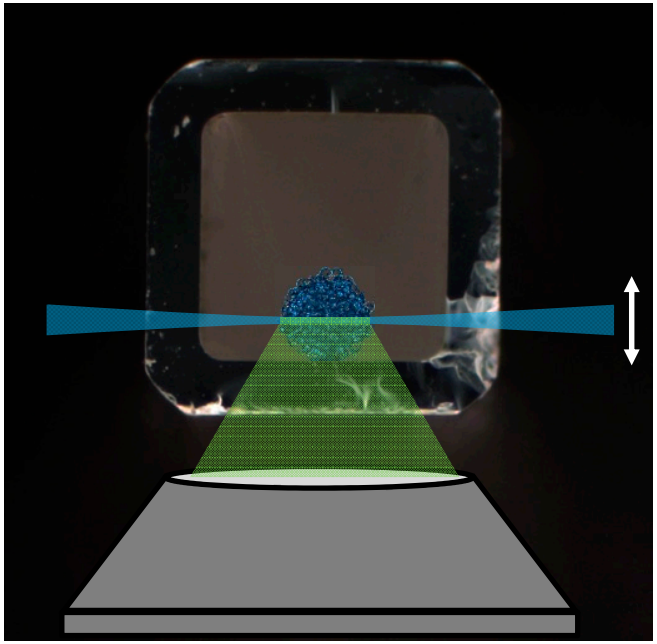
Light Sheet Fluorescence Microscopy (LSFM)

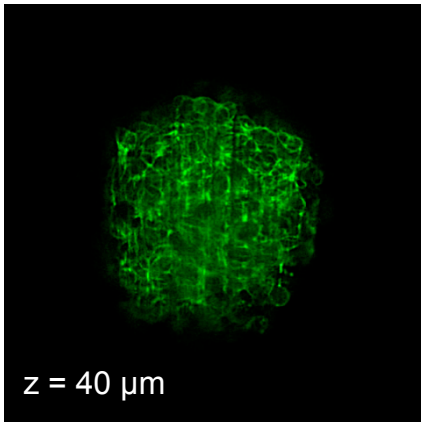


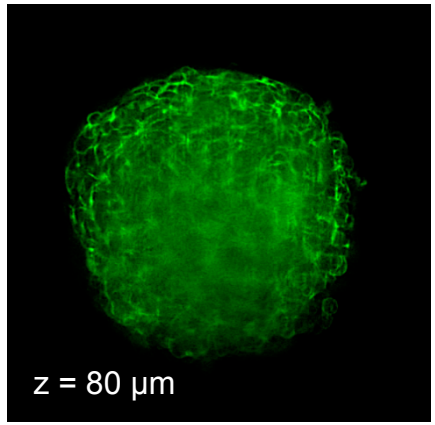


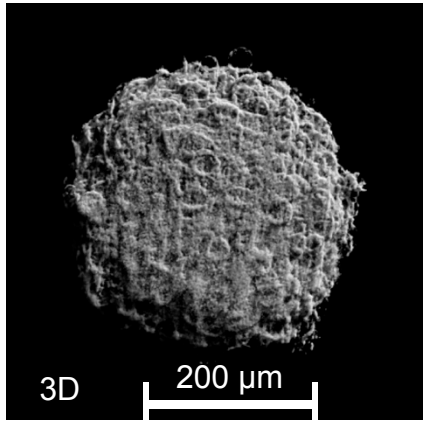
T. Bruns, S. Schickinger, R. Wittig and H. Schneckenburger, "Preparation strategy and illumination of 3D cell cultures in light sheet-based fluorescence microscopy," *J. Biomed. Opt*. 17, 101518 (2012).

Light Sheet Fluorescence Microscopy (LSFM)
(CHO-GFP-Mem)





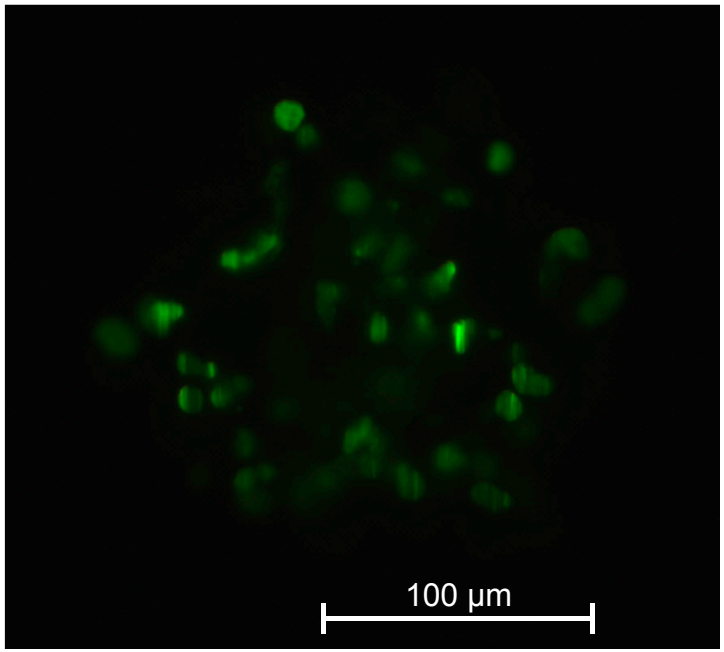




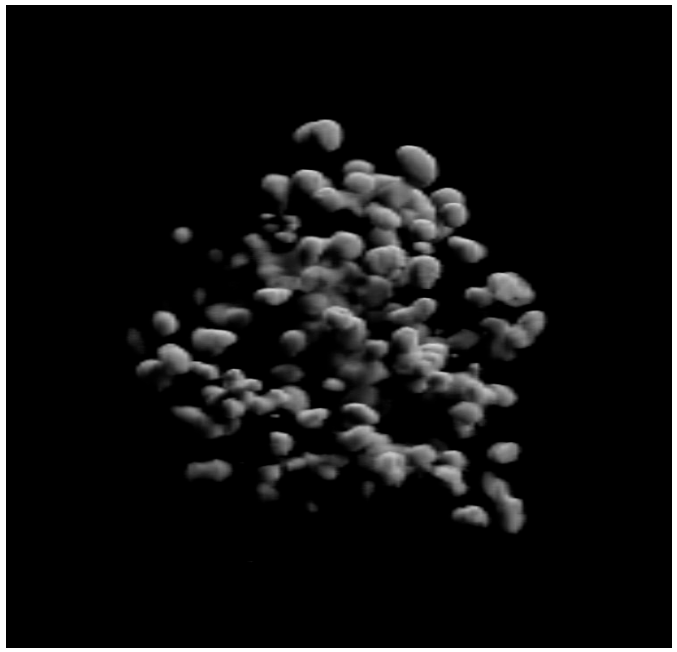
Selected parameters:
Beam waist: $\Delta z = 5–10$ µm
Beam width: $\Delta y \approx 8$ mm
Focal depth: $\Delta x \approx 150–200$ µm

LSFM Application: 3D Imaging of Necrotic Cells

(Rotenone: 1 µM, 3 h; CellTox: 2 h; λ_{ex} = 470 nm, $\lambda_d \geq$ 515 nm)



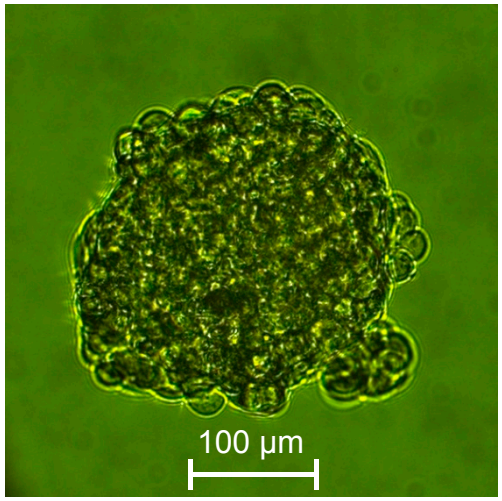
Single Plane: z = 50 µm; d ≤ 10 µm



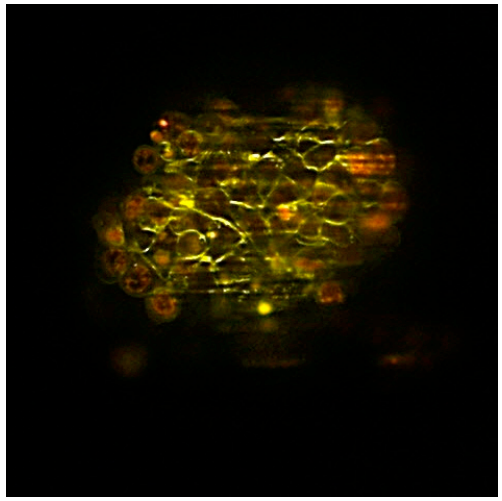
3D Reconstruction

LSFM Application: Uptake / Interaction of a Cytostatic Drug

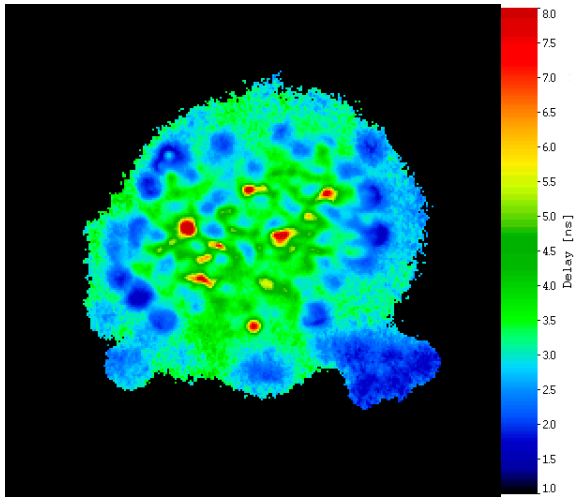
MCF-7 Breast Cancer Cells, Doxorubicin: 8 µM, 6 h, λ_{ex} = 470 nm, $\lambda_d \geq$ 515 nm



Transillumination



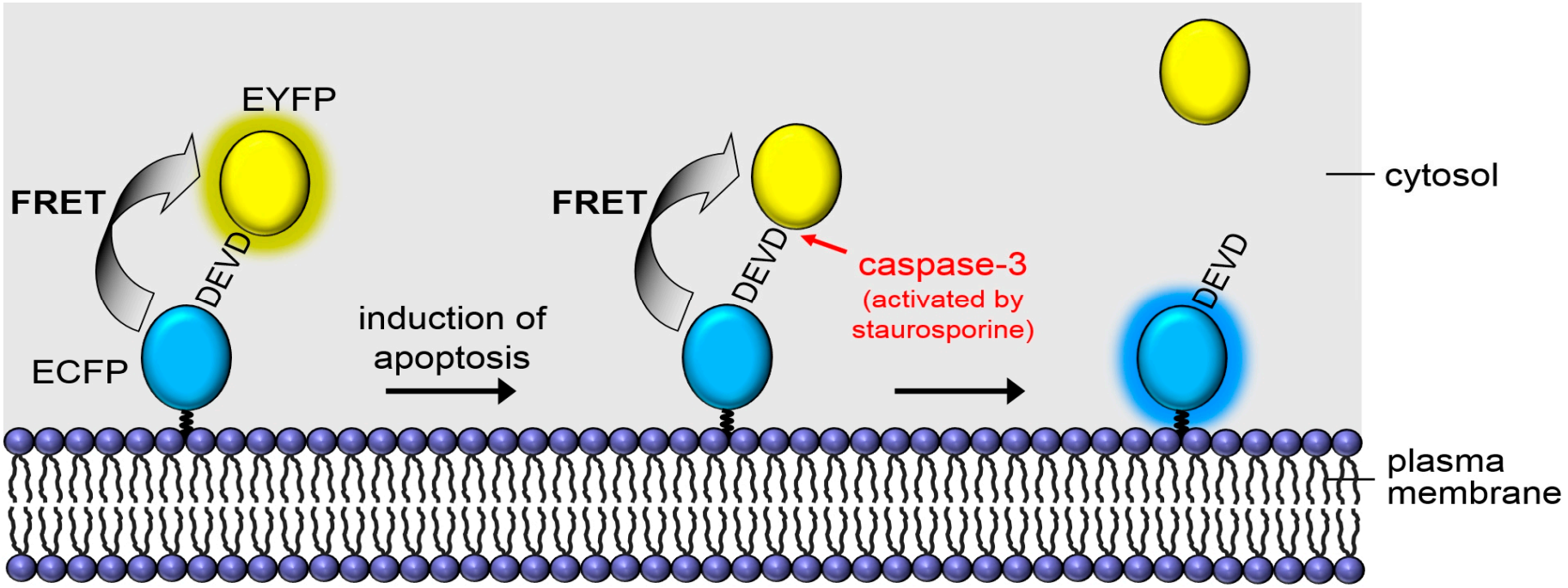
Fluorescence (single plane)



Fluorescence Lifetime

Fluorescence lifetime imaging (FLIM) is used to probe intermolecular Interactions of doxorubicin and to identify a degradation product

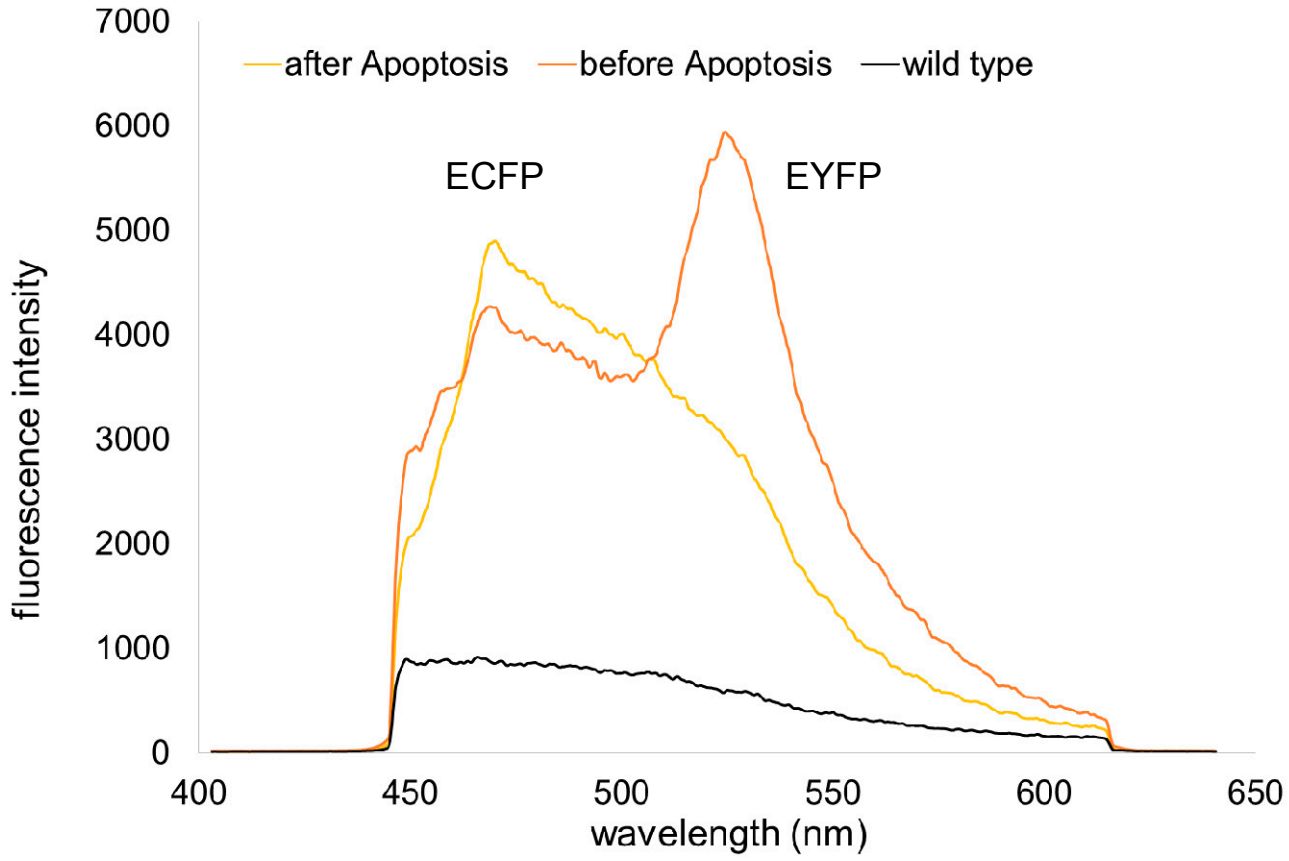
Förster Energy Transfer (FRET) Based Sensor for Apoptosis



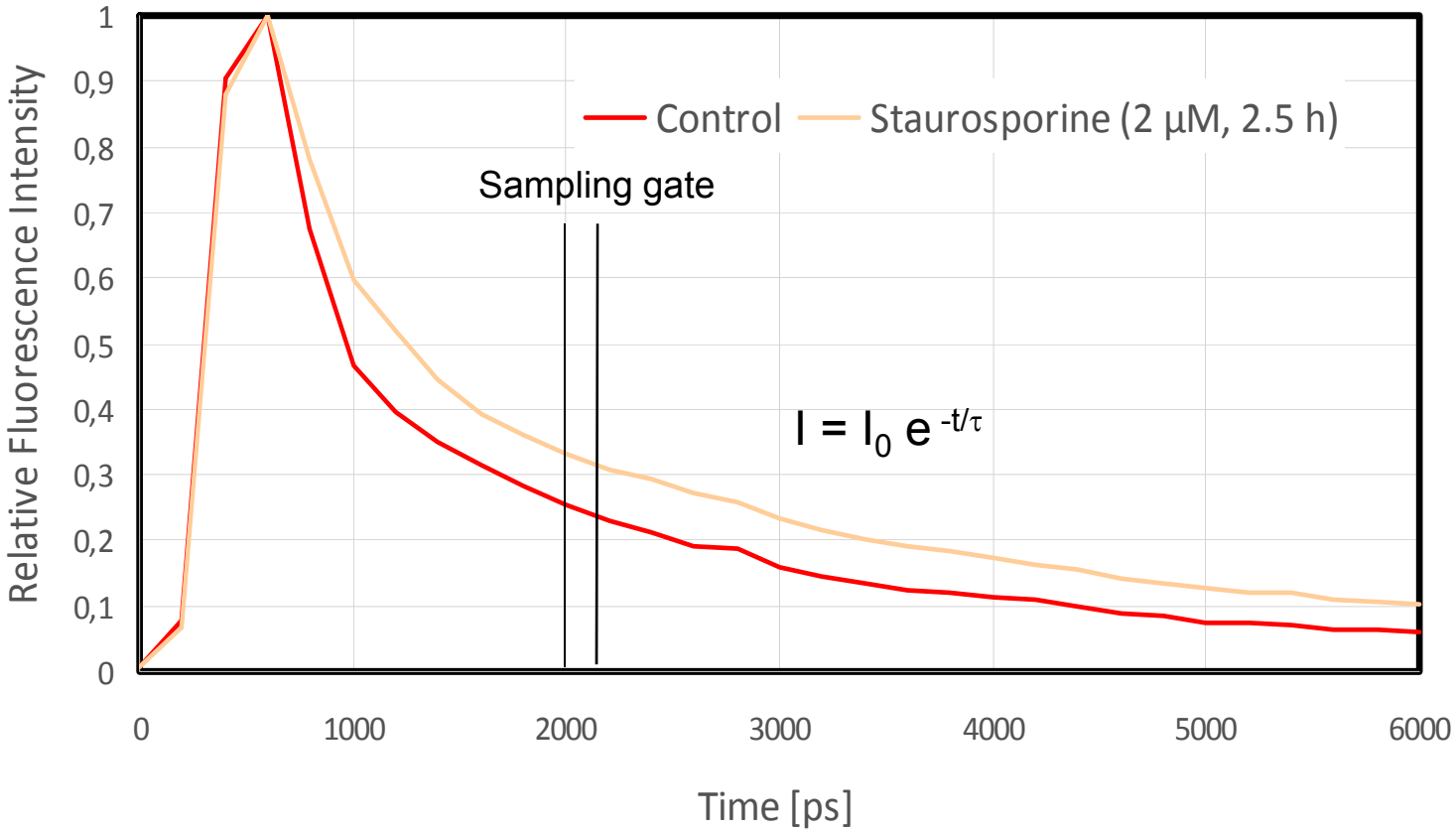
Non-radiative energy transfer from enhanced cyan fluorescent protein to enhanced yellow fluorescent protein via a cleavable peptide linker DEVD

Fluorescence Spectra prior to and subsequent to Apoptosis

λ_{ex} = 391 nm (ECFP)

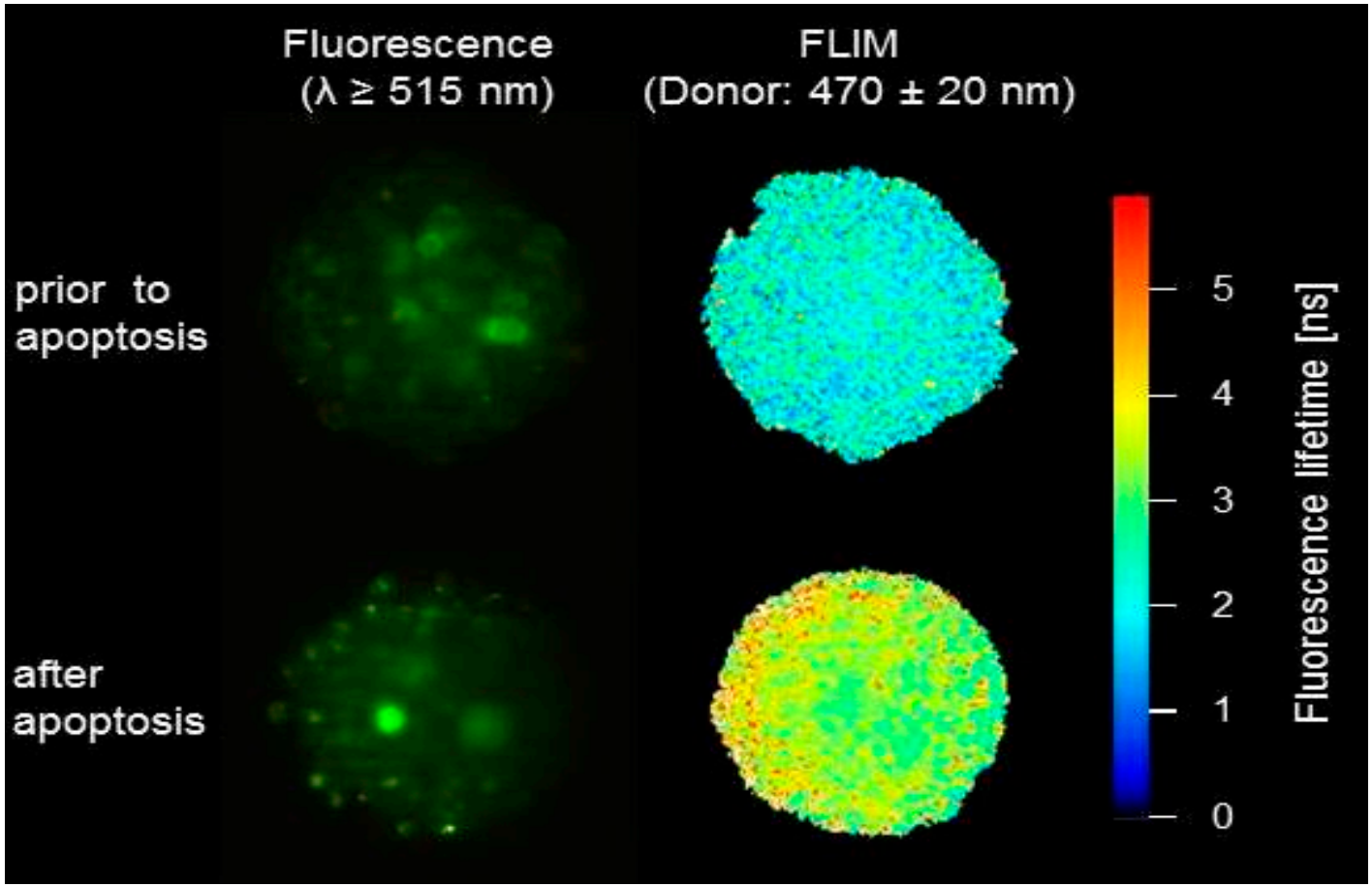


Fluorescence Decay Profiles prior to and subsequent to Apoptosis
- HeLA-Mem-ECFP-DEVD-EYFP -



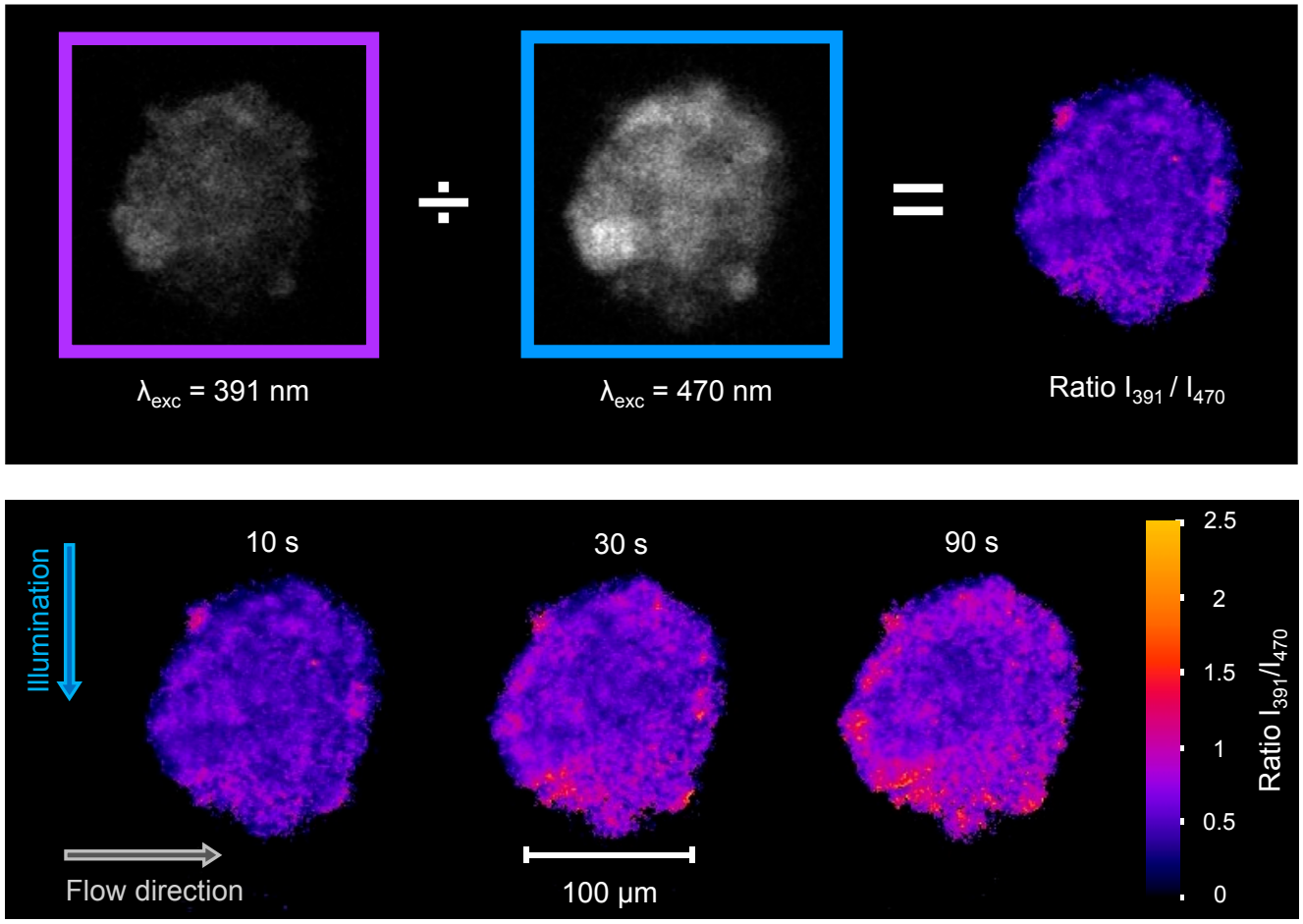
FRET-Based Membrane Assiciated Sensor for Apoptosis

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



LSFM Application: Redox Imaging upon Addition of H_2O_2

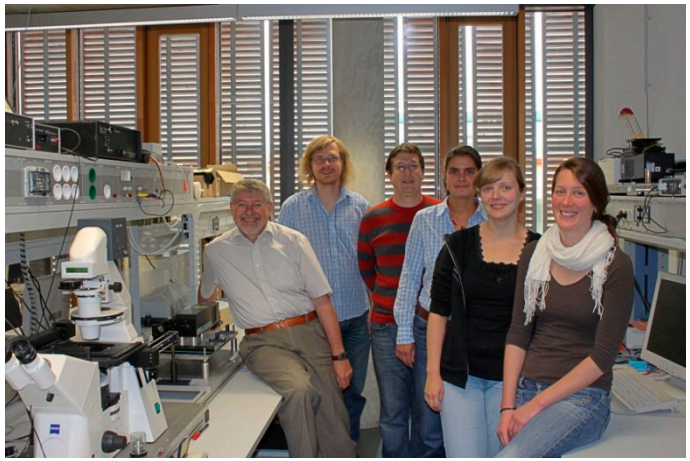
U251-MG glioblastoma cells with redox sensitive Grx1-roGFP2



Summary

- Light exposure in microscopy is limited due to phototoxicity
- Maximum light doses for cell survival - dependent on wavelengths and fluorescence markers – are typically in the range of 5–200 J/cm² corresponding to 50 s – 2000 s of solar irradiance
- Light sheet fluorescence microscopy (LSFM) needs minimum light doses for 3d samples, e. g. multicellular tumour spheroids (examples including apoptosis and necrosis, uptake of cytostatic drugs, redox imaging)
- TIRFM needs minimum light doses for cell surfaces or membranes (examples including focal adhesions, cell-substrate topology)

Acknowledgment / Literature



Projects are funded by Land Baden-Württemberg, the European Union (Europ. Fonds für die Regionale Entwicklung) as well as Bundesministerium für Wirtschaft und Energie (ZIM, grant no. KF 2888104UW3).

The authors thank B. Angres and NMI Reutlingen for providing HeLa cells expressing the FRET sensor as well as R. Wittig (ILM Ulm) for his cooperation.

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- T. Bruns, S. Schickinger, H. Schneckenburger: Single plane illumination module and micro-capillary approach for a wide-field microscope, J. Vis. Exp. 15(90) (2014) e51993.
- S. Schickinger, T. Bruns, R. Wittig, P. Weber, M. Wagner, H. Schneckenburger: Nanosecond ratio imaging of redox states in tumour cell spheroids using light sheet based fluorescence microscopy, J. Biomed. Opt. 18(12) (2013) 126007.
- 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.