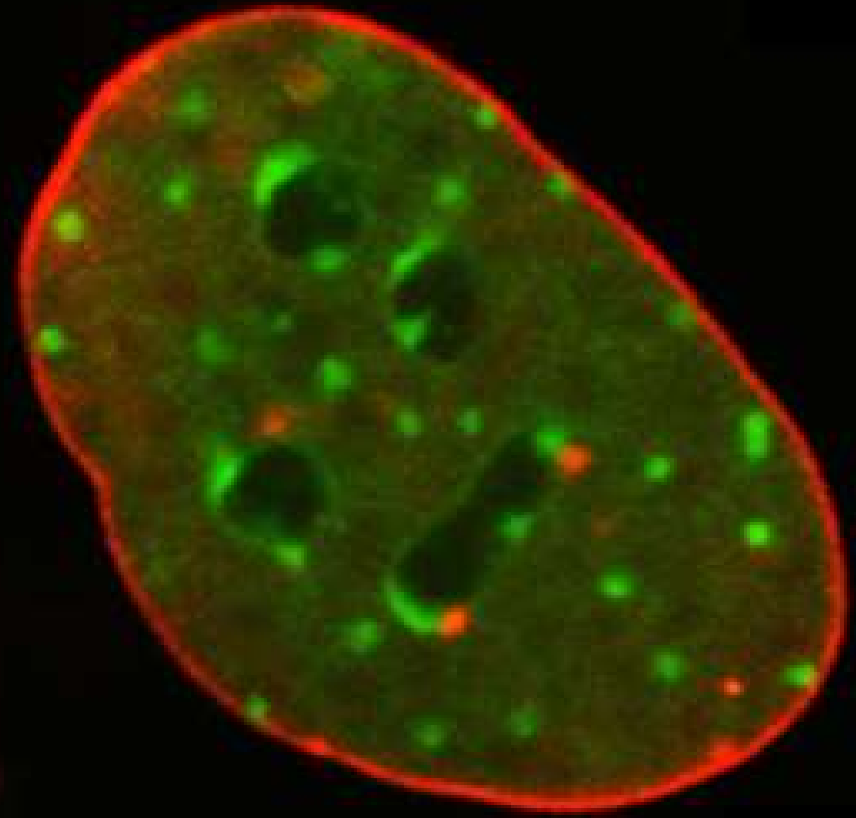
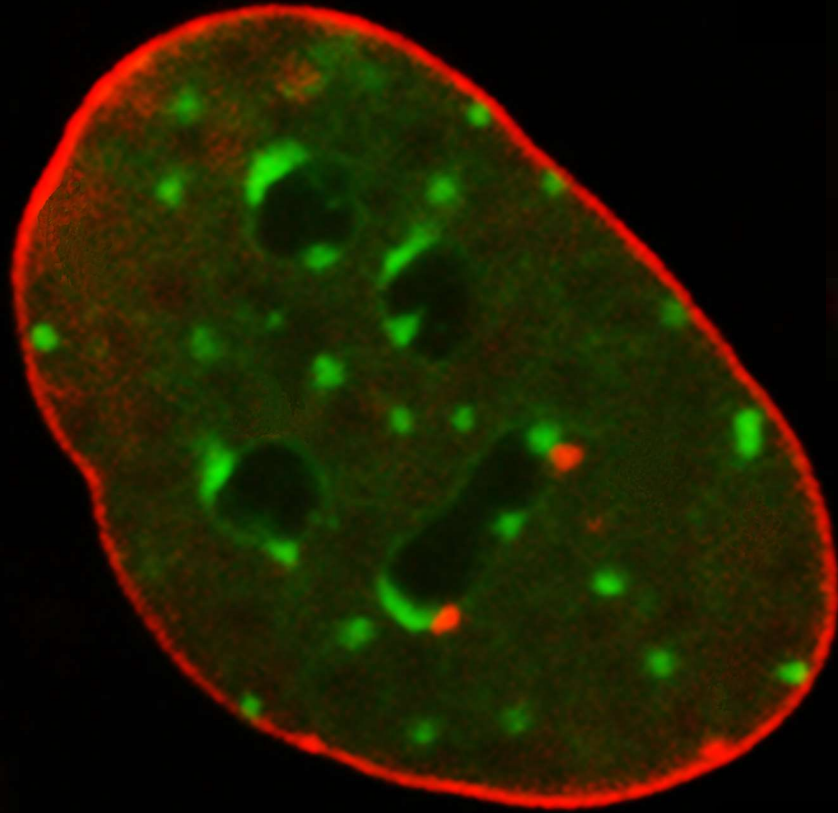


# *Reparace DNA a efekty HDAC a PRMT1 inhibitorů*

*Eva Bártová*

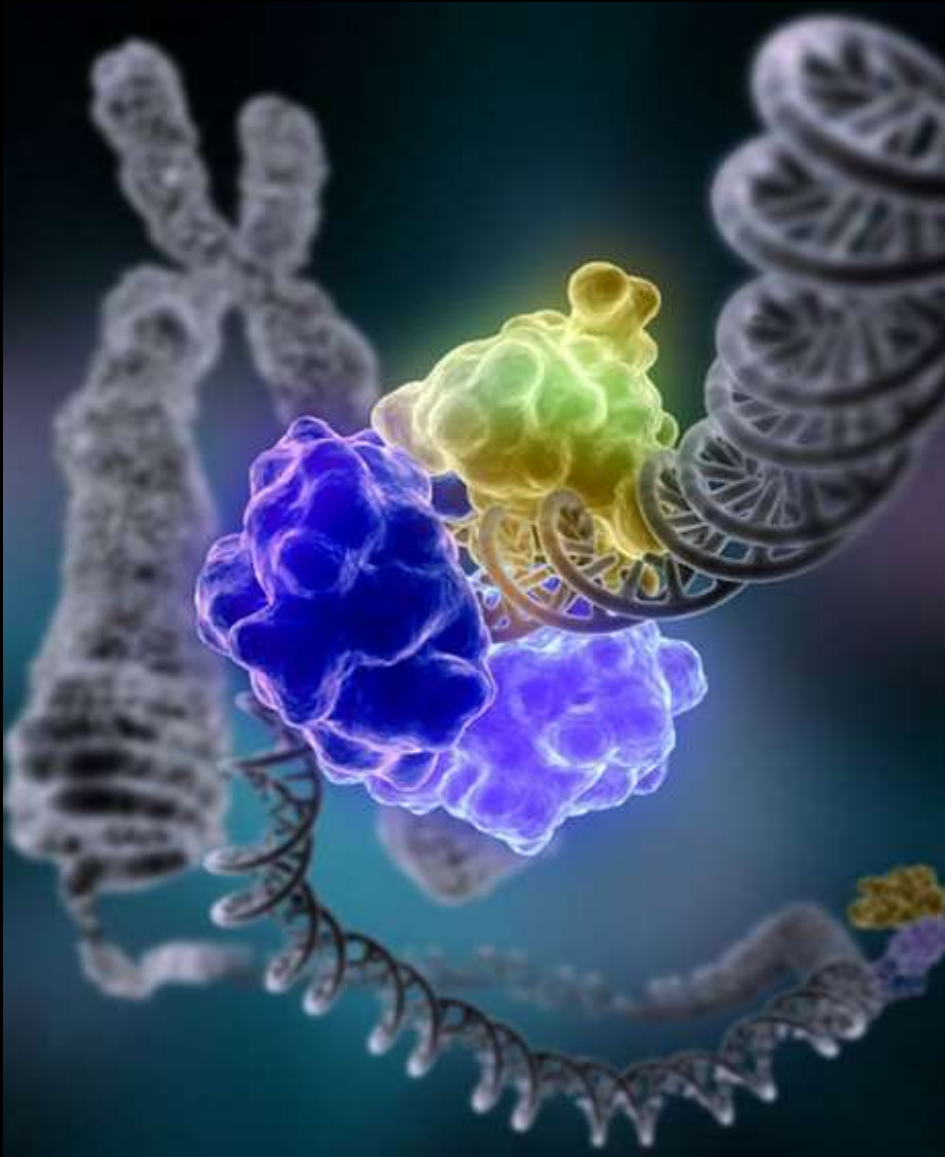
*Institute of Biophysics Academy of Sciences  
of the Czech Republic  
Brno*





*Game*

# DNA repair



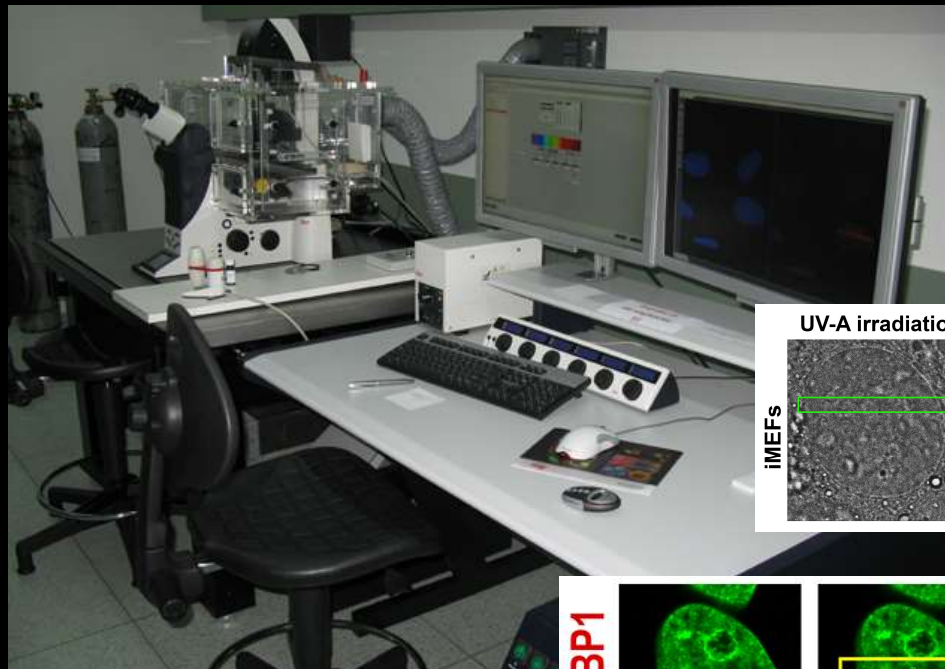
[http://commons.wikimedia.org/wiki/File:DNA\\_Repair.jpg](http://commons.wikimedia.org/wiki/File:DNA_Repair.jpg)

## Single-strand damage

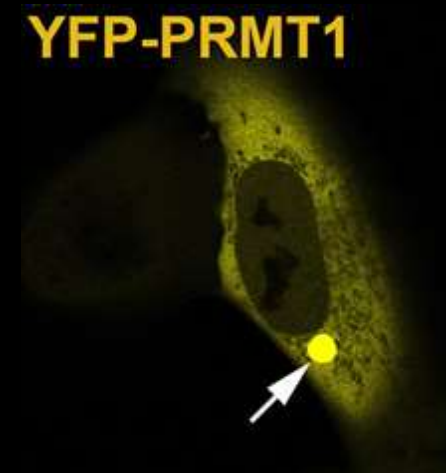
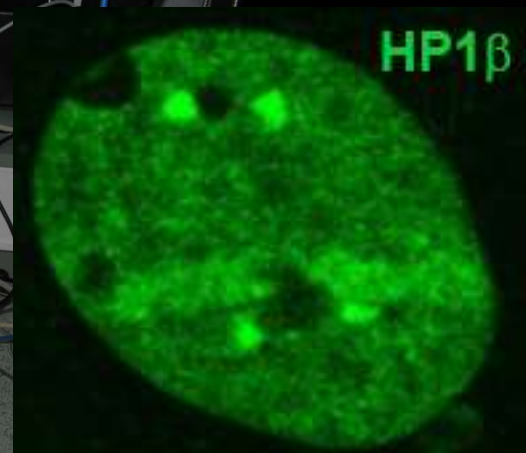
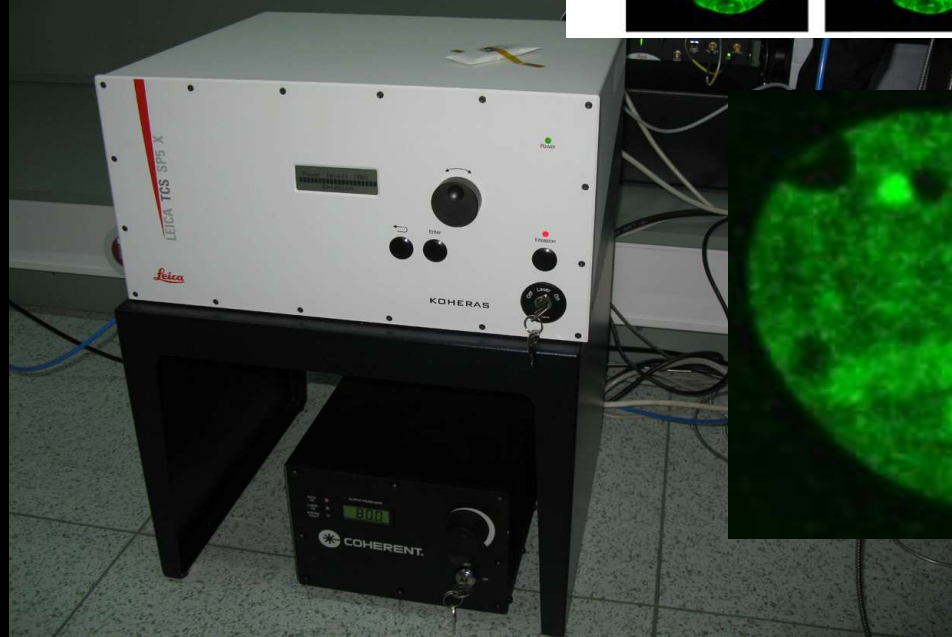
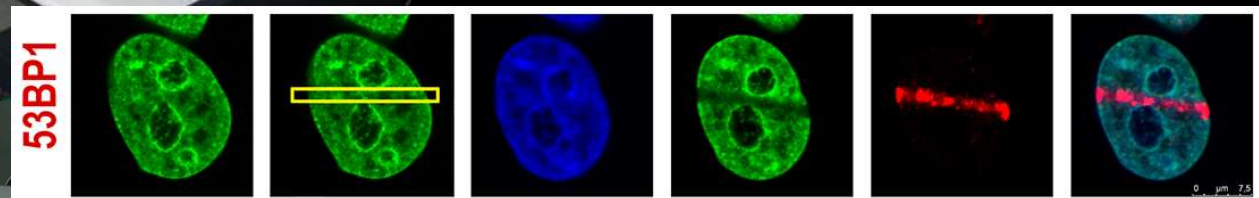
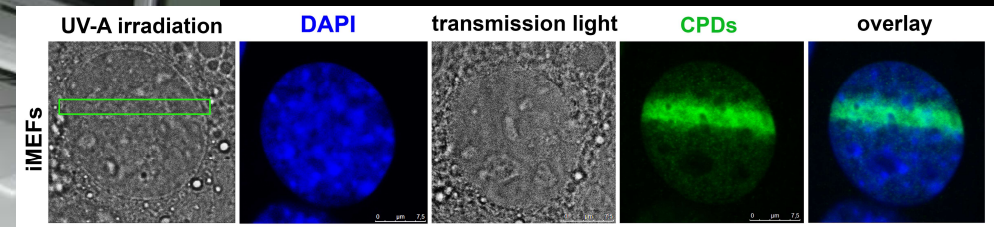
- ❖ Base excision repair (BER), which repairs damage to a single base caused by oxidation, alkylation, hydrolysis, or deamination.
- ❖ Nucleotide excision repair (NER), which recognizes bulky, helix-distorting lesions such as pyrimidine dimers and 6,4 photoproducts.
- ❖ Mismatch repair (MMR), which corrects errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides.

## Double-strand breaks

- ❖ non-homologous end joining (NHEJ)
- ❖ microhomology-mediated end joining (MMEJ)
- ❖ homologous recombination (HR)

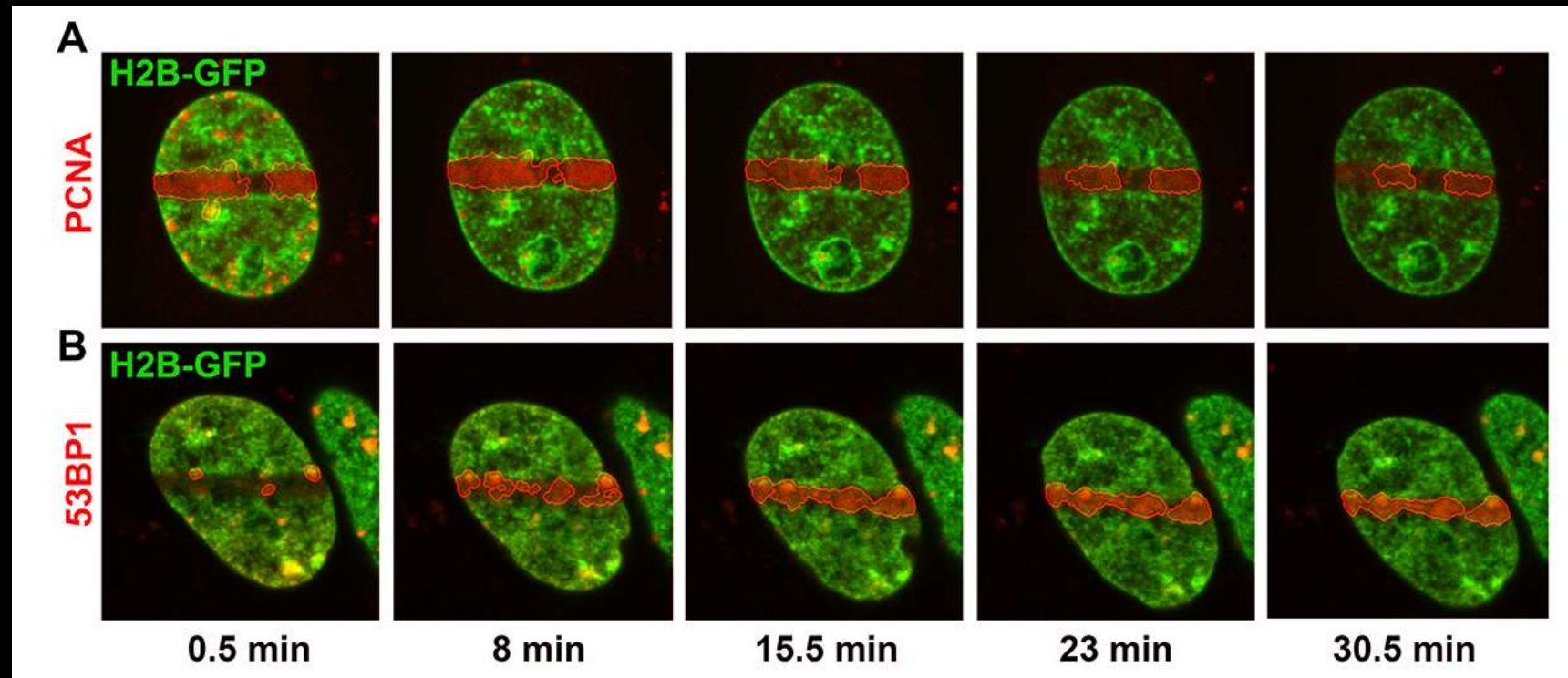


## Application of UV-lasers (355 nm and 405 nm) in DNA repair studies

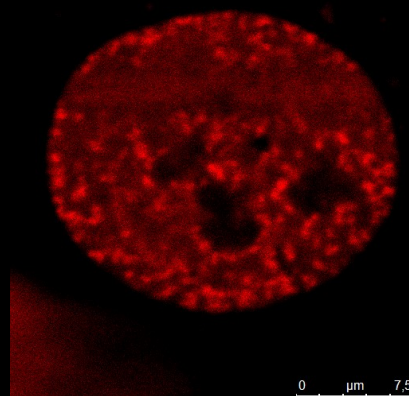


Experiments: Petra Sehnalová

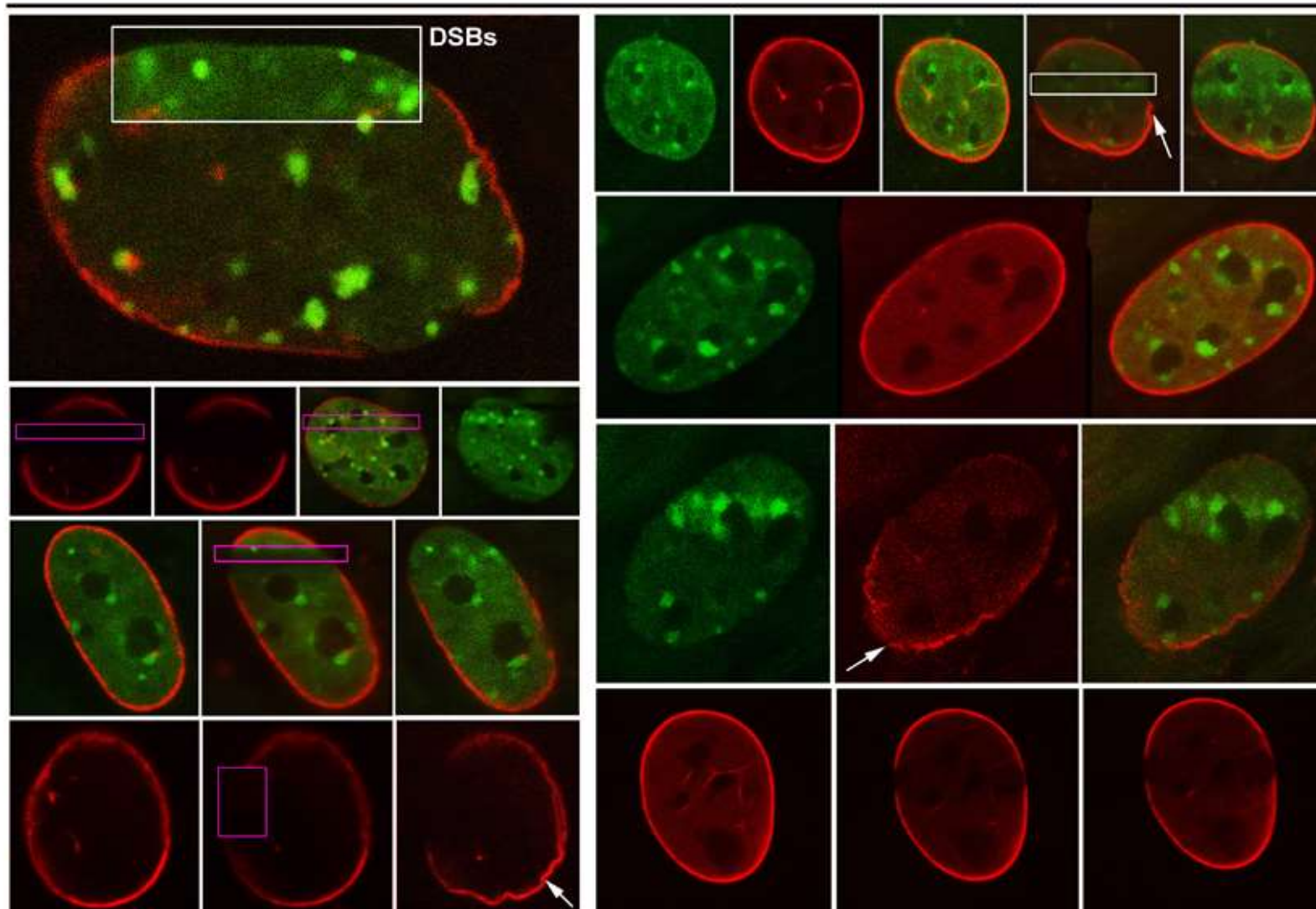




Experiments: Jana Suchánková and Gabriela Šustáčková

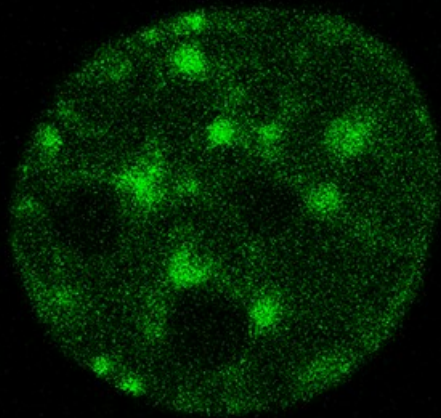


## GFP-HP1 $\beta$ / mCherry-Lamin A

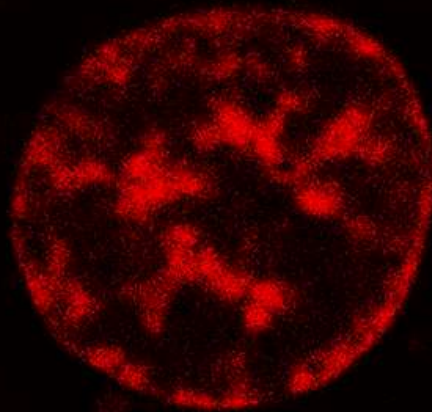


Experiments: Petra Sehnalová and Eva Bártová

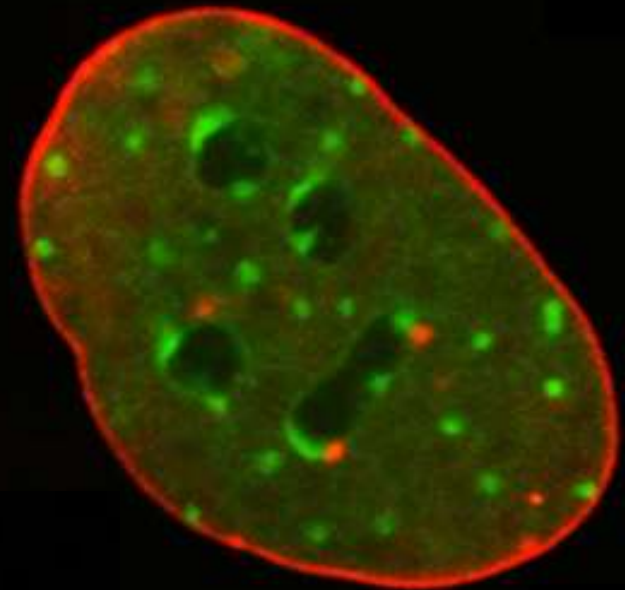
HP1 $\beta$



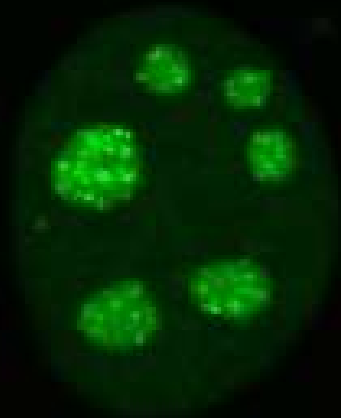
53BP1



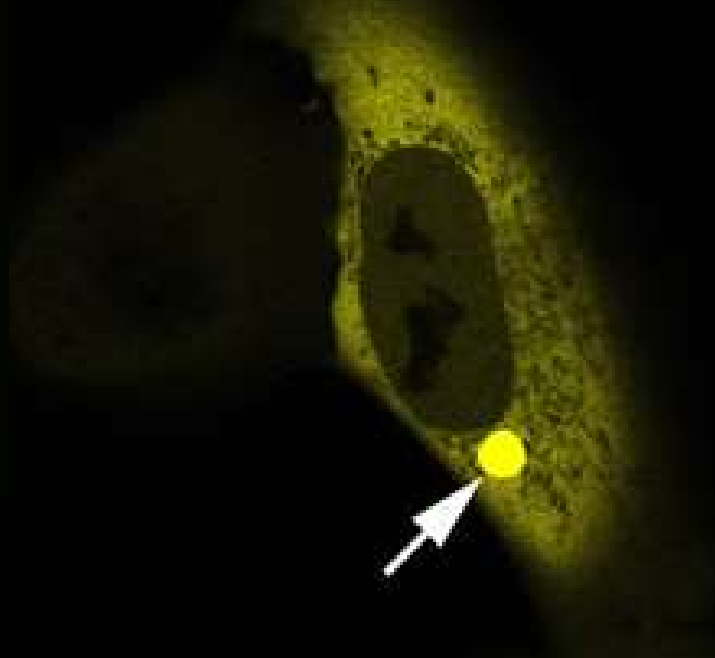
HP1 $\beta$  and A-type lamins



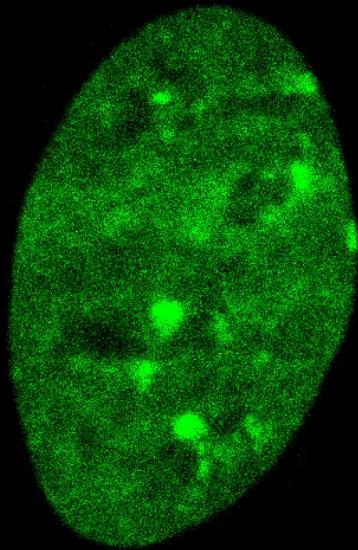
UBF1/2



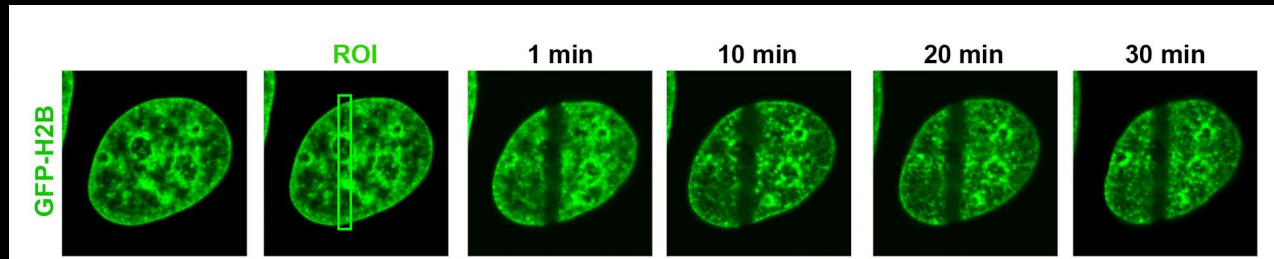
YFP-PRMT1



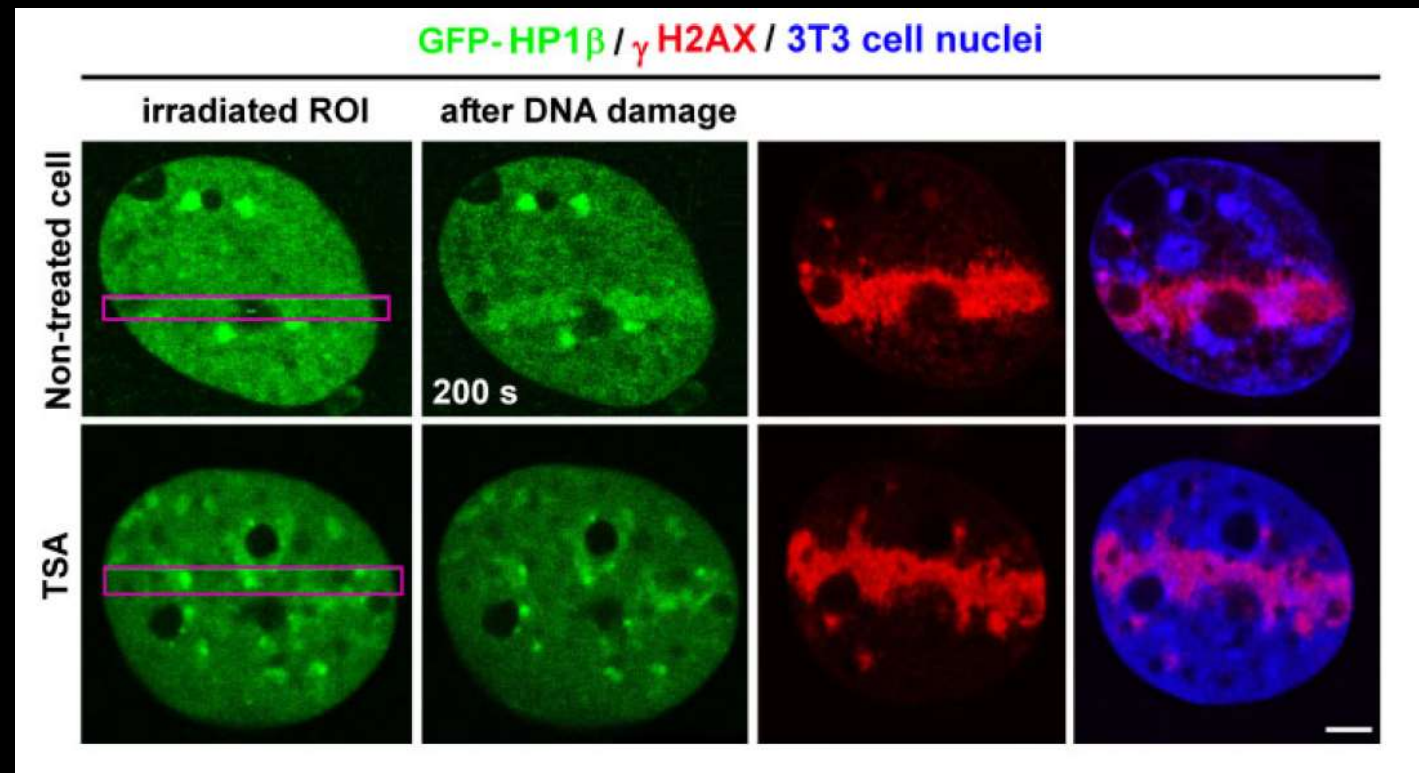




0  $\mu\text{m}$  5

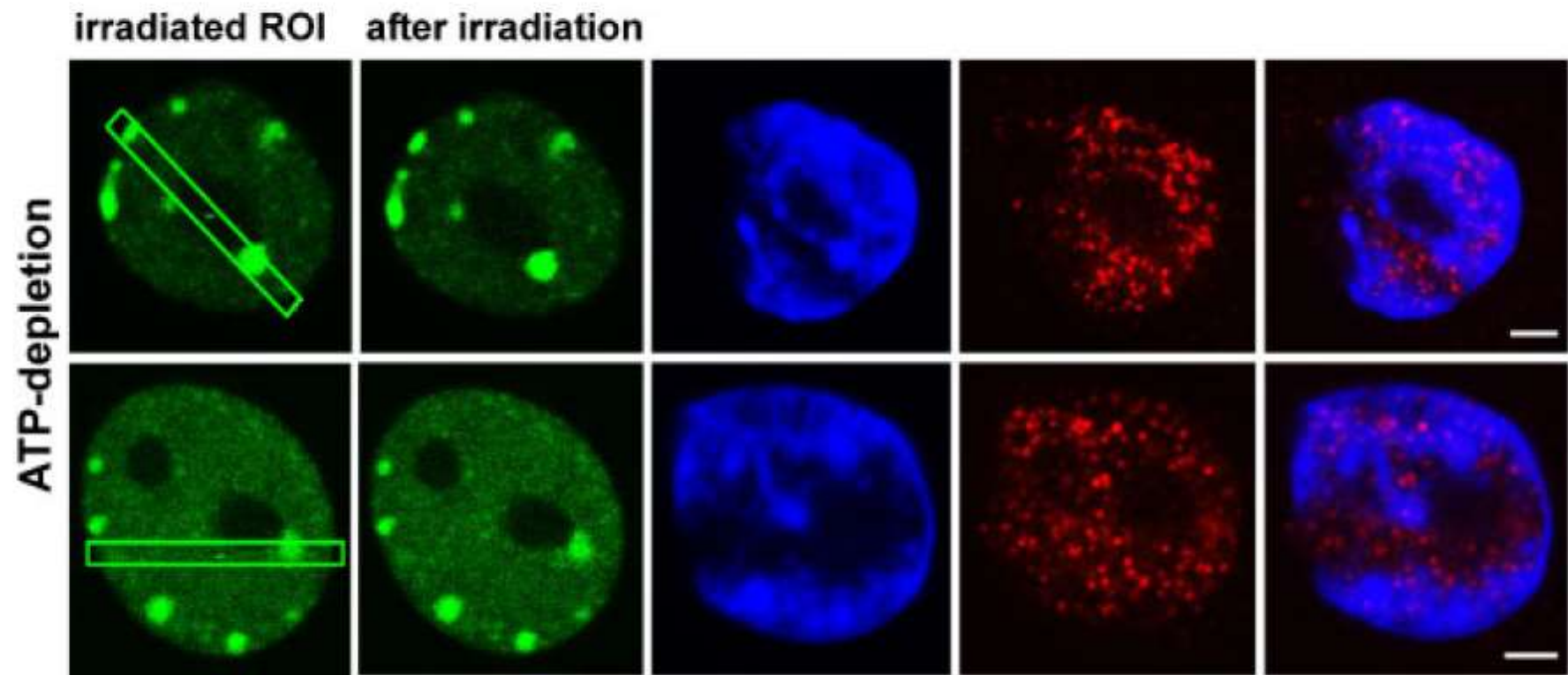


Šustáčková et al., JCP (2011)  
and experiments of Petra Sehnalová  
and Soňa Legartová



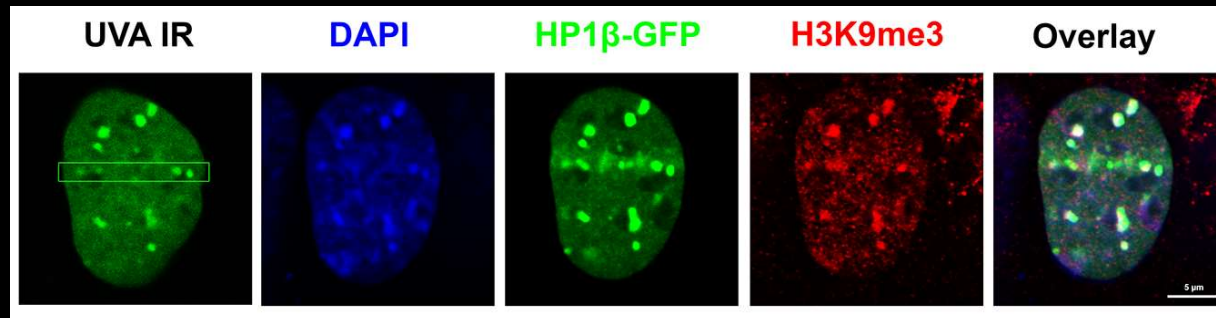
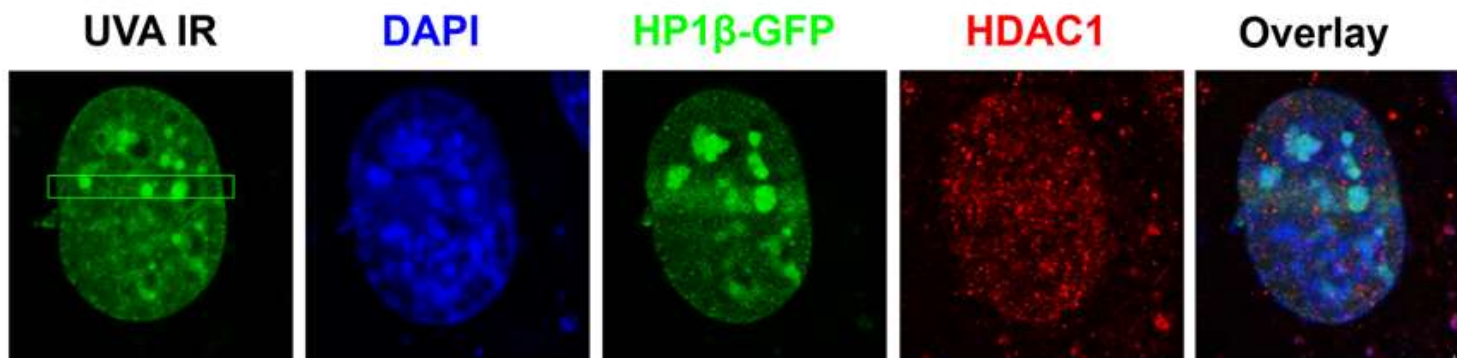
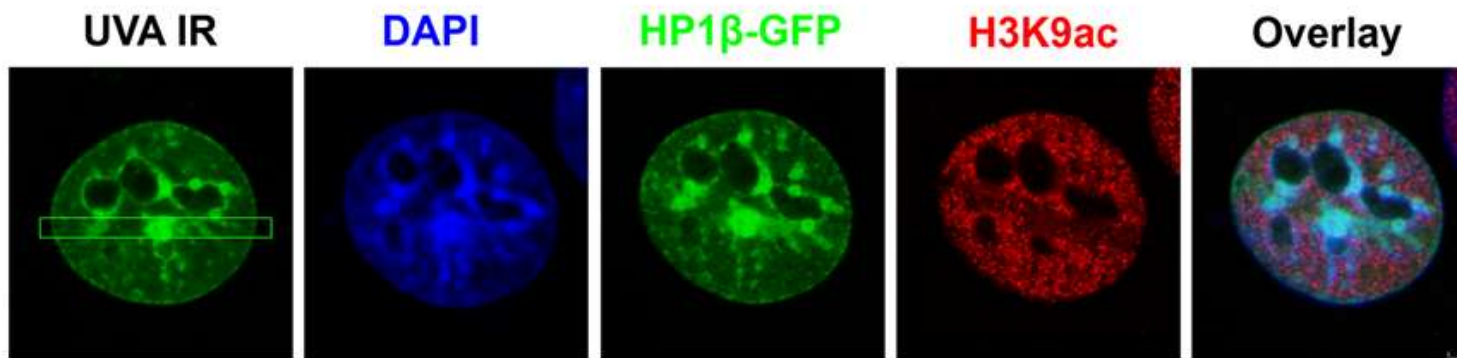


GFP-HP1 $\beta$  /  $\gamma$ H2AX / Nucleus



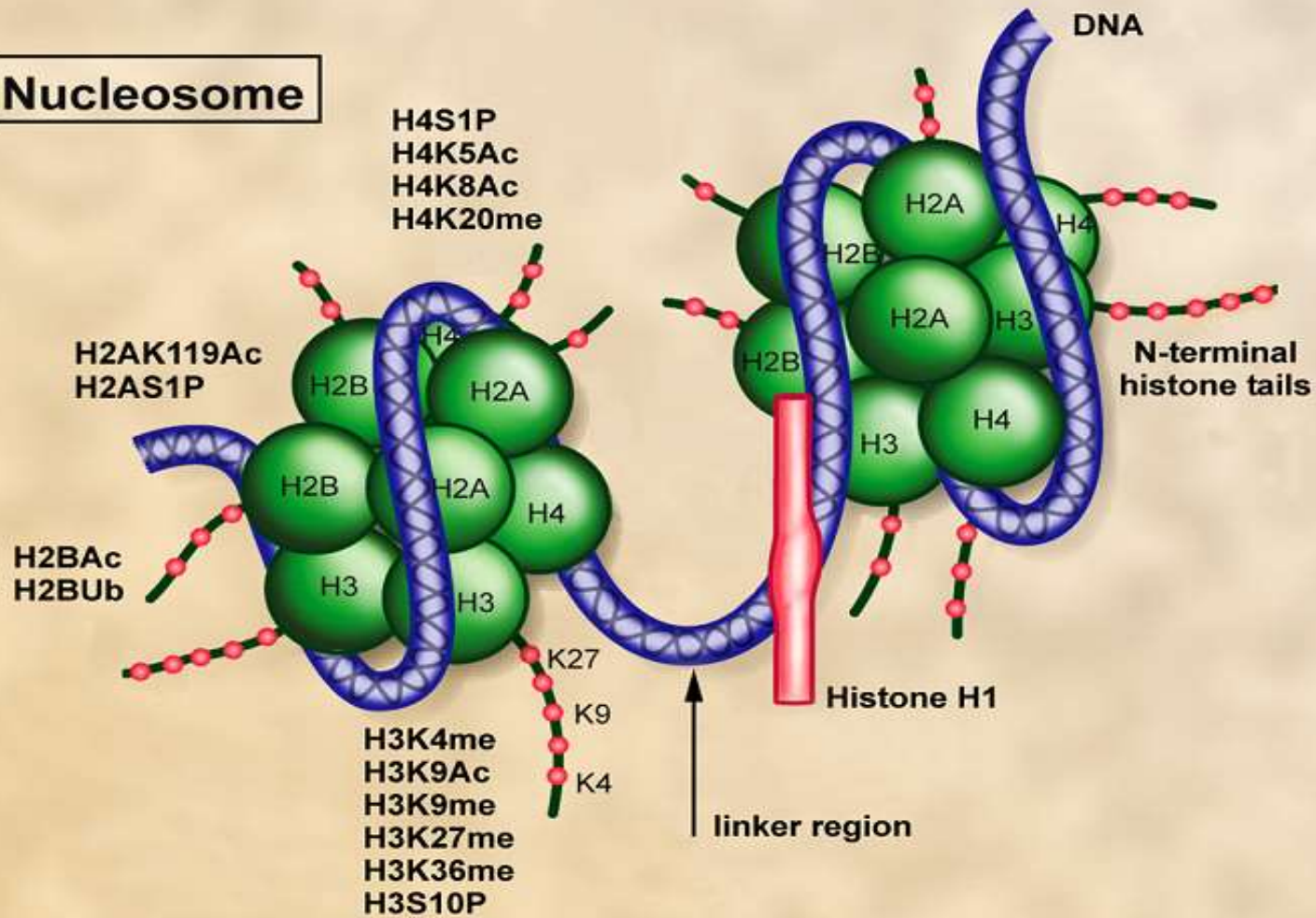
Šustáčková et al., JCP (2011)

## LMNA (wt) cells

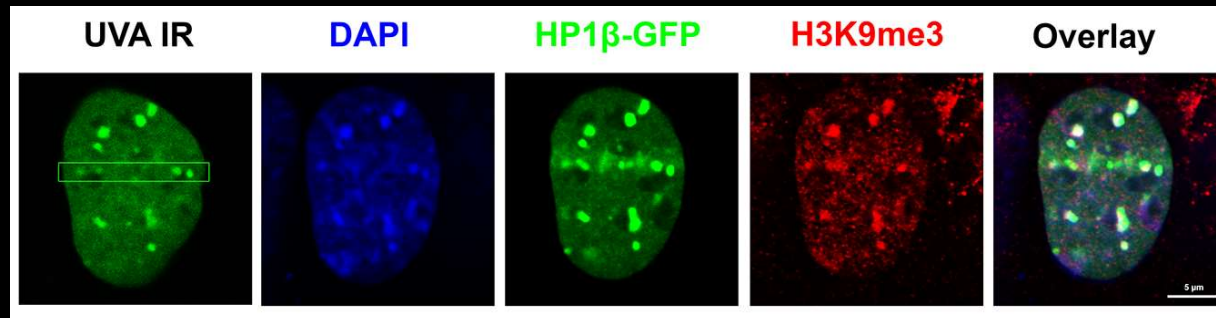
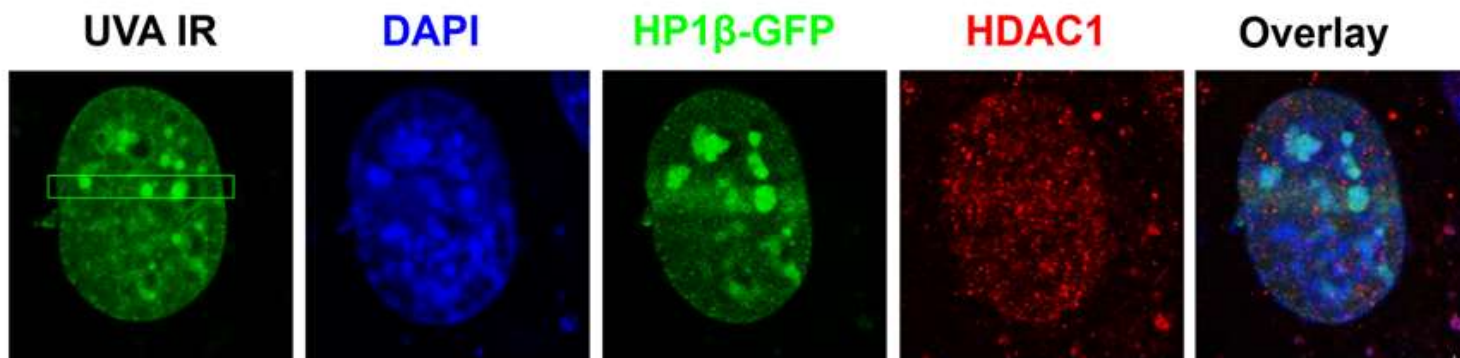
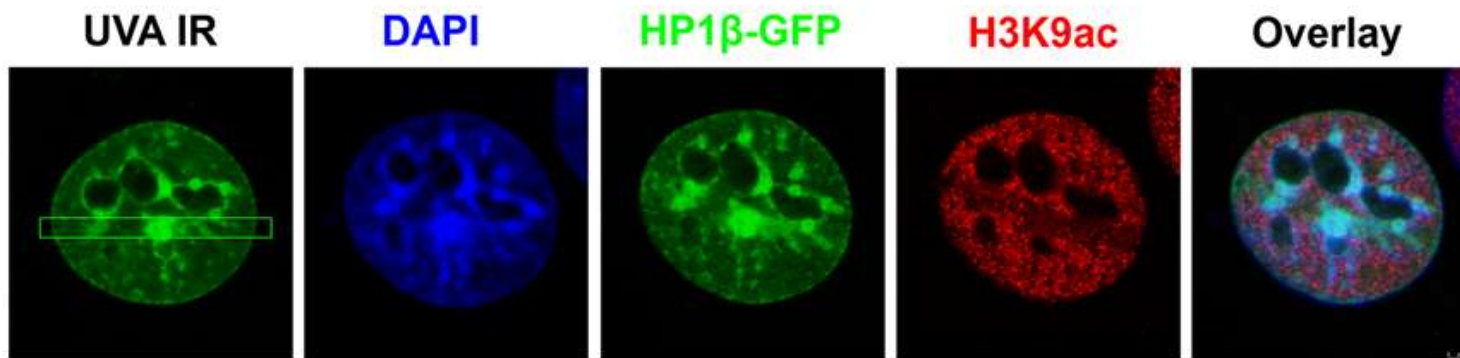


Experiments: Petra Sehnalová

# Nucleosome



## LMNA (wt) cells



Experiments: Petra Sehnalová



## PRMT1 arginine methyltransferase accumulates in cytoplasmic bodies that respond to selective inhibition and DNA damage

J. Suchánková,<sup>1</sup> S. Legartová,<sup>1</sup>  
P. Sehnalová,<sup>1</sup> S. Kozubek,<sup>1</sup> S. Valente,<sup>2</sup>  
D. Labella,<sup>2</sup> A. Mai,<sup>2</sup> C. Eckerich,<sup>3</sup>  
F.O. Fackelmayer,<sup>3</sup> D.V. Sorokin,<sup>4</sup>  
E. Bártová<sup>1</sup>

<sup>1</sup>Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

<sup>2</sup>Pasteur Institute - Fondazione Cenci Bolognetti, Department of Chemistry and Drug Technology, University of Rome La Sapienza, Italy

<sup>3</sup>Institute of Molecular Biology and Biotechnology, Department of Biomedical Research (IMBB-FORTH), Foundation for Research and Technology Hellas, Ioannina, Greece

<sup>4</sup>Faculty of Informatics, Masaryk University, Brno, Czech Republic

respond to DNA injury in the cell nucleus, and to treatment with various PRMT1 inhibitors.

### Introduction

Chromatin structure and function is controlled by many enzymes.<sup>1</sup> Protein arginine methyltransferases (PRMTs) methylate histones and other regulatory and structural proteins, with particular activity in the nucleus.<sup>2,3</sup> The PRMT family consists of 11 different methyltransferases (PRMT1-11) that control cellular processes such as transcription, RNA processing, nucleocytoplasmic shuttling of proteins, and DNA repair.<sup>4-7</sup> Reflecting these diverse functions, several PRMTs are located in both the cytoplasm and the nucleus, but display cell-type-specific differences in the ratio of nuclear *versus* cytoplasmic PRMTs' distribution.<sup>8</sup> Arginine methyltransferases in the nucleus act as epigenetic factors that induce transcriptional activation or silencing depending on the affected residue in core histones, and the symmetric or asymmetric nature of the methylation.<sup>9</sup> For example, PRMT1 and PRMT5 can both dimethylate arginine 3 of histone H4 (H4R3). However, PRMT5 methylates H4R3 symmetrically, which leads to silencing

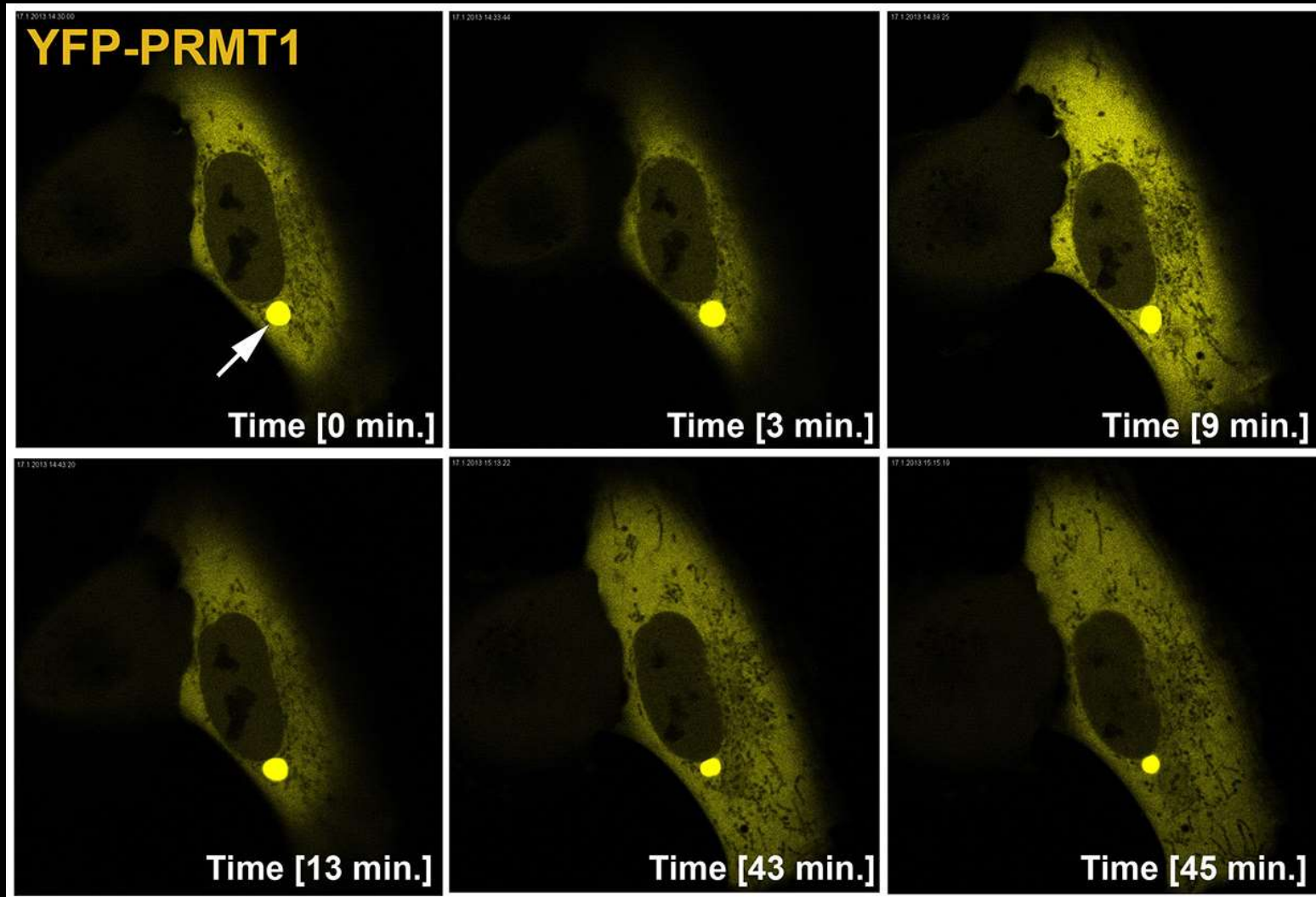
Correspondence: Eva Bártová, Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Královopolská 135, CZ-612 65, Brno, Czech Republic. Tel. +420.5.41517141 - Fax: +420.5.41240498. E-mail: bartova@ibp.cz

Keywords: Epigenetics, PRMTs, epi-drugs, arginine methylation, DNA repair.

Conflict of interests: the authors declare no potential conflict of interests.

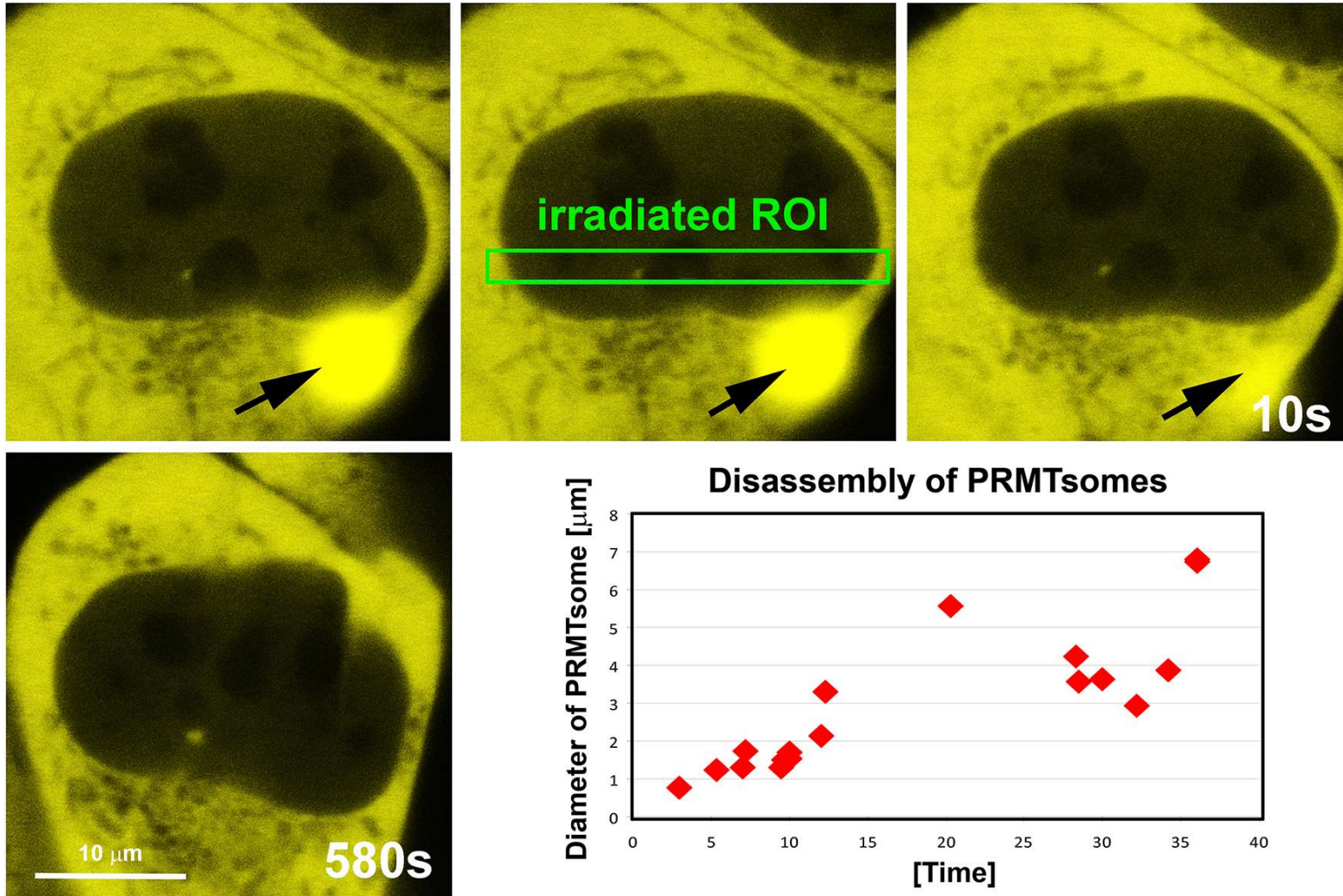
Funding: our work was supported by the following agencies: Grant Agency of Czech Republic (Grant N. P302/10/1022, P302/12/G157, and 13-07822S). EB, FOF, and AM are members of the EU-COST Action TD0905; EB is a principal investigator and coordinator of the EU Marie Curie Project PIRS-ES-GA-2010-269156-LCS. The postdoctoral fellowship of DVS was guaranteed by the Education for Competitiveness Operational Programme (ECOP), N. CZ.1.07/2.3.00/30.0030.

Contributions: JS, cell culture cultivation, treatments, plasmid DNA isolation, cell transfection, live cell studies after UV-A irradiation, immunofluorescence studies; SL, immunofluorescence and intermediated irradiation of the cells with  $\gamma$ -rays; SK, GACR projects P302/10/1022 and P302/12/G157 coordination; CE, provided YFP-PRMT1 plasmid DNA; FOF, manuscript contribu-

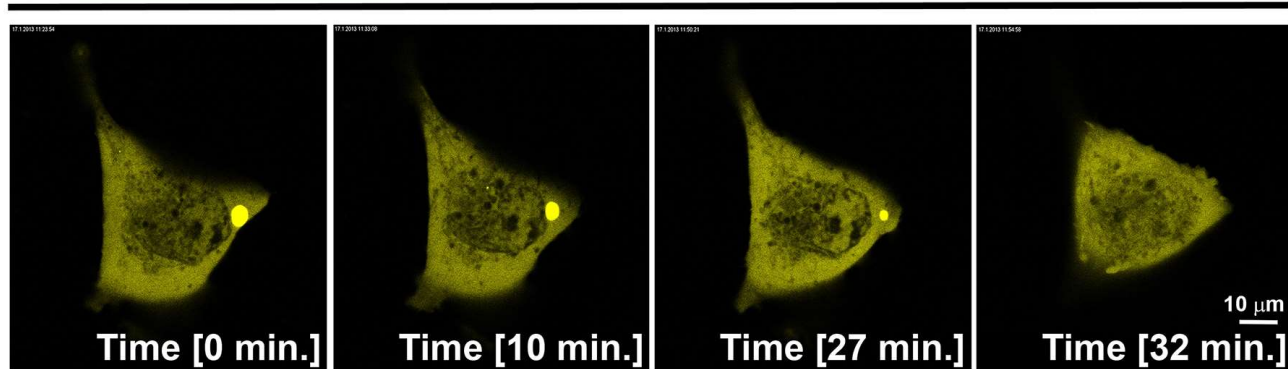




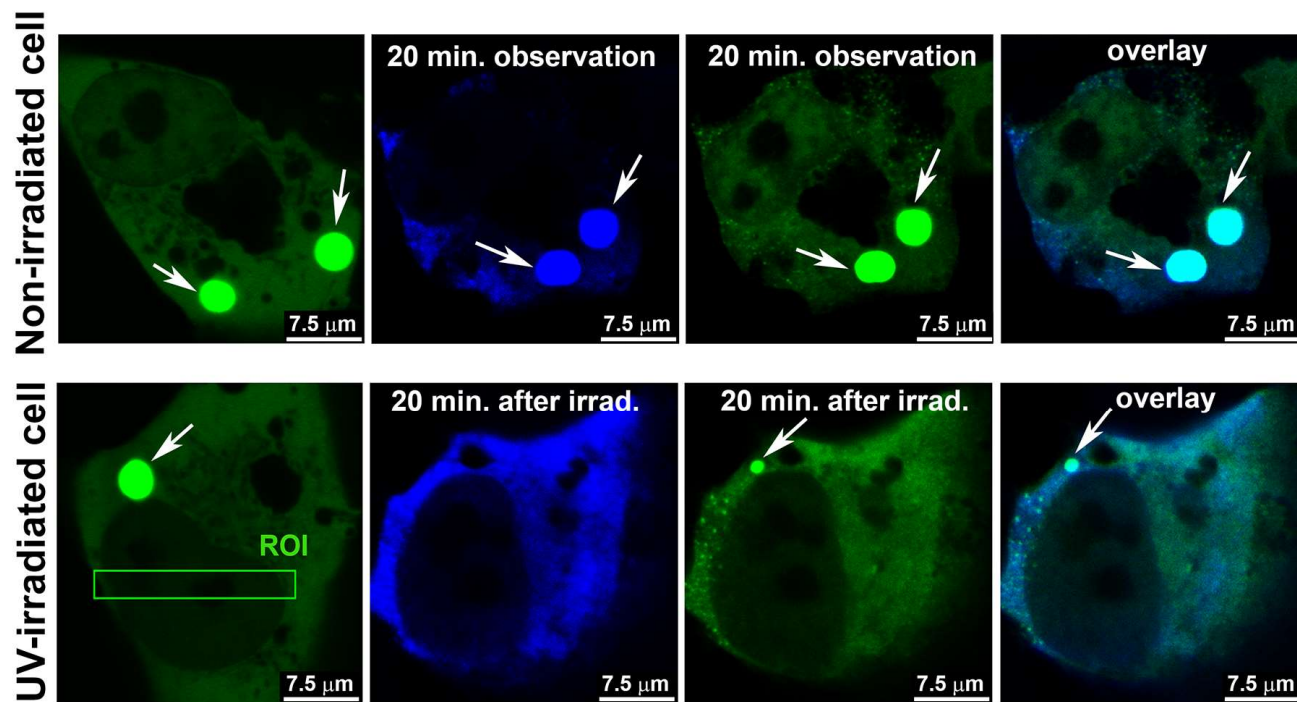
## YFP-PRMT1 in U2OS cells



## YFP-PRMT1 in U2OS cells / $\gamma$ -irradiation [5 Gy]

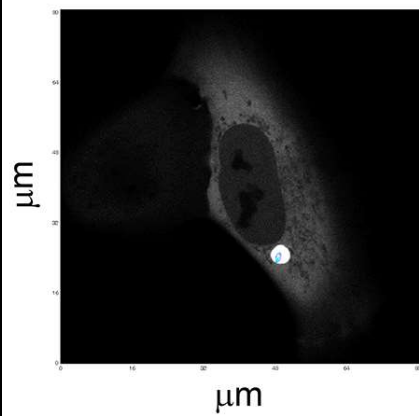


## YFP-PRMT1 / endogenous PRMT1

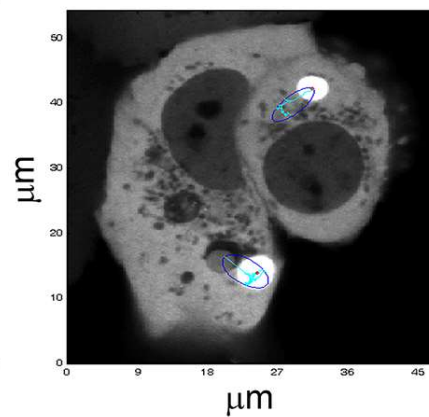




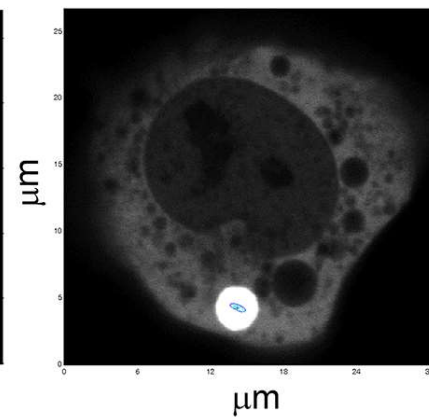
Non-treated cell



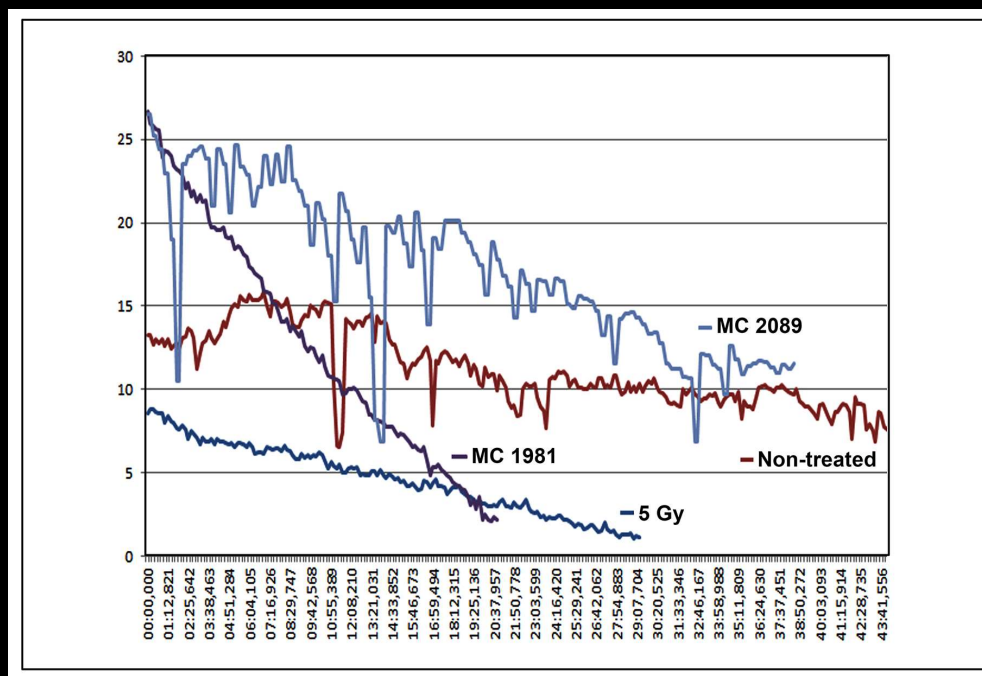
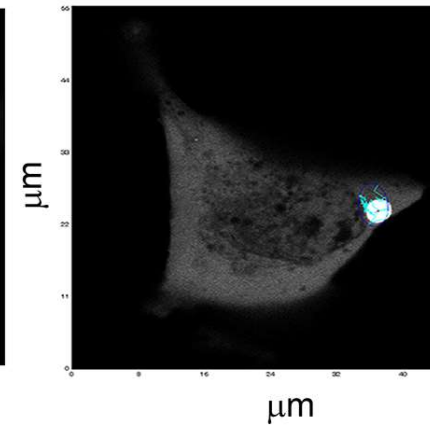
MC 1981



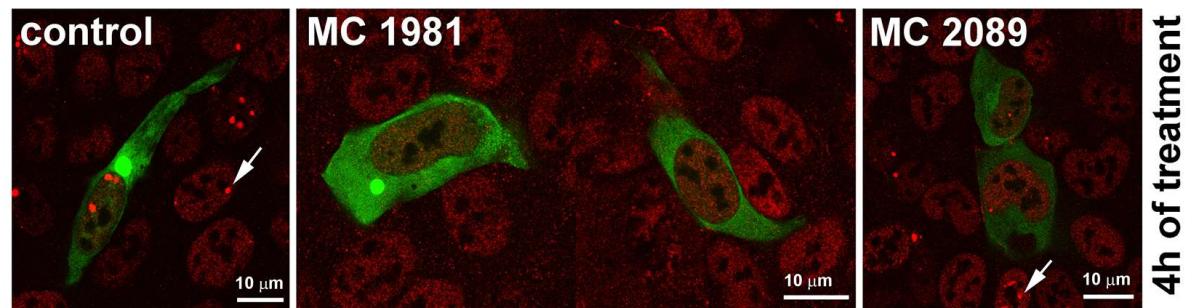
MC 2089



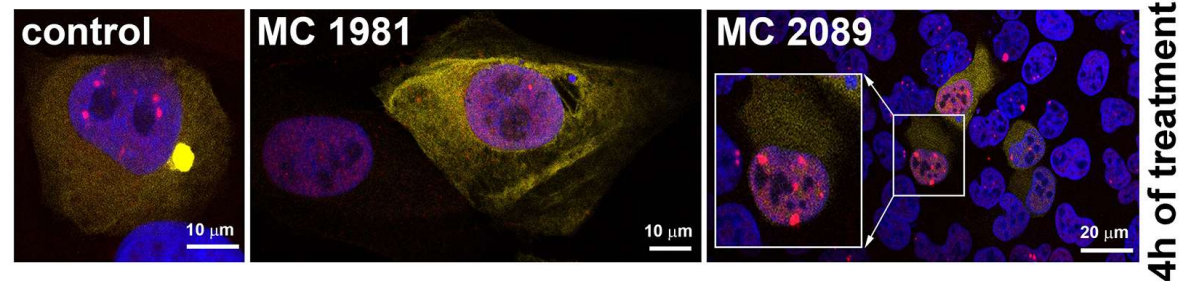
$\gamma$ -irradiation (5 Gy)



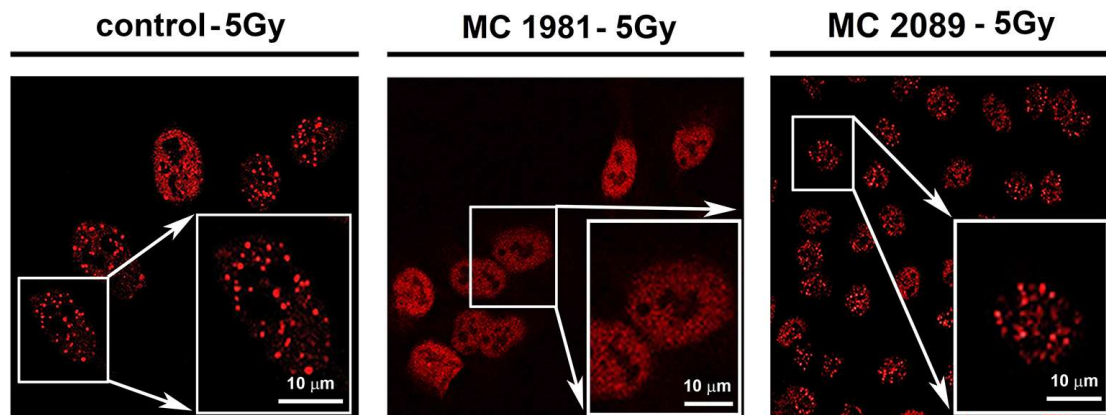
### YFP-PRMT1 / 53BP1 in U2OS cells



### YFP-PRMT1 / 53BP1 in U2OS cells

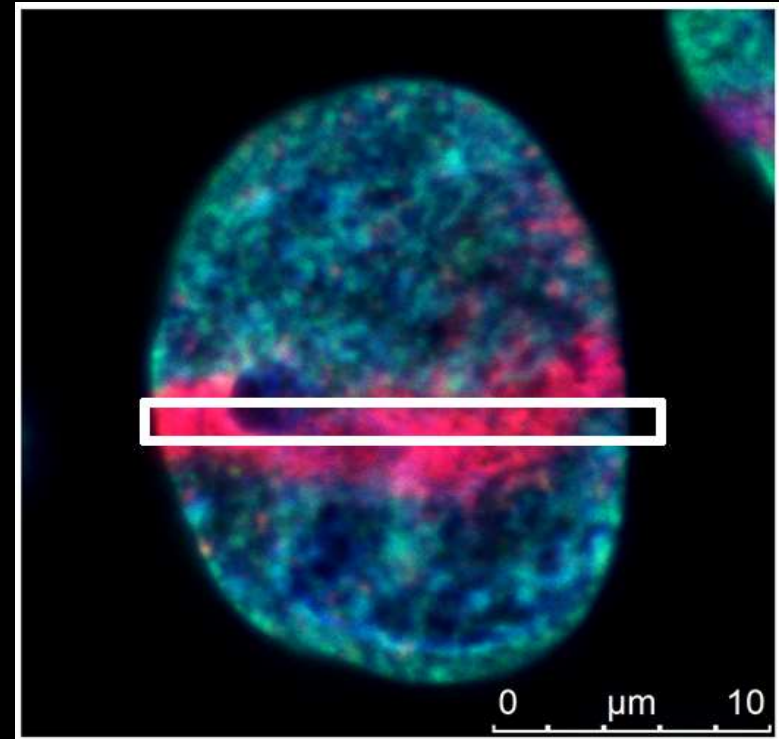


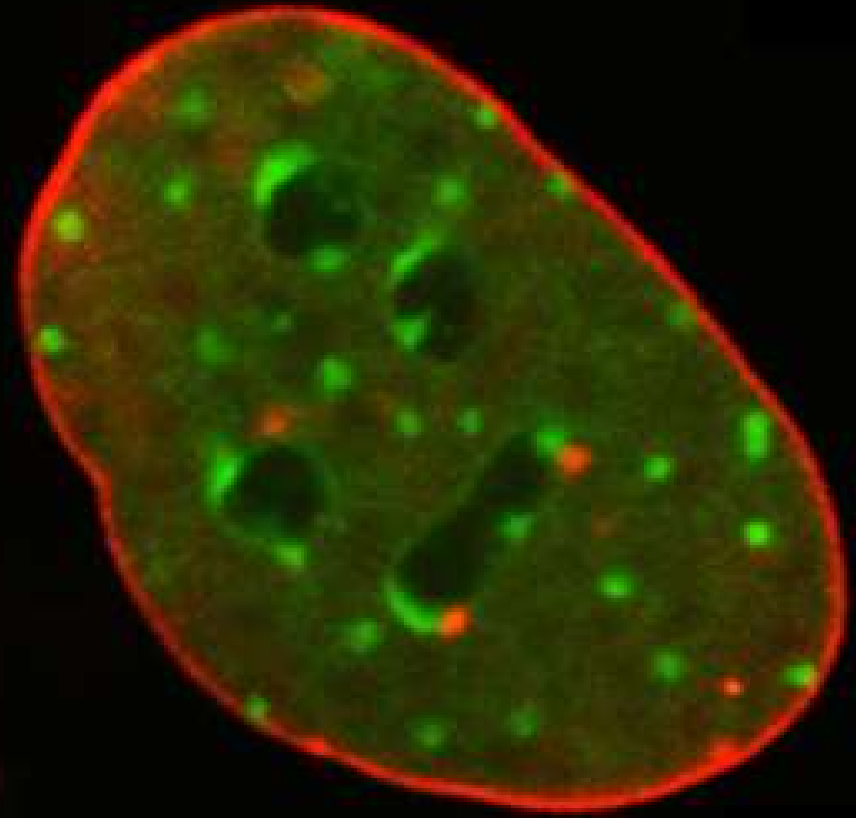
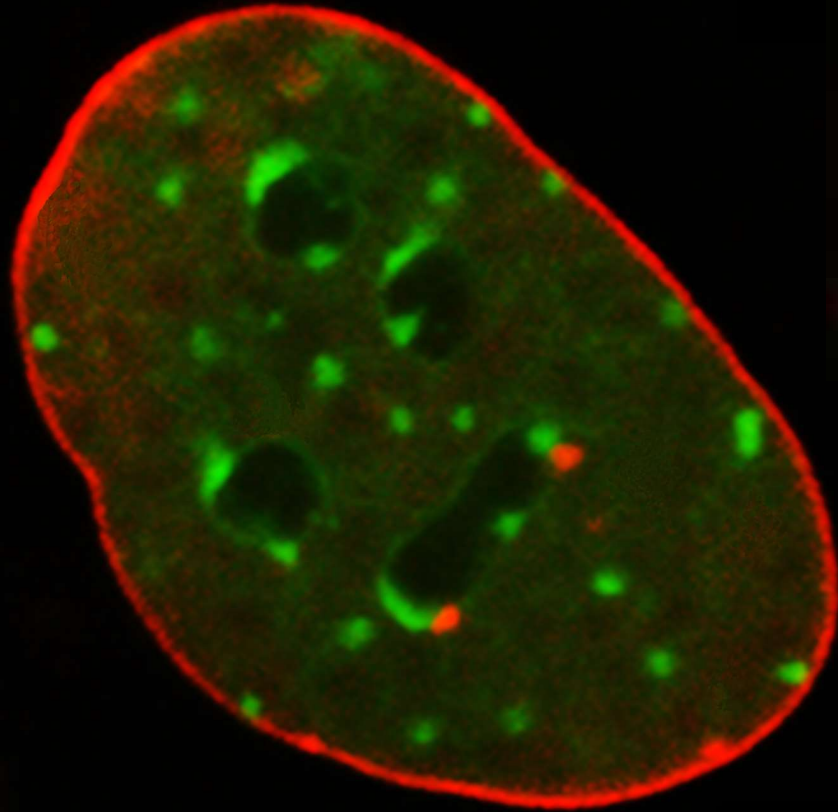
### 53BP1 in U2OS cells



## Conclusions

1. HP1 $\beta$  function at DNA lesions can be affected by HDAC inhibitors.
2. Irradiation of the cell nucleus by UVA lasers caused disappearance of PRMT1-positive cytoplasmic bodies
3. Disappearance of PRMT1-positive cytoplasmic bodies is really fast after  $\gamma$ -irradiation or the cell treatment by PRMT1 inhibitor MC 1981.
4. PRMT1 inhibitor MC 1981 prevents formation of 53BP1-positive irradiation-induced foci.

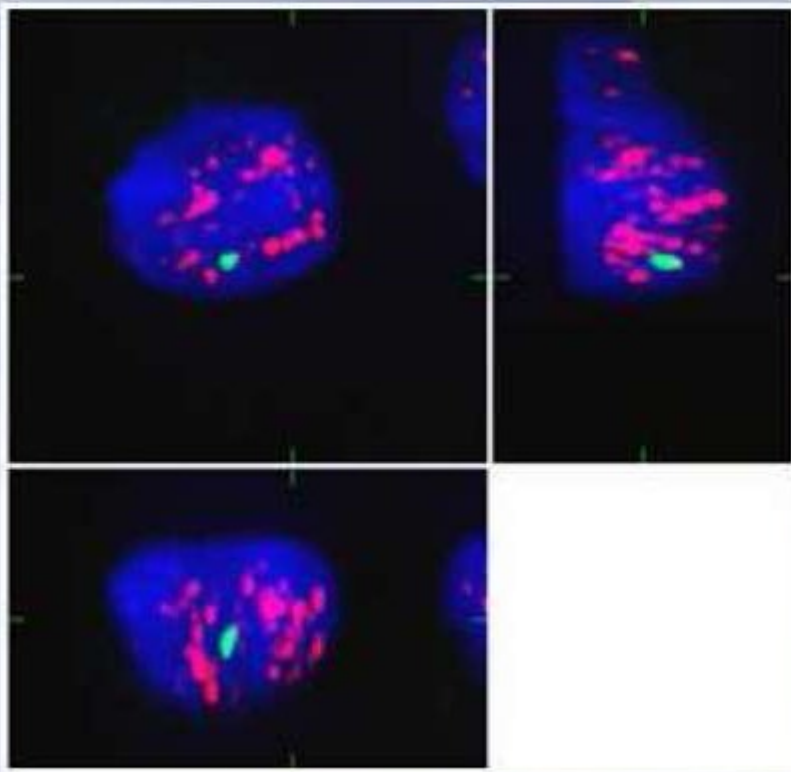
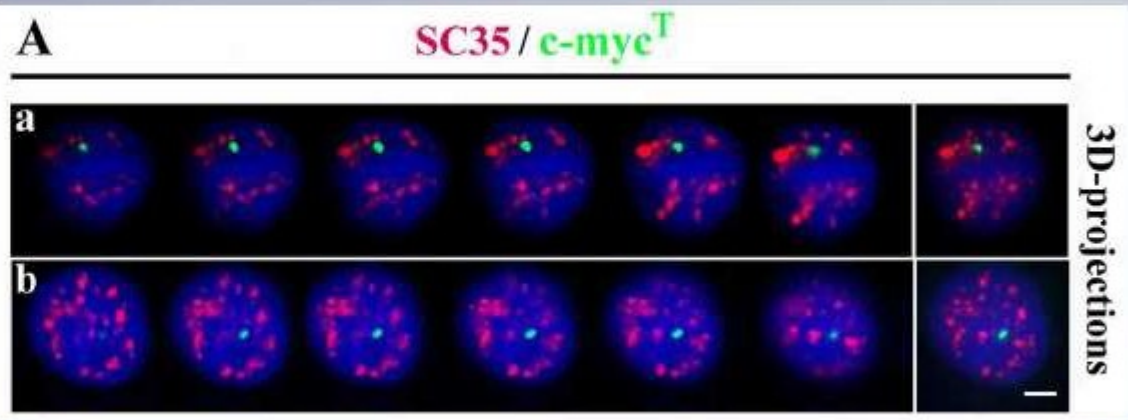




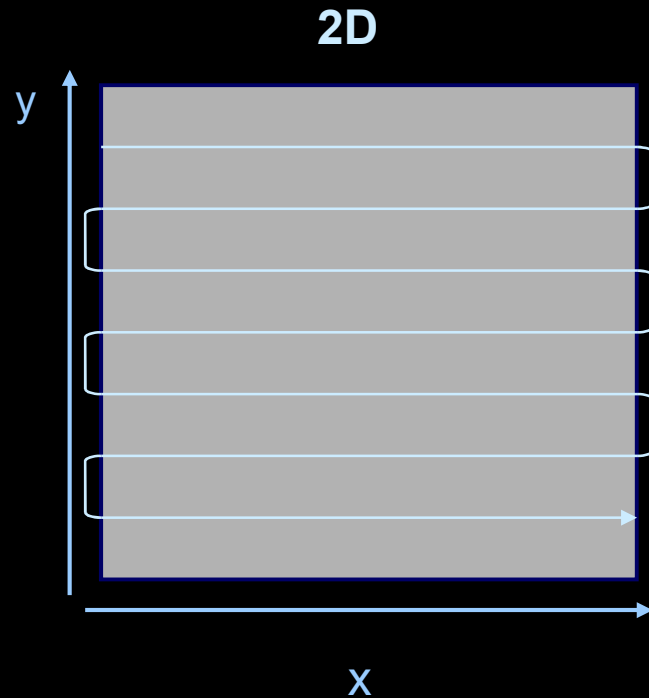
*Game and  
Methodology*



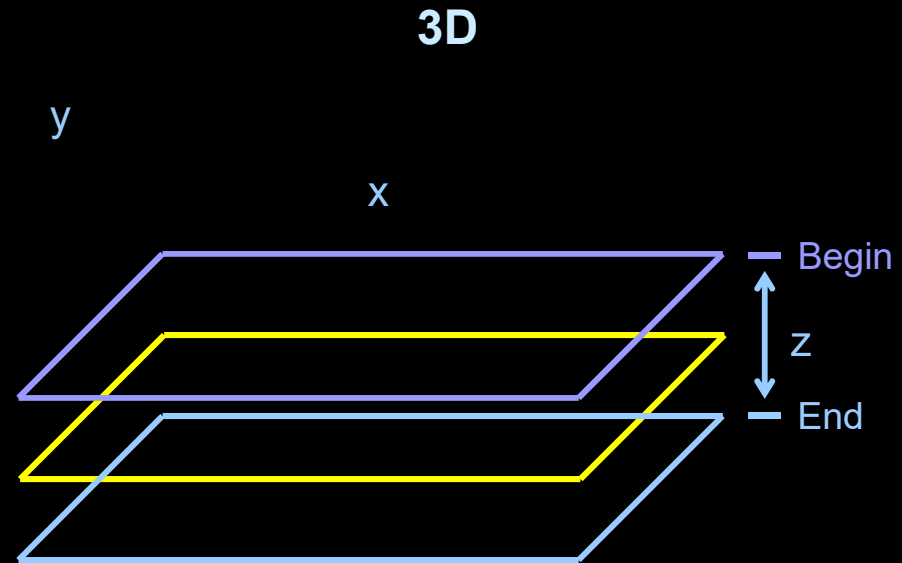
3D-projection



## Scanning in 2D and 3D by confocal microscope

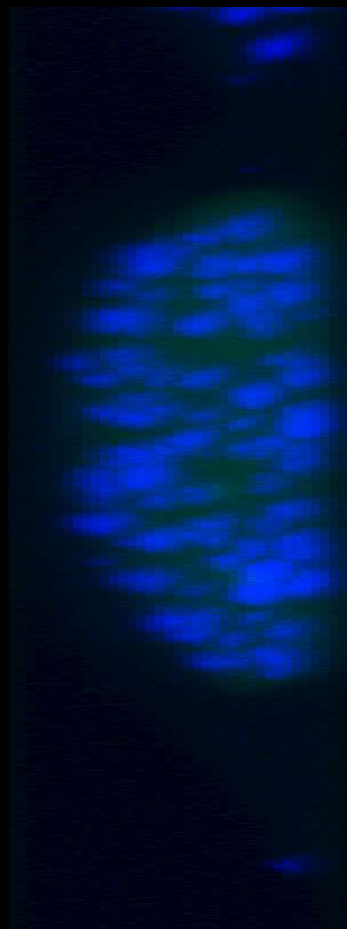
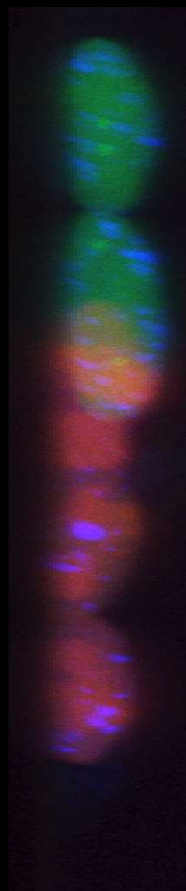


Laser beam moves firstly along x axis and then starts with new line in y axis.



Finishing scanning of one thin optical slice in xy plane, the scanning plane is moved in z axis to other slice

° pos=1



# Deconvolution

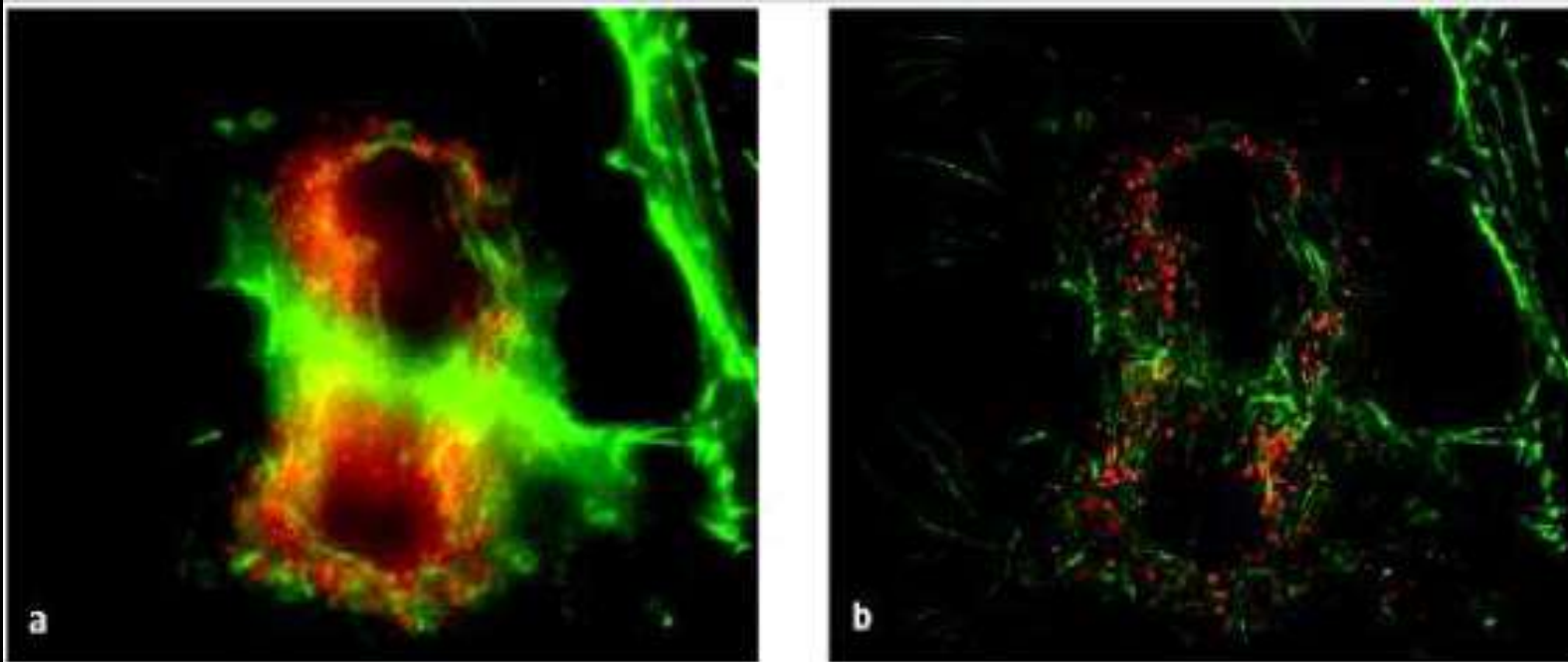


Fig. 3: Via deconvolution, artefacts can be computed out of fluorescence images. a). These artefacts are caused by the stray light from non-focused areas above and below the focus level. These phenomena, referred to as convolution, result in glare, distortion and blurriness. b). Deconvolution is a recognised mathematical procedure for eliminating such artefacts. The resulting image displayed is sharper with less noise and thus at higher resolution. This is also advantageous for more extensive analyses.

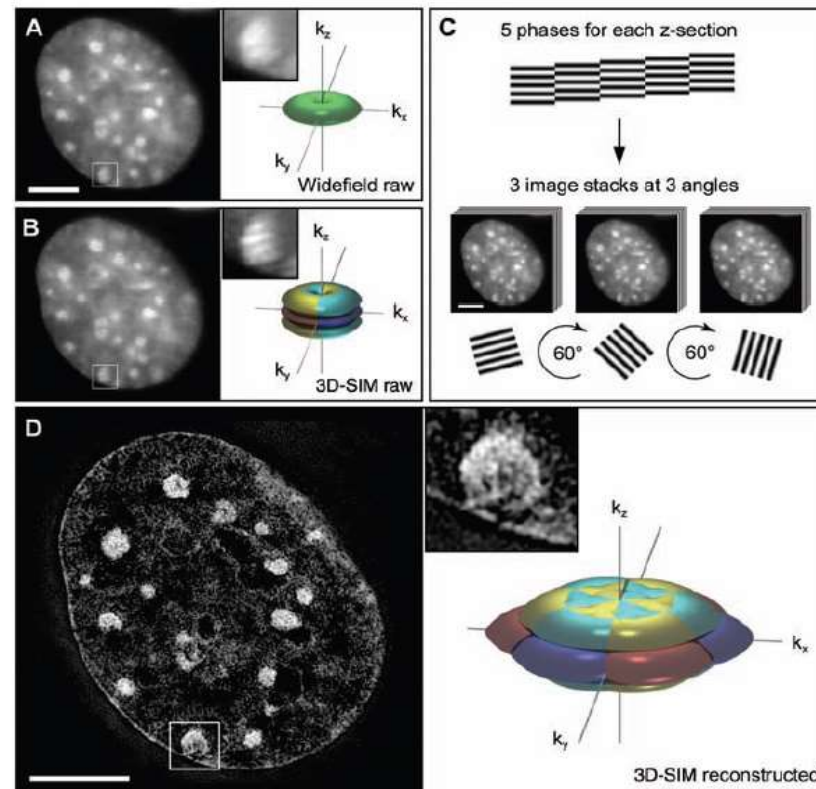
**Assuming linearity, convolution of the object and the imaging system PSF is affected by noise and produces a blurred image. Deconvolution restores the original object to an improved resolution and higher signal-to-noise ratio (SNR) level.**



# Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination Microscopy

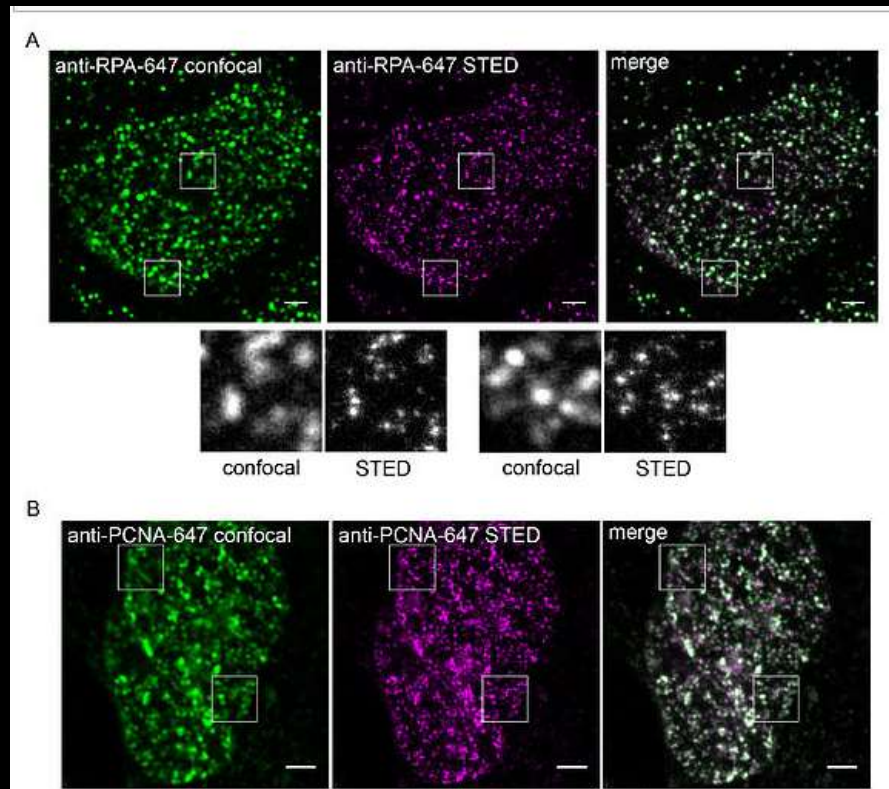
Lothar Schermelleh,<sup>1\*</sup> Peter M. Carlton,<sup>2\*</sup> Sebastian Haase,<sup>2,4</sup> Lin Shao,<sup>2</sup>  
Lukman Winoto,<sup>2</sup> Peter Kner,<sup>2</sup> Brian Burke,<sup>3</sup> M. Cristina Cardoso,<sup>4</sup> David A. Agard,<sup>2</sup>  
Mats G. L. Gustafsson,<sup>5</sup> Heinrich Leonhardt,<sup>1\*†</sup> John W. Sedat<sup>2\*†</sup>

**Fig. 1.** Subdiffraction resolution imaging with 3D-SIM. (A and B) Cross section through a DAPI-stained C2C12 cell nucleus acquired with conventional wide-field illumination (A) and with structured illumination (B), showing the striped interference pattern (inset). The renderings to the right illustrate the respective support of detection in frequency space. The axes  $k_x$ ,  $k_y$ , and  $k_z$  indicate spatial frequencies along the  $x$ ,  $y$ , and  $z$  directions. The surfaces of the renderings represent the corresponding resolution limit. The depression of the frequency support ("missing cone") in  $z$  direction in (A) indicates the restriction in axial resolution of conventional wide-field microscopy. With 3D-SIM, the axial support is extended but remains within the resolution limit. (C) Five phases of the sine wave pattern are recorded at each  $z$  position, allowing the shifted components to be separated and returned to their proper location in frequency space. Three image stacks are recorded with the diffraction grating sequentially rotated into three positions  $60^\circ$  apart, resulting in nearly rotationally symmetric support over a larger region of frequency space. (D) The same cross section of the reconstructed 3D-SIM image shows enhanced image details compared with the original image (insets). The increase in resolution is shown in frequency space on the right, with the coverage extending two times farther from the origin. Scale bars indicate  $5\ \mu\text{m}$ .

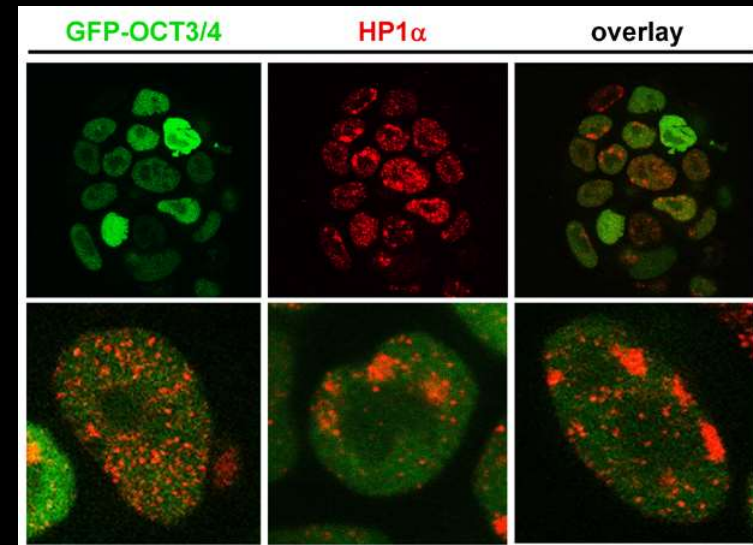


**Stimulated Emission Depletion microscopy, or STED microscopy, is a fluorescence microscopy technique that uses the non-linear de-excitation of fluorescent dyes to overcome the resolution limit imposed by diffraction with standard confocal laser scanning microscopes and conventional far-field optical microscopes.**

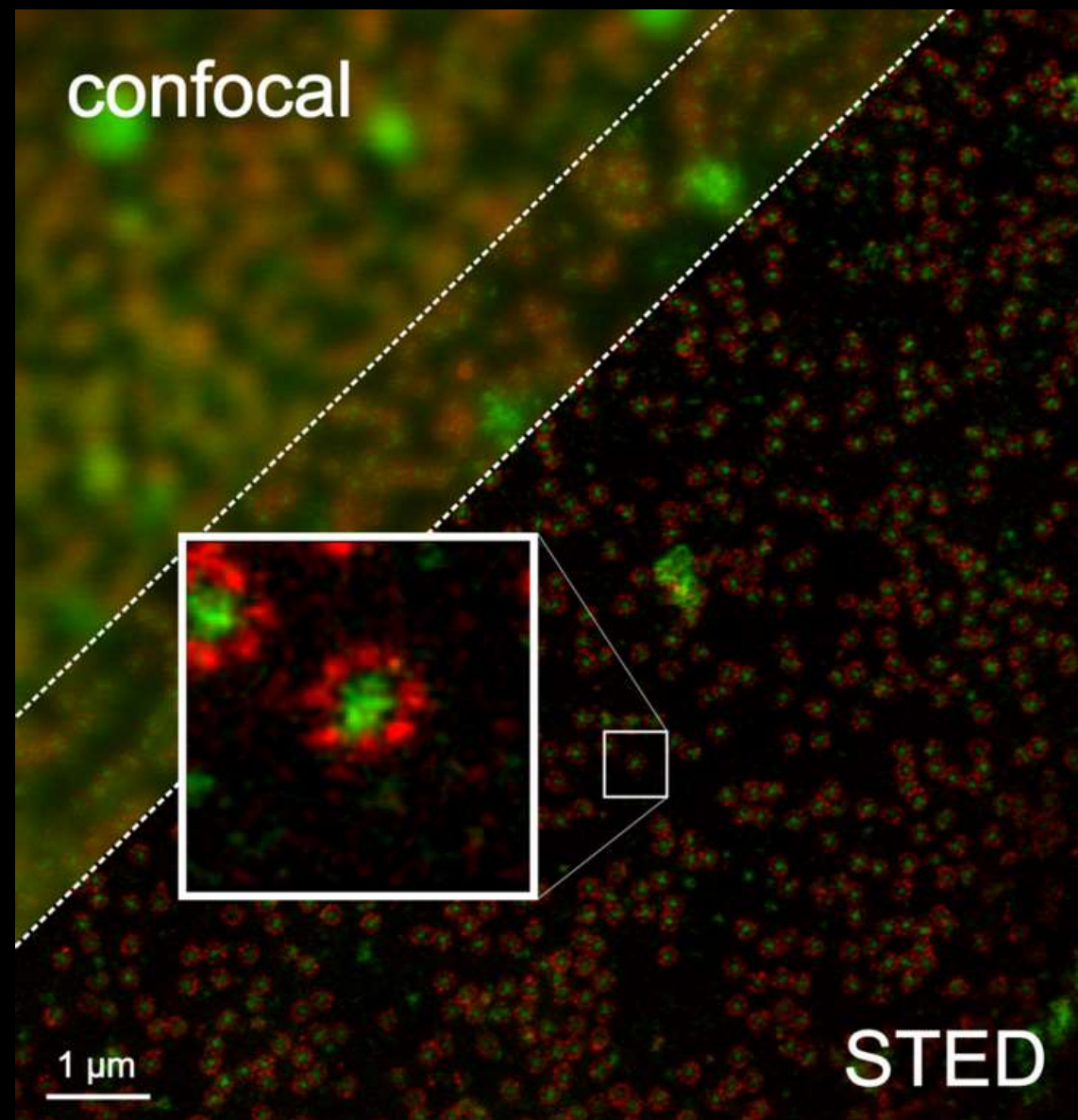
## **STED**



## **SP-5 LSCM**

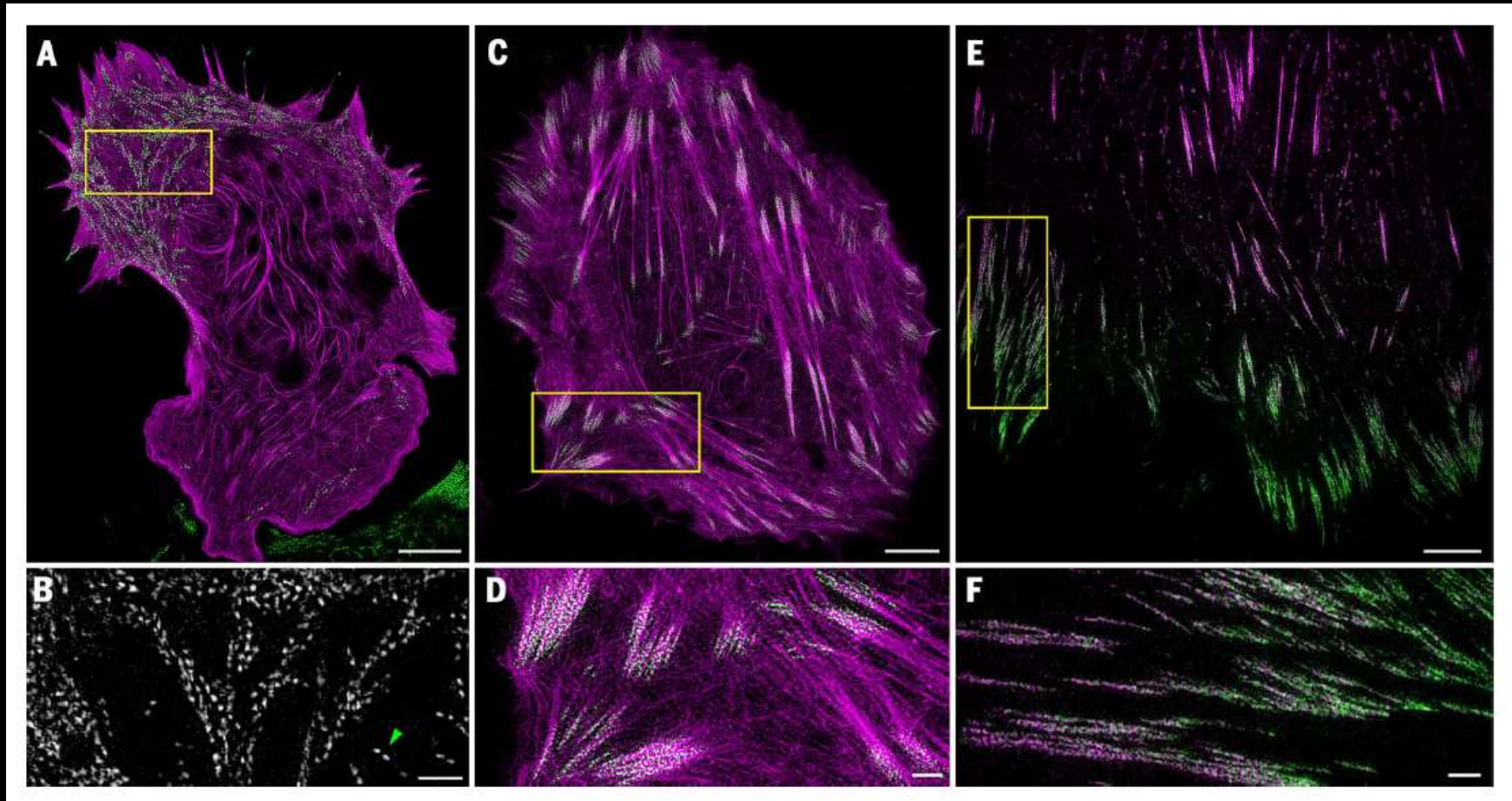








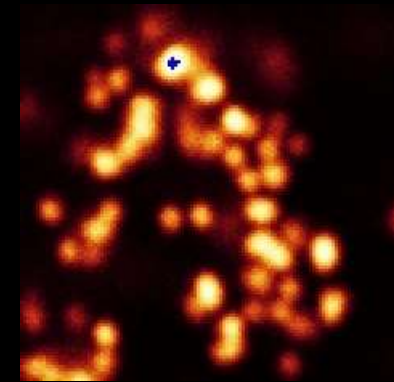
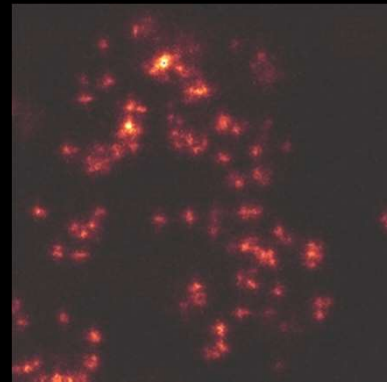
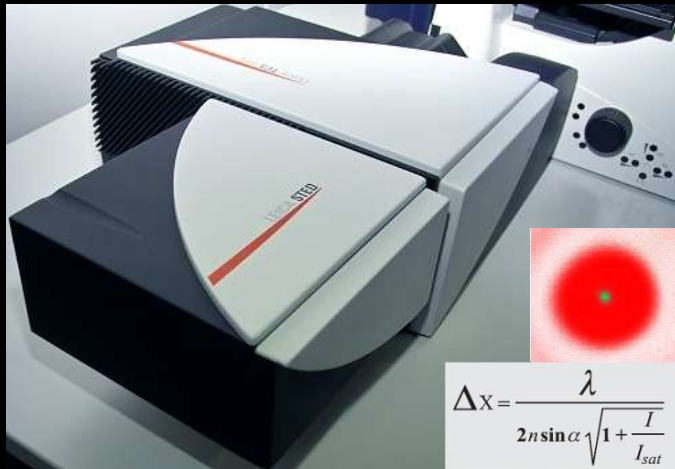
# High-speed live-cell imaging



Li et al., Science (2015)

# Leica STED – STimulated Emission Depletion SUPERRESOLUTION (subdiffraction) in xy plane

Hell, S. W. and J. Wichmann (1994). *Opt. Lett.*  
"Breaking the diffraction resolution limit by stimulated emission"



neurobiology  
membrane biology  
membrane rafts  
intracellular transport

Willig KI et al. *Nature* 2006

Sieber JJ et al. *Biophys J* 2006

Kittel RJ et al. *Science* 2006

Fitzner D et al. *EMBO J* 2006

Kellner RR et al. *Neuroscience* 2007

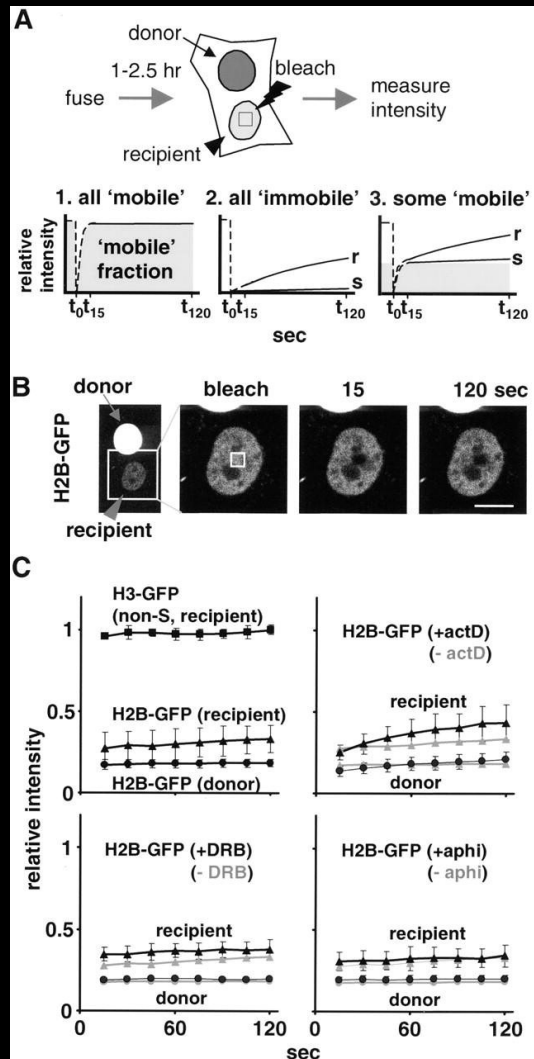
Lin W et al. *PNAS* 2007

Seebach J *Cardiovas. Res.* 2007

Sieber JJ *Science* 2007

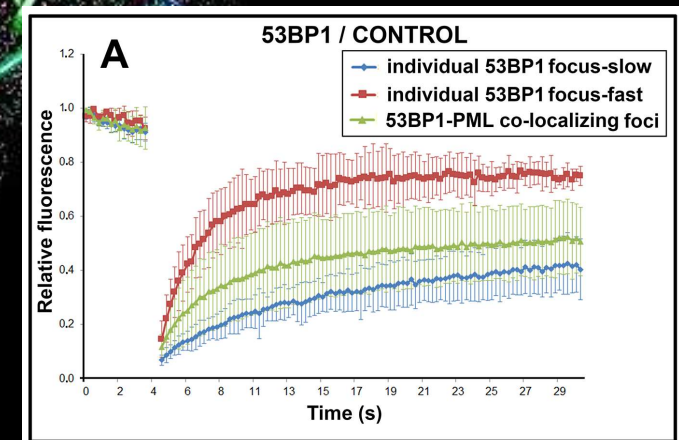
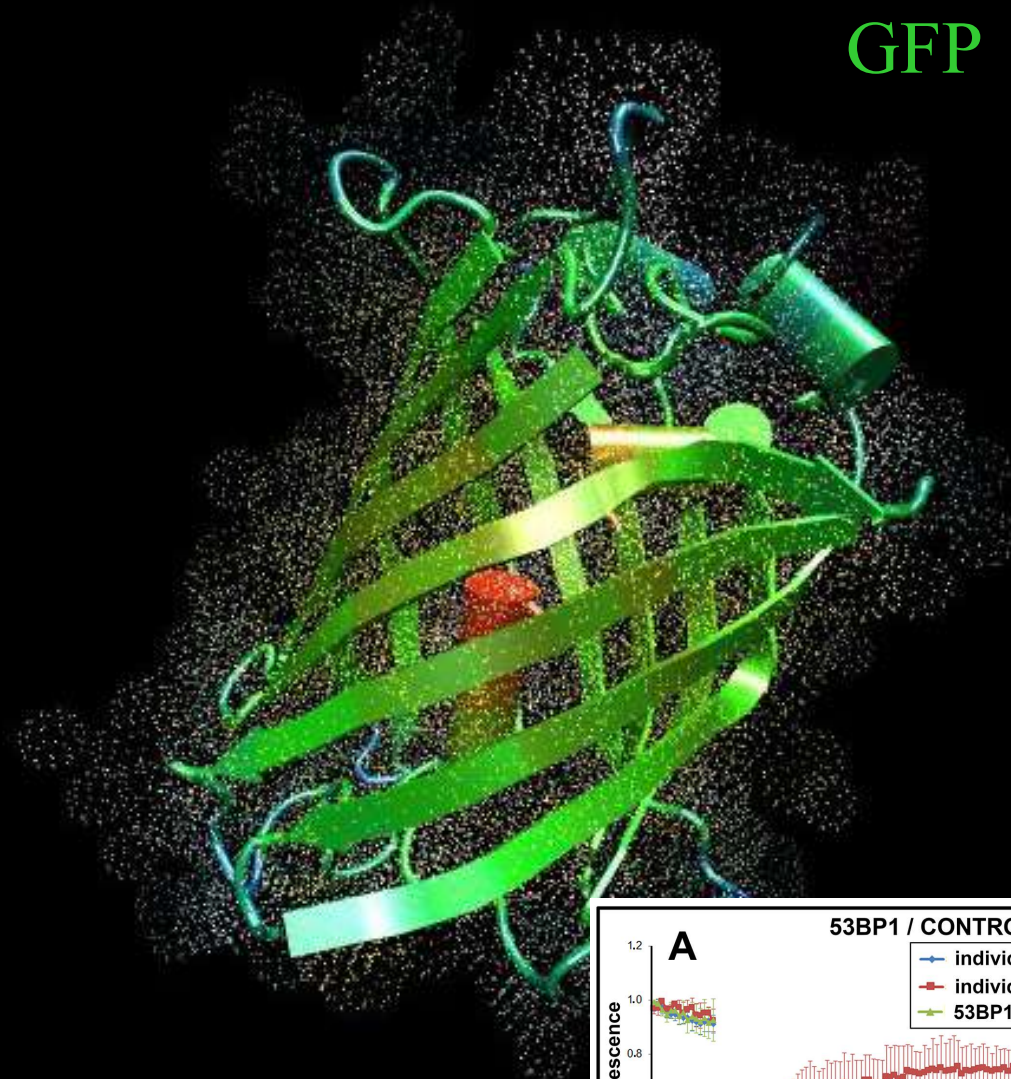
Unique





**JCB**

Kimura and Cook (2001)





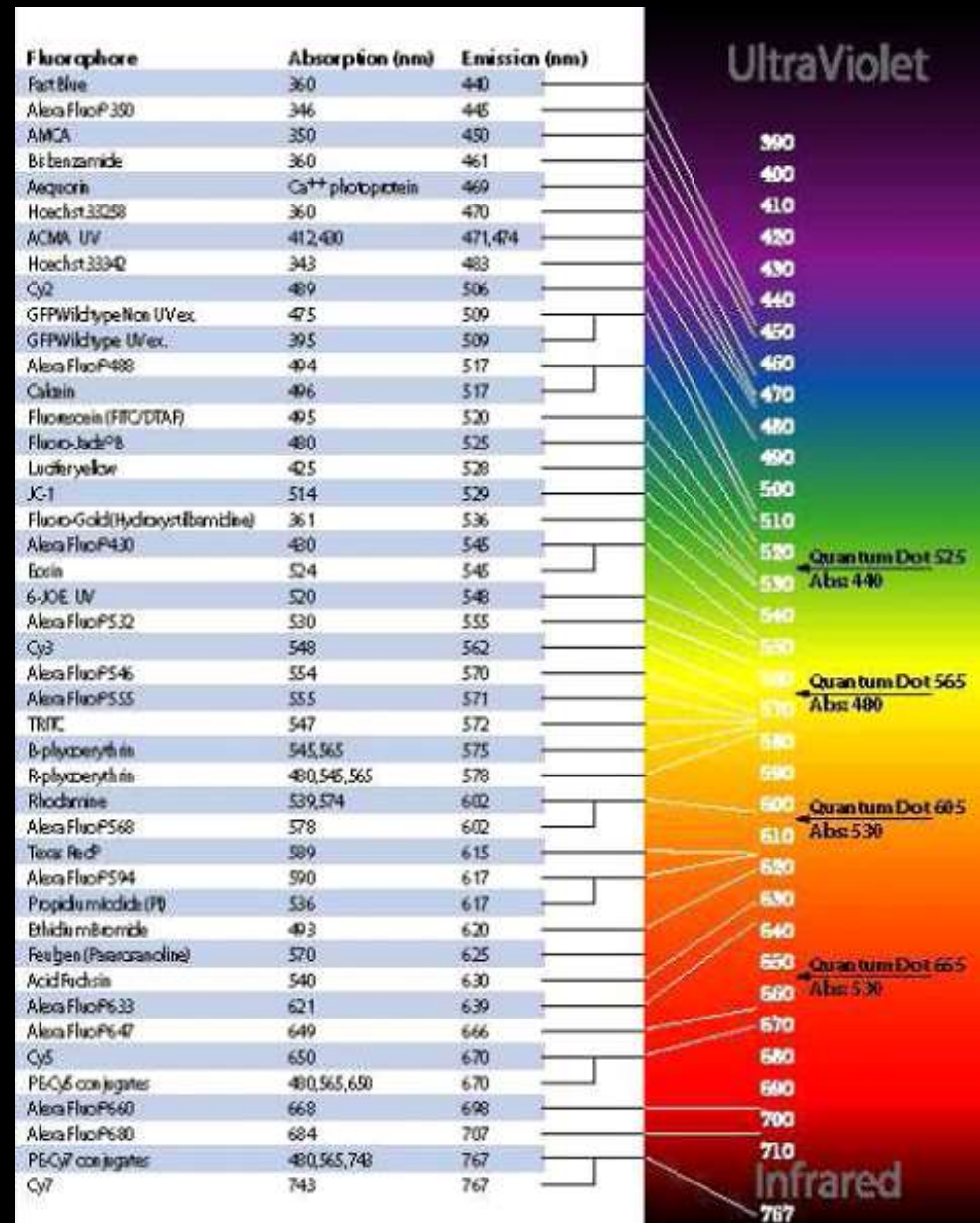
# Types of fluorochromes

Fluorochromes are essentially dyes, which accept light energy (e.g. from a laser) at a given wavelength and re-emit it at a longer wavelength. These two processes are called excitation and emission.

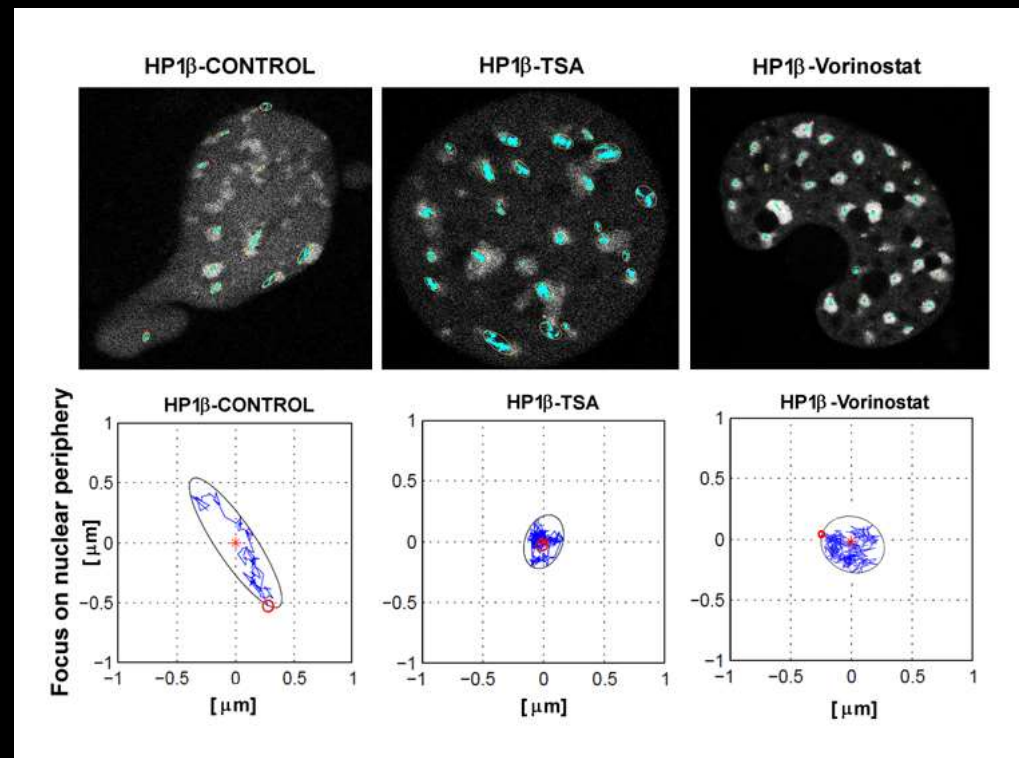
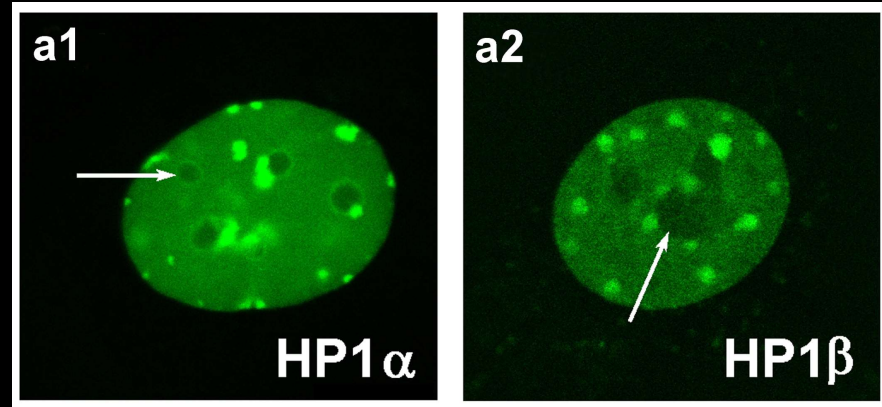
1. Fluorochromes conjugated with other molecule. Example represents quantum dots, used for ultra-sensitive nonisotopic detection.

2. Fluorochromes that binds directly to some structure. For example, DAPI or PI binds to DNA

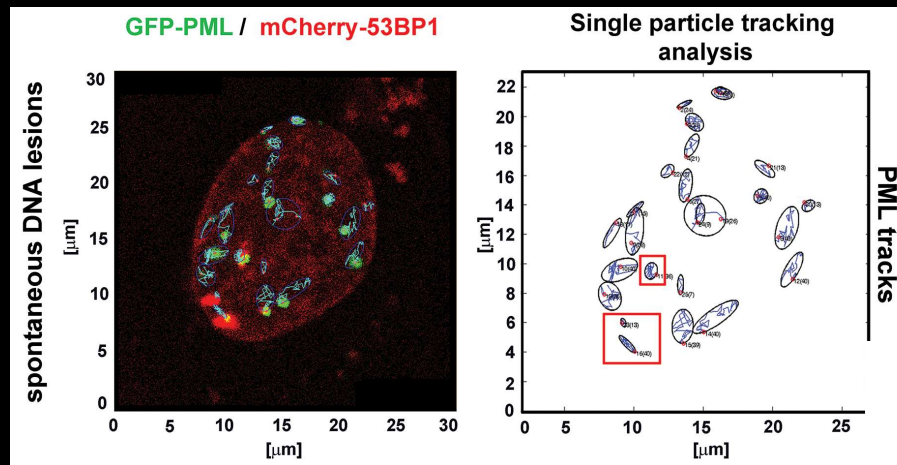
3. Fluorochromes produced by organism like *Aequorea victoria* (GFP) or octocoral *Dendronephthya sp.* (Dendra2)



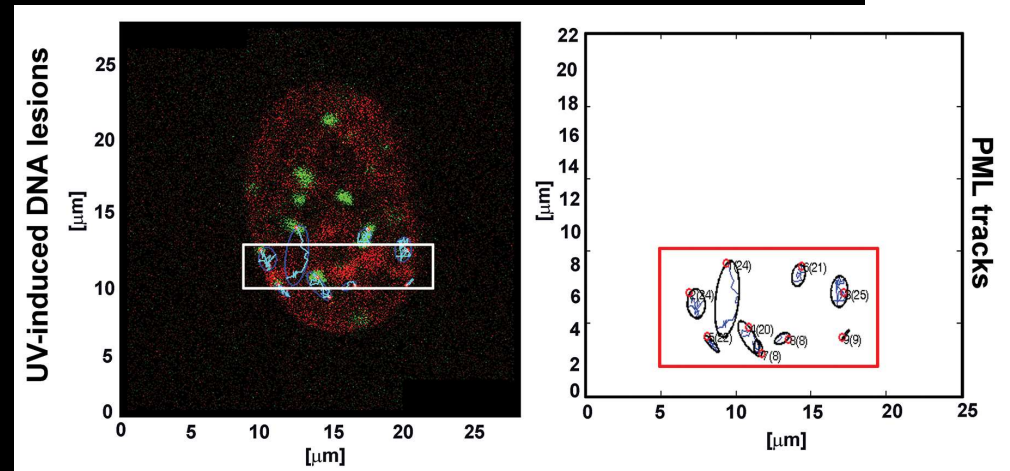
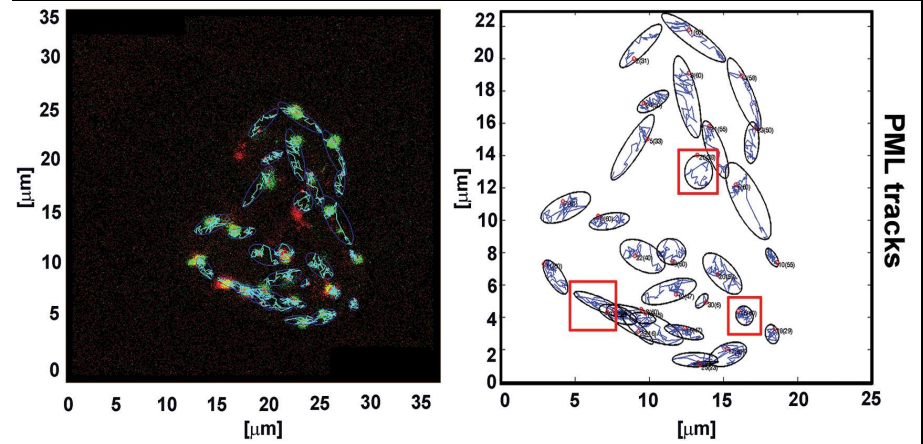
# Single particle tracking



Experiments of Lenka Stixová and Pavel Matula



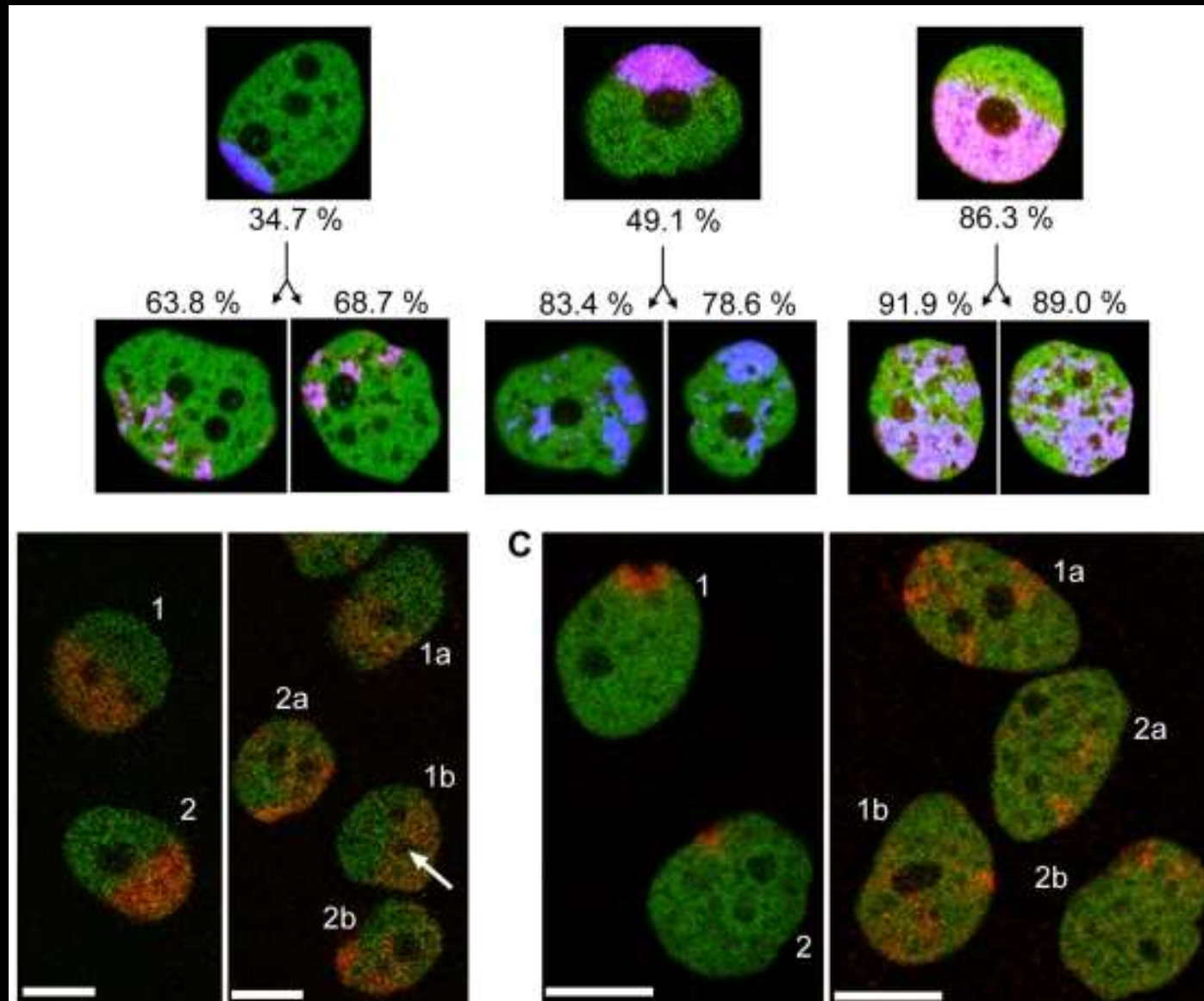
IRIF



Experiments of Veronika Foltanková and Dmitry Sorokin

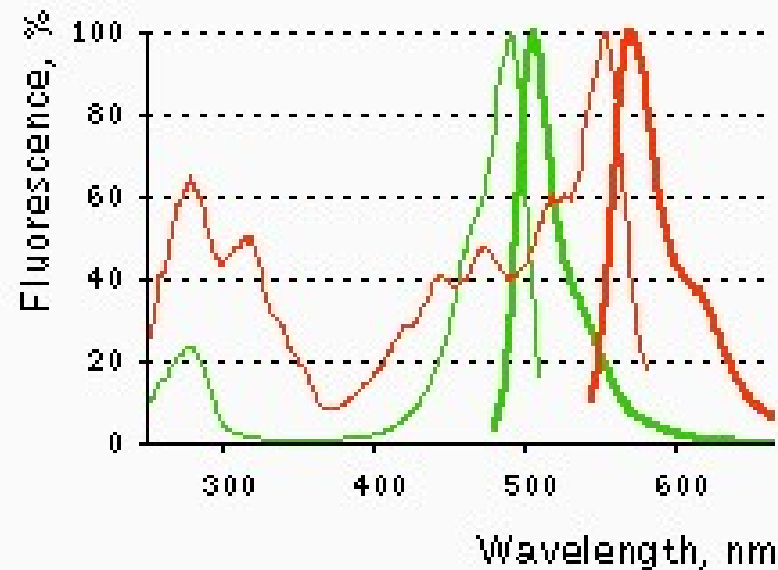


# Dendra2 photo-conversion





**Dendra2 is an improved version of a green-to-red photoswitchable fluorescent protein Dendra, derived from octocoral *Dendronephthya* sp. (Gurskaya *et al.*, 2006).**

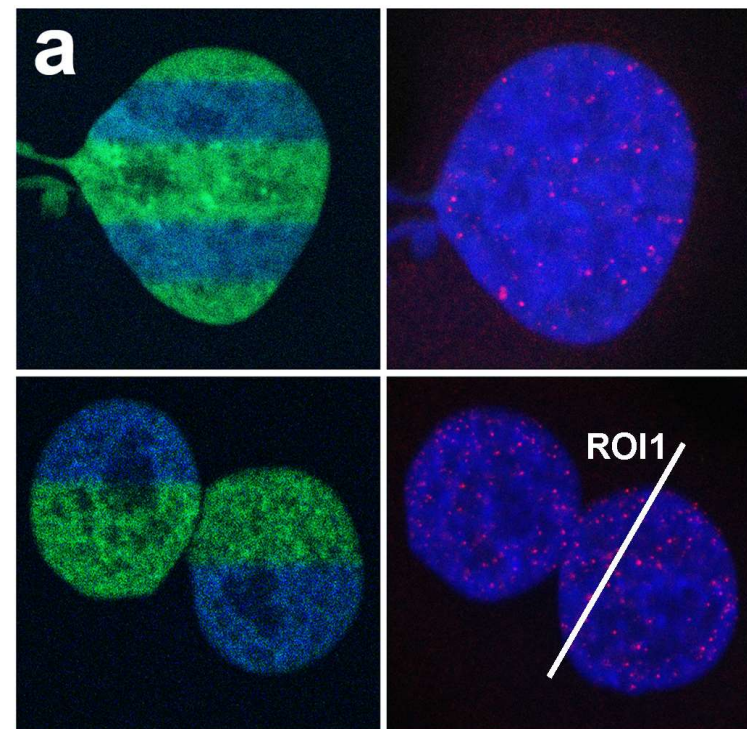


**Normalized excitation (thin line) and emission (thick line) for non-activated (green) and activated (red) spectra.**

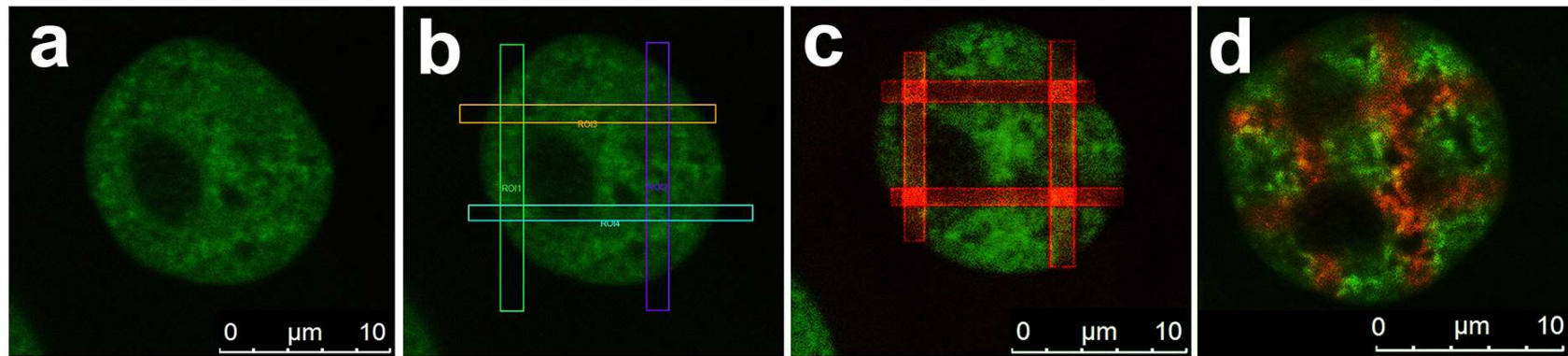
**Photoconversion by UV laser**

**H4-Dendra2**

**CPDs / Nucleus**



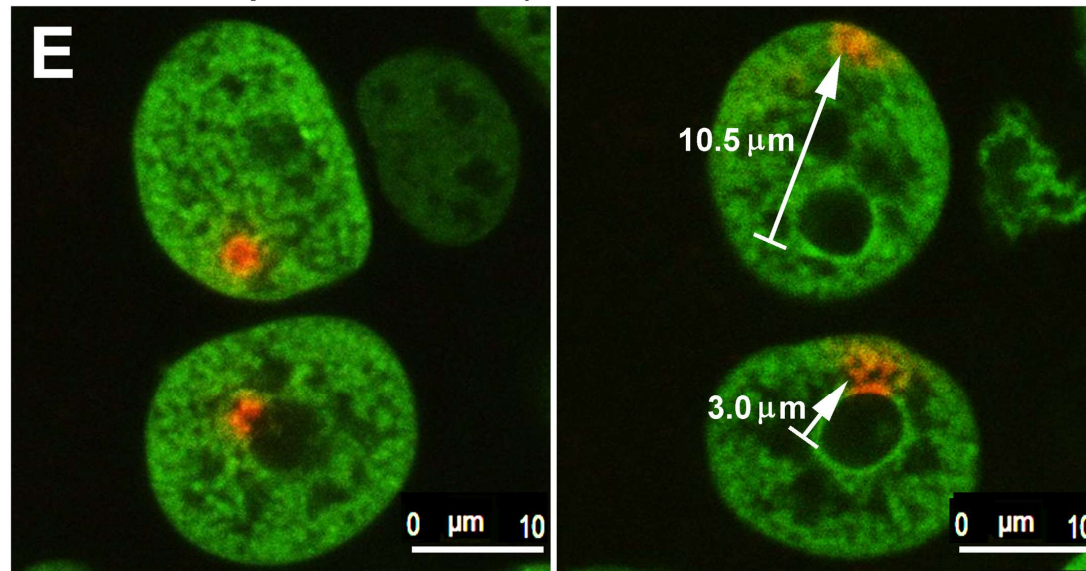
## Dendra2-H4 photoconversion



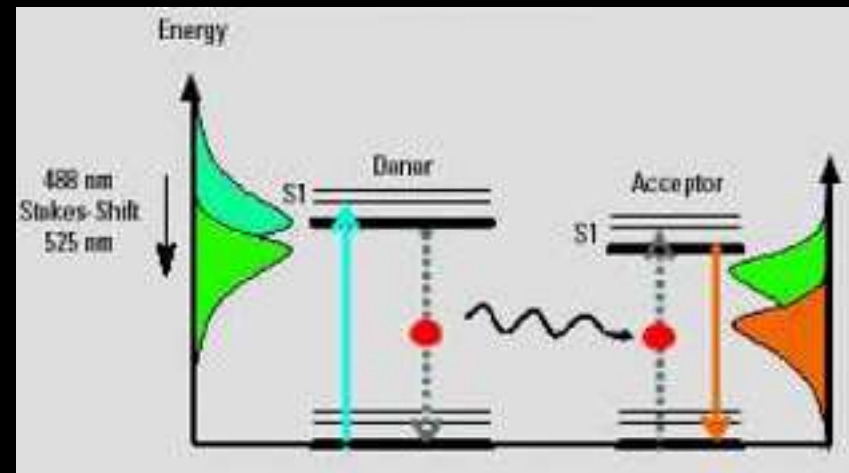
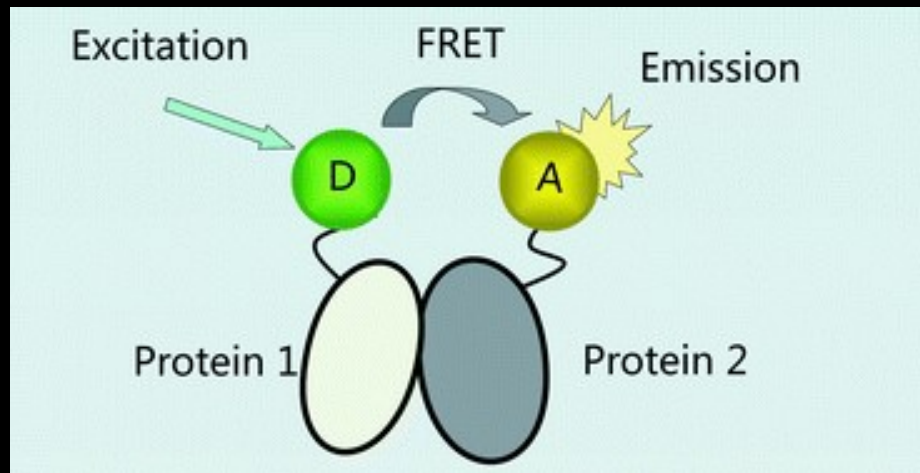
## H4-Dendra2 / H4-Dendra2

Actinomycin-D  
(0 min. after photoconversion)

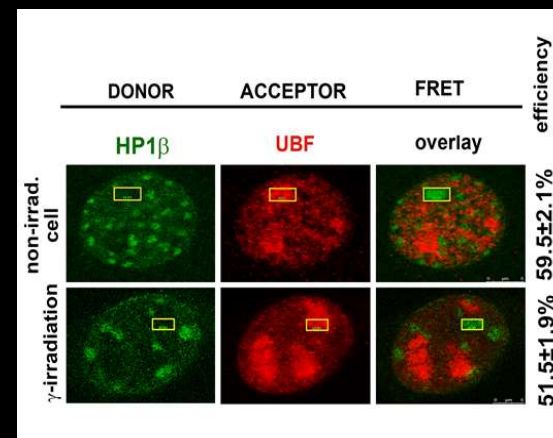
Actinomycin-D (90 min.)



**FRET (Fluorescence Resonance Energy Transfer)** is a technique for measuring interactions between two proteins *in vivo*. In this technique, two different fluorescent molecules (fluorophores) are genetically fused to the two proteins of interest.

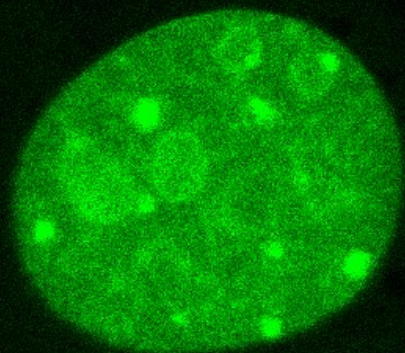


<http://www.rsc.org/publishing/journals/>



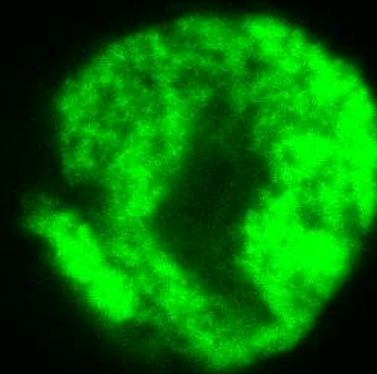


7/8/2010 9:30:30 AM



t=0.000sec

5/20/2010 2:01:52 PM



t=0.000sec

0  $\mu$ m 10

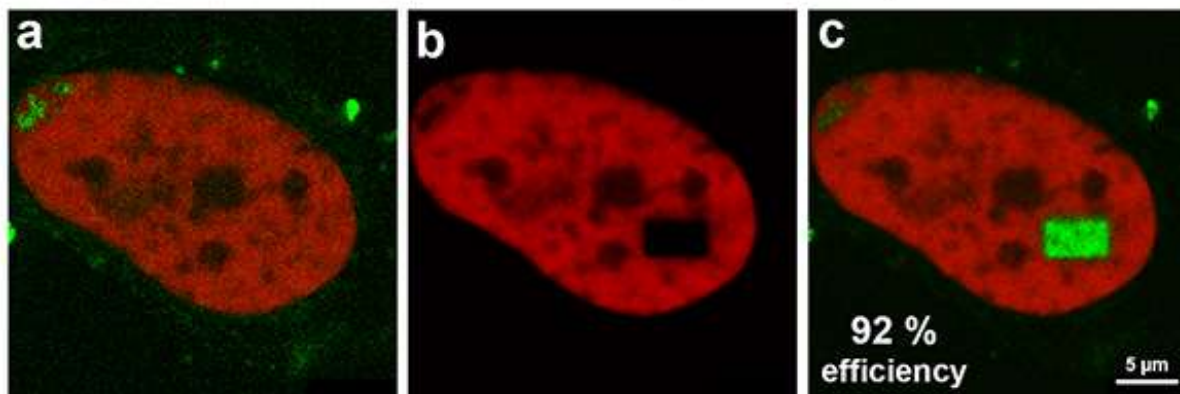
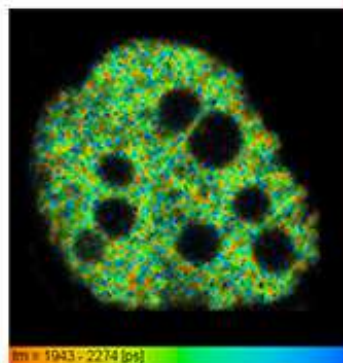
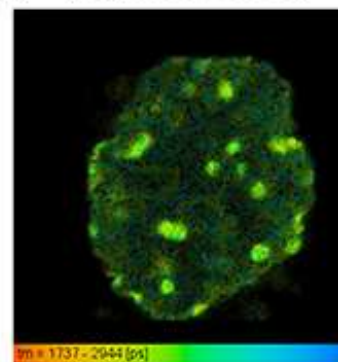


**A****FRET AB analysis****p53 / 53BP1**

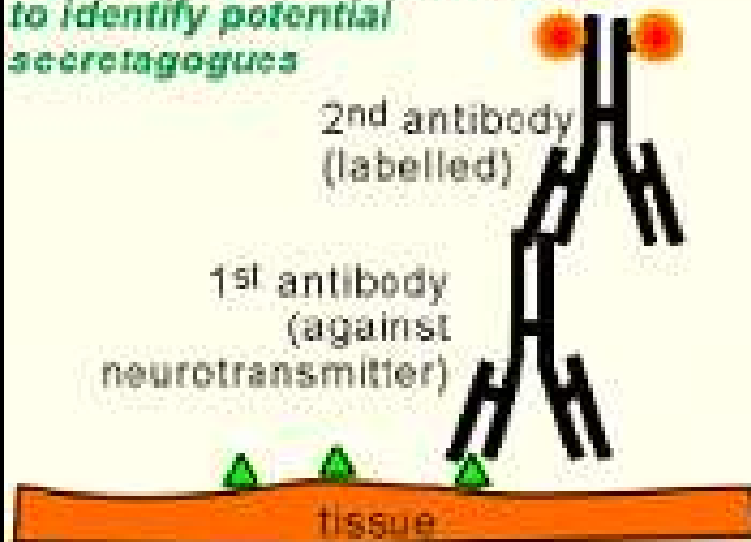
pre-bleach

bleaching

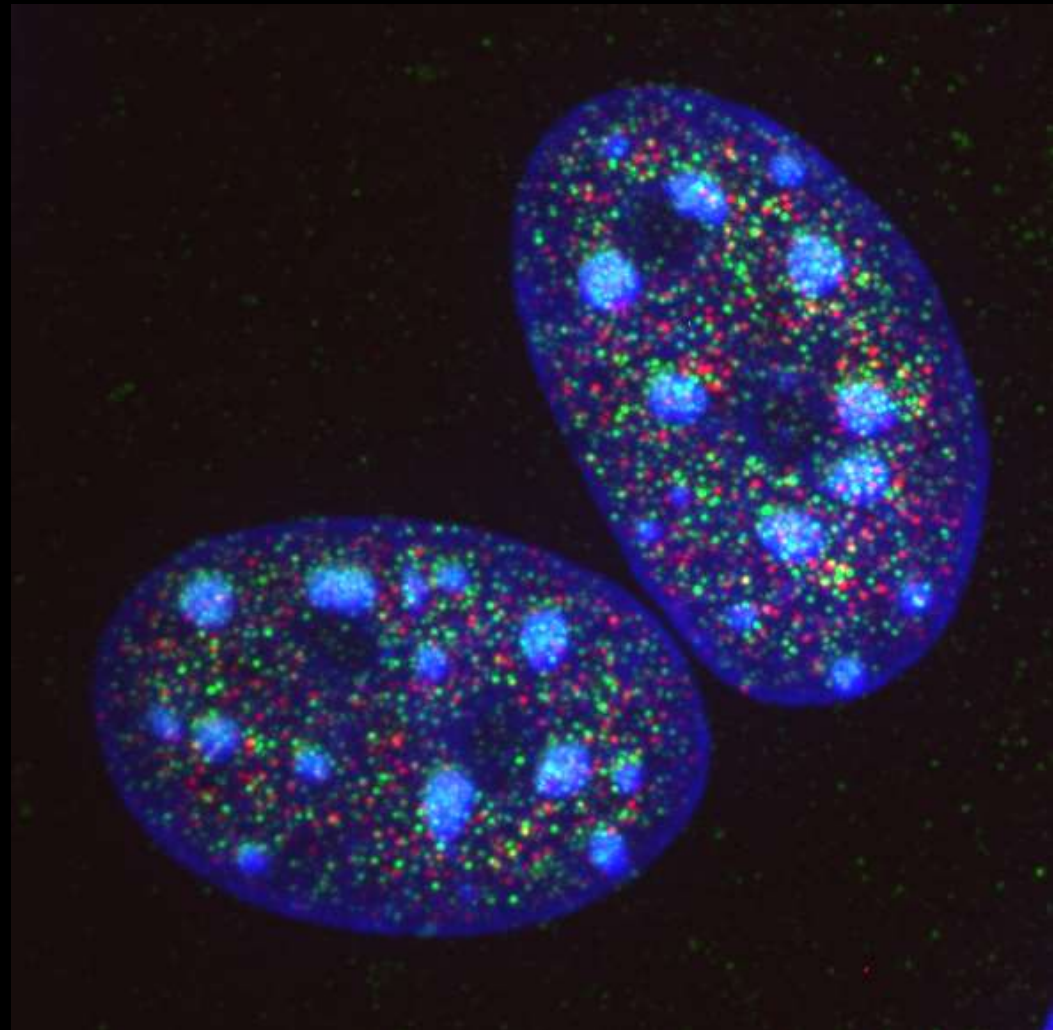
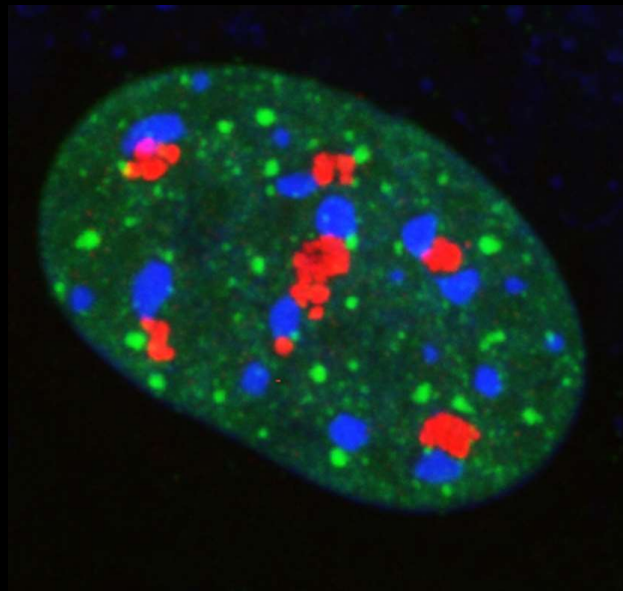
post-bleach

**B****FLIM-FRET analysis****a** p53 / 53BP1  
(positive control)lifetime  
histogram**b** HP1 $\beta$  / 53BP1  
(negative control)lifetime  
histogram

**Immunocytochemistry**  
to identify potential  
secretagogues

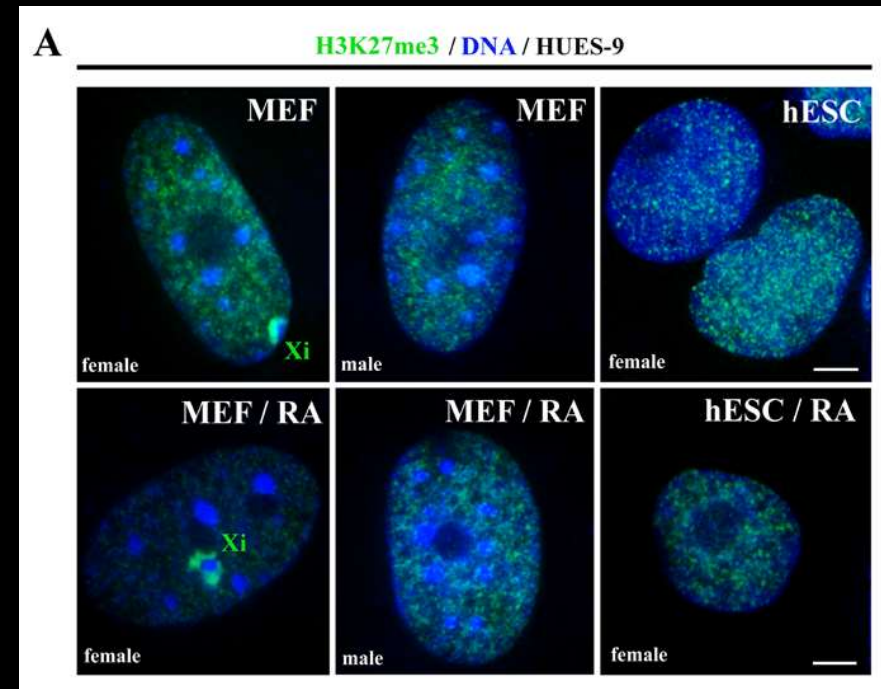
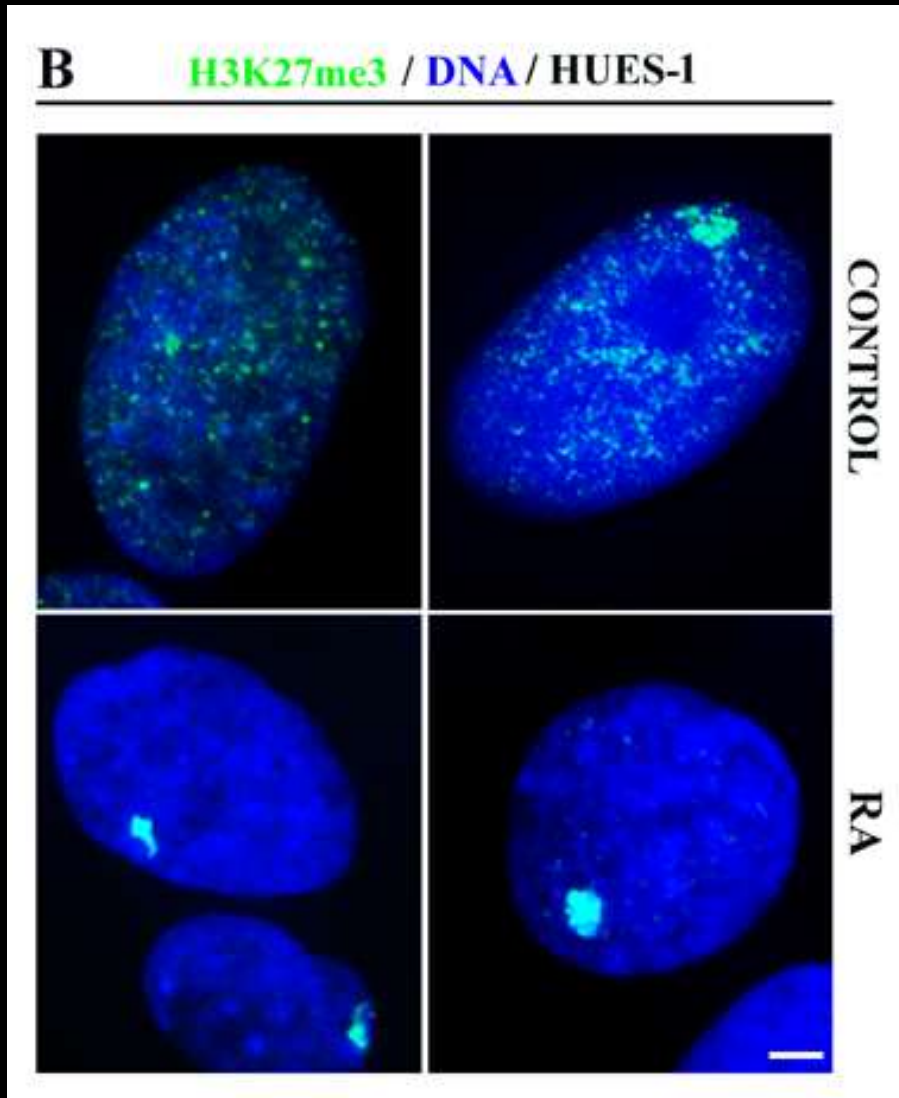


[http://www.celanphy.science.ru.nl/  
Bruce%20web/construction.htm](http://www.celanphy.science.ru.nl/Bruce%20web/construction.htm)



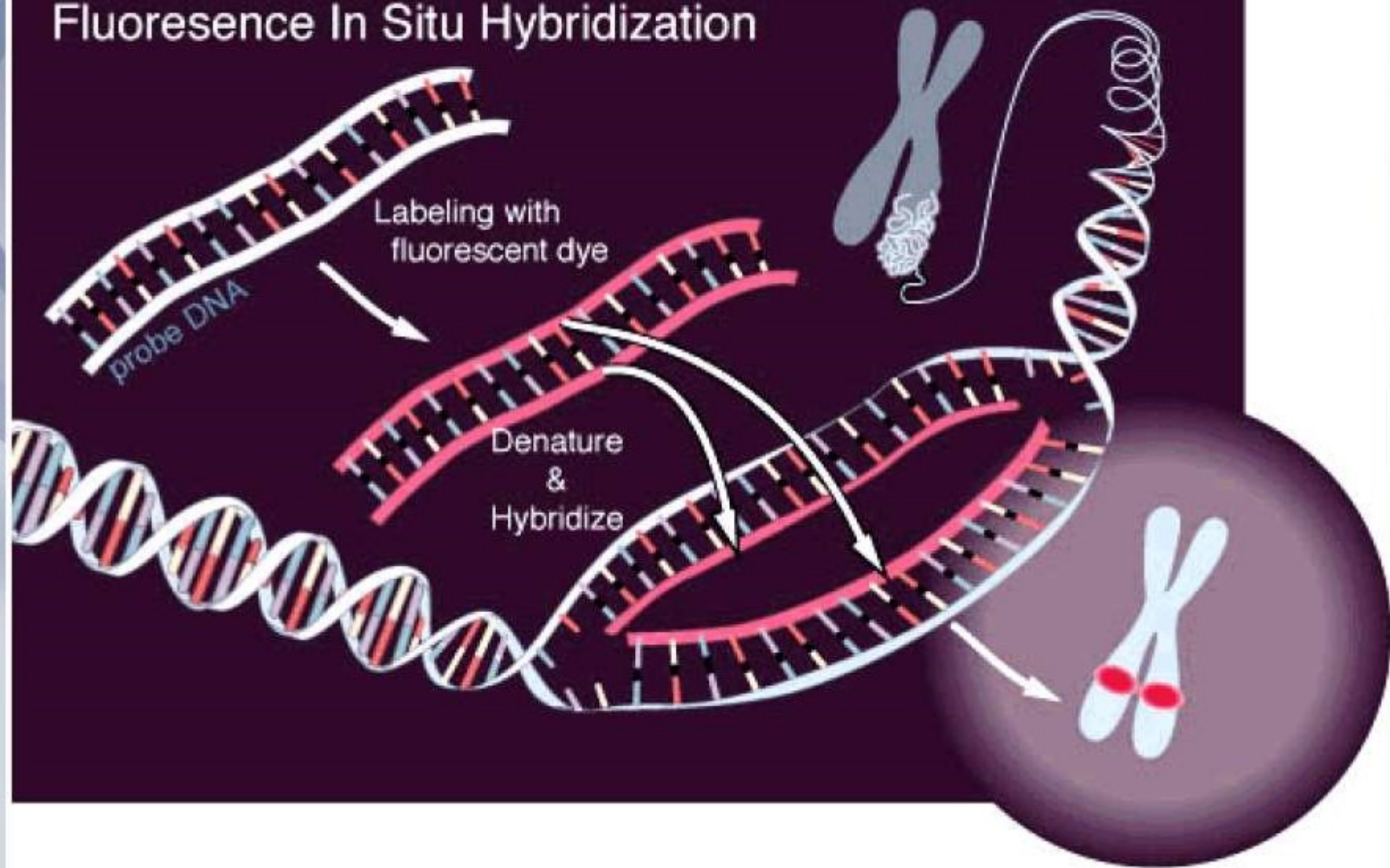
EB group, IBP, Brno

# Inactivation of X chromosome in hESCs in comparison to MEFs

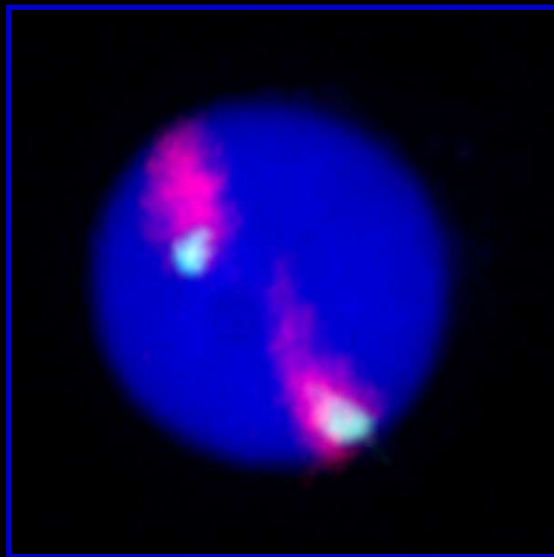
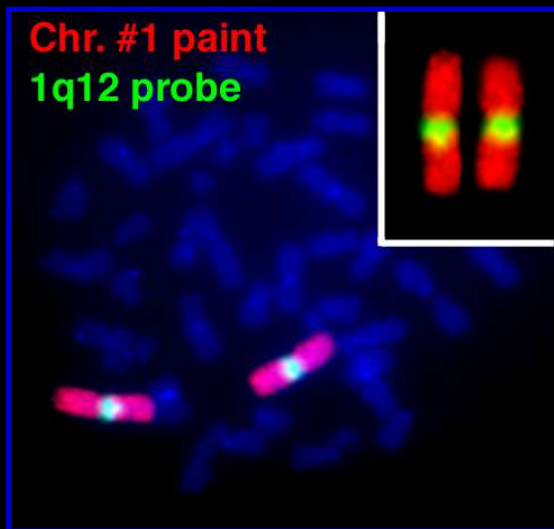




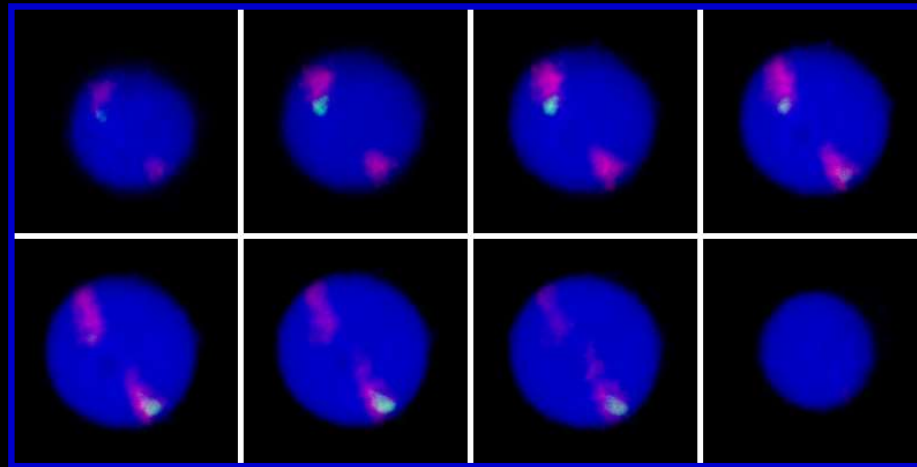
## Fluorescence In Situ Hybridization



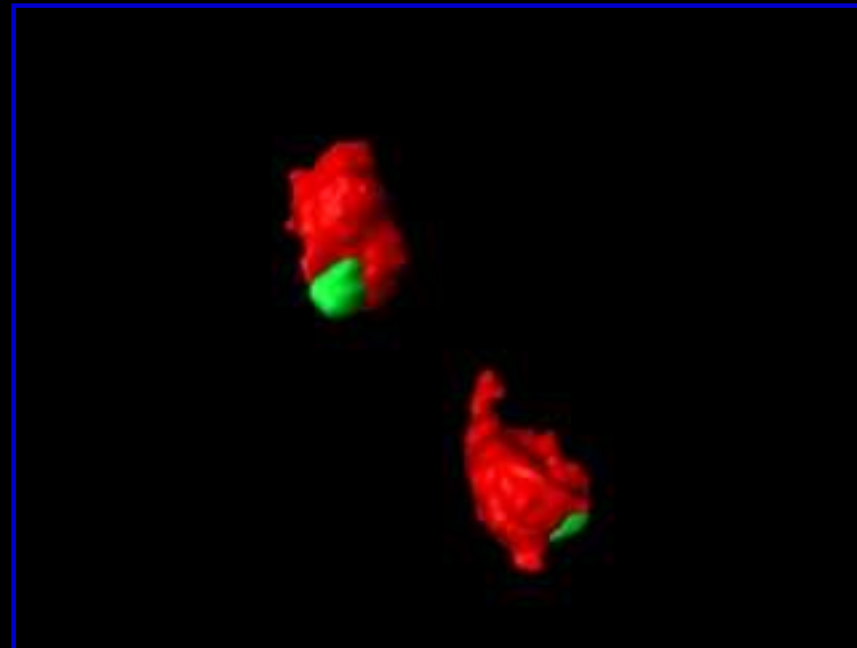
# 3D-FISH a konfokální mikroskopie



Maximální obraz  
Všech řezů

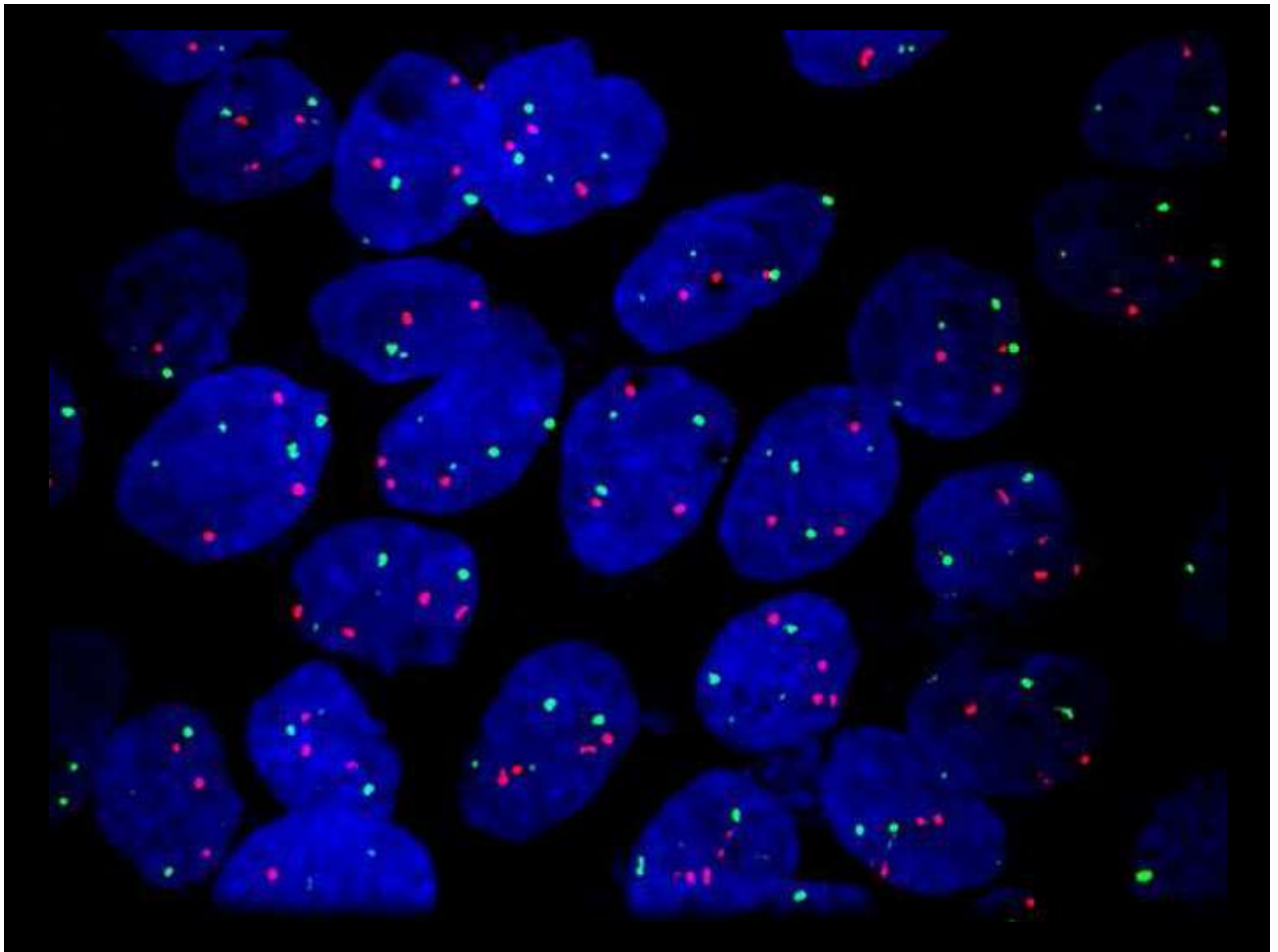


Galerie optických řezů



3D rekonstrukce CT

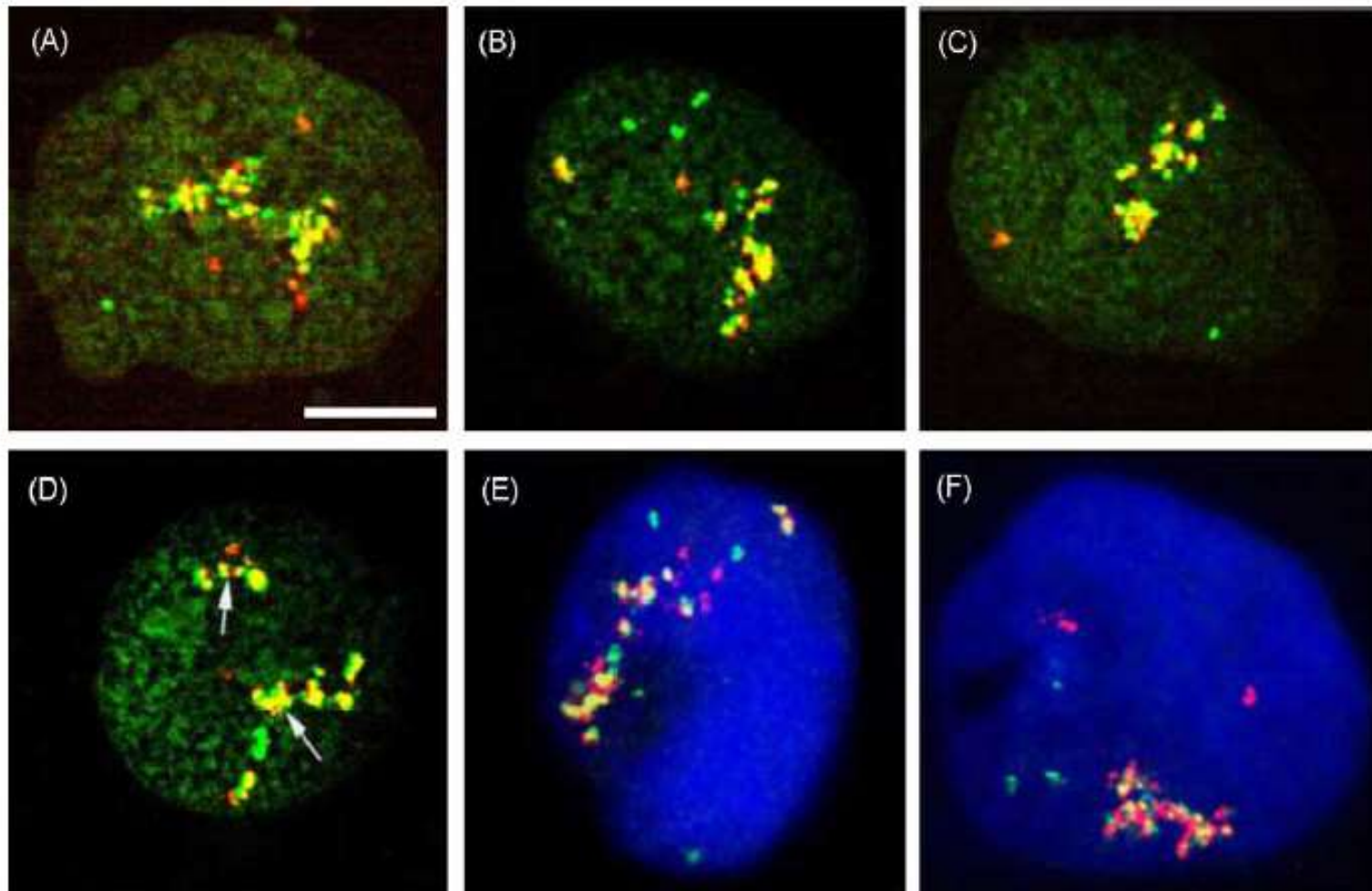
Weierich et al., (2003) in press





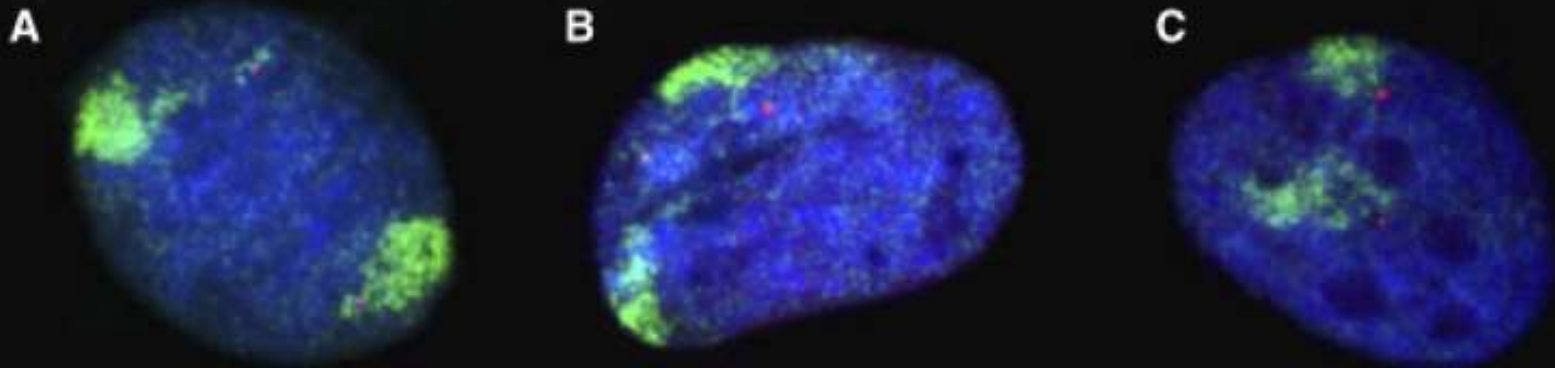
# K562 cells t(9;22)

*E. Bártová et al. / Leukemia Research 29 (2005) 901–913*



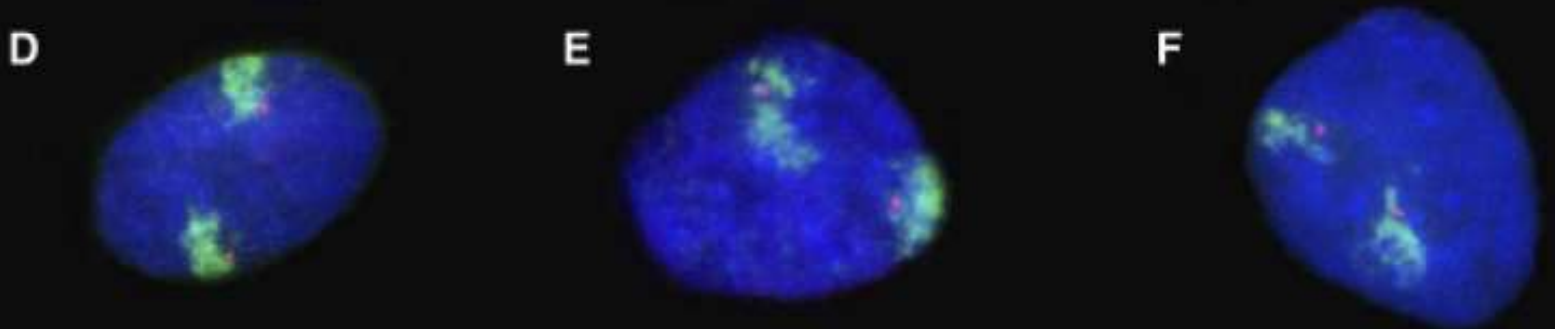
**Oct4 / HSA 6 in hES cells**

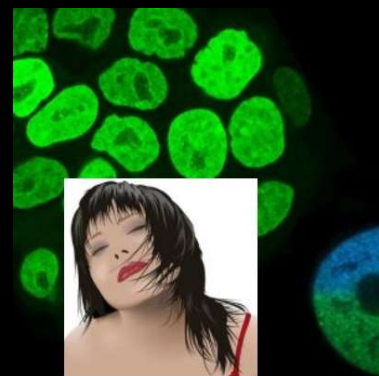
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**Oct4 / HSA 6 in hES cells - RA differentiated**

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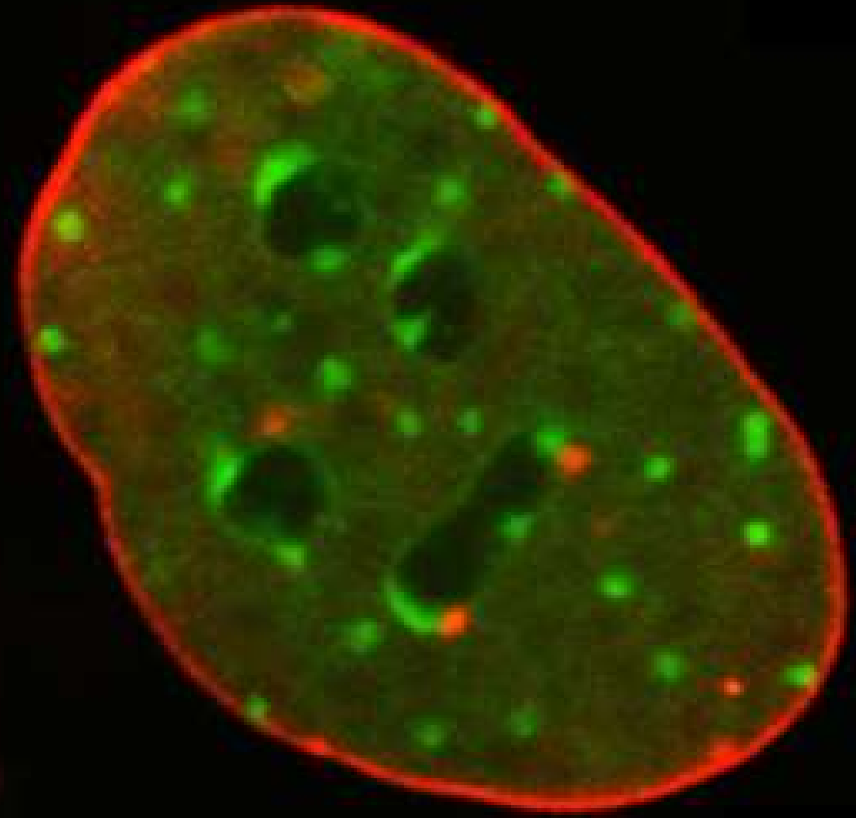
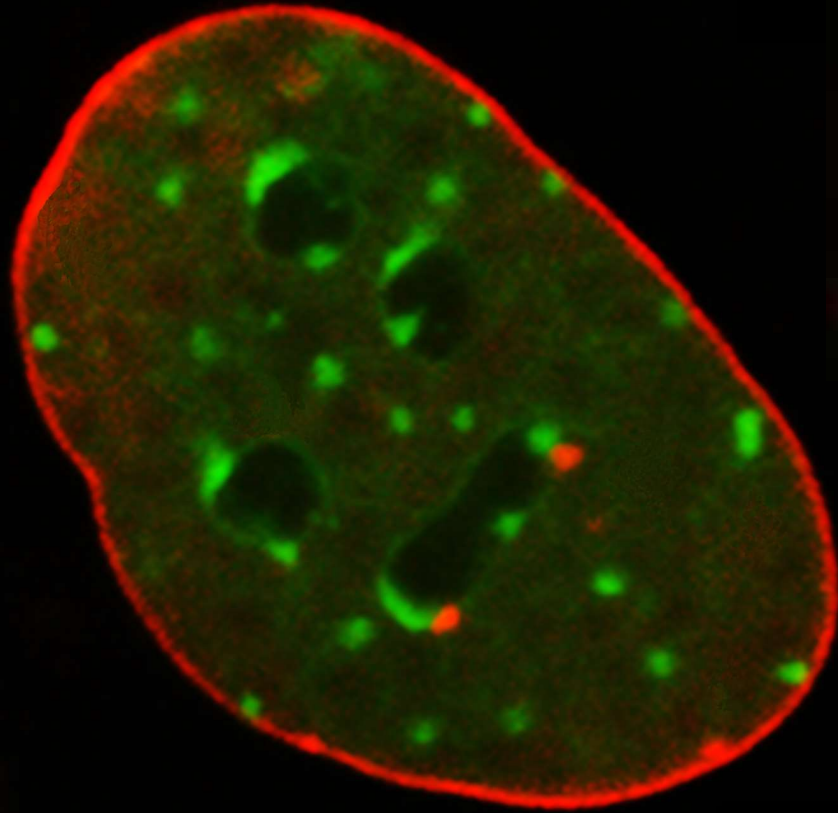




*Lenka Stixová, Sona Legartová,  
Petra Sehnalová, Jana Suchánková,  
Dmitry V. Sorokin*







*Game*