Reparace DNA a efekty HDAC a PRMT1 inhibitorů

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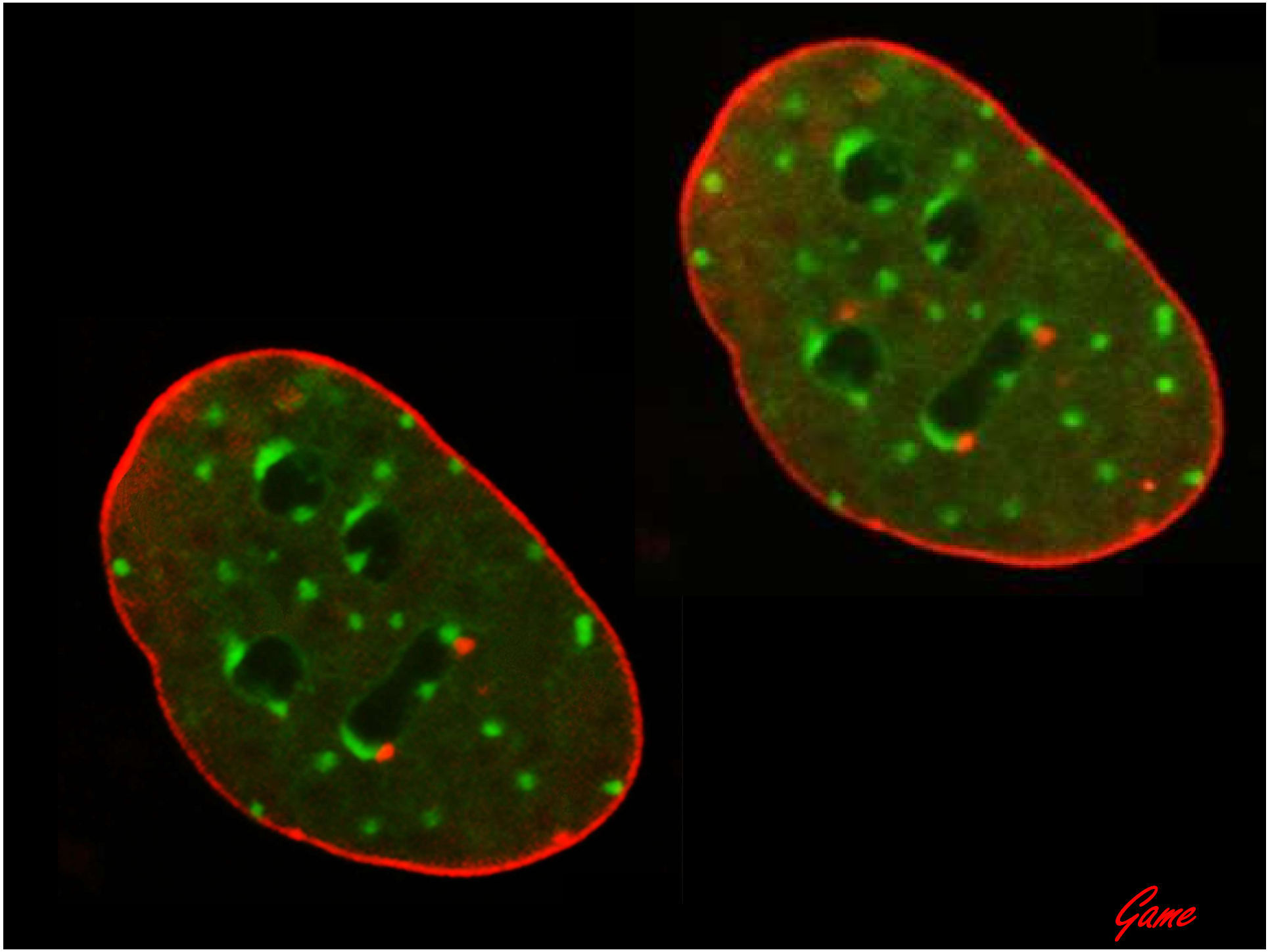
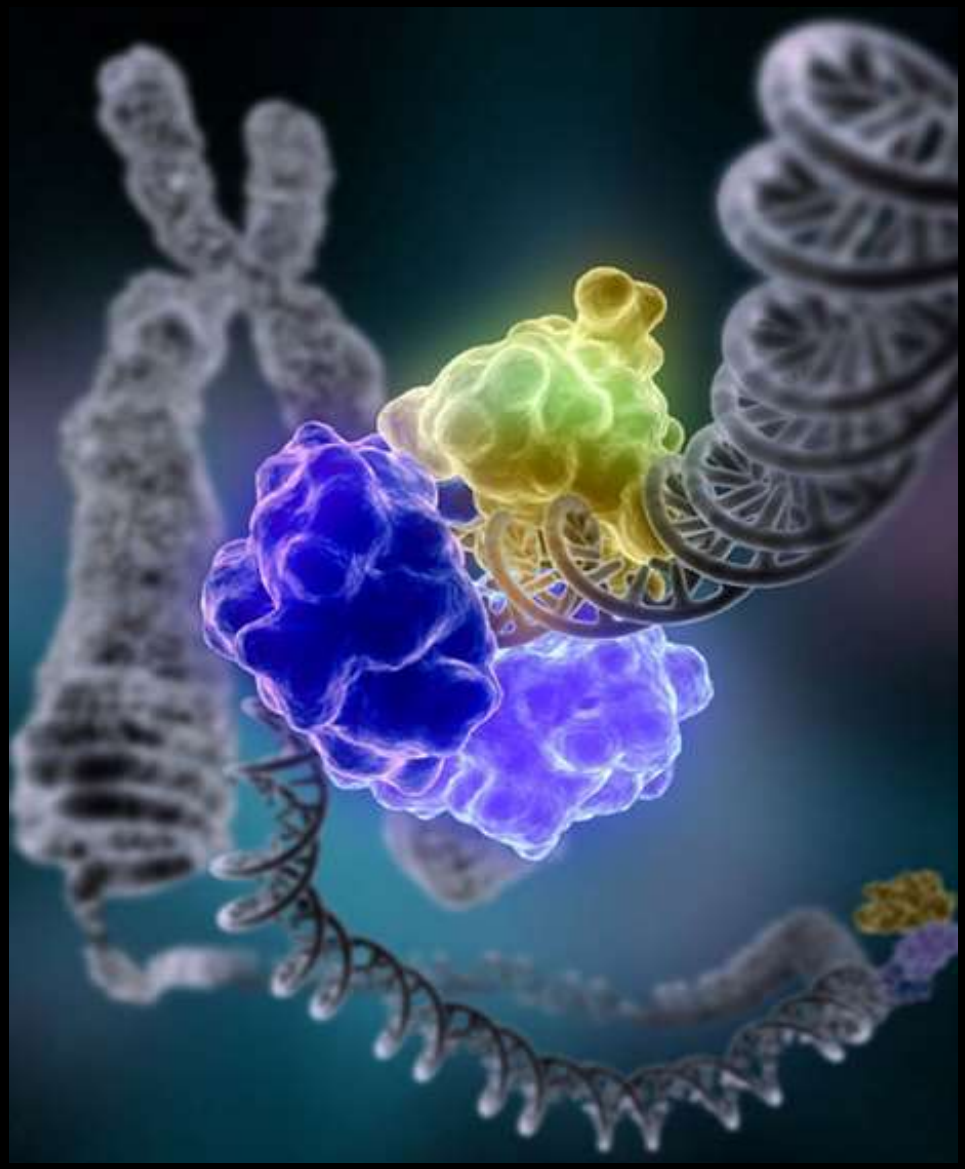

Game

DNA repair



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.

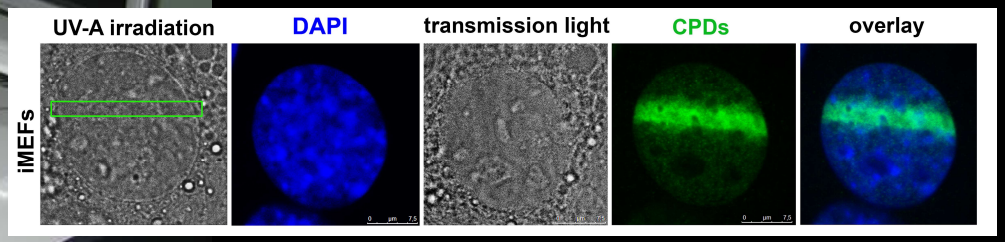
Double-strand breaks

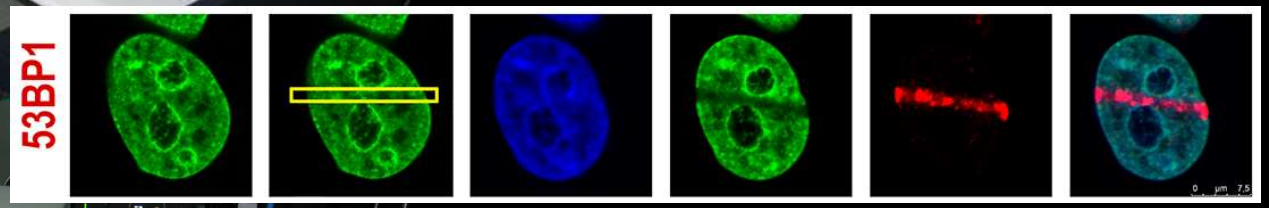
❖non-homologous end joining (NHEJ)

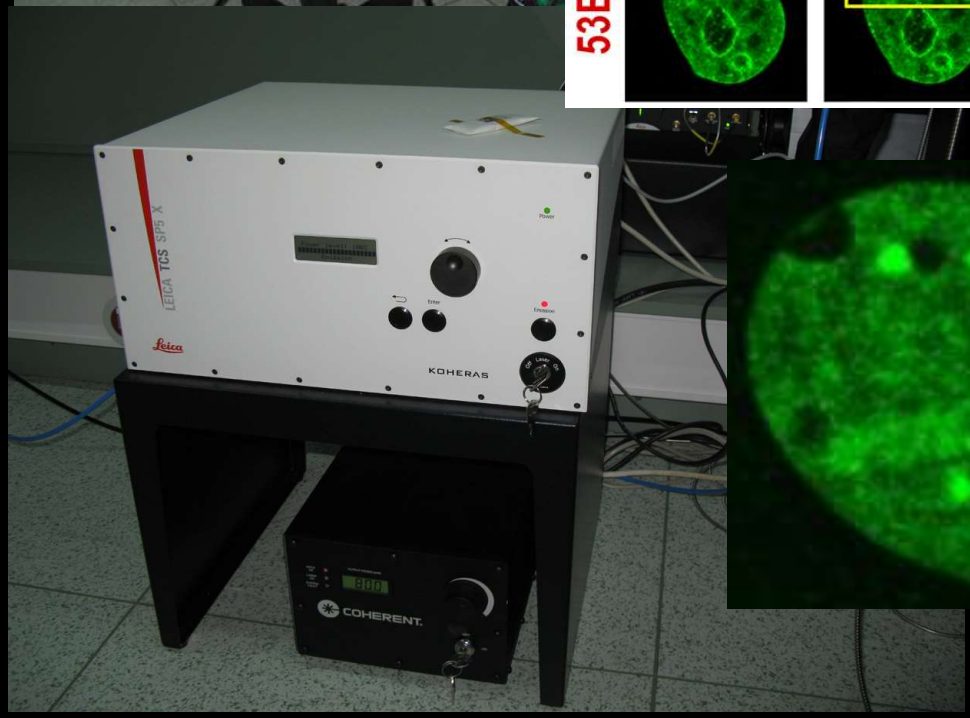
❖microhomology-mediated end joining (MMEJ)

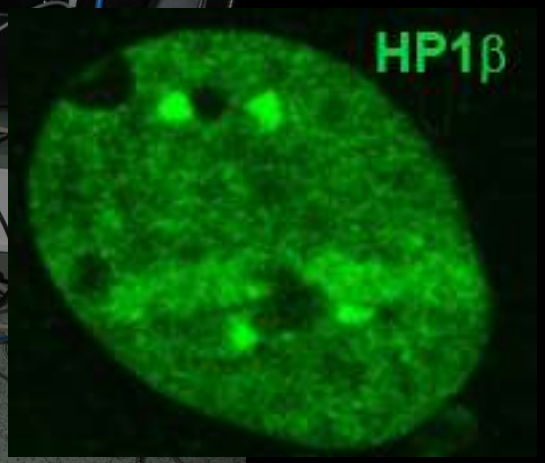
❖homologous recombination (HR)

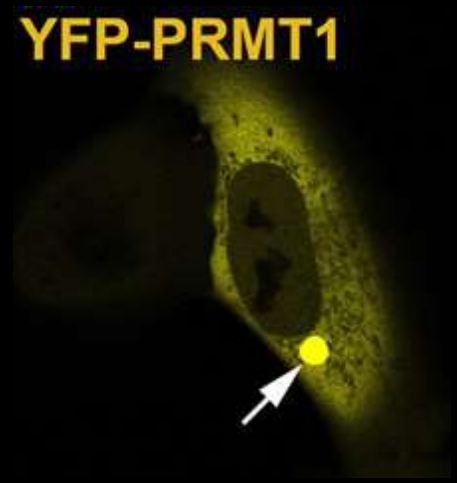
Application of UV–lasers (355 nm and 405 nm) in DNA reapair studies



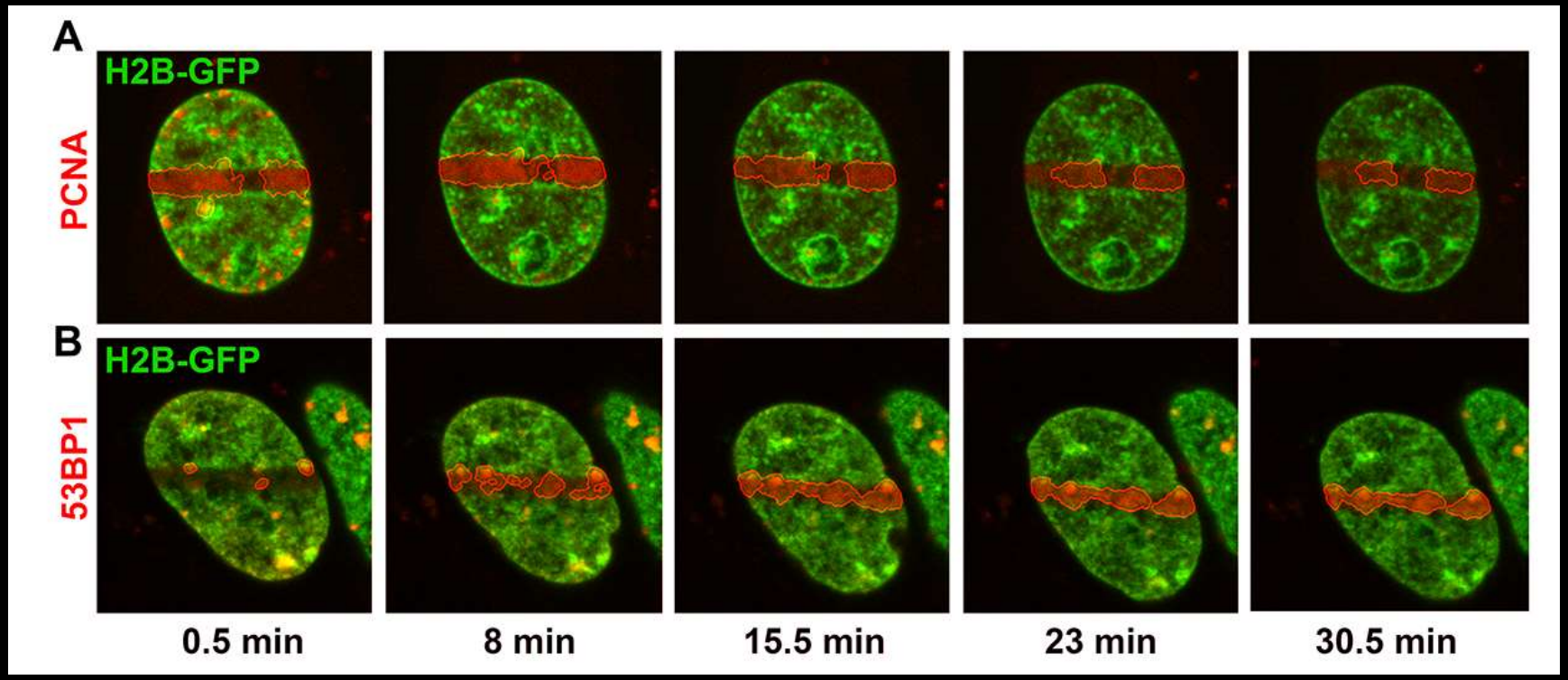




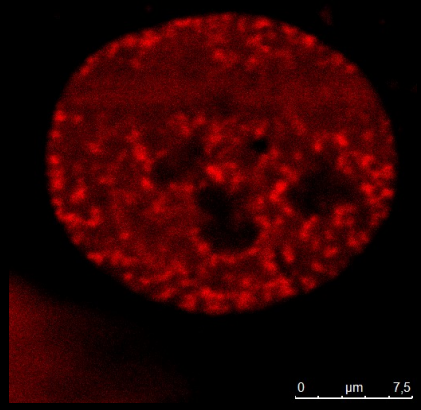




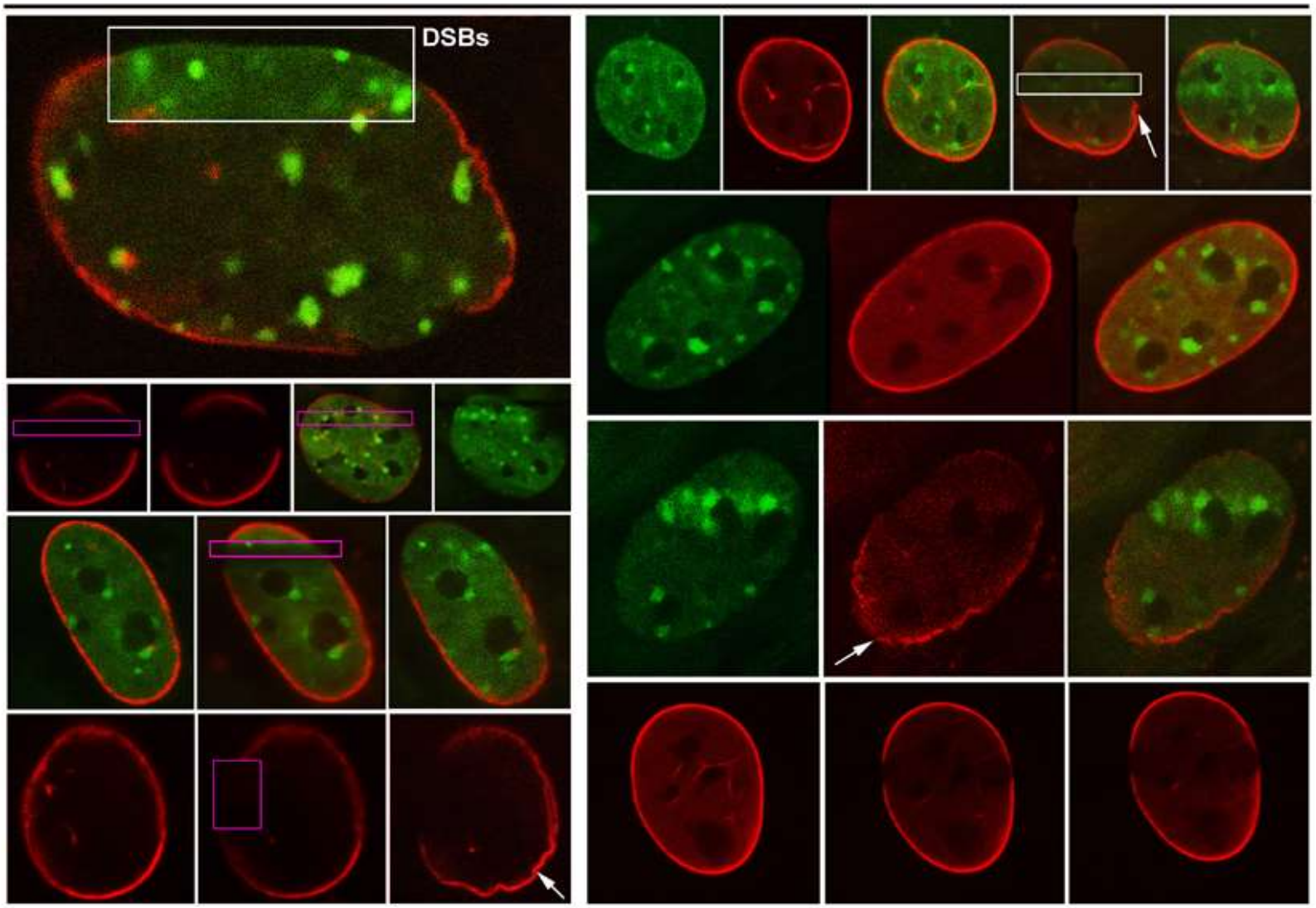
Experiments: Petra Sehnalová



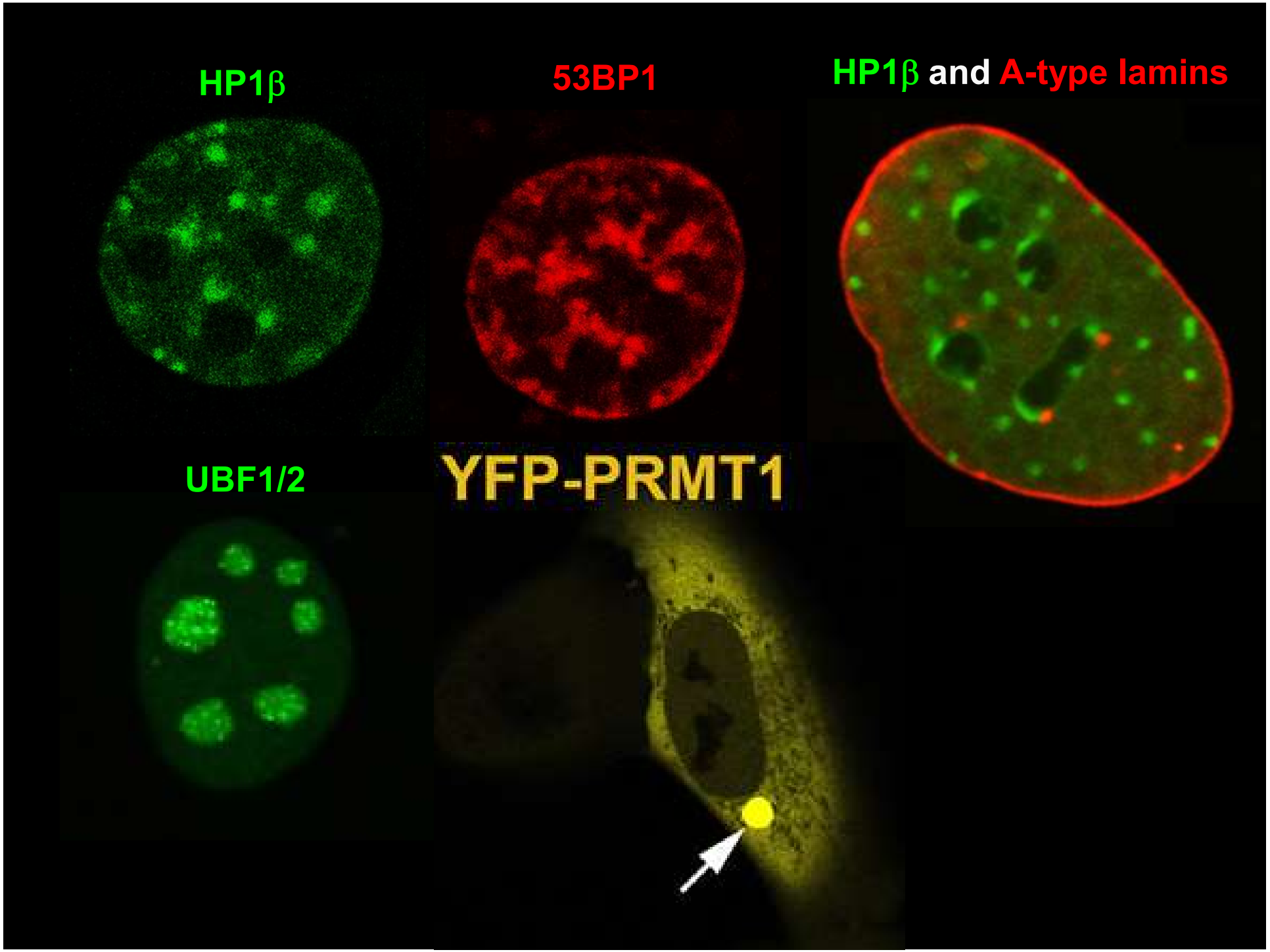
Experiments: Jana Suchánková and Gabriela Šustáčková

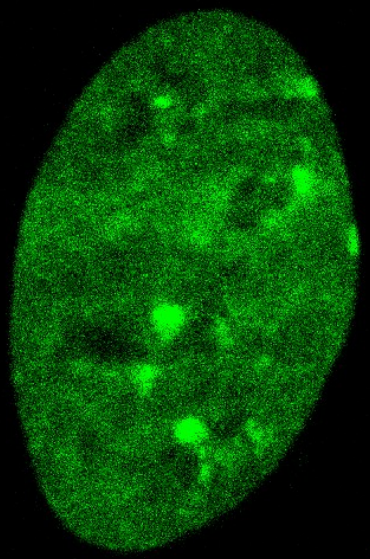


GFP-HP1β / mCherry-Lamin A

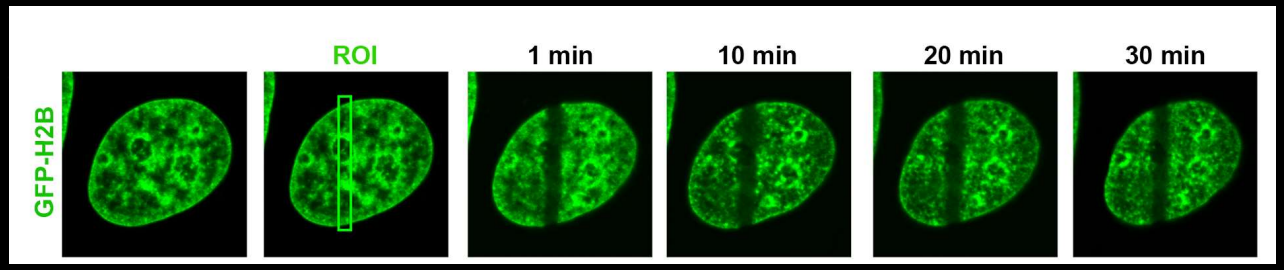


Experiments: Petra Sehnalová and Eva Bártová

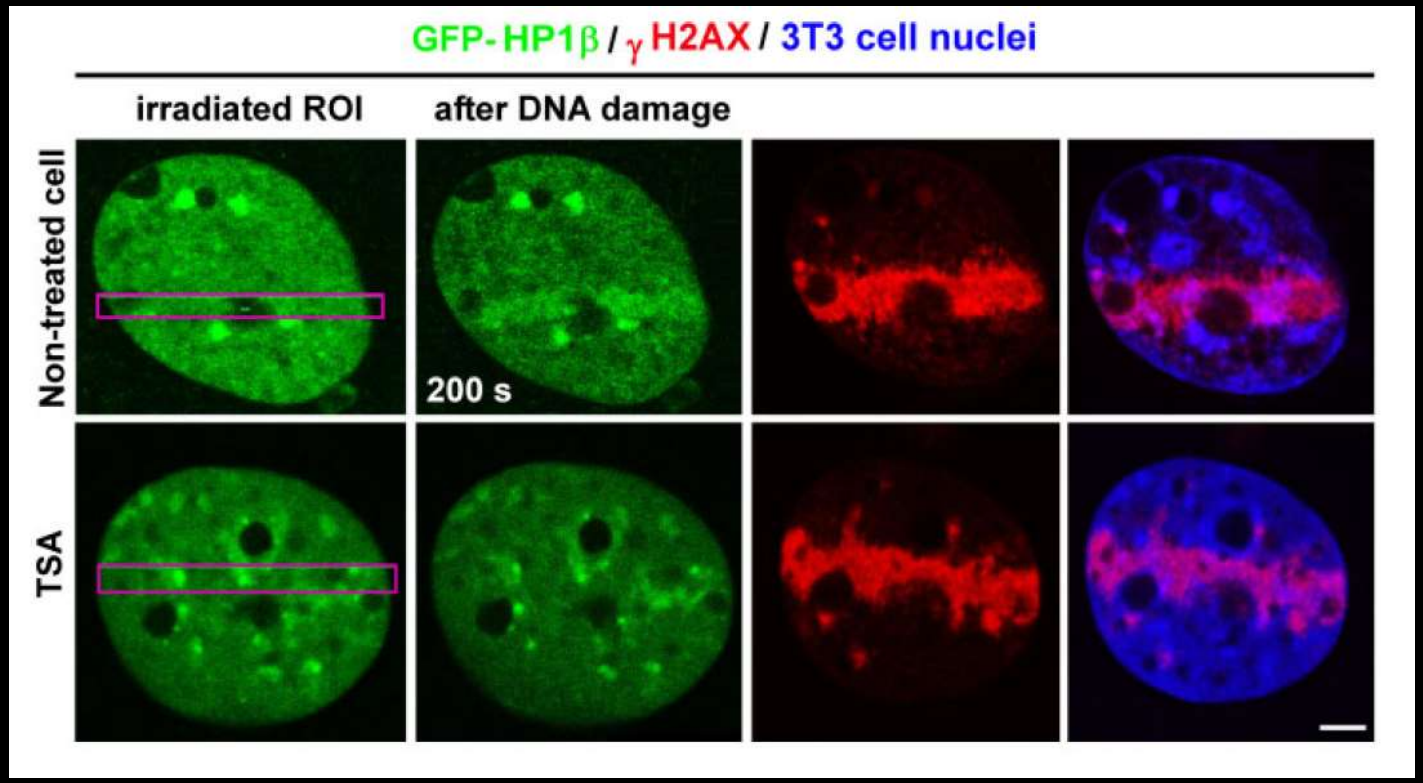

HP1β
53BP1
HP1β and A-type lamins
UBF1/2
YFP-PRMT1

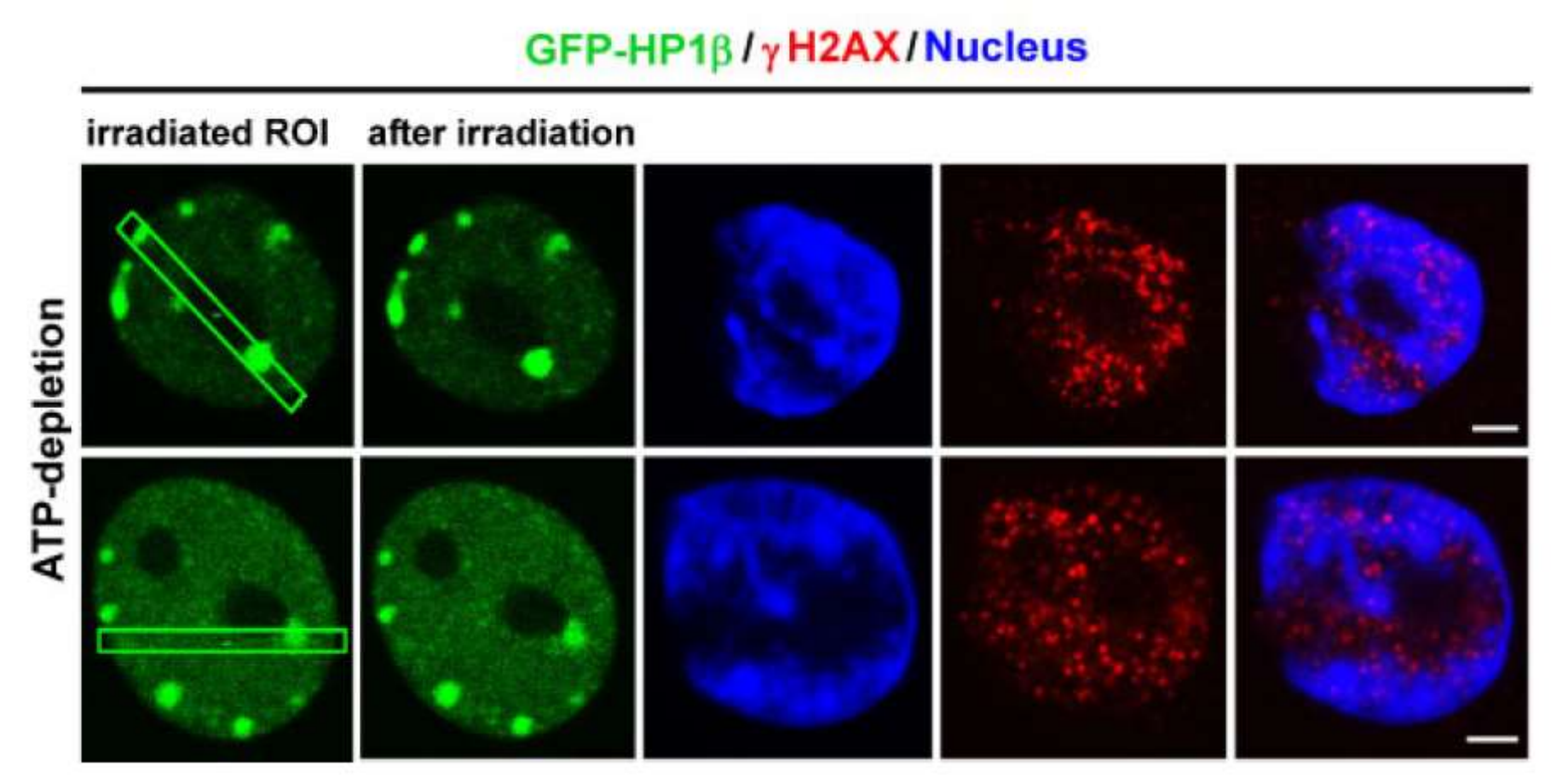


0 µm 5

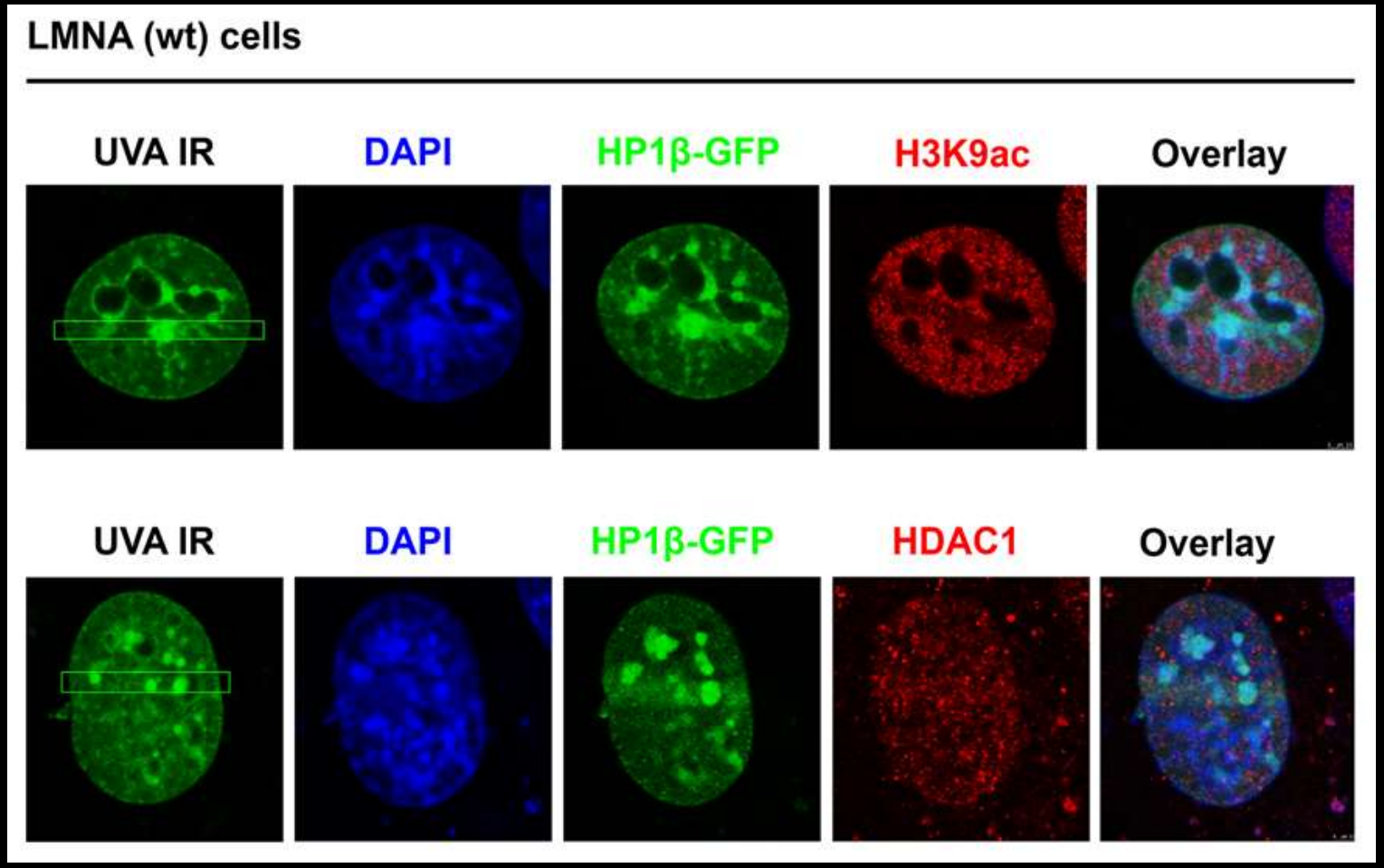


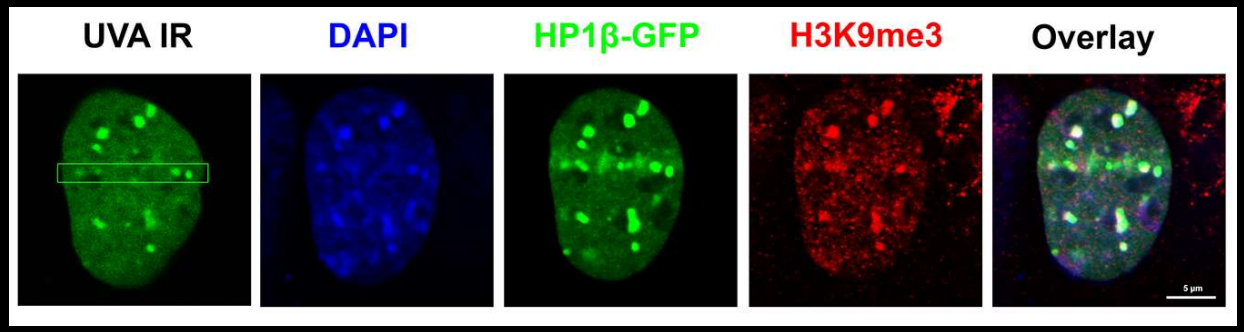
Šustáčková et al., JCP (2011)
and experiments of Petra Sehnalová
and Soňa Legartová



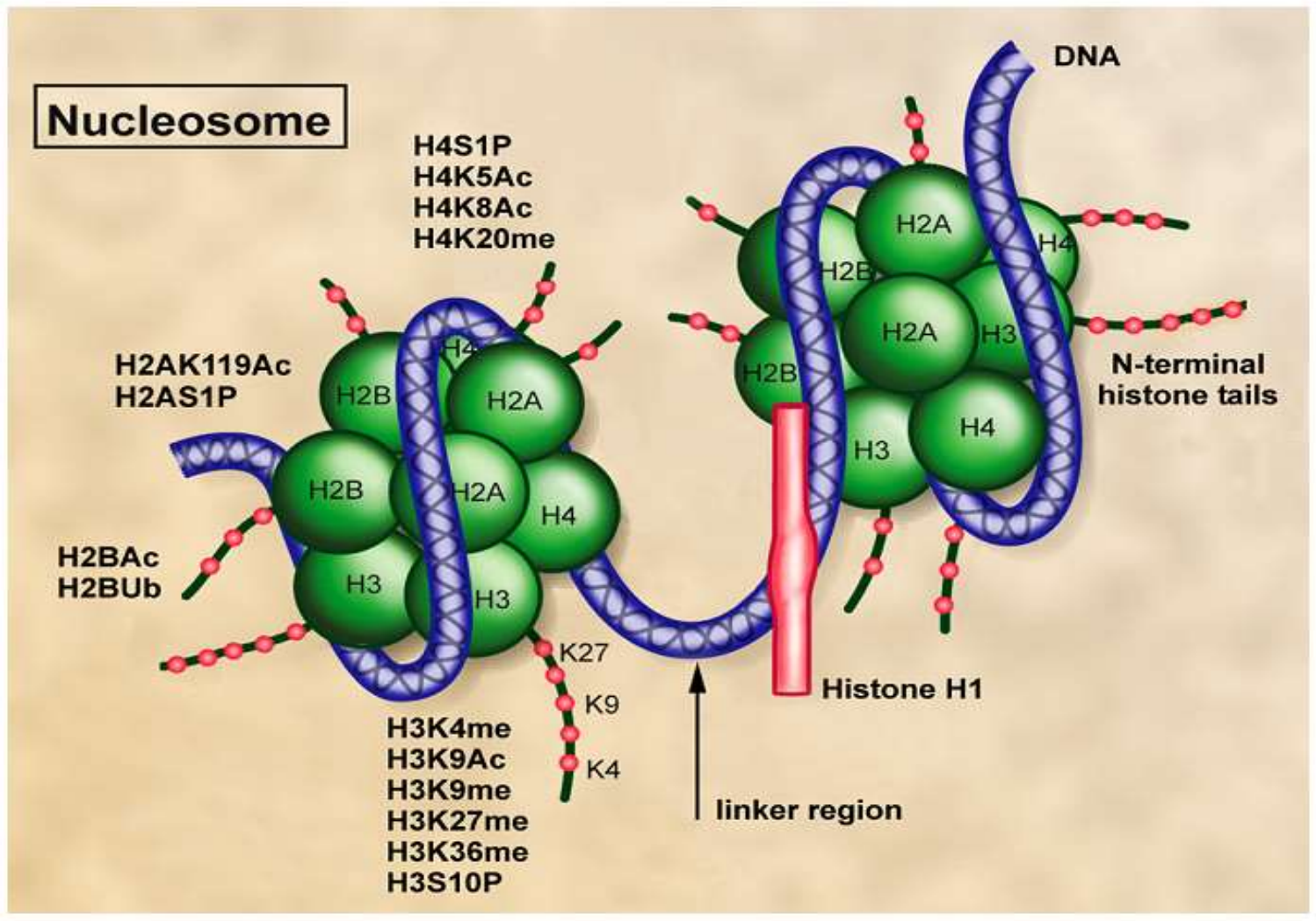


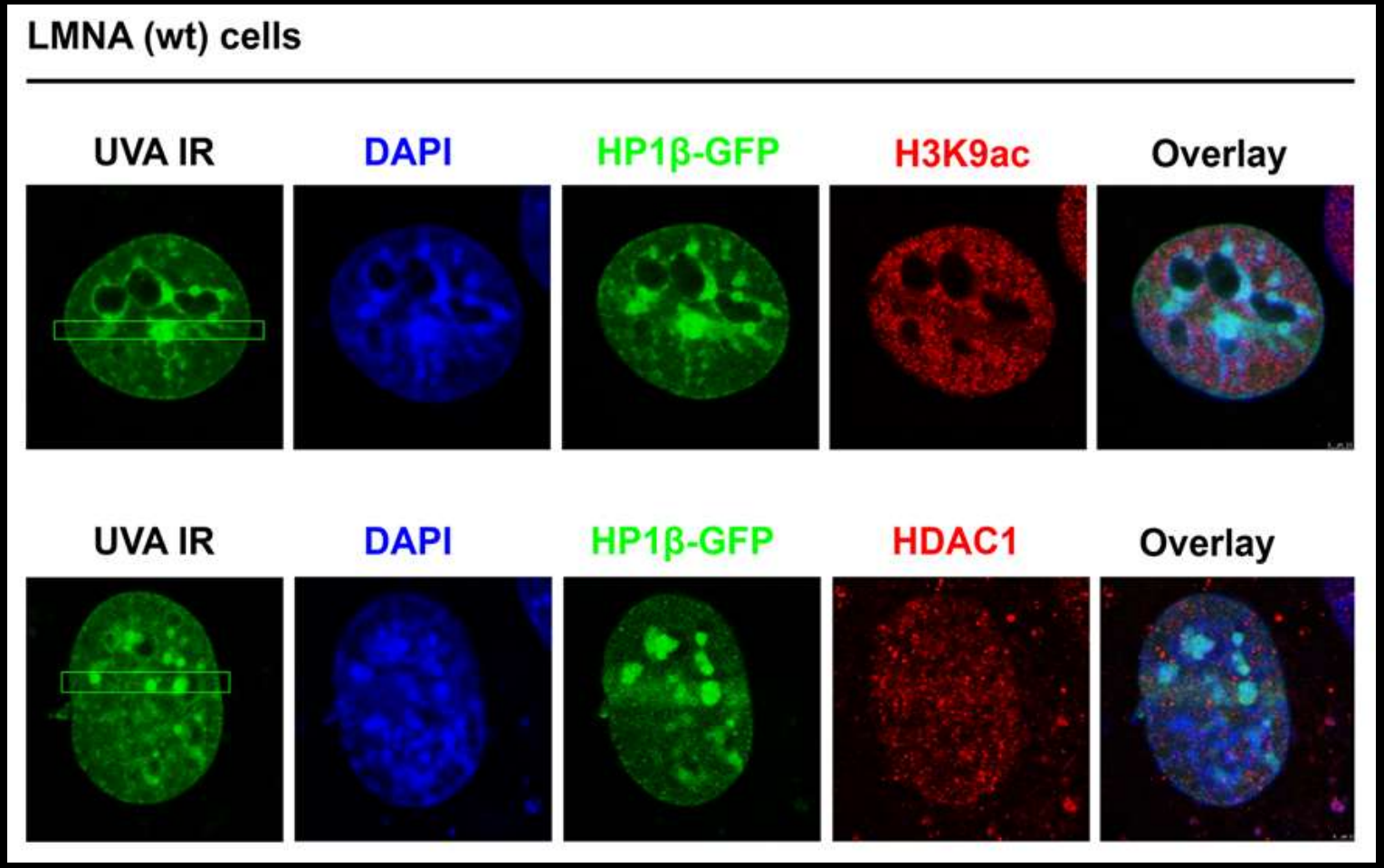
Šustáčková et al., JCP (2011)

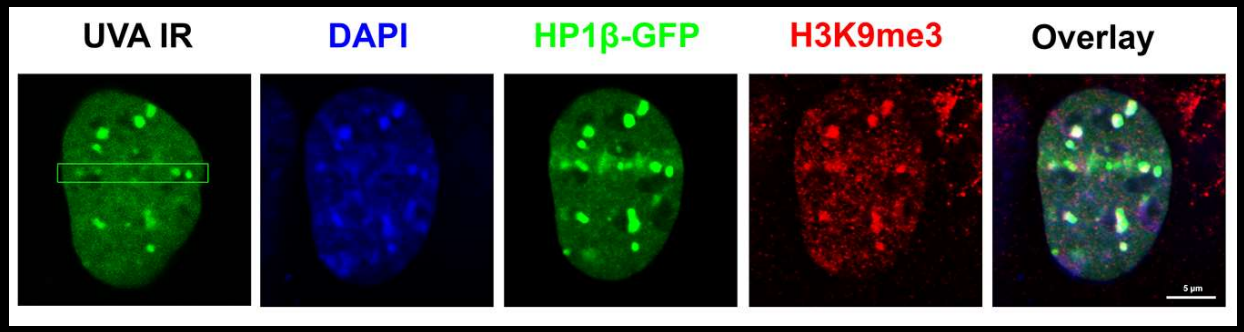




Experiments: Petra Sehnalová


Nucleosome
DNA
H4S1P
H4K5Ac
H4K8Ac
H4K20me
H2AK119Ac
H2AS1P
H2BAc
H2BUb
H2A
H2B
H3
H4
K27
K9
K4
H3K4me
H3K9Ac
H3K9me
H3K27me
H3K36me
H3S10P
Histone H1
linker region
N-terminal
histone tails





Experiments: Petra Sehnalová

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European Journal of Histochemistry 2014; volume 58:2389

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

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[4]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.[1] Protein arginine methyltransferases (PRMTs) methylate histones and other regulatory and structural proteins, with particular activity in the nucleus.[2,3] 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.[4-7] 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.[8] 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.[9] For example, PRMT1 and PRMT5 can both dimethylate arginine 3 of histone H4 (H4R3). However, PRMT5 methylates H4R3 symmetrically, which leads to silencing

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

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

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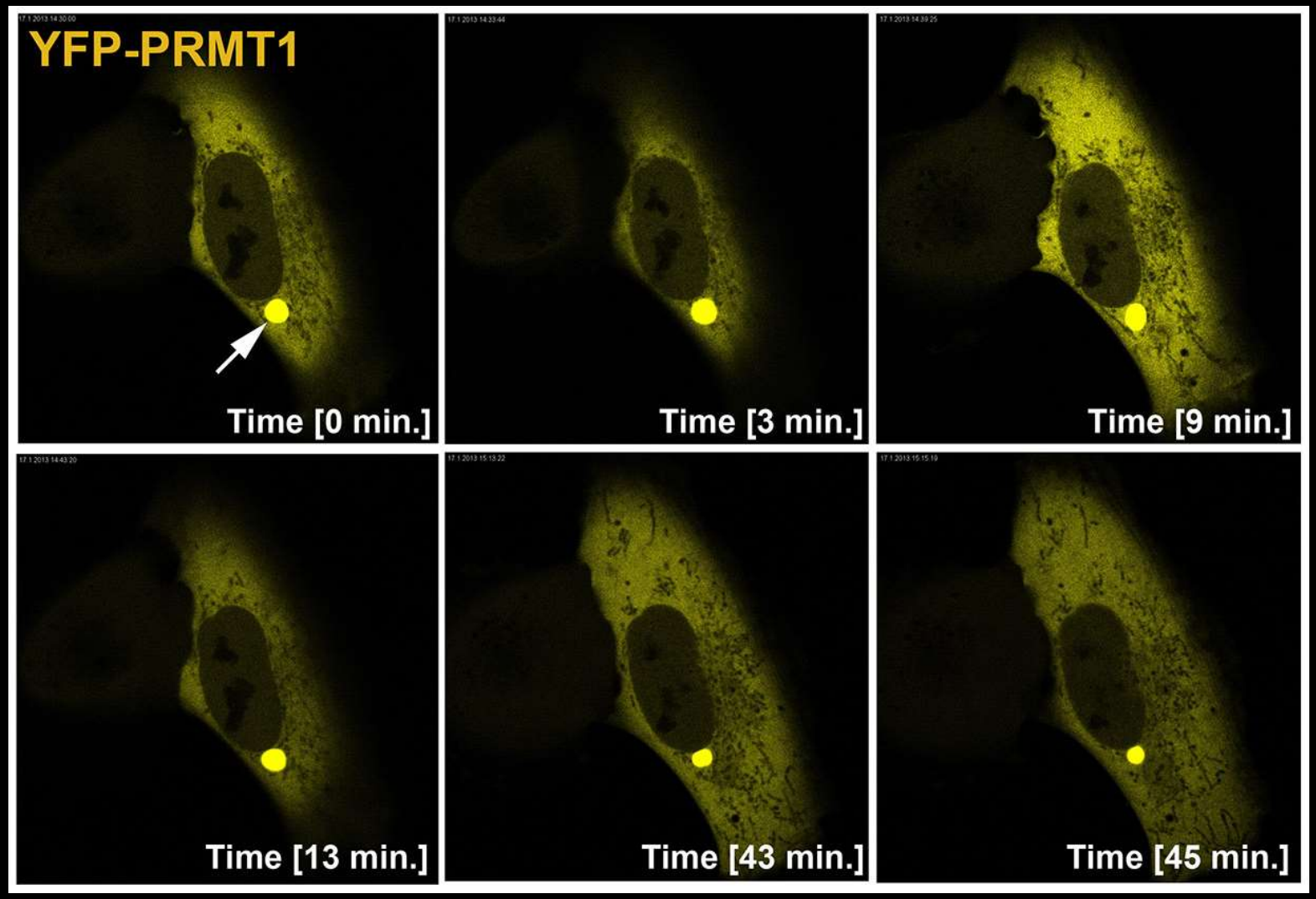
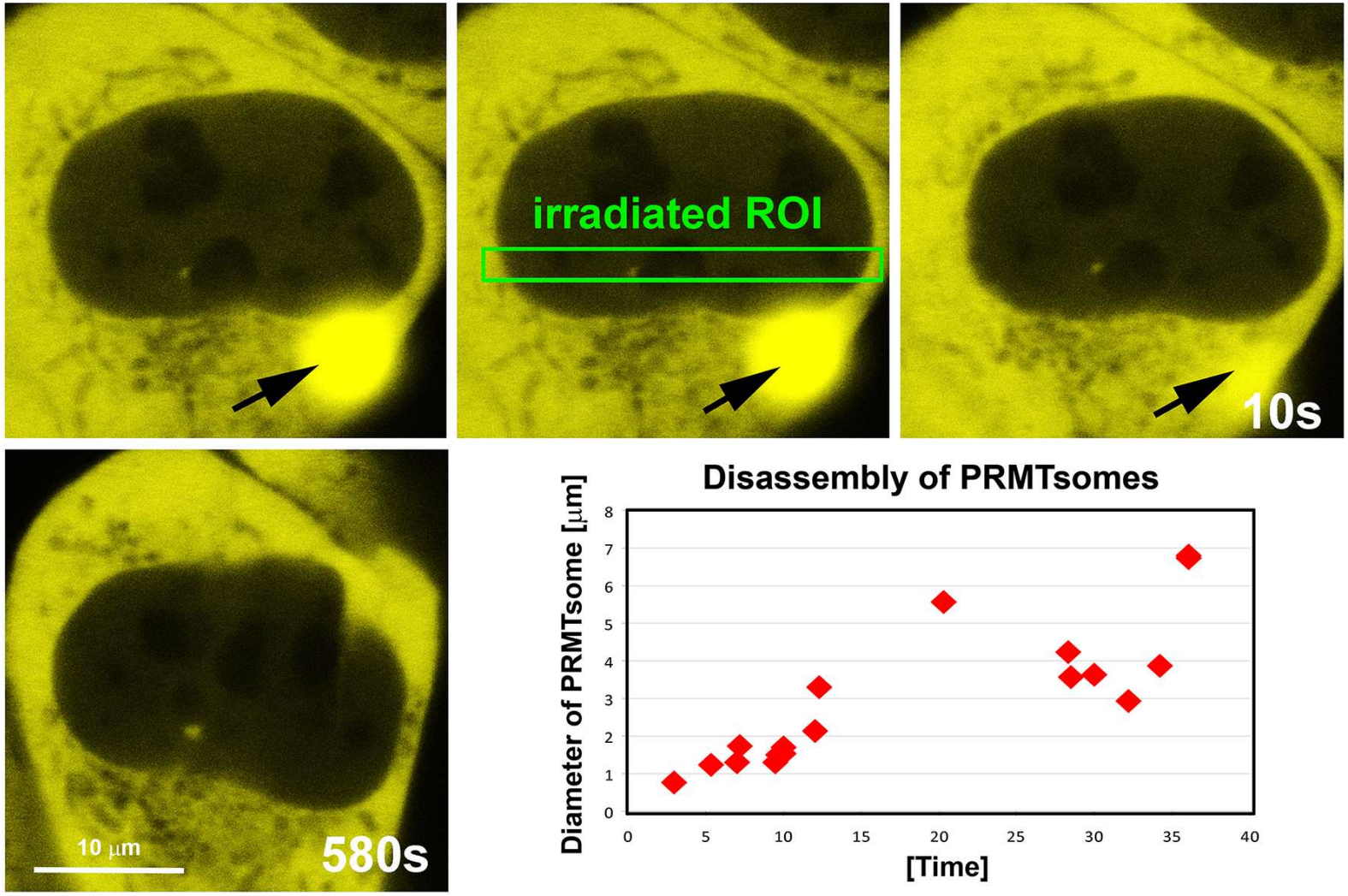
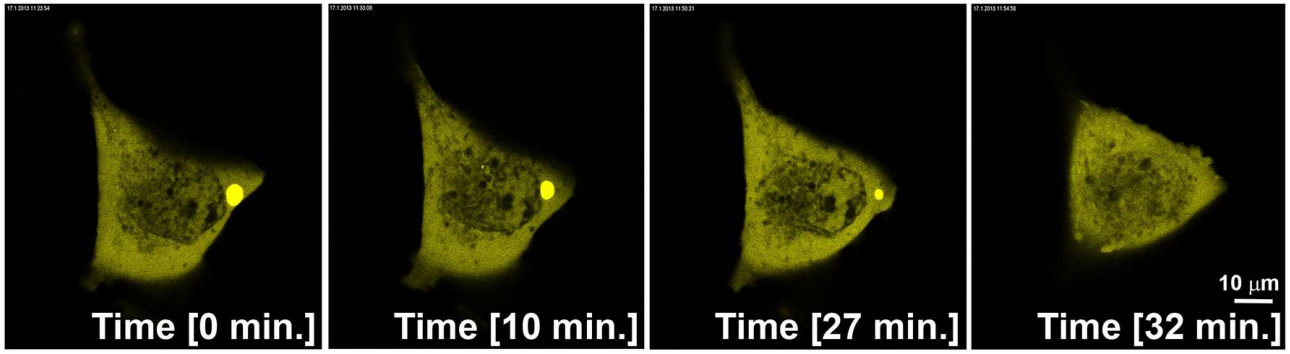
Contributions: JS, cell culture cultivation, treatments, plasmid DNA isolation, cell transfection, live cell studies after UV-A irradiation, immunofluorescence studies; SL, immunofluorescence and intermediated irradiation of the cells with γ-rays; SK, GACR projects P302/10/1022 and P302/12/G157 coordination;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


YFP-PRMT1
Time [0 min.]
Time [3 min.]
Time [9 min.]
Time [13 min.]
Time [43 min.]
Time [45 min.]

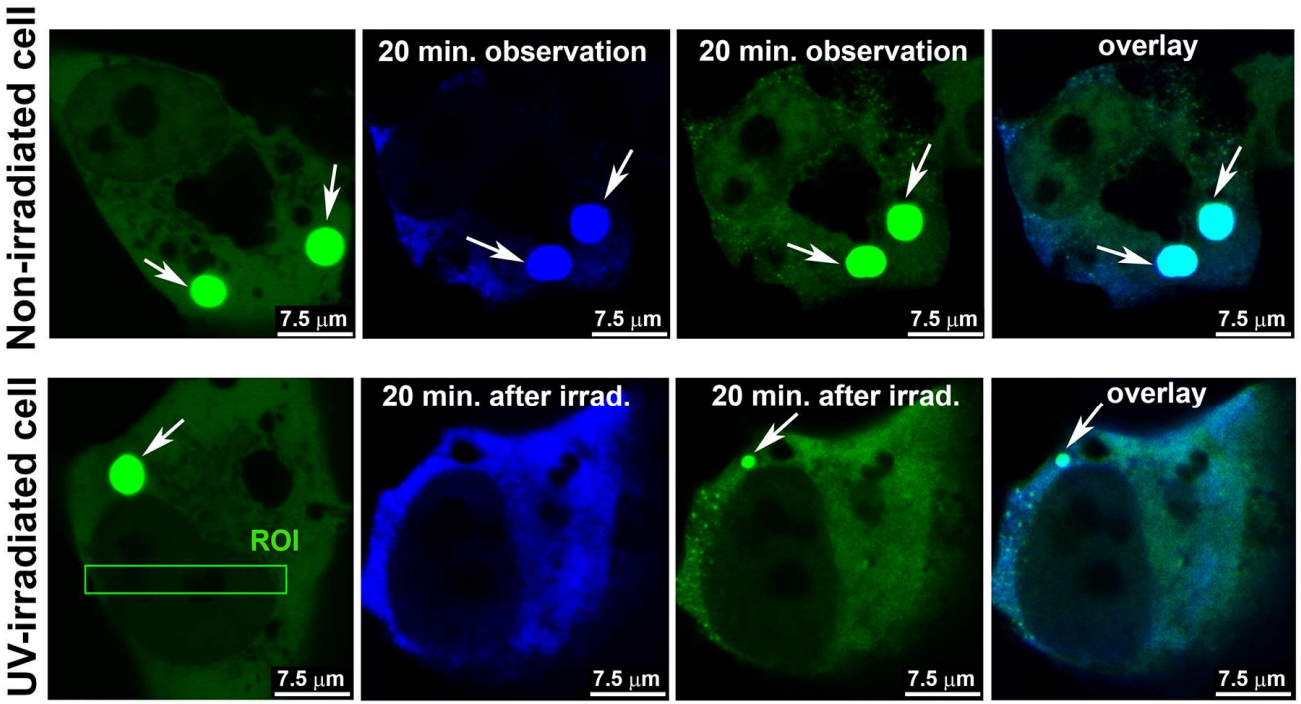
YFP-PRMT1 in U2OS cells

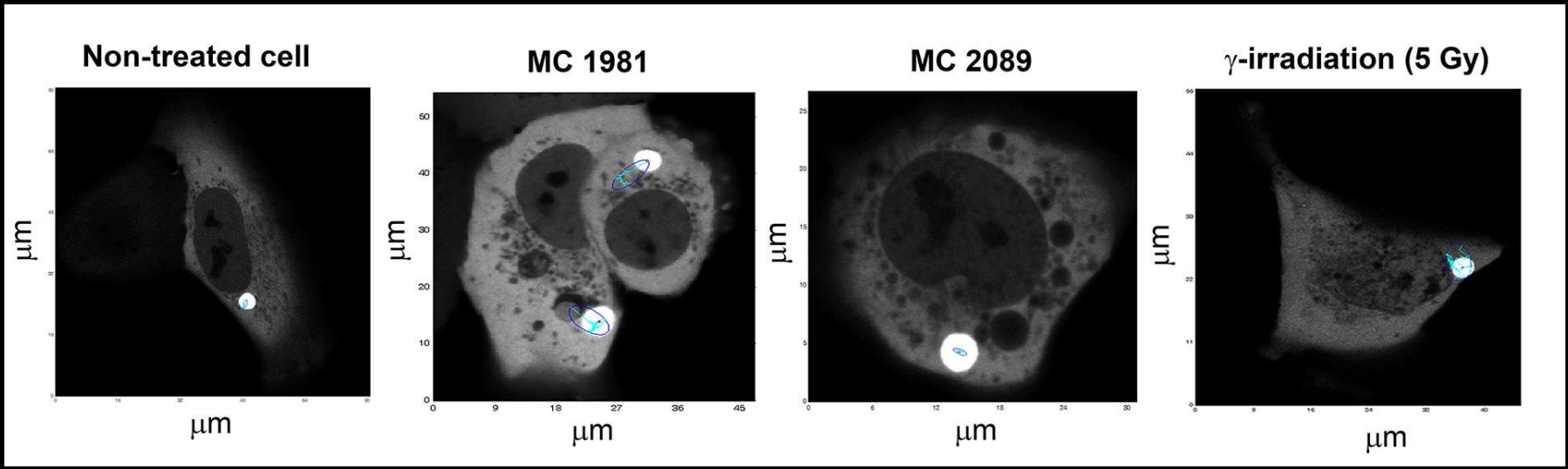


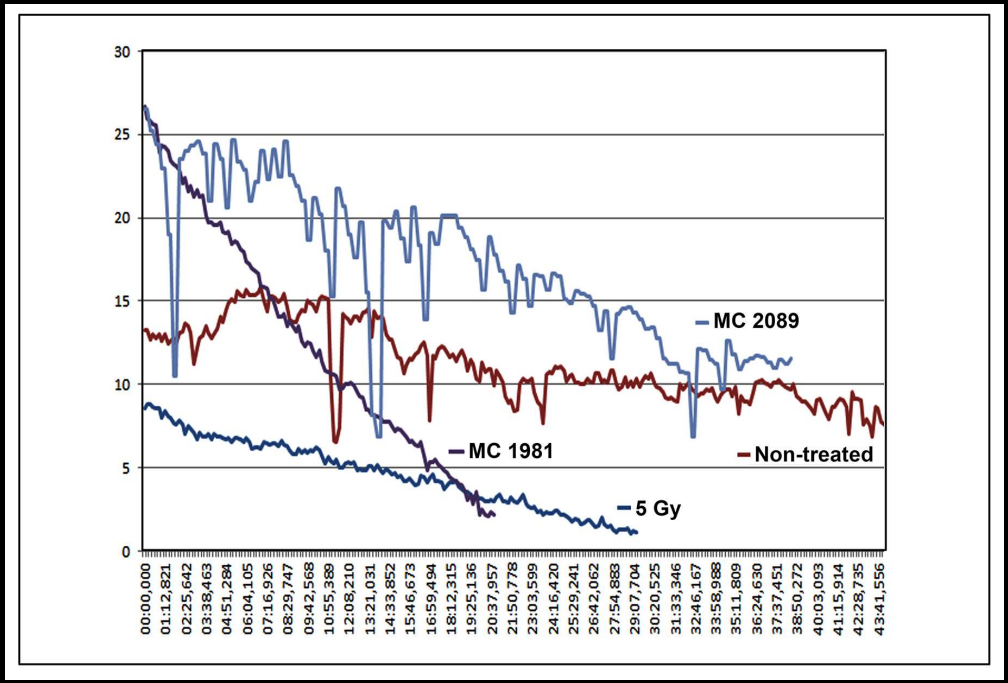
YFP-PRMT1 in U2OS cells / γ-irradiation [5 Gy]



YFP-PRMT1 / endogenous PRMT1




Non-treated cell
MC 1981
MC 2089
γ-irradiation (5 Gy)
µm
µm
µm
µm
µm
µm
µm
µm


30
25
20
15
10
5
0
MC 2089
MC 1981
Non-treated
5 Gy
00:00,000
01:12,821
02:25,642
03:38,463
04:51,284
06:04,105
07:16,926
08:29,747
09:42,568
10:55,389
12:08,210
13:21,031
14:33,852
15:46,673
16:59,494
18:12,315
19:25,136
20:37,957
21:50,778
23:03,599
24:16,420
25:29,241
26:42,062
27:54,888
29:07,704
30:20,525
31:33,346
32:46,167
33:58,988
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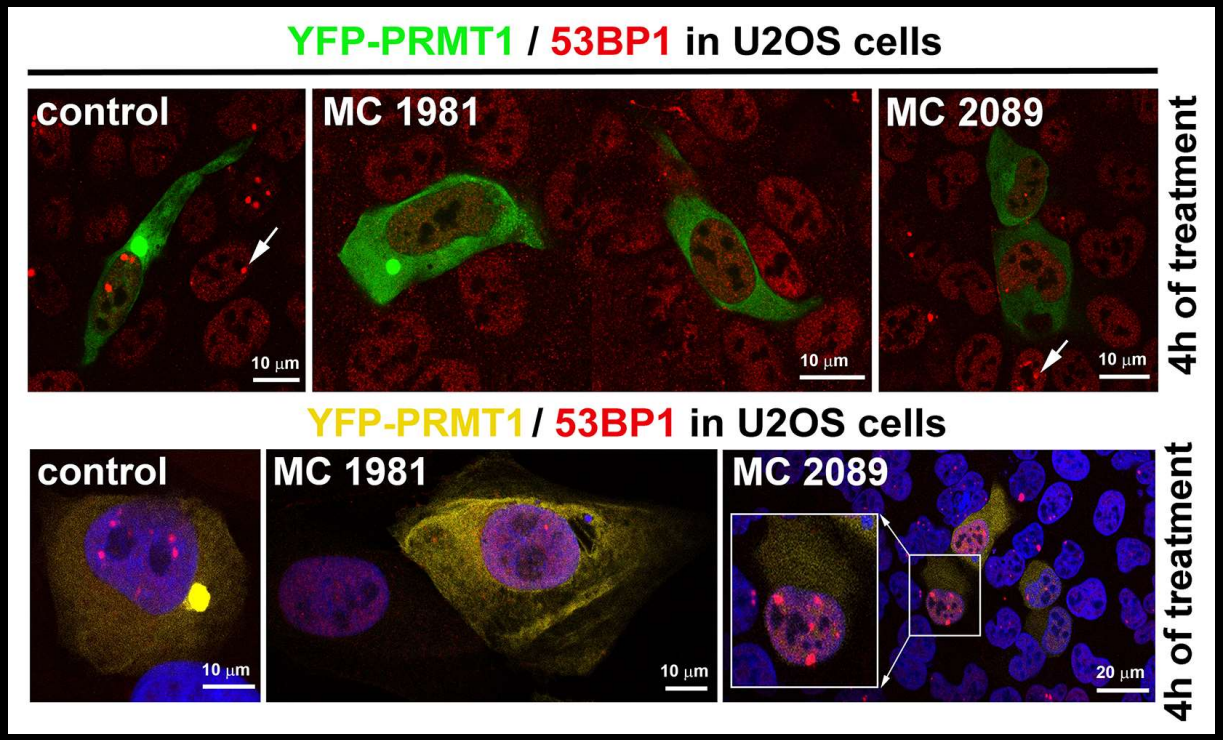
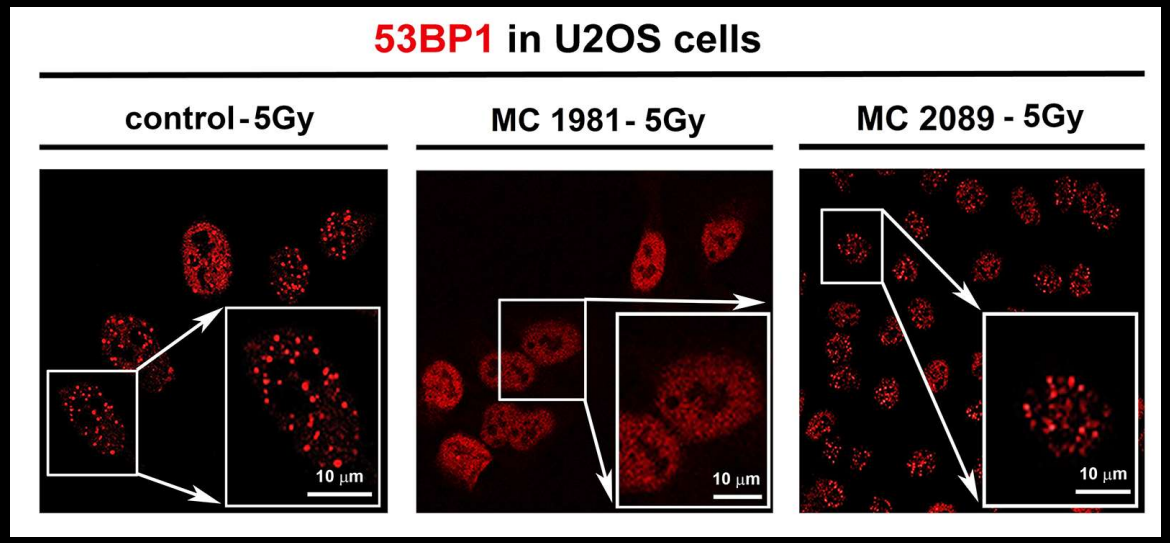
40:03,093
41:15,914
42:28,735
43:41,556


YFP-PRMT1 / 53BP1 in U2OS cells
control
MC 1981
MC 2089
10 µm
10 µm
10 µm
4h of treatment
YFP-PRMT1 / 53BP1 in U2OS cells
control
MC 1981
MC 2089
10 µm
10 µm
20 µm
4h of treatment


53BP1 in U2OS cells
control - 5Gy
MC 1981 - 5Gy
MC 2089 - 5Gy
10 µm
10 µm
10 µm

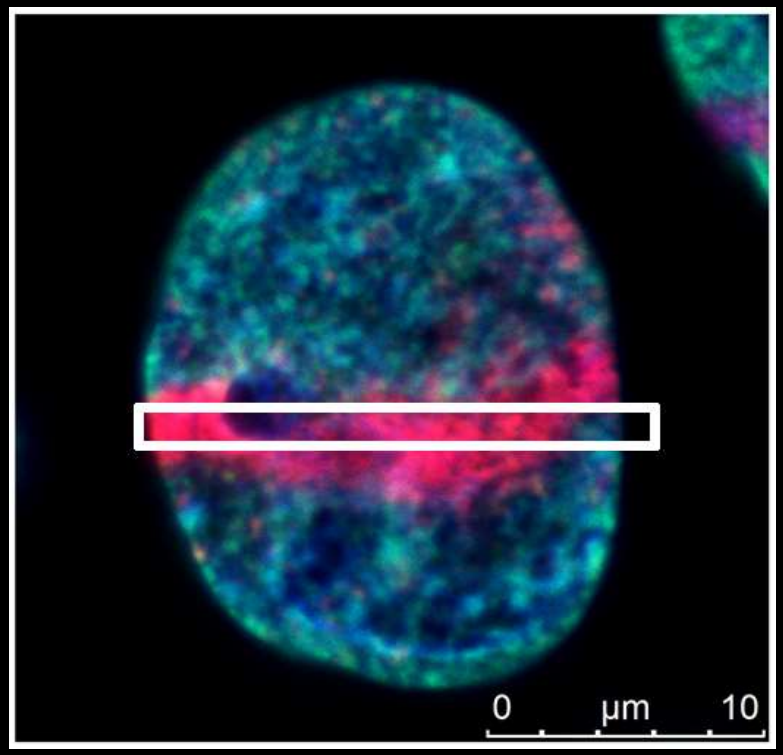
Conclusions

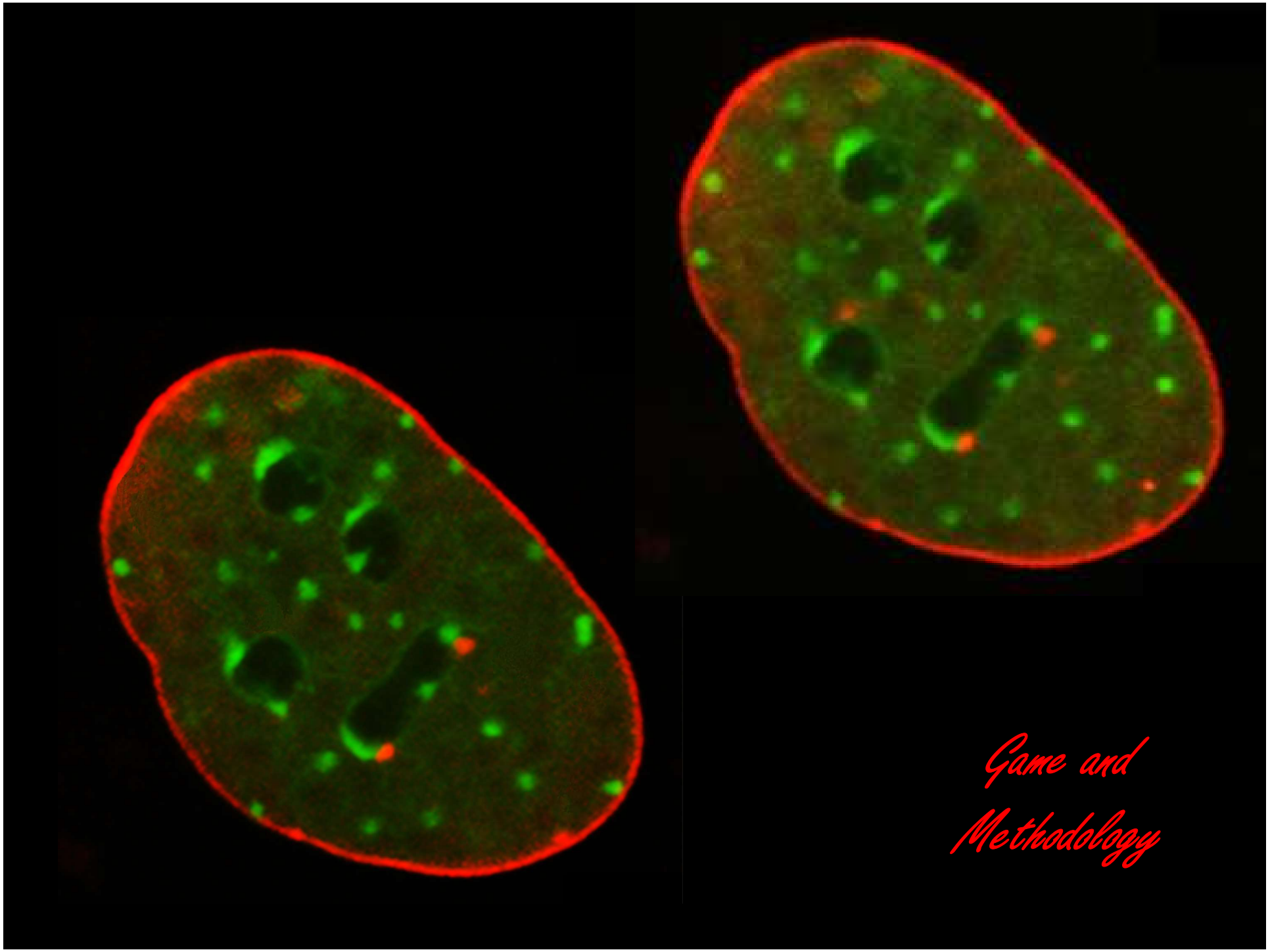
1. HP1β function at DNA lesions can be affected by HDAC inhibitors.

2. Irradiation of the cell nucleus by UVA lasers caused disappearance of PRMT1-positive cytoplasmic bodies

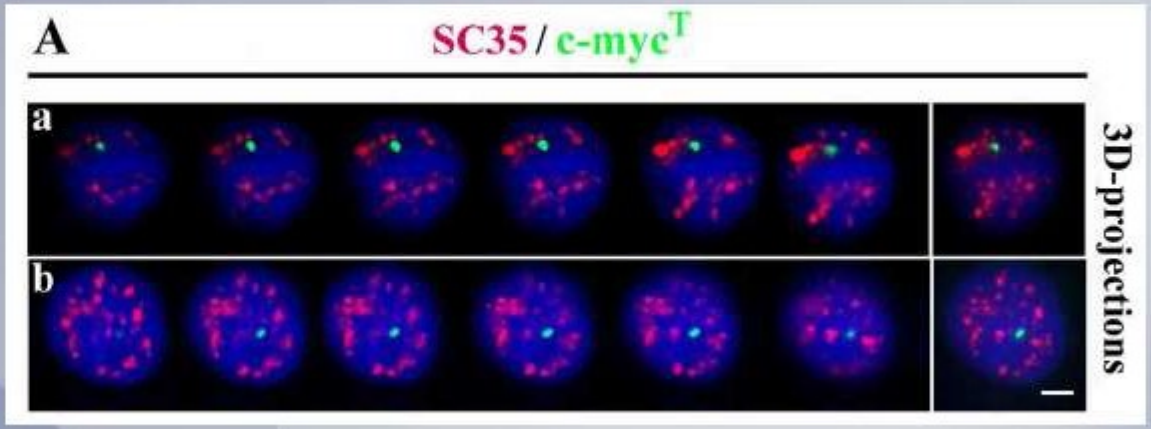
3. Disappearance of PRMT1-positive cytoplasmic bodies is really fast afte γ-irradition or the cell treatment by PRMT1 inhibitor MC 1981.

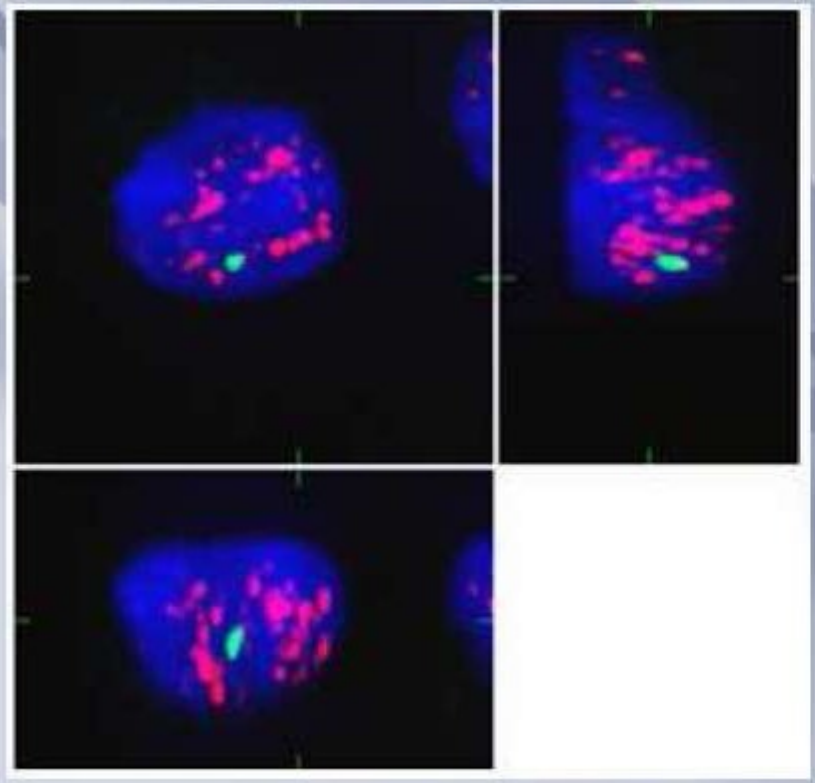
4. PRMT1 inhibitor MC 1981 prevents formation of 53BP1-positive irradiation-induced foci.



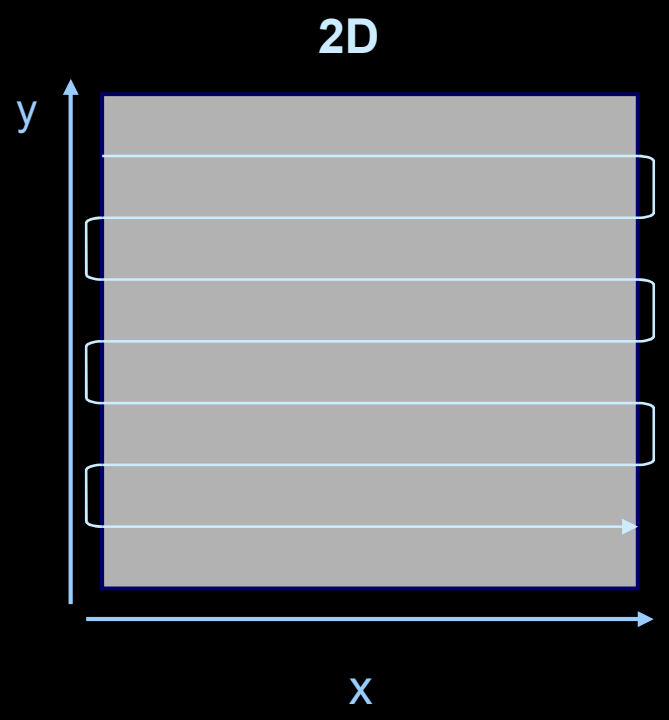

Game and
Methodology

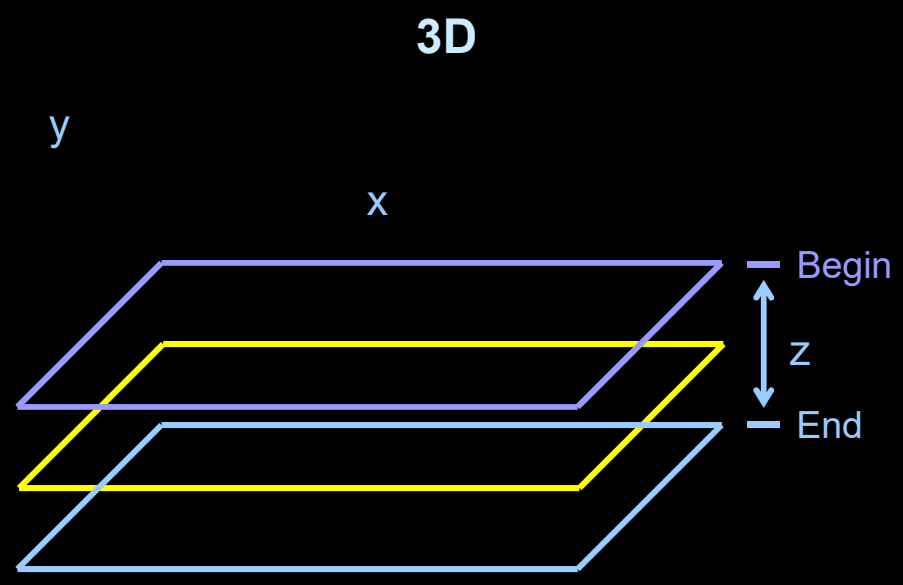
3D-projection





Scanning in 2D and 3D by confocal microscope

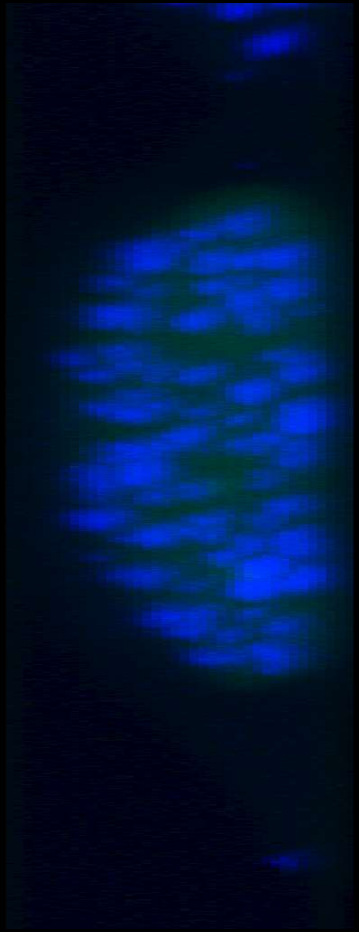




Laser beam moves firstly along *x* axis and then starts with new line in *y* axis.

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

° pos=1



Deconvolution

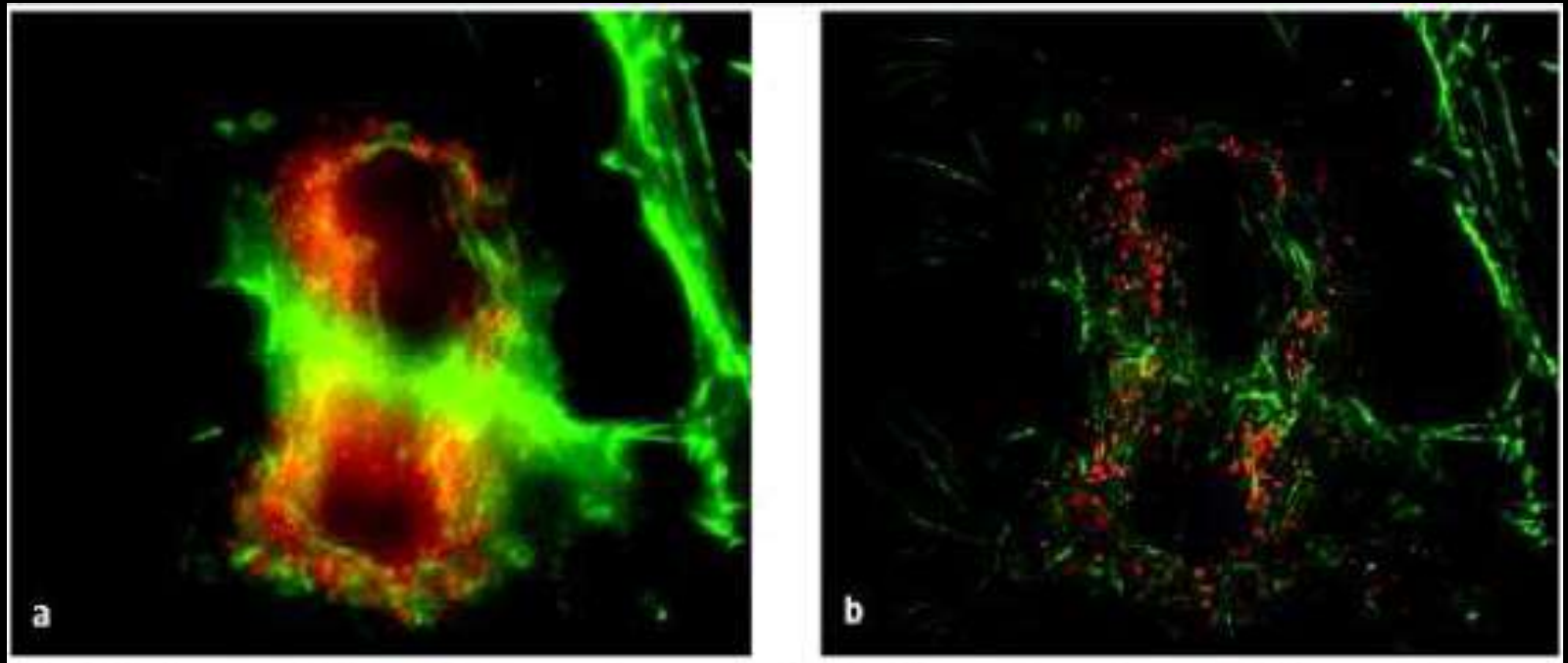


Fig. 3: Via deconvolution, artefacts can be computed out of fluorescence images. a). These artefacts are caused by the stray light from non-focused areas above and below the focus level. These phenomena, referred to as convolution, result in glare, distortion and blurriness. b). Deconvolution is a recognised 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,[1*] Peter M. Carlton,[2*] Sebastian Haase,[2,4] Lin Shao,[2] Lukman Winoto,[2] Peter Kner,[2] Brian Burke,[3] M. Cristina Cardoso,[4] David A. Agard,[2] Mats G. L. Gustafsson,[5] Heinrich Leonhardt,[1*†] John W. Sedat[2*†]

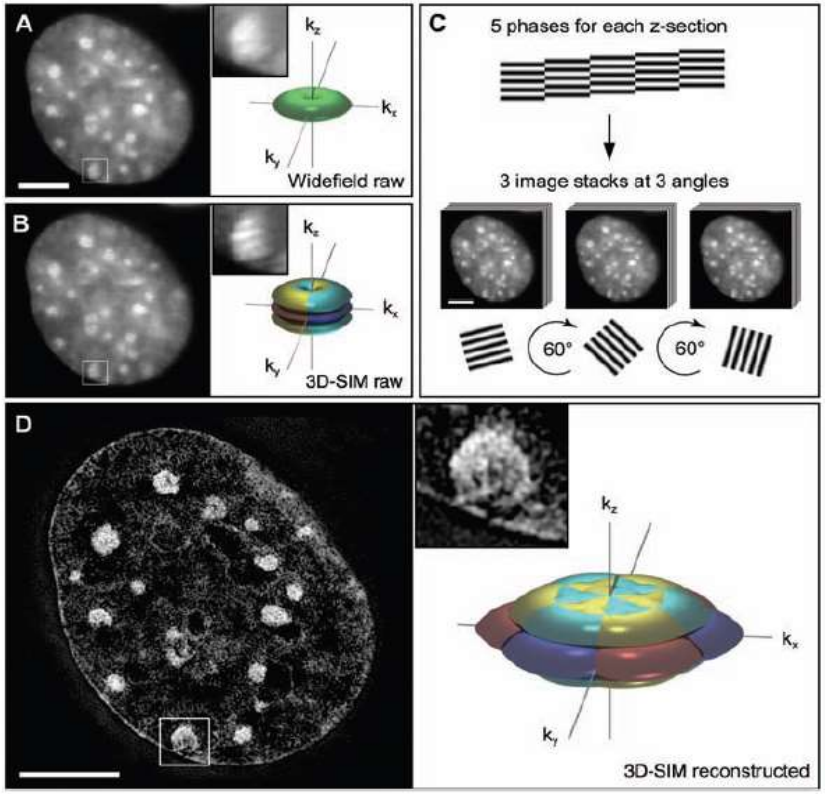


Fig. 1. Subdiffraction resolution imaging with 3D-SIM. (**A** and **B**) Cross section through a DAPI-stained C2C12 cell nucleus acquired with conventional wide-field illumination (A) and with structured illumination (B), showing the striped interference pattern (inset). The renderings to the right illustrate 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° 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 μm.

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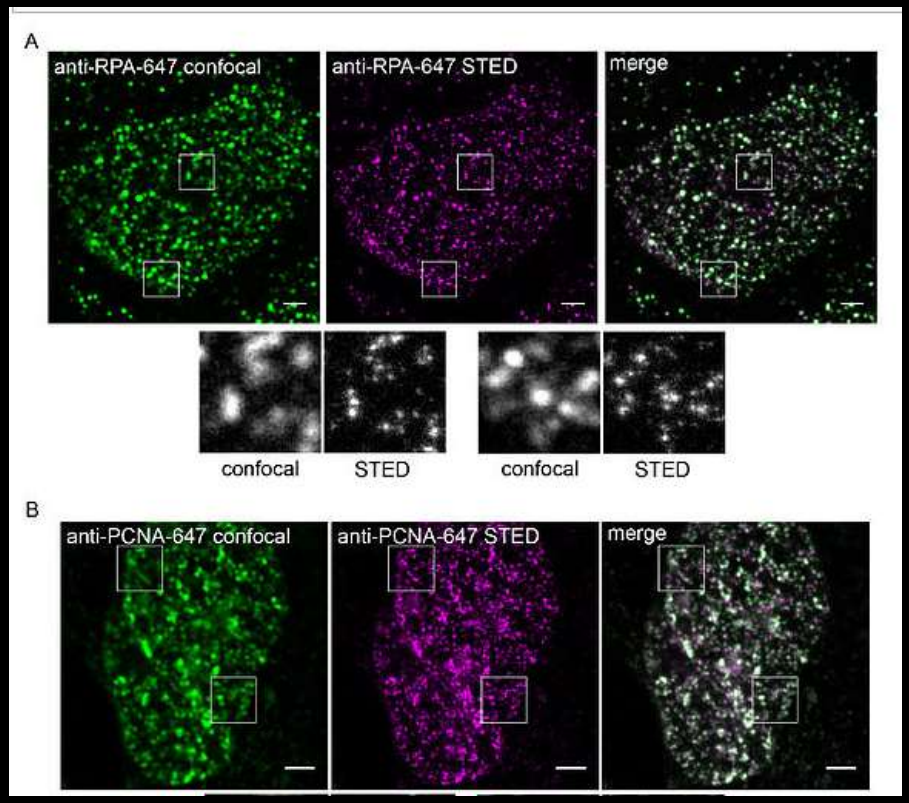
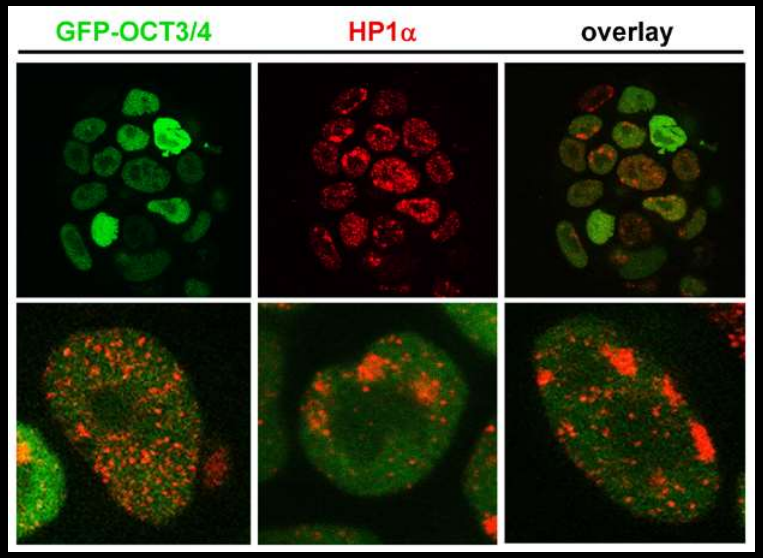
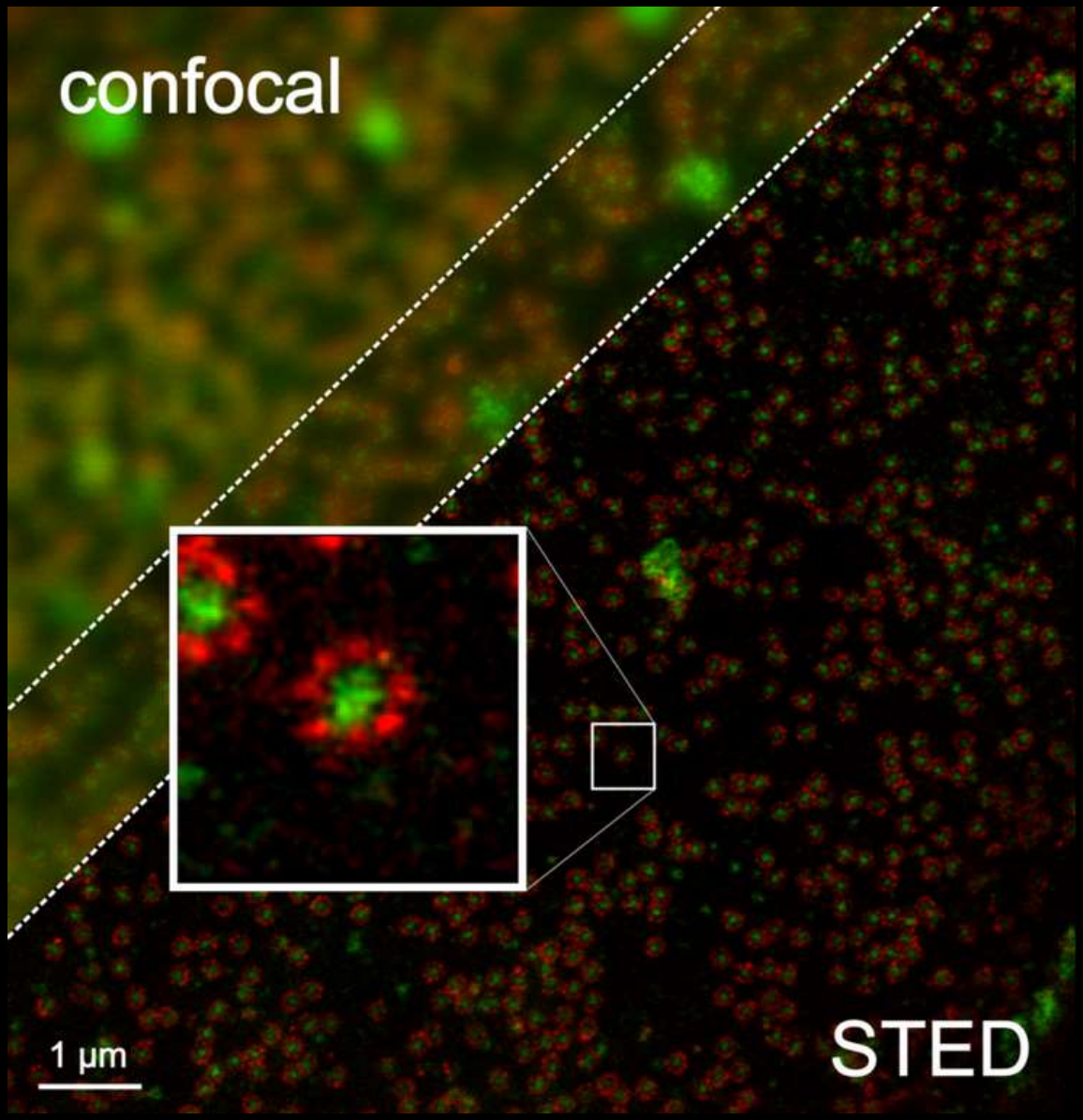
Stimulated Emission Depletion microscopy, or STED microscopy, is a fluorescence microscopy technique that uses the non-linear de-excitation of fluorescent dyes to overcome the resolution limit imposed by diffraction with standard confocal laser scanning microscopes and conventional far-field optical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

STED

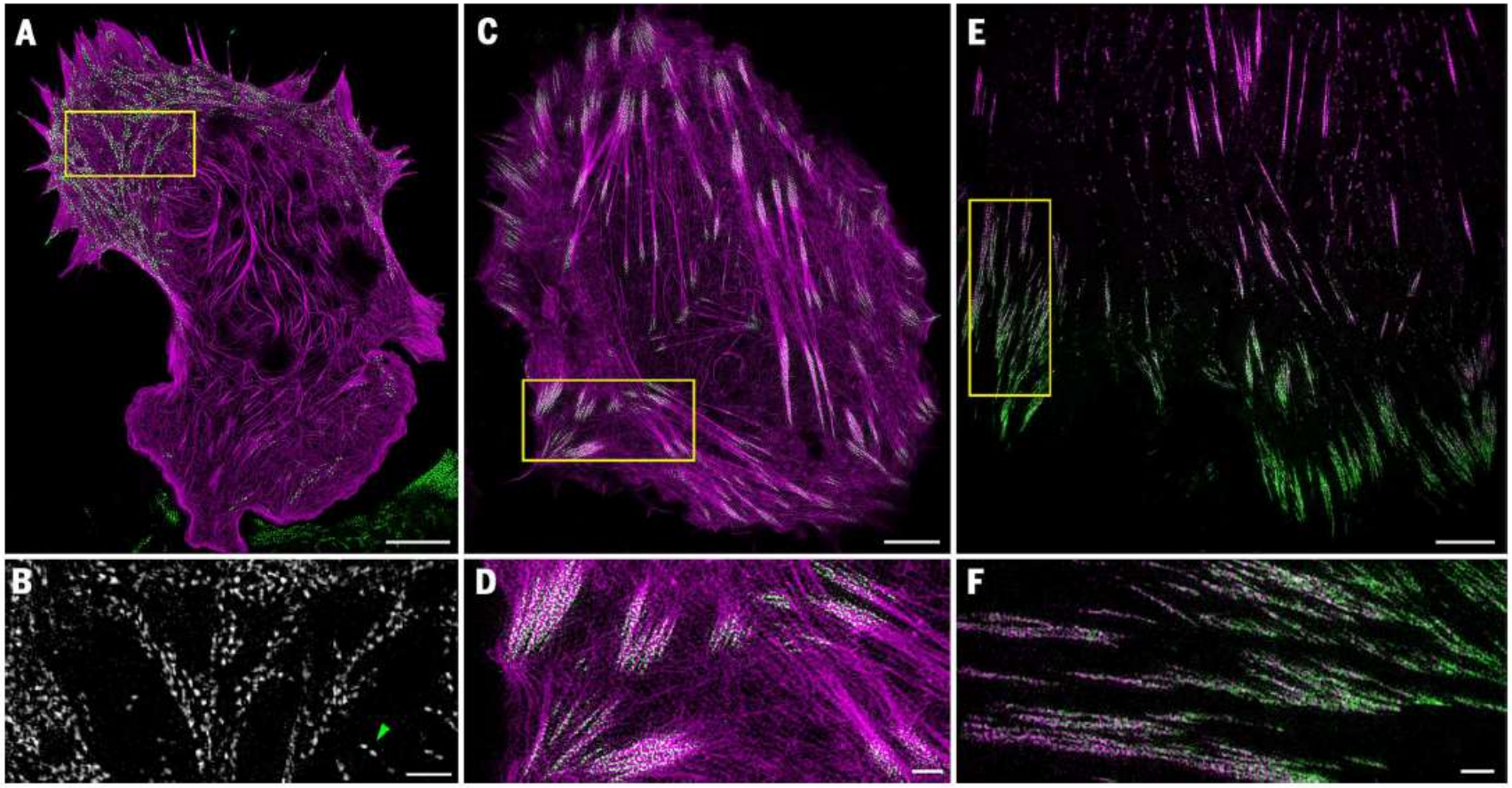


SP-5 LSCM




confocal
1 µm
STED

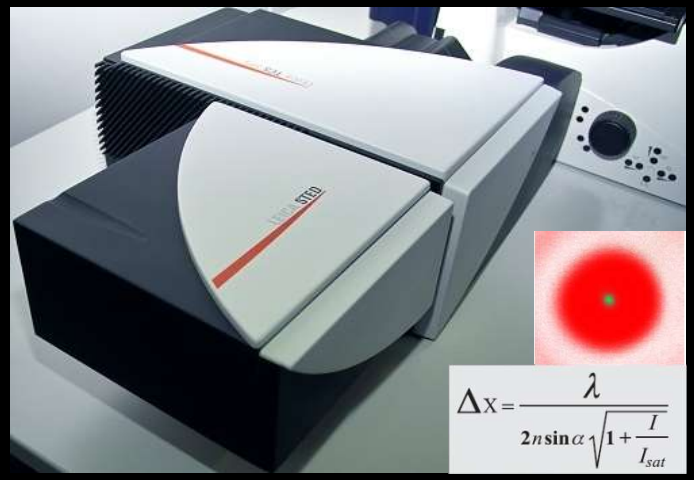
High-speed live-cell imaging

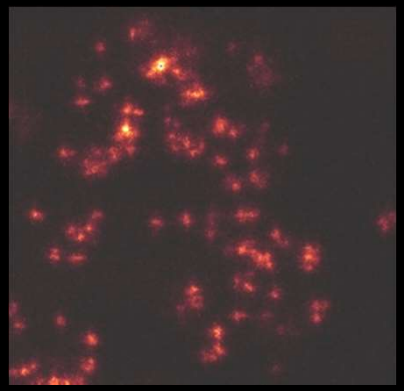


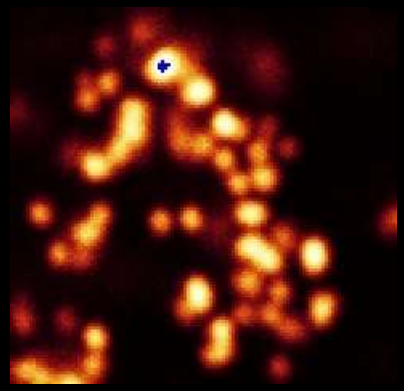
Li et al., Science (2015)

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"





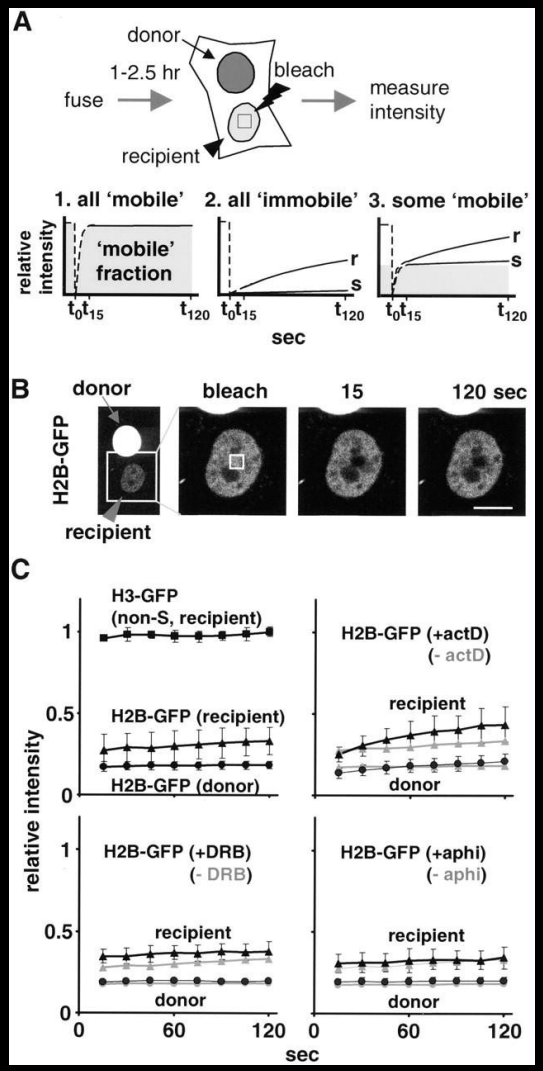


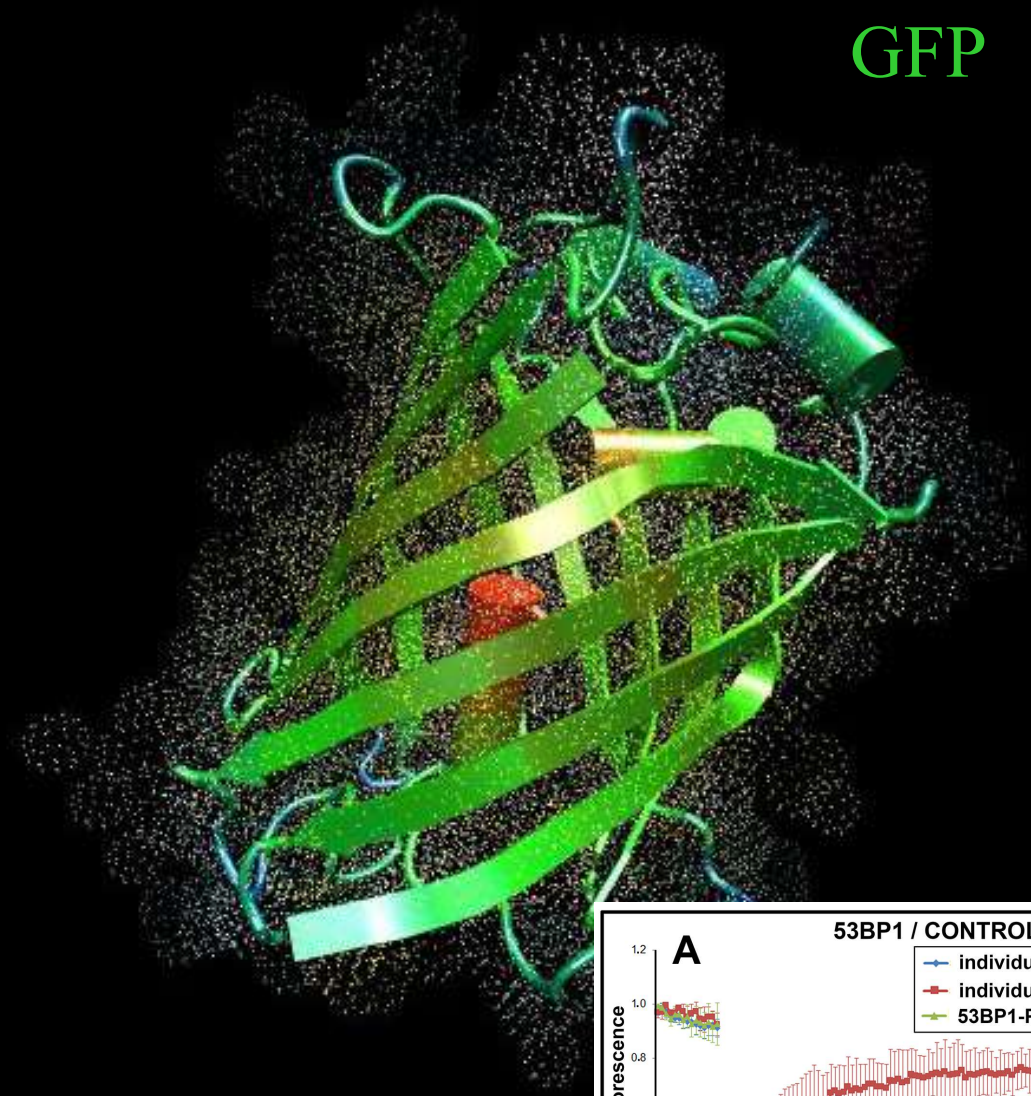
neurobiology
membrane biology
membrane rafts
intracellular transport

Willig KI et al. *Nature* 2006
Sieber JJ et al. *Biophy J* 2006
Kittel RJ et al. *Science* 2006
Fitzner D et al. *EMBO J* 2006

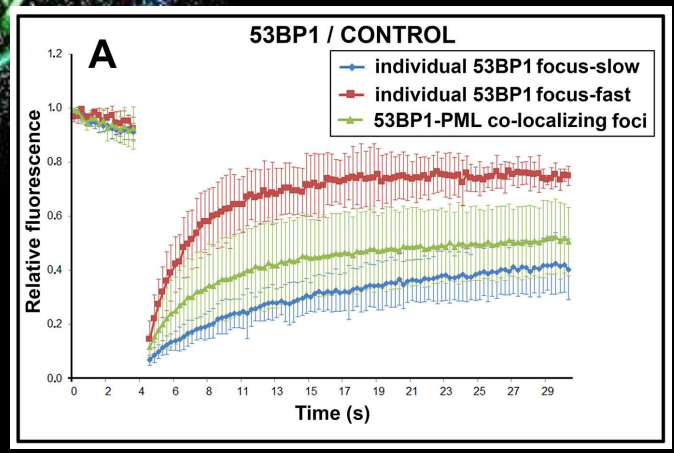
Kellner RR et al. *Neurosience* 2007
Lin W et al. *PNAS* 2007
Seebach J *Cardiovas. Res.* 2007
Sieber JJ *Science* 2007

Unique





FRAP



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.

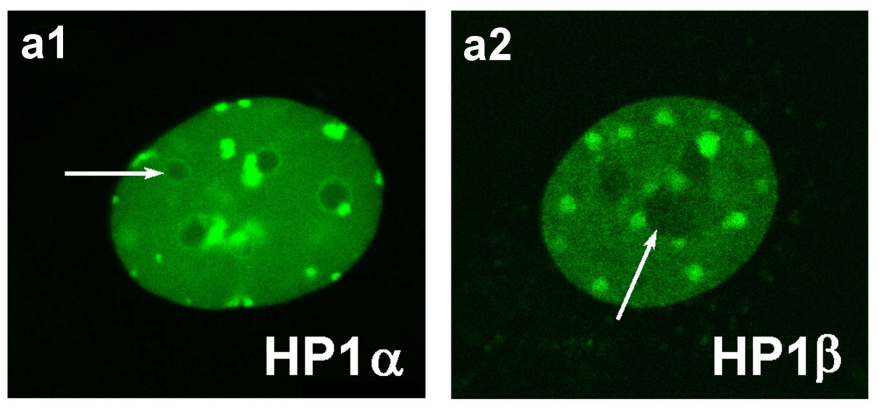
1. Fluorochromes conjugated with other molecule. Example represents quantum dots, used for for ultra-sensitive nonisotopic detection.

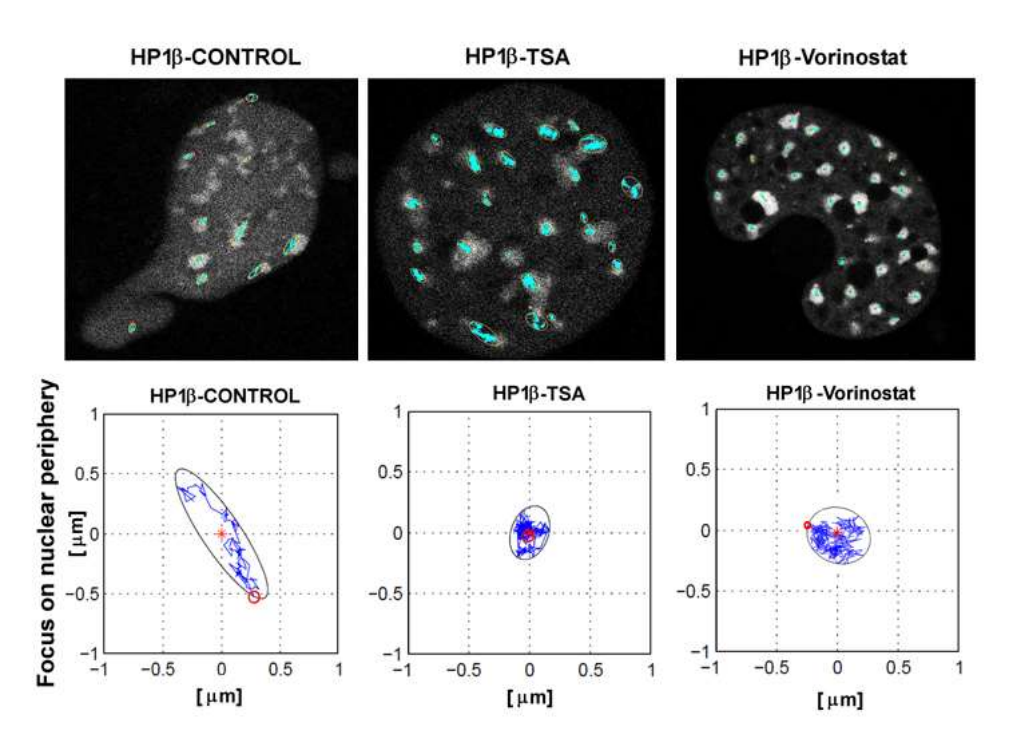
2. Fluorochormes that binds directly to some structure. For example, DAPI or PI binds to DNA

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

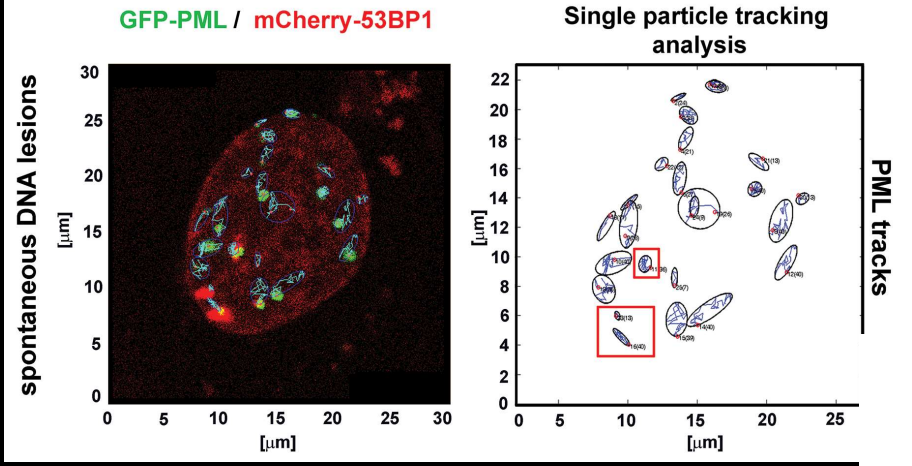
Fluorophore	Absorption (nm)	Emission (nm)
Fast Blue	360	440
Alexa Fluor 350	346	445
AMCA	350	450
Bisbenzamide	360	461
Aequorin	Ca^{++} photoprotein	469
Hoechst 33258	360	470
ACMA UV	412,400	471,474
Hoechst 33342	343	483
Cy2	489	506
GFP Wildtype Non UV ex.	475	509
GFP Wildtype UV ex.	395	509
Alexa Fluor 488	494	517
Calcein	496	517
Fluorescein (FITC/DTAF)	495	520
Fluoro-Jade® B	480	525
Lucifer yellow	425	528
JC-1	514	529
Fluoro-Gold (Hydroxystilbamidine)	361	536
Alexa Fluor 430	430	545
Eosin	524	545
6-JOE UV	520	548
Alexa Fluor 532	530	555
Cy3	548	562
Alexa Fluor 546	554	570
Alexa Fluor 555	555	571
TRITC	547	572
B-phycoerythrin	545,565	575
R-phycoerythrin	480,545,565	578
Rhodamine	539,574	602
Alexa Fluor 568	578	602
Texas Red®	589	615
Alexa Fluor 594	590	617
Propidium iodide (PI)	536	617
Ethidium bromide	493	620
Feulgen (Pararosaniline)	570	625
Acid Fuchsin	540	630
Alexa Fluor 633	621	639
Alexa Fluor 647	649	666
Cy5	650	670
PE-Cy5 conjugates	480,565,650	670
Alexa Fluor 660	668	698
Alexa Fluor 680	684	707
PE-Cy7 conjugates	480,565,743	767
Cy7	743	767

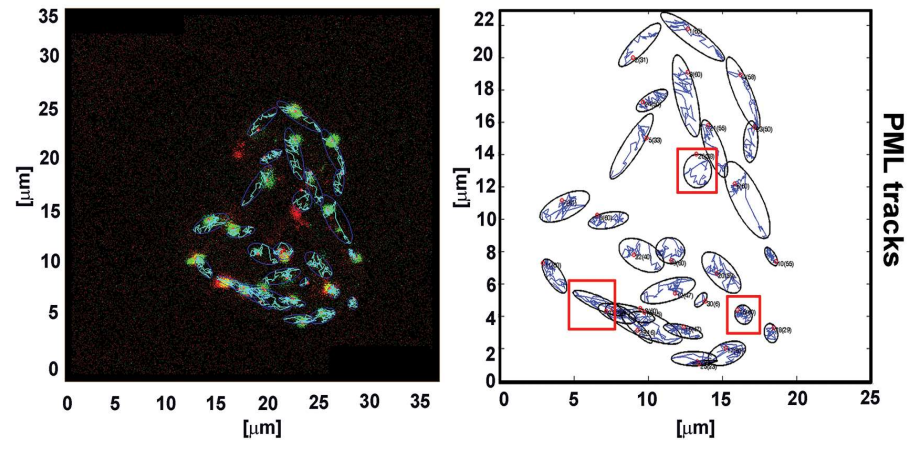
Single particle tracking

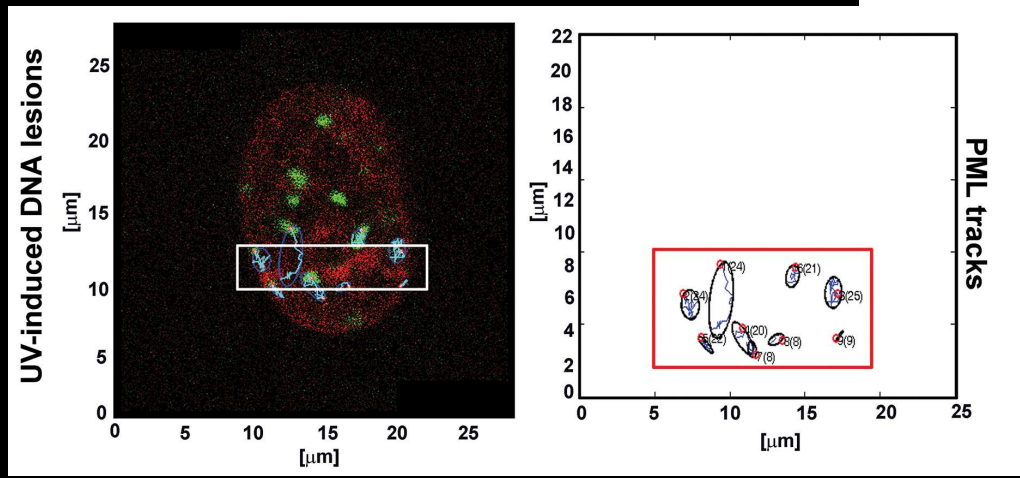




Experiments of Lenka Stixová and Pavel Matula

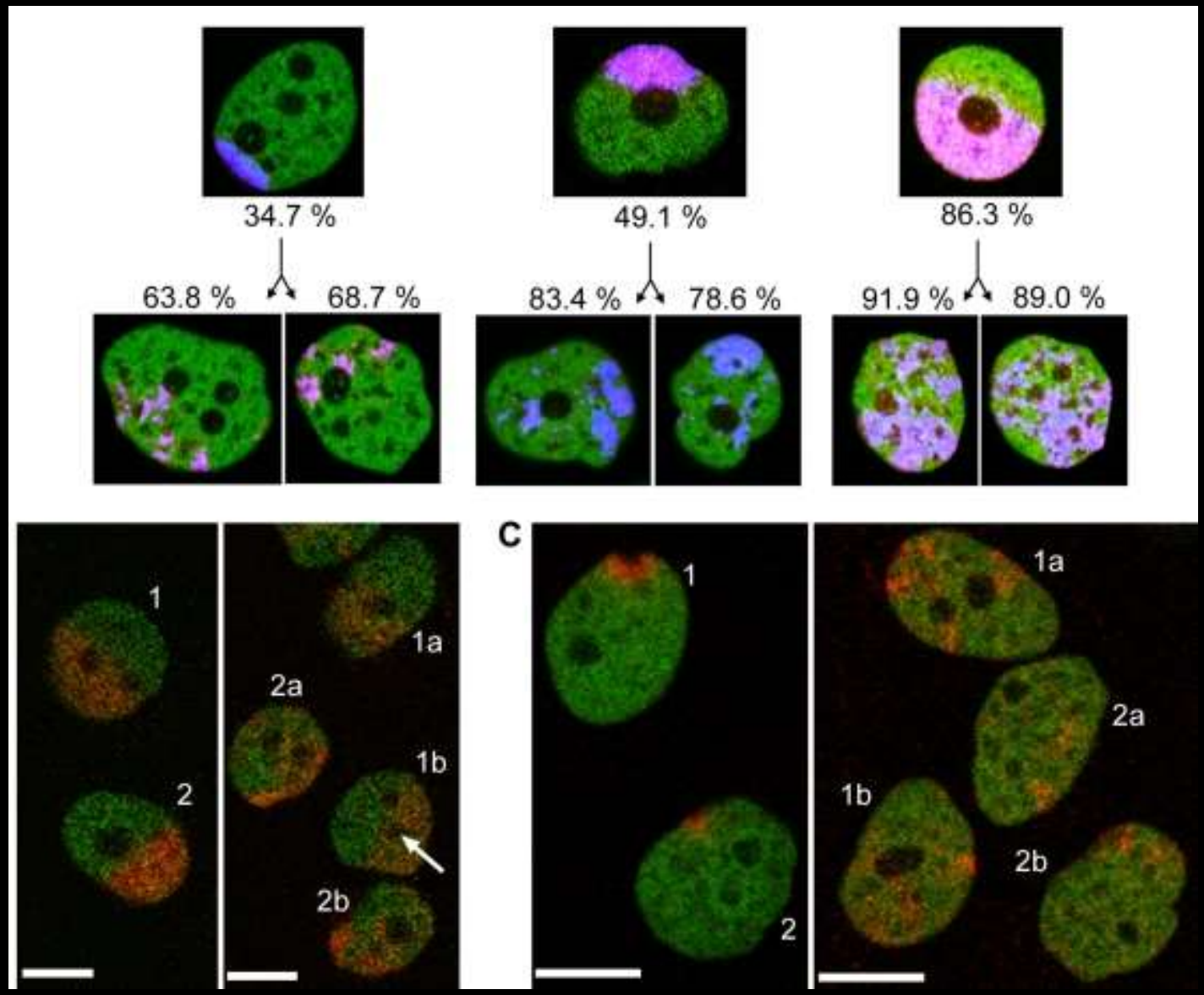




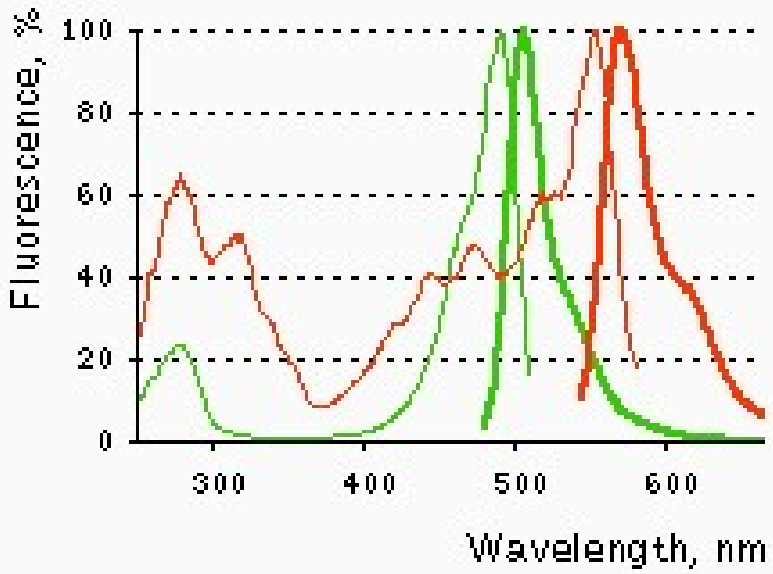


Experiments of Veronika Foltanková and Dmitry Sorokin

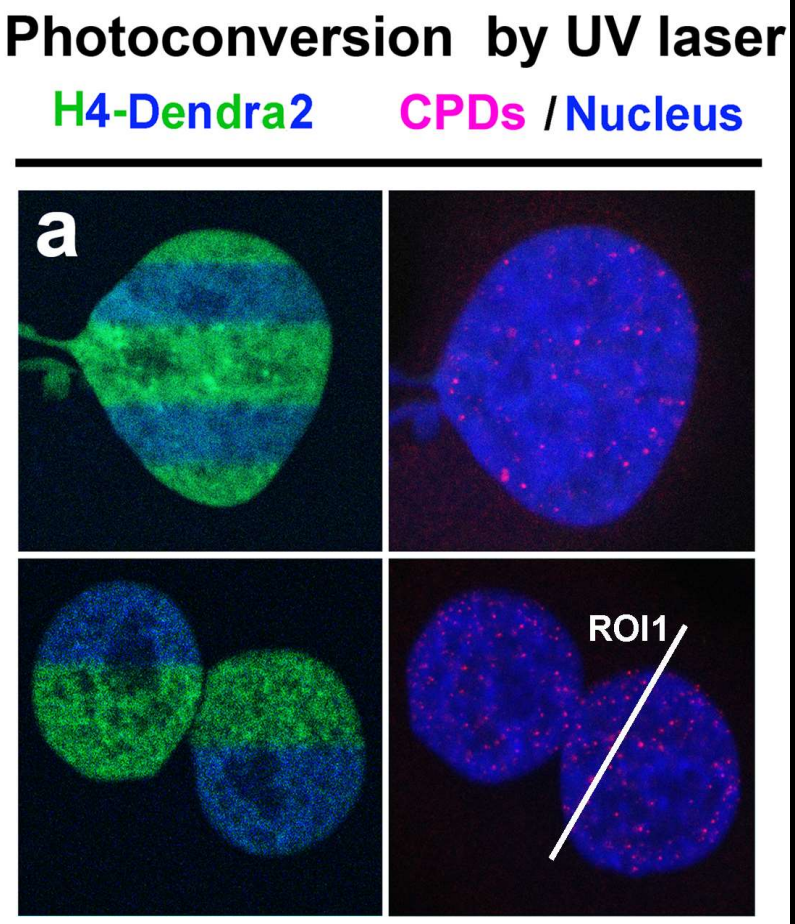
Dendra2 photo-conversion



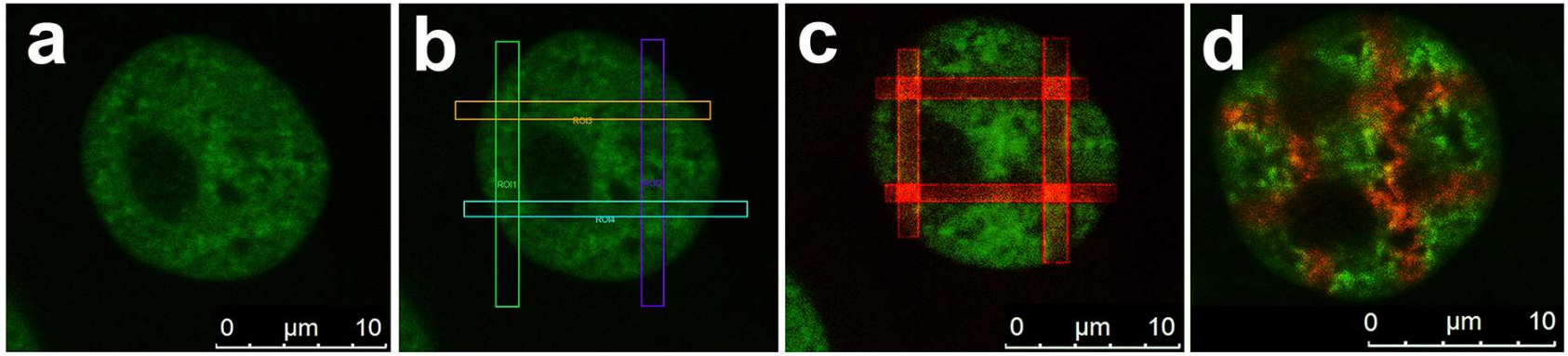
Dendra2 is an improved version of a green-to-red photoswitchable fluorescent protein Dendra, derived from octocoral *Dendronephthya* sp. (Gurskaya *et al.*, 2006).



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



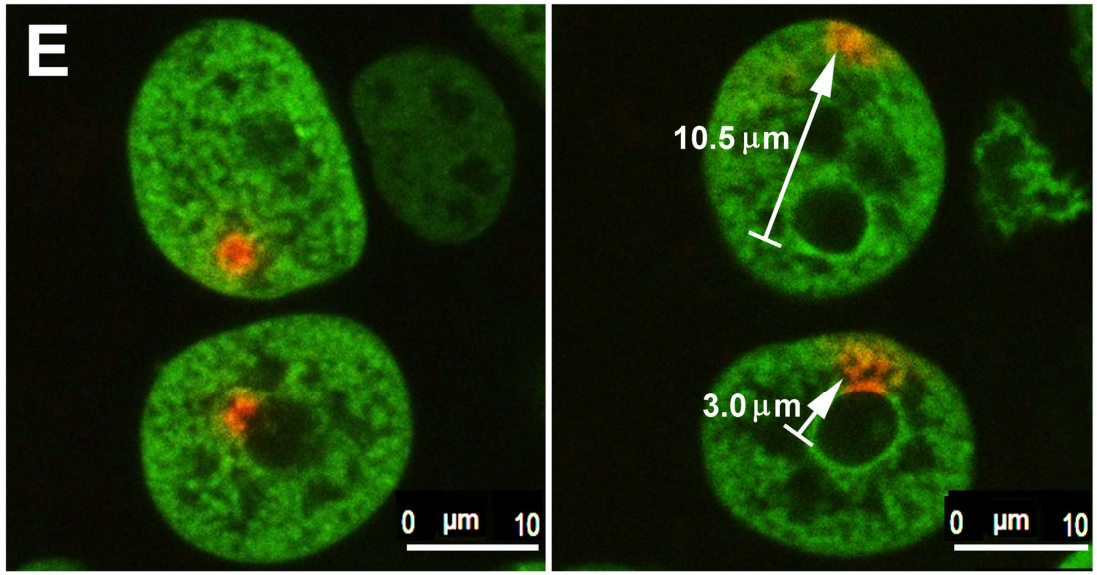
Dendra2-H4 photoconversion



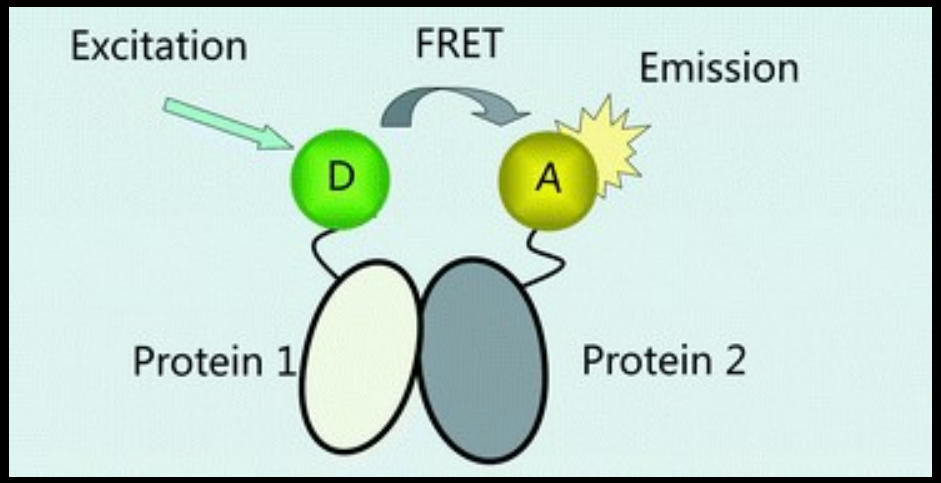
H4-Dendra2 / H4-Dendra2

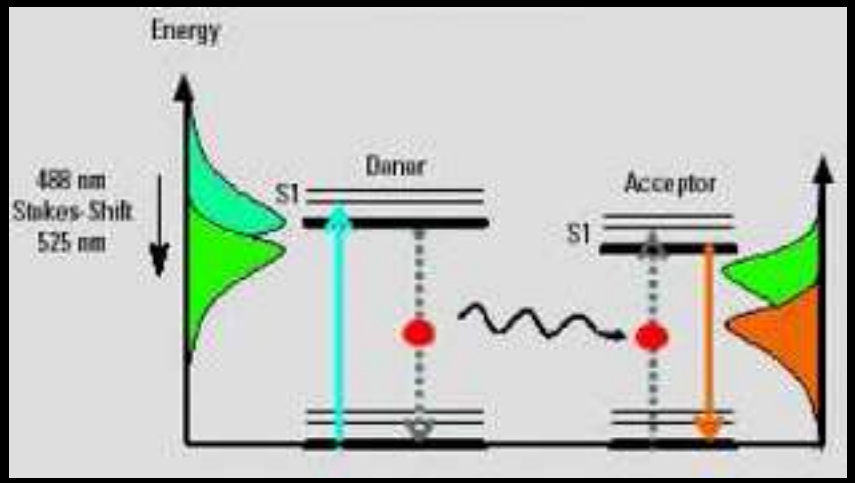
Actinomycin-D
(0 min. after photoconversion)

Actinomycin-D (90 min.)

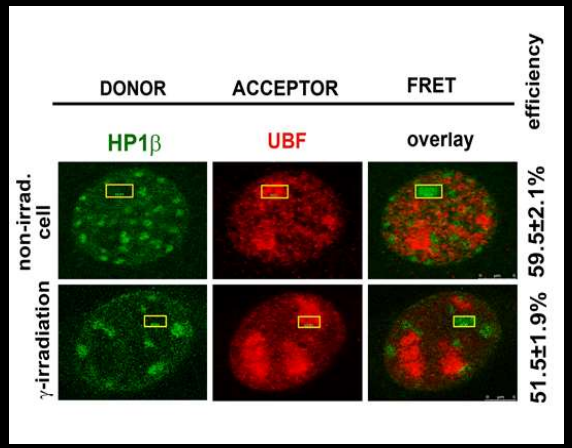


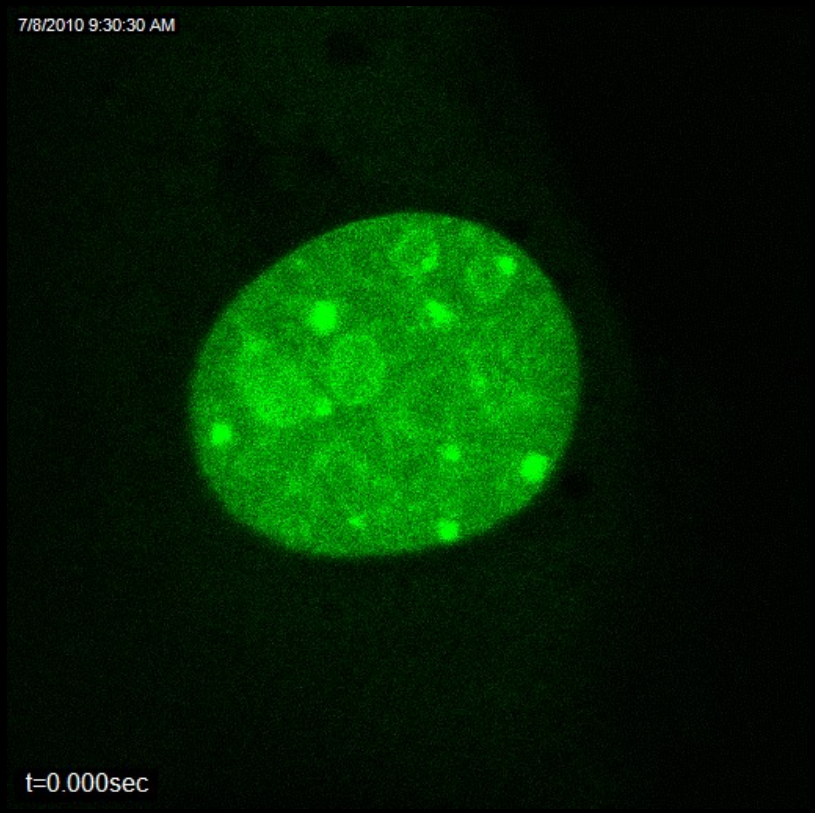
FRET (Fluorescence Resonance Energy Transfer) is a technique for measuring interactions between two proteins in vivo. In this technique, two different fluorescent molecules (fluorophores) are genetically fused the two proteins of interest.





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

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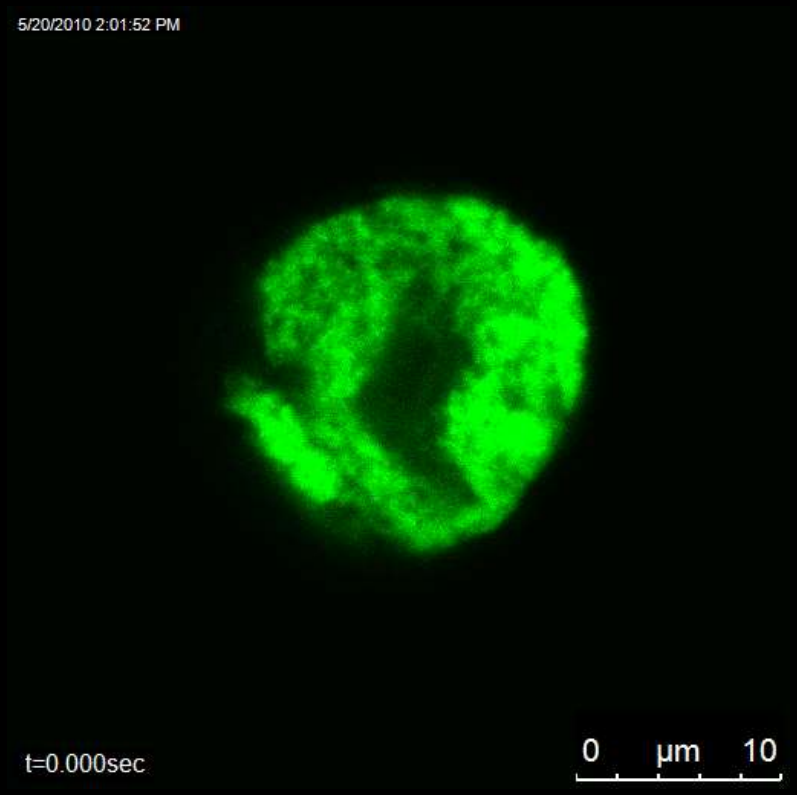
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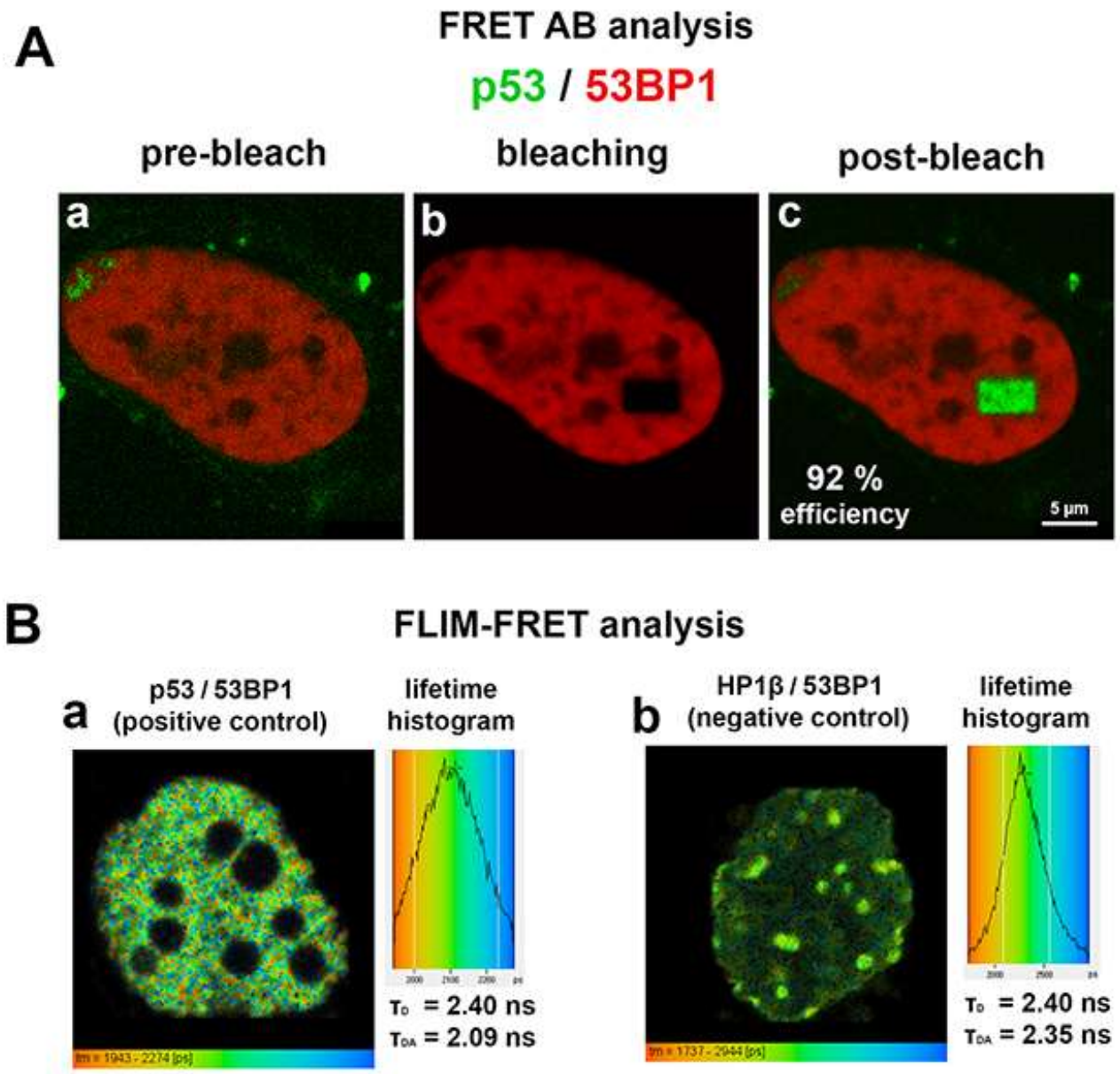
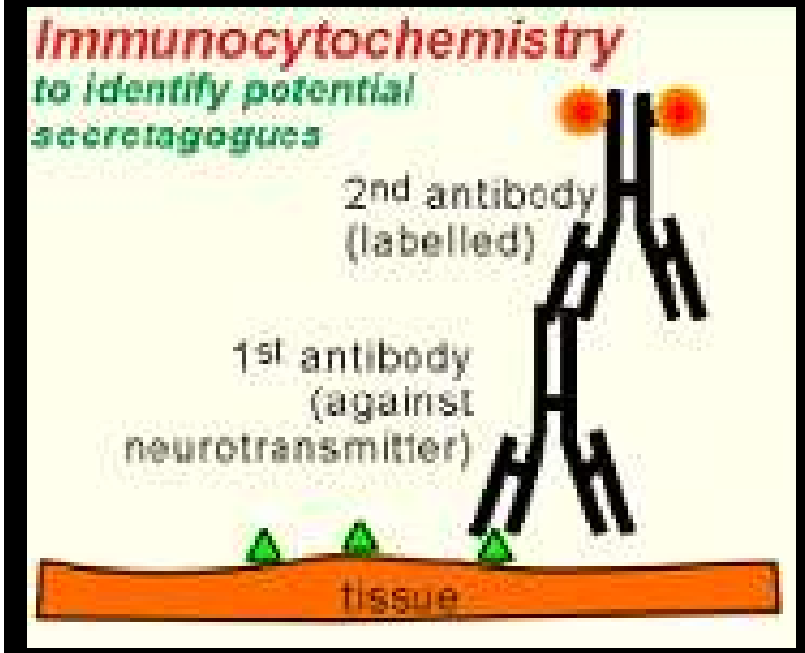
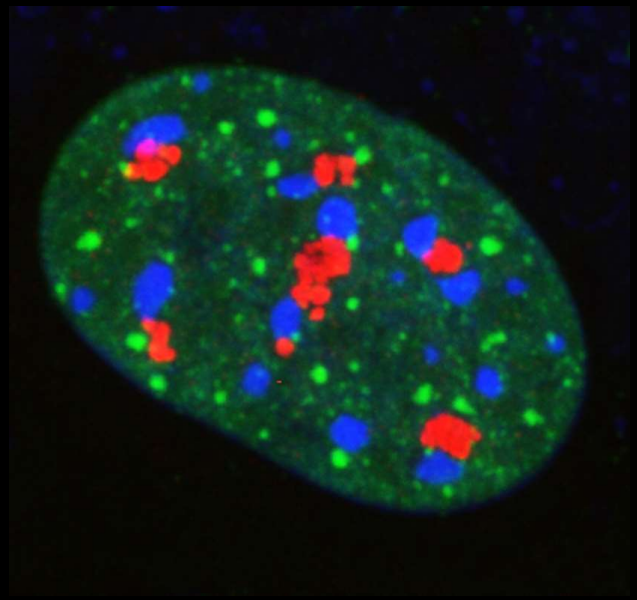
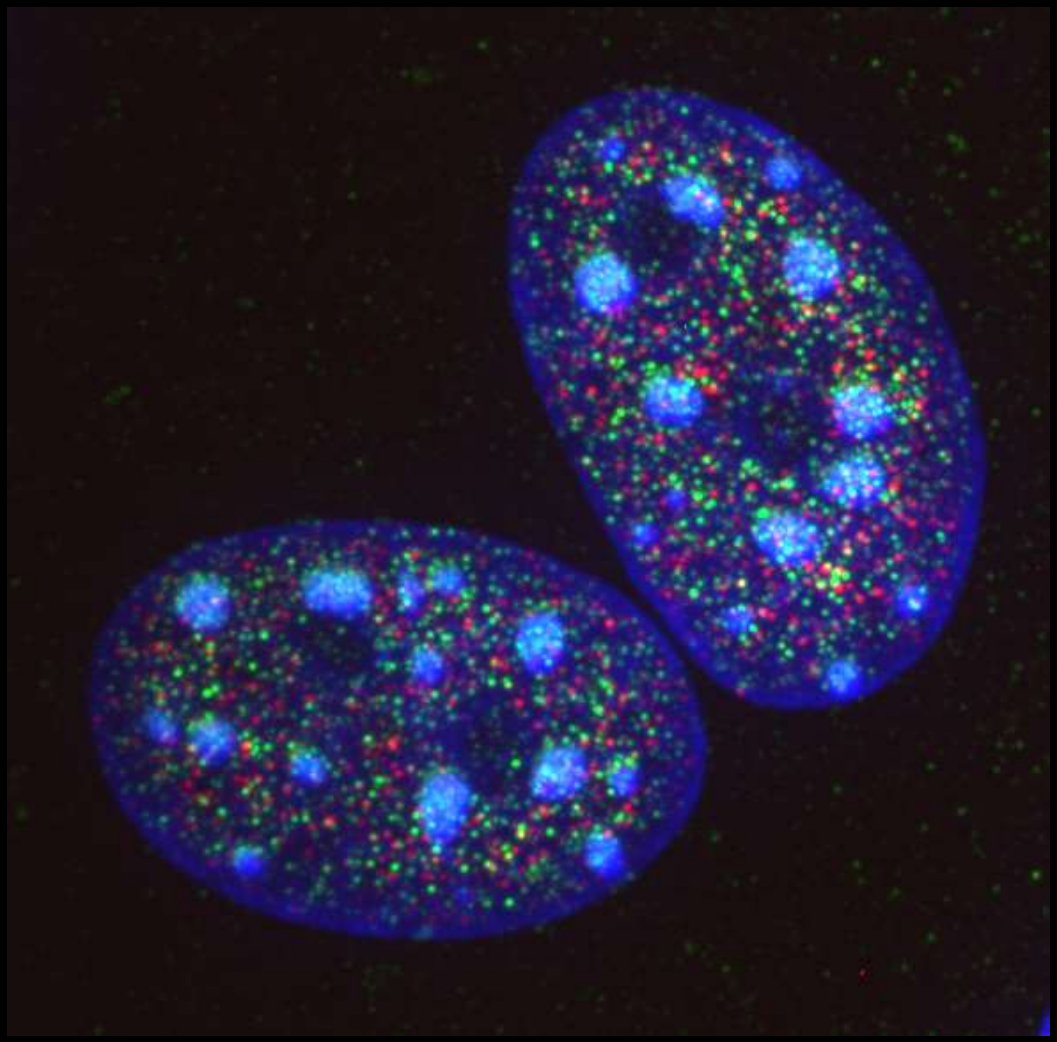

A
FRET AB analysis
p53 / 53BP1
pre-bleach
bleaching
post-bleach
a
b
c
92 %
efficiency
5 µm
B
FLIM-FRET analysis
a
p53 / 53BP1
(positive control)
lifetime
histogram
τD = 2.40 ns
τDA = 2.09 ns
b
HP1β / 53BP1
(negative control)
lifetime
histogram
τD = 2.40 ns
τDA = 2.35 ns



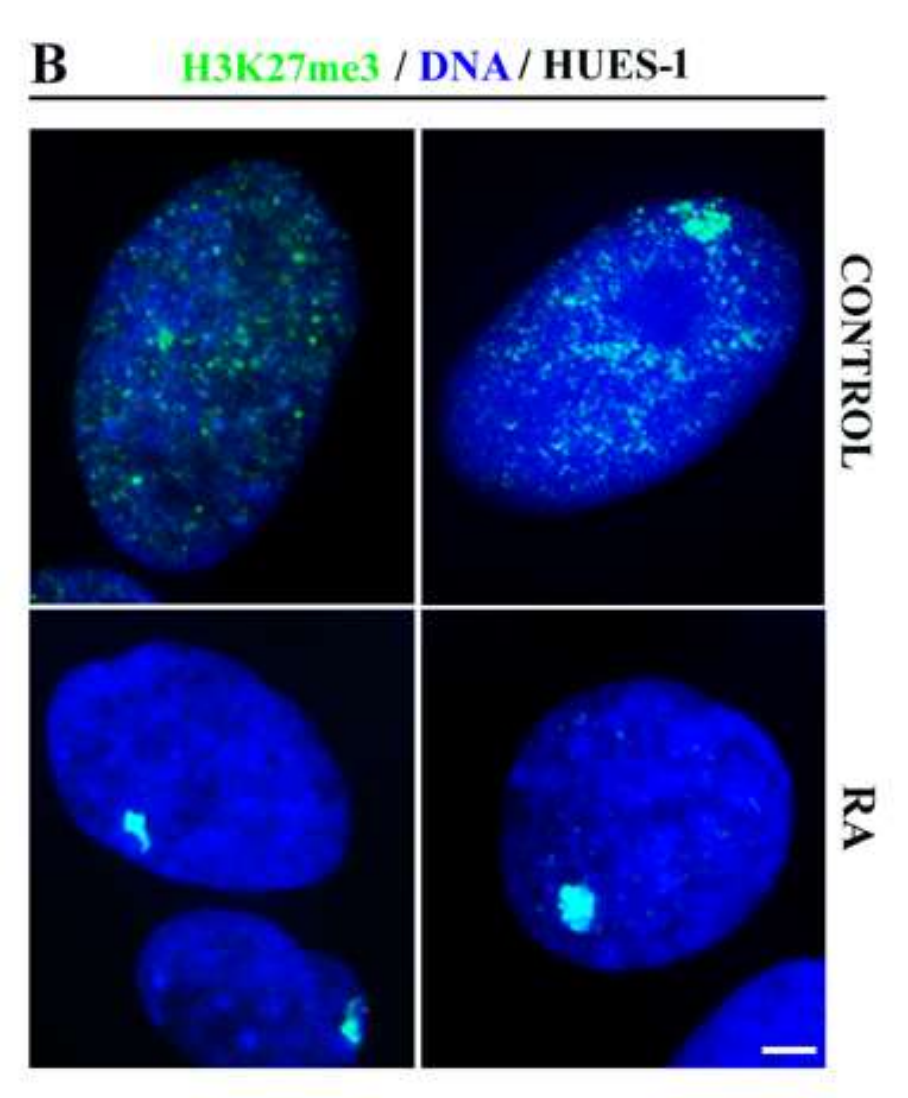
http://www.celanphy.science.ru.nl/
Bruce%20web/construction.htm

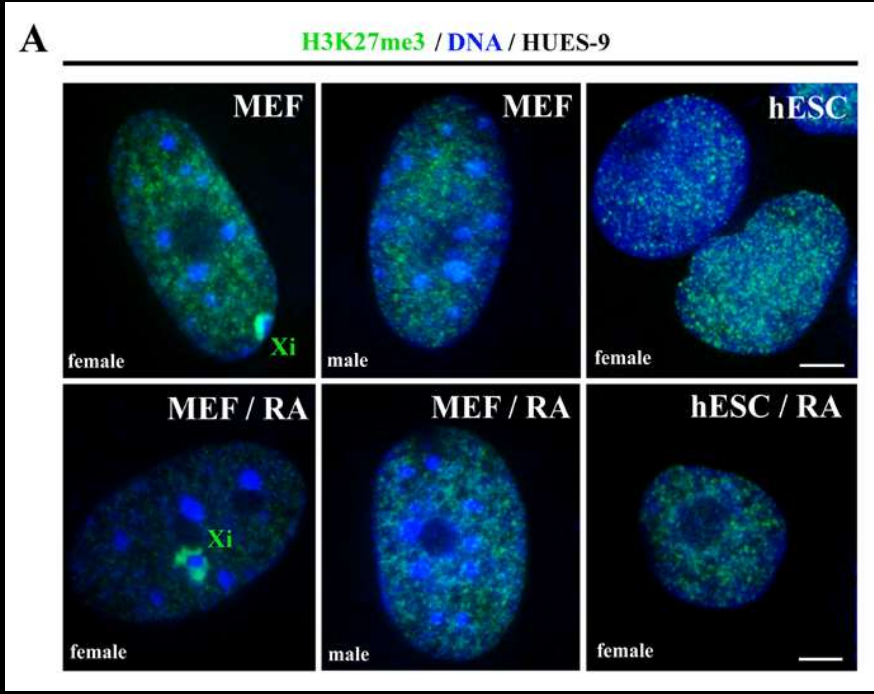


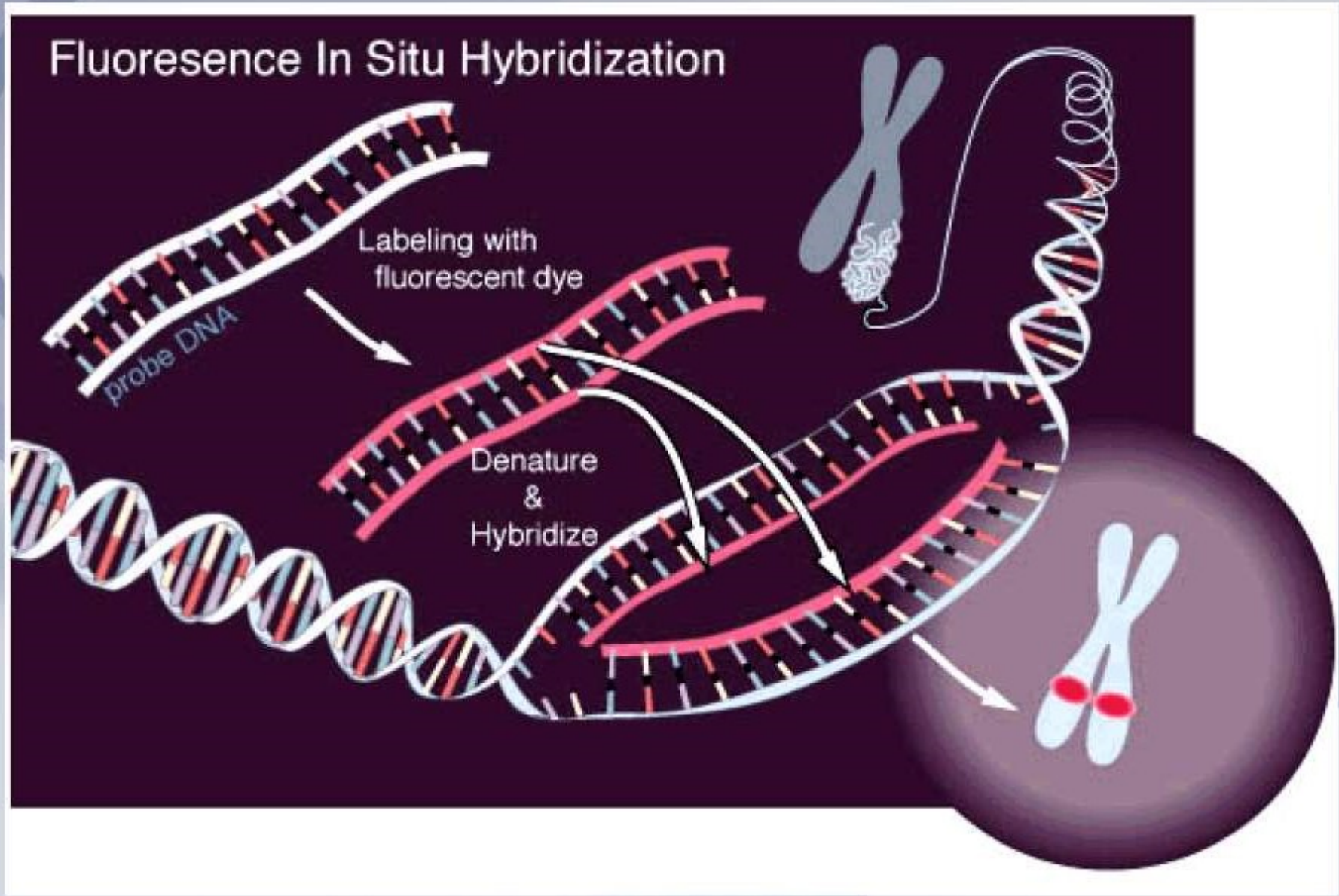


EB group, IBP, Brno

Inactivation of X chromosome in hESCs in comparison to MEFs

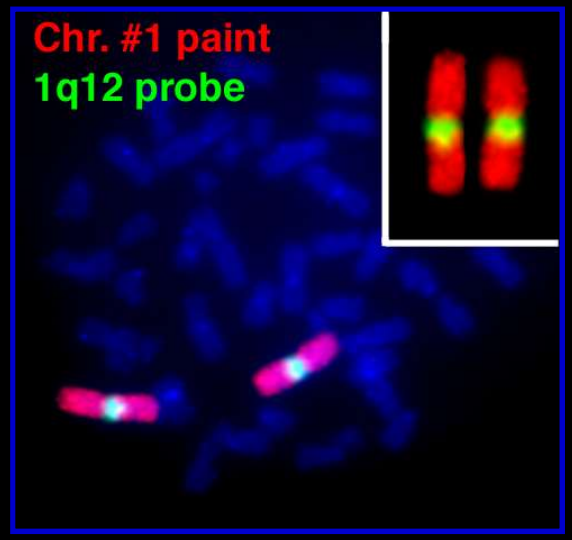


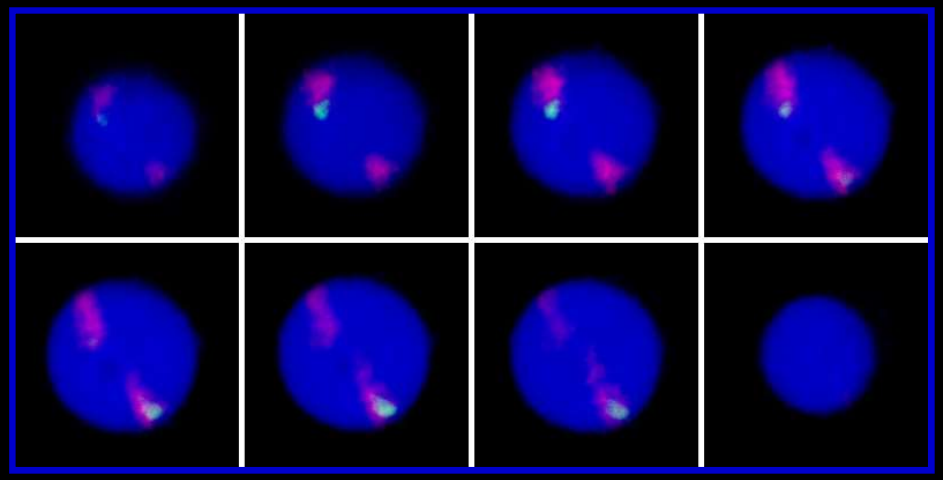




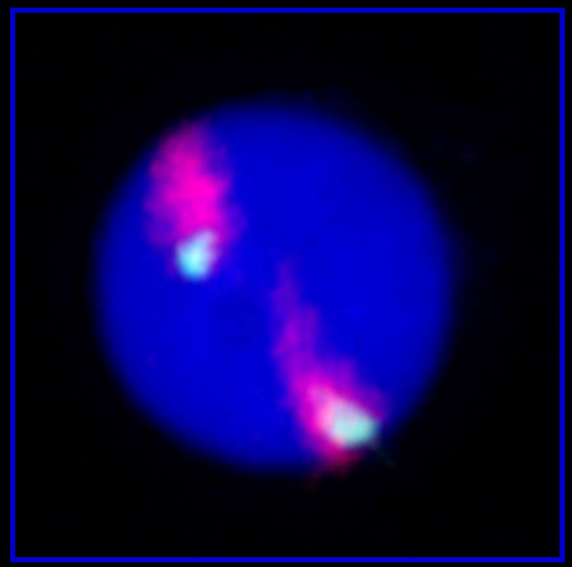
http://www.accessexcellence.org/RC/VL/GG/fish.html

3D-FISH a konfokální mikroskopie

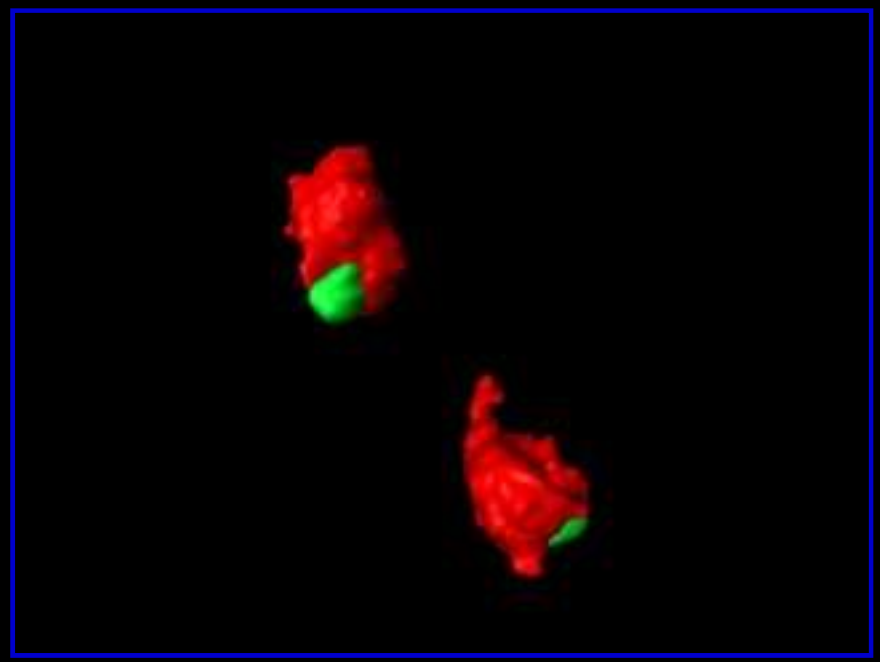




Galerie optických řezů

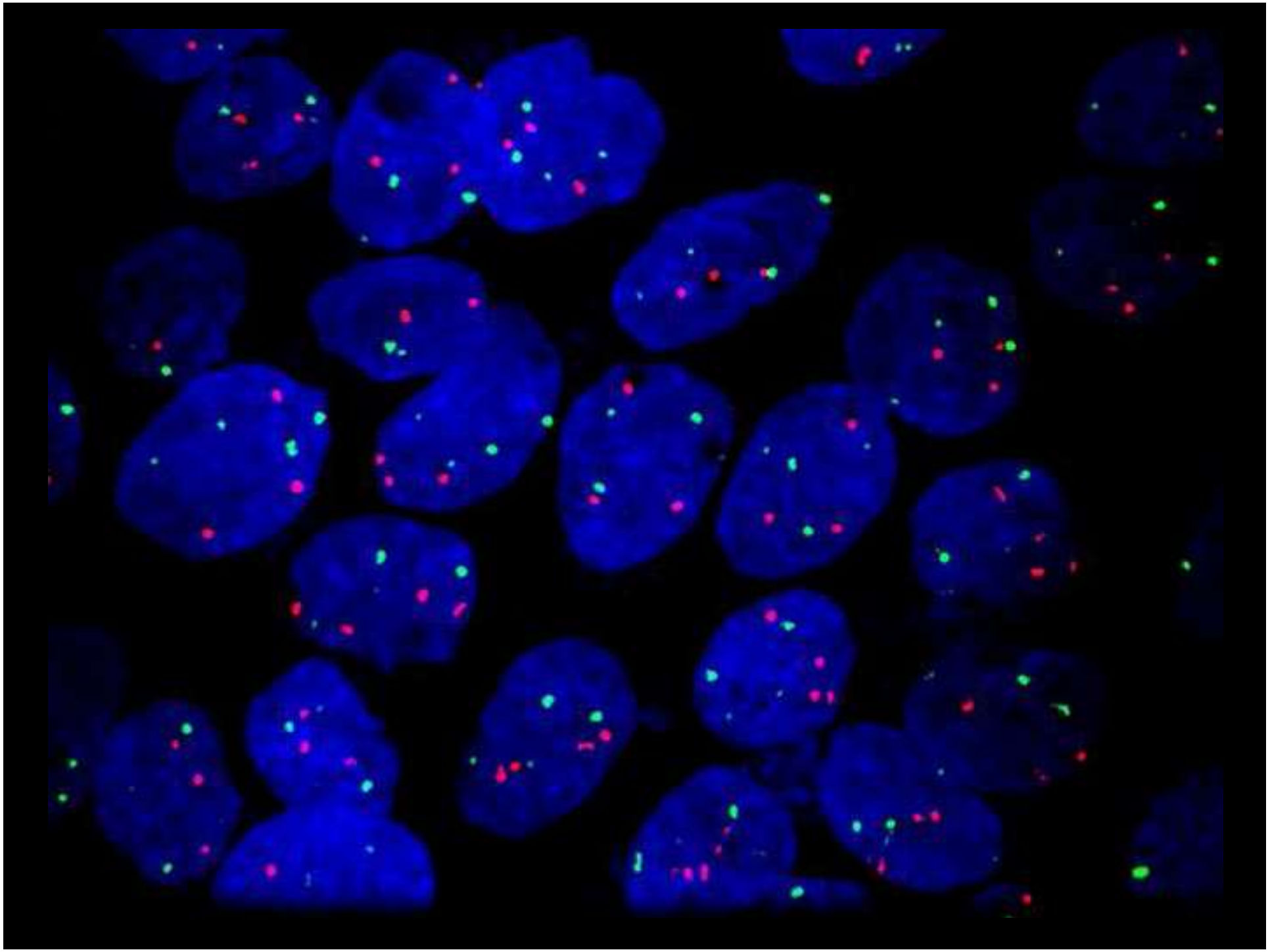


Maximální obraz
Všech řezů



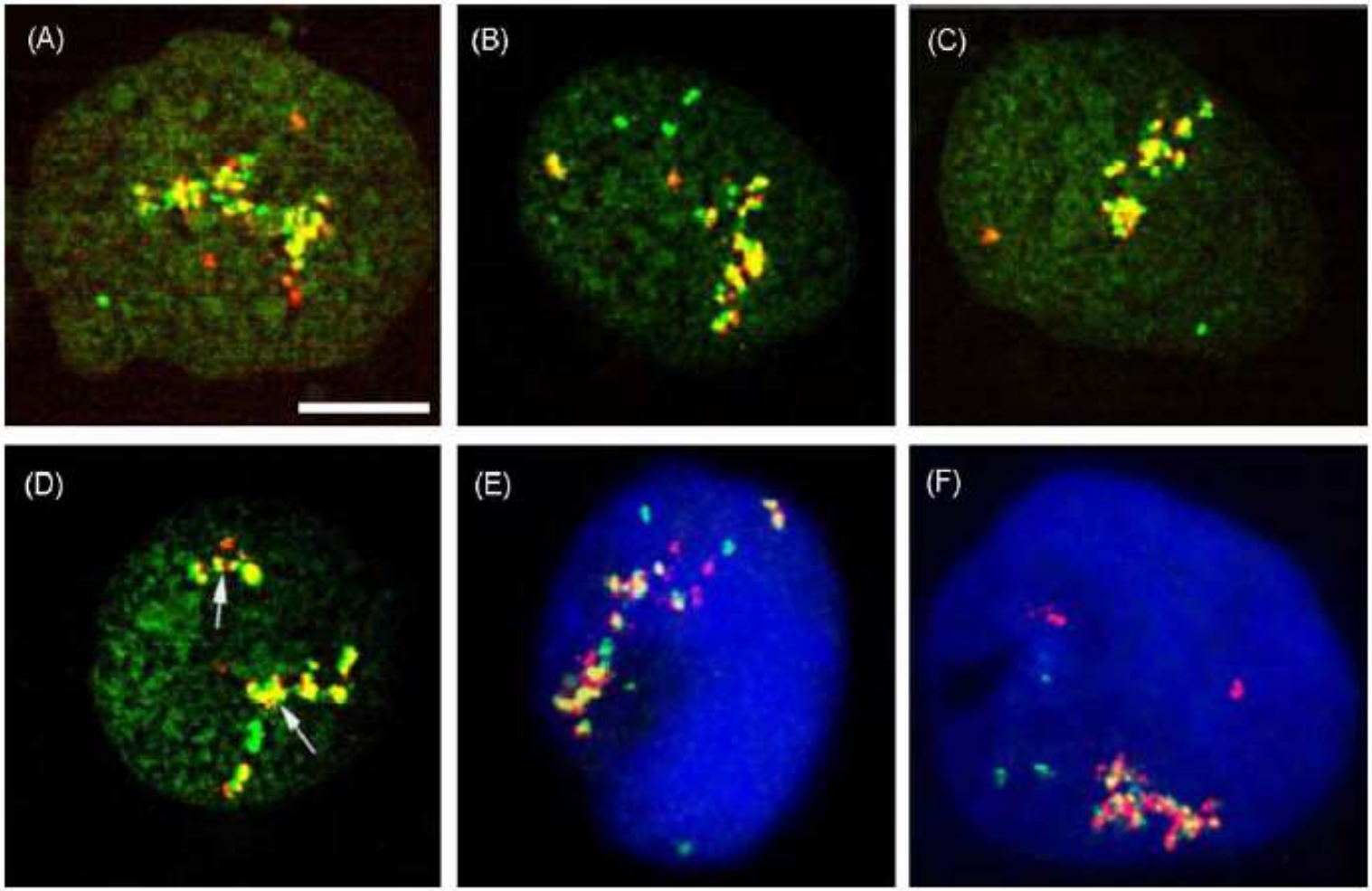
3D reconstrukce CT

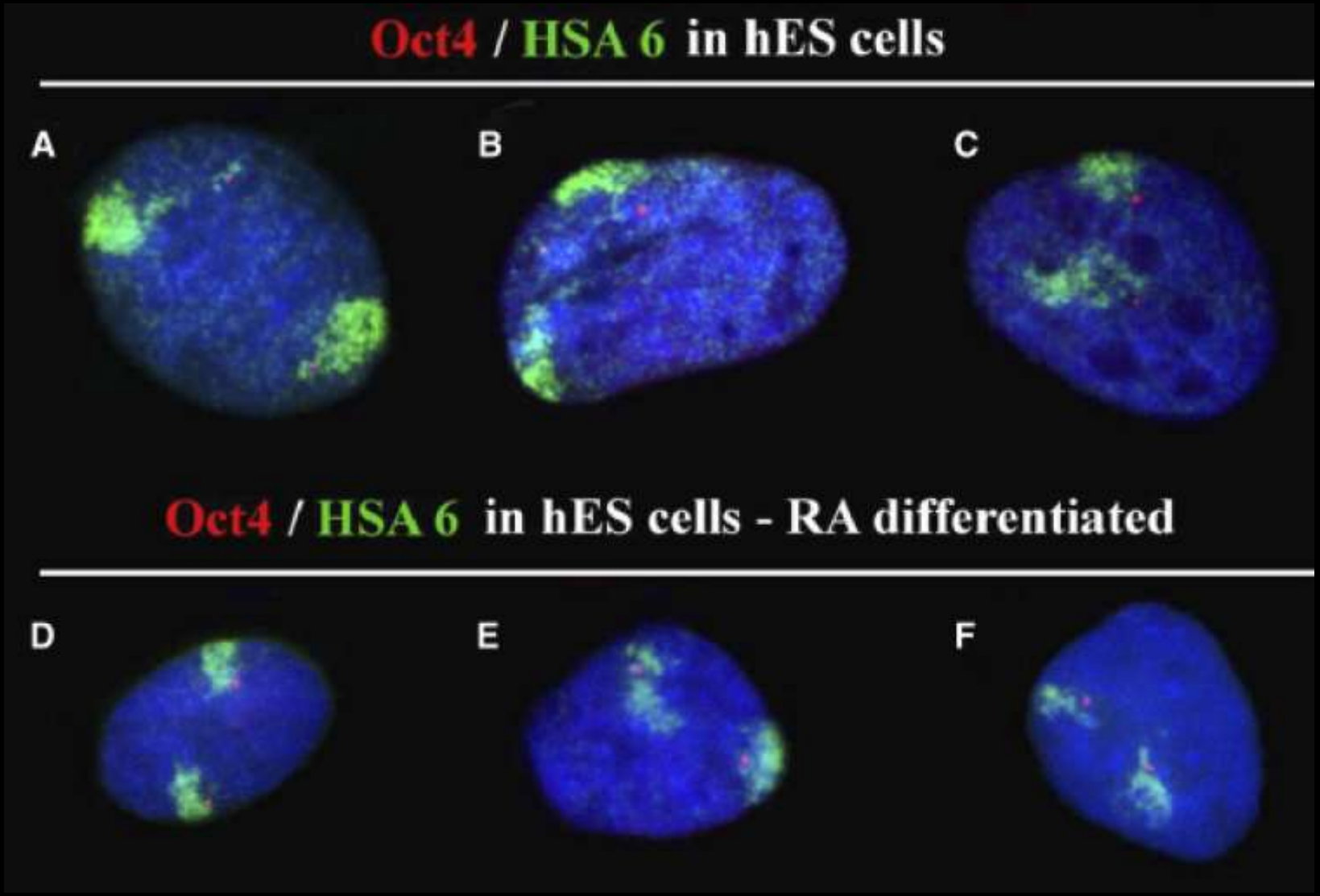
Weierich et al., (2003) in press



K562 cells t(9;22)

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

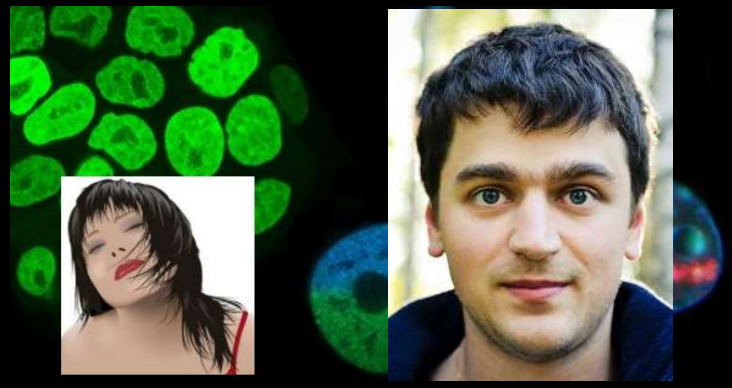




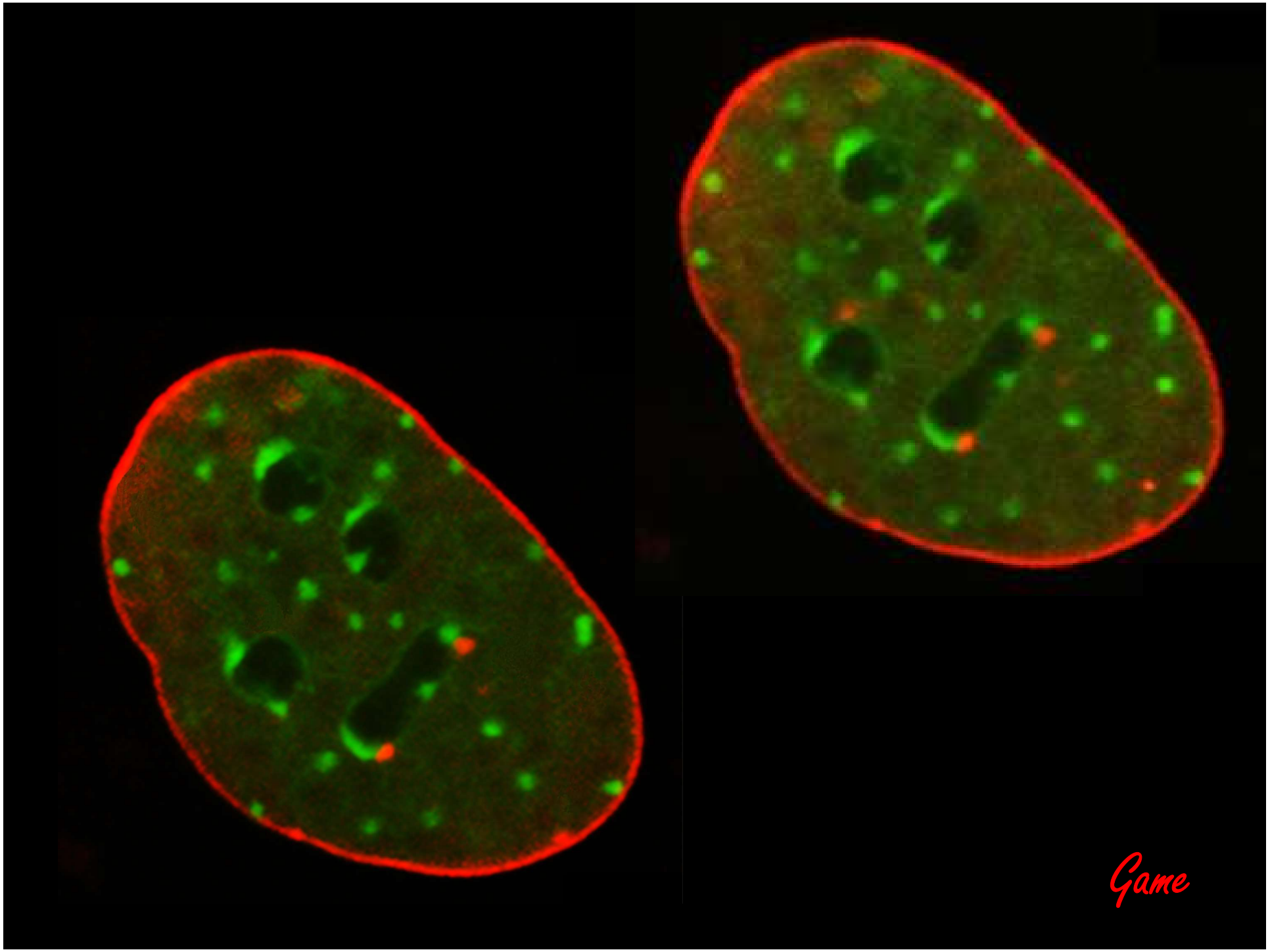
Bártová et al., Differentiation (2008)

GAČR

THE ACADEMY OF SCIENCES OF THE CZECH REPUBLIC



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