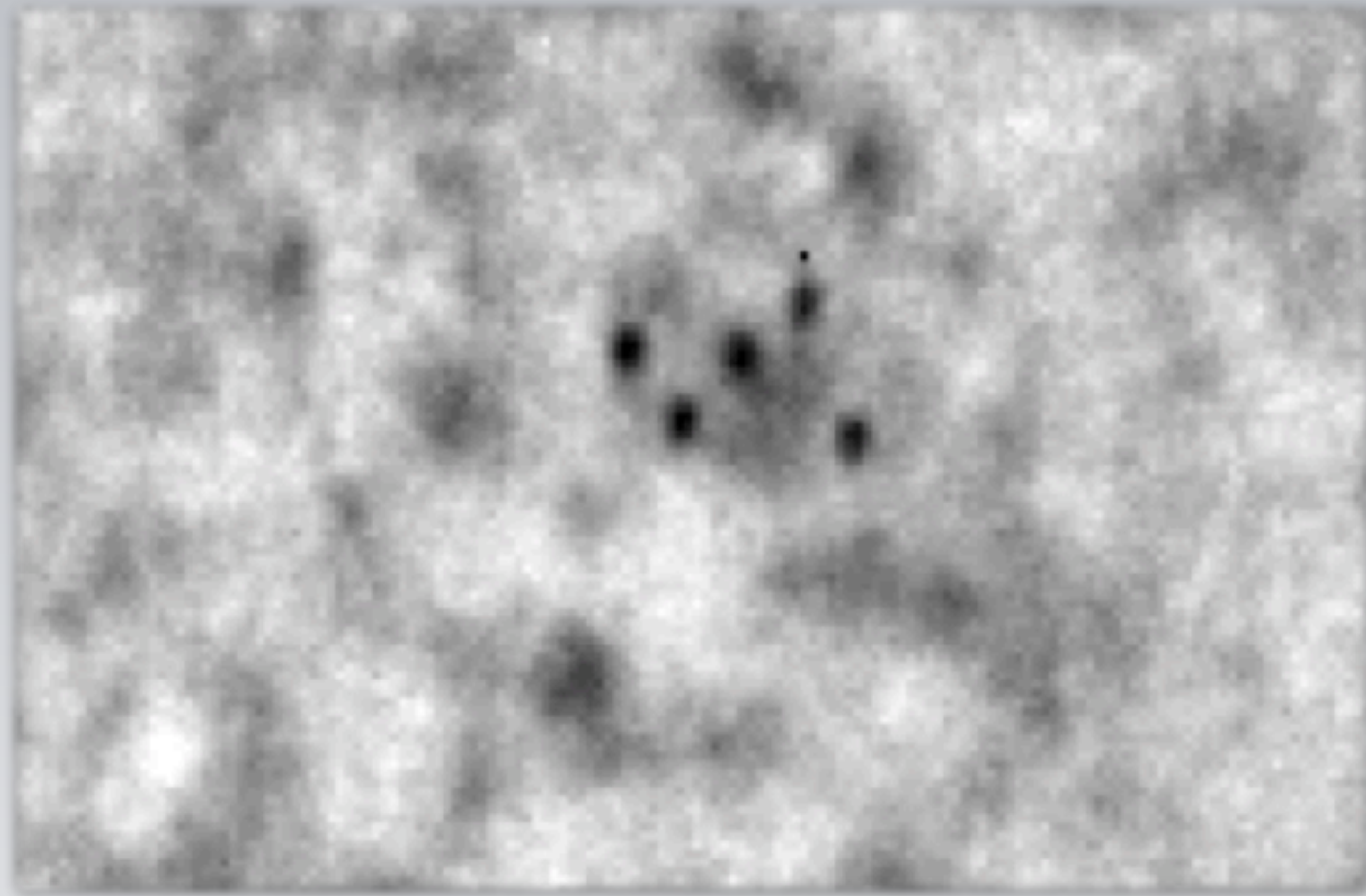


FACULTY OF PHYSICS UW

DNA damage and repair in cells exposed to high and low LET radiation

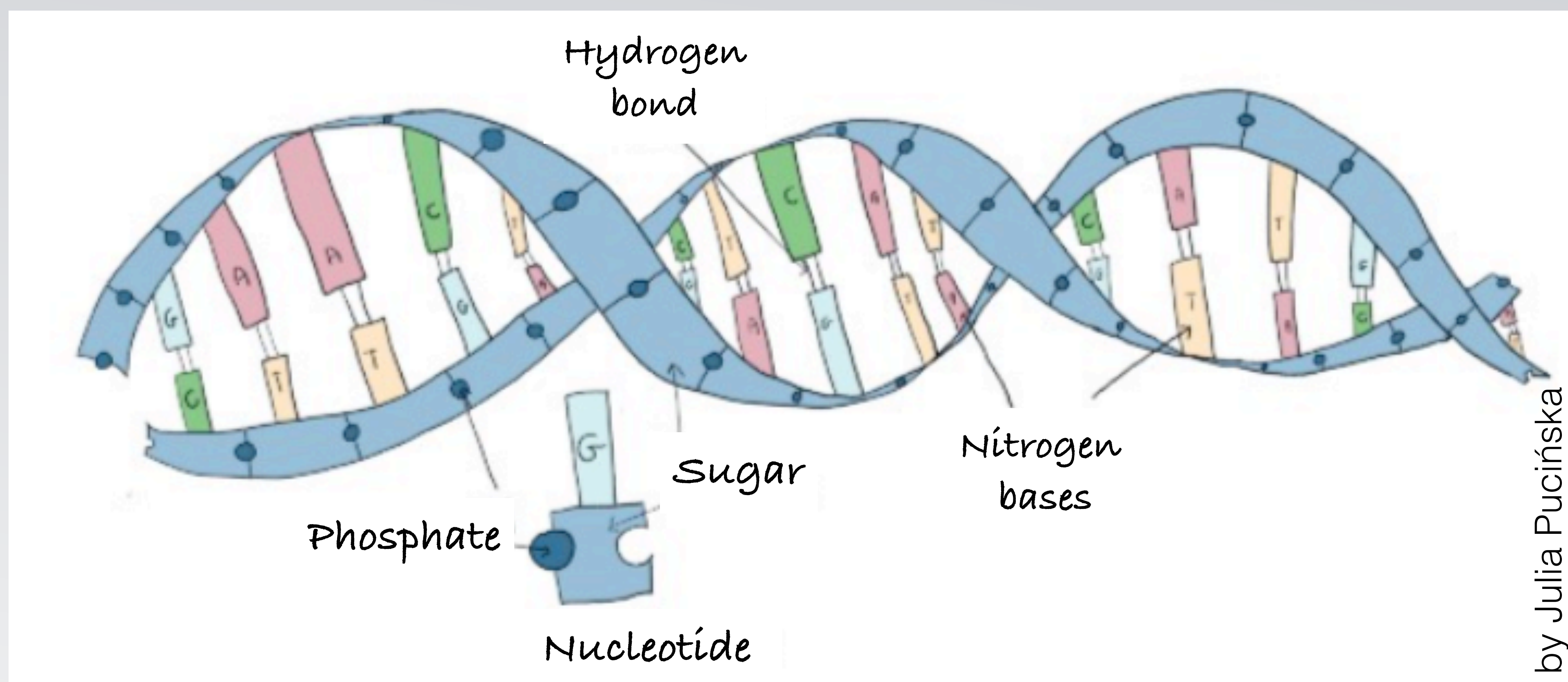
Beata Brzozowska

High Energy Physics Seminar, 6th of October, 2023

Medical physics @ Biomedical Physics Division

DNA damage and repair processes in cells exposed to ionising radiation

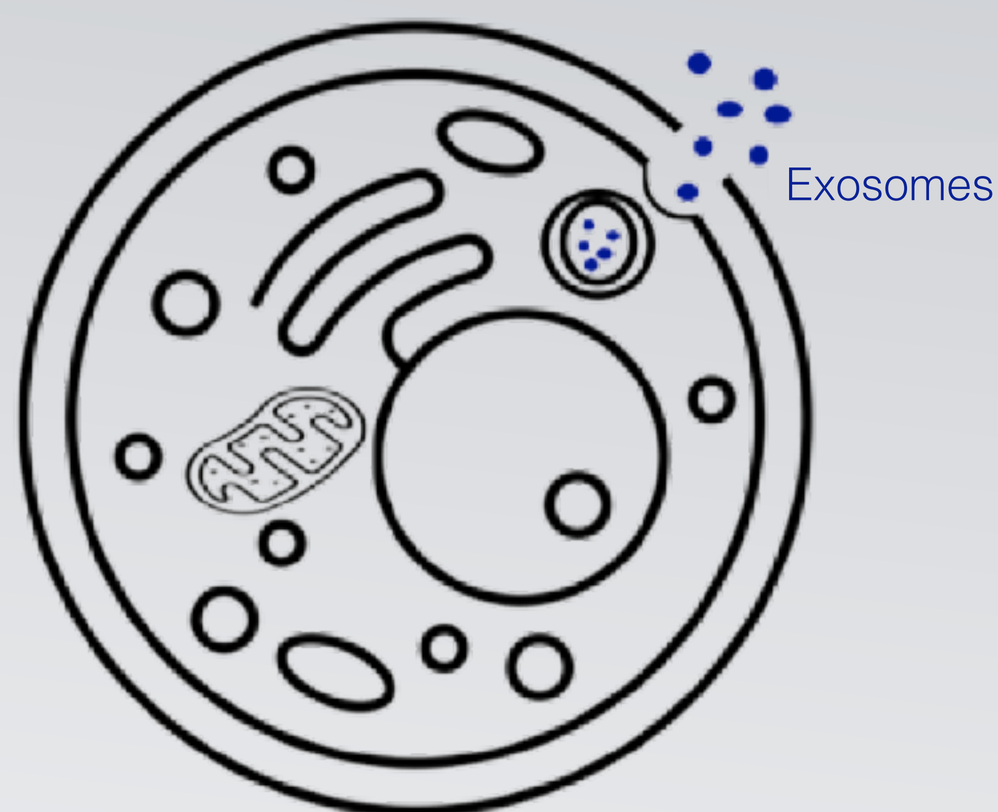
a few nm



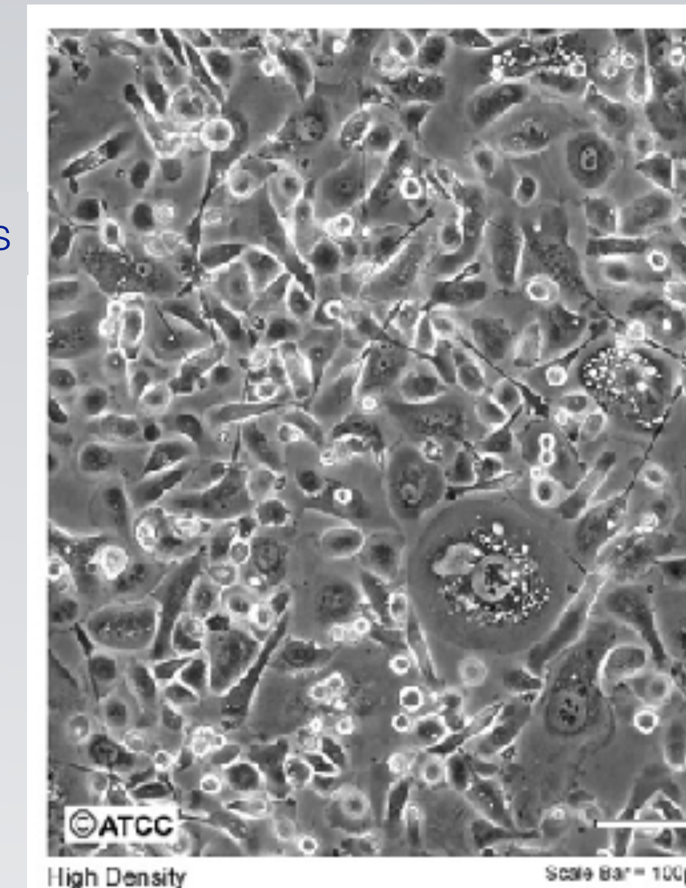
nanodosimetry

MC modeling

30-150 nm

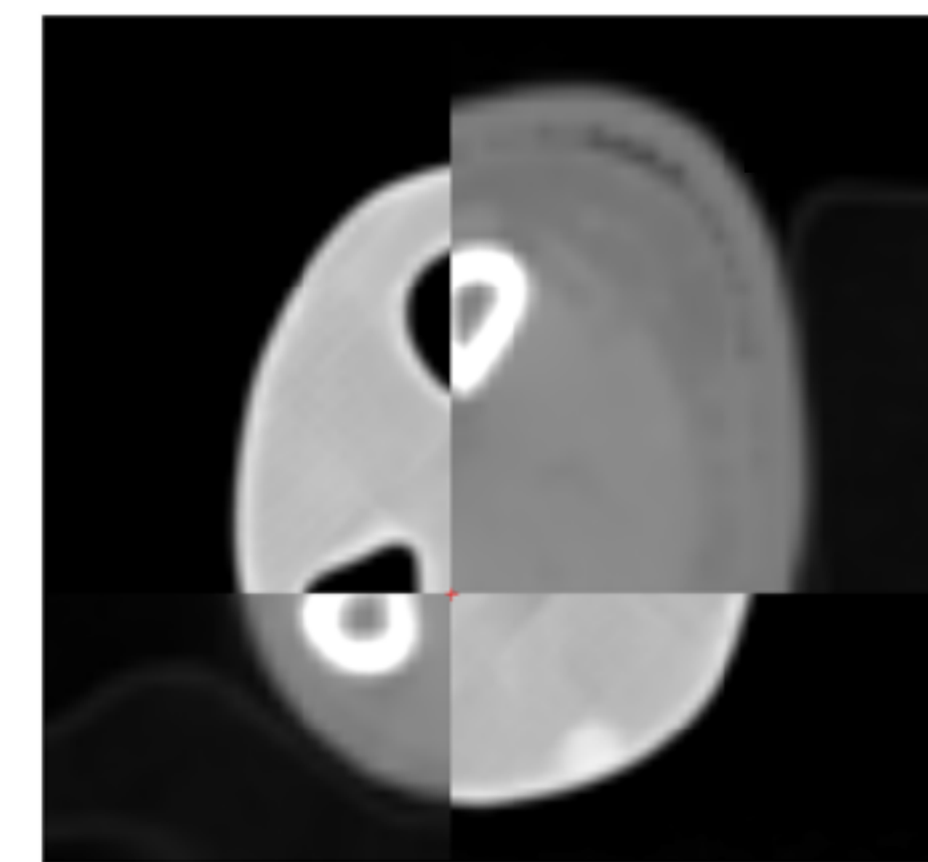


5-50 μm



radiobiology

5-15 cm



dosimetry in radiotherapy

Laboratories

in silico

in vitro

Outline

- Motivation: why mixed beams?
- DSB, SSB, GFP, γH2AX
- Focus analysis:

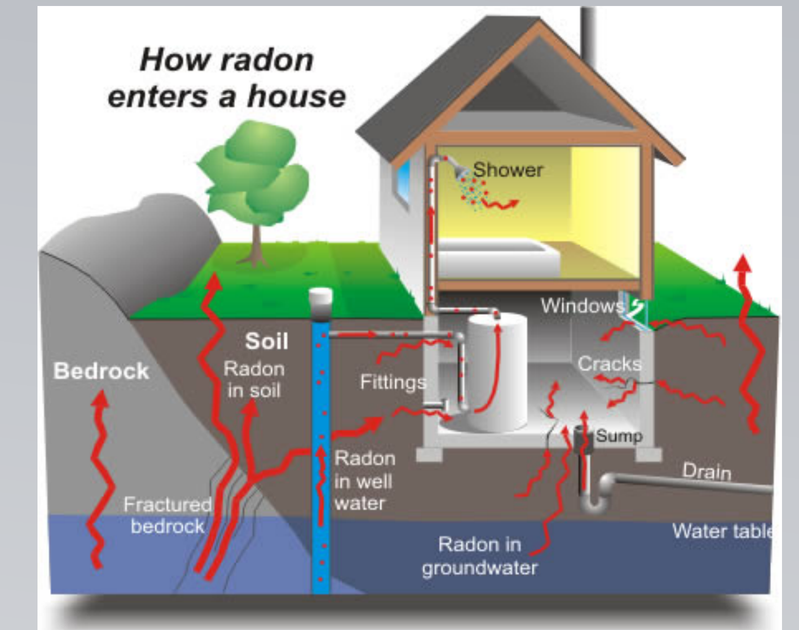
    A. fluorescence microscopy

    B. TEM

- Ongoing projects

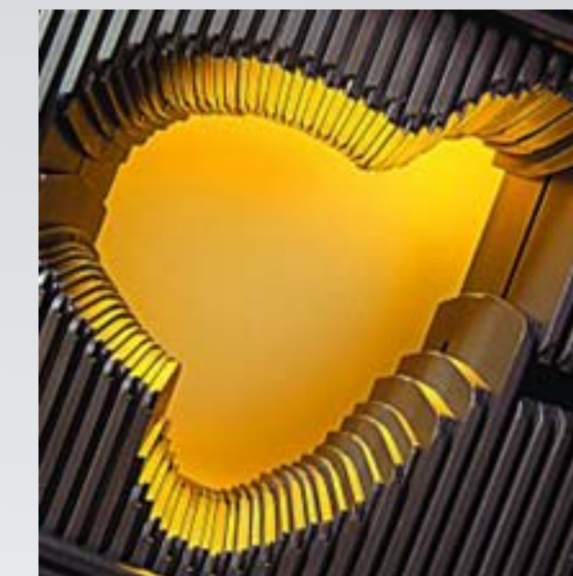
Who is exposed to mixed beams of radiation?

- People living in areas of high natural background radiation ($\alpha + \gamma$)

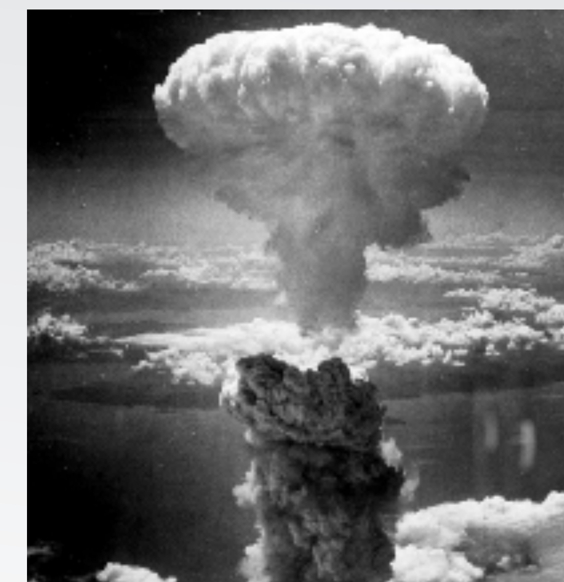


- Aeroplane passengers and astronauts ($n + p + \gamma$)

- Cancer patients treated with IMRT and proton therapy ($n + \gamma$)

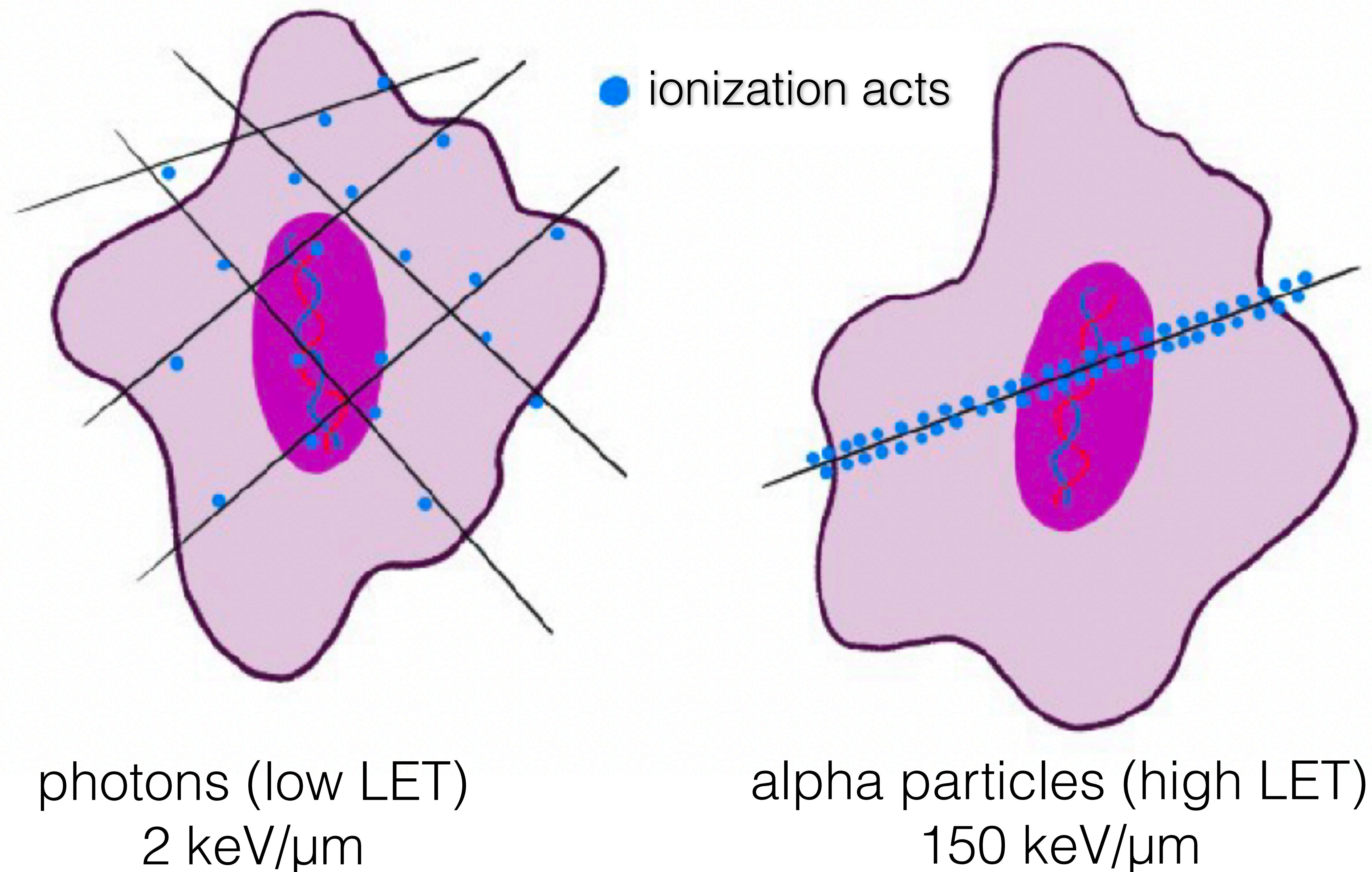


- People involved in radiation accidents

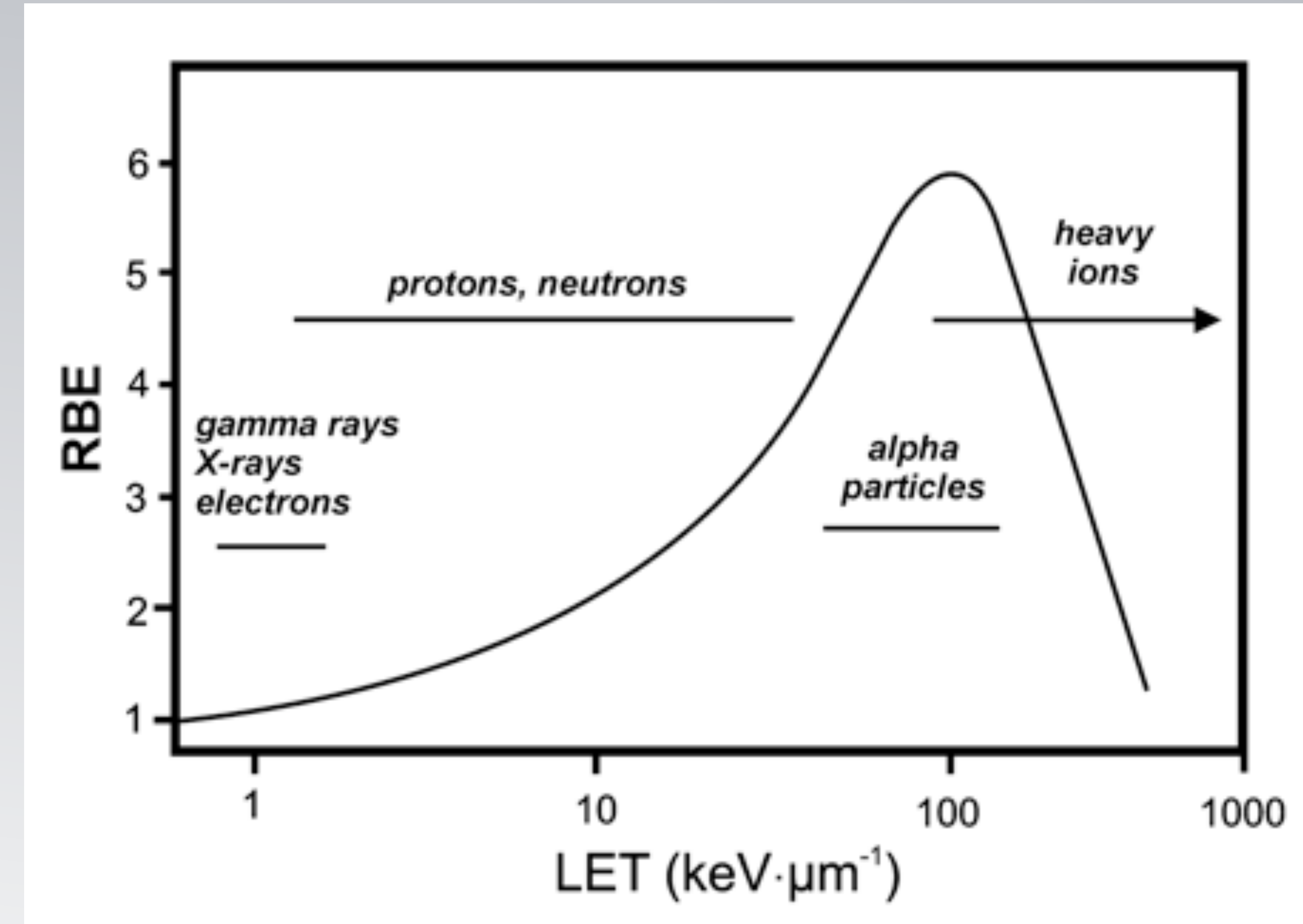


Biological effectiveness

Absorbed dose: $D = dE/dm$



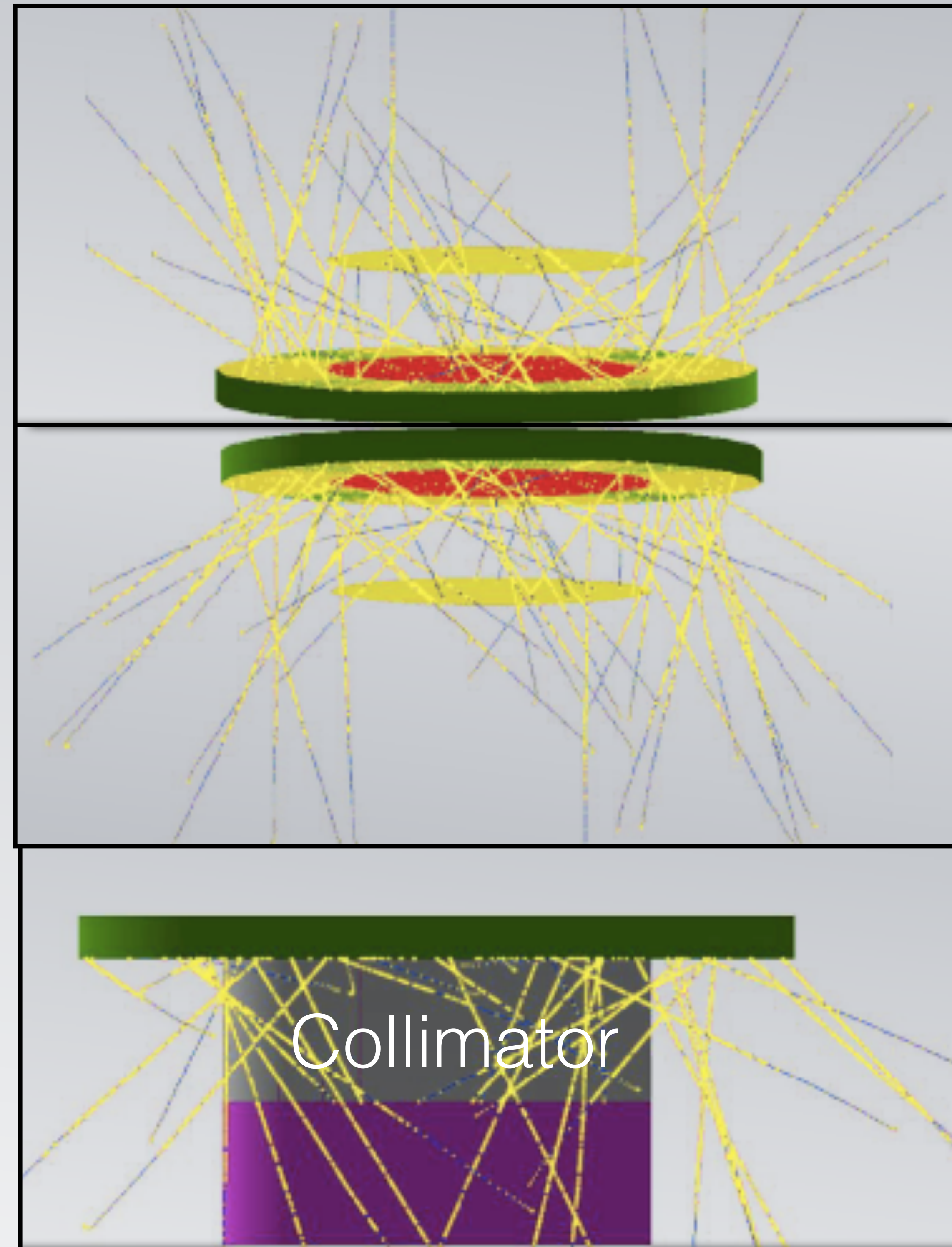
LET - Linear Energy Transfer

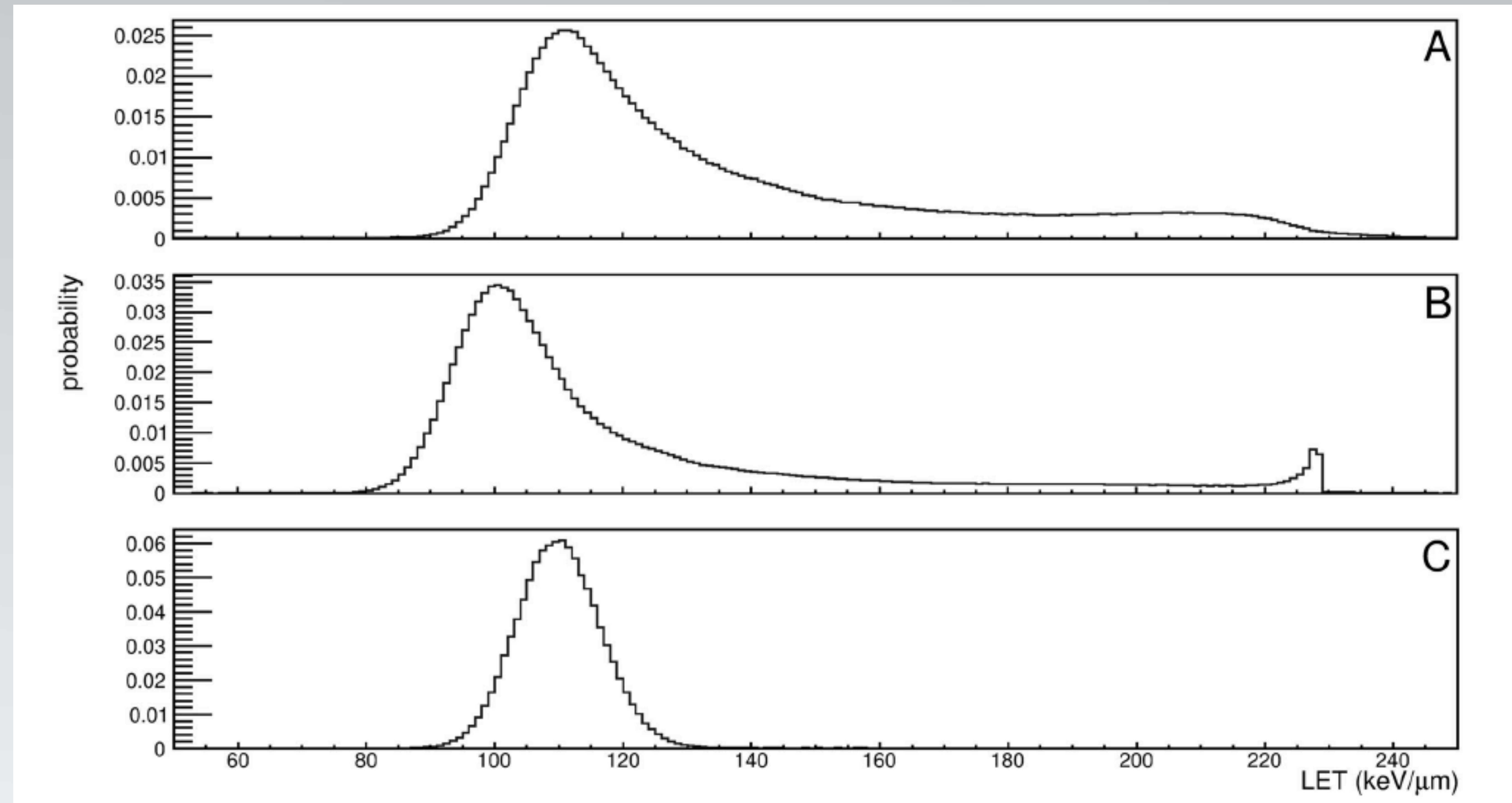


RBE - Relative Biological Effectiveness

LET distributions

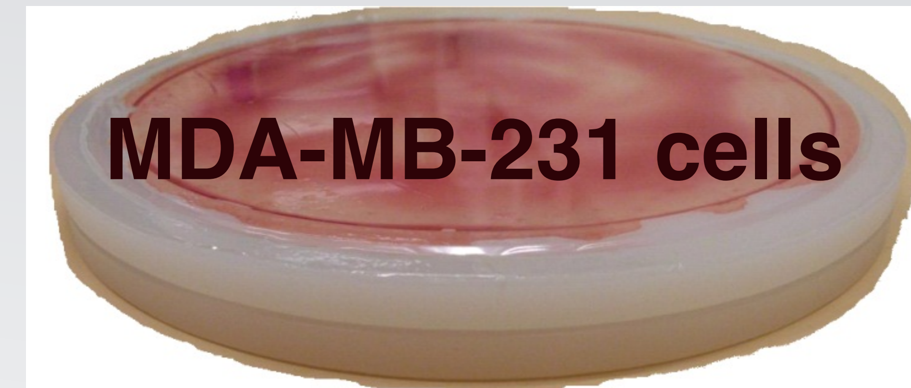
^{241}Am



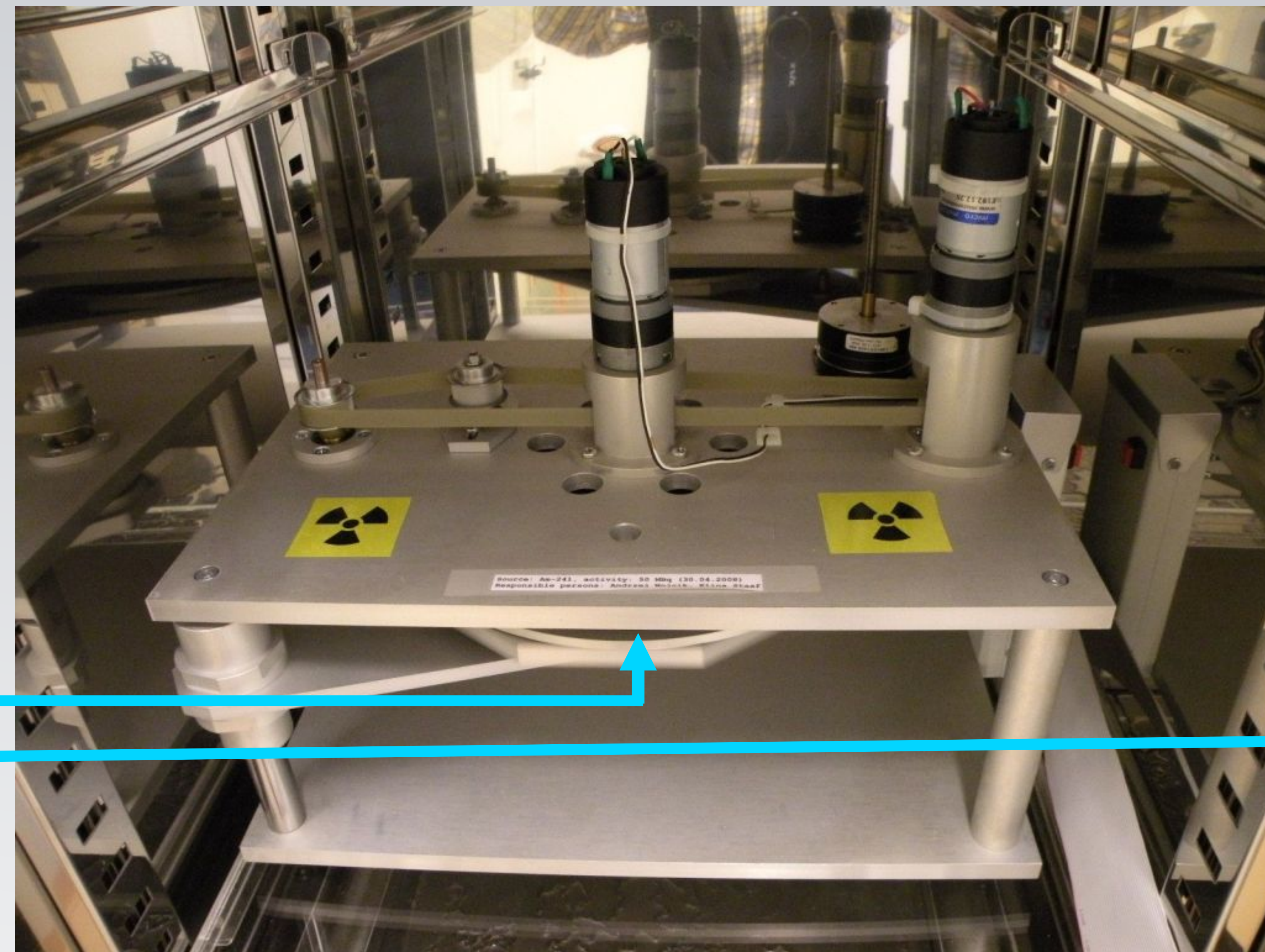


Tartas A., Filipek M., Pietrzak M., Wojcik A. i Brzozowska B. (2022). Modeling of dose and linear energy transfer homogeneity in cell nuclei exposed to alpha particles under various setup conditions, International Journal of Radiation Biology (https://doi.org/10.1080/09553002.2023.2161659)

Cell irradiation facilities

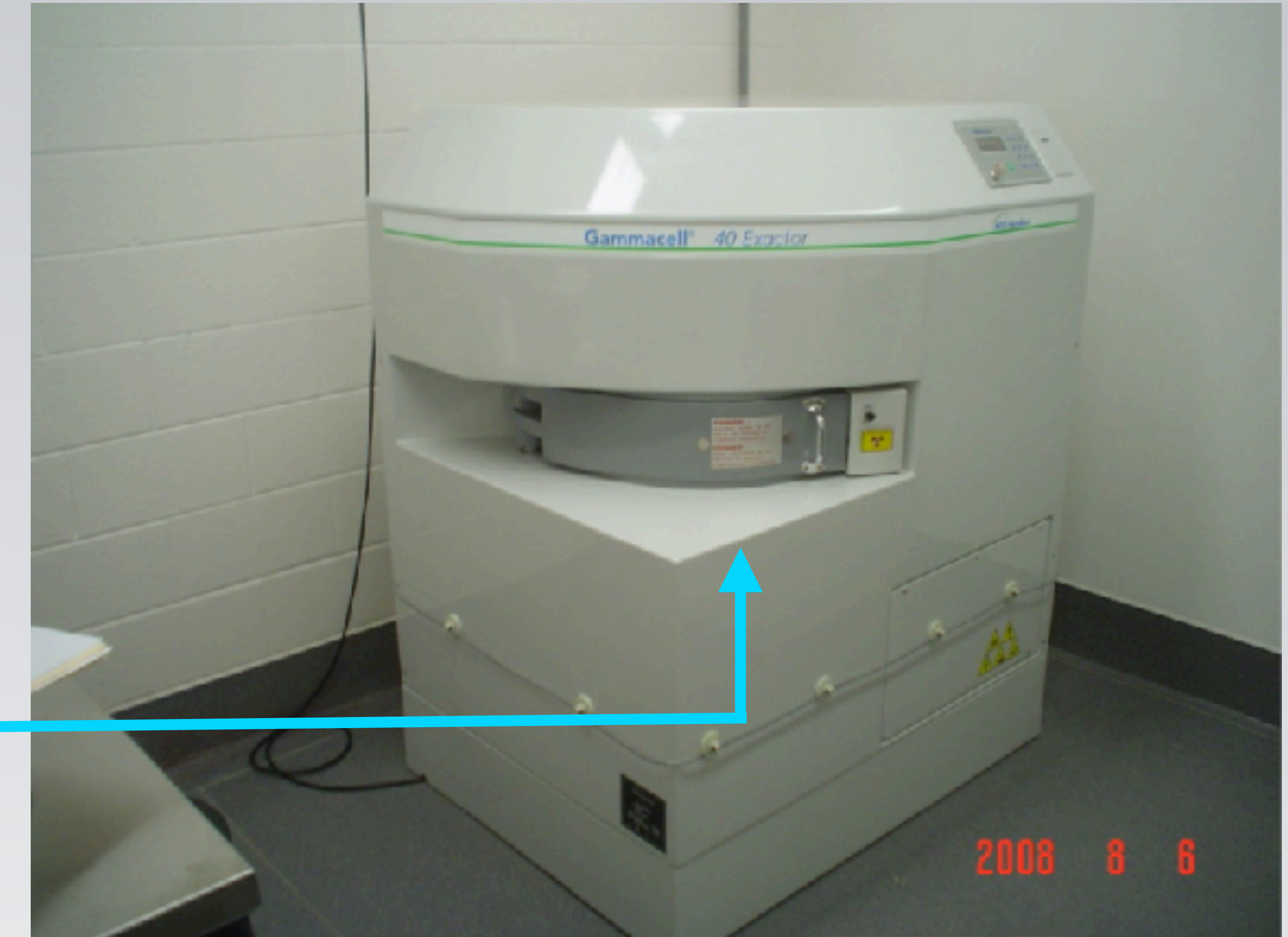


^{241}Am (0.22 Gy/min)



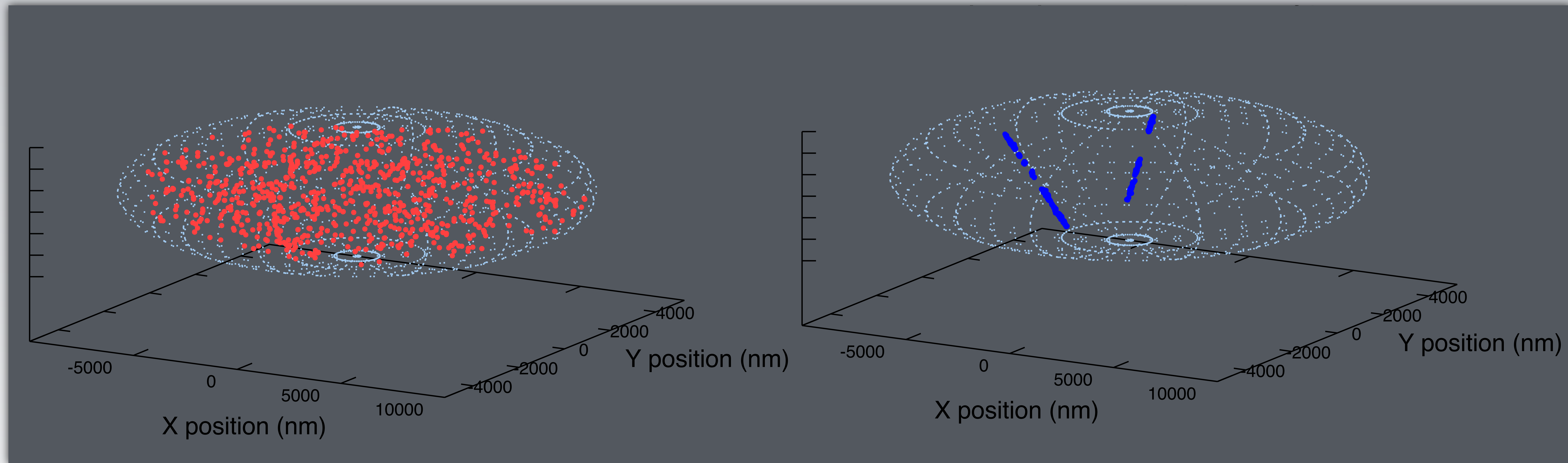
5 Gy of alpha particles
90 keV/μm

^{137}Cs (0.73 Gy/min)



5 Gy of photons
0.8 keV/μm

Ionisation distributions: α + γ

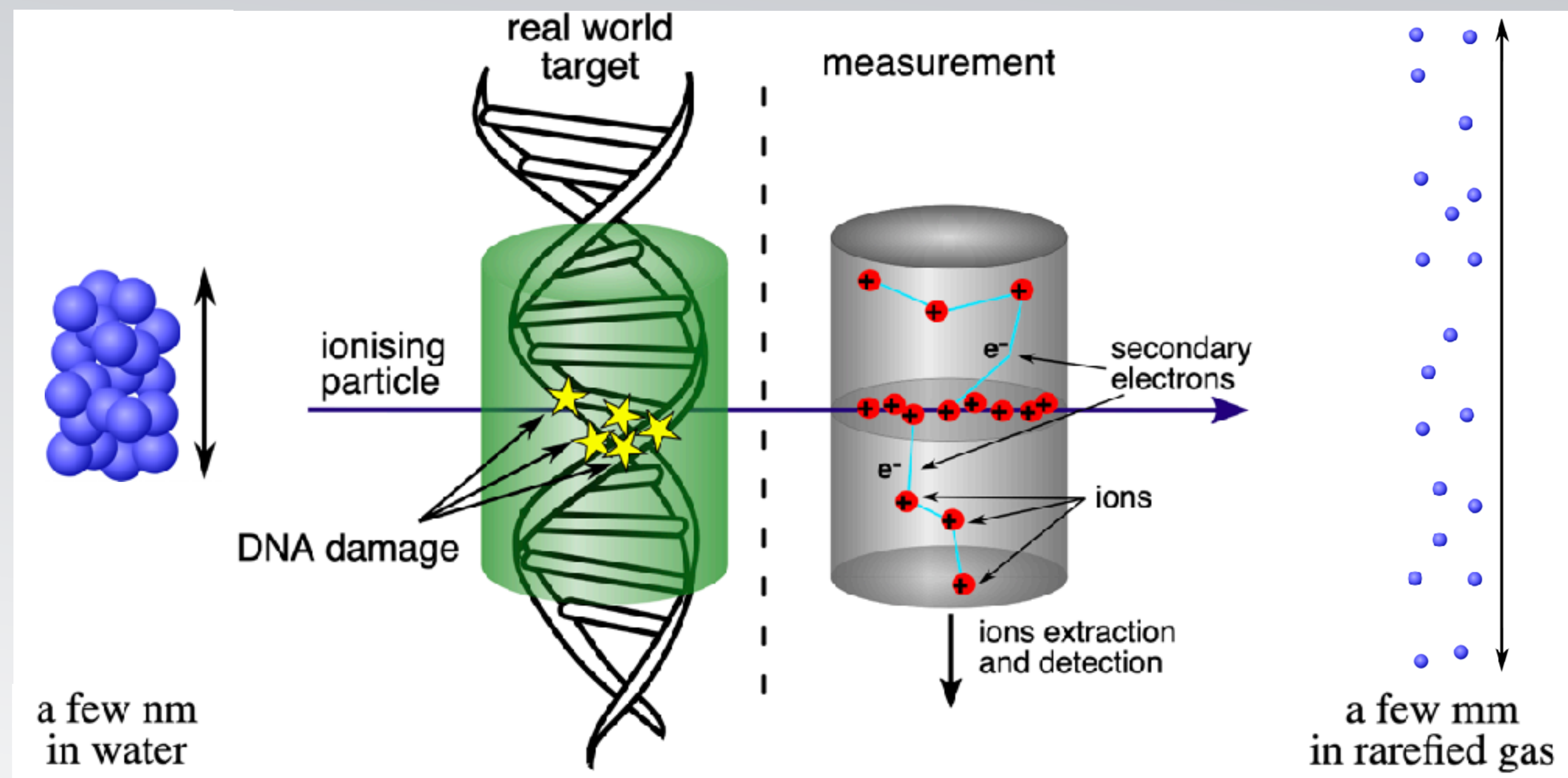


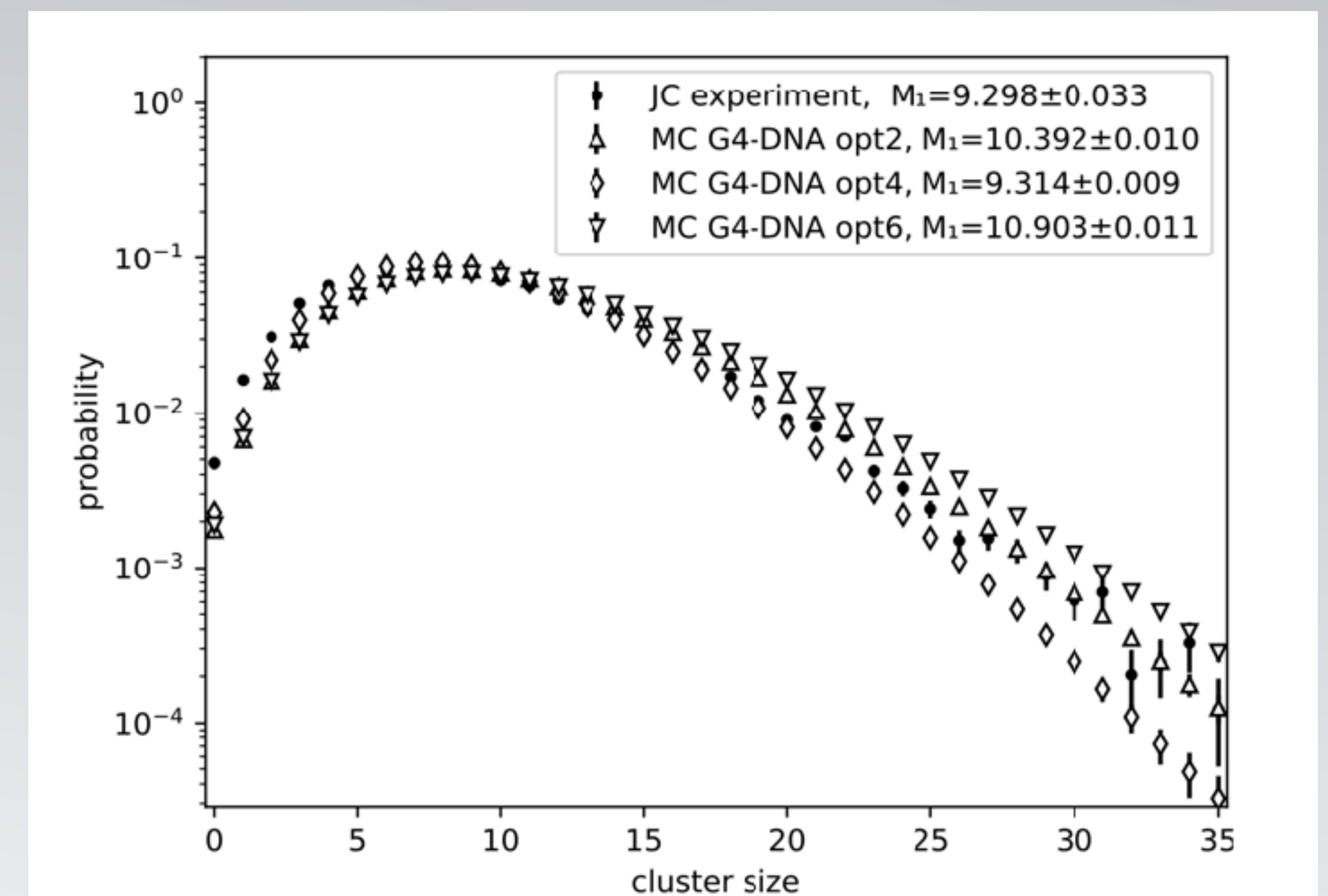
X-rays (0.25 Gy)

alpha particles (0.25 Gy)

MC simulations with PARTRAC

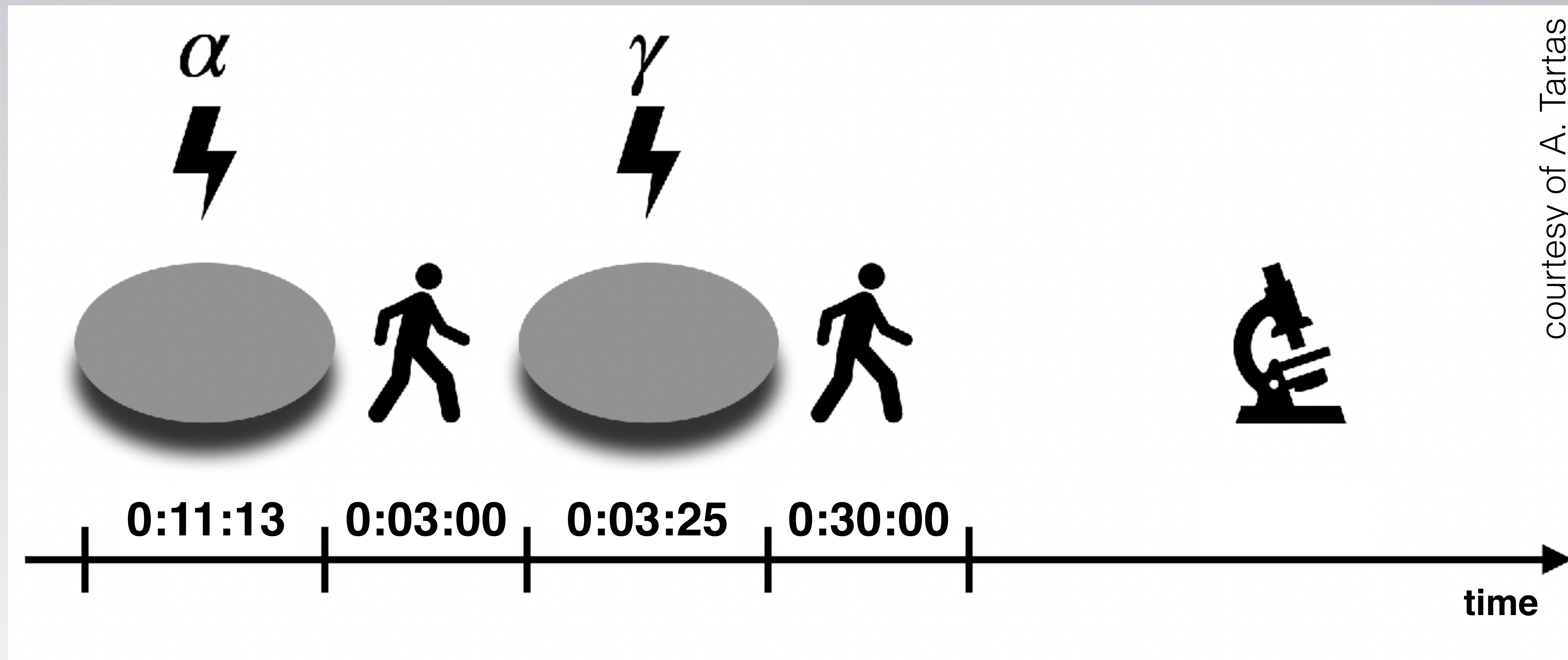
Nanodosimetry: measurements of ionisations





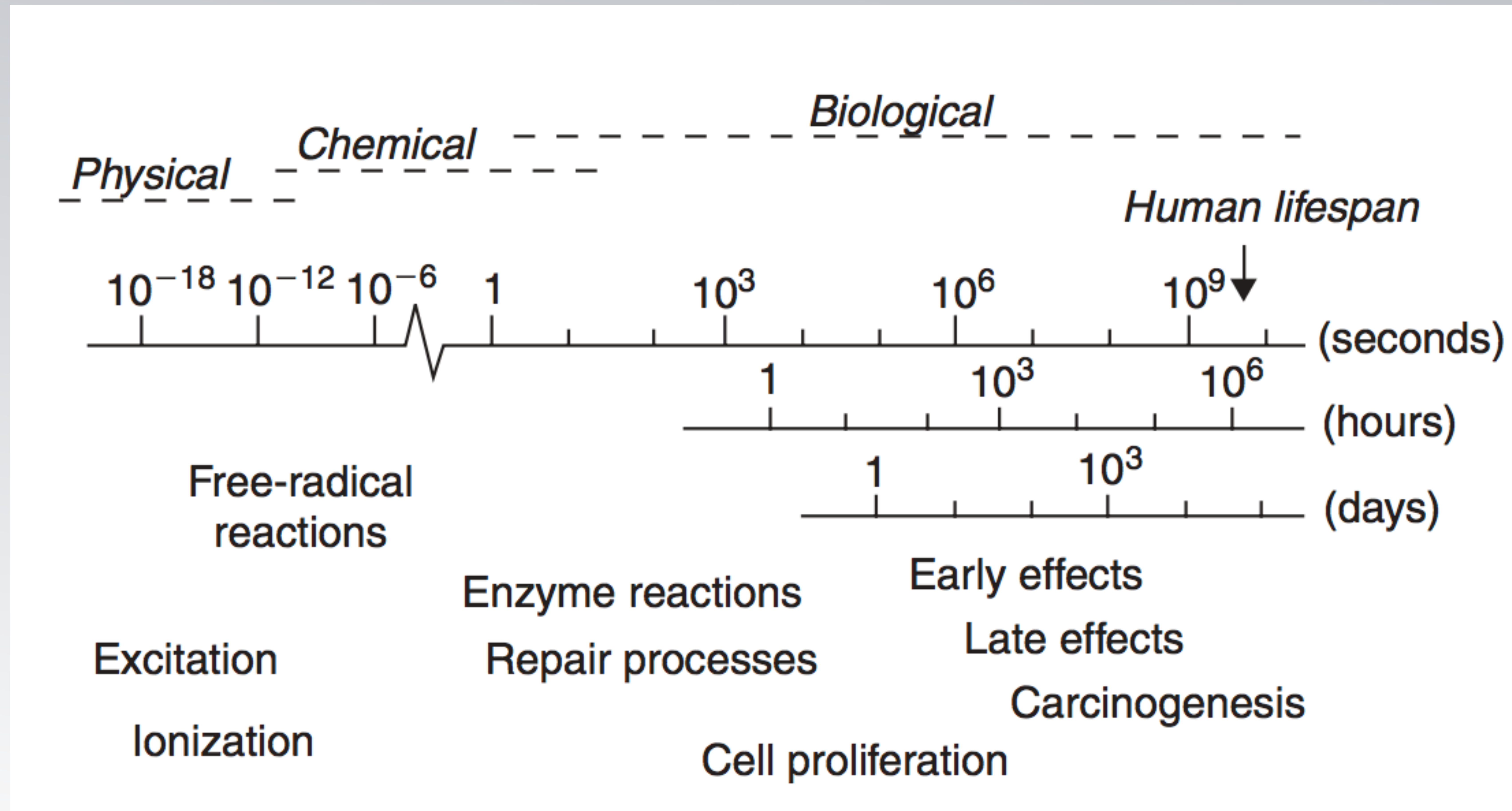
M. Pietrzak, M. Mietelska, A. Bancer, A. Rucinski, B. Brzozowska, Geant4-DNA modeling of nanodosimetric quantities in the jet counter for alpha particles, PMB 2021

Sequential mixed beam exposure

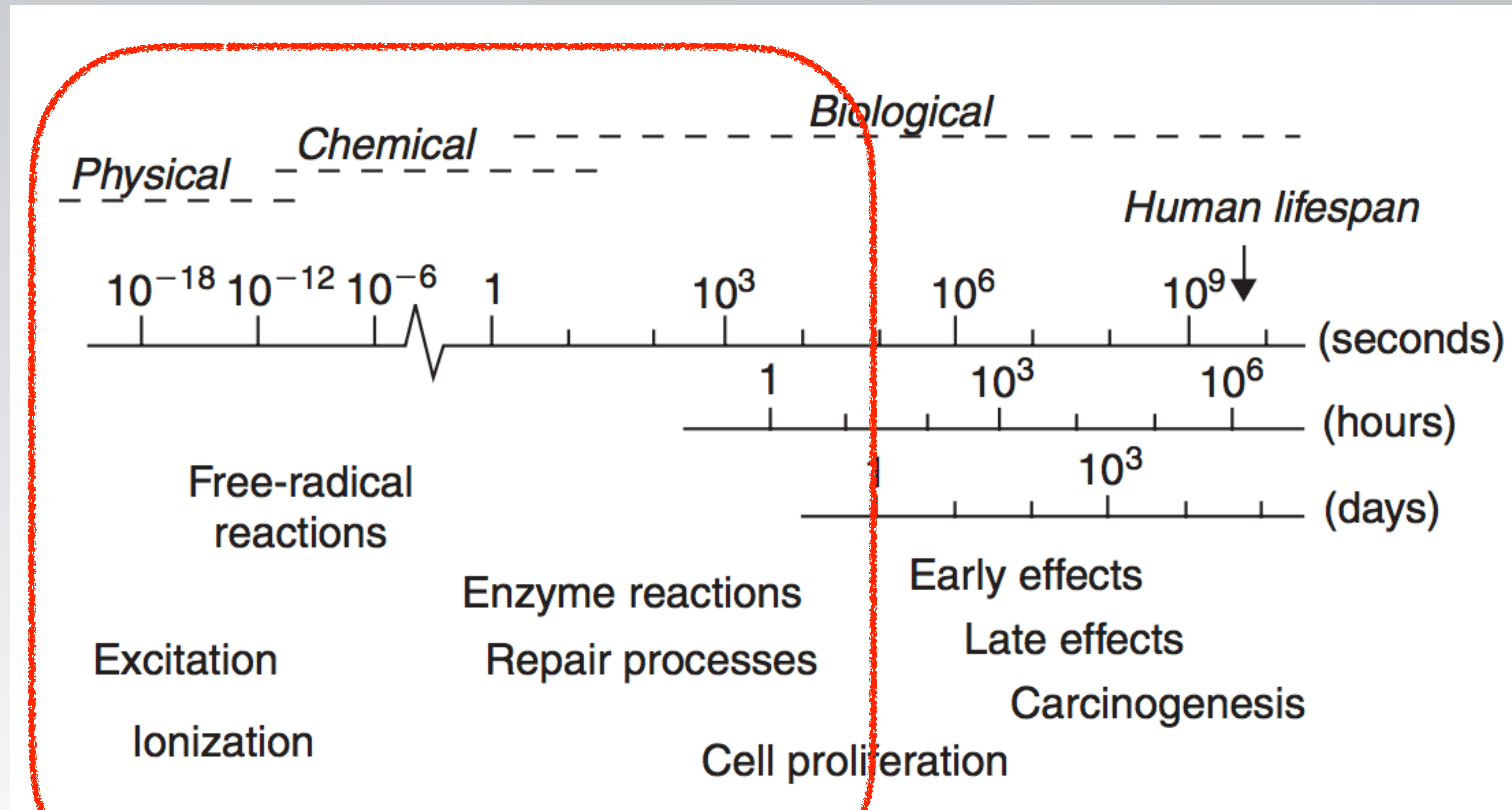


2.5 Gy of alphas + 2.5 Gy of photons = 5 Gy of mixed beams

Radiation exposure on biological systems

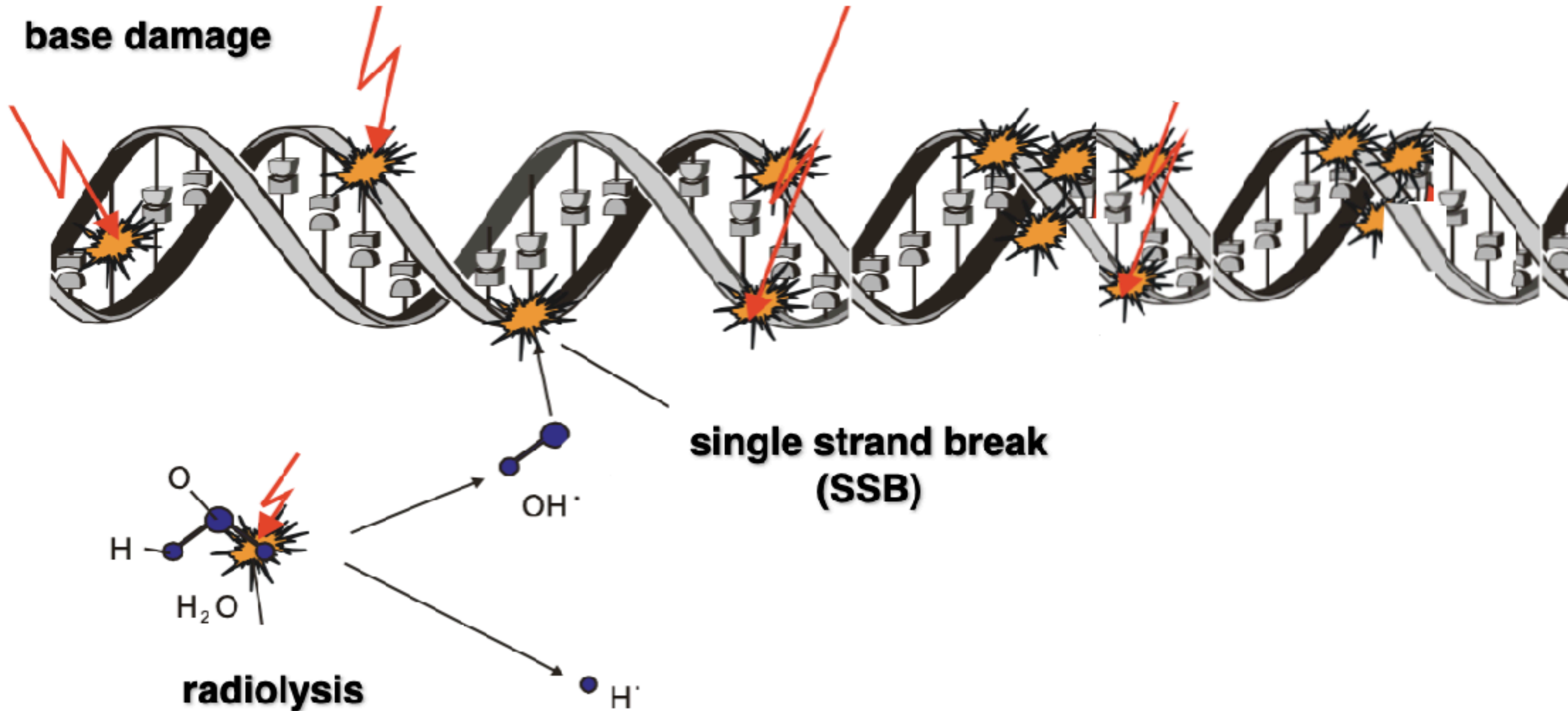


Radiation exposure on biological systems



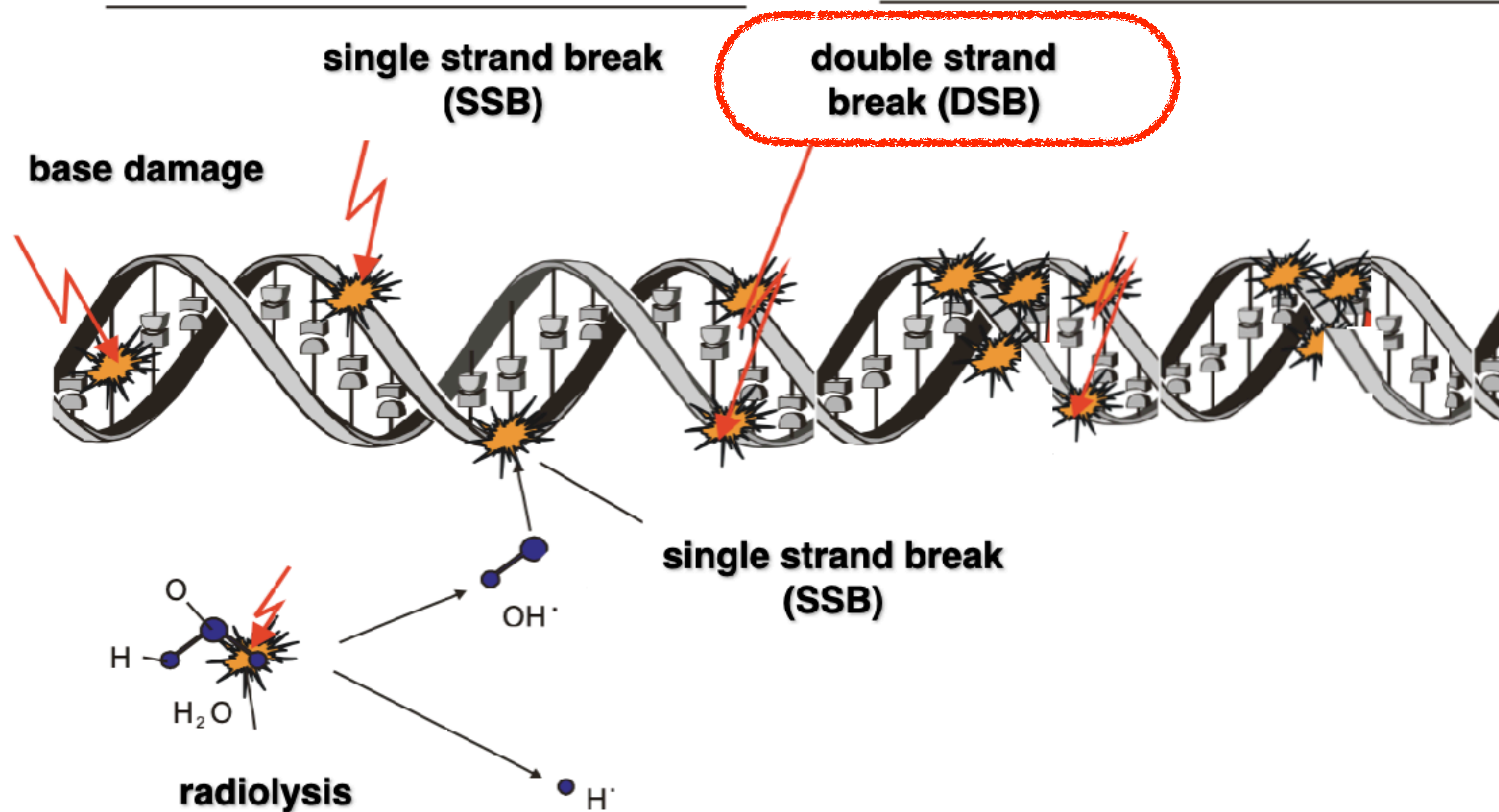
DNA damage

low LET high LET



DNA damage

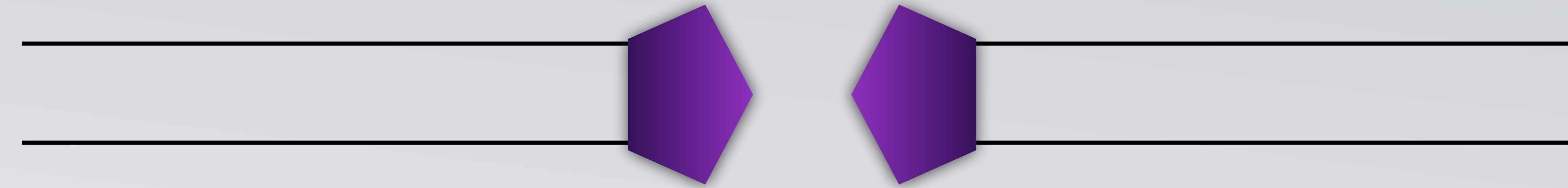
low LET high LET



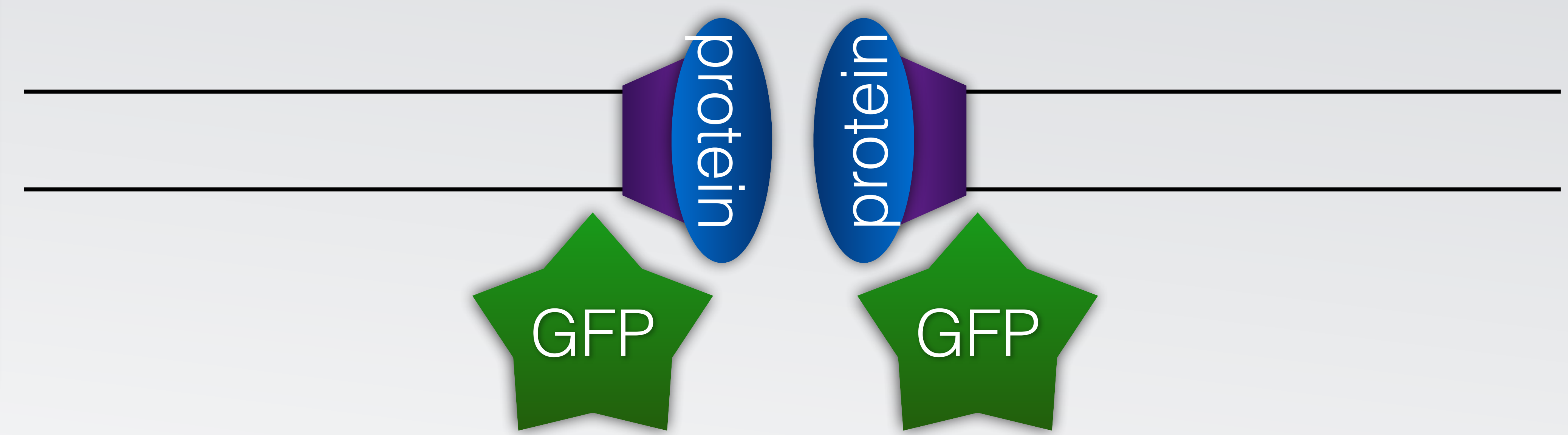
How to visualize DSB?

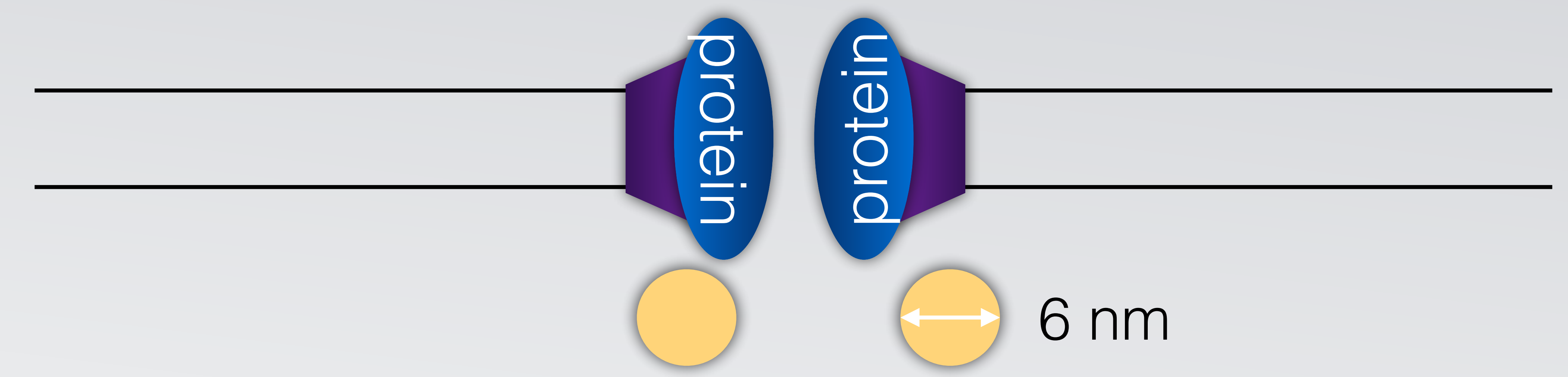
double strand break

}DNA strand



γH2AX analysis





TEM analysis

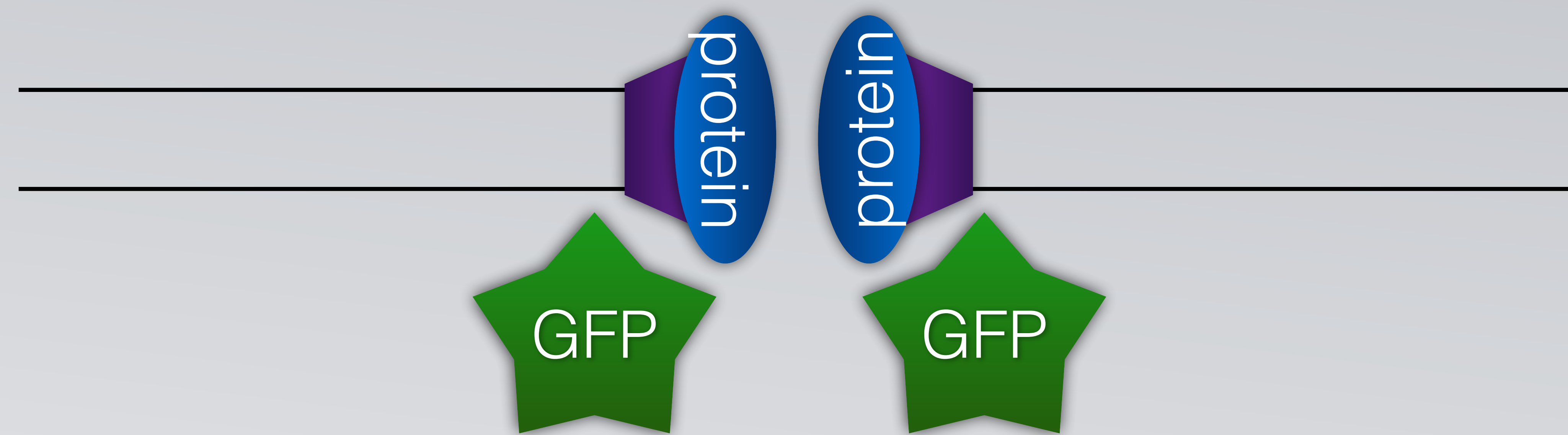
γH2AX immunofluorescence analysis

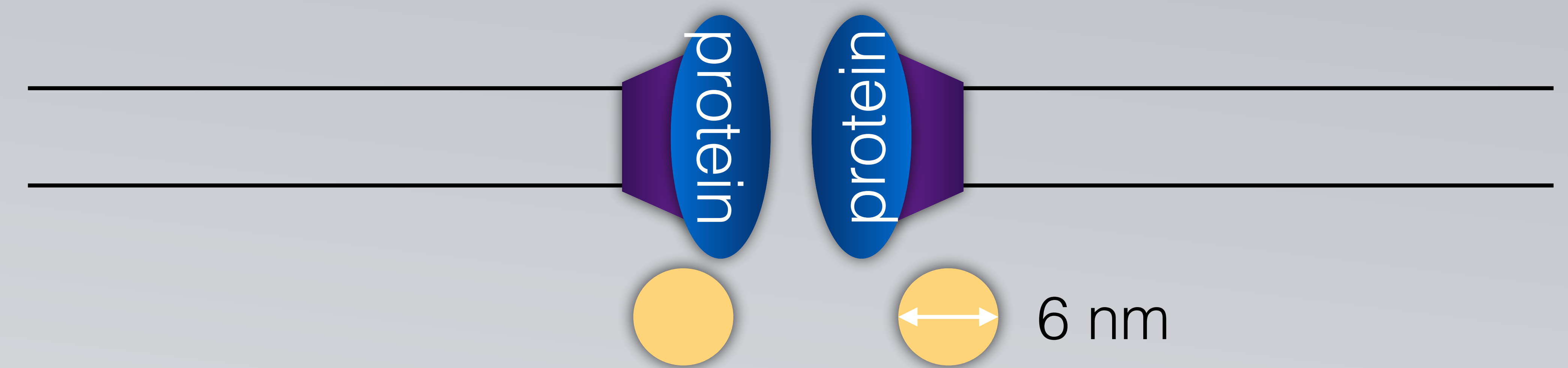
- At 30 min after each exposure ended, cells were washed with PBS and fixed using 70% EtOH for 10 min, then kept in PBS at 4°C until analysis.
- Analysis was performed according to an established protocol for immunofluorescence, where cells were permeabilised using 0.2% Triton-X in PBS, incubated with the same primary antibody as for TEM (1:800), followed by secondary anti-mouse IgG fluorescein isothiocyanate (FITC) (1:200, #F0257, Sigma-Aldrich), both in PBS containing 2% bovine serum albumin (BSA).
- Coverslips were mounted on an objective glass using Vectashield containing DAPI.
- Photos were taken with a 100x oil immersion objective using a Nikon Eclipse E800 fluorescence microscope (Nikon, Tokyo, Japan) coupled to a CCD camera, with the image analysis system ISIS (Metasystems, Althusheim, Germany).

TEM preparation procedure

- Cells were seeded on Thermanox plastic coverslips (13 or 25 mm in diameter (#174959/174985), Ted Pella, Inc., Rochester, NY, USA) in 12 or 6-well plates one day before radiation exposure.
- At 30 min after each exposure ended, cells were washed with phosphate-buffered saline (PBS) and fixed using 4% paraformaldehyde (Merck, Solna, Sweden) with 0.02% glutaraldehyde (Ted Pella) in 0.1 M sodium cacodylate buffer for 30 min.
- Thereafter, the fixation solution was removed, cells were washed in PBS, and then kept in PBS during transport (1.5-2 h).
- Dehydration was initiated upon arrival at the BioVis Facility (Uppsala University), followed by embedding, sectioning and immunostaining. Cells were dehydrated in graded EtOH (70, 95 and 99.9%) followed by a mixture of Lowicryl® K4M (Polysciences) and 99.9% EtOH (1:1) for 1 h and then changed to 100% Lowicryl and left overnight.
- The next day, the wells were filled with freshly prepared embedding media and then polymerized in UV-light at -35 ^{0}C for 72 h.
- Ultrathin sections (60-70 nm) of the cell layer were sectioned in a Leica UC7 ultramicrotome and placed on nickel grids.
- Prior to immunogold labelling, sections were blocked with 50 mM glycine in PBS for 15 min followed by blocking solution (Aurion, Wageningen, The Netherlands) for 30 min and then washed in 0.1% BSA-c (Aurion) in PBS 3 x 5 min. The sections were then incubated with primary anti-phospho-histone H2AX antibody Ser139 (Upstate/Millipore, #05-636) diluted 1:250 in 0.1% BSA-c for 2 hours. After washing in 0.1% BSA-c 6 x 5 min, the sections were incubated with an anti-mouse 6 (+/-3) nm gold conjugated secondary antibody diluted 1:80 (Aurion) for 1 hour. The sections were washed again in 0.1% BSA-c for 6 x 5 min and then in MilliQ water for 6 x 1 min.
- Finally, the sections were contrasted in 5 % uranyl acetate for 5 min. Upon completion, the TEM grids were sent to Kielce, Poland, for acquisition of images.

Focus analysis



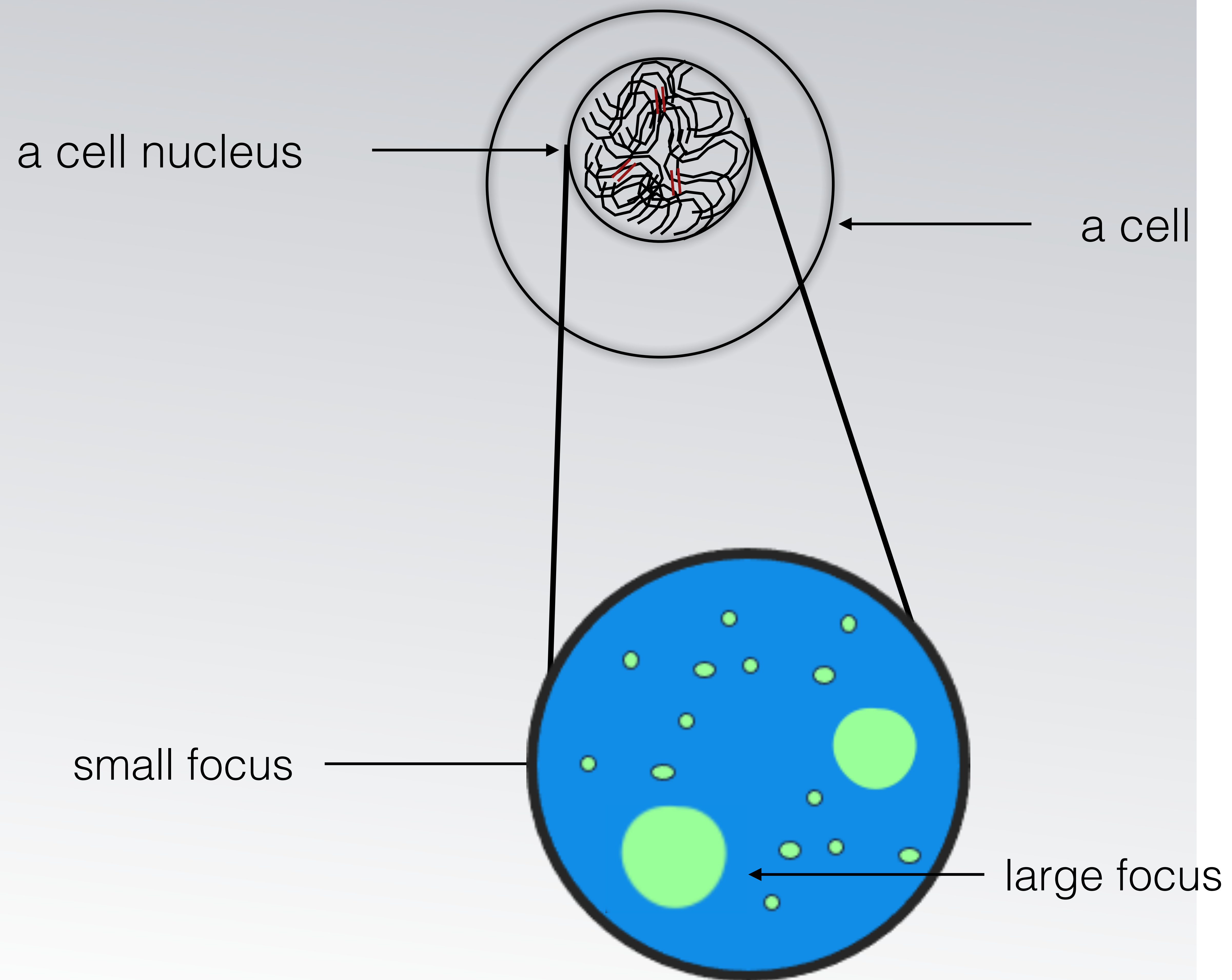


STEP 1: γH2AX analysis - focus frequency for the dose equal 5 Gy

STEP 2: TEM analysis - focus frequency for the dose equal 5 Gy

STEP 1: γH2AX analysis

Radiation-induced γH2AX foci



Control

X-rays

Alpha

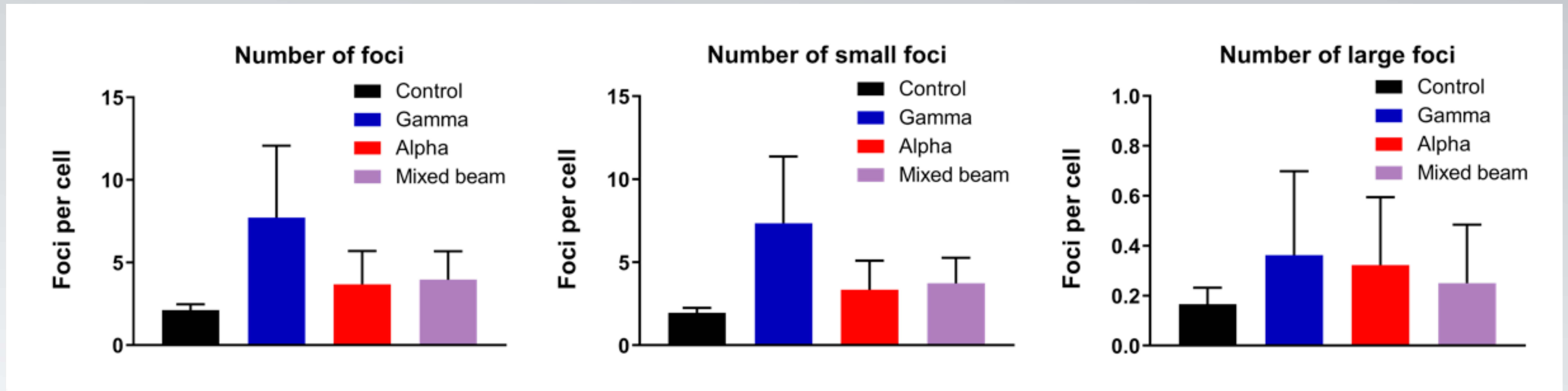
Mixed beam

100 cells per treatment

courtesy of J. Szczechowska

STEP 1: γH2AX analysis

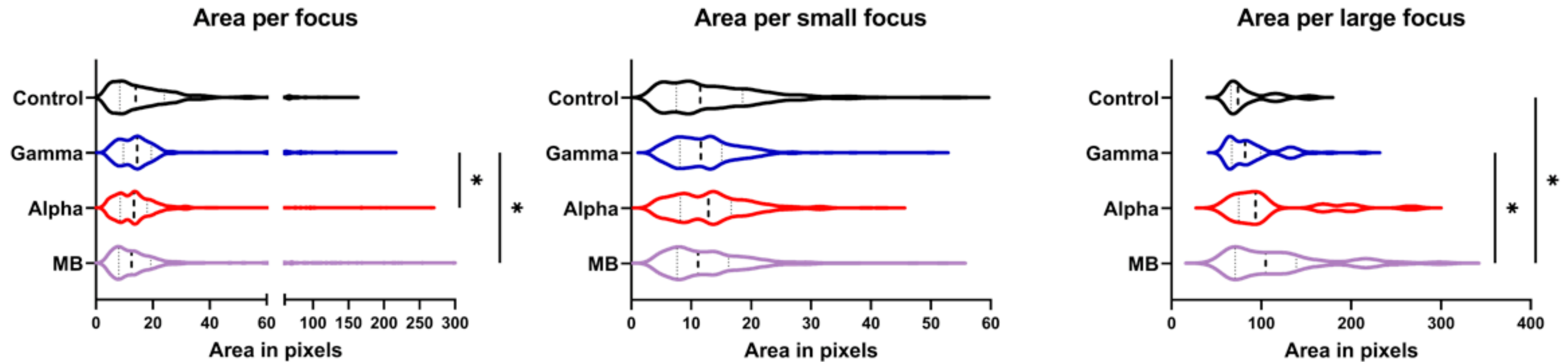
Number of γH2AX foci



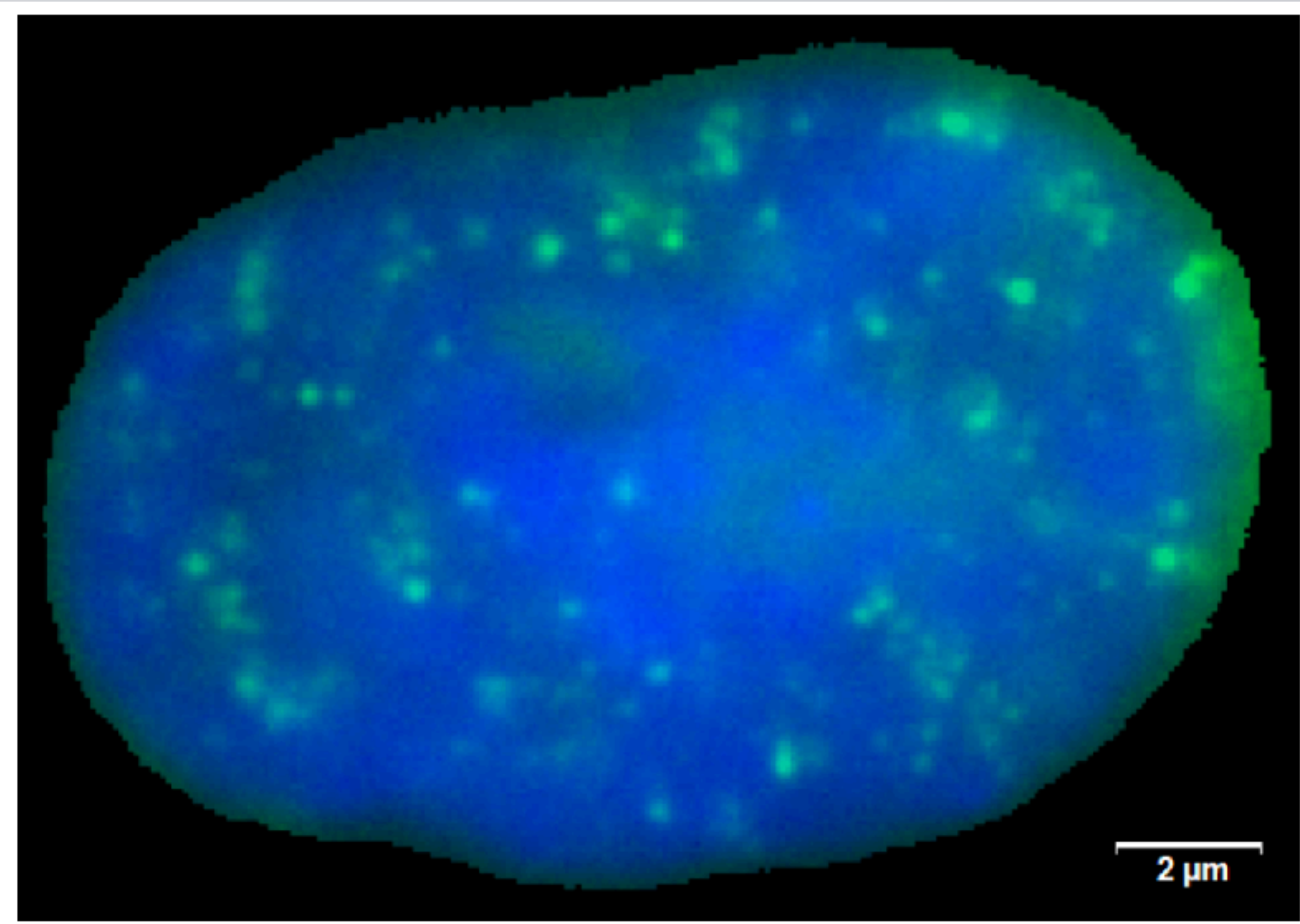
SF - likely containing simple DSB

LF - likely containing staggered complex DSB

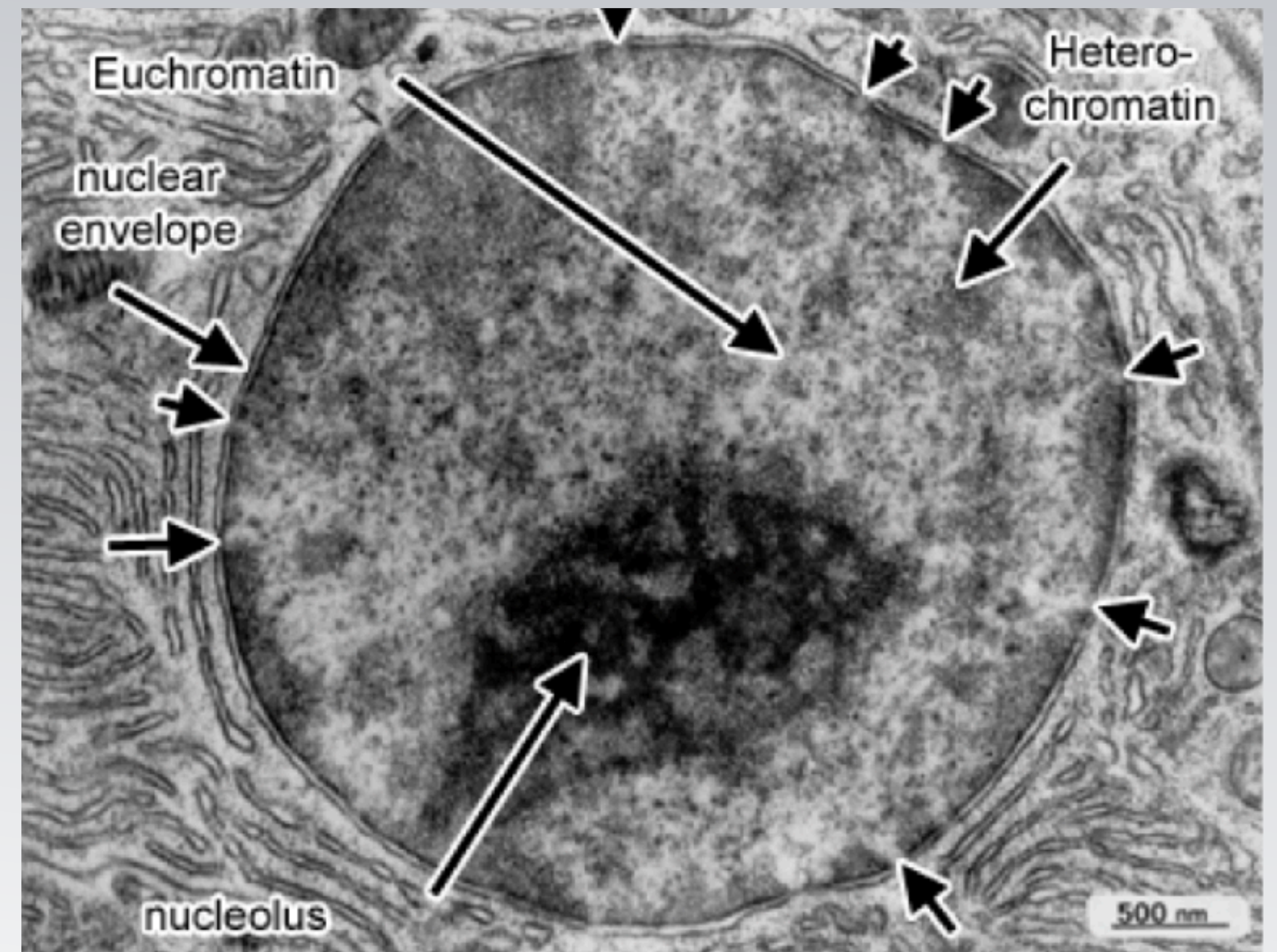
Size of γH2AX foci



Nucleus imaging



Fluorescence microscopy

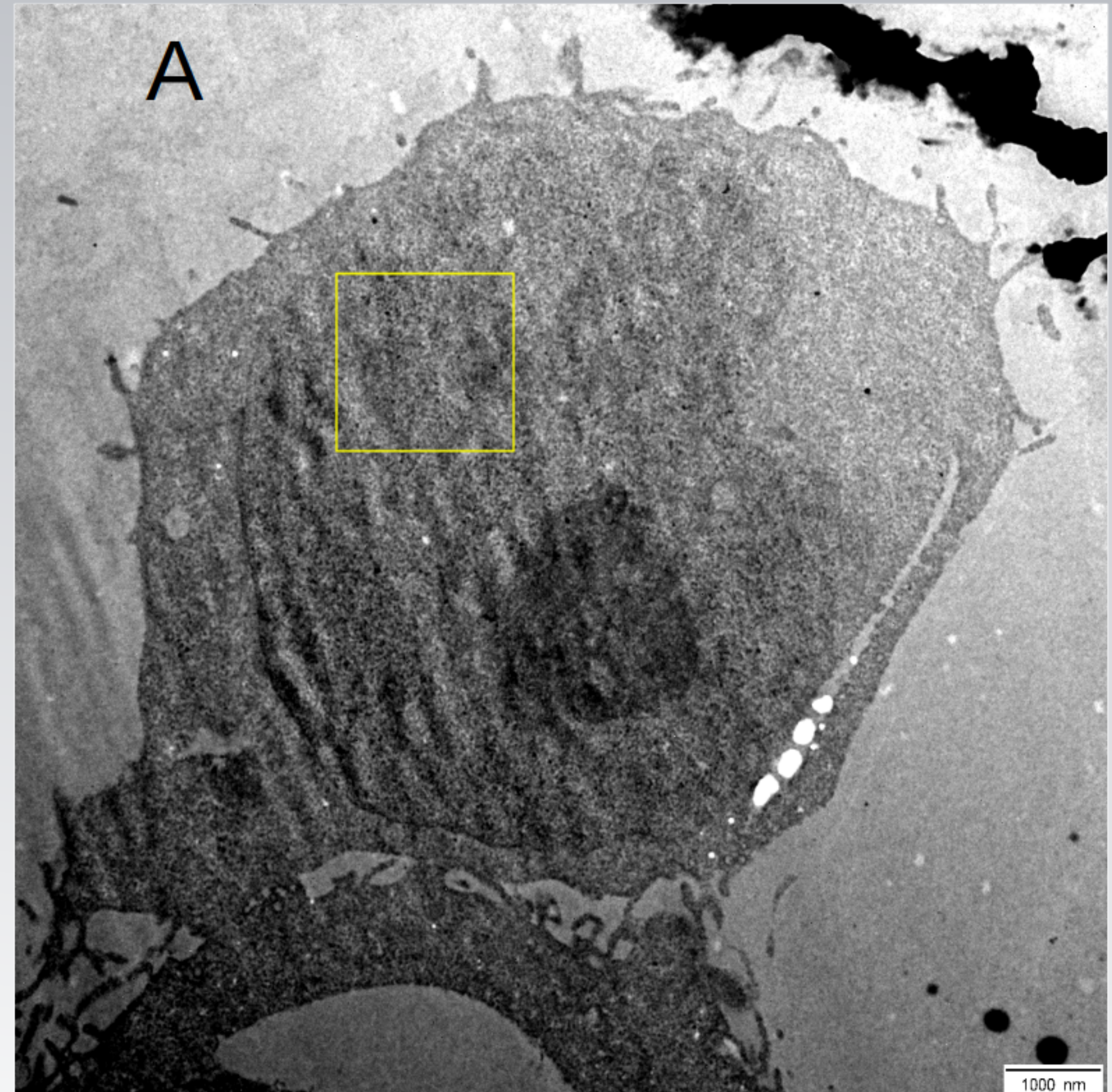


Transmission Electron Microscopy

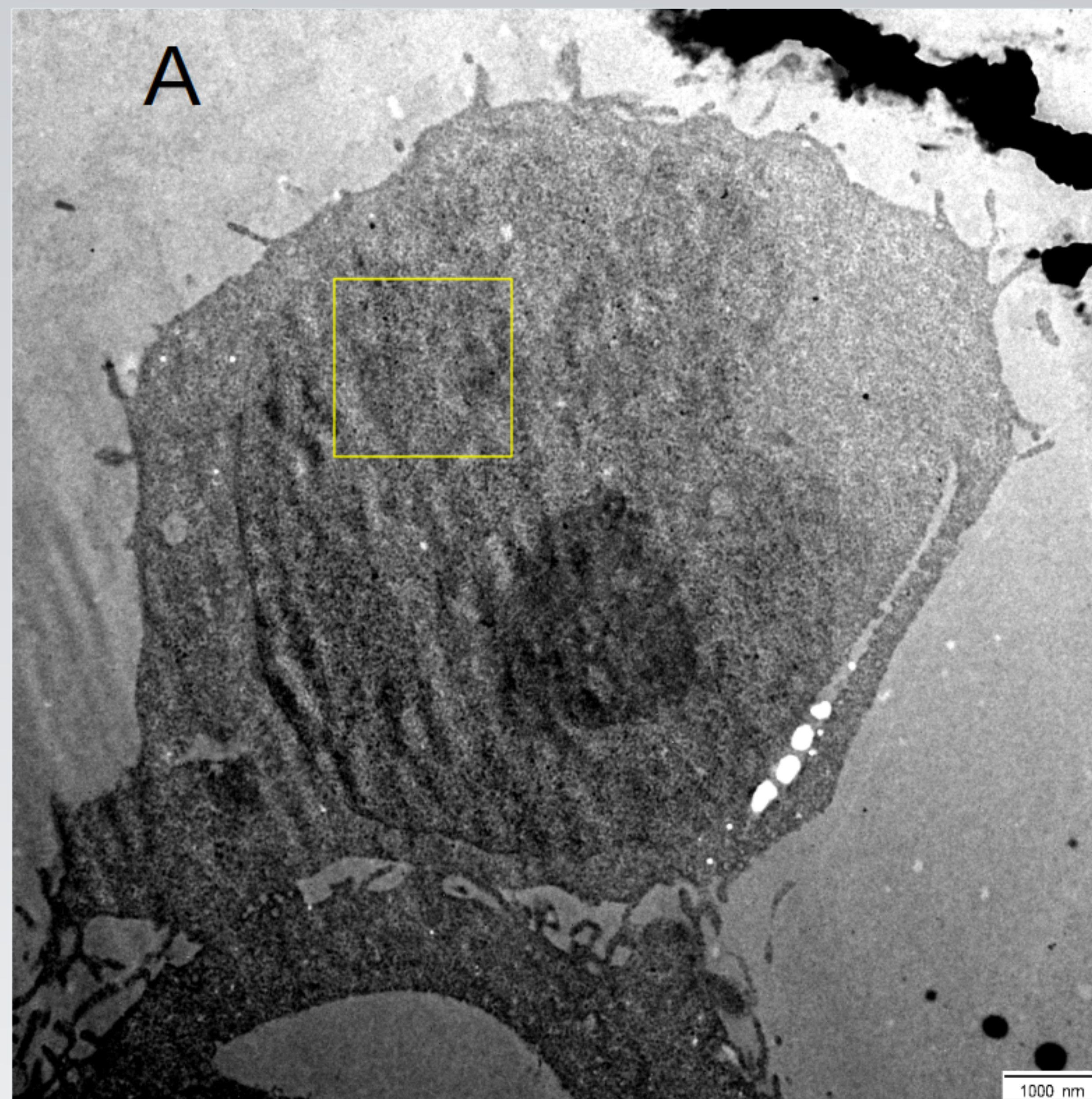
https://www.histology.leeds.ac.uk/cell/nucleus.php

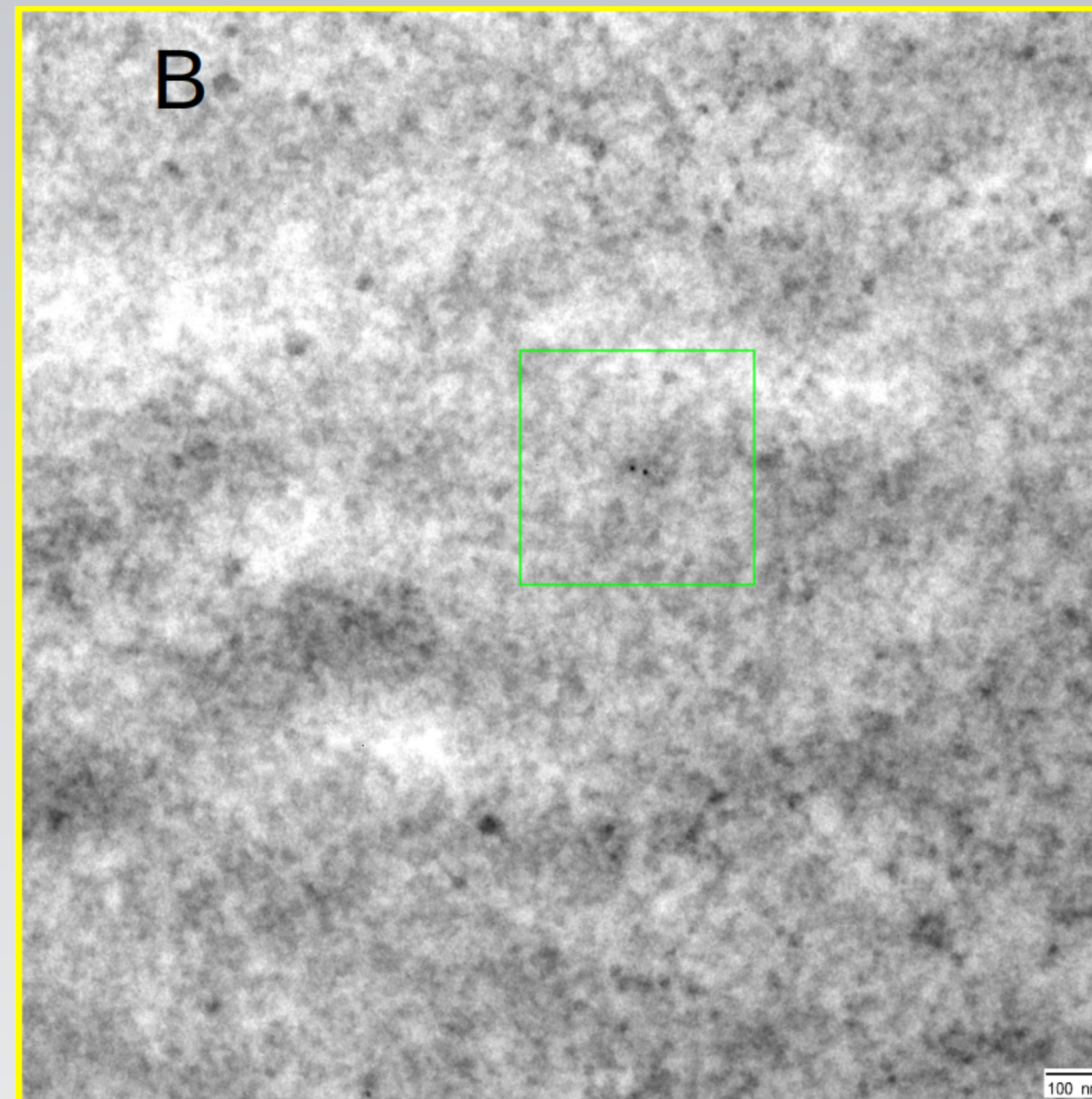
TEM imaging

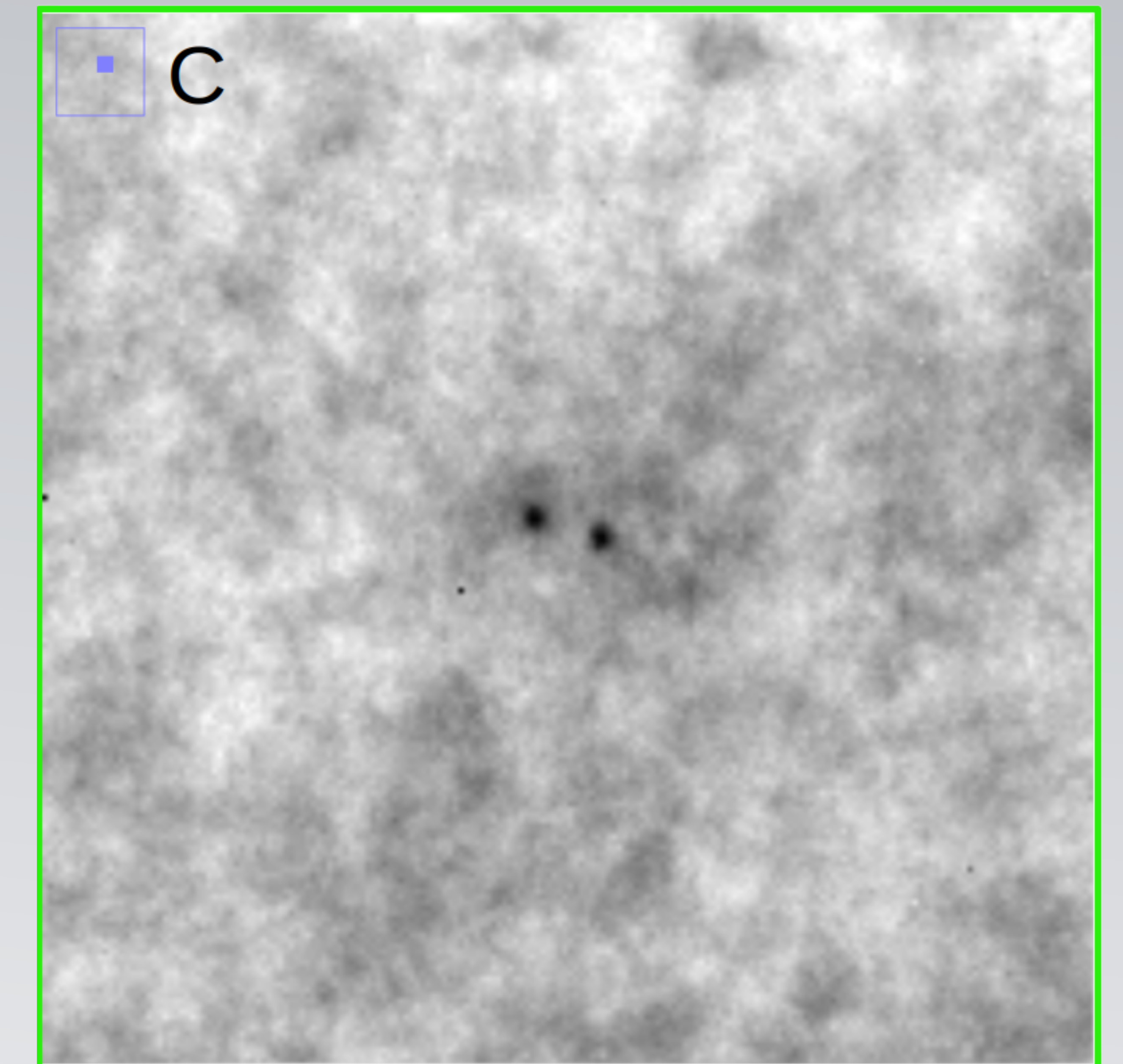
- 8000x magnification using a Tesla BS 500 electron microscope
- connection to a Sharp:Eye Dual Speed 1K-On-Axis CCD camera: 1.82 nm per pixel



TEM analysis procedure



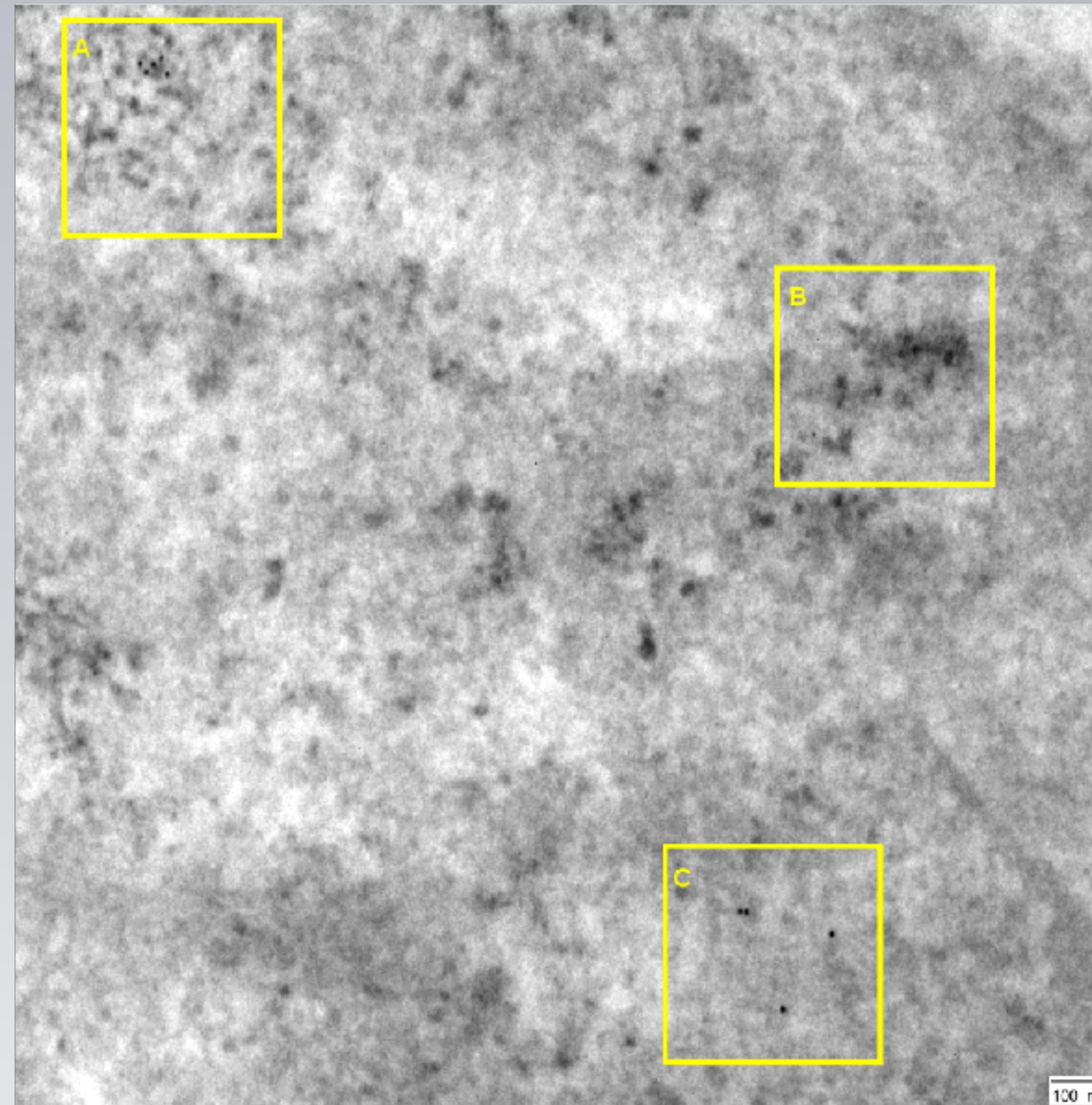


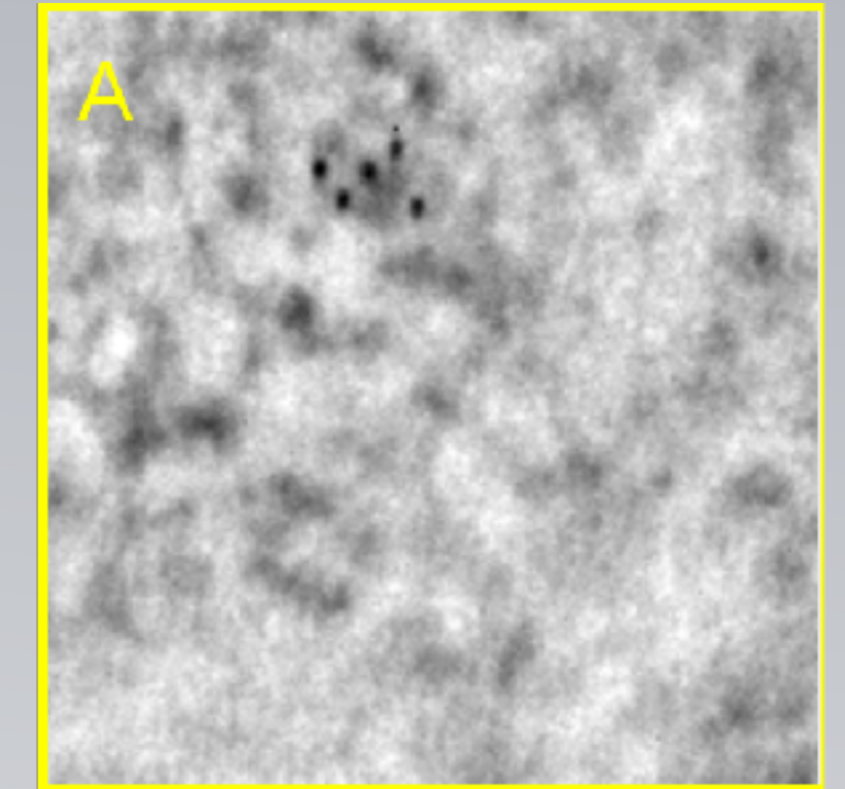


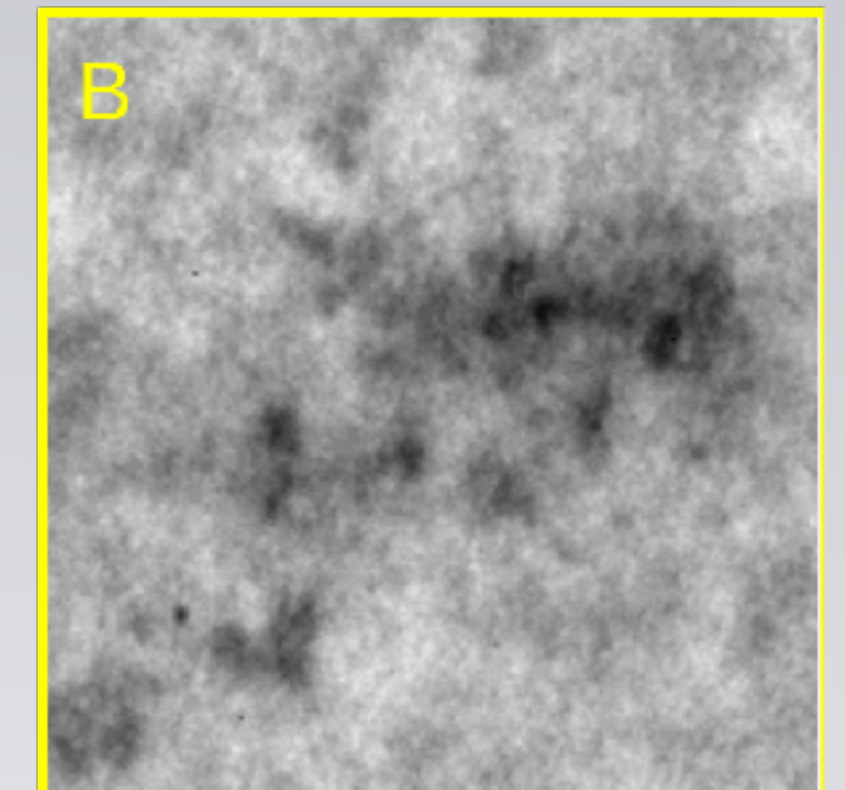
- 80 images were taken per radiation quality;
- Every picture was treated as a random representation of all investigated areas;
- Images were normalised to obtain a mean value of pixel darkness equal to 0 and a standard deviation equal to 1;

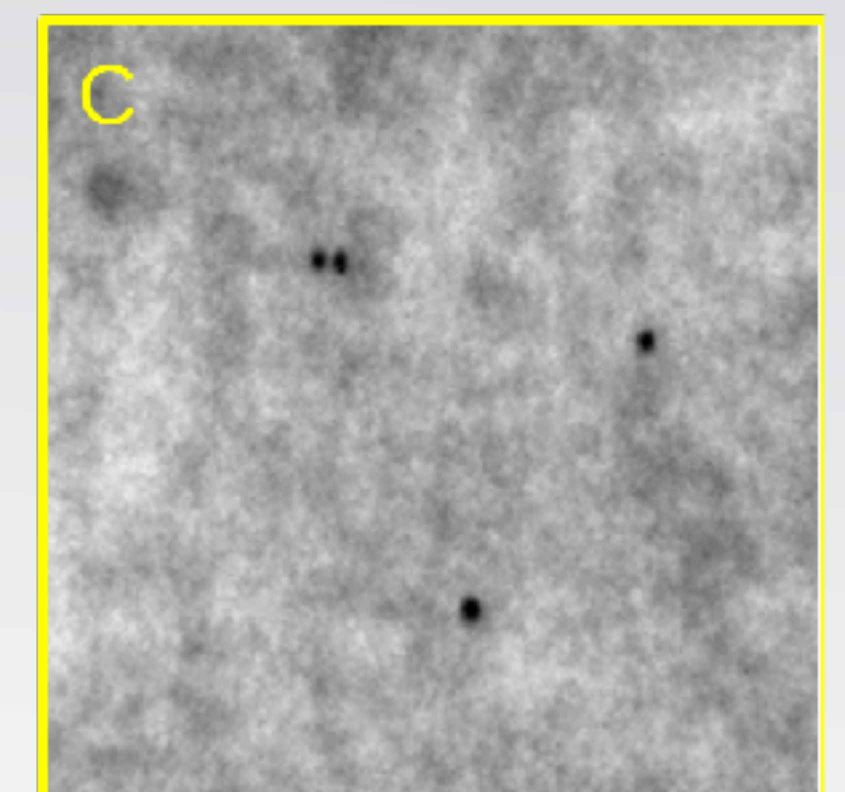
Bead selection criteria

1) it should look round independently on varying contrast settings,
2) it should be darker than other irregularities in the neighbourhood,
3) it should present itself as a circle of diameter of approximately 6 nm





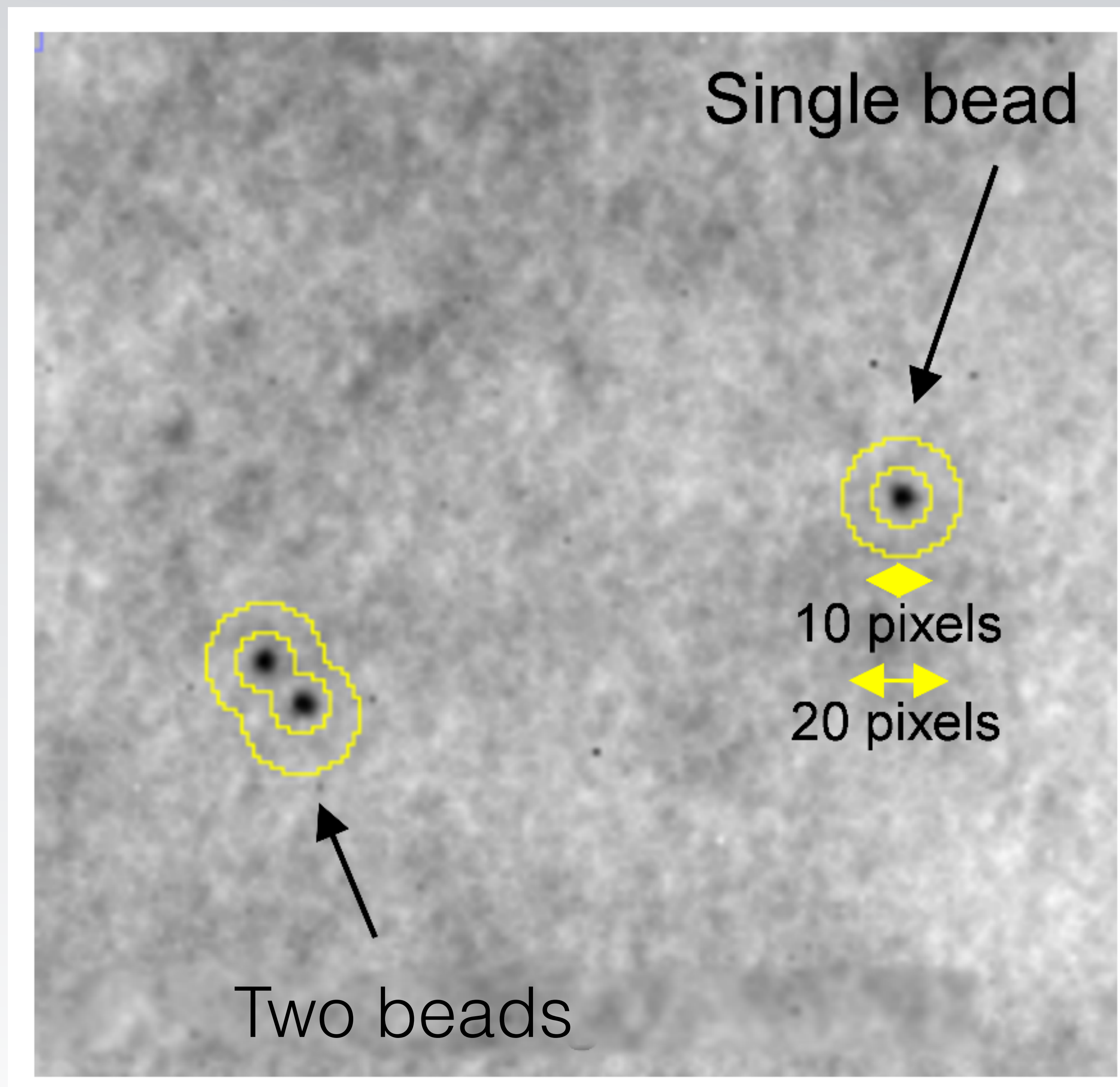


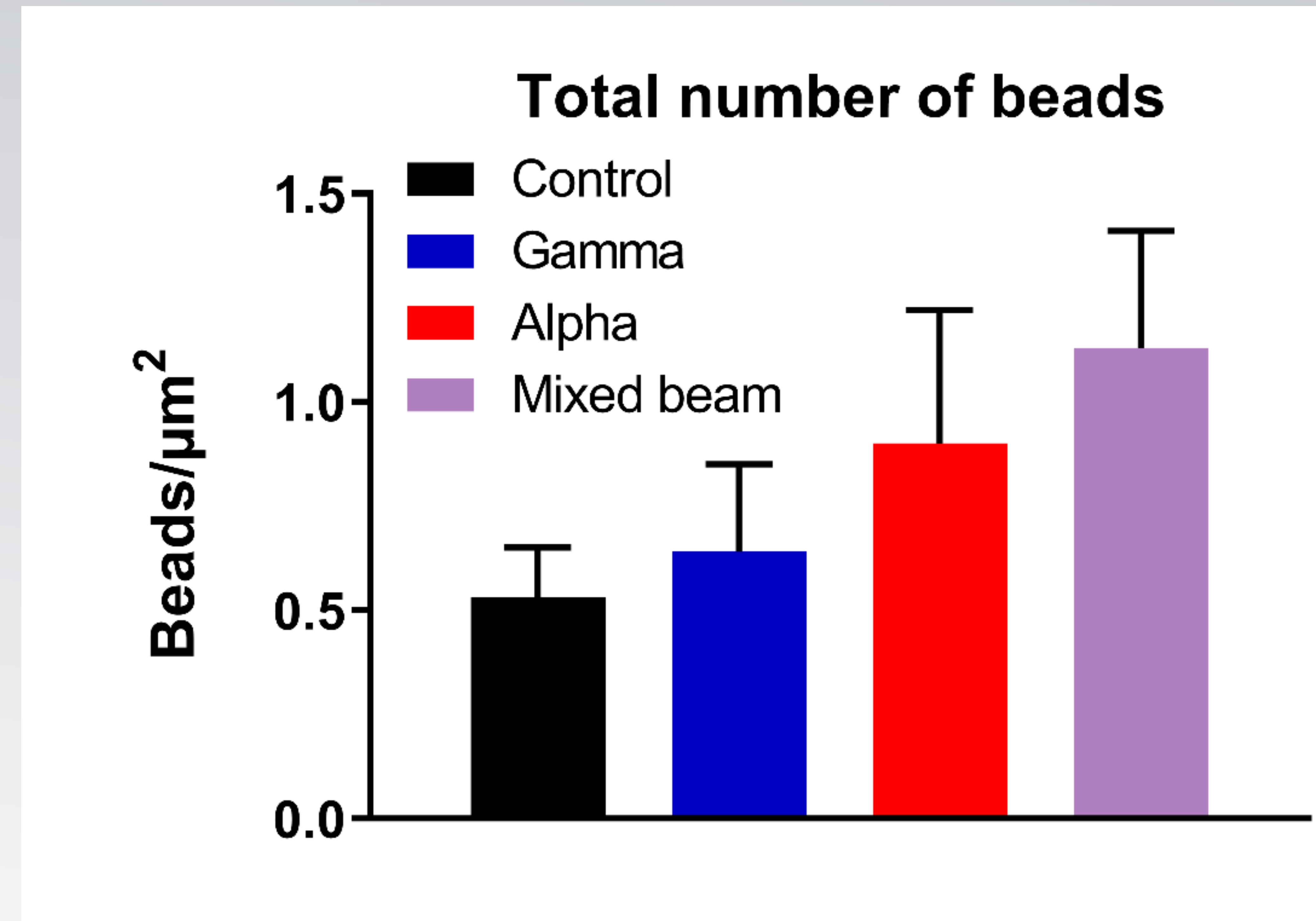


Enlarged areas:

A. 5 golden beads
B. None of the dark areas meet the criteria to be considered a bead
C. 4 golden beads

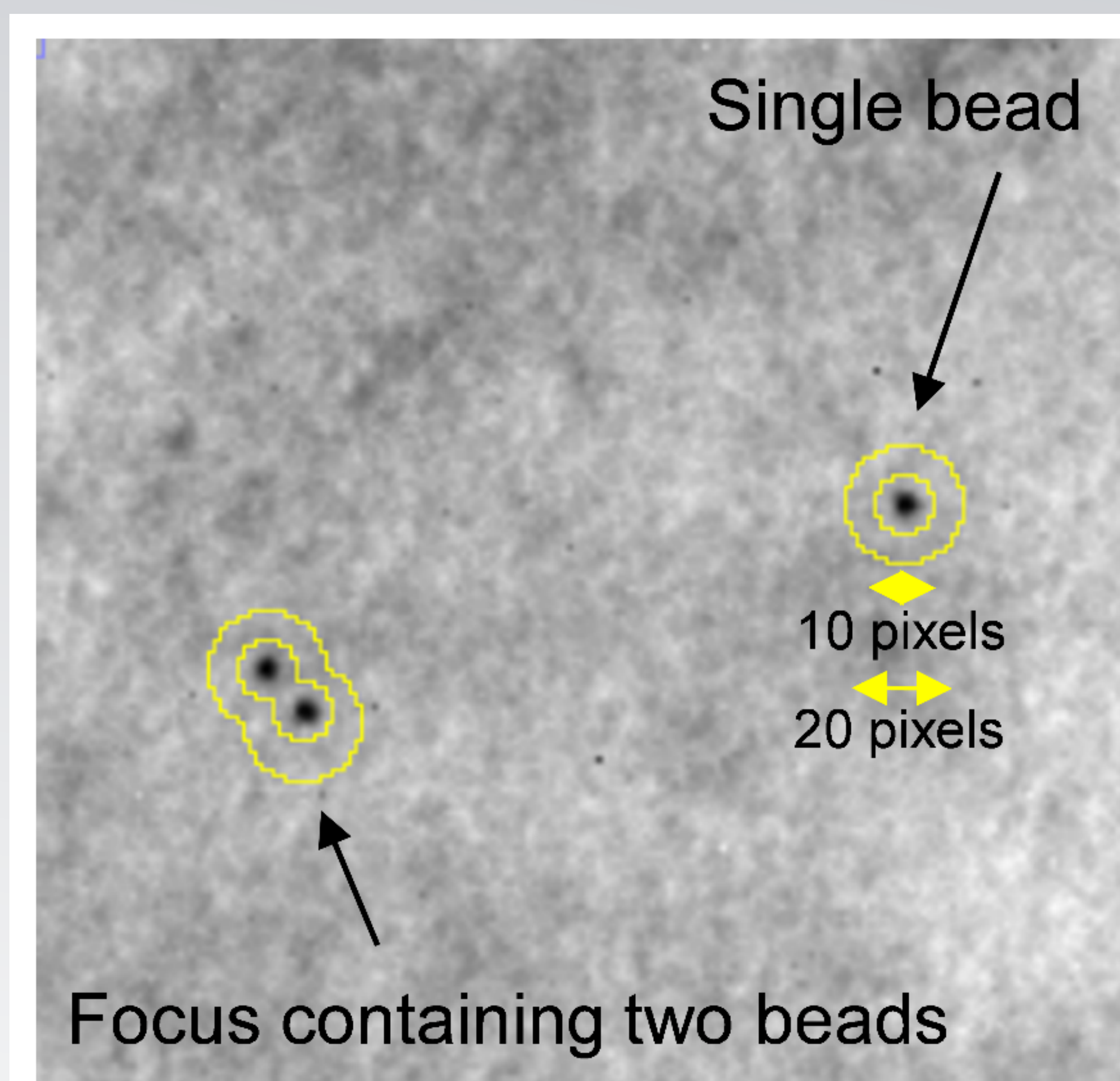
Number of beads per μm^2

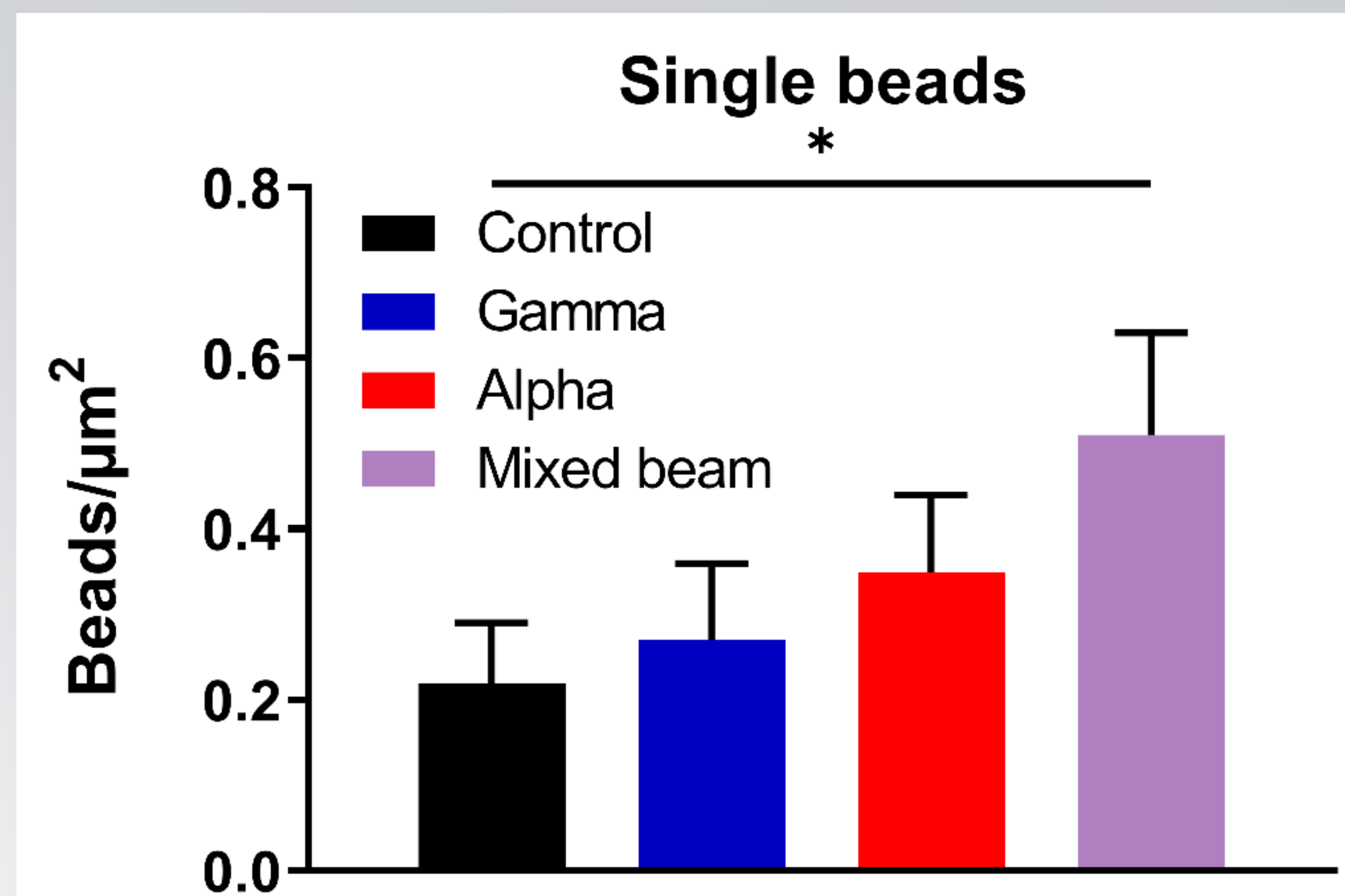


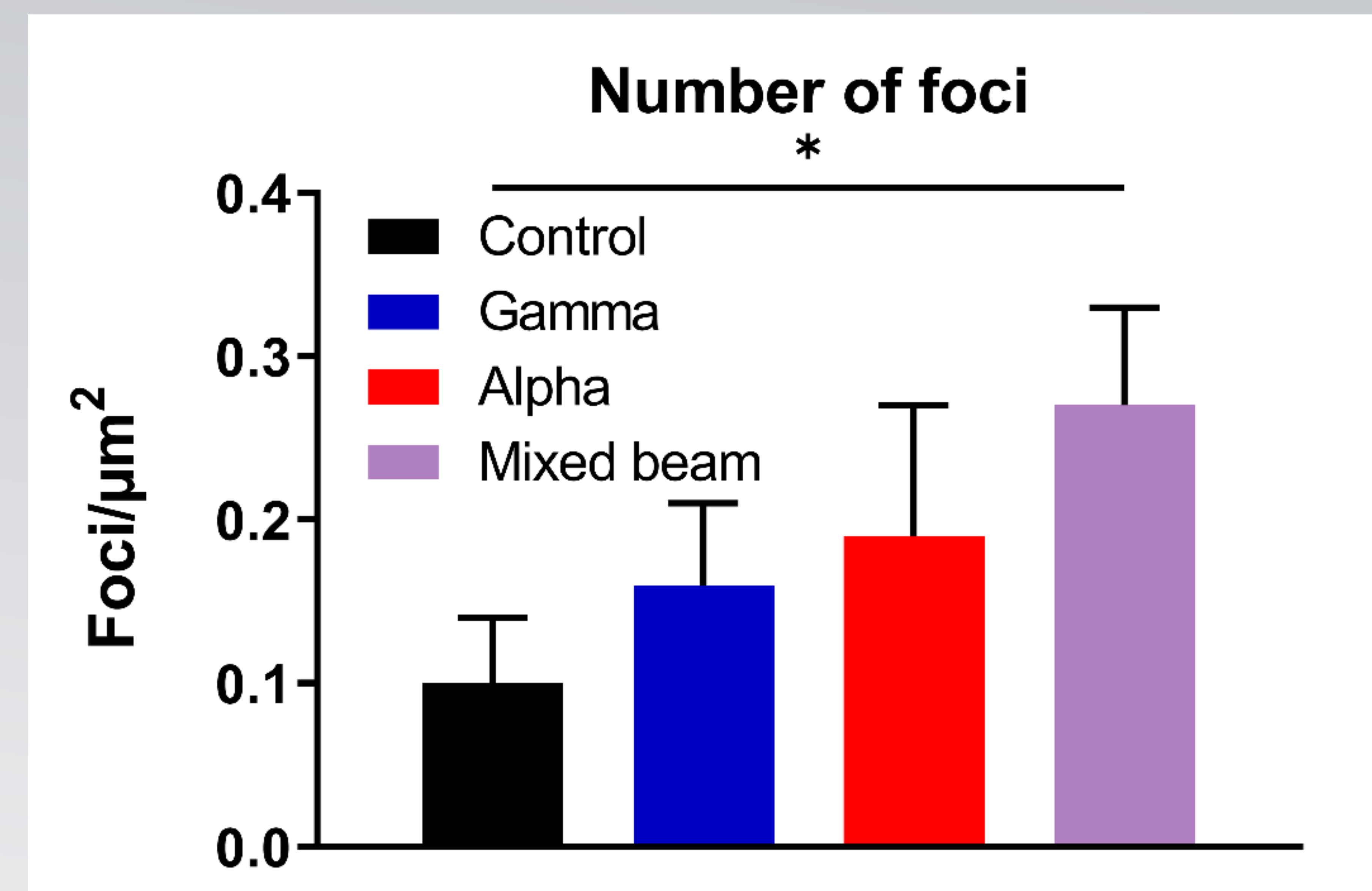


Beads vs foci

A circular mask of 20 pixel diameter (36 nm) was selected around each bead, from which the circle of 10 pixel diameter (18 nm) containing the bead was excluded.



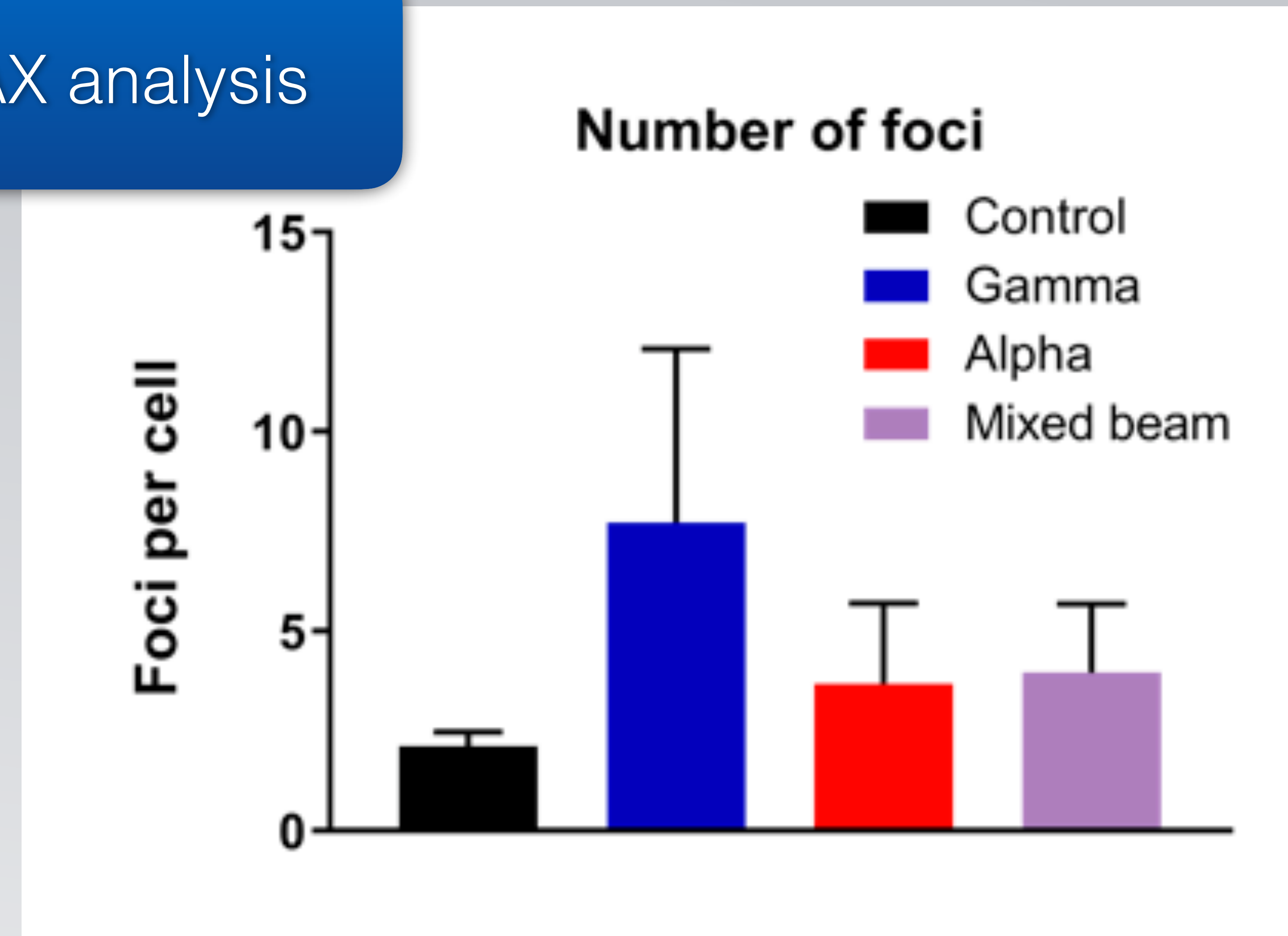




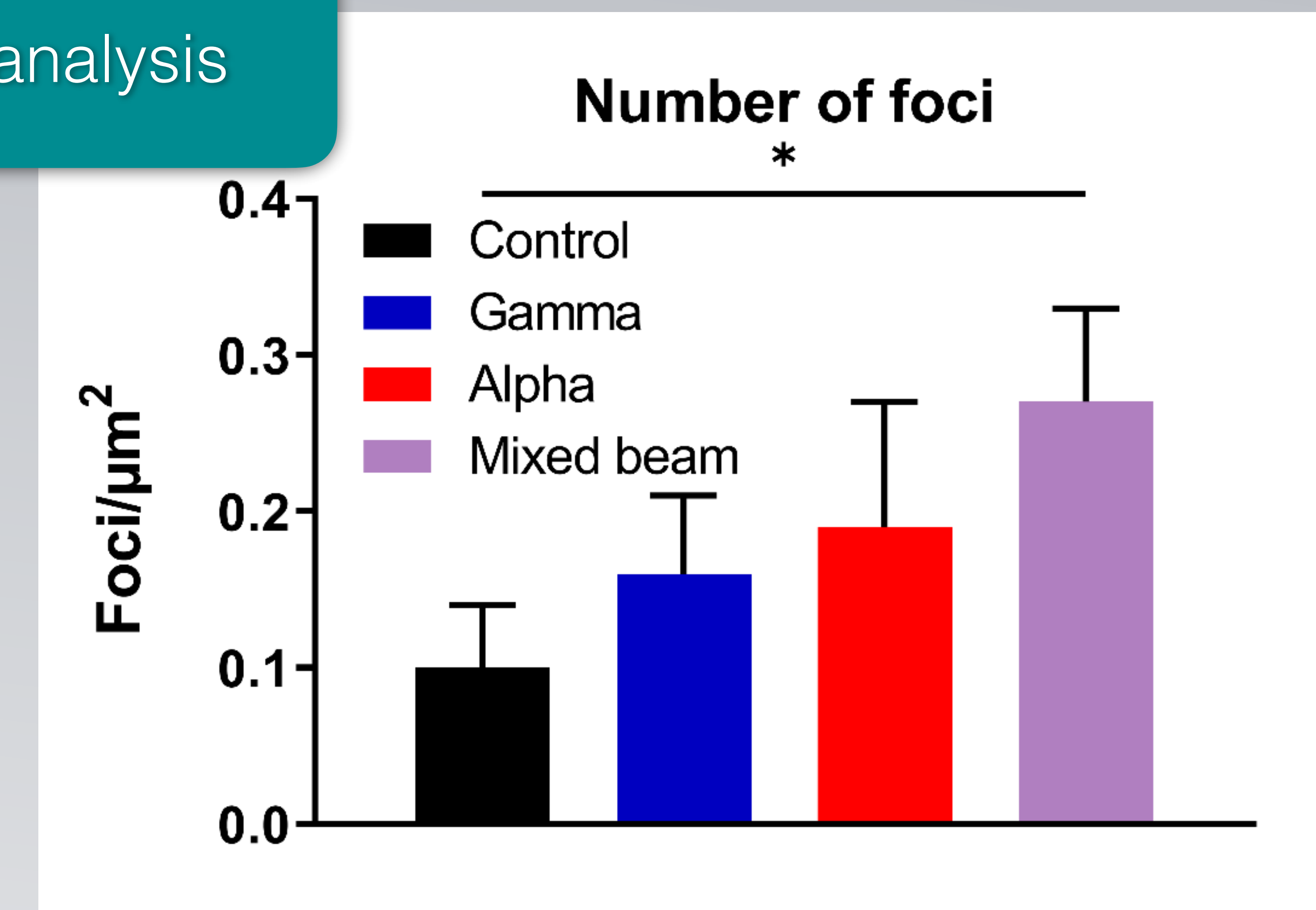
When the outer circles around one or more beads overlapped, the beads were treated as a focus.

Focus number and size

γH2AX analysis

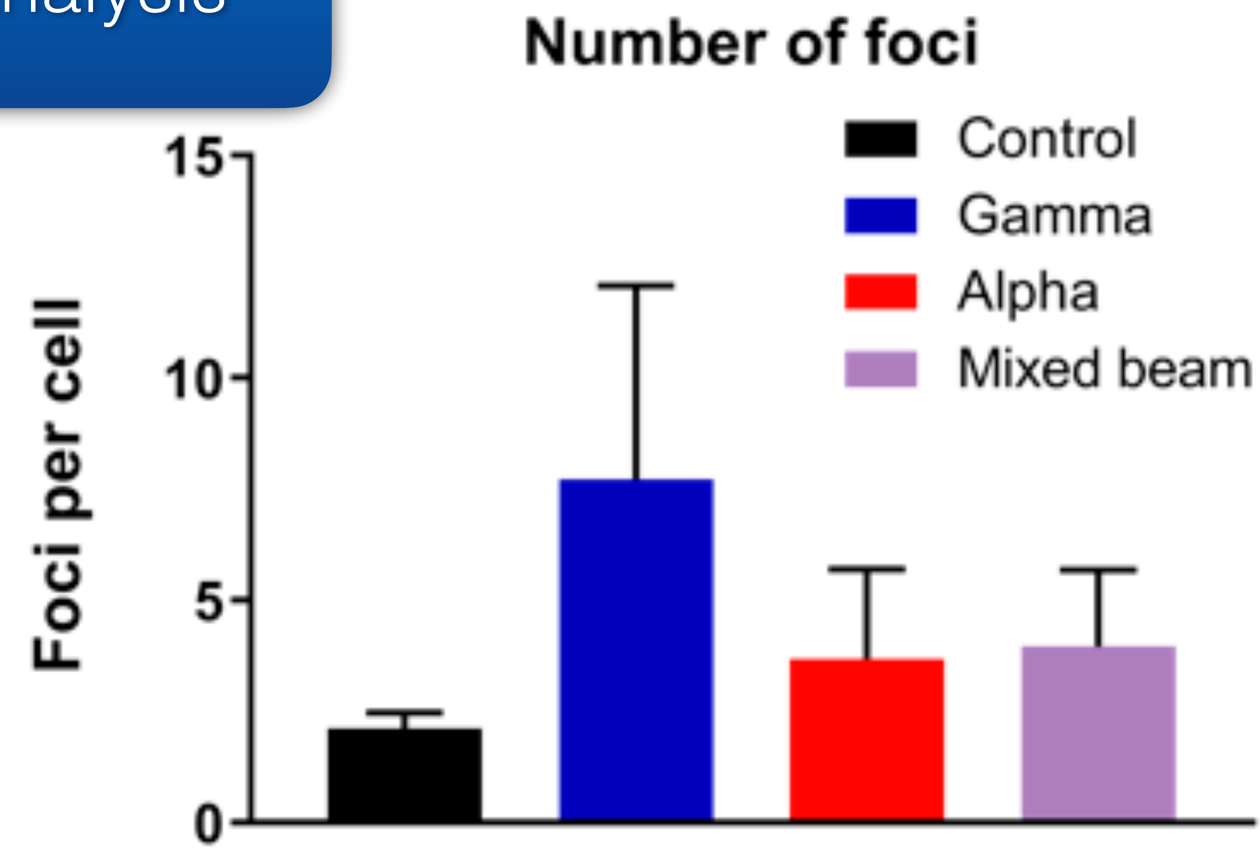


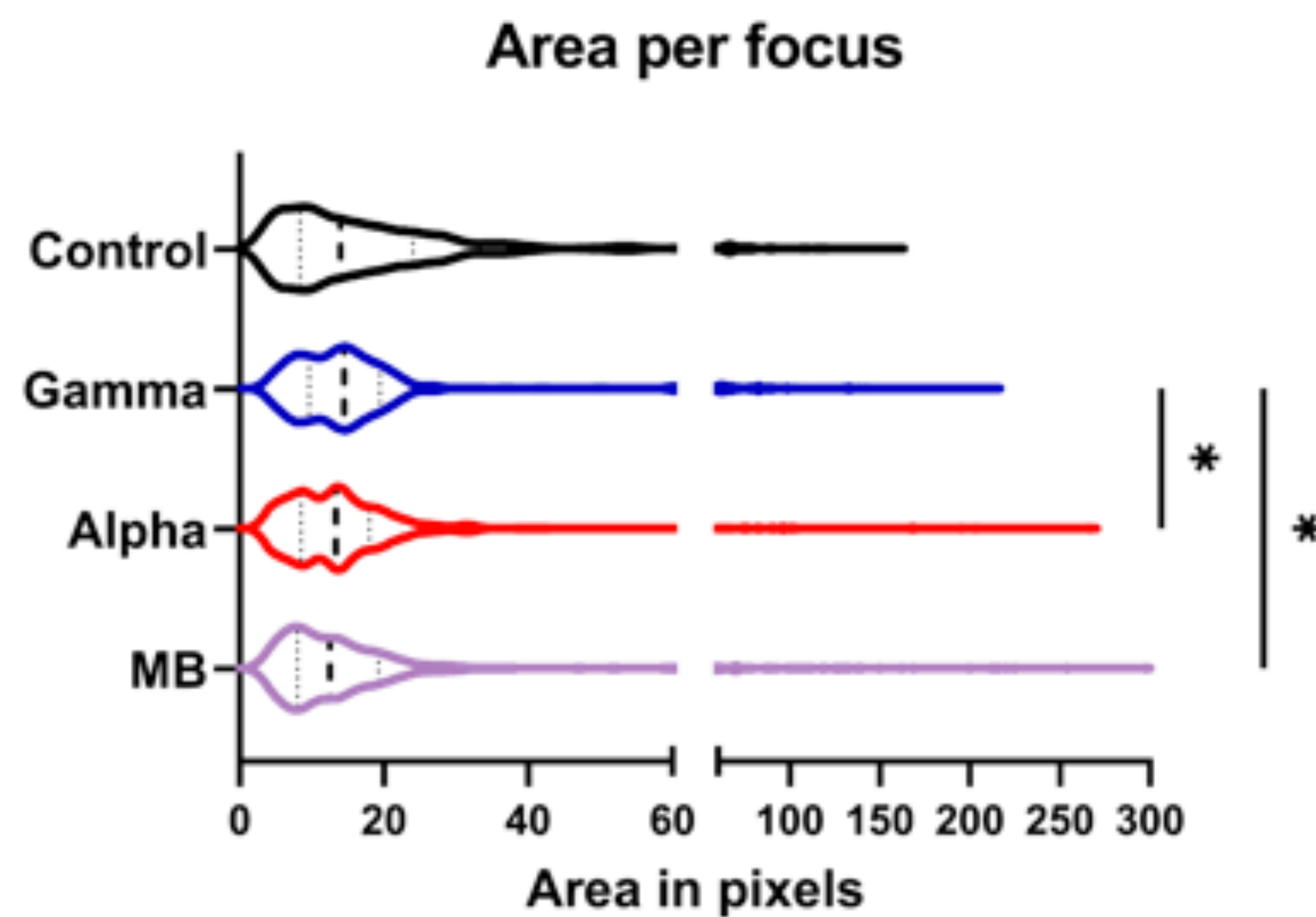
TEM analysis



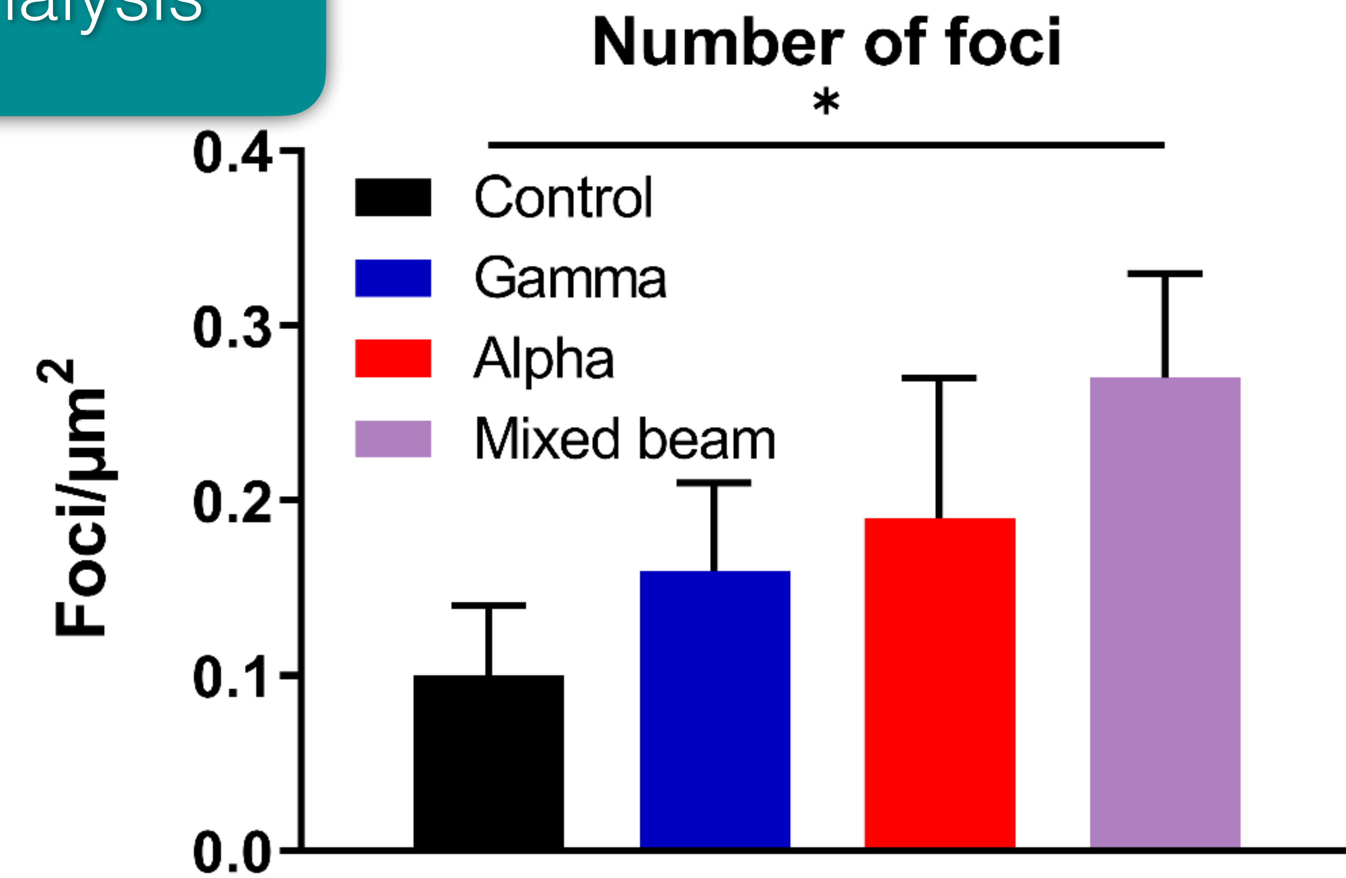
Focus number and size

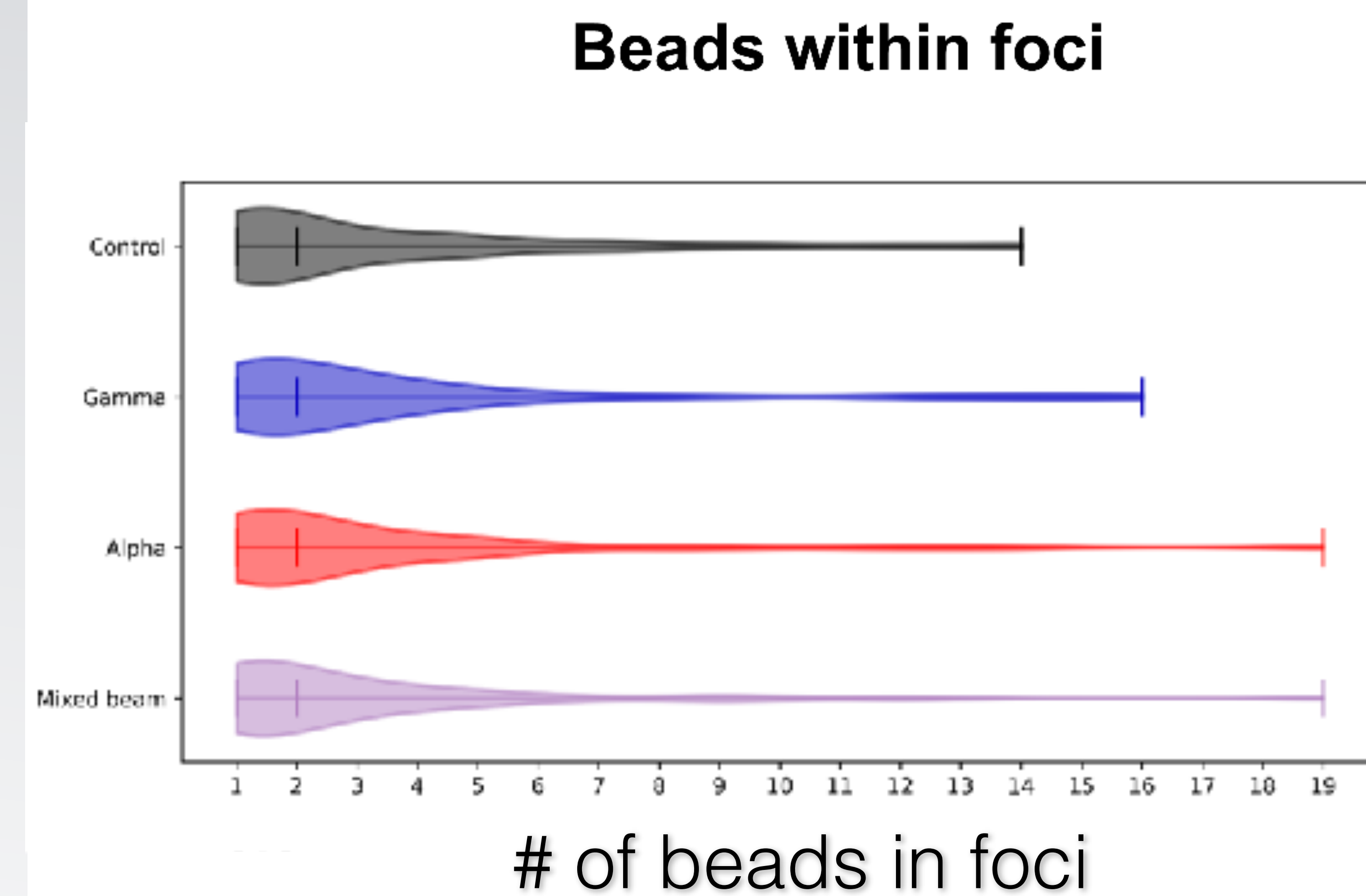
γH2AX analysis





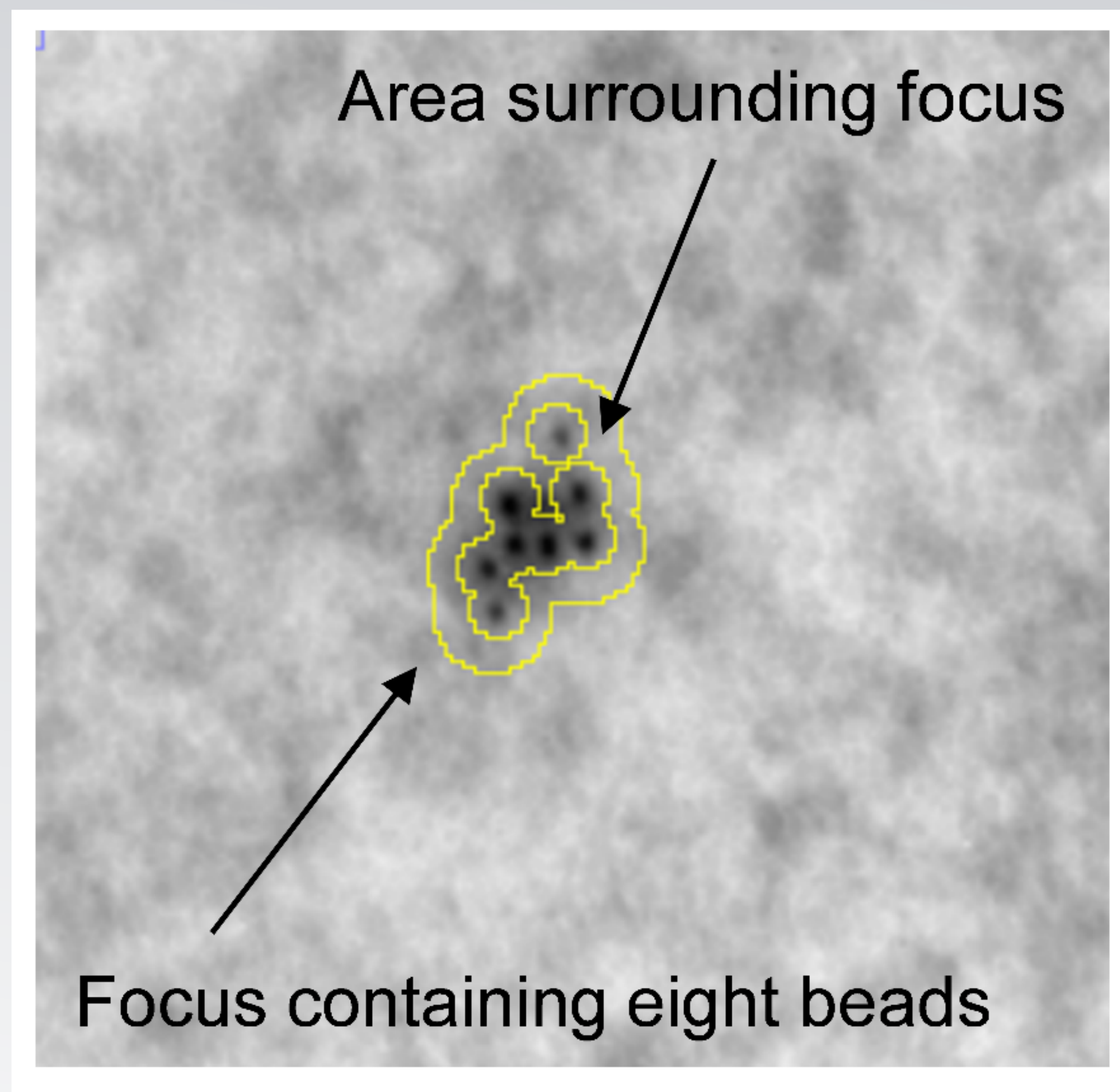
TEM analysis

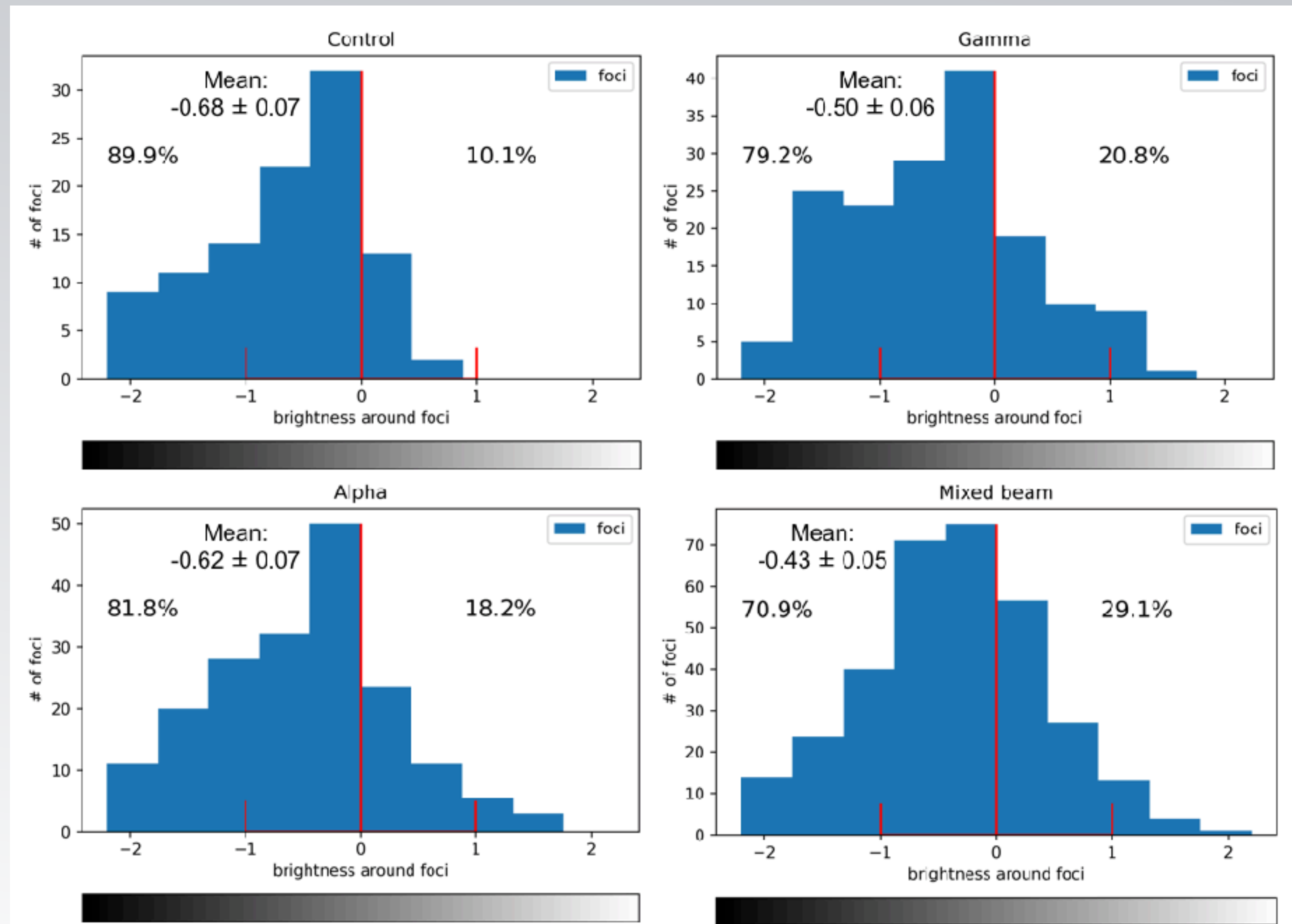




Focus localisation within chromatin

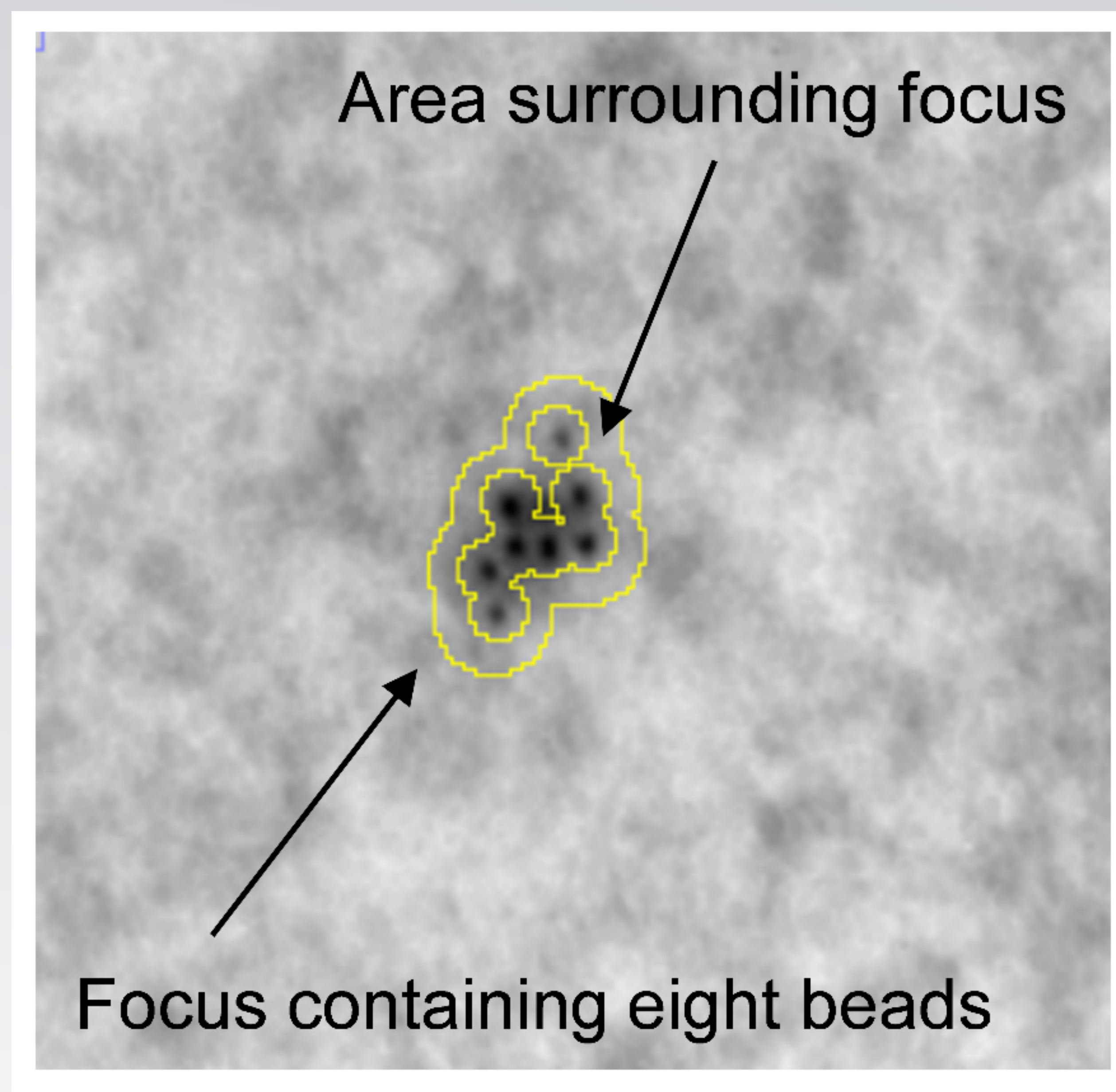
For each area surrounding a bead or focus (between the outer and inner ring), the average pixel intensity (brightness) was computed

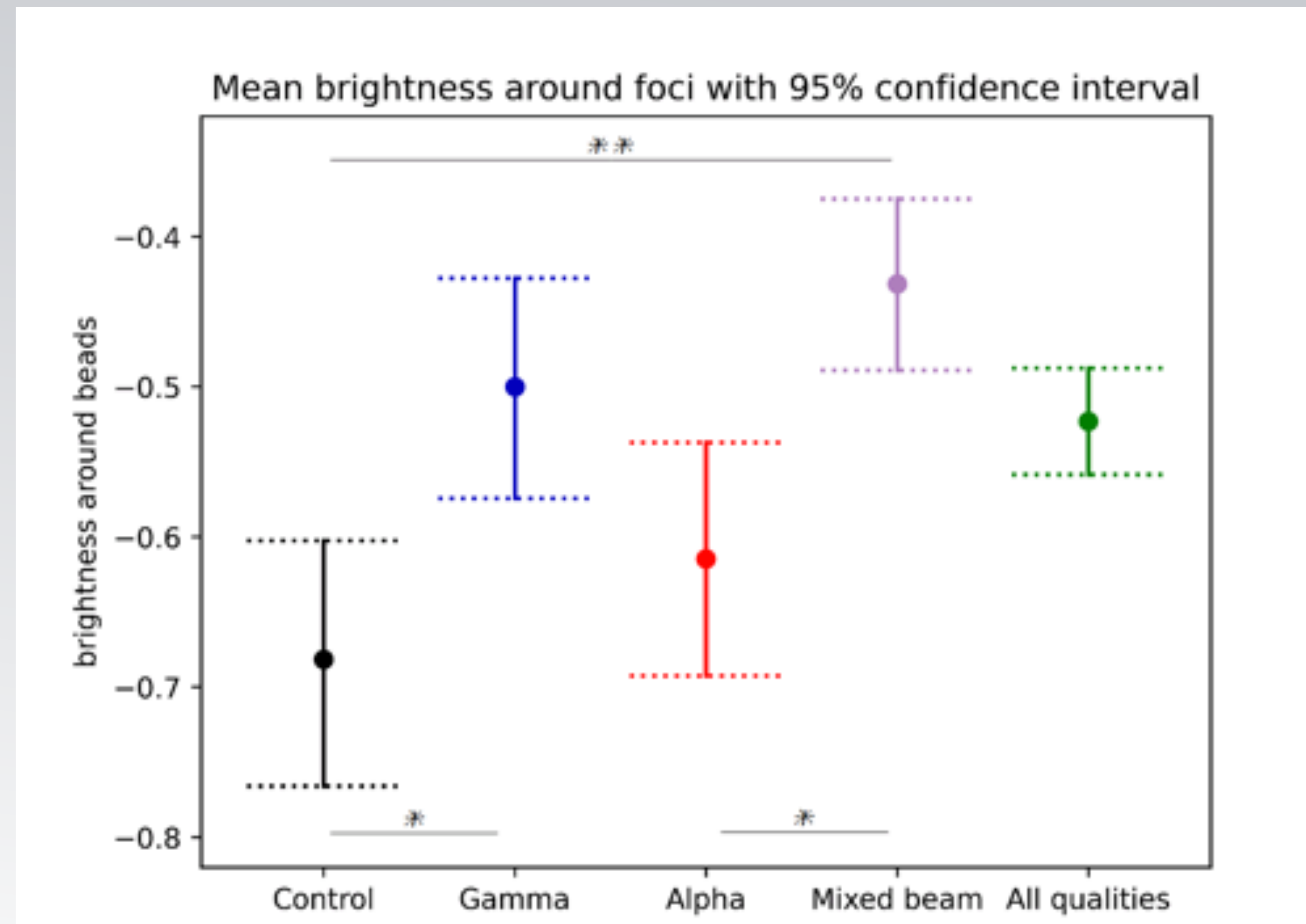


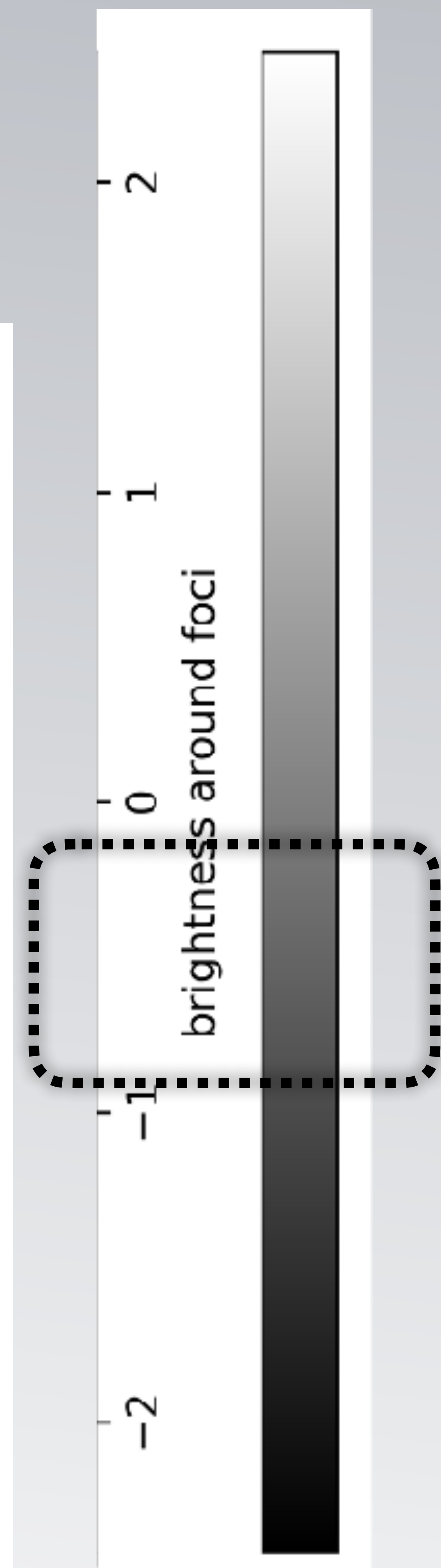


Focus localisation within chromatin

For each area surrounding a bead or focus (between the outer and inner ring), the average pixel intensity (brightness) was computed







Final conclusions

- Largest foci were induced by alpha and mixed beam exposure (γH2AX and TEM).
- Nanosized beads were most frequent after mixed beam exposure at 30 min post irradiation.
- Foci containing the highest number of beads, representing complex damage, remained in the areas of compact chromatin (this type of damage is remaining longer).
- TEM data at 30 min after exposure display a more prominent opening of the chromatin structure locally around foci after mixed beam exposure (the need of an extensive repair due to the excess damage induced).

Acknowledgments

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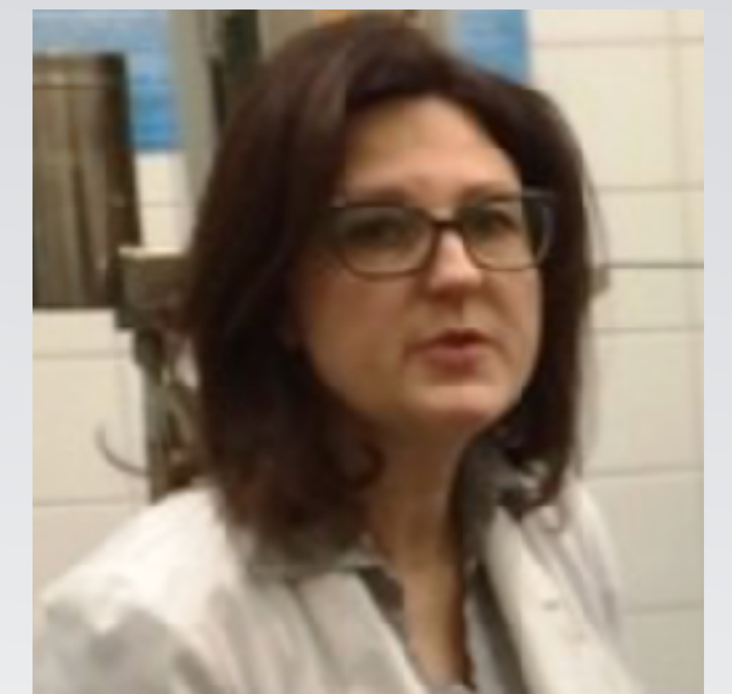
and Lei Cheng

Józef Ginter

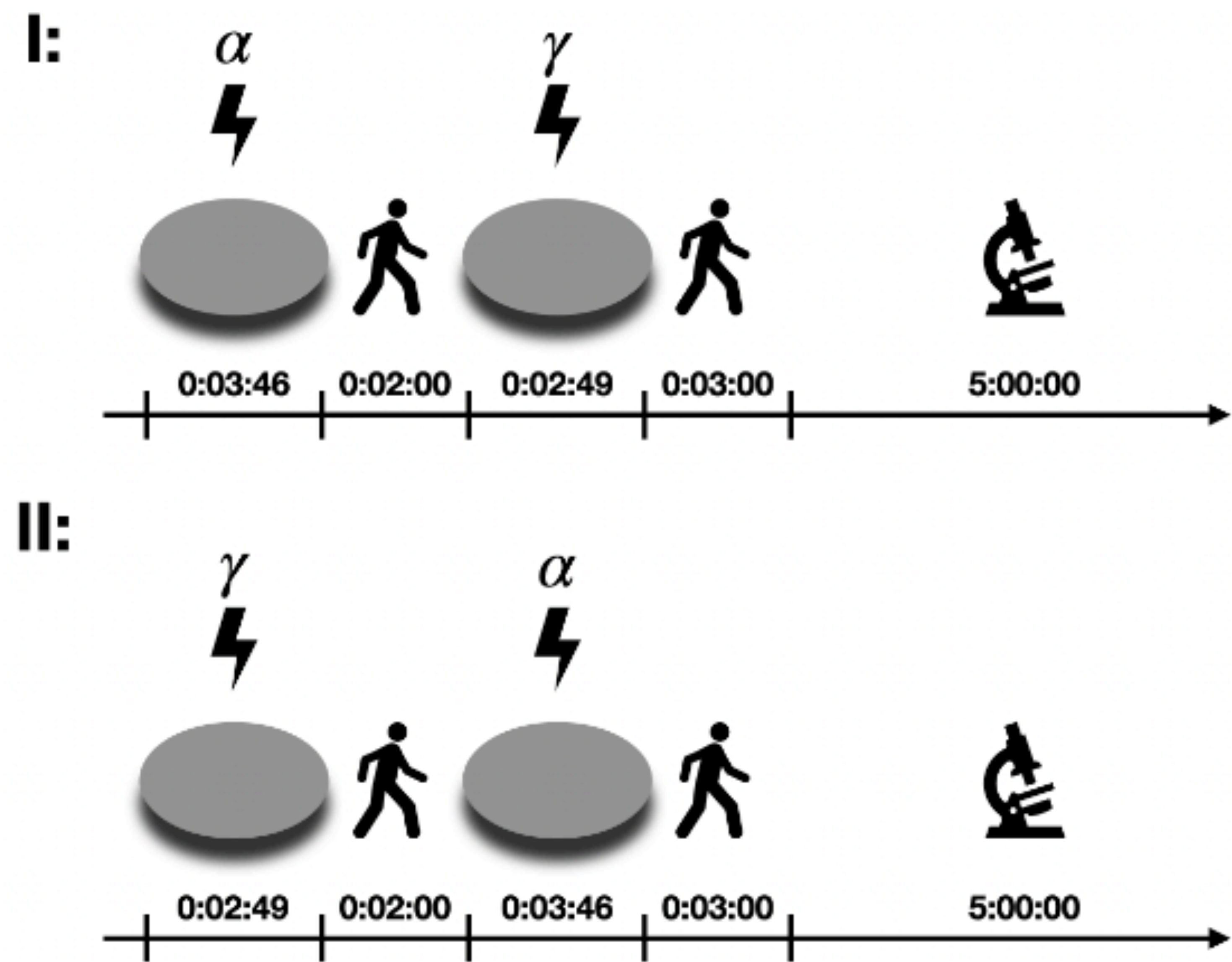
Katarzyna Życieńska

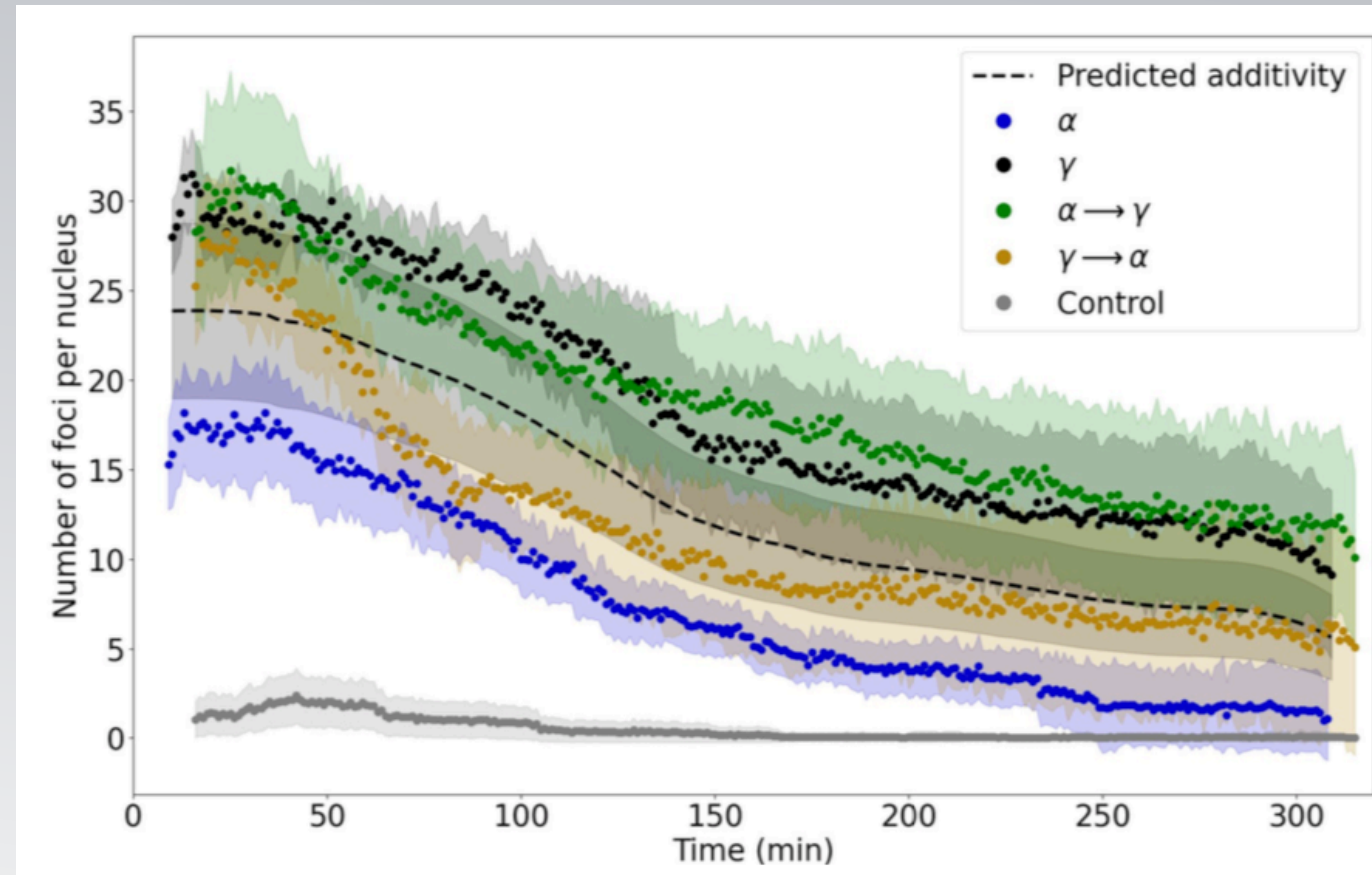
Anna Wieczorek



Małgorzata Łysek-Gładysińska

Does the order of irradiation matter: NBS1 focus dynamics





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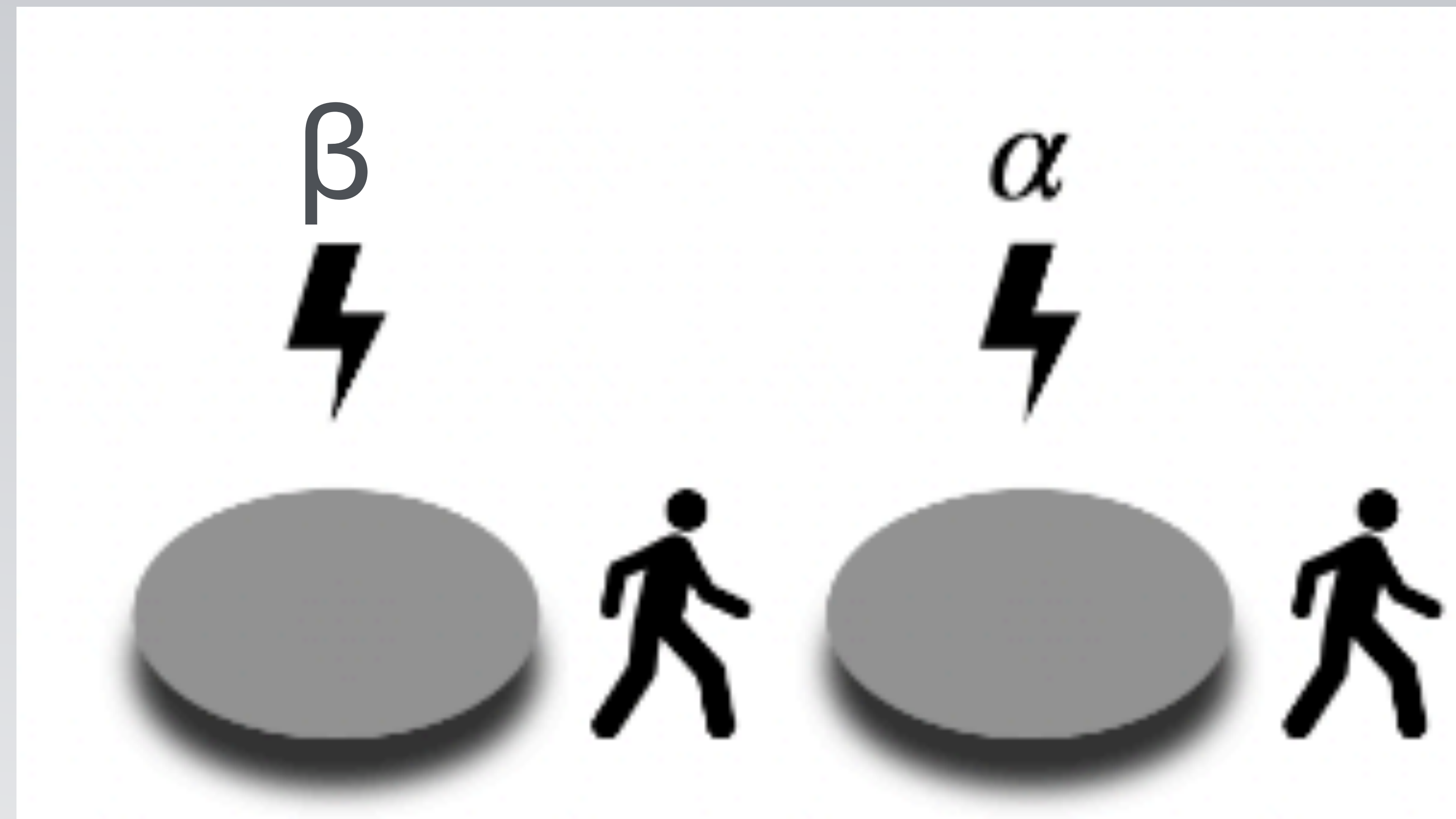
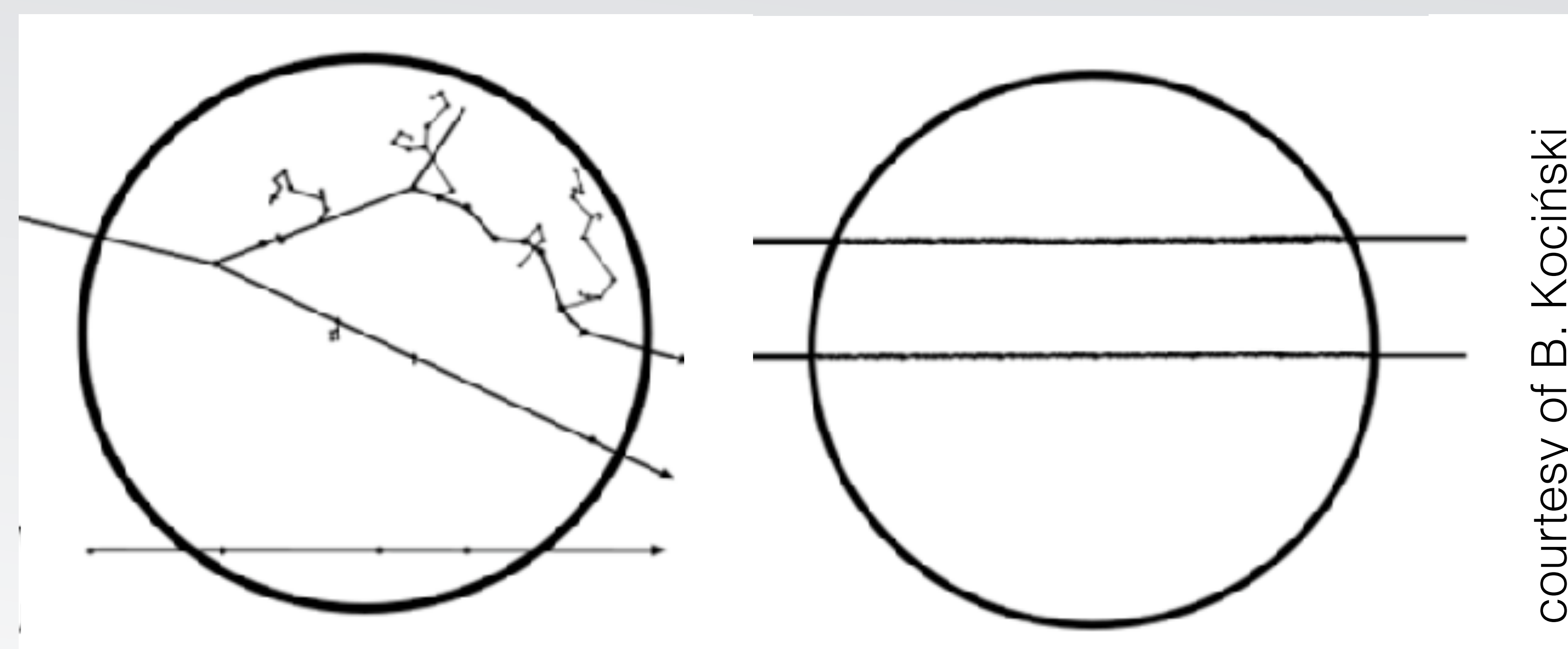
Tartas A., Lundholm L., Scherthan H., Wojcik A. i Brzozowska B. (2023). The order of sequential exposure of U2OS cells to gamma and alpha radiation influences the formation and decay dynamics of NBS1 foci, PLoS ONE 18(6): e0286902 (https://doi.org/10.1371/journal.pone.0286902)

Do cells adapt to radiation?





250 mGy

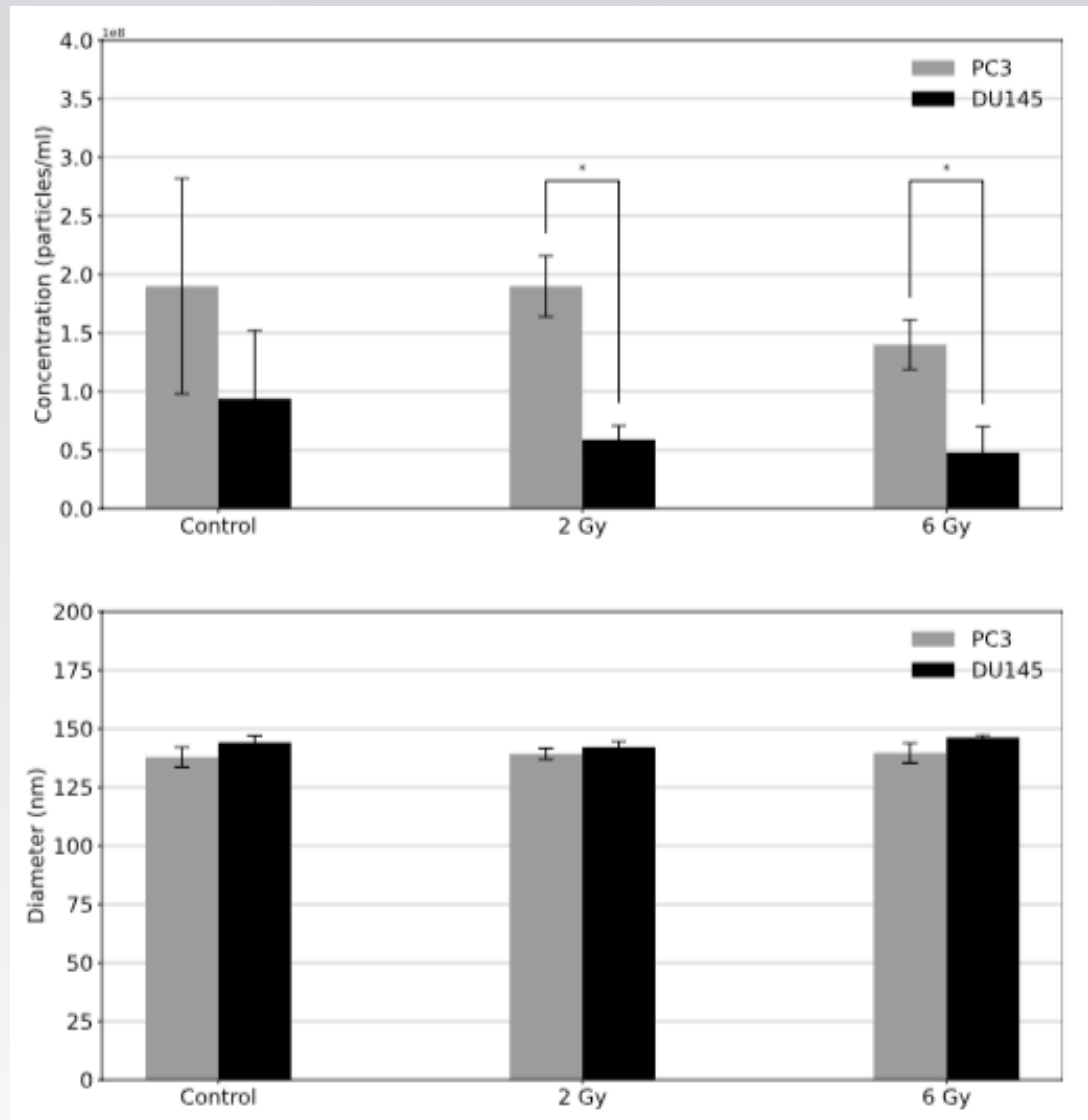
2 Gy

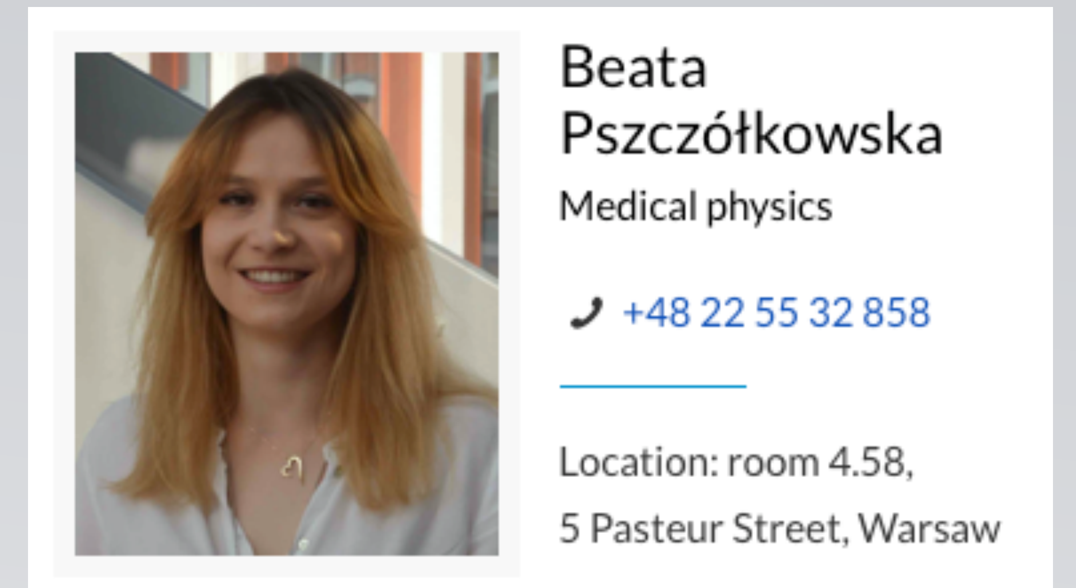
CELL SURVIVAL

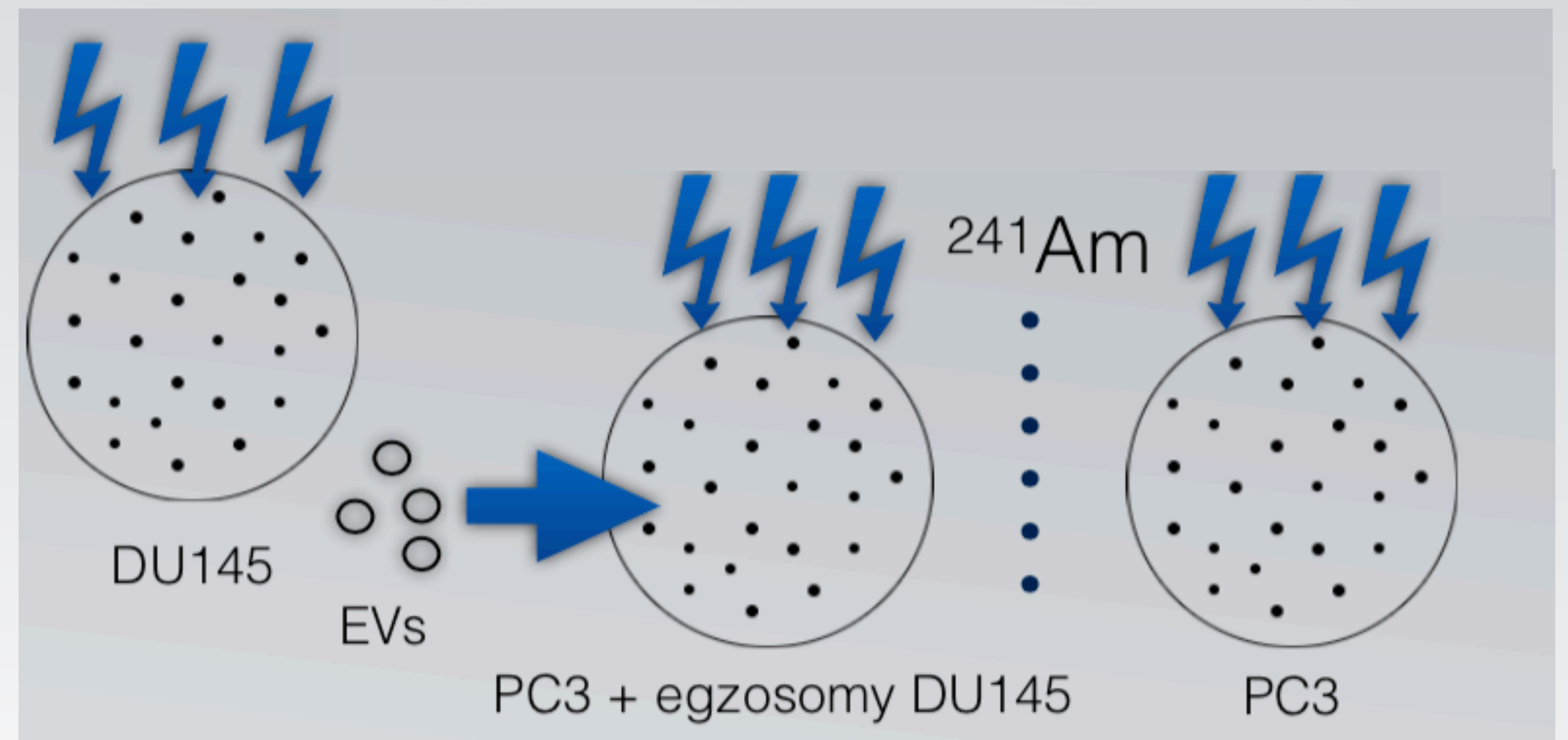
GAMMA-H2AX

FACULTY OF PHYSICS

Do exosomes make cells more radiosensitive?





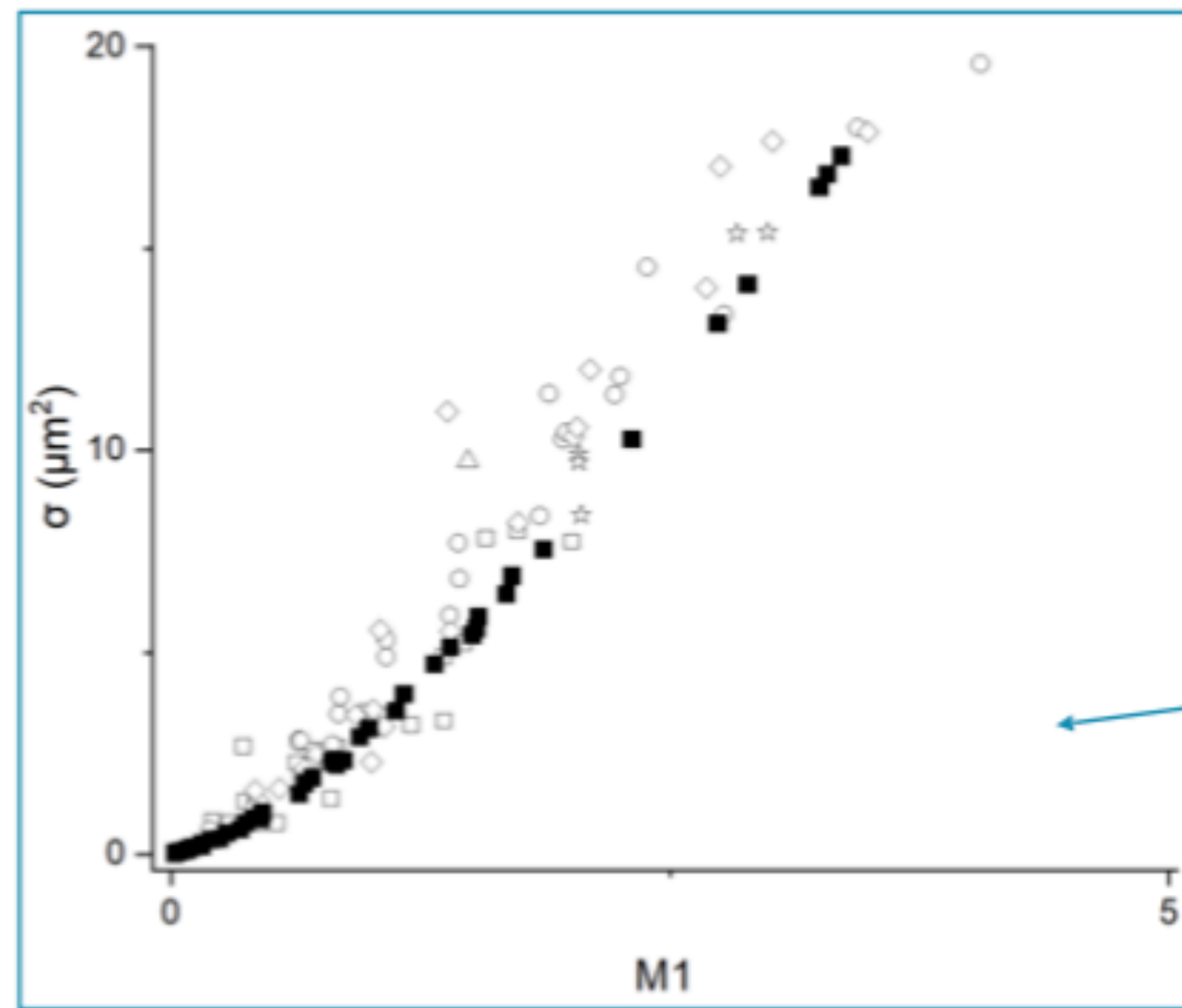


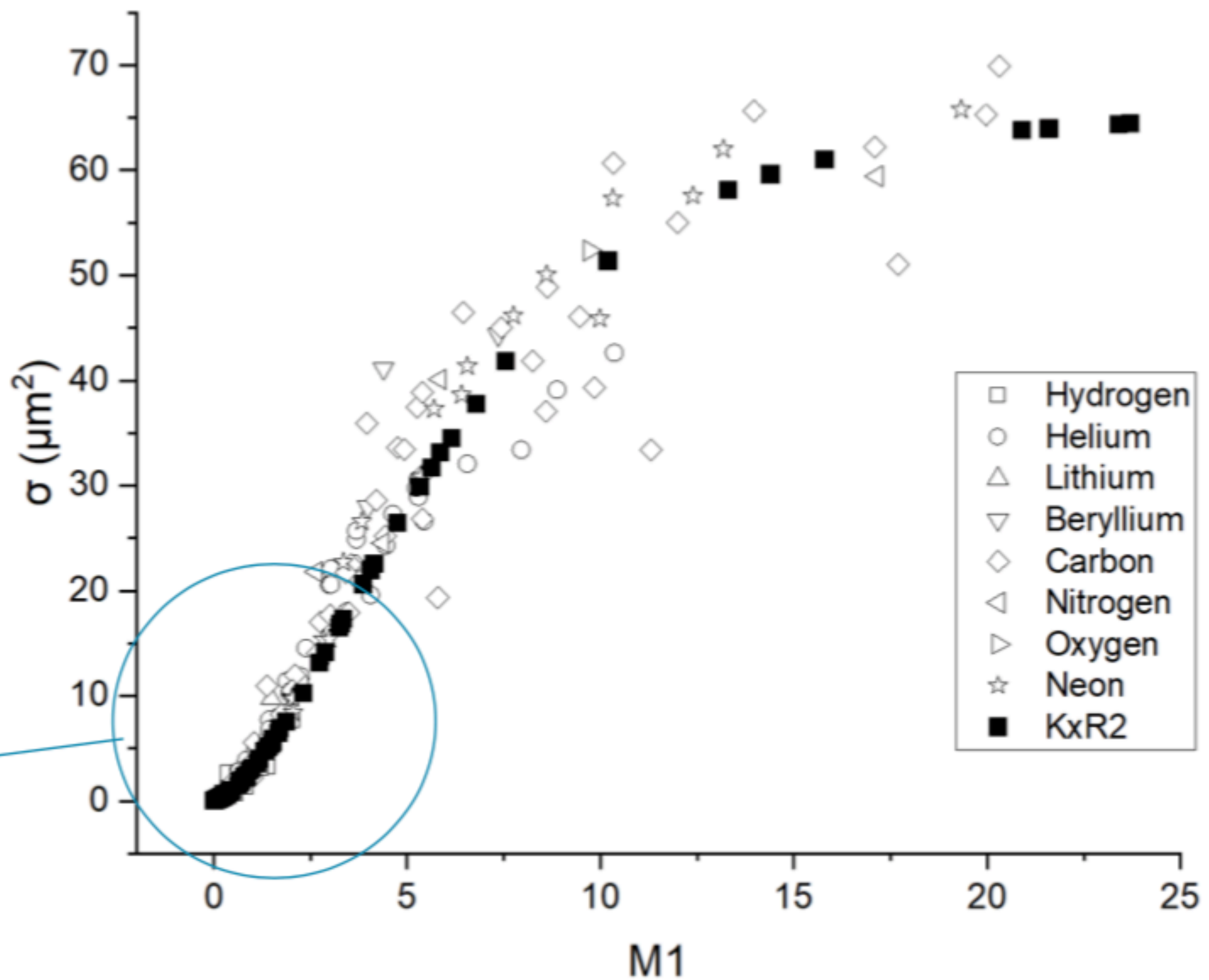
Pszczółkowska B., Olejarz W., Filipek M., Tartas A., Kubiak-Tomaszewska G., Żołnierzak A., Życieńska K., Ginter J., Lorenc T. i Brzozowska B. (2022). Exosome secretion and cellular response of DU145 and PC3 after exposure to alpha radiation. Radiation and environmental biophysics, 1-12

Can nanodosimetric quantities be used to describe DNA damage?

Geant4-DNA

PIDE





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M. Pietrzak, Nanodosimetric characteristic of carbon ion beam - experiments and Monte Carlo simulations, Doctoral thesis, 2023, Faculty of Physics, University of Warsaw

You can design and create, and build the most wonderful place in the world. But it takes people to make the dream a reality.

Biomedical Physics Division

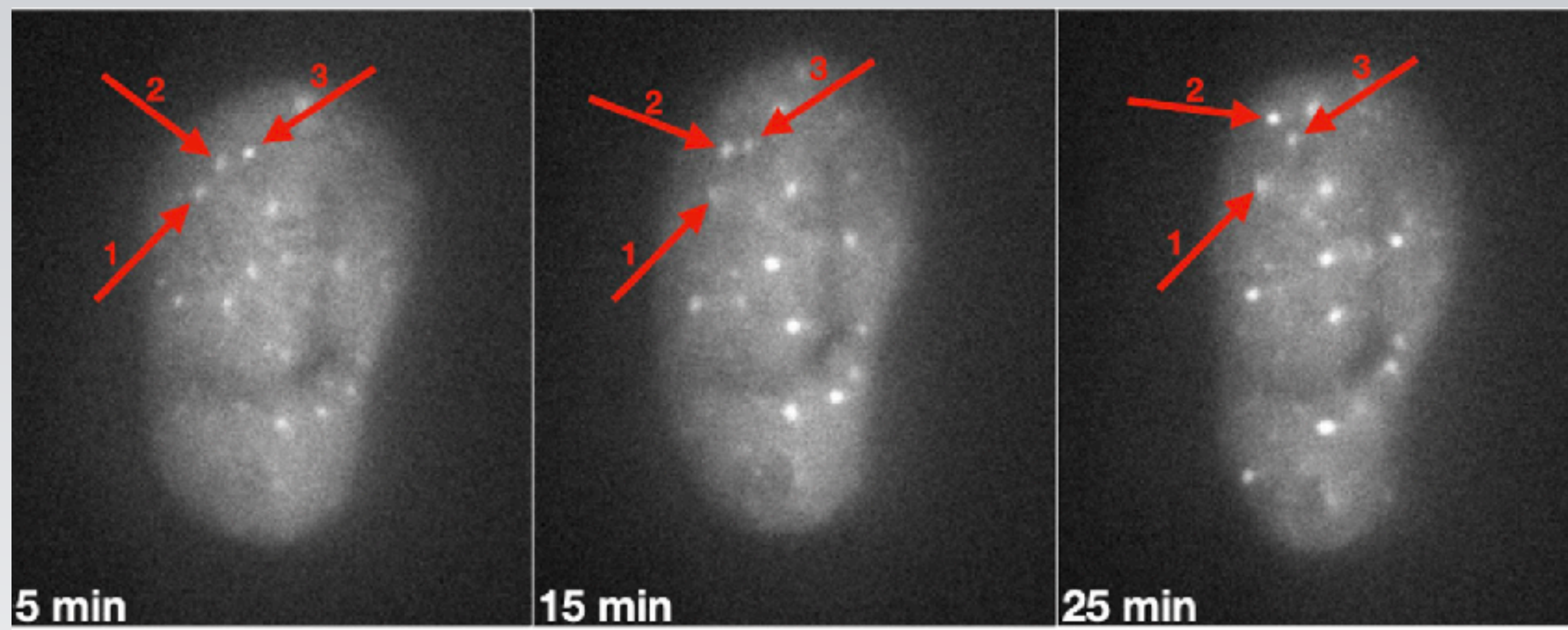
Division of Particles and Fundamental Interactions

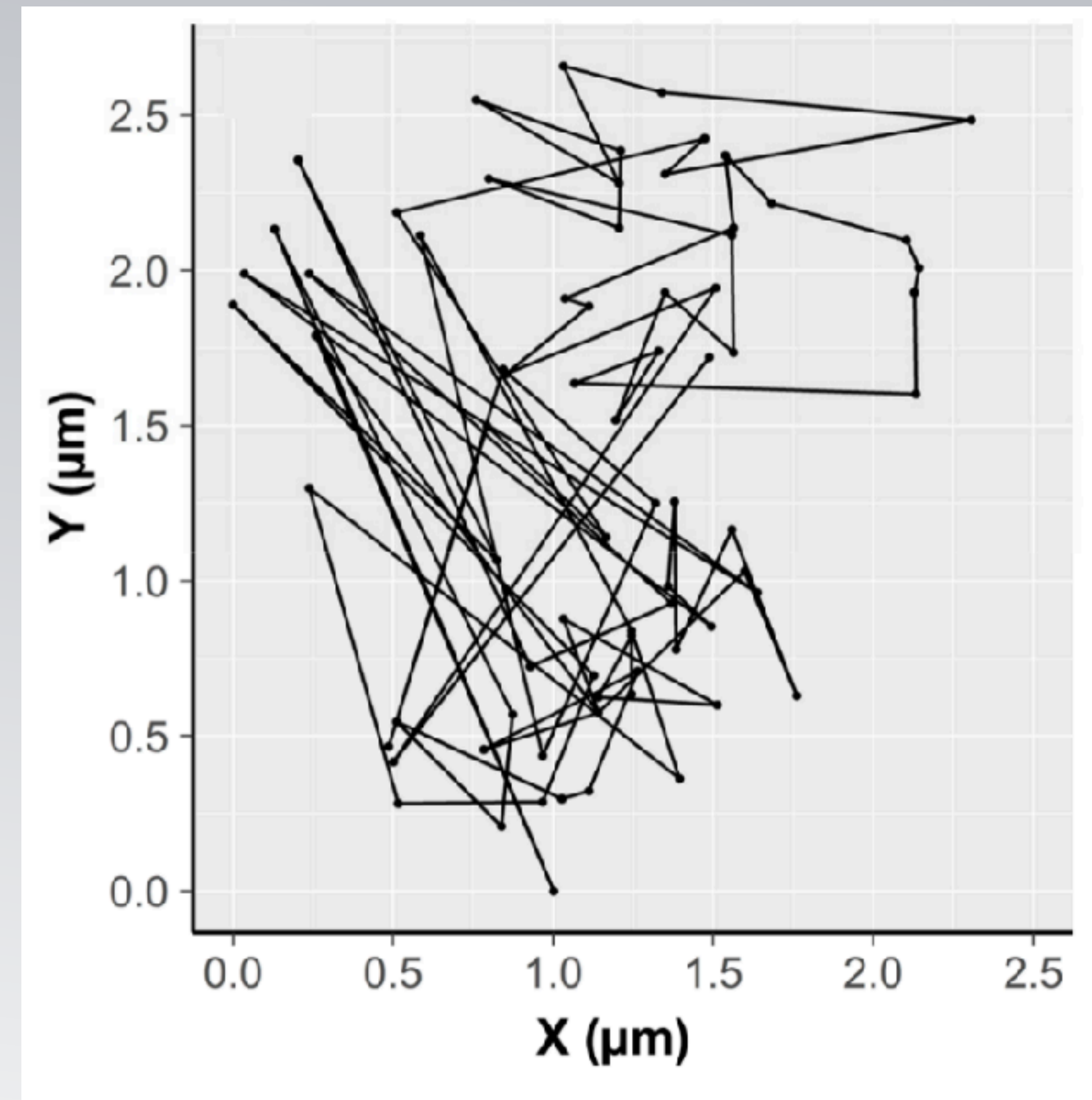
Thank you for your attention!

beata.brzozowska@fuw.edu.pl

Backup slides

Chromatin movement





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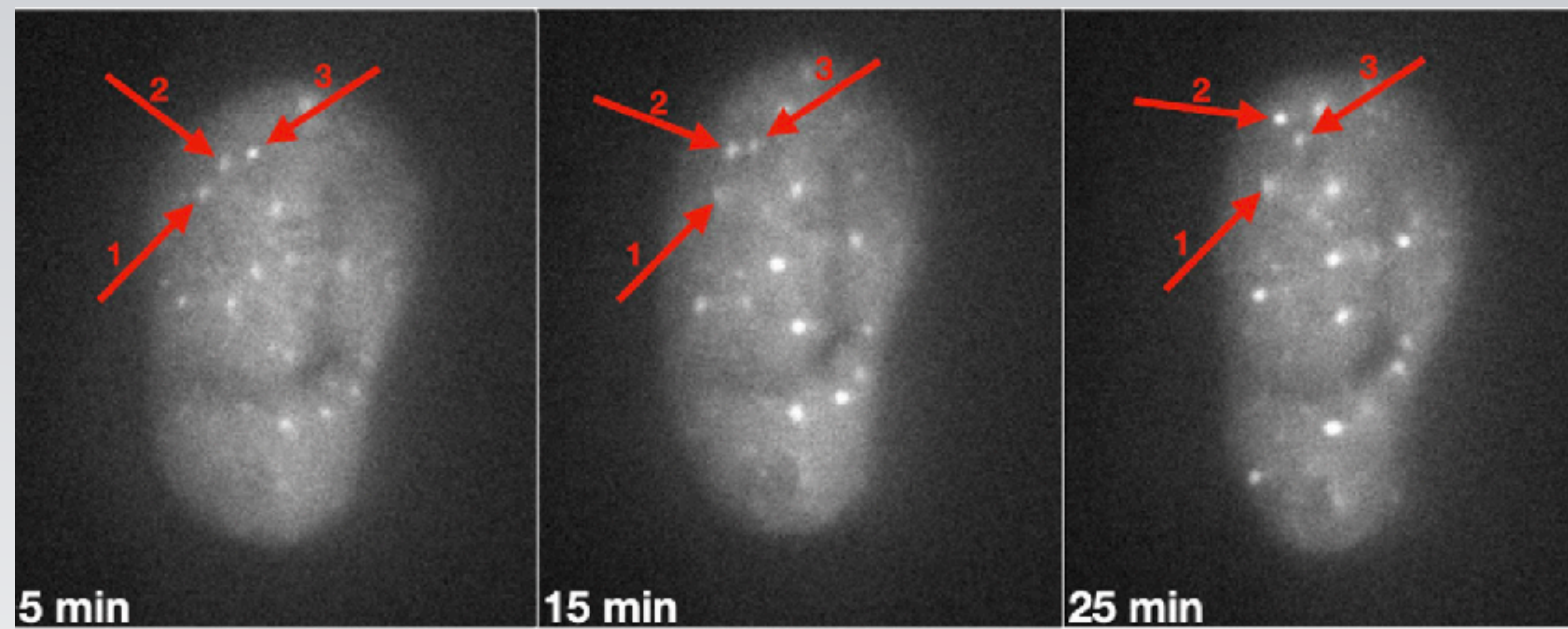
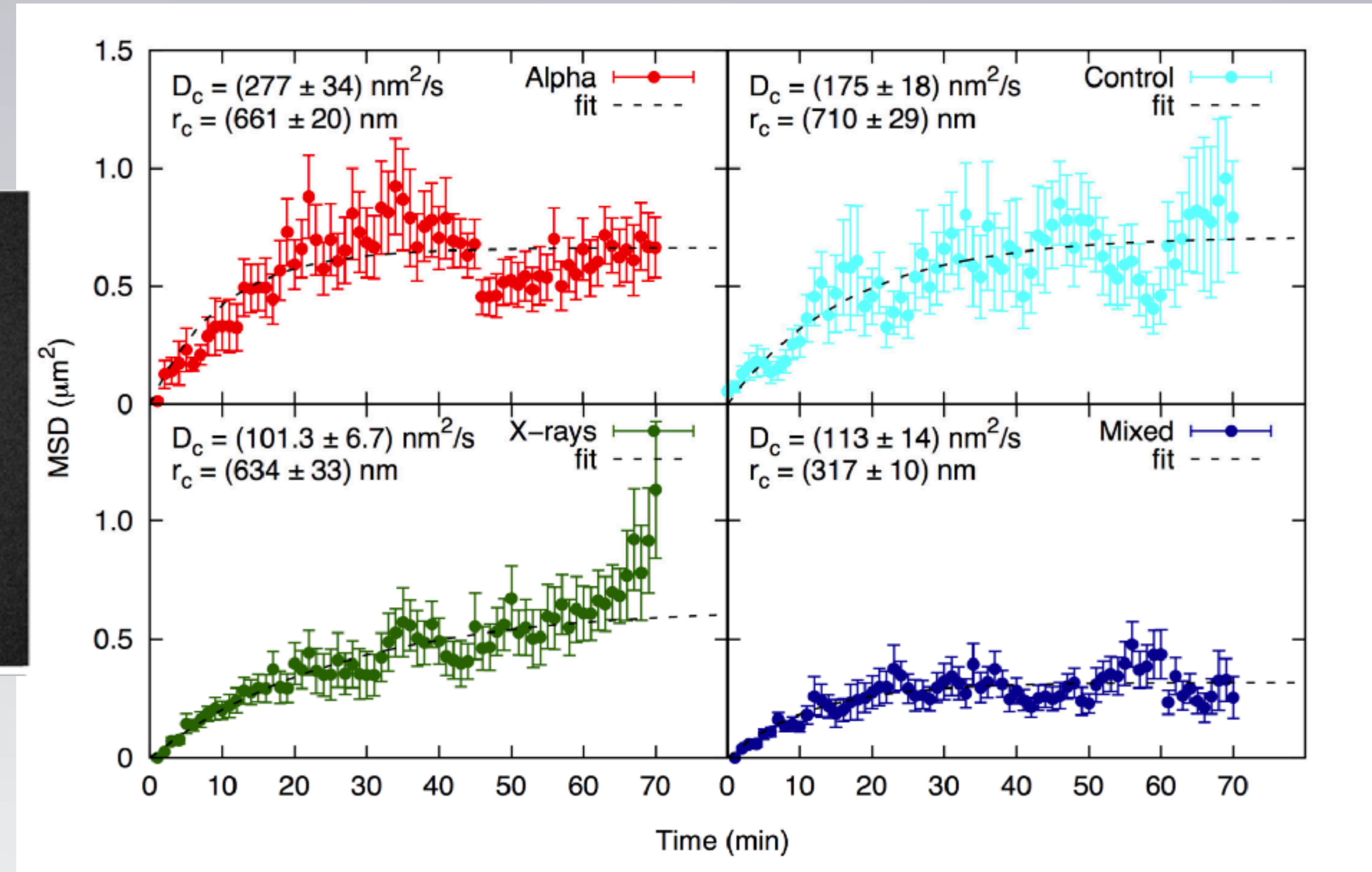
Chromatin movement

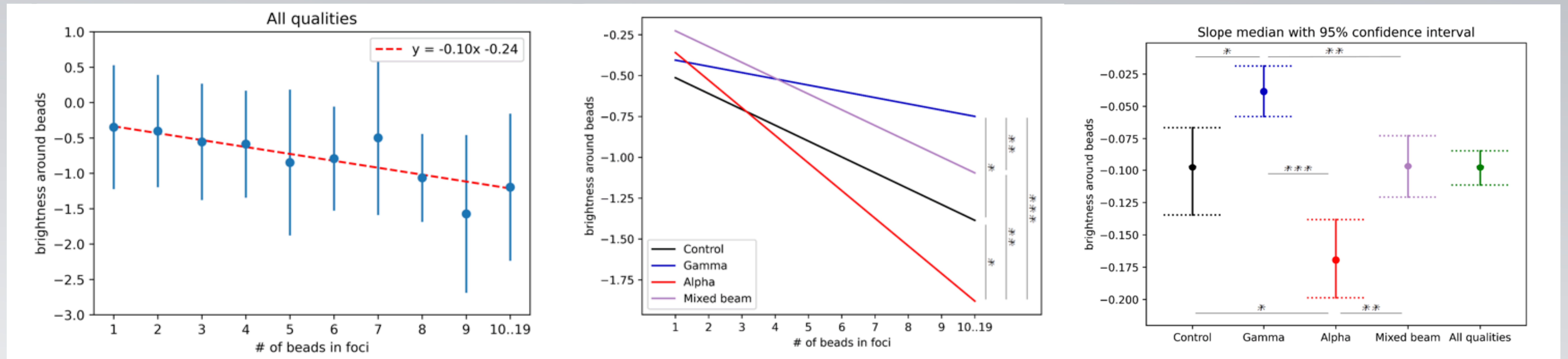




Sollazzo A, Brzozowska B, Cheng L, Lundholm L, Scherthan H, Wojcik A (2018) Live Dynamics of 53BP1 Foci Following Simultaneous Induction of Clustered and Dispersed DNA Damage in U2OS Cells. International Journal of Molecular Sciences 19(2): 519

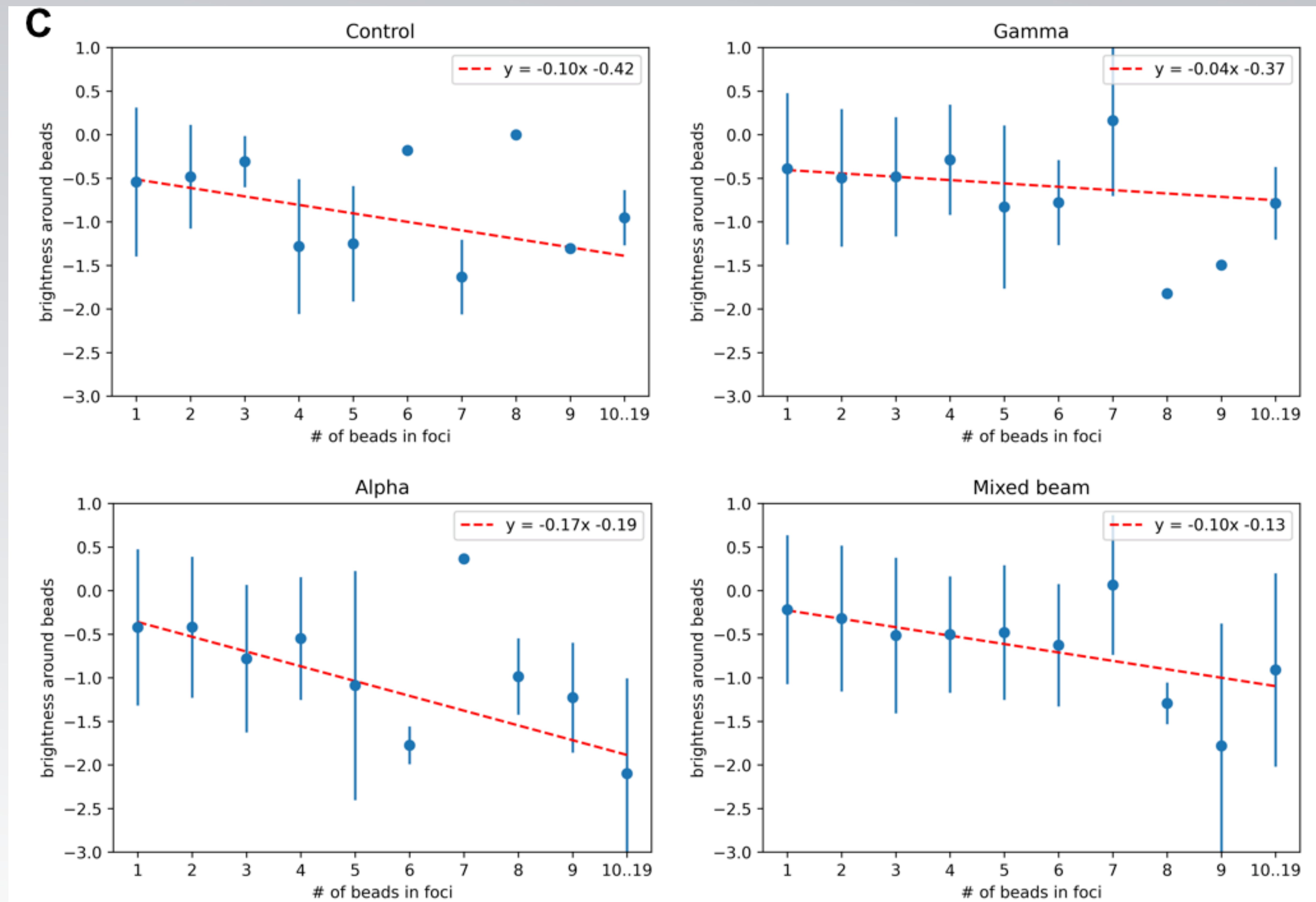
STEP 2: TEM analysis

Chromatin region vs complex DNA damage



- inverse correlation with focus size for lower levels of brightness (areas with more compacted chromatin)
- steepest linear slope was evident for alpha exposure (an indication of complex damage remaining within compacted chromatin areas at 30 min after exposure)

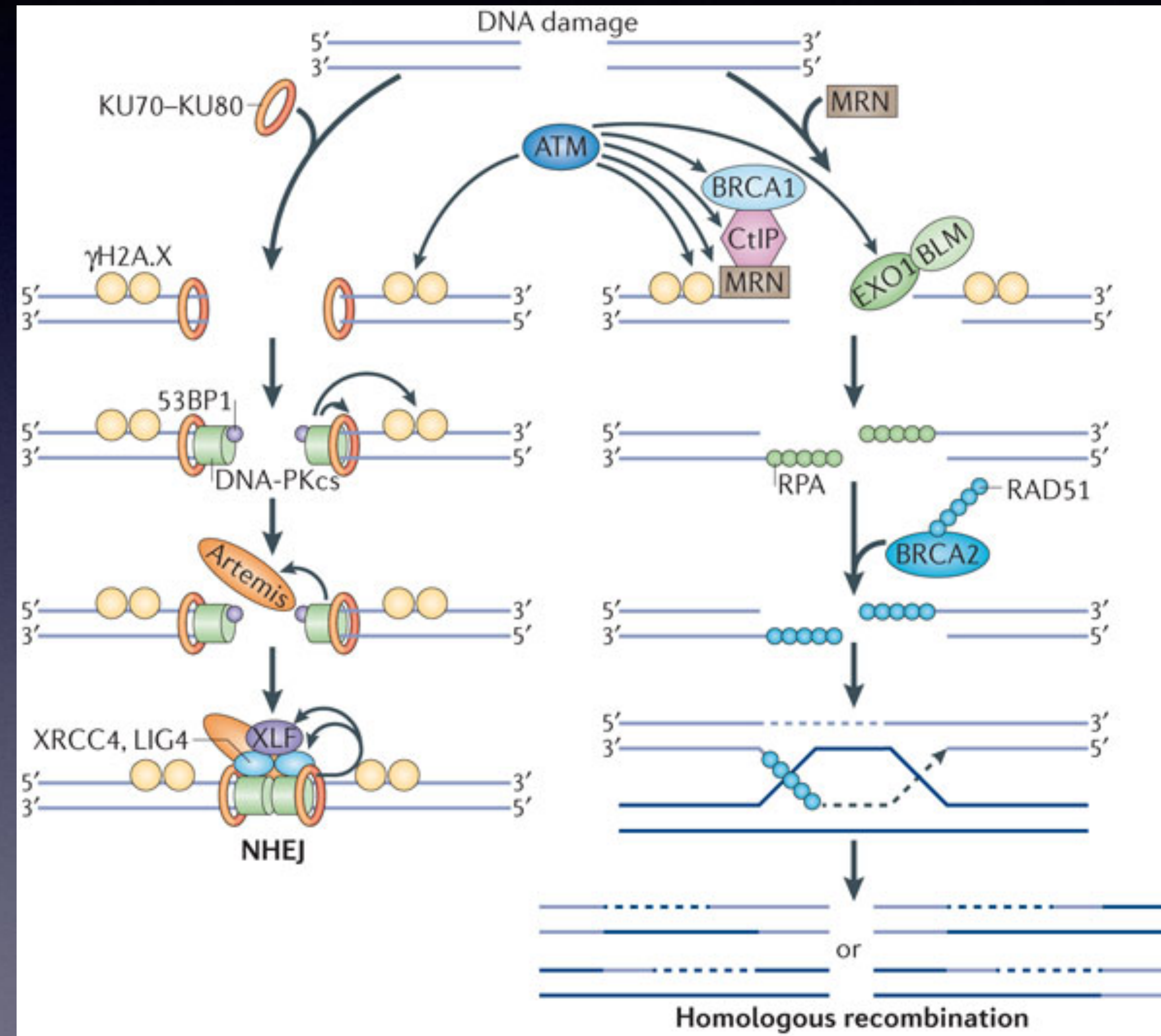
Focus location



Possible explanations

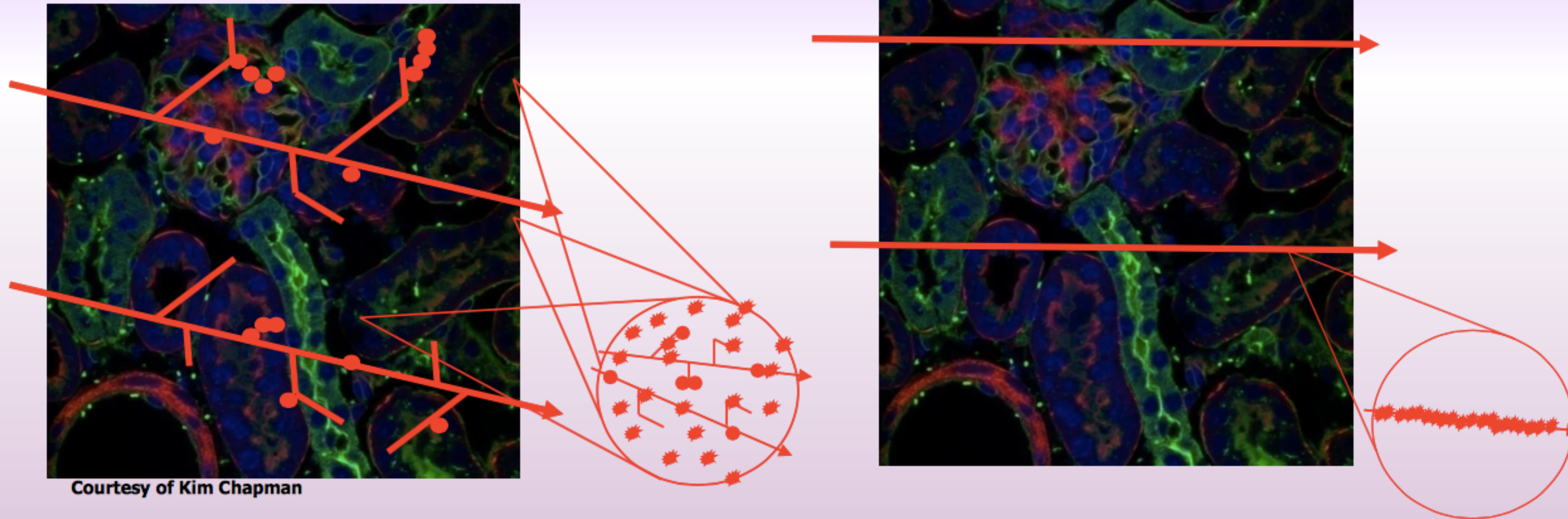
Damage response machinery	• additional damage induced by the second agent may not be repaired properly or in the usual time frame; • the pool of freely available repair proteins is depleted by high LET IR; • high LET exposure overwhelms DNA repair so that low LET-induced damage is not repaired properly;
Damage complexity	• high energy delivery in small subcellular volumes; • clustered DNA damage in cell nucleus; • high LET exposure leads to opened chromatin and more oxidative damage.

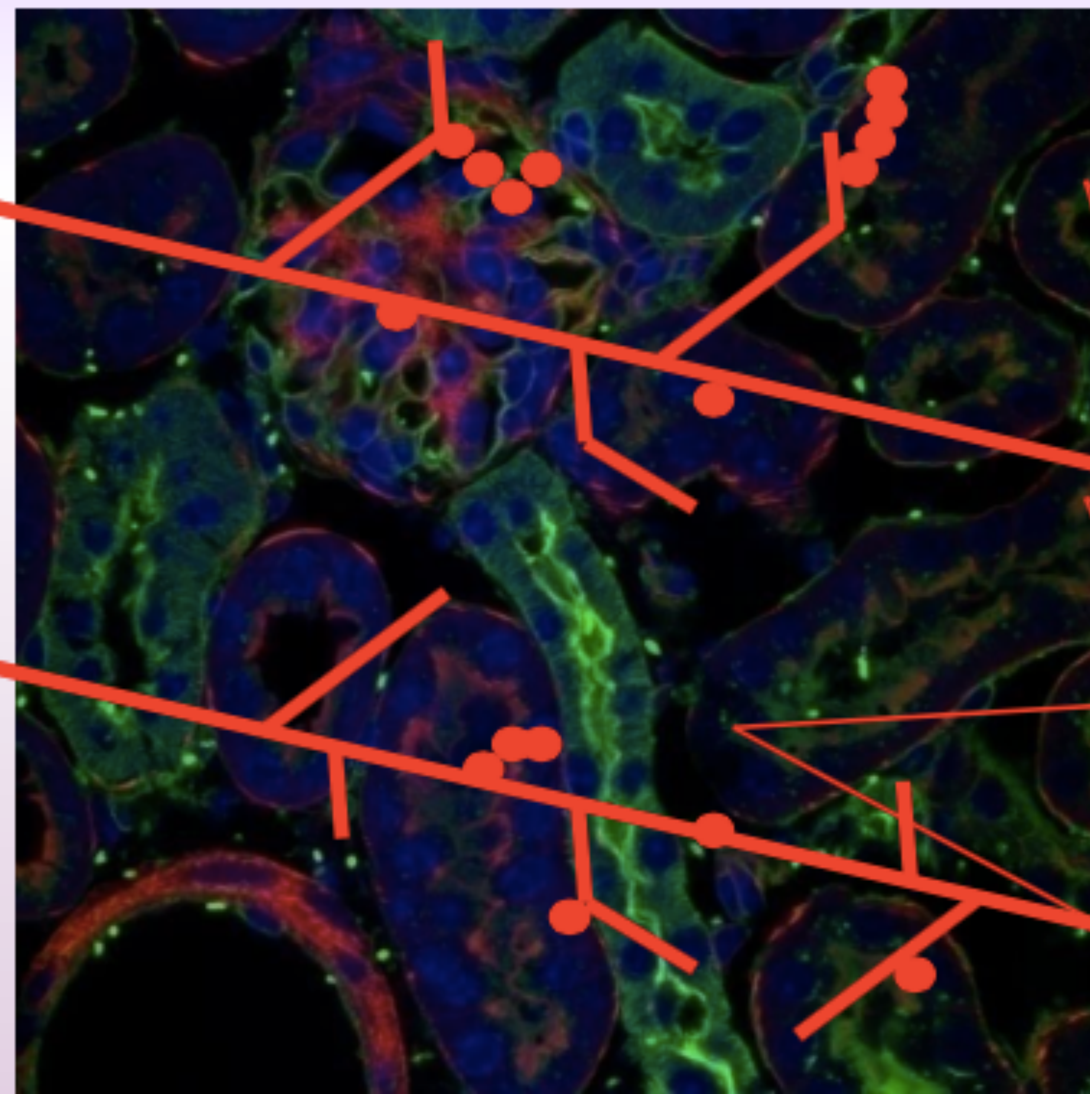
Proces naprawy DNA

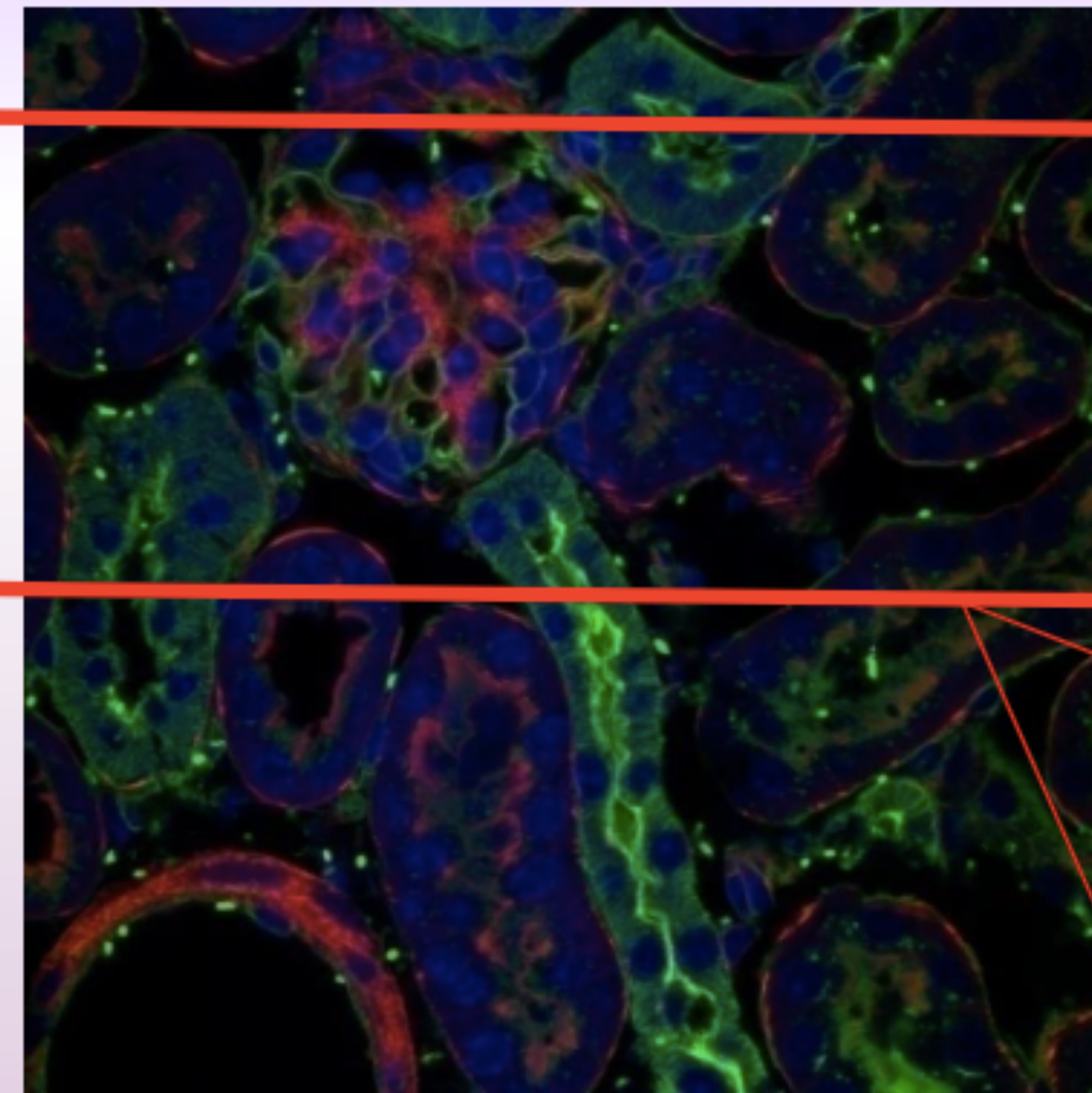


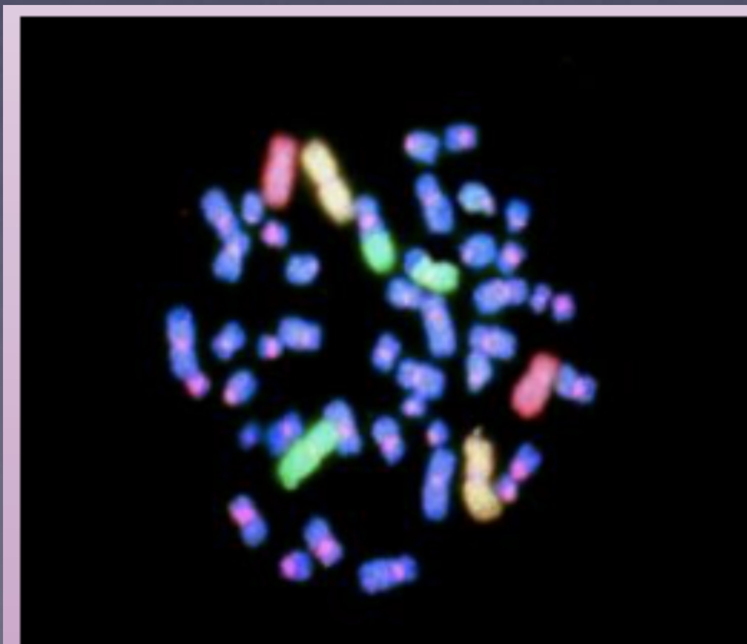
Nature Reviews Molecular Cell Biology **14**, 181-189 (March 2013)

Different quality radiation



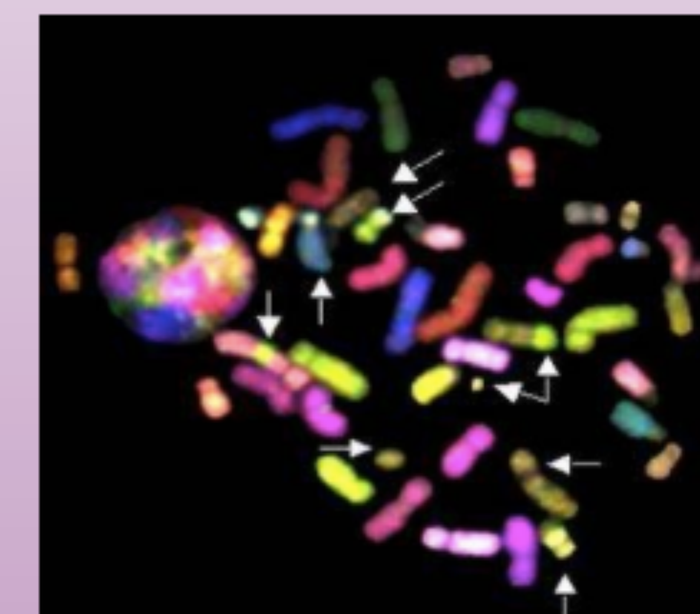




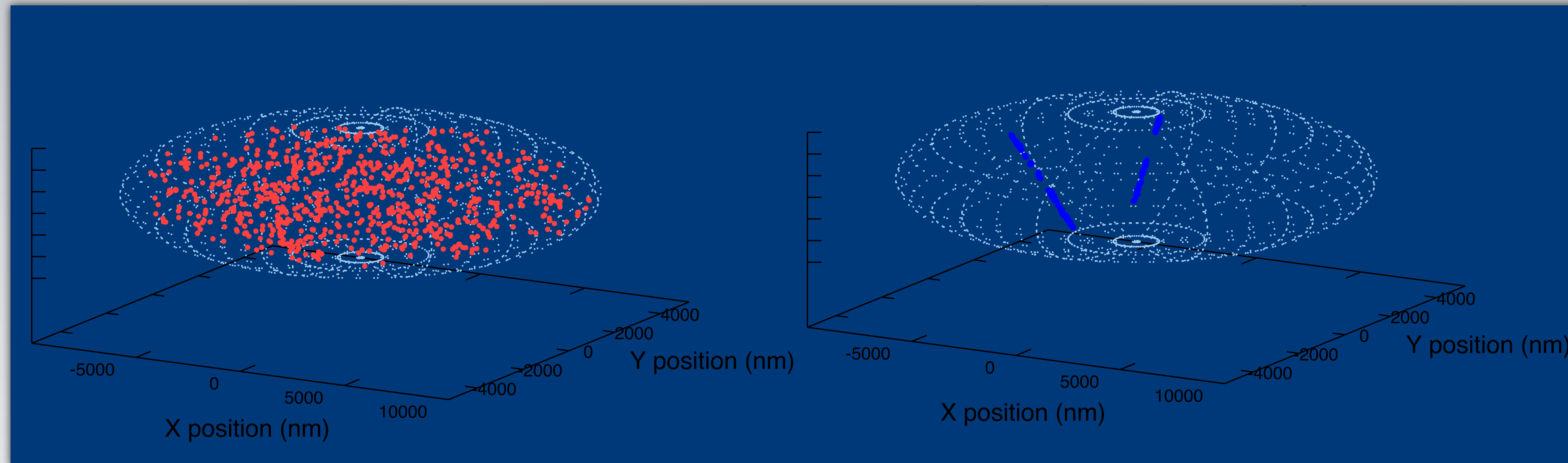

Courtesy of Paul Simpson

1 Gy corresponds to:

~1000 electron tracks	~2 alpha tracks
~40 DSB	~40 DSB
(*~20% complex*)	**(*~70% complex*)**


Courtesy of Rhona Anderson

Physical stage: mixed beams



photons (0.25 Gy) + alphas (0.25 Gy) = mixed beam (0.5 Gy)

Source	Dose (Gy)	DSB	SSB	SSB/DSB	Cluster damage
α particles	0.235	25.8	150.5	5.8	2.5
X-rays	0.253	13.5	241.3	17.9	0.2
Mixed beams	0.488	38.9	391.7	10.06	2.6