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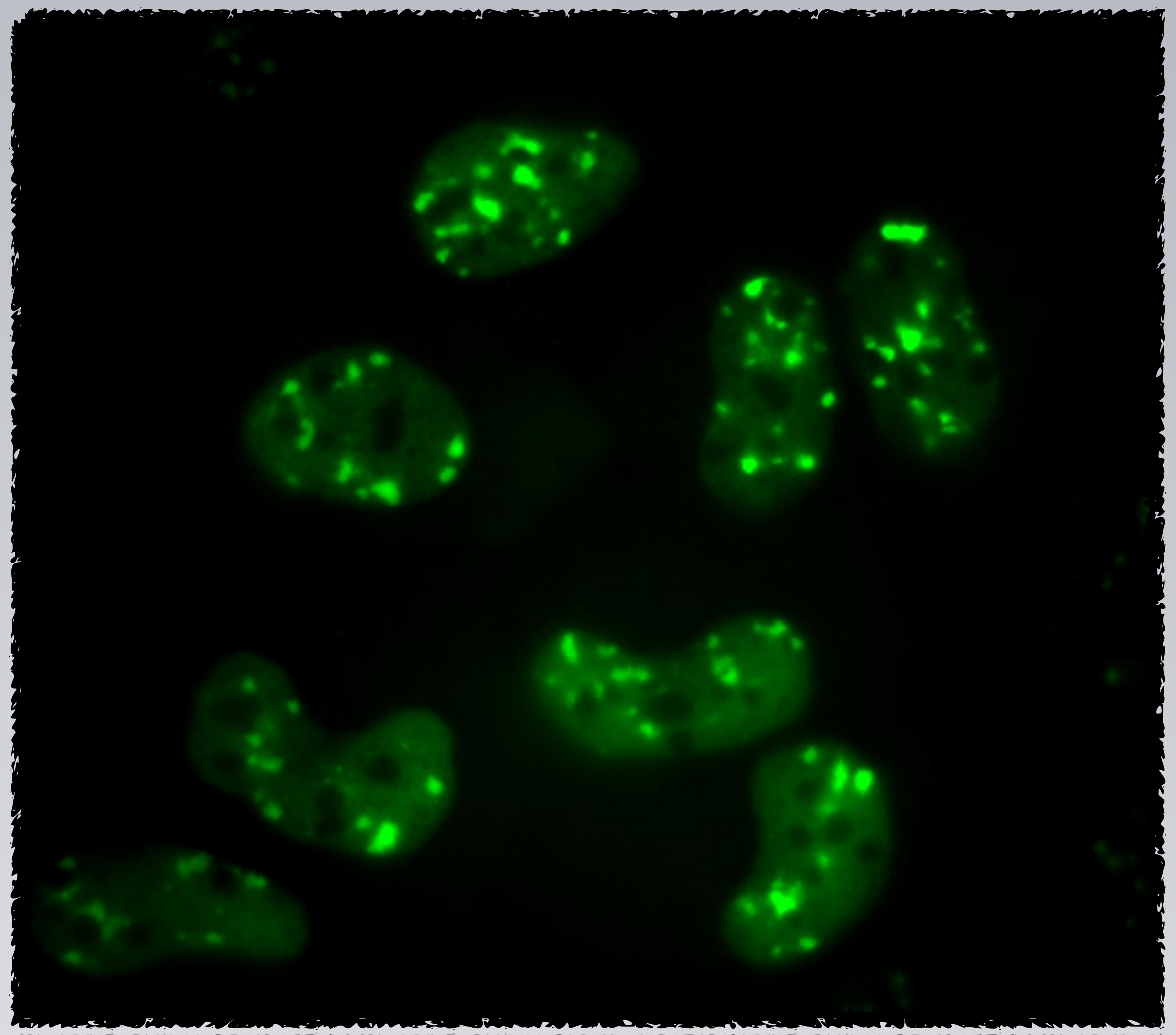

DNA damage and repair in cells exposed to radiation

Beata Brzozowska

Jerzy Pniewski and Leopold Infeld Colloquium, 4th of March, 2024 brain.fuw.edu.pl

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Neuroinformatics

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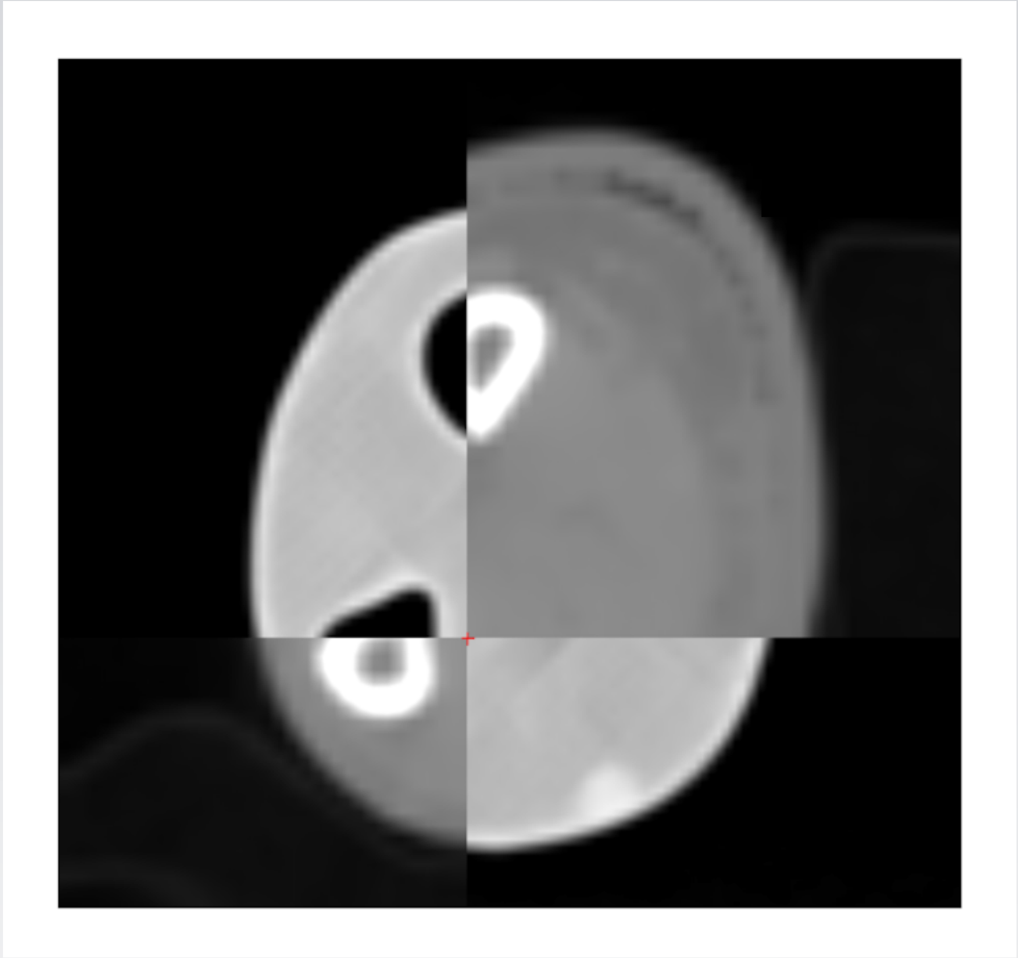
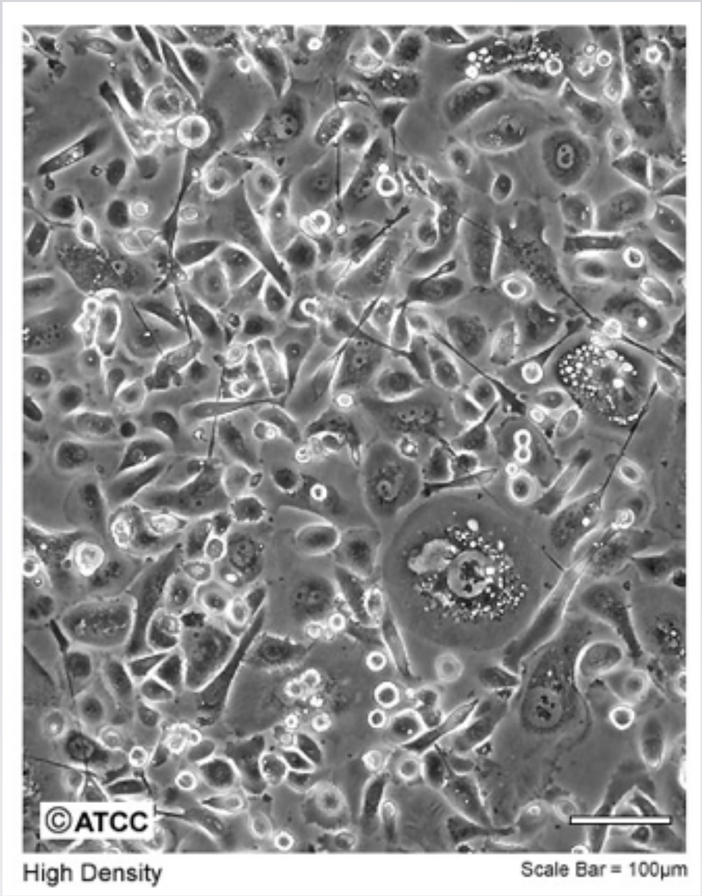
DNA damage and repair processes in cells exposed to ionising radiation

5-150 cm

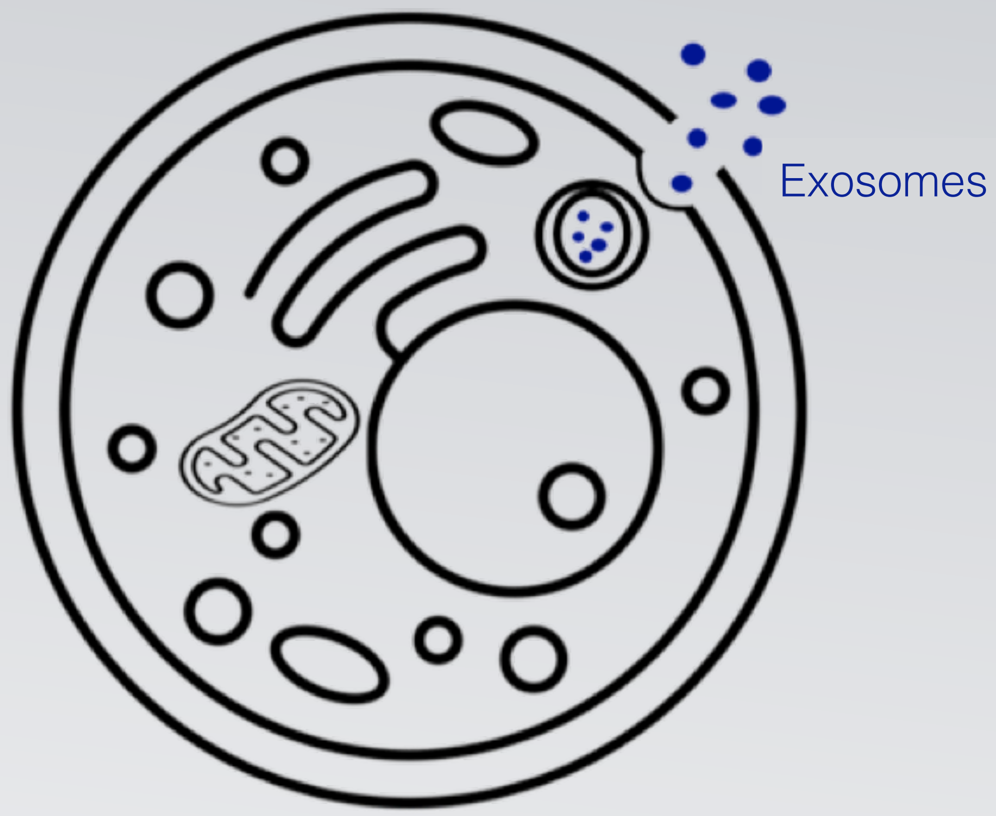


radiotherapy

5-50 µm

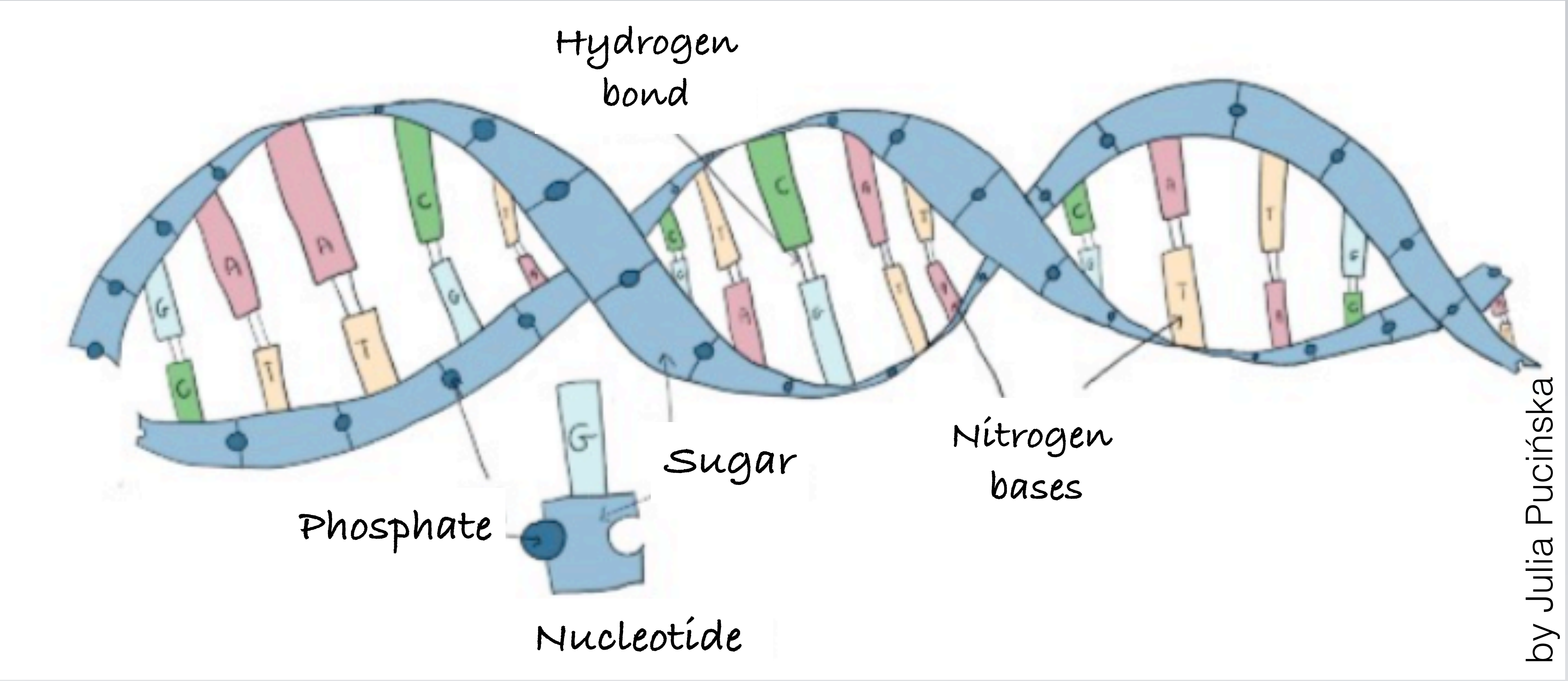


30-150 nm



radiobiology

a few nm

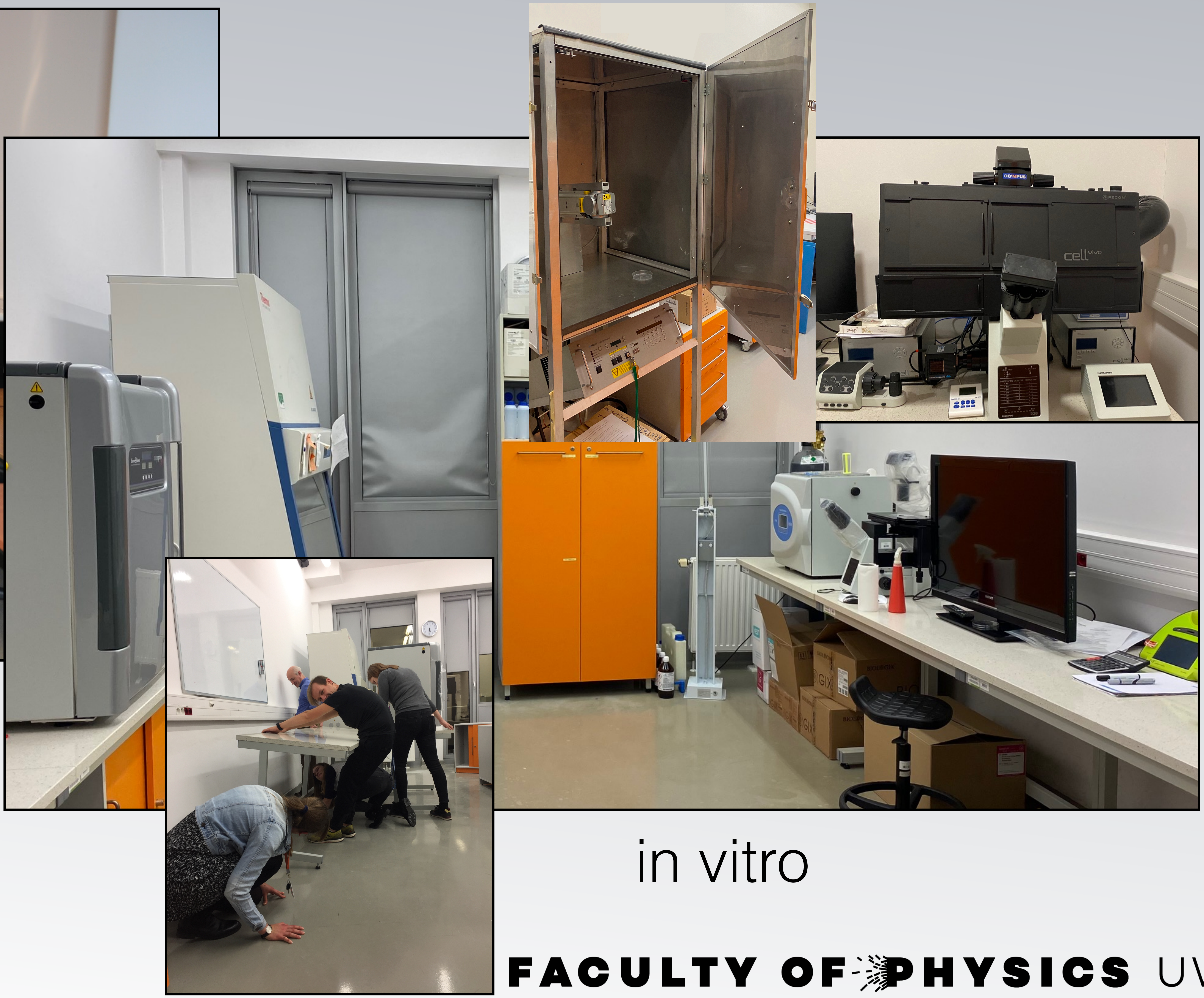


nanodosimetry

MC modeling

Laboratories

in silico



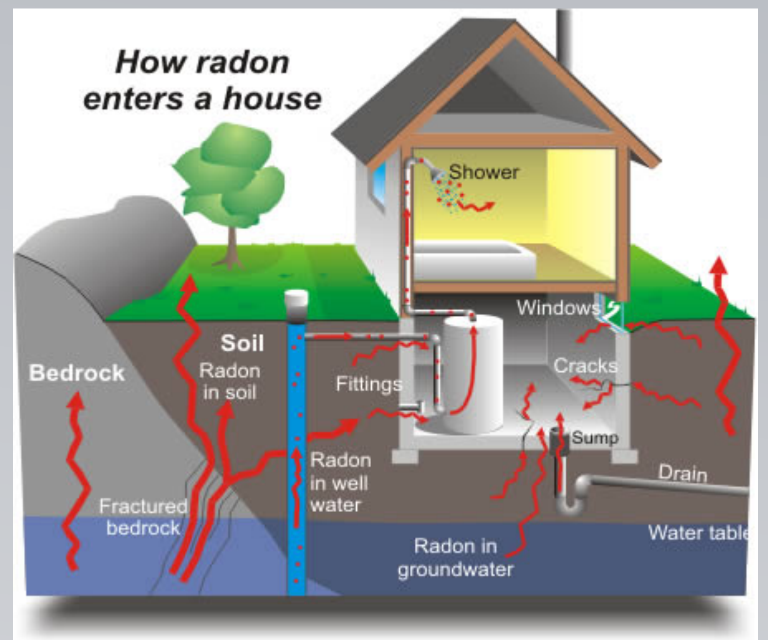
in vitro

Outline

- Motivation: why mixed beams?
- DSB, SSB, 53BP1, GFP
- Focus analysis:
  - A. Damage induction
  - B. Repair kinetics
- Ongoing projects

Who is exposed to mixed beams of radiation?

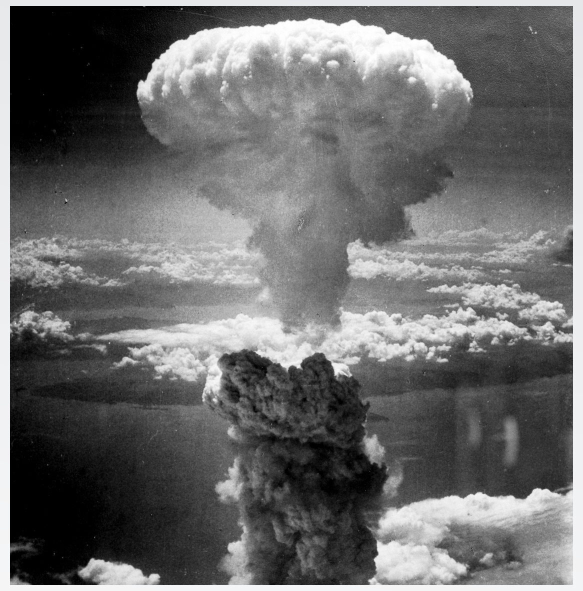
- People living in areas of high natural background radiation ($\alpha + \gamma$)



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

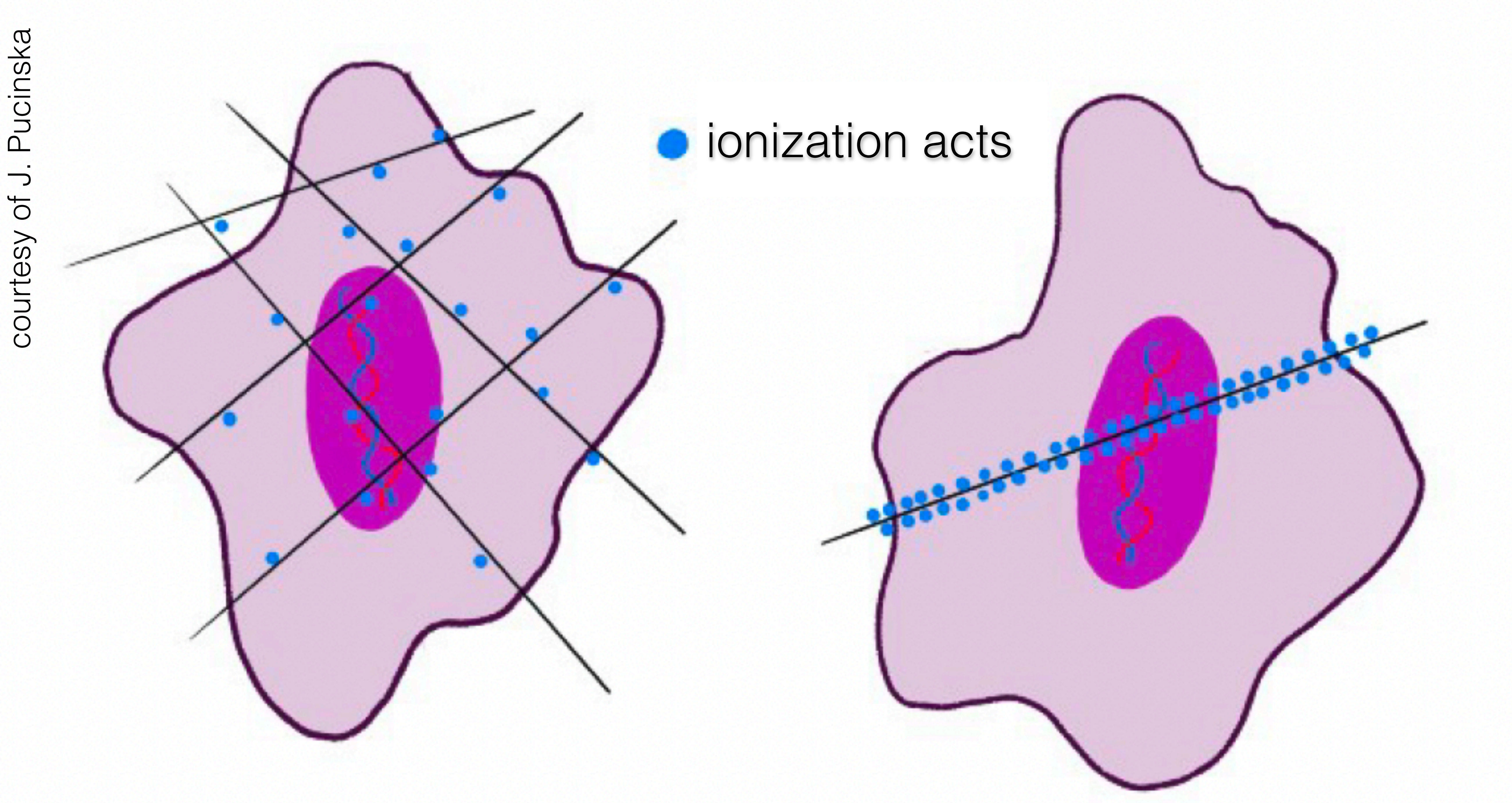
- Cancer patients treated with IMRT and proton therapy ($n + \gamma$)

- People involved in radiation accidents



Biological effectiveness

Absorbed dose: $D = dE/dm$

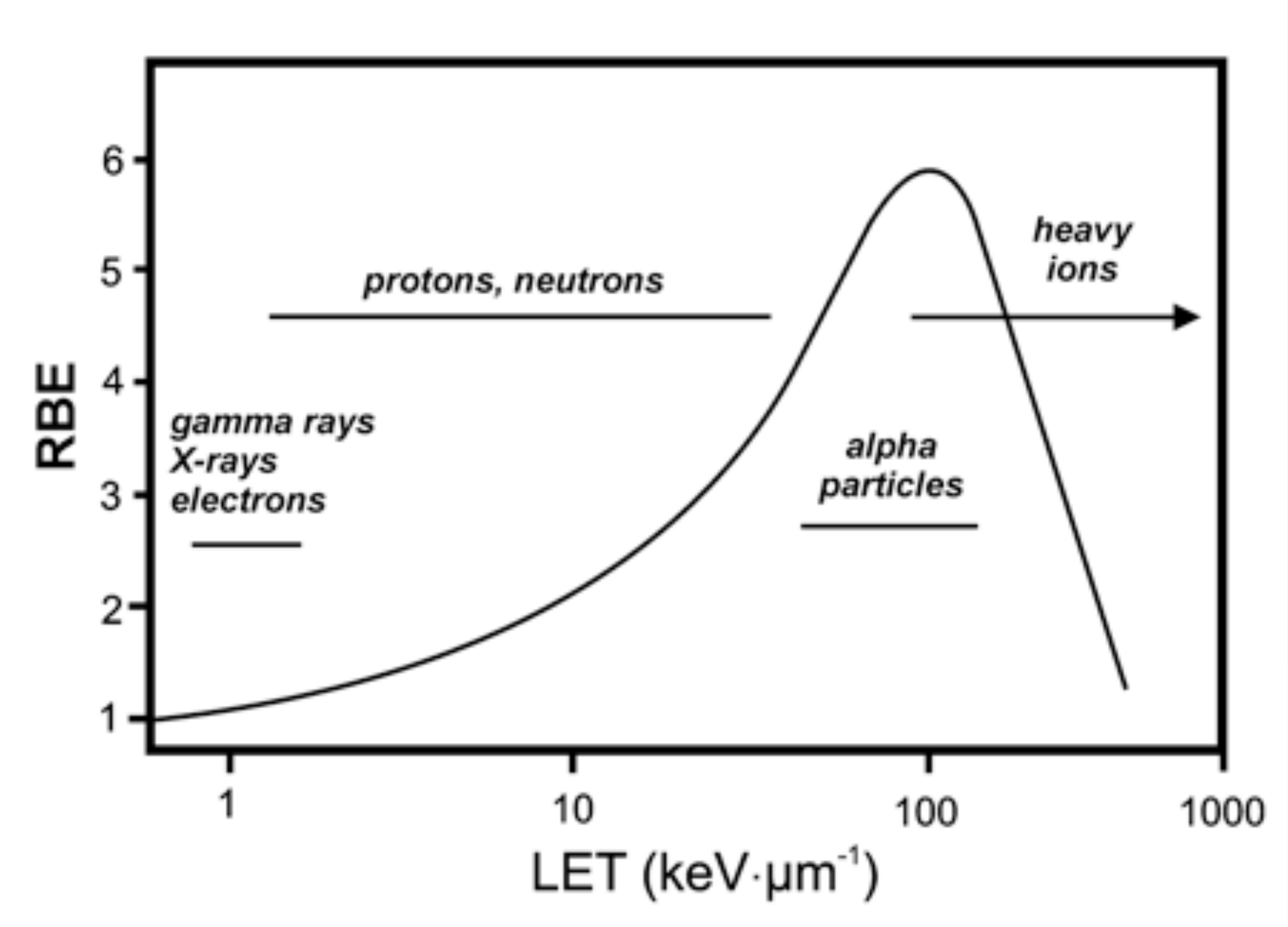


photons (low LET)
2 keV/µm

alpha particles (high LET)
150 keV/µm

LET (Linear Energy Transfer) = dE/dx

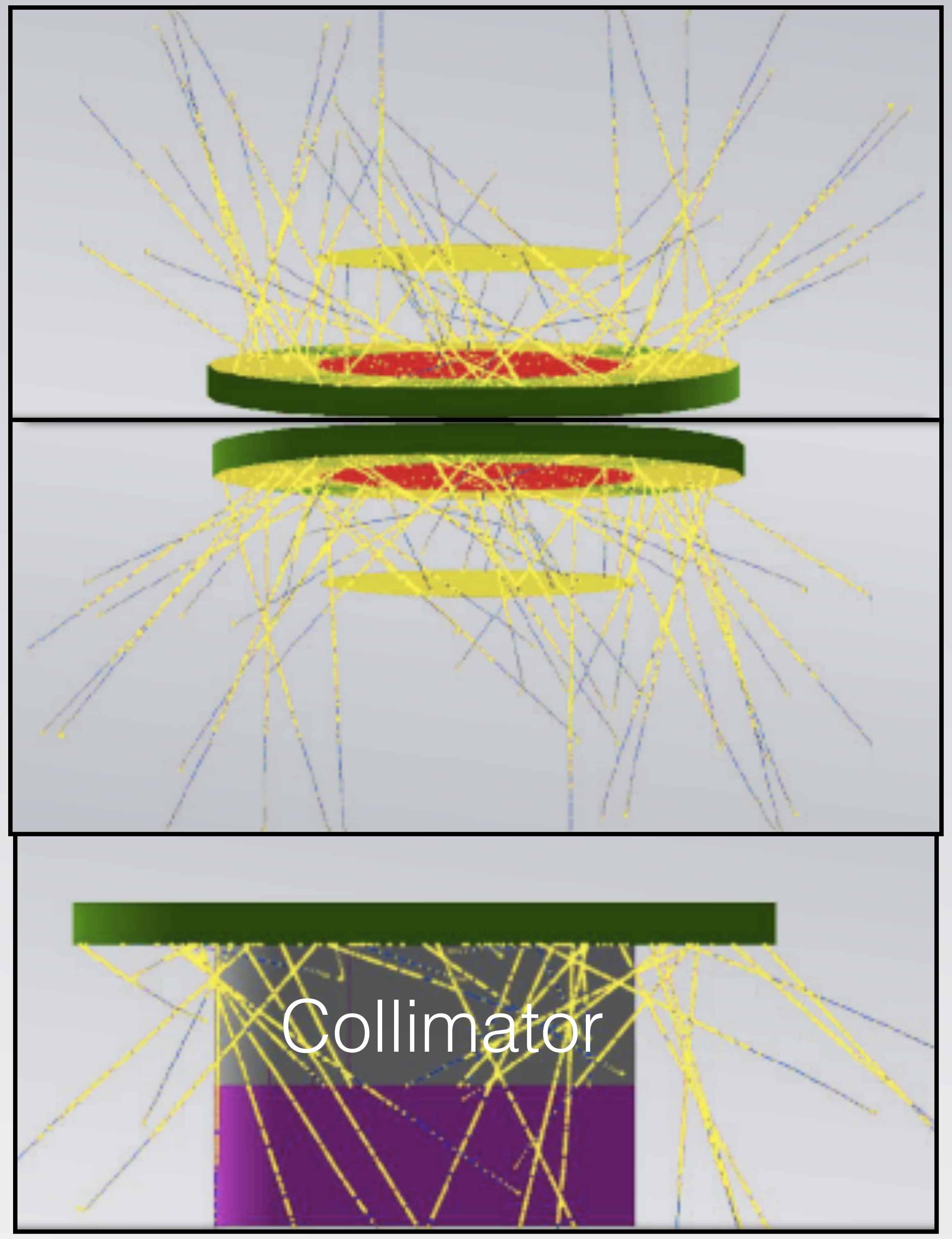
RBE - Relative Biological Effectiveness

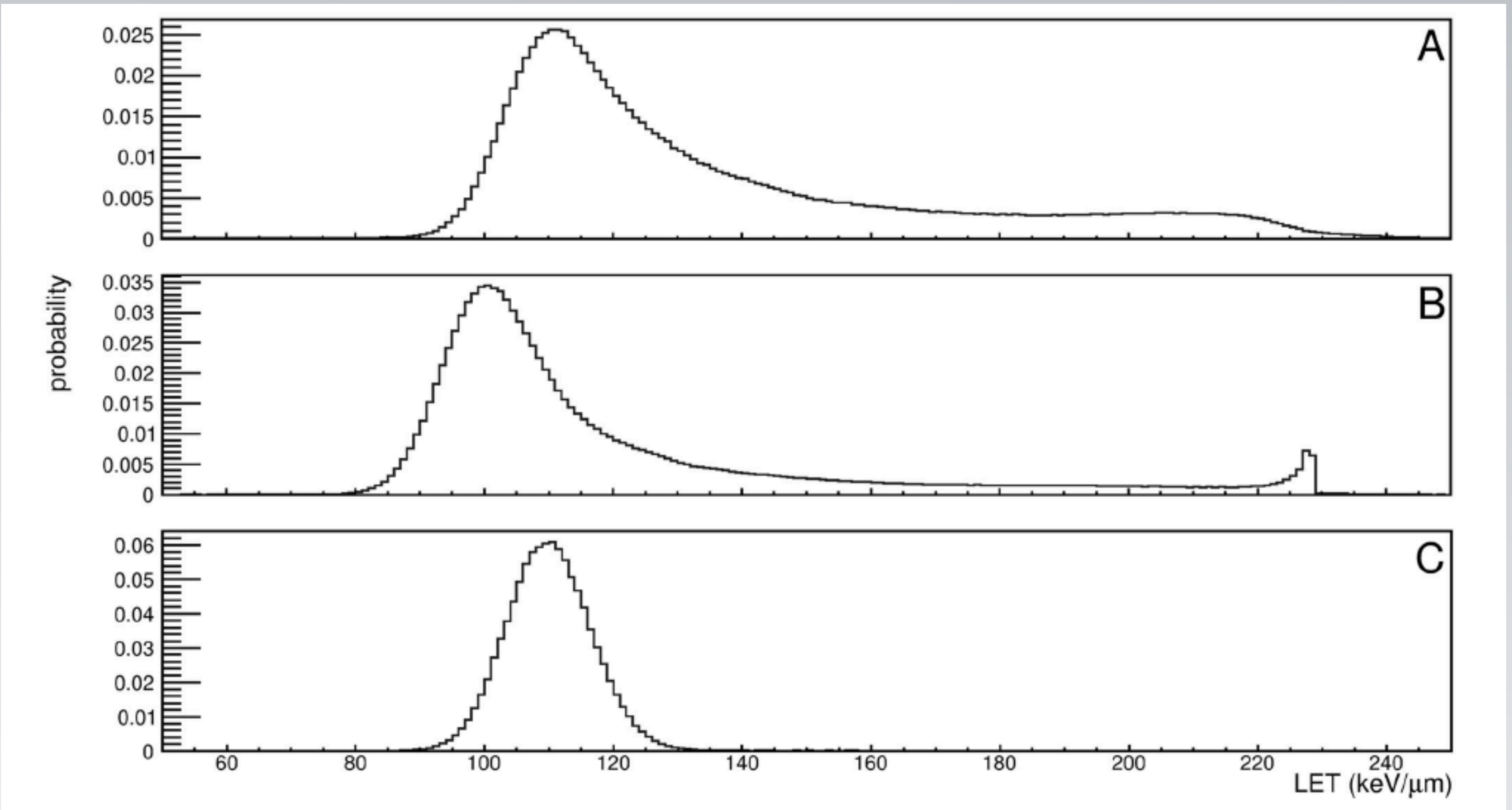


$RBE_{50\%SF} = D_{ref}/D_{test}$

LET distributions

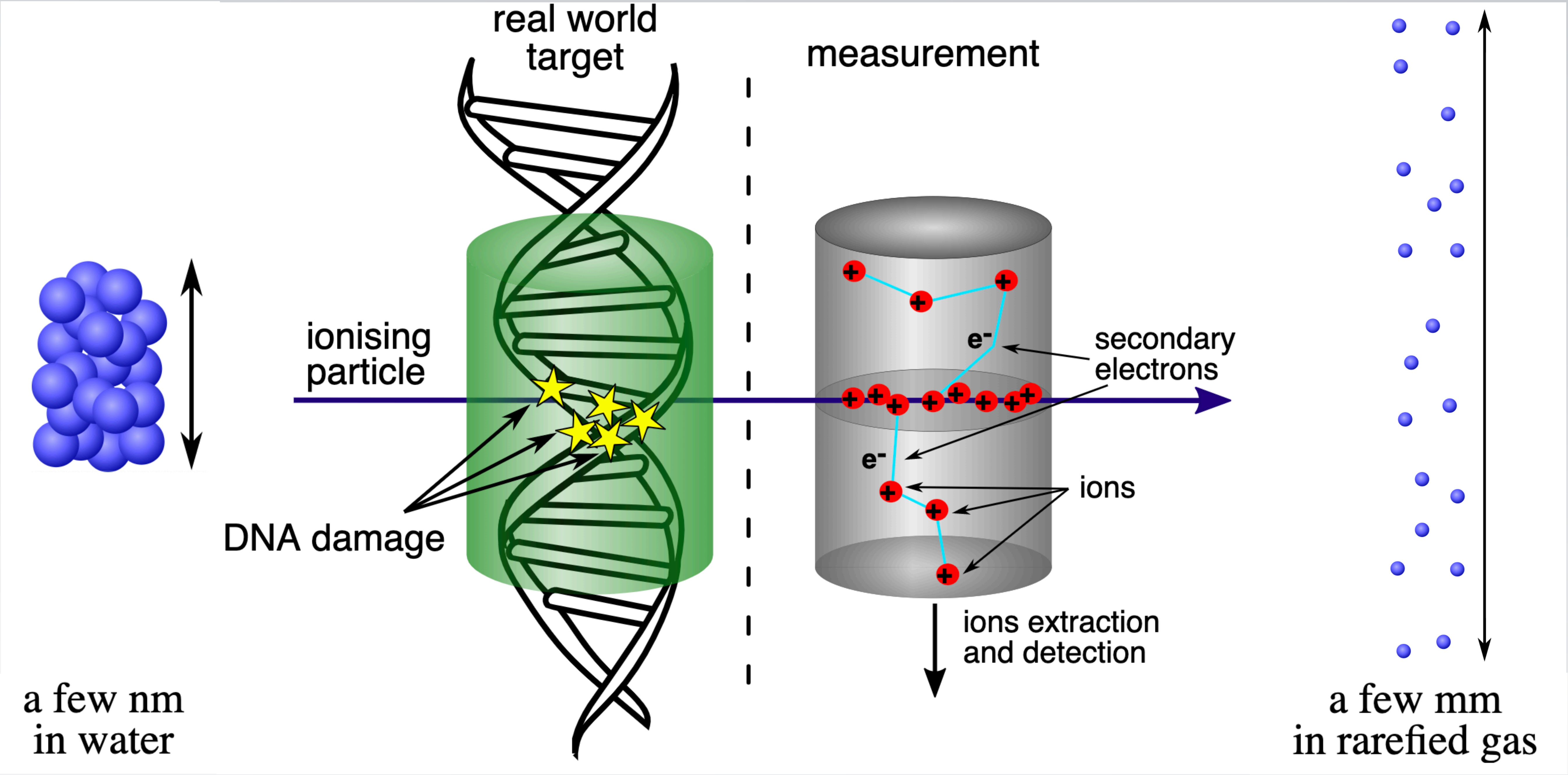
^{241}Am

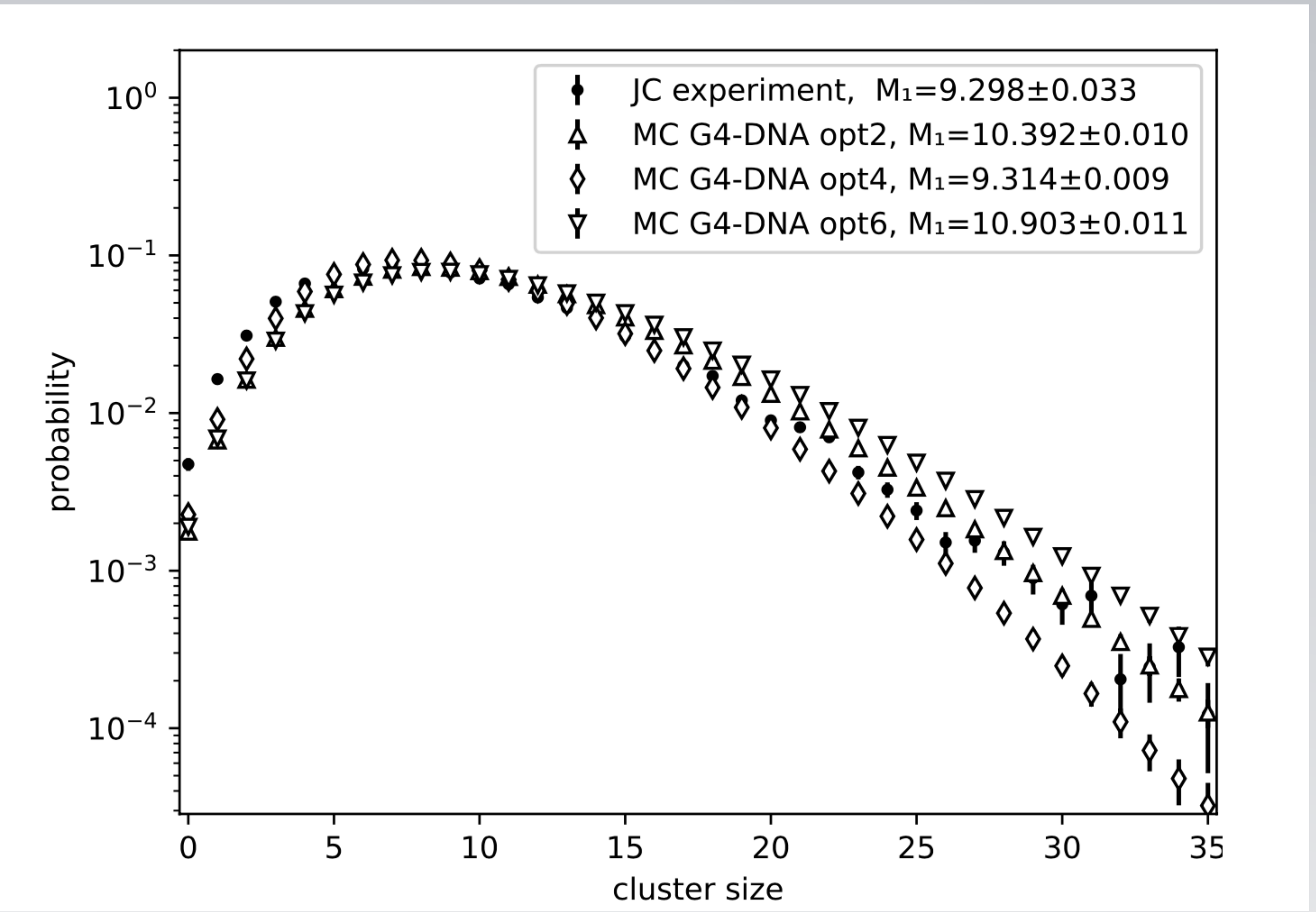




Tartas A., Filipek M., Pietrzak M., Wojcik A. i Brzozowska B. (2022). Modeling of dose and linear energy transfer homogeneity in cell nuclei exposed to alpha particles under various setup conditions, International Journal of Radiation Biology (https://doi.org/10.1080/09553002.2023.2161659)

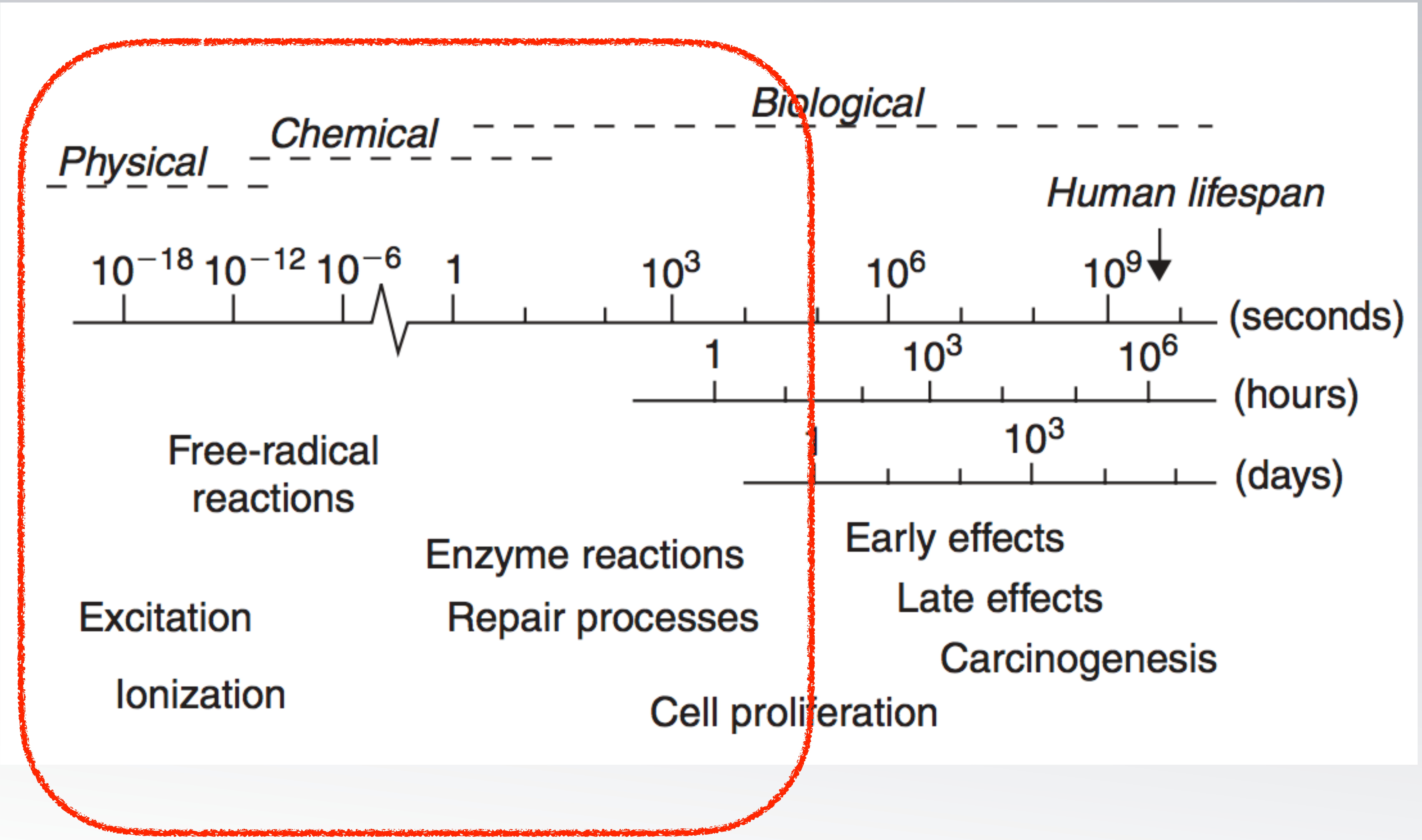
Nanodosimetry: measurements of ionisations





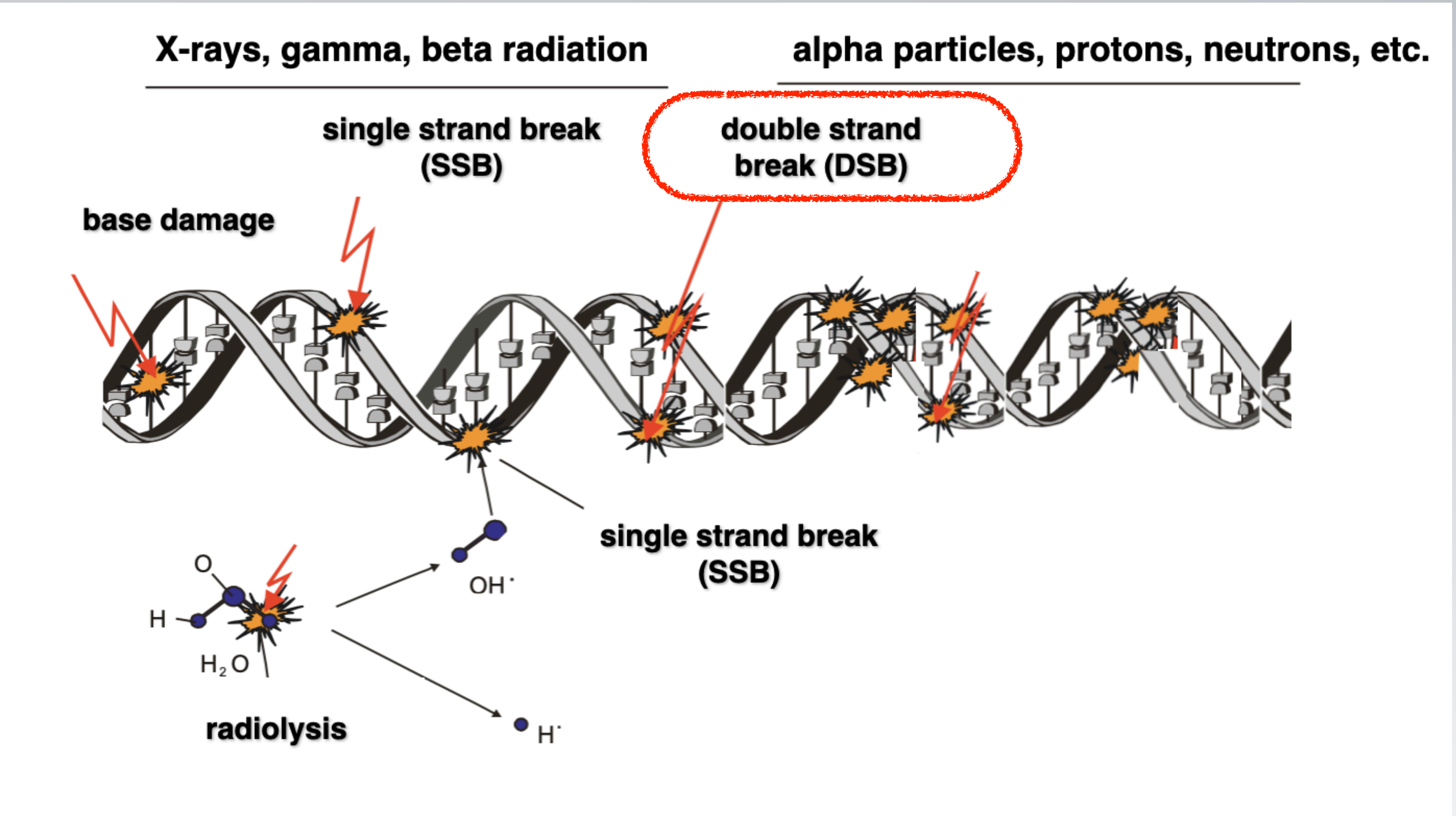
M. Pietrzak, M. Mietelska, A. Bancer, A. Rucinski, B. Brzozowska, Geant4-DNA modeling of nanodosimetric quantities in the jet counter for alpha particles, PMB 2021

Radiation exposure on biological systems



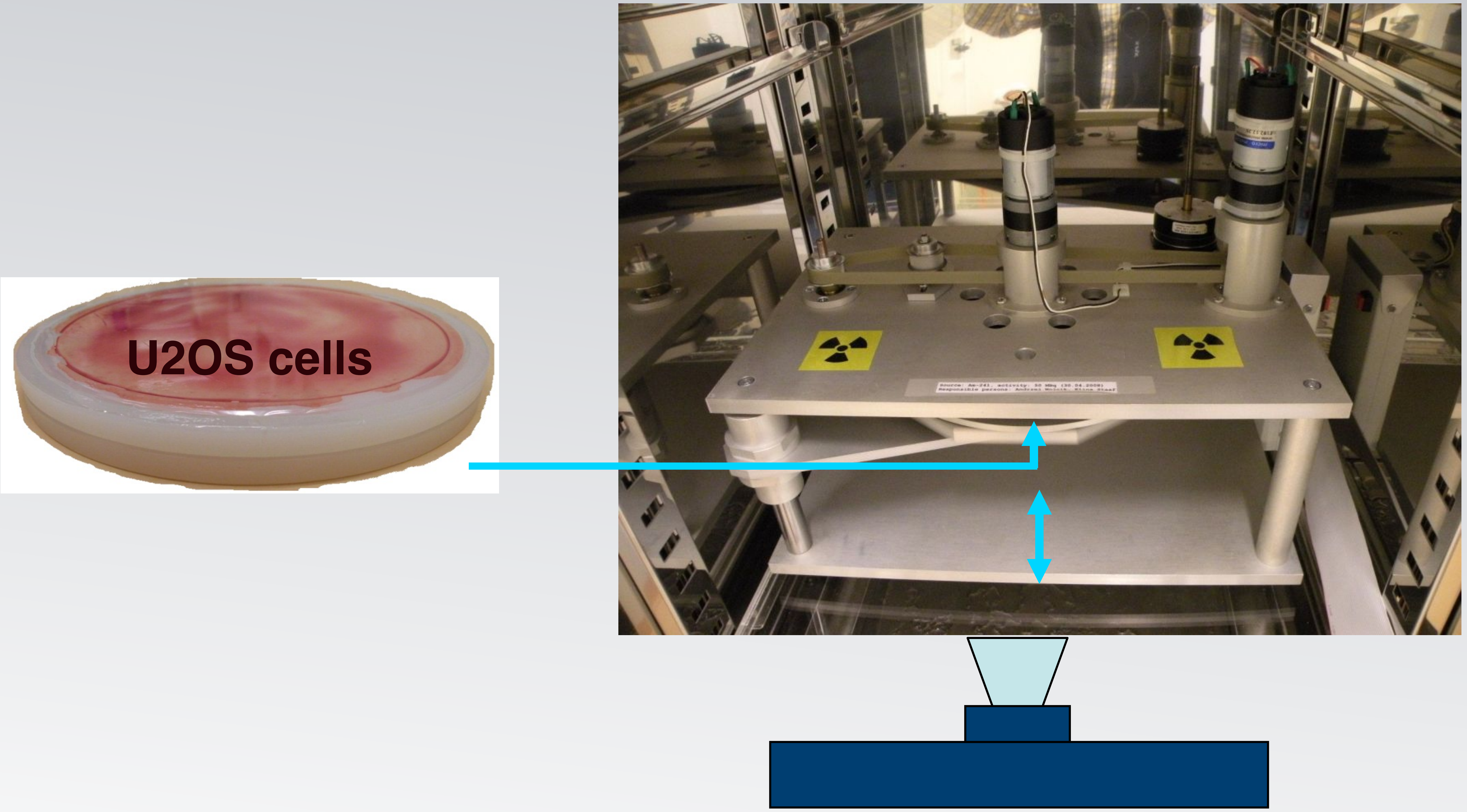
DNA damage

low LET high LET



Mixed beam exposure facility at Stockholm University

^{241}Am (0.21 Gy/min)

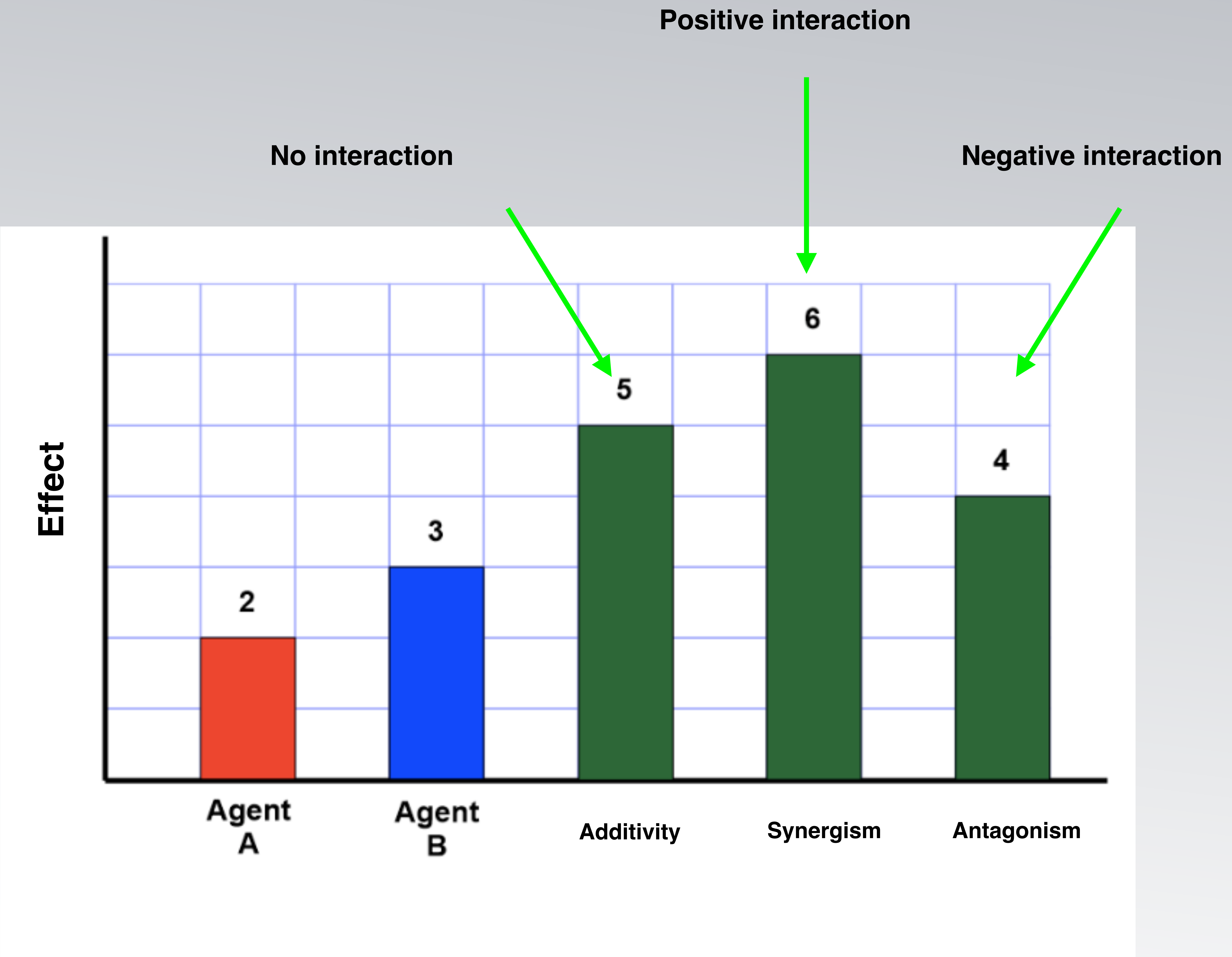


190 kV X-ray tube

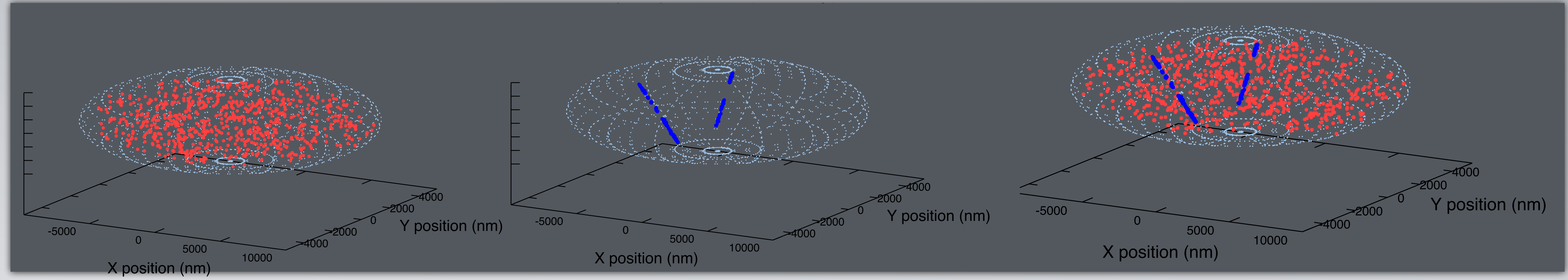
0.05 Gy/min

0.07 Gy/min

Synergism or additivity?



Mixed beam irradiation



photons (0.25 Gy) + alpha particles (0.25 Gy) = mixed beams (0.5 Gy)

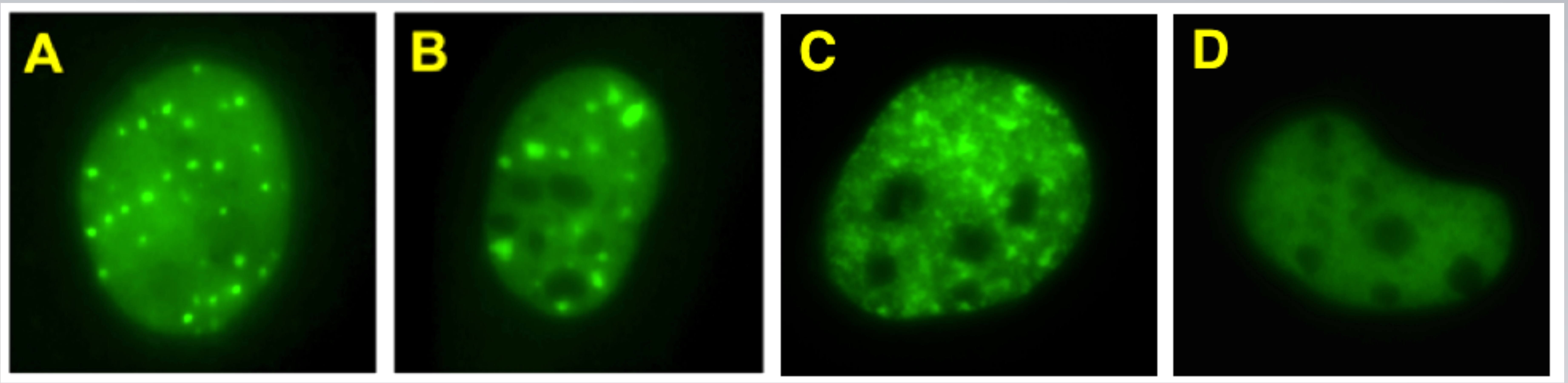
Source	Dose (Gy)	DSB	SSB	SSB/DSB
α particles	0.235	25.8	150.5	5.8
X-rays	0.253	13.5	241.3	17.9
Mixed beams	0.488	38.9	391.7	10.06

MC simulations with PARTRAC

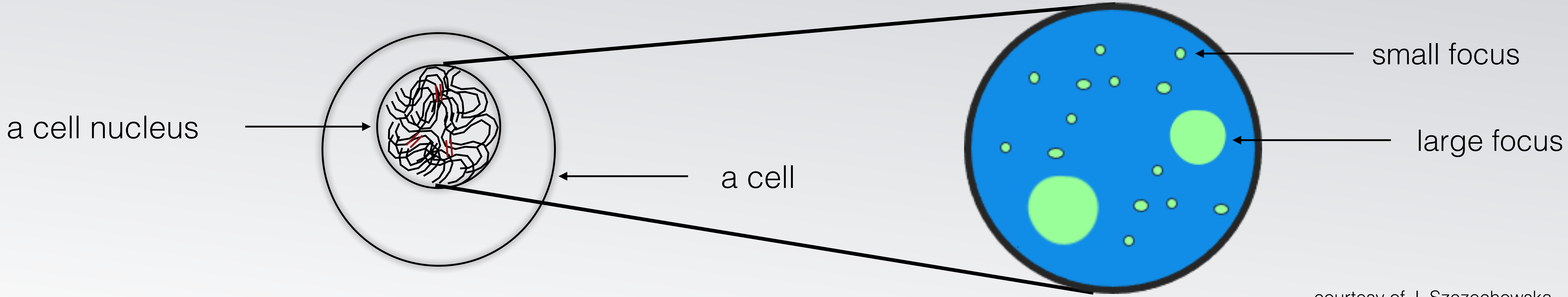
Brzozowska B., Tartas A. i Wojcik A. (2020). Monte Carlo modeling of DNA lesions and chromosomal aberrations induced by mixed beams of alpha particles and X-rays, Frontiers in Physics, Medical Physics and Imaging 8: 567864

How to visualize DSB?

Radiation-induced foci 53BP1



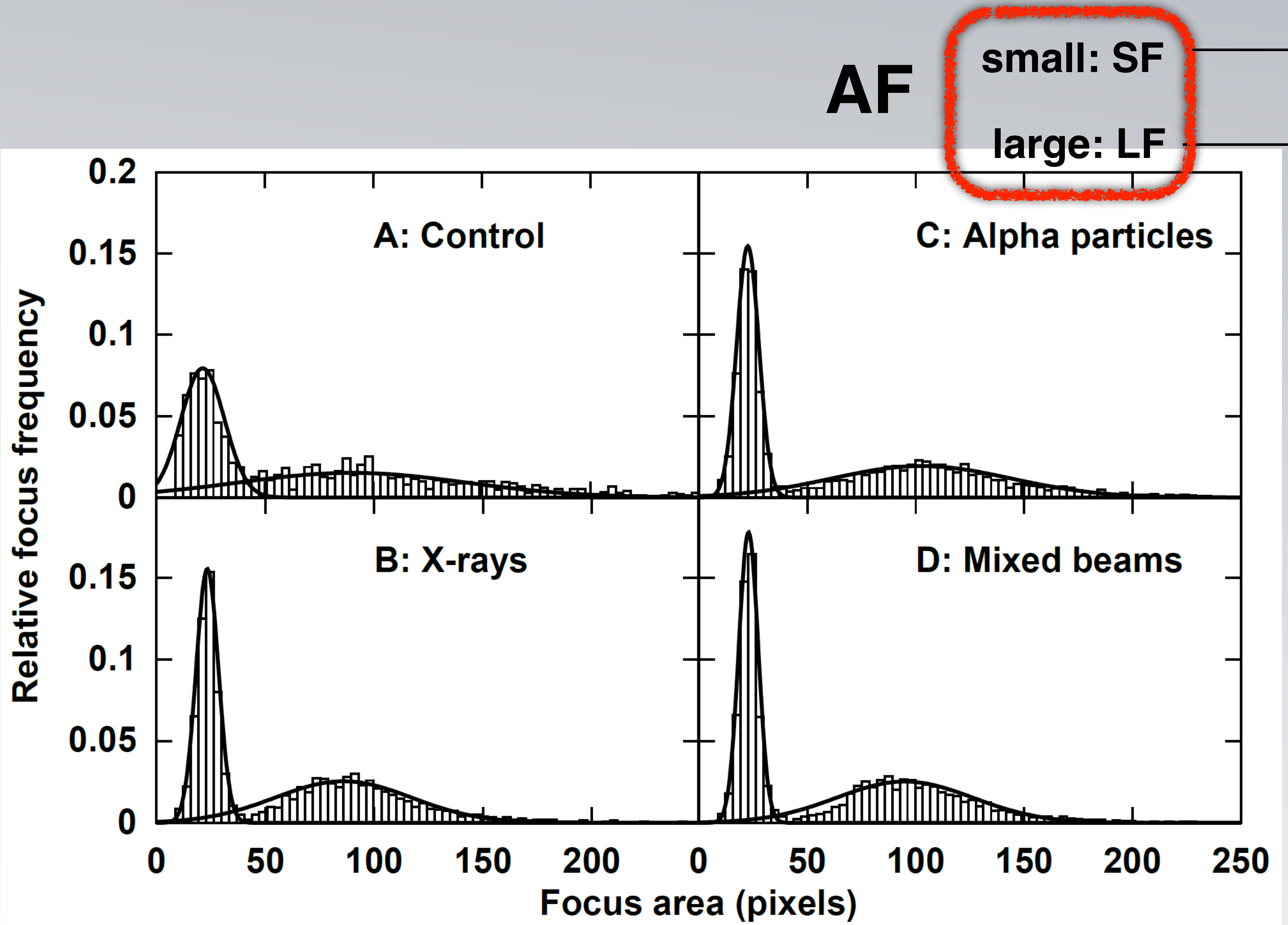
X-rays alpha particles mixed beams control cells (not irradiated)

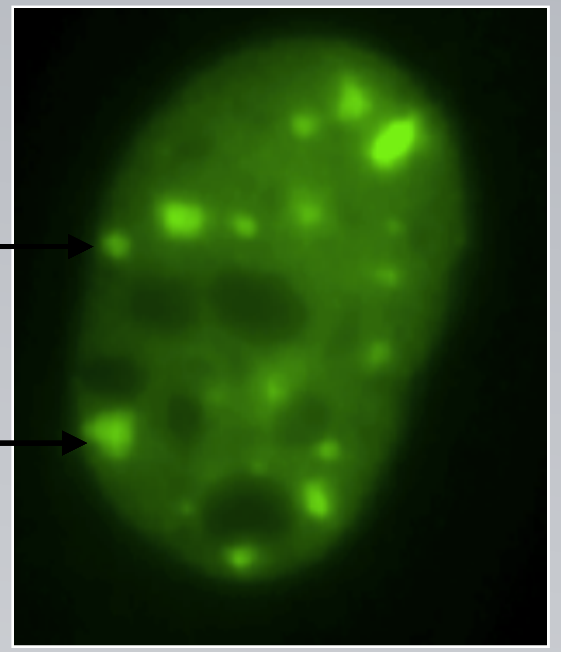


courtesy of J. Szczechowska

Sollazzo A., Brzozowska B., Cheng L., Lundholm L., Haghdoost S., Scherthan H. i Wojcik A. (2017). Alpha Particles and X Rays Interact in Inducing DNA Damage in U2OS Cells, Radiation Research 188(4): 400-411

Focus size





SF - likely containing simple DSB

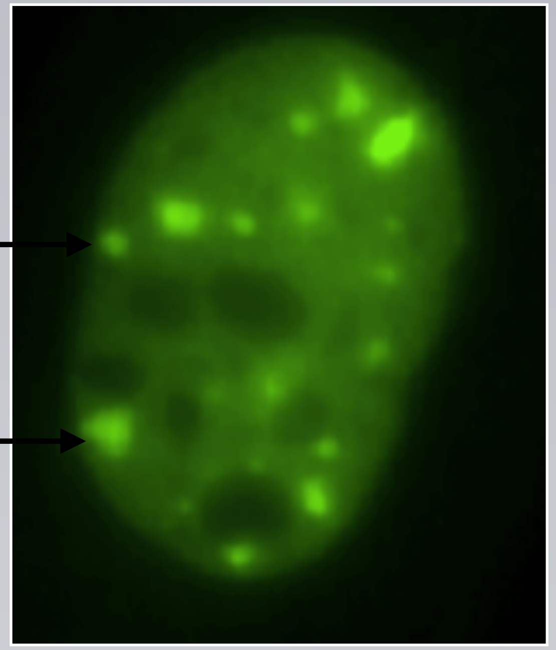
LF - likely containing staggered complex DSB

Focus analysis

AF

small: SF

large: LF



STEP 1: cell response on radiation - focus frequency as a function of dose measured 0.5 h after exposure

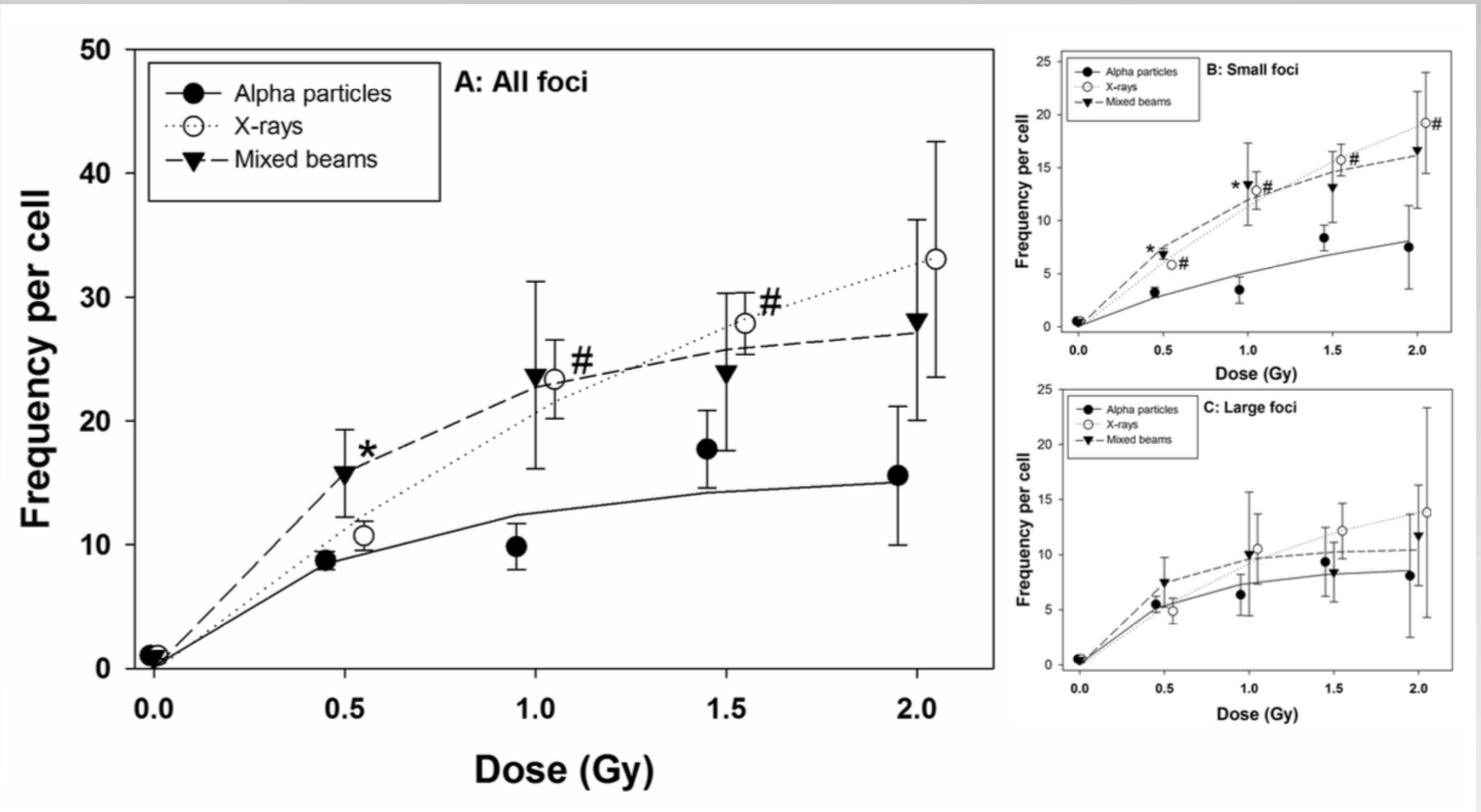
STEP 2: repair kinetics - focus frequency as a function of time for the dose equal 1 Gy

Dose response

STEP 1: cell response on radiation - focus frequency as a function of dose measured 0.5 h after exposure

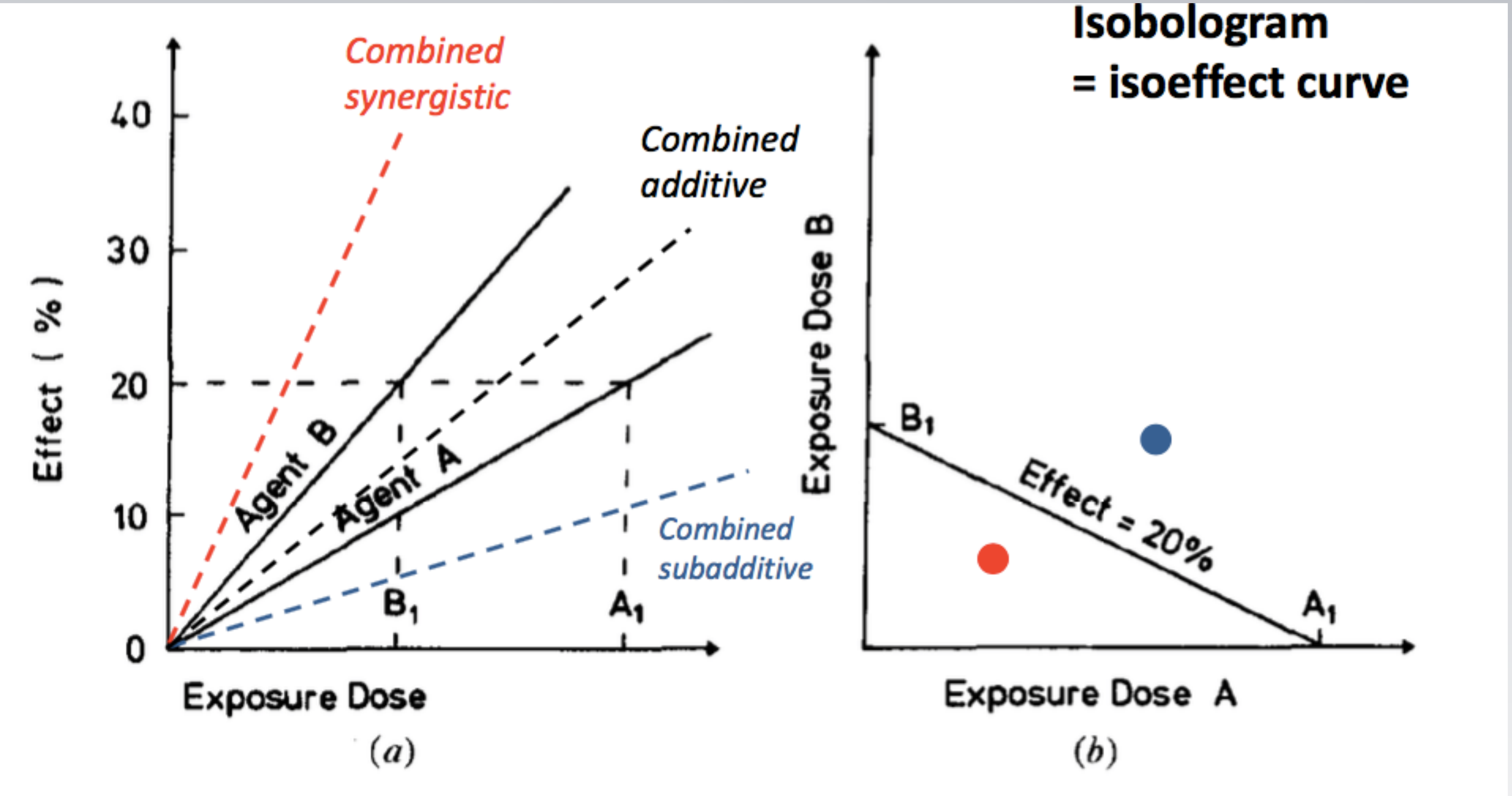
t = 0.5 h because the highest focus frequencies

Dose response in U2OS cells



saturation: focus confluency
alpha particles: lowest frequencies of foci (AF, SF)

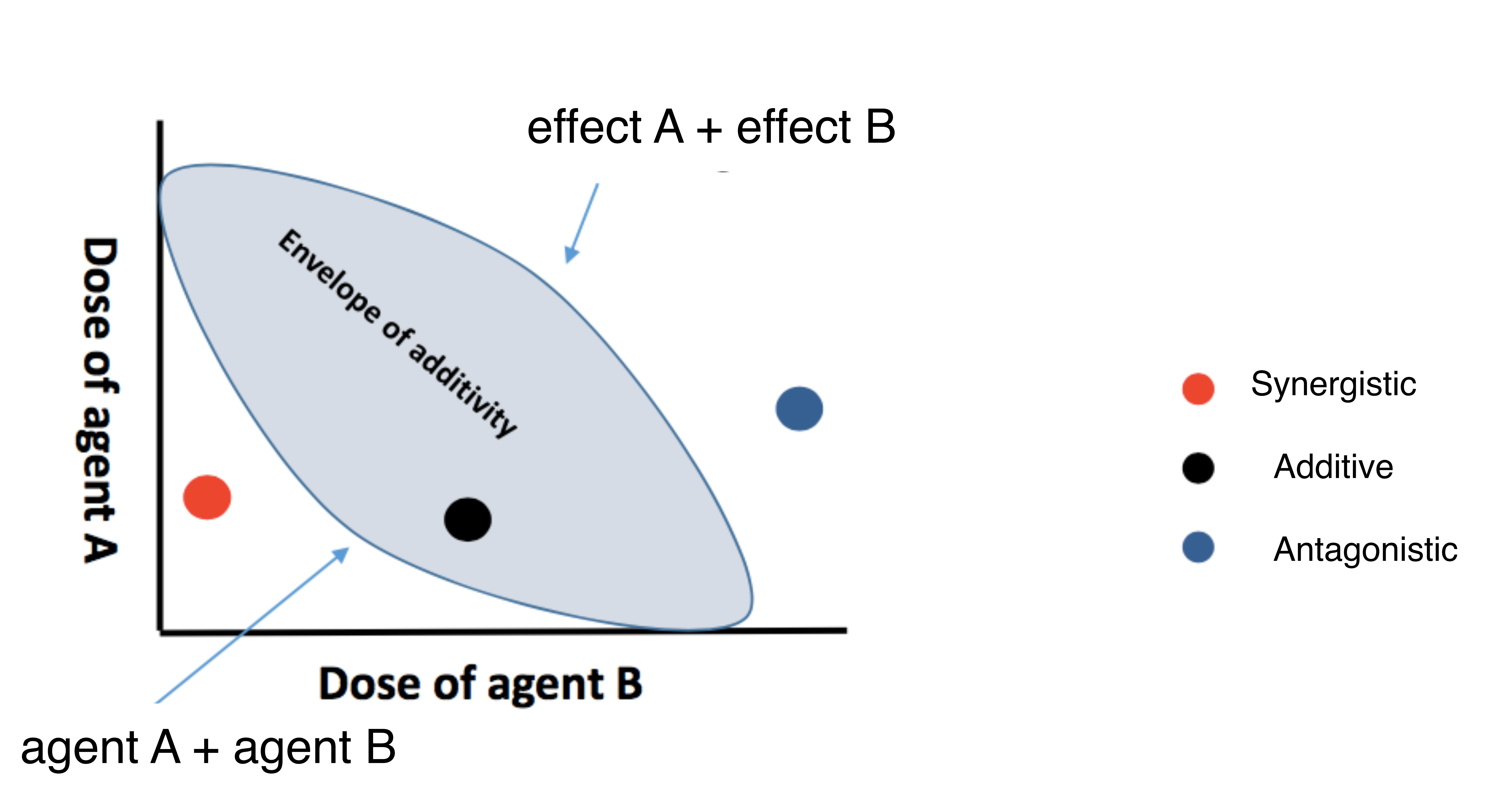
If the relationship is linear



C. Streffer and W-U. Müller. Dose-effect relationships and general mechanisms of combined exposures. IJRB 51:961-969, 1987.

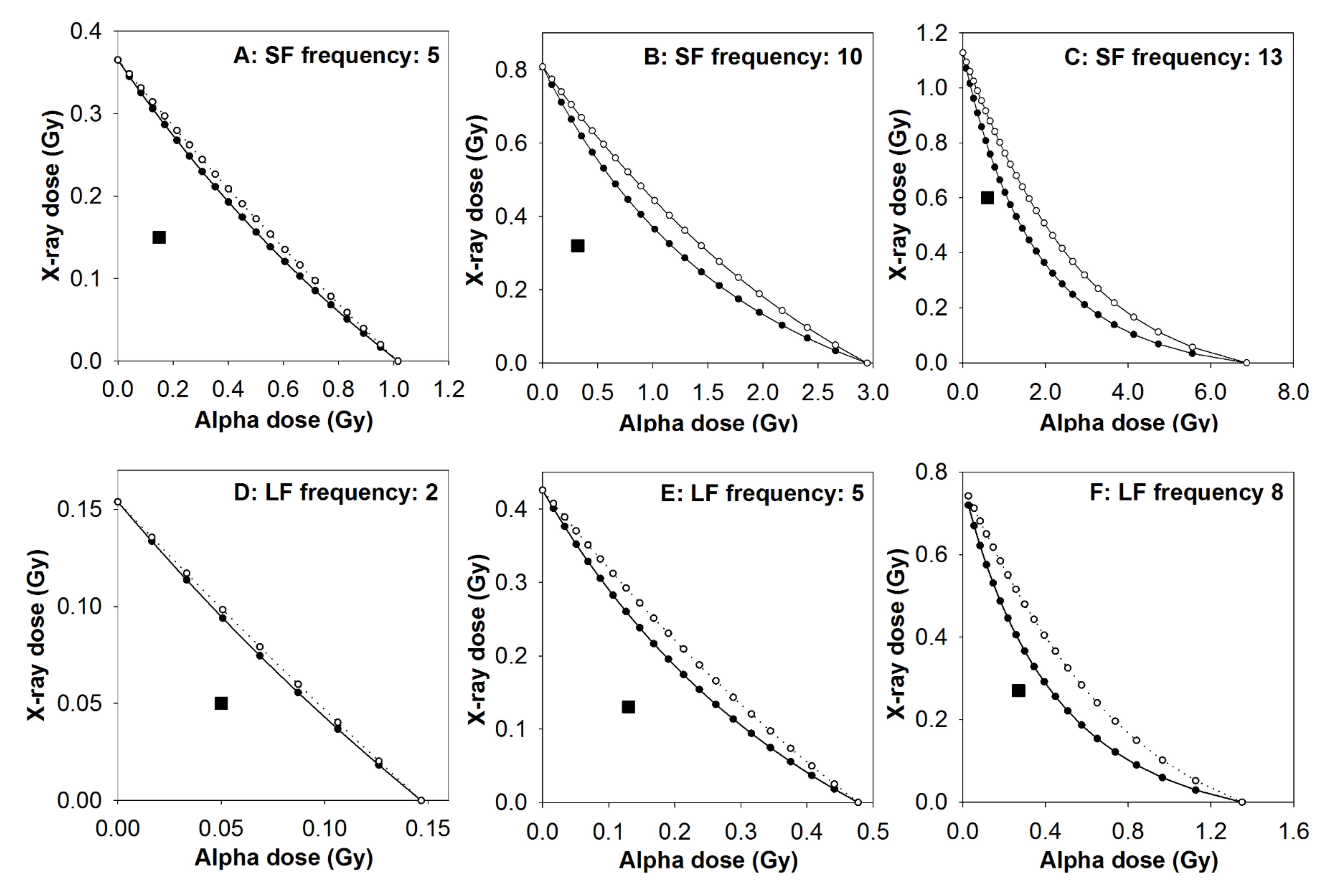
STEP 1: cell response on radiation

If the dose response is **not** linear



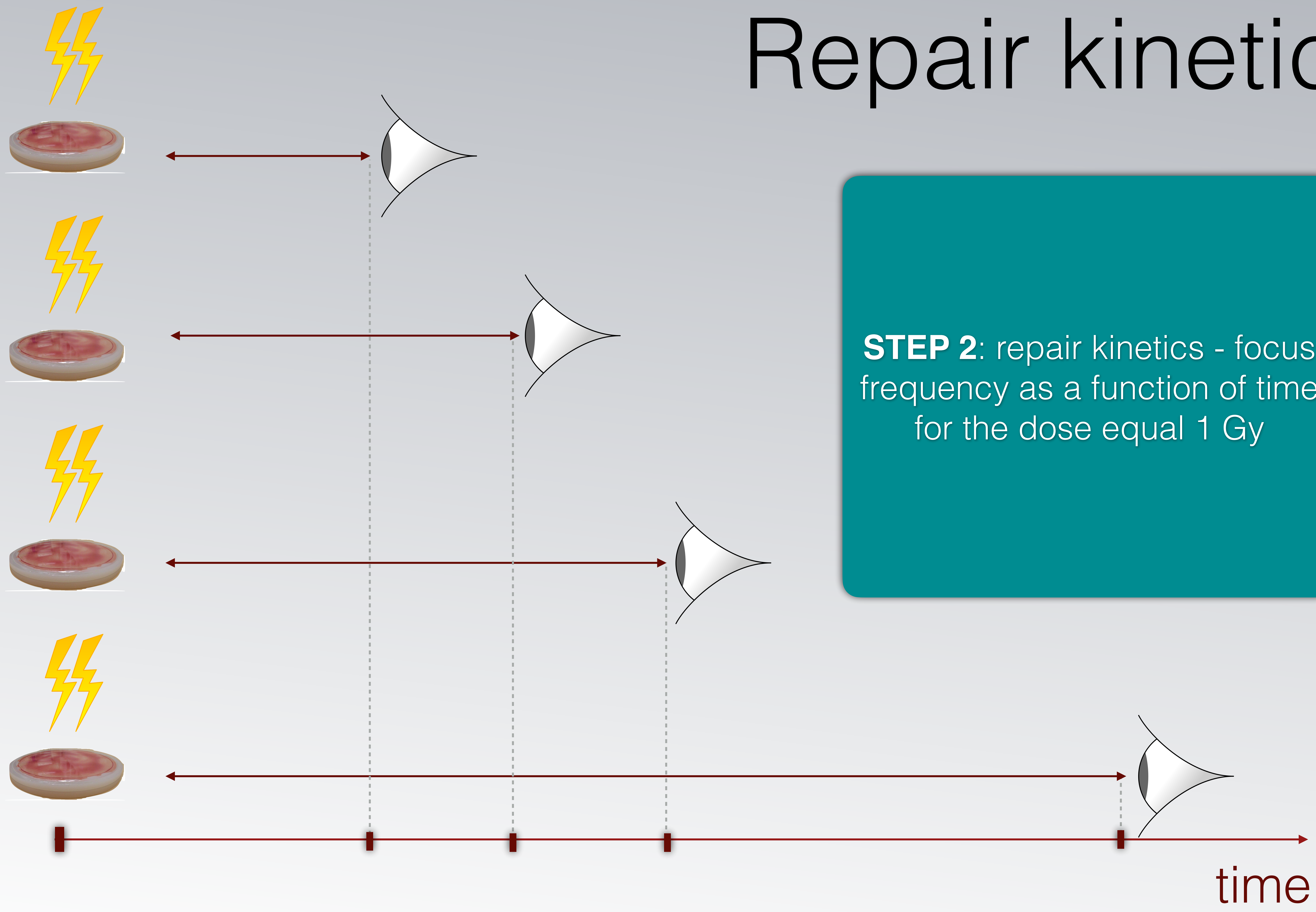
G.G. Steel and M.J. Peckham Int J Radiat Oncol Biol Phys 5:85-91, 1979

Envelopes of additivity for SF and LF



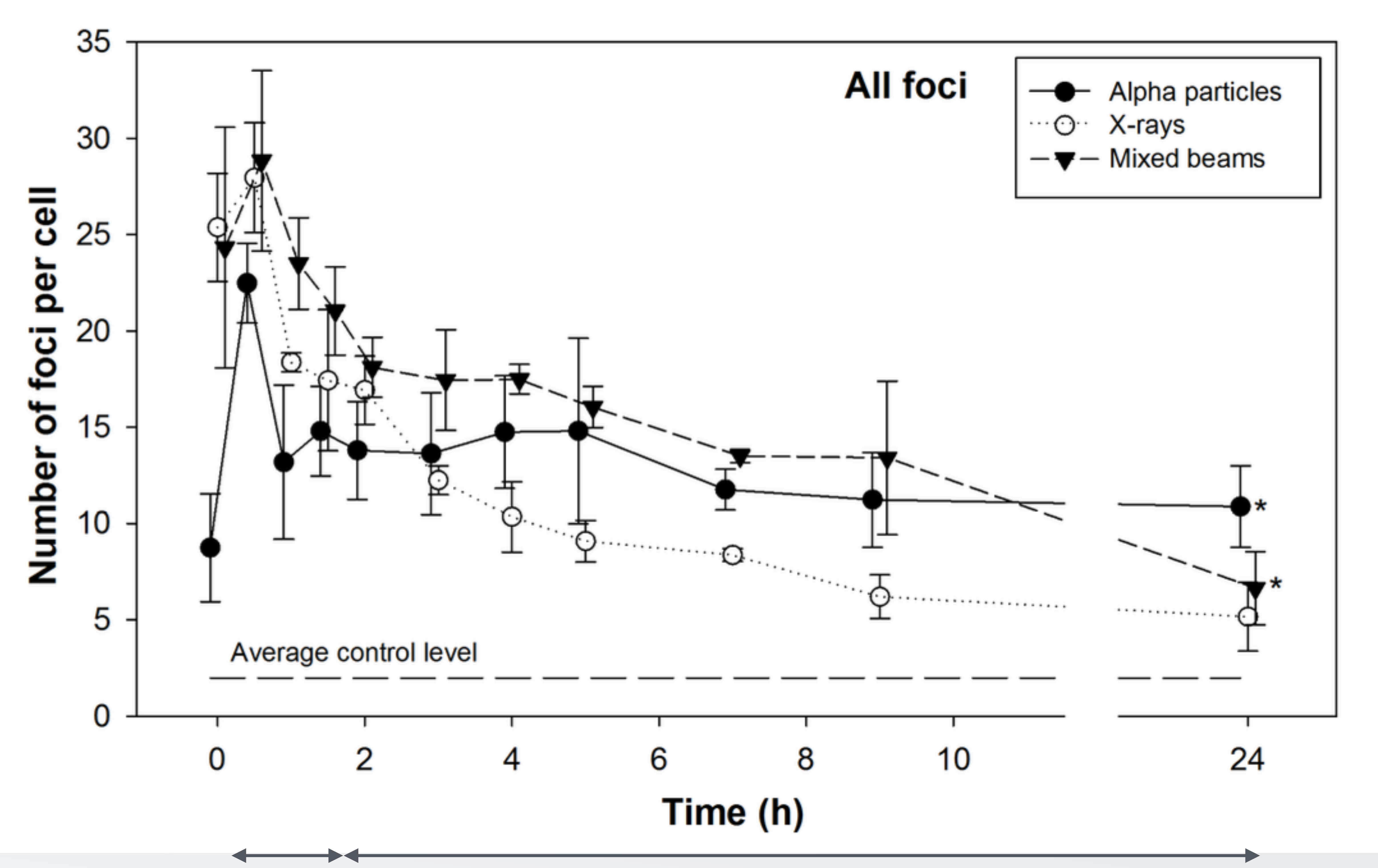
level of interaction is inversely related to the level of damage

Repair kinetics



STEP 2: repair kinetics - focus frequency as a function of time for the dose equal 1 Gy

Focus decay



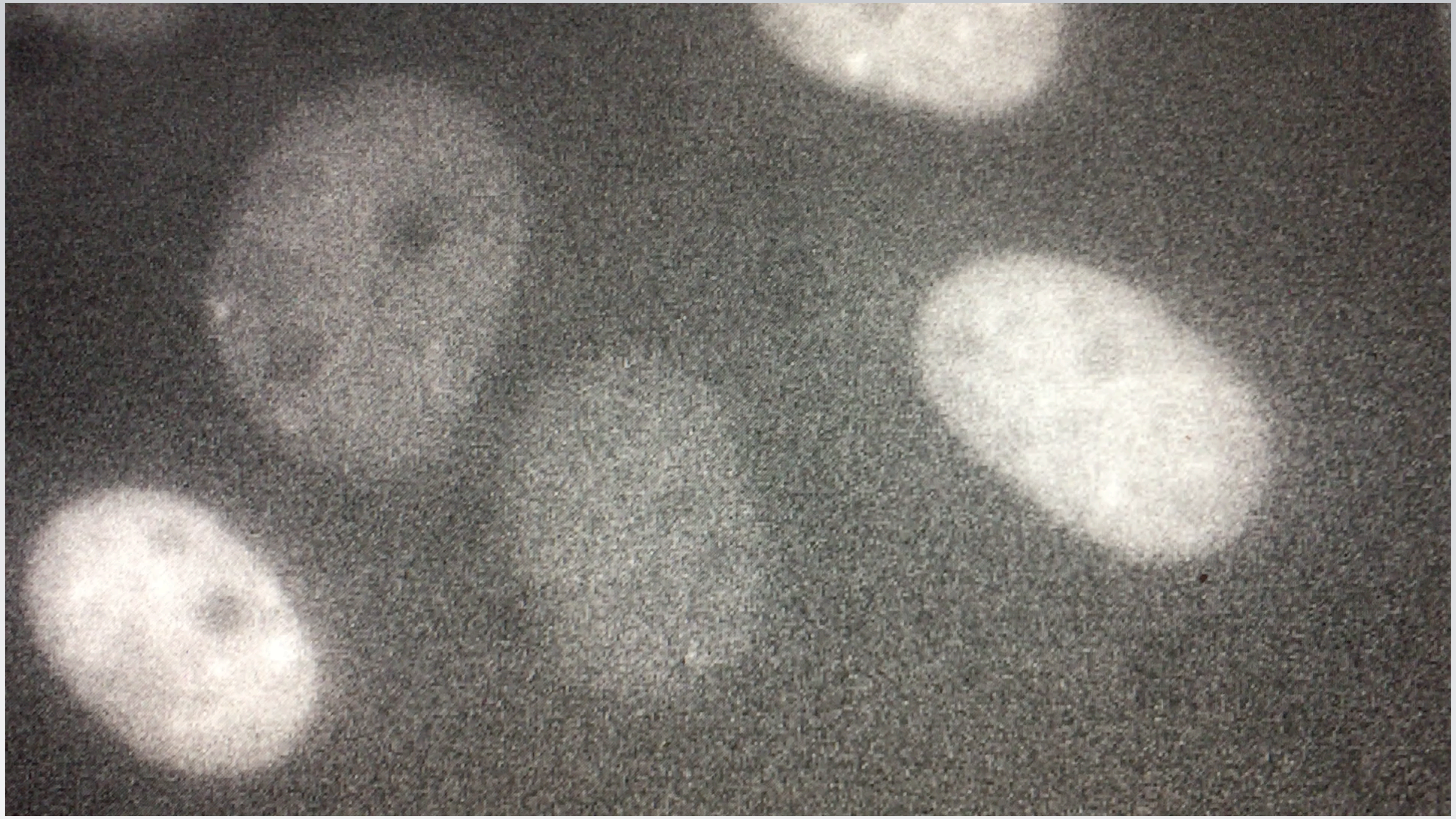
Fast repair

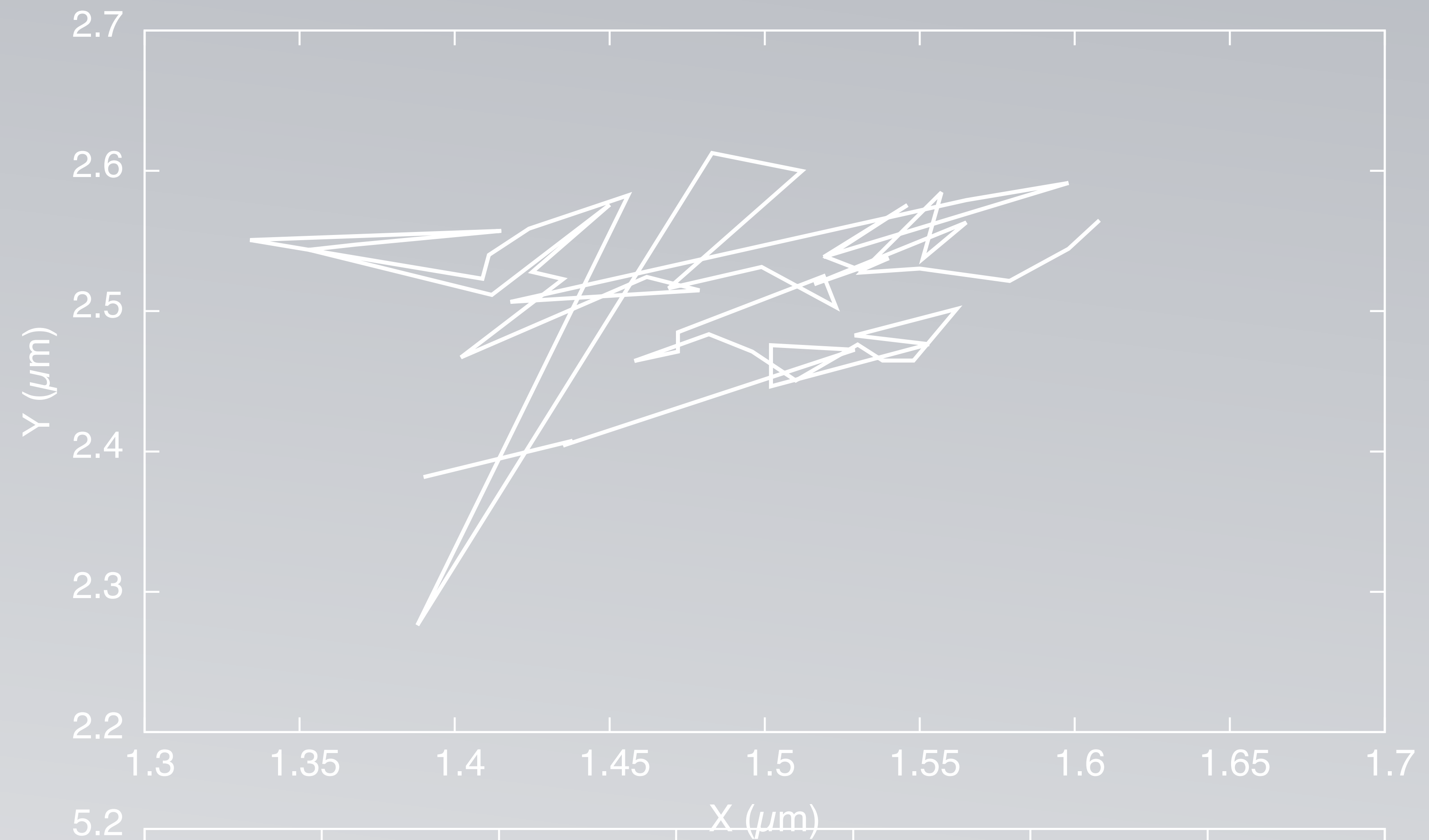
Slow repair

- focus appearance immediately after irradiation
- lower no. of foci for alpha particles at 30 min
- sharp decrease between 30 min and 1 h for alphas and X-rays (41%, 34%)
- slower decrease for mixed beams (18%)

control: 1.9 ±1.0
X-rays: 5.2 ± 1.8
alpha: 10.9 ± 2.1
mixed: 6.7 ± 1.9

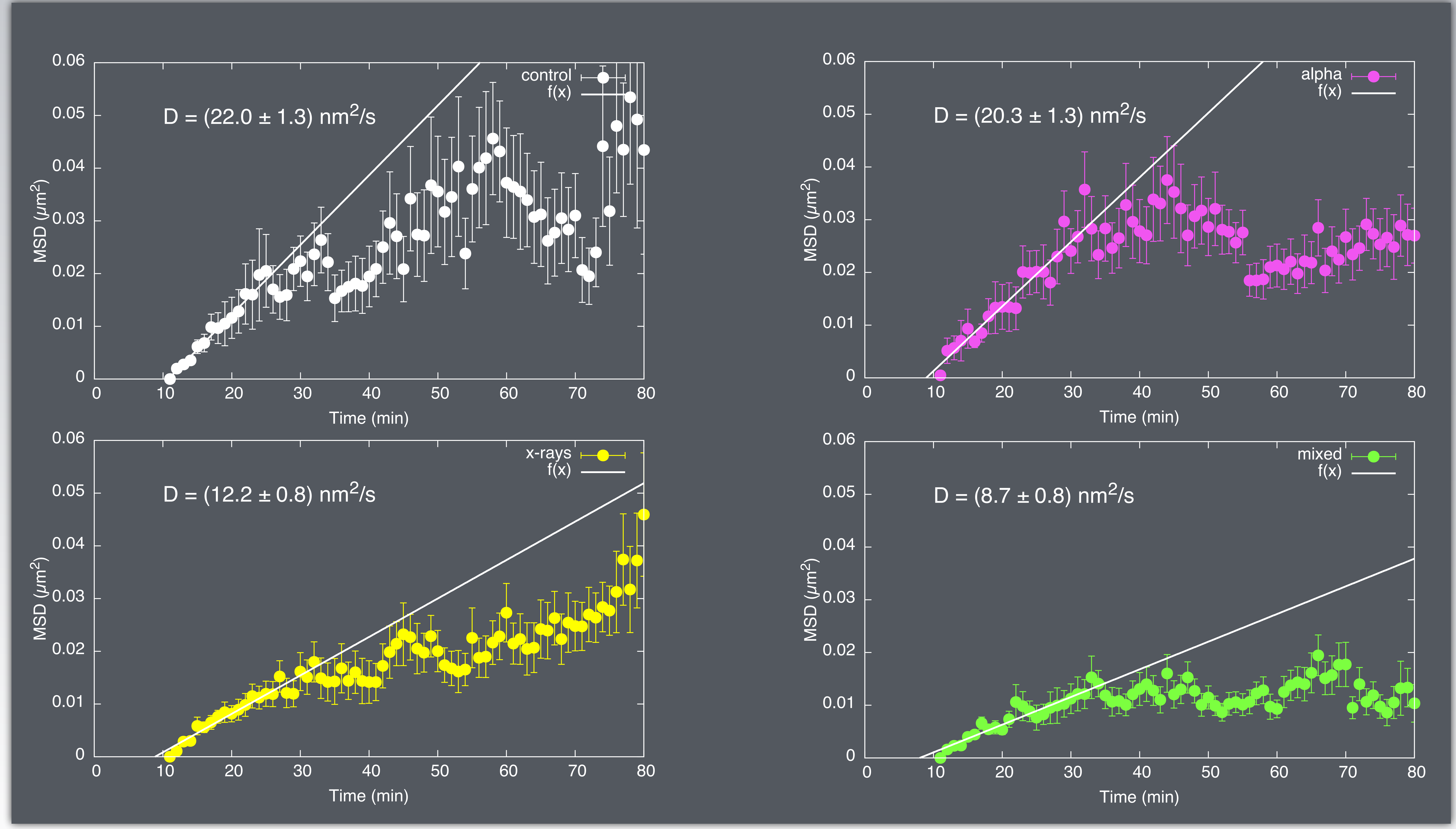
Live imaging





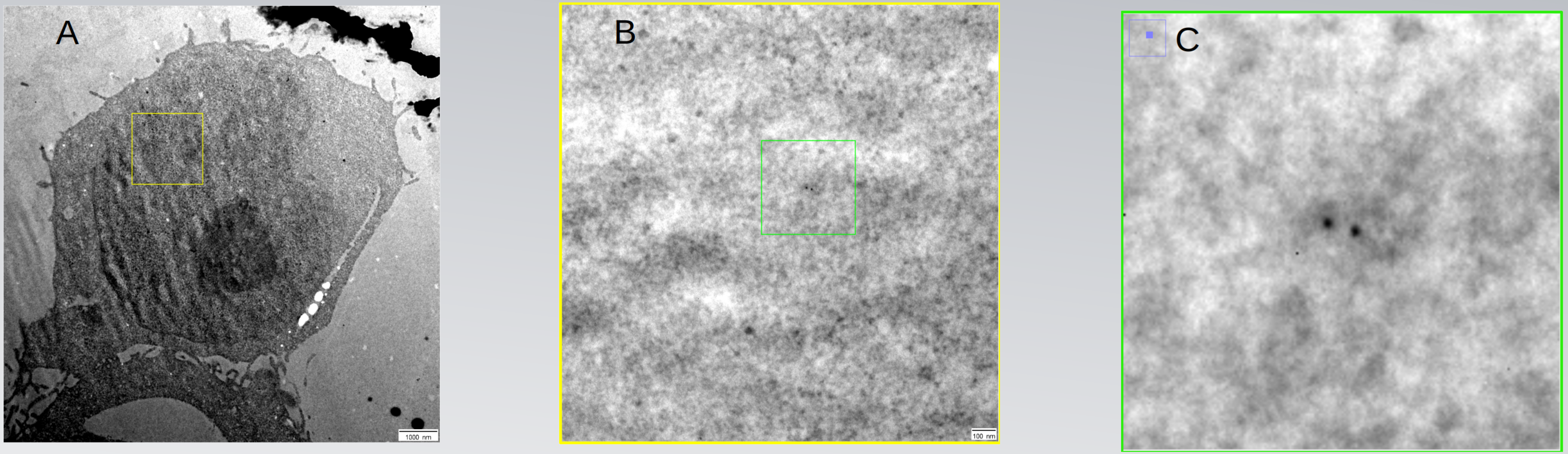
Sollazzo A., Brzozowska B., Cheng L., Lundholm L., Scherthan H. i Wojcik A. (2018). Live Dynamics of 53BP1 Foci Following Simultaneous Induction of Clustered and Dispersed DNA Damage in U2OS Cells, International Journal of Molecular Sciences 19(2): 519

Mean square displacement



How to visualize DSB?

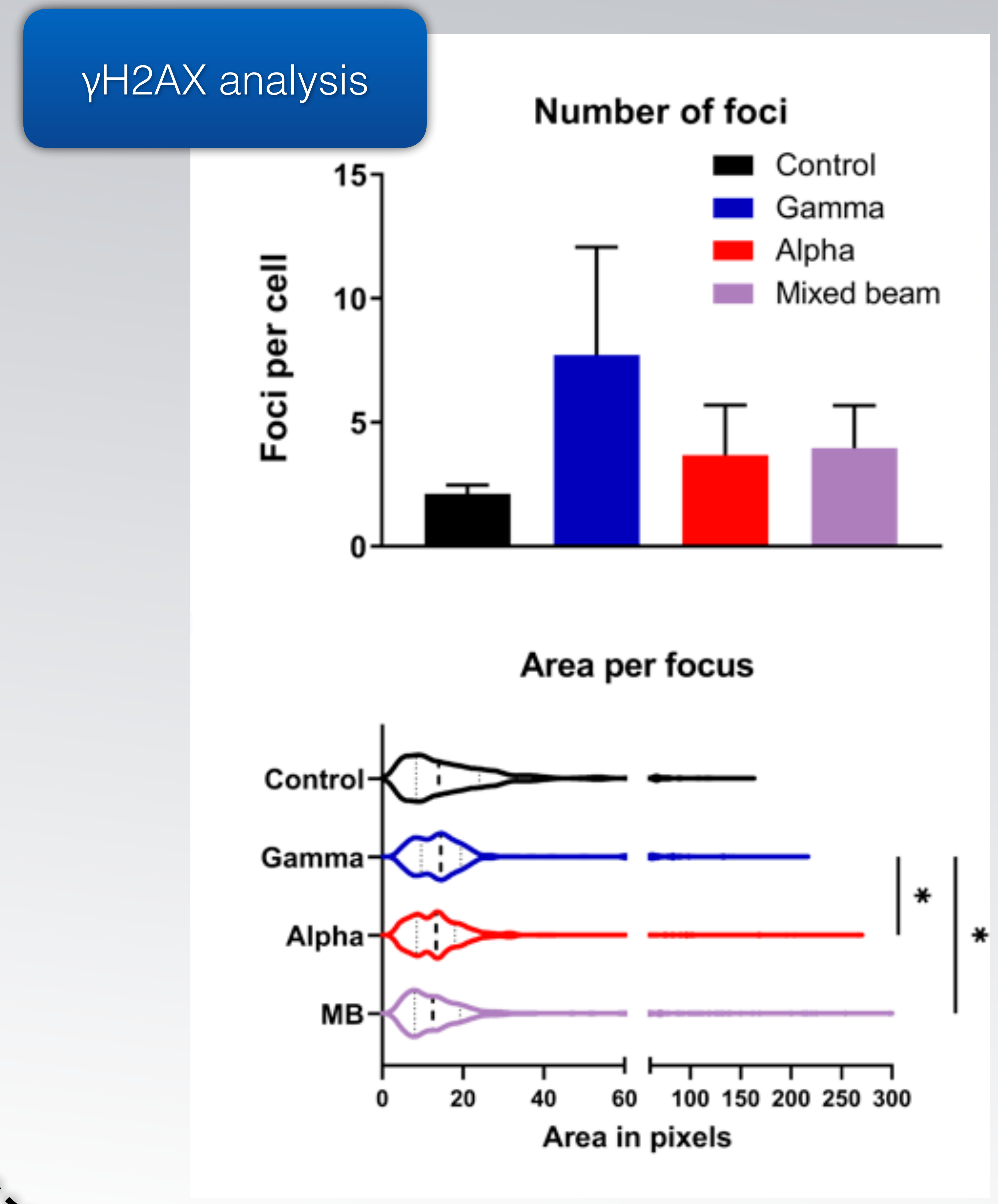
TEM analysis

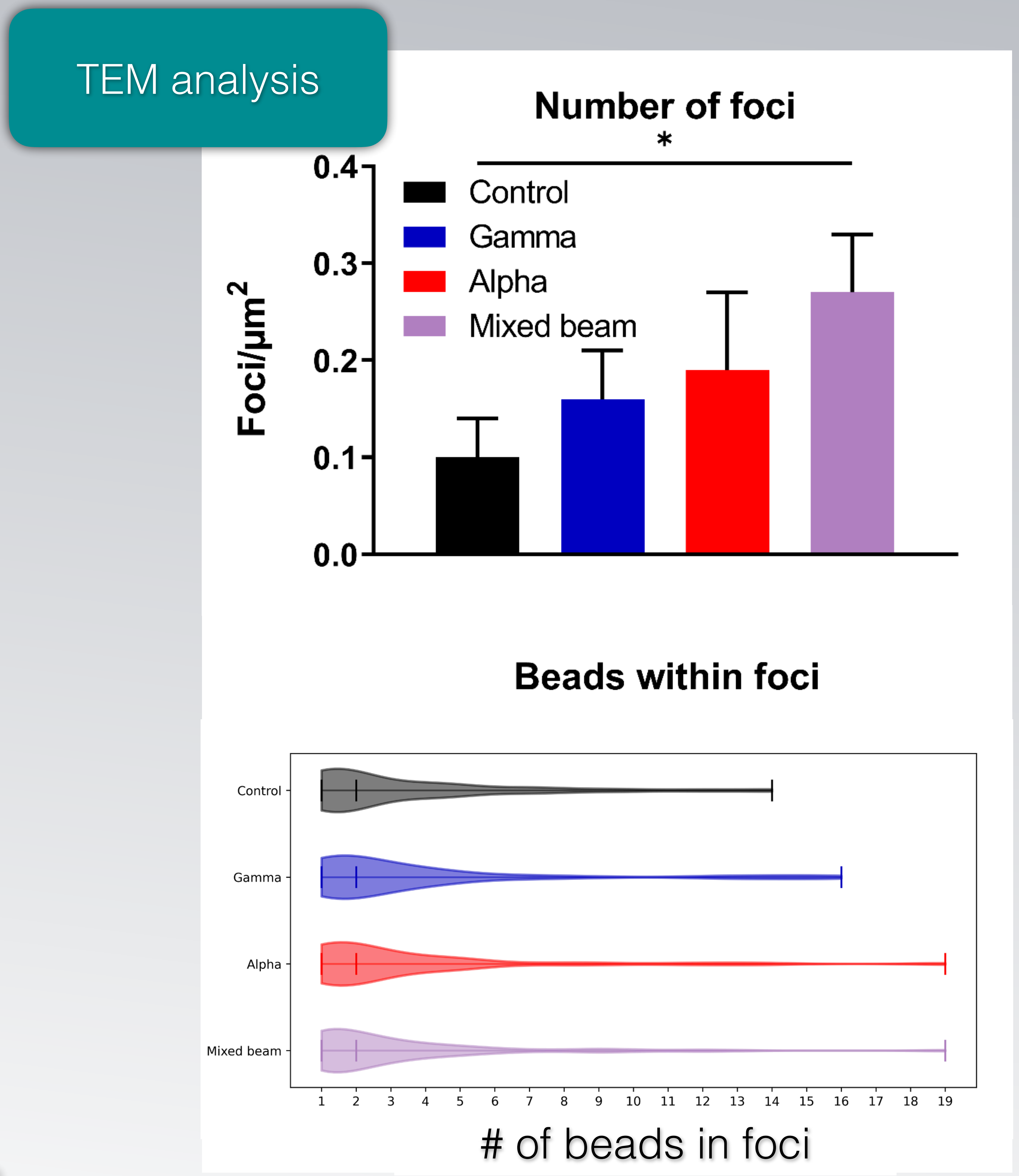


- 80 images were taken per radiation quality;
- Every picture was treated as a random representation of all investigated areas;
- Images were normalised to obtain a mean value of pixel darkness equal to 0 and a standard deviation equal to 1;

Akuwudike, P., López-Riego, M., Ginter, J., Cheng, L., Wieczorek, A., Życieńska, K., Łysek-Gładysińska, M., Wójcik, A., Brzozowska ,B. & Lundholm, L. (2023). Mechanistic insights from high resolution DNA damage analysis to understand mixed radiation exposure. *DNA repair*, *130*, 103554.

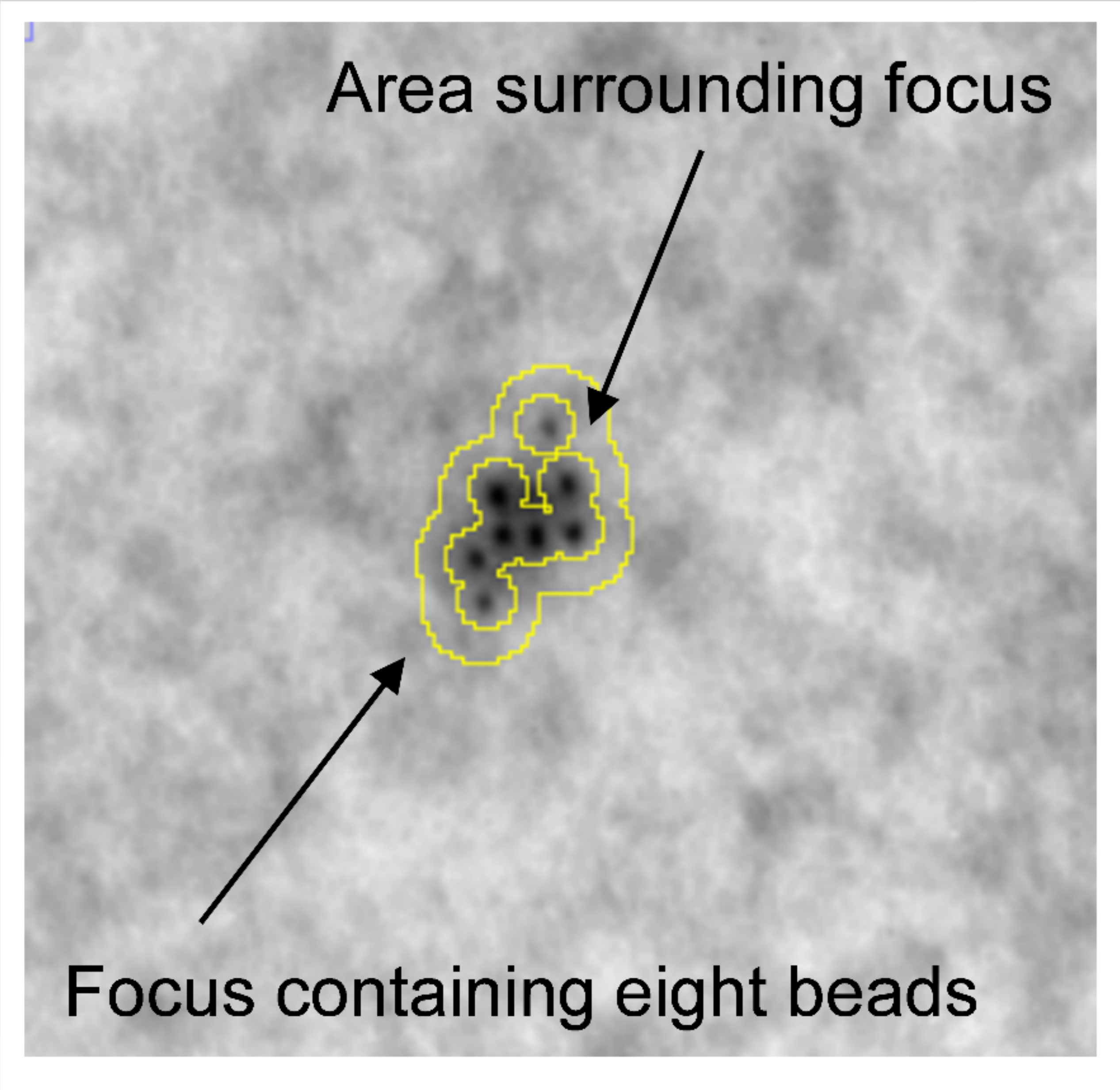
Focus number and size

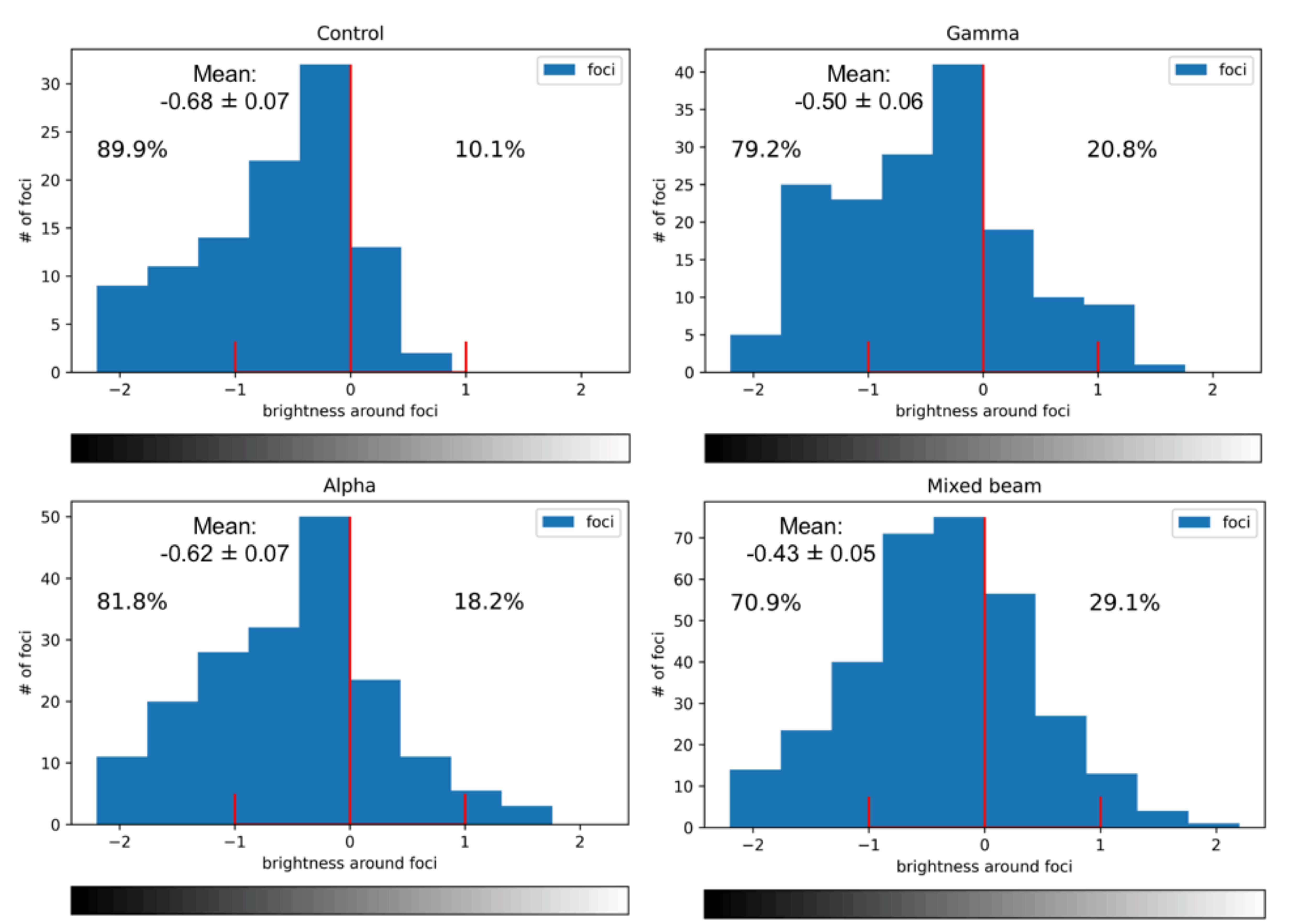




Focus localisation within chromatin

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





Conclusions

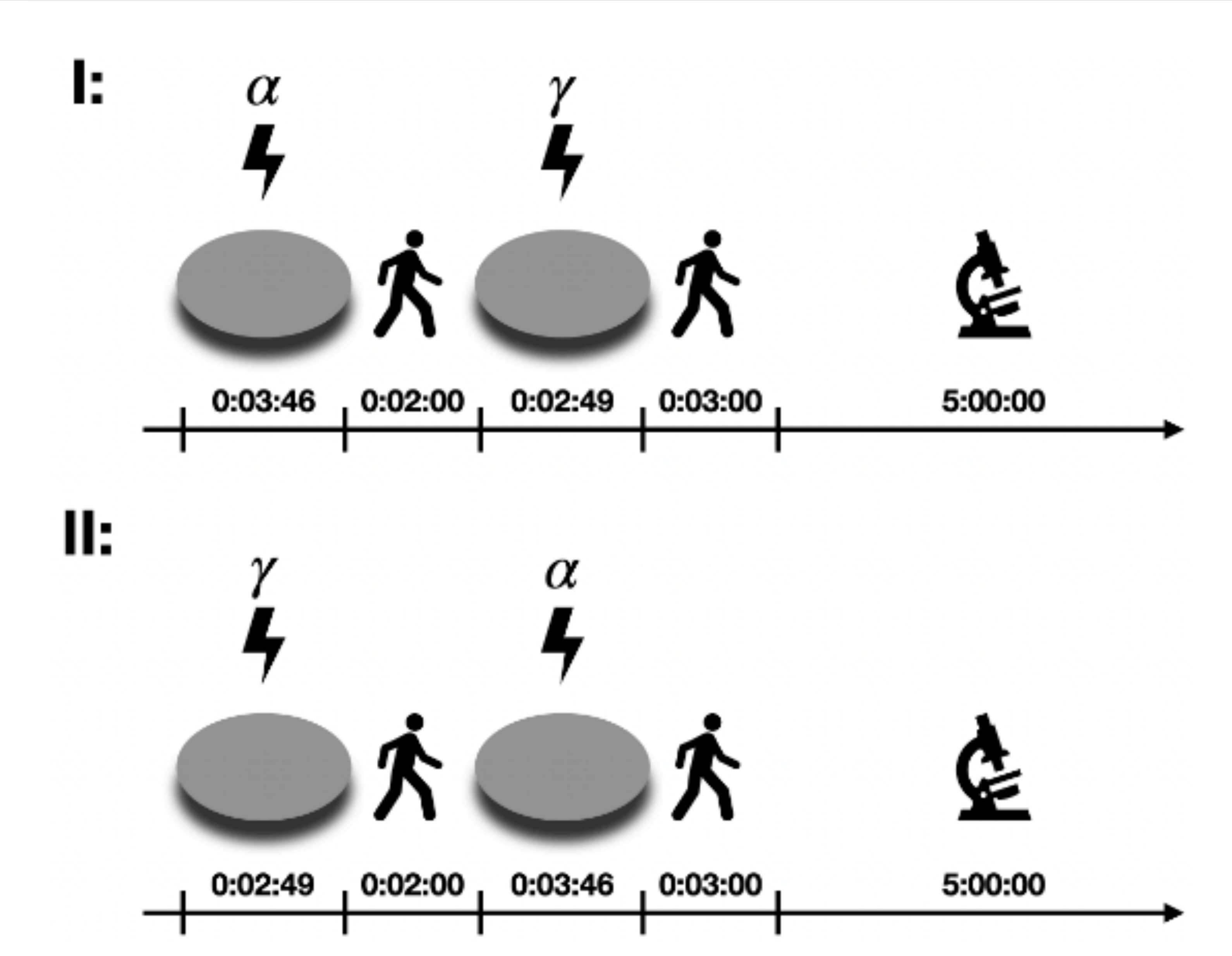
Damage response machinery	• additional damage induced by the second agent may not be repaired properly or in the usual time frame; • the pool of freely available repair proteins is depleted by high LET IR; • initial elimination of foci induced by mixed beams proceeded more slowly than expected;
Synergistic effect	• **high and low LET radiations interact to produce more DNA damage than expected from an additive action**; • the mixed beam effect should be considered when transferring cancer risk and radiation protection.

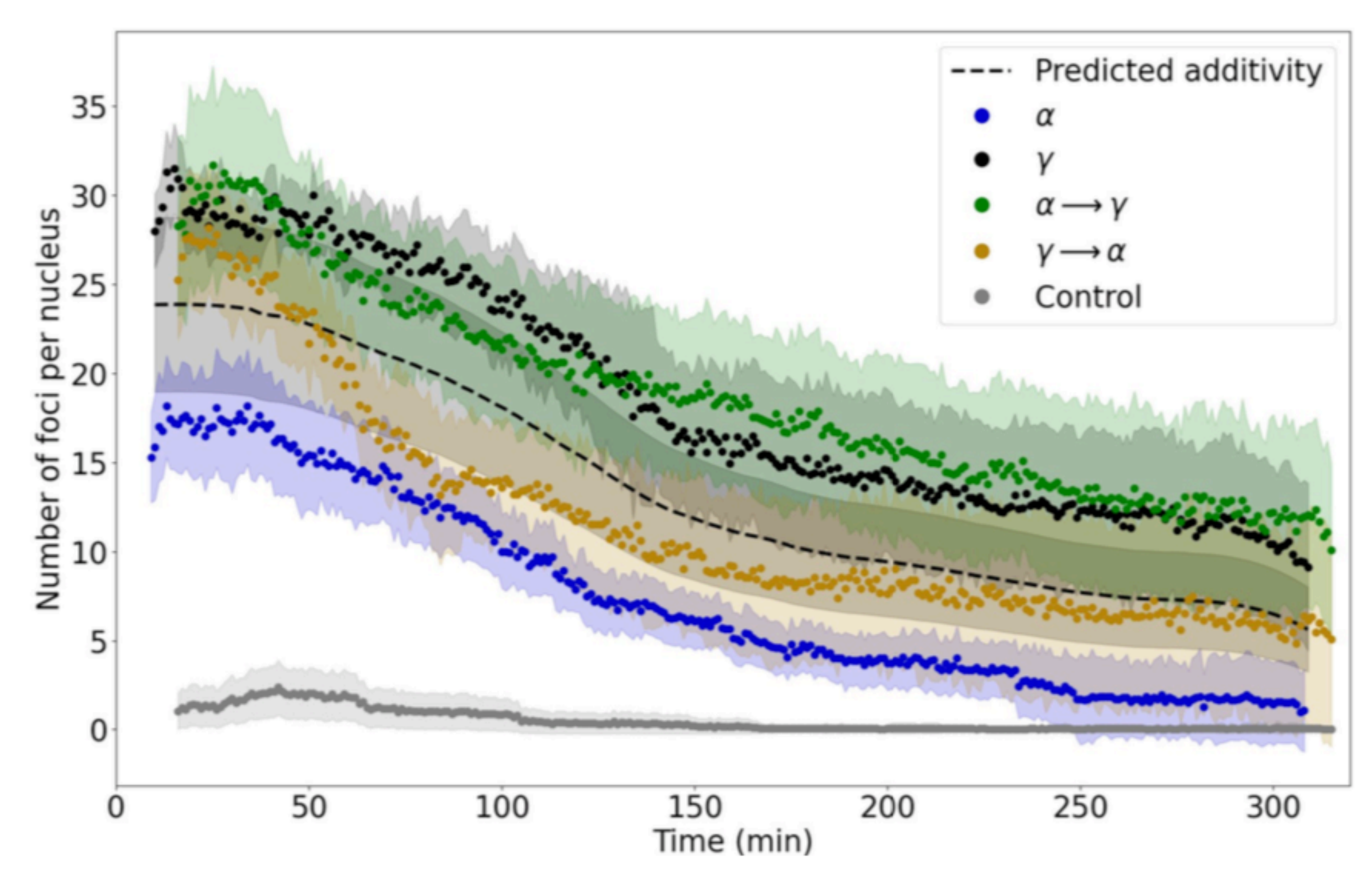
Stockholm University

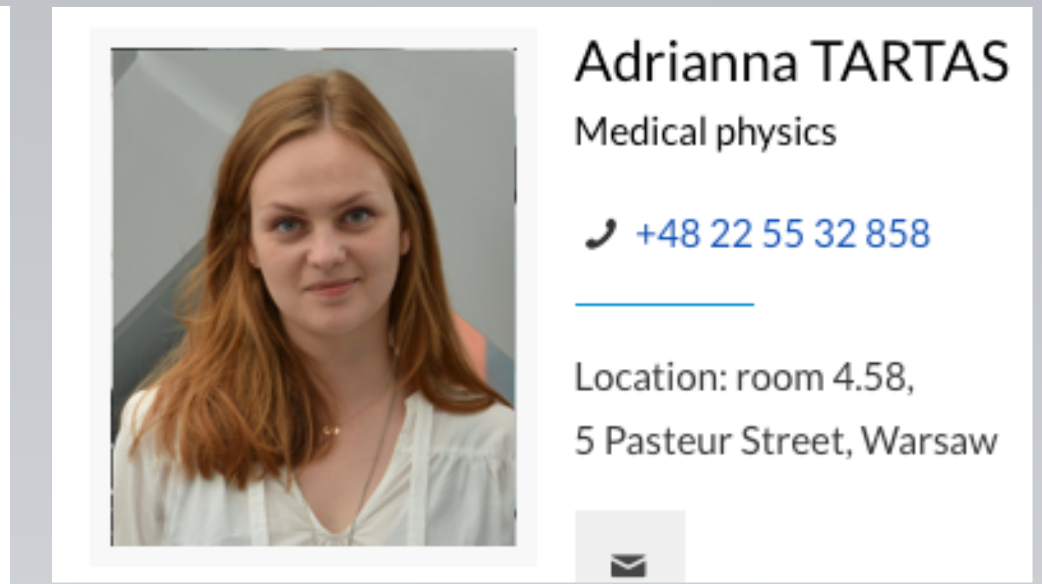
Department of Molecular Biosciences, The Wenner-Gren Institute

Andrzej Wójcik
Lei Cheng
Lovisa Lundholm
Alice Sollazzo

Does the order of irradiation matter: NBS1 focus dynamics







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)

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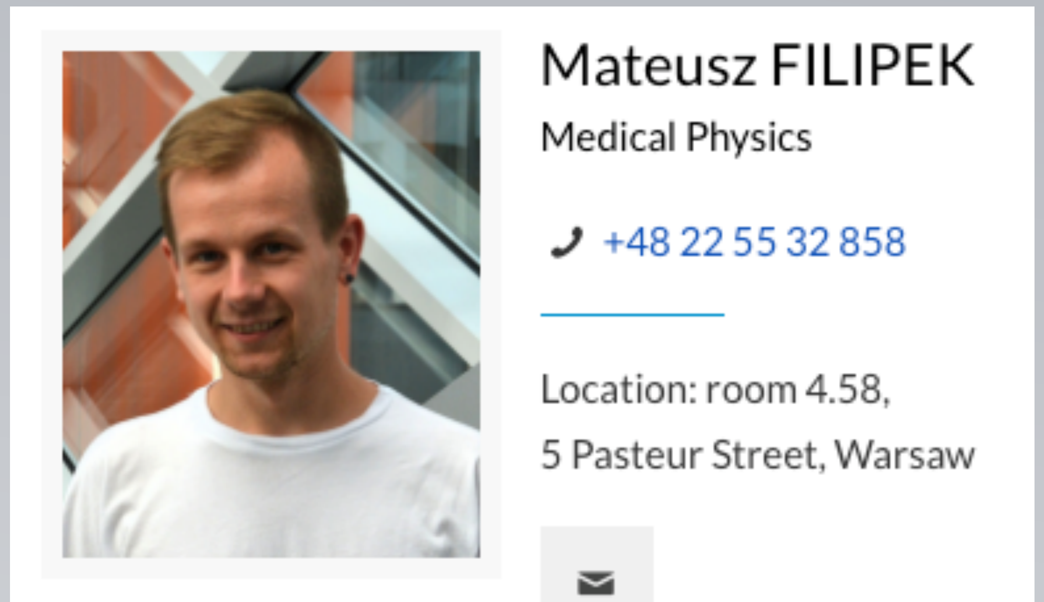
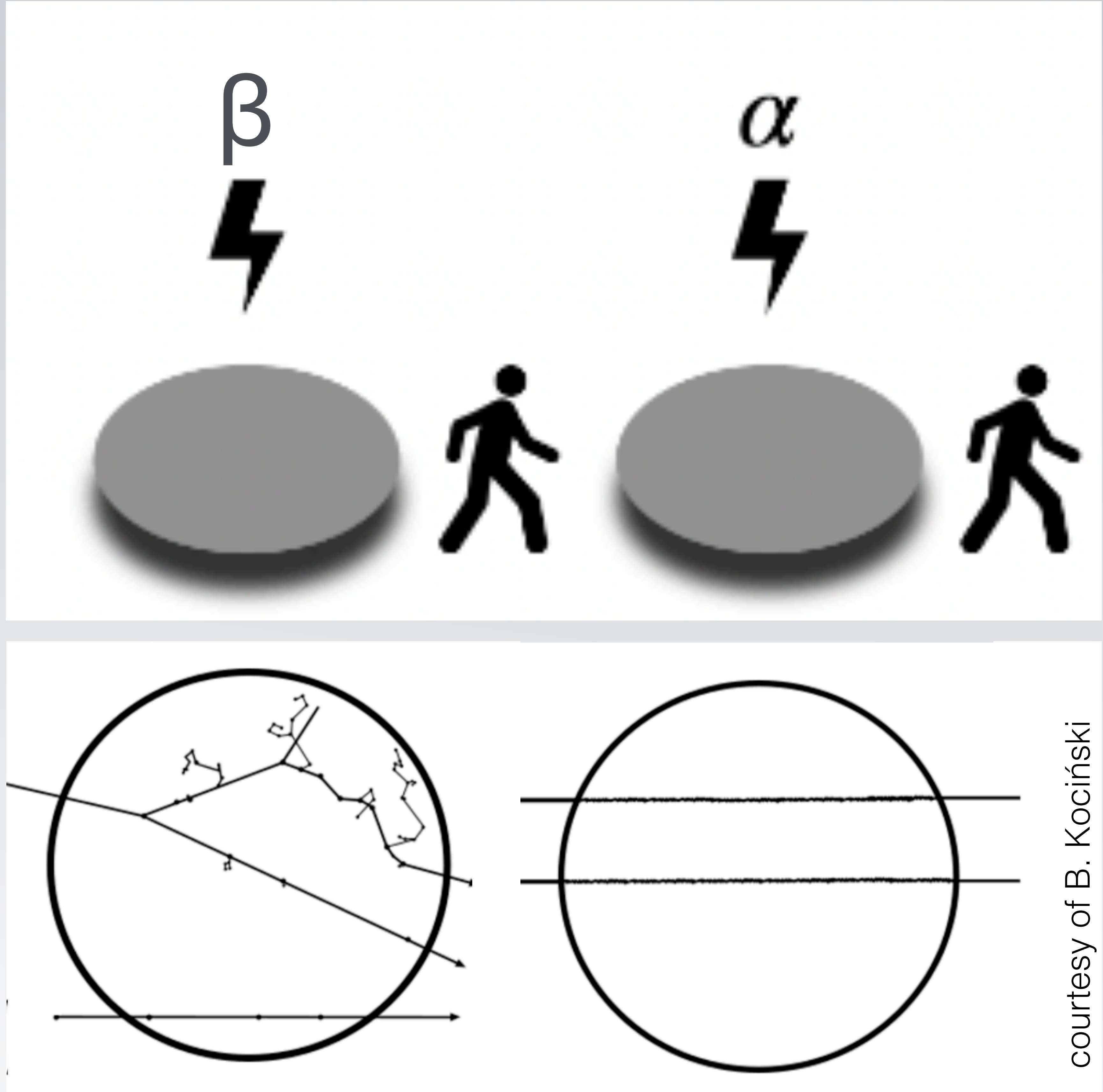
Do cells adapt to radiation?



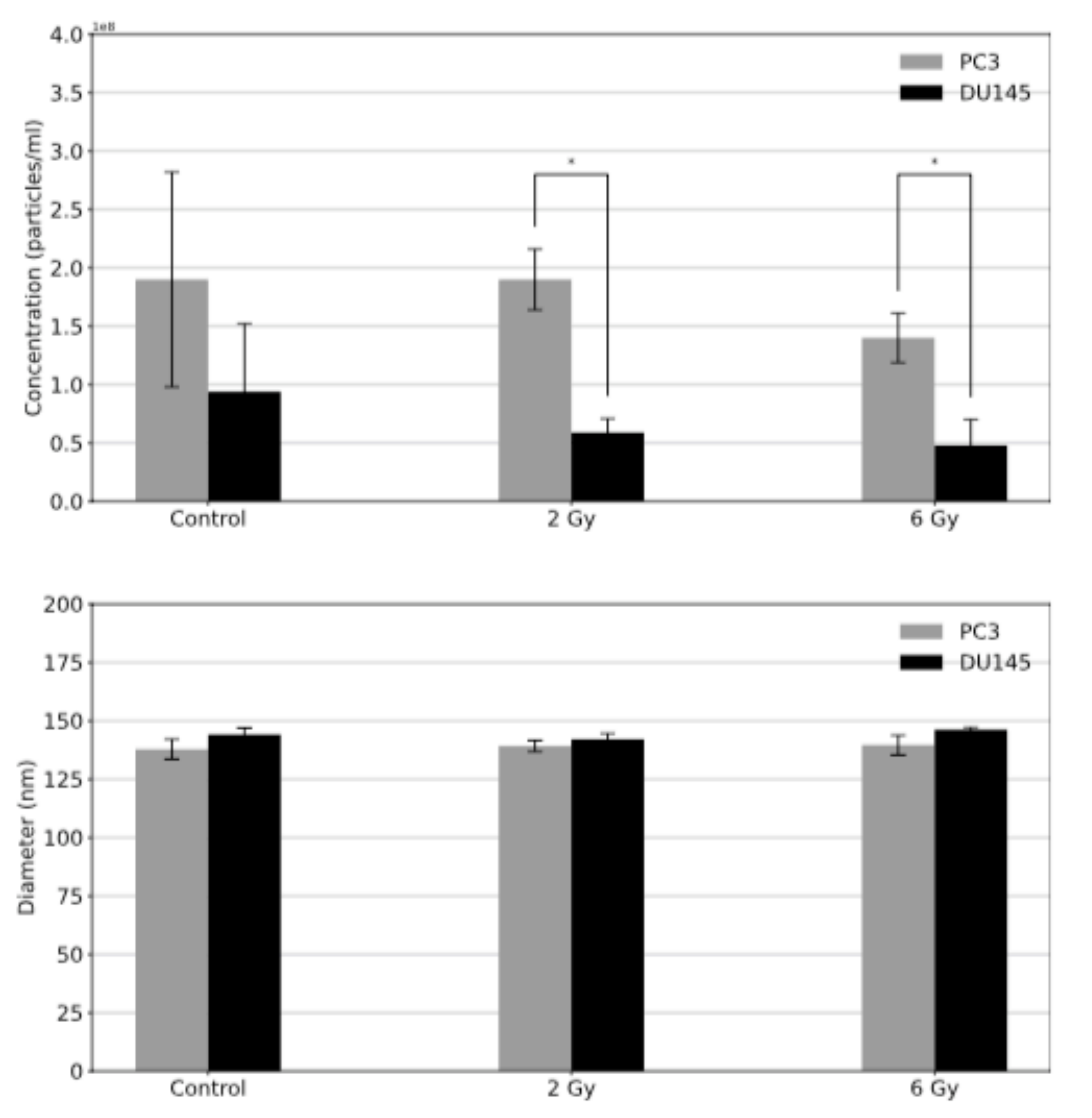


250 mGy   2 Gy

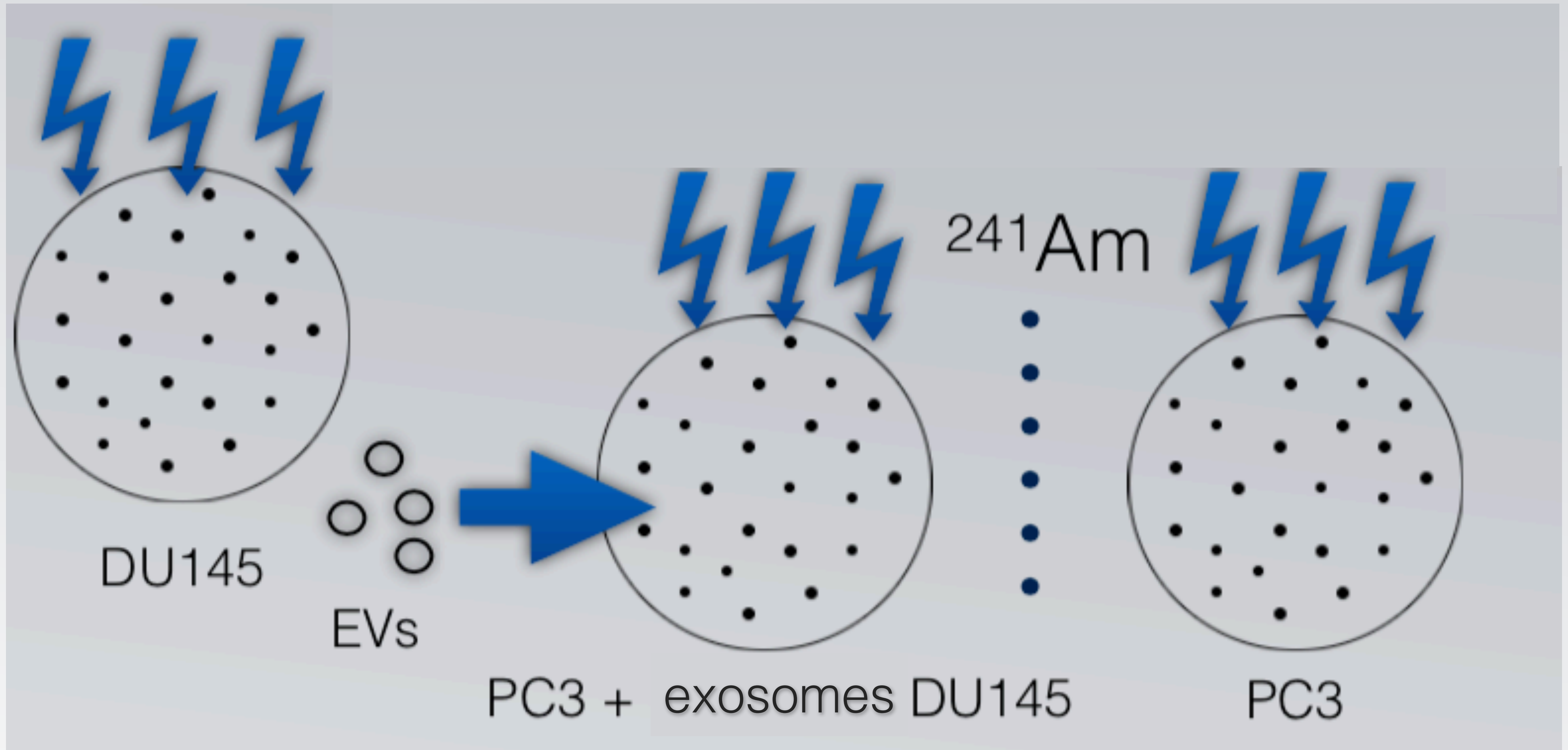
CELL SURVIVAL

REPAIR FOCUS ANALYSIS

Do exosomes make cells more radiosensitive?

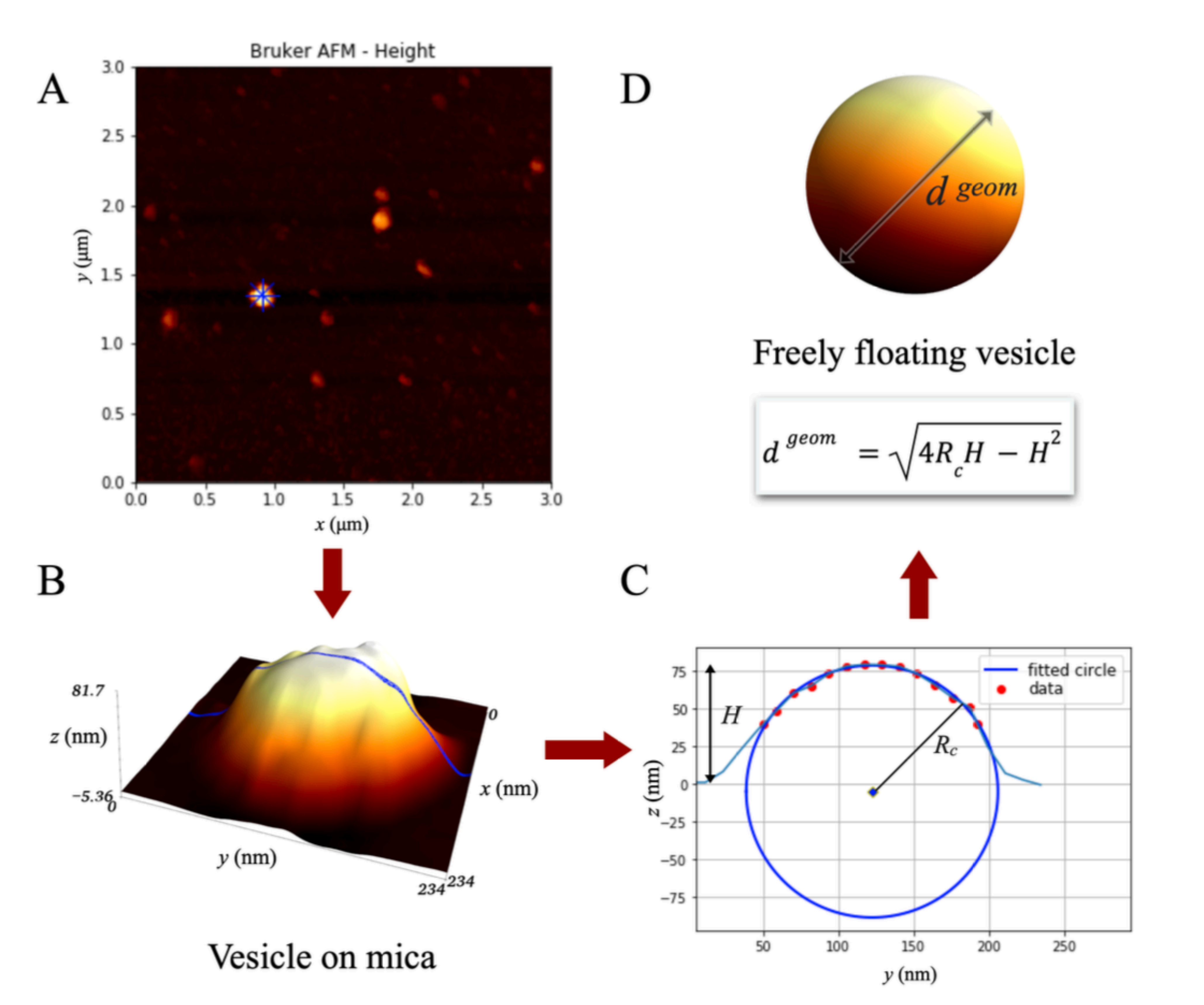


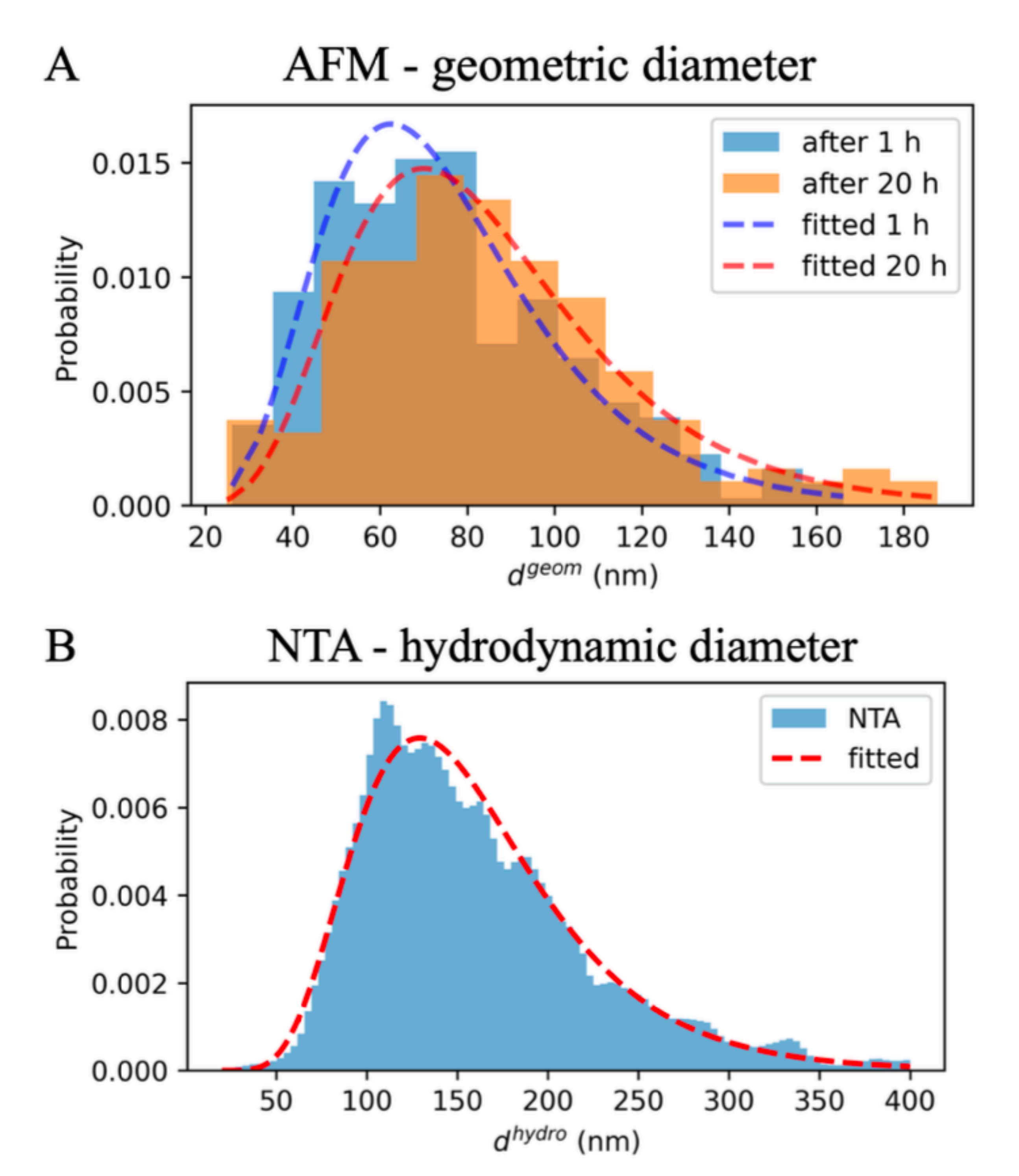
Beata Pszczółkowska
Medical physics
+48 22 55 32 858
Location: room 4.58,
5 Pasteur Street, Warsaw



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 we distinguish between exosomes from healthy and cancer cells?





Katarzyna ŻYCIEŃSKA
Medical physics
+48 22 55 32 855
Location: room 4.55, 5 Pasteur Street, Warsaw

Józef GINTER
Medical Physics
+48 22 55 32 867
Location: room 4.67, 5 Pasteur Street, Warsaw
Supervision

Życieńska, K., Pszczółkowska, B., Brzozowska, B., Kamiński, M., Lorenc, T., Olejarz, W., Sęk, S. & Ginter, J. (2022). Brownian Motion Influence on AFM Exosomes' Size Measurements. International Journal of Molecular Sciences, 23(17), 10074.

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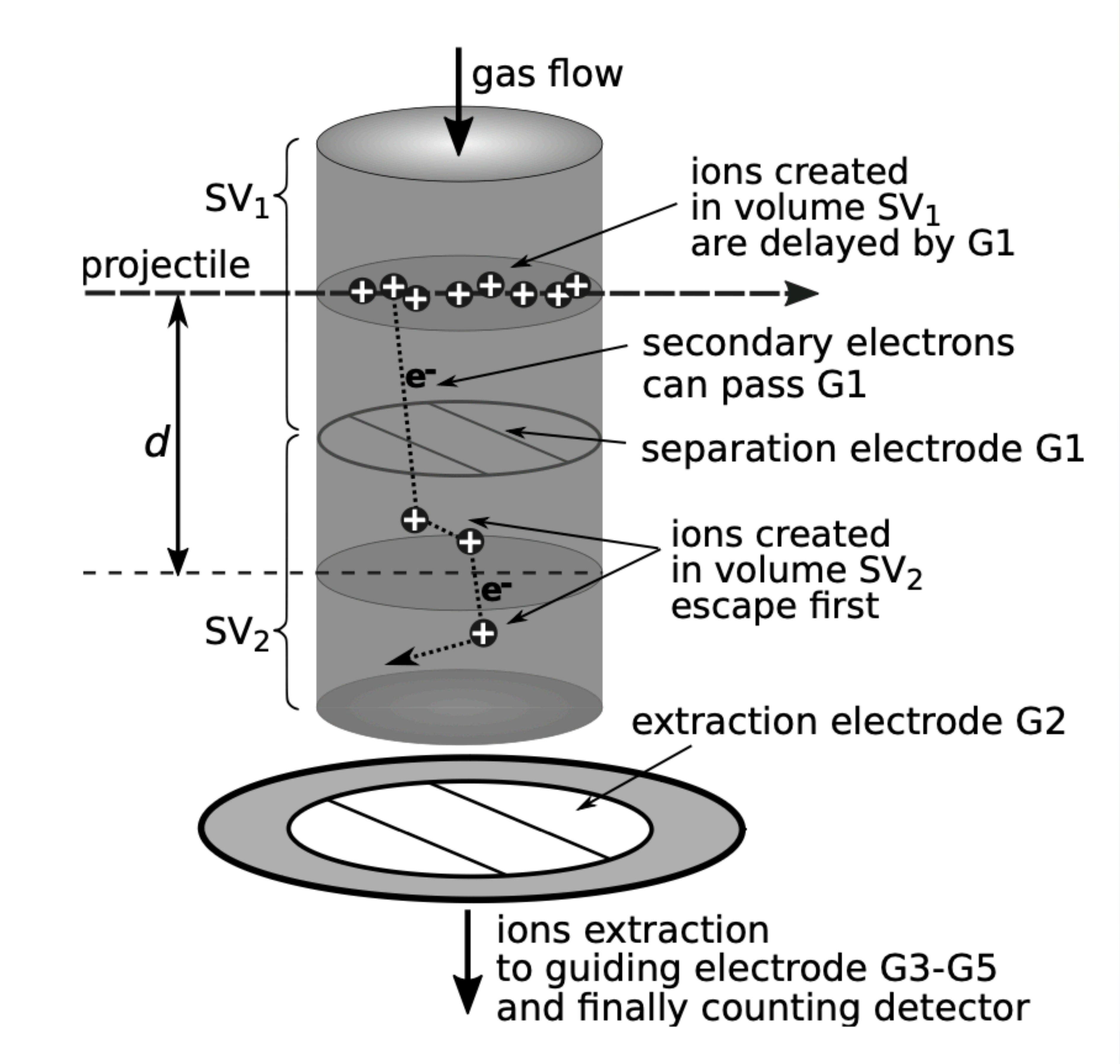
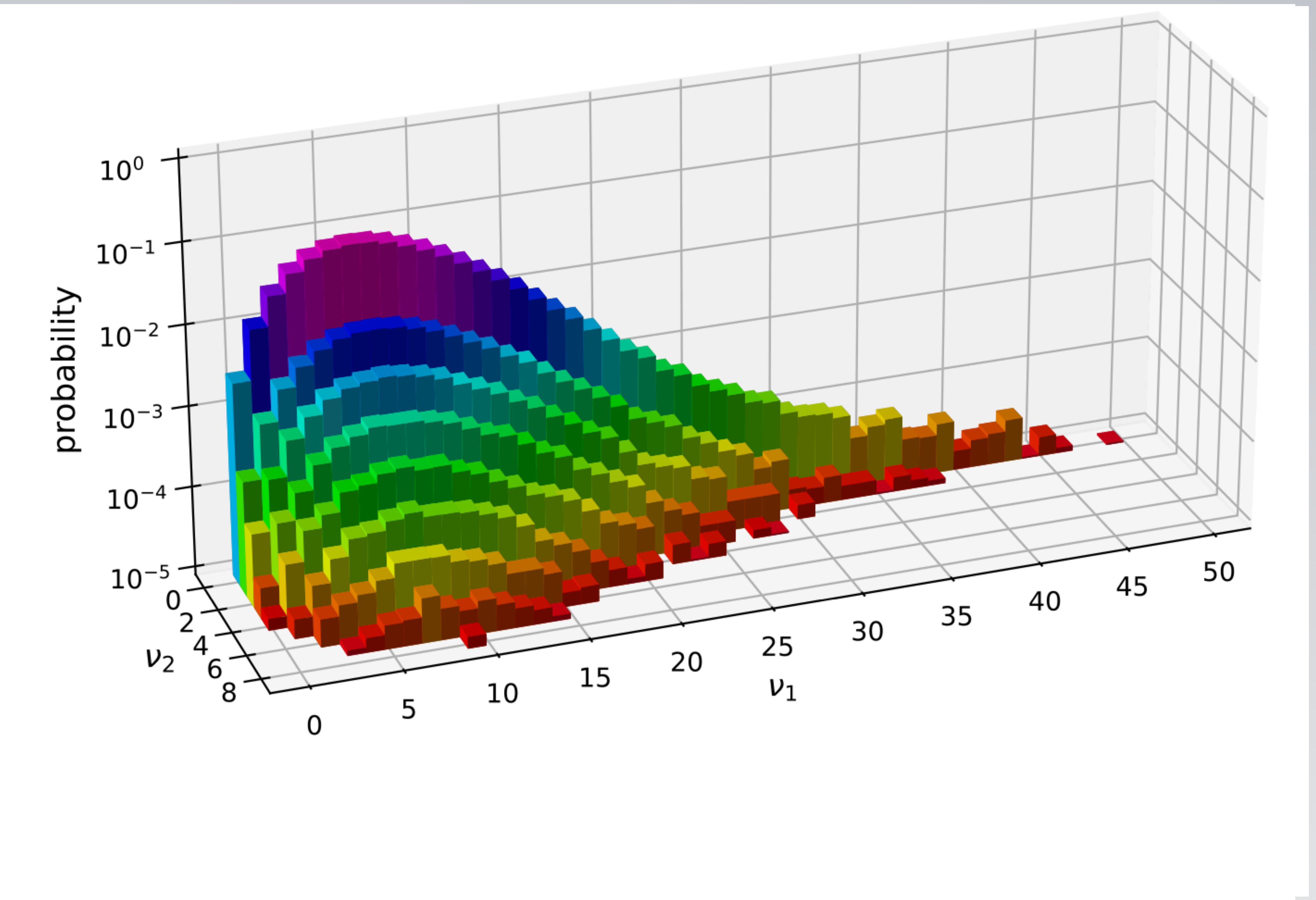
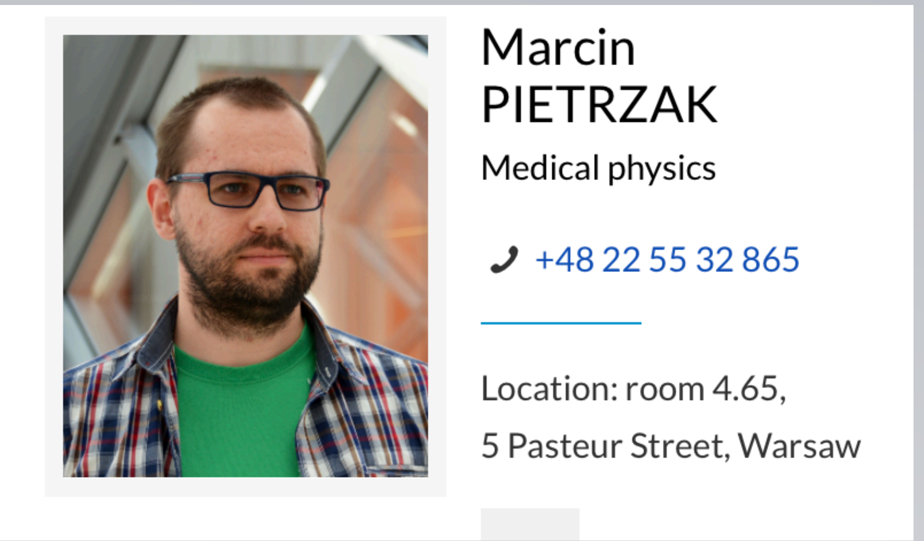
Can we measure spatial dependencies of ionisation events?



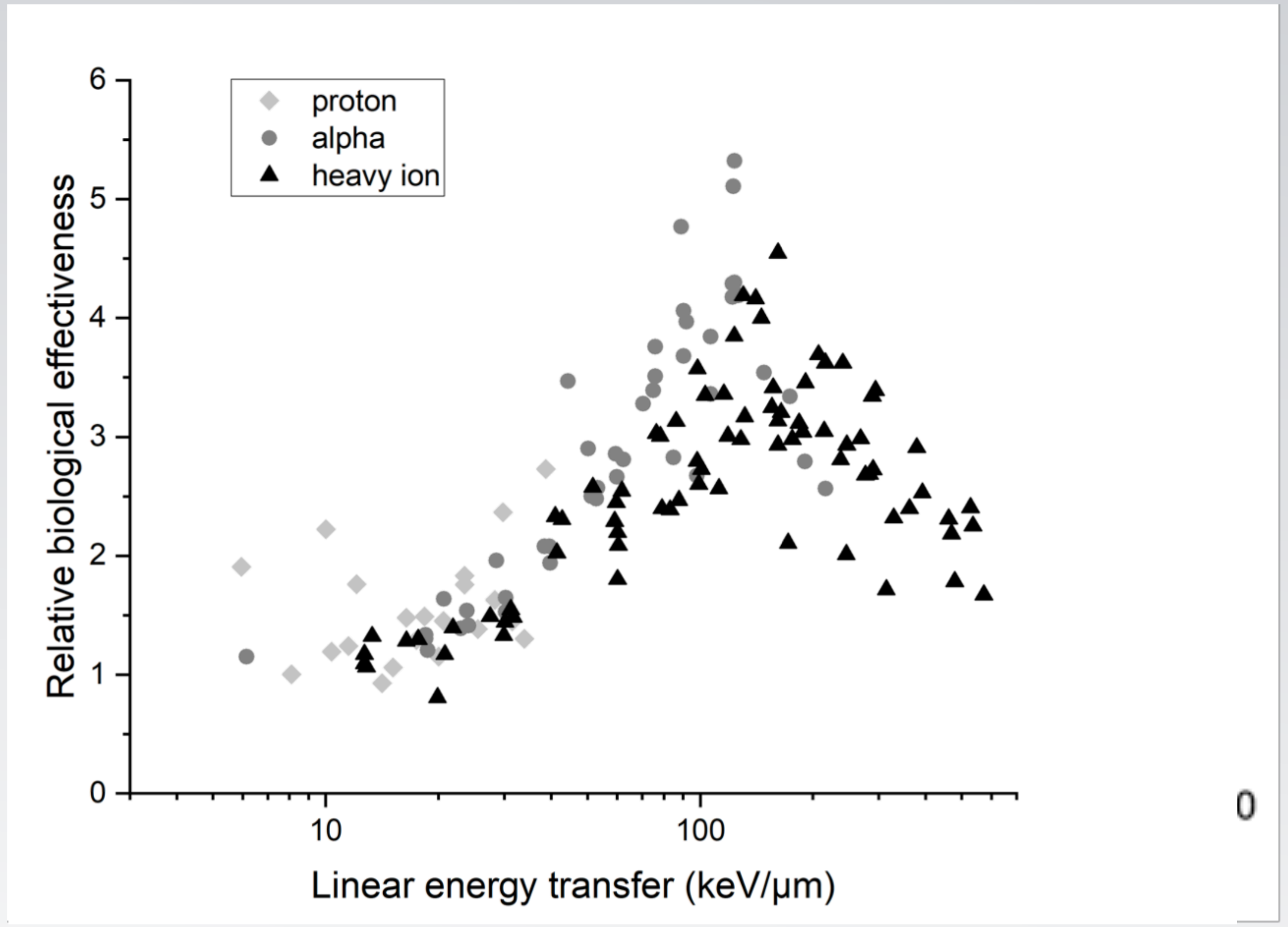


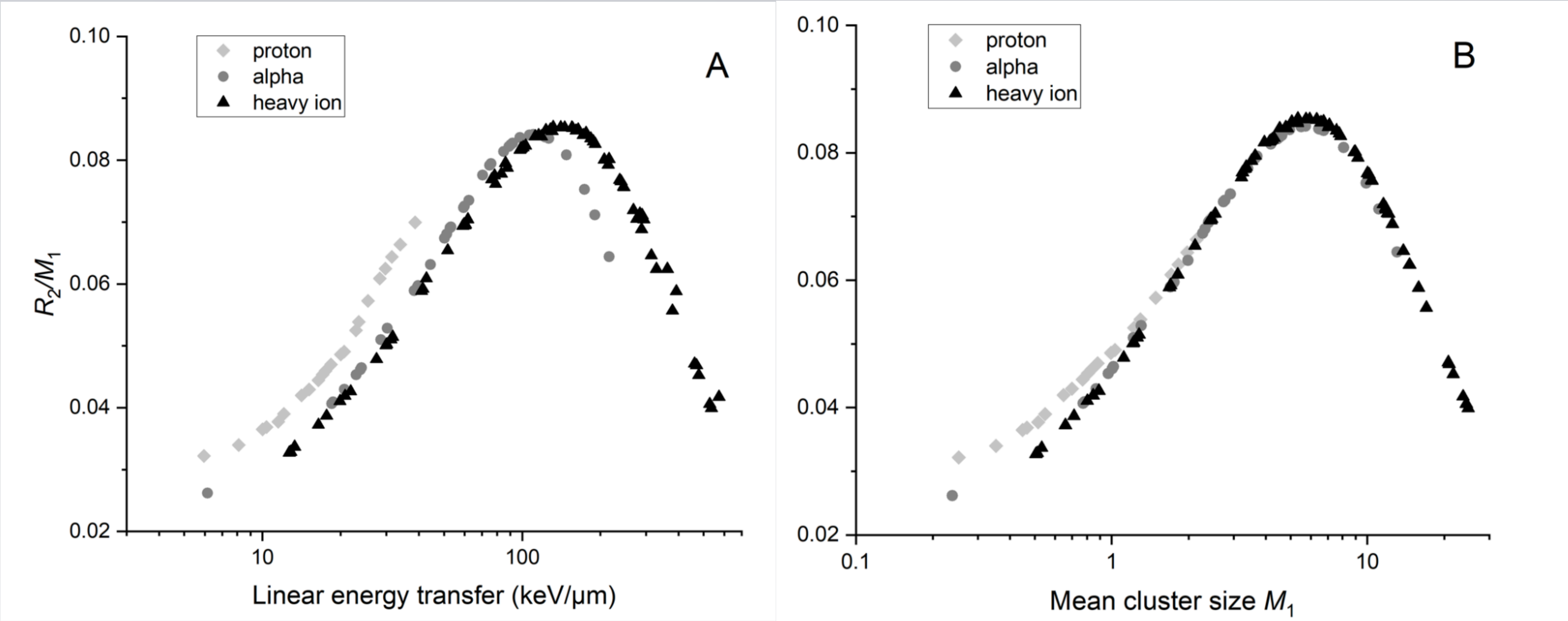


M. Pietrzak, Nanodosimetric characteristic of carbon ion beam - experiments and Monte Carlo simulations, Doctoral thesis, 2023, Faculty of Physics, University of Warsaw

Can nanodosimetric quantities be used to describe DNA damage?

Monika
MIETELSKA
Medical physics
+48 22 55 32 858
Location: room 4.58,
5 Pasteur Street, Warsaw





M. Mietelska, M. Pietrzak, A. Bancer, A. Ruciński, Z. Szefliński, B. Brzozowska, Ionisation detail parameters for DNA damage evaluation in charged particle radiotherapy: simulation study based on cell survival database, under preparation

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

Walt Disney

Thank you for your attention!

beata.brzozowska@fuw.edu.pl