
Using single-cell DNA sequencing as a dosimetric tool – An exploratory study

F. Mathew^{1*}, J. Yeo², J. Manalad¹, L. Galarneau¹, N. Ybarra¹, Y.C. Wang³,
P. Tonin⁴, I. Ragoussis³ and J. Kildea¹

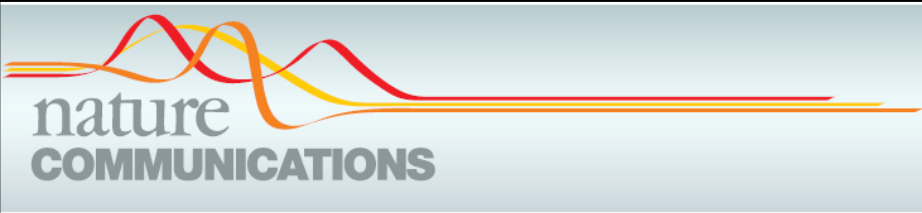
¹Medical Physics Unit, McGill University, Montreal, Canada

²Singapore Nuclear Research and Safety Initiative, National University of Singapore

³McGill Genome Centre, Montreal, Canada

⁴Research Institute-McGill University Health Centre, McGill University, Montreal, Canada

Mutation signature of radiation



Behjati et al. 2016

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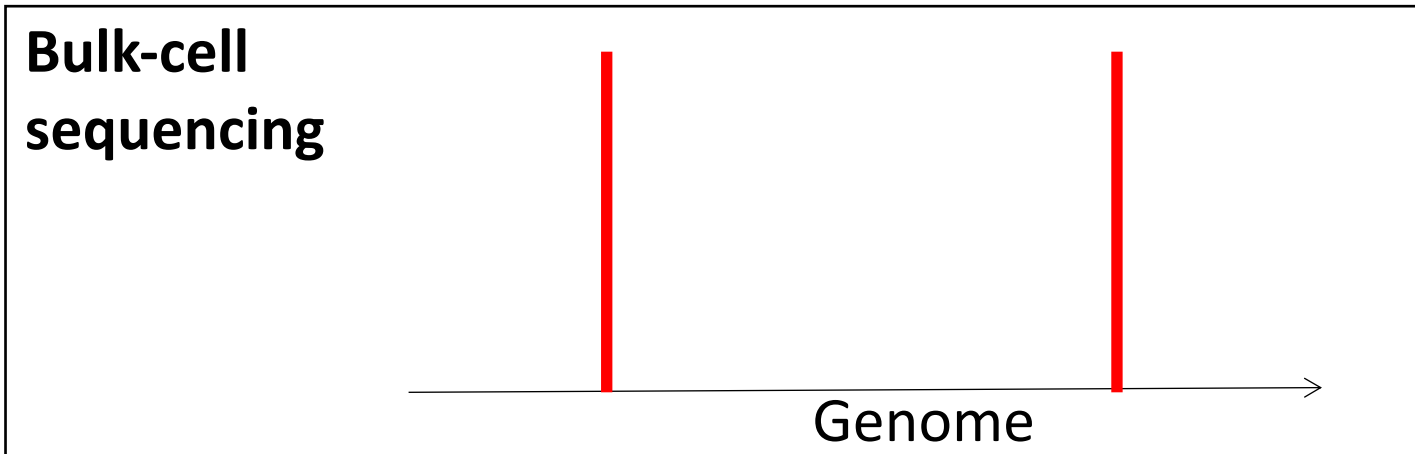
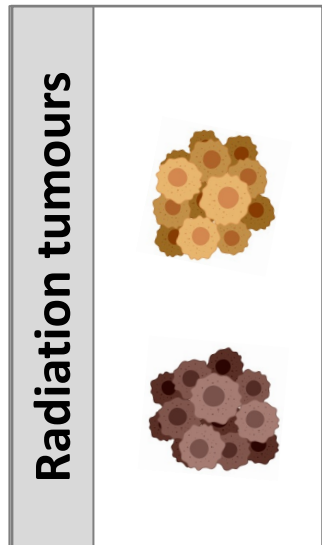
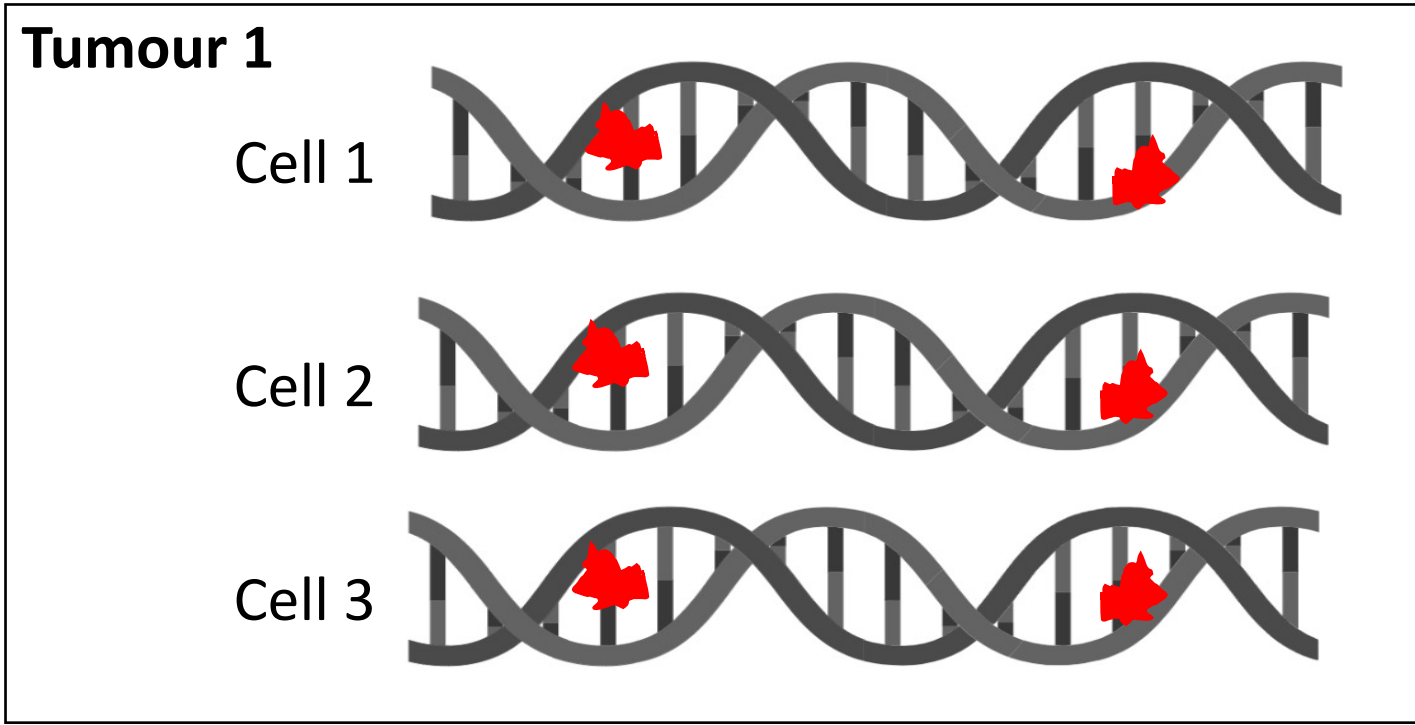
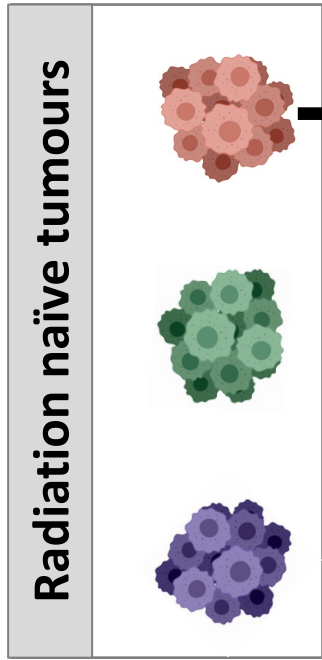
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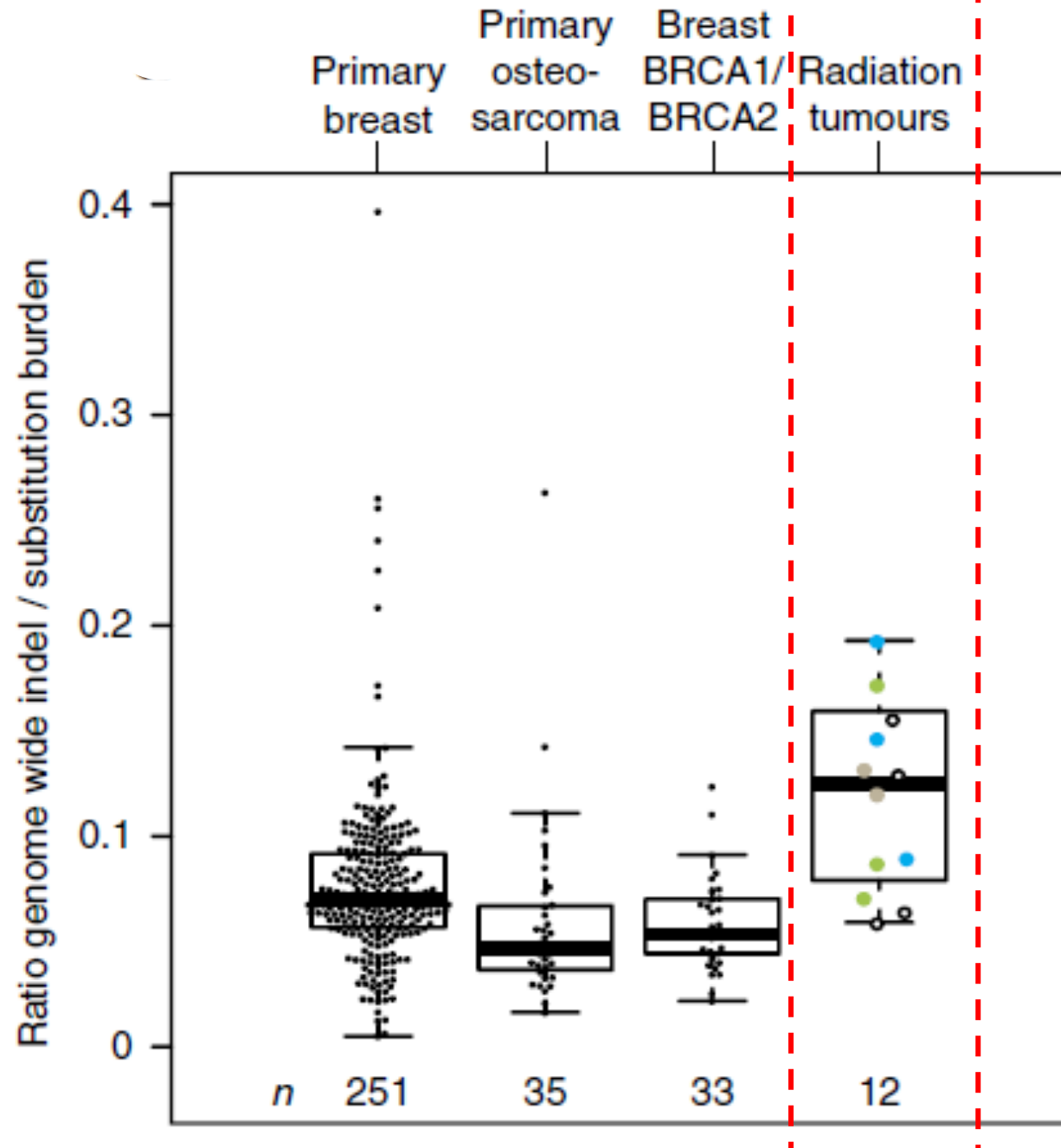
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Mutational signatures of ionizing radiation in second malignancies

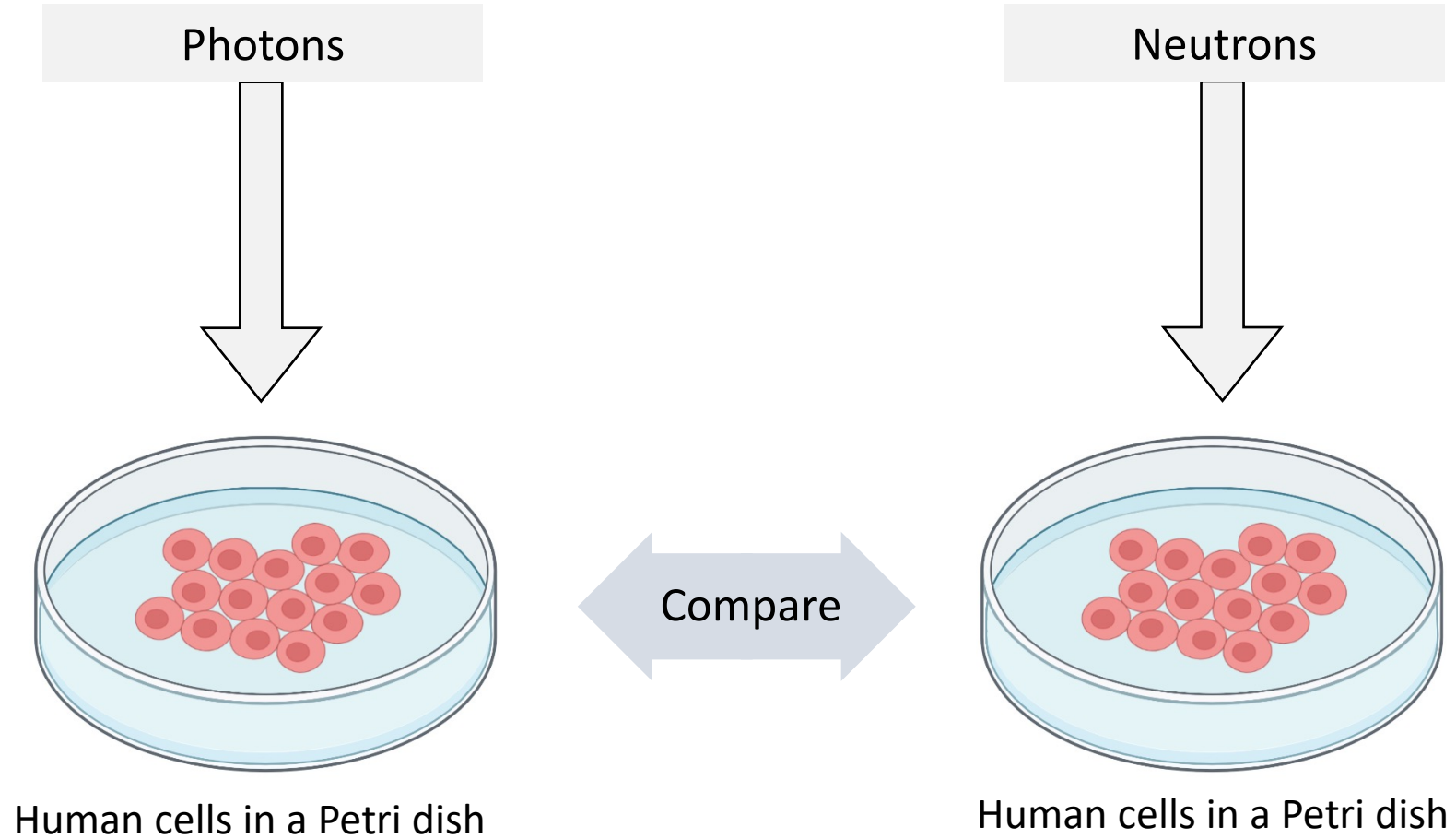
Sam Behjati^{1,2,*}, Gunes Gundem^{1,*}, David C. Wedge^{1,3}, Nicola D. Roberts¹, Patrick S. Tarpey¹, Susanna L. Cooke¹, Peter Van Loo^{4,5}, Ludmil B. Alexandrov¹, Manasa Ramakrishna¹, Helen Davies¹, Serena Nik-Zainal¹, Claire Hardy¹, Calli Latimer¹, Keiran M. Raine¹, Lucy Stebbings¹, Andy Menzies¹, David Jones¹, Rebecca Shepherd¹, Adam P. Butler¹, Jon W. Teague¹, Mette Jorgensen⁶, Bhavisha Khatri⁷, Nischalan Pillay^{6,7}, Adam Shlien^{1,8}, P. Andrew Futreal^{1,9}, Christophe Badie¹⁰, ICGC Prostate Group[†], Ultan McDermott¹, G. Steven Bova¹¹, Andrea L. Richardson^{12,13,†}, Adrienne M. Flanagan^{6,7}, Michael R. Stratton¹ & Peter J. Campbell^{1,14}

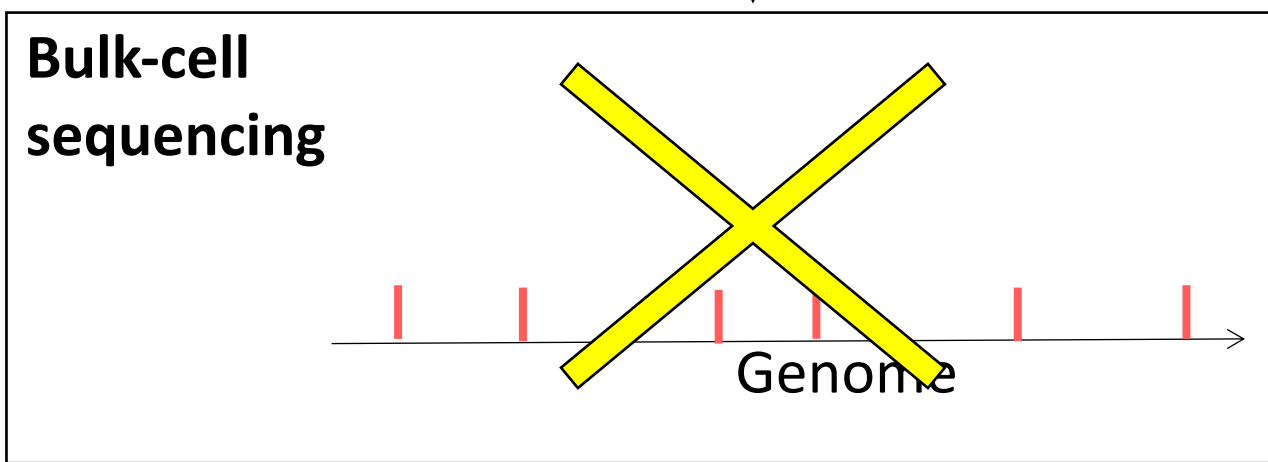
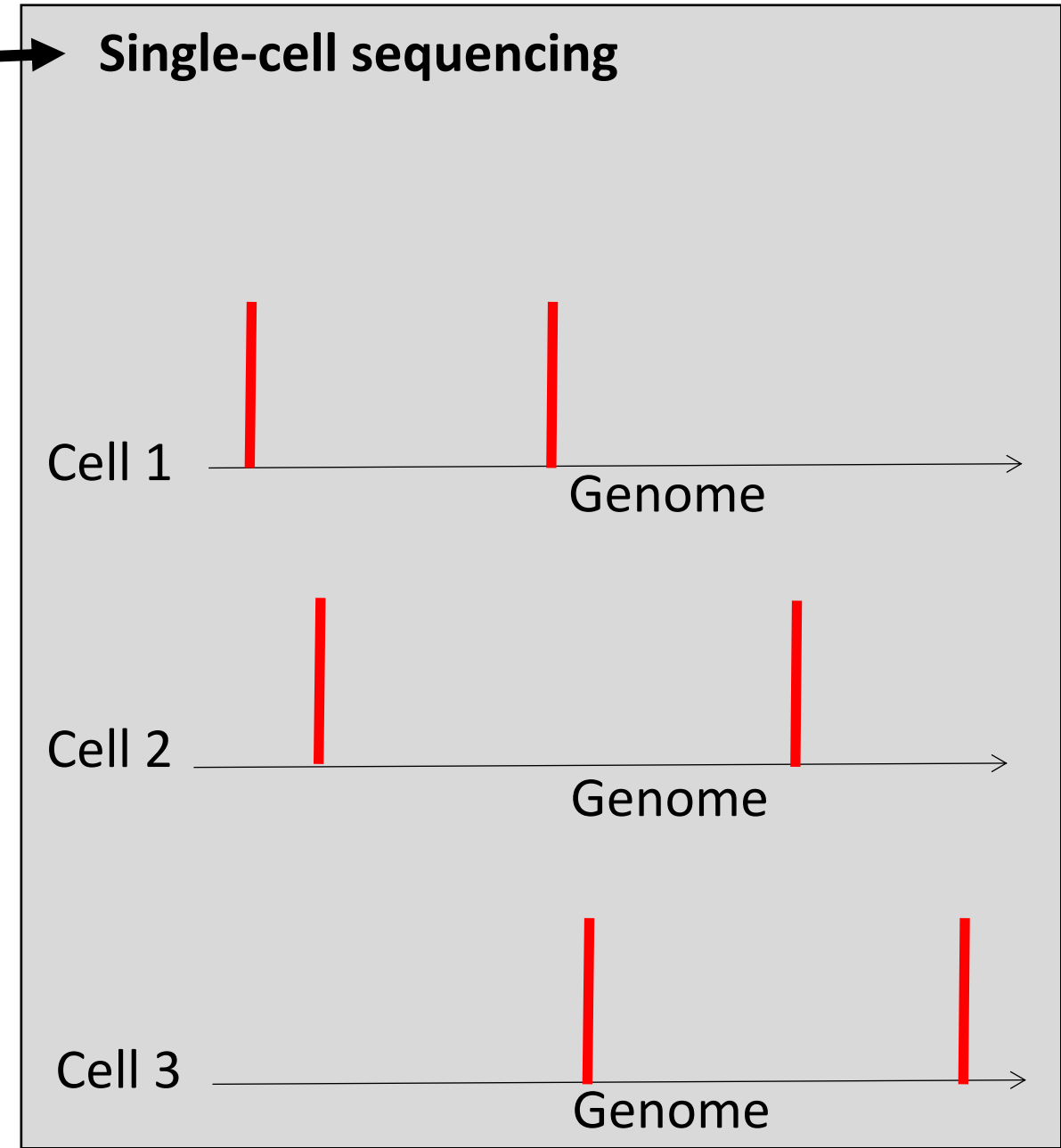
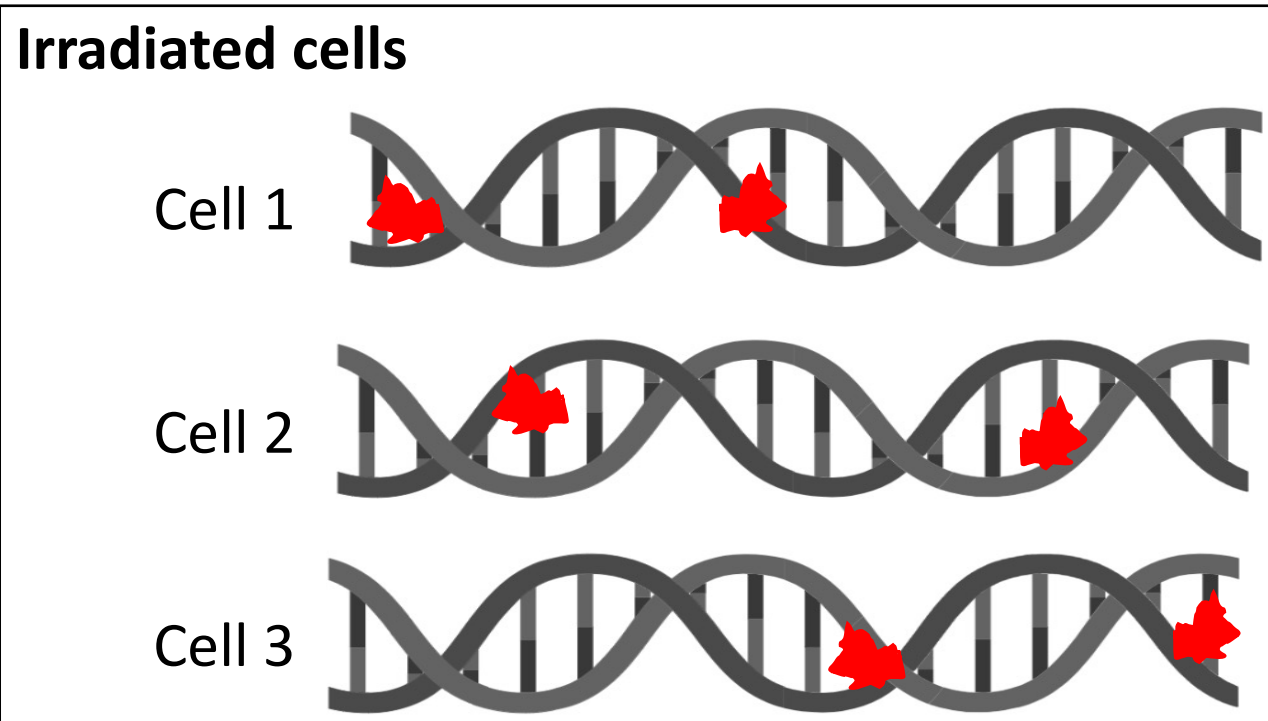
Behjati's samples





Our proposal

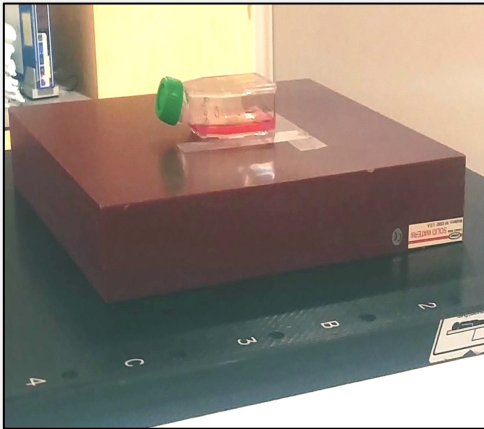




Our single-cell sequencing experiment

1

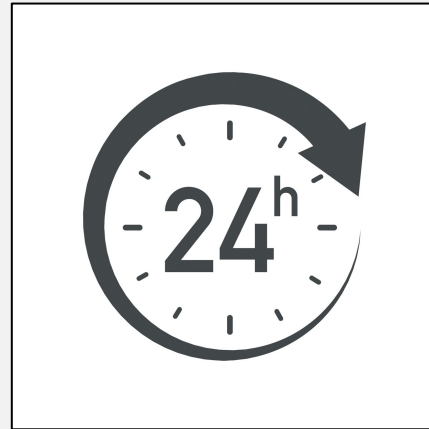
Irradiation



- B-lymphoblastoid cells
- 6 MV Photons
 - Control
 - 0.5 Gy
 - 1.5 Gy
 - 3.0 Gy

2

Incubation



24 hrs incubation for the cells to undergo one cell cycle (one repair cycle)

3

Sequencing



Single-Cell Whole Genome Sequencing

~500 cells per sample

4

Analysis

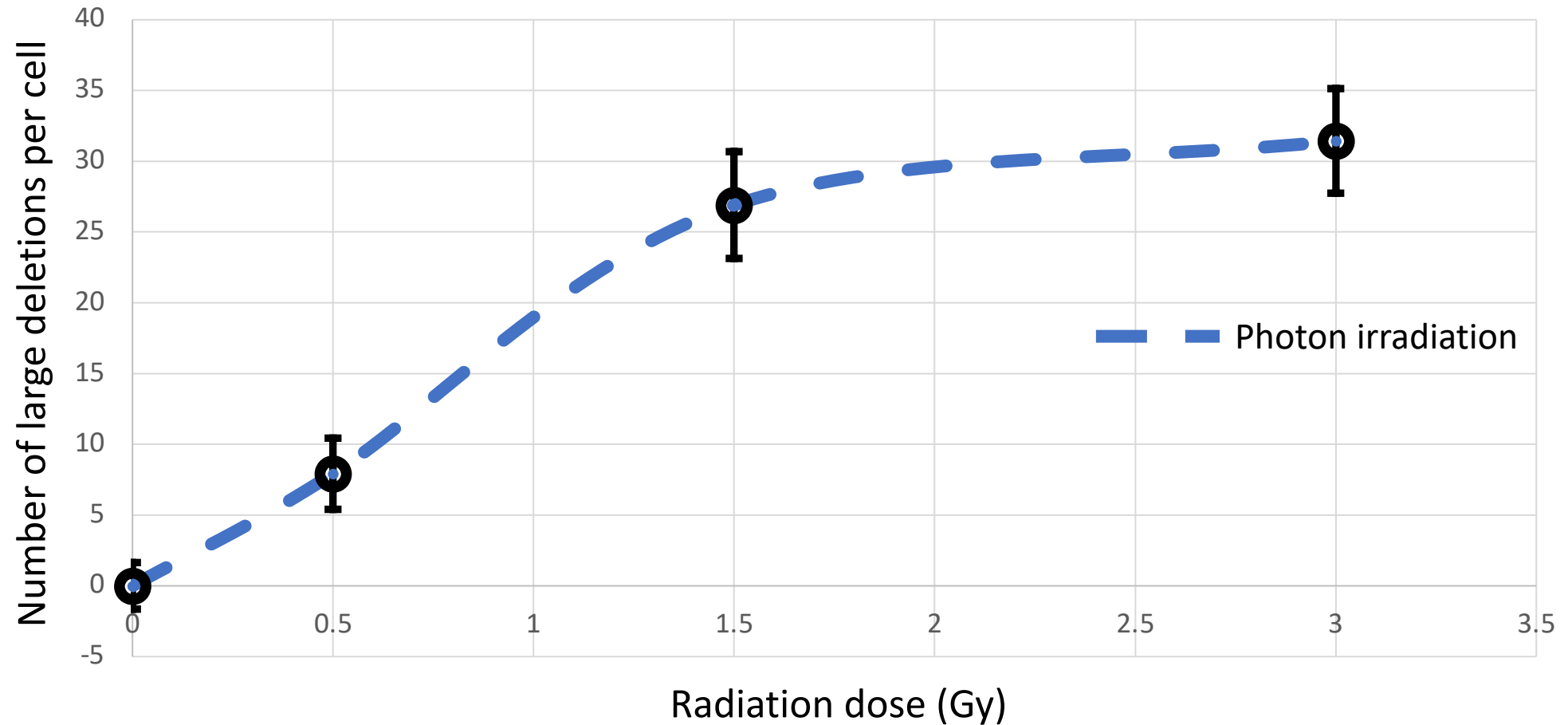


Mutation analysis of each individual cell sequenced.

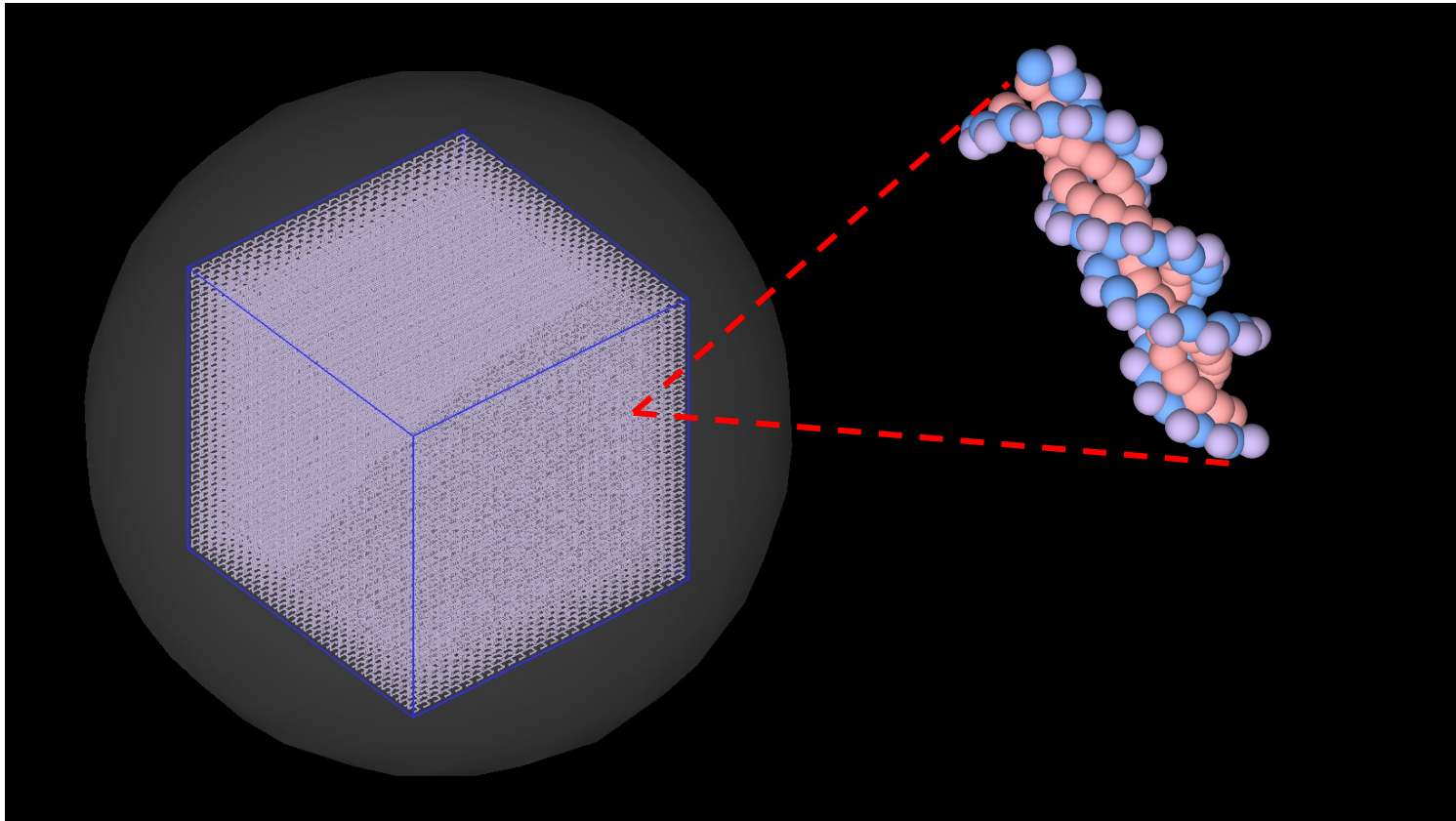
Large deletions and structural variations in the genome are measured

Preliminary result

Single-cell sequencing experiment result



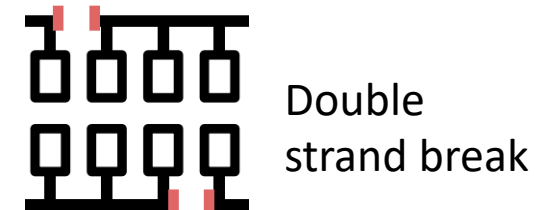
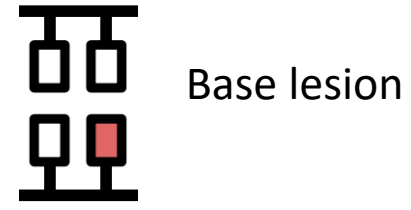
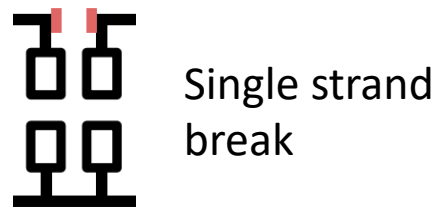
Our single-cell irradiation simulation



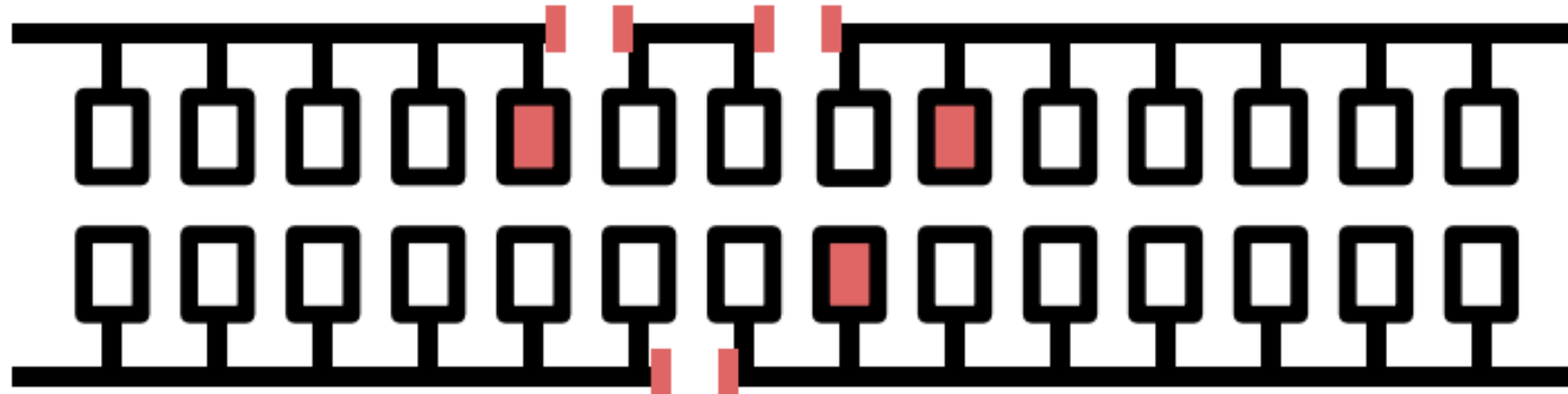
- Model built in TOPAS-nBIO simulation toolkit
- More than 100 cells were individual irradiated with doses:
 - 0.5 Gy
 - 1.5 Gy
 - 3.0 Gy
- Cluster of DNA damages (damages within 40bp from each other) that has at least one double strand break (DSB) is counted

Monte Carlo single-cell geometric nuclear DNA model

Poster by James Manalad (P2-17)

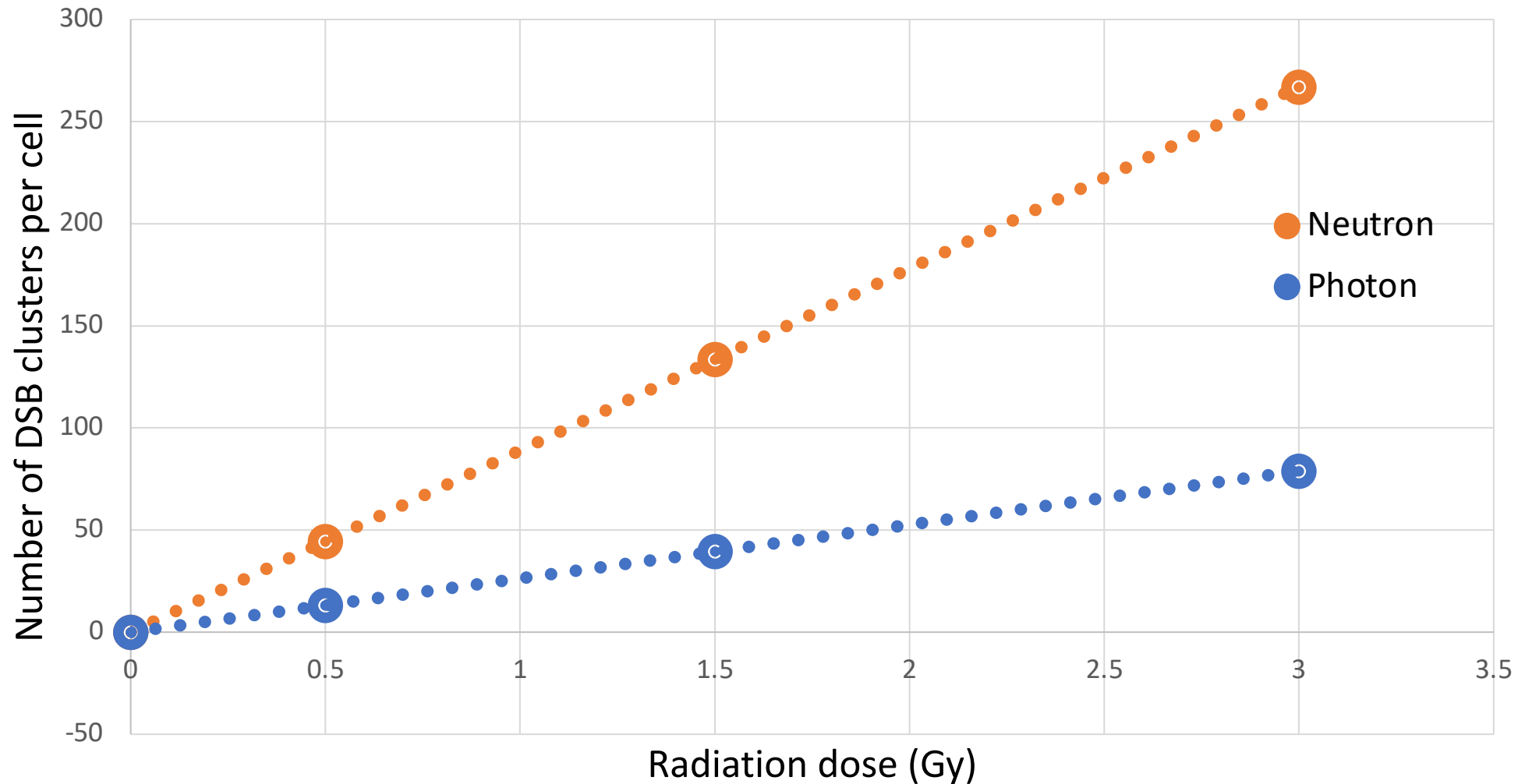


Double strand break cluster



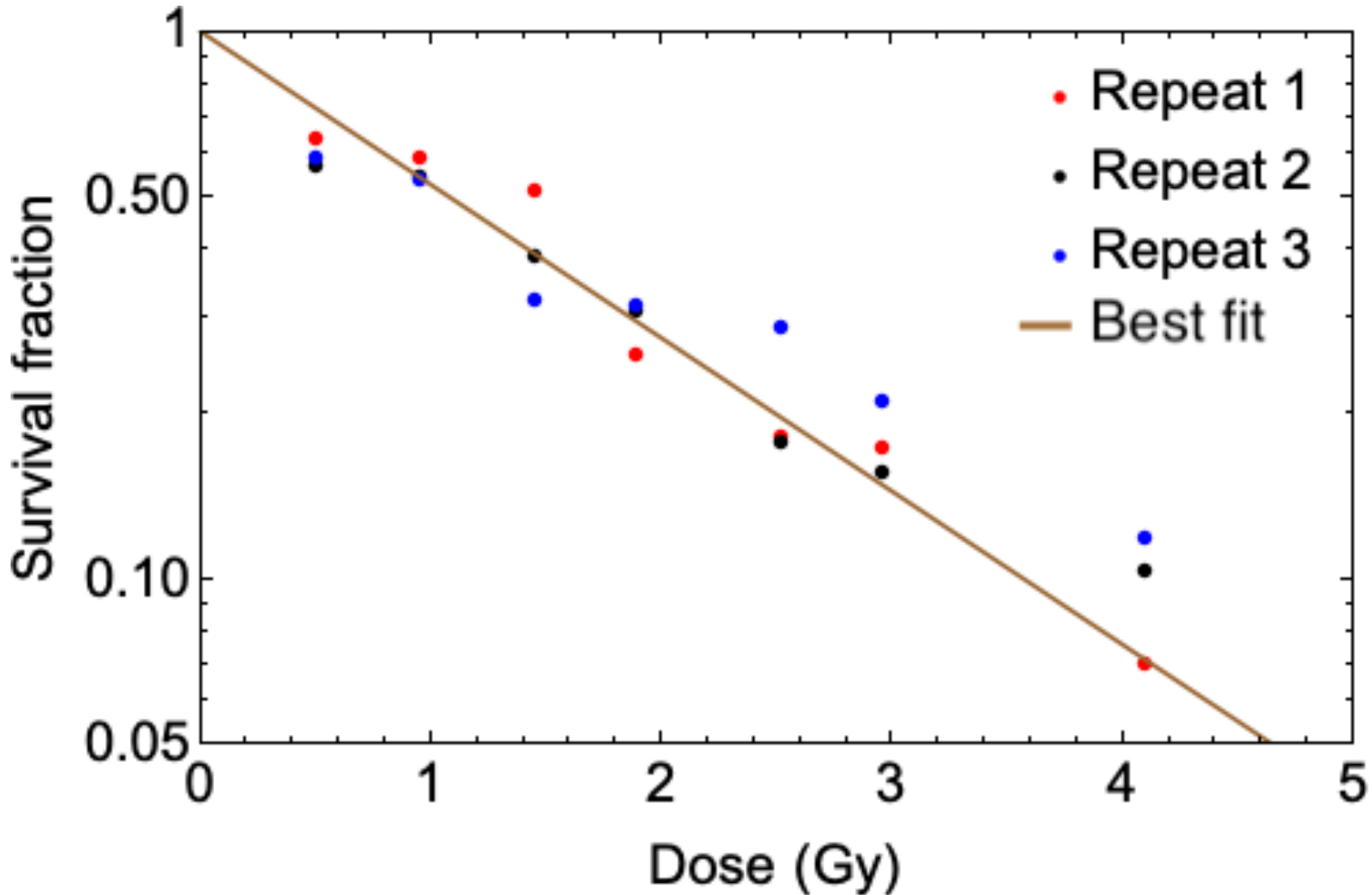
Montgomery et al. 2001

Single-cell irradiation simulation results

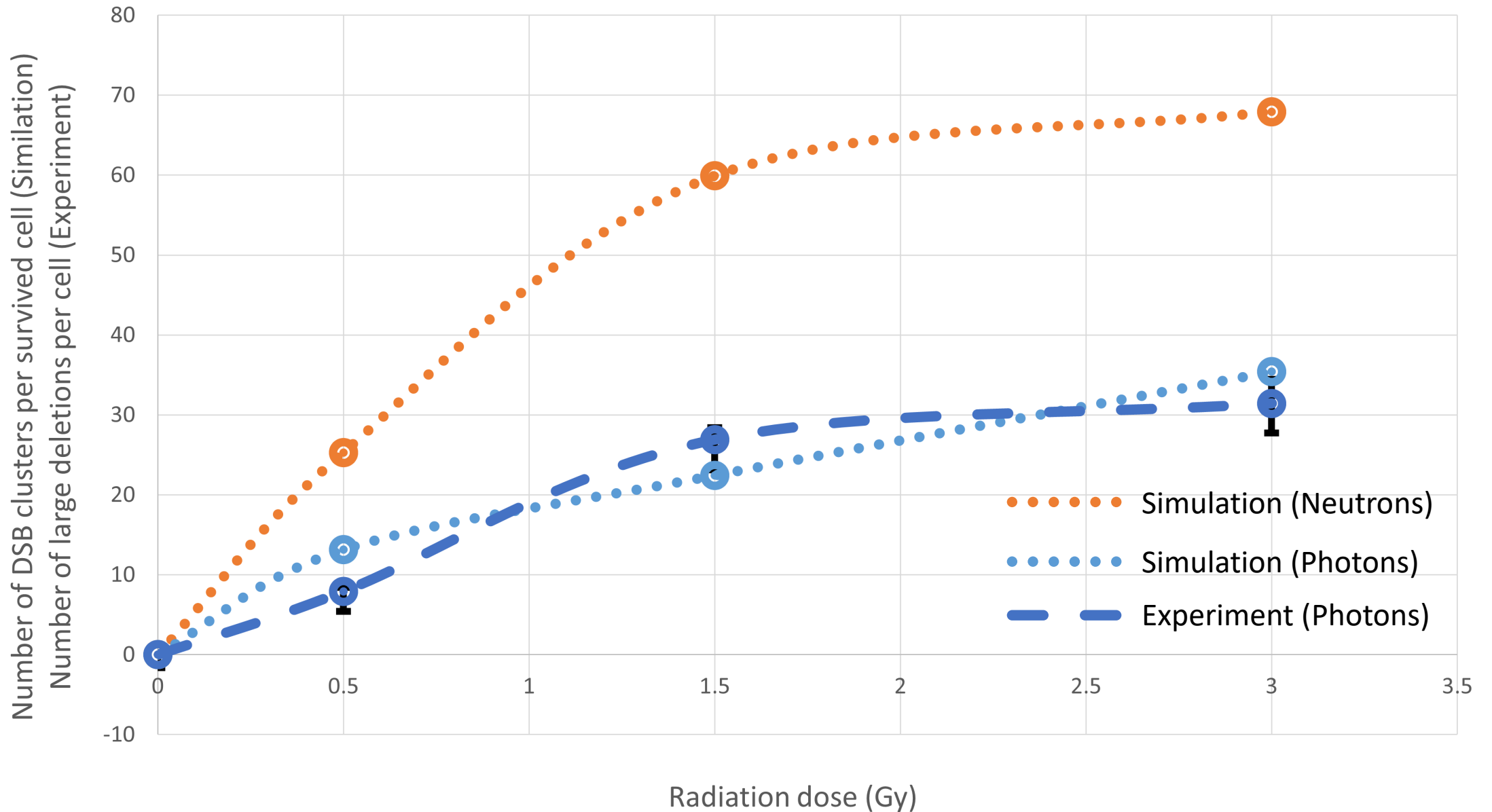


- Limitations:
1. No cell death
 2. No Repair

Survival curve of our cells



Simulation results adjusted for cell death



Summary

- We believe we will be able to discern radiation induced tumours according to the radiation quality based on the mutational patterns involved.
- We performed single-cell sequencing of irradiated cells and found a dose dependent increase in the number mutations across the genome.
- We also performed single-cell irradiation simulations and found agreement between simulation and experiment.
- Experiment needs to be repeated and the preliminary findings must be validated.

Potential applications of this work

- Learn about carcinogenesis and mutation signature of neutrons.
- Discern radiation type involved in biological data.
- Develop new bio-dosimetry techniques
- Develop predictive risk models.

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