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

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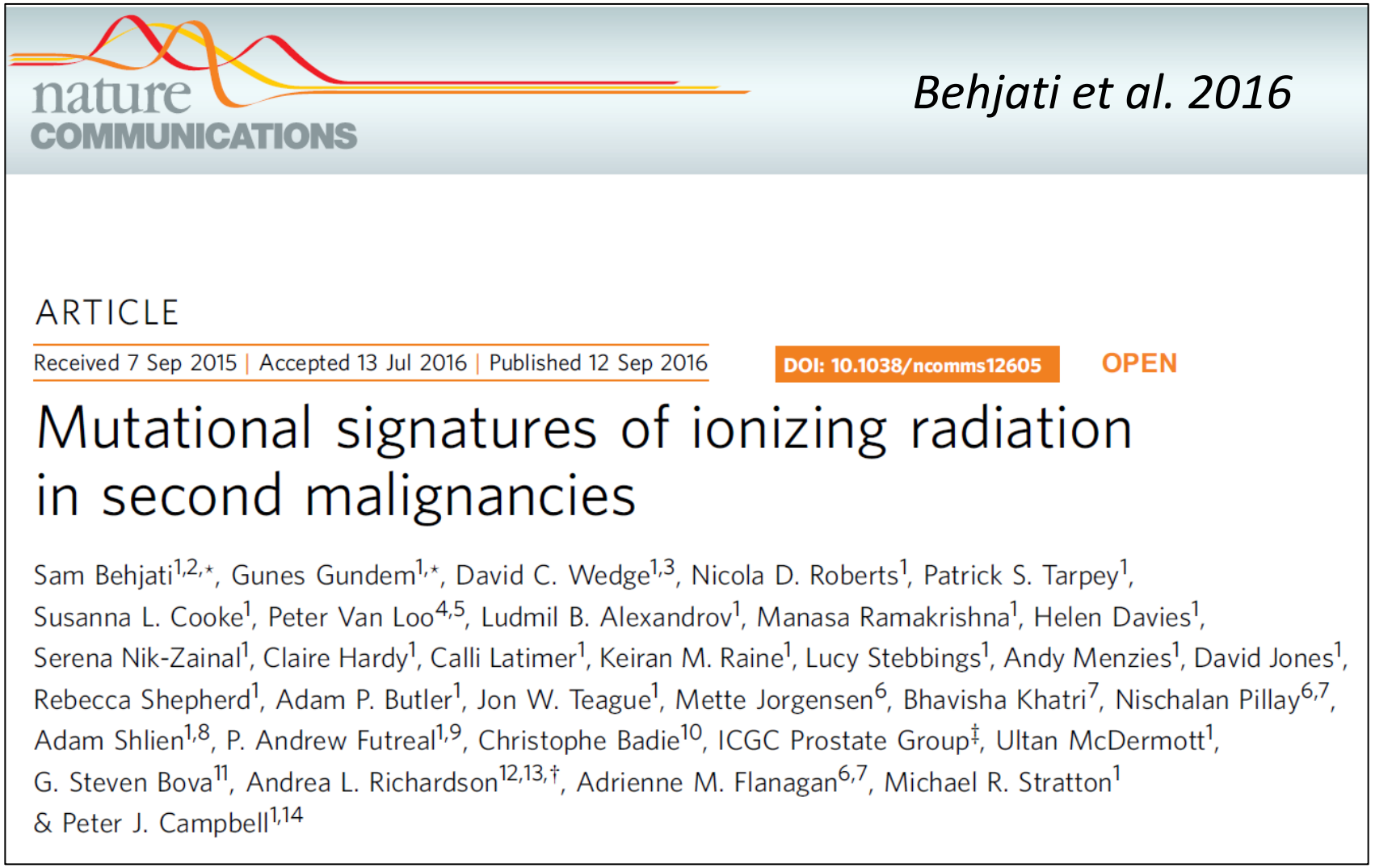
8 November 2022

McGill

Mutation signature of radiation



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Behjati et al. 2016

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Mutational signatures of ionizing radiation in second malignancies

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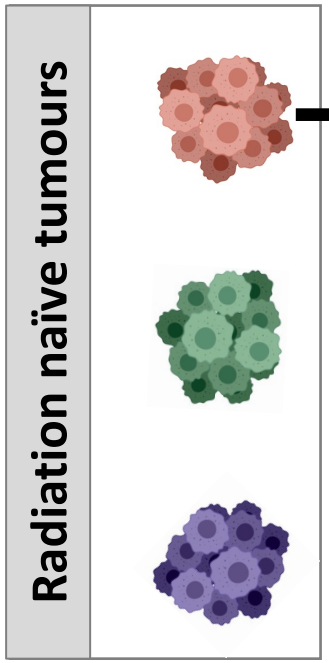
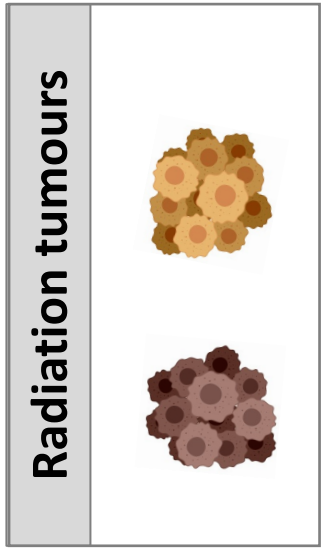
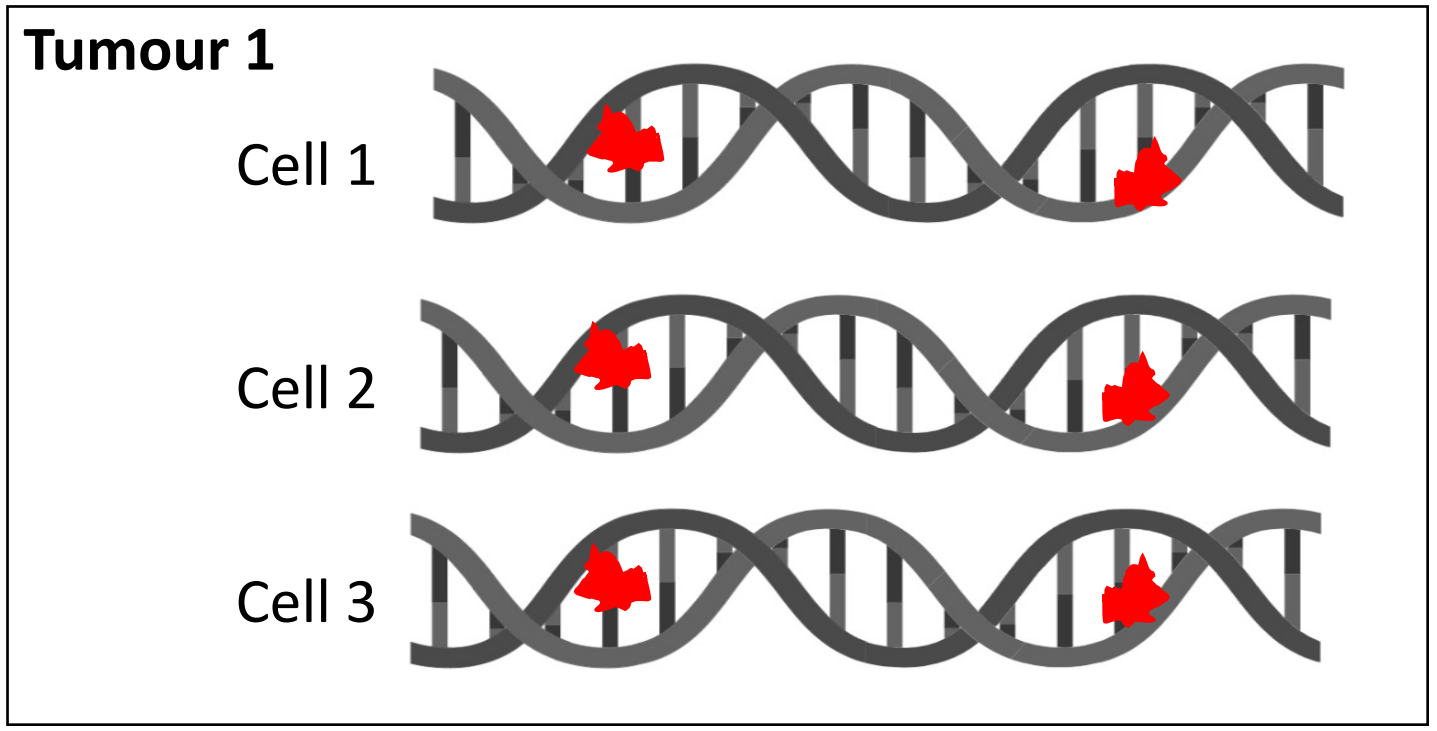
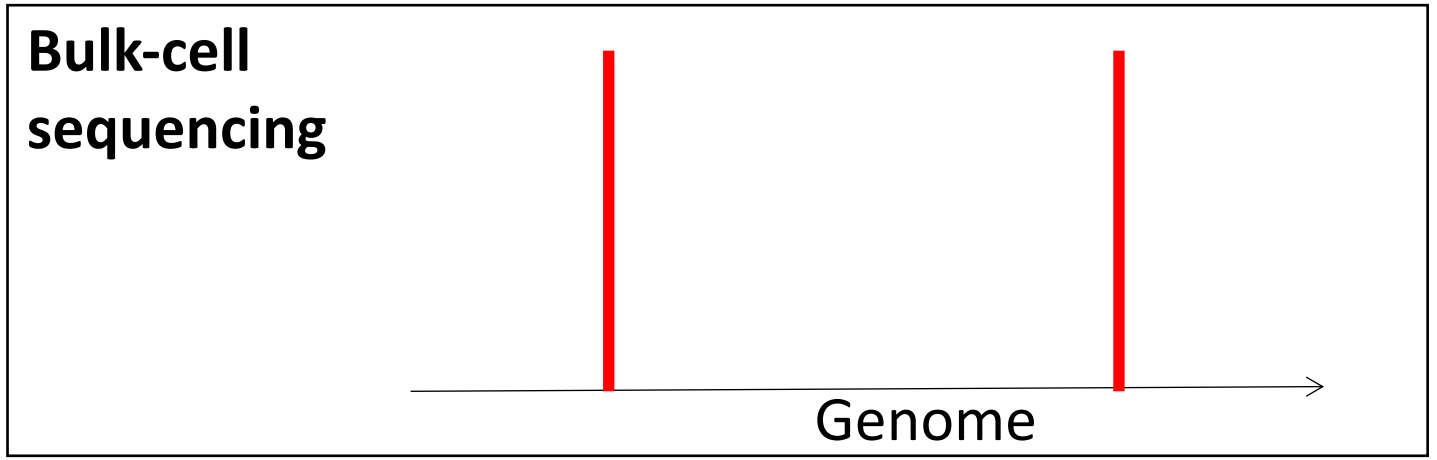
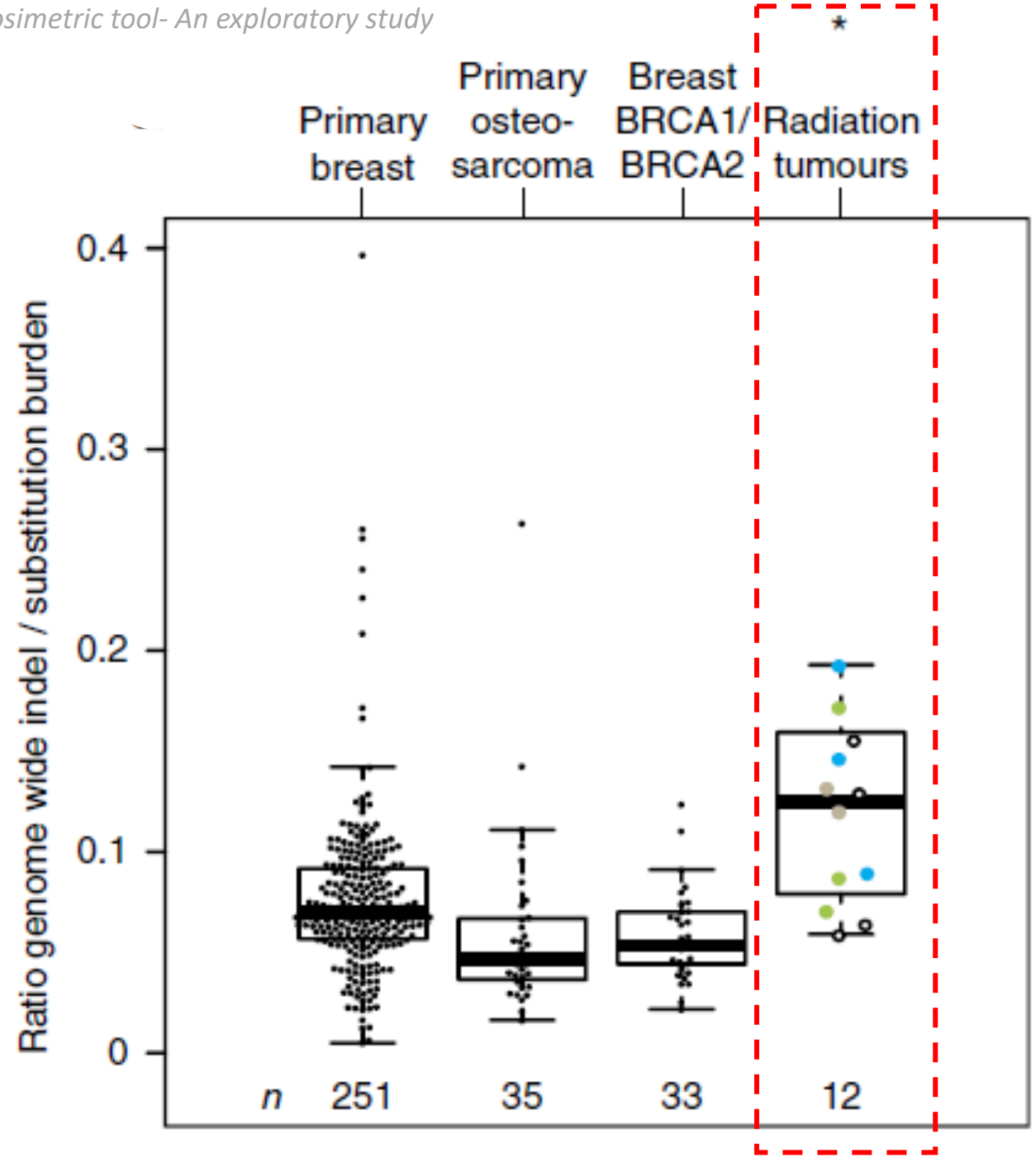
Behjati's samples











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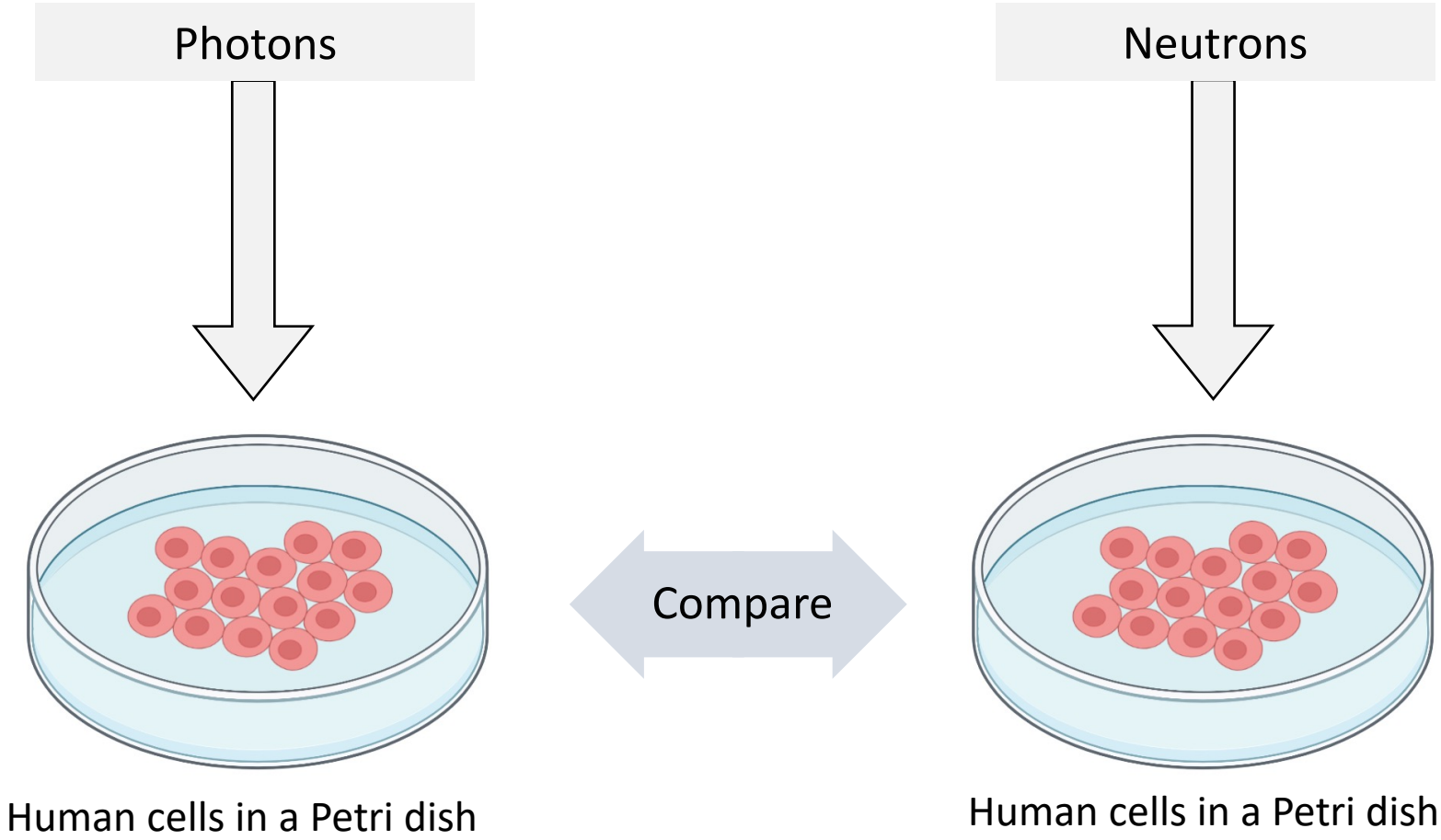
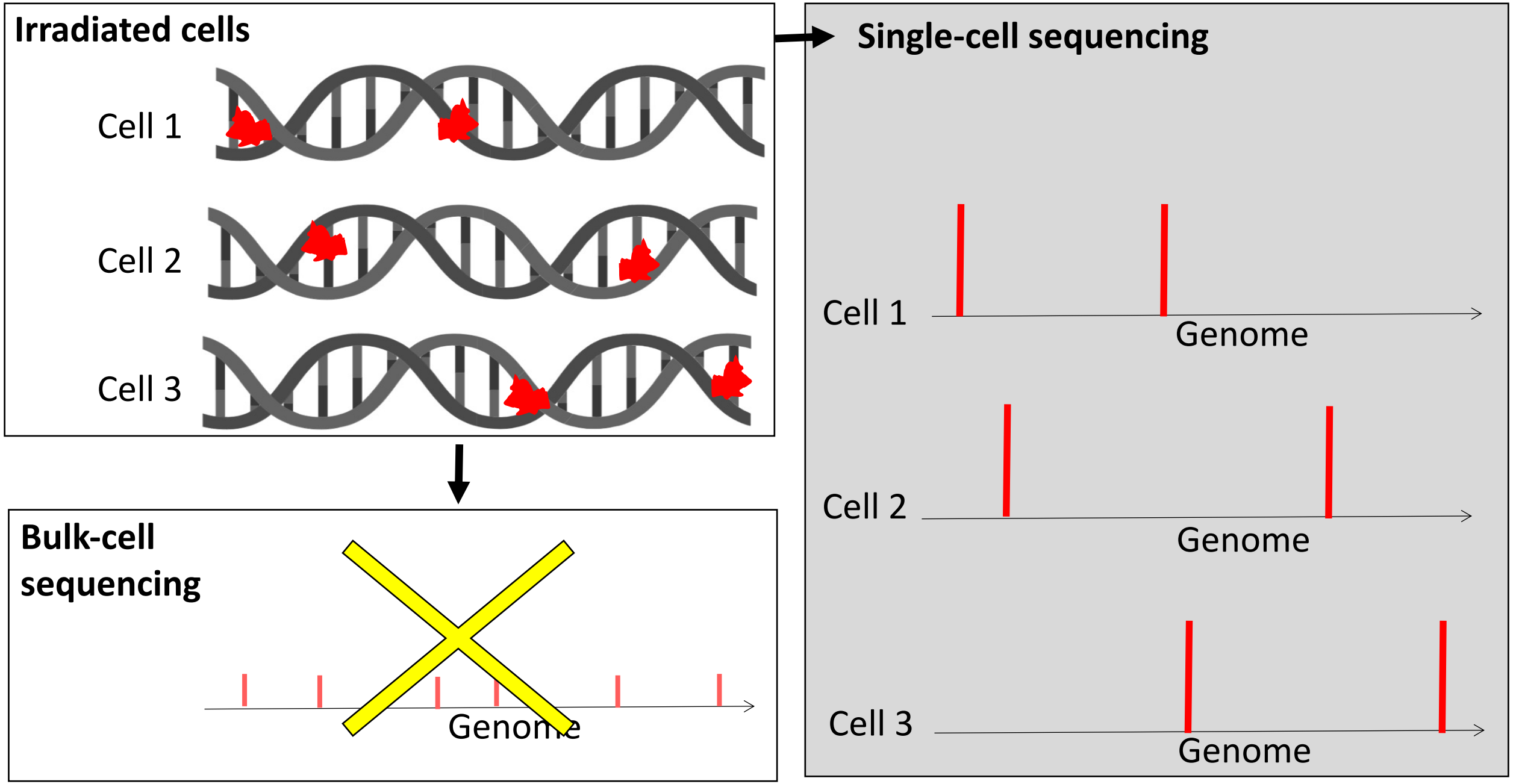
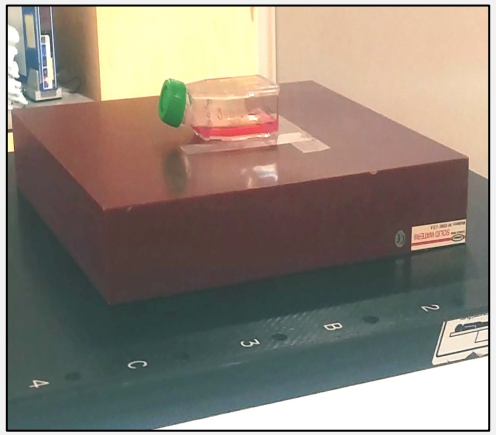
Our proposal




Irradiated cells
Cell 1
Cell 2
Cell 3
Bulk-cell sequencing
Genome
Single-cell sequencing
Cell 1
Genome
Cell 2
Genome
Cell 3
Genome

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

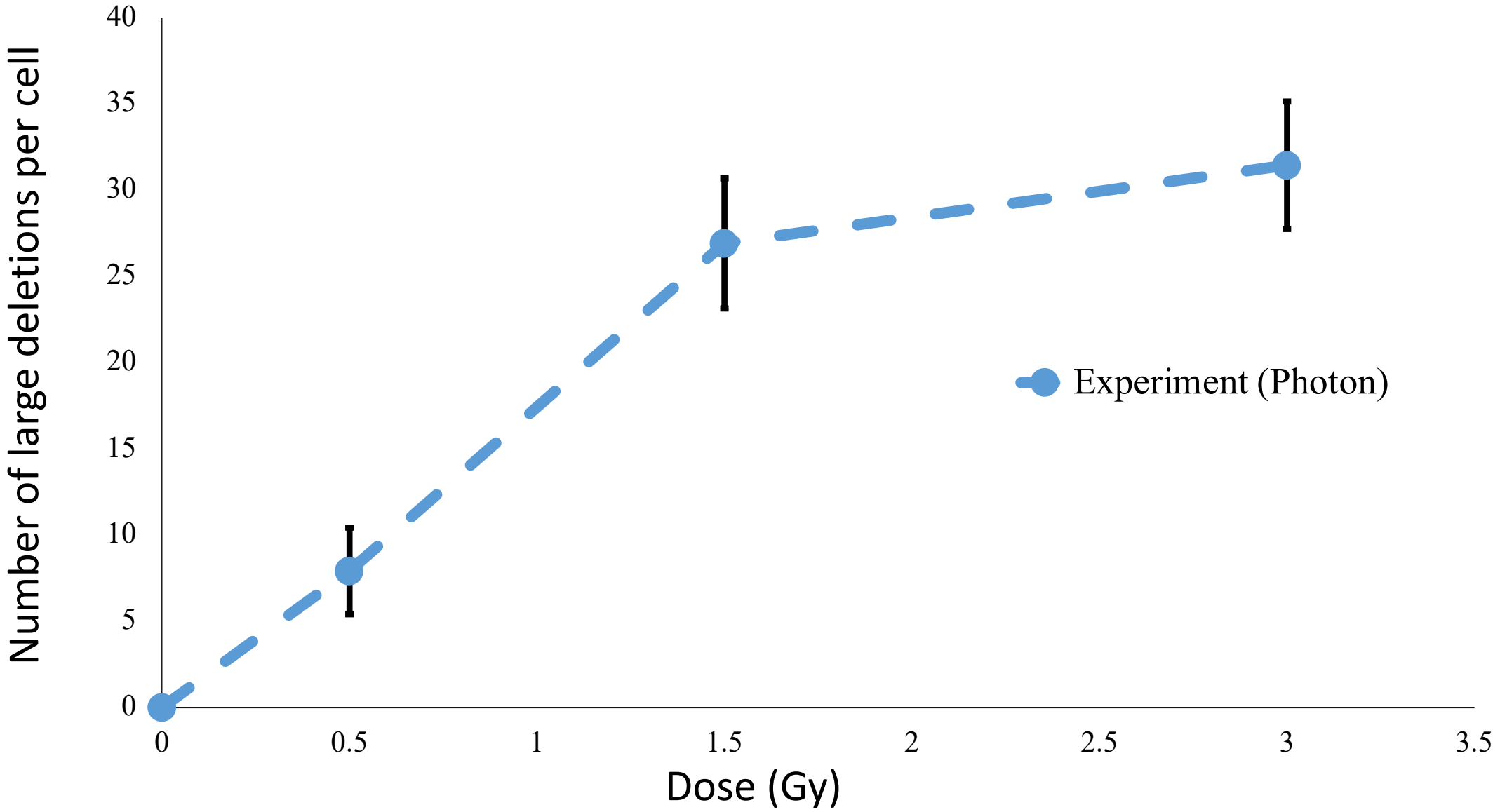
~500 cells per sample

4 Analysis

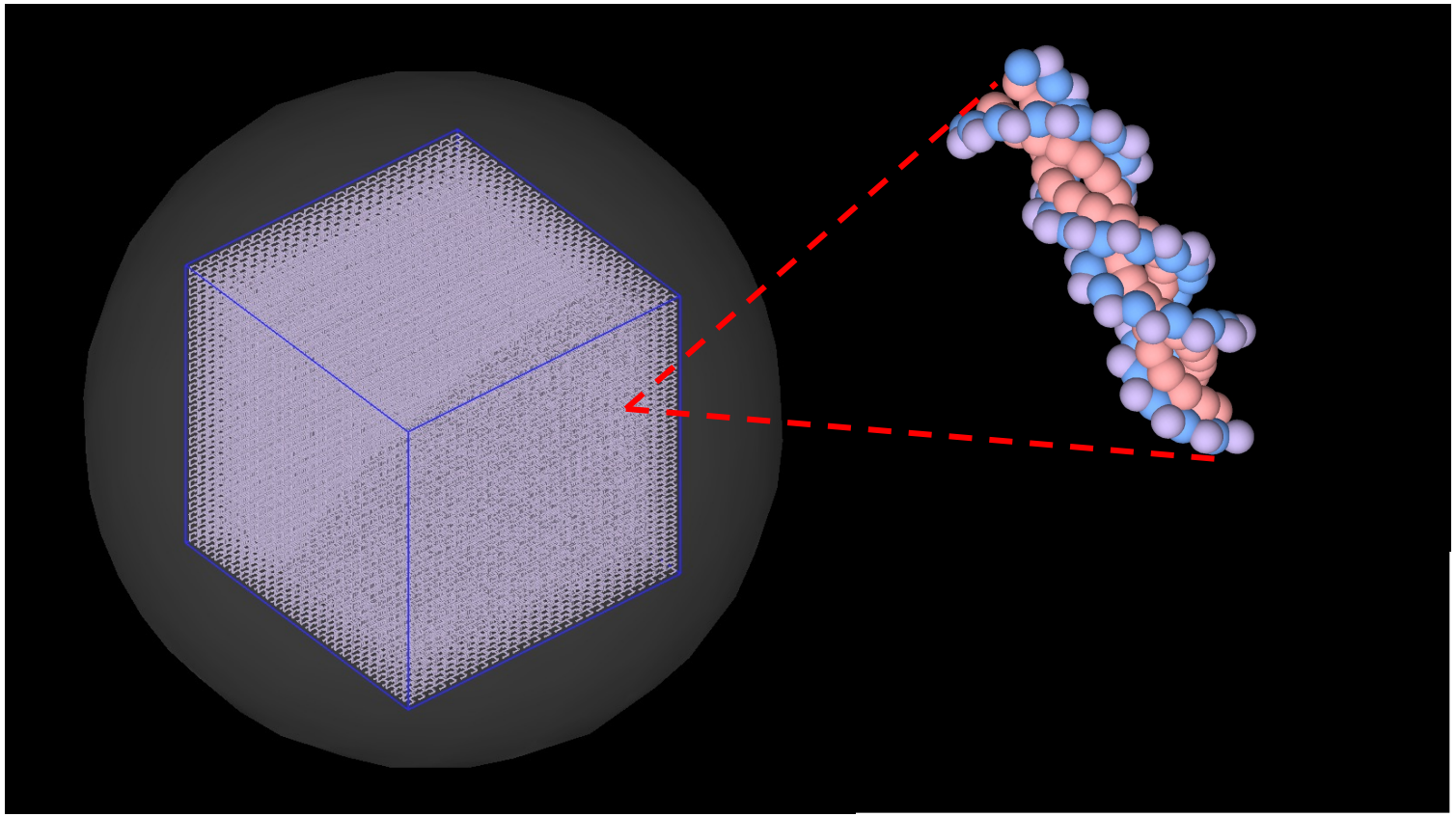
Mutation analysis of each individual cell sequenced.

Large deletions and structural variations in the genome are measured

Preliminary result

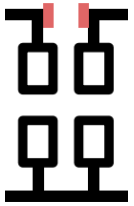


Our single-cell irradiation simulation

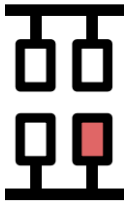


Monte Carlo single-cell geometric nuclear DNA model

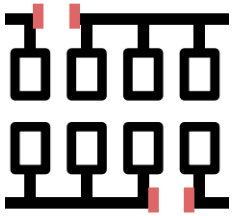
- Model built in TOPAS-nBIO simulation toolkit
- More than 100 cells were individual irradiated with doses:
  - 0.5 Gy
  - 1.5 Gy
  - 3.0 Gy
- Cluster of DNA damages (damages within 40bp from each other) that has at least one double strand break (DSB) is counted



Single strand break

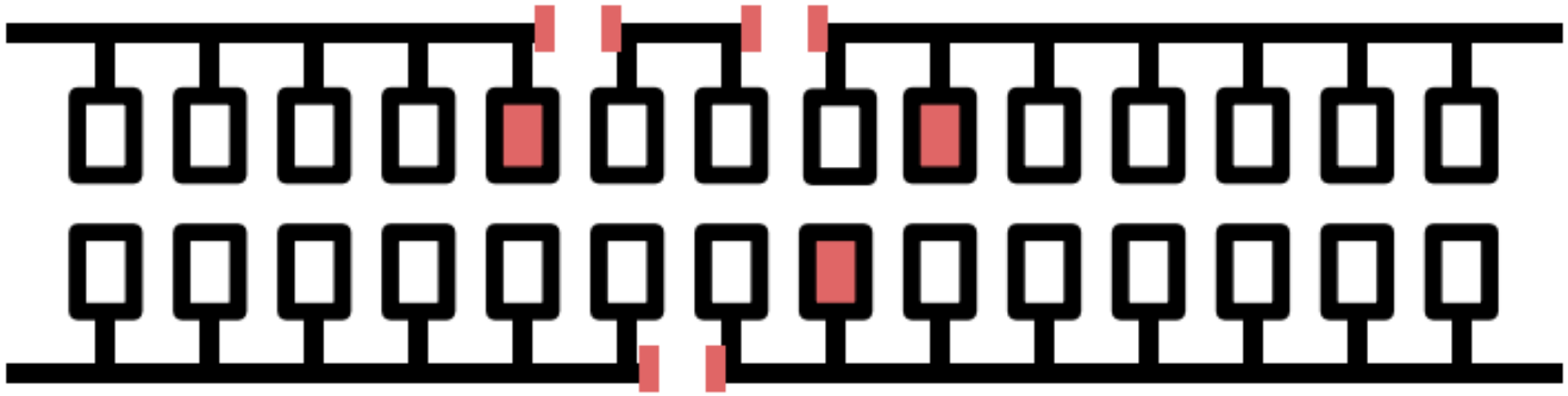


Base lesion

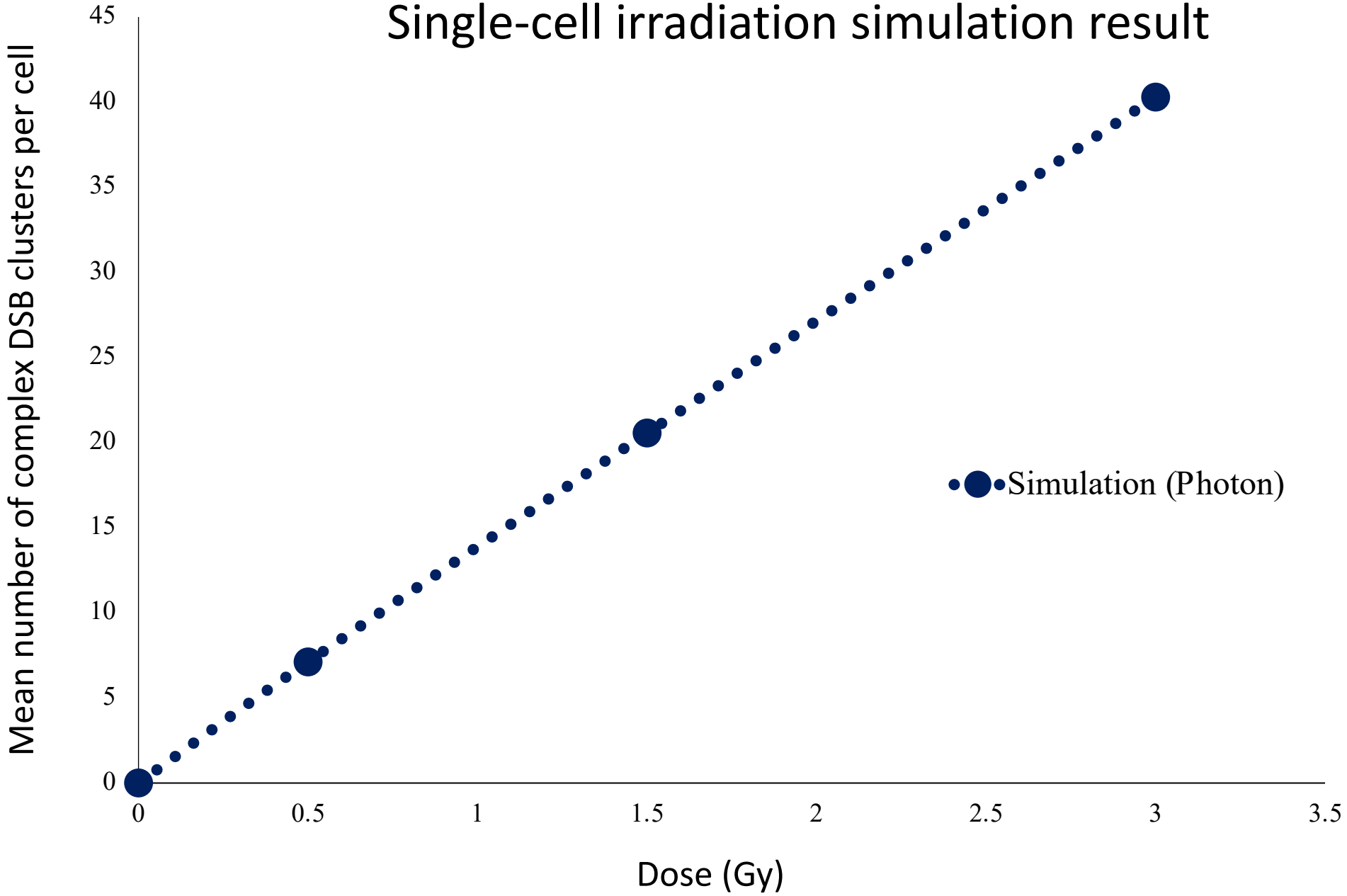


Double strand break

Double strand break cluster

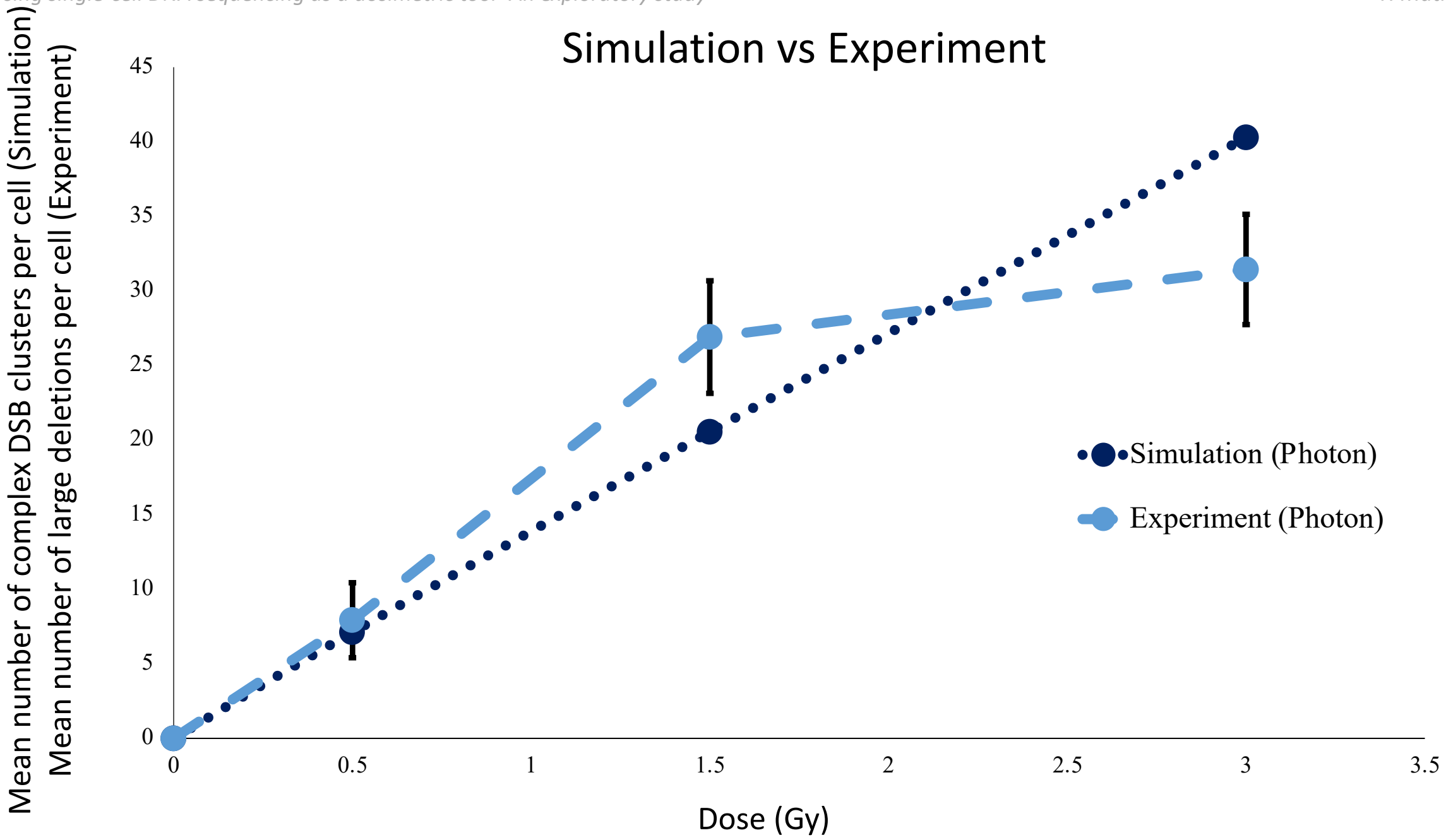


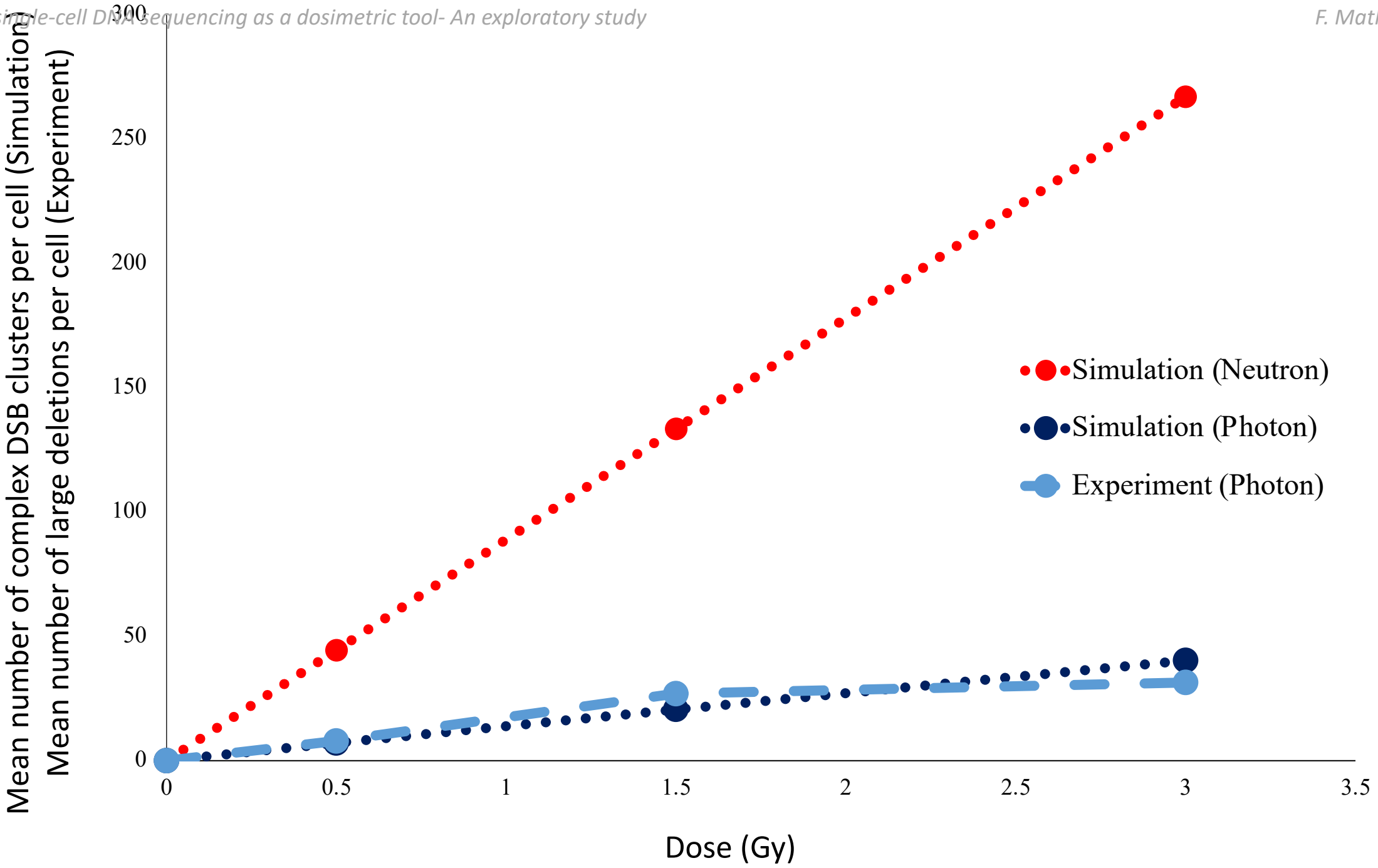
Montgomery et al. 2001



Limitations:

1. No cell death
2. No Repair


Simulation vs Experiment
Mean number of complex DSB clusters per cell (Simulation)
Mean number of large deletions per cell (Experiment)
45
40
35
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
Dose (Gy)
Simulation (Photon)
Experiment (Photon)


Mean number of complex DSB clusters per cell (Simulation)
Mean number of large deletions per cell (Experiment)
300
250
200
150
100
50
0
0
0.5
1
1.5
2
2.5
3
3.5
Dose (Gy)
Simulation (Neutron)
Simulation (Photon)
Experiment (Photon)

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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