LhARA Radiobiology Programme

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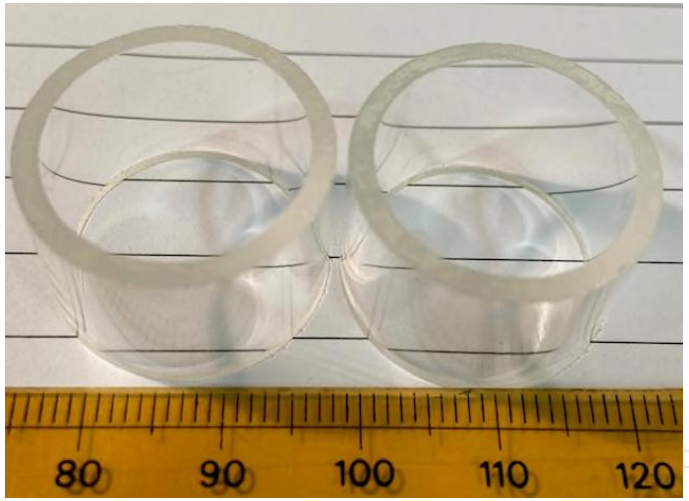
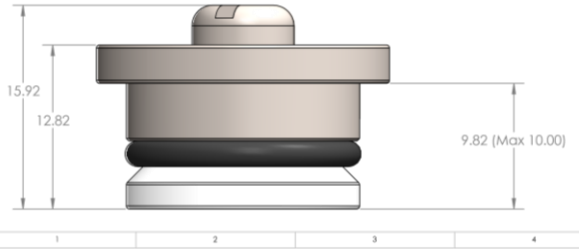
Radiobiology Set up at SCAPA

~10 MeV – grow cells on 2.5 µM Mylar in glass rings

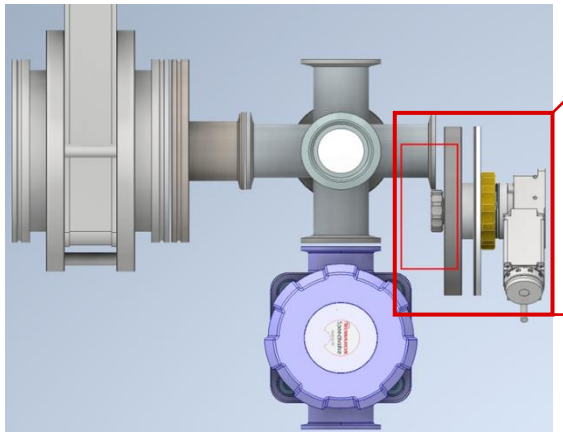


Internal diameter – 18.5 mm

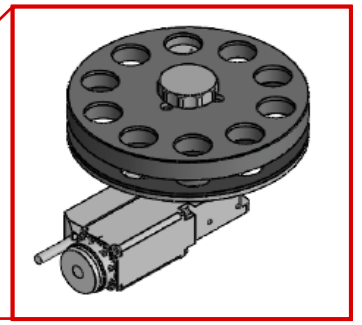
Outer diameter – 22.5 mm



Sealable lids



Glass ring holder



Leave a slot free for dosimetry?

CONV - 0.1Gy per pulse separated by 1 sec = **6Gy/min**

ULTRA-HIGH - 1-3Gy/pulse (2ns) = 0.5-1.5 GGy/sec (**~10^9 Gy/s**)

Initial radiobiology experiments at SCAPA

HeLa and FaDu cell lines

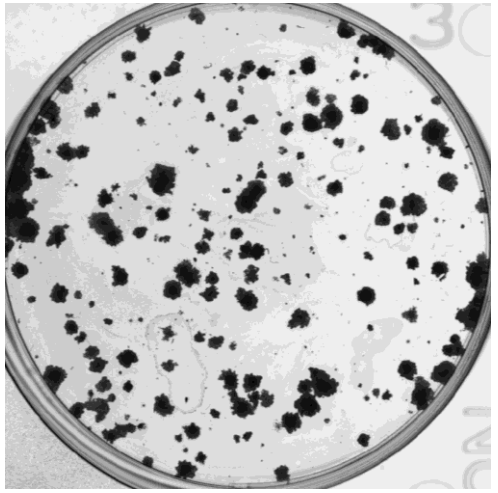
Clonogenic assays

ULTRA-HIGH – 1, 2, 3Gy – in triplicate

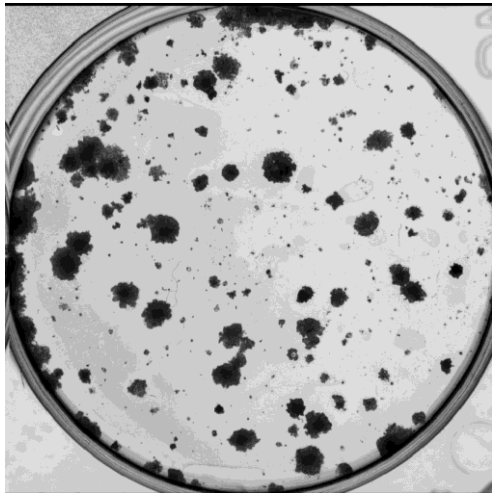
CONV – 1, 2, 3, 4, 6, 8Gy – in triplicate

Need at least 3 independent biological repeats

Control



4Gy



Clonogenic Optimisation

Control
200

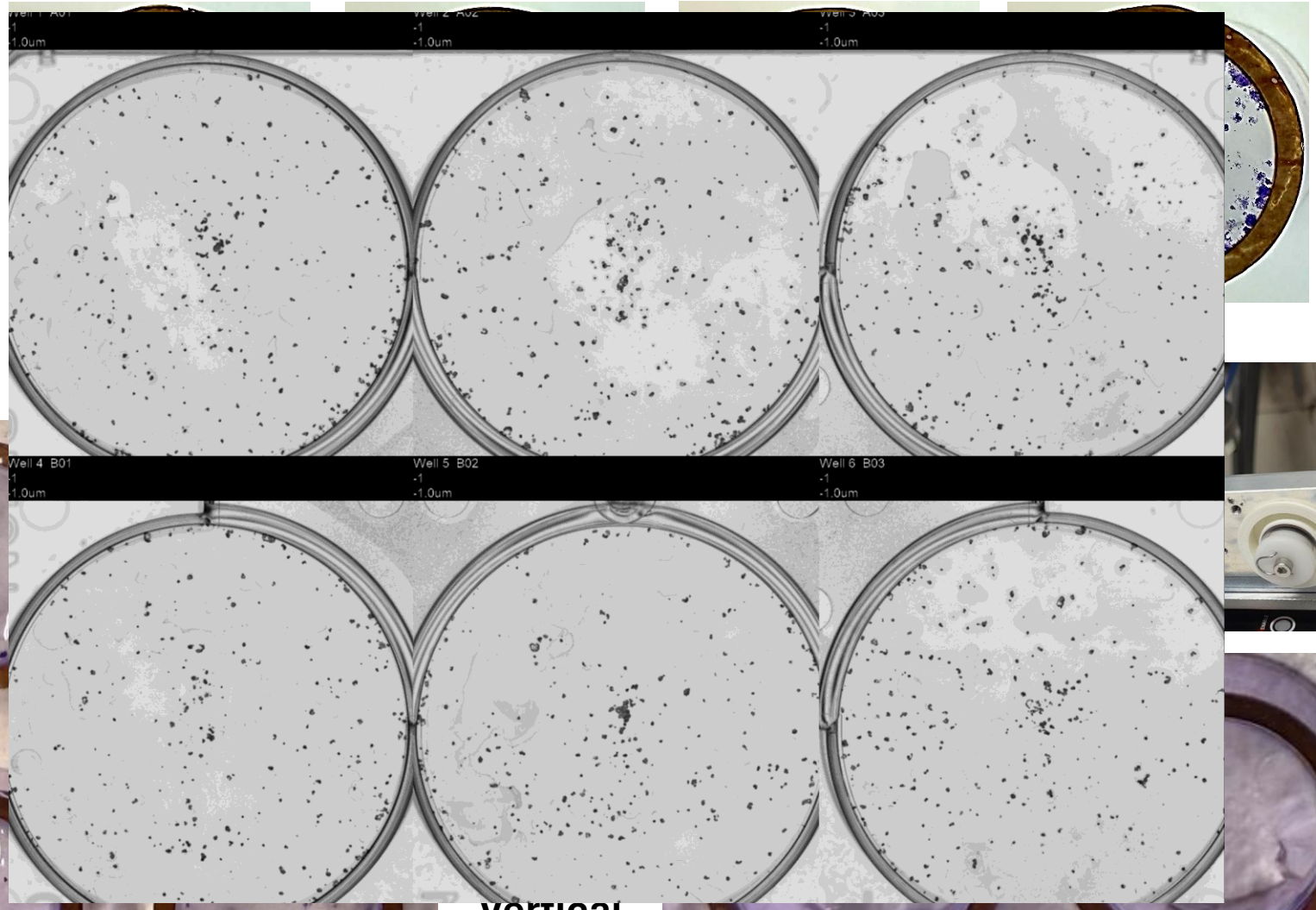
1Gy
200

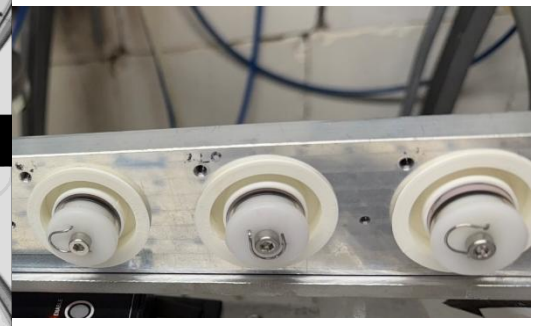
2Gy
800

4Gy
800

X-ray
Pre-plated

Horizontal

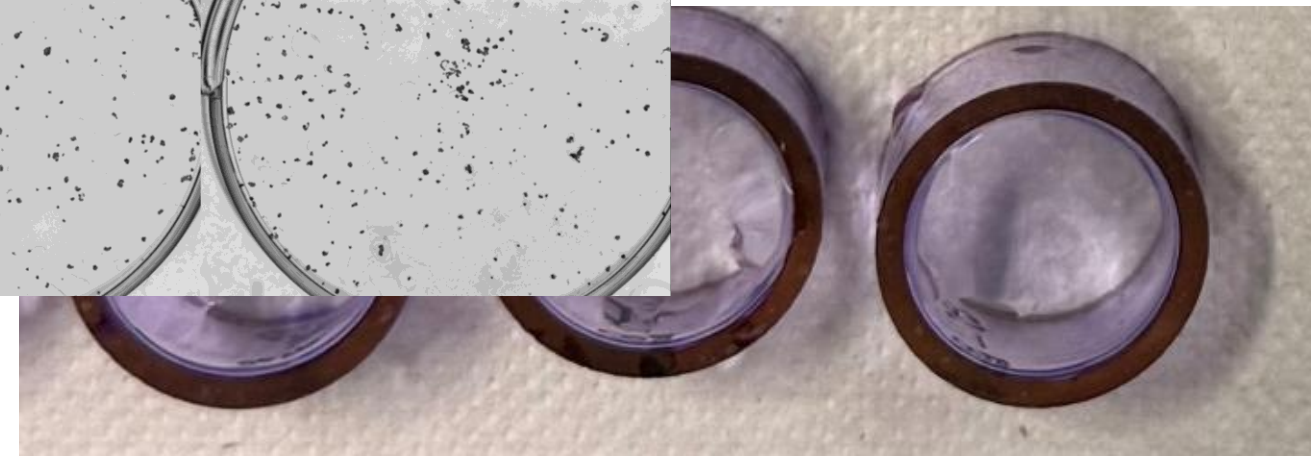




Control
(not mock IR)

Horizontal

Vertical

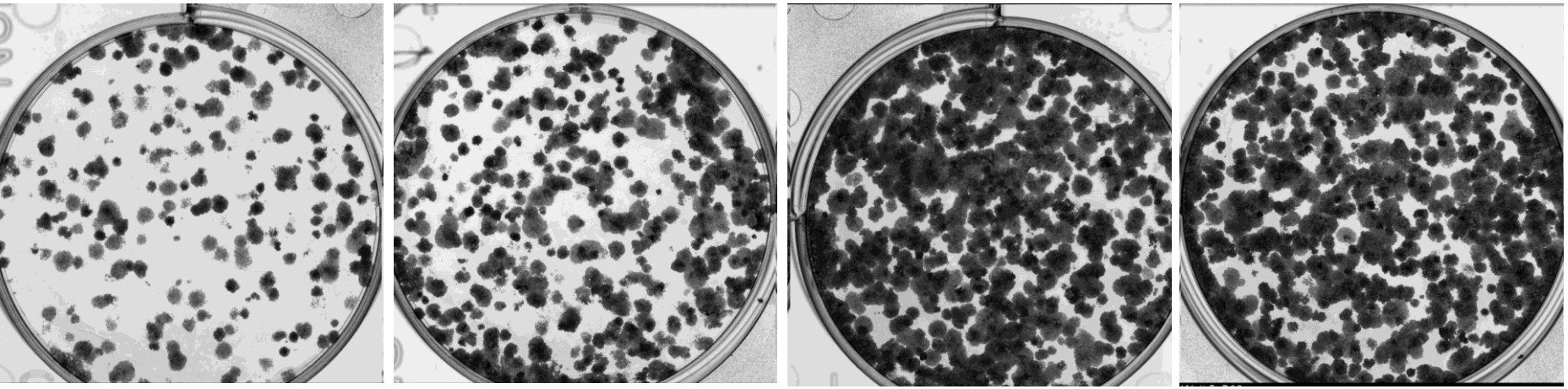


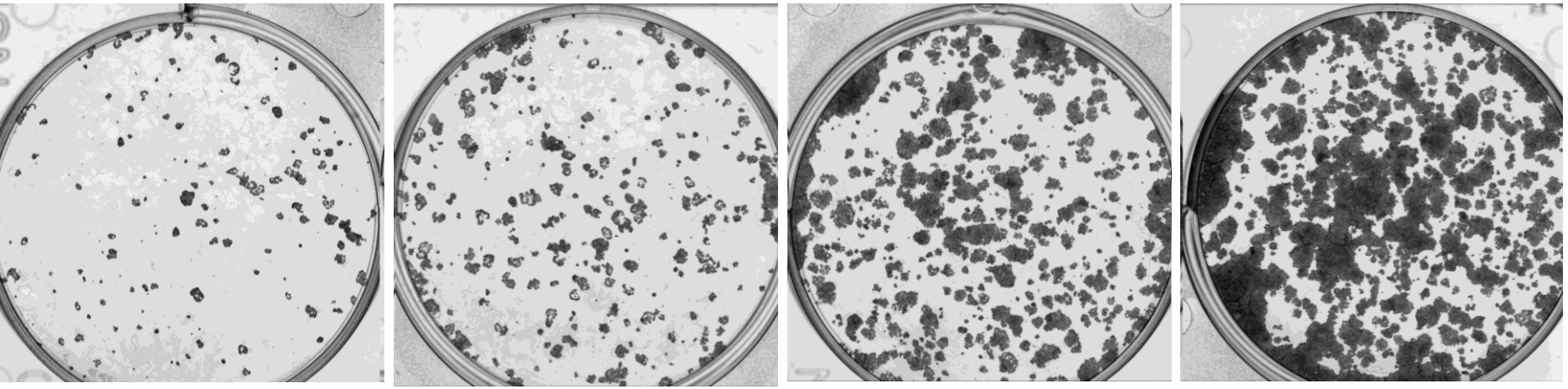
07.11.2025

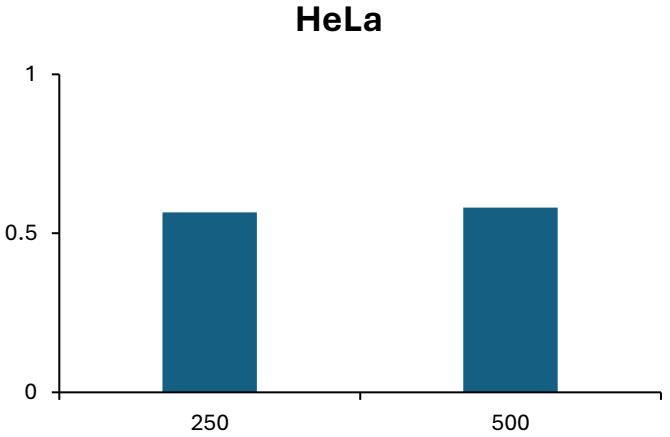
SCAPA Dry Run

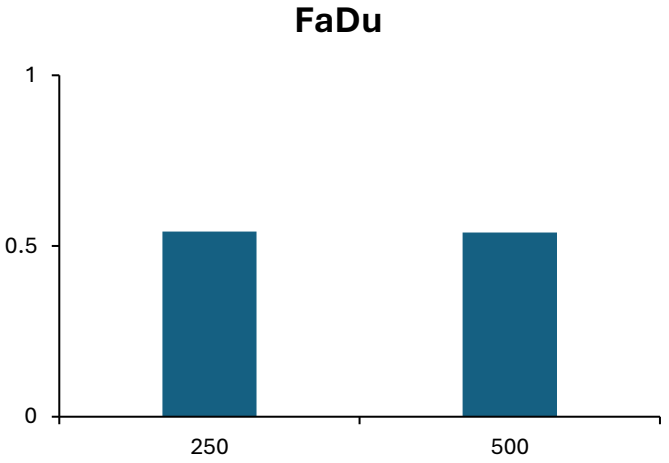
- Prepared for irradiation (lids/holder)
- Left vertically for 15-20min
- Replated

250 500 1000 2000









10 days post-seeding

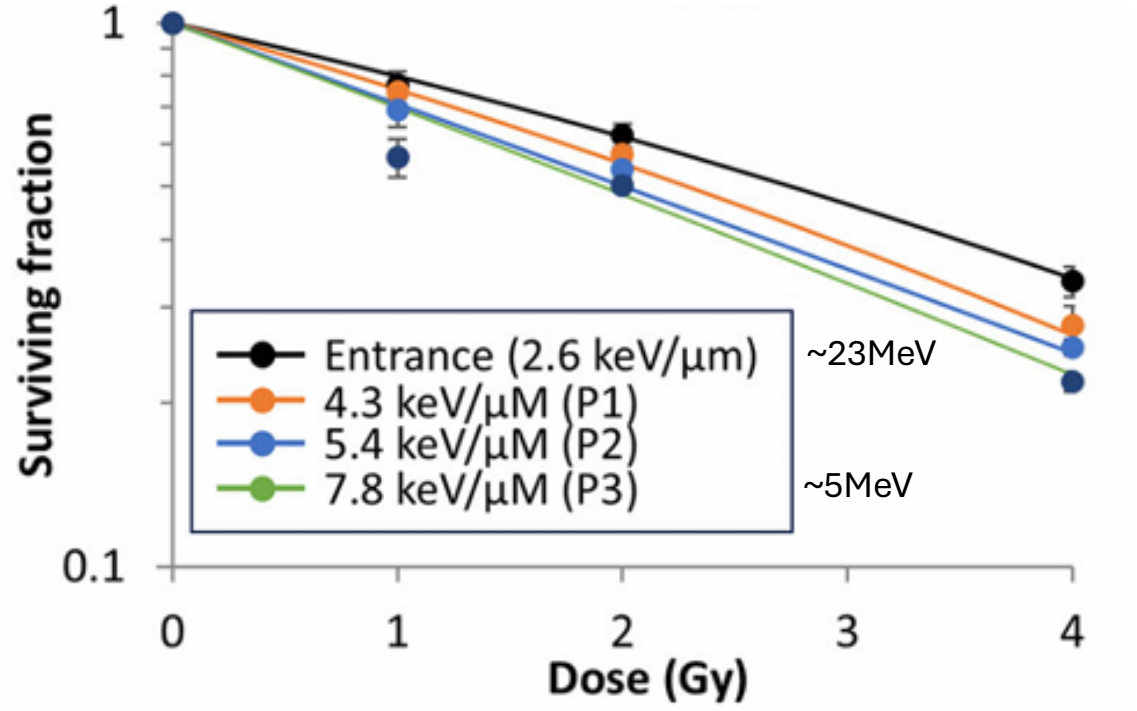
SCAPA Irradiation Plan

HeLa & FaDu
0Gy – 125, 250, 500
1Gy – 250, 500, 1000
2Gy – 500, 1000, 2000
4 Gy – 1000, 2000, 4000

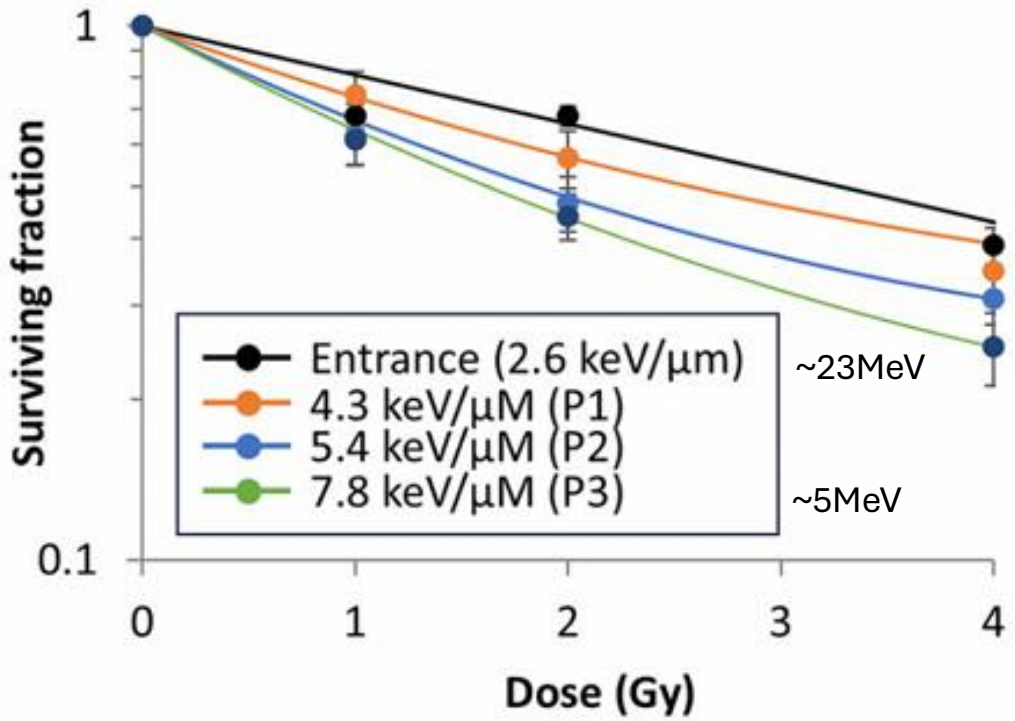
Day 1 – Dose titration 0, 1, 2, 4 Gy
Day 2 – Repeat
Day 3 – Determine shot-to-shot survival reproducibility (3x repeats of 2 Gy [4shots])

28MeV Proton Beam (MC-40 Cyclotron, Birmingham)

HeLa



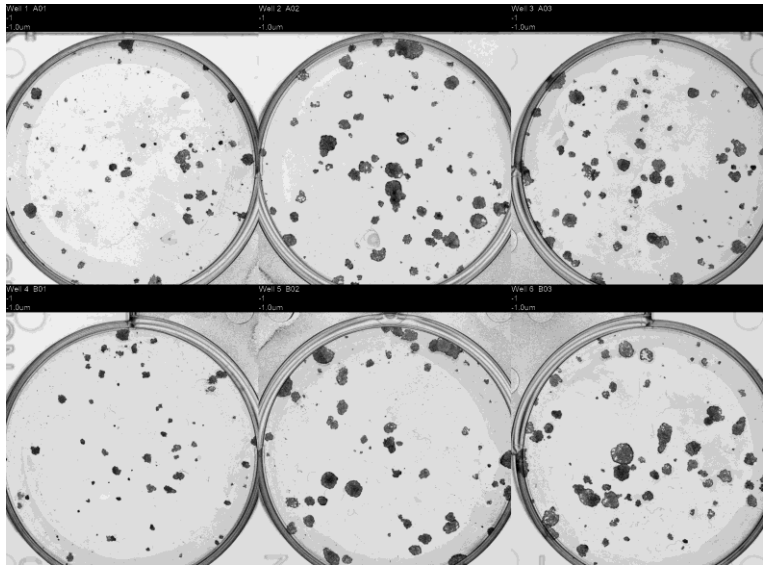
FaDu

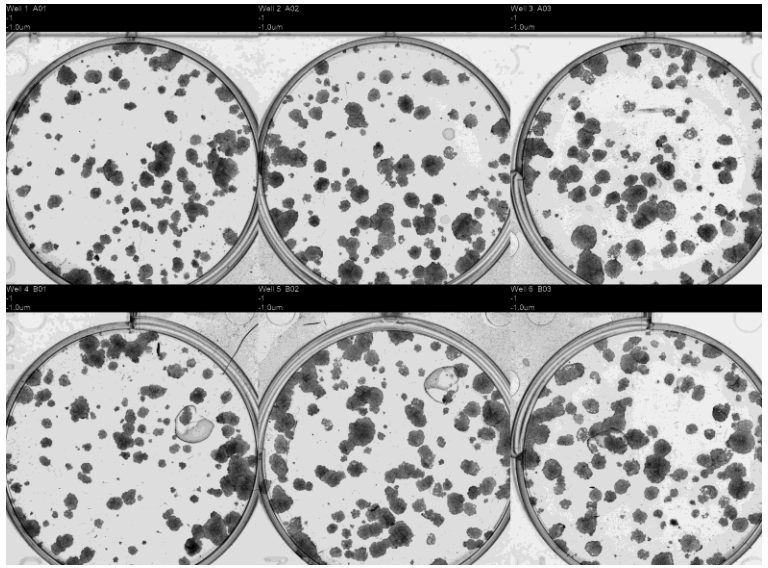


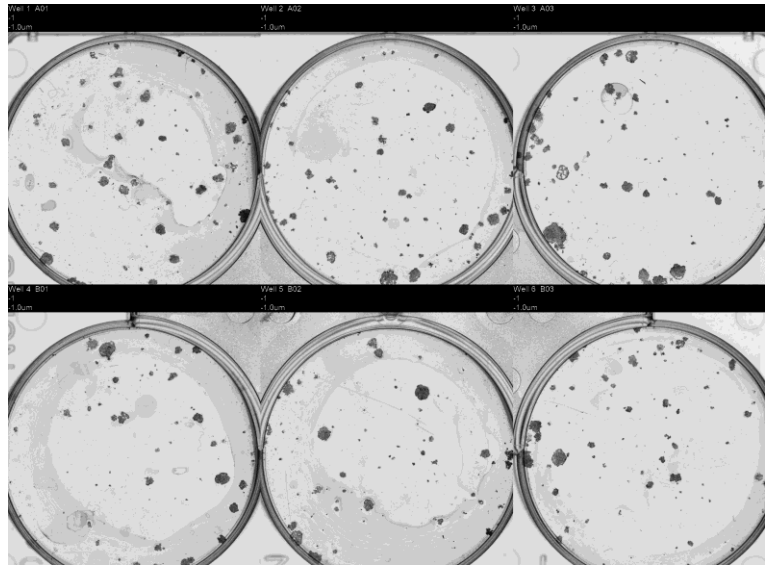
(Fabbrizi, et al. 2025)

SCAPA Irradiation Plates

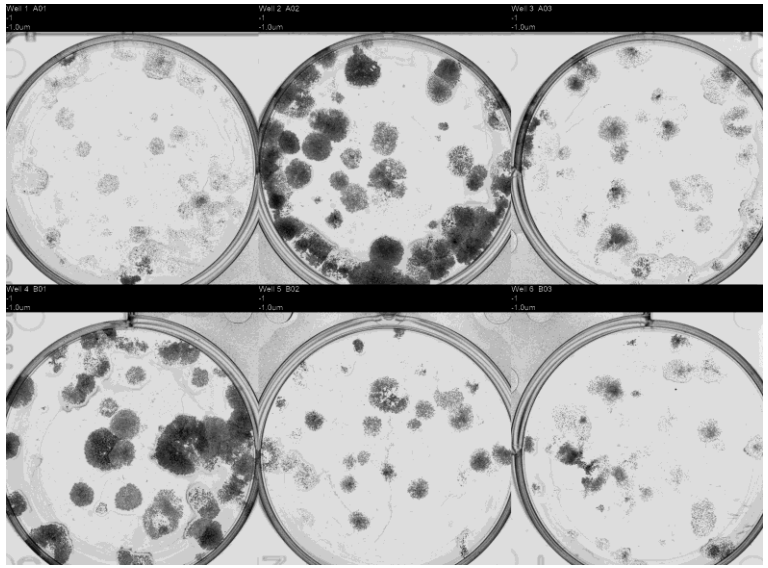
Dose Titration

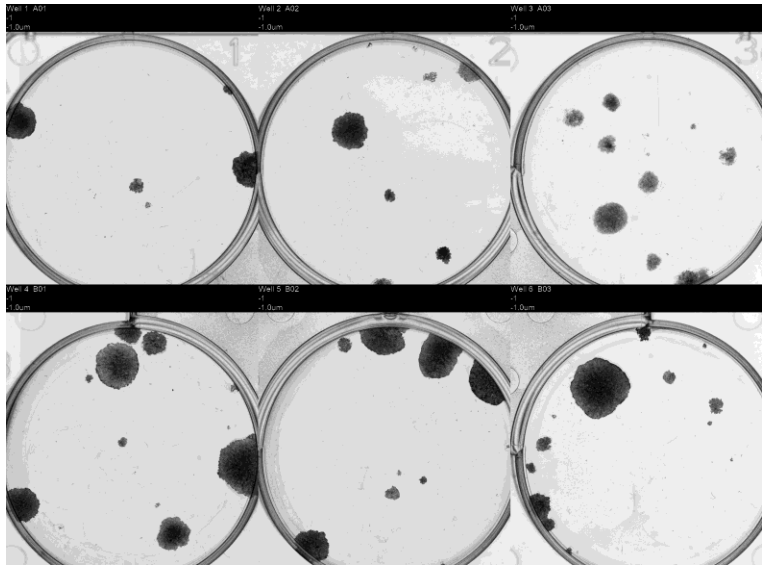


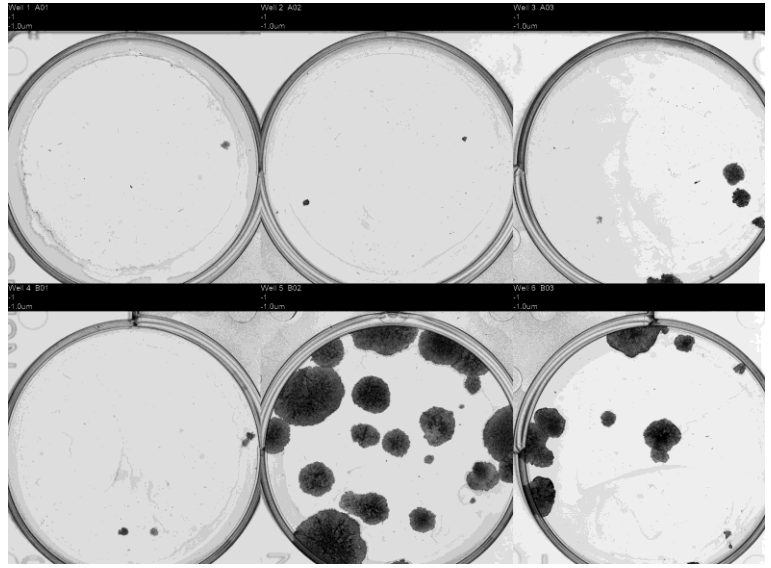




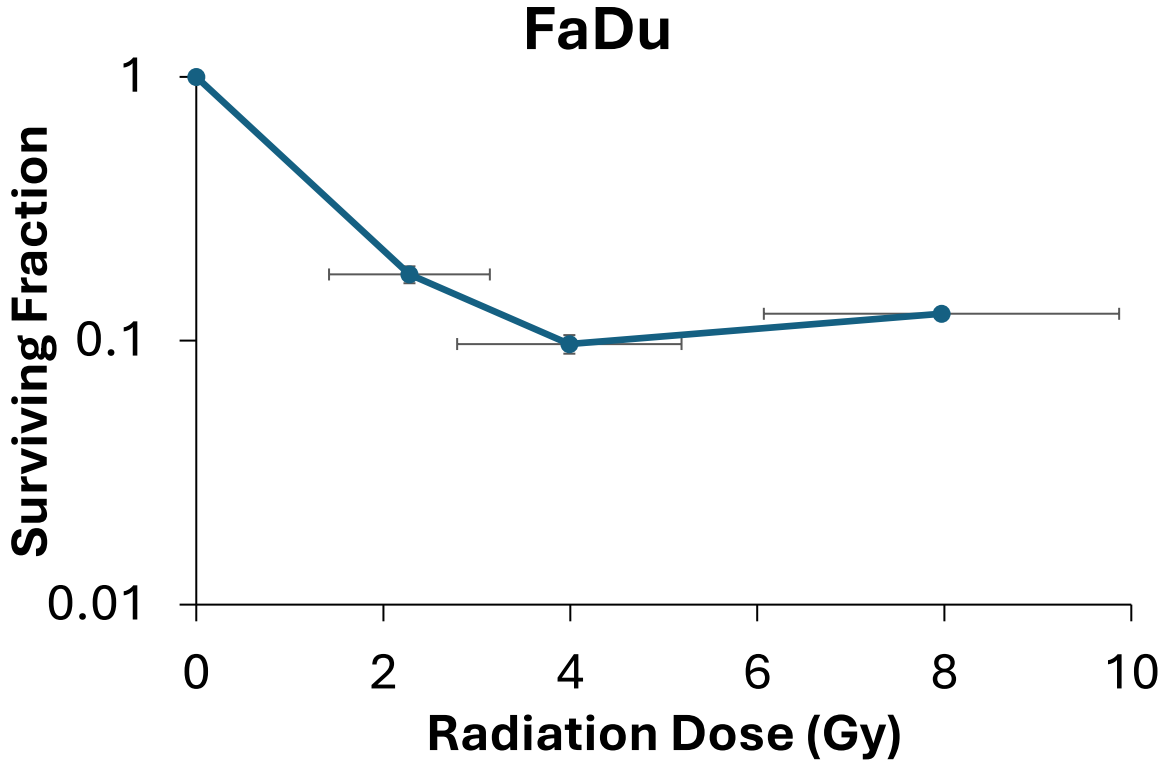
2Gy Shots





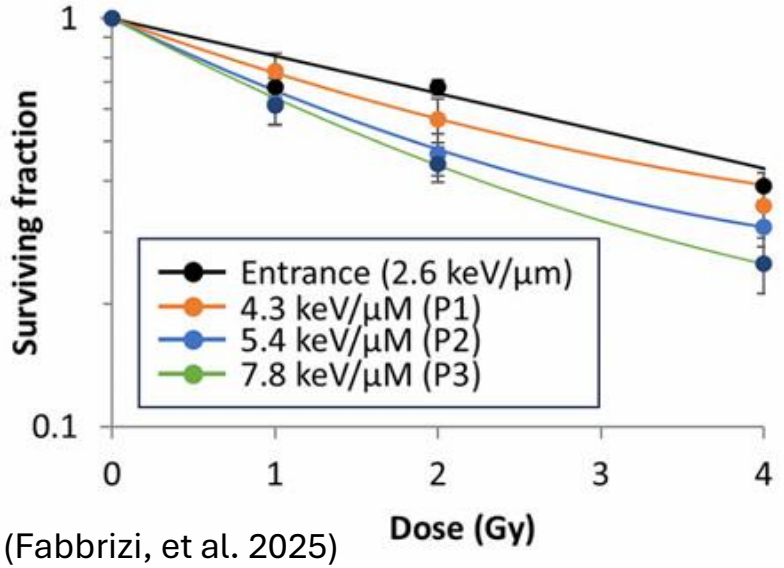


SCAPA FaDu Data…



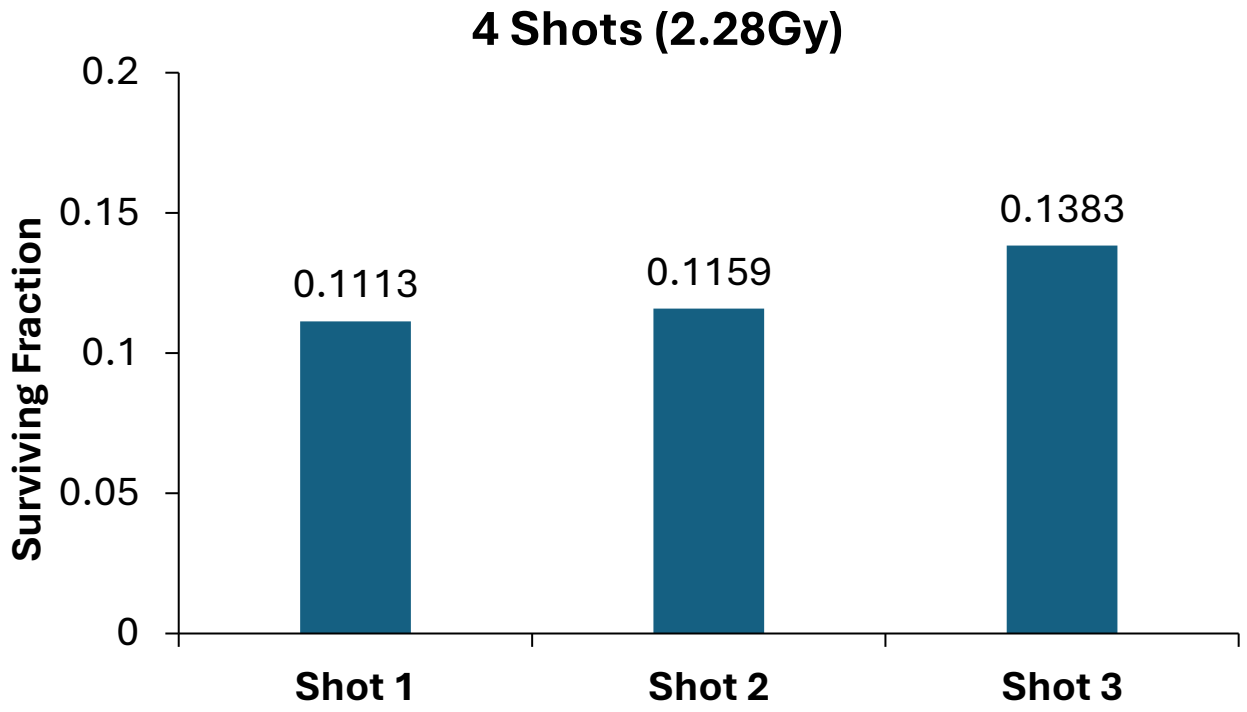
Average PE: ~12%

Shots	Mean Dose (Gy)	1sigma Min Dose (Gy)	1sigma Max Dose (Gy)
4	2.28	1.42	3.14
7	3.99	2.79	5.19
14	7.97	6.07	9.87



(Fabbrizi, et al. 2025)

SCAPA FaDu Data...



Average PE: ~11%

Shots	Mean Dose (Gy)	1sigma Min Dose (Gy)	1sigma Max Dose (Gy)
4	2.28	1.42	3.14

Discussion – ways to improve clonogenic formation…

- Reduce the irradiation time
  - How long cells can stay vertical (SCAPA ~1.5 h)
  - Reducing the time between shots from 20s to 5s
  - No RCF in the same carousel

Full systematic test, but a helium repeat done in – dishes were left out for 30mins and no colonies formed

- Dealing with potential causes: Temperature, CO2, lack of media, weak cell line
  - Temperature – Warm carousel
  - Drying out? – Rotate the carousel; modify lid design
  - Alternative cell line?

- Equipment
  - Monitor incubator – Thermostat; regular checking
  - Use Marie's lab – X-ray comparisons
  - Microscope

- Dose monitoring

Perform DNA damage and repair analysis simultaneously

Initial Radiobiology Experiments at SCAPA

DNA repair foci analysis

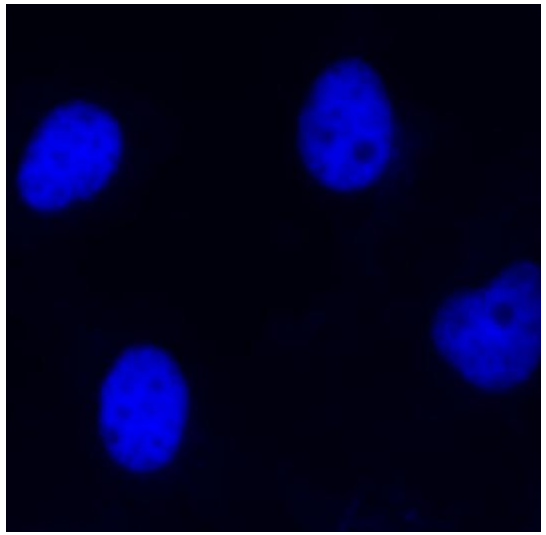
CONV 3Gy (?) – 1, 4, 8, 24 hr – in duplicate

ULTRA-HIGH 3Gy (?) – 1, 4, 8, 24 hr – in duplicate

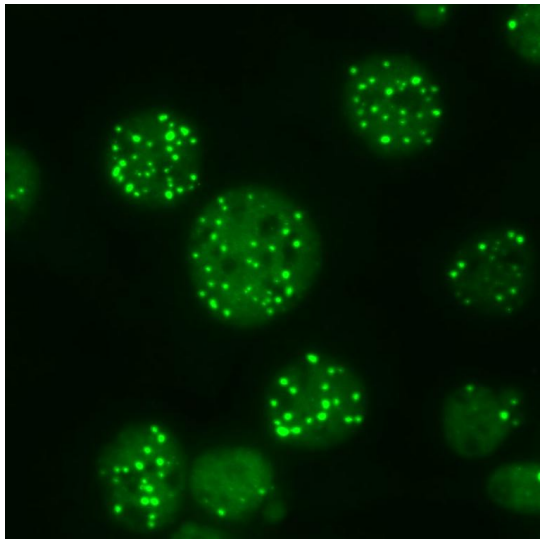
Need at least 3 independent biological repeats

Cells need to be fixed at certain timepoints (SCAPA) but can be processed later (Birmingham) – **Results in ~2 weeks**

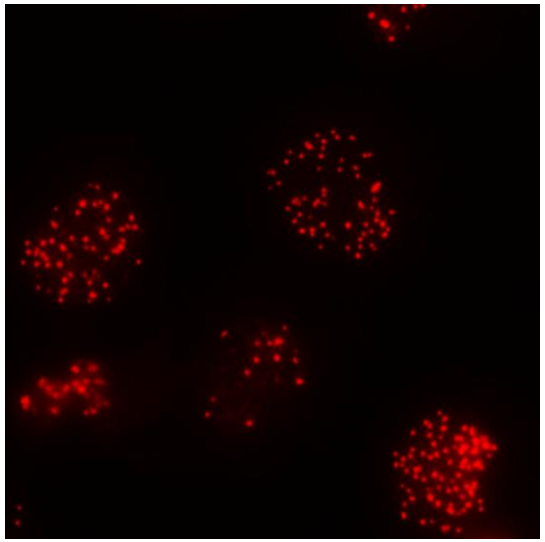
DAPI



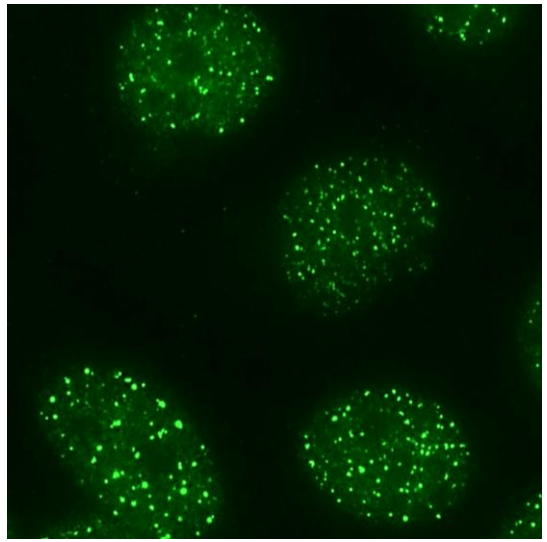
53BP1



γH2AX



RAD51

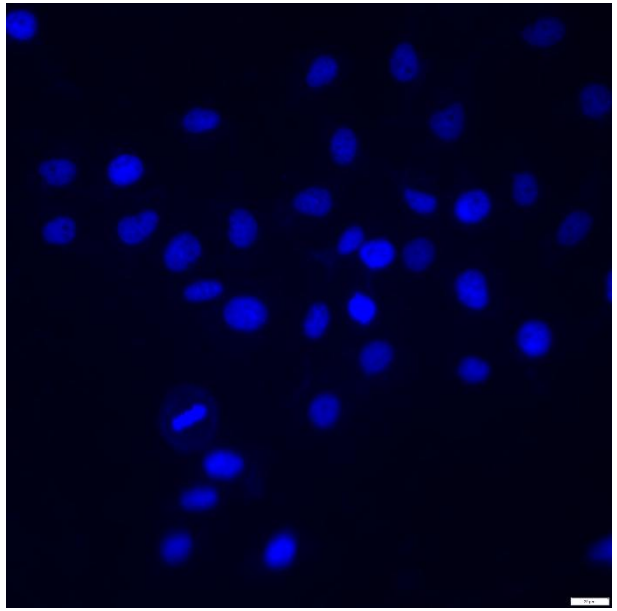


Radiobiology Preparations – **Mylar IF Optimisation**

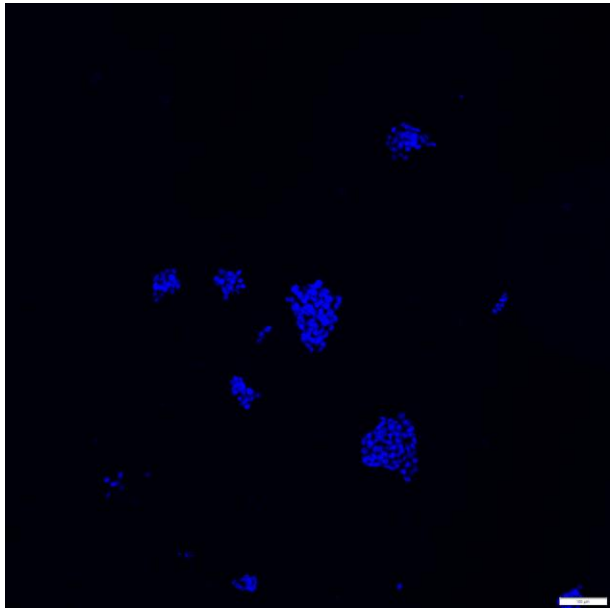
Protocol:

1. Culture cells as monolayer on mylar
2. IR and fix
3. Stain Primary/Secondary in glass rings
4. Add coverslip inside the ring with DAPI
5. Mount the mylar and coverslip onto a microscope slide with mounting media (without DAPI)
6. Leave to dry and cut around the coverslip

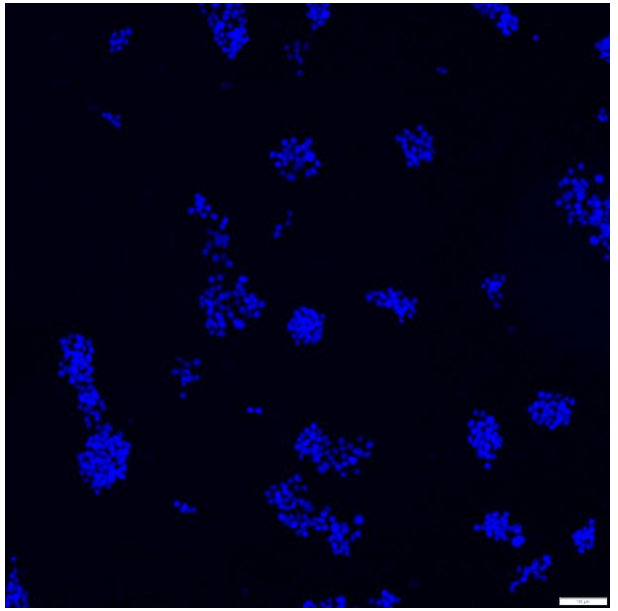
HeLa
50,000



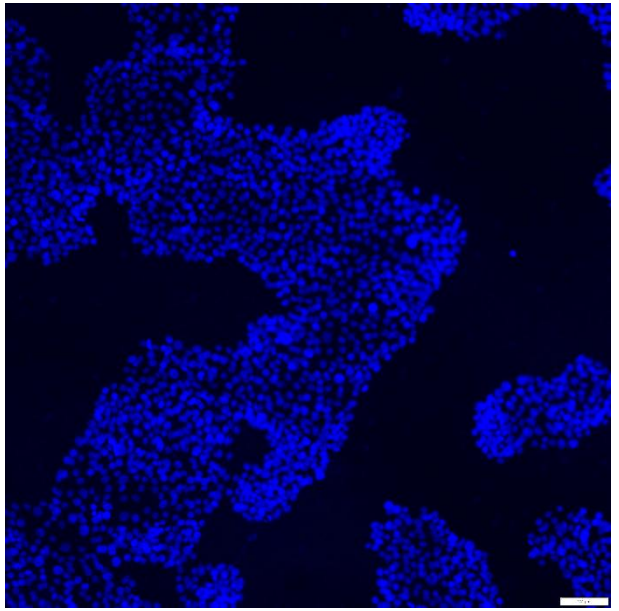
50,000



FaDu
100,000



200,000



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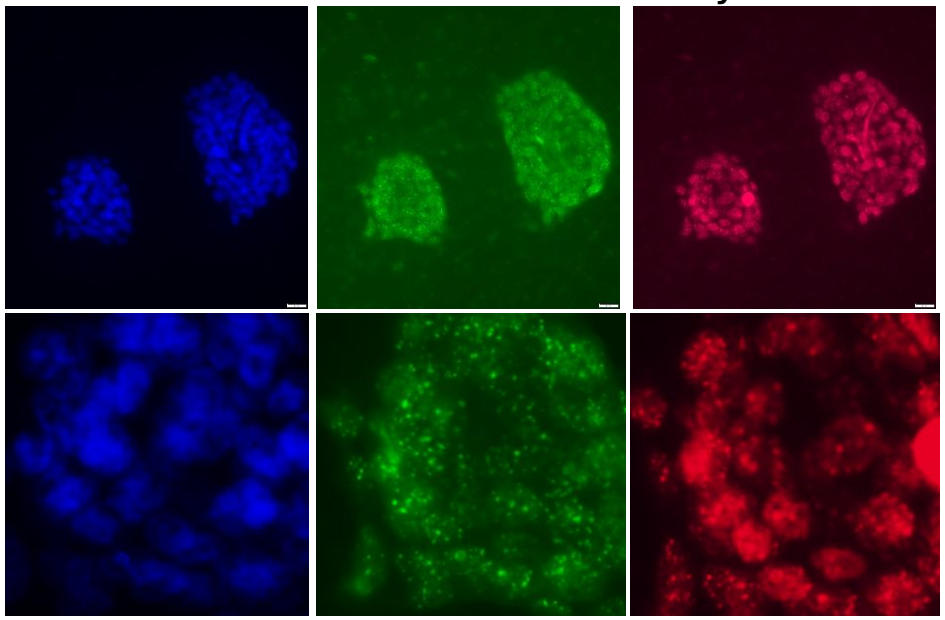
Radiobiology Preparations – **Mylar IF Optimisation**

FaDu

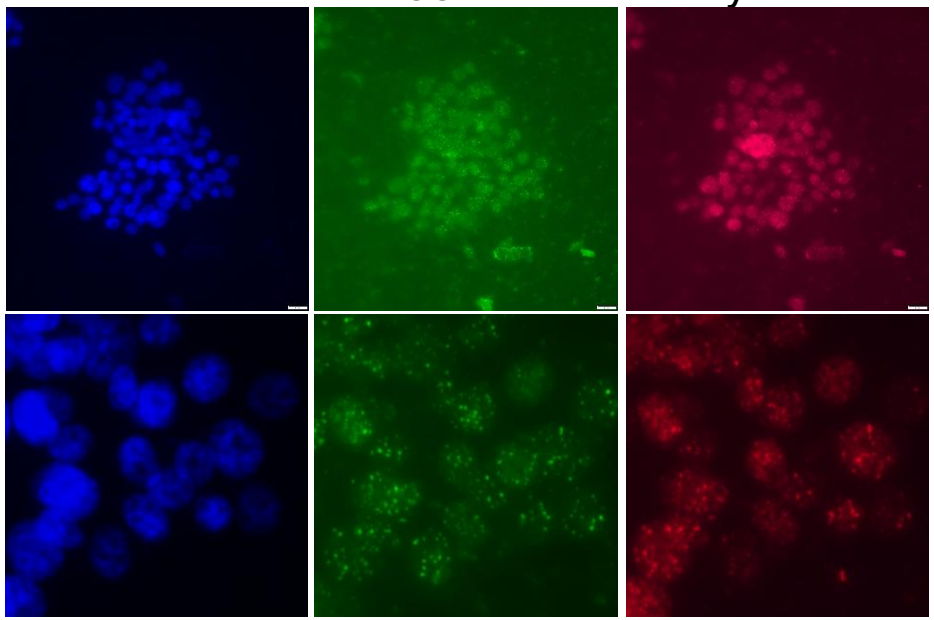
DAPI 53BP1 yH2AX

50,000
4Gy 4hr



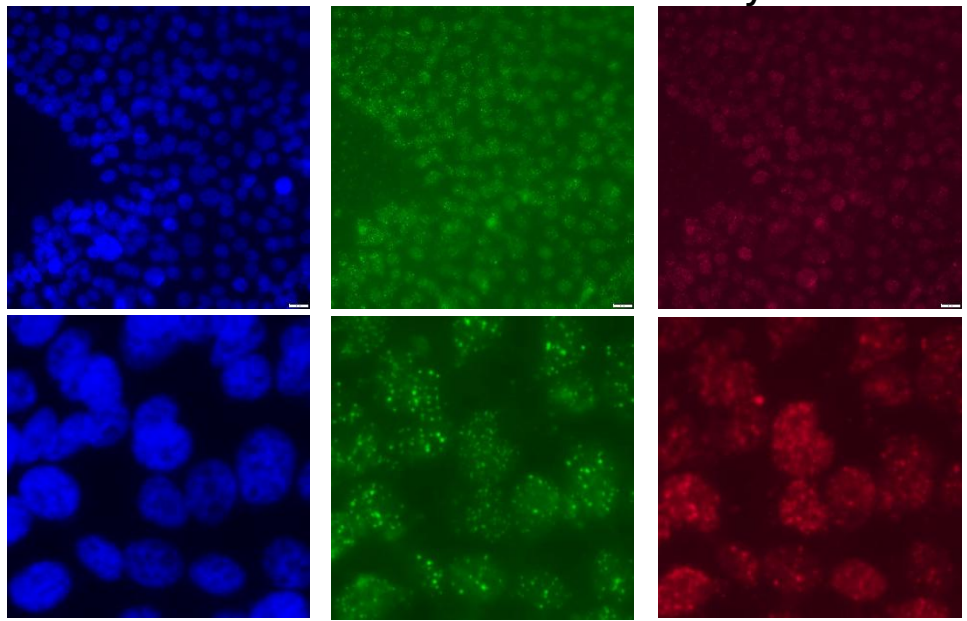
DAPI 53BP1 yH2AX

100,000
4Gy 4hr



DAPI 53BP1 yH2AX

200,000
4Gy 4hr



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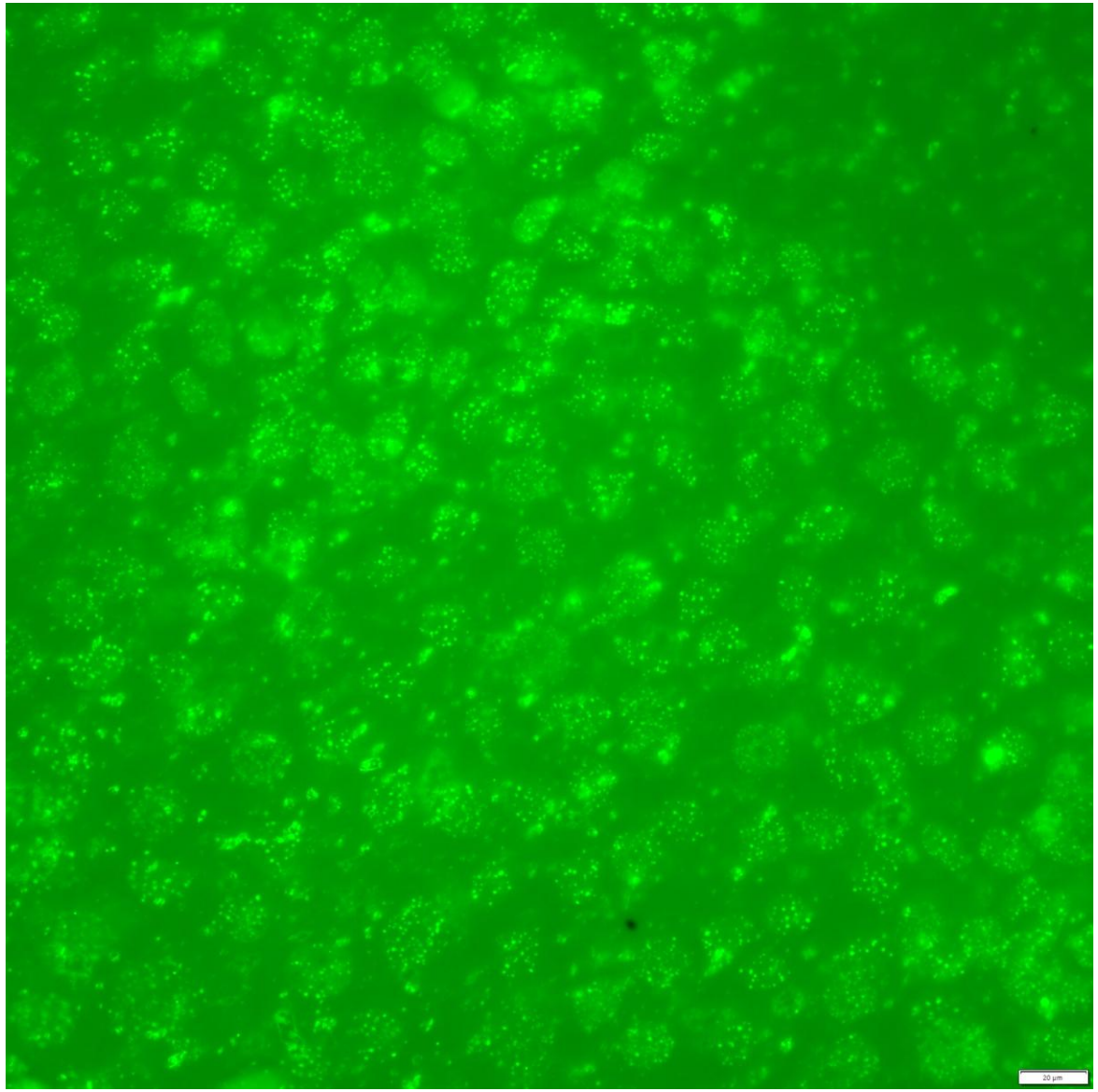
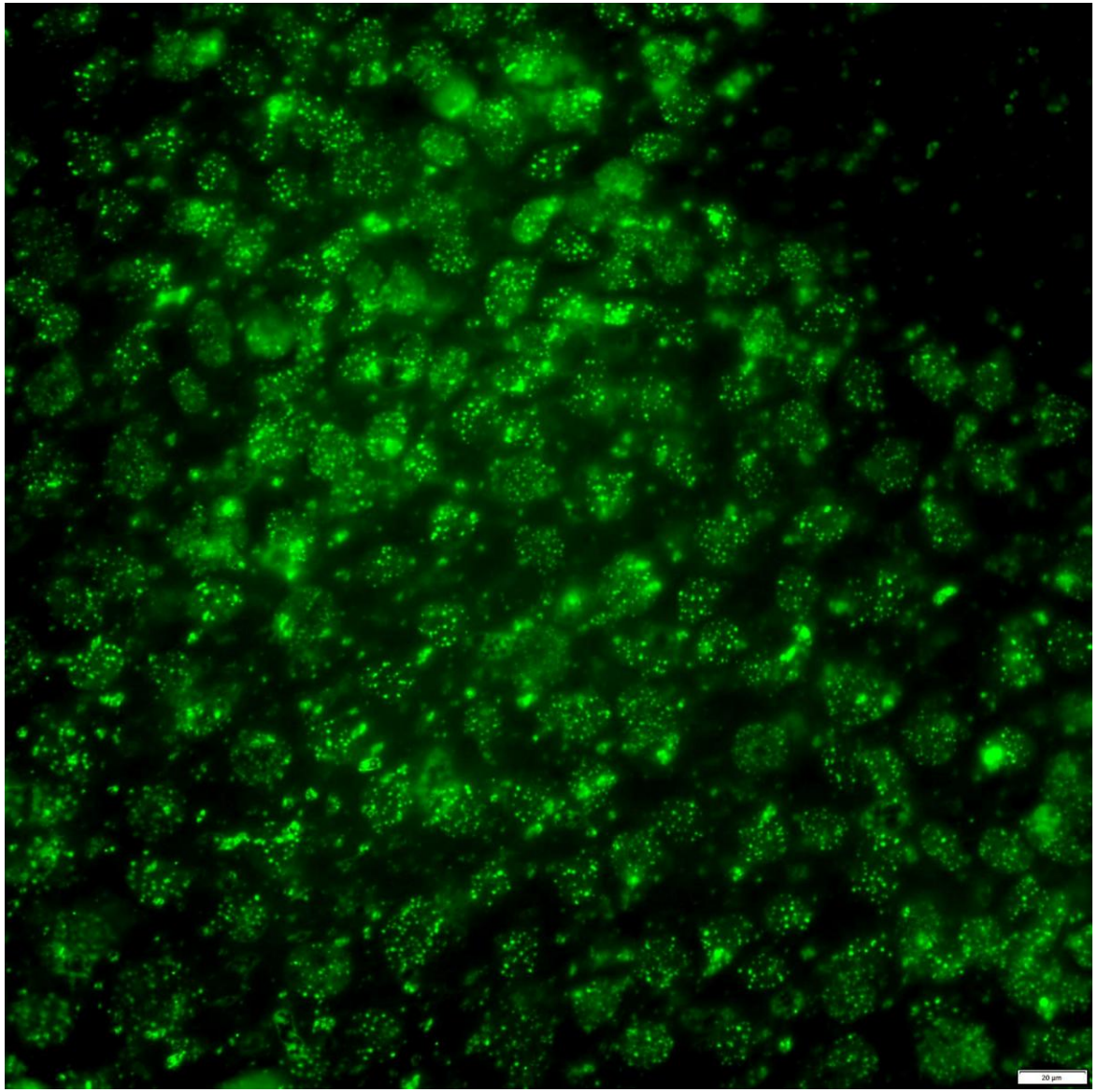
53BP1– 40x

FaDu 4hr 14 B_05



FaDu 4hr 14 B_05 – Reduced background



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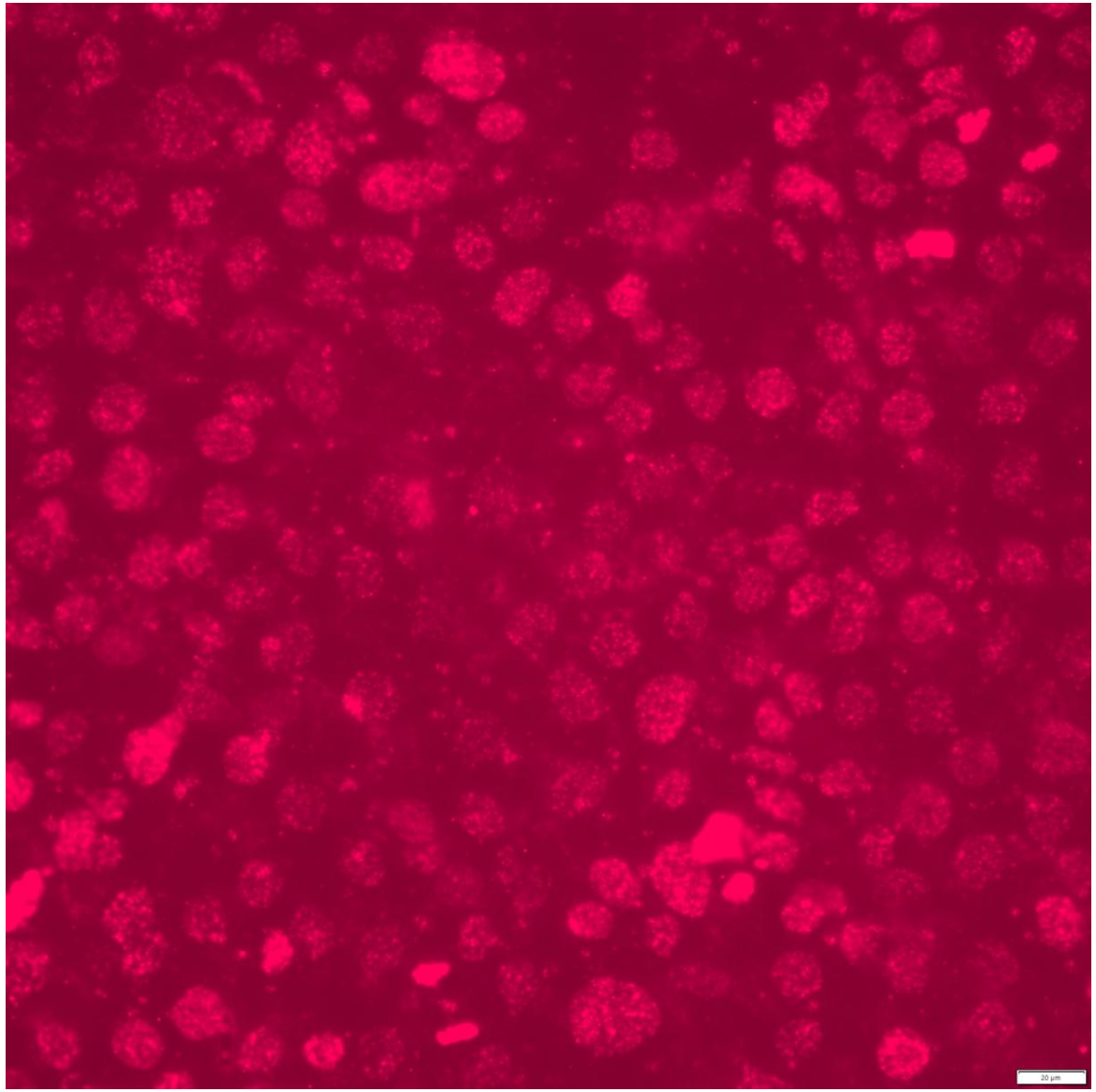
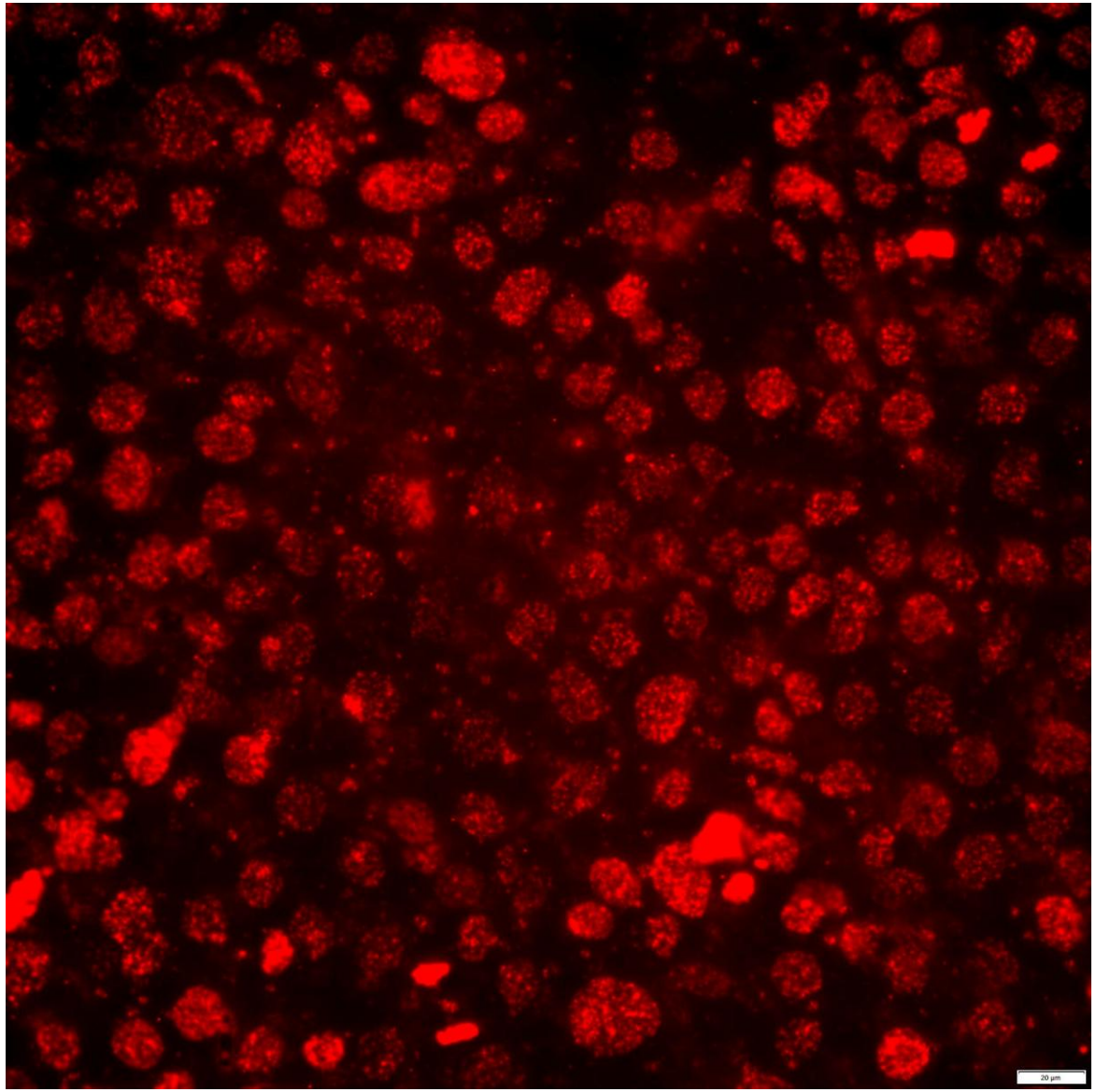
γH2AX – 40x

FaDu 1hr 11 A_03



FaDu 1hr 11 A_03– Reduced background



Background can only be reduced in post-processing – confocal could help?

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Radiobiology Preparations – **Cytospin IF Optimisation**

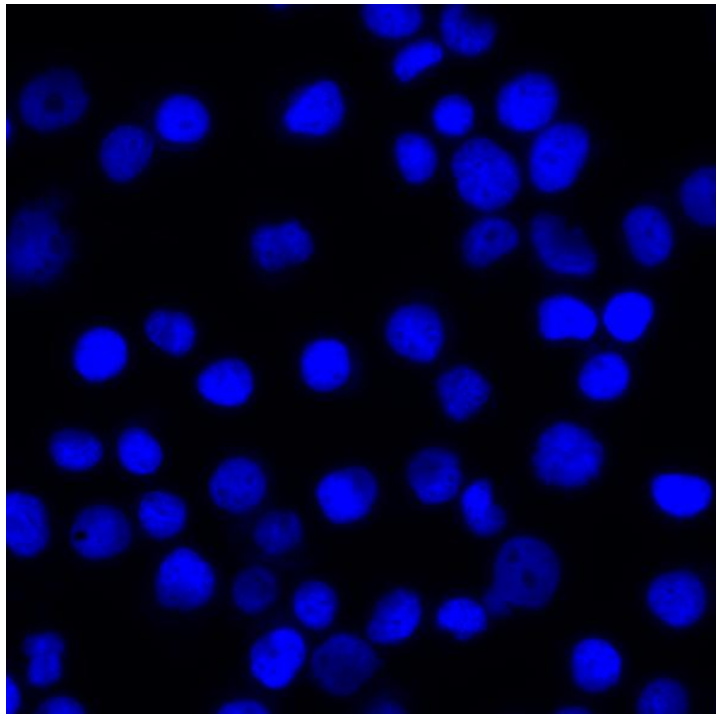
Protocol:

1. Seed cells as monolayer
2. Drug/IR
3. Trypsinise (0.25%) and neutralise cells (Media)
4. Count cells
5. Make dilution for known number of cells/ml – this depends on the cell line
6. Add microscope slide, filter paper and sample loader into cytospin clamp
7. Load 100ul of each sample on to respective slides
8. Spin – 1000rpm for 5min
9. Place slides in humidified chamber
10. PAP pen around cell area
11. Fix with 10% formalin for 10 min
12. PBS wash (x2)
13. Block with 200ul BSA (2%) for 1 hr at RT
14. Add 200ul Primary Ab overnight at 4ºC
15. PBS-T (0.1%) wash x3
16. Add 200ul Secondary Ab for 1hr at RT
17. PBS rinse
18. PBS wash for 10 min
19. Leave slides to dry
20. Add DAPI to microscope slide and cover with 13mm coverslip
21. Leave cells to dry
22. Store at 4ºC until imaging

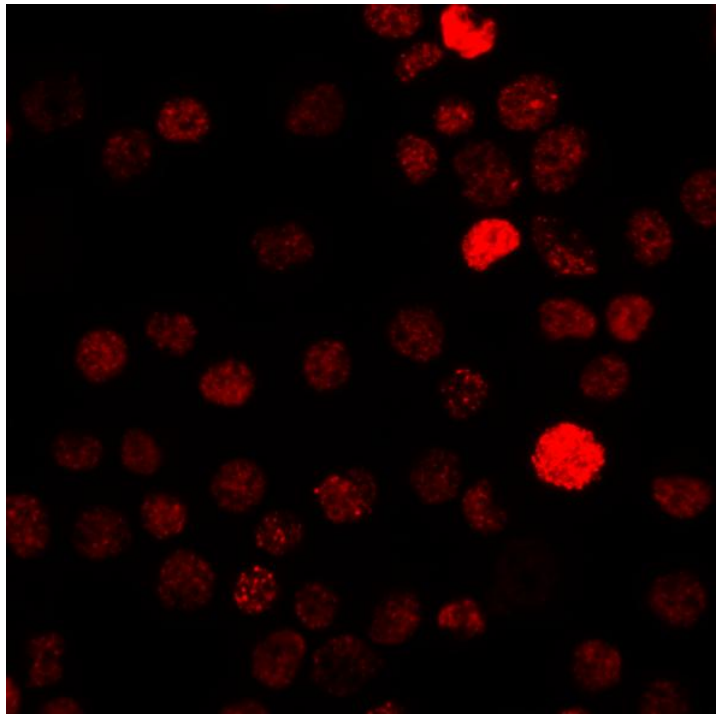
Note:- fixing after spinning helps cell retention, as fixing prevents cells from sticking to the slide

FaDu – 1h post-IR

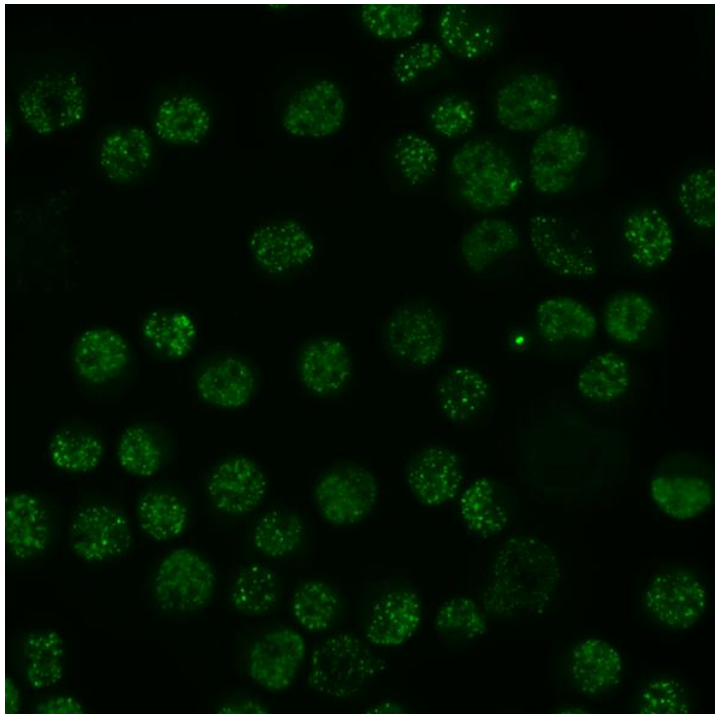
DAPI



yH2AX



53BP1



Completed optimisation for the number of cells required – 10,000 cells/100ul for FaDu (HeLa need optimising)

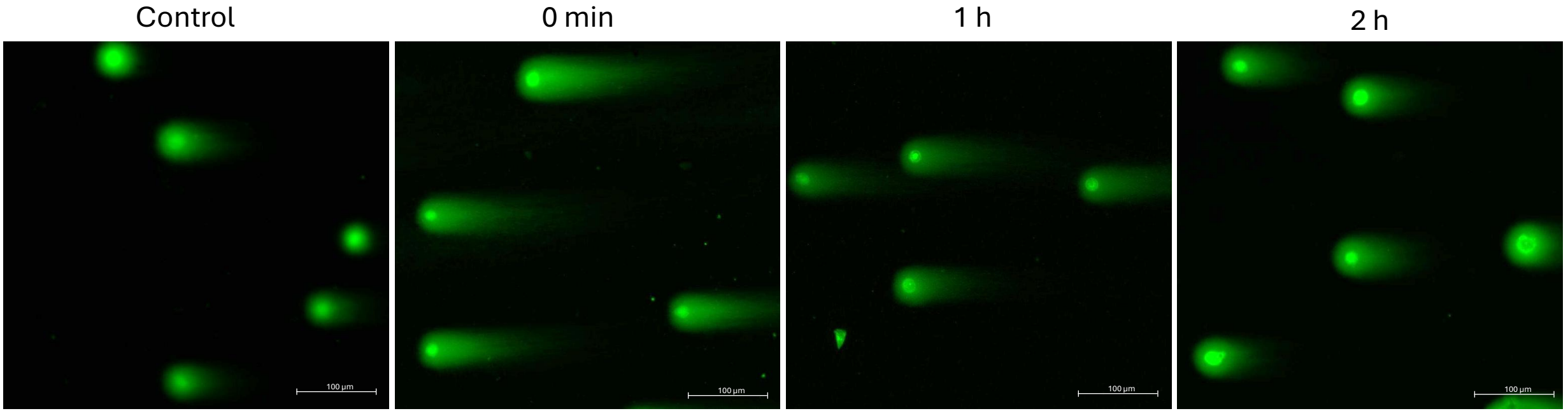
Initial Radiobiology Experiments at SCAPA

Comet Assays – *Neutral (DSB), Alkaline (SSB), Enzyme-modified (CDD)*

CONV 3Gy (?) – 0-4 h – in duplicate

ULTRA-HIGH 3Gy (?) – 0-4 h – in duplicate

Cells need to be lysed at certain timepoints and run electrophoresis (SCAPA) but can be stained, imaged and processed later (Birmingham) – **Results in ~2 weeks**



FaDu – Neutral Comet Assay (DSB) – 4Gy Protons (~7-10 keV/µm)

Conclusions

- Repeat SCAPA clonogenic assays – with new optimised conditions and X-ray comparisons on site
- Perform DNA damage analysis alongside clonogenic assays
- Perform biological comparisons utilising different beams (ELI, etc)