Radiobiology research at SCAPA - PoPLaR

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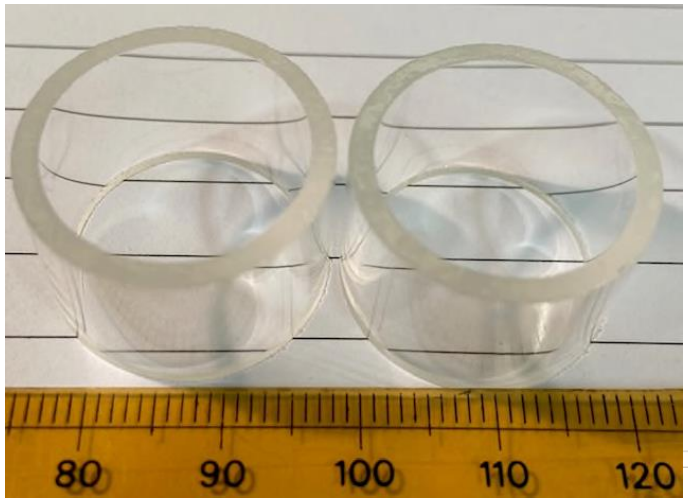
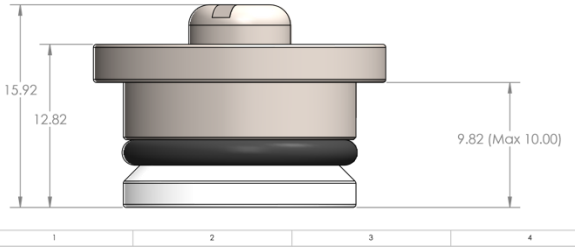
Radiobiology Set up at SCAPA

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



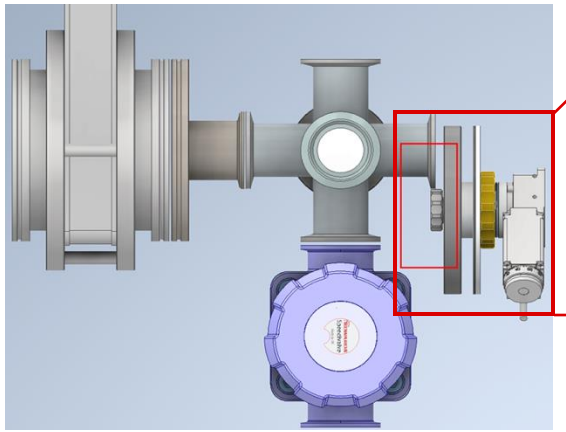
Internal diameter – 18.5 mm

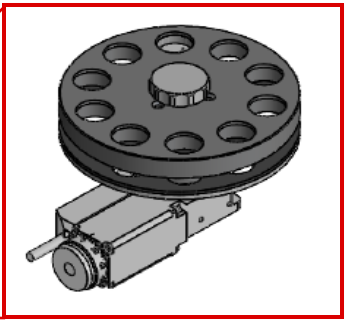
Outer diameter – 22.5 mm



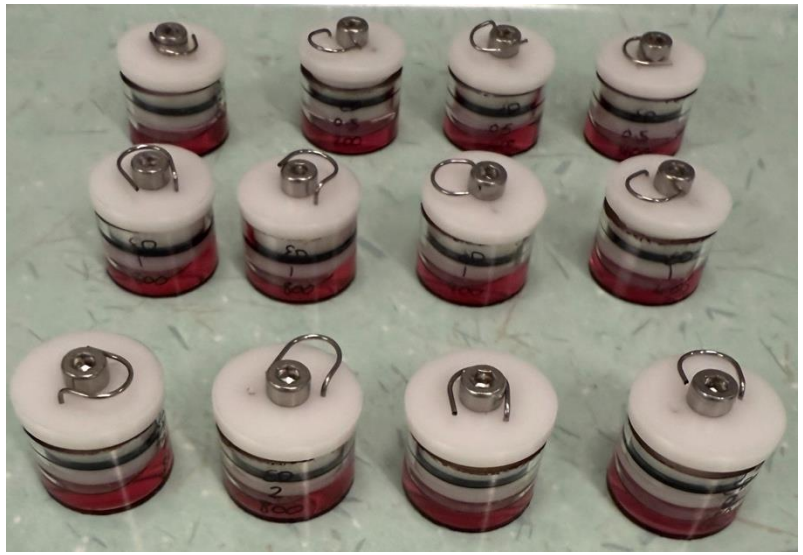
Sealable lids

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**)

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Initial Radiobiology Experiments at SCAPA

HeLa and FaDu cell lines

Clonogenic assays

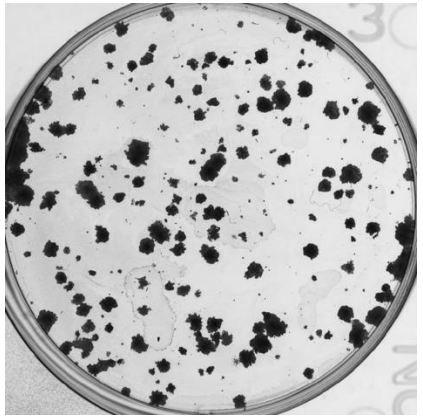
ULTRA-HIGH – 1, 2, 3Gy – in triplicate

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

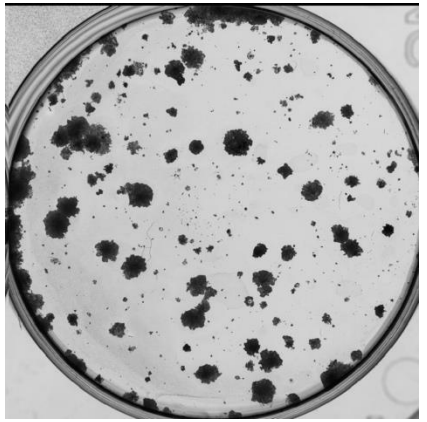
54 dishes to irradiate per experiment

Need at least 3 independent biological repeats

Control



4Gy



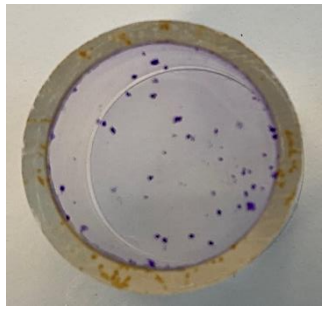
Timeline:

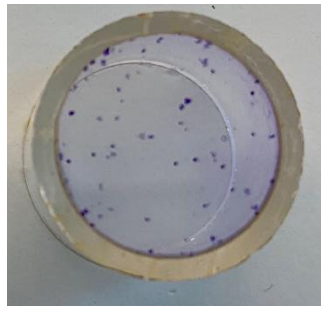
1. Thaw and culture cells at least one week before
2. Seed cells the day before (PM) irradiation (AM)
3. Stain colonies **7-10days** later (SCAPA) and analyse (Birmingham)
4. **Results in ~2 weeks**

Radiobiology Preparations 1 – **1.8cm Plating Efficiency**

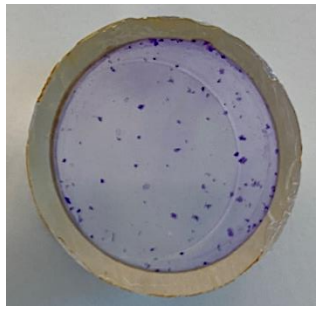
HeLa

100 Cells



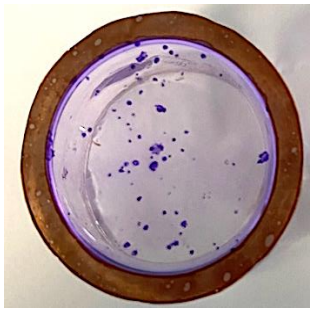


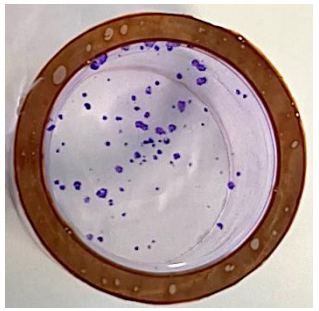
200 Cells



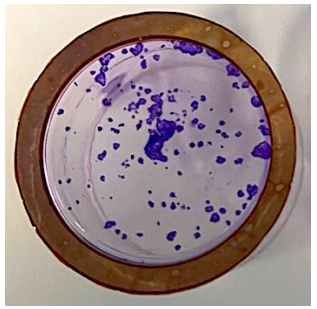
FaDu

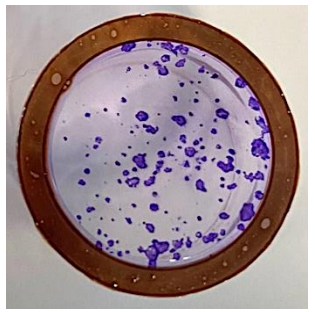
200 Cells

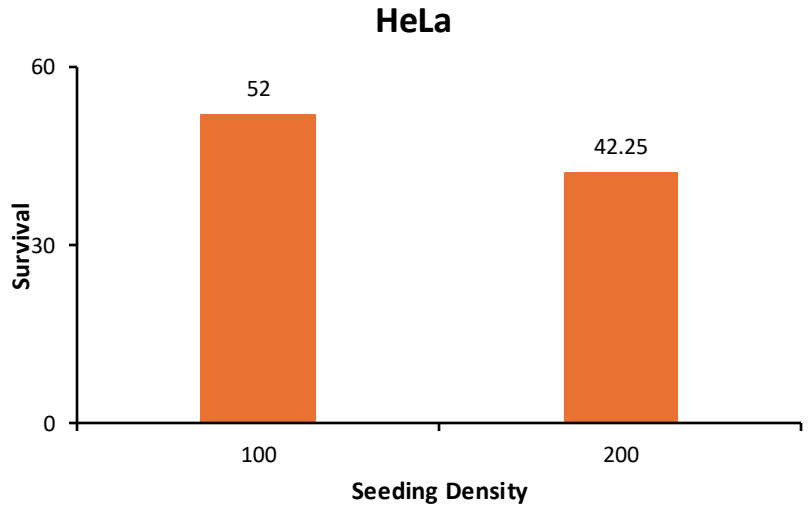


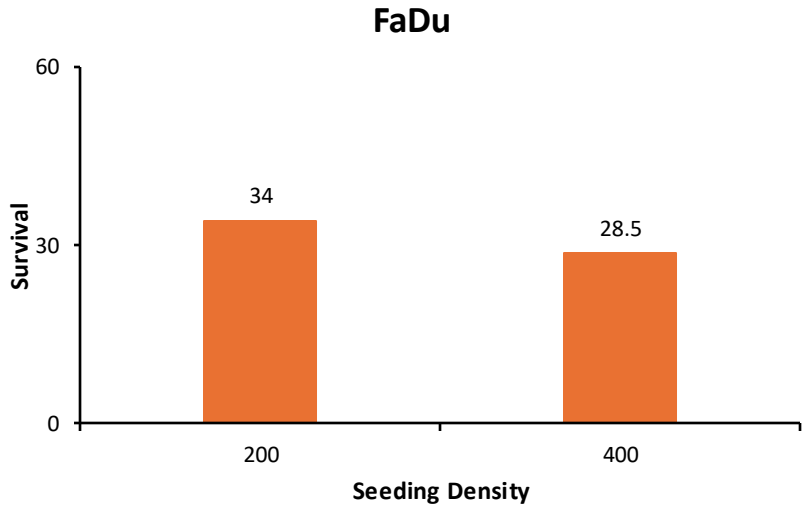


400 Cells









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Radiobiology Preparations 2 – **1.8cm Seeding Density Optimisation**

HeLa

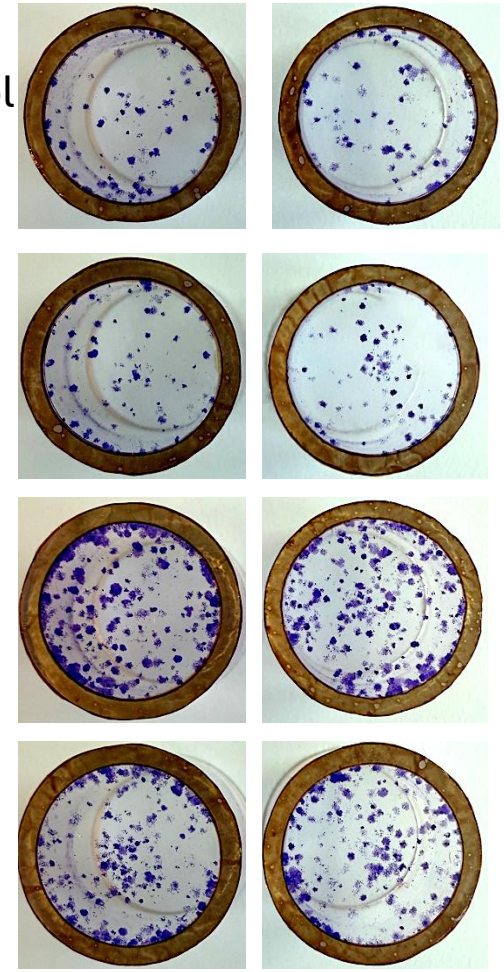
PE = ~40-50%

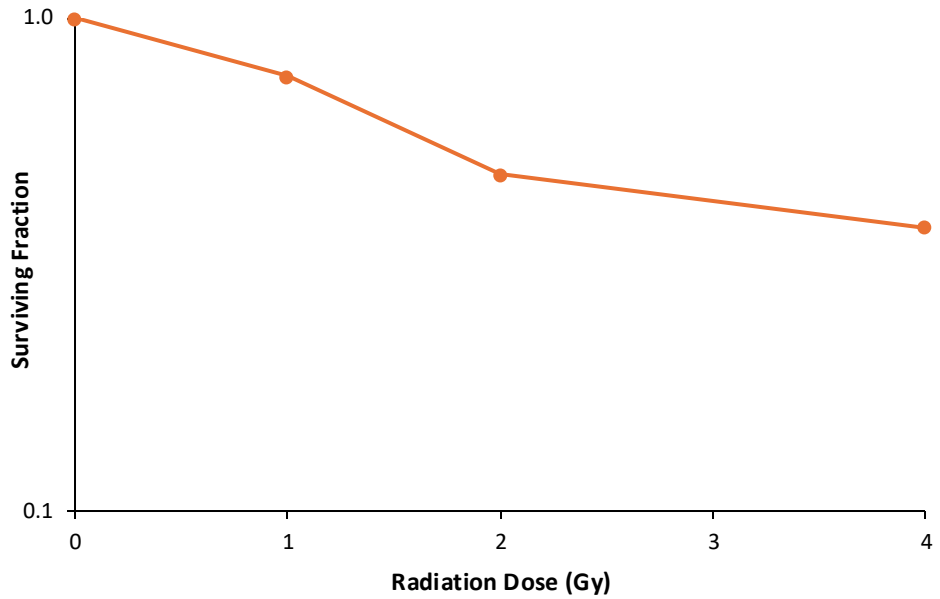
Control 200

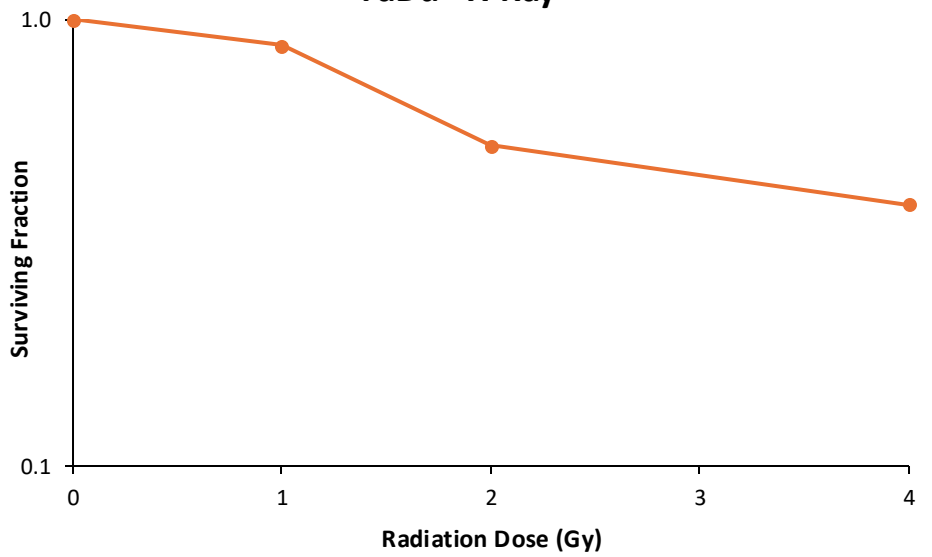
1Gy 200

2Gy 800

4Gy 800







FaDu

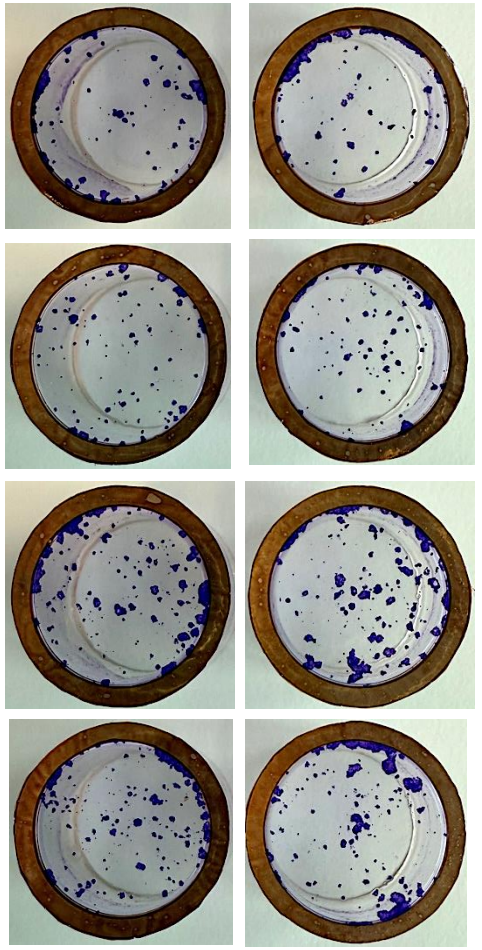
PE = ~30%

Control 400

1Gy 400

2Gy 800

4Gy 800



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Initial Radiobiology Experiments at SCAPA

DNA repair foci analysis

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

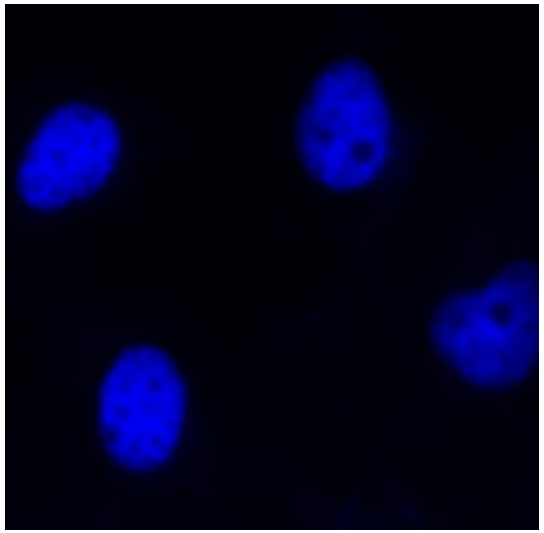
ULTRA-HIGH 3Gy (?) – 1, 4, 8, 24 hr – in duplicate

32 dishes to irradiate per experiment

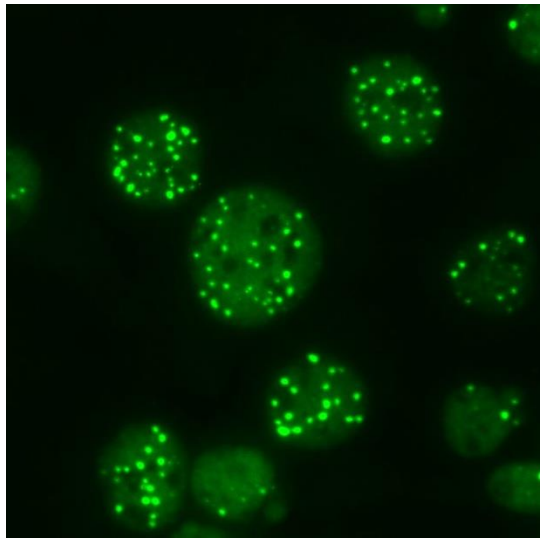
Need at least 3 independent biological repeats

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

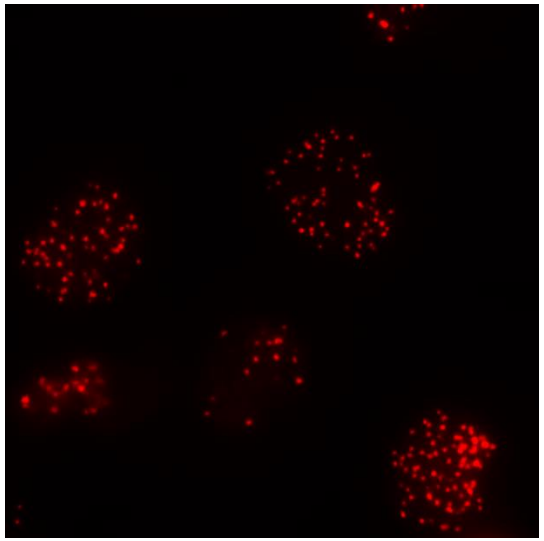
DAPI



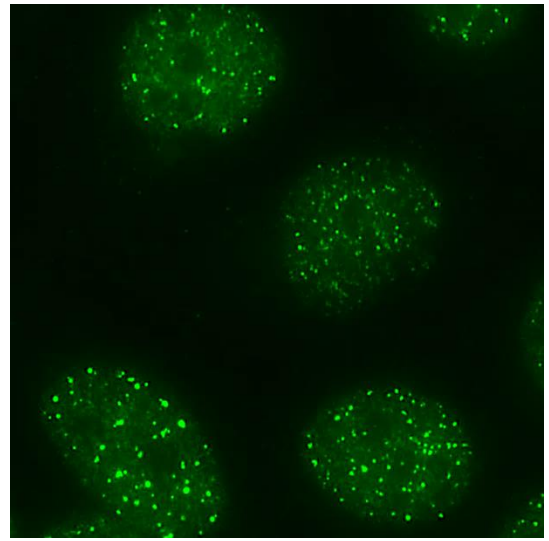
53BP1



γH2AX



RAD51



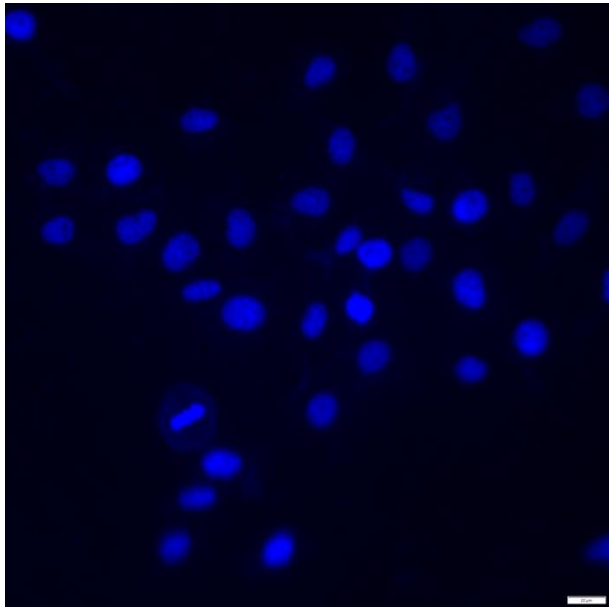
Radiobiology Preparations 3 – **Mylar IF Optimisation 1**

Protocol:

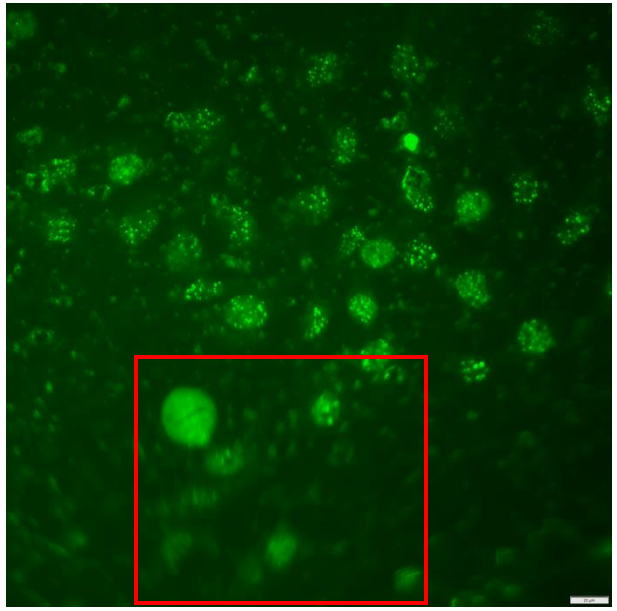
1. Culture cells as monolayer on mylar
2. IR and fix
3. Stain Primary/Secondary in glass rings
4. Cut out mylar and mount onto microscope slide (DAPI)
5. Use mounting media (without DAPI) on top to add coverslip (13mm) – to be able to use 60x oil immersion objective

HeLa

DAPI



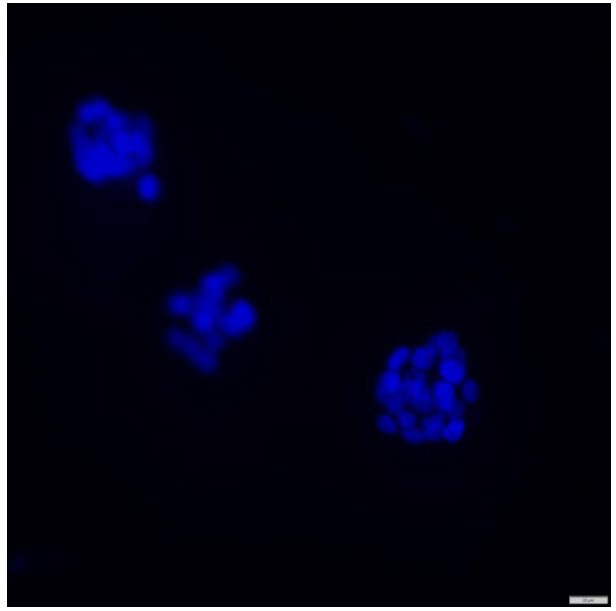
53BP1



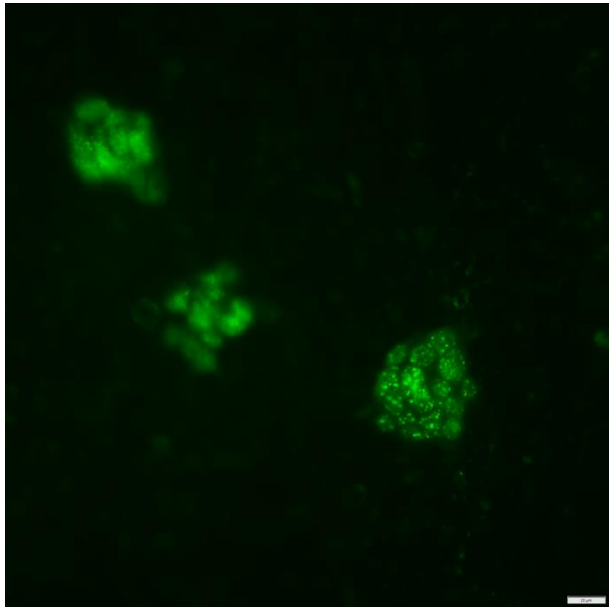
Wrinkles

FaDu

DAPI



53BP1



Colonies?

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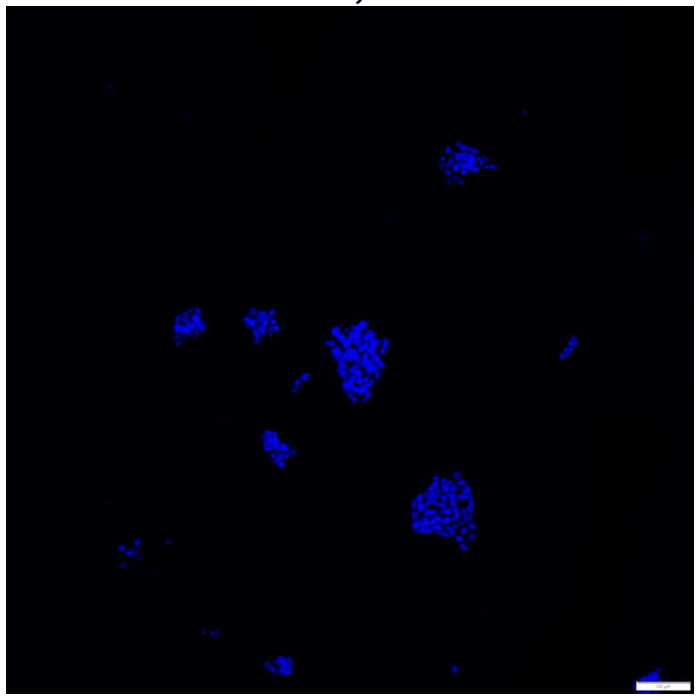
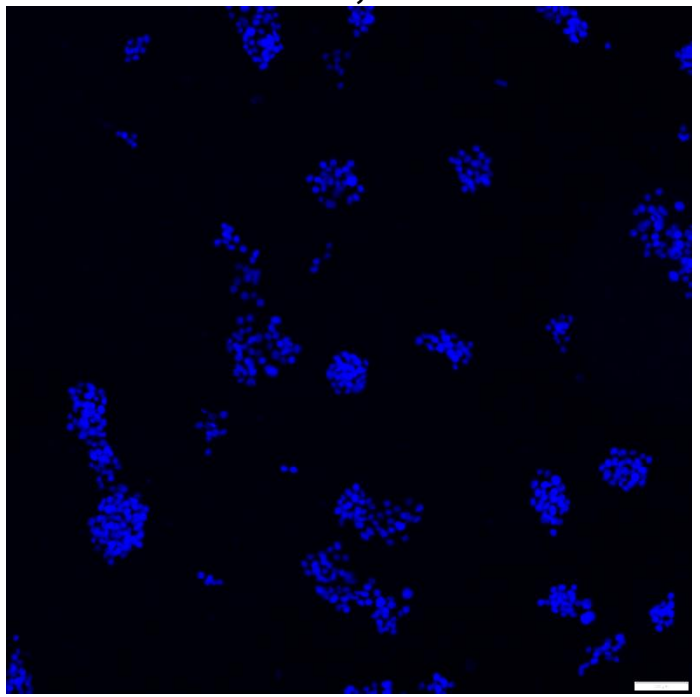
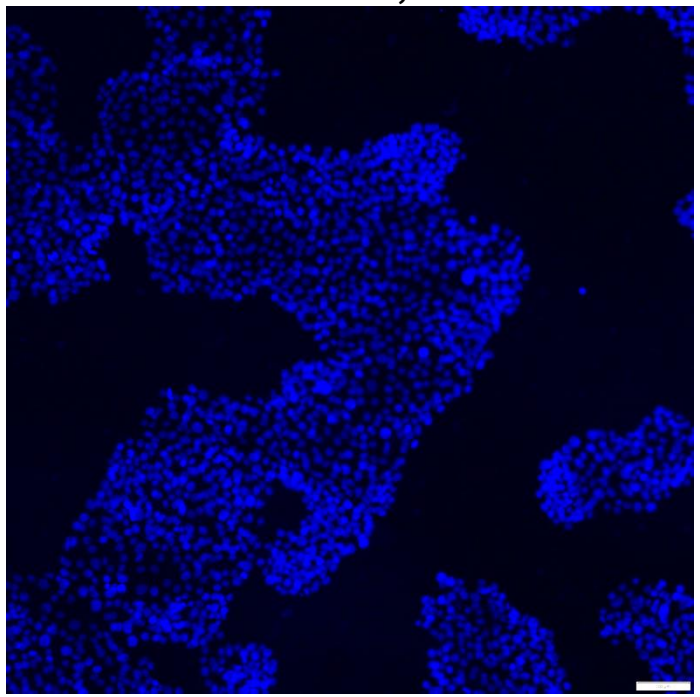
Radiobiology Preparations 3 – **Mylar IF Optimisation 2**

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

	50,000	100,000	200,000
FaDu			

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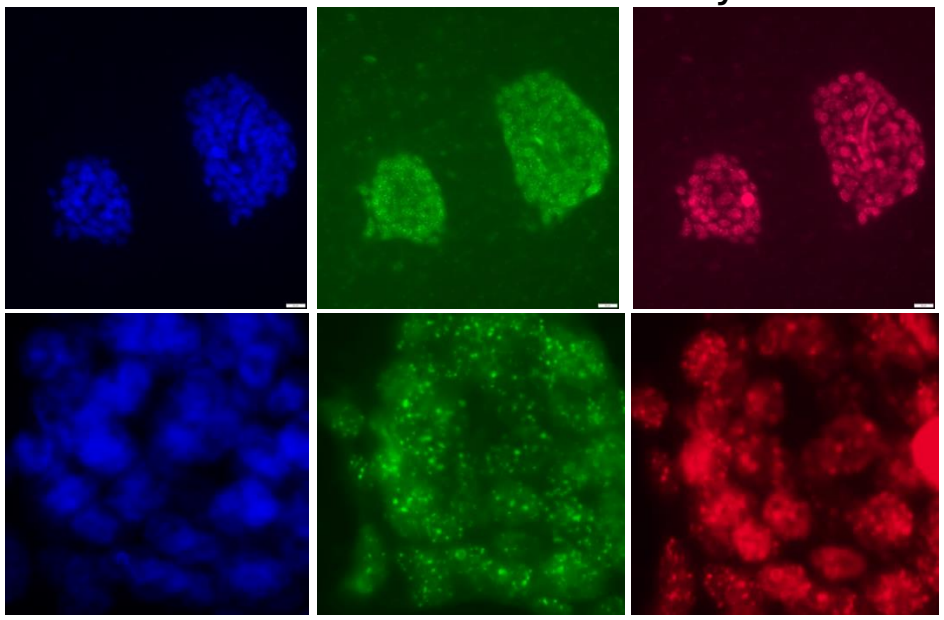
Radiobiology Preparations 3 – **Mylar IF Optimisation 2**

FaDu

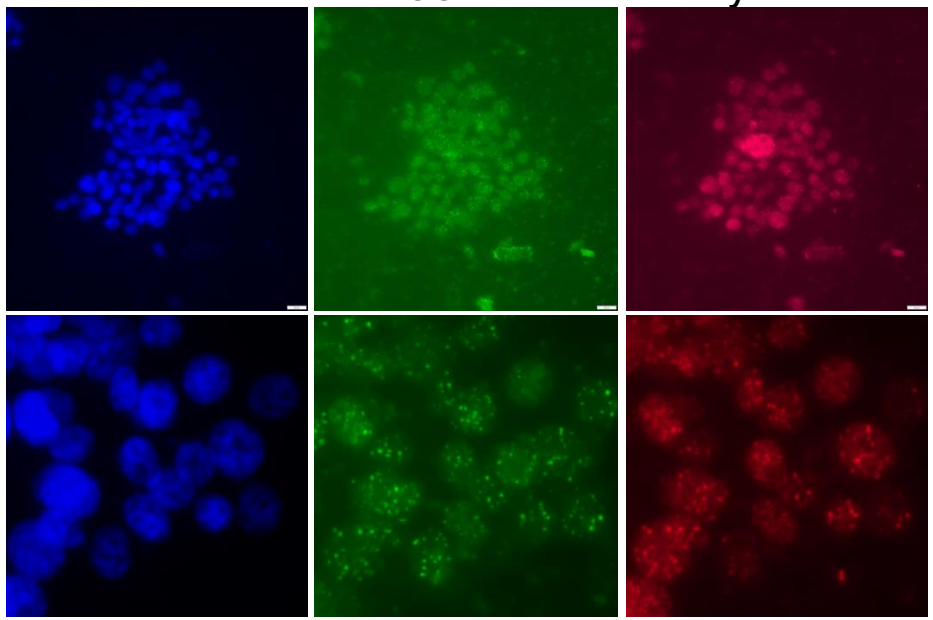
DAPI 53BP1 yH2AX

50,000
4Gy 4hr



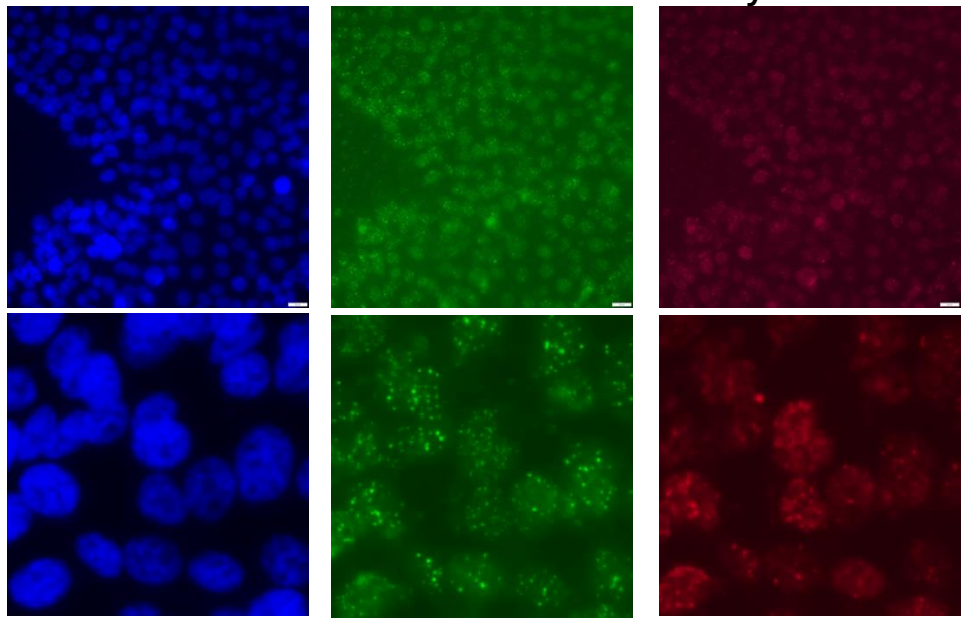
DAPI 53BP1 yH2AX

100,000
4Gy 4hr



DAPI 53BP1 yH2AX

200,000
4Gy 4hr



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Next Steps…

- 14 MeV vs 28 MeV comparisons – R1: 10th April
  (10 MeV, 4.8 keV/µm)
  Clonogenics and IF – 1.85cm Mylar dishes

- Glasgow Experiments; June-July 2025
  CONV - **6Gy/min**
  ULTRA-HIGH - **~10^9 Gy/s**

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