Targeted cell irradiation at the Surrey Vertical Beam

Surrey Ion Beam Centre

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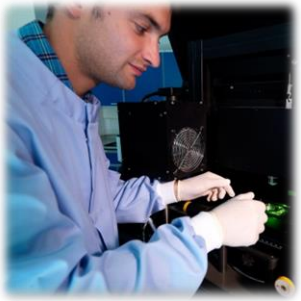
Introductions...



Charlie Jeynes
Surrey Ion Beam Centre

Anne-Catherine Wera
Surrey Ion Beam Centre
SPRITE

Karen Kirkby
Surrey Ion Beam Centre

The Surrey Vertical Beam

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Surrey Vertical Beam Facility

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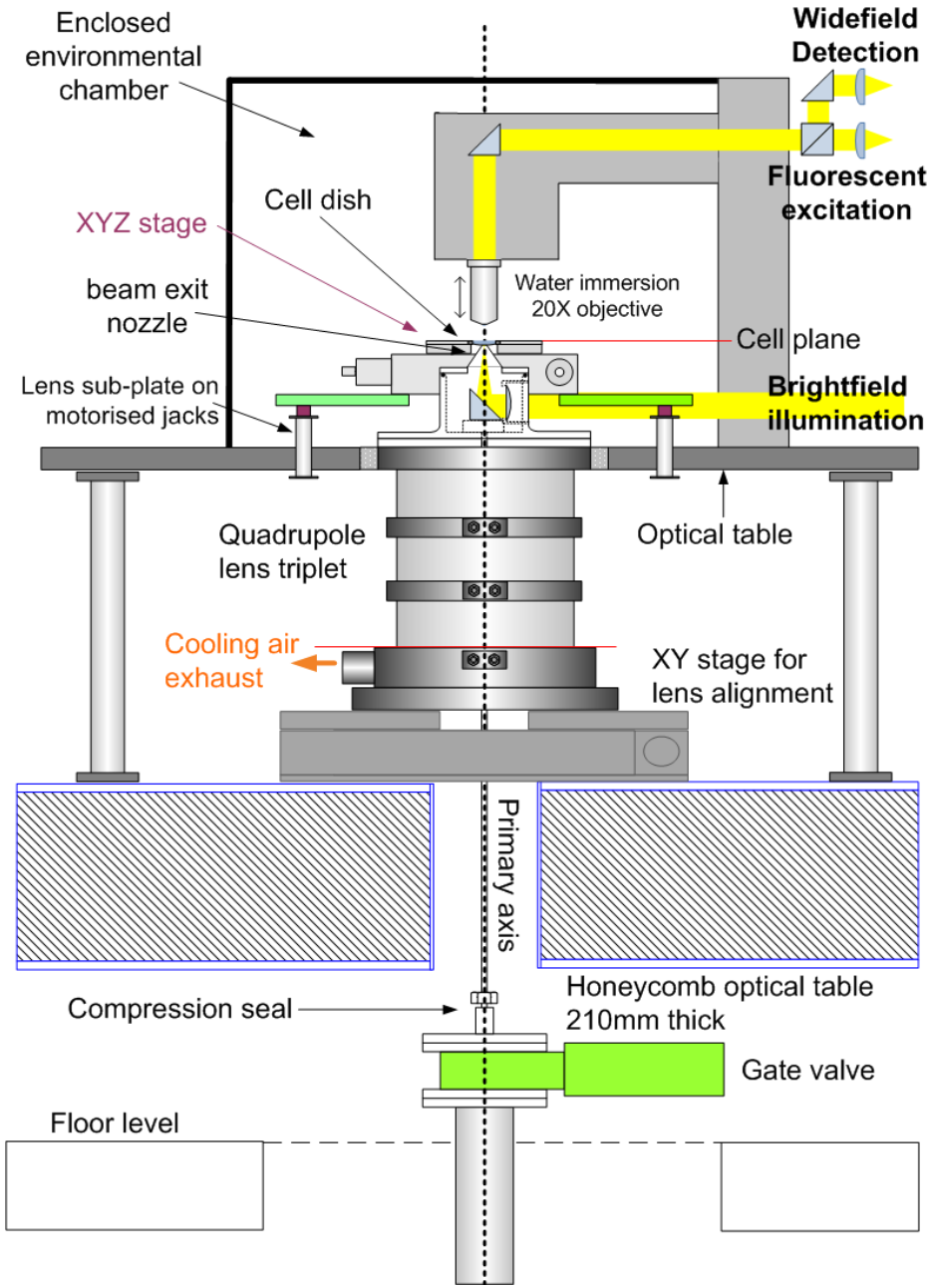
- OM-52 magnetic quadrupole focussing triplet.
- Ions from:
  - duo-plasmatron
  - sputter source.
- 2 MV Tandem
- Beams from H, to Ca

(Although Ca doesn't go very far through the cell!)



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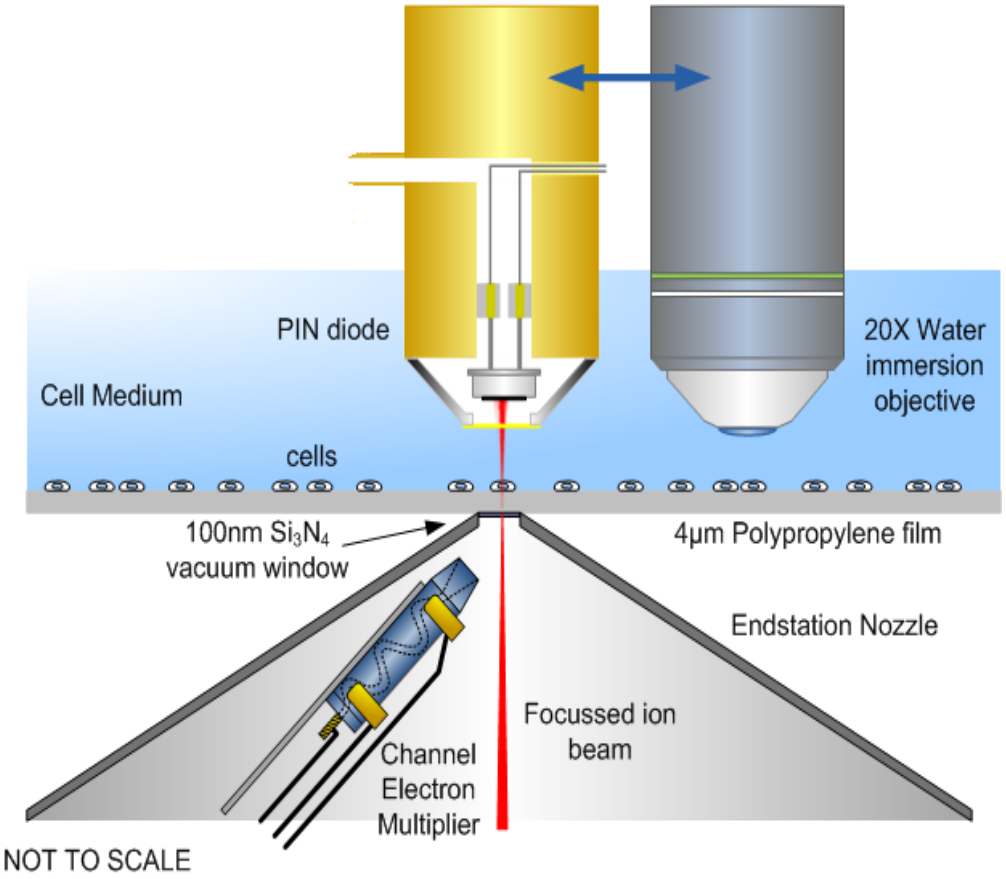
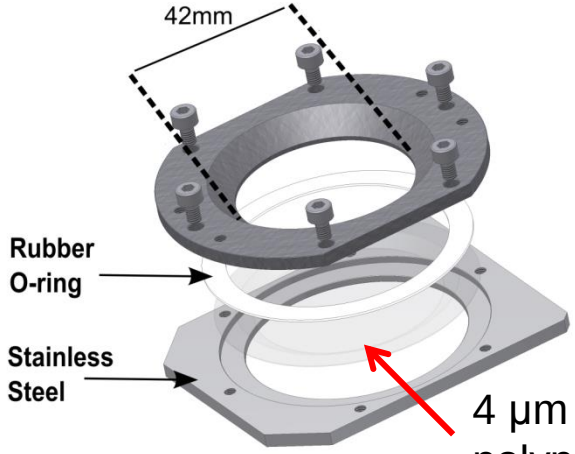
Microscope designed by Gray Cancer Institute, Oxford

Beam Nozzle and Cell Dish





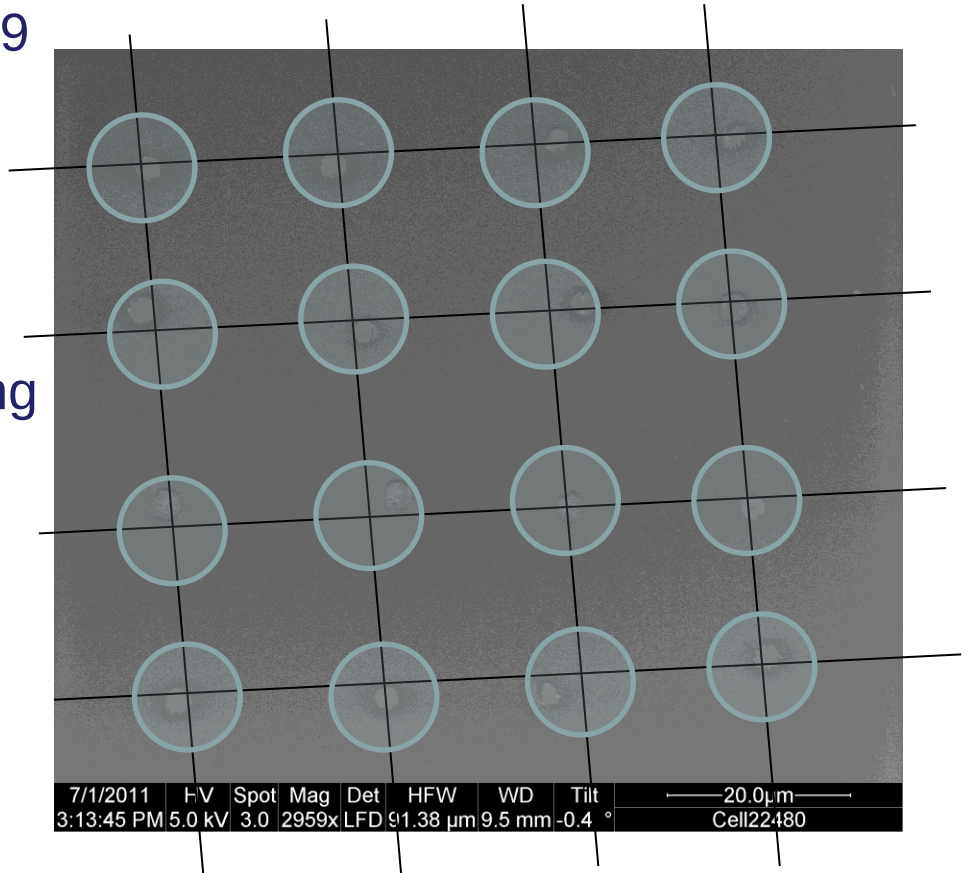
100 nm Si_3N_4 window

Targeting Accuracy

Do we hit what we aimed at?

- Single 3 MeV proton hits in CR-39
- Targeting within 5 um
- However, hit 100% of nuclei targeted, so good enough for radiobiology at present.
- STIM diode very good for counting single ions (>98% accuracy)

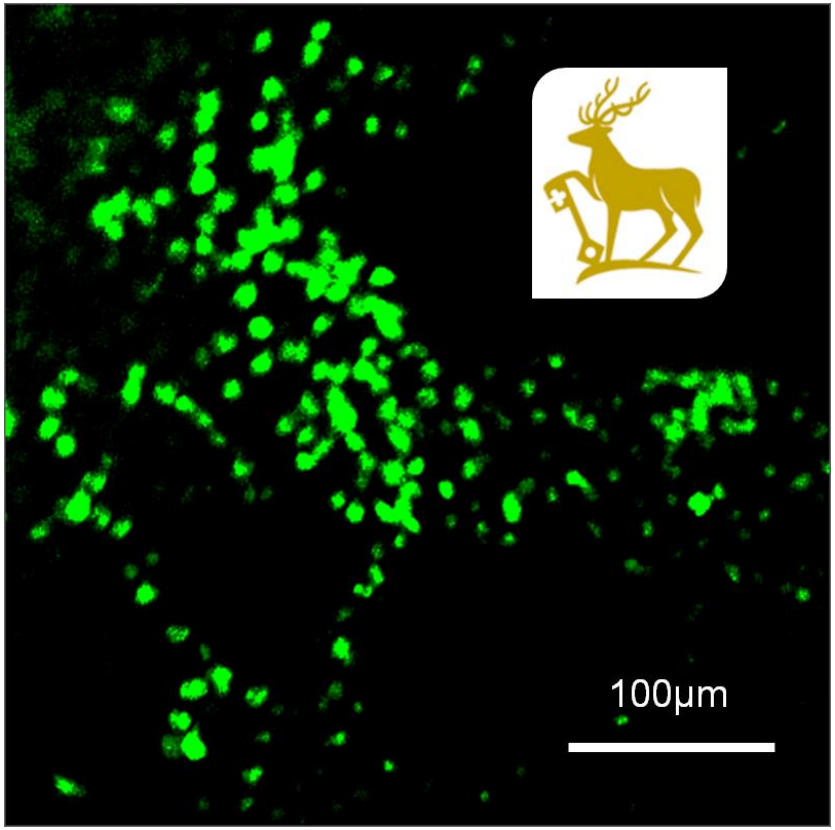
- Development of electrostatic scanning system will improve targeting to ~ 1 micron.



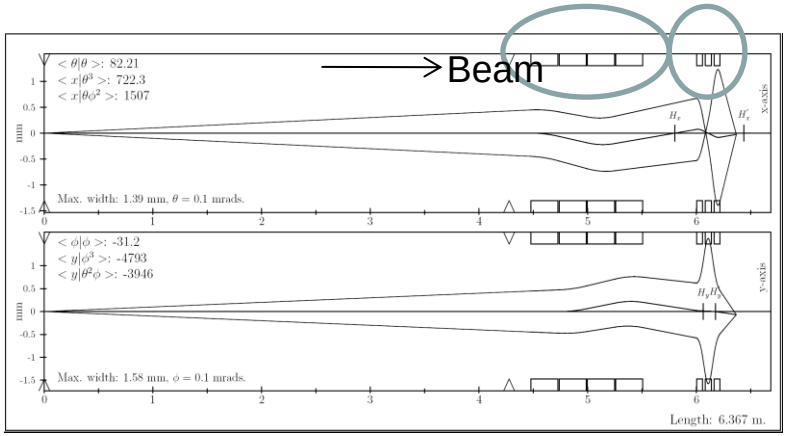
CR39 track etch, 20um spacing

= area of cell nucleus ~ 10um

Beam Scanning



- Electrostatic dog-leg scanning system scans into the principle planes of the lens for very low scanning aberration.



- Electrostatic dog-leg scanning system scans into the principle planes of the lens for very low scanning aberration.
- Can scan 500 um x 500 um
- Better accuracy than using stage to position cell over beam.

CHARM for cell recognition

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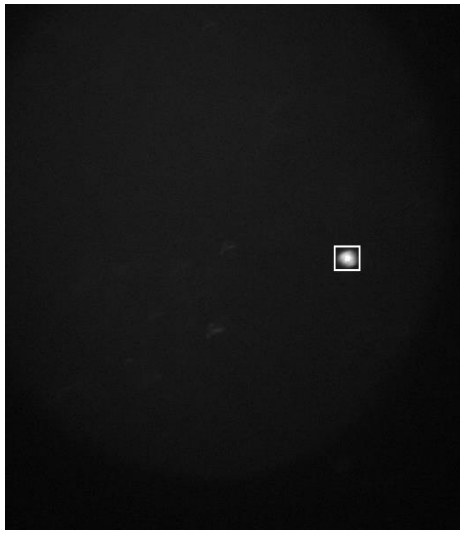
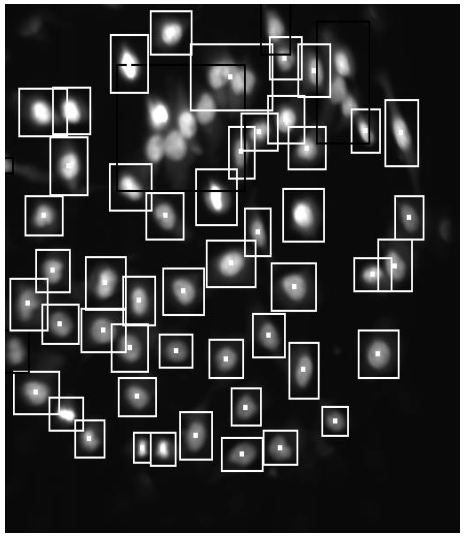
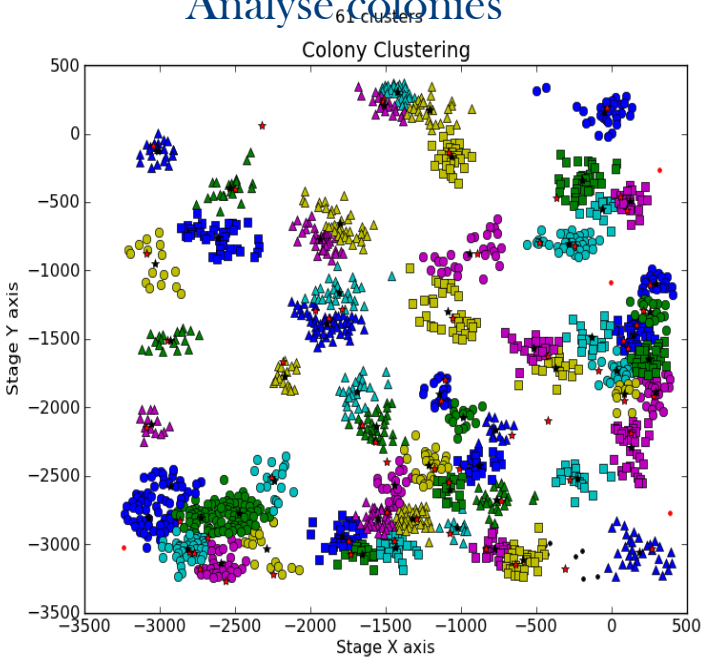
Day 1: Find Cells



Day 3-4: Count colonies



Analyse colonies



- Developed by Gray Institute for Radiation Oncology and Biology
- Uses many parameters to find cells:
  - Edge strength, size, circularity, intensity, etc.

- Sometimes struggles with clustered cells…

Targeted cells stained with the antibody anti-γH2AX to show double strand breaks where the beam has irradiated the cells
3.8 MeV H^+, 5 Gy irradiation

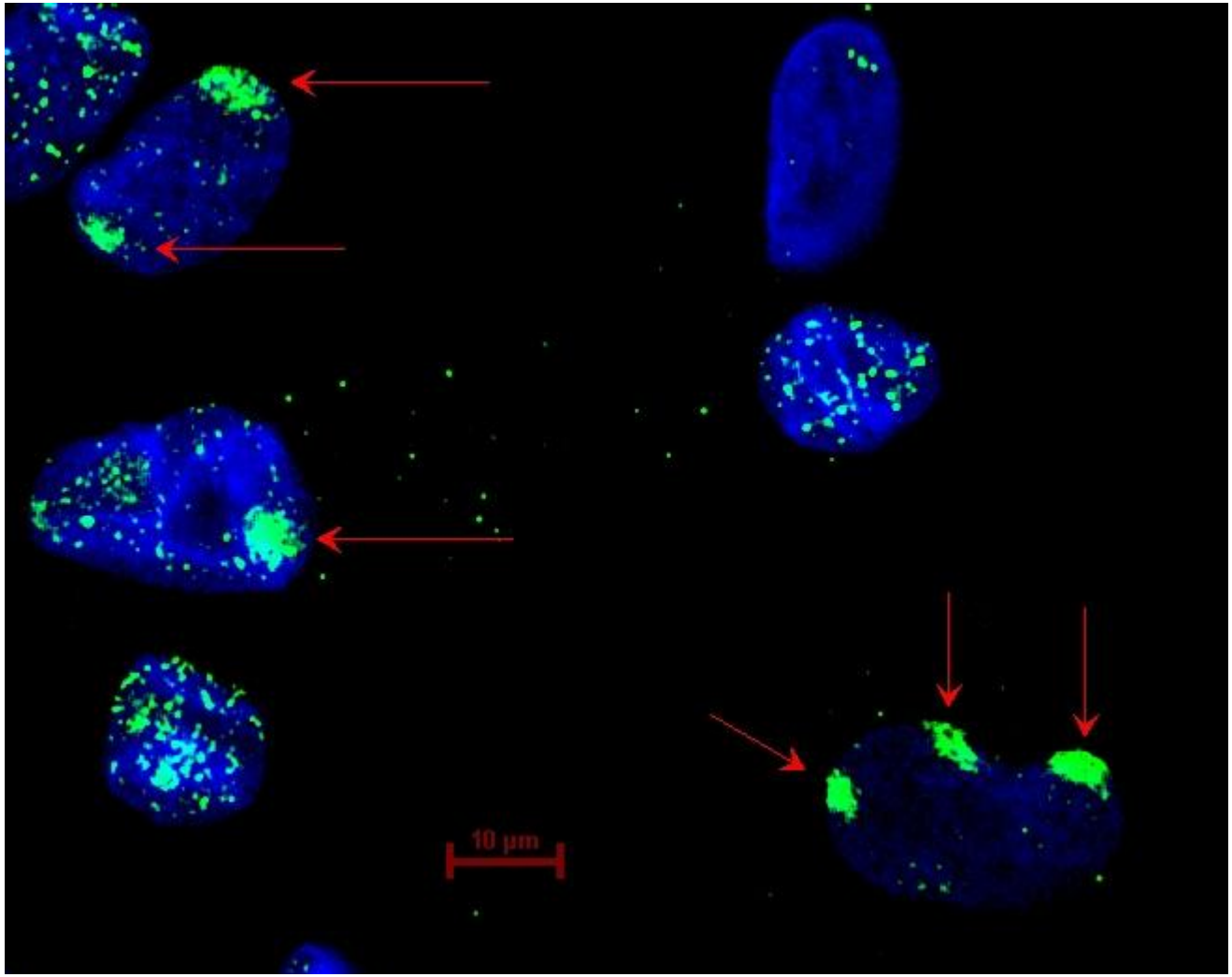


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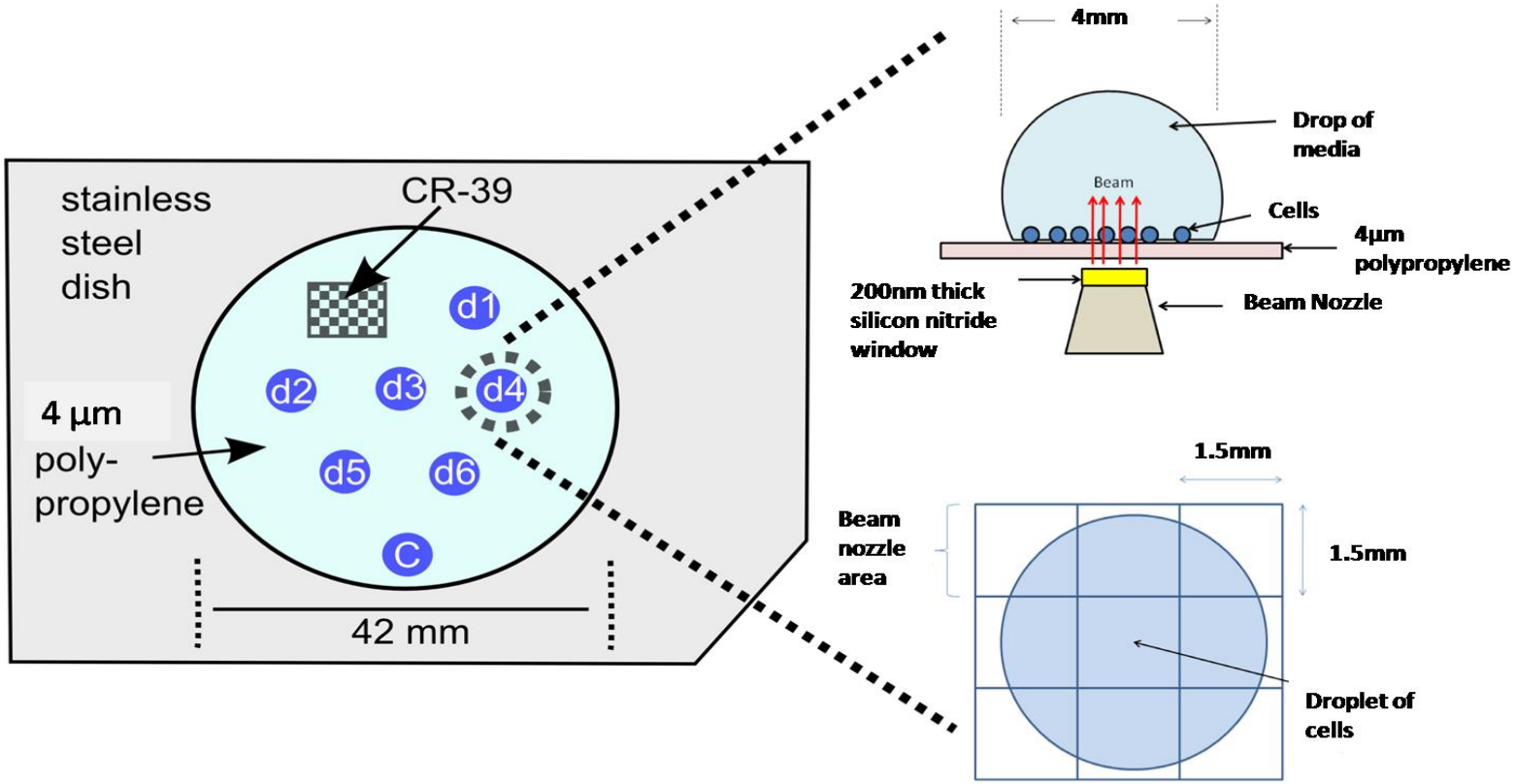
TABLE 1

The Range in Water (in Microns) of a Selection of Ions that can be Injected into the VNB as a Function of the Charge State after Stripping and Corresponding Energy Calculated in SRIM (*33*). The Energy is Constrained by the 2 MV Terminal. The Heaviest Element that can be Bent Up Tower by the 90° Magnet is Calcium

Charge state	1	2	3	4	5
Energy (MeV)	4	6	8	10	12
Ion					
^{1}H	241				
^{4}He	29.2	48.4			
^{7}Li		20.7			
^{12}C			11.4	13.8	16.5
^{16}O			9.3	10.9	12.5
^{20}Ne			8.8	10.0	11.2
^{35}Ci					8.6
^{40}Ca					8.0

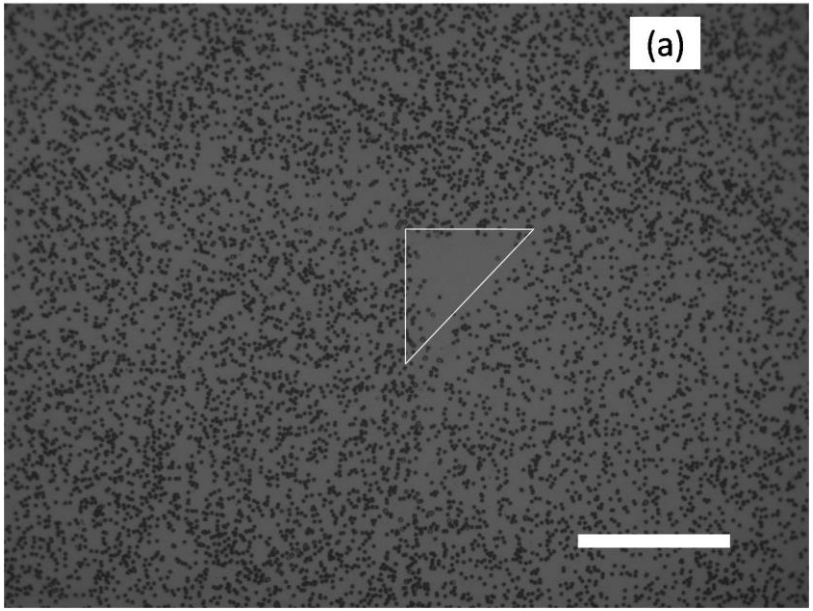
Merchant *et al.* (2012) Radiation Research

Broad-field irradiation

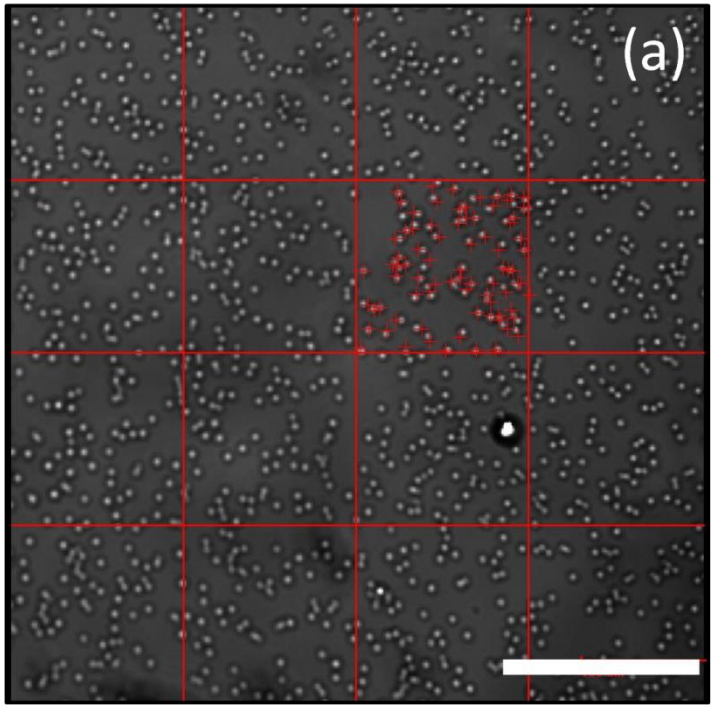


- Setting up the beam, checking stability and fluence of the beam takes about ½ day.
- Each "droplet" takes about 2 minutes to irradiate and so about 12 minutes to do a full dish of 6 "droplets". This does depend on the dose given.
- Also extra time is needed between dishes to check on the fluence of the beam.

Broad-field irradiation



Broad-field particle tracks in CR39 plastic



Uncertainties associated with "broadbeam" irradiation with charged particles

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Physics/ Dosimetry

$$\dot{D} = 1.6\, e^{-9}\, \frac{LET\,.\Phi}{\rho}\ (\mathrm{Gy/s})$$

LET: Linear Energy Transfer (keV/μm)

↳ Beam straggling & Variation across the cell thickness

Φ : Beam Flux (particles/cm^2.s)

↳ In-homogeneity & Beam current variation

AND POISSON STATISTICS

$$p(n) = \frac{(N\omega_0)^n}{n!} e^{-N\omega_0}$$

n: number of hit(s)
N <=> Φ
ω_0 <=> nucleus area

Uncertainties associated with "broadbeam" irradiation with charged particles

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What can we do?

LET: Beam straggling

> Mono-energetic beam produce by the accelerator, no scattering foil

Variation across the cell thickness

> Work with higher energy (3 MeV proton instead of 1 MeV proton)

> Calculation of the energy loss

Φ :In-homogeneity

> Homogeneity assessment with CR39

Beam current variation

> Check beam stability before irradiation

Uncertainties associated with "broadbeam" irradiation with charged particles

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What can we do?

Poisson statistics:

> Less important with higher fluences

Table 2 Contributions to the dose uncertainty

Unit	Average number of particles per cell		Standard deviation of particles assuming Poisson distribution		Dose uncertainty contribution due to Poisson statistics		Dose uncertainty contribution due to volume-averaged LET variation		Dose uncertainty due to flux variation (5 %)	
	Particles		Particles		Gy		Gy		Gy	
Average dose (Gy)	Protons	Alphas	Protons	Alphas	Protons	Alphas	Protons	Alphas	Protons	Alphas
0.5		2		1.41		0.35		0.004		0.03
1	28	4	5.52	1.85	0.20	0.46	0.003	0.008	0.05	0.05
2	56	8	7.43	2.87	0.27	0.72	0.006	0.016	0.10	0.10
3	84	12	8.98	3.45	0.32	0.87	0.008	0.024	0.15	0.15
4	112	16	10.26	3.99	0.37	1.00	0.011	0.032	0.20	0.20
5	138	20	11.52	4.47	0.41	1.12	0.014	0.040	0.25	0.25
6	166		12.73		0.46		0.017		0.30	

Poisson statistics LET Φ

Jeynes *et al.* (2012) Radiation and Environmental Biophysics

Uncertainties associated with "broadbeam" irradiation with charged particles

Biology

$$PE = \frac{N_{colonies}}{N_{cells}} \quad > \quad S = \frac{PE_D}{PE_{CTl}}$$

Plating Efficiency

↳ Number of cells seeded & Number of counted colonies

AND Biological system variation

What can we do?

Plating Efficiency:

Number of cells seeded

> Seed cells from the same solution, count them after seeding, ...

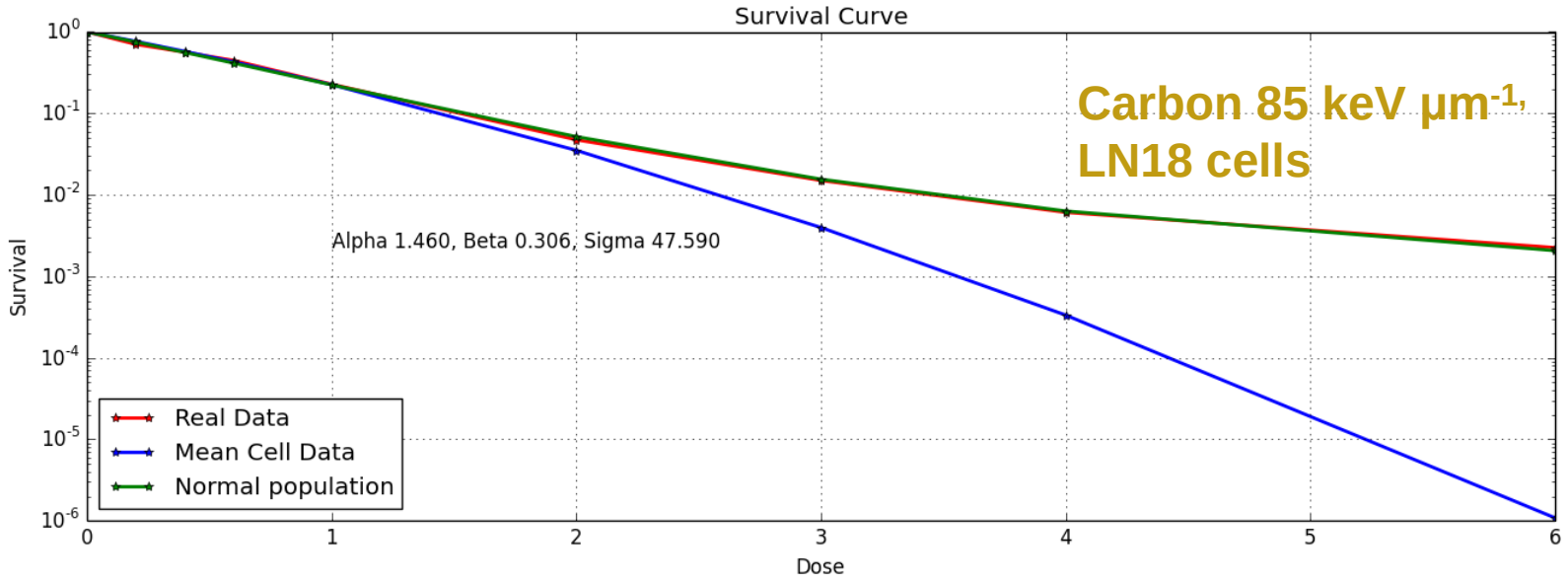
Colony counting

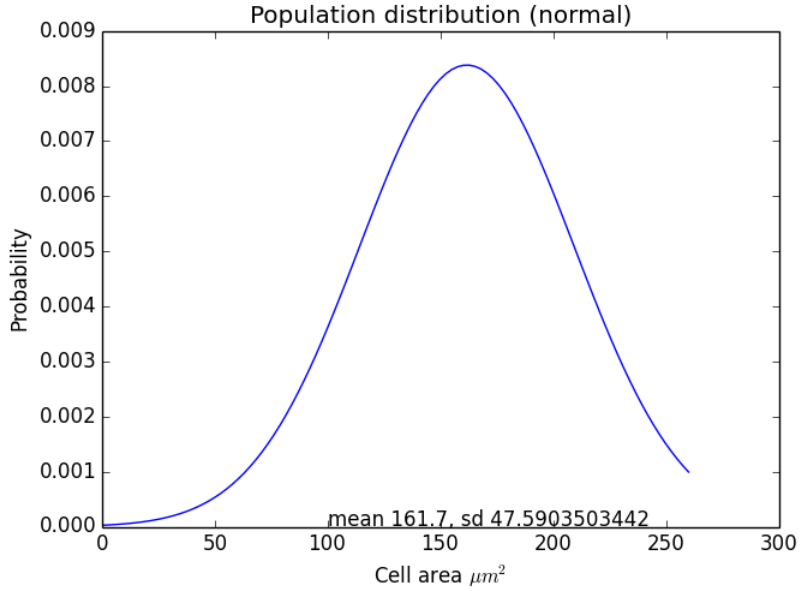
> Work with appropriate dilution

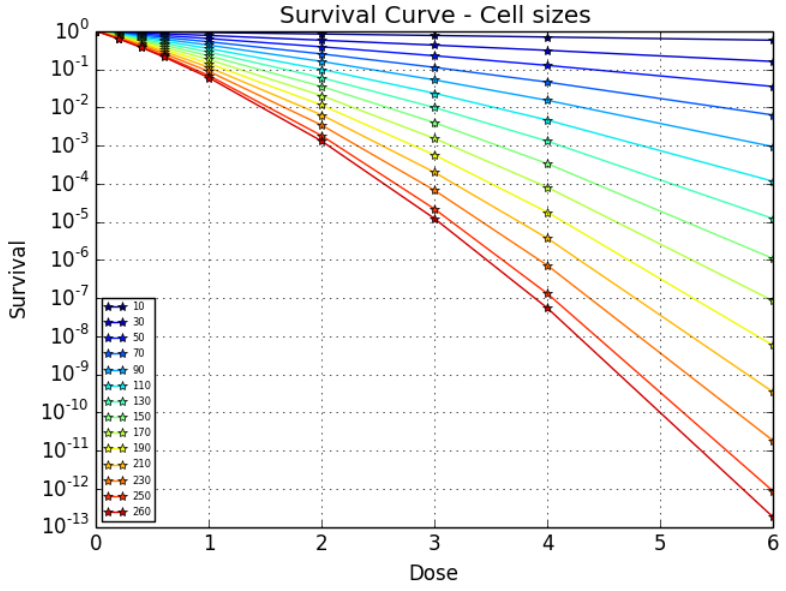
Biological system:

> Replicates

Analysis of Survival Curve considering cell size - Broadbeam







Wera *et al.* (2014) Phys. Med. Biol

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Thanks for listening!!