The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
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IAEA
International Atomic Energy Agency

Workshop on Biomedical Applications of High Energy Ion Beams

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Venue:
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ICTP, Trieste, Italy

Microbeam Technology

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Microbeam technology (in radiation biology)

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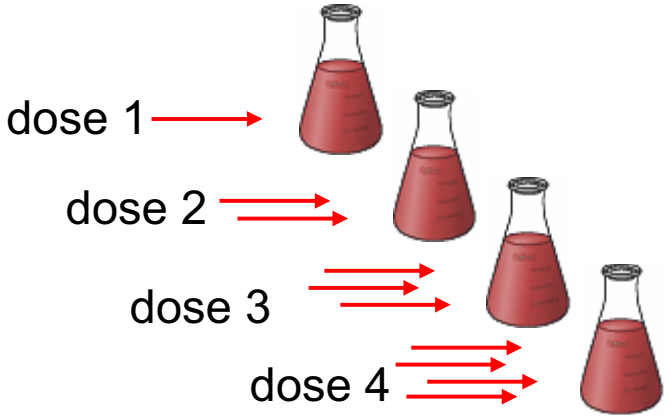
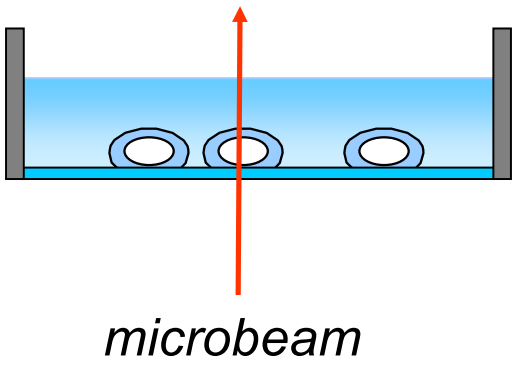
Microbeams in radiobiology

- Typically, cellular radiobiological effect is measured by irradiating and analysing **cell populations**.



- However, using a microbeam, it is now possible to irradiate **individual cells** within cell populations or tissues, or indeed selected parts of a cell.



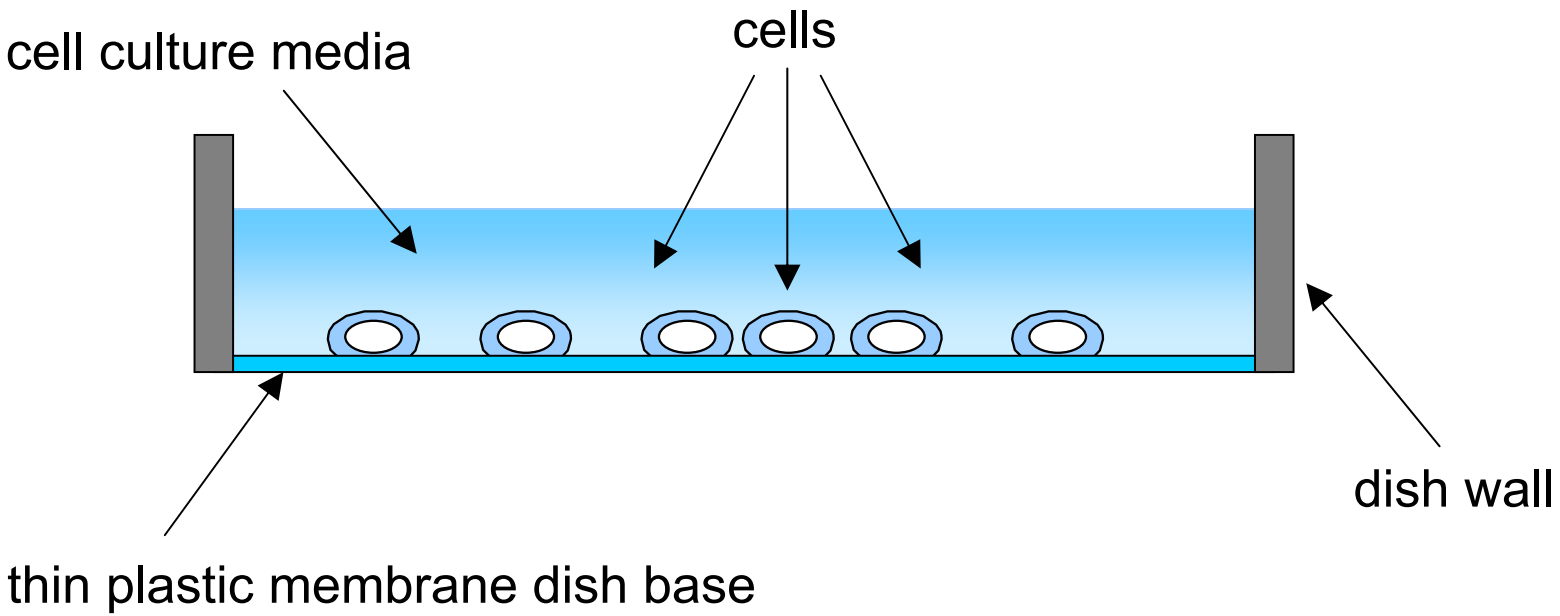
- This is very useful!

What is a microbeam?

- For radiation biology applications, a microbeam is a facility for aiming a micron-sized ‘beam’ of ionizing radiation at a biological target (for example, the nucleus of a cultured mammalian cell).
- Most microbeams use collimated or focussed energetic ions
- Microbeams have also be developed that use focussed low-energy X-rays and electrons
- Microbeams require methods for identifying and aligning the target to the radiation beam
- Precise dose delivery requires that the amount of delivered radiation to each target is controlled
- If many cells are to be irradiated, then the process of identifying, aligning and irradiating cells should be automated

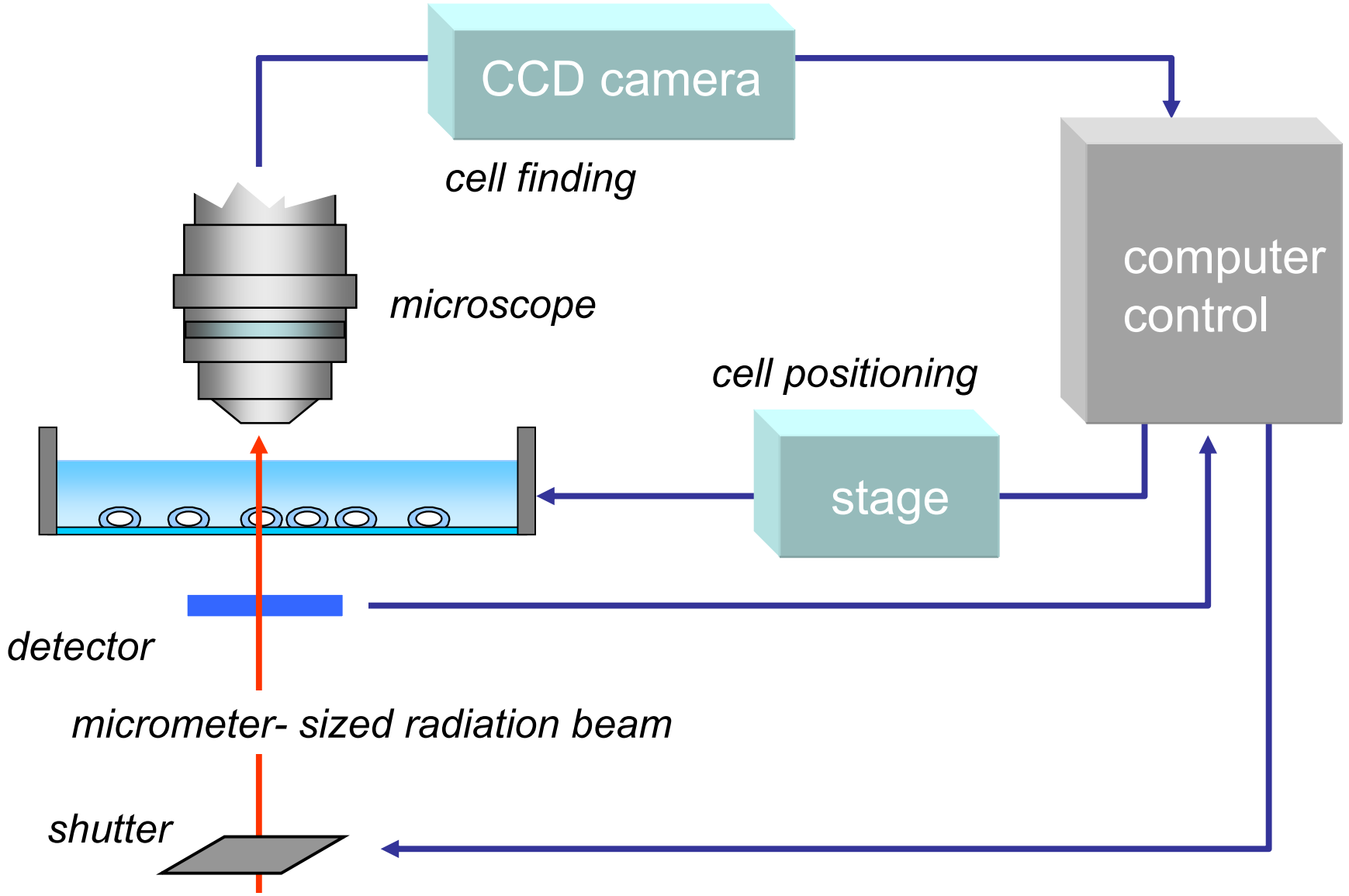
What is a microbeam?

- The radiations used in microbeam experiments have poor penetration in tissue (~few tens of microns), so specially designed cell dishes are needed



- Only cell types that attach to the plastic membrane can be used

What is a microbeam?



Rationale for microbeams

Advantages of microbeam methods:

- Can selectively irradiate individual cells within a cell population
- Can selectively irradiate individual parts of individual cells
- Can irradiate single cells with exact low doses (i.e. single ion)
- Single cell assay implicit in method

Advantages of non-microbeam (broad-field) methods:

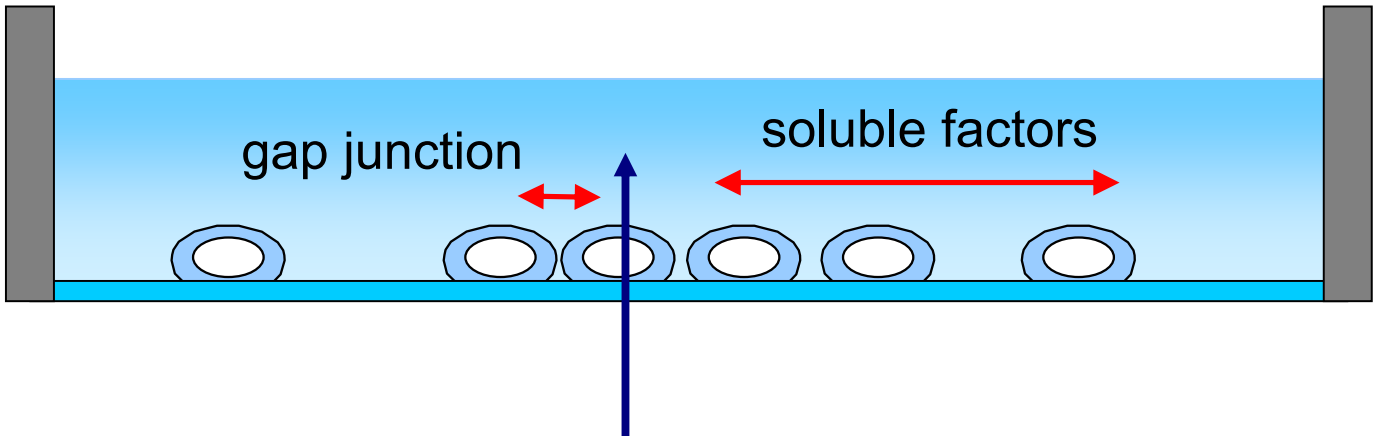
- Most irradiation equipment available commercially
- Well-established methods for irradiating biological samples
- Procedures often straightforward to implement and execute
- Can be used for experiments *in vitro* or *in vivo*
- Large sample volumes irradiated quickly

Rationale for microbeams

Irradiate individual cell (or cells) within a cell population

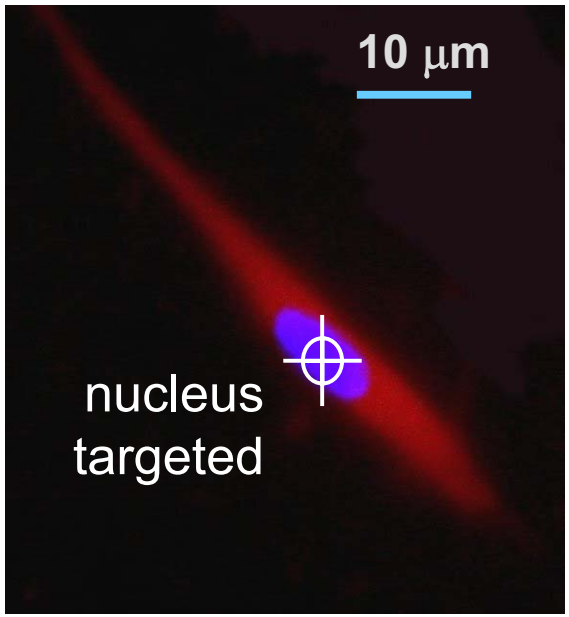
- The ‘method of choice’ for investigating the **bystander effect**

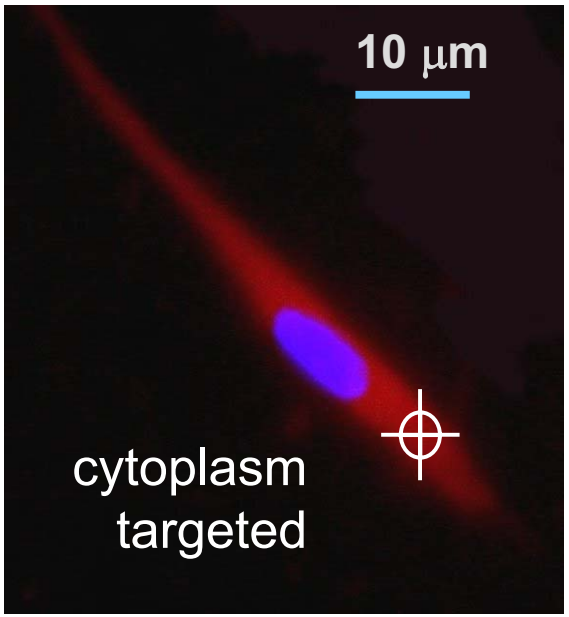
  - Cell-to-cell communication, either through gap-junctions, or soluble factors are important, and can be studied through microbeam experiments



Rationale for microbeams

Selectively irradiate individual parts of individual cells

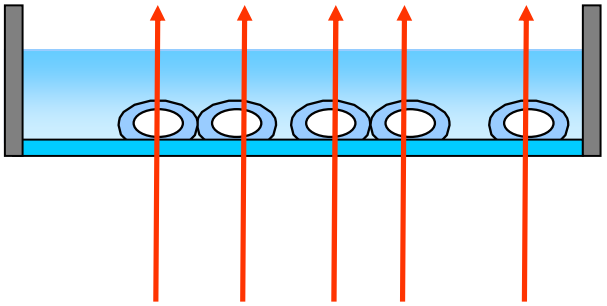




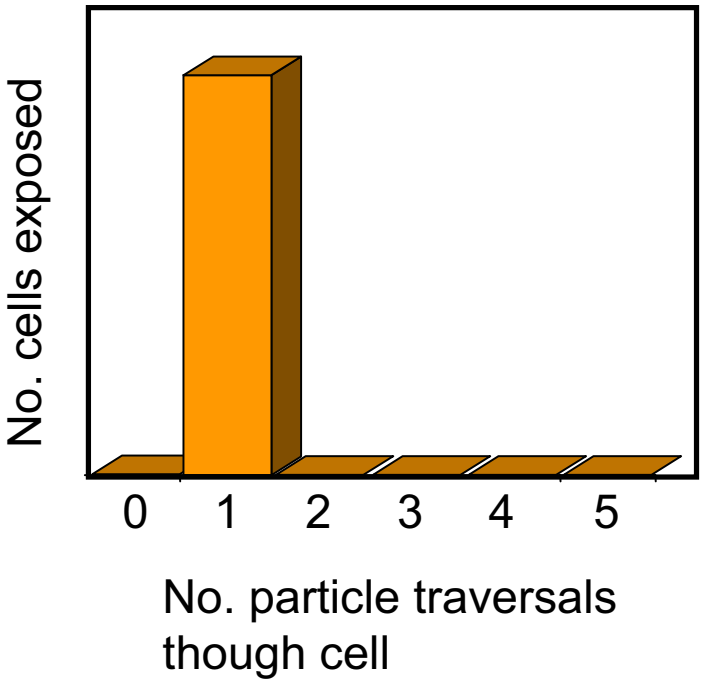
- 'Conventional wisdom' is that irradiating just the cytoplasm (no genomic DNA) is ineffective at low-to-moderate doses
- Microbeam experiments challenge conventional wisdom!

Rationale for microbeams

Irradiate single cells with a single particle: *microbeam*

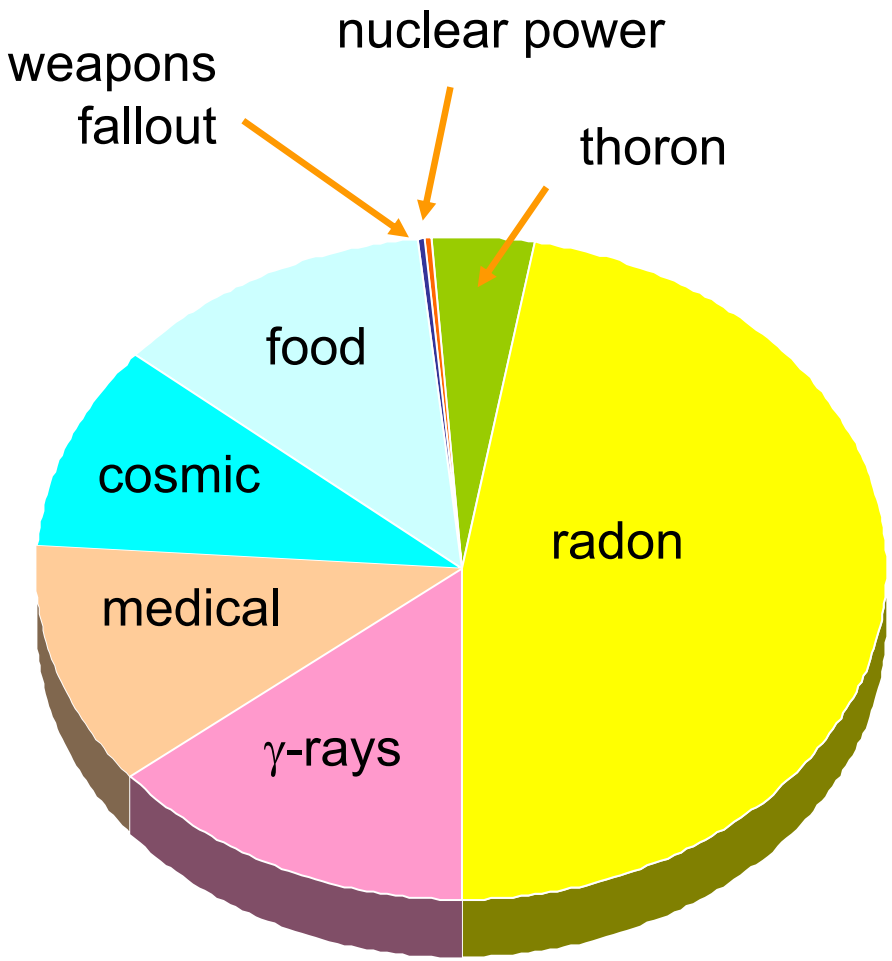


microbeam



- Microbeam: **exactly** of one particle per cell
- 100% traversed by exactly one particle

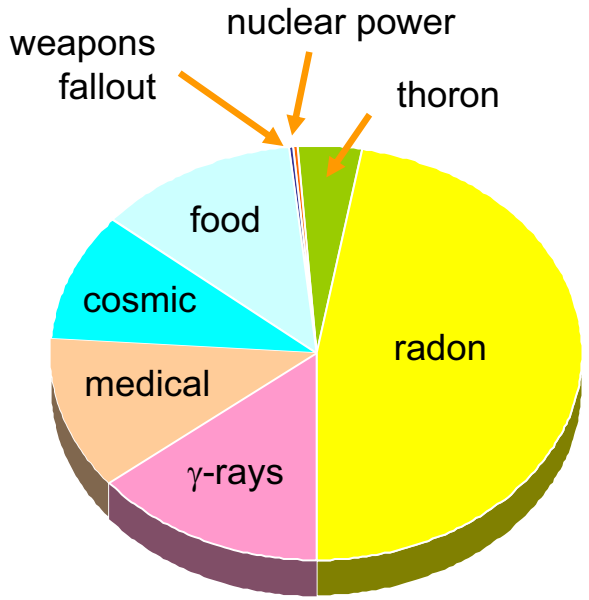
Radiation risk



- Radon daughters are inhaled and irradiate lung cells
- Radon daughters are α-particle emitters.
- In the south-west of England, estimates are that 1 in 20 lung cancers are due to radon

Average UK dose is 2.5 millisieverts per year

Radiation risk



A typical background dose 2.5 mSv per year is equivalent to:

~1 electron track / cell / year

~1 α-particle track / cell / century

However, one α-particle deposits **~several hundred-fold** greater dose (per cell) than one electron

- The probability of a cell being crossed by more than one α-particle is remote.
- Therefore, radon risk arises from a single α-particle traversal of a cell

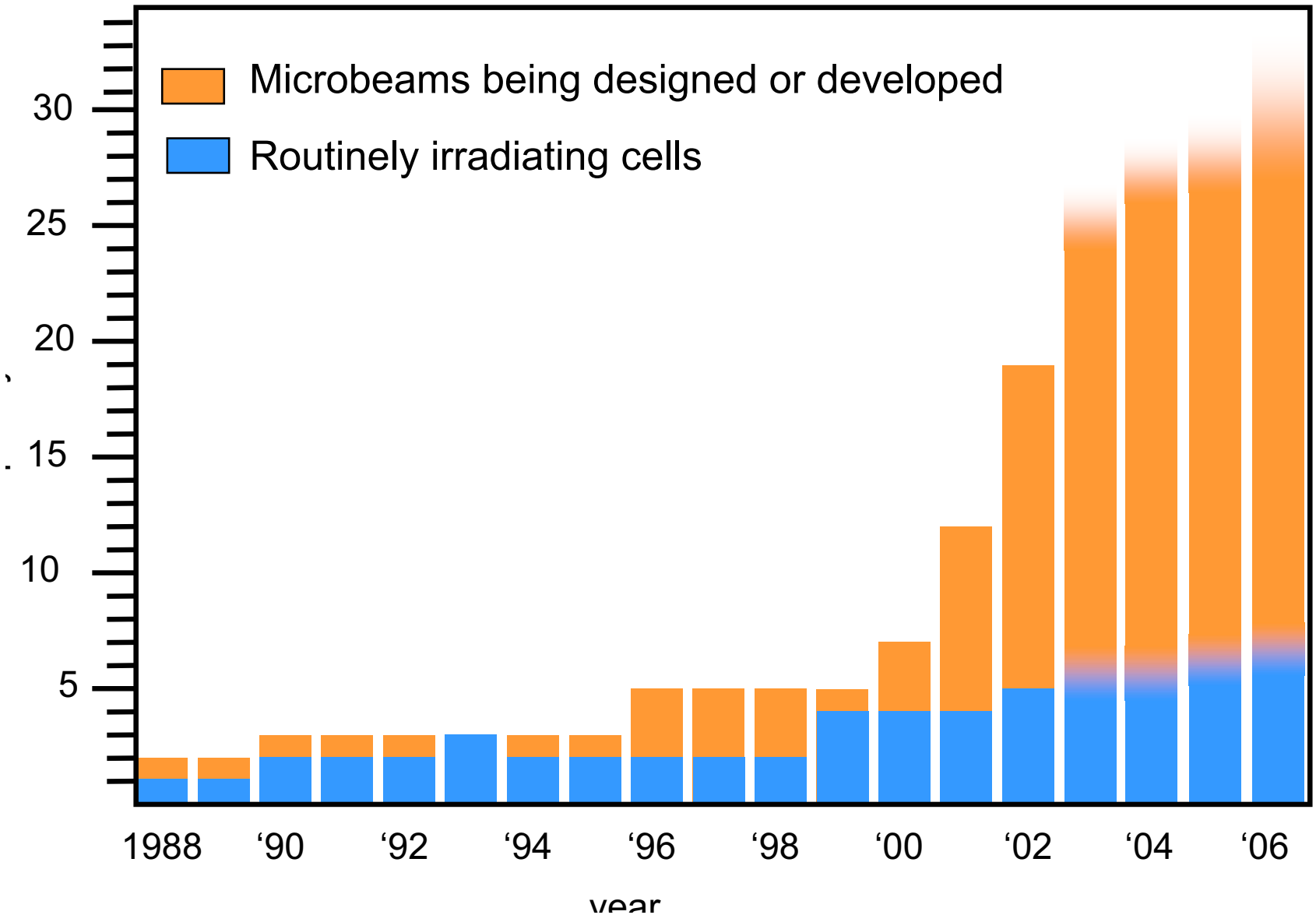
Microbeam specifications

Accuracy:	Need to reliably target the cell nucleus, or cytoplasm i.e. ~1-2 μm targeting accuracy
Detection:	Single particle delivery requires efficient real-time detection and shuttering
Imaging and alignment:	Ability to image target, log position and to precisely align target to microbeam. Ability to revisit cells
Speed:	Up 5,000 cells per dish; rapid, automated cell finding and irradiation essential
Environment:	'Cell-friendly' environment; cells in media, preferably vertical beamline

Development of microbeams Worldwide

- The first application of a charged-particle microbeam in radiobiology was a collimated system in 1953 (Zirkle and Bloom, 1953 *Science* **117**, 487)
- The first 'modern' microbeam was developed by Braby (Pacific North West, USA) in the late 1980s. This used vertically collimated light ions but was dismantled in the mid-1990s (Braby, 1992, *Scanning Microscopy*, **6**, 167)
- The second microbeam was developed at RARAF, New York in the early 1990s. This also uses vertically collimated light ions (*Geard et. al,* 1991, *Nucl. Instr. Meth.,* **B54,** 411)
- The third microbeam was developed by us at Gray Cancer Institute in the mid 1990s. Like the RARAF and PNL facilities. It also uses vertically collimated light ions. (Folkard et. al, 1997, *Int. J. Radiat. Biol.,* **72,** 375)

Development of microbeams Worldwide



Radiation Type (ions)

Particle sources for particle microbeams

- Typically, microbeams make use of particle accelerators
- It is possible to use an α-particle isotope source instead
- Most radiobiological microbeams use light ions

protons	p
helium ions	$^{3}He^{2+}$, $^{4}He^{2+}$

- Some microbeams use heavy ions i.e.

SNAKE Munich heavy ion microbeam:	protons – gold ions
GSI Darmstadt heavy ion microbeam:	carbon ions – uranium ions
JAERI Takasaki heavy ion microbeam:	argon ions, neon ions

Comparison of ‘advanced‘ ion facilities

mature

emerging

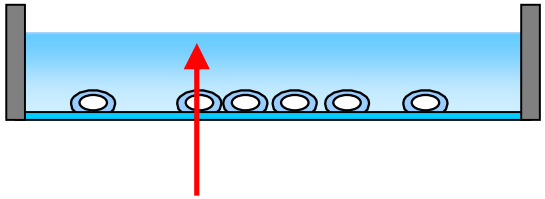
RARAF New York	H – Fe	5 MV singletron (>2006)
	He	4.2 MV VdG (<2006)
GCI, Northwood	H, He ions	4 MV VdG
JAERI Takasaki	heavy ions	90 MV AVF Cyclotron
SNAKE Munich	heavy ions	14 MV tandem
GSI Darmstadt	heavy ions	linear accelerator 11.4 MeV/u
PTB Braunschweig	H, He ions	20 MV cyclotron
INFN-LNL Legnaro	H, He ions	7 MV VdG
LIPSION Leipzig	H, He ions	3.5 MV singletron
CENBG Bordeaux	H, He ions	3.5 MV singletron
NIRS Chiba	H, He ions	3.4 MV HVEE tandem
IFJ Krakow	H, He ions	2.5 MeV VdG

Ion sources for particle microbeams

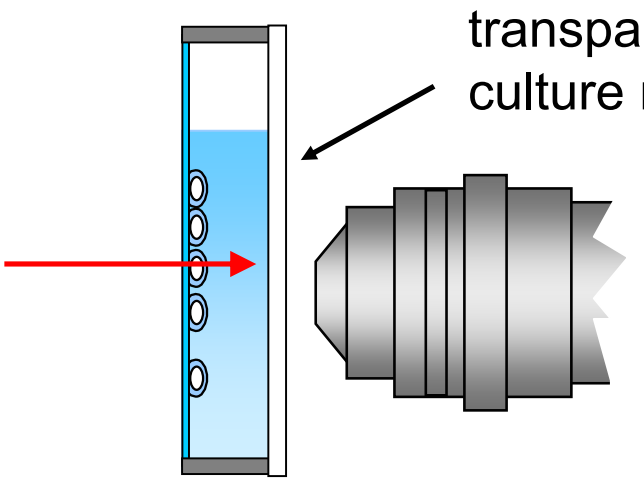
Protons	✓	Most penetrating for given accelerator
	✓	Useful LET range (10 – 40 keV μm^{-1})
	✓	Relevant to proton radiotherapy
	✗	Easily scattered
Helium ions	✓	Radiobiolically relevant to risk (radon)
	✓	Good penetration, less readily scattered
Heavy ions	✓	(Very) high LET studies
	✓	Particle radiotherapy, other than protons
	✓	Reduced scattering
	✗	Limited radiobiolical relevance

Microbeam orientation

- The preferred orientation for a radiobiological microbeam is **vertically up**

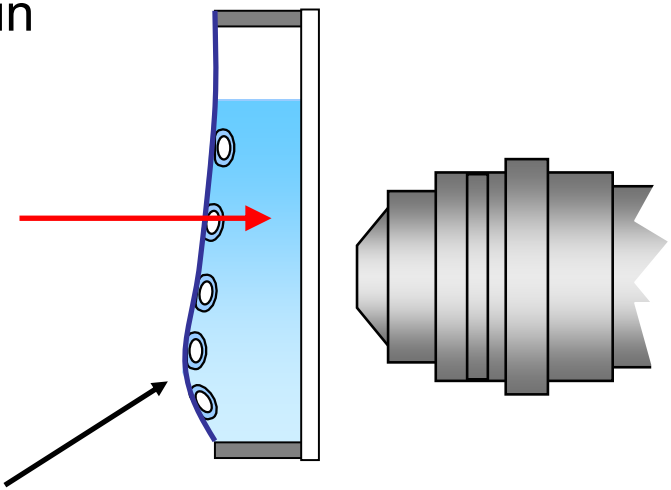


- Cell dish design more complex in other directions



transparent cap to contain culture media needed

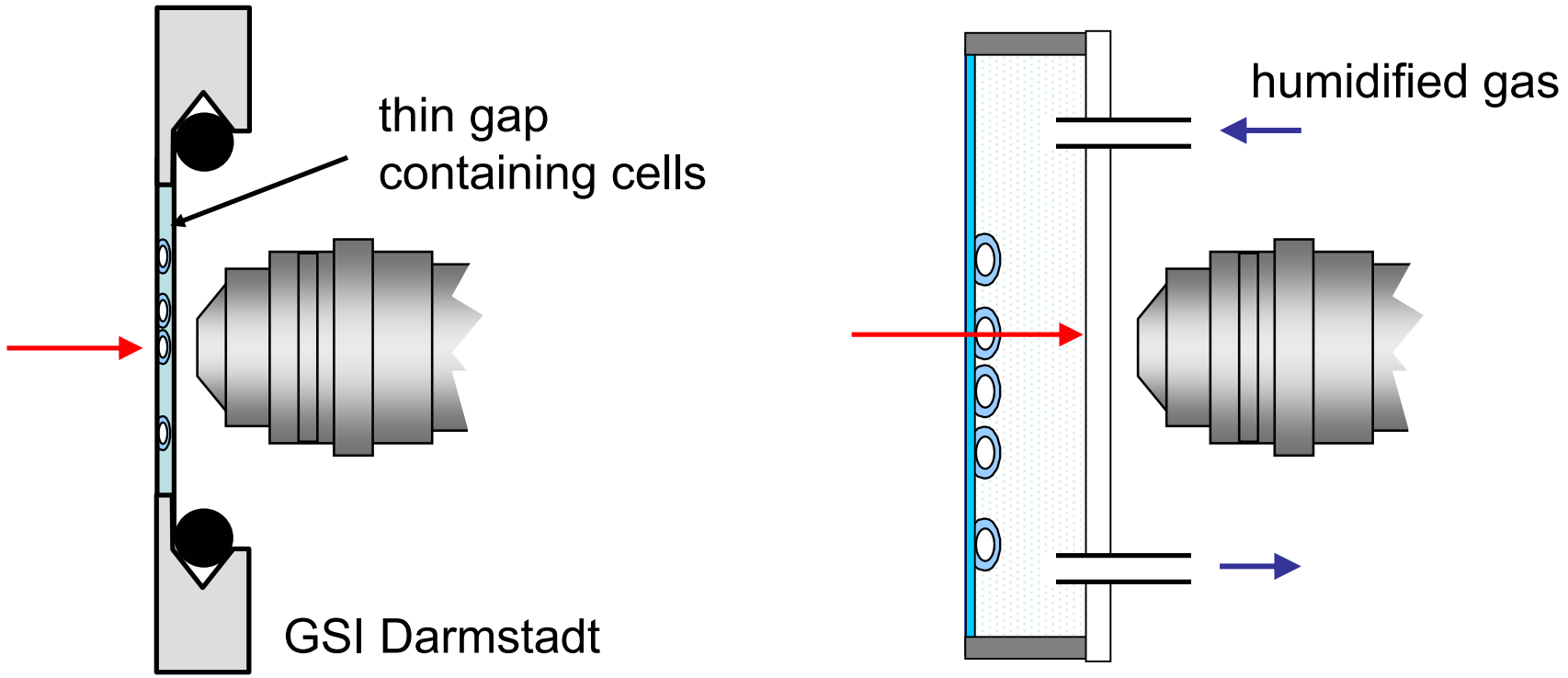
horizontal microbeam



gravity can cause plastic window to bow unpredictably

Microbeam orientation

- Nevertheless, several microbeams use a horizontal orientation
- Horizontally oriented systems usually developed around an existing horizontal beamline, or microbeam



Comparison of ‘advanced‘ ion facilities

mature

emerging

RARAF New York	He ions	vertically up
GCI, Northwood	H, He ions	vertically up
JAERI Takasaki	heavy ions	vertically down
SNAKE Munich	heavy ions	horizontal
GSI Darmstadt	heavy ions	horizontal
PTB Braunschweig	H, He ions	vertically down
INFN-LNL Legnaro	H, He ions	horizontal
LIPSION Leipzig	H, He ions	horizontal
CENBG Bordeaux	H, He ions	horizontal
NIRS Chiba	H, He ions	vertically up
IFJ Krakow	H, He ions	horizontal

Methods of producing a microbeam

A microbeam of ions can be formed by collimation or focussing

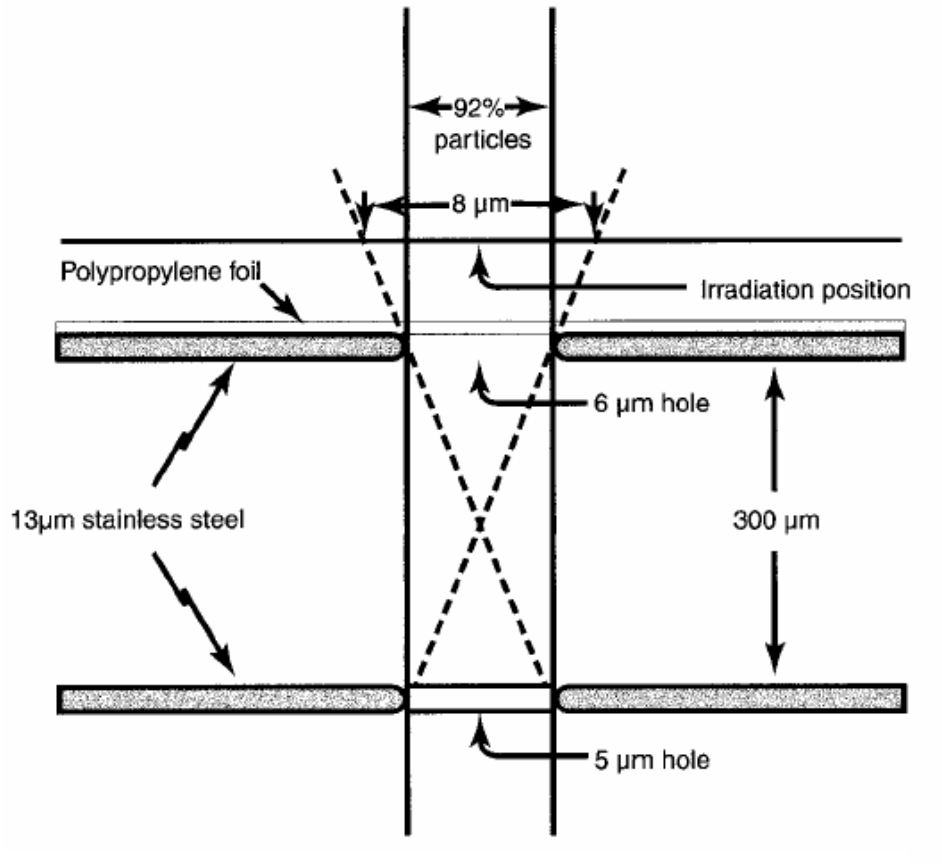
Advantages and disadvantages of collimation:

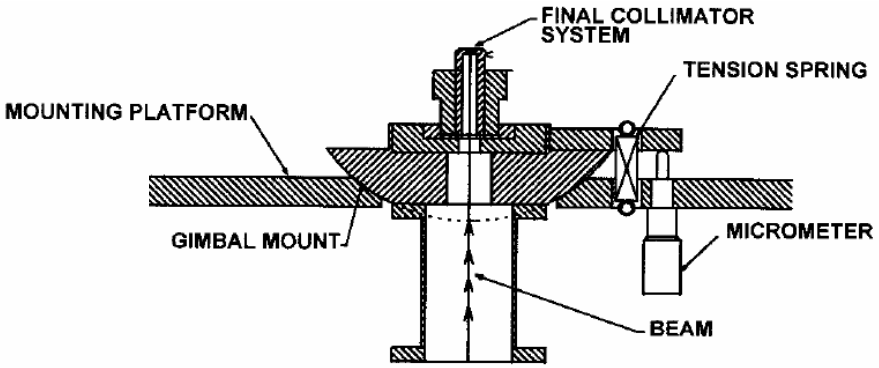
- ✓ Low-cost technology
- ✓ Can be compact
- ✗ Scattering limits spot size to a few microns

Advantages and disadvantages of focussing:

- ✓ Sub-micron spot sizes possible
- ✓ Steerable beam
- ✗ Greater space requirement
- ✗ Greater cost and complexity
- ✗ Power supplies required
- ✓ Existing microprobes available (but horizontal)

Methods of producing a microbeam: collimation

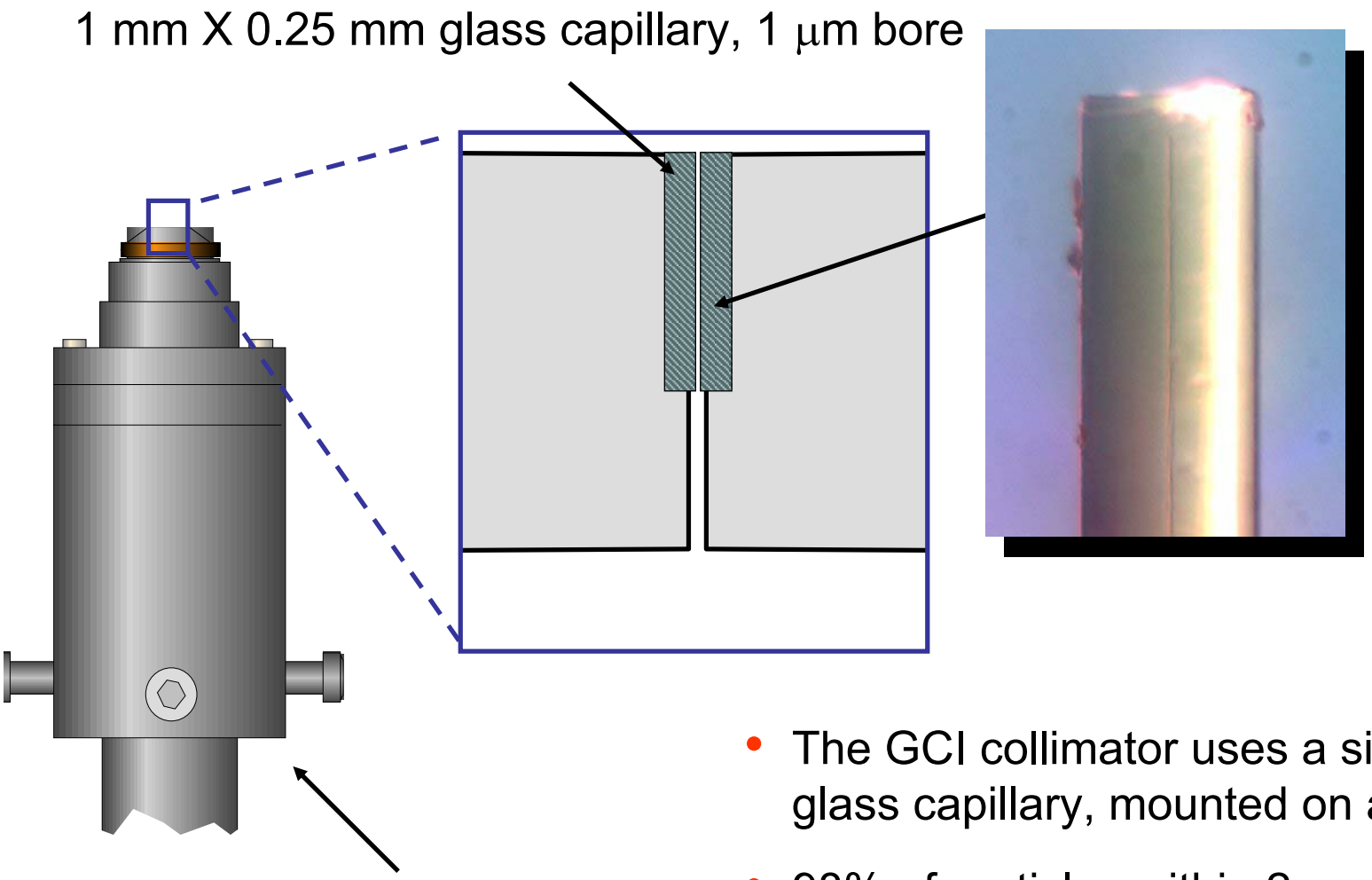




- The RARAF collimator uses two laser-drilled apertures separated by 300 µm and mounted on a gimbal
- 92% of particles within 3 µm radius

Randers-Pehrson et. al. 2001, *Radiat. Res.*, **156,** 210

Methods of producing a microbeam: collimation

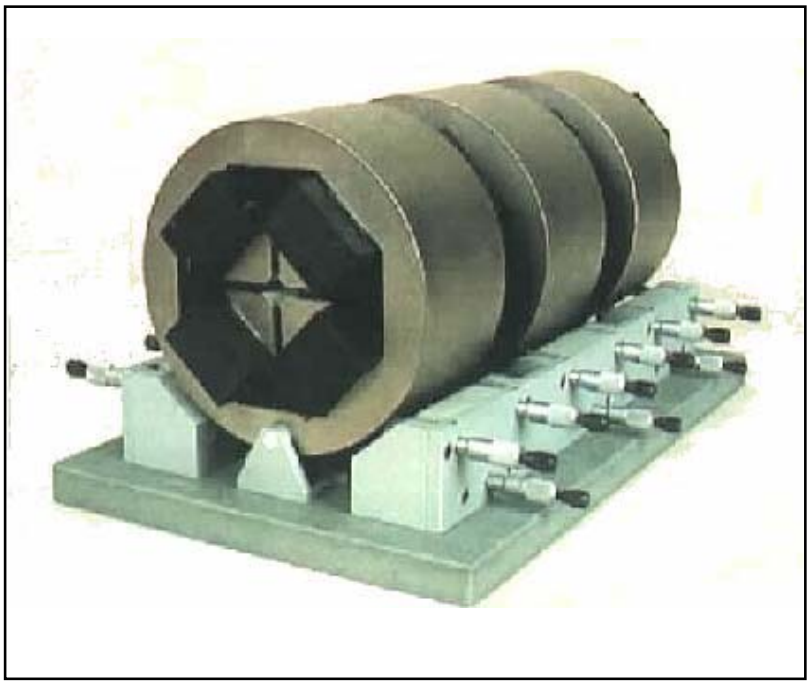


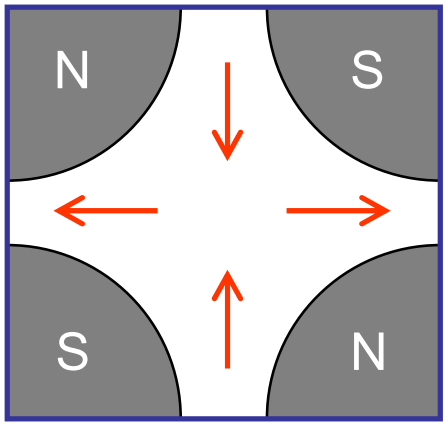
- The GCI collimator uses a single glass capillary, mounted on a gimbal
- 98% of particles within 2 μm radius

Methods of producing a microbeam: focussing

Focussing can be either **magnetic** or **electrostatic**

- Low energy beams can use magnetic solenoids
- At MeV energies, quadrupole lenses are used





Oxford Microbeams Ltd Triplet Lens system

Methods of producing a microbeam: focussing

Focussing can be either **magnetic** or **electrostatic**

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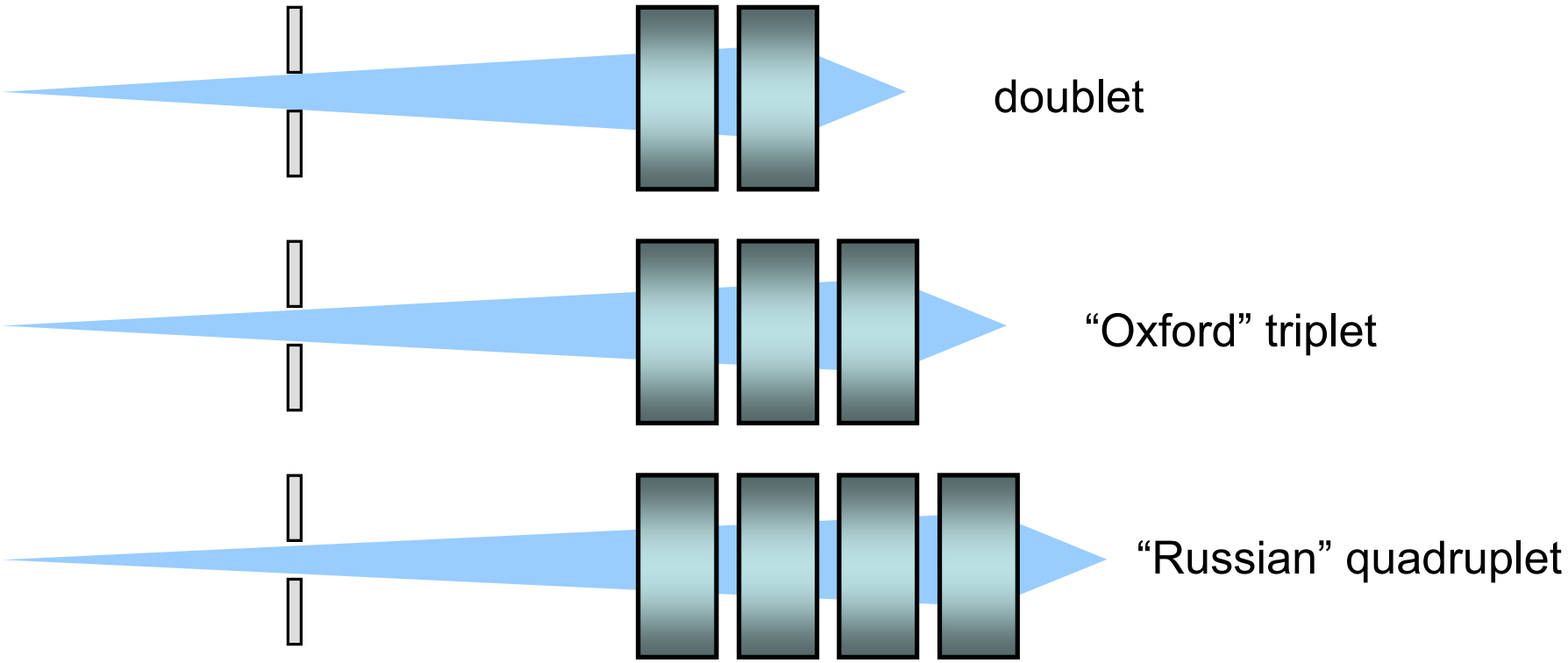


Methods of producing a microbeam: focussing

Electrostatic focussing

Advantages of electrostatic focussing:

- No hysteresis inherent in magnetic lenses, allowing easy change between differing LET beams.
- Stable voltage is more readily achieved than stable current
- The focal properties of electrostatic lenses depend only on the accelerating potential.

Comparison of ‘advanced‘ ion facilities

RARAF New York	He ions	collimation / focussing
GCI, Northwood	H, He ions	collimation
JAERI Takasaki	heavy ions	collimation
SNAKE Munich	heavy ions	focusing
GSI Darmstadt	heavy ions	focusing
PTB Braunschweig	H, He ions	focusing
INFN-LNL Legnaro	H, He ions	collimation
LIPSION Leipzig	H, He ions	focusing
CENBG Bordeaux	H, He ions	focusing
NIRS Chiba	H, He ions	focusing
IFJ Krakow	H, He ions	focusing

Particle detection

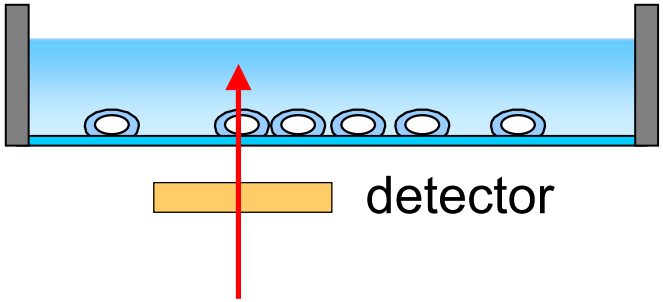
- An important requirement of an ion microbeam is that the number of particles incident on the target can be controlled
- Therefore an efficient particle detection and shuttering system is needed
- Shuttering is usually by fast electrostatic deflection of the beam

Possible methods of detections include:

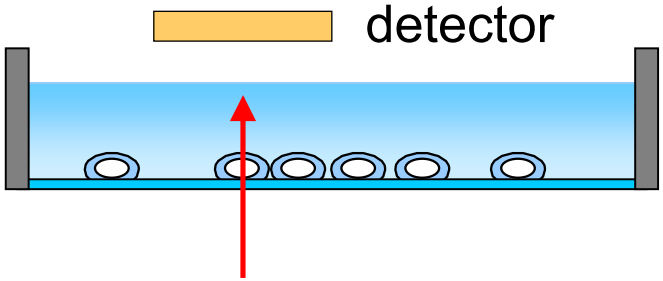
- Scintillation foils
- Gas proportional counters
- Solid-state diode detectors
- ‘Channeltron’ detectors
- ‘Track-etch’ plastic

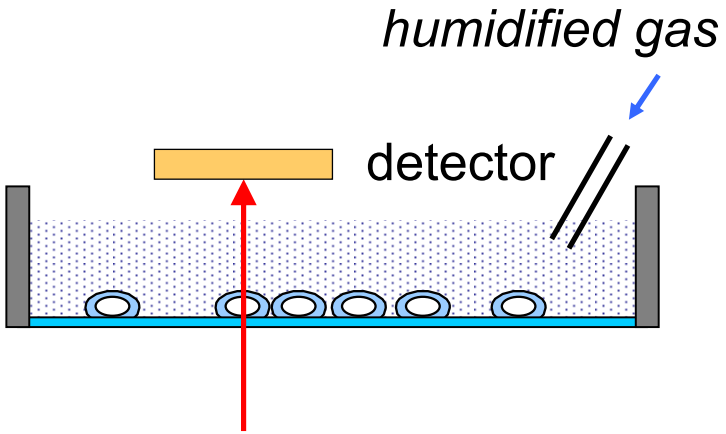
Particle detection

The detection problem…



- A detector placed before the sample has to be very thin.
- Even a thin detector will scatter the particles (reducing accuracy)

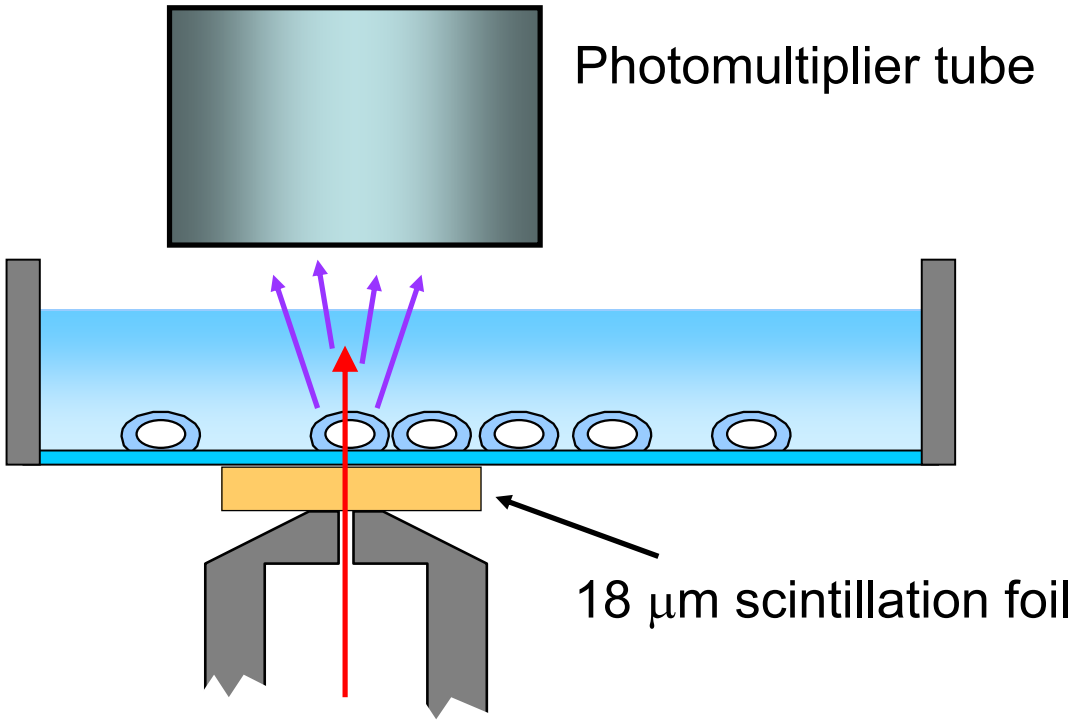




- To use a detector after the sample, the liquid is removed and the cells are bathed in humidified gas

Particle detection

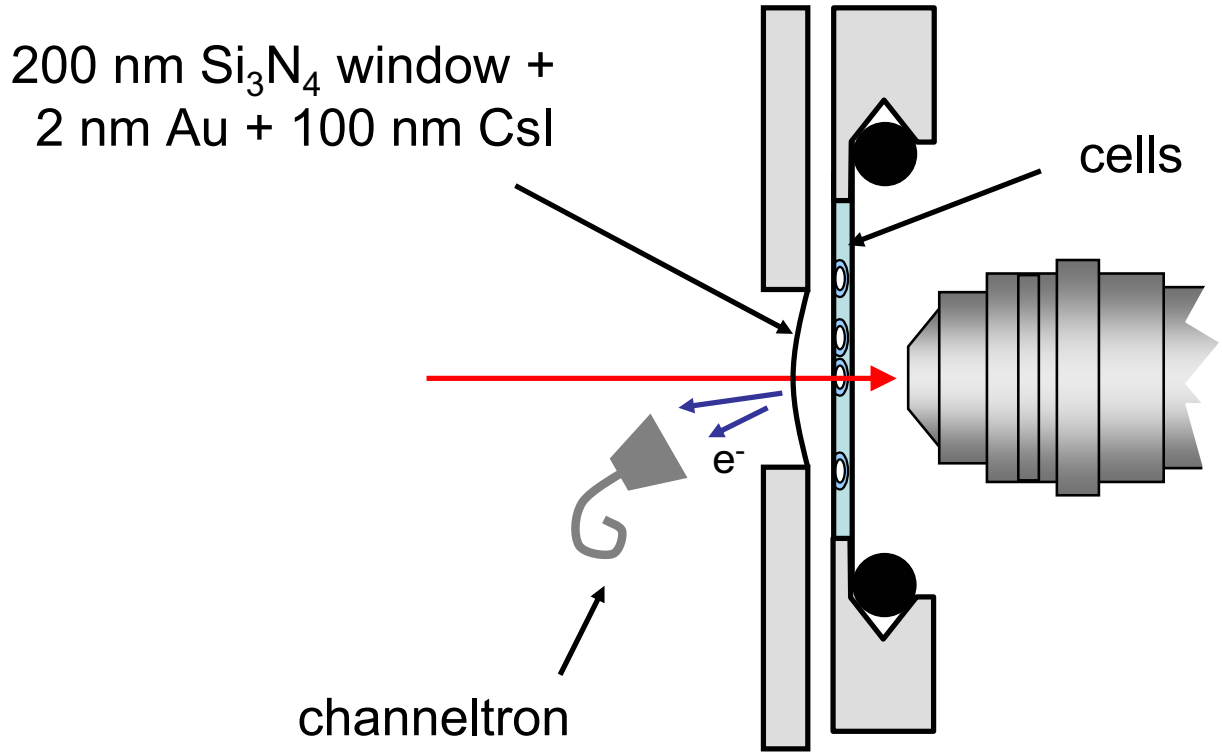
Examples: **Gray Cancer Institute**



Problem: Photomultiplier tube replaces microscope objective, therefore cannot view cells during irradiation

Particle detection

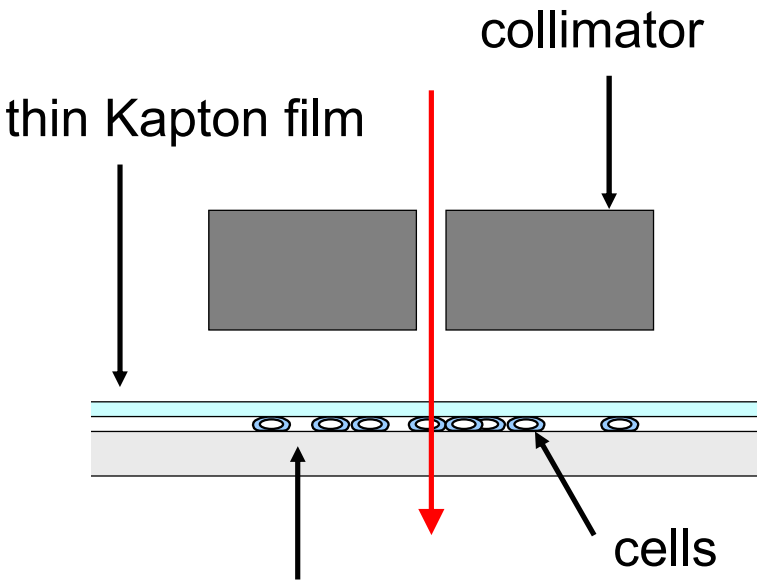
Examples: **GSI, Darmstadt**

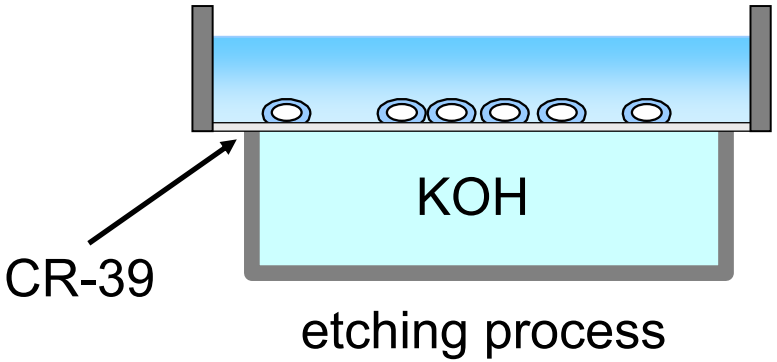


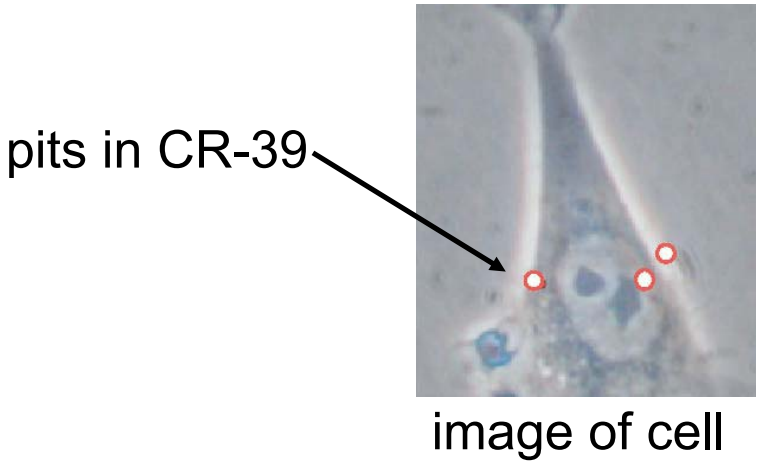
Problem: Not 100% efficient for light ions

Particle detection

Examples: **Takasaki heavy ion microbeam**



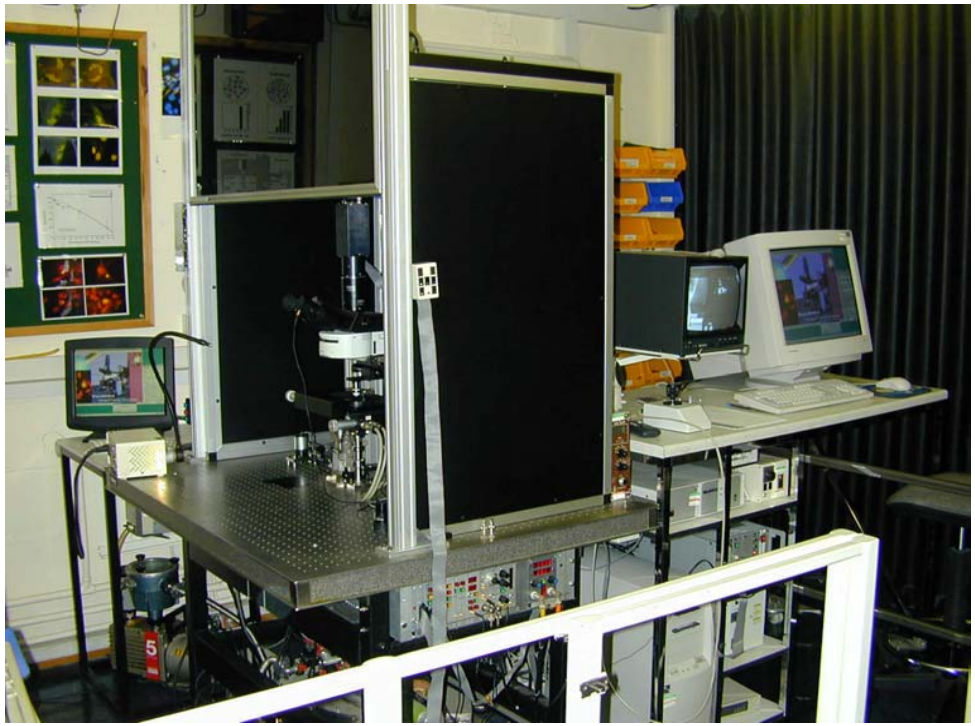




Problem: Retrospective detection; cannot be used to control the number of particle traversals

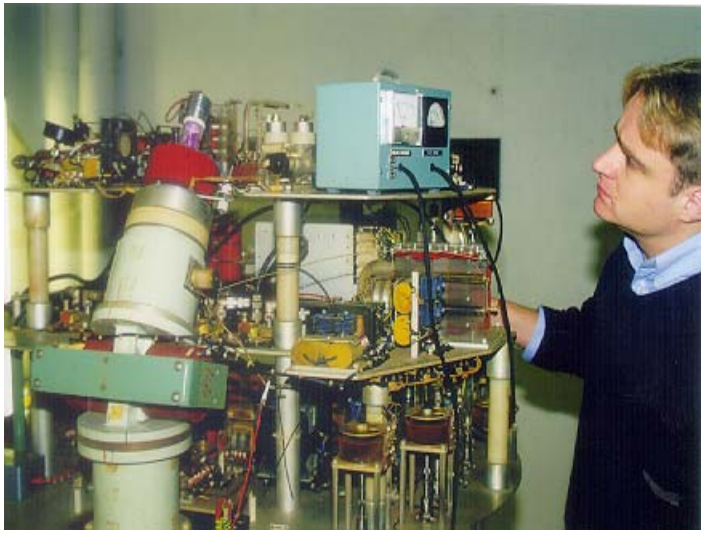
GCI charged-particle microbeam

Case study: The Gray Cancer Institute light ion microbeam

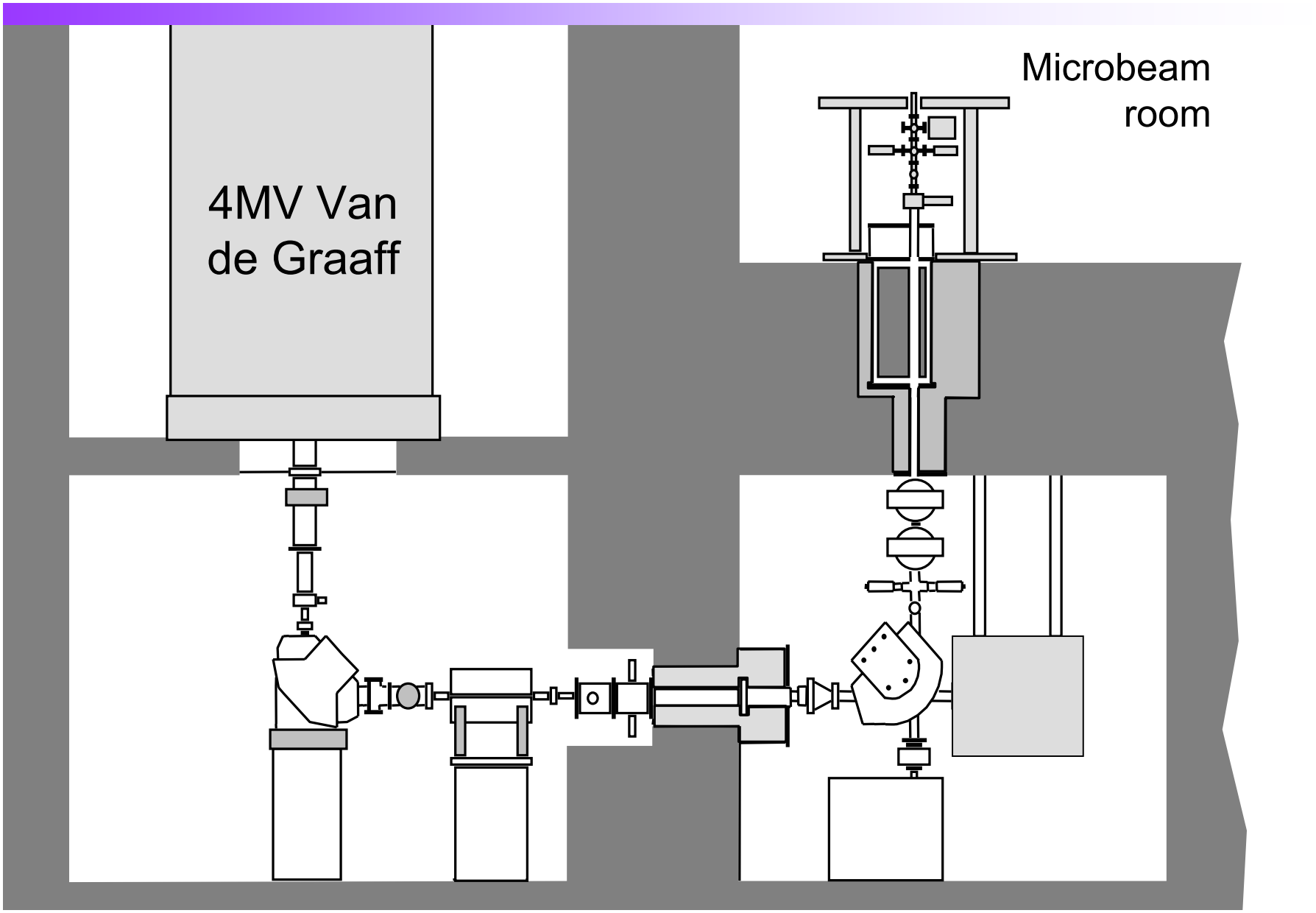


GCI charged-particle microbeam

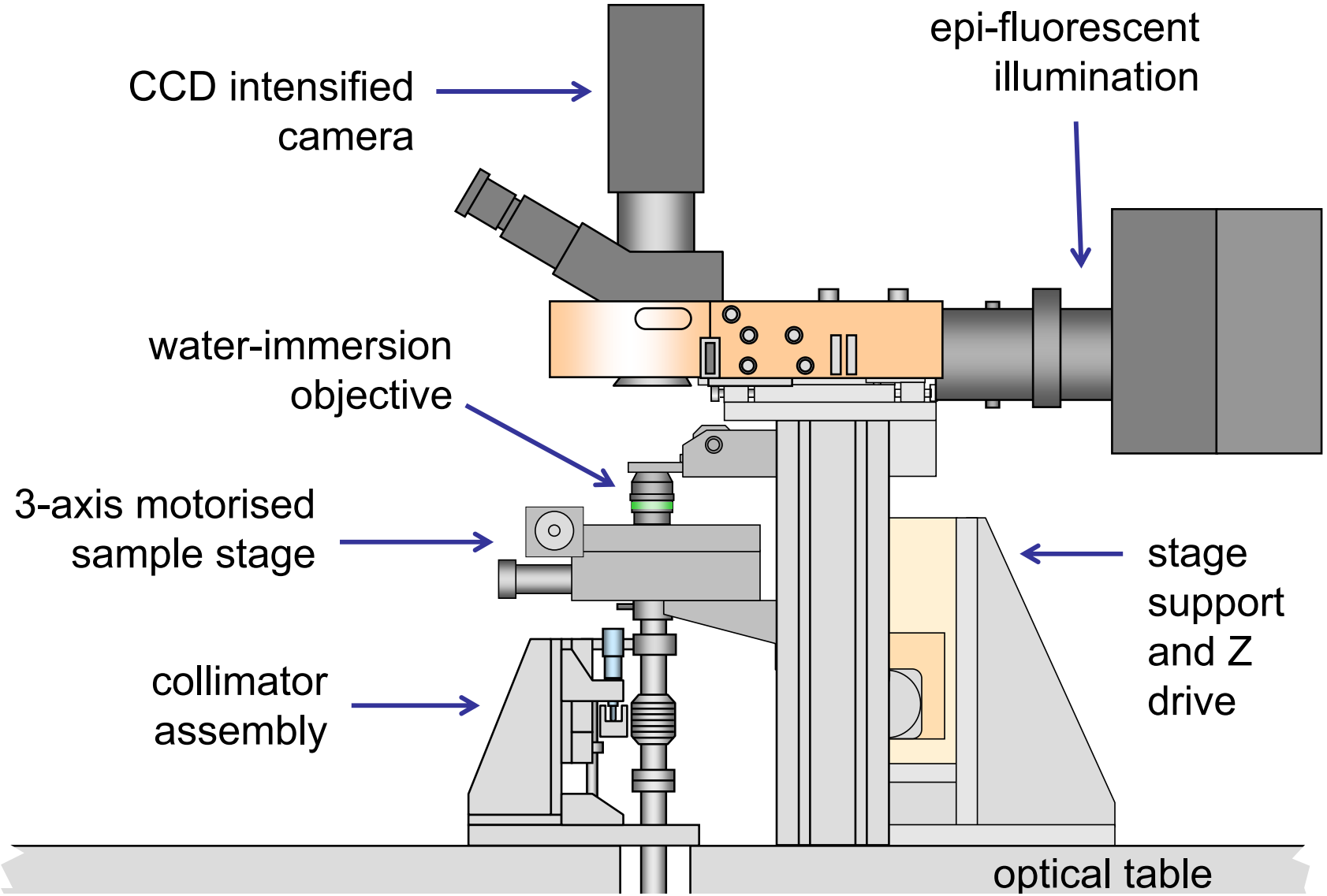
- The Gray Cancer Institute microbeam uses a 4 MV Van de Graaff accelerator
- It is used to accelerate protons and helium ions
- A dedicated vertical beamline has been added for use as a microbeam



GCI charged-particle microbeam

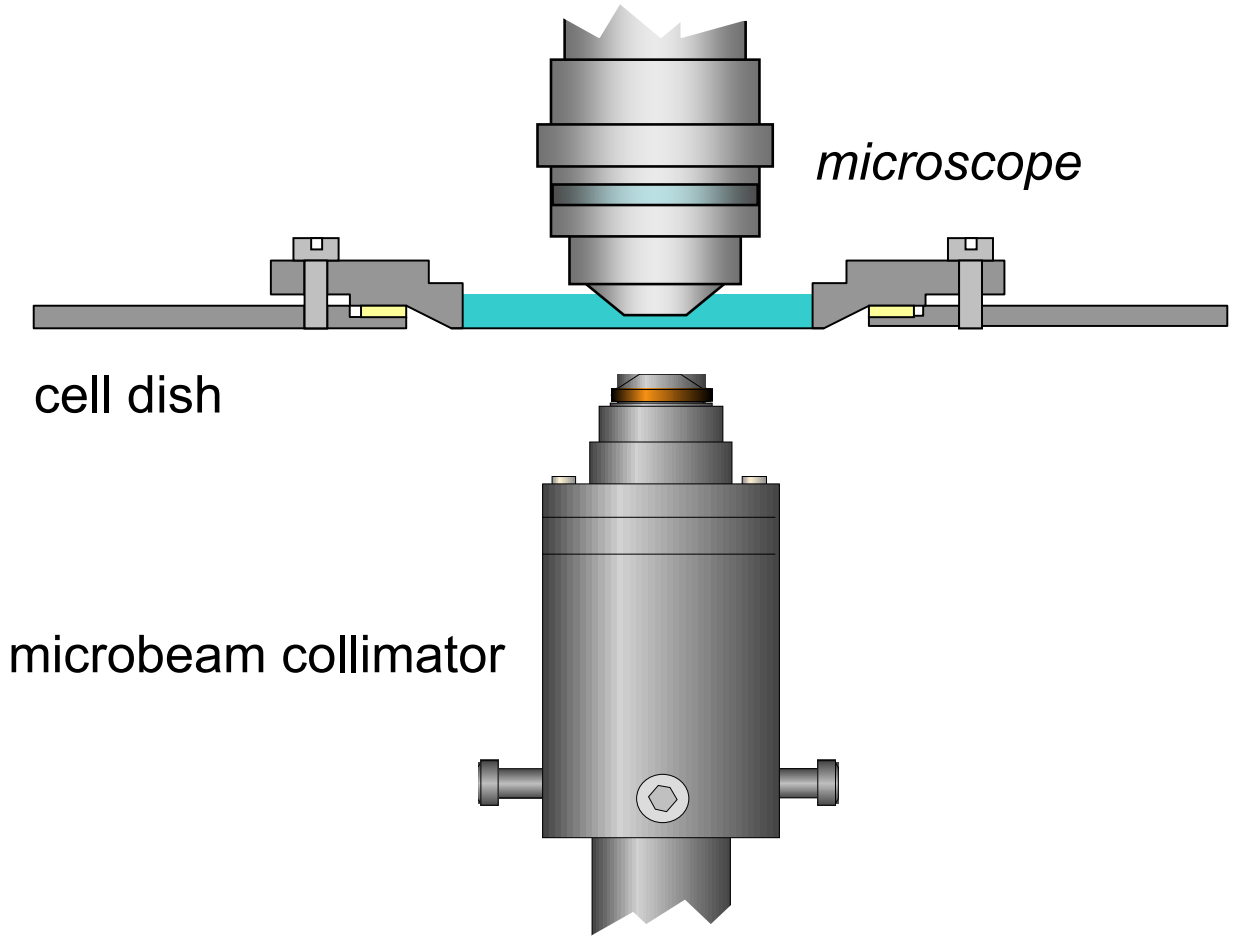


GCI charged-particle microbeam



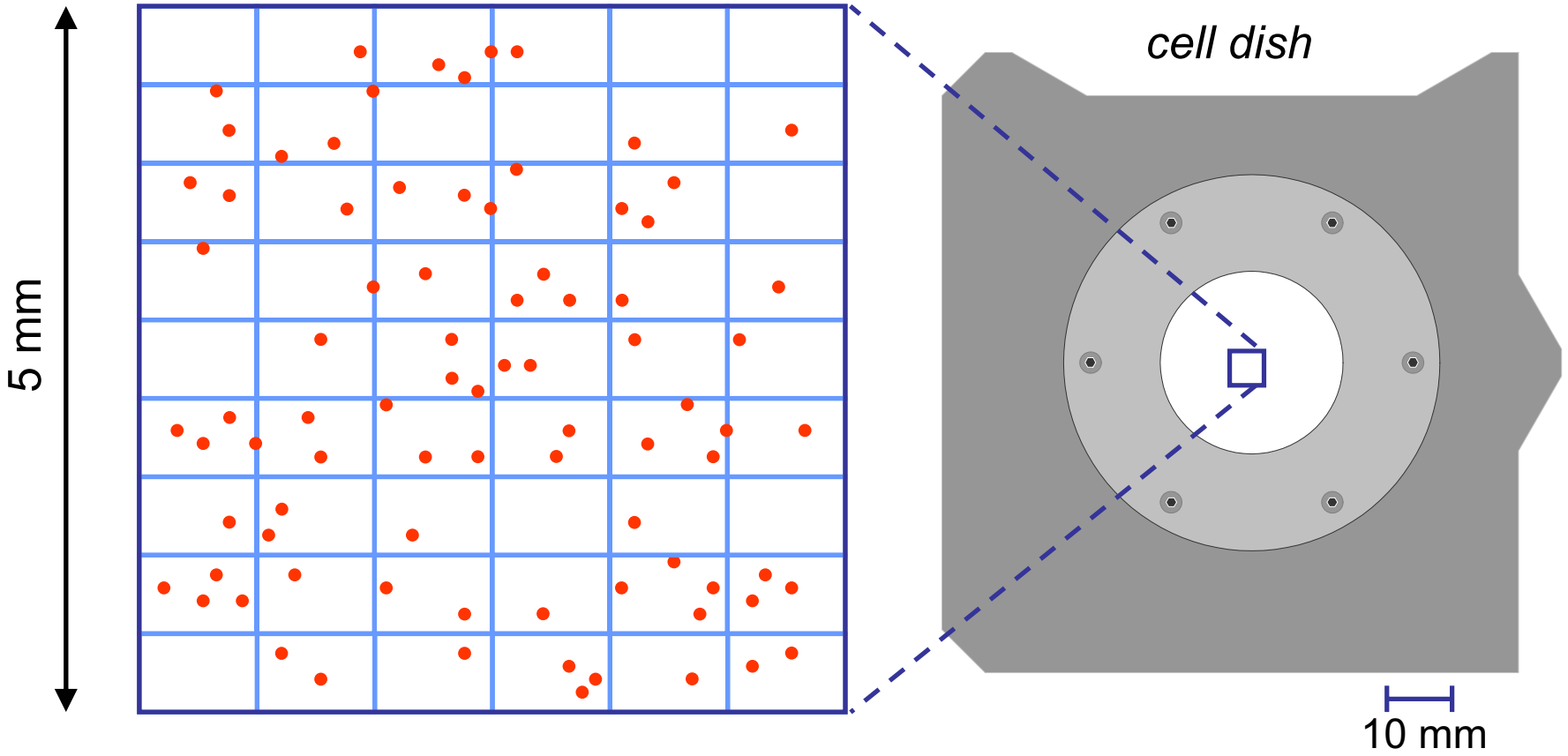
Cell finding step

- Stained cells are viewed using an epi-fluorescence microscope

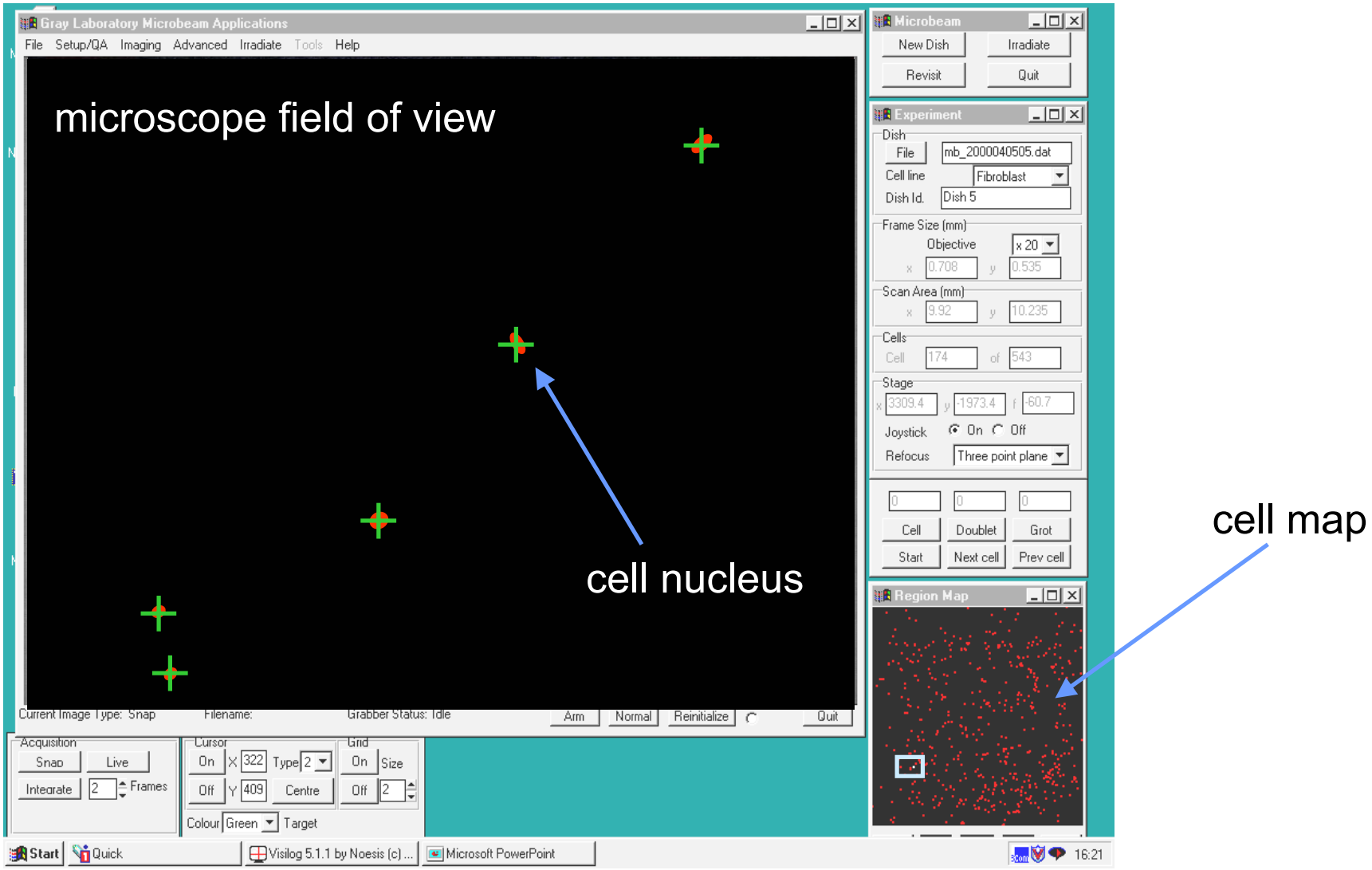


Cell finding step

- A map of cell co-ordinates is found by raster-scanning the dish and using image-processing to ascertain cell positions



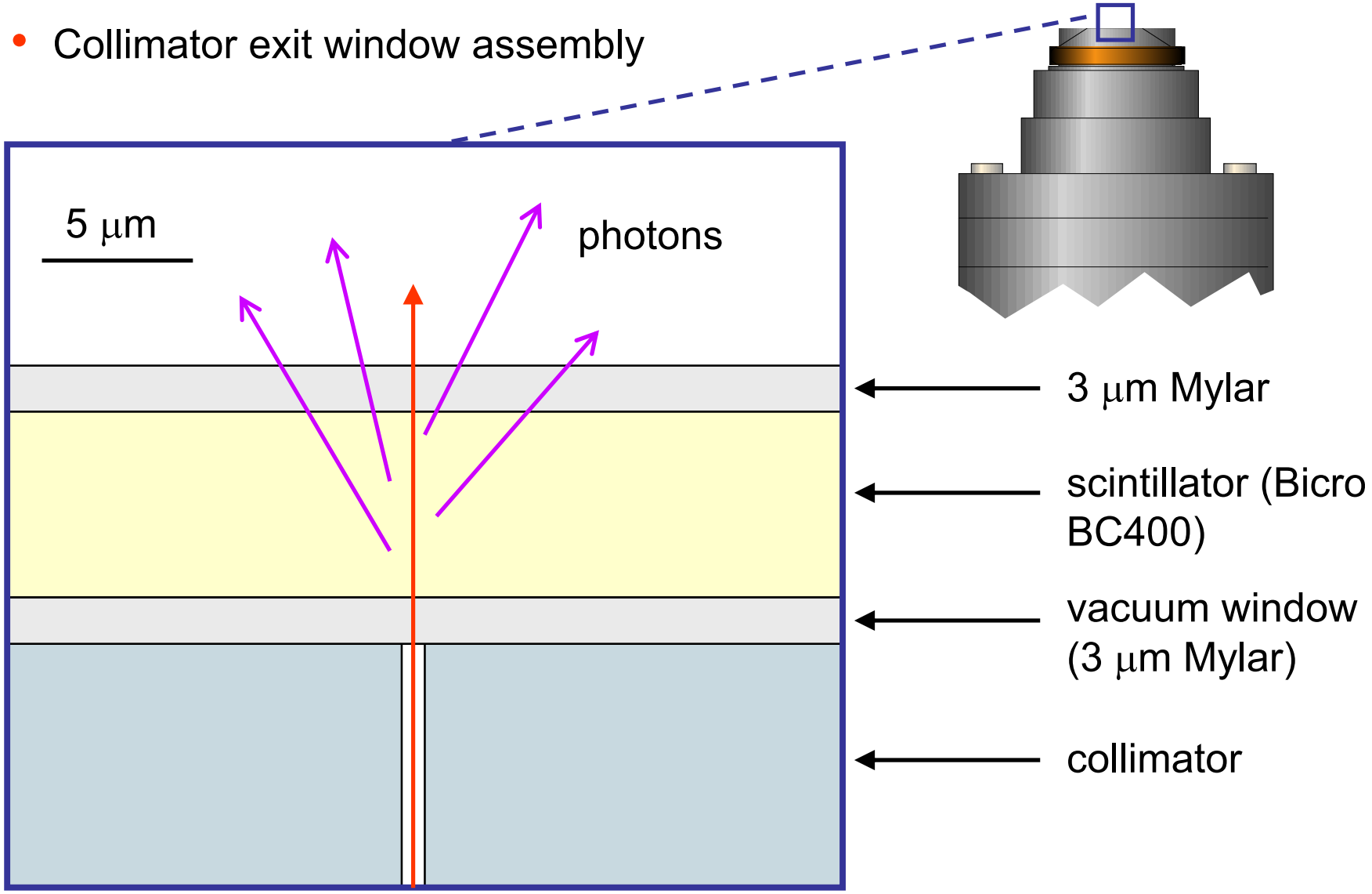
Cell finding step



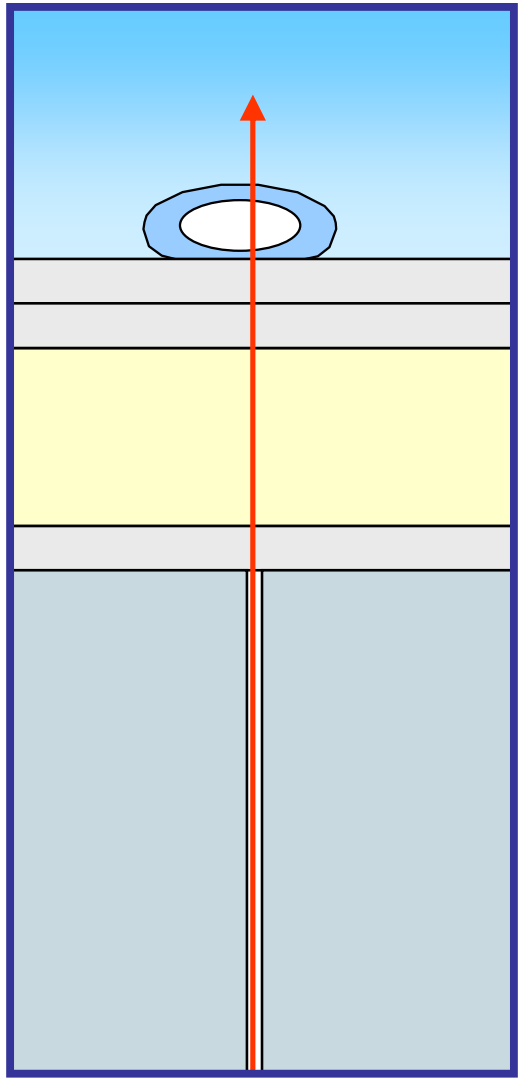
Typical screenshot during cell-finding step

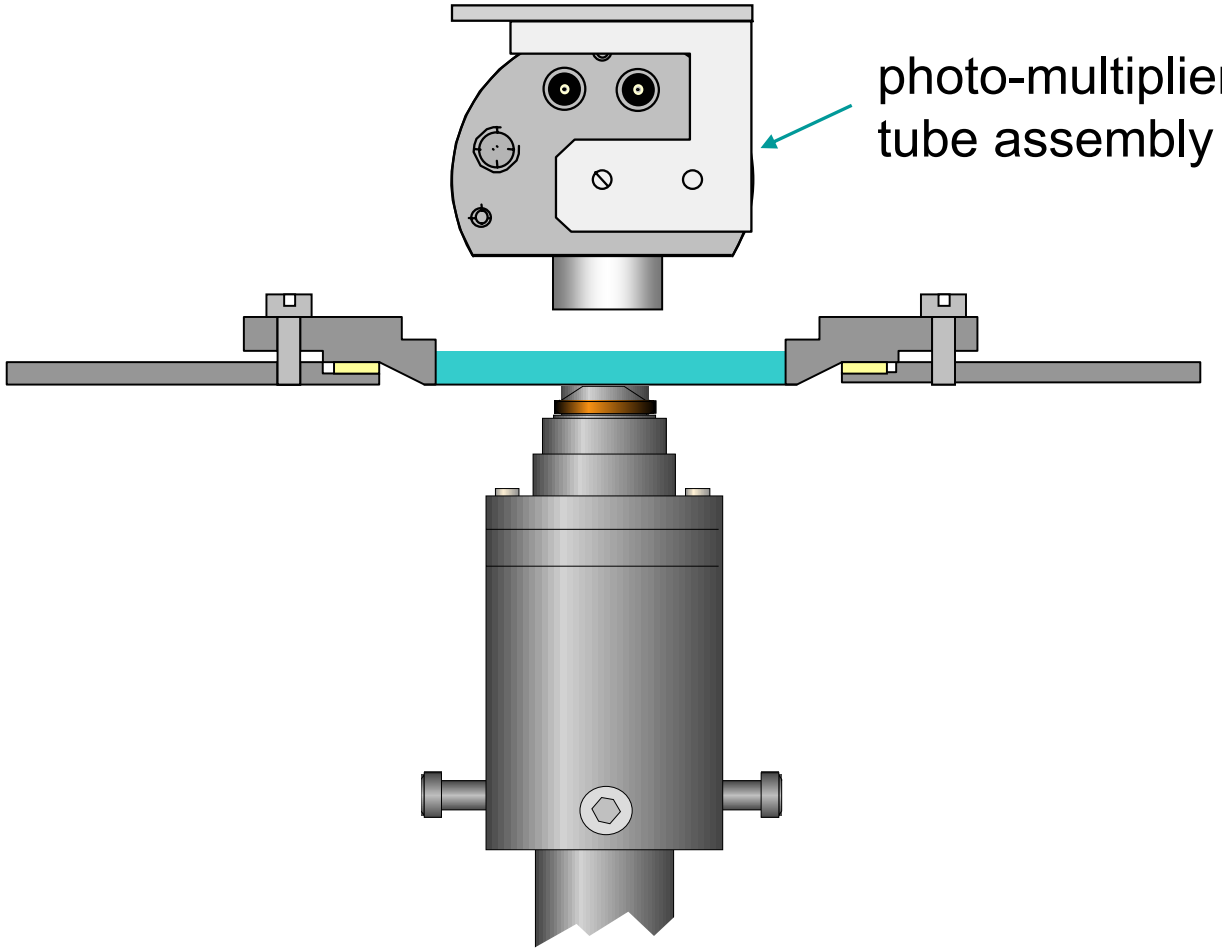
Irradiation step

- Collimator exit window assembly



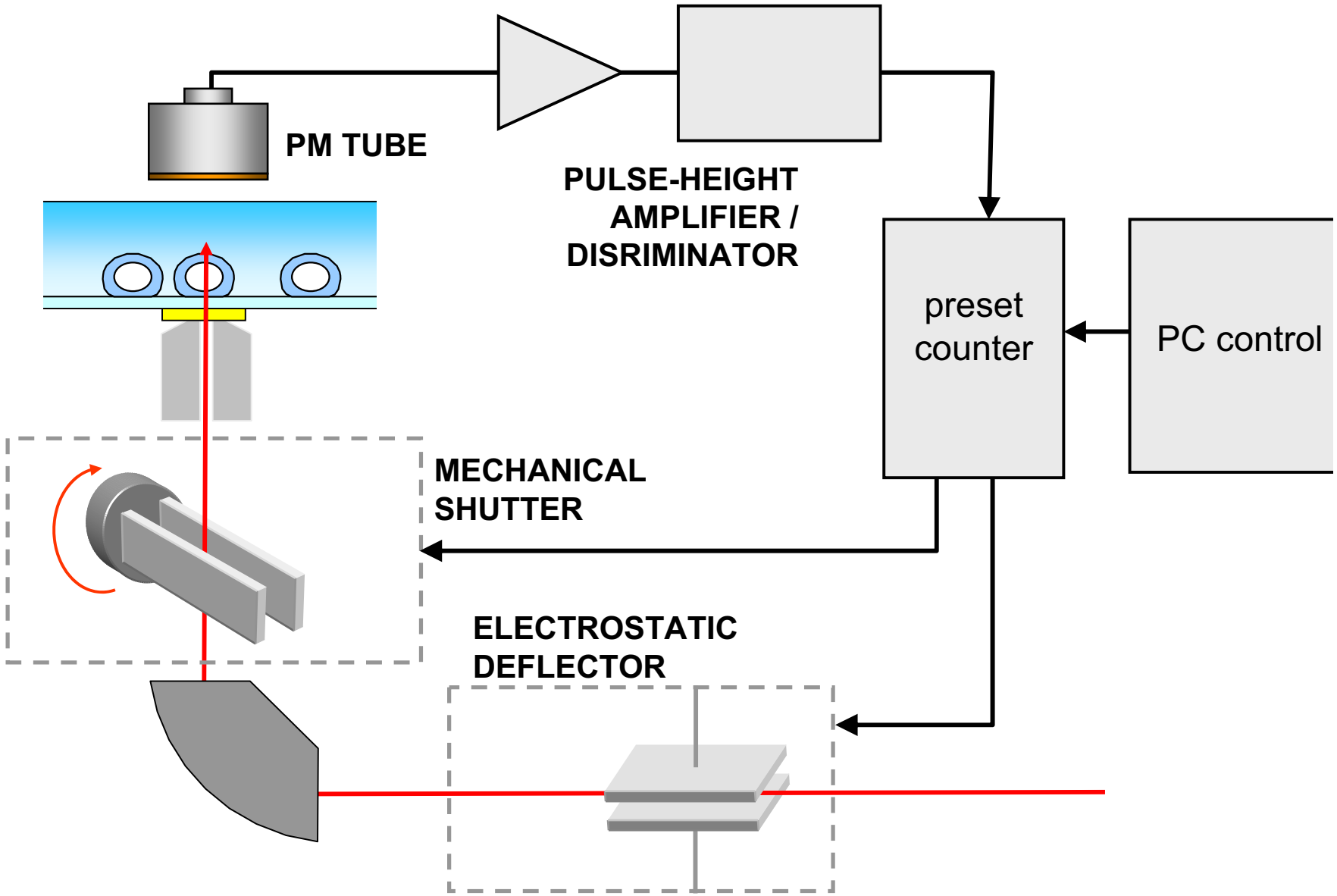
Irradiation step





Collimator assembly touches base of cell dish

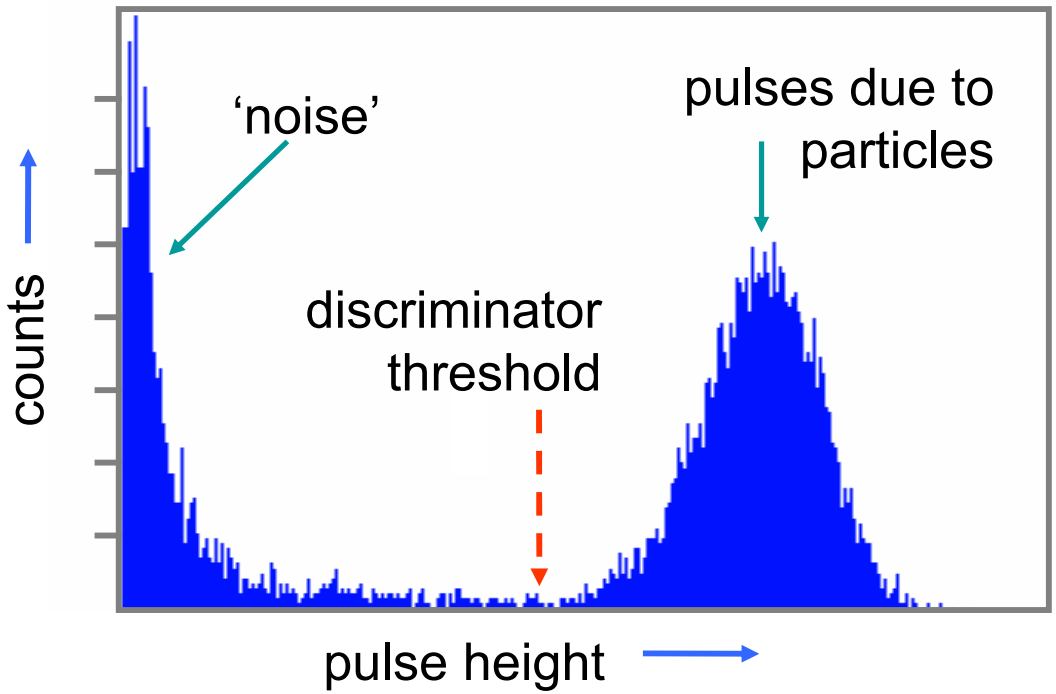
Irradiation step: Beam shuttering



Irradiation step: detector performance

Failure to correctly count particles can occur in two ways:

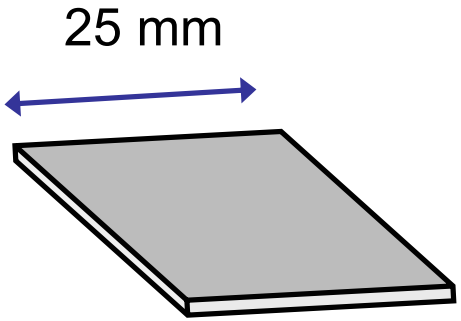
- Failure to detect particle traversal
- ‘False positives’



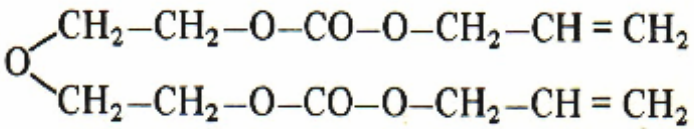
Photomultiplier tube pulse-height spectrum for many particles

Irradiation step: detector performance

- Detector performance can be assessed using CR-39 ‘track-etch’ plastic

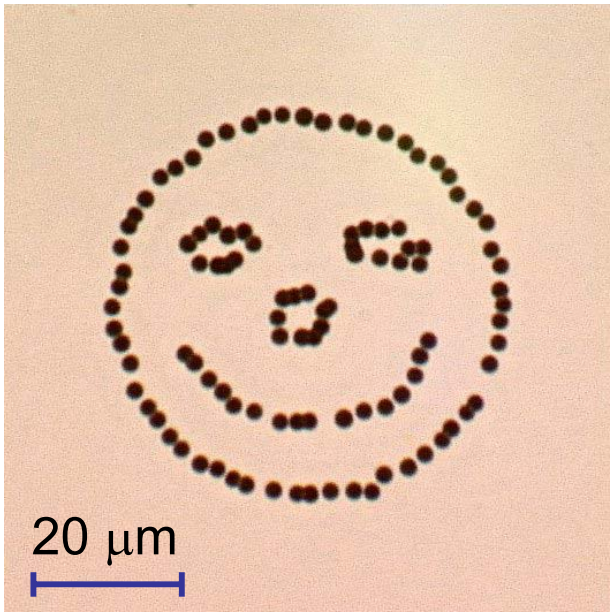


Polyallyl diglycol carbonate (PADC),

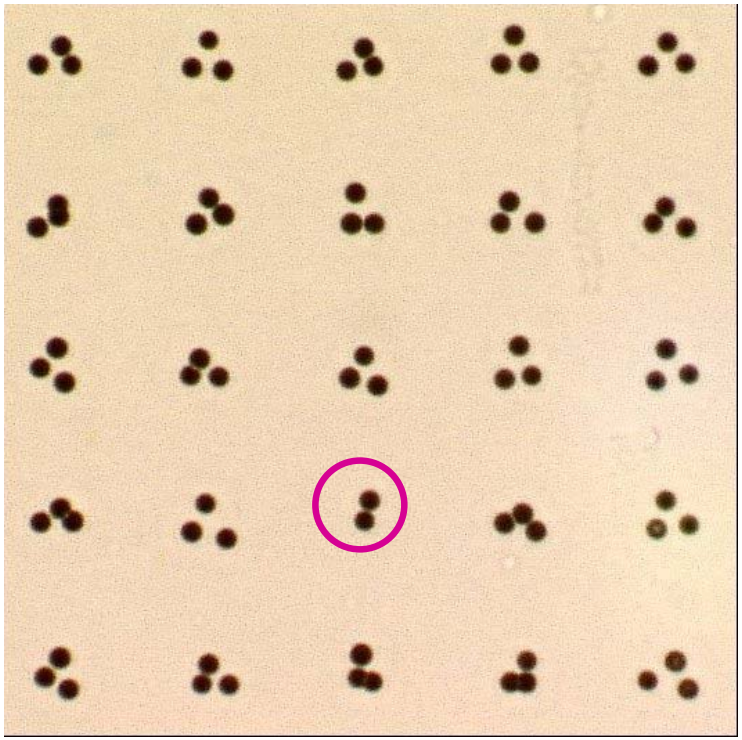


- CR-39 is a clear plastic, sensitive to the tracks of energetic particles

- After exposure, the tracks may be revealed by etching the material in a caustic alkali solution (i.e. NaOH).



Irradiation step: detector performance



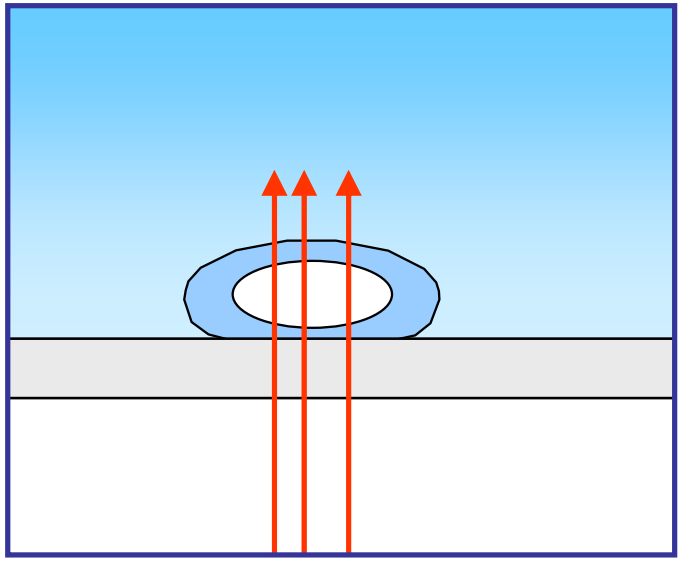
Exactly 3 pits per location,
on a 20 μm grid

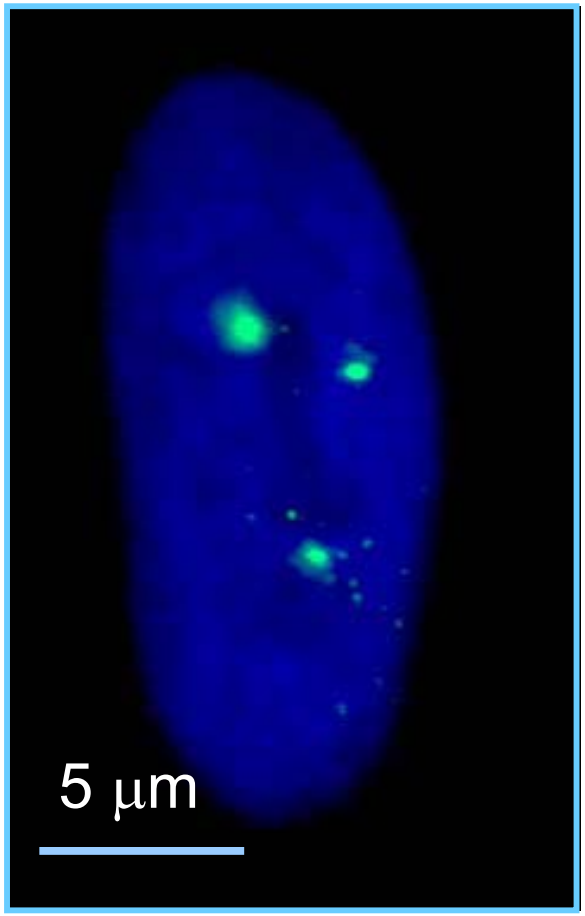
No. particles Delivered	Preset No. particles 1	2	3	4	5
0	0.8	-	-	-	-
1	99.2	0.9	-	-	-
2	-	99.1	2.5	-	-
3	-	-	97.5	2.2	0.3
4	-	-	-	97.2	3.1
5	-	-	-	0.6	96.3
6	-	-	-	-	0.3
7	-	-	-	-	-

Irradiation step: detector performance

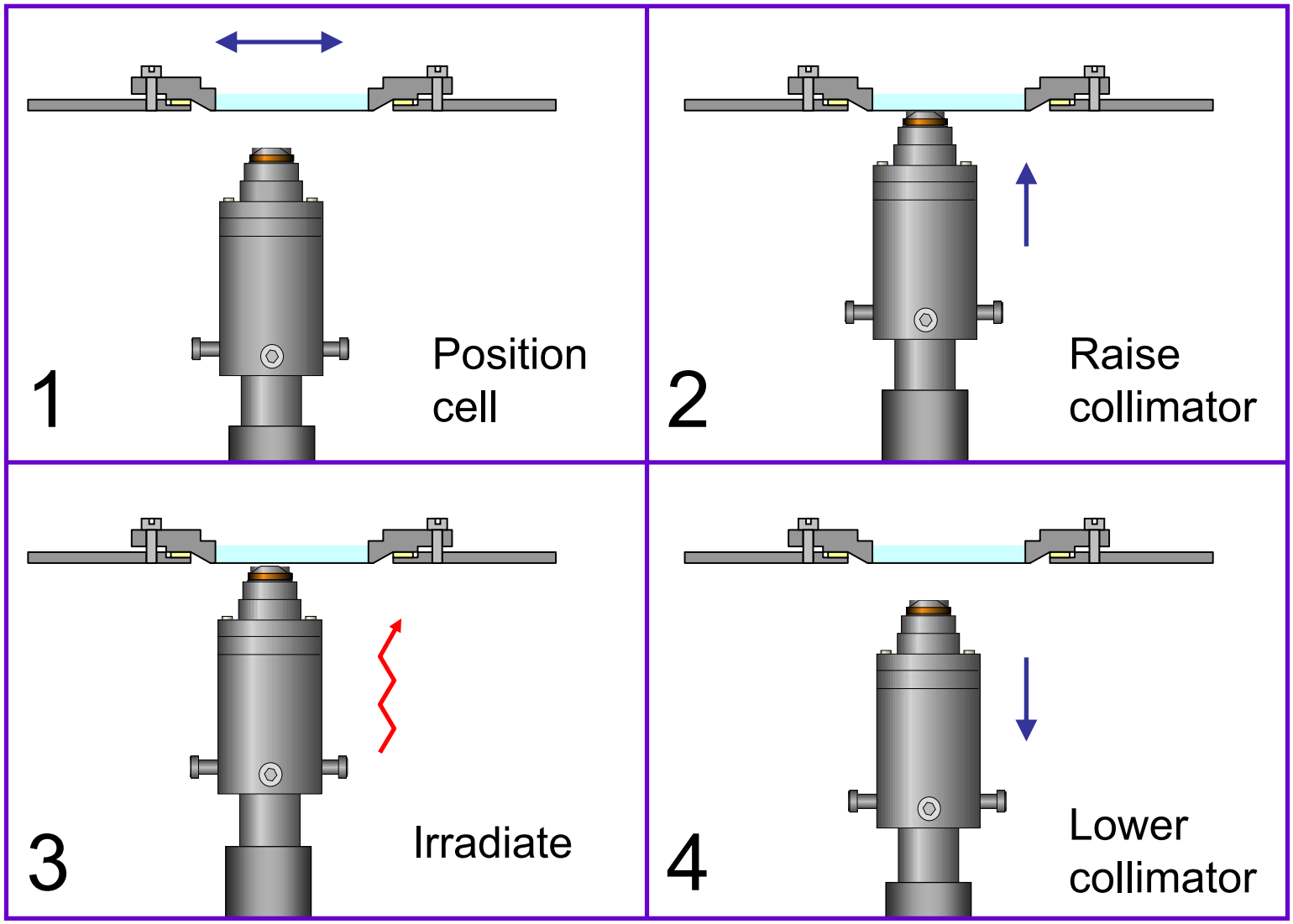
- Biological visualisation of detector performance

Three counted helium ions per cell



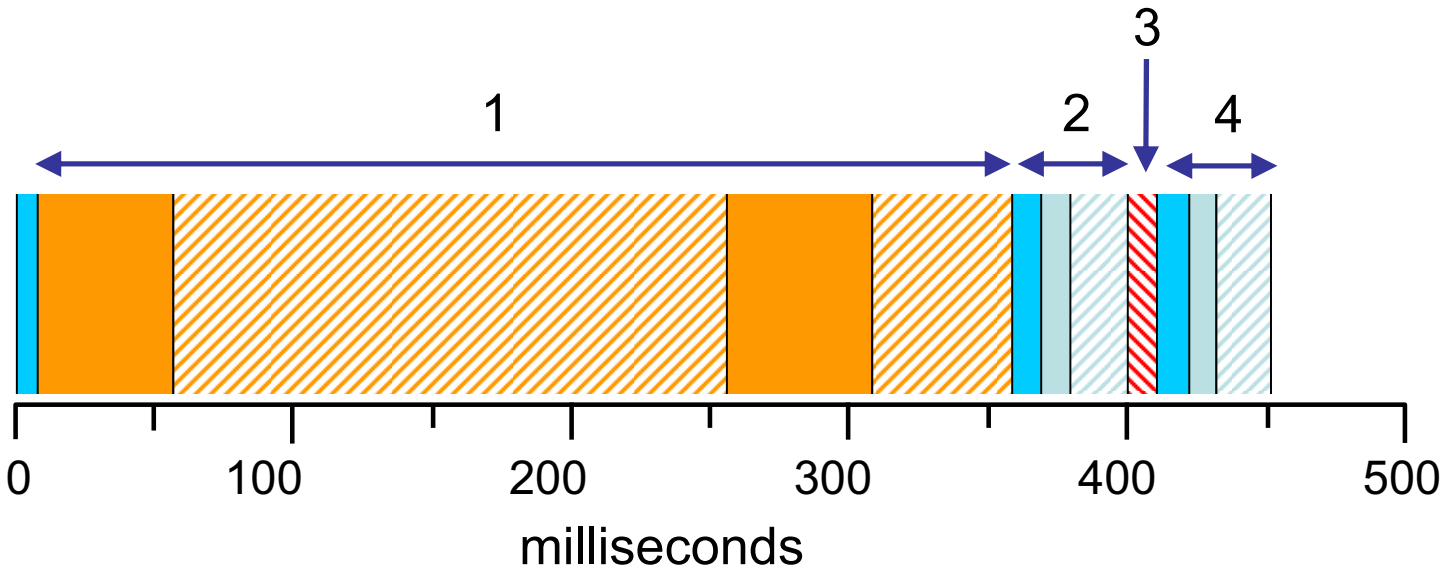


Irradiation step: cell throughput



Irradiation step: cell throughput

1. Position cell
2. Raise collimator
3. Irradiate
4. Lower collimator



read, write, handshaking
stage instructions
collimator instructions
irradiate
stage movement
collimator movement

Throughput: 450 milliseconds / cell (8,000 cells / hour)

Irradiation step: targeting accuracy

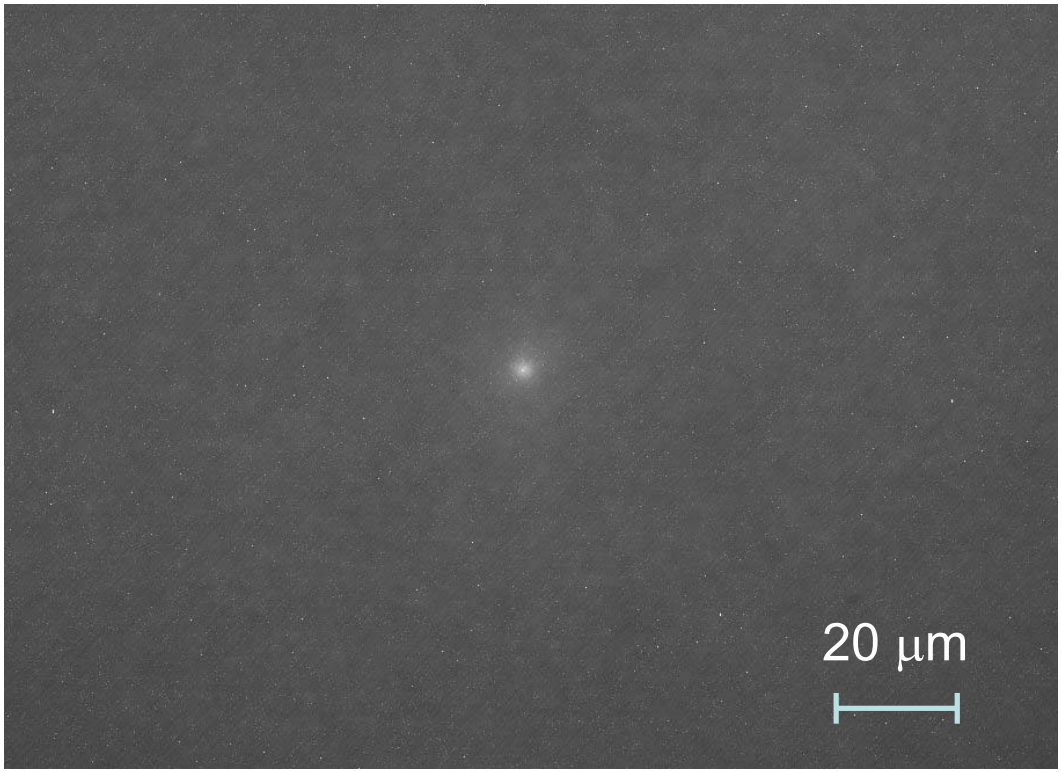
What is the particle microbeam targeting accuracy?

Targeting accuracy is determined by:

- Accuracy of **beam** identification and location
- Accuracy of **target** identification and location
- Target positioning accuracy
- Beam size

Beam identification & location

- CCD camera can be used to visualise the beam



- For every dish, the beam location is established after the cell-finding step and before the irradiation step
- ~1 μm uncertainty in beam location

Target identification & location

Target identification accuracy is determined by:

- Ability to visualize target
- Optical distortions in image

Countermeasures:

- *Optical distortions in image:* Measure distortion and apply a correction using a ‘look-up’ table OR move cell to irradiation position and find again

Target positioning accuracy

Target positioning accuracy is determined by:

- Mechanical stability
- Stage accuracy

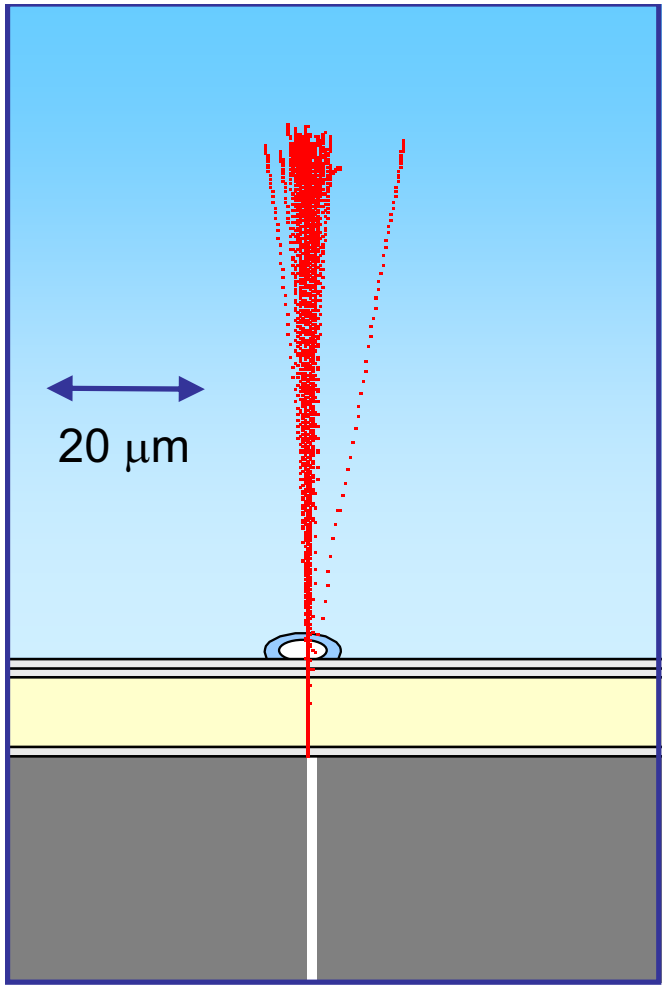
Countermeasures:

- *Mechanical stability:* vibration isolation, robust construction, temperature stability
- *Stage accuracy:* Stage is lead-screw driven, ensure anti-backlash measures are used. Use a stage with position sensors to provide ‘closed-loop’ position feedback

Beam size

Beam size is determined by:

- Collimator bore diameter
- Scattering in collimator, window and detector
- Collimator–to–cell distance



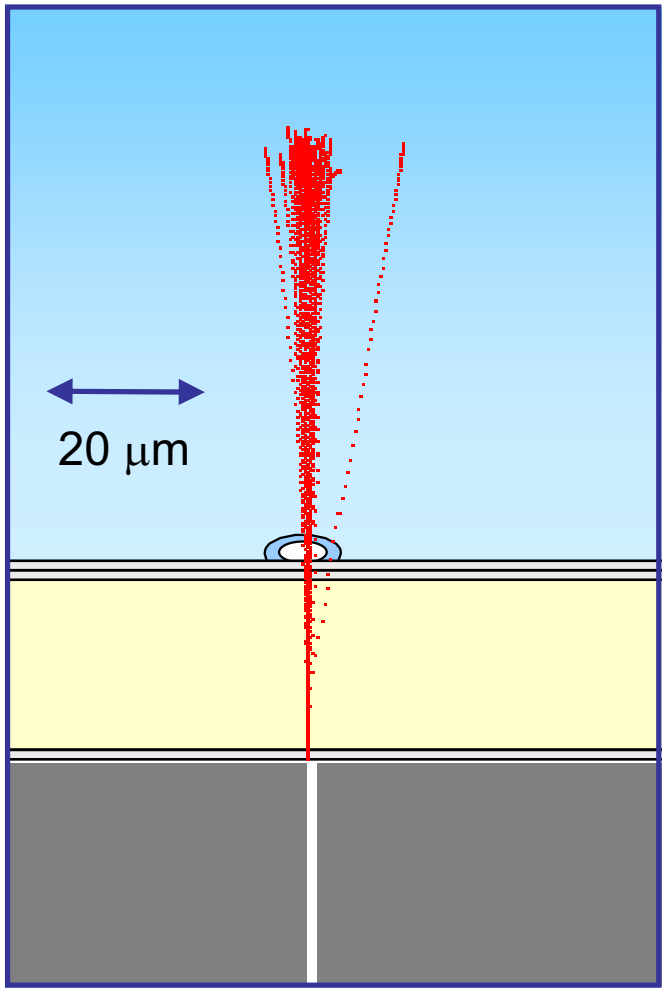
2.5 MeV proton (SRIM2003)

Beam size

Beam size is determined by:

- Collimator bore diameter
- Scattering in collimator, window and detector
- Collimator–to–cell distance

Thicker scintillator



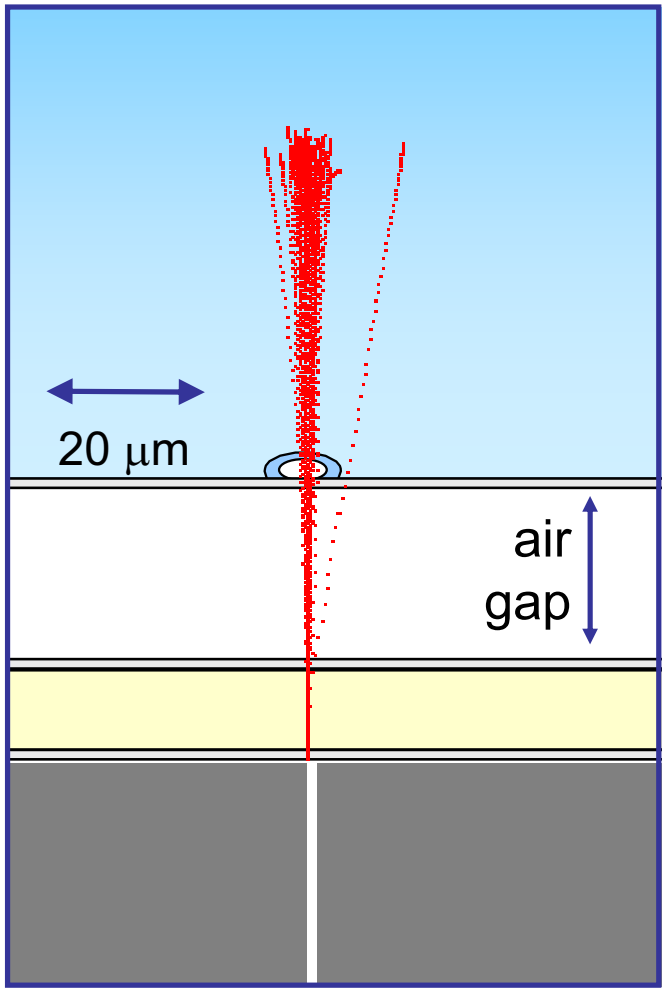
2.5 MeV proton (SRIM2003)

Beam size

Beam size is determined by:

- Collimator bore diameter
- Scattering in collimator, window and detector
- Collimator–to–cell distance

Increased collimator–to–cell distance

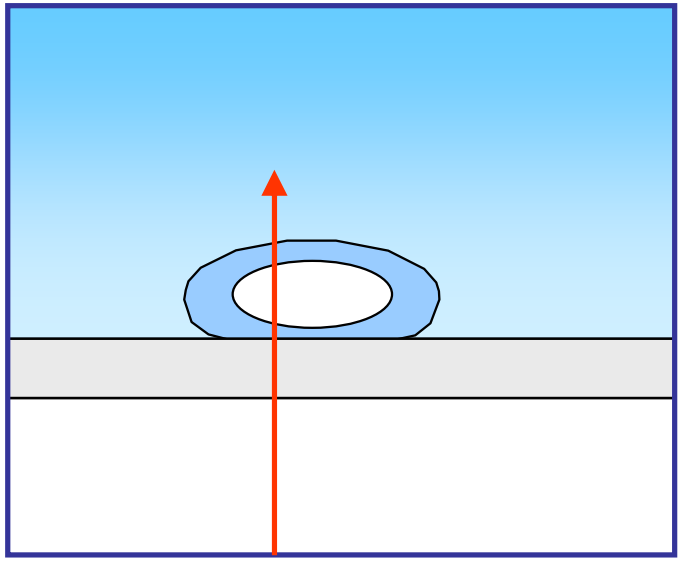


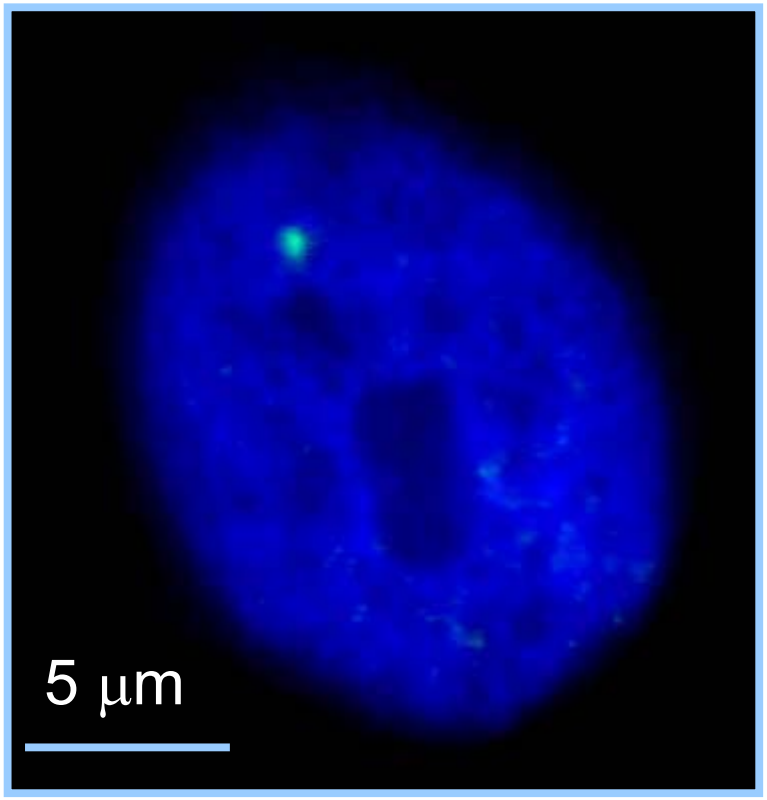
2.5 MeV proton (SRIM2003)

Measuring radiation damage to cells

- γ-H2AX assay: indicates the presence of DNA double strand breaks

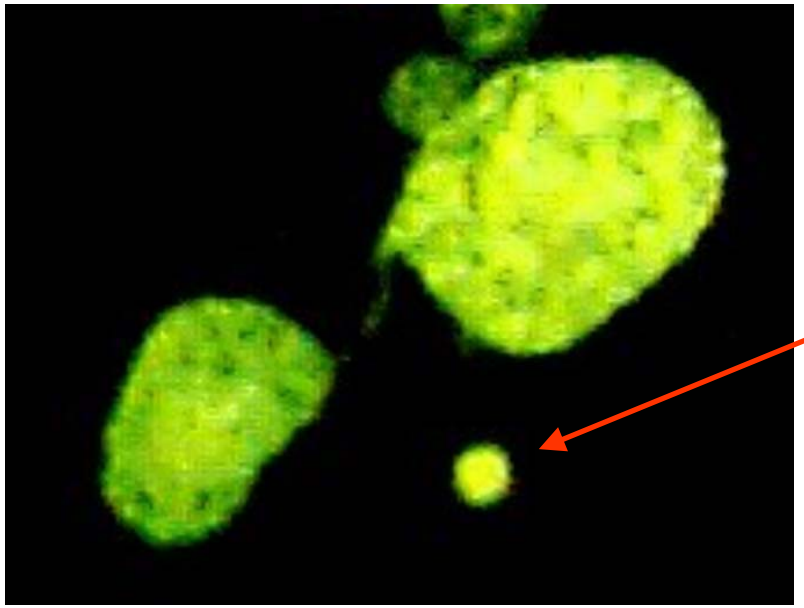
Single helium ion





Measuring radiation damage to cells

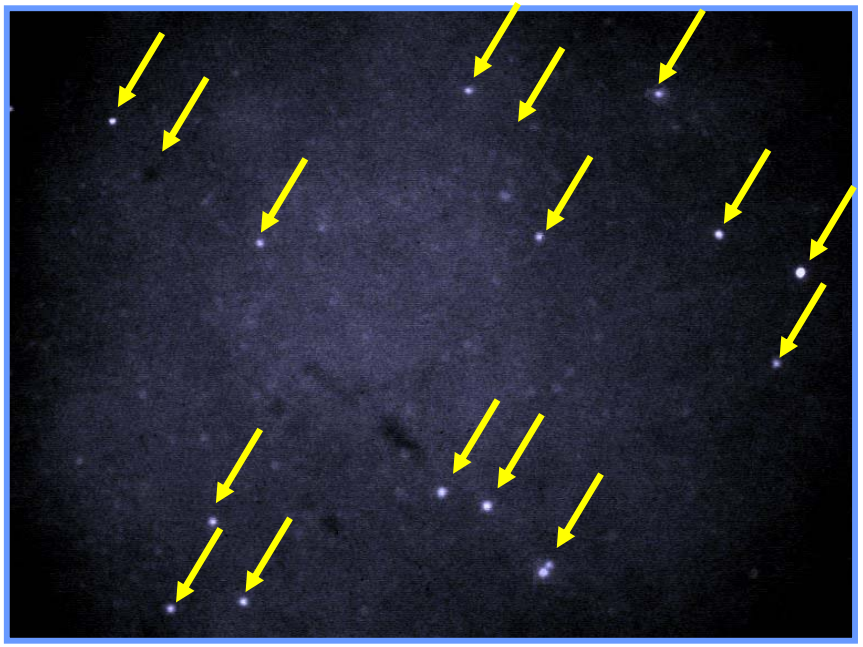
- Micronucleus assay



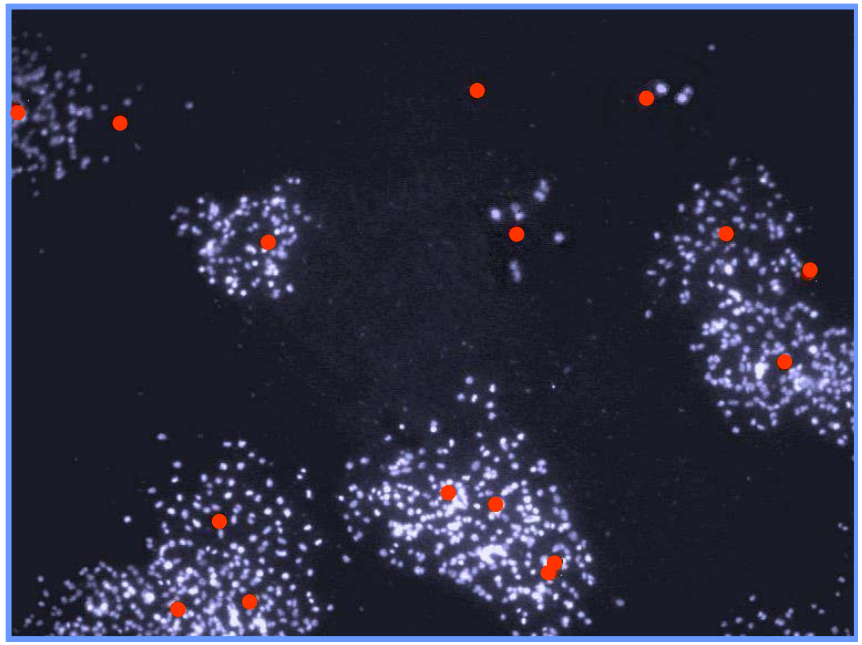
After irradiating cells, score bi-nucleate cells with micronuclei

Measuring radiation damage to cells

- Single cell clonogenic ‘survival’ assay

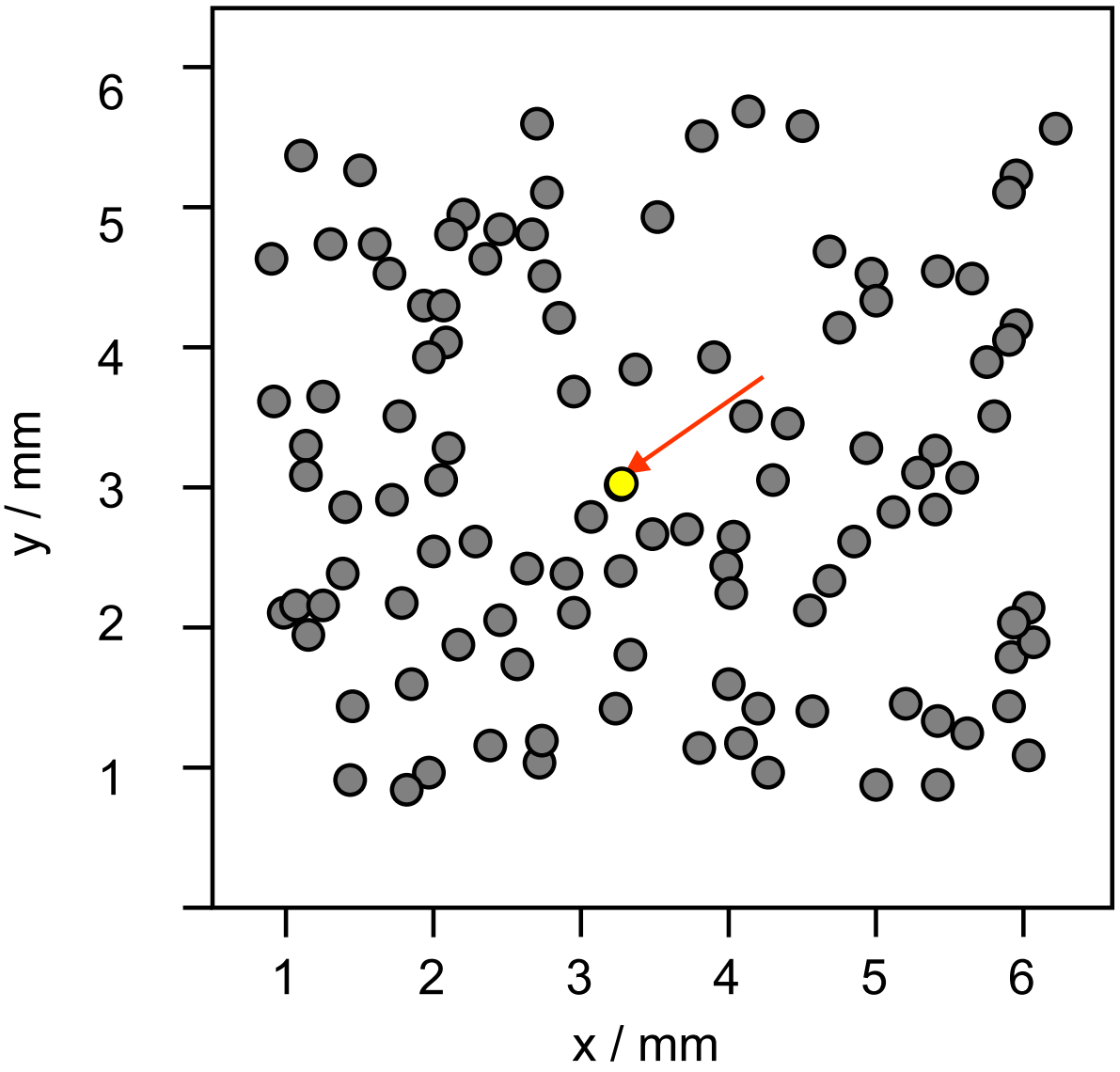


Target individual cells

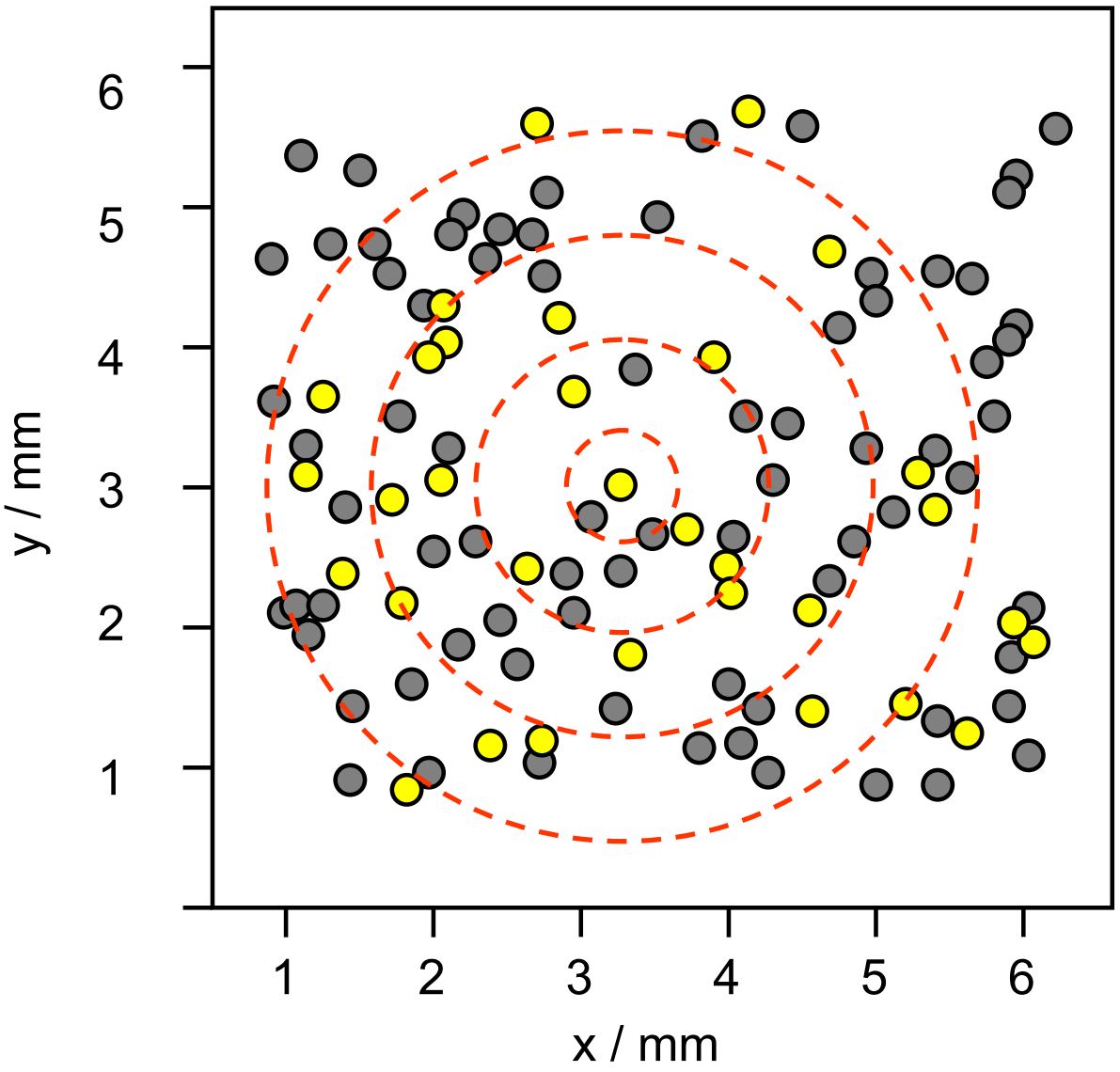


Revisit and score colonies

Measuring radiation damage to cells

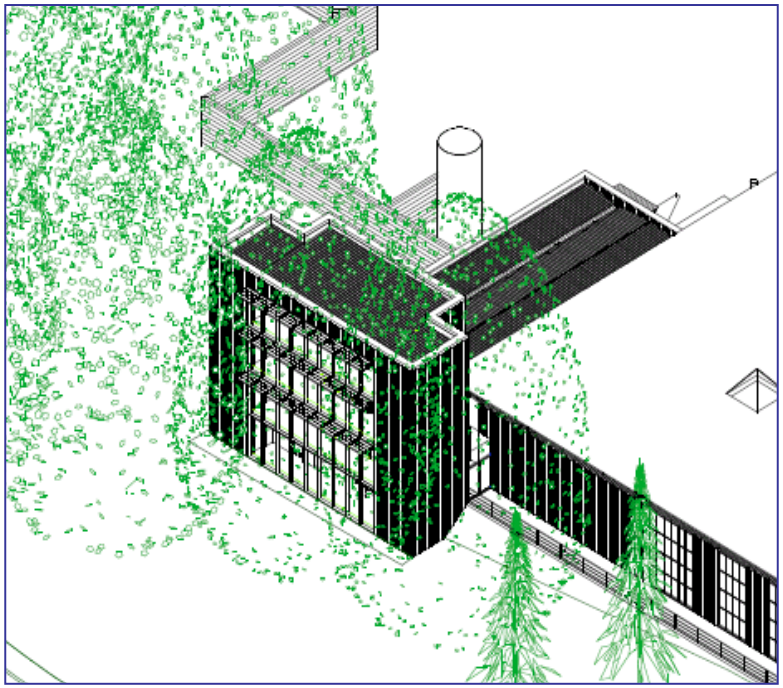


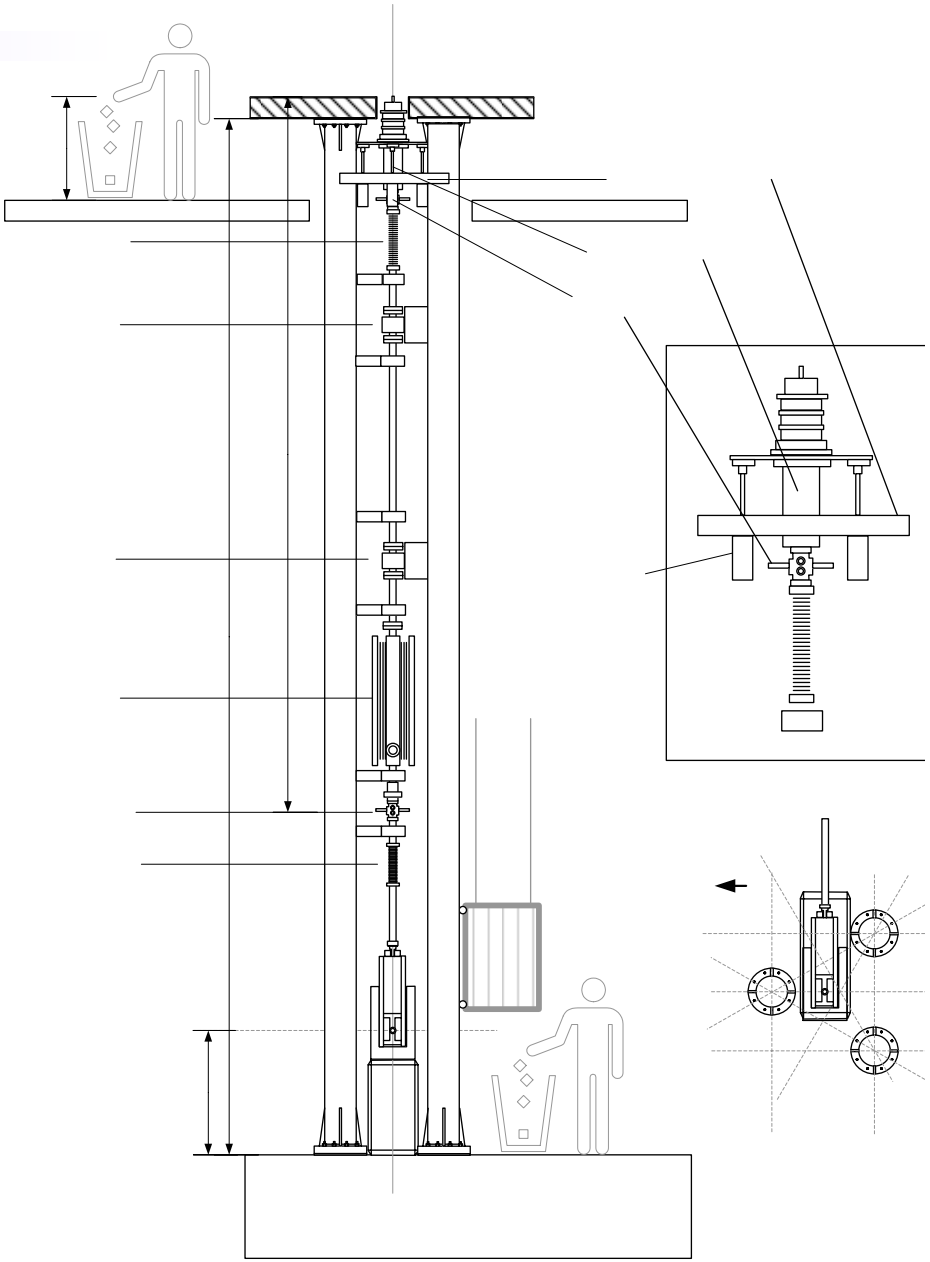
Measuring radiation damage to cells



The future

The University of Surrey / ROB vertical scanning nanoprobe

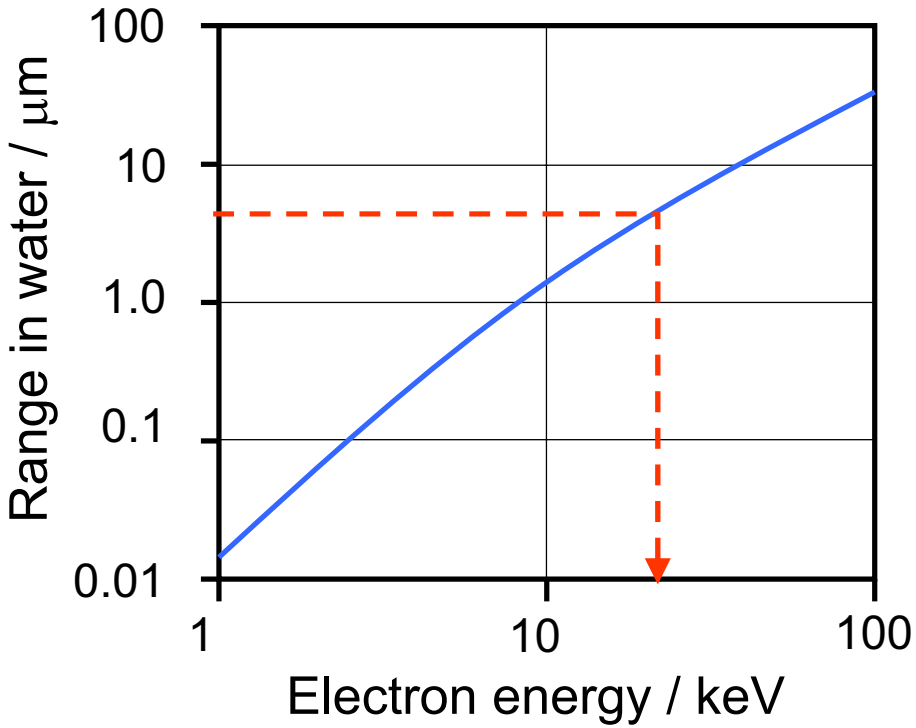


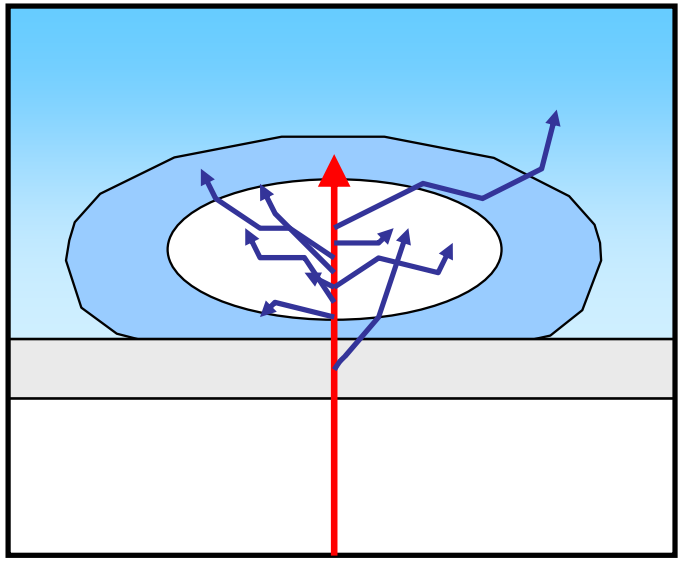


The competition

The focused electron microprobe

- Energetic electrons are relatively easy to generate and focus

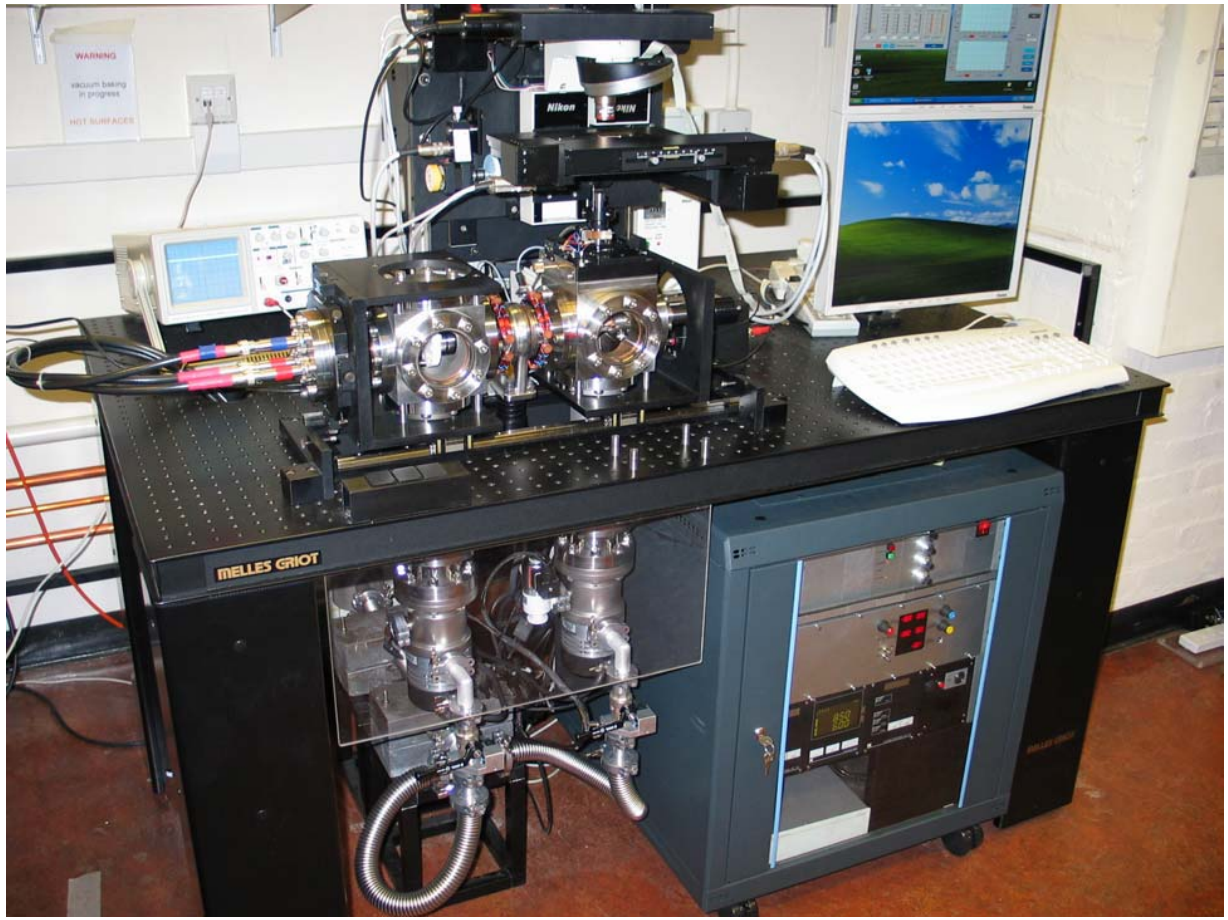




- For 5 μm thick cell, need ~25 kV electrons
- Problem: secondary electrons ranges ~cell diameter

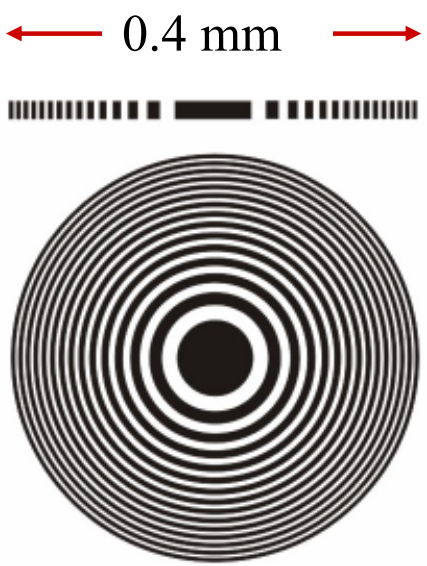
The competition

The focused low-energy X-ray microprobe

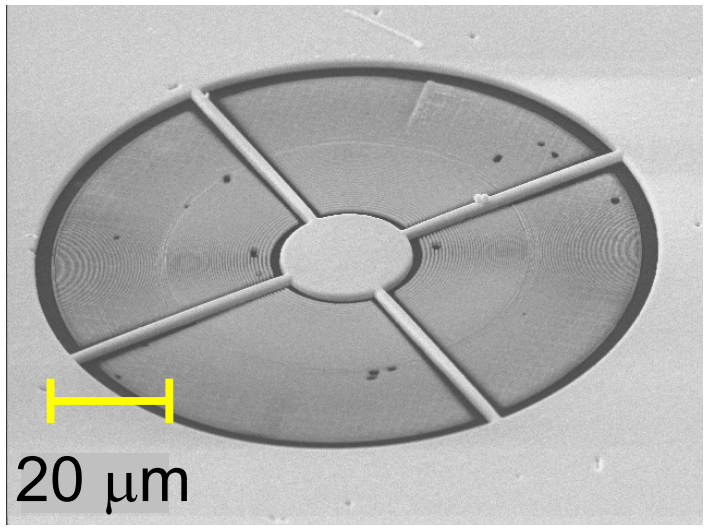


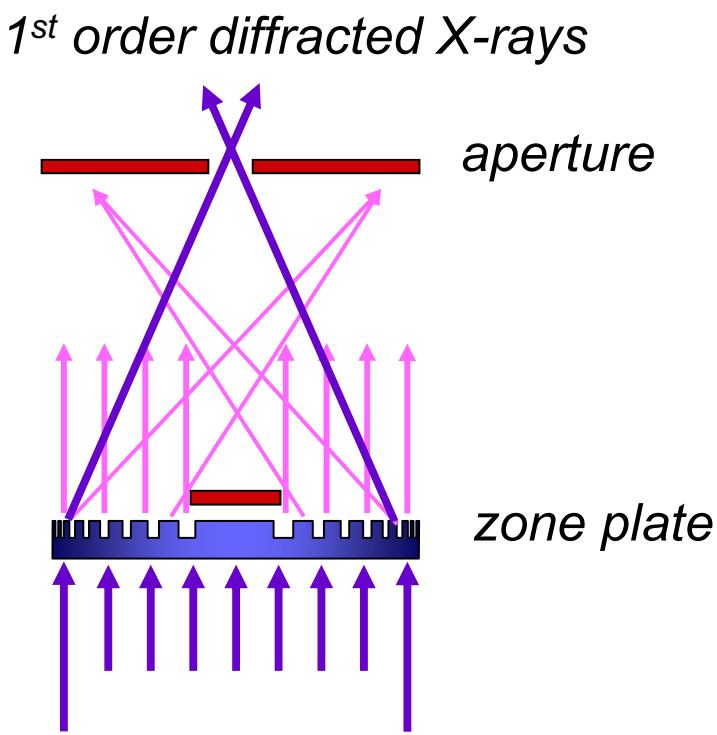
The Gray Cancer Institute low energy X-ray microprobe

Focussing low-energy X-rays



- Low-energy X-rays can be focussed using diffraction lenses called **zone-plates**
- Manufacture is highly specialised- limited to a few laboratories (i.e. King's College, London)





X-rays and charged-particles compared

X-rays

- Properties similar to hard X-rays and γ-rays
- Interact by photoelectric effect; no scattering
- Capable of very fine probes; less than 50nm
- Can deliver very low doses per cell
- Low-cost ‘bench-top’ sources possible

Charged-particles

- High LET single-particle source
- ‘Instantaneous’ dose-rate
- High cell throughputs
- Greater penetration in tissue possible
- Collimation ‘straightforward’