

CELLION

**Workshop Univ. Surrey / GCI
April 2004**

Track visualisation

GSI Material Sciences

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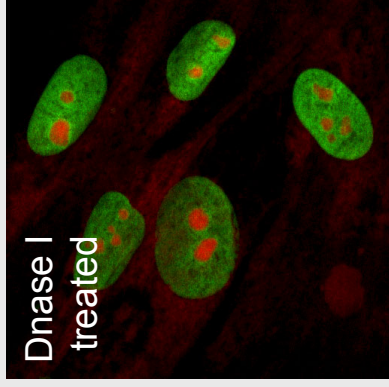
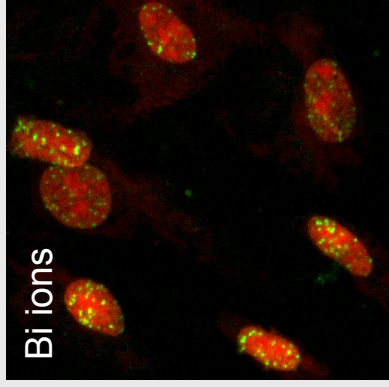
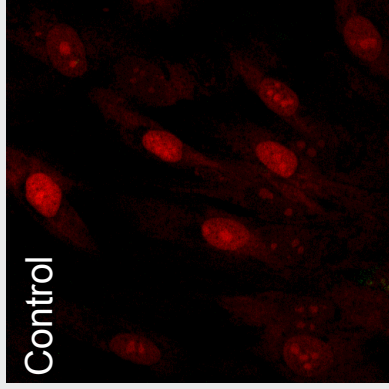
C. Fournier

B. Jakob

G. Taucher-Scholz

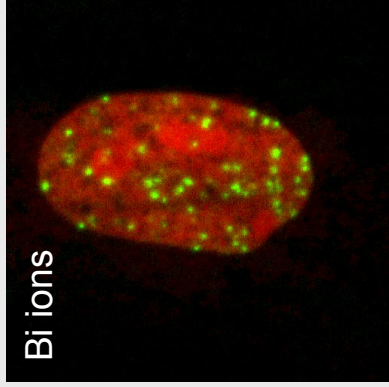
The basis of subnuclear track visualisation

**Particle irradiation: localized deposition of dose
localized production of DNA damage**



**Visualization of the
localized production of DNA DSBs
after Bi ion irradiation
and TUNEL assay (FITC-dUTP)**

Disadvantages: low sensitivity
stringent conditions

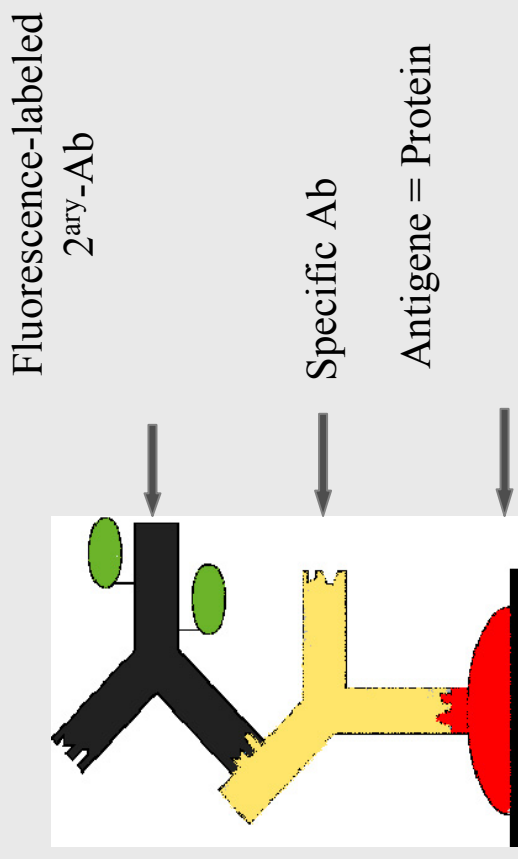


4.1 MeV/u Bi, 2×10^7 P cm⁻²

Track visualisation

Immunocytochemical detection of proteins recruited to sites of damage

- Cell permeabilisation
- Fixation
- Immunostaining
- DNA-counterstaining
- (confocal laser) microscopy

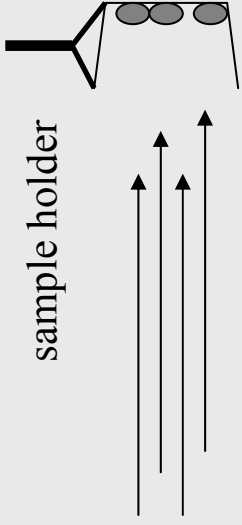


Requirements:

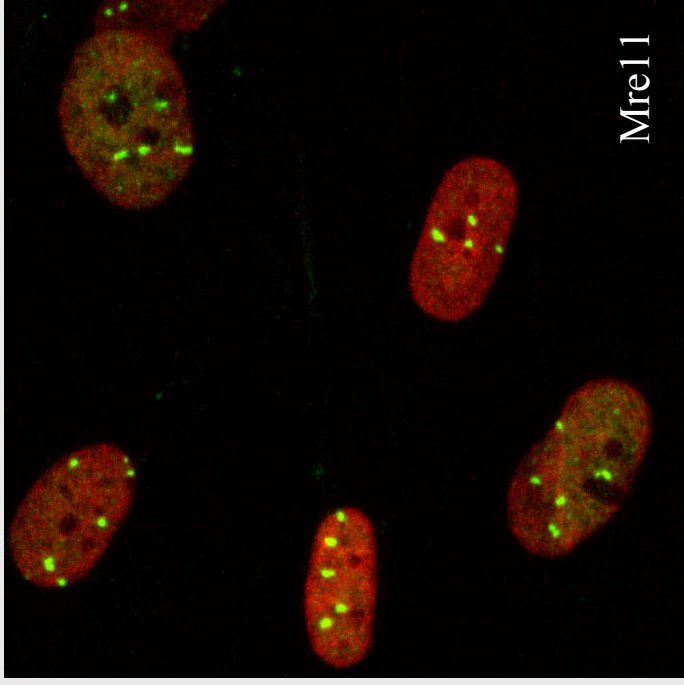
- Rapid concentration of proteins at damaged sites
- Specific response to IR-induced DNA damage
- Response in all cells (independent of cell cycle phase and cell status)

Biological visualization of single particle tracks (Mre11)

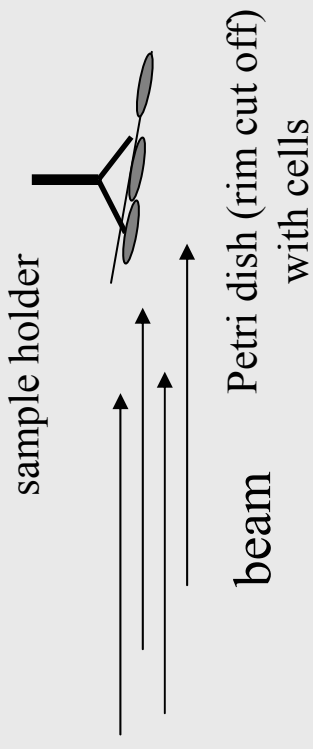
irradiation perpendicular to cell monolayer



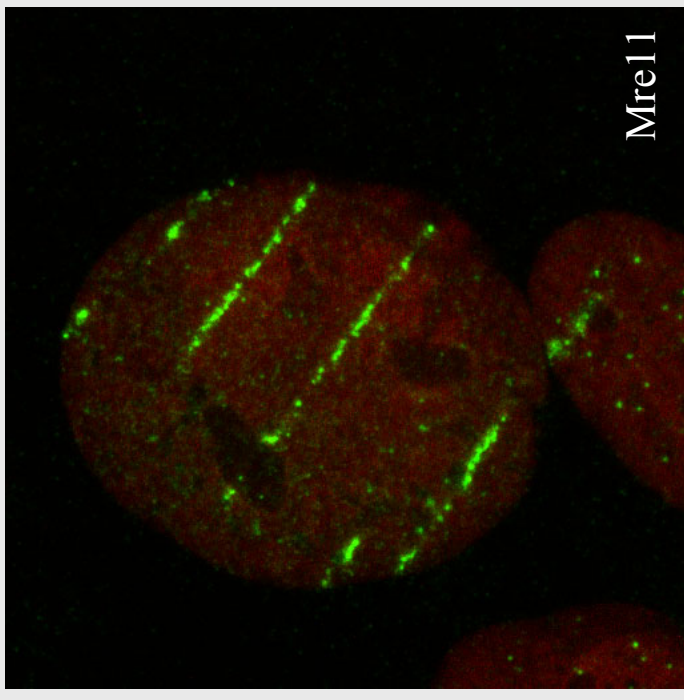
beam
Petri dish with cells



irradiation under a small angle ($< 5^\circ$)

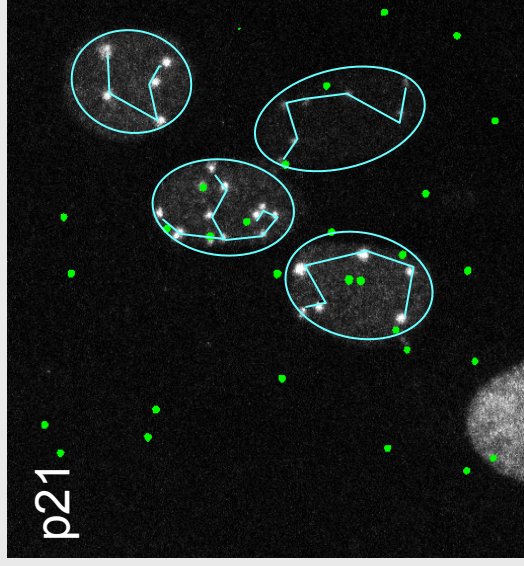


beam
Petri dish (rim cut off)
with cells

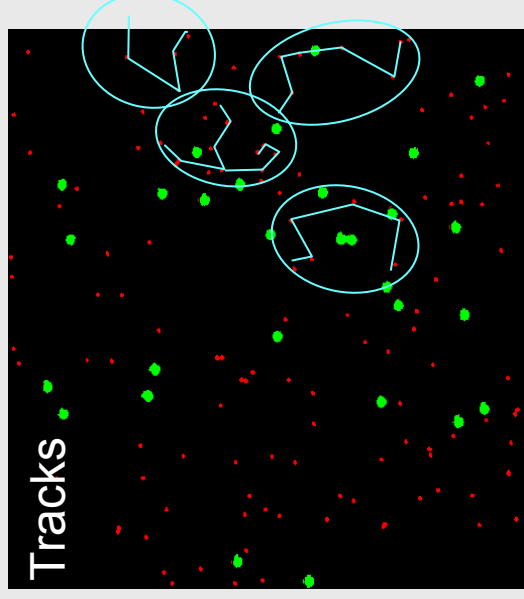
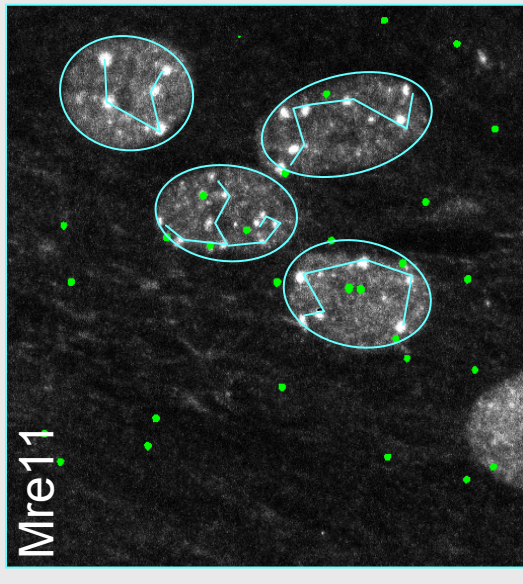


Correlation of protein foci with ion tracks

1st phase contrast + fluorescence image



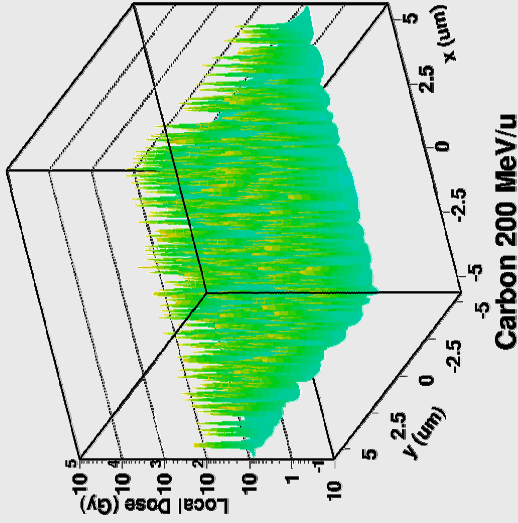
1st phase contrast + fluorescence image



Green: reference tracks

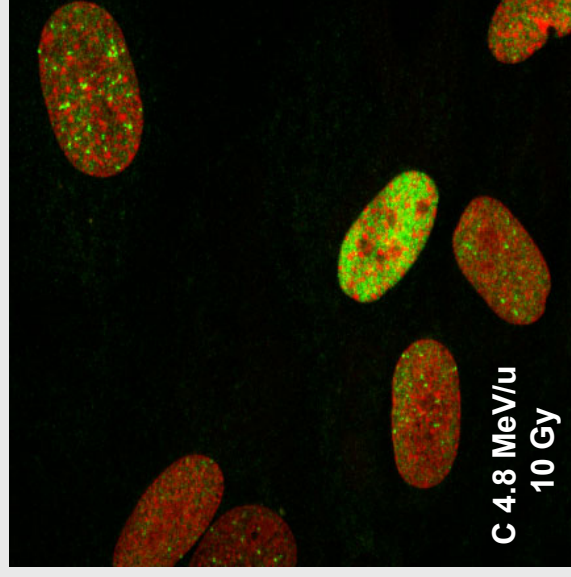
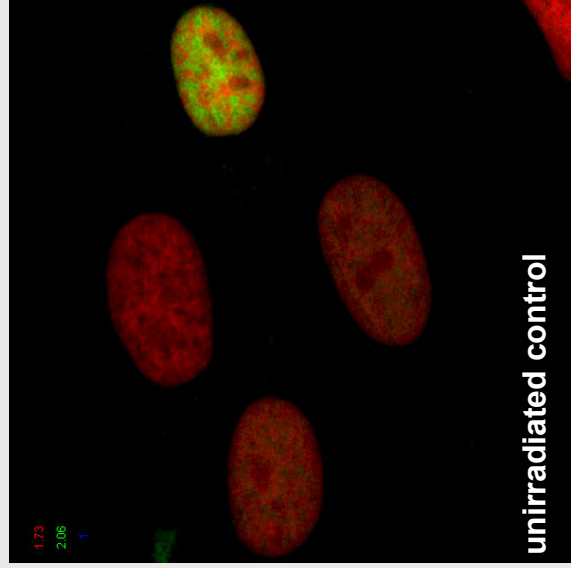
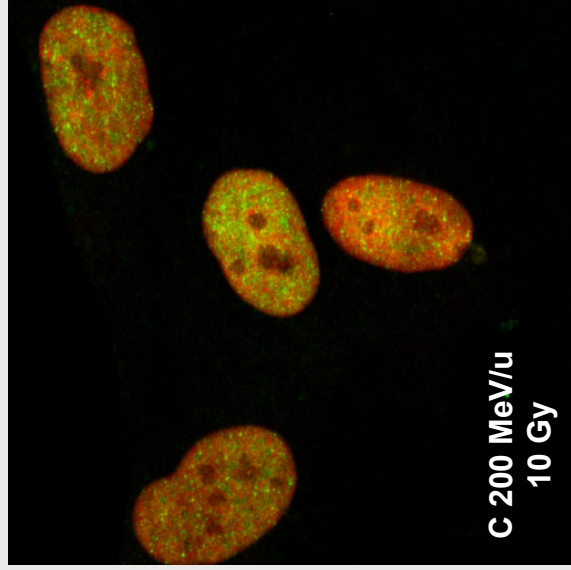
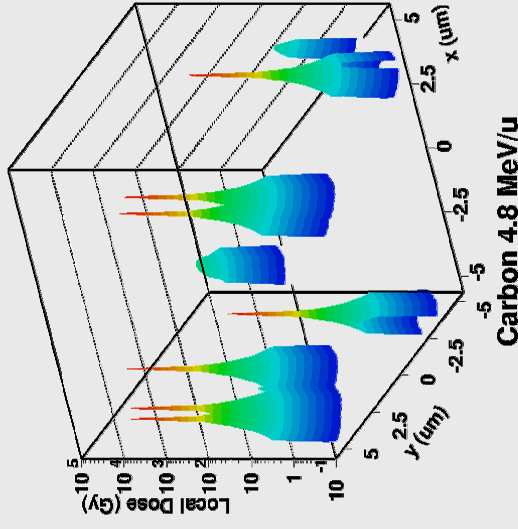
Red: nickel irradiation tracks

- p21: each focus corresponds to an irradiation track
not all cells show foci (e.g. S-phase)
- Mre11: all cells show foci
each irradiation track leads to a focus
additional foci may be detected (e.g. replication sites)



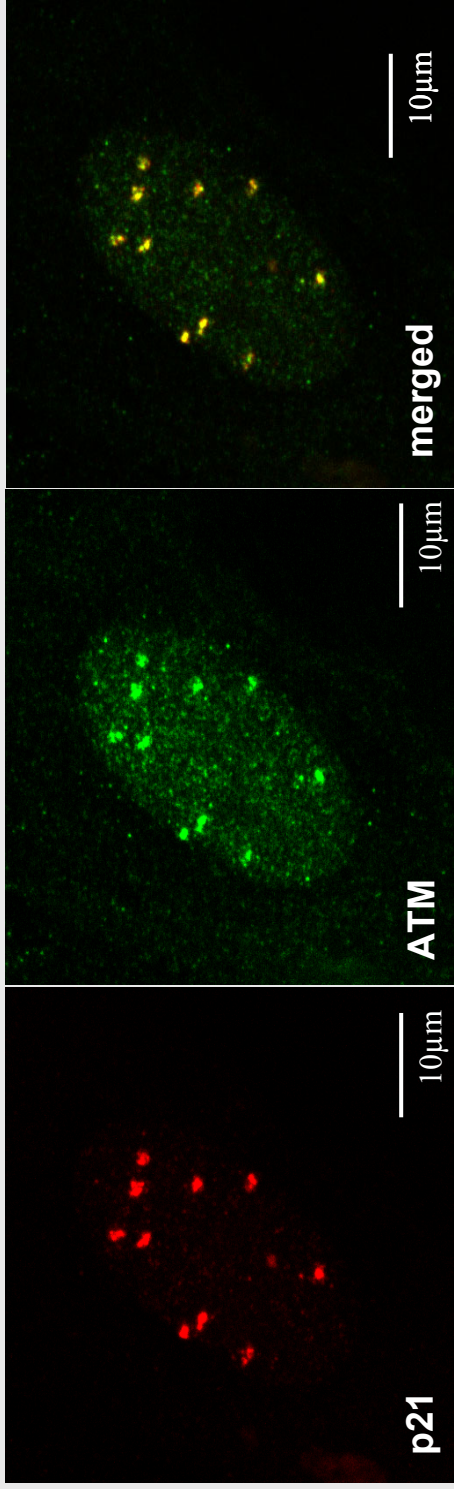
The pattern of
immunofluorescence
 corresponds to the
microscopic local
dose distribution

High and low energy
 carbon ion irradiation



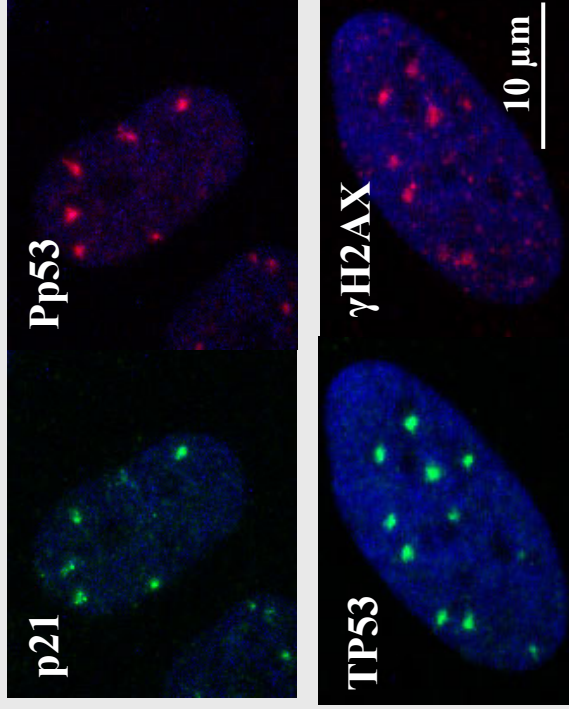
Human fibroblast nuclei immunostained for p21 following exposure to 10 Gy carbon ions

Co-localization of various proteins in foci at sites of ion traversal

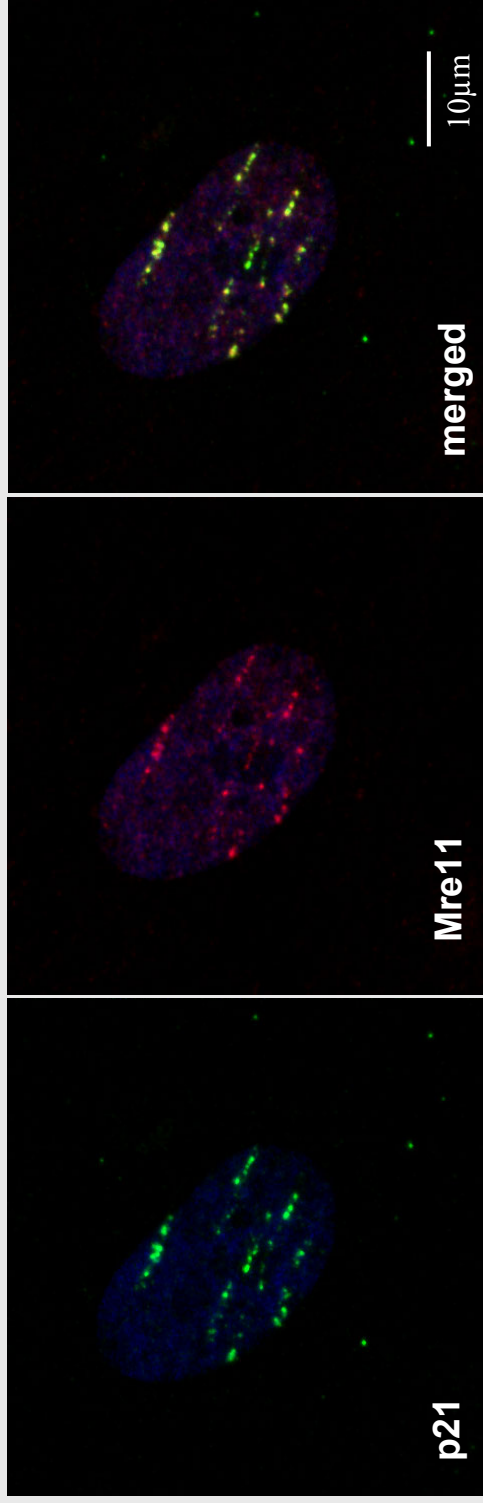


U ions
(3×10^6 p cm⁻²)
1 h post exp.

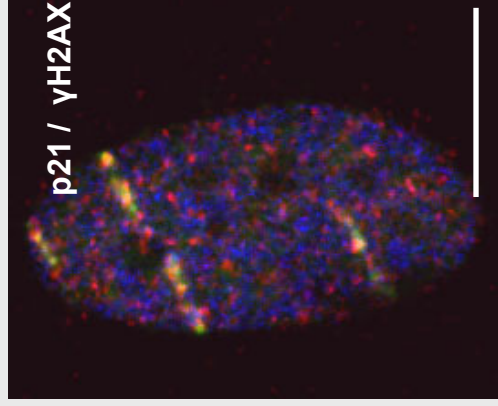
Colocalization of p21 and ATM to foci in nuclei of human fibroblasts



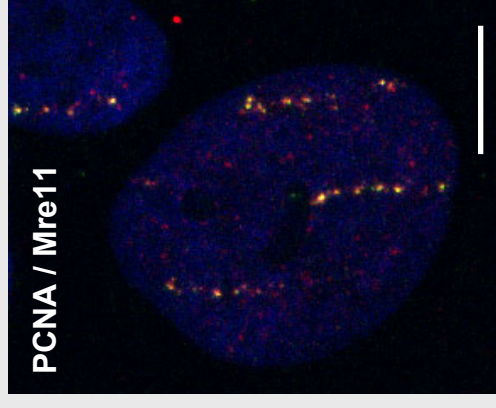
Co-localization of various proteins along radiation tracks



Incident beam at low angle: inhomogeneous protein streaks along carbon ion trajectories



C ions
($0.2 \times 10^7 \text{ p cm}^{-2}$)
1 h post exp.



Microbeam experiments:

Why are we interested in track visualisation?

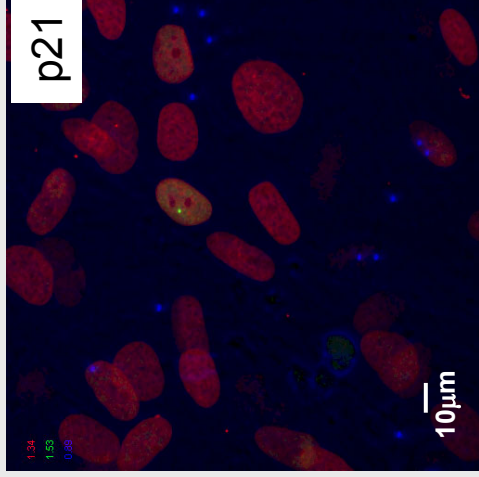
- Biological *in situ* verification of hits (many cells, 1 hit) – nuclear hits
- Estimate of hitting accuracy (many ions /point)
 - Analysis of cellular radiation responses
 - Studies on the response of proteins at damaged sites

Concerns in relation to single track visualisation:

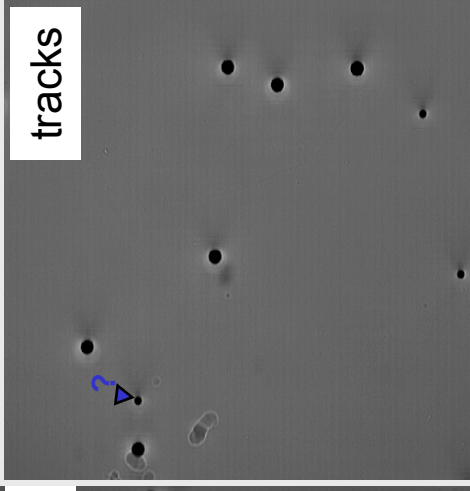
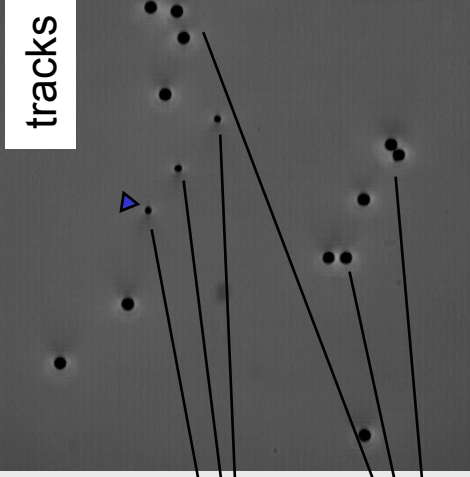
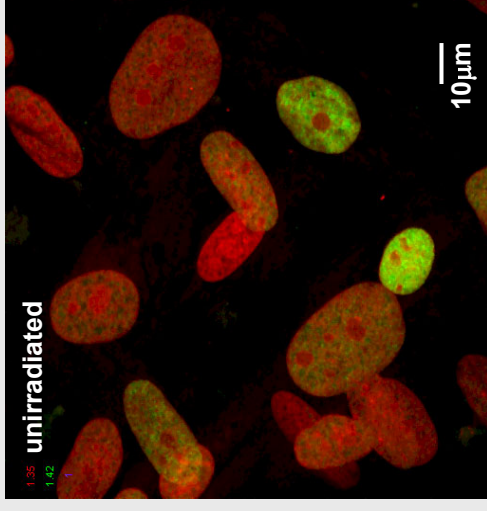
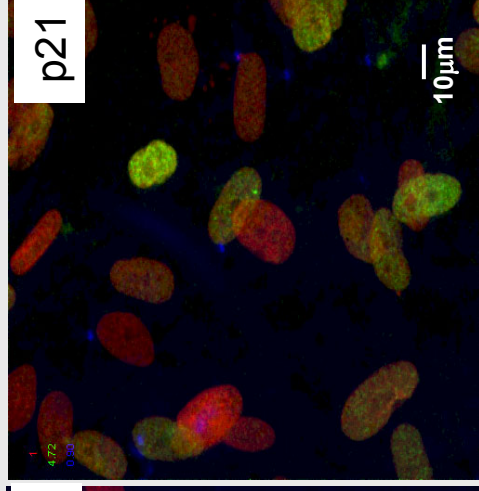
- Sensitivity of foci detection
- Background of foci in unirradiated cells
- Does every particle hit lead to focus formation in the irradiated cell?
-
- Do all cells respond (independent of cell cycle phase or cell status)
- Do bystander cells respond regarding foci formation?
- Kinetics of foci formation and persistence (postirradiation)

Low fluence irradiation of fibroblasts and retrospective track detection

15 min after irradiation



3 h after irradiation



Unirradiated control

Immunostaining:

p21 (green)

PI (red)

Zn 4.9 MeV/u

LET 4000 keV/μm

1×10^4 P/cm²

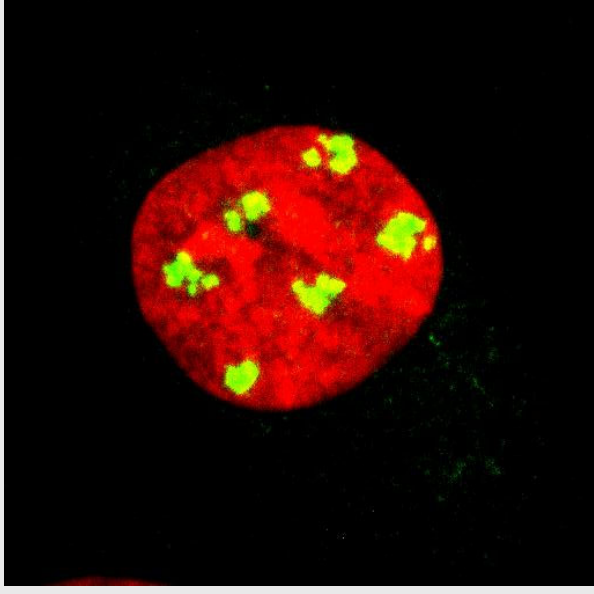
Image size: 160 x 160 μm

2.3 % of nuclei hit

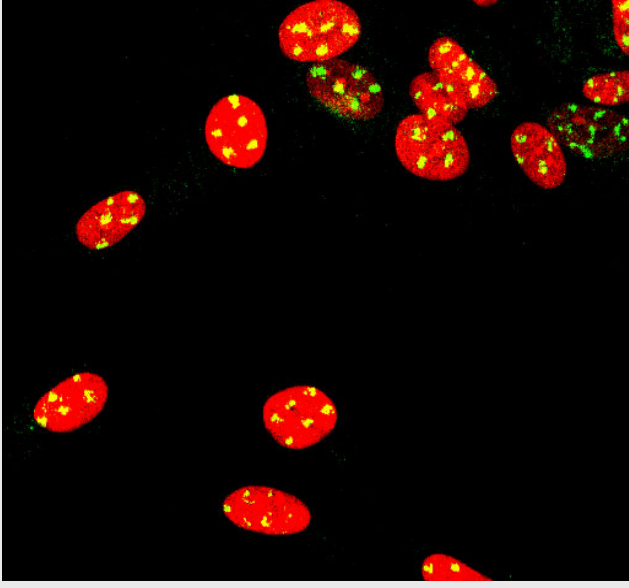
- Cell migration after longer times
- No p21 foci in bystander cells

Microbeam accuracy in cells

Pattern of 53BP1 foci
in fibroblasts nuclei after irradiation
with regularly spaced C ions ($7 \times 7 \mu\text{m}$)
4.8 MeV/u



Rotation of cells
after irradiation



Track visualisation: SNAKE (Munich), RAD51 staining

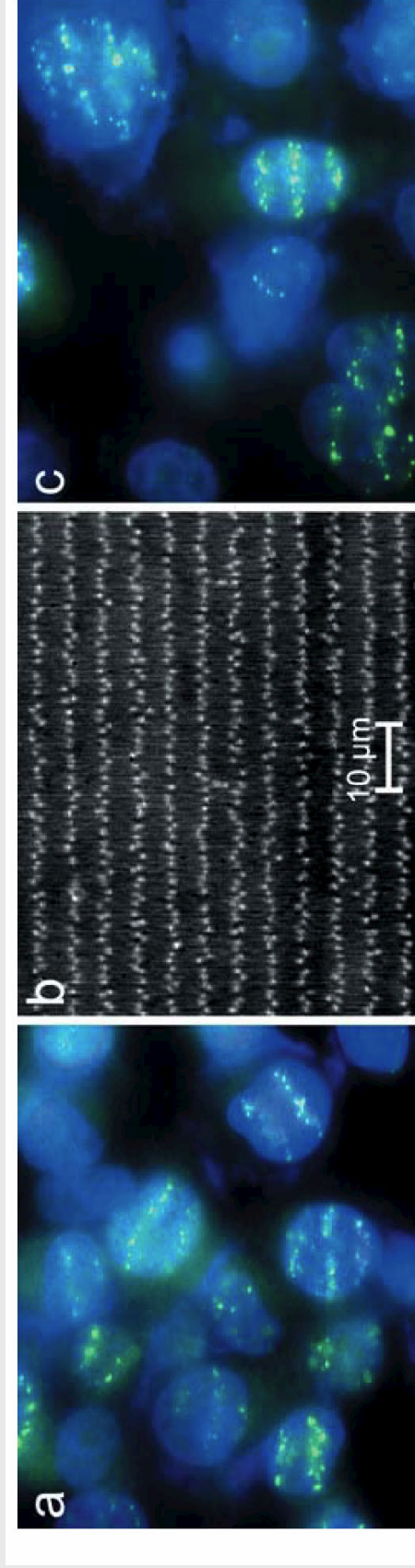


Fig. 8 Immunofluorescence micrograph of cells from irradiation sub-pattern III, with Rad51 foci depicted in green and DAPI staining of cell nuclei in blue **a**, **c**. For comparison, a cut-out of the nuclear track detector, which was irradiated with the same pattern,

is shown at the same magnification **b**. The *scalebar* is valid for all panels. Note an example for in-plane rotation in panel **a**, and shifted line pattern in the lower section of panel **c**

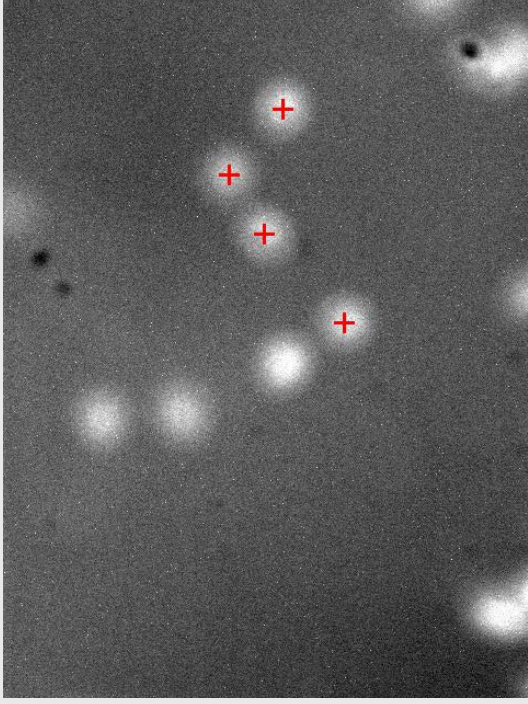
Hauptner et al., Radiat. Environ. Biophys., 2004

HeLa cells irradiated with 100 MeV O ions
1 µm lateral / 5 µm between lines
1 h postirradiation incubation

Targeting of single cells with defined number of ions

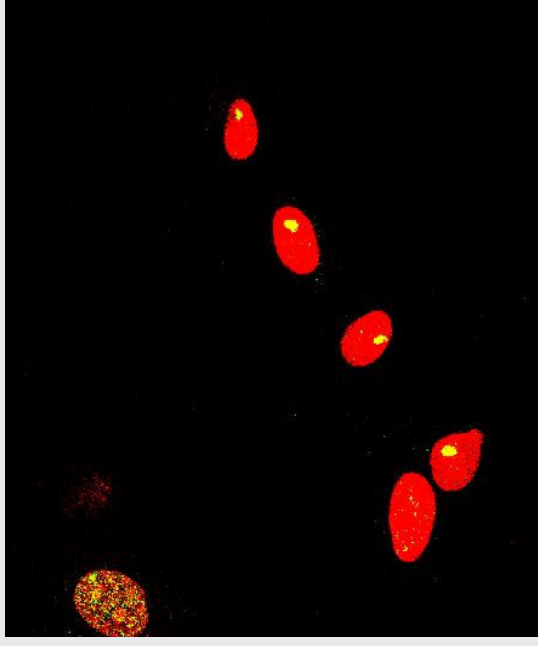
Irradiation:
carbon ions (4.8 MeV/u)

Detected and targeted cells



Cells targeted after cell detection
and irradiated with 16 C ions per spot

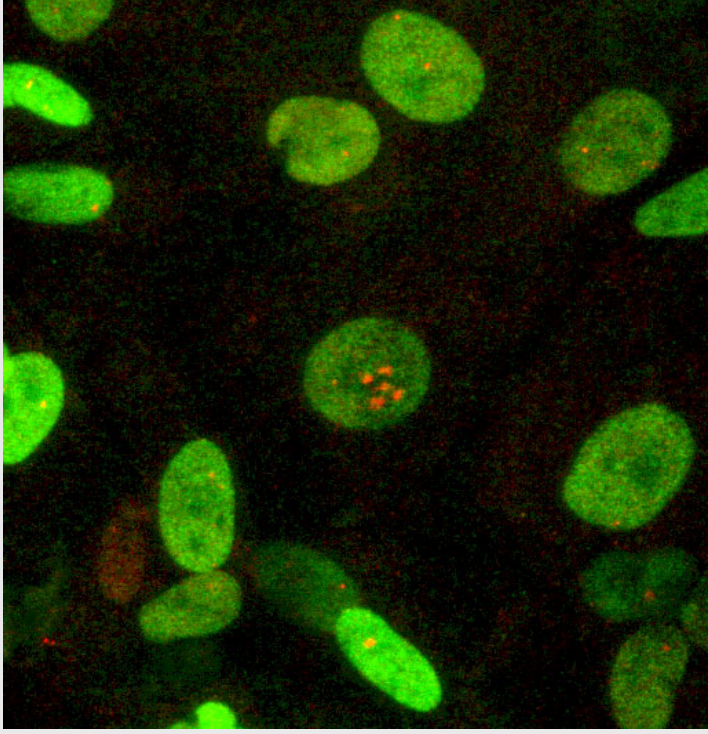
53BP1 foci
in irradiated fibroblast nuclei



53BP1 foci
after 1h immunostaining and
confocal microscopy
(notice cell migration)

Targeted irradiation of single cells

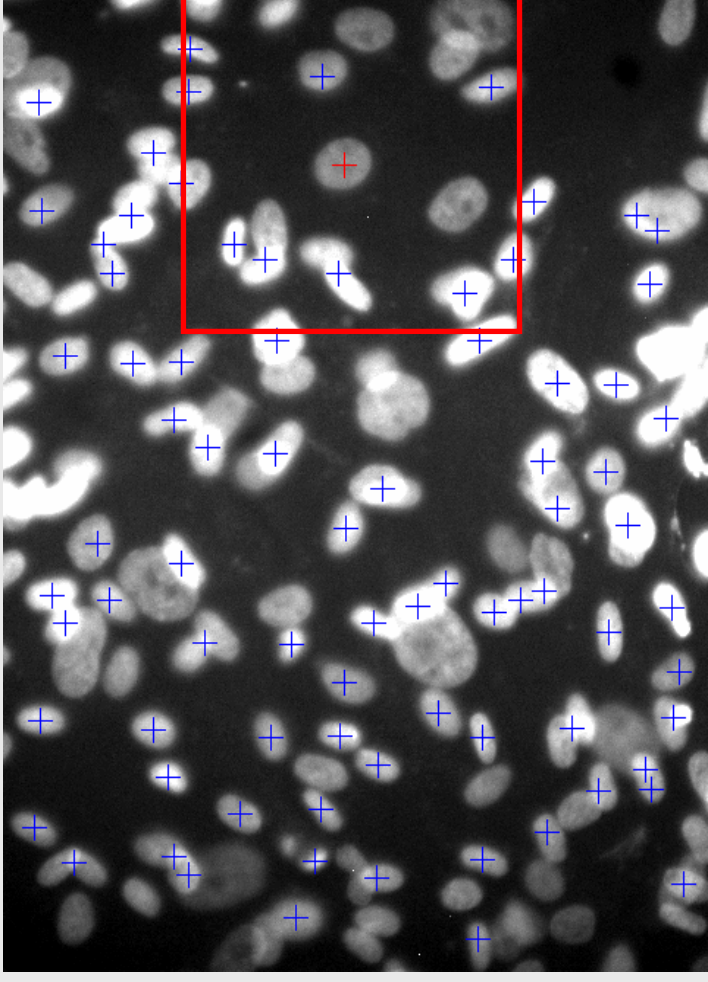
with 5 hits cross of 3 spots/axis
5 ions each at 3 μ m distance



Immunostaining **p21** / **53BP1**

1h post irradi

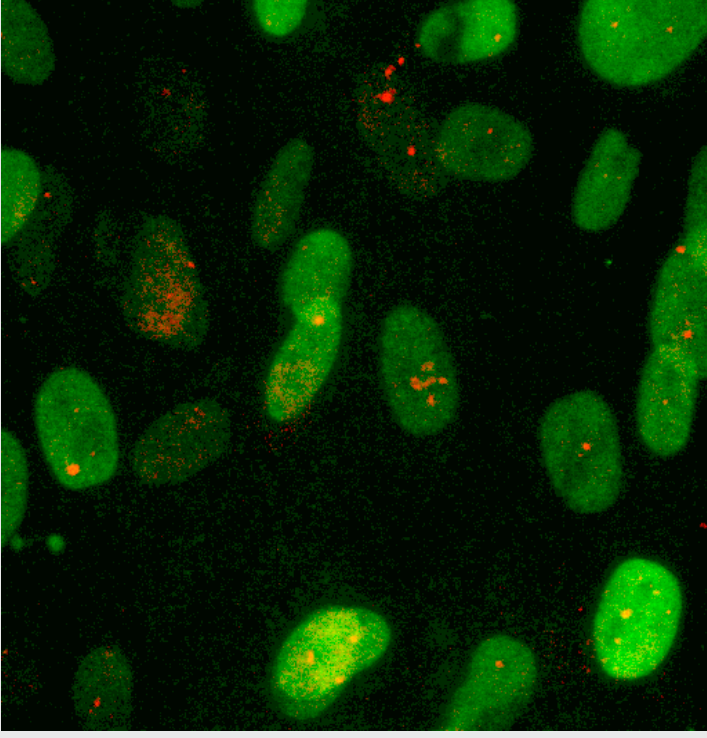
Very little no. of foci in non-hit cells
GSI



Detected and targeted cells

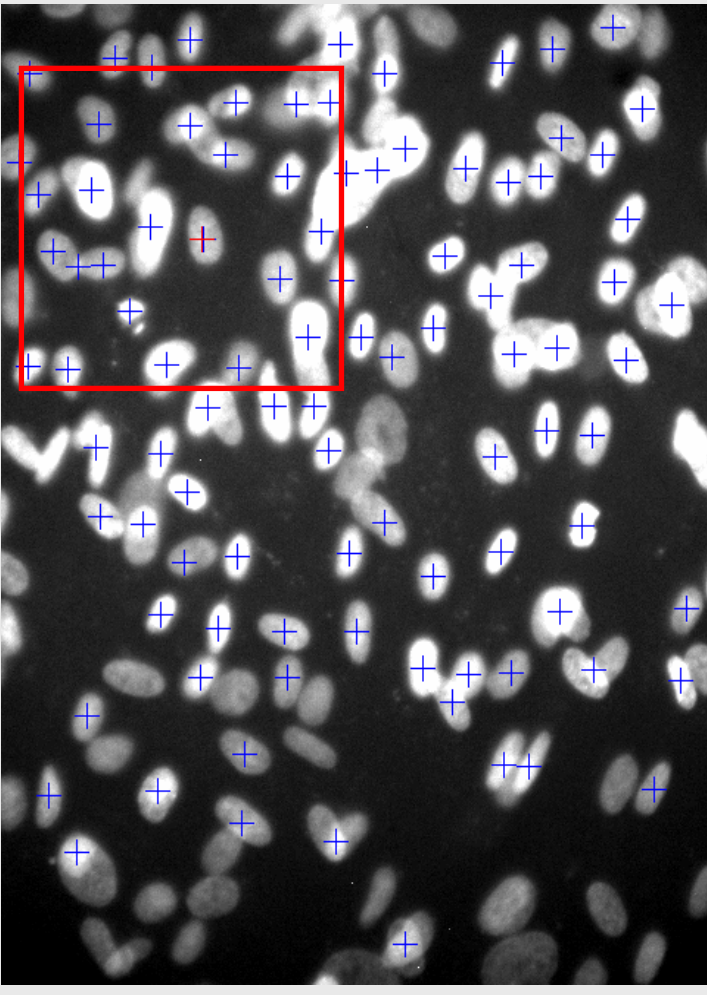
Targeted irradiation of single cells

with 5 hits cross of 3 spots/axis
5 ions each at 3 μ m distance



Immunostaining **p21** / **53BP1**

1h post irradiation



Detected and targeted cells

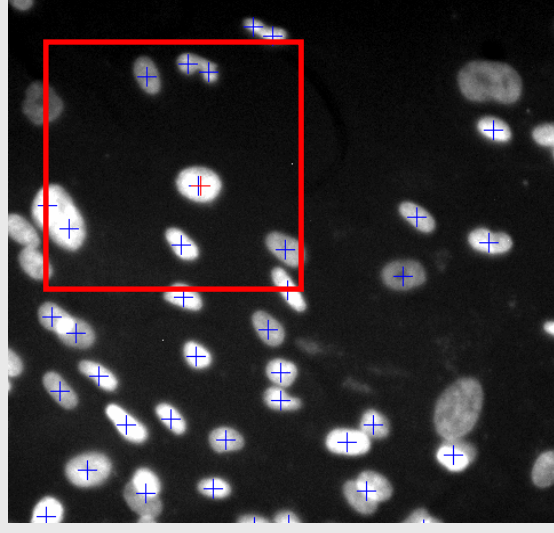
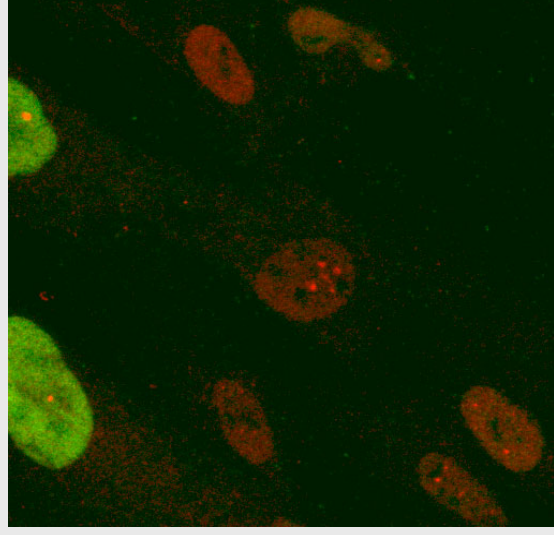
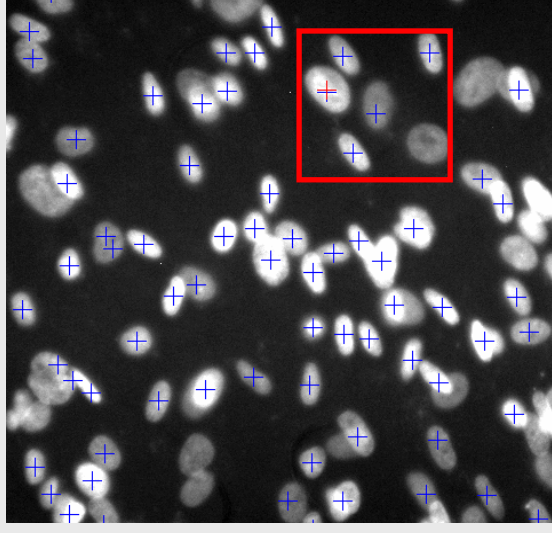
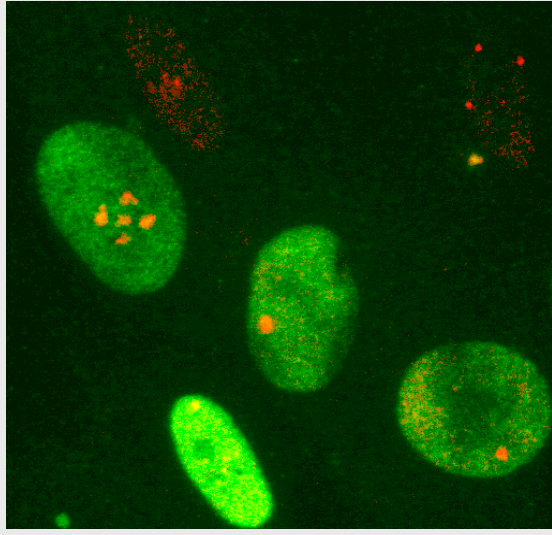
Considerable no. of foci in non-hit cells
GSI

Heterogeneous response of single irradiated cells

Single cell irradiation
with **carbon** ions

cross of 5 spots
5 ions each
3 μ m distance

p21 / **53BP1**
1h post irradi.



Summary

Development phase of biology and first microbeam experiments

- Cells can be grown to as single cells or to confluency in microbeam chambers.
- Hoechst and UV for cell detection are tolerated under the conditions applied.
- A regular pattern of ion traversals can be reproduced as a biological response.
- Defined single cells can be targeted with a determined number of heavy ions.
- **Track visualization is a useful tool to verify hit detection even after several hours post-irradiation incubation, but critical factors need to be considered.**
- Ongoing biological experiments (p21 induction in bystander cells).