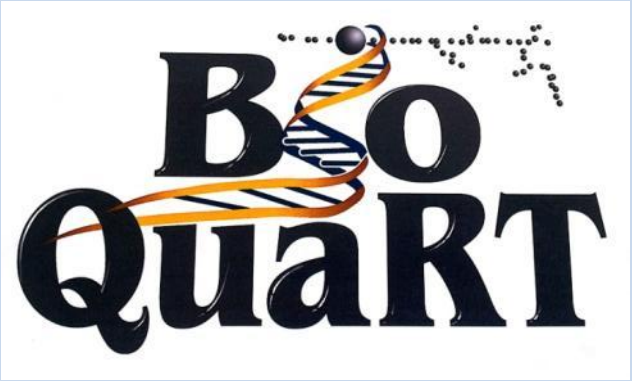
IRSN
INSTITUT
DE RADIOPROTECTION
ET DE SÛRETÉ NUCLÉAIRE

Faire avancer la sûreté nucléaire

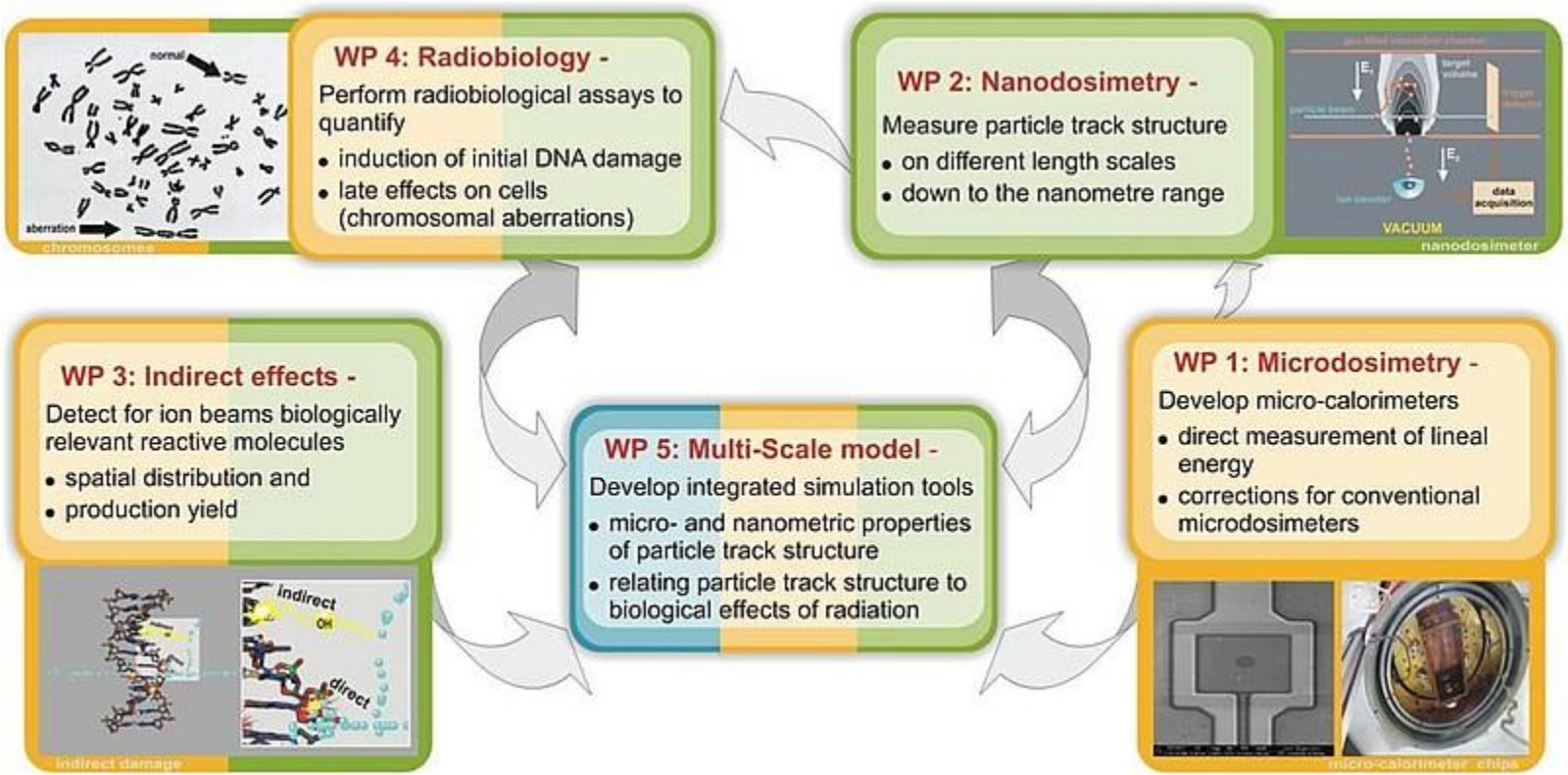
Benchmarking simulations by measured yields of initial DNA damage and late effects for ion beam irradiation of human and mammal cells.

IRSN/PRP-HOM/SRBE/LDB (France)
G. GONON, Pa. VOISIN and G. GRUEL
ENEA (Italy)
C. PATRONO, A. TESTA, M. PINTO
IST/ITN (Portugal)
O. MONTEIRO GIL
PTB (Germany)
U. GIESEN

BioQuaRT Midterm Dissemination Workshop
7 June 2013

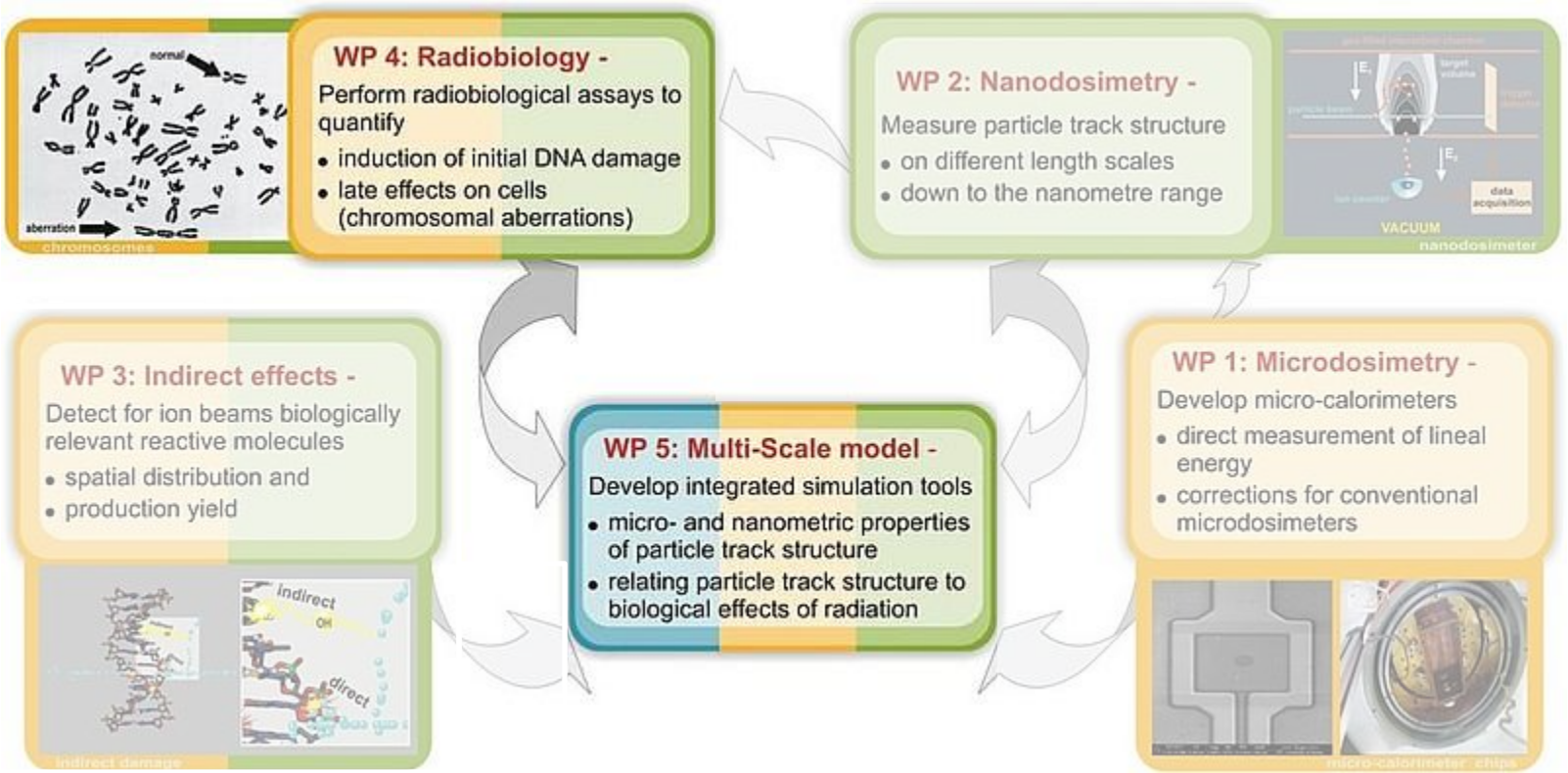


WP4: Radiobiology



Work package 4 focuses on the biological aspects of radiation damage and will produce data on initial DNA damage and late effects for ion beams of different radiation qualities.

WP4: Radiobiology



The biological data collected in this WP will be used for benchmarking with the multi-scale model developed in WP5.

Challenges of WP4

- To perform biological measurements that could “feed” the simulation

Challenges of WP4

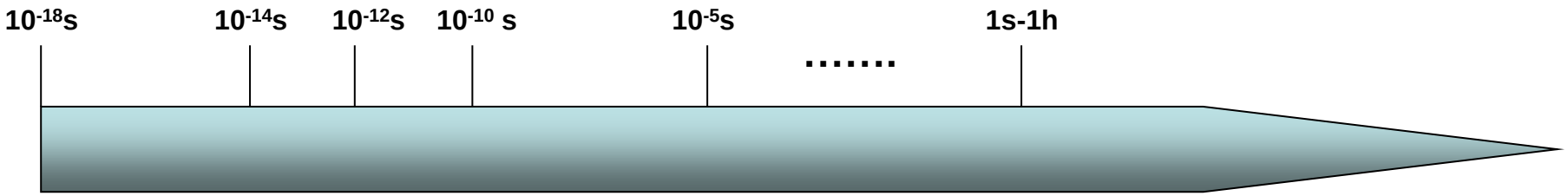
- To perform biological measurements that could “feed” simulations

    1. To choose relevant biological endpoints

Biological endpoint

↗ Link between physics events and biological events

- Post-irradiation timeline



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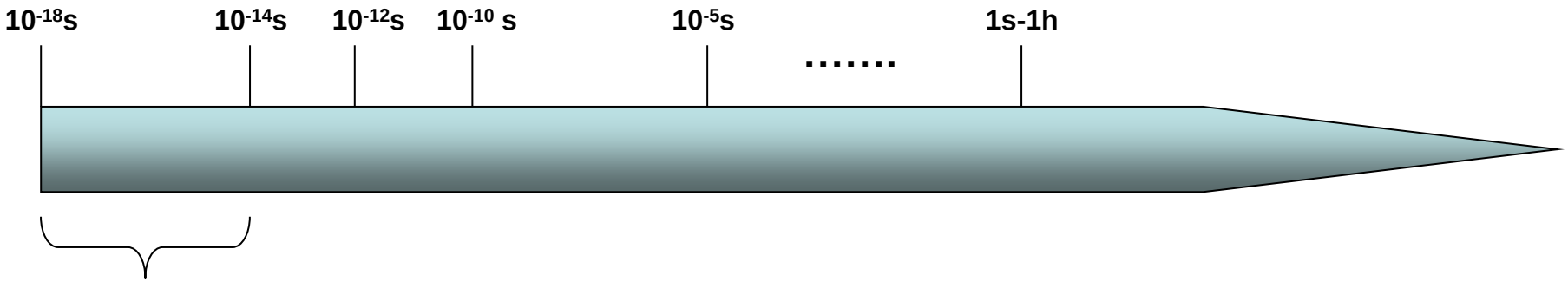

Physical stage

Monte carlo simulations micro/nano dosimetry data

Biological endpoint

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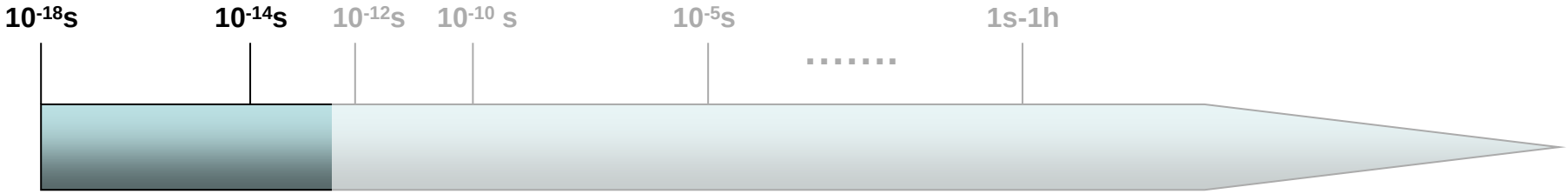

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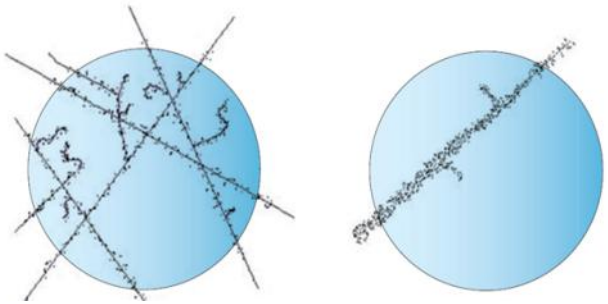

Track of ionizations

Biological endpoint

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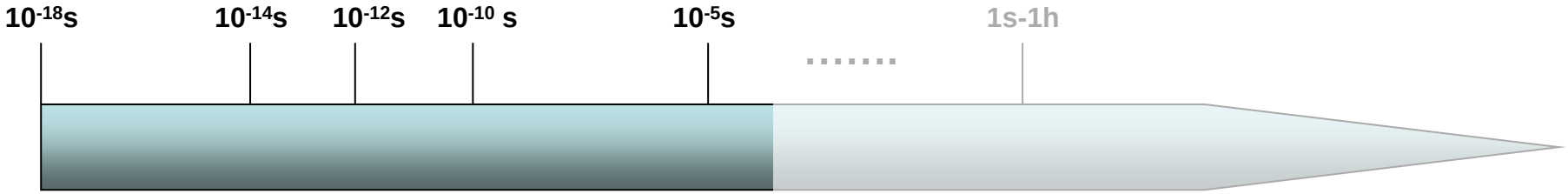
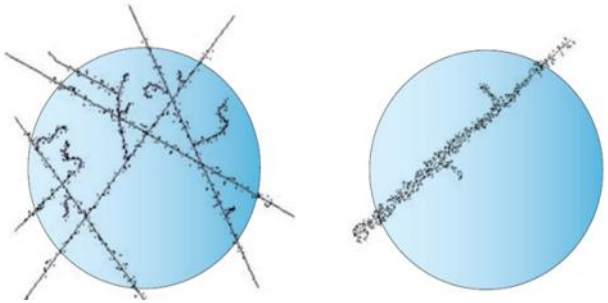
- Post-irradiation timeline



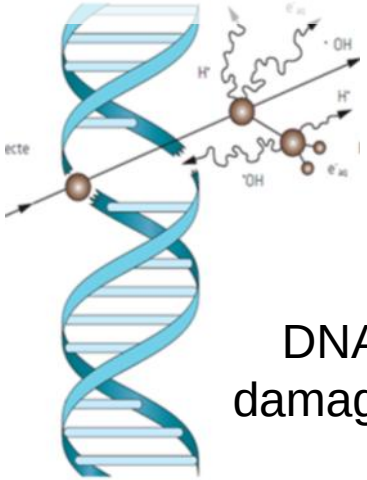
Physical stage

Monte carlo simulations: micro/nano dosimetry data



Track of ionizations

One type of damage among many others



DNA damages

Biological endpoint

↗ Link between physics events and biological events

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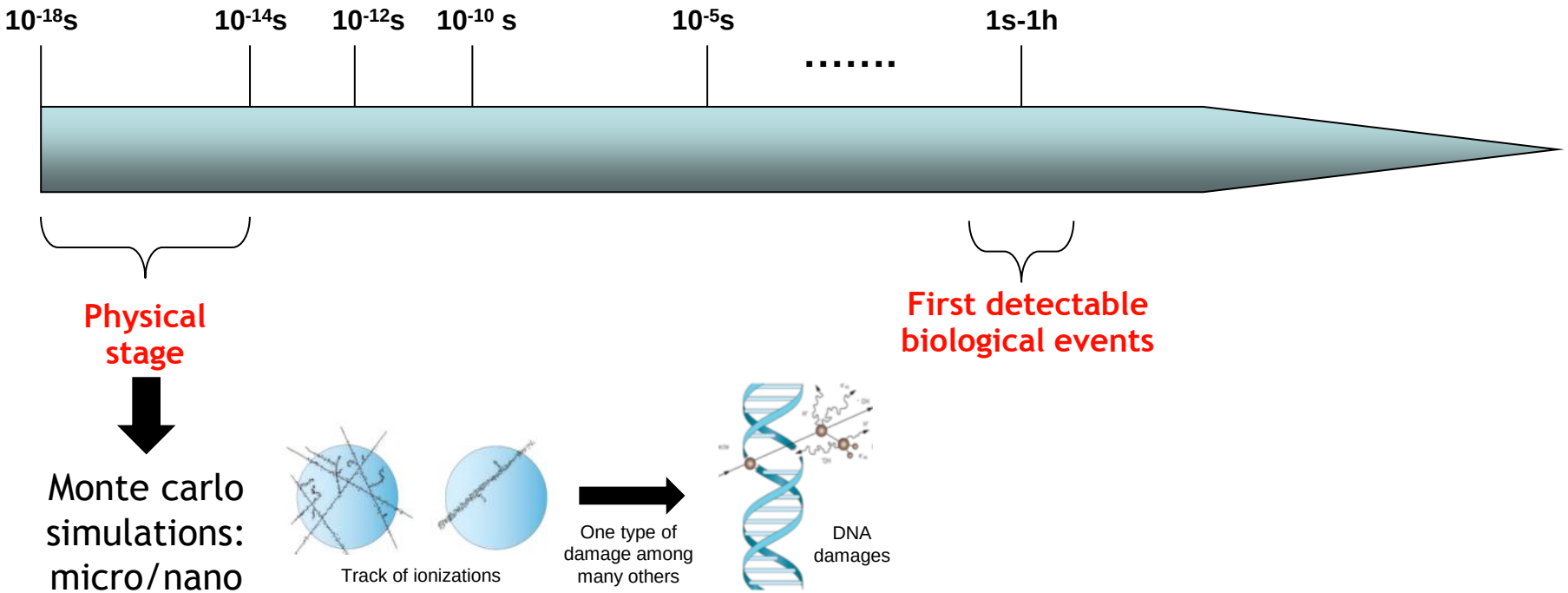

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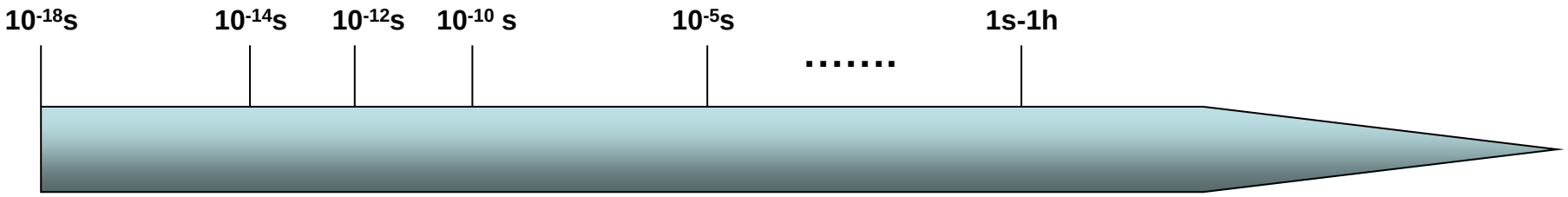

Physical stage

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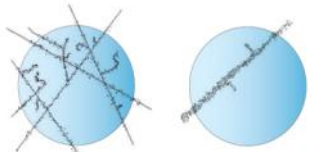

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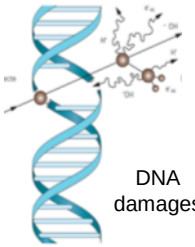


DNA damages

First detectable biological events

Signaling of DNA damage can be "easily" observed with a fluorescent labeling ->

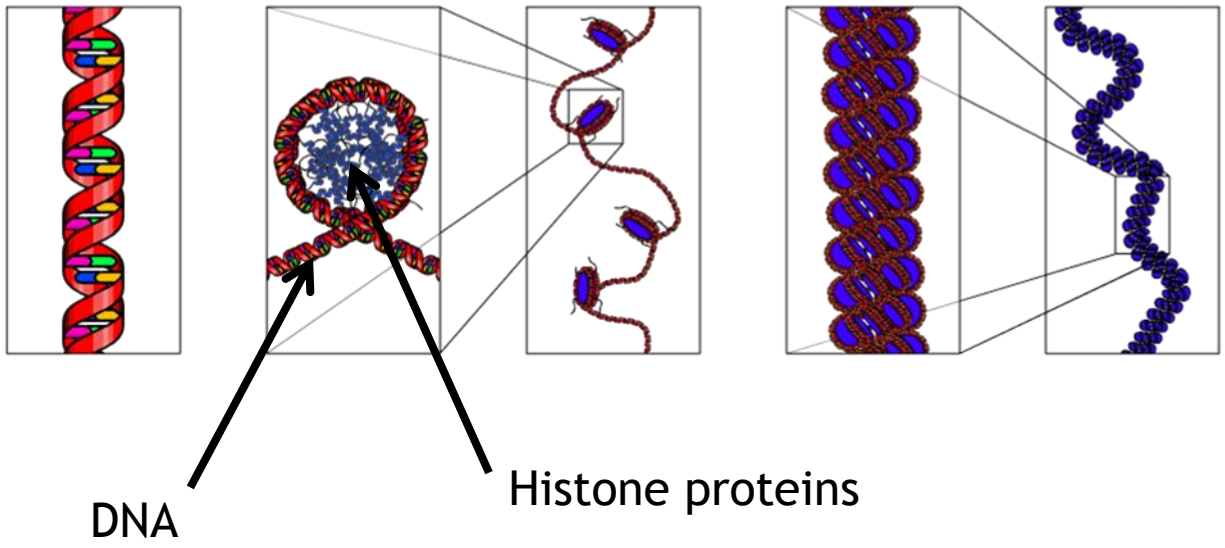
nuclear foci

Biological endpoint: early events

↗ Performed at IRSN (G. Gonon, Pa. Voisin and G. Gruel)

- Nuclear foci

In the cell nucleus, the DNA molecule is compacted around proteins named histones



Biological endpoint: early events

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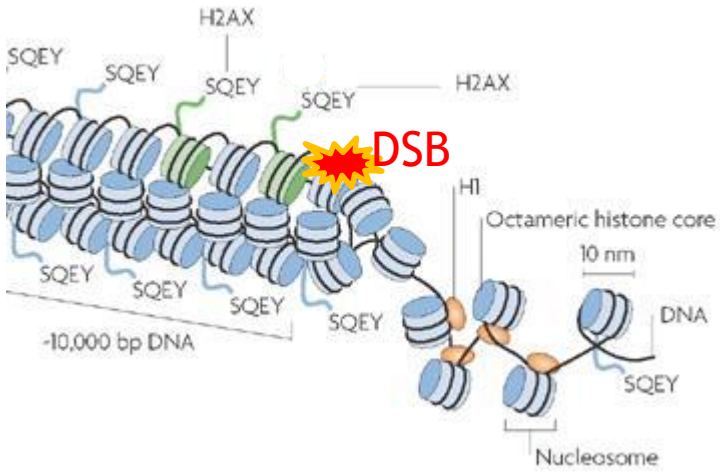
- Nuclear foci

When a double strand break occurs in the DNA molecule



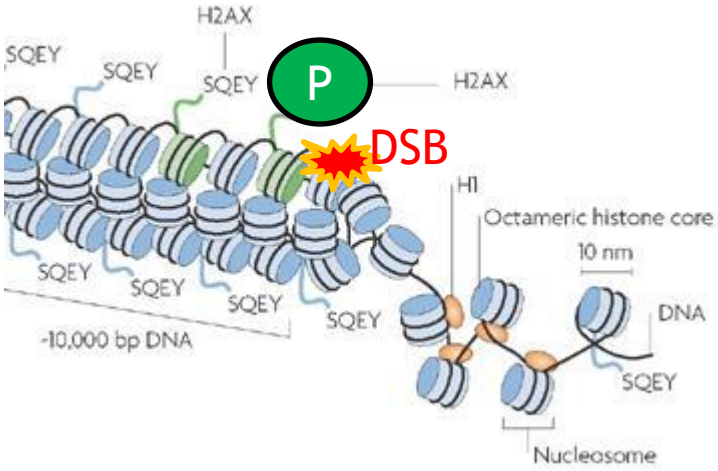
Bonner et al 2008

Biological endpoint: early events

↗ Performed at IRSN (G. Gonon, Pa. Voisin and G. Gruel)

Nuclear foci

Histone proteins can be modified around the damage (such as **γ-H2AX** foci) or certain proteins are relocalized at the site of DNA DSBs (such as **53BP1**)



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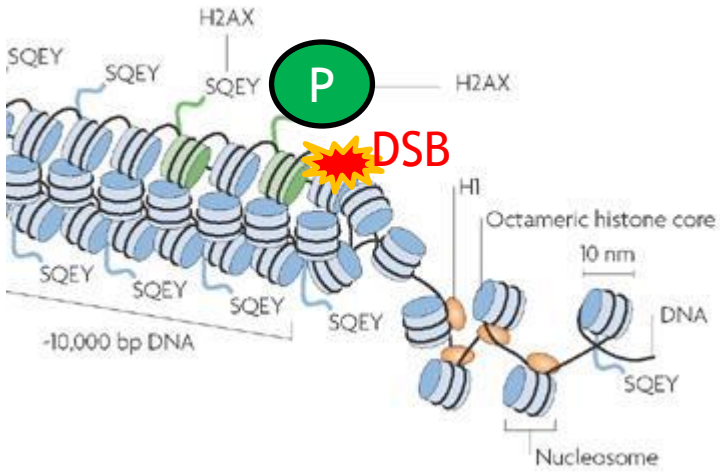
- Nuclear foci

And this modification and/or relocalization can be detected through a fluorescent labelling



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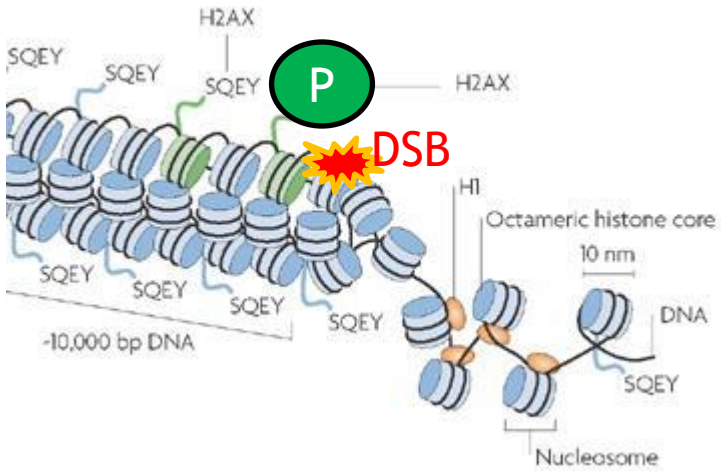
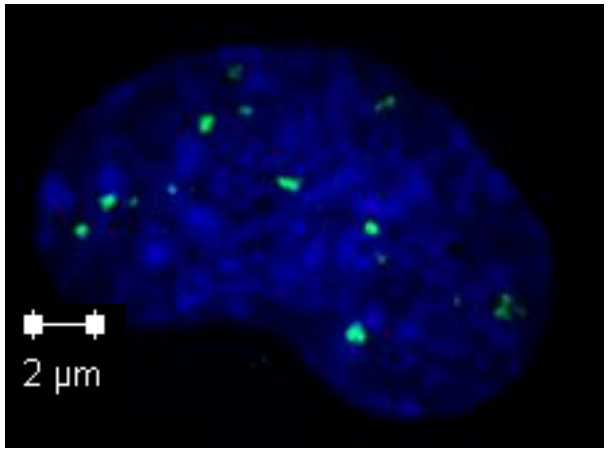


Bonner et al 2008

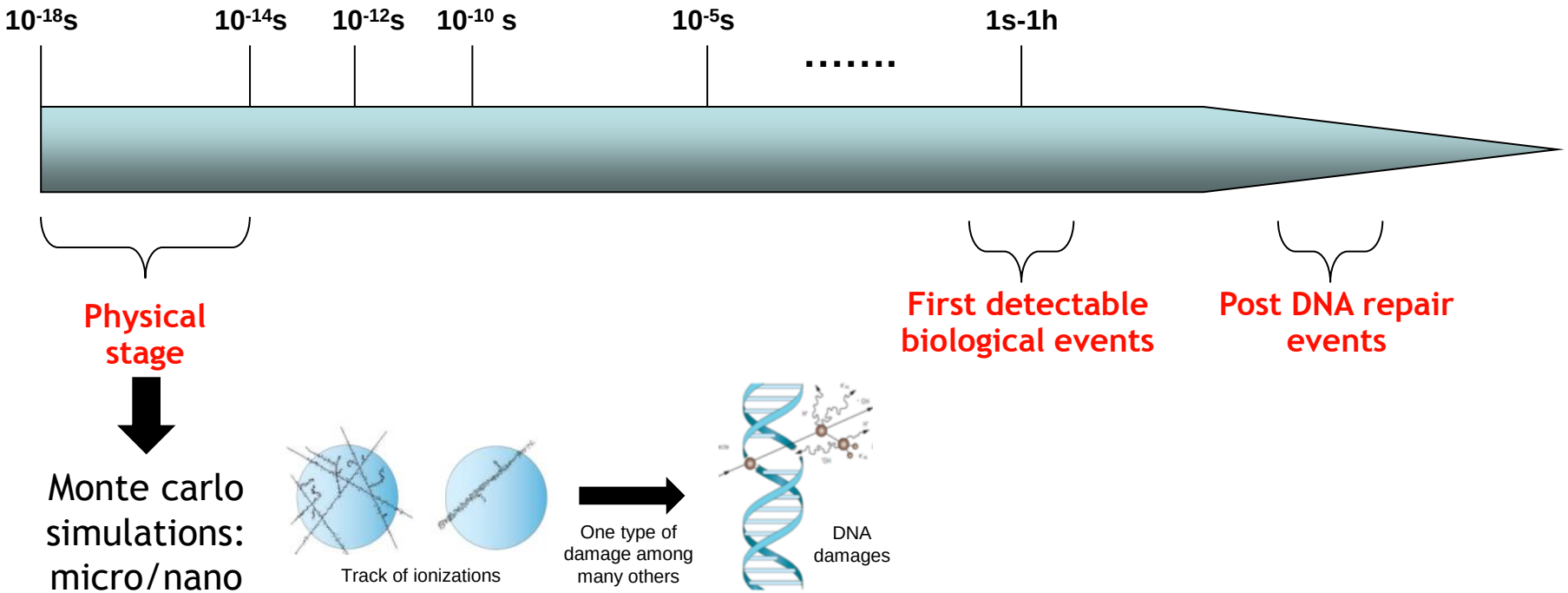


We can then analysed **quantity**, **quality** and **topology** of these foci

Biological endpoint

↗ Link between physics events and biological events

- Post-irradiation timeline



Biological endpoint

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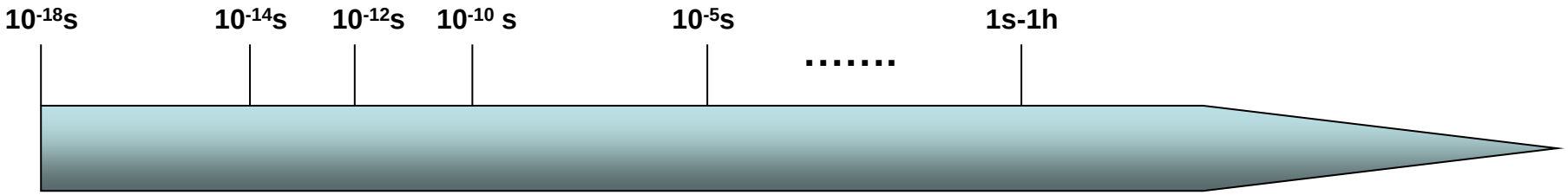
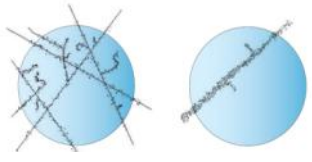


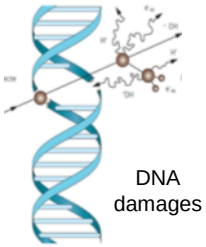
Physical stage

Monte carlo simulations: micro/nano dosimetry data



Track of ionizations

One type of damage among many others



DNA damages

First detectable biological events

Post DNA repair events

Observation of misrepair events ->

chromosome aberrations

Biological endpoint: late events

↗ Performed at ENEA (C. Patrono, A. Testa) and IST/ITN (O. Monteiro Gil)

Chromosome aberrations

The DNA ends may rejoin in different patterns from their original arrangement. The abnormalities that result are termed "chromosome aberrations" (CA) and may be visualized in specific stages of mitosis.

The frequency of CA is known to change with radiation dose and/or quality.

Biological endpoint: late events

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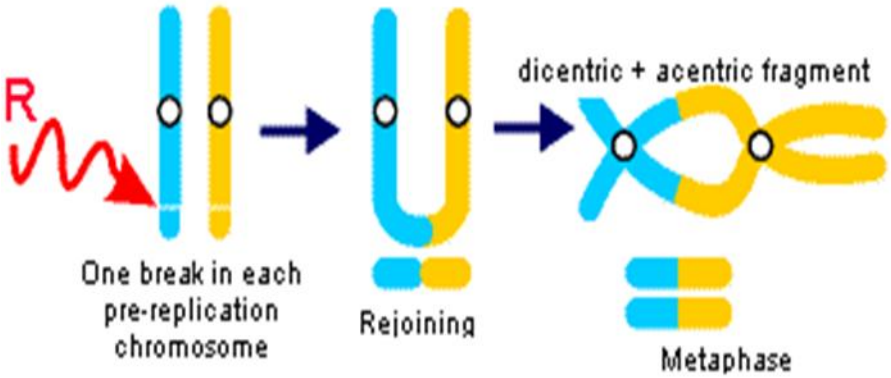
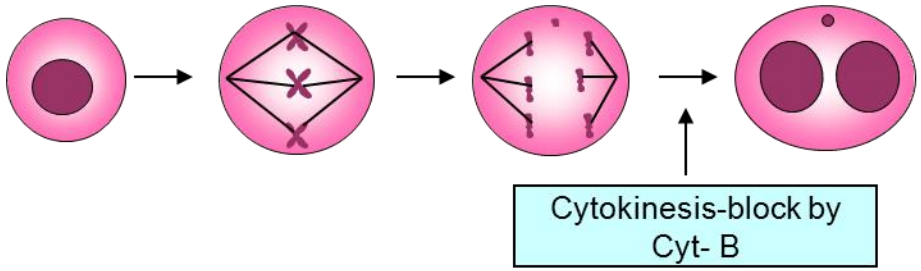
Chromosome aberrations

Two kinds of CA will be scored :

Dicentric chromosome



Micronuclei (MN)



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- Chromosome aberrations

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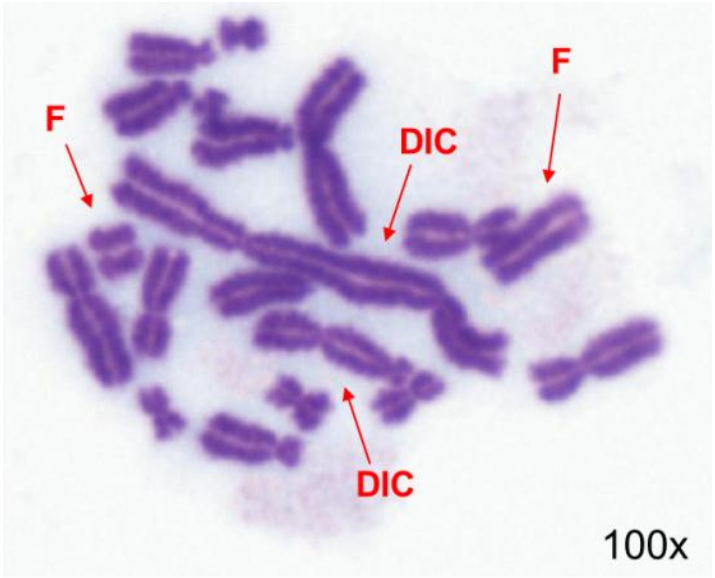

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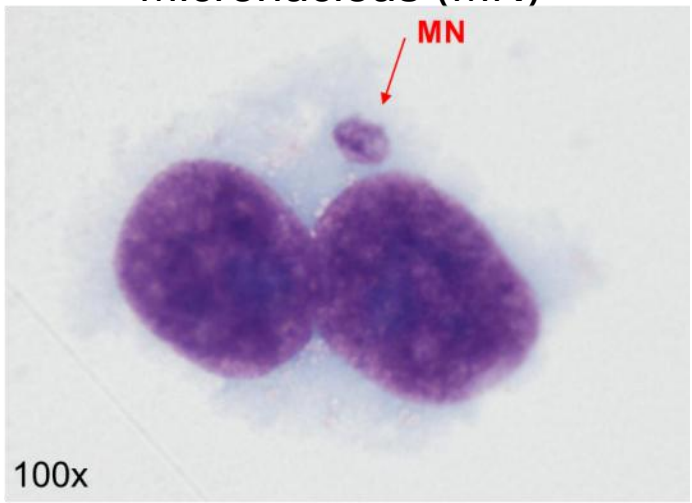


Observation on CHO-K1 cells after exposure to 20 MeV α particles

Challenges of WP4

- To perform biological measurements that could “feed” WP5

  1. To choose relevant biological endpoints

Challenges of WP4

- To perform biological measurements that could “feed” WP5

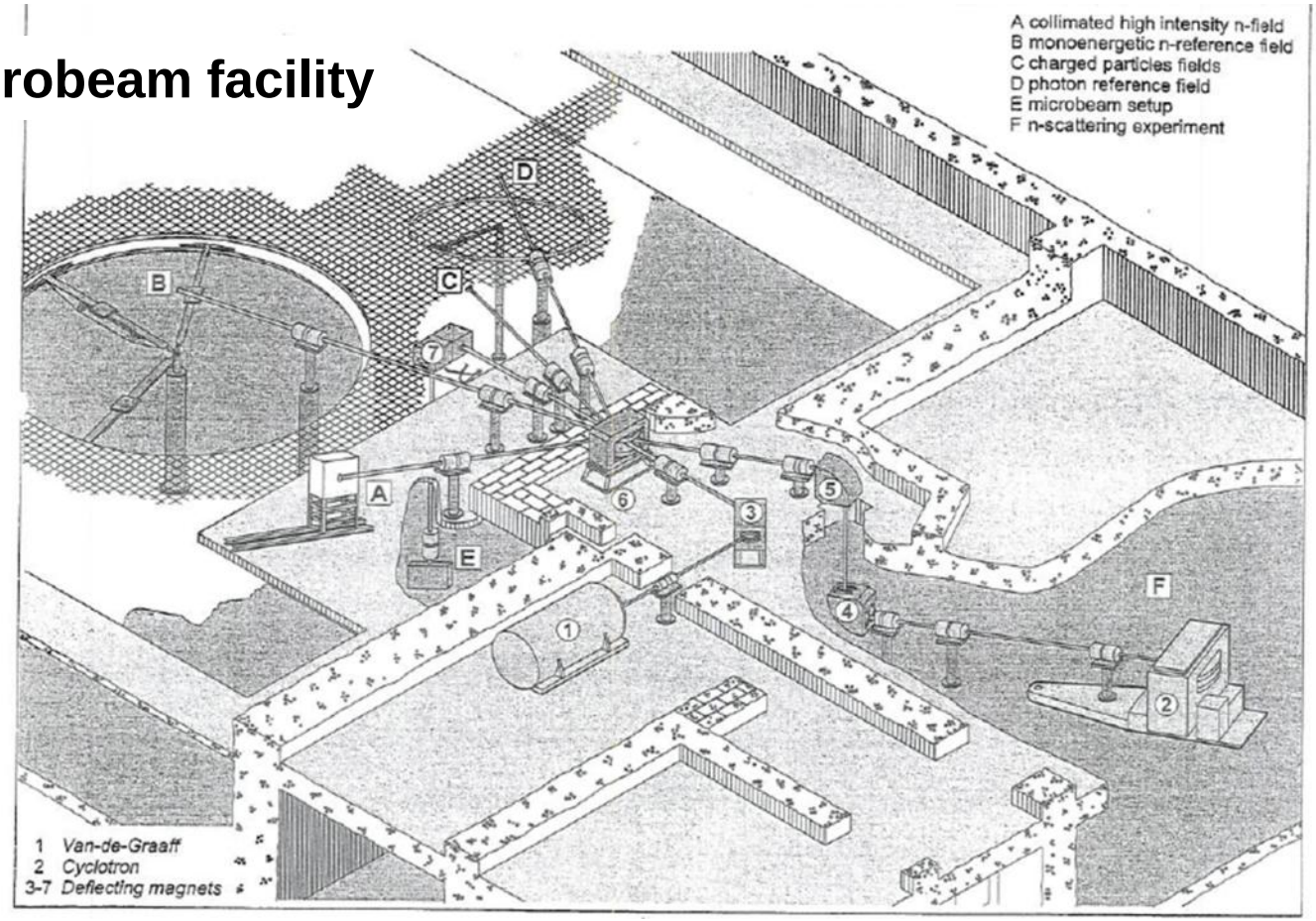
  1. To choose relevant biological endpoints

  2. To choose relevant irradiation conditions…

Microbeam irradiations

↗ Performed at PTB (U. Giesen)

PTB microbeam facility



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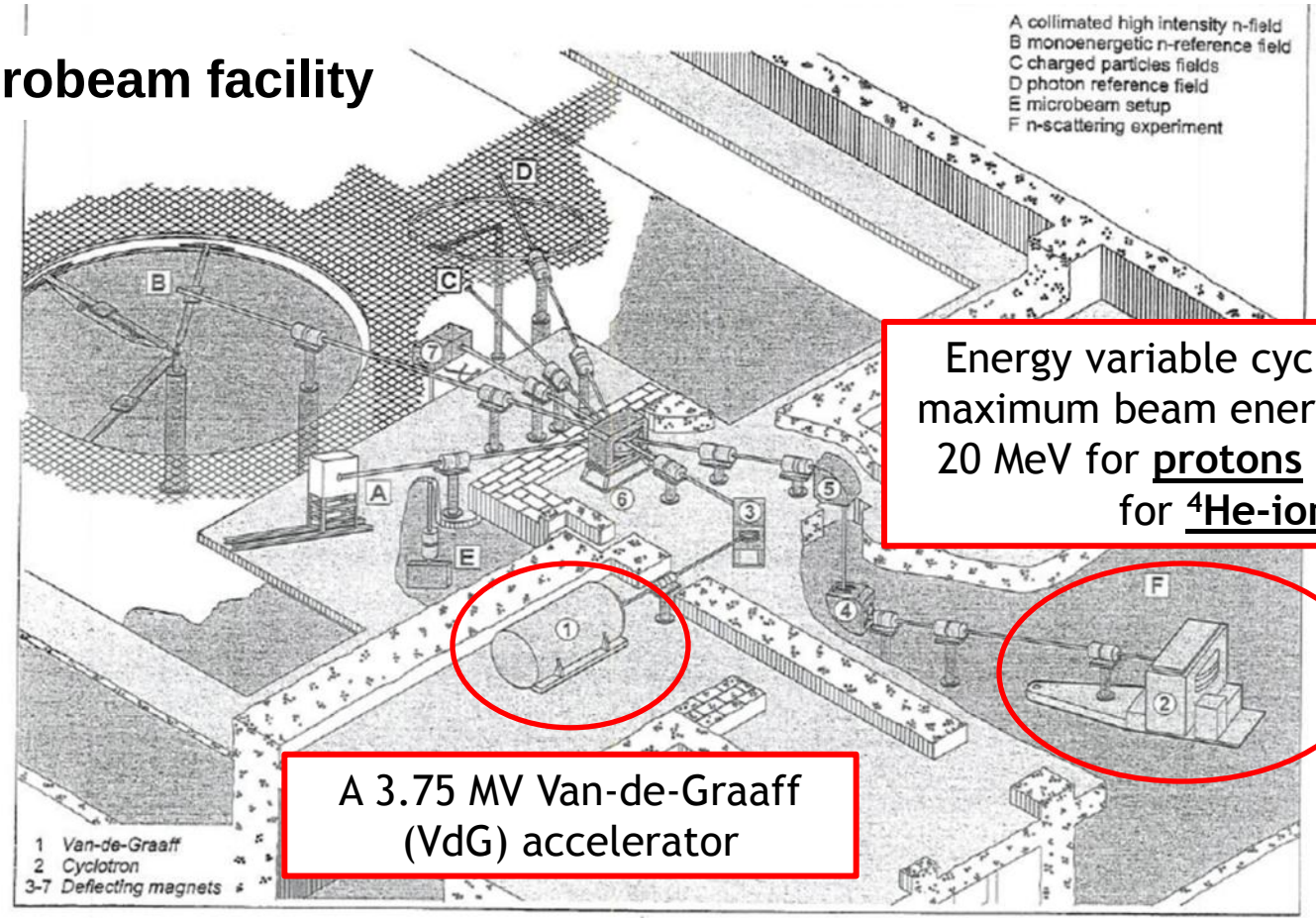

Energy variable cyclotron with maximum beam energies of up to 20 MeV for **protons** and 28 MeV for **^{4}He-ions**

A 3.75 MV Van-de-Graaff (VdG) accelerator

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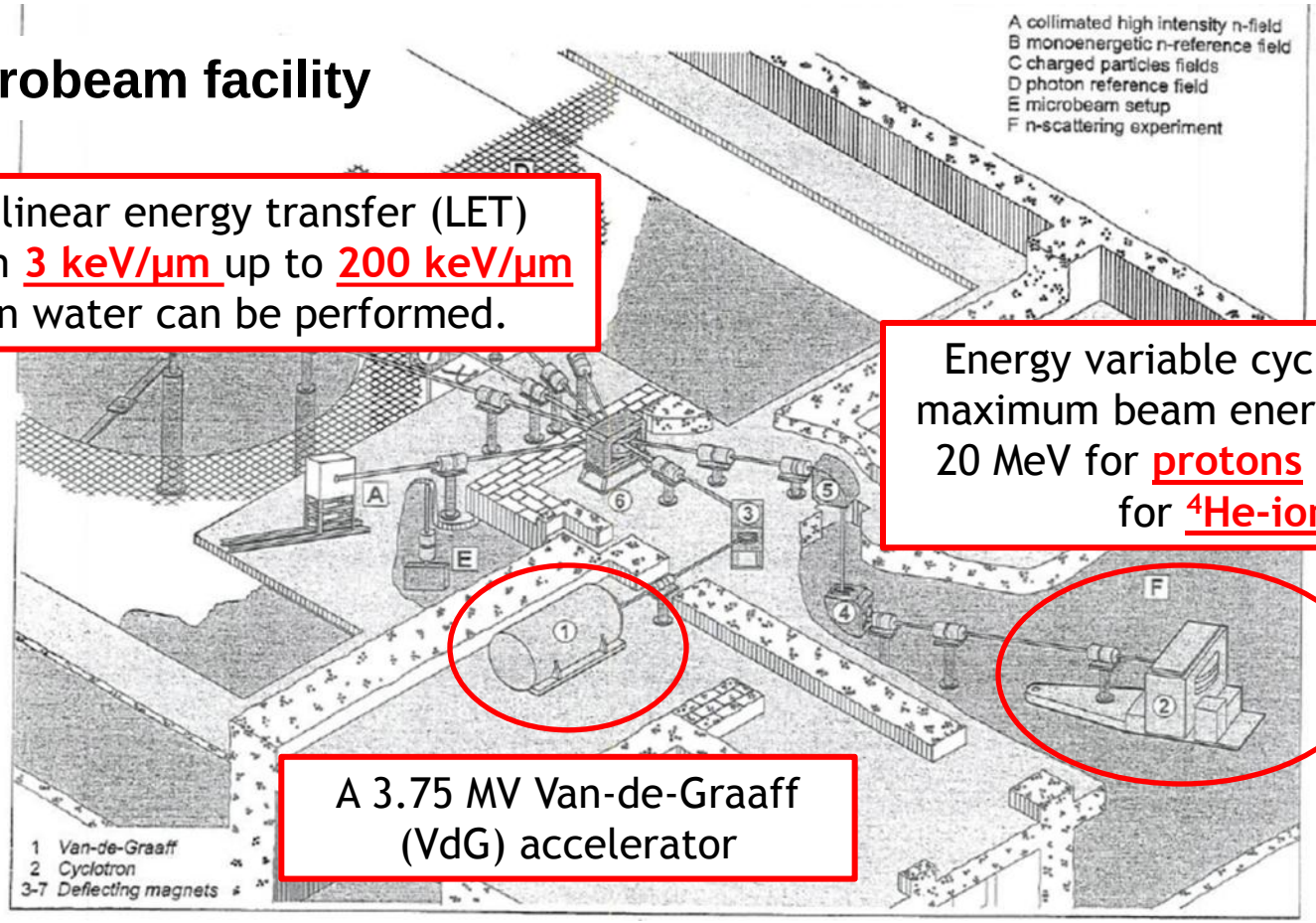

A linear energy transfer (LET) from **3 keV/µm** up to **200 keV/µm** in water can be performed.

Energy variable cyclotron with maximum beam energies of up to 20 MeV for **protons** and 28 MeV for **^{4}He-ions**

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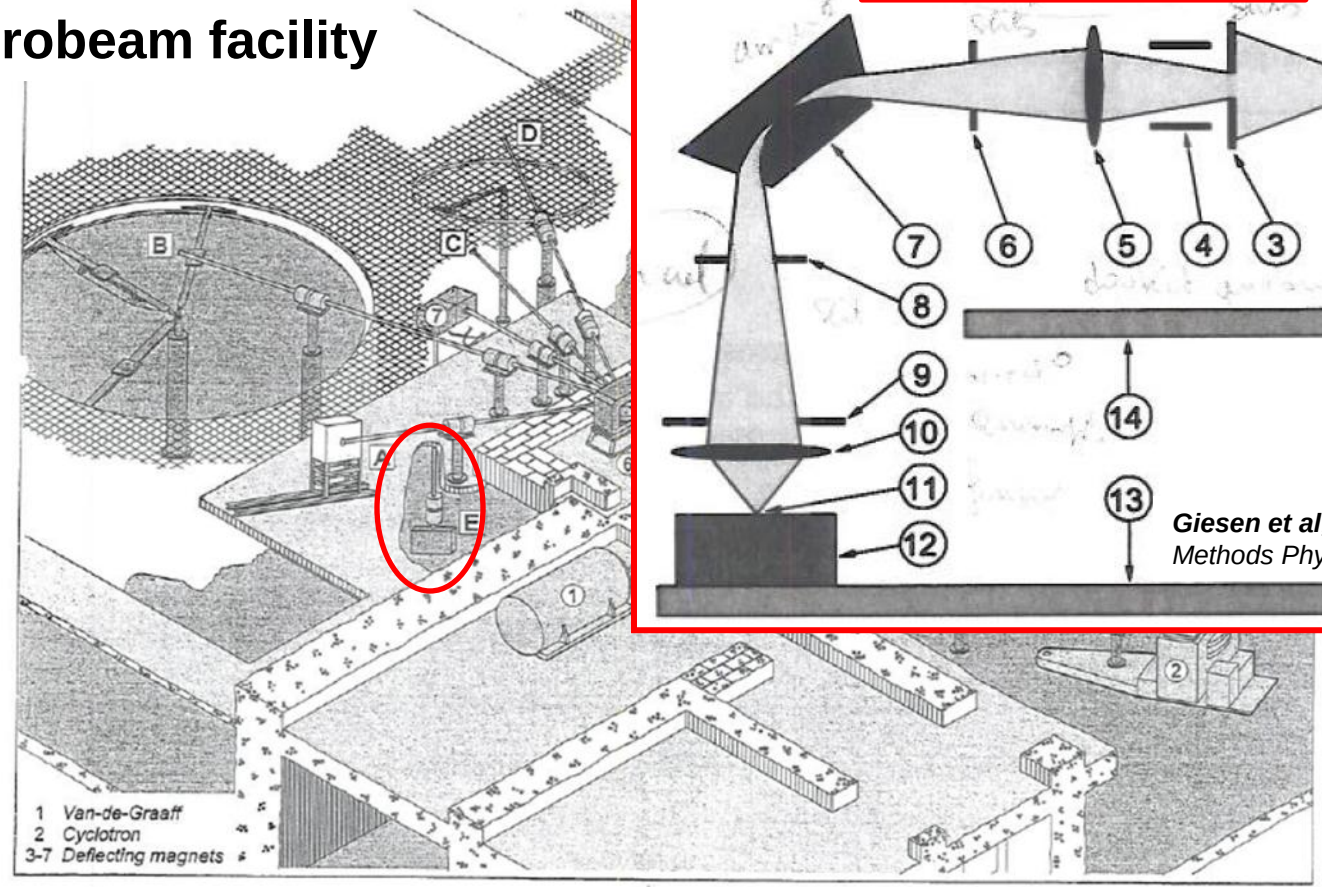

Microbeam line

***Giesen et al**, Nucl Instrum Methods Phys Res B, 2004*

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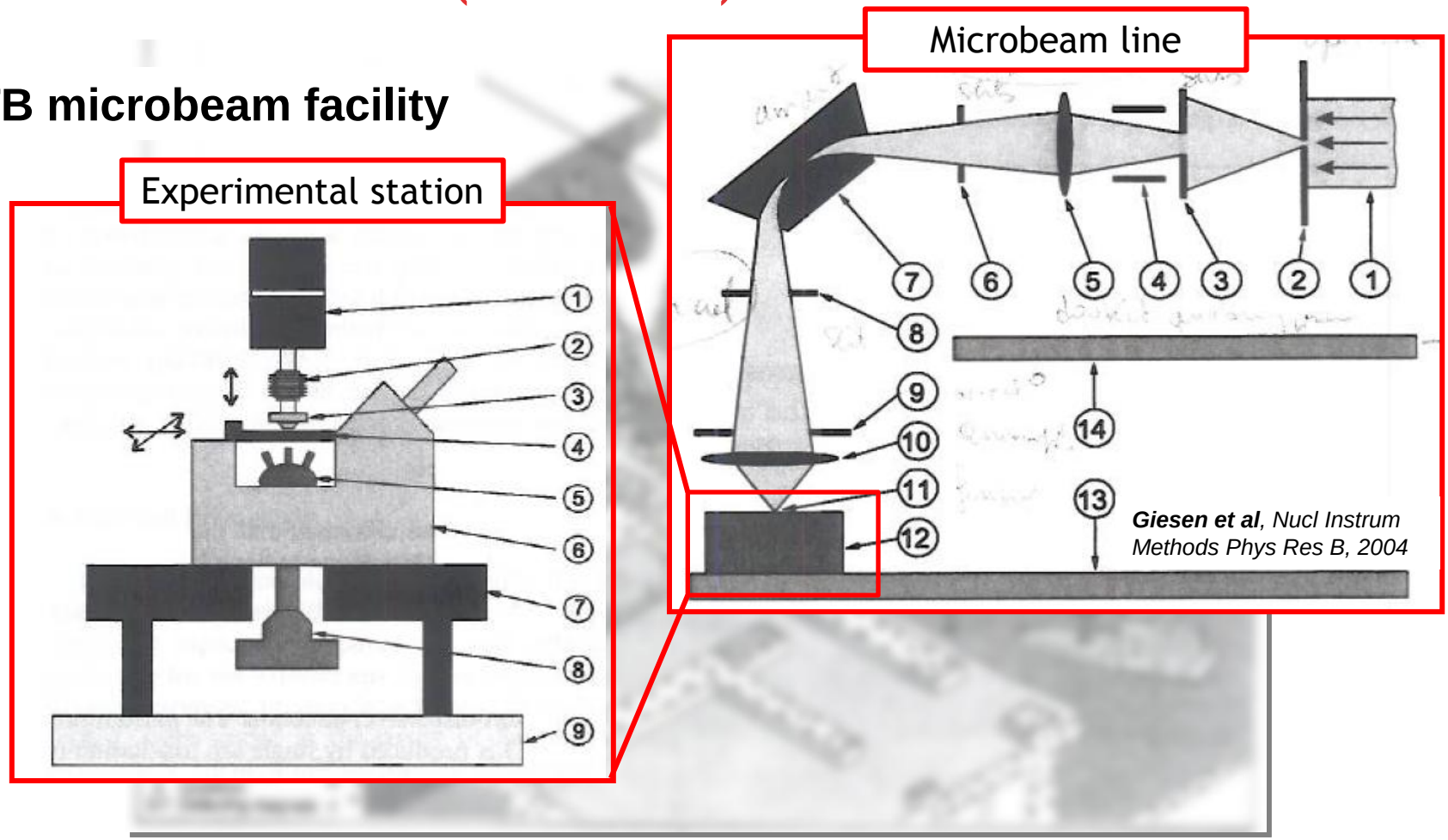

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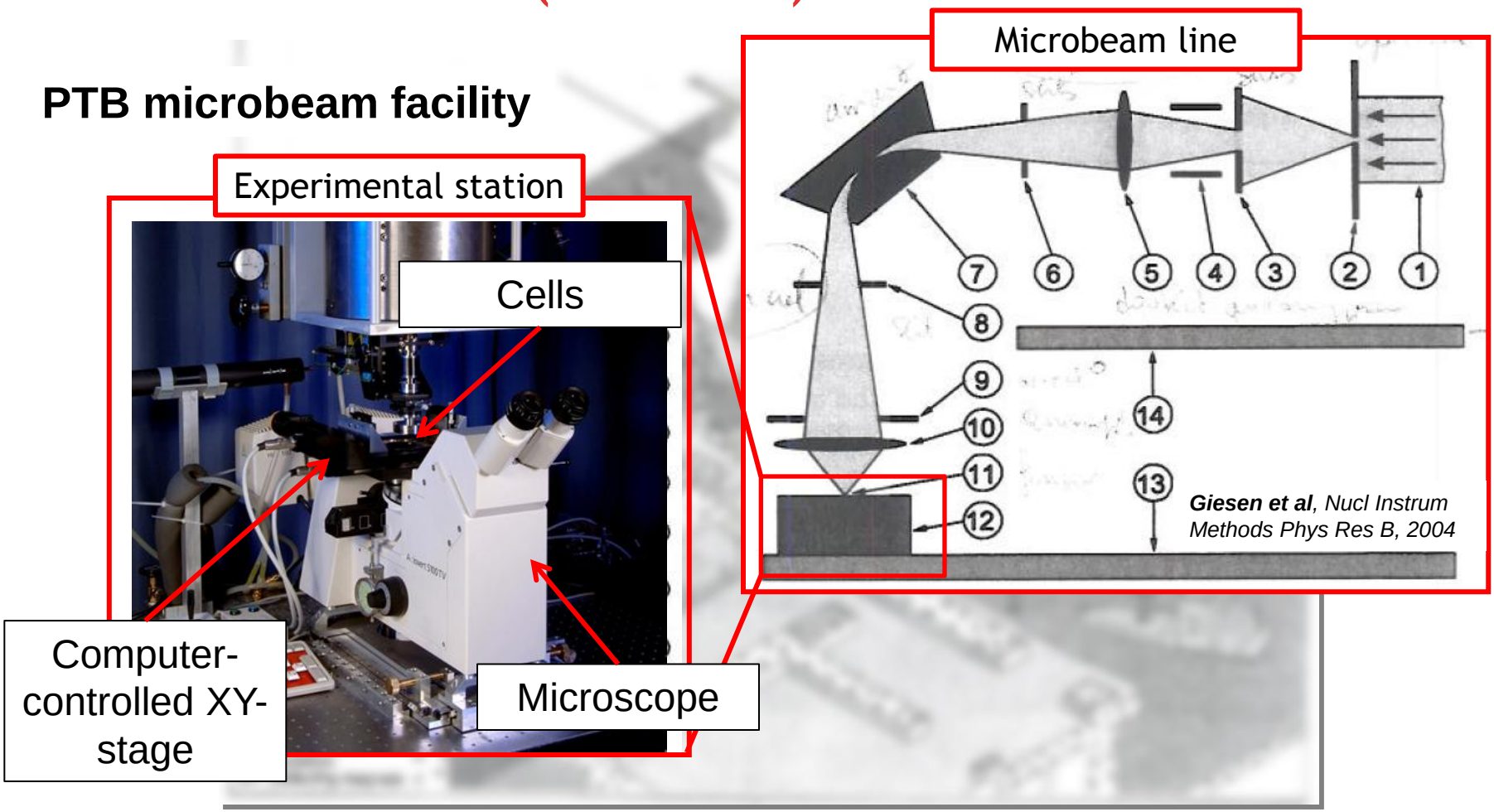
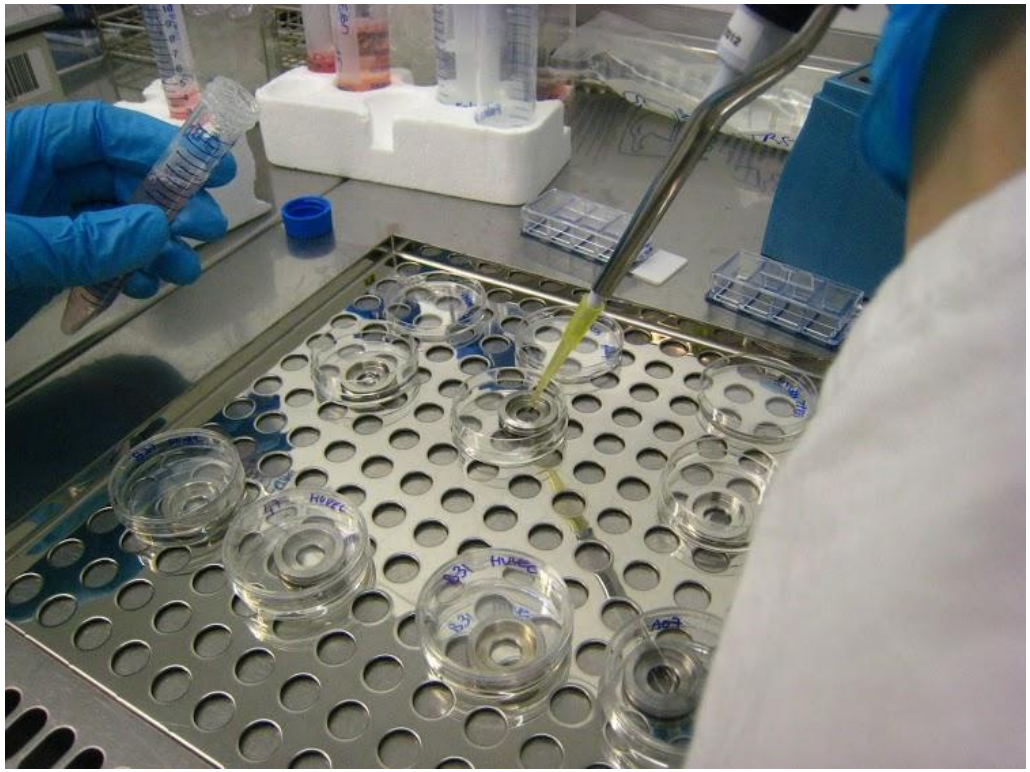
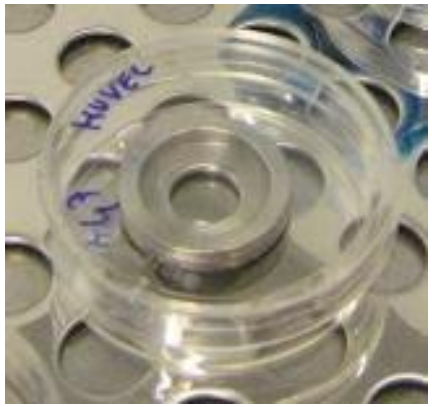


Microbeam irradiations

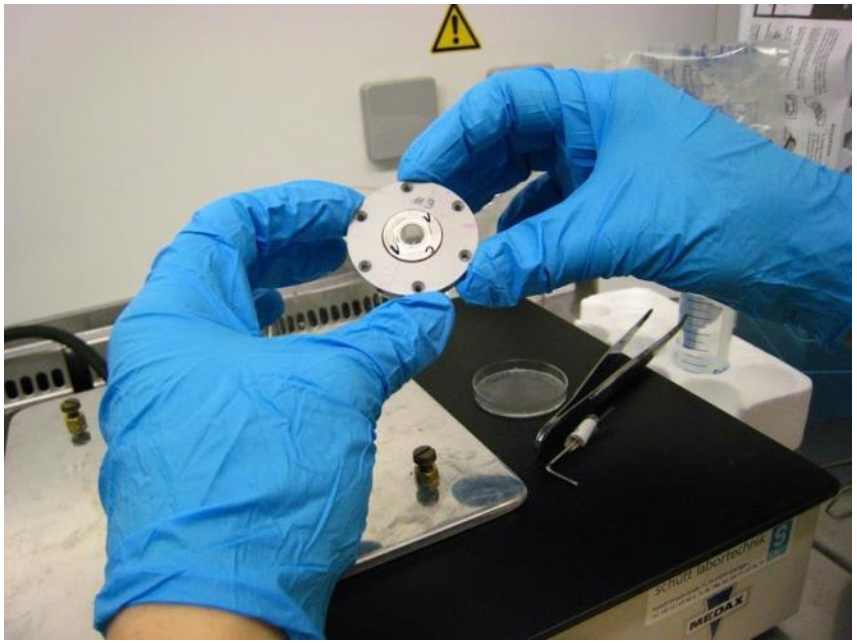
- Cell are plated in special dishes the day before





Microbeam irradiations

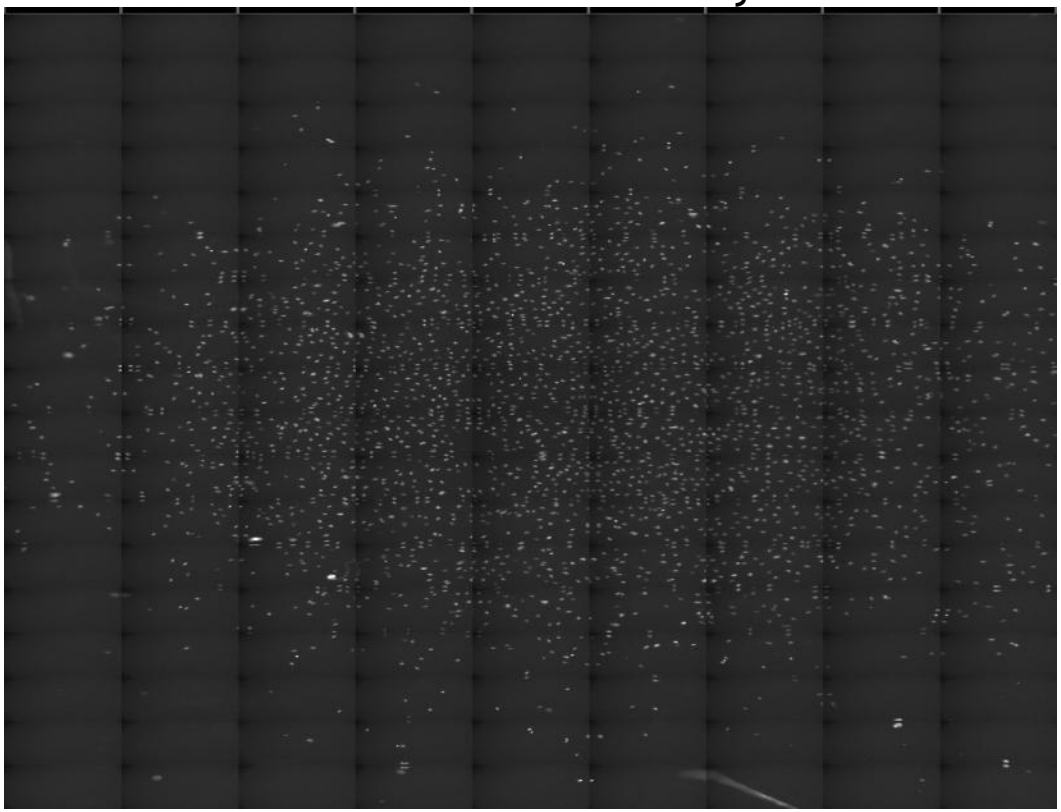
- The day of irradiation, cells are stained with Hoechst (Nucleus staining)
- Then, the dish is mounted in a special device

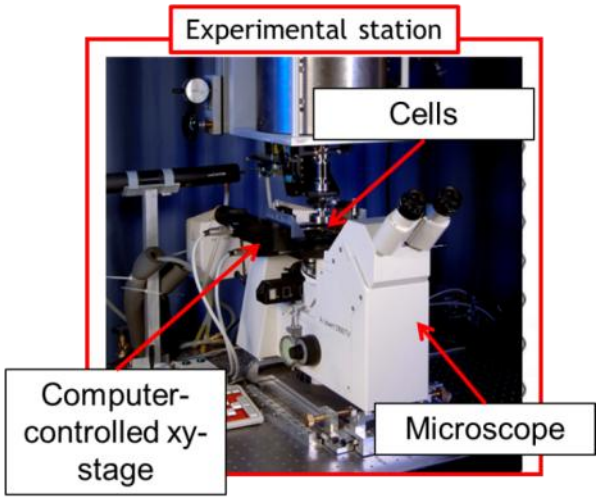


Microbeam irradiations

- Detection of cell nuclei

Scan of the cell monolayer





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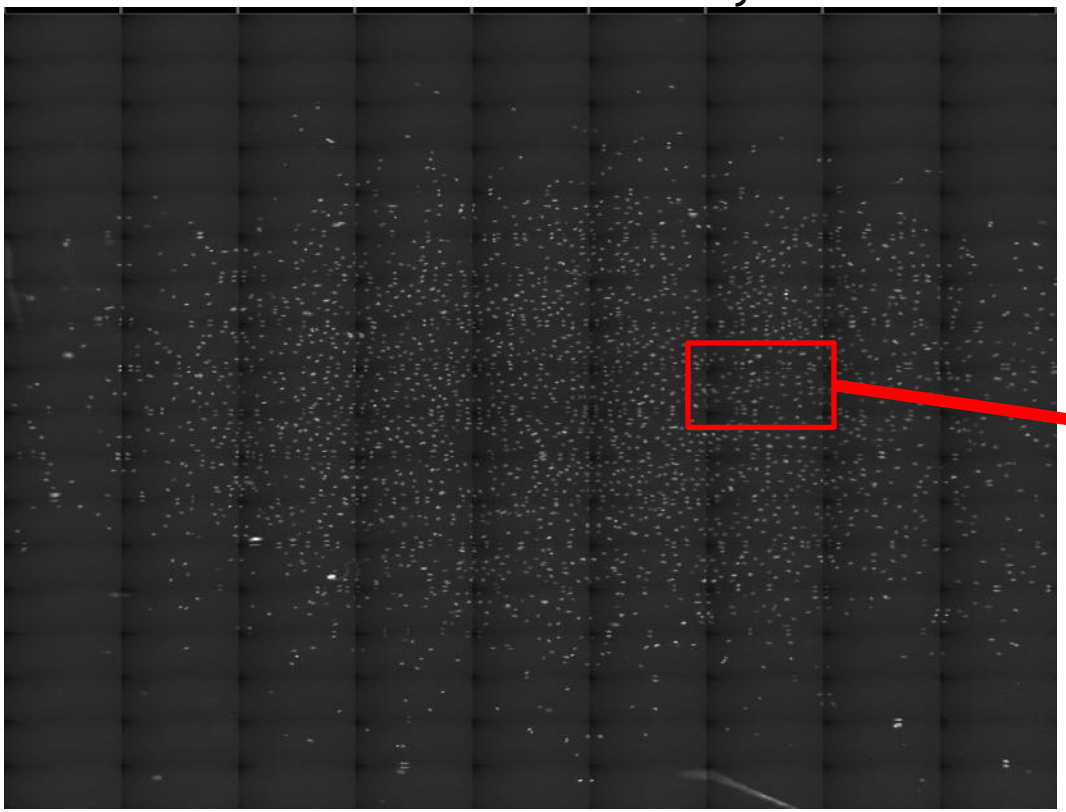
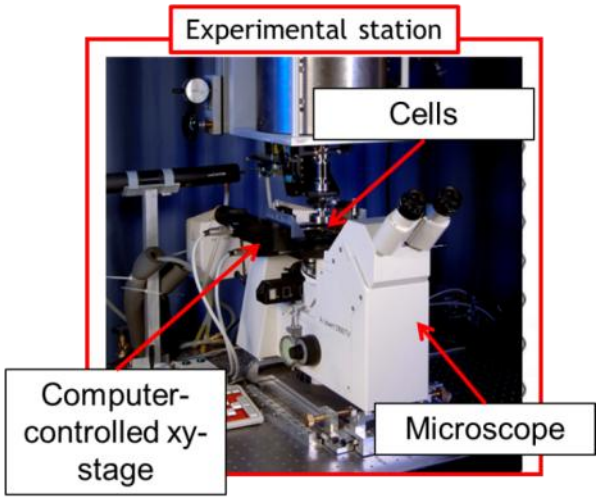
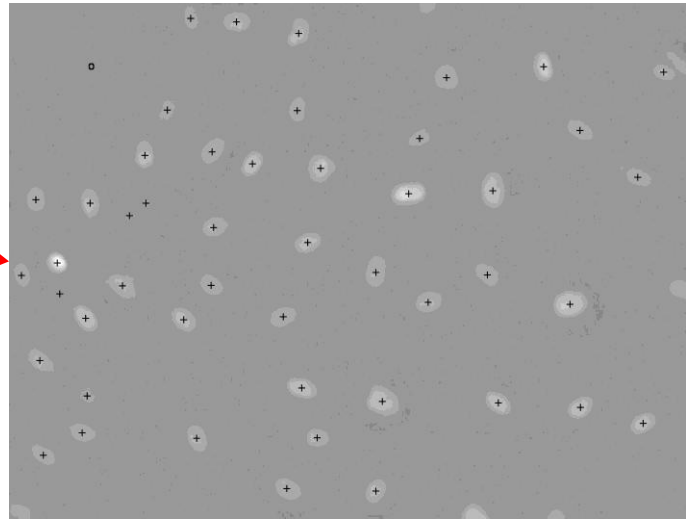


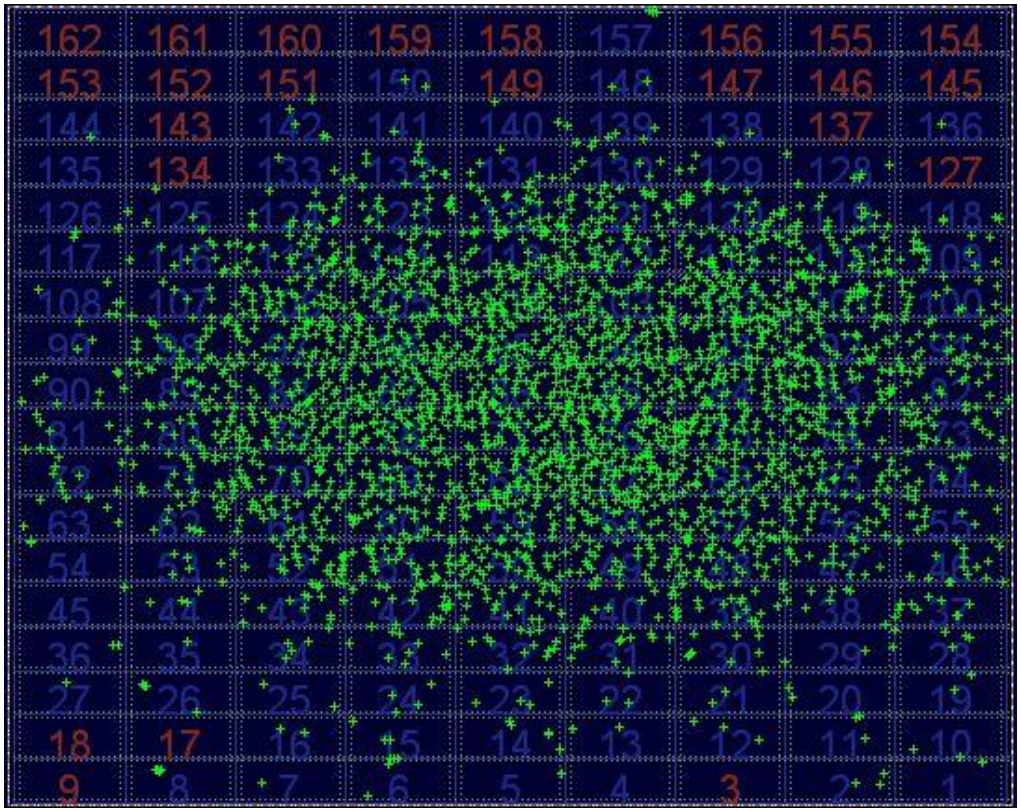


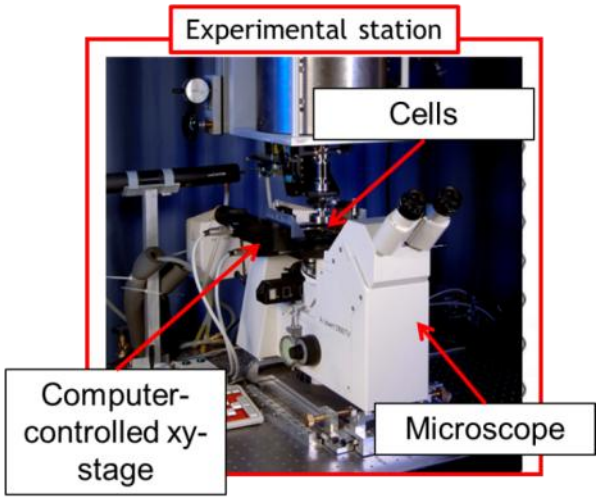


Automatic detection of the barycenter of each nucleus of the cell population

Microbeam irradiations

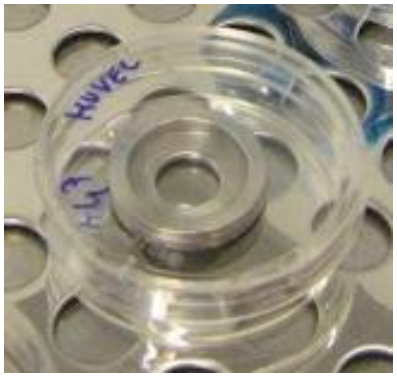
- Detection of cell nuclei



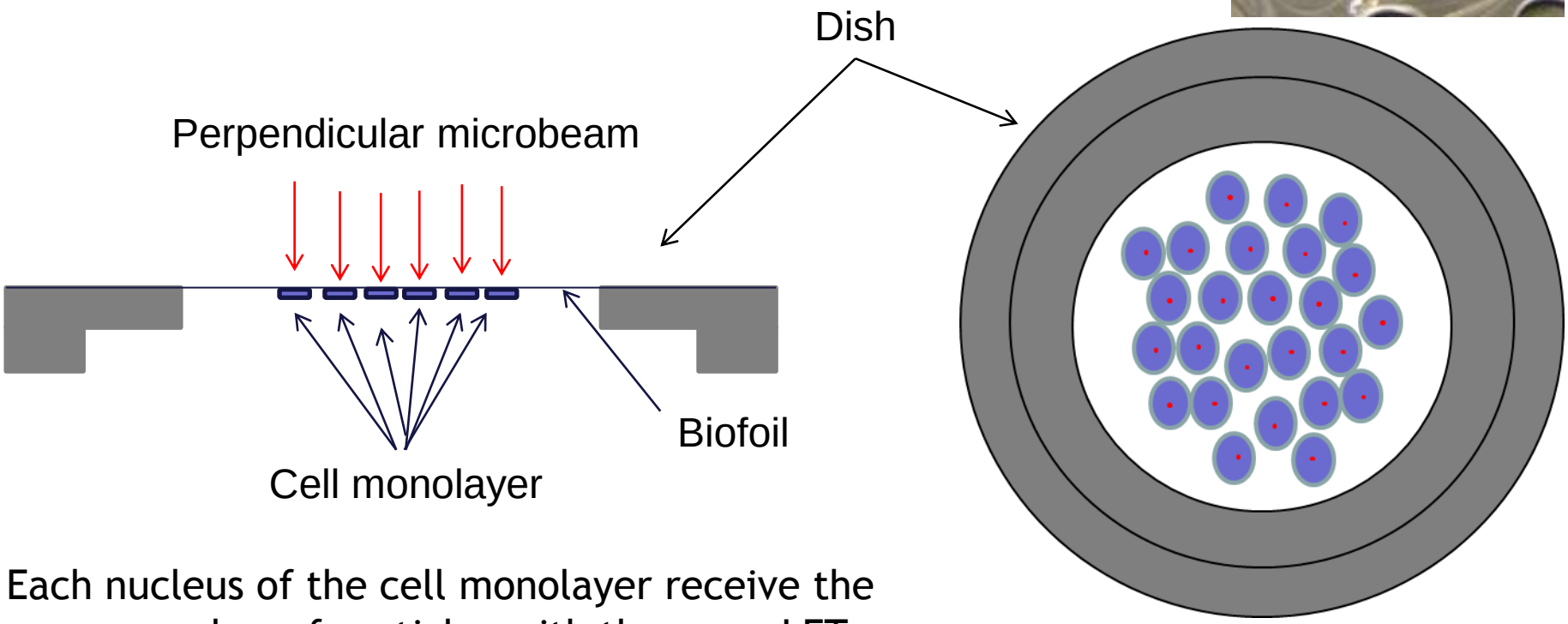


The coordinates of the “microbeam irradiation” are then computed.

Microbeam irradiations



- Geometry of the microbeam irradiations

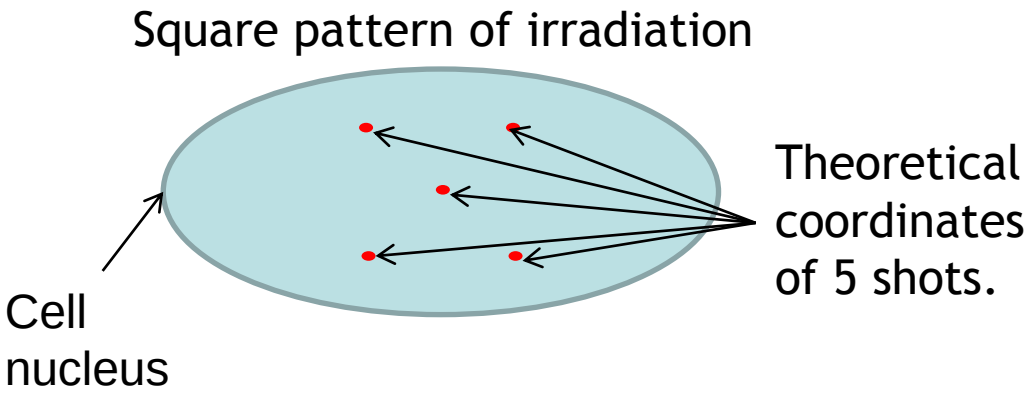


Each nucleus of the cell monolayer receive the same number of particles with the same LET.

Microbeam irradiations

- Types of irradiation already performed for “early events”

Alpha	Proton
8 MeV (160 keV/µm)	3 MeV (18 keV/µm)
20 MeV (37 keV/µm)	10 MeV (5 keV/µm)



Challenges of WP4

- To perform biological measurements that could “feed” WP5

  1. To choose relevant biological endpoints

  2. To choose relevant irradiation conditions…

Challenges of WP4

- To perform biological measurements that could “feed” WP5

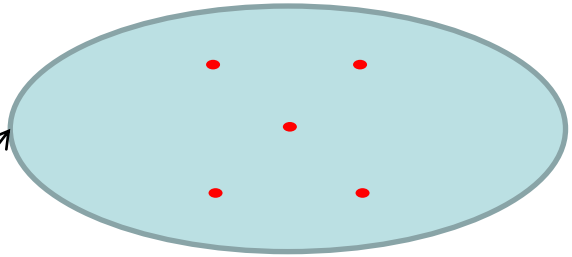
  1. To choose relevant biological endpoints
  2. To choose relevant irradiation conditions…
  3. …to be able to perform relevant measurements.

Measurements for early events

- Types of irradiation already performed for “early events”

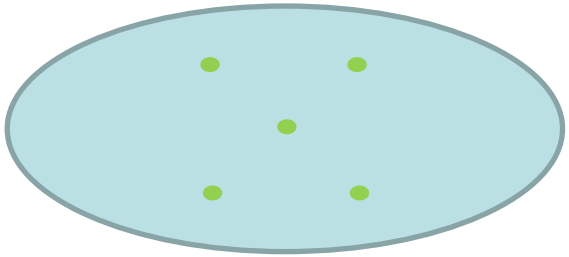
Alpha	Proton
8 MeV (160 keV/µm)	3 MeV (18 keV/µm)
20 MeV (37 keV/µm)	10 MeV (5 keV/µm)

Pattern of irradiation



Cell nucleus

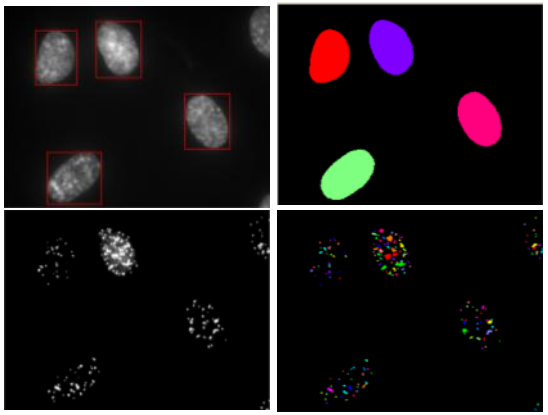
How many of resulting foci by cell nucleus ?



Measurements for early events

↗ Performed at IRSN (G. Gonon, Pa. Voisin and G. Gruel)

- Mass analysis



With the combination of
- high speed microscopy platform
- Automated image analysis

We can analyse quantity, quality and topology of foci **on a large population of nuclei (>2000)** exposed to the same irradiation condition

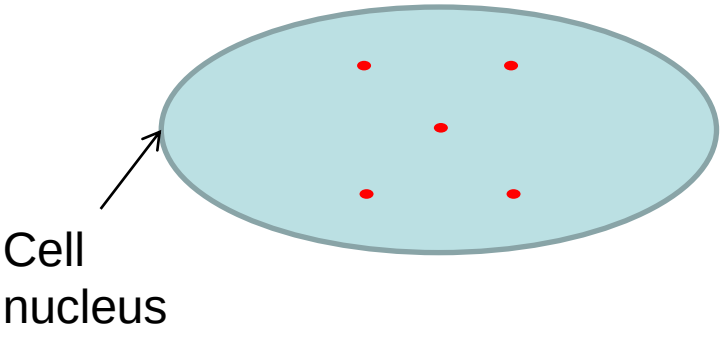
This provides a measure of the probability of the presence (or absence) of foci within a cell nucleus.

Measurements for early events

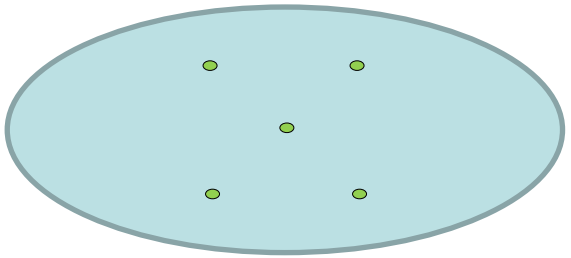
- Types of irradiation already performed for “early events”

Alpha	Proton
8 MeV (160 keV/µm)	3 MeV (18 keV/µm)
20 MeV (37 keV/µm)	10 MeV (5 keV/µm)

Pattern of irradiation



foci observed ?



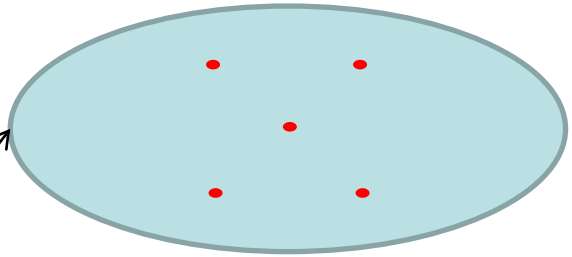
for ex: probability of hit: 5/5

Measurements for early events

- Types of irradiation already performed for “early events”

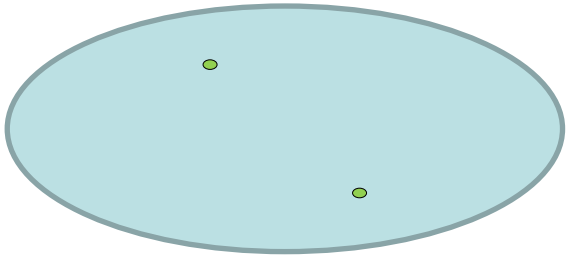
Alpha	Proton
8 MeV (160 keV/µm)	3 MeV (18 keV/µm)
20 MeV (37 keV/µm)	**10 MeV (5 keV/µm)**

Pattern of irradiation



Cell nucleus

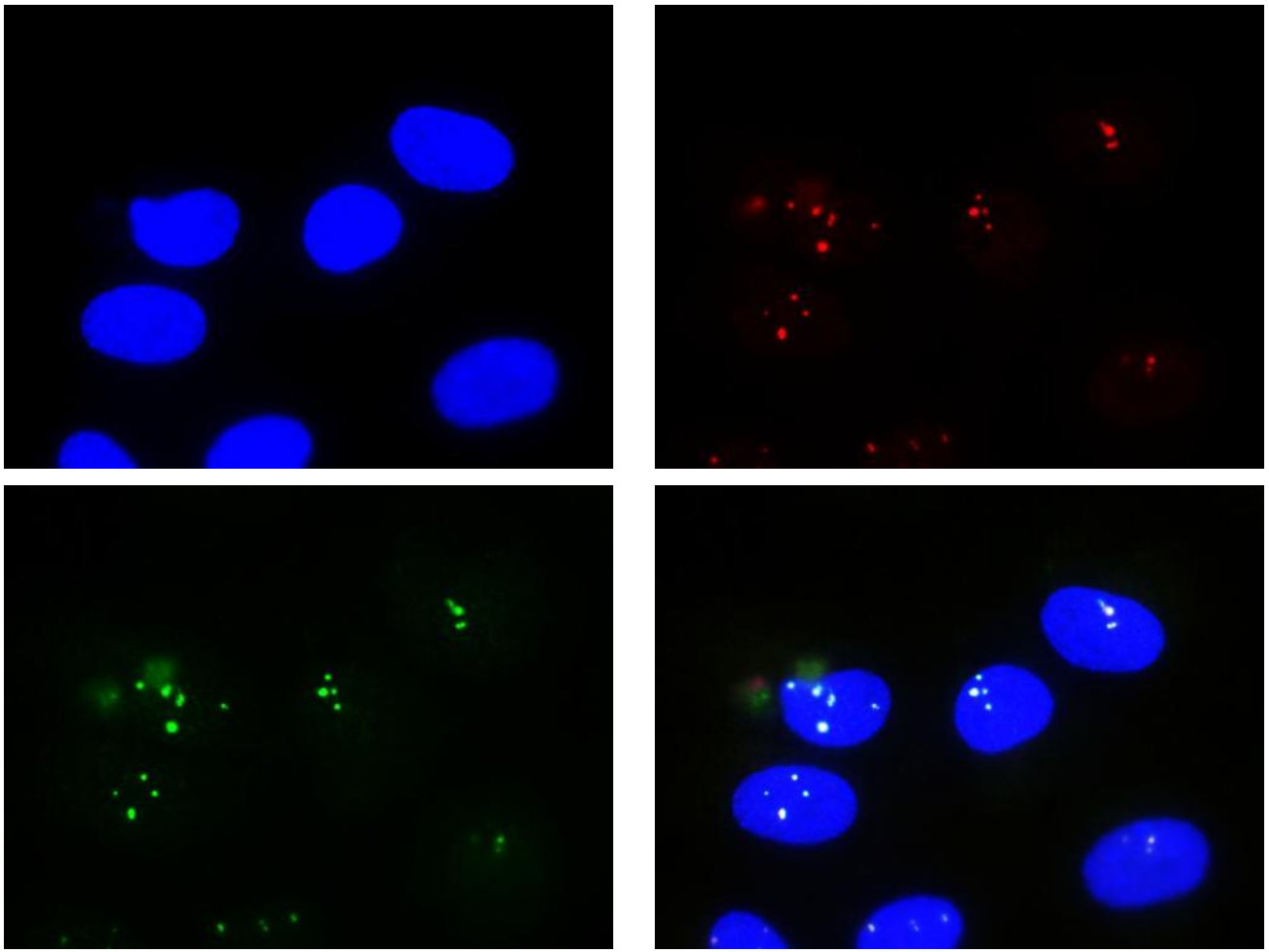
foci observed ?



for ex: probability of hit: 2/5

Measurements for early events

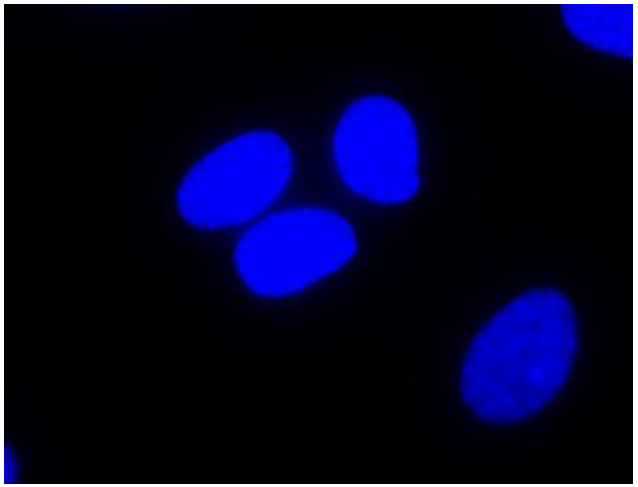
5α-irradiated dish
(20 MeV, 37keV/µm)



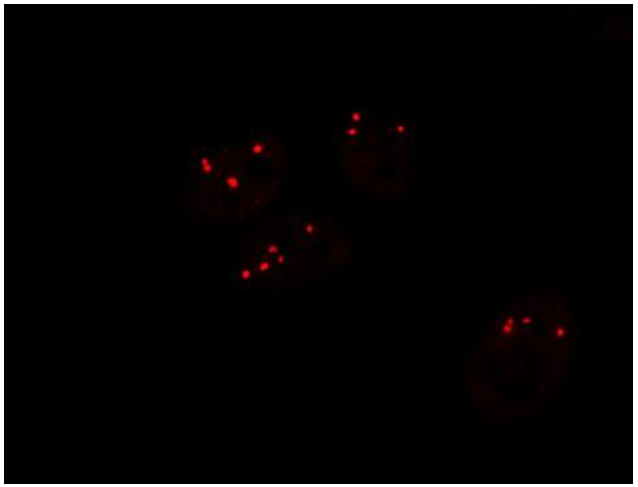
Measurements for early events

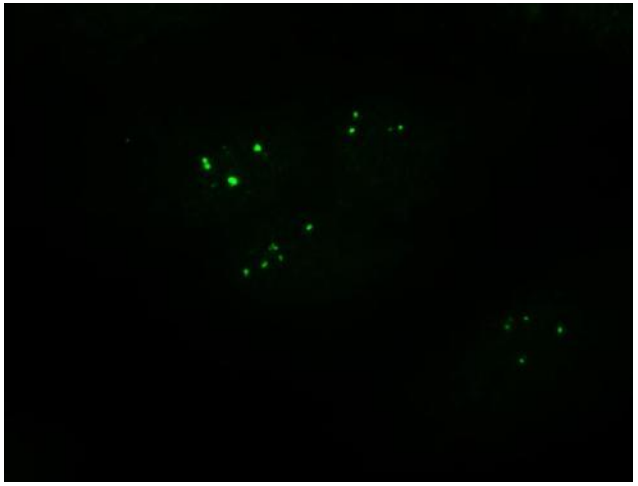
5α-irradiated dish
(20 MeV, 37keV/µm)

DAPI

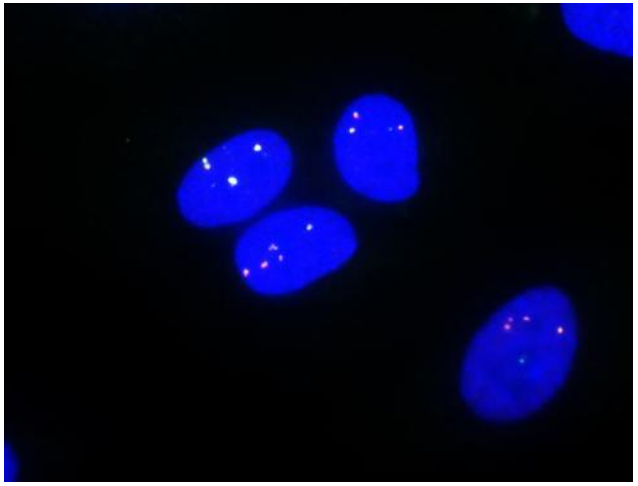


53BP1 (Tx-Red)





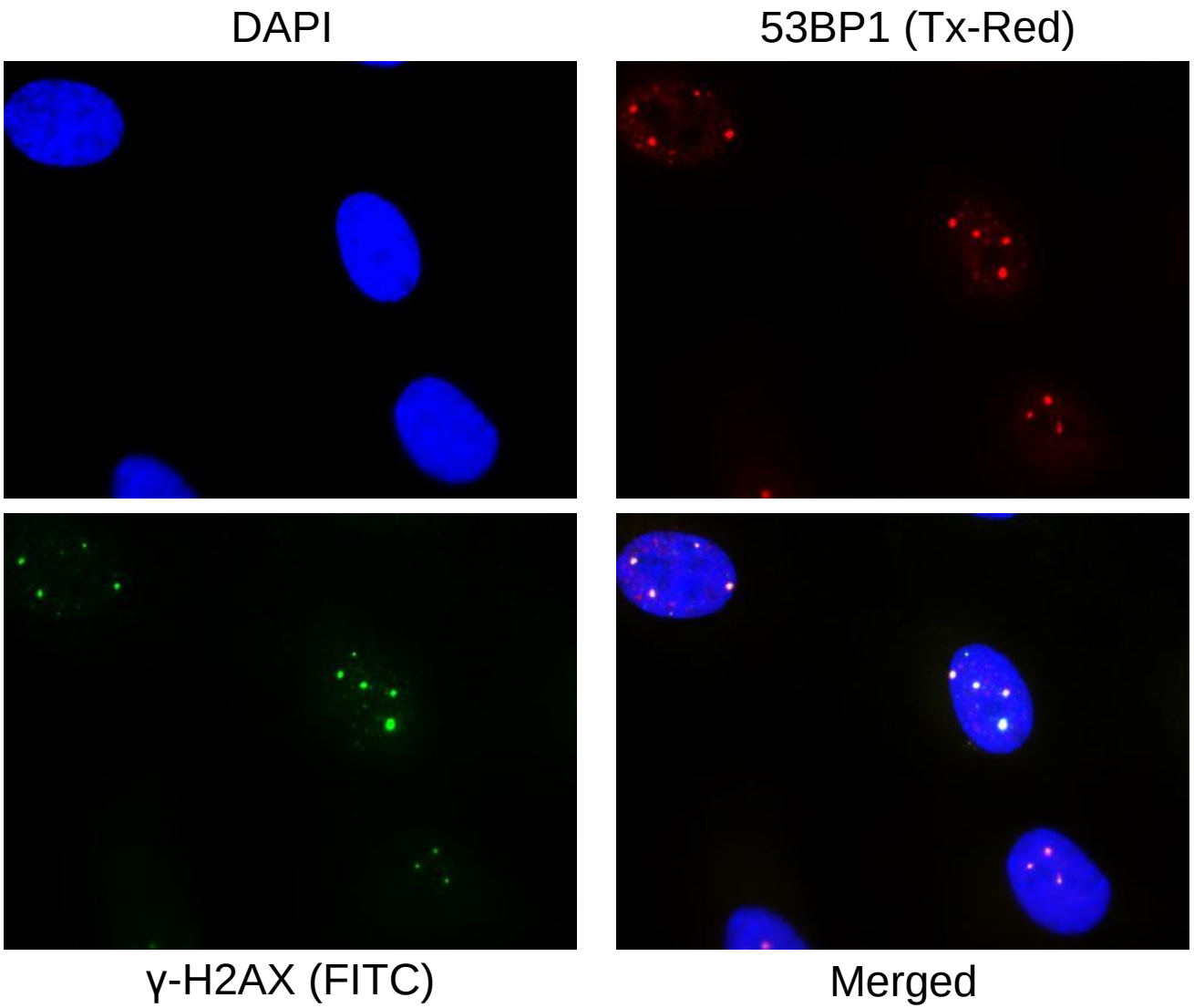
γ-H2AX (FITC)



Merged

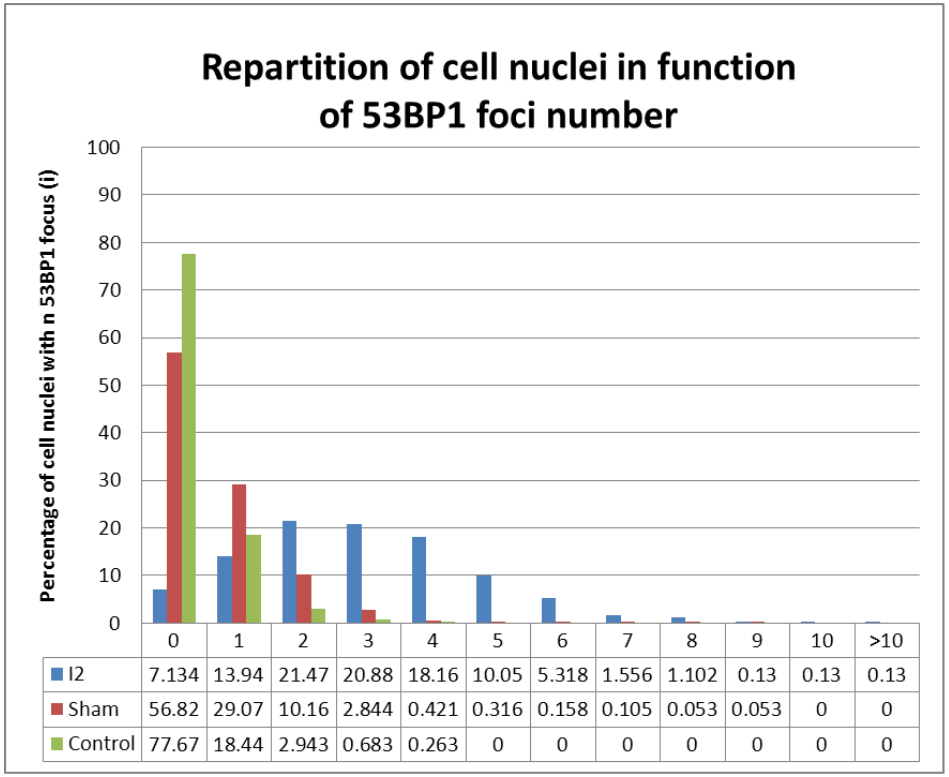
Measurements for early events

5α-irradiated dish
(20 MeV, 37keV/µm)

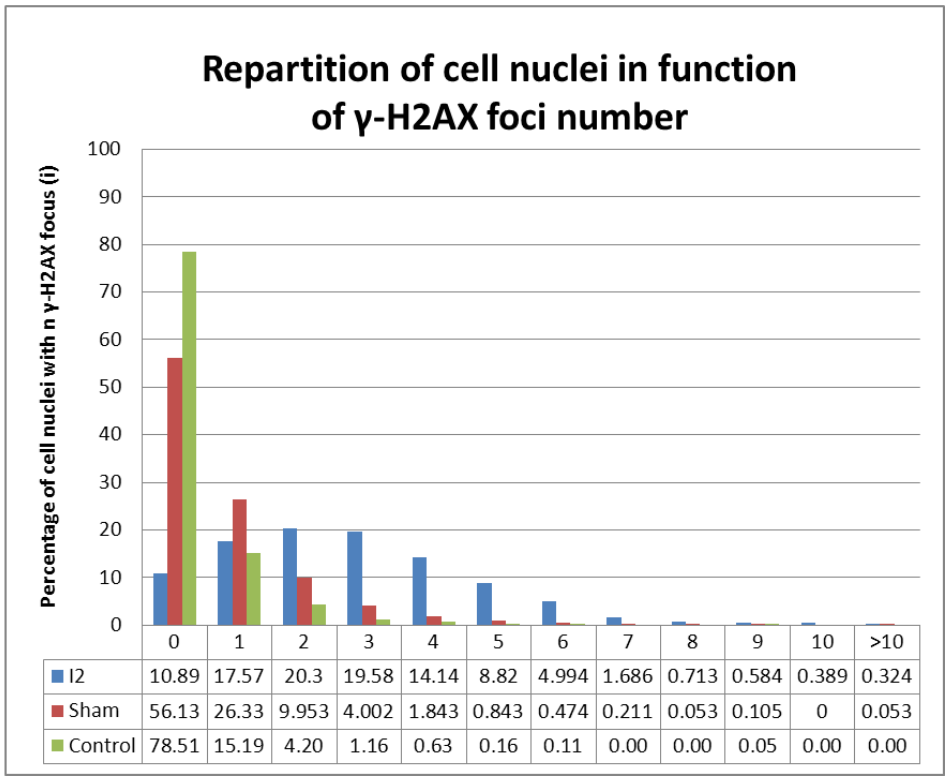


Mass Analysis

- HUVEC 5 alpha particles pattern (20 MeV, 37 keV/µm), Nov 28th, 2012 - more than 3000 analyzed



	0	1	2	3	4	5	6	7	8	9	10	>10
I2	7.134	13.94	21.47	20.88	18.16	10.05	5.318	1.556	1.102	0.13	0.13	0.13
Sham	56.82	29.07	10.16	2.844	0.421	0.316	0.158	0.105	0.053	0.053	0	0
Control	77.67	18.44	2.943	0.683	0.263	0	0	0	0	0	0	0



	0	1	2	3	4	5	6	7	8	9	10	>10
I2	10.89	17.57	20.3	19.58	14.14	8.82	4.994	1.686	0.713	0.584	0.389	0.324
Sham	56.13	26.33	9.953	4.002	1.843	0.843	0.474	0.211	0.053	0.105	0	0.053
Control	78.51	15.19	4.20	1.16	0.63	0.16	0.11	0.00	0.00	0.05	0.00	0.00

Challenges of WP4

- To perform biological measurements that could “feed” WP5

  1. To choose relevant biological endpoints
  2. To choose relevant irradiation conditions…
  3. …to be able to perform relevant measurements.

Challenges of WP4

- To perform biological measurements that could “feed” WP5

1. To choose relevant biological endpoints
2. To choose relevant irradiation conditions…
3. …to be able to perform relevant measurements.
4. To take into account the “uncertainties” of irradiation conditions and biological models

Considerations of “uncertainties”

- **Biological parameters**
  - Foci background

Considerations of “uncertainties”

Biological parameters

- Foci background
  - the stage of the cell cycle

Considerations of “uncertainties”

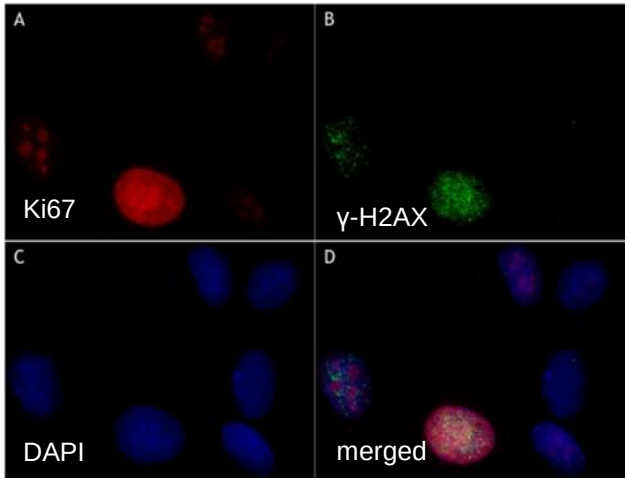
Biological parameters

Foci background

- the stage of the cell cycle

Cells in phase S, G_2 or M can be excluded from the analysis using a combination of several parameters measured on each nucleus as:

- Integrated fluorescence of DAPI (related to DNA quantity)
  and
- Integrated fluorecence of FITC (related to phosphorylation of H2AX due to DNA synthesis)



Considerations of “uncertainties”

Biological parameters

Foci background

- ~~the stage of the cell cycle~~ – SOLVED -
- Interaction between Hoechst staining (nuclei stain) and UV-scan

Considerations of “uncertainties”

Biological parameters

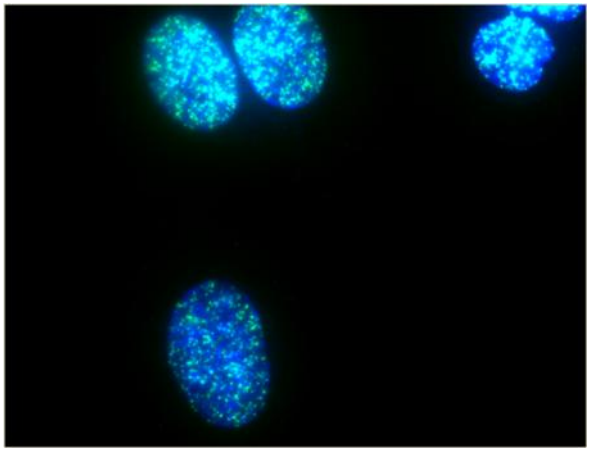
Foci background

- ~~the stage of the cell cycle~~ – SOLVED -
- Interaction between Hoechst staining (nuclei stain) and UV-scan

Illumination of live cells labelled with Hoechst produces γ-H2Ax foci

This was observed when Hoechst was excited with a **mercury lamp** combined with adequate filter.

This effect depends on **time** of illumination and **concentration** of Hoechst



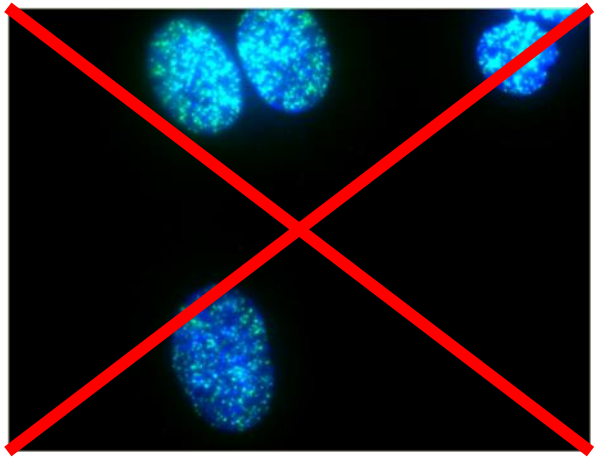
Considerations of “uncertainties”

Biological parameters

Foci background

- ~~the stage of the cell cycle~~ – SOLVED -
- Interaction between Hoechst staining (nuclei stain) and UV-scan

The replacement of the mercury lamp with new LED light source (Lumencor Spectra X) seems to totally remove the effect.



Considerations of “uncertainties”

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Foci background

- ~~the stage of the cell cycle~~ – SOLVED -
- ~~Interaction between Hoechst staining (nuclei stain) and UV-scan~~ – SOLVED-

Considerations of “uncertainties”

Biological parameters

- Foci background
  - ~~the stage of the cell cycle~~ – SOLVED -
  - ~~Interaction between Hoechst staining (nuclei stain) and UV-scan~~ – SOLVED-

Beam parameters

- Beam size
- Scintillator noise

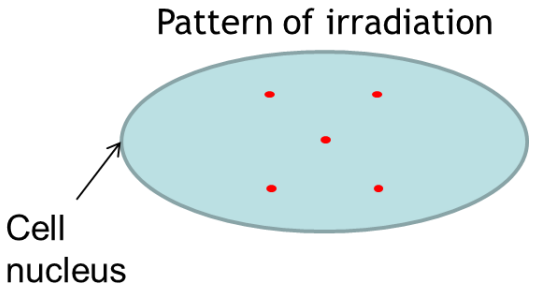
Considerations of “uncertainties”

Biological parameters

- Foci background
  - ~~the stage of the cell cycle~~ – SOLVED -
  - ~~Interaction between Hoechst staining (nuclei stain) and UV-scan~~ – SOLVED-

Beam parameters

- Beam size
- Scintillator noise

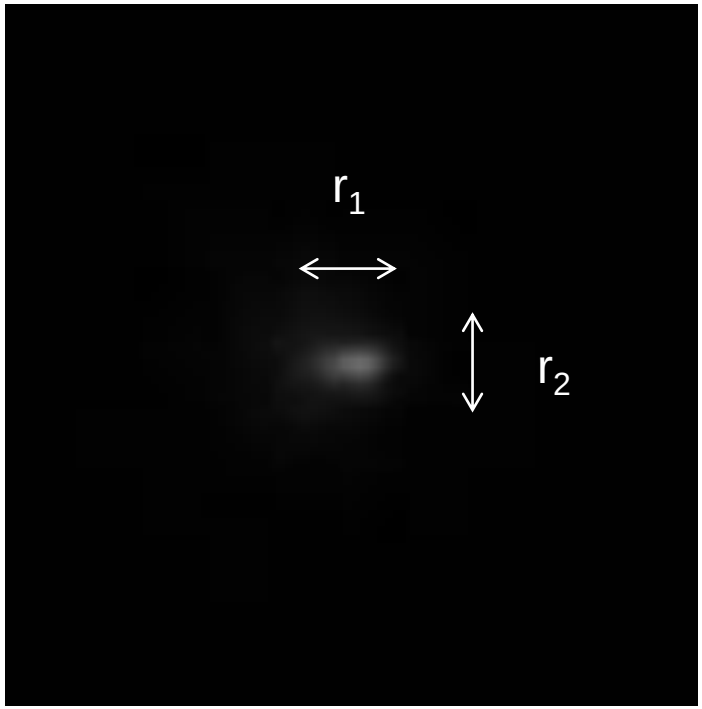


Could lead to the deformation of the theorical pattern of irradiation

Considerations of “uncertainties”

Beam parameters

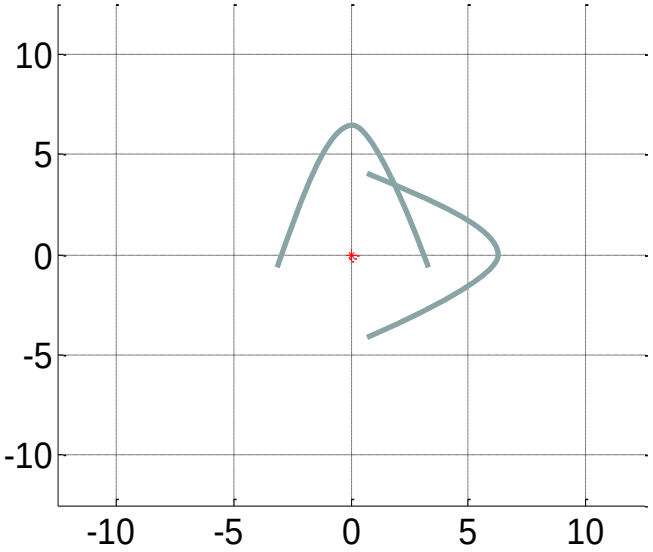
- **Beam size**: straddling effect and beam focalization
  - This could be estimated => the beam shape is measured for each microbeam set-up.



Considerations of “uncertainties”

Beam parameters

- **Beam size**: straddling of different layers and beam focalization
  - Coordinates of each hit of the pattern is a function of a Gaussian distribution in X and Y (μ=0, σ=r1/2.32 and σ=r2/2.32)



Considerations of “uncertainties”

Beam parameters

Scintillator Noise

- The noise of scintillator could be interpreted as a particle. This leads to the closure of the beam shutter even if no particle has been emitted. As a consequence no particle can be emitted instead of 1.

- At the contrary, the path of one particle through the scintillator could be interpreted as a noise. This leads to the non-closure of the beam shutter leaving another particle coming. As a consequence 2 particles can be emitted instead of 1.

Signal of the scintillator is saved for each irradiation: the overlap between background noise and real signal is estimated.

Considerations of “uncertainties”

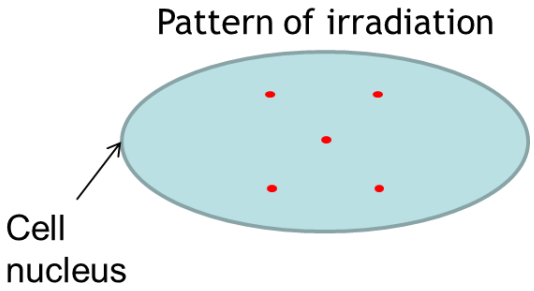
↗ Beam parameters

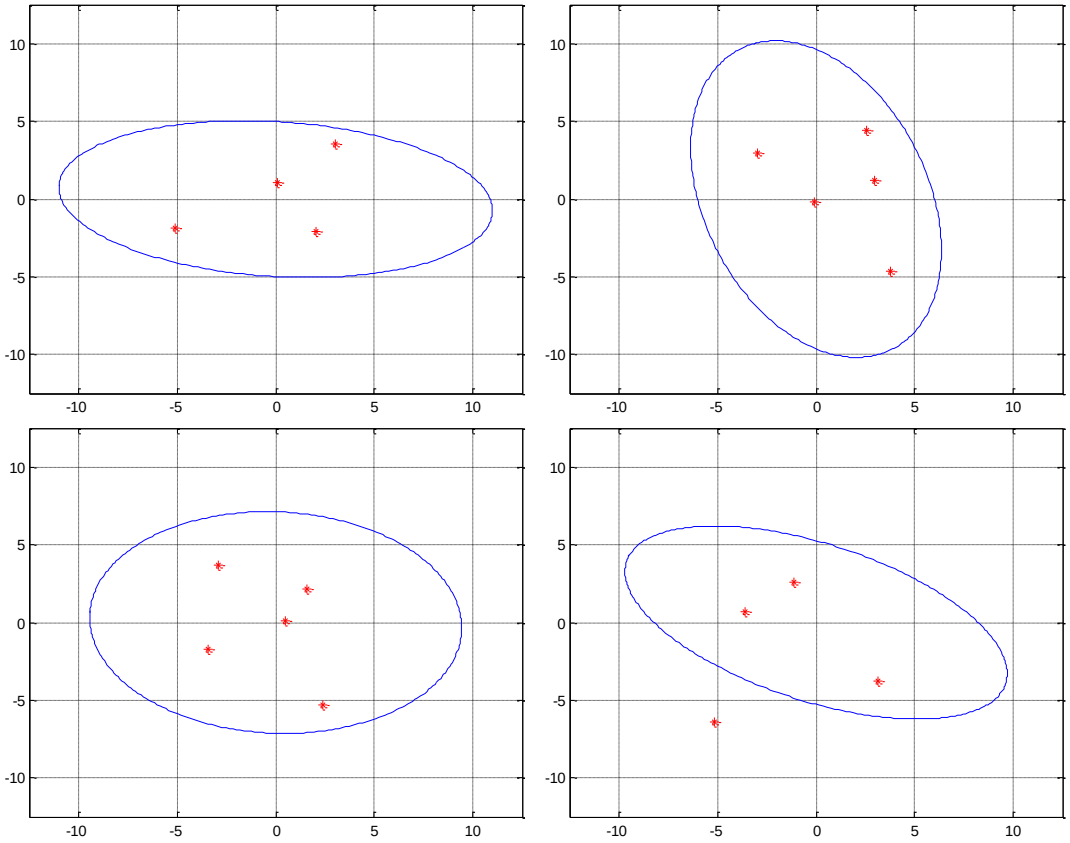
- We develop a script with Matlab to simulate how
  - The beam size
  - The scintillator noise
  - The nucleus dimension

  can affect the initial pattern of irradiation and then the foci distributions observed

Considerations of “uncertainties”

↗ Beam parameters





Considerations of “uncertainties”

Biological parameters

- Foci background
  - ~~the stage of the cell cycle~~ – SOLVED -
  - ~~Interaction between Hoechst staining (nuclei stain) and UV-scan~~ – SOLVED-

Beam parameters

- Beam size
- Scintillator noise

- Effects can be estimated -

Conclusion

It seems possible to remove at least a portion of the noise of the observed foci distribution among cell population.

The objective is to access to an accurate estimation of the probability of interaction between a given particle with a given LET and DNA.

With these kind of corrected measurements, it will be possible to compare biological results with Monte Carlo simulation by benchmarking.

Thank you