Challenges in radiobiology research with heavy ion beam in Poland

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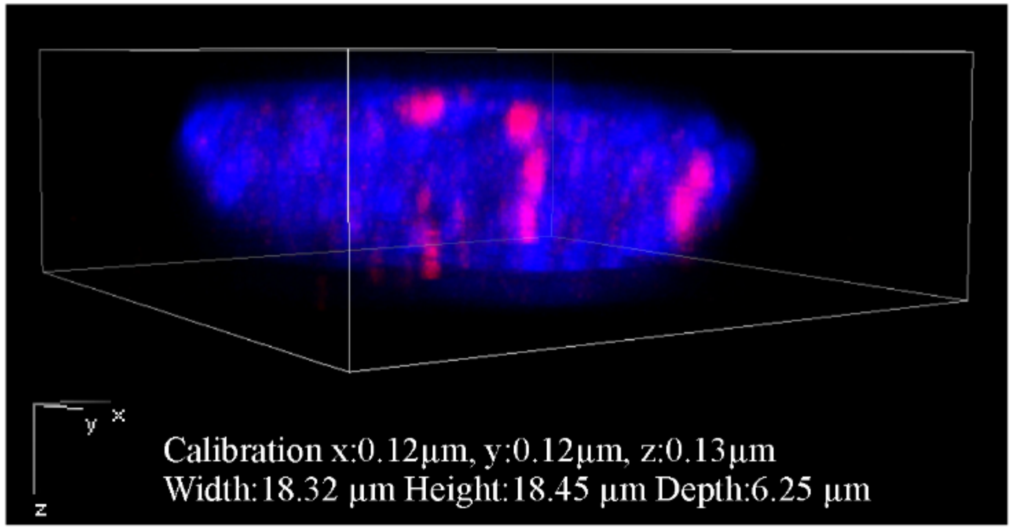
HIL Heavy Ion Laboratory

UNIVERSITY OF WARSAW

Conference "Przyszłość fizyki jądrowej niskich energii w Polsce a rozwój krajowej infrastruktury badawczej", 14-15 January 2019

Radiobiology



Research in radiation biology **develops knowledge about the effects of radiation** in cells, tissues, organs and organisms.

Discoveries and fundamental biological insights realized through these studies has led, for example, to innovation and progress in radiation oncology.

Basic insights from radiobiology can be applied to societally important topics such as carcinogenesis risk estimation from medical, occupational, or space travel radiation exposure and the development of medical treatments for radiation injury.

D.G. Kirsch et al., J. Natl. Cancer Inst. 110(4) (2018) 329

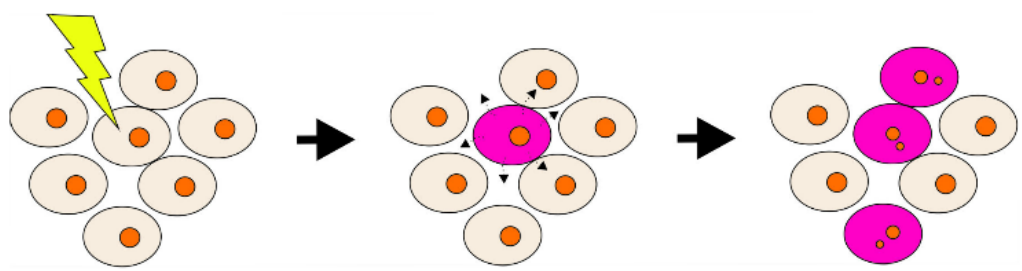
'Non-targeted' effects

In radiobiology 'target theory' is generally accepted.

It assumes that **direct** damage to the DNA helix is necessary to induce critical effects (either through direct ionization of the DNA, or through the action of reactive radical species from the ionization of water close to the DNA molecule).

But there is a growing interest in so-called **'non-targeted'** effects.

Non-targeted effects are those where cells are seen to respond **indirectly** to ionizing radiation and are in conflict with the conventional view of cellular radiation damage.



Studies of 'non-targeted' effects are benefiting most from the use of micro-irradiation techniques.

M. Folkard et al., Nucl. Instrum. Methods Phys. B 231 (2005) 189

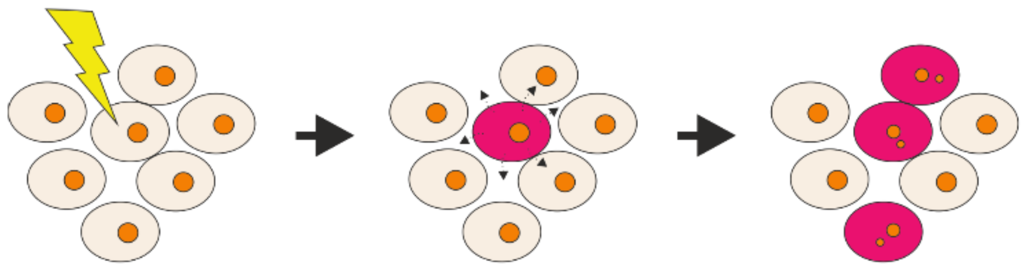
1947 r. - Kotval and Gray showed that α particles passing close (but not directly) through the chromatin induce chromosomal aberrations.

J.P. Kotval, L.H. Gray, Journal of Genetics, 48:135–154, 1947.

1968 r. - Plasma from in vitro irradiated blood transferred to lymphocytes from healthy donors causes chromosomal damage in them.

D. Scott, Cell Proliferation, 2:295–305, 1969.

Bystander effect



1992 r. - Nagasawa and Little irradiated Chinese Hamster Ovary (CHO) cells with a very low dose (0.3-2.5 cGy) of high-LET radiation, and observed sister chromatid exchanges in about 30% of the population, while directly irradiated was less than 1% of cells.

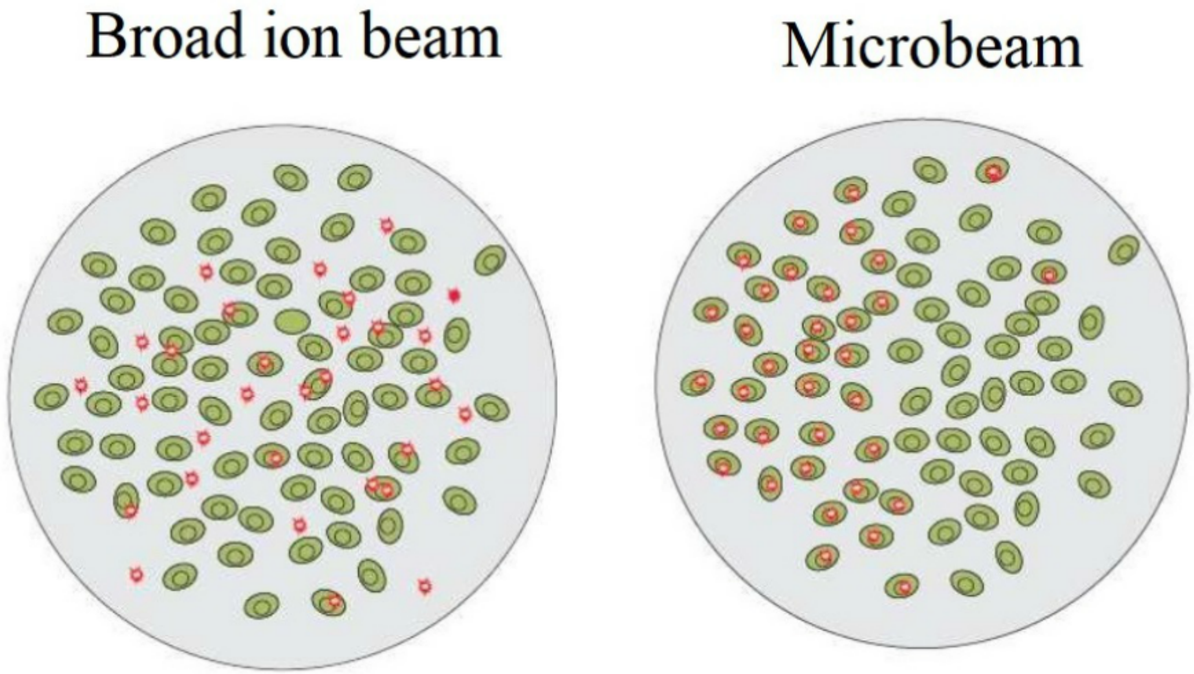
H. Nagasawa, J.B. Little, Cancer Research, 52:6394–6396, 1992.

1997 r. - Mothersill and Seymour observed that the medium in which cells were irradiated could reduce the survival of non-irradiated cells

C. Mothersill, C. Seymour. International Journal of Radiation Biology, 71(4):421–427, 1997.

1998 r. - Azzam et al. showed that signals can be transmitted via intercellular gap junctions between irradiated and non-irradiated cells.

E.I. Azzam et al. Radiation Research, 150(5):497–504, 1998.



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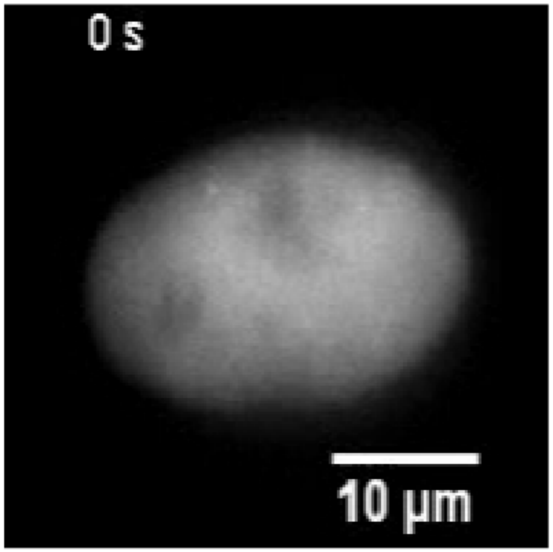
In radiobiology cellular experiments with **broad ion beam**, regardless of its horizontal or vertical orientation, **the number of ion tracks** registered in individual cell **varies over the population** of cells due to Poisson distribution.

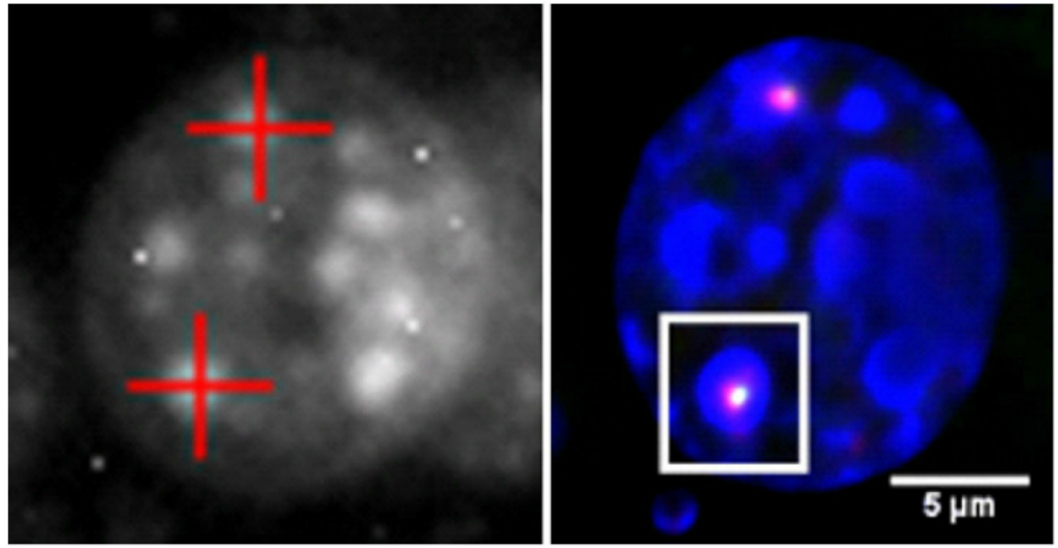
It is a serious problem for high-LET ions, where at low doses the fraction of cells in which no ion track was registered can be very high.

To overcome the problem and **target single cell** with a **predefined number of particles** the **microbeams** were designed in the last decade of the past century.

Single-ion microbeam facilities in Europe



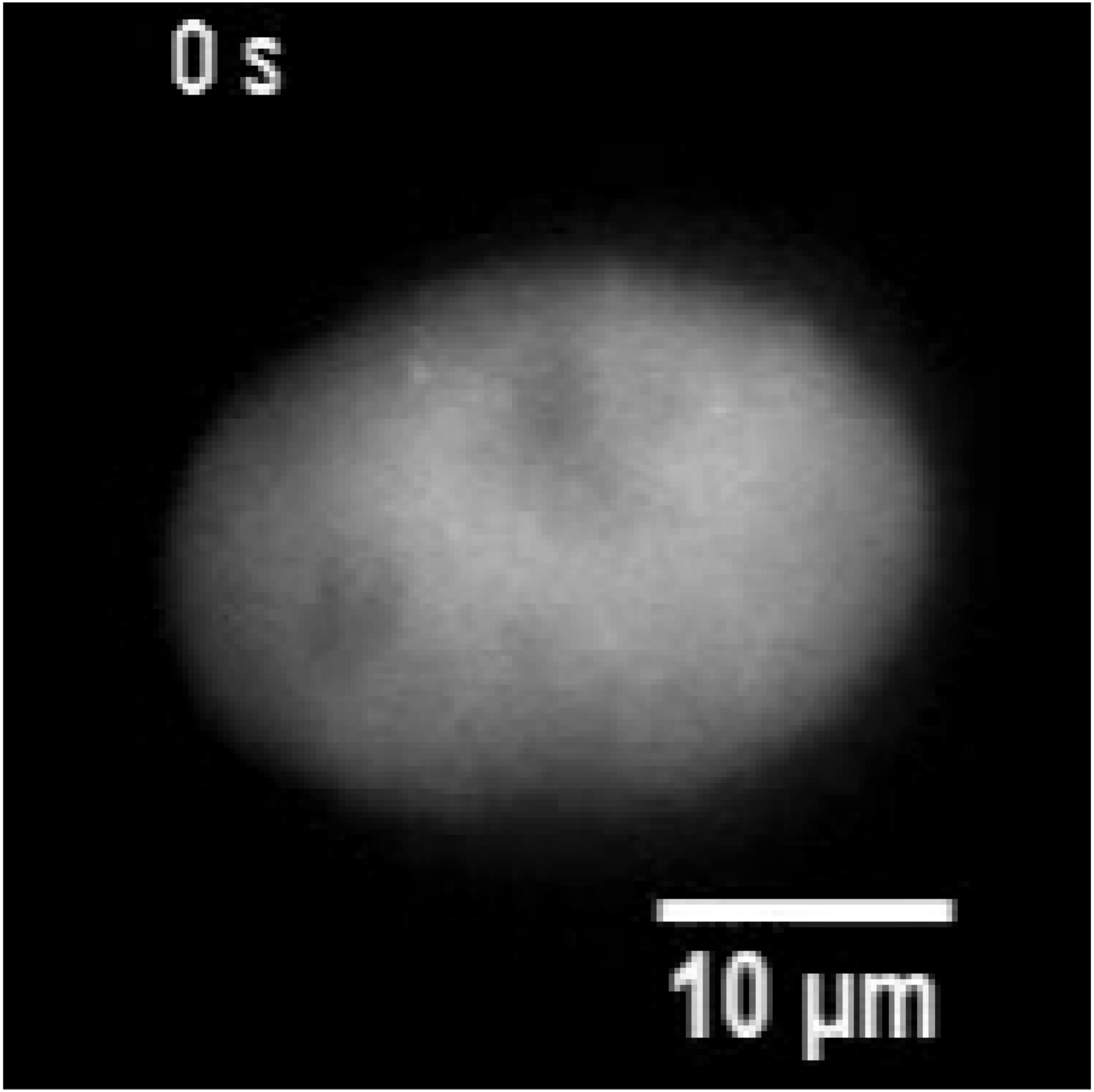
S. Bourret et al. Nuclear Instruments and Methods in Physics Research B 325, 27–34 (2014)



M.Durante, A.A.Friedl, Radiat. Environ. Biophys. 50:335–338 (2011)

Microbeam Facility	***Radiation type Energy/LET***	***Beam formation***	***Beam size on cell***	***Cell recognition***
Univ. of Surrey Guilford, **UK**	p, α, heavy ions	focusing	0.01 µm (in vaccum)	Fluorescence-based system Automated
Queen's Univ. Belfast, Northern Ireland, **UK**	X-ray 0.3-4.5 keV	zone plate	<1 µm	Fluorescence-based system Automated
GSI Darmstadt, **Germany**	from α to U-ions up to 11.4 MeV/n	focusing	0.5 µm	Fluorescence-based system Automated
LIPSION Leipzig, **Germany**	p, $^4He^{+,++}$ up to 3 MeV	focusing	0.5 µm	Unstained-cell recognition system Automated
SNAKE Munchen, **Germany**	from p to heavy ions 2-1000 keV/ µm	focusing	0.5 µm	Unstained-cell recognition system
PTB Braunschweig, **Germany**	p, α 3-200 keV/µm	focusing	<1 µm	Fluorescence-based system Automated
CEA-LPS Saclay, **France**	p, $^4He^{+}$ up to 3.75 MeV	microcollimation	10 µm	Fluorescence-based system Automated
CENBG Bordeaux, **France**	p, α up to 3.5 MeV	focusing	10 µm	Fluorescence-based system Automated
Lund NMP Lund, **Sweden**	p up to 3 MeV	focusing	5 µm	Unstained-cell recognition system Automated, in development
INFN-LNL Legnaro, **Italy**	p, $^3He^{+,++}$, $^4He^{+,++}$ 7-150 keV/µm	microcollimation	10 µm	Unstained-cell recognition system Semi-automated Automated, in development
IFJ Cracow, **Poland**	p up to 2.5 MeV	focusing	12 µm	Fluorescence-based system Automated
	X-ray 4.5 keV	mirrors	5 µm	

Based on: S. Gerardi J.Radiat.Res., 50:Suppl. A13-A20 (2009)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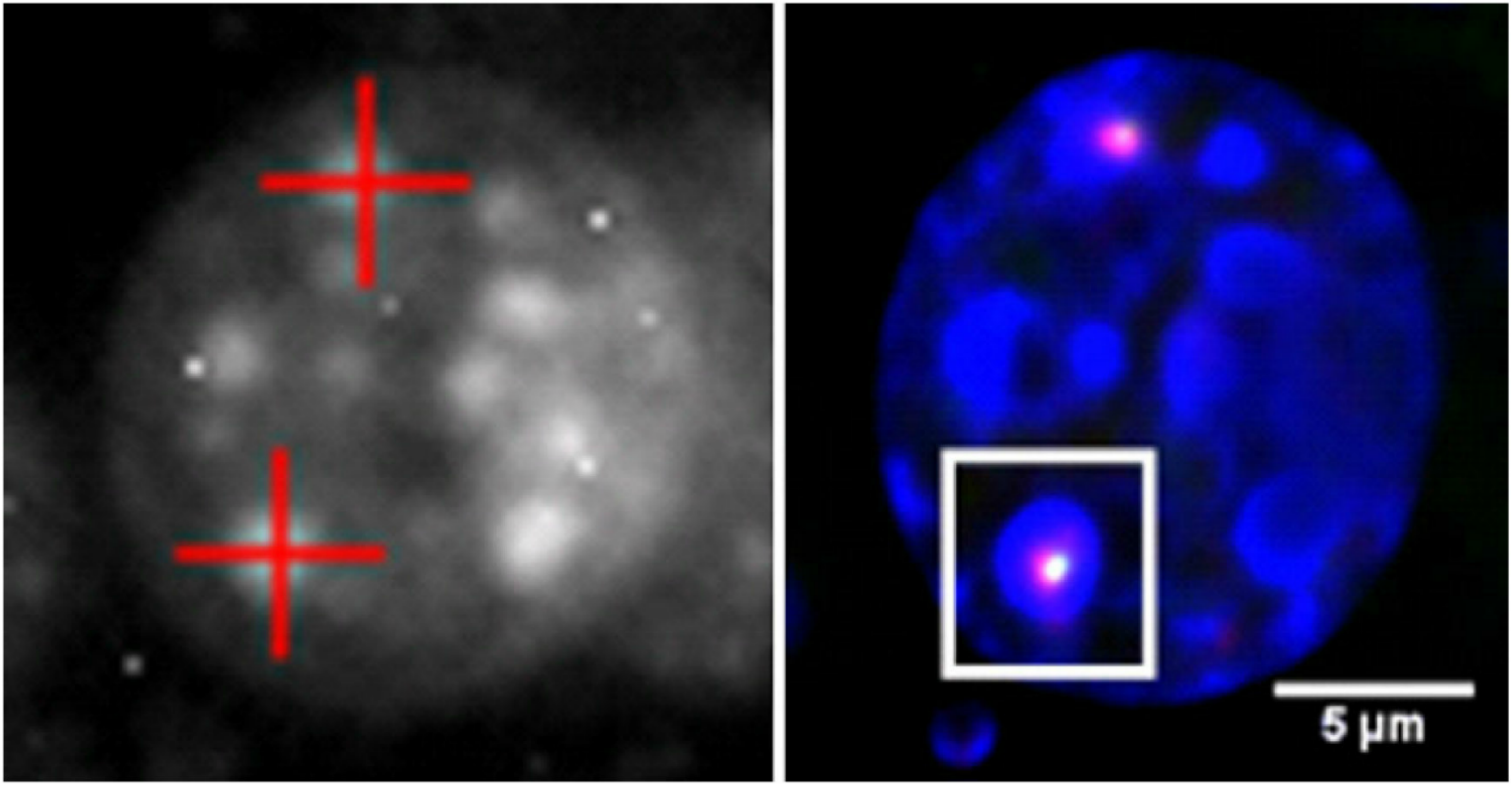
10 µm



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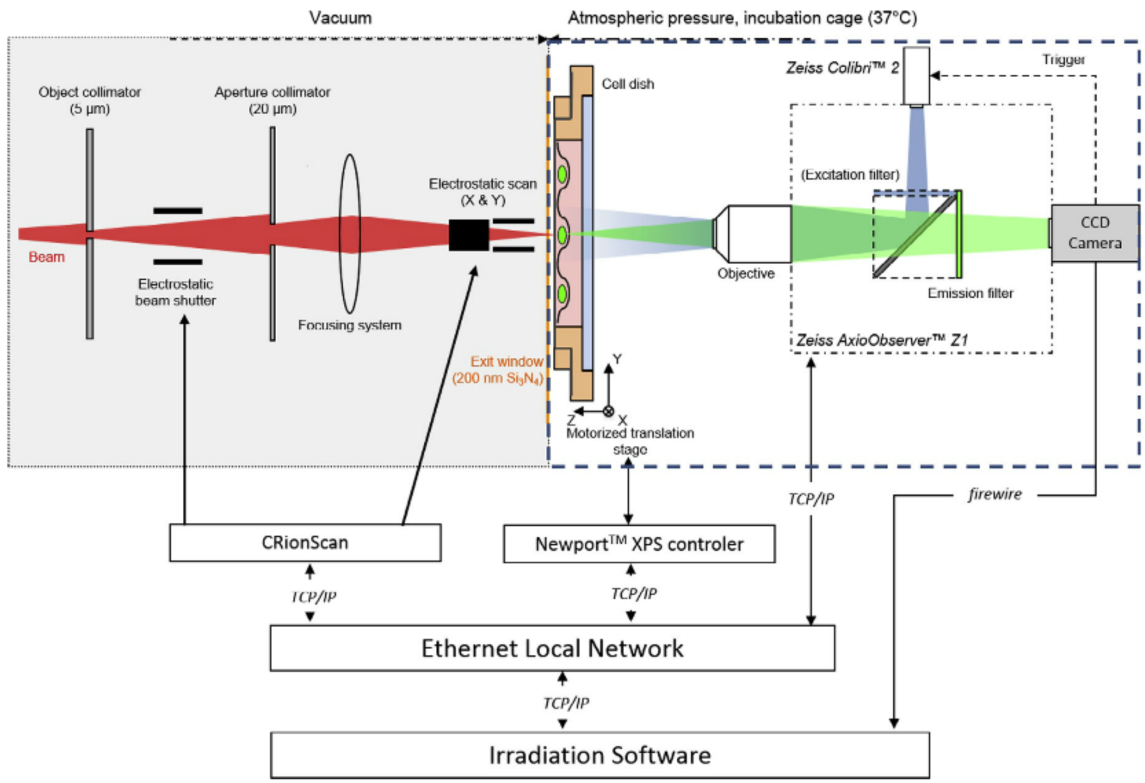
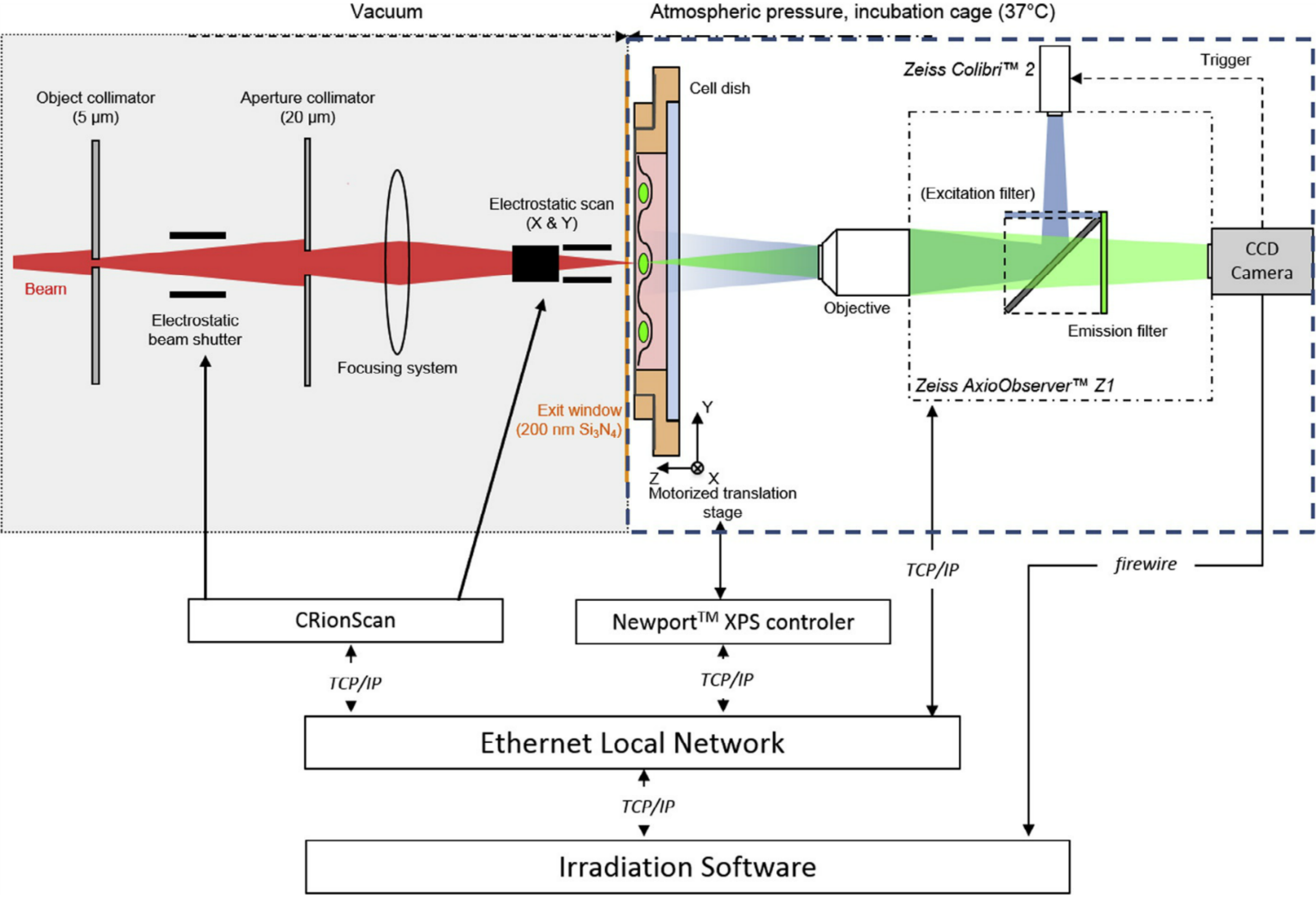
On-line live cell imaging



Scheme of the micro-irradiation set-up. The charged particles are focused in a micrometer spot using a triplet of magnetic quadrupoles and driven to the target cell under vacuum. They are extracted in air about 200 µm upstream the cell monolayer through a 200 nm thick Si_3N_4 window.

A microscope (Zeiss AxioObserver Z1) is positioned horizontally at the end of the beamline to visualize the cells and perform online time-lapse imaging. The cell dish can be positioned with 1 micrometer precision using a motorized translation stage. The whole experiment is controlled by a custom-made irradiation software addressing the instruments via TCP/IP messages.

S. Bourret et al. Nuclear Instruments and Methods in Physics Research B 325, 27–34 (2014)


Vacuum
Atmospheric pressure, incubation cage (37°C)
Object collimator
(5 µm)
Aperture collimator
(20 µm)
Electrostatic scan
(X & Y)
Cell dish
Zeiss Colibri™ 2
Trigger
(Excitation filter)
Beam
Electrostatic
beam shutter
Focusing system
Objective
CCD
Camera
Emission filter
Zeiss AxioObserver™ Z1
Exit window
(200 nm Si3N4)
Y
Z
X
Motorized translation
stage
TCP/IP
firewire
CRionScan
NewportTM XPS controler
TCP/IP
TCP/IP
Ethernet Local Network
TCP/IP
Irradiation Software

Radiobiological facilities with ion beams in Poland

Currently in Poland there are only two radiobiological facilities with ion beams:

Ion microbeam at
the Van de Graaf accelerator
(The Henryk Niewodniczanski
Institute of Nuclear Physics Polish
Academy of Sciences, Kraków)

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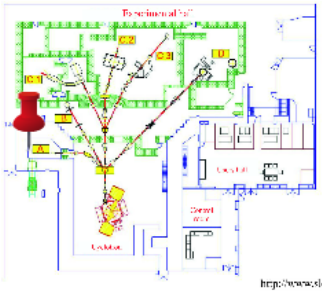
Warsaw cyclotron facility
with a horizontal broad ion beam
(Heavy Ion Laboratory at University of
Warsaw, Warszawa)

Experimental setup



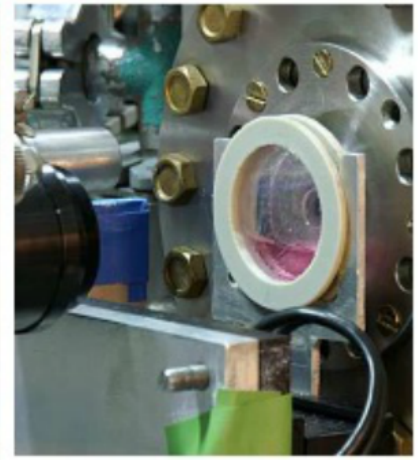
Experimental setup dedicated to the radiobiological studies is located at the A track Heavy Ion Laboratory, University of Warsaw

The Warsaw K=160 cyclotron provides beams of energies from 2 to 10 MeV/u and intensities up to a few hundreds pnA.

Almost all types of ions with high Linear Energy Transfer (LET) can be accelerated.

http://www.slcj.uw.edu.pl/

Układ mikrowiązki jonowej w IFJ PAN



Układ mikrowiązki jonowej działa w oparciu o akcelerator liniowy typu Van de Graaffa.

Napięcie przyspieszające: 2.5 MV

Wykorzystywane cząstki to jądra wodoru lub helu

Działanie układu i wyniki badań opisane zostały w publikacjach oraz pracach doktorskich

[1] O. Veselov, W. Polak, R. Ugenskiene, K. Lebed, J. Lekki, Z. Stachura, J. Styczeń, „Development of the IFJ Single Ion Hit Facility For Cells Irradiation", Radiation Protection Dosimetry

[2] Wojciech Polak - "Badanie reakcji komórek po naświetleniu pojedynczymi jonami„, praca doktorska,

[3] Oleksandr Veselov - "Irradiation of cells with targeted ions using optical automatic recognition"

Radiobiological facilities with ion beams in Poland

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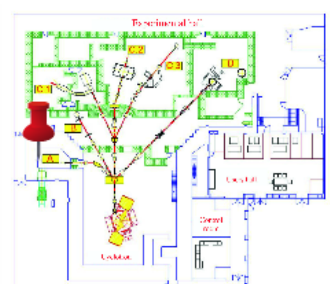

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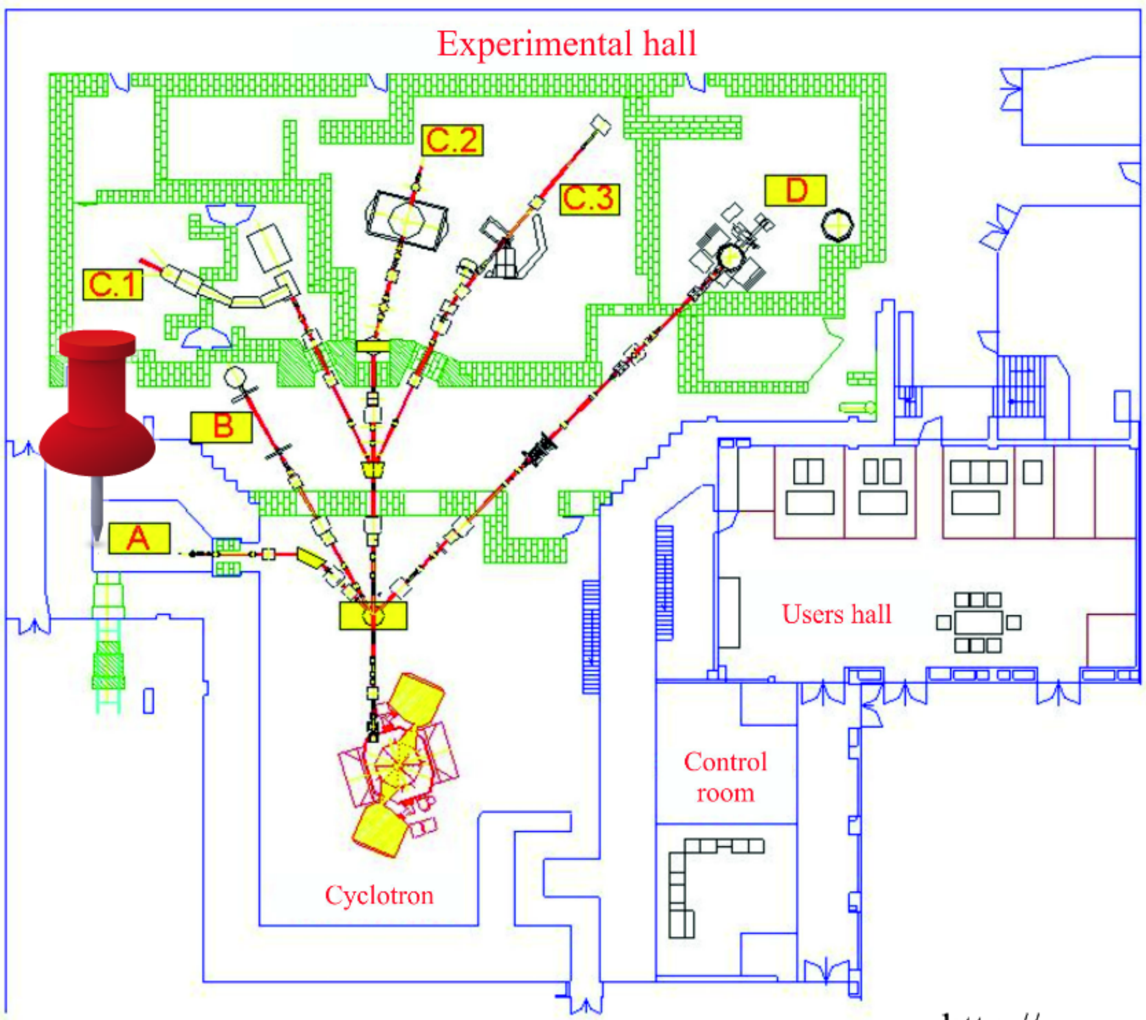

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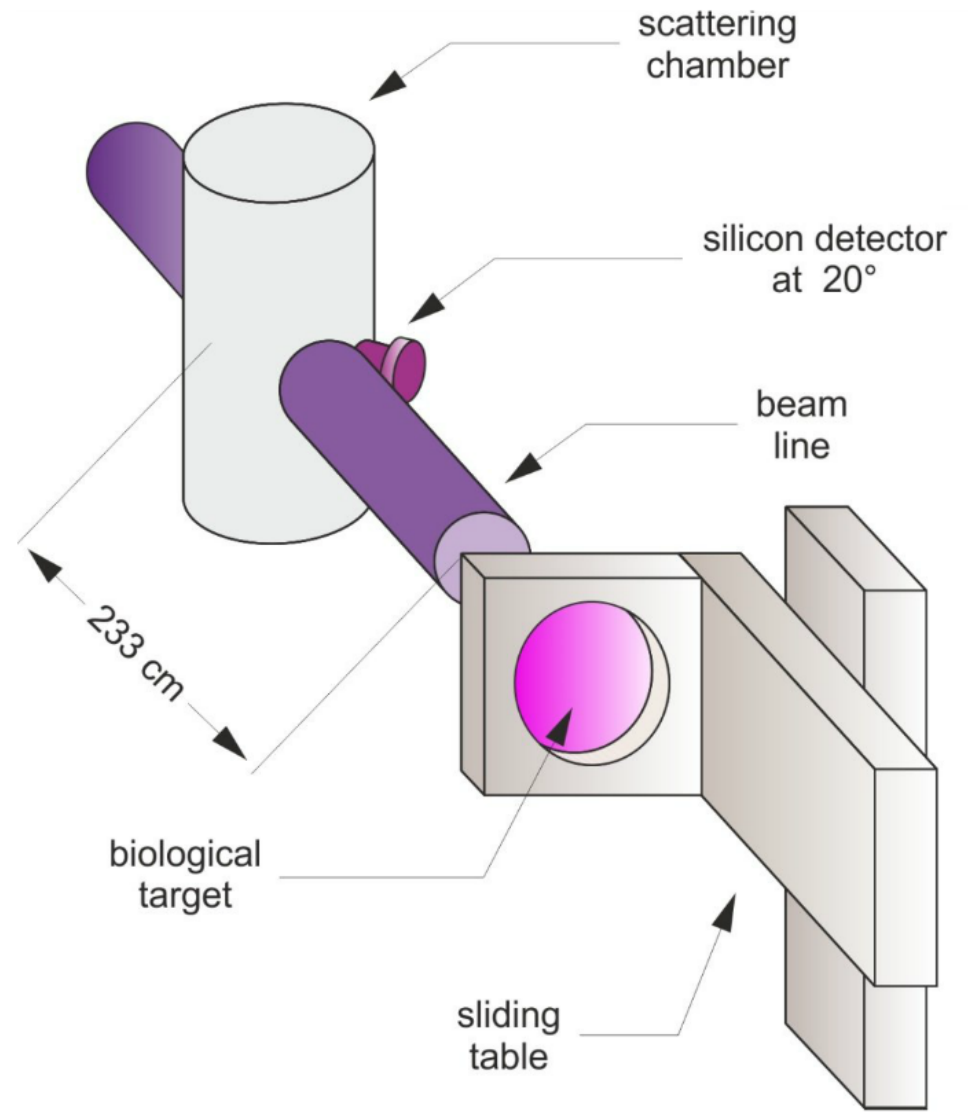
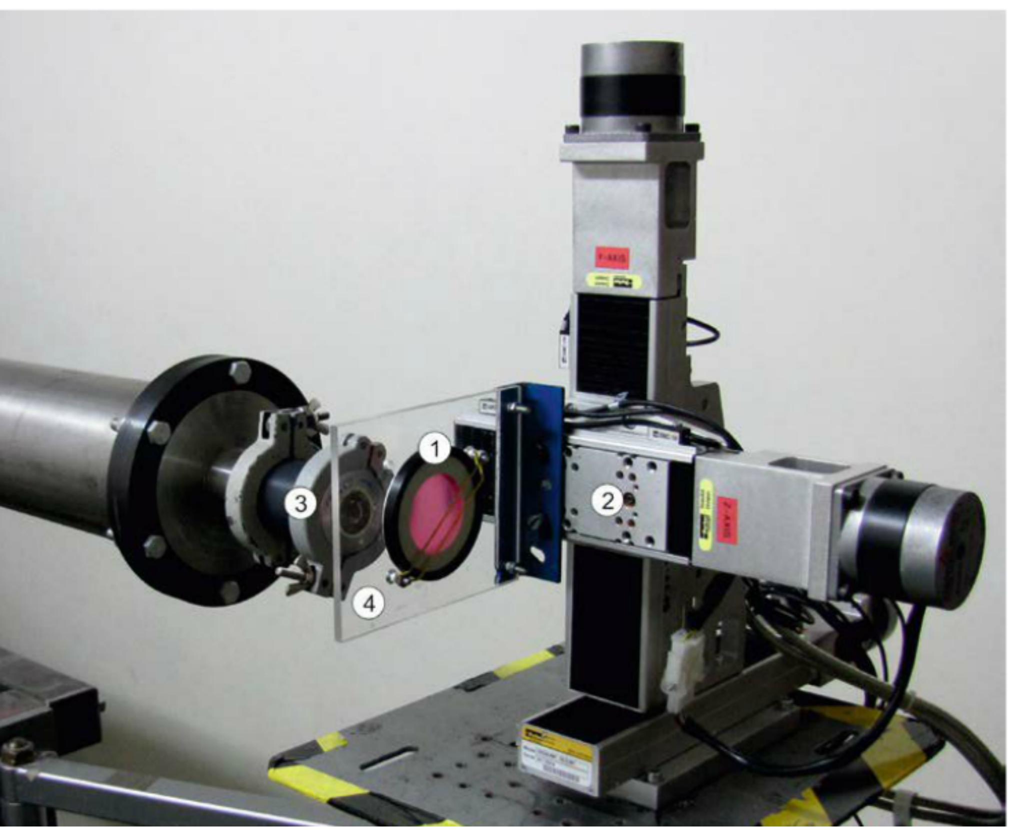
Experimental setup

- Beam is horizontal and stationary
- Biological target is fastened vertically on sample holder, mounted on x–y–z sliding table with remote control



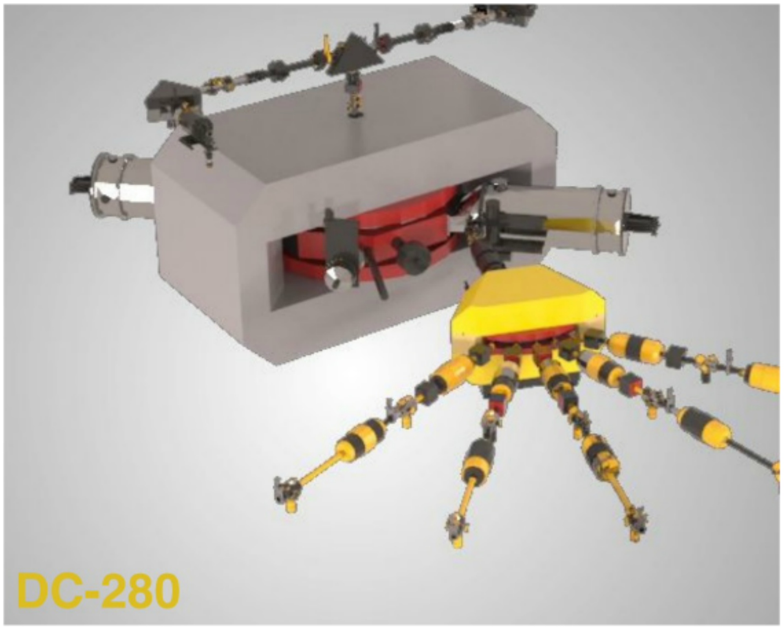


1. Biological target
2. Sliding table
3. Havar exit window
4. Sample holder

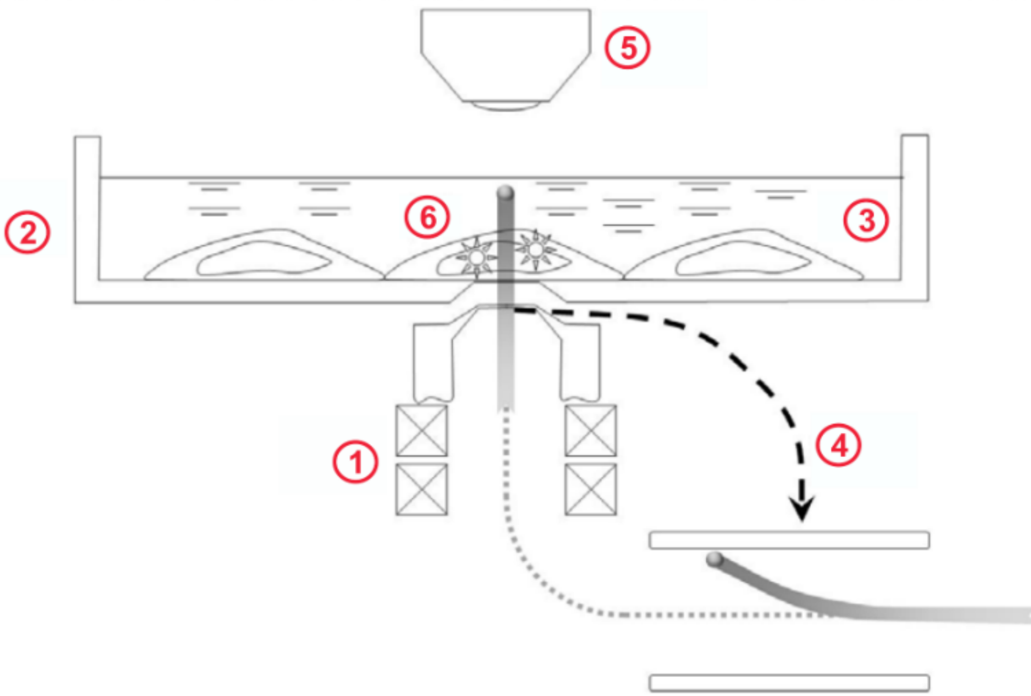
Plans for a new radiobiology research facility in Poland

New cyclotron at HIL will provide **higher intensity** of the ion beam, which will allow the construction of a **vertical ion microbeam** for radiobiological research.

In addition, **off-line** and **on-line** optical systems, including software responsible for automatic cell recognition will be installed.



An ideal experimental setup for radiobiology studies



(1) a sub-micrometer ion beam (<1 µm),

(2) a horizontal sample stage that can be operated under atmospheric pressure,

(3) targets consisting of a cell monolayer cultured with fresh medium and located in a standard culture environment,

(4) a device for precisely controlling the number of charged particles delivered to the target,

(5) a microscope for observing the targets optically,

(6) tools for observing changes in the target.

Based on: H.Khodja, Journal of Physics: Conference Series 261 (2011) 012012

Conclusions

1. **DNA damage and repair** caused by ionizing radiation with a high linear energy transfer (LET) **is of increasing importance** due to the clinical spread of radiotherapy using high energy carbon beams, as well as to assess the risk of cancer in astronauts in space.

2. Research on **DNA damage and repair mechanisms** using microbeams in the future will certainly be **one of the main areas** of interest for scientists.

3. In Poland, a **new device** dedicated to radiobiological research with an **ion microbeam** should be created. A very good opportunity to this will be the construction of a new cyclotron at HIL UW.


THANK YOU!