Radiobiological Studies
at the Joint Institute for Nuclear Research

Pavel Bláha
pavel.blahax@gmail.com

Laboratory of Radiation Biology

- **1959. First radiobiological experiments at JINR** – comparative evaluation of effects of protons and gamma on laboratory animals

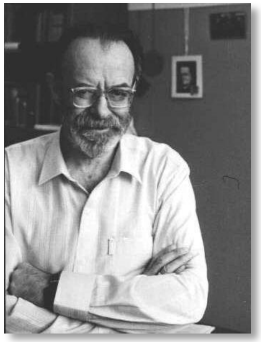
- **1964. Stationary laboratory** opened at the Laboratory of Nuclear Problems (LNP)

- **1978.** Establishment of the **Biological Research Sector** at the LNP – main aim: differences in the biological effectiveness of ionizing radiation with different physical characteristics

- **1988.** Establishment of the LNP's **Biophysics Department**

- **1995.** Establishment of JINR's **Department of Radiation and Radiobiological Research**

- **2005.** Establishment of JINR's **Laboratory of Radiation Biology**

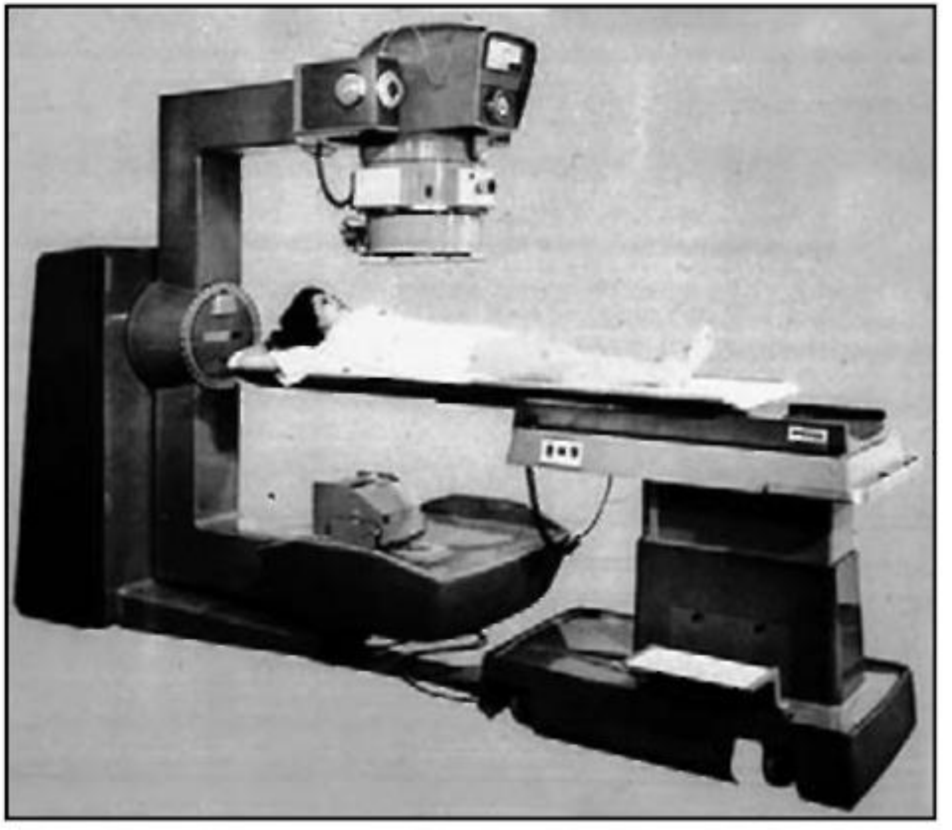


Prof. V.I. Korogodin, Head of the LNP's Biological Research Sector

Prof. E.A. Krasavin, Dr. Biol., Director of the Laboratory of Radiation Biology, JINR

Irradiation possibilities

- Gamma sources
  - Rokus-M (^{60}Co) →
- Protons
- Heavy charged particles



Phasotron (Synchrocyclotron) - LNP

Protons with energies up to 660 MeV – around the peak of the GCR protons

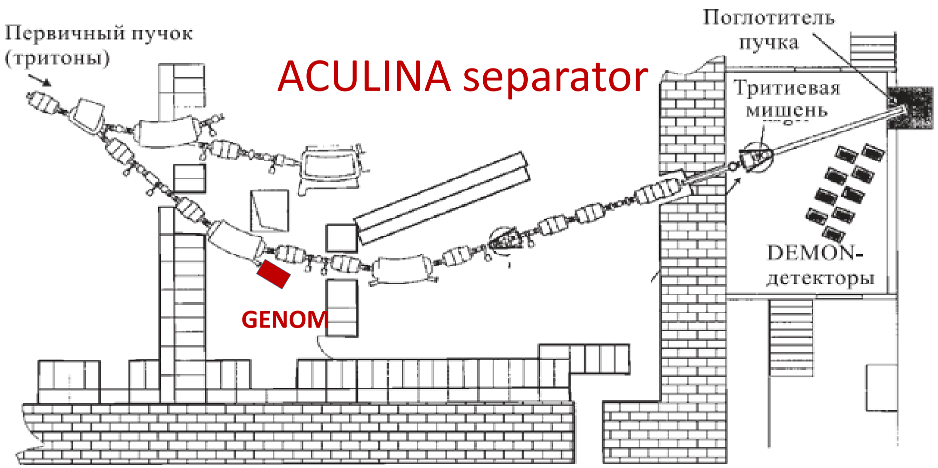
U-400M - LNR

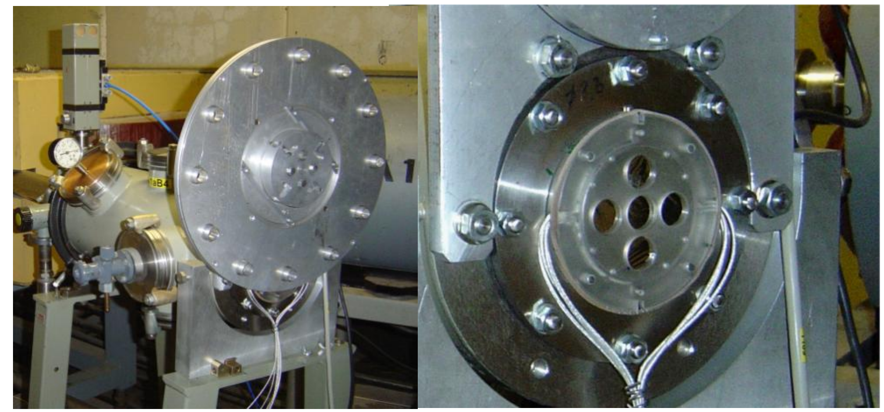
Heavy ions

$^{7}Li^{2+} \rightarrow {}^{86}Kr^{9+}$

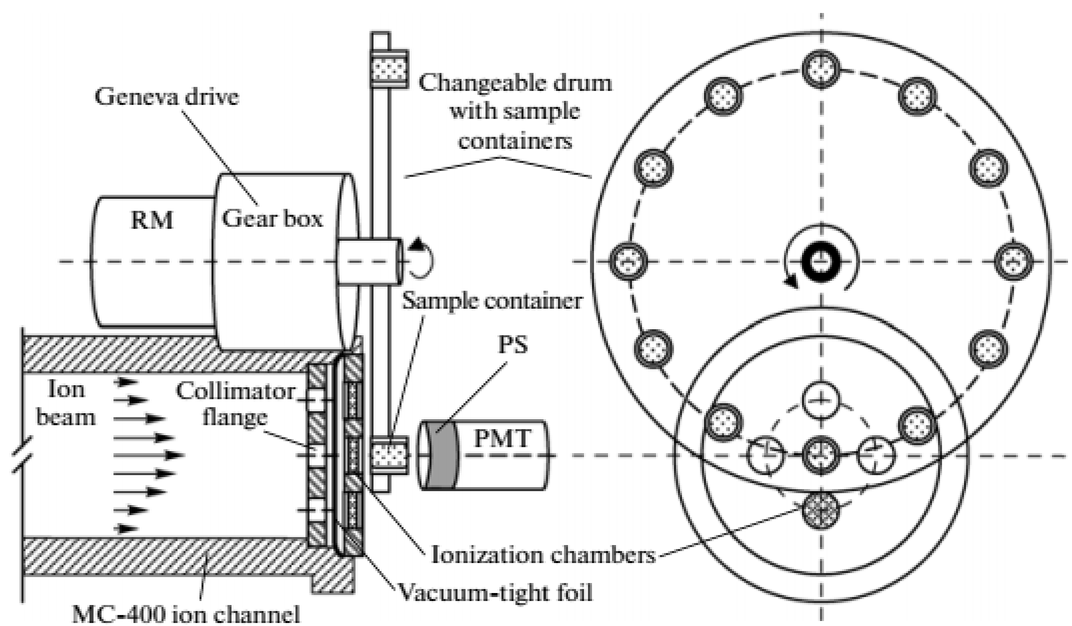
Energy around
50 MeV/nucleon

The LRB special stationary setup “Genome” at the U-400M



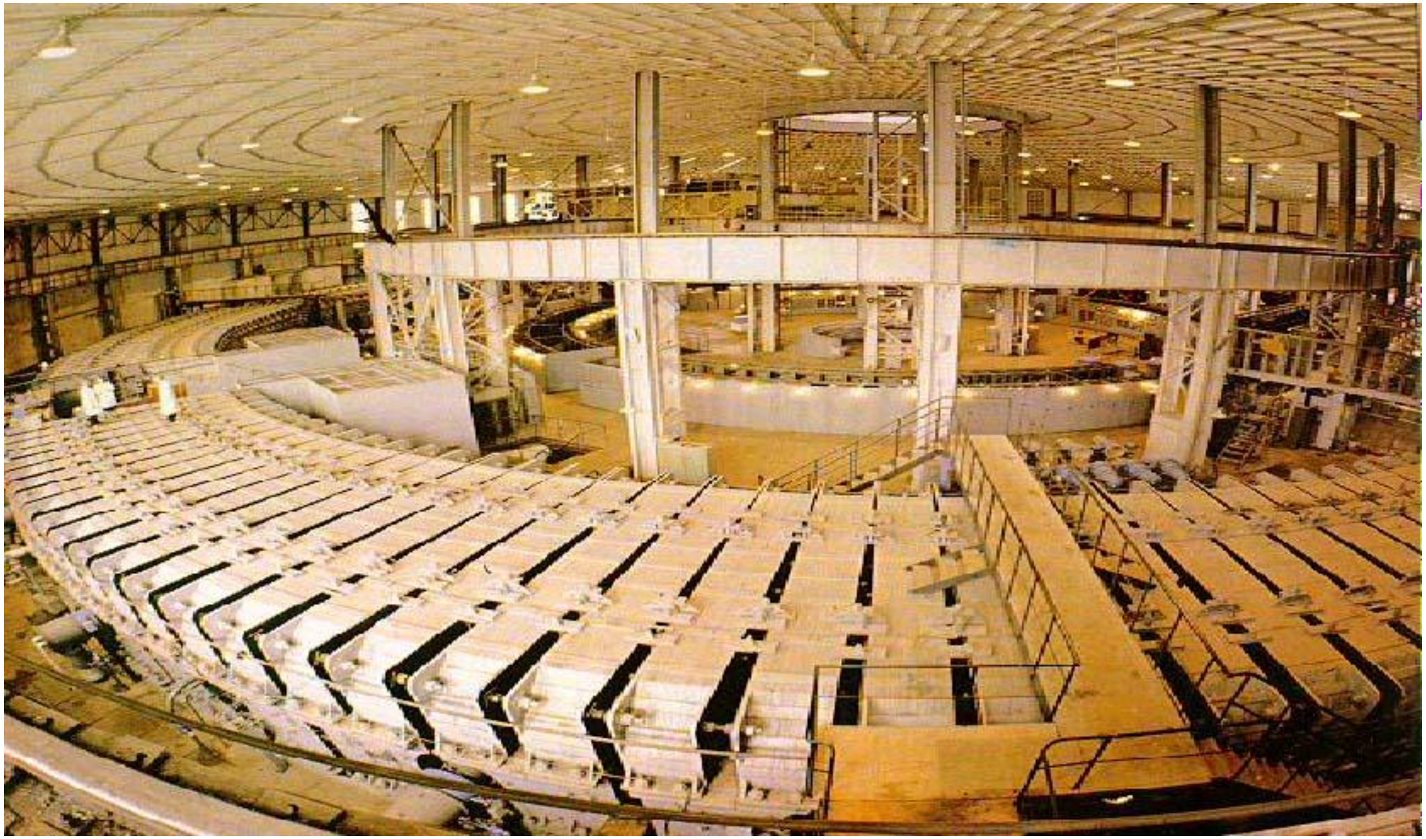


Fast automatic irradiation of thin biological samples (or small volumes of suspension) with high LET heavy ions in a wide range of absorbed doses



Bezbakh, et al., Particles and Nuclei Letters, 2 (179), 274-280, 2013

Synchrophasotron - LHEP



1957 - 2003
Acceleration of protons (up to 10 GeV/nucleon) and heavy ions

- Synchrophasotron, an accelerator built in Dubna in 1957, has become the biggest and the most powerful for his time. Its magnet weighs 36000 tons and is registered in the Guinness Book of Records as the heaviest in the world.

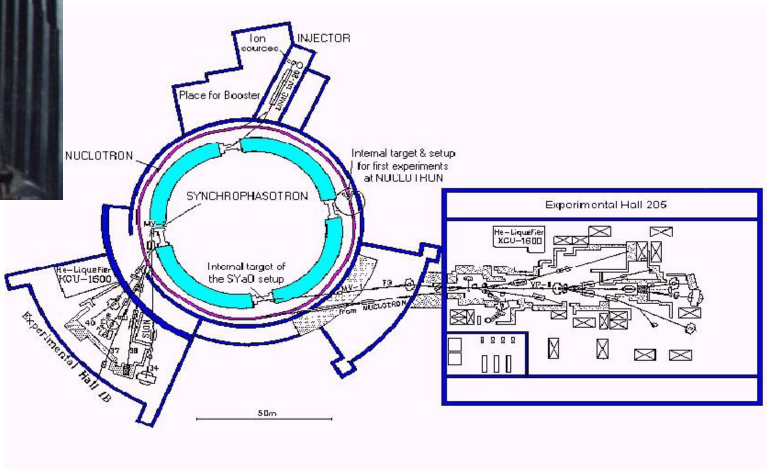
Nuclotron - LHEP

Heavy ions up to

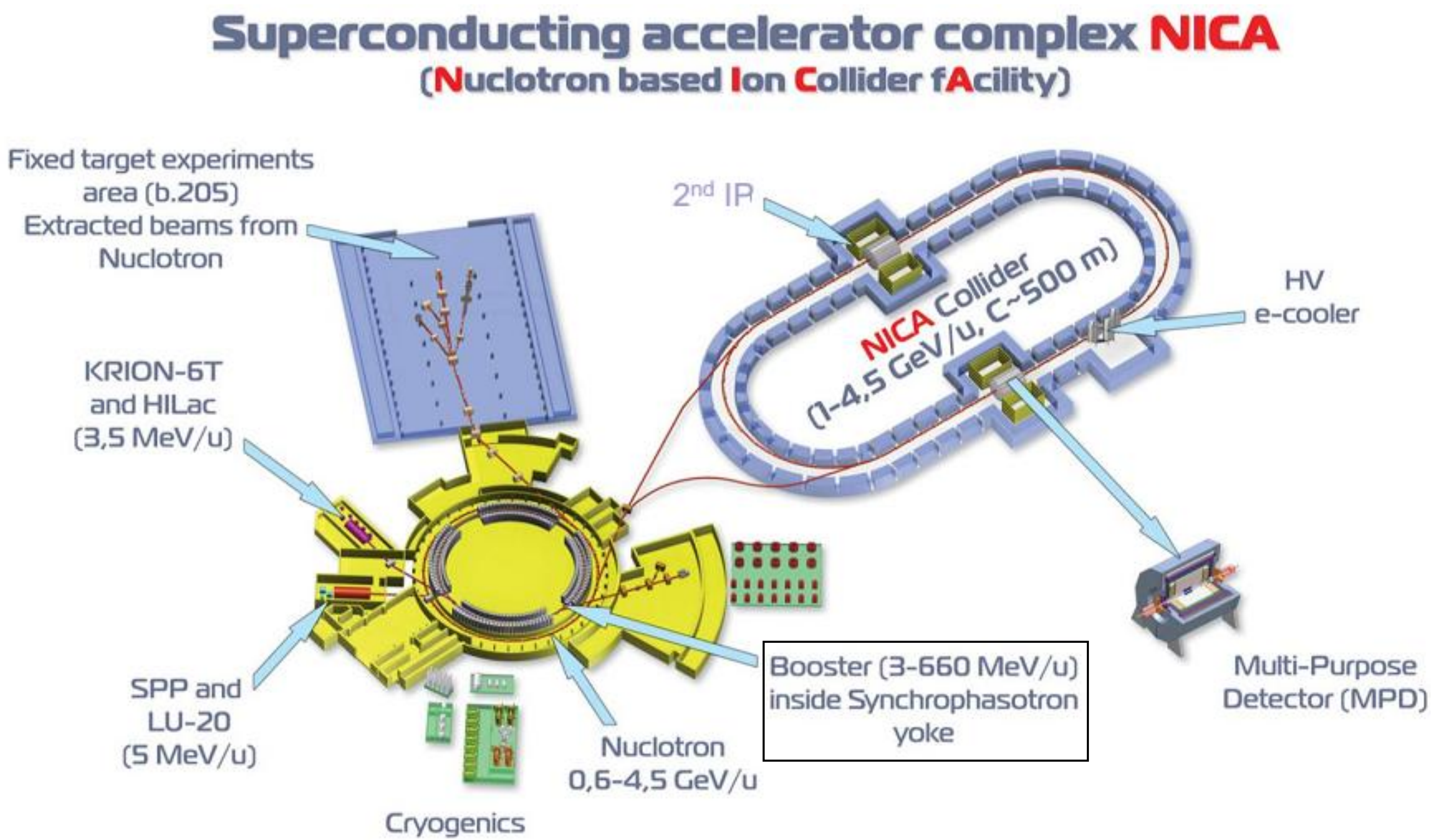
$^{197}Au^{79+}$

Energy:

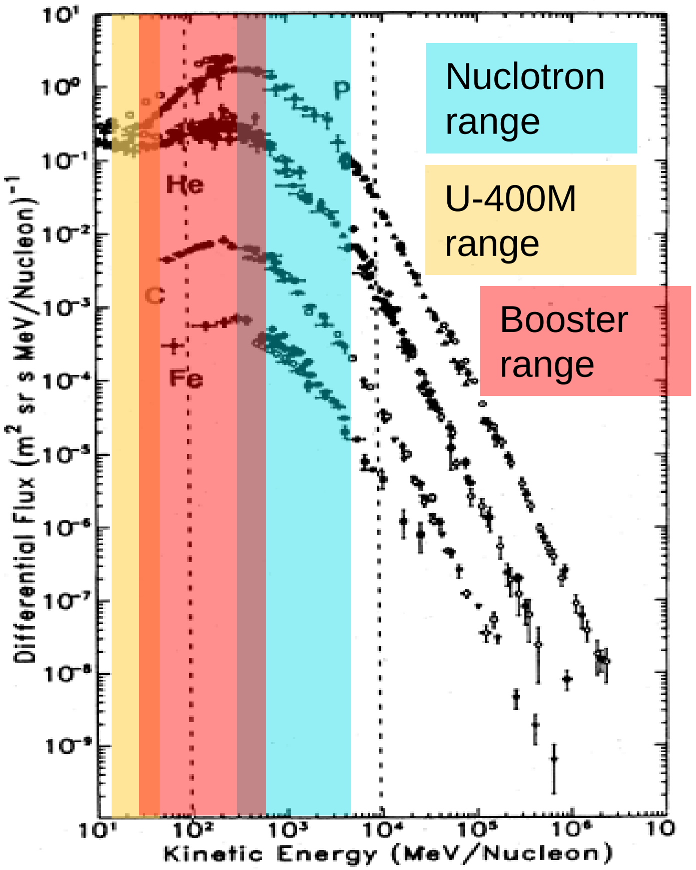
0,6 – 4,5 GeV/amu

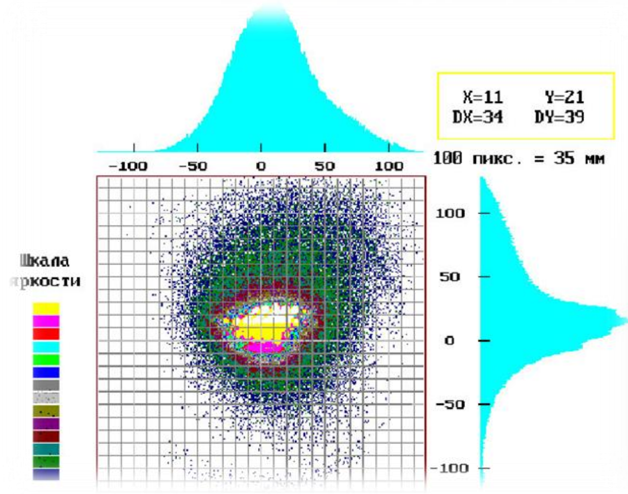


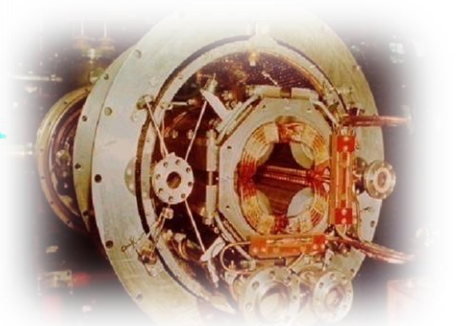
Future: Booster + NICA



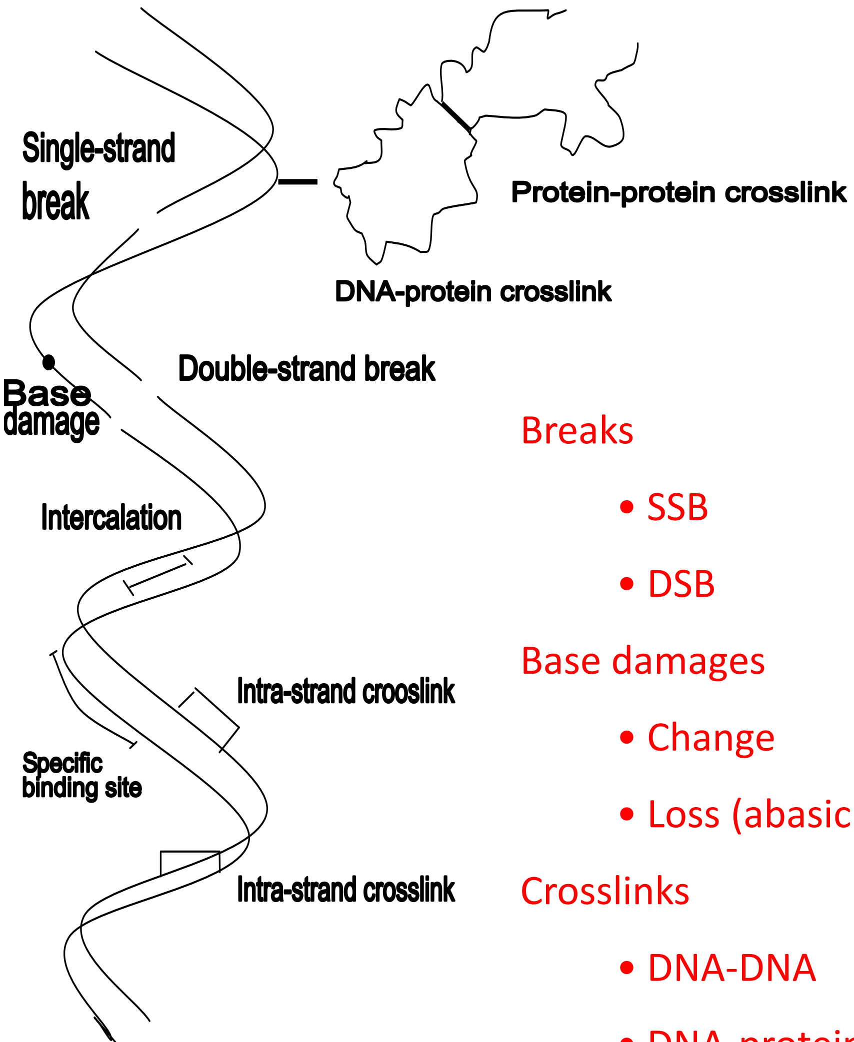
GCR and JINR's accelerators energy spectra







Why radiation? - Effects of ionizing radiation



Breaks
- SSB
- DSB

Base damages
- Change
- Loss (abasic sites)

Crosslinks
- DNA-DNA
- DNA-protein

Held, 2013 NSRSS

- Ionizing radiation is an **extraordinarily** efficient agent for causing biological effects.
- If the energy contained in **1 teaspoon** of water with temperature of **60°C** would be transformed to radiation – it would **kill** a grown up man!
- **Less than 1 microgram of Po-210** can kill a man! - more toxic than any known poison (Litvinenko case)

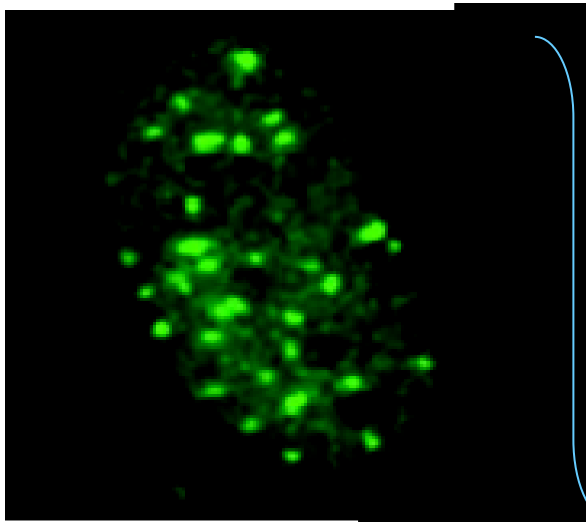
DNA is a Primary Target

- Radioisotopes in DNA are more lethal than when in RNA or protein.
- Microbeam experiments show cell nucleus to be more sensitive than cytoplasm.

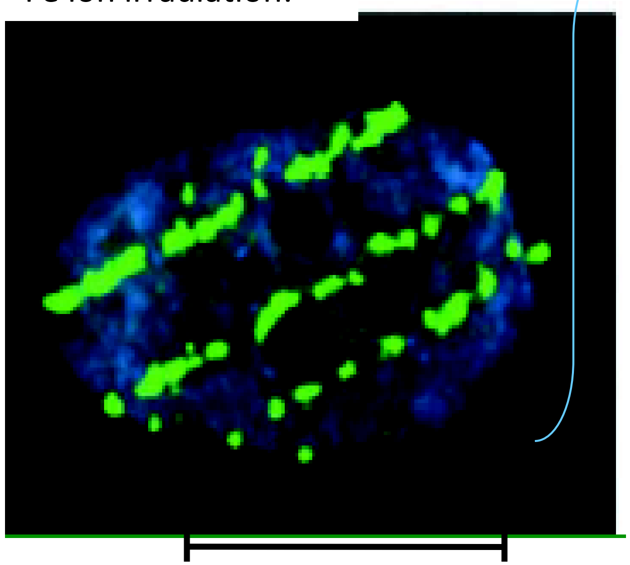
Why heavy ions?

Fluorescent foci marking DSB in cell nuclei

Gamma-ray irradiation:

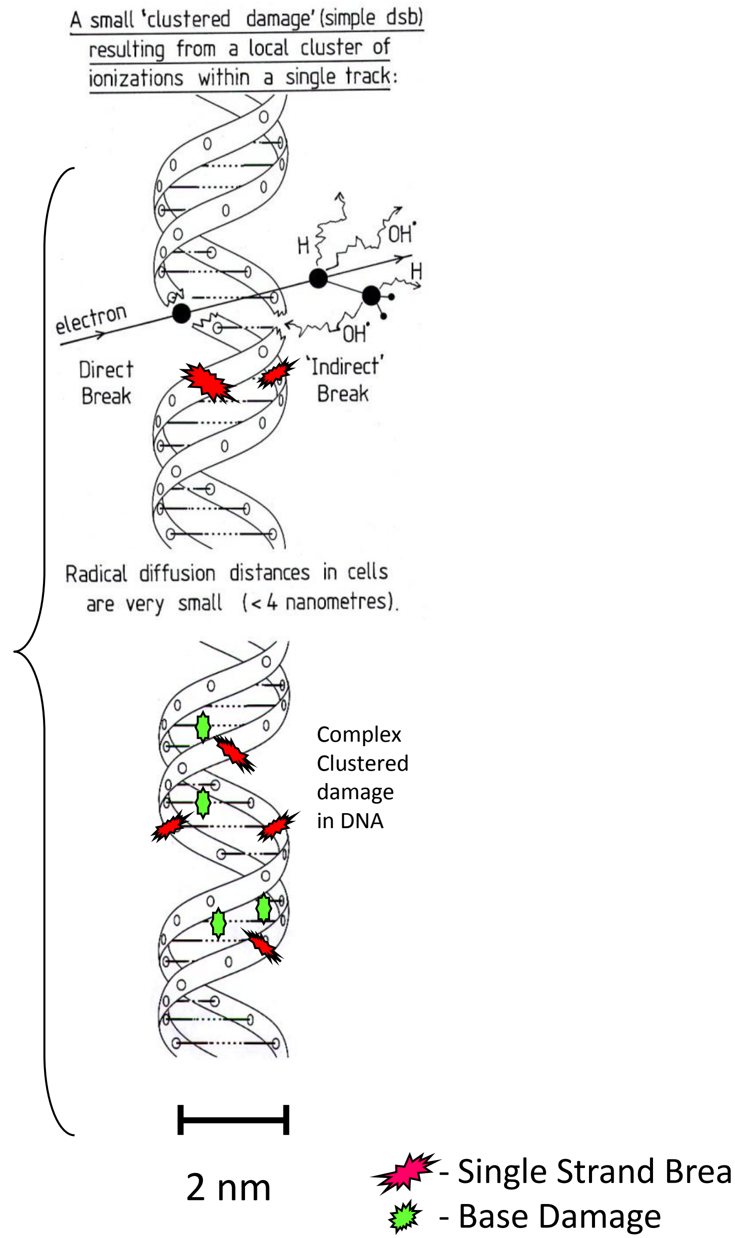


Fe ion irradiation:



10 µm

Magnification x 1600

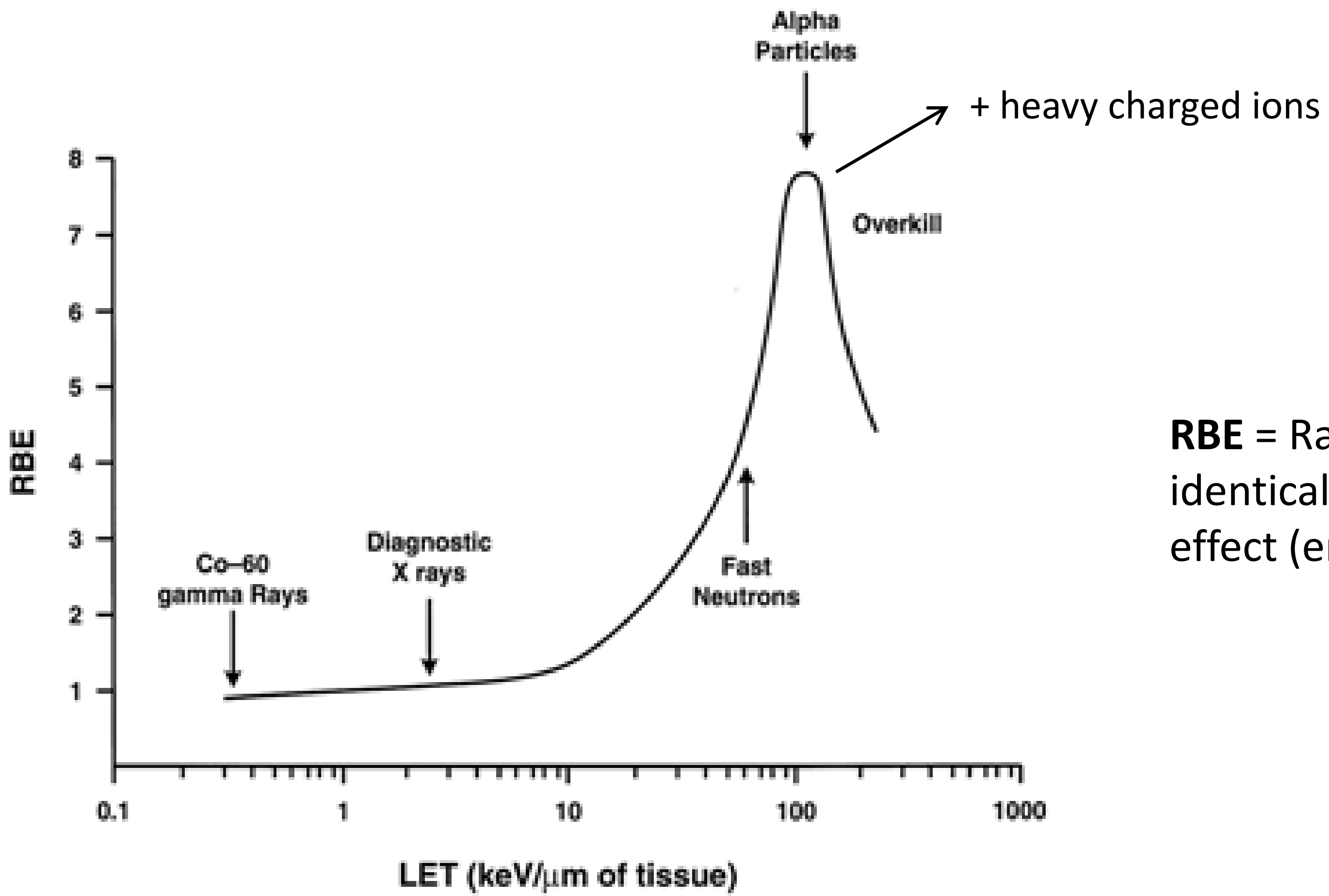


Goodhead, 2013 NSRSS

Dependence RBE on LET

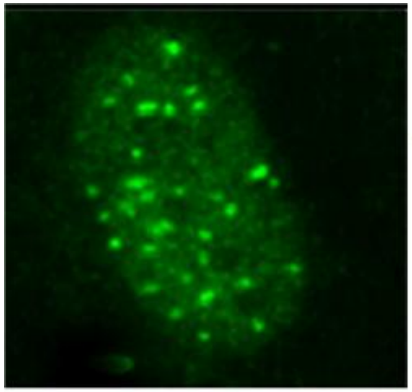
LET – Linear Energy Transfer [keV/μm]; **RBE** – Relative Biological Effectiveness

Endpoint = level of cell survival

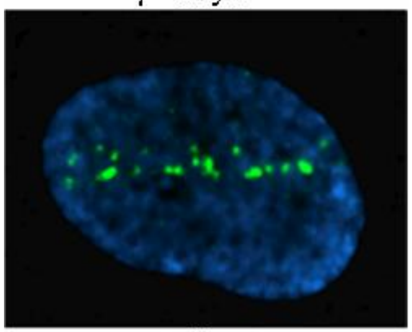


RBE = Ratio of doses for identical level of biological effect (endpoint)

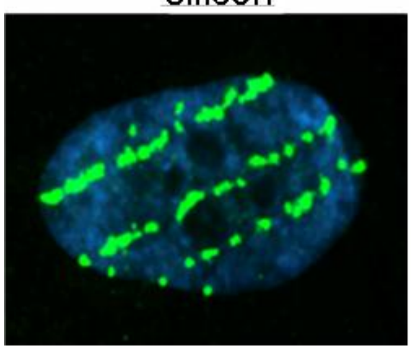
Heavy ions tracks



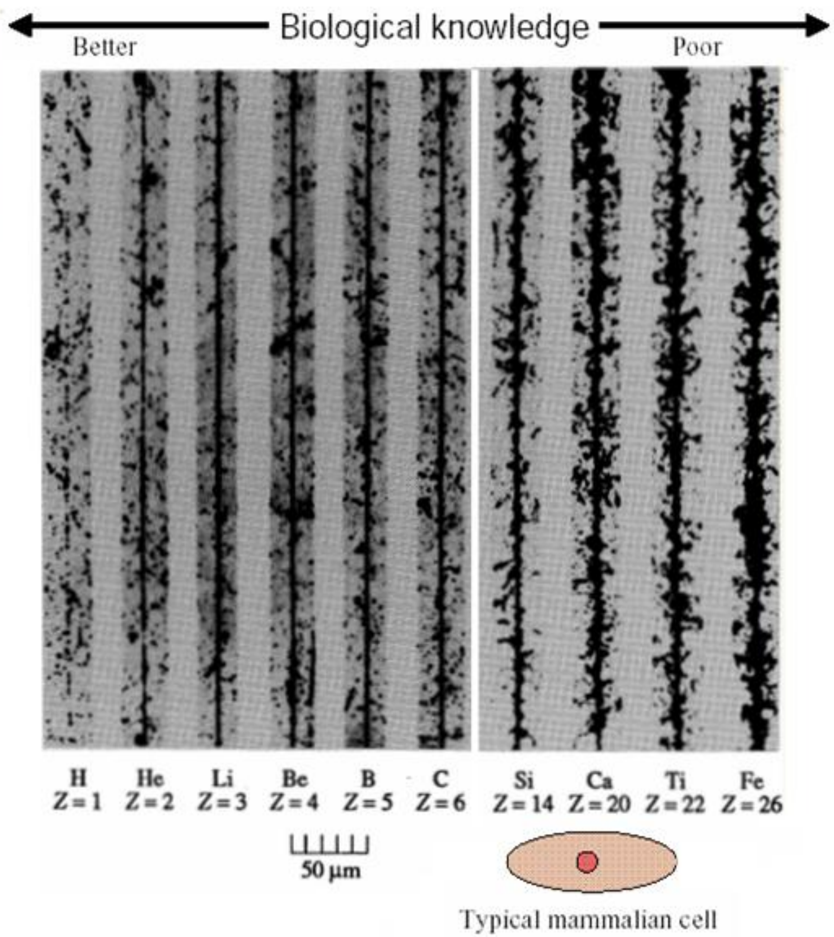
γ – rays

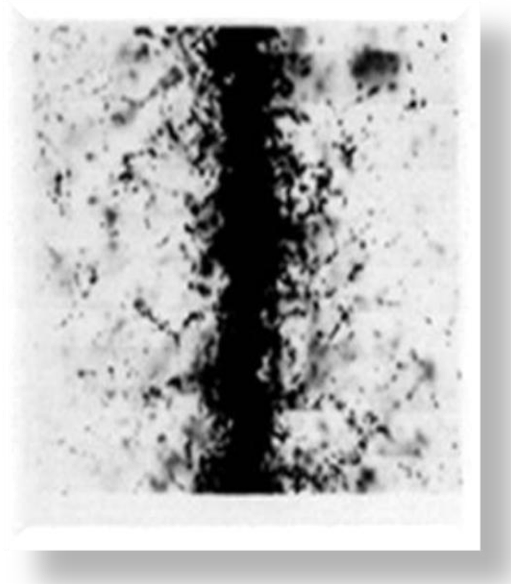


silicon



iron

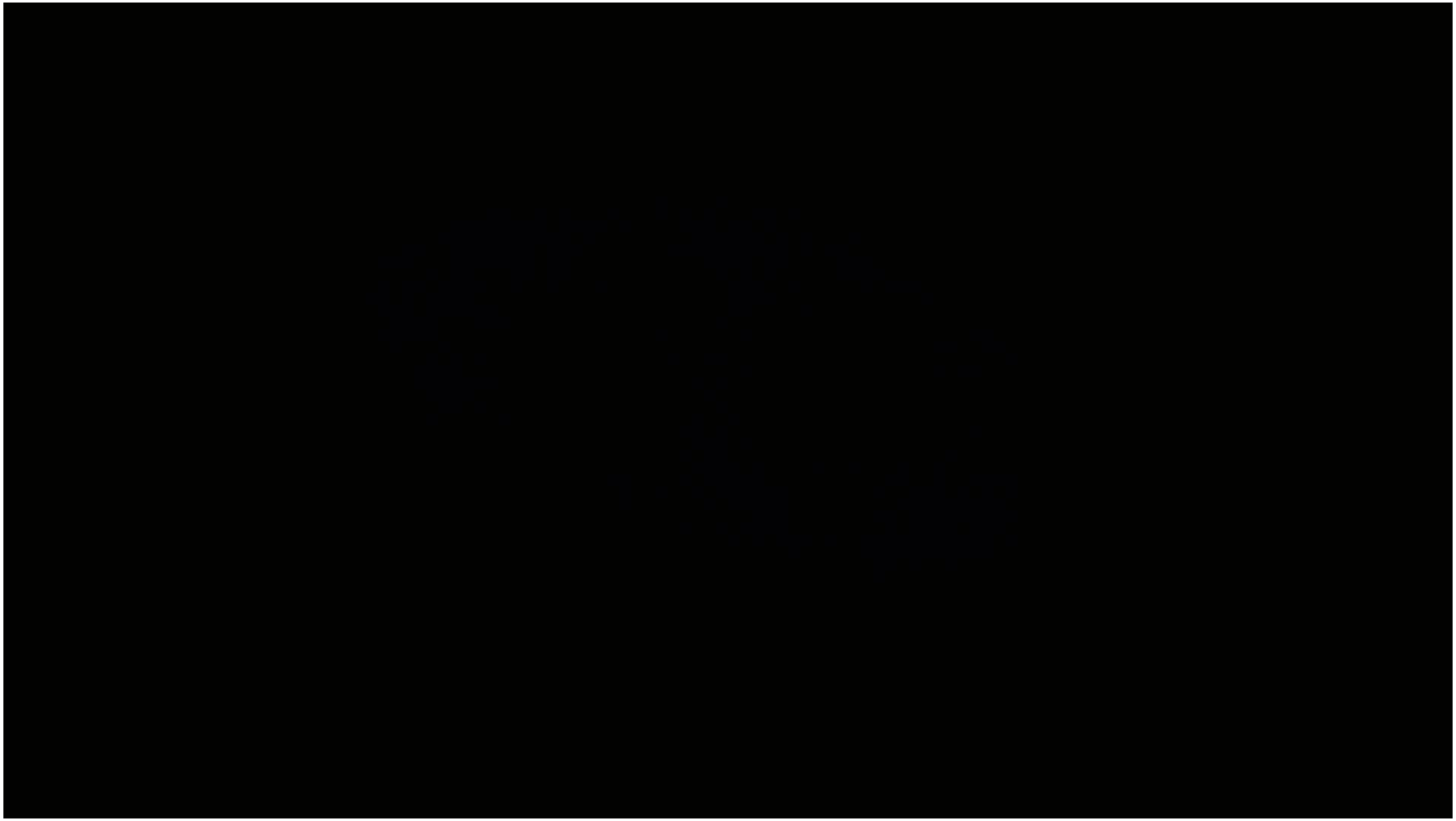




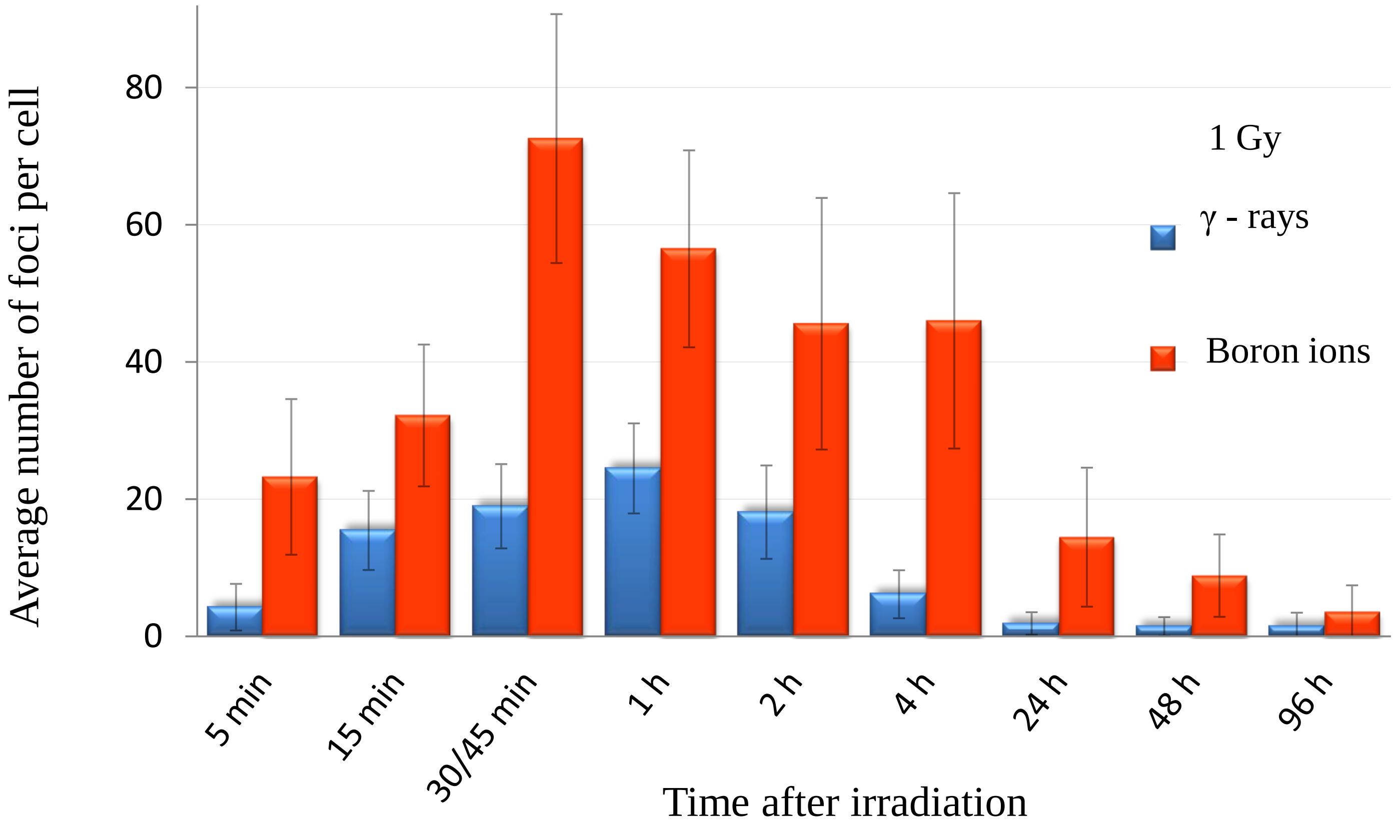
Z = 70

(Cucinotta & Durante, Lancet Oncol 2006)

Double-strand breaks (DSB) in DNA



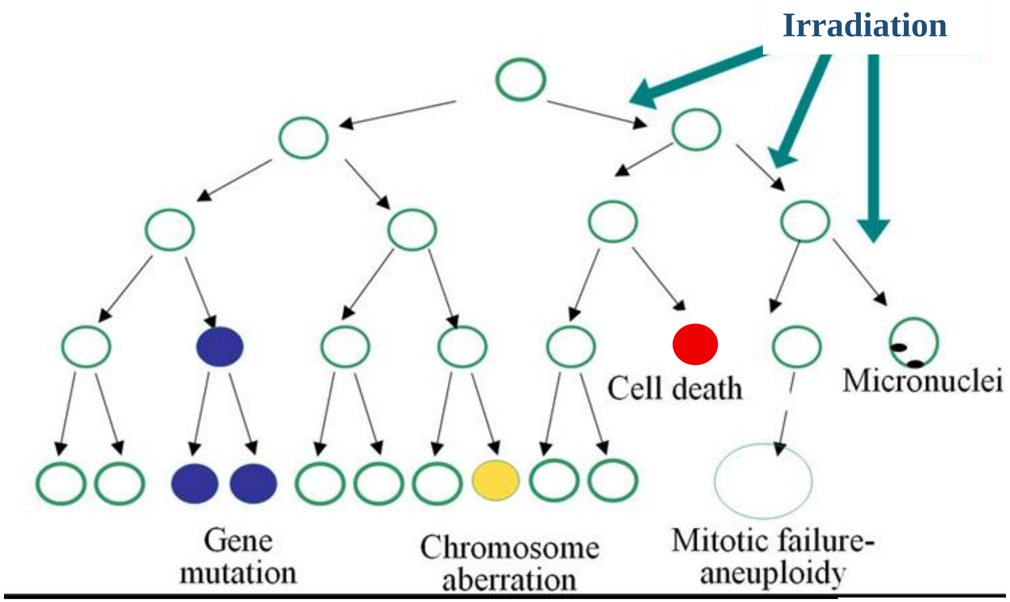
Damage and repair kinetics of DNA after irradiation



Radiation Genetics

Radiation-Induced Genomic instability

- IR-exposure can cause a persistent state of instability amongst surviving cells
- Late outcomes: delayed cell death, mutator phenotypes, non-clonal aberrations – observed in the progenies of irradiated cells

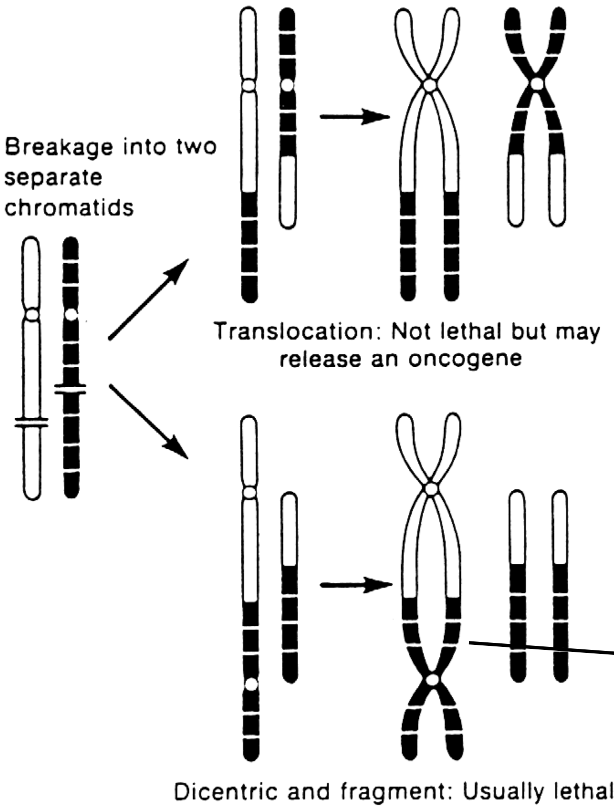


[U.S. DOE]

Ways to measure genomic instability:

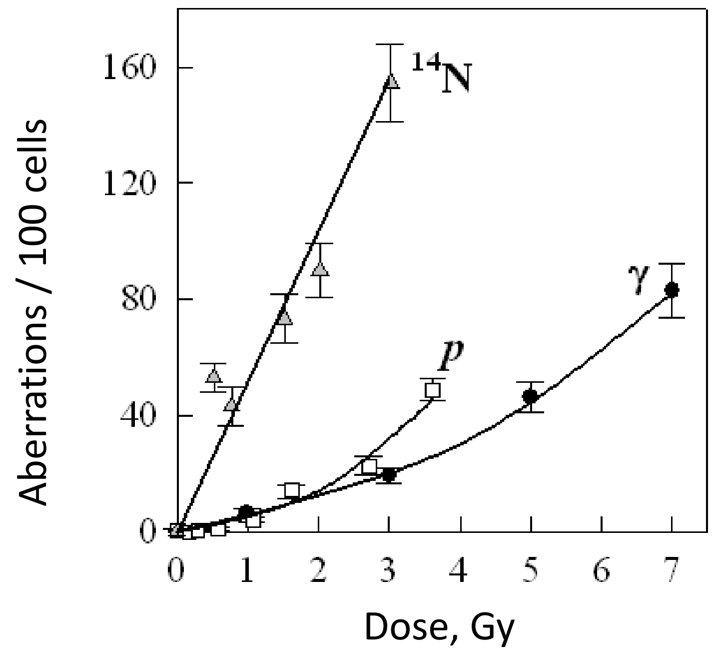
- **Delayed reproductive death**
- **Karyotypic heterogeneity**
- **Changes in mutation rates at specific loci**
- and others

Formation of chromosome aberrations after irradiation

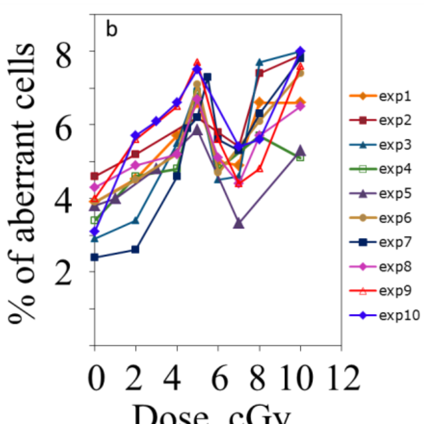


Stable aberration
Symmetrical (balanced) gene rearrangements

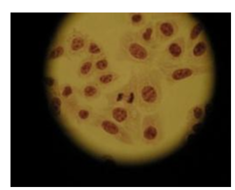
Unstable aberration
Asymmetrical
Fragment usually lost

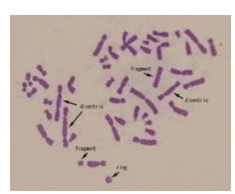


- Studying cytogenetic effects of low-dose γ-irradiation in human cells

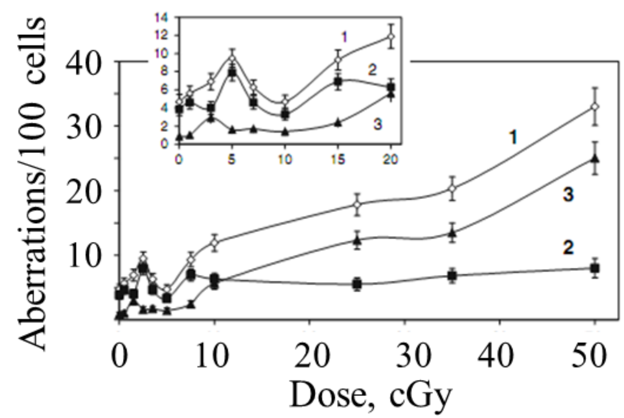


Mammary carcinoma cells

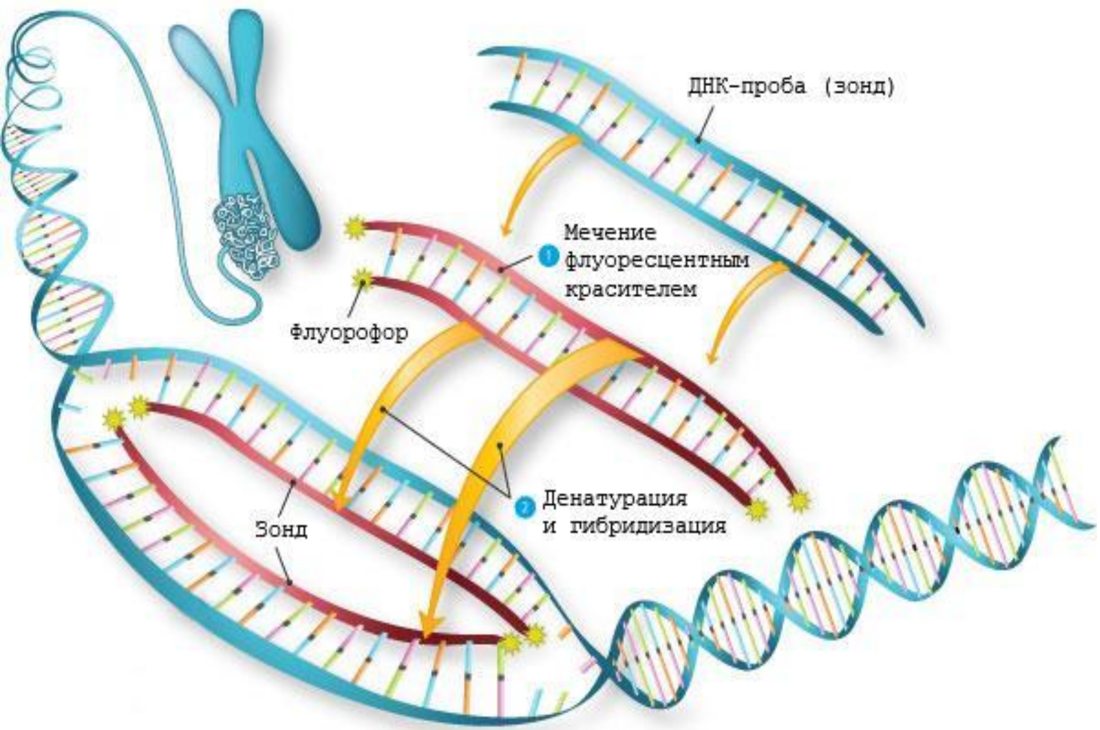


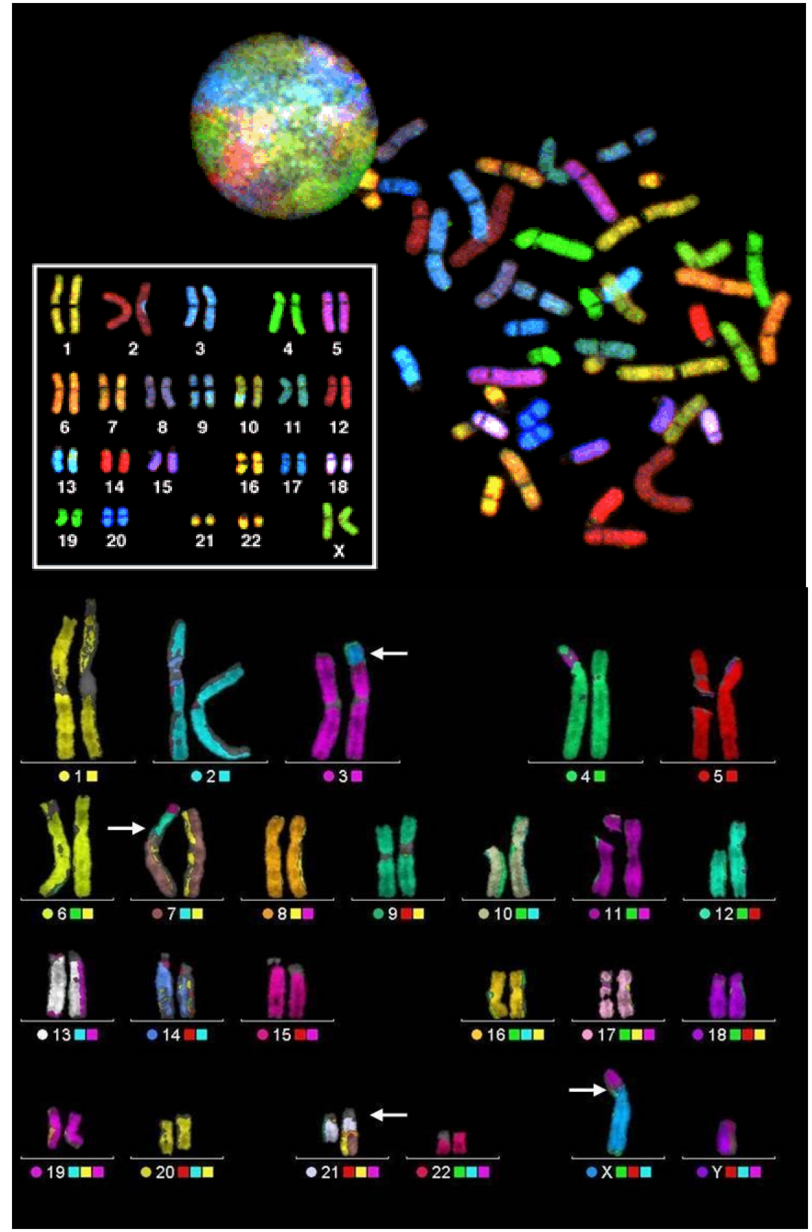


Human lymphocyte



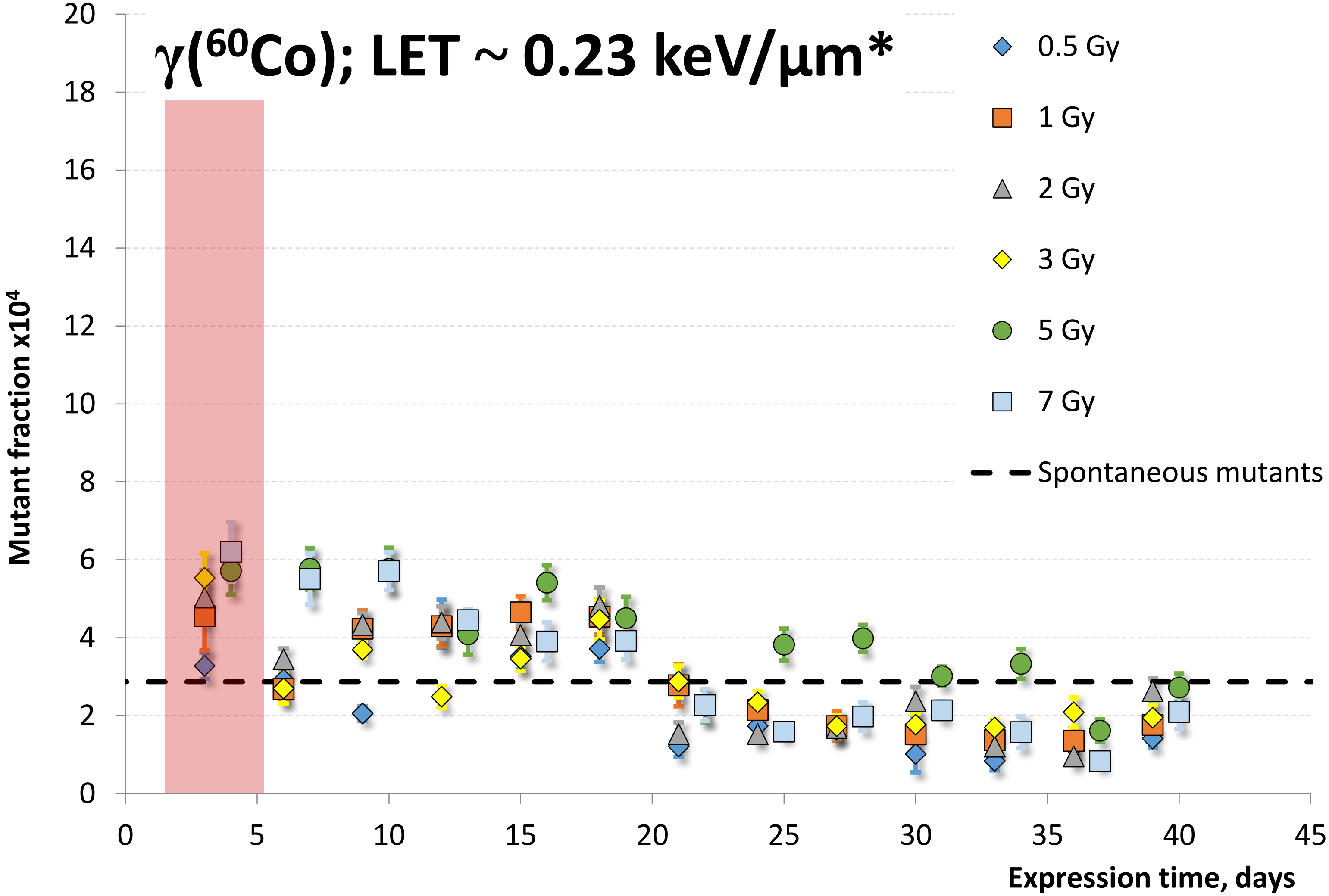
mFISH – multicolor Fluorescent in situ hybridization



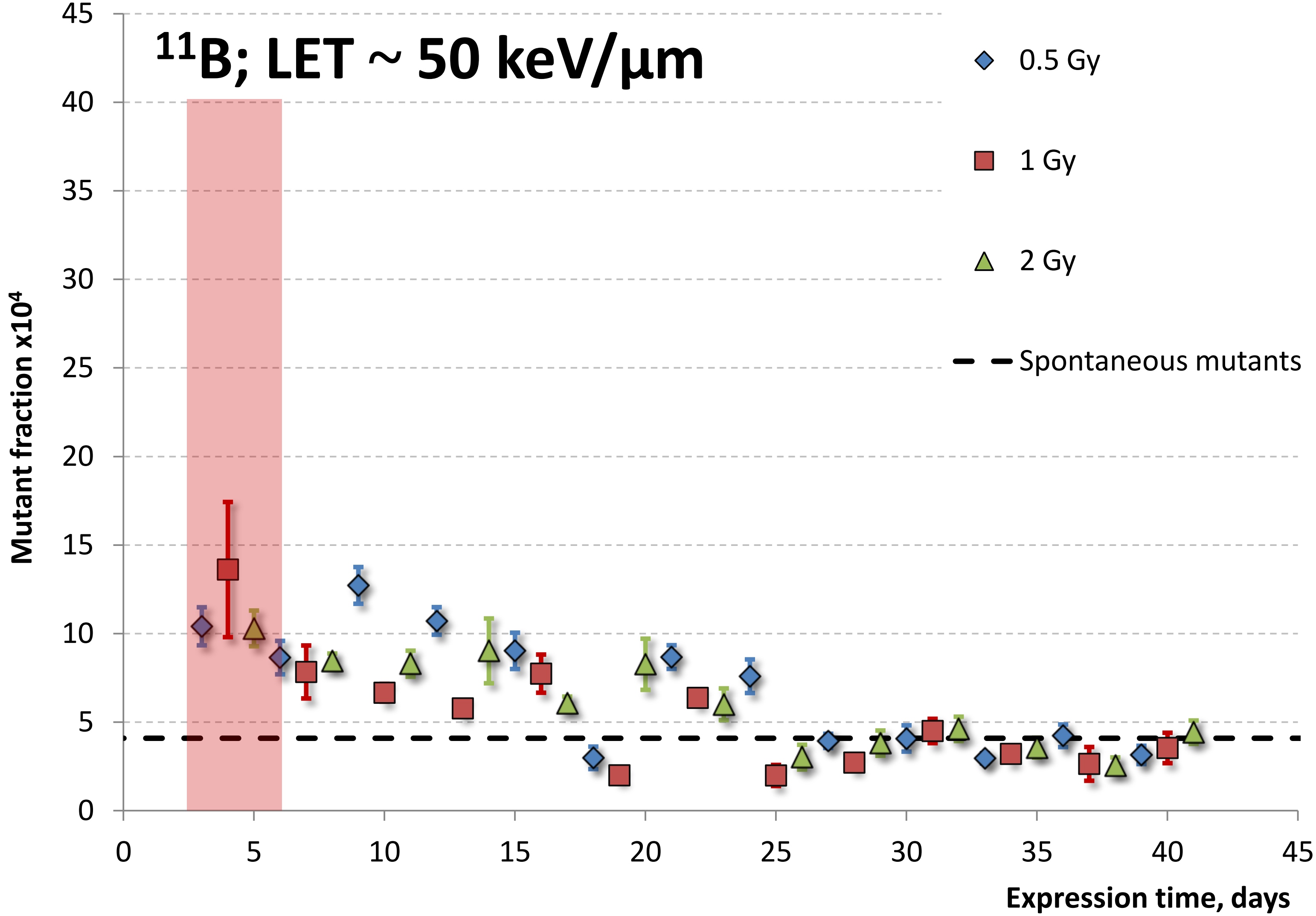


Translocations:
Chromosomes 3 and X
Chromosomes 7 and 21
Chromosomes 7, 12 and 15

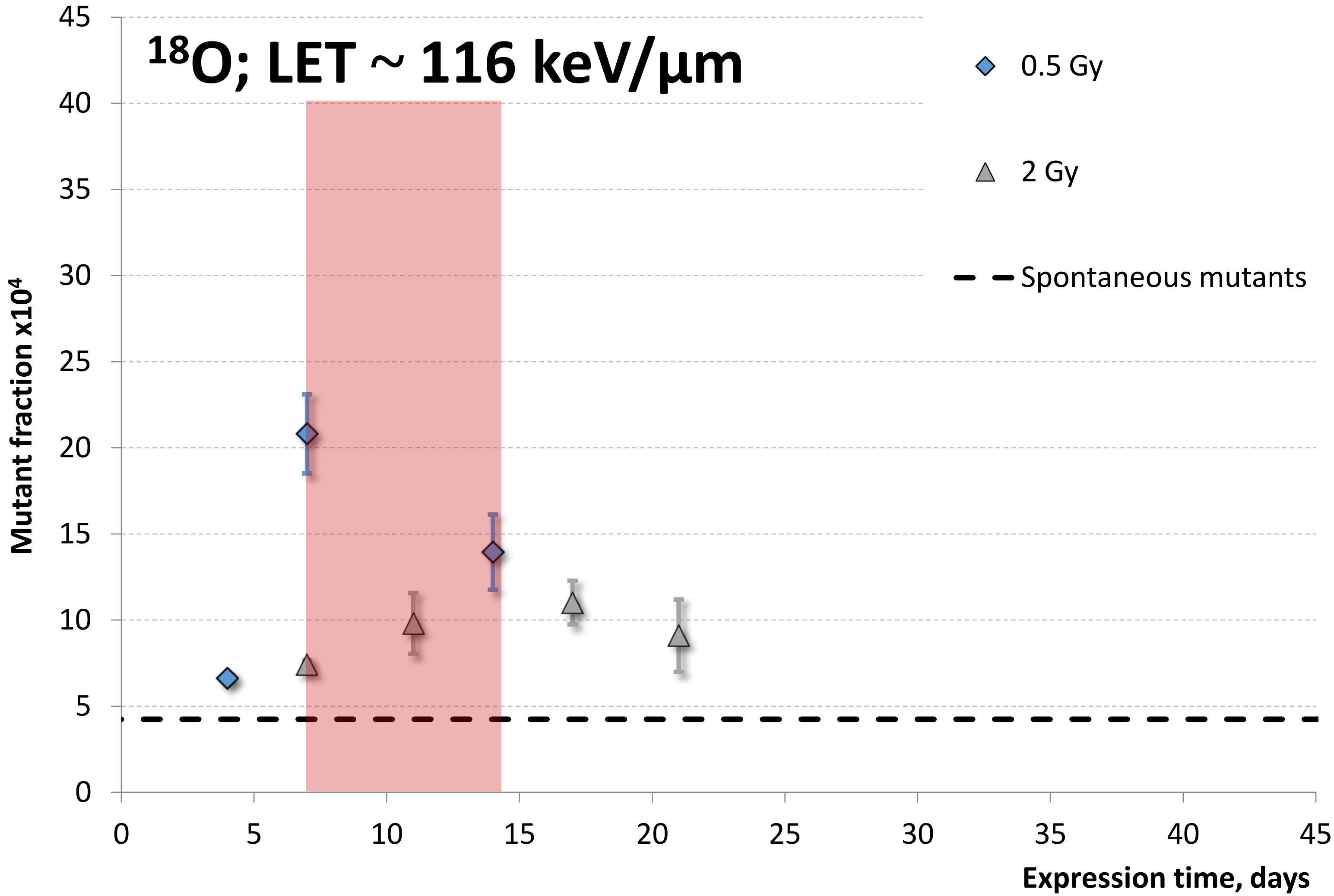
Mutagenesis – V79, HPRT gene



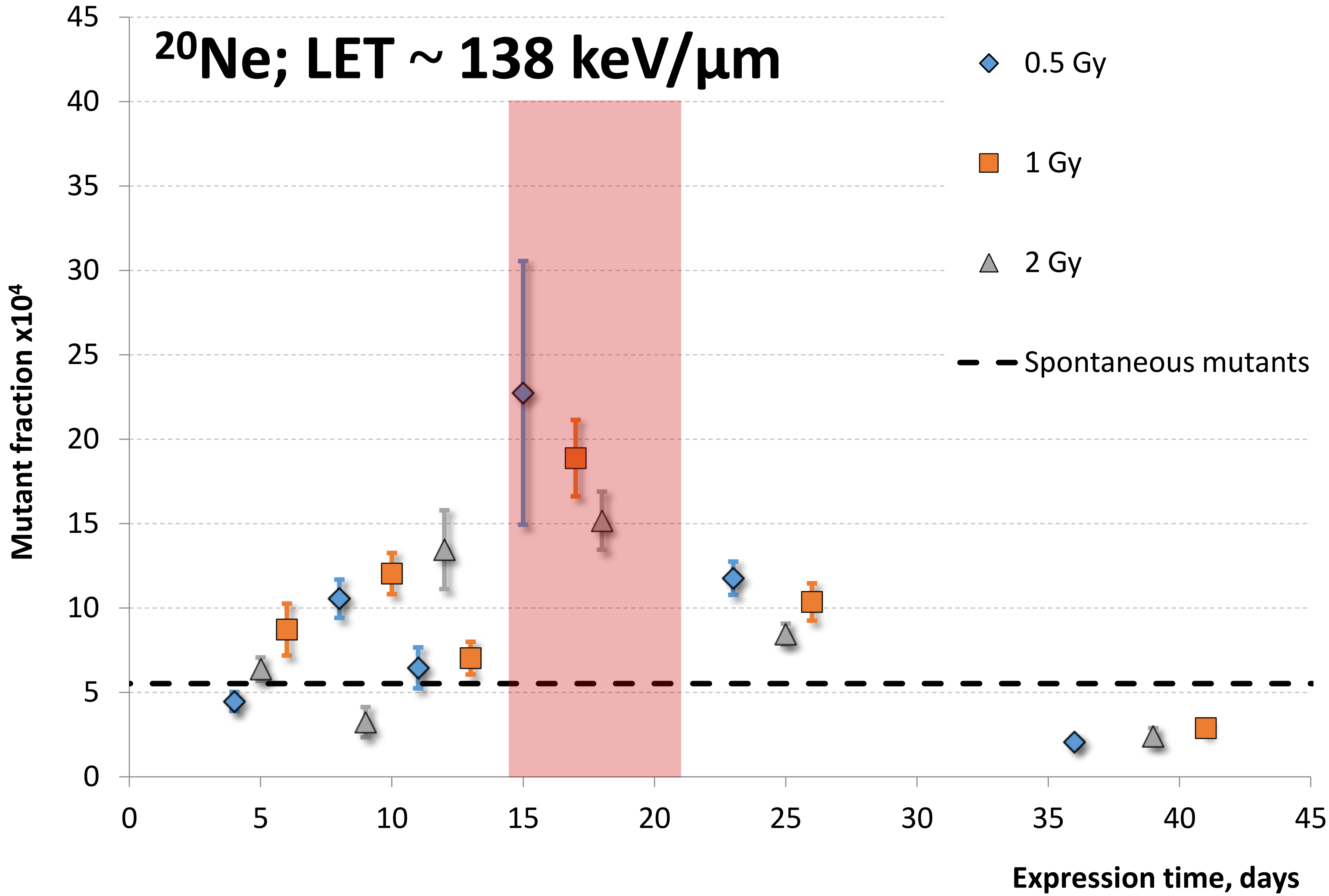
Mutagenesis – V79, HPRT gene



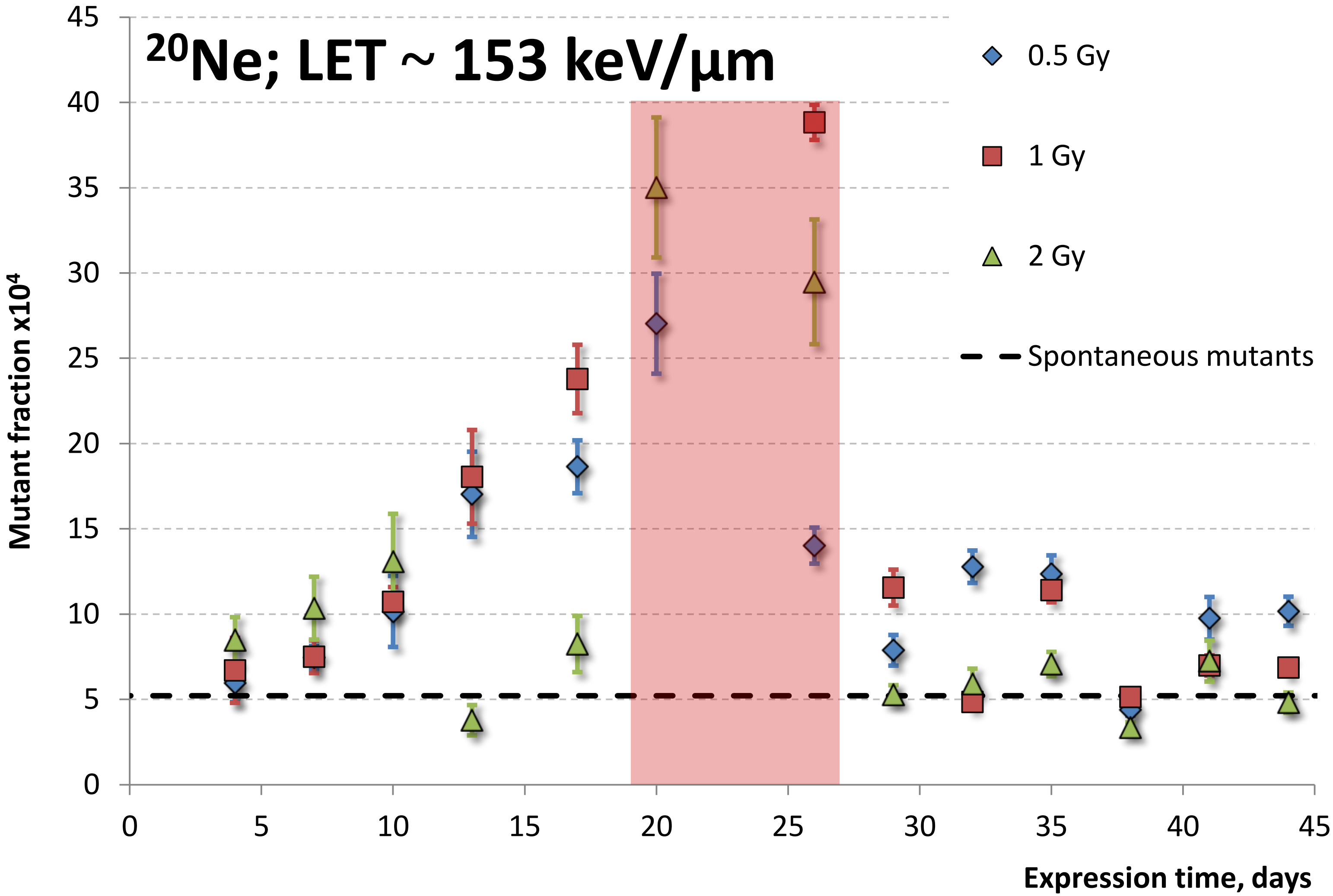
Mutagenesis – V79, HPRT gene

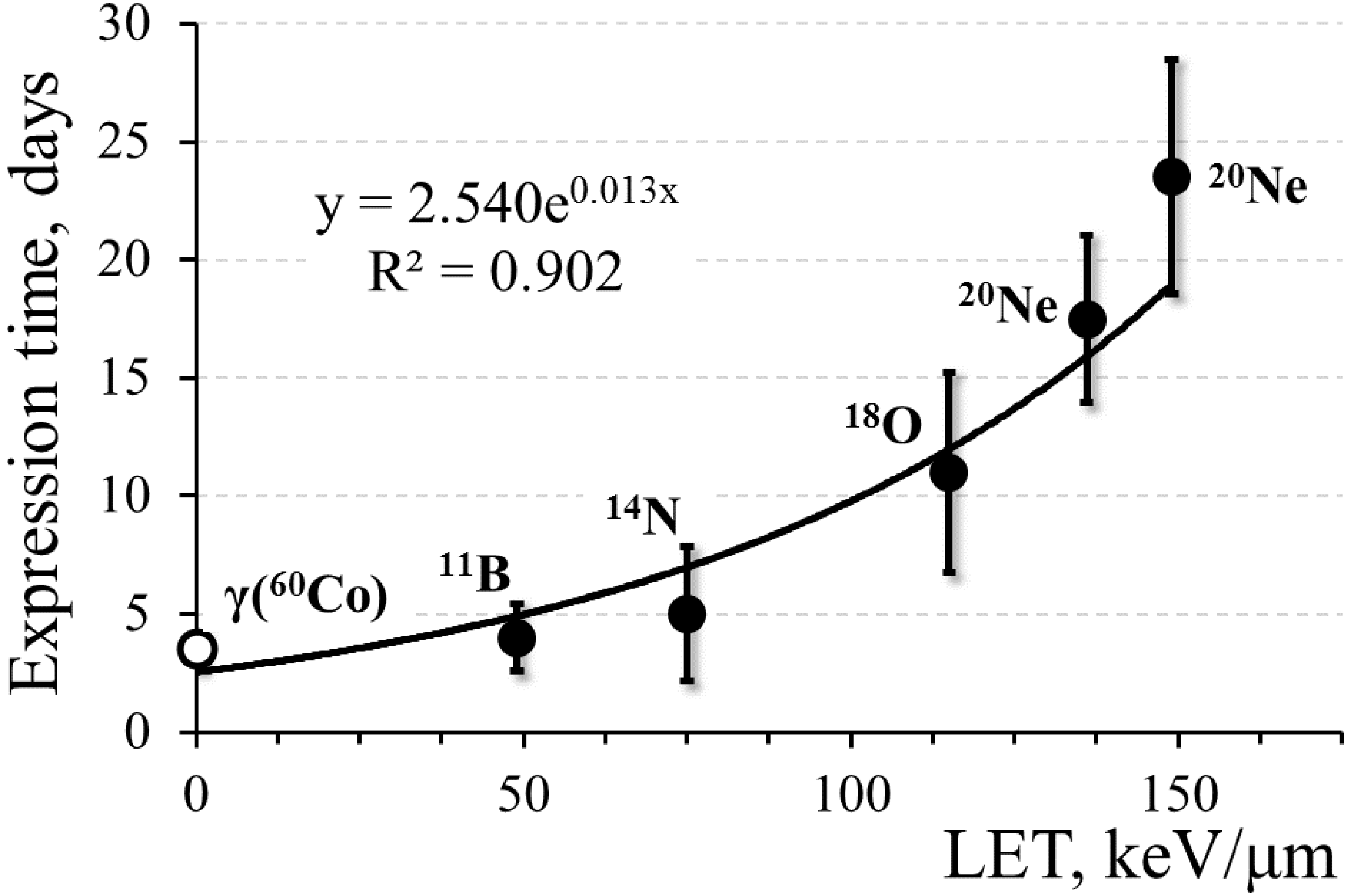


Mutagenesis – V79, HPRT gene



Mutagenesis – V79, HPRT gene

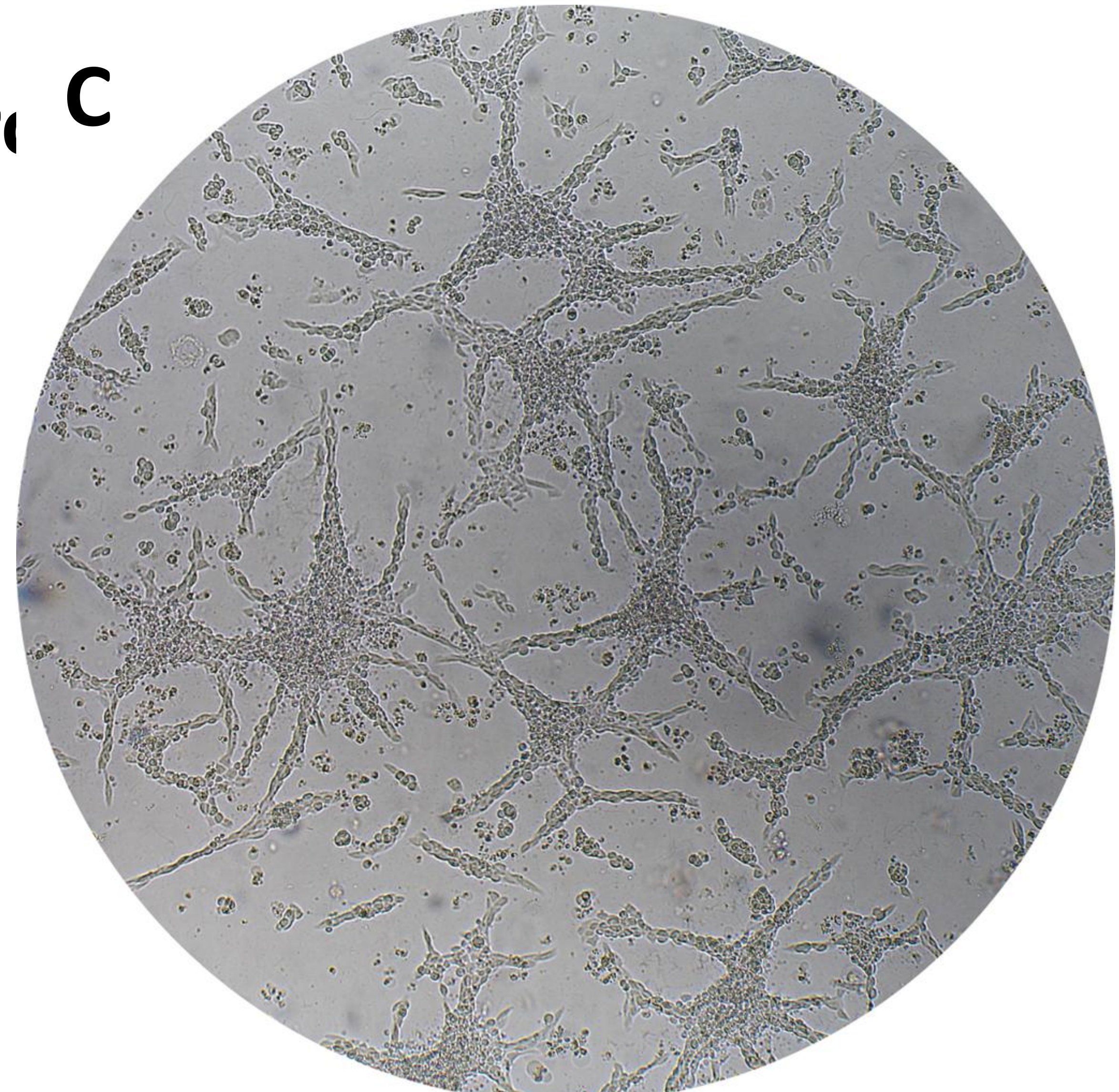




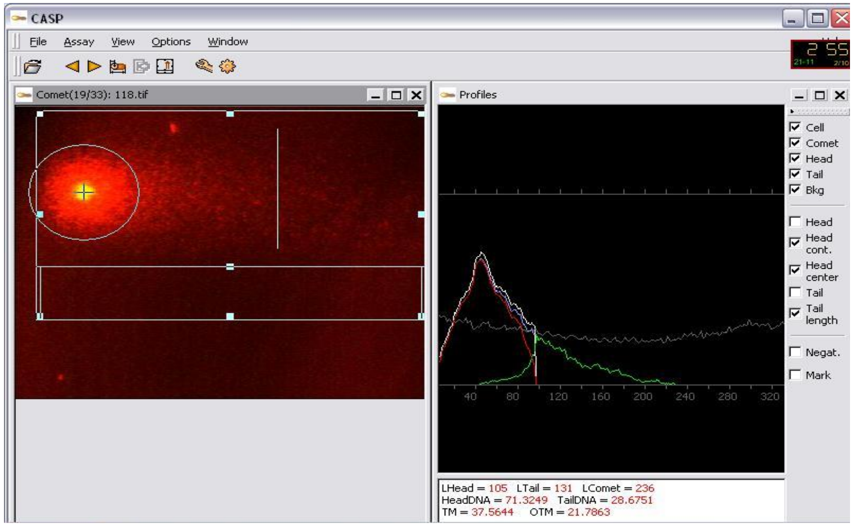
Expression time, days
30
25
20
15
10
5
0
y = 2.540e^0.013x
R² = 0.902
γ(60Co)
11B
14N
18O
20Ne
20Ne
0
50
100
150
LET, keV/μm

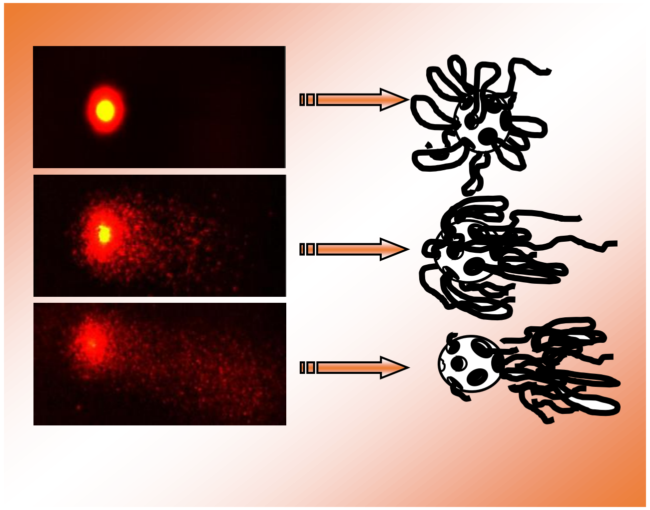
Morphological changes

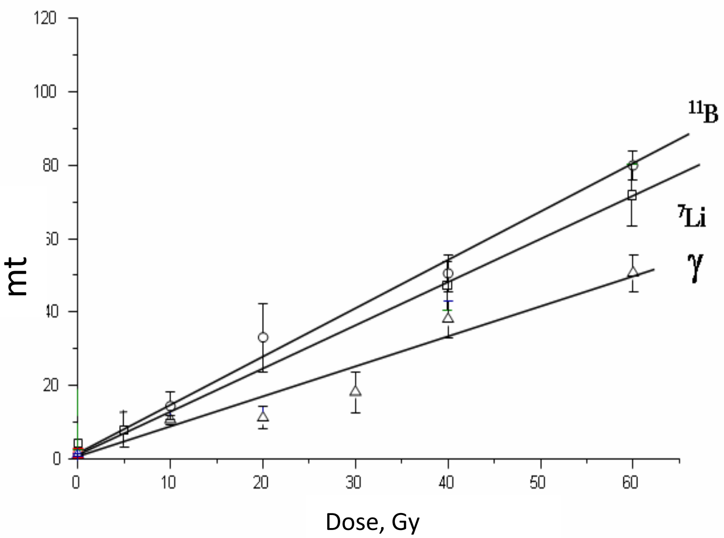
Contr(C

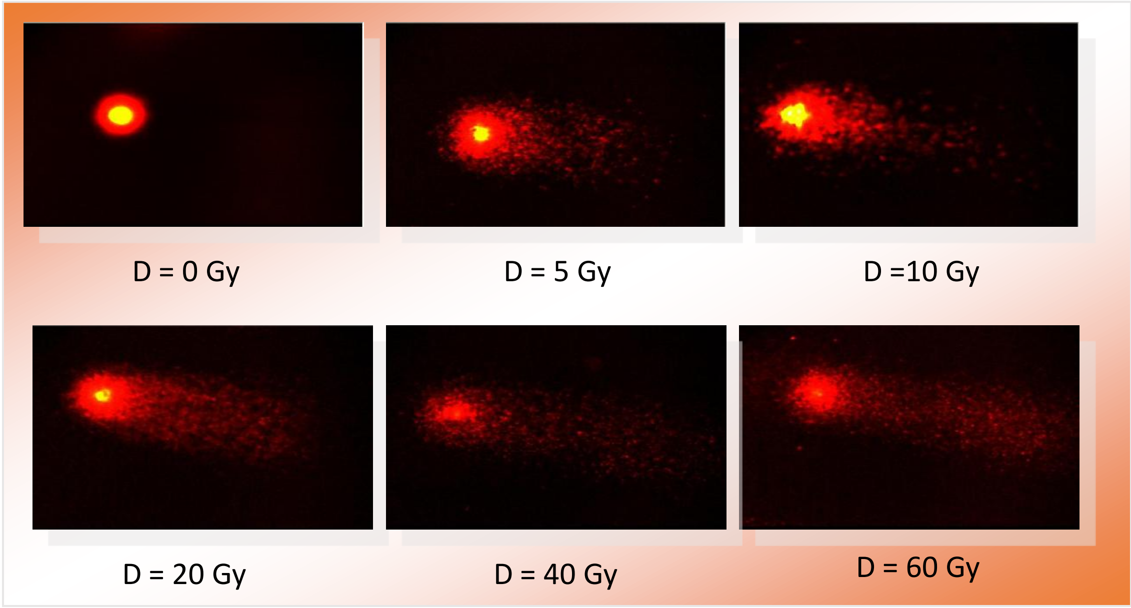


Comet assay method of DNA damage detection





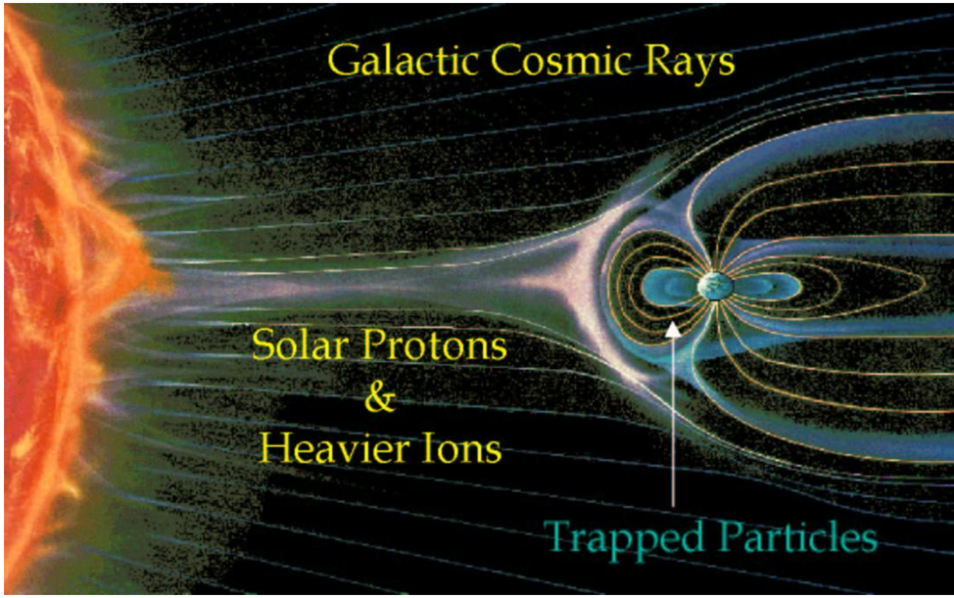




Space radiation

- **Galactic Cosmic Rays** (GCR) – high-energy protons and heavy ions
- **Solar Particle Events** – mainly low and medium-energy protons and electrons
  - Higly variable energy spectra
  - Rare "hard spectrum" events produce elevated fluxes up to ~ 1 GeV.
  - Main problem: **currently unpredictable**

- Trapped Radiation – in Low Earth Orbit
  - Van Allen Belts – trapped low energy protons and electrons



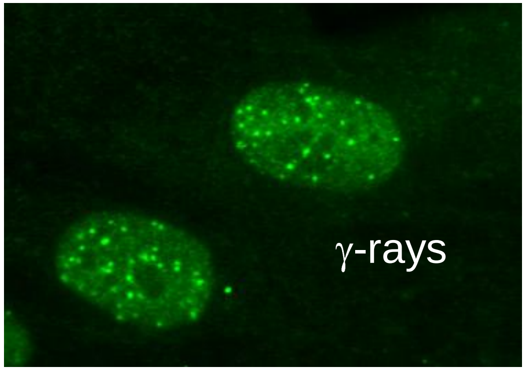
Zeitlinn, 2013 NSRSS

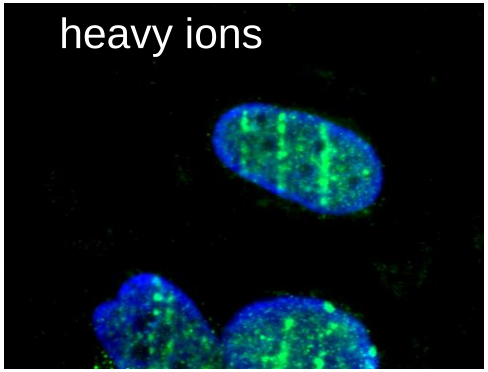
Why heavy ions are so important for space radiobiology?

The heavy ions of GCR (galactic cosmic rays) are the crucial factor of radiation risk for the astronauts during long-time interplanetary flights.

- The composition of GCR (~87 % are protons, ~11% - helium, 1-2 % - heavy ions). However, the contribution of heavy ions to the total equivalent dose of astronauts in the deep space is up to 60 %.
- GCR particles can have extremely high energy and LET and is very difficult to shield an astronaut from them.
- Shielding has excessive costs and will not eliminate galactic cosmic rays (+ **secondary radiation produced in shielding**)
- Unique damage to biomolecules, cells, and tissues occurs from HZE ions that is qualitatively distinct from the radiation on Earth
- **Exceptionally hard to simulate the GCR here on Earth** (extremely low dose-rates; mixture of heavy ions etc.)
- **No human data to estimate risk from heavy ions**

- Estimation of the dose for the Mars space travel (round-trip; **no time on the surface**) from the Curiosity mission:
    - Current technology, shortest round-trip: **0.66 0.12 Sv**
        - ➔ over the NASA limit = under these conditions no astronaut can fly to Mars

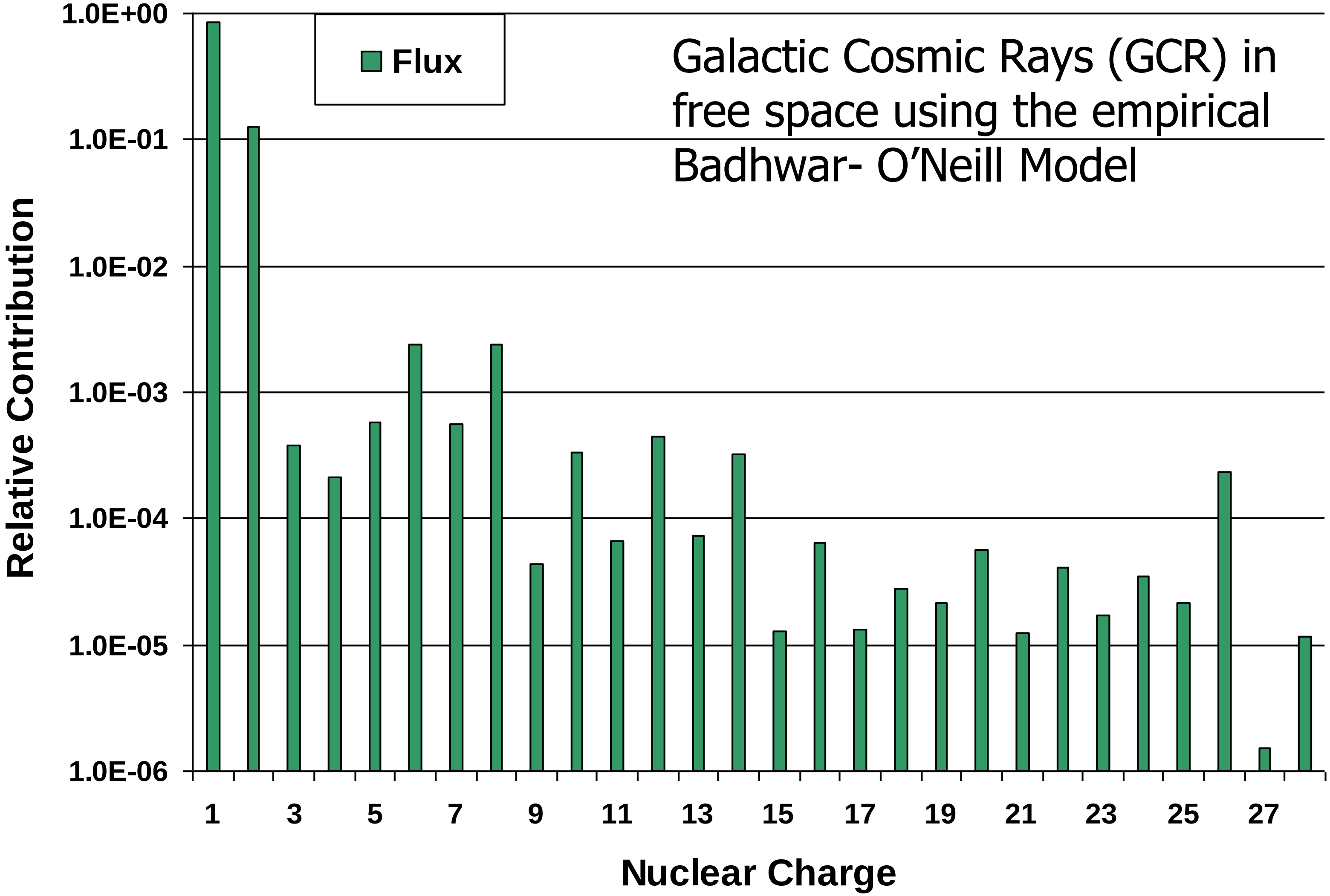




DSB in DNA due to irradiation of human cells by γ-rays and heavy ions.

Zeitlin et al., *Science*, 340, 2013

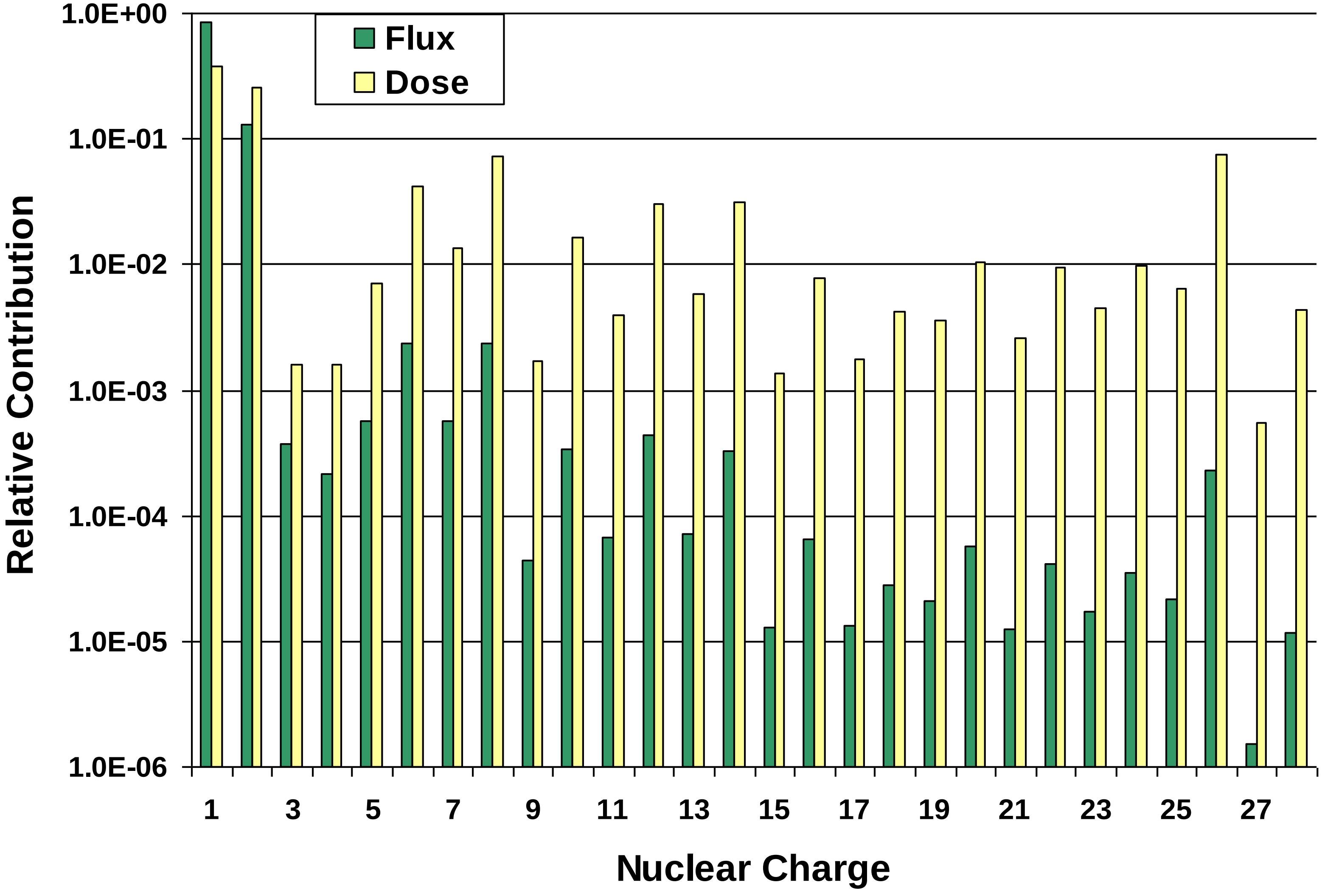
Galactic Cosmic Rays (GCR)



Badhwar-O'Neill GCR Model prediction for near solar minimum – for each species, integrate over energy.

Zeitlin, 2013 NSRSS

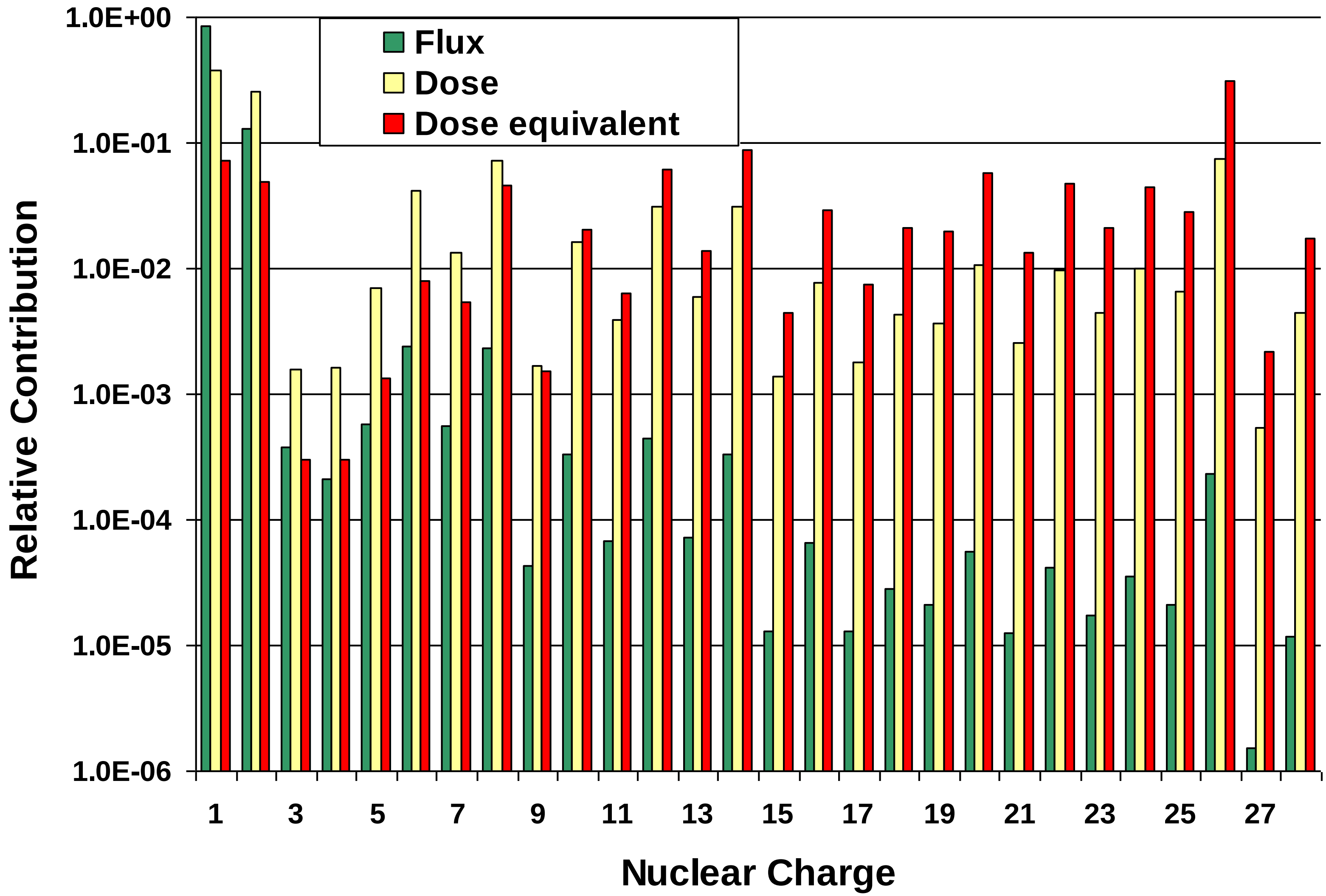
Galactic Cosmic Radiation (GCR)



Abundances weighted by Z^2.

Zeitlin, 2013 NSRSS

Galactic Cosmic Radiation (GCR)



Abundances weighted by $Z^2 \times \langle Q \rangle$.

Zeitlin, 2013 NSRSS

CNS Risks from Galactic Cosmic Rays (GCR)

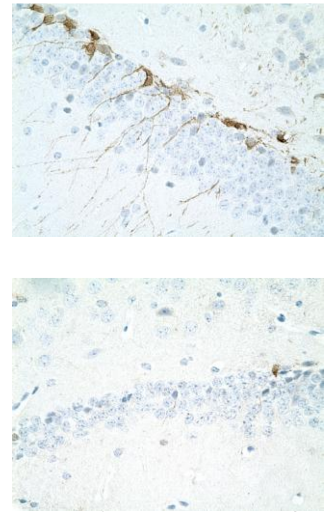
- **Retinal flashes** observed by astronauts (suggests single heavy nuclei can disrupt brain function).
  - Central nervous system (CNS) damage **by x-rays is not observed** except at very high doses
- In-flight cognitive changes and late effects similar to Alzheimer's disease are a concern for GCR.
- Cognitive tests in rats/mice show detriments at **doses as low as 10 mGy** (1 rad)
  - Studies have quantified rate of neuronal degeneration, oxidative stress, apoptosis, inflammation, and changes in dopamine function related to late CNS risks
- Large hurdle remains to establish significance in humans

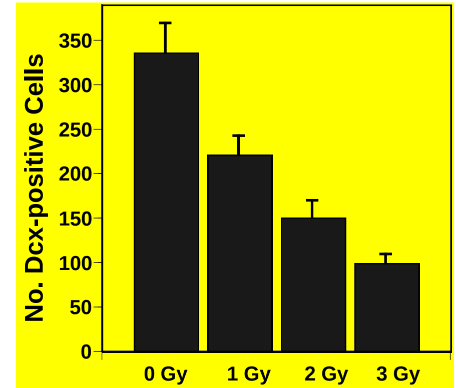
Mars mission

• 2 – 13% of cells would be hit by at least one Fe ion during a Mars mission.

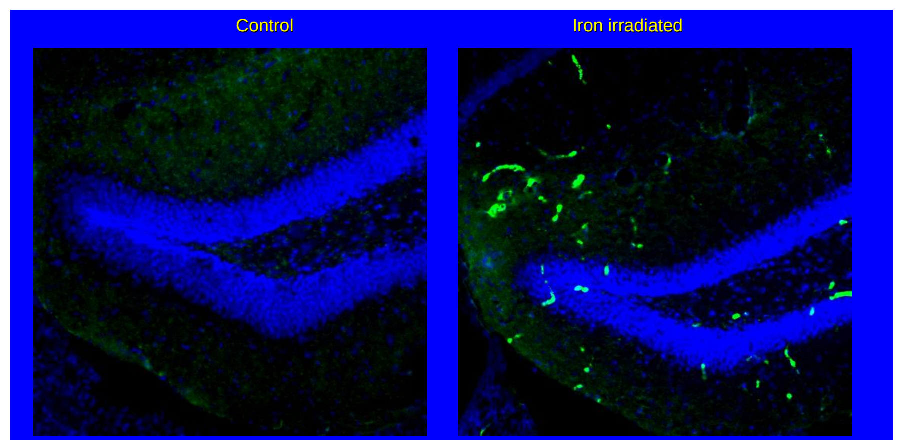
• 8 – 46% of cells would be hit by at least one particle with Z≥15 during a Mars mission.

• Every cell nucleus would be traversed by a proton once every 3





Reduction in number of neurons (neurodegeneration) for increasing Iron doses in mouse hippocampus (J. Fike, UCSF)



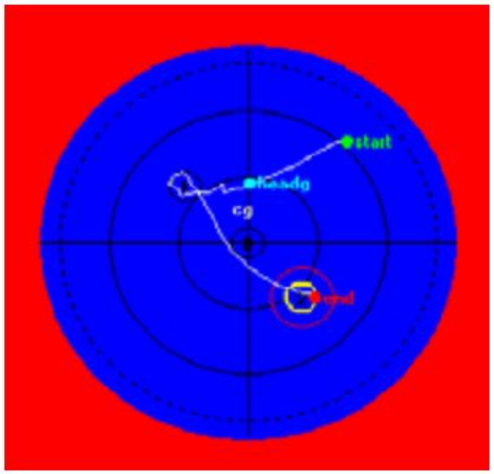
Oxidative Stress (Lipid peroxidation:4-Hydroxynonenal) is Increased in Mouse Hippocampus 9 Months After 2 Gy of ^{56}Fe Irradiation

Cucinotta, 2013 NSRSS

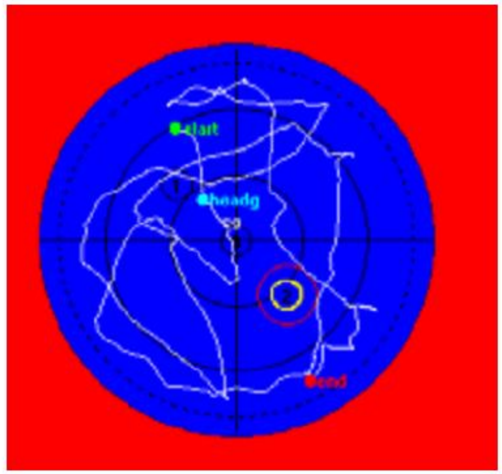
Cognitive test (Morris test)

^{56}Fe ions, 1 GeV/nucleon

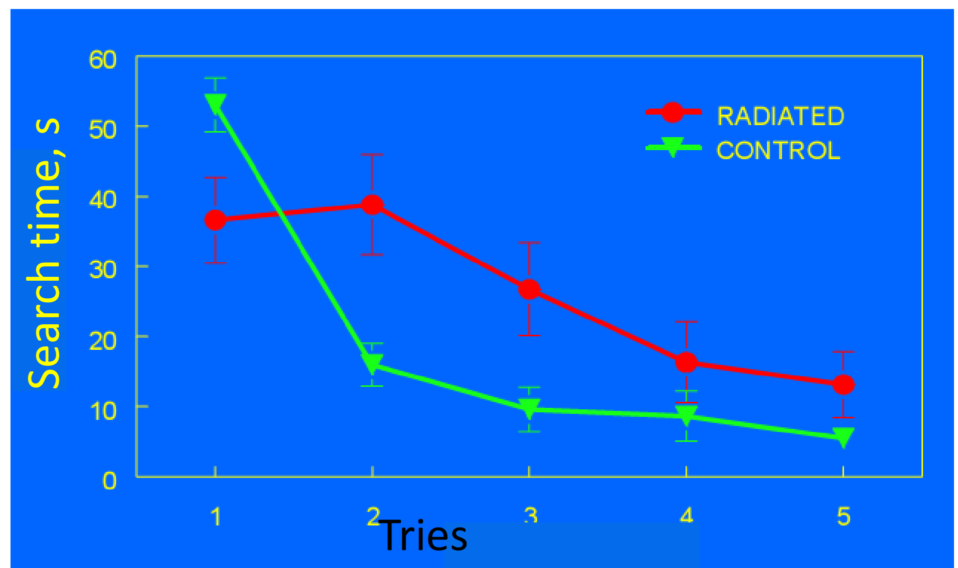
Control



1.5 Gy



1 month after irradiation



Rat 214-126
Morris Water Maze
Learning Test #1

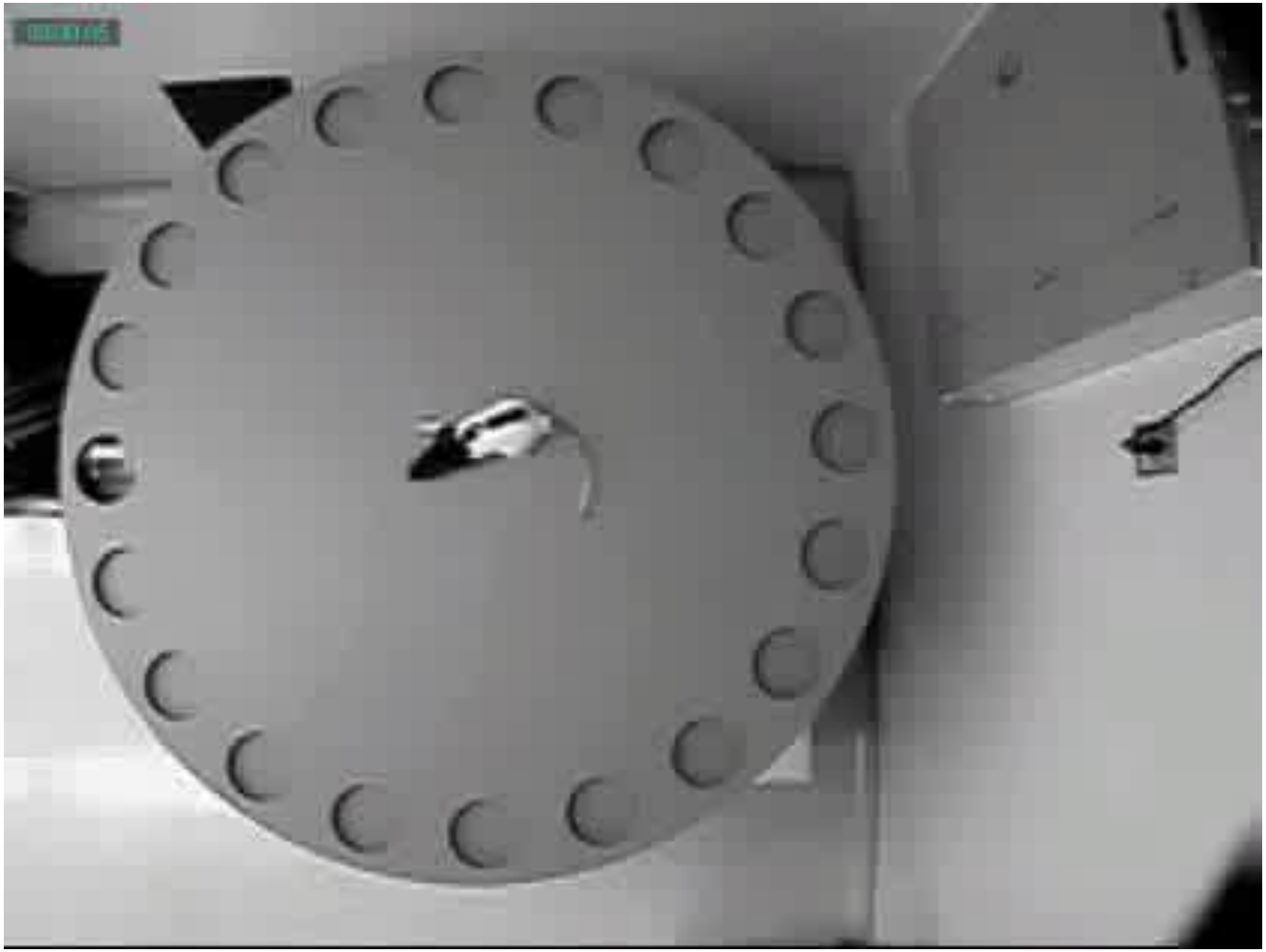
Tracking with:
Noldus Ethovision

Cyclotron Research Center
University of Liege

jepoirrier@ulg.ac.be
http://www.poirrier.be/~jean-etienne/

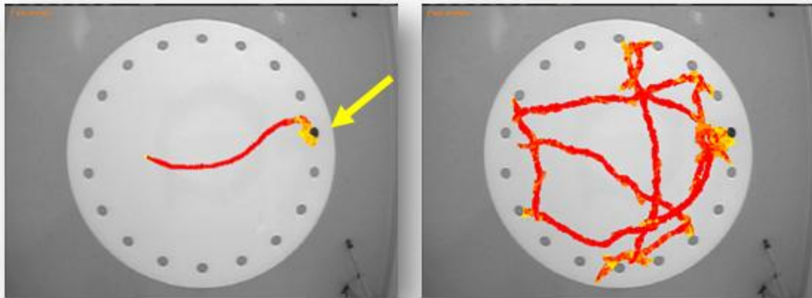
rat CRC

Barnes maze test

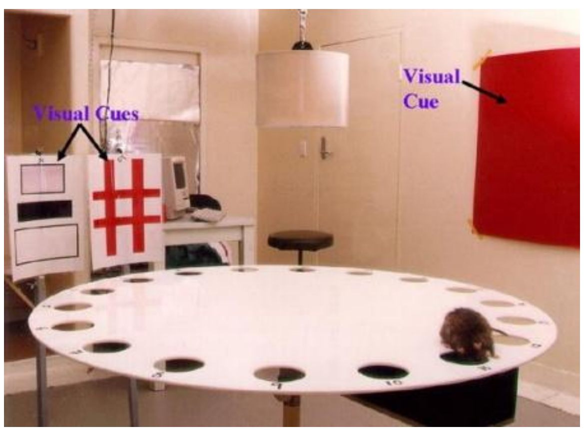


Impairment of spatial cognitive functions after exposure to ^{56}Fe

Control animal | Injured animal



Traces of Barnes maze performance until the mouse escape into the target hole (yellow arrow head)

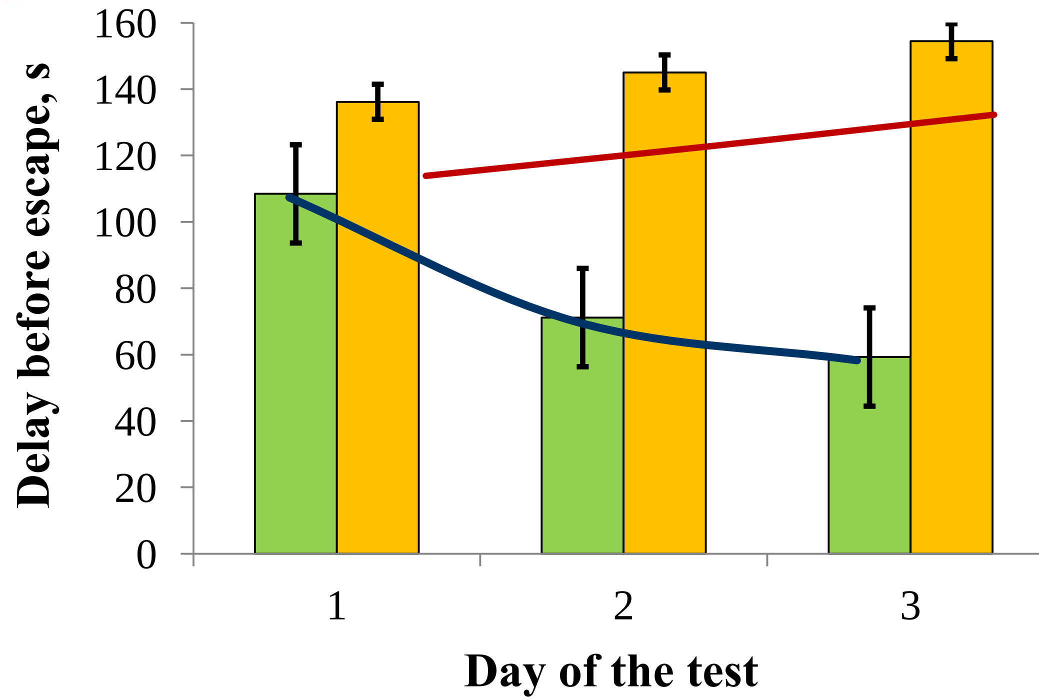


0.2 Gy

^{56}Fe, 1 GeV/n

$\Phi \approx 10^5$ /см^2

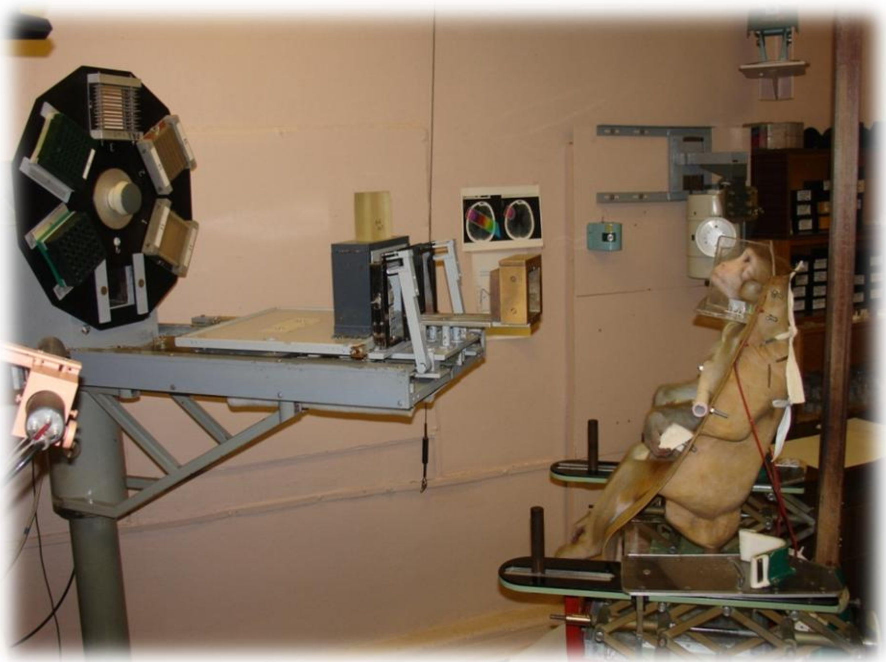
3 months after irradiation



R. Britten et al., 2012

First experiments with monkeys

Irradiation with a proton medical beam, 170 MeV



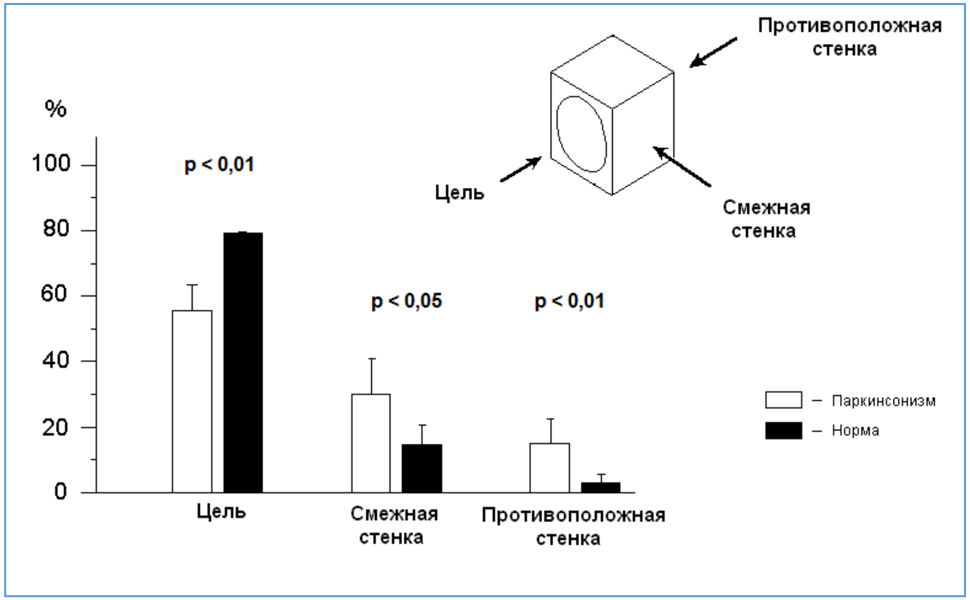
Irradiation with ^{12}C ions, 500 MeV/u, at the Nuclotron

Spatial perception in relation with Parkinson's disease

The right decision

Decision errors

With Parkinson's disease, the animals make more erroneous movements



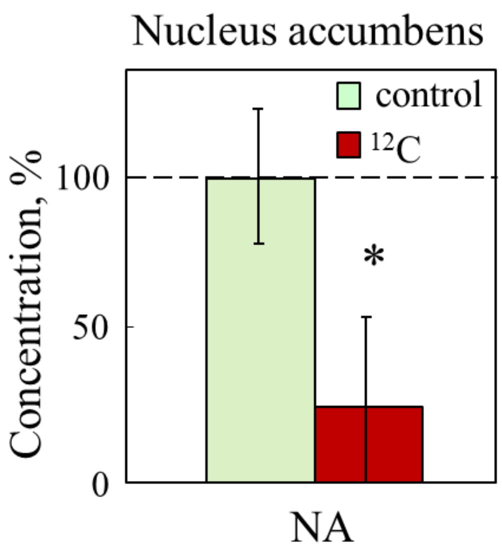
After proton irradiation (3 Gy), significant deviations from the norm were not detected

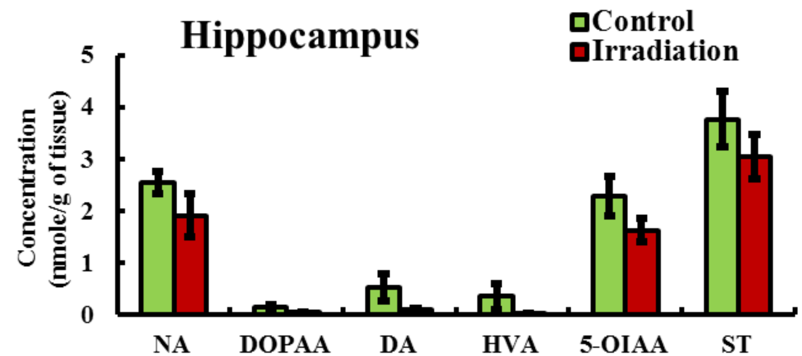
Neurotransmitters levels after irradiation

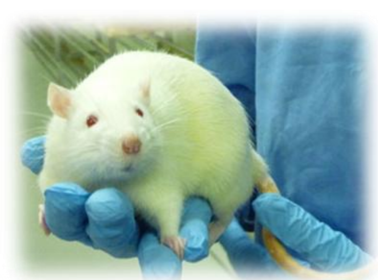
Irradiation with 1 Gy of 500 MeV/u carbon ions

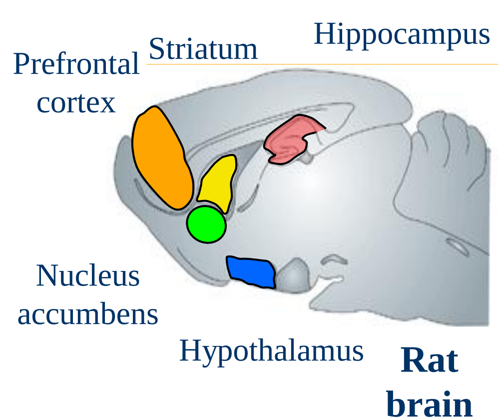
Radiation-induced decrease in the level of neurotransmitters is observed in the brain regions responsible for the *emotional and motivational state*

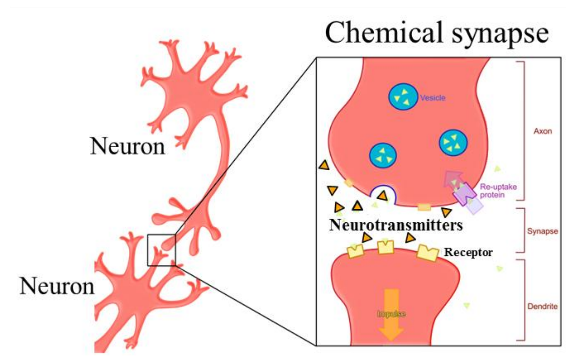
3 months after irradiation



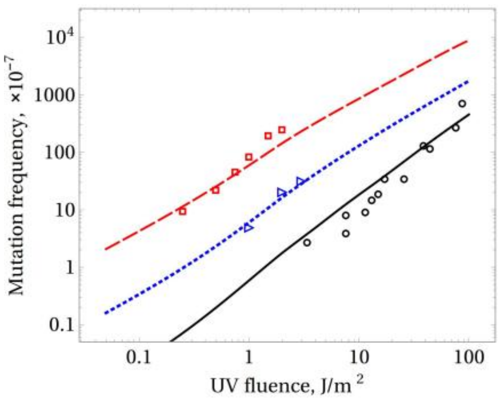


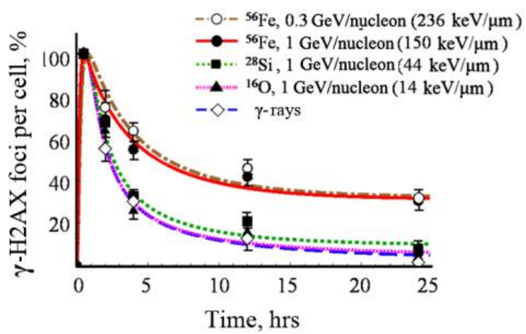






Mathematical modeling



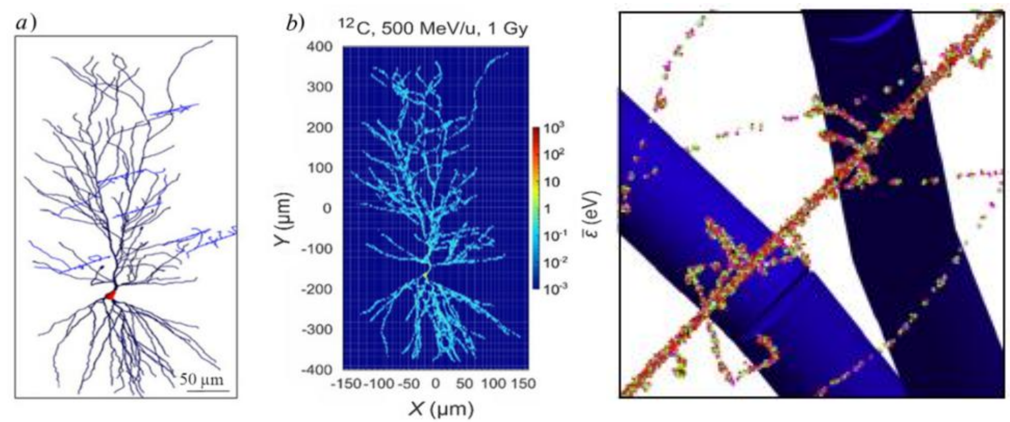


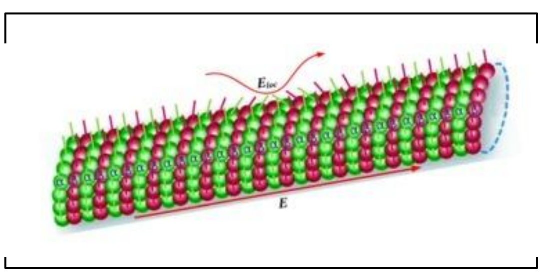
- ❑ Mathematical models of key radiation-induced DNA damage repair systems in bacterial cells were developed.
- ❑ Comprehensive computational study of radiation induced mutagenesis was performed.
- ❑ Detailed model of radiation-induced DNA double-strand break repair in mammalian and human cells was developed

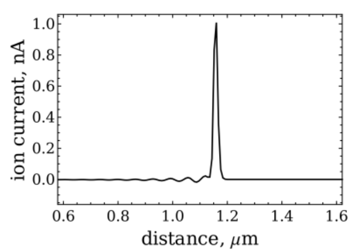
J. Theor. Biol. 2009, 2013, 2015

- ❑ Geant4-DNA toolkit was applied for the simulation of energy deposition processes in charged particle tracks and water radiation chemistry.
- ❑ The estimation of spatial energy and dose distributions, and the yield of radiolytic species was obtained within a single neuron and in a small neural network.

J. Radiat. Res. Appl. Sci. 2015,
Physica Medica. 2016.



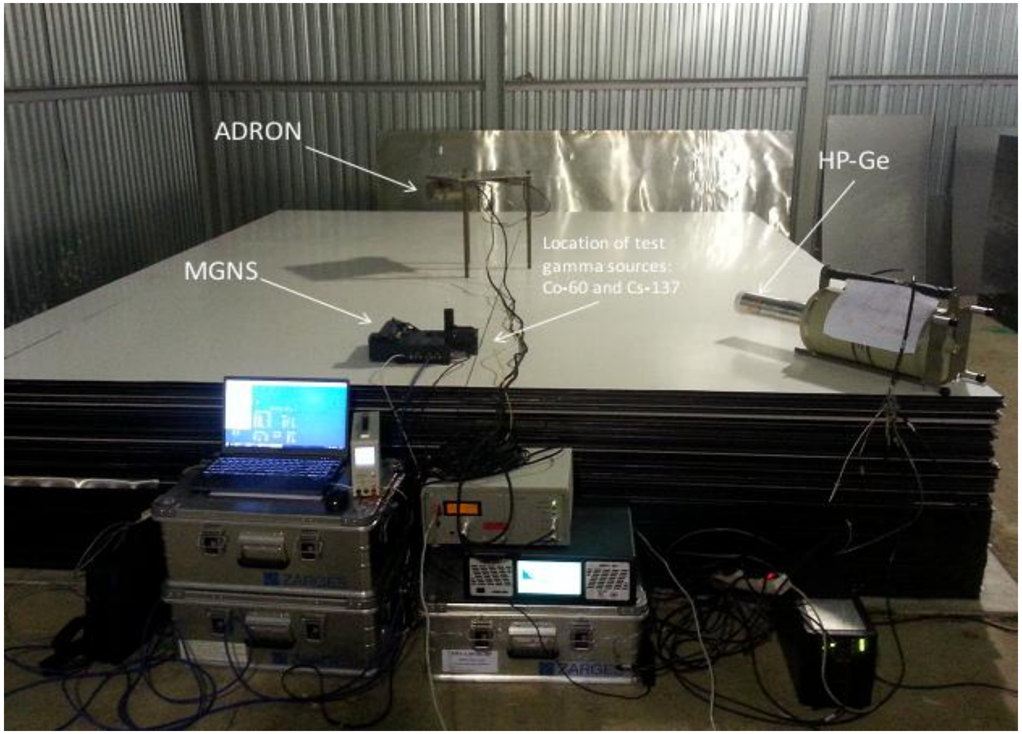




- ❑ Comprehensive models of intracellular signal transport phenomena were developed.
- ❑ The influence of low energy radiation on signals in molecular systems was studied.

Chaos. 2014, 2016, Appl. Math. Comp. 2016

Nuclear planetary science



In collaboration between the Space Research Institute (RAS) and FLNP (JINR), a ***special facility has been constructed*** at the LRB that can ***model planetary soil*** and allows testing prototypes of active neutron and gamma spectrometers.

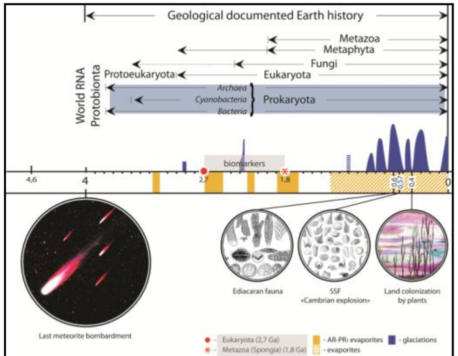
- The Dynamic Albedo of Neutrons (DAN) instrument is currently working on the Mars surface on board of NASA's Curiosity rover **(In cooperation with Space Research Institute RAS)**
  - Helped to find water on the Moon and Mars

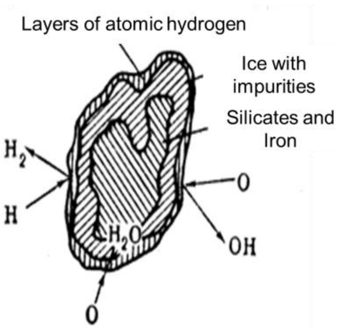
Astrobiology

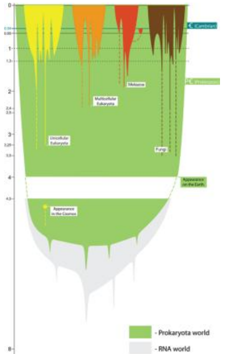
Theme: **"Research on Cosmic Matter on the Earth and in Nearby Space; Research on the Biological and Geochemical Specifics of the Early Earth“**

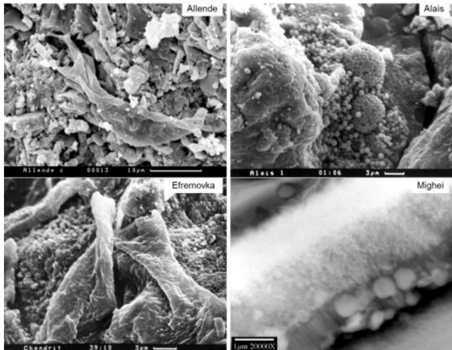
<u>Main fields of activity:</u>

- ❖ Biogeochemical studies of cosmic dust.
- ❖ Studies of biofossils and organic compounds in meteorites and ancient terrestrial rocks.
- ❖ Studies of cosmic matter with nuclear physics methods.

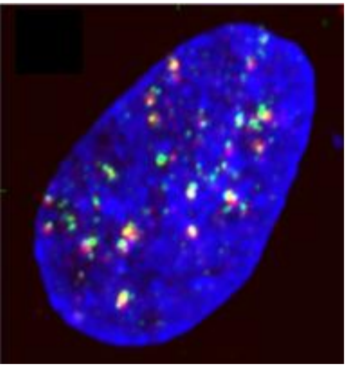
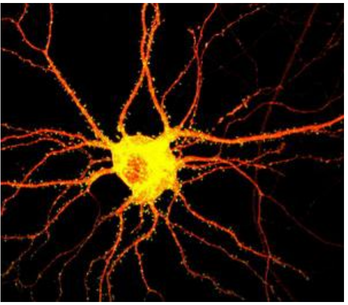




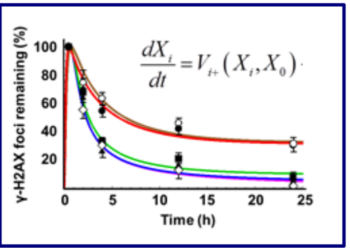




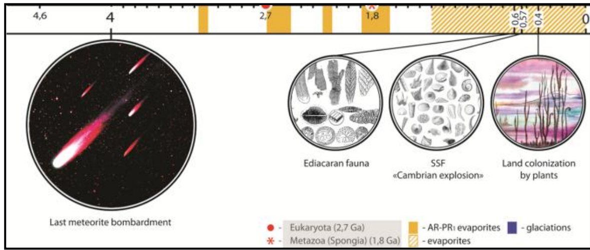
Outlook for radiobiological studies at JINR


- Radiobiology and radiation genetics.
- Studying the effects of heavy ion irradiation on the structures and functions of the central nervous system.
- Neurophysiology.
- Mathematical modeling of the radiation damage of the central nervous system.
- Ground-based experiments for space radiobiology.
- Action of heavy charged particles on eye structures: the lens and retina.
- Astrobiology.





Thank you for your attention!

pavel.blahax@gmail.com