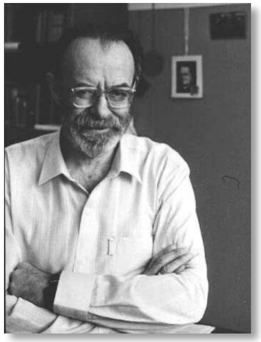
Radiobiological Investigations at Joint Institute for Nuclear Research

Pavel Bláha
Group of Radiation Cytogenetics
Laboratory of Radiation Biology, JINR
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
  - Phasotron

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



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

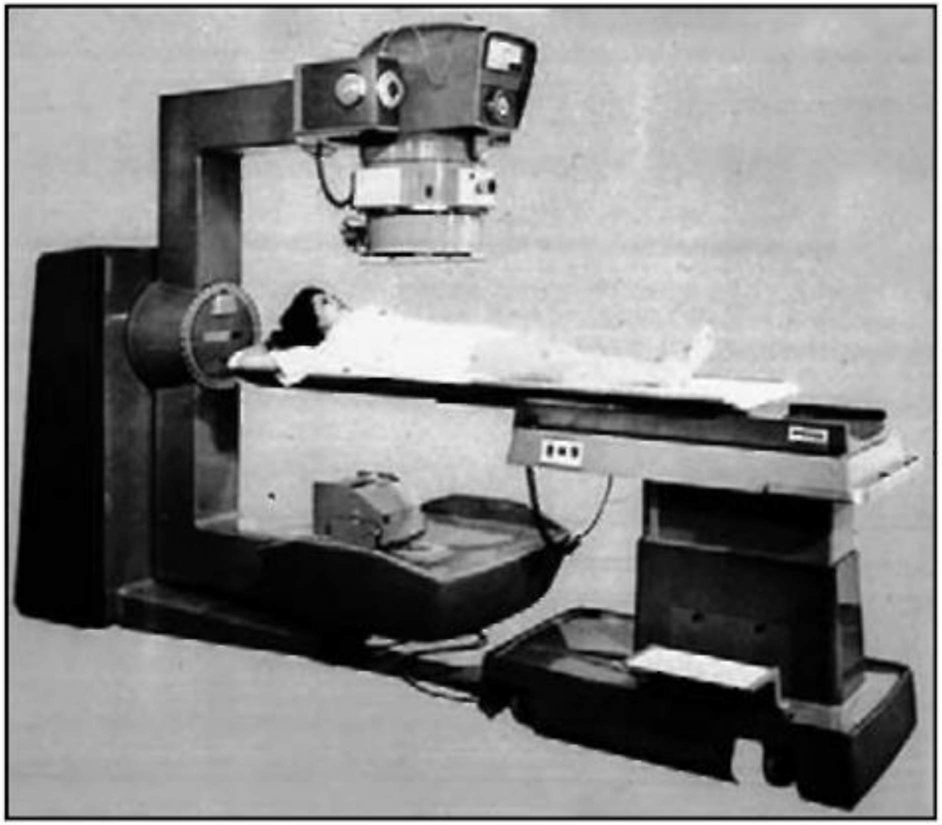
- **1995.** Establishment of JINR's **Department of Radiation and Radiobiological Research**

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

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

Irradiation possibilities

- Gamma sources
  - Rokus-M (^{60}Co) →
- Protons
  - Phasotron
- Heavy charged particles
  - U400M, Nuclotron



Phasotron (Synchrocyclotron) - LNP

Protons with
energies
up to 660 MeV

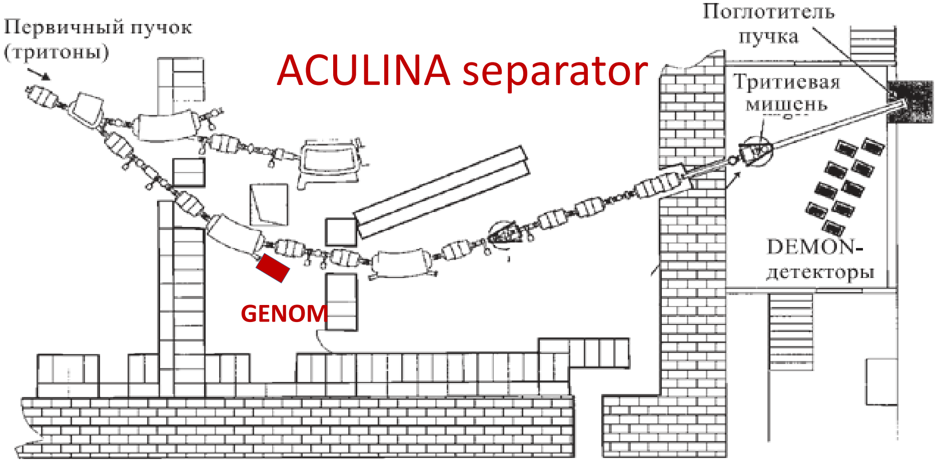
U-400M - LNR

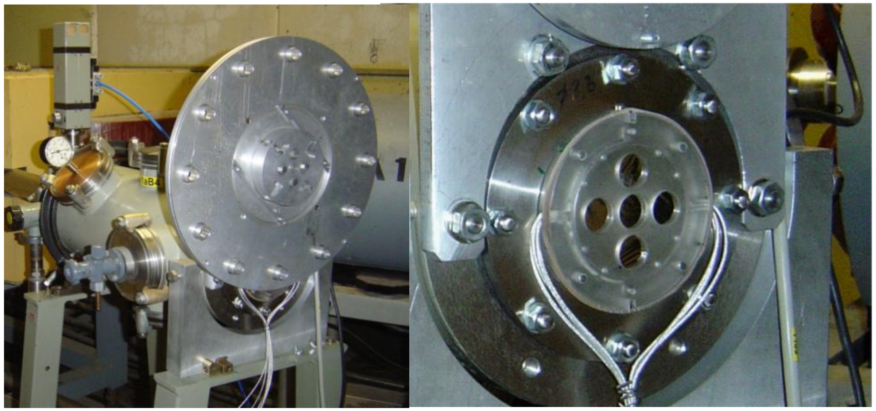
Heavy ions

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

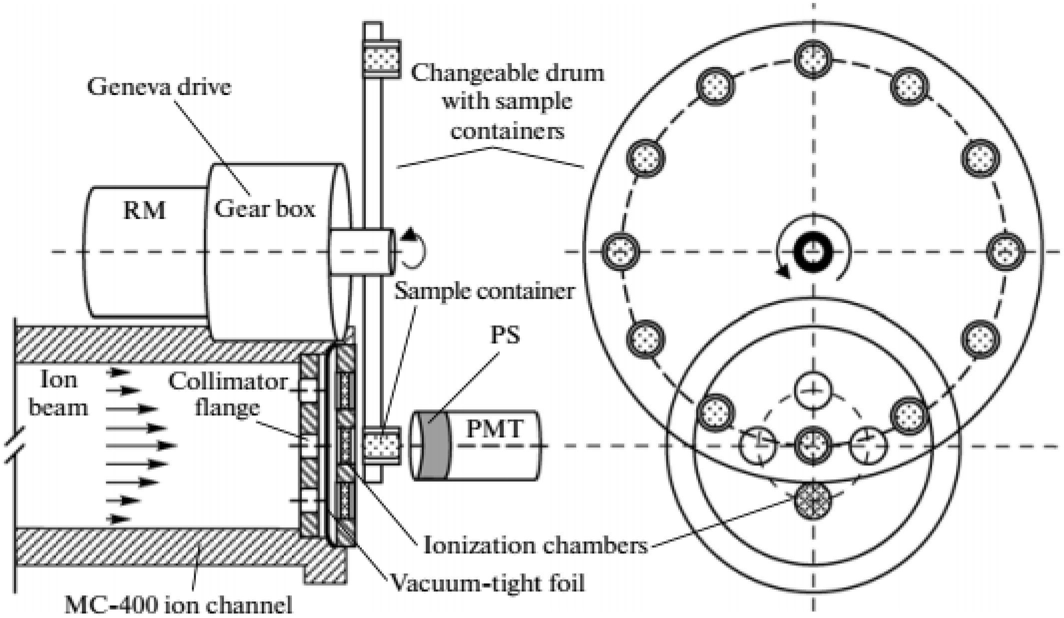
Energy around
50 MeV/nucleon

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



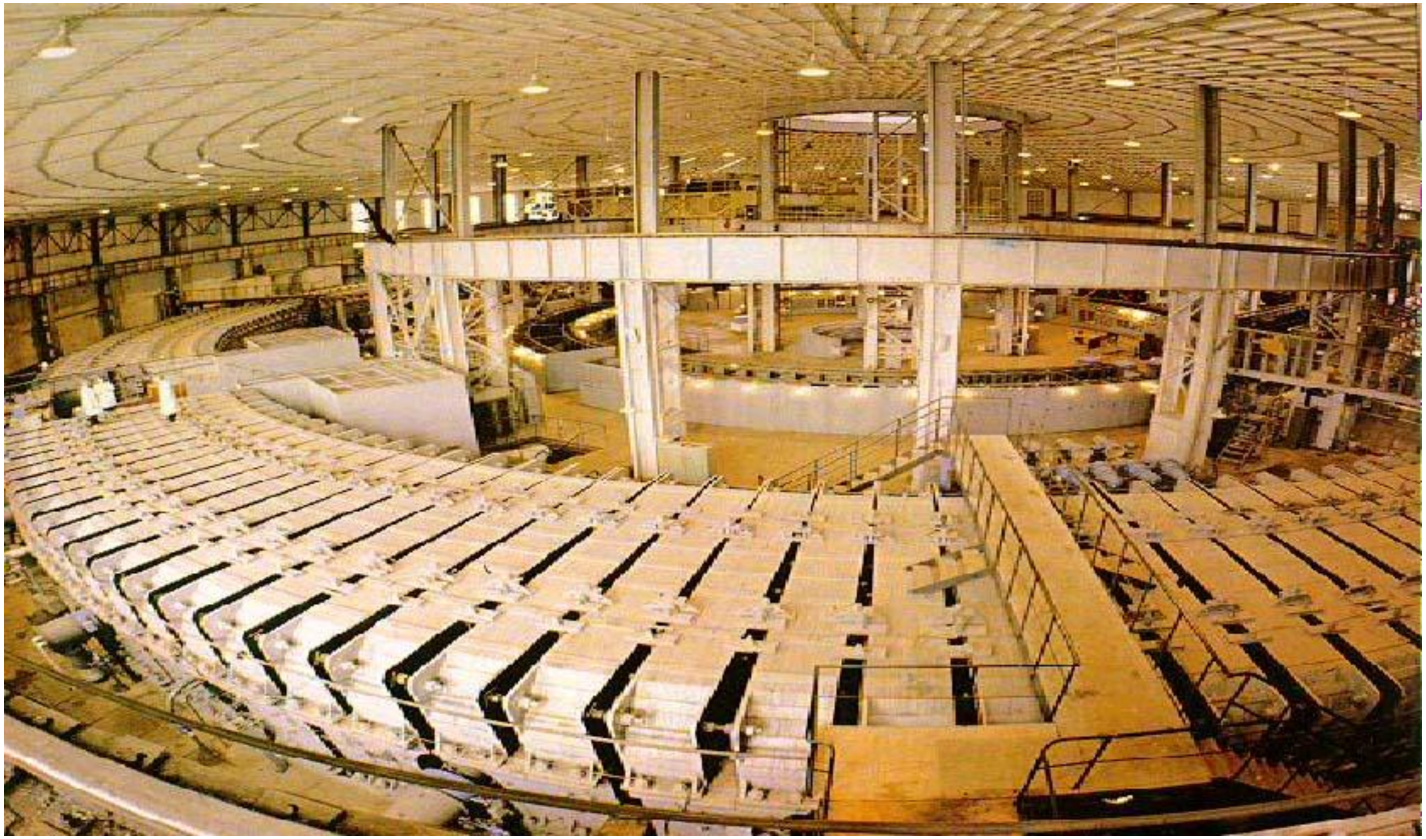


Fast automatic irradiation of thin biological samples (or small volumes of suspension) with high LET heavy ions in a wide range of absorbed doses



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

Synchrophasotron - LHEP



1957 - 2003
Acceleration of protons (up to 10 GeV per 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.

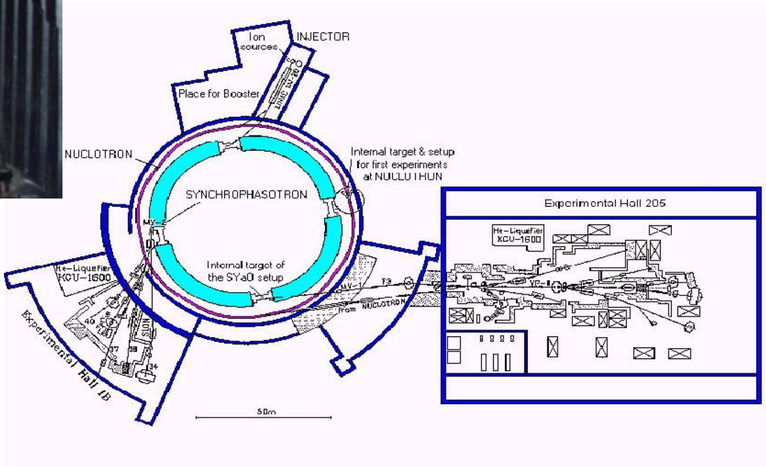
Nuclotron - LHEP

Heavy ions up to

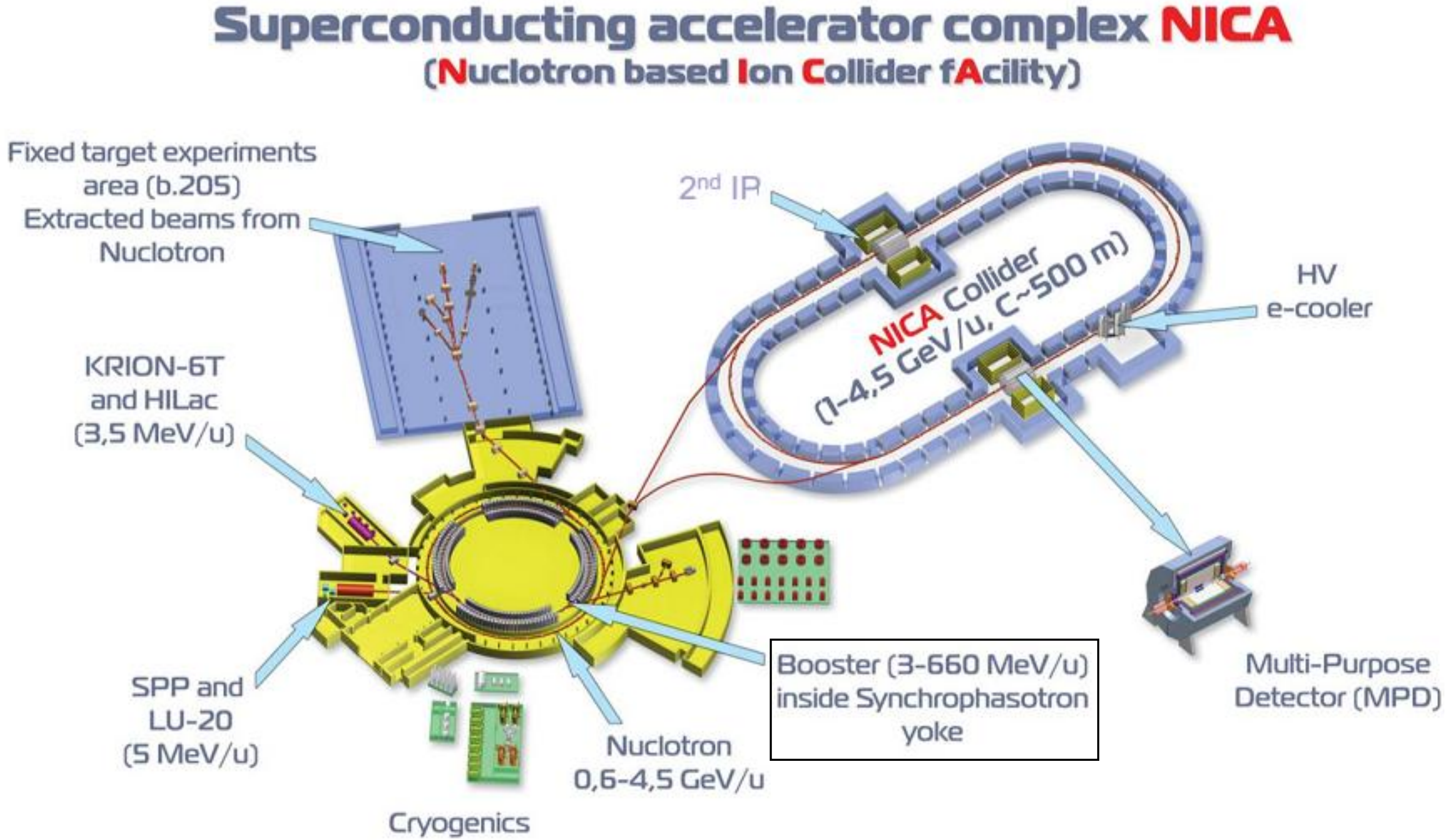
$^{197}Au^{79+}$

Energy:

0,6 – 4,5 GeV/amu



Future: Booster + NICA



Booster (3-660 MeV/u) inside Synchrophasotron yoke

Why ionizing radiation?

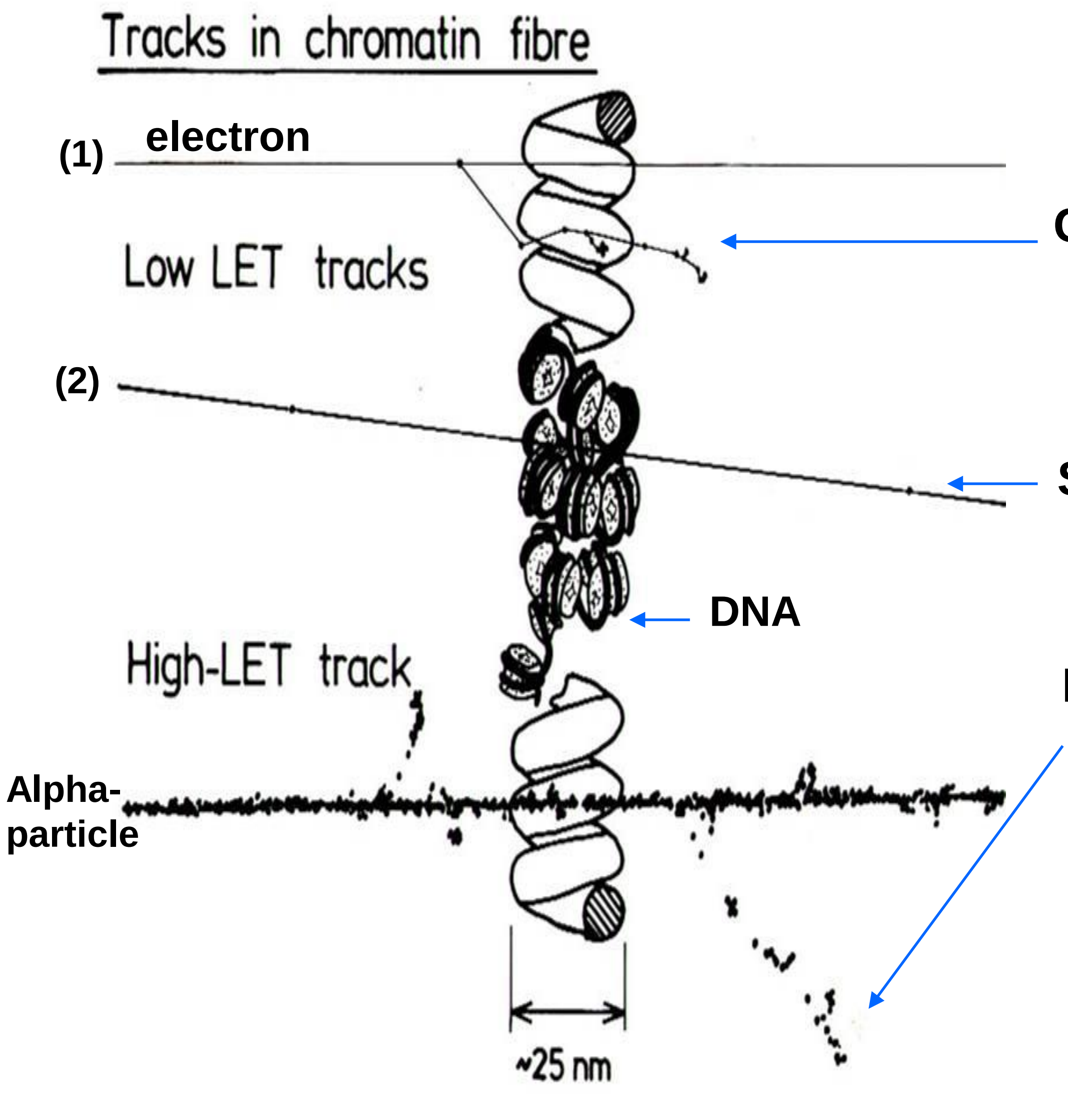
- Ionizing radiation is an **extraordinarily** efficient agent for causing biological effects

- Radiobiological paradox:
  - 1 teaspoon of tea (~ 5 g) at 85 °C will release around 1000 J of thermal energy in your body
    - ($E \sim 4{,}18 \times 10^3$ [J/kg*°C] * 5×10^{-3} [kg]* 48 [°C] ~ **1000 J**)

- $Dose = \frac{energy\ deposited}{mass\ of\ target}$
- 100 kg man
- D = 1000 J / 100 kg = **10 Gy** - twice of the lethal dose

- **Less than 1 microgram of Po-210** can kill a man! - more toxic than any known poison (Litvinenko case)

Radiation track structure



(1) electron

(2)

Clustered ionizations from low-energy electron

Single ionization

DNA

Delta-ray electron

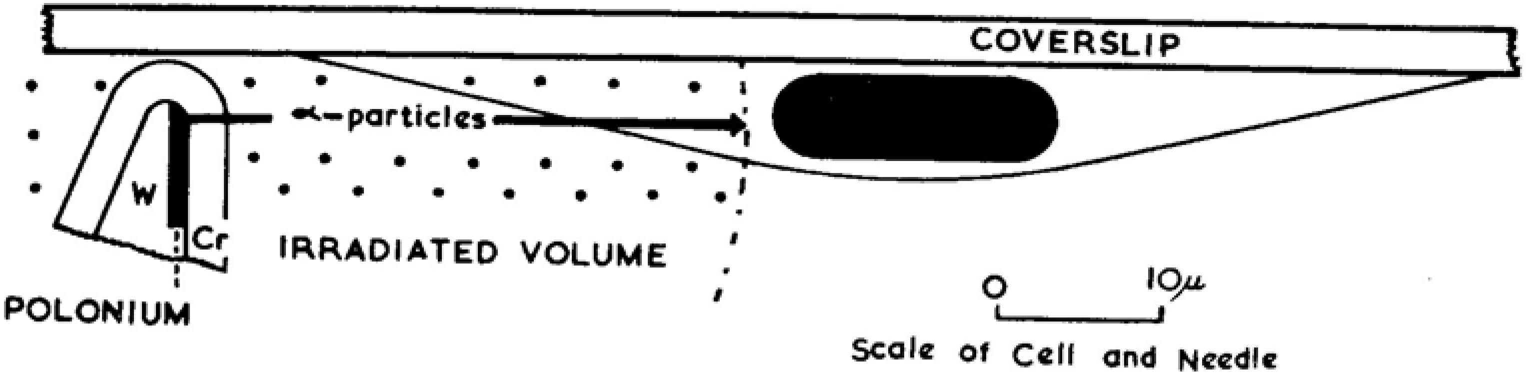
Alpha-particle

Non-random distribution of damage

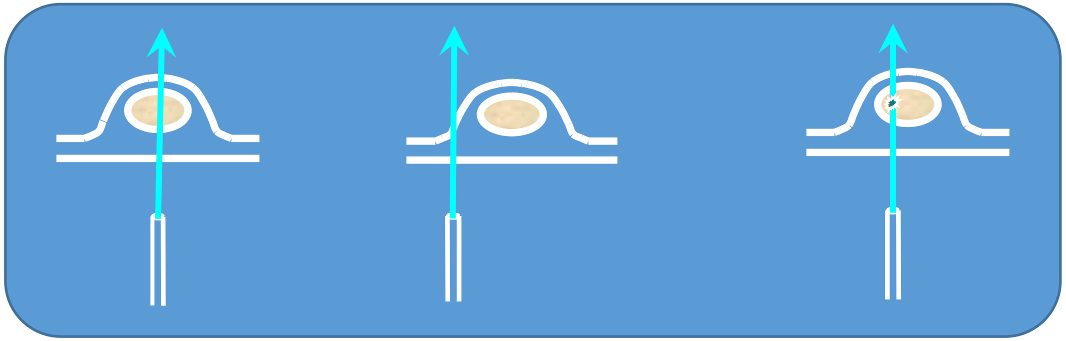
Goodhead, 2013 NSRSS

Primary target - DNA

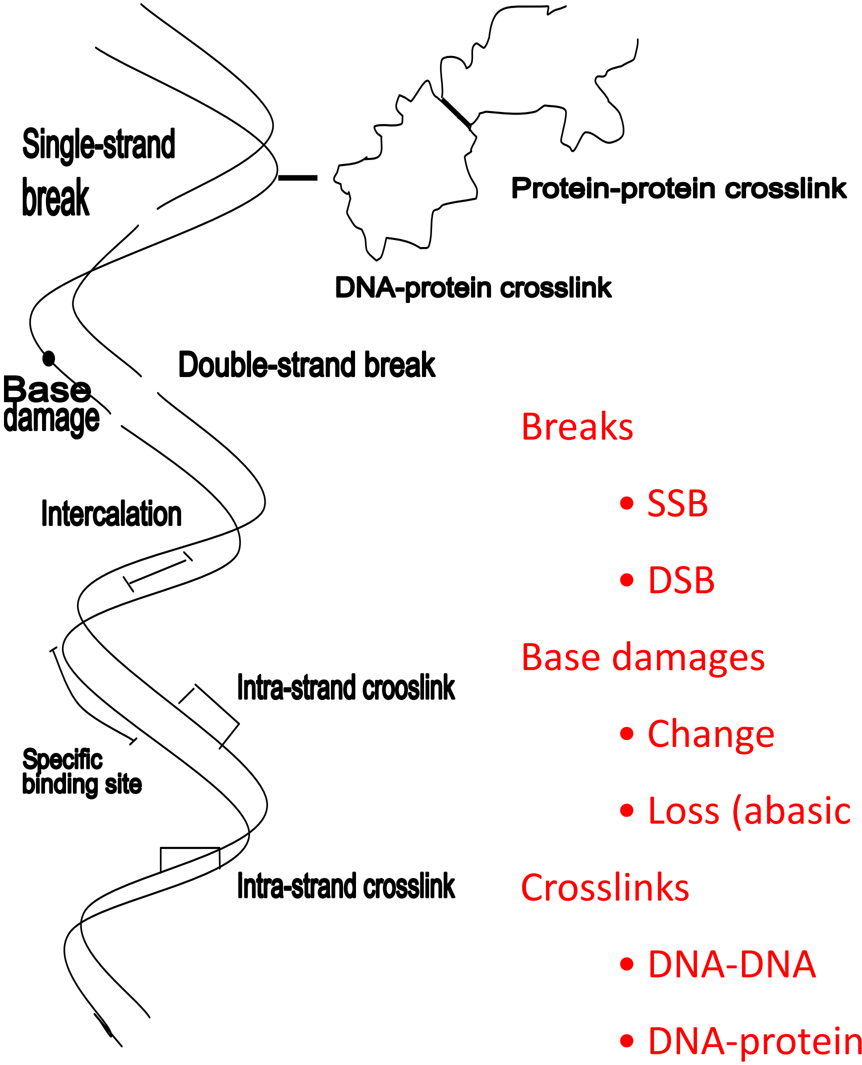
- Irradiation of the cell nucleus is much more effective in the **inactivation of cell proliferative capacity** in comparison to irradiation of cytoplasm
  - Polonium needle experiment - Munro T.R., Radiat. Res., 42 (451), 1970



  - Microbeam irradiation of the specific part of the cell



IR damage to DNA



Breaks

- SSB
- DSB

Base damages

- Change
- Loss (abasic sites)

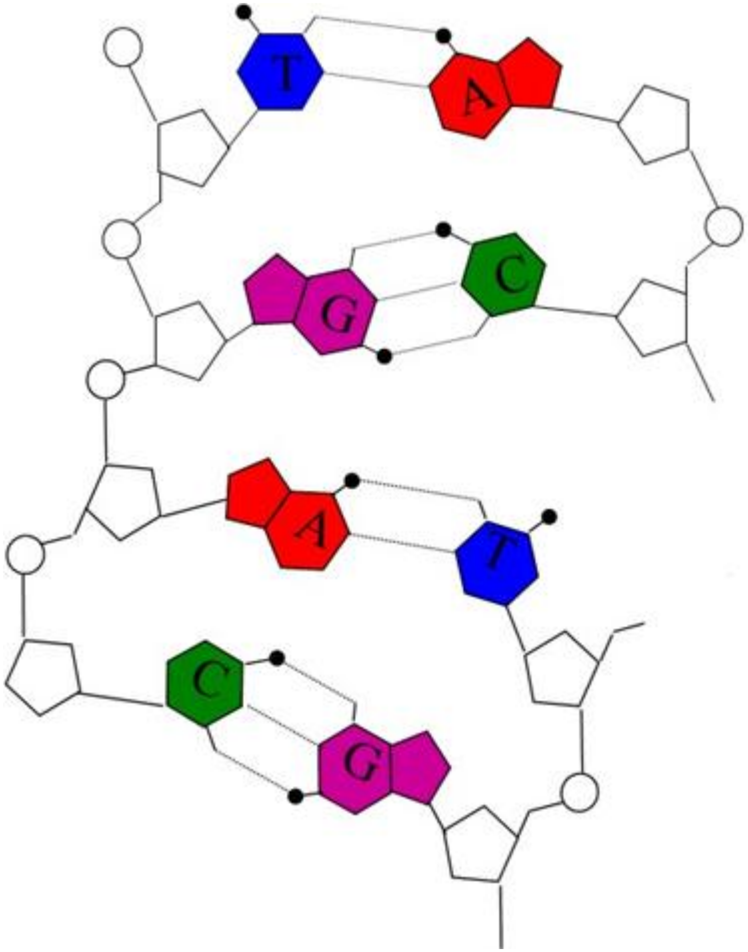
Crosslinks

- DNA-DNA
- DNA-protein

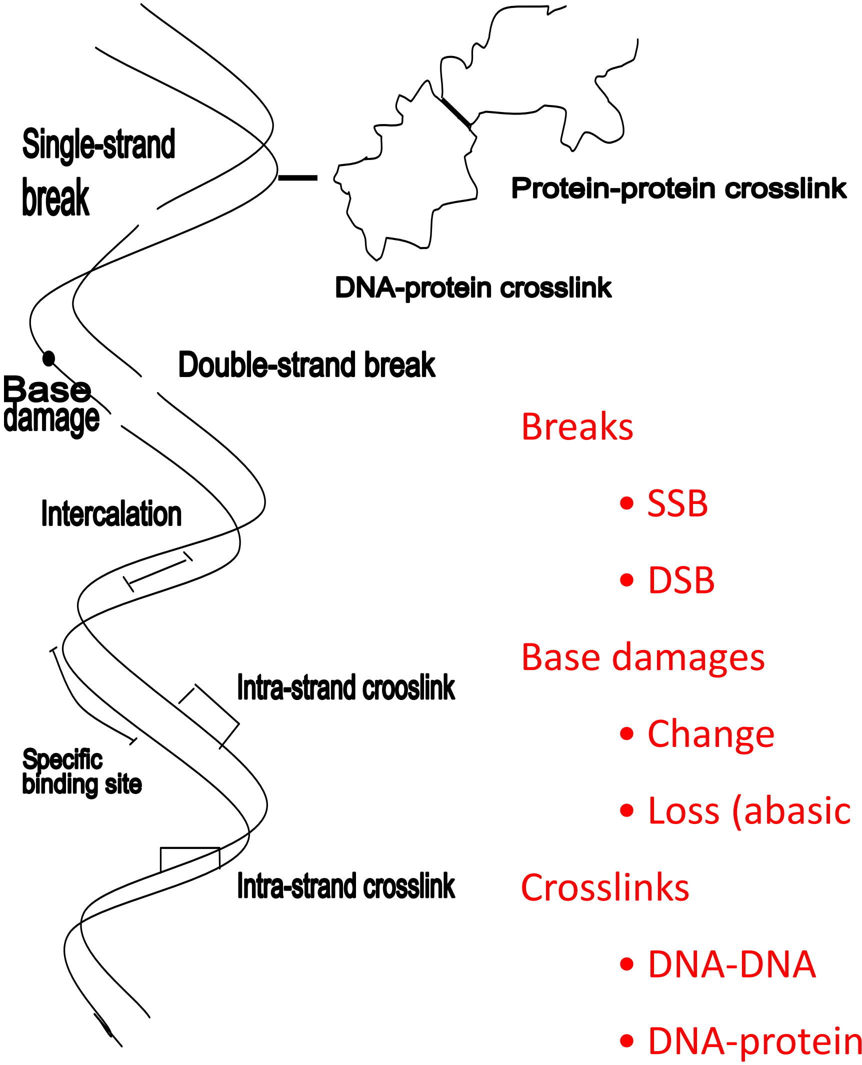
Held, 2013 NSRSS

Single Strand Break (SSB)

- easy to repair using the opposite strand as a template



IR damage to DNA



Breaks

- SSB
- DSB

Base damages

- Change
- Loss (abasic sites)

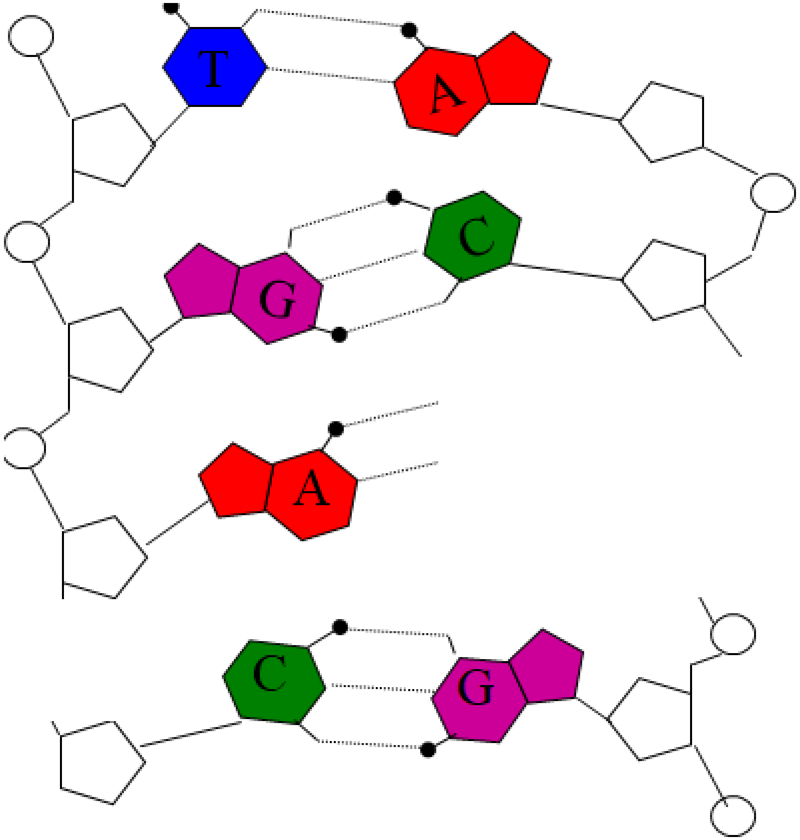
Crosslinks

- DNA-DNA
- DNA-protein

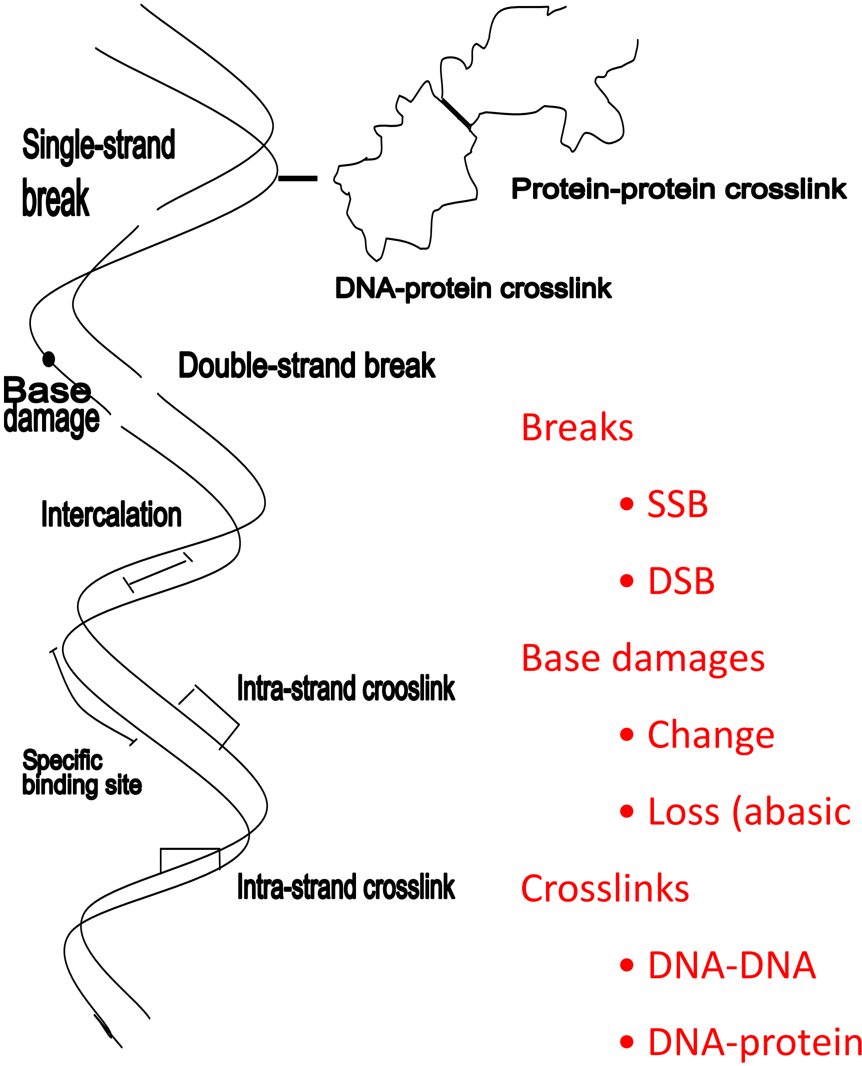
Held, 2013 NSRSS

Double Strand Break (DSB)

- Damage to both strands close to each other <20 bp
- Hard to repair
- Error prone



IR damage to DNA



Held, 2013 NSRSS

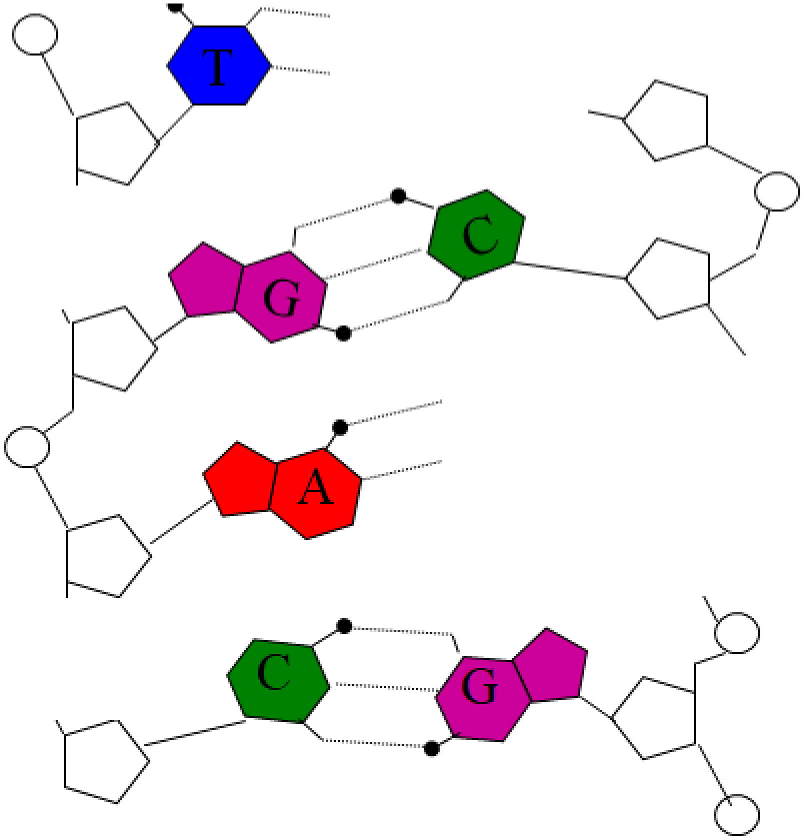
Breaks
- SSB
- DSB

Base damages
- Change
- Loss (abasic sites)

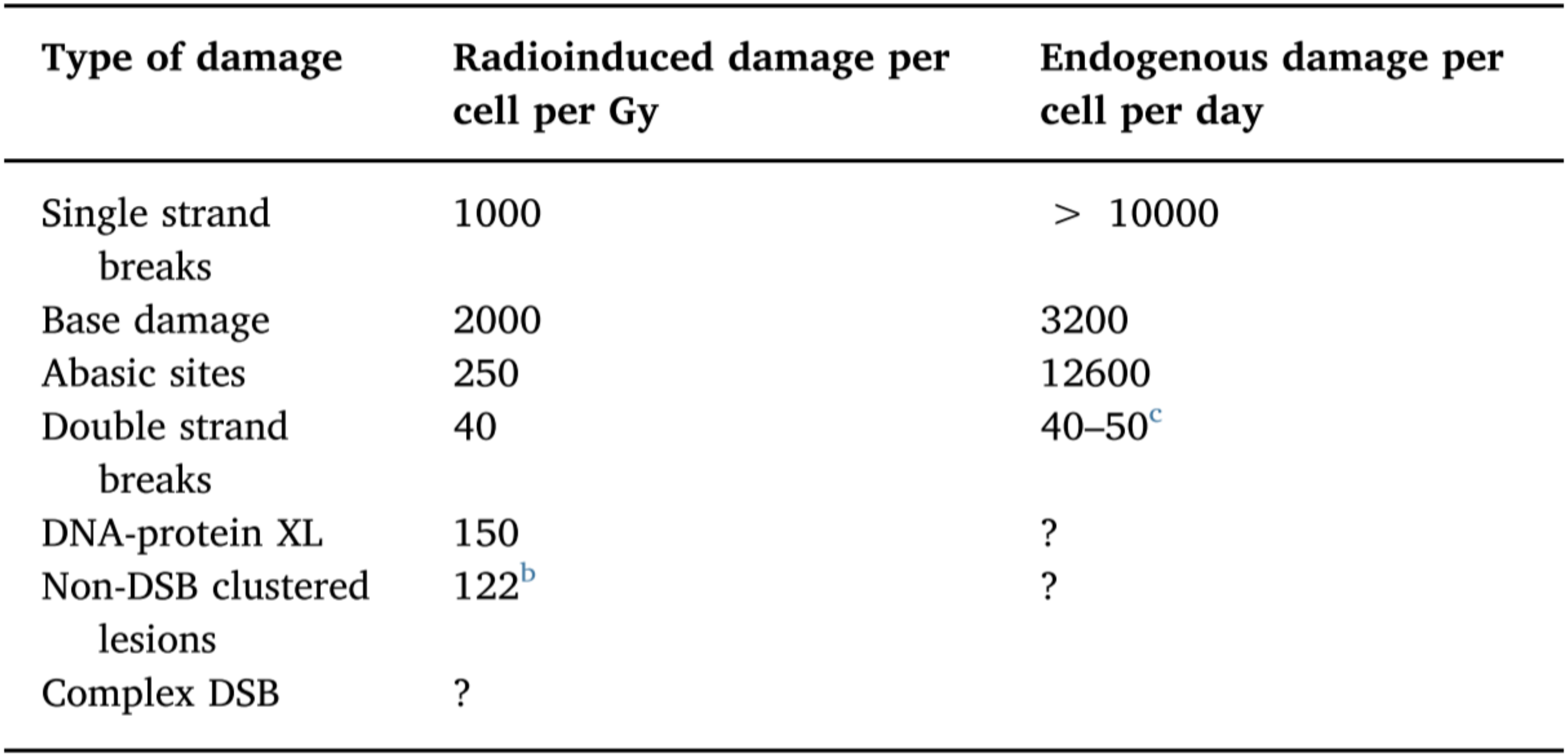
Crosslinks
- DNA-DNA
- DNA-protein

Cluster damage

- Multiple damages in a close proximity
- Very hard to repair
- Typically resulting in a loss of genetic information



DNA damage



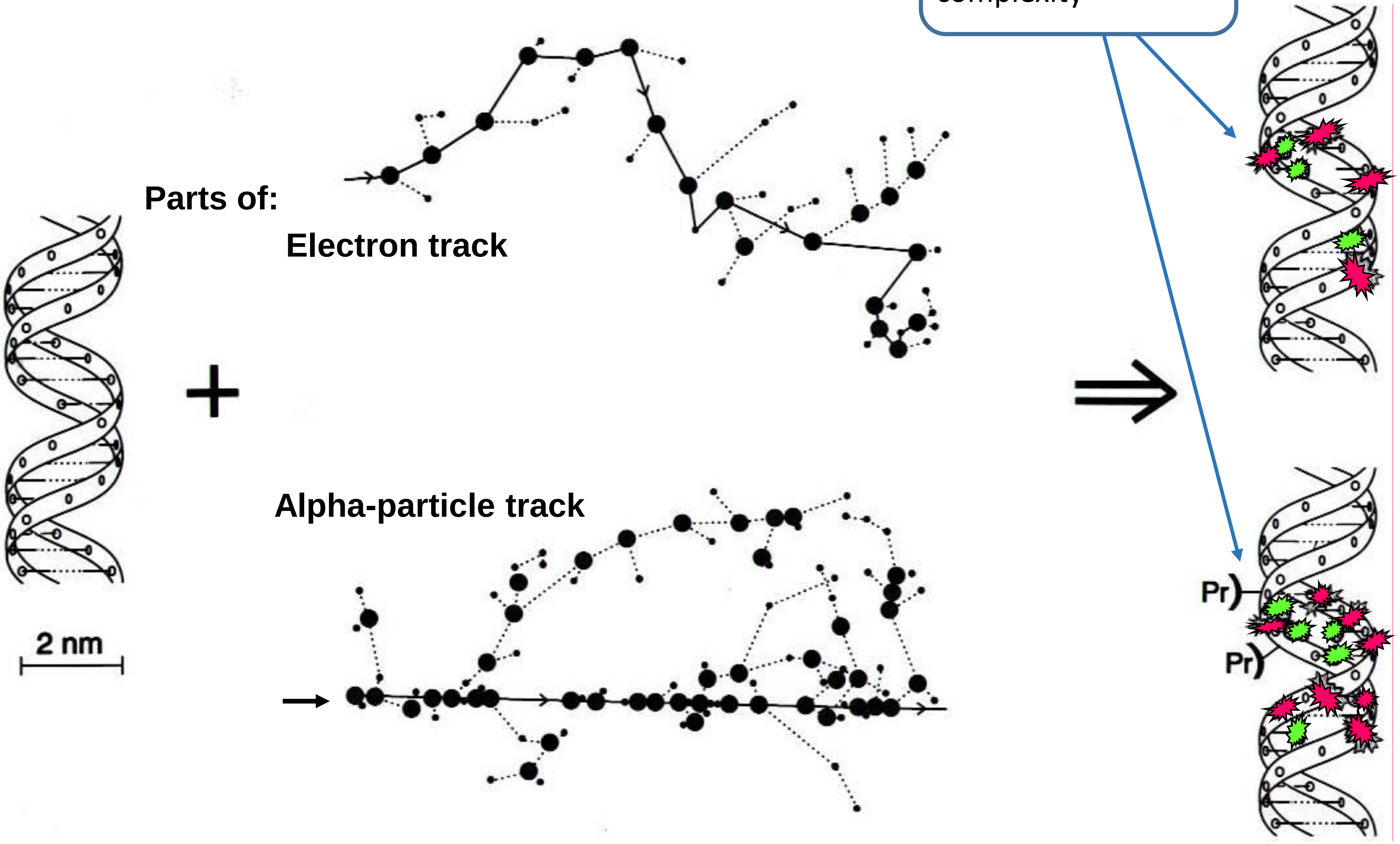
Type of damage	Radioinduced damage per cell per Gy	Endogenous damage per cell per day
Single strand breaks	1000	> 10000
Base damage	2000	3200
Abasic sites	250	12600
Double strand breaks	40	40–50[c]
DNA-protein XL	150	?
Non-DSB clustered lesions	122[b]	?
Complex DSB	?	

Sage E., Free Radical Biology and Medicine, 107, 2017

Why heavy charged particles?

- Basic research – different lesions caused to the living organisms
- Tool for modeling the biological effects of space radiation

Track differences



Clustered damage in DNA with different complexity

Parts of:

Electron track

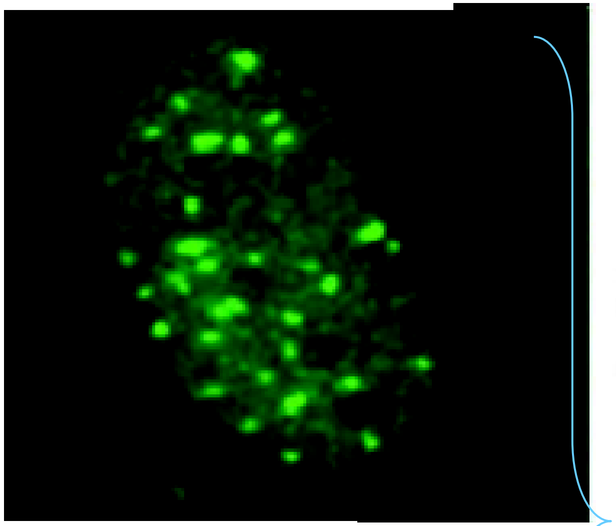
Alpha-particle track

Goodhead, IJRB, 65 (7), 1994

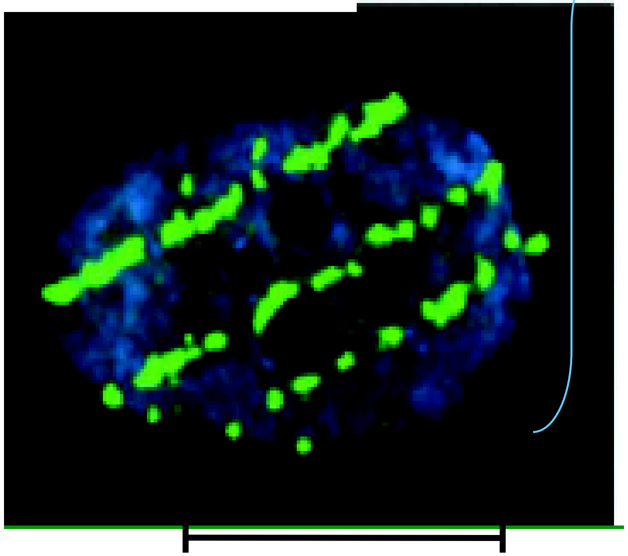
Gamma rays vs. heavy ions

Fluorescent foci marking DSB in cell nuclei

Gamma-ray irradiation:

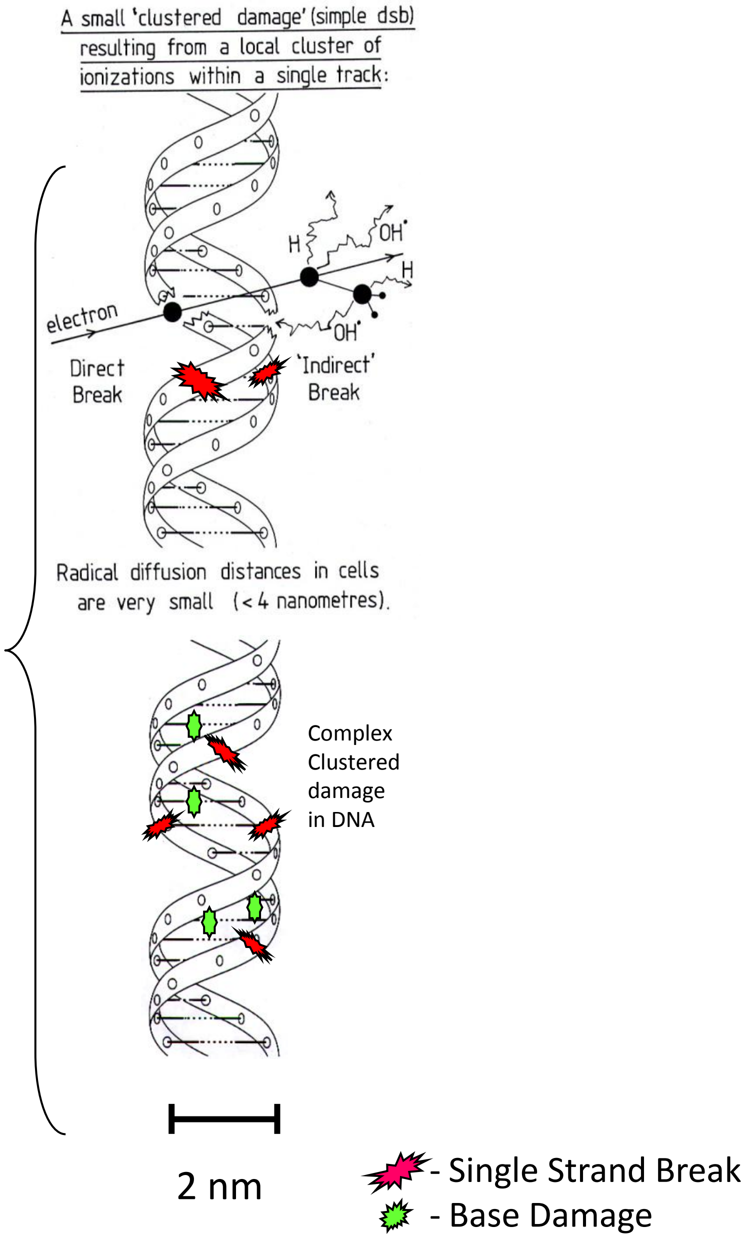


Fe ion irradiation:



10 µm

Magnification x 1600 →

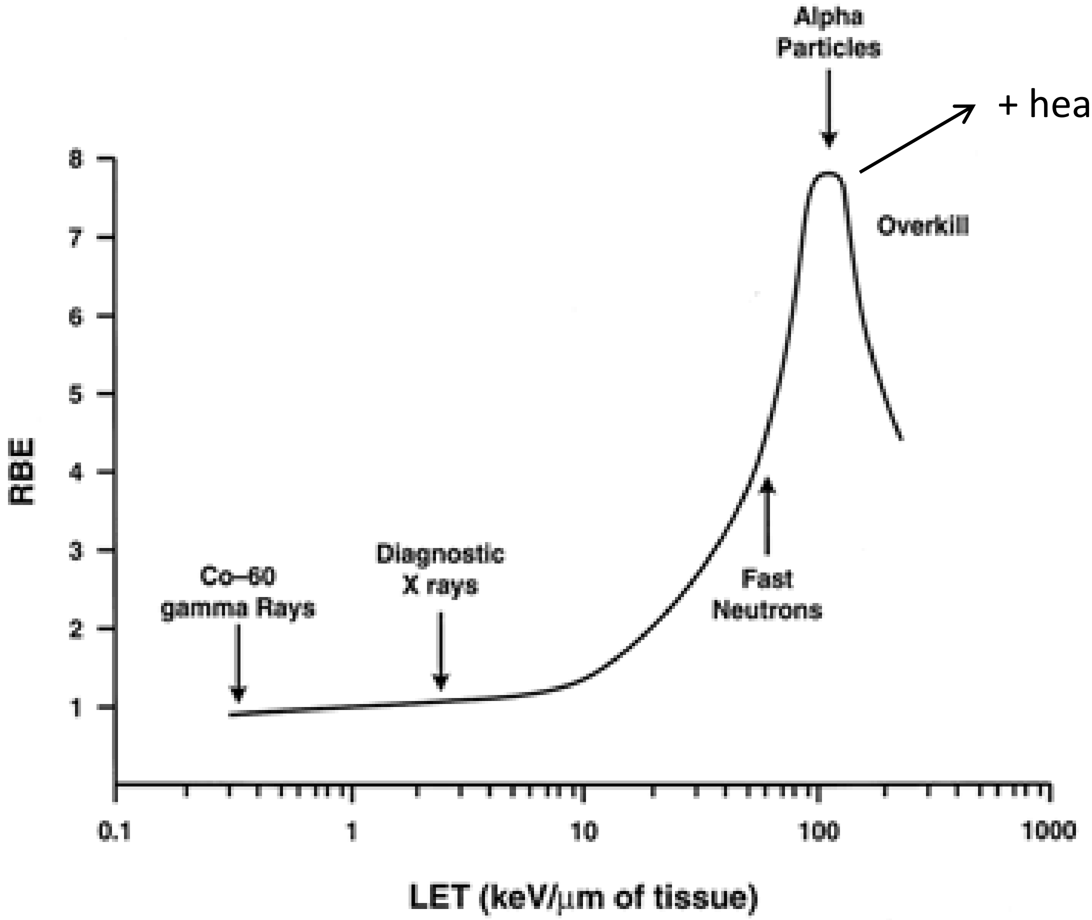


Goodhead, 2013 NSRSS

Dependence RBE on LET

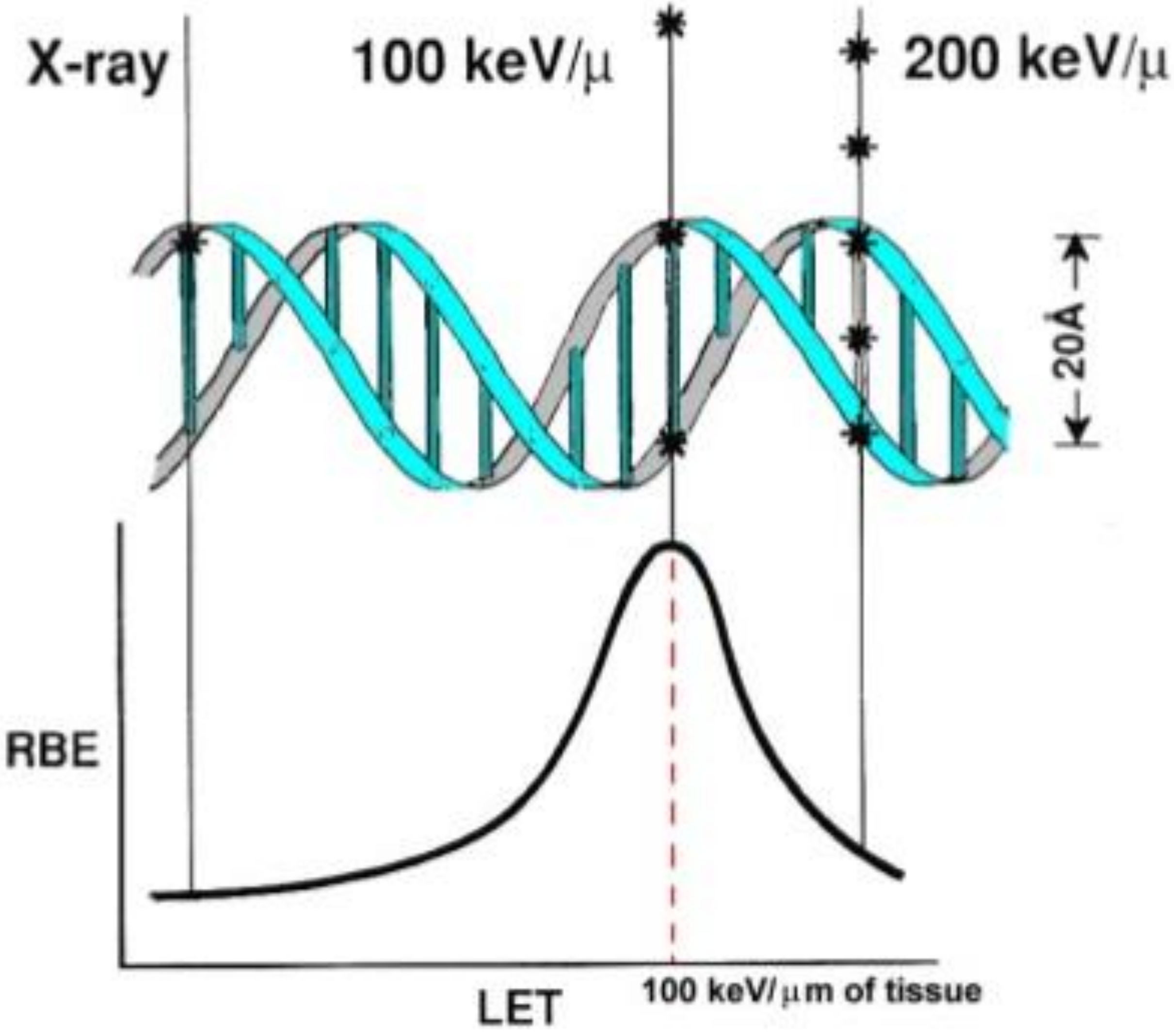
LET – Linear Energy Transfer [keV/µm]; **RBE** – Relative Biological Effectiveness

Endpoint = level of cell survival

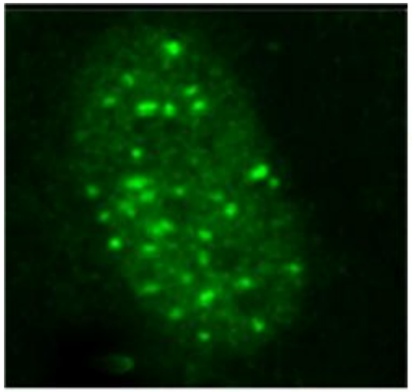


+ heavy charged ions

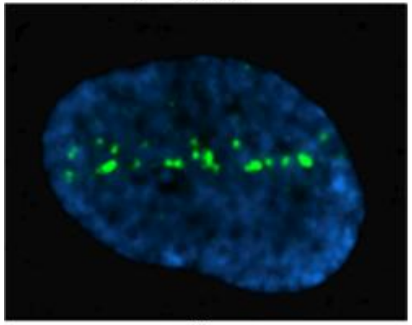
RBE = Ratio of doses for identical level of biological effect (endpoint)


X-ray
100 keV/μ
200 keV/μ
20Å
RBE
LET
100 keV/μm of tissue

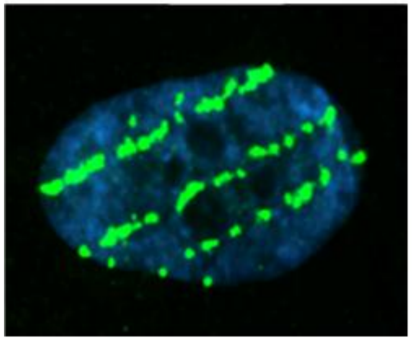
Heavy ions tracks



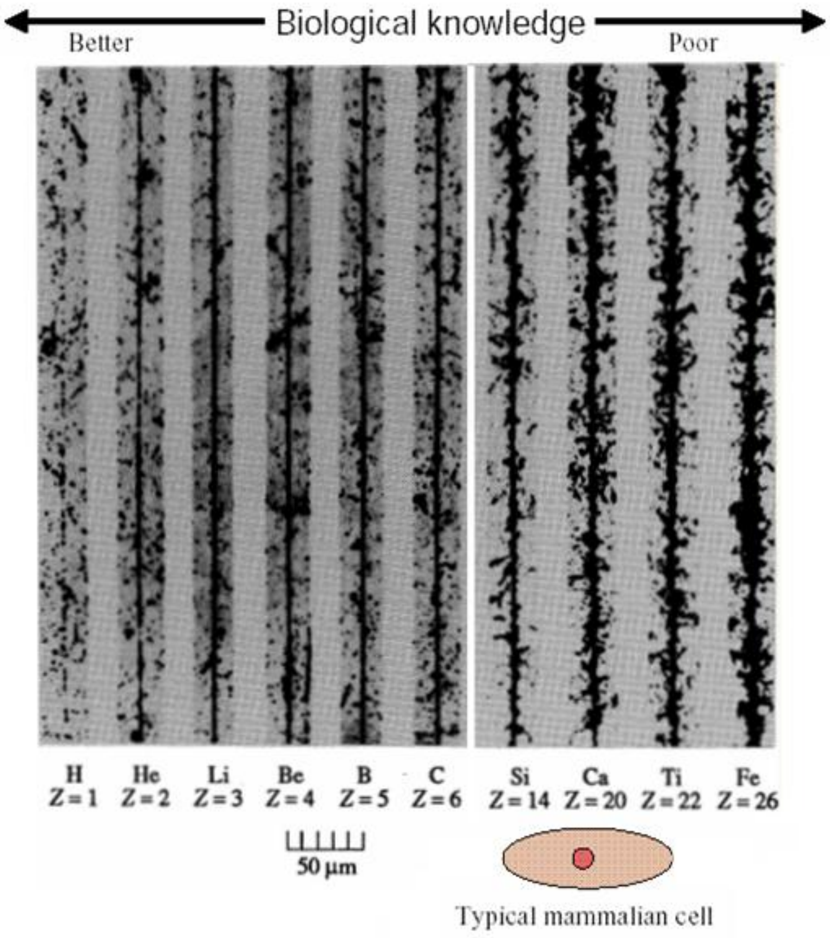
γ – rays



silicon



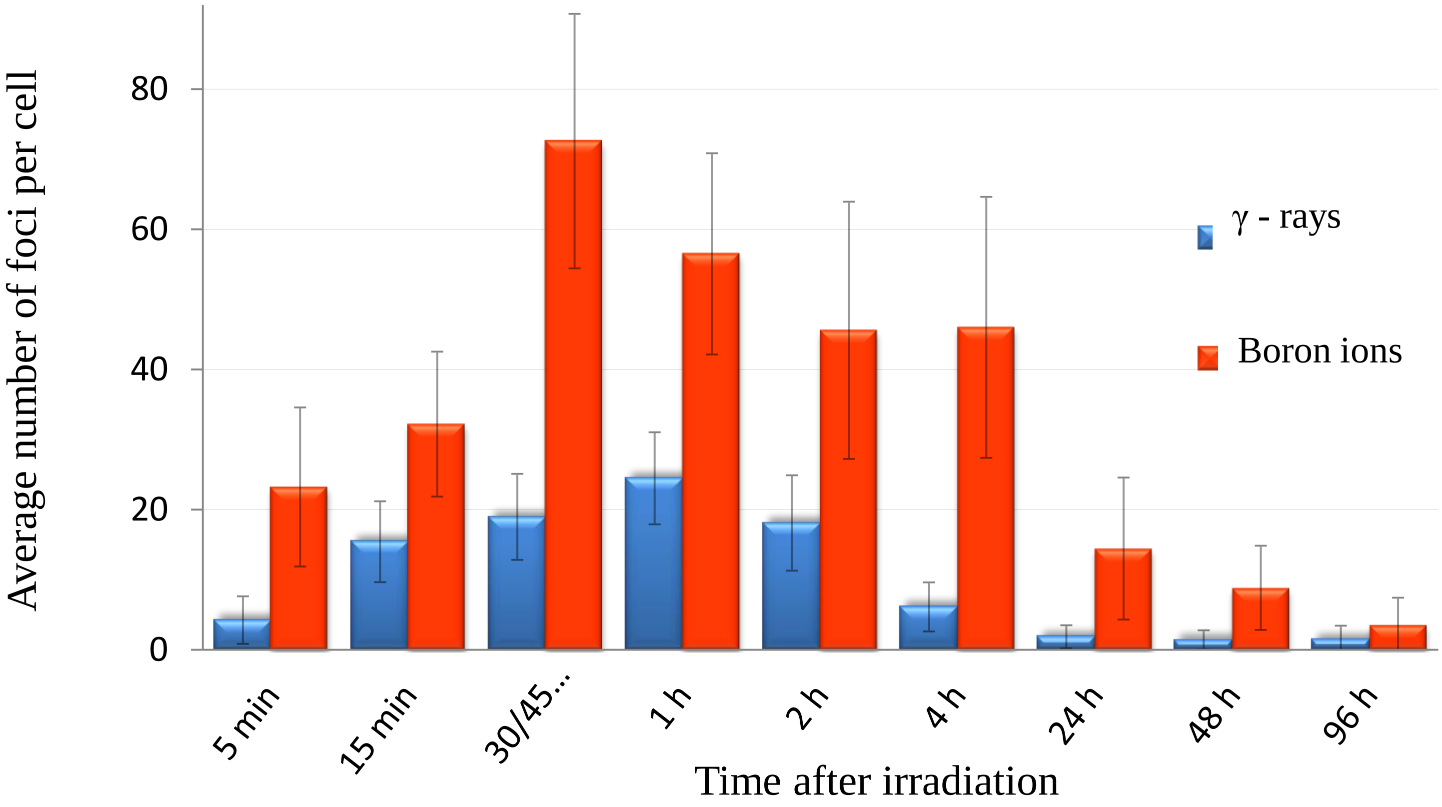
iron



Z = 70

(Cucinotta & Durante, Lancet Oncol 2006)

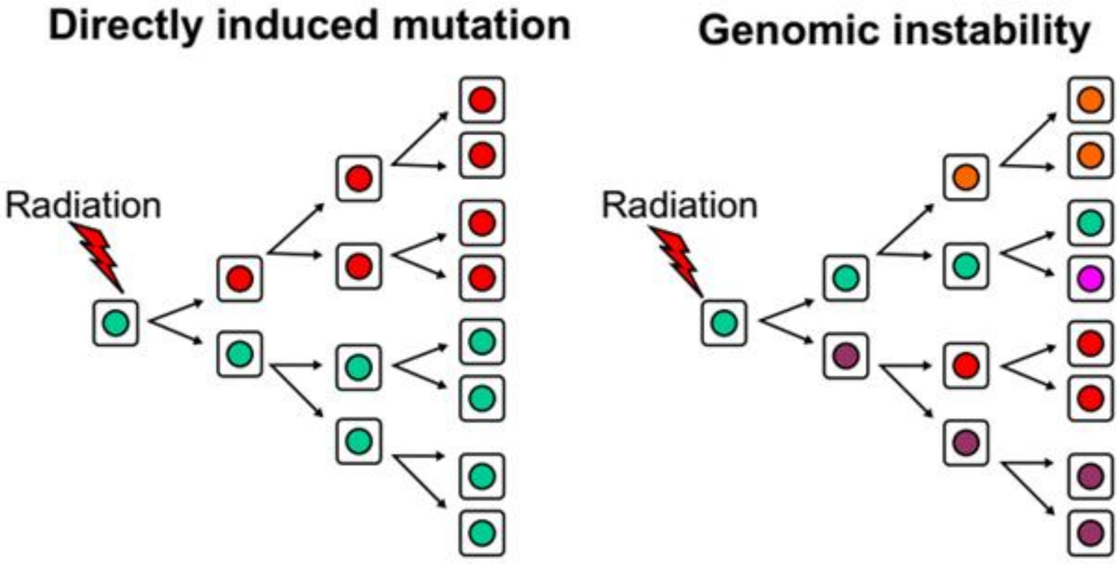
Damage and repair kinetics of DNA after irradiation



Radiation Genetics

Radiation-Induced **Genomic instability** – stochastic effect

- 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

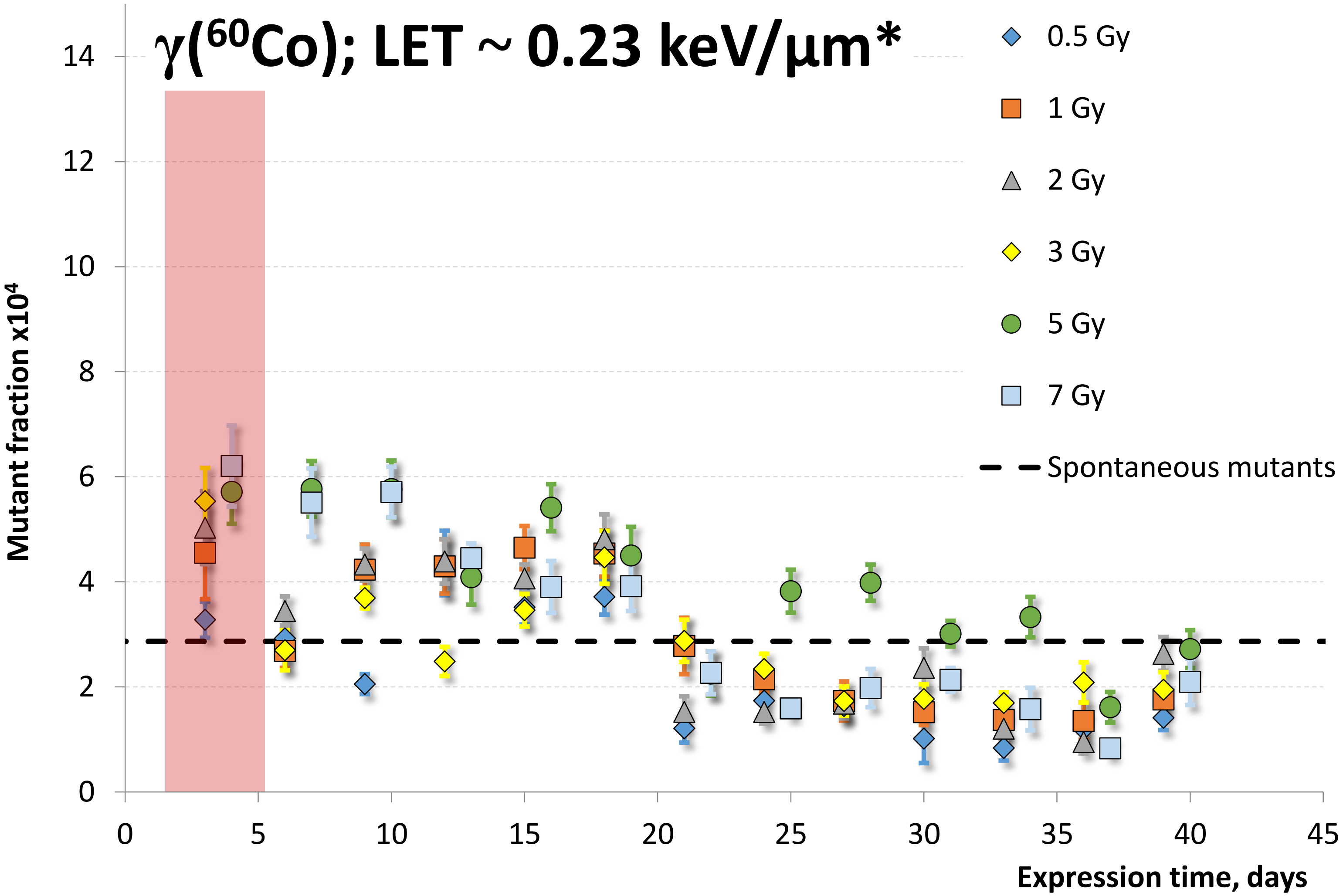


Modified from Streffer C. (2015)

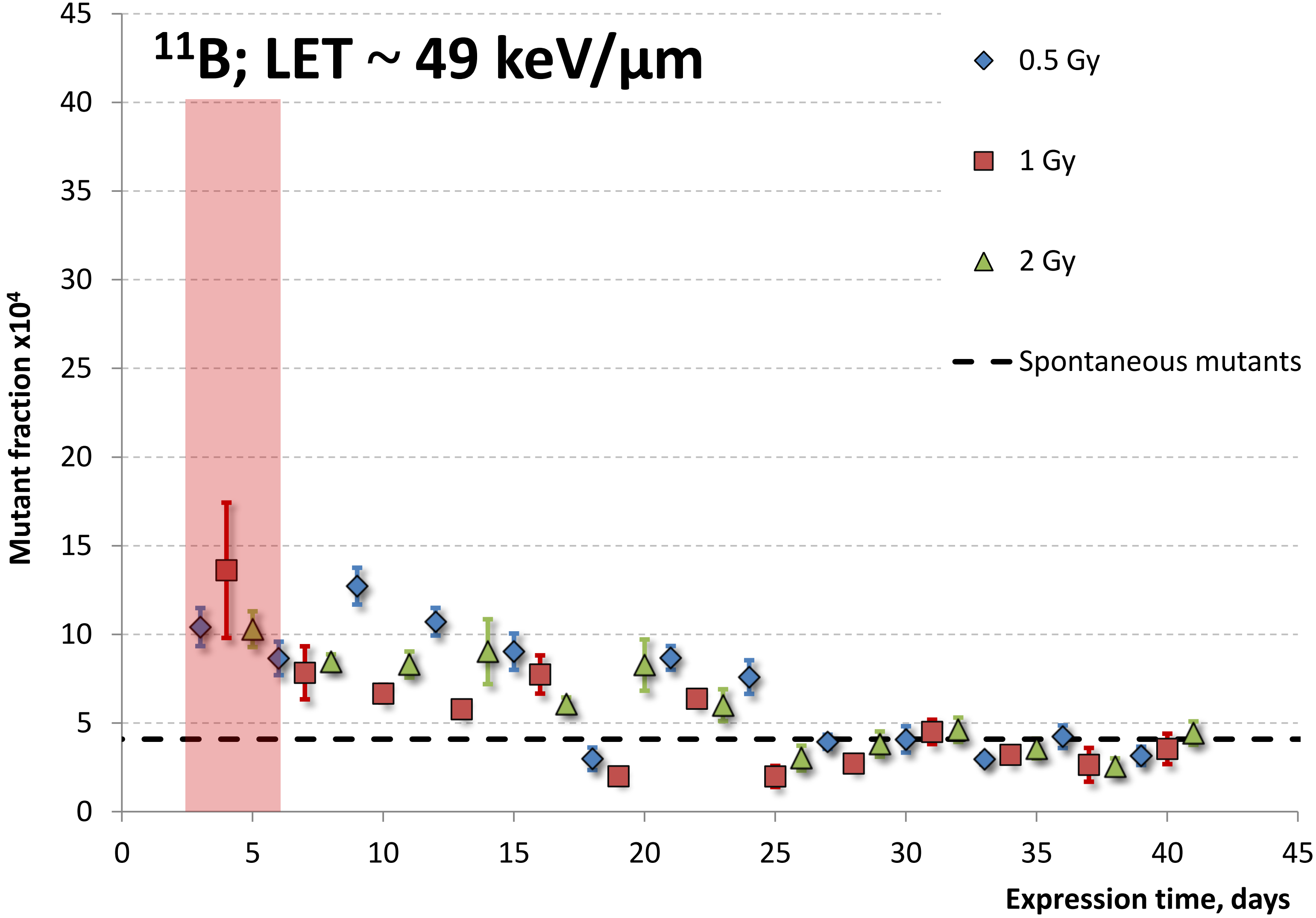
Ways to measure genomic instability:

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

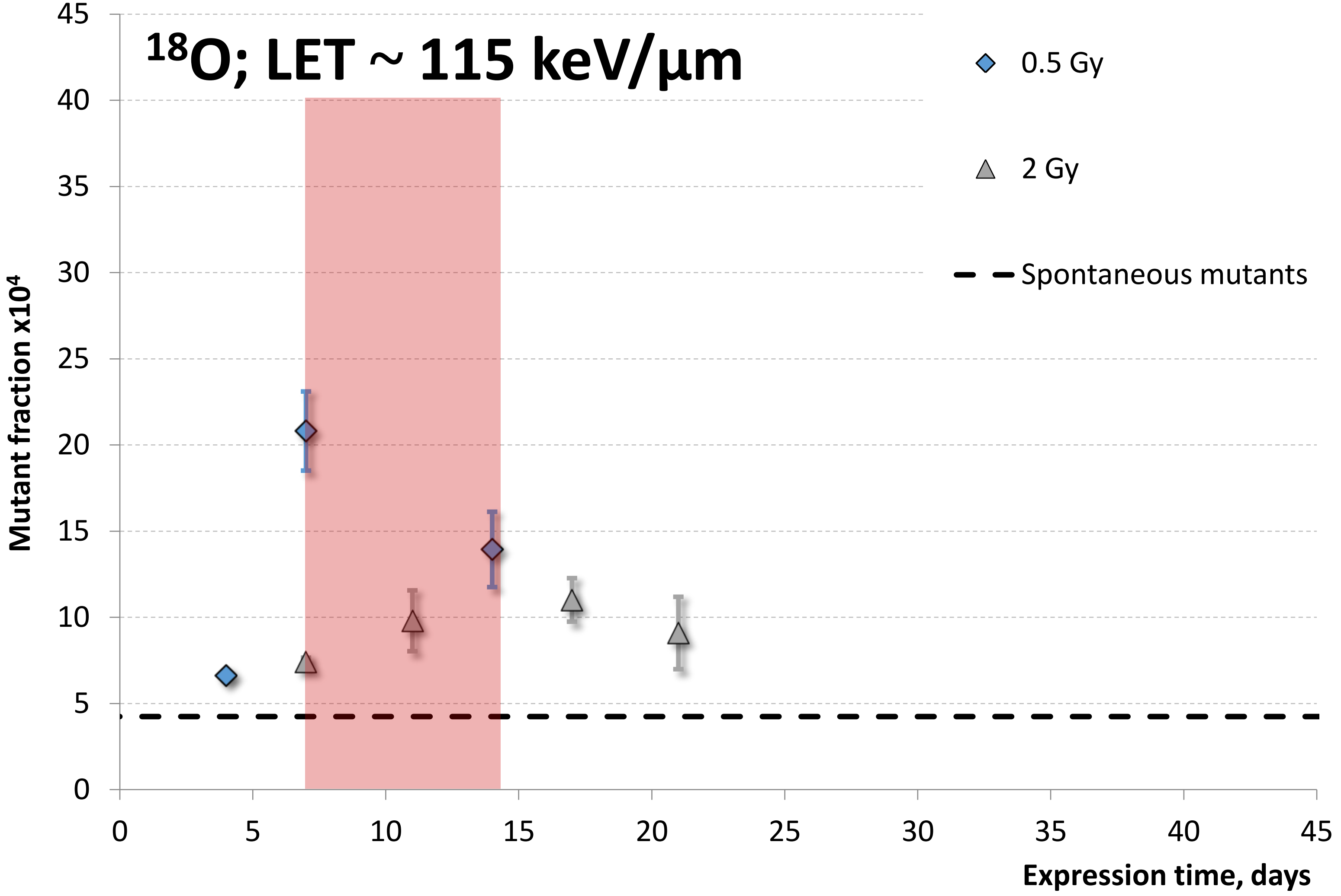
Mutagenesis – V79, HPRT gene



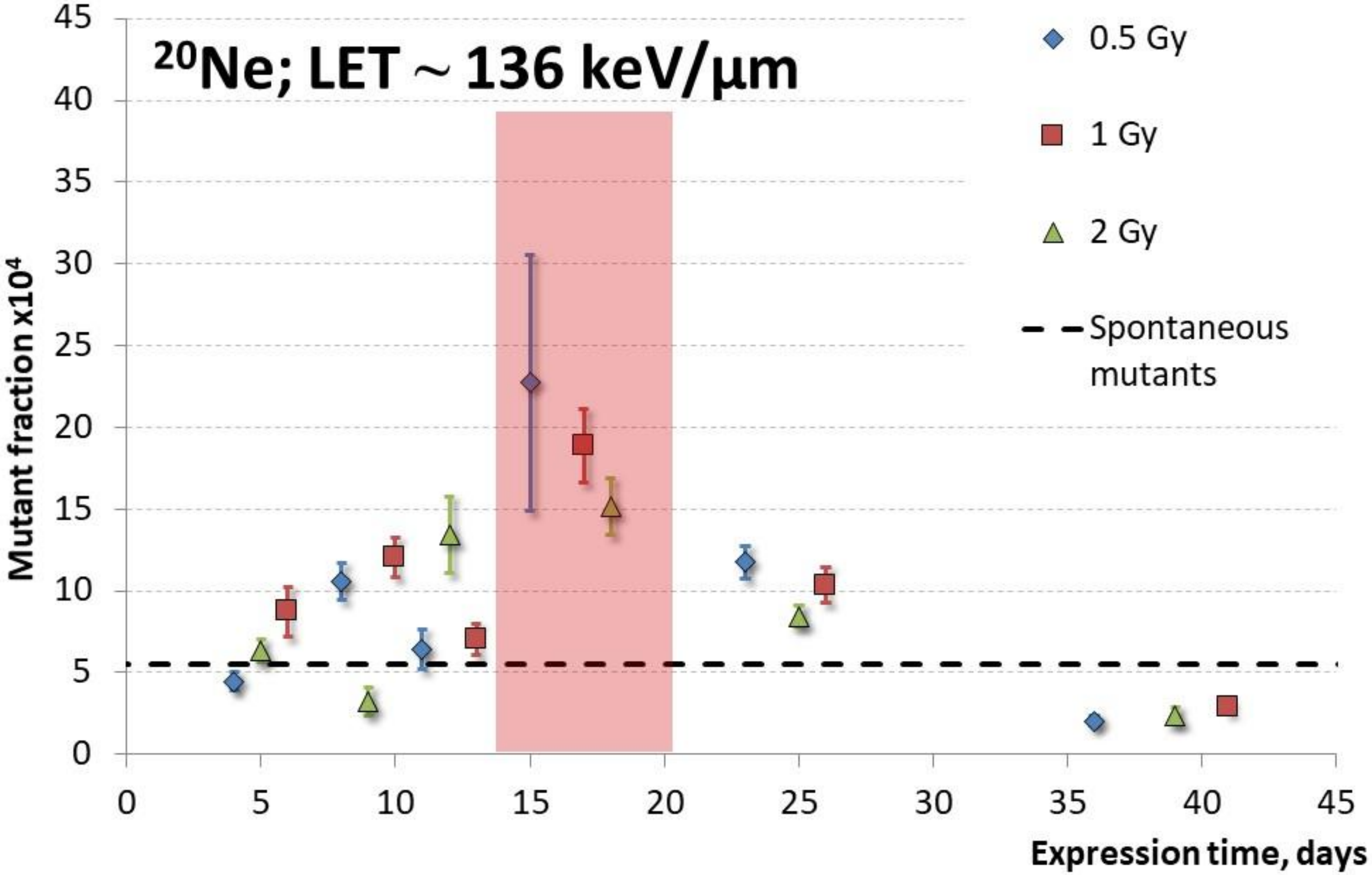
Mutagenesis – V79, HPRT gene



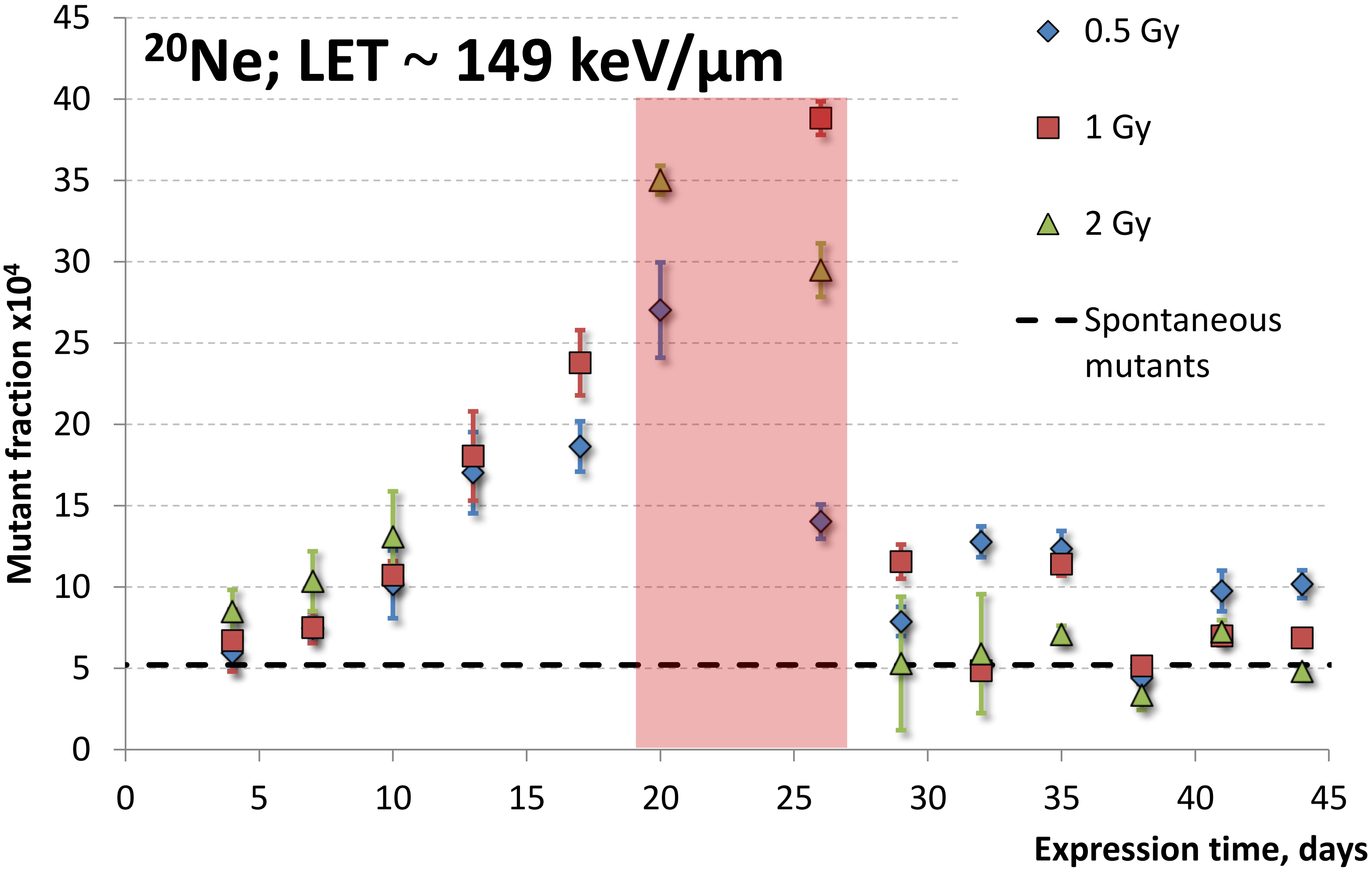
Mutagenesis – V79, HPRT gene

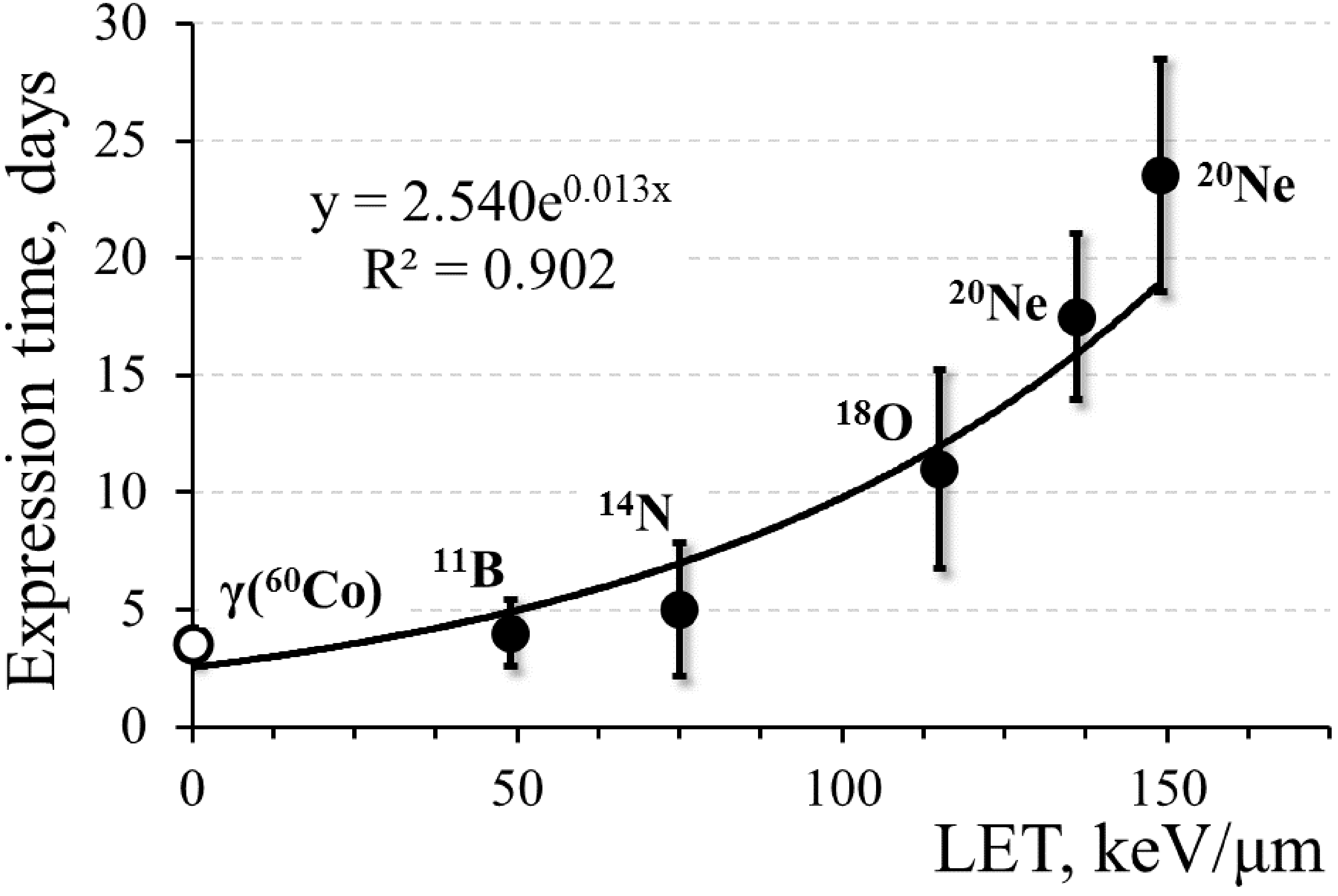


Mutagenesis – V79, HPRT gene



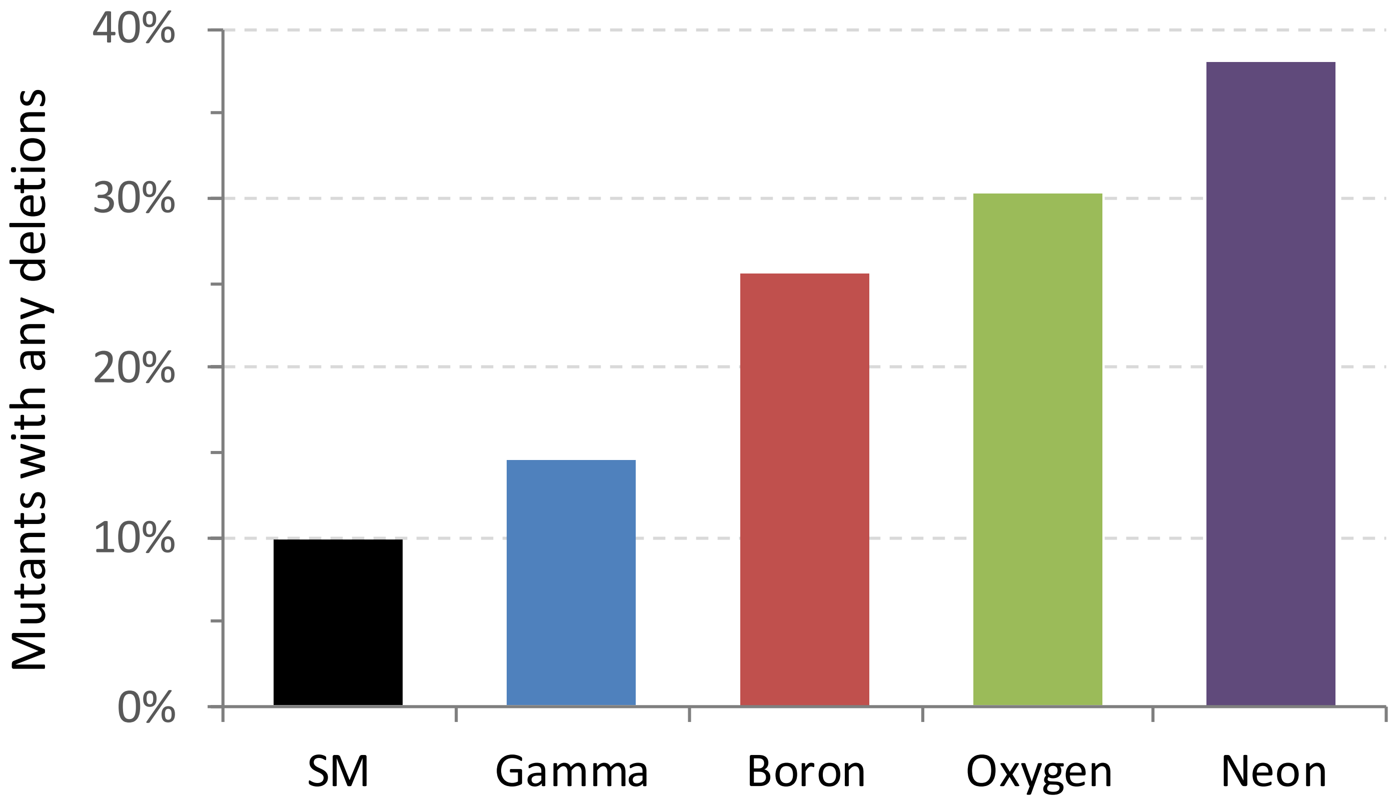
Mutagenesis – V79, HPRT gene





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

Structural changes, HPRT gene, V79

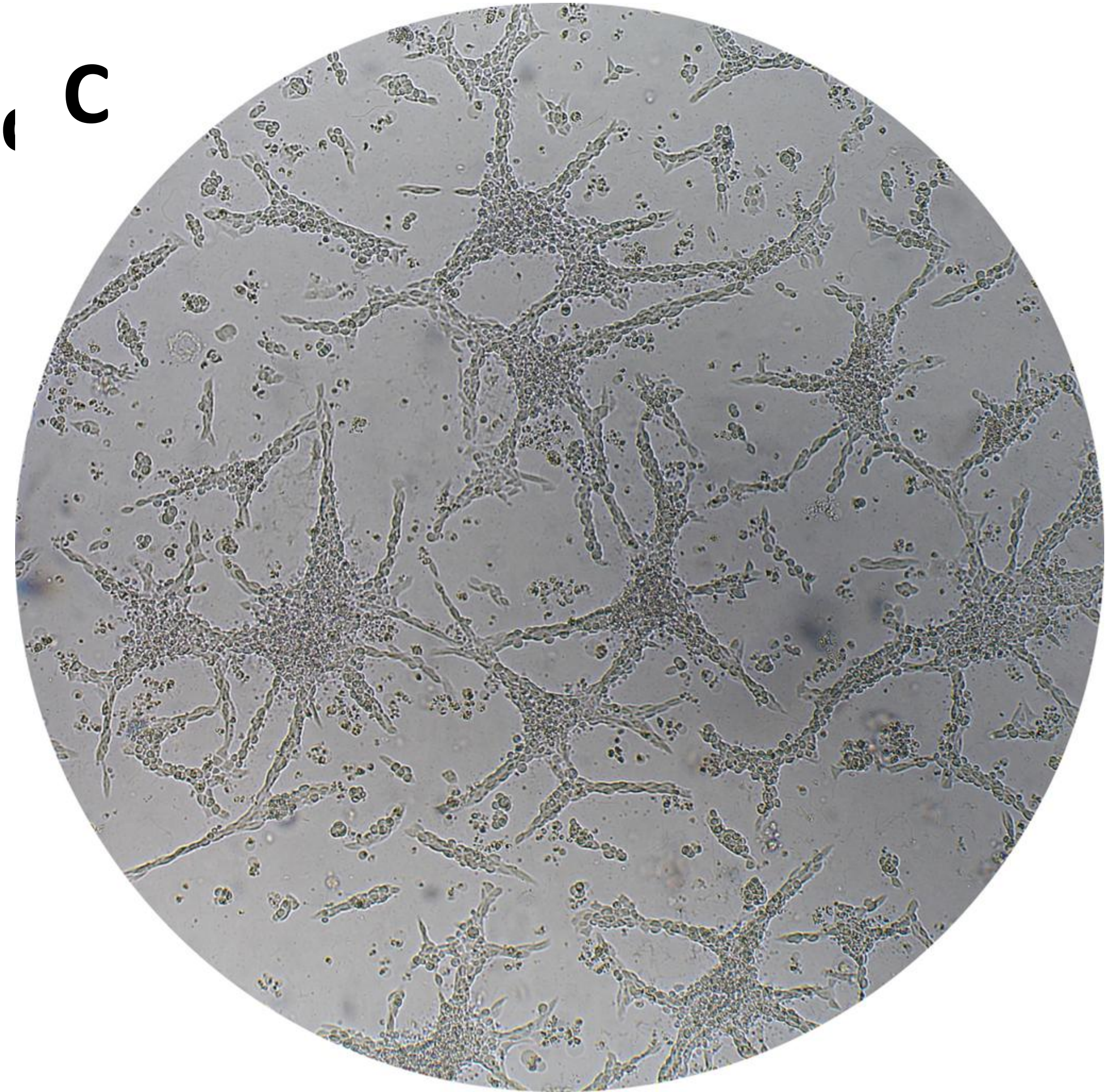


→ increasing LET

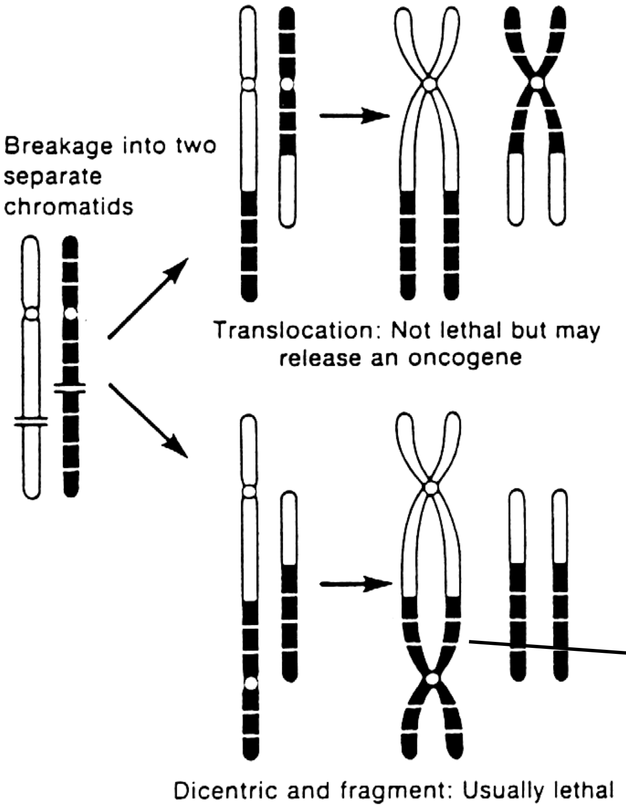
Morphological changes

Contr

C



Formation of chromosome aberrations after irradiation



Stable aberration

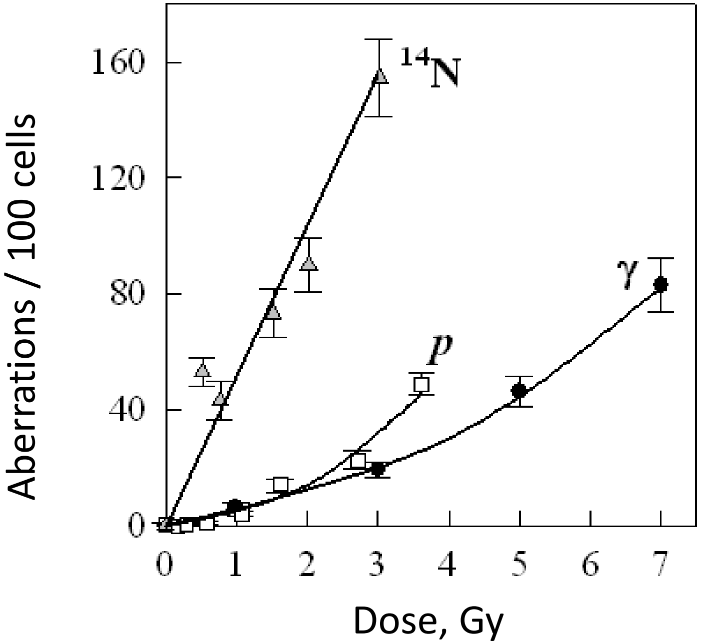
Symmetrical (balanced)

gene rearrangements

Unstable aberration

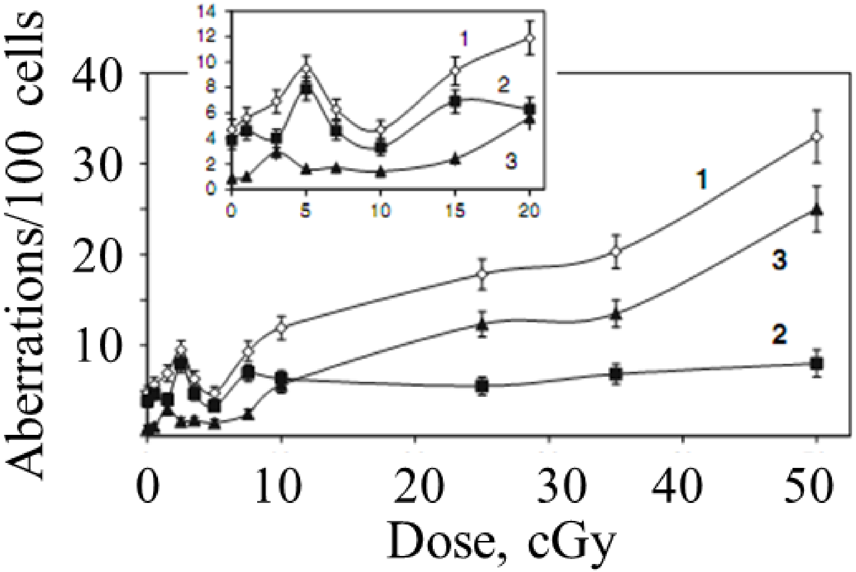
Asymmetrical

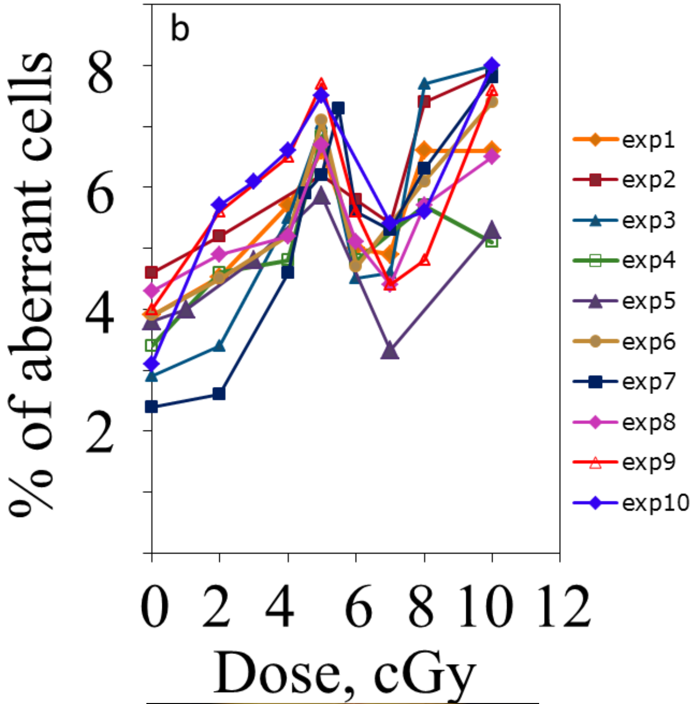
Fragment usually lost

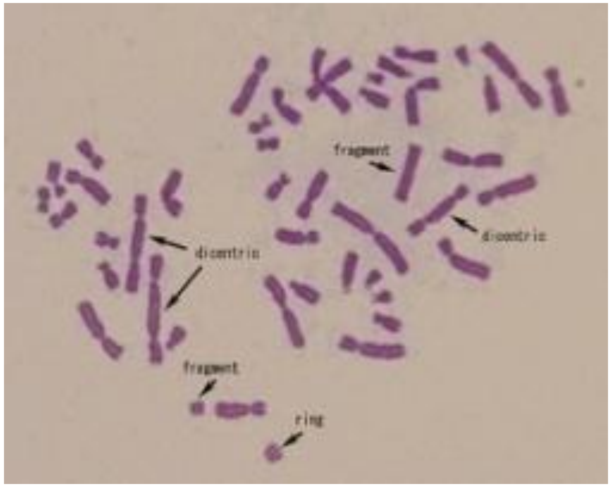


Formation of chromosome aberrations after irradiation

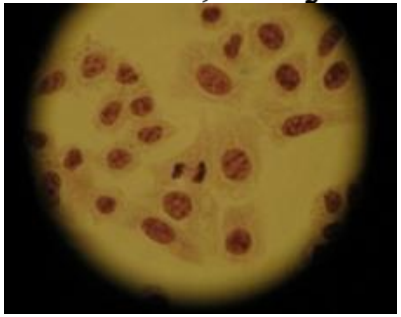
- Studying cytogenetic effects of low-dose γ-irradiation in human cells







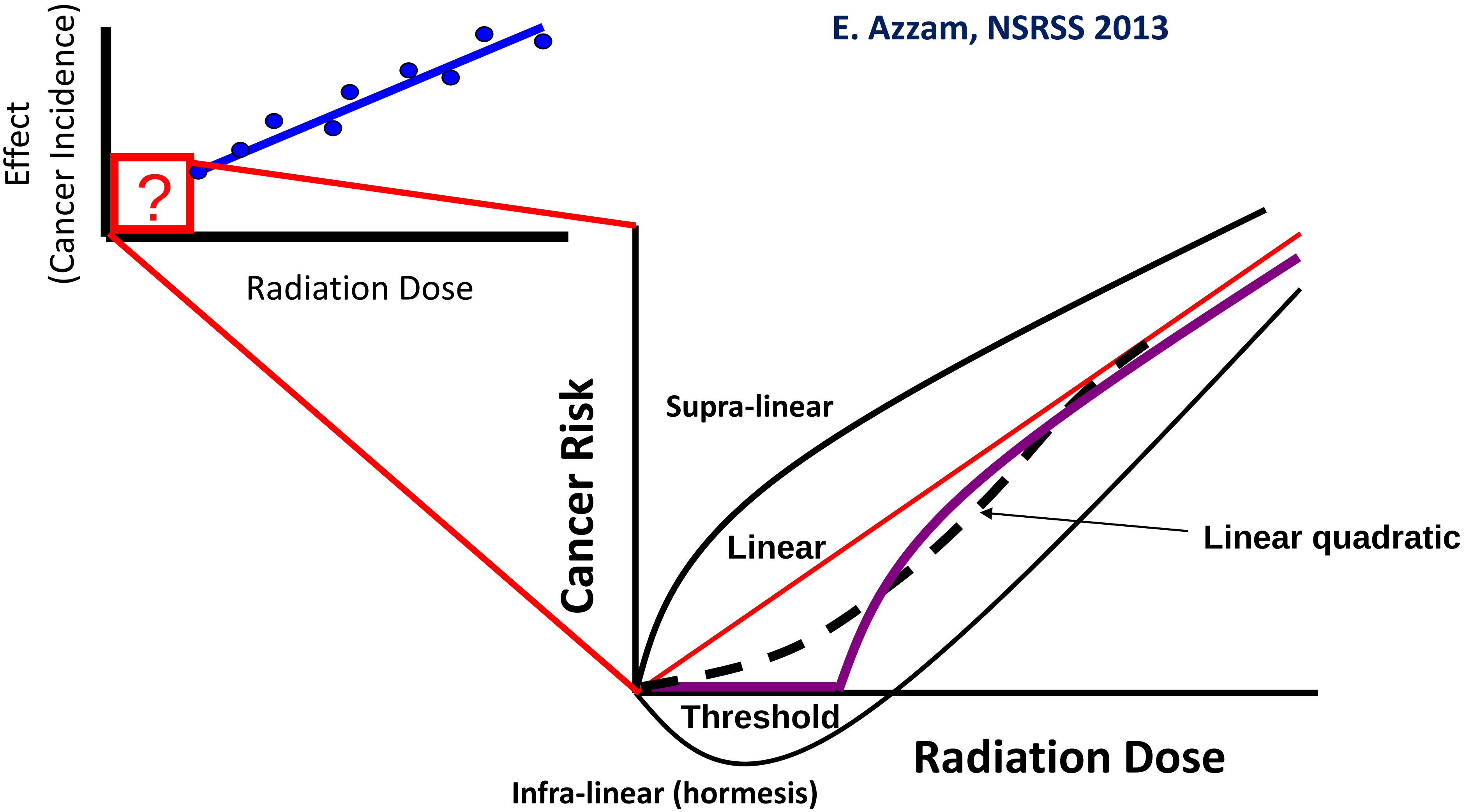
Human lymphocyte



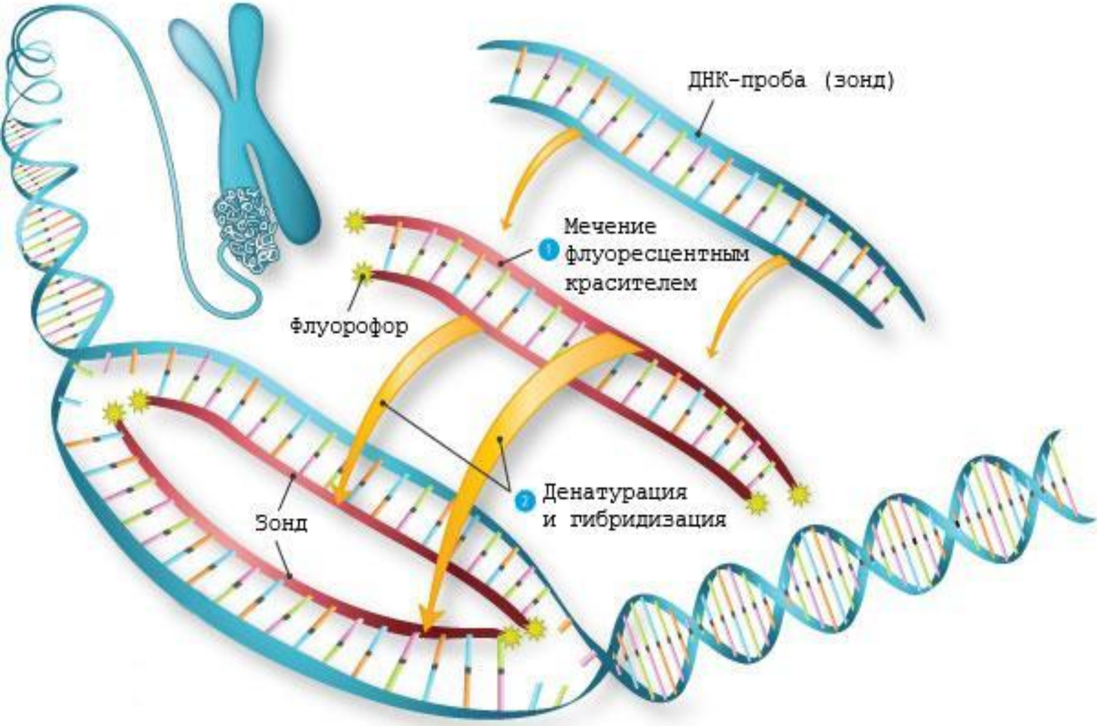
Mammary carcinoma cells

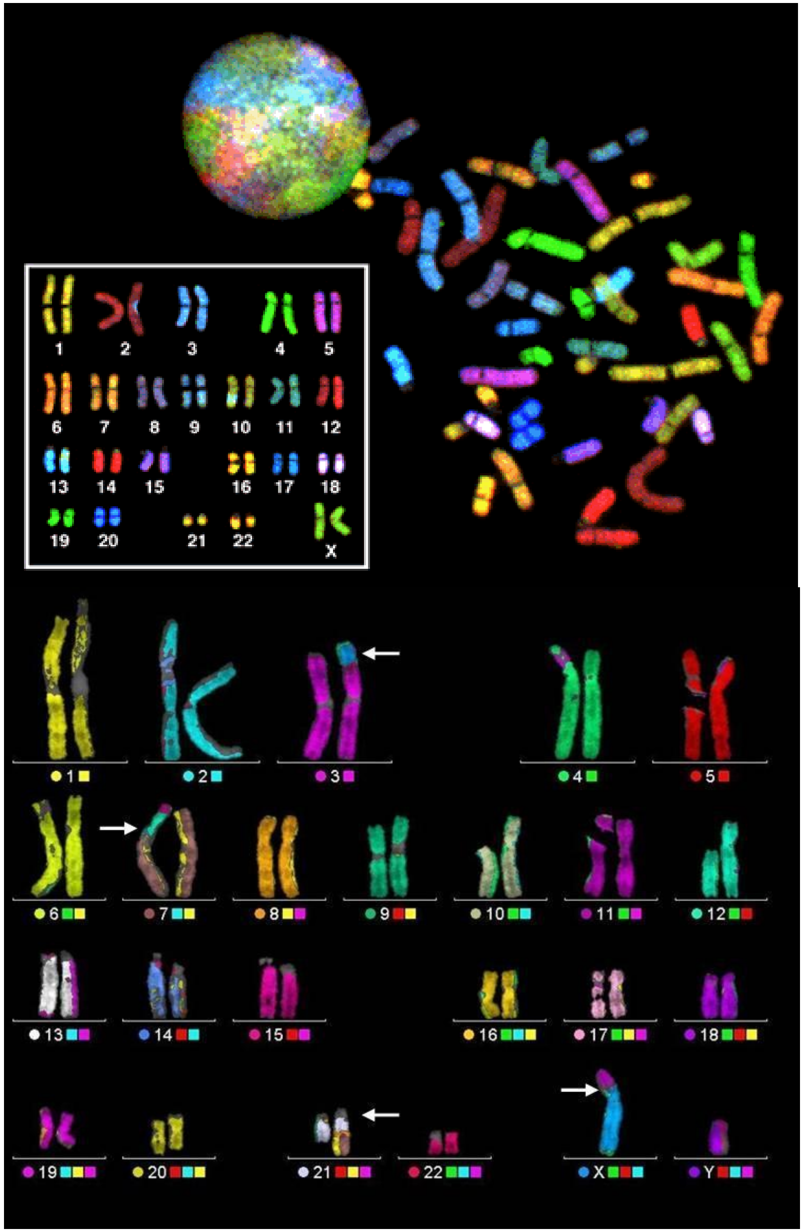
Linear no-treshold model – effects at low doses

E. Azzam, NSRSS 2013



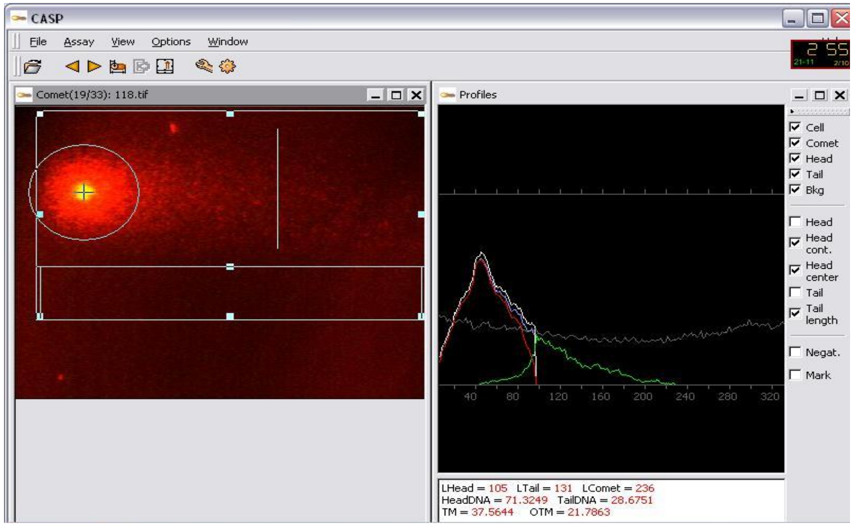
mFISH – multicolor Fluorescent in situ hybridization





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

Comet assay method of DNA damage detection

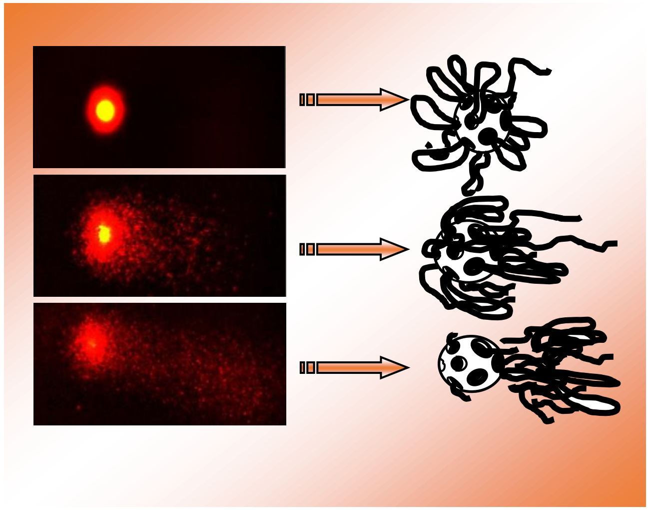


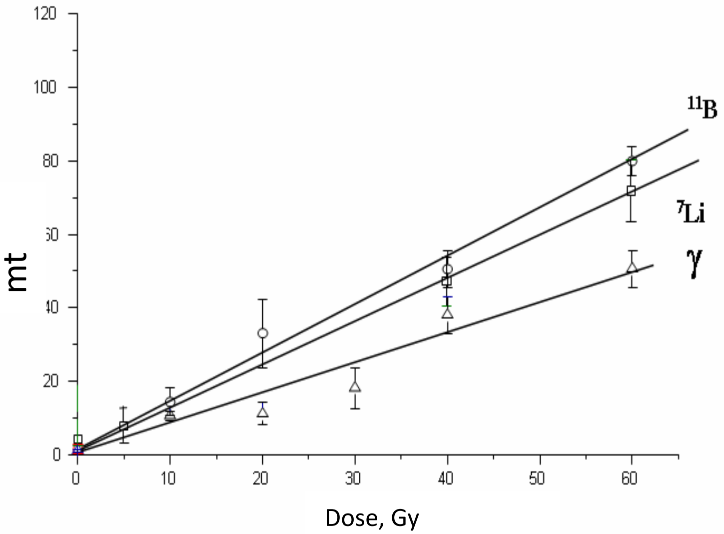
☑ Cell
☑ Comet
☑ Head
☑ Tail
☑ Bkg

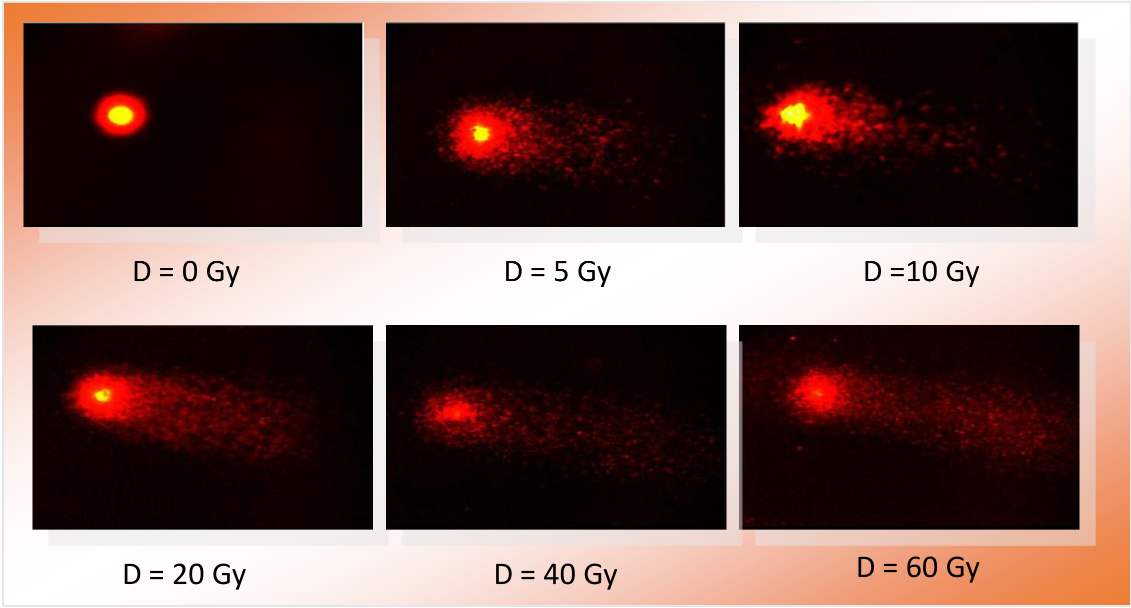
☐ Head
☑ Head cont.
☑ Head center
☐ Tail
☑ Tail length

☐ Negat.
☐ Mark

LHead = 105 LTail = 131 LComet = 236
HeadDNA = 71.3249 TailDNA = 28.6751
TM = 37.5644 OTM = 21.7863







D = 0 Gy

D = 5 Gy

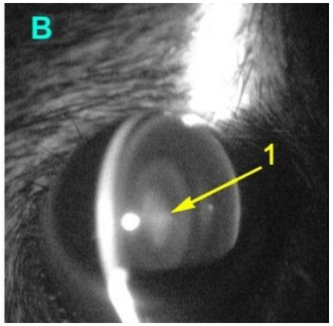
D =10 Gy

D = 20 Gy

D = 40 Gy

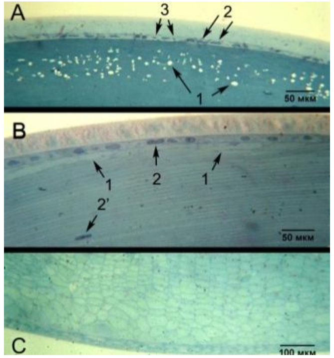
D = 60 Gy

Radiobiology of vision



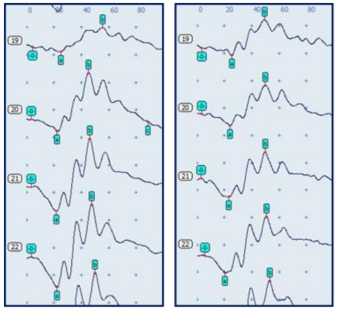
- Studying the mechanisms of radiation-induced cataract formation.

  It was shown that the molecular mechanism of the development of the senile and radiation-induced cataracts are the same.



- Studying mutagen-induced functional and morphological changes in the mammalian retina

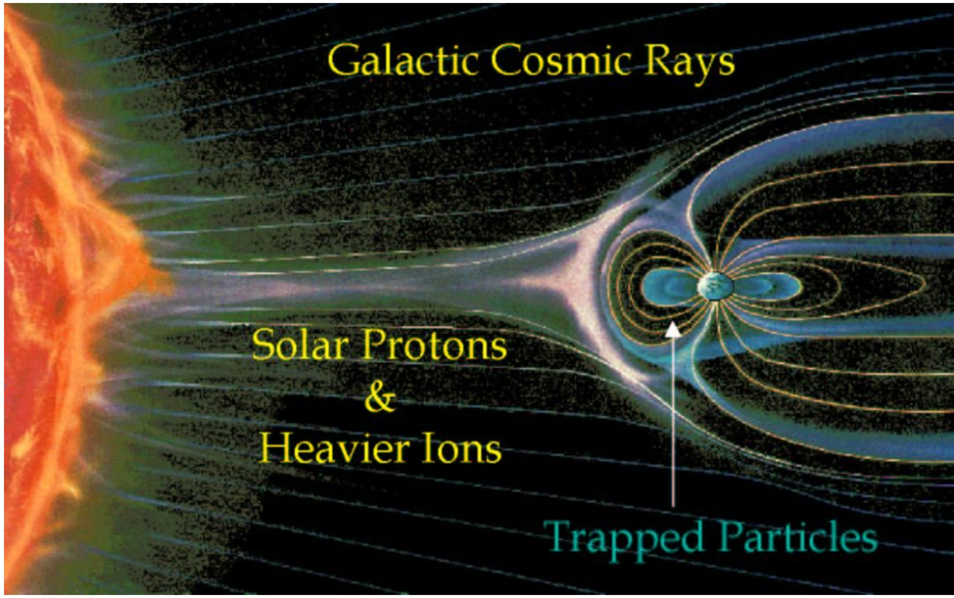
  A detailed study was performed of mouse electroretinograms taken in the absence and presence of the mutagen methylnitrosourea (MNU) in different concentrations. A threshold value for this mutagen was obtained.



control **24 h after MNU injection**

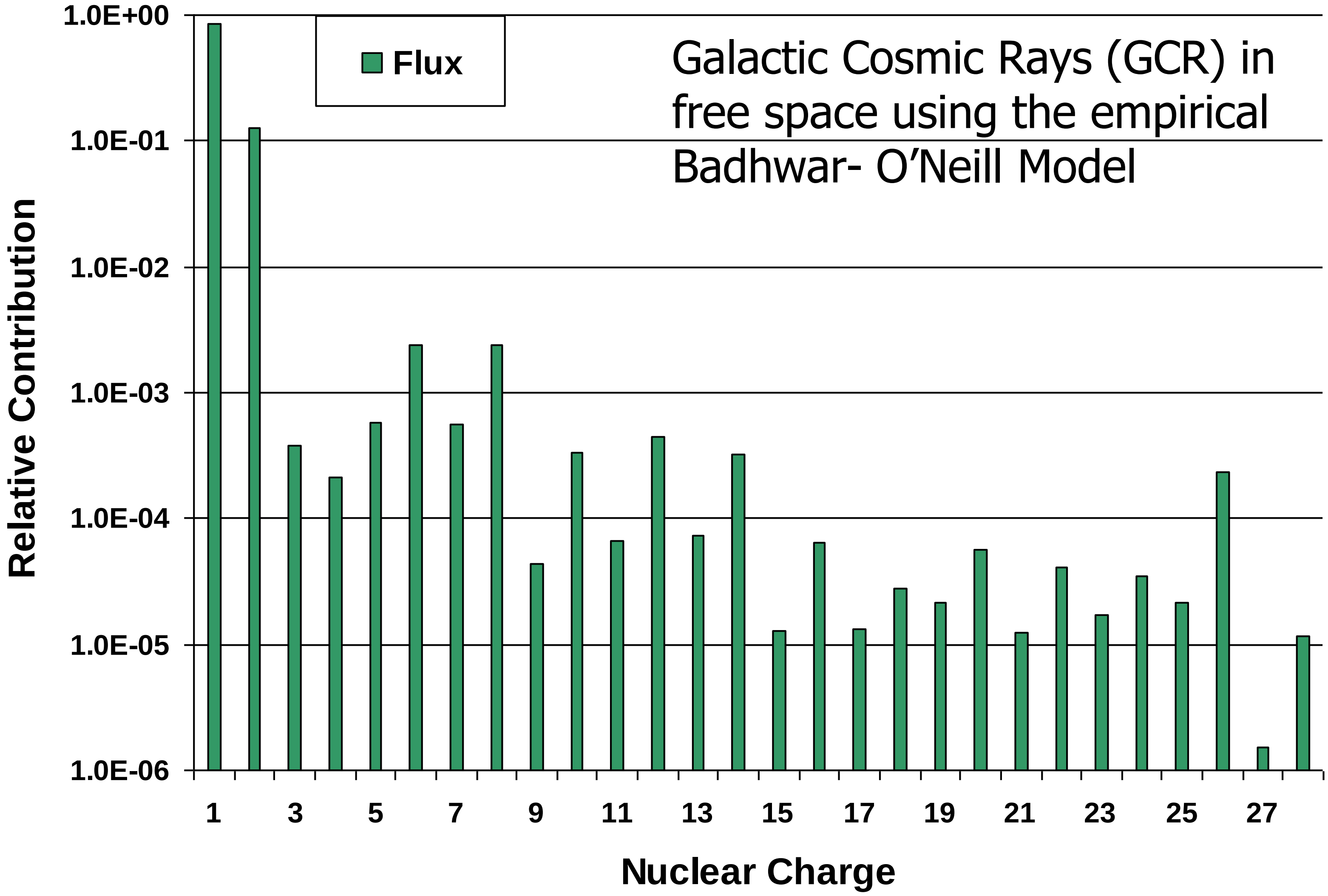
Space radiation

- **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

Galactic Cosmic Rays (GCR)

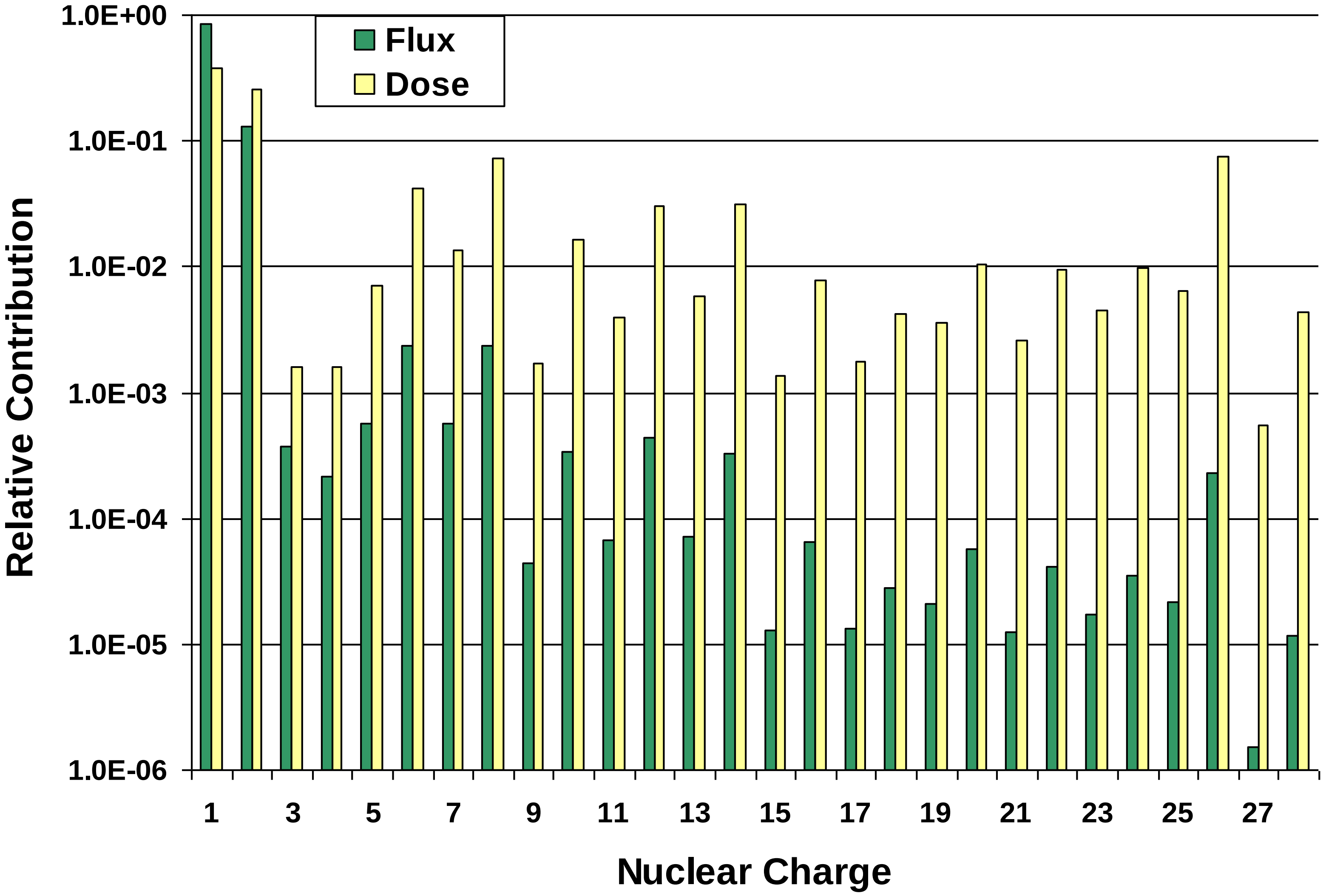


Galactic Cosmic Rays (GCR) in free space using the empirical Badhwar- O’Neill Model

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

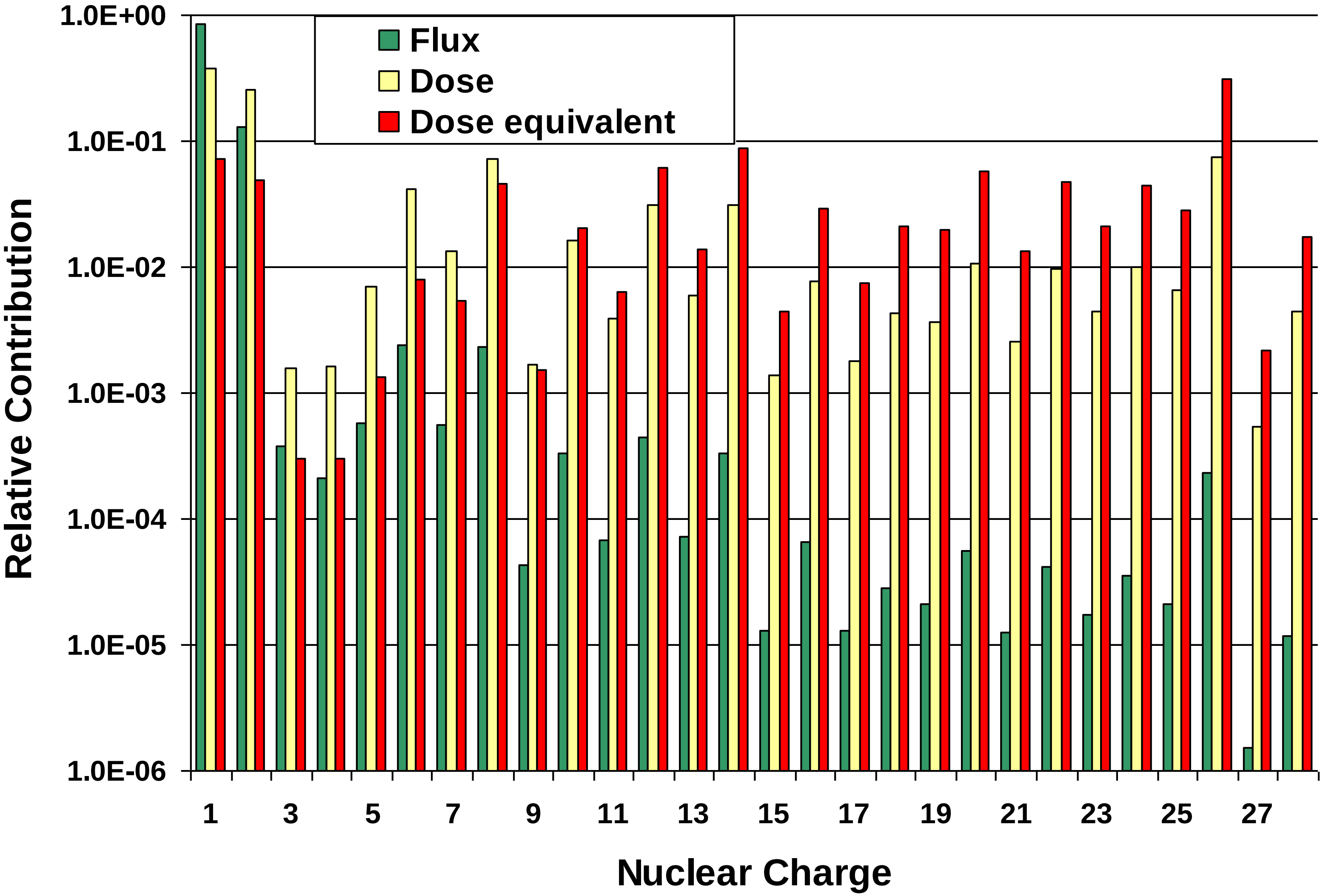
Zeitlin, 2013 NSRSS

Galactic Cosmic Radiation (GCR)



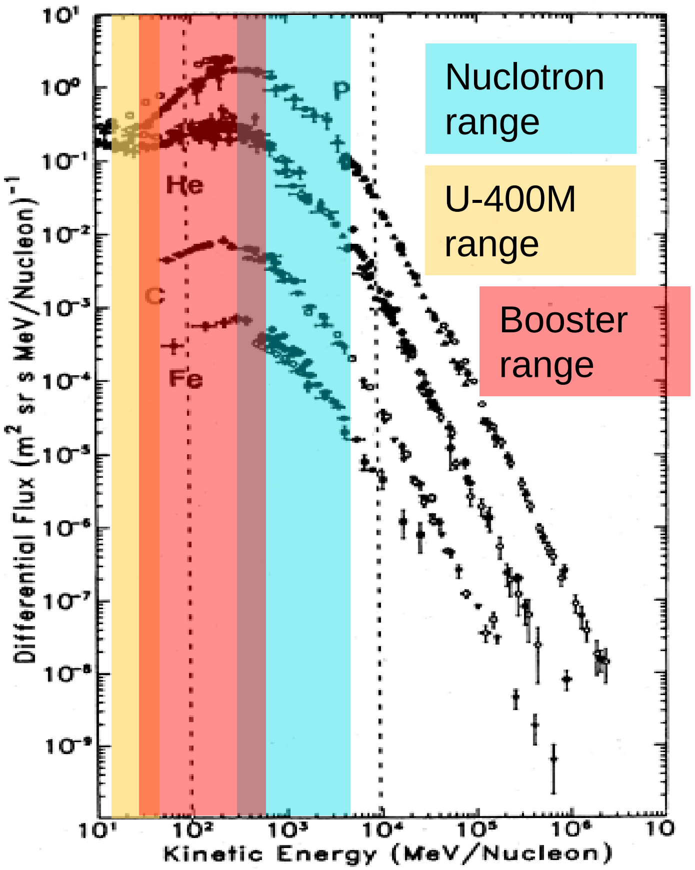
Zeitlin, 2013 NSRSS

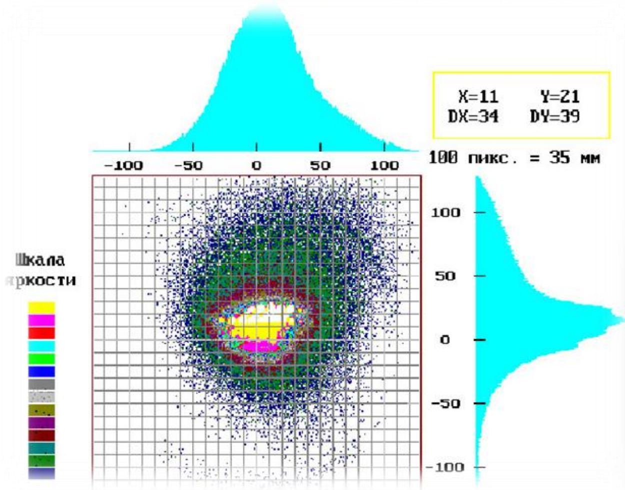
Galactic Cosmic Radiation (GCR)

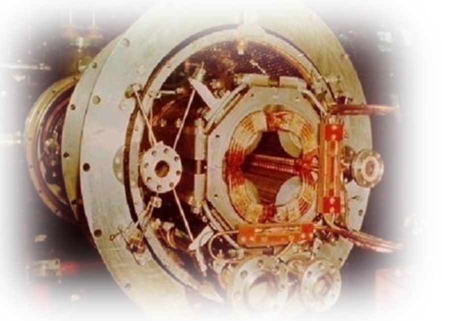


Zeitlin, 2013 NSRSS

GCR and JINR’s accelerators energy spectra







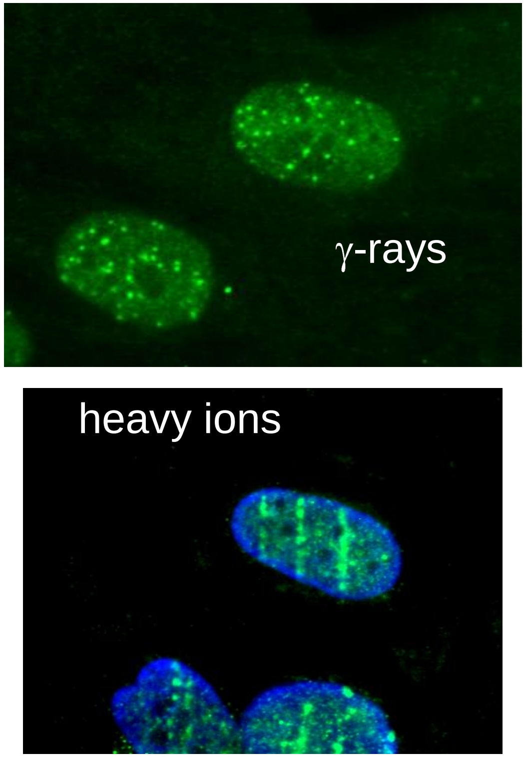
Why heavy ions are so important for space radiobiology?

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

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



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

CNS Risks from Galactic Cosmic Rays (GCR)

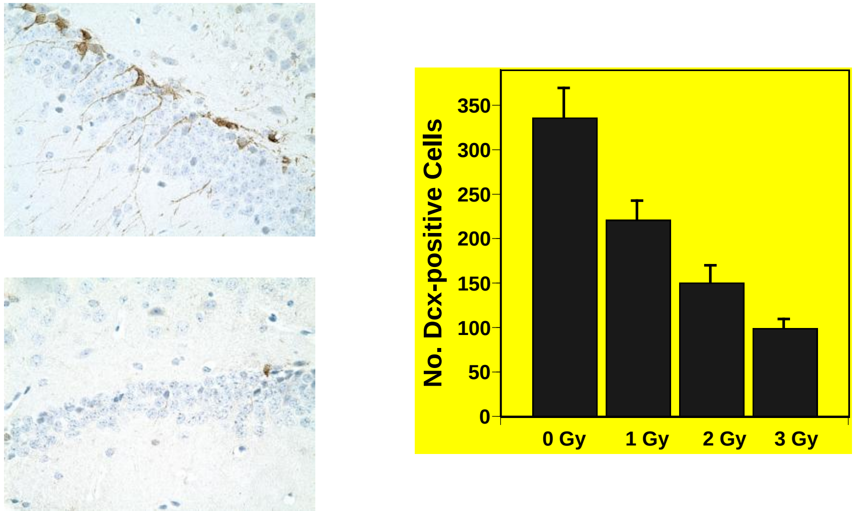
- **Retinal flashes** observed by astronauts (suggests single heavy nuclei can disrupt brain function).
    - Central nervous system (CNS) damage **by x-rays is not observed** except at very high doses
- In-flight cognitive changes and late effects similar to Alzheimer’s disease are a concern for GCR.
- 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

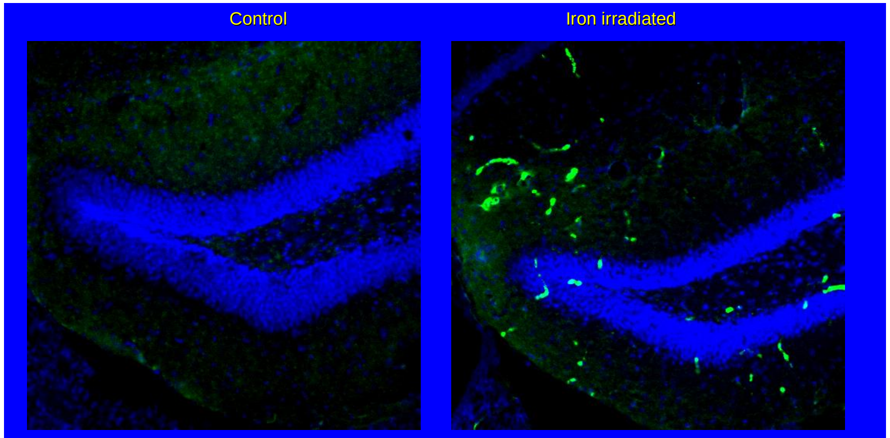
• 2 – 13% of cells would be hit by at least one Fe ion during a Mars mission.

• 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)



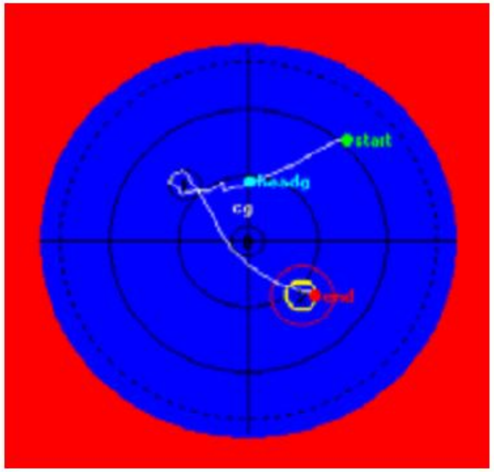
Oxidative Stress (Lipid peroxidation:4-Hydroxynonenal) is Increased in Mouse Hippocampus 9 Months After 2 Gy of ^{56}Fe Irradiation

Cucinotta, 2013 NSRSS

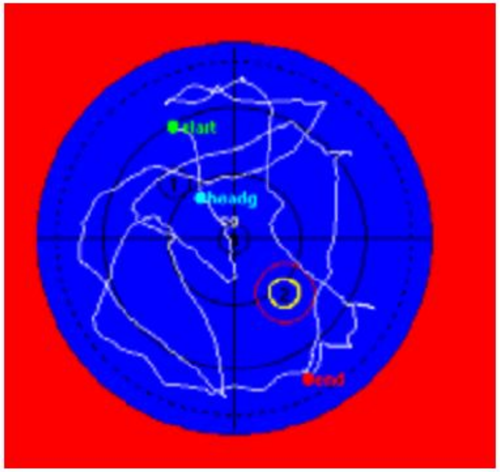
Cognitive test (Morris test)

^{56}Fe ions, 1 GeV/nucleon

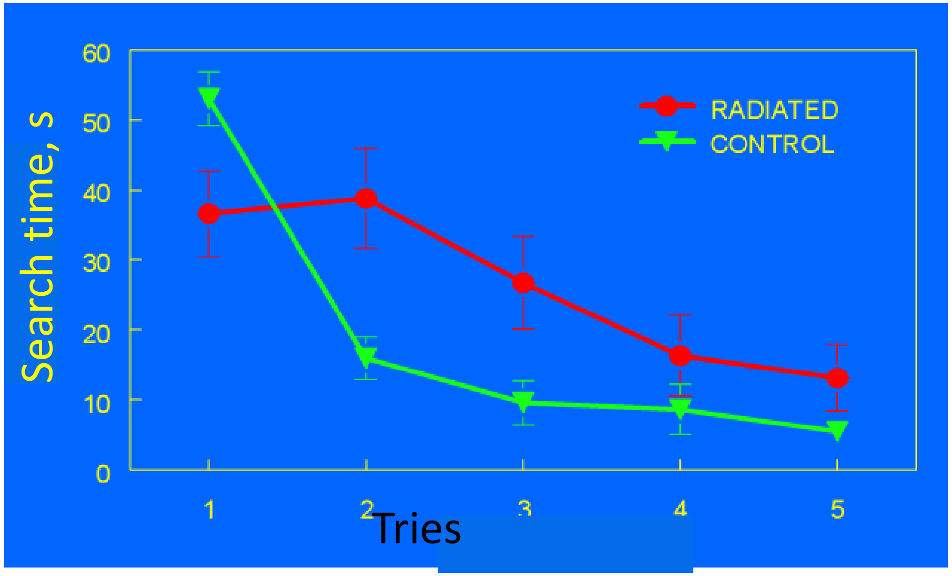
Control

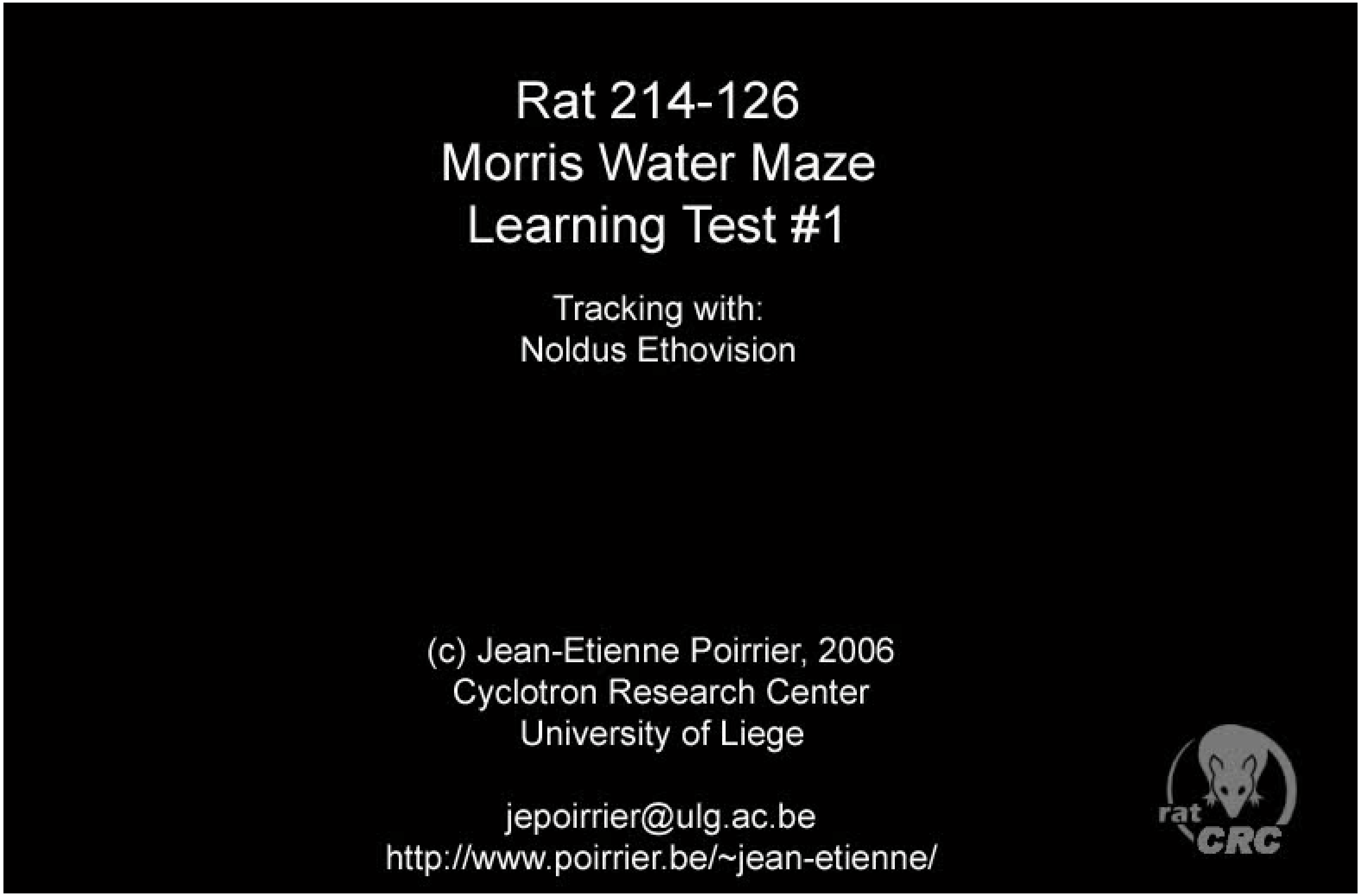


1.5 Gy



1 month after irradiation





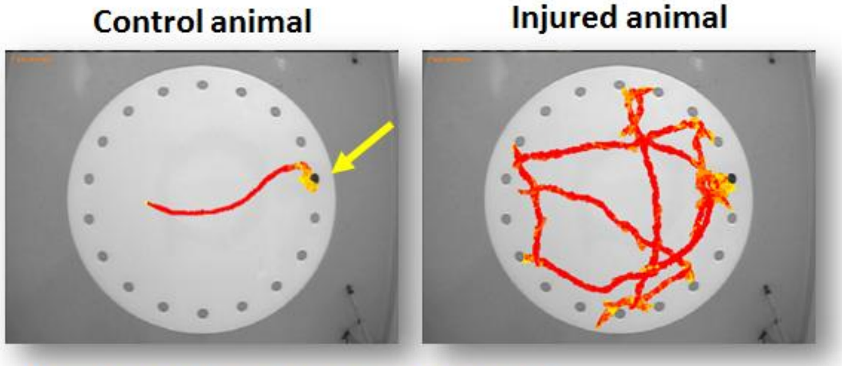
Rat 214-126
Morris Water Maze
Learning Test #1

Tracking with:
Noldus Ethovision

Cyclotron Research Center
University of Liege

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

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

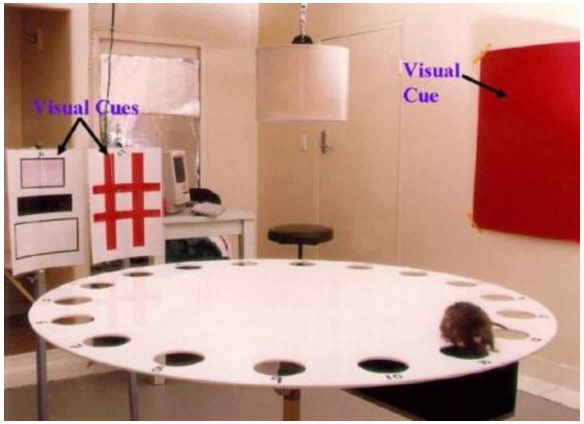


Traces of Barnes maze performance until the mouse escape into the target hole (yellow arrow head)

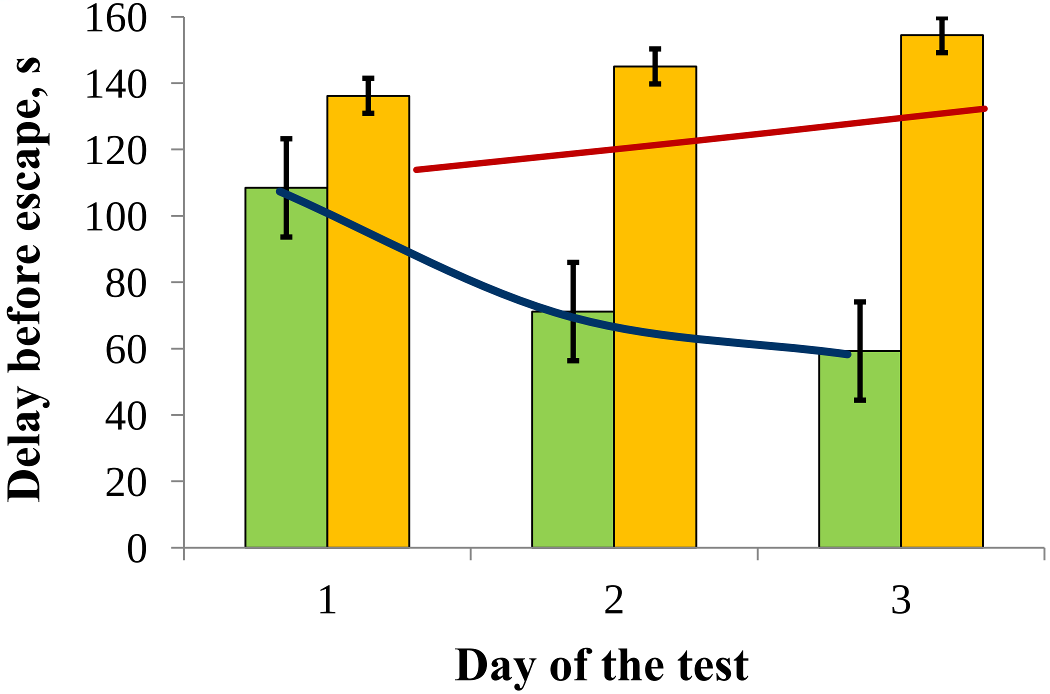
0.2 Gy

^{56}Fe, 1 GeV/n

$\Phi \approx 10^5$ /см^2

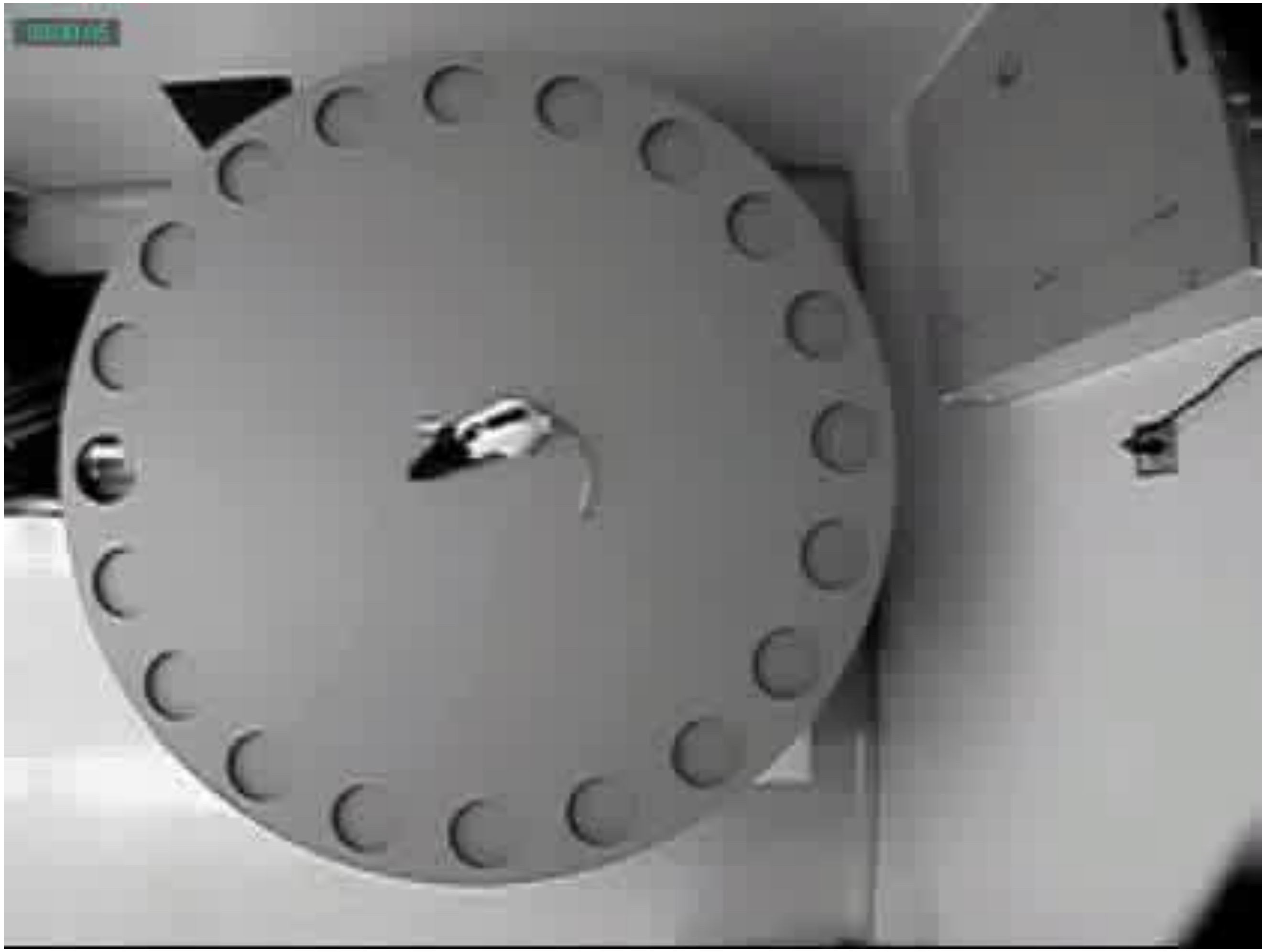


3 months after irradiation



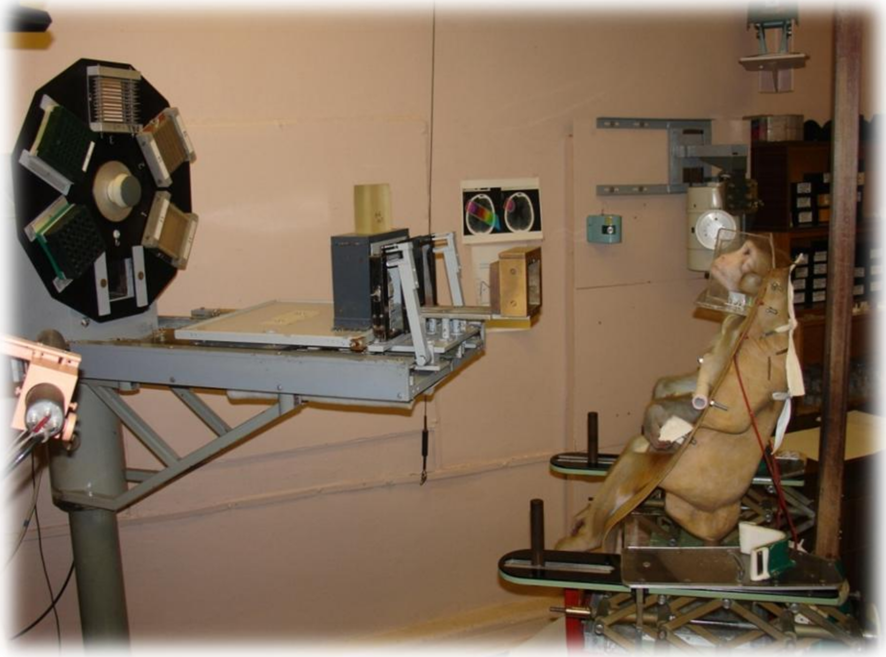
R. Britten et al., 2012

Barnes maze test



First experiments with monkeys

Irradiation with a proton medical beam, 170 MeV



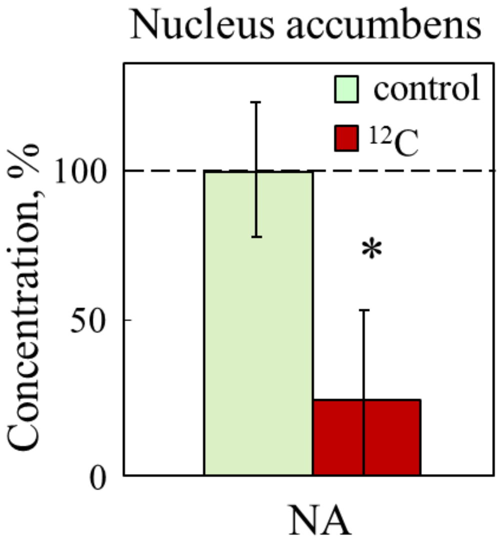
Irradiation with ^{12}C ions, 500 MeV/u, at the Nuclotron

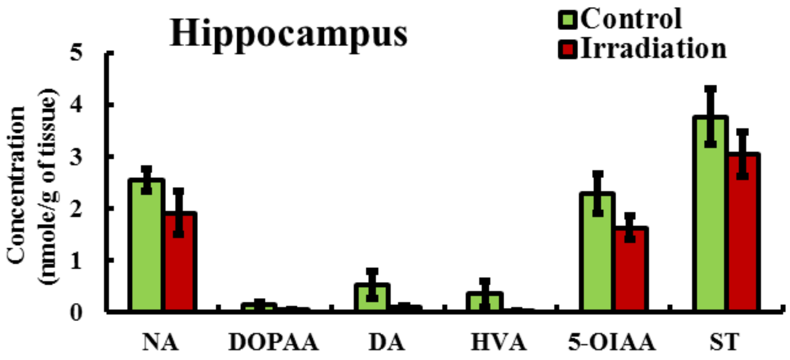
Neurotransmitters levels after irradiation

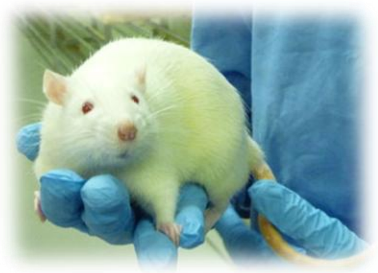
Irradiation with 1 Gy of 500 MeV/u carbon ions

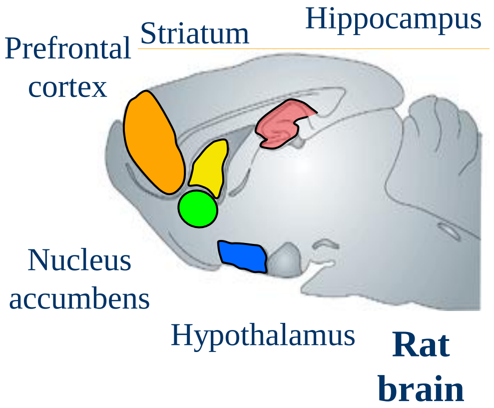
Radiation-induced decrease in the level of neurotransmitters is observed in the brain regions responsible for the *emotional and motivational state*

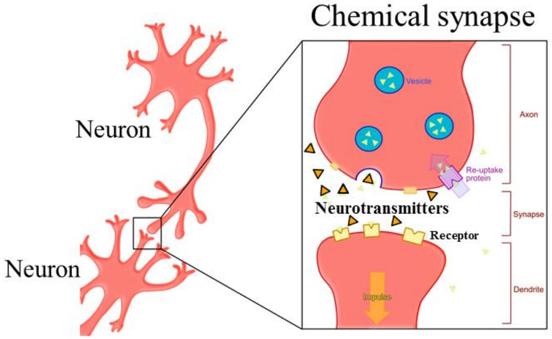
3 months after irradiation



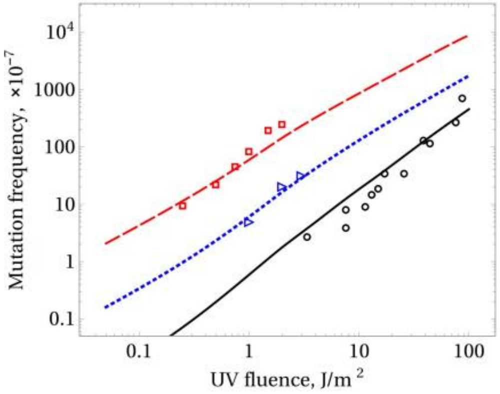


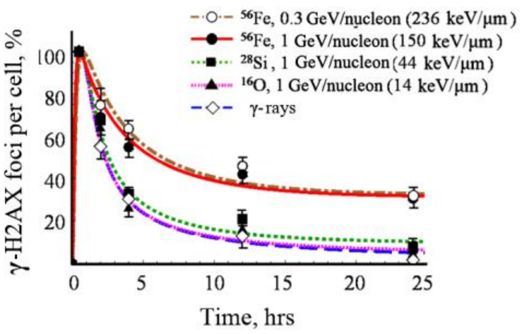






MATHEMATICAL MODELING



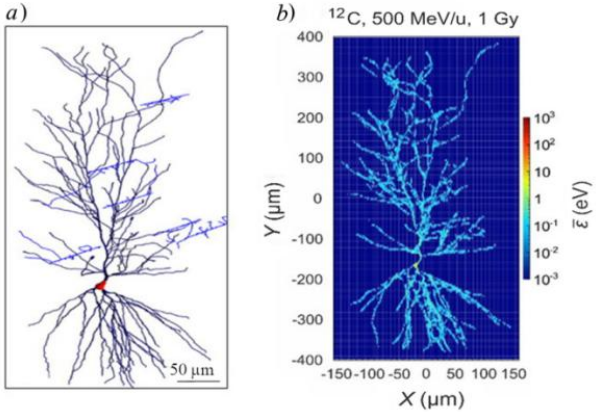


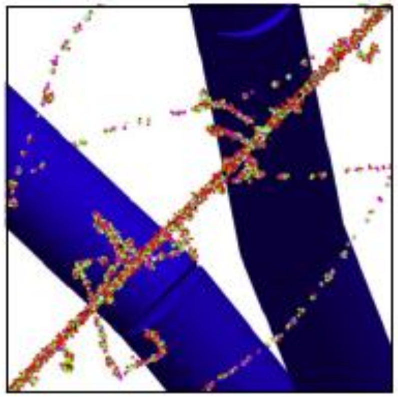
- Mathematical models of key radiation-induced DNA damage repair systems in bacterial cells were developed.
- Comprehensive computational study of radiation induced mutagenesis was performed.
- Detailed model of radiation-induced DNA double-strand break repair in mammalian and human cells was developed

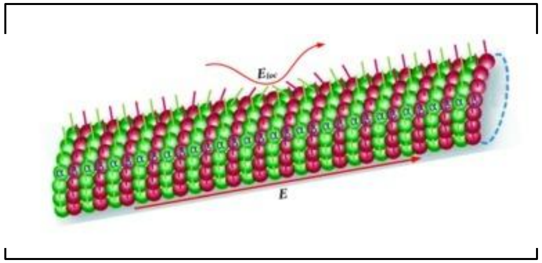
J. Theor. Biol. 2009, 2013, 2015

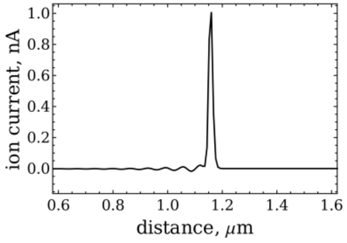
- Geant4-DNA toolkit was applied for the simulation of energy deposition processes in charged particle tracks and water radiation chemistry.
- The estimation of spatial energy and dose distributions, and the yield of radiolytic species was obtained within a single neuron and in a small neural network.

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





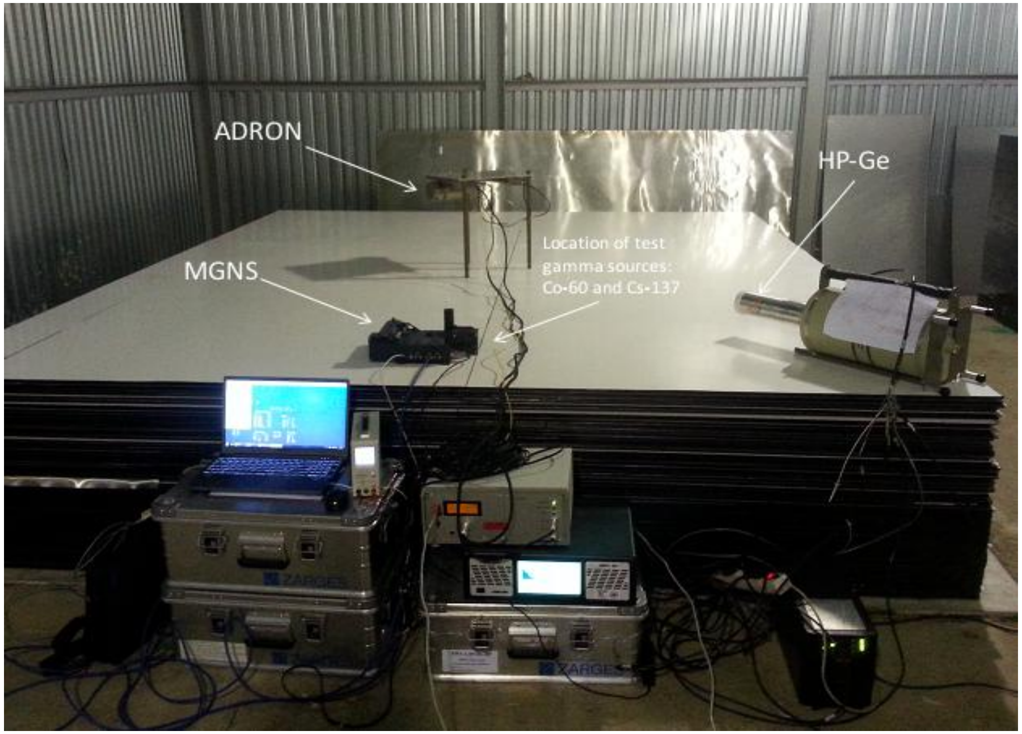




- Comprehensive models of intracellular signal transport phenomena were developed.
- The influence of low energy radiation on signals in molecular systems was studied.

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

Nuclear planetary science



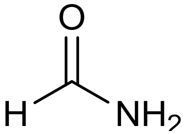
In collaboration between the Space Research Institute (RAS) and FLNP (JINR), a ***special facility has been constructed*** at the LRB that can ***model planetary soil*** and allows testing prototypes of active neutron and gamma spectrometers.

- 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) - **helping to find water**

Astrobiology

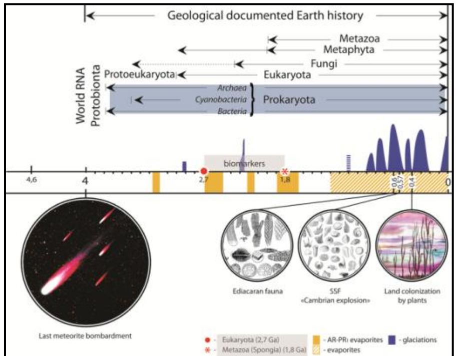
Theme: **"Research on Cosmic Matter on the Earth and in Nearby Space; Research on the Biological and Geochemical Specifics of the Early Earth“**

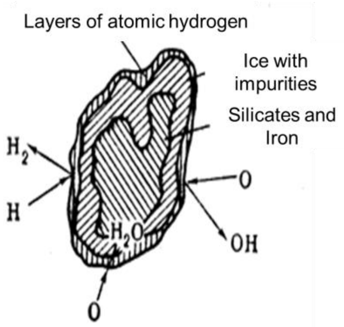
Main fields of activity:

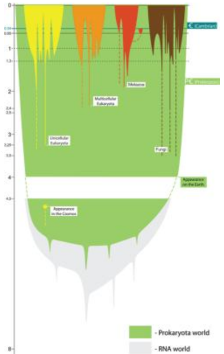


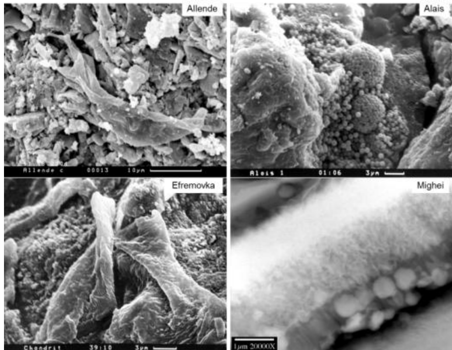
Formamide

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

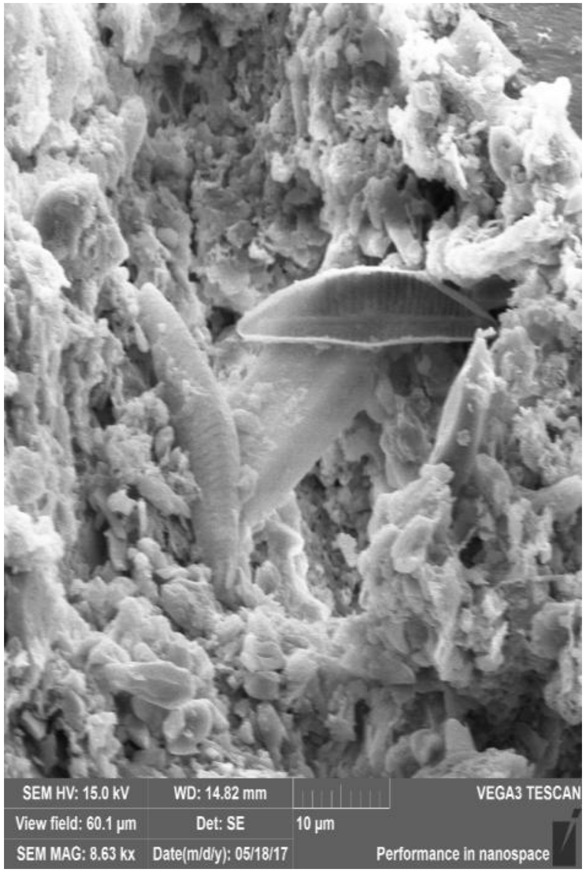




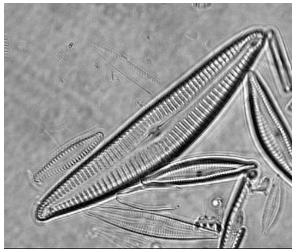


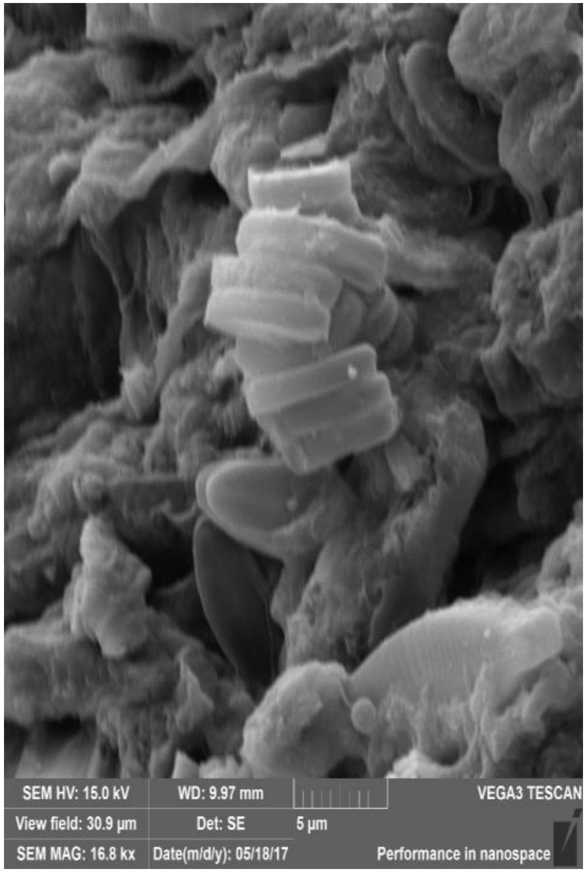


Astrobiology – biological objects on meteorites

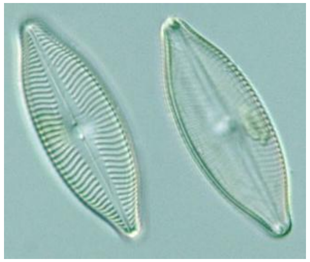


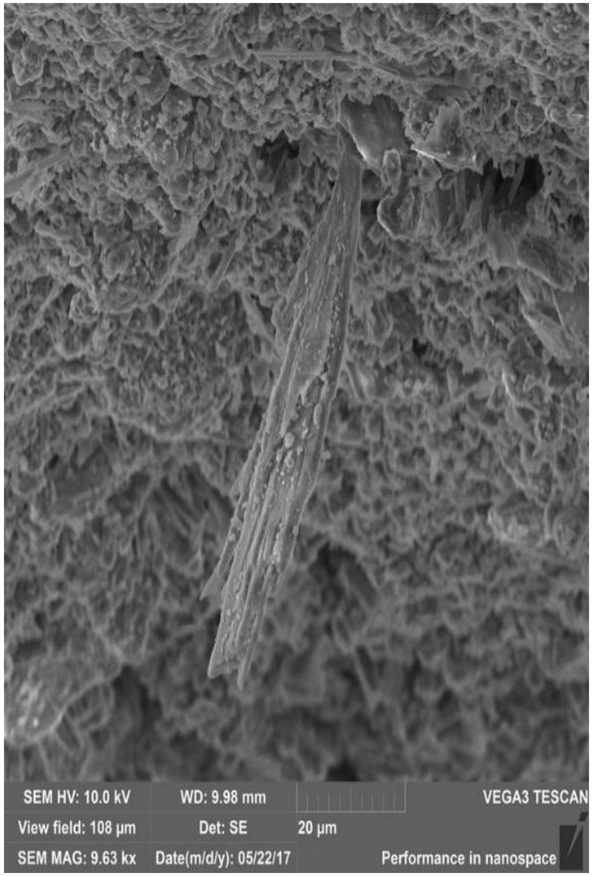
Diatom *Cymbellaceae*
Meteorite Polonnaruwa





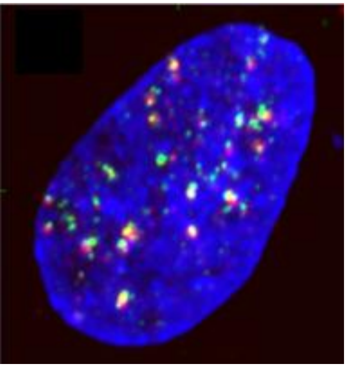
Diatom *Naviculaceae*
Meteorite Polonnaruwa

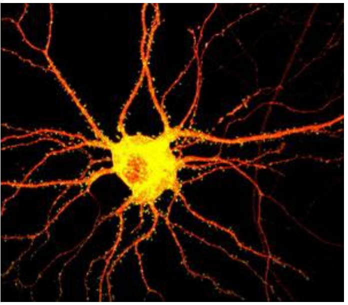




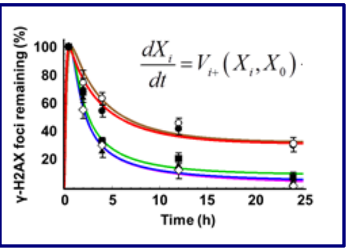
Part of bacteria (capsule)
Meteorite Orgueil

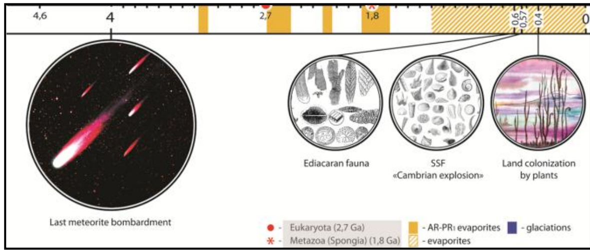
Outlook for radiobiological studies at JINR





- 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.

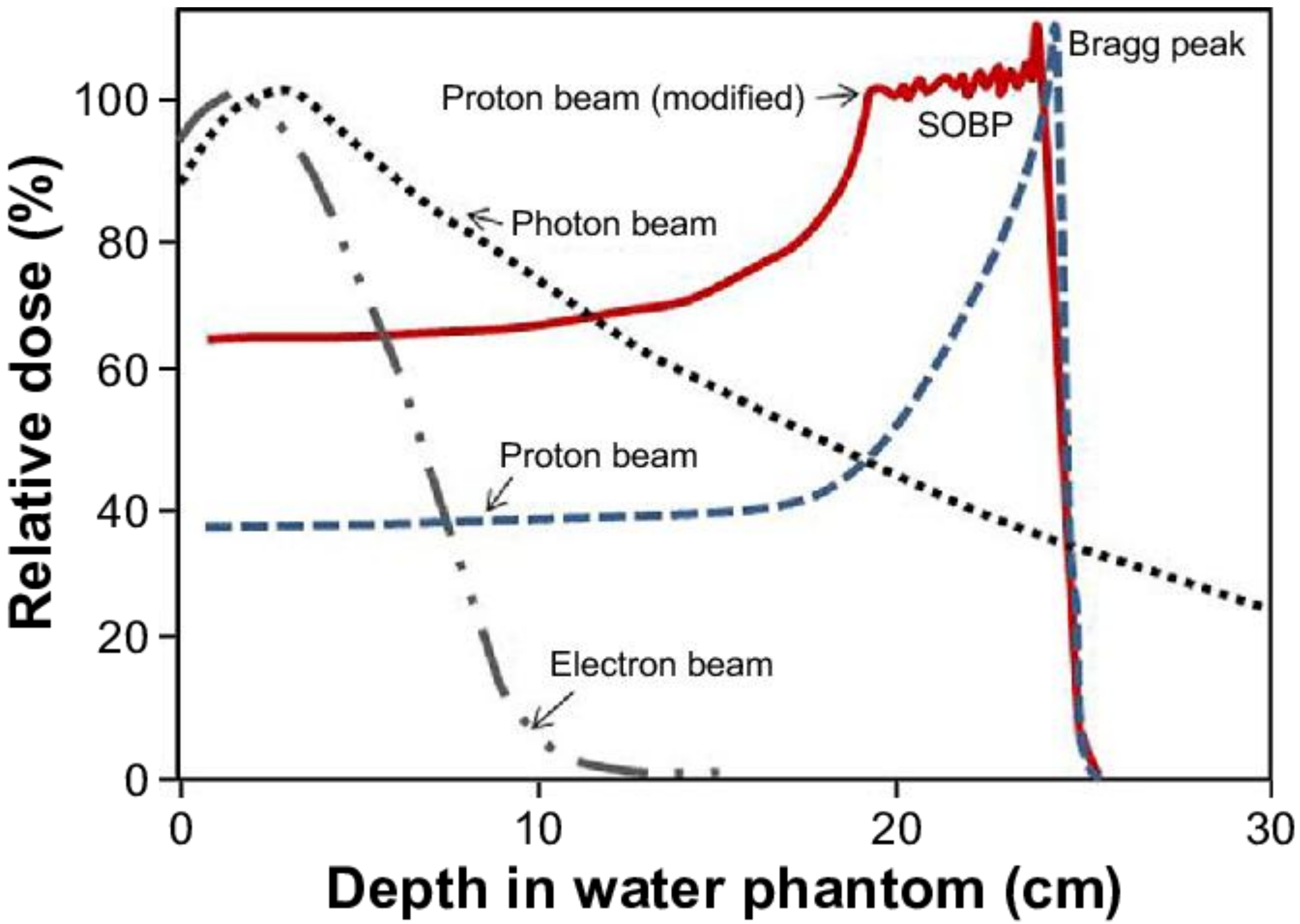




THANK YOU FOR YOUR ATTENTION!

pavel.blahax@gmail.com

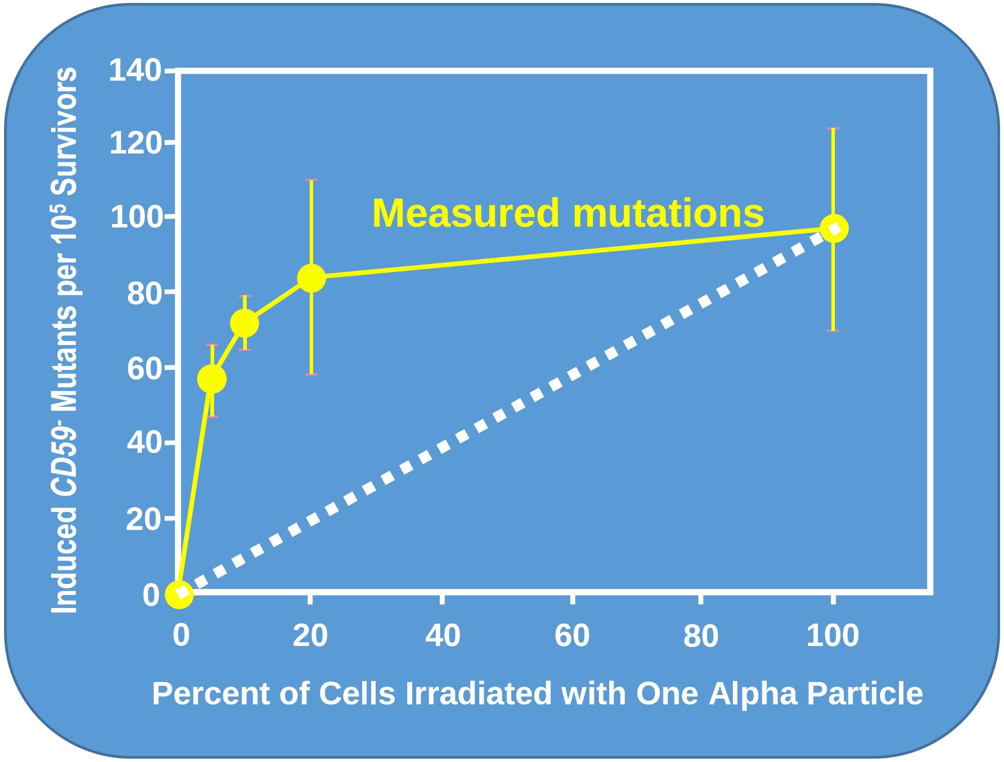
Dose deposition



Yamoah and Johnstone, OncoTargets and Therapy 2016:9 5721–5727

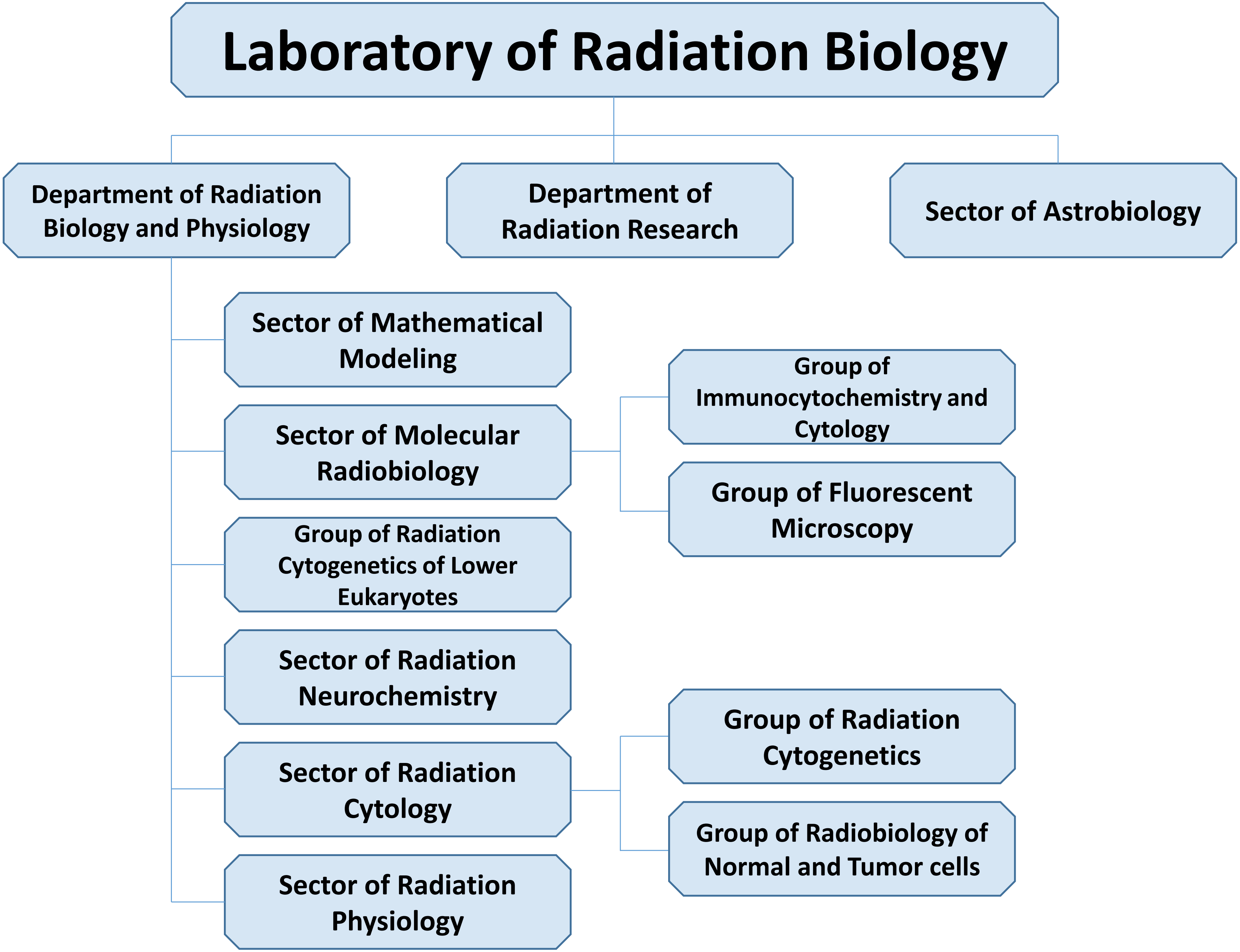
Supra-linear model – by-stander effect

- Microbeam studies – precise irradiation of selected part of the cell



- Where bystander responses have been quantitated, they have shown saturation
- Extrapolating linearly from low to very low doses could underestimate the risk at very low doses

Zhou et al PNAS <u>98,</u> 14410-5 (2001)



Laboratory of Radiation Biology

- Department of Radiation Biology and Physiology
  - Sector of Mathematical Modeling
  - Sector of Molecular Radiobiology
    - Group of Immunocytochemistry and Cytology
    - Group of Fluorescent Microscopy
  - Group of Radiation Cytogenetics of Lower Eukaryotes
  - Sector of Radiation Neurochemistry
  - Sector of Radiation Cytology
    - Group of Radiation Cytogenetics
    - Group of Radiobiology of Normal and Tumor cells
  - Sector of Radiation Physiology
- Department of Radiation Research
- Sector of Astrobiology