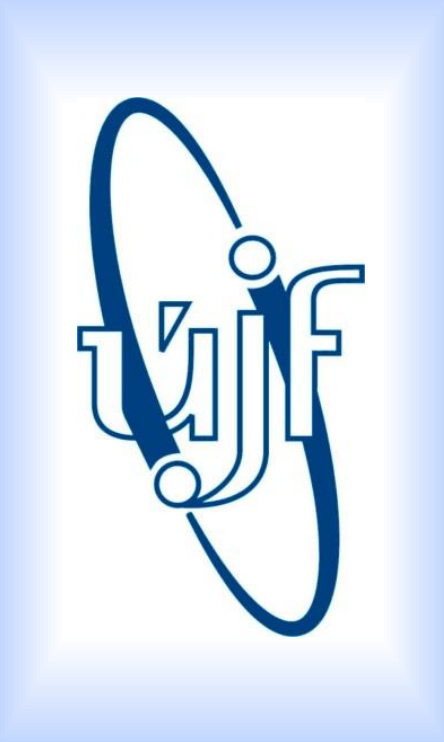
Biological effects of extremely low radiation background conditions



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Nuclear Physics Institute
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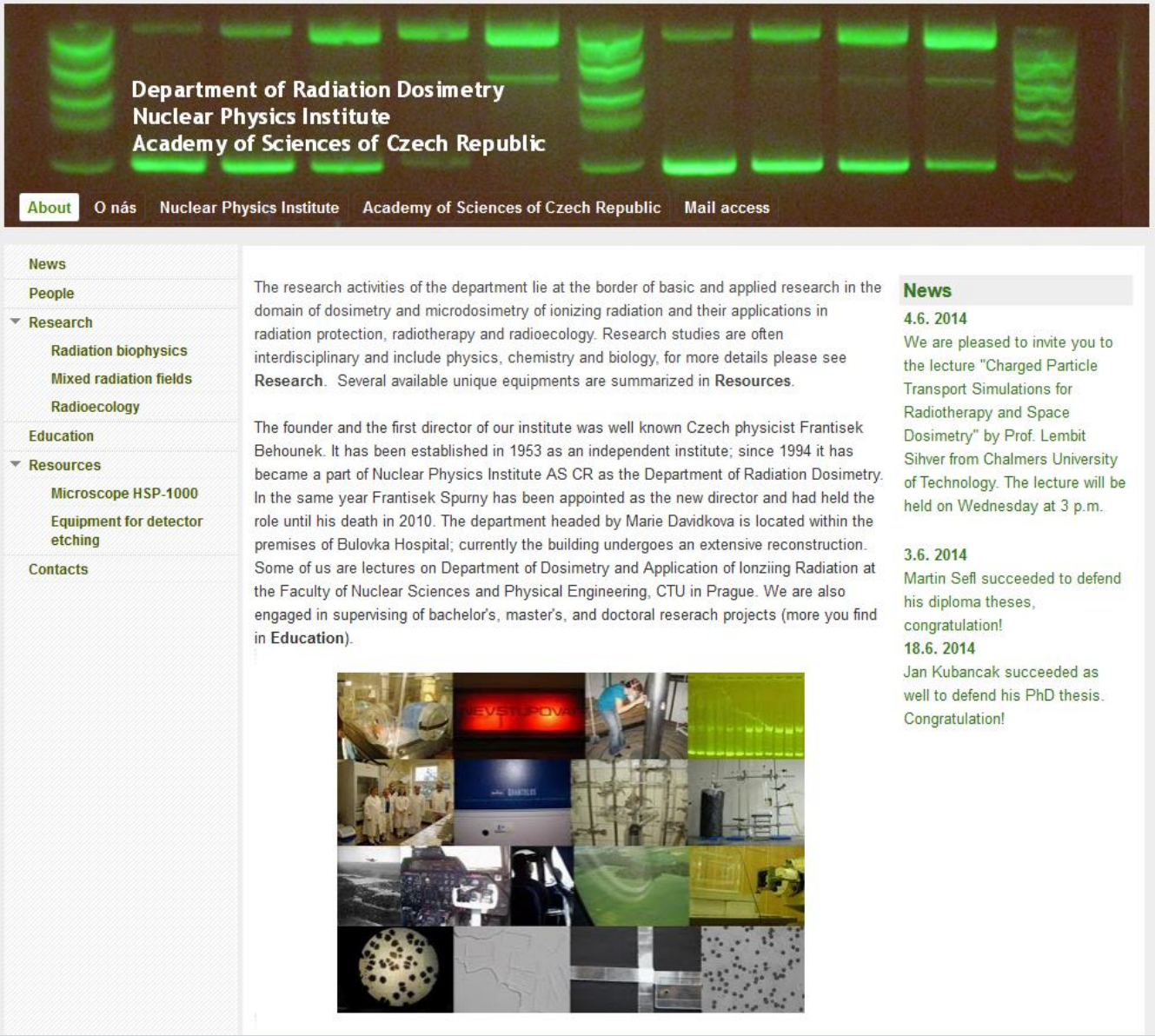
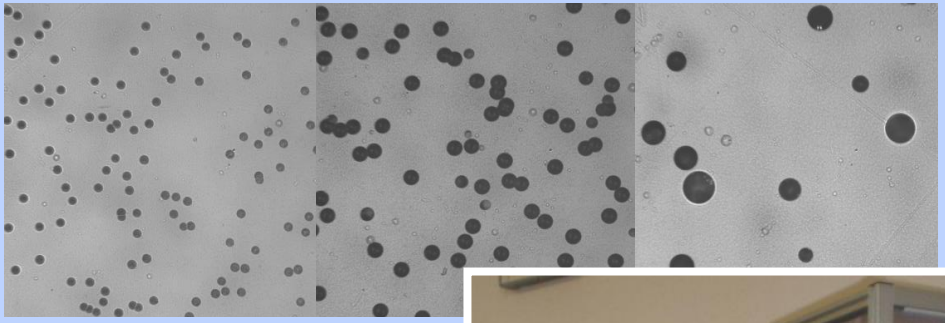
http://www.odz.ujf.cas.cz/



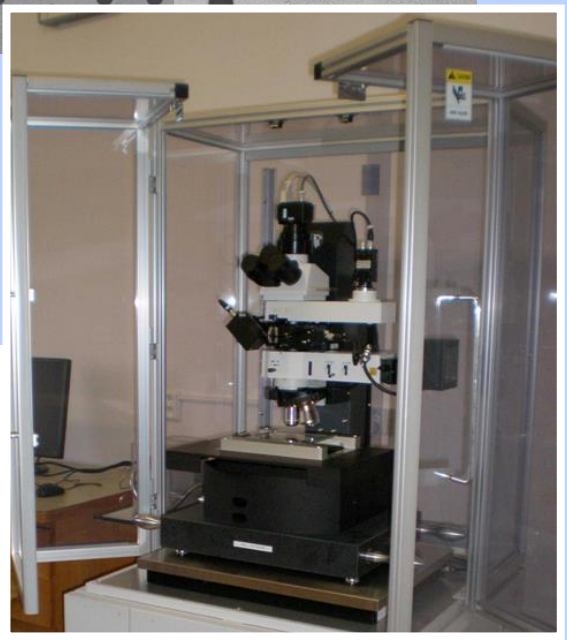
Development of new dosimetric methods for

- LET spectrometry
  (track-etched and semiconductor detectors)
- measurements in mixed radiation fields



Monitoring of cosmic radiation in space and at mountain observatories for research on

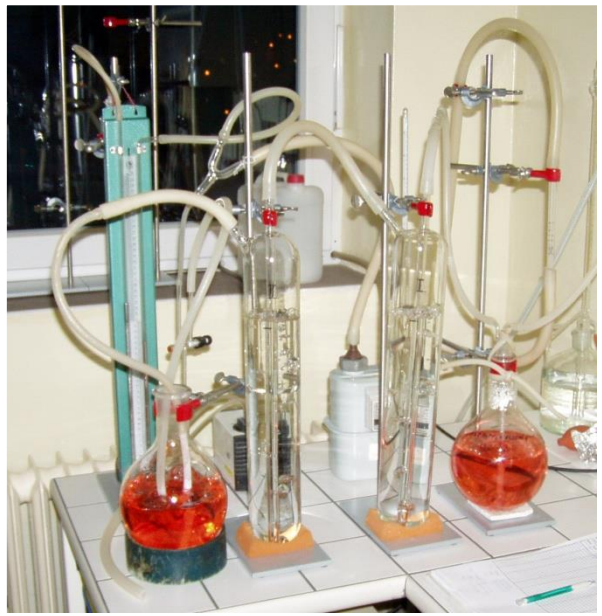
- space weather
- aircraft and spacecraft crew dosimetry



Radioecology and radiocarbon dating

- Radiocarbon dating laboratory CRL (in co-operation with Archaeological Institute ASCR)
- Fossil fuel combustion and atmospheric $^{14}CO_2$ and CO_2
- Past environmental changes and ^{14}C
- ^{14}C in the vicinity of NPPs and in reference areas

Low background liquid scintillation spectrometers QUANTULUS 1220



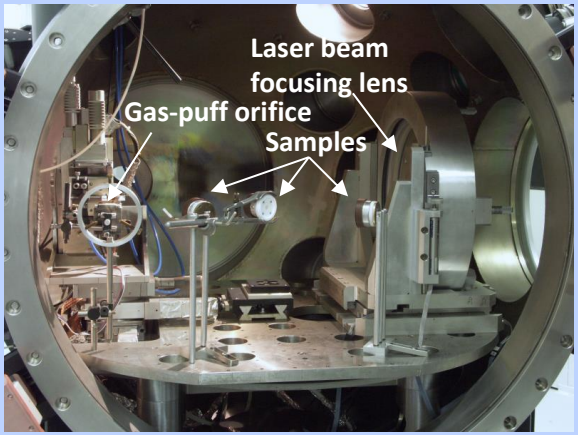
Monitoring of atmospheric $^{14}CO_2$

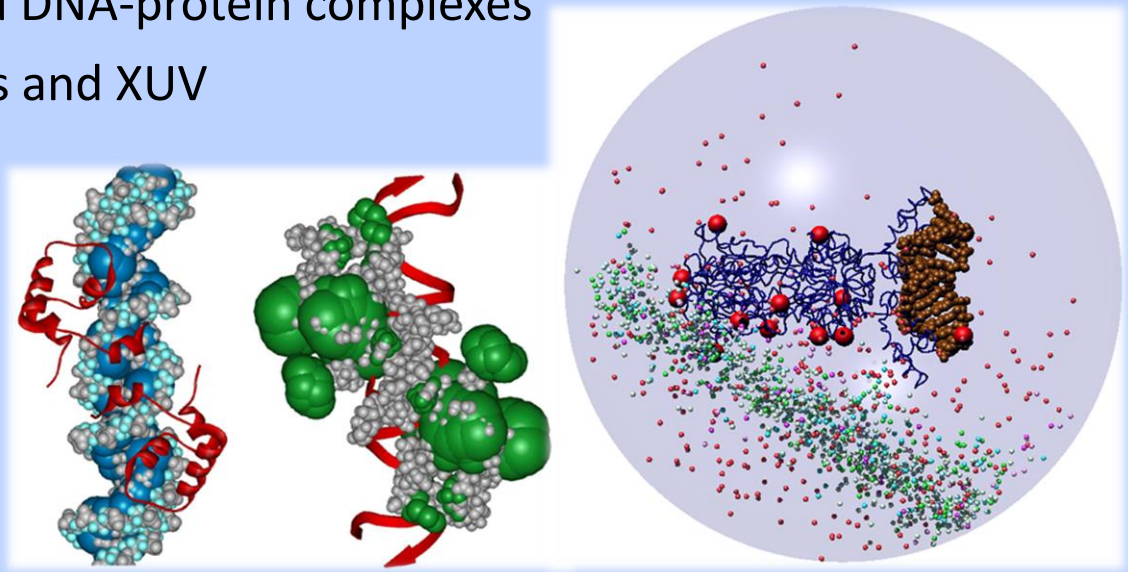
Sampling in the vicinity of NPP Temelín

- Development of new analytical methods
- Theory of formation of liquid scintillation pulse spectra

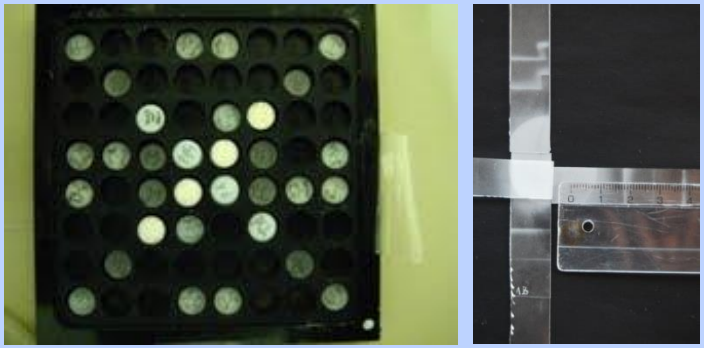
Research on biological effects of radiation

- Radiation damage to DNA and DNA-protein complexes
- Biological effects of soft X rays and XUV

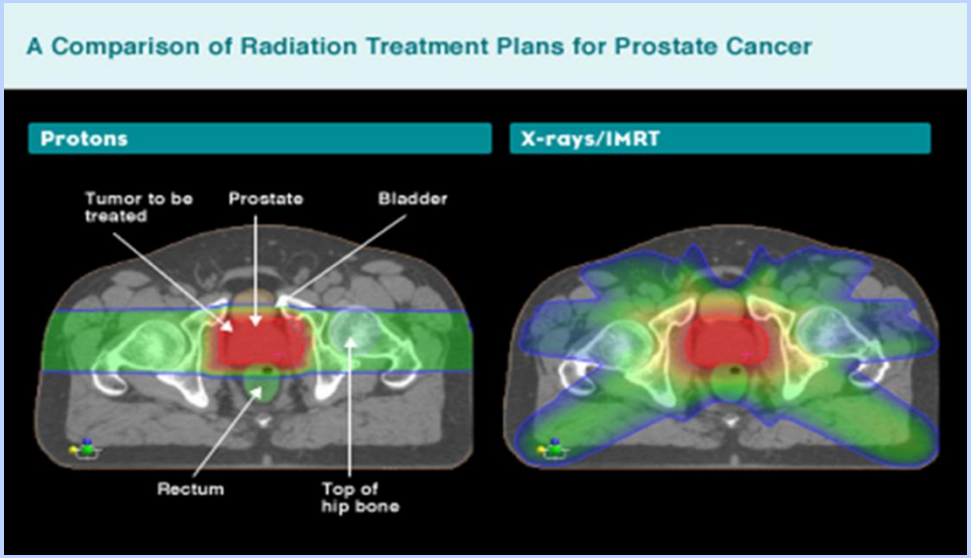




- Radiation therapy:
  - Induction of secondary cancers
  - Nuclear fragmentation

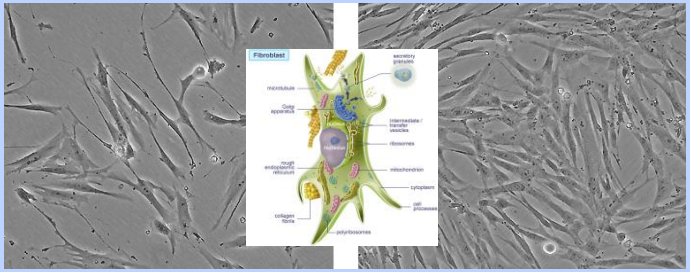


TLD and PADC in proton beam



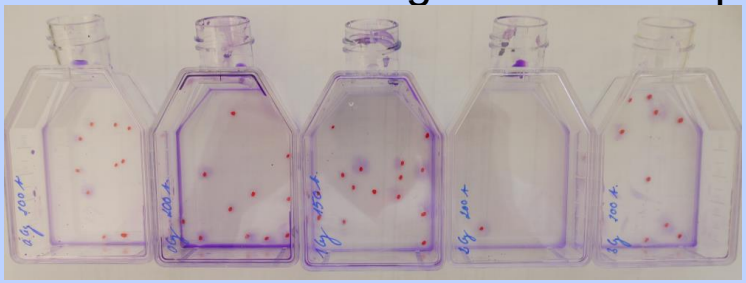
NPI rabiobiology program

First experiments using human normal neonatal skin fibroblasts.



Cells grown on a 2.5 µm mylar foil stretched on a plastic ring (Chemplex Industries, USA)

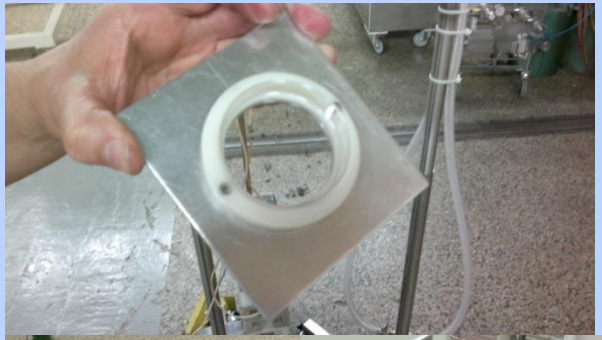
In some cases cells were grown in T25 flasks or on glass covered petri dishes.

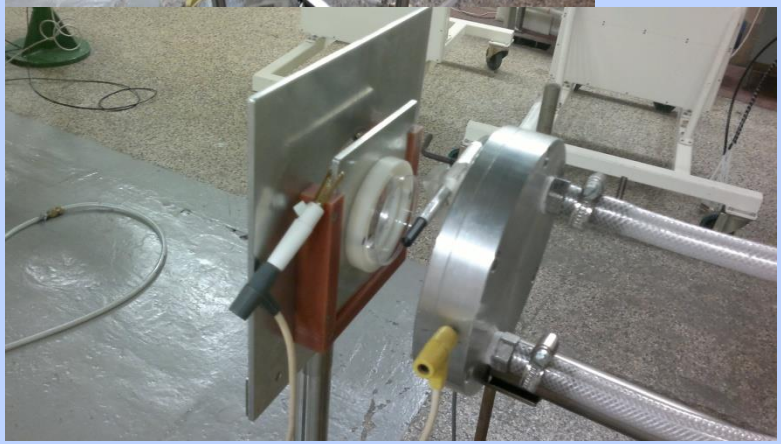


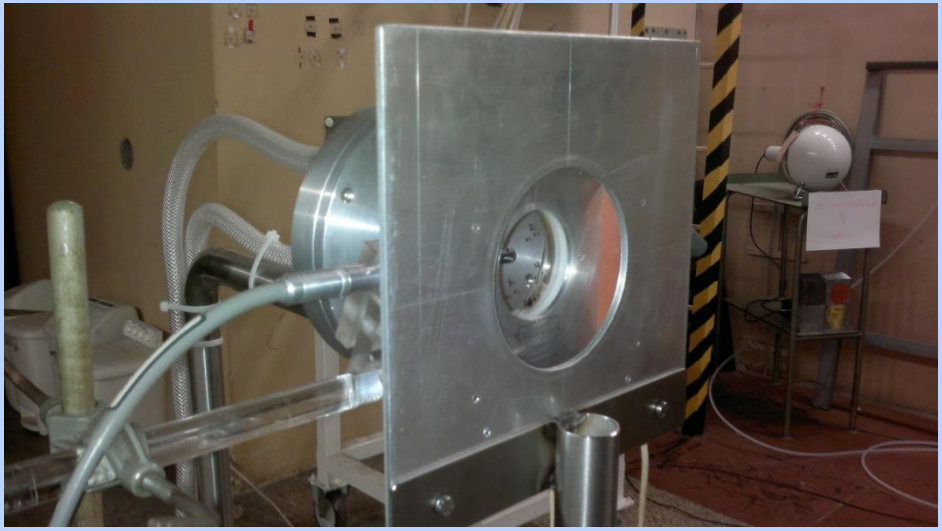
Cyclotron U-120M

Isochronous cyclotron U-120M located in the NPI

Current parameters of accelerated and extracted beams are: p+/ H−: 5.4–38 MeV, D+/ D−: 11–20.5 MeV, 3He+2: 16.2–55 MeV, 4He+2: 22–40 MeV

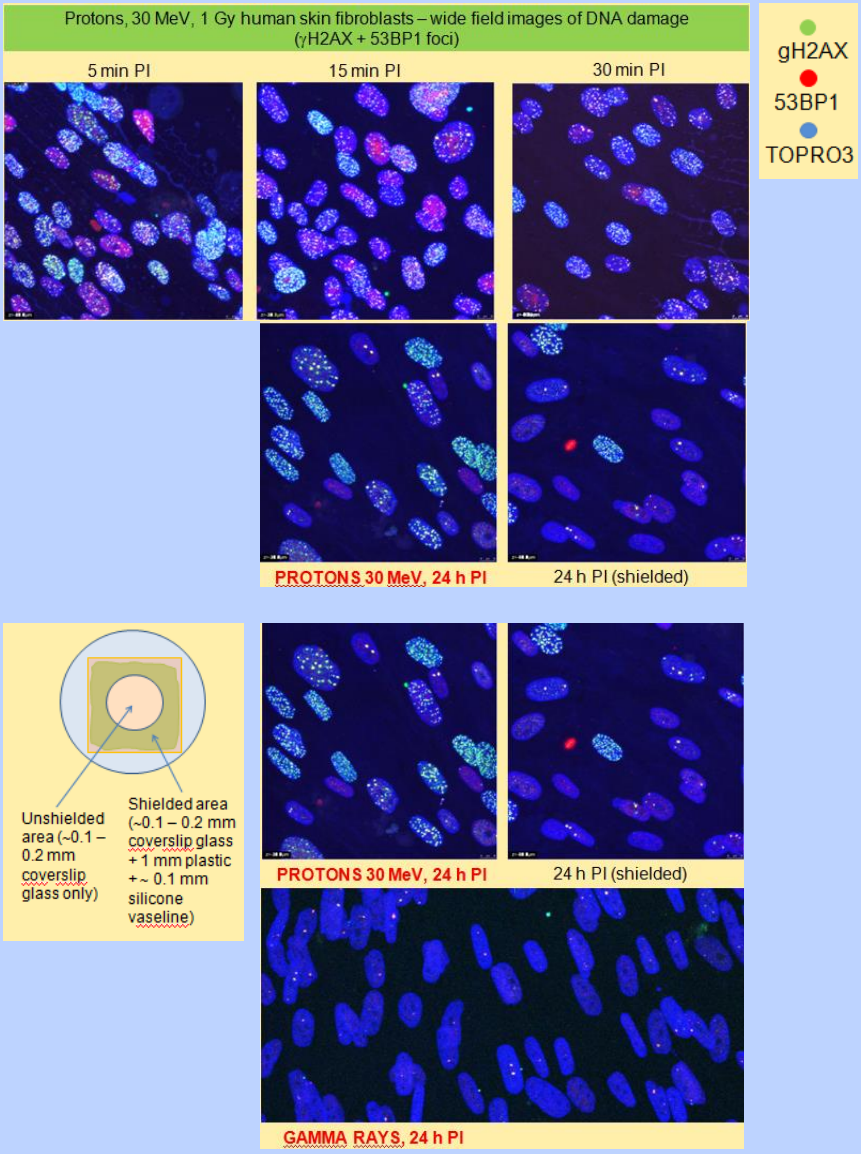


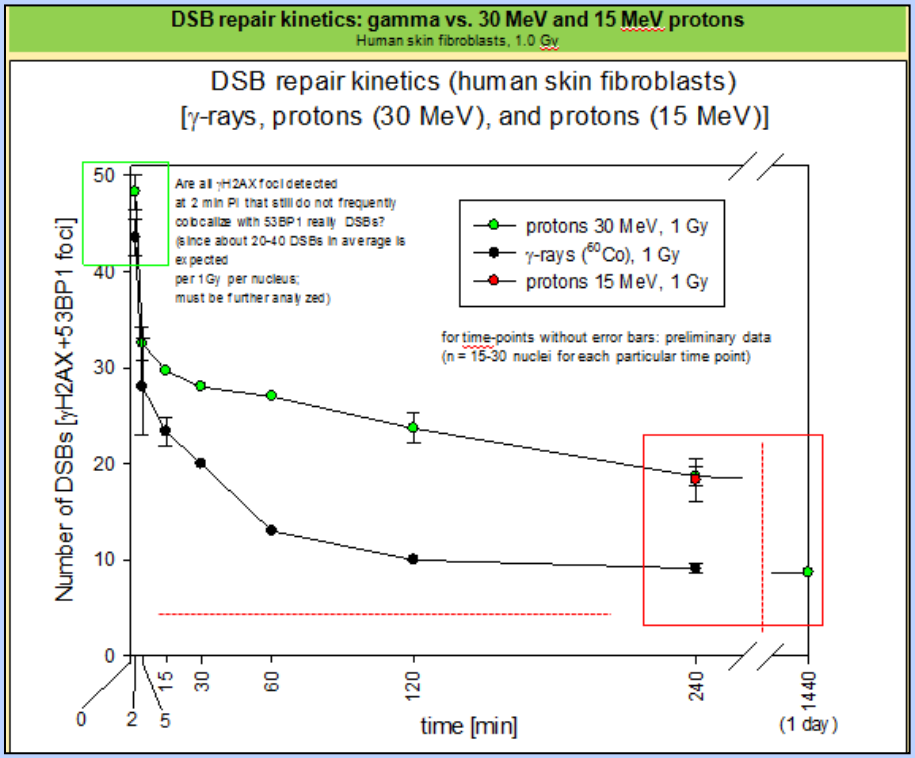


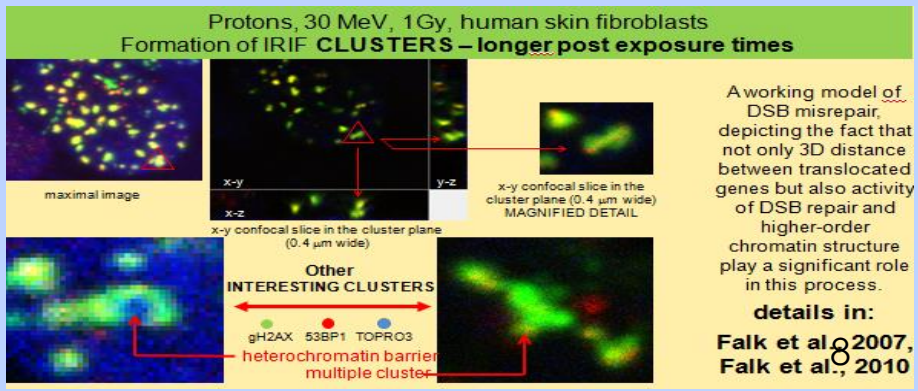


DSB induction and repair

cyclotron U-120M

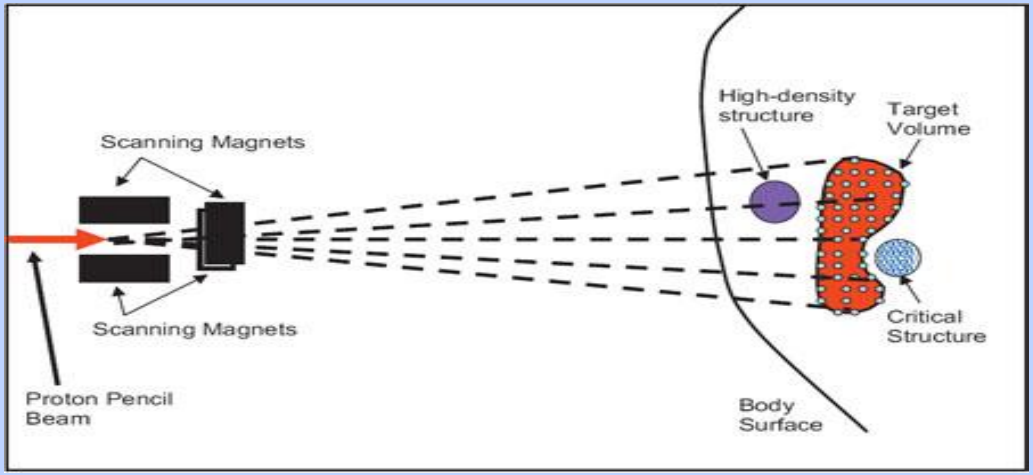




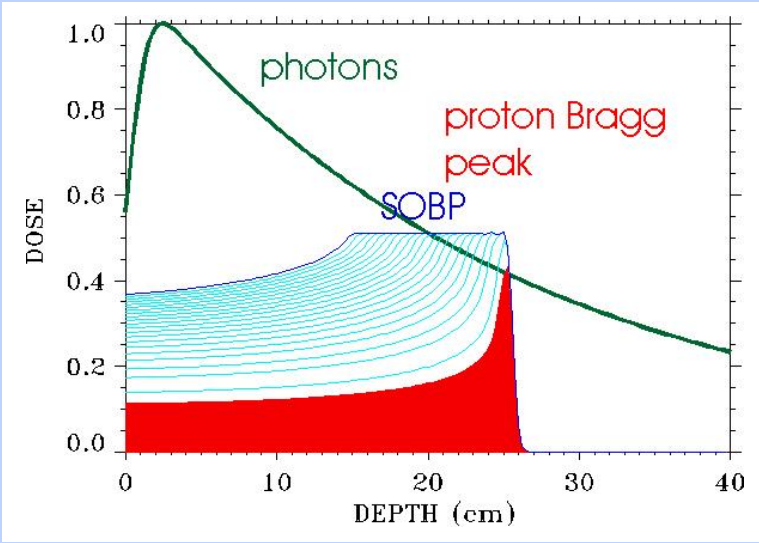


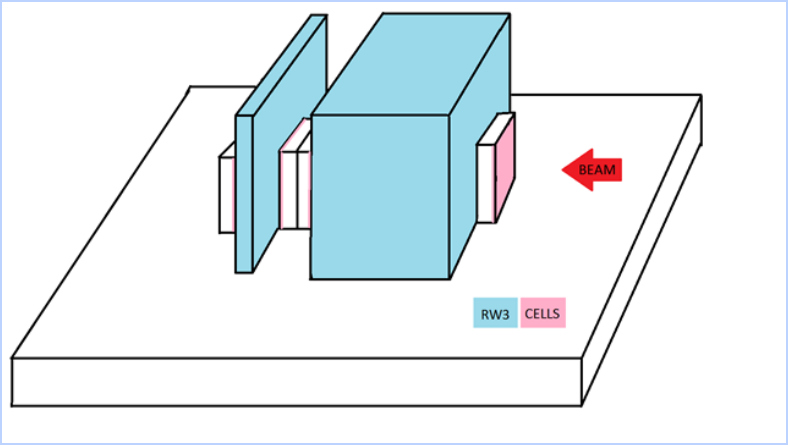
Proton Therapy Center Prague - Cyclotron IBA

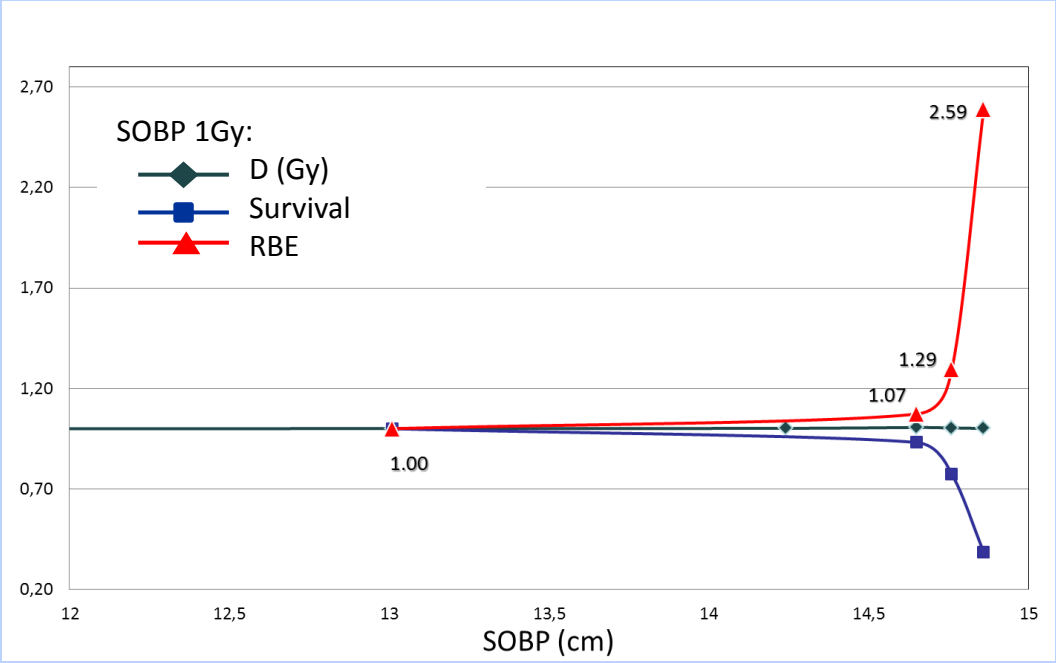
- 100-226 MeV
- PBS – Pencil Beam Scanning mode
- First patient on 12.12.12



RBE of proton SOBP

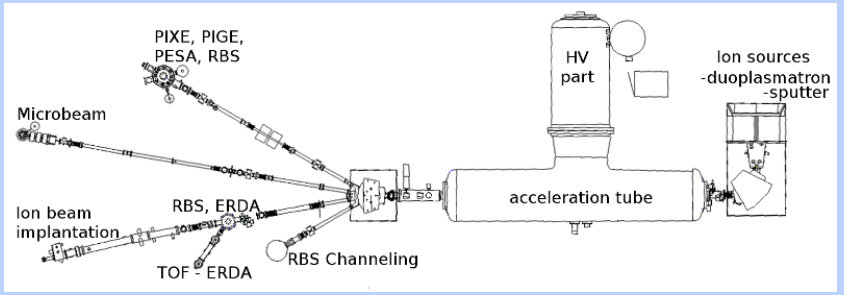


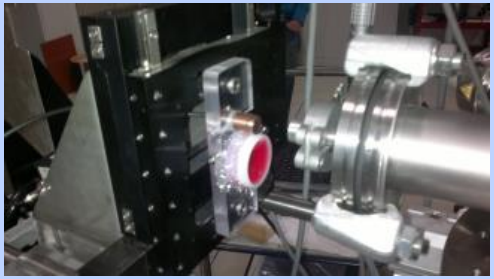


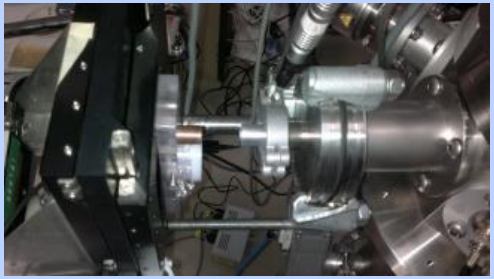


Tandetron 4130 MC

- Installed in NPI in 2005
- Accelerating ions of most of elements from H to Au with energies from 0.4-20 MeV and intensities up to tens of µA.
- The main laboratory accessories are devices for material characterization by standard nuclear analytical techniques (RBS, RBS-channeling, ERDA, ERDA-TOF, PIXE, PIGE, and Ion-Microprobe with 1 µm lateral resolution) and for high-energy implantation.







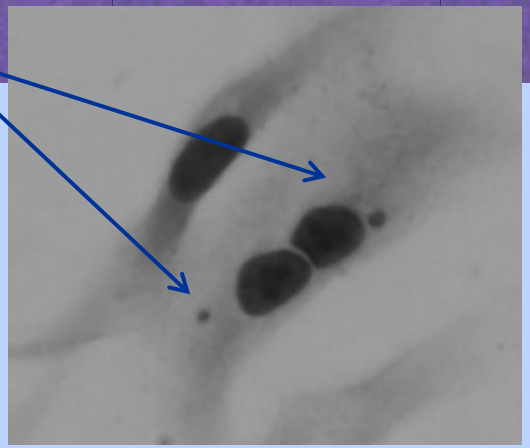
3 fluencies of 2 MeV protons

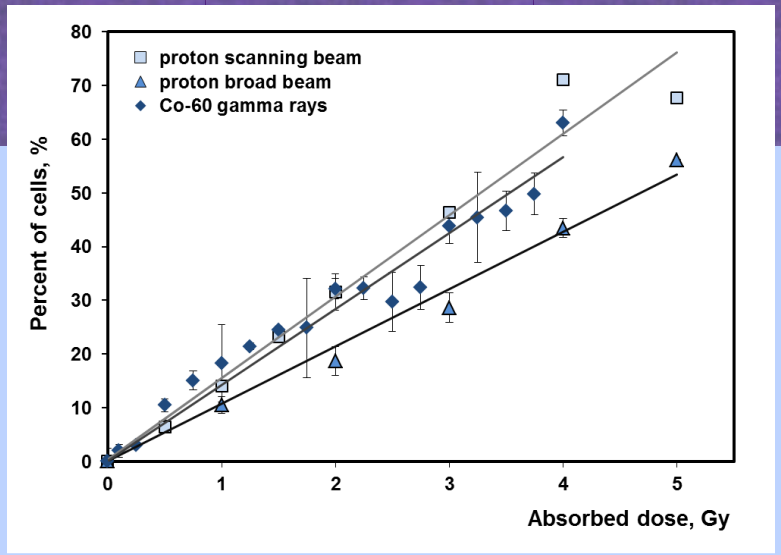
	beginning	end	mean	approx.dose
samples	protons/s			22min, 32x32mm
0	0	0	0	0 Gy
1	7900	7800	7850	0.1 Gy
2	37000	37000	37000	0.5 Gy
3	90000	85000	87500	1.2 Gy

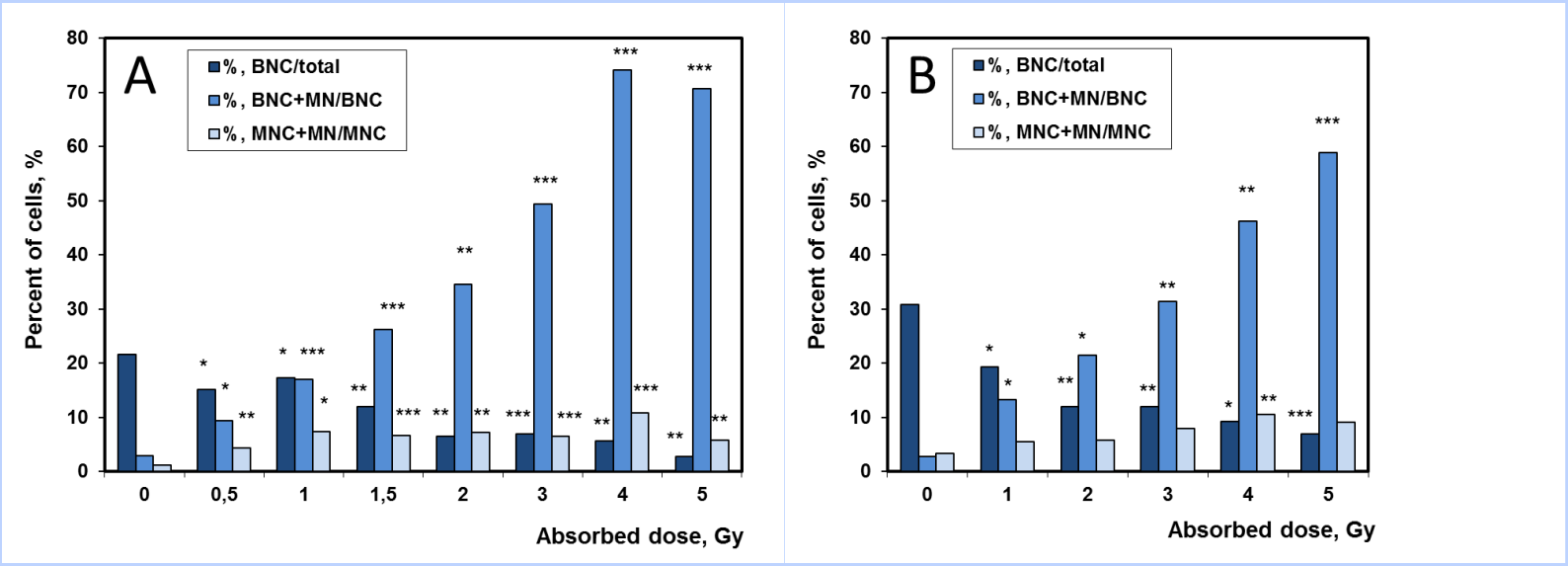
At the Tandetron facility, the cells were irradiated on 5 µm Mylar foils, because of the low range of the protons and alpha particles.

Special holder has been installed for the movement of the sample.

Micronuclei



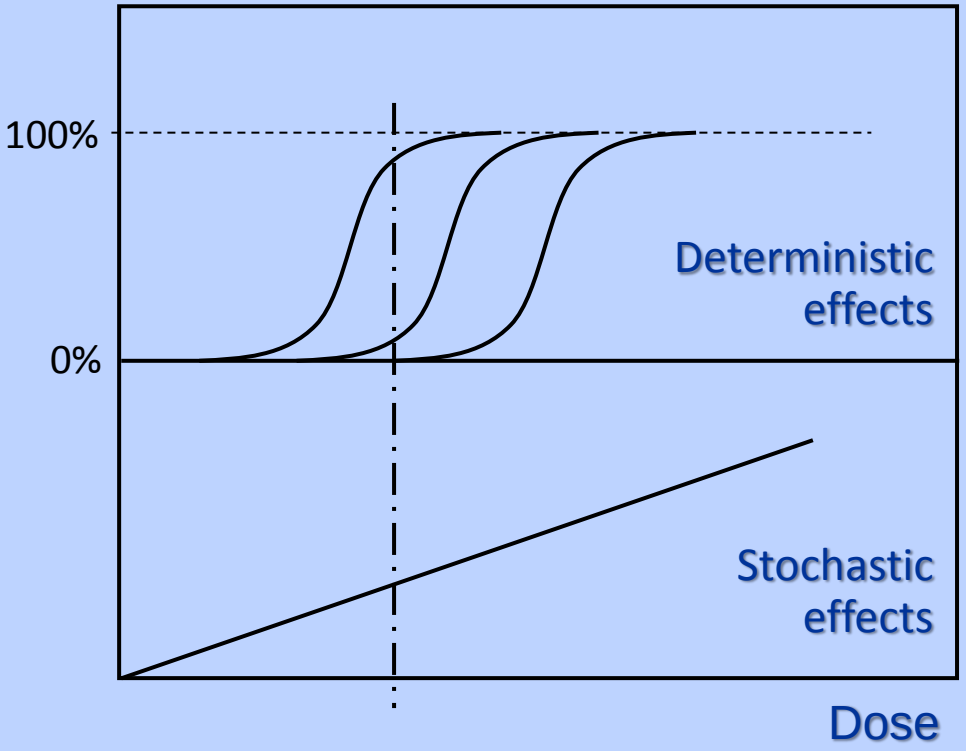




Micronuclei formation in human neonatal fibroblasts at first division after irradiation by 30 MeV proton beam A) proton scanning pristine beam, B) broad fixed beam. At least 1000 cells was scored and calculated %BNC from total cells, %BNC with MN from total BNC, and MNC with MN from total MNC. Proportion of binuclear cells reflects the cell division capacity, which decreases with increasing dose. Percent of binuclear cells with micronuclei was increasing with increasing absorbed dose (* - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$).

Deterministic and stochastic effects of IR

Deterministic effects develop due to cell killing by high dose radiation, appear above a given threshold dose, which is considerably higher than doses from natural radiation or from occupational exposure at normal operation, the severity of the effect depends on the dose, at a given high dose the effect is observed in severe form in all exposed cells, at higher doses the effect cannot increase.



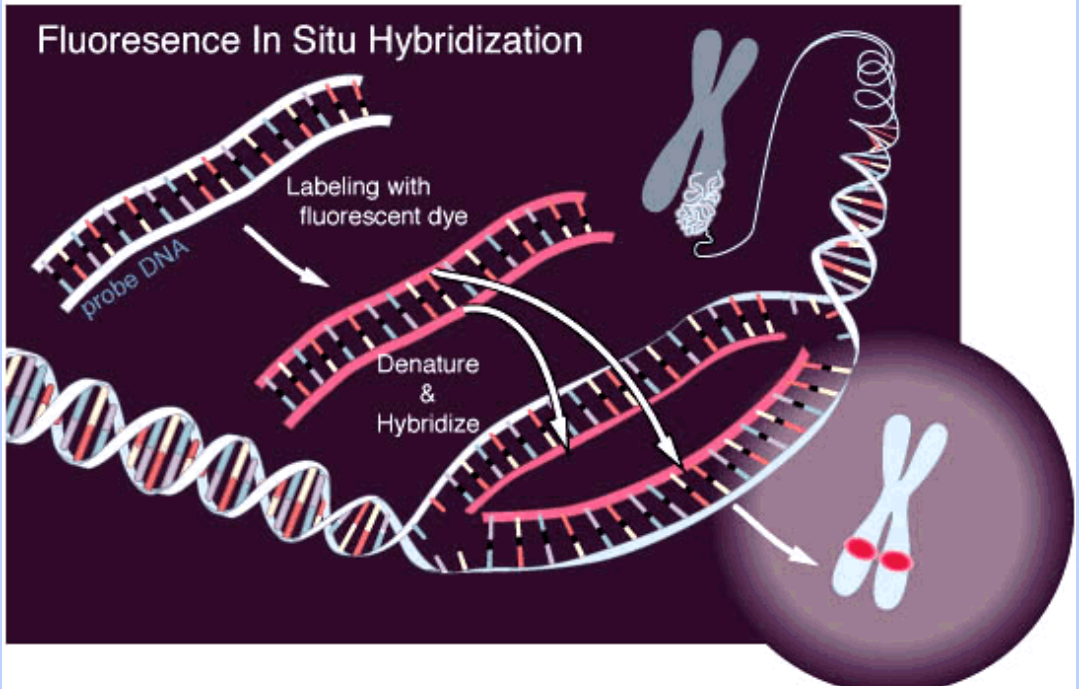
Stochastic effects develop due to mutation effects of low dose radiation, the threshold dose is not known accurately; it is observed that cancer of different location appears above different dose ranges, the severity of the effect does not depend on the dose, but the frequency of the appearance of the (probabilistic) effect in the exposed population group is dose dependent, (in most cases) linearly increasing with the dose.

- Cancer, stroke, hearth, gastrointestinal and respiratory diseases

Radiation damage at cellular level

- Stochastic effects of radiation are related to DNA damage and thus to effects at cellular level
- The processing and misrepair of radiation induced DSB and complex DNA damages are principally responsible for chromosome/gene alterations that manifest as chromosome aberrations and somatic cell mutations
- Mutations: from point mutations in single genes to deletions that encompass several physically linked genes
- Radiation induced spectrum of mutations differs from spontaneous mutations, mutations induced by UV and many chemical mutagens

Fluorescence *in situ* Hybridization (FISH)



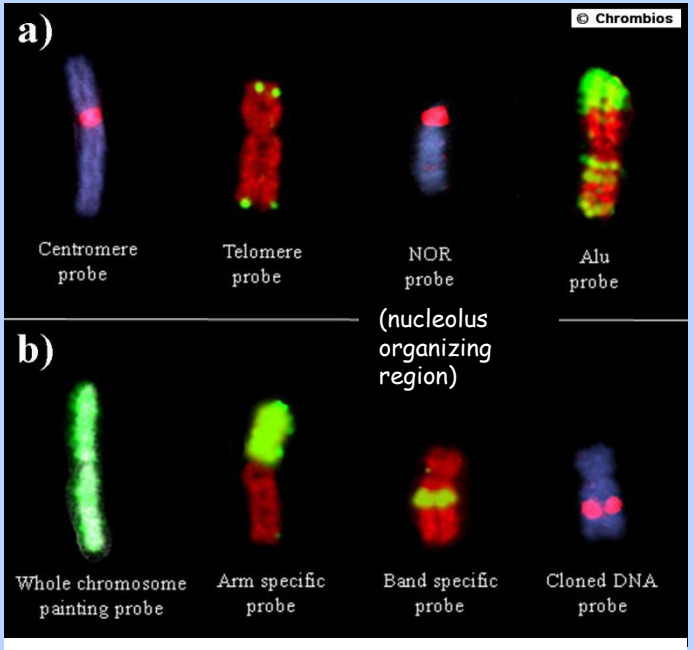
http://serc.carleton.edu/microbelife/research_methods/microscopy/fish.html

- FISH applies fluorescently labeled DNA probes to detect gene or chromosome abnormalities that are generally beyond the resolution of routine cytogenetics. The DNA is first *denatured*. The fluorescently labeled probe is added and hybridizes with the sample DNA at the target site as it *reanneals* back into a double helix. The probe signal can be seen through a fluorescent microscope.

The probes:
repetitive and "unique" sequences.

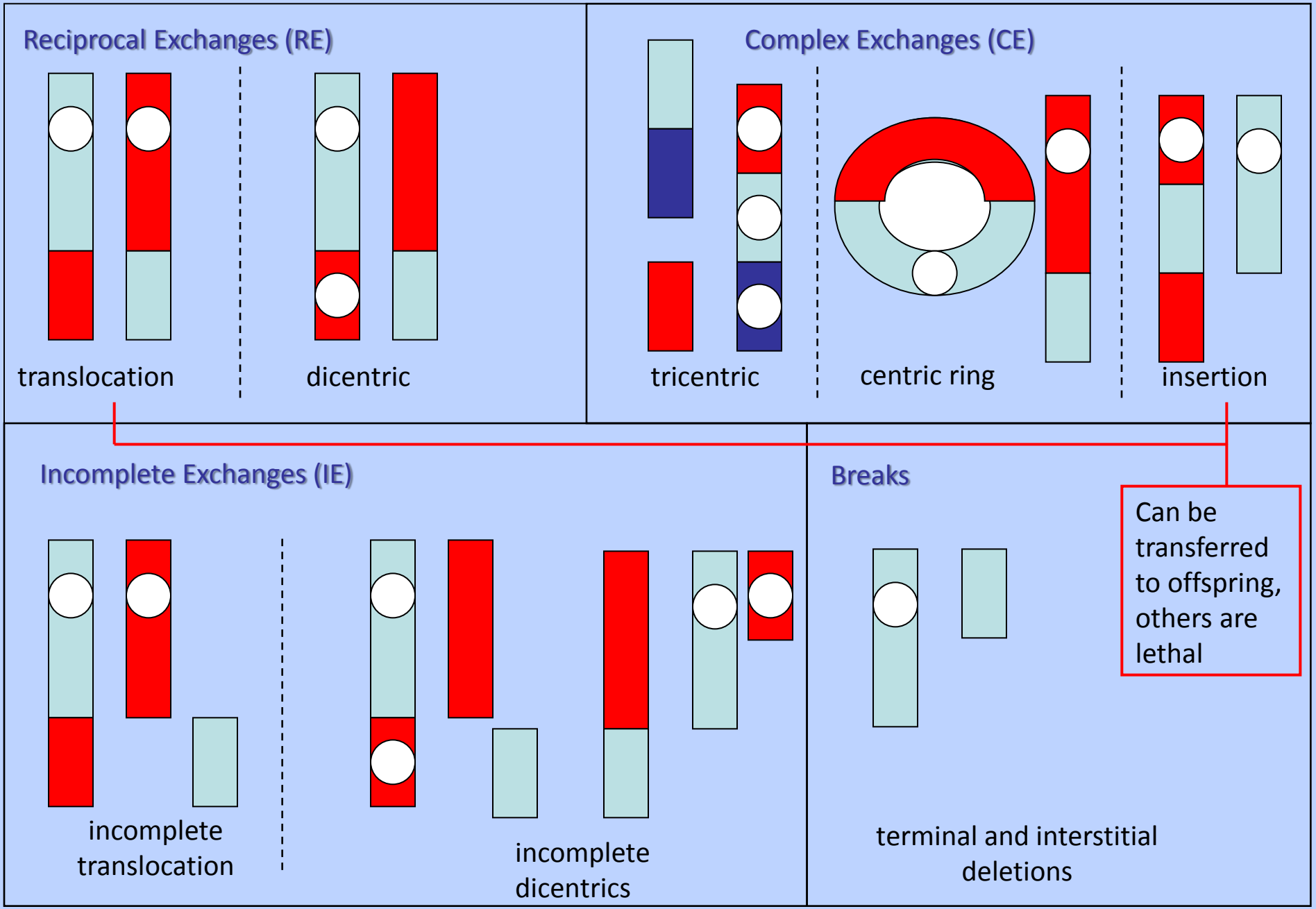
Targets:

- Metaphase chromosomes
- Interphase nuclei
- Extended chromatin fibers
- Entire Cells/RNA
- Tissue sections



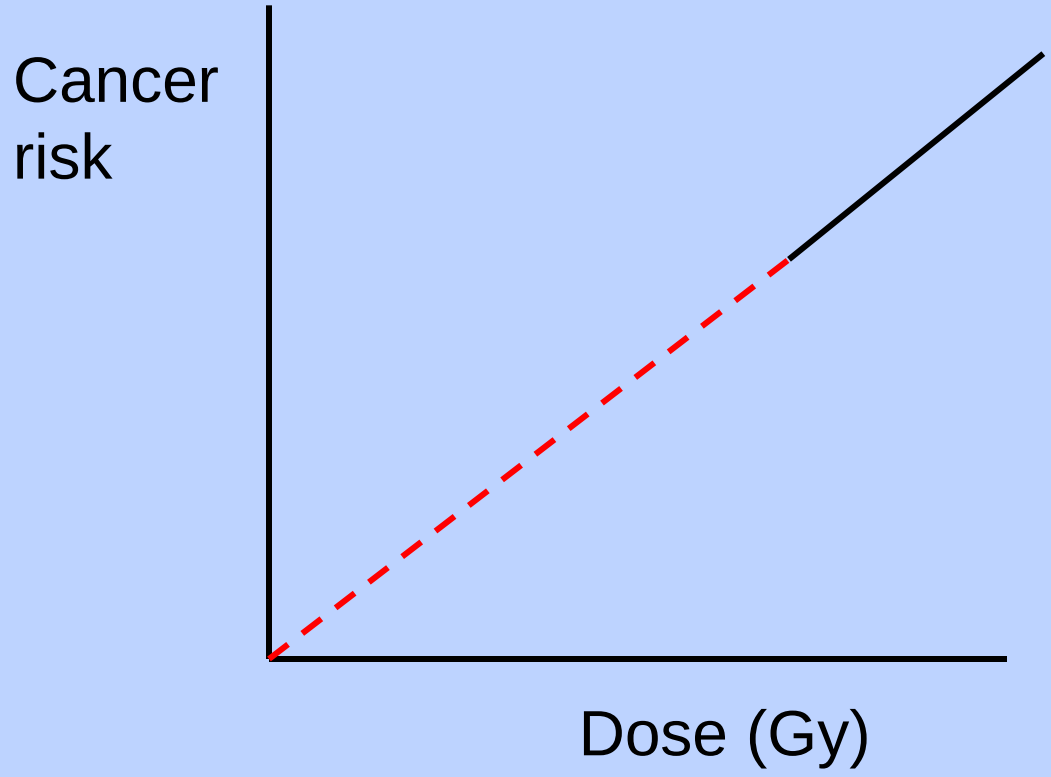
http://www.chrombios.com

Chromosome aberrations detected by FISH

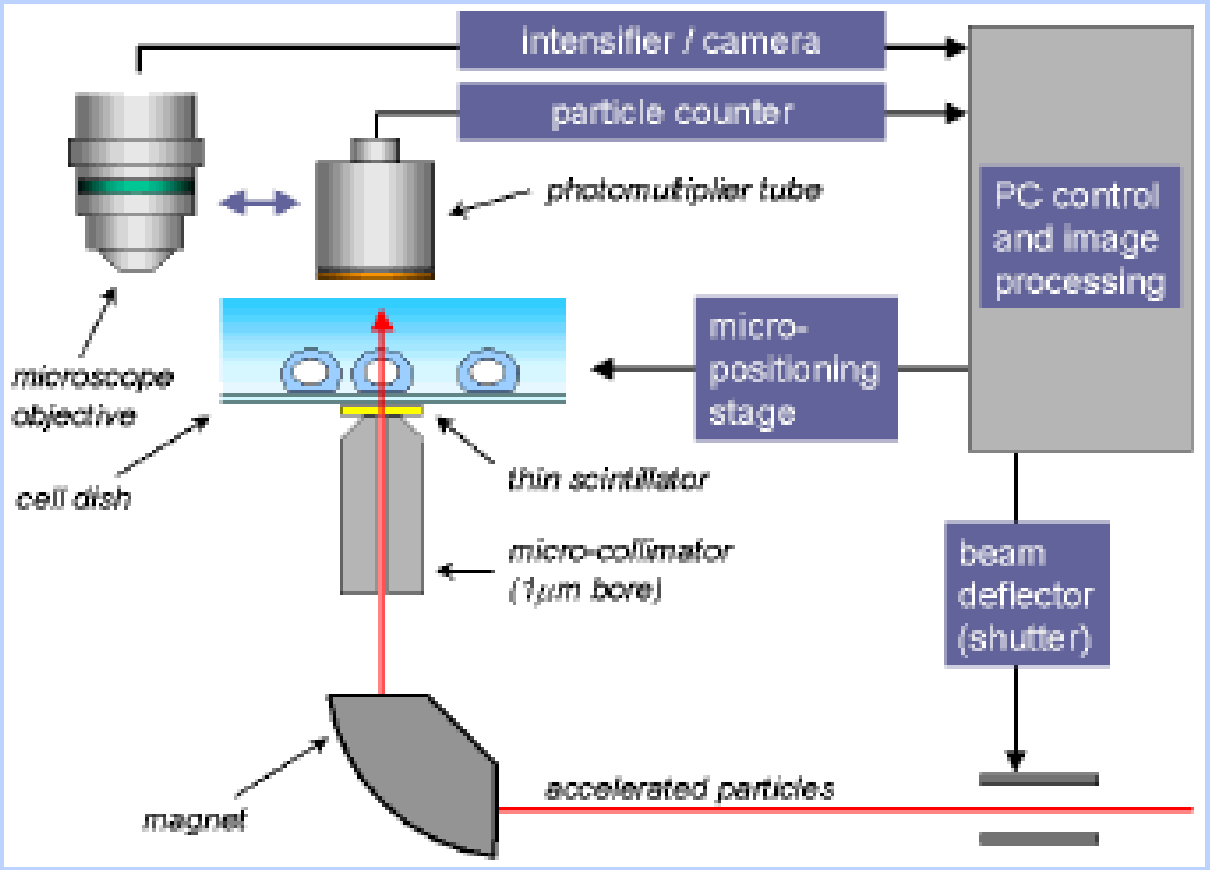


Dose-effect relationship

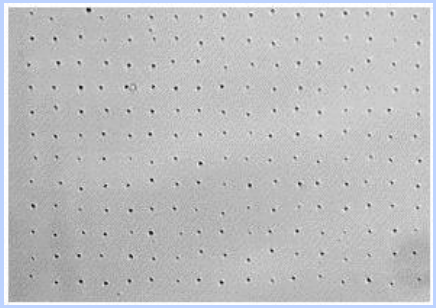
- Current understanding of mechanisms and quantitative data on dose and time-dose relationships support a linear dose response at low doses (i.e., LNT) for total cancer risk (ICRP 99 - Low-dose Extrapolation of Radiation-related Cancer Risk, 2005)



Microbeam irradiations



Gray Cancer Institute, report 2001

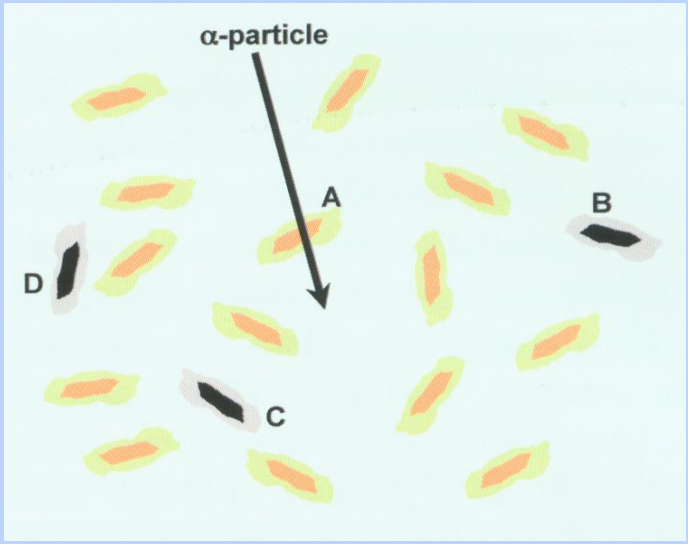


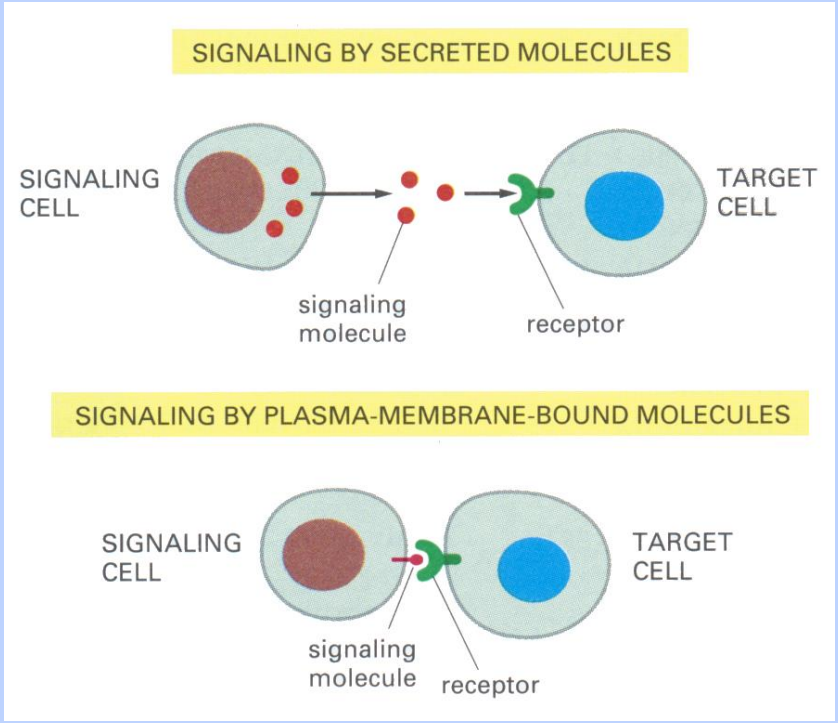
Track-etch plastic showing single hits by 3MeV protons, spaced at 20 micrometer intervals.

- precision sub-micron up to several microns
- up to 10000 cells per hour
- X and γ rays, electrons, ions up to argon nuclei

By-stander effects

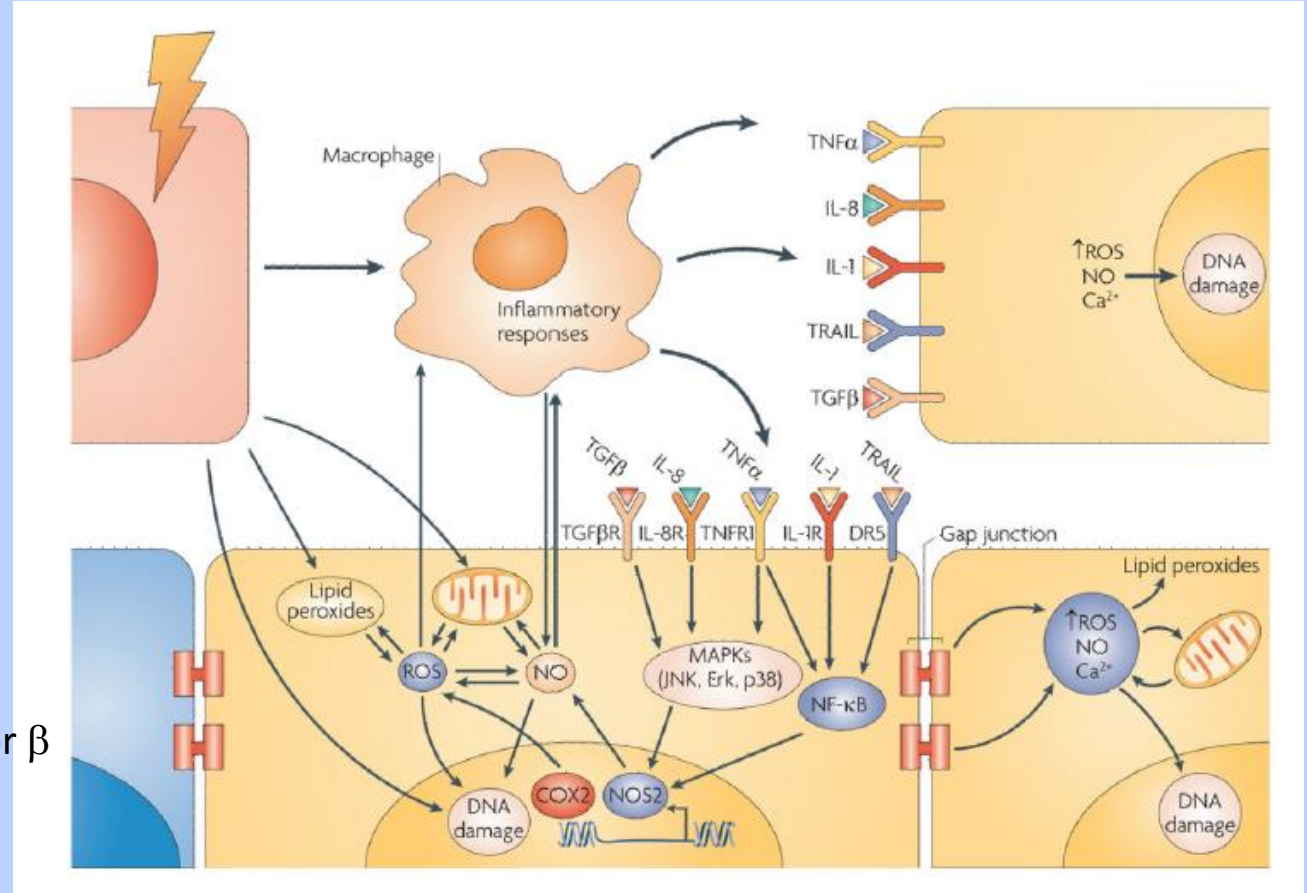
- Up to the early 1990's: important biological effects are produced only very close neighborhood of the tracks and within cell nucleus;
- Cellular responses induced via by-stander effect include chromosomal aberrations, mutations, cell death, apoptosis, malignant transformation and genomic instability.
- 1-30% of nonirradiated cells
- found in different cell systems and signaling mechanism appear to be involved (role of cytokines, ROS = reactive oxygen species)





Bystander signals

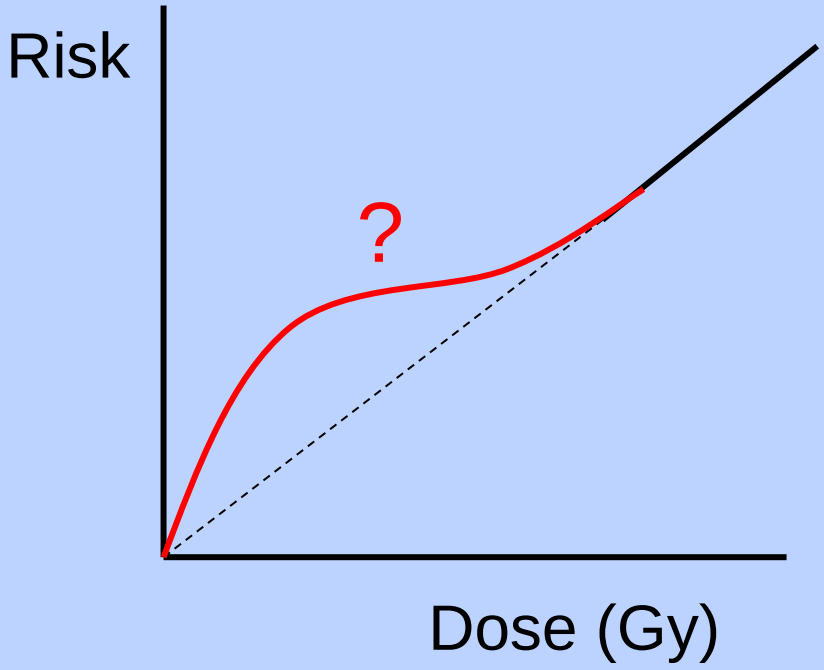
COX2 - cyclooxygenase 2
DR5 - death receptor 5
IL interleukin
JNK - Jun N-terminal kinase
NO - nitric oxide
NOS2 - NO synthase 2
ROS - reactive oxygen species
TGFβ transforming growth factor β
TGFβR - TGFβ receptor
TNFα - tumor necrosis factor α
TRAIL - TNF related apoptosis inducing ligand



Reprinted by permission from Macmillan Publishers Ltd: Prise and O´Sullivan, Nat. Rev. Cancer 9, 351, 2009, copyright 2009

- Through gap junctions and the cytokine signals into the extracellular matrix.
- **In vivo:** macrophages may be important mediators, which release bystander signals affecting nonirradiated cells. Cytokine-mediated signaling, signal transduction through MAPKs and nuclear factor κB alongside the production of reactive oxygen and nitrogen species.

Mutation frequency



Adaptive response and bystander effect

Adaptive response

= pre-exposing cell to a low priming dose appear to protect these cells from the deleterious effects of second, larger dose

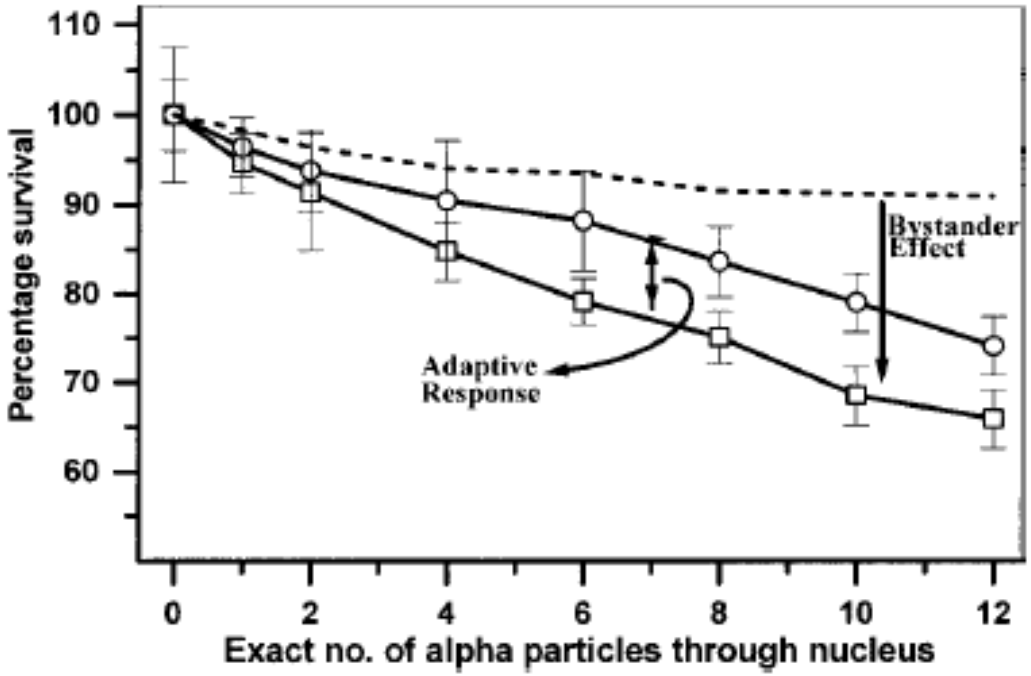
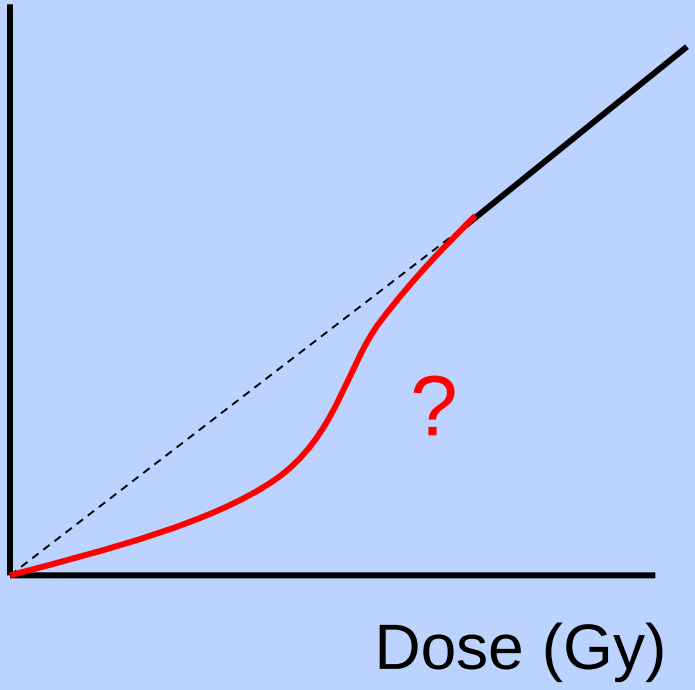


FIG. 1. The adaptive response and the bystander effect for cell survival in C3H 10T½ cells. The dotted line shows the percentage of cells that would be expected to survive when 10% of the cells are exposed to various numbers of α particles calculated from the survival curve for all cells irradiated. The squares show survival for various numbers of α particles, from 1 to 12, traversing 10% of the cell population. The extent to which this falls below the dotted line is an indication of the magnitude of the bystander effect. The circles show survival for cells exposed to 2 cGy of γ rays, 6 h before exposure to various numbers of α particles traversing 10% of the population. The extent to which the circles are above the squares reflects the adaptive response.

Sawant et al., Radiat. Res. 156, 177, 2001

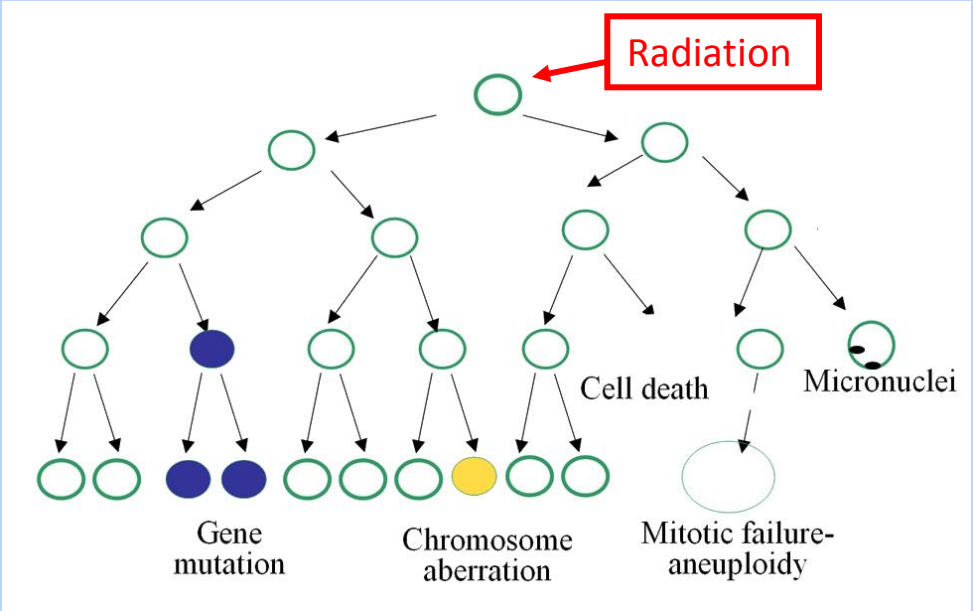
Adaptive response

- Both *in vivo* and *in vitro*
- Humans and animals
- In normal and tumour cells
- For different endpoints: chromosomal aberrations, micronuclei formation, gene mutations, cell killing
- Doses in the range 50 - 200 mGy, decline above 200 mGy, disappear above 500 mGy
- Hypothesis: enhance DNA repair ability and cellular antioxydant activity
- Adaptive response and apoptosis possibly constitute a complementary defense mechanism



Genomic instability

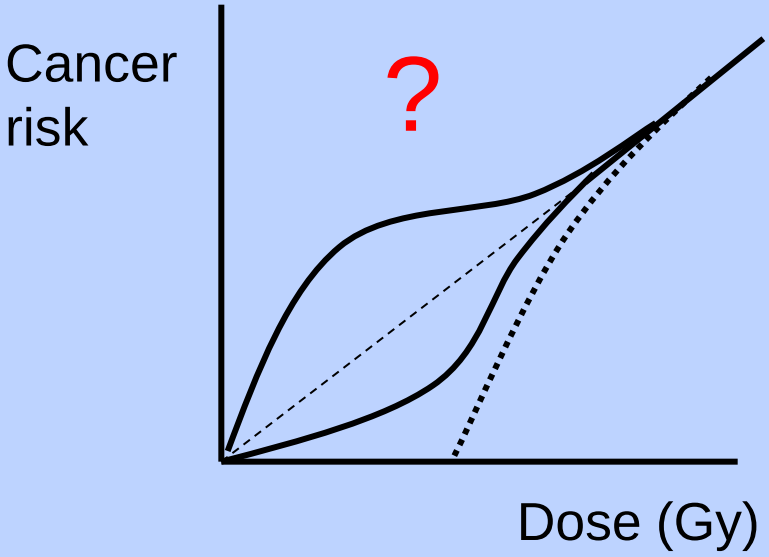
- Observed in number of different cellular systems whereby radiation exposure induces a type of instability in individual cells that is transmitted to their progeny leading to a persistent enhancement in the rate at which genetic changes arise in the descendants of irradiated cells after many generations of replication



- diverse endpoints: large-scale chromosomal rearrangements, deletions and aberrations, gene amplification (extra copies of specific DNA segments), aneuploidy (wrong number of chromosomes), micronucleus formation and gene mutation.
- The capacity of radiation to induce genomic instability depends to a large extent on radiation quality or linear energy transfer (LET) and dose.
- There appears to be a low dose threshold effect with low LET, beyond which no additional genomic instability is induced. Low doses of both high and low LET radiation are capable of inducing this phenomenon.

Conclusions

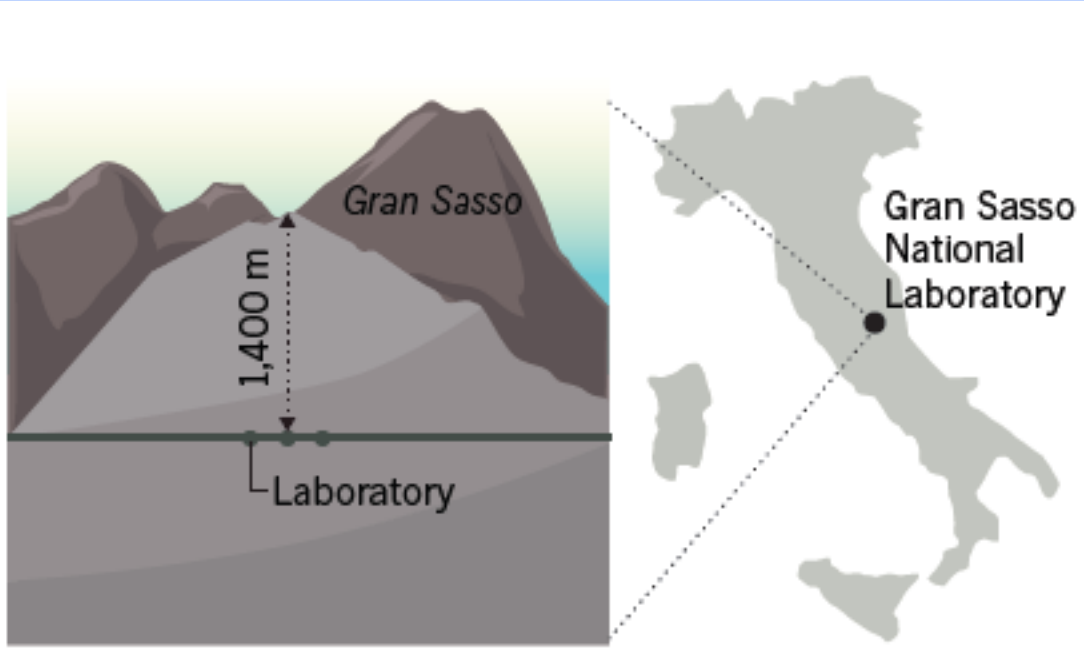
- Chromosome aberrations and somatic cell mutations suggest linear dose-effect relationship at low dose region.
- There exist the by-stander effect, adaptive response and genomic instability which may influence the nature of dose response relationship at low doses and low dose-rates.
- Dose response at low doses of ionizing radiation is at present uncertain and a simple extrapolation from high doses may not be appropriate.



- The better understanding of the mechanisms for these phenomena, the extent to which they are active *in vivo* and how they are interrelated is necessary before they can be confirmed as factors to be included in the estimation of potential risk to the human population of exposure to low levels of ionizing radiation.

Cell response to extremely low levels of IR

Gran Sasso National Laboratory INFN, Italy



Adopted from Nature 485, 435, 2012

First radiobiology experiments in LNGS (1992-3)

Satta et al. Mutation Research 347, 129-133, 1995

yeast cells *(Saccharomyces cereuisiae* D7)

120 generations

- 4 mSv in ISS, 0.6 mSv in LNGS

- cells treated by different concentrations of metyl methasulfonate (MMS, an alkylating agent and a carcinogen)

Induction of Ade-2 reciprocal recombinants or aberrant has been followed (single event of crossing-over at the Ade-2 locus provokes formation of a twin spot red-pink colonies, other recombinational rearrangements lead to red, pink or sectored colonies).

Mitotic intergenic recombination is a signature of DNA damage → cells cultivated at extremely low radiation background lose their ability to deal with DNA damage

LNGS vs ISS – human lymphoblastoid TK6 cells

Carbone et al. Radiat. Environ Biophys. 48, 189, 2009

- increase in micronuclei formation after 2 Gy X-rays

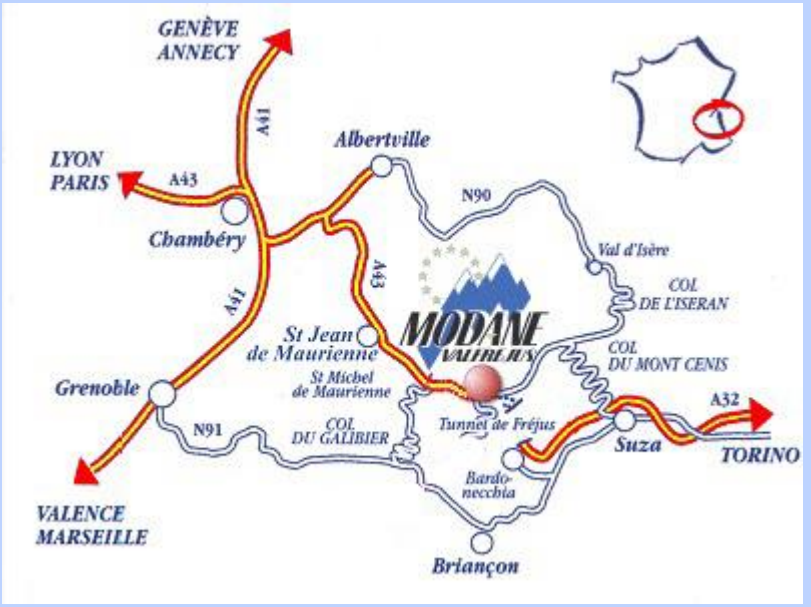
→ cells cultivated at extremely low radiation background lose their ability to deal with DNA damage

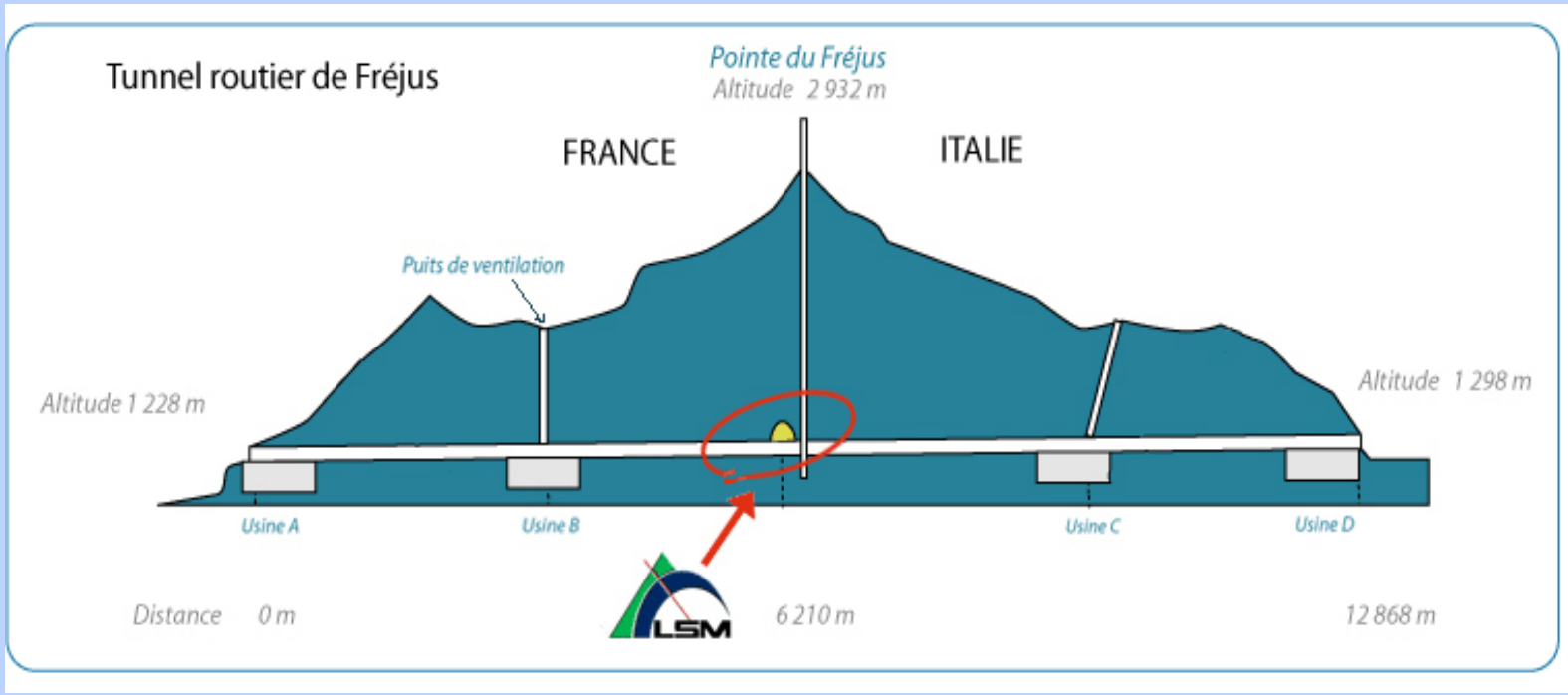
- decrease of antioxidant enzymatic activity after 6 months growth at the ISS and LNGS

- SOD - superoxide dismutase
- CAT - catalase
- SE-GPx - selenium-dependent glutathione peroxidase

Collaboration perspectives

Underground Laboratory Modane
(Laboratoire Souterrain de Modane, LSM)
CNRS/IN2P3 (LSM), France
(1700 m underground)





Adopted from http://www-lsm.in2p3.fr

Future perspectives

- Further decrease of natural radiation background level in the Underground Laboratory in Modane, France
- investigate the mechanisms involved in low dose radiation response of human/rodent cell cultures kept in conditions of extremely low levels of background ionizing radiation or in “reference” background radiation laboratories:
  - changes of the cell cycle regulation, cell proliferation and stability of the genome, gene expression of stress response enzymes

The main outcome of the project shall be determination of mechanisms regulated by low radiation doses which are necessary for physiological functioning and ability to cope with DNA damage.

Thank you for your attention