Cytogenetic signature of heavy charged particles: impact of LET and track structure

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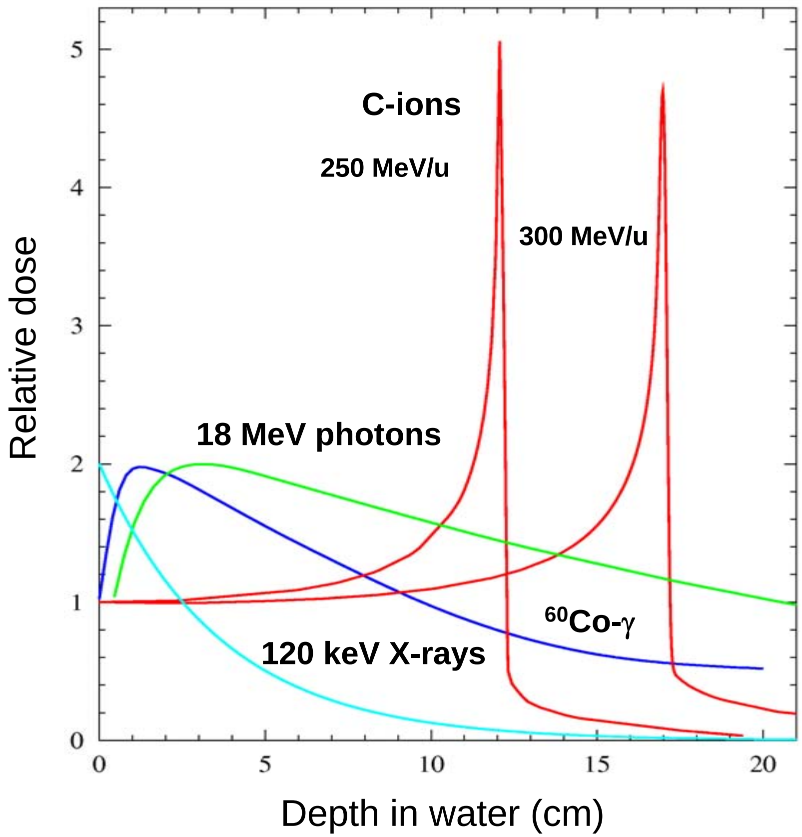
Heavy ion irradiation : characteristic features

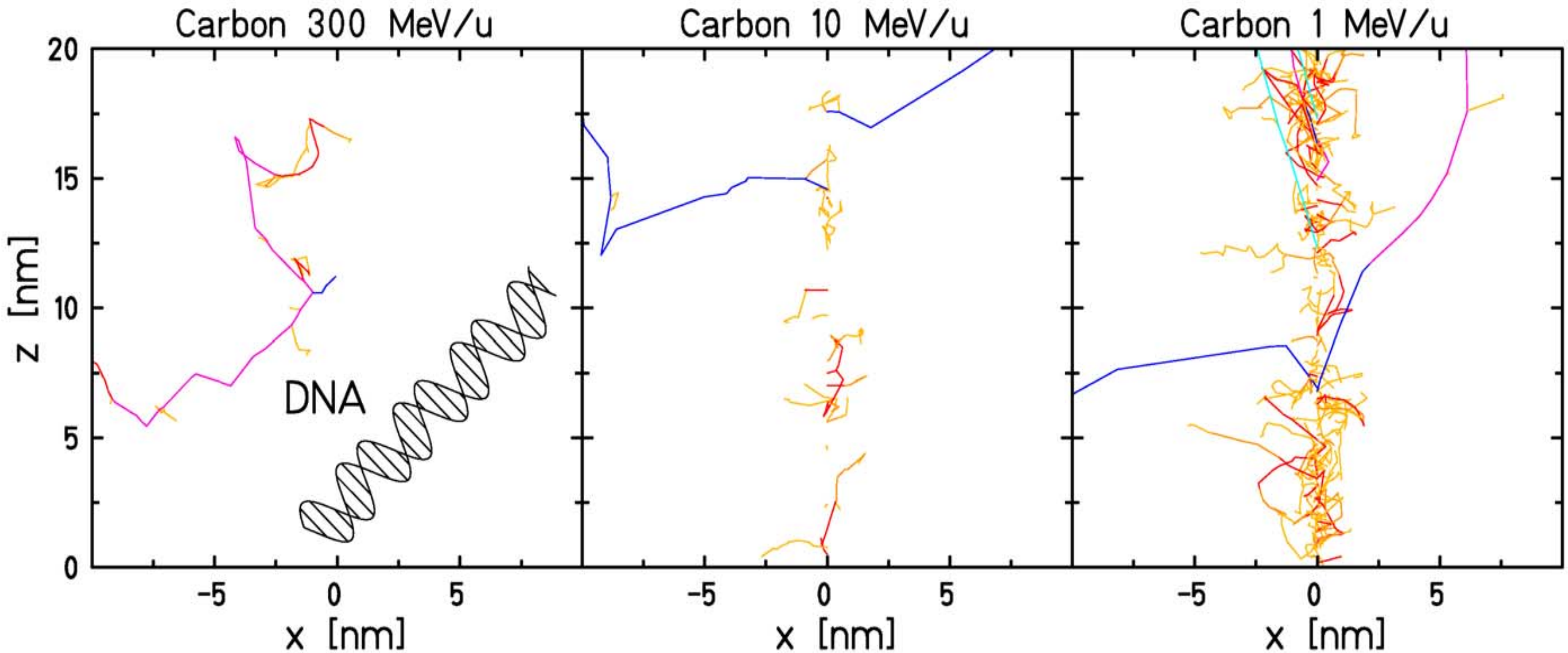
Cancer therapy with heavy ions

- ***Advantages: inversed dose-depth profile, greater biological effectiveness***
- ***~3000 patients in Japan and Germany***
- ***Questions: late effects***



GSI

Dose-distribution in nanometer scale



LET

13 keV/μm   170 keV/μm   720 keV/μm

(M.Krämer)   Linear energy transfer (LET)

$$\text{LET} = \frac{dE}{dx}$$

Heavy ion irradiation : characteristic features

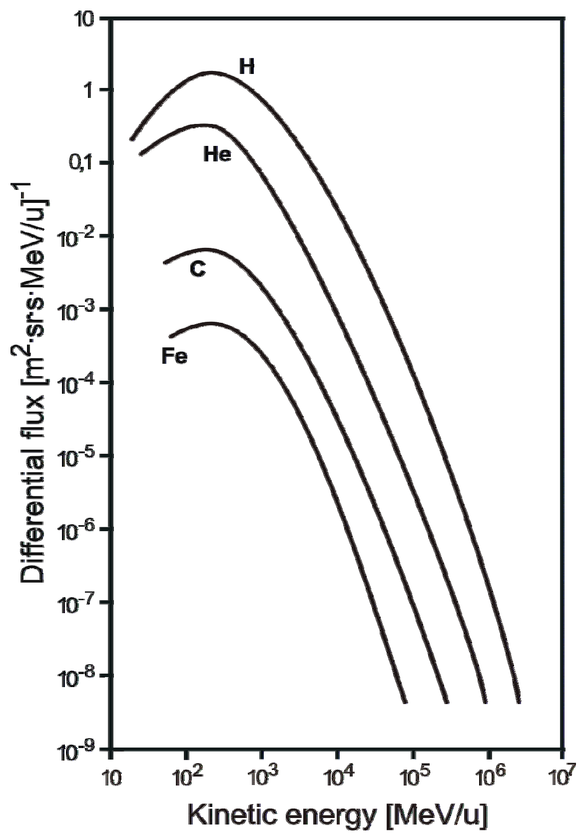
- *Quantity: >300 times higher dose*
- *Quality: particles*
- *Dose contribution of Fe-ions*

- *~500 % uncertainties in health risk for long-term space missions*

Radiation exposure in Space

$$Dose \propto Z_{eff}^2$$

Applied radiations
X-rays (LET value: 2 keV/μm)
990 MeV/u Fe-ions (155 keV/μm)
100 MeV/u C-ions (28.9 keV/μm)
11.6 MeV/u C-ions (175 keV/μm)



Energy spectrum of ions in galactic cosmic rays

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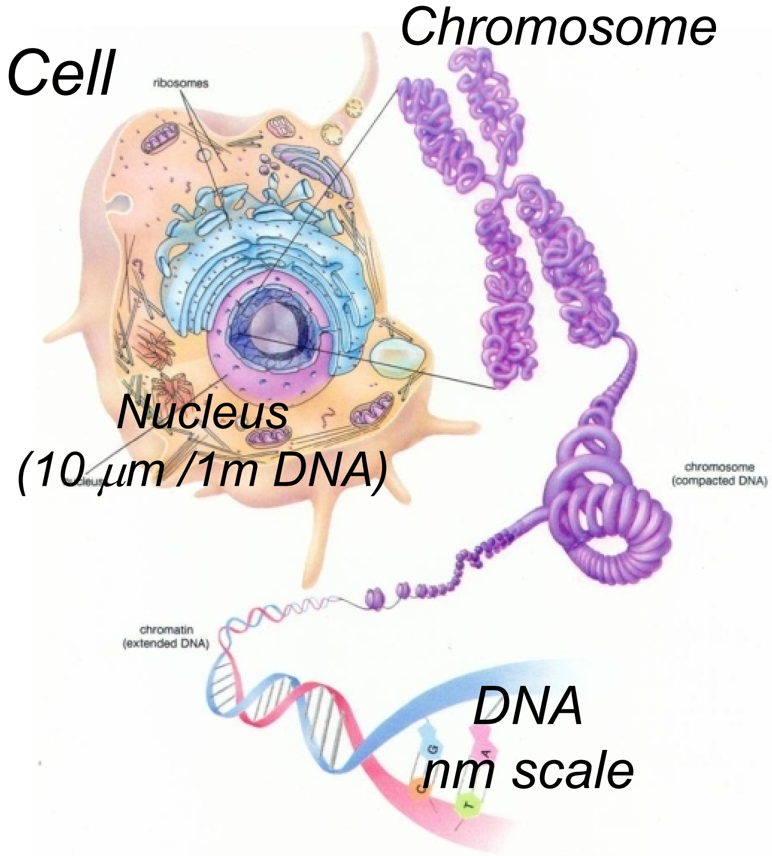
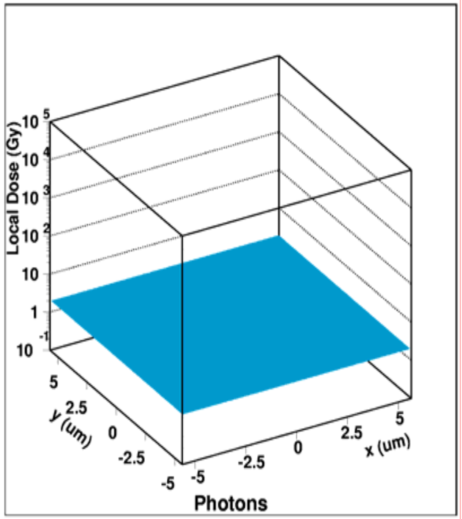
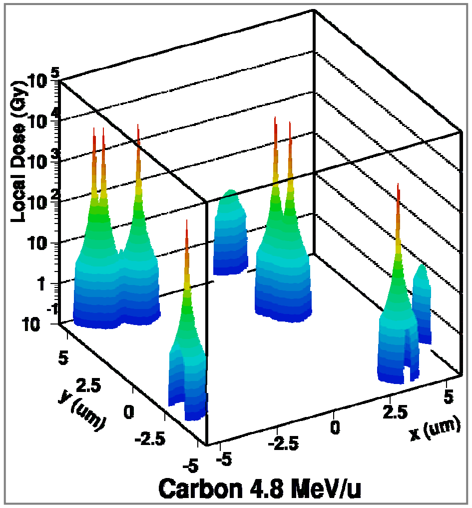
DNA DSB induction after heavy ion irradiation



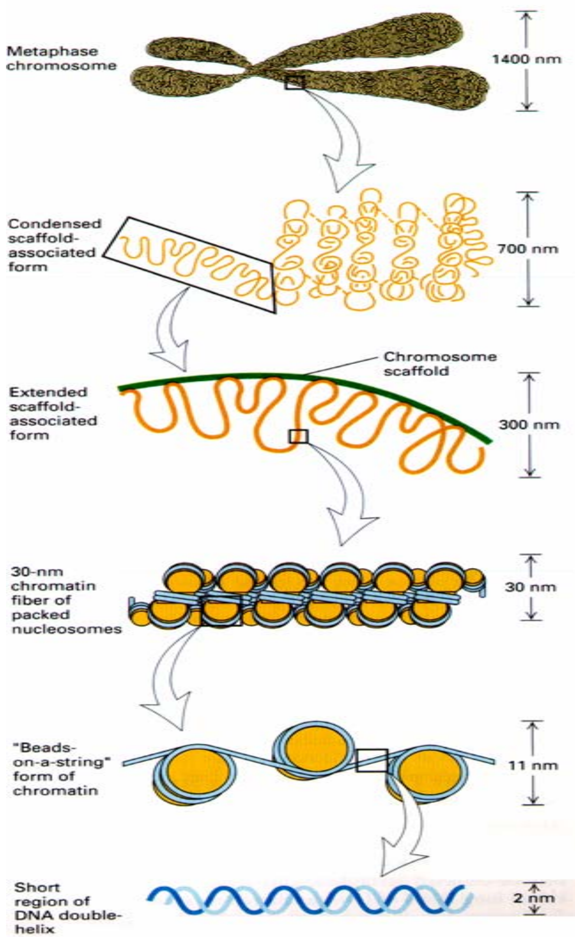


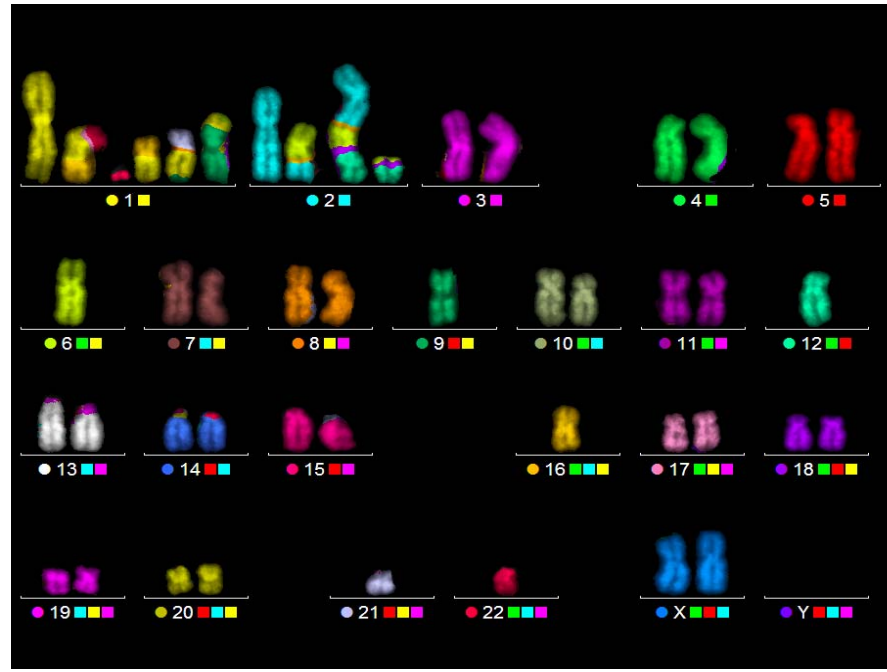


Inhomogeneous dose distribution

Primary lesions on the DNA molecule

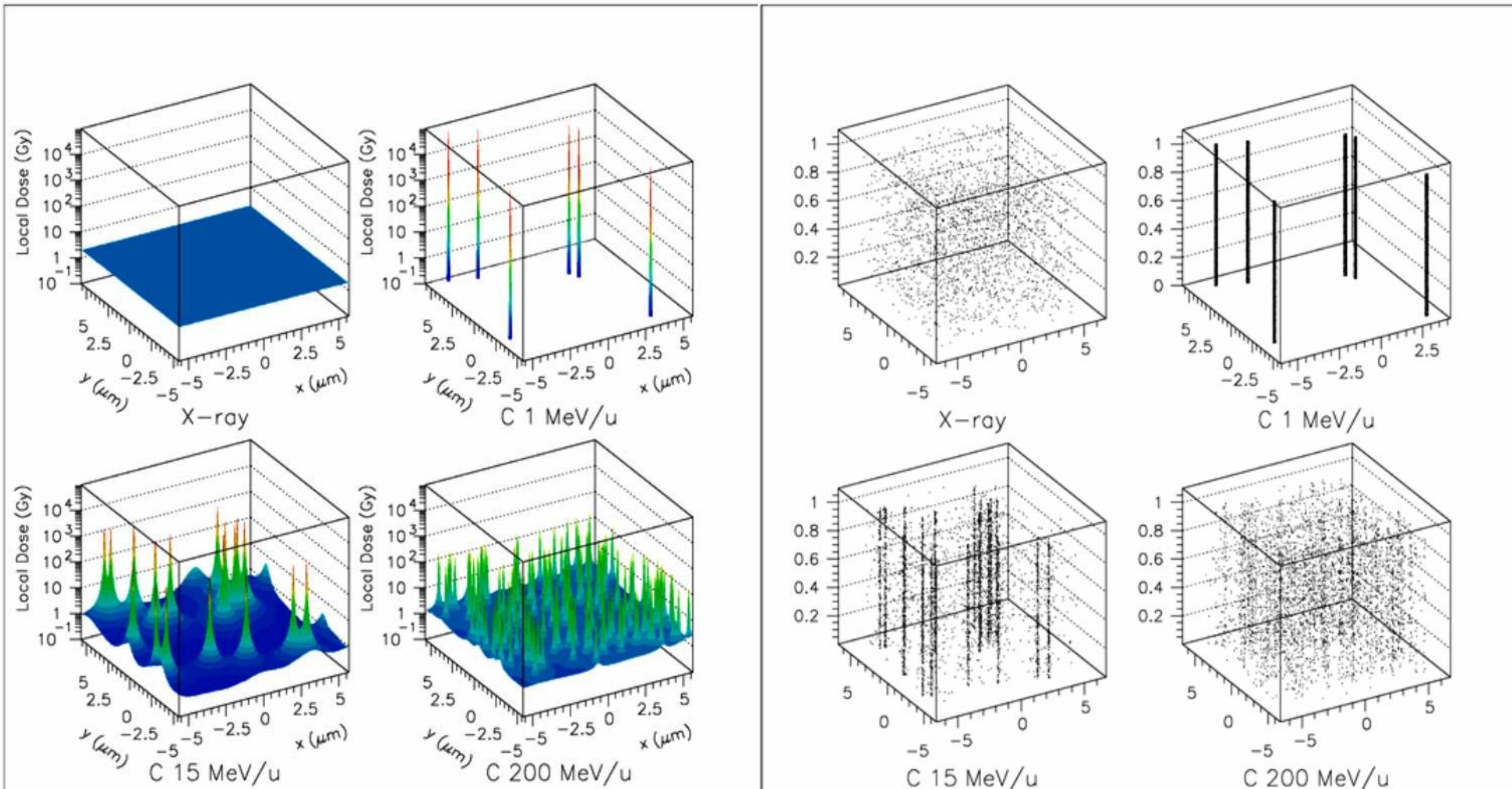
DNA hierarchical structure: from a polymer thread to spatial organisation of chromosomes





Average dose: 2 Gy, Yield: 200 events/Gy/Cell
LET (linear energy transfer): 16, 120, 720 keV/μm
(200, 15, 1 MeV/u C-ions)

(M. Scholz)

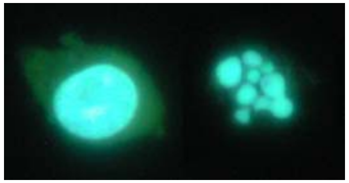


microscopic dose distribution → *event distributions*

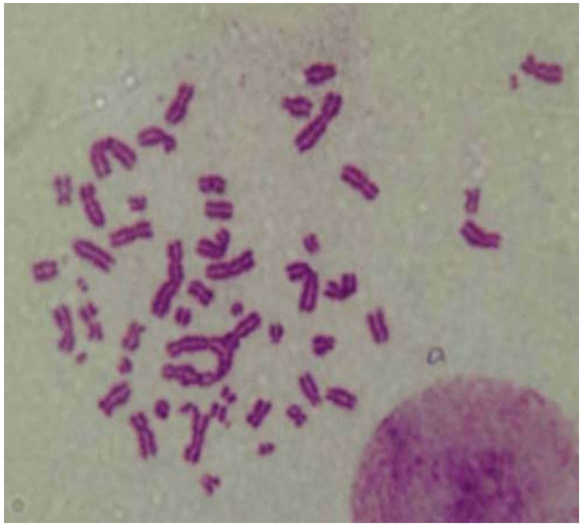
Chromosome aberrations

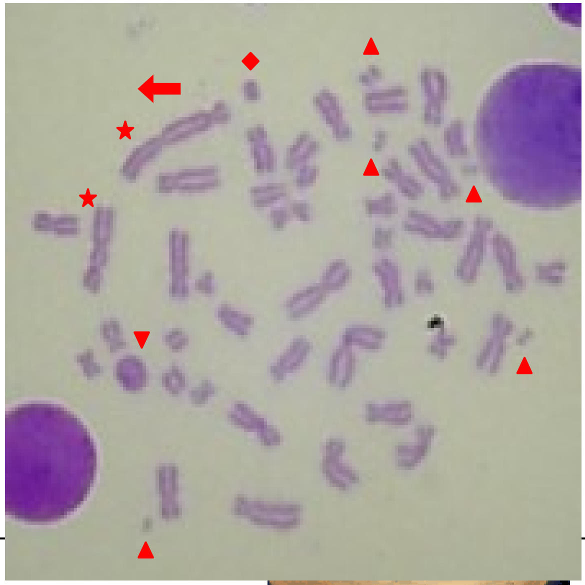
- *Originate from DNA damage (effect of induced DSBs processing)*
- *Important biomarker of cancer risk*
- *Used as "biological dosimeter"*
- *Model system: human peripheral blood lymphocytes*
- *Standard protocol: cell collection at a single time point (48 h)*
- *Open questions:*
  - *Cell cycle delay*
  - *Apoptosis*
  - *Inter/intra-donor variability*
  - *Radiation quality*

(Ritter, Lee, Nasonova)



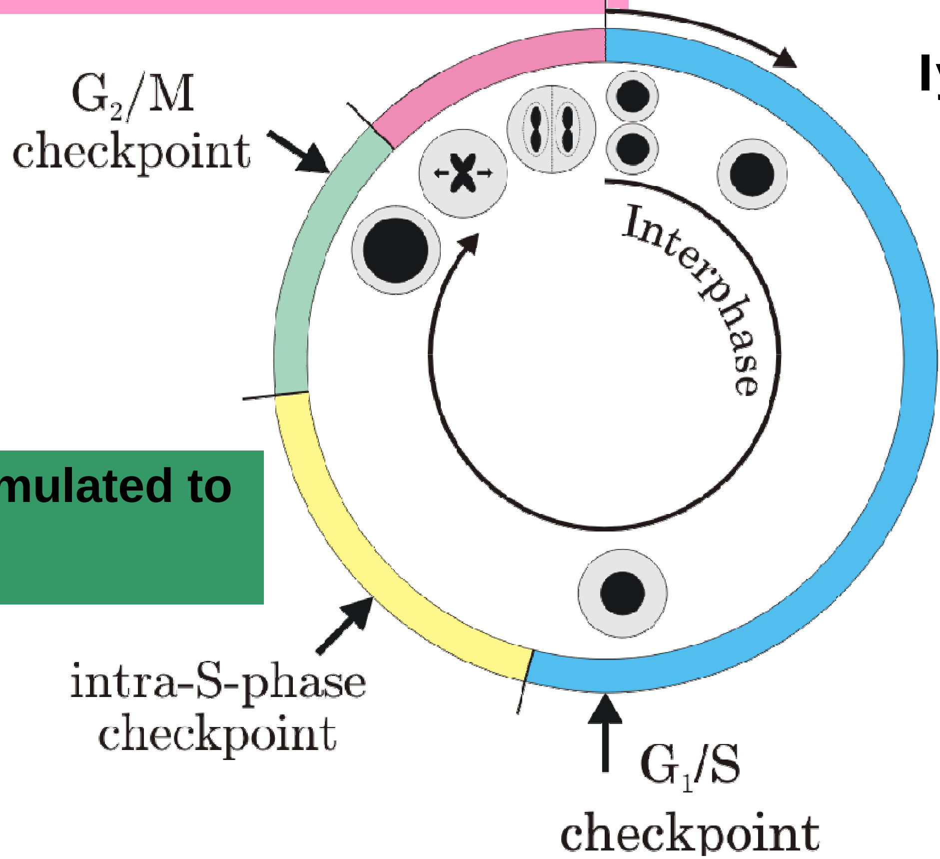
Normal Apoptotic





Standard experimental design: synchronous cells irradiated in G_0

Metaphase analysis (M)



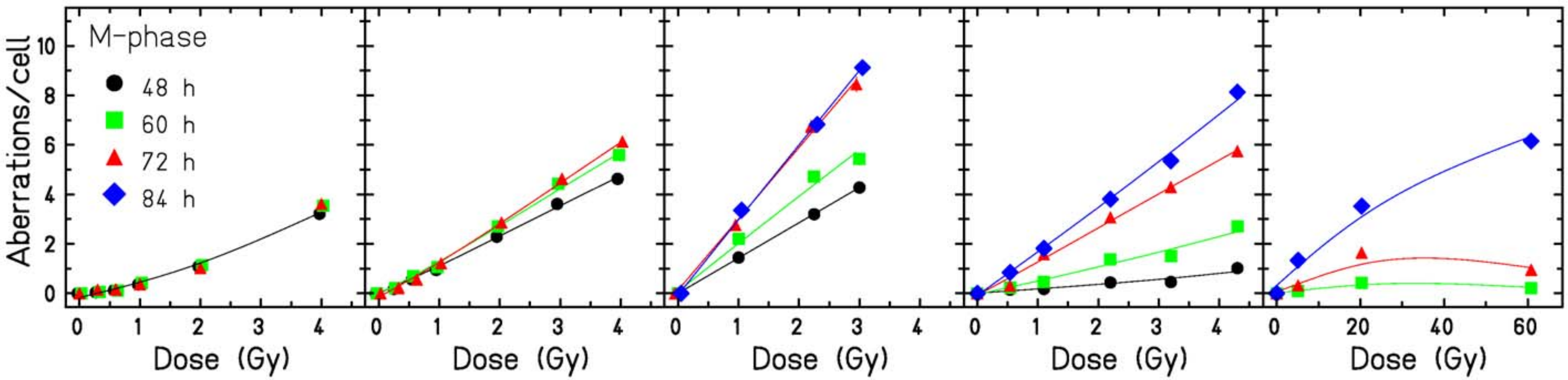
lymphocytes
G_0

+ PHA
+ BrdU

Irradiated cells are stimulated to enter the cell cycle

Time-course of aberrations in M-cells

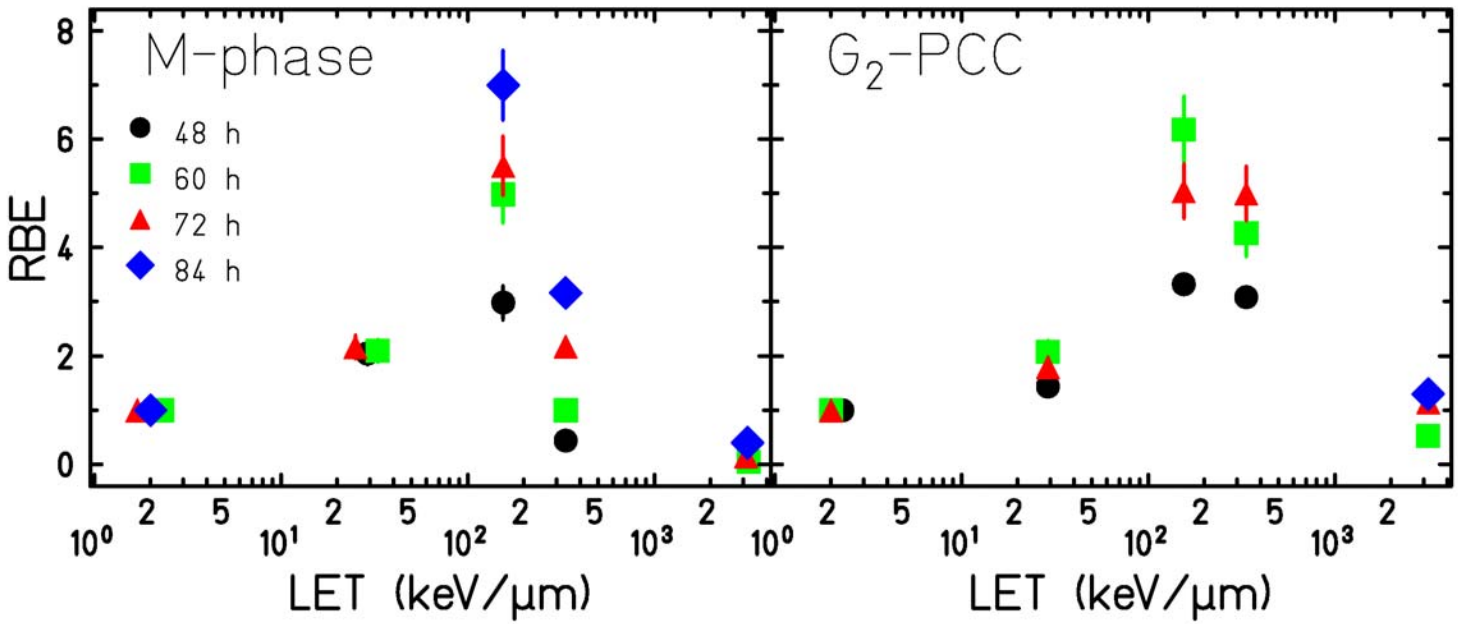
	X-rays	100 MeV/u C	990 MeV/u Fe	177 MeV/u Fe	4.1 MeV/u Cr
LET:	**2 keV/μm**	**29 keV/μm**	**155 keV/μm**	**335 keV/μm**	**3160 keV/μm**



Only first cycle metaphases were scored.

Lee et al. Adv. Space Res. 35 (2005)

RBE as a function of LET and time!



Analysis of damage at one sampling time will unavoidably result in an under- or overestimation of damage

Analysis of biological data and models involved

- ***The way out…***

  To interpret time-dependent yield of aberrations and their distribution-collection of cells over the complete time course of the first mitosis along with a mathematical analysis =integration of data (Scholz et al. 1998, Nasonova et al. 2001, Gudowska et al., 2005))

Random sums of random elements and compound Poisson distribution of lesions.

In brief: induction can be modelled as a CTRW (renewal process)…

$$x(t)=\sum_{j=1}^{n(t)} X_j$$

$$n(t)=\min\left\{n:\ \sum_{j=1}^{n} T_j > t\right\}$$

$$G_{x(t)}(s)=\left\langle e^{s x(t)}\right\rangle = G_{n(t)}\left[G_X(s)\right]$$

$$G_X(s)=\exp\left(\frac{\langle x\rangle}{\langle X_i\rangle}\left\{\exp\left(\langle X_i\rangle\left[e^{-s}-1\right]-1\right)\right\}\right)$$

$$\lambda=\langle n\rangle$$

$$\mu=\langle X_i\rangle$$

$$(x+1)P(x)=\langle x\rangle\sum_{k=0}^{x} C_k P(x-k)$$

$$C_k=\frac{\mu^k e^{-\mu}}{k!}$$

$$P(0)=\exp\left[\frac{\langle x\rangle}{\mu}\left(e^{-\mu}-1\right)\right]$$

- *Generally: counting Poisson process is time-dependent (cell-cycle delay of damaged cells)*
- *Intensity rate of secondaries X_j depends on local, spatial distribution of imparted energy*
- *The average number of aberrations scored in a population becomes time-dependent*

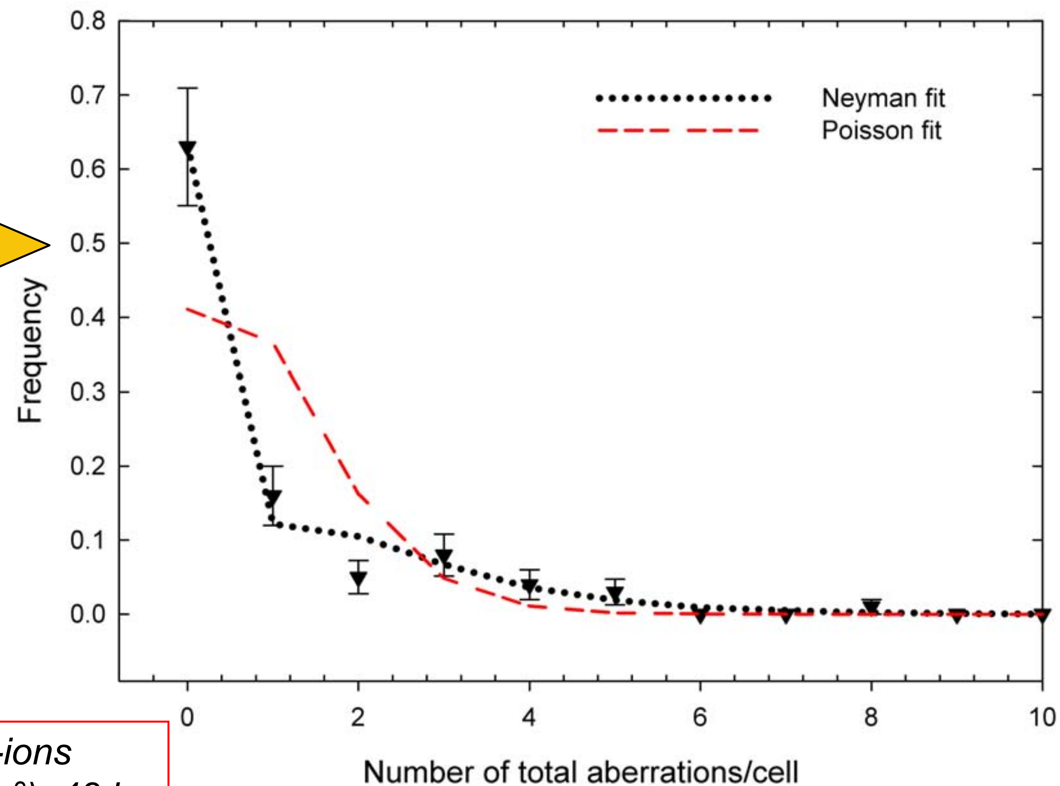
$\langle x\rangle=\lambda\mu$

Random sums of random elements and compound Poisson distribution of lesions.

Simple approximation reads…

$$P(x) = \frac{\mu^x e^{-\lambda}}{x!} \sum_{n=0}^{\infty} \frac{n^x}{n!} (e^{-\mu}\lambda)^n$$

1. *The number of particle traversals per cell nucleus n, $\lambda = \langle n \rangle$*
2. *The average number of aberrations induced by a particle hit μ*



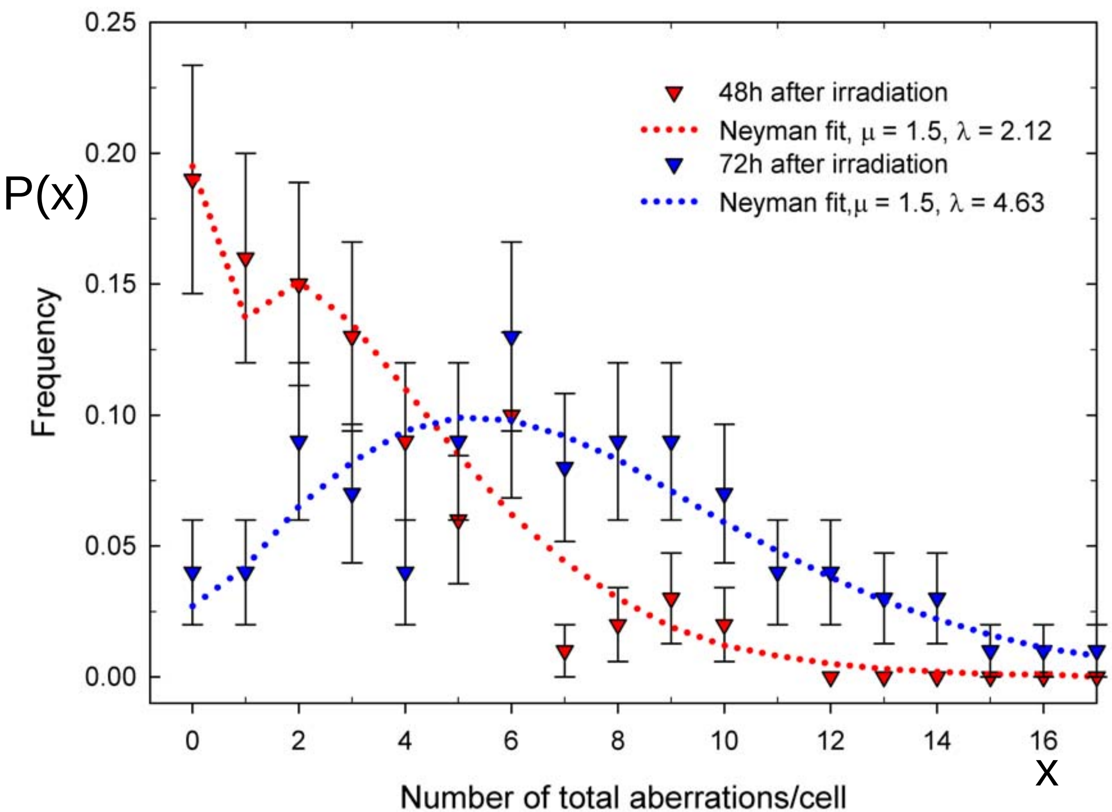
175 keV/μm C-ions
2 Gy (7.13x10^6 /cm^2), 48 h
0.89 aberrations per cell
1.78 particle hits/nucleus

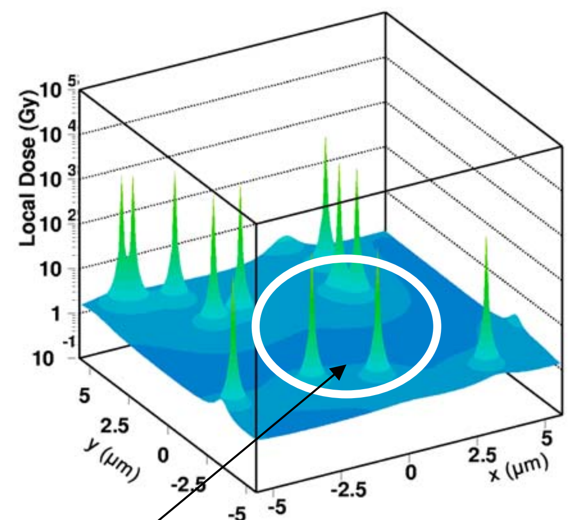
GSI

Frequency distribution of aberrations per cell

155 keV/µm Fe-ions, 2.3 Gy (9×10^6 /cm^2)

No hit: 11 %, 1 hit: 24 %, 2 hits: 27 %, 3 hits: 20 %, more: 18%





dimension of the cell nucleus

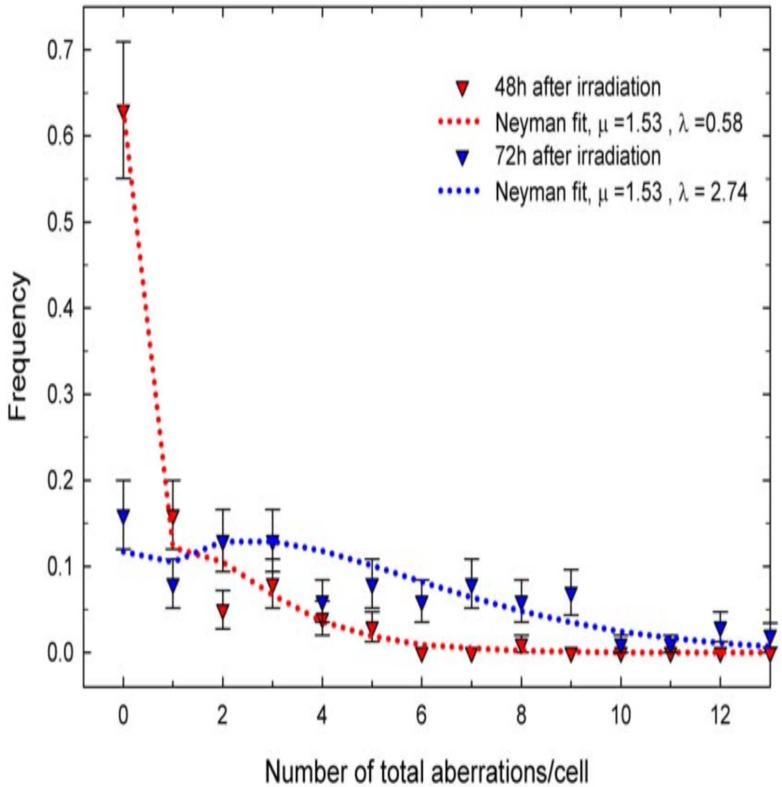
GSI

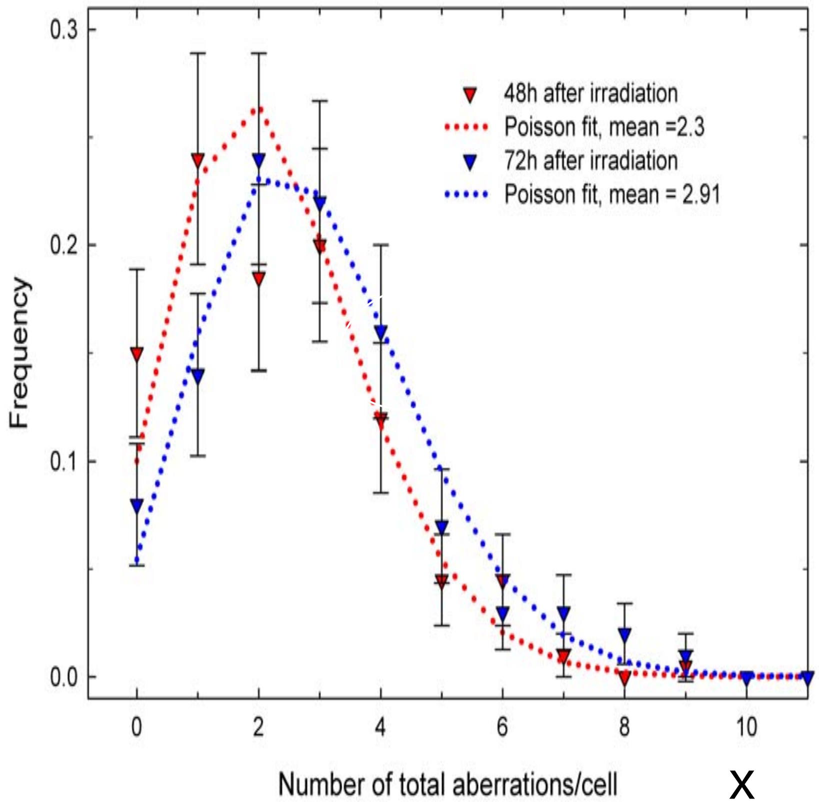
Frequency distribution of aberrations per cell: effect of LET

175 keV/µm C-ions, 2 Gy
(7.13x10[6] /cm[2])

29 keV/µm C-ions, 2 Gy
(43.1x10[6] /cm[2])

P(x)

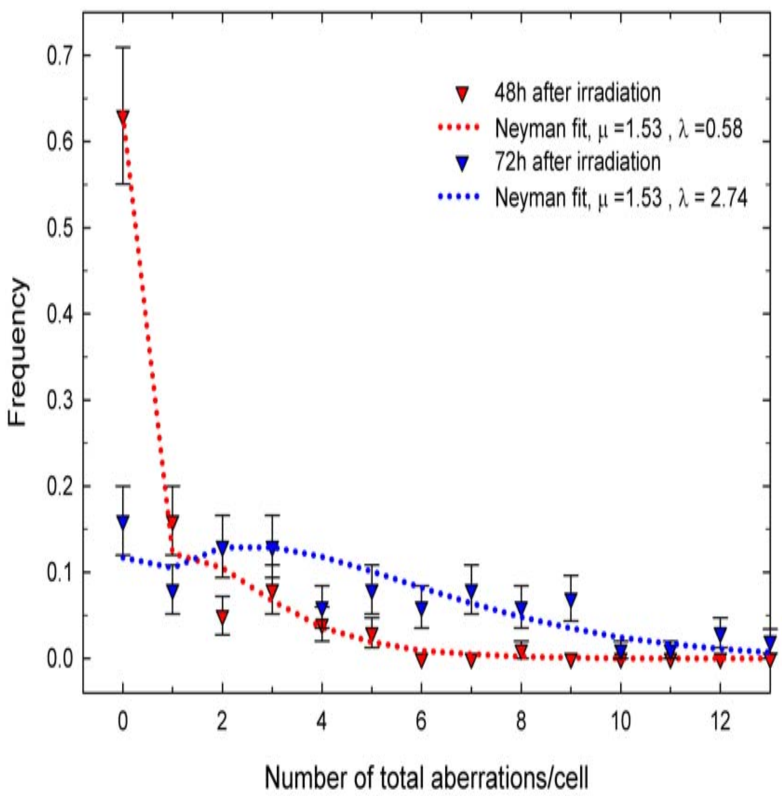




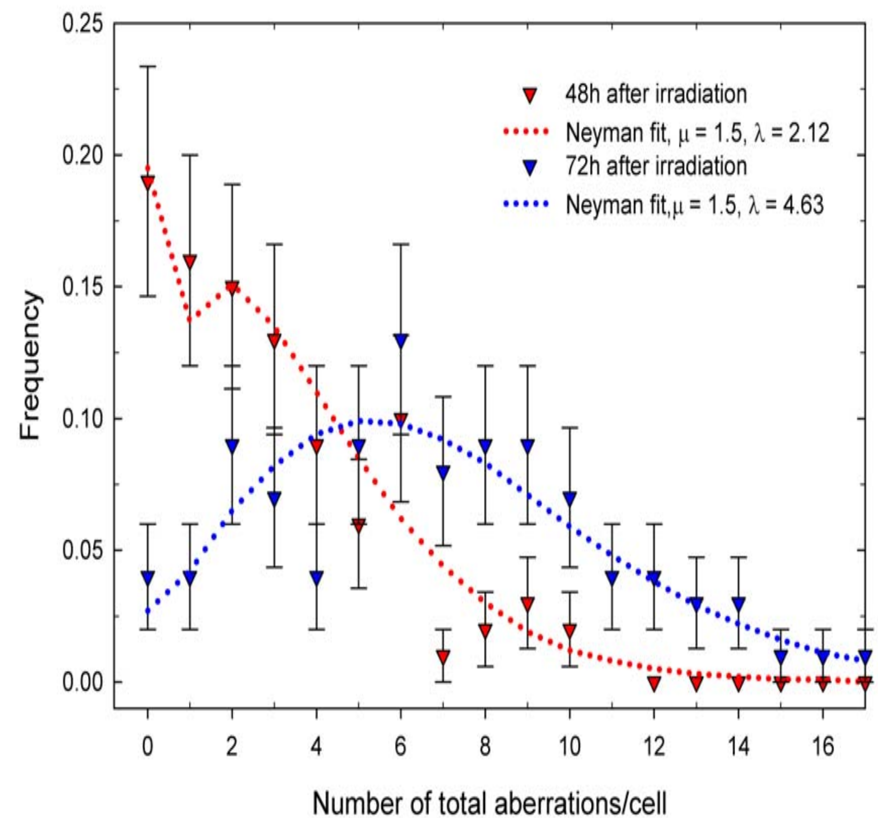
x

Frequency distribution of aberrations per cell: similar LET but different energy and track structure

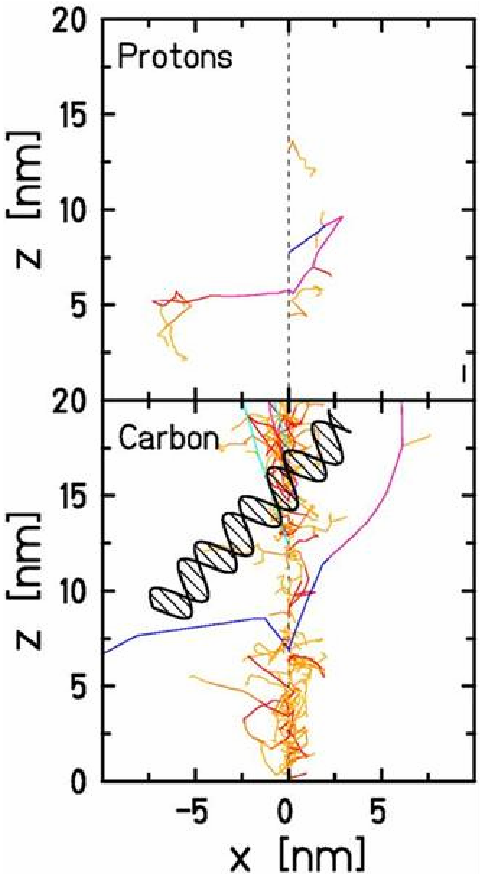
175 keV/μm C-ions, 2 Gy
(7.13×10^6 /cm^2)
Energy 11.6 MeV/n



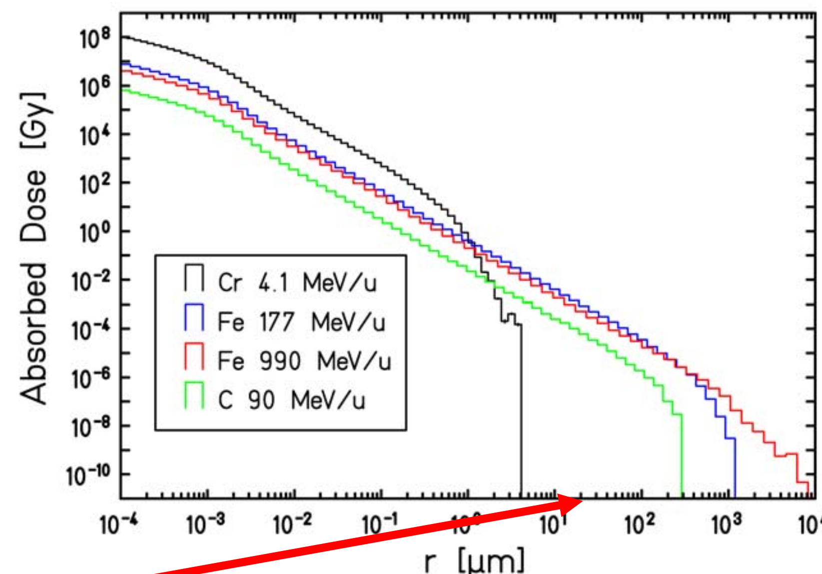
155 keV/μm Fe-ions, 2.3 Gy
(9×10^6 /cm^2)
Energy 1GeV/n



Back to fundamentals:
Physical characteristics of ion beams



Simulation of δ-electron emission by 1 MeV/u protons and C-ions.



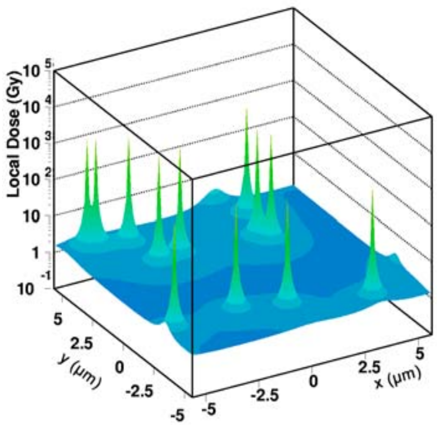
11.4MeV/n C-ions

(R_{max}=2.3µm)

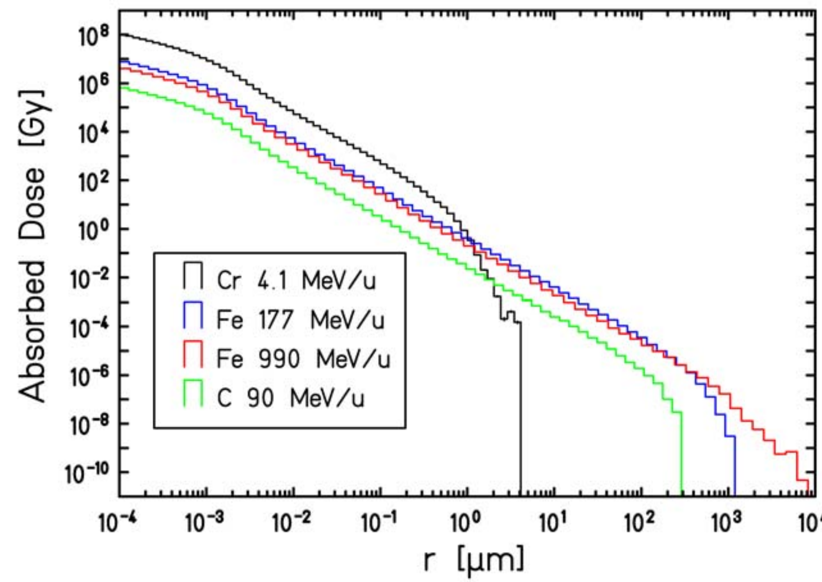
Local dose deposited in particle tracks.

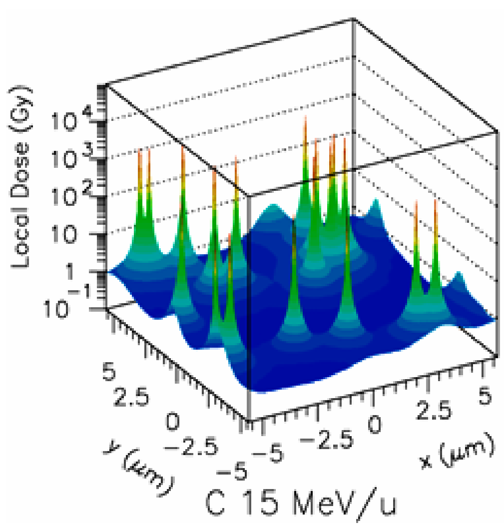
(M. Krämer, GSI)

Back to fundamentals: Physical characteristics of ion beams



Fe 990MeV/n





C 15MeV/n

Local dose deposited in particle tracks.

Total amount of aberrations/ aberrant cells (integration analysis)

$$N_k = \prod_{i=1}^{k} N_0 \left(1 + MI_i\right)$$

$$MI_i^* = MI_i \frac{N_k}{N_0} = MI_i \prod_{i=1}^{k} \left(1 + MI_i\right)$$

$$A_{tot} = \sum_i a_i MI_i^*$$

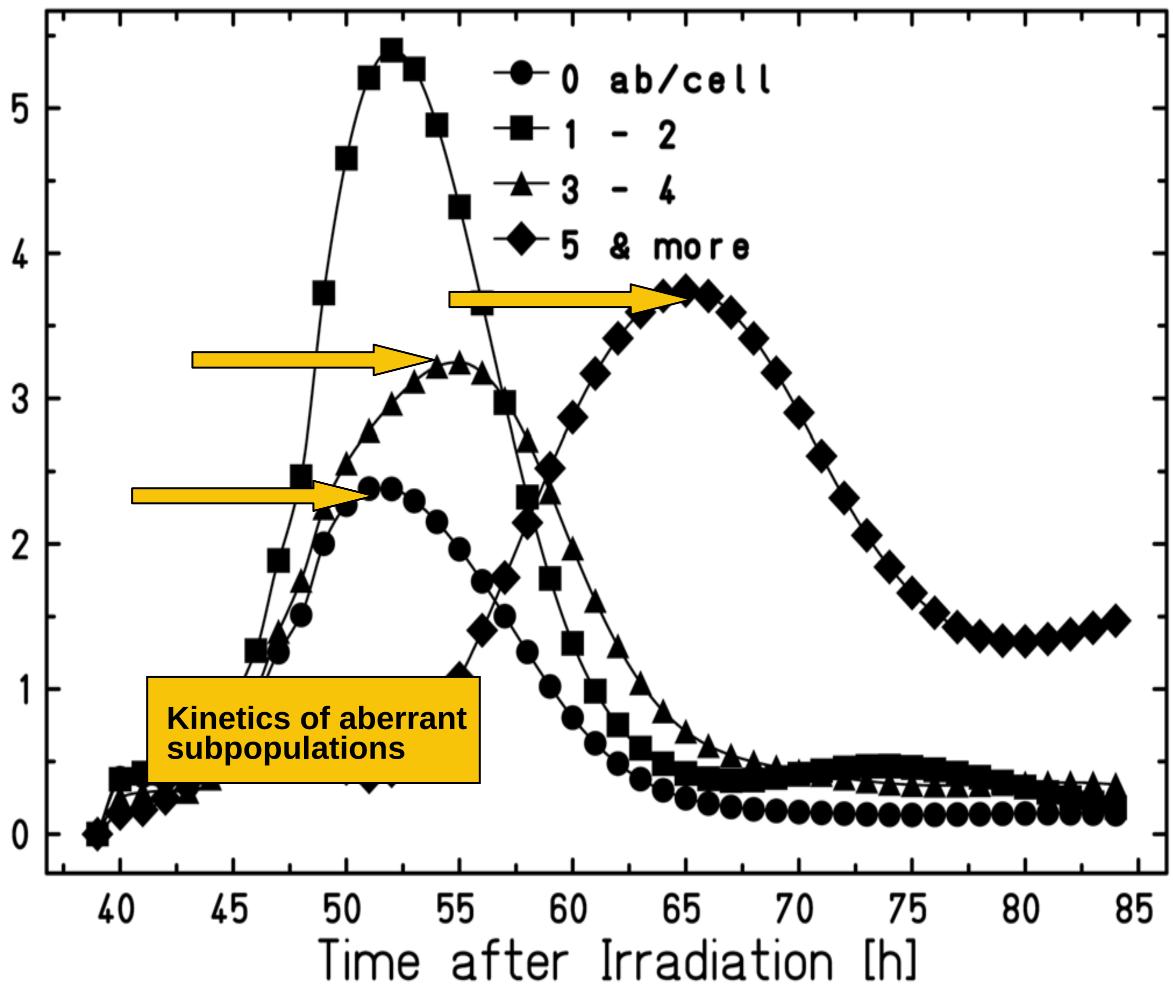
$$\Phi_{tot} = \sum_i f_i MI_i^*$$

- A_{tot} ***total no. of aberrations***
- a_i ***number of aberrations found at a time i***
- MI^*_i ***corrected mitotic index at a time I***
- Φ_{tot} ***total fraction of aberrant cells***

Reconstructed growth of population with respect to the starting number of cells

Cell kinetics in mitosis and cycle delay: lymphocytes
Fe 990 MeV/u, 2.23 Gy

Flux of aberrant cells (cells/h x 10^{-3})

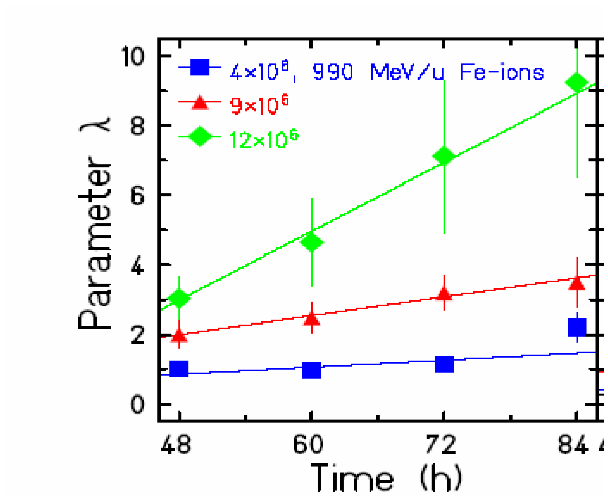


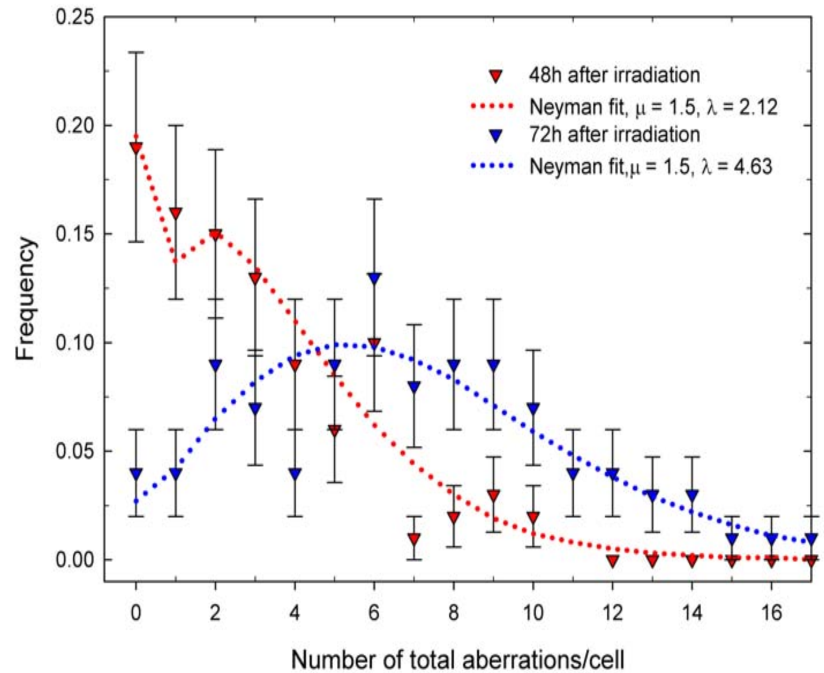
Modal values are shifted towards later times

Delay in mitosis can be correlated with a number of aberrations carried by a cell

Gudowska-Nowak et. el.
Int. J. Radiat. Biol. (2005)

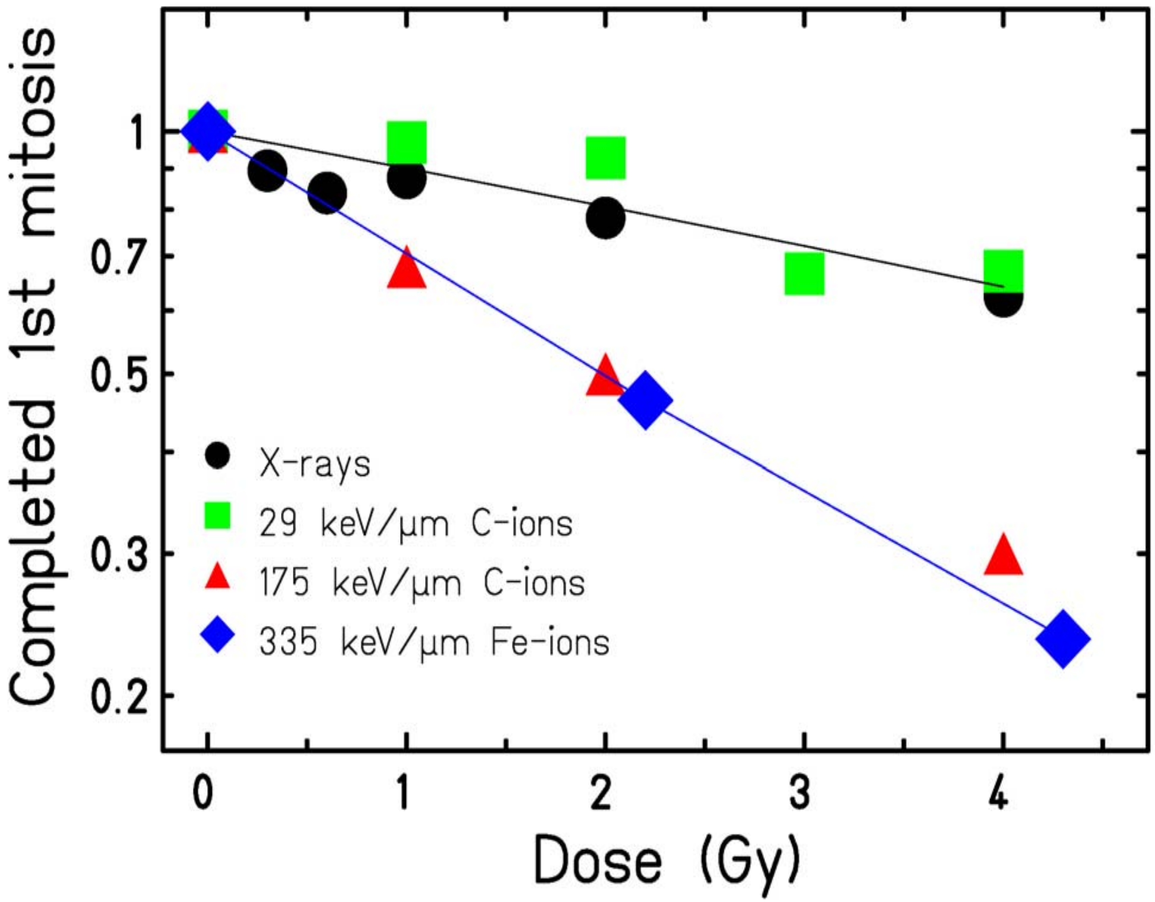
Detection of cell-cycle delay: time variation of parameter λ





Time-variation of „hits" (parameter λ)
reflects delay in mitosis of damaged cells

Cell kinetics in mitosis: integrated data for fraction of cells which completed mitosis up to 84h



Survival fractions in first mitosis

Note „efficiency" in the response for different LET values

The microscopic structure of energy deposition of heavy ions influences the production of aberrations

Low LET (2-30 keV/mm)

- Homogeneous dose deposition
- Aberrations are Poisson distributed
- Aberration yield does not change with time
- Standard metaphase method (48 h) is sufficient

- M- (or G2-assay) at several times should be assessed

High LET (150-3000 keV/mm)

- Inhomogeneous dose deposition
- Aberrations are distributed according to the compound-Poisson statistics
- Correlation between the number of particle hits and delay

SUMMARY

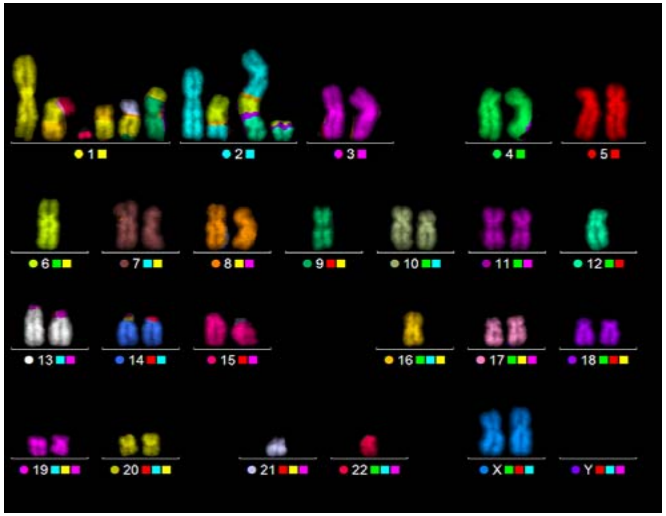
- ***Data clearly demonstrate that there is a selective delay of heavily damaged cells.***

- ***Cell cycle delays have to be taken into account for a realistic estimate of the cytogenetic effects of heavy ions.***

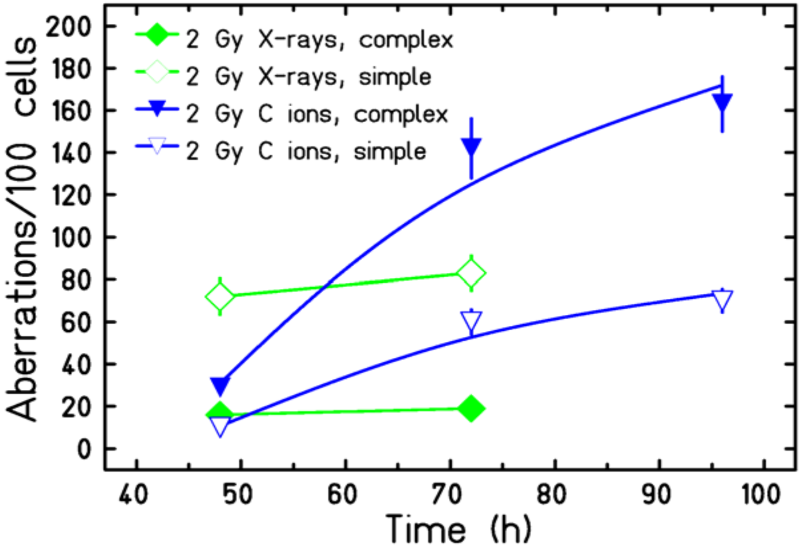
- ***Data measured at only time-point in metaphase or G2-phase cells insufficient: may mask real efficiency of particles.***

? Use of aberrations for risk assessment of high LET particles

2 Gy C-ions versus 2 Gy X-rays



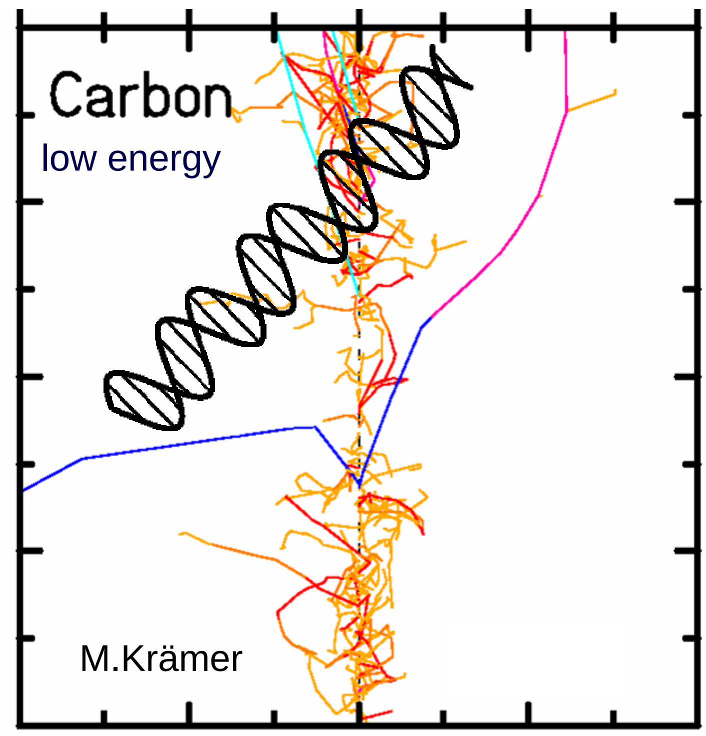
Complex aberrations

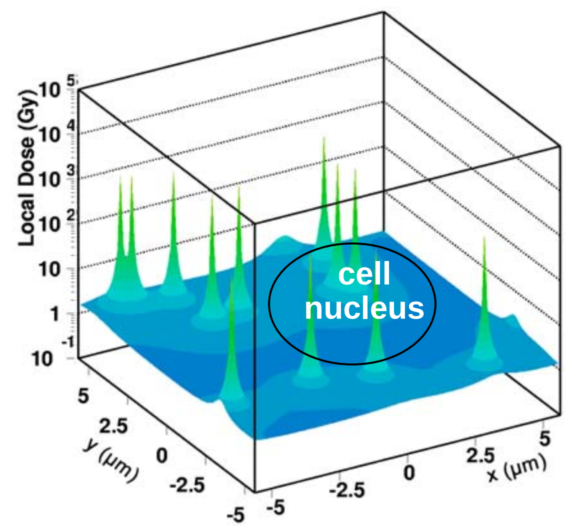


Time-course of aberrations detected by M-FISH

Higher biological effectiveness

Nanometer scale:

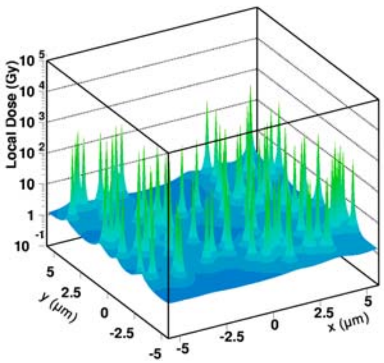




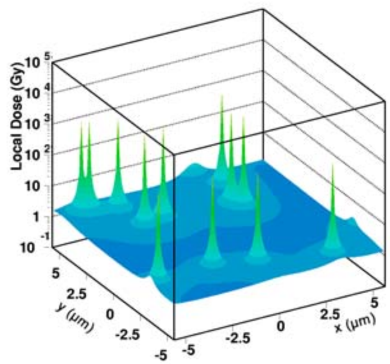
complex damage due to localized energy deposition

Microscopic dose distribution of X-rays or ions

90 MeV/u C-ions
29 keV/µm
2 Gy, 43*10⁶ /cm²



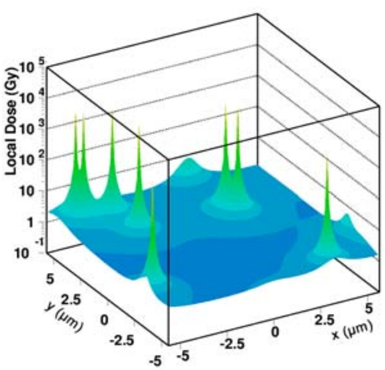
990 MeV/u Fe-ions
155 keV/µm
2.3 Gy, 9*10⁶ /cm²



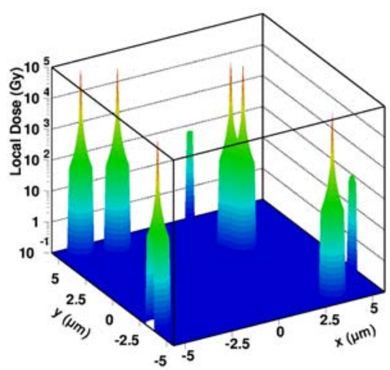
Particle fluence of 4*10⁶ ions/cm² corresponds to 1 particle hit in average per nucleus

(No hit: 37 %, 1 hit: 37 %, 2 hits: 18 %, more than 3 hits: 8 %)

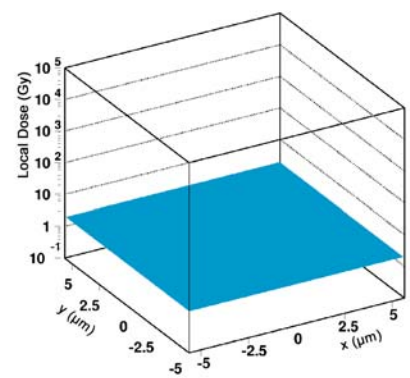
177 MeV/u Fe-ions
335 keV/µm
2 Gy, 3.7*10⁶ /cm²



4.1 MeV/u Cr-ions
3160 keV/µm
20.3 Gy, 4*10⁶ /cm²

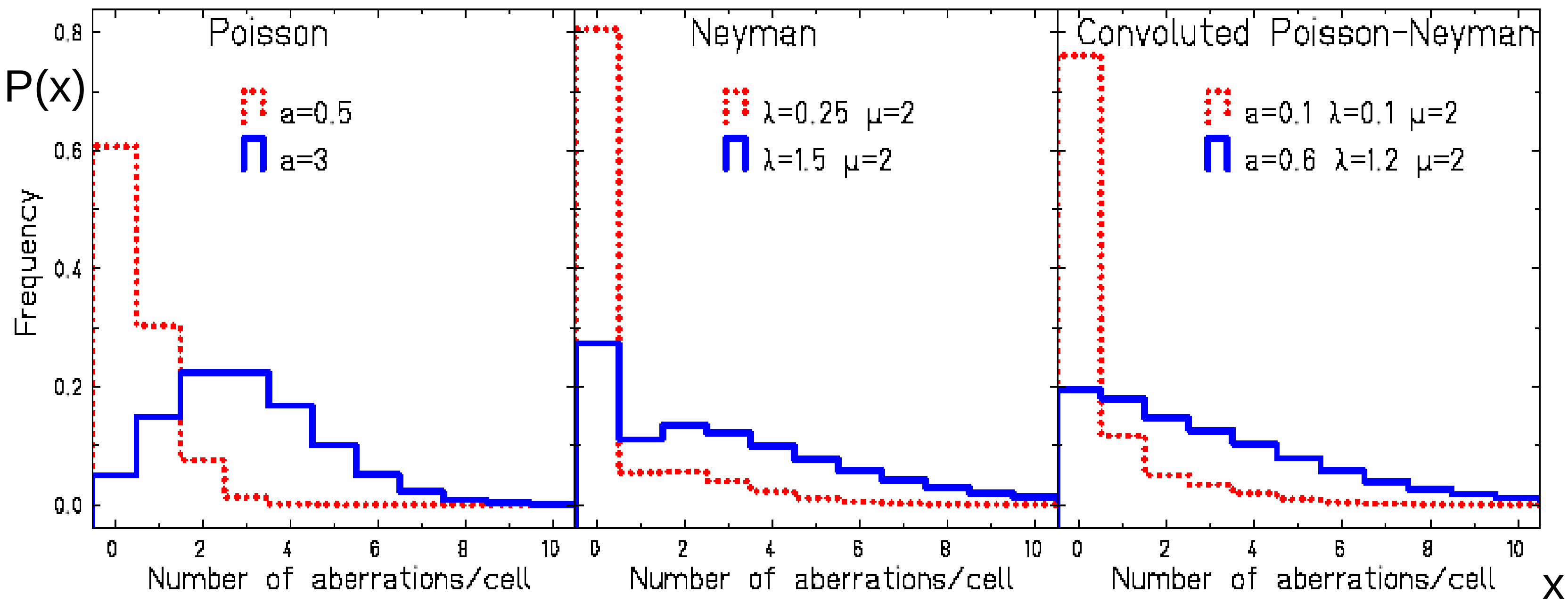


X-rays
2 keV/µm
2 Gy



Features of the statistics: overdispersion

comparison of frequency patterns



$$G_{N+P}(Z) = G_P(Z)G_N(Z) = exp[\lambda(e^{\mu(Z-1)} - 1) + a(Z-1)]$$

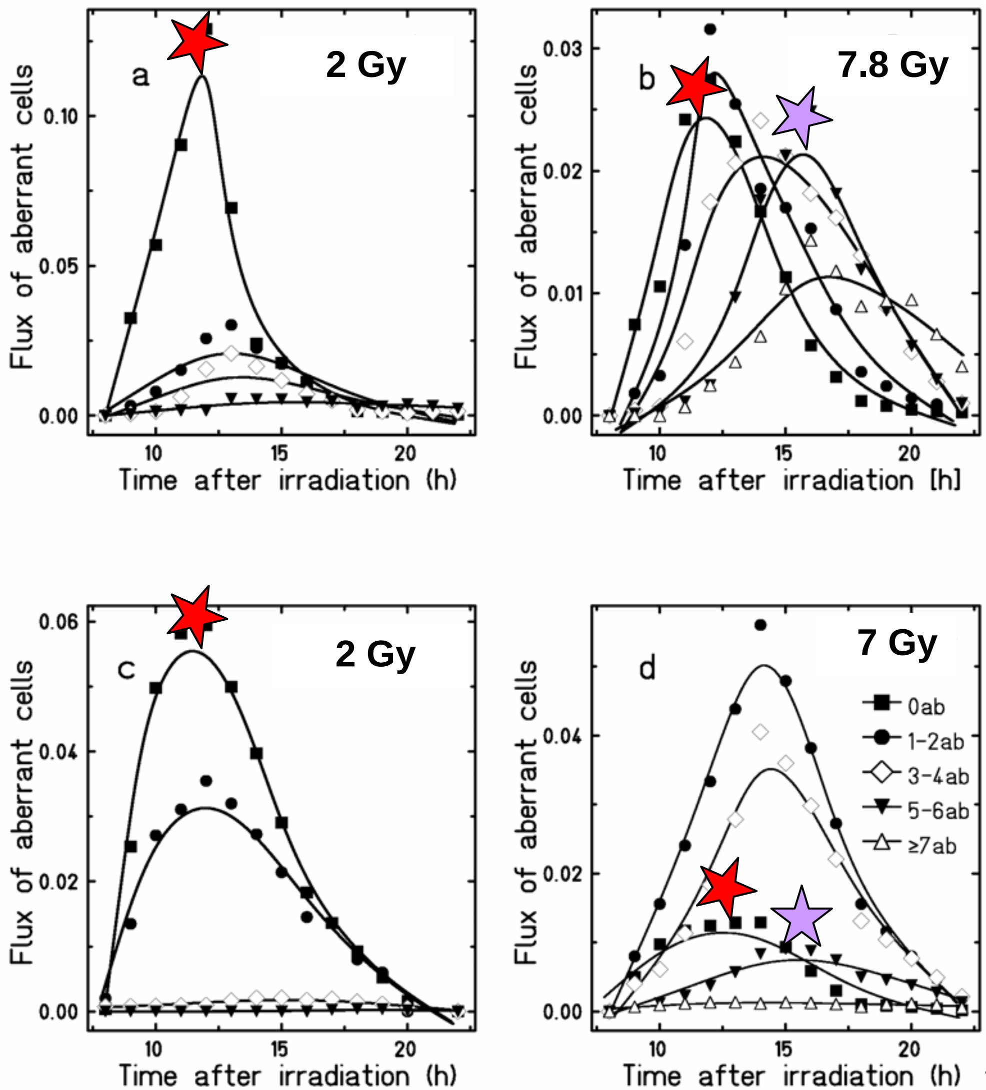
$$Z = \exp(isx)$$
$$G = \langle \exp(isx) \rangle$$

$$\frac{\sigma^2}{\langle X \rangle} = \frac{\lambda\mu^2 + \lambda\mu + a}{\lambda\mu + a} = 1 + \frac{\lambda\mu^2}{\lambda\mu + a}$$

Correlation between damage and delay times

Ar ions

X-rays

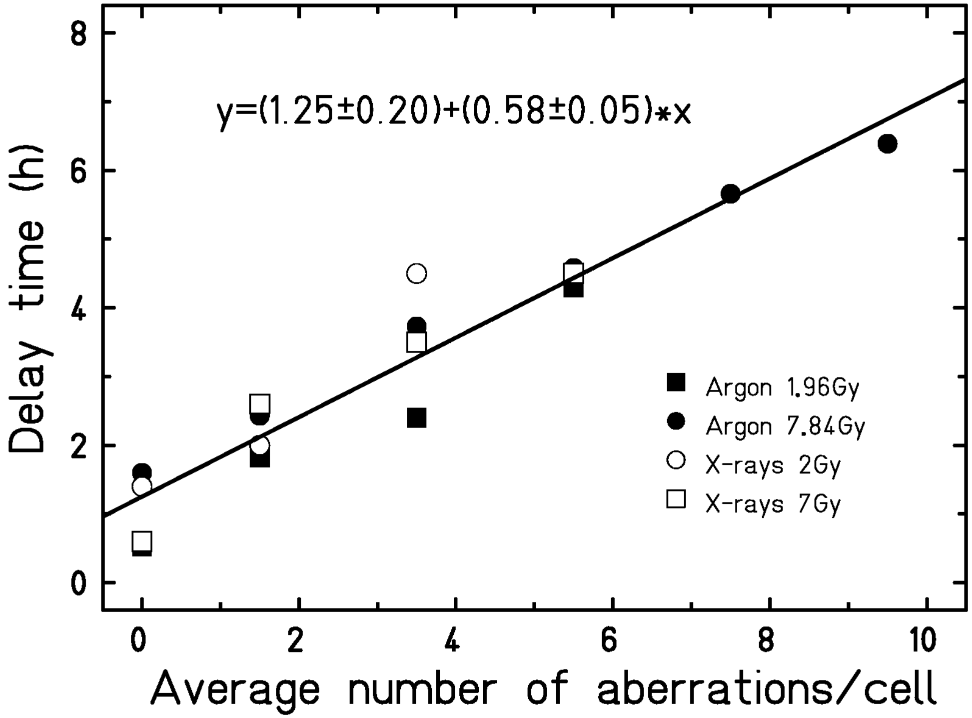


0 aberrations/cell

5-6 aberrations/cell

Gudowska-Nowak et al. IJRB (2005)

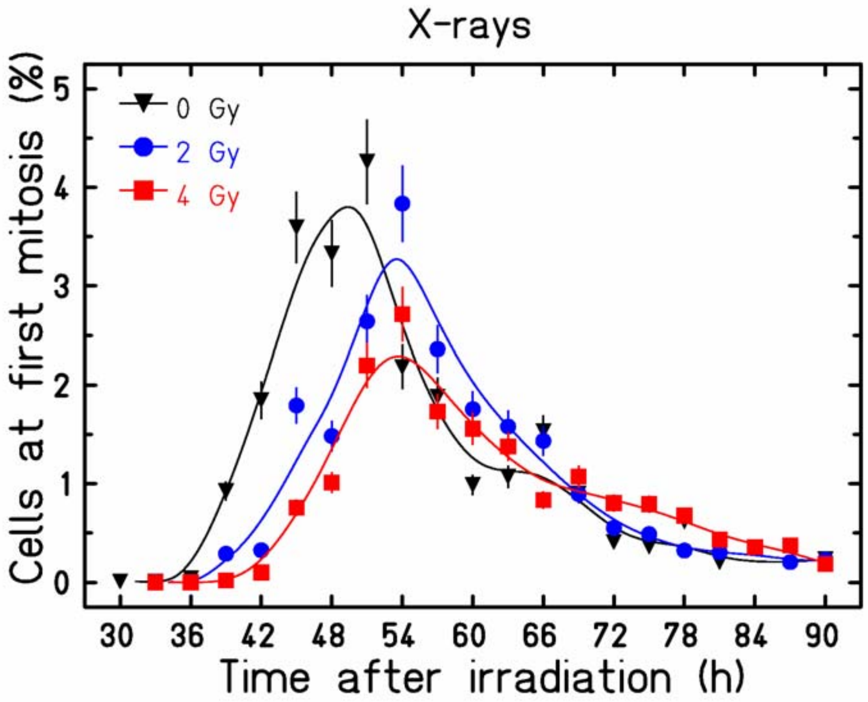
Correlation between damage and delay times

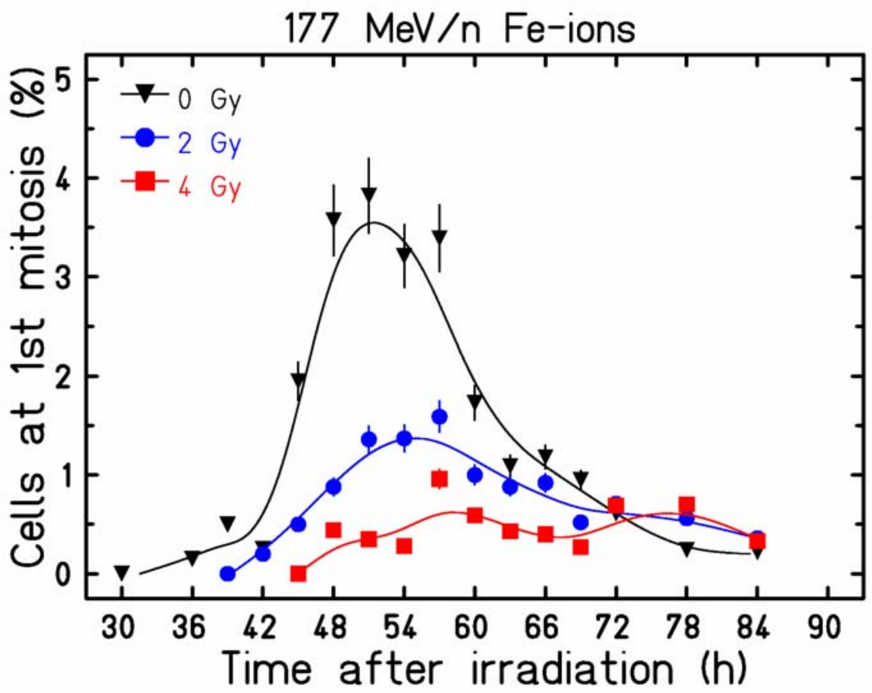


Direct correlation between the average delay time in entering 1st mitosis and the average number of aberrations carried by a cell.

Gudowska-Nowak et al. IJRB (2005)

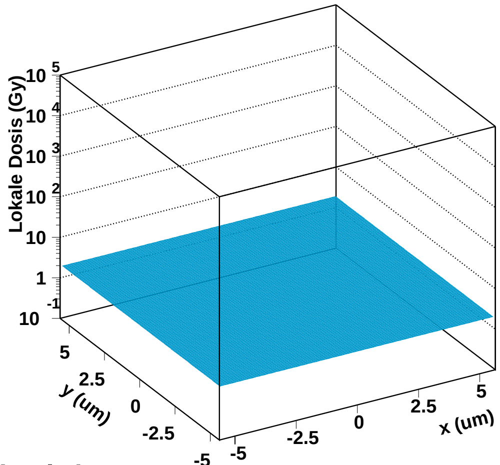
Mitotic delay as a function of dose



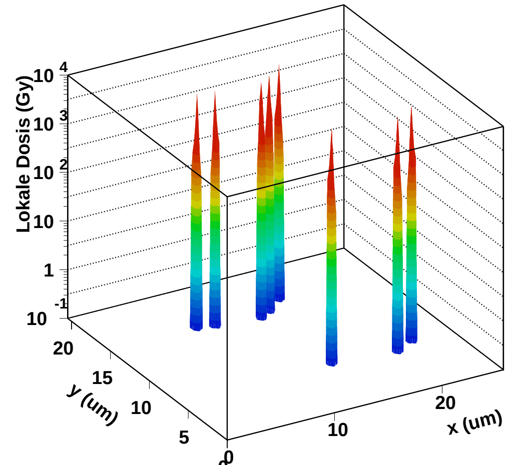


Lymphocytes of the same donor were exposed to either X-rays or Fe ions.

Dose-distribution in micrometer scale

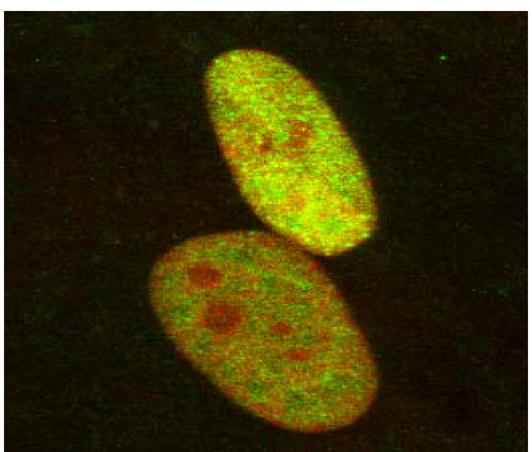


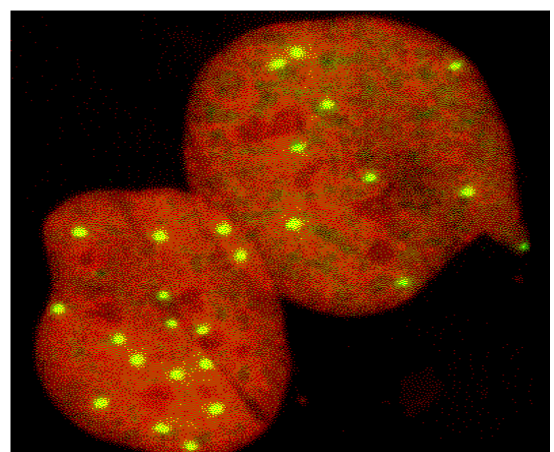
Photonen



Pb-Ionen, 3.1 MeV/u

(M. Scholz)





(G.Taucher-Scholz, B. Jacob)