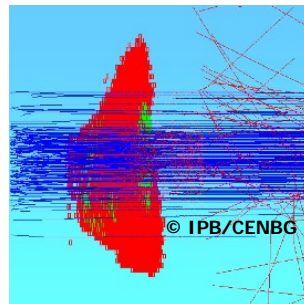


Microdosimetry in biological cells with the Geant4 Monte Carlo simulation toolkit

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Notice

- On behalf of the contributors to this project, we would like to **thank the organizers** for their invitation to present this invited talk
- This talk will cover
 - **N15-425 (poster)** : evaluation of phase effects in Geant4 microdosimetry models for particle interactions in water
 - **N40-5 (talk)** : microdosimetry in high resolution cellular phantoms using the very low energy electromagnetic extension of the Geant4 toolkit
 - **N40-6 (talk)** : cell survival modelling with the Geant4 Monte Carlo toolkit



Content

- **Science motivation and software context**
- **Specific activities involving Geant4**
 - Microdosimetry in high resolution phantoms
 - Chemical phase effects
 - Cell survival modelling
- **Conclusion**
- **Perspectives**



Context

- Understanding the **health effects of low doses of ionizing radiation** is the **challenge** of today's radiobiology research
- At the cell scale, recent results include the observation of
 - bystander effects
 - adaptative response
 - gene expression changes
 - genomic instability
 - genetic susceptibility
- ▶ May **question the validity of health risk estimation** at low doses of radiation
- ▶ Consequences for radiotherapy, environment exposure, future space exploration human missions...



Context

- Dedicated worldwide **experimental** facilities to investigate the interaction of ionizing radiation with living cells
 - radioactive sources
 - classical beams
 - **microbeams** : allows a perfect control of the deposited dose in the targeted cell
(ex. the CENBG irradiation facility in France)
- In support to the experimental activity, **modelling** of the radiation interactions at the cellular scale is an important activity in this field



Modelling radiation biology

- Modelling biological systems and Physics processes down to the nanometer / \sim eV scale is a **challenge**
- Several specialized Monte Carlo codes have been developed for radiobiology and microdosimetry

However

- Typically each one implementing models developed by its authors
- Limited application scope
- Not publicly distributed
- Legacy software technology (FORTRAN, procedural programming)



The Geant4 DNA extension

- **Alternative** approach : a powerful Monte Carlo code with
 - outstanding modelling capabilities (geometry, physics, interactivity...)
 - full flexibility for extension



The **Geant4** toolkit and the **Geant4 DNA extension**

- Full power of a **general-purpose** Monte Carlo system
- **Toolkit**: multiple modeling options, no overhead (use what you need)
- **Versatility**: from controlled radiobiology setup to real-life ones
- Open source, publicly released
- Modern software technology
- Rigorous software process
- Collaboration between ESA / INFN / IN2P3 (**open !**)



Motivation

For the first time a general-purpose Monte Carlo system is equipped with functionality specific to the simulation of biological effects of radiation

First development cycle : **Physics processes**

(Current) physics models in Geant4 DNA

Liquid water only

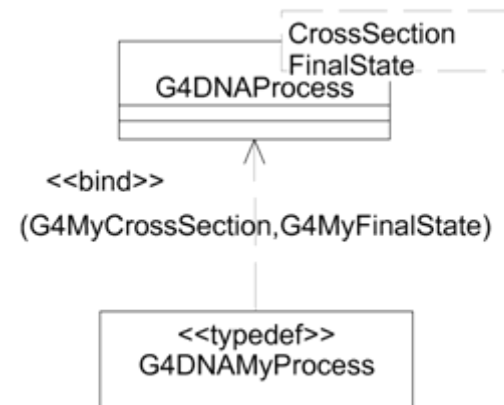
Down to the eV scale

	e	p	H	α , He+, He
Elastic scattering	> 7.5 eV Screened Rutherford	-	-	-
Excitation A ₁ B ₁ , B ₁ A ₁ , Ryd A+B, Ryd C+D, diffuse bands	7 eV – 10 keV Emfietzoglou	10 eV – 500 keV Miller Green 500 keV – 10 MeV Born	-	Effective charge scaling from same models as for proton
Charge Change	-	1 keV – 10 MeV Dingfelder	1 keV – 10 MeV Dingfelder	
Ionisation 1b ₁ , 3a ₁ , 1b ₂ , 2a ₁ + 1a ₁	7 eV – 10 keV Born	100 eV – 500 keV Rudd 500 keV – 10 MeV Born	100 eV – 100 MeV Rudd	

Novel software architecture

- Problem addressed with **novel software technology : policy-based** class design
(**new** in Monte Carlo simulation technology !)
- Physics processes configurable with a variety of model options
(cross section, final state)
- Open to extension and evolution

Physics processes as an empty shell that can be handled transparently by Geant4 kernel and can be configured by **template specialization** to acquire Physics properties.



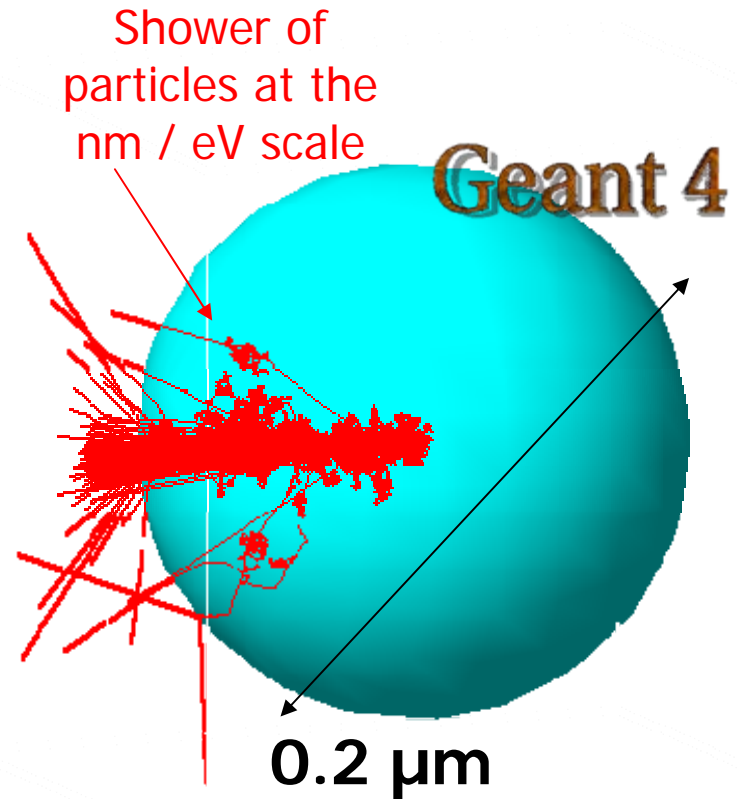


Geant4 DNA

- Physics paper will be published in TNS this December
 - “Geant4 physics processes for microdosimetry simulation: design foundation and implementation of the first set of models”
 - By S. Chauvie, Z. Francis, S. Guatelli, S. Incerti, B. Mascialino, P. Moretto, P. Nieminen and M. G. Pia
- Prototype already released in 2006
- Code publicly released in **December 2007**
- First applications are presented

Example: electron interactions

- Liquid water sphere
- 0.2 μm in diameter
- 1000 electrons shot from central point source
- 20 keV
- Physics processes :
 - Elastic scattering
 - Excitation
 - Ionisation



What about more realistic targets, like human cells ?



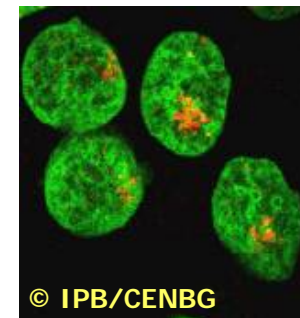
Microdosimetry in high resolution phantoms



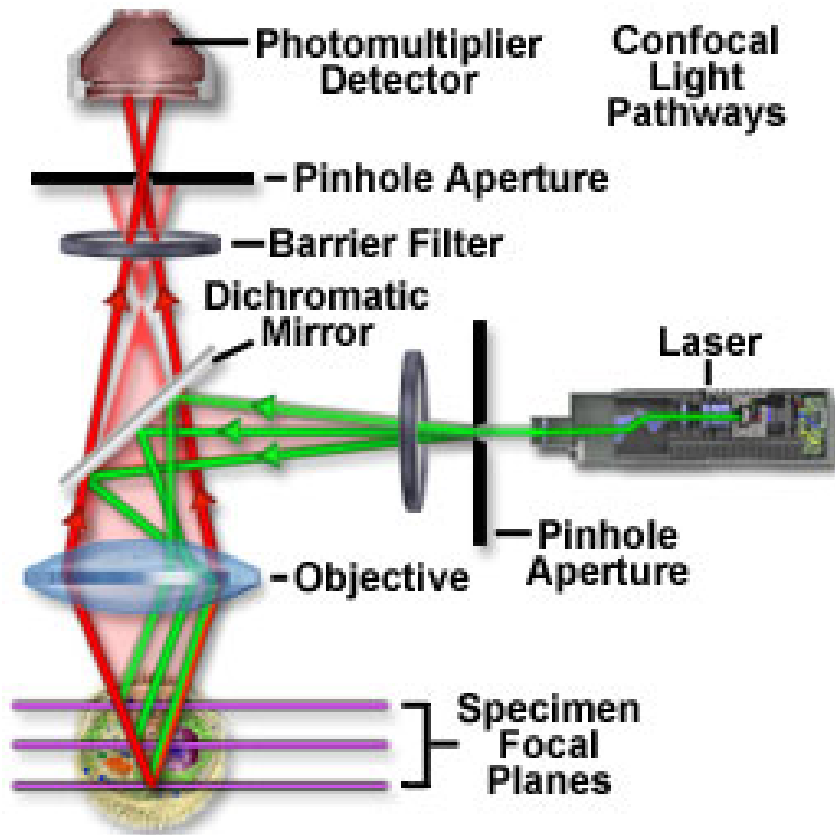
CENBG

AIFIRA irradiation facility in Bordeaux, France

- AIFIRA equipped with a cellular irradiation microbeam line
- 3 MeV proton or alpha beam
- **single cell & single ion mode**
- Targeting accuracy on living cells **in air** : a few μm
- Able to quantify DNA damages like double strand breaks



Realistic cellular geometries from confocal microscopy



Confocal microscopy offers **several advantages** over conventional widefield optical microscopy, in particular :

- ability to control **depth of field**
- **elimination of background information away from the focal plane** (that leads to image degradation)
- collect serial **optical sections** from thick specimens

Confocal microscopy

- We have acquired images of **human keratinocyte cells** HaCaT/(GFP-H2B)Tg, cell line used in single cell irradiation at CENBG
- Cells fixed after **4 hours or 24 hours** of incubation (cell irradiation conditions)

■ Staining

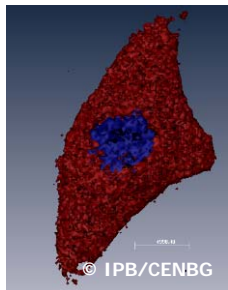
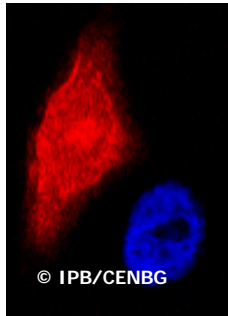
- **Nucleus** : Hoechst 33342 dye (DNA marker)
- **Cytoplasm** : propidium iodide, (RNA and DNA marker).
- **Nucleoli** (high chromatine and protein concentration regions): idem

■ Acquisition

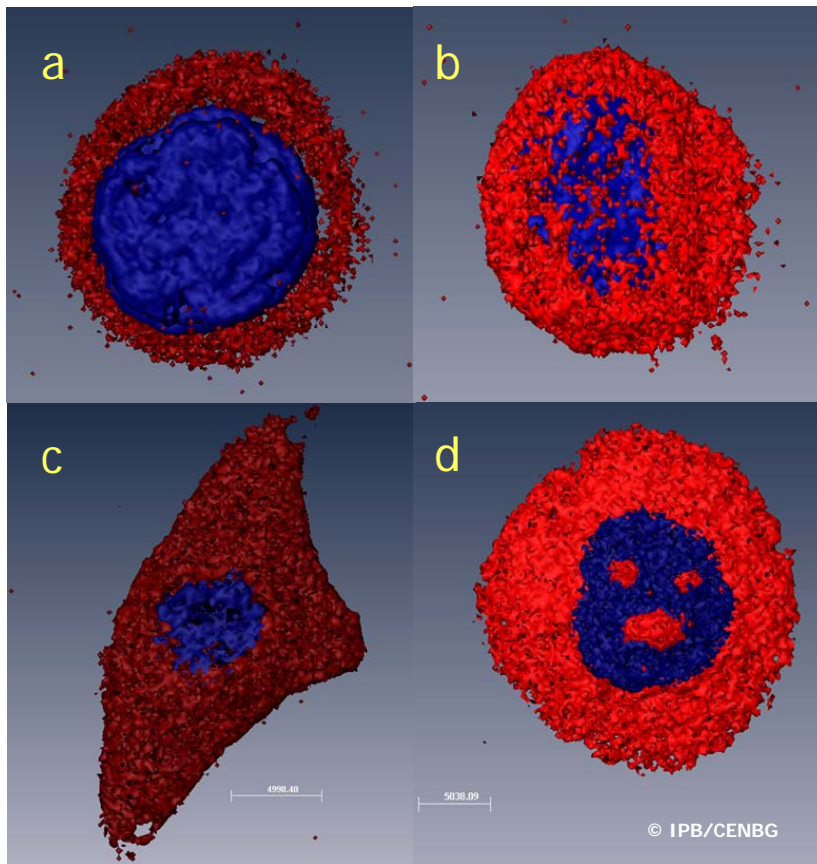
- 2D images acquired every **163 nm** with a Leica® DMR TCS SP2 confocal microscope
- several 2D resolutions, **up to 512 × 512 pixels**

■ Image reconstruction

- 3D stacking using the Leica Confocal Software®
- filtering and phantom geometry reconstruction with Mercury® Computer Systems, Inc. Amira® suite



Building cellular models



- Selection of four “phantoms” reconstructed from 128×128 2D confocal images.
- Incubation :
 - 4 hours for cells a and b
 - 24 hours for cells c and d
- The cytoplasm and nucleoli appear in red while the nucleus is shown in blue.



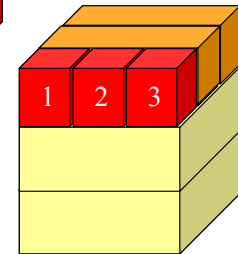
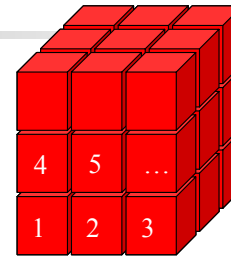
Geometry implementation

- Exploit the **advantage of a general purpose** Monte Carlo system
 - Powerful Geant4 geometry package
- Use of functionality **already existing** in the Geant4 toolkit
 - Parameterized volumes
 - Nested parameterization
- Direct benefit from investment in the Geant4 toolkit
- Realistic rendering of the biological systems
 - Important for overall realistic simulation results

**Not available in ad-hoc
Monte Carlo codes**


Cellular phantoms in Geant4

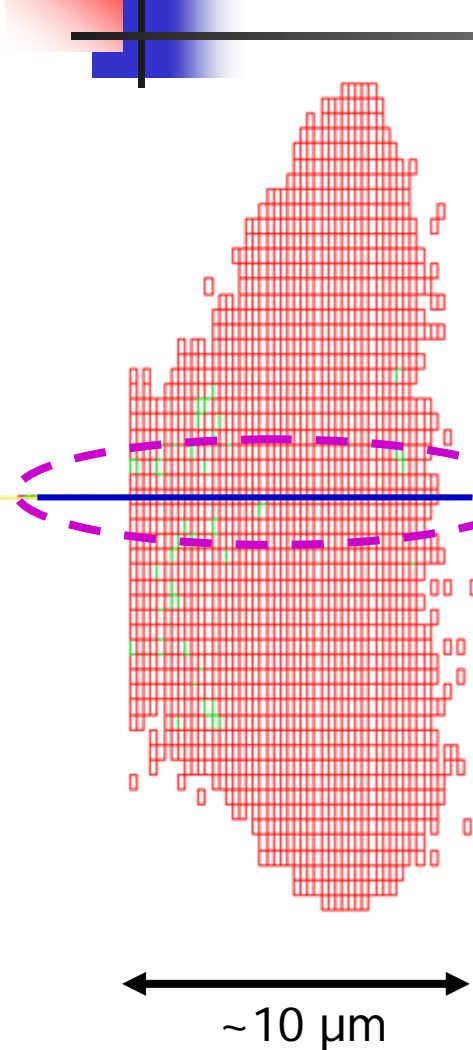
- Implemented either
 - as **parameterized** volumes
 - or **nested geometries**
(faster navigation above 64x64 resolution)



- Composition : liquid water
(compatible with Geant4 DNA models)
- These cellular models are publicly available in the Geant4 microbeam Advanced Example
 - [geant4/examples/advanced/microbeam](https://geant4.cern.ch/examples/advanced/microbeam)

Cellular phantom model

- 24h incubated cell
- Irradiated with a 2.37 MeV alpha+ beam
- 64 x 64 x 60 resolution
- $0.36 \times 0.36 \times 0.16 \mu\text{m}^3$ voxel size 



2.37 MeV
alpha+
particle
hitting the cell

Geant 4

cytoplasm

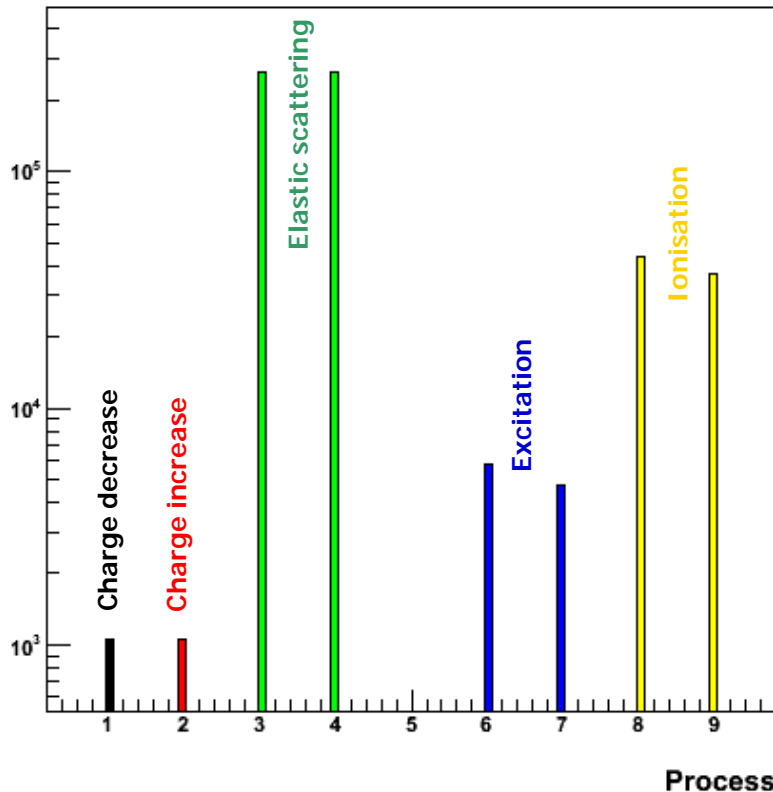
nucleus

Shower in cellular phantom

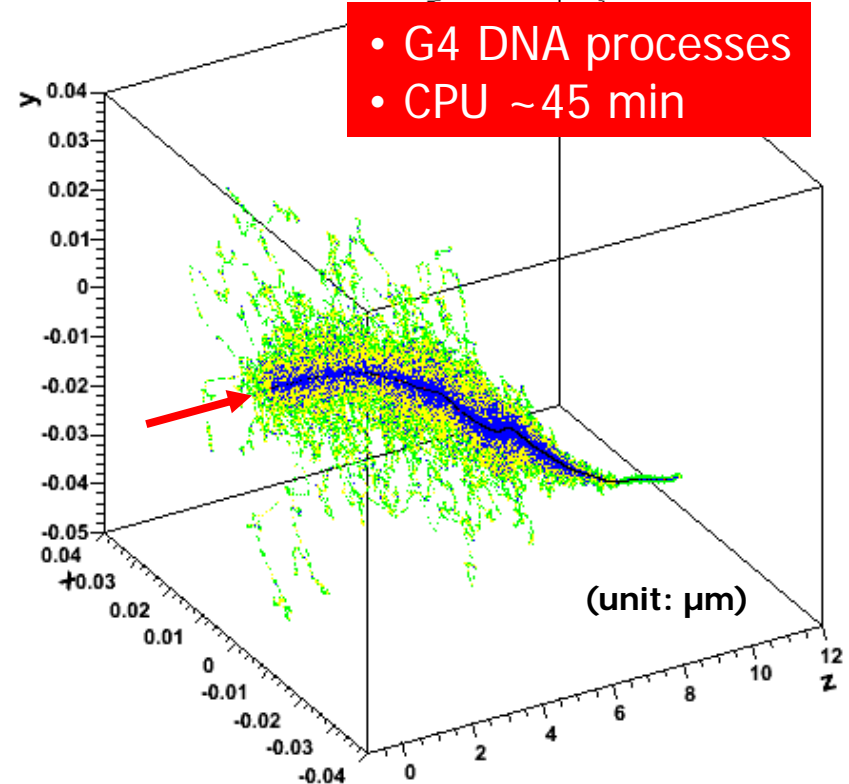
2.37 MeV alpha+ particle delivered on targeted cell
by the CENBG microbeam irradiation facility

ZOOM

G4DNA Processes



Track structure





Geant4 DNA capabilities

- With this Geant4 extension, it is now possible to study the energy deposit distribution produced by a primary particle and its secondaries **inside a cell**
- Access distributions in nucleus, cytoplasm, mitochondria,...
- But how well can the Geant4 DNA Physics models be trusted to produce these distributions ?



Phase effects



How accurate are Geant4-DNA physics models ?

- Both **theoretical** and **experimental** complexity in the very low energy régime
- **Theoretical calculations** must take into account the detailed dielectric structure of the interacting material
 - Approximations, assumptions, semi-empirical models
- **Experimental measurements** are difficult
 - Control of systematics
 - Practical constraints depending on the phase



Verification and Validation

- Verification

- Conformity with **theoretical models**
- Performed on all physics models

- Validation

- Against **experimental data**
- **Lack of** experimental data in liquid water in the very low energy range
- New measurements needed



Comparison against vapour/ice measurements

- Geant4 DNA : interacting medium is **liquid water**
 - Condensed phase is **more realistic** than the gas phase approximation
 - ...but **more difficult** to handle theoretically
- Relatively large amount of data exists in **vapour** at low energy
- A few measurements in **ice**
- For the comparison of Geant4-DNA models :
 - **Plausibility** of the physics results of the software
 - Insight into physical effects related to the **phase** of the interacting medium



A selection of results

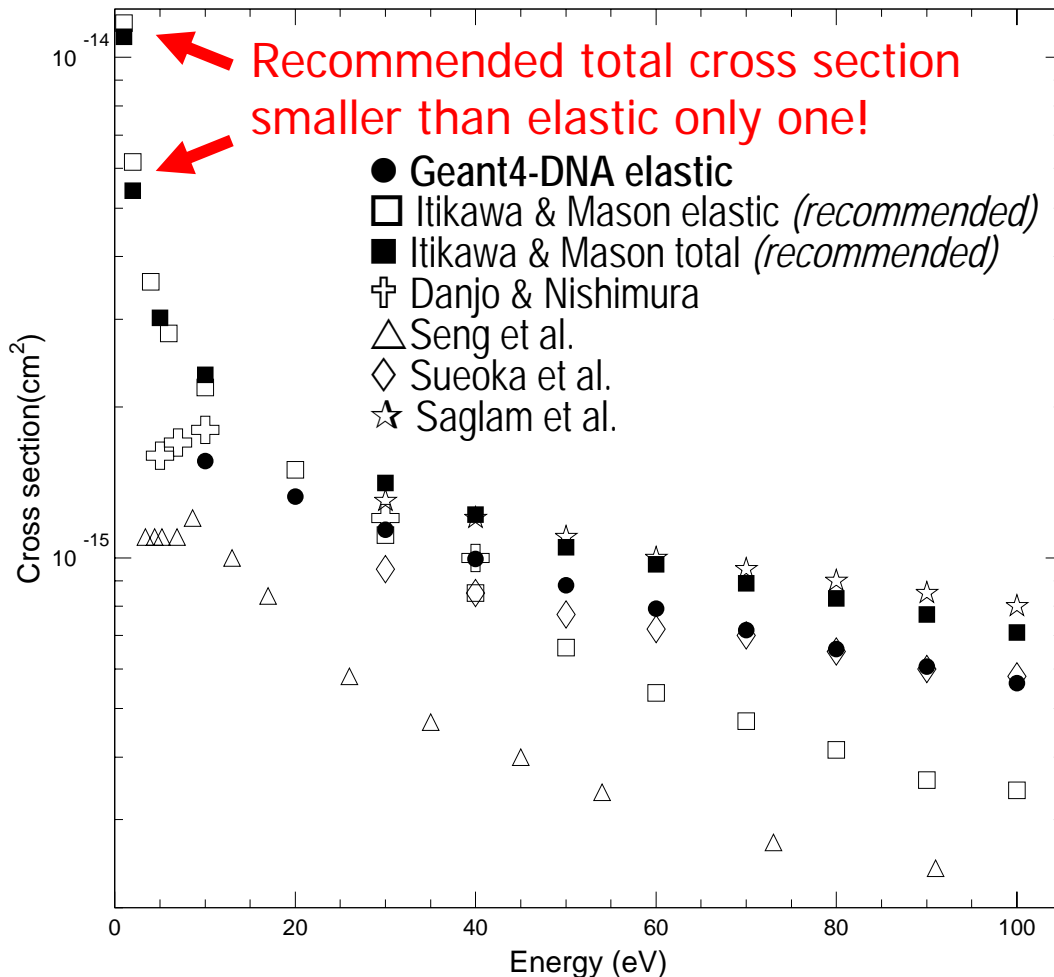
- Systematic comparison necessary
 - Survey of literature: extensive collection of experimental data (goal: all!)
 - No time to show all of them (\Rightarrow paper)
- Selection of a few interesting cases
 - Highlight the complexity of the experimental domain
 - Discuss physics modelling features



1) Electron elastic scattering

- **Theoretical challenge** to model accurately at very low energy
- Various theoretical approaches at different degrees of complexity
 - From simple **screened Rutherford** cross section to sophisticated **phase shift** calculations
- No experimental data in liquid water
- Geant4-DNA
 - Simple screened Rutherford cross section + semi-empirical model based on **vapour** data
 - Open to evolution along with the availability of new data

Electron elastic scattering: total cross section



- **Evident discrepancy of experimental data**
Geant4-DNA: better agreement with Danjo-Nishimura data
- **Puzzle** : inconsistency in recommended evaluated data from **Itikawa & Mason, J. Phys. Chem. Ref. Data, Vol. 34, No. 1, 2005, pp. 1-22**



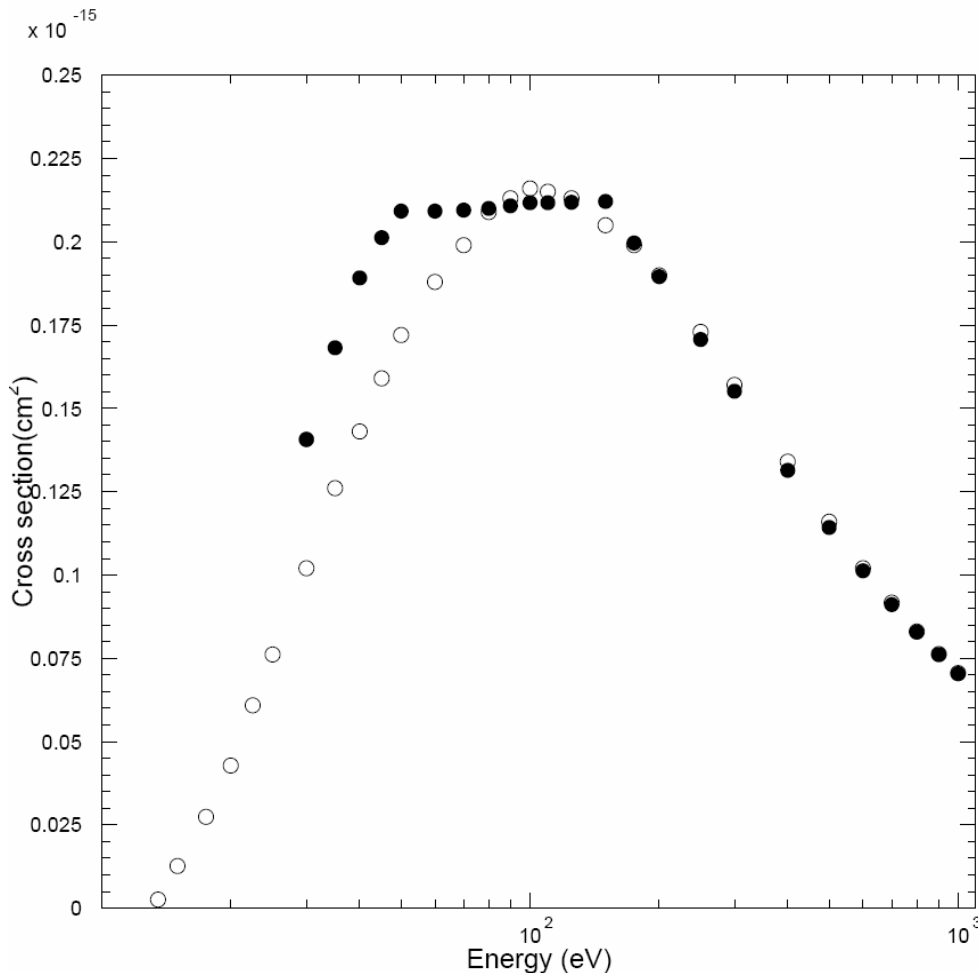
2) Electron ionisation

- **Semi-empirical model**

- D. Emfietzoglou and M. Moscovitch, "Inelastic collision characteristics of electrons in liquid water", NIM B, vol. 193, pp. 71-78, 2002

- Based on **dielectric formalism** for the valence shells ($1b_1$, $3a_1$, $1b_2$ and $2a_1$) responsible for condensed-phase effects
- Based on the **binary encounter approximation** for the K-shell ($1a_1$)

Electron ionisation: total cross section



- Note :
 - Geant4-DNA model: **liquid** water
 - Experimental data: **vapour**
- **Plausible** behaviour of the Geant4 implementation
- Phase differences are more significant **at lower energies**

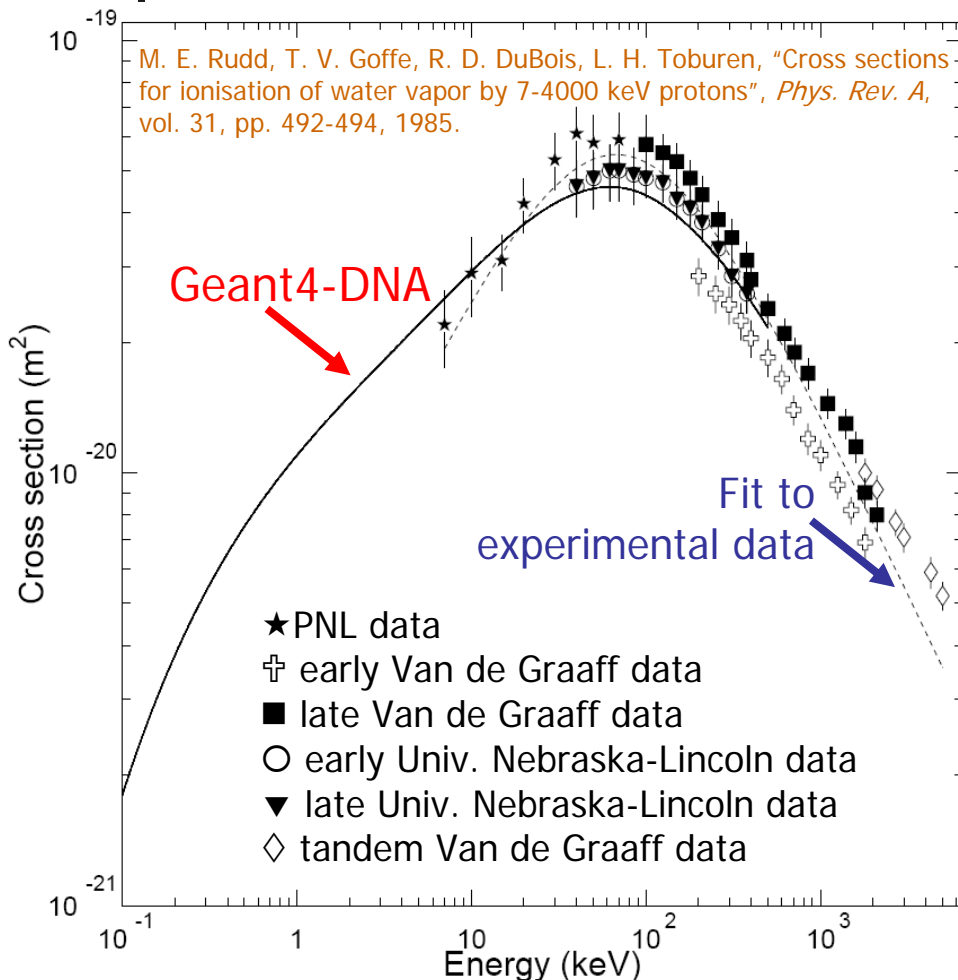


3) Proton ionisation

- Cross section based on two complementary models
 - a **semi-empirical analytical** approach for $100 \text{ eV} < E < 500 \text{ keV}$ with parameters specifically calculated for liquid water
 - a model based on the **Born theory** for $500 \text{ keV} < E < 10 \text{ MeV}$

• M. Dingfelder et al., "Inelastic-collision cross sections of liquid water for interactions of energetic protons", Radiat. Phys. Chem., vol. 59, pp. 255-275, 2000.
• M. E. Rudd et al., "Cross sections for ionisation of water vapor by 7-4000 keV protons", Phys. Rev. A, vol. 31, pp. 492-494, 1985.
• M. E. Rudd et al., "Electron production in proton collisions: total cross sections", Rev. Mod. Phys., vol. 57, no. 4, pp. 965-994, 1985.

Proton ionisation: total cross section



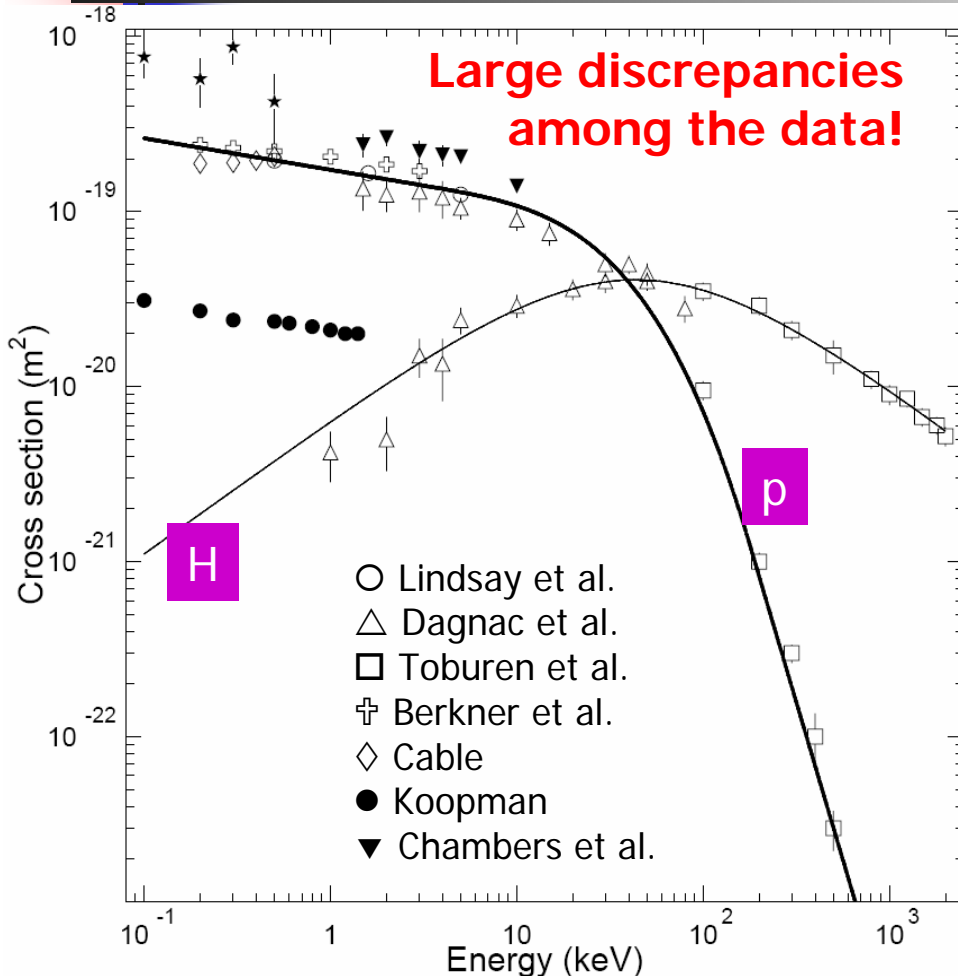
- Note :
 - Geant4-DNA model: liquid water
 - Experimental data: vapour
- All measurements performed by the same team
 - at different accelerators and time
- **Even data taken by the same group exhibit inconsistencies !**
 - Systematic is difficult to control in delicate experimental conditions
- Geant4-DNA models look plausible
- Hard to discuss phase effects in these experimental conditions!
 - Geant4 DNA model incompatible with exp. data (p value < 0.001), which are incompatible against one another
 - Data fit
 - compatible according to Cramer von Mises (p value = 0.1)
 - incompatible according to Anderson-Darling (p value < 0.001)

4) Proton and hydrogen charge change

- Cross section based on a **semi-empirical** approach
 - Described by an analytical formula
 - With parameters optimized from experimental data in **vapour**

- M. Dingfelder et al., "Inelastic-collision cross sections of liquid water for interactions of energetic protons", Radiat. Phys. Chem., vol. 59, pp. 255-275, 2000.
- B. G. Lindsay et al., "Charge transfer of 0.5-, 1.5-, and 5-keV protons with H₂O: absolute differential and integral cross sections", Phys. Rev. A, vol. 55, no. 5, pp. 3945-3946, 1997.
- R. Dagnac et al., "A study on the collision of hydrogen ions H⁺¹, H⁺² and H⁺³ with a water-vapour target", J. Phys. B, vol. 3, pp. 1239-1251, 1970.
- L. H. Toburen et al., "Measurement of highenergy charge transfer cross sections for incident protons and atomic hydrogen in various gases", Phys. Rev., vol. 171, no. 1, pp. 114-122, 1968

Charge change cross section: proton and hydrogen



- Note
 - Geant4-DNA model: liquid water
 - Experimental data: vapour
- Goodness-of-fit test
 - Geant4-DNA (liquid)
 - experimental data (vapour) [white symbols only]
 - Anderson-Darling
 - Cramer-von Mises
 - Kolmogorov-Smirnov
 - Kuiper
 - Watson
 - p-value > 0.1 from all tests

... but some data were used to optimise the semi-empirical model ! 35



Validation and evaluation of phase effects

- Geant4-DNA models (liquid) **look plausible** when compared to available experimental data (vapour)
- More experimental data are needed
 - In **liquid water** for model validation
 - In **vapour and ice** to study the importance of **phase effects** in modelling particle interactions with the medium
- Hard to draw firm conclusions about phase effects
 - Available experimental data (vapour and ice) exhibit **significant discrepancies** in many cases
 - Some of these data have been **already used to constrain or optimize** semi-empirical models

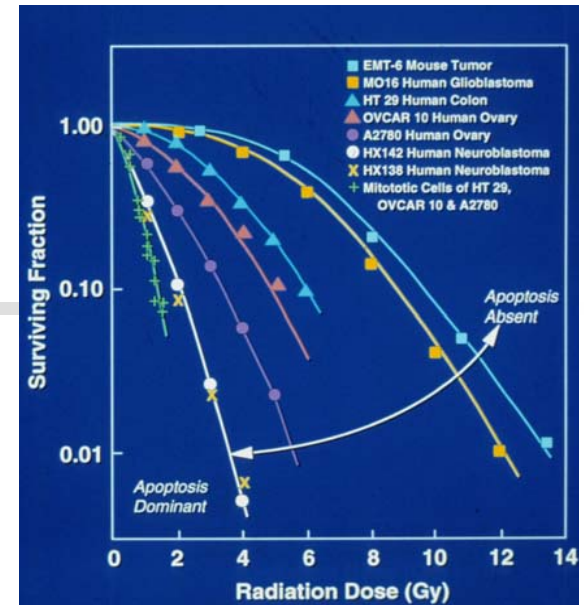


Changing scale

- Large effort for microscopic modelling of interactions and biological systems in Geant4
- Parallel approach : **macroscopic** modelling in Geant4
 - Concept of dose in a cell population
 - Statistical evaluation of radiation effects from empirical data
 - All this is possible in the same simulation environment (**toolkit**)

Cell survival

Courtesy E. Hall



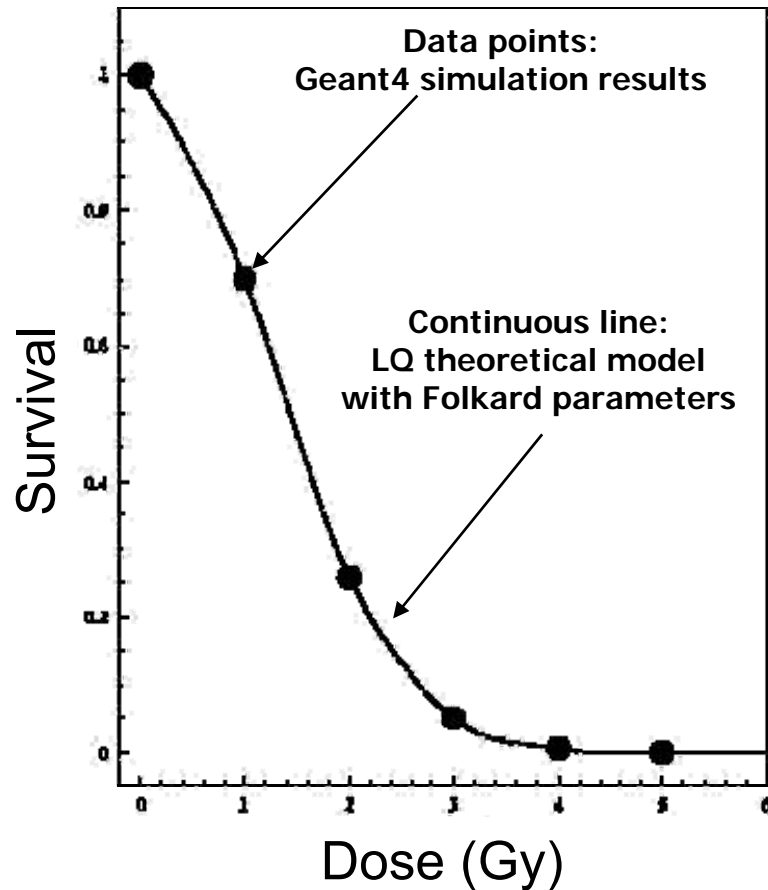
Human cell lines irradiated with X-rays



A macroscopic approach

- Example : modelling cell survival fraction in a population of irradiated cells
- Macroscopic cell survival models to be distributed in Geant4
(**advanced example** in preparation)

Example



- Computation of dose in a cell population and estimation of survival fraction
- Monolayer
- V79-379A cells
- Proton beam
- $E = 3.66 \text{ MeV/n}$
- LQ model

$$S = e^{-\alpha D - \beta D^2}$$

$$\alpha = 0.32$$

$$\beta = -0.039$$

Folkard et al, Int. J. Rad. Biol., 1996



Conclusion

- The Geant4 DNA processes are **publicly available**
- Can be applied to realistic cell geometries
- Full verification and comparison against vapour/ice will be published : evaluation of phase effects
- Thanks to the full flexibility of policy-based code design of Geant4 DNA, any interaction model can be **easily implemented in a few hours and automatically integrated in Geant4 !**
- **Not limited to radiation biology...**



Perspectives

■ Short term

- **Geant4 DNA processes**
 - will be available publicly in the December 07 release of Geant4
- **Microdosimetry advanced example**
 - will be available in the next Geant4 releases (December 07 or June 08)
 - illustrates how to use the Geant4 DNA Physics processes
- **Cellular survival advanced example**
 - in preparation
- Alternative Physics models thanks to the novel software design

■ Long term

- **Chemical phase** : production of radical species
- **Geometry phase** : DNA geometry modelling
- **Biological phase** : prediction of DNA damages (double strand breaks – fatal lesions -, DNA fragments...) after irradiation
- Other materials than liquid water : DNA bases, Silicon for space applications...
- Run on Grids



Thank you for your attention