

# Experimental activities of cellular irradiation at CENBG

Sebastien Incerti

representing the research of the  
Interface Physics – Biology group

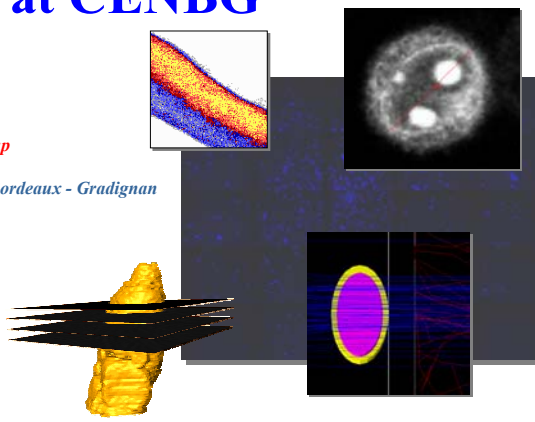
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CERN, Geneva  
July 26<sup>th</sup>, 2004



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# The CENBG lab



## Mixed Research Unit

- 18 researchers IN2P3 / CNRS
- 17 teacher-researchers Université Bordeaux 1
- 15 PhD students and post-docs
- 37 administrative and technical staff
- 2 technical staff ADERA for the ARCANÉ technology transfer cell

Univers, Noyaux et constituants élémentaires
Le laboratoire participe au développement de savoirs dans les domaines allant de l'infiniment petit jusqu'à l'infiniment grand : <ul style="list-style-type: none"> <li>- la stabilité et la cohésion nucléaire</li> <li>- les particules cosmiques de grandes énergies</li> <li>- le neutrino, particule la plus abondante de l'univers</li> <li>- les nouveaux processus d'excitation des noyaux atomiques avec des lasers de puissance</li> <li>- l'élaboration de modèles pour expliquer la structure des noyaux et des hadrons</li> </ul>

Environnement et Energie Nucléaire
Thèmes développés : <ul style="list-style-type: none"> <li>- les mécanismes de réponse lors de l'exposition à des rayonnements ionisants à faible dose</li> <li>- le comportement de la barrière cutanée comme protection contre l'exposition chronique à des nanoparticules</li> <li>- les très faibles radioactivités appliquées à la datation du vin</li> <li>- la caractérisation et le dosage de poussières fines en suspension dans l'air</li> <li>- le devenir des déchets nucléaires produits par des réacteurs de fission</li> <li>- l'énergie nucléaire du futur</li> </ul>

Biomédical
<ul style="list-style-type: none"> <li>- Etude des mécanismes de signalisation de la mort cellulaire programmée (apoptose)</li> <li>- Modélisation de l'interaction avec ADN à l'aide d'un code de simulation en développement</li> <li>- Etude de la pénétration transcutanée et des effets délétères à long terme de nanoparticules</li> <li>- Développement d'une nanosonde, ligne de faisceau de l'accélérateur AIFIRA, pour l'analyse chimique à deux et trois dimensions à l'échelle subcellulaire de métaux traces et de métaux lourds cytotoxiques</li> </ul>

For more information ... <http://www.cenbg.in2p3.fr>

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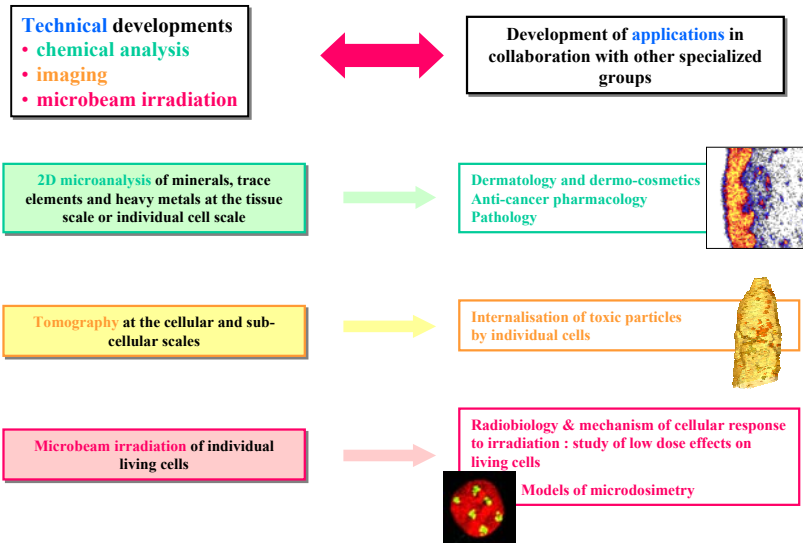
# The "Interface Physics Biology" group

12 Physicists & biologists

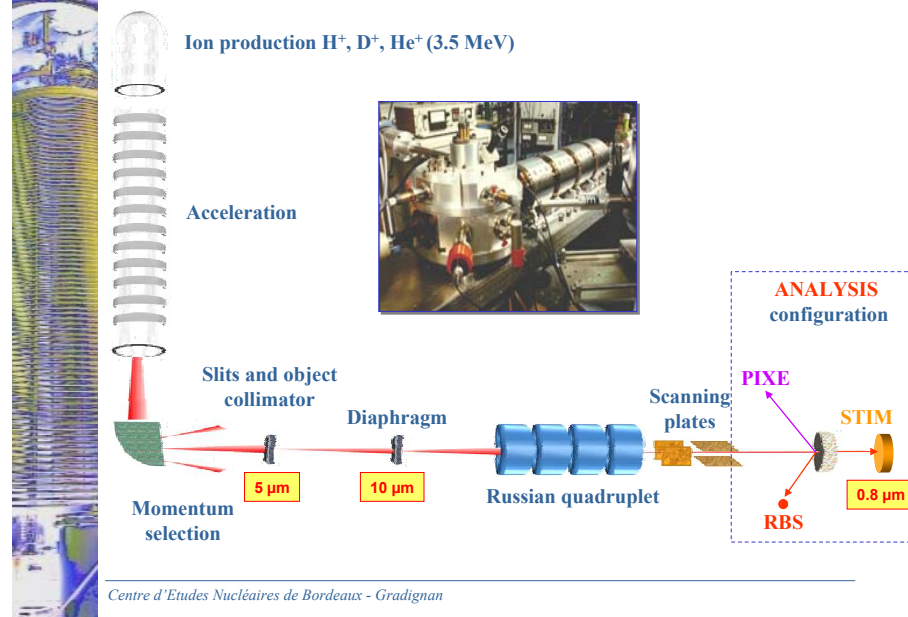
	<b>Philippe MORETTO</b> Professeur Université Bordeaux 1
	<b>Pierre AGUER</b> Directeur de recherche CNRS / IN2P3
	<b>Claire HABCHI</b> Maître de conférences Université Bordeaux 1
	<b>Sébastien INCERTI</b> Chargé de recherche CNRS / IN2P3
	<b>Philippe BARBERET</b> ATER Université Bordeaux 1 (2003 – 2004)
	<b>Yves BARBOTTEAU</b> Post-doc Pierre Fabre laboratory (2004 – 2005)
	<b>Etienne GONTIER</b> Post-doc CEE NANODERM (2002 – 2005)
	<b>Markus HEISS</b> Post-doc CEE CELLION (2004 – 2005)
	<b>Richard SMITH</b> Post-doc CNRS (2003 – 2004)
	<b>Maria-Dolores YNSA</b> Post-doc Spanish Ministry of Research (2003 – 2004)
	<b>Duy Thuy NGUYEN</b> Doctorant French Ministry of foreign affairs (2003 – 2005)
	<b>Thomas POUTHIER</b> Doctorant BDI CNRS / COGEMA (2003 – 2006)

Studies of the consequences of  
**environmental stress at the  
biological tissue and cell scales**

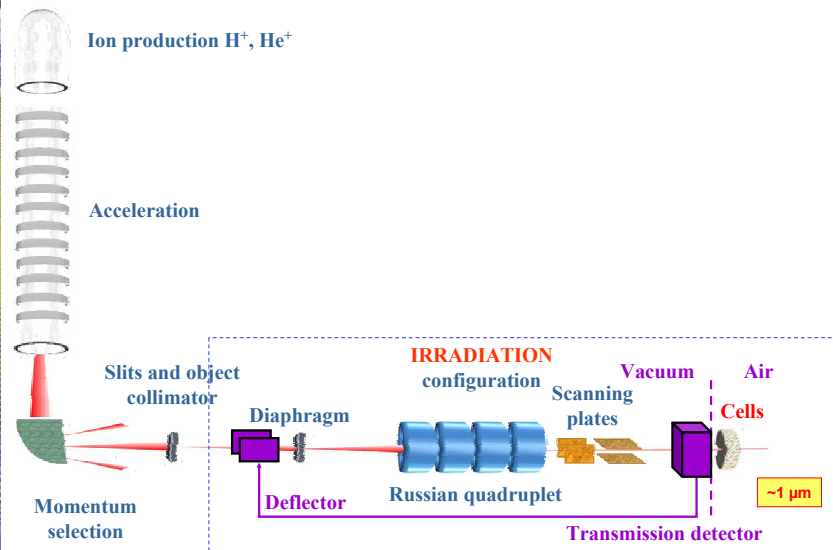
## Three research axes



## The microbeam line : ANALYSIS configuration



## The microbeam line : IRRADIATION configuration



A better understanding of the **risk** to human health caused by **low doses** of **ionising radiation**

## From high doses to low doses

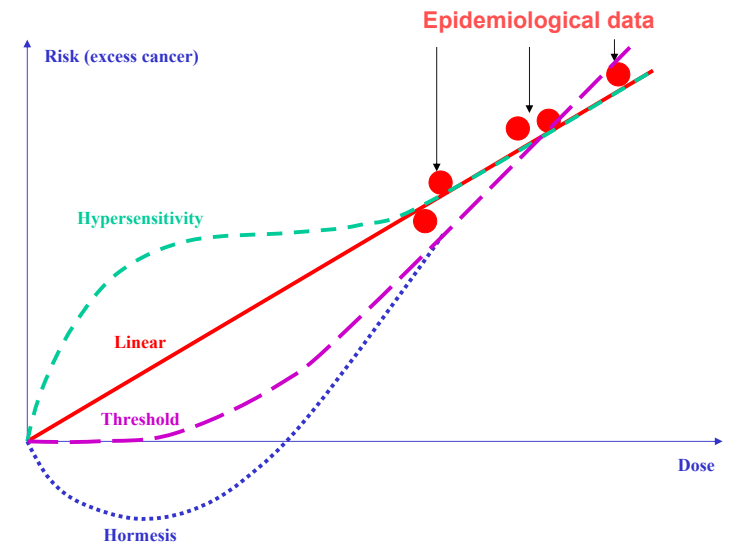
Estimates of radiation risk at low doses (environmental and natural exposures, typically < 0.2 Sievert) are generally derived from epidemiological studies of human populations who have been exposed to high doses of radiation.

For exposures to weak doses, where a true risk has been neither proven nor disproven, the probability of developing stochastic<sup>\*</sup> effects is, by agreement, considered as being proportional with the dose received.

The quantification of the relation between the dose and the effect is, in this context, established by **extrapolating** from what has been observed with higher doses down to the levels that apply in most environmental and natural exposures.

\* : random and deferred in time

## Low dose extrapolation : 4 hypothesis



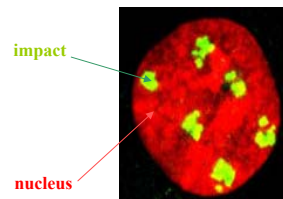
## Unique features of single particle microbeams

A single particle microbeam delivers a **predetermined exact number** of charged particles through a particular cell and makes it possible to choose whether the particles traverse the **nucleus or the cytoplasm**.

**Adherent cells** cultured as a monolayer on thin polymer foils and maintained in their culture medium are irradiated. The cell nuclei or any other subcellular target may be automatically identified on line using **fluorescence microscopy** associated with a **cell recognition system**.

The response of well-identified individual cells or their progeny may be **compared to non-irradiated cells** (by inspecting the cells after division).

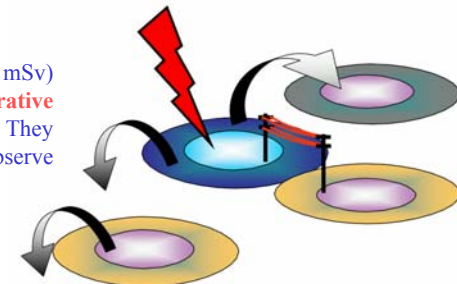
Damage repair may be located by **protein expressions sites** and **correlated** to particle tracks using fluorescence microscopy.

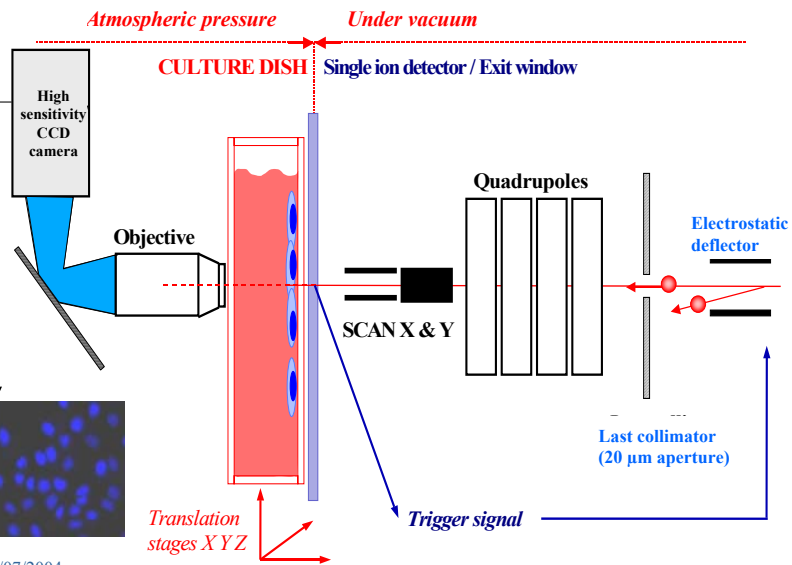


## Ionisation effects : non-targeted and delayed effects

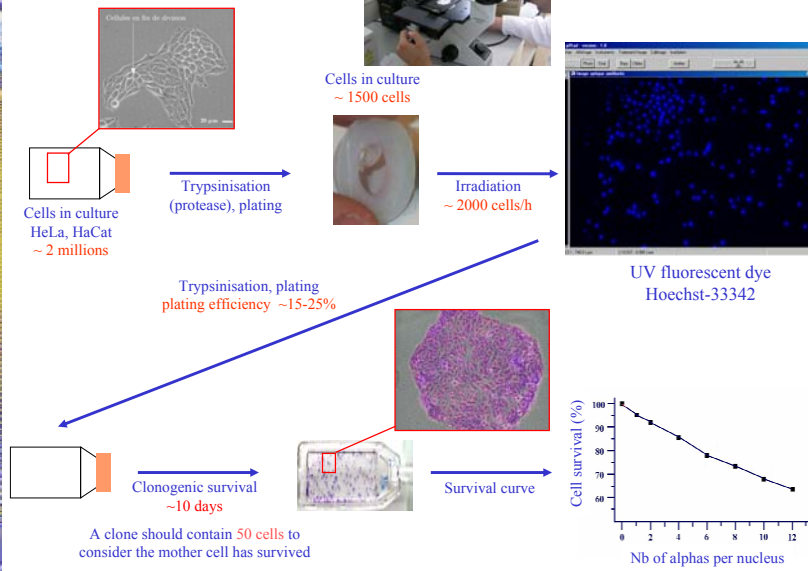
- **Bystander** or collective response
- **Adaptive** responses
- Genomic instability (delayed response on daughters)
- Low-dose hypersensitivity
- Gene induction (P53 increase, ...)
- Inverse dose-rate effect
- Cellular signalling (cytoplasmic targets)

Low dose irradiation (<200 mSv) could imply **mechanisms operative but hidden at higher doses**. They are extremely difficult to observe experimentally.





6/07/2004  
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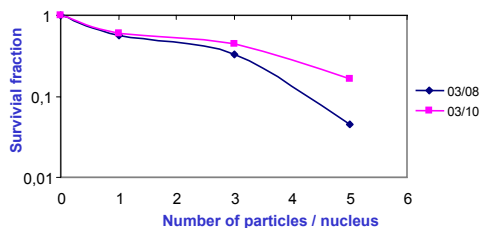
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First survival curve – March 2004

A preliminary survival curve for 3 MeV α ...

$$SF = \frac{\text{Number of clones}}{\text{Number of irradiated cells} \times \text{plating efficiency } PE}$$

$$PE = \frac{\text{Number of clones (non irradiated)}}{\text{Number of mother cells (non irradiated)}} \quad 15-25 \%$$



$$S(D) = e^{-\alpha x} = e^{-\frac{x}{D_0}}$$

$D_0$ : mean lethal dose  
(survival rate of 1/e @ 37%)

- $D_0$  extracted from fit close to 2-3 particles per nucleus
- in agreement with macrobeam estimation of  $D_0$
- in agreement with the literature

Need for a reliable simulation tool

WHY A SIMULATION TOOL ?

Technical challenge : to deliver the beam ion by ion, in air, keeping a spatial resolution compatible with irradiation at the cell level, i.e. below 10 μm

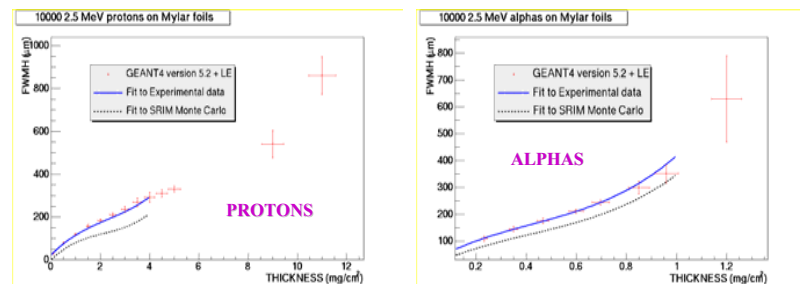
A simulation tool will help :

- understand and reduce **scattering along the beam line** as much as possible : collimator, diaphragm, residual beam pipe pressure...
- understand and reduce **scattering inside the irradiation chamber** : single ion detector, beam extraction into air, cell culture layer...
- predict **ion transport** (ray tracing) in the beam line magnetic elements to study image distortions due to intrinsic and parasitic aberrations
- **dosimetry** and comparison with **macrobeam irradiation** or **source irradiation**

with high flexibility and integration.



# Testing GEANT4 at the micrometric scale



- horizontal error bars : ± 5% experimental uncertainty on the foil thickness value
- vertical error bars combine statistical fluctuations obtained by varying the number of incident particles in the simulation and systematic fluctuations of the FWHM values due to the ± 5 % error on the foil thickness ; they range from 1% to 4% for protons and from 5% to 7% for alphas.
- ICRU\_R49p and ICRU\_R49He electronic stopping power tables used (G4hLowEnergyIonisation)
- Important issue on cuts :**
  - Default cutValue in PhysicsList.cc : **100 μm and above**
  - Max step length in target foil logic volume (UserLimits) in DetectorConstruction.cc : **foil thickness / 10**
  - low energy EM and standard packages give same results in the measured region of thickness

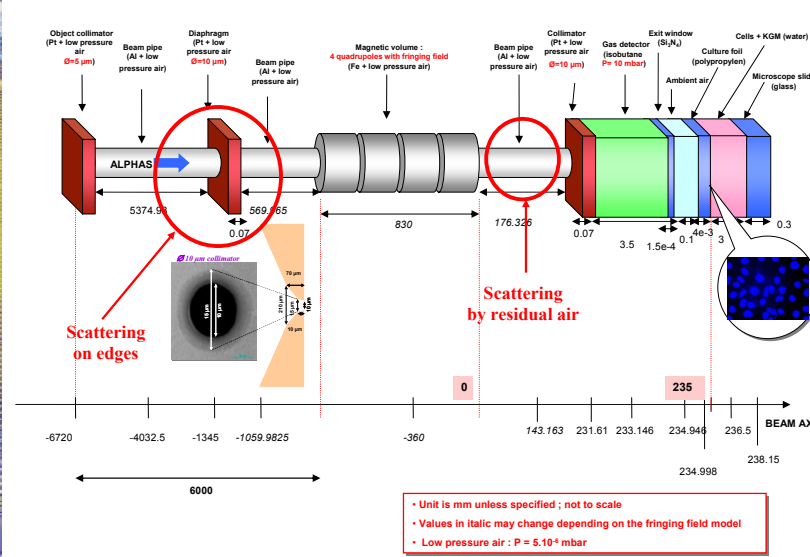
**Reference**

Simulation of ion propagation in the CENBG microbeam line using GEANT4  
 S. Inceri et al., Nucl. Instr. And Meth. B 210 (2003) 92-97

The MSC model used in GEANT4 belongs to the class of condensed simulations. The model is based on Lewis' MSC theory and uses model functions to determine the angular and spatial distributions after a step. The functions have been chosen in such a way as to give the same moments of the (angular and spatial) distributions as the Lewis theory.

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# The irradiation line setup defined in GEANT4

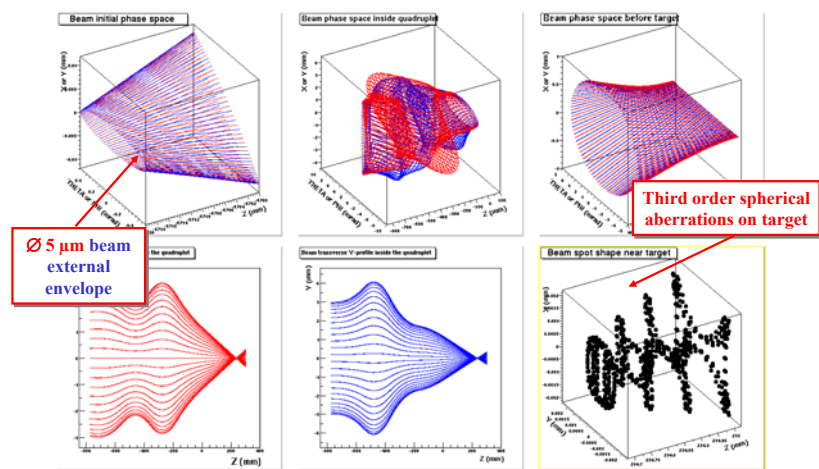


• Unit is mm unless specified ; not to scale  
 • Values in *italic* may change depending on the fringing field model  
 • Low pressure air : P = 5.10<sup>-4</sup> mbar

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# Beam Optics with GEANT4

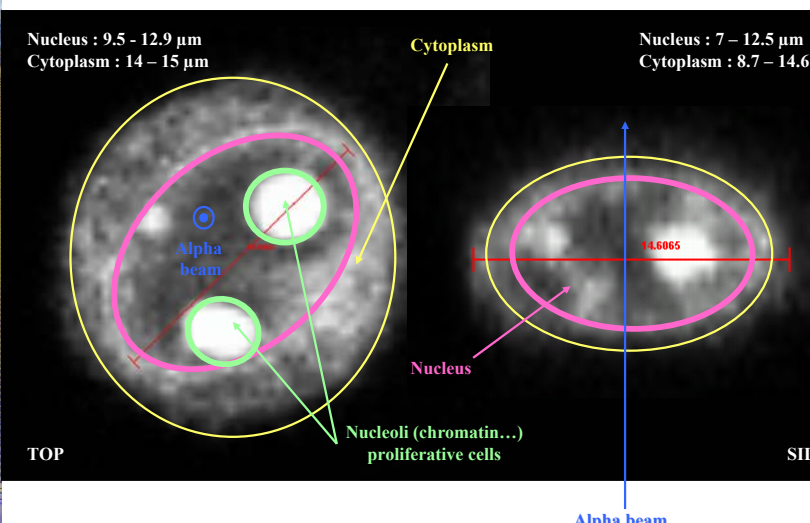
$$\frac{x(\sigma)^2 (\mu\text{m}^2)}{2.5^2} + \frac{q(\sigma)^2 (\text{mrad}^2)}{0.5^2} = 1$$

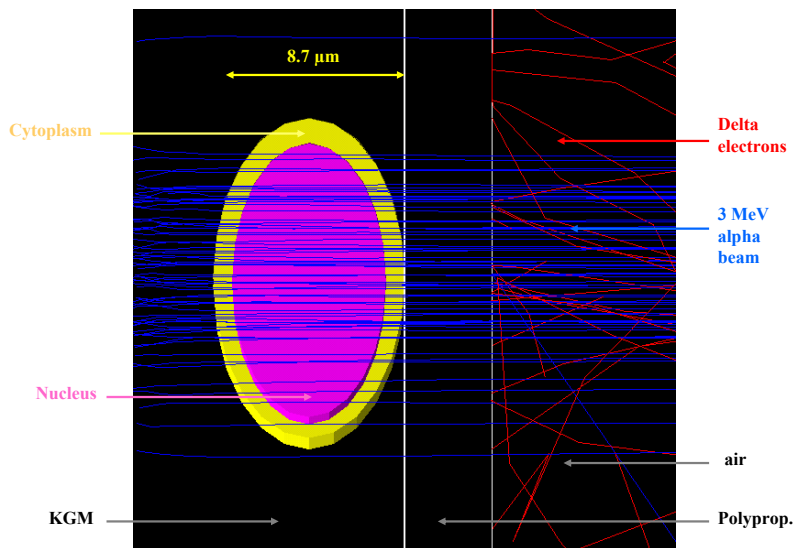


∅ 5 μm beam external envelope

Third order spherical aberrations on target

# HaCat cell modelling from confocal microscopy



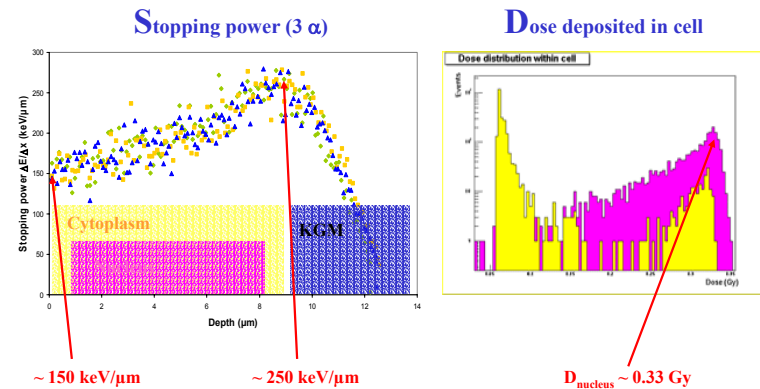


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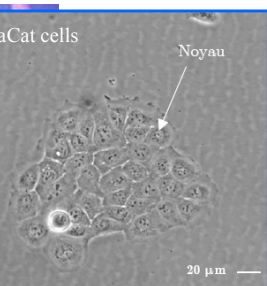
$$\langle T \rangle \pm s_T = 2.37 \pm 0.01 \text{ MeV on target}$$

Most of the energy loss occurs in the polypropylene foil (~18.4 %) and in the Si<sub>3</sub>N<sub>4</sub> window (~1.7%)



Deliver doses to cellular nuclei up to a few tenths of a Gray.

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## Infrared microscopic characteristics of radiation-induced damage

In collaboration with N. Gault, J.L. Lefaix, J.L. Poncey, CEA DSV, Fontenay aux Roses

**Fourier Transform Infrared Micro-spectroscopy (FT-IR)** : absorption spectrum sensitive to the biochemical changes in the various subcellular compartments (DNA, lipids, carbohydrates and proteins). These spectra provide pertinent information on molecular composition and conformational structure. It has been recently shown that FT-IR may be useful for assessing the process of apoptosis at the molecular level.

### Objectives

FT-IR study of radio-induced alterations in individual apoptotic cells after selective irradiation of cellular compartments with MeV alphas.

### Cells

**HaCaT**, permanent epithelial cell line obtained by spontaneous transformation of human adult keratinocytes.

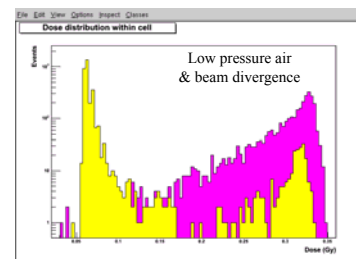
### Different stages

- Validation of the clonogenic survival curves by alpha irradiation of HaCat cell nuclei.
- Comparison of microbeam irradiation with classical alpha irradiation.
- Selective irradiation of nuclear compartments and FT-IR analysis of individual cells for different dose levels.

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## Comparison of micro- VS macro-beam irradiation

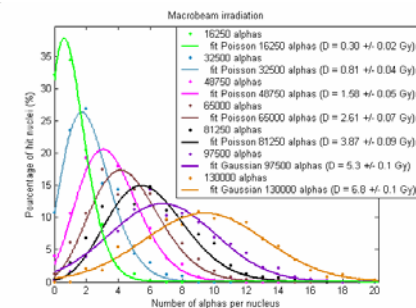
### Microbeam irradiation 1 alpha / nucleus



■ dose distribution in the nucleus  
■ dose distribution in the cytoplasm

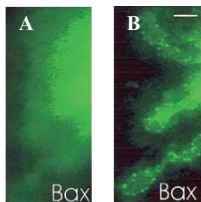
0.33 Gy most probable dose for exactly 1 alpha/nucleus (3 MeV, CENBG)

### Macrobeam irradiation



0.3 Gy average dose for an average of 1 alpha/nucleus (8 MeV, CEA-DPTA)



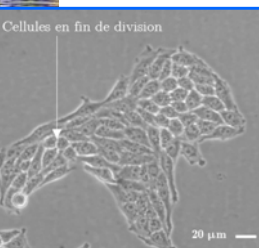


In collaboration with F. Ichas, European Institute for Chemistry and Biology, INSERM EMI-9929, University Bordeaux II

**Ionising radiations may induce nuclear, cytoplasmic signalisation :**  
Bax (pro-apoptotic) protein redistribution and formation of Bax clusters at the mitochondrial periphery as a function of time :  
15 min (A : diffuse), 6h30 (B : relocated)

## Objectives

- Characterization of **intracellular transduction** induced by irradiation using **dynamic fluorescent microscopy** performed on individual cells.
- Comparison of **nuclear and cytoplasmic irradiation**.



Cells  
HeLa cell line

## Method

Exploration of the mitochondrial molecular events by fluorescent protein (GFP - green, TRMR - red : tetramethylrhodamine methyl ester) in variable irradiation conditions with MeV alpha particles.

• **Protein relocation during apoptosis** : F. Ichas, IECB & INSERM E347, Université Victor Segalen - Bordeaux II

• **Bystander effect, adaptative response and FT-IR** with N. Gault, J.L. Lefaix, J.L. Poncy, CEA/DSV Fontenay-aux-Roses, using the **macrobeam** for cellular irradiation at CEA-DPTA (Bruyères-le-Châtel).

• **Marie Curie European network** in the framework of the 6<sup>th</sup> PCRD : **CELLION - studies on cellular response to targeted single ions using nanotechnology**, four years from 2004

• **European COoperation** in the field of Scientific and Technical research **COST Action P9 : RADAM Radiation DAMAGE in biomolecular systems**, 15 EU countries, four years from 2004, **WG5 : track structure in cells**.

**PARTRAC software** (Werner Friedland, Peter Jacob, Herwig G. Paretzke GSF-Institute of Radiation Protection)



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## To summarize : our activities with GEANT4

• **Validation** of GEANT4 simulations at micrometer and nanometer scales (actual microbeam & AIFIRA nanobeam line in 2005)

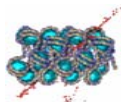
• **Beam optics** (ray tracing for the design of the new nanobeam line)

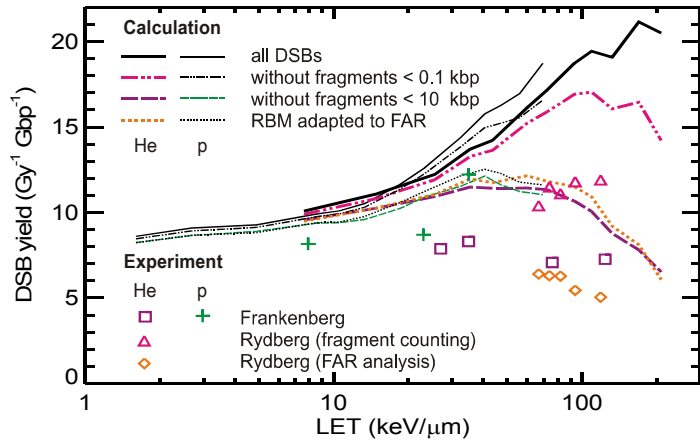
• Experimental **setup optimization** (scattering) and **validation of irradiation method** (micro- VS macro-beam and source irradiation)

• **Microdosimetry** for simple cellular geometries

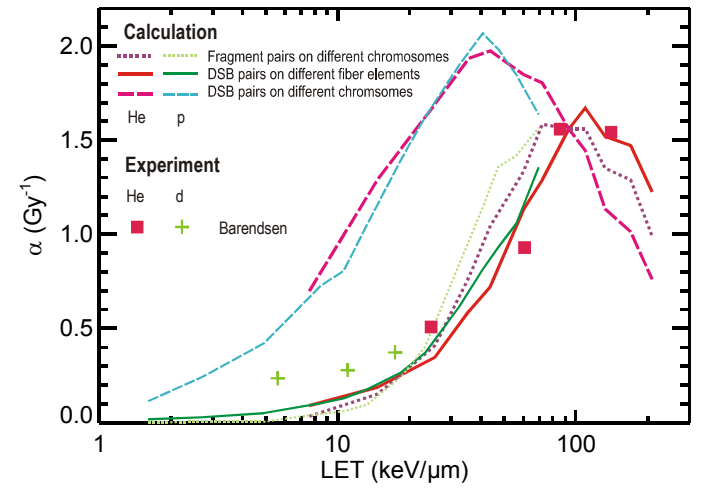
but we would like to go beyond and join the GEANT4 DNA project to quantify the damages (simulation/measurement of SSB and DSBs, ...)

**The CENBG and its experimental upcoming facility for irradiation at the nanometer scale – AIFIRA – will provide many opportunities to test G4 and its extensions at this scale**



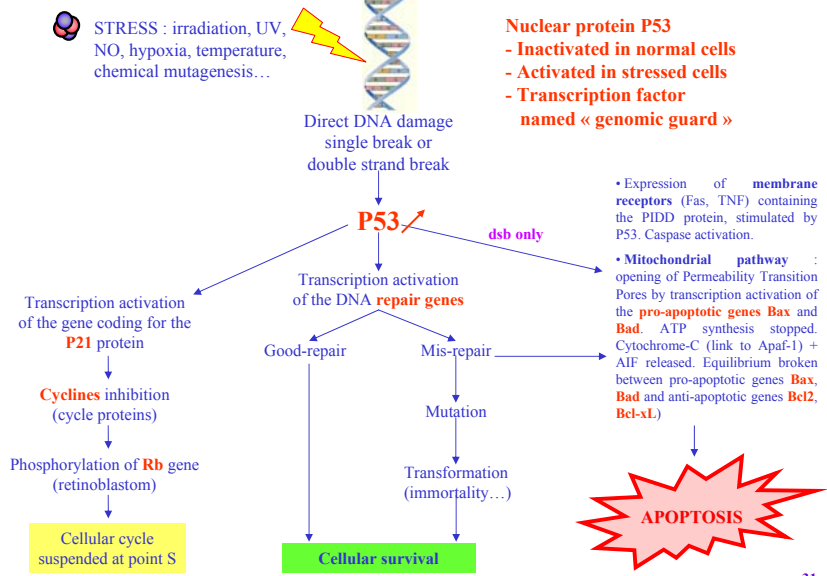


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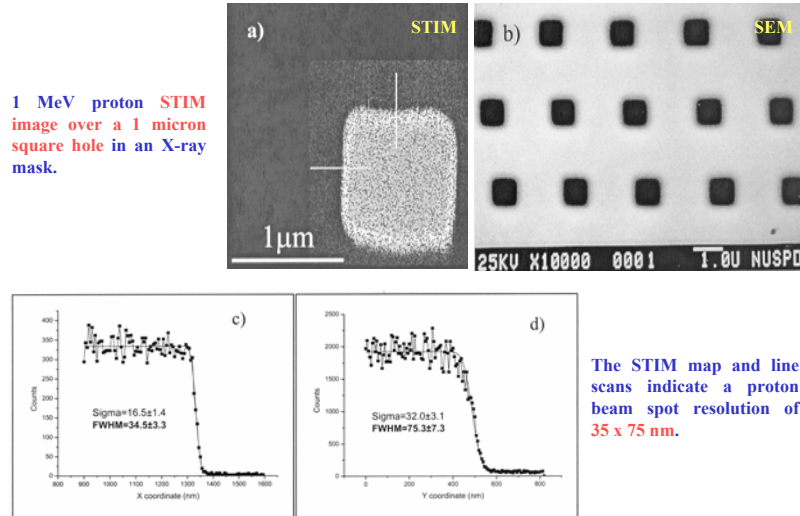
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**Ionisation effects : targeted effects standard model**



**AIFIRA : nanobeam resolution**

Resolution test using the Oxford Microbeam OM52 miniature lenses.





The first generation of microbeams were developed during the 90s :

- Gray Laboratory, **UK** (p,  $^3\text{He}$ )
- RARAF, Columbia University, New York, **USA** (p,  $\alpha$ )
- Texas A&M University, **USA** (p,  $\alpha$ )
- TIARA, JAERI, Takasaki, **Japan** (heavy ions)

These facilities are **collimated** beam lines :

- Irradiation rate limited by the **mechanical displacement** of the well in the beam
- Spatial resolution limited by beam **scattering** on collimator edges

Other lines are under development : INFN Legnaro, **Italy** ; MIT Boston, **USA**...

Other facilities are under development and use **focused** beam lines :

- The beam is quickly directed on target through electrostatic deflection
- Better spatial resolution

They are :

- GSI Darmstadt, **Germany** (heavy ions)
- PTB Braunschweig, **Germany** (p,  $\alpha$ )
- CENBG, **France** (p,  $\alpha$ )
  - first irradiation of cell line on October 30th, 2002
  - first survival curve on March 8th, 2004

A focused line is also under development at Columbia University, NY, **USA**.