Towards the characterization of single-cell molecular response to ionizing radiation

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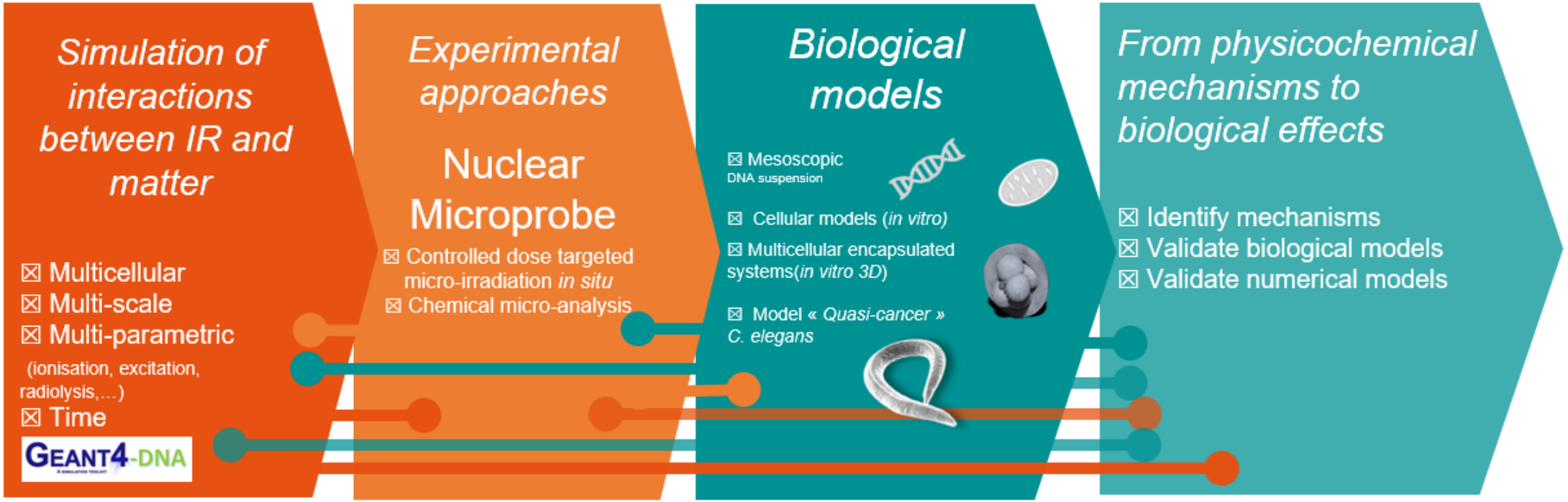
From the dose to the biological response

- The iRiBio team aims to study the interactions between **Ionizing Radiation** and **Living matter** through a combination of multiple approaches :



- The recent inclusion of **radiolysis** in Geant4-DNA enables the possibility of characterizing the full scope of radio-induced damage at the **cellular scale**

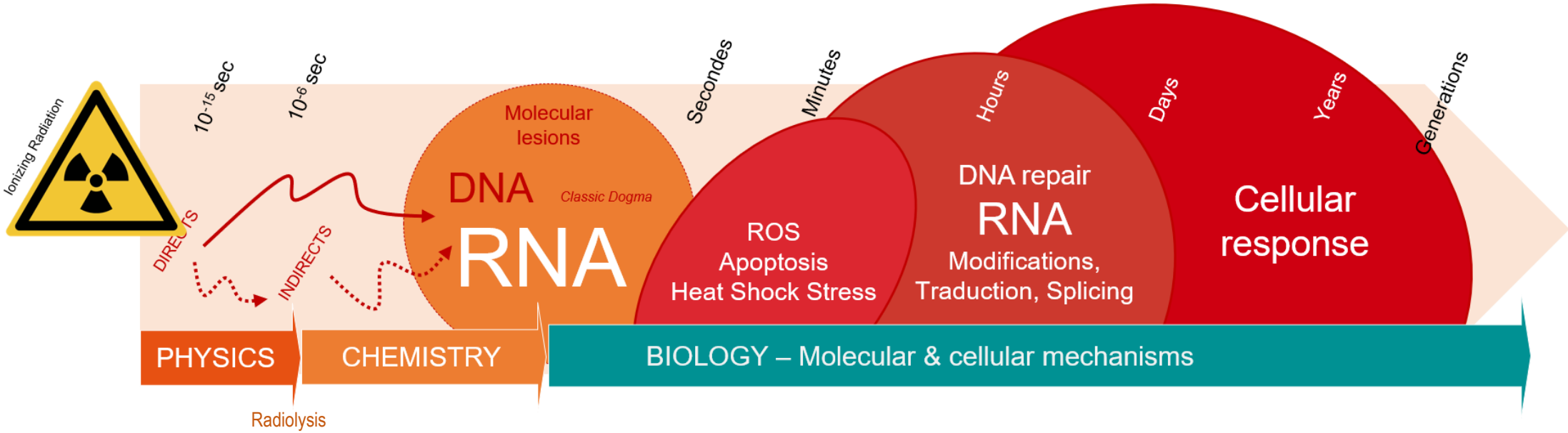
There is a need for a well-defined biological response to Ionizing Radiation to complement the simulations and models defined

Exploring outside the **classic dogma** of radiobiology

DNA is the focus of most radiobiology studies due to the important risks of non-repaired damage

- Permanent cellular damage/aging (non-replicating cells)
- Cell apoptosis
- Adoption of tumoral behavior (replicating cells)

Analysis of RNA molecules offers insight on the **molecular and cellular mechanisms** involved in the **Ionizing radiation - induced response**



Designing a **reproducible** single-cell irradiation pipeline

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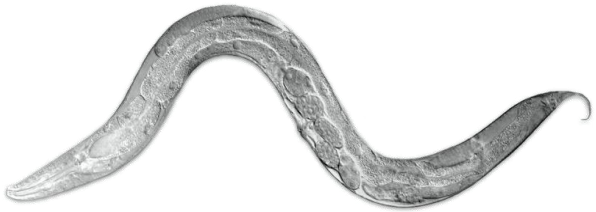
Irradiation method

Microbeam line connected to a particle accelerator

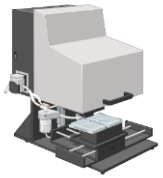
In vivo model



Caenorhabditis elegans, a well-defined popular model in biological studies

Analysis methods

Confocal microscopy

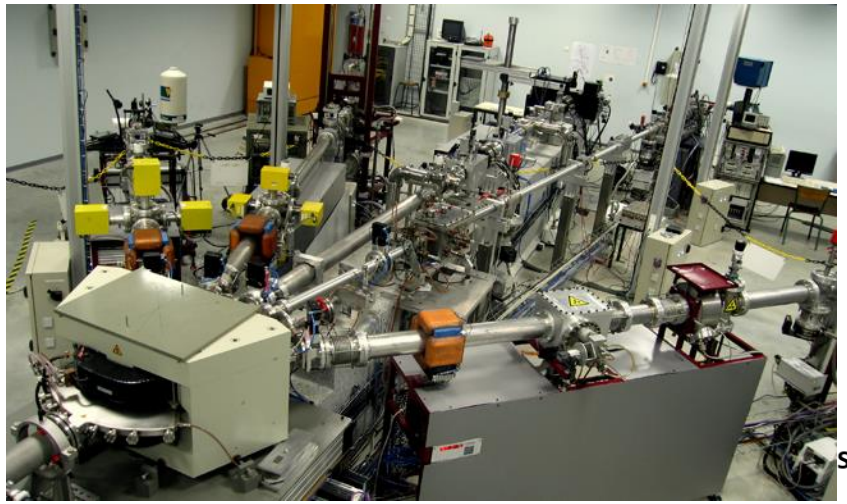


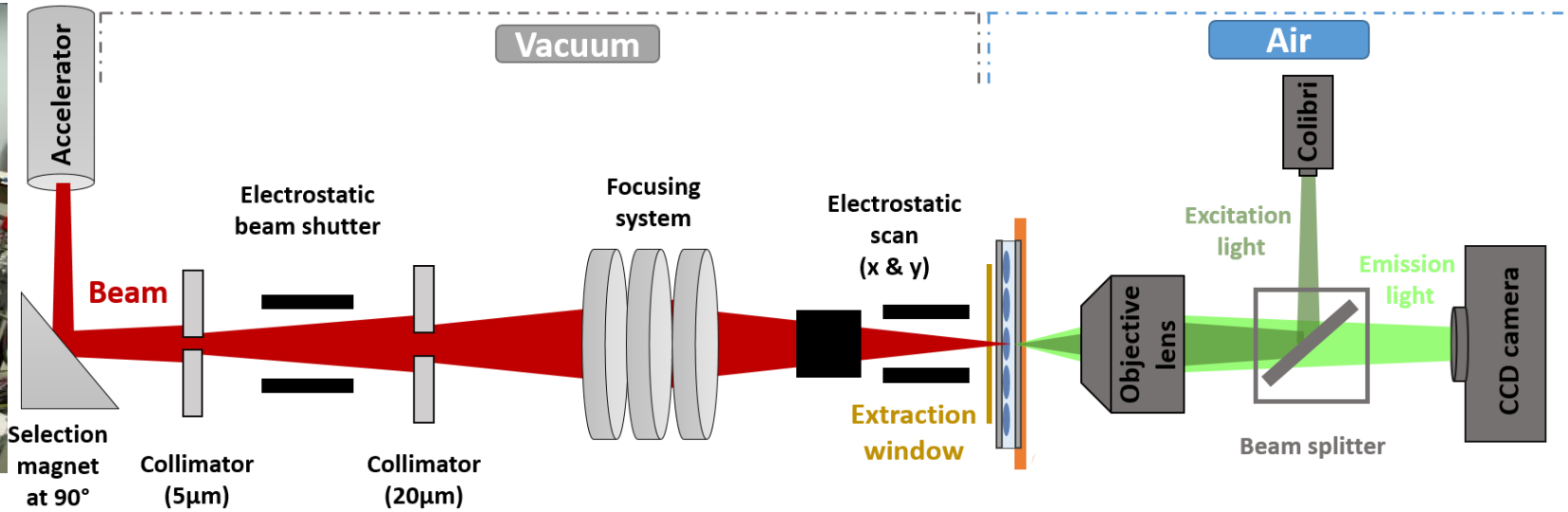
Flow cytometry

3rd generation sequencing

Targeting **selected cells** at micrometric precision

Nuclear **Microbeam** (AIFIRA facility, Gradignan)





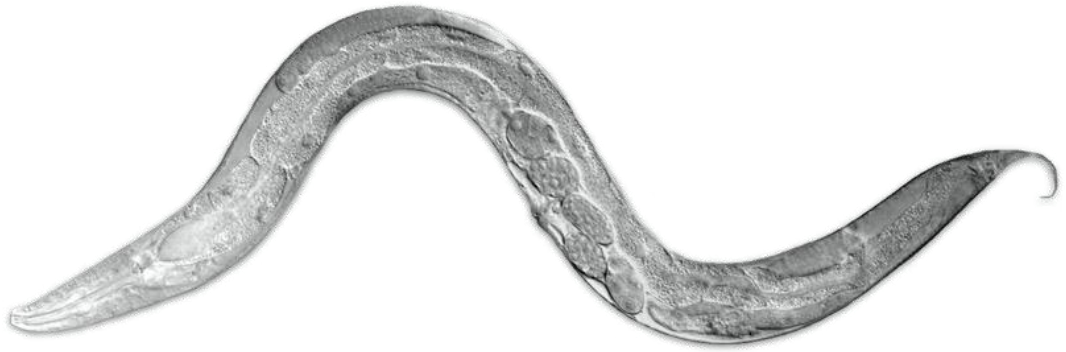
- Charged particles up to 3 MeV (protons, α particles)
- ~1µm spatial resolution
- Real-time fluorescence microscopy

An immobile and standardized model is required to work in association with this technique.

Barberet *et al* (2015), Muggiolu *et al* (2017), Torfeh *et al* (2019)

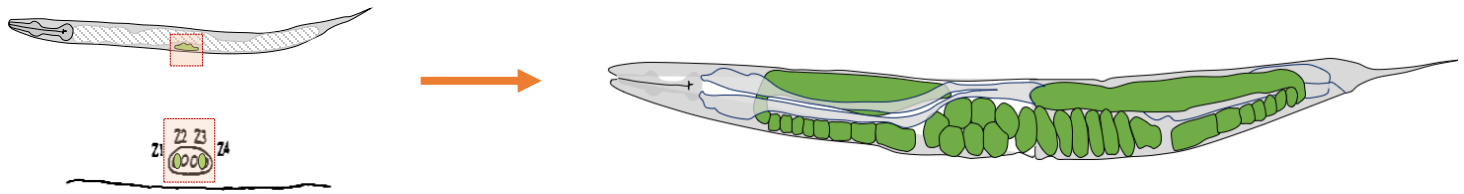
Caenorhabditis elegans

- Fast development ► Quick “long-term” impact
- Hermaphrodite autonomous reproduction ► No genetic mixing
- Reversibly **immobilizable** ► Precise targeting
- Synchronizable ► **Homogenous** populations of individuals



- Fluorescent reproductive system
- 2 fluorescent cells at L1 stage

GZ264 strain



L1 stage

Adult stage

Short-term and **long-term** response in a limited time frame

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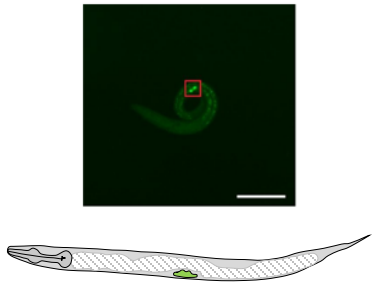
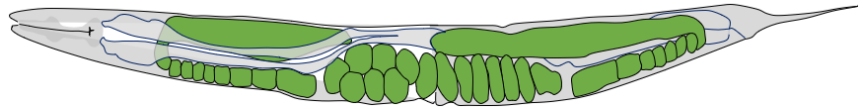




Irradiation
(3, 30, 300 Gy)

3 hours

Short term response

3rd generation sequencing

- Cellular pathways activated / inhibited
- Bases modifications

3 days

Long term response

Confocal microscopy

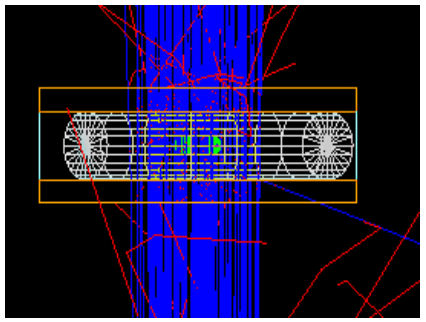
- Observable phenotypes of reproductive system

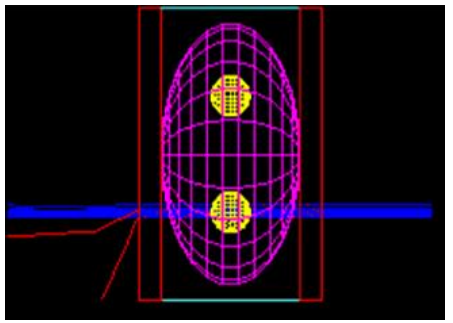
Flow cytometry

- Developmental delays
- Impact on reproductive system development

Determination of **delivered dose** using GEANT4 A SIMULATION TOOLKIT

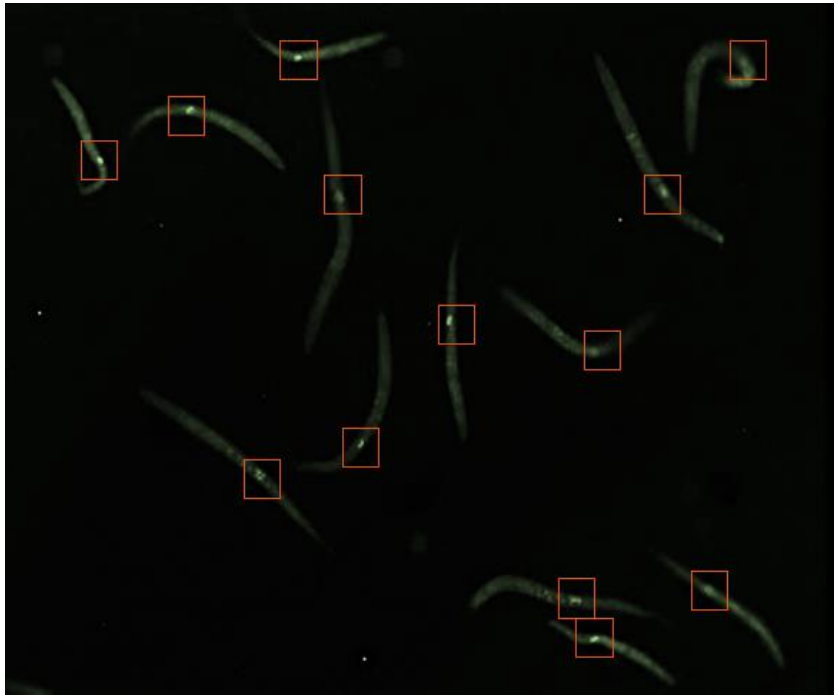
- Simulation of the Z1-Z4 cells region and its irradiation with protons
- Interaction probabilities between 3 MeV protons and targeted cells
- Calculation of average number of protons necessary to deliver intended dose (3, 30 and 300 Gy)





Proof of concept : a correct deposit of dose

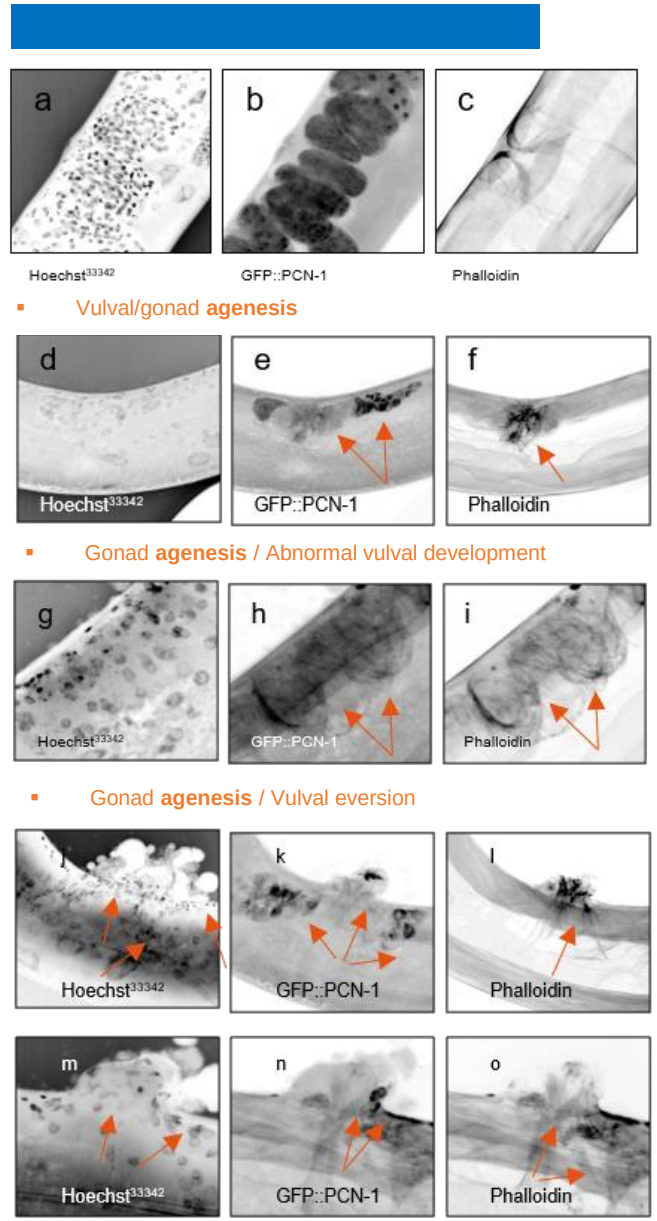
- Manual targeting of Z1-Z4 (synchronized L1 immobilized worms) and irradiation at 300 Gy



- Fluorescent marking for microscopy:
  - Hoechst : DNA
  - GFP : PCN-1 (reproductive system)
  - Phalloidin : actin (cellular structure)

- Presence of a vulval eversion phenotype previously described in mutagenesis studies (Seydoux et al, 1993)

Confocal microscopy



Quantifying **developmental delays**

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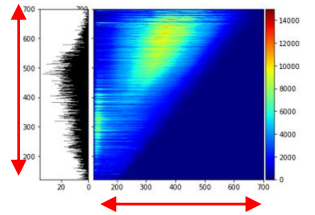


Flow cytometry

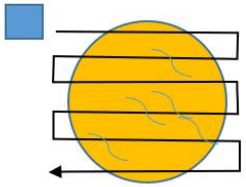
Organism-wide irradiations
Large populations
>2000 worms / expriment

Adapting our experiments to high-troughput techniques

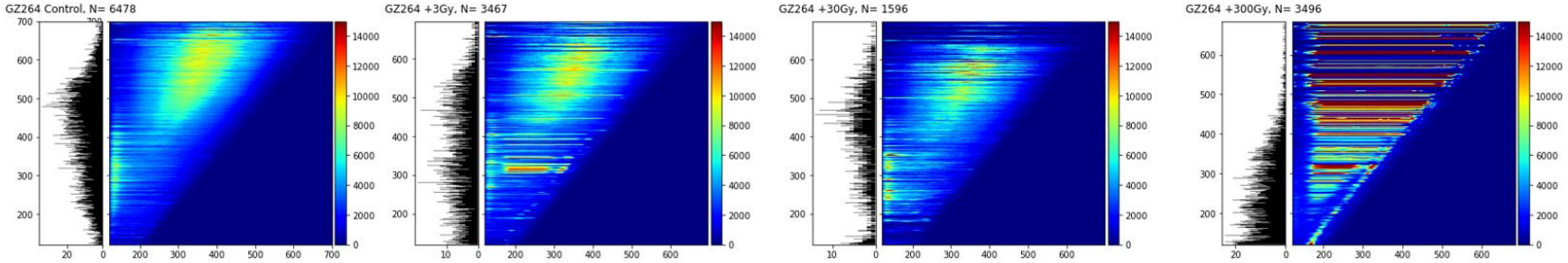
Reaching statistical significance

Cell-specific irradiations
Small populations
2-500 worms / experiment

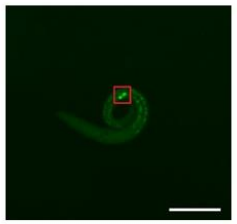
Non-targeted Micro-irradiation



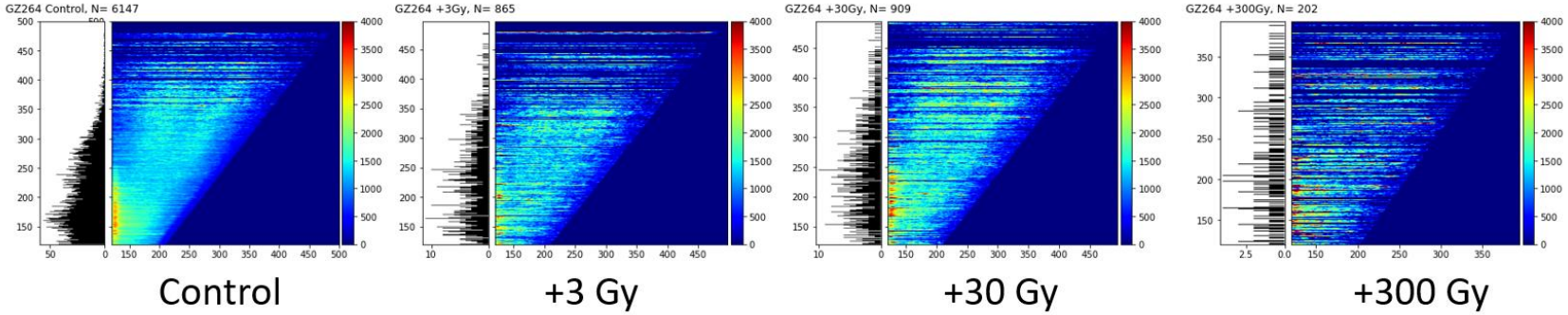
Protons 3 MeV
1 mm² + scan



Targeted Micro-irradiation



Protons 3 MeV
Diam = 4 µm
+ targeting
(20x20µm²)



Transcriptome analysis of **cellular expression**

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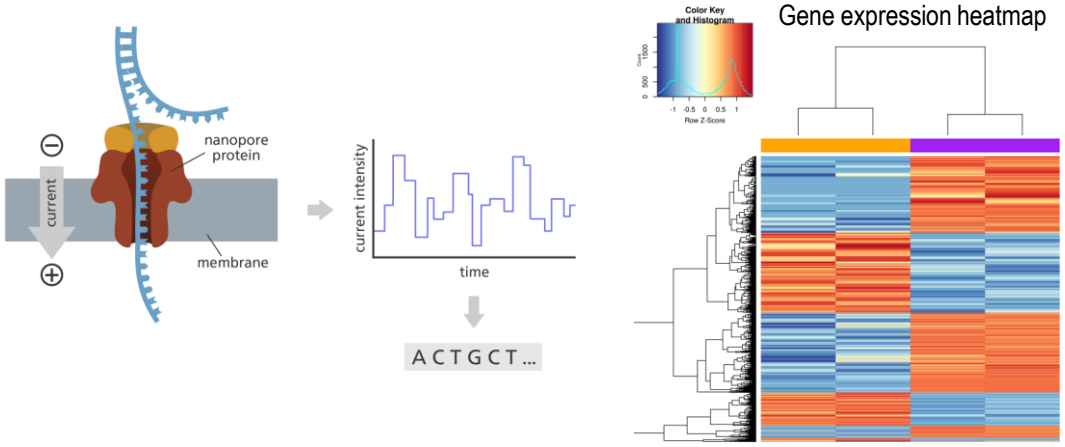
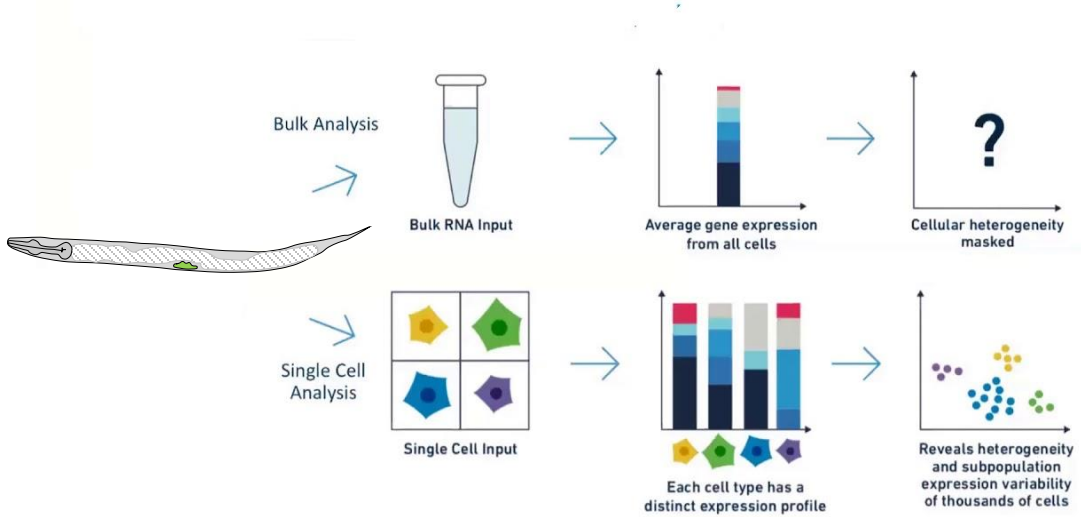
3rd generation sequencing (MinION)



- Direct-RNA sequencing
- Differential expression analysis / Base modifications (technology still in development)
- Sufficient amounts of RNA required
- Bioinformatics tools developed and tested on starved worms

Bulk / Single-cell sequencing



- Organism-wide response / Cell-specific response
- Non-targeted irradiation / Targeted irradiation

Summary of project progress

- A **reproducible** pipeline of selected cell irradiation on a **homogenous population** of **in vivo** worms has been setup and validated by direct observation of **radio-induced damage** on targeted regions.

- Results from non-targeted (organism-wide) irradiations resulting in **developmental delays** have proved our capacity to analyze our worms population in Flow cytometry

- Bioinformatics tools have been setup for transcriptome analysis

- Multiple samples are being produced in order to reach **sufficient quantities** for use in the **high-troughput** techniques of Flow cytometry and Transcriptome sequencing

Thank you for your attention !