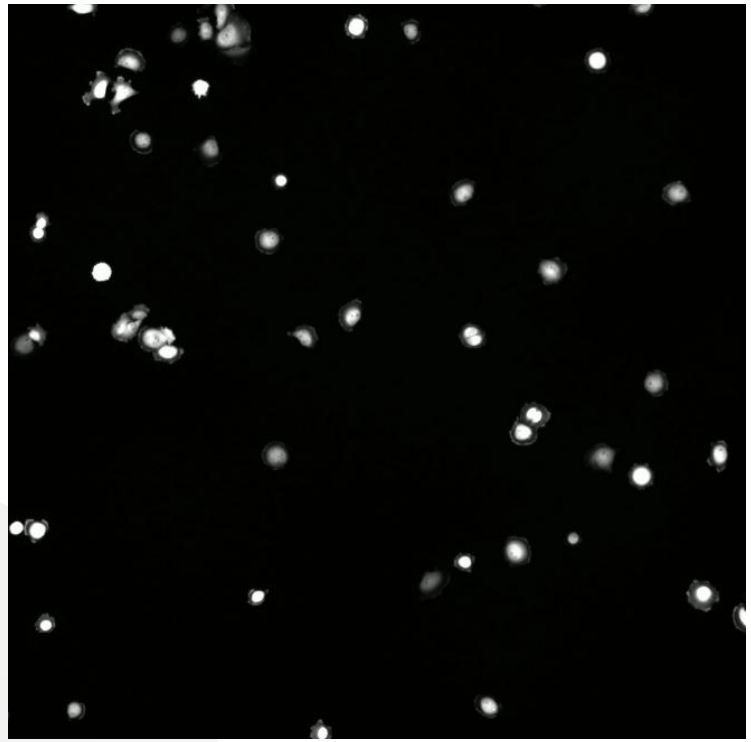
Videomicroscopic analysis of individual cellular dynamics induced by X-ray irradiation

1. Context: impact of irradiation on a cellular population *in vitro*

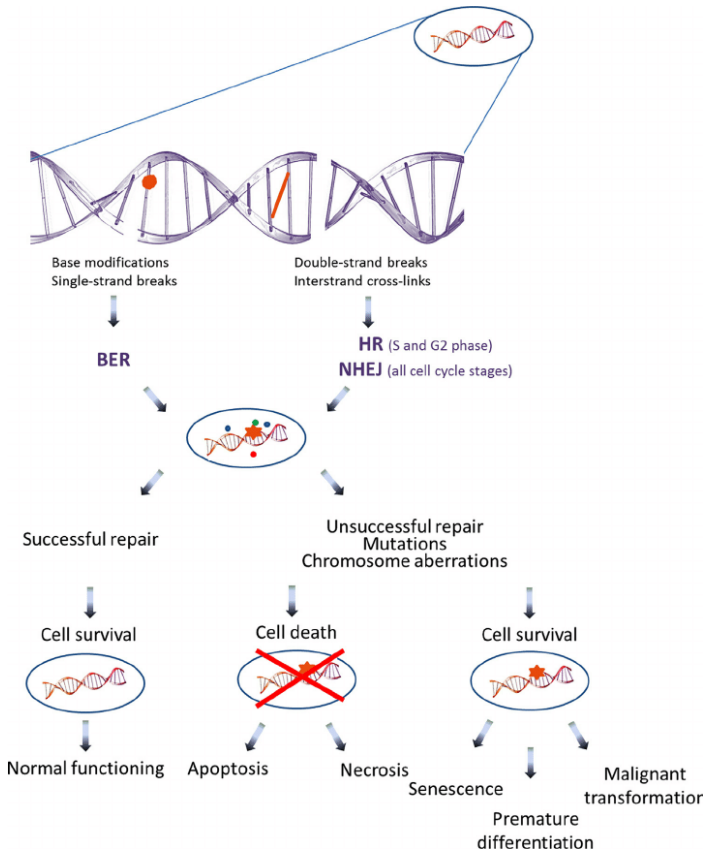
2. Methodology
   - Experimental protocol
   - Development of the tracking algorithm

3. Results
   - Preliminary results
   - Analytical perspectives



contact: josephine.courouble@ijclab.in2p3.fr

1. Context



Arena and all (2014)
Acta Astronautica 104. 419-431

Global analysis:

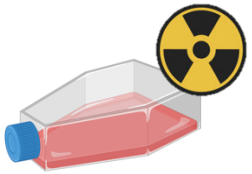
- Cell counting
- Confluence
- Clonogenic assays
- MTT assays
- …

Videomicroscopy aims:

- Single-cell tracking
- Time-lapse analysis
- Study of multiple parameters (cell division, behavior, etc.)

2. Experimental protocol

Cell model: MCF-7 **GFP**

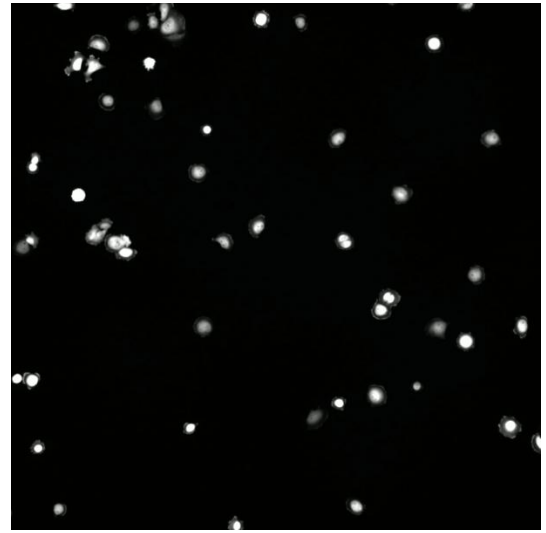


X-rays irradiation
320 keV
0-20 Gy

5.10^3 cells/mL

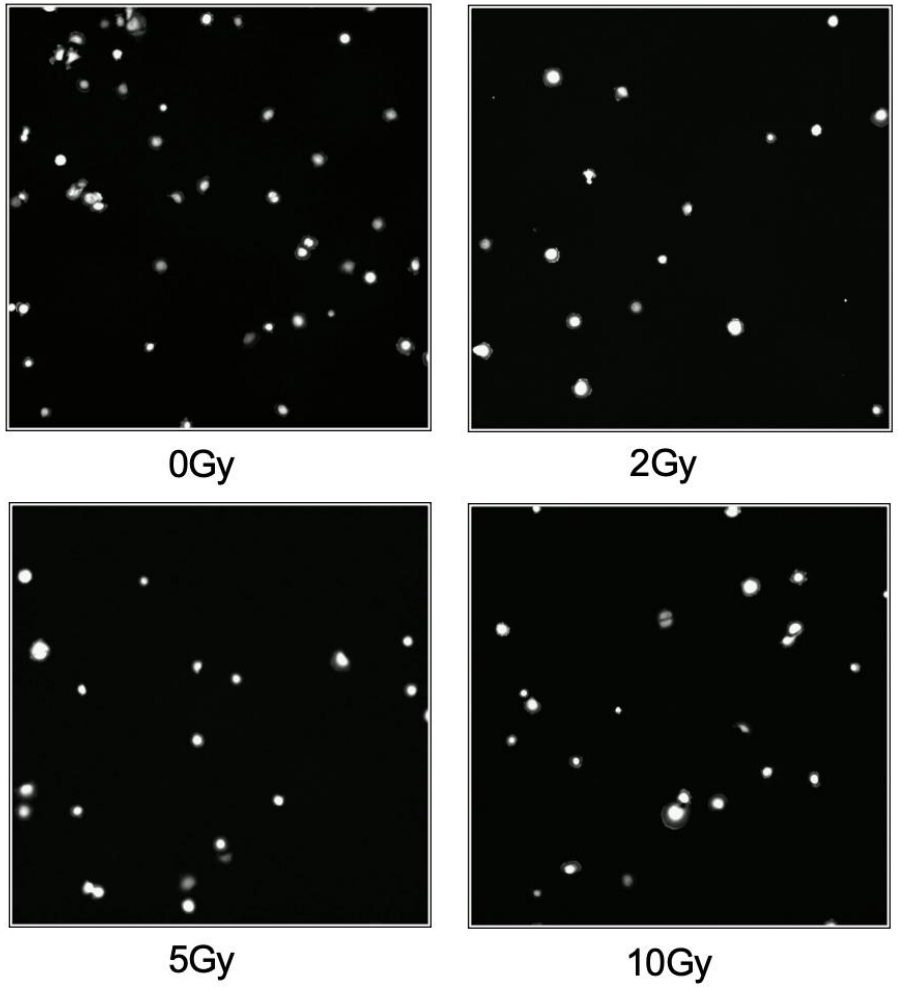
Inverted Microscope Nikon Eclipse TS2R
Camera Hamamatsu Orca Flash 4.0LT

4 to 6 days
1 photo/30min



1330µm

2. Development of the tracking algorithm



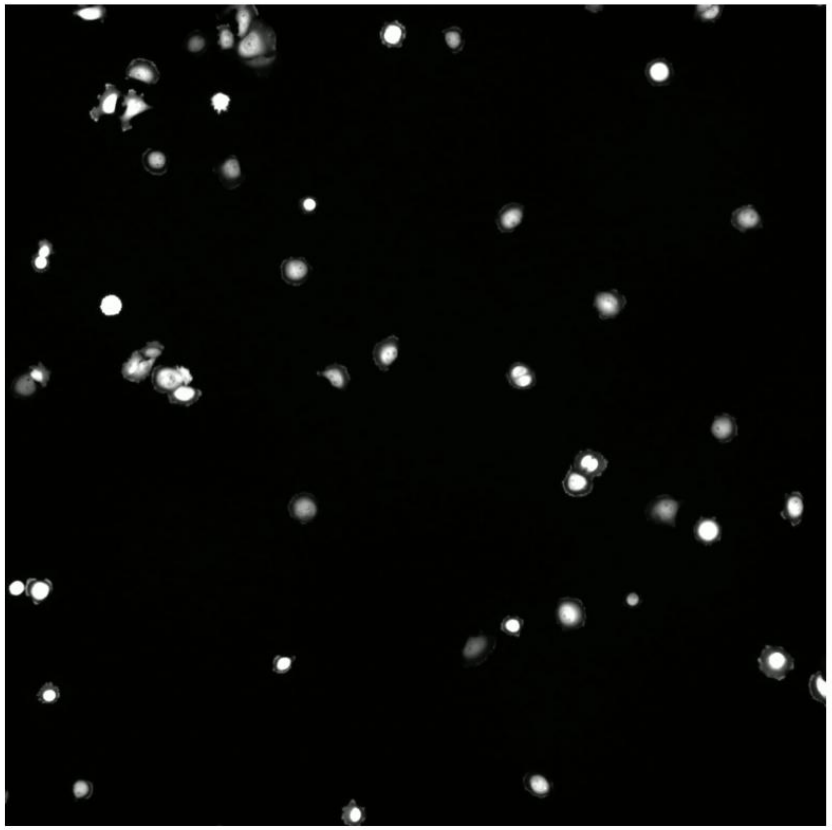
Main objectives:

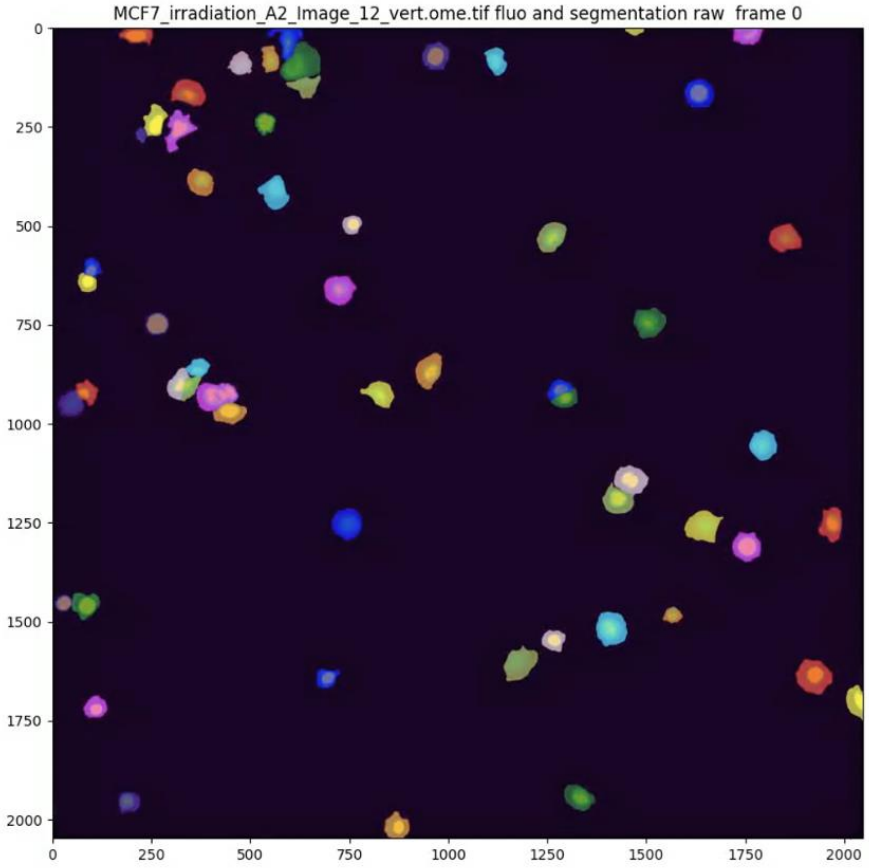
- Identify cells
- **Track** the cells over time
- Identify **mitosis**
- Define **key parameters** representative of individual cell behavior

3 scales of study:

- Cellular population
- Individual cell
- Cell lineage tree

2. Development of the tracking algorithm





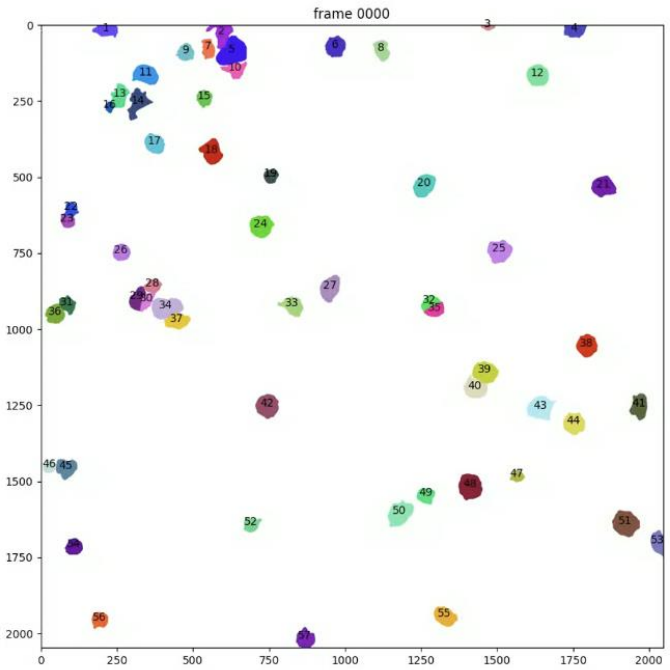
Step 1: Segmentation

Cellpose librairie:

- Train a model for each experimental condition
- Segment the cells

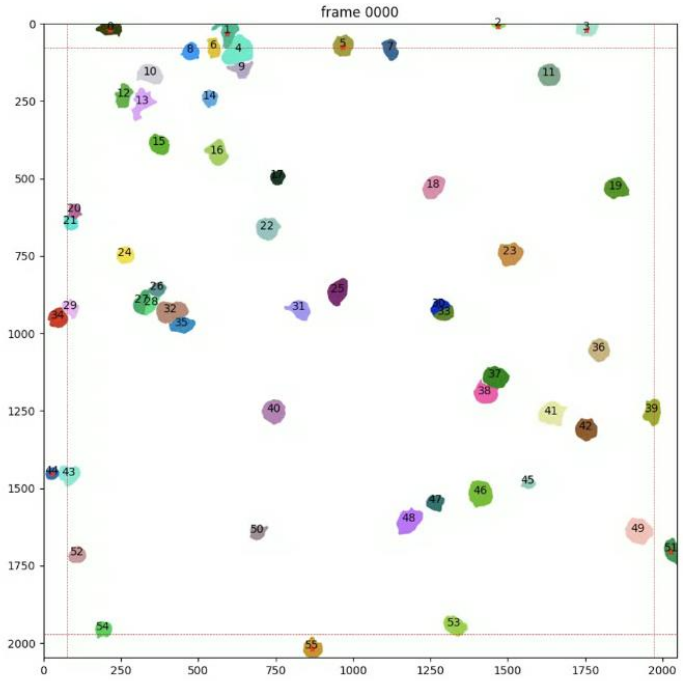
https://github.com/MouseLand/cellpose

2. Development of the tracking algorithm



Step 2: Tracking

- Correct Cellpose segmentation errors
- Assign a label to each cell
- Track the cells over time



Step 3: Linking

- Individual data
- Identify mitosis
- Characterize individual cell behavior

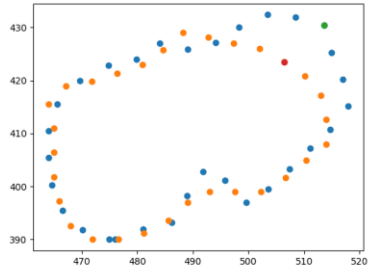
GitLab

https://gitlab.in2p3.fr/josephine.courouble/celltrack

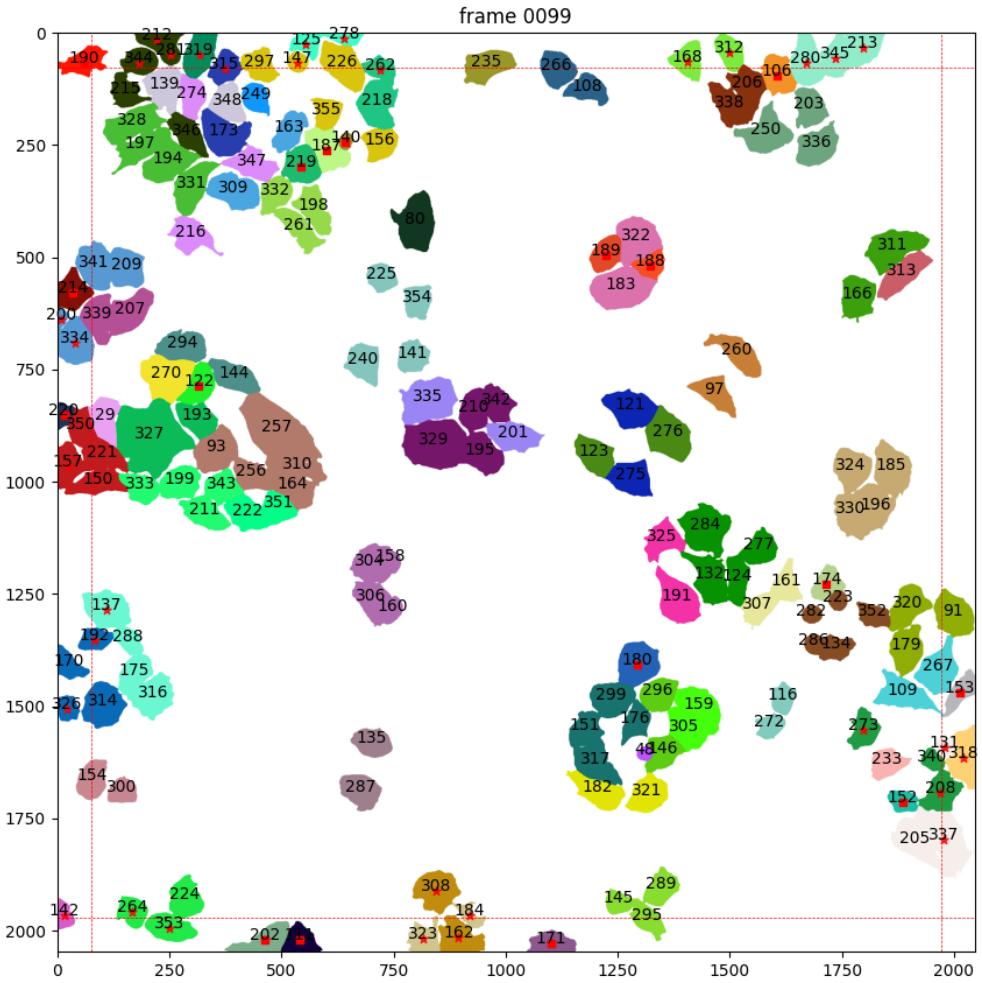
To track cells and cell divisions, a weight-based system is used:

- **distance**
- **contour** similarity
- (**time** since the last division)

A set of **cells matching** is generated: **minimizes total weight**



2. Development of the tracking algorithm



Individual cells data

- Assigned cell label
- Presence of the cell:
  - First/last frame
- Frame by frame individual data:
  - Position x/y
  - Surface
  - Fluorescence

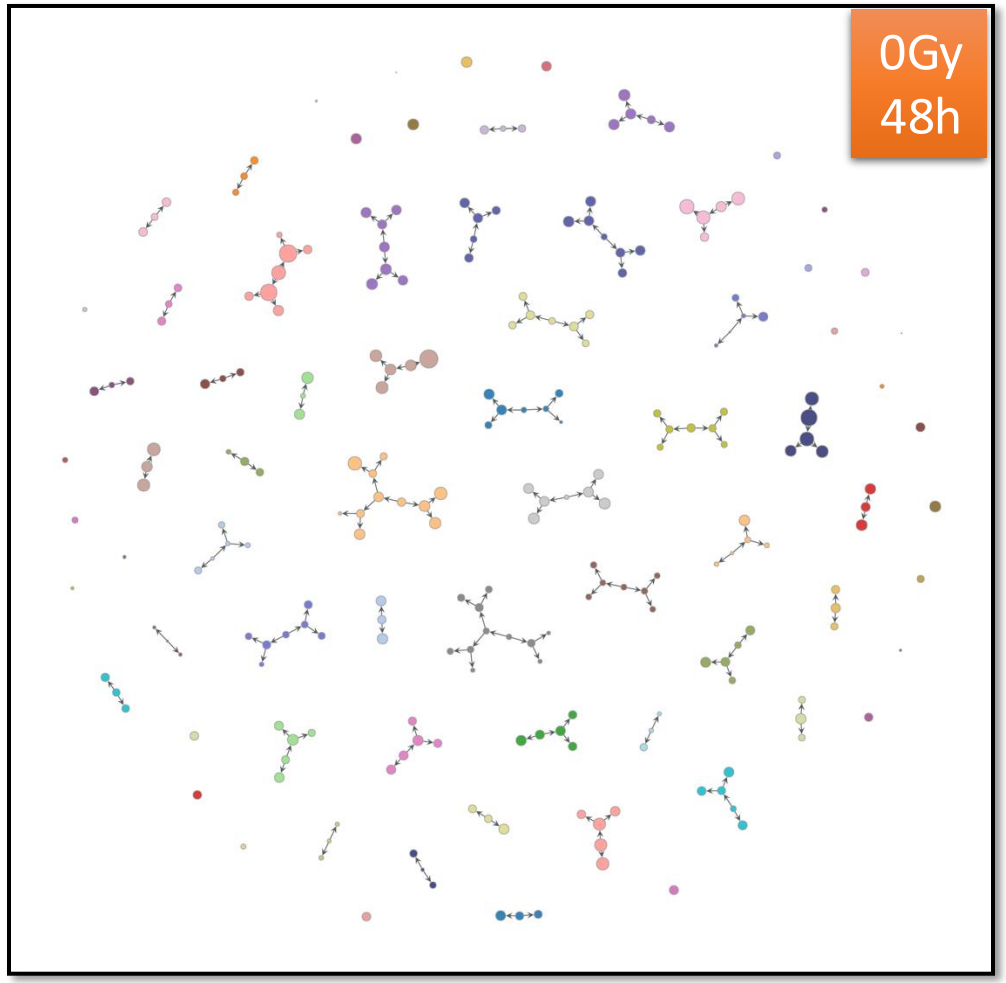
Data

- Cell appearance:
  - Initial
  - Mitosis
  - Spontaneous
- Cell disappearance:
  - End
  - Mitosis
  - Death
- Tracking reliability:
  - Edge appearance
  - Edge disappearance
  - Edge passage
  - Too short lifespan
  - Cell too recent

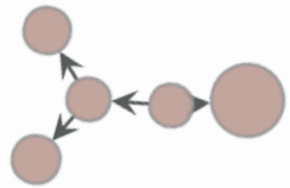
Flags

Control sample

Lineage tree scale

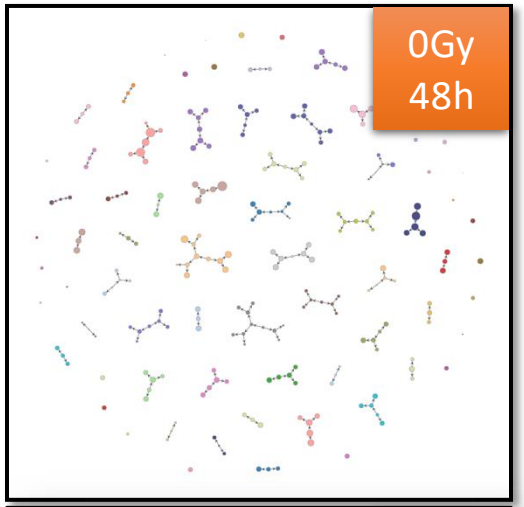


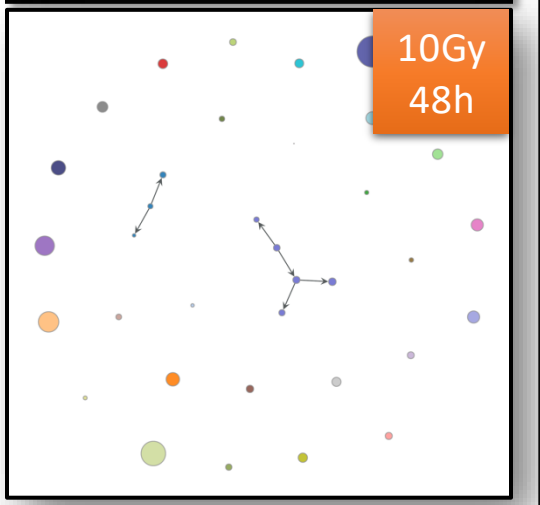
Cell representation by lineage tree

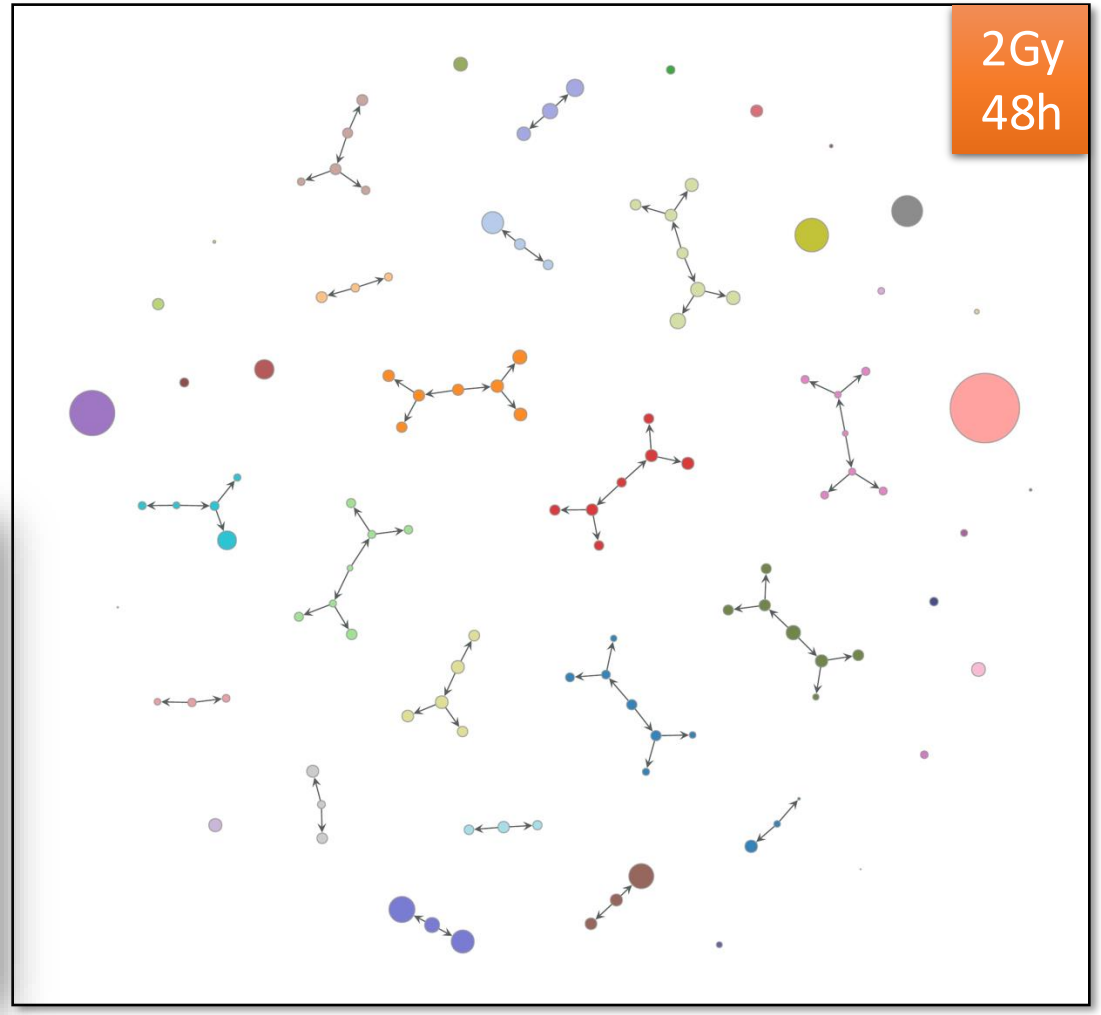


2Gy X-rays irradiation

Lineage tree scale

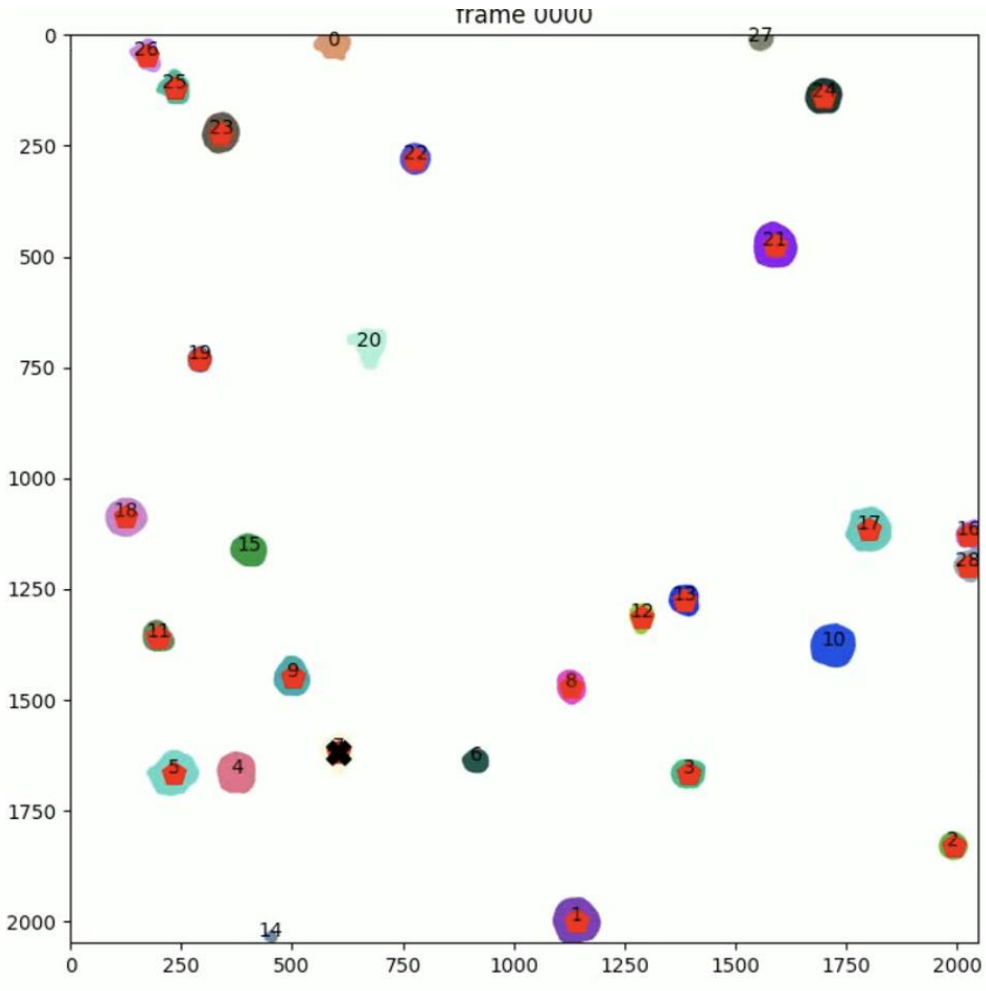






- Less cells
- Smaller trees
- More senescent cells

Categories

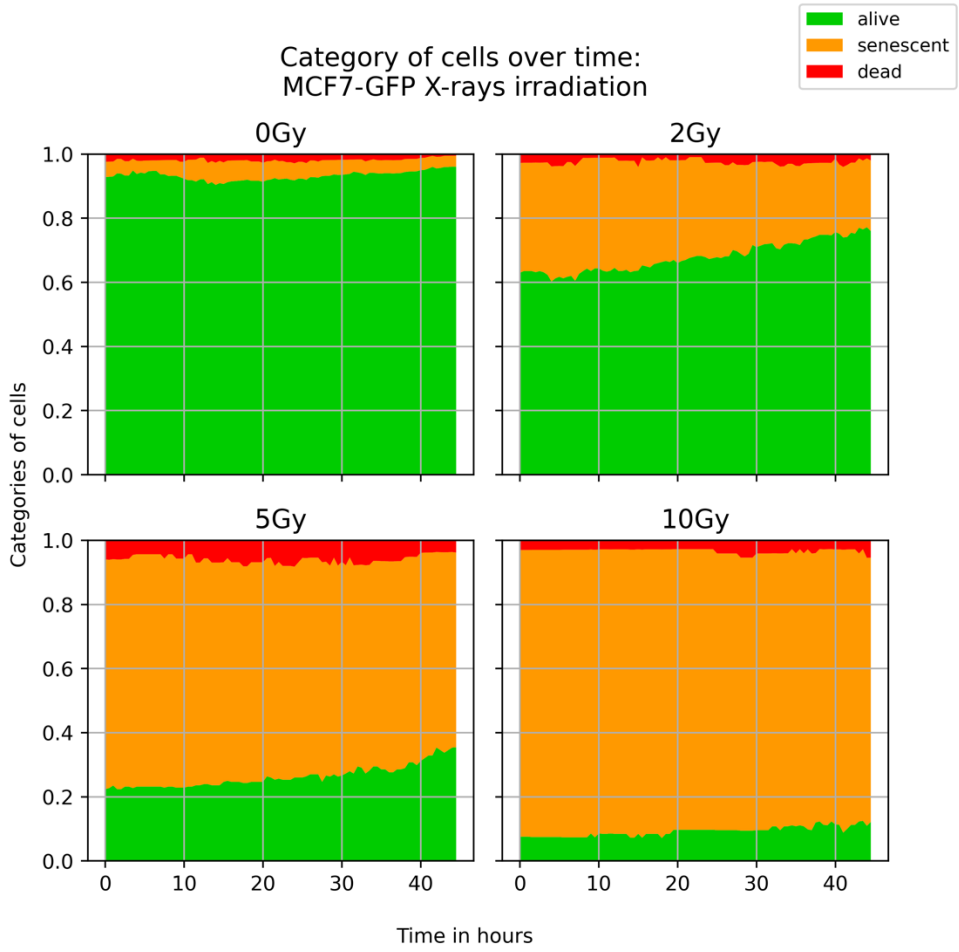


Classify cells into 3 categories:

- **Living** cells
- **Senescent** cells
- **Dead** cells

Categories evolution

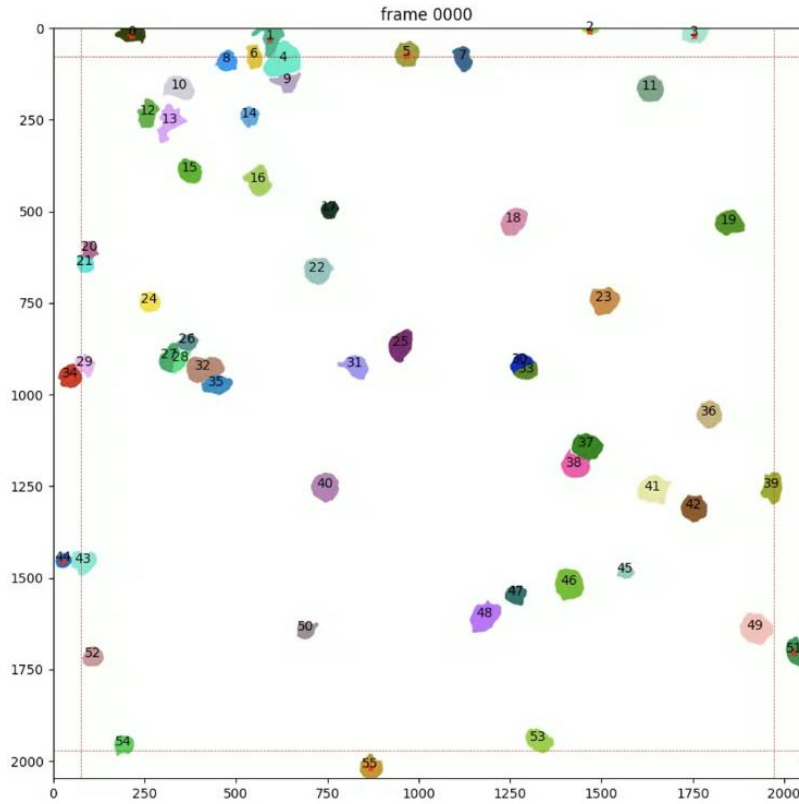
Individual scale



Conclusion

- **Algorithm tested** on real data, efficient tracking
- **Results show** irradiation impact on
  - cell population
  - lineage tree
  - individual cells

- **Future work:**
  - Analyse **lineage trees**
  - Define keys **parameters/correlations**
  - Investigation of **cell interactions:** Bystander effect
  - Explore **experimental conditions**: radio-amplifying nanoparticles



Thank you for your attention

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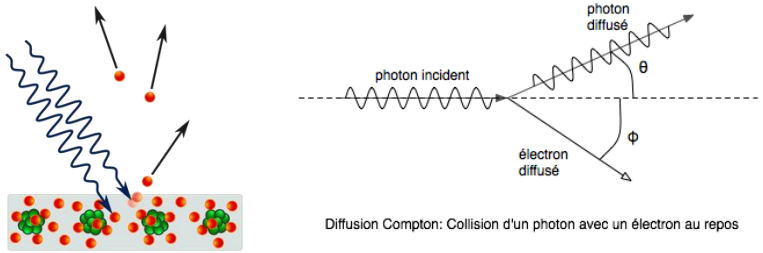
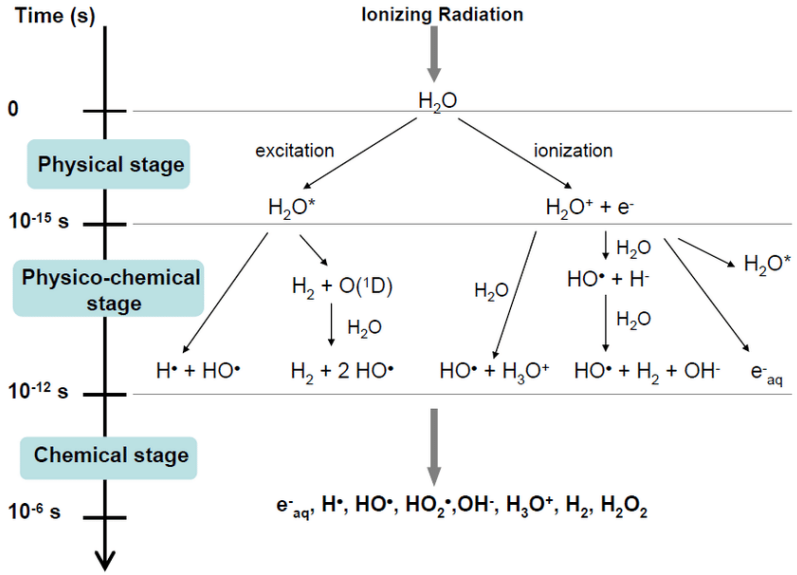
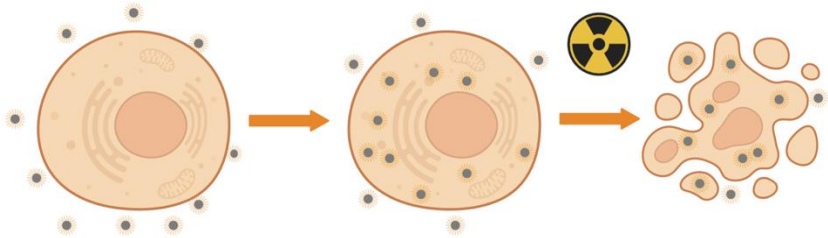
Back-up

Back-up



Diffusion Compton: Collision d'un photon avec un électron au repos



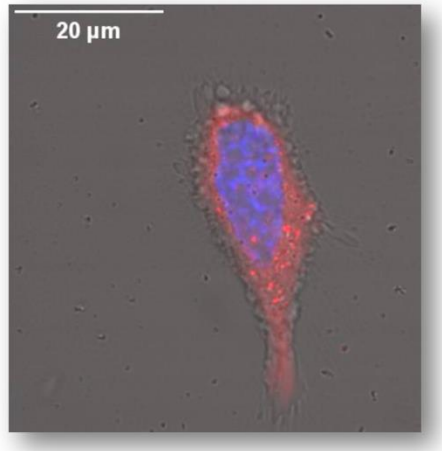


1) Exposition to NPs

2) Internalization of NPs within the cytoplasm

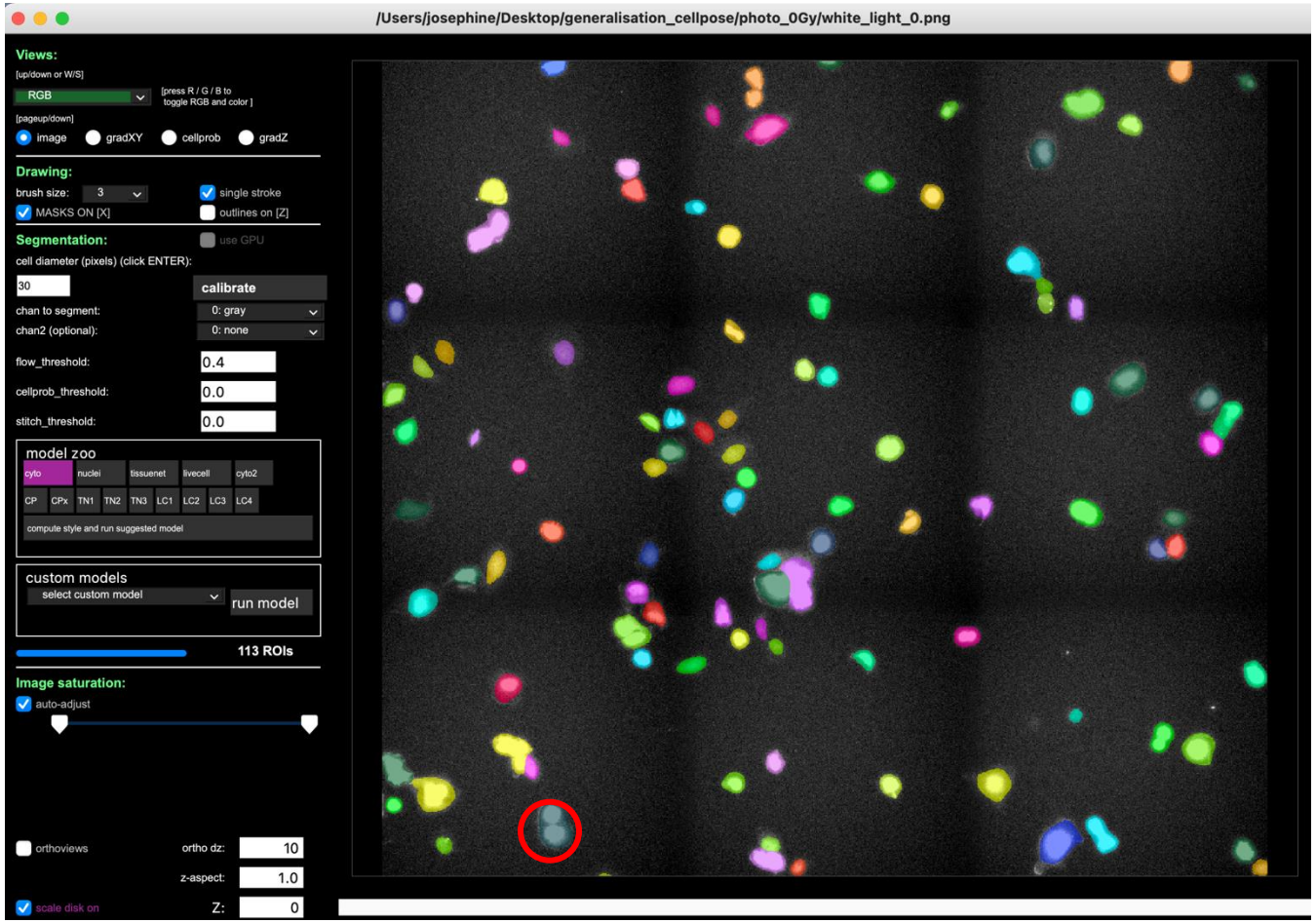
3) Irradiation

Schema of internalization of NPs and irradiation of a cancerous cell



Micrography of Hela cell exposed to NPs BiPt@$(NH_2)_2$-PEG marked with rhodamine

Back-up


/Users/josephine/Desktop/generalisation_cellpose/photo_0Gy/white_light_0.png
Views:
[up/down or W/S]
RGB
[press R / G / B to toggle RGB and color]
[pageup/down]
image
gradXY
cellprob
gradZ
Drawing:
brush size:
3
single stroke
MASKS ON [X]
outlines on [Z]
Segmentation:
use GPU
cell diameter (pixels) (click ENTER):
30
calibrate
chan to segment:
0: gray
chan2 (optional):
0: none
flow_threshold:
0.4
cellprob_threshold:
0.0
stitch_threshold:
0.0
model zoo
cyto
nuclei
tissuenet
livecell
cyto2
CP
CPx
TN1
TN2
TN3
LC1
LC2
LC3
LC4
compute style and run suggested model
custom models
select custom model
run model
113 ROIs
Image saturation:
auto-adjust
orthoviews
ortho dz:
10
z-aspect:
1.0
scale disk on
Z:
0

GitLab https://gitlab.in2p3.fr/josephine.courouble/celltrack

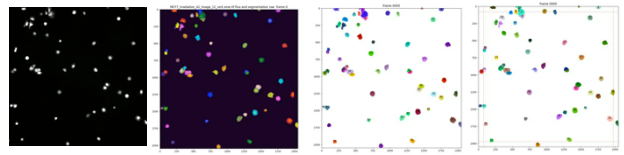
INPUT

- Images .tiff
- Configuration file:
  - Execution parameters (file names, time between two images…)
  - Biological parameters of cell line (usual surface area, usual division time…)

```
./celltrack.py --config config/test.yaml --doAll
```

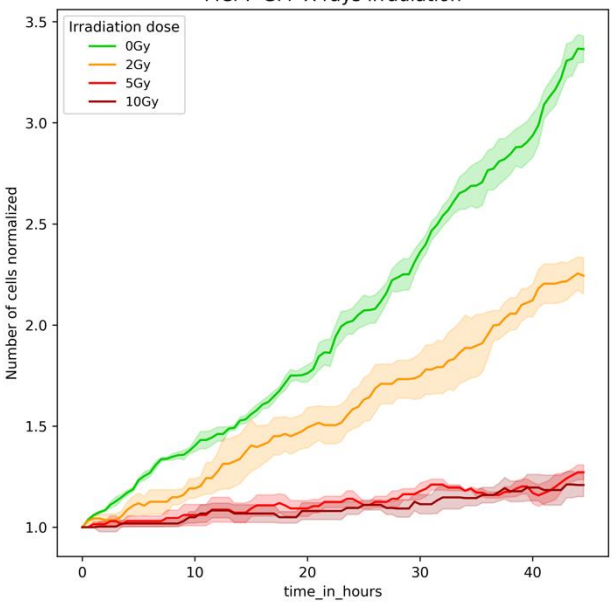
OUTPUT

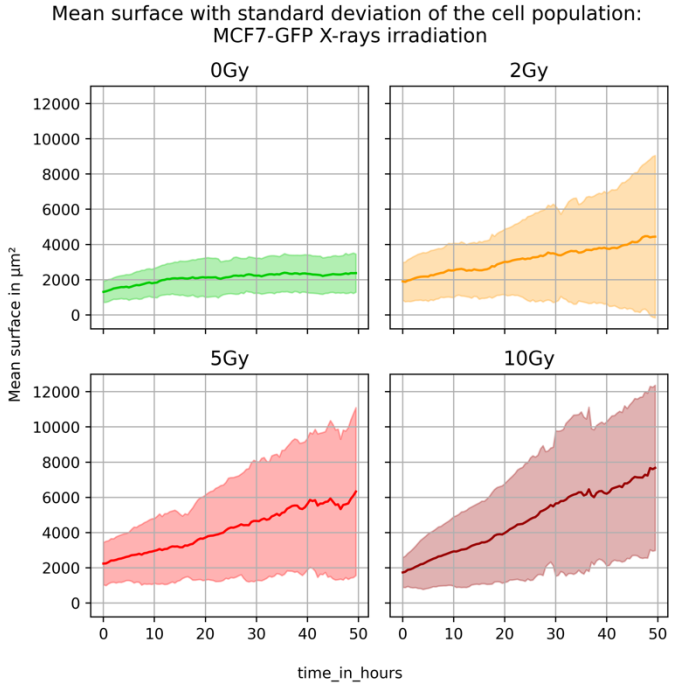
- Table containing individual cellular data
- Matrices containing labeled segmentation
- Videos allowing user visual verification at each step of the analysis



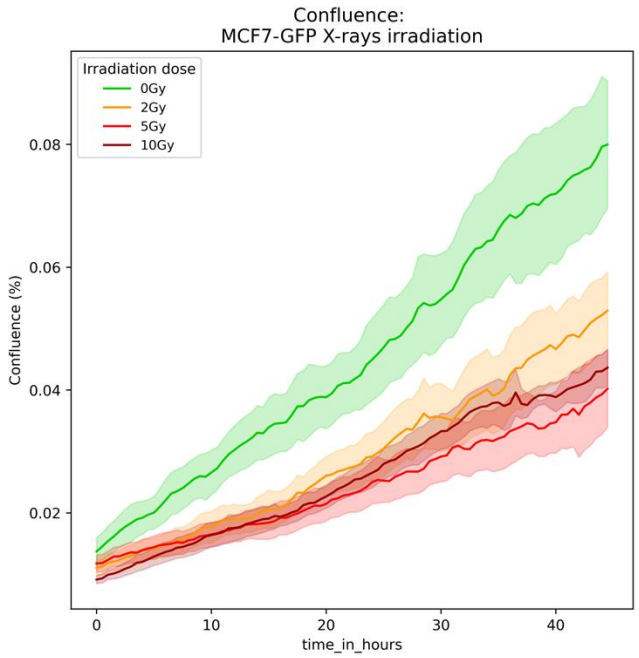
Celltrack_Analysis

Number of cells + individual area





Confluence
(Fraction of area occupied by cells)



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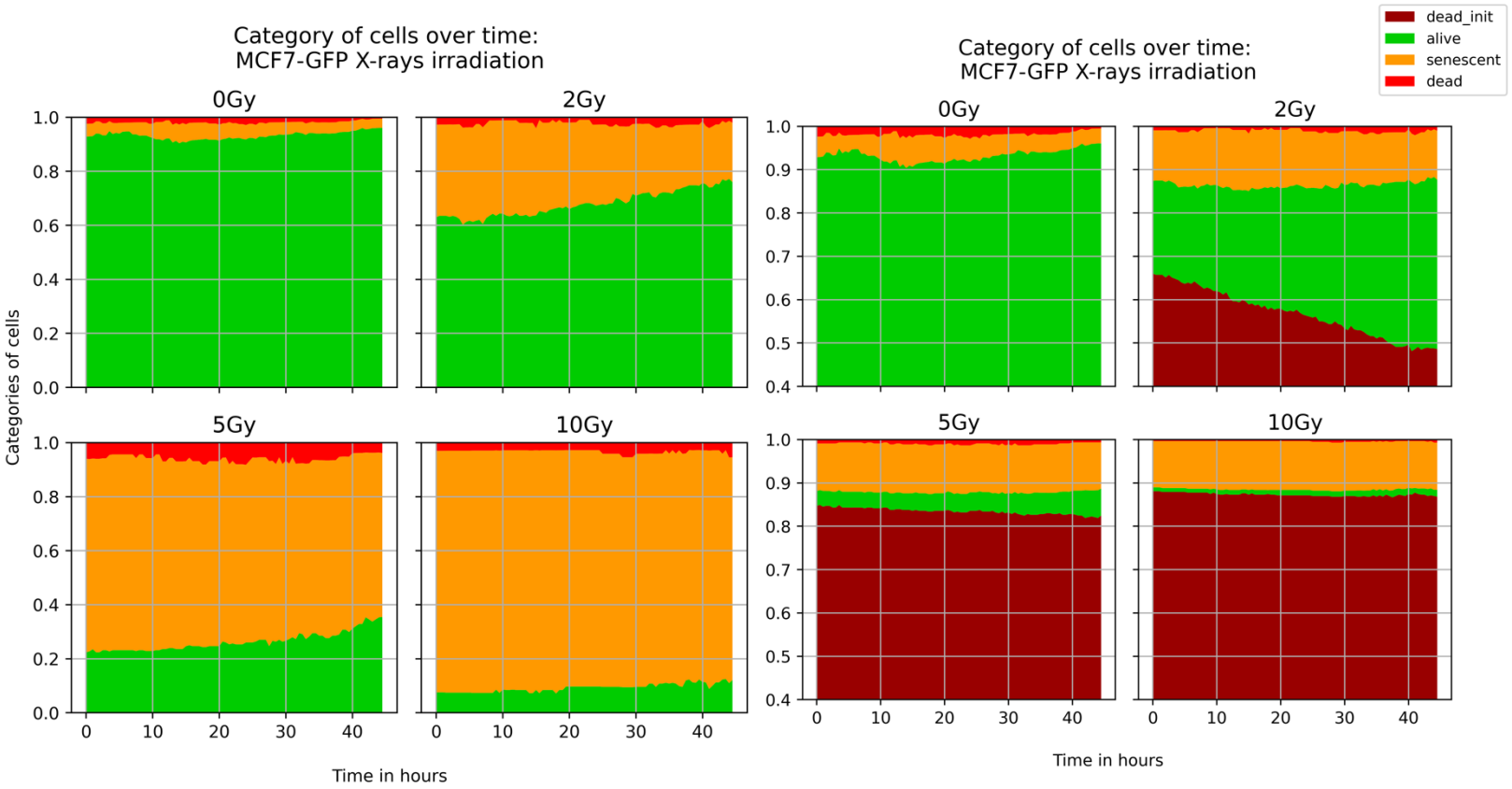
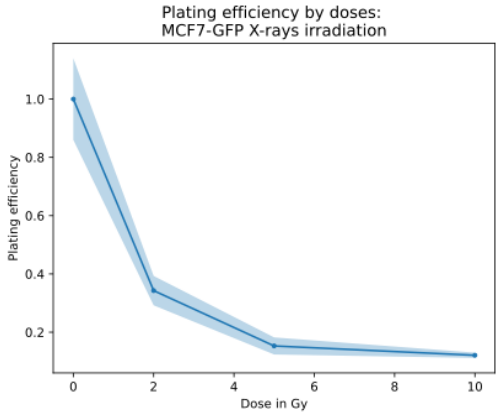
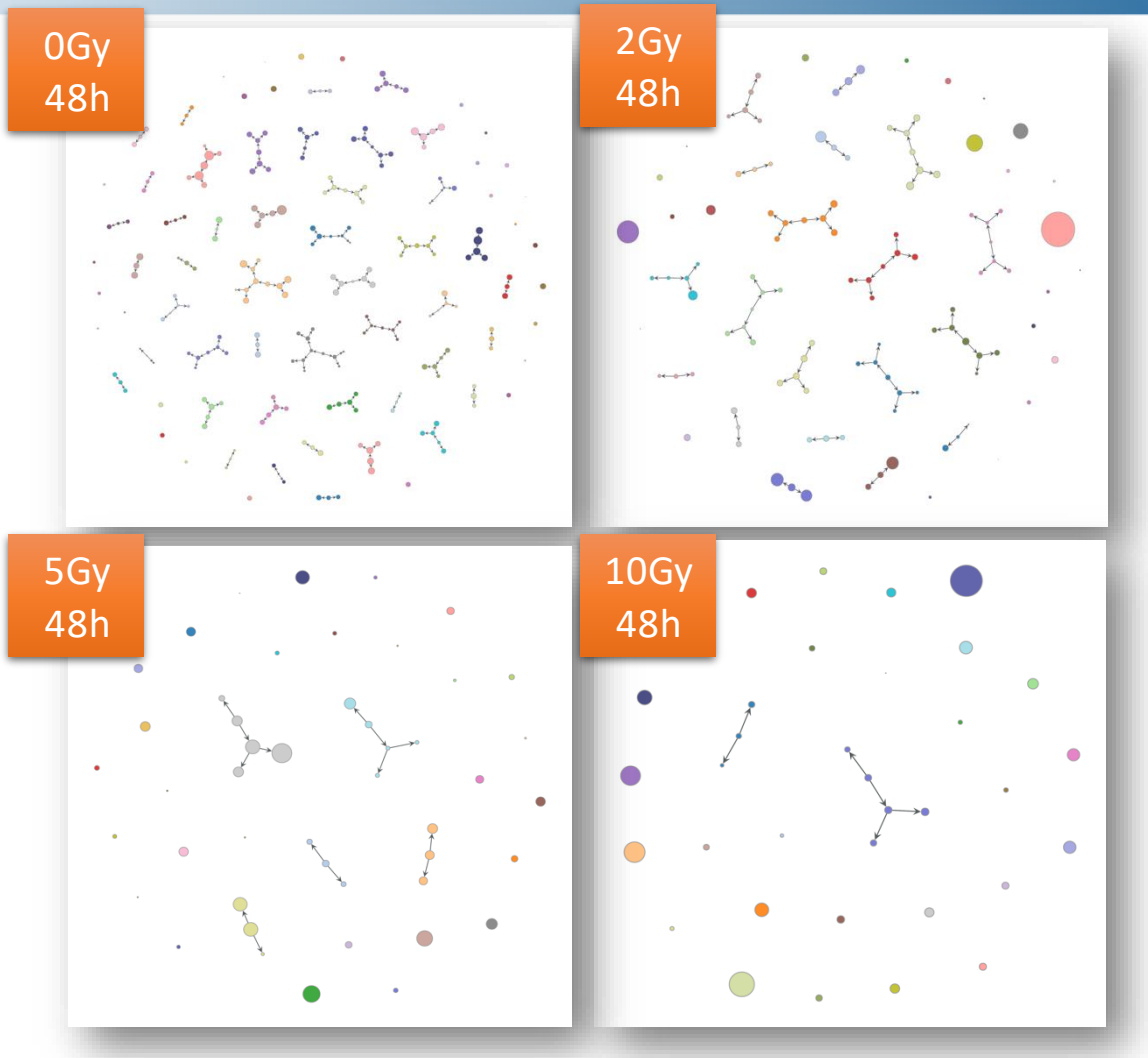




$$\text{dead\_init} = \frac{\text{nbr\_cells\_init} \times (1 - \text{plating\_efficiency})}{\text{plating\_efficiency}}$$

BACK-UP: Cell lineage

Lineage tree scale



Lineage tree scale