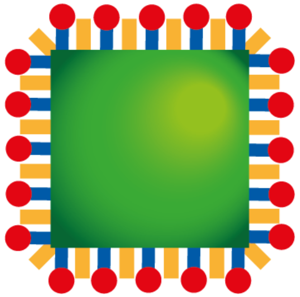
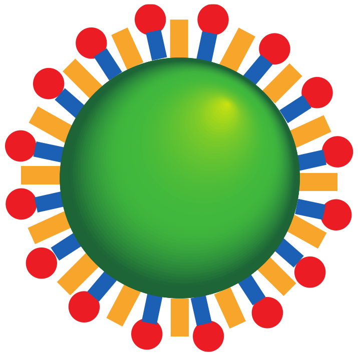


The **PANCAKE** project

Proton therapy **A**ssisted by **N**ano Pd-DDS as **CA**ncer cell **K**ill**E**rs

Magdalena Parlinska, *IFJ PAN*
Olivier Seksek, *IJCLab*



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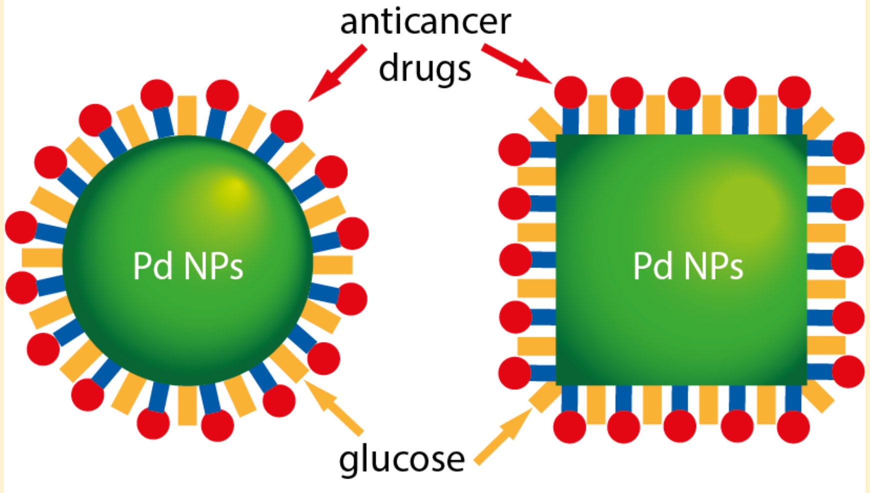
Idea: The addition of Pd-DDS to cancer cells and subsequent proton irradiation leads to higher cell mortality than irradiation, NPs or drug addition alone.



Synthesis of Pd DDS = Pd nanospheres or nanocubes functionalized by glucose and with anticancer drugs immobilized on the NPs surface

Experiment: In situ long-term, real-time holotomographic microscopy observations of Pd NPs, drugs and Pd-DDS effects in cells: (i) morphological changes, (ii) absorption dynamics, (iii) interaction, (iv) accumulation sites in 3D prior and after proton irradiation.

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Idea: The radioamplification by Pd-DDS of the proton irradiation (at subletal doses) may also lead to a change in the individual cell behavior inside an homogeneous or heterogeneous cell population.

Experiment: In situ long-term, real-time videomicroscopy data acquisition of Pd NPs, drugs and Pd-DDS effects in cells: (i) morphological changes, (ii) migration dynamics, (iii) interaction, (iv) proliferation, (v) bystander effects.

Data analysis and processing: machine learning algorithms development in order to obtain an emulator to predict cell behavior.

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Holotomography superresolution microscope
Nanolive 3d cx-a

NON-INVASIVE 3D CHARACTERIZATION

Live cell imaging in physiological conditions without any bleaching or phototoxicity

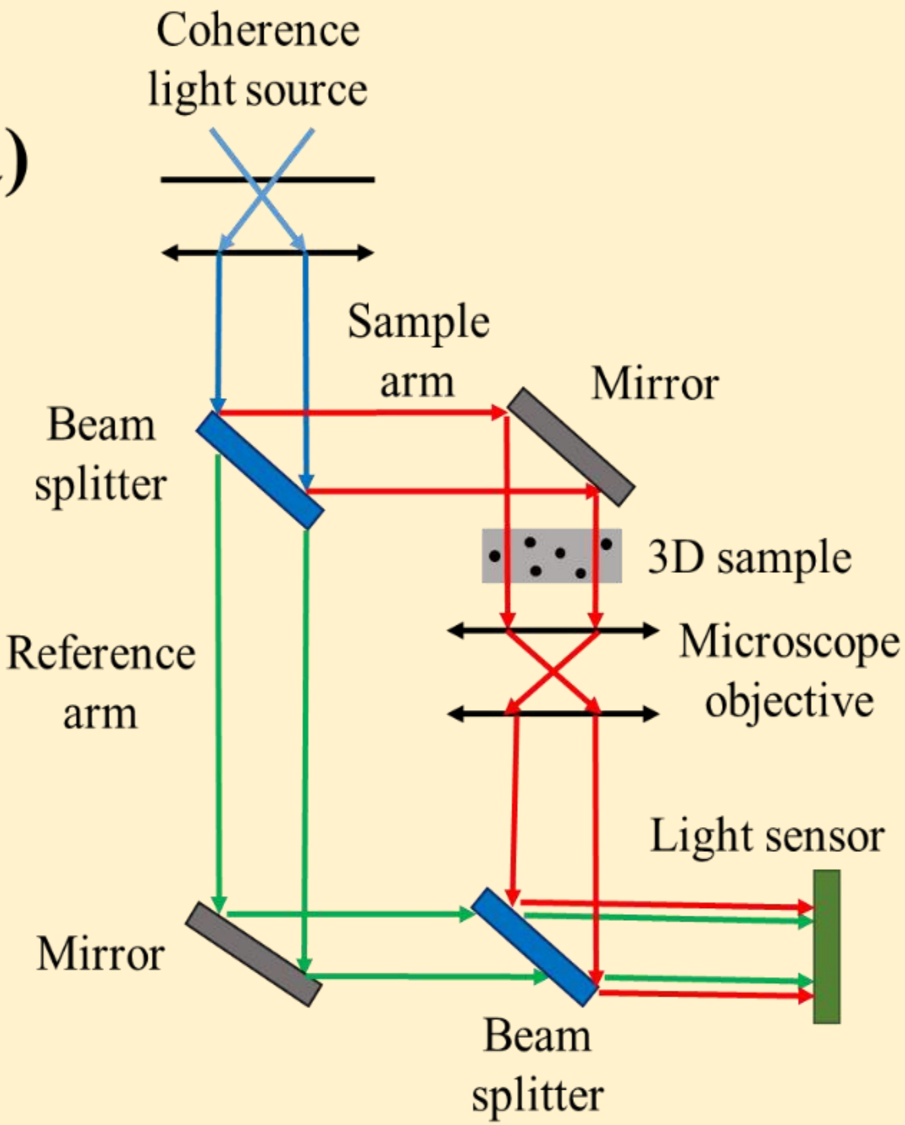
LABEL-FREE 4D CONTINUOUS OBSERVATION

Measurement of cell processes from seconds to weeks

MULTIPLEXING

High resolution and high sensitivity characterization of multiple cell organelles based on their refractive index (RI)

a)



b)

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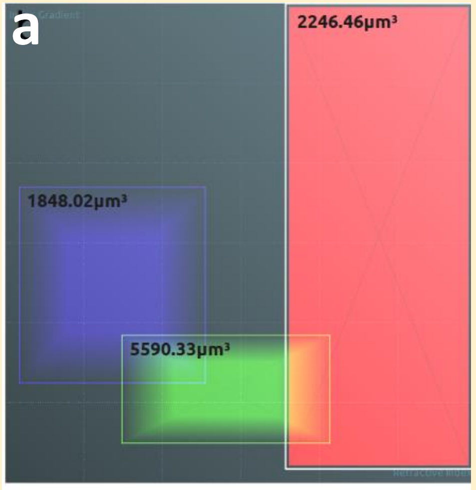
a) Distribution of RI with the value of RI volume corresponding to individual structures: **nanoparticles**, **nuclei and cytoplasm** and **cell membrane**



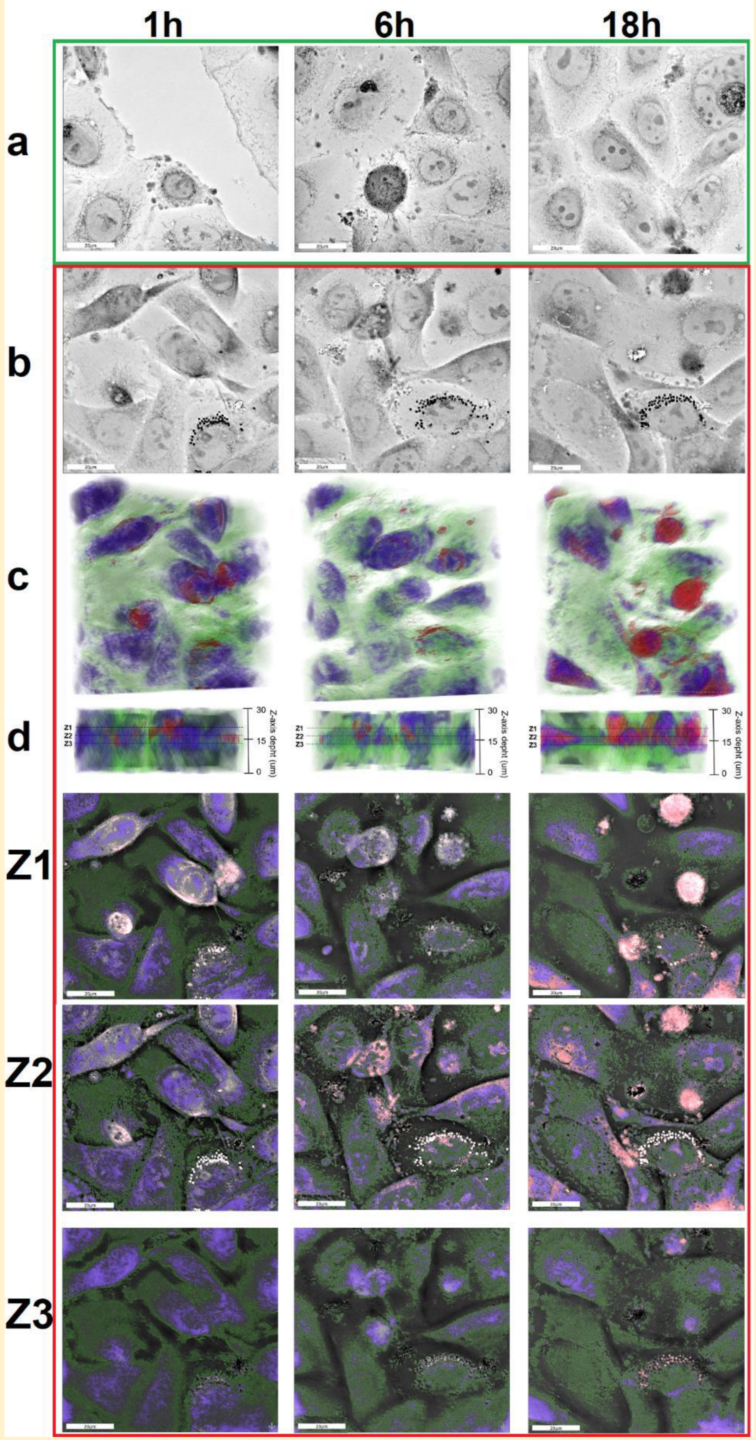
b) Holotomographic images of SW480 colon cancer cells cultured a) without and b) with Pd NPs taken after 1 h (left column), 6 h (middle column) and 18 h (right column).

c) Reconstructed 3D holotomographic images based on the RI value of: Pd NPs (red color), nuclei (blue color) and cell membrane (green color).

d) Z-axis reconstruction of holotomographic images with indicated thickness of slices approximately 13µm (Z1), 17µm (Z2) and 21.6 µm (Z3),

Z1, Z2 and **Z3** panels show holotomographic images with marked RI of **Pd NPs**, **nuclei** and **cell membrane**.

Scale bar ≈ 20 µm.



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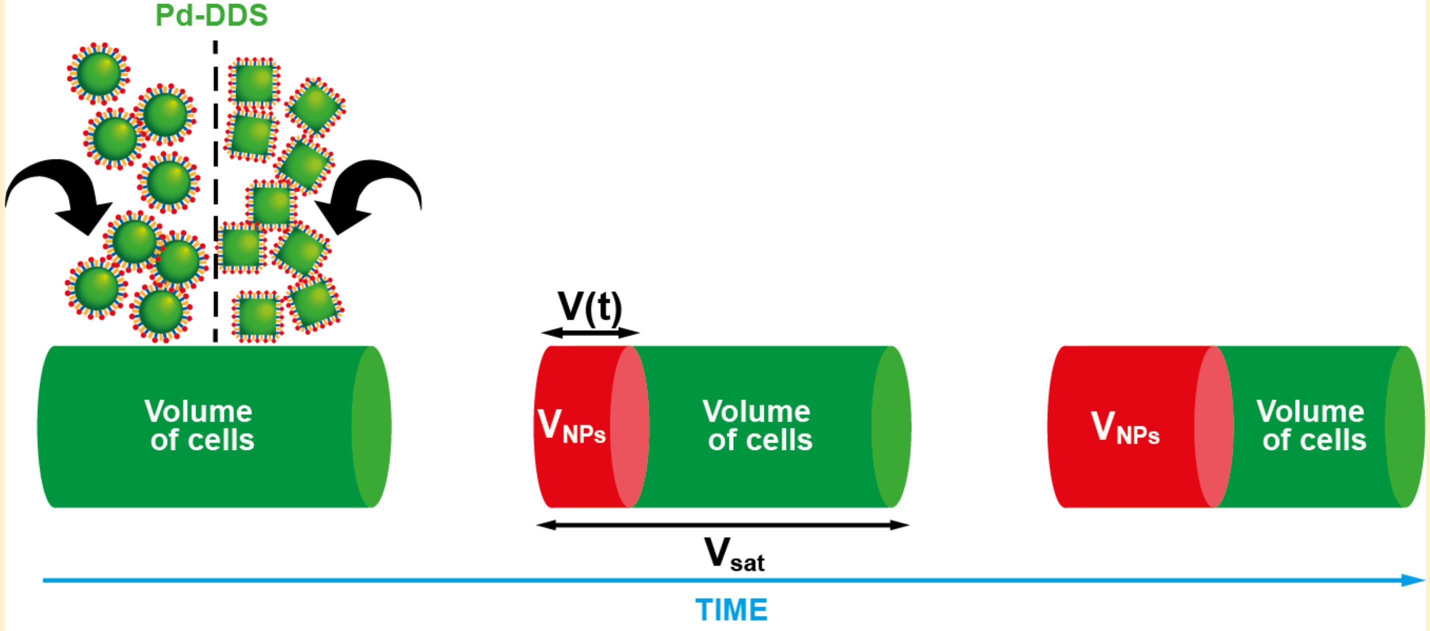
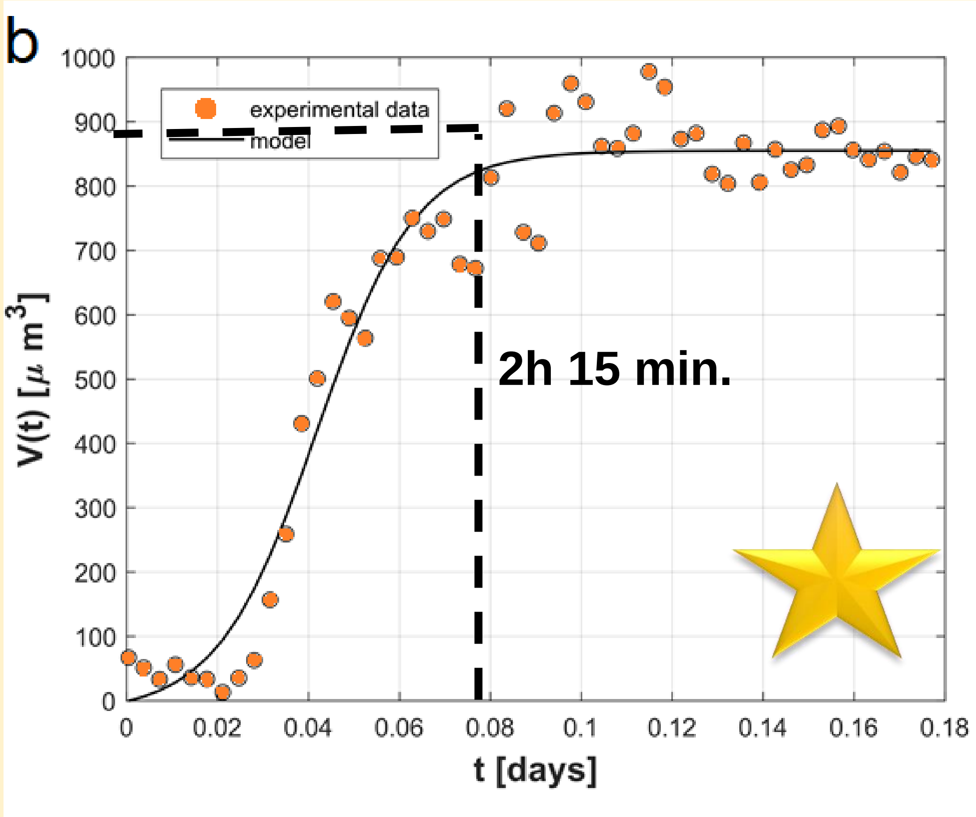
Nanoparticles absorption model

$$\frac{dV(t)}{dt} = S\left(V_{SAT} - V(t)\right) + D\frac{V(t)}{V_{SAT}}\left(V_{SAT} - V(t)\right)$$





Conditions:

- $V(0) = 0$
- $t \geq 0.$

$$V(t) = V_{SAT}\frac{1 - e^{-(S+D)t}}{1 + \frac{D}{S}e^{-(S+D)t}}$$

The correlation of the **model** with **experimental data** is equal to 96% for Au nanostars, thus it fulfils its role, as a physical description of the phenomenon of kinetics of NPs absorption by glioblastoma U118 cells.

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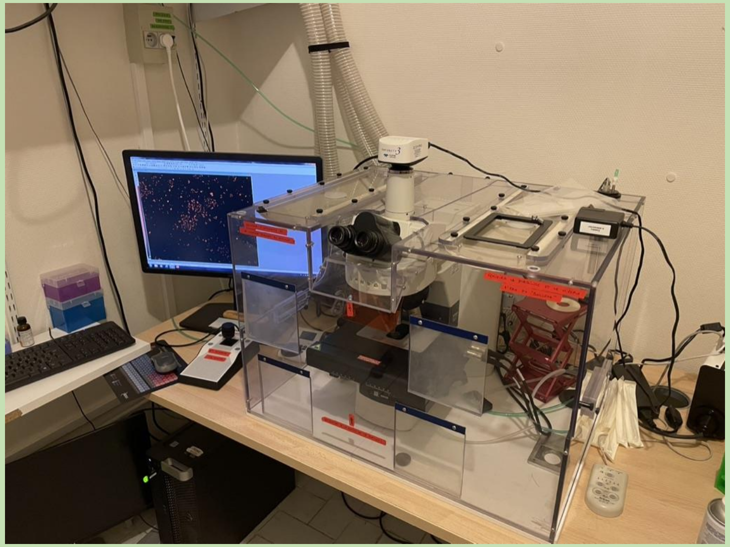
Long-time videomicroscopy monitoring of cell growth on in vitro models

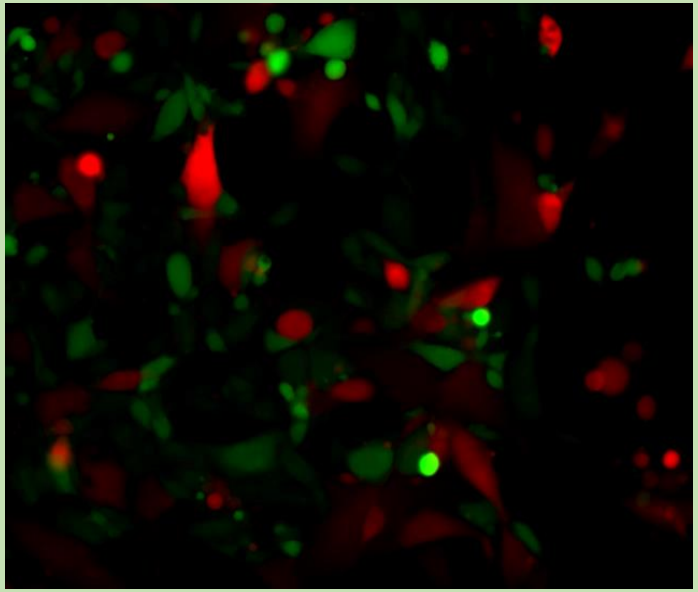


EXPERIMENTAL SET-UPS

Live cell imaging in physiological conditions on long-time processes

CELL MODIFICATION

Cell transformation in order to obtain various fluorescent sub-populations



Videoimaging of two-population F98 mouse glioma cells: green (GFP-transfected) non-irradiated and red (RFP-transfected) irradiated (X-ray, 20 Gy) cells.

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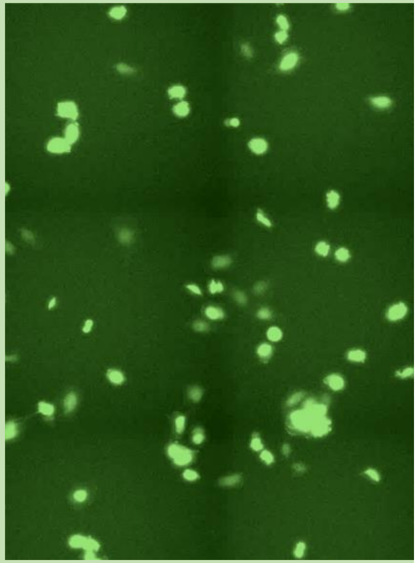
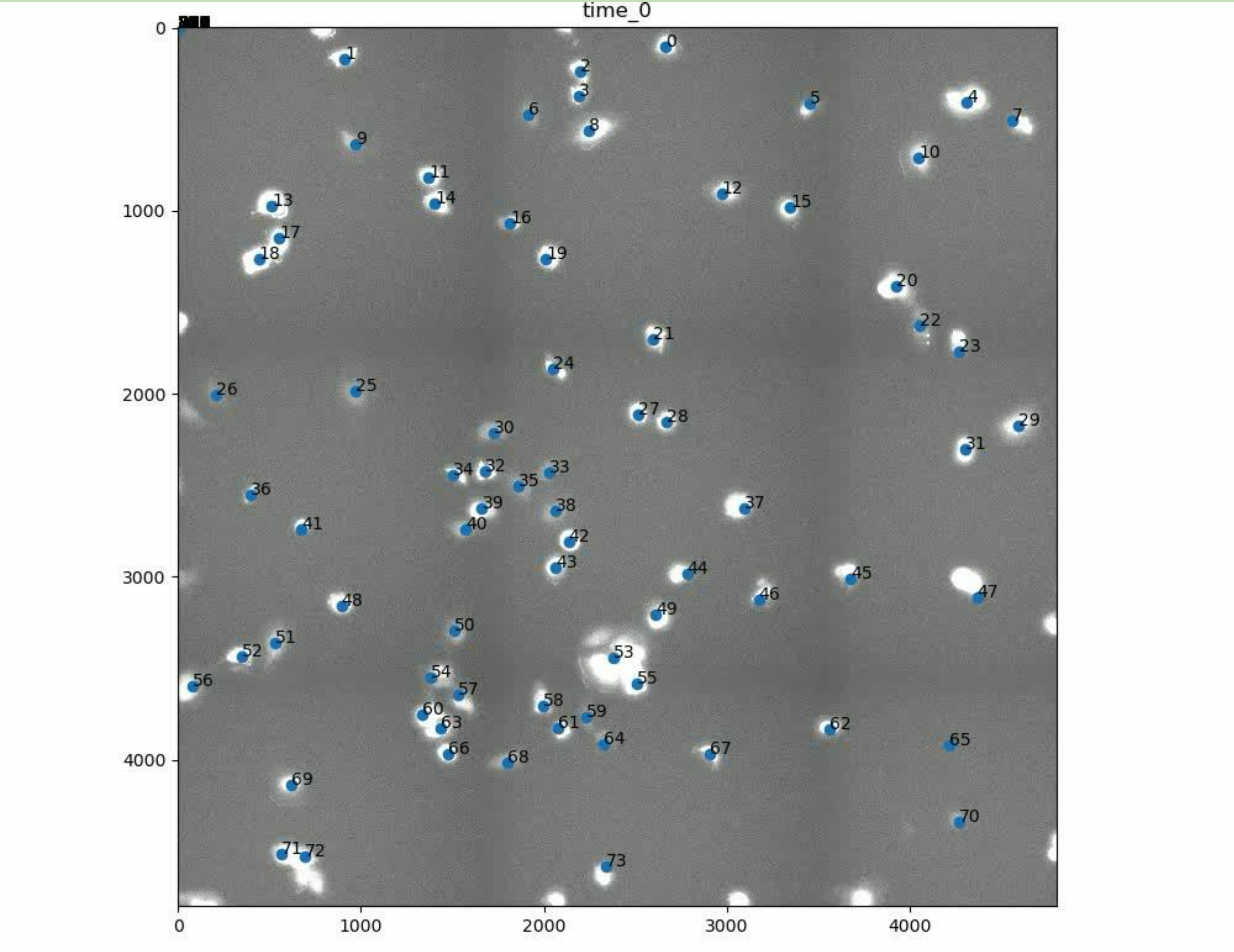
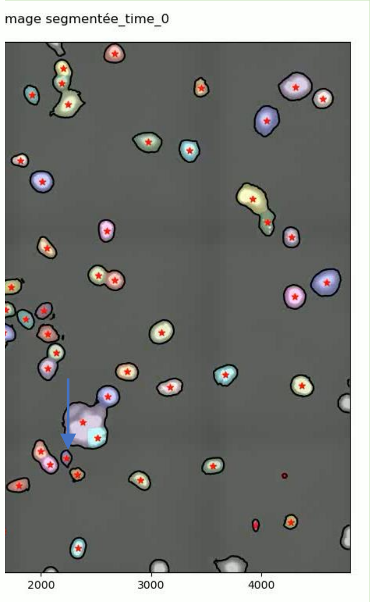
Data analysis for individual cell recognition

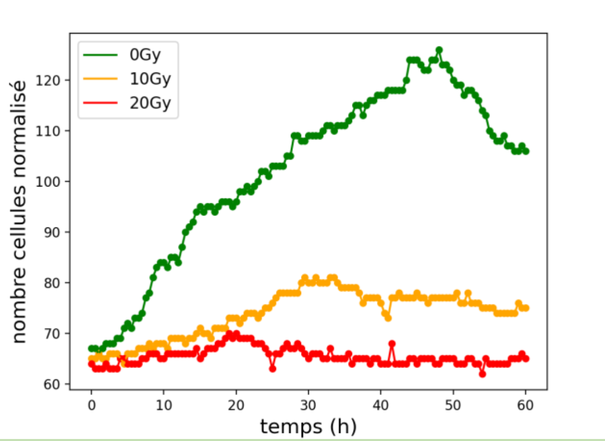






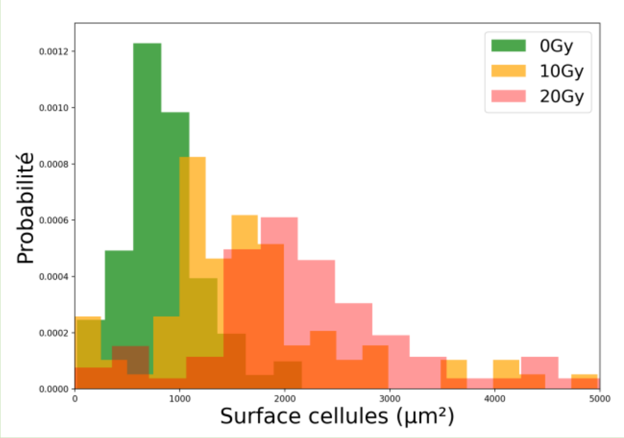
Examples of preliminary data

X-RAY Irradiation
MCF-7 cells (single population)



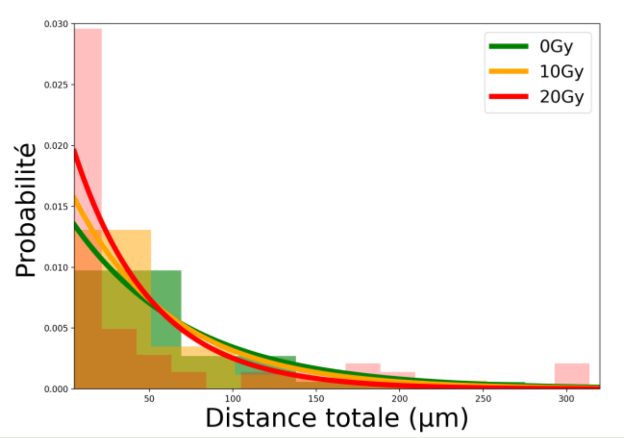
Cell number

0Gy: 59%
10Gy: 15%
20Gy: < 1%



Individual cell Surface (µm^2)

0Gy: 897µm^2
10Gy: 1644µm^2
20Gy: 2210µm^2



Individual cell distance travelled (µm)

0Gy: 70µm
10Gy: 59µm
20Gy: 47µm

→ **Data processing to emulator for cell behavior prediction**

IFJ PAN team

First name / Family name	Function (Researcher, Engineer etc)	Role in the pre-project	% of participation
Magdalena Parlinska	Senior researcher	Project leader, morphological evaluation of Pd NPs using TEM	30
Joanna Depciuch-Czarny	Senior researcher	Synthesis of Pd NSs & NCs, functionalization NPs with anticancer drugs, Nanolive imaging	40
Bartosz Klębowski	Researcher	Immobilization of glucose on Pd NPs, cell & spheroid culture, proton beam irradiation	40
PhD student	student	Synthesis of Pd NCs & NSs, cell and spheroid culture	70

Budget

Type of expenses	Amount [Euro]
Equipment	-----
Consumables	6 000 (consumables for cell culture, spheroids culture, palladium salts, other chemicals, plastics, glassware, TEM consumables etc.)
Travels	2 000
Total	8 000

IJCLab team

First name / Family name	Function (Researcher, Engineer etc)	Role in the pre-project	% of participation
Olivier Seksek	Senior researcher	Project leader.	30
Joséphine Courouble	PhD student (10/23-09/26)	Data acquisition (with irradiation and NPs conditions) and data analysis.	50
Delphine Crépin	Science engineer	Cell lines production and characterization	20
Loick Ridou	Technician	Cell line production and culture (2D and 3D)	20
Stéphane Plaszczynski	Senior researcher	Data analysis.	15

Budget

Type of expenses	Amount
Equipment	-----
Consumables	6000 (consumables, cells, transport…)
Travels	2000
Total	8000