On going activities for my master thesis

in the context of:

ISOLPHARM_ADMIRAL experiment

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Agenda

- Analysis of the *in vitro* effects of the «cold» solutions of Ag, Pd and $\mathrm{Ag\text{-}Pd}$
- Calculation of the cellular $\mathrm{S\text{-}value}$ by mean of Monte Carlo simulations in $\mathrm{Geant4}$
- Analysis of the *in vitro* radiobiological effect of $^{111}\mathrm{Ag}$

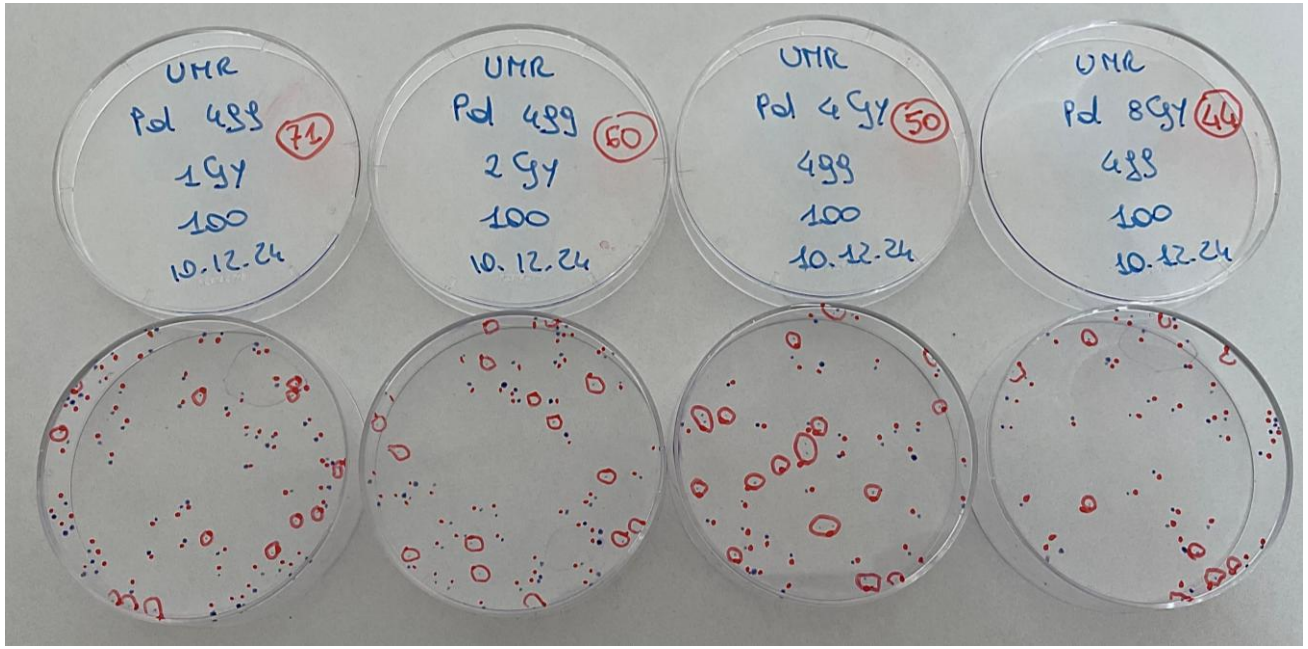
Are Ag, Pd and Ag-Pd cytotoxic?

Materials and methods

Cell survival assay on UMR-106 cell line.

Three Petri for each condition of:

- Initial cell seeding;
- Ag, Pd and Ag-Pd concentration
- Incubation period (Time point)



Are Ag, Pd and Ag-Pd cytotoxic?

Materials and methods

Time point	V_{Ag} [μl]	C_{Ag} [μM] [3]	A_0 [kBq/ml] (*)
4 days	13 ± 0.2	4.02	59
	27 ± 0.2	8.34	119
	54 ± 0.2	16.69	239
	108 ± 0.2	33.37	478
6 days	10 ± 0.2	3.09	43
	20 ± 0.2	6.18	87
	39 ± 0.2	12.05	174
	78 ± 0.2	24.10	347

Time point	V_{Pd} [μl]	C_{Pd} [nM] [4]	A_0 [kBq/ml] (*)
4 days	13 ± 0.2	0.53	59
	27 ± 0.2	1.10	119
	54 ± 0.2	2.20	239
	108 ± 0.2	4.40	478
6 days	10 ± 0.2	0.41	43
	20 ± 0.2	0.81	87
	39 ± 0.2	1.59	174
	78 ± 0.2	3.18	347

Time point	V_{Ag+Pd} [μl]	C_{Ag} [μM]	C_{Pd} [nM]	A_0 [kBq/ml] (*)
4 days	13 ± 0.2	2.00	0.26	59
	27 ± 0.2	4.17	0.55	119
	54 ± 0.2	8.34	1.10	239
	108 ± 0.2	16.69	2.20	478
6 days	10 ± 0.2	1.55	0.20	43
	20 ± 0.2	3.09	0.41	87
	39 ± 0.2	6.03	0.79	174
	78 ± 0.2	12.05	1.59	347

Are Ag, Pd and Ag-Pd cytotoxic?

Materials and methods

To evaluate the cell proliferation capability…

Plating efficiency (PE):

$$\mathrm{PE} = \left.\frac{\text{cell colonies}}{\text{cells seeded}}\right|_{\mathrm{CTR}} \times 100$$

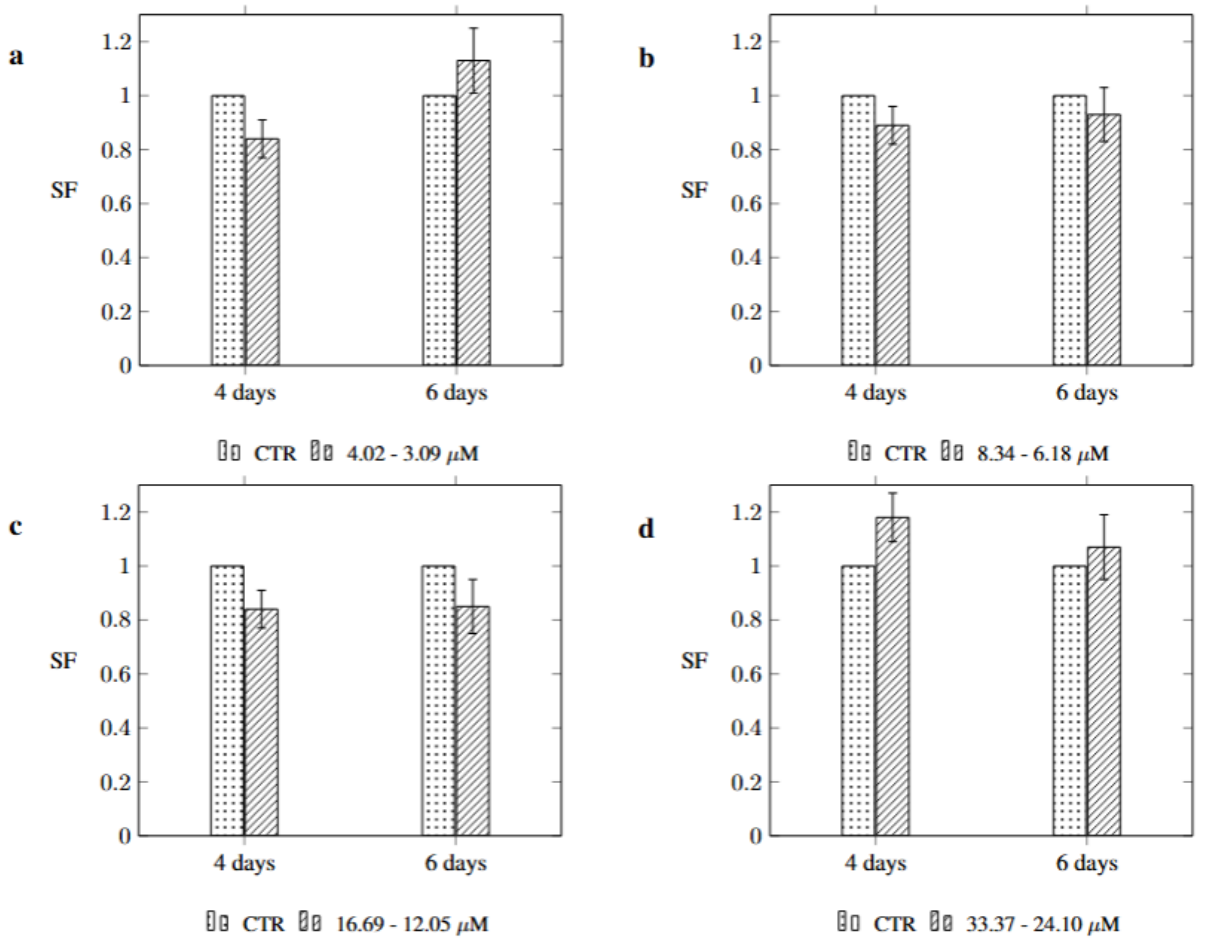
Survival fraction (SF):

$$\mathrm{SF} = \left.\frac{\text{cell colonies}}{\text{cells seeded}}\right|_{\text{treatment}} \times \frac{100}{\mathrm{PE}}$$

Are Ag, Pd and Ag-Pd cytotoxic?

Results: Ag effects

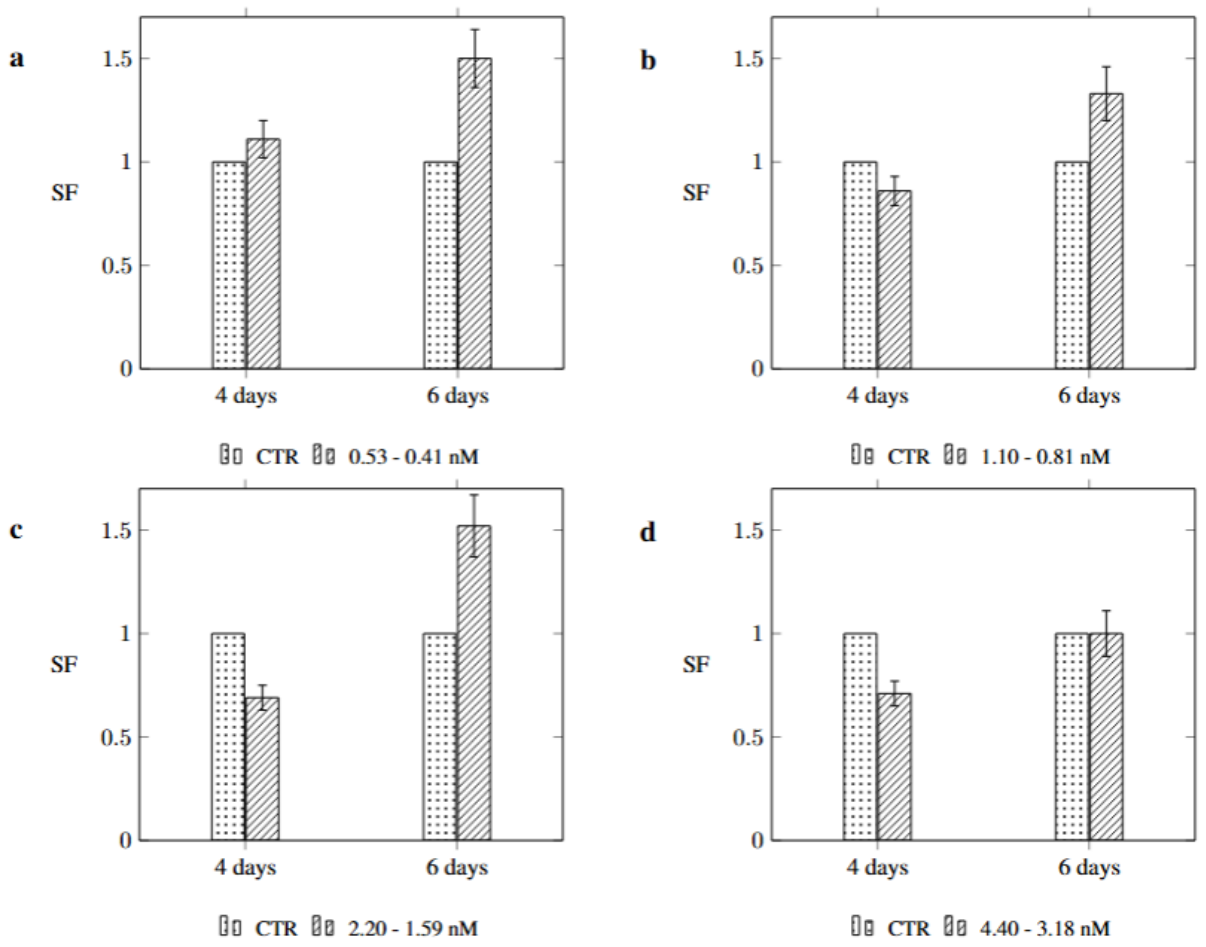
Time point	C_{Ag} [μM]	SF
4 days	4.02	0.84 ± 0.07
	8.34	0.89 ± 0.07
	16.69	0.84 ± 0.07
	33.37	1.18 ± 0.09
6 days	3.09	1.13 ± 0.12
	6.18	0.93 ± 0.10
	12.05	0.85 ± 0.10
	24.10	1.07 ± 0.12



Are Ag, Pd and Ag-Pd cytotoxic?

Results: Pd effects

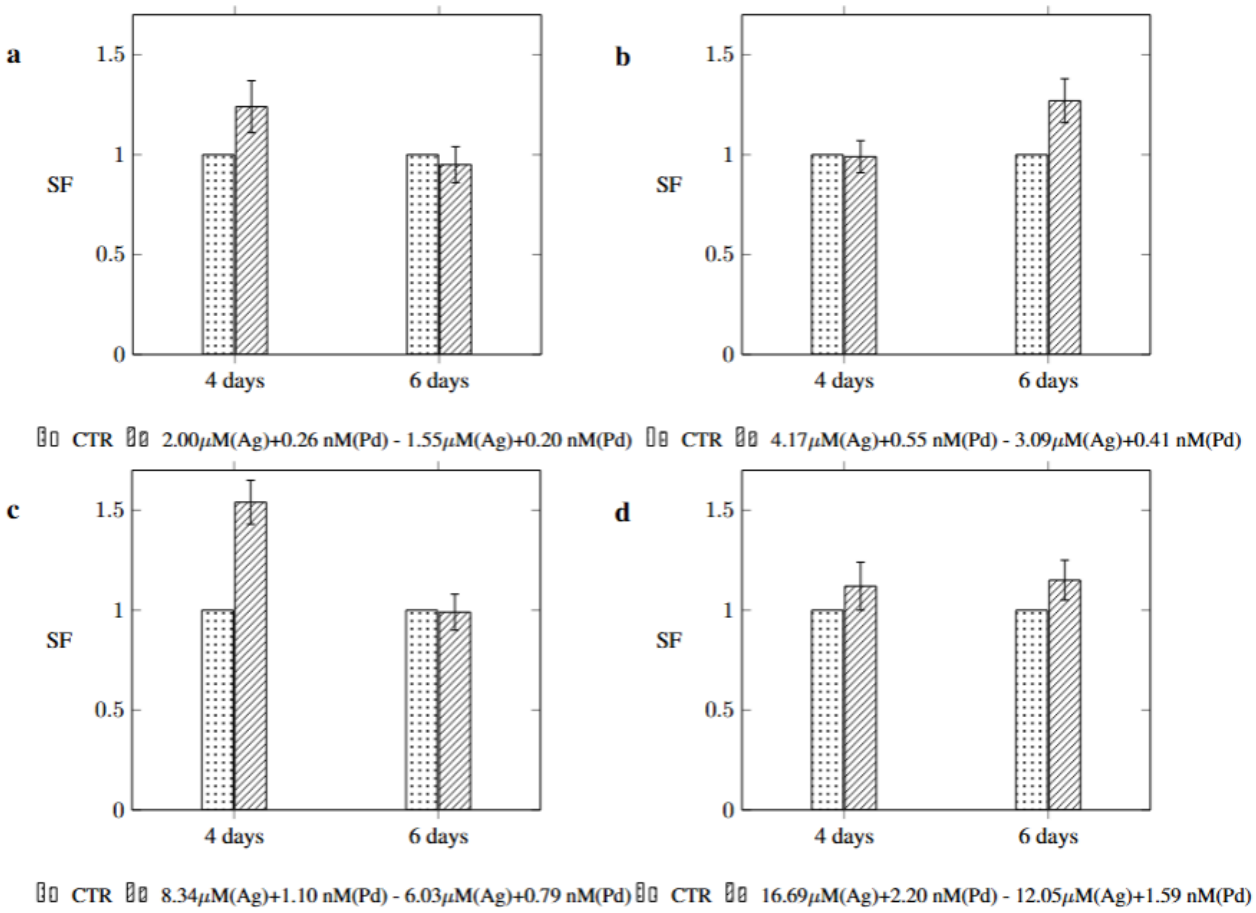
Time point	C_{Pd} [nM]	SF
4 days	0.53	1.11 ± 0.09
	1.10	0.86 ± 0.07
	2.20	0.69 ± 0.06
	4.40	0.71 ± 0.06
6 days	0.41	1.50 ± 0.14
	0.81	1.33 ± 0.13
	1.59	1.52 ± 0.15
	3.18	1.00 ± 0.11



Are Ag, Pd and Ag-Pd cytotoxic?

Results: Ag-Pd effects

Time point	C_{Ag} [μM]	C_{Pd} [nM]	SF
4 days	2.00	0.26	1.24 ± 0.13
	4.17	0.55	0.99 ± 0.08
	8.34	1.10	1.54 ± 0.11
	16.69	2.20	1.12 ± 0.12
6 days	1.55	0.20	0.95 ± 0.09
	3.09	0.41	1.27 ± 0.11
	6.03	0.79	0.99 ± 0.09
	12.05	1.59	1.15 ± 0.10



Are Ag, Pd and Ag-Pd cytotoxic?

Discussion: Ag effects in the literature

Silver is a well-known antimicrobial agent.

Reference values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):
- MIC: $(2{,}78 \times 10^{-7} - 1{,}16 \times 10^{-5})$ M
- MBC: $(2{,}41 \times 10^{-6} - 9{,}32 \times 10^{-5})$ M.

But, are there any toxic effects?

From literature ≪sub-cytotoxic≫ concentrations = concentrations $< 10^{-4}$ M.

Ag concentrations in the present study are ≪ threshold for cytotoxcity.

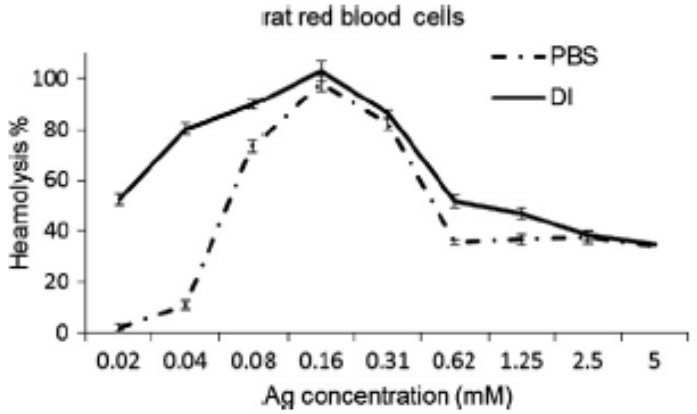
Are Ag, Pd and Ag-Pd cytotoxic?

Discussion: Ag effects

1) The trend of the survival fraction does not follow a monotonic profile neither for increasing Ag concentration nor for increasing time point.

Possible explanation:
Higher SF at higher Ag^+ concentrations and longer time points caused by the increasing tendency of Ag to form the precipitating and less toxic AgCl.

2) Lower cell viability for intermediate concentrations is not something new.



Similar profile, found in literature, albeit for different cell process, cell line and orders of magnitude of Ag concentration.

Are Ag, Pd and Ag-Pd cytotoxic?

Discussion: Pd effects in the literature

In vivo :

LD_{50} [mg/kg]	Administration route
5 (*)	Intravenous
6	Intratracheal
70	Intraperitoneal
200	Oral

LD_{50} measured on Charles-River CD1 rats in mg/kg of body weight.

In vitro :

Reference toxic concentrations for the 50% of cell population = $(134 - 360)$ µM.

↓

Pd concentrations in the present study are five orders of magnitude lower than these reference values.

(*) Reached in the CAPiR experiment.

Are Ag, Pd and Ag-Pd cytotoxic?

Discussion: Pd effects

1) For the 4 days incubation, the decreasing SF is compatible with increasing Pd and, so, Pd^{+} concentration. The promoted cell proliferation in the lowest Pd concentration condition could be due, on the other hand, to the hormesis.

2) The 6 days incubation seems to promote cell proliferation much more. It could arise from the adaptive response.

Overall, especially for the long-term contact conditions, no toxicity can be stated to exist in the analyzed situation.

Are Ag, Pd and Ag-Pd cytotoxic?

Discussion: Ag-Pd effects

There is no monotonic behaviour of the SF as function of the concentration and time point.

More complex mechanisms could characterize the chemical interactions among Ag and Pd solutions and the culture medium.

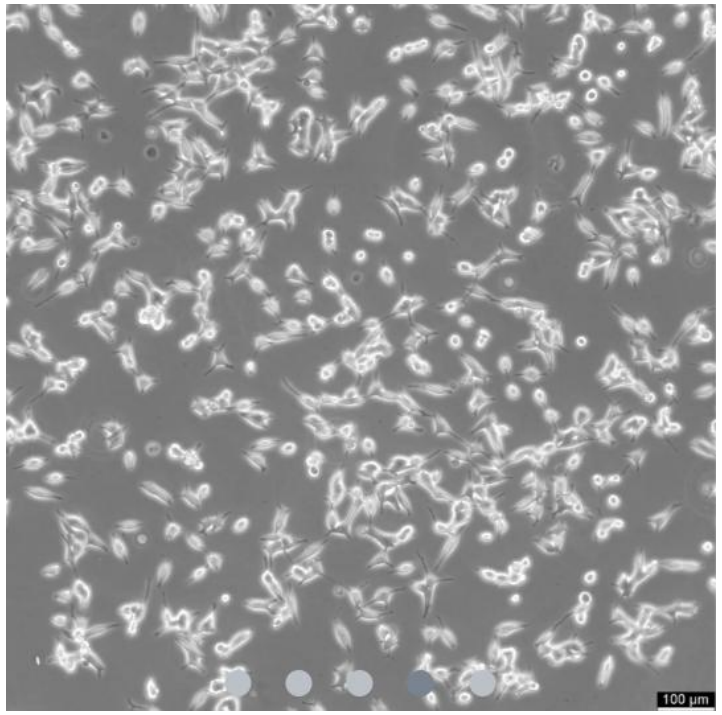
HOWEVER…

Once combined, Ag and Pd so not show any clear sign of toxicity *in vitro*.

Calculation of the cellular S-value by mean of Geant4

Geometry implementation: the cell

Cell line simulated = Lymph Node Carcinoma of the Prostate (LNCaP) cell, derived from a metastatic lesion in a lymph node of a prostate cancer patient. (*)



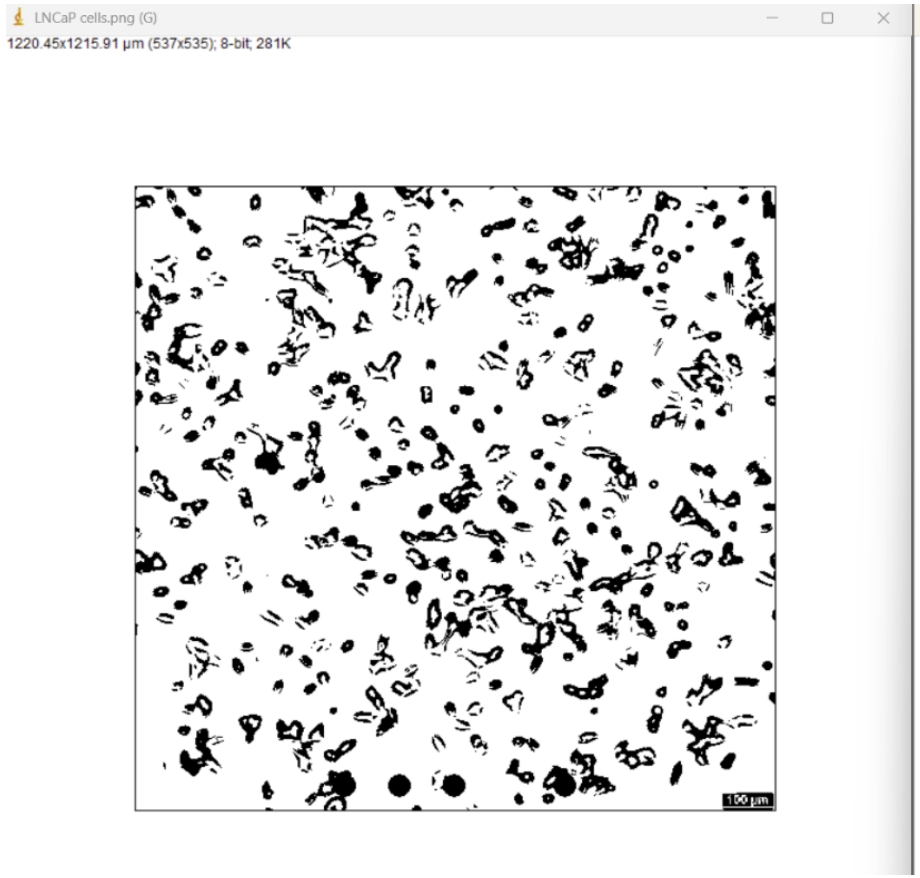
(*) They express a mutated form of the androgen receptor (AR) gene → prostate cells are hypersensitive to even low levels of androgens → higher PSA expression. Thanks to their androgen-sensitive growth, they serve as a cornerstone in understanding the role of androgens in cancer progression and prostate cancer.

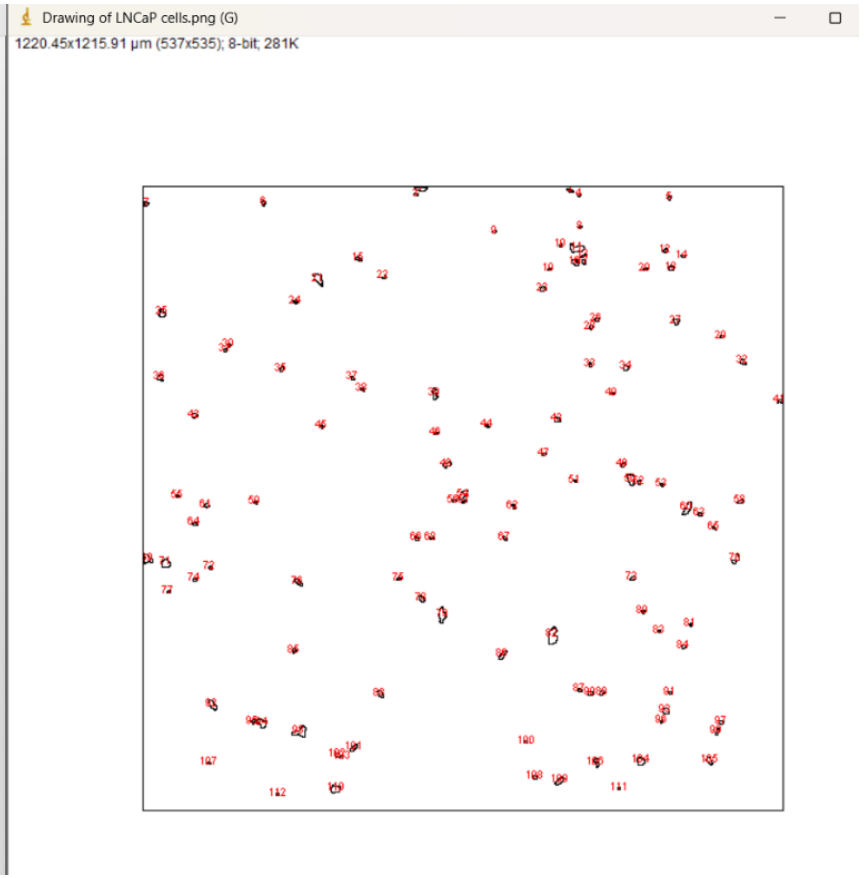
Calculation of the cellular S-value by mean of Geant4

Geometry implementation: the cell

Using ImageJ, the diameter of the cell nucleus has been estimated to be:

$$d_{nucleus} = 9.0\ \mu m$$



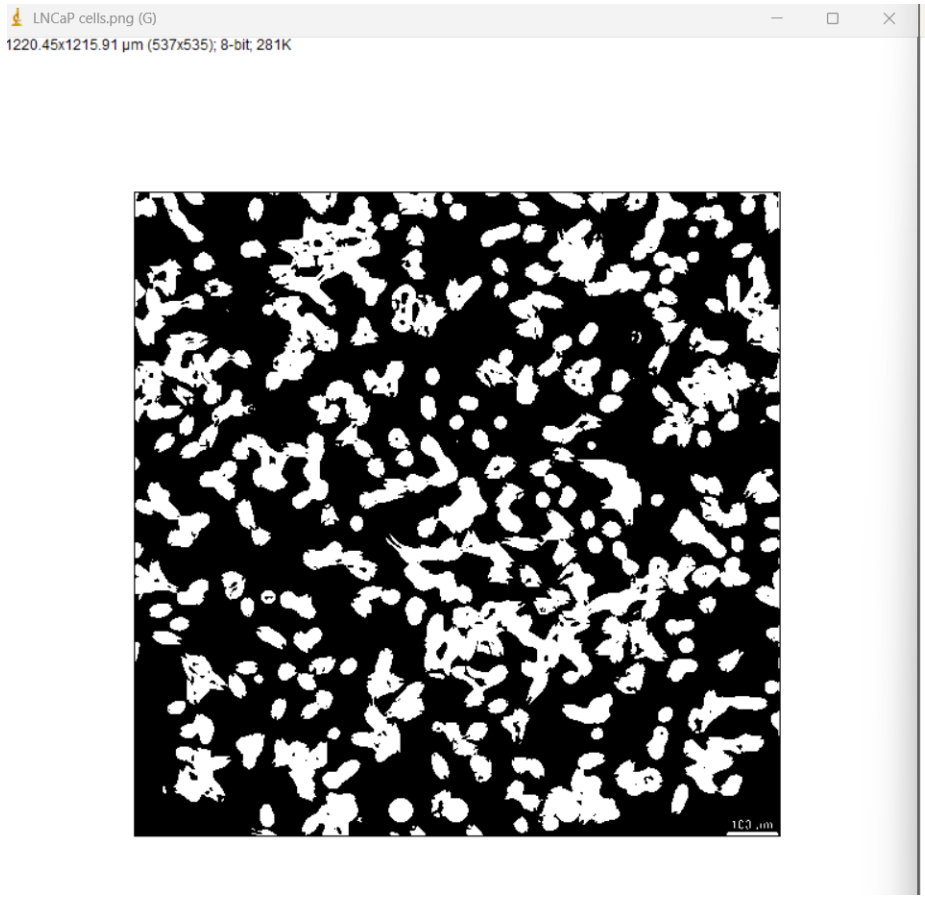


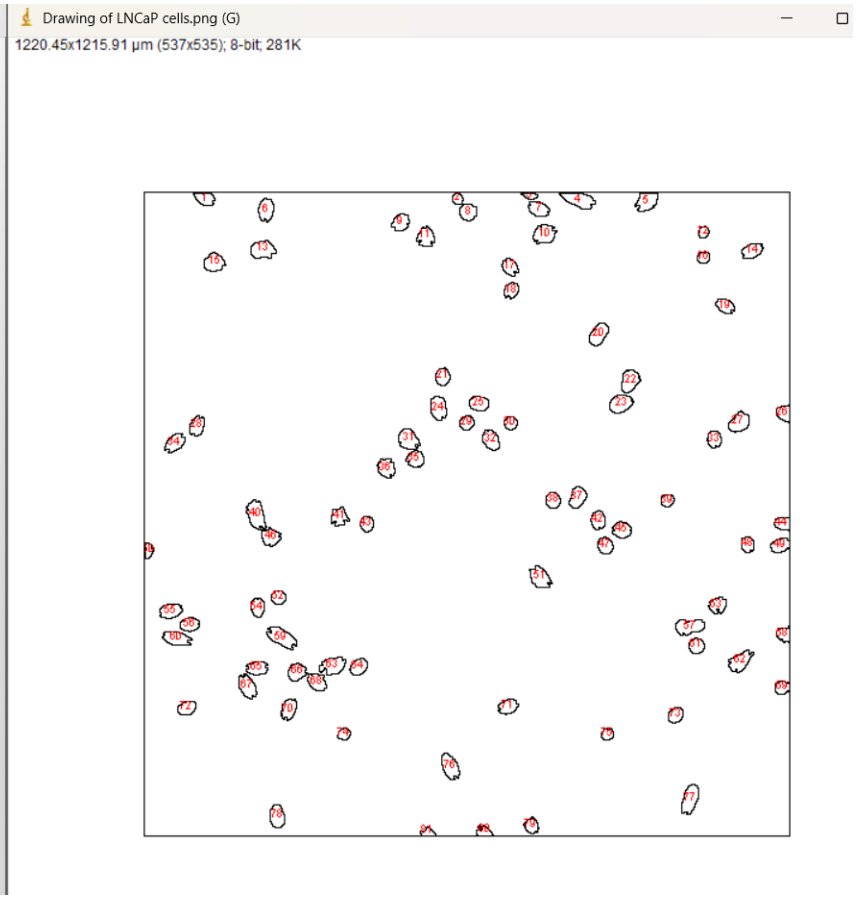
Calculation of the cellular S-value by mean of Geant4

Geometry implementation: the cell

Using ImageJ, the diameter of the cell has been estimated to be:

$$d_{cell} = 31.4\ \mu m$$



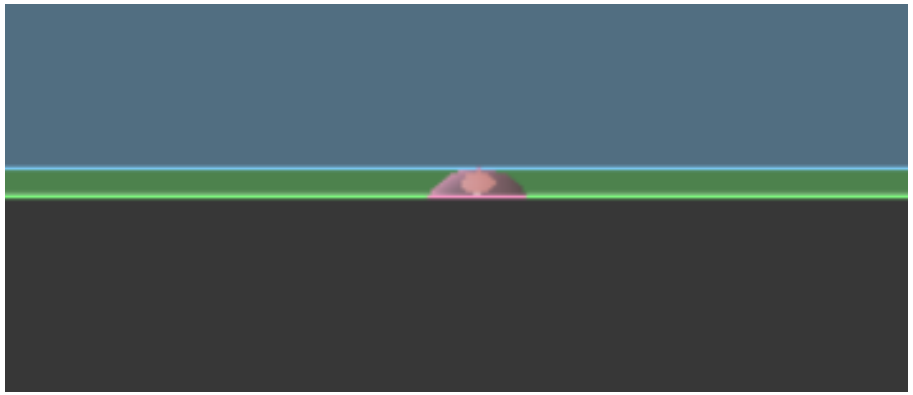


Calculation of the cellular S-value by mean of Geant4

Geometry implementation: the cell

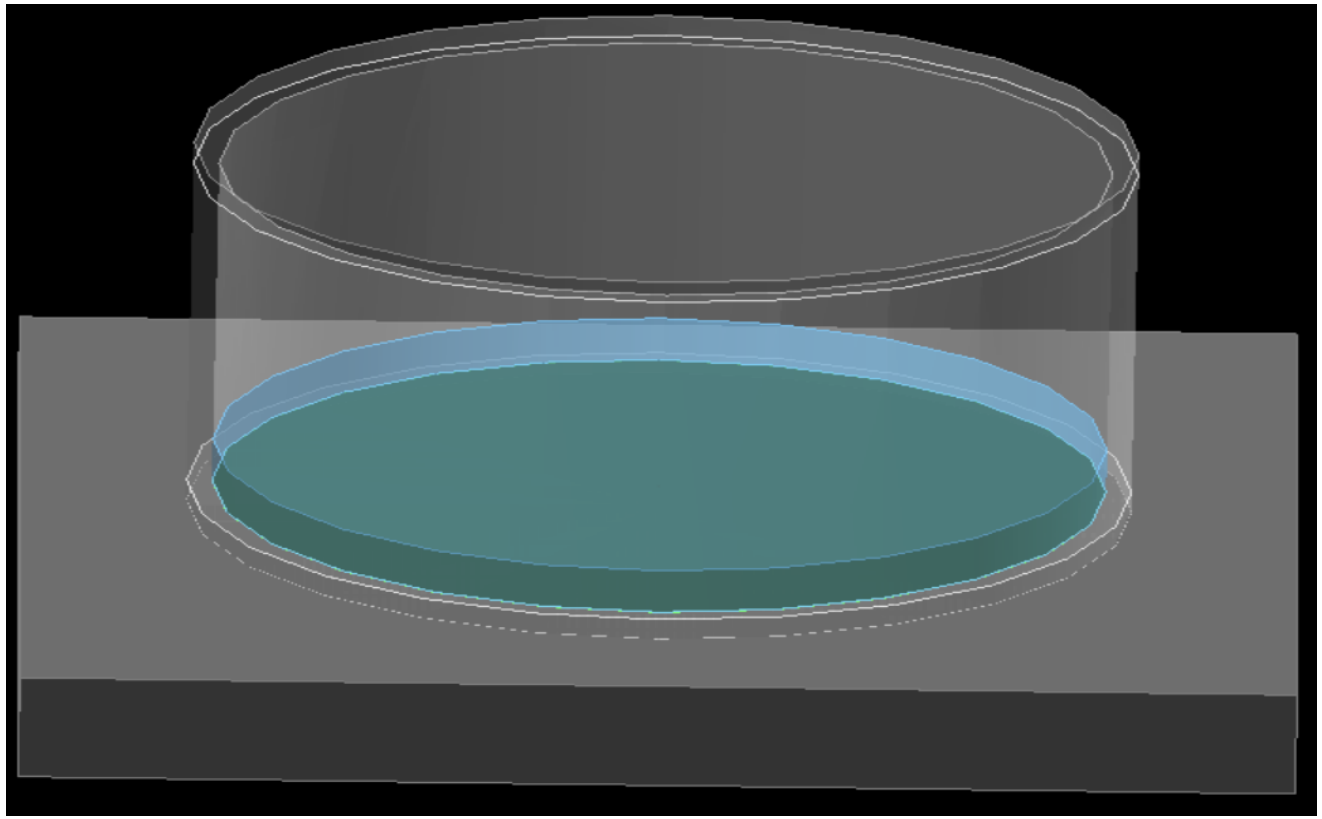
How I modelled the adherent cell:

Where the cell is in the culture medium:



Calculation of the cellular S-value by mean of Geant4

Geometry implementation: the P35 dish, support and culture medium



For the P35 dish:

- $\text{diameter} = 35\ \text{mm}$
- $\text{thickness} = 1\ \text{mm}$
- $\text{height} = 16\ \text{mm}$
- material = polystyrene

For the support plane:

- $\text{height} = 5\ \text{mm}$
- material = stainless-steel

For the culture medium:

- $\text{height}_{\text{bottom}} = 10\ \mu\text{m}$
- $\text{height}_{\text{top}} = 2.09\ \text{mm}$
- material = water

Calculation of the cellular S-value by mean of Geant4

Data scoring and next purposes

I am presently working on the implementation of the classes aimed to the data scoring.

What to calculate in the next weeks:

Self-absorbed dose

Source
Membrane
Whole cell
Cytoplasm

Target
Nucleus
Whole cell

Cross-absorbed dose 1→2

Source (*)
Membrane 1
Whole cell 1

Target
Nucleus 2
Whole cell 2

Environmental dose

Source
Surrounding environment

Target
Nucleus
Whole cell

(*) As shown by Emma Raniero in her master thesis, in this evaluation the membrane distribution and the cytoplasm one give the same results.

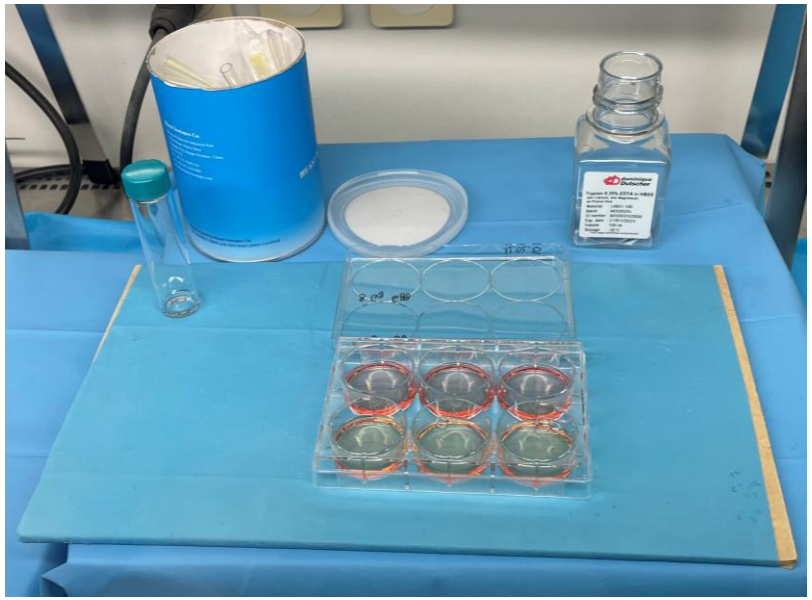
Cell survival assay for ^{111}Ag

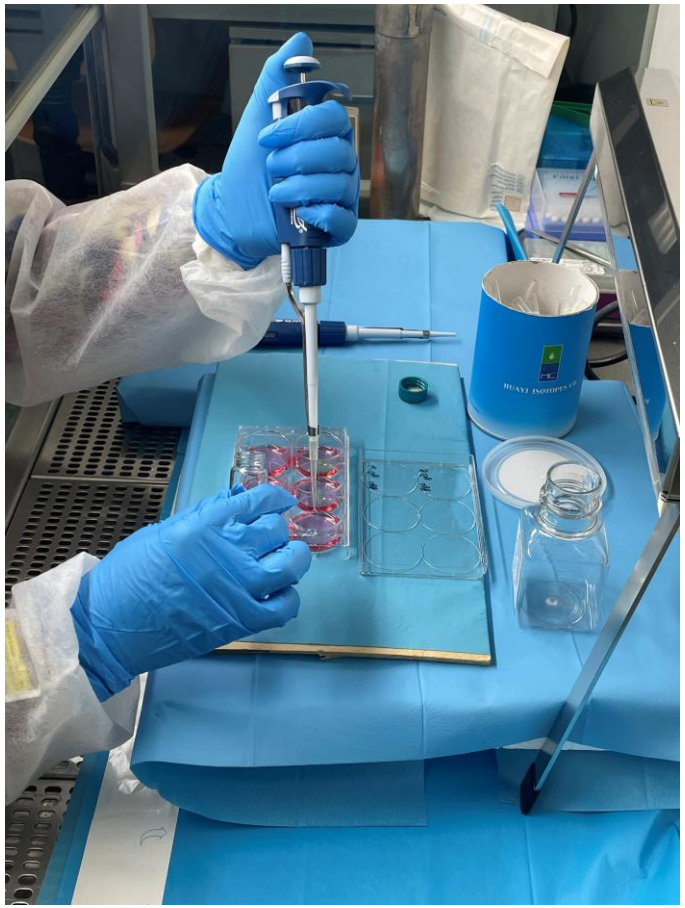
Data analysis and modelling

In the next weeks, I will also analyze the results obtained from the clonogenic measurements done on UMR-106 cells to study the radiobiological effect of ^{111}Ag.

Two models will be used to fit the data:

1) The linear quadratic (LQ) Model
2) The Induced-Repair (IndRep) Model.





Thank you!