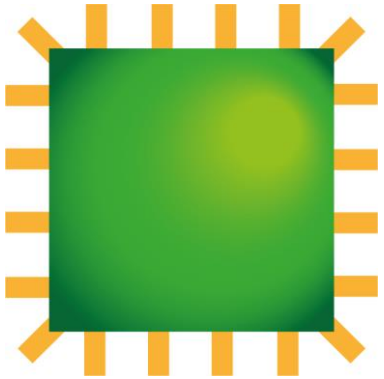
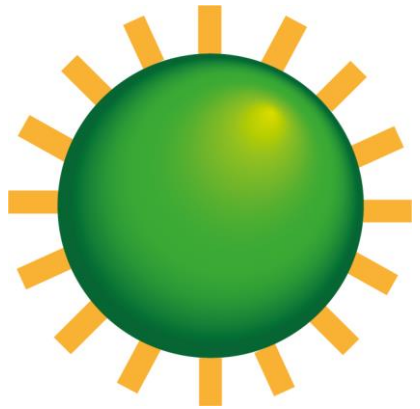


The **PANCAKE** project

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

Magdalena Parlinska, IFJ PAN
Olivier Seksek, IJCLab



IFJ PAN team

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

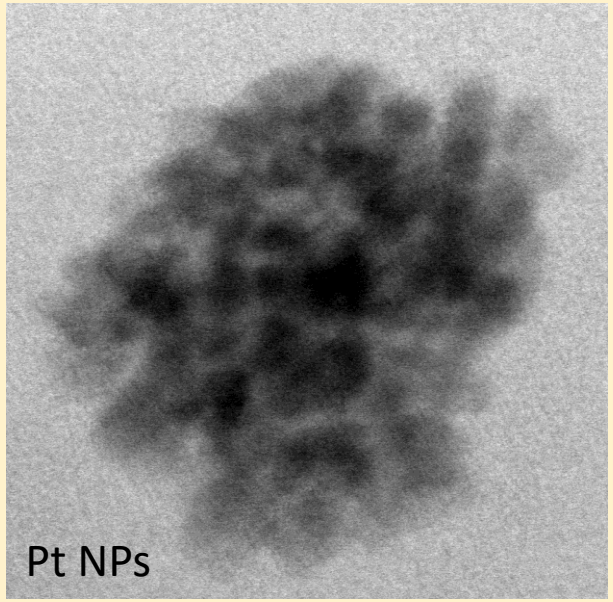
IJCLab team

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

IFJ PAN

Joséphine

Idea: The **addition of Pt NPs** to cancer cells and subsequent **proton irradiation** leads to **higher cell mortality** than **irradiation without and with Pd NPs.**



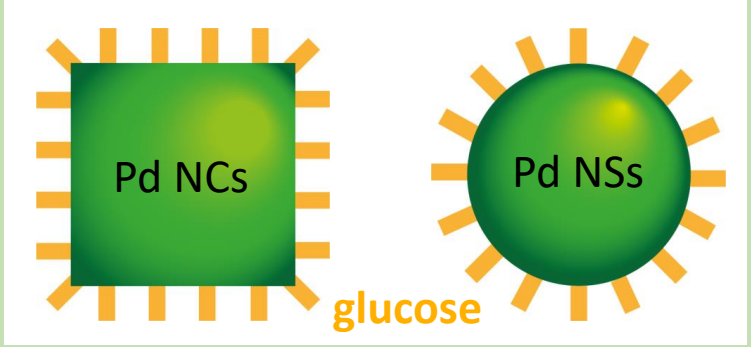
Experiment: In situ long-term, real-time holotomographic microscopy observations of Pd-DDS effects in cells: (i) morphological changes, (ii) absorption dynamics, (iii) interaction, (iv) accumulation sites in 3D prior and after proton irradiation.

IJCLab

Bartosz

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

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



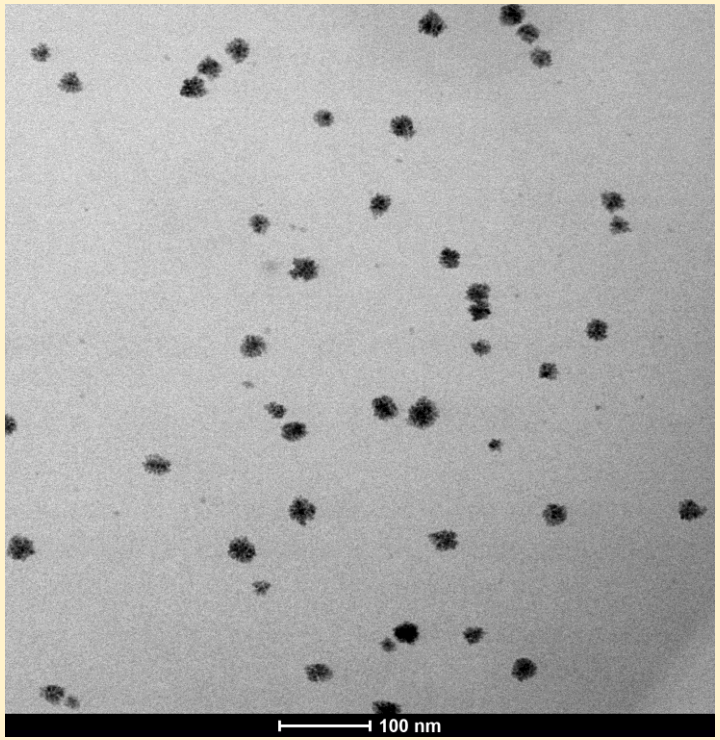
Experiment: In situ long-term, real-time videomicroscopy data acquisition of Pd NPs, drugs and Pd-DDS effects in cells: (i) morphological changes, (ii) migration dynamics, (iii) interaction, (iv) proliferation, (v) bystander effects.

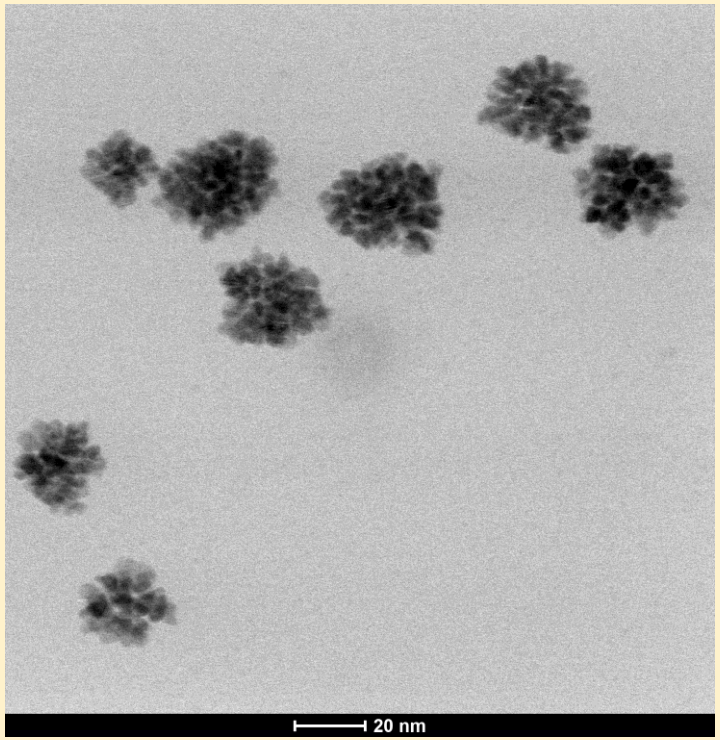
Data analysis and processing: machine learning algorithms development in order to obtain an emulator to predict cell behavior.

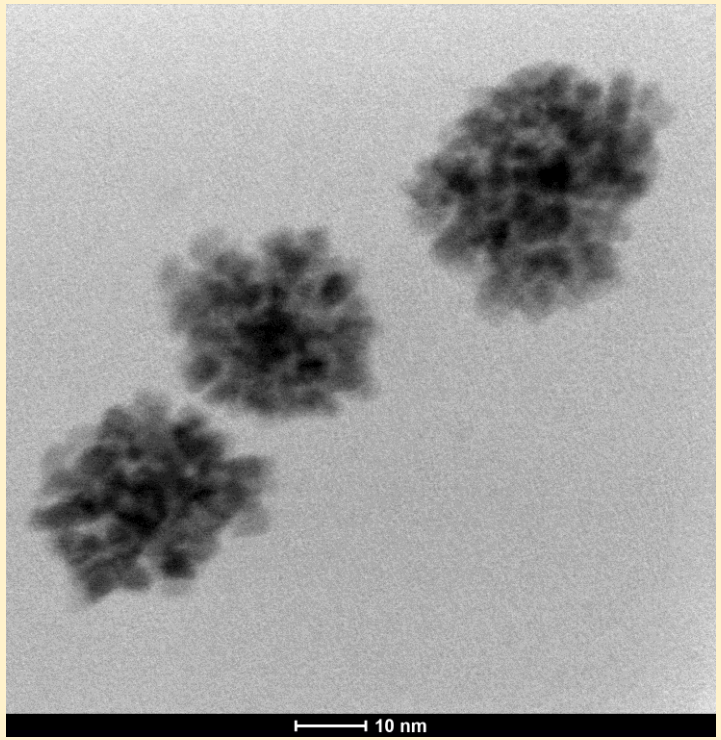
Results obtained during the visit of Joséphine Courouble @ IFJ PAN 9. – 27.09.2024

Two months ago, Joséphine send us her cell lines, which were cultured by Bartek to be ready for measurements

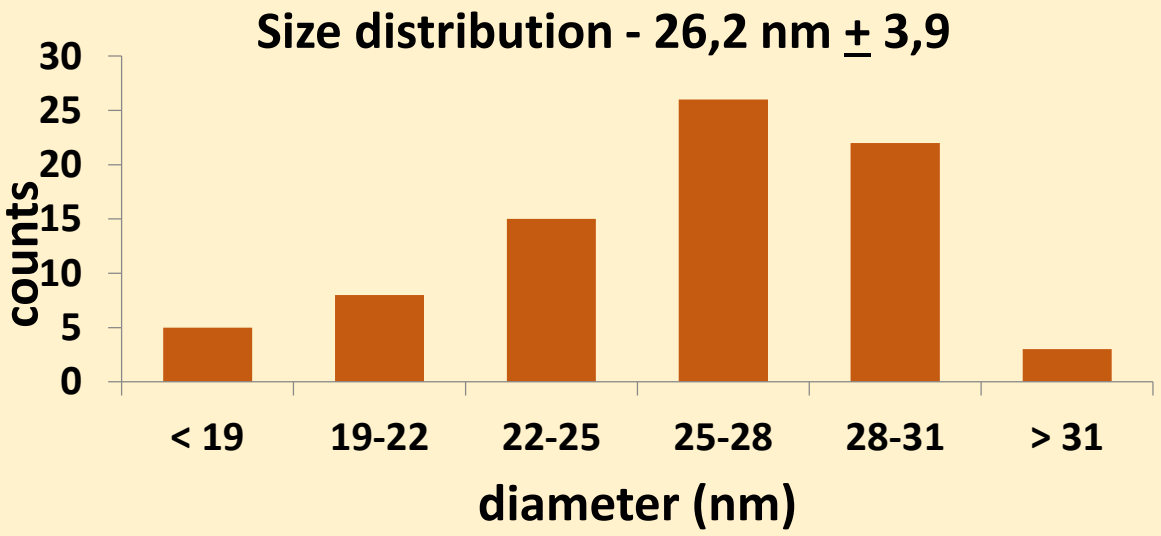
Morphology and size distribution of Pt NPs







TEM images of the porous, flower-shaped Pt NPs



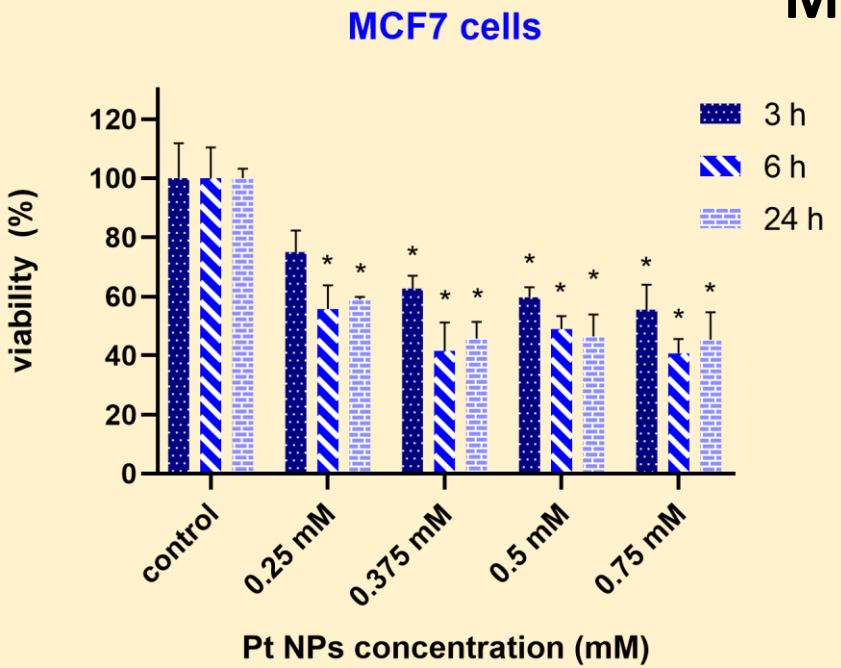
2 cell lines of breast cancer MCF7 and MDA-MB 231

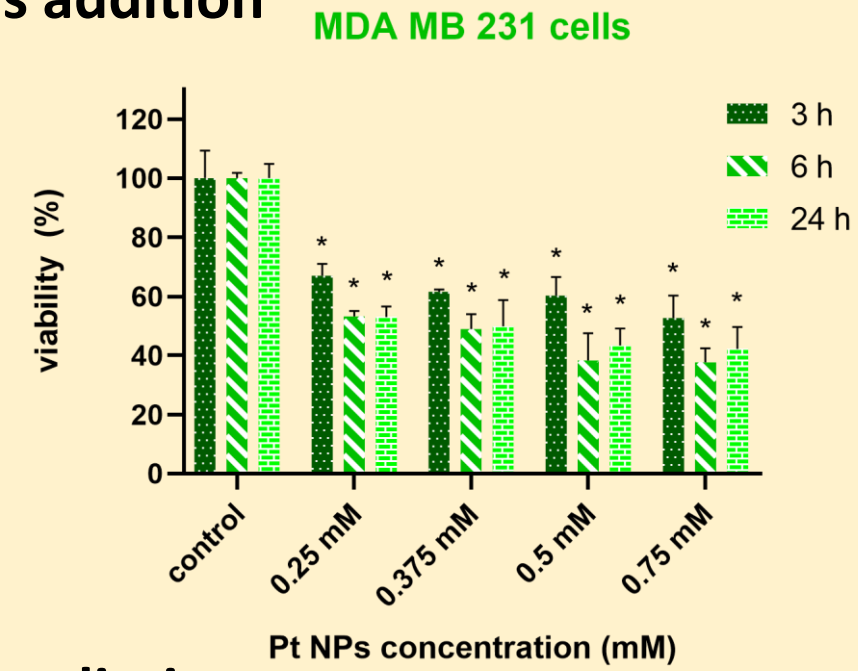
IJC Lab: X-ray irradiation with Pt NPs

IFJ PAN: proton irradiation with Pt NPs

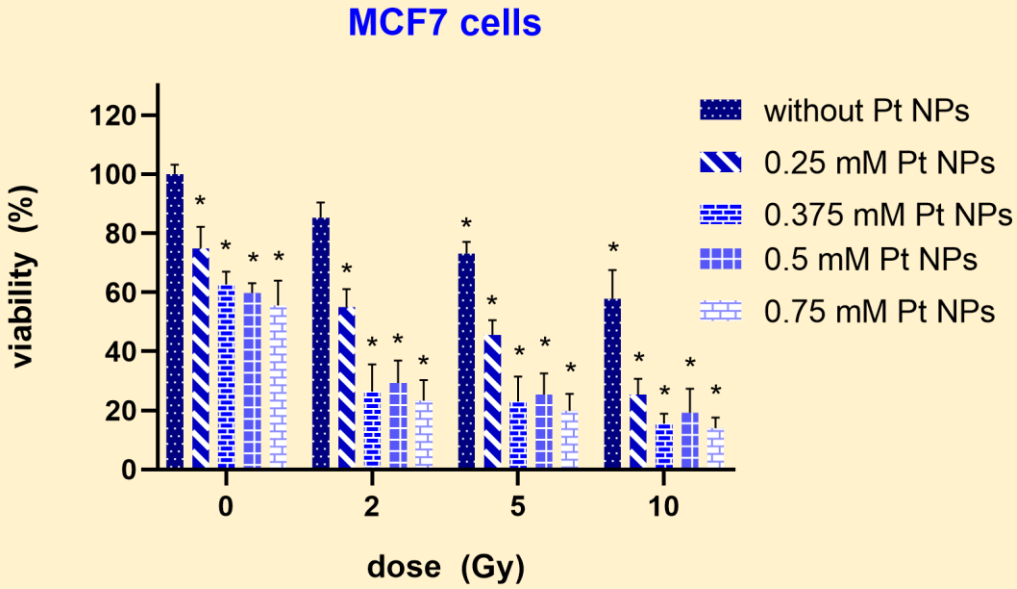
4 concentrations of Pt NPs:
0.25 mM, 0.375 mM, 0.50 mM and 0.75 mM

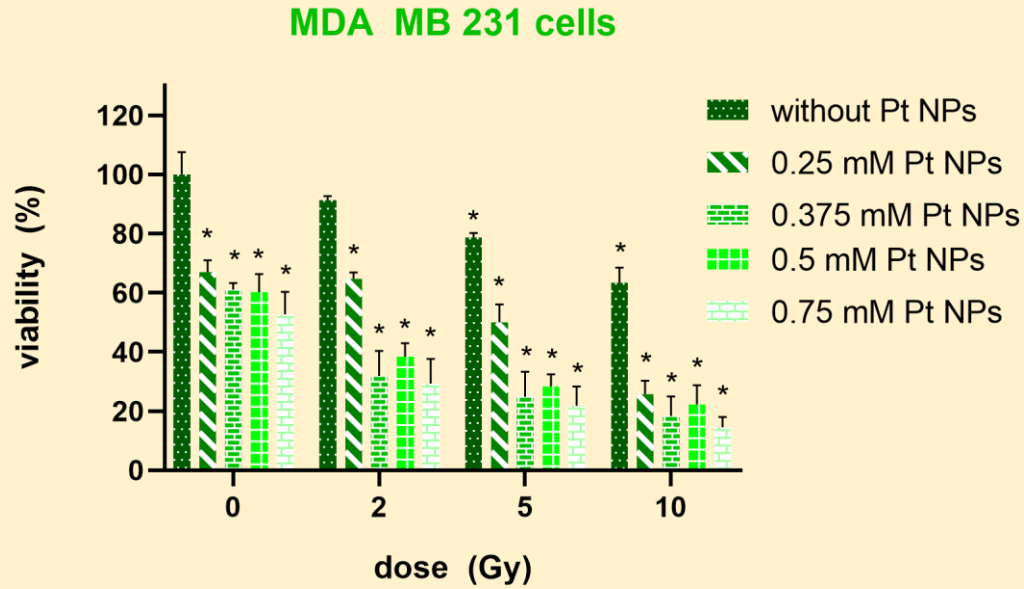
MTS results – after NPs addition





MTS results – after proton irradiation





IFJ PAN

Holotomography superresolution microscope Nanolive 3d cx-a

NON-INVASIVE 3D CHARACTERIZATION

Live cell imaging in physiological conditions without any bleaching or phototoxicity

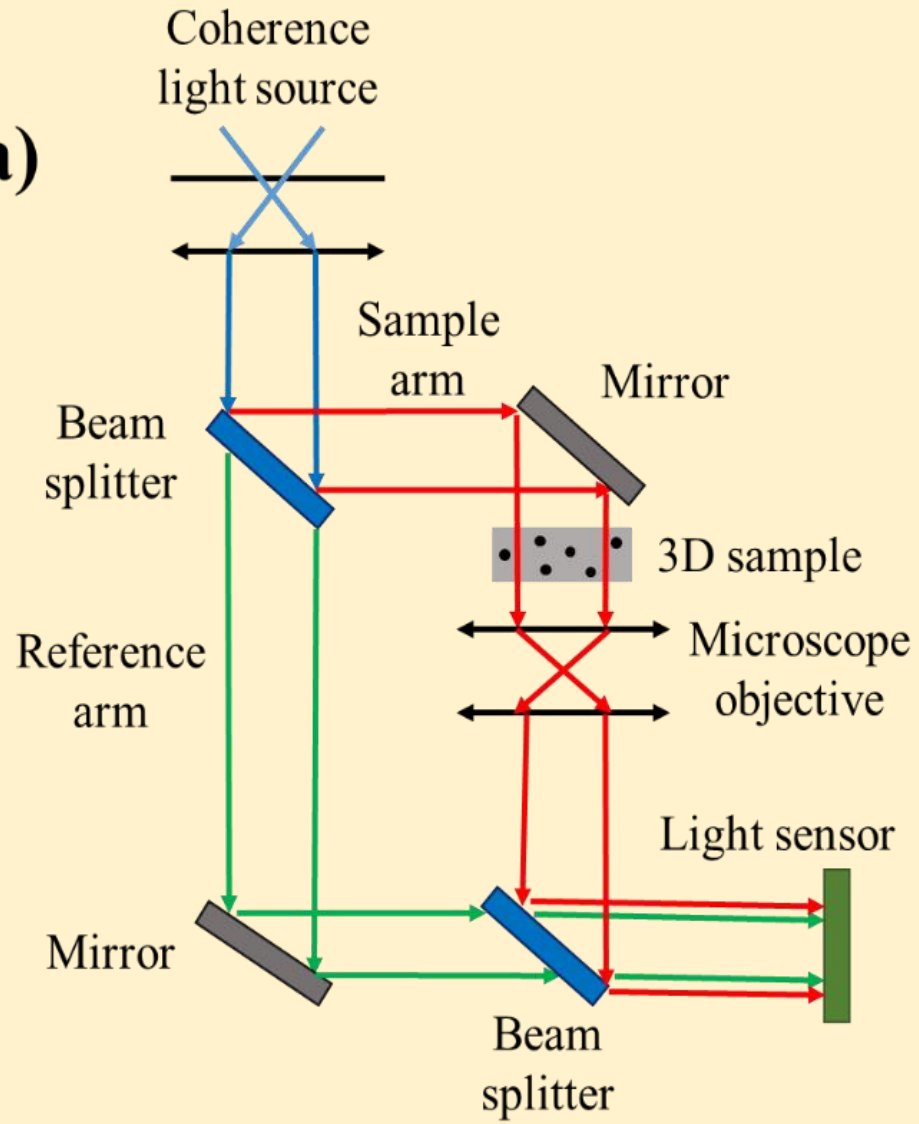
LABEL-FREE 4D CONTINUOUS OBSERVATION

Measurement of cell processes from seconds to weeks

MULTIPLEXING

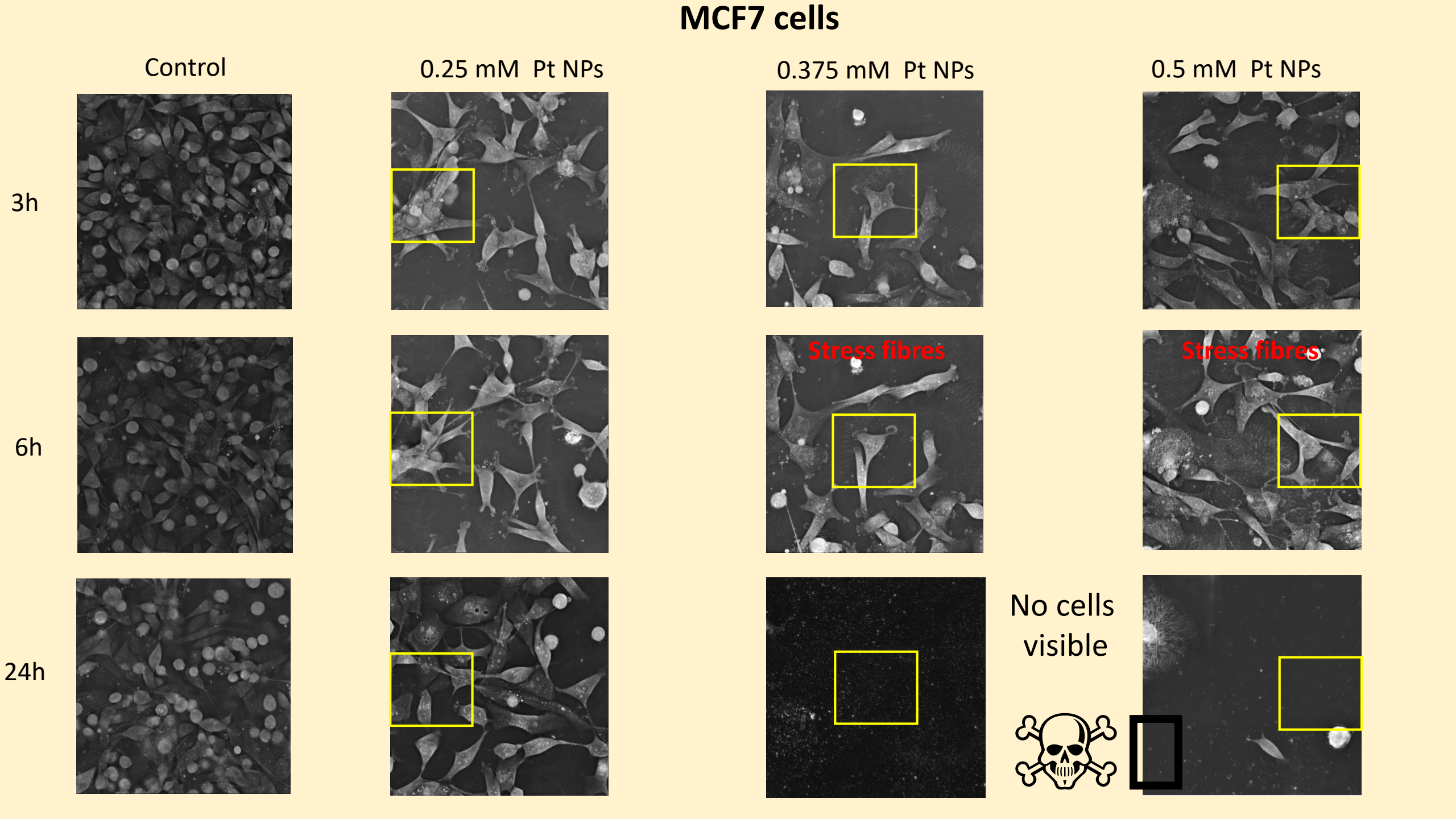
High resolution and high sensitivity characterization of multiple cell organelles based on their refractive index

a)



b)

MCF7 cells

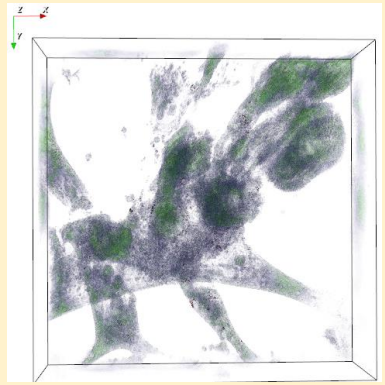

Control
0.25 mM Pt NPs
0.375 mM Pt NPs
0.5 mM Pt NPs
3h
6h
24h
Stress fibres
Stress fibres
No cells visible

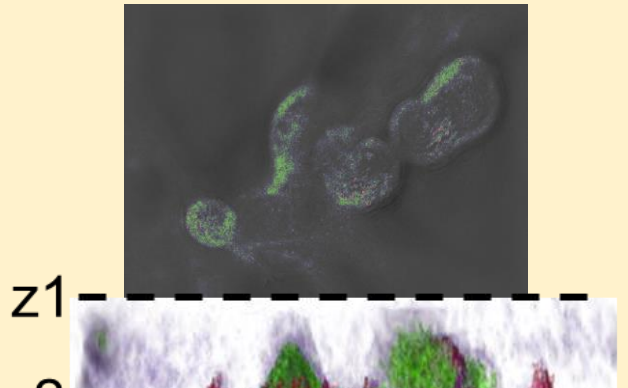
MCF7 cells + 0.25 mM Pt NPs

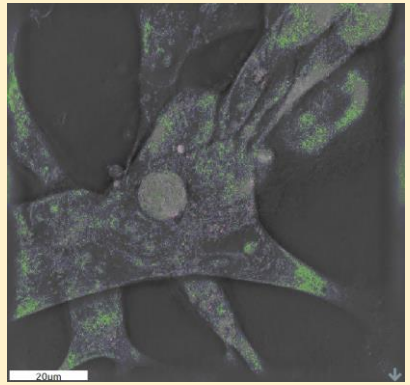
Pt NPs cell membrane
cytoplasm & cell nucleus

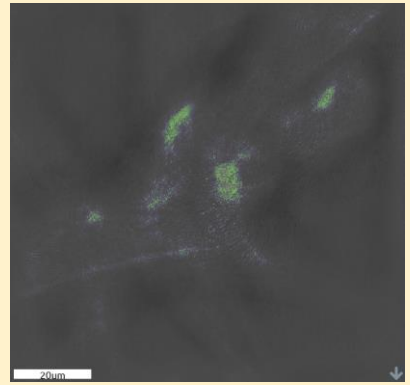
RI distribution | Z1 | Z2 | Z3

3h

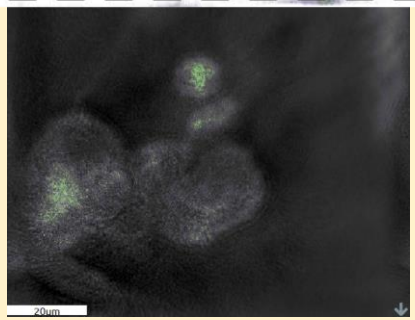


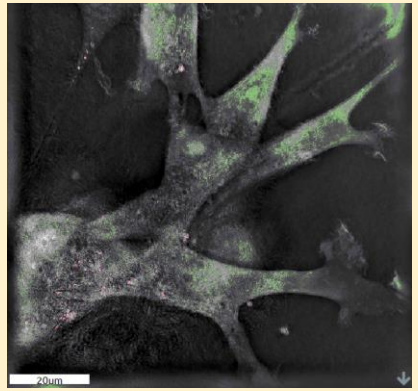


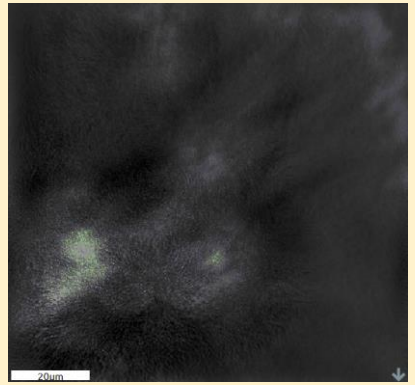




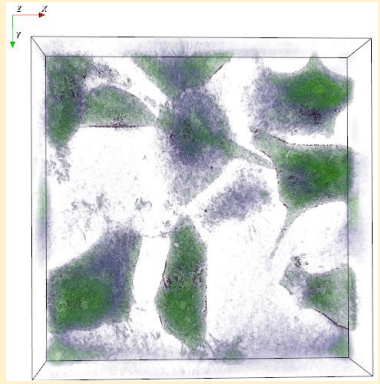
6h

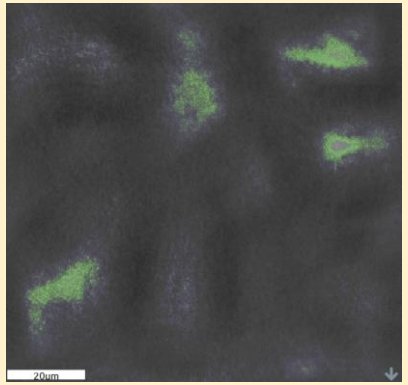


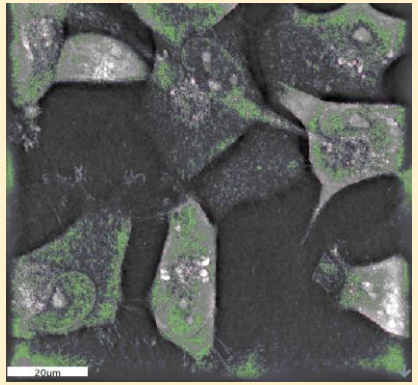


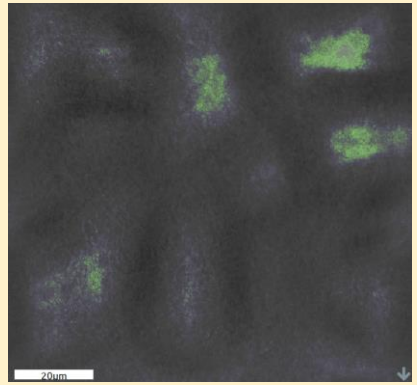


24h

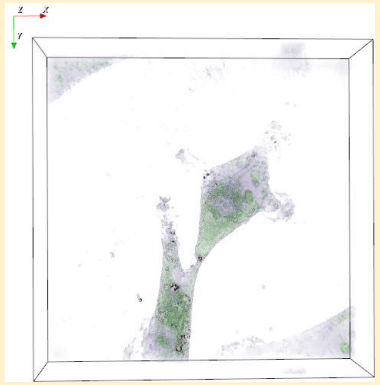
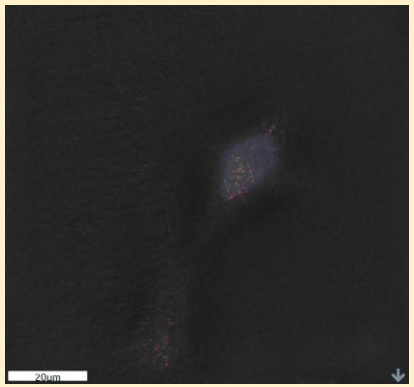
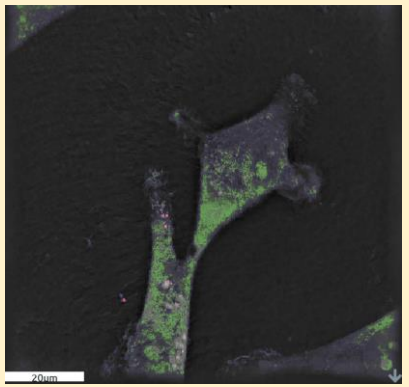
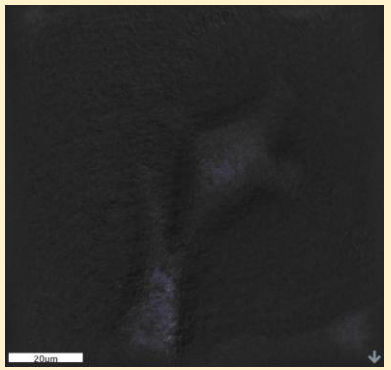
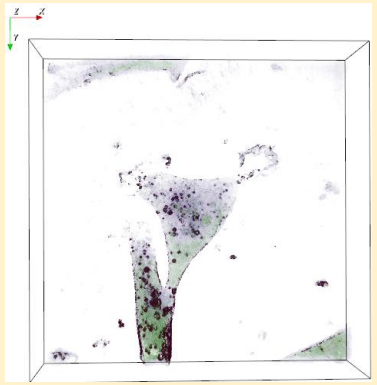
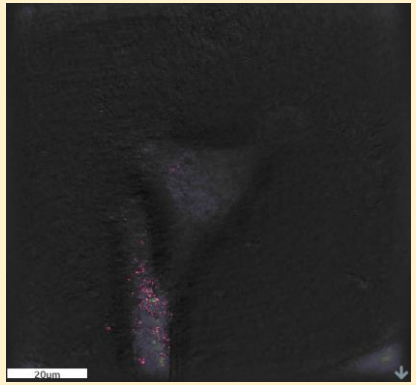
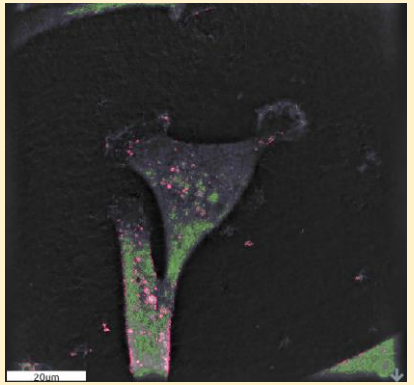
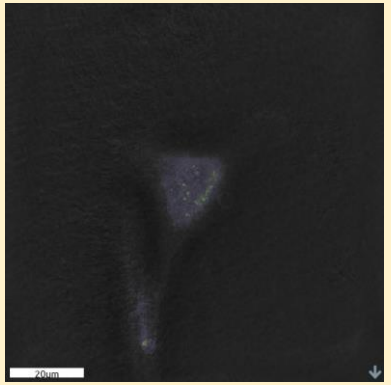
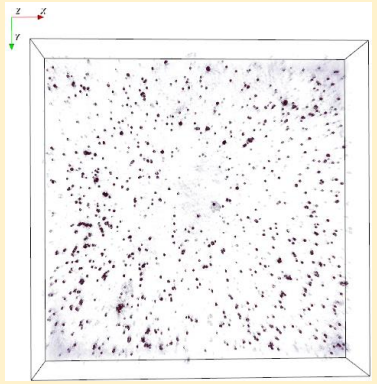
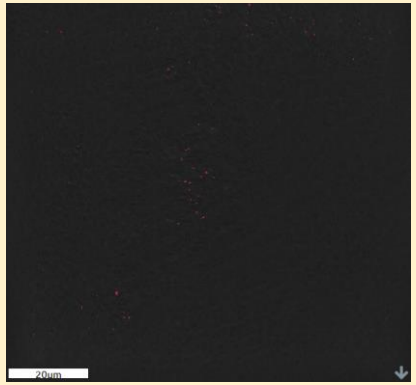
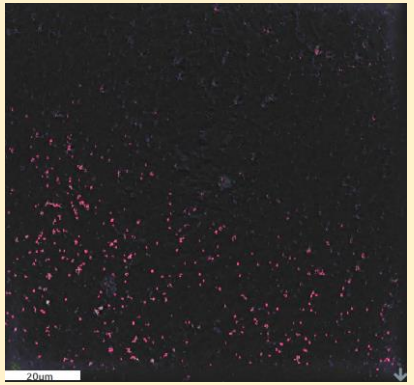
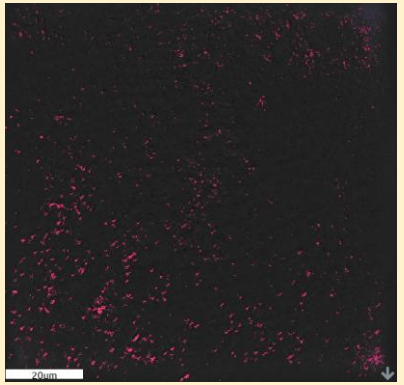




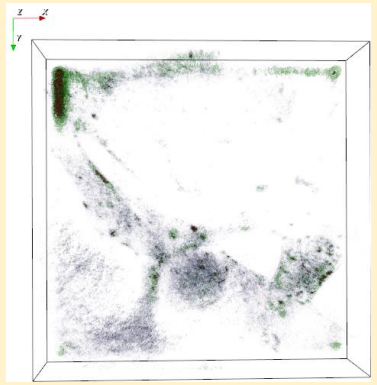
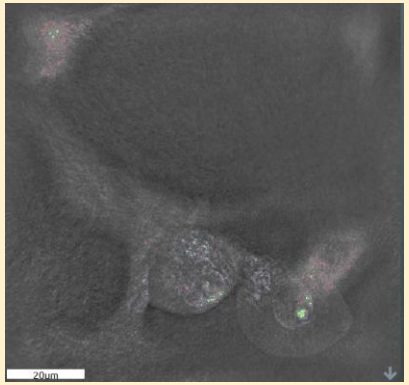
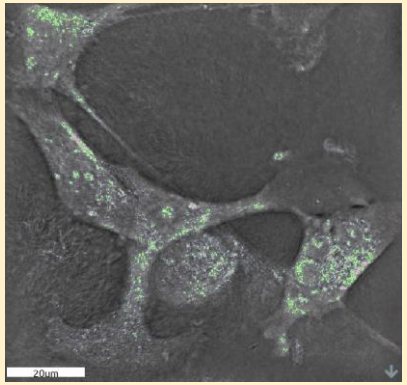
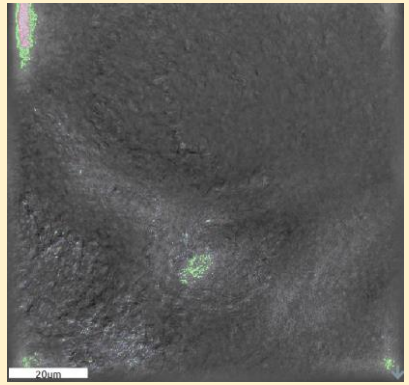
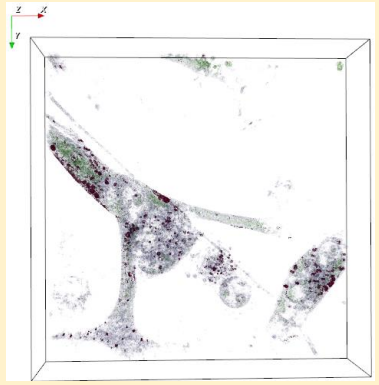
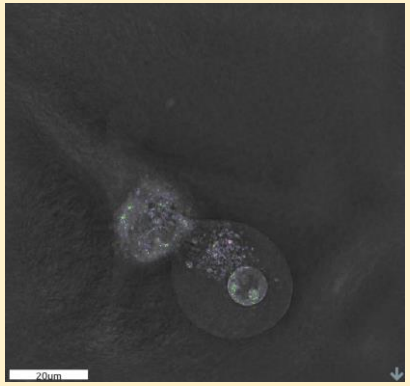
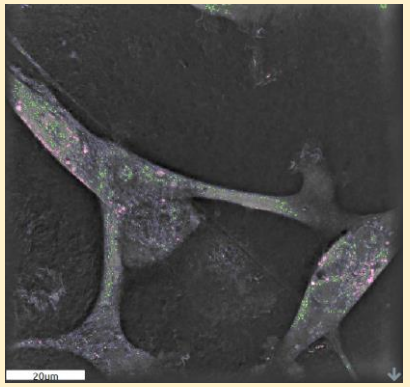
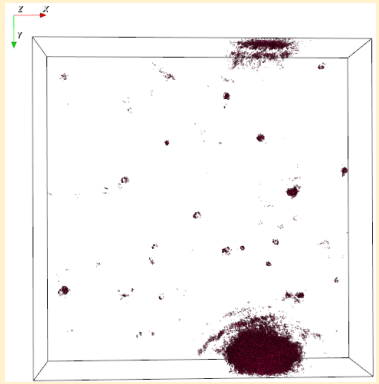
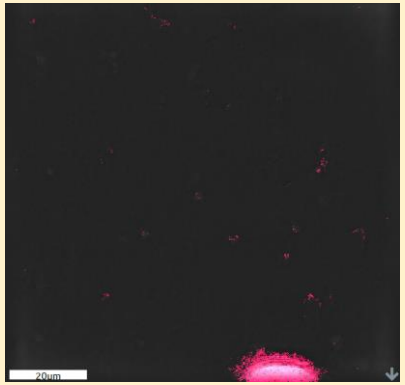




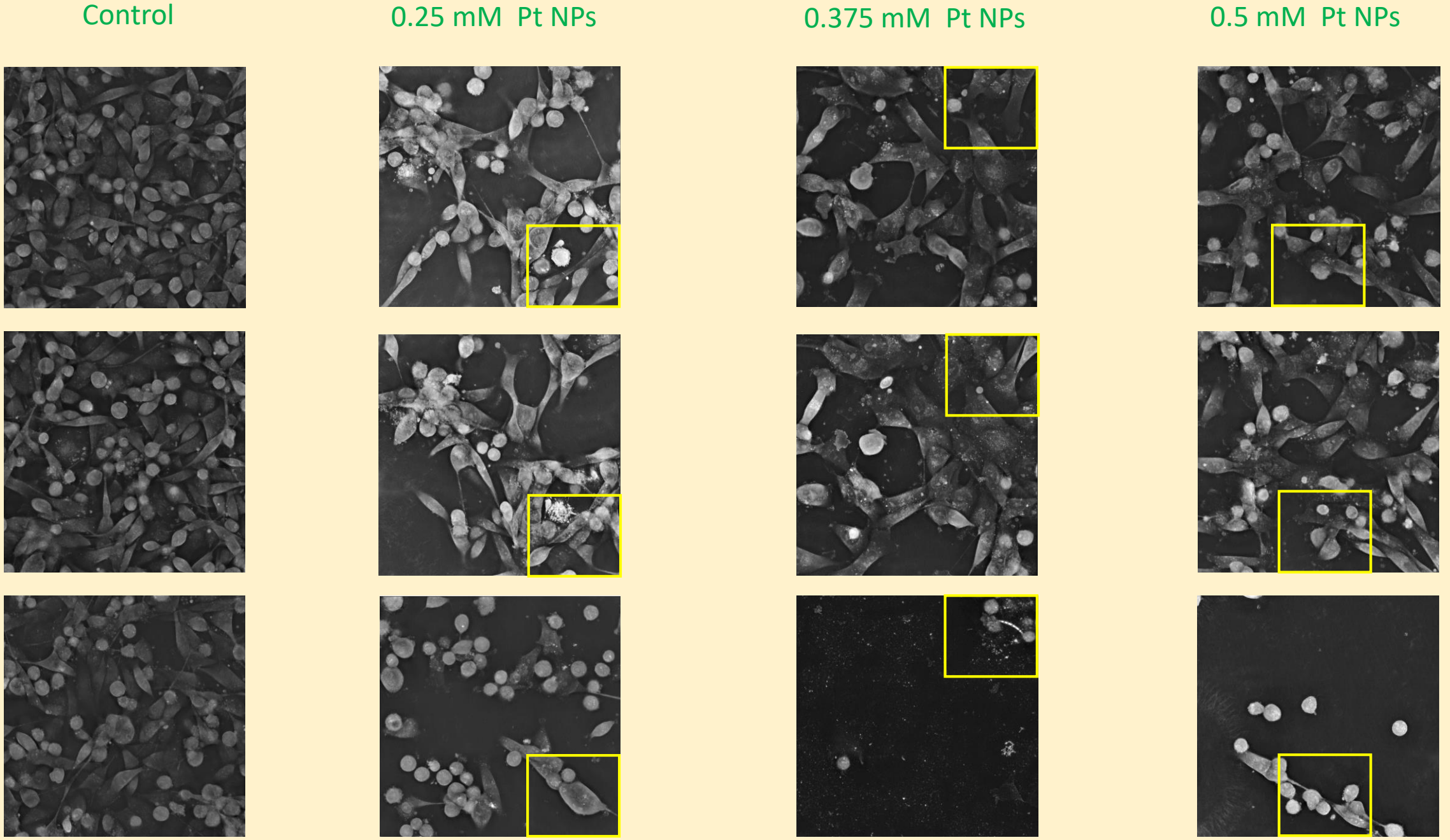
MCF7 cells + 0.375 mM Pt NPs

	RI distribution	Z1	Z2	Z3
3h				
6h				
24h				

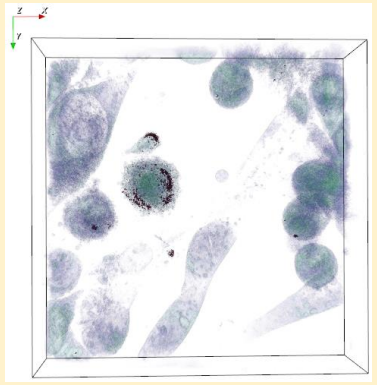
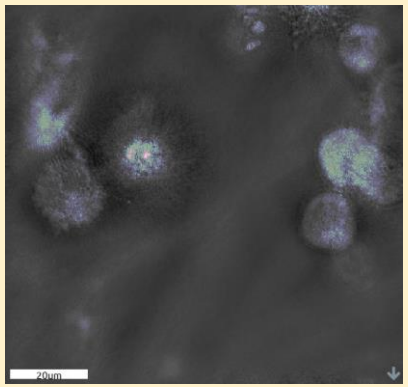
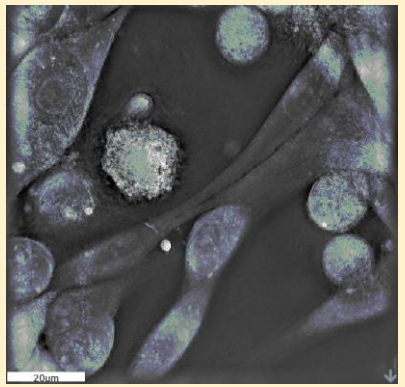
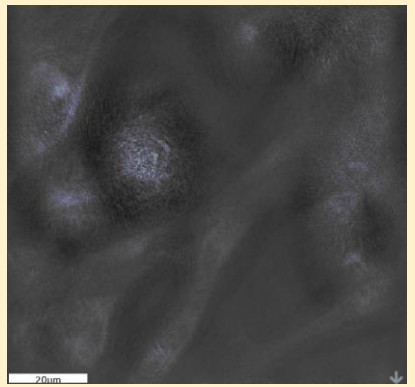
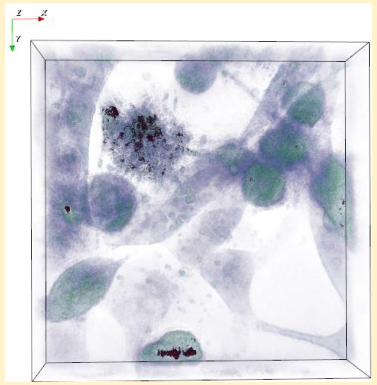
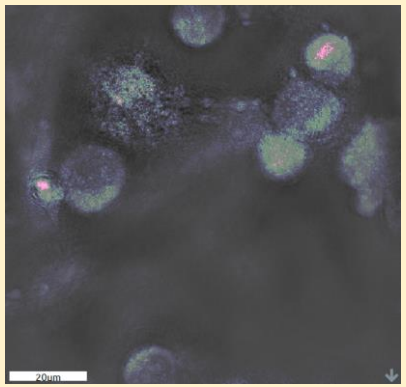
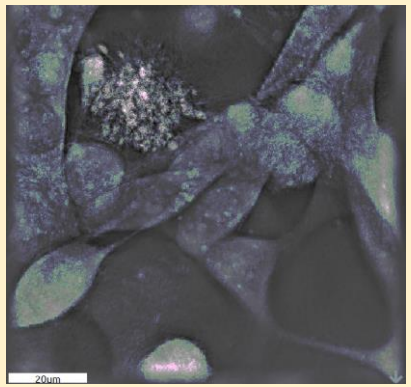
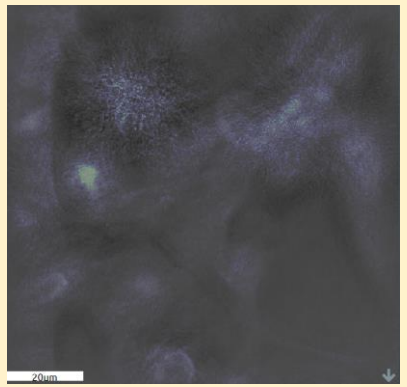
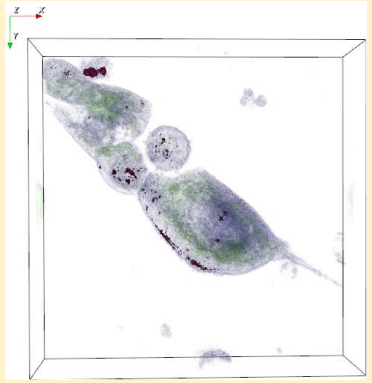
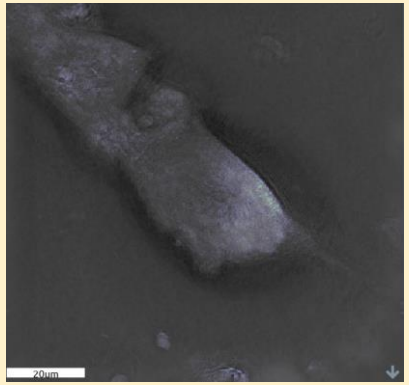
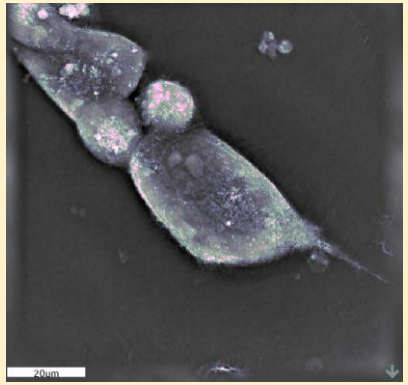
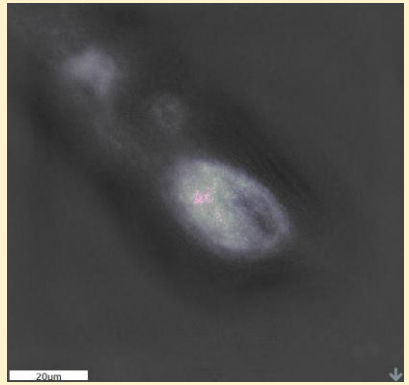
MCF7 cells + 0.5 mM Pt NPs

	RI distribution	Z1	Z2	Z3
3h				
6h				
24h				

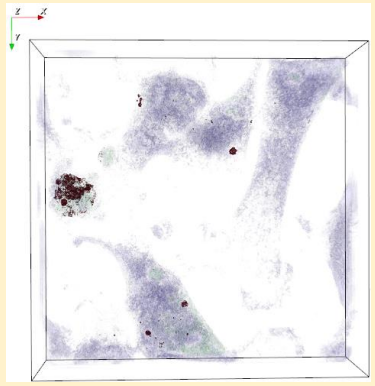
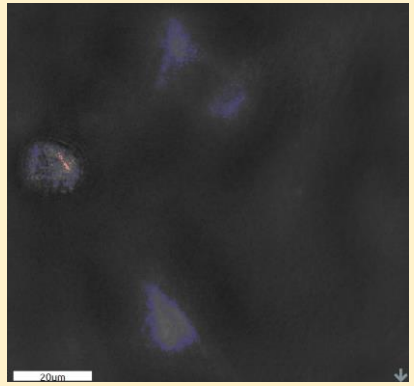
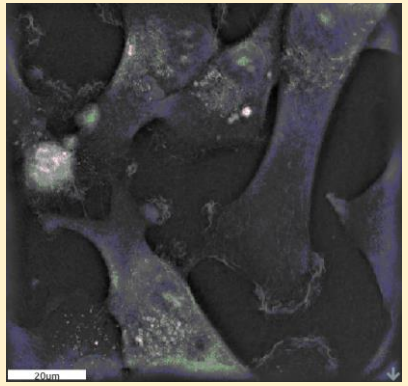
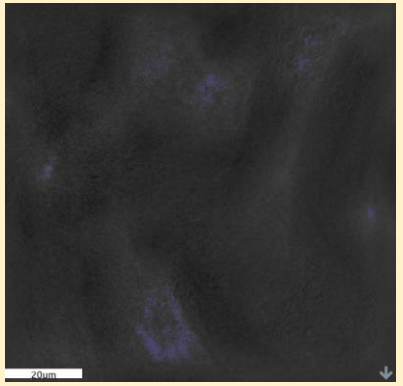
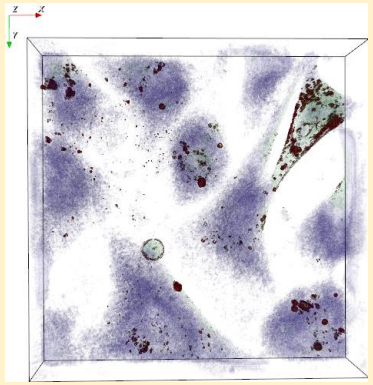
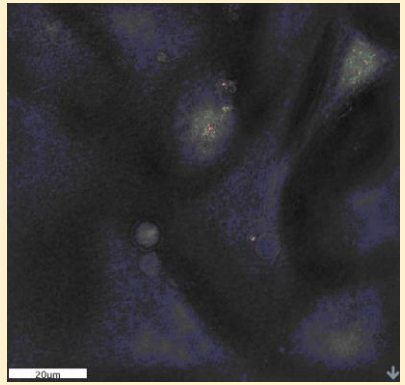
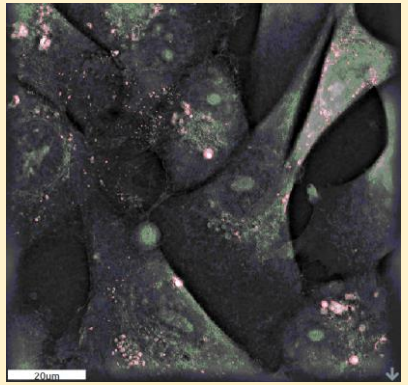
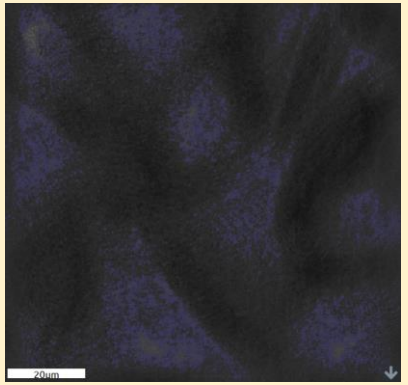
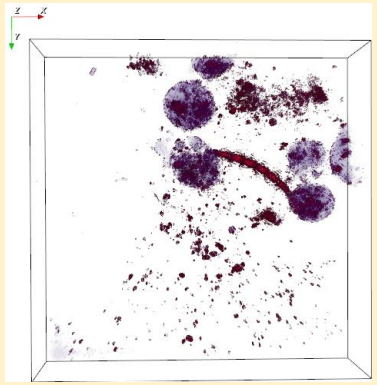
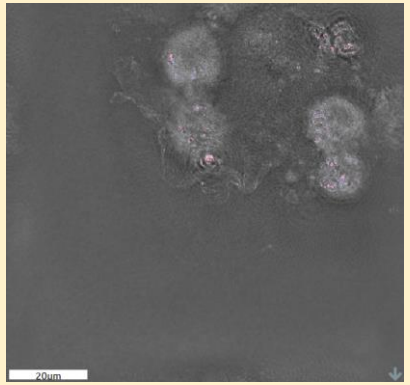
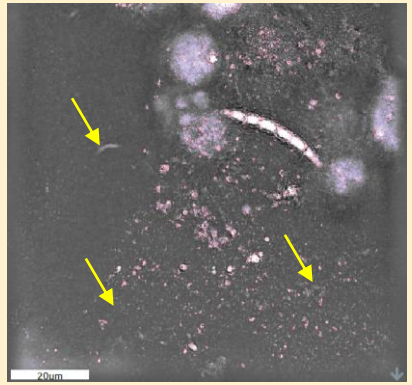
MDA-MB 231 cells



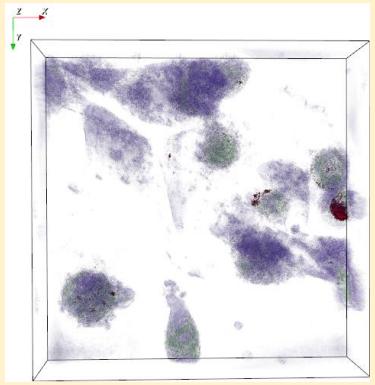
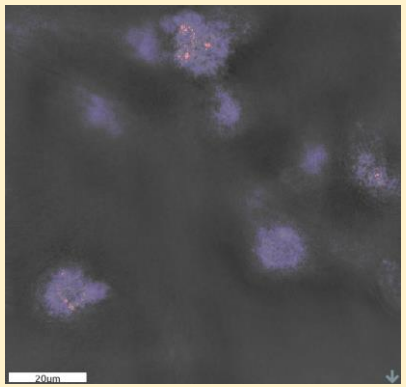
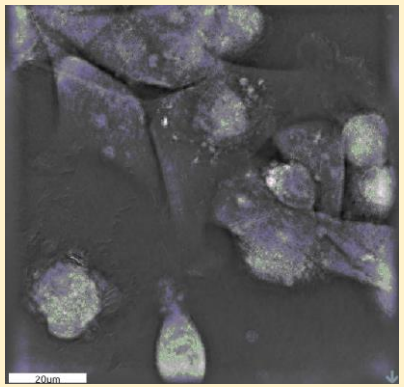
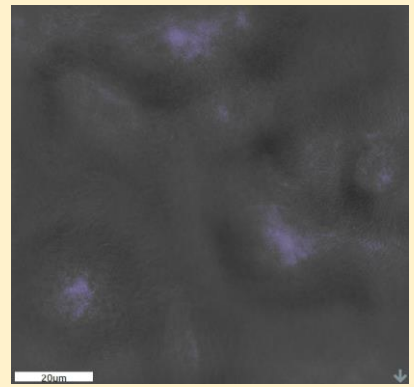
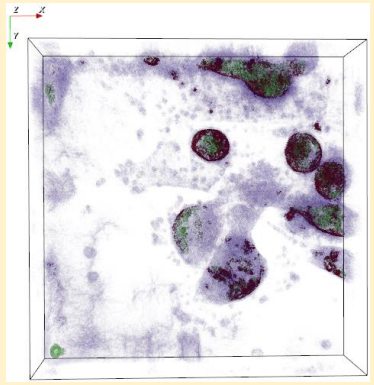
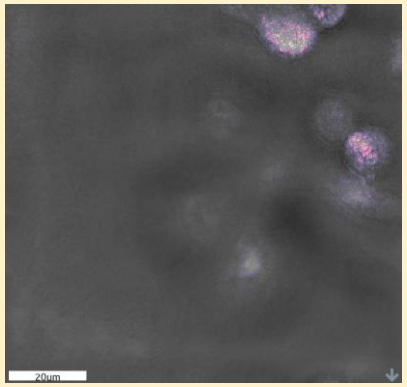
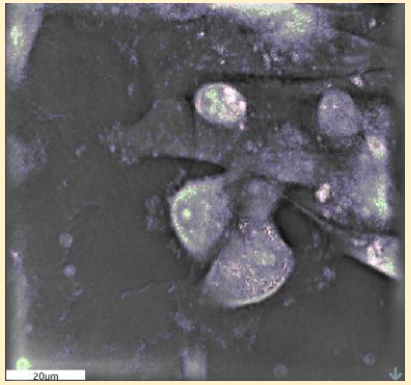
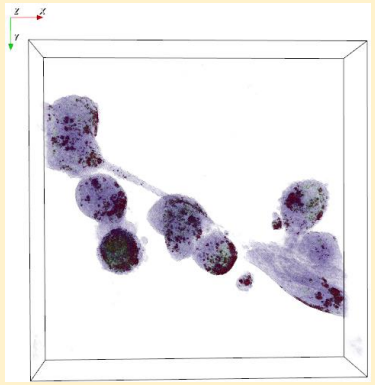
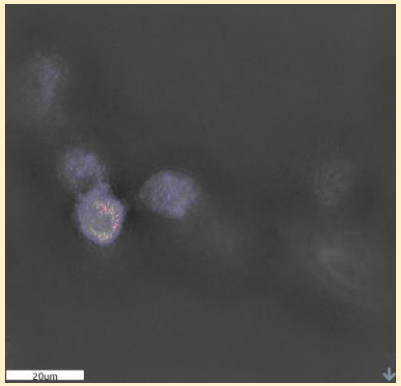
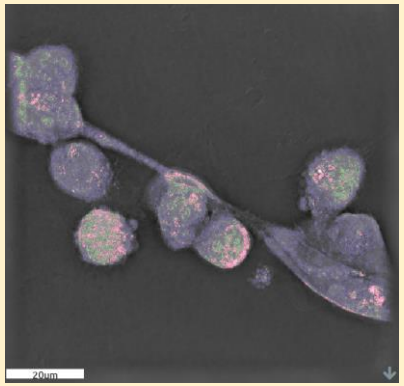
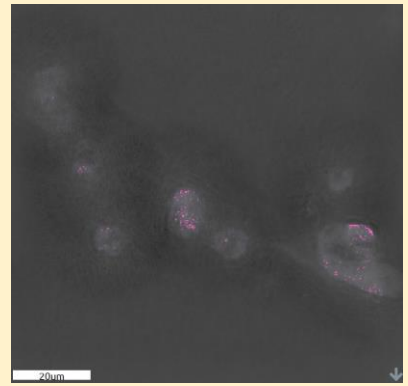
MDA-MB 231 cells + 0.25 mM Pt NPs

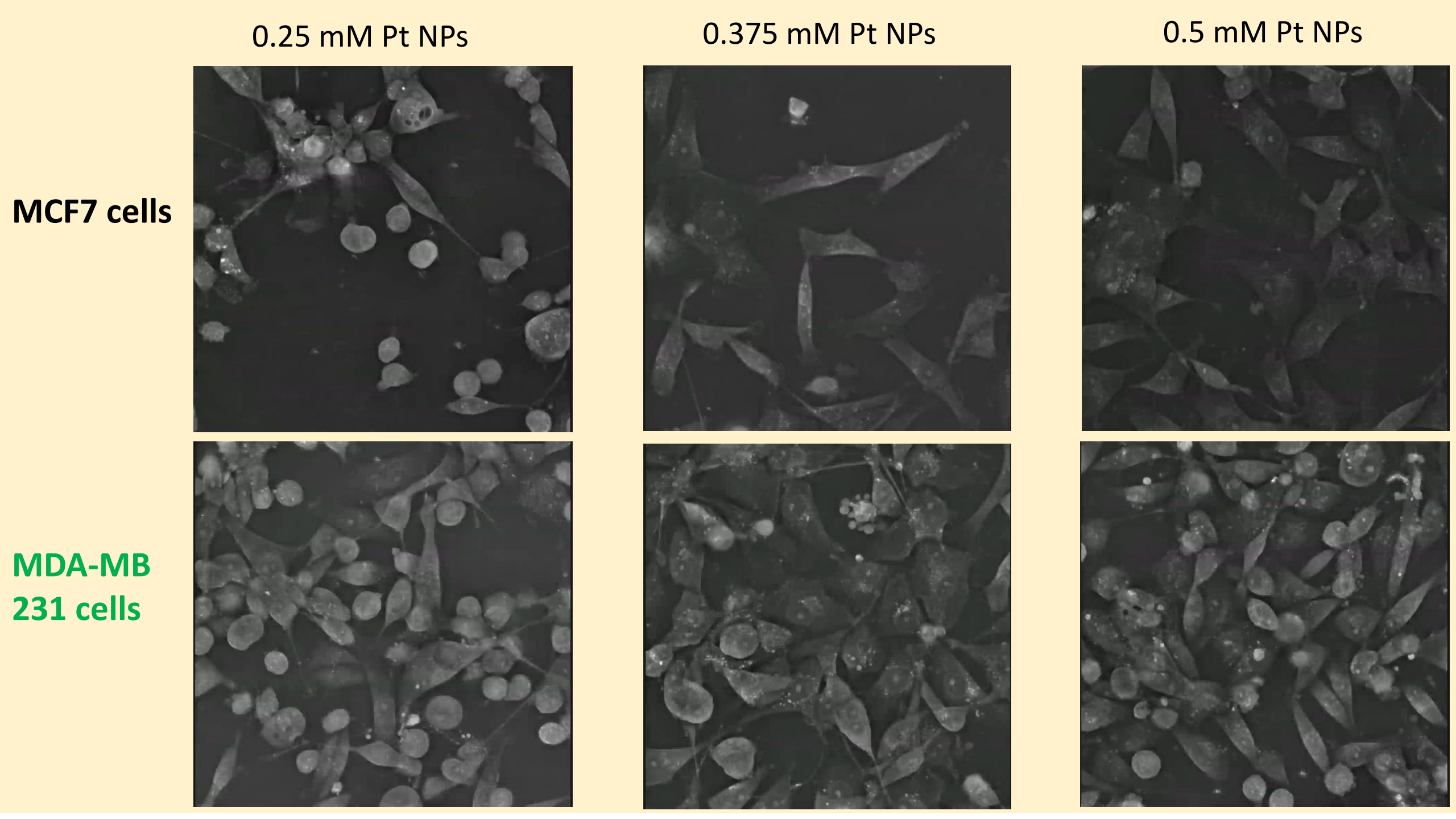
	RI distribution	Z1	Z2	Z3
3h				
6h				
24h				

MDA-MB 231 cells + 0.375 mM Pt NPs

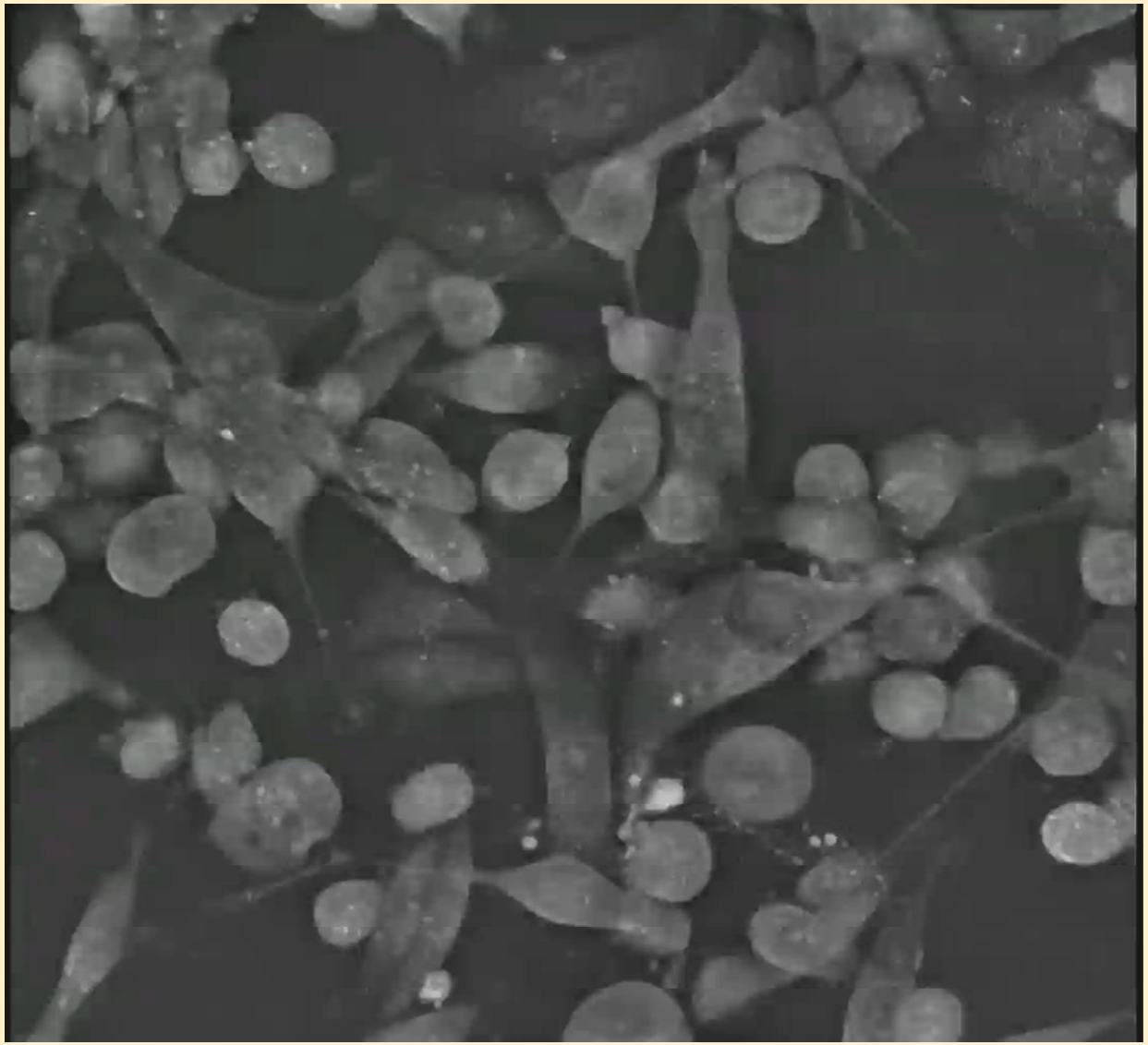
	RI distribution	Z1	Z2	Z3
3h				
6h				
24h				

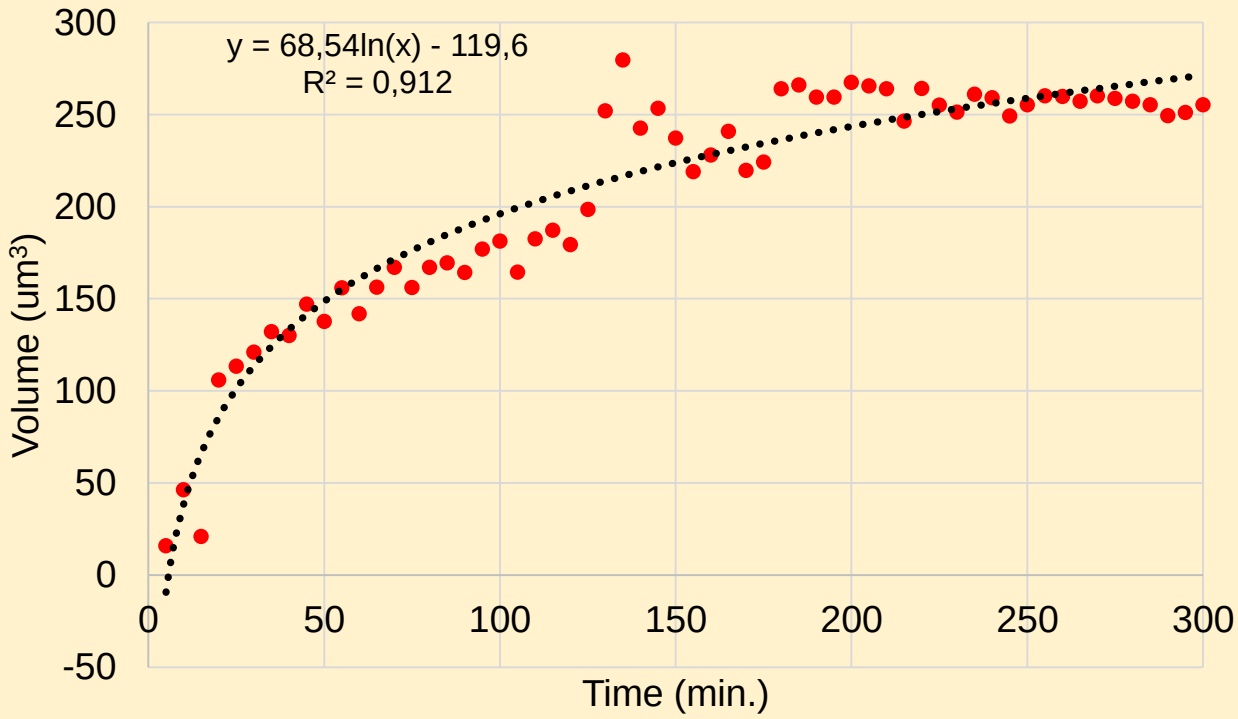
MDA-MB 231 cells + 0.5 mM Pt NPs

	RI distribution	Z1	Z2	Z3
3h				
6h				
24h				


0.25 mM Pt NPs
0.375 mM Pt NPs
0.5 mM Pt NPs
MCF7 cells
MDA-MB 231 cells

MDA-MB 231 cells + 0.25 mM Pt NPs





$$\frac{dV(t)}{dt} = S(V_{SAT} - V(t)) + D\frac{V(t)}{V_{SAT}}\left(V_{SAT} - V(t)\right), \qquad (1)$$

$$V(t) = V_{SAT}\frac{1 - e^{-(S+D)t}}{1 + \frac{D}{S}e^{-(S+D)t}}, \qquad (2)$$

Depciuch J., et al., Small 2024, 20, 2400778 Model: size & shape

Model further development:
NPs concentration and cell line type

Future plans

Dr. Bartosz Klębowski @ IJCLab 21.10 – 8.11.2024

Three months ago, Bartek send to IJCLab his cell lines, which were cultured and modified by the group of Olivier to be ready for videomicroscopy measurements

Planned measurements:

- Culture of transfected LN229 and U118 glioma cells
- X-ray irradiation of glioma cells cultured with/without spherical and cubic Pd NPs
- Analysis of control and irradiated cells using videomicroscopy

We plan to publish 2-3 publications.
Next year, we plan to submit a common project, where IJCLab (dr. Oliver Seksek) will be as a consociate.

Thank you