"Studies of spheroids formed from melanoma cell lines by means of microCT and Positron Annihilation Lifetime Spectroscopy (PALS)"

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European Funds Smart Growth

FNP Foundation for Polish Science

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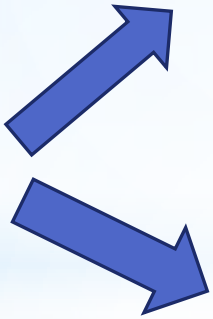
My research plan

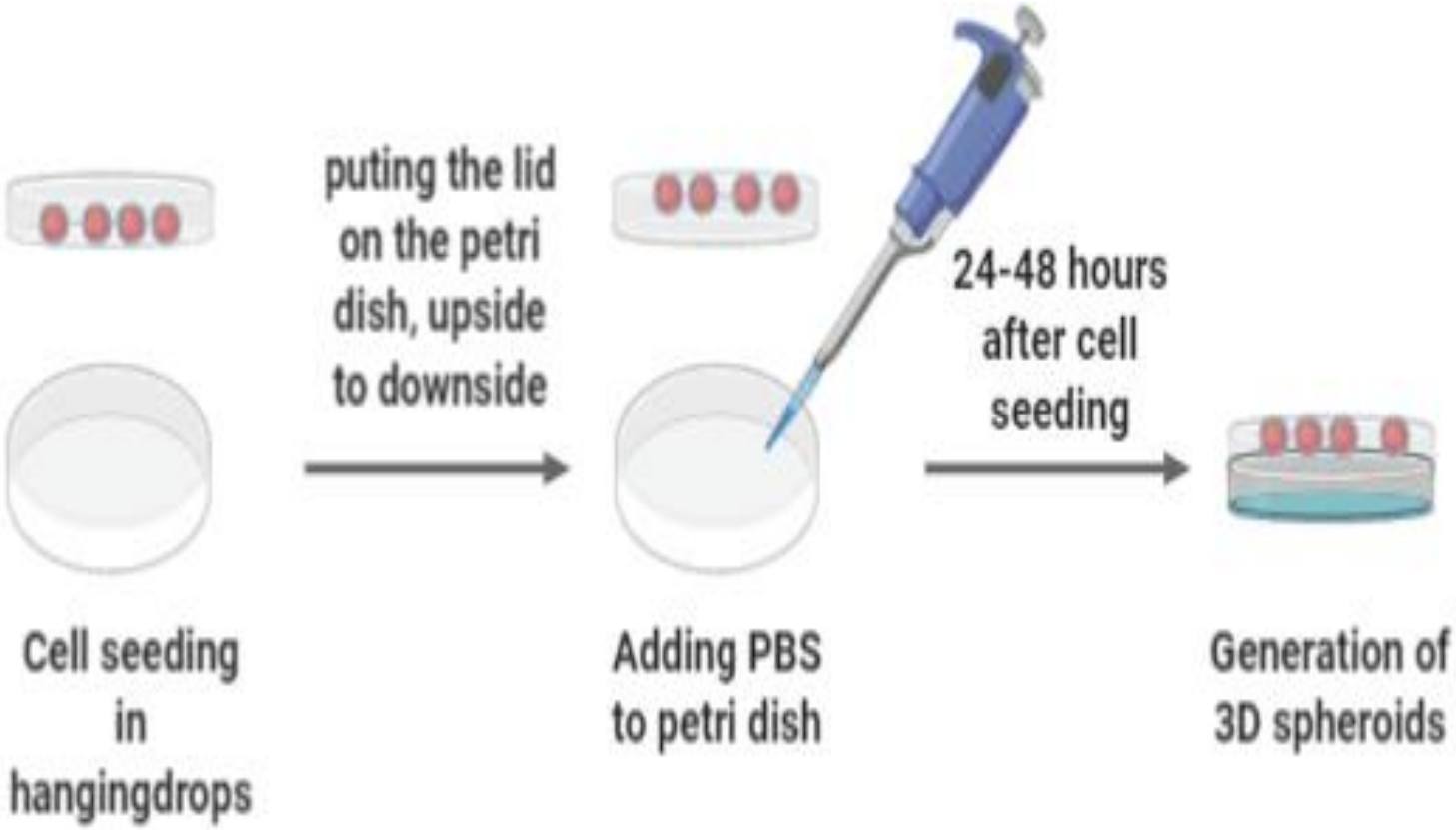


Biological experiments on Multi cellular tumor spheroids(MCTS)

Physical experiments onMulti cellular tumor spheroids(MCTS)

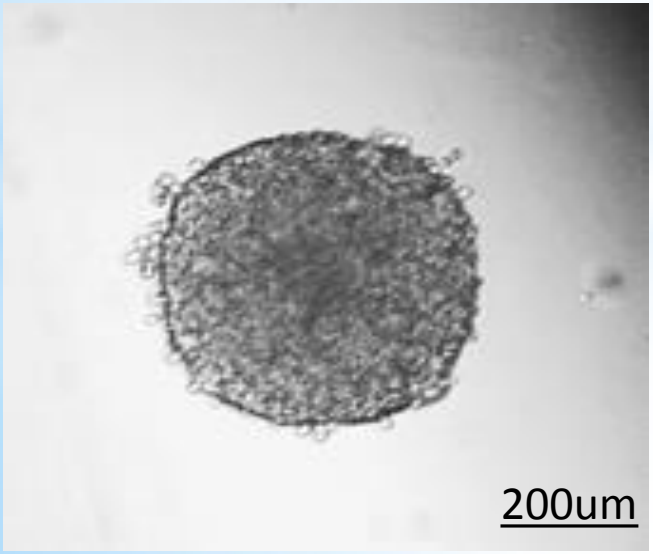
Cell line	Originate	Characteristic	Formation Method	Initial cell number	Volume of each drop
WM266	Malignant tumor	Metastatic	1.Hanging Drop 2. 5D Microplate	500,1000,1500 $1 * 10^{6}$	15 ul 0.5 ml per well
wm115	Primary tumor	Non metastatic	1.Hanging Drop 2. 5D Microplate	500,1000,1500 $1 * 10^{6}$	15 ul 0.5 ml per well
Melano cyte	Normal skin cells	Normal skin cells	5D Microplate	$1 * 10^{6}$	0.5 ml per well

Hanging drop method



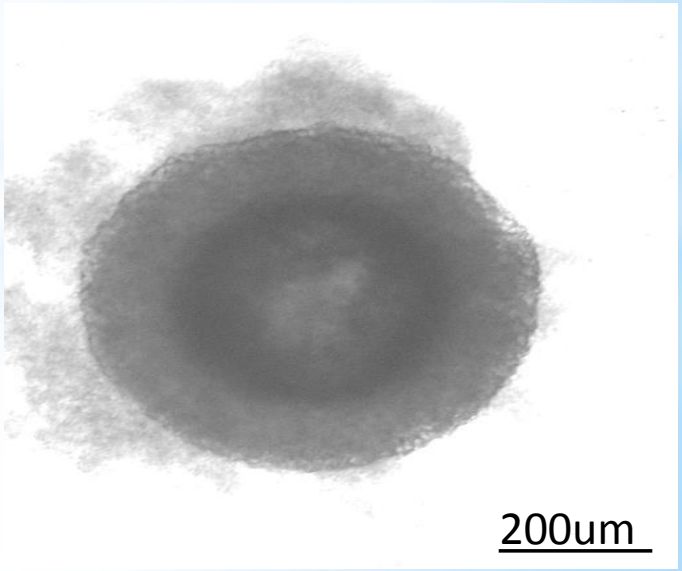
What is Spheroids?

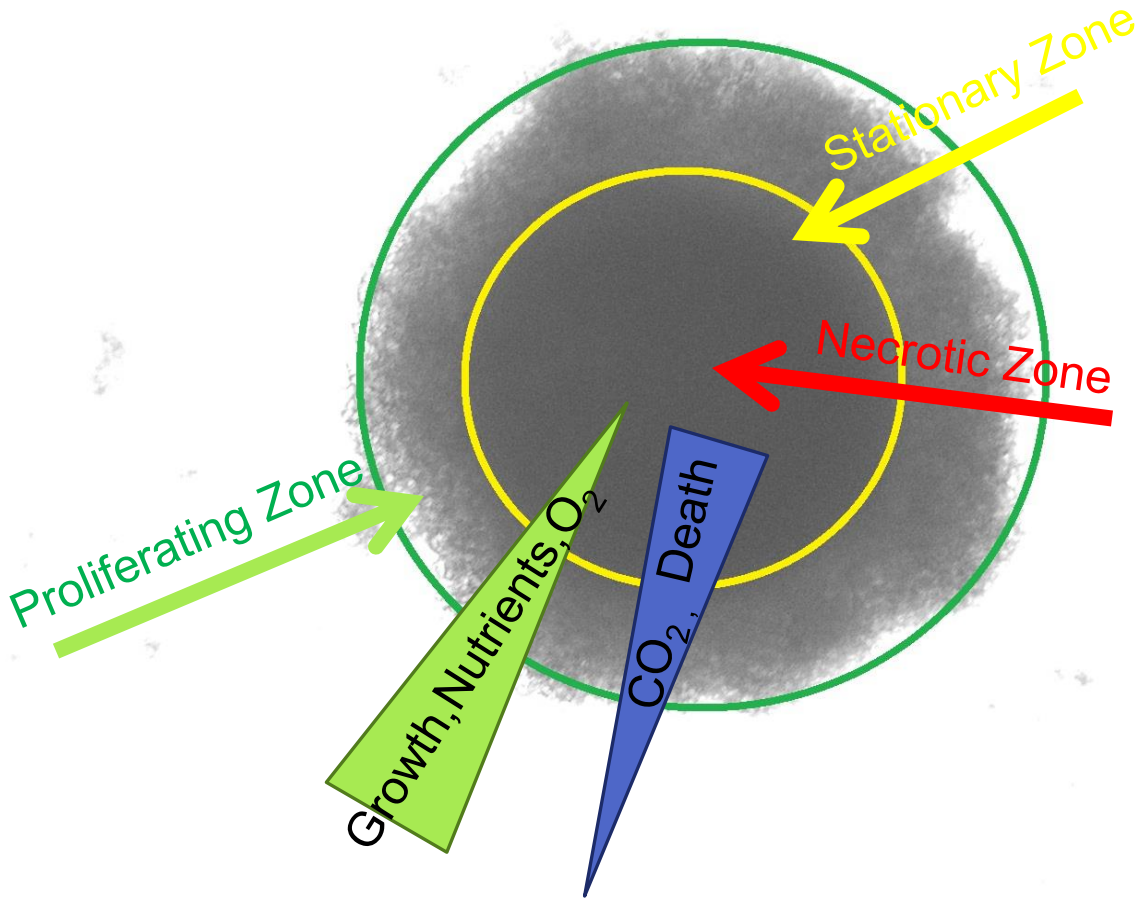
48 hours



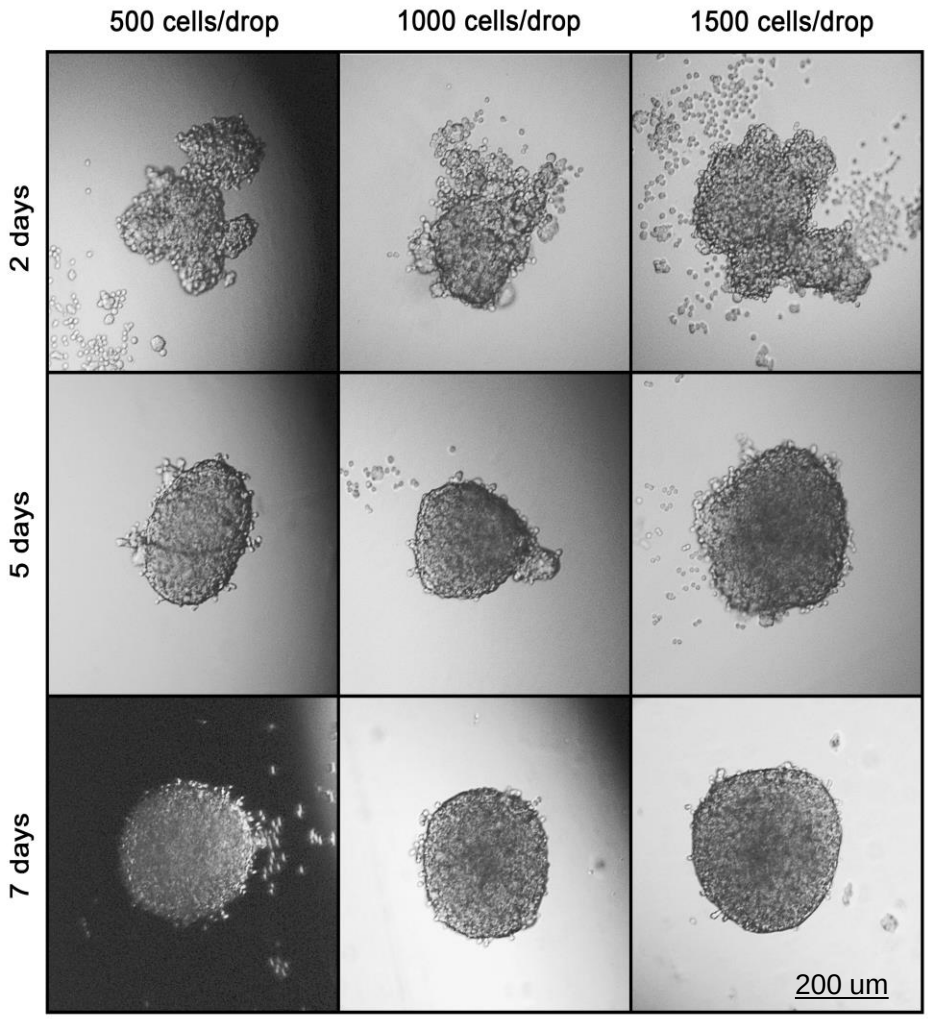
culturing

7 days

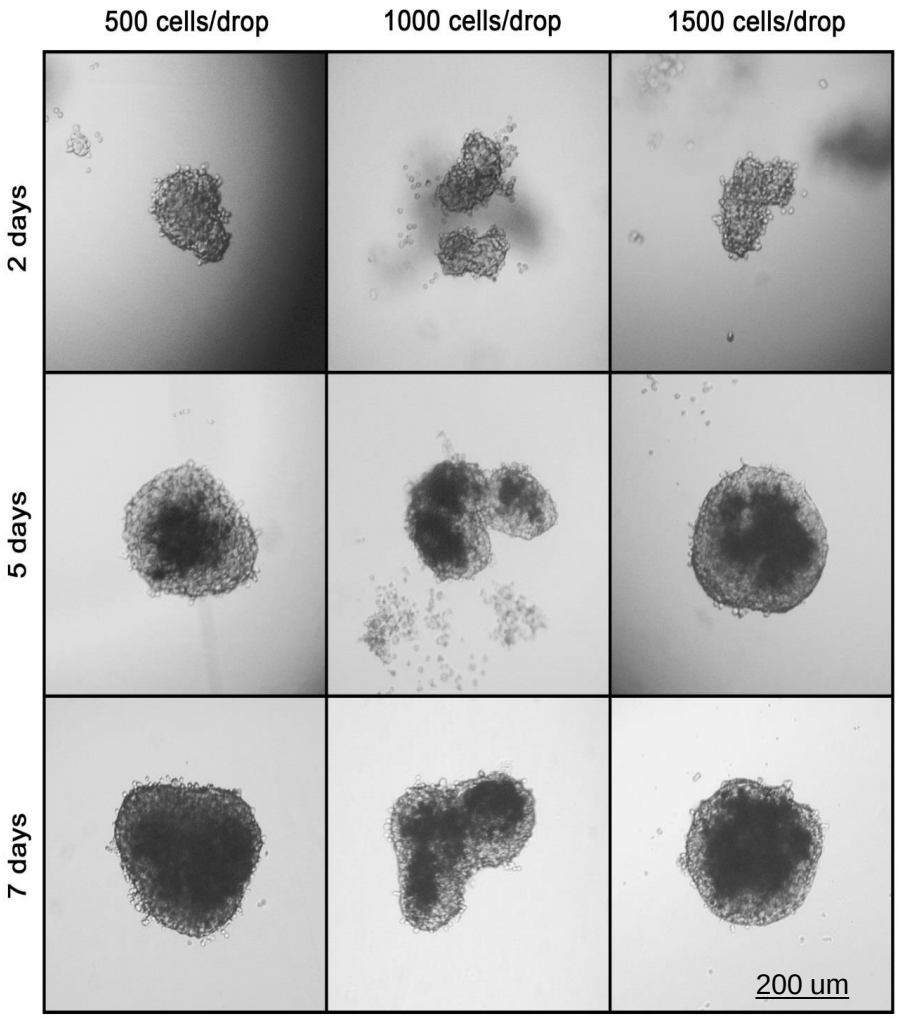




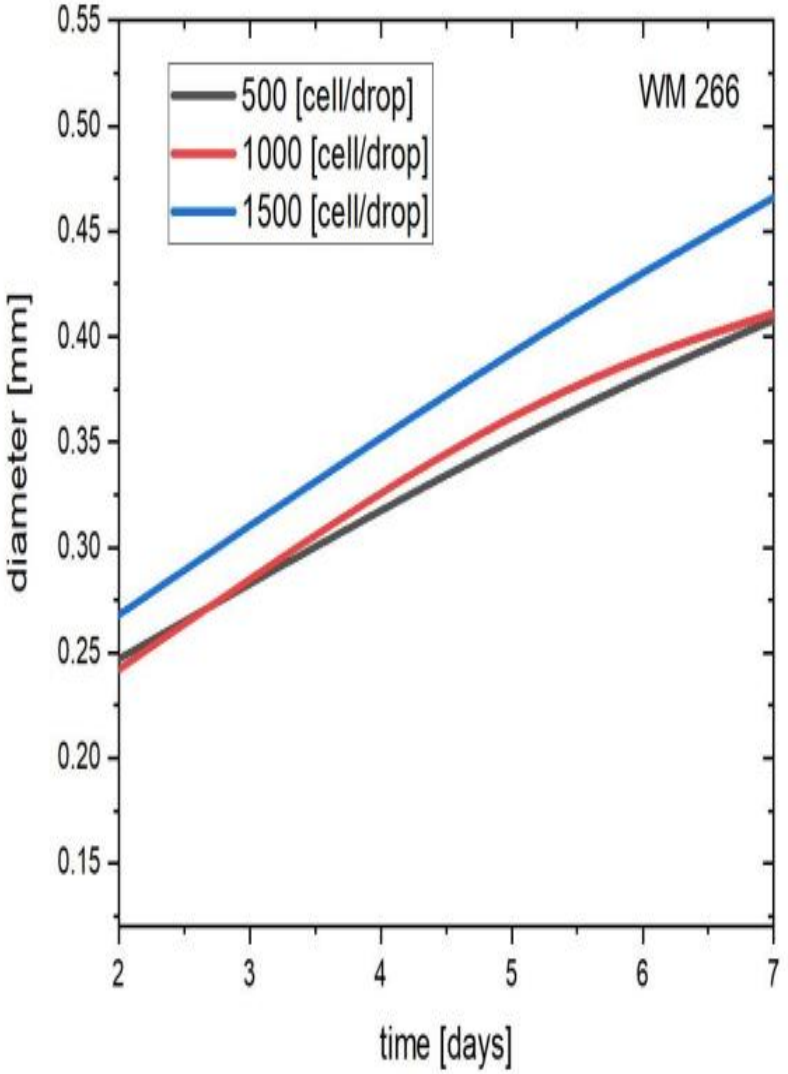
Microscopic images of spheroids

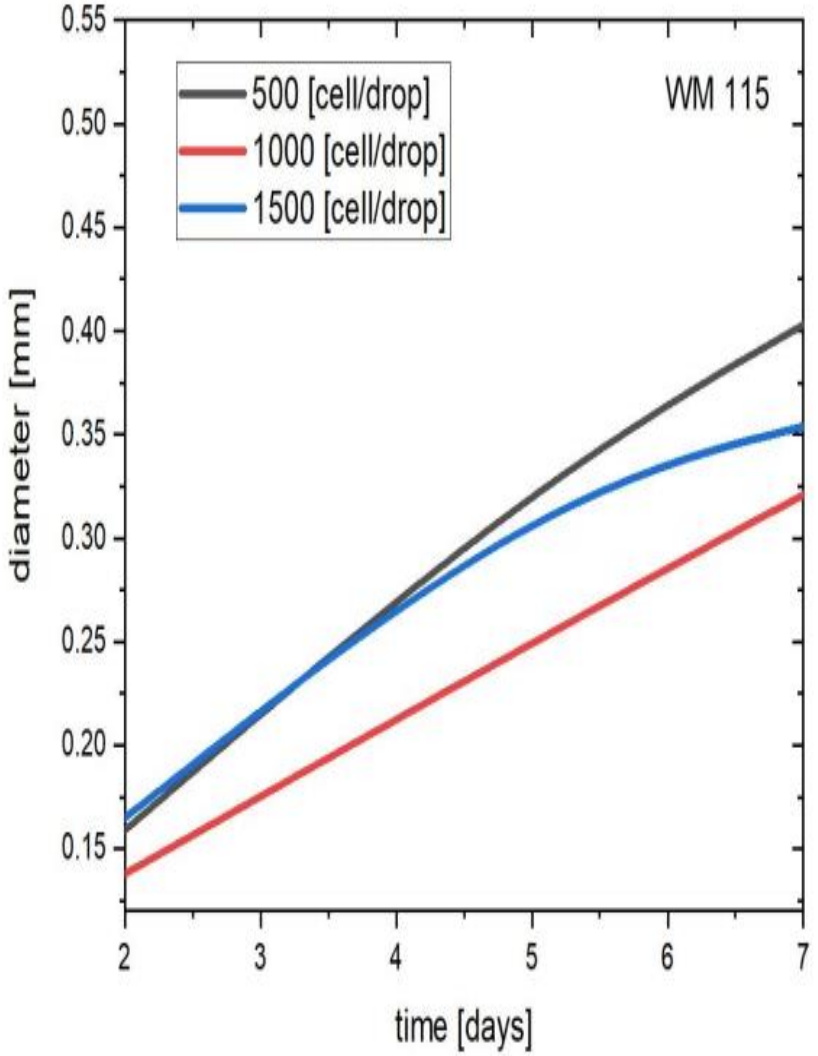


WM266



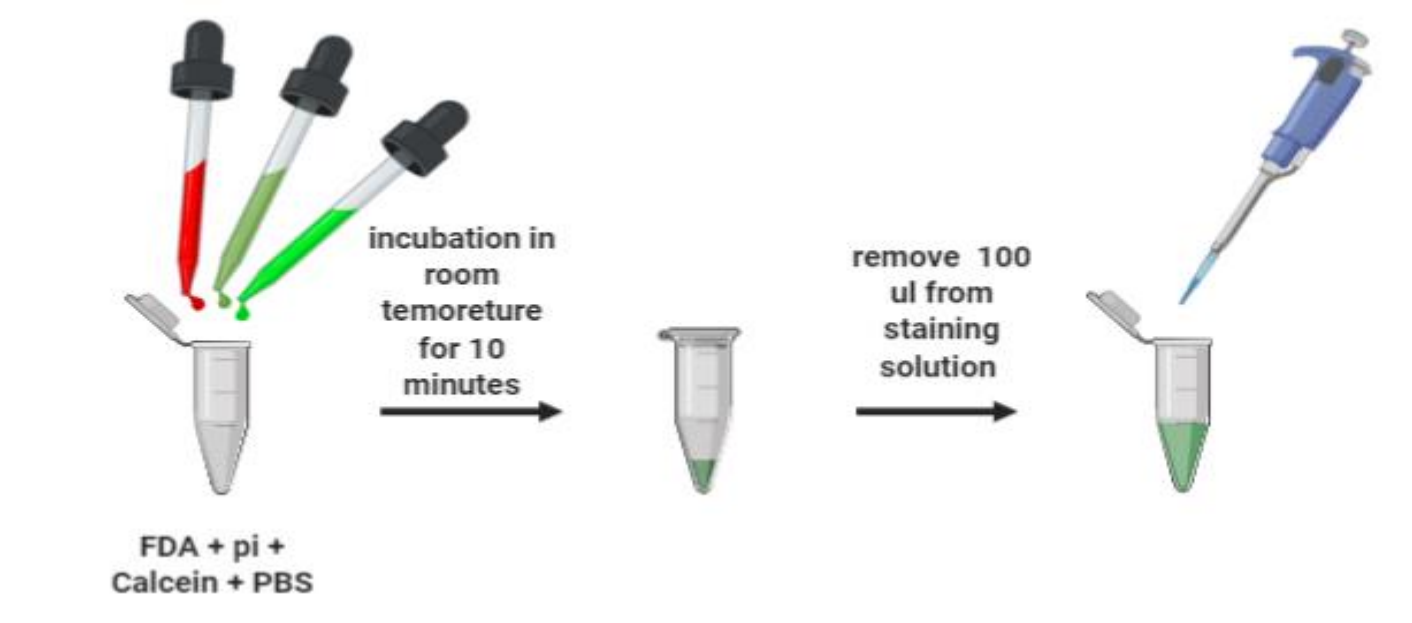
WM115


WM 266
500 [cell/drop]
1000 [cell/drop]
1500 [cell/drop]
diameter [mm]
time [days]
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
2
3
4
5
6
7

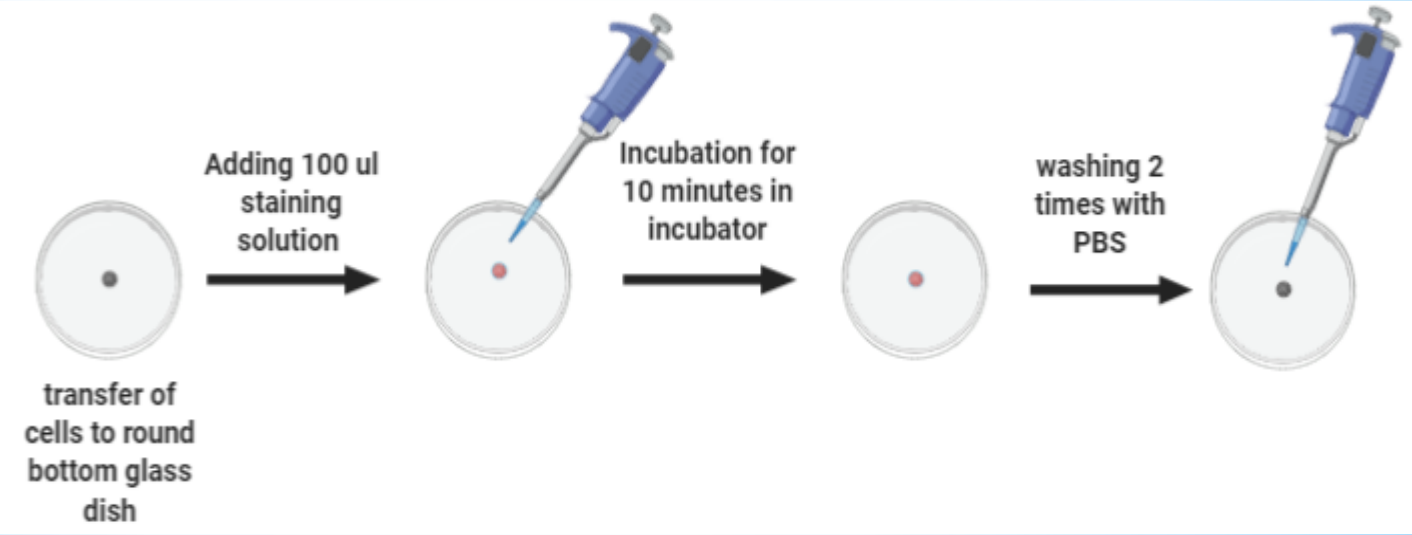

WM 115
500 [cell/drop]
1000 [cell/drop]
1500 [cell/drop]
diameter [mm]
time [days]
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
2
3
4
5
6
7

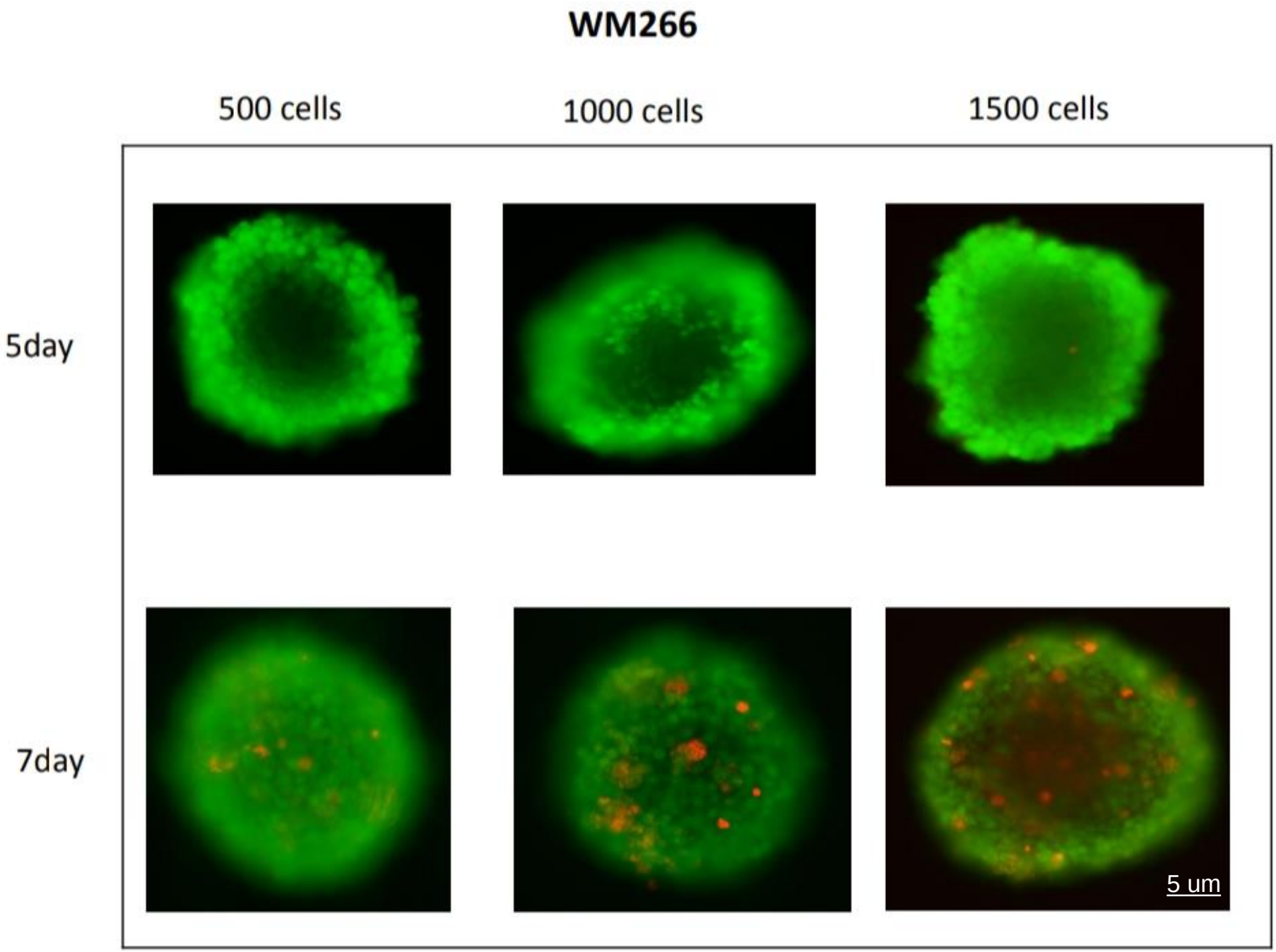
Viability test with Fluorescence Microscope

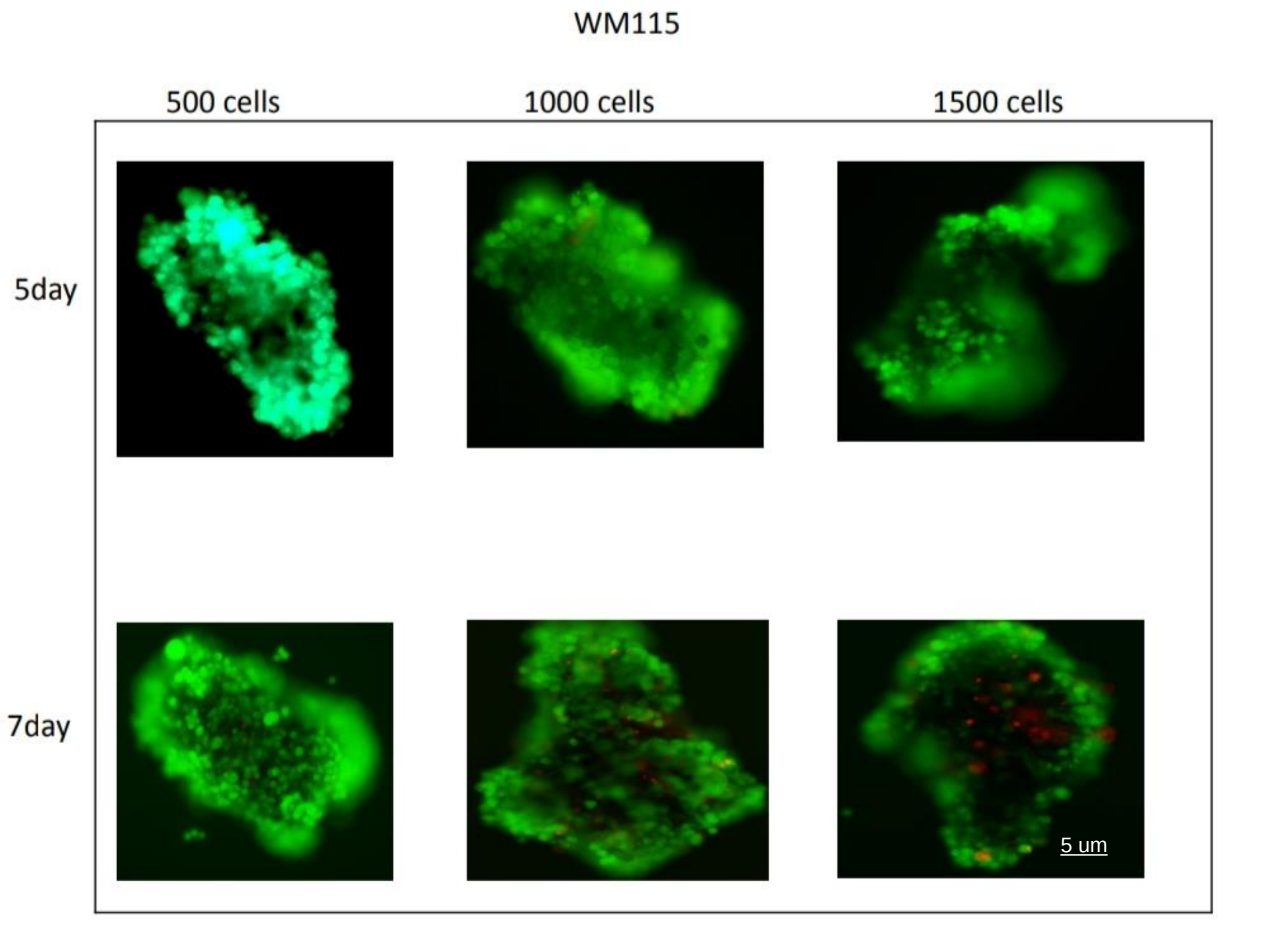
Step 1.Preparation of staining solution



Step 2.staining single spheroid

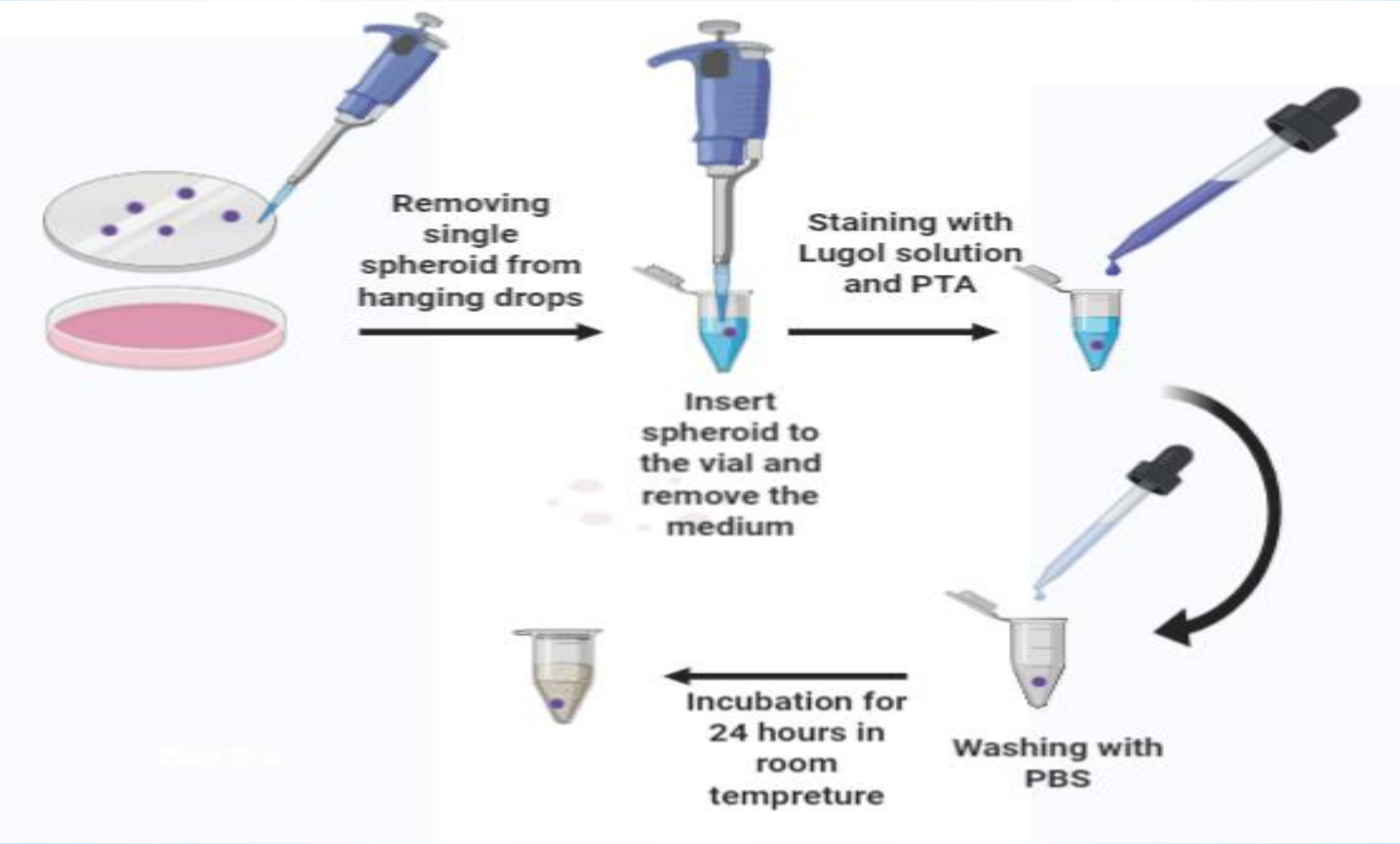



WM266
500 cells
1000 cells
1500 cells
5day
7day
5 um

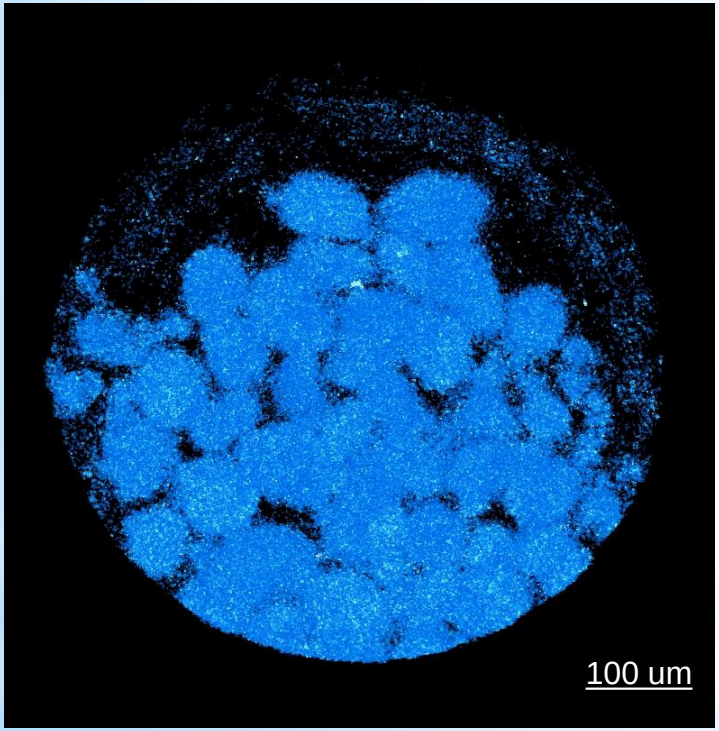

WM115
500 cells
1000 cells
1500 cells
5day
7day
5 um

Micro tomography

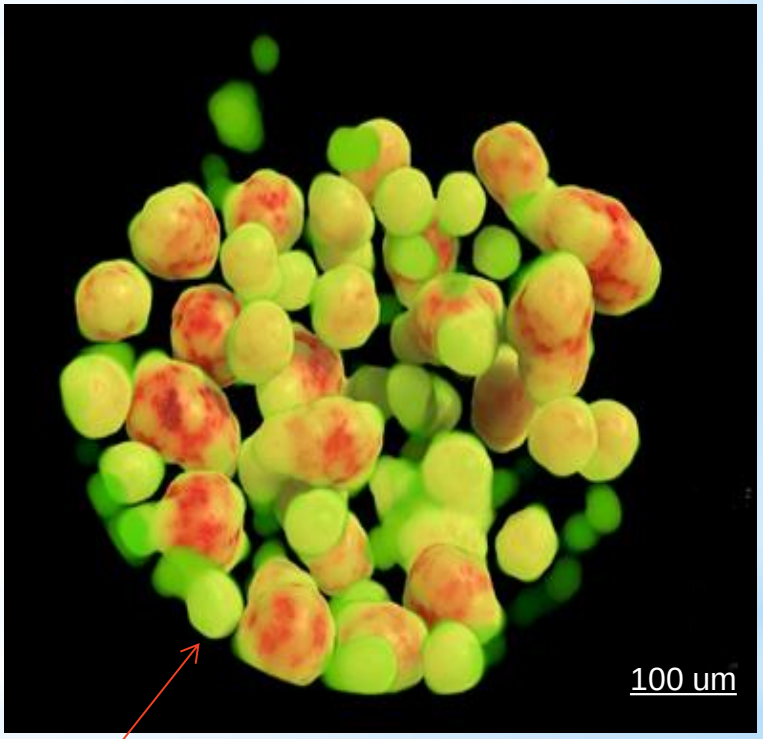
Step. 1: staining with Lugol



Micro tomography images of WM266 cell line in 7th day after cell seeding

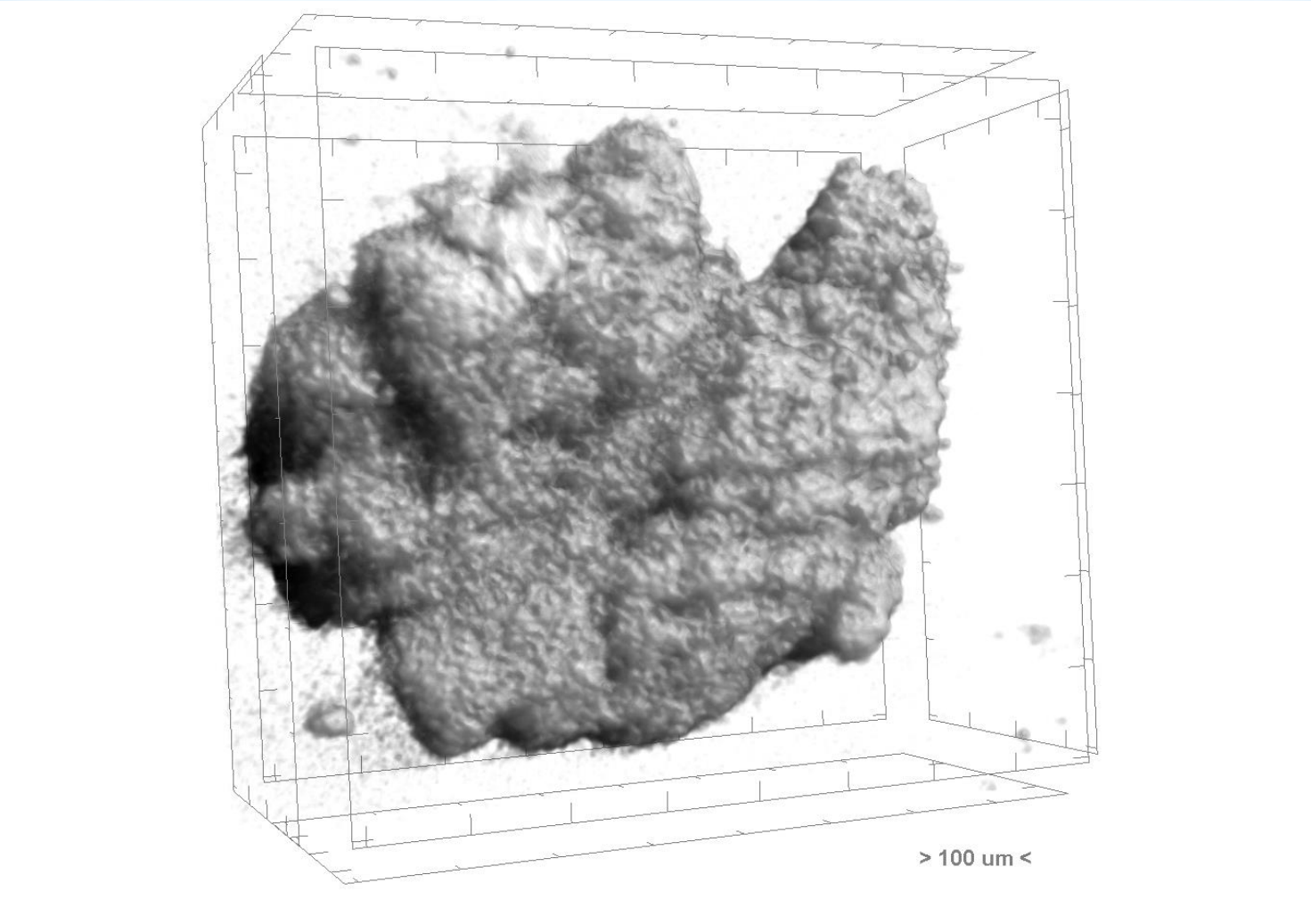


After reconstruction

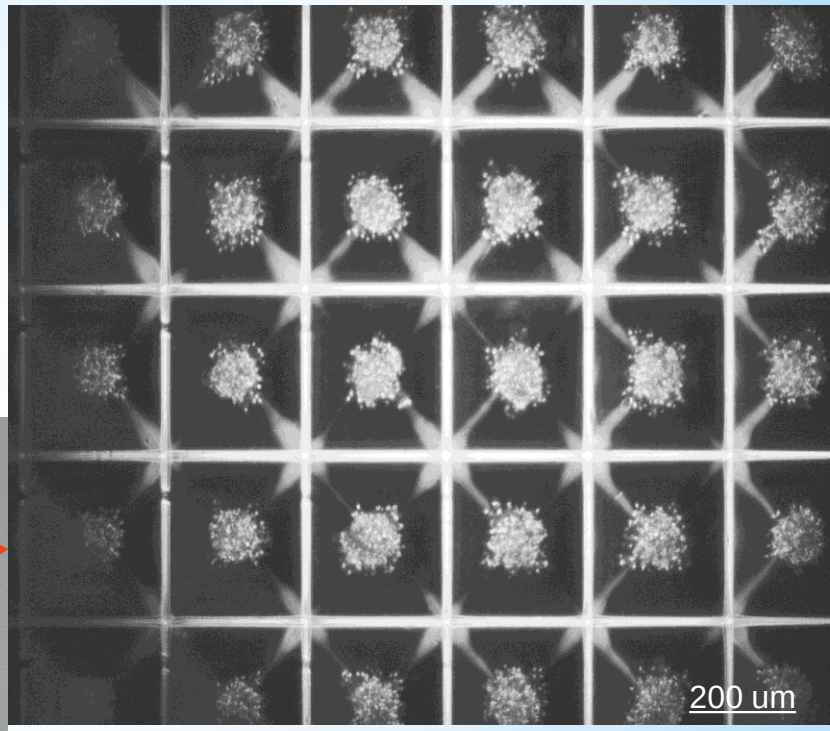


Spheroid cluster

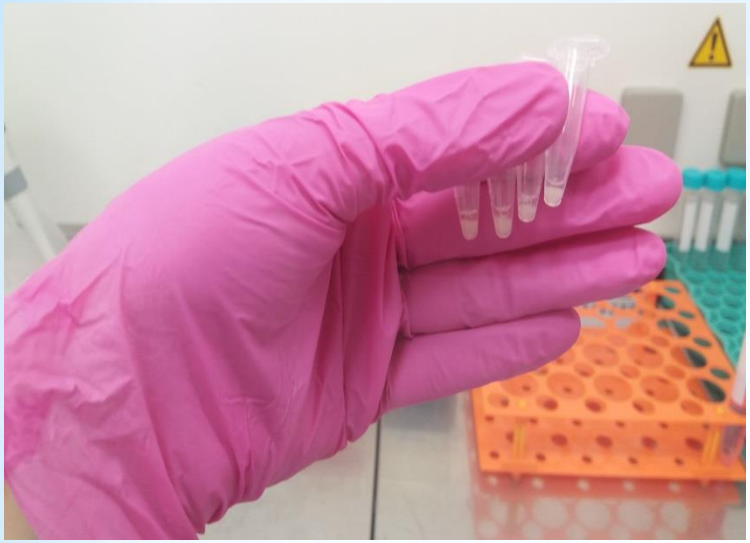
Micro tomography images of WM115 cell line in 7^{th} day after cell seeding

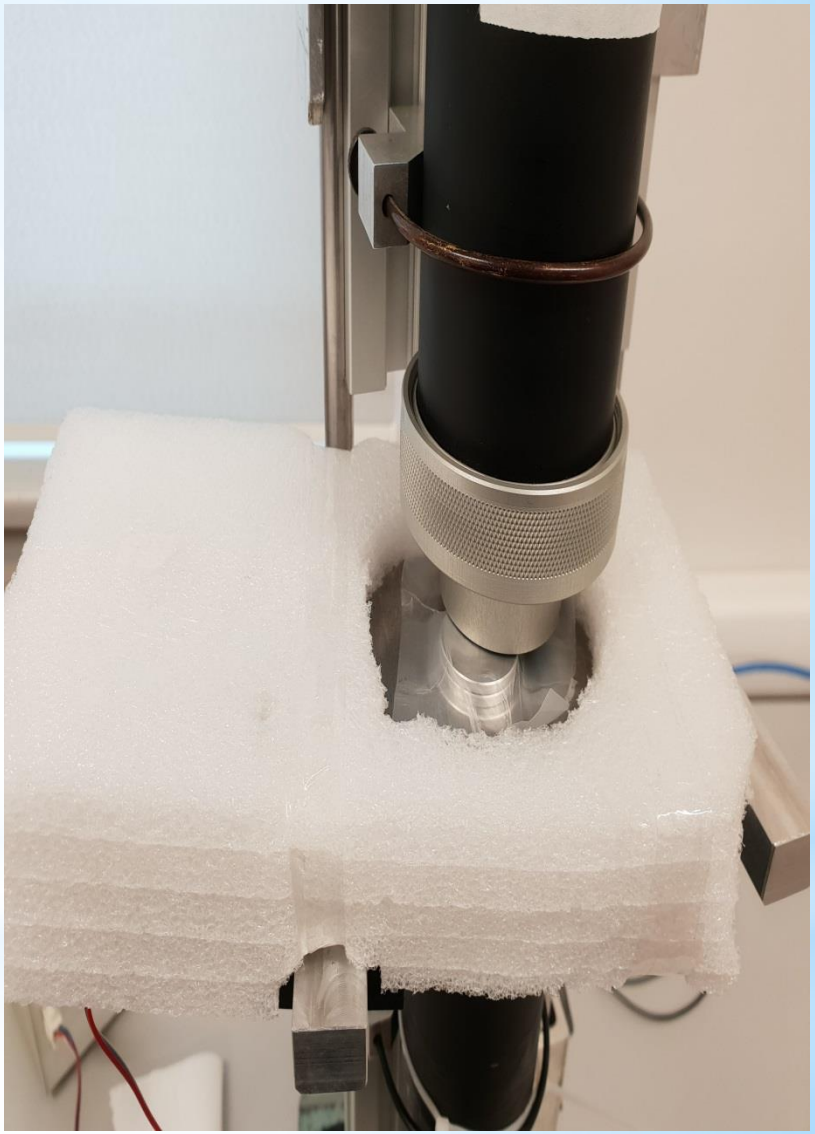


5D microplate



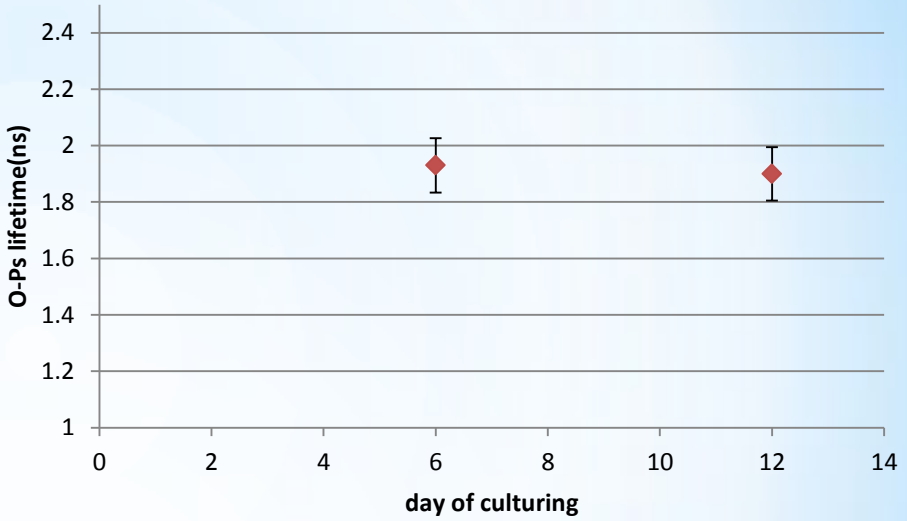
PALS measurement in Spheroids

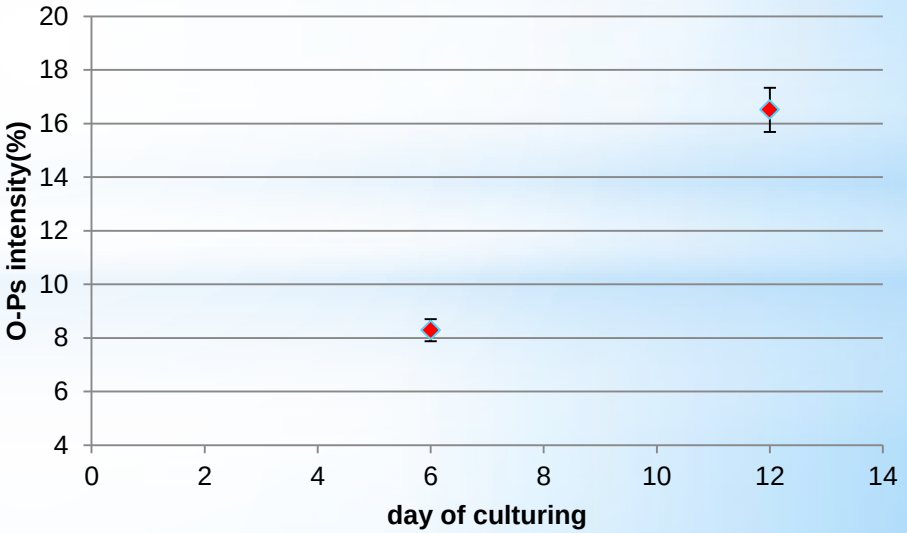




PALS measurement in WM266 spheroids

day	6	12
Number of spheroids	18000	36000





Conclusion:

In this study I selected different density of cells to evaluate their growth and size during the time. Working on 3D cell culture enable us to control the size and volume of spheroids as cancer cell model ,evaluate the cancer cells in different stages and recognize the behavior of cells in the real tumor.

Micro tomography has been already used for imaging animal tissues and biological samples but in this study we use X-ray micro-CT for 3D cell cultures as a new research on cancer cells. Micro-MRI has also used for generation of images of different type of tissues but it is a costly method that needs expensive equipment.

PALS measurement is a new method to identify a new indicator for diagnosis cancer that 3D spheroids can be good models for getting results similar to real tumor cells.

limitations:

1.larg number of spheroids is needed.

2.Preserving spheroid structure during experiments.

3.Maintenance of spheroids for long time with high viability probability can be challenging.


Thanks For
Your Attention