

Supplementary Material

1. Supplementary Data

1.1. SALSA configuration file

In the following the configuration file used to describe the organotypic model in SALSA is reported.

MODEL:

Cell Types:

Cell Line = organoid

1 = Dead

2 = Cancer_Quiescent

3 = Cancer_Replication

4 = Fibroblasts

5 = Mesothelial

Rules:

0 = 1->0, $a \cdot (\text{TIME} - \text{TD})$

1 = 1->1, $1 - (a \cdot (\text{TIME} - \text{TD}))$

2 = 1->0, environment (Glc)

3 = 1->0, environment (O2)

4 = 2-> 1, $b \cdot (\text{AGE}) / (\text{Glc} + \text{O2}) - f \cdot \text{DRUG1}$

5 = 2-> 3, $c \cdot (\text{Glc} + \text{O2}) + d \cdot \text{D0}$

6 = 2-> 2, $1 - (b \cdot (\text{AGE}) / (\text{Glc} + \text{O2}) - f \cdot \text{DRUG1}) - (c \cdot (\text{Glc} + \text{O2}) + d \cdot \text{D0})$

7 = 2->0.5*U, environment (Glc)

8 = 2->0.5*U, environment (O2)

9 = 3->2, $b \cdot (\text{AGE}) / (\text{Glc} + \text{O2})$

10 = 3->3+3, $a \cdot \text{TLD} + c \cdot (\text{O2} + \text{Glc}) - g \cdot \text{DRUG1}$

11 = 3->0+3, $e / (\text{D0} + \text{AGE})$

12 = 3->3, $1 - (b \cdot (\text{AGE}) / (\text{Glc} + \text{O2})) - (a \cdot \text{TLD} + c \cdot (\text{Glc} + \text{O2}) - g \cdot \text{DRUG1}) - (e / (\text{D0} + \text{AGE}))$

13 = 3->U, environment (Glc)

14 = 3->U, environment (O2)

15 = 4 ->4, 1

16 = 4->0.5*U, environment (Glc)

17 = 4->0.5*U, environment (O2)

18 = 5 ->5, 1

19 = 5->0.5*U, environment (Glc)

20 = 5->0.5*U, environment (O2)

Scaffold:

material = organoid

side [cm] = 0.6

layers = 10

porosity [%] = 87

INITIAL CONDITIONS:

Cell Types:

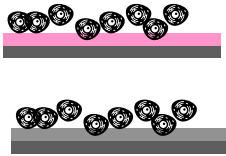
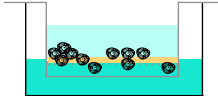
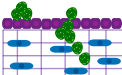
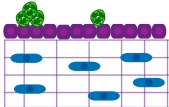
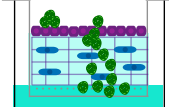
1 = 0
2 = 14
3 = 0
4 = 14
5 = 71
total=28K

Scaffold:

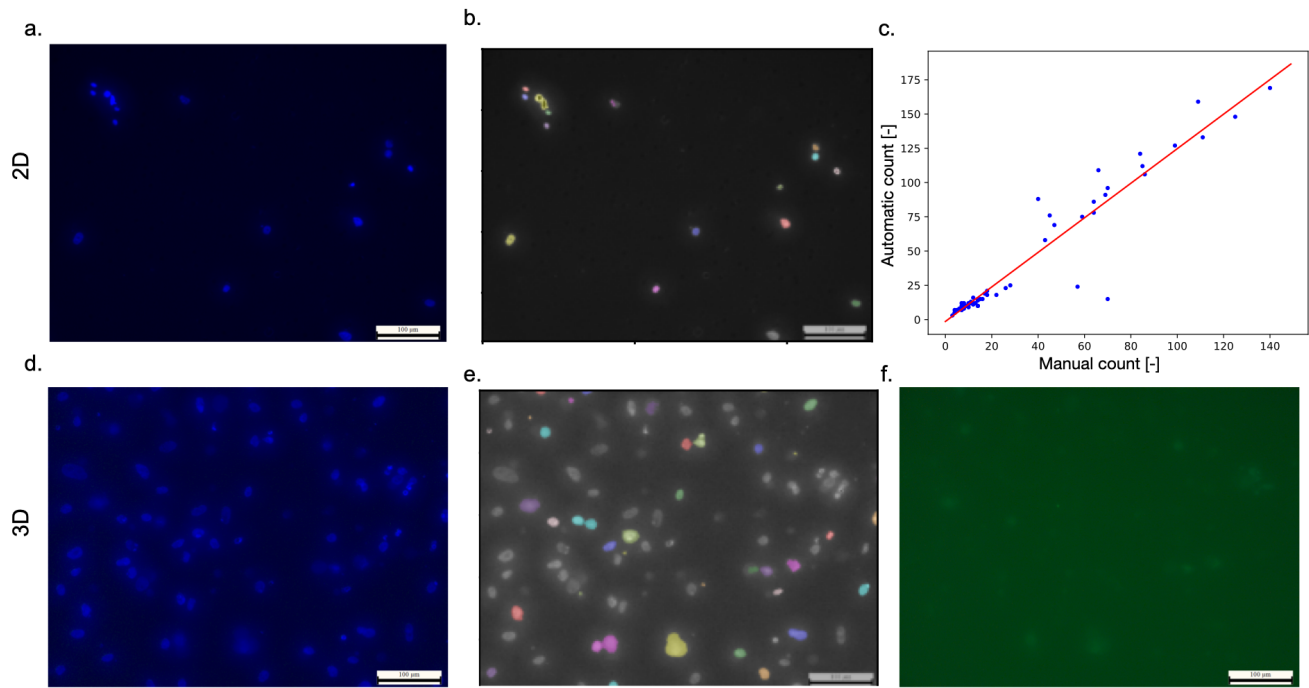
seeding = Organoid
iterations = 72
media = RPMI
media replace frequency [day]= 3
volume per scaffold [ml] = 0.2
flow rate [ml/h] = 0

Treatment:

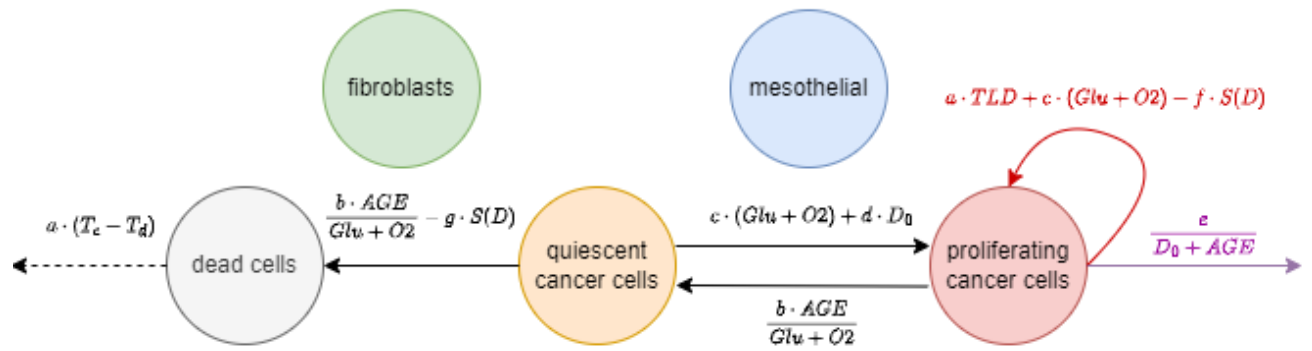
ID = 1
name = **drug_name**
dose [ug/ml] = **drug_dose**
treatment = [0]
type = media

	Adhesion	Invasion	Response to treatment
 2D monolayer	 • PEO4 cells are seeded on a collagen or BSA coating • @ 2, 3, 4 h unattached cells are washed away • Quantification using crystal violet staining	 • PEO4 cells are seeded on a porous membrane (grey dotted line) coated with Matrigel. • Cells invade through the membrane following a nutrients gradient (different shades of blue) • Cells on the other side of the membrane are stained with DAPI and imaged.	 • PEO4 cells are seeded in a 96-well plate (partially shown above). • Different concentrations of the drug are added. • Cell metabolism is measured to provide an estimate of the number of live cells
 3D multi-spheroid  3D organotypic model	 • PEO4 cells are seeded on the organotypic model • @ 2, 3, 4 h unattached cells are washed away • Quantification using crystal violet staining	 • PEO4 cells are seeded on the organotypic model laid over a porous membrane (grey dotted line). • Cells invade through the structure following a nutrients gradient (different shades of blue) • Cells on the other side of the membrane are stained with DAPI and imaged.	 • 3D multi-spheroids of PEO4 cells are printed in a 96-well plate (partially shown above). • Different concentrations of the drug are added. • Cell metabolism is measured to provide an estimate of the number of live cells

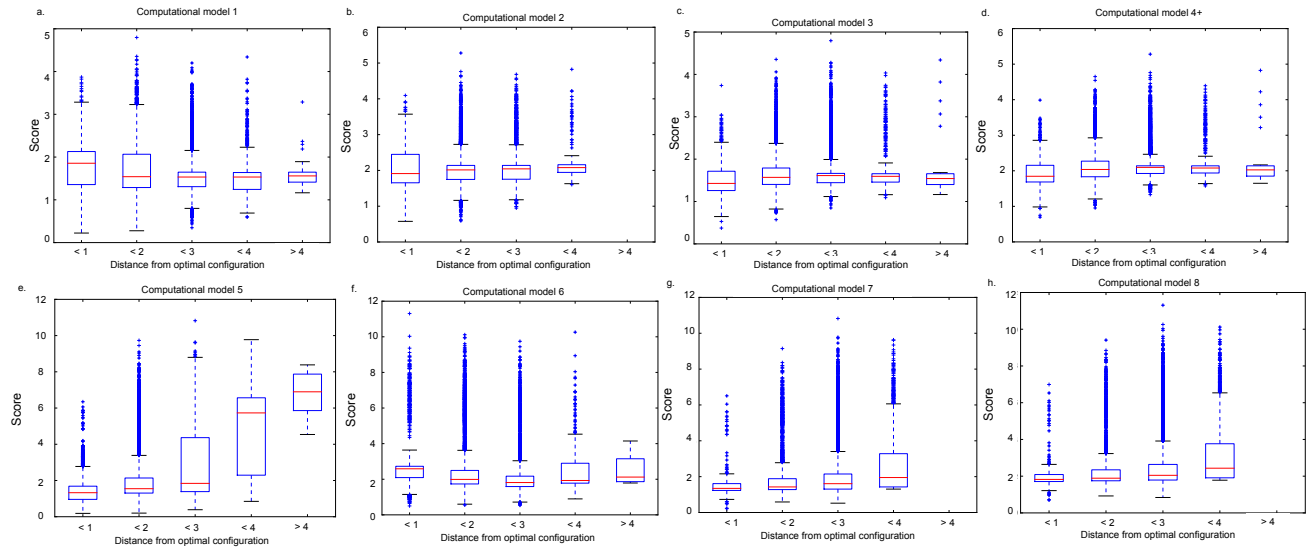
Supplementary Figure 1: Summary of the experimental analysis conducted in this work. For each considered experimental model (2D monolayer, 3D multi-spheroid and 3D organotypic model) a schematic representation of adhesion, invasion and response to treatment assays is shown together with a brief description of the main steps of these protocols



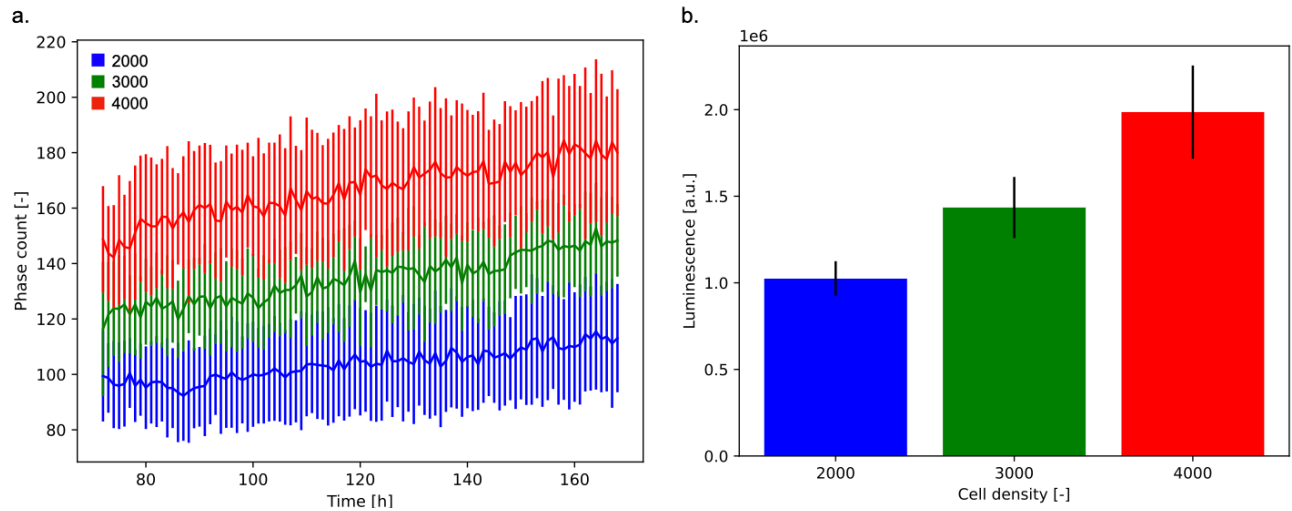
Supplementary Figure 2: Software for the analysis of the images from transwell invasion assays. a. Representative image from the 2D dataset. The cells' nuclei are stained with DAPI. b. Segmentation of the image in a. Colour overlays identify individual cells. c. Comparison between the manual and automatic counts obtained on the same images ($R^2 = 0.95$). All the images in the 2 dataset were used for this analysis. d. Representative image of the stained nuclei for the 3D dataset. e. Segmentation of the image in d. In this case only cells recognised as cancer were overlayed with different colors. f. GFP channel for the image in d. Only cancer cells express this fluorescent protein.



Supplementary Figure 3: Diagram outlining the behaviour of the simulated cells upon introduction of drug treatment. This is a modified version of Figure 1b, in which the proliferation probability and the rate of cell death have been modified to include the effect of cisplatin/paclitaxel. The definition of the terms and variables in this figure are in the label of Fig.1., Tab. 1 and the methods section.



Supplementary Figure 4: Representation of the score distribution for each computational model as a function of the distance from the optimal configuration. The experimental data used for the calibration of each model are summarised in Tab 2, while Tab. 3 shows the parameters for each model. The score was computed as shown in Eq. 3 while the formula for the distance from the optimal configuration is in Eq. 4



Supplementary Figure 5: Analysis of cell growth in the 3D multispheroid model. a. Tracking of the number of cells (phase count) obtained from images stacks acquired using an Incucyte S3 Live Cell Analysis System over a period of 7 days. b. End point measurements with the CellTiter-Glo 3D kit. The results obtained with the latter are equivalent to the endpoint measurements in the latter.