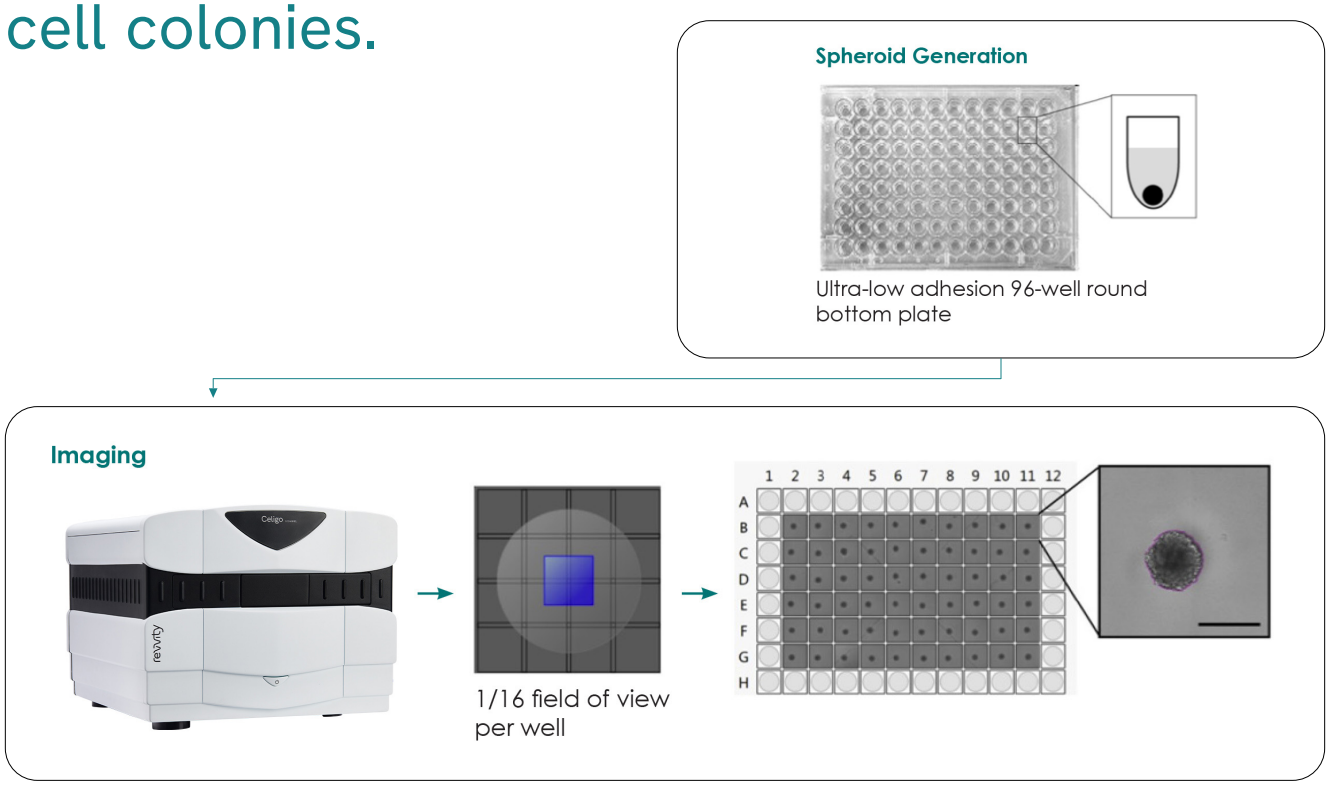
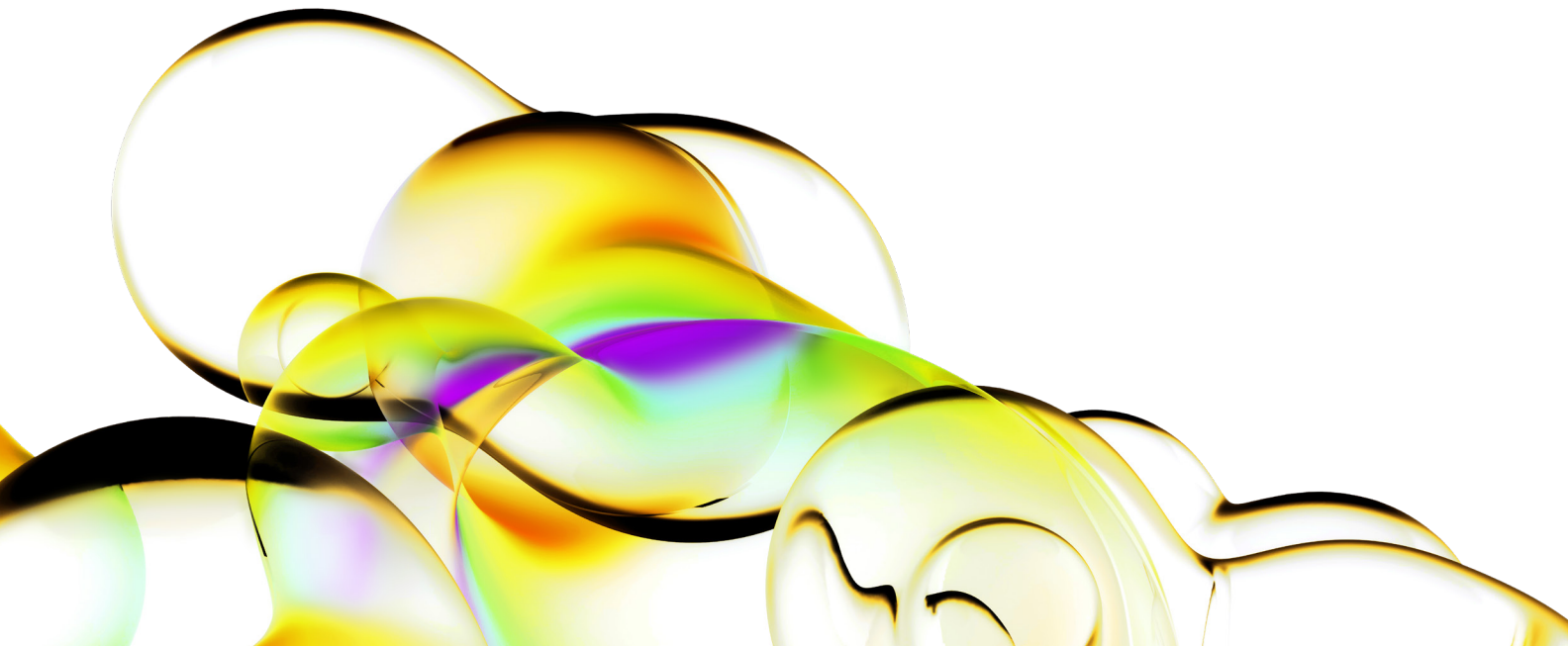
Automated imaging and analysis of 3D tumor spheroids and cancer stem cell colonies.

3D Tumor Spheroid Functional Assays

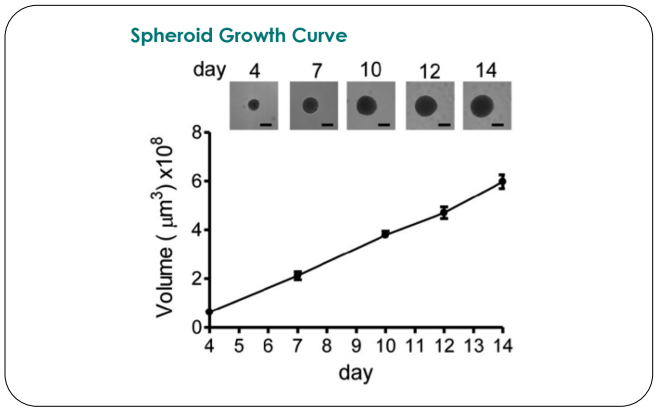
The Celigo™ imaging cytometer has been developed to fully automate image and analysis of tumorspheres, embryoid bodies and cancer stem cell colonies. It has been used in the development of 3D tumor spheroid-based functional assays for target validation and drug evaluation.

Data courtesy of Dr. Maria Vinci, Cancer Research UK, Cancer Therapeutics Unit, The Institute of Cancer Research.



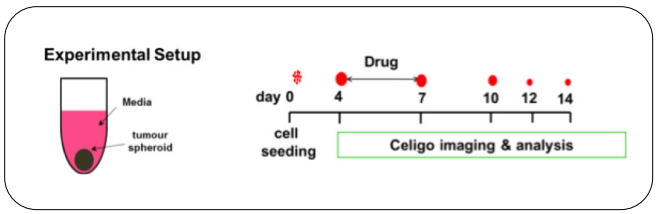


- Identify and count spheroids in flat or U-bottom wells
- Reduce cumbersome manual morphology measurements and improve data reproducibility
- Monitor growth of spheroids over time
- Screen numerous spheroid plates per day, 96, 384 well plates
- Scan an ultra-low adhesion 96-well plate in 8 minutes
- Measure viability using dual-fluorescence assays
- Multi-parameter analysis : counts, size, short and long diameter, est. volume, perimeter, area and more.

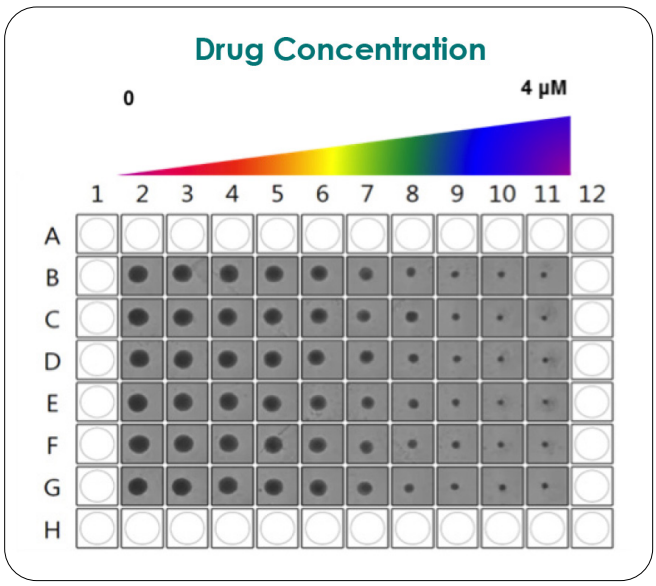


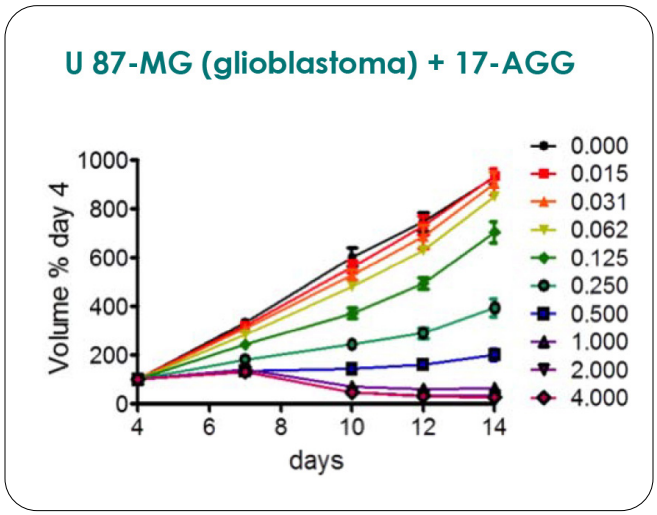
Tumor spheroid growth inhibition assay

4-day old spheroids were treated with compounds for 72 hours. Drug induced concentration-dependent growth inhibition was obtained.



- Day 0-4: form spheroids
- Day 4-7: drug treatment
- Day 4-14: measure spheroid size

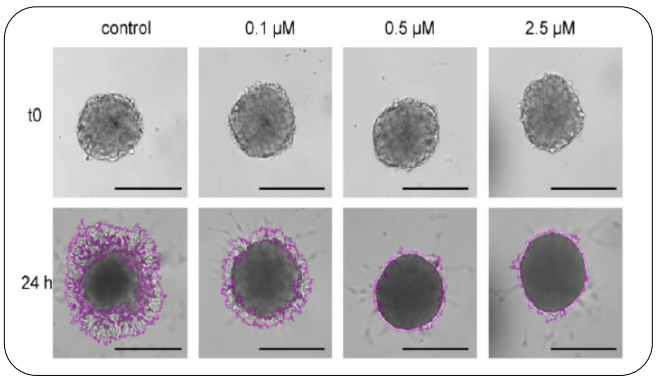


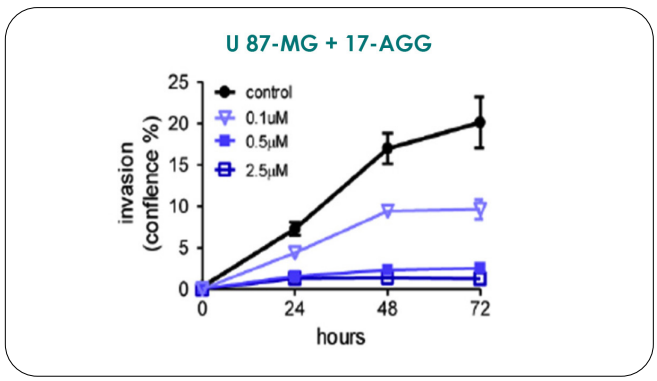


Vince et al. BMC Biology 2012, 10:29, March 2012

Invasion into Matrigel assay

- Day 0-4: form spheroids
- Day 4: add Matrigel® to provide a semi-solid gel-like matrix
- Day 4-7: use Celigo image cytometer to determine the area occupied by individual cells or cell clusters

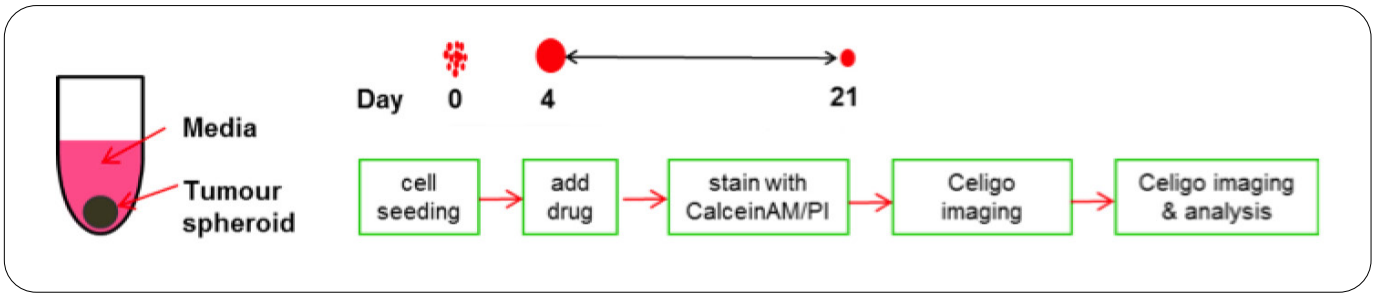


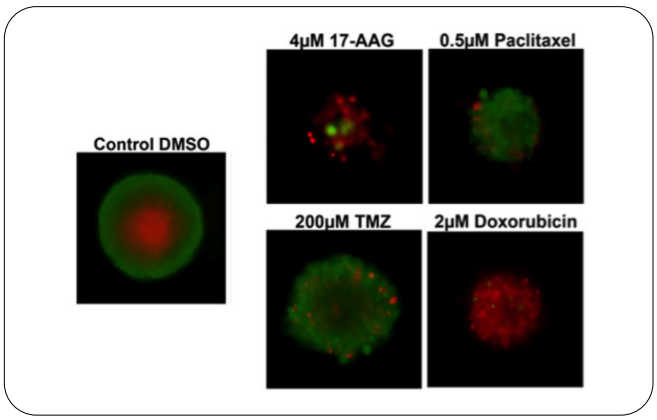


Two-fluorescence viability assay for 3D tumor spheroids

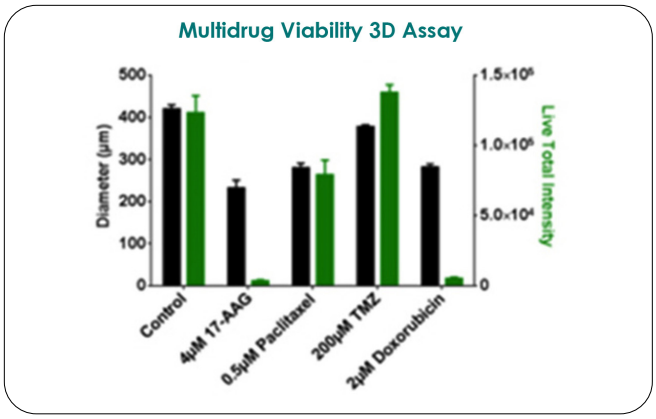
3D cultures were treated with multiple drugs (17-AAG, Paclitaxel, TMZ or Doxorubicin) and stained for viability after 21 days.

Live/dead stains used were Calcein AM (green), Propidium Iodide (red) to measure live and dead cells on days 4, 7, 10, 14, and 17. Plates containing spheroids were imaged on the Celigo image cytometer.





- 17-AAG decreased sphere size and caused the most significant cell death
- Paclitaxel decreased sphere size but maintained a signifiicant number of live cells
- Temozolomide caused no significant decrease in sphere size and did not cause cell death
- Doxorubicin decreased sphere size and caused significant cell death



Spheroid diameter (black) and total live fluorescent intensity (green) after drug treatment.