Improved robustness for 3D spheroid screening using High Content Analysis

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Introduction

To mimic the initial avascular stages of solid tumors *in vivo*, spheroids need to develop hypoxia and subsequent necrosis in their center. Indeed, due to the presence of quiescent cells, spheroids are more resistant to certain compounds compared to cells grown as 2D monolayers and therefore considered a more relevant model for drug screening. Widely used methods to obtain multicellular spheroids involve culturing malignant cells in anchorage-independent growth conditions, generating a single spheroid per well and have poor compatibility with high throughput and HCS approaches.

We have developed a simple method to grow **multiple homogenous spheroids per well** in 96 well plate format. Spheroids are attached to the glass bottom and are directly compatible with conventional biochemical readouts as well as **High Content Analysis** without troublesome plate transfer. Our method is fully compatible with automation and allows spheroids to develop up to 600 µm in diameter with interwell CVs of 5% or less**.**

Background

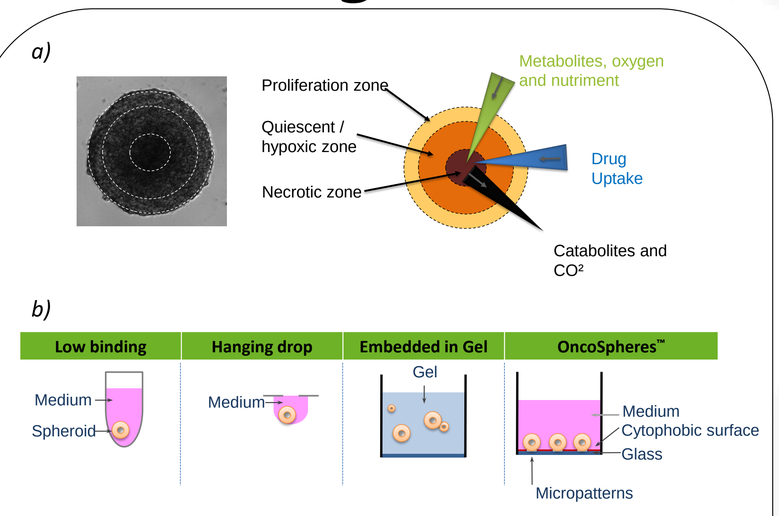
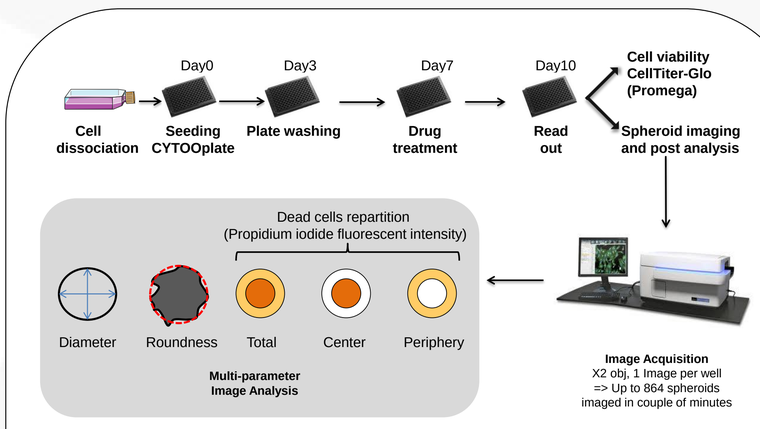


Figure 1: Mature spheroid and drug uptake
a) Scheme of spheroid organization and its implication in nutrient, O^2 and CO^2 trafficking as well as drug availability. All cell conditions need to be considered for drug development. b) Summary of available spheroid culture techniques compared to CYTOO OncoSpheres™ model.

Methods



Compatible with various cell lines: T47D & MCF7 (Breast), HCT116 & HT29 (Colon), SKOV3 (Ovary), …

Figure 2: Workflow from spheroid growth to data analysis.
Wetlab work as well as image acquisition and data analysis are facilitated by using fixed spheroids compared to floating ones.

Robust, homogenous and reproducible multiple spheroids growth and maturation

Production of Multiple and Homogenous spheroids

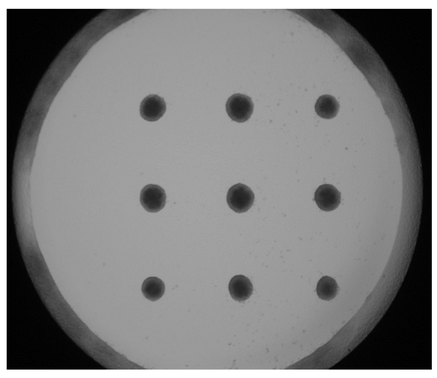


Figure 3: Typical well at 2x magnification.
2x Brightfield acquisition HT-29 spheroids after 12 days in culture (Operetta, Perkin-Elmer).
Multiple spheroids on same z level allow fast and robust image acquisition.

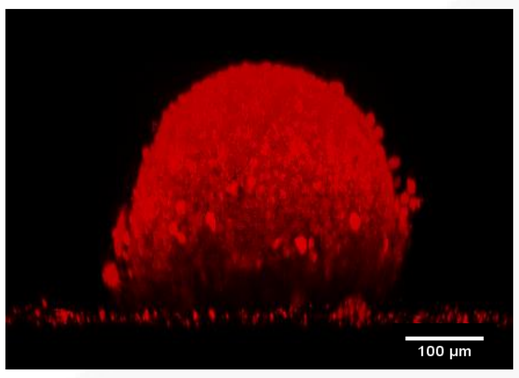


Figure 4: Multiphoton view of spheroid.
Tridimensional reconstruction from multiphoton scanning micrographs of a representative HT-29 spheroid at 14 days.
Spheroid is about 400 µm in diameter in all directions.

Linear and homogenous growth

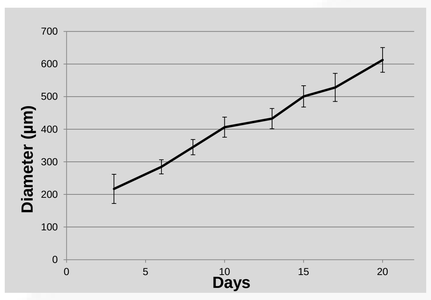


Figure 4: Control of growth over time
Growth kinetics of HT-29 spheroids.Structures are continuously growing for up to 20 days, reaching a size of 600µm.

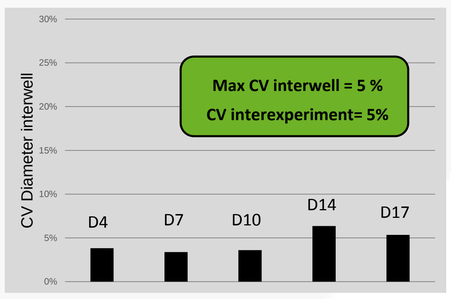


Figure 5: Low coefficient of variation
Calculated CV (coefficient of variation) of measured spheroids diameter. Maximum observed CV's are 5% between wells of a same plate as well as different plates, regardless of the age of spheroids.

Spheroids have all characteristics of avascular tumors

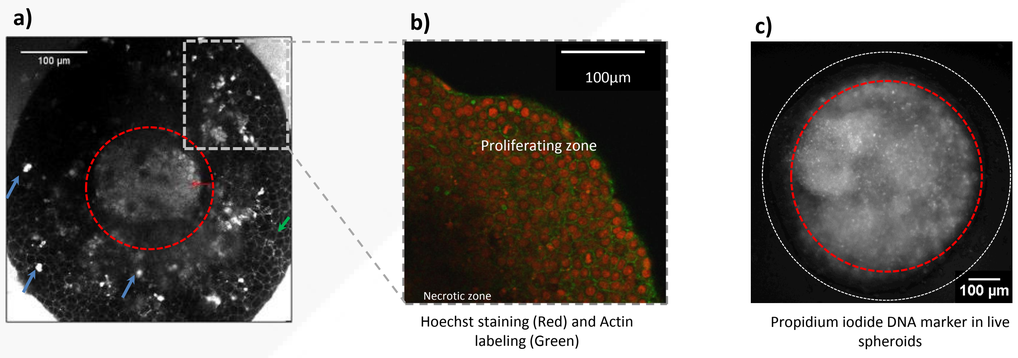


Figure 6: Morphology of a representative HT-29 spheroid.
a) Cross-section imaged from SRB (sulforhodamine B) labeling include a peripheral proliferating area highlighted by typical SRB exclusion in intercellular environment (green arrow) and a necrotic center zone revealed by SRB diffusion (Red arrow). Interestingly, necrotic cells are present in the proliferating area (blue arrow) . b) The peripheral proliferating area contains dividing cells, as observed by Hoechst staining of metaphasic plates (red) and rich actin network (green). c) The necrotic center zone reveled by using propidium iodide staining.

HCA compatibility facilitates characterization of MOA

Compatibility with standard viability readouts

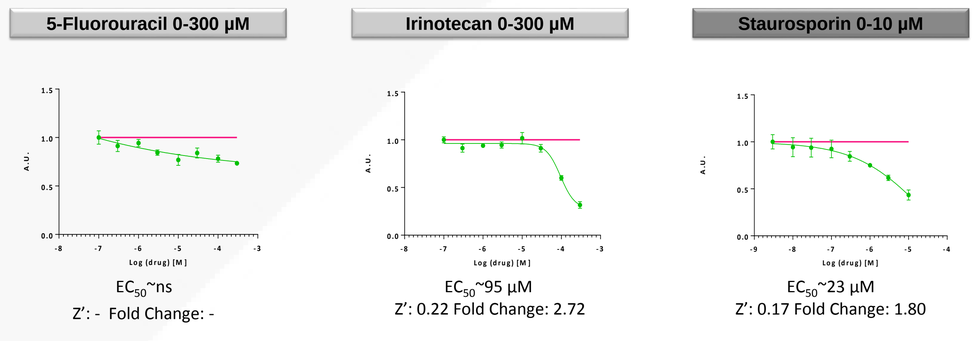


Figure 7: CellTiter Glo analysis and EC50 establishment

A new model fully compatible with HCS

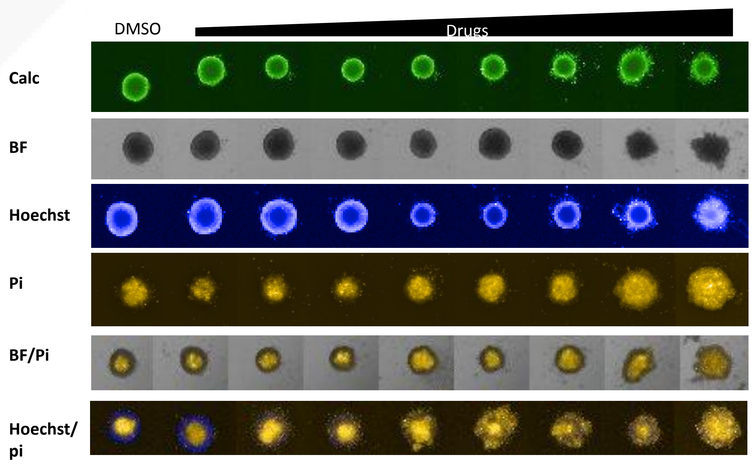


Figure 8: Illustration of labelings and image acquisition available with spheroids treated with different drugs in dose response.
Calc: calcein. Pi: Propidium iodide. BF: Brightfield

Characterization of drug MOA

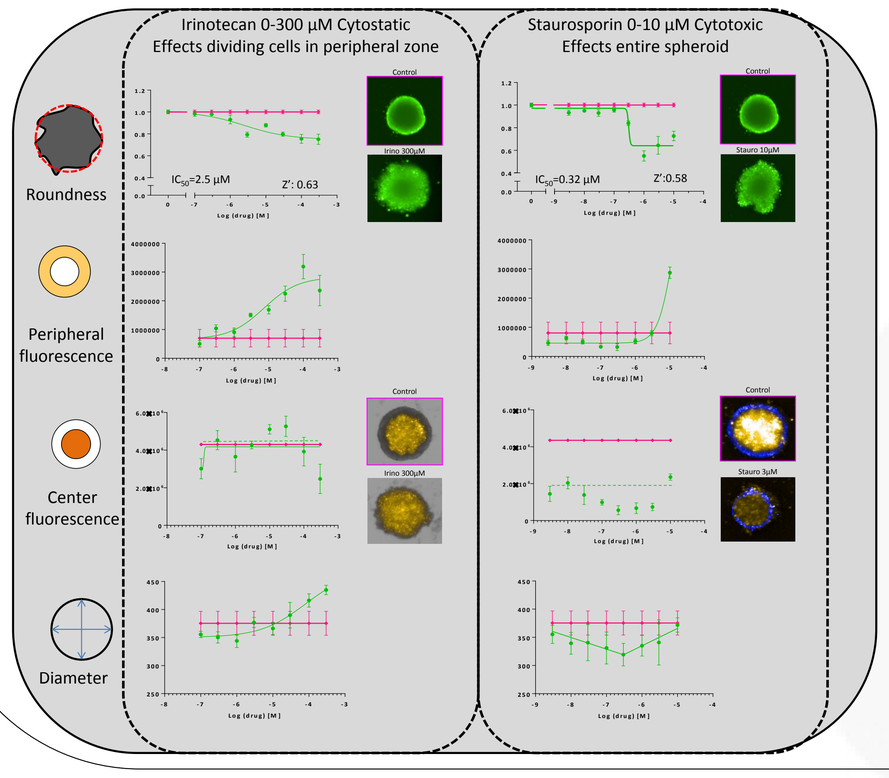


Figure 9: Validation of Oncospheres™ for image-based HCA and direct insight into spheroid response and compound MOA.
Differences in local effects of a cytostatic versus a cytotoxic compound can be distinguished. Z' is >0.5 for several parameters. n=27 spheroids in 3 wells

Key advantages

- New HCS solution with functional spheroids
- Robust and automated assay
- Mature spheroid model
- More spheroids per data point per screen with CV <5%
- Applicable to HCS IC50 curve determination
- Directly compatible with imaging and HCA

Conclusion

Altogether, the production method presented allows production of **multiple spheroids** per well which translates into high assay robustness. The full compatibility with **High Content Analysis** opens up new avenues toward the development of innovative and more pertinent readouts that will contribute to a higher definition of compound Mode Of Action.