3D quantification of *in toto* immunofluorescence on spheroids

Immunodetection • 3D Cell Models • Light Sheet • Spatial Distribution • Oncology

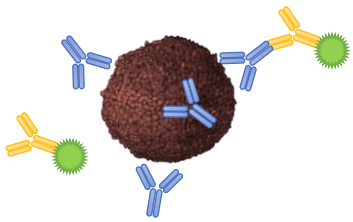
YOUR NEEDS

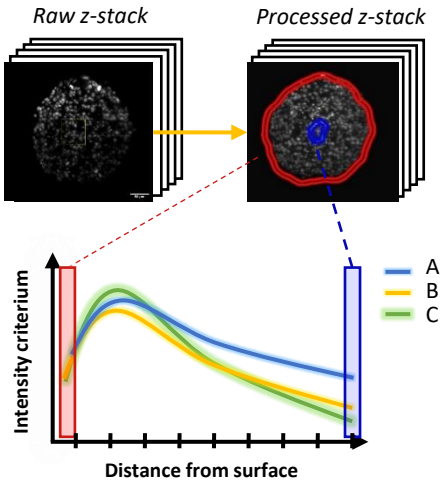
- Detect, localize and quantify proliferation markers in the 3D sample
- Assess the antiproliferative activity of compounds in 3D cell models

OUR SOLUTIONS

- Handling from 3D cell culture to 3D light sheet microscopy
- *In toto* immunofluorescence labeling
- Quantification and multi-parametric analysis

General Procedure





Spheroids production in ULA 96-well plates

- Numerous cell lines available or on request

In toto spheroids labeling and handling for microscopy:

- Tunable in-house protocol
- Consecutive steps of permeabilization, saturation and antibody incubation best suited for 3D samples
- Possibility of complementary dye staining if required
- Optical clearing

Image acquisition:

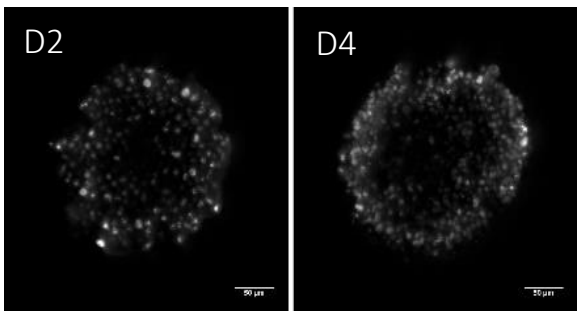
- Light sheet microscopy at 5X magnification

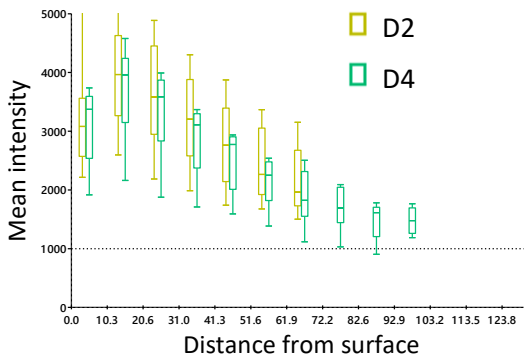
Image processing:

- Automatic 2D or 3D quantification of the signal distribution within the sample, based on the raw z-stack acquisition:
  1. Image registration for multichannel data conditioning.
  2. Spatial distribution analysis using sample-based concentric rings, from surface to center of spheroid.
  3. Numerical output can be any type of criterium: mean / median intensity, texture index, extracted feature.
- Interactive visualization with advanced customizable media.

Application example: spatial analysis of a proliferation marker

- MCF-7 spheroids were labeled by *in toto* immunofluorescence with an antibody against KI67 to identify proliferating cells. Clearing was then performed before light-sheet imaging. Bottom-left images illustrate the detection of KI67 protein on spheroids at day 2 and 4 after seeding.
- The fully automatic image processing algorithm allows to highlight a strong surface proliferation, starting from day 2 of culture *(bottom-right graph).* A decreasing proliferation gradient is observable from surface to center of the spheroid as growth progresses until day 4.





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