Analysis of tumor spheroids growing in Kugelmeiers sphericalplates 5D

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SYNENTEC

BACKGROUND

Over the last years, it became obvious that the complex nature of cancer is not reflected in the widely used two-dimensional (2D) monolayer cell culture systems. Therefore, three-dimensional (3D) cell culture models became increasingly popular. One of these models is based on the formation of multicellular tumor spheroids. Spheroids strikingly mirror the 3D cellular context of *in vivo* tumors. In order to become an alternative to 2D systems, it must be possible to generate 3D spheroids with a homogenous size to obtain comparable and reproducible results.

To solve this challenge, Kugelmeiers developed the sphericalplate 5D. This plate contains a patented microstructure (US Pat. 8,911,690 B2) enabling scientists to grow 9000 single spheroids or cell aggregates within only one 24-well plate. In this application note, we introduce a method for imaging multicellular tumor spheroids grown in the sphericalplates 5D using a NYONE® Scientific SC4 Cell Imager and SYNENTEC´s image analysis YT-software®.

For this purpose, two different cell lines, HepG2 and HCT116, were used. First of all, handling of the sphericalplates 5D was established. After that, the formation of spheroids was imaged over five days. Lastly, the spheroids were treated with TRAIL or paclitaxel and the drug's effects were visualized and evaluated by Live/Dead-staining using Calcein AM and Propidium iodide.

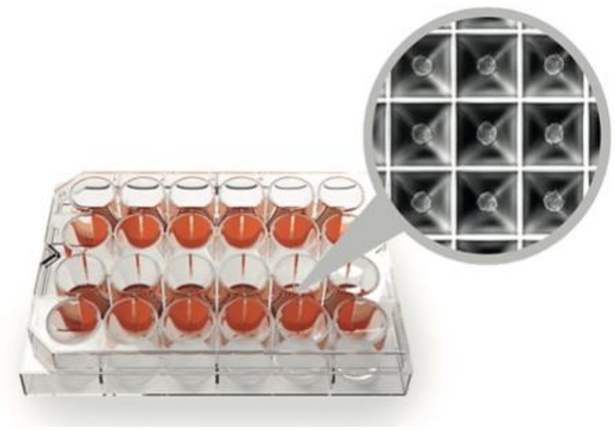
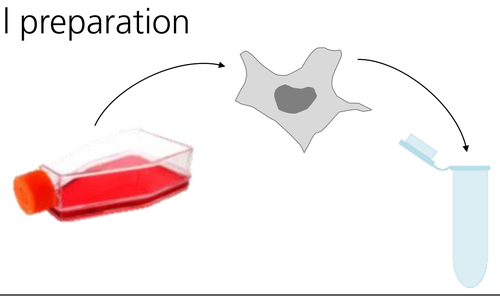


Fig. 1: Image of the Kugelmeiers 5D sphericalplate.
The image shows the sphericalplate 5D and a close-up image of the microwells containing spheroids. This special plate supports Superior spheroid clustering, without cell adhesion thanks to high-end ultra-low attachment nanocoating. And because of the fusion of geometry with biology - every cell finds its niche. Physiological environment is given thanks to rounded bottoms.

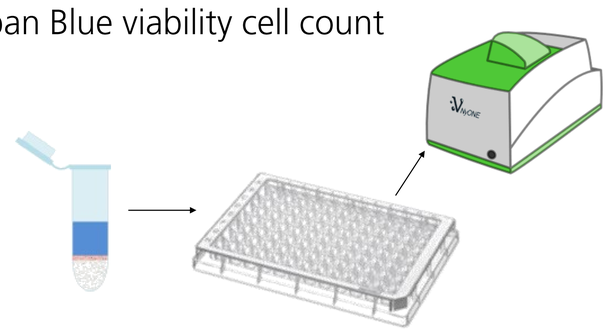
Experimental workflow

Step

Cell preparation

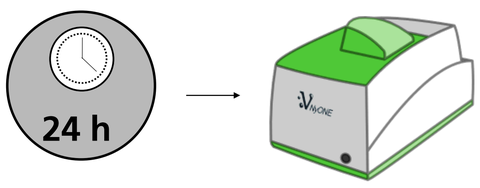


Trypan Blue viability cell count

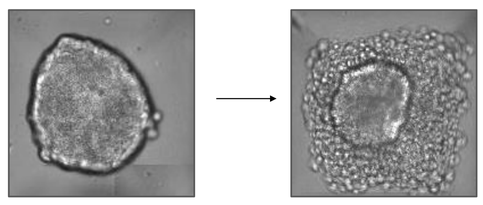


Preparing Kugelmeiers sphericalplate 5D

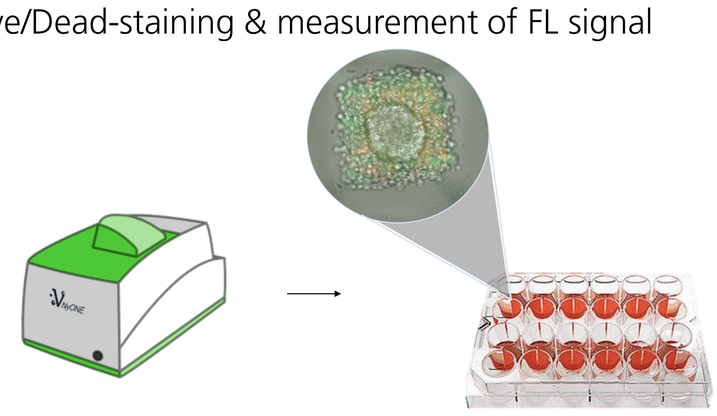
d0 and daily measurement



Spheroid treatment



Live/Dead-staining & measurement of FL signal



Results

Before processing **After processing**

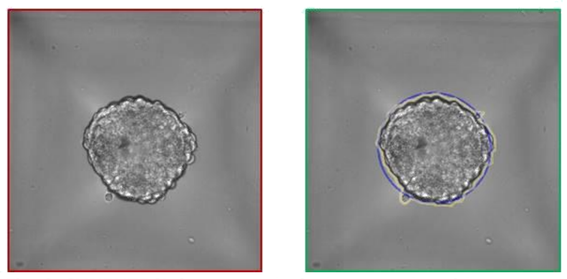


Fig. 2: SYNENTEC's image analysis detects spheroids.
Brightfield image of spheroids from cancer cells. Single Cell Cloning application in YT-software® recognizes confluence (yellow line) and calculates a „blob" (blue line) representing the spheroid's area.

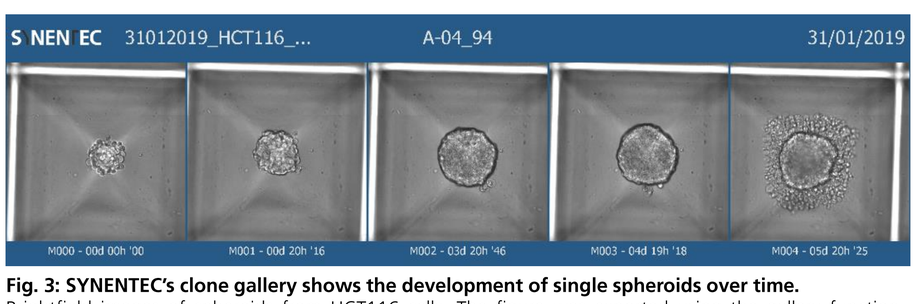


Fig. 3: SYNENTEC's clone gallery shows the development of single spheroids over time.
Brightfield images of spheroids from HCT116 cells. The figure was exported using the gallery function. Additionally to the images of each measurement, it contains information about the experiment name, well and spheroid number as well as measurement dates.

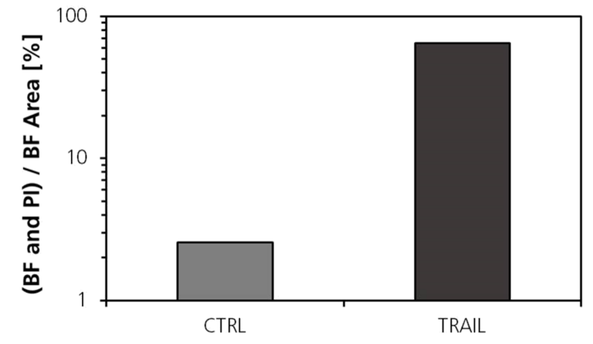


Fig. 4: TRAIL increased the size of the PI-positive area.
Spheroids generated from HCT116 cells were treated with 100 ng/ml of TRAIL for 24 h and stained with Calcein AM and PI. The fluorescence was imaged with NYONE® and the images analyzed with Confluence (2F) application. The data was exported and analyzed with SigmaPlot. Here, the percentage of the PI-positive area is shown for untreated (CTRL) or TRAIL-treated cells. The mean of duplicate wells is shown.

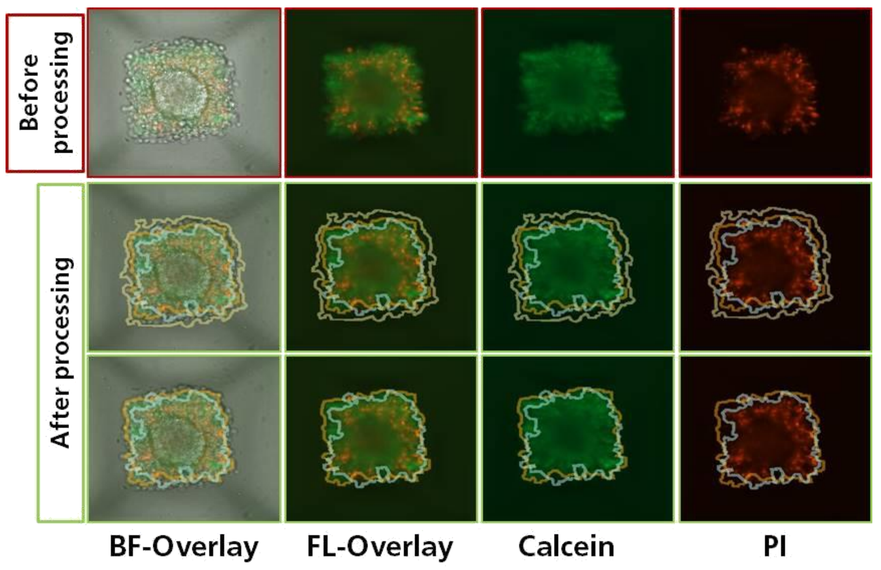


Fig. 5: Live/Dead assay analysis in Confluence (2F) wizard of YT -software®.
Brightfield (BF) and fluorescent (FL) images of spheroids from HCT116 cells after FL staining. The upper pictures show raw images. The second and third row show graphic overlays based on results from BF (yellow), Calcein (orange) and PI (blue) image processing.

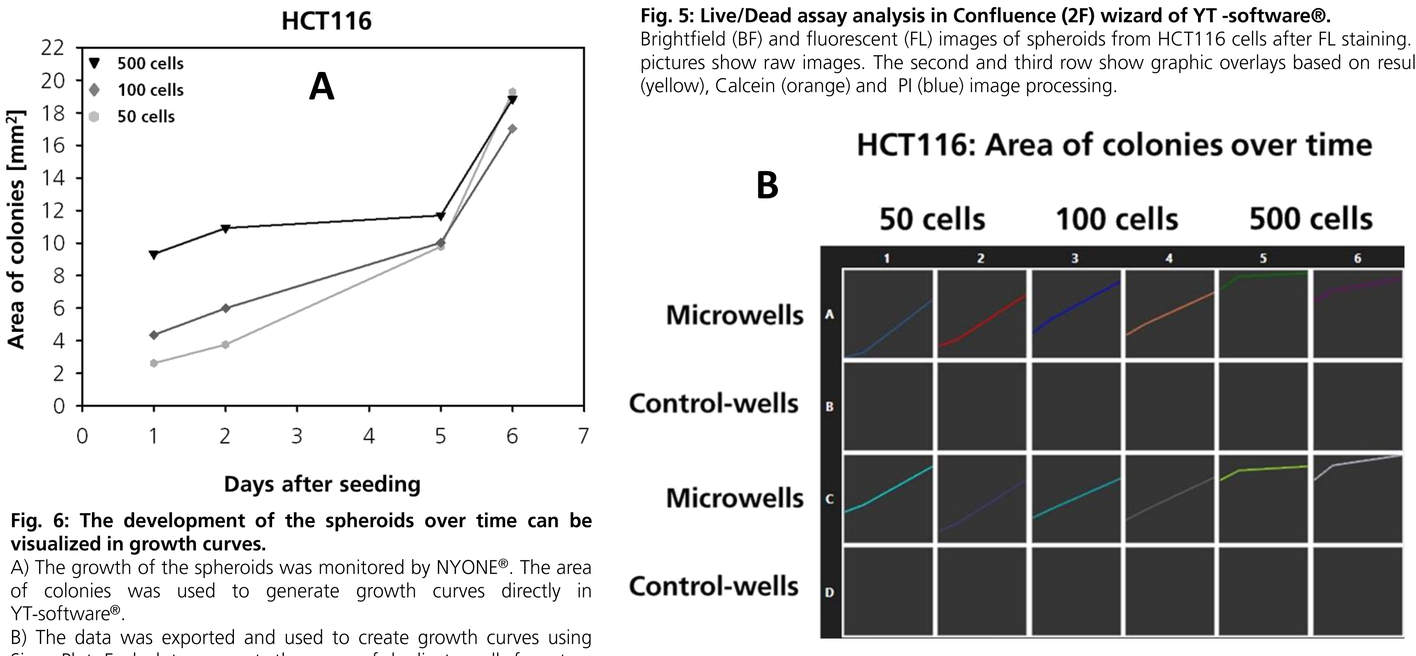


Fig. 6: The development of the spheroids over time can be visualized in growth curves.
A) The growth of the spheroids was monitored by NYONE®. The area of colonies was used to generate growth curves directly in YT-software®.
B) The data was exported and used to create growth curves using SigmaPlot. Each dot represents the mean of duplicate wells from two independent experiments.

CONCLUSIONS USING KUGELMEIERS SPHERICALPLATE 5D IN COMBINATION WITH SYNENTECS SIENTIFIC IMAGING SYSTEMS & YT-SOFTWARE®

- very potent and easy-to-use tool for working with 3D spheroid systems
- it is possible to grow a 3D spheroid cell culture model in a few days
- starting the measurement from template only takes minutes, just like the data handling and export which is made very easy and automatically
- Laser-based autofocusing is extremely precise and adjustable to ensure optimal imaging of samples
- 16 bit sCMOS camera with highest level in quantum efficiency allows detection of even weak fluorescence signals and the spheroids are protected by short exposure times
- Image processing across every channel is a powerful and robust tool to generate meaningful data

ACKNOWLEDGEMENT
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