Time Lapse Imaging of Spheroids – zenCELLowl Incubator Microscope

Simone Ruckdäschel[1], Stefanie Michaelis[2], Joachim Wegener[1,2]

[1]Institute of Analytical Chemistry, Chemo- & Biosensors, University of Regensburg

[2]Fraunhofer Research Institution for Microsystems and Solid State Technologies EMFT, Munich

MCF-7 cells, cultivation and spheroid generation

MCF-7 cells were kindly provided by the research group of Prof. Göpferich (University of Regensburg). Cells weregrown in Minimum Essential Medium Eagle, supplemented with fetal calf serum (10 % (v/v)), penicillin (100 µg/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and pyruvate (1 mM). Cells were split once per week in a ratio of 1:20 and cultivated at 37 °C with 5 % CO_2. MCF-7 spheroids were prepared by self aggregation of suspended cells in an agarose-coated 96-well plate (6000 cells/well) supported by orbital shaking at 37 °C with 5 % CO_2 over seven days.

Experimental set-up

Time lapse imaging of spheroids on adhesive or non-adhesive surfaces was performed using 24-well plates (Eppendorf, Catalog no. 0030722116) in two different experimental set-ups at 37 °C with 5 % CO_2.

A first experimental set-up was designed to monitor the *Proliferation of Spheroids*. Spheroids (1 day old, 6000 cells/well) were placed into the wells of a 24-well plate coated with agarose (1.5 % (w/v) in medium,200 µL/well) in order to prevent the spheroids from adhesion. The spheroids´ growth behavior was observed over four days using the zenCELLowl with the following settings: *Total Time Lapse Imaging*: 96 h, *Interval*: 10 min, *Focus*: ~500–600, *Exposure*: -7, *Illumination*: 30, *Brightness*: 16. The second experimental set-up was designed to monitor the *Adhesion and Outgrowth of Spheroids*. Here, spheroids (7 days old, 6000 cells/well) were placed into the wells of an uncoated, tissue culture treated 24-well plate. The spheroids´ adhesion was observed by the zenCELLowl with the following settings: *Total Time Lapse Imaging*: 48 h, *Interval*: 10 min, *Focus*: ~400–500, *Exposure*: -7, *Illumination*: 30, *Brightness*: 26.

The recorded images of each experiment were processed and analyzed using ImageJ (Wayne Rasband, NIH) and the zenCELLowl software.