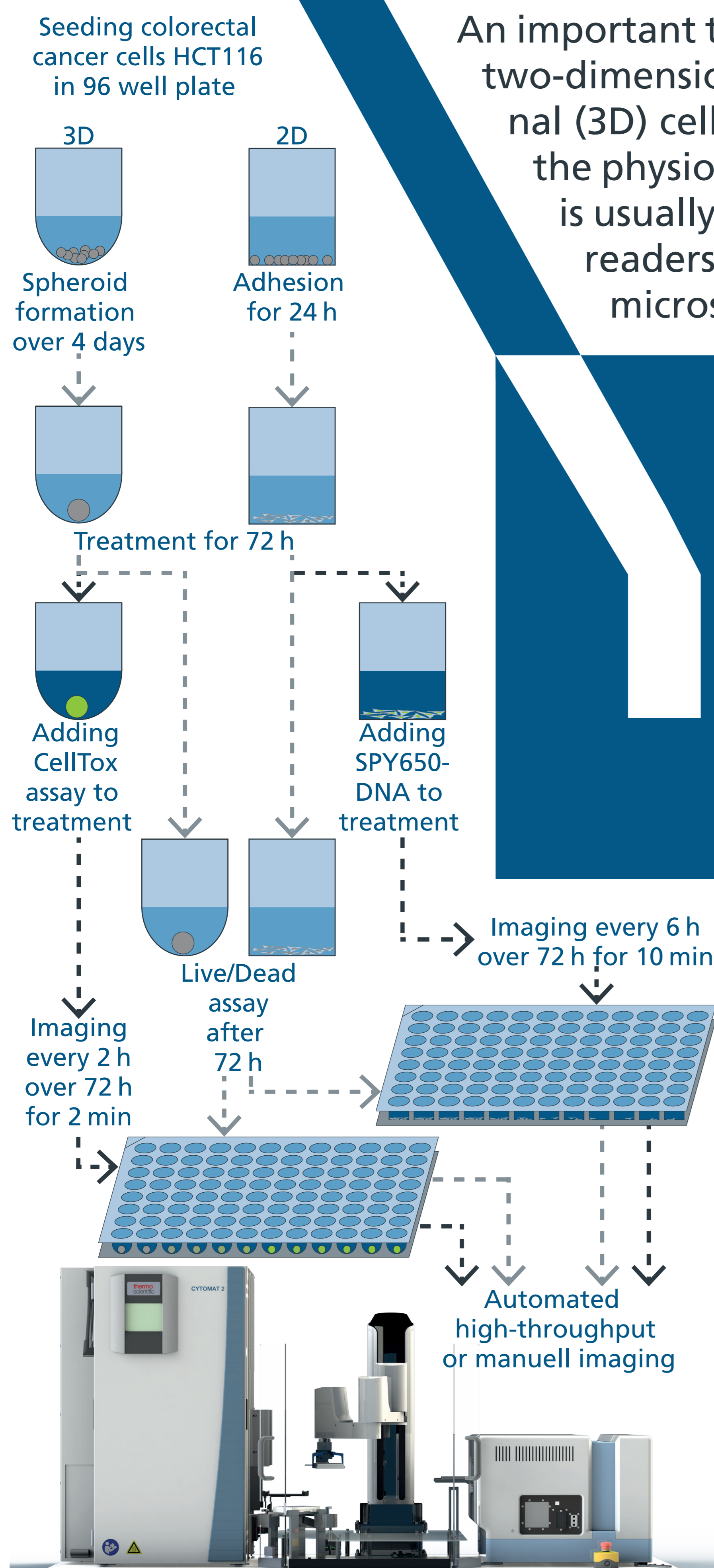# SYNENTEC

Analyzing Cytotoxicity in a 2D and 3D Colorectal Cancer Model using an Automated High Content Imaging System

Schaefer, W.[1]; Hedemann, N.[2]; Willms, A.[1]; Werdelmann, B.[1]; Stoehr, M.[1]; Schulze, K.[1]; Sebens, S.[3]; Geisen, R.[1] & Pirsch, M.[1]

[1]SYNENTEC GmbH, Elmshorn, Germany; [2]Department of Gynaecology and Obstetrics, CAU + UKSH, Kiel, Germany; [3]Institute for Experimental Cancer Research, CAU + UKSH, Kiel, Germany

Method

Seeding colorectal cancer cells HCT116 in 96 well plate

3D — Spheroid formation over 4 days

2D — Adhesion for 24 h

Treatment for 72 h

Adding CellTox assay to treatment

Adding SPY650-DNA to treatment

Live/Dead assay after 72 h

Imaging every 2 h over 72 h for 2 min

Imaging every 6 h over 72 h for 10 min

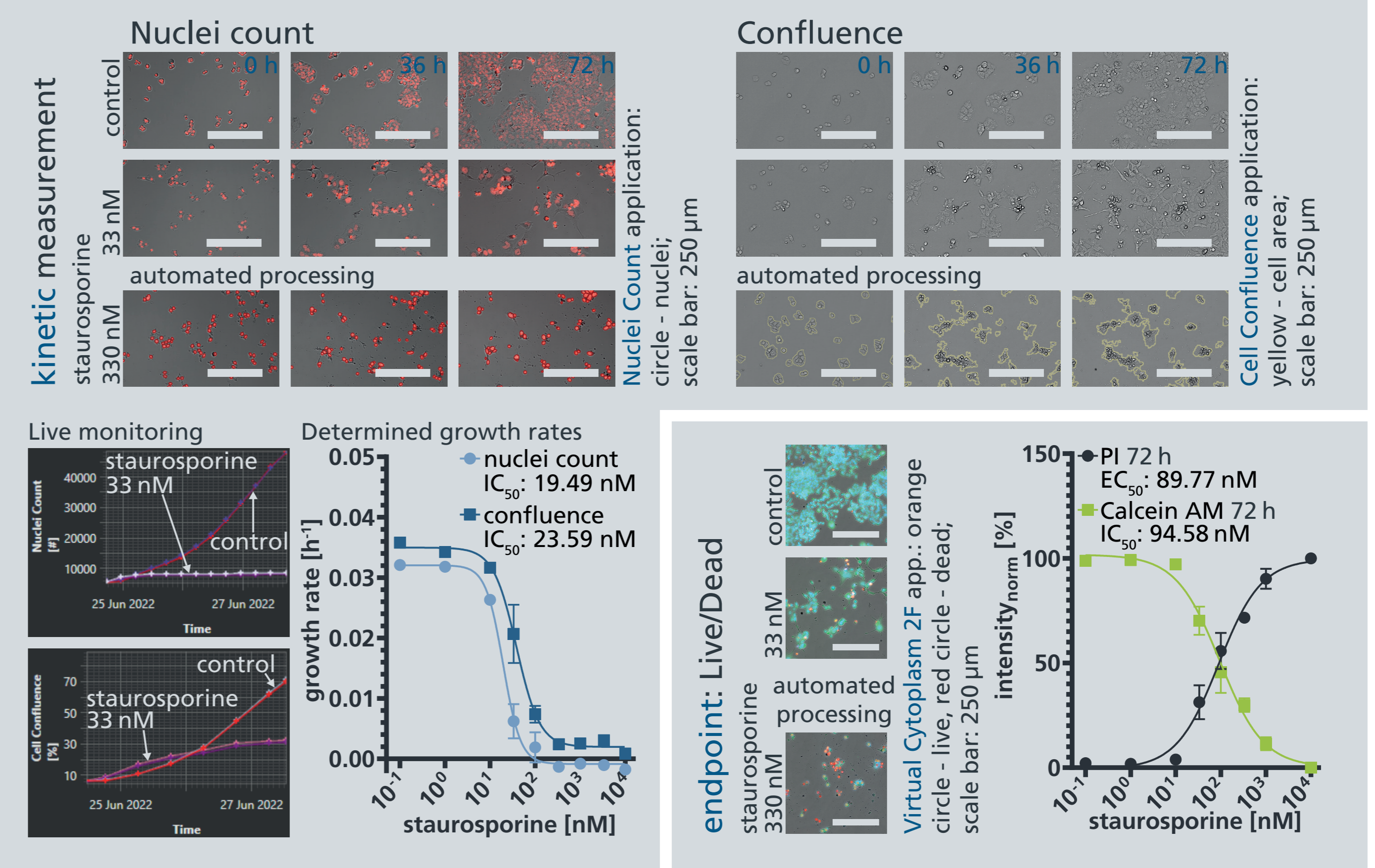Automated high-throughput or manuell imaging

Introduction

An important tool in the field of cancer research and drug discovery is cell culture. Conventionally, two-dimensional (2D) cell culture models have been used, but in the last years, three-dimensional (3D) cell culture models like spheroids have gained more interest. 3D models better mimic the physiology of tissues and tumors, but the generation, application and evaluation of assays is usually more difficult. Therefore, either simple one-parameter endpoint assays using plate readers are used as a read-out for high-throughput screening. Or confocal or time-lapse microscopes are used for multi-parameter high content analysis.
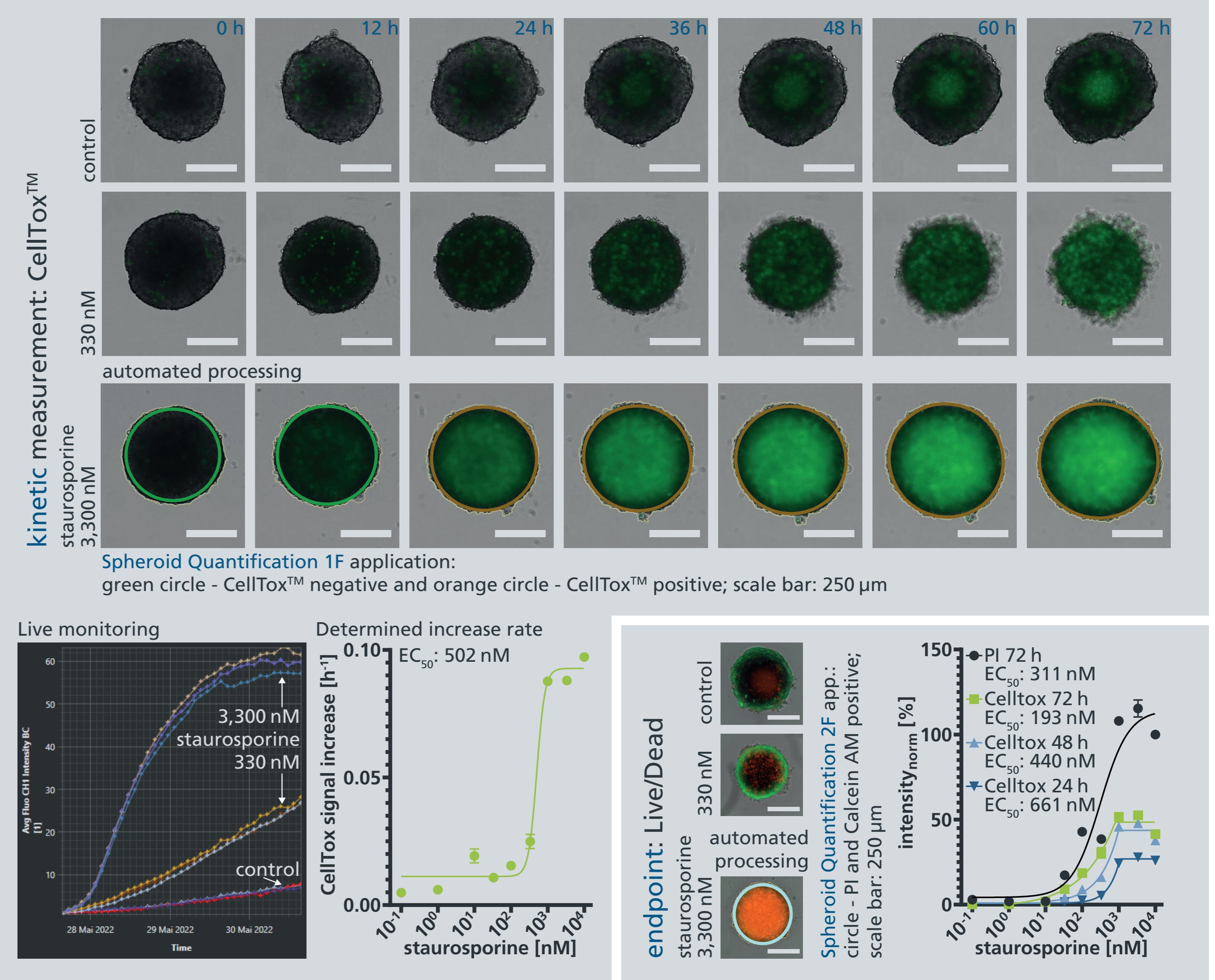
Here, we used SYNENTEC's automation system to analyze effects of the drug staurosporine in 2D and 3D cell models of colorectal cancer cells (HCT116) over time.

Results

2D measurement

kinetic measurement

Nuclei count — control, staurosporine 330 nM, staurosporine 33 nM, automated processing — 0 h, 36 h, 72 h — Nuclei Count application: circle - nuclei; scale bar: 250 µm

Confluence — control, staurosporine 330 nM, staurosporine 33 nM, automated processing — 0 h, 36 h, 72 h — Cell Confluence application: yellow - cell area; scale bar: 250 µm

Live monitoring — staurosporine 33 nM, control

Determined growth rates — nuclei count IC$_{50}$: 19.49 nM; confluence IC$_{50}$: 23.59 nM

endpoint: Live/Dead — control, staurosporine 330 nM, staurosporine 33 nM, automated processing — Virtual Cytoplasm 2F app.: orange circle - live, red circle - dead; scale bar: 250 µm — PI 72 h EC$_{50}$: 89.77 nM; Calcein AM 72 h IC$_{50}$: 94.58 nM

3D measurement

kinetic measurement: CellTox™ — control, staurosporine 330 nM, staurosporine 3,300 nM, automated processing — 0 h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h — Spheroid Quantification 1F application: green circle - CellTox™ negative and orange circle - CellTox™ positive; scale bar: 250 µm

Live monitoring — 3,300 nM staurosporine, 330 nM, control

Determined increase rate — CellTox signal increase rate EC$_{50}$: 502 nM

endpoint: Live/Dead — control, staurosporine 330 nM, staurosporine 3,300 nM, automated processing — Spheroid Quantification 2F app.: circle - PI and Calcein AM positive; scale bar: 250 µm — PI 72 h EC$_{50}$: 311 nM; Celltox 72 h EC$_{50}$: 193 nM; Celltox 48 h EC$_{50}$: 440 nM; Celltox 24 h EC$_{50}$: 661 nM

3D and 2D analysis with our automation system:

- The system enables convenient and timesaving high-throughput imaging
- Cytotoxic effects can be monitored directly over time
- YT-SOFTWARE® analyzes many different parameters
- Time charts and heat maps provide a quick result overview
- Various assays can be performed
- 3D can be easily compared to 2D