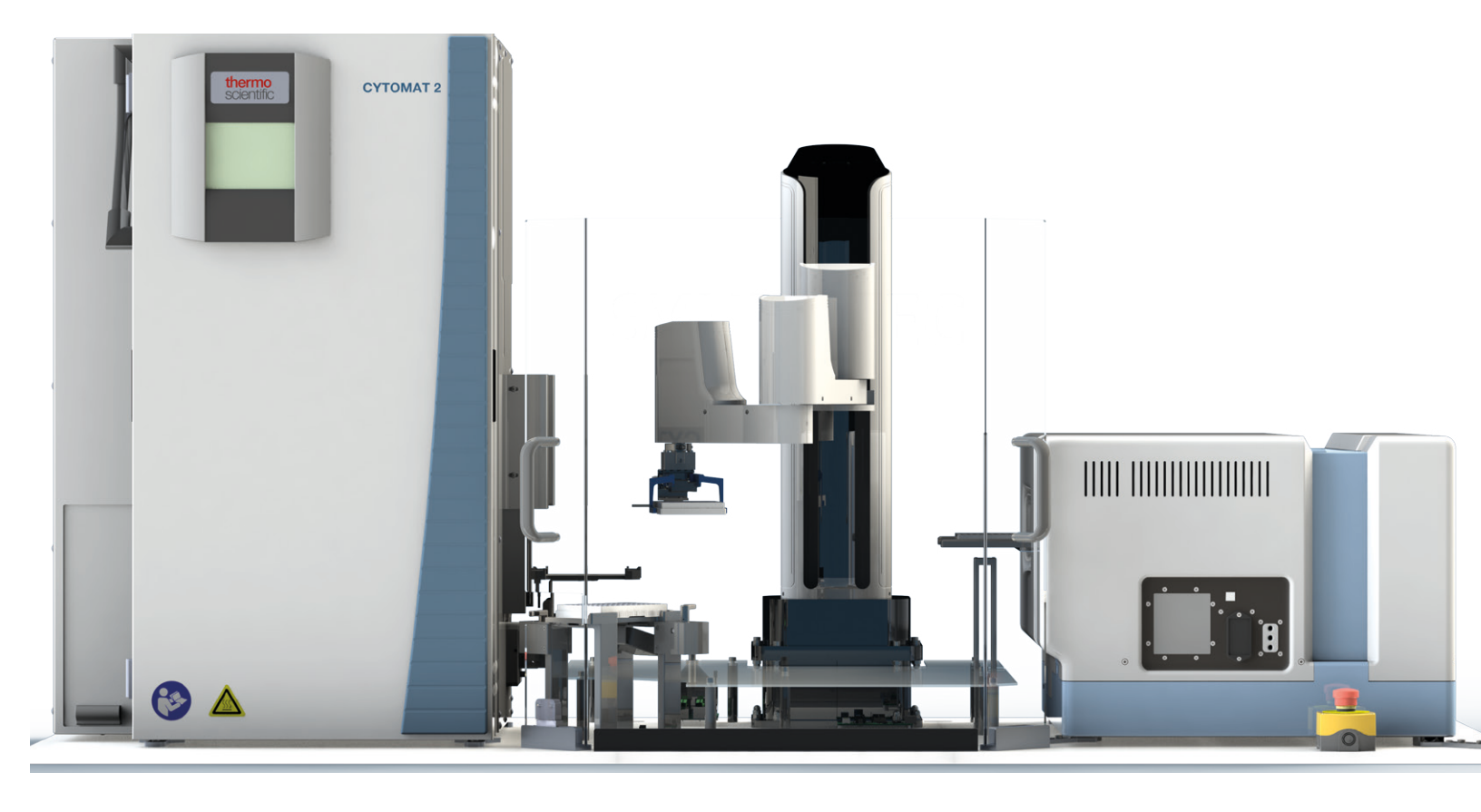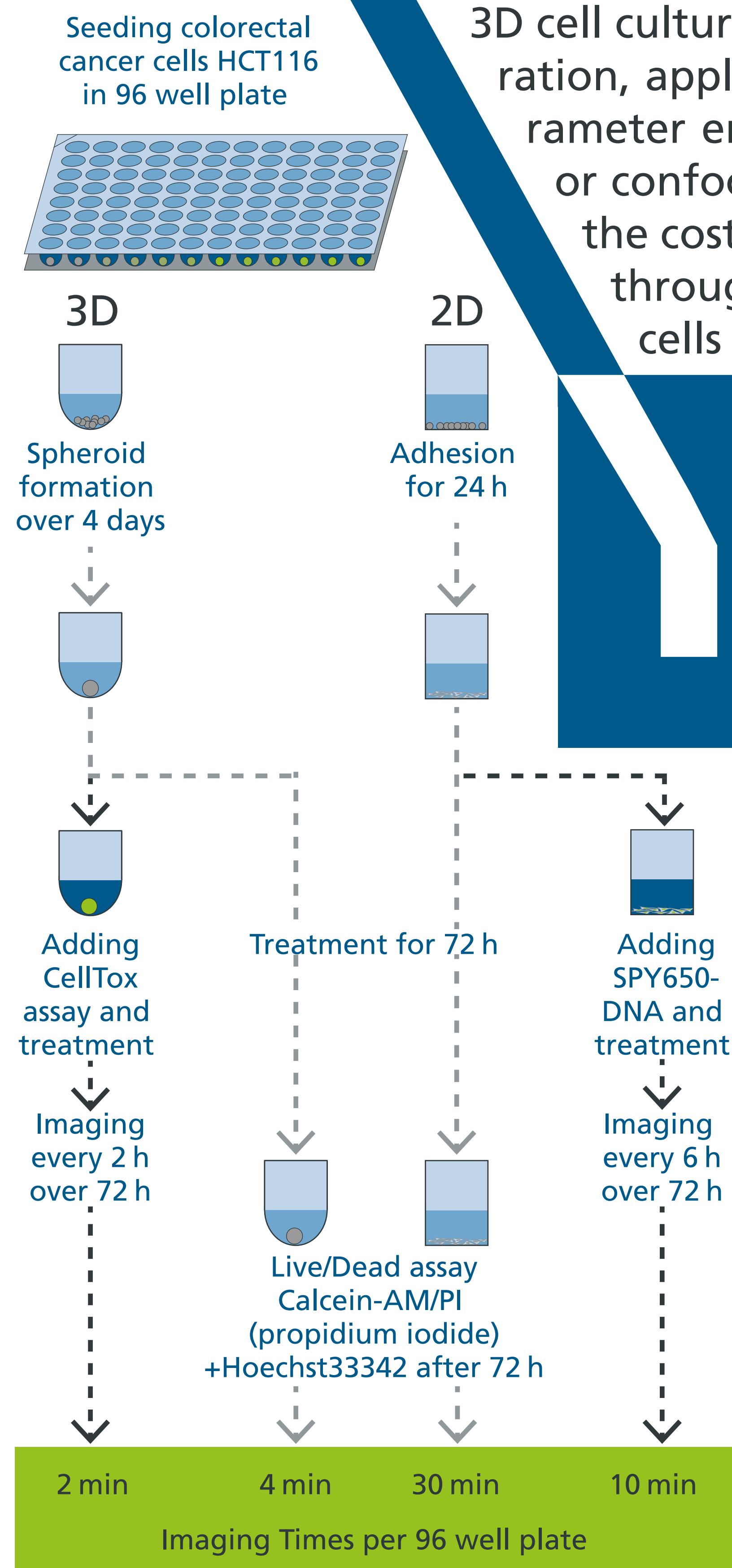# SYNENTEC

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

Adding CellTox assay and treatment

Imaging every 2 h over 72 h

2D

Adhesion for 24 h

Treatment for 72 h

Adding SPY650-DNA and treatment

Imaging every 6 h over 72 h

Live/Dead assay Calcein-AM/PI (propidium iodide) +Hoechst33342 after 72 h

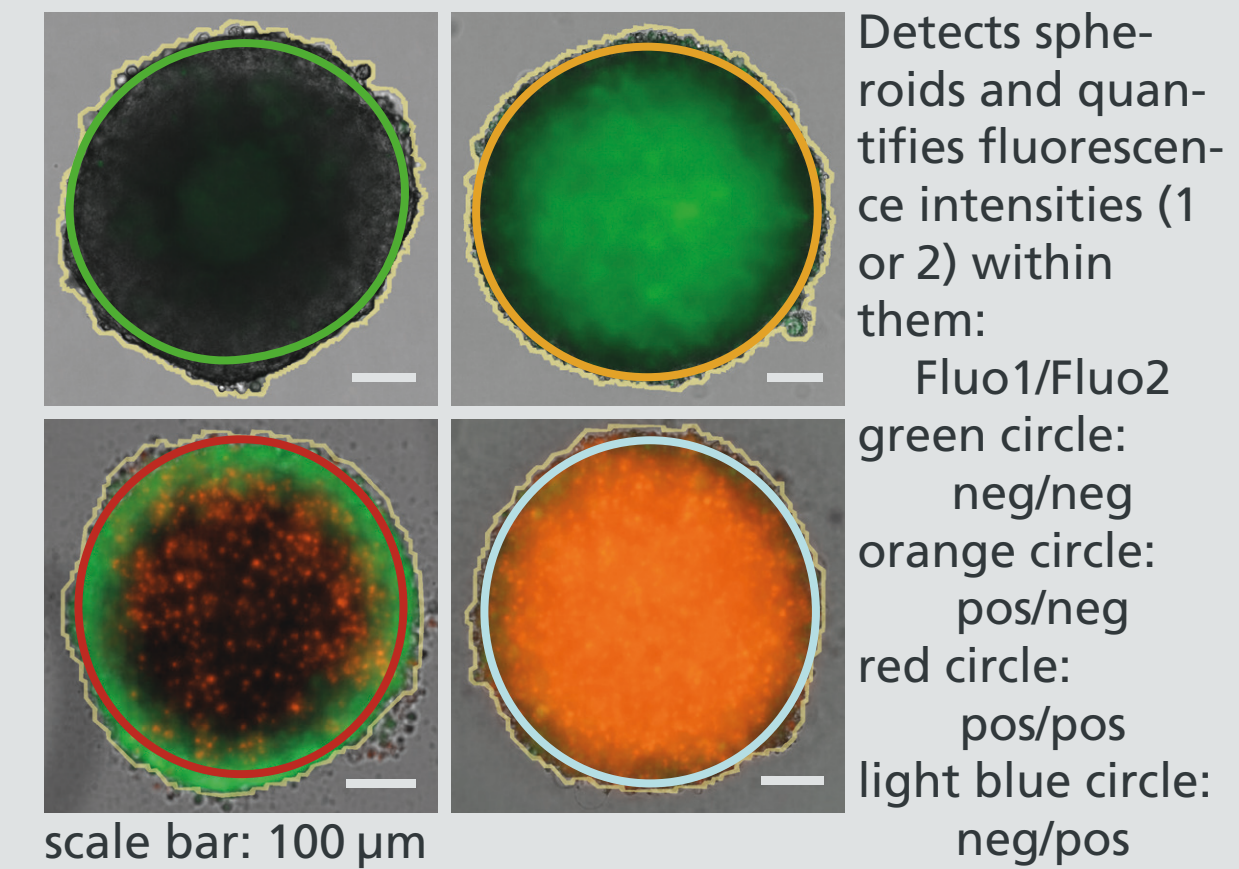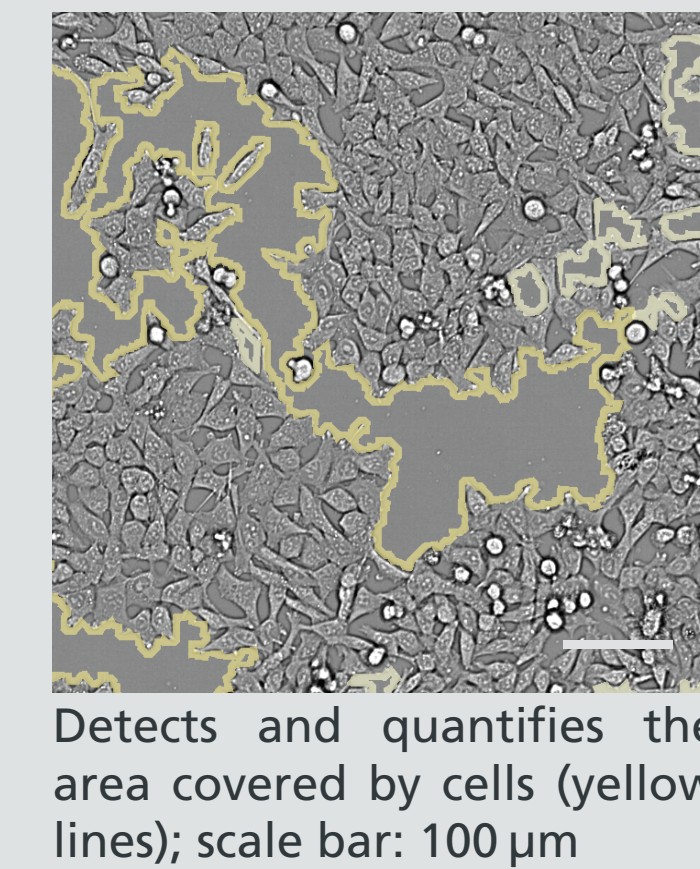| 2 min | 4 min | 30 min | 10 min |

Imaging Times per 96 well plate

Introduction

3D cell culture models better mimic the physiology of tissues and tumors than 2D models, 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, often at the cost of throughput. Here, we describe the analysis of cytotoxicity in tumor spheroids in a high-throughput manner using our automation system. We generated spheroids of colorectal cancer cells (HCT116), treated them with the drug staurosporine, added CellTox™ Green and automatically imaged them every two hours. Imaging of a 96 well plate took around 2 min enabling high throughput. Additionally, we performed end-point and 2D analysis.
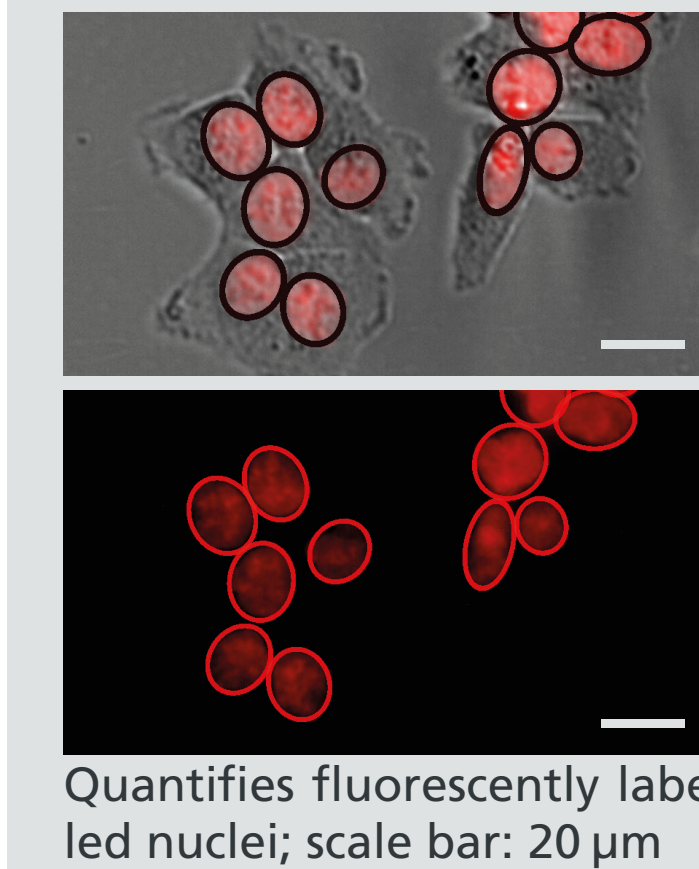
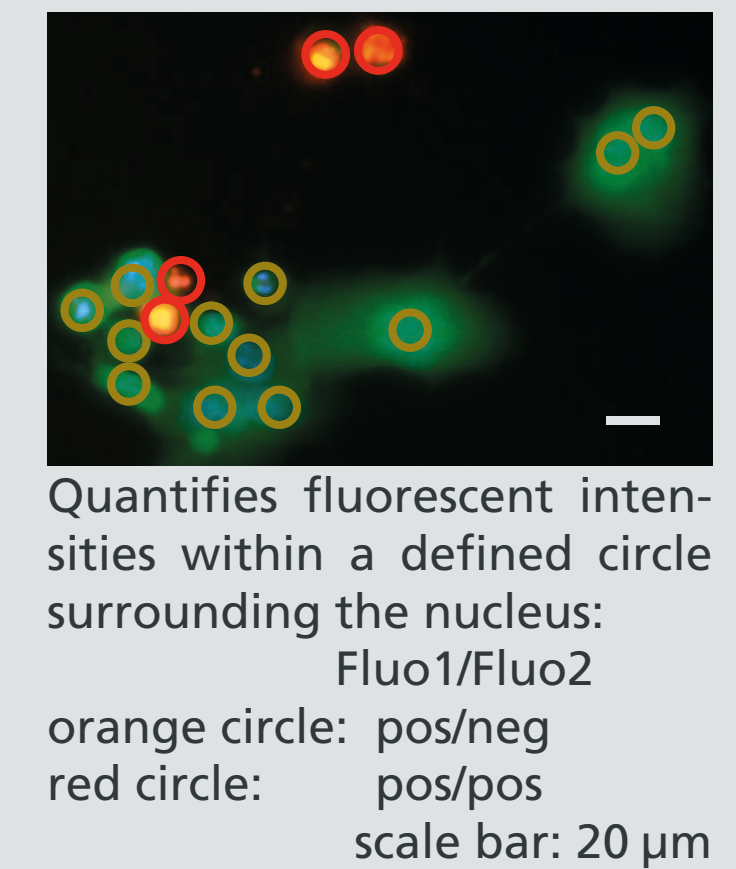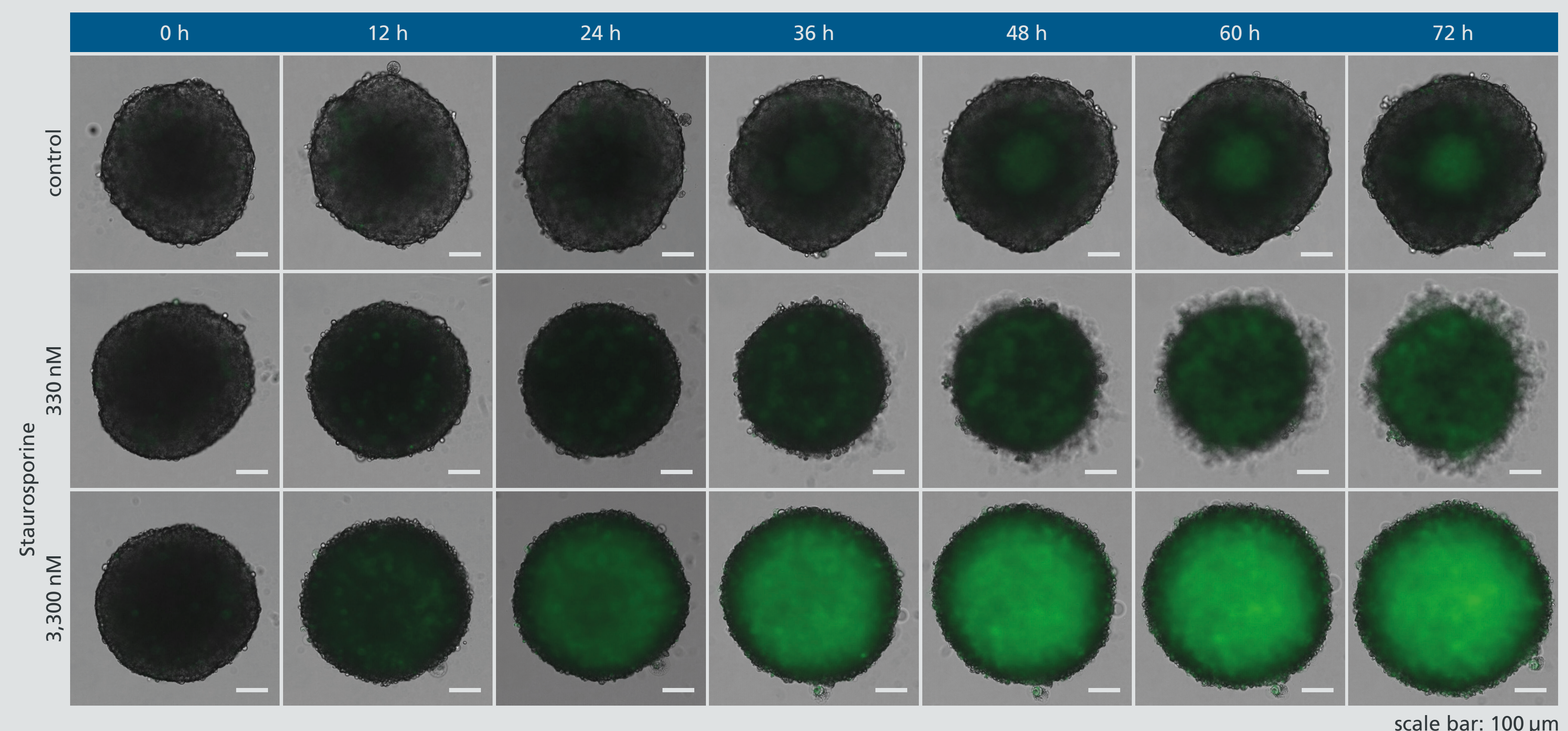## Applications

Spheroid Quantification (1F/2F)

Detects spheroids and quantifies fluorescence intensities (1 or 2) within them:
Fluo1/Fluo2
green circle: neg/neg
orange circle: pos/neg
red circle: pos/pos
light blue circle: neg/pos
scale bar: 100 µm

Cell Confluence

Detects and quantifies the area covered by cells (yellow lines); scale bar: 100 µm

Nuclei Count

Quantifies fluorescently labeled nuclei; scale bar: 20 µm

Virtual Cytoplasm

Quantifies fluorescent intensities within a defined circle surrounding the nucleus:
Fluo1/Fluo2
orange circle: pos/neg
red circle: pos/pos
scale bar: 20 µm

Results

kinetic measurement: CellTox™

3D measurement

| | 0 h | 12 h | 24 h | 36 h | 48 h | 60 h | 72 h |

control
Staurosporine 330 nM
Staurosporine 3,300 nM

scale bar: 100 µm

Live monitoring
3,300 nM Staurosporine
330 nM
control
Avg. Fluo CH1 Intensity BC [-]
Time [day]

Determined rates
EC50: 502 nM
CellTox signal increase [h⁻¹]
Staurosporine [nM]

endpoint measurement: Live/Dead

control
Staurosporine 330 nM
Staurosporine 3,300 nM
Brightfield   Calcein-AM   PI   Merge
scale bar: 100 µm

PI 72 h EC50: 311 nM
CellTox 72 h EC50: 193 nM
CellTox 48 h EC50: 440 nM
CellTox 24 h EC50: 661 nM
intensity norm [%]
Staurosporine [nM]
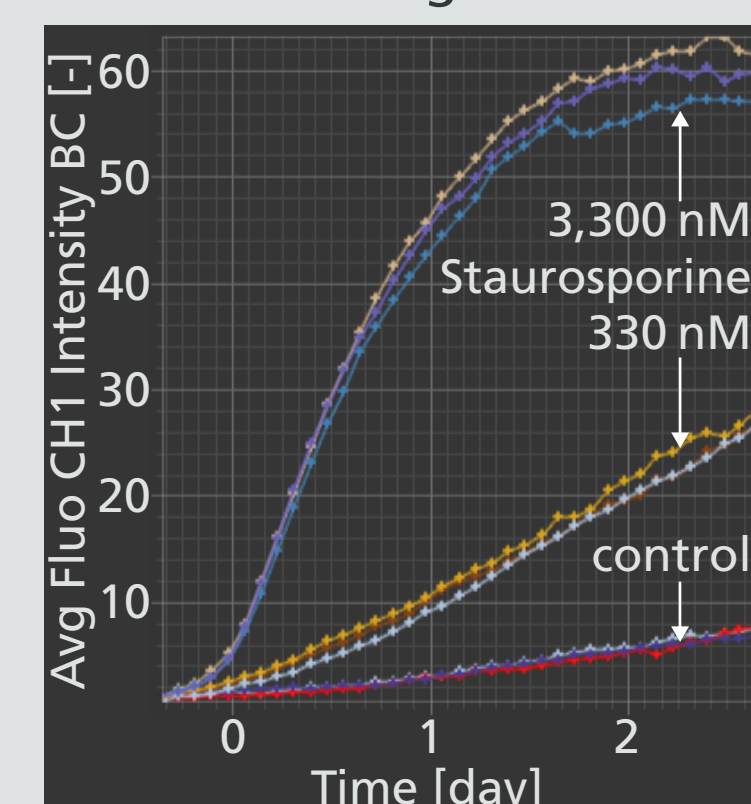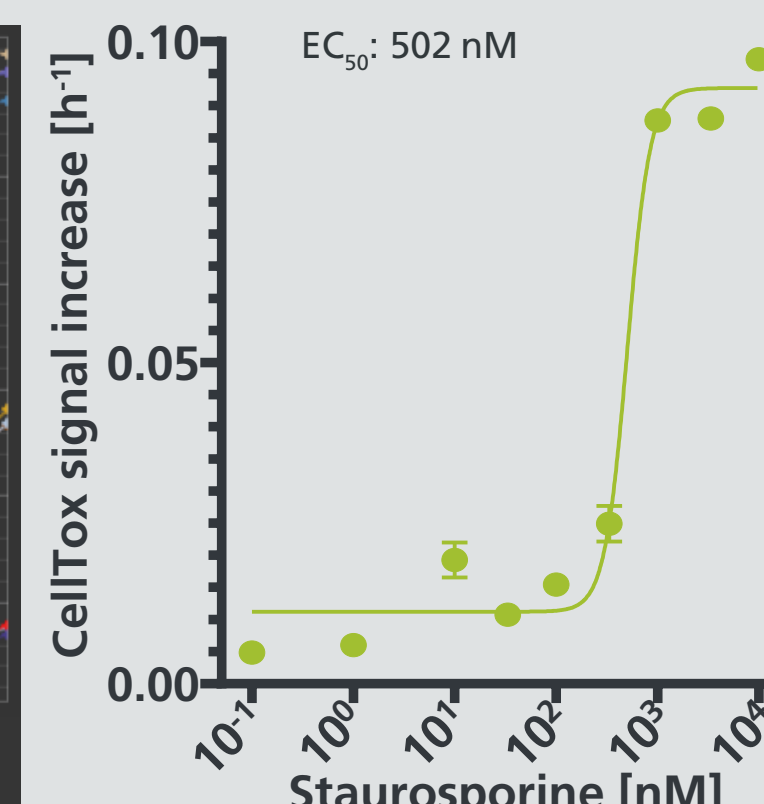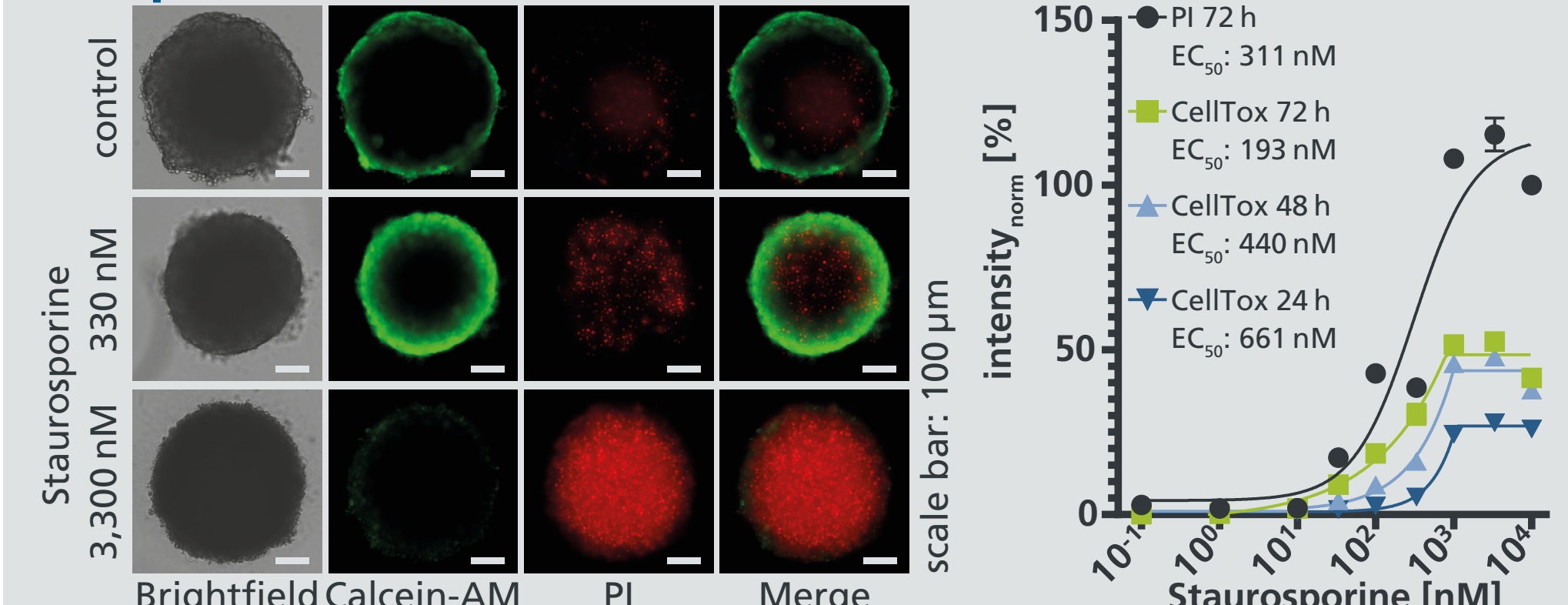
kinetic measurement: SPY650-DNA

2D measurement

control
33 nM Staurosporine
Nuclei Count [#]
Time [day]

control
Staurosporine 33 nM
Cell Confluence [%]
Time [day]

nuclei count IC50: 19.49 nM
confluence IC50: 23.59 nM
growth rate [h⁻¹]
Staurosporine [nM]

endpoint measurement: Live/Dead

control
Staurosporine 33 nM
Staurosporine 330 nM
Brightfield   Calcein-AM   PI   Merge
scale bar: 100 µm

PI 72 h EC50: 89.77 nM
Calcein AM 72 h IC50: 94.58 nM
positive cells [%]
Staurosporine [nM]
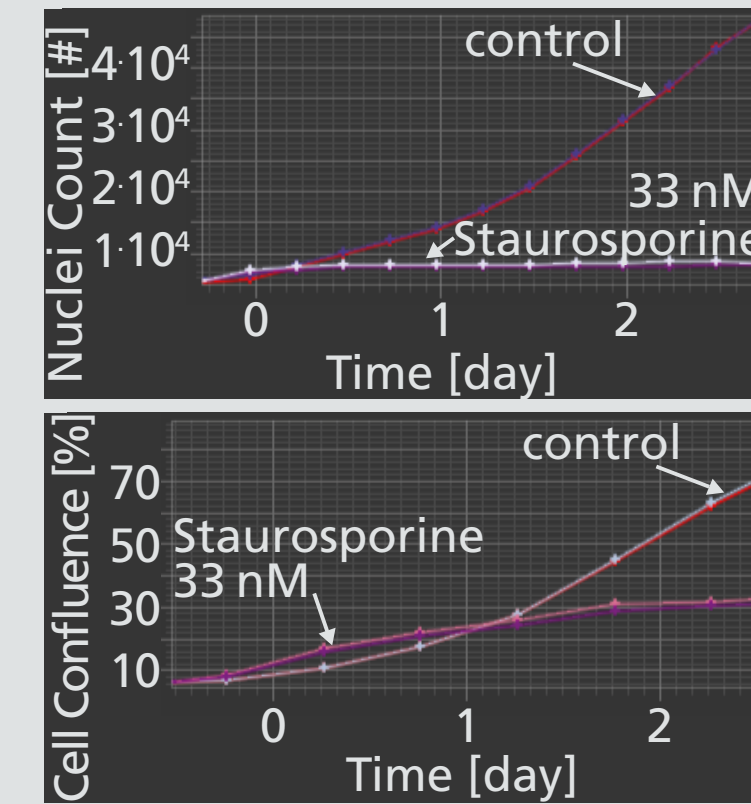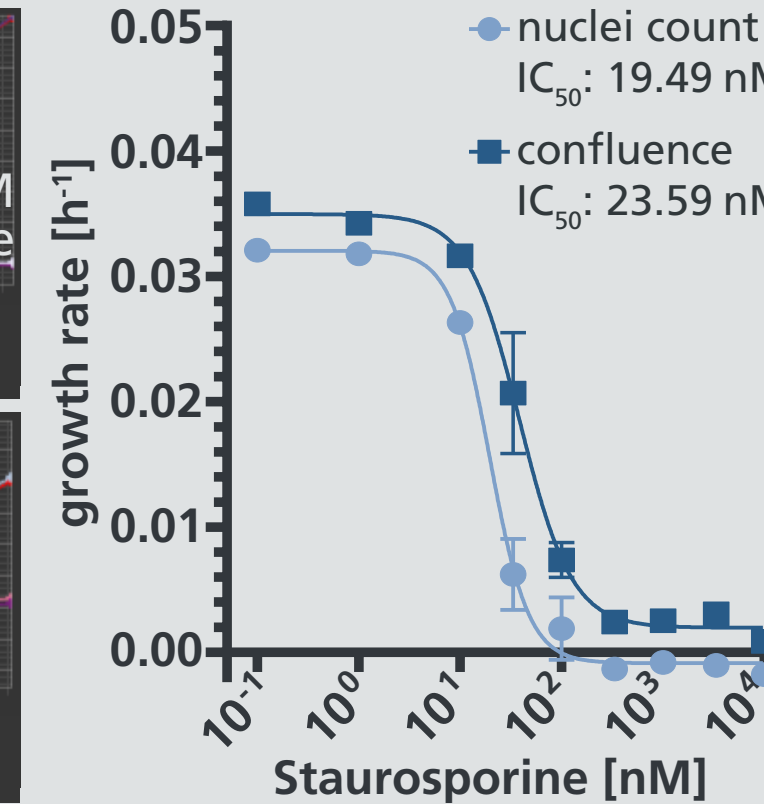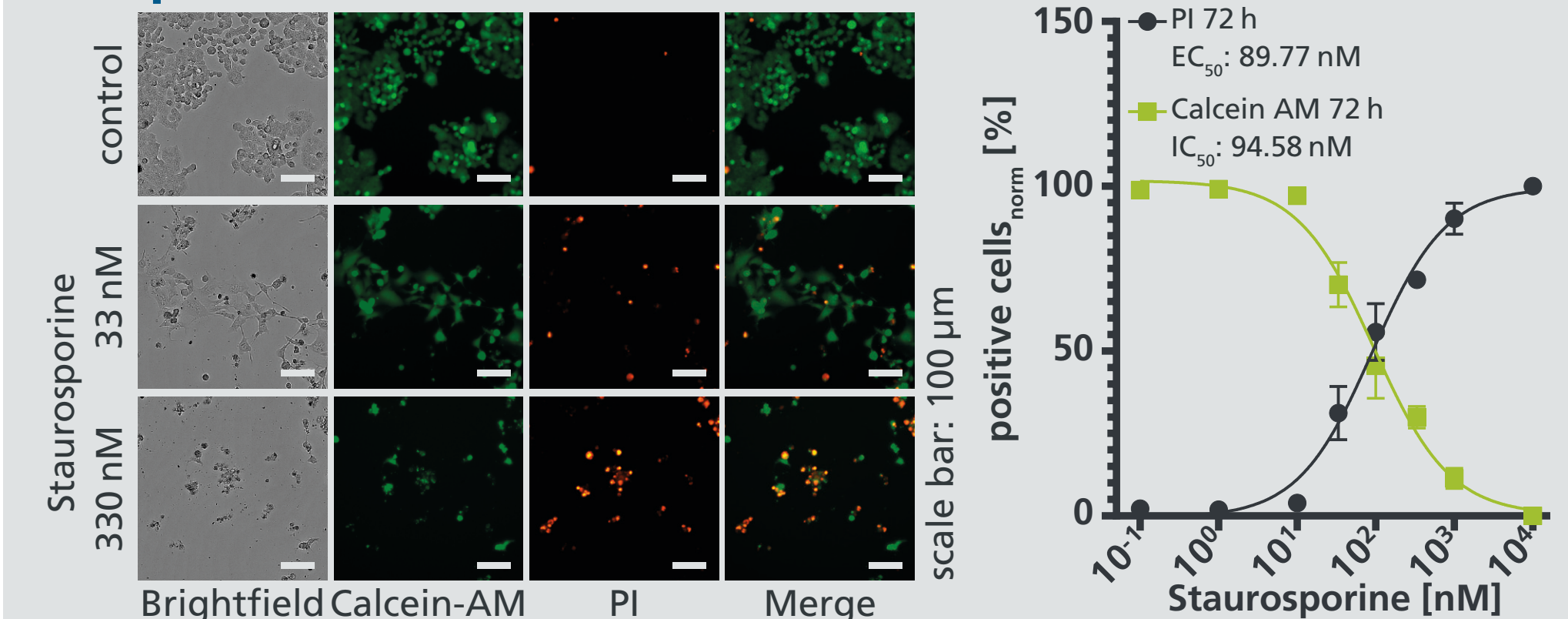
3D and 2D analysis using our automation system:

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