APPLICATION NOTE

Apoptosis assay using AI-driven fully automated ECLIPSE Ji

ECLIPSE Ji with Smart Experiment software enables seamless run from image acquisition to analysis and graph creation. Pre-trained Artificial Intelligence (AI) and pre-defined imaging processes automatically optimize image acquisition and analysis condition settings, providing visualized data and EC_{50} information with simple operation. Apoptosis is a type of programmed cell death. Abnormalities in apoptosis cause various diseases such as cancer, autoimmune diseases, and neurological disorders. Many cancer cells acquire resistance to apoptosis, avoiding cell death and continuing to proliferate. Apoptotic cells can be detected by fluorescence dependent on the activity of Caspase-3/7, a marker for apoptosis. This application note introduces an example of using the Apoptosis module of Smart Experiment to visualize the dose-dependent induction of apoptosis by staurosporine in cancer cells and quantify the effect of the drug by calculating the EC_{50}.

Keywords: Apoptosis, caspase activity, anticancer drug research, pharmacological test, automatic setting, EC_{50}, dose-response curve

Experimental overview

Add compound

CellEvent™ Caspase-3/7 Stain

Hoechst Stain

Cell Seeding Culture		Compound Treatment	
24h	30min	48h	30min

Fully automated imaging

Apoptosis

✓ Capture
✓ Image analysis
✓ Data visualization
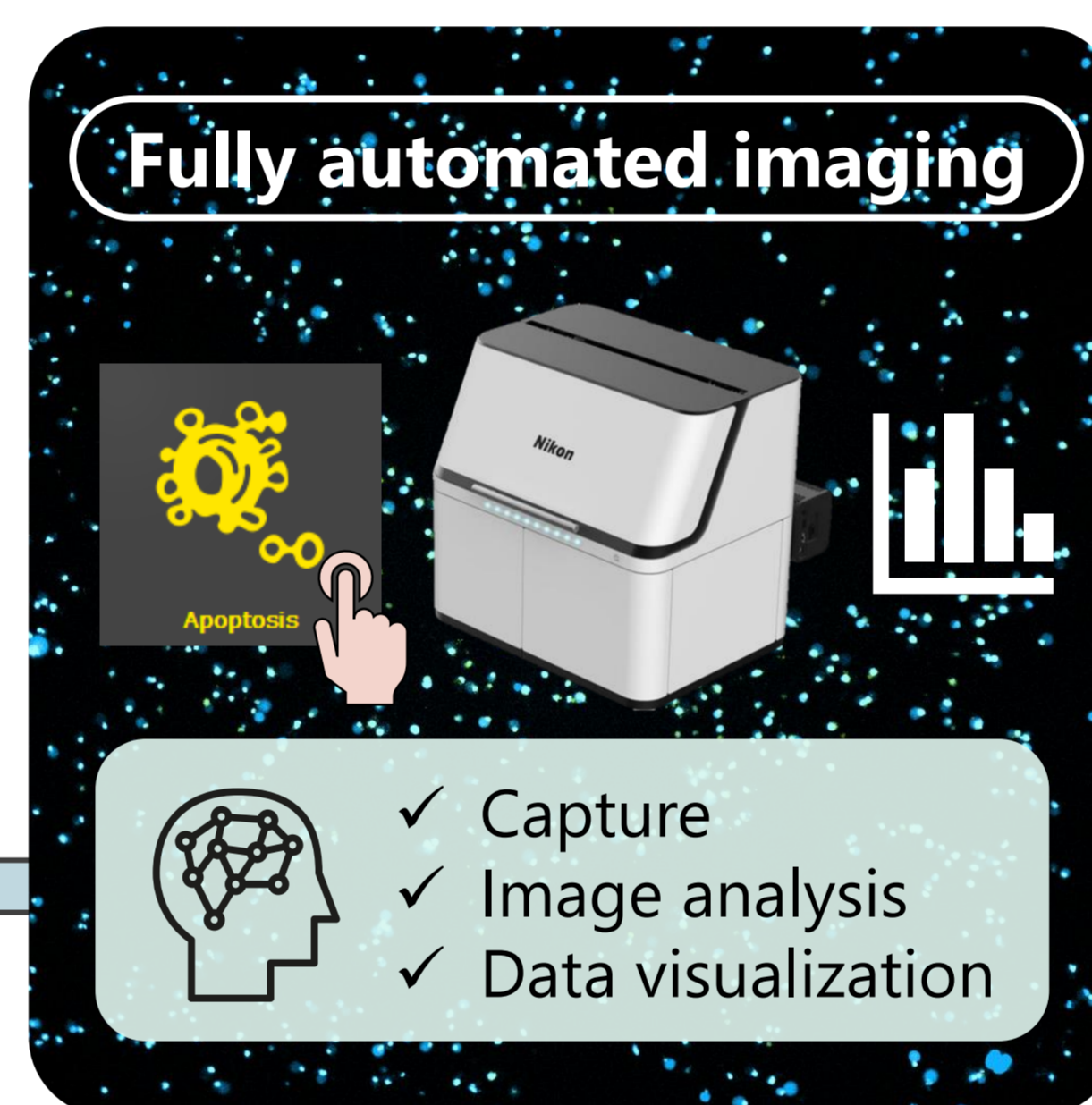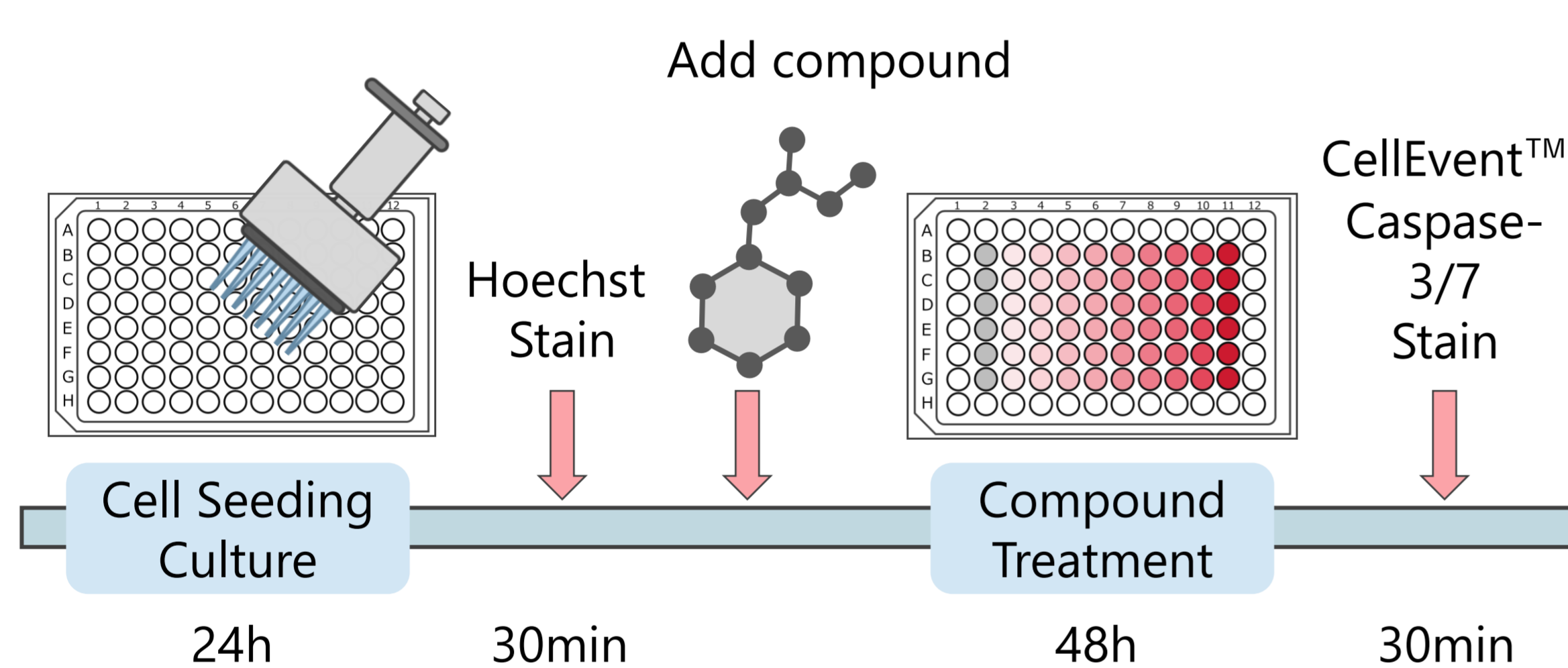
● **Key features**
✓ Fully automated from image acquisition to analysis and graphing
✓ Measuring the percentage of the number of apoptotic cells
✓ Easily quantify drug response
✓ Automatically create dose-response curve
✓ Automatically calculate EC_{50} /IC_{50}
✓ Automatically calculate Z'-factor

(1) HeLa cells were seeded in 96-well plates and cultured for 24 hours. (2) Cells were stained with media containing Hoechst 33342 for 30 minutes. (3) The test substance staurosporin was diluted to 10 different concentrations in medium, added to each well, and treated for 48 hours. (4) Cells were stained with CellEvent™ Caspase-3/7 for 30 minutes. (5) Without changing the medium, the well plate was placed on ECLIPSE Ji, and image acquisition and analysis were run automatically by selecting the Apoptosis icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells	Hoechst 33342	352/461
Nucleus of Apoptotic cells	CellEvent™ Caspase-3/7	502/530
Magnification	**Field of view**	
10X	1.76 x 1.76 mm	

Table. 1: Detection regions, fluorescence labels, and image acquisition conditions

Results

Top: Brightfield image
Bottom: Merged image

Fluorescence images

Masks

Hoechst 33342   All cells   10 μm

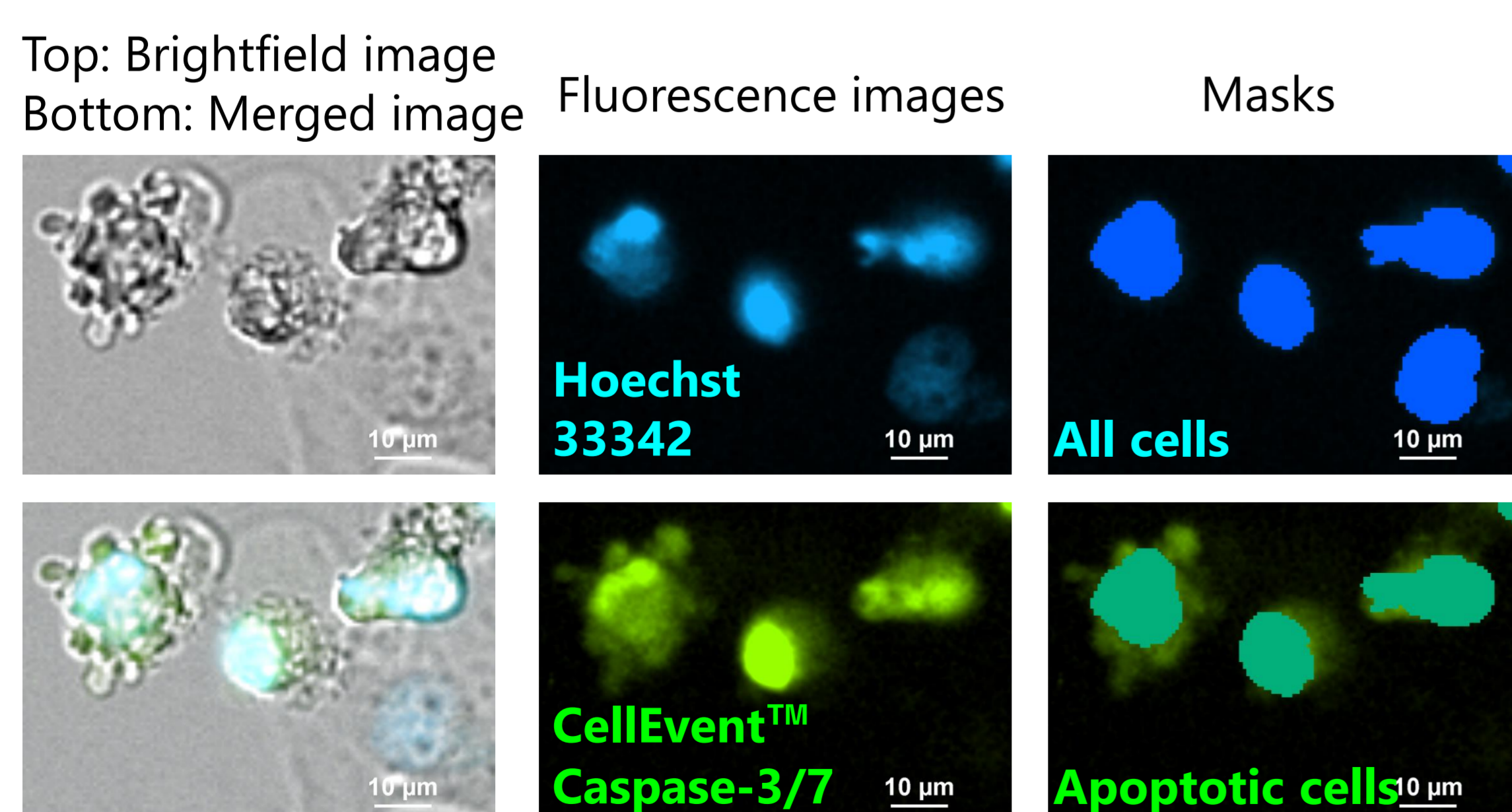CellEvent™ Caspase-3/7   Apoptotic cells   10 μm

Fig. 1: Images and masks of HeLa cells
Upper left: brightfield image, Lower left: merged image of brightfield and fluorescence, Center: fluorescence images, (Top: Hoechst 33342 (blue: nuclei of all cells), Bottom: CellEvent™ Caspase-3/7 (green: nuclei of apoptotic cells)), Right: masks (blue mask: nuclei of all cells, green mask: nuclei of apoptotic cells)
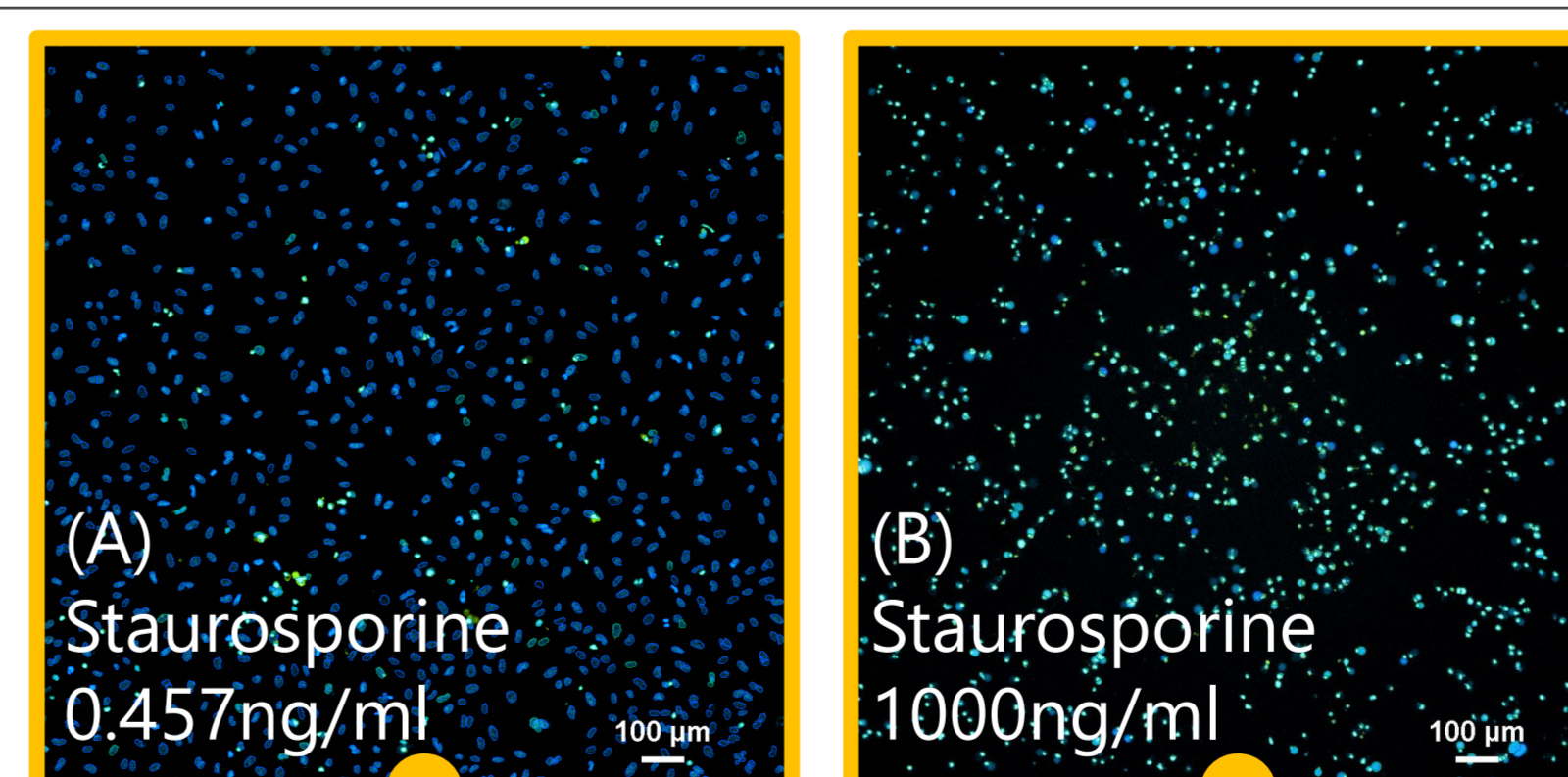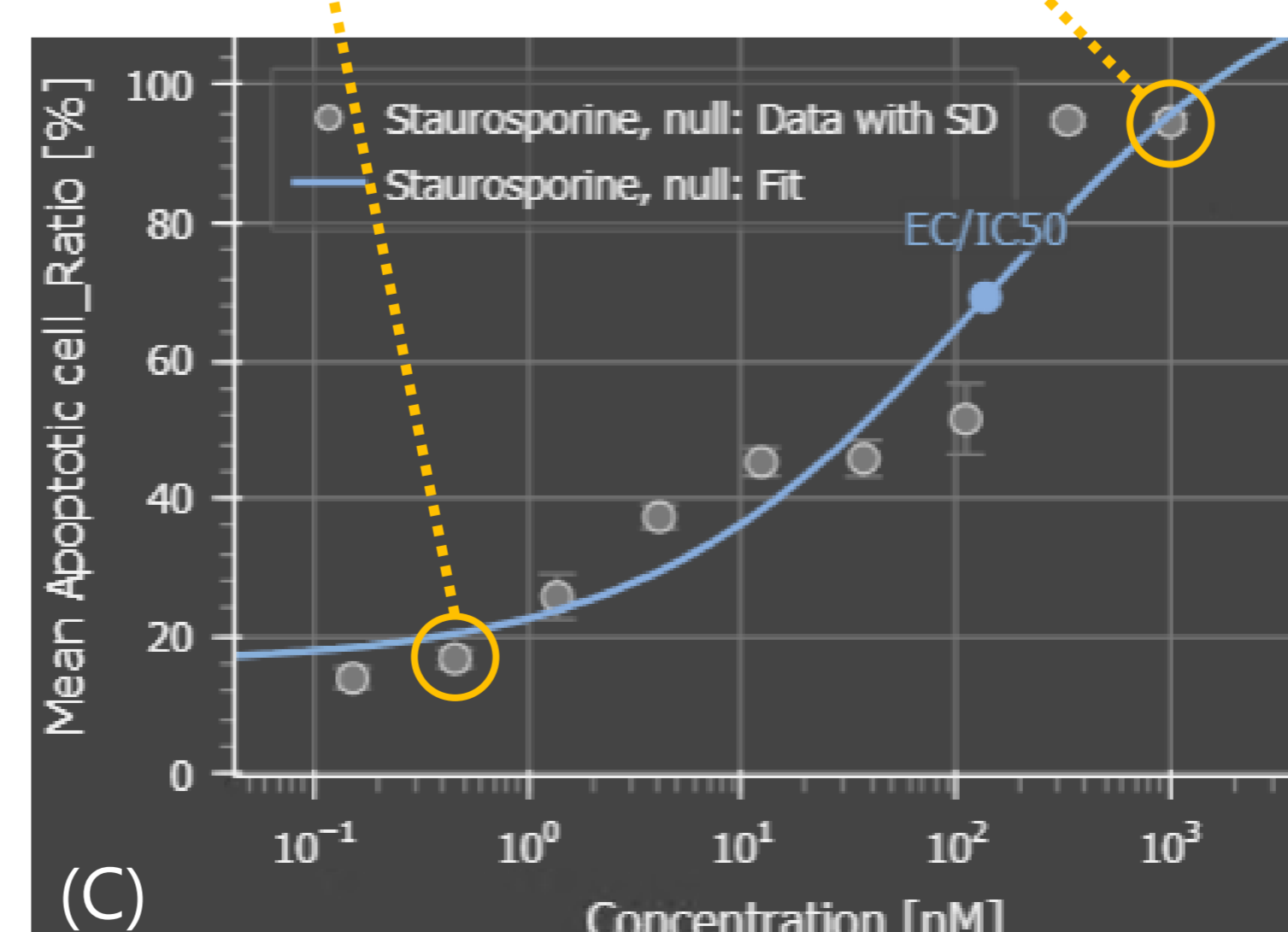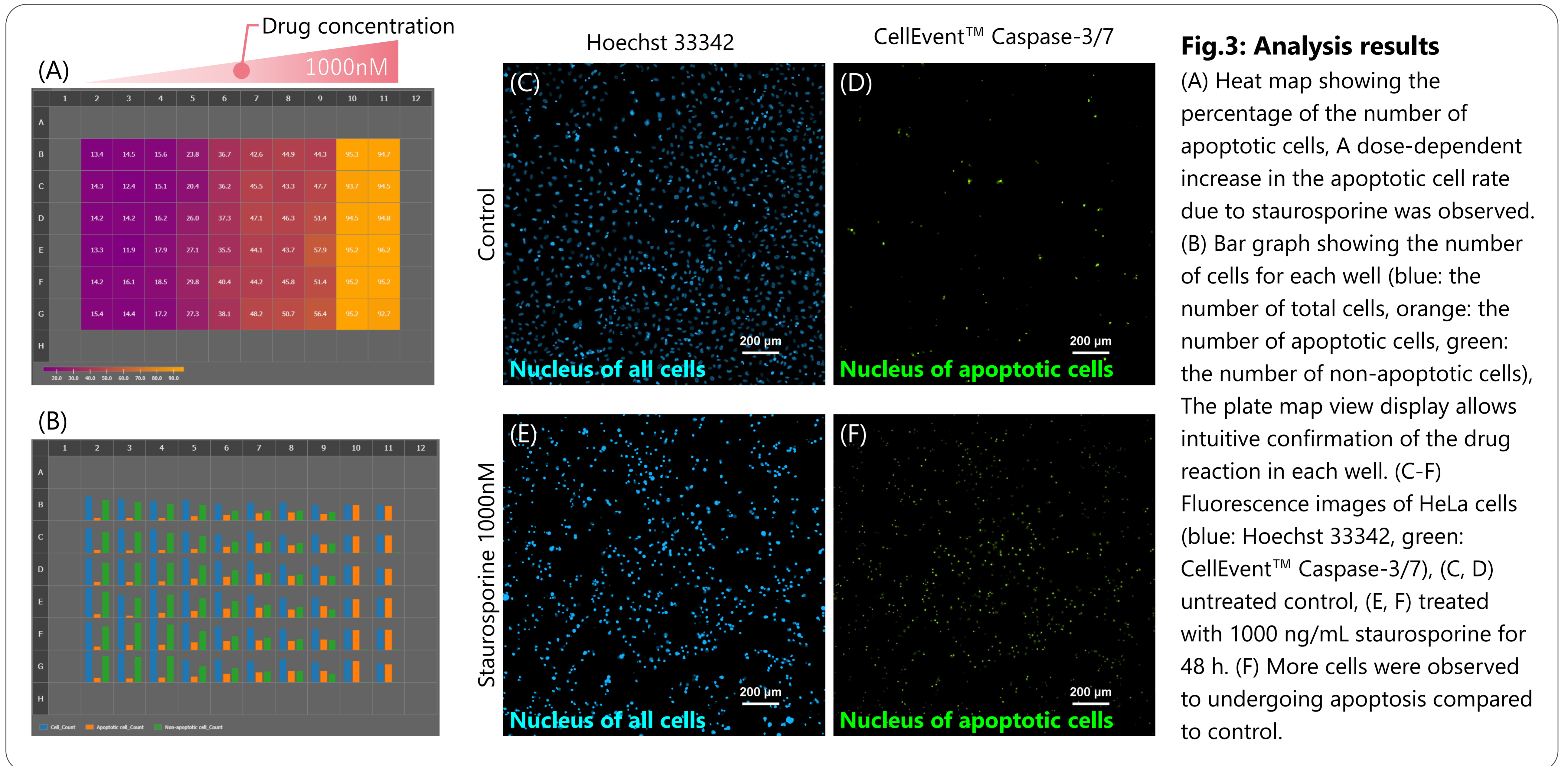

(A) Staurosporine 0.457ng/ml   (B) Staurosporine 1000ng/ml   100 μm

(C) Mean Apoptotic cell_Ratio [%] vs Concentration [nM]
- Staurosporine, null: Data with SD
- Staurosporine, null: Fit
- EC/IC50

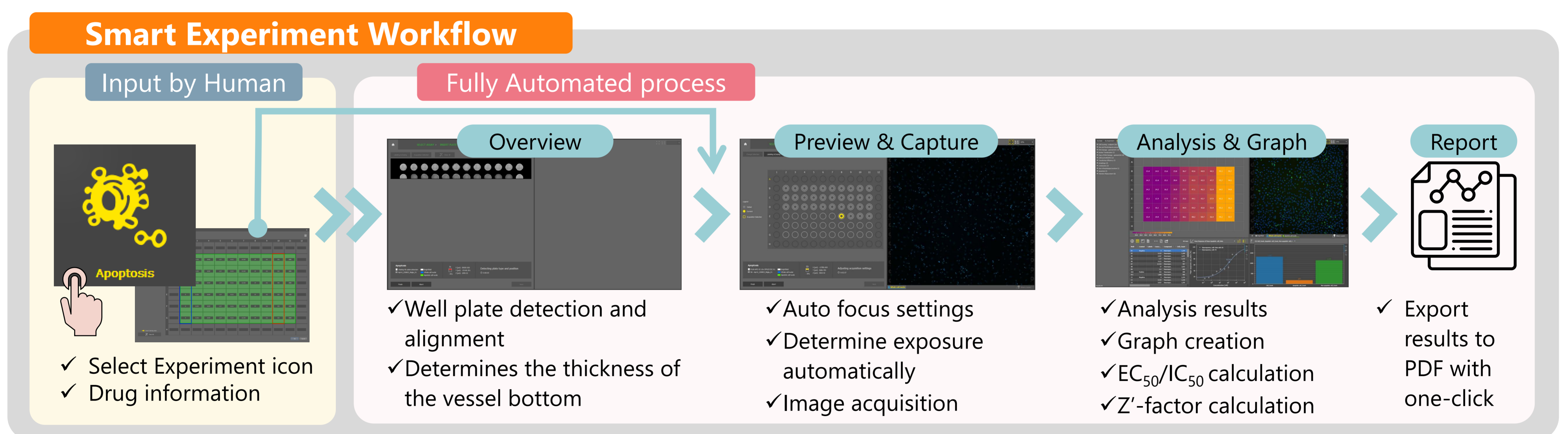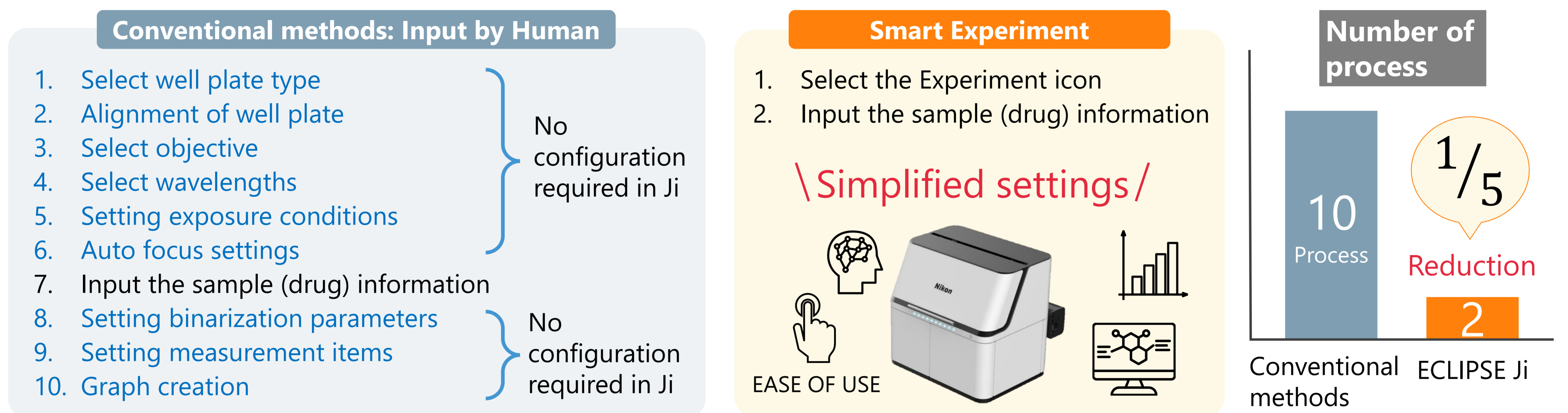**Fig. 2: Images of apoptotic HeLa cells and dose-response curve of staurosporine.**
(A, B) Fluorescence image with overlaid mask outline, (blue mask: nuclei of all cells, green mask: nuclei of apoptotic cells), Scale bar: 100 μm. (C) Dose-response curve showing a dose-dependent increase in the ratio of apoptotic cells with staurosporine (X-axis: drug concentration (log), Y-axis: ratio of the number of apoptotic cells), EC_{50} = 137.182 nM, Z'-factor = 0.95.

Results

Fig.3: Analysis results
(A) Heat map showing the percentage of the number of apoptotic cells, A dose-dependent increase in the apoptotic cell rate due to staurosporine was observed. (B) Bar graph showing the number of cells for each well (blue: the number of total cells, orange: the number of apoptotic cells, green: the number of non-apoptotic cells), The plate map view display allows intuitive confirmation of the drug reaction in each well. (C-F) Fluorescence images of HeLa cells (blue: Hoechst 33342, green: CellEvent™ Caspase-3/7), (C, D) untreated control, (E, F) treated with 1000 ng/mL staurosporine for 48 h. (F) More cells were observed to undergoing apoptosis compared to control.

Smart Experiment automates experiment and reduces the number of process

Conventional methods: Input by Human

1. Select well plate type
2. Alignment of well plate
3. Select objective
4. Select wavelengths
5. Setting exposure conditions
6. Auto focus settings

} No configuration required in Ji

7. Input the sample (drug) information
8. Setting binarization parameters
9. Setting measurement items
10. Graph creation

} No configuration required in Ji

Smart Experiment

1. Select the Experiment icon
2. Input the sample (drug) information

＼Simplified settings／

EASE OF USE

Number of process

10 Process — Conventional methods

$1/5$ Reduction

2 — ECLIPSE Ji

Smart Experiment Workflow

Input by Human
- ✓ Select Experiment icon
- ✓ Drug information

Fully Automated process

Overview
- ✓ Well plate detection and alignment
- ✓ Determines the thickness of the vessel bottom

Preview & Capture
- ✓ Auto focus settings
- ✓ Determine exposure automatically
- ✓ Image acquisition

Analysis & Graph
- ✓ Analysis results
- ✓ Graph creation
- ✓ EC_{50}/IC_{50} calculation
- ✓ Z'-factor calculation

Report
- ✓ Export results to PDF with one-click

Summary

- ✓ The dose-dependent increase in the percentage of apoptotic cells caused by staurosporine treatment was confirmed.
- ✓ The percentage of apoptotic cells were measured, and a dose-response curve was automatically created, and the EC_{50} was calculated.
- ✓ Smart Experiment can be run fully automatically from image acquisition to analysis and graph creation.

- ✓ It is a simple operation to place the well plate on Ji, select the Apoptosis icon, and input the sample information. Under the conditions of this experiment, it took approximately 20 minutes from the start of imaging to the graph display.
- ✓ A pretrained "CellFinder.ai" finds the optimal focal plane, there is no need to set tedious autofocus.
- ✓ Researchers can concentrate on more creative research activities by leaving tedious setting tasks to AI.

Sample preparation protocol

HeLa cells are seeded in a 96-well plate at a density of 6x10e3 cells/well and culture for 24 hours at 37℃ in a 5% CO_2 incubator. Cells are stained with media containing 2 µg/ml Hoechst 33342 for 30 minutes at 37℃ in a 5% CO_2 incubator. Dilute Staurosporin to concentrations of 0 nM, 0.152 nM, 0.457 nM, 1.41 nM, 4.11 nM, 12.3 nM, 37 nM, 111 nM, 333 nM, and 1000 nM in observation medium (10% FBS, 4 mM L-Glutamine, 1% Pc/Smin in FluoroBright DMEM), and add those to plate as 6 replicates of each concentration. The cells are treated with staurosporin for 48 hours at 37℃ in a 5% CO_2 incubator. Cells were then treated with 10 µl of CellEvent™ Caspase-3/7 Detection Reagent diluted 40x with observation medium to each well and place at room temperature for 30 minutes. To prevent detachment of dead cells, well plates were placed on ECLIPSE Ji without changing media to run image acquisition and analysis.

Tips

Apoptosis assay is an assay that measures the percentage of the number of apoptotic cells to the total cell count. Generally, DAPI cannot stain the nuclei of living cells. Please stain the nuclei of all cells with Hoechst 33342, not DAPI. In addition, washing after drug treatment detaches dead cells from the bottom of the well plate, reducing the accuracy of quantitative analysis. Therefore, image acquisition should be performed without washing or fixing the cells after drug treatment.

Materials and reagents

Cell Culture	
Cell Line	HeLa (RIKEN RCB0007)
Growth medium	MEM + 10%FBS + 1%Pc/Sm
Culture vessel	EZVIEW® Culture Plate B (Glass Bottom Plate) Microplate 96 well (AGC techno glass (IWAKI), 5866-096)

Test substance	
Compound	Staurosporin
Test concentration	Negative control: 0 nM, Positive control: 333 nM To make a dose-response curve, design required concentration points as follows: (0) 0 nM, (1) 0.152 nM, (2) 0.457 nM, (3) 1.41 nM, (4) 4.11 nM, (5) 12.3 nM, (6) 37 nM, (7) 111 nM, (8) 333 nM, (9) 1000 nM
Plate map example	(plate map — rows A–H, columns 1–12) A: b b b b b b b b b b b b B: b (0) (1) (2) (3) (4) (5) (6) (7) (8) (9) b C: b (0) (1) (2) (3) (4) (5) (6) (7) (8) (9) b D: b (0) (1) (2) (3) (4) (5) (6) (7) (8) (9) b E: b (0) (1) (2) (3) (4) (5) (6) (7) (8) (9) b F: b (0) (1) (2) (3) (4) (5) (6) (7) (8) (9) b G: b (0) (1) (2) (3) (4) (5) (6) (7) (8) (9) b H: b b b b b b b b b b b b "b" : blank well

Reagents

Product name	Product number	Supplier
MEM (Minimum Essential Medium)	11095080	Thermo Fisher Scientific
FluoroBrite™ DMEM	A1896701	Thermo Fisher Scientific
Staurosporine from Streptomyces sp.	S5921	Sigma-Aldrich
-Cellstain®- Hoechst 33342 solution	H342	Dojindo Laboratories
CellEvent™ Caspase-3/7 Detection Reagents	L3224	Thermo Fisher Scientific
Dimethyl sulfoxide (DMSO)	276855	Sigma-Aldrich

☐ **Compatible vessel***

· 24, 48, 96 well plate

＊Compatible with glass and polystyrene bottom well plates. If cell adhesion to the bottom is a priority over image quality, use well plates with polystyrene bottoms.

Reference

Chae, HJ, *et al.*, Molecular mechanism of staurosporine-induced apoptosis in osteoblasts. *Pharmacol Res* 42 (4):373-81 (2000).

Product information

Smart Imaging System ECLIPSE Ji

ECLIPSE Ji is an AI-Driven, fully automated imaging system. By using it in combination with NIS-Elements SE, image acquisition, analysis, and graph creation can be run seamlessly and automatically. It is equipped with "CellFinder.ai", which uses AI to find the optimal focal plane in autofocus settings that normally require advanced human judgment. Various trained AIs are implemented in the image acquisition and analysis process. This greatly reduces the number of steps for setting and optimization and makes it easier for everyone to get results.
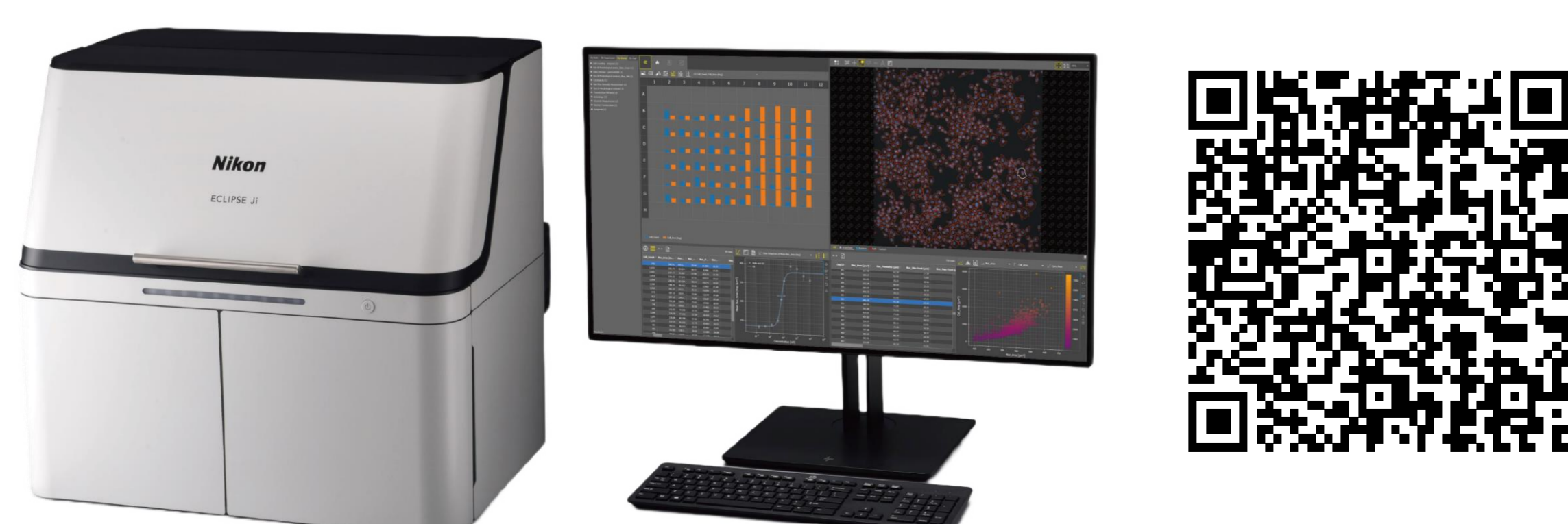
Imaging Software NIS-Elements SE Apoptosis (Option)

✓ Fully automated from image acquisition to analysis and graph display.
✓ The percentage of apoptotic cells can be measured.
✓ One-click reports can be created and output with PDF including images, analysis results, dose-response curves, and EC_{50}/IC_{50} calculation results.
✓ Cellular imaging and analysis with Ji is easier and more comfortable.
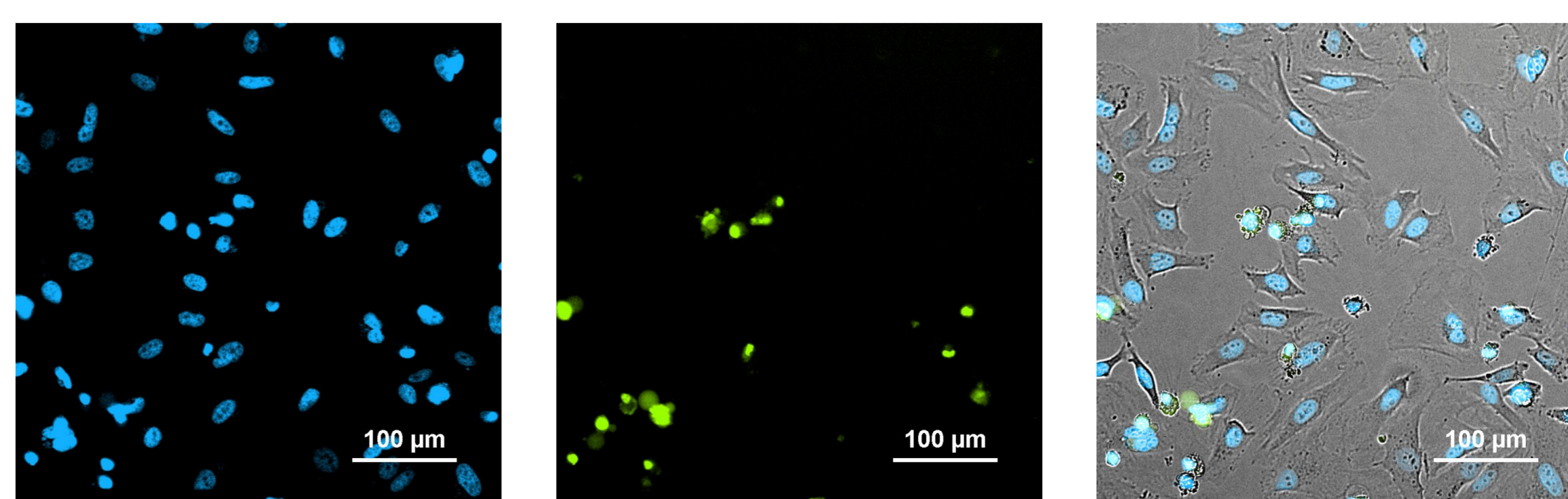
Fluorescence image
Hoechst 33342
Nuclei of all cells

Fluorescence image
CellEvent™ Caspase-3/7
Nuclei of apoptotic cells

Merged image
Brightfield +
fluorescence image