Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

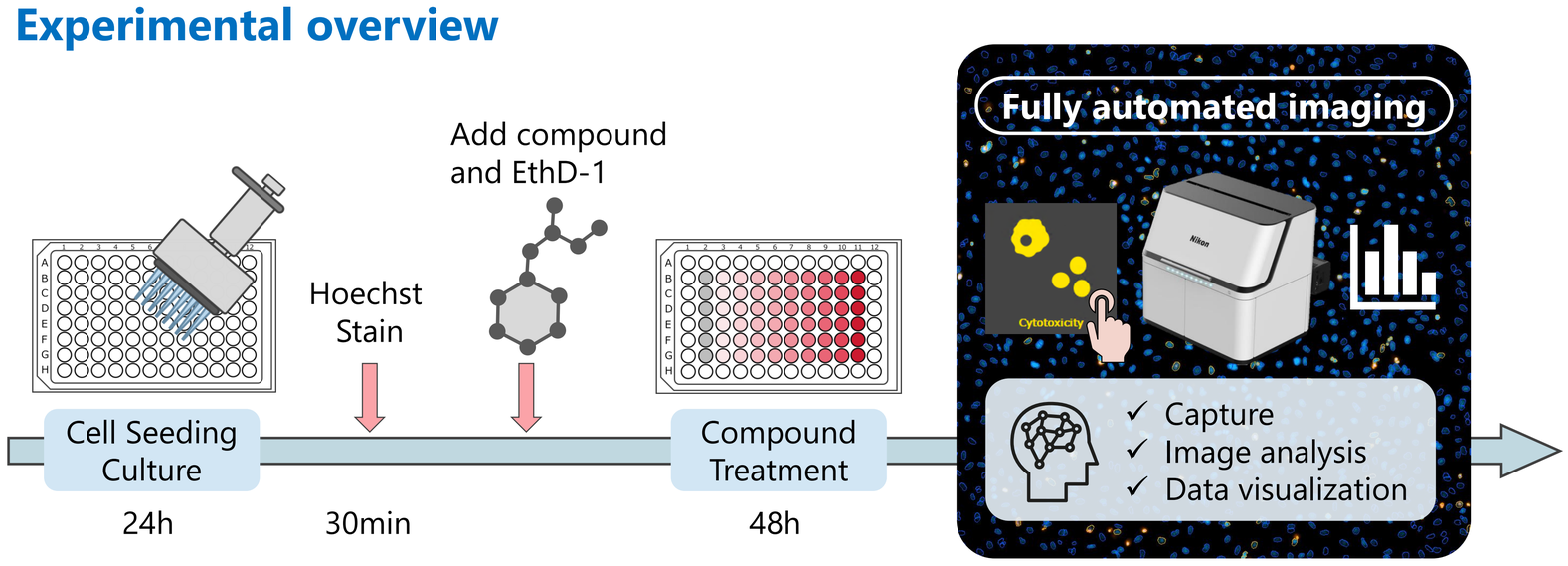
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Cytotoxicity

Cytotoxicity assay using AI-driven, fully automated ECLIPSE Ji

ECLIPSE Ji with Smart Experiment software enables seamless experiments from image acquisition to analysis and graph creation. Pre-trained Artificial Intelligence (AI) and pre-defined imaging processes automatically optimize image acquisition and analysis settings, providing visualized data and EC_{50} information with simple operation. Cytotoxicity analysis is a versatile assay used in a variety of biological research, such as studying the effects of pharmacological compounds, development of culture media and scaffolds for cell culture, or elucidation of cellular physiology. This application note introduces an example of using the Cytotoxicity module of Smart Experiment to visualize the dose-dependent induction of cell death due to staurosporine and quantify the effect of the drug by calculating the EC_{50}.

Keywords: Cytotoxicity, Live / Dead, Cell viability, automatic setting, EC_{50}, dose-response curve

Experimental overview



- **Key features**
  - ✓ Fully automated from image acquisition to analysis and graphing
  - ✓ Measuring the ratio of live and dead 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 then exposed to media containing Hoechst 33342 and incubated for 30 minutes. (3) Cells were then treated for 48 hours with the test substance staurosporin was diluted to 10 different concentrations in media containing EthD-1. (4) Without changing the media, the well plate was placed on ECLIPSE Ji and image acquisition and analysis were run automatically by selecting the Cytotoxicity assay icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells	Hoechst 33342	352/461
Nucleus of dead cells	Ethidium homodimer-1	528/617
Magnification	**Field of view**	
10X	1.76 x 1.76 mm	

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

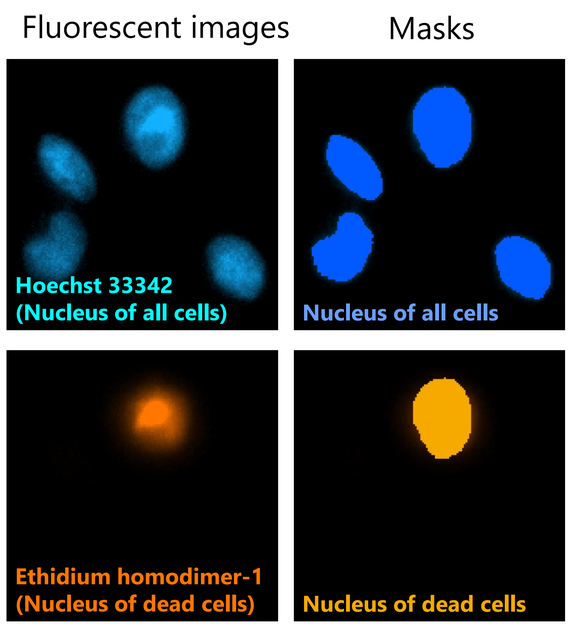


Fig. 1: Fluorescent images and masks
Left: Fluorescence images, Hoechst 33342 (blue: nucleus of all cells), Ethidium homodimer-1 (orange: nucleus of dead cells), Right: Masks

Results

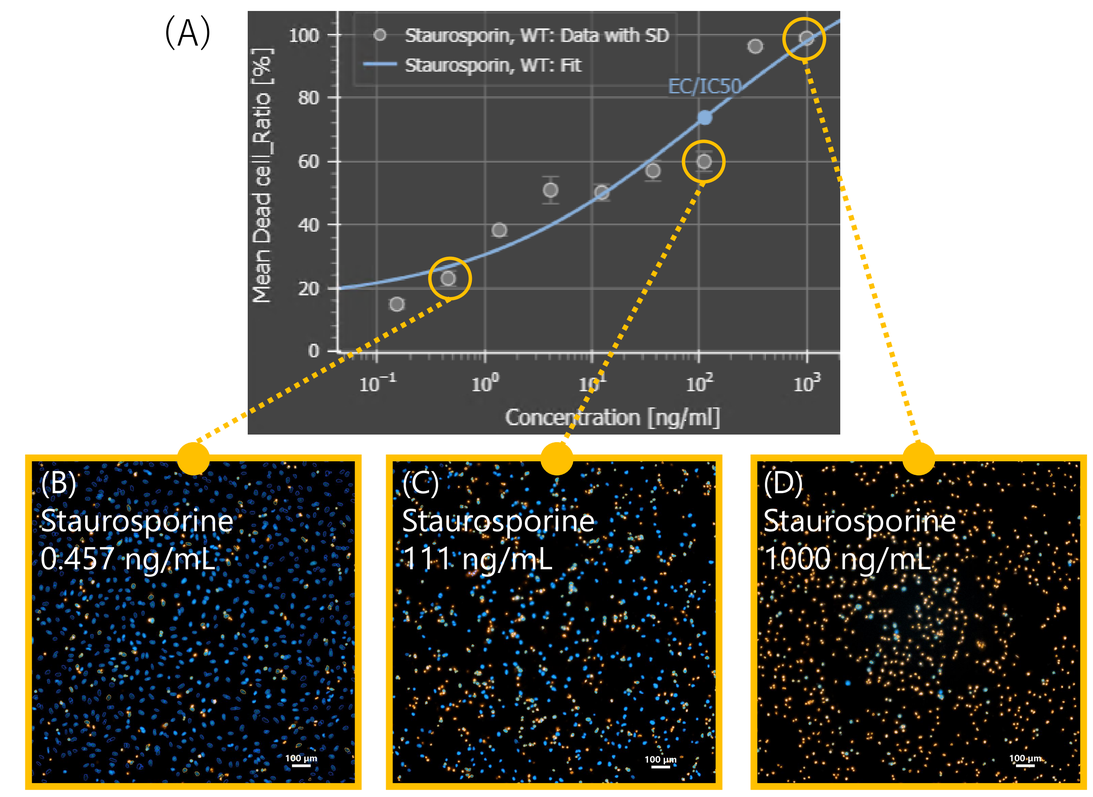


Fig. 2: Dose-response curve and images of HeLa cells treated with staurosporine for 48 hours.
(A) Dose-response curve showing a staurosporine -dependent cell death (X-axis: drug concentration (log), Y-axis: ratio of dead cells), EC_{50} = 112.716ng/ml, Z'-factor = 0.90, (B, C, D) Masks outline overlaid on merged fluorescence images (Blue mask: nuclei of all cells, Orange mask: nuclei of dead cells), Scale bar: 100µm

Results

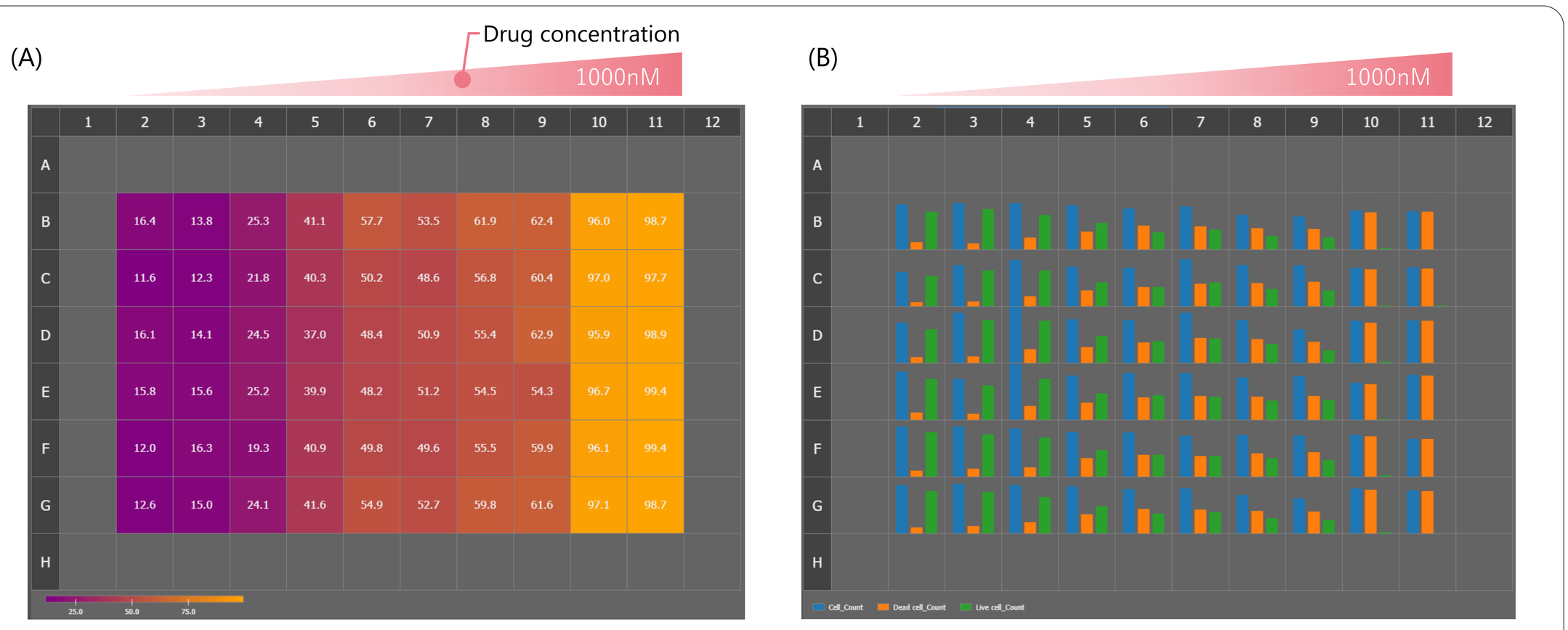


Fig.3: Analysis results

(A) Heat map showing ratio of dead cells

(B) Bar graph showing the number of cells for each well (blue: number of total cells, orange: number of dead cells, green: number of live cells),
The plate map view display allows intuitive confirmation of the drug reaction in each well.

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

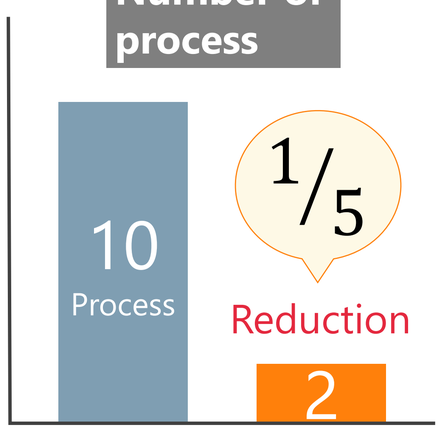
No configuration required in Ji

Smart Experiment

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

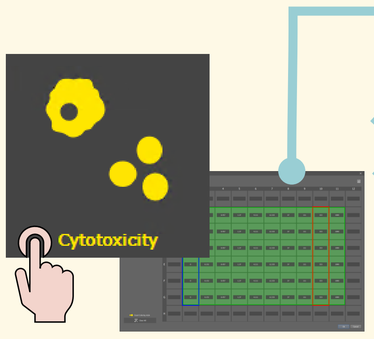
\Simplified settings/

Number of process



Smart Experiment Workflow

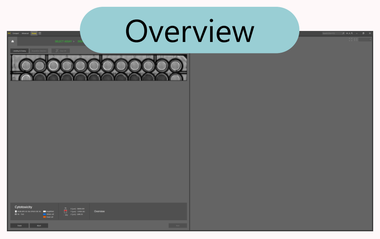
Input by Human



✓ Select Experiment icon
✓ Drug information

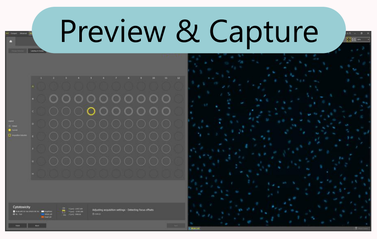
Fully Automated process

Overview



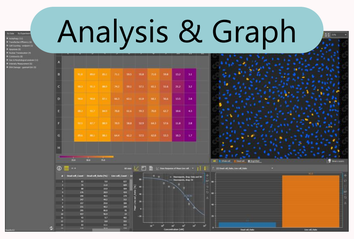
✓Well plate detection and alignment
✓Determines the thickness of the vessel bottom

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 and save in PDF format with one click

Summary

- ✓ The dose-dependent increase in the ratio of dead cells with staurosporine was confirmed.
- ✓ The ratio of live and dead cells were measured, a dose-response curve was automatically created, and the EC_{50} was calculated.
- ✓ Smart Experiment can be run automatically from image acquisition to analysis and graph creation.
- ✓ It is a simple operation to place the well plate on Ji, select the Cytotoxicity 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 even on thick plastic-bottomed multi-well plates.
- ✓ 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 were stained with media containing 2 µg/ml Hoechst 33342 for 30 minutes at 37℃ in a 5% CO_2 incubator. Staurosporin diluted in FluoroBright DMEM contained 4µM EthD-1 at 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, 1000 nM was added to 6 replicate wells. The cells are treated staurosporin for 48 hours at 37℃ in a 5% CO_2 incubator. To prevent detachment of dead cells, well plates were placed on ECLIPSE Ji without changing media to run image acquisition and analysis.

Tips

Cytotoxicity assay is an assay that measures the ratio of live and dead cells. 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	Falcon® 96 well Clear Flat Bottom TC-treated Culture Microplate (Corning, 353072)
Test substance	
Compound	Staurosporin
Test concentration	Negative control: 0 nM, Positive control: 1000 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	

	1	2	3	4	5	6	7	8	9	10	11	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
LIVE/DEAD™ Viability/Cytotoxicity Kit, Ethidium homodimer-1 (EthD-1)	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.

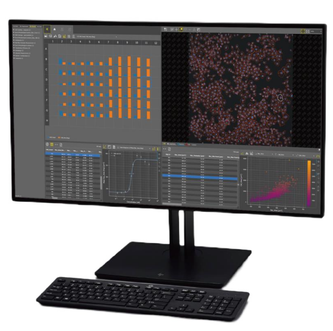
Reference

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 SmartExperiment Basic Set Cytotoxicity

- ✓ Fully automated from image acquisition to analysis and graph display.
- ✓ The ratio of live and dead cells can be analyzed.
- ✓ 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.