

## APPLICATION NOTE

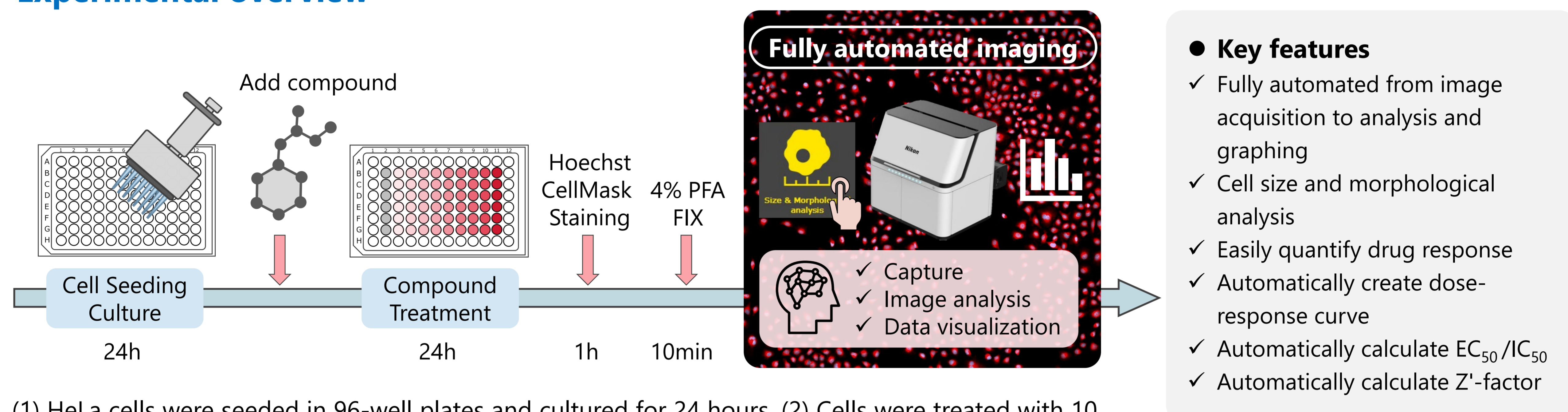
 Smart Imaging System ECLIPSE Ji  
 Imaging Software NIS-Elements SE  
 Size & Morphological analysis

# Cell size and morphological analysis is easier and more comfortable with the 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. Cell size and morphological analysis is a versatile assay used in a variety of biological research, such as studying the effects of pharmacological compounds, the development of culture media and scaffolds for cell culture, or elucidation of cellular physiology. This application note introduces an example of using the Size & Morphological analysis module of Smart Experiment to visualize the dose-dependent increase in cell area caused by camptothecin treatment and to quantify the effect of the drug by calculating the  $EC_{50}$ .

Keywords: cell size analysis, morphological analysis, automatic setting,  $EC_{50}$ , dose-response curve

## Experimental overview



(1) HeLa cells were seeded in 96-well plates and cultured for 24 hours. (2) Cells were treated with 10 different concentrations of camptothecin for 24 hours. (3) Nuclei and cell membranes were stained, and cells were fixed. (4) The well plate was placed on ECLIPSE Ji and image acquisition and analysis was run automatically by selecting the Size & Morphological analysis icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells	Hoechst 33342	352/461
Cell region	CellMask™ Deep Red	649/666
Magnification	Field of view	
10X	1.76 x 1.76 mm	

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

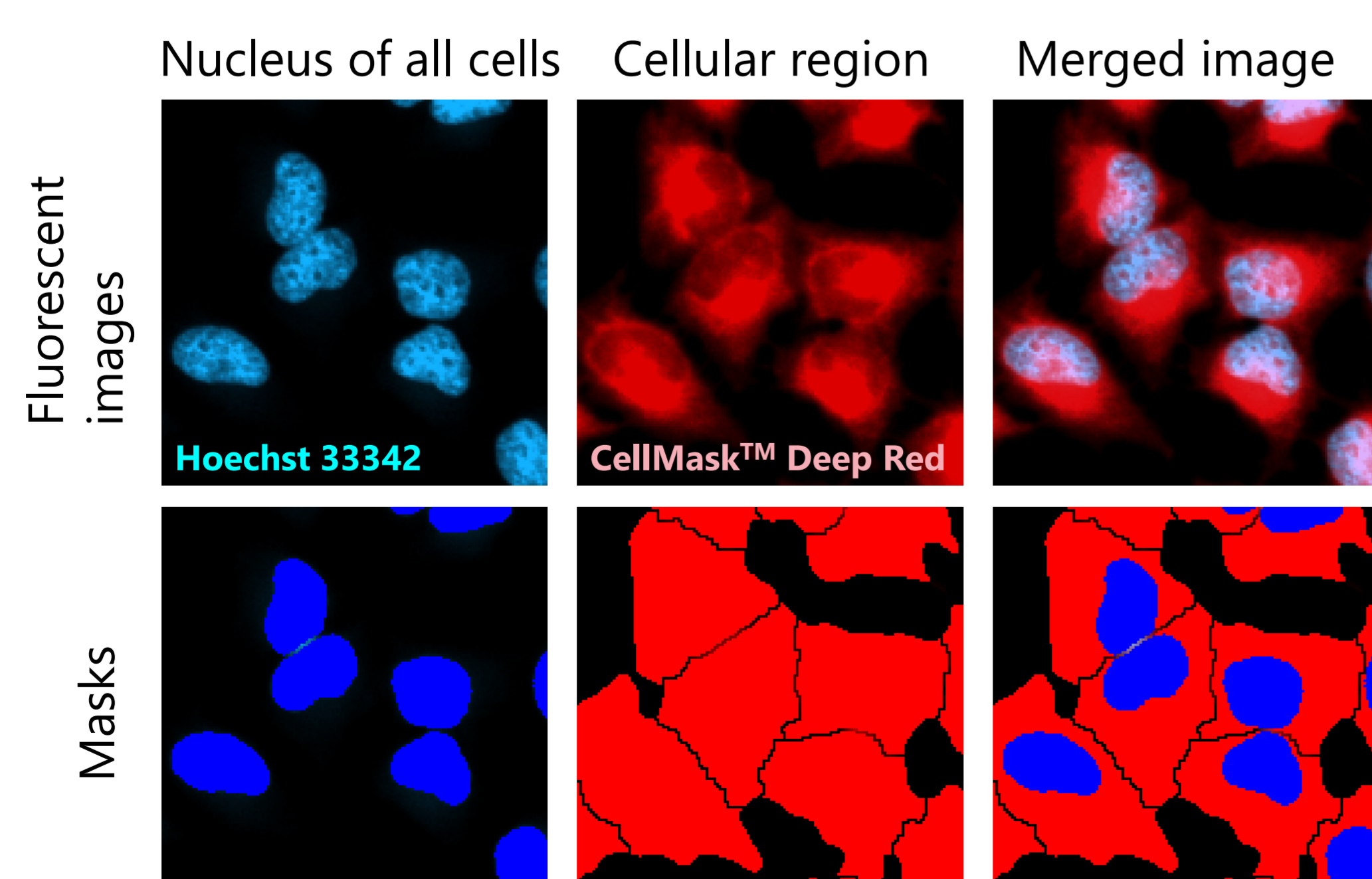


Fig. 1: Binarization and segmentation

Upper row: Fluorescence images, Hoechst 33342 (blue: nucleus of all cells), CellMask™ Deep Red (red: cellular region), Merged image (right). Lower row: Mask images, (blue: nucleus masks, red: cellular region masks), Merged masks (right)

## Results

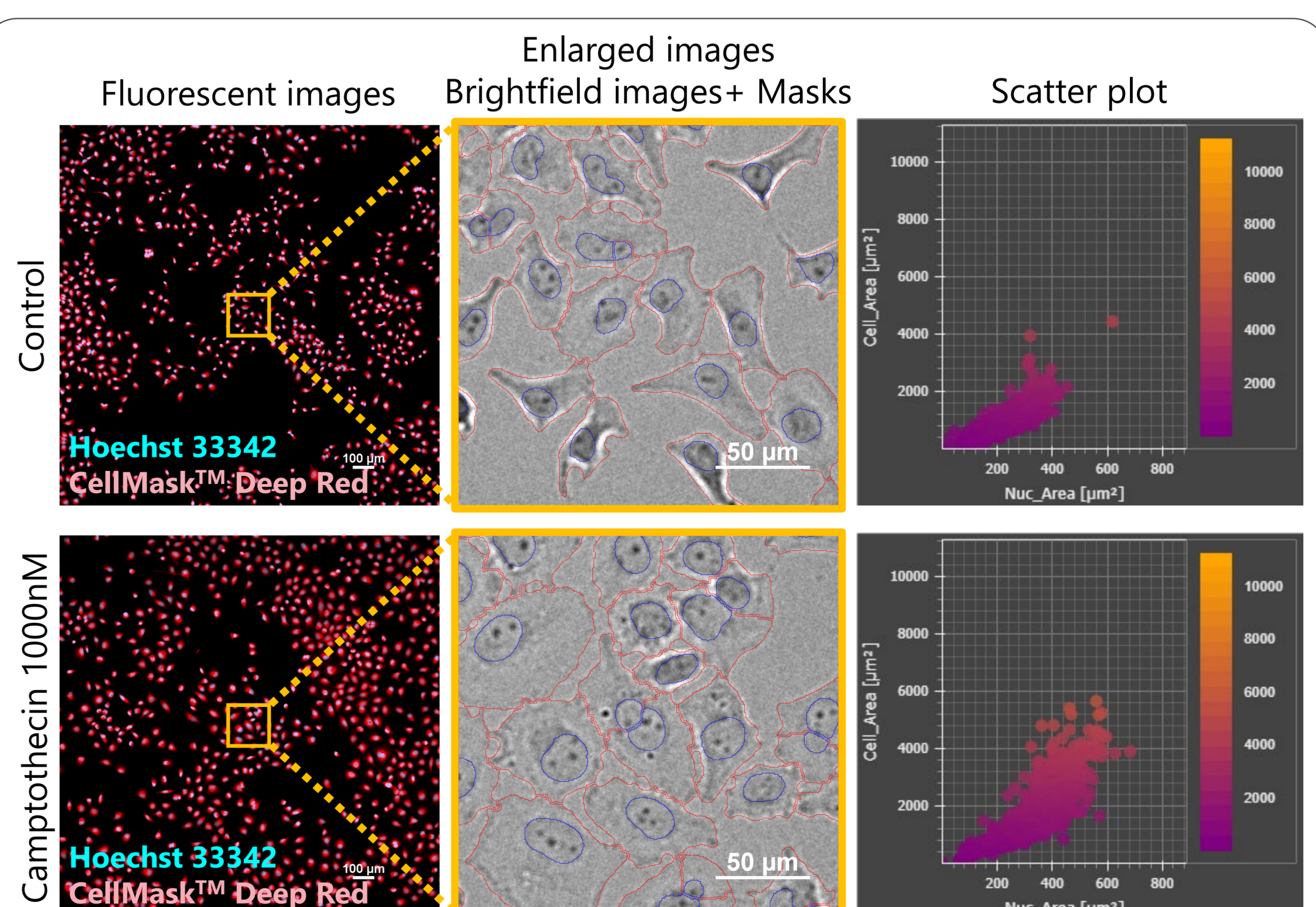
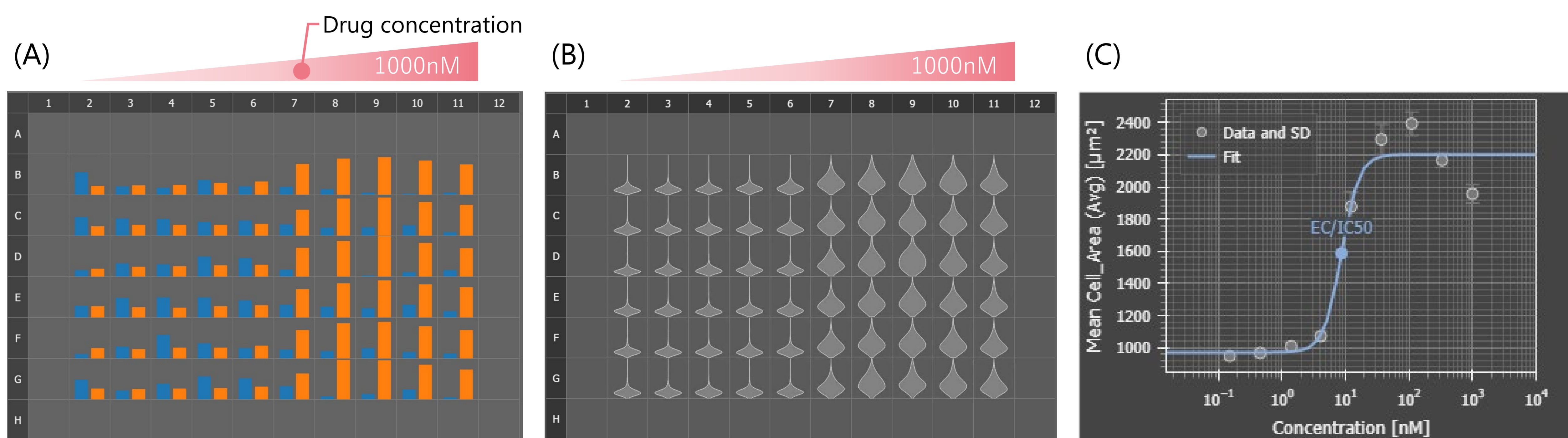


Fig. 2: Masked images and analysis results of HeLa cells treated with control and camptothecin 1000nM for 24 hours.

Upper row: control, Lower row: camptothecin 1000 nM, Left: Fluorescent images (scale bar: 100  $\mu$ m), Center: Enlarged images, Brightfield with outline of masks (blue: nucleus masks, red: cellular region masks), scale bar: 50  $\mu$ m), Right: scatter plot of analysis results (X-axis : Nucleus area, Y-axis: Cell area). The cell area increased in wells treated with 1000 nM of camptothecin.



## Results and discussion



**Fig. 3: Analysis results of cell number and cell area (average), dose-response curve for camptothecin**

(A) Bar graph (blue: cell number, orange: cell area per cell (average)), average cell area (orange) increased significantly in the wells with the drug concentration above 12.3 nM (columns 7-11). In addition, the number of cells (blue) was less compared to the control (column 2), and cell proliferation was suppressed. (B) Violin plot (cell area per cell (average)), The plate map view display allows intuitive confirmation of the drug reaction in each well. Numeric data can be viewed in the heatmap display and data table. (C) Dose-response curve showing a dose-dependent increase in cell area due to camptothecin, (X-axis: drug concentration (log), Y-axis: mean area per cell),  $EC_{50} = 8.716$  nM,  $Z'$ -factor = 0.64.

### Drug info

#### Pharmacology of Camptothecin, Mechanism of action:

Camptothecin is an inhibitor of DNA topoisomerase I, an enzyme involved in DNA replication. Camptothecin inhibits functions related to cell division, such as DNA replication and tubulin depolymerization, so it suppresses cell proliferation and has antitumor activity. Therefore, cell division stops and the phenomenon of cell size increase is observed.

## 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
  7. Input the sample (drug) information
  8. Setting binarization parameters
  9. Setting measurement items
  10. Graph creation
- No configuration required in Ji
- No configuration required in Ji

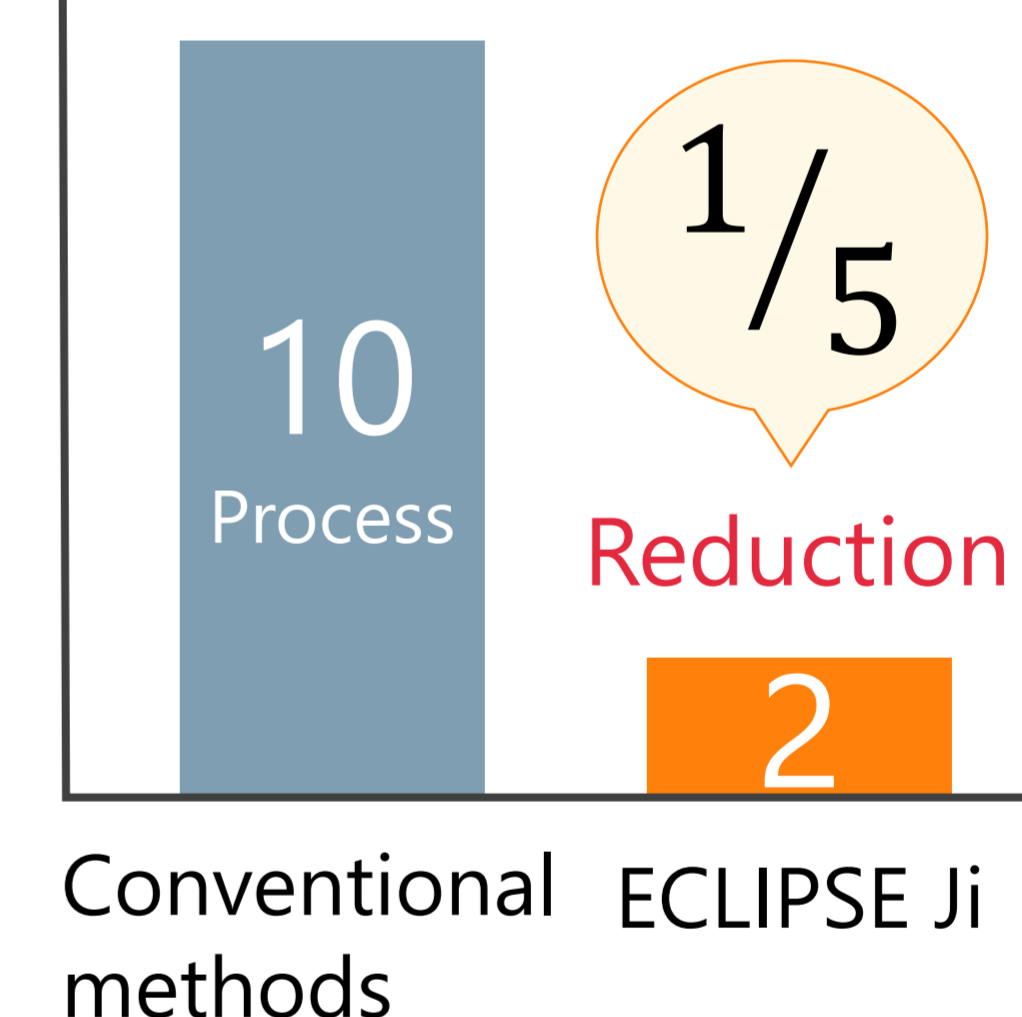
### Smart Experiment

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

\ Simplified settings /

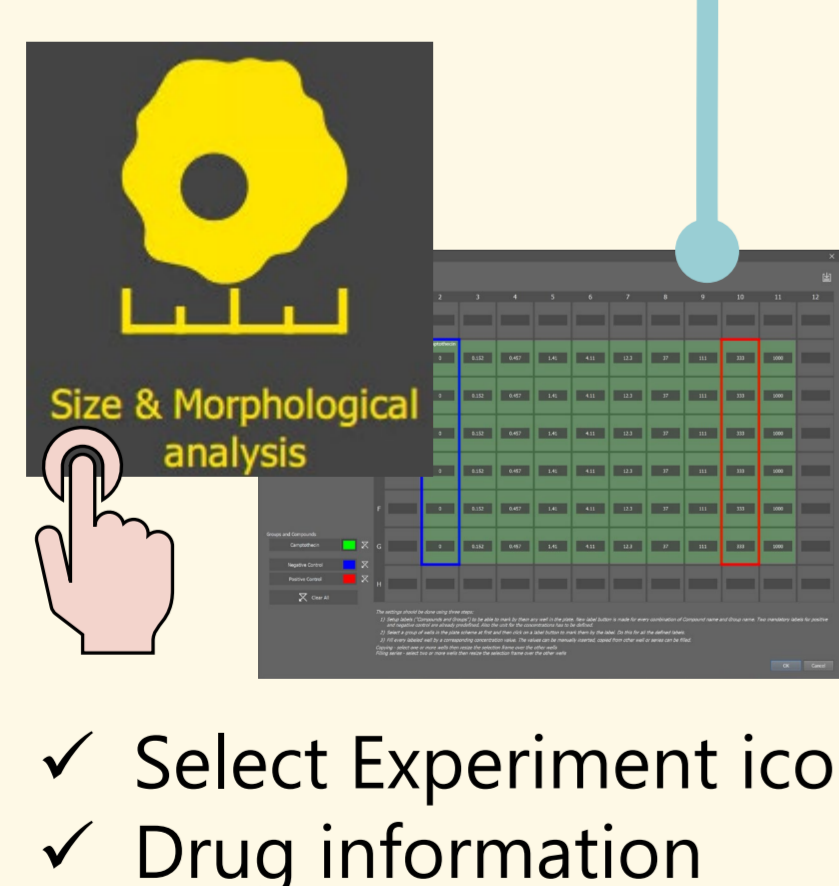


### Number of process



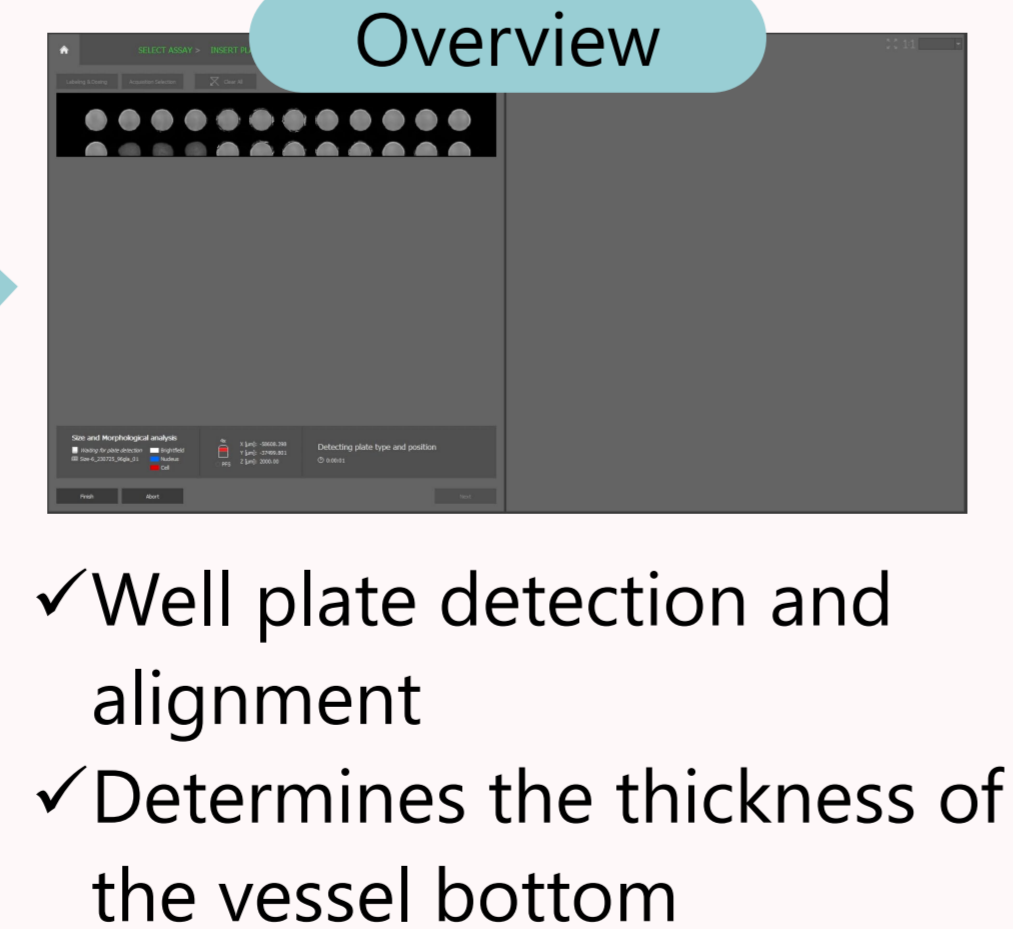
## Smart Experiment Workflow

### Input by Human

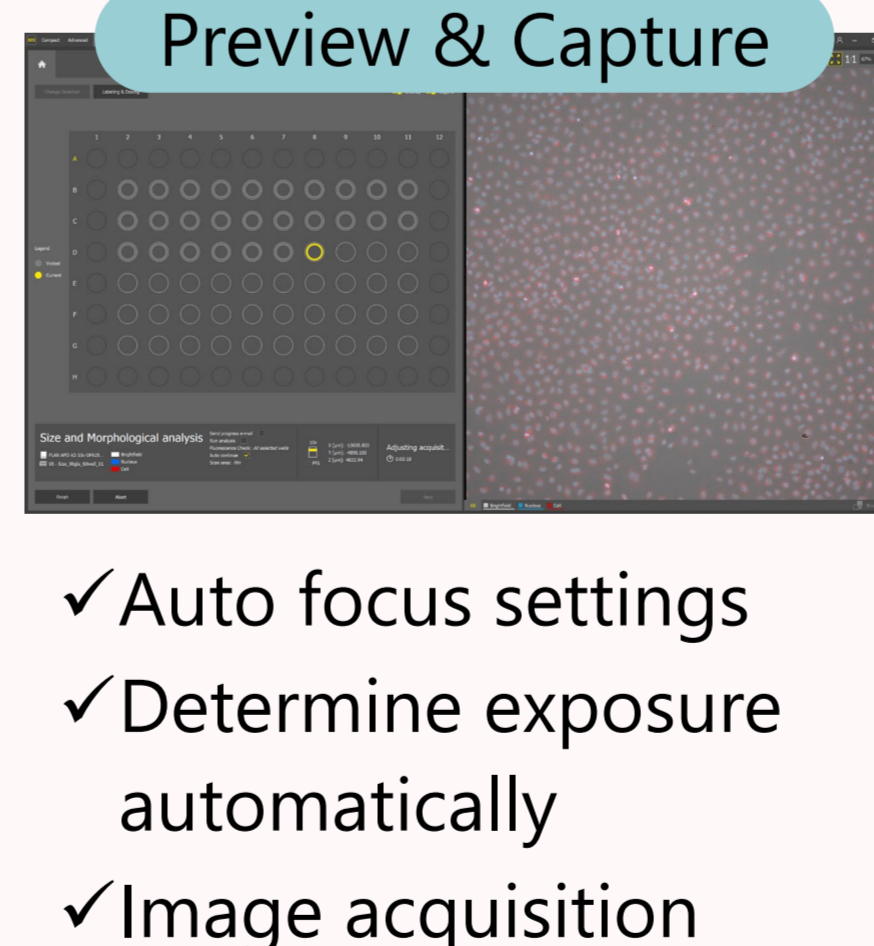


### Fully Automated process

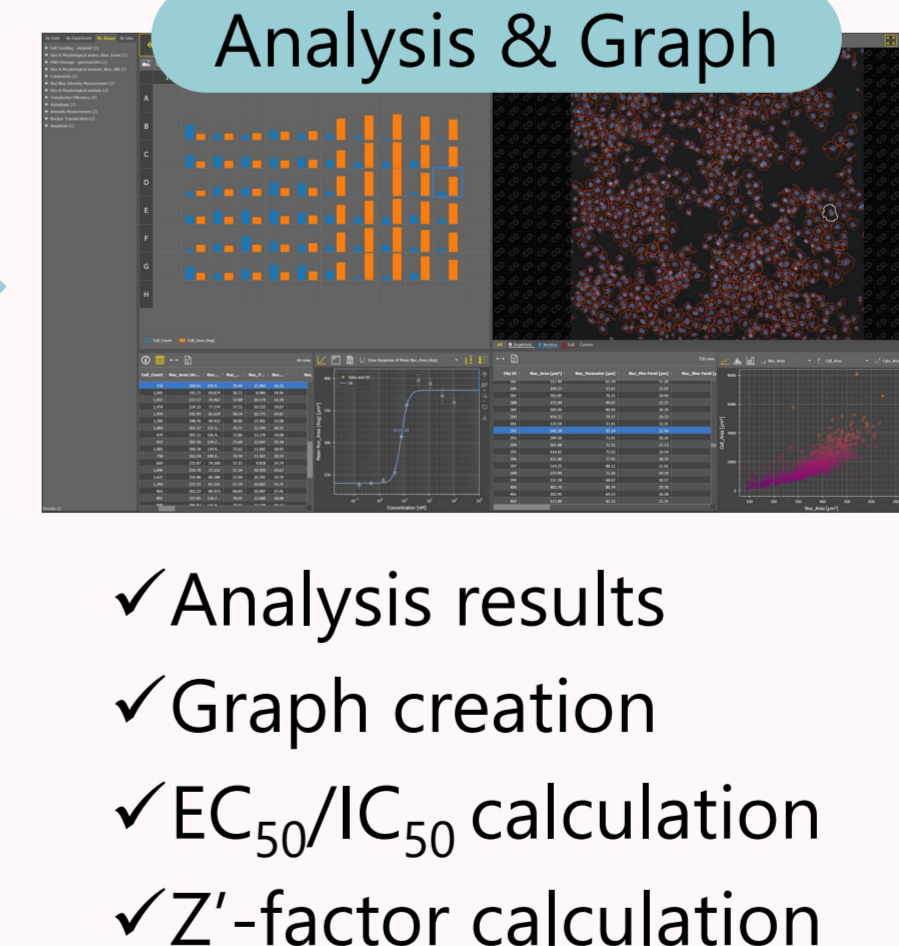
#### Overview



#### Preview & Capture



#### Analysis & Graph



#### Report



Conventional methods require many settings to optimize image acquisition and analysis conditions. Smart Experiment automates the process of image acquisition, analysis, and visualization, greatly reducing the number of manually-selected settings and optimization steps.

Smart Experiment is equipped with "CellFinder.ai", which uses AI to find the optimal focal plane by autofocus settings that otherwise require advanced human judgment. This process is simple only selecting the Experiment icon and input the sample (drug) information is required. This easy-to-use software allows even first-time users to learn how to operate it in a short time.



## Summary

- ✓ The dose-dependent increase in cell area and suppression of cell proliferation by camptothecin were confirmed.
- ✓ Cell size was measured, a dose-response curve was automatically created, and the EC<sub>50</sub> was calculated.
- ✓ Smart Experiment can be run fully automatically from image acquisition to analysis and graph creation.
- ✓ A pretrained "CellFinder.ai" finds the optimal focal plane, there is no need to set tedious autofocus.
- ✓ It is simple to place the well plate on Ji, select the Size & Morphological analysis icon, and input the sample information. Under the conditions of this experiment, the automated routine took approximately 17 minutes from the start of imaging to graph display.
- ✓ Researchers can concentrate on more creative research activities by leaving tedious setting tasks to AI.

## 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	Camptothecin																																																																																																																					
Test concentration	Negative control: 0 nM Positive control: 333 nM To make a dose-response curve, design required concentration points as follows: Ex: (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	<table border="1"> <thead> <tr> <th></th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> <th>5</th> <th>6</th> <th>7</th> <th>8</th> <th>9</th> <th>10</th> <th>11</th> <th>12</th> </tr> </thead> <tbody> <tr> <th>A</th> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> </tr> <tr> <th>B</th> <td>b</td> <td>(0)</td> <td>(1)</td> <td>(2)</td> <td>(3)</td> <td>(4)</td> <td>(5)</td> <td>(6)</td> <td>(7)</td> <td>(8)</td> <td>(9)</td> <td>b</td> </tr> <tr> <th>C</th> <td>b</td> <td>(0)</td> <td>(1)</td> <td>(2)</td> <td>(3)</td> <td>(4)</td> <td>(5)</td> <td>(6)</td> <td>(7)</td> <td>(8)</td> <td>(9)</td> <td>b</td> </tr> <tr> <th>D</th> <td>b</td> <td>(0)</td> <td>(1)</td> <td>(2)</td> <td>(3)</td> <td>(4)</td> <td>(5)</td> <td>(6)</td> <td>(7)</td> <td>(8)</td> <td>(9)</td> <td>b</td> </tr> <tr> <th>E</th> <td>b</td> <td>(0)</td> <td>(1)</td> <td>(2)</td> <td>(3)</td> <td>(4)</td> <td>(5)</td> <td>(6)</td> <td>(7)</td> <td>(8)</td> <td>(9)</td> <td>b</td> </tr> <tr> <th>F</th> <td>b</td> <td>(0)</td> <td>(1)</td> <td>(2)</td> <td>(3)</td> <td>(4)</td> <td>(5)</td> <td>(6)</td> <td>(7)</td> <td>(8)</td> <td>(9)</td> <td>b</td> </tr> <tr> <th>G</th> <td>b</td> <td>(0)</td> <td>(1)</td> <td>(2)</td> <td>(3)</td> <td>(4)</td> <td>(5)</td> <td>(6)</td> <td>(7)</td> <td>(8)</td> <td>(9)</td> <td>b</td> </tr> <tr> <th>H</th> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> <td>b</td> </tr> </tbody> </table> <p>"b" : blank well</p>		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
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## Sample preparation protocol

- 1) HeLa cells are seeded in a 96-well plate at a density of 4.5x10<sup>3</sup> cells/well and culture for 24 hours at 37°C in a 5% CO<sub>2</sub> incubator.
- 2) Camptothecin is diluted with medium to concentrations of 0, 0.152, 0.457, 1.41, 4.11, 12.3, 37, 111, 333, 1000 nM, and each concentration of test substance solution is added to each 6 wells. The cells are treated for 24 hours with camptothecin at 37°C in a 5% CO<sub>2</sub> incubator.
- 3) Replace with medium containing 5 µg/ml CellMask™ Deep Red and 2 µg/ml Hoechst 33342 and stain for 1 hour at 37°C in a 5% CO<sub>2</sub> incubator.
- 4) Fix cells in 4% PFA for 10 minutes at room temperature.
- 5) Wash cells twice with PBS.

Reagents		
Product name	Product number	Supplier
(S)-(+)-Camptothecin, >=90% (HPLC), powder	C9911	Sigma-Aldrich
-Cellstain® - Hoechst 33342 solution	H342	Dojindo Laboratories
CellMask™ Deep Red Plasma Membrane Stain	C10046	Thermo Fisher Scientific, Invitrogen™
MEM (Minimum Essential Medium)	11095080	Thermo Fisher Scientific
Fetal bovine serum (FBS)	10437028	Thermo Fisher Scientific
Penicillin-Streptomycin (Pc/Sm) (10,000 U/ml)	15140122	Thermo Fisher Scientific
16%-Paraformaldehyde Aqueous Solution (16% PFA) *Dilute by 4% with PBS before use	11850-14	Nacalai Tesque
Dimethyl sulfoxide (DMSO)	276855	Sigma-Aldrich

### ☐ Compatible vessel\*

- 24, 48, 96 well plate

\* Compatible with glass and polystyrene bottom well plates. If image quality is a priority, use glass-bottom well plates.

## Reference

Futamura, Y, et al., Morphobase, an Encyclopedic Cell Morphology Database, and Its Use for Drug Target Identification. *Chemistry & Biology* **19** 1620-1630 (2012)

## 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 Size & Morphological analysis

- ✓ Fully automated from image acquisition to analysis and graph display.
- ✓ Cell counting, nucleus and cell morphology analysis.
- ✓ One-click reports can be created and output with PDF including images, analysis results, dose-response curves, and EC<sub>50</sub>/IC<sub>50</sub> calculation results.
- ✓ Cellular imaging and analysis with easier and more comfortable.