APPLICATION NOTE

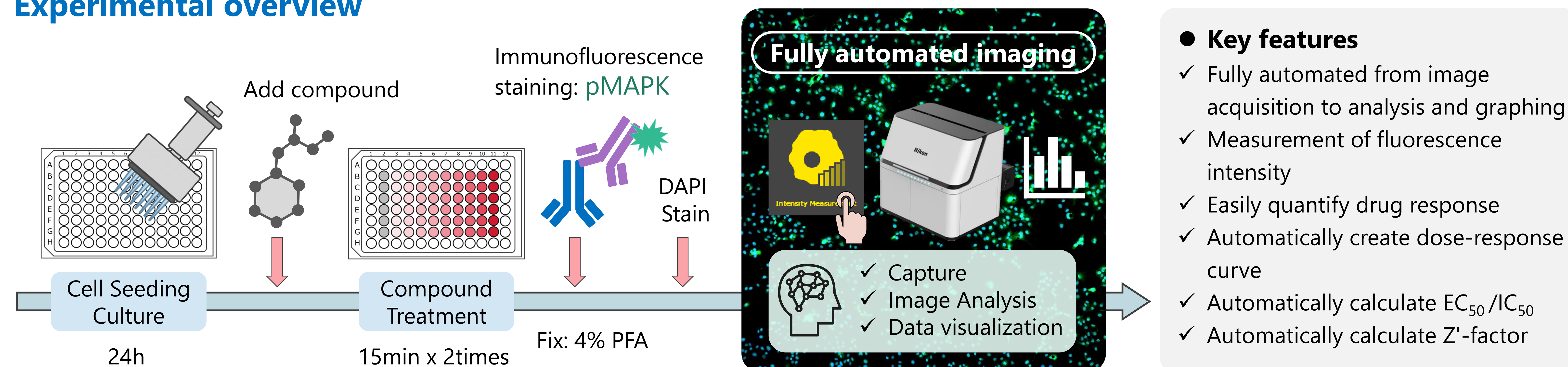
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Intensity Measurement

Pre-trained AI-powered label-free detection of cell regions from brightfield images and measurement of fluorescence intensity at the cellular level

ECLIPSE Ji with Smart Experiment software enables seamless operation 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. Fluorescence intensity measurement is used in a wide range of applications, from basic research of cell biology to drug discovery, including quantification of protein and enzyme activity. This application note introduces an example of using the Intensity Measurement module of Smart Experiment to detect cell regions label-free from brightfield images and measure the fluorescence intensity at the cellular level. We also present an example of quantifying the drug's effect by automatically visualizing the dose-dependent decrease in fluorescence intensity derived from pMAPK and calculating the IC_{50}.

Keywords: fluorescence intensity, protein quantification, drug discovery, anticancer drug research, automatic setting, IC_{50}, dose-response curve

Experimental overview



(1) HeLa cells were seeded in 96-well plates and cultured for 24 hours. (2) The test substance U0126 was diluted to 10 different concentrations, added to each well and treated for 15 minutes. (3) Cells were transferred to growth medium containing each test concentration of U0126 and 10 ng/ml PMA and incubated for 15 minutes. (4) Cells were fixed with 4% PFA. Membrane permeabilization was performed with 0.2% Triton X-100. (5) Immunofluorescence staining was done with Rabbit anti pMAPK antibody and Goat anti-rabbit IgG H&L Alexa Fluor™ 488. Nuclei were stained with DAPI. (6) The well plate was placed on ECLIPSE Ji and image acquisition and analysis was run automatically by selecting the Intensity Measurement icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells	DAPI	345/455
Cell region	None (Detect from brightfield image using AI)	Brightfield
Target molecule (pMAPK)	Rabbit anti-pMAPK (primary), Goat anti-rabbit IgG H&L Alexa Fluor™ 488 (secondary)	495/519
Magnification	**Field of view**	
10X	1.76 x 1.76 mm	

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

Point

Label-free

Pre-trained AI

AI automatically identifies **cell regions** from brightfield

No additional cell membrane staining is required for cell area identification with AI identifies cell areas. This allows for a wider range of experiments as the fluorescence wavelength can be used to detection fluorescent proteins.

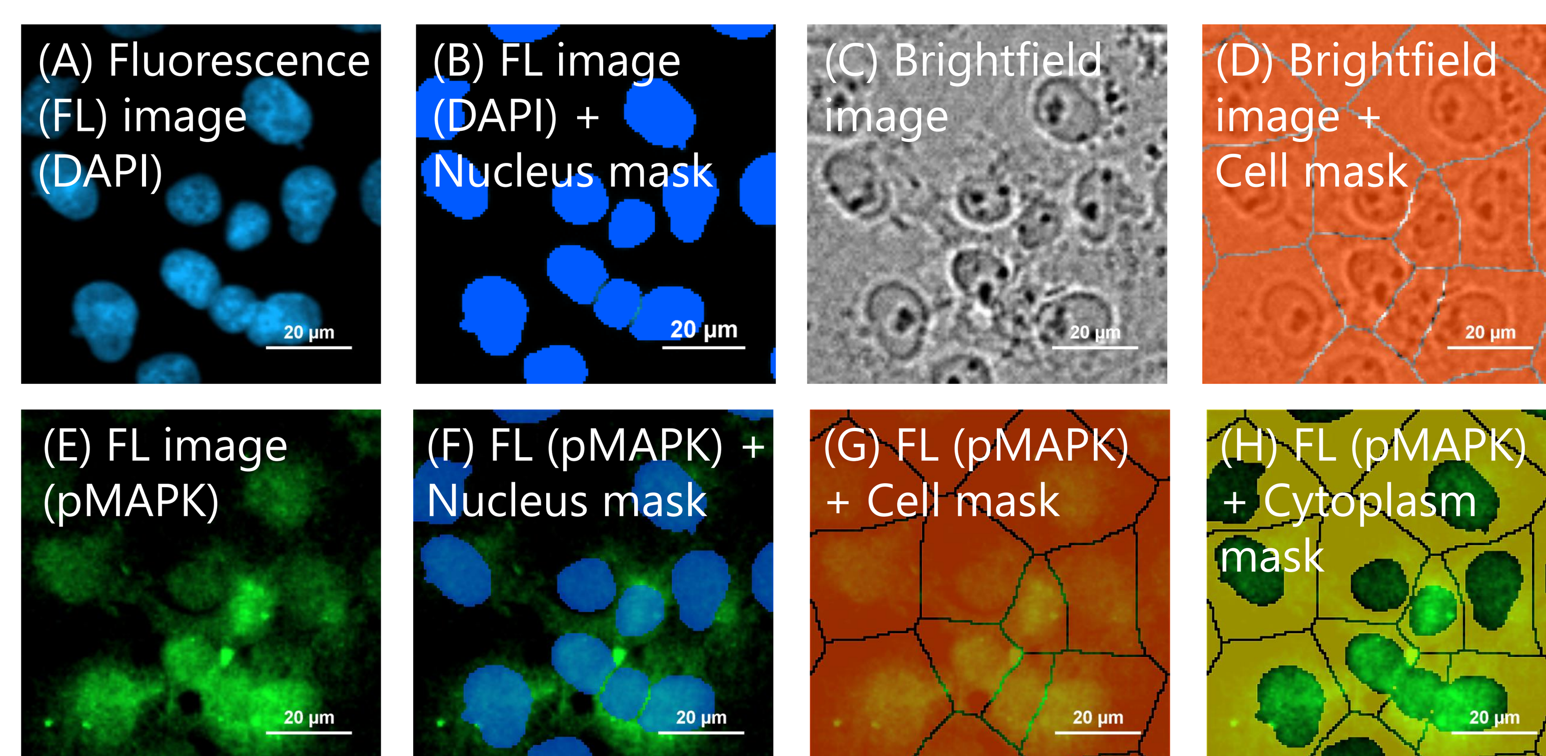


Fig. 1: Binarization method and target molecule measurement
Nucleus region (A) detected by DAPI is binarized to create a nucleus mask (B). A trained AI identifies the background and cell regions from the brightfield image (C). Create a cell mask (D) by segmenting the cell region using the nucleus mask (B). A cytoplasm mask (G) is created by subtracting the region of the nucleus mask (B) from the region of the cell mask (H). The intensity of immunofluorescence-stained pMAPK-derived fluorescence was measured in each mask region (nucleus, cell, cytoplasm) from the fluorescence image (E), and expression level of the target protein was quantified (F, G, H). Scale bar: 20 µm

Results

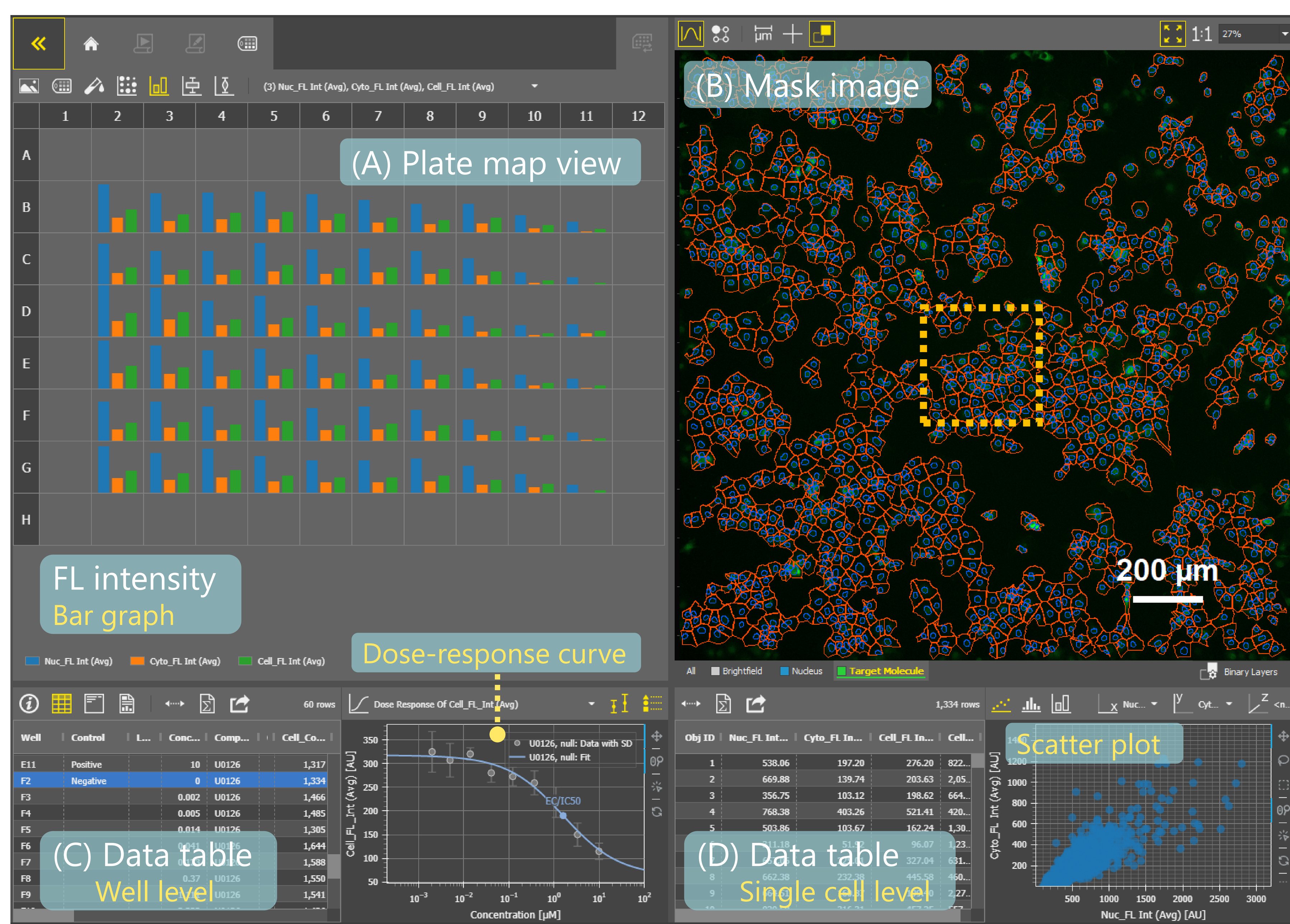


Fig. 2: Fluorescence intensity measurement results, Software with easy-to-analyze GUI
Heatmaps, bar graphs, boxplots, and violin plots displays in the plate map view allow you to intuitively confirm the analysis results for the entire plate. The data table provides well-level (C) and single-cell level (D) information. Dose-response curves, scatter plots, histograms, and bar graphs can be displayed. The bar graph (A) in the plate map view shows a drug concentration-dependent decrease in the mean pMAPK-derived fluorescence intensity in the nuclear, cellular, and cytoplasmic masked regions. In this experiment, the IC_{50} based on pMAPK-derived fluorescence intensity in the cell mask region was 1.665. Scale bar: 200 µm

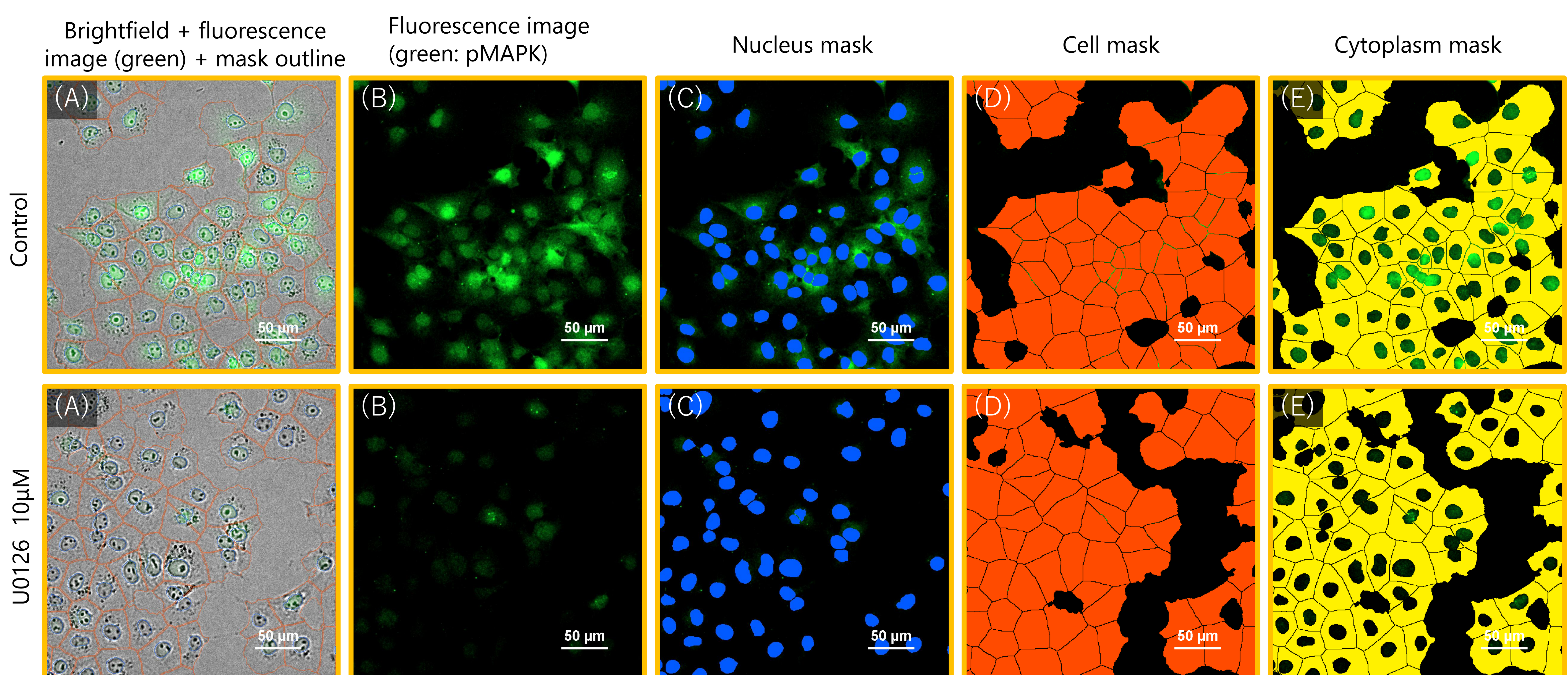


Fig. 3: Images of control (untreated) and HeLa cells treated with U0126 for 30 minutes, masks overlaid on fluorescence image
Enlarged image created by opening the image file in advanced mode and cropping the area of the orange dotted frame (0.31 mm × 0.31 mm) in Fig. 2. Upper row: control (untreated), lower row: treated with U0126 10 µM for 24 hours, (A) Merged image of brightfield and fluorescence (green: pMAPK) overlaid with mask outline (blue: nucleus mask, red: cell mask), (B) Immunofluorescence image of pMAPK, (C-E) Fluorescence image (green: pMAPK) overlaid with each mask, (C) blue: nucleus mask, (D) red: cell mask, (E) yellow: cytoplasm mask , Scale bar: 50 µm

Summary

- ✓ The trained AI was able to automatically identify cellular regions from brightfield images and measure the mean fluorescence intensity derived from pMAPK in the cell mask region from the fluorescence channel.
- ✓ The dose-dependent decrease in pMAPK-derived fluorescence intensity with U0126 could be confirmed.
- ✓ Smart Experiment can be run fully automatically from image acquisition to analysis and graph creation.
- ✓ A dose-response curve is automatically created and the IC_{50} can be calculated.
- ✓ This procedure was divided into a few simple steps: place the well plate on Ji, select the Intensity Measurement 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 other research activities by leaving tedious tasks to AI.

Sample preparation protocol

1) COS7 cells are seeded in a 96-well plate at a density of 1x10e4 cells/well and cultured for 24 hours at 37℃ in a 5% CO_2 incubator.
2) U0126 diluted with medium concentrations of 0, 0.002, 0.005, 0.014, 0.041, 0.123, 0.370, 1.111, 3.333 and 10µM, and each concentration of pre-treatment medium is added to each of the 6 wells. Cells are treated with pre-treatment medium for 15 minutes at 37° C in a 5% CO_2 incubator.

3) Pretreatment medium is removed and treatment medium is added (growth medium containing each test concentration of U0126 and 10 ng/ml PMA) to each of the 6 wells. Cells are treated for 15 minutes in a 37° C, 5% CO2 incubator.
4) 4% PFA is added to the wells and leave at room temperature for 10 minutes to fix the cells.
5) Wash cells 3 times with PBS.
6) Add 0.2% Triton X-100 in PBS to the wells and leave at room temperature for 15 minutes for membrane permeabilization.
7) Wash cells 3 times with PBS.
8) Blocking with 3% BSA in PBS to cells and leave at room temperature for 30 minutes.
9) Replace with PBS containing primary antibody (1:200) diluted in blocking solution and leave at room temperature for 2 hours.
10) Wash cells 3 times with PBS.
11) Replace with PBS containing secondary antibody (1:500) diluted in blocking solution and leave at room temperature for 1 hour.
12) DAPI (2 μg/ml) is added to the wells and leave at room temperature for 5 minutes.
13) Wash cells 3 times with PBS.

Materials and reagents

Cell Culture	
Cell Line	COS7 (RIKEN RCB0539)
Growth medium	DMEM (Low Glucose) + 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	U0126
Test concentration	Negative control: 0 μM Positive control: 10 μM To make a dose-response curve, design required concentration points as follows: Ex: (0) 0 μM, (1) 0.002 μM, (2) 0.005 μM, (3) 0.014 μM, (4) 0.041 μM, (5) 0.123 μM, (6) 0.370 μM, (7) 1.111 μM, (8) 3.333 μM, (9) 10 μM
Plate map example	(see plate map below)

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
U0126, Selective MKK inhibitor	ab120241	Abcam
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb (primary antibody)	4370	Cell Signaling Technology
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	A-11008	Thermo Fisher Scientific, Invitrogen™
DAPI Solution	62248	Thermo Fisher Scientific
Dulbecco's modified Eagle's Medium (DMEM)	11885084	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
Phorbol 12-myristate 13-acetate (PMA)	P8139	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

Favata, MF, et al., Identification of a Novel Inhibitor of Mitogen-activated Protein Kinase Kinase *Journal of Biological Chemistry* **273** 18623-18632 (1998)

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 Smart Experiment Basic Set Intensity Measurement

- ✓ Fully automated from image acquisition to analysis and graph display.
- ✓ Fluorescence intensity of nucleus, cell, and cytoplasm regions can be analyzed fully automatically and easily.
- ✓ 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.