Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

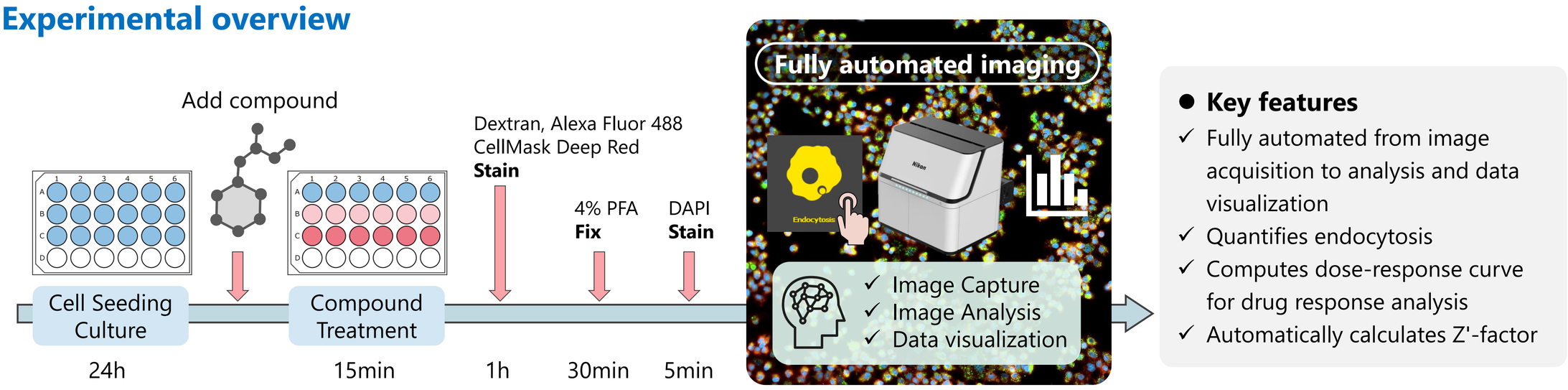
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Endocytosis（Option）

Analysis of endocytosis using the AI-driven, fully automated smart imaging system ECLIPSE Ji

Endocytosis is a fundamental cellular process in which extracellular materials are taken up by cells and utilized or degraded. It is also a known route of infection for small pathogens and viruses. Observing endocytosis provides useful insights on cellular responses to a wide variety of substances such as drug candidates or pathogens. The ECLIPSE Ji digital inverted microscope equipped with NIS-Elements Smart Experiment (SE) software enables automated, seamless imaging workflows from acquisition to analysis and data visualization. This application note introduces the Endocytosis SE module, which was used to quantify the endocytic activity of A431 cells by measuring the fluorescence intensity and distribution of fluorescently labeled dextran.

Keywords: infectious disease research, endocytosis, pharmacological testing, drug discovery

Experimental overview



(1) A431 cells were seeded in 24-well plates and cultured for 24 hours. (2) Cytochalasin D, the test substance, was adjusted to concentrations of 0µM, 3µM and 10µM and added to each well for a 15-minute incubation period. (3) Media was changed to contain Dextran, Alexa Fluor™ 488 (final concentration: 100 µg/mL), Cytochalasin D and CellMask™ Deep Red (final concentration: 5 µg/mL), and incubated for 1 hour. (4) Cells were fixed with 4% PFA. (5) Nuclei were stained with DAPI (final concentration: 2 µg/mL). (6) The well plate was placed on ECLIPSE Ji and image acquisition and analysis were run automatically by selecting the Endocytosis icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nuclei	DAPI	341/452
Puncta	Dextran, Alexa Fluor™ 488	495/519
Cell region	CellMask™ Deep Red	649/666
Magnification	**Field of view**	
20X	0.88 x 0.88 mm	

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

Results

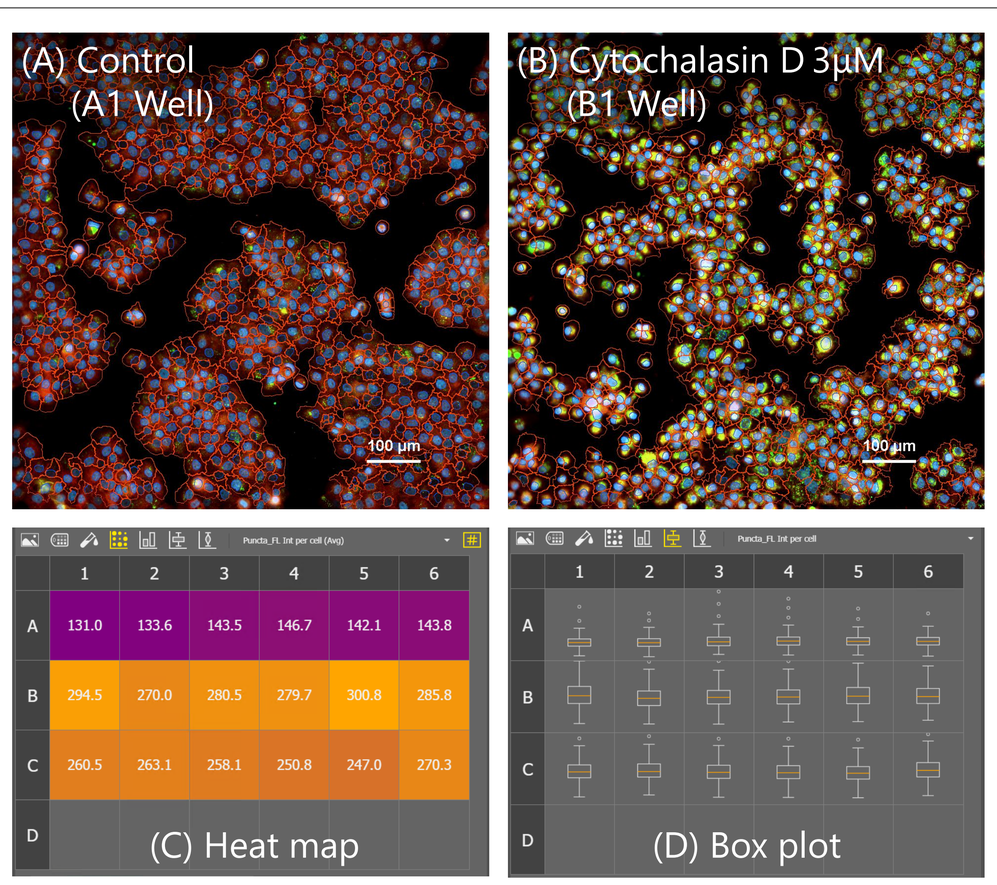


Fig. 1: Binarization and fluorescence intensity measurement of Dextran, Alexa Fluor™ 488

Nuclei were detected by DAPI and binarized to create a nucleus mask. A cell mask was created using the fluorescence image of CellMask™ Deep Red. The fluorescence intensity of accumulated Dextran, Alexa Fluor™ 488 within the cell mask region was measured.

(A, B) Fluorescent image of A431 cells overlaid with mask outlines. (Blue: DAPI, Green: Dextran, Alexa Fluor™ 488, Red: CellMask™ Deep Red, Blue mask: Nucleus, Red mask: Cell mask), Scale bar: 100 µm.

(C, D) Analysis results of the average fluorescence intensity of accumulated Dextran, Alexa Fluor™ 488 per cell are displayed for each well. The concentrations of Cytochalasin D are 0µM, 3µM, and 10µM from the top of the well plate map. Compared to the control, the average fluorescence intensity of accumulated Dextran, Alexa Fluor™ 488 taken into A431 cells was greater in the presence of Cytochalasin D at concentrations of 3µM or higher.

Summary

- ✓ The average fluorescence intensity of accumulated Dextran, Alexa Fluor™ 488 within the cellular regions was measured.
- ✓ The presence of Cytochalasin D induced a greater uptake if Dextran, Alexa Fluor™ 488 into cells.
- ✓ The ECLIPSE Ji Smart Experiments can be performed automatically from image acquisition to analysis and data visualization.
- ✓ This procedure was divided into a few simple steps: place the well plate on the Ji, select the Endocytosis icon, then input the sample dosage information.
- ✓ A pretrained "CellFinder.ai" finds the optimal focal plane - there is no need to manually set autofocus.
- ✓ Researchers can concentrate on other research activities by leaving tedious tasks to AI.

Materials and reagents

Cell Culture	
Cell Line	A431
Growth medium	DMEM (HG) + 10% hi-FBS
Culture vessel	EZVIEW® Culture Plate LB (Glass Bottom Plate) Microplate 24 well (AGC techno glass (IWAKI), 5826-024)
Test substance	
Compound	Cytochalasin D
Test concentration	Negative control: 0µM Positive control: 3µM A: 0µM, B: 3µM, C: 10µM
Plate map example	1 2 3 4 5 6 A 0 0 0 0 0 0 B 3 3 3 3 3 3 C 10 10 10 10 10 10 D b b b b b b Unit : µM “b” : blank well

Reagents		
Product name	Product number	Supplier
Cytochalasin D	C8273	Sigma-Aldrich
Dextran, Alexa Fluor™ 488; 10,000 MW, Anionic, Fixable	D22910	Thermo Fisher Scientific
CellMask™ Deep Red Plasma Membrane Stain	C10046	Thermo Fisher Scientific
DAPI Solution (1mg/mL)	62248	Thermo Fisher Scientific
Dulbecco’s Modified Eagle’s Medium - high glucose (DMEM (HG))	D5796	Sigma-Aldrich
Fetal Bovine Serum (FBS)	172012	Sigma-Aldrich
CultureSure DMSO (DMSO)	031-24051	Fujifilm-Wako
Penicillin-Streptomycin (Pc/Sm) (10,000 U/mL)	15240122	Thermo Fisher Scientific
16%-Paraformaldehyde Aqueous Solution (16% PFA) *Dilute by 4% with PBS before use	11850-14	Nacalai Tesque
DPBS, no calcium, no magnesium (PBS (-))	14190144	Gibco™
D-PBS (+) Preparation Reagent (Ca, Mg solution) (100x) (100x Ca, Mg solution)	02492-94	Nacalai Tesque

☐ Compatible vessel*

- 24, 96 well plate

＊Compatible with only glass bottom well plates.

Reference

L He, *et al.*, Contrasting roles for actin in the cellular uptake of cell penetrating peptide conjugates. *Scientific Reports* **8**, Article number: 7318 (2018)

Product information

Smart Imaging System ECLIPSE Ji

The AI-driven, fully automated ECLIPSE Ji digital inverted microscope equipped with NIS-Elements Smart Experiment (SE) software enables automated, seamless imaging workflows from acquisition to analysis and data visualization. It utilizes the pre-trained AI function "CellFinder.ai“ to find the optimal focal plane, which is more reproducible than manually setting the autofocus plane based on human judgement. Various pre-trained AI routines are implemented in the image acquisition and analysis process. This greatly reduces the number of steps for setting optimization and ultimately increases the accessibility of microscopy as a research tool.

Imaging Software NIS-Elements SE Endocytosis（Option）

- ✓ Fully automated from image acquisition to analysis and data visualization.
- ✓ Fluorescence intensity in cellular regions based on the distribution of Dextran labeled with a fluorescent dye can be easily analyzed automatically.
- ✓ One-click reports can be created and exported as PDFs including images and analysis results.