Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

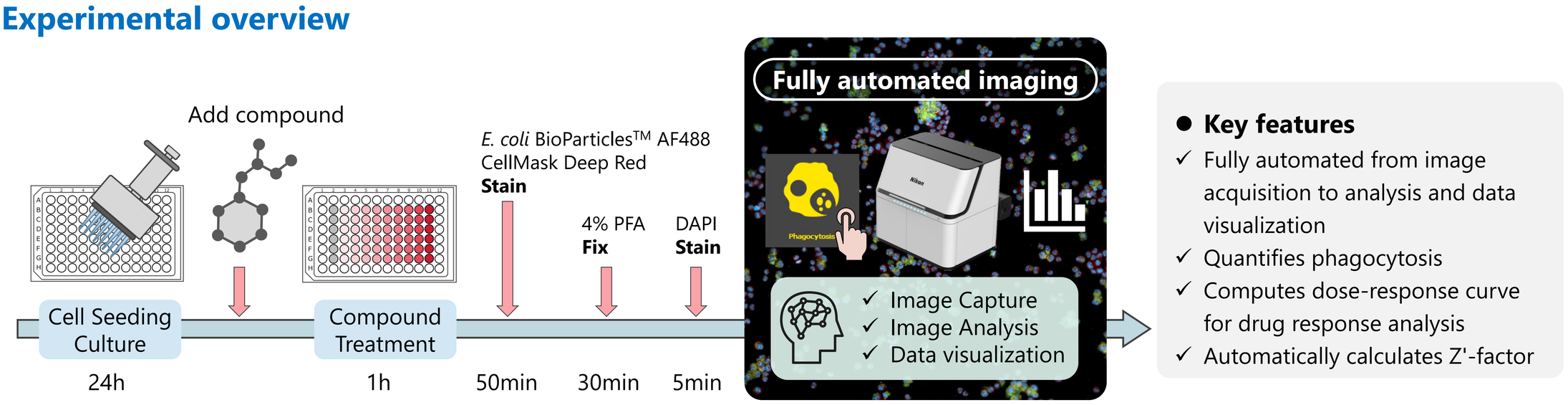
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Phagocytosis（Option）

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

Phagocytosis is the process by which immune cells take in and degrade extracellular foreign substances, such as dead cells and bacteria. 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 Phagocytosis SE module, which was used to quantify the phagocytic activity of J744.1 cells by measuring the fluorescence intensity and localization of fluorescently labeled *E. coli* (K-12 strain).

Keywords: Immunology research, phagocytosis, pharmacological testing, drug discovery

Experimental overview



(1) J774.1 cells were seeded in 96-well plates and cultured for 24 hours. (2) Cells were treated with 10 different concentrations of Cytochalasin D for 1 hour. (3) Media was changed to contain *E. coli* BioParticles™ AF488 containing Cytochalasin D (final concentration: 2.0x10e6 BioParticles/well) and CellMask™ Deep Red (final concentration: 5 µg/mL), followed by a 50-minute incubation period. (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 Phagocytosis icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nuclei	DAPI	360/460
Bioparticles	*Escherichia coli* (K-12 strain) BioParticles™, Alexa Fluor™ 488 conjugate	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 and discussion

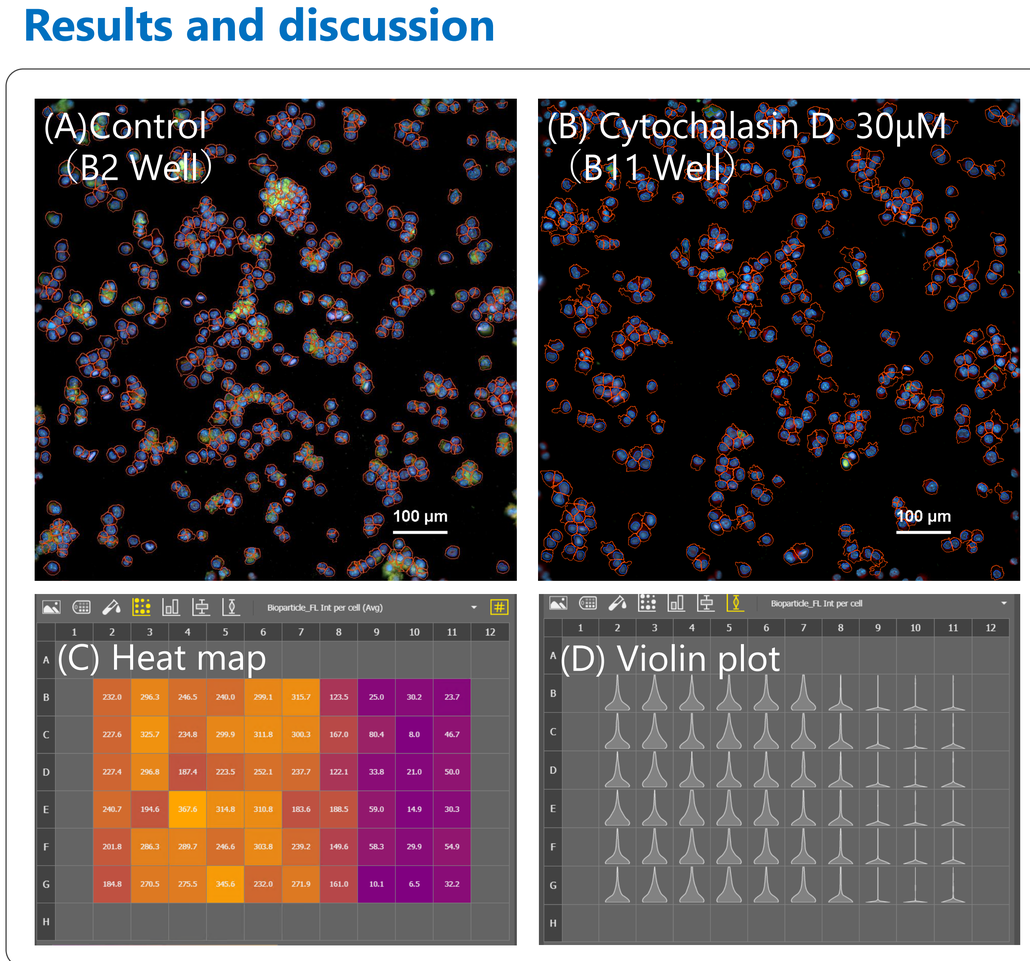


Fig. 1: Binarization and fluorescence intensity measurement of *E. coli* BioParticles™ AF488

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 *E. coli* BioParticles™ AF488 within the cell mask region was measured. (A, B) Fluorescence image of J774.1 cells overlaid with mask outline. (Blue: DAPI, Green: *E. coli* BioParticles™ AF488, 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 *E. coli* BioParticles™ AF488 per cell are displayed for each well. Cytochalasin D concentrations are, from left to right in the well plate map, 0µM, 0.003µM, 0.01µM, 0.03µM, 0.1µM, 0.3µM, 1µM, 3µM, 10µM, and 30µM. Compared to control, the fluorescence intensity of *E. coli* BioParticles™ AF488 taken into cells decreased in wells where the concentration of Cytochalasin D was 3 µM or higher. This result indicates that Cytochalasin D inhibits the uptake of fluorescently labeled E. coli into J774.1 cells.

Summary

- ✓ The average fluorescence intensity of accumulated fluorescently labeled *E. coli* within cellular regions was measured.
- ✓ It was observed that Cytochalasin D inhibits the uptake of fluorescently labeled *E. coli* into J774.1 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 Phagocytosis 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	J774.1
Growth medium	RPMI 1640 + 10% hi-FBS
Culture vessel	EZVIEW® Culture Plate B (Glass Bottom Plate) Microplate 96 well (AGC techno glass (IWAKI), 5866-096)
Test substance	
Compound	Cytochalasin D
Test concentration	Negative control: 0μM Positive control: 10μM To make a dose-response curve, design required concentration points as follows: Ex: (0) 0 μM, (1) 0.003 μM, (2) 0.01 μM, (3) 0.03 μM, (4) 0.1 μM, (5) 0.3 μM, (6) 1 μM, (7) 3 μM, (8) 10 μM, (9) 30 μM
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
Cytochalasin D	C8273	Sigma-Aldrich
Escherichia coli (K-12 strain) BioParticles™, Alexa Fluor™ 488 conjugate	E13231	Thermo Fisher Scientific
CellMask™ Deep Red Plasma Membrane Stain	C10046	Thermo Fisher Scientific
DAPI Solution (1mg/mL)	19178-91	Nacalai Tesque
RPMI 1640 with L-Gln, liquid	30264-85	Nacalai Tesque
Fetal Bovine Serum (FBS)	172012	Sigma-Aldrich
Dimethyl sulfoxide (DMSO)	276855	Sigma-Aldrich
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 well plate
- 96 well plate

＊Compatible with only glass bottom well plates.

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 Phagocytosis（Option）

- ✓ Fully automated from image acquisition to analysis and data visualization.
- ✓ Fluorescence intensity in cellular regions based on the presence of fluorescently-labeled *E. coli* (K-12 strain) can be easily analyzed automatically.
- ✓ One-click reports can be created and exported as PDFs including images and analysis results.