Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

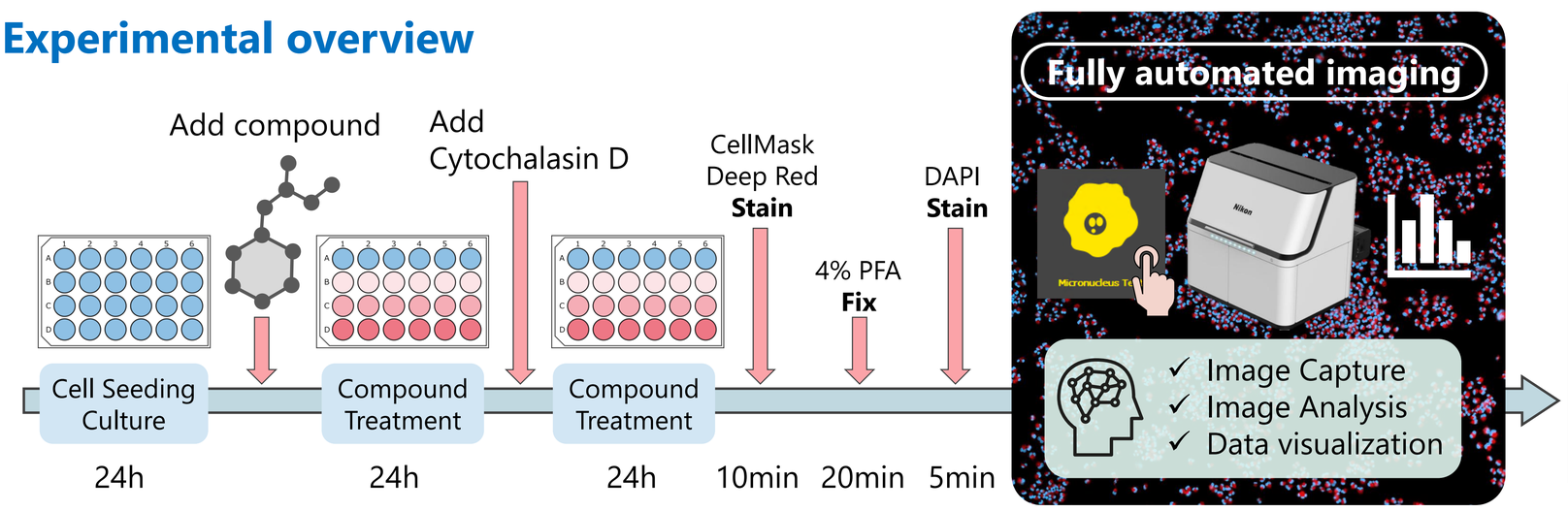
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Micronucleus Test（Option）

Calculating the percentage of cells with micronuclei using the AI-driven, fully automated smart imaging system ECLIPSE Ji

Genotoxic substances are known to induce chromosomal aberrations which cause micronuclei to form during cell division. Detection of these micronuclei via microscopy is useful for characterizing the genotoxicity of drug candidates and other compounds. 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 Micronucleus Test SE module, which was used to determine the percentage of cells containing micronuclei in response to the genotoxic compound Mitomycin C.

Keywords: Genotoxicity, toxicology, toxicity testing, toxicity evaluation, safety testing, pharmacological testing, drug discovery

Experimental overview



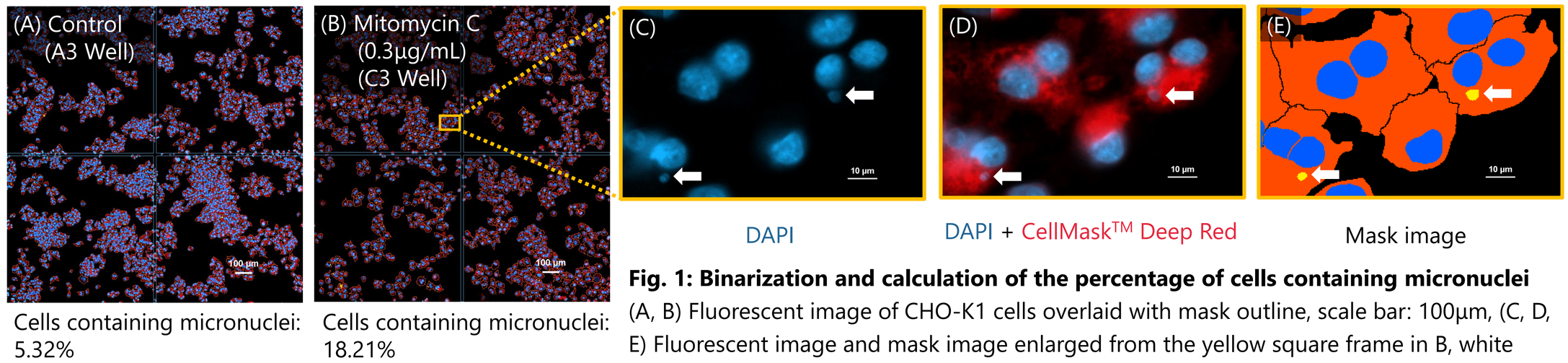
- **Key features**
  - ✓ Fully automated from image acquisition to analysis and data visualization
  - ✓ Automatically quantifies the ratio of micronuclei formation in cells
  - ✓ Computes dose-response curve for drug response analysis
  - ✓ Automatically calculates Z'-factor

(1) CHO-K1 cells were seeded in 24 well plates and cultured for 24 hours. (2) The test substance Mitomycin C was adjusted to concentrations of 0µg/mL, 0.1µg/mL, 0.3µg/mL, and 1µg/mL, and added to each well for a 24-hour treatment period. (3) The media was changed to contain Cytochalasin D (final concentration: 6µM) and incubated for 24 hours. (4) CellMask Deep Red (final concentration: 5µg/mL) was added followed by a 10-minute incubation period. (5) Cells were fixed with 4% PFA. (6) Nuclei were stained with DAPI (final concentration: 2µg/mL). (7) The well plate was placed on ECLIPSE Ji and image acquisition and analysis were run automatically by selecting the Micronucleus Test icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells and micronuclei	DAPI	341/452
Cell region	CellMask™ Deep Red	649/666
Magnification	**Field of view**	
20X	0.88 x 0.88 mm Tiling image (2X2) FOV: 1.75 x 1.75 mm	

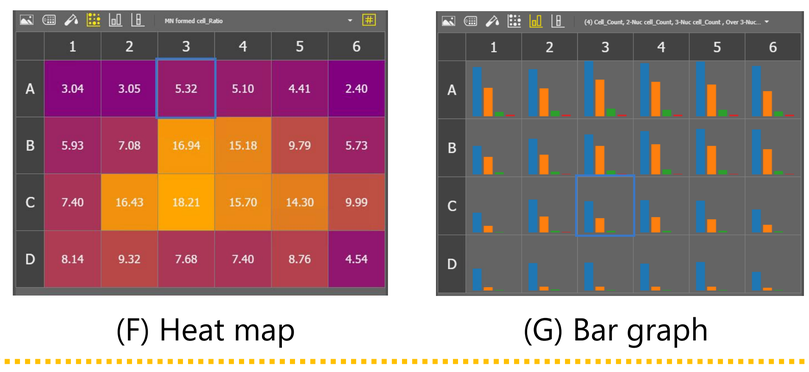
Table. 1: Detection regions, fluorescence labels, and image acquisition conditions

Results



Cells containing micronuclei: 5.32%

Cells containing micronuclei: 18.21%



(F) Heat map

(G) Bar graph

Fig. 1: Binarization and calculation of the percentage of cells containing micronuclei
(A, B) Fluorescent image of CHO-K1 cells overlaid with mask outline, scale bar: 100µm, (C, D, E) Fluorescent image and mask image enlarged from the yellow square frame in B, white arrow : Micronucleus, scale bar: 10µm, (E) The nuclear mask (blue) and micronucleus mask (yellow) are based on the nuclear region detected by DAPI, while the cell mask (red) is based on the cellular region detected with CellMask™ Deep Red. (F) Heatmap showing the percentage of cells with micronuclei relative to total cell count. Wells treated with Mitomycin C resulted in a higher ratio of cells containing micronuclei compared to control wells in row A. (G) Bar graph showing the number of multinucleated cells after inhibiting cell division with cytochalasin D. Mitomycin C treatment causes micronuclei formation rather than additional, proper nuclei (blue: total number of cells, orange: number of cells with two nuclei, green: number of cells with three nuclei) , red: number of cells with 4 or more nuclei)

Summary

- ✓ The ratio of the number of cells with micronuclei relative to the total number of cells was calculated.
- ✓ Micronuclei were significantly more prevalent in wells treated with the genotoxic compound Mitomycin C compared to untreated control wells.
- ✓ 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 Micronucleus Test 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	CHO-K1 (JCRB9018)
Growth medium	Ham's F-12 + 10% hi-FBS
Culture vessel	EZVIEW® Culture Plate LB (Glass Bottom Plate) Microplate 24 well (AGC techno glass (IWAKI), 5826-024)
Test substance	
Compound	Mitomycin C (MMC)
Test concentration	Negative control: 0µg/mL Positive control: 1µg/mL A: 0µg/mL, B: 0.1µg/mL, C: 0.3µg/mL, D: 1µg/mL
Plate map example	1 2 3 4 5 6 A 0 0 0 0 0 0 B 0.1 0.1 0.1 0.1 0.1 0.1 C 0.3 0.3 0.3 0.3 0.3 0.3 D 1 1 1 1 1 1 Unit :µg/mL

Reagents		
Product name	Product number	Supplier
Mitomycin C (MMC)	M4287	Sigma-Aldrich
Cytochalasin D	C8273	Sigma-Aldrich
CellMask™ Deep Red Plasma Membrane Stain	C10046	Thermo Fisher Scientific
DAPI Solution (1mg/mL)	62248	Thermo Fisher Scientific
Ham's F-12 Nutrient Mix	11765054	Gibco™
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.

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 Micronucleus Test（Option）

- ✓ Fully automated from image acquisition to analysis and data visualization.
- ✓ The ratio of cells with micronuclei can be easily analyzed automatically.
- ✓ One-click reports can be created and exported as PDFs including images and analysis results.