Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

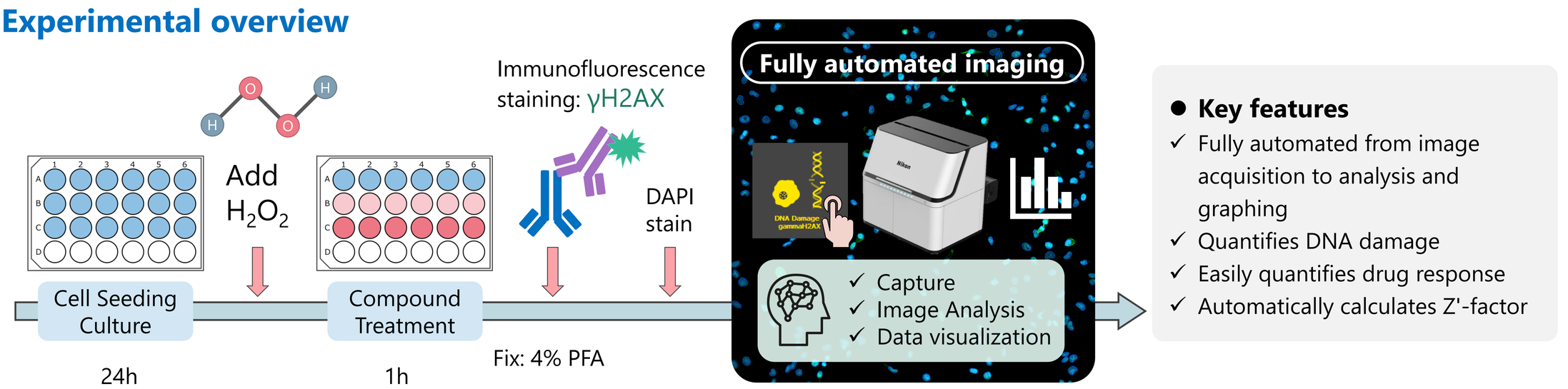
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
DNA Damage - gammaH2AX (Option)

Analysis of DNA Damage using AI-driven fully automated ECLIPSE Ji

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 procedures automatically optimize image acquisition and analysis condition settings, providing visualized data in a simple, streamlined process. γH2AX is a marker of DNA damage and DNA repair. It is known that histone protein H2AX is phosphorylated to form γH2AX at the site of DNA double-strand break (DSB), which is involved in DNA repair. This application note introduces an example of using the DNA Damage - gammaH2AX module of Smart Experiment to quantify DNA damage by measuring fluorescence intensity derived from immunofluorescence-stained gammaH2AX in the nucleus region.

Keywords: DNA damage, γH2AX, drug discovery, toxicology, toxicity testing, toxicity evaluation, safety pharmacological study, automatic setting

Experimental overview



(1) HeLa cells were seeded in 24-well plates and cultured for 24 hours. (2) Hydrogen Peroxide (H_2O_2) was prepared with increasing medium concentrations of 0, 0.1mM, and 0.3mM, and each concentration of medium is added to each well and treated for 1 hour. (3) Cells were fixed with 4% PFA. Membrane permeabilization was performed with 0.1% Triton X-100. (5) Immunofluorescence staining was done with Anti-gamma H2A.X (phospho S139) antibody and Goat Anti-Rabbit IgG H&L Alexa Fluor® 488. Stain nuclei with DAPI. (6) The well plate was placed on ECLIPSE Ji and image acquisition and analysis was run automatically by selecting the DNA Damage - gammaH2AX icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells	DAPI	345/455
DNA damage (γH2AX)	Anti-gamma H2A.X (phospho S139) (primary), Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (secondary)	495/519
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 method and γH2AX fluorescence intensity measurement
Nucleus regions detected by DAPI are binarized to create a nucleus mask (A). The fluorescence intensity of immunofluorescence-stained γH2AX-derived in the region of the nucleus mask are measured (B) to quantify the amount of γH2AX. Upper fluorescence image: control HeLa cells, lower fluorescence image: HeLa cells treated with H_2O_2 for 1 hour (blue: DAPI, green: γH2AX immunofluorescence staining image, (A, B) fluorescence image overlaid with mask outline, (C, D) Measurement results of mean fluorescence intensity derived from γH2AX. Scale bar: 100 µm

Summary

- ✓ The mean fluorescence intensity derived from γH2AX (DNA damage) in the nucleus mask region detected by fluorescence could be measured.
- ✓ The dose-dependent increase in γH2AX-derived fluorescence intensity with H_2O_2 could be confirmed.
- ✓ A Smart Experiment can be performed fully automatically from image acquisition to analysis and data analysis.
- ✓ This procedure was divided into a few simple steps: place the well plate on the Ji, select the DNA Damage - gammaH2AX icon, then input the sample information. Under the conditions of this experiment, it took approximately 20 minutes from the start of imaging to the final results.
- ✓ 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.

Materials and reagents

Cell Culture	
Cell Line	HeLa (RIKEN RCB0007)
Growth medium	MEM + 10%FBS + 1%Pc/Sm
Culture vessel	EZVIEW® Culture Plate LB (Glass Bottom Plate) Microplate 24 well (AGC techno glass (IWAKI), 5826-024)
Test substance	
Compound	Hydrogen Peroxide (H_2O_2)
Test concentration	Negative control: 0 mM Positive control : 0.3 mM
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 b b b b b b "b" : blank well

Reagents		
Product name	Product number	Supplier
Hydrogen Peroxide (H_2O_2)	084-07441	Fujifilm-Wako
Anti-gamma H2A.X (phospho S139) antibody	ab11174	Abcam
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (secondary antibody)	ab150077	Abcam
DAPI Solution	62248	Thermo Fisher Scientific
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
Bovine Serum Albumin (BSA)	A4919	Sigma-Aldrich
Polysorbate 20 (Tween-20)	103168	MP Biomedicals
Triton™ X-100	T8787	Sigma-Aldrich
DPBS, no calcium, no magnesium (PBS)	14190144	Thermo Fisher Scientific

☐ Compatible vessel*

- 24-well plate
- 96-well plate

＊Compatible with only glass bottom well plates.

Reference

Löbrich, M, *et al.*, gammaH2AX foci analysis for monitoring DNA double-strand break repair: strengths, limitations and optimization. *Cell Cycle* **9** 662 -9 (2010)

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 DNA Damage - gammaH2AX (Option)

- ✓ Fully automated from image acquisition to analysis and graph display.
- ✓ γH2AX-derived fluorescence intensity in the nucleus region detected by fluorescence can be analyzed easily and fully automatically.
- ✓ 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.