Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

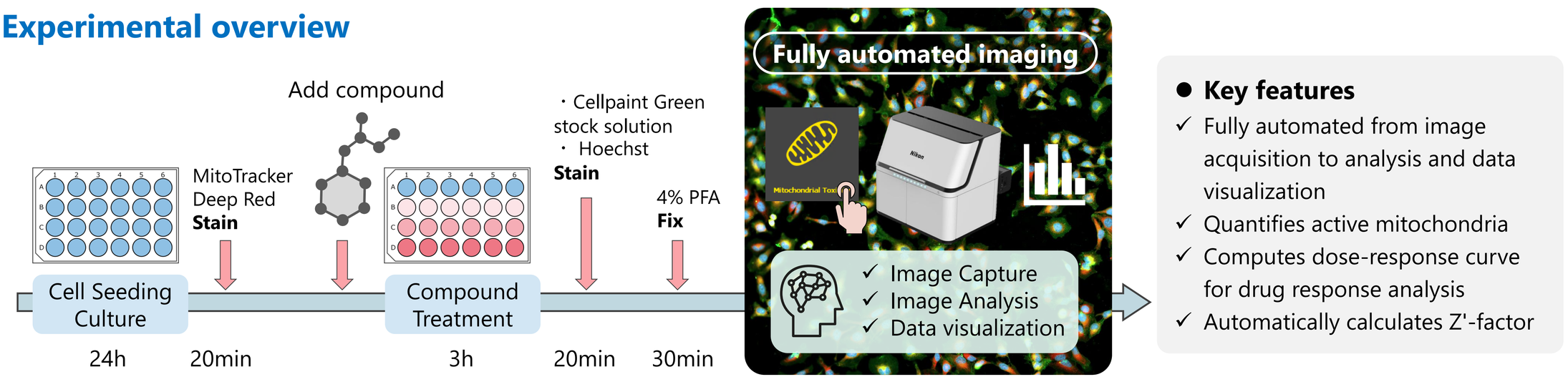
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Mitochondrial Toxicity（Option）

Mitochondrial toxicity analysis using the AI-driven, fully automated smart imaging system ECLIPSE Ji

Mitochondria are important organelles that produce energy, and mitochondrial dysfunction is known to be involved in metabolic abnormalities and the development of various diseases. Mitochondrial membrane potential-dependent fluorescent dyes provides unique insights related to mitochondrial health. 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 Mitochondrial Toxicity SE module, which was used to analyze drug-induced mitochondrial toxicity by measuring the fluorescence intensity of a dye that stains healthy mitochondria.

Keywords: Mitochondrial toxicity, toxicology, toxicity testing, toxicity evaluation, pharmacological testing, drug discovery

Experimental overview



- **Key features**
- ✓ Fully automated from image acquisition to analysis and data visualization
- ✓ Quantifies active mitochondria
- ✓ Computes dose-response curve for drug response analysis
- ✓ Automatically calculates Z'-factor

(1) HeLa cells were seeded in 24 well plates and cultured for 24 hours. (2) Media was changed to contain MitoTracker™ Deep Red (final concentration: 1µM) and incubated for 20 minutes. (3) The mitochondrial activity-disrupting compound FCCP was adjusted to concentrations of 0µM, 25µM, 50µM, and 100µM, added to the appropriate wells, and incubated for 3 hours. (4) Cells were stained with Cellpaint Green stock solution (2µl/mL) and Hoechst (2µg/mL)) and incubated for 20 minutes. (5) Cells were fixed with 4% PFA. (6) The well plate was placed on ECLIPSE Ji and image acquisition and analysis were run automatically by selecting the Mitochondrial Toxicity icon.

Detection region	Fluorescence label	Ex/Em (nm)
Nuclei	Hoechst 33342	350/461
Cell region	Cell Navigator® Cell Plasma Membrane Staining Kit *Green Fluorescence*	497/505
Healthy mitochondria	MitoTracker™ Deep Red FM - Special Packaging	644/665
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 MitoTracker™ Deep Red FM

Nuclei were detected by Hoechst 33342 and binarized to create a nucleus mask. A cell mask was created using the fluorescence image of the Cell Navigator® Cell Plasma Membrane stain. The fluorescence intensity of MitoTracker™ Deep Red FM within the cell mask regions was measured.

(A, B) Fluorescent image of HeLa cells overlaid with mask outline. (Blue: DAPI, Green: Cell Navigator® Cell Plasma Membrane, Red: MitoTracker™ Deep Red FM, Blue mask: Nucleus, Green mask: Cell mask), Scale bar: 100 µm.
(C, D) Analysis of average fluorescence intensity of MitoTracker™ Deep Red FM per cell for each well. The concentrations of FCCP are 0µM, 25µM, 50µM, and 100µM from the top row of the well plate map. In wells with the concentration of FCCP were 25µM or higher, the average fluorescence intensity of MitoTracker™ Deep Red FM was decreased. This indicates that FCCP reduces the number of healthy, active mitochondria that can be labeled by MitoTracker™ Deep Red FM.

Summary

- ✓ The average fluorescence intensity of MitoTracker™ Deep Red FM within cellular regions was measured.
- ✓ The average fluorescence intensity of MitoTracker™ Deep Red FM was decreased in wells treated with concentrations of FCCP 25µM or higher.
- ✓ 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 Mitochondrial Toxicity 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	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	Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP)
Test concentration	Negative control: 0µM Positive control: 50µM A: 0µM, B: 25µM, C: 50µM, D: 100µM
Plate map example	1 2 3 4 5 6 A 0 0 0 0 0 0 B 25 25 25 25 25 25 C 50 50 50 50 50 50 D 100 100 100 100 100 100 Unit : µM

Reagents		
Product name	Product number	Supplier
FCCP	0453	R&D Systems, Inc.
MitoTracker™ Deep Red FM - Special Packaging	M22426	Thermo Fisher Scientific
Cell Navigator® Cell Plasma Membrane Staining Kit *Green Fluorescence*	22682	AAT Bioquest
Hoechst 33342 Solution(1mg/mL)	19172-51	Nacalai Tesque
MEM (Minimum Essential Medium)	11095080	Gibco™
Fetal Bovine Serum (FBS)	10437028	Thermo Fisher Scientific
Dimethyl sulfoxide (DMSO)	276855	Sigma-Aldrich
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™

☐ 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 Endocytosis（Option）

- ✓ Fully automated from image acquisition to analysis and data visualization.
- ✓ Fluorescence intensity in cellular regions based on MitoTracker™ Deep Red FM can be easily analyzed automatically.
- ✓ One-click reports can be created and exported as PDFs including images and analysis results.