Shedding New Light On **MICROSCOPY**

APPLICATION NOTE

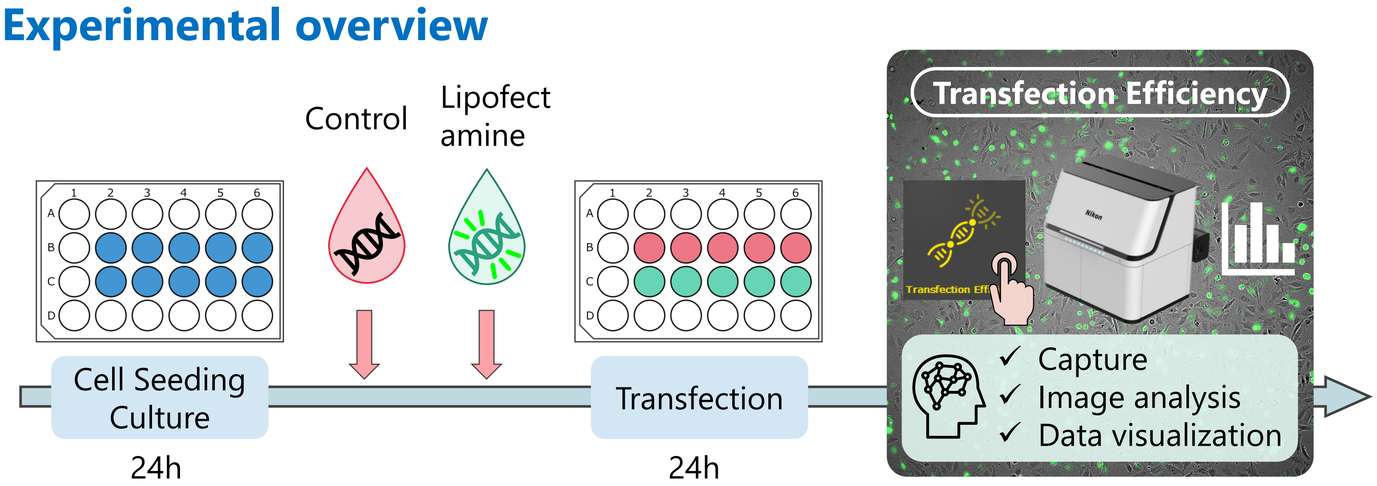
Smart Imaging System ECLIPSE Ji
Imaging Software NIS-Elements SE
Transfection Efficiency

AI-powered optimization of transfection efficiency by nucleus and cellular region detection from brightfield images

ECLIPSE Ji with Smart Experiment software enables seamless run from image acquisition to analysis and graph creation. Pre-trained Artificial Intelligence (AI) and pre-defined imaging processes automatically optimize image acquisition and analysis settings, allowing you to obtain numerical data and graphs with simple operations. Transfection is a technique for introducing nucleic acids (DNA or RNA) into cells. Target proteins can be expressed and visualized with fluorescence to analyze protein expression level. However, the optimal concentration of transfection reagent and amount of nucleic acid varies from cell to cell. Therefore, it is necessary to optimize transfection conditions by measuring the percentage of cells expressing the fluorescent protein out of the total number of cells observed. This application note introduces a method for evaluating transfection efficiency with minimal damage to cells by detecting nuclei and cellular regions from brightfield images using the Transfection Efficiency module of Smart Experiment.

Keywords: Transfection efficiency, gene transfer, optimization, label-free, Artificial Intelligence (AI), automatic setting

Experimental overview



- **Key features**
- ✓ Fully automated from image acquisition to analysis and graphing
- ✓ Label-free detection that identifies nuclei and cell regions from bright-field images using pre-trained AI
- ✓ Measure the percentage of fluorescent-expressing cells

(1) HeLa cells were seeded in 24-well plates and cultured for 24 hours. (2) A control solution mixed with plasmid DNA (GFP expression vector) and a transfection reagent (Lipofectamine) solution were added to each well. Medium was added to each well at 500 µl/well and cells were treated for 24 hours. (3) The well plate was placed on ECLIPSE Ji and image acquisition and analysis was run automatically by selecting the Transfection Efficiency icon.

Point

Pre-trained AI

Automatic identification of **nuclei** and **cellular regions** from brightfield images

Detection region	Fluorescence label	Ex/Em (nm)
Nucleus of all cells	None (Detect from bright-field image using AI)	Brightfield
Cell region	None (Detect from bright-field image using AI)	Brightfield
Target protein	GFP	488/509
Magnification	**Field of view**	
10X	1.76 x 1.76 mm	

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

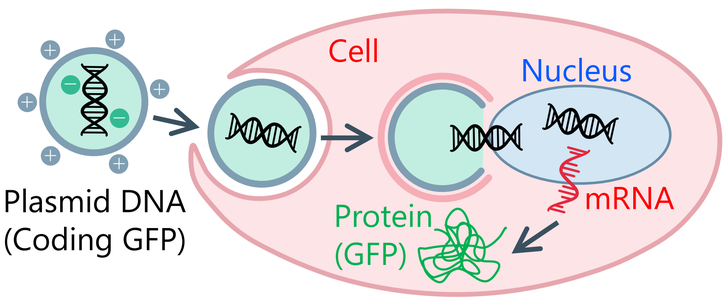


Fig.1: Schematic diagram of gene transfer with lipid-based transfection reagents

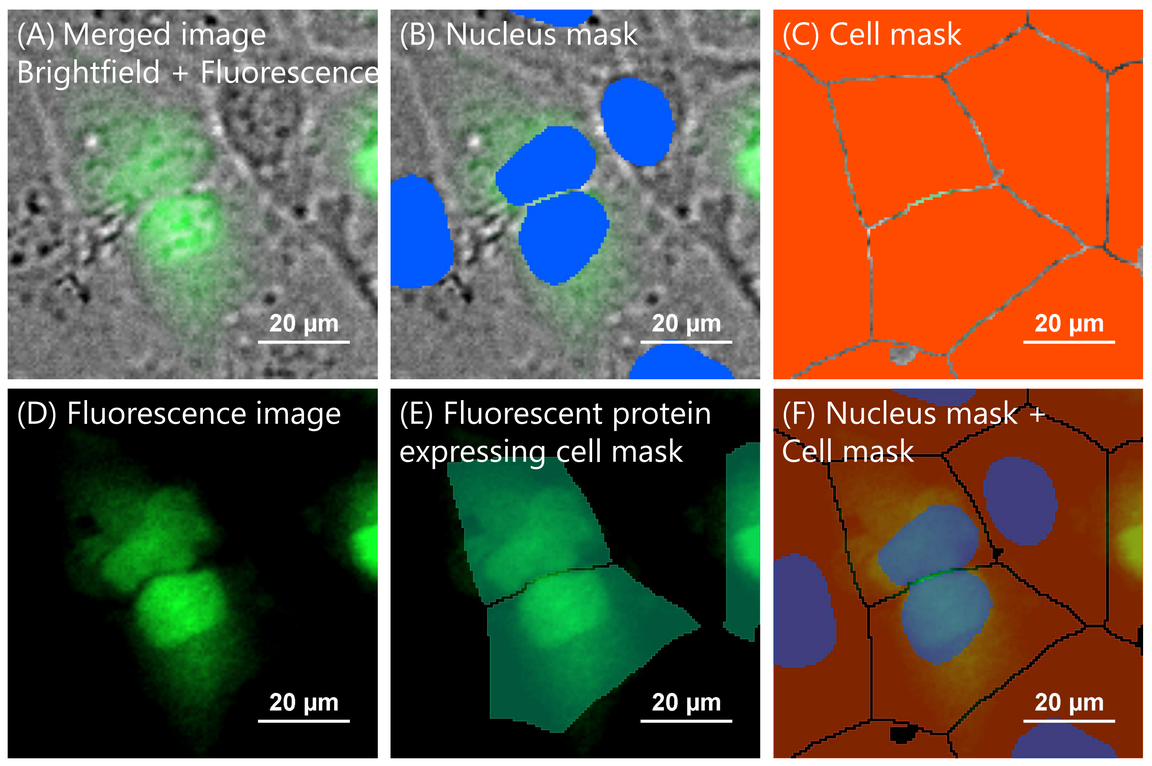


Fig. 2: Binarization and segmentation
Upper row: Merged image of brightfield + Fluorescence, Lower row: Fluorescence image, (B, C) Nucleus mask (blue) and cell mask (red) are created from the brightfield image by a trained AI. (E) A mask of cells expressing the fluorescent protein (green) is created from the overlapping region of the cell mask (red) and the GFP-expressing region. (F) Fluorescence image with overlaid nucleus masks (blue) and cell masks (red), Scale bar: 20µm

Results

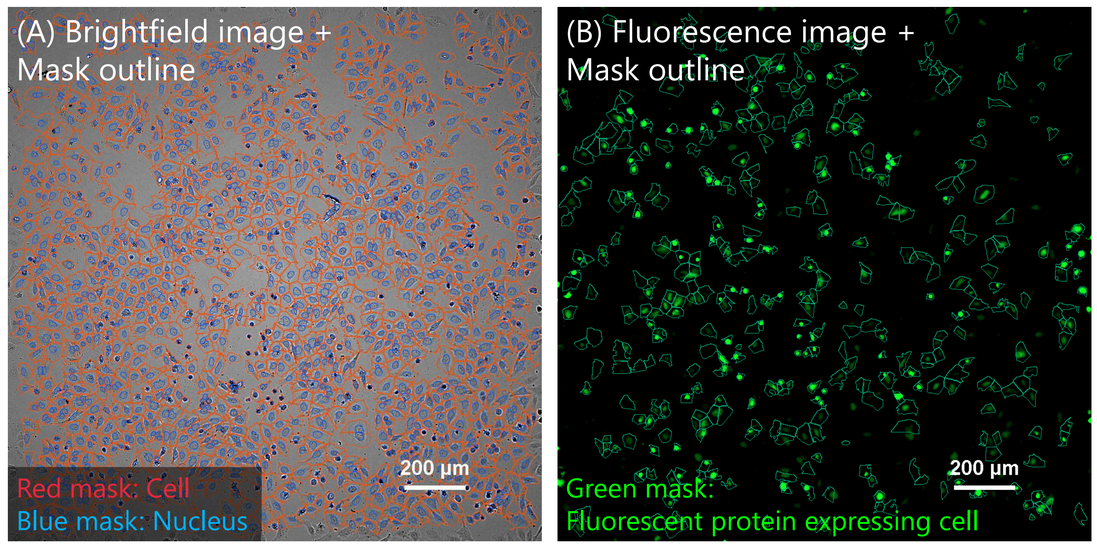


Fig. 3: Images of HeLa cells treated with Lipofectamine (0.75 µl/well) for 24 hours

(A) Brightfield image overlaid with mask outline, (B) Fluorescence image overlaid with mask outline, Blue mask: Nucleus, Red mask: Cell region, Green mask: Fluorescent protein expressing cell, The total number of cells: 1,450, The number of fluorescent protein expressing cell : 406, Fluorescent protein expressing cell rate (transfection efficiency): 28%, Scale bar: 200µm,

Formula: Fluorescent expressing cells ratio[%] = (Number of fluorescent protein-expressing cells / Total number of cells) X 100

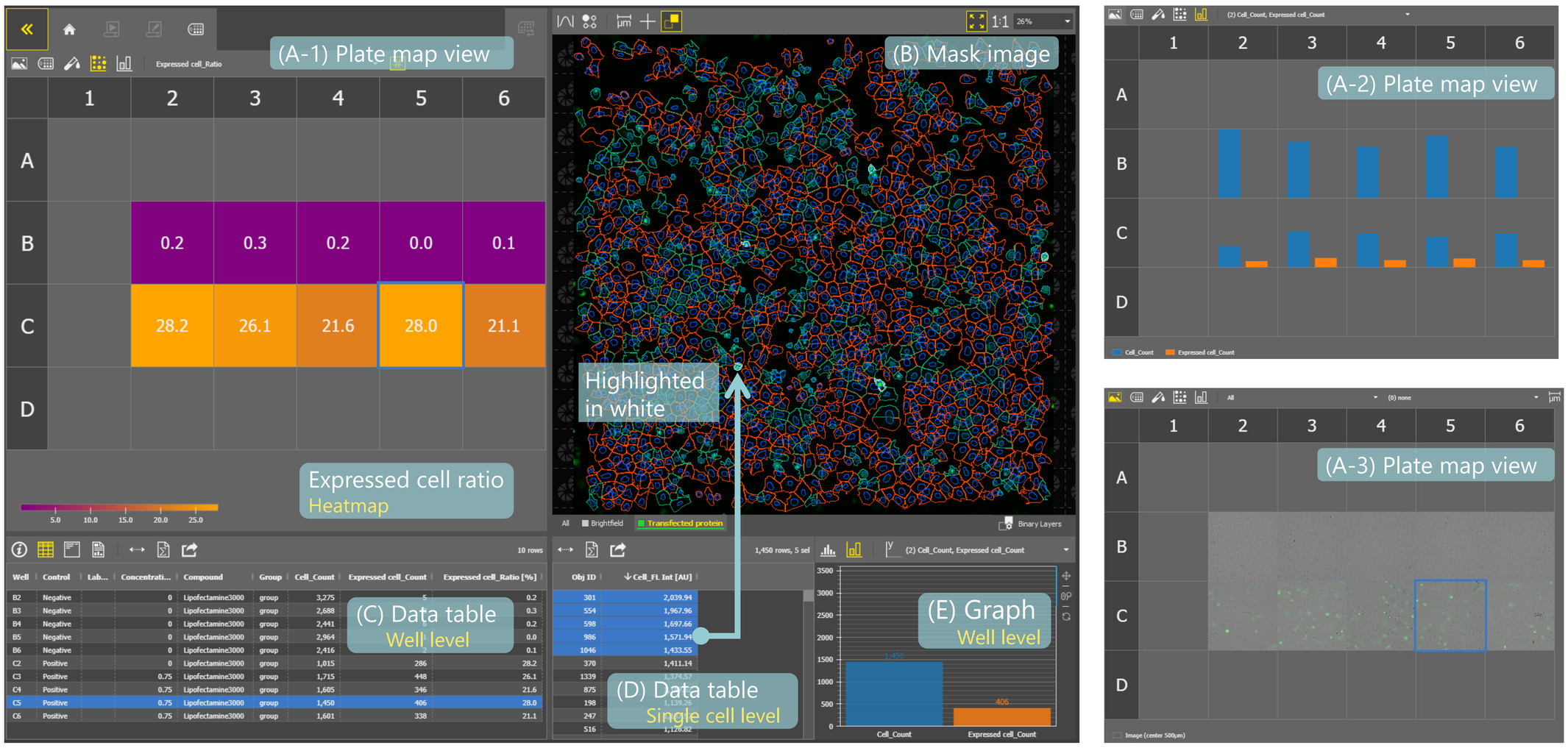


Fig. 4: Transfection efficiency analysis results, Software with easy-to-analyze GUI

The heat map (A-1) and bar graph (A-2) displays in the plate map view allow you to intuitively confirm the analysis results for the entire plate. Thumbnail images (A-3) can also be displayed in the plate map view. The data table provides well-level (C) and single-cell level (D) information. Data and images are linked so that when you select the objects in the single-cell level data table (D), the selected cells are highlighted with a white mask on the image (B). The single-cell level data table (D) provides fluorescence intensity information for individual cells. (E) Well level data can be displayed graphically.

Summary

- ✓ Smart Experiment can be run fully automatically from image acquisition to analysis and graph creation.
- ✓ Transfection Efficiency module uses pre-trained AI to identify nucleus and cell region from brightfield images, enabling label-free counting of all cells. In addition, fluorescence-expressing cells can be identified from fluorescence images and the percentage of fluorescence-expressing cells can be measured.
- ✓ AI technology for label-free identification of nucleus and cellular region from brightfield images without staining the cells provides maximum flexibility in downstream applications.
- ✓ It is a simple operation to place the well plate on Ji, select the Transfection Efficiency icon, and input the sample information.
- ✓ Researchers can concentrate on more creative research activities by leaving tedious setting tasks to AI.

Sample preparation protocol

1) HeLa cells are seeded in a 24-well plate at a density of 9x10e4 cells/well and culture for 24 hours at 37℃ in a 5% CO2 incubator.
2) Make sure the confluency is around 80-90%.
3) Mix 23.5 µl/well of Opti-MEM and 0.75 µl/well of Lipofectamine for positive control. Prepare 25 µl/well of Opti-MEM for negative control.
4) Mix Opti-MEM 25 µl/well, P3000 1 µl/well and Plasmid 500 ng/well.
5) Mix the solution prepared in step 3 and the plasmid solution prepared in step 4 in a 1:1 ratio and allow to place at room temperature for 5 minutes.
6) Add 500 µl/well of medium for observation (10% FBS + 4 mM L-Glutamine + 1% Pc/Sm in FluoroBright DMEM) to the mixture in step 5.
7) Replace the growth medium of the cells cultured overnight with 500 µl/well of the test medium prepared in step 6, and culture the cells for 24 hours at 37℃ in a 5% CO2 incubator.
8) Place the well plate in ECLIPSE Ji and run Transfection Efficiency to measure the percentage of GFP-expressing cells.

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	Lipofectamine® 3000 (Lipofectamine)
Test concentration	Negative control: (0) 0 µl/well Positive control: (1) 0.75 µl/well
Plate map example	1 2 3 4 5 6 A b b b b b b B b (0) (0) (0) (0) (0) C b (0.75) (0.75) (0.75) (0.75) (0.75) D b b b b b b "b" : blank well

Reagents		
Product name	Product number	Supplier
MEM (Minimum Essential Medium)	11095080	Thermo Fisher Scientific
FluoroBrite™ DMEM	A1896701	Thermo Fisher Scientific
Lipofectamine™ 3000 Transfection Reagent	L3000008	Thermo Fisher Scientific
Opti-MEM™ I Reduced Serum Medium	31985062	Thermo Fisher Scientific
Plasmid DNA (coding GFP in this experiment)	-	-
L-Glutamine (200 mM)	25030081	Thermo Fisher Scientific

☐ Compatible vessel*

- 6, 12, 24 well plate

＊Compatible with glass and polystyrene bottom well plates. If cell adhesion to the bottom is a priority over image quality, use well plates with polystyrene bottoms.

Reference

For the transfection sample preparation protocol, refer to the manufacturer's Thermo Fisher Scientific L3000008 Lipofectamine™ 3000 Transfection Reagent protocol.

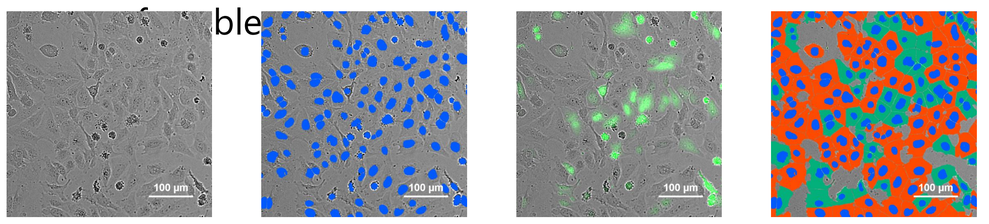
Product information

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 SmartExperiment Basic Set Transfection Efficiency

- ✓ Fully automated from image acquisition to analysis and graph display.
- ✓ Measure the percentage of fluorescence-expressing cells
- ✓ 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 [illegible]



Brightfield image | Brightfield image +Mask (blue: Nucleus mask) | Brightfield + fluorescence image (GFP) | Mask (Green: Fluorescent protein expressing cell mask

Smart Experiment automates experiment and reduces the number of process

Conventional methods: Input by Human

1. Select well plate type
2. Alignment of well plate
3. Select objective
4. Select wavelengths
5. Setting exposure conditions
6. Auto focus settings

→ No configuration required in Ji

7. Input the sample (drug) information
8. Setting binarization parameters
9. Setting measurement items
10. Graph creation

→ No configuration required in Ji

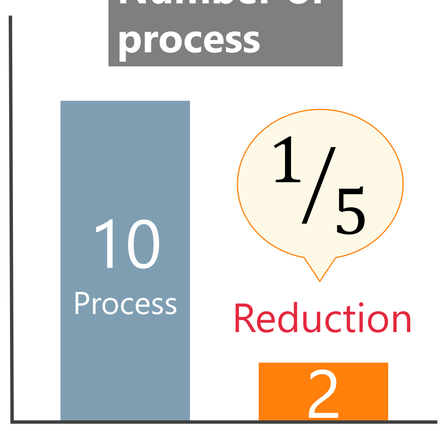
Smart Experiment

1. Select the Experiment icon
2. Input the sample (drug) information

\Simplified settings/

EASE OF USE

Number of process



Smart Experiment Workflow

Input by Human

Select Experiment icon
Sample information

Fully Automated process

Overview → Preview & Capture → Analysis & Graph → Report