APPLICATION NOTE

General Analysis 3 image analysis software module

Validation of control cells in phenotypic screening and selection of analysis methods according to cell morphology

Phenotypic screening is a cell-based, automated, high-throughput assay for identifying compounds, peptides and siRNA that cause morphological changes in model cells. New drugs can be identified and validated by quantifying changes of cell shape, cell death, organelles, and proteins labeled with fluorescent probes from microscope images of control cells and drug-treated cells. Have you ever experienced measurement results that show similar results to control cells even if you can observe morphological changes in drug treated cells, and being unable to numerically clarify the difference in morphological changes due to the effect of a drug? Have you ever had trouble selecting cell morphology parameters and analysis methods? In order to analyze differences in phenotypes resulting from the effect of a drug, it is necessary to consider the variability of control cells and the characteristics of the cells. This application note introduces an example wherein cell regions are identified using the General Analysis 3 optional image analysis module of the NIS-Elements imaging software, and multiple morphological parameters are used to analyze three cell types showing different morphologies, as well as how to select the optimal analysis method for each cell morphology.

Creation of a cell mask from a brightfield image (using *Homogeneous Area Detection*)

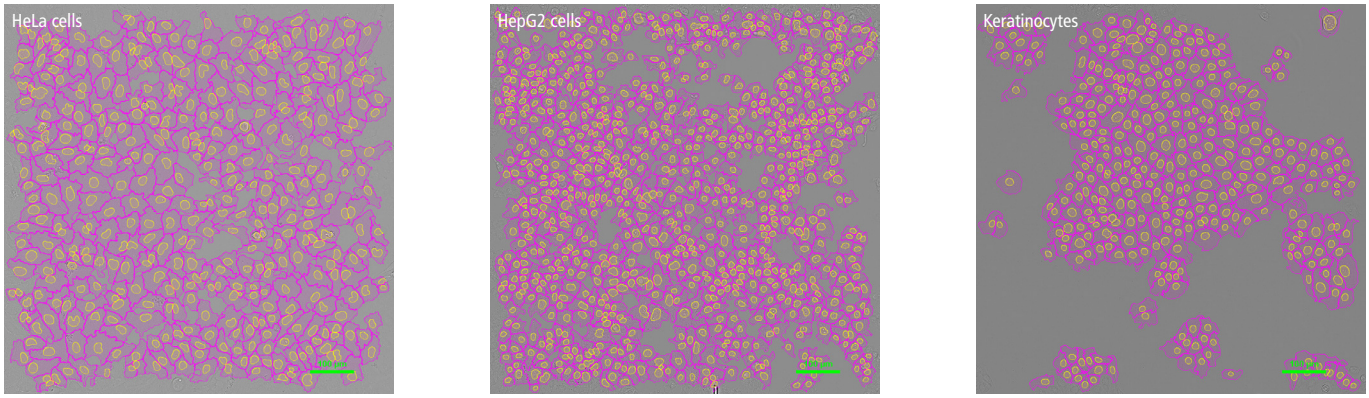

Fig.1: Brightfield images overlaid with cell masks (pink) and nucleus masks (yellow) Objective: 20X, Scale bar: 100 µm

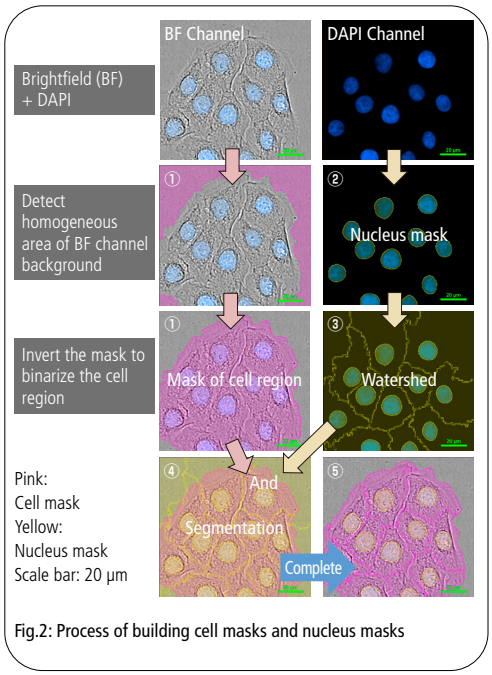

Fig.2: Process of building cell masks and nucleus masks

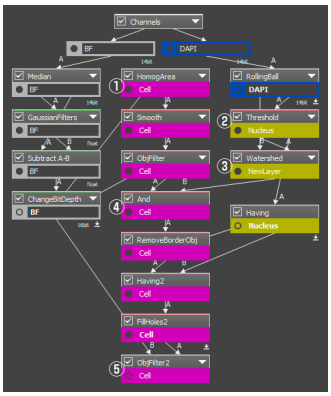

Fig.3: Building of analysis blocks in General Analysis 3

Homogeneous Area Detection of the General Analysis 3 image analysis module is a function for detecting the background homogeneous region of a brightfield image. Using this function, the background region of the brightfield image was binarized and inverted to create a mask of the cell region ①. The DAPI-stained nucleus region in the fluorescent channel image was binarized to create a nucleus mask ② and divided using the watershed function ③, then individual cells were segmented ④. Thus, a cell mask and nucleus mask were created ⑤. This allows effective use of fluorescent wavelengths for the analysis of organelles and specific proteins, rather than having to perform green or red fluorescent staining for only identifying cell regions.

Methods

HeLa cells, HepG2 cells, and keratinocytes seeded and cultured on a 96-well plate were fixed and nucleus-stained with DAPI. Brightfield and DAPI images were captured using the Ti2-E inverted microscope, DS-Qi2 monochrome camera, D-LEDI fluorescence light source, and a 20X objective, and a cell morphology analysis recipe was created with General Analysis 3 of NIS-Elements. Image analysis was performed on BatchGA3 and graphs were created in Microsoft Excel®.

Results

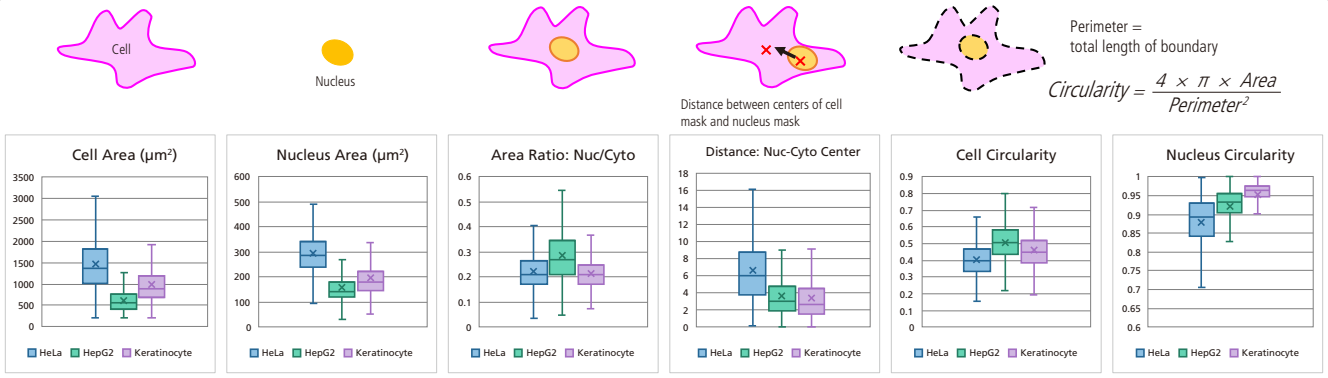

Fig.4: Cell morphology parameters and analysis results

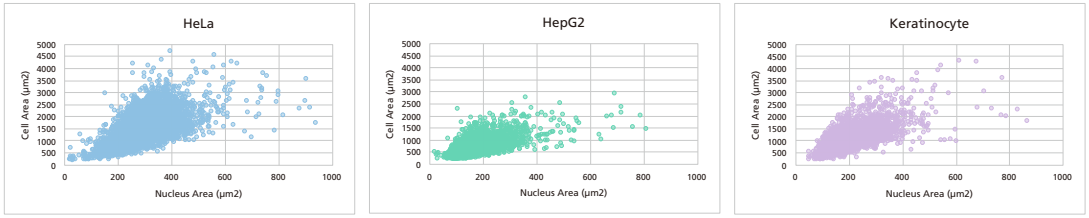

Fig.5: Scatter plot
X-axis: Nucleus area,
Y-axis: Cell area
Total number of analyzed cells:
HeLa: 4,735 cells
HepG2: 4,826 cells
Keratinocyte: 4,717 cells

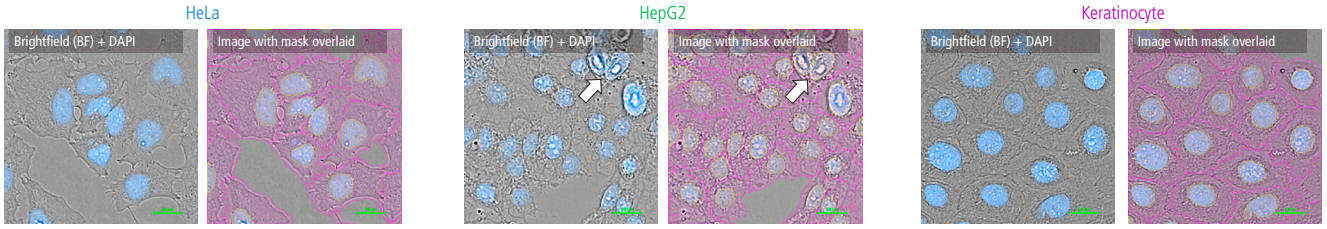

Fig.6: Enlarged images and features of regions showing typical cell morphology

Scale bar: 20 μm

Characteristics of cell morphology and selection of image analysis methods

Table.1: Comparison of cell morphology

	Lamellipodium	Cell boundaries	Size of cytoplasm and nucleus	Shape of nucleus	Area ratio of nuclei/cytoplasm	Location of nuclei	Morphology
HeLa	Large	Unclear	Large	Ellipse	Minor variability	Major variability	Irregular
HepG2	Small	Unclear	Small	Round	High ratio of nucleus area	Internuclear distance is close	Irregular
Keratinocyte	Small	Clear	Middle	Round	Minor variability	Minor variability Located in the center of cytoplasm	Regular

HeLa cells spread flat lamellipodia, and their individual cell areas have large variability (Fig.4, Fig.5). The lamellipodia region has low contrast, so it is difficult to detect the pericellular region with high accuracy. The cell area of HeLa cells is small in regions with high cell density and large in regions with low cell density [Ref.1]. Therefore, when the confluency of control cells is high at the endpoint of drug effect evaluation, it is difficult to clarify the difference between the phenotypes of control cells and drug treated cells using cell area as the parameter. If you use cell area as the parameter of a HeLa cell drug efficacy test, it is necessary to evaluate efficacy under the condition of relatively low cell density.

HepG2 cells have a high nucleus/cytoplasm area ratio (Fig. 4), and cells tend to aggregate (Fig. 6, white arrow), so nucleus analysis is difficult. The boundaries between cells is unclear, but the edges between the background region and the cell region are clear, so quantitative analysis of the total cell area in the field of view is easy (Fig.6).

Keratinocytes have a short distance between the center of the nucleus and that of the cell, and the areas of the cell and the nucleus are slightly large (Fig. 4). The nucleus circularity is high and the variability in cell morphology is very low (Fig. 4). Since the distance between the nuclei of adjacent cells is long, morphological analysis of the nucleus is easy (Fig. 6).

Summary

Table 2: Comparison of ease of image analysis

	HeLa	HepG2	Keratinocyte
Cell count, nucleus morphological analysis	Good	Fair	Excellent
Cell area, cell morphological analysis	Poor	Good	Excellent

- Using the Homogeneous Area Detection function, it is possible to generate cell masks from brightfield images and perform cell morphology analysis.
- By evaluating the variability of control cells, the optimum analysis parameters can be selected.

Reference

1. Application Note "Low Phototoxicity Long-Term Live Cell Apoptosis Assay Using Label-Free Live Cell Imaging" SW_app_VC_e_08

Product Information

General Analysis 3 optional image analysis module of NIS-Elements imaging software

By combining analysis blocks, cell regions can be easily binarized and measured. This allows flexible image analysis according to your purpose.

CFI Plan Apochromat Lambda D 20X

Images with uniform brightness and sharpness right up to the edge of the large 25 mm field of view can be obtained. Since deterioration in light intensity at the image periphery is minimal, highly reliable results can be obtained during quantitative analysis.