APPLICATION NOTE

**ECLIPSE Ti2-E Inverted Microscope**
***NIS.ai* AI Module for Microscopes (Convert.ai)**

# Label-Free Morphological Analysis Based on Deep Learning Using Primary Cells

Conventionally, immortalized cell lines that proliferate indefinitely have been used for *in vitro* efficacy and pharmacological testing as well as safety tests during drug discovery. However, it has been found that established cell lines have accumulated mutations in the genes of normal cells and have lost the properties of the original tissue. Therefore, there is a need for *in vitro* studies using normal cells that reflect the nature of the original tissue and patient-derived primary cells. Nonetheless, since normal cells divide only a limited number of times, it is difficult to obtain sufficient cell numbers for cell-based assays. In recent years, it has become possible to analyze the morphology of live cells without labeling by means of image processing technology that utilizes deep learning, enabling the acquisition of a large amount of information from a small number of cells. This application note introduces an example that verifies that a digitally stained image generated from brightfield images of keratinocytes, which are human epidermal cells, using Convert.ai of NIS.ai is more effective at distinguishing cell regions than fluorescence images, and enables label-free morphological analysis of cells.

Keywords: Artificial intelligence, deep learning, digital stain, label-free, image analysis, cell morphology analysis, drug discovery

## Overview of the Experiment

After keratinocytes were fixed, their nuclei were fluorescently stained with Hoechst33342, and their tubulin stained with fluorescent antibodies. Brightfield images and fluorescence images (nuclei = Ex/Em 385/460, tubulin = Ex/Em 621/697) were captured at 36 points using the Ti2-E inverted microscope, DS-Qi2 monochrome camera, D-LEDI fluorescence illuminator and a 20X objective. Using the brightfield images as source images and the fluorescence images as ground truth images, 28 images were used for training to build a trained model (Fig. 1). The trained model was applied to the eight images that were not used for training to create inference (digitally stained) images (Fig. 1). A recipe to binarize the nucleus and cell regions was created with GA3, by defining areas where an intensity value of 5,000 or more in the blue fluorescence channel (Hoechst) as nuclear regions, and areas where an intensity value of 2,000 or more in the red fluorescence channel (tubulin) as cell regions (Fig. 2). The same GA3 recipe was applied to both the fluorescence and digitally stained images to create a nuclear mask and cellular mask. The accuracy of the recipe was verified by comparing the ground truth fluorescence images and the results of cell morphology analysis of the AI-inferred images (Fig. 3, Fig. 4).

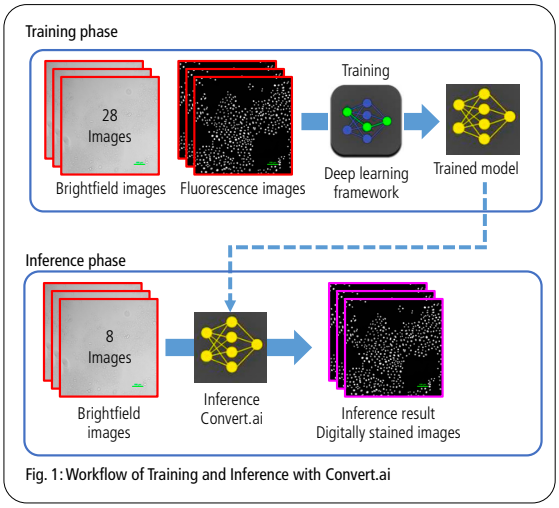


Fig. 1: Workflow of Training and Inference with Convert.ai

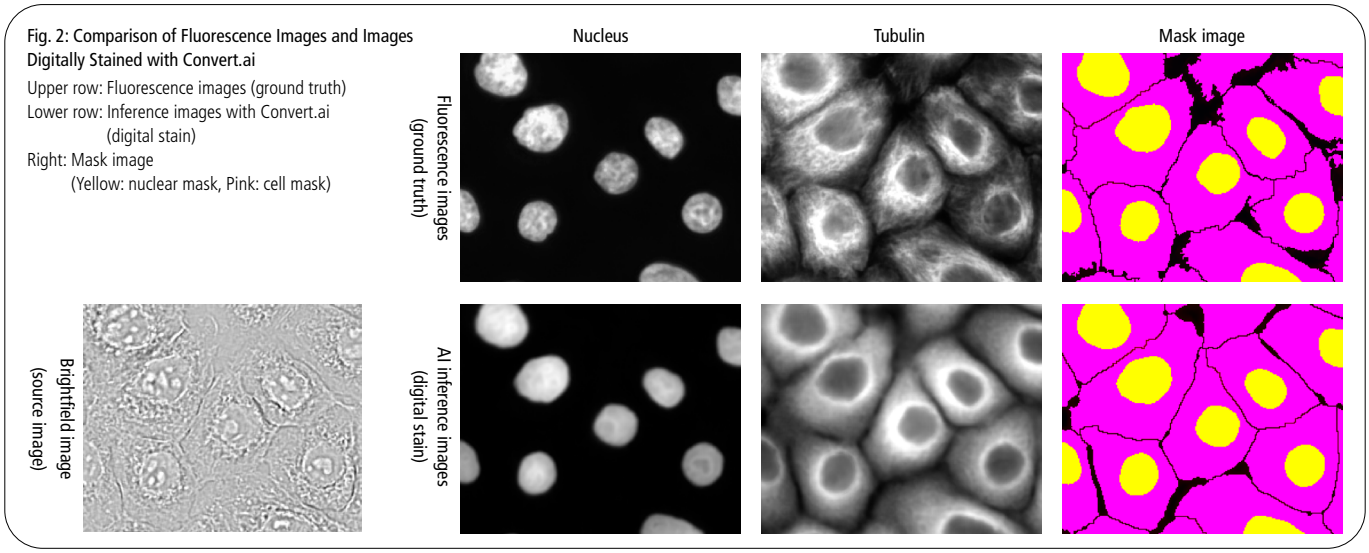


Fig. 2: Comparison of Fluorescence Images and Images Digitally Stained with Convert.ai
Upper row: Fluorescence images (ground truth)
Lower row: Inference images with Convert.ai (digital stain)
Right: Mask image
(Yellow: nuclear mask, Pink: cell mask)

## Result

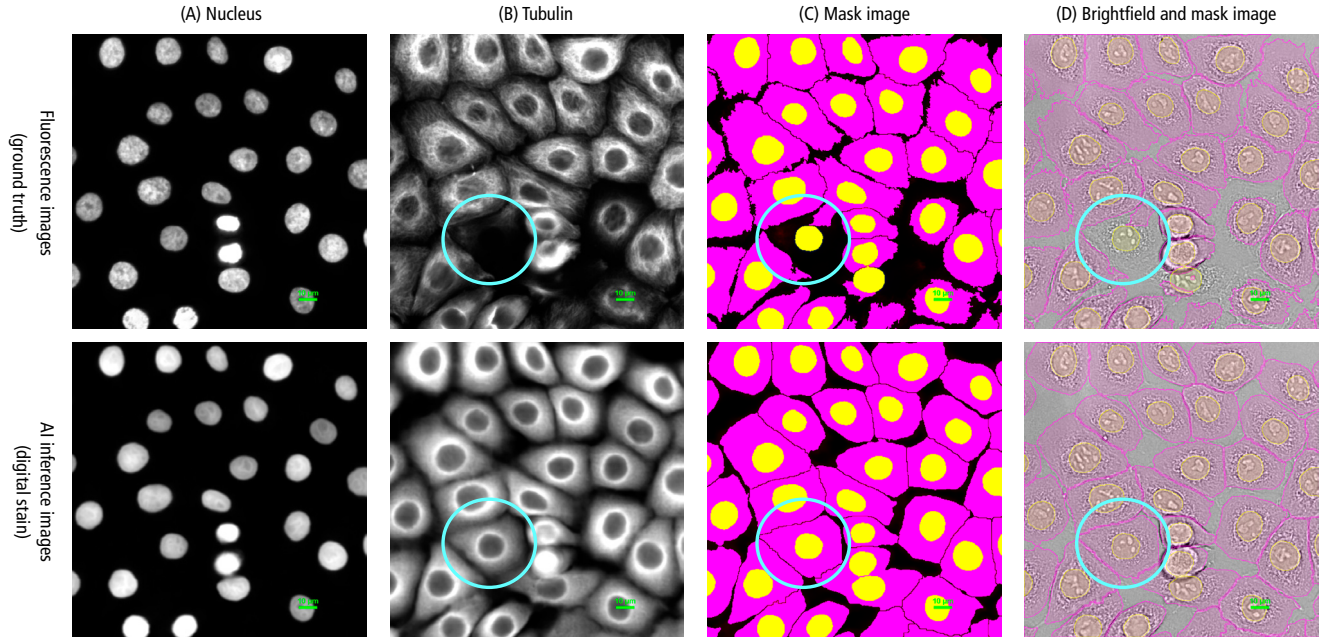


Fig. 3: Comparison of Fluorescence Images and Images Digitally Stained with Convert.ai

From left: (A): nucleus, (B): tubulin, (C): mask image (yellow: nuclear mask, pink: cell mask), (D): brightfield image overlaid with mask image
Upper row: fluorescence images (ground truth), lower row: inference image with Convert.ai (digital stain), scale bar: 10 µm

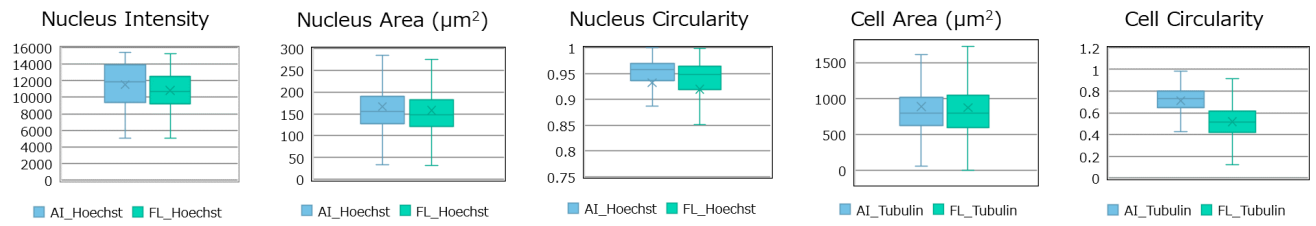


Fig. 4: Comparison of Cell Morphology Analysis Results Obtained by Means of Fluorescence Images and Images Digitally Stained with Convert.ai

Boxplot of analyzed results from about 5,800 cells. Blue: analyzed results of inference image using Convert.ai, green: analyzed results of fluorescence image

Through analysis of about 5,800 cells, it was found that the area and circularity of nuclei obtained using AI inference images were similar to those obtained from the ground truth fluorescence images, indicating that highly accurate morphological analysis of the nucleus was possible using digitally stained images (Fig. 4). However, the fluorescence intensity of the nuclei could not be visualized accurately with a digitally stained image using Convert.ai (Fig. 3-A, Fig. 4). Also, inference using Convert.ai from a brightfield image could not visualize the fine fibrous structure of filamentous tubulin (Fig. 3-B). On the other hand, the AI inference image could generate smoother images over the entire cell area than fluorescence images, enabling segmentation along cell contours and more accurately binarizing cell area and circularity than fluorescence images (Fig. 3-D, Fig. 4). The digitally stained images that inferred cell area from brightfield images could visualize cell regions even for cells (light blue circled area) that did not express tubulin (Fig. 3-D).

*Fluorescent antibody staining for actin and the small molecule compound CellMask™ Deep Red are also widely used to generate training data for cell area detection and digital staining. Please select the appropriate reagent for your cells.

| | AI inference image | Fluorescence image |
|---|---|---|
| Features | Identification of nuclei and cell region, cell morphology analysis, low phototoxicity | Quantitative analysis of specific molecules and proteins, phototoxicity |
| Nuclear mask | ★★★★ | ★ |
| Cell mask | ★★★★★ | ★★★ |
| Fluorescence intensity | N/A | ★★★★★ |
| Organelle | ★ | ★★★★★ |

Table 1: Suitability for Quantitative Analysis in Live Cell Imaging

Low phototoxicity label-free cell analysis with Convert.ai is suitable for the morphological analysis of nuclear and cell regions. Quantitative analysis by conventional fluorescence staining is suitable for the analysis of fluorescence intensity and morphological analysis of organelles.

## Summary

- Nuclear and cellular regions can be identified from a digitally stained image generated by Convert.ai, enabling label-free morphological analysis of cells.
- Since using Convert.ai allows recording of changes over time with live cells, more information can be obtained from fewer cells, enabling cost reduction.
- Individual cells can be segmented by specifying nuclear and cell regions from the image inferred by Convert.ai. Therefore, high content imaging of live cells with minimal fluorescence excitation and low phototoxicity is possible, in combination with quantitative analysis of fluorescence intensity and proteins using conventional fluorescent staining.
- Label-free cell morphology analysis with live cell imaging does not require cell fixation and dyeing, enabling improvement of operating efficiency.

## Product Information

### *NIS.ai* AI Module for Microscopes (Convert.ai)

The NIS.ai module of NIS-Elements AR imaging software can improve image processing and analysis workflows using deep learning. Convert.ai of NIS.ai can distinguish nuclear regions without dyeing, enabling quantitative analysis in live cells, a process which is difficult using conventional methods.