APPLICATION NOTE

NIS.ai AI module for microscopes

Digital Staining for Cell-Cycle Analysis through a Combination of Fucci and NIS.ai.

Chemical staining is very common for microscopic observation of cell samples. However, staining living cells with chemical dyes even temporarily perturbs cell physiology and is not applicable to vulnerable cells with assured quality for clinical use. For this reason, in recent years, an increasing number of machine learning techniques that detect specific structures and proteins from unstained samples have been developed (Reference 1). A clear understanding of cell cycle regulation is crucial for many therapeutic studies that use stem cells, and from a medical perspective, in implantation of human-induced pluripotent stem cell-derived tissues, for example, a reliable and non-invasive method for monitoring the cell cycle in an unstained manner is really required (Reference 2, 3). This application note introduces a digital staining technique based on the NIS.ai module and Fucci technology that allows researchers to infer cell cycle phases from unstained images, such as phase contrast images, and classify them into 5 phases: G1, G1/S, late S, G2, and M. This work was done with the cooperation of Dr. Atsushi Miyawaki and Dr. Asako Sakaue-Sawano of the Laboratory for Cell Function Dynamics, RIKEN Center for Brain Science.

Experiment Overview

An AI model for inferring the cell cycle from unstained images was constructed by using the NIS.ai microscope AI module. NIS.ai is a deep learning technology based on neural networks (Fig. 1). One of its functions, Convert.ai, is expected to infer the pattern of fluorescence signals from unstained images to generate digitally stained images. Fucci technology in principle harnesses the cell-cycle-dependent degradation of human Cdt1 and Geminin, and is currently composed of two variants with different cell-cycle-resolving capabilities: Fucci(SA) and Fucci(CA). While Fucci(SA) highlights the cell-cycle transition from G1 to S phase with high color contrast, Fucci(CA) distinguishes interphase boundaries between G1, S, and G2 phases (Reference 4, 5). In this study, Fucci(SA)5 and Fucci(CA)5 were used; the suffix "5" indicates that a red-emitting fluorescent protein (AzaleaB5) and a green-emitting fluorescent protein (h2-3) are used for fluorescence labeling (Reference 6). HeLa cells constitutively expressing Fucci(SA)5 and Fucci(CA)5 were imaged separately, but their AI models were combined later for more effectively dissecting phases within an interphase.

Training

Fluorescence images were obtained from HeLa cells that expressed Fucci(CA)5 or Fucci(SA)5 and used as training images. Convert.ai was trained to output digitally stained images of Fucci according to input images, which were acquired mostly by phase contrast microscopy. Trained models based on Fucci(CA)5 and Fucci(SA)5 are called Convert.ai(CA) and Convert.ai(SA), respectively.

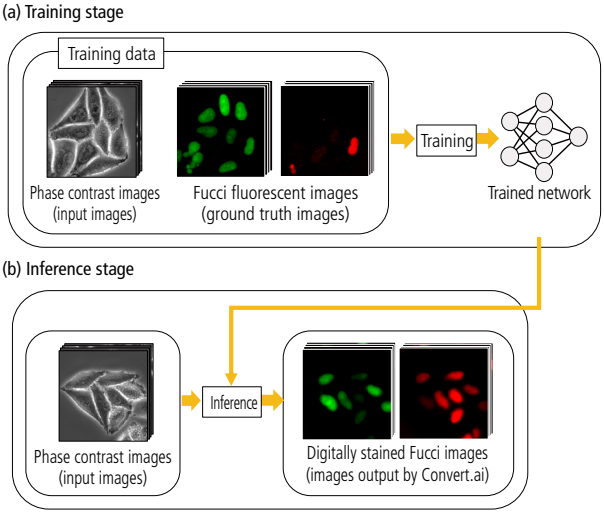

Fig. 1: Overview of AI model for digital staining

Convert.ai consists of a training stage (a) and an inference stage (b). Multiple fluorescence images of HeLa/Fucci cells were used for training data.

(a) In the training stage, 1000 images were prepared. The training images are a set of Fucci-green and -red fluorescence images and phase contrast images.

(b) In the inference stage, a new phase contrast image is input to the trained Convert.ai, which outputs a digitally stained Fucci image. The trained Convert.ai can be repeatedly applied to new datasets.

Cells:
- HeLa/Fucci(CA)5: RCB4919, RIKEN BioResource Research Center
- HeLa/Fucci(SA)5: RCB4917, RIKEN BioResource Research Center

Microscope: Eclipse Ti2-E
Objective: CFI S Plan Fluor ELWD ADM 20XC
CMOS camera: ORCA-Flash4.0 V3 (Hamamatsu Photonics)
Image acquisition: 40-hour time-lapse imaging at 30-minute intervals in 60 locations
AI training condition: 3,000 iterations with 1,000 sets of images

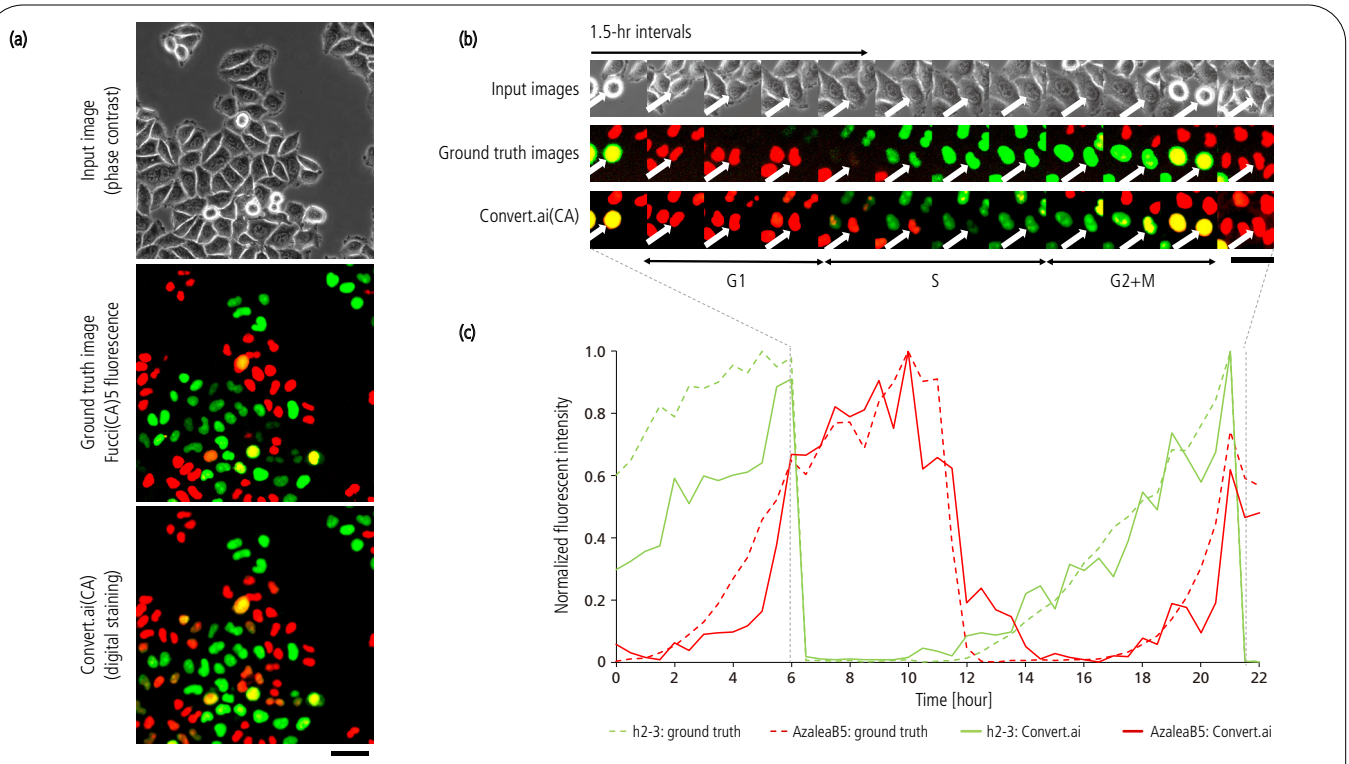

Fig. 2: Generation of digitally stained Fucci(CA)5 images with Convert.ai(CA)

(a) Comparison of the digitally stained image by Convert.ai(CA) and a ground truth image. Input image: phase contrast image input to trained Convert.ai(CA). Scale bar: 50 µm.

(b) An example of cell cycle phase inference for phase contrast images from a series of time-lapse imaging data. Each image is extracted and arranged at 1.5-hour intervals. Scale bar: 50 µm.

(c) Temporal profiles of h2-3 (green) and AzaleaB5 (red) fluorescence intensity in cell indicated by arrowhead in (b). Dotted lines indicate data from ground truth and solid line indicates data from digital staining. The tracking function of the NIS-Elements software and a digital staining model for cell nucleus detection were used for cell tracking. Refer to Application Note "Highly accurate and non-invasive cell counts utilizing machine learning" for the digital staining model used for cell nucleus detection.

Conversion of phase contrast images into digitally stained Fucci images

By use of a completely new input, i.e., training-unrelated phase contrast images, Convert.ai(CA) made correct inference to successfully create an output, a digitally stained image of Fucci(CA)5. The performance was explicitly confirmed by matching with a ground truth (Fig. 2a). Cell-cycle related transitions of fluorescence intensity typical of Fucci were identified in the output images, indicating the capability of the trained Convert.ai to infer the cell cycle (Fig. 2b, 2c).

Population analysis of digitally stained Fucci images

The cell-cycle phasing performance of Convert.ai(CA) was statistically examined by comparing the cell cycle profile with that of the ground truth (Fig. 3). Individual cells were plotted as green (h2-3) versus red (AzaleaB5) fluorescence intensities of digitally stained images (Fig. 3a, right). Some representative cells identified to be in G1, S, and G2+M phases in the ground truth profile (Fig. 3a, left) were localized in the Convert.ai(CA) profile (Fig. 3a, right), and substantial matching was confirmed between the two cell cycle profiles. Also, the relative population size of cells in G1, S, and G2+M phases was nearly the same in both the ground truth and Convert.ai(CA) (Fig. 3b).

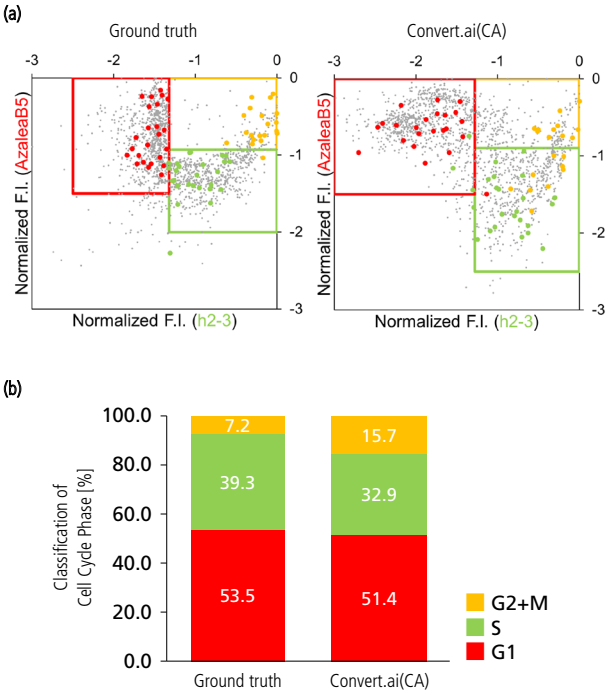

Fig. 3: Classification analysis of the cell cycle using digitally stained Fucci(CA)5 images

(a) Scatter plots of h2-3 (green) and AzaleaB5 (red) fluorescence intensity values in individual cells. The axes are logarithmic. The left panel shows data from a ground truth (Fucci(CA)5) and the right shows data from digital staining (Convert.ai(CA)). The G1 phase is shown in the red frame, the S phase is shown in the green frame, and the G2+M phases are shown in the yellow frame. Typical cells from each cell cycle phase in the ground truth data are annotated, and the corresponding data in the digital staining images are plotted.

(b) Population analysis for the cell groups defined and classified in (a).

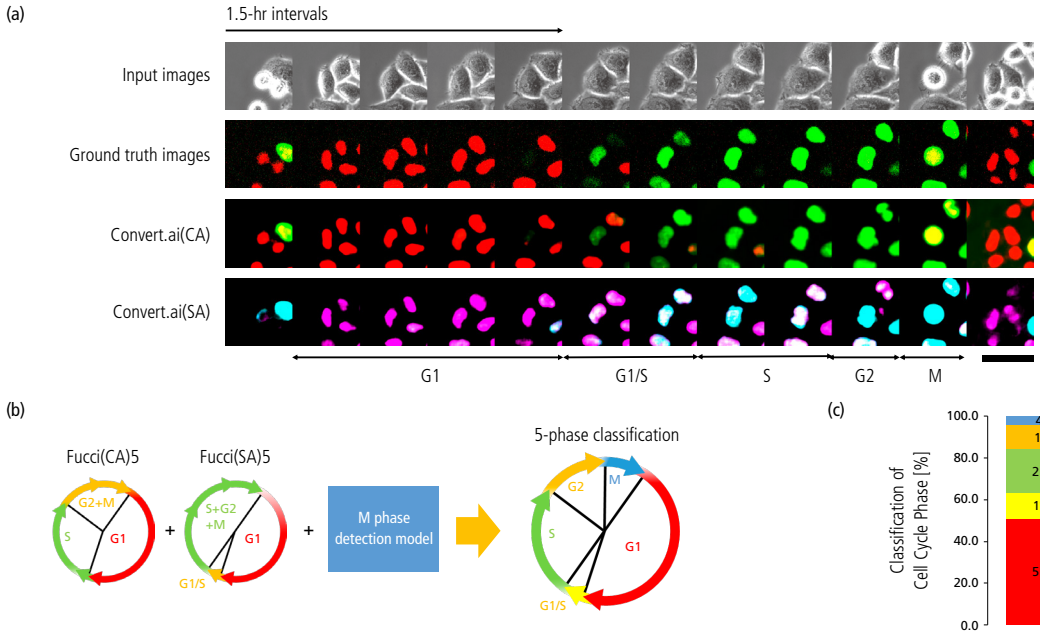

Fig. 4: 5-phase classification of the cell cycle

(a) Generation of digitally stained Fucci(CA)5 and Fucci(SA)5 images with each Convert.ai function. HeLa/Fucci(CA)5 cells were used for input images. Each image is extracted and arranged at 1.5-hour intervals. Scale bar: 50 µm.

(b) Illustration of a classification analysis of the 5 phases of a cell cycle. Each cell cycle phase of an individual cell can be identified by applying the Fucci(CA)5 and Fucci(SA)5 digital staining models, and M phase detection model (Segment.ai) to the phase contrast image.

(c) Population analysis for the cells classified by method (b) for each cell cycle phase.

Further resolving cell cycle phases

A combination of Convert.ai(SA) with Convert.ai(CA) was found to dissect an interphase into four phases: G1, G1/S, late S, and G2. The remaining phase, M, involves a drastic change in morphology and is easily identifiable by a phase contrast image-based Segment.ai model. The assembly of NIS.ai models enables 5-phase classification of the cell cycle (Fig. 4), enabling researchers to analyze cell cycle regulations in a quantitative and comprehensive manner.

Summary

With the help of Fucci technology, NIS.ai makes it possible to infer and classify each phase of the cell cycle simply by use of a phase contrast image as an input. This is expected to be a useful tool in the field of regenerative medicine, which requires living cell samples be evaluated without staining.

This experiment was jointly conducted by RIKEN, the Institute of Physical and Chemical Research, and the System Development Department of Nikon Corporation.

References

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Product information

NIS.ai AI module for microscopes

The NIS.ai image processing/analysis module, which extends the NIS-Elements imaging software, contains three AI modules: Enhance.ai, Convert.ai, and Segment.ai.

Convert.ai, which was used in the experiment described in this application note, can learn to generate a digitally stained image that approximates a fluorescent image from unstained cell images such as phase contrast and differential interference contrast images. Since it enables long-term time-lapse imaging without fluorescent staining, it enables minimally invasive analysis with less damage from excitation light.

Segment.ai can learn to segment only the target object. It provides new solutions for cases in which target extraction is difficult with conventional binarization, and cases that require manual classification.