APPLICATION NOTE

NIS.ai AI Module for Microscopes (Segment.ai)

Highly accurate segmentation of cell areas based on DIC images using deep learning

Quantification of cell migration and cell confluency is important in biological and medical research on cellular functions. A scratch assay is used to quantitatively measure the speed of cells migrating to a cell-free area (gap) that is physically produced. This is a common technique for evaluating cell migration in cell development and differentiation, as well as in the invasion and metastasis of cancer cells. However, manually processing unstained sample images for quantification takes an immense amount of time. In addition, the Wound Healing function, a special application for scratch assays in the NIS-Elements imaging software, shows roughly correct results, but it has limited accuracy in terms of detailed detection.
In this Application Note, we introduce examples of quantification of a scratch assay using the NIS.ai module of NIS-Elements. These examples proved that NIS.ai can make more accurate inferences compared to the existing Wound Healing function based on a small number of training images.

Materials

Cells and reagents

- HeLa cells
- Minimum Essential Medium Eagle With Earle's salts (Sigma-Aldrich, M4655)
- FluoroBrite™ DMEM (Thermo Fisher Scientific, A1896701)
- Fetal Bovine Serum, qualified, USDA-approved regions (Thermo Fisher Scientific, 10437028)
- L-Glutamine (200mM) (Thermo Fisher Scientific, 25030081)
- Penicillin-Streptomycin (10,000 U/mL) (Thermo Fisher Scientific, 15140122)
- Trypsin-EDTA (0.25%), phenol red (Thermo Fisher Scientific, 25200072)
- DPBS, no calcium, no magnesium (Thermo Fisher Scientific, 14190144)
- EZVIEW®CulturePlateLB (AGCTECHNO GLASS, 5816-006)
- Platinum Soft Filter Tips - 10µL (BMBio, WF-10RS)

Observation devices and software

- Microscope: Ti2-E (Nikon)
- Objective: CFI S Plan Fluor ELWD 20xC, NA 0.45 (Nikon)
- CMOS: ORCA Flash4.0 V3 (Hamamatsu)
- Imaging software: NIS-Elements (Nikon)
- Stage Top Incubator: STX series (TOKAI HIT)

Methods

HeLa cells were seeded into a 6-well plate at a confluency of around 70%, and cultured for approximately one day. A 10µL pipette tip for micropipetting was used to scratch a linear wound through these cells. The cells were washed in DMEM three times.
DIC images (16-bit, 2048×2044 pixels) of the field of view including the scratched region were captured in 10-minute intervals for 32 hours while maintaining the environment at 37°C, 5% CO_2 using a Stage Top Incubator.
As training data, 16 images with varying cell densities of a field of view different from that for the data for inference were selected from time-lapse images and used. To save labor in annotation, Wound Healing, an existing application of NIS-Elements, was used to roughly create binary data, which was then modified manually (Fig. 1). The Segment.ai function of NIS.ai was used to create a model by learning from the training data 1000 times, and inferences were made.
The areas [µm^2] of the scratched regions were derived from segmented images with NIS.ai and segmented images with Wound Healing only. The ratio of each of these areas to the area of the scratched region on the manually created ground truth images was calculated and compared.

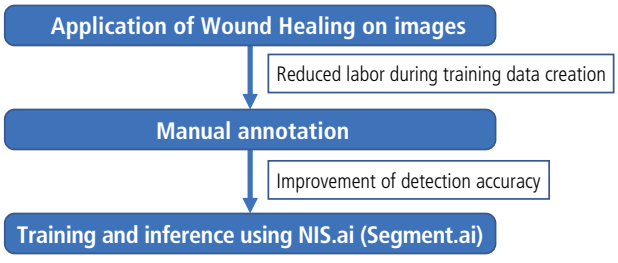

Fig. 1
Workflow for creating training data and purpose of each process

Results

It was shown that NIS.ai could detect the boundaries of the scratched region more accurately than Wound Healing (Fig. 2, a and b). In inference and measurement with long-term time-lapse images, the results using NIS.ai achieved a 99.69% match with the ground-truth results on average, while the results using Wound Healing fluctuated significantly. This confirmed that NIS.ai could be used for accurate quantification of scratch assays (Fig. 3, a and b). It also confirmed that NIS.ai enables accurate detection even at time points where the results using Wound Healing showed a significant error (Fig. 3, c, d and e).

Summary

The advantages of image processing using NIS.ai are as follows.

- Allows segmentation based on unstained images with no phototoxicity.
- Allows a trained model to be established with less training data.
- Allows more accurate segmentation compared to existing image processing.
- Effective for a large number of images such as long-term time-lapse images.
- Allows labor-saving creation of training data in combination with Wound Healing.

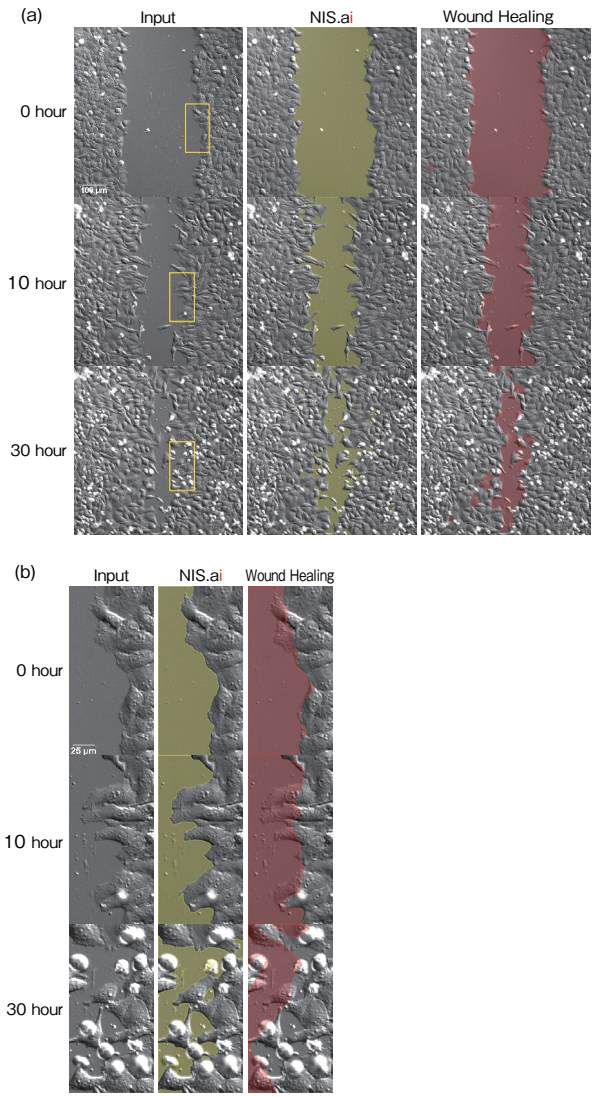

Fig. 2
(a) Results of detection of the scratched region at 0th, 10th, and 30th hours
(b) Enlarged views of the yellow rectangle regions in the Input image

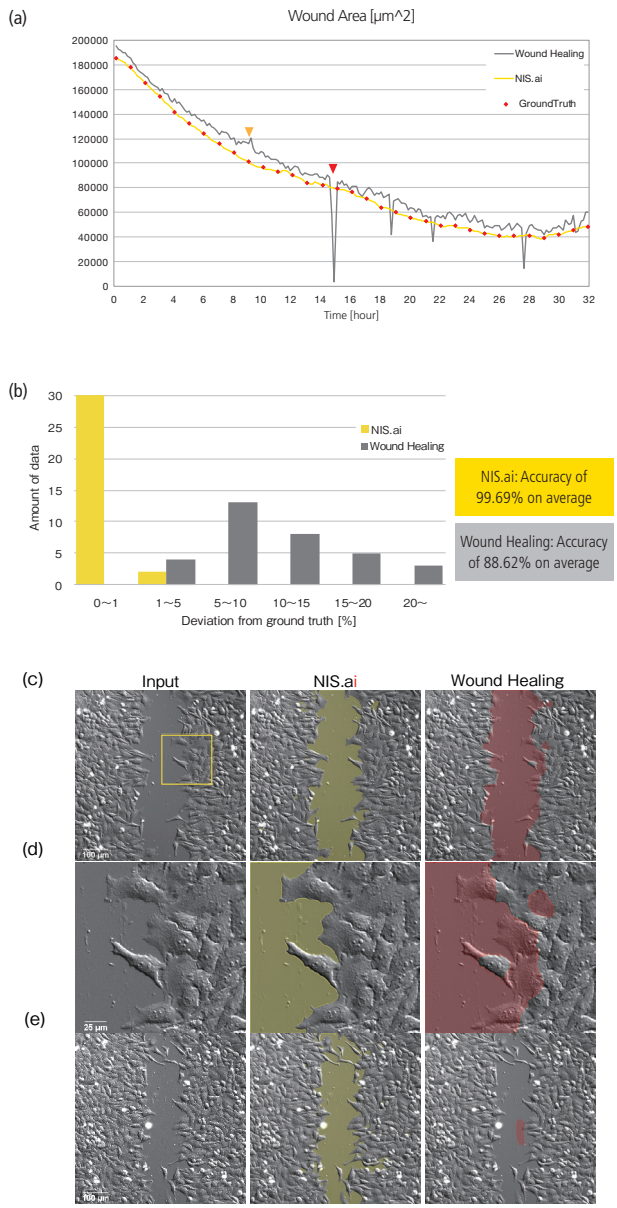

Fig. 3
(a) Change in the scratched area over time
—: NIS.ai (Segment.ai), —: Wound Healing, ◆: Ground truth masked manually (33 points created at one hour intervals). ▼: Time point with a significant (>20%) difference between results using NIS.ai and Wound Healing, ▼: Time point where the results using Wound Healing produced a significant error
(b) Deviation of the quantification using NIS.ai/Wound Healing from quantification using ground-truth data
(c) Area detected at the time point indicated by the ▼ in Graph (a)
(d) Enlarged views of the yellow rectangle regions in the Input image
(e) Area detected at the time point indicated by the ▼ in Graph (a)

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Product Information

NIS.ai AI Module for Microscopes (Segment.ai)

The NIS.ai module of NIS-Elements imaging software utilizes deep learning to learn from training data, improving image processing and analysis workflows. The Segment.ai function of NIS.ai can be used to easily extract target cells from differential interference or phase contrast images containing a mixture of cells with different shapes, enabling quantitative measurement.