APPLICATION NOTE

NIS.ai AI module for microscopes (Enhance.ai)

Reduction of phototoxicity and clarification of fluorescent images by Enhance.ai with high S/N technology using AI

For a detailed study of subcellular events, time-lapse imaging using a fluorescent microscope is indispensable; however, for fluorescent observation, the phototoxic effects of excitation light are a concern. For this reason, technology that enables accurate quantitative analysis while preventing photobleaching and cell damage, using the lowest excitation light intensity or shortest exposure time possible, is required.
Mr. Kentaro Kobayashi, from the Research Institute for Electronic Science at Hokkaido University, reduced the phototoxicity of excitation light and evaluated the results based on a quantification of the length of mitochondria in a cell using Enhance.ai, one of the NIS.ai AI modules for the NIS-Elements imaging software. This application note introduces experimental examples.

Issues of existing fluorescent time-lapse observation

As fluorescent observation, especially time-lapse observation, can visualize the ever-changing details in a cell in real time, it is an integral technology for the elucidation of subcellular events. The development of microscope technology has also contributed to facilitating time-lapse observation. A superior video of fluorescent time-lapse images gives a great visual impression and even attracts those who are not familiar with biology.
However, for clear fluorescent imaging, it is necessary to obtain sufficient fluorescent signals, which requires irradiation of strong excitation light or long exposure times. Photobleaching of fluorescent dyes and the effects of phototoxicity caused by the production of active oxygen species cannot be prevented, due to repetitive irradiation by excitation light. As a result, the reliability of quantitative evaluation declines considerably with the decrease in fluorescent brightness, or the biological phenomenon being observed is affected by cell damage[1,2]. Moreover, a target may move during a long exposure time, making a clear image unobtainable.
For these reasons, often in past time-lapse observations, image data gradually darkened due to photobleaching, or only dark and unclear fluorescent images were obtained.

Enhance.ai can obtain a clear image while reducing noise

Enhance.ai, developed by Nikon, is a new image processing function using deep learning. It makes it possible to generate a bright fluorescent image from a dark fluorescent image by training the network in advance with pairs of dark fluorescent images and bright fluorescent images (Figure 1. (a)).

Generally, if a dark fluorescent image is enhanced by contrast adjustment, noise is also enhanced and the image becomes unclear because the difference between fluorescent signals and noise is small; however, using Enhance.ai, an image with a high S/N ratio can be obtained. In the image obtained by enhancing the contrast of the original dark fluorescent image, noise is also enhanced and the image cannot be observed well, but in the image processed by Enhance.ai, only the fluorescent signals are enhanced and individual mitochondria can be clearly observed (Figure 1. (b)).

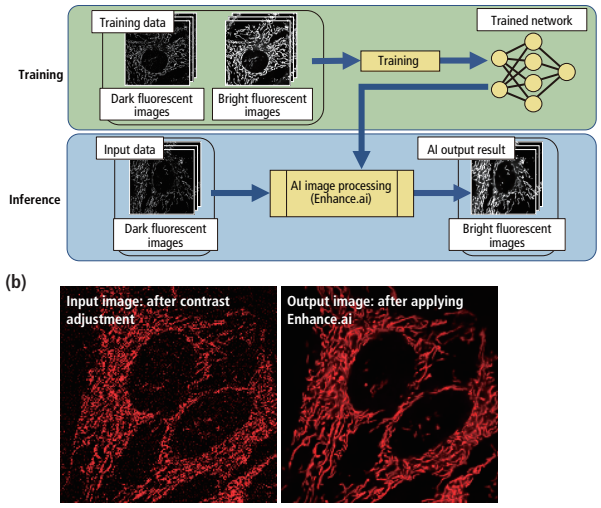

Figure 1.
(a): Workflow of training and inference using Enhance.ai. A bright image is generated by applying Enhance.ai to an image obtained by a low power laser. Enhance.ai training condition: a training model is constructed using 20 pairs of fluorescent images of mitochondria respectively obtained by a high power laser and a low power laser.
(b): Input image for which only contrast is adjusted (left), output image obtained by applying Enhance.ai (right)

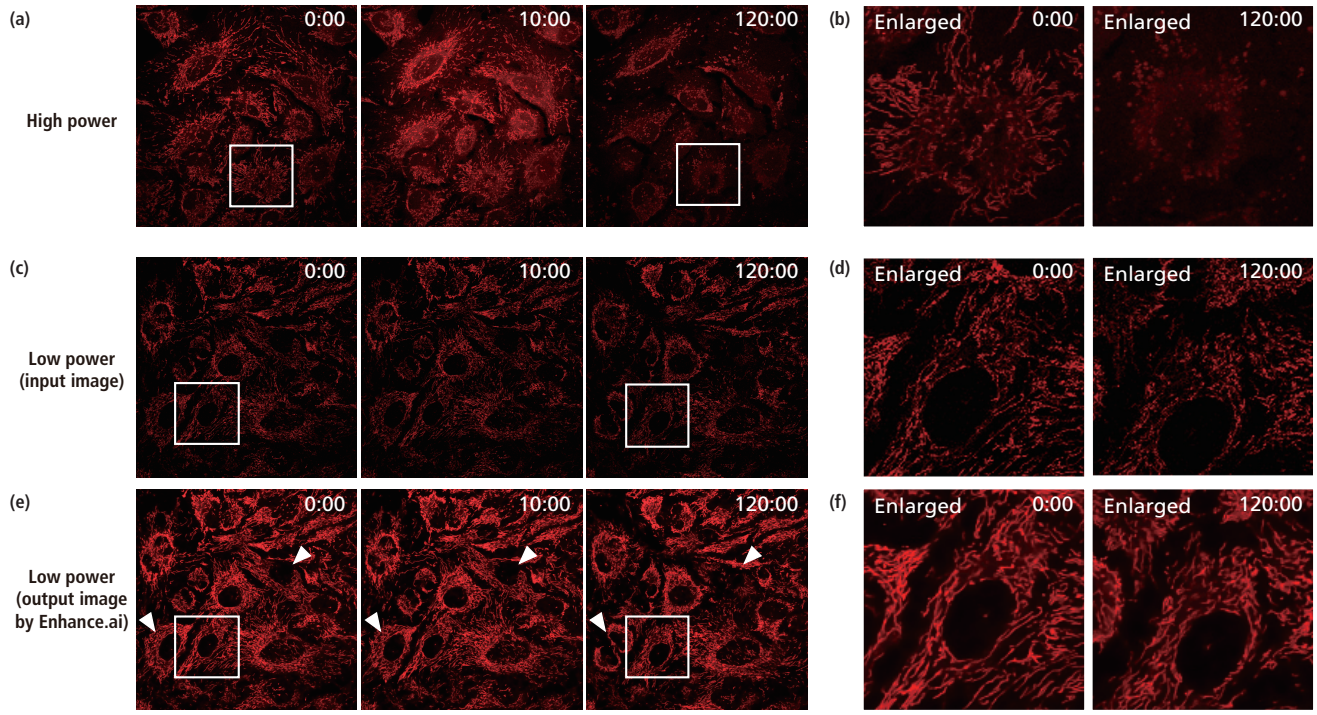

Figure 2
(a): Time-lapse images captured with high power laser at 0, 10, and 120 minutes
(c): Time-lapse images captured with low power laser at 0, 10, and 120 minutes
(e): Output image with image (c) input, and subjected to Enhance.ai, Δ is a dividing cell

(b)(d)(f): Enlarged images of ROI in images (a), (c), and (e)
Sample: HeLa cells labeled with MitoTracker™ Red CMXRos (Thermo Fisher Scientific, M7512)
Acquisition device: Ti-E inverted microscope + A1R si confocal system, CFI Apochromat Lambda S 60X Oil objective, STXG-WSKMX stage incubator (Tokai Hit Co. Ltd.)
Laser intensity: High 1.4%, Low 0.1%, imaging time: 120 minutes at 10-second intervals
Images courtesy of Mr. Kentaro Kobayashi, Research Institute for Electronic Science, Hokkaido University (images were obtained at Hokkaido University Nikon Imaging center)

Enhance.ai reduces cell damage during time-lapse imaging

Mitochondria produce ATP and are closely involved in supplying energy within cells. For this reason, temporal changes in form and behavior of mitochondria within cells can act as a barometer for checking the status of cells. Time-lapse imaging is an effective method for acquiring these phenomena.[3]

The important point in such cases is to eliminate the effects of phototoxicity due to excitation light as much as possible. During observation by way of relatively strong excitation light irradiation, it was confirmed that phototoxicity in mitochondria became noticeable from about 10 minutes after start of observation, and that fragmentation progressed after 120 minutes, leading to complete destruction.
This implies that the damage due to excitation light considerably affected the mitochondria and functioning of cells (Figure 2. (a-b)). On the other hand, during observation with minimum excitation light, individual mitochondria became very clear when Enhance.ai was applied, and fragmentation or photobleaching rarely occurred even after 120 minutes (Figure 2. (c-f)).
In addition, cell division was completed smoothly during the 120 minutes of observation, confirming maintenance of the same cell functions as those of a regular case (△ shown in Figure 2. (e)).

These results show that using Enhance.ai can minimize the effects of phototoxicity and photobleaching caused by excitation light, and enables the acquisition of clear images even during time-lapse imaging.

Evaluation of phototoxicity effect reduction by quantification of the length of mitochondria in cells

Next, temporal changes in mitochondria were quantitatively evaluated. When segmentation of mitochondria was performed using the General Analysis quantification function of NIS-Elements, mitochondria morphology was correctly acquired in images after Enhance.ai processing, making measurement possible (Figure 3. (a)).

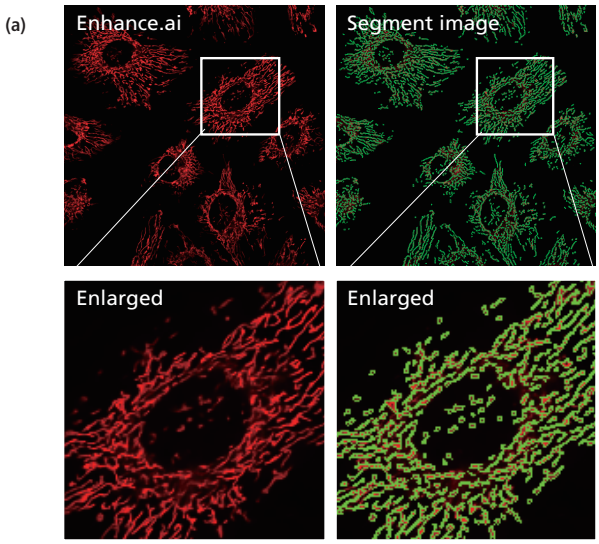

Figure 3
(a): Left column: Output result of Enhance.ai (upper) and enlarged image (lower), Right column: Binary mask (green) that detected mitochondria from the images on the left

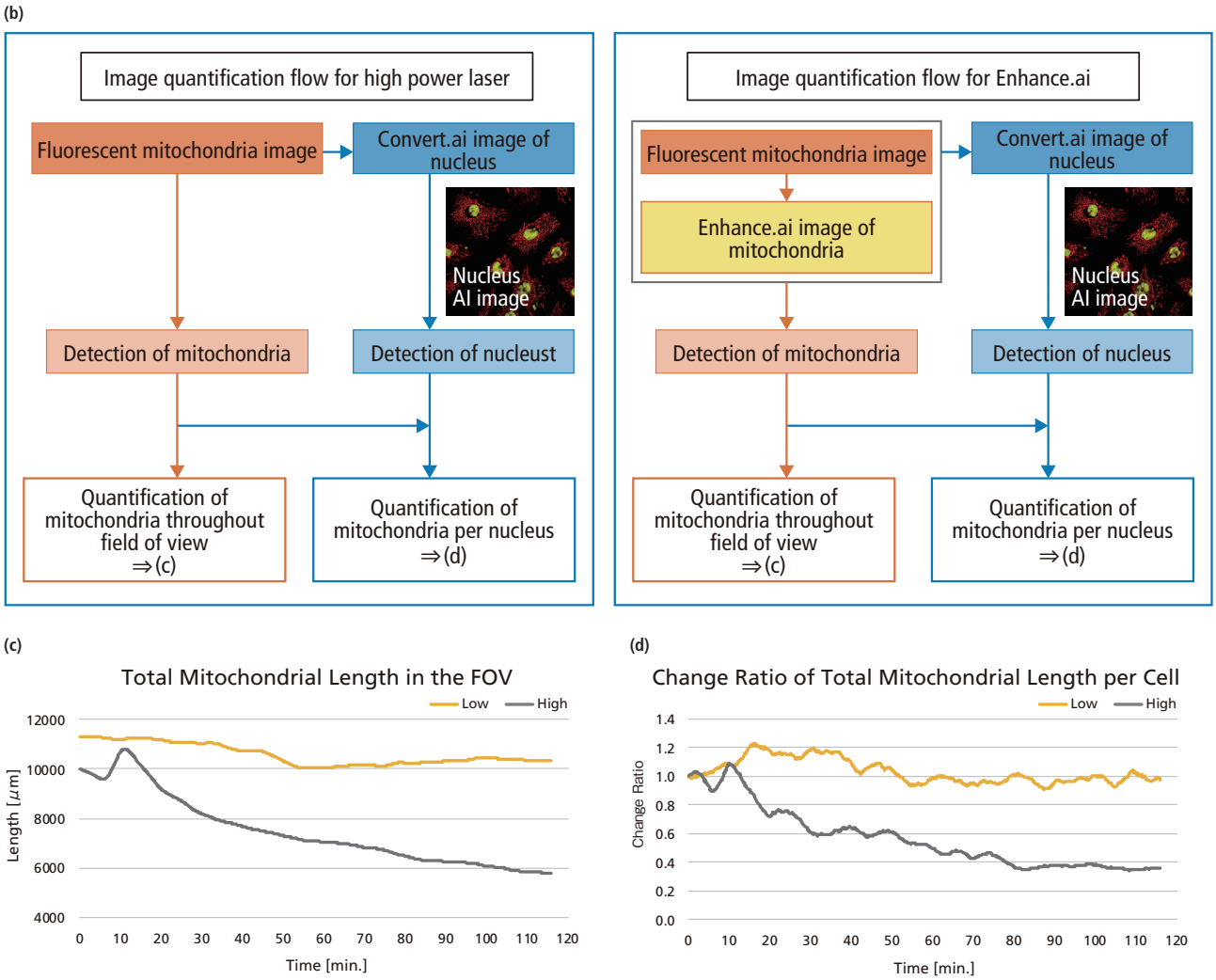

Figure 3

(b): Mitochondria quantification workflow, Convert.ai training condition: Training model is constructed using 20 pairs of fluorescent images of mitochondria and nuclei (labeled with Hoechst 33342 (Thermo Fisher Scientific, H3570)).

(c): Temporal changes [min.] in the sum [μm] length of mitochondria within the field of view. A movement average (n = 25) was obtained for the values of every point.

(d): Temporal changes [min.] in the rate of change calculated from the sum length of mitochondria per cell. A movement average (n = 25) was obtained for the values of every point.

The sum length of mitochondria within the field of view and the sum length of mitochondria per cell were calculated, and each temporal change was measured. The number of cells within the field of view was calculated by inference of the nucleus area in the fluorescent mitochondria image using the Convert.ai function of NIS.ai (Figure 3. (b)). During observation with strong excitation light, fragmentation and destruction of mitochondria progressed immediately after the start of observation and the effects of phototoxicity were apparent. Under these conditions, it is not possible to correctly measure the behavior of cells. On the other hand, in the case of observation with weak excitation light, values were mostly stable over 120 minutes of observation, proving that correct measurement is possible even under minimal excitation light conditions (Figure 3. (c-d)).

Conclusion

As shown above, Enhance.ai enables short-interval observation at short intervals and long-term time-lapse observation, which were previously difficult due to the effects of excitation light. As a concrete example, although vesicle transport in cells is an extremely high-speed phenomenon, and molecular-level observation is difficult because fluorescent signals are weak, Enhance.ai is expected to contribute to these studies. [4,5]

Moreover, NIS-Elements enables easy and accurate quantitative evaluation. Therefore, effective use of Enhance.ai is expected to be able to visualize life phenomena that could not be easily evaluated with fluorescent microscopes.

Reference literature

1) Laissue et al. Nature Methods. 2017; 14(7): 657-661.
2) Knight et al. Am J. Physiol. Cell Physiol. 2003; 284: 1083-1089
3) Mehta et al. J. Biol. Chem. 2019; 294(10): 3385-3396
4) Tojima et al. J. Cell Science. 2019; 132: jcs231159
5) Rosendale and Perrais. Int. J. Biochem. Cell Biol. 2017; 93: 41-45

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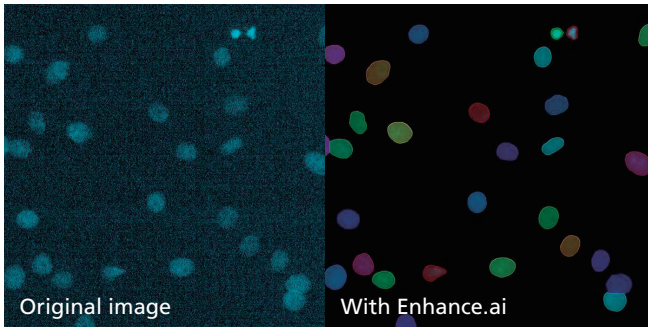
Product information

To obtain highly reliable measurement and analytical results, it is necessary to extract only the area of concern with a high degree of accuracy. Nikon's NIS.ai microscope AI module dedicated to NIS-Elements imaging software automates image processing using deep learning. NIS.ai converts images during or after image acquisition into images that enable accurate detection of only the area of concern, with reduced phototoxicity. As "Enhance.ai", "Convert.ai", and "Segment.ai" can enable a network to learn image processing using the user's original training images, the optimum result can be obtained for each application.

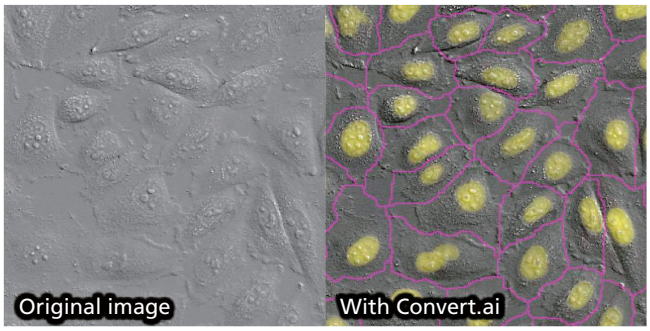
Enhance.ai

Enables a network to be trained to generate high-contrast and high S/N images from images acquired with weak signals. As accurate segmentation is possible using a fluorescent image obtained with a short exposure time or weak excitation light, analysis can be achieved with reduced phototoxicity.



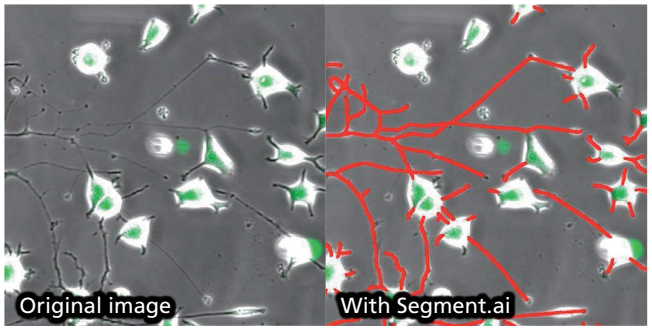
Convert.ai

Enables a network to be trained to generate fluorescent images from unstained cell images in phase contrast, differential interference contrast, and other types of images. As this makes long-term time-lapse imaging possible without fluorescent staining, non-invasive analysis that does not damage cells due to excitation light can be realized.



Segment.ai

Enables a network to be trained to generate images in which only the target cells are identified from images that contain a variety of cells. As conventional binarization cannot classify cells of a specific shape or size, manual classification is necessary; however, Segment.ai enables automatic learning-based classification.



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