AI 画像処理技術を活用したミトコンドリアの詳細解析

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Detailed Analysis of Mitochondria using AI Image Processing Technology

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ミトコンドリアは主要な細胞内小器官の一つであり，エネルギー代謝を司るため基礎研究分野のみならず創薬開発等の応用分野で幅広く研究されている．ミトコンドリアの研究では，ミトコンドリアを蛍光分子で標識し解析する手法が主流であり，動態の解析にはタイムラプス撮影が不可欠である．しかし，蛍光観察では蛍光分子の励起を行うことにより，光毒性によるミトコンドリア活性や形態への影響，蛍光の褪色が生じ得る．また，ミトコンドリアは微細な構造であるため，定量解析を行う場合には鮮明な画像を用意する必要がある．このため，可能な限り低い励起光強度や短時間露光の条件で，細胞へのダメージや蛍光褪色を回避し，鮮明な画像を得る技術が求められている．

顕微鏡画像統合ソフトウェア NIS-Elements は artificial intelligence（AI）の一種である深層学習技術を用いた画像処理機能（NIS.ai）を搭載している．本稿では，低い励起光強度で取得した不明瞭な画像から明瞭な画像を生成する機能（Enhance.ai）を用いることで，ミトコンドリア解析における光毒性を回避できる効果を確認した．さらに，焦点面外から発せられる蛍光シグナルを除去する機能（Clarify.ai）もミトコンドリアの定量解析に有用であることを示した．これらの技術により，従来よりも正確かつ詳細なミトコンドリア動態解析が可能になり，現象解明への寄与が期待される．

Mitochondria, which are one of the major organelles for controlling energy metabolism, have been extensively studied in the fields of basic and applied research of biology, drug discovery research, and so on. Mitochondrial studies generally utilize the method of staining with fluorescent molecules while time-lapse imaging is indispensable for analyzing mitochondrial dynamics. However, in fluorescence experiments, the excitation of fluorescent molecules may affect mitochondrial activity and morphology owing to phototoxicity and fluorescence photo bleaching. In addition, owing to the fine structure of mitochondria, it is necessary to prepare a clear image when performing quantitative analysis. Therefore, a technique for obtaining clear images which avoids damage to cells and fluorescence photo bleaching under the conditions of excitation light intensity and short exposure as low as possible is desired.

The NIS-Elements imaging software for microscope, is implemented with an image processing function using deep learning technology (NIS.ai). In this study, we confirmed the effect of avoiding phototoxicity in mitochondrial analysis by using the function (Enhance.ai) to generate a clear image from an unclear image acquired using low excitation light intensity. Furthermore, it was shown that the function for removing blurred light from outside the focal plane (Clarify.ai) is also useful for the quantitative analysis of mitochondria. These technologies enable more accurate and detailed mitochondrial dynamics analysis than before, and are expected to contribute to the elucidation of phenomena.

Key words ライフサイエンス，顕微鏡，画像処理，深層学習，ミトコンドリア
life science, microscopy, image analysis, deep learning, mitochondria

1 Introduction

In the life sciences, digital image processing is commonly used for specimen images acquired using biological microscopy.

Recently, various image processing techniques that use deep learning technology have been investigated. These techniques have been proposed to improve the image quality of the acquired images and are expected to be applied to various research fields because these can facilitate imaging under conditions that are less toxicity to biological specimens. Additionally, these techniques are likely to improve the efficiency of quantitative analysis.

NIS-Elements is an imaging software for microscope systems. It provides microscope and camera control, image processing, analysis, and reporting functions. Nikon implemented NIS.ai modules as image-processing function based on deep learning technology in NIS-Elements. In this paper,

we focused on mitochondrial research and introduce several effective applications of NIS.ai functions along with Enhance.ai, a function that generates a clear image from a blurred image taken under conditions that minimize the effects of staining and other treatments on biological specimens.

2 Importance and Challenges of Mitochondrial Research

Mitochondria, which are intracellular organelles, play an important role as sites for producing the energy used by living organisms. Accordingly, mitochondrial activity and quality maintenance mechanisms are implicated in various diseases. Hence, these mechanisms have attracted considerable attention in areas ranging from basic research to drug discovery and applied research [1].

Time-lapse observation using a microscope enables the real-time visualization of biological phenomena. Therefore, microscopic time-lapse imaging is an indispensable technique for mitochondrial research, where changes in morphology and behavior over time are indices for analysis [2]. However, fluorescence staining is used to visualize mitochondria, and a high excitation light intensity and long exposure time are required to obtain clear fluorescence images for quantitative analysis. In time-lapse imaging, repeated exposure to excitation light inevitably causes photobleaching and phototoxicity owing to the production of reactive oxygen species [3]. This can decrease the accuracy of structure detection and quantification owing to photobleaching or damage the cells, which may affect the biological phenomena under observation [4]. By contrast, if the excitation light intensity is suppressed and the exposure time is shortened to avoid phototoxicity and photobleaching, obtaining clear images with a detectable mitochondrial morphology becomes difficult. Hence, a technique is needed to obtain clear images under the lowest possible excitation light intensity and a short exposure time while avoiding damage to cells and the photobleaching of fluorescence.

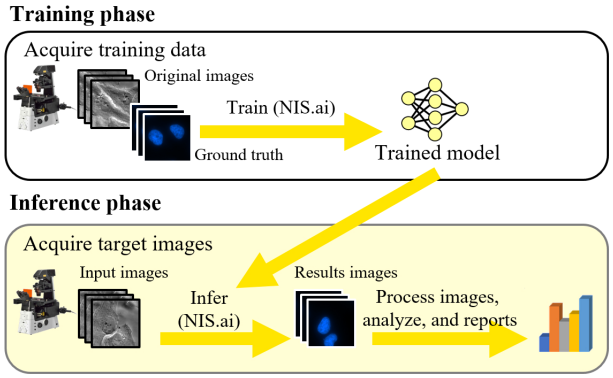

Fig. 1 Training and inference workflow using NIS.ai

3 AI Image-Processing Technology and Time-lapse Morphological Analysis of Mitochondria

NIS.ai uses convolutional neural networks, a type of deep learning, and employs supervised learning, which requires training data. Notably, Enhance.ai can be trained by users. Specifically, processing with Enhance.ai requires training, which consists of two phases: a training phase, where a trained model is further trained using training data prepared by the user, and an inference phase, where target images are output from the target data (input image) using the result of the training (Fig. 1).

The training data is a pair of fluorescence images. One image has an undesirably low signal/noise ratio (S/N) and a small difference in fluorescence intensity between the background and signal regions, whereas the other image has a high-S/N and is clear. The resulting training model is applied to the low-S/N fluorescence image, which is processed to obtain a high-S/N fluorescence image. The left fluorescence image in Fig. 2 is a low-S/N fluorescence image of the labeled mitochondria, which was enhanced by contrast adjustment, resulting in a blurred image with significant noise. When Enhance.ai is applied to the image, a high-S/N

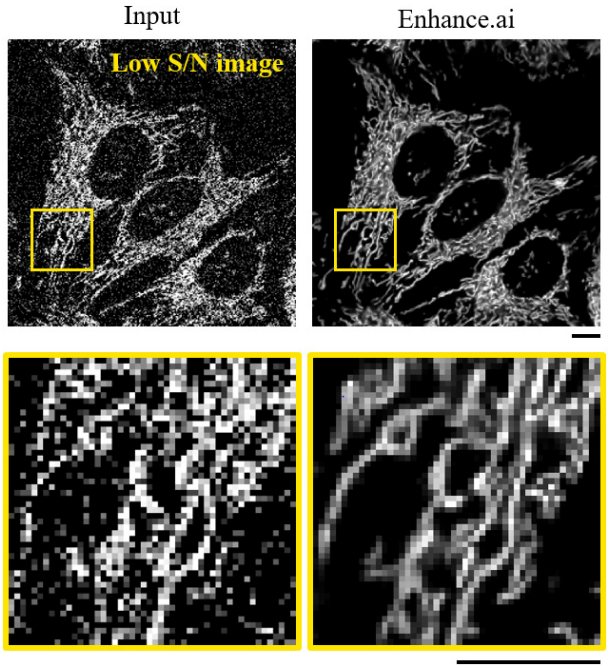

Fig. 2 Improved image S/N using Enhance.ai
Input: Low-S/N fluorescence image input to Enhance.ai. Enhance.ai: Inference results using Enhance.ai. The lower panel shows the enlarged image of the yellow rectangle in the upper panel. Scale bars are 10 μm.

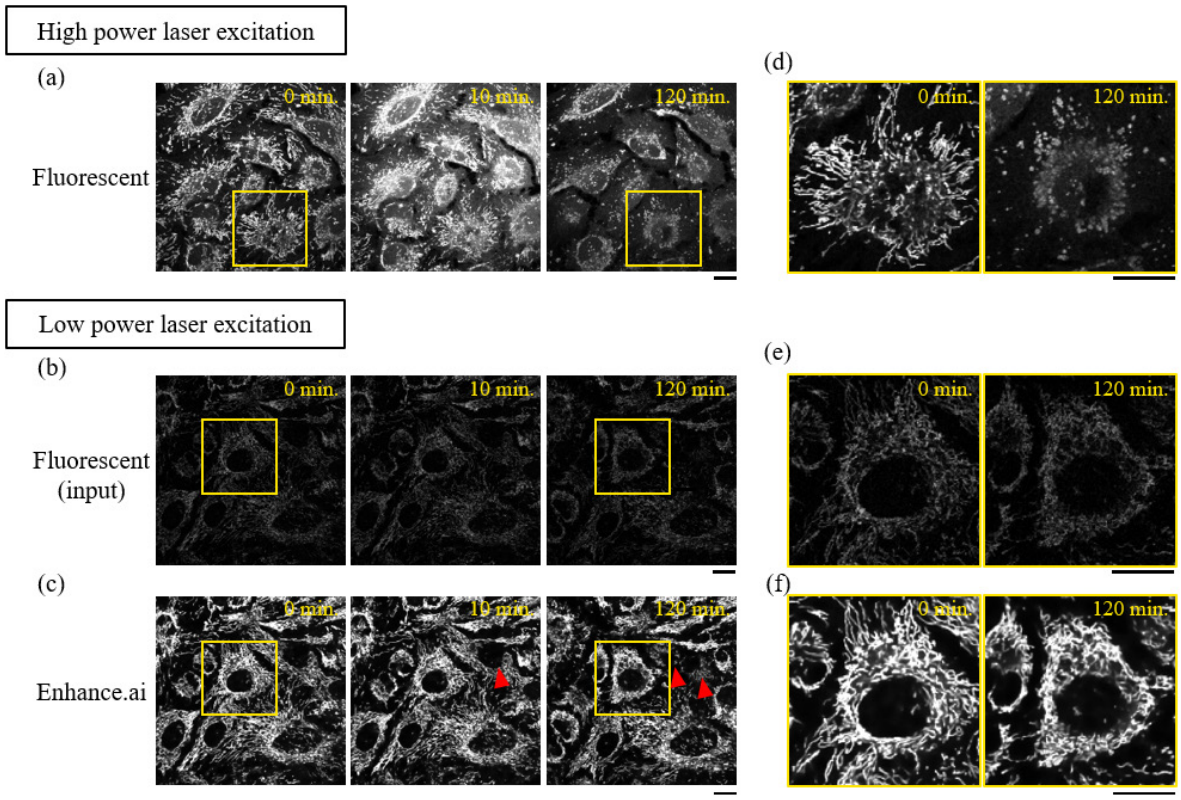

Fig. 3 Comparison of mitochondrial morphology under fluorescent excitation and Enhance.ai output

(a-c) Time-lapse images at 0 min (left), 10 min (center), and 120 min (right) acquired every 10 s. Scale bars are 20 μm.

(a) Fluorescence image of MitoTracker Red CMXRos (Thermo Fisher Scientific, Waltham, US) excited by a high-power laser.

(b) MitoTracker Red CMXRos fluorescence image excited by a low-power laser, used as input image to Enhance.ai. The image contrast setting was increased up to 4-times compared to (a).

(c) Output image using Enhance.ai with (b) as input image. Contrast is adjusted with (a). Red arrowheads indicate cells undergoing division.

(d-f) Magnified images of the yellow rectangle in (a-c), respectively. Scale bars are 20 μm.

image, in which individual mitochondria can be observed, is obtained, as shown in the right image in Fig. 2.

To verify the effects of using Enhance.ai combined with a low excitation-light intensity for fluorescence to study mitochondrial morphology, a confocal microscope was used to acquire two types of time-lapse images every 10 s for 120 min. High-S/N images were obtained by adjusting the intensity of the laser, which served as the excitation light, and low-S/N images were obtained by setting the laser intensity to the minimum. Enhance.ai was applied to the low-S/N images.

When the laser intensity was set to obtain high-S/N images, mitochondrial phototoxicity appeared approximately 10 min after the observation started, and fragmentation progressed to complete destruction after 120 min (Fig. 3(a), (d)). This is understood to be due to the phototoxic effect of the excitation light on the mitochondria and their fundamental cellular function. However, when fluorescence images were obtained with the laser intensity set to the minimum, the images were indistinct (Fig. 3(b), (e)), but Enhance.ai made individual mitochondria very clear (Fig. 3(c), (f)). No changes in image quality due to mitochondrial fragmentation or photobleaching occurred even 120 min after the observation started. Additionally, cell division progressed during the 120 min of observation (Fig. 3(c), red arrowheads), suggesting that the cells maintained their normal functions.

Next, the changes in mitochondrial morphology over time were evaluated quantitatively (Fig. 4). For quantitative analysis, the image processing and measurement functions of NIS-Elements, i.e., General Analysis 3, were used. By using the images enhanced by Enhance.ai for image analysis, mitochondrial morphology could be accurately identified and measured (Fig. 5). As measurement items, we calculated the total length of mitochondria, within the image field in which the mitochondrial region was detected, along with the rate of change in the total length of mitochondria per cell. Additionally, the changes were observed over time. The number of cells in the field of view was quantified by detecting the cell nucleus region in the fluorescent image of mitochondria using the Convert.ai function, which is an NIS.ai functions.

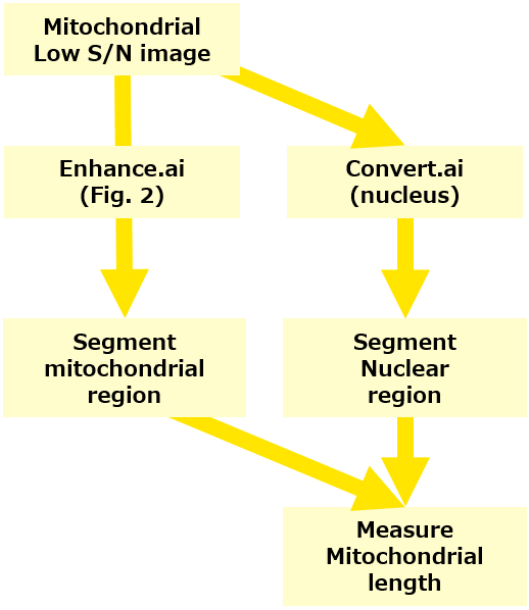

Fig. 4 Flow of mitochondrial analysis using Enhance.ai

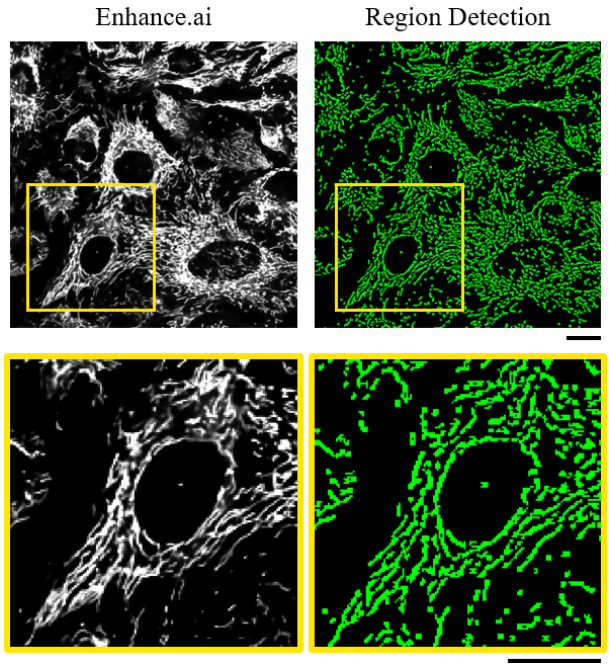

Fig. 5 Mitochondrial region detection using Enhance.ai output image

Enhance.ai: Enhance.ai output results. Region detection: Mitochondrial regions detected using the General Analysis function. The bottom image is a magnified image of the yellow rectangle in the upper panel. Scale bars are 20 μm.

The results showed that when the laser intensity was high, mitochondrial morphology began to change approximately 10 min after the observation started, quantitatively indicating the effect of phototoxicity. By contrast, when the images were acquired with minimal laser intensity and Enhance.ai was used, mitochondria length remained constant throughout the 120 min of observation (Fig. 6).

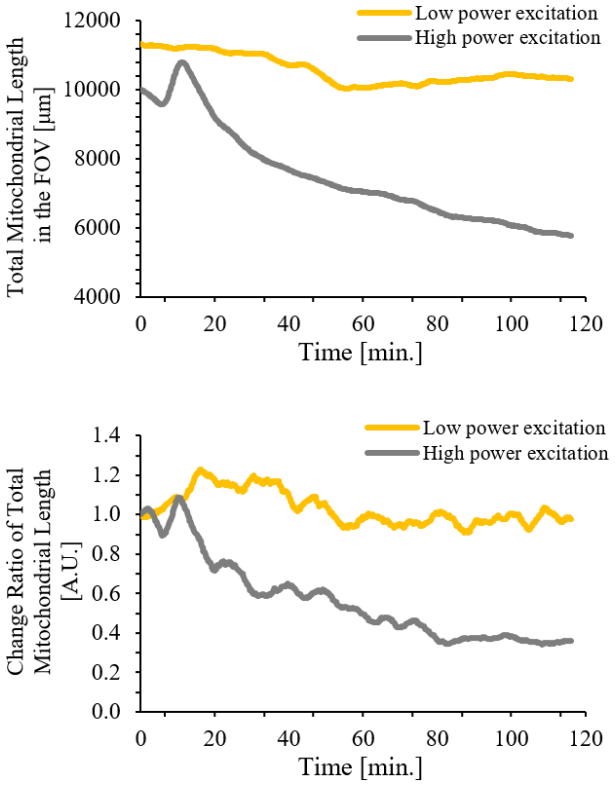

Fig. 6 Quantification of changes in mitochondrial morphology over time

Graph showing the change in total mitochondrial length [μm] (top) and the rate of change of the total mitochondrial length [A.U.] in the field of view. Enhance.ai was used for the images acquired with low-power fluorescence excitation (yellow) and high-power fluorescence excitation (gray). The time interval is 10 s.

These results show that the Enhance.ai function can be used to avoid the effects of phototoxicity on mitochondria and to analyze their morphology quantitatively.

Overall, Enhance.ai is an effective tool for assessing mitochondrial quality over extended periods of time and for analyzing the detailed dynamics of mitochondria and related molecules. We expect it to be used in basic research on mitochondria and drug discovery.

4 Other Examples of Application of AI Functions to Mitochondrial Analysis

Clarify.ai, a function of NIS.ai, is an image quality improvement function that removes out-of-focus fluorescence without training.

Compared to the images captured with a confocal microscope, images acquired with a CMOS camera using a conventional epifluorescence microscope may blur owing to the leakage of fluorescence from outside the focus. Mitochondria are extended and distributed not only in the XY direction but also in the Z direction in the cell, which can cause

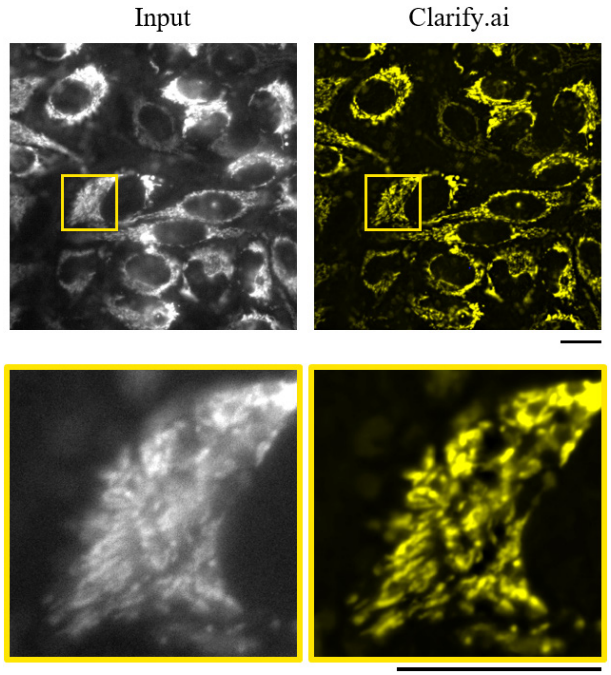

Fig. 7 Mitochondrial fluorescence image sharpening using Clarify.ai
Input: An image with severe out-of-focus light leakage.
Clarify.ai: Inference results using Clarify.ai. The lower panel shows the enlarged image of the yellow rectangle in the upper panel. Scale bars are 20 μm.

leakage of fluorescence from outside the focus. When mitochondria images containing multiple out-of-focus fluorescent components are acquired, it is difficult to accurately detect mitochondria regions through image processing. By inputting such images to Clarify.ai, clear, in-focus images can be obtained (Fig. 7). This makes it possible to detect and quantify mitochondrial regions without using microscopic observation methods with high Z-resolution, such as confocal microscopy.

Additionally, NIS.ai has a Segment.ai function that directly extracts target regions from image input and a Convert.ai function that can be used to output fluorescent images from unstained image input.

By training from fluorescent images and hand-drawn extraction regions, e.g., Segment.ai enables the extraction of complex morphological structures, such as mitochondria, without utilizing advanced image analysis techniques (Fig. 8). Additionally, by training Convert.ai with a set of bright-field images and fluorescence images, it is possible to generate fluorescence images from bright-field images of label-free specimens (Fig. 9). Although such digital staining techniques are not suitable for all specimens and target intracellular structures [5], they can reduce phototoxicity and eliminate toxicity caused by staining reagents in applicable specimens completely.

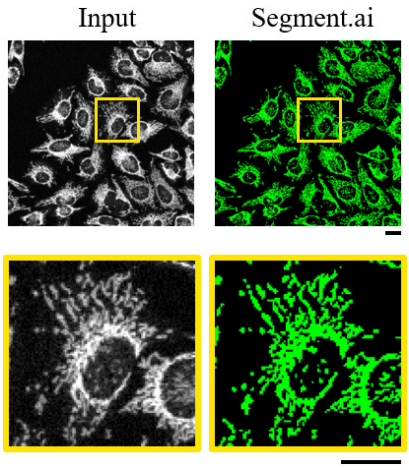

Fig. 8 Detection of mitochondrial regions using Segment.ai
Input: Fluorescence image input to Segment.ai
Segment.ai: Mitochondrial regions detected by Segment.ai.
The lower panel is a magnified image of the yellow square region in the upper panel. Scale bars are 20 μm.

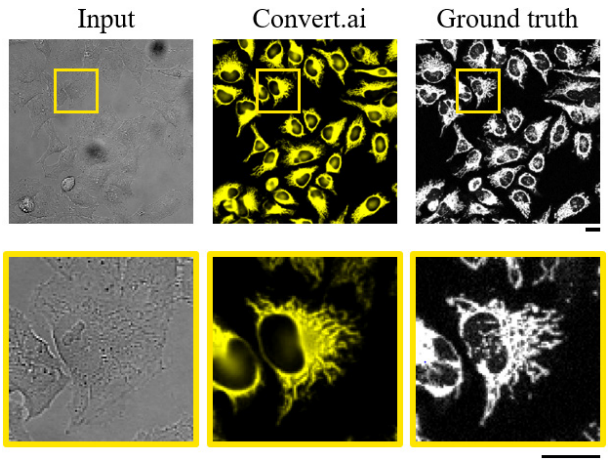

Fig. 9 Mitochondrial fluorescence images generated from transmitted-light images using Convert.ai
Input: Transmitted bright-field image input to Convert.ai. Convert.ai: Mitochondrial fluorescence image generated by Convert.ai. Ground truth: Ground truth fluorescence image. The lower panel is a magnified image of the yellow rectangle in the upper panel. Scale bars are 20 μm.

5 Overview of Materials

The cells, microscopes, and NIS.ai functions used in the experiments described above are summarized in Table 1.

Table 1　Summary of experimental materials

	Fig. 2, 3	Fig. 7	Fig. 8	Fig. 9
Cell	HeLa cells			
Microscope	Eclipse Ti2-E			
Image acquisition	Confocal microscopy (A1R)	Epifluorescence microscopy (CMOS)	Confocal microscopy (A1R)	Confocal microscopy (A1R)
Objective magnification	60x	100x	20x	20x
NIS.ai	Enhance.ai	Clarify.ai	Segment.ai	Convert.ai
Iterations	1000	Without training	1000	1000
Input	Fluorescence	Fluorescence	Fluorescence	Bright-field
Output	Fluorescence	Fluorescence	Detected region	Fluorescence

6 Conclusion

NIS.ai enables high-S/N images to be obtained without strong excitation light irradiation or long exposures. This simplifies performing time-lapse observations and analyses, which would have been difficult using traditional methods over short intervals or extended periods of time. In addition to the example of mitochondria, we expect this technique to be applied to research that requires capturing high-speed phenomena, such as intracellular vesicular transport [6], [7]. Furthermore, as this system can remove light blur from the area beyond the focus area, a problem that has hindered the detection of fine structures, we expect it to help improve the efficiency of the analysis.

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