APPLICATION NOTE

AX/AX R with NSPARC Confocal based Super Resolution Microscope powered by NIS .ai

Supercharging your NSPARC time-lapse by processing with NIS Enhance.ai

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Membrane trafficking is a typical high-speed imaging application in the life sciences. Using cellular membrane trafficking as an example, we show that Enhance.ai, a deep learning module, can be combined with the Nikon Spatial Array confocal detector (NSPARC) to speed up time-lapse imaging. This method can acquire 2D data five times faster than 3D data, while providing a better visualization than 2D deconvolution. As a result, this method can be used to increase throughput and reduce phototoxicity. Since the trained network is not a generalizable solution for deconvolution, a training procedure is necessary. This implies that custom AI tools can be integrated with the latest detector technology for enhanced results.

Keywords: high resolution, time-lapse, organelle, ER, lysosome, AI, contact sites, deconvolution, pixel reassignment, Alzheimer's Disease, acquisition optimization

Introduction

The flow of membrane material between endomembrane compartments and the plasmalemma is essential for protein transport and other macromolecules inside and outside the cell. This process is critical for proper cell function and has been implicated in several diseases, including Alzheimer's Disease [1] .

In the membrane trafficking field, membrane contact sites (MCS) have recently become of increasing interest [6;7]. These areas of close connections between organelles are highly preserved throughout evolution [2], and it is believed they play a crucial role in facilitating signaling. Appropriate functioning of the endoplasmic reticulum (ER) heavily relies on these contact sites for lipid synthesis and establishes close connections with various organelles, including mitochondria, Golgi, endosomes, lysosomes, peroxisomes, chloroplasts, and the plasma membrane [3;4;5].

Measuring and tracking these organelles, and more specifically, MCSs in living cells, typically requires a high-speed imaging device with high resolution to reveal the movements of the endomembranes.

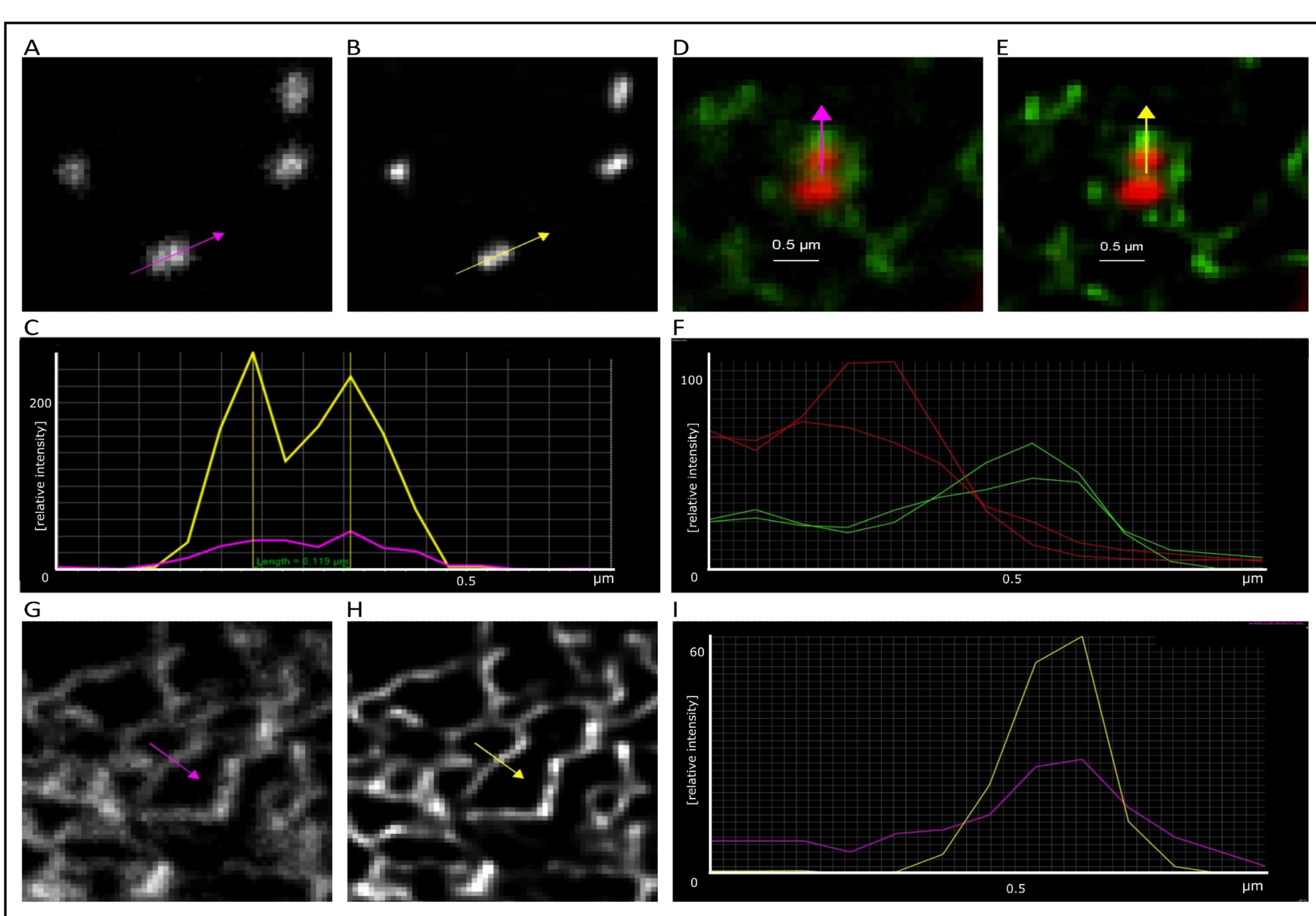

Figure 1: NSPARC images of GATTAquant sample and biological sample

A) GATTAquant DNA Origamis 120B sample (120nm spacer-AF488) imaged with NSPARC detector and PLAN APO λD 100x OIL.

Comparison between raw data (A) and processed (B) results (3D deconvolution, Richardson-Lucy, 100 iterations).

C) Intensities of A and B at locations indicated by magenta and yellow arrows, respectively. Similar quantifications as in A, B, and C have been performed with biological samples, where D and E depict the ER and lysosomes of a mouse embryonic fibroblast, and F shows the intensity distribution at the magenta and yellow arrows in D and E. The colors in the graph refer to the color channels in D and E. Processed NSPARC data have higher peaks than the raw data. Similarly, G and H compare enhanced and raw data. The intensity distribution is marked by the respective arrows.

High-resolution time-lapse imaging of membrane contact sites

Here, we are exploring the contact sites between ER and lysosomes related to their dysregulation with respect to Alzheimer's Disease [1], focusing on contact site duration, size, and number. At present, this is challenging with current imaging methods, but new technology developments open the door to improving the traditional trade-offs in microscopy that have imposed limits on accessing these small, fast-paced, short-lived biological processes.

For this purpose, we used the NSPARC detector on a Nikon Ti2 AX confocal microscope together with the NIS Enhance.ai module. NSPARC utilizes an ultra-low-noise detector array to capture a two-dimensional image at each scanned point. The Image Scanning Microscopy (ISM) method enhances the signal-to-noise ratio through increased signal levels using the reassignment method, allowing for imaging with lower excitation power.

The NSPARC detector consists of an array of 25 elements, allowing for oversampling of the standard single airy unit emission from the confocal plane. This two-dimensional data is promptly utilized to capture ultra-fine structural details that are typically not captured using conventional detection methods.

To achieve optimal visualization results, pixel reassignment is combined with a deconvolution process, resulting in a two-step standard procedure, leading to impressive outcomes (**Fig 1**). However, using the NSPARC optimally restricts the maximum speed benefits from the resonant scanner since oversampling is required for optimal deconvolution and sufficient photon collection for optimal reassignment.

Moreover, this effect is exacerbated if one aims for ideal super-resolution results using 3D deconvolution, as multiple z-planes are required. Reducing the number of frames will speed up the imaging. However, this may result in only 2D deconvolution being applicable, which often leads to less satisfactory results as compared to 3D deconvolution. In the 3D deconvolution case, the 3D shape of the point spread function and hence information from more planes can be utilized (**Fig. 2**). This is important for these short-lived contact sites since the accurate depiction of organelle distribution and high imaging speed are crucial.

Hence, the question is, how can we improve this situation to optimize the imaging of fast cellular processes? In other words, can we reduce the frames in the z-direction, which are needed for 3D deconvolution, and thus speed up the imaging process to gain temporal resolution without compromising the imaging quality for the specific task of imaging quick membrane events?

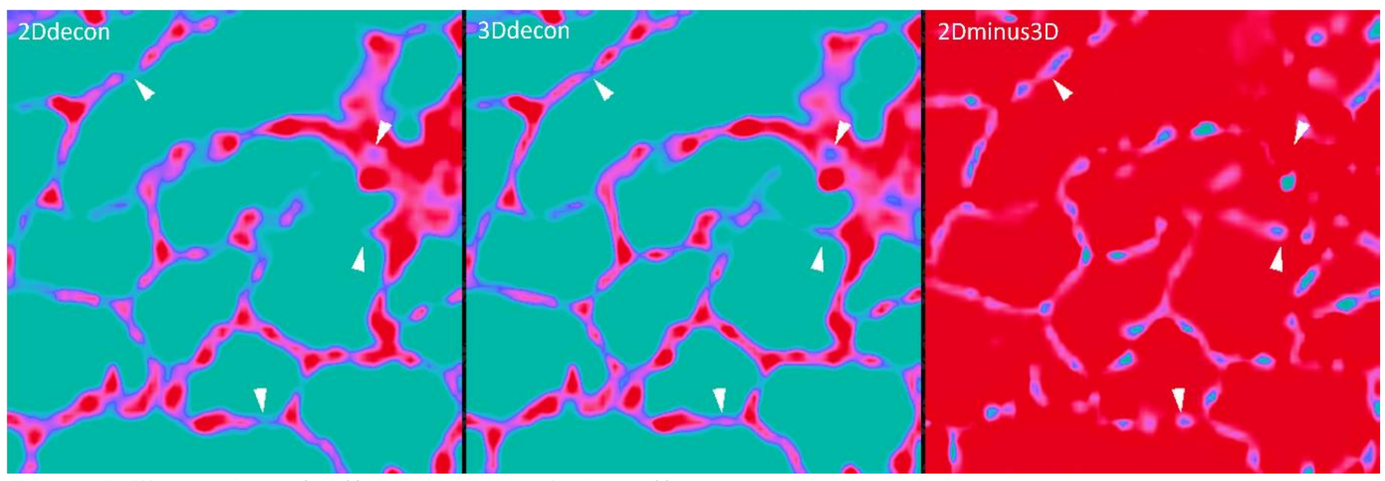

Figure 2: Illustration of different deconvolution effects
Differences between 2D deconvolution (left) and 3D deconvolution (middle) (Richardson-Lucy, ten iterations, medium noise). Right panel: 2D minus 3D.

For this purpose, we devised an innovative solution of training NIS Enhance.ai, a neural network, on 3D deconvolved data to enhance our 2D datasets as these can be acquired faster than 3D data, hence gaining imaging speed.

As such, Enhance.ai is a deep learning module that uses deep convolutional neural networks to improve the quality of captured images. The module uses a supervised learning approach, which involves acquiring a dataset with pairs of images captured in two settings – low quality and high quality (ground truth). The network is trained to transform the low-quality image into a high-quality image, making it a flexible tool suitable for any specific use case and image modality.

Result

We trained NIS Enhance.ai to confirm that it can mimic the results of 3D deconvolution on a 2D dataset and to enhance NSPARC data for improved speed and quality in imaging ER-lysosome contact sites.

The intention was to transform the 2D deconvolution process with AI into a 3D one, containing high spatial frequencies and keeping structural detail, leveraging Enhance.ai as an easy-in-reach and ready-to-use commercial tool for data acquired on a commercial microscope. Training is straightforward and does not require complex knowledge of math or neural networks. All parameters and hyperparameters are calculated automatically without user interaction.

After training (for more information, see the Material and Method section), the network can be used to enhance the quality of other similar images, increasing throughput and reducing phototoxicity on samples. Enhancing samples is fast, taking seconds to minutes depending on the dataset size and GPU card performance.

While the above is a general description of how NIS Enhance.ai works, here our goal for the enhance module was to achieve two objectives:

1. Enhance the contrast/signal in the image to resemble images acquired with higher laser power
2. Mimic 3D deconvolution so that we can use it on 2D datasets and gain speed

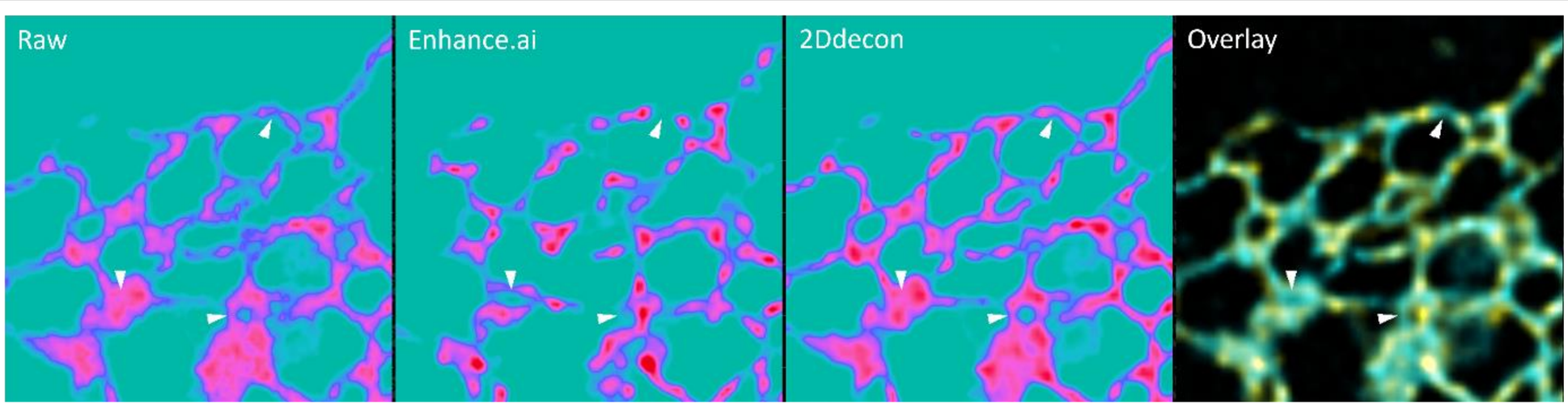

Figure 3: Compromised Enhance.ai results compared to 2D deconvolution result
Illustrating mismatch/differences (fltr: raw / Enhance.ai (mimic 3D decon + enhanced contrast) / 2D decon (Richardson-Lucy, 10 iterations, medium noise) / overlay Enhance.ai (yellow) and 2Ddecon (cyan)

To obtain the training dataset, we acquired each channel twice: first, we used the actual acquisition settings (prevent bleaching and enable fast imaging). Second, we used the settings we would ideally use to obtain a high-quality/contrasted image (with high laser power).

However, the sample's high motility limited our acquisition options. Unfortunately, acquiring two consecutive frames without sample movement is impossible, and using this dataset to train Enhance.ai creates processing artifacts, which compromise the data (**Fig. 3**).

Therefore, we limited ourselves to training Enhance.ai to only mimic 3D deconvolution on a 2D dataset. We used a 3D dataset for training, but the resulting AI network will be applied only to 2D datasets. A depiction of the concrete steps carried out can be found in **Fig. 4**.

Figure 4: Schematic depiction of the steps applied in our case

The acquisition speed for a 2D 2-channel time-lapse was 273ms/frame whereas the speed for the 3D case, with 3 planes and 2 channels was 1.462s/frame. This means that 2D data can be acquired with a fivefold speed increase as compared to 3D data, while Enhance.ai provided a better visualization than the 2D deconvolution, albeit less good than 3D deconvolution.

Although we gain speed, it is unlikely that a neural network would recapitulate a full 3D deconvolution in any general way. Therefore, we wanted to assess the divergence with the 2D deconvolution in this specific trained case. In addition, we verified that the network doesn't hallucinate and leads to artificial thinning of structures, which is sometimes observed using extreme filtering strategies.

For this purpose, we used image pairs and applied a Fourier Ring Correlation measure. Fourier Ring Correlation measures the degree of correlation between two images at different spatial frequencies [8] and is sometimes used to estimate the resolution/distribution of spatial frequencies in given image pairs. This is an estimation as the slight movement within the time-lapse images of the image pairs can affect the measurement. However, as all comparisons are affected by the error, it can be applied here as a relative measure. As expected, **Fig. 5** shows that 2D deconvolution yields an improved frequency distribution compared to the raw image. In addition, when compared to 2D deconvolution, Enhance.ai does not lead to surprising frequency distributions. Consequently, we analyzed the images to test for differences in the results stemming from the image content. We analyzed the time series for track length and area contacts/track.

Fig. 5B shows that the results follow a general trend with increasing resolution/frequencies in the image and are in line with the expectations.

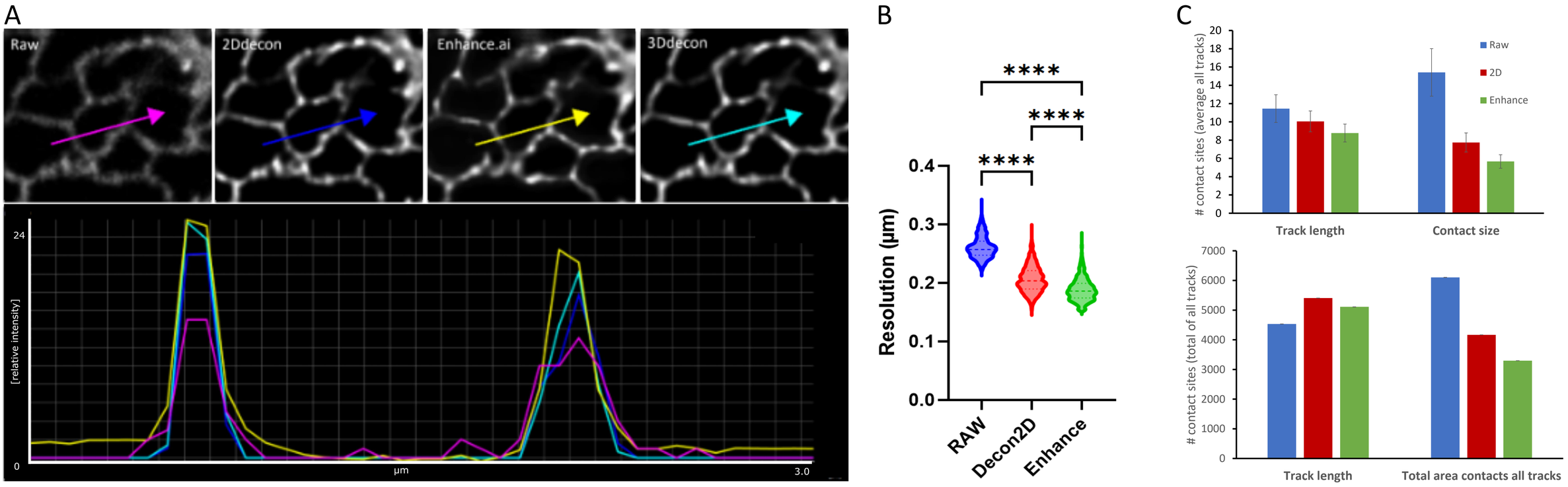

Figure 5: Validation of Enhance.ai network A) Comparison of raw NSPARC data (magenta), 2D deconvolved (blue), Enhance.ai (yellow) result, and 3D deconvolved image (Richardson-Lucy, 10 iterations, medium noise). B) Spatial frequencies in the raw, 2D deconvolution and Enhance.ai images. C) Quantification of imaging results using tracking in GA3. Top graph shows average values, bottom graph summed results.

Conclusion and Outlook

Here, we show how Enhance.ai can be combined with NSPARC imaging and applied to a question on membrane trafficking.

This deep learning module uses deep convolutional neural networks to 'boost' and speed up imaging in cellular membrane trafficking data, acquired with the NSPARC detector. In our case, the 2D data can be acquired five times faster than 3D data, and while Enhance.ai was not as effective as 3D deconvolution, it provided better visualization than 2D deconvolution.

Enhance.ai uses a supervised learning approach that transforms various use cases and image modalities with a limited number of images required. Once trained, the network can enhance similar images.

The results here show that using our approach can increase throughput and reduce phototoxicity. However, most importantly, we can use it to speed up the acquisition process in the context of NSPARC imaging and overcome the limitation of using 3D input for deconvolution to obtain optimal imaging results. Our analysis of image quality (**Fig. 5B**) and image content (**Fig. 5C**) show that the results and quality are not compromised in our concrete example. While Enhance.ai can be applied on similar images, the trained network is not a generalizable solution for deconvolution, and in comparison, to 3D deconvolution, a training procedure needs to be applied.

Overall, our data indicate how the custom use of AI tools readily available in NIS can be combined with the latest-generation detectors to yield superior results, promising new insights in disease-relevant applications.

Material and Methods

Cells

Live imaging was performed on wild-type mouse embryonic fibroblasts expressing sec61-GFP and treated with LysotrackerTM Deep Red to assess the dynamics of the endoplasmic reticulum and lysosomes, respectively. More specifically, the data generated at high resolution and speed allows for both a qualitative and quantitative assessment of the organelles' shape and distribution and the communication between them at membrane contact sites. Images were acquired on a Nikon Ti2 AX confocal equipped with an NSPARC detector. We used a Nikon PLAN APO λD 100x lens with 1.45 NA (oil immersion) for acquisition.

Enhance

Enhance.ai was trained following the general recommendations.

For efficiency, an NVIDIA GPU card is recommended for optimal performance. Enhance.ai typically requires 20-100 images acquired under the same imaging conditions and sample type. Images should be acquired at different ROIs to ensure variability and mutual alignment between low- and high-quality channels.

Image augmentation is done automatically before training to expand the dataset and train a more robust network. During training, sub-volumes of data are sampled from the training dataset and fed into the neural network. The network extracts features from these images, processes them, and reconstructs the ground truth image from the corresponding low-quality image. Over time, the network gets better at enhancing images.

Analysis

All analysis was performed using NIS-elements 6.02.03.

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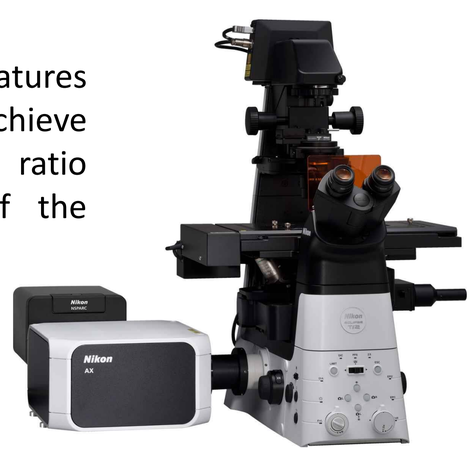
Product information

AX/AX R with NSPARC Confocal-based Super Resolution Microscope

The super-resolution detector NSPARC features an array of 25 subunit detectors to achieve higher resolution and signal to noise ratio without comprising the functionality of the conventional AX/AX R confocal microscope.



Product information is here

NIS.ai Module for Microscopes (Enhance.ai)

The NIS.ai module of NIS-Elements imaging software utilizes deep learning to learn from data training, improving image processing and analysis workflow. Enhance.ai function of NIS.ai enables a network to be trained to generate high-contrast and high Signal-To-Noise (S/N) images from images acquired with weak signals.

General Analysis (GA)/General Analysis 3 (GA3) analysis module

An optional software for customizing the automation of image analysis. GA3 can customize automation of analytical and statistical processing of complex workflows such as 3D volume imaging and 4D tracking.