Volume EM analysis

Large-scale quantification of human cytomegalovirus particles in a PFIB-SEM volume

Introduction

Electron microscopy (EM) is capable of generating highly detailed images of biological specimens; these contain vast amounts of information and can be leveraged to answer critical biological questions. By combining EM with advanced image analysis, you can also extract quantitative and spatial information from these complex datasets.

Specifically, the analysis of large 3D EM volumes enables the visualization and quantification of cellular structures within their spatial context (Figure 1). This offers critical insights into the organization, interaction, and ultrastructural architecture of cells.

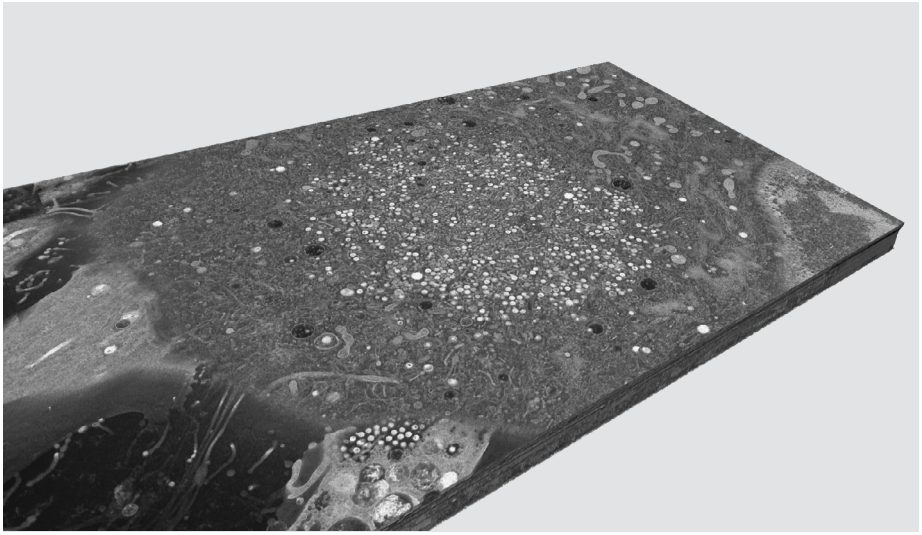

Figure 1. 3D volume rendering of a human cytomegalovirus (HCMV)-infected fibroblast cell, with a voxel size of 6 x 6 x 20 nm and total volume of 540 µm³. Sample provided courtesy of Dr. Clarissa Read (Ulm University) and Prof. Jens von Einem (Ulm University Hospital).

Technical challenges

The analysis of large 3D datasets can often be labor and time intensive; this is particularly true for the segmentation and separation of biological structures, as well as the quantification of cellular features. Furthermore, image quality frequently needs to be enhanced through pre-processing steps such as contrast improvement, noise reduction, and background removal in order to maximize reliable visualization and analysis.

AI tools and techniques

Recent advances in AI are transforming the analysis of these large 3D microscopy datasets. This includes image enhancement tools that can improve image quality by reducing noise and increasing contrast, as well as AI-based segmentation tools that can automate structural identification.

Thermo Scientific™ Amira™ Software integrates such advanced AI-driven denoising and segmentation capabilities, enabling you to process large datasets more efficiently in order to reveal complex 3D cellular architectures with minimal manual intervention. By accelerating image processing and segmentation workflows, AI helps you obtain meaningful biological insights faster and with greater confidence.

Application example

A 3D dataset of an HCMV-infected fibroblast cell was acquired using a Thermo Scientific™ Helios Hydra™ PFIB-SEM (plasma focused ion beam scanning electron microscope) and the Thermo Scientific™ Spin Mill Bio™ Method. An oxygen plasma FIB was used to reduce charging. The dataset covered a total volume of 540 μm^3 with a voxel size of 6 × 6 × 20 nm. This approach enabled the visualization of the viral assembly compartment, as well as thousands of individual viral particles, all within the cellular context.

Image processing and analysis were performed using Amira Software (Figure 2). The acquired 3D image stack was first registered and denoised. A subset of the images was manually annotated to identify both naked and enveloped capsids, as well as the cytoplasmic viral assembly compartment (cVAC) membrane. These annotations were used to train a 2D U-Net–based pixel segmentation model, which was subsequently used to segment the entire dataset.

Following segmentation, viral particles were quantified and characterized. A distance map was also generated from the cVAC and viral particle segmentation labels. This produced a spatial representation in which each pixel (that belongs to a viral particle) was assigned a value corresponding to its shortest distance from the cVAC membrane. This could be used to quantitatively analyze the particle distribution relative to the cVAC.

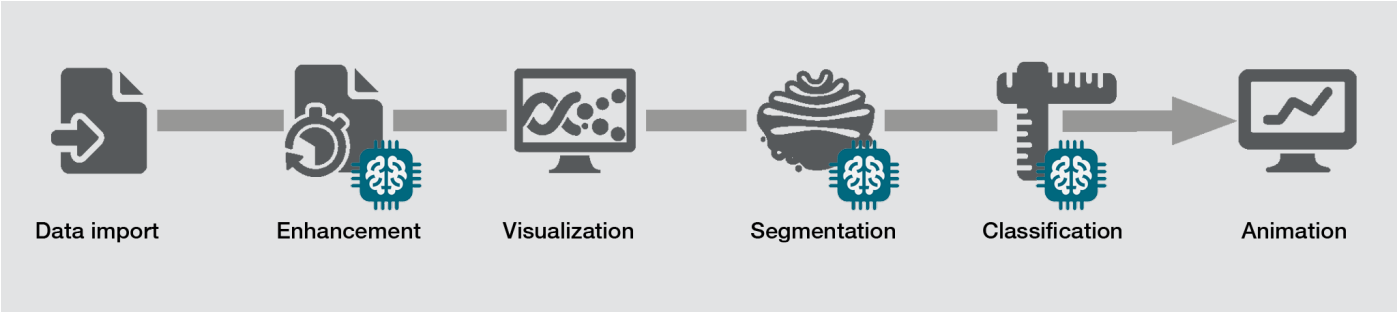

Figure 2. The Amira Software workflow used in this application note, highlighting data import as well as pre-processing, segmentation, and quantitative analysis.

Results

Quantification of viral particles

Using AI deep learning–based segmentation, more than 3,000 viral particles were identified within a single infected cell, enabling the discrimination of two particle populations: enveloped and naked capsids. Specifically, 2,927 enveloped capsids and 501 naked capsids were detected (Figure 3).

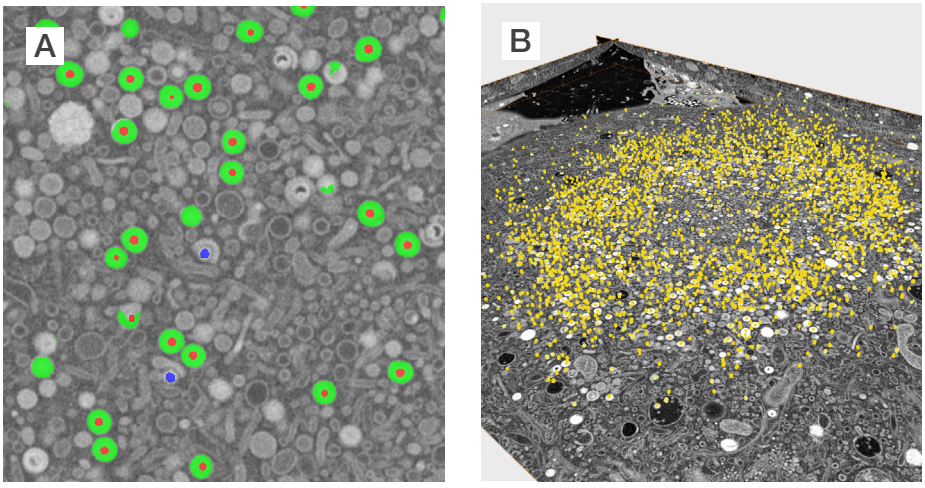

Figure 3. Visualization of HCMV particle segmentation and quantification results. (A) 2D image showing the identification of naked (blue) and enveloped (green and red) capsids. (B) Detection of more than 3,000 viral particles, shown in 3D. Sample provided courtesy of Dr. Clarissa Read (Ulm University) and Prof. Jens von Einem (Ulm University Hospital).

Spatial distribution of viral particles within a cell

A quantitative analysis of particle distance from the cVAC boundary was performed (Figure 4). Distances are color-coded, with warm colors indicating particles located farther from the cVAC and cool colors indicating particles in close proximity to the cVAC. Corresponding box plots show the distribution of these measured distance values.

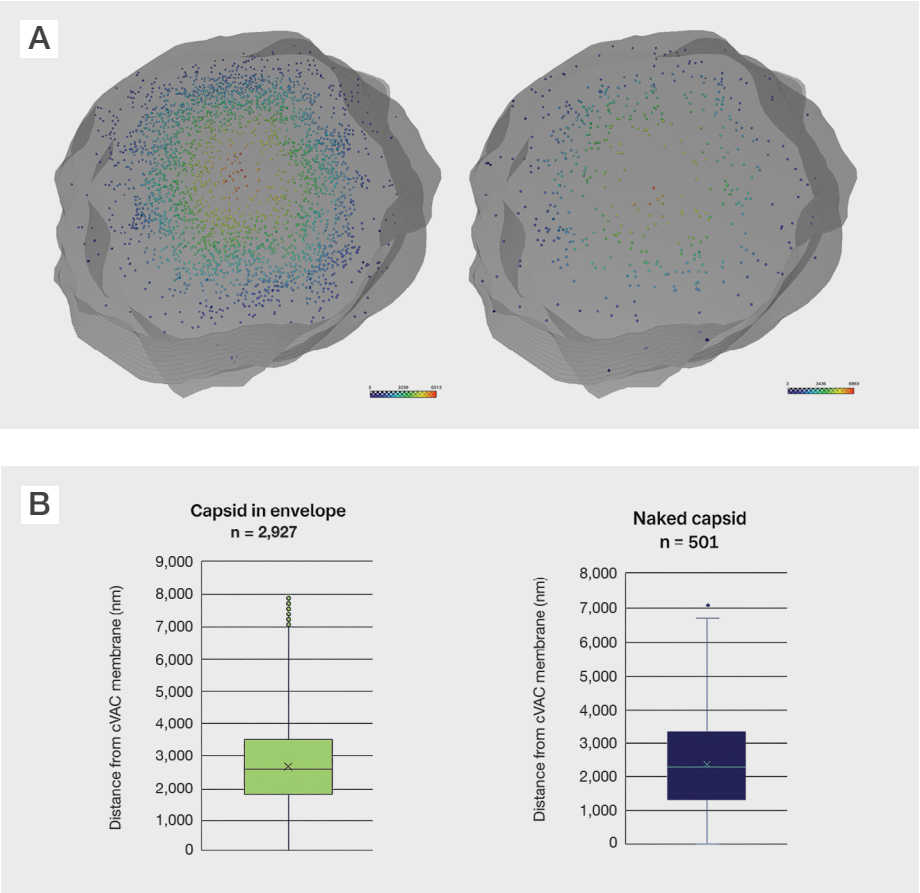

Figure 4. Quantitative spatial analysis of HCMV particle distribution relative to the cVAC. (A) Enveloped and naked capsids are color-coded according to their distance from the cVAC membrane (shown in grey). (B) Corresponding box plots showing the distribution of the measured distances.

Conclusion

This study demonstrates how AI-powered segmentation tools in Amira Software enable the efficient analysis of large PFIB-SEM datasets. Using a limited set of manual annotations, a 2D U-Net model was trained to segment viral particles and cellular structures throughout an entire fibroblast volume.

The resulting quantitative analysis identified more than 3,000 viral particles and revealed their spatial distribution relative to the cVAC. Amira Software can facilitate the characterization of viral assembly, maturation, and intracellular trafficking for HCMV particles, and has the potential to support the analysis of many other biological datasets. These results highlight the potential of AI-assisted workflows to accelerate ultrastructural analysis and the extraction of biologically meaningful information from complex 3D EM datasets.

Learn more about volume EM data analysis with Amira Software at **thermofisher.com/amira**