Tutorial: AI Assisted Segmentation in the segmentation+ workroom

Before starting the tutorial, make sure you have the Deep Learning Python environment installed. If not, you can follow our tutorial How to Install the Deep Learning Python Environment.

Introduction

The AI Assisted Segmentation tool helps users to produce segmentations on complex images with minimal effort. This interactive approach uses a shallow neural network to provide results as fast as possible, with the AI Assisted Segmentation tool doing most of the work segmenting the image. It is particularly well suited to speeding up the processing of electron microscopy images but can also be used with other 3D datasets.

In this tutorial, the AI Assisted Segmentation tool is used to interactively segment and reproduce the results of the EMD-8594 cryo-EM image that was provided in [1]. First, a minimal part of the image is labelled, and patches added that will be used to train the AI-assisted segmentation tool. After the first result, the user can gradually improve the segmentation using the AI Assisted segmentation again or the other tools available in the Segmentation+ workroom.

Scenario: segmentation of the cryo-EM EMD-8594 image

In this image, it is possible to segment several structures of interest, such as vesicles, Mitochondria, Ribosomes and Microtubules (see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5623144/figure/F1/ for more details). From an annotation that includes all these structures, the AI Assisted tool is capable of automatically segmenting each of them. Opening the "CryoEM_Segmentation.hx" project, you will find a proposed annotation including a label image and a corresponding patch set for each structure of interest (Mitochondria, Vesicles, Microtubules, Ribosomes). The project also contains the final segmentation with all the structures (see Figure 5).

In this tutorial, we will be focusing on microtubule segmentation, to give you a good understanding of this AI-based tool.

Manual Segmentation of Microtubules

Using the brush tool, we will manually select and label microtubule structures to provide information for the AI Assisted Segmentation.

1. Load emd_8594.to-ushort00.tif.am (found in *CryoEM_Segmentation-files* of the Xtra*)*
2. Open the Segmentation+ workroom.
3. Select the XY data viewer (the viewer positioned on slice 40).
4. Select the brush tool and brush some microtubules as illustrated in Figure 1.
    - Click on the brush tool button again to de-select the tool and return to image navigation.

5. Select “Material1” in the Segmentation panel, then click “Add to” in the “Assign Selection to Material” panel.

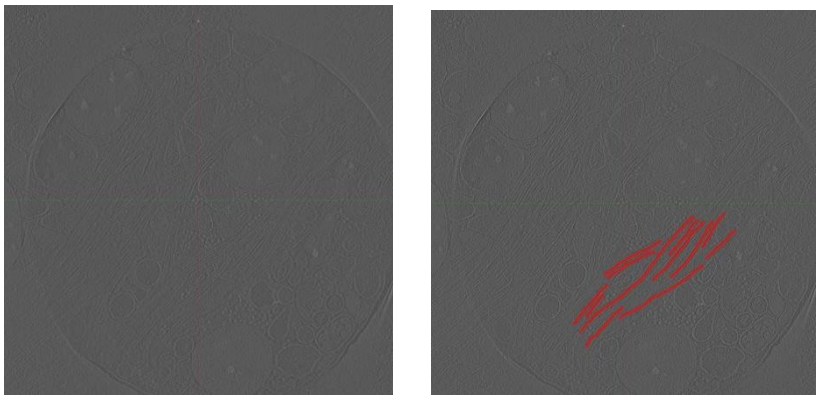


Figure 1: (left) emd_8594.to-ushort00.tif.am image; (**right)** Microtubule selection using the Brush tool.

Defining regions of interest with Patch Sets

Using patches (square areas that are used to train the AI model), we will define regions of interest that will be used to train the shallow model: 10 patches will contain labelled microtubules (Material 1) and several patches will contain counter examples (areas without microtubules). Creating a patch set changes the user interface:

- A patch set appears in the Segmentation panel below “Material 1”.
- The “Patch Set Navigation” panel opens below the “Assign Selection to Material” panel. This panel contains a slider to allow the user to navigate between patches.
- The “Add Patches” tool is accessible in the Viewer toolbar as well as in the Patch Set Navigation panel.

To create the patch set:

1. Click “Create New Patch Set”.
2. In the dialog, keep the default patch size of 64 and click Ok.
3. Select the “Add Patches” tool.
4. Click on the image to add patches with labelled microtubules inside (Figure 2).
    - If a patch contains microtubules that have not been labelled, label them following steps 4 and 5 above, or use the patch suppression.
    - To delete a patch, deactivate the “Add Patches” tool; press Esc to switch to the interactive mode, click on the border of the patch to select it, then press Delete.
    - Press Ctrl + Z to remove the last added patch.

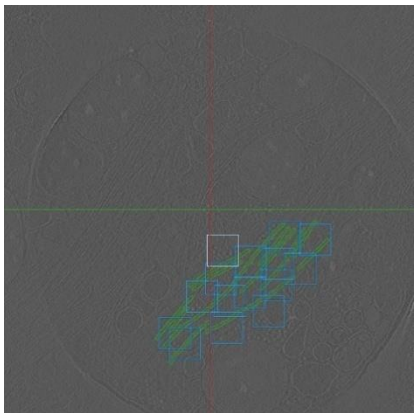


Figure 2: Addition of Patches with labelled microtubules in green

5. Add around 40 counter examples patches (Figure 3). Only add patches in areas without any microtubules, otherwise the deep learning model will have difficulty learning to identify the microtubules.

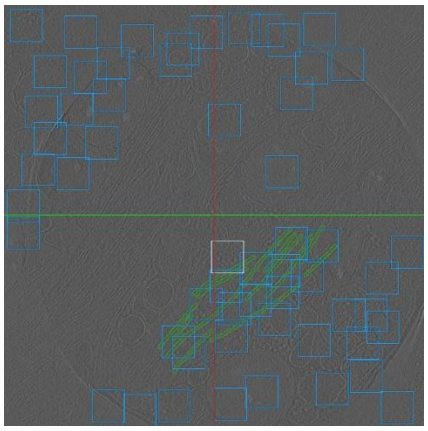


Figure 3: Addition of Patches without microtubules to provide the deep learning model with counter examples.

6. Select the AI Assisted Segmentation tool in the Automated Segmentation Tools panel, then click “Compute Label”.

The “Compute Label” process starts, and the progress bar displays the different steps to produce the result: the AI Assisted Segmentation initialization, the training stage of the shallow deep learning model, and the stage of prediction. At the end, the label image displays in the viewer as shown in Figure 4.

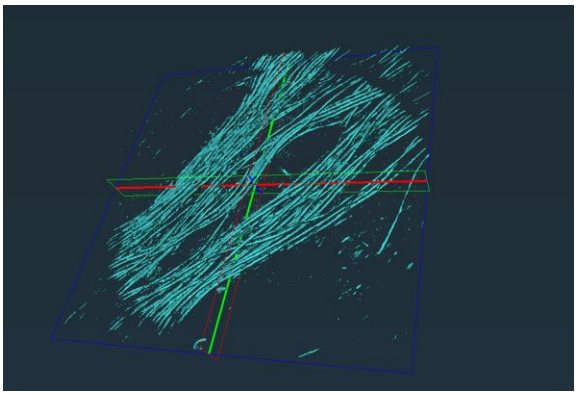


Figure 4: Segmentation obtained using the AI Assisted Segmentation tool

It is possible to improve this initial segmentation using other tools available in the "segmentation+" workroom. Post-processing tools, such as "Remove Islands" or "Smooth Label," can be utilized. Additionally, interactive tools like the "Pick" tool are very convenient for manually correcting the segmentation.

As discussed in the introduction, the AI Assisted Tool can segment all the structures of interest inside the processed image, starting from a complete annotation (refer to Figure 5 on the left). The resulting segmentation is shown Figure 5, on the right.

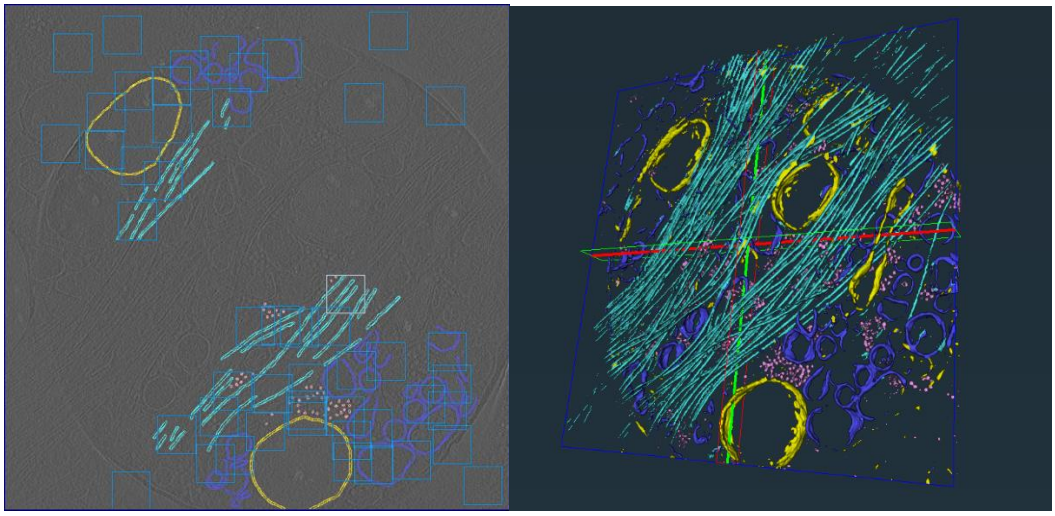


Figure 5: (left) Complete annotation of all the structures in emd_8594.to-ushort00.tif.am, **(right)** final segmentation of the four structures of interest.

References

[1] Muyuan Chen, Wei Dai, Stella Y Sun, Darius Jonasch, Cynthia Y He, Michael F Schmid, Wah Chiu & Steven J Ludtke, Convolutional neural networks for automated annotation of cellular cryo-electron tomograms, Nature Methods 14; 983-985, 2017.