

Tutorial: AI Assisted Segmentation in the Segmentation+ Workroom

Introduction

The AI Assisted Segmentation tool helps users to segment complex images with minimal effort. This interactive approach allows you to select from a range of models with the AI Assisted Segmentation tool doing most of the work segmenting the image.

In this tutorial, we use the AI Assisted Segmentation tool to interactively segment and reproduce the results of the EMD-8594 cryo-EM image provided in [1]. First, we label a minimal part of the image, then add ROIs that will be used to train the AI-assisted segmentation tool. After the first result, you 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 can automatically segment each of them. Opening the “CryoEM_Segmentation_2024_2.hx” project included in the Xtra, you will find a proposed annotation including a label image and a corresponding ROI 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 focus 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 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.

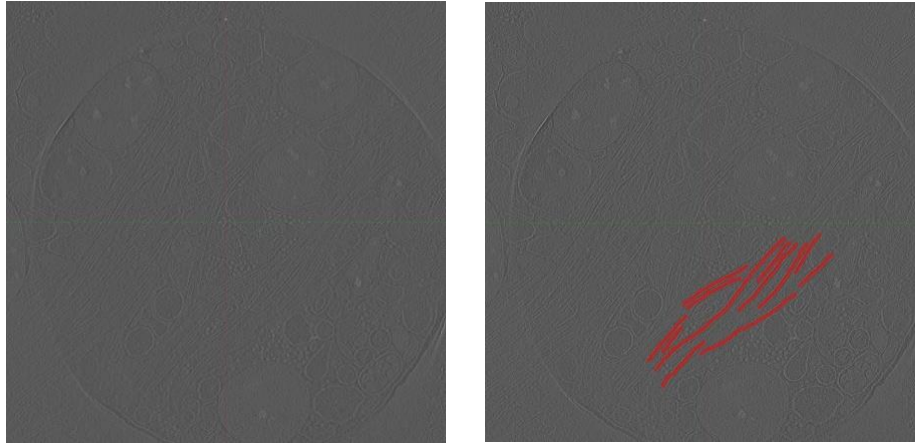


Figure 1: (left) emd_8594.to-ushort00.tif.am image; (right) Microtubule selection using the Brush tool.

Defining regions of interest with ROI Sets

Using ROIs, we define regions of interest that will be used to train the shallow model: we will define 2 ROIs in this example. Creating an ROI set changes the user interface:

- The ROI tab is opened, and an ROI set appears in the tree view.
- The “ROI Set Navigation” panel opens below the “Assign Selection to Material” panel. This panel contains a slider to allow the user to navigate between ROIs.

To create the ROI set:

1. Click “Create New ROI Set”. The ROI tab opens, and the Create New ROI button displays in the Segmentation panel.
2. Click “Create new ROI” twice to create 2 ROIs in the XY viewer.
3. Deactivate any interactive tools (such as the Brush Tool) then click in the viewer and press Esc to activate interactive mode.
4. Select each ROI then resize them to select the areas of interest.

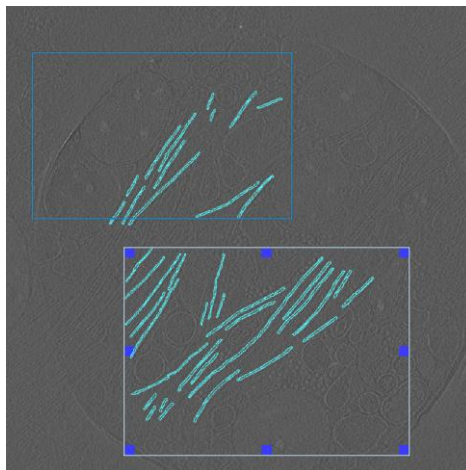


Figure 2: Definition of 2 ROIs with labelled microtubules in blue.

Make sure you label all the microtubules inside the defined ROIs to provide the most relevant information to train the model.

Additional actions:

- Select an ROI in the viewer then press delete to remove it.
- Press Ctrl + Z to Undo the last ROI action.
- Press Ctrl + Y to Redo the last ROI action.

Use the AI Assisted Tool to train a model and segment the entire image

1. Select the AI Assisted Segmentation tool in the Advanced Tools panel.
2. To create a model, click the  button.

The AI Model Selection window opens. The default model is VGG16, but other models are available: Shallow, VGG19, ResNet18, Generic UNet. After you create a model, it remains accessible through this window for reuse.

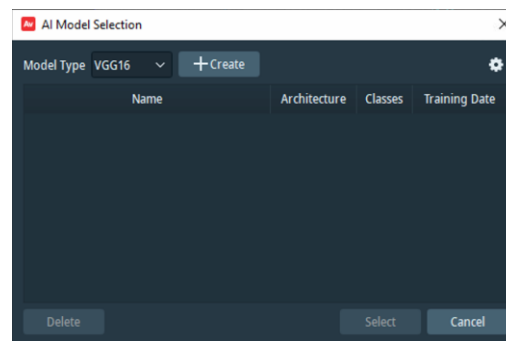


Figure 3: Deep learning model creation window. The default model is VGG16, but other models are available: Shallow, VGG19, ResNet18, Generic UNet. Once a model is created, it remains accessible through this window for reuse.

3. Keep the default VGG16 model and click “Create”. The window closes and parameters and buttons appear in the AI Assisted Segmentation tool panel. By reopening the “AI Model Selection” window, the created model is now visible in the model list.
4. Click “Launch Training” to start the model training. The progress bar indicates the different training steps.
5. Once the training is finished, click “Compute Preview” to check the model performance on a single slice.
6. Click “Compute Label” to segment the entire image.

At the end of the prediction process, the labeled image displays in the viewer as shown in Figure 4.

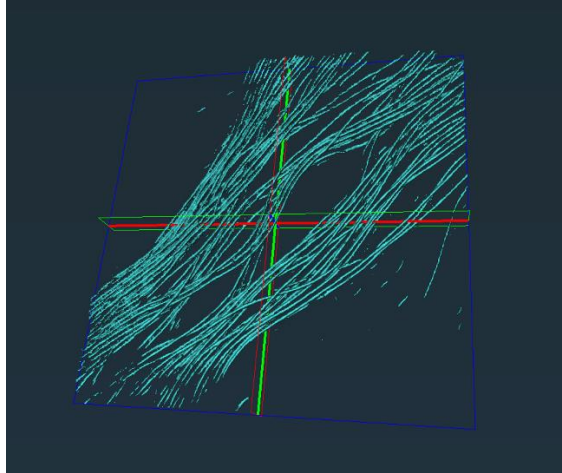


Figure 4: Segmentation of Microtubules obtained using the AI Assisted Segmentation tool.

You can improve this initial segmentation using other tools available in the "Segmentation+" workroom. You can use post-processing tools, such as "Remove Islands" or "Smooth Label", to clean up the label image. 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.

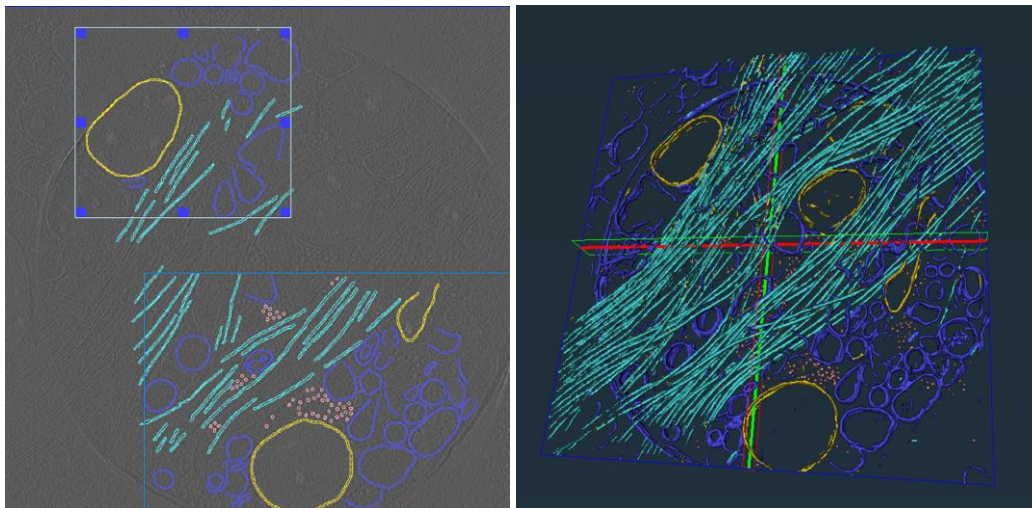


Figure 5: (left) Complete annotation of all the structures in emd_8594.to-ushort00.tif.am, **(right)** final segmentation of the four structures of interest.

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.