Mitochondria segmentation in EM data with Napari-Empanada

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Segmentation of Electron microscopy images

- Electron microscopy images stained with heavy metals
- Grey scale images containing dozens of organelles & subcellular structures

YES

Highly integrated understanding re. biological function

BUT

Major image analysis challenge!

- Specific identification of different organelle types in electron microscopy is based on some combination of the electron density, contrast, shape, and relative location of each structure

Empanada

1. **Purpose**: provide essential nutrients, energy for bodily functions, and support for growth, repair, and overall health
2. **Key Feature**: type of baked or fried turnover consisting of pastry and filling, common in Spain, other Southern European countries, North African countries, Latin American countries, and the Philippines.
3. **Dataset**:



Napari-Empanada

1. **Purpose**: Empanada-napari plugin democratizes deep learning image segmentation for electron microscopy (EM) researchers
2. **Key Feature**: Comes pre-loaded with **MitoNet**, a pre-trained specialist machine learning model for mitochondria instance segmentation in EM images.
3. **Dataset**:
   - Pre-trained on ~1.5 million unlabeled cellular EM images.
   - Fine-tuned on ~135,000 labeled mitochondrial instances.

Napari-Empanada Background

1. **Documentation:** https://empanada.readthedocs.io/en/latest/getting_started/install.html
2. **MitoNet publication:** https://www.sciencedirect.com/science/article/pii/S240547122200494X

Installation

1. Install Anaconda Navigator
2. Install Napari

```
conda update -n base conda
conda create -n empanada-ws-env python=3.9
conda activate empanada-ws-env
conda install -c conda-forge napari pyqt
```

3. Install Empanada-napari plugin

Note: do NOT install plugin through Napari GUI

```
pip install empanada-napari
```

Usage – Case 1

1. Demo Dataset: https://mitoem.grand-challenge.org/MitoEM/
   - ~ 26 GB → tiny crop
2. 3D Inference:
   - Segmentation Confidence Thr: 0.50
   - Centre Confidence Thr: 0.10
   - Centres Min Distance: 3

Usage – Case 2

1. Demo Dataset 2: Cell Gatan 3View Serial Block Face Cell
2. Load data
3. Scale data as voxels are anisotropic:

```
viewer.layers[Image_name'].scale = [5, 1, 1]
```

4. 3D Inference:
    - Segmentation Confidence Thr: 0.35
    - Centre Confidence Thr: 0.75
    - Centres Min Distance: 5

Usage – Case 2

- Segmentation is ok but room for improvement

→ Pick fine tuning patches

  - # Patches for annotation: 1
  - Patch size (px) = 224
  - Delete initial dataset and labels
  - Use 2D inference for a “head start”
    - Get initial segmentation for “clean up”
    - Tick [Output to layer]
    - On third slice of flip book
  - Run 2D inference → segmentations output to labels layer

Usage – Case 2

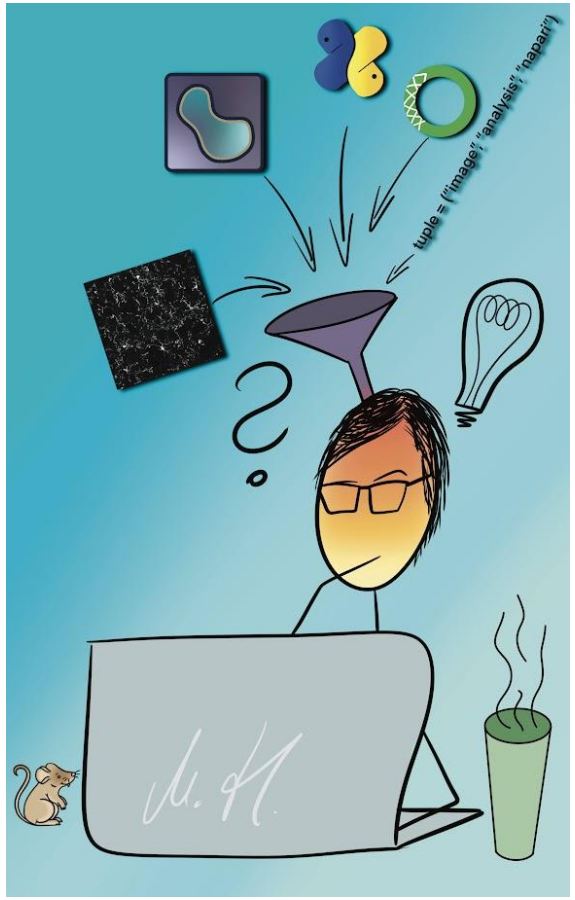
- Paint and erase labels on third slice only
  - Duplicate labels layer (if required)
  - Make edits on original labels layer
  - Select label using dropper [4]
  - Erase [1], draw[2], fill [3] as required
    - See documentation for merging, splitting of labels
  - Save training patches and labels

Usage – Case 2

- Fine tune model – ideally on GPU leveraging hardware
  - Training directory: annotated images
  - Finetunable layers:
    - **None**: Select this option if the model did fairly well during the initial inference on your data.
    - **Stages 1 - 4**: Select between these options depending on how well the model did on the initial inference.
    - **All**: Select this option if the model did not perform well on your data. This option will take more time but could offer better results depending on your specific task.

Summary

- Plugin designed to simplify the application of deep learning models for electron microscopy (EM) image segmentation.
- suited for researchers aiming to perform instance segmentation of mitochondria, using pre-trained model MitoNet
- Ideal for large-scale vEM data sets
- Leverage pre-trained deep learning models without coding experience
- Fine tune pre-trained models to custom model



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