

How to re-train the classifier

Annotating New Images

To train the model to perform better for your specific use case, begin by selecting relevant images of the same cell type and acquisition conditions as those you plan to analyze.

Pre-processing

Before annotating the images, the following steps are necessary. They can all be performed by creating a project folder for your images and using the “MyoFuse.ipynb” file as described in the “MyoFuse : How to Use” documentation.

1. **Channel Splitting:** Separate the channels of your original images to create individual files for nuclei and myotubes.
 2. **Nuclei Segmentation:** Perform segmentation of the nuclei using the custom Cellpose model.
 3. **Image Normalization:** Perform normalization of myotubes images to prepare them for training.
 4. **File Preparation:** Transfer the resulting “Images” and “Masks” folders into a new folder with a name of your choice to prepare them for annotation.
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Annotation

1. **Loading Images:** Open the Svetlana annotation plugin and load your images by selecting the folder you created in the pre-processing step.
2. **Parameters:** Keep the parameters unchanged:
 - **Patch size:** 200 pixels
 - **Number of labels:** Unchanged
3. **Annotation Process:**
 - Use your keyboard for annotation:
 - Press **1** if the nucleus is located outside a myotube.
 - Press **2** if the nucleus is located inside a myotube.
 - Follow the method described in the article, using the local loss of myotube-specific fluorescence as the basis for your annotations.
4. **Saving Progress:** Save your annotations regularly. If needed, you can resume labeling later by using the Svetlana folder generated when you first saved your annotations.

Important: Insert additional images into the initial folder before starting the annotation process. While you are not required to annotate all the images, you won't be able to add new ones later if you want to improve the results.

Training

1. **Loading Data:** Load your data by selecting the “label” file from the “Svetlana” folder.
2. **Custom Network:** Use the “Load custom network” option and select the “MyoFuse.pth” file.
3. **Training Parameters:**
 - **Data Normalization:** Use “Min-Max normalization.”
 - **Class Weights:** Keep the “Apply Class Weights” option activated.
 - **Data Augmentation:** Activate all options in the “Data Augmentation Type” section for optimal results.
4. **Evaluation:** After training, assess the model’s accuracy using the prediction module with new images that were not used for training. These new images must be pre-processed like the training images (channel splitting, segmentation, normalization). Test different models saved during the training process (found in the “Svetlana” folder).

Prediction

Once the model achieves satisfactory accuracy, analyze your images using the prediction module. Alternatively, you can replace the model path in the complete Python script with the path to your new model. Please refer to the "MyoFuse : How to use" guide if needed.