MyoRegenTrack: Quantifying Recovery Process of Skeletal Muscle on HE-Stain via Learning from Day Label Proportion
Supplementary Material

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Limitation of our softwares

When inputting the Day 0 images, which should contain only Myofibers, of tissues with inadequate freezing before CTX injection into the proposed software, obvious misclassification results were obtained. This indicates that the proposed software cannot adequately handle such unexpected domains.

Inference Pipeline

An overview of the proposed software is presented.

Applying software for laminin to HE-stained images

The results of applying OpenCSAM[1], developed for laminin-stained images, to HE-stained images are presented.

Classifier of SVM by 4 features

To supplement the classification performance discussed in the introduction using a Support Vector Machine (SVM)

Comparison of glycerol and CTX by using Pseudo-Label

Evaluate the recovery progress of mice injected with CTX and glycerol using a model trained with suspected labels. Focusing on the results from Day 5, it becomes apparent that an incorrect Recovery Score might be calculated if an error occurs in the predicted class.

References

1. Desgeorges, T. *et al.* Open-CSAM, a new tool for semi-automated analysis of myofiber cross-sectional area in regenerating adult skeletal muscle. *Skeletal Muscle* **9**, DOI: https://doi.org/10.1186/s13395-018-0186-6 (2019).
2. Encarnacion-Rivera, L., Foltz, S., Hartzell, H. C. & Choo, H. Myosoft: an automated muscle histology analysis tool using machine learning algorithm utilizing fiji/imagej software. *PLoS ONE* **15**, e0229041, DOI: https://doi.org/10.1371/journal.pone.0229041 (2020).
3. Stringer, C., Wang, T., Michaelos, M. & Pachitariu, M. Cellpose: a generalist algorithm for cellular segmentation. *Nat. Methods* **18**, 100–106, DOI: https://doi.org/10.1038/s41592-020-01018-x (2021).

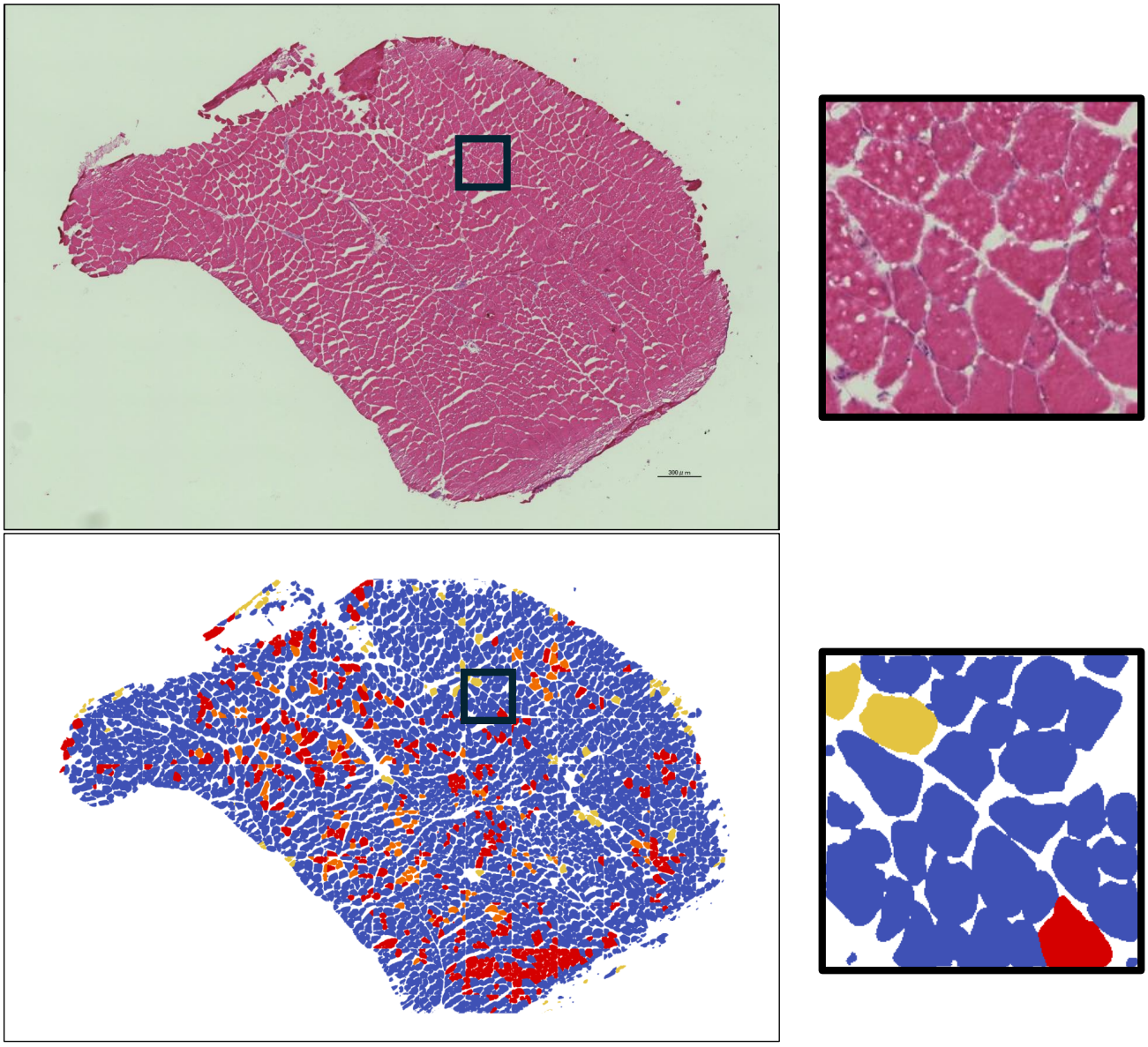

Figure 1. Results of inputting the pre-injection Day 0 images, which had inadequate freezing treatment, into the proposed software. Red corresponds to Myofiber, Yellow to Small Myotube, Blue to Myoblast, and Orange to Large Myotube.

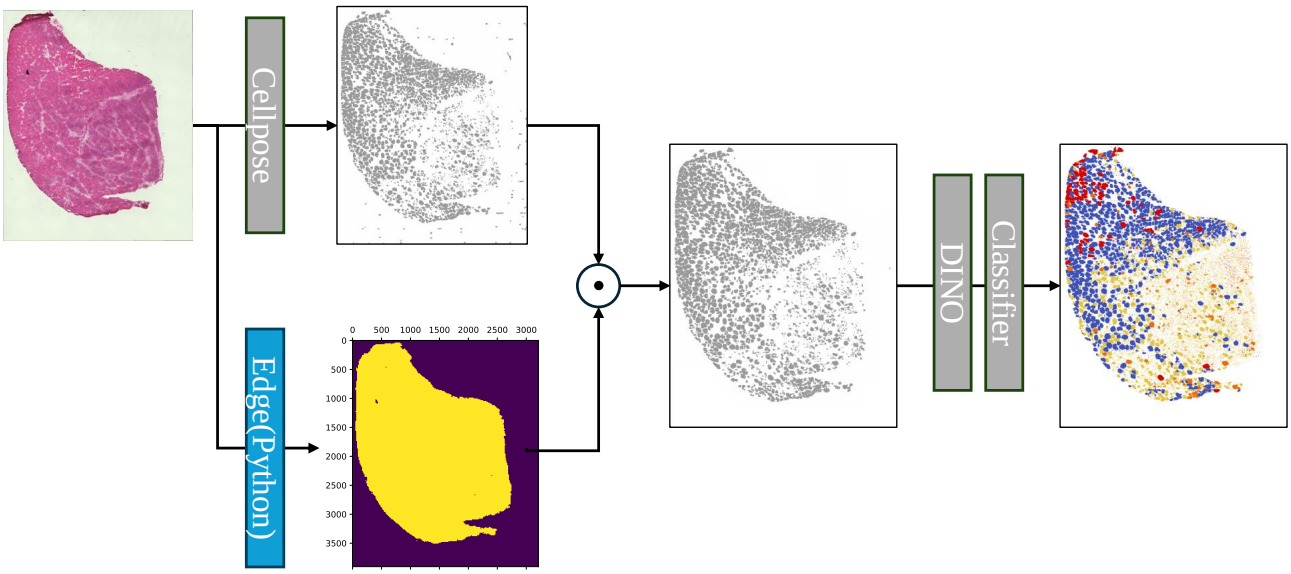

Figure 2. When input into Cellpose, the images are clipped to 256×256 [pixel], and when input into DINO and the Classifier, they are clipped to 64×64 [pixel] according to the procedure in Fig. 5(a) of the main text. Parallel to being input into Cellpose, the input images are processed by Python software for edge detection to extract masks of the stained image regions. The results from Cellpose and the edge detection are combined using the Hadamard product, resulting in segmentation data of only the stained regions. Bypassing these through DINO and the Classifier, the segmented data for each cell is color-coded according to its class and outputted.

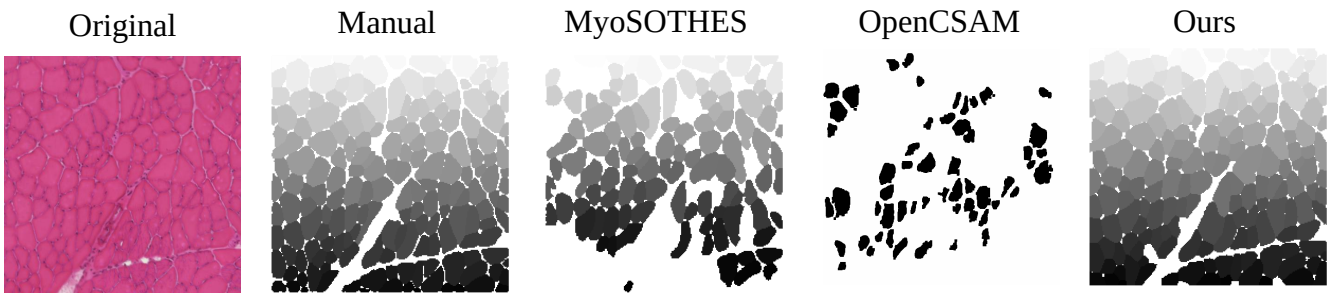

Figure 3. The input consists of 256×256 [pixel] images. Manual refers to segmentation results performed manually. The parameters for MyoSOTHES and Ours are the same as described in the main text. For OpenCSAM, the default parameters were used.

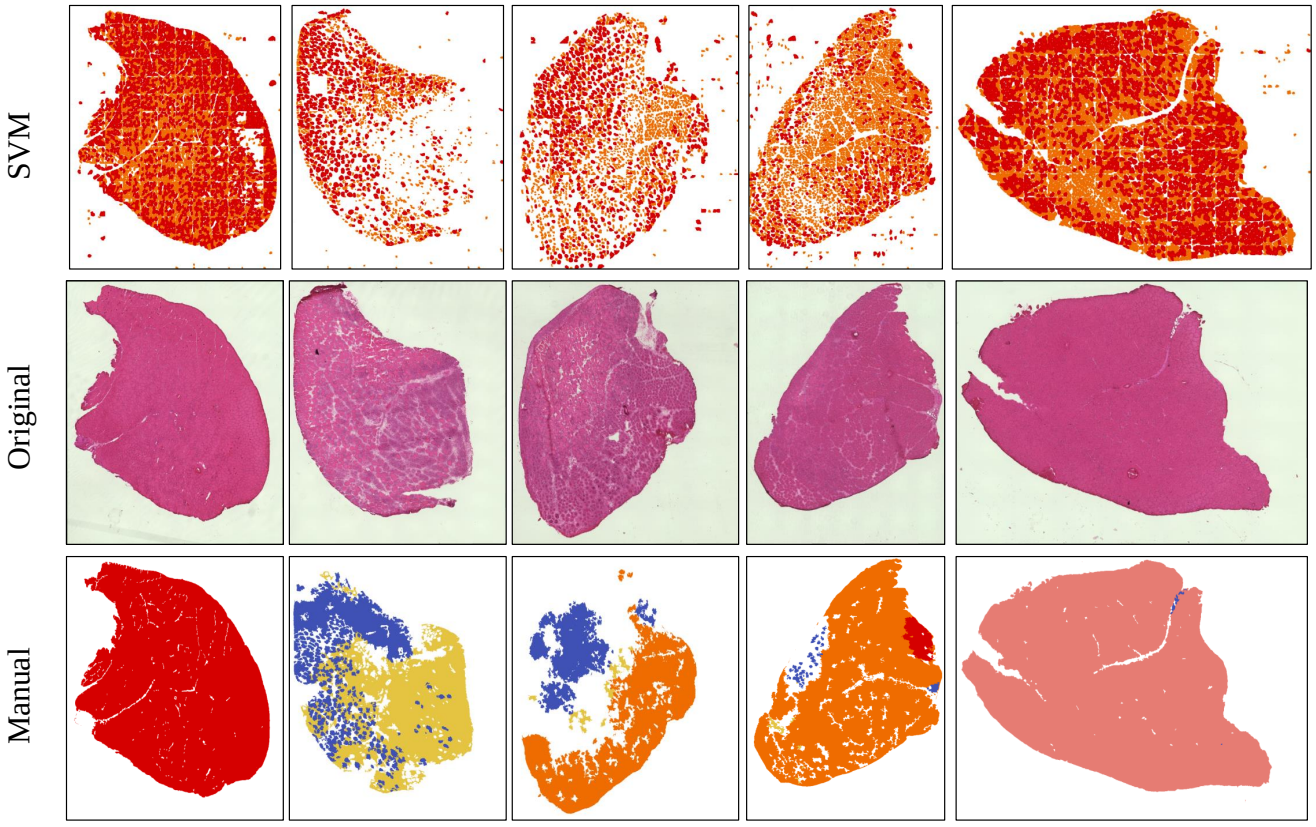

Figure 4. The classification results using a Support Vector Machine (SVM) are presented. The results are based on four features—Area, Circularity, Minimum Feret, and Feret Aspect Ratio—obtained from the segmentation in Myosoft[2], along with the date proportion data to generate pseudo-labels for SVM training and inference. The colors correspond to Red: Myofiber, Yellow: Small Myotube, Blue: Myoblast, and Orange: Large Myotube

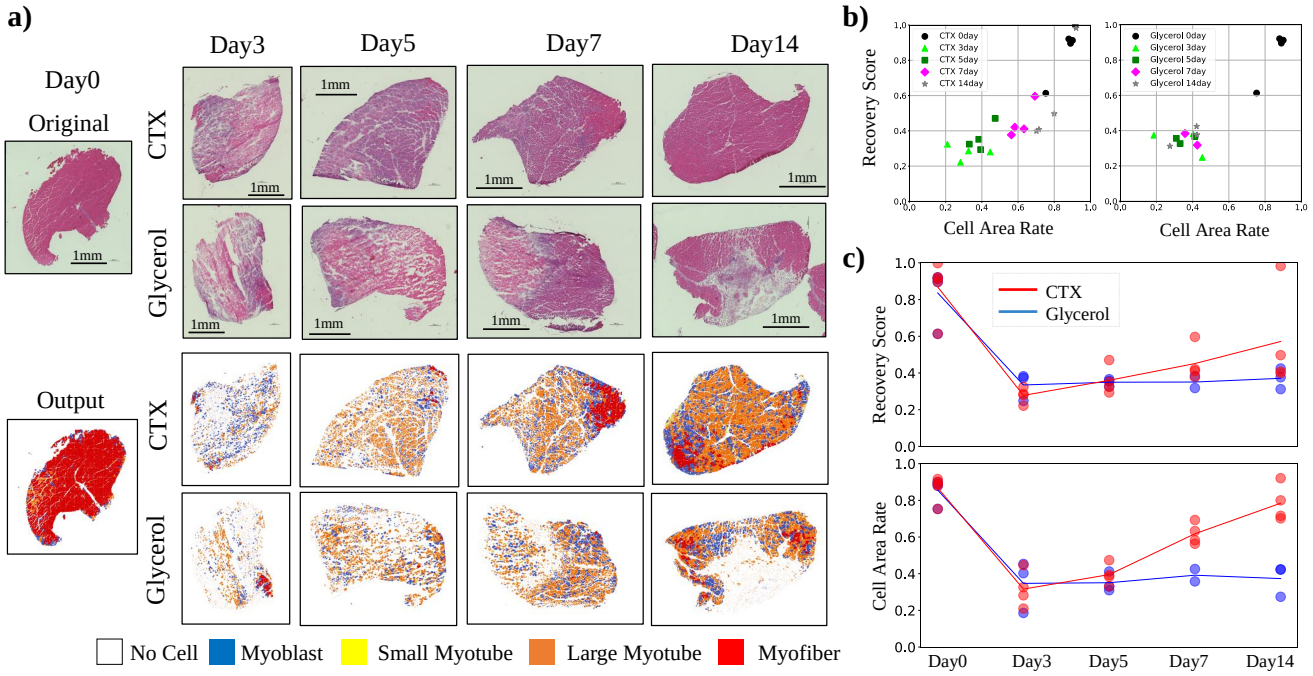

Figure 5. **a)** Day 0 represents the tissue before injection. Days 3 and beyond indicate the number of days elapsed since the injection of CTX or glycerol, which induces necrosis and recovery in the tissue. The output displays the results by pseudo-label model, where each color corresponds to Red: Myofiber, Yellow: Small Myotube, Blue: Myoblast, Orange: Large Myotube, and white indicates areas where no cells were detected.
b, c) We count the pixels of each color within the edge-detected tissue and compute the proportion $\hat{p}$ of each color relative to the Cell Area (Segmentation Area). The Recovery Score was calculated for each image. Each point in this figure corresponds to one WSI. For the Cell Area, the area ratio of cells detected by Cellpose[3] to the muscle tissue region $\frac{SegmentationArea}{StainArea}$ was calculated. Note that since Day 0 is before injection, the data for CTX and glycerol are the same.